Review

Spatially resolved electrical impedance methods for cell and particle characterization

Electrical impedance is an established technique used for cell and particle characterization. The temporal and spectral resolution of electrical impedance have been used to resolve basic cell characteristics like size and type, as well as to determine cell viability and activity. Such electrical impedance measurements are typically performed across the entire sample volume and can only provide an overall indication concerning the properties and state of that sample. For the study of heterogeneous structures such as cell layers, biological tissue, or polydisperse particle mixtures, an overall measured impedance value can only provide limited information and can lead to data misinterpretation. For the investigation of localized sample properties in complex heterogeneous structures/mixtures, the addition of spatial resolution to impedance measurements is necessary. Several spatially resolved impedance measurement techniques have been developed and applied to cell and particle research, including electrical impedance tomography, scanning electrochemical microscopy, and microelectrode arrays. This review provides an overview of spatially resolved impedance measurement methods and assesses their applicability for cell and particle characterization.

Keywords:
Electrical impedance spectroscopy / Electrical impedance tomography / Impedance / Microelectrode arrays / Scanning electrochemical microscopy

1 Introduction

Electrical impedance (EI) is commonly used for the characterization of particles and biological samples such as cells and tissue. EI measurements have been used to determine basic particle properties like size [1] and concentration [2], as well as to resolve cell characteristics like cell type [1] and viability [3]. The popularity of EI sensing methods relies on the fact that they enable simple, marker-free, and non-invasive sample characterization. Another advantage of EI-based characterization methods that contribute to their broad applicability is the large availability of low-cost impedance measurement devices like LCR-meters and lock-in amplifiers, as well as the possibility to build such equipment from scratch in the lab [4]. EI measurements rely on electrodes placed in contact to, or near a specimen. For cell, tissue, and particle characterization, such electrodes can also be miniaturized. Miniaturized electrodes for impedance measurements can be integrated into most experimental setups, which again makes impedance-based characterization broadly relevant. Furthermore, simultaneous optical and electrical sample characterization is possible by utilizing transparent electrodes, which elevates the quality and quantity of acquired data [5].

The temporal resolution of impedance measurements depends on the excitation frequency, the bandwidth of analog signal processing, and the sampling rate of the signal. To monitor the impedance of fast processes, high-frequency measurements need to be used. For most applications, the temporal resolution of the measurement is not a limiting factor.
factor. For example, impedance sensing at the timescales on which most cell and tissue related processes take place (ms to several hours) is not difficult to achieve [6]. Some applications like cell counting in high-throughput cytometry require faster sensing, but even in such cases, the temporal resolution of the impedance measurement is typically not a limitation [7]. In contrast to temporal resolution, the spatial resolution associated with most common impedance-based techniques is very limited. The simplest workaround to the limited spatial resolution is to reduce the sample volume, using for example microfluidic channels to confine cells. A reduction in channel dimensions reduces the volume analyzed and results in more information about the location of the sample within the analyzed volume. The most common application of this strategy is single cell confinement for characterization in Coulter counters [1,2]. In such cases, the resolution is limited by the smallest attainable channel dimensions. In very small channels however, other problems arise such as fouling and clogging, making this an ineffective strategy towards increasing the spatial resolution of impedance measurements.

Spatially resolved sample characterization allows for the study of heterogeneous structures such as cell layers, biological tissue, or polydisperse particle mixtures and the investigation of localized sample properties or localized events in complex heterogeneous structures/mixtures. Most commonly, spatial resolution in cell and particle research is achieved using light microscopy. Other methods including electron microscopy, computer tomography and magnetic resonance imaging are also used, although less frequently. Optical characterization can provide information about many sample properties such as size, condition and morphology and is therefore indispensable for a variety of applications [8]. Impedance measurements are performed in a much lower frequency range compared to visible light, which allows for the characterization of different properties like sample polarization and conductivity.

Recently, a few impedance-based characterization methods with the ability to map/image impedance have been developed and demonstrated. These techniques rely on scanning probes, electrode arrays, and tomography and have been used to study various processes. Similar to optical techniques, impedance measurements have been used to monitor cell/tissue growth [9] and migration [10] in cell cultures. However, impedance mapping also enables the study of localized events in samples that might not be observable optically. Some examples of such events include electroporation [11,12], lysis [13], cell attachment [14], and drug response [15]. Another advantage of impedance mapping is the ability to spatially characterize non-optically transparent samples, such as cells grown in scaffolds [16].

Scanning probe techniques are used to map impedance using small electrodes, by scanning the area above the surface of samples submerged in a conductive liquid. The resolution of scanning probe techniques thereby depends on the electrode dimensions, the distance between the electrode and sample, and the conductivity of the liquid. Spatial resolution in array-based methods is achieved through localized measurements between different electrodes. To increase the sensitivity of array-based impedance measurements, the sample is placed on the surface of the electrode or in close proximity to it. For tomographic impedance measurements, circular electrode configurations surrounding the sample are most commonly used. Different excitation and sensing configurations are used to acquire datasets that can be utilized to reconstruct the distribution of impedance within the sample volume and create an image.

The topic of bio-impedance measurements has been previously extensively reviewed [17–20]. Reviews on the technical aspects of scanning electrode techniques [21,22] and bio-sensing using electrode arrays [23–25] have also been published without a focus on impedance measurements. Electrical impedance tomography has also been reviewed for medical and industrial applications [26–28]. However, the aforementioned reviews do not focus on spatially resolved impedance measurements. Here, we review the most popular techniques used for spatially resolved impedance measurements, specifically for applications in cell and particle characterization. A theoretical introduction to impedance and its measurement principles is also given to provide the necessary foundation for understanding the techniques discussed. The three most promising spatially resolved impedance measurement techniques are thoroughly reviewed, namely scanning electrochemical microscopy (SECM), micro-electrode arrays (MEAs), and electrical impedance tomography (EIT). A critical comparison of all discussed techniques is given in terms of their measurement principles, suitability for cell and particle studies, and their applications. Finally, an outlook on future applications is also discussed.

2 Background

Electrical impedance measurements are used to quantify the resistance of a material to an injected electrical stimulus. In a DC circuit, Ohm’s law is used to describe the relationship between resistance (R), voltage (V), and current (I). In the case of an AC circuit, the same general equation relates the voltage and current phasors, but resistance is, in this case, replaced by impedance (Z). Unlike DC resistance, AC impedance is complex and has a real and an imaginary part. The real part of complex impedance is simply resistance, whereas the imaginary part arises from inductive and capacitive effects and is referred to as reactance. This relation can be expressed as:

$$Z(\omega) = \frac{V(\omega)}{I(\omega)} = Z_{\text{real}}(\omega) + j Z_{\text{imag}}(\omega). \quad (1)$$

where $j$ is the imaginary unit ($j^2 = -1$) and $\omega$ is radial frequency. As evident by Eq. (1), impedance is a function of frequency. The magnitude of impedance is given by:

$$|Z| = \sqrt{Z_{\text{real}}^2 + Z_{\text{imag}}^2} \quad (2)$$

In addition to its magnitude, AC impedance is also characterized by its phase. When a circuit is driven with DC, there is
no distinction between impedance and resistance. The phase of complex impedance is given by:

\[
\varphi = \arctan \left( \frac{Z_{\text{mag}}}{Z_{\text{real}}} \right)
\]  

(3)

There are two approaches to recording an impedance spectrum. The first approach is to use an input potential of a single frequency and sweep the frequency over time. The second approach is to apply multiple frequencies at the same time and record the output impedance spectrum. The resulting impedance spectra can be presented in the form of Bode-Diagrams or in the form of Nyquist-Plots.

### 2.1 Particle, cell, and tissue impedance

When an electric field is applied across a conductive material, free charges begin to move in response to the field creating a net electric current. A material’s ability to conduct electric current can be described in terms of its electrical conductivity. In the case of dielectric materials, an external electric field causes the displacement of fixed charges. The molecules gain electric dipole moment and the material is polarized. A measure of the ability of a material to interact with an electric field and become polarized is described in terms of its electric permittivity. Variations in the electrical conductivity and/or permittivity of a material result in variation in the overall electric current, which can be measured as a change in impedance.

In suspensions, the presence of particles or cells leads to local variations in conductivity and/or permittivity and therefore an impedance response can be recorded. The dielectric properties of cells and tissues are frequency-dependent or dispersive [20]. Dielectric/permittivity dispersion arises from different polarization mechanisms and can be grouped into \(\alpha\), \(\beta\), \(\delta\), and \(\gamma\) dispersion as shown in Fig. 1.

In biological samples, \(\alpha\) dispersion occurs at low frequencies (below a few kHz), \(\beta\) dispersion occurs at frequencies from tens of kHz to tens of MHz, \(\delta\) and \(\gamma\) dispersion occurs at frequencies in the GHz range. Depending on the frequency range impedance measurements are performed at, different processes/properties can be probed indicated in Fig. 1. This is because different polarization mechanisms respond to different characteristic frequencies.

The mechanisms that result in permittivity dispersion have been extensively studied. Dielectric studies in the low frequency range can be challenging mainly because electrode polarization at these frequencies is also significant (see Section 2.2). For that reason, the mechanisms behind \(\alpha\) dispersion in biological samples are the least well understood. In general, \(\alpha\) dispersion has been found to arise from processes like low-frequency relaxation in colloidal particle suspensions and cell membrane permeability that allows ion transport [29]. \(\beta\) dispersion is known to arise mainly from the interfacial polarization of biological membranes. Additionally, polarization effects due to particle size and morphology are manifested as \(\beta\) dispersion, which makes this frequency regime very relevant for the study of cells. \(\delta\) and \(\gamma\) dispersion is mainly a result of polarization due to the reorientation of water molecules. Impedance measurements used to characterize cells and particles are therefore typically performed at frequencies between a few Hz to several MHz.

Several equivalent circuit models have been developed and are well established for the description and analysis of impedance measurements. Relaxation effects are represented by series or parallel resistor–capacitor circuits [30]. Commonly used equivalent circuits for cells and particles in suspensions are shown in Fig. 2, where \(R_s\) and \(C_s\) are the resistive and capacitive components of the solution respectively, and \(R_p\) and \(C_p\) those of the particle. Cells are modelled using the components \(C_m\) and \(R_m\) to represent the membrane, and \(R_i\) and \(C_i\) the cytoplasm.

### 2.2 Electrodes

One critical component of impedance measurement setups are the electrodes. In most cases, the cells or particles under investigation are suspended in an ionic liquid. For impedance measurements, a conductive electrode needs to contact the liquid. Upon contact, the electrons in the metal attract the positive ions in the solution, which form a charge layer along the metal/liquid interface. A second layer of ions subsequently forms on the first surface charge due to Coulombic attraction, electrically screening the first layer. This dielectric double layer gives rise to electrode polarization effects and can be theoretically described using the Helmholtz-Gouy-Chapman-Stern model. In addition to the compact Helmholtz double layer, a diffuse outer layer that consists of excess ions is formed, often referred to as the Gouy-Chapman-Stern layer [31]. The two double layers can be represented in an equivalent circuit in terms of two capacitors connected in series.

In addition to double layer formation, metal/liquid contact also induces redox electrochemical reactions due to charge transfer across the interface. The diffusive nature of this faradaic process can be modeled using a chargetransfer resistance (\(R_{CT}\)) and a Warburg impedance (\(Z_W\)).
A simplified, commonly used equivalent circuit was introduced by Randles to model electrodes in contact with a liquid [32]. Suitable electrode materials have to be selected according to the experimental conditions. Electrodes can be classified as ideally polarizable or non-polarizable. Non-polarizable electrodes exhibit no polarization due to the absence of an electrical double layer, which allows faradic current to pass freely. Ideally polarizable electrodes allow no faradic current flow. For DC and low frequency measurements, faradic current is essential for current conduction, therefore non- or low-polarizable electrode materials such as Ag/AgCl are used. Under AC and high frequency conditions, the impedance of the capacitive current path through the double layer declines, making polarizable electrodes like Au and Pt preferable [33]. When high electrode transparency is required, indium tin oxide is commonly used [5].

Impedance measurements can be performed using configurations of two, three, or four electrodes. When two electrodes are used, impedance is measured between a working electrode and a counter electrode. While the two-electrode configuration is commonly used due to its simplicity, it does not allow for the decoupling between contributions from the sample and contribution due to electrode polarization. Data accuracy is improved by using non-polarizable electrodes and through mathematical corrections [34]. Three-electrode configurations allow for the measurement of the working electrode potential against a reference electrode, which carries a stable and known potential. Most commonly, four-electrode configurations are used for impedance measurements for the characterization of biological tissue [35]. Current is applied to the sample through a pair of excitation electrodes, and voltage is measured using the two sensing electrodes. The sensing electrodes are connected to high-impedance inputs, which minimize the flow of parasitic current and therefore the effects of electrode polarization [33]. Parasitic effects in the measurement circuit and electrode misplacement can compromise the measurement result in four-electrode configurations [36].

### 2.3 Impedance measurement principle

Impedance is measured by applying an excitation signal to a sample in the form of voltage or current and measuring the amplitude and phase of the response voltage or current. The amplitude of the excitation should be large enough to ensure good signal-to-noise ratio, but small enough to ensure that non-linear effects in the sample are not affected or induced. Joule heating due to the sensing current has to also be considered at all frequencies, while effects relying on polarization are most relevant in the DC and low frequency range. Direct effects on biological samples like electroporation require strong electric fields and are not expected to occur during most sensing applications [37]. However, some cell types like neuronal axons [38, 39] and cancer cells [40, 41] can be affected by small electric fields. Such cell specific effects have to be considered when electrical measurements are performed.

Different measurement techniques, including bridge circuits, lock-in, and signal mixing techniques, are available and are used in LCR-meters, impedance spectrometers, and vector network analyzers [17, 42]. Most impedance measurements rely on lock-in techniques since they allow for flexible setup configuration and fast scanning. The real and imaginary components of the sample response signal are measured via phase-sensitive detection, also known as lock-in amplification. For measurement, a sinusoidal input signal is applied to the sample, here given in the form of a current signal:

\[
I_{in} = I_{in0} \sin(\omega_{in} t + \varphi_{in})
\]  

(4)

In case of a linear response, the resulting voltage will have the form:

\[
V_{meas} = V_{max} \sin(\omega_{in} t + \varphi_{in} + \varphi_{sample}),
\]

(5)

where \(\varphi_{sample}\) is the phase shift introduced by the sample and \(V_{max}\) is the amplitude of the measured voltage. To extract the real and imaginary output components, quadrature amplitude modulation is used for signal processing. The measured output voltage is multiplied by a sinusoidal (or rectangular) signal in-phase with the input signal, and a quadrature signal that is \(\pi/2\) out of phase with the input signal. The multiplication is carried out either in the analogue domain via direct conversion mixing or phase sensitive rectification, or in the digital domain in the form of multiplication in digital signal processing. Lock-in amplification is frequency selective and that makes this technique a valuable tool for signal recovery in noisy environments. Most spatially resolved impedance characterization methods discussed in this review rely on lock-in amplification.
3 Spatially resolved impedance measurements

The three most promising spatially resolved impedance measurements are based on scanning probe, micro-electrode, and tomographic impedance measurements. The experimental setups required to perform impedance measurements using these methods vary dramatically and so do their technical characteristics. When deciding which method to use, one should consider the technical characteristics and applicability of that method against the particular requirements of the system under study. Spatial resolution using impedance measurements can be achieved in one, two, or three dimensions. The number of dimensions required to fully characterize a specimen also depends on the requirements and nature of the system under study. For example, spatially resolved impedance measurements in microfluidic environments often only require 1D or 2D measurements since the flow transport properties add another temporal dimension. In the case of cell cultures and tissues, two or three sensing dimensions are necessary to fully characterize cell layers and tissue structures. In the following sections, the working principles, technical characteristics and research applications of SECM, MEA, and EIT are reviewed in detail.

3.1 SECM

Various approaches to scanning probe impedance measurements were demonstrated for samples immersed in liquid with the most commonly used being SECM [43]. SECM is based on an amperometric measurement performed using a single microelectrode scanning over the surface of a sample. SECM experiments are performed in a solution that contains a redox couple: a reducing species and its corresponding oxidized species. When the probe is far from the surface of the sample, the oxidizing species is reduced at the probe due to the applied potential, and diffusion limited steady-state current is produced. When the probe approaches an insulator, regeneration of oxidized species is inhibited and a decrease in diffusion-limited current is observed. A conductive surface oxidizes the reduced species formed at the probe, resulting in an increase in current. In addition to the sample material, the probe-to-sample distance and the concentration of electro-chemically active species in the liquid result in changes in current. SECM can therefore be used to map surface compositions, monitor variations in concentration, map the topography of a surface, and acquire information regarding the conductivity of a surface [44].

Depending on the experiment, the use of a redox mediator is not always favourable since it can affect the chemistry of the system under study. In absence of a redox mediator, AC amperometric measurements cannot be used to acquire distance feedback. AC measurements can be alternatively used to probe the impedance of the system [45]. In AC-SECM, a sinusoidal signal is applied to the DC bias, which allows for complex sample characterization. Properties like conductivity and electrochemical activity have been mapped using AC-SECM in the absence of a redox mediator [46]. In order to decouple current fluctuations due to changes in topography (distance fluctuation) from fluctuations due to changes in electrical properties, shear force sensing and intermittent contact approaches were also implemented for distance control [47, 48]. Based on these approaches a wide range of scanning probe measurements were developed for different applications. Recently, AC-SECM measurements were also combined with fast Fourier transform impedance spectroscopy for faster spectral imaging [49].

3.1.1 SECM working principle

The experimental setup of SECM resembles that of other scanning probe microscopy techniques. The electrode position is controlled using a motorized xyz-stage with sub-micrometer resolution, often accompanied by a piezo stage for a high-resolution z-positioning [50]. The scanning probe electrode is sealed in a glass capillary for insulation and only a tip with sub-micrometer diameter is exposed and used for scanning. For impedance measurements using AC-SECM, two- and three-electrode configurations are also commonly used (see Section 2.2). A fourth electrode is used in some setups to establish contact to the sample [51, 52]. The DC potential is controlled via a potentiostat and a low-amplitude AC excitation is added to the DC operating point [46, 51]. The output current amplitude and phase are detected using lock-in amplification (see Section 2.3). A schematic of the AC-SECM experimental setup is shown in Fig. 3.

Several SECM operating modes exist including constant-height, -distance, -current, and -impedance [53]. Constant-height mode scanning is performed with the tip at a constant z-height. The measured signal is a combination of distance dependent feedback and signal from changes/ variations in liquid/sample conductivity. Signal resolution improves when the tip is located closer to the sample surface. This makes scanning samples with an unknown or changing topology susceptible to collisions with the tip. This problem can be overcome by operating in constant-distance mode; however, a distance-dependent feedback signal is required. Since current and impedance show a strong distance dependency, a commonly used approach to distance control is to use impedance as the feedback signal to control the z-height [54]. This approach works well as long as the liquid and sample conductivity remain constant within the scan area. A more precise way to control distance is to use mechanical feedback, like a piezo-based shear force sensor [47].

The size of the scanned area determines the spatial resolution of SECM measurements, which in turn depends on the size of the scanning probe. The diameter of the scan area (D) can be approximated using Eq. (6) [55].

$$D = a + 1.5d,$$

where $a$ is the conductive electrode diameter and $d$ is the probe-to-sample distance. Eq. (6) indicates that for high
spatial resolution, both the diameter of the electrode and the probe-to-sample distance need to be minimized. Commonly used scanning distances are in the range of one electrode diameter (hundreds of nanometers to tens of micrometers) or below [47]. Another parameter that affects the resolution and sensitivity of the measurement is the ratio between the overall probe diameter (conductive core + insulation) to the core diameter, referred to as RG ratio. It was found that tips with small RG result in higher resolution, while a larger RG increases sensitivity [56].

3.1.2 Applications of AC-SECM

AC-SECM has been intensively used for the analysis of biological materials such as proteins, DNA, antibodies, and cells [22, 53]. Due to the many parameters affecting the measured impedance discussed above, analysis of an isolated single property can be challenging. One example of AC-SECM used to probe cell morphology was given by Kurulugama et al. [57]. A combination of constant-current and constant-impedance SECM was used to study PC12 cells in vitro. By operating at constant-distance mode, cell topology could be detected via a change in impedance by moving the tip across the cell. Detailed images of single cell topology were acquired using this technique and are shown in Fig. 4.

Constant-height AC-SECM imaging were also performed by Diakowski et al. for the topological mapping of living COS-7 cells in cell culture medium [14]. Stationary measurements (no scanning) were also used to study the cellular activity, metabolic activity in response to stimulants, and changes in the height of single cells. By monitoring the temporal response during stationary measurements, cell wall oscillations could be monitored with sub-micron resolution.

Beyond cells, AC-SECM has been used for surface and particle characterization. For example, Rincon et al. used AC-SECM to characterize the semiconducting properties of TiO₂ nanostructures under different bias by mapping the surface [58]. The ability of AC-SECM to differentiate between materials based on their conductivity was also demonstrated using chalcopyrite and pyrite particles with diameters between 50–70 µm embedded in epoxy resin [59]. A difference in the current feedback allowed for differences in material to be detected and 2D images of the material surface properties to be acquired. The experimental details used for cell and particle characterization in research work discussed in this section are summarized in Supporting Information Table S1.

3.2 MEAs

Patterned MEAs can be used for localized impedance sensing. Microelectrodes are typically patterned using photolithography and can be integrated into biosensors, organ-on-chip platforms, and microfluidics used to study particles, cells, and biological tissues. Advances in microelectronics have enabled the development of robust, high-resolution MEAs for biological investigation. In an early example, MEAs were used to map the electrical activity of embryonic chicken heart cells [60]. Further development led to small 8 × 6 or 8 × 8 MEAs, which are now an established tool for studying electrically active cells in cultures or sliced tissues [23]. In these simple arrays, each individual sensing electrode is wired to external measurement circuitry. This wiring takes up additional space and limits electrode density. The sensing resolution in MEAs is defined by the spacing between the individual electrodes. To achieve high sensing resolution and high electrode counts, MEAs have been developed which use integrated electronics to perform multiplexing [61]. Additional integration of signal conditioning [62, 63], and in some cases even data conversion circuits, has led to fully integrated designs that rely on few external components [64, 65]. These devices are manufactured using complementary metal-oxide-semiconductor (CMOS) technology, and
the electronics are covered with a biocompatible passivation layer, leaving only the sensing electrodes directly exposed to the sample environment. As not all electrode materials are CMOS compatible, a post-fabrication step is often required to cover the CMOS-compatible aluminum or copper contacts with biocompatible metals like Au, Pt, or Pt-black [66–68] for sensing electrodes. Electrode polarizability needs to also be taken into account according to the experiment requirements, as discussed in Section 2.2. Modern MEA designs use transistor-based arrays, with large numbers of electrodes and on-chip stimulation [69,70]. An example of an 8 × 8 MEA with integrated analog signal processing and analog to digital conversion is shown in Fig. 5.

3.2.1 Impedance sensing MEAs

Specialized MEAs with higher system complexity are required for impedance mapping, since an excitation signal must be provided and both amplitude and phase must be measured. Impedance sensing MEAs typically differ from other types of MEA in their electrode count, signal detection principle, and analog-to-digital conversion circuitry (Fig. 5). What follows is a summary of several recent developments relating to the architecture of impedance sensing MEAs. In ref. [71], Hassibi and Lee demonstrated a differential sensor array for impedance spectroscopy, voltammetry, potentiometry, and field-effect sensing that completely relies on a standard CMOS integrated MEA. In ref. [72], the application of the lock-in principle for pixel-based arrays in CMOS integration is demonstrated. Manickam et al. [73] presented a fully integrated 10 × 10 MEA for impedance measurements of biological samples, which uses one large electrode in the buffer above the sample for excitation. In this MEA, each pixel includes a trans-impedance amplifier to convert the measured current and an IQ-Mixer for phase sensitive detection of that signal. Row and column multiplexing are integrated on the chip, while signal processing and analog-to-digital conversion are performed on external components. In ref. [67], another impedance sensing approach is presented within a multi-modality sensor array incorporating 144 electrodes arranged in nine blocks of 4 × 4. While in impedance-sensing mode, each electrode in the array can be configured either for voltage excitation or for current sensing. One IQ detection channel is integrated and the sampling is realized with an external DAQ-board. The highest resolution impedance sensing MEAs contains a total of 59 760 electrodes, multiplexed to 32 impedance readout and 2048 extracellular action-potential
Figure 5. Exemplary setup of an $8 \times 8$ MEA with integrated analog signal processing and analog to digital conversion. The sensing area is marked in blue and a common reference electrode is used. The sensing frequency is provided via an external signal and $N$ signal-processing channels are interfaced by a DAQ card for signal recording [66].

recording channels [66,74]. The voltage excitation signal was provided by one large reference electrode. Each impedance readout channel consists of a trans-impedance amplifier, an IQ-mixer, and low pass filters. On-chip waveform generation is controlled via a serial interface, and the output data is transferred by an 8-bit parallel interface to the data acquisition system. An overview of the different array specifications discussed above is given in Supporting Information Table S2.

3.2.2 Applications of MEAs in cell and tissue characterization

Several studies enabled by the use of impedance sensing MEAs are discussed below. The impedance mapping function integrated into the multi-modality sensor array was used to observe the attachment and detachment of human ovarian cancer cells from the electrodes [67]. The detachment process was triggered by Accutase™ in the cell medium, and was observed in low-resolution ($7 \times 7$ pixels) impedance images as a localized decrease in measured impedance. High-resolution (59320 electrodes) impedance mapping was demonstrated for cell aggregates of mouse embryonic stem cells (Fig. 6) [66]. Using the same MEA design, slices of mouse brain tissue were also investigated and compared to an optical microscope image. Different cell layers were clearly distinguishable in the brain impedance maps, since the areas with high cell density showed greater local impedance. Sub-cellular resolution was demonstrated in these impedance images, and the electrical activity in an individual neuron was recorded.

3.3 EIT

Electrical impedance tomography is a non-invasive imaging technique in which the electrical impedance of samples is inferred from electrical measurements and is used to create tomographic images. In EIT, the impedance distribution of the sample is reconstructed based on electrical measurements within an electrode array. The boundary conditions are measured at different excitation sites within the array by applying certain current patterns (most commonly alternating current) and measuring the resulting voltages. From the boundary data, an impedance distribution map of the sensing volume can be reconstructed (see Section 3.3.2). Most applications of EIT are in the medical field [75–77], but other applications like industrial process monitoring have also been explored [28,78].

3.3.1 Working principle

The most common electrode configuration used for EIT is circular. Here we use the circular electrode configuration to present the working principle of EIT, but the sensing principle can be transferred to differently shaped arrays. A general setup for an EIT system using eight electrodes arranged in a circular configuration is shown in Fig. 7A. Multiplexers are used to control the excitation and sensing positions, which allows for sample volume mapping. Typically, a pair of electrodes is used to apply a small AC current for excitation, while the resulting voltage is measured between two other electrodes. Both the excitation location and the sensing location are changed sequentially during the measurement. At every excitation location, as many voltage measurements as necessary are performed in order to fully describe the resulting potential distribution. By shifting the excitation site, the complete potential distribution at the boundary is mapped. An example of some basic excitation patterns is given in Fig. 7B. The most common excitation patterns utilize adjacent or opposing electrodes for sensing, but alternating electrodes approaches have also been investigated [79].
Different excitation configurations result in different sensitivity distributions within the sensing volume. In contrast to SECM and MEAs, determining the resolution of EIT images is not straightforward as it depends on image reconstruction algorithms (see Section 3.3.2) and the method used for the calculation [80]. Additionally, resolution is affected by many parameters such as the electrode configuration, the excitation and sensing pattern, and signal-to-noise ratio. For maximum resolution, parallel excitation and measurement on all electrodes can be employed for more complex excitation and sensing patterns. That, however, also increases the complexity of the electrical hardware setup [81, 82].
Measurement data are stored in the form of a matrix, which contains the boundary conditions at each excitation site and are further used for image reconstruction.

### 3.3.2 Image reconstruction

Image reconstruction in electrical impedance tomography can be formulated as an inverse problem; mapping boundary conditions to sample properties. The conductivity $\sigma$ inside a domain $\Omega$ is determined from voltage measurements, given current injection, on the boundary $\partial\Omega$. Assuming no interior current, the following set of equations describes the system:

$$\nabla \cdot \sigma \nabla \phi = 0 \text{ on } \Omega \quad (7)$$

Neumann boundary conditions, with boundary current $I$:

$$\sigma n \cdot \nabla \phi = I_{\text{on}} \text{ on } \partial\Omega \quad (8)$$

Dirichlet boundary conditions, with boundary voltage $V$:

$$\phi = V \text{ on } \partial\Omega, \quad (9)$$

where $V$ is the electric potential. Boundary voltages measurements for given current injections, approximate the Neumann-to-Dirichlet (NtD) map $\Lambda$, mapping $I$ to $V$. The inverse problem amounts to finding $\sigma$ given $\Lambda$. Under certain assumptions on the functions $A$, $V$, $I$, and the domain $\Omega$, $\sigma$ can be uniquely identified [83, 84]. In practice, experimental data are noisy, $\Lambda$ is approximated, and the inverse problem is solved by considering the forward problem of voltage prediction given known currents, and optimizing $\sigma$ to fit the measurements [79]. We review standard approaches to solving the forward and inverse problem. We compare these with recent developments in neural network-based solutions to the inverse problem. Here, the theory behind the inverse problem is not treated exhaustively, but more information can be found in [85].

#### Classical approaches: the forward problem

Many classical approaches to the inverse problem rely on solving the forward problem [79, 85]. For the forward problem, $\sigma$ and $I$ in Eq. (7) are given, and Dirichlet boundary conditions are determined. Therefore, any method solving partial differential equations on a domain $\Omega$ may be applied. A common choice is finite element methods [86].

#### Classical approaches: regularized least squares

Naively, $\sigma$ can be estimated by following optimizing the objective given $V$ and $I$:

$$\min_{\sigma} \| V_{\text{ex}} - \Lambda_{\sigma}^\text{fw}(I) \|_2, \quad (10)$$

where $\Lambda_{\sigma}^\text{fw}$ is the NtD map for a given $\sigma$ determined by solving the forward problem, and $V_{\text{ex}}$, $I$, are the experimental voltage and current, respectively. As the inverse problem is ill-conditioned, optimization-based methods need to be regularized to converge to a result [79, 85]. Optimization algorithms mostly differ in their discretization of the problem, as well as the regularization used [79, 85, 87–89]. While a naive discretization works, it is computationally costly [79, 85]. More sophisticated, multigrid methods were shown to provide comparable accuracy at lower cost.

#### Classical approaches: D-bar

Another approach maps the inverse problem to an unphysical Schrödinger equation [90]. A change of variables is performed for Eq. (7), replacing $\sigma := \Delta \sqrt{\sigma}/\sqrt{\sigma}$ and $\phi := \sqrt{\sigma}\phi$. This results in a Schrödinger-like equation:

$$-\Delta \tilde{\phi} + q_0 \tilde{\phi} = 0 \quad (11)$$

This equation can be solved using special solutions $\mu(z, k) = e^{izk} \psi(z, k)$, referred to as complex geometrical optics solutions. These solutions solve the following equation:

$$\overline{\nabla} \mu(z, k) = \frac{1}{4\pi k} f(k) \exp \left( i \left( zk + \overline{zk} \right) \right) \mu(z, k), \quad (12)$$

where $f(k)$ is the scattering transform. This type of equation is called a D-bar equation. The scattering transform can be related to the NtD map, via:

$$f(k) = \int_{\Omega} e^{ik\Lambda} (\Lambda - \Lambda_0) \psi(z, k) dz \quad (13)$$

A tractable approximation:

$$f(k) = \int_{\Omega} e^{ik\Lambda} (\Lambda - \Lambda_0) e^{ikz} dz \quad (14)$$

may be plugged into the D-bar equation to yield approximate solutions [90]. This method is robust and fast enough for real-time imaging [91].

#### Neural network approaches

As the inverse problem is underdetermined and ill-conditioned [92], it presents considerable difficulty to solution by algebraic techniques. While the above methods provide solutions, they are plagued by high computational cost and low quality of reconstructed images [93]. In similar domains, neural networks have shown promise in providing better results, faster than classical methods [94–96]. The inverse problem can be treated using neural networks, as large amounts of data needed for training can be generated by solving the forward problem. Here, we review the state-of-the-art in neural network-based approaches for EIT.

#### Neural network approaches: neural finite elements

An early line of approaches harnessing neural networks for the inverse problem discretizes the measured domain into elements on a triangular mesh. An artificial neural network then solves the inverse problem for each element [92, 97, 98]. These methods are simple to implement without a dedicated machine-learning framework.
Figure 8. Overview of the U-Net architecture. A given input image is progressively down-sampled and compressed across the down-sampling path. Learned filters are applied to each pixel in the image, computing its next down-sampled representation. Each step saves its intermediate down-sampled image data. Once the input is sufficiently compressed, it is successively transformed and up-sampled along the up-sampling path by combining outputs of the previous up-sampling steps with the saved outputs of the down-sampling path (skip connections). This architecture allows for the translation of images with similar semantic content and, in the case of EIT, synthesis of sharper solutions to the inverse problem.

Neural network approaches: neural post-processing
Convolutional neural networks have proven to be powerful tools for image generation and enhancement [95, 96]. They enable the generation of higher-quality versions of corrupted images [94], as well as image super-resolution [96]. Similarly, the D-Bar method improves when combined with convolutional neural networks [99]. For this, a U-Net neural network (Fig. 8) is used [100], which has proven to work well for image enhancement and medical image translation [101]. U-Net compresses an input image, reducing spatial resolution, then constructs an output image by increasing resolution and incorporating information from intermediate compression steps [100]. The resulting combination of D-Bar and U-Net-based image enhancement qualitatively and quantitatively outperforms D-Bar. Other classical reconstruction algorithms have also been combined with neural networks for post-processing, resulting in large improvements in quality at negligible runtime cost [102–104].

Pure neural network approaches
Another approach foregoes classical reconstruction algorithms entirely, using neural networks to reconstruct images directly from experimental results [105, 106]. Tan et al. trained a convolutional neural network over voltage measurements to solve the inverse problem. Li et al. pre-trained stacked autoencoders on simulated data [105, 107] and used those for image reconstruction.

3.3.3 Applications of EIT in cell and tissue characterization
One of the first applications of EIT in tissue imaging was to monitor conductivity changes during electroporation of rat liver tissues [108]. A parallel electrode array was used to study migration, growth, and permeabilization of human epithelial stem cells [9, 10]. The advantage of this geometry is that spatial resolution is determined along the thickness of the electrode, while inhomogeneities are averaged along the electrode length. Printed circuit boards have been used as an accessible and cost-effective way to manufacture electrode arrays of different configuration and small sizes (mm range) [16, 109, 110]. One such 16-electrode array was used for the study of Physarum polycephalum cells grown on a thin layer of agar gel in a cylindrical PDMS chamber [109]. A similar electrode configuration was used to demonstrate 3D image reconstruction of cell pellets using different algorithms and experimental parameters [110]. EIT for monitoring cell cultures in hydrogels and scaffolds has also been demonstrated [16].

To improve imaging results, time-difference EIT and frequency-difference EIT were also investigated. In time-difference EIT, input voltages for image reconstruction were generated by a voltage difference between a reference measurement and the actual measurement. This allowed for the monitoring of temporal changes in the measurement volume. Frequency-difference EIT uses two excitation signals of different frequencies at the same time. The measured voltage difference resulting from the two frequencies is used for image reconstruction. This technique is particularly suitable for monitoring cell cultures since cell membranes are known to filter out low frequency currents and allow high frequency currents to pass through ($\beta$-dispersion). Frequency-difference EIT has been used to visualize $\beta$-dispersion in breast cancer cells (Fig. 9) [16]. Additionally, the effects of drugs on breast cancer cells were investigated using 16-electrodes arranged in two concentric circles around one ground electrode [15]. A cell pellet was placed in the sensor volume and changes in impedance due to membrane permeabilization were used to image the process. An overview of discussed applications along with their experimental details is given in Supporting Information Table S3.

4 Concluding remarks
Spatially resolved impedance measurements have the potential to revolutionize tissue, cell, and particle characterization by enabling 2D and 3D property mapping. When it comes to cell cultures, this review discussed applications of such techniques in growth, viability, migration, attachment, and drug response. Applications of impedance measurements in...
ex vivo tissue characterization for composition and process monitoring have also been discussed. In particle research, impedance-based mapping for the characterization of particle distribution, surface composition, and electrical properties are some of the applications covered.

Despite the potential impact of spatially resolved impedance measurement techniques, achieving high-resolution along with signal processing for the isolation of sample properties can be challenging tasks. In all cases, high-resolution requires small sensing volumes, achieved by minimizing the electrode size and electrode-to-sample distance.

Three impedance measurement techniques that allow for the collection of spatially resolved data have been described in this review, namely AC-SECM, MEAs, and EIT. A general overview of these techniques with regard to applications and specifications is given in Table 1 and Supporting Information Tables S1, S2, and S3. AC-SECM can achieve high-resolution, but fast process monitoring is not possible since data acquisition requires scanning over an area. Depending on the mode of operation, topology and impedance distribution can be mapped across a surface resulting in a 3D image of electrical properties. The biggest disadvantage of AC-SECM is the dependency of measured impedance on the electrode-to-sample distance. When probing a sample of an unknown surface morphology, the source of the impedance signal can be difficult to interpret. Strategies on how to bypass this problem and the assumptions they entail were discussed in detail in Section 3.1.1. Overall, the measured impedance in AC-SECM can be affected by many parameters, which makes monitoring isolated properties challenging.

MEAs used for impedance mapping can achieve good spatial resolution at moderate imaging times. The functionality of impedance sensing using MEAs is however limited due to the static, planar electrode layout. Impedance sensing of samples directly attached to the electrode surface or in close proximity to it can only provide 2D information collected along the sensing plane. This makes MEAs most suitable for studying cells grown on the surface of the electrode, or samples that can be placed directly on top of the array. Another limitation is the rather large MEA chip size, which does not allow for efficient integration into advanced cell culturing devices like microfluidics and organ-on-chip platforms. Advanced MEA platforms offer a high level of integration that limits the amount of required external devices and opens up possibilities for use in commercially available impedance-sensing biochips.

The application of EIT to particle and cell research provides a very flexible and highly accessible technique for spatially resolved impedance characterization. In contrast to SECM and MEA, EIT is capable of three-dimensional impedance mapping. At its current state of development, the major drawback of EIT is the limited achievable resolution and the sensitivity variation within the sensing volume. Another limitation results from the fact that impedance maps need to be reconstructed from acquired data, which makes this technique prone to measurement and reconstruction errors. These drawbacks limit the applications of EIT, the capability of 3D mapping however makes this technique attractive for applications in cell, tissue, and particle research.

Each impedance measurement technique presented in this review can be used for the characterization of a range of different samples. Even though there are no hard lines we can draw when it comes to describing applications, the advantages and disadvantages of each technique described above, along with their technical characteristics, can give an indication on which technique is best suited for which samples. For example, the ability to perform AC-SECM directly in a cell culture dish makes this technique popular for the characterization of established cell cultures or the monitoring of the cell culturing process [14, 57]. The high spatial resolution and the ability to characterize surfaces also makes AC-SECM commonly used for the characterization of sample morphology/topology, especially when samples are expected to be flat [58].

Similarly, the application of MEAs for impedance mapping is limited to samples that can either be attached to or brought in direct contact with the electrode array. MEAs are commonly used to study cells that can be cultured directly on
Table 1. Summary/comparison of common measurement characteristics and applications

| AC-SECM [21] | MEA [23] | EIT [15, 16, 110] |
|--------------|----------|------------------|
| **Operation principle** | Surface scanning | Sensing at each electrode sensing between two electrodes | Reconstruction of impedance distribution based on boundary measurements |
| **Electrodes** | | | |
| Common materials | Pt, carbon fiber | Au, Pt, Pt-black, Al | Au, Ti-Pt |
| Size | ~1 μm–25 μm | ~10 μm–100 μm | ~1 mm |
| Number | 1 scanning electrode | up to 59 760 | typically 16 |
| Pattern | Single tip | Rectangular array | Circular array |
| **Impedance sensing** | | | |
| Principle | 2, 3 electrodes | 2 point electrodes | 4 point electrodes |
| Excitation | Voltage | Voltage | Current |
| Output | Topology, 2D surface impedance map | 2D impedance map | 2D, 3D impedance map |
| Typical resolution | ~1 μm | ~20 μm | 0.1–1 mm |
| Resolution limitations | Actuator step size resolution, microelectrode size | Electrode spacing | Electrode configuration, image reconstruction, sensing parameters |
| **Most common applications** | | | |
| Cells & tissues | Layer and surface characterization (topology) | Cell attachment, tissue slices | Cell pellets, cells in scaffolds |
| Particles | Particles on surfaces | / | / |
| **Advantages & disadvantages** | | | |
| Advantages | - High resolution | - High integration level | - Simple electrode setup |
| | - Imaging in culture dish | - Mass production | - High sampling rates |
| | - Surface topology mapping | | - 3D and 2D imaging |
| Disadvantages | - Limits in integration | - Limited to planar samples | - Limited resolution |
| | - Slow due to scanning | - Only 2D characterization | - Reconstruction artefacts |

5 Outlook

Due to the ability of microfluidics to simulate dynamic in vivo conditions, many branches of biology and pharmacy began to utilize such platforms for cell and tissue cultures. Additionally, microfluidics offer enormous control over the experimental conditions, which also makes them popular for synthesis and characterization of particles. We believe that the combination of microfluidic-based platforms like organ-on-chip with impedance mapping can allow researchers to tap into previously unattainable knowledge. Such advanced platforms would be valuable for studies on the effect of drugs on living tissue, disease dissemination through cellular monolayers, or the study of transport across tight junctions. Currently, many organ-on-chip and microfluidic cell cultures are equipped with trans-epithelial/endothelial electrical resistance electrodes for the electrical characterization of the sample. However, trans-epithelial/endothelial electrical resistance measurements provide no spatial information, which makes them a suboptimal tool for the study of complex, localized processes.

All techniques presented in this review are under ongoing development and could be optimized for use in microfluidic-based platforms. Nevertheless, the current development objective is to achieve higher data resolution...
and/or reduced sampling times. Although high resolution is important, we believe that development efforts should be more focused towards miniaturization. A few isolated attempts have been made to integrate impedance mapping in microfluidics without much success or follow-up studies. For example, some soft-probe SECM approaches have been demonstrated in microchannels but the scanning probe nature of this technique makes integration fundamentally challenging. From an integrability and potential impact point of view, EIT is the most interesting technique for use in microfluidics. Microelectrodes can be deposited in or integrated into microchannels and 3D impedance maps of cells under different conditions can be reconstructed. The combination of EIT with machine learning algorithms for image reconstruction (another aspect for EIT that has not been extensively studied) makes this platform an even more powerful tool for decoding the secret life of cells. A similar approach to electrode integration can be used for MEAs on-chip; the 2D nature of data is however somewhat limiting the potential impact of this technique.

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