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Mixed-Mode Microsystems for Biological Cell Actuation & Analysis

Keith R. Muir

Dedicated to
Margaret Helena Muir
1949–2017
Abstract

Personalised medicine is widely considered to be the future of global healthcare, where diagnosis, treatment, and potentially even drug development, will become specific to, and optimised for, each individual patient. Traditional population based cell studies suppress the influence of outlier cells that are frequently those of most clinical relevance. Hence single-cell analysis is becoming increasingly important in understanding disease, aiding diagnosis and selecting tailored treatment; but remains the preserve of biomedical laboratories far from the patient. Current instruments depend upon cell-labelling to identify the cell type(s) of interest, which require that these be chosen \textit{a-priori} and may not be those most clinically relevant. Furthermore, cell-labelling is fundamentally subjective, requiring highly-skilled operators to decide upon the validity of each and every test. Therefore, new test methods need to be developed to enable the widespread adoption of single-cell analysis.

The passive electrical properties of biological cells are known to be indicative of the specific cell type, but no technology has demonstrated their comprehensive measurement within a mass-manufacturable device. This work aims to show that biologically meaningful information can be obtained in the form of identifiable “cell signatures” through broadband frequency measurements spanning 100 kHz to 100 MHz that exploit the properties of differential electric fields.

This hypothesis is tested through the design, implementation and experimental testing of a dedicated microsystem that integrates two novel designs of electrical sensor within a standard, mass-manufacturable Complementary Metal-Oxide Semiconductor microelectronics technology. One sensor measures the absolute electrical environment above a single sense electrode. The other measures the difference in electrical environment between a pair of electrodes, with view to provide information regarding the suspended cell only, through rejecting the common signal due to its suspending medium. Both sensors are shown capable of detecting individual biological cells in physiological solution, and the differential sensor capable of identifying individually-fixed red blood cells, cervical cancer HeLa cells, and three diameters of homogeneous polystyrene micro-beads of comparable size, all while suspended in physiological saline.

These results confirm the hypothesis that differential electric fields provide greater distinction of suspended cells from their environment than existing electrical methods. This finding shows that electrode polarisation arising from proximity to liquids, and particularly physiological media, can be overcome through fully-differential electrical cell sensing. However, misalignment between cells and sensor electrodes limits the sensitivity achieved with the microsystem. Methods to overcome such alignment issues should be investigated in future work, along with higher frequency measurements beyond those presented here.
Lay Summary

This work investigates improvements for measuring individual biological cells for identifying their specific type without the need for choosing ahead of time what cell type(s) to look for, potentially eliminating biased decision making as required by all existing cell analysis instruments.

Cells represent the building blocks of biology and identifying what cell type a sample contains is an essential process in medical diagnostics and treatment management. Today, cell types are identified using complex instruments that use chemical labels to identify cell type(s) as chosen by their operator. If the operator does not choose correctly, potentially vital information can be lost, negatively affecting diagnosis and treatment management. The accuracy of these labels is also fundamentally limited. Consequently, existing cell analysis instruments require highly-skilled operators and are typically located in dedicated biomedical laboratories far from patients.

This work extends an alternative technique that uses the intrinsic electrical differences between cell types to identify individual cells without operator bias. The chosen technique is explored through two novel designs of electrical sensors integrated within a dedicated microchip. Two biological cell types are identified from their unique electrical signatures without the use of cell labelling and achieved with minimal sample processing. However, the misalignment of individual cells to each sensor limits the absolute sensitivity of the microchip and future implementations will need overcome this issue, to explore the ultimate capabilities of the technique.

Overall, the obtained results indicate the potential for the unbiased identification of cell types from their intrinsic electrical properties achieved with highly-scalable microchip technology, capable of supporting widespread adoption in future healthcare.
Declaration of originality

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own.

__________________________________________
Keith R. Muir
In writing a book, one accumulates a great amount of indebtedness.

A. von Hippel [1]
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<tr>
<td>AC</td>
<td>Alternating Current</td>
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<td>ADC</td>
<td>Analogue-to-Digital Converter</td>
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<td>AED</td>
<td>Absolute Electric Displacement (Sensor)</td>
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<td>ams</td>
<td>austriamicrosystems (foundry)</td>
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<tr>
<td>ASIC</td>
<td>Application Specific Integrated Circuit</td>
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<td>AZ</td>
<td>Auto-Zero</td>
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<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>CBCM</td>
<td>Charge Based Capacitance Measurement</td>
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<tr>
<td>CD</td>
<td>Cluster of Differentiation</td>
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<tr>
<td>CHO</td>
<td>Chinese Hamster Ovary (cell)</td>
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<td>CM</td>
<td>Claussius-Mossotti (factor)</td>
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<tr>
<td>CMOS</td>
<td>Complementary Metal-Oxide Semiconductor</td>
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<tr>
<td>CPE</td>
<td>Constant Phase Element</td>
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<tr>
<td>CSV</td>
<td>Comma Separated Value (file)</td>
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<td>CVC</td>
<td>Coulter Volumetric Counter</td>
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<tr>
<td>DC</td>
<td>Direct Current</td>
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<td>DEP</td>
<td>Dielectrophoresis</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DS</td>
<td>Dielectric Spectroscopy</td>
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<tr>
<td>EROT</td>
<td>Electro-rotation</td>
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<td>FACS</td>
<td>Fluorescence-Activated Cell Sorting</td>
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<td>FC</td>
<td>Flow Cytometry</td>
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<td>FET</td>
<td>Field-Effect Transistor</td>
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<tr>
<td>FFF</td>
<td>Fluid-Flow Fractionation</td>
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<td>FFT</td>
<td>Fast Fourier Transform</td>
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<td>FIFO</td>
<td>First-In, First-Out (memory)</td>
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<td>FMT</td>
<td>Fast M-Transform</td>
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<td>FPGA</td>
<td>Field-Programmable Gate Array</td>
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<td>FSR</td>
<td>Full Scale Range</td>
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<tr>
<td>HDL</td>
<td>Hardware Description Language</td>
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<td>IC</td>
<td>Integrated Circuit</td>
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<tr>
<td>IRF</td>
<td>Instrument Response Function</td>
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<tr>
<td>IS</td>
<td>Impedance Spectroscopy</td>
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<td>ISFET</td>
<td>Ion-Sensitive Field-Effect Transistor</td>
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## Abbreviations

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<td>ITO</td>
<td>Indium Tin Oxide</td>
</tr>
<tr>
<td>LHS</td>
<td>Left Hand Side</td>
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<td>LPF</td>
<td>Low-Pass Filter</td>
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<tr>
<td>LTI</td>
<td>Linear Time Invariant</td>
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<td>LVDS</td>
<td>Low-Voltage Differential Signalling</td>
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<td>MACS</td>
<td>Magnetic-Activated Cell Sorting</td>
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<td>MEMS</td>
<td>Micro Electro-Mechanical System</td>
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<td>MMT</td>
<td>Maxwell’s Mixture Theory</td>
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<td>MOS</td>
<td>Metal Oxide Semiconductor (transistor)</td>
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<td>MPW</td>
<td>Multi Project Wafer</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MSB</td>
<td>Most Significant Bit</td>
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<td>MSPS</td>
<td>Mega Sample per Second</td>
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<td>μTAS</td>
<td>Micro-Total Analysis System</td>
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<td>NMOS</td>
<td>N-channel MOS</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PC</td>
<td>Personal Computer</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed Circuit Board</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PD</td>
<td>Photodiode</td>
</tr>
<tr>
<td>PMOS</td>
<td>P-channel MOS</td>
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<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
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<td>RED</td>
<td>Relative Electric Displacement (Sensor)</td>
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<tr>
<td>Redox</td>
<td>Reduction-Oxidation (reaction)</td>
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<td>RF</td>
<td>Radio Frequency</td>
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<td>RHS</td>
<td>Right Hand Side</td>
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<td>RMS</td>
<td>Root Mean Square</td>
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<td>SAW</td>
<td>Surface Acoustic Wave</td>
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<tr>
<td>SNR</td>
<td>Signal-to-Noise Ratio</td>
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<tr>
<td>SPAD</td>
<td>Single Photon Avalanche Diode</td>
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<td>SRAM</td>
<td>Static Random Access Memory</td>
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<tr>
<td>SSM</td>
<td>Single-Shell Model</td>
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<td>SUT</td>
<td>Sample Under Test</td>
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<tr>
<td>USB</td>
<td>Universal Serial Bus</td>
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Chapter 1

Introduction

1.1 Overview

Personalised medicine is widely considered to be the future of global healthcare [6, 7, 8], where diagnosis [9], treatment [10], and potentially even drug development [11], will become specific to, and optimised for, each individual patient. Traditional population based cell studies suppress the influence of outlier cells [12] that are frequently those of most clinical relevance [13]. Hence single-cell analysis is becoming increasingly important in understanding disease, aiding diagnosis and selecting tailored treatment: but remains the preserve of biomedical laboratories far from the patient. Current instruments depend upon cell-labelling to identify the cell types(s) of interest, which require that these be chosen a-priori and may not be those most clinically relevant. Furthermore, cell-labelling is fundamentally subjective, requiring highly-skilled operators to decide upon the validity of each and every test. Therefore, new test methods need to be developed to enable the widespread adoption of single-cell analysis.

The passive electrical properties of biological cells are known to be indicative of specific cell types, but no technology has demonstrated their comprehensive measurement within a mass-manufacturable device. This work aims to show that biologically meaningful information can be obtained directly from single cells, without labelling, by measuring their passive electrical properties. Specifically, that identifiable “cell signatures” can be obtained through broadband frequency measurements that exploit the properties of differential electric fields to probe single cells in physiological solution. These measurements are performed with a dedicated microsystem that exploits highly-scalable and mass-manufacturable microelectronics technology, which overcomes the practical limitations that have prevented existing single-cell analysis methods from widespread adoption in general medical practice.

Potential applications of this technology include many areas across biomedical research, food science, agriculture, and in particular human healthcare, where there are applications in the diagnosis, monitoring and treatment of various diseases. For example, detection of invasive (infectious) material (e.g. bacteria, fungi, circulating cancer cells), monitoring of a cellular response to drug treatments (e.g. antibiotics, chemotherapy, immunity), or treatment of illnesses that require cell harvesting from a donor (e.g. bone marrow transplants before and after chemotherapy), or from the patient (e.g. autologous cell therapies). The following sections explore the importance of single-cell analysis and existing methods for this purpose.
1.2 Biology by Units: Single-Cell Analysis & Actuation

Flow Cytometry (FC) [14, 15], is the state-of-the-art in single-cell analyses, with applications across microbiology [16], cell membrane potential studies [17], programmed cell death (apoptosis) [18], and bacterial analysis [19], with sensitivity down to single molecules [20] and even in-vivo applications reported [21]. FC originates in the 1950s with the Coulter Volumetric Counter (CVC) [22, 23], which exploits the intrinsic DC resistance of biological cell membranes. The CVC flows individual cells in physiological saline solution one at a time through an orifice slightly larger in diameter than the largest expected cell. A DC current is flowed between two immersed electrodes either side of the orifice and the voltage measured across them. As a cell transits the orifice, current is occluded in direct proportion to the cell’s size, resulting in a resistance change between electrodes recorded as a voltage pulse [24]. The CVC therefore provides measurement of cell size through cellular DC resistance, referred to as the Coulter Method which became, and remains, synonymous with blood cell counting.

Fulwyler [25] first applied the Coulter Method in 1965 to sort individual cells from their flowing stream using electrostatic inkjet technology invented by Sweet the same year [26]. Considering that the cell is the unit of biology, the ability to isolate individual cells from a sample enables analyses to be conducted at the highest level of biological resolution.

Modern FC began in 1972 with the invention of Fluorescence-Activated Cell Sorting (FACS) by Herzenberg et al. [27]. FACS identifies different cell types (phenotypes) via the specific binding (labelling) of fluorescent molecules (probes) coated with a surface chemistry (antigen) complementary to that expressed by the phenotype of interest (antibody), illustrated in Figure 1.1(a). Individual cells are flowed in series past a laser that excites the probe and the corresponding fluorescence emission is recorded. Increases in fluorescence intensity indicate a cell expressing the particular label has been detected, which is optionally deflected into a collection vessel using electrostatic sorting [28]. Modern FACS systems can sort sub-micron particles including individual bacterium [29]. FACS has become synonymous with Flow Cytometry and used interchangeably in the field. The terms are kept separate here, FC referring to analysis and FACS as analysis and sorting.

FC hardware has advanced over recent years to achieve flow rates exceeding 10,000 events/s [30, 31] and support up to 16 individual fluorescence channels excited by up to 6 individual laser wavelengths, spanning deep ultra violet to infrared. However, while FC data capture is rapid, standardising FC data interpretation has multiple challenges [32] and requires highly-skilled operators experienced in the specific biology of each sample and the hardware constraints of each cytometer, to design an effective cytometry experiment. Primarily, FC is limited by non-specific binding, where labels attach to cells other than the phenotype of interest, illustrated in Figure 1.1(b). Non-specific binding requires flow cytometrists to make subjective decisions
regarding whether to include, or exclude, individual events from the data analysis. Diagnostics require the same analysis to be performed time after time, in a standardised, repeatable and reliable test. Consequently, FC has been limited in its adoption as a diagnostic technique, and today remains the preserve of biomedical laboratories far from the patient.

1.3 Benefits of Miniaturisation

The capability advances in FC (and hence FACS) have come in parallel, and in part due to, the miniaturisation of fluidic and electronic components. Miniaturisation by definition enables components to be reduced in scale, which for electronic components for example, will increase their performance through reducing parasitic effects due to routing and device area. Miniaturisation can also bring forth physical phenomena not present at larger length scales. In fluid flow for example, a flow can either be turbulent or laminar depending upon the dominance of inertial to viscous forces, the ratio of which is defined by the dimensionless Reynold’s (Re) number:

\[
Re = \frac{\rho u L}{\mu},
\]

where \( \rho \) [kg/m\(^3\)] is the density of the flowing fluid with dynamic viscosity \( \mu \) [kg/(m.s)], \( u \) [m/s] the flow speed and \( L \) [m] the characteristic length of the flow system. Broadly, \( Re \leq 2,000 \) indicates a laminar flow regime, where viscous forces dominate and the flow is smooth and continuous. For water \((\rho = 10^3 \text{ and } \mu \sim 10^{-3})\) at room temperature, laminar flow occurs for small \( u \) or \( L \), the latter made possible through miniaturisation, and termed microfluidics for characteristic lengths beneath approximately one millimetre. Blood flow around the body is an example of microfluidics. Indeed, blood cells experience a different regime to larger particles in suspension, due to their microscopic size. For instance, a sphere with radius \( r \) [m], surface
area $A \text{ [m}^2\text{]}$ and volume $V \text{ [m}^3\text{]}$, has a surface area to volume ratio:

$$A = 4\pi r^2; \quad V = \frac{4}{3}\pi r^3; \quad \Rightarrow \frac{A}{V} = \frac{3}{r};$$

which means the surface of a 1 $\mu$m radius particle will be, compared to its volume, 1,000 times that of a 1 mm radius particle. Hence, surface effects dominate microscopic biological cells in suspension [33] and reduce chemical reaction times, increasing the throughput of devices that exploit such processes, particularly so for small sample volumes. Miniaturisation is further capable of achieving far greater geometric field gradients than possible otherwise. Modern microfabrication technology can readily pattern features separated by fractions of a single micron. As geometric field gradients are inversely proportional to distance, microfabricated devices can achieve gradients several orders of magnitude greater than macroscopic devices.

1.4 Advances in Single-Cell Studies: the Role of Microelectronics

Microfabricated flow devices have been used for FC studies to achieve small footprints [34, 35, 36] aided by miniaturised detector technologies [37, 38, 39]. Cell sorting within microfluidic devices is reviewed extensively in [40].

Miniaturisation through microfabrication can be further enhanced through the use of active microelectronic integration, which provides three main technical benefits compared to passive microfabricated devices:

1. **Miniaturisation:** due to the inherent use of microfabrication technology.
2. **Integration:** signal processing circuitry can be integrated within immediate proximity to sensors to increase sensitivity and Signal to Noise (SNR) ratio.
3. **Parallelism:** multiple identical circuit elements can be integrated into two-dimensional arrays providing a large sensing area and physical redundancy.

Complementary Metal-Oxide Semiconductor (CMOS) technology has become ubiquitous in microelectronics. CMOS-integrated devices provide a route to the manufacturing scale and low-cost affordability required for personalised medicine devices. The capability of integrating different sensor modes and signal processing electronics into a single CMOS device has been exploited in numerous biological applications, as reviewed by Adiguzel and Kulah [41]. Of these, acoustic, chemical, light-based, magnetic and electrical modes seem most suited to single-cell analysis and actuation. These are briefly reviewed in the following section.
1.5 Applicable Sensing and Actuation Modes

1.5.1 Acoustic Methods

Miniaturisation has permitted the handling of individual cells via piezoelectric driven tweezers \[42\], however the greatest adoption of mechanically-induced cell sensing and actuation arises from acoustics. Acoustic sensing and actuation arises from the transduction of an oscillating mechanical force with a solid container, to generate a standing wave within the contained sample. While acoustic based sensing has been reported for measuring the concentration of \textit{E. coli} and \textit{P. aeruginosa} fungi \[43\], the actuation of cells through Surface Acoustic Wave (SAW) technology has more widely been adopted \[44\]. Typically, SAW devices induce a standing wave within the suspending fluid (creating one-dimensional low pressure regions) as opposed to the micro-particle or cell directly \[45\]. SAW has been applied in conventional FC for two- and three-dimensional sample stream focussing \[46, 47, 48\]. Two-dimensional SAW has also been used in static volumes for so-called acoustic tweezers \[49\]. Acoustic induced cell sorting has also been reported in microfluidic sample flows \[50, 51\].

However, while acoustic and SAW technology in particular provide an intrinsic means of patterning fluids, they do not provide information regarding the suspended cells. Hence, acoustic methods alone are not well suited for single cell analysis.

1.5.2 Chemical Methods

Chemical methods are referred to here as sensors which require a chemical reaction with which to perform the sensing function. The combination of microfluidic flow and large surface area to volume ratios has been exploited in miniaturising common laboratory tasks into so-called Micro-Total Analysis Systems (\(\mu\)TAS) \[52, 53\]. These combine multiple functions from sample preparation to chemical analyte analysis within single microfabricated devices. However, these functions are typically focussed upon genomic or proteomic processes employing techniques such as DNA amplification through Polymerase Chain Reaction (PCR) \[54\], and not the study of the outcome of these processes.

Conversely, the Ion-Sensitive Field Effect Transistor (ISFET), formed from a standard Field Effect Transistor (FET), provides a means of direct molecule sensing. Historically, ISFET sensors were used for pH sensing, where an exposed FET gate dielectric would absorb \(H^+\) ions, modifying its work function and resulting in a change in transconductance recorded as a corresponding change in current through the ISFET \[55\]. Such an architecture required custom microfabrication and was replaced with standard CMOS-compatible topologies \[56, 57, 58\]. Molecule specificity can be tailored through post-processing CMOS devices with a different dielectric, such as \(P^+\) for bacterial detection \[59\]. The ISFET has been most widely adopted in
genomics, with entire genome studies made possible through highly-parallel integration (1.2M element arrays) in 0.35\(\mu\)m CMOS [60].

Regarding actuation, chemical methods have been mostly demonstrated at the molecular level [61], due to their dependence upon Reduction-Oxidation (Redox) reactions, and hence are not suited to actuating single-cells. Hence, while \(\mu\)TAS systems have received considerable attention and ISFETs are immediately applicable to biological analyte sensing, chemical methods are less well suited to analysing whole cells. ISFETs are inherently absorptive and hence become fouled with use, reducing the total sample volume measurable with each device. ISFETs are also reliant upon specific binding, as per FC, and hence are similarly limited by non-specific binding (shown in Figure [1.1]).

### 1.5.3 Light-based Methods

Electromagnetic radiation in the form of light plays an intrinsic role in biology, due to the evolution of animal cells from plants, and their ability to extract energy from light, via photosynthesis [62]. Hence optical (and near-optical) wavelengths are most compatible with biology. The invention of CMOS-integrated image sensors by Renshaw, Denyer, Wang and Wu [63, 64] enabled the widespread adoption of optical wavelength imaging technology, exemplified by the smartphone camera. CMOS image sensors have been used to image cells in microfluidic flow [65], fluorescence intensity [66] and the integration of sub-nanosecond timing circuitry enables fluorescence-lifetime cytometry to be performed upon individual cells [67, 68, 69, 70], opening the possibility of fluorescence-lifetime FACS [71].

Light-based cell actuation methods are extensively reviewed by Jonáš and Zemánek [72]. Of these, the invention of Optical Tweezers (OT) by Ashkin reported in 1970 [73] is most prevalent, showing that particles can be manipulated by light due to their index of refraction. In other words, by their intrinsic material properties. However, OT require a laser beam be focussed for each particle to be manipulated. And while optical cell sorting has been reported [74], the particle flow rate is six orders of magnitude beneath that of modern FACS systems.

Whilst light-based sensing methods are rapidly benefiting from microelectronic integration, image analysis alone is insufficient to phenotype similar cell types. Alternatively, cell labelling can be exploited, but suffers from non-specific binding as per conventional FC (see Figure [1.1]). Furthermore, while CMOS-integration has enabled miniaturisation of image sensors, separate light sources are still necessary, leading to complex mechanical assemblies to align and focus light onto the specific site of interest. Consequently, while light-based sensing and actuation are immediately relevant to biological analysis applications, they are not yet a complete solution for advancing personalised medicine in themselves.
1.5.4 Magnetic Methods

Magnetic Resonance Imaging (MRI) has become a staple of modern medical diagnostics, but MRI scanners remain expensive due to their physical size and cryogenic cooling requirements. Despite their hardware restrictions, advances in MRI output data analysis have been shown to detect single particles for cellular imaging [75]. The high field gradients made possible through miniaturisation have tremendous scope to employ MRI for this purpose. Fan et al. [76] report a desktop MRI system for three-dimensional cell imaging. Micro Electro-Mechanical System (MEMS) processing is exploited and combined with front-end electronics integrated in standard 0.18μm CMOS. MRI is based upon Nuclear Magnetic Resonance (NMR), which Liu et al. [77, 78] demonstrate in a handheld microsystem for biomolecular detection, integrating receiver coils and front-end signal processing in Radio-Frequency (RF)-CMOS.

Magnetic-labelling of target cells (the direct equivalent of fluorescence labelling used in FC) has been employed to detect cancer cells using NMR [79, 80]. Direct magnetic sensing and classification of such labels has been reported in an integrated microfluidic and 0.18μm [81] and 65nm CMOS devices [82].

Magnetic separation of Red Blood Cells (RBCs) from whole blood was first reported in 1975 by Melville, Paul and Roath [83, 84]. This method exploits the intrinsic susceptibility of RBCs to magnetic fields due to their iron content bound within haemoglobin. Magnetic-labelling became a standard cell separation technique through the optimisation of high-gradient sorting columns made by Miltenyi [85], giving rise to Magnetic-Activated Cell Sorting (MACS), now used in diagnostic devices [86]. Further miniaturisation efforts have been made through the combination of SiGe microelectronics and microfluidics for magnetic cell separation [87].

However, whilst magnetic cell separation has been widely adopted, it is again dependent upon cell labelling. RBCs are unique amongst all other cells in their intrinsic magnetic permeability - all others are indistinguishable from vacuum [2]. This is exploited by MRI for non-destructive medical imaging. Magnetic methods therefore suffer the same limitations as conventional FC, they require cell labelling and hence are limited by non-specific binding.

1.5.5 Electrical Methods

Electricity and biology have been connected since the discovery of the former by Galvanni in his famous experiments with frog muscles in the late 18th Century. His research was used by Volta to build the first electrical battery capable of delivering a known current to a circuit: the voltaic pile. Consequently, electrochemical experiments exploiting redox reactions were the first adopted for the electrical analysis of materials. Faraday attributed redox reactions to the basis of electrochemistry, were charge carriers provided by a battery alter the chemistry of
Introduction

the sample under test, resulting in current flow through that sample. Electrochemical sensing is a well established technique for measuring the electrical activity of a sample with modern implementations reported in 0.18μm CMOS [88, 89, 90]. However, electrochemical sensing is fundamentally destructive. Therefore, electrochemical methods are not well suited to single cell sensing with view to sample re-use, as per FACS.

Nonetheless, electrochemical sensing provides a method of analysing the intrinsic properties of a sample, without use of extrinsic sample labelling, as per the majority of techniques reviewed above. Indeed, the Coulter Method provides such an intrinsic measurement, by exploiting the DC resistance of cellular membranes. The AC impedance of cell suspensions had first been investigated in the mid 19th Century and found to decrease with increasing frequency [91, 2]. Single cell impedance measurements of RBCs at DC and 20MHz were first described by Thomas et al. in 1974, and soon extended to identify numerous mammalian cell types using a modified Coulter Volumetric Counter by Hoffman and Britt [3, 92]. The advent of modern microfabrication has enabled the miniaturisation of such devices and the field of microfluidic Impedance Spectroscopy (IS) [93].

Microfluidic IS has been shown capable of identifying, with single cell resolution, RBCs [94], the major white blood cell groups [95, 96], and various tumour cell types [97, 98], with the greatest cell discrimination achieved across a frequency band spanning approximately 100kHz to 100MHz [2]. IS therefore provides a method for identifying cells immediately relevant for diagnosis, across a band of frequencies readily accessible by modern electronics, and without the need for extrinsic cell labelling.

Where microfluidic IS senses the response of suspended cells to an applied electrical stimulus (field), Dielectrophoresis (DEP) uses the stimulus to exert a force upon individual cells for the purpose of actuation [99, 100]. DEP has shown distinguishable frequency dependent behaviour regarding cell viability [101], apoptosis [102], cancer type [103, 104] and stem cell differentiation [105]. The DEP force is proportional to the gradient of the applied electric field and therefore directly benefits from miniaturisation and microelectronic integration, with single cell actuation demonstrated upon devices implemented in standard 0.35μm CMOS [105, 106, 107].

Therefore, electrical methods have the capability to sense, identify, count and actuate individual biological cells due to their intrinsic electrical properties, exploiting phenomena that directly benefit from miniaturisation and microelectronic integration.

1.6 Thesis Aims

This work aims to show that biologically meaningful information can be obtained directly from individual cells, without cell labelling, by measuring their passive electrical properties across
the 100 kHz to 100 MHz frequency band. Specifically, that identifiable “cell signatures” can be obtained through broadband frequency measurements that exploit the properties of differential electric fields to probe single cells in physiological solution. This hypothesis is tested through the design, implementation and experimental testing of a dedicated microsystem that integrates two novel designs of electrical sensor within a standard CMOS microelectronics technology. Measurements are performed to assess whether these sensors are capable of conveying information regarding cell size, cell type, and density; suggesting that with future prototype refinement and algorithmic development, characteristic “cell signatures” can be generated that could aid the observer to make better decisions faster, and draw relevant conclusions about the contents of a particular biological sample, without making decisions about that sample a-priori.

1.7 Objectives

The primary objectives of this work are:

- To specify, design, build and test a dedicated microsystem capable of measuring the interaction between electric fields and single biological cells in physiological liquid suspension, for frequencies spanning 100 kHz to 100 MHz.

- To derive a specification to target with the microsystem design.

- To incorporate two novel electrical sensor designs:
  
  1. **Absolute electric sensor**: to sense the entire electrical environment in proximity to a single electrode, consisting of the suspended cell and its suspending medium.
  
  2. **Differential electric sensor**: to sense the difference in electrical environment between a pair of electrodes, with view to provide information regarding the suspended cell only, by rejecting the common signal due to its suspending medium.

- To design and implement a sample stimulus method for the above sensors, capable of generating electric fields containing the frequencies of interest in a repeatable manner.

- To test the microsystem and assess its capabilities regarding its potential for:
  
  - Operation when exposed to different liquids, particularly physiological media such as saline.
  
  - Detecting polystyrene micro-beads, to assess the impact of size and particle location upon the sensor responses for micro-particles of identical material.

  - Detecting individual biological cells in physiological solution.
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– Distinguishing different cell types across a number of parameters (e.g. cell size, location, density, etc.).
– Determining whether there is an optimal band of frequencies for performing the above measurements.

Further objectives are:

• To understand the capabilities and limits of the developed microsystem.
• To elucidate the early potential for the application of “cell signatures” for characterising individual cells, to aid the predictive capabilities of the system.
• To understand areas where the microsystem will need to improve regarding signal detection and analysis.
• To utilise the opportunity of manufacturing a dedicated microsystem to integrate additional sensing and actuation technologies where possible.

1.8 Contribution to Knowledge

This work describes the integration of two novel designs of electrical sensor within a standard, highly-scalable CMOS technology. One sensor measures the absolute electrical environment above a single sense electrode. The other measures the difference in electrical environment between a pair of electrodes, with view to provide information regarding the suspended cell only, through rejecting the common signal due to its suspending medium. Measurements are reported with these novel sensors upon unlabelled single cells in physiological saline solution across an unprecedented number of frequency points, performed within a single measurement.

1.9 Thesis Structure

Chapter 2 reviews the background and state-of-the-art in the electrical sensing and actuation of individual biological cells, identifying Dielectric Spectroscopy (DS, related to IS) and DEP as the most capable for the purpose of this work. Two novel architectures of DS sensor are presented with their feasibility and potential performance assessed via modelling in Chapter 3. Chapter 4 presents the design and implementation of a dedicated microsystem integrating these two sensor architectures with two photonic sensors and DEP actuation circuitry. Chapter 5 presents the results obtained from the microsystem which are discussed in detail in Chapter 6. Conclusions drawn from this work are presented and future work considered in Chapter 7.
Chapter 2
Literature Review

2.1 Introduction

This work is concerned with the study of biological matter through the use of electricity, specifically to the interaction between individual biological cells in physiological liquid suspension and electric fields. The general concepts of electrical analysis through sensing and actuation are first introduced, taking the macroscopic approach of von Hippel [1], which describes the interaction of matter with electric and magnetic fields of any frequency. The magnetic properties of biological cells are practically identical to that of vacuum [2] (with the exception of iron within red blood cells [83]), meaning the electric behaviour of biological cell suspensions can be treated independently from magnetism. Any matter will exhibit energy storage and dissipative effects, described by the definition of complex permittivity \( \varepsilon^* \), which is frequency dependent. In the case of biological cell suspensions, \( \varepsilon^* \) exhibits dispersions in the frequency domain, attributed to specific relaxation phenomena from DC to approximately 10 GHz, which are briefly described. Practical implications of measuring biological cell suspensions are then considered, before the state-of-the-art in single cell electrical sensing and actuation is reviewed across the aforementioned dispersion frequency band.

2.2 General Concepts

2.2.1 The Passive Electrical Properties of Biological Cells: A Brief Summary

All biological cells are active electrochemical entities, where molecules are continually processed through chemical reactions consuming and releasing energy as part of a healthy cellular metabolism [62]. Electrically-active cells, such as nerve and muscle, concentrate ions to produce spikes of electricity to initiate further processes: such as transmission of a nerve impulse, or contraction of a muscle. While these active electrical cellular properties are immediately relevant to the health and function of an organism, their passive electrical properties are of interest here. Passive properties refer to those that exist in the absence of electrical activity arising from the cell itself and hence are relevant to every kind of cell.

The passive electrical properties of biological cells are extensively reviewed by Schwan [102], Pethig and Kell [110] and Foster and Schwan [111]. In very brief summary, broadly two cell types exist:
Eukaryotic cells have membrane-bound internal cellular components (organelles) including a nucleus, suspended within a saline internal environment (cytoplasm) bound within another outer membrane. All mammalian cells are eukaryotic.

Prokaryotic cells lack any membrane-bound organelles, but have a cytoplasm encased within a thicker outer cell wall. All bacteria are prokaryotic.

The physiological boundaries of membranes or cell walls present interfaces between the cell and its surroundings, and internally within the cell itself. Most cells typically exist suspended within a charge rich environment (such as Na\(^+\) and Cl\(^-\) in saline solution) of high electrical conductivity, but electricity does not move freely through cells. Consequently, biological cells are not ideal conductors but exhibit dielectric properties. Indeed, saline exhibits dielectric properties in addition to its high conductivity. It therefore becomes useful to investigate the response of dielectrics to an applied electric field (E-field), which provides the stimulus necessary with which the passive electrical properties of biological cells can be sensed and hence analysed.

2.2.2 Induced Dipoles and Dispersions

Any material that is not an ideal conductor will exhibit dielectric properties, such is the case for biological cells. Dielectrics become polarised in an electric field, giving rise to induced dipoles, in broadly three ways [II]:

- **Electronic polarisation**: the displacement of the positively charged nucleus from the surrounding negatively charged electron cloud,
- **Atomic polarisation**: displacement of positive and negatively charged atoms bound within a molecule,
- **Orientational polarisation**: molecules bound within a material aligning with the applied E-field.

In addition, charge carriers typically exist within a dielectric that are free to migrate through the material. When these are impeded in their motion by boundaries between materials, or by becoming trapped within a material, or cannot be efficiently charged and discharged at electrodes, a space charge arises which acts to distort the applied E-field. This gives rise to a fourth polarisation mechanism, which increases the observed permittivity of a material:

- **Interfacial polarisation**: due to charge accumulation at dielectric boundaries.

All four of these polarisations exist at zero frequency (DC), but their behaviour is also time (and hence frequency) dependent. When an external E-field is applied across a dielectric material,
its application induces dipoles within that material, giving rise to the polarisations listed above. These induced dipoles are originally disordered but experience a torque to align themselves with the applied E-field. This torque (or dipole moment) is directly proportional to the induced charges and separation between them, illustrated in Figure 2.1.

![Figure 2.1: Torque T [N.m] acting upon an electric dipole formed by two equal and opposite charges Q [C]. Force F [N] arises from electrostatic attraction to the applied electric field E [V/m], rotating the dipole through angle $\theta$ [rad] until parallel to the field. After von Hippel [I].](image)

It becomes convenient to represent a non-ideal dielectric by its complex permittivity $\varepsilon^*$, defined as:

$$\varepsilon^* = \varepsilon' - j\varepsilon''$$

(2.1)

where $\varepsilon'$ is the real permittivity of the dielectric, representing all energy storative effects exhibited by the material and $j = \sqrt{-1}$. $\varepsilon''$ is termed imaginary permittivity, but is more intuitively understood through its relationship to dielectric conductivity $\sigma$:

$$\sigma = \omega\varepsilon''$$

(2.2)

where $\omega$ [rad.s$^{-1}$] is angular frequency. $\varepsilon''$ represents all energy consumptive effects exhibited by the material, which can be actual Ohmic conductivity due to flowing charge carriers, or the energy loss associated with the friction accompanying orientation of dipoles [II]. Debye [1] first explained orientational polarisation as a relaxation mechanism, where larger dipole molecules will take longer to respond to an applied E-field than small molecules. This time dependence gives rise to a frequency dependence, or dispersion, defined by:

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j\omega\tau}$$

(2.3)

where $\varepsilon_s$ and $\varepsilon_\infty$ are respectively defined as the static permittivity (at zero frequency) and the permittivity observed at a frequency much greater than the period of the dispersion, $\tau$ [s].
Equation (2.3) accurately represents the dielectric properties of gases, but less so for condensed matter, such as liquids. Cole \cite{113} extended Debye’s expression for condensed matter, by introducing a broadening of dispersion time constants through parameter $\alpha$:

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_\infty - \varepsilon_\infty}{1 + (j\omega \tau)^{1-\alpha}}. \quad (2.4)$$

Schwan \cite{2} defined three broad dispersions for biological cells in suspension (illustrated in Figure 2.2) and attributed to them the following terms:

- **$\gamma$-dispersion**: attributed to atomic polarisation of water molecules, occurring in the 1 GHz to 10 GHz frequency band,
- **$\beta$-dispersion**: attributed to orientational polarisation of dipoles induced within the cell, occurring between 100 kHz to 100 MHz band, and
- **$\alpha$-dispersion**: attributed to interfacial polarisation between the cell membrane and its suspending medium, occurring beneath 100 kHz.

![Figure 2.2: Generalised frequency dependence of the real permittivity of biological cell suspensions. Dashed line: idealised dispersions with single, discrete time-constants (Debye). Solid line: dispersion broadening in practice (Cole). After Schwan \cite{2}.

While illustrative, the dispersions described above are generalisations only and in practice broaden across one another as shown in Figure 2.2. Particularly so for complex biological samples which have multiple dielectric interfaces, such as between the cell membrane and its suspending medium.

---

\footnote{Care must be taken in interpreting $\alpha$. It modifies the Debye expression to better fit experimental data \cite{113} and is frequently represented in equivalent circuits as an impedance ($Z$) with real and reactive components. However, while the magnitude of $Z$ may be frequency dependent, the resulting phase angle from equation (2.4) is not. Instead, a Constant Phase Element (CPE) is frequently used in the literature and considered to hold physical meaning. However, as Cole and Cole \cite{113} state, “It must be understood that the use of $Z$ is merely one way of expressing the experimental facts and that it and its real and imaginary parts have no conventional meaning”. Hence the use of $Z$ and $\alpha$ are purely for mathematical convenience.}
suspension medium, the membrane and cytoplasm, and between the cytoplasm and internal organelles. These dielectric interfaces all contribute to orientational polarisation and hence are mostly apparent in the \( \beta \)-dispersion region, which appears of particular interest for identifying different cell types from one another (phenotyping). Dielectric polarisation and how such polarisation can be represented in physical space, is considered further in the following section.

### 2.2.3 Dielectric Polarisation

Complex permittivity \( \varepsilon^* \) enables the passive electrical properties of biological cells to be represented through a non-ideal capacitance. The simplest arrangement of which is the parallel-plate capacitor, comprised of two conductors (electrodes) separating the Sample Under Test (SUT). A dielectric SUT acts to increase the measured capacitance between the electrodes when compared to vacuum, due to charge carriers within the dielectric neutralising those applied to the electrodes which would otherwise contribute to the external E-field. Faraday first defined this phenomenon of **dielectric polarisation** [114], viewed as the action of dipole chains forming under the influence of the applied E-field, shown schematically in Figure 2.3.

**Figure 2.3:** Schematic representation of dipole polarisation. After von Hippel [1].

The above treatment of the total applied charge being neutralised by bound charges within the dielectric can be extended into space by attributing the following field vectors:

- **\( \mathbf{D} \) [C/m\(^2\)]:** the *electric displacement*, represents the total (or true) charge measurable upon an electrode,

- **\( \mathbf{E} \) [V/m]:** the *electric field intensity (or strength)*, represents the free charge available to contribute to an electric-field across the dielectric, and

- **\( \mathbf{P} \) [C/m\(^2\)]:** the *electric polarisation*, represents the bound charge that neutralises applied charges at the electrode-dielectric interface.
Derivations of the above field vectors from total, free and bound charge densities upon capacitor electrodes are given in Appendix A. They are related to each other through expression (2.5) and illustrated in Figure 2.4:

\[ D = \varepsilon_0 E + P \]  

where \( \varepsilon_0 [\text{F/m}] \) is the permittivity of vacuum, and whose dimensions define those for the above field vectors [I].

2.2.4 Electrode-Sample Interface

Dielectric polarisation is inevitable when an electrode comes into contact with a dielectric, due to bound charge carriers. When the dielectric is a liquid, its molecules are readily available to contribute to electrode polarisation. Physiological liquids are electrolytic, with ample free charge carriers necessary to sustain an isotonic environment for suspended cells [62]. When such a liquid comes into contact with a charged electrode, two key phenomena occur:

- **Electrode polarisation**: interfacial polarisation occurs at the electrode-sample interface. Polar molecules (such as water) align to the applied E-field and their charged ends are attracted to oppositely charged electrodes. Molecules closest to each electrode condense upon them, defined as the *Helmholtz layer* [115], which attract further molecules from the solution to form the diffuse *Guoy-Chapman* layer [116], increasing the measurable real permittivity between electrodes. Combined, these phenomena are referred to as the *Electric Double Layer* (EDL) or *Stern Layer*, and contribute to the \( \alpha \)-dispersion.
**Redox reactions**: Free charge carriers on each electrode exchange with oppositely charged carriers in solution. Reduction-Oxidation (Redox) reactions occur at the electrode-sample interface and a current flows between electrodes. This electrochemical reaction defines *Faradaic* current and forms the basis of all electrochemical sensors [117].

EDL formation is illustrated in Figure 2.5 and frequently modelled as an ideal capacitance [118], due to the increase in real permittivity observed between electrodes. But Faradaic current can still flow [119], permitting potentially damaging reactions at the electrode-sample interface to occur [120].

![Figure 2.5:](image)

**Figure 2.5**: (a) Electric Double Layer (EDL) or Stern Layer formation through polar water molecules condensing upon a charged electrode. (b) Electric potential (ψ [V]) distribution across EDL, where $d_1$ and $d_2$ are the Helmholtz and Guoy-Chapman layers, respectively.

The EDL represents the decrease in free charge density available to probe the sample (via E) from the increase in bound charge density (P) required to neutralise the abundance of polar molecules in a liquid sample. The presence of free charge carriers within an electrolytic sample gives rise to redox reactions at the electrode-sample interface and Faradaic current flow, which will consume non-inert electrode materials. The combination of these effects pose a challenge in electrode material choice, with gold, platinum, carbon-black and silicon-carbide popular choices due to their various material and electrical properties [118, 121].

### 2.2.5 Displacement Current

Alternatively, a dielectric material can be placed in series between each electrode and the electrolytic SUT to prevent Faradaic current flow of free charge carriers across the electrode-sample interface. This approach exploits dielectric polarisation to condense bound charge carriers upon the dielectric-sample interface, which in turn produce an electric displacement (D) across the SUT. Maxwell [122] defined the change of D with respect to time as *displacement current*:
\[ i_D = \frac{dD}{dt} \cdot A, \]  
(2.6)

where \( i_D \) [A] is the effective current arising from the change in electric displacement \( D \) [C/m\(^2\)] across the SUT with electrode area \( A \) [m\(^2\)] in time \( t \) [s]. Displacement current \( i_D \) can therefore be used in place of conventional current, provided that the stimulus generating the electric displacement is alternating with respect to time.

Having defined complex permittivity \( \varepsilon^* \) and the behaviour of dielectrics when exposed to electric fields and in contact with electrolytes, we can now consider how these are represented via circuit analysis methods.

### 2.3 Circuit Analysis Techniques

The microscopic size of individual mammalian cells means the frequency bands of interest defined in Section \[ \text{Section 2.2.2} \] have wavelengths sufficiently long as to apply Ohm’s Law and hence standard circuit analysis techniques. Note that this assumes that \( \varepsilon^* \) is linear (i.e. its value is independent of the applied E-field intensity), and that induced potentials across the cell membrane are beneath the action potential of any excitable cells (i.e. \( \varepsilon^* \) represents the passive electrical properties of the cell and its suspending medium). Circuit analysis techniques are readily applied by representing real permittivity \( \varepsilon' \) in terms of electrical capacitance \( C(\varepsilon') \) [F], and real conductivity \( \sigma \) in terms of resistance \( R(1/\sigma) \) [\( \Omega \)] or its reciprocal, conductance \( G(\sigma) \) [1/\( \Omega \)]. The vector sum of which leads to complex impedance \( Z(\omega) \), illustrated in Figure 2.6.

![Figure 2.6: Complex vector representation of frequency dependent impedance \( Z(\omega) \) at frequency \( \omega_i \).](image)

\( Z(\omega) \) of biological cell suspensions is frequency dependent due to the frequency dependence of \( \varepsilon^* \) and its components \( \varepsilon' \) and \( \sigma \), as shown in Figure \[ \text{Figure 2.2} \]. Hence, we obtain an impedance spectrum with respect to frequency:

\[ Z(\omega) = \sum_{i=f_1}^{i=f_2} \left[ R(1/\sigma_i) + jC(\varepsilon'_i) \right] = \sum_{i=f_1}^{i=f_2} Z(\omega_i), \]  
(2.7)
where \( f_1 \) and \( f_2 \) [Hz] represent the minimum and maximum measured frequencies within the impedance spectrum, respectively. Choosing the values of \( f_1 \) and \( f_2 \) will determine which dispersion is likely to be the dominant factor in \( Z(\omega) \), as shown in Figure 2.2. We therefore observe that assessing the complete frequency dependence of a biological cell suspension can be achieved through impedance spectroscopy.

2.3.1 Impedance Spectroscopy

Impedance Spectroscopy (IS) is performed most simply by placing a SUT between two electrodes and either: applying a known current \( i \) [A] between electrodes and measuring the resulting voltage \( V \) [V] across them, or vice-versa. Where ionic conduction of free charge carriers dominate (as in the case for electrolytic samples in direct contact with electrodes), IS becomes electrochemical impedance spectroscopy \([2,3]\). The resulting complex impedance \( Z \) [\( \Omega \)] of the SUT is then found from:

\[
Z_{IS}(\omega) = \frac{V(\omega)}{i_c(\omega)},
\]

(2.8)

where \( V \) [V] is the known (measured) voltage across electrodes, \( i_c \) [A] is the measured (known) conventional current flowing between electrodes and \( \omega \) [rad.s\(^{-1}\)] is angular frequency. IS has been applied to biological cell suspensions since the early 20\(^{th}\) Century \([9,1,24]\).

2.3.2 Dielectric Spectroscopy

When a dielectric is placed in series between the electrodes and sample, a conventional flow of free charge carriers is prohibited, preventing electrochemical reactions from occurring. Instead, when the applied stimulus changes with respect to time, an effective (displacement) current flows (see Section 2.2.5). The use of displacement current as opposed to conventional current is used here to define Dielectric Spectroscopy (DS). DS measures the effective impedance \( Z \) [\( \Omega \)] of a SUT in the same manner as IS, with displacement current \( (i_d \) [A]) replacing conventional current \( (i_c \) [A]):

\[
Z_{DS}(\omega) = \frac{V(\omega)}{i_d(\omega)},
\]

(2.9)

Note that \( Z_{DS} \) and \( Z_{IS} \) will not be equal due to the difference in dielectric polarisation at the SUT interface: free charge carriers can exchange in IS, but not in DS. DS measurements will also have a different frequency response to those made with IS, due to the stimulus and measurement electrodes being effectively capacitively-coupled to the SUT through the series dielectric layer. DC measurements can still be performed, provided the polarisation of the series dielectric is accounted for \([125]\), in a similar manner as electrode polarisation in IS \([118]\).
2.4 Sample Interface Methods

2.4.1 Electrode Configurations

Electrical sensing requires at least two electrodes to define a known potential-difference across a sample and hence an electric field gradient along which conventional (or displacement) current can flow. Alternatively, a known conventional (or displacement) current can be applied to the SUT and the resulting voltage upon the electrodes measured. The latter case is most widely adopted since voltage outputs can be sensed by high-impedance circuitry that minimises the impedance load seen by the SUT. Both electrodes in this 2-electrode system will experience polarisation, due to bound charges within the sample being attracted to the potentials imposed upon the driven (working) electrode and those arising upon the second (counter) electrode, as shown in Figure 2.7. Such electrode polarisation will act to screen free charge carriers available for conventional (or displacement) current flow through the sample.

![Diagrams of 2-electrode systems: (a) voltage-driven, (b) current-driven.](image)

Two methods are frequently used to overcome the reduction in measured signal due to electrode polarisation arising from the 2-electrode system. Figure 2.8(a) shows the 3-electrode system, which places a third (reference) electrode within the sample to remove the working electrode polarisation from the measured signal. However, the output voltage is still referenced to an arbitrary potential, such as that upon the the counter electrode (e.g. ground), and hence will experience the same polarisation. The 4-electrode system overcomes this limitation by introducing a second reference electrode to the sample, shown in Figure 2.8(b). Comparing the potential difference across the two reference electrodes renders the measured output voltage independent of any arbitrary potential, resulting in minimal attenuation in measured output signal due to electrode polarisation.

Which electrode setup provides the best output is application dependent. However, in the 3- and 4-electrode systems, the reference electrode(s) must be exposed to a similar environment to the working and counter electrodes, to provide an accurate reference.
2.4.2 Electrode Functionalisation

Electrode materials can be chosen for their ability to bond with particular molecules to support functionalisation, whereby a material is deposited upon the electrode with specificity toward a target molecule, such as H\(^+\) and K\(^+\) ions for ISFETs \cite{56, 55, 60, 59, 126}. When the chosen material is biologically-active, it becomes a biosensor \cite{127, 117, 128}. Reported biosensor applications include DNA probes for genome sequencing \cite{55, 129, 130, 60}, protein identification \cite{131}, lipid bilayers for ion channel studies \cite{132}, and antigens targeting specific cellular antibodies for phenotyping \cite{113}.

Biosensors are frequently referred to as “label-free” since the cell sample requires no pre-processing. However, they are nonetheless limited in their specificity, as per cell-labelling, by non-specific binding (see Section 1.1) and the associated need to choose a-priori what cell phenotype to target. Biosensors physiologically modify the sample under test which may be undesirable, and they are fundamentally absorptive, meaning the underlying sensor becomes fouled with use. This poses particular issues for large sample volumes and compounded if the underlying sensor is microfabricated, where the arising sensor cost will be directly proportional to its sensitive area. A biosensor is ultimately a transducer that has a certain transfer function and efficiency less than unity. Direct measurements of the interaction of biological cells with an applied E-field are instead possible and considered in Sections 2.5 through 2.7.

2.4.3 Geometric Cell Constant

The above electrode configurations can be applied in any geometry, however their physical alignment directly affects the equivalent capacitance and / or resistance measured between electrodes. A “cell constant” can be used to modify a unit geometric capacitance and / or resistance (equivalent to a parallel-plate approximation) by a dimensionless value to account for the sensor electrode geometry. This is an unhelpful term considering its application. Instead, the term geometric cell constant will be used throughout this work to avoid confusion.
Literature Review

Having described electrode arrangements and their optional functionalisation, we now consider how these electrodes connect the SUT to external circuitry.

2.4.4 Circuit Interface

The difference in electrical impedance arising from bulk measurements of whole blood was first studied by Fricke in 1925 and its equivalent capacitance found to be on the order of a few picofarads \([133, 134]\), which is on the order of parasitic shunt capacitance arising from electrical connections. Consequently, interfacing circuits that minimise such parasitic loading of the SUT are necessary, and broadly two types have been applied.

Bridge Methods

Bridge methods are based upon the Wheatstone Bridge with reactive components used to extend its application into the frequency domain. Illustrated in Figure 2.9, the bridge comprises four branches, split into two pairs across which the output voltage is measured. The first branch acts as a reference with components of known and variable impedance. The second branch is used for measurement with the SUT forming one half branch. The bridge is initially balanced with no sample present by adjusting the reference impedance until the output voltage measures zero. The sample is then introduced, unbalancing the bridge, and the reference impedance adjusted until the output voltage again measures zero. The SUT impedance is equal to the adjustment in reference impedance necessary to rebalance the bridge.

![Figure 2.9: Modified Wheatstone Bridge to measure SUT impedance. After Hoffman and Britt.](image-url)
The bridge method has enabled impedance measurements up to 4.5 MHz upon bulk cell samples \cite{133} and individual red blood cells in a modified Coulter volumetric counter \cite{3}. However, while accurate, the bridge method requires balancing for every measured frequency, which will require a finite period to achieve. Consequently, the bridge method is not well suited to broadband sensing, particularly so for high sample flow rates as per conventional flow cytometry.

**Direct Sensing**

Direct sensing connects the SUT electrodes directly to sensing circuitry, typically through an impedance buffer that presents a high-impedance load to the SUT, shown schematically in Figure 2.10. Direct sensing is made possible through miniaturisation of electronic components and the corresponding reduction in parasitic shunt impedances, to minimise attenuation of the SUT signal due to impedance loading.

![Direct sensing circuit](image)

**Figure 2.10:** Direct sensing method: the SUT sensor electrodes are connected directly to impedance buffering circuitry that presents a high-impedance (low attenuation) load $C_L$ to the SUT.

Direct sensing is particularly relevant to charge-based sensing techniques such as capacitive sensors \cite{135,136,137,131,126} and dielectric spectroscopy in general, since the series dielectric as necessary in DS will present a high-impedance load to the SUT.

2.4.5 **Stimulus and Sensing Methods**

The passive electrical properties of a SUT can only be analysed through sensing its response to an applied stimulus, which can be DC, AC or both. Impedance and dielectric spectroscopy require the applied stimulus to contain frequency components spanning the band of interest (defined between $f_1$ and $f_2$ in expression (2.7), such as approximately 100 kHz and 100 MHz for $\beta$-dispersion measurements). The chosen stimulus method directly affects the signal processing method necessary to extract the desired impedance data. Two types of AC stimulus methods are commonly employed and introduced briefly below.
**Frequency Domain**

Broadband AC measurements can be performed by sweeping sinusoids across the individual frequencies of interest. However, this proves difficult to achieve in practice due to noise. Electrode polarisation, which acts to attenuate the sensed signal, is inevitable in the highly-conductive environment of physiological fluids. Gawad et al. [138] estimate that a 15 $\mu$m diameter polystyrene micro-bead will exhibit a 25% variation in impedance in a 20×20×20 $\mu$m$^3$ sensing volume. This variation reduces to beneath 1% for 5 $\mu$m diameter particles.

The sensor signal of interest is therefore often of comparable magnitude to the noise within the sensing volume. Demodulation techniques as illustrated in Figure 2.11 are frequently used to overcome this problem. The SUT is stimulated with a known (reference) current with frequency $f_r$ [Hz], derived from reference voltage $V_r$ [V] applied across a precision resistor. The SUT output voltage is input into two frequency mixers, which multiply the recorded sensor signal ($V_s$ at frequency $f_s$) by the reference signal ($V_r$) and its 90° phase-shifted counterpart. Each mixer outputs a voltage with two frequency components, comprised of the difference ($f_s - f_r$) and sum ($f_s + f_r$) of its input frequencies. Since the recorded sensor signal has the same frequency as the reference ($f_s = f_r$), the difference term cancels to DC. A Low-Pass Filter (LPF) removes the sum component, and two DC voltages appear at the in-phase ($X$) and quadrature ($Y$) outputs. The magnitude ($R$ [V]) and phase ($\theta$ [rad.$s^{-1}$]) of the output voltage with respect to the reference input is converted to polar form in the usual way:

$$R = \sqrt{X^2 + Y^2}; \quad \theta = \tan^{-1}(Y/X). \quad (2.10)$$

This demodulation approach exploits phase detection and is referred to as the lock-in technique. *Impedance analysers* take this one step further, outputting the sample impedance at frequency $f_s$ by dividing the output voltage by the reference current.
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While demodulation (and its associated lock-in and impedance analyser techniques) maximises the sensitivity achievable from sweeping individual frequencies, the need for a dedicated mixer and LPF per frequency makes broadband measurements impractical [139]. Instead, repeated measurements are necessary at every frequency step, requiring up to 10 s for sweeps between 100 Hz and 1 MHz [140]. Hence, demodulation techniques are not well suited for the rapid acquisition of broadband measurements, particularly considering that conventional flow cytometry is capable of analysing up to 10,000 events/s [31].

Time Domain

The second type of AC stimulus are time domain methods that exploit Fourier transformation [141]. A perfect impulse contains an infinite sum of frequency components, but are impossible to realise in practice. Approximations of time domain impulses can be used, which have a finite bandwidth limited by the stimulus source. These include rectangular, Gaussian and sinc pulses. However, while they contain multiple frequency components, the energy of each frequency is distributed unevenly around the fundamental, leading to attenuation at low and high frequencies, reducing the achievable SNR [142]. Chirp waveforms can also be used, however these again have a non-uniform energy distribution, with a crest factor (ratio between signal peak and RMS) of $\sqrt{2}$ [143].

![Figure 2.12: Broadband impedance measurement estimation using time-domain m-sequence method.](image)

Alternatively, rectangular pseudo-random Maximum Length Sequences (MLS, or m-sequences) exhibit a crest factor of 1 and a flat spectral density, similar to noise, which is ideal for broadband frequency measurements. Illustrated in Figure 2.12, m-sequences are cyclical, rectangular
Literature Review

Waveforms readily generated in digital electronics, and have been used for measuring the impulse response of audio systems since the 1960s [144]. Transfer function estimates are made by stimulating the SUT with an m-sequence containing the desired frequency components (see below), and cross-correlating the recorded output with the input reference. The maximum and minimum measurable frequencies are limited by Nyquist and the sequence order, respectively:

\[ f_{\text{max}} = \frac{f_s}{2}, \]
\[ f_{\text{min}} = \frac{f_s}{2^{(m-1)}}, \]

where \( f_s [\text{Hz}] \) is the sampling frequency, and \( m \) the sequence order, defined by the number of registers used to synthesise the m-sequence. For instance, a 9-bit m-sequence generated at 200 MHz will contain 256 frequency components spanning 781.25 kHz to 100 MHz spaced equally in 781.25 kHz steps. M-sequences have one more 1 than 0 terms, which gives rise to a DC offset, which is avoided through use of AC-coupled DS measurements. They also assume the SUT to be Linear Time Invariant (LTI), meaning the sample has not changed during the analysis period. This assumption is satisfied provided the analysis period (equal to \( 1/f_{\text{min}} \)) is small compared to the rate at which the sample changes. These properties have been exploited for single cell IS measurements upon flowing cells using the m-sequence method [145, 146].

Background Summary

The preceding sections have introduced complex permittivity as a means of describing the passive electrical properties of biological cells in suspension. Any dielectric will become polarised in an electric-field, with four dominant polarisation phenomena occurring. These polarisations are frequency dependent, giving rise to dispersions across broad frequency bands, with wavelengths long enough to permit the use of standard circuit analysis techniques. The concepts of total, bound and free charge were introduced and attributed the field vectors of electric displacement, electric field intensity (or strength), and polarisation. Their roles at the electrode-sample interface were then considered for dielectrics with complex permittivity and electrolytic materials with conductive electrodes. Electrodes can be configured in broadly four ways and stimulated in either the frequency- or time-domain, the response to which will contain the sensor signal(s) of interest.

We now use these concepts to explore the literature for methods used in the electrical sensing and actuation of biological cells in liquid suspension, across the \( \alpha, \beta \) and \( \gamma \)-dispersions.
2.5 Methods for $\alpha$-dispersion Studies

This section reviews methods for the study of biological cell suspensions at frequencies beneath 100 kHz.

2.5.1 Resistance Based Methods

Discrete Devices

The Coulter Volumetric Counter (CVC) introduced in Section 1.1 was the first automated cell counting instrument. Based upon the Coulter Method first patented in 1949 \[22\], the CVC exploits the intrinsic DC resistance of biological cell membranes to count individual cells suspended in physiological saline as they transit through an orifice. A DC current is flowed between two immersed electrodes (typically inert Pt) located either side of the orifice and the voltage measured across them; as a cell transits the orifice, current is occluded in direct proportion to the cell’s size as illustrated in Figure 2.13, resulting in a resistance change between electrodes recorded as a voltage pulse \[23\].

![Figure 2.13](image-url)

**Figure 2.13:** Coulter volumetric method: (a) empty orifice filled with saline (DC conductivity $\sigma_s$) constrains E-field; (b) presence of cell within orifice (volume $v(r)$ [m$^3$], where $r$ is particle radius [m], DC conductivity $\sigma_c < \sigma_s$ [S]) occludes E-field lines proportional to its volume through interfacial polarisation, increasing orifice resistance, measurable as an increase in voltage across electrodes.

Specifically, the DC resistance measured by the CVC arises from interfacial polarisation due to charge accumulation at the boundary between the conductive suspending saline solution and the dielectric cell membrane. Hence, the CVC measures complex permittivity $\varepsilon^*$ at zero frequency. While the recorded pulse height is ideally a direct function of the particle size alone, the pulse height, shape and duration is dependent upon the particle trajectory \[147\], limiting the absolute size measurement possible with the CVC. Combined with the similarity in size of the immune cells \[148\], the CVC has been restricted to automated blood cell counting. However, label-free cell phenotyping studies have been reported on CVCs modified to measure the AC impedance of samples within the $\beta$-dispersion band, considered further in Section 2.6.
Integrated Devices

Despite the absence in cellular specificity arising from cell size, the Coulter Method has found adoption in modern nanopore technology for DNA analysis [149]. Nanopores exploit a biological or synthetic membrane through which nanoscale holes (nanopores) have been constructed, with diameters matching specific DNA nucleotides [150]. Typically, a 3-electrode potentiostat sensing arrangement is used that applies a DC voltage across a highly-parallel array of nanopores and the global change in resistance (or equivalently, conductance) arising from the sample is measured as a change in current. The sensing arrangement requires potentiostat feedback to accurately control the applied voltage, with conductance recordings of 1.16 nS reported between 500 mHz and 20 kHz for individual DNA molecules upon solid state nanopores, connected to an instrumentation amplifier input stage implemented in 0.35 $\mu$m CMOS [151, 152].

Although nanopores have shown single molecule sensing resolution, the inability to integrate sensing functions within the nanopore membrane fundamentally limits the approach to individual molecules and not whole cells, and hence is not applicable to this work.

Hassibi and Lee report a reconfigurable 3-electrode biosensor architecture for potentiometry, electrochemical IS and ion sensitive field effect transistor operation (ISFET, see Section 1.5.2), implemented in 0.18 $\mu$m CMOS [89, 90]. A 10×5 array of 160×120 $\mu$m² pixels each contain two parallel 60×60 $\mu$m² working and reference electrodes formed within the topmost metal layer. The working electrode is functionalised with Bovine Serum Albumin (BSA) and the counter electrode left as bare Al. Each are connected to a dedicated instrumentation amplifier and share a global counter electrode connected to a common-mode voltage. The device is shown capable of measuring the presence of BSA from DC to 10 kHz. Manickam et al. report a similar 3-electrode biosensor arrangement applying the lock-in technique implemented in 0.35 $\mu$m CMOS and comprised of a 10×10 element array of 40×40 $\mu$m² electrodes, post-processed with gold and a capture probe [131]. The system has been shown capable of identifying unique electrochemical impedance spectra between 750 Hz to 100 kHz for single- and double-stranded DNA, BSA and Phosphate Buffered Saline (PBS). The latter device requires a separate, discrete reference electrode and both devices are fundamentally limited by non-specific binding due to their reliance upon biosensor electrode coatings.

Impedance imaging at DC has been reported upon a 4×4 element array of 100 $\mu$m² electrodes formed in 0.35 $\mu$m CMOS and post processed with a gold coating [153], and two-dimensional impedance imaging at 10 kHz demonstrated upon passive microfabricated Pt electrodes using an impedance analyser for observing cell migration and stratification of human epithelial stem cells in real time [140]. However, the limited resolution and use of expensive custom microfabrication and CMOS post processing steps makes these approaches unattractive for this work.
2.5.2 Capacitance Based Methods

Direct Charge Sensing
Charge based sensing methods provide a measure of the dielectric properties separating a pair of electrodes through electric displacement $D$. Recalling that capacitance $C$ [F] is defined as the proportion between charge $Q$ [C] and electric potential $V$ [V], capacitance based sensors are readily employed for measuring $D$. Examples include a $32 \times 32$ element biosensor array of $7 \times 7 \, \mu m^2$ functionalised electrodes at $15 \, \mu m$ pitch implemented in $0.35 \, \mu m$ CMOS for DNA detection \[126\]. A functionalised sensor electrode is reset to a known starting potential, the DNA sample introduced, and resulting change in charge measured with respect to a known polysilicon capacitor located within each pixel. An output voltage is generated through an in-pixel buffer connected to a global current sink. The authors report a sensitivity of 10 DNA base pairs, however the biosensor method is fundamentally limited by non-specific binding.

Charge Sharing
Prakash et al. \[136, 137, 154\] describe CMOS-integrated sensors for cell adhesion studies, applying a charge-sharing technique first reported for fingerprint sensing \[155\], where six $40 \times 40 \, \mu m^2$ electrode array senses the change in charge induced upon them as cells adhere to the passivation dielectric above each electrode. Implemented in $0.5 \, \mu m$ CMOS with no post processing necessary, the authors report sub-fF sensitivity. The same technique has been extended to measure 300 nm magnetic beads in direct contact with post-processed copper electrodes formed in a $256 \times 256$ element sensor array with $0.6 \times 0.72 \, \mu m^2$ pixel area in 90 nm CMOS \[156\]. The latter work demonstrates the capability of the charge sharing technique to sense particles much smaller than individual cells (and indeed individual bacterium), and the opportunity presented by methods compatible with standard CMOS scaling, reporting sensor elements approaching the size of an individual virus.

Charge Amplification
Manaresi et al. present a charge amplification method for capacitance measurements of single cells above a $320 \times 320$ element array of sense electrodes spaced at $20 \, \mu m$ pitch, formed in the top metal layer of a standard $0.35\mu m$ CMOS process, with reference to a separate conductive top-plate forming the second conductor, separated $100 \, \mu m$ from the electrode array \[135\]. The authors report sensing $10 \, \mu m$ diameter polystyrene beads, Yarrowia lipolytica yeasts and human erythro-leukaemia K562 cells, achieving a measured capacitance resolution of $0.42fF$. Each electrode is furthermore capable of actuating cells via dielectrophoresis (see Section 2.6.3). However, the reported measurements were performed in low-conductivity mannitol solution and relied upon a 9V step being applied to the top-plate, which is DC contact with the sample, risking Faradaic current flow. The complexity of aligning such a top-plate with the sensor array makes this architecture unattractive.
Charge Integration

The first CMOS-integrated DNA polymerisation sensor is claimed by Anderson et al. [157]: implemented in 0.18 μm CMOS, a charge integration scheme to convert induced charge to output voltage is employed across a 5×5 element array of post-processed electrodes spaced at 500 μm pitch. Whilst the authors report a 25 fC limit of detection, the method requires long integration periods (>1 s) and a large 30 pF feedback capacitor per sensor, resulting in a 300×300 μm² sensor area that is approaching fifty times that of an individual biological cell, and hence is not well suited to rapid single-cell analyses.

Charge integration is employed by Stagni et al. who report a 14.3% shift in capacitance arising from DNA immobilisation upon an 8×16 array of circular gold electrodes with 200 μm diameter, post-processed upon 0.5 μm CMOS [123, 158]. The authors integrate analogue-to-digital conversion via charge-to-frequency conversion, where the voltage arising from charge integrated upon the sense electrodes is compared to reference voltages, which when exceeded, trigger a comparator to change state. The comparator output is used to reset a digital counter running synchronous to the applied current stimulus. The presented system is an elegant solution, particularly as it exploits a fully-differential architecture to avoid referencing an absolute capacitance (which would be poorly defined). However, the measured capacitance resolution is directly proportional to the current stimulus, which will be noise limited beneath 1 μA [158].

Charge Based Capacitance Measurement (CBCM)

Charge Based Capacitance Measurement (CBCM) was first developed to accurately measure metal interconnect shunt capacitances in microelectronic processing, with 16 aF sensitivity claimed from implementation in 0.5 μm CMOS [159]. CBCM measures the difference in charging (and discharging) current consumed between two matched CMOS inverters, one connected to the unknown capacitance and the other to a reference capacitor. 10 μm to 50 μm polyamide particle sensing in 10 m/s air flows (the latter contributing a capacitance change of 60 aF) has been reported from 20 × 20 μm electrodes integrated in 0.8 μm CMOS [160].

CBCM has been extended to measure various biological liquids (including deionised water and methanol) at 100 Hz [161] and 10 kHz [162] through integrating ΣΔ analogue-to-digital conversion with one reference and three sense electrodes, implemented in 0.18 μm CMOS and combined with microfluidics. The same device has been post-processed with inclusion of a biosensor layer to target specific analytes [163]. Stagni et al. apply CBCM with post-processed gold deposition and biosensor functionalisation to demonstrate a 20% shift in measured capacitance arising from DNA immobilisation upon 1×1 mm² sense electrodes, implemented in 0.5 μm CMOS, operating over a frequency range of 10 Hz to 100 Hz [130]. While the above biosensors are inherently restricted by non-specific binding, the differential nature of CBCM is attractive, as the measured capacitance does not depend upon an absolute reference, but rather the absence versus presence of a particle.
The CBCM method has been further applied in the form of current integration to measure the capacitance change arising from immobilisation of H5N1 virus strands upon post-processed, DNA functionalised electrodes measuring $80 \times 80 \mu m^2$ at 90 $\mu$m pitch, integrated in 0.35 $\mu$m CMOS [164]. A differential version of the same sensor architecture is further reported to achieve a sensitivity increase of 57.8 dB, equivalent to a fifty-fold decrease in limit of detection of DNA bases [165]. However, the biosensor nature of these devices and reference electrode being physically distant to the sense electrodes (>500 $\mu$m) limits the applicability of this approach for single-cell analyses.

2.5.3 Electrowetting-on-Dielectric

Electrowetting-on-Dielectric (EWOD) is an attractive method for highly-parallel, single cell actuation, and potentially, sorting. EWOD operates by modifying the surface tension between a fluid packet and its surroundings through application of a voltage across the meniscus formed between the two media [166], illustrated in Figure 2.14. Translation, separation and merging of fluid packets can be achieved depending upon the electrode pattern [3], with actuation of physiological media demonstrated [167]. Integration in CMOS has the potential to manufacture multiple electrodes into two-dimensional arrays [168], integrate sensor circuitry [169] and potentially, to define fluid packets upon the same scale as individual cells.

![Figure 2.14: Electrowetting-on-Dielectric (EWOD) theory of operation: the surface tension between the fluid packet and its surroundings is modified by application of a voltage across the meniscus formed between the two media, resulting in a change of contact angle with the substrate, and motion in the direction of the applied voltage. From Cho, Moon and Kim [3].](image)

However, EWOD typically requires voltages higher than those compatible with standard CMOS, and a top-plate electrode aligned parallel to the actuating electrode array to aid fluid packet actuation and prevent evaporation. These limitations have been overcome to demonstrate EWOD actuation of deionised water droplets on standard 5 V CMOS without use of a top-plate [108], through using oil as the surrounding medium. However, the latter was achieved through a complicated sample pre-processing procedure. The same authors also report highly parallel single cell actuation using dielectrophoresis, considered further in Section 4.6.3.
2.5.4 $\alpha$-dispersion Methods Discussion

Capacitance based methods provide a direct measure within the $\alpha$-dispersion and are attractive due to their voltage output, that is readily sensed via high impedance (and hence low loss) circuitry. Of those, direct charge sensing and charge amplification are the most relevant, as they make no assumption as to the frequency dependence of the measured capacitance. Indeed, the majority of reviewed capacitance based sensing schemes assume the measured capacitance to be frequency independent, however the complex permittivity of biological cell suspensions is frequency dependent; rendering these methods incapable of broadband measurements. Furthermore, $\alpha$-dispersion methods do not provide information directly upon the sample itself, but rather its interaction with its environment, due to the dominance of interfacial polarisation at low frequencies, and particularly so for microscale electrodes [118]. Hence, the $\alpha$-dispersion is not of immediate relevance to this work.

Regarding actuation, EWOD appears an attractive means of actuating multiple, discrete fluid packets in a highly-parallel manner; however the method requires actuation voltages in excess of those tolerated by standard low-voltage CMOS processes, or complex sample preparation procedures. Consequently, EWOD is not considered further for this work.

2.6 Methods for $\beta$-dispersion Studies

This section reviews methods for the study of biological cell suspensions across the 100 kHz to 100 MHz band.

2.6.1 Bulk Sensing Methods

Fricke and Morse first describe bulk measurements of whole blood suspensions in 1925 from 800 Hz to 4.5 MHz using an impedance bridge [133, 134], observing the internal conductivity of Red Blood Cells (RBC) as 0.17% of NaCl saline solution. Their electrolytic cell comprised of platinum black electrodes within a glass container, split into two semi-spherical halves to provide a simple geometric cell constant approximation. The blood sample was in DC contact to the electrodes and static, experiencing sedimentation due to gravity. Stimulus was applied and measurements performed immediately after stirring the sample suspension to avoid sedimentation, and interestingly, the authors report a non-random alignment of the sample after stirring stopped. While the authors do not attribute the cause of this alignment, it may be an early example of dielectrophoresis (DEP, see Section 2.6.3). The same effect was also observed in suspensions of cream [139].
Literature Review

Schwan and Ferris in 1968 [170] report bulk measurements of the effective permittivity of yeast suspensions using a 4-electrode bridge arrangement. Similar approaches employing impedance analysers (see Section 2.4.5) are used for the study of bulk suspensions of *Saccharomyces (S.) cerevisiae* yeast at various stages of fermentation [171, 172, 173], blood [174] and mouse fibroblasts [175]. The lattermost reports a greater decrease in complex permittivity magnitude than expected for mouse fibroblasts across the β-dispersion, which the authors attribute to villus protrusions from the cell membrane (used by the adherent cell type to attach itself to its surroundings). This finding indicates the difficulty in interpreting the Cole-Cole parameter α in expression (2.4), and furthermore, the use of a single value (termed membrane capacitance) as a means of identifying cell types: the authors report membrane capacitance variations between 1.7 F/cm^2 to 4.0 F/cm^2 for the same cell type, depending upon the batch of cells [175].

Microfabricated Devices

Microfabricated devices have been shown capable of identifying healthy and cancerous white blood cells using time domain IS [176]. Bulk impedance of yeast (*S. cerevisiae* and *S. pombe*) from 50 kHz to 5 MHz while undergoing actuation via dielectrophoresis at 6.7 MHz [177], and similarly biosensor layers for bulk capturing CD34+-/- expressing T-cell cancers [104] have also been reported. The measured impedances increase in proportion to cell alignment with the electrodes. This suggests that DEP alignment for static samples is an attractive means of increasing measurement repeatability and potentially, resolution.

2.6.2 Single Cell Sensing Methods

Single cell impedance measurements of RBCs using a Coulter Counter (see Section 2.5.1) modified with an impedance bridge to support a 20MHz frequency measurement were first reported by Thomas et al. in 1977 [178]. The authors define electrical opacity as the ratio of AC impedance (attributed to the interior of the cell) to DC resistance (attributed to the cell’s volume), and demonstrate a distribution of opacity measurements for RBCs. Hoffman and Britt [3] extend this method through use of an impedance bridge and demodulation to show distinct electrical opacity distributions for 15.7 µm diameter polystyrene micro-beads and RBCs, with 1.0 MHz AC stimulus. The same authors extend their modified Coulter Counter to distinguish mouse and human lymphocytes, chick and human RBCs, Chinese Hamster Ovary (CHO) cells and colon tumour cells by their electrical opacity at DC and 4.5 MHz [92]. The authors reduce the opacity distributions to single values by defining relative electrical opacity, where \( V_{\text{rf}} \) and \( V_{\text{dc}} \) [V] refer to the radio-frequency (4.5 MHz) and DC components of the cells and micro-beads, respectively:

\[
\text{Relative Opacity} = \frac{|V_{\text{rf}}(\text{cells})|/|V_{\text{dc}}(\text{cells})|}{|V_{\text{rf}}(\text{beads})|/|V_{\text{dc}}(\text{beads})|}.
\] (2.13)
Microfabricated Devices

Microfluidic IS systems are reviewed extensively by Cheung [93] and Chen [98]. Notable devices include that by Ayliffe et al. [179] that incorporates 36 $\mu m^2$ platinum electrodes within a 4 $\mu m$ tall by 10 $\mu m$ wide microchannel etched in a quartz substrate. DC IS measurements at (10, 100, 500) kHz and 1 MHz are performed via an external impedance analyser and shown to distinguish polymorphoneuclear leukocytes from RBCs, with a pronounced dispersion present. Gawad et al. report a microfluidic IS device formed in glass with platinum electrodes measuring 20$\times$20 $\mu m^2$, spaced 20 $\mu m$ apart, that exploits rotational symmetry: two devices are anodically bonded together to form a 20 $\mu m$ tall microchannel with two pairs of parallel-plate electrodes. Demodulation is used to distinguish latex micro-beads of 5 $\mu m$ and 8 $\mu m$ diameter from each other and RBCs using 1.72 MHz and 15 MHz frequency components, in physiological saline solution, at flow rates up to 100 events/s [180].

An identical device is used to distinguish healthy and individually fixed RBCs from micro-beads [92] and the major white blood cell groups of monocytes, lymphocytes and neutrophils through opacity measurements performed at 503 kHz and 1.7 MHz, using an external impedance analyser [95, 96]. The same system has also discriminated activated from non-activated T-cell lymphocytes (expressing CD4+ antibodies) through the use of antigen coated polystyrene microbeads through IS measurements spanning 500 kHz to 10 MHz [181]. Similar devices have demonstrated sheathless operation through DEP sample focussing [182], integrated two-dimensional acoustic focussing [183], cell morphology detection [184] and three-dimensional electrodes, used for discerning healthy from cancerous T-cells across six demodulated frequencies spanning 100 kHz to 27 MHz [185].

The aforementioned devices all rely upon external impedance analysers to perform the actual IS measurements. Gawad, Sun and colleagues report the use of time domain m-sequence stimulus (introduced in Section 2.4.5) for single cell impedance measurements, demonstrating RBC impedance spectra containing 512 frequencies spanning 1.95 kHz to 500 kHz, achieved in physiological saline using a 10th order sequence with a 1 MHz sampling frequency [143, 145, 146]. The sampling frequency was limited through use of an external data acquisition board, and the parasitic shunting capacitance of the microfabricated device [143, 138].

CMOS integrated Sensors

Chen et al. present 96$\times$96 element array of 25$\times$25 $\mu m^2$ gold electrodes integrated in 0.18 $\mu m$ CMOS for electrochemical IS measurements of MCF-7 breast cancer cells, recording a 20% increase in impedance with respect to background KCl solution at 200 kHz [186]. The 3-electrode technique is employed, with an Indium Tin Oxide (ITO) coated transparent counter electrode aligned parallel to the CMOS device. A similar approach has been taken with addition of a biosensor layer upon a 10$\times$10 element array of 50$\times$50 $\mu m^2$ gold electrodes integrated in
0.35 μm [131]. Demodulation is applied on-chip with stimulus spanning 500 Hz to 5 MHz and numerous salt buffers and DNA spectra shown.

Lee et al. present a CMOS integrated impedance cytometer for the analysis of single cells in flow between 20 kHz and 1 MHz [132]. Exploiting 3D particle focussing, the impedance difference arising from two pairs of microfabricated electrodes (located upon another device) is sensed through a demodulation technique similar to that described previously, but integrated on-chip. ΣΔ error correction is employed to minimise the drift of electrode DC voltage over time. The authors report discrimination between RBCs and individually fixed RBCs from the pulse height data alone, with no impedance spectra shown.

2.6.3 Dielectrophoresis

The electromotive force, the motion of conductive bodies in close proximity to flowing electric currents, has been well documented since Faraday [114], and the formation of induced dipoles within a dielectric when exposure to an external electric field since Maxwell [122]. Electrophoresis (EP) exploits the latter to measure the electrical affinity of materials and benefits from miniaturisation due to the high E-field strengths made possible at the microscale [125, 188]. Dielectrophoresis (DEP) arises due to the torque experienced by an induced dipole to align itself with the applied E-field, as illustrated in Figure 2.1. When the E-field is non-uniform such that a geometric gradient exists, a net torque arises such that a dielectric particle will experience an electromotive (electrokinetic or ponderomotive [189, 190]) force, first termed DEP by Pohl in 1951 [191]. When exposed to an AC electric field, a spherical particle with radius $r$ [m] will experience a net DEP force $\mathbf{F}_{\text{DEP}}$ [N] averaged over the period of the AC excitation:

$$\langle \mathbf{F}_{\text{DEP}} \rangle = 4\pi r^3 \varepsilon_p^* \Re \left[ f_{CM}^* \nabla |\mathbf{E}|^2 \right],$$

where $\varepsilon_p^*$ [F/m=C/m²] is the absolute complex permittivity of the particle, $\mathbf{E}$ [V/m=N/C] the applied electric field strength, and $f_{CM}^*$ the Claussius-Mossotti (CM) factor, defined as:

$$f_{CM}^* = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*},$$

where $\varepsilon_m^*$ [F/m] is the absolute complex permittivity of the suspending medium. The difference in particle and medium complex permittivities defines whether the suspended particles experience an attractive (positive) or repulsive (negative) DEP force towards regions of high E-field density, which will vary with the frequency according to the frequency dependence of the particle and medium complex permittivities, illustrated in Figure 2.15.
Figure 2.15: DEP force \( F \) [N] experienced by a particle with complex permittivity \( \varepsilon_p^* \), suspended in a liquid medium with complex permittivity \( \varepsilon_m^* \), when exposed to non-uniform electric field \( E \) [V/m]. (a) Negative DEP (nDEP, where \( \varepsilon_p^* < \varepsilon_m^* \)). (b) Positive DEP (pDEP, where \( \varepsilon_p^* > \varepsilon_m^* \)).

**Microfabricated Methods**

Microfabrication has enabled DEP actuation to be applied across a wide range of biological samples, as reviewed by Pethig [100, 192] and Hughes [193]. Gascoyne and Vykoukal [194] further review the variation in DEP cross-over frequency (where samples transition from experiencing pDEP to nDEP, or vice versa), which has been exploited with integrated biosensor electrode coatings and impedance analysis for capturing rare peripheral blood mononuclear cells [104]. In addition to particle translation with p/nDEP, particles experience a rotational force termed electrorotation (EROT) when exposed to a rotating electric field, generated from quadrature stimulation on typically semi-circular electrodes. This approach has identified viable from non-viable \( S. \) cerevisiae yeast from their EROT behaviour in 0.5 mS/m glucose solution [101]. Quadrature stimulus can also be applied to translate particles across an electrode array, termed travelling-wave DEP (TW-DEP). TW-DEP has been shown capable of identifying T-cell lymphocytes at different stages of their life cycle due to their direction of travel with respect to the TW field in 40 mS/m solution [195].

The greatest potential application for DEP (and its related electrokinetic phenomena) in biology arises from its integration with microfluidics, to enable label-free cell sorting, such as separating human breast cancer cells from RBCs and T-cell lymphocytes in flowing 56 mS/m sucrose suspension [113]. Termed Fluid Flow Fractionation (FFF), DEP cell actuation has been demonstrated to trap cells for enrichment applications of yeast (in 30 \( \mu \)S/m solution) [196], mouse fibroblasts (10 mS/m) [197] and cancerous monocytes (33 mS/m), at flow rates equivalent to 0.25 m/s (comparable to conventional flow cytometry) [198]. Microfabrication on transparent substrates enables fluorescently labelled samples to be focussed [199] and sorted with DEP in 0.3 S/m solution flowing at 300 \( \mu \)m/s [200]. Similar DEP focussing has been demonstrated with integrated impedance sensing in static [177] and flow environments [95, 201, 202]. Label-free enrichment of cancerous peripheral blood mononuclear cells (PBMCs) at 3,300 events/s from
whole blood re-suspended in 30 mS/m glucose solution has also been reported [203]. However, the above devices all require custom microfabrication processes including gold and platinum electrode deposition, and external excitation sources.

**CMOS integrated Actuators**
The passive microfabricated devices described above are all formed by a single metallisation layer, which limits the possible electrode arrangements to those which support one-dimensional DEP actuation only. CMOS integration overcomes this problem by providing multiple metal layers for two-dimensional arrays and multiplexing circuitry integration, for independent electrode addressing. Hunt et al. exploit these features to perform highly-parallel, single cell DEP actuation of *S. cerevisiae* yeast cells upon a 128×256 element array of 11×11 μm² electrodes, formed in the top most metal layer of a standard 0.35 μm CMOS process [108]. Yeast was re-suspended in 100 μS/m mannitol buffer and actuated via a 5 V peak-to-peak square wave excitation at 1 MHz. The sample was AC coupled to the electrode array via the passivation dielectric deposited as standard during CMOS manufacturing, preventing electrochemistry.

A further benefit of CMOS microelectronics is the capability to integrate sensor circuitry. Manaresi et al. apply this for integrating photodiodes (PD) with DEP actuation circuitry within a 320×320 element array with 20 μm electrode pitch, implemented in 0.35 μm CMOS [106, 204]. The same group integrate a charge amplification based capacitive sensor at the same pitch [135] and demonstrate optical and capacitive sensing of *S. cerevisiae* and *Yarrowia lipolytica* yeast cells suspended in low conductivity 280 mM mannitol solution, with 3.3 V peak-to-peak excitation at 1 MHz. The CMOS electrode array is AC coupled to the sample across the CMOS passivation layer, but requires an additional electrode aligned parallel to, and spaced 100 μm away from, the array for DEP actuation and capacitive sensing.

**2.6.4 β-dispersion Methods Discussion**
The preceding sections have reviewed numerous methods for sensing and actuating individual biological cells in suspension across the β-dispersion band, which have demonstrated a wide variety of cell phenotyping and sorting applications. Regarding sensing, broadband measurements are desirable to observe an impedance spectrum as opposed to a single value of capacitance, to identify subtle differences between similar cells. Time-domain dielectric spectroscopy using digital m-sequence stimulus combined with CMOS integrated sensors appear to have tremendous scope for rapid, broadband analysis across the entire 100 kHz to 100 MHz frequency band, and as to date unreported in the literature. Single cell actuation via DEP has been demonstrated upon CMOS integrated devices, but all those reported require the cell sample to be re-suspended in low conductivity media (e.g. mS/m), compared to that of physiological saline (1.2 mS/m).
Such re-suspension risks cell loss and removes the cell further from its in-vivo environment. However, Sudsiri et al. show the effect of increasing medium conductivity upon EROT experiments, observing an exponential drop off in force with increasing medium conductivity (and no actuation occurring for conductivities above 1 S/m) [205]. The high electric field gradients made possible through microfabrication and hence CMOS integration may well overcome this restriction, particularly for large particles, as seen by the DEP force dependence upon these parameters in expression (2.14). Broadband impedance measurements using m-sequence stimulus and single-cell actuation via DEP are therefore immediately relevant to this work.

2.7 Methods for γ-dispersion Studies

This section reviews methods for the study of biological cell suspensions across the 100 MHz to 10 GHz band.

2.7.1 Discrete Methods

A microfabricated co-planar waveguide formed from gold electrodes deposited upon a quartz substrate beneath a microfluidic channel is reported by Grenier et al. [206]. Microwave measurements are performed across 1 GHz to 40 GHz (using an external network analyser) upon human umbilical vein endothelial cells as they transit the waveguide. An average 20% decrease in permittivity is observed when cells transit the waveguide. A similar waveguide approach is taken to trap individual cancerous B-cells above gold electrodes for interrogation across the same bandwidth; a maximum capacitive contrast (defined as the difference in capacitance observed when cells are present within the trap and not) of 0.53 fF is obtained at 5 GHz [207]. The authors observe the capacitive contrast for cells is significantly lower than that of polystyrene micro-beads, attributing the reduction due to the abundance of water within cells. A further macroscale waveguide approach for mm-waves has been applied for measuring yeast concentration during fermentation across narrow bands centred at 2.6-3.95 GHz, 8.2-12.4 GHz, and 33-50 GHz [208]. However, the above waveguides are fixed in their measurement frequency by their physical shape, making broadband measurements infeasible in practice.

Microfabricated resonant transmission lines have been shown to achieve 650 zF capacitance resolution across a 50 Hz bandwidth centred at 1.5 GHz, applied to sensing 6 μm and 8 μm polystyrene micro-beads and CHO cells [209] and T-cell lymphocytes, suspended in physiological saline, while in microfluidic flow [210]. A similar device has been configured as a reflectometer to detect 5.7 μm polystyrene beads and CHO cells achieving 1.25 aF sensitivity across a similar 50 Hz bandwidth centred at 2 GHz applying the lock-in technique [211]. While resonant and reflection techniques can achieve capacitance sensitivities approaching two orders
of magnitude greater than the capacitive sensors reviewed in Section 2.5, they require external analyser hardware with part per million accuracy to support the delicate calibration necessary for such sensitivities [211]. This limits their applicability for broadband measurements, compounded by their dependence upon expensive custom microfabrication.

Open-ended coaxial reflection methods have also been reported [212] including blood measurements across 1 MHz to 3 GHz in a parallel-plate capacitor sample arrangement [213]. The greatest distinction between cells is apparent within the 1 kHz to 10 MHz region, indicating greatest distinction achieved with \( \beta \)-dispersion measurements. Indeed, \( \gamma \)-dispersion measurements spanning 500 MHz to 40 GHz show little distinction between cell types due to the dominance of water relaxations within the frequency band.

### 2.7.2 Integrated Methods

CMOS integration provides an alternative to waveguides, by enabling radio-frequency measurements to be performed directly on-chip with minimal loss due to small shunt capacitances. Complex permittivity measurements spanning 0.625 GHz to 10 GHz in eight discrete steps are reported via frequency mixing with on-chip inductors integrated in 0.18 \( \mu \)m CMOS [214]. A similar LC tank approach is used with integrated oscillator arrays tuned at 60 GHz and 120 GHz for dielectric measurements of bound water relaxation in 65 nm CMOS [215].

Mohammad et al. report a ring-oscillator based capacitive sensor that achieves \( \sim 14 \) aF sensitivity at 0.7 GHz and 1.4 GHz by observing the beat frequency arising through the capacitive loading of two tuned ring-oscillators, implemented in 0.35 \( \mu \)m CMOS [216, 217]. The device is shown to detect 10 to 15 \( \mu \)m diameter polystyrene micro-beads and CHO cells at the applied frequencies. A similar frequency shift approach combines tuned LC resonators and a Voltage Controlled Oscillator (VCO) to sense the complex permittivity of methanol-ethanol and xylene-ethanol mixtures across 7 to 9 GHz frequency band implemented in 90nm CMOS [218]; the same approach is extended to three tuned LC resonators across 1 to 6 GHz band for de-ionised water and PBS analysis implemented in 0.18 \( \mu \)m CMOS [219]. The frequency difference approach is again demonstrated at 6.5 GHz, 11 GHz, 17.5 GHz and 30 GHz for measurements upon single cells in a microfluidic integrated system achieving 0.32 aF sensitivity in 65 nm CMOS [220].

\( \gamma \)-dispersion Method Discussion

The above resonator based approaches are all limited by the need for a dedicate, tuned circuit per analysis frequency, making broadband resonance measurements impractical. Integration in CMOS can overcome some of these difficulties via frequency difference methods exploiting tuneable oscillators, such as VCOs. However, their absolute tuneable range is similarly
restricted by their physical design, making broadband measurements similarly impractical. A further limitation of $\gamma$-dispersion measurements arises from the dominance of bound water relaxation within the frequency band, fundamentally limiting the difference observed between biological cells and their suspending medium [207]. Indeed, no actuation methods are reported in the $\gamma$-dispersion band, due to the resonance of water and risk of microwave sample heating. Hence, the $\gamma$-dispersion is not of immediate relevance to this work.

2.8 Summary

This chapter has reviewed the study of biological cells in liquid suspension through the use of electricity, and specifically, when exposed to electric fields. Their passive electrical properties were briefly summarised and found to be predominantly dielectric. Complex permittivity $\varepsilon^* = \varepsilon' + j\omega\sigma$ provides a convenient way of representing materials with energy consumptive and dissipative qualities, represented by real permittivity $\varepsilon'$ and real conductivity $\sigma$, respectively.

Dipoles are induced in a dielectric when exposed to an external electric field and give rise to four forms of polarisation: electronic, atomic, orientational and interfacial. These polarisation mechanisms dominate at different frequencies, resulting in frequency-dependent dispersions. Biological cell suspensions exhibit three dominant dispersions, termed by Schwan [2] as $\alpha$, $\beta$ and $\gamma$. The $\alpha$-dispersion occurs at low frequency (beneath 100kHz) and is due to interfacial polarisation between the cell membrane and its suspending medium; the $\beta$-dispersion occurs at intermediate frequency (between approximately 100kHz and 100MHz) and attributed to orientational polarisation of dipoles within the cell; and the $\gamma$-dispersion is attributed to atomic polarisation of water molecules, occurring at high frequency (above 1GHz).

Impedance spectroscopy provides a method of measuring $Z(\omega)$ across the band of angular frequency $\omega$ that covers the $\alpha$, $\beta$ and $\gamma$-dispersions, but risks electrochemical reactions occurring at the electrode-sample interface through Faradaic current flow. Dielectric Spectroscopy alleviates this problem by placing a dielectric in series between the electrode and sample, replacing conventional current flow by displacement current. Sample interfacing methods were then considered and 2-, 3- and 4-electrode arrangements introduced to respectively reduce the effect of electrode polarisation upon the measured sample impedance. Methods of interfacing circuitry with the Sample Under Test (SUT) were introduced and direct sensing found to be most appropriate for rapid, broadband impedance measurements. Stimulus methods in the frequency- and time-domains were considered, with m-sequence stimulus deemed similarly appropriate for this work.

Methods were then reviewed for the analysis and actuation of biological cell suspensions across the $\alpha$, $\beta$ and $\gamma$-dispersion bands. The $\alpha$-dispersion provides little information regarding the
sample directly, due to the dominance of electrode polarisation at low frequencies. However, direct charge sensing and charge amplification methods were deemed immediately relevant for this work. Electrowetting-on-Dielectric (EWOD) was introduced as a potential means of actuating discrete packets of fluid in a highly-parallel manner, but deemed limited by its dependence upon high actuation voltages and complex parallel-plate conductor assemblies.

Methods operating across the $\beta$-dispersion were shown capable of distinguishing numerous cell types across a wide range of applications, however a broadband of measurements are desirable to observe an impedance spectrum as opposed to a single value of capacitance, to identify subtle differences between similar cells. Most reported implementations require custom microfabrication or CMOS post-processing, and are reliant upon external lock-in amplifiers or impedance analysers. Dielectrophoresis was introduced and shown capable of highly-parallel actuation of individual cells without extrinsic cell labelling, and directly benefits from CMOS integration.

Methods spanning the $\gamma$-dispersion were then introduced and found to be limited in their cell phenotyping capabilities due to the dominance of bound water molecules in across the $\gamma$-dispersion band. Indeed, no actuation methods were found operable in this band due to the risk of microwave heating the sample due to said bound water.

Recalling the purpose of this work is to analyse and actuate cells without the use of extrinsic cell labelling, with view for use in rapid diagnostics, dielectric spectroscopic measurements spanning the $\beta$-dispersion frequency band appear the most relevant for this purpose. Time-domain dielectric spectroscopy using digital m-sequence stimulus combined with CMOS integrated sensors appear to have tremendous scope for rapid, broadband analysis across the entire 100 kHz to 100 MHz frequency band, and as to date unreported in the literature. The next chapter investigates the feasibility of such sensors.
3.1 Introduction

Chapter 2 concluded that a gap exists within the literature for Dielectric Spectroscopic (DS) sensors integrated in Complementary Metal-Oxide Semiconductor (CMOS) microelectronics, capable of measuring frequencies across the $\beta$-dispersion band of approximately 100 kHz to 100 MHz, introduced in Section 2.2. This chapter assesses the feasibility and potential performance of two sensor architectures: the first aims to be sensitive to the absolute electrical environment above a single electrode, to provide information regarding individual micro-particles and their suspending medium. The second architecture aims to be sensitive to the difference in electrical environment above a pair of electrodes, to cancel the common component of the suspending medium, and hence provide information upon the suspended particle only.

Microelectronics technology provides the capability of integrating identical circuit elements into two-dimensional (2D) array structures upon the same scale as individual micro-particles, such as biological cells. A highly-parallel array of sensing circuit elements benefits from physical redundancy (where manufacturing defects within one region of the array can be avoided by addressing another region of the array), and a physically greater sensitive area than possible with one sensor circuit element alone. The use of CMOS microelectronics permits multiplexing and signal processing circuits to be integrated beneath each sensor element, reducing the total number of off-chip connections necessary and maximising the sensitivity of recorded signals.

The chapter begins by assessing a unit geometry that models the interface between an array of CMOS-integrated electrodes and liquid sample media. A Basic Sensing Element (BSE) geometry has been defined and an equivalent circuit proposed. Analytical expressions relating the BSE’s geometric and material property parameters to equivalent circuit elements are derived for a medium containing no sample. A micro-particle is added to the BSE and equivalent circuit elements modified accordingly. Parallel combinations of the BSE are used to define two DS sensor architectures and expressions derived for their equivalent circuits. These circuits are then used to assess the feasibility and potential performance of each sensor, and help guide their implementation within a dedicated microsystem described in Chapter 4.
3.2 General Approach

Figure 3.1(a) represents a simplified geometry for sensing individual biological cells with CMOS-integrated electrodes. Particle $p$ is suspended in a dielectric medium (together referred to as the “sample”) above an array of sensor electrodes, separated from them by an isolating dielectric layer. The isolating dielectric layer serves two purposes: (i) to prevent free charge carrier exchange at the electrode-sample interface (preventing electro-chemical reactions from occurring), and (ii) to provide a high-impedance region upon which to store charge carriers arising from interactions between the sample and an applied electric field. It is assumed that each electrode is capable of sensing and stimulus, i.e. each electrode is capable of applying an arbitrary potential to the isolating dielectric layer immediately above itself.

Figure 3.1: Simplified model geometry: particle $p$ is suspended within a dielectric medium, separated from sensing electrode array via an isolating dielectric layer. (a) 3D geometry. (b) Reduced 2D geometry upon surface $S$.

Noting that the geometry in Figure 3.1(a) is rotationally symmetric, surface $S$ can be defined such that the 3D geometry can be reduced to 2D, as shown in Figure 3.1(b). Furthermore, the array is composed of identical sensing elements, the minimum unit of which is a pair of adjacent electrodes. This unit is termed the Basic Sensing Element (BSE) and considered in the following section. Multiple BSEs can be connected in parallel to model the interaction of numerous electrodes with a suspended particle. An equivalent circuit is first derived for an empty BSE, with component values related to design parameters, to permit simulations of the BSE’s frequency response. Two sensor architectures based upon the BSE are then developed and their potential performance explored.
3.2.1 Basic Sensing Element

The equivalent circuit of an empty BSE (where no particle is present) is shown in Figure 3.2(a). It consists of a parallel pair of planar electrodes, each with width \( w \) [m], thickness \( t \) [m] and spaced \( g \) [m] apart. The electrodes are separated from each other, and the sample medium, by the isolating dielectric layer. This layer will be the passivation dielectric deposited during standard CMOS manufacturing, assumed to be planar, homogeneous and crystalline in structure, giving rise to dispersions in the GHz region. The sample medium is similarly assumed to be homogeneous and to wet the entire isolating dielectric layer surface, leaving no voids. The sample medium will contain free charge carriers such as salt molecules, again leading to dispersions in the GHz region. Recalling the interest of this work is measuring frequencies within the \( \beta \)-dispersion, we can simplify the frequency behaviour of the BSE by ignoring frequencies and hence dispersions arising within the GHz region. Consequently, the isolating dielectric and sample medium are modelled as ideal capacitors, with homogeneous and linear permittivities.

![Figure 3.2: (a) Basic Sensing Element equivalent circuit when no particle is present. (b) Coordinate systems used to derive capacitances \( C_x \), \( C_y \) and \( C_s \). Electric field lines shown for \( V_1 > V_2 \).](image)

Figure 3.2(b) shows the coordinate systems used to derive analytic expressions for the shunt capacitance \( C_x \) [F] between adjacent electrodes, the coupling capacitance \( C_y \) [F] between the electrodes and sample interface, and \( C_s \) [F] the suspending medium capacitance sensed between electrodes. The following simplifying assumptions have been made:

1. **3D geometry can be reduced to 2D:** the simplified geometry shown in Figure 3.1(a) is rotationally symmetric, with a repeating unit of two parallel electrodes. Surface \( S \) is chosen to be symmetrical through the electrode pair centre, enabling the 3D geometry to be reduced to 2D.
2. **Separation of electric displacement components**: assumes the total electric displacement incident upon each electrode can be reduced to 2D, with independent horizontal ($D_x$) and vertical ($D_y$) components.

3. **Electric fields vary in one dimension only**: i.e. each capacitance can be treated as a parallel-plate capacitor, which holds for large plate areas in close proximity to one another.

The parallel-plate approximation for $C_s$ is illustrated in Figure 3.3. In rectangular co-ordinates, the electric-field probing the sample appears as a fringing-field between electrodes, whereas in cylindrical co-ordinates the field can be considered to vary in one cylindrical axis ($\rho$) only. This approximation greatly simplifies the geometry used to derive $C_s$ and holds for electrodes with large area placed in close proximity to one another.

![Figure 3.3](image.png)

**Figure 3.3**: $C_s$ derivation as parallel-plate capacitor through co-ordinate transform, showing E-field lines (solid) and isopotential lines (dashed).

Applying the above simplifying assumptions, analytical expressions relating $C_x$, $C_y$ and $C_s$ to the BSE’s geometric parameters shown in Figure 3.2 (a) are derived using the procedure outlined below [221]. Full derivations for each capacitance are given in Appendix B.

1. **Define problem geometry, coordinate system and boundary conditions**: for the three regions (denoted $i = 1$ – 3) shown in Figure 3.2 (b). Identify the isopotentials to calculate the capacitance between, and apply an arbitrary potential difference $V_0$ [V] across them as boundary conditions.

2. **Solve for potential between isopotentials**: permittivities $\varepsilon_x$, $\varepsilon_y$ and $\varepsilon_s$ are assumed to be ideal, permitting no direct current flow. Hence, there are no free charge carriers within these regions and the potential difference $V_i$ [V] across them can be calculated using Laplace’s equation $\nabla^2 V_i = 0$.

3. **Calculate electric-field between isopotentials**: given potential difference $V_i$ solve for $E_i$ [V/m] using $E_i = -\nabla V_i$. 

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4. **Calculate electric-displacement between isopotentials:** given \( E_i \) and assuming the dielectric between the isopotentials is isotropic (homogeneous in all directions) and linear (i.e. its relative permittivity \( \varepsilon_i [1] \) is independent of the arbitrary applied potential difference \( V_0 \)), solve for \( \mathbf{D}_i \) [V/m] using \( \mathbf{D}_i = \varepsilon_i \mathbf{E}_i \).

5. **Evaluate electric-displacement at one isopotential:** the total electric displacement (\( \mathbf{D} \)) incident upon the isopotential is equivalent to that upon its surface (\( \mathbf{D}_S \)). Recalling the simplifying assumptions above, the surface electric displacement is equivalent to its normal component only, i.e. \( \mathbf{D} = \mathbf{D}_S = D_n \mathbf{n} \), where \( \mathbf{n} [1] \) is a unit vector normal to the isopotential surface.

6. **Evaluate charge density at either isopotential:** by recognising that charge density \( \rho_S \) [C/m\(^2\)] is equivalent to the normal component of the electric displacement, \( D_n \).

7. **Find charge upon each isopotential:** by performing a surface integration over the isopotential surface, \( Q \) [C] = \( \int_S \rho_S dS \).

8. **Solve for capacitance between isopotentials:** using \( C_i \) [F] = \( |Q| / V_0 \), noting that the arbitrary choice of applied potential difference \( V_0 \) used to calculate \( Q \) cancels.

Applying the above procedure to regions \( i = 1 \sim 3 \) in Figure (b) yields the following expressions for the components shown in Figure (a):

\[
C_x = \frac{\varepsilon_0 \varepsilon_x w t}{g} \tag{3.1}
\]

\[
C_y = \frac{\varepsilon_0 \varepsilon_y w^2}{d} \tag{3.2}
\]

\[
C_s = \frac{\varepsilon_0 \varepsilon_s w}{\pi} \tag{3.3}
\]

where \( \varepsilon_0 \) is the permittivity of vacuum (8.854\( \times \)10\(^{-12} \) [F/m]) and recalling that the geometric parameters \( w \) [m], \( t \) [m] and \( g \) [m] are the electrode width, thickness and spacing, respectively, and \( d \) [m] the isolating dielectric layer thickness. Having derived these component expressions, the presence of a particle within the BSE is now considered.

**3.2.2 BSE with Solid Particle**

The addition of a particle within the basic sensing element acts to displace a finite volume of the suspending medium, modifying its permittivity \( \varepsilon_s \) between electrodes and hence changing \( C_s \). Frequency dependence is modelled by replacing \( \varepsilon_s \) with complex particle permittivity \( \varepsilon_s^* \). Separating the permittivity and conductivity components yields real permittivity \( \varepsilon_p \) and real
Modelling conductivity $\sigma_p$, represented by equivalent circuit elements $C_p$ [F] and $R_p$ [\Omega] in Figure 3.4(a). The parallel combination of capacitance $C_p$ and resistance $R_p$ forms the most general case for a first-order dispersion. $C_p$ represents any energy absorptive mechanism and $R_p$ any energy consumptive mechanism within the particle, without making any assumptions as to the origins of these mechanisms.

**Figure 3.4:** (a) BSE equivalent circuit when particle is present. (b) Geometric parameters used to derive equivalent particle capacitance $C_p$ and resistance $R_p$. Electric field lines shown for $V_1 > V_2$.

Charge carriers at the interface between the particle and suspending medium will act to cancel one another, leading to interfacial polarisation. While finite element methods [180, 138, 222] and finite difference methods [223] have been developed to solve the geometric problem shown in Figure 3.4(b), Maxwell [224] first described the interfacial polarisation arising from spherical inclusions within an otherwise homogeneous dielectric medium as a change in effective permittivity of that medium. Extended first by Wagner [225], and then by Fricke [91, 226] for non-spherical inclusions, Maxwell’s Mixture Theory (MMT) states the effective particle permittivity $\varepsilon_p$ becomes [2, 141]:

$$
\varepsilon_p = \varepsilon_m \left( \frac{2\varepsilon_m + \varepsilon_i}{2\varepsilon_m + \varepsilon_i} - 2p(\varepsilon_m - \varepsilon_i) \right),
$$

(3.4)

where $\varepsilon_p$ is the effective permittivity of a suspension of spherical inclusions with permittivity $\varepsilon_i$ occupying a small volume fraction $p$ within a medium with original permittivity $\varepsilon_m$. Considering the particle as a single inclusion and reducing the 3D geometry to 2D, the particle becomes a circle with area $A_i$ [m$^2$] suspended within a semi-circular area of medium $A_m$ [m$^2$] above the electrodes, with radius $w + (1/2)g$ [m]. Volume fraction $p$ then becomes:

$$
p = \frac{A_i}{A_m} = \frac{2r^2}{(1/4)g^2 + w^2 + gw},
$$

(3.5)
Particle capacitance $C_p(\varepsilon_p)$ is then calculated from Equation (3.3) by setting $\varepsilon_s = \varepsilon_p$. Particle resistance $R_p(\sigma_p)$ is determined similarly. Substituting conductivity for permittivity within Equation (3.4) yields the effective conductance of the solution containing a spherical particle. Recalling the definition of resistance $R = (1/\sigma)L/A \, [\Omega]$, where $\sigma \, [S/m]$ is the conductivity of a region with length $L \, [m]$ through area $A \, [m^2]$. Referring to Figure 3.4(b), substituting $A = A_m$ for the semi-circular sensing region above the electrodes and $L = (w + g)/2$ as the centre-line through the sensing region, the effective resistance of the region becomes

$$R_p = \frac{1}{\sigma_p} \frac{\pi (w + g)}{2w^2}.$$  

(3.6)

$C_p$ then models all energy storage effects and $R_p$ all energy consumptive effects due to the presence of a particle, without making prior assumptions as to the origins of these effects.

### 3.2.3 BSE with Membrane-Bound Particle

The MMT method can be extended to yield the effective permittivity of spherical particles surrounded by thin membranes, such as biological cells, by the Single-Shell Model (SSM). The effective complex permittivity of a whole cell ($\varepsilon^*_c$) becomes a function of the complex permittivities of the membrane ($\varepsilon^*_m$) and cytoplasm ($\varepsilon^*_c$) $[141, 227]$:

$$\varepsilon^*_c = \varepsilon^*_m + \frac{2(1 - \nu) + 1(1 + 2\nu)E}{(2 + \nu) + (1 - \nu)E},$$  

(3.7)

where geometric factor $\nu = (1 - d_m/r)$, with $d_m \, [m]$ the membrane thickness, and $r \, [m]$ the outer radius of the whole cell, and intermediate parameter $E = \varepsilon^*_c/\varepsilon^*_m$. The effective real permittivity $\varepsilon_p$ due to the presence of a membrane-bound particle within the BSE is found by substituting real permittivity $\varepsilon^*_c = \varepsilon^*_i$ into Equation (3.4). Effective conductance $\sigma_p$ is calculated similarly. Particle capacitance $C_p$ is then calculated using Equation (3.3) by setting $\varepsilon_s = \varepsilon_p$, with particle resistance $R_p$ calculated from Equation (3.6).

Having defined the BSE, its equivalent circuit and related its component values to geometric and material properties of a sample above planar microelectrodes, the next section describes how these are used in developing two topologies of electric displacement sensor.
3.3 Absolute Electric Displacement (AED) Sensor

The Absolute Electric Displacement (AED) Sensor provides a method for sensing changes in complex permittivity of micro-particles and their suspending medium. The AED Sensor comprises two BSEs in parallel, with a common centre electrode, shown in Figure 3.5(a). It applies the 3-electrode approach to measure the change in permittivity between the left and right electrodes as a charge upon the centre output electrode. Presenting a high-impedance load \( C_L [F] \) to the output electrode converts this change in charge to an output voltage, \( V_o [V] \).

![Figure 3.5: AED Sensor small-signal equivalent circuits for (a) no particle and (b) with particle.](image)

Changes in medium and particle permittivities are sensed by the AED Sensor via the total electric displacement \( D_o [C/m^2] \) incident upon its output electrode, where \( D_o = D_x + D_y \). \( D_x \) arises from shunt electric-field between adjacent electrodes horizontally through the isolating dielectric layer. \( D_y \) arises from the fringing-field penetrating the sample vertically through the isolating dielectric layer. Now recalling that:

\[
D_o(\varepsilon^*) = \varepsilon_0 E_o(\varepsilon^*) + P_o(\varepsilon^*)
\]

where \( \varepsilon^* \) represents the frequency dependent complex permittivity of the sample, introduced in Section 2.2.2. Electric-field \( E_o [V/m] \) represents the free charge density upon the output electrode arising from changes in complex permittivity between the left and right electrodes, and polarisation \( P_o [C/m^2] \) the bound charge density upon the output electrode. \( P_o \) is assumed to change in proportion to the medium permittivity alone, and \( E_o \) due to \( \varepsilon^* \), represented as capacitance \( C_p \) and resistance \( R_p \) in Figure 3.5(b), as described previously. The AED Sensor is therefore sensitive to changes in the suspending medium and the suspended particle, giving insight into the complete electrical environment above its three electrodes.
Nodal analysis of the equivalent AED Sensor circuits shown in Figure 3.5 yields the following voltage transfer function:

\[
\frac{V_o}{V_i} = \frac{C_y(1/Z) + C_x[(1/Z) + sC_y + sC_{eq}]}{[2C_x + C_y + C_L][(1/Z) + sC_y + sC_{eq}] - sC_y^2} \tag{3.9}
\]

where \( s = j\omega \) is the Laplace operator, \( j = \sqrt{-1} \), \( C_{eq} = C_s C_y / (C_s + C_y) \) [F] and all other capacitances are related to design parameters as described in Sections 3.2.1 and 3.2.2. Sample impedance \( Z \) is explained in the following sections.

### 3.3.1 AED Sensor with No Particle

Figure 3.5(a) shows the equivalent circuit for the AED Sensor when no particle is present. In this case, sample impedance \( Z = (1/sC_{eq}) \) [F] and Equation (3.9) simplifies to:

\[
\frac{V_o}{V_i} = \frac{C_{eq} C_y + C_x (2C_{eq} + C_y)}{(2C_x + C_y + C_L)(2C_{eq} + C_y) - C_y^2}. \tag{3.10}
\]

Note the absence of \( s \) terms (and hence frequency dependance) in Equation (3.10). The AED Sensor is therefore expected to exhibit only changes in output voltage magnitude for different sample media. Figure 3.6 plots the AED Sensor response of Equation (3.10) for varying sample permittivities \( \varepsilon_s \). The greatest medium sensitivity is achieved when the difference in sensor response is greatest to changes in \( \varepsilon_s \), observed for electrodes with a large width and gap around 20% of the electrode width.

**Figure 3.6:** AED Sensor magnitude response for various sample permittivities \( \varepsilon_s \) versus (a) electrode width \( w \) with fixed gap \( g = w/10 \), and (b) electrode gap \( g \) as percentage of fixed width \( w = 20\mu m \).
3.3.2 AED Sensor with Particle

Figure 3.5(b) shows the equivalent circuit for the AED Sensor when a particle is present. In this case, sample impedance $Z = (1/sC_y) + R_p/(1 + sR_pC_p) [\Omega]$. Figure 3.7 shows the frequency-dependent AED Sensor magnitude response for the single-shell cell model suspended in water.

First order dispersions are observed in the megahertz region, as anticipated by the $\beta$-dispersion described in Section 2.2.2. Increasing cell radius with respect to electrode width yields the greatest shift in dispersion frequency. The greatest shift in magnitude post-dispersion arises for smallest cell radii. Larger particles can therefore be sensed by their dispersion frequency, and smaller particles via the change in magnitude post-dispersion.

3.3.3 AED Sensor Summary

This section has presented the AED Sensor architecture intended to give insight into the complete electrical environment above its electrodes. An equivalent circuit for the AED Sensor has been developed and demonstrated to be sensitive to changes in medium permittivity (Section 3.3.1) and changes in both cell radius and complex permittivity (Section 3.3.2). The greatest magnitude responses from empty media are achieved for large electrode widths and small gaps. The greatest magnitude responses from micro-particles are achieved at high frequency and for electrode widths greater than the micro-particle radius.
3.4 Relative Electric Displacement (RED) Sensor

The Relative Electric Displacement (RED) Sensor provides a method for sensing changes in complex permittivity in suspended micro-particles while rejecting changes in the complex permittivity of their suspending medium. The RED Sensor comprises three BSEs in parallel, with two common centre electrodes, shown in Figure 3.8(a). It applies the 4-electrode approach to measure the change in permittivity between the leftmost and rightmost electrodes as a change in the difference in charge upon the centre pair of output electrodes. Presenting high-impedance load $C_L$ [F] to the output electrodes converts this change in charge to two output voltages, $V_{oP}$ and $V_{oN}$ [V].

The RED Sensor can sense changes in medium and particle permittivities via the total electric displacement $D_{oP}$ and $D_{oN}$ [C/m$^2$] incident upon its output electrodes. Recalling that:

$$D_{1}(\varepsilon^*_1) = \varepsilon_0 E_1(\varepsilon^*_1) + P_1(\varepsilon^*_1) \quad (3.11)$$
$$D_{2}(\varepsilon^*_2) = \varepsilon_0 E_2(\varepsilon^*_2) + P_2(\varepsilon^*_2) \quad (3.12)$$

where subscripts 1 and 2 denote the $P$ and $N$ output electrodes, respectively, $\varepsilon^*$ [F/m] the complex permittivity seen above each electrode, and $\omega$ the angular frequency. Electric-field $E$ [V/m] represents the free charge upon the output electrode arising from changes in permittivity between the input and output electrode pairs, and polarisation $P$ [C/m$^2$] the bound charge upon the output electrodes. Assuming the electrodes are in close proximity to one another, such as the case for microelectrodes, the bound charge upon both output electrodes will be the same:

$$P_1 = P_2, \quad (3.13)$$

![Figure 3.8: RED Sensor small-signal equivalent circuits for (a) no particle and (b) with particle.](image-url)
Modelling

which become common terms in Equations (3.11) and (3.12). Hence the contribution from bound charges arising from the suspended medium are cancelled by the RED Sensor by observing the difference in output electric displacements:

\[ D_1(\varepsilon_1^1) - D_2(\varepsilon_2^2) = \varepsilon_0|E_1(\varepsilon_1^1) - E_2(\varepsilon_2^2)|, \]

which corresponds to the differential transfer function \((V_{oP} - V_{oN})/(V_{iP} - V_{iN})\). Free charges (\(E\) components) change due to \(\varepsilon^*\), represented as capacitance \(C_p\) and resistance \(R_p\) in Figure 3.8(b), as described previously. The RED Sensor is therefore sensitive to changes in the suspended particle while rejecting its suspending medium, giving specific insight into the particle’s electrical characteristics.

3.4.1 RED Sensor with No Particle

Figure 3.8(a) shows the equivalent circuit for the RED Sensor when no particle is present. Nodal analysis yields the following transfer function:

\[ \frac{V_{oP} - V_{oN}}{V_{iP} - V_{iN}} = \frac{(C_y + C_{eq} + 2C_s)C_x + C_{eq}C_y}{(C_y + C_{eq} + 2C_s)(3C_x + C_y + C_L) - C_y^2}, \]

where \(C_{eq} = C_sC_y/(C_s + C_y) [F]\) and all other capacitances are related to design parameters as described in Sections 3.2.1 and 3.2.2. Figure 3.9 plots the RED Sensor response of Equation (3.15) for varying sample permittivities \(\varepsilon_s\).

Figure 3.9: RED Sensor magnitude response for various sample permittivities \(\varepsilon_s\) versus (a) electrode width \(w\) with fixed gap \(g = w/10\), and (b) electrode gap \(g\) as percentage of fixed width \(w = 20 \mu m\).
Modelling

Note the absence of $s$ terms (and hence frequency dependance) in Equation (3.15). The RED Sensor is therefore expected to exhibit only changes in output voltage magnitude for different sample media, as per the AED Sensor described in Section 3.3. Compared to the AED Sensor response, the RED Sensor has greater rejection of the medium permittivity. Figure 3.9(a) shows a 4dB decrease in magnitude for 1$\mu$m electrode width, which decreases with increasing $\varepsilon_s$, reaching a peak medium rejection around 20$\mu$m electrodes. Figure 3.9(b) shows the greatest medium rejection is achieved for electrodes separated with a gap equal to 30% their width.

3.4.2 RED Sensor with Particle

Figure 3.8(b) shows the equivalent circuit for the RED Sensor when a particle is present. Nodal analysis yields its transfer function:

$$\frac{V_oP - V_oN}{V_iP - V_iN} = \frac{AC_x + sC_{eq}C_y}{A(3C_x + C_y + C_L) - sC_y^2}, \quad (3.16)$$

where $A = sC_y + sC_{eq} + (2/Z)$ and sample impedance $Z = (1/sC_y) + R_p/(1 + sR_p C_p) \quad [\Omega]$. Figure 3.10 shows the frequency-dependent RED Sensor magnitude response for the single-shell cell model suspended in water. The magnitude response rejects at lower frequencies, where the electric-field will preferentially avoid the suspended cell. Post-dispersion, the electric-field penetrates the cell, leading to the differences in magnitude response recorded for varying cell radii. Smaller particles yield the greatest overall sensor response.

![Figure 3.10: RED Sensor magnitude response for single-shell cell model suspended in water ($\varepsilon_s = 80$). Cell radii are proportional to fixed electrode width ($w = 18\mu$m) and spacing ($g = 2\mu$m). Cell membrane has thickness $d_{mem} = 100$nm, permittivity $\varepsilon_{mem} = 12$ and conductivity $\sigma_{mem} = 1 \times 10^{-12}$, with cytoplasm permittivity $\varepsilon_{cp} = 80$ and conductivity $\sigma_{cp} = 1.2 \times 10^{-5}$.

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Modelling

3.4.3 RED Sensor Summary

This section has presented the RED Sensor architecture, intended to give specific insight into the electrical characteristics of a cell above its electrodes. An equivalent circuit for the RED Sensor has been developed and demonstrated to be less sensitive to changes in medium permittivity compared to the AED Sensor (Section 3.4.1), with greatest sensitivity to changes in cell radius and complex permittivity (Section 3.4.2). The greatest rejection of empty media responses is achieved for small electrodes with small gaps. The greatest sensitivity to suspended micro-particles is achieved at high frequency for micro-particles with radii greater than half the electrode width. Sensitivity is also achieved for smaller particle radii, but little dispersion is observed micro-particles with a diameter equal to the electrode width.

3.5 Summary

This chapter has presented an approach to modelling micro-particles suspended in liquid media above an array of micro-electrodes. A Basic Sensing Element (BSE) geometry has been defined and an equivalent circuit proposed. Analytic expressions have been derived for the equivalent circuit elements, with respect to the geometric parameters of the BSE. Connecting two BSEs in parallel yields the Absolute Electric Displacement (AED) Sensor, designed to be sensitive to the entire electrical environment (micro-particle and suspending media) above one sensor electrode. An equivalent circuit was derived for the AED Sensor, and simulations performed to demonstrate its sensitivity to various permittivities of suspending media and micro-particle radii. The AED Sensor is predicted to be most sensitive to empty media for large electrode widths and small gaps, and most sensitive to micro-particles at high frequency, when their radius is less than the electrode width.

Connecting three BSEs in parallel yields the Relative Displacement (RED) Sensor, designed to sensitive to the difference in electrical environment (micro-particle only) between a pair of electrodes. An equivalent circuit was derived for the RED Sensor and simulations performed to demonstrate its sensitivity or otherwise to various permittivities of suspending media and micro-particle radii. The RED Sensor is predicted to reject empty media best when its electrodes are small with small gaps, and most sensitive to micro-particles at high frequency for micro-particles with radii greater than half the electrode width.

Chapter 4 details the implementation of these two sensor architectures within a dedicated CMOS-based microsystem, ahead of experimental trials with biological cells in Chapter 5.
4.1 Introduction

The following chapter describes the design and implementation of a dedicated microsystem that implements the Absolute Electric Displacement (AED) and Relative Electric Displacement (RED) Sensor architectures investigated in Chapter 3, for the purpose of rapid, time-domain dielectric spectroscopy (DS) measurements upon, and dielectrophoresis (DEP) actuation of, single-cells in physiological suspension, spanning the $\beta$-dispersion band of frequencies between 100 kHz and 100 MHz. The system comprises four main components:

1. **Application Specific Integrated Circuit (ASIC):** that implementes the AED and RED Sensors within a standard Complementary Metal-Oxide Semiconductor (CMOS) microelectronics process, formed into highly-parallel arrays to maximise the sensitive area of the device (described in Section 4.3).

2. **Printed Circuit Boards (PCB):** in two forms: a module PCB to support the ASIC and a motherboard PCB to provide signal processing circuitry including analogue-to-digital conversion, digital control in the form of a Field-Programmable Gate Array (FPGA) and Universal Serial Bus (USB) connectivity to a Personal Computer (PC, Section 4.4).

3. **Firmware:** implemented within the FPGA to generate the time-domain stimulus for DS sensing, capture data in real time, and configure the ASIC as defined through software control (Section 4.5).

4. **Software:** running on a separate PC to provide user configuration of the microsystem, initiate and receive data capture from the microsystem, and to perform subsequent data post-processing for presentation to the user, or saving plots to file (Section 4.6).

The final microsystem is presented in Section 4.7 and its performance regarding single-cell sensing and actuation assessed through experimental trails, detailed in Chapter 5.
4.2 Global Constraints

4.2.1 Microelectronics Technology

The context of this work is to assess the potential of DS sensors and DEP actuators for the purpose of phenotyping, and potentially sorting, single-cells within a device that can demonstrate potential for widespread adoption in future healthcare. Consequently, technology scalability and affordability are key design parameters. The use of CMOS microelectronics has the potential to achieve these parameters, due to its now almost ubiquitous application. Nonetheless, CMOS microelectronics is an expensive technology to prototype, and custom CMOS processes would escalate prototyping costs yet further. Consequently, this work exploits Multi Project Wafer (MPW) prototyping that provides a number of standard CMOS process options, but without the option of custom layers or microfabrication steps.

4.2.2 Light Sensitive Detectors

The expense of CMOS prototyping also encourages us to maximise the use out of each ASIC. Recalling the sensor modes considered in Section 1.5, light sensitive detectors are immediately relevant to biological analysis applications. As such, two light sensor topologies are included within the ASIC: a metallurgical photodiode (PD) and a custom single-photon avalanche diode (SPAD) detector from [228]. Figure 4.1(a) shows the PD, which exploits the light sensitivity exhibited by any semiconducting junction to output an analogue voltage ($V_o$) directly proportional to the light flux incident upon its surface. Figure 4.1(b) shows the SPAD, formed from a reversed biased junction diode that undergoes avalanche breakdown when a photon strikes its surface; resistor $R_q$ converts the resulting pulse into a voltage, adjusted to overcome digital circuit thresholds, producing digital output $OUT$, whose pulse rate is directly proportional to the incident photon flux.

![Cross sections through two CMOS-integrated light detectors: (a) a standard photodiode, and (b) a single-photon avalanche diode (SPAD).](image)

**Figure 4.1:** Cross sections through two CMOS-integrated light detectors: (a) a standard photodiode, and (b) a single-photon avalanche diode (SPAD).
4.2.3 ASIC Process Choice

The specific CMOS process with which to manufacture the microsystem ASIC must accommodate both budgetary and technical constraints. The cost of any microelectronic device is directly proportional to its area, and while CMOS scaling (termed Moore’s Law) provides for decreasing costs per transistor, the cost of an MPW prototype is a function of the specific CMOS process and the absolute area of the manufactured device. Considering that the ASIC will need interface with liquid suspensions that need introduced to, and removed from, the device, a large device area is preferred. This encourages the use of less expensive, more mature technologies, and standard process options.

The ASIC must also satisfy technical constraints. The SPAD structure described previously requires use of a deep n-well to form its light sensitive junction diode, illustrated in Figure 4.1(b), which are not included as standard in mature CMOS processes. Deep n-wells are however available in high-voltage process options. The austriamicrosystems (ams) foundry provide a high-voltage process option for their 0.35 μm technology. This is an attractive option, as the ams high-voltage (H35) option is built upon their standard low-voltage (C35) technology, permitting use of standard transistors for implementing circuitry in addition to the SPAD structure. 0.35 μm CMOS technology has been applied in a wide range of applications for single-cell studies, as reviewed in Sections 2.5 through 2.7. Most notably, in direct charge sensors [126], charge amplification methods [135] and integrated with DEP actuators [106, 107, 108, 135]. However, while the 0.35 μm process is a mature and hence less expensive technology, the high-voltage option increases the device cost per area.

Consequently, the microsystem ASIC is implemented in high-voltage 0.35 μm (H35) CMOS from ams, manufactured via the Europractice MPW service with a cost optimal area of 7 mm².

Having introduced the global constraints applying to the microsystem, its major components are described in the following sections.
4.3 Application Specific Integrated Circuit

The following sections first define the specifications used to design the ASIC, the considerations behind its common elements (including sample and sensor interfaces), and the specific design and implementation of each sensor element, before the output signal path and integrated ancillary circuitry are presented.

4.3.1 Specifications

The primary purpose of the ASIC is to implement the Absolute Electric Displacement (AED) and Relative Electric Displacement (RED) Sensor architectures introduced in Sections 3.3 and 3.4, respectively, to assess their performance for phenotyping single biological cells in liquid solution without the use of extrinsic sample labelling. These sensors require the sample to be stimulated in a similar manner as used for sample actuation with dielectrophoresis (DEP), hence the opportunity to provide for both is capitalised upon. Thirdly, the ASIC’s functionality is maximised through integrating light sensors in the form of photodiodes (PD) and single-photon avalanche detectors (SPAD) detectors as described in Section 4.2. These design criteria and more are reflected in the following specifications:

1. **Technology:** 0.35 μm high-voltage CMOS with 4 metal and 2 polysilicon routing layers.
2. **Resolution:** the device should be capable of sensing and actuating individual biological cells in liquid solution.
3. **Sensor modes:** to implement both AED and RED Sensor architectures, combined with light sensors in the form of PDs and SPADs.
4. **Actuation modes:** to include methods for sample actuation with DEP.
5. **Bandwidth:** the electric sensors and actuators should be capable of operating across the $\beta$-dispersion, spanning 100 kHz to 100 MHz, inclusive.
6. **Parallelism:** each sensor and actuator element should be incorporated into highly-parallel array structures, to maximise the active device area exposed to the sample.
7. **Integration:** sensors and actuators should be combined where possible.
8. **Provision for sample flow:** the active arrays should be made compatible for subsequent alignment and attachment to an external microfluidic system.
9. **Area:** the gross device area should not exceed 7 mm².

Having defined the target specifications for the ASIC, its common features are described in the following section.
4.3.2 Electrode Design

Electrodes formed in the topmost metal layer of the H35 CMOS process are used across the ASIC, to minimise the total thickness of passivation dielectric between the sample and electrode surface, and hence maximise its coupling capacitance $C_y$ (defined in Section 3.2.1). Recalling the above specifications to integrate both sensing and actuating functions, the electrode size requires careful consideration to maximise the performance of both. Square electrodes are used throughout to maximise symmetry as per the assumptions made in developing the sensor models, described in Sections 3.3 and 3.4. In addition to the global constraints outlined previously, the use of standard CMOS limits the available design variables to the electrode width $w$ and spacing (or gap) $g$, with height fixed by the process metal layer thickness.

Sensor Considerations

The AED Sensor is intended to be sensitive to the entire electrical environment above its single sensor electrode. Considering its models developed in Section 3.3, Figure 3.7 shows the greatest signal change arising from the presence of a micro-particle above the AED Sensor occurs for electrode widths comparable to the size of the micro-particle. Figure 3.6(a) considers the AED Sensor response when no micro-particle is present, with distinction between media increasing directly in proportion to electrode width (likely due to the associated increase in $C_y$), and peak distinction for gaps beneath 30% of the electrode width, shown in Figure 3.6(b). Hence, the optimal AED Sensor electrode is of comparable size to the biological cell suspended above it, with a gap between 30% and 40% of its width.

The RED Sensor is intended to sense suspended micro-particles only, and reject the signal due to their suspending medium, common to its two sensor electrodes (see Section 3.4). Figure 3.10 shows the greatest sensitivity is achieved for micro-particles of comparable size to the RED Sensor electrode(s). The sensor signal should reject maximally when no micro-particles are present; defined here as the minimal difference in signal arising from empty media. Figure 3.10(a) shows this occurs for small electrodes, likely due to the decrease in coupling capacitance $C_y$. Figure 3.10(b) indicates that small electrode gaps yield the most similar sensor output signal. Hence, the optimal RED Sensor electrode(s) are those of comparable size to the biological cell suspended above it (as per the AED Sensor), but with gaps beneath 30% of their width.

Actuator Considerations

Regarding sample actuation with DEP, the force experienced by a particle due to DEP is given in expression (2.14) and shown to be directly proportional to the square magnitude of the applied electric field, and linearly to its geometric gradient. The latter is impacted by electrode design and indicates that electrodes with small gaps are optimal to maximise the DEP force.
Process Considerations

The topmost metal layer within the high voltage H35 CMOS process has a minimum spacing of 2 μm [229], which is four times the minimum spacing of its lower metal layers. This (relatively) large spacing is due to the top metal layer thickness, fixed at 2.8 μm and approximately four times that of the lower metal layers [230]. The thick top metal is to support high current densities arising from the expected high voltage applications of the H35 process.

The thick top metal also complicates the passivation dielectric(s) to be used as the isolating dielectric for DS measurements. Whereas the low-voltage ams process passivation comprises of two 1 μm thick layers of silicon dioxide and nitride, the H35 process adds an additional 5 μm thick polyimide layer [230]; whose permittivity would reduce the expected value of $C_y$ (and hence the expected sensor signal) by a factor of five. The polyimide layer must therefore be removed by a single post-processing step of O$_2$ plasma ashing. Note however that such post-processing would not be required if a standard low-voltage CMOS process were used instead.

Electrode Dimensions and Component Values

The above considerations lead to a final square electrode with dimensions of 20 μm pitch and 2 μm gaps, corresponding to 10% of the 18 μm width per edge. This pitch is comparable to those used by devices incorporating photodiode and capacitance based sensors with DEP actuators [106, 107, 135, 231], chosen to support a wide range of mammalian cell sizes. The same electrode dimensions are used across the entire ASIC to permit direct comparisons between the AED and RED Sensor architectures. The resulting values for the equivalent circuit components introduced in Sections 3.3 and 3.4 are given in Table 4.1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Value [fF]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_x$</td>
<td>0.87</td>
</tr>
<tr>
<td>$C_y$</td>
<td>7.23</td>
</tr>
<tr>
<td>$C_s$</td>
<td>3.04</td>
</tr>
</tbody>
</table>

Table 4.1: Equivalent circuit component values for manufactured electrode dimensions, for a suspending medium with real permittivity $\varepsilon_r = 60$.

4.3.3 Electrode and Pixel Interface

An individual electrode forms the minimum repeating unit used across the ASIC. A pixel is defined here as the minimum element necessary to form each sensor. A pixel may contain one, two or four electrode units, as used in the AED Sensor, RED Sensor, and SPAD Sensor pixels (described in Sections 4.3.5, 4.3.6 and 4.3.7), respectively. Each electrode supports the following design criteria:
System Design

- **Operation as an excitation source**: to connect DEP actuation or DS stimulus signals to each electrode.

- **Independent configuration**: to permit arbitrary excitation patterns to be applied across the array for sample actuation or sensor stimulus.

- **Excitation persistence**: to continue applying the configured excitation signal while other electrodes are configured and pixel outputs sensed.

- **Reset to a common potential**: to provide a known starting condition and avoid arbitrary potential differences across the array from interfering with DS sensing.

To support these criteria, a 2:1 multiplexer is integrated beneath each electrode permitting one of two input signals \(V_{SIG,A}\) and \(V_{SIG,B}\) to be connected to the electrode metal, as defined by an addressable 2 bit memory, providing four states as shown in Table 4.2. The memory is configured by two global digital inputs, **EXCITE** and **SELECT**.

<table>
<thead>
<tr>
<th>State</th>
<th><strong>EXCITE</strong></th>
<th><strong>SELECT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reset</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sense</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Excite (V_{SIG,A})</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Excite (V_{SIG,B})</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 4.2**: Global electrode configuration truth table, where the “Sense” state is specific to each sensor type, described in their relevant sections.

Each electrode memory bit is stored within a 1 bit Static Random Access Memory (SRAM) made uniquely addressable through series NMOS switches connected to row (ROW) and column (COL) address lines, shown schematically in Figure 4.2 [106, 107, 135]. Memory input \(A (\overline{A})\) is stored upon memory output \(Q (\overline{Q})\) when both \(ROW\) and \(COL\) are high. Complementary inputs \(A\) and \(\overline{A}\) are necessary to ensure robust operation of the SRAM (to ensure sufficient voltage is seen by the SRAM to overcome its static noise margin [232] after the series NMOS source-drain voltage drops). While this requires twice the routing and an additional two NMOS switches per SRAM, it nonetheless consumes significantly less area than a digital register, and does not require refreshing as per a more compact dynamic random access memory.

4.3.4 **Excitation Signal Interface**

Excitation signals comprise those used for DEP sample actuation and DS sensor stimulus. In both cases, differential signalling is supported to maximise the electric field magnitude for actuation, and provide complementary stimulus for sensing. Four global input signals \(V_{SIG[3:0]}\)
support two pairs of differential signals and full quadrature for stimulus and actuation purposes, the latter enabling electro-rotation (EROT) and travelling-wave DEP.

A pair of 2:1 multiplexers selects two of the four global inputs to be distributed to a row of electrodes (choosing $V_{SIG.A}$ and $V_{SIG.B}$, described previously). To support the persistence in excitation signal required by each electrode, the row multiplexer control bits ($RSIG._SEL[1:0]$) are stored in digital registers addressed by $ROW$ and protected behind an active-high clock, $RSIG._CLK$. The excitation signal path is purely analogue to provide maximum flexibility while minimising any switching interference that would arise from digital buffering. Each multiplexer is formed from 5 V transmission gates (T-gates, parallel NMOS and PMOS switches) to permit rail-to-rail operation and hence maximise the available electric field strength available for sample actuation, or sensor stimulus.

Having introduced the common electrode and pixel addressing strategies, the three sensor pixel designs implemented within the ASIC are detailed in the following sections.
4.3.5 AED Sensor Pixel

The Absolute Electric Displacement (AED) Sensor pixel implements the AED Sensor architecture investigated in Section 3.3. The pixel comprises of one electrode, a photodiode (PD), a pair of multiplexers and a pair of analogue impedance buffers, whose connectivity is defined by the configuration of the 2 bit pixel memory, shown conceptually in Figure 4.3 and in Table 4.3. The pixel employs a direct charge sensing approach for DS sensing similar to that reported by Musayev et al. [126], where the charge upon electrode $E[i, j]$ (where $i$ and $j$ represent the row and column address of the pixel, respectively) is connected to an output impedance buffer, whose output is sent off-chip through the AED Sensor array output signal path (described in Section 4.3.8). Optical sensing is performed simultaneously by the PD whose output voltage is connected to an identical impedance buffer, routed to a parallel output signal path. Excitation is achieved through the common 2:1 multiplexer architecture described in Section 4.3.3.

![AED Sensor pixel schematic](image)

**Figure 4.3:** AED Sensor pixel concept: the 2 bit memory configuration defines whether the electrode $E[i, j]$ is connected to one of two excitation signals, or an addressable output impedance buffer.

<table>
<thead>
<tr>
<th>$E[i, j]$ state</th>
<th>EXCITE</th>
<th>SELECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excite ($V_{SIG_B[i]}$)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Excite ($V_{SIG_A[i]}$)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sense ($M_1$ gate)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Reset ($GND$)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4.3:** AED Sensor truth table showing electrode state upon $ROW[i]$.

Figure 4.3 shows the AED Pixel schematic that employs 5 V transistors throughout to maximise excitation signal swing. Both multiplexers are formed from T-gates whose complementary se-
Figure 4.4: AED Sensor pixel schematic shown in sensing configuration. External signals are shown in capitals and internal signals in lower case, where $s$ ($\bar{s}$) and $e$ ($\bar{e}$) refer to the stored in-pixel configuration state asserted by global inputs $EXCITE$ and $SELECT$, respectively. The 2 bit Control Bus comprises of both global inputs and their complements.

Select bits are generated within the 2 bit in-pixel SRAM memory. When configured for sensing (as shown in Figure 4.4), electrode $E[i, j]$ is connected directly to $M_1$, whose gate dielectric shares the charge present upon the electrode. Impedance buffering is achieved through transistor $M_1$, configured as a source-follower, where charge condensed upon its gate modifies its transconductance, resulting in a change in current through row-selectable switch $M_2$. This is converted to output voltage $V_{PD[j]}$ through an NMOS load attached to the base of the output column bus at the array level (not shown). DC bias is provided to $M_1$ via a pull-up resistor formed by long-channel device $M_3$. The same architecture is employed for the PD, where $M_5$ is configured as a source-follower to provide impedance buffering, addressed through row-selectable output switch $M_6$. The AED sensor is reset to $GND$ through pull-down switch $M_4$, and similarly the PD reset to $AVDD$ through pull-up switch $M_7$. Excitation is performed by asserting $EXCITE$ high, with $SELECT$ asserting which of $V_{SIG.A}$ or $V_{SIG.B}$ is attached to $E[i, j]$ as shown in Table 4.3.

Source-follower $M_1$ presents electrode $E[i, j]$ with a 10 fF capacitive load to ground due to its gate capacitance. Additional shunt capacitances are also present due to the drain terminals of $M_3$, $M_4$ and both devices within the series T-gate. These capacitances are minimised through sharing terminals within the pixel layout, to minimise charge division and hence signal attenuation, while maximising the source-follower bandwidth.
System Design

Figure 4.5: AED Sensor pixel layout shown with 20×20 μm² bounding box: (a) layers from Metal 1 downwards, (b) layers from Via 1 upwards. Layer colours shown in key.

The AED Sensor layout is shown in Figure 4.5 and measures 20×20 μm². The PD is visible to the top right of the pixel, whose transistors are symmetrical about the vertical centre-line. The impedance buffering devices and row-selectable switches are located at the top of the pixel, away from the 2 bit SRAM and excitation T-gates to minimise coupling onto the analogue outputs. Their drain terminals are shared to minimise capacitive loading of the electrode as described previously. Global configuration bits are routed horizontally in metal 3 (MET3) and orthogonal to the analogue outputs, routed vertically on metal 2 (MET2), to minimise capacitive coupling. A power grid is employed routing through metals 1, 2 and 3 to avoid DC drops across the sensor array. Electrode $E[i, j]$ is formed in topmost metal 4 (MET4).

Array Integration
The AED Sensor pixel is tiled into columns containing 32 pixels and an array of 64 columns, to form a 32×64 pixel array measuring 640×1,280 μm².
4.3.6 RED Sensor Pixel

The Relative Electric Displacement (RED) Sensor pixel implements the sensor architecture investigated in Section 3.4. The pixel comprises of two electrodes located upon adjacent columns within the same row, a fully-differential charge amplifier with capacitive feedback, two pairs of multiplexers and two 2 bit SRAM memories. The RED Sensor implements a fully-differential charge amplification scheme, shown conceptually in Figure 4.6, based upon the differential charge amplifier method presented by Romani et al. [135]. The fully-differential scheme presented here aims to reject the electrode polarisation assumed to be common to both pixel electrodes, and hence provide information regarding suspended cells, and not their suspending medium. This is made possible through the use of complementary electric fields, stimulating the sample through adjacent RED Sensor pixel electrodes. Each electrode within the pixel is independently configurable for excitation, as described in Section 4.3.3, whereas a master-slave relationship is employed for sensing. Once electrode $E[i, (j + 1)]$ is configured for sensing, its internal $SELECT$ state is controlled by that within the master $E[i, (j + 0)]$ half of the pixel.

![Figure 4.6: RED Sensor pixel concept: fully-differential charge amplification scheme.](image)

<table>
<thead>
<tr>
<th>$E[i, (j + 1)], E[i, (j + 0)]$ state</th>
<th>$EXCITE[(j + 1), (j + 0)]$</th>
<th>$SELECT[(j + 1), (j + 0)]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excite ($V_{SIG,B[i]}$)</td>
<td>[1, 1]</td>
<td>[1, 1]</td>
</tr>
<tr>
<td>Excite ($V_{SIG,A[i]}$)</td>
<td>[1, 1]</td>
<td>[0, 0]</td>
</tr>
<tr>
<td>Auto-Zero</td>
<td>[0, 0]</td>
<td>[X, 1]</td>
</tr>
<tr>
<td>Sense</td>
<td>[0, 0]</td>
<td>[0, 0]</td>
</tr>
</tbody>
</table>

Table 4.4: RED Sensor truth table showing electrode states upon $ROW[i]$. 67
Figure 4.7: RED Sensor pixel schematic shown in sensing configuration. External signals are shown in capitals and internal signals in lower case, where \( e[i, j] \) and \( s[i, j] \) refer to the stored in-pixel configuration state asserted by global inputs EXCITE and SELECT, respectively, within the unique electrode address given by row index \( i \) and column index \( j \). The 4 bit Control Bus comprises of both global inputs and their complements for both pixel electrodes, as per truth Table 4.4.

Figure 4.7 shows the RED Sensor pixel schematic that employs 5 V transistors throughout to maximise excitation signal swing. The fully-differential amplifier is a dual-cascoded telescopic topology with internal common-mode feedback, achieved with NMOS transistors operating within the linear region at the base of each branch, connected to their respective branch outputs. A cascoded topology is essential to eliminate the Miller Effect, where feedback capacitance \( C_F \) as seen by the sense electrodes would be amplified by the open-loop gain of the amplifier, attenuating the sensed charge (and hence sensor signal) by the same proportion. \( C_F \) is a 24.5 fF polysilicon capacitor optimised to occupy the available area in layout for good matching between pixel branches and to minimise DC output voltage drift arising from leakage current.

The loop gain of each amplifier branch is \( C_y/C_F \), where \( C_y = 7.23 \) fF (given in Table 4.1) is the electrode-to-sample coupling capacitance across the isolating dielectric layer. Hence, the amplifier output voltage appearing upon the gates of \( M_{1a,b} \) will be 0.295 times that upon the iso-potential within the sample immediately above the sense electrode. This attenuation is a design trade-off to achieve the component matching essential between amplifier branches, particularly so considering the small values in absolute terms. NMOS devices \( M_{1a,b} \) are configured as source-followers with switches \( M_{2a,b} \) providing row selection of the differential pixel output voltages on to the RED Sensor array output signal path (described in Section 4.3.8).
System Design

differential pixel output voltage is connected to output buses common to each column of pixels through addressing the master pixel address of \( E[i, (j + 0)] \).

An Auto-Zero (AZ) scheme is implemented via an NMOS switch around feedback capacitor \( C_F \) to cancel DC offset voltages arising across each capacitor due to residual charge storage. It is essential that both amplifier branches are brought out of AZ simultaneously, to ensure minimal voltage difference at the amplifier inputs at the start of sensing. This is achieved through the master-slave arrangement where the internal state of \( SELECT \) within electrode \( E[i, (j + 0)] \) controls that within \( E[i, (j + 1)] \), when the latter is put into its sense state (\( EXCITE \) asserted low). The AZ state of both branches \( (AZ[i, k]) \) is controlled by \( s[i, (j + 0)] \), where \( k \) refers to the column address common to both branches of the pixel, generated by boolean OR of adjacent array column addresses \( COL[j + 0] \) and \( COL[j + 1] \), performed at the column-level, not shown in Figure 4.7.

Each amplifier receives four bias voltages distributed across each row of pixels, gated by \( ROW[i] \) at the row-level (not shown) to minimise power consumption across the array. To further reduce power consumption and potential interference arising from other pixels, pull-down switches (connected to \( acol[k] \)) are employed to collapse the input voltages of amplifiers upon columns not addressed within the powered row. Similarly, interference from amplifiers within rows not addressed, but whose outputs are connected to the output signal bus common to each column, have their inputs collapsed by switches connected to \( arow[k] \).

RED Sensor Pixel Layout

The RED Sensor pixel layout is shown in Figure 4.8 and measures \( 40 \times 20 \) \( \mu \)m\(^2\). The layout is symmetric about the vertical centre line, with the sole exception of the AZ switch connections, whose mismatch is minimised to a \( 0.8 \times 0.5 \) \( \mu \)m\(^2\) area of polysilicon. Each half of the pixel is split into three vertical columns: i) amplifier branch, (ii) output source-follower and feedback capacitor, and (iii) excitation multiplexers and SRAM memory. These halves are mirrored symmetrically through the vertical centre line, with digital configuration bits distributed across each row of pixels sharing contacts between pixels on adjacent columns. Similarly, pixels on adjacent rows are mirrored to share contacts within the amplifier and output source-followers. Excitation signals \( V_{SIG,A} \) and \( V_{SIG,B} \) are distributed across each row of pixels and screened by static digital configuration bits \( EXCITE \) and \( SELECT \) (and their complements) to avoid coupling interference onto the four analogue amplifier bias voltages also distributed horizontally across each row of pixels. A grid power supply is employed to avoid DC voltage drops across the array, with sensor electrodes formed in the topmost MET4 layer.

Array Integration

The RED Sensor pixel is tiled into columns containing 32 pixels (each containing two electrodes) and an array of 32 columns, to form a \( 32 \times 32 \) pixel array measuring \( 640 \times 1,280 \) \( \mu \)m\(^2\).
Figure 4.8: RED Sensor pixel layout shown with $40\mu m \times 20\mu m$ bounding box: (a) layers from metal 1 downwards, (b) layers from via 1 upwards. Layer colours shown in key.
4.3.7 SPAD Sensor Pixel

The SPAD Sensor pixel implements the SPAD topology shown in Figure 4.1 with integrated active quench and gating circuitry. The deep n-well of the SPAD structure sets a minimum distance between adjacent SPADs at 36 μm, to prevent short circuiting neighbouring SPADs through deep n-well diffusion. This distance exceeds the 20 μm electrode pitch. Consequently, the SPAD pixel is implemented with one SPAD centred within a 2×2 electrode sub-array, shown schematically in Figure 4.9. The SPAD is deactivated through $M_1$ receiving active-high $GATE[k]$, where $k$ is the SPAD array address, connected to MSB of the 2×2 sub-array address. The SPAD is enabled with de-assertion of $GATE[k]$ and actively quenched through long-channel PMOS $M_2$. Optional fast quenching is applied through de-assertion of active-low $FQ[k]$ on $M_3$. Glitches appearing upon the addressed SPAD output are prevented during this period through assertion of active-high $MASK[k]$. Excitation for each electrode within the pixel is achieved through the common 2:1 multiplexer architecture described in Section 4.3.3.

![Figure 4.9: SPAD Sensor pixel schematic with active and optional fast quench circuitry.](image)

The SPAD deep n-well further places a minimum distance to surrounding PMOS devices. Subsequently, the CMOS active quench and gating circuits (formed from pull-up PMOS devices, a boolean NOR gate and digital tri-state output buffer, respectively) must all be located to the periphery of the pixel, as visible in Figure 4.11. Low voltage 3.3 V devices are used throughout for compatibility with standard digital blocks included with the H35 process.

Array Integration

The SPAD Sensor array comprises 32×32 elements and sub-arrays of 2×2 electrodes, to form a 32×32 pixel array measuring 1,280×1,280 μm².
Figure 4.10: SPAD Sensor pixel layout shown with 40\(\mu\)m\(\times\)40\(\mu\)m bounding box: (a) layers from metal 1 downwards, (b) layers from via 1 upwards. Layer colours shown in key.
4.3.8 Output Signal Path

The analogue outputs from the AED and RED Sensor arrays are multiplexed at the base of each column, containing an NMOS load to convert their source-follower output currents into voltages. A single pair of column outputs are selected through the asserted $COL[j]$ address ($acol[k]$ for the RED Sensor) and connected to the gate of a 750 $\mu$m wide PMOS source-follower preceding the output bond-pads. The pre-pad source-follower is biased through a current mirror supplied with an external 5 mA current ($IBIASP5mA$), and designed to be capable of driving a 1 $V_{pp}$ signal on to a 1 pF load at 100 MHz, to enable dielectric spectroscopy measurements across the entire $\beta$-dispersion frequency band. The digital SPAD pixel output is buffered on-chip prior to output via a digital bond-pad.

4.3.9 Ancillaries

**Binary-to-Decimal Decoders**

Binary-to-decimal decoding is used to address the 8,192 total electrodes across the three ASIC sensor arrays, separated into 6 bit column address selected through $COLSEL[5:0]$ and 7 bit row address, selected through $ROWSEL[6:0]$. The decoders are implemented from low-voltage 3.3 V digital blocks provided by the H35 process. Their unique outputs are stored in digital registers clocked with $ADRS_{CLK}$, to prevent glitch addresses from being selected during array configuration. The 3.3 V outputs are internally level-shifted to 5 V for interfacing with the AED and RED Sensor pixel arrays.

**Bias Voltage Generator**

The AED and RED Sensor arrays require analogue bias voltages to set the DC operating point of the pixel sensors and the output signal path described previously. These are generated from a global bias generator comprising of a telescopic branch similar to those used in the fully-differential amplifier. The biasing branch receives one 50 $\mu$A reference current ($IBIASP50\mu A$) and a common-mode reference voltage ($V_{CM}$), typically set at 2.5 V.

**SPAD Timing Generator**

The SPAD quenching and masking signals $GATE$, $\overline{FQ}$ and $MASK$ are optionally generated on-chip with respect to a timing generator comprised of 120 CMOS inverter delays described elsewhere [68].
4.3.10 Top-Level

Figure 4.11 shows the top-level layout and micrograph of the final microsystem ASIC with its sensor arrays highlighted. Bond-pads are restricted to two opposite sides of the device to provide for a planar flow path across the sensor arrays without interruption from protruding bond-wires. Digital control and configuration connections are located on opposite sides from the analogue sensor outputs, bias lines and analogue power supplies, to minimise digital shoot-through switching current interfering with analogue signals.

Figure 4.11: Microsystem ASIC: (a) Top-level layout and (b) Micrograph. Array regions highlighted, where “S” refers to sensors, and “E” the total number of electrodes within the highlighted array.
4.4 Printed Circuit Boards

The microsystem employs two custom printed circuit boards (PCBs), referred to as the module and motherboard, shown schematically in Figure 4.12. The module acts as interface between the ASIC and motherboard, the latter providing signal conditioning circuitry for ASIC inputs and outputs, under digital control form of an Opal Kelly XEM3010 module [233], comprised of a Xilinx Spartan-3 Field-Programmable Gate Array (FPGA) [234] with Universal Serial Bus (USB) connection to a PC. The custom components are briefly detailed further below.

4.4.1 Module

The module measures 30×30 mm². It provides for chip-on-board wire-bonding and enables ready ASIC replacement with a fresh device should it become fouled with use. The module connects directly to the motherboard through a pair of 2×30 pin low-profile surface-mount headers to minimise the capacitive load seen by the ASIC output bond-pads.

4.4.2 Input Signal Conditioning

The input signal conditioning block receives excitation signals from either the FPGA or an external source via two SMA connections (not shown). Each source is optionally amplified to 5 V on-board. Multiplexers under FPGA control determine which source connects to the four global ASIC input pins $V_{SIG[3:0]}$ and broken out to headers for observation by oscilloscope.

4.4.3 Output Signal Conditioning

The 3.3 V digital SPAD output connects directly from the module to FPGA and analogue PD output $V_{PD}$ connects to an 8 bit, 1 Msample/s (MSPS) analogue-to-digital converter (ADC) with a serial 3.3 V output (AD7478 [235]). The AED and RED Sensor outputs are processed by dedicated paths comprised of a fixed-gain, low-distortion differential amplifier (AD8351 [236]), a programmable-gain amplifier (PGA870 [237]) and an 8 bit, 250 MSPS, pipeline ADC (AD9480 [238]). The ADC outputs use low-voltage differential signalling (LVDS) to minimise switching noise upon the motherboard, and outputs a data-synchronous LVDS clock, used in firmware to eliminate the round-trip delay through the microsystem (see Section 4.5.2).

The output signal path is AC-coupled throughout due to the inherently AC nature of DS sensor signals. This permits each device to operate at its optimal DC bias point, and eliminates the need for any DC adjustment of sensor signals through e.g. chopping, etc.
4.4.4 Power Supplies and Bias References

The motherboard generates five unique power supplies from optionally external sources or from the 5 V USB bus voltage output from the XEM3010. In addition to the ASIC 5 V analogue supply AVDD5V0, a 3.3 V analogue supply (AVDD3V3) is generated on-board to supply the AD9480 ADCs. Digital supplies VDD3V3 support all digital control interfaces between the ASIC, FPGA and most on-board components. A further 5 V digital supply VDD5V0 is used by the ASIC to level-shift its digital interface lines internally to 5 V. SPAD reverse bias voltage \( V_{BD} \) is generated by a switching regulator (LT1615 [239]) with adjustable output voltage. All necessary bias references for the on-board components and ASIC (IBIASP5mA, IBIASP50uA and \( V_{CM} \)) are generated on-board by variable resistor adjustable low-dropout regulators, or optionally input from external sources.

4.4.5 Layout

Both module and motherboard PCBs use a 6 metal layer stack-up with 0.125 mm minimum track width and spacing. Signals are routed as microstrips on the top and bottom layers with continuous ground planes on the layers immediately beneath. Analogue and digital routing are physically distant to avoid interference coupling. The internal two layers are reserved for power supply planes and analogue bias lines. The motherboard measures 150 × 100 mm².

Figure 4.12: Motherboard schematic.
4.5 Firmware

The microsystem firmware is written in Verilog hardware description language (HDL) and used to configure the Xilinx Spartan-3 FPGA on-board the XEM3010 module and communicate to a controlling PC, described in Section 4.4. The firmware performs three interrelated functions:

- **System configuration**: asserts non time critical signals within the FPGA and upon the motherboard that remain constant throughout an experiment.

- **Array configuration**: asserts the desired electrode pattern and excitation/sensing configurations between data captures.

- **Data capture**: timing critical operation where data output from the on-board ADC must be sampled and stored within the FPGA, and communicated across USB, without corruption.

The above functions are all under software control through a common Host Interface (HI) block provided by Opal Kelly. The implemented firmware architecture is shown in Figure 4.13 and described in the following sections.

4.5.1 Memory Considerations

The implemented architecture arises from the need to conduct dielectric spectroscopy (DS) sensing experiments within the minimum possible time period. It is inevitable that micro-particles in liquid suspension will move over time, be that through sedimentation in static volumes, or due to microfluidic flow, introducing a substantial source of measurement error. Furthermore, the use of m-sequence stimulus for DS measurements requires that the system under test be linear time invariant (LTI). This assumption holds provided that our measurement period is short by comparison to any change within the sample.

The simplest method to achieve short sensing or measurement periods is to capture data from the whole sensor array and write it to FPGA memory, to be read out across the USB to file once the experiment is over. The most parallel sensor array upon the ASIC comprises 2,048 sensors and outputs 8 bit data from the ADC for every m-sequence step. The Xilinx Spartan-3 XC3S1500 FPGA on-board the XEM3010 has 32 dedicated block random access memory (BRAM) tiles, each with 16 kbit capacity. The maximum number of m-sequence steps per sensor that could be stored is:

\[
\left[ \frac{\text{steps}}{\text{sensors}} \right] = 32 \left[ \frac{\text{blocks}}{\text{blocks}} \right] \times 16 \times 1024 \left[ \frac{\text{bits}}{\text{blocks}} \right] \times \frac{1}{8} \left[ \frac{\text{bits}}{\text{blocks}} \right] \times \frac{1}{2048} \left[ \frac{1}{\text{sensors}} \right] = 32.
\]
Recalling that Fourier transformation requires two data inputs per every output, were every sensor output to be stored in one fill of FPGA memory, the total number of measurement frequencies would be reduced to 16; which is not particularly broadband. Alternatively, the FPGA memory could be filled and read-out repeatedly until the desired number of steps per sensor are achieved. While a streaming approach (where data is written to firmware and read into software simultaneously) could be taken, it would maximise the digital switching noise upon the motherboard during data capture. Hence, streaming has not been employed, but rather a sequential sequence of write, read, and repeat.

4.5.2 Timing Accuracy

A further requirement of m-sequence stimulus is clock cycle accurate timing, due to the high auto-correlation and low cross-correlation properties of the m-sequence \[144\]. Encoded sensor output data must be cross-correlated with the input m-sequence to extract the impulse information required for Fourier transformation into the frequency domain. This requires that the output signal arising from a particular m-sequence step be cross-correlated with the corresponding step in the original m-sequence. The output would otherwise be inaccurate, hence the necessity of clock cycle accurate timing. However, the total propagation delay from FPGA output, through the sensor ASIC and return back to the FPGA, is unknown and potentially variable. An absolute timing constraint cannot then be used.

Instead, a source-synchronous architecture has been employed, made possible by the choice of ADC. The AD9480 has separate sample and data output clocks, which share the same frequency, but need not have any set phase relationship \[238\]. Consequently, by synchronising the m-sequence generation with the ADC sample clock, and synchronising the FPGA memory to the ADC output clock, we can eliminate the round-trip propagation delay through the microsystem.

4.5.3 Architecture

Figure 4.13 shows the main firmware blocks and their respective clock domains.

ADC Receivers

Both DS sensor ADCs have a dedicated receiver module that operates synchronous to the ADC output data clock, via a digital clock manager (DCM) hardware block within the FPGA. The interface block receives ADC data and its output clock as 3.3 V LVDS, fixing bit-flips that arise from the motherboard routing \[240\], and outputs into the FPGA BRAM memory block, configured as a first-in first-out (FIFO) memory. FIFO reading and writing is controlled by the memory controller block, that outputs directly to an Opal Kelly block-throttled USB pipe \[241\].
Figure 4.13: Firmware architecture where HI refers to USB host interface module from Opal Kelly.

Excitation Controller
The excitation controller generates and outputs m-sequence stimulus of selectable order and quadrature DEP actuation signals in the form of digital clocks with 50:50 mark:space ratio and programmable frequency, up to half the global reference clock (see below) frequency. The excitation controller module is synchronous to the global reference clock domain.

Array Controller
Array controller consists of finite state machines to assert upon a target ASIC electrode array a user defined configuration, communicated from software during the system configuration phase outlined earlier. In sensing experiments, array controller configures the same stimulus pattern across a user defined pattern of electrodes, to permit direct comparisons between pixels to made. Only one pixel configuration is asserted at a time, to avoid cross talk between stimulus sources and adjacent sensors. Array controller is synchronous to the reference clock domain.

Host Interface
The Opal Kelly host interface (HI) module provides USB communication in the form of input and output pipes (for bulk data transfers), wires (to assert individual bits) and triggers (one clock cycle long pulses) [241]. All USB communication must coordinate with the HI module, which operates off a dedicated 48 MHz HI clock that requires one DCM.
System Design

Master Controller
The master controller module provides global oversight and a common interface for the entire firmware. The ADC sample clocks are generated from the module and synchronised with the m-sequence generator in the excitation controller module. FIFO data writing is coordinated between the ADC receiver and array controller modules, to ensure the desired electrode configuration has been asserted, and the same number of clock cycles elapsed, before m-sequence stimulus is enabled via excite controller. FIFO reading is coordinated between ADC receiver and HI modules. Master controller is synchronous to the reference clock domain.

Reference Clock
Master controller receives a clock from the XEM3010’s on-board phase-locked loop (PLL) from which it generates a reference clock used for array configuration and excitation stimulus. The ADC receiver and HI modules operate within independent clock domains. Appropriate clock synchronisation strategies are employed whenever crossing between clock domains. Timing closure was only achieved for a reference clock frequency of 50 MHz, limiting the maximum measurable frequency to 25 MHz, one quarter that of the ASIC specification target (see Section 4.3.1).
4.6 Software

The Python programming language is used throughout for the microsystem software and performs broadly two types of task, detailed in the following sections:

- **Online tasks**: are time-dependent and performed while a sensing experiment is ongoing. These include controlling the flow of data between the firmware and output data file.

- **Offline tasks**: are performed once the experiment is complete and so less time-dependent. These include post-processing the output data file to extract the information of interest.

4.6.1 Online Tasks

Online tasks are those conducted while a sensing experiment is ongoing. Actuation experiments produce no output, other than a prompt indicating the desired electrode pattern and actuation signals have been applied to the target electrode array upon the ASIC. Whereas sensing experiments generate data that must be communicated from the firmware, across the USB, and into data files without corruption. Data is transferred across the USB at 48 MHz, set by the host interface provided by Opal Kelly [233]. However, the FPGA has insufficient memory to store data from an entire sensor array scan. While the master microsystem reference clock is of comparable frequency (50 MHz, see Section 4.5), a streaming approach is avoided to minimise digital switching noise during sensing periods.

Instead, once the FPGA memory has been filled, data is read out as fast as possible across the USB, before subsequent sensing is permitted to occur. This is necessary to minimise test time and hence the impact of any environmental drift within the sample (or microsystem) during the sensing period. The software indicates to the firmware when it is ready to receive data (which would be corrupted if the firmware were to attempt communication beforehand), then bulk read across the USB and written into a Comma Separated Value (CSV) file, where each column represents an m-sequence permutation, and every row an individual sensor. Note that the firmware can throttle these bulk reads and withhold transferring data for an unspecified period, which the software must accommodate without dropping out.

4.6.2 Offline Tasks

System Configuration

The software configures the microsystem through a test script which prompts the user to enter key parameters regarding the experiment to be conducted, such as the sample type, suspending medium and sensor array to target. These are then communicated to the firmware which, in turn,
System Design

applies the necessary configurations to the motherboard (see Section 4.4). Configuration parameters include the chosen excitation signal source, m-sequence order, number of m-sequence iterations to apply to the ASIC before data is captured (to ignore transient settling behaviour within the sample), and the PGA gain setting to be used within the output signal conditioning path. The automated data capture protocols implemented within firmware are initiated with a single USB trigger event.

Data Processing

Unlike the frequency-domain analysis methods described in Section 2.4.5, time-domain m-sequence output data are not in immediately useful form. They must first be converted into a form compatible with Fourier transformation, from which the desired frequency domain data are retrieved. M-sequence encoded data output from the ASIC must first be cross-correlated with the input m-sequence before Fourier transformation [144, 243]. However, cross-correlation is computationally intensive, requiring \( n^2 \) operations, where \( n \) is the number of captured data points (equivalent to the m-sequence length, \( 2^{m-1} \), where \( m \) is the sequence order). Hence, cross-correlation becomes exponentially more intensive with every bit increase in m-sequence length. Instead, a transformation can be performed that achieves the necessary cross-correlation, but does so within only \( n \cdot \log_2 n \) computations, termed the Fast M-Transform (FMT). The FMT exploits the Hadamard transform to rapidly calculate the measured impulse response from the encoded m-sequence data [244], expressed in (4.1) as:

\[
\Psi' = \frac{1}{2^{m-1}} \pi_1 H \pi_2 \eta,
\]

(4.1)

where \( \Psi' \) is the estimated output spectrum of the system under test, \( m \) the sequence order, \( H \) the sparse Hadamard matrix, \( \eta \) the measured m-sequence encoded response, and \( \pi_{1,2} \) are respectively, encode and decode matrices, necessary to reorder the m-sequence encoded response into that compatible with the Hadamard matrix. Fenimore describes a method where \( \pi_1 = \pi_2 \), which requires that the input m-sequence be naturally-ordered, that is that the decoded output spectrum is phase-aligned with the input sequence [245]. The above FMT algorithm has been implemented using the Python numerical package, Numpy.

Once the FMT has been applied, the desired frequency-domain data is retrieved through the Fast Fourier Transform (FFT) and saved to another CSV file. The FFT protocol is performed with Numpy, which is used to explore the resulting output data. A further Python package, matplotlib, is then used to present graphical plots to the user, or save plots to file. The figures presented in Chapter 5 are all generated in this way.
4.7 Summary

This chapter described the design and implementation of a dedicated microsystem including the AED and RED Sensor architectures investigated in Chapter 3, for the purpose of rapid, time-domain dielectric spectroscopy (DS) measurements upon, and dielectrophoresis actuation of, single-cells in physiological suspension. The microsystem targeted a specification to operate across the $\beta$-dispersion band of frequencies spanning 100 kHz and 100 MHz.

Section 4.3 described the implementation of an ASIC implemented in high-voltage 0.35 $\mu$m CMOS that implements both AED and RED Sensor designs into highly-parallel pixel arrays with combined stimulus and DEP actuation circuitry under each and every electrode. Mixed-mode sensing has been supported through the integration of photodiodes and SPADs within the same ASIC. Its support module and motherboard PCBs were introduced (Section 4.4) and the microsystem’s firmware and software described in Sections 4.5 and 4.6, respectively. A sourcesynchronous firmware architecture was employed to achieve the clock cycle accurate timing made necessary through the use of m-sequence stimulus. A consequence of this architecture is a maximum operating frequency of 50 MHz, setting a maximum measurable frequency of 25 MHz, one quarter of the target specification. The final microsystem assembly is shown in Figure 4.14.

![Figure 4.14](image)

**Figure 4.14:** (a) Photograph of final microsystem assembly with £1 coin for scale. (b) Microsystem within the experimental setup including stereo microscope with light source and external 5 V analogue power supply.
Chapter 5
Results

5.1 Introduction

Chapter 4 described the design and implementation of dedicated microsystem for the assessment of dielectric spectroscopy measurements performed upon individual biological cells in liquid suspension across the $\beta$-dispersion band of frequencies, spanning approximately 100 kHz to 100 MHz, with the Absolute Electric Displacement (AED) and Relative Electric Displacement (RED) sensor architectures described in Sections 4.3.5 and 4.3.6, respectively. The microsystem has also been designed with the capability of moving (actuating) micro-particles in liquid suspension through four mechanisms: positive (p-) and negative (n-) Dielectrophoresis (DEP), Travelling-Wave DEP (TW-DEP) and Electro-Rotation (EROT). Combining these capabilities into a single device provides the potential to move micro-particles to specific locations across the microsystem Application Specific Integrated Circuit (ASIC) electrode array, by means of attraction, repulsion, translation and rotation, respectively.

This chapter presents the results obtained from the microsystem to assess its capabilities in actuation and sensing experiments performed with polystyrene micro-particles, and live yeast cells and individually-fixed biological cells, suspended in de-ionised water and physiological saline solution.

Section 5.2 describes the design of actuation experiments, with results presented for each method in Sections 5.3, 5.4 and 5.5, respectively. De-ionised water (DiH$_2$O) and Phosphate-Buffered Saline (PBS) are chosen as suspending media for their low and high conductivity, respectively. Polystyrene micro-beads and *Saccharomyces Cerevisiae* yeast cells are chosen as exemplar micro-particles for their similar size and difference in structure: micro-beads are solid while the yeast cells have a thick cell membrane. The yeast cell membrane should yield a different frequency-dependent behaviour than the solid micro-bead, and withstand the significant osmotic pressure of suspension in DiH$_2$O. Section 5.6 describes the design of sensing experiments used to assess the functionality and performance of the AED and RED Sensor architectures. Section 5.7 presents data obtained from the AED Sensor array, and Section 5.8 data obtained from the RED Sensor array.

The experimental results are summarised in Section 5.9 before discussed in detail in Chapter 6.
5.2 Design of Actuation Experiments

5.2.1 System Configuration

The microsystem detailed in Chapter 4 includes three separate electrode arrays each capable of multiplexing two of four global input signals onto each individual electrode. Physically separated across the ASIC, each electrode array integrates an identical multiplexer architecture. Each array has a different architecture supporting 5V\textsubscript{pp}, 3.3V\textsubscript{pp} and 2.5V\textsubscript{pp} actuation signal swings. The following section describes the setup and configuration of these arrays to move micro-particles using different forms of Dielectrophoresis (DEP) and Electro-Rotation (EROT).

Electrode Patterns

The DEP force is proportional to the magnitude and gradient of the applied electric-field, as shown in Equation (2.14). The field intensity depends upon the absolute voltage swing (set by the multiplexing architecture under each electrode) and the proximity of neighbouring electrodes connected to different electrical potentials. The ASIC connects all its electrodes to ground when in reset, requiring individual electrodes to be addressed and their multiplexers configured to connect an actuation signal to the electrode metal. Consequently, the DEP force exerted upon the sample depends upon the pattern of configured electrodes and the signal connected to them. Three stimulus patterns are used to generate Positive (p) / Negative (n)-DEP, Travelling-Wave (TW)-DEP and EROT, as shown in Figure 5.1.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5_1.png}
\caption{Electrode patterns and resultant forces (for particles with greater permittivity than their suspending medium) used for sample actuation with DEP: (a) p/n-DEP, (b) TW-DEP and (c) p/n-DEP and EROT. Each pattern represents a tile repeated across each electrode array. Labels indicate actuation signal phase, with 0°, 90°, 180° and 270° components and clear electrodes indicate no signal applied.}
\end{figure}

Actuation Signals

Square-wave actuation signals with 50:50 mark:space ratio were generated directly from the microsystem Field Programmable Gate Array (FPGA) with 3.3 V\textsubscript{pp} swing and programmable frequency up to 25MHz (see Section 4.5). Four phases of actuation signal with 0°, 90°, 180° and 270° components enable TW-DEP and EROT particle actuation depending upon the chosen electrode patterns shown in Figure 5.1.
5.2.2 Media Selection

Two media have been used to explore the capability of DEP sample actuation using the system, namely de-ionised Water (DiH$_2$O) and Phosphate-Buffered Saline (PBS).

**De-ionised Water**

De-ionised Water (DiH$_2$O, $\sigma = 1 \times 10^{-9}$ [S/m]) was chosen to maximise the DEP force experienced by suspended particles. The DEP force is proportional to the magnitude of the applied E-field. A low conductivity suspending fluid such as DiH$_2$O has few free charge carriers to neutralise the applied E-field, and consequently the DEP force. However, the absence of free charge carriers results in a highly hypotonic solution, generating significant osmotic pressure across biological cell membranes.

**Phosphate-Buffered Saline**

Phosphate-Buffered Saline (PBS, $\sigma = 1.2$ [S/m]) was chosen for biological relevance: its high conductivity arises from high numbers of free charge carriers, which present an isotonic environment for biological cells. However, the abundance of free charge carriers within PBS will readily neutralise the applied E-field, and hence the DEP force. The choice of DiH$_2$O and PBS present the two extremes in terms of DEP force magnitude and osmotic pressures experienced by the sample.

5.2.3 Particle Selection

Two particle types have been chosen to investigate the capability of the ASIC to actuate micro-particles using all four DEP methods, including 10 $\mu$m diameter polystyrene micro-beads, and *Saccharomyces Cerevisiae* yeast cells.

**Polystyrene Micro-Beads**

Polystyrene micro-beads with 10 $\mu$m diameter were chosen due to their comparable size to a large number of biological cells (including the yeast cells used here), and for their material composition. Polystyrene has low permittivity ($\varepsilon = 3.5$), resulting in a negative valued Clausius-Mosotti Factor (see Equation (2.15) in Section 2.6.3) when suspended in DiH$_2$O, and consequently will experience n-DEP. Their highly regular shape and homogeneous structure permits direct comparisons to be made between electrode arrays and direct comparison to the expression for DEP force, derived for spherical particles.

**Yeast**

*Saccharomyces Cerevisiae* yeast cells were chosen due to their tolerance to high osmotic pressures. Fungal cell walls are significantly thicker and stronger than the lipid bilayer of mammalian cells, permitting fungus to withstand hypotonic suspending solutions (such as DiH$_2$O) and the resulting expansion arising from the cell absorbing water molecules to balance the os-
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motic pressure across its membrane. Cerevisiae cells are also spherical and visually measured by microscope to be approximately 10\(\mu\)m in diameter, matching the size of the polystyrene micro-beads described above. The DEP force is proportional to the cubic power of particle size, hence slight differences in particle size will similarly vary the DEP force experienced by the particle. The dispersion expected across the cell membrane will lead to significantly increased effective permittivity for the yeast cells by comparison to the polystyrene beads, resulting in p-DEP.

The 10 \(\mu\)m diameter micro-beads and cultured, live yeast cells were suspended in DiH\(_2\)O and PBS with a concentration of 1\(\times\)10\(^6\) cells/ml. This concentration was found empirically through observation to yield reasonable particle coverage across the electrode array.

5.2.4 Actuation Test Protocol

Experimental Setup
16 \(\mu\)L aliquots were deposited by pipette directly upon the ASIC array and allowed to sediment. A microscope coverslip was placed on top of the reservoir encapsulation to prevent evaporation and permit microscope observation of the scene above the array. A still micrograph was taken to record the start position of each micro-particle. An actuation pattern shown in Figure 5.1 was configured across each array, and actuation signals enabled and applied to the electrodes simultaneously. An initial frequency of 25 MHz was used, corresponding to the highest actuation frequency. Micrographs were taken at regular 2 s intervals to record any micro-particle movement. The applied frequency was divided in integer multiples of two every 16 s to assess the effect of applied frequency upon the DEP force experienced by the micro-particles. This frequency division was continued until the lowest actuation frequency of 10 kHz was reached.

Cleaning
A cleaning protocol was developed that first withdraws as much sample as possible from the array with a pipette. DiH\(_2\)O is then deposited and a non-porous electronics cleaning wand used to scrub any remaining particles off the sensor array. The fouled DiH\(_2\)O fluid is then withdrawn and any remaining fluid removed by compressed air. This procedure is repeated until no particles are visible upon the sensor array.
5.3 Particle Actuation with DEP

Figure 5.2 shows 10 μm diameter polystyrene beads suspended in DiH₂O being patterned across the ASIC electrode arrays via n-DEP. A random distribution of beads was first deposited onto the electrode array and actuation signals applied with the pattern shown in Figure 5.1(a). The beads showed the greatest sensitivity to an actuation signal with 1.0 MHz frequency. The micrograph shown in Figure 5.2 was taken after 60 s of 1.0 MHz actuation. Beads outwith the electrode arrays remain un-patterned as visible around the sample reservoir edges and over regions of grounded (un-patterned) electrode. The ASIC is therefore capable of micro-particle actuation via n-DEP for non-biological particles suspended in DiH₂O.

![Figure 5.2: (a) Micrograph showing 10 μm diameter polystyrene micro-beads being patterned via n-DEP across the ASIC electrode arrays. Actuation signal is 3.3 V_{pp} square-wave with 1.0 MHz frequency, applied with the electrode configuration pattern shown in Figure 5.1(a). (b-d) Close-ups on electrode array regions integrating different actuation signal multiplexing circuits, annotated 1-3, respectively.](image)

The procedure described above was repeated for 10 μm polystyrene beads suspended in PBS. No particle movement was observed after 20 s for each actuation frequency, spanning 25 MHz to 10 kHz in 250 steps. This indicates the ASIC is incapable of generating sufficient force via DEP to move cellular-sized micro-particles when suspended in PBS.
5.4 Particle Actuation with TW-DEP

Figure 5.3 shows micrographs captured of the ASIC with 10 μm polystyrene beads suspended in DiH₂O being conveyed across its electrode arrays via TW-DEP, when exposed to a 3.3 V<sub>pp</sub> square-wave actuation signal with 1.0MHz frequency, applied with the electrode configuration pattern shown in Figure 5.1(b). A random distribution of beads is shown in time step 1, with time steps 2 and 3 corresponding to 120 s and 720 s, respectively after the actuation signals were activated. The array in Figure 5.3(c) has been completely cleared within 120 s, whereas the other two still have beads remaining upon them after 720 s.

Figure 5.3: Micrographs showing 10 μm diameter polystyrene micro-beads conveyed via TW-DEP across the electrode arrays. Bead motion follows applied force $F_{TW}$. Micrographs correspond to architecture of actuation signal multiplexer used in each electrode array: (a) 3.3 V<sub>pp</sub> series NMOS switches, (b) 5.0 V<sub>pp</sub> series NMOS switches, and (c) 5.0 V<sub>pp</sub> transmission gates. Actuation signal is 3.3 V<sub>pp</sub> square-wave with 1.0MHz frequency, applied with the electrode configuration pattern shown in Figure 5.1(b). Time steps (1-3) correspond to 0 s, 120 s and 720 s after actuation signals were activated.

The procedure described above was repeated for 10 μm polystyrene beads suspended in PBS. No particle movement was observed after 720 s of actuation signal application with signal frequencies spanning 25 MHz to 10 kHz across 250 steps. This indicates the ASIC is incapable of generating sufficient force via TW-DEP to move cellular-sized micro-particles suspended in PBS.
5.5 Particle Actuation with Combined DEP and EROT

Figure 5.4 shows yeast cells patterned across the ASIC electrode array that integrates a 5.0 V transmission gate (t-gate) multiplexer per electrode. The yeast cells are initially randomly distributed before being focused to the centre of each $2 \times 2$ electrode sub-group with the electrode pattern shown in Figure 5.1(c), indicating that yeast cells experience p-DEP at the applied actuation frequency of 5.0 MHz, found to yield the minimum response time to the applied actuation signals. Furthermore, the cells are observed to rotate in the direction of increasing phase shift of the actuation signal, demonstrating the capability of the ASIC to manipulate biological particles suspended in DiH$_2$O with p-DEP and EROT simultaneously.

![Micrographs showing yeast cells patterned across ASIC electrode array with integrated 5.0 Vpp t-gate multiplexer.](image)

**Figure 5.4**: Micrographs showing yeast cells patterned across ASIC electrode array with integrated 5.0 V$_{pp}$ t-gate multiplexer. (a) Electrode actuation pattern applied to array (as per Figure 5.1(c)). (b-f) Micrographs at 0 s, 30 s, 60 s, 80 s and 240 s after actuation applied, respectively. Actuation signal is 3.3 V$_{pp}$ square-wave with 5.0 MHz frequency.

The procedure described above was repeated for yeast cells suspended in PBS. No particle movement was observed after 720 s since actuation signals were applied, with signal frequencies spanning 25 MHz to 10 kHz across 250 steps. This indicates the ASIC is incapable of generating sufficient force via p-DEP or EROT to move yeast cells suspended in PBS.
5.6 Design of Sensing Experiments

The microsystem detailed in Chapter 4 integrates the Absolute Electric Displacement (AED) and Relative Electric Displacement (RED) sensor architectures into two dedicated, highly-parallel arrays, physically separated upon the ASIC (see Figure 4.11). The following sections describe the design of experiments performed upon these two sensor arrays, to assess their response to different media phases (gas and liquids), and particle complex permittivities. The same test protocols are used for each sensor array to provide direct comparisons between the sensor architectures.

5.6.1 System Configuration

Sensor Array Pattern
The ASIC integrates 2,048 AED and 1,024 RED sensors within their dedicated arrays. Each AED sensor comprises one electrode and each RED sensor comprises two electrodes, occupying one row and two adjacent columns. Data is captured from sensors across the entire array, bar a perimeter of two sensor addresses in width, to avoid non-uniformities at the array edges. This perimeter prevents use (for either stimulus or sensing) of the outermost rows and columns of each array, where sensor mismatch will be greatest.

Stimulus Pattern
A consistent stimulus pattern is used relative to each addressed sensor. AED Sensors are stimulated from the rows immediately above and below the addressed sensor, with the same phase of m-sequence, shown in Figure 5.5(a). RED Sensors are stimulated differentially with 0° (P) and 180° (N) m-sequence components applied to both columns on the rows immediately above and below the addressed sensor, respectively, as shown in Figure 5.5(b). These patterns generate the greatest sensor signal response by maximising the stimulus signal applied across the sample in closest proximity to each addressed sensor, while minimising direct coupling of the stimulus signal across the addressed sensor due to the common row-based routing (see Section 4.3.4).

Stimulus Signal
Both the AED and RED sensors are sensitive to the interaction of the sample to an applied stimulus. An 11 bit m-sequence with 50 MHz synthesis clock is used throughout, requiring 2,047 cycles of the master 50 MHz clock to complete one iteration (see Section 4.5). Two complete iterations are used. The first permits transient charging to occur across the isolating dielectric layer and sample medium, and the second to record data.

Manual Gain Adjustment
The analogue output signal path on-board the Support PCB contains a Programmable-Gain Amplifier (PGA) with gain settings from -11.5 dB to +20 dB in 0.5 dB steps. The PGA
Results

Figure 5.5: Stimulus electrode patterns for (a) AED Sensor and (b) RED Sensor arrays. $P$ represents the 0° phase, and $N$ the 180° phase component of the stimulus m-sequence. $S$, $S_P$ and $S_N$ represent the addressed AED Sensor, and positive (master) and negative (slave) sides of the RED Sensor, respectively.

Gain is manually adjusted to maximise the sensor output signal received by the Analogue-to-Digital Converter (ADC), while avoiding saturation.

Data Acquisition

M-sequence encoded data is captured in real-time sequentially from each sensor address (see below). At each sensor address, data is converted to digital via the ADC on-board the motherboard PCB and stored in Block RAM memory on-board the Field Programmable Gate Array (FPGA). Once full, the BRAM memory is read-out to a data file via USB (see Sections 4.4 and 4.5). This procedure is repeated until data has been captured from all enabled sensors. Data is then post-processed offline, first by decoding the m-sequence encoded data, and then by Fourier Transformation to the frequency domain (see Section 4.6).

5.6.2 Media Selection

Three media have been used to explore the capability of the AED and RED sensor architectures, namely air, de-ionised water (DiH$_2$O) and Phosphate-Buffered Saline (PBS).

Air: By default, the sensor ASIC is exposed to air when no other media has been applied. Experiments performed in air provide a measure of the shunt capacitance between sensor electrode edges, as this path has lower impedance than between the electrode faces (since $\varepsilon_{\text{air}} = 1.2$ compared to the intermetal layer dielectric silicon dioxide $\varepsilon_{\text{SiO}_2} = 3.9$ [231]).

DiH$_2$O: ($\varepsilon_{\text{H}_2\text{O}} = 80$ at 1 MHz) has approximately sixty times the permittivity of air and consequently provides a measure of the coupling between electrode faces, as necessary for particle sensing. DiH$_2$O avoids the additional complication of free charge carriers absorbing the electric-field between sensor electrodes.

PBS: is the most biologically-relevant medium due to the abundance of free charge carriers, essential to provide an isotonic environment for mammalian cells.
5.6.3 Particle Selection

Five types of micro-particles have been used to explore sensitivity to particle size and material composition, including three diameters of polystyrene micro-beads, and two species of mammalian cells.

Polystyrene Micro-Beads
Micro-beads labelled 10.0 μm, 15.0 μm and 20.0 μm in diameter (actual diameters 11.00 μm, 15.66 μm and 18.50 μm, respectively) were chosen to explore the sensitivity to particle size for identical material composition. The specific diameters correspond respectively to 0.55, 0.78 and 0.93 times the 20.0 μm electrode pitch common to each ASIC array (see Section 5.3.7). Micro-beads are not representative of biological cells however, due to their homogeneous structure and hence absence of internal dielectric boundaries, such as the cell membrane. Frequency dependent dispersions are therefore unlikely with micro-beads.

Individually-Fixed Mammalian Cells
Ideally, experiments with live biological cells would be used to investigate the effectiveness of the AED and RED sensors. However, handling live cells requires infrastructure that was not available for this work. Individually-fixed cells have instead been used, where formaldehyde displaces water within each cell, fixing their morphology and preventing decay. Individually-fixed cells therefore provide a reasonable proxy for live cells.

Mature Red Blood Cells (RBC) were chosen due to their size and absence of a nucleus. RBCs have a lipid-bilayer cell membrane containing haemoglobin to transport oxygen. Interfacial polarisation at this membrane will give rise to a frequency-dependent dispersion. The absence of a nucleus lends the RBC to being approximated by the single-shell model (see Section 3.2.3), providing a convenient reference. RBCs are on average 7.5 μm in diameter, well within the fixed electrode pitch of 20 μm. However, their prolate spheroid shape will contribute to signal variation due to their orientation.

Cervical cancer HeLa cells are similar in size to RBCs with 8.5 μm diameter and share a lipid bilayer membrane. Unlike the RBCs, HeLa cells contain a nucleus, which will exhibit a secondary dispersion through interfacial polarisation.

In summary, polystyrene micro-beads provide measurements of identical material composition with changing size to be investigated, and individually-fixed RBC and HeLa cells provide for single and double dispersions, respectively, for similarly sized particles.
5.6.4 Sample Preparation

Micro-beads suspended in DiH$_2$O were bought from Polysciences and homogeneous single-cell samples of individually-fixed RBC and HeLa cells, suspended in PBS, were prepared and donated for this work. The micro-beads were resuspended in PBS after centrifugation and draining of their native DiH$_2$O media.

The ASIC presents a reservoir above the sensor arrays for depositing sample directly upon the electrode array. The ideal test condition is a monolayer of particles aligned in a consistent position relative to each activated sensor, with sufficient distance between particles to avoid those located on neighbouring electrodes from interacting with each other. Interaction here refers to affecting the sensed signal due to the presence of another particle, or screening the applied stimulus. Particle coverage across the sensor array will be proportional to particle diameter. Whereas the electrode array is fixed in hardware, particle concentration can be changed to yield the target particle coverage across the array. Equation (5.1) calculates the target particle concentration $C_t$ [particles/ml] for the desired array coverage for a given particle diameter $d$ [m] with initial concentration:

$$C_t = s \frac{4wh}{V\pi d^2}$$  \hspace{1cm} (5.1)

where $s$ [particles] is the fraction of the array to be covered, $w$ [m] the sensor array width, $h$ [m] the sensor array height, and $V$ [m$^3$] the sample reservoir volume above the array. Equation (5.1) assumes particles are spherical and that all particles eventually sediment upon the array. The value $s=0.2$ was found empirically to yield optimal array coverage. The sample reservoir volume was similarly found to be 16 µl.

5.6.5 Test Protocols

Instrument Response Function

The intrinsic noise response, or Instrument Response Function (IRF), of the system is recorded across each sensor array by applying m-sequence stimulus to the ASIC, while leaving all stimulus electrodes inactive. The IRF is recorded in the ambient medium, air.

Media

The array response in the presence of air, DiH$_2$O and PBS is recorded across each array to provide a background measurement as reference for particle sensing. A 16 µl aliquot of one liquid medium is applied to a previously cleaned sensor array, and a glass coverslip applied to seal the sample reservoir and prevent evaporation. Data is obtained from one row of sensors and used to manually adjust the PGA gain setting to maximise the signal seen by the ADC.
Data is then captured from all sensors within the activated sensor array pattern as described in Section 5.6.1. This process is applied for both DIH$_2$O and PBS. Ambient measurements in air require no sample deposition and are captured upon a similarly cleaned sensor array.

**Particles**

The sample deposition procedure described above is applied for each particle type. A 3 min period is allowed to elapse ahead of capturing data, to provide sufficient time for particles to sediment upon the sensor array. A microphotograph is then taken for reference and data capture enabled. This procedure is repeated for each particle type with the array cleaned between sample depositions.

**Cleaning**

A cleaning protocol was developed that first withdraws as much sample as possible from the array with a pipette. DIH$_2$O is then deposited and a non-porous electronics cleaning wand used to scrub any remaining particles off the sensor array. The fouled DIH$_2$O fluid is then withdrawn and any remaining fluid removed by compressed air. This procedure is repeated until no particles are visible upon the sensor array.

**5.6.6 Design of Sensing Experiments Summary**

The preceding section has described the system configuration, media and particle selection, sample preparation and test protocols used to obtain the results to be presented in the following sections. Stock solutions of 11.00 μm, 15.66 μm and 18.50 μm polystyrene micro-beads suspended in PBS were prepared to yield a desired coverage of sample across the sensor array. Individually-fixed single-cell suspensions of RBCs and HeLa cells were similarly prepared. Aliquots of each were deposited, one at a time, upon a clean sensor array. A microphotograph was taken after 3 mins and data capture enabled. The raw data is stored to file and the system cleaned before repeating the above procedure for the next particle type.

**Data presentation**

Magnitude data is presented with solid lines indicating the mean of the recorded responses at each frequency bin, and coloured regions one standard variation surrounding that mean. Magnitude data has been low-pass filtered to smooth high-frequency switching noise with a first-order discrete Butterworth response with cut-off at 10 MHz for visibility.
5.7 AED Sensor Results

This section presents results from the AED Sensor array. Magnitude data are first presented for different media, including air, DiH$_2$O and PBS, to set background references upon which particle data will be added. Data from three diameters of polystyrene micro-bead are then presented, followed by fRBC and HeLa cells, to demonstrate the performance of the sensor for solid and membrane-bound particles, with varying complex permittivities. The results are then assessed and discussed in Chapter 6.

5.7.1 AED Media Sensing

Figure 5.6 (a) shows the sensor array response to air, with and without stimulus, to measure the intrinsic noise within the system, and Figure 5.6 (b) the responses due to DiH$_2$O and PBS.

![AED Sensor array magnitude responses for (a) air with and without stimulus, (b) liquid DiH$_2$O and PBS. Solid lines show the mean response, coloured regions show one standard deviation.](image)

Figure 5.6: AED Sensor array magnitude responses for (a) air with and without stimulus, (b) liquid DiH$_2$O and PBS. Solid lines show the mean response, coloured regions show one standard deviation.
Referring to Figure 5.6(a), the intrinsic noise within the AED Sensor array begins around $-10$ dB, descending to a floor centred about $-22.5$ dB before rolling off above $10$MHz, indicating the sensor bandwidth. The response to air is $12$ dB larger, centred around $-10.5$ dB before similarly rolling-off above $10$MHz, converging toward the noise floor at the maximum measured frequency of the $25$MHz. The variation within the noise and air responses are similar at approximately $17$ dB.

Referring to Figure 5.6(b), the liquid media of DiH$_2$O and PBS share a similar magnitude response at frequencies beneath $100$ kHz before diverging, with the DiH$_2$O response around $5$ dB greater than PBS, before rolling-off above $10$ MHz. Variation within the responses are similar at frequencies beneath $10$ kHz before similarly diverging, with the DiH$_2$O response reaching three times that of PBS across the measured frequency band.

Comparing the results of Figures 5.6(a) and (b), shows that DiH$_2$O has approximately the same response as air above $10$ kHz. The difference between liquid media responses and the intrinsic noise floor is approximately $17$ dB across the measured frequency band.

### 5.7.2 AED Solid Particle Sensing

The following section presents data obtained from solid polystyrene micro-bead particles with varying diameters. The chosen plotting method described below is designed to account for the uncontrolled nature of the scene above each sensor array, while showing the consistency or otherwise between individual sensors. Each plot has three sections, described below.

Section (a) shows micrographs from 5 Regions of Interest (ROI) containing a $3 \times 3$ sub-array of AED Sensors, surrounded by a one sensor deep “moat”. Each ROI is chosen to contain ideally one particle with the moat used to avoid the applied stimulus pattern shown in Figure 5.5(a) from being interfered with by neighbouring particles.

Section (b) presents the magnitude response recorded from each ROI. The mean from each ROI is plotted as a line surrounded by a filled region showing one standard deviation of recorded magnitude responses from that ROI. This process is repeated for all 5 ROIs, to show the statistical variation across each ROI and between ROIs.

Section (c) presents the magnitude response recorded from each ROI less the background response of PBS containing no particles, and hence the relative response of the AED Sensor array. The plot is formatted as per that in section (b), where the mean for each ROI response is plotted as a line surrounded by a filled region indicating one standard deviation about that mean.

Figures 5.7, 5.8 and 5.9 show AED sensor magnitude responses from $11.00 \, \mu m$, $15.66 \, \mu m$, and $18.50 \, \mu m$ diameter micro-beads, all suspended in PBS, respectively.
Figure 5.7: AED Sensor array magnitude responses from 11.00 μm diameter polystyrene micro-beads. (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.8: AED Sensor array magnitude responses from 15.66 μm diameter polystyrene micro-beads. (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.9: AED Sensor array magnitude responses from 18.50 μm diameter polystyrene micro-beads. (a) Micrographs of 3×3 sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Comparing the micrographs in section (a) of Figures 5.7, 5.8 and 5.9 to the fixed 20 μm pitch of the background electrode array, confirms the change in diameter of each micro-bead sample. Comparing the absolute magnitude responses in section (b) of Figures 5.7, 5.8 and 5.9 shows a consistent magnitude pattern for each micro-bead diameter. The response starts at approximately $-22.5 \, \text{dB}$ and plateaus to $-15 \, \text{dB}$ at 100 kHz, before rolling-off above 8 MHz to $-32.5 \, \text{dB}$ at 25 MHz. The spread of recorded responses is within 10 dB for each micro-bead diameter, with a subtle negative shift in magnitude for increasing micro-bead diameter.

The relative magnitude responses shown in section (c) of Figures 5.7, 5.8 and 5.9 begin at $-10 \, \text{dB}$ before decreasing to $-32.5 \, \text{dB}$ at 300 kHz. A consistent peak is apparent across ROIs and micro-bead diameters at 800 kHz. The responses continue to decrease above 800 kHz toward a minimum of $-35 \, \text{dB}$ at 25 MHz. The gradient of relative magnitude decrease with increasing frequency appears to increase with micro-bead diameter.

### 5.7.3 AED Cell Sensing

The following section presents data obtained from individually-fixed Red Blood Cells (RBCs, Figure 5.10) and HeLa cells (Figure 5.11), suspended in PBS. The same plotting format is used as described in previous section, with identical plot limits to permit direct comparisons between the data.

Comparing the micrographs in section (a) of Figures 5.10 and 5.11 to the fixed 20 μm pitch of the background electrode array, confirms the fRBCs have a diameter of approximately 8 μm (corresponding to one third of the fixed electrode pitch), and HeLa cells a diameter approaching 20 μm. Note these diameters are similar to those of the micro-beads described in Section 5.7.2.

Comparing the absolute magnitude responses in section (b) of Figures 5.10 and 5.11, both the fRBCs and HeLa responses begin around $-22.5 \, \text{dB}$, settling around $-15 \, \text{dB}$ at 100 kHz, before rolling-off above 8 MHz. The spread of recorded responses is within 10 dB for each cell type. The fRBC response is most different from that of the HeLa cells at frequencies above 20 MHz, with a 5 dB difference apparent at 25 MHz.

The relative magnitude responses shown in section (c) of Figures 5.10 and 5.11 better shows this difference in response between cell types. A distinct “hump” is apparent in the fRBC responses above 10 MHz, not visible within the HeLa responses or those for micro-beads. The gradient of relative magnitude decrease is also subtly different between cell types, with the HeLa gradient larger than that of the fRBCs. Indeed, the observed gradient is different between cell types and to those recorded for micro-beads.
Figure 5.10: AED Sensor array magnitude responses from individually-fixed Red Blood Cells (fRBC). (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.11: AED Sensor array magnitude responses from individually-fixed cervical cancer (HeLa) cells. (a) Micrographs of 3×3 sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
5.7.4 AED Sensing Results Comparison

Sections 5.7.2 and 5.7.3 have presented data recorded for solid micro-beads and individually-fixed mammalian cells, respectively. This section compares these results to each other and to the AED Sensor model developed in Section 3.3.

Figure 5.12: Summary of AED Sensor responses for 11.00 μm, 15.66 μm, and 18.50 μm polystyrene micro-beads, individually-fixed RBCs and HeLa cells. (a) Measured data. (b) Model predictions.

Figure 5.12(a) shows the average (mean) relative magnitude response measured across ROIs for each particle type. The measured responses share a similar trend, decreasing in magnitude with increasing frequency. The magnitude difference between micro-bead diameters and cell types is much smaller than this overall characteristic. The most obvious differences are at frequencies beneath 100 kHz, where the micro-bead responses are consistently 2.5 dB smaller than those from cells. However little difference is observed between micro-bead diameters and between cell types. Figure 5.12(b) shows the responses predicted by the AED Sensor model developed in Section 3.4. Equations (3.4) and (3.7) are used to calculate the effective permittivities for the solid micro-beads and membrane-bound cells. These values are then used to calculate the component values in Equation (3.9). The background response for PBS is calculated using Equation (3.10) and subtracted to yield the plots in Figure 5.12(b).
The model predictions of Figure 5.12(b) do not match the measured responses shown in Figure 5.12(a). This appears due to the magnitude of load capacitance $C_L$, necessary to match the absolute magnitude of the measured data, dominating the other circuit elements which represent different particles.

![Figure 5.13: Summary of relative opacity responses from the AED Sensor array. (a) Measured data. (b) Model predictions.](image)

The preceding plots have attempted to summarise the data obtained with the AED Sensor array, however only subtle differences are visible upon an otherwise large “carrier” characteristic. A more illustrative plot of AED Sensor responses is shown in Figure 5.13(a), which shows the relative change in magnitude with frequency, all relative to that for the smallest (11.00 μm diameter) micro-bead. Relative Opacity (see Section 2.6.2) provides a comparison of how opaque a particle is to the applied electric-field, relative to an 11.00 μm polystyrene micro-bead. Figure 5.13(a) shows the 15.66 μm and 18.50 μm micro-beads have similar relative opacities to one another below 10 MHz, before diverging. The greatest divergence is observed for the greatest difference in bead diameter. Regarding the cell types, the relative opacity plot shows an overall difference from that of micro-beads, and each other, for frequencies above 10 MHz. These results indicate that the AED Sensor is most sensitive to differences in micro-particles at higher frequencies, but that the presented model is inaccurate at predicting such behaviour.
5.8 RED Sensor Results

This section presents results from the RED Sensor array. Magnitude data are first presented for different media, including air, DiH$_2$O and PBS, to set background references upon which particle data will be added. Data from three diameters of polystyrene micro-bead are then presented, followed by fRBC and HeLa cells, to demonstrate the performance of the sensor for solid and membrane-bound particles, with varying complex permittivities. The results are then assessed and discussed in Chapter 6.

5.8.1 RED Media Sensing

Figure 5.14 (a) shows the sensor array response to air, with and without stimulus, to measure the intrinsic noise within the system, and Figure 5.14 (b) the responses due to DiH$_2$O and PBS.

![Figure 5.14](image)

**Figure 5.14:** RED Sensor array magnitude responses for (a) air with and without stimulus, (b) liquid DiH$_2$O and PBS. Solid lines show the mean response, coloured regions show one standard deviation.
Referring to Figure 5.14(a), the intrinsic noise within the RED Sensor array begins around $-10$ dB, descending to a floor centred about $-35$ dB at 25 MHz. The response to air is initially $-17.5$ dB (7dB smaller than the null stimulus condition) and increases to a plateau of $-7.5$ dB between 100 kHz and 6 MHz, before rolling off to $-12$ dB at 25 MHz. The peak Signal to Noise Ratio (SNR) of 27.5 dB is reached at 6 MHz. The variation within the noise and air responses are similar at approximately 17 dB.

Referring to Figure 5.14(b), the liquid media of DiH$_2$O and PBS share a similar magnitude response at frequencies beneath 100 kHz before diverging, with the DiH$_2$O response around reaching a maximum 15 dB greater than that for PBS between 1 MHz and 10 MHz. The PBS response remains within a 5 dB range across the measured frequency band. Both DiH$_2$O and PBS responses begin to converge above 10 MHz.

Comparing the results of Figures 5.14(a) and (b), shows that DiH$_2$O has approximately 2 dB smaller response to air above between 1 MHz and 10 MHz. The SNR for DiH$_2$O peaks at 30 dB at 8 MHz, with PBS reaching a peak SNR of 18 dB at 10 MHz.

5.8.2 RED Solid Particle Sensing

The following section presents data obtained from solid polystyrene micro-bead particles with varying diameters. The chosen plotting method described below is designed to account for the uncontrolled nature of the scene above each sensor array, while showing the consistency or otherwise between individual sensors. Each plot has three sections, described below.

Section (a) shows micrographs from 5 Regions of Interest (ROI) containing a $3 \times 3$ sub-array of RED Sensors (recalling that each RED Sensor occupies two adjacent electrode columns), surrounded by a one sensor deep “moat”. Each ROI is chosen to contain ideally one particle with the moat used to avoid the applied stimulus pattern shown in Figure 5.5(b) from being interfered with by neighbouring particles.

Section (b) presents the magnitude response recorded from each ROI. The mean from each ROI is plotted as a line surrounded by a filled region showing one standard deviation of recorded magnitude responses from that ROI. This process is repeated for all 5 ROIs, to show the statistical variation across each ROI and between ROIs. Section (c) presents the magnitude response recorded from each ROI less the background response of PBS containing no particles, and hence the relative response of the RED Sensor array. The plot is formatted as per that in section (b), where the mean for each ROI response is plotted as a line surrounded by a filled region indicating one standard deviation about that mean.

Figures 5.15, 5.16 and 5.17 show RED sensor magnitude responses from 11.00 μm, 15.66 μm, and 18.50 μm diameter micro-beads, all suspended in PBS, respectively.
Figure 5.15: RED Sensor array magnitude responses from 11.00 μm diameter polystyrene micro-beads. (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.16: RED Sensor array magnitude responses from 15.66 μm diameter polystyrene micro-beads. (a) Micrographs of 3×3 sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.17: RED Sensor array magnitude responses from 18.50 μm diameter polystyrene micro-beads. (a) Micrographs of 3×3 sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Comparing the micrographs in section (a) of Figures 5.15, 5.16 and 5.17 to the fixed 20 μm pitch of the background electrode array, confirms the change in diameter of each micro-bead sample. Comparing the absolute magnitude responses in section (b) of Figures 5.15, 5.16 and 5.17 shows a consistent magnitude pattern, beginning at −35 dB and increasing with frequency for each micro-bead diameter. While the majority of responses are similar, ROI[4] in Figure 5.18 and ROI[5] in Figures 5.16 and 5.17 are obvious outliers. The variability is similar between all recorded responses at approximately 7.5 dB.

The relative magnitude responses shown in section (c) of Figures 5.15, 5.16 and 5.17 begin around −10 dB before decreasing to a minimum of −35 dB at 3 MHz. Above 3 MHz, an increase in magnitude response with increasing micro-bead diameter becomes apparent, with variability in the magnitude response for each micro-bead diameter.

### 5.8.3 RED Cell Sensing

The following section presents data obtained from individually-fixed Red Blood Cells (RBCs, Figure 5.18) and HeLa cells (Figure 5.19), suspended in PBS. The same plotting format is used as described in previous section, with identical plot limits to permit direct comparisons between the data.

Comparing the micrographs in section (a) of Figures 5.18 and 5.19 to the fixed 20 μm pitch of the background electrode array, confirms the fRBCs have a diameter of approximately 8 μm (corresponding to one third of the fixed electrode pitch), and HeLa cells a diameter approaching 20 μm. Note these diameters are similar to those of the micro-beads described in Section 5.8.2.

The absolute magnitude responses in section (b) of Figures 5.18 and 5.19 share a similar magnitude response characteristic below 3 MHz, increasing from −37.5 dB at 24.4 kHz to −22.5 dB at 200 kHz, before diverging. The HeLa cell responses further increase to a maximum of −16 dB at 25 MHz compared to the fRBC response which decreases by 2 dB above 3 MHz, reaching a maximum difference of 11 dB between cell types at 25 MHz. The fRBCs have the greatest variation across ROIs with two (ROI[3, 5]) outlier groups whose magnitude is consistently 7.5 dB greater than those from the other ROIs.

The relative magnitude responses shown in section (c) of Figures 5.18 and 5.19 shows the greatest differences in observed magnitude shift occur above 3 MHz, with the fRBC responses reaching −24 dB and the HeLa responses −32.5 dB at 25 MHz. The fRBC responses appear to begin plateauing at 25 MHz, whereas the HeLa cell responses have a subtle “hump” with a peak of 2.5 dB at approximately 22 MHz before decreasing again to −35 dB at 25 MHz. Indeed, the greatest differences observed between micro-bead and cell responses is at higher frequencies, as per the AED Sensor responses presented in Section 5.7.2 and 5.7.3.
Figure 5.18: RED Sensor array magnitude responses from individually-fixed Red Blood Cells (RBC). (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Figure 5.19: RED Sensor array magnitude responses from individually-fixed cervical cancer (HeLa) cells. (a) Micrographs of $3 \times 3$ sensor sub-array Regions of Interest (ROI): thin lines indicate ROI perimeter, thick line stimulus perimeter. (b) Recorded absolute magnitude data, (c) relative magnitude data. Lines show mean for each ROI, colour fill indicates one standard deviation about the mean.
Results

5.8.4 RED Sensing Results Comparison

Sections 5.8.2 and 5.8.3 have presented data recorded for solid micro-beads and individually-fixed mammalian cells, respectively. This section compares these results to each other and to the RED Sensor model developed in Section 3.4.

Figure 5.20: Summary of RED Sensor responses for 11.00 \( \mu \)m, 15.66 \( \mu \)m, and 18.50 \( \mu \)m polystyrene micro-beads, individually-fixed RBCs and HeLa cells. (a) Measured data. (b) Model predictions.

Figure 5.20 (a) shows the average (mean) relative magnitude response measured across ROIs for each particle type. Below 3MHz, the measured responses share a similar trend of decreasing in magnitude with increasing frequency. Above 3 MHz, the difference in aggregate response between particle types becomes evident. The 11.00 \( \mu \)m micro-beads continue their trend toward \(-35\) dB at 25 MHz, followed by HeLa cells \((-32\) dB), 15.66 \( \mu \)m beads \((-31\) dB), 18.50 \( \mu \)m beads \((-28.5\) dB) and the maximum response recorded for fRBCs \((-26\) dB) at 25 MHz. Figure 5.20 (b) shows the responses predicted by the RED Sensor model developed in Section 3.4. Equations (3.4) and (3.7) are used to calculate the effective permittivities for the solid micro-beads and membrane-bound cells. These values are then used to calculate the component values in Equation (3.16). The background response for PBS is calculated using Equation (3.15) and subtracted to yield the plots in Figure 5.20 (b).
Results

The model predictions of Figure 5.21(b) do not agree with measurement beneath approximately 1 MHz, but show agreement to within 3.5 dB above 1 MHz. Predicted values at 25 MHz are inline with measurements but 3.5 dB greater. This overestimate appears to arise from the value used for $C_L$ in Equations (3.16) and (3.15). It appears the load capacitance within the RED Sensor pixel (see Section 4.3.6) is approximately twice that estimated from the MOSFET gate and drawn feedback capacitances within the sensor pixel.

![Graph of Relative Opacity](image1)

**Figure 5.21:** Summary of relative opacity responses from the RED Sensor array. (a) Measured data. (b) Model predictions.

The preceding plots have attempted to summarise the data obtained with the RED Sensor array. A more illustrative plot of RED Sensor responses is shown in Figure 5.21(a), which shows the relative change in magnitude with frequency, all relative to that for the smallest (11.00 μm diameter) micro-bead. Relative Opacity (see Section 2.6.2) provides a comparison of how opaque a particle is to the applied electric-field, relative to that of an 11.00 μm polystyrene micro-bead. Figure 5.21(a) shows the RED Sensor model accurately predicts the responses for 15.66 μm and HeLa cells, however it underestimates those for the 18.50 μm micro-beads and fRBCs. These results indicate that the RED Sensor is most sensitive to differences in microparticles at higher frequencies, and not only their size, but their entire complex permittivity.
5.9 Summary

This chapter has presented the results obtained from the MINIFC microsystem for both the actuation and sensing of micro-particles suspended in de-ionised water and saline solution.

Actuation

The microsystem has demonstrated a unique capability of moving (actuating) polystyrene micro-beads of 11.00 μm diameter via repulsion (nDEP) and translation (TW-DEP), and *Saccharomyces Cerevisiae* yeast cells via attraction (pDEP) and rotation (ER) upon a single device, requiring no top-plate sample biasing electrode. This capability was demonstrated for suspensions in De-Ionised water (DiH$_2$O) when exposed to a 3.3 V$_{pp}$ actuation signal with 50:50 mark:space ratio across a programmable frequency range of 25 MHz to 10 kHz in 250 steps. Micro-beads were observed to respond fastest at 1.0 MHz, and yeast cells to 5.0 MHz actuation signal frequencies. Suspensions in Phosphate-Buffered Saline (PBS) were also attempted, but no particle actuation was observed to occur. PBS represents a biologically-relevant medium in which yeast cells were isotonic. In contrast, yeast cells appear swollen in DiH$_2$O, indicating a hypotonic state. The osmotic stress induced by the absence of free charge carriers in DiH$_2$O makes this an unattractive medium to use for mammalian cell sensing.

Sensing

Sensing experiments were designed and applied to the Absolute Electric Displacement (AED) and Relative Electric Displacement (RED) sensor architectures integrated within the MINIFC microsystem. Magnitude responses were recorded across a 24.4 kHz to 25 MHz band in linear steps of 24.4 kHz via an 11 bit m-sequence with 3.3 V$_{pp}$ swing. Background measurements were performed with no stimulus applied to record the intrinsic noise-floor across each sensor array when exposed to Air. Responses for Air, DiH$_2$O and PBS were recorded with full stimulus applied and Signal to Noise Ratio (SNR) spectra obtained. In PBS, a peak SNR of 17 dB at 1 MHz, and 27.5 dB at 6 MHz, were obtained for the AED and RED sensor arrays, respectively.

Samples of 11.00 μm, 15.66 μm and 18.50 μm polystyrene micro-beads, and individually-fixed Red Blood Cells (fRBC) and cervical cancer (HeLa) cells, were prepared and suspended in PBS. Each sample was applied one at a time to a cleaned device. The absence of micro-particle actuation in PBS presents a problem in aligning said micro-particles with individual sensors, which will contribute additional variation in the results obtained across each sensor array. A statistical approach was therefore taken. For each sample type, data was recorded from 5 Regions of Interest (ROI) comprising a 3×3 sensor sub-group, ideally containing one particle. Each sub-group is surrounded by a one sensor deep “moat” to prevent neighbouring particles interacting with those above the addressed sensor, or screening the applied stimulus.
The AED Sensor was found capable of distinguishing micro-beads from cells, and the two cell types from one another, when suspended in PBS. However, the absolute magnitude of these differences was found to be within 0.5 dB of each other, much smaller than anticipated by the modelling presented in Section 3.3. The RED Sensor was found capable of distinguishing micro-beads from each other and the two cell types, and the two cell types from one another. Magnitude shifts between particles were in the order of 5 dB, and accurately predicted by the model developed in Section 3.4. While magnitudes were accurately predicted, differences between measured and predicted frequency response were identified. These results are discussed in detail in Chapter 6.
6.1 Introduction

Chapters 3 through 5 have presented the modelling, design and implementation, and experimental application of the developed microsystem, respectively. Through critical discussion, this chapter assesses the microsystem’s intended purpose: to actuate and sense individual biological cells in liquid suspension. Of particular interest is the sensing capability or otherwise of the microsystem to the β-relaxation of individual biological cells (arising from interfacial polarisation between dielectric boundaries), and hence whether those cells can be identified from their suspending medium and each other.

This assessment concentrates upon the architecture of circuitry used to achieve micro-particle actuation and sensing, the experimental setups used to assess their functionality and performance, the results obtained from the experiment presented in Chapter 5, and their significance. Section 6.2 discusses the actuation capabilities or otherwise of the microsystem, and Sections 6.3.3 and 6.4.3 discuss the AED Sensor and RED Sensor, respectively. Finally, global observations are made and conclusions drawn, presented in summary in Chapter 7.

6.2 Actuation

Dielectrophoresis (DEP) and the related phenomena of electro-rotation (EROT) present an attractive means of actuating micro-particles specifically due to their frequency response, providing a complementary capability to micro-particle analysis via Dielectric Spectroscopy. Sections 5.3 to 5.5 describe experiments conducted to assess the capability of the microsystem in moving particles via attraction (pDEP), repulsion (nDEP), translation (TW-DEP) and rotation (EROT) to time-varying electric fields. The following sections discuss the circuit architectures implemented within the microsystem’s Application-Specific Integrated Circuit (ASIC), before discussing the results of the experiments conducted with the microsystem.

6.2.1 Actuation Architecture

The microsystem integrates a novel architecture of actuation circuitry in CMOS that can pattern any one of four global analogue excitation signals to any electrode on the ASIC (see Section
Previously reported CMOS implementations of DEP sample actuation circuits have only provided two global input signals, enabling attractive (pDEP) and repulsive (nDEP), only \cite{106,107,135,108}. The microsystem’s capability of full quadrature sample excitation permits similar use of n/pDEP (demonstrated in Section 5.4), but also sample translation via Travelling-Wave DEP (TW-DEP, demonstrated in Section 5.4) and combinations of these methods with particle rotation via EROT (demonstrated in Section 5.5).

The microsystem has the highest reported actuation signal bandwidth for a CMOS integrated DEP device. Hunt et al. \cite{108} reports a maximum bandwidth of 1.8 MHz and Manaresi et al. \cite{106,204,135} a maximum actuation frequency of 1 MHz. Section 5.6 demonstrated actuation of *Saccharomyces Cerevisiae* yeast cells at 5.0 MHz, using a combination of pDEP and EROT. Further implementations in \cite{106,204,135} all require use of a conductive top-plate to bias the suspending medium, located between 85 \(\mu\text{m}\) and 100 \(\mu\text{m}\) from, and parallel to, the active electrode array. The microsystem requires no such top-plate, drastically simplifying the manufacturing and hence cost of any device based upon the presented architecture.

Passive DEP device implementations such as \cite{103,196,105} fix which DEP method they are compatible with at design time by their physical electrode layout, which defines the geometry of electric field applied across the sample. The DEP force is proportional to gradient of the applied electric field \((\nabla |E|, \text{see Equation (2.13)})\). Interdigitated electrodes are typically used for p/nDEP, where \(|\nabla |E|\) is concentrated as stripes between adjacent digits connected to 0\(^\circ\) and 180\(^\circ\)-phase components of the applied actuation signal, respectively. TW-DEP can be achieved provided the interdigitated electrode layout is compatible with full quadrature actuation signals, whereas EROT requires crossed electrodes to concentrate \(|\nabla |E|\) to a point between electrodes. CMOS-based implementations can avoid this limitation by integrating highly-parallel 2D arrays of electrodes (e.g. \(320 \times 320\) elements \cite{135}) which are individually programmable and configurable. \(|\nabla |E|\) can then be reconfigured on-the-fly across highly-parallel electrode arrays.

However, passive systems are typically more flexible than CMOS implementations due to their wider voltage range compatibility than possible with low-voltage active circuitry. A further benefit of passive systems previously reported is the capability of superimposing actuation signals, not possible with the digital-only CMOS implementations reported to date. For example, Pethig et al. \cite{195} reports superimposing pDEP and TW-DEP signals onto interdigitated passive electrodes to sort different cell fractions. The microsystem has demonstrated a similar such capability combining pDEP and EROT (see Section 5.5), and is furthermore compatible with complex actuation signals, due to the analogue nature of its actuation circuitry, with the additional benefit of arbitrarily programmable electrode patterning on-the-fly.

Overall, the actuation architecture of the microsystem has greater programmability than passive devices, with the greatest flexibility of all reported CMOS devices to date. However, these
benefits are a trade-off with voltage swing and absolute sample volume, due to the cost and manufacturing limitations on the physical area of CMOS devices. For similar reasons, passive devices are much better suited to integration with microfluidics, enabling larger sample volumes to be used, and greater cell numbers to be analysed per test, than were possible in this work. Where the optimum trade-off lies will depend upon the particular application in mind.

6.2.2 Design of Actuation Experiments

Micro-beads provide a convenient sample to demonstrate nDEP due to their homogeneous structure and permittivity beneath that of the suspending medium (yielding a negative Clausius-Mossotti (CM) factor as described see Section 2.6.3). Conversely, the cytoplasm of biological cells has a permittivity comparable to that of liquid suspending media, and can experience both attractive and repulsive DEP (positive and negative CM factors). Saccharomyces Cerevisiae yeast cells were chosen for their tolerance of high osmotic pressures, due to the absence of free-charge carriers within De-Ionised Water (DiH2O).

Two suspending media were chosen for actuation experiments, namely DiH2O and Phosphate-Buffered Saline (PBS). DiH2O is poorly conductive (σ ~ 10^{-12} S/m) and will yield the greatest DEP force due to high electric-field strength across suspended micro-particles. However, its absence of free-charge carriers will present an acutely hypotonic environment for suspended biological cells. Conversely, PBS is highly conductive (σ ~1.2 S/m) and should present an isotonic environment for suspended cells, as they would experience in-vivo. The combination of micro-beads, yeast cells, DiH2O and PBS enables a wide spread of CM factors to be assessed with the microsystem.

6.2.3 Micro-Particle Attraction and Repulsion (p/n-DEP)

Figure 5.2 shows 10 μm diameter polystyrene micro-beads suspended in DiH2O being repelled via nDEP from regions of high |∇E| across the three electrode arrays integrated within the ASIC. Regions of Interest (ROI) numbered 1-3 are shown, corresponding to the AED Sensor array (see Section 4.3.5), RED Sensor array (see Section 4.3.6), and SPAD Sensor array (see Section 4.3.7), respectively. The beads were initially randomly distributed across the entire field of view and the micrographs shown were taken 60s after the 3.3 V_{pp} actuation signal was applied. The micro-beads have successfully been repelled to the desired electrode pattern shown in Figure 5.1(a) for all three arrays within the 60 s period. Figure 5.4 shows live Saccharomyces Cerevisiae yeast cells being attracted via pDEP to the centre of 2×2 subgroups of electrodes patterned as per Figure 5.1(c). These results demonstrate the programmability of the ASIC and its capability to attract and repel individual micro-beads and yeast cells using p/nDEP.
6.2.4 Micro-Particle Translation and Rotation (TW-DEP and EROT)

Figures 5.3 and 5.4 show 10 μm polystyrene micro-beads and live yeast cells being translated and rotated by TW-DEP and EROT, respectively. The negative CM factor of polystyrene micro-beads prevents their rotation with EROT, but enables translation with TW-DEP. Combined, these results demonstrate the capability of the microsystem to actuate micro-particles using two phenomena previously unreported upon a CMOS-based device. Further novelty is achieved with combination of p/nDEP as described in the previous section.

6.2.5 Limitations upon Micro-Particle Actuation with DEP

While the microsystem has demonstrated its capability to move micro-particles across its electrode arrays, two distinct limitations have been discovered. Namely, the timescales necessary to achieve such movement, and the inability to move particles suspended in PBS.

In relative terms, a marked difference in time is necessary to assert p/nDEP compared to TW-DEP and EROT. Comparing the timescales across Figures 5.2, 5.3 and 5.4 show that p/nDEP can assert a pattern within one minute, with negligible difference observed across the three electrode arrays. TW-DEP and EROT require significantly longer, in the order of several minutes to achieve the patterns shown in Figures 5.3 and 5.4. This occurs due to reduction in DEP force when adjacent electrodes are not driven complementarily (compare electrode pattern (a) and (b) in Figure 5.1), reducing the electric-field gradient ($\nabla |E|$) between electrodes (see Equation (2.14)). Increasing the actuation voltage (and hence $|E|$) can overcome this reduction in force, but absolute limits will be imposed by the voltage tolerance of the chosen CMOS process, by the isolating dielectric breakdown strength or the activation energy of electrode-to-sample interfaces in passive implementations.

In absolute terms, the timescales required by CMOS compatible devices in the literature also require several minutes to assert micro-particle patterns with p/nDEP (see [106, 204, 108]). While these CMOS-based devices integrate highly-parallel electrode arrays capable of moving several hundred cells simultaneously, the absolute time required to achieve micro-particle patterning is greater than 6 orders of magnitude longer than the sensing period required by the AED and RED Sensor architectures described in this work. The above timescale issues are also compounded by the limitations upon the suspending media.

The second limitation of particle actuation with DEP is the need for low-conductivity suspending media. The experiments described in Sections 5.3 through 5.5 have all been conducted with DiH$_2$O. When conducted with PBS, no particle motion was recorded after application of actuation signals, even after significant time (>720 s). This arises from the free-charge carriers present within PBS screening the actuation charges applied to electrodes, drastically reducing
Discussion

the electric-field strength through the sample and hence the DEP force experienced by microparticles within the sample. Low-conductivity suspending media present an acute problem for studying biological cells: the absence of free-charge carriers results in hypotonic solutions, causing suspended cells to absorb water, swell up, and eventually rupture under the osmotic pressure across their membranes. All DEP and EROT experiments reported in the literature require biological cells to be resuspended in low-conductivity media (in the order of mS, reported in the references of review papers [100, 192]). Substitute molecules are used to overcome the osmotic pressure problem, including sugar solutions such as mannitol [106, 204, 135] and sucrose and glucose [105]. The question arises as to whether cells suspended in sugar solution bear any resemblance to cells suspended in saline solution, as is the case in vivo.

6.2.6 Actuation Discussion Summary

The microsystem has been shown capable of actuating individual micro-particles when suspended in DIH$_2$O via all three DEP mechanisms (p/nDEP and TW-DEP), and combined with EROT. The use of 10 µm diameter polystyrene beads and live Saccharomyces Cerevisiae yeast cells indicate that individual cell actuation of similarly sized mammalian cells is also possible. However, two distinct limitations have been found. Firstly, the timescales necessary to achieve micro-particle actuation, and secondly, the inability to actuate micro-particles when suspended in PBS. Overall, the above limitations mean that particle actuation via p/nDEP, TW-DEP or EROT are not practical for use in biologically-relevant media. This presents an acute limitation on the practical use of DEP for biological cell actuation within the presented microsystem or otherwise. The sensing experiments conducted in this work are therefore performed without the benefit of micro-particle actuation.

6.3 AED Sensor

6.3.1 AED Sensor Architecture

The AED Sensor uses a single electrode to sense the total electrical environment (suspended micro-particles and suspended medium) in close proximity to that electrode. Changes in the electric displacement incident upon the electrode arise from the complex permittivity of the sample above the electrode when exposed to stimulus signal(s). This change is measured as a change in charge $dQ$, which is effectively a change in capacitance $dC$ when a fixed stimulus voltage is applied across the sensing volume (see Section 3.2.1). The literature reports, and the model developed in this work predicts, sensitivity on the order of fF and below are necessary to distinguish individual cells from the background suspending medium, and potentially lower still to differential between cell types.
Discussion

Passive devices may benefit from the absence of a ground plane in close proximity to the sensor electrodes (as is the case with CMOS integrated circuits), limiting the shunt capacitance to ground, and hence the charge division upon the device output. However, any external signal conditioning circuitry, such as analogue to digital conversion, will present a capacitive load on the order of pF to the sensor output \[238\]. Considering that the sensed signal is sub-fF in size, this will create a charge division in the order of 40 dB. Such small capacitances are highly susceptible to thermal or switching noise sources, leaving the sensor signal vulnerable to corruption from the external electronic environment. ICs with active gain circuitry in immediate proximity to sense electrodes avoids this issue entirely, provided sufficient care is taken regarding device layout to minimise switching noise coupling through common IC connections.

Compared to other CMOS-based implementations of capacitive biological cell sensors (e.g. \[135, 160, 246, 162\]), the AED Sensor is the simplest topology reported, requiring a single transistor buffer, a single biasing device and addressing switch (see Section 4.3.5). However, the simplicity of the source-follower topology comes at the expense of harmonic distortion (due to the asymmetry of its voltage transfer function) and lack of gain. The absence of feedback around the source-follower prevents oscillation, but results in a gain less than unity, and therefore attenuates the input voltage, reducing the absolute sensitivity of the AED Sensor.

6.3.2 Design of AED Sensor Experiments

Section 6.2 has discussed the inability of DEP to move micro-particles when suspended in PBS. This is unfortunate for sensing experiments, as micro-particles could have been aligned to sensors with DEP to minimise any measurement variation due to micro-particle to electrode alignment. While DEP actuation was shown possible in DiH\(_2\)O, determining the functionality of the AED Sensor in PBS is of significant novelty, and hence the absence of particle alignment is considered a reasonable limitation to demonstrate the capability of the AED Sensor in PBS.

The measurement approach taken in Section 5.7 of extracting similar ROI across the AED Sensor array provides a reasonable means of comparing data obtained from otherwise similar micro-particles. Hence, the greatest contribution of measurement variation will likely arise from the misalignment of the suspended micro-particles with the sensor electrodes. Future means of minimising this source of measurement variation is discussed in Section 6.5.

Section 5.7 presents magnitude data captured from air, DiH\(_2\)O and PBS when no particles are present. Each medium is exposed to an 11 bit m-sequence with 3.3 \(V_{pp}\) swing sampled at 50 MHz, yielding 1,023 frequency-domain data points at multiples of 24.4 kHz, spanning a frequency band of 24.4 kHz to 25 MHz. This represents a fifty fold increase in maximum measured frequency for m-sequence analysis of biological cells \[143\], reaching the band of frequencies where \(\beta\)-relaxation is expected to occur.
6.3.3 AED Sensor Results

Medium Sensing
Figure 5.6(a) shows magnitude data captured from the AED Sensor array when exposed to air with and without stimulus, the latter to provide a measure of the intrinsic noise floor within the system. Figure 5.6(b) shows magnitude data for DiH$_2$O and PBS. Overall, the characteristic for liquid DiH$_2$O and PBS is less than that for gaseous air. This result is initially counter-intuitive, considering the real permittivity of water ($\varepsilon = 80$) is approximately sixty times that of air ($\varepsilon = 1.2$ at 1 MHz), which means the electric-field coupled through DiH$_2$O is sixty times stronger than that for air. This result is an illustration of electrode polarisation (see Section 2.2.4), i.e. the neutralising of applied stimulus charge carriers by bound charges within the sample liquid. This effect is further increased by the free charge carriers present within PBS, as seen in Figure 5.6(b). Nonetheless, the PBS response is above the noise-floor, with a Signal-to-Noise Ratio (SNR) in excess of 10 dB between 150 kHz to 25 MHz, indicating that the AED Sensor can sense micro-particles suspended in PBS.

Further consideration of Figure 5.6(b) shows that all recorded magnitude responses begin to roll-off above 6 MHz. The consistency of this cut-off frequency between samples indicates that 6 MHz is the AED Sensor bandwidth, which is approaching an order of magnitude lower than the designed bandwidth of 50 MHz. This suggests the AED Sensor output signal path has not been optimised. While this bandwidth limits the sensitivity of the AED Sensor to $\beta$-relaxation frequencies, its bandwidth is an order of magnitude greater than previously reported CMOS-integrated capacitive cell sensors [135, 165, 126].

Micro-Particle Sensing
Section 5.7.2 presents AED Sensor magnitude data captured from polystyrene micro-beads with 11.00 $\mu$m, 15.66 $\mu$m and 18.50 $\mu$m diameters suspended in PBS, shown in Figures 5.7, 5.8 and 5.9, respectively. Polystyrene micro-beads were chosen for their identical material properties to assess the effect of particle radius upon the AED Sensor response. Overall, the AED Sensor response is dominated by that of the suspending medium PBS for frequencies above 300 kHz. Beneath this frequency, the effective impedance of the suspended micro-beads will be greater than that of the suspending PBS. Consequently, the electric-field will preferentially avoid the micro-beads, giving rise to the greatest magnitude response at low-frequency. This result is consistent with the literature [24, 92, 181, 138, 94, 5]. However, the above treatment that micro-beads occlude electric-field at low frequencies only holds if the geometry surrounding the micro-bead acts to constrain the electric-field, such as the Coulter orifice [23]. The AED Sensor has no such geometric constraint. This result indicates that the derivation of $C_s$ in Section 3.2.1 is inaccurate: the electric-field varies in more than the $\rho$-axis alone, but likely in the $z$-axis too.
A difference in magnitude response is observed between micro-bead diameters, but much smaller than anticipated by model simulations shown in Figure 5.7. Figure 5.12 shows the average response recorded across five ROIs per micro-bead diameter. These average responses are within 0.5 dB of each other across the measured frequency band. This attenuation in micro-particle response is most likely due to electrode polarisation. A contributing factor will be the inability to align particles optimally with the sense electrodes, leading to measurement variation, the mean average of which may act to reduce the net recorded magnitude response presented here.

Figure 5.13 shows the relative electrical opacity of micro-beads with respect to that of the 11.00 μm diameter micro-beads. Electrical opacity provides a measure of how opaque a particle appears to an applied electric-field. Figure 5.13(a) shows that the 15.66 μm and 18.50 μm diameter micro-beads have similar electrical opacity spectra to that of the reference 11.00 μm micro-beads. This result indicates that the AED Sensor is capable of identifying the presence of micro-beads when suspended in PBS, but it is not capable of absolute particle sizing in the presented microsystem architecture. However, this may be possible by applying a geometric constraint to limit the variation of the electric-field within the z-axis above the sensor volume, as described above.

**Biological Cell Sensing**

Figures 5.10 and 5.11 in Section 5.7 show the AED Sensor magnitude response for fRBCs and fHeLa cells suspended in PBS, respectively. Comparing the relative magnitude responses (subplot (c) in the above Figures) to each other, the overall frequency characteristics are similar below 10 MHz, above which the responses diverge. The fRBC response increases by approximately 3 dB with respect to the fHeLa response, peaking around 12.5 MHz, before returning to a magnitude similar to the fHeLa response, due to the bandwidth of the AED Sensor. This indicates that the greatest discrimination between cell types is achieved at frequencies above 10 MHz, corresponding to the β-relaxation.

Comparing the micro-bead responses to those of the cells, shows the cell responses have a greater overall magnitude up to 6 MHz, as visible in the aggregate mean responses taken across ROIs shown in Figure 5.12(a). These differences are most obvious in the Relative Opacity responses shown in Figure 5.13, which show a high-pass characteristic. The micro-bead and cell opacities are similar at the reference frequency of 24.4 kHz, before diverging above 100 kHz. The micro-beads approach a relative opacity of unity, indicating similar opacity to that of the reference 11.00 μm diameter micro-beads, discussed above. This demonstrates the AED Sensor is capable of distinguishing individually-fixed cells from micro-beads.
Comparing the two cell type responses, fRBCs show the aforementioned peak at 12.5 MHz, indicating the AED Sensor is capable of distinguishing the two cell types from one another. Considering the diameters of the fRBC and fHeLa cells (\(\sim 7.5 \, \mu m\) and 15 \(\mu m\), respectively) are similar to those of the chosen micro-bead samples, the relative opacity results show the AED Sensor is not sensing particle size alone, but sensitive to the complex permittivity of the suspended particles. This sensitivity appears to increase with frequency into the \(\beta\)-relaxation range, but masked by the bandwidth of the AED Sensor implementation.

Table 6.1 shows the effective micro-particle capacitances \(C_p\) calculated from Maxwell’s Mixture Theory (MMT) and the Single-Shell Model (SSM), described in Sections 3.2.2 and 3.2.3, respectively. The micro-bead capacitances are beneath 1 fF, with the membrane-bound cell samples approximately four to seven times greater. Observing the relative opacity measurements from Figure 5.13, it appears the minimum measurable particle capacitance of the AED Sensor is \(\sim 1 \, fF\) above 1 MHz. This corresponds to one sixth the minimum measured capacitance (6 fF) reported by direct charge sensing at DC with CMOS [126], for sensing DNA when suspended in low-conductivity DiH\(_2\)O (low signal loss due to electrode polarisation). By comparison, this work has demonstrated 1 fF resolution of individual biological cells suspended in highly-conductive PBS (high signal loss) across 1,024 frequencies between 24.4kHz and 25 MHz.

The difference in recorded magnitude responses between micro-beads and individually-fixed cells cannot therefore be attributed to particle radius alone, demonstrating that the AED Sensor architecture is sensitivity to the complex permittivity of the micro-particles. The AED Sensor has therefore been shown capable of identifying micro-beads and two cell types when suspended in PBS, and differentiate the cell types from one another, by using their difference in \(\beta\)-relaxation response.

### 6.3.4 Measurement Comparison with Models

Section 5.3 made the assumption that the suspending liquid would have a transfer characteristic independent of frequency within the measurable range. Figure 5.6 indicates this assumption holds within the measurement passband, but that the AED Sensor has a significantly lower

<table>
<thead>
<tr>
<th>Sample</th>
<th>(C_p , [fF])</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.0 (\mu m)</td>
<td>0.5</td>
<td>MMT</td>
</tr>
<tr>
<td>15.66 (\mu m)</td>
<td>0.75</td>
<td>MMT</td>
</tr>
<tr>
<td>18.5 (\mu m)</td>
<td>0.9</td>
<td>MMT</td>
</tr>
<tr>
<td>fRBC</td>
<td>3.87</td>
<td>MMT + SSM</td>
</tr>
<tr>
<td>fHeLa</td>
<td>3.73</td>
<td>MMT + SSM</td>
</tr>
</tbody>
</table>

Table 6.1: Calculated micro-particle capacitances and modelling method.
bandwidth than that intended. Comparing the predicted values for background media in Figure 5.6(a) with those measured in Figure 5.6, shows that liquid DiH$_2$O ($\varepsilon = 80$) has a transfer function approaching 7.5 dB lower than that predicted for the 18 $\mu$m wide AED Sensor electrode. This reduction is most likely due to an underestimate in the load capacitance ($C_L$) seen by the electrode, which would similarly explain, at least in part, the reduction in observed sensor bandwidth.

Figure 5.12(a) shows the aggregate ROI mean responses recorded for the various micro-beads and cells. Figure 5.12(b) shows the results predicted from the AED Sensor transfer function of Equation (3.9), less the background response of Equation (3.10). The model does not match measurement. Indeed, the value of $C_L$ necessary to approximate the measured magnitude response is four times greater (40 fF) than that predicted (10 fF). Consequently, the effect of suspended particles within the AED Sensor transfer is being attenuated by at least a factor of four. It appears the assumption that $C_y$ accurately represents electrode polarisation at the isolating dielectric layer interface is wrong, and a series double-layer capacitance (see Section 2.2.4) is also necessary.

The relative opacity measurements and model predictions shown in Figure 5.13 appear to have the same limitation. However, the measured difference in relative opacities between micro-beads and cells cannot be explained by micro-particle radius alone. This implies the AED Sensor is sensitive to the complex permittivity of suspended particles and not just their size as per the Coulter method [23, 3, 92].

### 6.3.5 AED Sensor Discussion Summary

The AED Sensor is capable of sensing micro-beads and individually-fixed biological cells when suspended in PBS via Dielectric Spectroscopy, due to their difference in $\beta$-relaxation responses.

The AED Sensor architecture requires only three low-voltage transistors and is therefore highly scalable to smaller CMOS geometries. However, the absolute differences in voltage recorded by the sensor were 20dB lower than predicted by the sensor model developed in Section 3.3. This discrepancy arises from the absence of a double-layer capacitance within the equivalent circuits shown in Figure 3.5, which indicates the AED Sensor experiences electrode polarisation due to bound and free charge carriers upon its sense electrode.

The AED Sensor has a calculated minimum capacitance measurement resolution of 1fF, equivalent to six-fold increase from previous direct-charge sensing implementations in CMOS. Potentially greater sensitivity and discrimination between cell types appears possible with increasing sensor bandwidth. Overall, further work is necessary to determine the absolute limit of detection of the AED Sensor architecture for single cell analysis.
6.4 RED Sensor

6.4.1 RED Sensor Architecture

The RED Sensor uses a pair of adjacent sense electrodes to sense the difference in electric displacement between those electrodes, to cancel the contribution of the suspending medium, and provide information upon the suspended micro-particles only. The RED Sensor utilises the physics of complementary electric-fields, enabling a fully-differential architecture to be used, which looks at the difference between these fields. Its fully-differential nature provides for twice the gain of the single-ended AED Sensor discussed in the previous section. The RED Sensor pixel architecture is detailed in Section 4.3.6. It implements a fully-differential amplifier with capacitive feedback, where the input capacitance is formed by the sensor volume ($C_p$) in series with the isolating dielectric layer ($C_y$ which separates the electrodes from the sample medium), and feedback capacitance ($C_F$) is made by a pair of poly-poly capacitors integrated in-pixel.

Reported implementations of biological cell sensors that use a differential architecture compare a measurement branch (which the sample modifies) with a reference branch (unmodified by the sample) [135, 90, 162, 165], to cancel electrode polarisation, sensor drift and other environmental effects common to both electrodes. However, these reference branches are by definition not exposed to the same environment as the measurement branch. Indeed, some implementations use purely electronic components as their reference (such as a poly-poly capacitor in [135]), or they are located significant distances from the measurement electrode (e.g. >2 mm in [165]). Hence the reference branches of existing differential biological cell sensors are not accurate references.

The fully-differential architecture of the RED Sensor is designed to overcome this issue by observing the difference between two identical electrodes in immediate proximity to one another. In other words, each electrode is being used as the reference for the other. Environmental or drift signals are cancelled by taking the difference in signal detected upon both electrodes. This assumes the greatest difference between the sensor electrodes is due to the presence of a micro-particle. $C_F$ was set at 24.5 fF to minimise leakage-induced drift across the data capture period. However, $C_y$ was calculated at 7.34 fF, meaning the sensor circuit has a gain of only 0.3, and hence acts to attenuate the sensor signal. Negligible drift was observed across all measurements within the sensing period, permitting smaller values of $C_F$ to be used, and potentially, enabling signal gain in-pixel. Feedback capacitance $C_F$ must be on the same order as the change in capacitance $C_p$ of the sample, to avoid charge division and destruction of the sensed signal, which would simply not be possible with passive circuits requiring interconnect to external signal processing circuits, as discussed in Section 6.3.1.
6.4.2 Design of RED Sensor Experiments

RED Sensor experiments were performed with the same setup as used for AED Sensor experiments described in Section 5.6 and discussed in Section 6.3.2. Micro-particle misalignment is a more pronounced issue with the RED Sensor, due to its differential transfer function, and capacity to cancel signals that occur on opposite sides of the sensor. Care must therefore be taken when interpreting aggregate averages across sensor sites as done in Section 5.8.

6.4.3 RED Sensor Results

Medium Sensing

Figure 5.14(a) shows the differential magnitude data captured from the RED Sensor array when exposed to air with and without stimulus, the latter to provide a measure of the intrinsic noise floor within the system. Figure 5.14(b) shows magnitude data for DiH₂O and PBS, which have a peak SNR of 30 dB and 17.5 dB, respectively, indicating the RED Sensor can sense micro-particles when suspended in PBS.

The RED Sensor is based upon the assumption that the polarisation experienced by its two sense electrodes is identical (see Equation (3.13) in Section 3.4) and hence can be cancelled by observing the difference in signal between its electrodes. The differential responses shown in Figure 5.14 would be highly attenuated and independent of frequency if this assumption were true. This is not the case for DiH₂O, whose differential response is 6dB greater than its absolute response observed by the AED Sensor (shown in Figure 5.6) and changes in excess of 23 dB across the measured frequency band. Conversely, the differential PBS response is lower in magnitude than the absolute response recorded by the AED Sensor, and varies less than 10 dB with frequency. This result is likely due to the abundance of free charge carriers present in PBS (σ ~1.2 S/m) compared to DiH₂O (σ ~1 \( \mu \)S/m), that can readily respond to screen the applied stimulus. These additional neutralising charge carriers will attenuate the applied stimulus and sensed charges, reducing the overall magnitude response of PBS with respect to DiH₂O, as observed in Figure 5.14(b). Hence the assumption that \( P_1 = P_2 \) holds for PBS and applies to DiH₂O for frequencies above 1MHz, which lies within the \( \beta \)-dispersion range. This indicates that the RED Sensor is rejecting electrode polarisation within the \( \beta \)-dispersion band of frequencies.

Micro-Particle Sensing

Section 5.8.2 presents RED Sensor differential magnitude data captured from polystyrene micro-beads with 11.00 \( \mu \)m, 15.66 \( \mu \)m and 18.50 \( \mu \)m diameters suspended in PBS, shown in Figures 5.18, 5.19 and 5.20, respectively. Polystyrene micro-beads were chosen for their identical material properties to assess the effect of particle radius upon the RED Sensor response. The
responses are similar beneath 3 MHz before diverging, with the magnitude shift proportional to the micro-bead diameter, and hence its volume. This indicates the RED Sensor is capable of distinguishing polystyrene micro-beads from the suspending PBS. Outlier spectra are apparent for certain ROIs, which were not apparent for the AED Sensor. These outliers demonstrate the sensitivity of the RED Sensor to micro-particle position above its electrodes, as visible in the micrographs shown in Figures 5.15(a), 5.16(a) and 5.17(a).

Biological Cell Sensing
Figures 5.18 and 5.19 in Section 5.8 show the RED Sensor magnitude response for fRBCs and fHeLa cells suspended in PBS, respectively. Comparing the relative magnitude responses (subplot (c) in the above Figures), the overall frequency characteristics are similar below 10 MHz, above which the responses diverge. The fRBC response plateaus to a peak difference of 7.5 dB at 25 MHz, whereas fHeLa responses are seen to peak around 7 MHz, before dropping off up to 25 MHz. The fRBC behaviour is indicative of a first-order dispersion centred around 10 MHz, as would be expected from the $\beta$-relaxation, as described in Section 2.2.2. This suggests the RED Sensor is detecting the interfacial polarisation of the fRBC membranes.

The peaking observed within the fHeLa responses could indicate a secondary dispersion [173, 247], centred around 20 MHz, but whose upper limit is not visible within the measured frequency band. If so, this would be consistent with interfacial polarisation of the nuclear membrane. However, further measurements with greater SNR are necessary before such an assertion can be confirmed. This behaviour is most visible in Figure 5.20(a), which compares the aggregate spectra obtained across ROIs for each micro-particle type. The dispersion of the fRBC response occurs at a frequency around 10 MHz, which is comparable to the 4.5 MHz used by Hoffman et al. to identify live RBCs [3, 92].

Figure 5.20(a) shows the aggregate mean magnitude responses for each micro-particle type and Figure 5.20(b) shows model results generated from Equations (3.15) and (3.16), with effective micro-particle capacitances as shown in Table 6.1. Comparing the measured and model results shows the RED Sensor has a capacitance sensitivity of $\sim$0.5 fF at 25 MHz, comparable to the 0.42 fF sensitivity reported by Romani et al. [135] for single-ended charge amplification integrated in 0.35 $\mu$m CMOS with 20 $\mu$m pitch electrodes. However, the Romani et al. measurements were performed in low-conductivity (and hence low signal loss) mannitol sugar solution and required the solution be biased through DC connection to an external top-plate, with an applied voltage step of +/-9 V. The use of mannitol solution implies that biological sensing was not possible in PBS, indicating that while the capacitance sensitivity is similar to this work, the biological sensitivity per unit capacitance is significantly less. The RED Sensor has been shown capable of identifying micro-beads and two cell types from each other when suspended in PBS, across 1,023 individual frequencies.
Figure 5.20(a) further shows the RED Sensor is sensitive to more than the micro-particle size alone. As visible within the micrographs shown in subplot (a) of Figures 5.15 through 5.18, fRBCs have a diameter of \(\sim 7.5 \mu m\), whereas its aggregate response is 8dB greater than that of 18.50 \(\mu m\) diameter micro-beads. These results show fRBC and fHeLa cells can be distinguished solely by their \(\beta\)-dispersion response when suspended in PBS, a result not previously reported.

Relative opacity measurements shown in Figure 5.21(a) confirm this assertion, as the frequency response of fRBCs and fHeLa cells is seen to differ from those of 15.66 \(\mu m\) and 18.50 \(\mu m\) micro-beads, and from those predicted by the modelling results of Figure 5.21(b). Comparing the RED and AED Sensor responses in Figures 5.21 and 5.17, respectively, the change in recorded magnitude response is in excess of 6dB for each particle type. Were the RED Sensor solely providing a differential form of the AED Sensor measurement, a twofold (6 dB) increase in measured magnitude would be apparent. Since the magnitude differences between the AED and RED Sensor results are greater than 6 dB, and considering the difference in frequency response between the sensor measurements, it can be concluded that the RED Sensor is providing a different and complementary measurement to that of the AED Sensor.

### 6.4.4 Measurement Comparison with Models

The fully-differential architecture of the RED Sensor is designed to reject the signal components common to the sensor’s two electrodes, which were assumed to share identical electrode polarisation. The accuracy of the model results predicted for the RED Sensor in Sections 3.4 and 5.8 demonstrate that this assumption holds, particularly for PBS, and increasingly so with increasing frequency for DiH2O. The absence of a double-layer capacitance to model electrode polarisation has no effect upon the differential response of the RED Sensor, providing further evidence that the above assumption \(P_1 = P_2\) holds.

The accuracy of the modelling results supports the simplifying assumption that the electric-field \(E\) between electrodes is proportional to the cylindrical \(\rho\)-axis only. However, the variation in measurements across ROI demonstrate that the RED Sensor is sensitive to micro-particle position above its electrodes, which shows the assumption that \(E(\rho)\) does not hold. Future modelling should account for the micro-particle position with respect to the sensor electrodes, and that \(E\) changes in at least two dimensions, in the \(\rho\)- and \(z\)-axes as shown in Figure 3.2.

### 6.4.5 RED Sensor Discussion Summary

The RED Sensor is capable of distinguishing micro-beads of varying diameters and two types of individually-fixed biological cells from each other, when suspended in PBS via Dielectric Spectroscopy, due to their different \(\beta\)-relaxation behaviours. The fully-differential nature of sensor
architecture rejects electrode polarisation, increasingly so with increasing frequency. The results obtained indicate that greater distinction between micro-particles has been achieved than previously reported due to the RED Sensor’s unique architecture and increased frequency resolution across the $\beta$-relaxation frequencies. However, the implemented sensor circuit attenuates and further work is necessary to determine the absolute limit of detection of the RED Sensor architecture. Potentially greater sensitivity and discrimination between cell types is possible with increasing sensor bandwidth and in-pixel gain.

6.5 General Observations

6.5.1 Architecture Improvements

The AED Sensor bandwidth is much lower than anticipated. This indicates greater parasitic capacitance is present within the pixel and/or output signal path, most likely within the ASIC. Reducing these parasitic capacitances is possible through improvements within the AED Sensor pixel topology and layout, in addition to the signal path off-chip. Buffering at the column level with a high gain-bandwidth amplifier would further increase the measured bandwidth, and potentially provide gain on-chip to increase the overall SNR of the microsystem.

The AED and RED Sensor output signal paths use an 8 bit ADC with 1 V Full Scale Range (FSR), corresponding to a minimum convertible signal of 3.9 mV with peak SNR of 47 dB. The motherboard (see Section 4.4) incorporates up to 40 dB of gain, reducing the minimum convertible signal to 39 $\mu$V, which corresponds to twice input-referred noise of the ADC, and hence is measurable. Increasing the ADC resolution (e.g. to 16-bits) would drastically reduce the minimum measurable signal, and consequently the resolution of both sensor architectures.

6.5.2 Stimulus Improvements

The stimulus pattern used for both AED and RED Sensor measurements is applied to electrodes on the rows immediately next to the addressed sensor, and upon the same column(s), as shown in Figure 5.5. These patterns maximise the value of $C_x$ within each sensor’s transfer function (see Sections 4.4 and 4.5). Considering $C_x$ appears upon both the numerator and denominator of each sensor’s transfer function, it has the effect of masking the signal contribution due to the suspended particle (represented within $Z$ in Equations (3.9) and (3.16)), resulting in “wasted” signal. By using alternative stimulus patterns that minimise the contribution from $C_x$, the “useful” signal measured by each sensor can be maximised, and potentially greater sensitivity achieved.
M-sequence stimulus signals have been used throughout. M-sequences present a convenient means of deterministically measuring a broadband of frequencies within a minimal time period. Their pseudo-random yet deterministic nature, combined with low cross-correlation and high auto-correlation [244], enables signals beneath a system’s noise-floor to be measured. Combining m-sequence stimulus with changes in applied stimulus patterns (that minimise $C_x$ as described above) has the potential to further increase sensitivity of the system beyond that presented here. Further noise-reducing improvements can be made by including an anti-aliasing filter on the ADC input, to avoid harmonics of the m-sequence stimulus from folding back into the measured frequency band.

### 6.5.3 Experimental Setup and Results Interpretation

The inability of microsystem to actuate micro-particles suspended in PBS via DEP presents a problem in characterising the limit of detection for the AED and RED Sensor architectures. Positional changes with respect to the sensor electrode(s) will contribute measurement variation between sensor locations across each array, on top of the natural variation of interest expected between individual cells. Hence, direct comparisons between sensor locations is difficult to achieve. The statistical approach taken in Sections 5.7 and 5.8 is one possible approach to overcome the effect of micro-particle to sensor misalignment. However, the RED Sensor is particularly sensitive to such mis-alignment, as its differential nature can lead to some sensor locations yielding a positive signal, and others a negative signal, which cancel when averaged together. The relative sizes of these contributions will add another source of variation to the RED Sensor measurement. Ensemble averaging is one method of reducing this unwanted measurement variation. Performing multiple measurements from each sensor site can reduce the noise-floor by $1/\sqrt{n}$ where $n$ is the number of samples (E.g. $n = 4$ reduces measurement noise by factor of 2).

Integrating microfluidics that generate laminar flow above the microsystem is one possible solution to align micro-particles with the columns (or rows) of each sensor array. Although such an alignment will have mechanical tolerances, a highly-parralel 2D sensor array provides many opportunities for a micro-particle to be analysed. A Linear Time-Invariant (LTI) approximation (as required for m-sequence analysis) is possible if the data capture period is small with respect to the movement of a micro-particle above each sensor. Assuming data capture within $1/10^{th}$ of the 20$\mu$m electrode pitch satisfies LTI, the microsystem would be compatible with a 0.1m/s sample flow rate, comparable to that used in flow cytometry today [31]. A further benefit of microfluidic sample flow is the potential of signal integration to occur across multiple sensor sites inline with the sample flow, enabling noise-floor reduction through ensemble averaging, and potentially increased sensitivity. However, many engineering issues will need overcome to integrate microfluidics within the microsystem.
A final consideration in determining the absolute sensitivity of the AED and RED Sensor arrays is the use of individually-fixed cell samples. The samples used in this work have been fixed in formaldehyde. Such fixation acts to displace the cell cytoplasm with formaldehyde, to avoid sample degradation via oxidation. This process effects permanent physiological changes to the cell, including a $\sim 20\times$ reduction in cytoplasm permittivity. Such a reduction in permittivity acts to minimise the effective permittivity of the suspended cell (see Equation (3.7)) and the value of $C_p$ within each sensor’s transfer function. Hence individually-fixed cells are physiologically different and less electrically defined than live cells in suspension. The microsystem may therefore have greater sensitivity for live cells than the individually-fixed cell measurements presented in this work.

6.6 Summary

This chapter has presented a critical discussion of the capabilities or otherwise of the microsystem to actuate and sense individual biological cells and polystyrene micro-beads in the biologically-relevant medium of PBS.

6.6.1 Actuation Summary

Micro-particle actuation was discussed in Section 6.2, and detailed that the ASIC as is the first reported device capable of actuating micro-particles using all four DEP-related phenomena of attraction (pDEP), repulsion (nDEP), translation (TW-DEP) and rotation (EROT). The microsystem further benefits from the absence of a conductive top-plate to bias the suspending medium, which requires highly accurate alignment, complicating manufacturing and hence cost of the overall microsystem. Polystyrene micro-beads with 10 $\mu$m diameter and *Saccharomyces Cerevisiae* yeast cells were used to demonstrate micro-particle actuation when suspended in DiH$_2$O. However, no actuation was achieved when these micro-particles were suspended in PBS. The critical discussion concluded that the abundance of free-charge carriers present in PBS, which are necessary for an isotonic cellular environment, act to screen the applied electric-field to such an extent as to make individual micro-particle actuation with DEP and/or EROT impossible. The consequence of this finding was that the following sensing experiments were conducted without the benefit of micro-particle alignment to sensor electrodes.

6.6.2 AED Sensor Summary

Regarding micro-particle sensing, the microsystem integrates two architectures of electric displacement sensor: the AED Sensor, designed to be sensitive to the entire electrical environment above one electrode (comprising a micro-particle and its suspending medium), and the
Discussion

RED Sensor, designed to be sensitive to the difference in electrical environment between a pair of electrodes (and hence sensitive only to the micro-particle). AED Sensor results were discussed in Section 6.3.3, and concluded that the presented sensor architecture is capable of identifying individually-fixed RBCs and HeLa cells from each other, and comparably sized polystyrene micro-beads, when suspended in PBS. Their magnitude responses appear most defined at higher frequencies, however such distinction was limited by the measured sensor bandwidth (~6 MHz), which was significantly lower than designed (50MHz). This was attributed to additional loading within the ASIC. Absolute micro-particle sizing was not apparent with AED Sensor and potential improvements to achieve so were outlined, such as a geometric constraint to focus electric-field lines around micro-particles at low frequencies.

6.6.3 RED Sensor Summary

Section 6.3.3 discussed results obtained with the RED Sensor, which was found capable of identifying 11.00 μm, 15.66 μm and 18.50 μm diameter polystyrene micro-beads from each other, when suspended in PBS. Furthermore, the sensor was found capable of identifying individually-fixed RBCs and HeLa cells from each other, and the comparably sized micro-beads. Again, the greatest difference between magnitude responses was observed at higher frequencies. The RED Sensor results were matched well by the models developed in Section 3.4, and demonstrate the RED Sensor is sensitive to micro-particle complex permittivity, and not just their size. The change in frequency response and magnitude increases observed between the RED and AED Sensors, indicates the RED Sensor is providing more than a differential AED Sensor response, and thus a different and complementary measurement to that of the AED Sensor.

6.6.4 General Observations Summary

General observations regarding improvements to the microsystem architecture, applied stimulus and experimental setup and interpretation were discussed in Section 6.5. These concluded that a means of aligning cells to sensor electrodes, such as microfluidics, would drastically decrease the observed variation between measurements. A higher resolution ADC would enable exploration of the absolute limit of detection for each sensor architecture; different stimulus patterns could be used to maximise the contribution of the cell to sensed signals; ensemble averaging could be used to reduce the observed noise-floor, and that live cells are likely to provide a greater signal distinction than the individually-fixed cells used in this work.
Chapter 7
Conclusions

7.1 Overview

This work has aimed to demonstrate that biologically meaningful information can be obtained directly from individual cells, without employing cell-labelling (eliminating subjective a-priori decision making), by measuring their passive electrical properties. Specifically, that identifiable “cell signatures” can be obtained through broadband frequency measurements between 100 kHz and 100 MHz that exploit the properties of differential electric fields to probe single cells in physiological solution. This hypothesis was tested through the design, implementation and experimental testing of a dedicated microsystem that integrates two novel designs of electrical sensor within a standard Complementary Metal-Oxide Semiconductor (CMOS) microelectronics technology. The following sections summarise the conclusions drawn with respect to the aims and objectives of this work, outlined in Sections 1.6 and 1.7, respectively.

The passive electrical properties of individual biological cells in liquid suspension can be represented by complex permittivity, whose real (imaginary) component describes the energy storage (dissipative) behaviour of the sample, without prior assumptions as to their origin. These components give rise to an effective cellular capacitance and resistance, which are most completely assessed through measuring their response to a broadband of frequencies, termed Impedance Spectroscopy (IS, see Section 2.2). IS measurements can however be affected by electrochemical reactions arising at the interface between the electrolytic suspension media and conductive measurement electrodes; a dielectric boundary placed in series between the electrodes and sample prohibits free charge carrier exchange and hence electrochemical reactions. This approach is termed Dielectric Spectroscopy (DS).

The complex permittivity of biological cells varies with frequency due to dipoles of various sizes being induced within the sample when exposed to an external electric field. These dipoles arise via dielectric polarisation, of which cells exhibit three dominant types: atomic (displacement of positive from negative charged ends of a molecule), orientational (alignment of induced dipoles with the applied field) and interfacial (due to boundaries between regions of different dielectric properties). These polarisations are attributed to frequency-dependent dispersions and respectively termed the $\gamma$-dispersion (occurring between 1-10 GHz), $\beta$-dispersion (100 kHz to 100 MHz) and $\alpha$-dispersion (less than 100 kHz). The $\beta$-dispersion provides the greatest information between different cell types and differences between cells of the same type.
This work has presented two novel DS sensor architectures for the broadband electrical analysis of individual biological cells in solution across the $\beta$-dispersion band, integrated into highly-parallel arrays within an Application Specific Integrated Circuit (ASIC) implemented within a standard 0.35 $\mu$m CMOS technology (see Section 4.3). CMOS microelectronics provides the capability to manufacture circuits at the same scale as individual biological cells, and provides for integration of signal processing circuitry in immediate proximity to each sensor. These traits respectively enable smaller magnitude and broader frequency signals to be measured than possible with passive microfabricated devices.

The ASIC was supported by a dedicated Printed Circuit Board (PCB) and encapsulated to enable experimental testing of liquid De-Ionised Water (DiH$_2$O, whose low conductivity provides for greatest sensor signal, but a non-physiological cellular environment), Phosphate-Buffered Saline (PBS, highly conductive physiological solution) and various polystyrene micro-beads on the same scale as individually fixed Red Blood Cells (fRBC) and cervical cancer HeLa cells (fHeLa), all suspended in PBS. The results obtained are described in Chapter 5 and discussed in detail in Chapter 6. The following sections present the conclusions drawn from this work.

7.2 $\beta$-Dispersion Cell Signatures captured in Real-Time

Chapter 2 reviewed the literature and concluded that the $\beta$-dispersion (spanning approximately 100 kHz to 100 MHz) contains the most relevant information for identifying and classifying cells of different type and differences between cells of the same type. Lower frequency $\alpha$-dispersion measurements are most sensitive to cell size (see Section 2.5), however cell size is not alone sufficient to identify different cell types, while higher frequency $\gamma$-dispersion (1-10 GHz) data are dominated by the atomic polarisation of water molecules (see Section 2.7).

A gap was identified in the literature for broadband DS analysis of individual biological cells performed across the $\beta$-dispersion from 100 kHz to 100 MHz in one measurement. A substantial body of evidence exists for DS measurements of biological cell suspensions at one to four frequencies [2, 92, 180, 132], extending up to a maximum of sixteen [174, 75, 96, 104], but are all passive microfabricated devices that use a separate impedance analyser system, which may require multiple iterations to capture the desired frequency data (discussed in Section 2.4.5). They are similarly limited in their minimum detectable signal due to parasitic capacitance upon the sensor electrodes. Previously reported CMOS-integrated systems, that alleviate such parasitic effects, are also limited in their frequency resolution across the $\beta$-dispersion band, with 8 frequencies the largest number reported [131] and bandwidths limited to beneath 1MHz [187].

Alternatively, time-domain methods that exploit Fourier transformation can be exploited, but have been limited in their bandwidth beneath 500 kHz [143, 145, 146].
Conclusions

This work has demonstrated the use of m-sequence stimulus for time-domain DS measurements across the lower reaches of the β-dispersion band, capturing 1,024 individual frequency steps between 24.4 kHz to 25 MHz, inclusive, within a 40.96 μs measurement period. This is simultaneously the greatest number of individual frequency points recorded, and highest bandwidth measurements reported, for single-cell analysis using m-sequence stimulus.

However, the maximum recorded frequency in this work (25 MHz) is one quarter of that targeted (100 MHz). This arises from the tight timing constraints required by the m-sequence analysis method, which were only achieved for a global firmware clock frequency of 50 MHz (see Section 4.5). Furthermore, the bandwidth of the presented electrical sensor architectures were limited to beneath 10 MHz (one tenth of that targeted by the design), arising from a poor design of signal output path integrated within the ASIC. Optimising this output path and migrating to more modern data converter and FPGA hardware can readily achieve higher frequency analysis using m-sequences, as indicated is necessary for greater distinction between cell types (see below). Indeed, m-sequence stimulus methods provide the greatest scope to achieve highly granular “cell signatures” within the minimum possible measurement time period, maximising sample throughput and hence the information extracted from a sample.

7.3 Single-Cell Identification in Physiological Suspension

The two electrical sensor architectures presented in this work are respectively named the Absolute Electric Displacement (AED) sensor, designed to measure absolute electrical environment above a single sense electrode, and Relative Electric Displacement (RED), designed to measure the difference in electrical environment between a pair of electrodes, with view to provide information regarding the suspended cell only, through rejecting the common signal due to its suspending medium. This section concludes the results obtained from both sensor architectures.

Sections 5.7.1 and 5.8.1 respectively presented the effect of various media upon the AED and RED sensors. Phosphate-buffered saline (PBS) is shown to attenuate the magnitude response of both when compared to de-ionised water (DiH₂O), attributed to the abundance of free charge carriers present in PBS and absent in DiH₂O. The attenuation effect was seen to reduce the overall sensitivity of the AED sensor, whereas the RED sensor magnitude response appears to increase above approximately 10 MHz (despite the bandwidth of the sensor acting to further attenuate the magnitude response at these frequencies). Hence, the assumption made that the RED sensor acts to reject electrode polarisation appears correct, and increasingly so for higher frequencies. This suggests that higher frequencies above 10 MHz are preferable for measuring cells suspended in physiological saline.
Sections 5.7 and 5.8 respectively presented the results obtained from 11.00 μm, 15.66 μm and 18.50 μm diameter polystyrene micro-beads, and individually-fixed Red Blood Cells (fRBCs) and cervical cancer (fHeLa) cells, all suspended in PBS. The AED Sensor was found capable of identifying micro-beads from cells, and fRBCs from fHeLa above 10MHz. The RED Sensor was found capable of identifying each diameter of micro-bead from cells, and distinguish between fRBCs and fHeLa above 3MHz. The RED Sensor responses were typically 10dB greater in magnitude than the AED Sensor. The difference in the obtained “cell signatures” is maximised when their magnitude response with respect to frequency is plotted relative to those of the 11.00 μm beads (termed relative electrical opacity, shown in Figures 5.12 and 5.20 for the AED and RED sensors, respectively). The greatest difference in magnitude response between cell types (and micro-beads) again are apparent for higher frequencies.

The AED Sensor magnitude responses do not match the model developed in Section 3.3; this discrepancy is attributed to the absence within the model of an electric double layer capacitance to model electrode polarisation (see Section 2.2.4), separate to that of the isolating dielectric layer capacitance. Consequently, future models should account for this additional source of signal attenuation.

The RED Sensor magnitude responses are well matched by the model developed in Section 3.4 demonstrating that the underlying assumption that electrode polarisation can be cancelled through a fully-differential measurement holds. They further demonstrate that the RED Sensor is sensitive to the complex permittivity of each micro-particle (and cell), and not just their size.

In addition, the difference in magnitude and frequency responses recorded between the sensor architectures indicates that the RED Sensor is providing more than a purely differential AED Sensor response. Therefore, the AED and RED sensors are providing different and potentially complementary information regarding the complex permittivity of the sample, which may be of biological relevance.

7.4 Physiological Liquids Prohibit Cell Actuation with DEP

Sections 5.3 through 5.5 described the highly-parallel actuation polystyrene micro-beads and Saccharomyces Cerevisiae yeast cells upon the microsystem ASIC using all four Dielectrophoresis (DEP) related phenomena of attraction (pDEP), repulsion (nDEP), translation (TW-DEP) and rotation (EROT), when suspended in DiH₂O. However, no actuation was achieved when these micro-particles were suspended in physiological PBS. This was again attributed to the abundance of free-charge carriers present in PBS (absent in DiH₂O), which while necessary for an isotonic cellular environment, act to screen the applied charge carriers necessary to generate sufficient E-field strength through the sample as to permit actuation via DEP or EROT.
Section 2.6.3 reviewed the literature and discovered that all previously reported DEP and EROT actuation experiments require micro-particles to be resuspended in low-conductivity (low free charge carrier concentration) liquids, with sugar-based additives necessary to achieve an isotonic cellular environment. While such re-suspension is straightforward and requires minimal sample handling, the question then arises as to whether cells suspended in sugar solution bear any resemblance to cells suspended in saline solution, as is the case in-vivo.

Therefore, while DEP and EROT have been used extensively for cellular actuation, they are not relevant to physiological suspensions. Whether this poses a fundamental limitation on their wider deployment, or merely limits their use to re-suspended samples, will depend upon the individual experiment to be conducted.

### 7.5 Micro-Particle Misalignment Restricts Achievable Sensitivity

The measurements reported in Sections 5.7 and 5.8 were limited in determining the absolute sensitivity of the AED and RED sensor architectures due to micro-particle misalignment with sensor (and stimulus) electrodes. The absence of micro-particle actuation via DEP in PBS meant that micro-particles were randomly distributed across each sensor array, leading to a substantial source of measurement variation not due to the underlying sensors. Choosing specific regions of interest and averaging multiple measurements were used to minimise this effect.

However, the misalignment of micro-particles to sensor electrodes will need overcome to ascertain the absolute sensitivity (and consequently, specificity) possible with DS for biological cells suspended in physiological solution. Indeed, the assumption that the electric field above the sensor array is independent of its \( z \)-axis position (see Section 3.2.1) was wrong. Therefore, to maximise the effectiveness of DS measurements, the sample must be constrained in three-dimensions: aligned to electrodes within the sensor plane, and limited in its height above the sensor array.

### 7.6 Sensor Electrode Area Trade-Off with Cell Size

Chapter 3 derived an analytic model from first principles for a single, spherical micro-particle with complex permittivity suspended in liquid solution above an array of micro-electrodes. A minimum possible 2-electrode sensor geometry was defined as a Basic Sensing Element (BSE) and used to construct the AED and RED sensor architectures. These models enabled exploration of the effect of electrode width and spacing with respect to the real permittivity of solution, and micro-particle radius, upon the magnitude response of each sensor architecture. Electrode widths comparable to micro-particle diameter were found to maximise the magni-
Conclusions

tude response of both sensor architectures, with little influence due to micro-particle presence observed at frequencies beneath 1MHz. This frequency insensitivity arises from the isolating dielectric layer between the sensor (and stimulus) electrode(s), which forms a capacitance in series with the sample ($C_y$), and hence an effective high-pass filter. Reducing the electrode area reduces this series capacitance and hence increases the cut-off frequency of the filter.

A design optimisation is therefore necessary to set the cut-off frequency arising from the series dielectric layer below the minimum frequency of interest (~100 kHz for $\beta$-dispersion measurements). Reducing the isolating dielectric layer thickness, or increasing its permittivity, can increase $C_y$, however these parameters are in practice limited by manufacturing reliability and compatibility with standard CMOS-processes. Post-processing CMOS has been applied for this purpose \[60\], but adds complexity and hence cost. Instead, the electrode width is the most accessible design parameter, limited by the microfabrication capabilities of the chosen process.

As mentioned in Section \[7.3\], the AED sensor model did not fit the measured data, whereas the RED sensor model did. This was attributed to the AED sensor model failing to account for electrode polarisation in the form of an electric double layer capacitance, which should be included in future models. Furthermore, an appropriate geometric cell constant (see Section \[2.4.3\]) should be applied that accounts for the variation in sensor measurements to the $z$-axis position of the sample with respect to the sense (and stimulus) electrodes.

7.7 Future Work

Live Cells

The experiments conducted in this work were all performed on biological cells individually fixed in formaldehyde to permit use outwith a biological laboratory. While providing a convenient biological proxy for live cells, formaldehyde fixation greatly affects their physiology and dielectric properties: the membrane becomes rigid and water (real permittivity $\varepsilon \approx 60$) is displaced for formaldehyde ($\varepsilon \approx 3$), effectively encapsulating each cell within a bubble of low permittivity, leading to interfacial polarisation and signal attenuation at the cell to solution interface. Live cells will further be actively interacting with their environment introducing a time-dependent response, which may contain additional, biologically relevant information.

Phase Measurements

This work has concentrated exclusively upon the frequency-dependent magnitude response of individual cells in suspension. Phase measurements can readily be calculated from the complex transfer function recorded from each cell. The linear nature of phase measurements may make them more sensitive to subtle changes in permittivity compared to the magnitude response.
Conclusions

Frequency Measurements up to 1GHz
Whilst the $\gamma$-dispersion does not appear to provide any increase in distinction between cell types, measurements between 10 MHz and 1 GHz may provide further information due to the apparent decrease in the attenuation effect observed with PBS for frequencies above approximately 10 MHz. Indeed, the greatest distinction between “cell signatures” was achieved for frequencies greater than 10 MHz, indicating that higher frequency measurements may provide simultaneously greater distinction between cell types, and greater measured signal (and hence increasing SNR for increasing frequency).

Electrodes Smaller than Individual Cells
The sensor architectures and measurement techniques presented in this work are directly compatible with standard CMOS technology scaling, leading to smaller sensor circuits, and comparatively higher operating frequencies. By integrating electrodes smaller than individual cells, it may be possible to sense the contribution in sensor signals due to sub-cellular components (organelles). Indeed, direct sensing of smaller molecules such as bacteria ($\approx 1 \mu m$ in scale) and potentially even virus ($\approx 100nm$ in scale) may be possible through employing CMOS scaling, as the work by Rothberg et al. \cite{60} and Widdershoven et al. \cite{156} point towards. Such capabilities would open a wealth of applications across biological research and clinical diagnostics.

Improved Analytical Models
The results obtained from the RED Sensor model well matched the experimental data, however the AED Sensor model did not. This appears due to the model excluding the presence of an Electric Double Layer (EDL) between the isolating dielectric and sample solution. While the differential nature of the RED Sensor cancelled this common component, the AED Sensor does not. Hence, future analytical models should account for the EDL. Further, the models developed in this work do not account for micro-particle position with respect to the sensor electrode, which is a dominant effect in experimental measurements. A sensor volume constant \cite{3, 180, 140, 5} that modifies the recorded sensor signal due to particle alignment should be defined and employed.

Absolute Specificity and Sensitivity
Further work is necessary to explore the limit of detection, sensitivity and specificity of each cell type, but the microsystem presented in this work provides a basis upon which to design optimised devices for the purpose of rapid, label-free screening of individual biological cells in physiological suspension in a compact device, implemented in standard, highly scalable CMOS microtechnology.
Conclusions

Improved ASIC Signal Path

The output signal path integrated within the ASIC appears to limit the bandwidth of the entire system. Improvements can be made within the pixel design (see Sections 4.3.5 and 4.3.6), column buses, pre-pad buffer and output signal path on the printed circuit board (PCB, see Section 4.4). Regarding the pixels, migrating the design from 5 V transistors to 3.3 V, where a greater signal transconductance is achieved per unit area would reduce the shunt capacitance seen by each sensor electrode. This can be further decreased by minimising the number of devices attached to the sensor electrode node. The pixel output can similarly be improved through the use of 3.3 V transistors to increase the bandwidth and minimise the load seen by each column bus. Signal buffering at the column level could further increase bandwidth, as would an optimised pre bond-pad buffer. Indeed, the latter could form an amplifier to provide low-noise gain on-chip, before communication to the PCB. The PCB signal processing could be improved through low-noise amplification and a higher resolution, higher sample frequency analogue-to-digital converter (ADC) than used here; provided an improved firmware design can achieve the timing constraints necessary for higher frequency m-sequence stimulus.

Indeed, the above improvements can be further capitalised upon through migration to more modern CMOS processes than the 0.35 \( \mu \)m technology used in this work.

7.8 Summary

This work has demonstrated that biologically meaningful information in the form of identifying different “cell signatures” for individually-fixed RBCs and HeLa cells, and three diameters of polystyrene micro-bead, can be obtained without employing cell-labelling. Measurements were performed in physiological saline solution across 1,024 frequencies between 24.4 kHz and 25 MHz inclusive, within a measurement period of 40.96 \( \mu \)s. Single-cell resolution was demonstrated with two novel electrical sensor designs implemented within a dedicated microsystem employing standard microelectronics technology.

This work has demonstrated the wider potential of dielectric spectroscopy for label-free, single-cell analysis of biological cells in physiological solution, eliminating subjective \textit{a-priori} decision making, as required by all existing single-cell analysis methods. Furthermore, the use of standard mass-manufacturable microelectronics technology opens the possibility for dielectric spectroscopy to be adopted as an effective, single-cell analysis method, in affordable devices for widespread use in future general medical practice.
Appendix A

Electric Field Vectors

The action of bound and free charge carriers can be extended into space via the field vectors:

1. D the electric displacement,
2. E the electric field intensity,
3. P the electric polarisation.

This appendix describes how these field vectors are derived.

A capacitor with vacuum as its dielectric will store a charge \( Q \) [C] between its plates, when connected to voltage source \( V \) [V]:

\[
Q = C_0 V
\]  

(A.1)

where \( C_0 \) [F] represents the vacuum or geometrical capacitance. When filled with a dielectric, its capacitance will increase proportional to the permittivity of the dielectric:

\[
C = C_0 \frac{\varepsilon' \varepsilon_0}{\varepsilon_0} = C_0 \kappa'
\]  

(A.2)

where \( \varepsilon' \) represents the real permittivity described previously in Section 2.2 and \( \varepsilon_0 \) is the permittivity of vacuum. Their ratio \( \kappa' \) is defined as the real relative permittivity of the dielectric and is related to complex permittivity by the complex relative permittivity \( \kappa^* \):

\[
\kappa^* = \frac{\varepsilon'}{\varepsilon_0} = \kappa' - j\kappa''.
\]  

(A.3)

Filling a capacitor with a dielectric will act to increase its capacitance (and hence its stored charge capacity) through neutralising charges at its electrode surfaces, which would otherwise contribute to the E-field across the capacitor \([11]\). This phenomenon is called dielectric polarisation and can be visualised as per Figure 2.3, where the action of dipole chains formed under the influence of an external E-field bind counter-charges with their free ends on each conductor surface.
Considering expressions (A.1) and (A.2) in terms of the applied voltage across our capacitor, we find that:

\[ V = \frac{Q}{\kappa' C_0}, \]  

(A.4)

which can be interpreted as only a fraction of the total charge \( Q \) is available to contribute to the voltage \( V \) across the capacitor. This fraction is defined as free charge \( Q(1/\kappa') \), whereas the remainder, or bound charge \( Q(1 - (1/\kappa')) \), is neutralised by the polarisation of the dielectric.

The distribution of these charges in space is described through the use of field vectors. The measurable, or total charge, \( Q \) concentrated upon the capacitor is distributed across the surface of each electrode with a density \( s \) [C/m\(^2\)]:

\[ Q = \int_A sdA. \]  

(A.5)

The measurable charge density \( s \) is represented by the vector \( D \), the electric flux density or electric displacement, whose normal component is equal to the surface charge density:

\[ sdA \equiv (D \cdot n)dA = D_n dA, \]  

(A.6)

where \( n \) is a unit vector aligned normal to the capacitor electrode surface. Similarly, the free charge density \( s(1/\kappa') \) is attributed vector \( E \), the electric field strength or field intensity:

\[ s \left( \frac{1}{\kappa'} \right) dA \equiv \varepsilon_0 (E \cdot n)dA = \varepsilon_0 E_n dA \]  

(A.7)

and the bound charge density \( s(1 - (1/\kappa')) \) is attributed vector \( P \), called the polarisation, as

\[ s \left( 1 - \frac{1}{\kappa'} \right) dA \equiv (P \cdot n)dA = P_n dA. \]  

(A.8)

The relationship between the total, free and bound charge carriers and their representation by the electric displacement \( D \), electric field strength \( E \) and polarisation \( P \), respectively, is found by combining expressions (A.6), (A.7) and (A.8) and illustrated in Figure A.1:

\[ D = \varepsilon' E, \]  

(A.9)

\[ P = D - \varepsilon_0 E = (\varepsilon' - \varepsilon_0) E. \]  

(A.10)

Both electric displacement \( D \) and polarisation \( P \) have dimensions of charge per unit area by their defining equations, but the dimensions of \( E \) have yet to be defined, as they depend upon
the dimensions of the permittivity, whose dimensions have yet to be chosen. This provides the possibility of extending the electric field intensity $E$ into space by stating that an electric probe charge $Q$ experiences a force (defined by vector $F$, illustrated in Figure A.1) when inserted into an electrostatic field of intensity $E$ [1]:

$$F = QE.$$  \hspace{1cm} (A.11)

Therefore, the electric field strength $E$ becomes equivalent to the *force per unit charge* acting upon a detector charge, and permittivity obtains the dimensions of:

$$[\varepsilon] = \left[ \frac{\text{charge per unit area}}{\text{force per unit charge}} \right]$$ \hspace{1cm} (A.12)

which reduces to [F/m]. By defining the permittivity in this way, it is possible to show that polarisation $P$ is equivalent to the *electric dipole moment per unit volume* of the capacitor dielectric. Further, the above dimensions for $E$ are equivalent to *potential gradient* [V/m], which are the dimensions most widely associated with electric field strength.
Appendix B
Component Derivations

This appendix derives the expressions for the major Basic Sensing Element equivalent circuit components: shunt capacitance $C_x$ [F] between adjacent electrodes, the coupling capacitance $C_y$ [F] between the electrodes and sample interface, and $C_s$ [F] the capacitance arising from the suspending medium between electrodes, as shown schematically in Figure 3.2(a). The simplifying assumptions and procedure used to derive these expressions are outlined below.

Figure B.1: Coordinate systems used to derive capacitances $C_x$, $C_y$ and $C_s$. Surfaces referring to boundary conditions are shown in bold, with the corresponding coordinate value given for each region (annotated 1–3) as shown.

Simplifying assumptions

1. **3D geometry can be reduced to 2D**: the simplified geometry shown in Figure 3.1(a) is rotationally symmetric, with a repeating unit of two parallel electrodes. Surface $S$ is chosen to be symmetrical through the electrode pair centre, enabling the 3D geometry to be reduced to 2D.

2. **Separation of electric displacement components**: assumes the total electric displacement incident upon each electrode can be reduced to 2D, with independent horizontal ($D_x$) and vertical ($D_y$) components.
3. **Electric fields vary in one dimension only:** i.e. each capacitance can be treated as a parallel-plate capacitor, which holds for large plate areas in close proximity to one another.

**Procedure**

1. **Define problem geometry, coordinate system and boundary conditions:** for the three regions (denoted \( i = 1 - 3 \)) shown in Figure B.1. Identify the isopotentials to calculate the capacitance between, and apply an arbitrary potential difference \( V_0 \) [V] across them as boundary conditions.

2. **Solve for potential between isopotentials:** permittivities \( \varepsilon_x, \varepsilon_y \) and \( \varepsilon_z \) are assumed to be ideal, permitting no direct current flow. Hence, there are no free charge carriers within these regions and the potential difference \( V_i \) [V] across them can be calculated using Laplace’s equation \( \nabla^2 V_i = 0 \).

3. **Calculate electric-field between isopotentials:** given potential difference \( V_i \) solve for \( E_i \) [V/m] using \( E_i = -\nabla V_i \).

4. **Calculate electric-displacement between isopotentials:** given \( E_i \) and assuming the dielectric between the isopotentials is isotropic (homogeneous in all directions) and linear (i.e. its relative permittivity \( \varepsilon_i \) [1] is independent of the arbitrary applied potential difference \( V_0 \)), solve for \( D_i \) [V/m] using \( D_i = \varepsilon_i E_i \).

5. **Evaluate electric-displacement at one isopotential:** the total electric displacement (\( D \)) incident upon the isopotential is equivalent to that upon its surface (\( D_S \)). Recalling the simplifying assumptions above, the surface electric displacement is equivalent to its normal component only, i.e. \( D = D_S = D_n n \), where \( n \) [1] is a unit vector normal to the isopotential surface.

6. **Evaluate charge density at either isopotential:** by recognising that charge density \( \rho_S \) [C/m\(^2\)] is equivalent to the normal component of the electric displacement, \( D_n \).

7. **Find charge upon each isopotential:** by performing a surface integration over the isopotential surface, \( Q \) [C] = \( \int_S \rho_S dS \).

8. **Solve for capacitance between isopotentials:** using \( C_i \) [F] = \( |Q| / V_0 \), noting that the arbitrary choice of applied potential difference \( V_0 \) used to calculate \( Q \) cancels.

The capacitances derivations for each region shown in Figure B.1 are given below.
Component Derivations

Region 1: $C_x$ represents the shunt capacitance between electrode side walls (thickness $t$ [m]) through the passivation dielectric, with real permittivity $\varepsilon_x$ [F/m]. The geometry is rectangular, so cartesian coordinates are chosen:

$$\nabla V = \frac{\partial V}{\partial x} n_x + \frac{\partial V}{\partial y} n_y + \frac{\partial V}{\partial z} n_z = 0.$$  

Step 1: Applying the simplifying assumption of separation of electric displacement components, we assume the arbitrary voltage $V_0$ applied between electrodes varies with $x$ only, i.e. $V_0(x)$ (noting the full derivatives):

$$\nabla^2 V \rightarrow \frac{d^2 V}{dx^2} = 0.$$  \hspace{1cm} (B.1)

Step 2: Multiplying expression (B.1) through by $dx$ and integrating twice with respect to $x$ yields an expression for the potential across Region 1:

$$\frac{dV}{dx} = \int 0 dx = A$$  \hspace{1cm} (B.2)

$$V = \int A dx = Ax + B.$$  \hspace{1cm} (B.3)

Applying boundary conditions $V = 0$ at $x = 0$ and $V = V_0$ at $x = g$, expression (B.3) finds $B = 0$ and $A = V_0/g$. Substituting these back into expression (B.3) yields:

$$V = \frac{V_0}{g} x.$$  

Step 3: Electric field $E$ [V/m] is found from the negative gradient of potential $V$ in expression (B.1) with respect to the variable axis $x$, where $n_x$ is the unit vector in the $x$-direction:

$$E = -\nabla V = -\frac{d}{dx} V n_x = -\frac{V_0}{g} n_x.$$  

Step 4: Calculate electric displacement $D$ [V/m] between isopotentials, recalling the assumption that $\varepsilon_x$ is an isotropic and linear dielectric:

$$D = \varepsilon E = -\varepsilon_x \frac{V_0}{g} n_x.$$  

Steps 5 and 6: evaluate $D$ at either isopotential to calculate surface charge density $\rho_S$ [C/m$^2$]
at the electrode surface:

\[ \rho_S = D \big|_{x=0} = -\varepsilon_x \frac{V_0}{g}. \]

Step 7: total charge \( Q \) [C] upon the electrode side wall is the surface integral of \( \rho_S \), where \( S \) \([m^2]\) is the electrode side wall area:

\[ Q = \int_S \rho_S dS = \int_S -\varepsilon_x \frac{V_0}{g} dS = -\varepsilon_x \frac{V_0}{g} S. \]

Step 8: finally, the capacitance \( C_x \) [F] between electrode side walls is then:

\[ C_x = \frac{|Q|}{V_0} = \varepsilon_x \frac{V_0 S}{g} = \varepsilon_x \frac{S}{g}, \]

yielding the well known expression for a parallel-plate capacitor. Substituting electrode area \( S \) for its geometric parameters results in our final expression for \( C_x \):

\[ C_x = \varepsilon_x \frac{wt}{g}. \] (B.4)

Region 2: \( C_y \) represents the coupling capacitance \( C_y \) [F] between the electrodes and the suspending medium. The procedure is identical to that for \( C_x \) with the boundary condition that \( V_0 \) is applied between the electrode and an isopotential projected on to the isolating dielectric layer immediately above itself.

Region 3: \( C_s \) represents the capacitance arising from the suspending medium between electrode isopotentials projected on to the isolating dielectric layer, with real permittivity \( \varepsilon_s \) [F/m].

Step 1: While \( C_s \) appears as a fringing capacitance in rectangular coordinates, by applying the simplifying assumption that the electric field varies in one dimension only, we can consider \( C_s \) as a parallel-plate capacitor in cylindrical coordinates:

\[ \nabla V = \frac{\partial V}{\partial \rho} n_\rho + \frac{1}{\rho} \frac{\partial V}{\partial \varphi} n_\varphi + \frac{\partial V}{\partial z} n_z. \] (B.5)

Applying the simplifying assumption of separation of electric displacement components, we assume arbitrary potential \( V_0 \) applied between electrodes varies with \( \varphi \) only, i.e. \( V(\varphi) \):

\[ \nabla^2 V \to \frac{d^2 V}{d\varphi^2} = 0. \] (B.6)
Step 2: Multiplying expression (B.6) through by \(d\varphi\) and integrating twice with respect to \(\varphi\) yields an expression for the potential across Region 3:

\[
\frac{dV}{d\varphi} = \int 0 \, d\varphi = A
\]  

(B.7)

\[
V = \int A \, d\varphi = A\varphi + B.
\]  

(B.8)

Applying boundary conditions \(V = V_0/2\) at \(\varphi = 0\) and \(V = -V_0/2\) at \(\varphi = \pi\), expression (B.8) finds \(B = V_0/2\) and \(A = V_0/\pi\). Substituting these back into expression (B.8) yields:

\[
V = V_0 \left( \frac{\varphi}{\pi} + \frac{1}{2} \right).
\]

Step 3: Electric field \(\mathbf{E} \, [\text{V/m}]\) is found from the negative gradient of potential \(V\) in expression (B.5) with respect to the variable axis \(\varphi\), where \(\mathbf{n}_\varphi\) is the unit vector in the \(\varphi\)-direction:

\[
\mathbf{E} = -\nabla V = - \frac{1}{\rho} \frac{d}{d\varphi} V \mathbf{n}_\varphi = \frac{-V_0}{\rho \pi} \mathbf{n}_\varphi.
\]

Step 4: Calculate electric displacement \(\mathbf{D} \, [\text{V/m}]\) between isopotentials, recalling the assumption that \(\varepsilon_x\) is an isotropic and linear dielectric:

\[
\mathbf{D} = \varepsilon \mathbf{E} = -\varepsilon_s \frac{1}{\rho \pi} V_0 \mathbf{n}_\varphi.
\]

Steps 5 and 6: evaluate \(\mathbf{D}\) at either isopotential to calculate surface charge density \(\rho_S \, [\text{C/m}^2]\):

\[
\rho_S = \mathbf{D}|_{\varphi=0} = -\varepsilon_s(V_0/\rho \pi).
\]

Step 7: charge \(Q \, [\text{C}]\) is the surface integral of \(\rho_S\), where \(S \, [\text{m}^2]\) is the isopotential surface area:

\[
Q = \int_S \rho_S dS = \int_S -\varepsilon_s \frac{V_0}{\rho \pi} dS = -\varepsilon_s \frac{V_0}{\rho \pi} S.
\]

Step 8: the capacitance \(C_x \, [\text{F}]\) between isopotentials through the suspending medium is then:

\[
C_s = \frac{|Q|}{V_0} = \varepsilon_s \frac{V_0 S}{\rho \pi} \frac{1}{V_0} = \varepsilon_s \frac{S}{\rho \pi},
\]

and substituting \(S = w^2 \, [\text{m}^2]\) and \(\rho = w \, [\text{m}]\), we find the expression for \(C_x \, [\text{F}]\):

\[
C_s = \varepsilon_s \frac{w}{\pi}.
\]  

(B.9)
Appendix C
AED Sensor Transfer Function

Figure C.1: AED Sensor small-signal equivalent circuits for (a) no particle and (b) with particle.

Figure C.1 shows the equivalent circuits used to derive the transfer function for the AED Sensor, using the component expressions derived in Appendix B. The leftmost electrode applies stimulus voltage $V_i$ across the solution to node voltage $V_s$, the central output electrode node with voltage $V_o$, and the rightmost electrode connected to ac-ground. In both (a) and (b), the electrode potentials see solution capacitance $C_s$ in series with isolating dielectric layer capacitance $C_y$, with equivalent capacitance

$$C_{eq} = \frac{C_s C_y}{C_s + C_y}.$$  \hfill (C.1)

When a particle is present above the sensor, $C_s$ is replaced by the parallel combination of $C_p$ and $R_p$, in series with $C_y$. Its equivalent impedance then becomes

$$Z = \frac{1}{sC_y} + \frac{R_p}{1 + sC_p R_p}.$$  \hfill (C.2)

The above relationships are used in the following derivation, which uses the equivalent circuits shown in Figure C.1.
Applying Kirchhoff’s Current Law (KCL) at $V_o$:

$$(V_i - V_o)sC_x + (0 - V_o)sC_L + (V_s - V_o)sC_y + (0 - V_o)sC_x = 0.$$  \hfill (C.3)

Applying KCL at node voltage $V_s$:

$$(V_i - V_s)\frac{1}{Z} + (V_o - V_s)sC_y + (0 - V_s)sC_{eq} = 0.$$  \hfill (C.4)

Solving for $V_s$ in Equation (C.3):

$$sV_o(C_x + C_L + C_y + C_x) = sV_iC_x + sV_sC_y,$$  \hfill (C.5)

noting that the $s$ terms cancel:

$$\Rightarrow V_s = \frac{V_o}{C_y}(2C_x + C_y + C_L) - \frac{V_iC_x}{C_y}. \hfill (C.6)$$

Substituting $V_s$ into Equation (C.4):

$$V_i \left(\frac{1}{Z}\right) = V_s \left(\frac{1}{Z} + sC_y + sC_{eq}\right) - sV_oC_y$$

$$= \left[\frac{V_o(2C_x + C_y + C_L) - V_iC_x}{C_y}\right]\left(\frac{1}{Z} + sC_y + sC_{eq}\right) - sV_oC_y. \hfill (C.7)$$

Multiplying through by $C_y$ and collecting $V_i$ terms on the LHS and $V_o$ terms on the RHS:

$$V_i \left[C_y \left(\frac{1}{Z}\right) + C_x \left(\frac{1}{Z} + sC_y + sC_{eq}\right)\right] = V_o \left[(2C_x + C_y + C_L) \left(\frac{1}{Z} + sC_y + sC_{eq}\right) - sC_y^2\right].$$

Solving for the transfer function expression $V_o/V_i$ yields:

$$\frac{V_o}{V_i} = \frac{C_y(1/Z) + C_x[(1/Z) + sC_y + sC_{eq}]}{(2C_x + C_y + C_L)[(1/Z) + sC_y + sC_{eq}] - sC_y^2},$$  \hfill (C.9)

as per Equation (5.34). The response of the AED Sensor when no particle is present is found by substituting $Z = 1/(sC_{eq})$ into Equation (C.9):

$$\frac{V_o}{V_i} = \frac{sC_yC_{eq} + sC_x(2C_{eq} + sC_y)}{s(2C_x + C_y + C_L)(2C_{eq} + C_y) - sC_y^2}.$$  \hfill (C.10)
Note that $s$ is a common factor on both the numerator and denominator and hence cancel, as stated by the assumption that the suspending medium alone has no frequency dependence.

**Dimensional Analysis**

Dimensional analysis is essential to confirm the validity of expressions by comparing the units upon the LHS of an expression, with those upon its RHS. Dimensional analysis of Equation (C.10) shows:

\[
\left[ \frac{V_o}{V_i} \right] = \frac{[F^2 + F^2]}{[F^2 - F^2]} = 1,
\]

which shows the RHS dimensions of the AED Sensor’s voltage transfer function when no particle is present is equal to that expected on the LHS ($[V]/[V] = 1$), confirming that Equation (3.10) is valid.

The response of the AED Sensor when a particle is present is found by substituting $Z = (1/sC_y) + R_p/(1 + sR_pC_p)$. First, let us consider the two terms in $Z$ separately. Both include the Laplace Operator $s = j\omega$, where $j = \sqrt{-1}$ and $\omega$ [rad.$s^{-1}$] is angular frequency. Recalling that radians are dimensionless by definition, $[s] = s^{-1}$. Now considering that:

\[
R = \frac{V}{I} \Rightarrow [\Omega] = \left[ \frac{[V]}{[A]} \right] = \left[ \frac{V \cdot s}{C} \right],
\]

\[
C = \frac{Q}{V} \Rightarrow [F] = \left[ \frac{C}{V} \right],
\]

since $[A] \equiv [C \cdot s^{-1}]$, the $R_pC_p$ product is found to have units of seconds, confirming its expected units for a time constant. Therefore:

\[
[Z] = \frac{1}{[s^{-1} \cdot F]} + \frac{[\Omega]}{1 + [s^{-1} \cdot s]} = \left[ \frac{s}{F} \right] + [\Omega].
\]

Substituting the expressions for the units of $R$ (C.12) and $C$ (C.13) into the above, we find that:

\[
[Z] = \left[ \frac{V \cdot s}{C} \right] + \left[ \frac{V \cdot s}{C} \right] = [s \cdot F^{-1}].
\]
Substituting the above units for $Z$ back into Expression (C.9) and recalling $[s] = s^{-1}$, finds that

$$\frac{V_o}{V_i} = \frac{[F][s^{-1} \cdot F] + [F][s^{-1} \cdot F]}{[F][s^{-1} \cdot F] - [s^{-1} \cdot F^2]}$$

$$\equiv \frac{[F^2 \cdot s^{-1}] + [F^2 \cdot s^{-1}]}{[F^2 \cdot s^{-1}] - [F^2 \cdot s^{-1}]}$$

$$\Rightarrow \frac{V_o}{V_i} = 1$$

which shows the RHS dimensions of the AED Sensor’s voltage transfer function when a particle is present is equal to that expected on the LHS ($[V]/[V] = 1$), confirming that Equation (3.9) is valid.
Appendix D

RED Sensor Transfer Function

Figure D.1: RED Sensor small-signal equivalent circuits for (a) no particle and (b) with particle.

Figure D.1 shows the equivalent circuits used to derive the transfer function for the RED Sensor, using the component expressions derived in Appendix B. Differential stimulus input voltage $V_{iD} = V_{iP} - V_{iN}$ is applied across the sample between the left- and right-most stimulus electrodes, where $V_{iP}$ and $V_{iN}$ represent the $0^\circ$ and $180^\circ$ phase components of the input voltage, respectively. Differential output voltage $V_{oD} = V_{oP} - V_{oN}$ is sensed upon the inner left and right electrodes. In both (a) and (b), the electrode potentials see solution capacitance $C_s$ in series with isolating dielectric layer capacitance $C_y$, which has an equivalent capacitance of

$$C_{eq} = \frac{C_s C_y}{C_s + C_y}.$$  \hfill (D.1)

When a particle is present above the sensor, $C_s$ is replaced by the parallel combination of $C_p$ and $R_p$. Its equivalent impedance then becomes

$$Z_p = \frac{R_p}{1 + sC_pR_p}.$$  \hfill (D.2)

The above relationships are used in the following derivation, which uses the equivalent circuits shown in Figure D.1.
Applying Kirchhoff’s Current Law (KCL) at $V_{oP}$:

$$(V_{iP} - V_{oP})sC_x + (0 - V_{oP})sC_L + (V_{sP} - V_{oP})sC_y + (V_{oN} - V_{oP})sC_x = 0, \quad (D.3)$$

and again at $V_{oN}$, yields:

$$(V_{iN} - V_{oN})sC_x + (0 - V_{oN})sC_L + (V_{sN} - V_{oN})sC_y + (V_{oP} - V_{oN})sC_x = 0. \quad (D.4)$$

Applying KCL at node voltage $V_{sP}$:

$$(V_{iP} - V_{sP})sC_{eq} + (V_{oP} - V_{sP})sC_y + (V_{sN} - V_{sP}) \frac{1}{Z_p} = 0, \quad (D.5)$$

and again at $V_{sN}$, yields:

$$(V_{iN} - V_{sN})sC_{eq} + (V_{oN} - V_{sN})sC_y + (V_{sP} - V_{sN}) \frac{1}{Z_p} = 0. \quad (D.6)$$

Solving (D.3) and (D.4) for $(V_{oP} - V_{oN})$:

$$(D.3) \Rightarrow (V_{oP} - V_{oN})sC_x = V_{iP}(sC_x) - V_{oP}(sC_x + sC_L + sC_y) + V_{sP}(sC_y) \quad (D.7)$$

$$(D.4) \Rightarrow (V_{oP} - V_{oN})sC_x = -V_{iN}(sC_x) + V_{oN}(sC_x + sC_L + sC_y) - V_{sN}(sC_y) \quad (D.8)$$

and adding (D.8) to (D.7) finds:

$$2(V_{oP} - V_{oN})sC_x = (V_{iP} - V_{iN})sC_x - (V_{oP} - V_{oN})(sC_x + sC_L + sC_y) + (V_{sP} - V_{sN})sC_y. \quad (D.9)$$

Solving (D.5) and (D.6) in terms of $V_{sP}$ and $V_{sN}$, respectively:

$$(D.5) \Rightarrow V_{sP}(sC_y) = -V_{sP}(sC_{eq} + \frac{1}{Z_p}) + V_{oP}(sC_y) + V_{iP}(sC_{eq}) + V_{sN} \left( \frac{1}{Z_p} \right) \quad (D.10)$$

$$(D.6) \Rightarrow V_{sN}(sC_y) = -V_{sN}(sC_{eq} + \frac{1}{Z_p}) + V_{oN}(sC_y) + V_{iN}(sC_{eq}) + V_{sP} \left( \frac{1}{Z_p} \right) \quad (D.11)$$

and subtracting (D.11) from (D.10) to solve in terms of $(V_{sP} - V_{sN})$,

$$(V_{sP} - V_{sN})sC_y = -(V_{sP} - V_{sN})(sC_{eq} + \frac{2}{Z_p}) + (V_{iP} - V_{iN})sC_{eq} + (V_{oP} - V_{oN})sC_y. \quad (D.12)$$
Collecting all \((V_{sP} - V_{sN})\) terms on the LHS yields:
\[
(V_{sP} - V_{sN})(sC_y + sC_{eq} + \frac{2}{Z_p}) = (V_{iP} - V_{iN})sC_{eq} + (V_{oP} - V_{oN})sC_y. \tag{D.13}
\]

Setting \(A = (sC_y + sC_{eq} + \frac{2}{Z_p})\) and substituting expression (D.13) for \((V_{sP} - V_{sN})\) into (D.9):
\[
2(V_{oP} - V_{oN})sC_x = (V_{iP} - V_{iN})sC_x - (V_{oP} - V_{oN})(sC_x + sC_y + sC_L) \\
+ \left[(V_{iP} - V_{iN})\left(\frac{sC_{eq}}{A}\right) + (V_{oP} - V_{oN})\left(\frac{sC_{eq}}{A}\right)\right]sC_y
\]
and collecting \((V_{oP} - V_{oN})\) on the LHS, and \((V_{iP} - V_{iN})\) on the RHS yields:
\[
(V_{oP} - V_{oN})\left(3sC_x + sC_y + sC_L - \frac{(sC_y)^2}{A}\right) = (V_{iP} - V_{iN})\left(sC_x + \frac{s^2C_{eq}C_y}{A}\right).
\]

Multiplying through by \(A\) and solving for \((V_{oP} - V_{oN})/(V_{iP} - V_{iN})\) finds:
\[
\frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{sC_xA + s^2C_{eq}C_y}{A(3sC_x + sC_y + sC_L) - sC_y^2}. \tag{D.14}
\]

Dividing the numerator and denominator by \(s\) yields the differential transfer function for the RED Sensor as given in Equation (3.16):
\[
\frac{V_{oD}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{AC_x + sC_{eq}C_y}{A(3C_x + C_y + C_L) - C_y^2}. \tag{D.15}
\]

The RED Sensor response when no particle is present is found by substituting \(Z_p = 1/(sC_s)\). Transfer function (3.16) then becomes:
\[
\frac{V_{oD}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{C_x(sC_y + sC_{eq} + 2sC_s) + sC_{eq}C_y}{(sC_y + sC_{eq} + 2sC_s)(3C_x + C_y + C_L) - sC_y^2}. \tag{D.16}
\]

noting the common factor of \(s\) on the numerator and denominator cancels:
\[
\Rightarrow \frac{V_{oD}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{C_x(C_y + C_{eq} + 2C_s) + C_{eq}C_y}{(C_y + C_{eq} + 2C_s)(3C_x + C_y + C_L) - C_y^2} \tag{D.17}
\]

which yields the RED Sensor transfer function for no particle present as per Equation (3.15).
**RED Sensor Transfer Function**

### Dimensional Analysis

Dimensional analysis of Equation (D.17) for the no particle case shows:

\[
\frac{V_{0D}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{[F^2 + F^2]}{[F^2 - F^2]} = 1,
\]

which shows the RHS dimensions of the RED Sensor’s differential voltage transfer function when no particle is present is equal to that expected on the LHS \((V - V)/(V - V) = 1\), confirming that Equation (3.15) is valid.

Dimensional analysis of Equation (D.15) requires expanding intermediate parameter \(A = (sC_y + sC_{eq} + 2/Z_p)\) and recalling expression (D.2) that \(Z_p = R_p/(1 + sR_pC_p)\), where \(s = j\omega\) is the Laplace Operator, \(j = \sqrt{-1}\) and \(\omega \text{ [rad/s]}\) is angular frequency. Recalling that radians are dimensionless by definition, \([s] = s^{-1}\), and the units of \(Z_p\) can be found as:

\[Z_p = \frac{[\Omega]}{[1 + s \cdot s^{-1}]} = [\Omega].\]

The relationship between \([R]\) and \([C]\) (given in (C.12) and (C.13), respectively), shows that \([\Omega] = [s \cdot F^{-1}]\). Expanding \(A\) and substituting back into (D.15) yields:

\[
\frac{V_{0D}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{(sC_y + sC_{eq} + 2/Z_p)C_x + sC_{eq}C_y}{(sC_y + sC_{eq} + 2/Z_p)(3C_x + C_y + C_L) - sC_y^2},
\]

which, when collecting like units, has the following dimensions:

\[
\frac{V_{0D}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{[F \cdot s^{-1}] + [F \cdot s^{-1}] + [\Omega^{-1}][F] + [s^{-1} \cdot F^2]}{[F \cdot s^{-1}] + [F \cdot s^{-1}] + [\Omega^{-1}][F] - [s^{-1} \cdot F^2]}
\]

\[= \frac{[F^2 \cdot s^{-1}] + [F^2 \cdot s^{-1}] + [F \cdot \Omega^{-1}] + [F^2 \cdot s^{-1}]}{[F^2 \cdot s^{-1}] + [F^2 \cdot s^{-1}] + [F \cdot \Omega^{-1}] - [F^2 \cdot s^{-1}]}.
\]

And recalling that \([\Omega^{-1}] = [F \cdot s^{-1}]\), the above reduces to:

\[
\frac{V_{0D}}{V_{iD}} = \frac{(V_{oP} - V_{oN})}{(V_{iP} - V_{iN})} = \frac{[F^2 \cdot s^{-1}] + [F^2 \cdot s^{-1}]}{[F^2 \cdot s^{-1}] - [F^2 \cdot s^{-1}]} = 1.
\]

which shows the RHS dimensions of the RED Sensor’s differential voltage transfer function when a particle is present is equal to that expected on the LHS \((V - V)/(V - V) = 1\), confirming that Equation (3.16) is valid.
References


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