

Developing Label-free Imaging Techniques to Study Biological and Energy Conversion
Processes

by

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ABSTRACT

Obtaining in situ characteristics of Lithium metal batteries (LMBs) is extremely important for understanding basic surface reactions involved in solid electrolyte interphase (SEI) formation, lithium nucleation/plating and thus overall cycling performance improvement of the battery cell. This thesis demonstrates a new characterization technique based on a principle that is completely different from the conventional EC detection technologies, plasmonic-based electrochemical imaging (PECI). It images local reactions (both faradaic and non-faradaic) without using a scanning microelectrode. Utilizing the reflectivity from surface plasmon resonance (SPR), PECI is fast and non-invasive, and its signal is proportional to incident light intensity, thus does not decrease with the area of interest.

SEI layer formation dynamics as well as its correlation with the afterwards lithium plating and nucleation have been successfully characterized in the form of spatial resolved electrochemical current images at various fixed potentials and local cyclic voltammetry methods are developed and demonstrated with real samples. Fast imaging rate (up to 106 frames per second) with $0.2 \times 3 \mu\text{m}$ spatial resolution have been achieved in both tradition electrolyte (1M LiPF_6 in EC/DMC) and engineered electrolyte systems, including highly concentrated electrolyte (4M LiFSI in DME) , and additive added electrolytes. An advanced localized high concentration electrolyte composed of 1M LiTFSI in 1,2 DME-TTE have also been characterized in support of the discovery of advanced ether-based electrolyte performances.

This dissertation also describes a related but different research project that develops a facile method to test the possibility of metal plasmon induced by intrinsic lithium on non-plasmon surfaces. A third project of this dissertation is to develop a method to provide local insights on oxygen evolution reaction electrocatalyst design and material discovery using total internal reflection.

The last part constitutes the expansion of conventional microscope to single cell impedance and cancer metabolism screening. Different phases of cell-substrate adhesion were successfully extracted via a conductive polymer (PEDOT:PSS) and using HeLa cell line. Using a facile imaging method, the metabolic pathway switch

has also been observed in the HeLa cell line in the presence of glucose transporter inhibitor and drug dosage for 14 hours.

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I. ADVANCED ENERGY PRACTICAL CHARACTERIZATION

1.1 In situ characterization of lithium metal battery

1.1.1 Introduction to lithium metal battery

As the market of smart and portable devices as well as electric vehicles is experiencing a historical expansion, the world-wide demand for high energy density of storage grows in concert with it, as the more functionality and durability of the devices requires a sufficient energy supply to drive those devices. Since the introduction of LiCoO_2 as a positive electrode material by Dr. John Goodenough in 1980,¹ the popular rechargeable batteries based on Li-ion intercalation with positive and negative electrode materials have been widely deployed in current consumer devices.² However, cells based on intercalation chemistry can provide only limited energy density, which is problematic in light of growing demands for large-scale storage. With such ever increasing emergence of new application markets, lithium metal anodes with ultra-high theoretical specific capacity (3860 mAh g^{-1}) and low reduction voltage ($-3.04 \text{ V vs. S.H.E.}$) has been considered “Holy Grail” in advanced rechargeable batteries.³⁻⁵ However, the parasitic dendrite growth which leads to reversibility and brings safety issues is the inherent hinder that prevents more practical and general application of lithium metal batteries (LMBs).⁶⁻⁹ This barrier yielded a low CE (Columbic Efficiency) and safety issues thus needs a considerable amount of attention. In order to solve this issue, enormous research efforts including electrolyte engineering,^{7, 10} coating artificial protective layer,¹¹⁻¹⁵ and electrolyte additives¹⁶ have been employed, most of which are focusing on promoting the underlying solid-

electrolyte-interphase (SEI) film, because it is well accepted that the SEI is of vital importance in LMBs as this layer serves as transportation vial for Li ions and electron insulator at the same time.¹⁷ They all achieved some superior results regarding the repeatability of cycling and columbic efficiency.

However, the superior number of repeatable cycles reported in the literature were usually due to unlimited amount of lithium and electrolyte in the coin cell configurations. Thus the exact suppression of Li dendrites and long lifespan of practical Li batteries are still challenged with limitations,¹⁸ which may lead to unwarranted claims unless careful attention is paid to fundamental performance characteristics. Therefore, it is reasonable to believe that a more in-depth exploration of the SEI related characteristics helps to acquire a more comprehensive understanding of the initial stages in practical LMBs and to tackle the cycling and safety issues.

1.1.2 Characteristics of lithium metal plating/stripping and electrode-electrolyte interphase

A comprehensive picture of the lithium metal battery performance can be divided into several parts, including both physicochemical properties like columbic efficiency and surface impedance after exposure to prolonged cycling experiments, and structural properties including surface morphology as well as the dendrite growth situation (thickness, density, etc). Therefore, it will be fundamental to provide a definition and common practices of these characteristics before any real characterization are being conducted on the electrode.

Columbic efficiency and cyclic performance. One of the traditional and widely adopted characteristics that almost each battery research group implements is the

coulombic efficiency and number of repeatable cycles of charging and discharging with reasonable energy retention. Coulombic efficiency (CE),¹⁹ also called faradaic efficiency or current efficiency, describes the charge efficiency by which electrons are transferred in batteries between electrodes. CE is the ratio of the total charge extracted from the battery to the total charge put into the battery over a full cycle. It is usually used to estimate the cycling life of LIB/LMB because CE reflects the loss of Li⁺ during each cycle,²⁰ therefore the reversibility of the battery can be quantified through this parameter. However, CE itself is not sufficient to demonstrate the performance of the cell being tested since it does not provide spatial or localized information across the cell and we have no way to find out the morphology or the in-situ dendrite growth situation in operando. We will still spend sufficient pages on talking about the cyclic performance of the battery cell and use it as a reference, but we will be using another kind of technique which does provide the extra spatial distributions.

Surface electrochemical impedance. All batteries lose performance and experience degradation issues during cycles of charging and discharging.²¹ For example, loss of the alkali metal and consumption/decomposition of electrolytes while battery cells are working are common issues the current state-of-art battery systems are facing. In addition, a non-negligible deterioration of ionic transport through components and across interfaces resulting from the above two reasons gave the battery performance a big challenge to sustain continued cycling.²² All these issues will induce a gradual increasing electrochemical impedance of electrode surface, thus by quantifying the electrochemical impedance we could obtain a general idea how bad the battery has been cycled and how much life it still sustains. However, this

parameter itself does not sufficiently provide localized information or morphology information. Its results also typically average out individual location characteristics. Also, knowing how bad the battery has become sometimes does not satisfy the researchers, as most of the time people are interested in how well the battery can perform, instead of how bad the system has degraded into.

Solid electrolyte interphase formation and morphology. One of the hottest topics regarding lithium metal battery has been lying around discovering the solid electrolyte interphase formation composition, dynamics and morphology. And many groups even start to develop artificial SEI protection layers to protect the lithium metal anode from being corroded.^{11, 14, 23, 24} As Li metal can react with most organic solvents, a surface film is formed during the initial charging/discharging processes. In 1979, Peled firstly realized the electrically insulating and ionically conductive interface and named it as the solid electrolyte interphase (SEI).¹⁷ This layer is typically with a thickness of ~20nm and incorporates various organic and inorganic components, resulting from consumption of anode and electrolyte, and effectively prevents the further physical contact between Li and solvent.²⁵ This mixture and even complicated behaviors of SEI made it fundamentally important to the improvement and development of stable and safe lithium metal battery.

Lithium nucleation and dendrite formation. As stated above, all batteries degrade upon prolonged time of cycling. Despite superior electrochemical performances of metallic lithium anodes, it suffers from constant dendrite formation coming from non-uniform lithium nucleation. Enormous efforts have been made to tackle the dendrite growth,^{6, 7, 9, 10, 16, 26, 27} since it is the main issue that is causing the

low columbic efficiency and safety issues in most LMBs. A uniform and mechanically strong enough lithium deposition is usually desired for most applications. Therefore, this PhD research will also spend a significant number of pages trying to characterize and visualize the lithium nucleation and dendrite formation and will demonstrate the feasibility of surface plasmon generation caused by mossy and non-uniform lithium deposition. Metallic lithium growth and dissolution process will also be directly observed to serve as a supplement to the pre-formed SEI layer characteristic.

In summary, providing a facile and easy to use technique to measure these properties in operando and in-situ would console the actual concerns of battery performances and thus promote the development of novel configurations of batteries. In the next section, we will review some of current methodologies and techniques that have been used to assess the dynamics and performance metrics of lithium metal batteries including both *ex situ* and *in situ* methods and provide some potential comparison and application insights.

1.1.3 State-of-art lithium metal battery characterization methods

To establish the basic structure models of SEI formation and lithium plating behaviors, the correlated formation mechanisms, researchers have already developed characterization tools including XPS, Raman, IR and EDS, etc. In this section, some of the most state-of-art techniques that have been proven successful will be discussed and briefly reviewed.

XPS. As a surface quantification technique, X-ray photoelectron spectroscopy (XPS) is a powerful surface analysis tool widely adopted among almost every battery materials researches.^{28, 29} It can be used to identify the elements that exist within a

material or covering its surface, as well as quantifying the amount of material on the surface by virtue of obtaining electron binding energy out of the surface. Recently, XPS has been employed in the analysis of SEI layer growth during the charging and discharging cycles for highly concentrated aqueous electrolyte solutions.³⁰ Such spectroscopic analysis tool provided superior spatial resolution as well as high precision of identification. However, a typical XPS machine is composed of multiple components, including X-ray source, ultra-high vacuum chamber with proper shielding and to make it work in operando, special designed sample holder needs to come to play, which made its wide and simple application a little bit handicapped.

EIS. Electrochemical impedance spectroscopy (EIS)¹⁹ is a non-destructive technique which provides a considerable amount of information in a relatively short period of time, while preserving integrity of the battery electrodes.²¹ It allows in situ dynamic measurements during battery cycling as well as ex situ measurements at various states of charge and discharge. The EIS is particularly sensitive to systems that contain several impedance elements, including bulk components and interfaces, which makes it well-suited to study a multi-component device such as a battery. However, the spectroscopy obtained via EIS typically does not enclose the localized information, neither the surficial morphology nor the exact compositions of the electrode, which are two most important aspects of lithium metal battery metrics.

FT-IR. As of spectroscopy analysis, Fourier Transform Infrared Spectroscopy (FT-IR) needs to be mentioned. It employs a Michelson interferometer to measure the absorption fingerprints at multiple wavelengths of the sample, and it has been intensively implemented in the characterization of the components in lithium-ion

batteries.³¹⁻³³ In the sense of spectrum of SEI layer and lithium metals, FTIR experience the difficulty of generating enough absorption or emission signal given the intrinsic composition of SEI layer onsite and metallic components. Therefore, a typical FTIR spectrometer would be generally hard to characterize the in-situ battery performances under practical applications.

Cryo-EM. Transmission electron microscopy (TEM) has been employed into the field of battery material investigations.³⁴⁻³⁶ However, under practical conditions where pristine SEI needs to be reserved, due to the intrinsic artifacts introduced by this microscopic technique, neither the conventional ex-situ TEM nor the recently developed in situ TEM setups could 100% preserve the probing alkali elements and alkali containing compounds, which would make the observation results deviate from reality and could lead to misleading results. Therefore, a Nobel prize winning cryogenic-electron microscopy (cryo-EM) has become viral since it could provide superior resolution of electrode material while ³⁷preserving the electrochemical state of SEI and the dendrites.^{38, 39} All in all, this method is still part of the ex situ family of characterization techniques and thus facing low adoption rate in situ for battery systems. On the other hand, the liquid nitrogen requirement to preserve the electrode surface as well as the requirement for a special sample holder limits its wide laboratory adoption.

Since the need for a solid understanding of electrode material characteristics in operando is surging, there is currently a lot of focus on in situ methods to characterize the anode surface, as summarized in this review.⁴⁰ Notably, C. Jin *et al* made an effort of trying to recover the dead lithium to compensate the loss of metal anode by means

of series of iodine redox reactions,⁴¹ this signals another historic demanding call for in situ characterization methods to provide a comprehensive picture of such processes that appeared. Therefore, the most state-of-art in situ characterization methods in their application sense in the lithium battery is going to be discussed.

In-situ AFM. Thanks to the cantilever being incorporated in most atomic force microscopy (AFM), this super-resolute imaging technique allows direct probing of SEI formation and structure onsite. For example, Arthur v. Cresce *et al*⁴² implemented AFM in situ combined with an ex-situ XPS analysis tool to provide a solid understanding of the SEI live formation and composition information. Their analysis paved the way of in situ precision measurement of the SEI formation dynamics in most Li ion battery graphite site. However, using AFM would still be an invasive method even if the expected results were claimed to be minimal destructive to the surface. And for composition analysis, XPS, the ex-situ tool had to come to play to make it complete regarding the surface components identification. So, even techniques like in-situ AFM⁴³ could provide the true topography of the SEI components and identify the effects of the additive, this technique alone is usually limited by the single image scan speed which restricts the overall throughput of the technique. Therefore, given those benefits, if a non-invasive method could be introduced to the real-time characterization of the lithium metal battery systems, while providing qualitative information and quantitative information simultaneously, it would make this in situ analysis of battery materials step even further.

ToF-SIMS. Since the early adoption of Time-of-flight Secondary Ion Mass Spectrometry in 1994,⁴⁴ its wide application in the characterization of lithium metal

battery components has been expertise by Dr. Zihua Zhu, including the comprehensive understanding of SEI layer formations and dynamics in the lithium ion batteries due to its intrinsic surface sensitivity property.⁴⁵ An electric double layer and a more concrete layer composition information in operando could be determined by liquid SIMS, which provided a more dynamic picture of how the SEI layer formed and the key underlying function of it. To gain a further understanding of the SEI layer beyond simulation alone, a most recent implementation of ToF-SIMS in combination with XPS was reported in 2021.⁴⁶ Though a more thorough picture of the dynamics were obtained, the too-complex whole system made it hard to operate in situ nor non-invasive, not to say providing an intact electrode surface information. Therefore, ToF-SIMS combined with XPS itself is superior to most current analytical techniques, but still not ideal for easy and accessible lab adoption.

In-situ Raman. One of the most popular techniques for qualitative and quantitative analysis tool is to use Raman scattering and measure the spectrum of Raman shift over a broad range of wavelengths. Raman has already been implemented by Hy, S *et al*⁴⁷ to provide a solid information on how the SEI layer is formed on electrode surface regarding the application of lithium ion battery. Given the capability of measuring the surface components precisely, we will implement the same protocol and utilize an easy to manipulate Raman detection vessel thanks to the generous offer from Dr. Zheng Fan, University of Houston.

SECM. There are chemical imaging methods developed to study the localized catalytic activities. For example, the powerful scanning electrochemical microscopy (SECM) can obtain local electrochemical information by scanning a microelectrode

across the electrode surface. It has been implemented into the measurement of the local electrochemical reaction activities⁴⁸ and also used to probe the electrochemical reactions on a single nanoparticle with 15 nm diameters by Allen Bard^{49, 50} and on sub- 10 nm scale particles by Michael Mirkin.⁵¹ SECM has also been powerful enough to be utilized in the measure the double layer effects in voltametric measurements⁵² and molecule uptake in live cells.⁵³ Although possessing such powerful capabilities, it needs to scan over the surface one point by one point, which limits its fast applications on the catalyst screening.

PECM. Plasmonic electrochemical microscopy has been developed by N.J. Tao's group.^{54, 55} This ultra-surface sensitive technique can map the localized electrochemical catalytic efficiency at once using the imaging cameras by measuring the subtle refractive index change happening on the electrode surface.⁵⁵ However, a thin layer of plasmonic metal, such as gold, silver, or copper, is needed, which makes itself not applicable for imaging the in situ oxidation reactions, such as OER, as the reactions will damage the plasmonic sensing surface and introduce the artifacts on the images.

Observing the real SEI formation behavior in a working cell will definitely facilitate the understanding of dendrite nucleation and growth. So, in-operando characterization measures is highly needed. Other in situ methods being in use but not so popular include wide angle X-ray scattering (WAXS), small-angle X-ray scattering (SAXS),⁵⁶ neutron reflectometry⁵⁷ and so on. They either requires complex instrumentation, or too bulky to perform inside lab-level medium size gloveboxes, thus limiting their wide adoption in most research labs. Therefore, this Ph.D. research

dissertation will not spend the primary focus on improvement of these techniques, even though sometimes they yield promising results.

In summary, tons of methods have been implemented to study the interphase and electrochemical compositions in the lithium batteries. But they are limited by either lack of in-situ composition information and spatial resolution (horizontally or vertically), or even the lack of simplicity to implement. Also, during the lithium plating and stripping process, not only dendrites grow on the electrode surface, insulating SEI also accumulates on new lithium surfaces of previous cycles, making the characterization work even harder to reach the goal due to the complicated structure of the layer. Therefore, this PhD research will focus on improving the short board of traditional characterization techniques and will be providing new insights about the lithium metal battery charging and discharging behaviors and performance metrics by using a combination of spatial resolution and qualitative identification of surface composition methods, which will in the end build the comprehensive picture of lithium metal anode properties. The research result is expected to promote the development and true efficacy of electrolyte additives, and formation of artificial SEI which are two main hot topics of lithium metal battery research focus.

In addition to overcoming the temporal or spatial resolution limitations, this PhD research also aims to target the surface sensitivity and instrumental complexity of current state-of-art ex-situ and in-situ characterization techniques, such as cryo-TEM (Transmission Electron Microscopy), SIMS (Secondary ion mass spectrometry) and XPS (X-ray photoelectron spectroscopy). A surface ultra-sensitive imaging technique will be implemented to measure the dynamics of lithium nucleation process as well as

the SEI protective layer formation. Time dependent lithium nucleation process will be examined by virtue of lithium plasmon generated by the different amount of deposited metallic lithium on the electrode, enhancing the capability of the mentioned surface detection technique. Traditional transmission microscope images will also be captured to provide the lithium deposition dynamics beyond the surface vicinity, and an innovative in-situ Raman detection kit is implemented to give insights on the electrode surface in situ to identify compositions on the go. Hopefully, we could build a comprehensive picture of what, when, and how the lithium metal battery operation activities can be improved, and the expected outcomes would be easily adapted to other battery related trials, for example, study of the electrolyte additive's effect, and development of other innovative lithium metal anodes. This PhD research will highlight the key strategies of pushing forward the systematic characterization of lithium metal batteries, thereby promoting the future design of metallic battery systems to accommodate the societal demand for energy storage. In the next section, another relevant but not directly related project which involves the metal oxide oxygen evolution reaction catalysts imaging will be introduced and discussed.

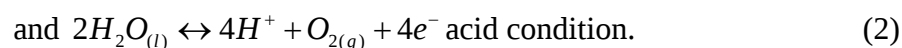
1.2 Advanced screening in catalytic oxygen evolution reaction (OER)

1.2.1 Introduction to Oxygen Evolution Reaction

The growing need to store large amounts of energy produced from renewable sources has recently directed substantial R&D efforts.⁵⁸ It is also anticipated that within the next 15 years, the energy demand will be twofold.⁵⁹ Among most renewable energy resources, water electrolysis which converts the water molecule into hydrogen

and oxygen have been outstanding because of its source's wide accessibility and reusability.

In the process of water splitting, oxygen evolution reaction (OER) is the core reaction for generation the reusable energy source oxygen and hydrogen. And the OER is highly pH dependent, where in acid conditions, two water molecules are oxidized into four protons and oxygen molecule and in alkaline conditions, hydroxyl groups are oxidized and transformed into H₂O and O₂ in the form of the following reactions as



Compared to half reaction of hydrogen evolution reaction (HER) as



where two electrons were involved in the transformation, OER demands transfer of four electrons, and kinetically favorable OER process occurs through multi-step reactions. Therefore, it is the OER that is reaction rate determining and making the overall water splitting process sluggish and results in large overpotential.

To overcome the energy barrier of OER, electrocatalysts that possess low overpotential as well as high activity and condition stability have already been playing an important role.^{60, 61} Currently, noble metal based OER catalysts stay on top of the list for facilitation of the oxygen evolution, including a high performing RuO₂ and IrO₂-based materials. They have achieved an overpotential as low as 250 mV at current density of 10mA/cm².⁶² But due to the high cost and their relatively low stability under higher anodic potentials, alternatives of these precious metals are in the

horizon. Fe⁴⁺ based quadruple perovskite which stands for the huge family of perovskite catalysts has also been implemented into the reinforcement of oxygen evolution reaction catalyst through the covalent bonding network, and have achieved a high activity that exceeds that of state-of-art OER catalysts such as BSCF and RuO₂ with a low overpotential 310 mV at 0.5 mA/cm².⁶³

1.2.2 Transition metal based OER catalysts

Besides these mixed-metal delicate structured OER catalysts, noble-metal-free first line transition metal including the Fe/Co/Ni *etc.*, is another set of candidates that can provide sufficient catalyst efficiency while maintaining a good level of stability under different pH and temperature conditions.^{64, 65} Spinel transition metal oxides is widely studied towards OER due to their low cost and promising OER activity. For example, a detailed comparison between oxides including CoAl₂O₄, ZnCo₂O₄ and Co₃O₄ have been conducted and demonstrated that different covalent bonds of the Co oxidative state could lead to different catalytic activities.⁶⁶

Besides Spinel oxides, more conventional metal oxides and metal oxyhydroxides are also prevalent in the purpose of reducing the energy barrier or lowering the overpotential, either through delicate structure design or doping of intrinsic efficient alloy materials to make a composite.⁶⁷⁻⁷¹ All of these transition metal based OER catalysts have the common goal to facilitate the OER process, and so many catalyst distinctions have brought the urgent need of deeper exploration and comprehensive understanding of the catalysts. In recent years, high-level theoretical tools and computational studies have led to significant progress in the atomic level understanding of the OER and electrocatalyst behavior. In parallel, several

experimental studies have explored new catalytic materials with advanced properties and kinetics on a technical relevant level. Part of this dissertation section employs a facile reflection imaging based screening system to study the cobalt catalytic OER as well NiFeIr alloy based OER.⁷² Localized electrochemical current density, overpotential and correlated metal oxidation maps were established and plotted. In support of the common knowledge that metal oxide catalysts are usually location dependent and surface structure heterogenous, we also find that the location complimentary phenomenon between the current density and the overpotential exists, while getting the corresponding metal oxidation map gave us the capability to demonstrate this distinct relationship between the oxides and the overpotential as well. The discussed reflection optical imaging method could find many applications in catalyst activity measurement and screening.

II. PRINCIPLE OF CHARACTERIZATION TECHNIQUES

2.1 Surface Plasmon Imaging

2.1.1 Introduction to Surface Plasmon Resonance

Surface plasmon resonance (SPR) is a label-free, real-time and highly sensitive optical surface sensing technique with numerous applications in a variety of disciplines. SPR can be used to probe refractive index changes that occur within the vicinity of a sensor surface. Thus, any physical phenomenon at the surface that alters the local refractive index will lead to a response and can be quantified.

Over the last three decades, SPR has attracted a great deal of research efforts. Many research reports have appeared describing advancements in SPR technology and its applications, including intensity interrogation, angle interrogation and wavelength interrogation.⁷³ SPR technology has been commercialized and become a widely accepted tool for characterizing and quantifying biomolecular interactions⁷⁴⁻⁷⁷ and surface impedance imaging.^{78, 79} More recently, label free detection of metallic nanoparticle have also been in hot research focus.⁸⁰ Many applications of SPR technique have been developed already, and the list is expanding rapidly. The rapid development of SPR is because the technology is label free, real time, versatile and highly sensitive, among which label-free and in-situ detection is the most important advantage of SPR for detecting and studying biomolecular activities. This is because label-based technologies, such as fluorescence detection, is mainly end-point detection, which provides no kinetic information of the reaction/binding process, and

the use labels may alter the native properties of the molecules and often lead to misleading results.

Surface plasmon resonance is a charge-density oscillation that may exist at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric. The charge density wave is associated with an electromagnetic wave, the field vectors of which reach their maxima at the interface and decay evanescently into both media. This surface plasma wave (SPW) is a TM-polarized wave (magnetic vector is perpendicular to the direction of propagation of the SPW and parallel to the plane of interface). Therefore, we used p-polarized light to excite the surface plasmon. Given by the tuning capability of the propagation constant described by

$$\beta = k \sqrt{\frac{\epsilon_m n_s^2}{\epsilon_m + n_s^2}} \quad (4)$$

where k denotes the free space wave number, ϵ_m the dielectric constant of the metal ($\epsilon_m = \epsilon_{mr} + i\epsilon_{mi}$), and n_s the refractive index of the dielectric,⁸¹ one can finely detect the minimal surface refractive change Δn_s by measuring the β across the vicinity of the sensing surface. As may be concluded from Eq. (1), at different optical wavelengths, this condition is fulfilled by several metals including gold, silver and sodium. When the incident angle is bigger than critical angle, although incident light is totally internal reflected, a component of this light, which is called evanescent wave, will generate, and penetrates into the less dense medium. The wave vector of the evanescent field along the x direction is given by

$$K_{ev} = K_i \sin \theta = \frac{\omega_0}{c} n_g \sin \theta \quad (5)$$

where K_i is the wave vector of incident light, ω_0 is the frequency of incident light, n_g is the refractive index of the dense medium glass, θ is the angle of incidence light.

In practice, the surface plasmon is affected by changes in the dielectric constant of materials in contact with the metal film. The dielectric constant, ε , is a dimensionless quantity that is proportional to the square of the refractive index of the sample. As the refractive index of sample region changes, it will alter the coupling efficiency of the light into the plasmon mode, which can be monitored by observing the SP coupling angle, the incident beam angle giving maximum SP coupling, θ_R , denoted by

$$\sin(\theta_R) = \sqrt{\frac{\varepsilon_m \varepsilon_s}{(\varepsilon_m + \varepsilon_s) \varepsilon_g}}, \text{ where } \varepsilon_s \text{ is the dielectric constant of sample and } \varepsilon_g \text{ is the}$$

dielectric constant of dense medium, glass. Under this matching condition, the evanescent wave of incoming light can couple with the free oscillating electrons (plasmons) in the metal film where $K_{sp} = K_{ev}$. When matched, the reflectivity under different sample refractive index is shown in the below picture,

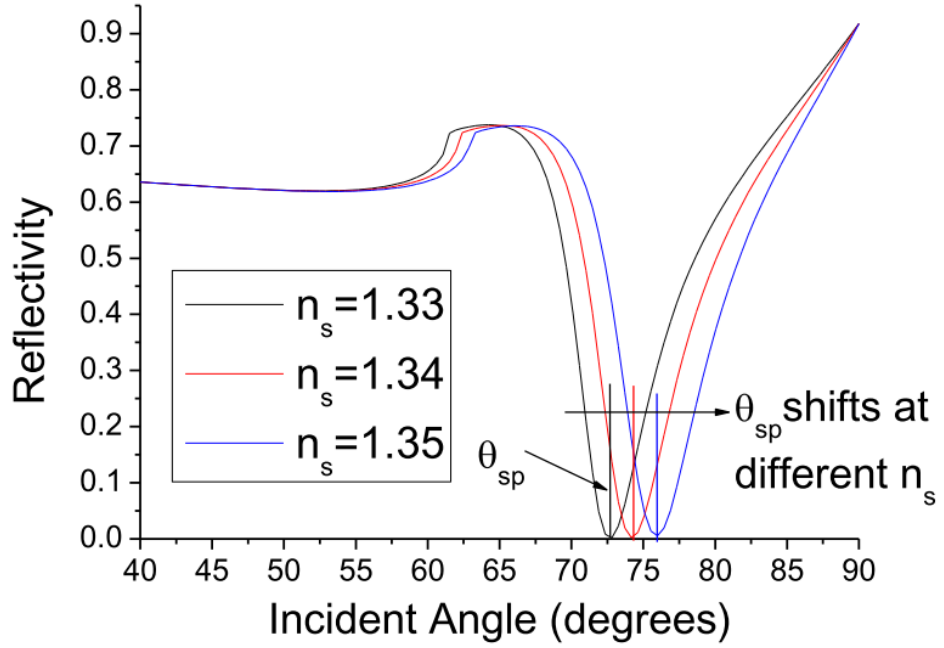


Fig. 2.1 SPR curves. Different refractive indices have different resonance angle (θ_{sp}).

By using a popular Kretschmann SPR configuration⁸² shown below Fig 2.2(a) on the left, a schematic drawing of the real imaging setup is shown below on the right Fig 2.2(b).

Therefore, via collection of the reflectivity image sequence using the Pike camera depicted in Fig. 2.2(c), we were able to gain the localized information/reaction happening on the metal surface, which is the surface plasmon generating metal layer. In our case, a polarized 680 nm laser beam is used as the light source where it penetrates the substrate glass (SF11, $n = 1.778$) all the way through the sensing surface and gets reflected through the 100X objective and collected by the CCD camera. Whenever there is enough reaction related refractive index change happens within the vicinity of ~ 200 nm, i.e., the penetration depth of the surface evanescent wave, this

delicate change could be represented by the decreased/increased light intensity collected by the CCD.

Using this setup, transitional electrochemical reactions during the initial stage of lithium metal battery charging and discharging as well as spatial resolved SEI morphologies on the cathode have been synchronized in concert to provide a whole picture of the SEI formation dynamics in both traditional carbonate electrolyte and highly concentrated electrolyte alternatives. Alterations of other lithium salt anions were also fully studied to provide a comprehensive picture of the lithium metal battery characterization. Four distinct reaction regions could be identified in the process of SEI formation, and our technique can be used to provide solid quantitative information for the development of safer lithium metal anodic materials. These four distinct regions and their corresponding signals interpretation will be discussed in the next chapter.

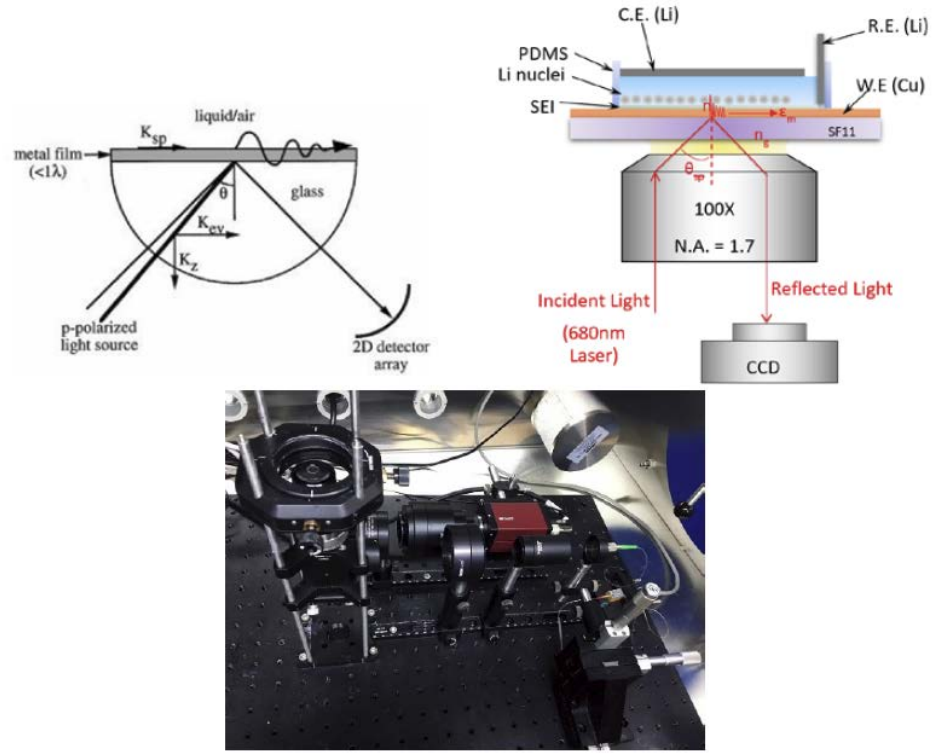


Fig. 2.2 Principle of SPR Imaging. (a) Kretschmann configuration of SPR; (b) Schematic drawing of the SPR setup utilized in lithium metal battery characterization; (c) Real in situ characterization setup picture.

In the next section, a brief description of our intensity interrogation mode SPR thickness detection and imaging principles is going to be discussed.

2.1.2 Simulation: From SPR signal to thickness

The metal plasmonic surface was chosen as Cu since it is minimizing the alloy formation with deposited lithium metal thus introducing a minimal surface destruction, as reported.^{83, 84} On top of this metal plasmonic sensing layer, we wish to establish a direct relationship between the reflectance signal and the thickness of the material deposited. Therefore, we utilized the free open-source surface plasmon simulation software Winspill that is a PC based and computes the reflectivity of optical multiplayer system based on Fresnel equations and matrix formalism. By altering the

deposition layer thickness while fixing the refractive index of the layer constant, we were able to obtain a nearly linear relationship between the change of reflectance and the change of thickness below.

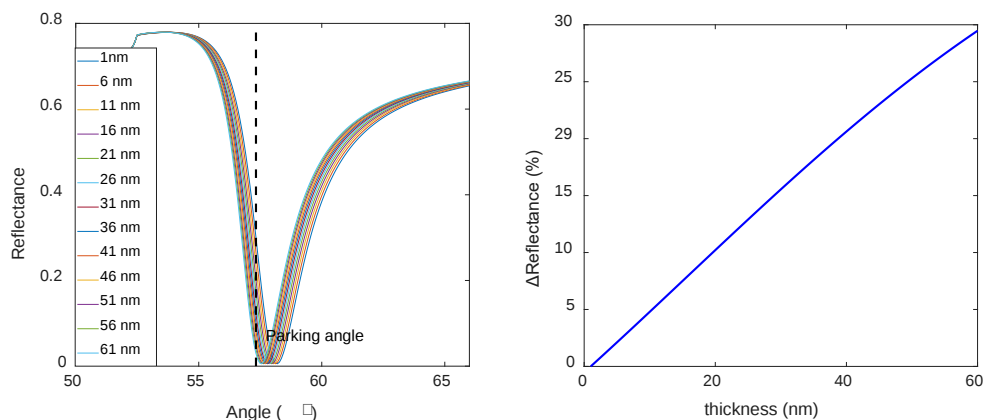


Fig. 2.3 From optical to thickness. (a) Angle simulation of reflectivity under different thickness of SEI; (b) Calibration curve between reflectance vs. thickness at parked angle indicated by dash line in (a).

From the above optical vs. thickness simulation curve, we could readily use the obtained image sequence intensity to quantify how much of the material has been deposited. Note that since our incident angle is fixed at $\sim 30^\circ$ of the total internal reflection intensity, the SPR imaging technique could identify subtle refractive index changes as small as 10^{-6} refractive index unit. A recent study⁸⁵ even brought this detection limit down to 10^{-8} which demonstrates the capability of SPR to perform ultra-surface sensitive measurements.

2.2 In situ Raman Spectroscopy

Raman spectroscopy is a vibrational technique that can provide quantitative and qualitative information about both organic and inorganic compounds on a battery electrode surface. Therefore, in the past decades, Raman spectroscopic studies on graphite electrodes have been reported, where the Li intercalation process in and out

from the cathode was the major research focus. SEI formation,^{47, 86} lithium plating/growth^{87, 88} and lithium ion concentration⁸⁹ were also achievable through this quantifiable technique.

Therefore, for qualitative compositional analysis purpose while maintaining a high level of quantitative characterization capability, we utilized a commercial HORIBA Raman Imaging and Spectroscopy iHR550[®] with a center wavelength of 642 nm, accompanied with a homemade lithium battery imaging kit shown in the picture below:

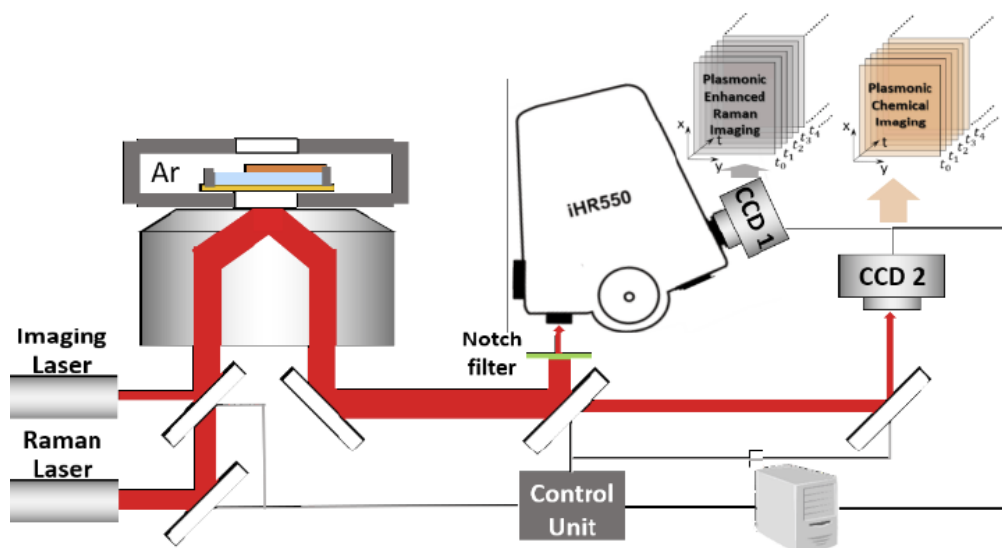
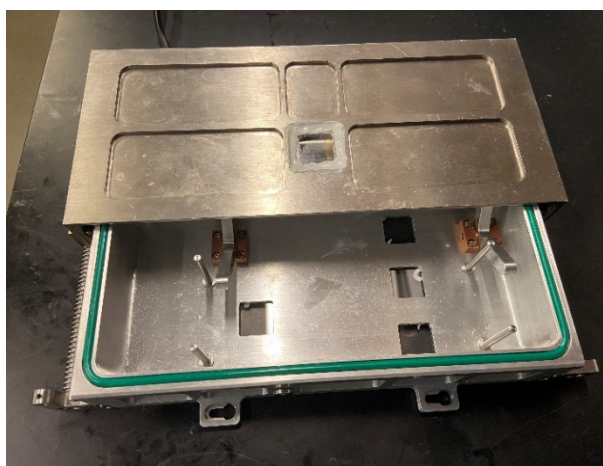


Fig. 2.4 In situ Raman characterization kit. a) home built Raman detection vessel; b) in situ Raman detection system block chart.

Using the home-built Raman detection vessel, we could fill the vessel with inert gas argon to maintain the electrode condition to be pristine. The level of air tightness for the vessel is capable of maintaining 90% of the Argon gas in 2 hours, which is suitable for in situ CV analysis for lithium metal battery electrodes. Directly transporting the detection vessel from the Argon filled glovebox to the outside Raman detection system, we could obtain both the Raman fingerprint lines and the location images simultaneously by virtue of the Fig. 2.4b setup. Thus, in situ images as well as real time Raman signals could be synchronized to allow for detailed analysis of the electrode surface components formed during the battery operation conditions.

Given the capability of running electrochemical experiments in situ, including cyclic voltammetry and constant current lithium plating, it would be easy enough to identify the real surface composition difference with and without corresponding SEI formation as well as the lithium metal deposition through the underlying window. Expected results and analysis will be discussed in later sections.

2.3 In situ Transmission microscopy

As for supplemental projects to this dissertation, including the cancer metabolism and cell impedance imaging, we plan to utilize the current commercialized bright field inverted microscope and accompanied with different objectives for different purposes. Below figure shows the picture of the microscope we were using.



Fig.2.5 Picture of bright field inverted microscope. Olympus IX-83.

Note that using this transmission microscope, it will also make the characterization comprehensive if we could also see the dendrite growth dynamics beyond the ~ 200 nm surface vicinity range, since typically after multiple cycles of charging/discharging, the electrode surface would have already been covered with a lot of as-deposited lithium and that would be over $1\ \mu\text{m}$ thickness range. Therefore, a supplemental light source on top of the SPR schematic shown below is provided to see lithium metal deposition dynamics beyond the surface vicinity.

2.4 Reflection Microscope

Our reflection optical imaging method is based on a TIR (total internal reflection) setup shown in Fig. 2.6a. The light beam from an LED light source was collimated first, as indicated by the red cylinder, and then it is illuminated onto the working electrode through a 60-degree triangle prism with a refractive index of 1.78. By capturing the reflected light intensity coming out from the working electrode, the CCD camera can obtain spatial intensity distribution across the entire working electrode and catalyst surface. During the electrochemical reactions, the reaction products'

concentration will increase and the refractive index of the electrolyte will decrease near the sensing/electrode surface, according to the Fig. 2.6b below where Winspall simulation of reflectivity under different refractive index change values.

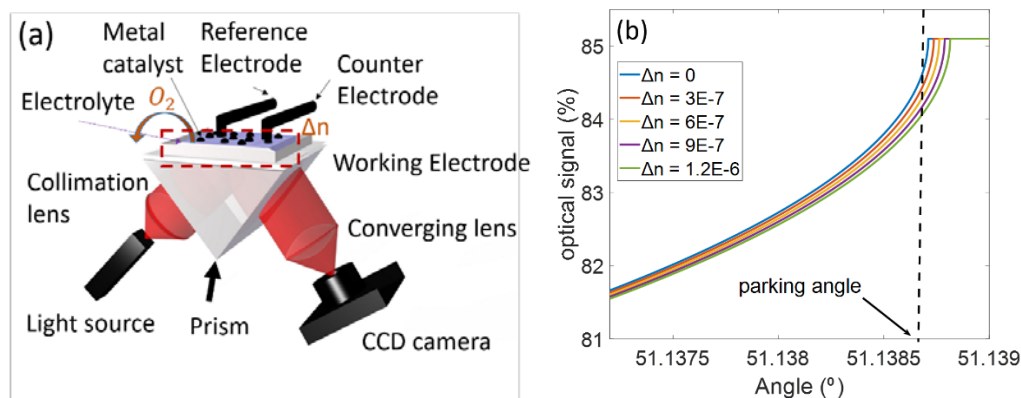


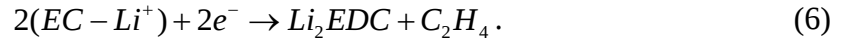
Fig. 2.6. Principle demonstration. (a) Schematic drawing of the catalyst screening setup; (b) Reflectance responses to refractive index changes (Δn). The calculation is performed with Winspall and the electrolyte refractive index is set to be $n=1.33$. The figure shows the reflectance responses with different refractive index changes $\Delta n = 0, 3E-7, 6E-7, 9E-7, 1.2E-6$.

It is also demonstrated that our reflection optical microscope is super sensitive that it has the capability of detection subtle refractive index changes as small as 10^{-8} RIU (Refractive index unit). This detection limit will be discussed later in the section 6.1.

III. IMAGING SEI FORMATION DYNAMICS

3.1 Introduction to SEI formation mechanism

In the characterization process of this research, a traditional electrolyte system containing the lithium hexafluorophosphate (LiPF_6) in common solvent (ethylene carbonate (EC)⁺ dimethyl carbonate (DMC)) will be implemented, and the typical reduction potential of organic solvent is below 1 V.²⁵ Note that having been such a hot research topic, the model of SEI formation on the lithium ion battery anode below this potential has already been established.⁹⁰ According to most research papers, the majority belief lies that the SEI layer is structured out of two separate layers, where the inner layer of SEI is mainly comprised of inorganic components, including LiF , Li_2O and Li_2CO_3 .^{45, 91} The outer layer mainly comes from the component from the reduction of organic solvents and forms a partially soluble component lithium ethylene dicarbonate (LiEDC).⁹² This is the major soluble product⁹³ comes from the reduction of EC in the presence of high concentration of this solvent, according to the following reaction path:



In combination of these two layers, the SEI thus plays an important role as an electron insulator while maintaining a good conductor for lithium ion simultaneously. According to the coulombic interaction mechanism,⁹⁴ before the formation of either the organic or the inorganic layer, due to the potential polarization, an electric double layer forms at the electrode/electrolyte interface caused by the self-assembly of solvent molecules. This is double layer has also been directly detected by real-time mass spectrometric characterization via in-situ liquid-SIMS and will also be spatially

quantified in this chapter to provide a comprehensive picture of this complex and fundamental layer in most lithium metal battery systems.

3.2 Experimental design

Being the performance tuning center and the critical layer, which lays the foundation of lithium metal plating on top of it, solid electrolyte interphase remains the key characteristics of lithium metal batteries, even all non-aqueous liquid battery systems. How well this layer forms, how easy it breaks down and the lasting life of it will all eventually turn into a preferred/worsened battery performance. Almost a ton of research papers have been published, on how to improve this electrically insulative but ionic conductive layer by 1) designing a more compact and uniform and anti-break SEI, 2) providing some artificial SEI layer to serve as the protection, and 3) electrolyte engineering, including mixture solvents and additives. Therefore, the following questions must be answered for each characterization technique:

- 1) What's the function of SEI? Can the characterization provide information or results from these functions?
- 2) How does the SEI form during charging/discharging? SEI formation dynamics and mechanisms.
- 3) What's the morphology of this SEI? How thick is the layer and how mossy is it?
How does it impact the later step of lithium deposition?
- 4) What composition of SEI is formed during the operando? Are there any correlations between these compositions that are fundamental to the battery performance?
- 5) Its failure mechanism, mainly mechanically breaking after long periods of cycling, which lays the road for the lifetime of the battery.

Having been quantified by electrochemical quartz crystal microbalance (EQCM) and in situ gas analysis, the traditional SEI formation process on graphite site was made clearer than before by T. Liu et al that the SEI formation in the presence of

cyclic and acyclic carbonate electrolytes originates preferably from the reduction of the cyclic carbonates, e.g., ethylene carbonate (EC).⁹⁵

However, enough in situ characteristics including both electric double layer and the SEI layer afterwards in the application of lithium metal systems still remain unclear. In this PhD research, primary focus is going to focus on eliminating the complex system requirement while providing a non-invasive method to quantify the SEI formation, including the dynamics with potential, thickness, and composition allocation as well as spatial resolution of the non-uniform SEI layer on electrode (if any). Optical signal trends synchronized with recorded voltage and electrochemical current simultaneously would offer the capability of seeing the time axed responses of electrolyte reduction reactions accompanied with applied potential and the corresponding electrochemical current. Such reaction specific information could not be easily obtained by cyclic voltammetry alone or other traditional characterization means. Sufficiently sampled image sequences showing the surface morphology would also serve as a supplemental spatial information expressor so that the localized formation of SEI aggregates/bulks can be clearly seen, thus the overall performance calculated from either CV curves or current holding trials can be easily attributed.

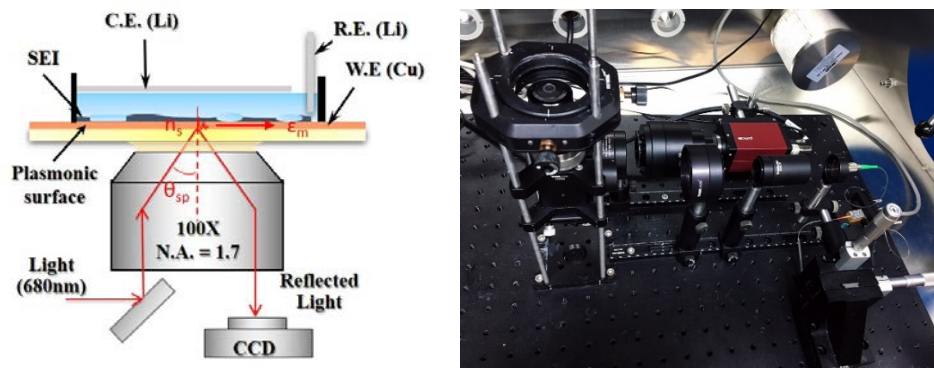


Fig. 3.1. Experimental setup for measurement of SEI. (a) schematic drawing of the setup; (b) real picture of the measurement setup.

By using a popular Kretschmann configuration shown above Fig 3.1a on the left, and by measuring the reflected light intensity on the CCD camera side, we could gain the localized information/reaction happening on the metal surface, which is the surface plasmon generating layer.

The counter electrode (C.E.) was placed in such a way that the uniformity of the E field when applying potential to the working electrode can be maintained. The whole electrochemical cell sits on the 100X Olympus Objective (N.A. = 1.7) by passing through a 47 nm Cu coated SF11 glass ($n=1.7736$) assisted with proper matching oil. The laser light we used to excite Surface Plasma is 680 nm. Applying the Fresnel's equation, the light reflecting back is depended on the surface plasmon condition. The critical angle for SPR is larger the Total Internal Reflection (TIR). The control of the incident position can be done by motor controller (Kinesis K-Cube). CCD camera was used to collect the 2D spatial map of the refraction light intensity. Then, we can synchronize the optical intensity from camera and CV from electrochemical workstation in real-time by using the data acquisition card (National Instruments USB-6009). Since the sensitivity of reactions within 200 nm above the Cu film vertically, the refractive index change of the dielectric medium within this range will cause a the SPR excitation curve shift along the X axis (Y: Reflection intensity, X: Incident Angle). Moreover, the slope of the point at the same position on the curve largely contributes to the surface sensing sensitivity. Therefore, the thickness of the Copper film is designed for excitation of the sharpest cross-section extinction SPR peak, which can be proved by open-source simulation software (Winspall). All the

substrates are rinsed in 1M HCL solution for 5 min and washed by deionized water, excessive ethanol as well as Acetone. Drying them with Nitrogen blow and attach PDMS reservoir to the Cu film surface by Epoxy Resin, followed by an overnight stand to allow Epoxy to fully cure.

Electrolyte Preparation. There are several electrolytes we used for our characterization. The products we bought from Sigma-Aldrich are 1M LiPF_6 in Ethylene Carbonates (EC): Dimethyl Carbonates (DMC) (1:1 in volume ratio), In addition, we have a courtesy supply of the as-prepared 4M Lithium bis(fluorosulfonyl)imide (LiFSI) in 1,2 dimethoxyethane (DME) from Dr. Wu Xu, Pacific Northwest National Laboratory. All electrolyte solutions were prepared and stored in Argon-filled Glovebox (MBraun Labstar, both H_2O and O_2 level are less than 0.5 ppm).

Electrochemical experiments. The electrochemical workstation we used is CHI 660e (CH Instruments, Inc). Our working electrode is Cu thin film, Counter and Reference electrode are Lithium (from Sigma Aldrich, cleaning Li surface by doctor blade). The reaction is triggered by CV scans starting from 2.6 V vs. Li/Li^+ to 0.3 V vs. Li/Li^+ , as well as another set of CV scan which covers lower potential 0.05 V. We chose this potential window to avoid the oxidation of Cu film when at ~ 2.8 V Li/Li^+ and UPD lithium nucleation occurrence close to 0 V vs. Li/Li^+ . The open circuit potential for all the electrolytes we used before experiments are between 3.2 V to 3.3 V vs. Li/Li^+ . The potential referred in this paper are all versus Li/Li^+ .

3.3 Characterization results

During the lithium plating process, a series of electrochemical reactions occur before lithium cation gets reduced into lithium metal on the cathode. Based on the plasmonic signal measured by SPRM during this process and synchronization obtained between electrochemical current and voltage, we can divide the cathodic reaction path into four regions (Fig. 3.2b). Region 1 starts right off the scanning potential starting point, and reaches a minimum at ~ 1.9 V, with a corresponding current peak at ~ 2 V. Region 2 captures the tail of region 1 and follows a slow increasing pattern. Region 3 experiences a major increasing trend over the potential window between 0.9 V and 0.3 V, and reaches a local maximum after passing the potential window lower bound and scanning back to 0.3 V. This region is accompanied by a major electrochemical reduction peak that is repeatable over three cycles (Fig. 3.2e), corresponding to the EC reduction of electrolyte components and formation of the eventual SEI. The last Region 4 is showing a decreasing trend of the plasmonic signal when potential keeps increasing after 0.3 V and touches the potential window upper bound. During this signal decreasing period, the accompanying electrochemical current stays above zero, advocating itself to be the partial oxidation of the previously formed organic SEI components.

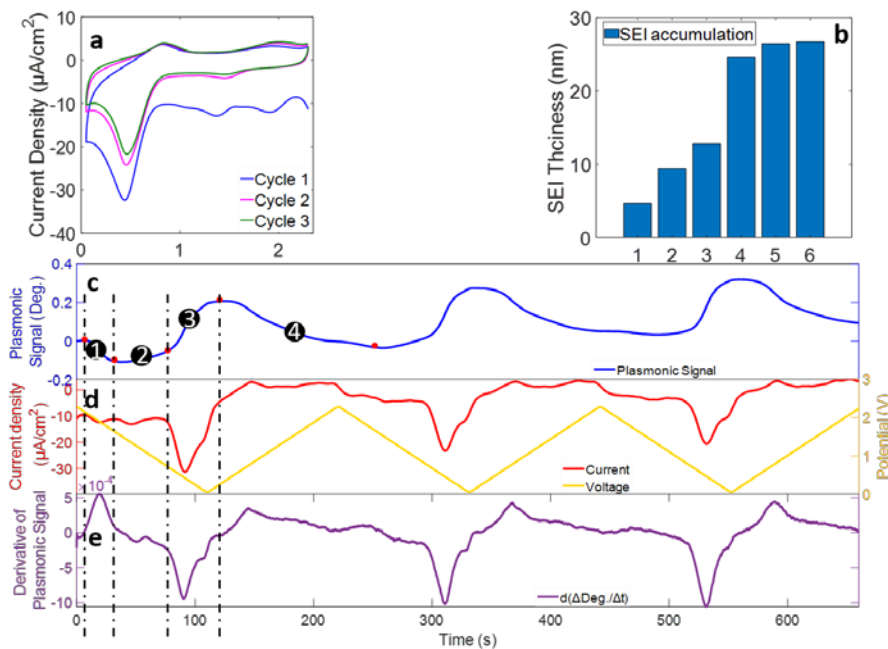
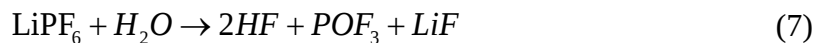


Fig. 3.2 Primary results of SEI characterization. (a) The Cyclic Voltammetry of three cycles using the LP40 electrolyte (1M LiPF₆ in EC/DEC (v:v, 1:1)), starting from 2.6V to 0.3V, scan rate is 5 mV/s. (b) SEI layer thickness accumulation. (c)-(e) Synchronization of SPR optical signal synchronized with electrochemical current, Derivative of SPR intensity, Current, Voltage over the time domain.

As explained in chapter II, in our SPRM system, the critical angle shifts due to the vicinal refractive index change on the plasmonic surface, causing a reflectivity change. This vicinal refractive index alteration could be caused by these four processes, including LiF formation, electric double layer formation/repel and Li⁺ solvation, major EC reduction and precipitation, or other carbonate reduction and dissolution of outer organic layer of SEI previously formed on the electrode. They will be individually characterized and quantified in this section.

3.3.1 LiF formation

In the presence of trace amount of H₂O as a catalyst for hydrolysis of PF₆⁻ anion,²⁶ LiF will form and precipitate, according to either chemical reaction Eq. (7) or electrochemical reaction Eq. (8) as



According to these two equations, the formation of LiF constitutes towards the battery performance as the fundamental protective layer. This could also be directly read from the CV curve in Fig.1e, where a reduction peak only shows up in the first half cycle, while the rest of CV scans only showed the reversible redox peaks around 0.3 V-0.5 V, which is the major reduction of the EC/DEC dual solvent.

Correspondingly, we could find that the initial irreversibility from the plasmonic response, where Region 1 was labelled. The plasmonic signal depth of this dip, represents the amount of decomposition of the fluoride related salt, i.e., the initial formation of LiF at around 1.9 V, of which the potential can be almost perfectly matched with literatures.^{26, 96-98} The purple line on the bottom Fig. 3.2e is the derivative of the plasmonic signal, of which we use to correlate with electrochemical current and voltage windows plotted in Fig. 3.2c. Note that compared to the red curve which represents the electrochemical current, our plasmonic based characterization technique identified the irreversible LiF formation at ~1.9 V, where this peak was buried under in the electrochemical current, meaning this technique could be used to track the transitional electrochemical reactions happening in the battery cell more precisely.

The formation of LiF at high voltage (~1.9 V, Fig. 3.2b) can also be proved by simulation of the refractive index of LiF compound within the vicinity of experimental electrolyte using Winspall. The refractive index of loosely formed LiF at the first

formation state with a refractive index $n = 1.32$,⁹⁹ refractive index of LiF films as a function of time is significantly lower than that of the surrounding electrolyte ($n=1.404$, experimental calibrated), so the actual coupling efficiency of surface plasmon will decrease, resulting in the critical angle to shift according to Eq (5), and finally causes a decreased reflectance collected by CCD camera in Fig. 3.2a.

To confirm the above hypothesis, we prepared a control electrolyte with 50 ppm water. The plasmonic signal of the control electrolyte and the pristine electrolyte were plotted side by side in Fig. 3.3a, where the orange curve located in the bottom of the whole figure showed a good trend of initial decreasing dip within the first 40 seconds, while the sample without the H₂O content, even though the initial dip still exists, it experienced a shorter time of deposition and less amount. This is also accompanied by simultaneous LiF signal increase in the CV (Fig. 3.3b) at 1.9 V. This could facilitate our claim that the first section of plasmonic signal dip comes from the hydrolysis of LiPF₆ salt and this initial layer is typically what most inorganic and organic SEI layer relies on, and is essential to achieve high coulombic efficiencies.

3.3.2 Electric double layer formation

After 1st initial dip, the Cu surface is nicely passivated with an initial layer of LiF and other inorganic components, such as Li₂O and Li₂CO₃.⁹⁰ At lower potential between 1.9 V and 1 V as section 2 labelled in Fig. 3.2b, the collected plasmonic response changes to a slightly lower level caused by electric double layer formation. The pair of corresponding electrochemical current curve and derivative curve within this voltage window represents the repeating electric double layer formation, as within

this potential window on CV (Fig. 3.2e), no obvious redox peaks exist except for the first cycle, meaning only superficial charge transfer and their corresponding accumulation are involved.

To eliminate the possibility that even the micro ampere amount of electrochemical current comes from other possible side reactions, we controlled the potential window to stay within 1.9 V to 1 V (Fig. 3.3f), and we find that the plasmonic signal relaxes to its original level after the potential is released at ~140 s, meaning during the first 140 s, no surface accumulates that can cause a permanent refractive index change to have been detected. Therefore, we supplemented a further experimental proof beyond hypothesis that this preceding process is well established before any reductive decomposition of the electrolyte component,^{45, 100, 101} and by using the intrinsic plasmonic based tool discussed, it's easy to quantify how much and how durable this electric double layer is.

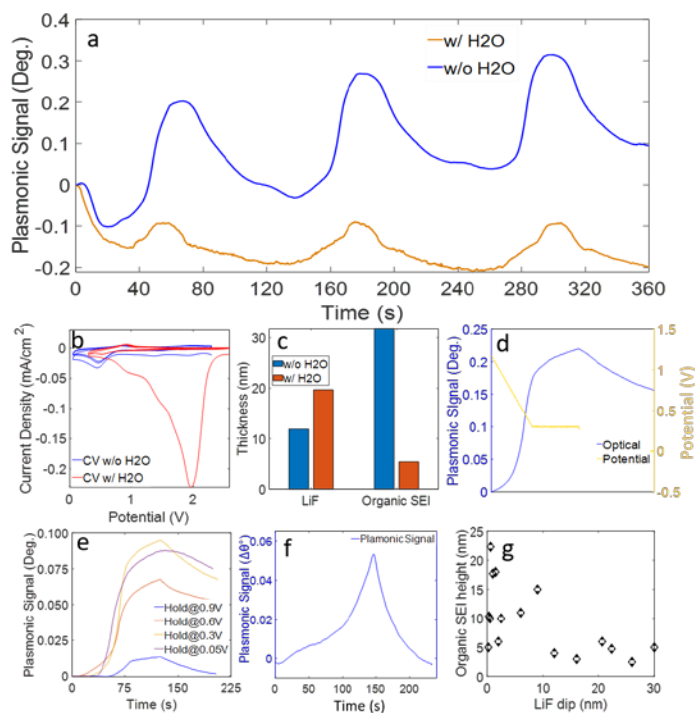


Fig. 3.3. Foundations of the SEI formation dynamics. (a) 1M LiPF₆ in EC/DEC electrolyte with (orange) and without (blue) water content. (b) Corresponding CVs. (c) First cycle thickness growth of LiF and organic SEI. (d) Sweep, hold (@0.3 V) and release using water free electrolyte. (e) Comparative studies of sweep & hold at different potentials. (f) From 1.9V to 1 V and release at ~140s. (g) Organic SEI thickness vs. LiF thickness.

3.3.3 Major SEI formation

As potential moves to even lower and reaches the controlled lower bound ~ 0.05 V and goes back ~ 0.3 V, we observed a major growth of optical signal level, with a relatively faster growing compared to electric double layer formation section 2. This stage of fast growing is caused by the major EC reduction between 0.9 V and 0.3 V, which mainly comes from the cyclic carbonate reduces to linear carbonate radicals and solvates the lithium cation to form the upper layer of SEI.^{92, 102} With the highest steep growing happening around 0.3 V, where the fastest reaction was achieved,^{103, 104} the

reduction reaction forms products like Li_2CO_3 with low EC concentration, and $(\text{CH}_2\text{OCO}_2\text{Li})_2$, with high EC concentration.¹⁰⁵ We chose 0.3 V as a reference and swept the electrode from open circuit potential to 0.3 V and hold the potential at 0.3 V for approximately 120 s (Fig. 3.3d yellow line). Afterwards, we release the potential hold and make the electrode freestanding. Following the yellow line which represents the potential sweep, the blue optical response curve showed an obvious fast increment at the initial stage, while later when the potential was held constant, we see that the plasmonic signal indicates that the SEI layer is still densifying at this vicinity.

However, in a big contrast to Fig. 3.3f where plasmonic signal comes back to original level after releasing the potential, we did not find the similar trend in the plasmonic signal plotted in Fig. 3.3d, where the outer organic SEI experienced only a partial recovery, meaning there are reaction-caused accumulates on the electrode while holding the potential at 0.3 V.

We also controlled the negative electrode potential to stay at 0.9 V, 0.6 V, 0.3 V and 0.05 V, respectively, to study how the densification of the SEI evolves under different potentials. While a potential at ~ 0.9 V did not induce any accumulation, a potential at ~ 0.3 V gives the cathode more capability to decompose the carbonate and assemble a more densified interphase on the electrode. This SEI densification difference is caused by the intrinsic behavior that at the potential of 0.3 V, seen from the Fig. 3.2a CV curve, usually experiences a lot more reductions of the cyclic carbonate reduction and Li^+ solvation, while at other potentials, these reactions are either minimized or diminished according to the recorded electrochemical current. This comparison also serves as an additional evidence that our plasmonic imaging

technique is able to capture the most delicate reaction mechanisms and represent it in a visible and intuitive way.

Interestingly, by performing organic SEI component accumulation using CV technique with different amount of water contented electrolyte and comparing the organic component thickness and LiF thickness, we found a seemingly inverse relationship between the amount of LiF deposited on the electrode surface and the amount of SEI layer accumulated on the same electrode. As can be seen from the scatter plot located in Fig. 3.3g, the more LiF deposited on the electrode, the less organic SEI layer gets deposited. We propose that this relationship can be utilized to control the SEI growth structure, thus tuning the inorganic proportion while maintaining a desired proportion of outer layer of SEI. Therefore, a controlled SEI structure that promotes a high battery cycling performance can be readily benchmarked and quantified.

3.3.4 Organic SEI dissolution

As has been discussed by T. Liu et al,⁹⁵ the composition of SEI layer is usually amorphous and messy, generally comprised of an inner inorganic layer and an outer organic layer. During the charging/discharging operation cycles, both two layers play key roles in maintaining a good Li^+ conductor while electron insulator at the same time. The inner layer usually is composed of Li_2O , Li_2CO_3 , LiOH and LiF , while the outer layer is usually organic with more soluble contents, like $\text{LiOCO}_2\text{C}_2\text{H}_5$, $\text{LiOCO}_2\text{CH}_3$ and LiEDC , with a relatively higher solubility, as has been discussed and demonstrated by Ken Tasak et al^{93, 106}, and LiEDC can be re-oxidized to form both

solid and gaseous products.⁹⁵ Given these facts, we believe that section 4 is caused by outer organic layer's oxidation, and it can be accelerated via applying an oxidizing potential on the working electrode, as shown a decreasing trend in Fig. 3.2b.

Note that in the obvious decreasing trend located in Fig. 3.2b section 4, even though the overall trend is decreasing, the speed/slope of decreasing was not necessarily to be as the same as while it was increasing. This could be confidently explained by the thermodynamically unfavorable behavior of the LiEDC to be oxidized compared to the reduction process,⁹⁵ so the SEI accumulates tend to have more retention ability on the electrode surface rather than being stretched away. This could also be caused by the mossy structure of the as-deposited SEI on the electrode, preventing the material from being oxidized. And since section 4 also involves the electric double layer expelling at a later stage within the potential window, a slower decrease of plasmonic signal is expected.

3.3.5 Analysis of Organic SEI vs. LiF

Having completed the four distinct regions of SEI formation, we were able to establish a comprehensive model of the SEI formation dynamics in the traditional carbonate-based electrolytes. However, this model is not yet 100% complete until I noticed a seemingly reverse relationship between the LiF and the organic SEI shown below:

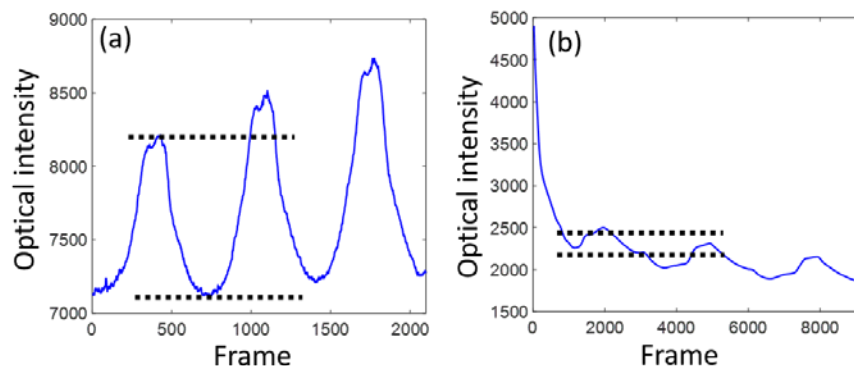


Fig. 3.4. Representative trials of SEI characterization. (a) Optical signal with less LiF; (b) Optical signal with more LiF. Organic SEI represented by black dash line.

Obviously telling from the above comparison curve, the optical signal of organic SEI (black dash line) in the electrolyte with less LiF in Fig. 3.4a was much bigger than the counterpart signal in the electrolyte with more initial LiF. Actually, this reverse relationship could be repeated with even more experimental trials. To prove this reverse relationship hypothesis, I placed more attention to the relationship and conducted similar experiments. The 2-D plot between organic SEI vs. LiF signal is shown in the scatter plot:

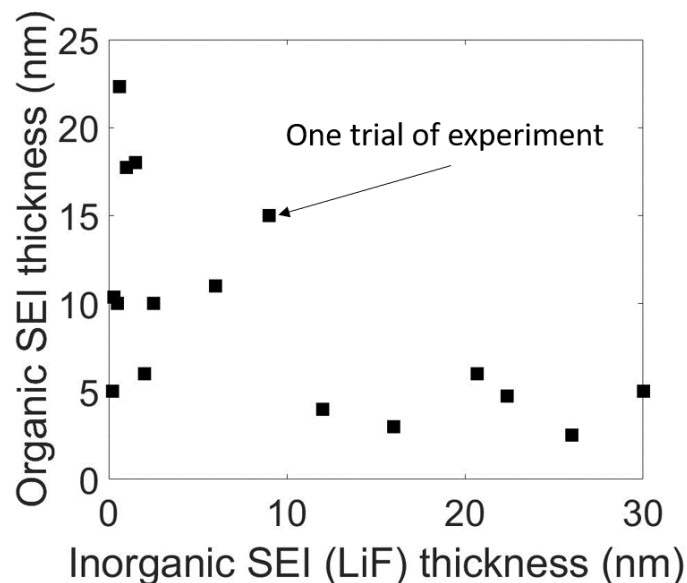


Fig. 3.5. Scatter plot of multiple trials of SEI characterization between organic SEI vs. Inorganic.

where each black dot in the scatter plot represents one single trial of experiment. This scatter plot is good enough to tell us that there is indeed some reversal relationship between these two signals, where the initial deposition of LiF signal is low, the corresponding organic SEI signal is high, and vice versa. We propose this reverse relationship is caused by the tuning capability of the SEI structure: when the amount of inorganic layer majority (LiF) is taking its part, the parasitic consumption of the electrolyte could be minimized, resulting in a less organic SEI layer. On the other hand, if there is not enough of performance ensuring initial layer deposited on the electrode surface, the continuous consumption of the electrolyte will happen, which we do not necessarily desire.

Therefore, using our plasmonic method, this subtle and hard-to-characterize reverse relationship could be identified, and it could be used to control the structure of the deposited SEI, so that desired amount of inorganics could be obtained to ensure the repeatability of the battery cell.

3.3.6 Analysis of electric double layer among electrolytes

On top of these obtained optical signal regarding the SEI formation dynamics, I found that the situation of the electric double layer formation before the major SEI is also not quite the same. As the following figure shows,

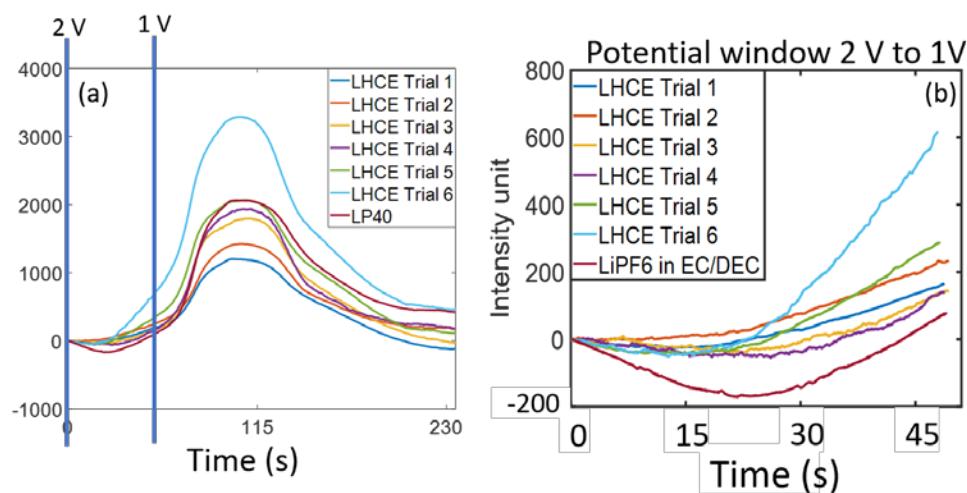


Fig. 3.6. Comparative plot of electric double layer formation among different electrolytes. (a) Optical response of the first cycle for localized high concentration electrolyte (LHCE) vs. traditional carbonate electrolyte (1M LiPF₆ in EC/DEC, LP40); (b) Zoom in of the observation window from 2 V to 1 V.

where within the same observation window (2 V to 1 V), the red curve Fig. 3.6b showed the initial signal decrease at ~ 15s, whereas for the advanced electrolyte, the formation of the electric double layer (represented by signal increase on plasmonic surface) was triggered almost instantaneously with the sweeping potential. This is because in the carbonate electrolyte, the PF₆⁻ anion experiences the hydrolysis and the product HF will react with solvated Li⁺ to form the LiF precipitation at 1.9 V. While for the LHCE, no PF₆⁻ anion is present in the electrolyte, therefore the LiF formation is minimal. This distinction also fertilized our method's capability to capture the transitional chemical reactions in battery systems.

3.4 Qualitative information acquisition using Raman spectroscopy

One of the most important question that is being asked for SPR technology, is what exactly the surface compositions are there on the sensing plasmonic surface. Therefore, we collaborated with Dr. Zheng Fan from College of Technology at University of Houston, to construct an in situ vessel for spectrum analysis. The real picture of the detection vessel is as follows:

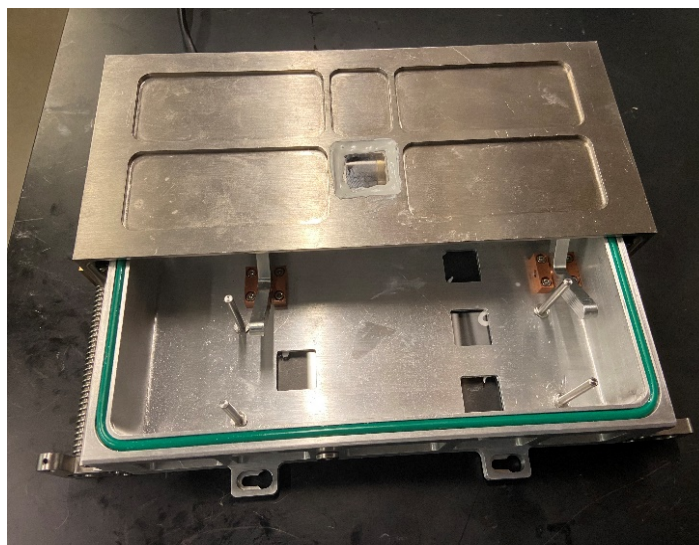


Fig. 3.7 Real picture of in situ Raman detection vessel.

where the electrode leads and observation windows were reserved for the connection of the in situ configuration of the battery cells. This in situ Raman detection vessel is also air tightened enough to accommodate the effective cycling of lithium metal battery trials.

Using the detection vessel, we performed the in situ formation of LiF on the Copper plasmonic surface by sweeping the potential inside the Argon filled glovebox. After that, we place the vessel on the Horiba Raman imaging system accompanied with a central wavelength laser of 642 nm, so that multiple regions of LiF deposited

Raman signals of the sensing substrate were acquired, with sufficient comparison of the background without any LiF compositions.

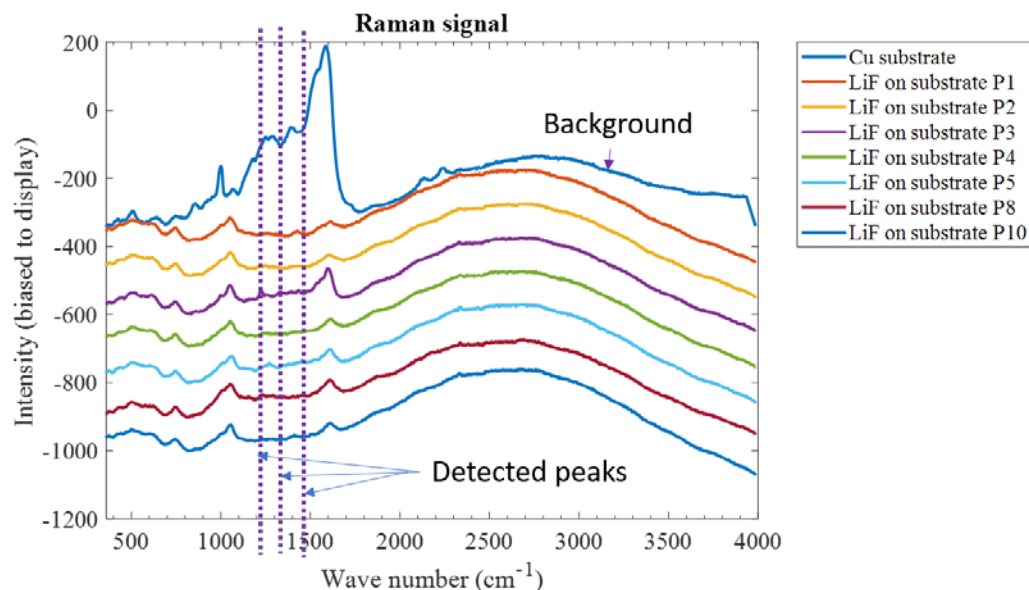


Fig. 3.8. Raman shift peak locations of surface SEI components.

As can be told from the Raman signal plot above, note that most of the majority peaks located on the spectrum come from the background fluorescence, including the peak at 1600 cm⁻¹, the peak at 1050 cm⁻¹ and the peaks at smaller wavenumbers. Though buried under these background peaks, we could still see the optical shift three peaks at ~ 1250 cm⁻¹, 1350 cm⁻¹ and 1430 cm⁻¹. Having the background signal on hand and plotted side by side, we have enough confidence that these three peaks are caused by the LiF formation on the substrate, especially for substrate P3.

However, due to the fact that there is not yet enough readily available Raman peaks database, these detected three Raman peaks need more solid evidence to support

the hypothesis. In the future, a further enhancement of the Raman signal to noise ratio will be needed.

3.5 Conclusion

In this chapter, I have established a comprehensive model and characterized the formation process of the solid–electrolyte interphase on Cu cathode at the nanoscale. Four distinct regions of the whole CV scan range have been identified and quantified. (1) LiF formation at 1.9 V (Fig. 3.3b); (2) electric double layer formation (Fig. 3.3f); (3) initial and major EC reduction at 0.6 V-0.3 V (Fig. 3.3d e); (4) lithium alkyl carbonates produced by EC reduction are partially re-oxidized during anodic scan above 0.3 V. An overall accumulation of SEI thickness ~28 nm after six cycles of CV based SEI formation was observed, and individual spatial non-uniformity has been detected as well. Change of electrolyte seems to alter the SEI accumulation speed, and further investigation is needed to explain the origin of this effect. We hope that this level of detail can provide useful guidelines for designing and tailoring better interphases for new battery chemistries.

IV. CHARACTERIZATION OF SEI FORMATION DYNAMICS IN ADVANCED ELECTROLYTE SYSTEM

4.1 Highly concentrated electrolytes

As well discovered, Lithium Ethylene Dicarboxylate (LiEDC) is the major SEI component in SEI layer,⁹² and most of the studies have been focused on improving this LiEDC rich interphasial layer's performance, like putting an engineered artificial passive layer^{9, 11-15, 23, 24, 107-120} on the electrode, adding electrolyte additives^{16, 121} to facilitate the layer compactness and alternating the solvent from carbonate to ether based solvents.^{122, 123} Subsequently, we would also like to benchmark the transitional reactions during the SEI formation process for these advanced engineered electrolytes. We swap the electrolyte system to a highly concentrated alternative, where the major lithium salt has been changed to 4M LiFSI and the solvation agent is DME, which is proven to be more effective on forming a compact and stable SEI layer.¹²⁴⁻¹²⁸ From the Fig. 4.1 synchronization plots, we have noticed several differences from traditional electrolytes as follows: (1) Additional electrochemical current peaks at high potential (>1.5 V); (2) The slope change of each cycle from the electric double layer to major SEI formation is also more drastic than traditional carbonate electrolyte response, meaning that given the abundance of lithium cation solvated into FSI⁻ anion,¹²⁹ the turnover from electric double layer to faradaic reaction became more favorable. These differences further demonstrated that the high concentration electrolyte's superior performance potential compared to carbonates, as it could preserve more lithium ionic

solvation into the salt anion and promote thermodynamically favorable motions of these ion movements inside the electrolyte.

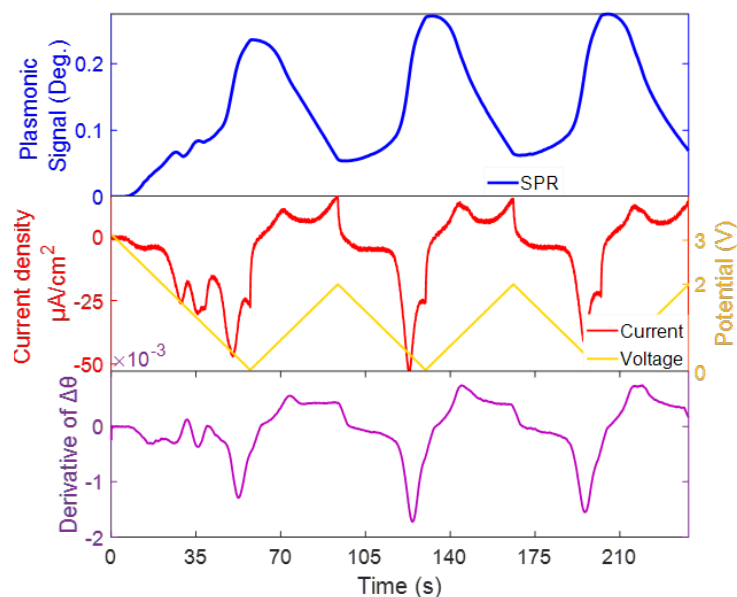


Fig. 4.1 SEI formation dynamics of high concentrated electrolyte (HCE, 4M LiFSI in DME).

4.2 Localized highly concentrated electrolytes

One noticeable alternative electrolyte system that proves to be coulombic efficient and repeatable is using ether-based electrolyte solvent instead of traditional carbonate based solvent. Yet few of these purely ether-based electrolyte systems possesses superior performance under more stringent conditions, including high voltage and stability of the SEI layer formed on lithium metal anodes. Therefore, multiple alternatives of more advance engineered electrolytes containing either bis(2,2,2-trifluoroethyl) ether (BTFE) or the ,1,2,2-tetrafluoroethyl-2,2,3,3tetrafluoropropyl ether (TTE) as the co-solvent.¹³⁰ These advanced electrolytes have proven to be more coulombic efficient and with high cycling efficiency. However, currently there is yet

to be sufficient in situ characterization methods for these electrolytes which can provide quantifiable information as well as electrode images simultaneously, even SEM images have been obtained and the surface structure of these electrolyte containing electrodes have superior morphologies.

Therefore, in this section, I am going to present the characterization technique discussed and perform the in-situ benchmarking on this kind of advanced electrolyte.

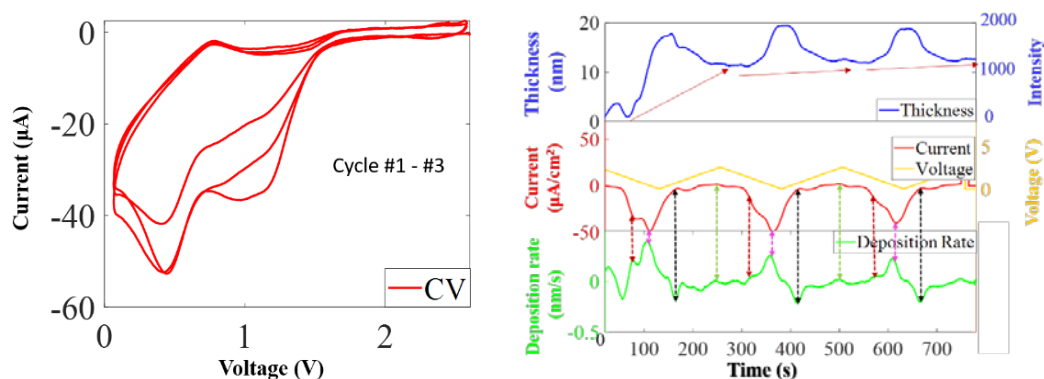


Fig. 4.2. SEI characteristics of localized high concentration electrolyte (LHCE). (a) CV of SEI characterization process in LHCE. (b) Representative characteristics of LHCE SEI accumulation.

As the above Fig. 4.2a shows, in the CV, we could see that it poses similar redox peaks with EC/DMC based electrolytes, and with good current correspondence, as the Fig. 4.2b shows between the green curve and the red curve. This gave us the possibility to quantify the reactions in situ.

However, the most important difference lying in this advanced electrolyte is the significant irreversibility meaning the initial deposition was much bigger than the traditional electrolyte. Compared to the traditional carbonate electrolytes in Fig. 3.2, where the SEI accumulation keeps accumulating with a minimal step each cycle, this localized electrolyte showing this first-cycle-only irreversibility signals that the

composition of the electrolyte constitutes the superior performance on the SEI formation, of which the first cycle's irreversibility is most believed to be the intrinsic behavior of the layer. This could be caused by the synergistic decomposition of the electrolyte solvents in the presence of both DME and the TTE and thus needs further investigation to solidify.

4.3 Electrolyte additives effect on the SEI formation

One of the most popular electrolyte additive to enhance the performance of the carbonate electrolyte cycling is Fluoroethylene Carbonate (FEC).¹³¹ Proven to be supportive in the process of lithium ion solvation and facilitate the formation of the most important SEI composition LiF, FEC could also be utilized to promote the controlled structure of SEI. Therefore, we supplemented our characterization study via the extra 10% FEC being added to the traditional carbonate electrolyte, 1M LiPF₆ in EC/DEC. Using a similar electrochemical workstation and electrode configuration as before (R.E and C.E: Lithium foil, W.E.: Copper thin film, 47 nm), we found that there is a huge difference between the electrolyte with and without the FEC additive, as shown in the below figure:

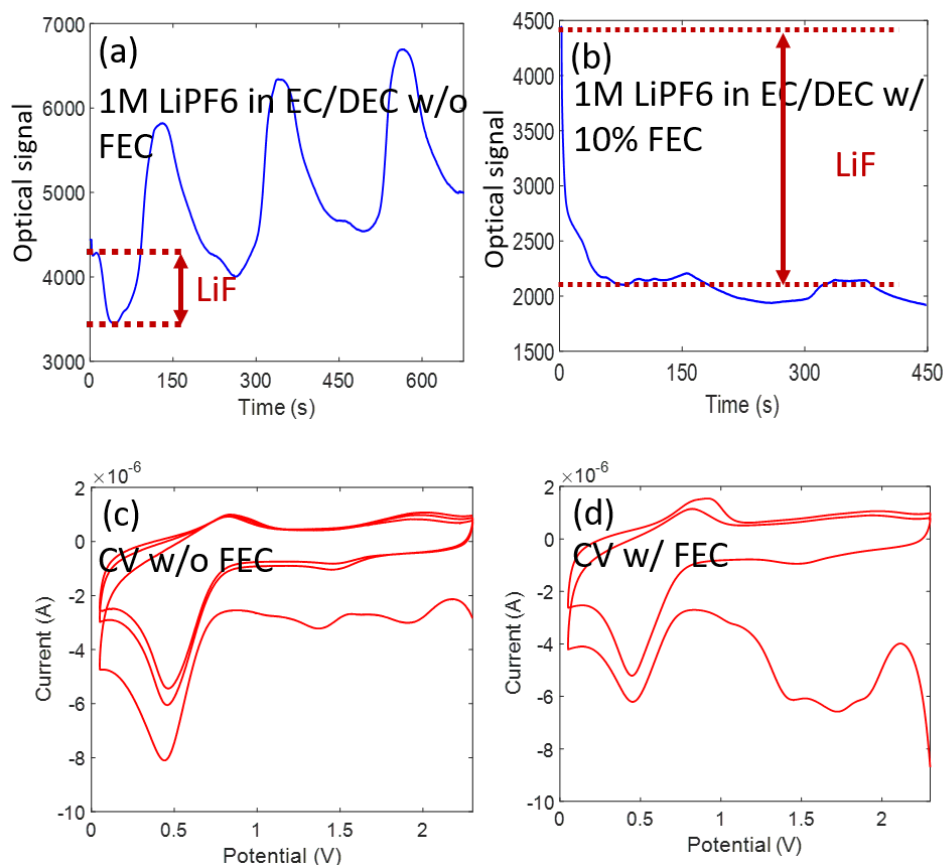


Fig. 4.3. FEC as the alternate additive to carbonate electrolyte. (a)optical signal without FEC; (b) optical signal with 10% FEC; (c) CV of electrolyte without FEC;(d) CV of electrolyte with 10% FEC.

From the above comparison figure, we could see that as the electrolyte is pure without any additive, the initial LiF optical signal was minimal, with the following SEI formation 3 cycles being similar with the previous characterization results, even with some electrochemical current peaks showing up in the CV under Fig. 4.3c.

However, with the extra added 10% FEC to the electrolyte system, not only does the electrochemical current on CV showed a much bigger reduction peak around 1.6 V, but also the optical signal on the top Fig. 4.3b showed the incomparable signal decrease before the first cycle of organic SEI formation. This is caused by the reduction of the FEC (mainly comes from the cyclical de-fluorination) into the product

of LiF and precipitate onto the electrode surface, causing the adjacent refractive index to drop below the electrolyte. This is also a good aspect of the additive effect, as the addition of fluoride element to the electrolyte actually promoted the dense layer of LiF formation, while preserving the electrolyte from being further consumed, thus could lead to a higher charging/discharging performance.

In addition to FEC, water has also been proven to be effective on the formation of the initial layer of LiF on the electrode surface. Therefore, we continued with our characterization study via the extra 50 ppm H₂O being added to the traditional carbonate electrolyte, 1M LiPF₆ in PC. Using a similar electrochemical workstation and electrode configuration as before (R.E and C.E: Lithium foil, W.E.: Copper thin film, 47 nm), we found that there is a huge difference between the electrolyte with and without the water additive, as shown in the below CV:

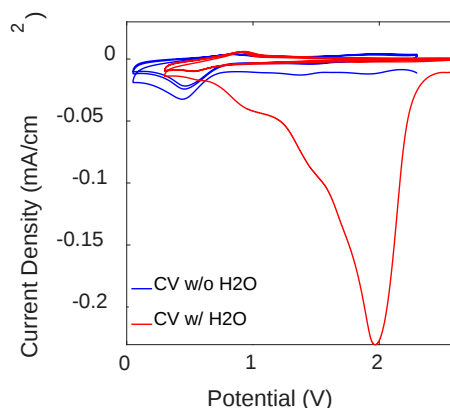


Fig. 4.4. CV of electrolyte with and without water additive.

where the blue curve represents the CV for the electrolyte without the water additive, and the red curve represents the CV for the electrolyte with the 50 ppm H₂O added to the electrolyte. We could see that with the extra amount of water additive, the red CV gave us a huge reduction peak at ~ 2 V, corresponding to the formation of LiF.

And with this electrochemical current distinction being held on hand, we plotted the corresponding optical responses of these two electrolytes below:

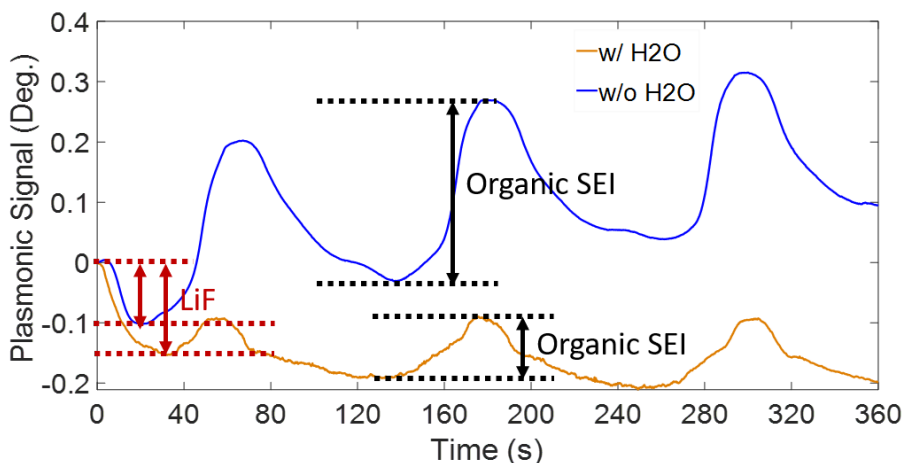


Fig. 4.5 Optical signal comparison between electrolyte with and without H₂O additive.

where the LiF signal was much bigger on the plasmonic signal with water additive, while the LiF signal was smaller for the pure electrolyte counterpart. The corresponding optical signal of the organic SEI was also identified to be quite different from each other, as the reverse relationship being discussed earlier in chapter III.

4.4 Conclusion

In this chapter I have characterized the SEI process of the solid–electrolyte interphase on Cu cathode at the nanoscale with some advanced electrolyte systems, including high concentration electrolyte, localized high concentration electrolyte and lithium perchlorate in EC/DMC. Given the capability of detecting similar SEI accumulation trends over multiple CV cycles using lithium foil as counter and reference electrode, we also noticed some key differences in these electrolytes which prove to be a good sign for the advanced performance of these electrolytes. For example, in the high concentration electrolytes (HCE), some additional

electrochemical reaction current peaks have been observed in the optical signal and the transition from the electric double layer to major SEI formation dynamic was also different from the traditional carbonate electrolytes. On the other hand, the initial deposition or irreversible and inorganic SEI layer on the cathode surface showed up more drastically and could be verified with the acquired optical signal with both water and FEC additive treated electrolyte. In summary, the change of electrolyte components seems to alter the SEI formation dynamics fundamentally, and further investigation is needed to explain the origin of this effect.

V. IMAGING LITHIUM NUCLEATION DYNAMICS

5.1 Introduction to lithium nucleation

A combination of the traditional EIS and SEM provided some new insights on lithium dendrite formation in lithium metal anodes, but the surface morphology was captured after multiple trials of constant current lithium depositions, therefore there is no way to know how the surface grow in situ and before maturing. On the contrary, a lot of measures have been taken to provide a practical lithium metal anode, including self-healing, artificial SEI, electrolyte flow control, additives, electrolyte engineering, and so on. Such great efforts put the urgent need of a solid and comprehensive approach of dendritic structural and compositional characterization to the next level. A cutting-edge synchrotron hard X-ray microtomography was used to look at the dendrite formation underneath the lithium electrode, where Katherine J. Harry et al concluded that preventing dendrite formation in polymer electrolytes depends on inhibiting the formation of subsurface structures in the lithium electrode. Such insightful progress even makes the demand of in-situ characterization technique even higher, that's to say, if we could directly observe the subsurface structures during the lithium metal battery operation, we are generating the benchmarking tool for this prevention target.

Theoretically, all batteries degrade upon prolonged time of charging/discharging cycling. Despite superior electrochemical performances of metallic lithium anodes, it suffers from constant dendrite formation coming from non-uniform lithium nucleation. Enormous efforts have been made to tackle the dendrite growth,^{1, 2, 132-136} since it is the main issue that is causing the low columbic efficiency and safety issues in most

lithium metal batteries (LMBs). A uniform and mechanically strong enough lithium deposition is usually desired for most applications. More recently, a novel technique which utilized the short oxidative pulse to introduce the lithium deposition sites on top of the pre-formed SEI has been studied and proven to be more effective towards formation of a compact and uniform lithium deposition.¹³⁷ Therefore, taking all these aspects into account, in order to capture these transitional electrochemical reactions which induced dendrite formation, the characterization technique should possess the following characteristics:

- 1) **Real time and fast tracking** of the nucleation process, as the smallest structural change and morphology evolution could happen within sub second level during battery charging/discharging;
- 2) **Non surface destructive**, noninvasive, since the real operation conditions would be better preserved rather than altered, and the surficial process usually involves intermediates demanding good surface conservation;
- 3) **Reasonable spatial resolution** to see the dendrites/nuclei as small as possible, so that subtle structural change that contributes the overall briskness could be identified.

In this chapter, significant number of pages will be focused on characterization and visualization of the lithium nucleation and dendrite formation. Surface plasmon signal caused by mossy and non-uniform lithium deposition will be studied and benchmarked. Pre-formation of SEI layer before the metallic lithium growth and stripping process will also be directly observed to serve as a supplement to the major characteristic of the post formed metallic lithium.

5.2 Experimental design

In order to capture the transitional processes of lithium metal nucleation/plating according to those requirements stated in the last chapter, we plan to utilize the as-developed surface plasmon imaging system below to perform the imaging of these negative potential induced reactions.

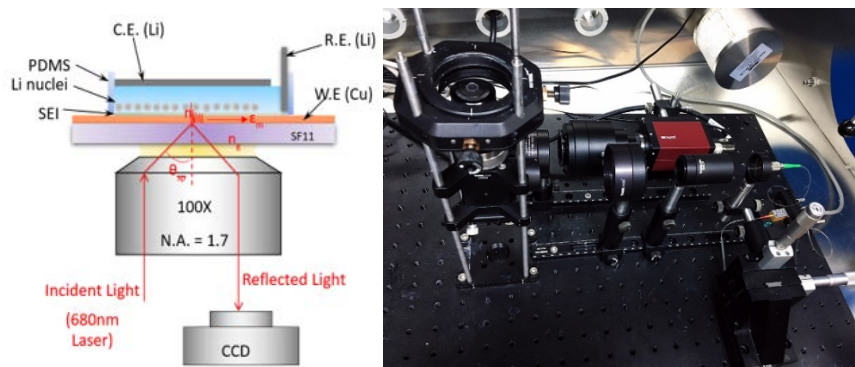


Fig. 5.1 Experimental setup for measurement of lithium nucleation. (a) Schematic drawing of the setup; (b) real picture of the measurement setup.

By using the same Kretschmann configuration shown above Fig 5.1a on the left, and by measuring the reflected light intensity on the CCD camera side, we could gain the localized lithium nucleation information happening on the metal surface, which is the surface plasmon generating layer. Such plasmonic signal could reach the detection range as deep as 200 nm, which could be used to establish the relationship between the pre-formation of SEI on the electrode and the post-formation of the lithium particles.

The counter electrode (C.E.) was placed in parallel with the working electrode so that the uniformity of the E field when applying potential to the working electrode can be maintained. The whole electrochemical cell sits on the 100X Olympus Objective (N.A. = 1.7) by passing through a 47 nm Cu coated SF11 glass ($n=1.7736$) assisted with proper matching oil. The laser light we used to excite surface plasma is 680 nm.

Applying the Fresnel's equation, the light reflecting back is depended on the surface plasmon condition. The critical angle for SPR is larger the Total Internal Reflection (TIR). The control of the incident position can be done by motor controller (Kinesis K-Cube). CCD camera was used to collect the 2D spatial map of the refraction light intensity. Then, we can synchronize the optical intensity from camera and CV from electrochemical workstation in real-time by using the data acquisition card (National Instruments USB-6009). Since the sensitivity of reactions within 200 nm above the Cu film vertically, the refractive index change of the dielectric medium within this range will cause a the SPR excitation curve shift along the X axis (Y: Reflection intensity, X: Incident Angle). Therefore, the thickness of the Copper film is designed for excitation of the sharpest cross-section extinction SPR peak which can be proved by open-source simulation software (Winspall). All the substrates are rinsed in 1M HCL solution for 5 min and washed by deionized water, excessive ethanol as well as Acetone. Drying them with nitrogen gas flow and attach PDMS reservoir to the Cu film surface by Epoxy Resin, followed by an overnight stand to allow Epoxy to fully cure.

Electrolyte Preparation. There are several electrolytes we used for our nucleation characterization. The products we bought from Sigma-Aldrich are 1M LiPF_6 in Ethylene Carbonates (EC): Dimethyl Carbonates (DMC) (1:1 in volume ratio). All electrolyte solutions were prepared and stored in Argon-filled Glovebox (MBraun Labstar, both H_2O and O_2 level are less than 0.5 ppm).

Electrochemical experiments. The electrochemical workstation we used is CHI 660e (CH Instruments, Inc). Our working electrode is Cu thin film, Counter and

Reference electrode are Lithium (from Sigma Aldrich, cleaning Li surface by carbon steel blade). The reaction is triggered by CV scans starting from 2.6 V vs. Li/Li⁺ to lowest -0.7 V vs. Li/Li⁺. We chose this potential window to avoid the oxidation of Cu film when at ~ 2.8 V Li/Li⁺. The open circuit potential for all the electrolytes we used before experiments are between 3.2 V to 3.3 V vs. Li/Li⁺. The potential referred in this paper are all versus Li/Li⁺.

5.3 Results of lithium nucleation

To establish the model of lithium nucleation, sand's time model has been proposed to describe the whole process for metallic dendrites as

$$\tau = \pi D \left(\frac{eC_0}{2Jt_a} \right) \quad (9)$$

where D is the electrolyte diffusion constant, e is the elementary charge, C_0 is the initial concentration of the electrolyte and J is the effective current density and t_a is the anionic transport number. Therefore, to provide a more comprehensive picture given this formula, different current density will be chosen as the J is inversely proportional to the initiation time of dendritic nucleation(dynamics). And changing D , the diffusion constant, mainly through tuning different electrolyte composition/concentration will also benefit making the whole characterization complete and more accurate.

Using the traditional 1M LiPF₆ in EC/DMC electrolyte system, a time dependent lithium nucleation optical signal along with the corresponding potential and current is synchronized below:

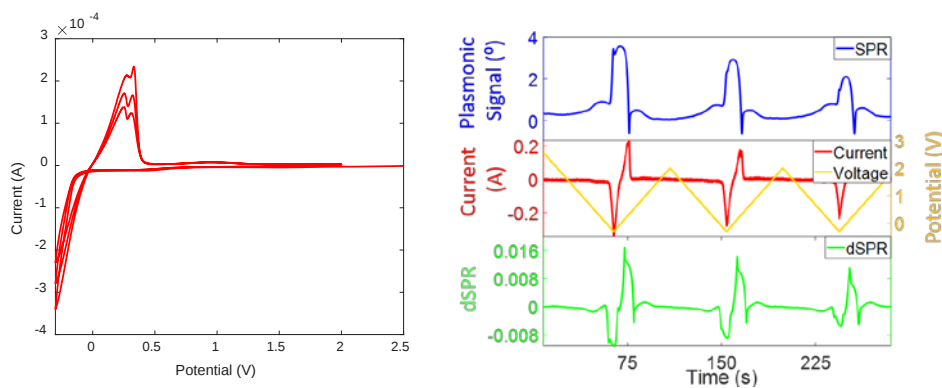


Fig. 5.2. Lithium nucleation characteristics. Cycle #1-#3. (a) CV curve for the lithium nucleation. 2.5 V to -0.3 V. Scan rate:0.05 V/s, R.E/C.E: lithium foil, W.E: Cu. Electrolyte: 1M LiPF₆ in EC/DMC. (b) Optical signal characteristics of lithium nucleation. Blue curve: optical; yellow curve: potential; green curve: derivative for the blue optical signal; red curve: electrochemical current.

As can be seen from the above lithium nucleation curves, since the potential covers more negative potential points beyond zero volts compared to SEI formation, we could see the plasmonic nucleation signal after the potential has passed zero volt, with a very good corresponding electrochemical current and the derivative optical located in the bottom of Fig. 5.2b. Note that on the blue curve, the sharp signal sitting at the potential window below zero is caused by the reduction of lithium cation into lithium atom. Notably, before and after this huge signal, we also notice that there are shoulders on both directions of the nucleation signal, representing the reversible SEI layer formation before and after the lithium nucleation. This transitional reaction response has thus been identified from the pre-step for lithium nucleation.

Going on with the lithium nucleation, we found that the symmetry was enhanced, as shown below in Fig. 5.3 where both symmetric shoulders lie on both sides of the nucleation signal. We think this is a good sign as more nucleation cycles progress, the underlying protection layer still forms to prohibit the parasitic consumption of the electrolytes.

At the same time, the baseline of the nucleation signal also increases as the number of cycles increase, meaning the as-deposited lithium metal on the electrode surface could not be 100% stripped over elongated cycling. This parasitic phenomenon actually causes the lithium battery to degrade over periods of charging and discharging, and thus we believe under practical conditions, a better and more compact SEI layer should be there to protect the lithium anode.

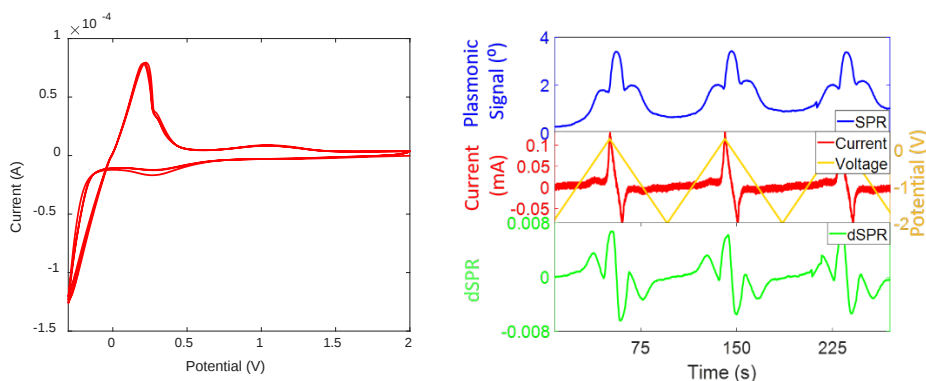


Fig. 5.3. Lithium nucleation characteristics. Cycle #4-#6. (a) CV curve for the lithium nucleation. 2.6 V to -0.7 V. Scan rate: 0.05 V/s, R.E/C.E: lithium foil, W.E: Cu. Electrolyte: 1M LiPF₆ in EC/DMC. (b) Optical signal characteristics of lithium nucleation. Blue curve: optical; yellow curve: potential; green curve: derivative for the blue optical signal; red curve: electrochemical current.

We could also extract different images over the entire cycle and try to compare them side by side to provide the capability of spatial resolution. In the below figure, different stages of lithium nucleation points have been plotted according to the potential points.

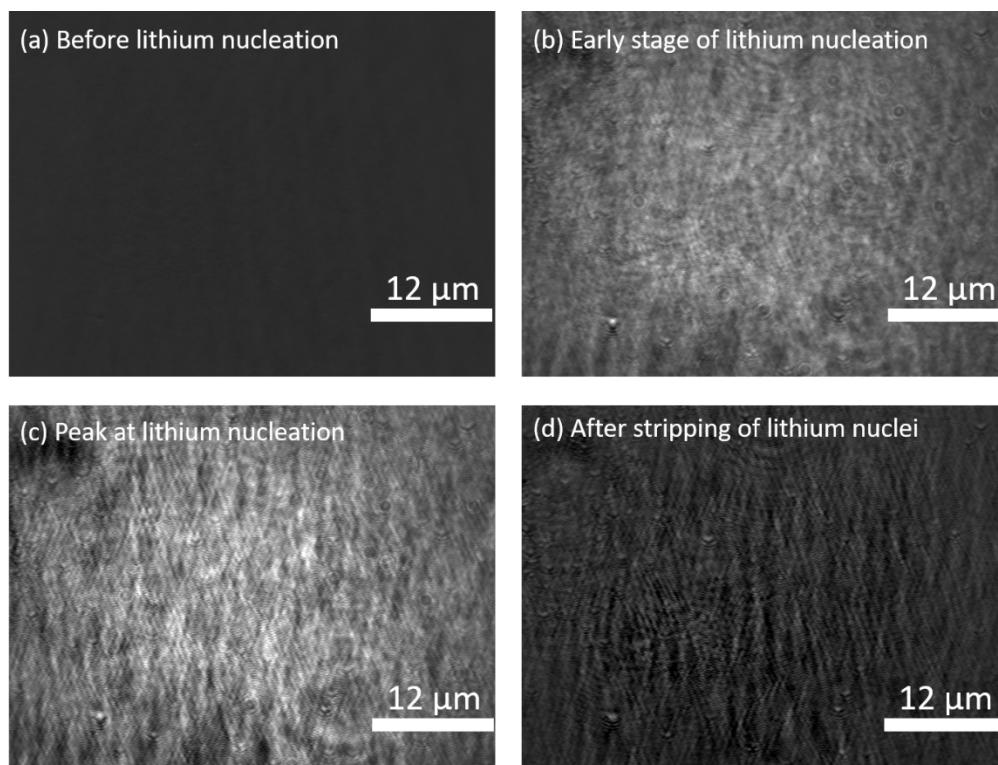


Figure 5.4 Lithium nucleation process images over the entire cycle.

We can see that during the entire cycle of lithium nucleation, the electrode surface experienced several stages of non-uniformity:

- 1) First, before the onset of lithium nucleation, Figure 5.4a showed a very clean surface image of the electrode, where no particles were there;
- 2) Second, when the potential touches the point of 0 V, some sites on the electrode began to form the initialized nuclei, non-uniformity has started to show up;
- 3) When the potential reaches the lowest point where the working electrode was set to be -0.3 V, a lot of clusters and individual non-uniformities were there, accompanied with highest optical reflectivity;
- 4) After the completion of lithium stripping, the optical intensity went back to lower level, however, at this point, the image intensity is not 100% equal to

figure 5.4a, as there are some residuals staying on the electrode surface, representing the non-perfect reversibility of the battery system.

With such spatial resolved lithium nuclei image sequence over one CV cycle, the transitional electrochemical reactions on the electrode surface were successfully identified. However, since the non-uniformity is so big on the surface, a better and more compact underlying SEI protection layer is desired so that the lithium metal battery's cycling efficiency could be further enhanced.

5.4 Conclusion

In this chapter, I have characterized the process of the lithium metal nucleation beyond the solid–electrolyte interphase on Cu cathode at the nanoscale. While CV scan range was moderate (>-0.4 V), there is an obvious pre-formation of the SEI for these cycles, and this preformed layer could be recovered when the scan potential was reversed. However, with a much lower potential point which is below -0.5 V, the formation of the lithium nuclei seems to destroy the SEI signal very much and thus not fully recoverable. This formation process has been proved by Fig. 5.4 where multiple potential points' images were plotted and corresponded. The reversible SEI layer forms before the actual lithium plating, and under different plating current densities (0.2 mA/cm² and 5 mA/cm²) were also identified and plotted against time to provide the picture of the in-situ process. We hope that this level of detail can provide useful guidelines for designing and tailoring better lithium plating conditions for new battery chemistries.

VI. HIGH-THROUGHPUT SCREENING OF OXYGEN EVOLUTION REACTION ELECTRO-CATALYSTS

Quick and easy access to clean and reusable energy is demanded for decades since 21st century.¹³⁸ Confronting the overwhelming energy resources human can get, chemically transferring one molecule to the target molecule is always of paramount importance, as during the molecule formation process, energy transfer is usually involved. For example, due to its wide distribution on the planet, water splitting using electricity to converge and store the energy in the clean hydrogen is a hot research focus for years. Recently a new nanostructure based on Ni₃S₂ nanowires@N-doped graphene-like carbon layers supported by nickel foam has been demonstrated to electrocatalytic split the water at a low overpotential 271 mV and 134 mV for oxygen and hydrogen respectively.¹³⁹ On top of the discovery of these advanced electrocatalysts, strain engineering for nanostructures has also gained momentum in recent years; and it has proven to be powerful in tuning the surface electronic structure and the catalytic properties of nano water splitting catalysts.¹⁴⁰⁻¹⁴⁴

The overall water splitting process aims to functionalize two simultaneous reactions: the hydrogen evolution reaction (HER), which takes place at the cathode, and the oxygen evolution reaction (OER) at the anode. However, due to the charge transfer barrier where the generation of one oxygen molecule requires in total four electrons transfer, the OER will usually determine the reaction rate comparing to the HER.^{59, 145-147} Therefore, efficient OER catalysts to lower the overpotential are critical. For the past decade, multiple metals and metal oxides catalysts have been developed for OER. Up till now, Ru-based and Ir-based materials, basically RuO₂ and IrO₂, carry

the best performance for OER, which yields an overpotential as low as 250 mV at current density of 10 mA/cm².⁶² However, under high potential (> 2V), RuO₂ and IrO₂ metal can be further oxidized and side reactions can interfere with the reaction cell. Also, these noble metals are very expensive, making themselves not ideal for wide application of water splitting catalysts. Therefore, low-cost and abundant transition-metal (such as Fe, Co, Ni)-based electrocatalysts have come to the stage acting as alternatives for the OER catalysts due to their relatively stable activity in alkaline media while maintaining a good performance of lowering the overpotential and higher current density.

Many research has been conducted regarding developing transition metal oxide electrocatalysts for water splitting, because of their earth-abundance, low price, and high activity towards OER.⁶⁴ In these studies, very low over-potential at high current density were achieved, some low overpotential even reached as low as 0.73 eV regarding the Gibbs free energy.¹⁴⁸ Fenglei, et al also achieved a low over potential of 312 mV at 10 mA/cm² by a self- templated fabrication of CoO-MoO₂ nanocages.¹⁴⁹ An even lower overpotential 264 mV at 20 mA/cm² was also achieved via vanadium heteroatoms doping into NiS₂ nanosheets.¹⁵⁰

However, the catalytic efficiencies were mostly evaluated by the traditional electrochemical voltammetry, which average and smooth out the responses from the entire electrode surfaces.^{59, 60, 151-157} On the other hand, the transition metal oxide catalyst show heterogeneity on their surface structures,^{65, 147, 151, 156, 158-160} and the catalytic efficiency is highly location dependent.¹⁶¹⁻¹⁶⁴ C. Xiang et al ¹⁶⁵ published their work by imaging the bubble intensities on active sites to demonstrate the

efficiency of the electrocatalysts. They acquired the bubble intensity slope as well as a bubble figure of merit, which successfully demonstrated the non-uniformity of the catalytic activity on the electrode surface. In addition, successful detection of the most active transition metal oxides OER catalyst relies on high throughput in situ material screening.¹⁶⁶⁻¹⁶⁸ Therefore, an imaging-based method that can map the localized catalytic reaction activities is critical for catalyst evaluation and screening.

There are chemical imaging methods developed to study the localized catalytic activities. For example, the scanning electrochemical microscopy (SECM) can measure the local electrochemical reaction activities⁴⁸ and has been used to probe the electrochemical reactions on a single nanoparticle with 15 nm diameters by Allen Bard^{49, 50} and on sub- 10 nm scale particles by Michael Mirkin.⁵¹ SECM has also been powerful enough to be utilized in the measure the double layer effects in voltametric measurements⁵² and molecule uptake in live cells.⁵³ Although powerful, it needs to scan over the surface one point by one point, which limits its fast applications on the catalyst screening. Plasmonic electrochemical microscopy (PECM) has been developed by N.J. Tao's group.^{54, 55} This technique can map the localized electrochemical catalytic efficiency at once using the imaging cameras.⁵⁵ However, a thin layer of plasmonic metal, such as gold, silver, or copper, is needed, which makes itself not applicable for imaging the in situ oxidation reactions, such as OER, as the reactions will damage the plasmonic sensing surface and introduce the artifacts on the images.

In this work, we developed a reflection optical imaging method to measure the catalytic activity of OER. The OER catalyst was synthesized on the ITO or FTO

electrode surface and the OER generates a large amount of oxygen which will decrease the refractive index of the electrolyte. The decrease of refractive index will change the reflection intensity of the electrode surface and be captured by the CCD camera. The intensity changes on different locations of the images reflect the localized reaction activity. The use of ITO or FTO electrode as current collector instead of plasmonic sensing surface in PECM allows us to image the oxidation reaction without damaging the optical imaging signals. To demonstrate the principle, we electroplated cobalt thin film (10-15 nm) onto the ITO electrode and studied its OER catalytic activities. The current density and the over-potential maps were imaged using the reflection optical imaging method. We found that the catalytic activities are not uniformly distributed across the surface. To further understand this non-uniformity, we have also extracted the Co oxidation map. It showed that the hot spots of the catalytic activities on the electrode are correlated to the Co oxidation responses. More Co oxidation will lead to higher OER current and lower overpotentials. We have also studied the OER reactions of NiFeIr alloy and demonstrated the potential capability of this method for high through transition metal alloys screening for OER.

6.1 Catalytic efficacy screening principle

Our reflection optical imaging method is based on a TIR (total internal reflection) setup shown in Fig. 7.1a. The light beam from an LED light source was collimated first, as indicated as the red cylinder, and then is illuminated onto the working electrode through a 60-degree triangle prism with a refractive index of 1.78. By capturing the reflected light intensity coming out from the working electrode, the CCD camera can obtain spatial intensity distribution across the entire working electrode and

catalyst surface. During the electrochemical reactions, the reaction products' concentration will increase, and the refractive index of the electrolyte will change near the sensing/electrode surface.

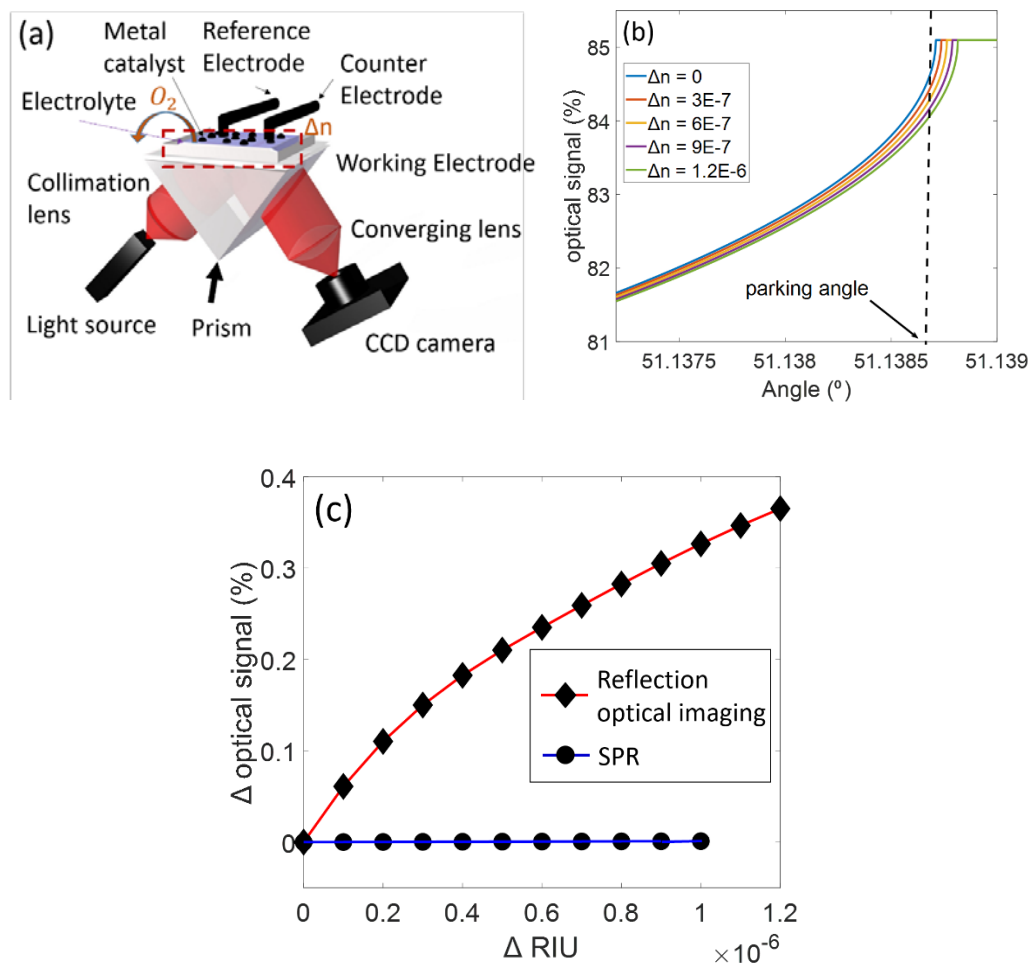


Fig. 6.1 OER screening principle demonstration. (a) Schematic drawing of the screening setup, 1M KOH electrolyte, W.E.: ITO glass with catalyst, R.E.: Ag/AgCl Sat' KCL, C.E.: platinum wire; (b) Reflectance responses to refractive index changes (Δn). Electrolyte refractive index 1.33. (c) Sensitivity of reflectance imaging (red curve) compared with SPR resonance (blue curve).

To measure and image these small refractive index changes during the reaction, we chose our incident light angle close to the critical angle ($\sim 51.139^\circ$ in our system). Fig. 7.1b shows the reflectance changes (%) near the critical angles. When the refractive

index changes near the sensing/electrode surface, it will generate relatively big reflectance changes (Fig. 7.1b) near the critical angle. Based on this observation, the incident light angle (parking angle) is selected to be close to the critical angle to get the biggest sensitivity. This is different comparing with the surface plasmon resonance (SPR) based sensing methods, where the incident light angle is bigger than the critical angle and the evanescent wave is generated and utilized. Although sensitive, the penetration depth of SPR (~ 200 nm) will limit its sensing range for the chemical reaction products detection. When we park our incident angle very close to the critical angle, the incident light beam will partially be refracted into the electrolyte and the reflected light intensity will be very sensitive to the refractive index change in the electrolyte. Fig. 7.1c shows the sensitivity curves for our system and the SPR method. We can see that our system shows much better sensitivity compared with SPR. This is because the refracted light penetrates the electrolyte much thicker than that of SPR setup and could capture more subtle reactions. In Fig. 7.1c, we learned that 1.2×10^{-6} of refractive index unit (RIU) change will result in approximately 0.4% reflectance response. Based on the noise level of our imaging system, our detection limit is $\sim 2.39 \times 10^{-8}$ RIU (see Supplementary Information for reference). This sensitivity is enough for measuring the OER reactions.

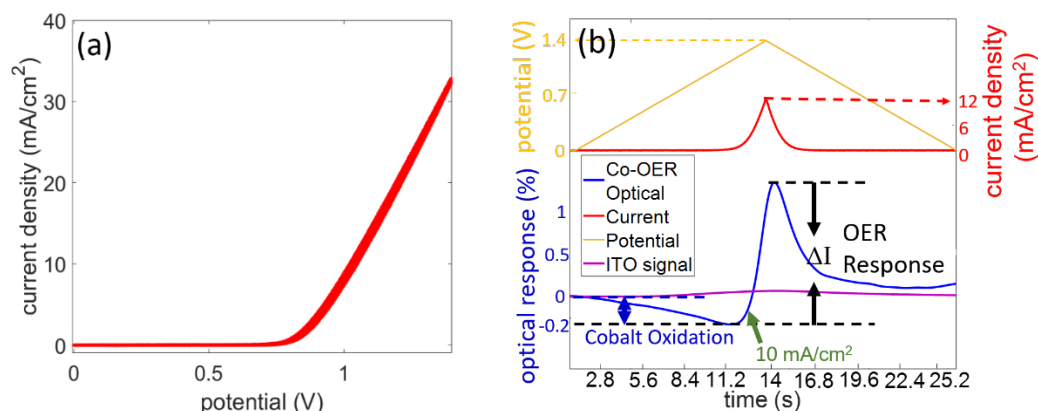


Fig. 6.2 Measure the OER with reflection optical imaging method. (a) Traditional cyclic voltammetry of Co-catalytic OER, 0-1.4V, scan rate 0.1 V/s; (b) Optical responses of OER reaction. Yellow curve: Potential swept along time axis; red curve: current density recorded; blue curve: Co-catalytic OER response; purple curve: bare ITO optical response.

6.2 Experimental

Reagents and chemicals. Potassium hydroxide (KOH), cobalt chloride (CoCl₂), nickel sulfate (NiSO₄) and iron sulfate (FeSO₄) were purchased from Sigma Aldrich Products. KOH solution was prepared by dissolving potassium hydroxide into DI water reaching a final concentration of 1 M. The catalytic salt solution CoCl₂, NiSO₄ and FeSO₄ were prepared using DI water reaching a final concentration of 0.1 M, 9 mM and 9 mM, respectively. Supporting electrolyte H₂SO₄ and (NH₄)₂SO₄ were also purchased from Sigma Aldrich.

Electrochemical measurements. Oxygen evolution reaction was performed on a CHI 1205C electrochemical workstation (CH Instruments) in 1M KOH electrolyte at 25 ± 1 °C, using a three-electrode system that consisted of an Ag/AgCl (saturated KCl, CH Instruments, Inc) electrode as the reference electrode, and platinum wire (Alfa Aesar Inc) as the counter electrode. Indium tin oxide (ITO) film covered glass slip (Thickness #1, Structure Probe, Inc.) was chosen as the working electrode to minimize the background interference while maintaining sufficient conductivity. Potential

window was chosen between 0 and 0.9 V for catalytic OER with a scan rate of 0.1 V/s and 0.95 V for NiFe catalytic OER with a scan rate of 0.2 V/s.

Catalyst material loading. Single cobalt catalyst and alloy catalysts were loaded onto the ITO substrate by electrochemical plating via galvanostatic technique. For electroplating the cobalt metal on the ITO, we used chronoamperometry working at -1 V for 0.1 s vs. Ag/AgCl (in saturated KCl). Electrolyte solution was injected into the electrolyte cell after complete rinsing by DI water to minimize the interference of other ions. For electroplating the NiFeIr alloy on the ITO, we used the same method at -1.1 V accompanied with pulsed current technique with repeated 0.1 s pulses at -20 mA/cm² to minimize hydrogen bubble generation during the electroplating process.

Optical imaging. Allied Vision Pike camera was used to record the reflectance changes during the electrochemical reactions. The frame rate of the camera is 106.6 fps. Electrochemical current and voltage were synchronized with optical response using a DAQ card (6008, NI Instruments) in order to allow for in situ analysis of local reactions.

6.3 Cobalt induced catalytic OER screening

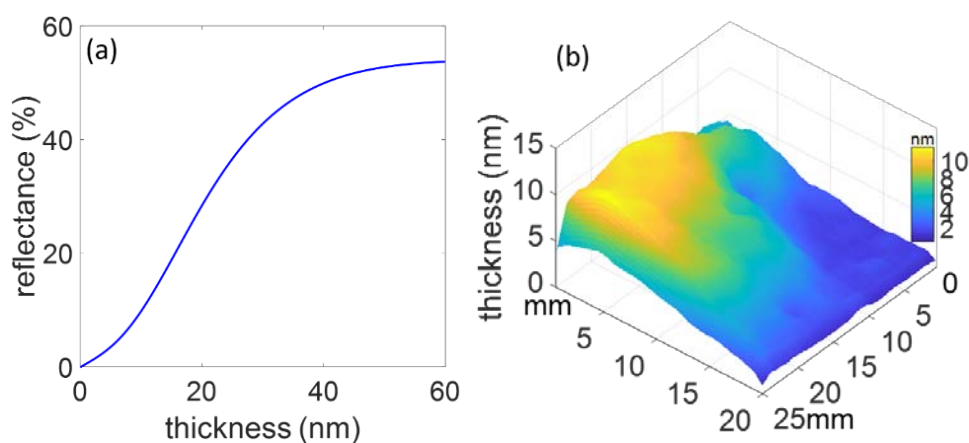
To demonstrate the capability of our method to measure the OER, Co was deposited on the ITO electrode and mounted onto our system (Fig. 7.2a). 0.1 M NaOH was used as electrolyte and the CV scan was performed while we image the reflected light intensity distributions using the CCD camera. We scanned the potential from 0 V to 1 V (vs. Ag/AgCl/Sat. KCl reference electrode) and the corresponded CV is shown in Fig. 7.2a. Fig. 7.2b shows the applied potential (yellow curve on top), the corresponded measured current (red curve in the middle) and the optical reflectance

(blue curve on the bottom) obtained from the same CV measurement. The reaction current starts to increase from 0.85 V which is caused by the OER. Correspondingly, the optical response also shows the increase of reflectance (blue curve in Fig. 7.2b) starting from the same potentials. This is caused by the refractive index decreases of the electrolyte due to the large amount of oxygen generation from the reaction. The optical responses reach the maximum around 1V and decreases when we scanned back. We have also plotted the optical responses on an ITO electrode without Co catalyst (red curve in Fig. 7.2b). The bare ITO electrode itself cannot generate the big OER current. Therefore, the OER response from the Co catalyst sample (blue curve in Fig. 7.2b) is about 20 times bigger than that on bare ITO electrode (red curve in Fig. 7.2b). This further demonstrated that our reflection optical imaging method could measure the OER responses.

Preceding the actual oxygen evolution, we also observe that the reflectivity decreases when the potential is scanned from 0 V to 0.85 V (blue curve in Fig. 7.2b). This is due to the oxidation of the Co catalyst to the cobalt oxide. The transition metal catalysts are always oxidized to their oxide forms before the start of the OER and the oxidation status of the catalysts will determine the catalytic efficiency of OER. Therefore, the capability to image the oxidation process of the transition metals is very important. The reflectance of the electrode surface will decrease when the transition metal is oxidized to the metal oxides.

We have simulated the reflectance responses vs. the thickness of Co layer (Fig. 7.3a). From this curve, we can map the deposition thickness of the Co layer. Fig. 7.3b shows the thickness of deposited Co catalyst layer, and the thickness could vary

anywhere from 2 nm to 10 nm. Note that this non-uniformity is intentionally introduced during the electrochemical deposition process of Co onto the ITO. This further demonstrated the needs to measure the localized OER and the catalyst oxidation responses. In addition, the derivative of the optical reflectance responses (dI/dt) should be proportional to the current of the catalyst oxidation and reduction (see supporting materials for more discussion). We have plotted the dI/dt of Co oxidation and reduction responses in Fig. 7.3c and the CV responses in Fig. 7.3d. Two redox peaks are shown in both figures which are corresponded to the oxidation of cobalt and reduction of cobalt oxide. The good correlation verify that our reflection optical imaging method can be used to study the oxidation process of the transition metals.



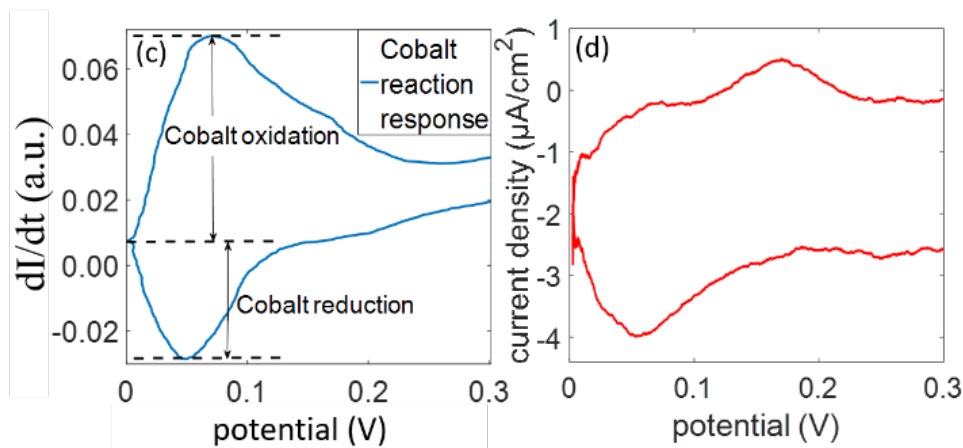


Fig. 6.3. Measure the metal oxidation responses using the reflection optical method. (a) Cobalt thickness calibration curve; Different thicknesses of cobalt will generate different reflectivity on the ITO electrode; (c) dI/dt Time derivative of cobalt oxidation and reduction responses; (d) Cobalt redox electrochemical current.

To provide quantitate signal of catalytic efficiency, we translated optical signals (reflectivity changes) to the electrochemical current density through the calibration experiments. We have synthesized different thicknesses of the Co catalyst onto the ITO electrode, and the OER is performed and measured with our optical system and potentiostat at the same time. The reflectivity change caused by the OER and the corresponding current density is plotted in Fig. 7.4, and the curve shows a good linear correlation between the optical responses to the current density. This means we can convert out optical signals to the electrochemical reaction current for OER.

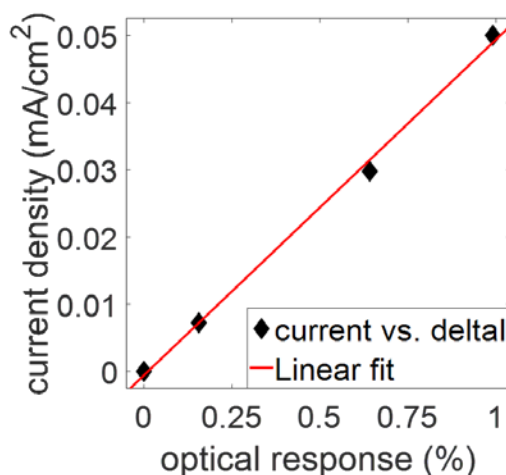


Fig. 6.4 Calibration from optical response to current density.

OER current density map. Non-uniformity of catalytic responses at different locations of the electrodes is critical for us to understand the heterogeneity of the catalytic reactions. The optical imaging could provide the localized information without any scanning. We have recorded the reflectance change maps at different time points (3 frame per second) during the entire OER process. For each individual pixel, we plotted the optical responses during the OER and a response curve that is similar to the blue curve in the Fig. 7.2b is obtained. The OER current density at that pixel was extracted from its optical response curve and converted to the electrochemical current density by using the calibration curve (Fig. 7.4). For example, the optical response (ΔI) that is correlated to the OER current can be calculated by subtracting the minimum from the maximum optical intensity on the optical response curve, such as the amplitude (ΔI) between two black arrows in Fig. 7.2b (on blue curve).

We calculated the localized OER current using this procedure for each pixel and the current density maps is shown in Fig. 7.5a. First, the OER current density is location dependent. The current density at different locations varies from 20 mA/cm² to 80 mA/cm². Second, there are some catalytic current hot spots, such as the locations

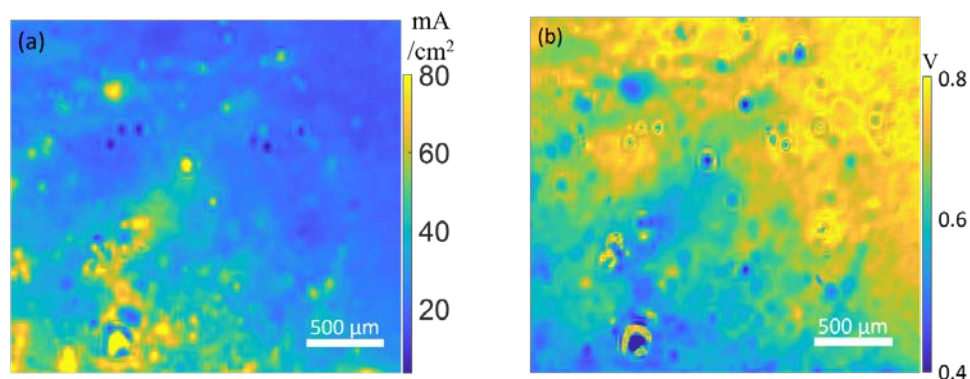
pointed out by the white arrows and circled by the white dashed line. The big OER responses is due to the thicker deposition of Co catalyst at those locations.

Overpotential. To further understand the non-uniformity of the OER current responses, we have also extracted the overpotential (η) map of this reaction. The current response curve was converted from the optical response curve at each pixel, and 10 mA/cm² was chosen as the threshold current density to define the overpotential. As shown in Fig. 7.5b, the overpotential is also highly location dependent, ranging from 0.4 V to 0.8 V vs. Ag/AgCl reference electrode. Correspondingly to the hot spots in the OER current map (Fig. 7.5a), we can also see the low overpotential spots in Fig. 7.5b (pointed by white arrows and circled by the white dashed line). The high catalytic activity will lead to a lower overpotential. The highest oxygen evolution current obtained was approximately 70 mA/cm² at the location where the lowest overpotential $\sim$ 0.7 V was achieved.

Cobalt oxidation map. We have also created the Cobalt oxidation map by calculating the optical intensity's derivative (dI/dt). Similar to Fig. 7.2c, the maximum Cobalt oxidation response at each pixel is plotted in Fig. 7.5c. The regions and spots that have bigger response represent the bigger Cobalt oxidation signals. The hot spots and regions in Fig. 7.5a and 7.5b are all shown up in Fig. 7.5c. This proves that the high OER catalytic activities in those regions are due to the more catalytic material depositions. The observation matches the mechanism developed by Norskov, Rossmeisl et al¹⁶⁹ very well, where metal is acting as the adsorbent for oxygen generation. The more cobalt metal gets oxidized, the more adsorbent it provides to the

solution, thus the higher activity of OER is achieved. That's why we see the correlating pattern between maps very clearly.

Prior to the effective catalytic effect of cobalt towards the oxygen evolution, as shown in Fig. 7.2 blue curve, we propose the optical intensity drop between 0 and 0.7 V to be the oxidation of cobalt since oxidation of cobalt metal¹⁷⁰ to cobalt oxyhydroxide would make the Co/Co-oxides density concentrated near the surface, which induces a refractive index increment.¹⁷¹ According to Fresnel's law, the reflectance will decrease in response to the refractive index change. Therefore, calculated from the optical response over the entire surface and depending on the reaction direction, we could obtain an oxidation/reduction response of cobalt (Fig. 7.2c. Fig. 7.2d), which subsequently contributes to the catalytic sites. This process is fundamental to the oxygen evolution regarding the chemical activity.



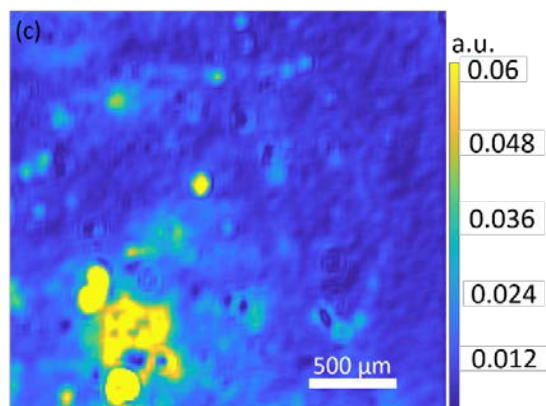


Fig. 6.5 Localized cobalt-catalytic OER images. (a) OER response localized map; (b) over-potential response localized map; (c) cobalt oxidation response localized map.

6.4 Nickel-Iron alloy transition metal induced catalytic OER screening

To test the potentials of our reflection optical imaging method for OER catalyst screening, we synthesized the NiFeIr alloy onto the ITO electrode, as NiFeIr transitional alloy catalysts were heavily studied towards the OER due to its high performance of providing the surface adsorption on the electrode surface during the oxidation process. Note that the main component of this catalyst is NiFe and we only have a trace amount of Ir. CVs were recorded between 0 and 1 V vs. Ag/AgCl in saturated KCl to achieve sufficient oxygen generation. As reported by Louie, Mary W. et al.¹⁷² this redox peaks pair is due to the oxidation of Nickel and not correspond to the oxidation of Iron, but the potential position was right shifted by the existence of Iron doping. Starting around 0.6 V, the overall current begins to substantially increase signaling that the OER is being triggered.

Utilizing a similar principle demonstration strategy as cobalt oxides (Fig. 7.2), we managed to measure both the OER response, overpotential and metal catalyst

oxidation as defined in Fig. 7.6. The nickel oxidation response could be benchmarked as the optical signal decrease from the starting point of potential sweep to the ending point of the minimum, whereas the OER response follows right after this ending point and reaches the maximum at potential ~ 0.9 V. Similarly, the 10 mA/cm^2 correlated overpotential was obtained by finding the underlying potential at this current density and transformed according to our electrolyte pH value and subtracted the equilibrium potential. Using this information, we have obtained the OER current density map (Fig. 7.7a), overpotential map (Fig. 7.7b) and metal oxidation map (Fig. 7.7c).

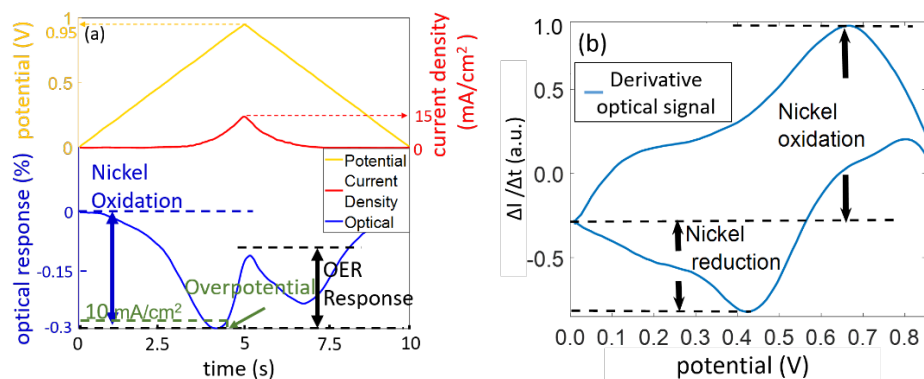
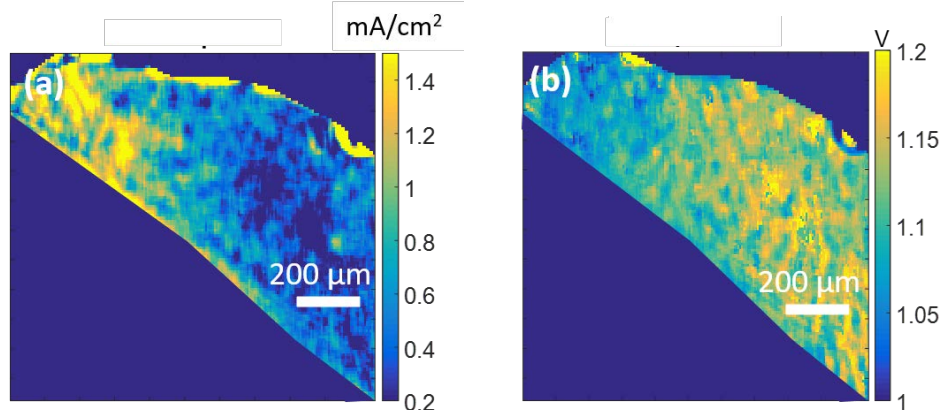


Fig. 6.6. The OER responses of NiFeIr electrode. (a) Typical NiFe-OER optical response along with principle definitions; (b) derivative nickel reduction and oxidation optical response.



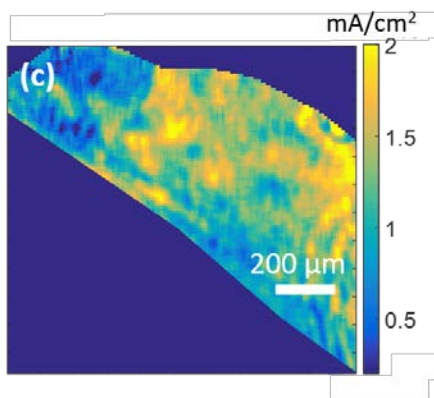


Fig. 6.7. Localized NiFeIr catalytic response maps, upper right and lower left corners are the reservoir region, so we block them out. (a) OER response; (b) over-potential response; (c) nickel metal oxidation response.

We find that the localized OER current has a non-uniform distribution across the whole chip, as the Fig. 7.7a shows. The left part of the substrate gave us much higher OER response as compared to the rest of the substrate, since it is showing a much brighter intensity response caused by non-uniform distribution of electroplated NiFe alloy. By comparing Fig. 7.7a and 7.7b, they are also complementary with each other, with respect to the catalytic performance non-uniformly distributed. However, contrary to the cobalt catalyst oxidation, our map here is complementary with the OER response, suggesting the contribution of nickel oxidation is taking effect towards the oxygen evolution reaction, which is proving the mechanism proposed by Louie, Mary W.,¹⁷² where 50% composition of Iron would make the nickel redox peaks right shifted and gradually overlapping with the OER current. The less nickel oxidation peaks found, the more OER response to the metal catalyst loading.

6.5 Conclusion

In conclusion, we have developed and demonstrated a novel reflection optical microscope to measure and map the localized OER responses. The electrochemical

reactions generate products concentration changes which lead to a local refractive index changes. We use a CCD camera to record and map this reflectivity changes during the reaction. From this measurement, we have obtained Faradaic current and the over-potential maps. From the images, we found that the localized catalytic reaction activities are quite different at different locations. This is due to the non-uniformity created by the catalyst synthesis process and demonstrate the importance to use optical imaging method map the localized catalytic efficiency. The oxidation and reduction of cobalt oxide can be imaged with our reflection optical imaging method as well. In addition, the NiFeIr alloy's OER catalytic activities have been studied as well. The proposed reflection optical imaging method could find many applications in catalyst activity measurement and screening.

VII. CHARACTERIZATION OF THE METABOLIC PATH SWITCH UPON DEPLETION OF GLYCOLYSIS IN CANCER CELLS

7.1 Introduction to metabolic cancer treatment and benchmarking

Chemotherapies targeting metabolism have been effective cancer treatments for decades, and the success of these therapies suggests that a therapeutic window exists to target malignant metabolism.¹⁷³ Unlike normal cell and normal tissues, proliferating cancer cells have been proven to be utilizing a different metabolic pathway compared to oxidative phosphorylation (OXPHOS). Instead, under low oxygen microenvironments, glycolysis with the transported glucose takes the main part for the cancer cell ATP generation as an alternative for the normal mitochondrial respiration, known as the famous Warburg effect.¹⁷⁴ Warburg effect states that instead of using enzymes to carry out the electron transfer during mitochondrial oxidation/reduction process, glucose is broken down into pyruvate thus Acetyl CoA and ultimately enters the TCA cycle to provide energy, given the schematic process of it below:

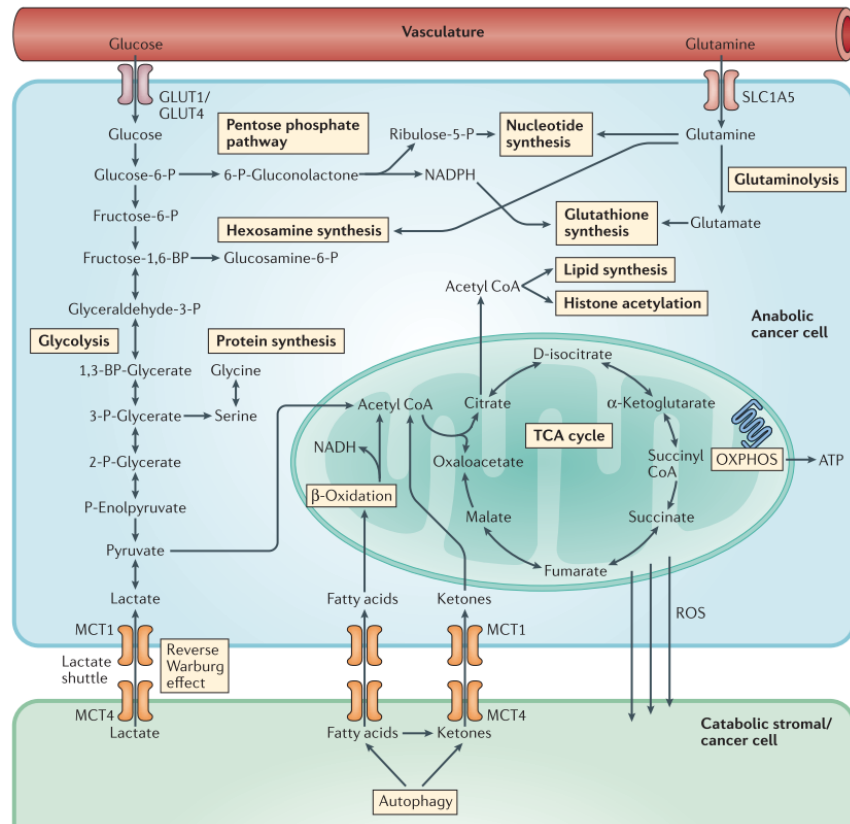


Fig. 7.1. Metabolic adaptation of cancer cells. Reverse Warburg effect occurs as malignant cells take up lactate, free fatty acids, and ketones released by surrounding catabolic cells, which can be used to replenish TCA-cycle intermediates and facilitate OXPHOS. (Reproduced from U. E. Martinez-Outschoorn, M. Peiris-Pages, R. G. Pestell, F. Sotgia and M. P. Lisanti, *Nat Rev Clin Oncol* 14 (1), 11-31 (2017).)

Therefore, a lot of glucose-targeting metabolic treatment drugs have been developed to inhibit the glucose being transported into the cyto-plasm, including 3PO,¹⁷⁵ WZB-117¹⁷⁶ and STF-31,¹⁷⁷ of which the 5 μ M STF-31 have been proven to reduce the cancer cell survival in 10 days.

Given these achievements, researchers have brought this question to more and more clinical trials.^{178, 179} However, one major question has yet to be discussed thoroughly, which is the drug efficacy in different individual cells. In order to achieve single cell screening/analysis, a lot of research efforts have been put into microfluidic devices for the purpose.^{180, 181} But these recent advances are typically limited by the

requirement for delicate manipulation of each single cell, and sometimes cell debris can be captured instead of the real biological live cell. Low throughput is another fundamental limitation on these techniques.

In this report, we wanted to see the effect of STF-31, which was first introduced by Denise A. Chan et al.¹⁷⁷ This drug specifically binds the GLUT1, the principal glucose transporter protein on the cell membrane and thus inhibits the glucose uptake of the cancer cells. As a primary metabolism pathway, glycolysis is prevalent in most cancer cells. Therefore, with GLUT1 being targeted, the cell's major energy supply is shut off upon injection of the drug. So, in response to the drug dosage, the intact function of OXYPHOS could start again due to a lack of glucose supply, in order to gain energy for cell activities. Herein, we reported an algorithm to study the individual cell response to the glucose inhibition drug and hopefully we should observe the switch process over time. By virtue of our method, the individual cell activities trend over time immediately after drug dose was obtained. It is clearly shown that the cells experienced an initial increase in cell activity then slow down over 14 hours, which proves that our method can observe the pathway switch. Cell heterogeneity and long time cell-cell variation have also been identified with sufficient activity level trends. Experimental details are described below.

7.2 Label-free metabolic imaging technique

High throughput transmission imaging-based analysis of single cancer cell metabolism activity is the primary technique, and the setup is shown in section 7.2.1. Analysis of the cell activities were conducted via a facile algorithm and the research

results have been demonstrating the capability of capturing the metabolic switch between OXPHOS and glycolysis which is going to be discussed further.

7.2.1 Cell activity capturing setup

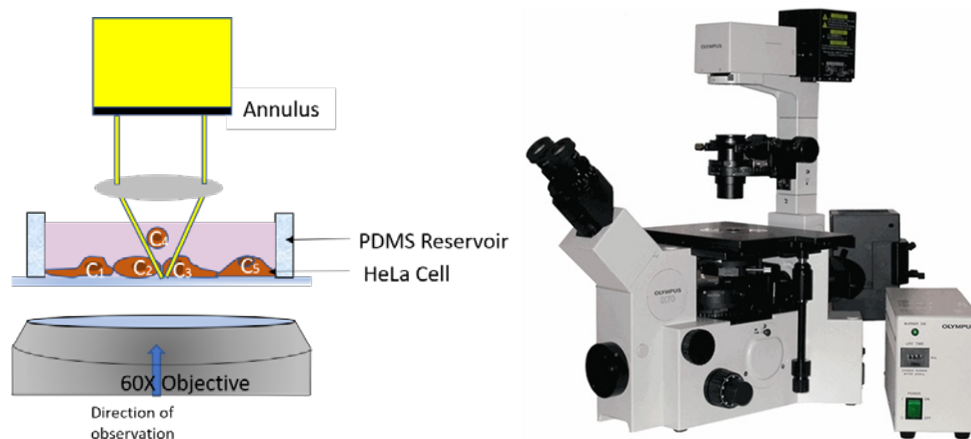


Fig. 7.2. Inverted brightfield microscope for label free cell metabolism screening. (a) Schematic drawing of the screening setup. (b) Picture of Olympus Inverted Microscope IX-70.

Our cell line was chosen to be the popular HeLa cell line which proves to be metabolism effective. We use the *ThermoFisher Scientific* Microbiological Incubators to maintain the good conditions for the new cell line to grow and proliferate. Once our HeLa cell culture is ready (allowed 2 weeks for full confluency on the glass coverslip), we fully replace the complete medium with drug dose with 20 μM STF-31. And for each time point, we captured cell metabolism for 5 min, and at the silent period when no signal was recorded, the cells are kept within favorable incubation conditions (moisturized cell incubator with 5% CO_2 and kept at 37.1 $^{\circ}\text{C}$). The total duration of cell metabolic activity measurement duration lasts for ~ 14 h.

As for the Fig. 8.2 showing, at each time point, we placed the live HeLa cell containing reservoir with the complete medium/drug dosed medium under the inverted microscope's focus. Then we record the cell activity via the transmitted microscope

images over the desired duration of time. The captured image sequence can be then utilized for the arithmetic calculation of cell activities which is going to be discussed below.

7.2.2 Theory

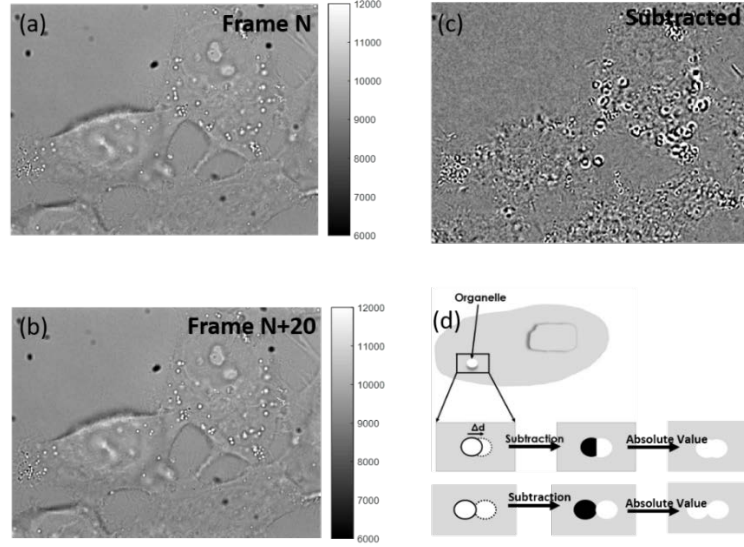


Fig. 7.3 Principle of metabolism benchmarking technique. (a) A random sampled frame of cells with timestamp N; (b) Twenty frames later than the as-sampled frame; (c) Subtraction result of the sampled two frames; (d) Cell activity calculation principle.

In order to capture the real cell activity, we used the cell organelle motions as a standard metric to determine the cell activity level, because the cell organelle movements are directly related to the metabolic activities.¹⁸²⁻¹⁸⁴ Therefore, we could use the cell motion activity to serve as our measurement of metabolic activity according to the following

$$S \approx \pi r^2 \frac{\Delta d}{2r} \bullet I \approx \pi r^2 \frac{\Delta d}{2} \bullet c = \frac{\pi}{2} \Delta d \bullet r^3 \quad (10)$$

$$\text{and } E = F \bullet \Delta d = (F_g + F_d) \bullet \Delta d \approx F_g \bullet \Delta d \sim \Delta d \bullet r^3 \quad (11)$$

where the S represents the measured optical movement signal by subtraction of two frames using the principle depicted in Fig. 8.3d, and the E is the current energy consumption by the cell. We could use these two adjacent equations to establish a linear relationship between the cell metabolic activity and the optical signal within each frame. As can be seen in Fig. 8.3d, within a fixed time period, a larger movement of each cell organelle will induce a bigger difference before and after the subtraction, causing a bigger integration of intensity signal. In this way we could directly utilize the measured optical intensity to capture the current activity level of the HeLa cell. The subtraction frame length was chosen to be 20 after careful comparison between different frame lengths below where subtraction of 20 point starts to converge while without losing too much of small movements (Frame rate was 6.5fps, ~ 3 s). However, this choice of subtraction length was just made beyond intuition, and needs further more solid experimental support to make a more accurate choice. Fortunately, as long as we use the same subtraction frame length over the whole image sequence, we will be able to capture the cell activity before and after drug treatment as well as perform the cell-cell heterogeneity analysis.

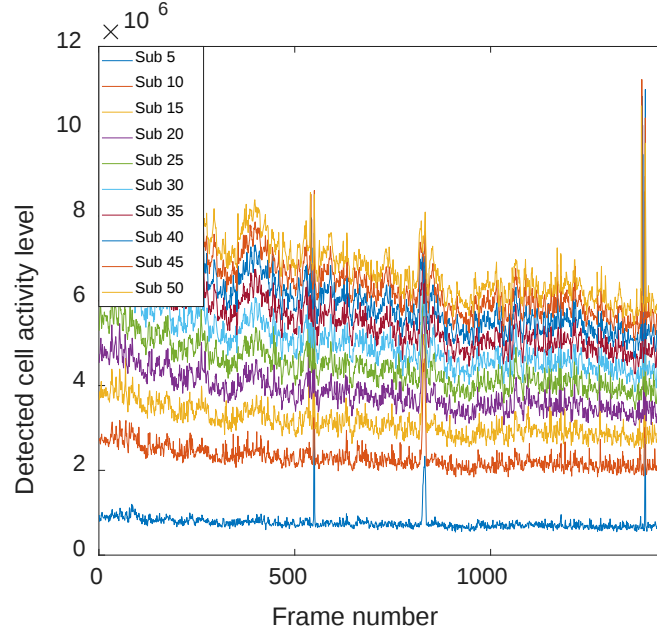


Fig. 7.4 Choice of subtraction frame length.

7.2.3 Demonstration of measurement capability via ATP depletion and PFA cell fixation

Given different cell activity conditions, the cell organelle motion level would also be quite different from one another, and this cross-cell line heterogeneity should be able to be captured by our transmission microscope screening method. To confirm this hypothesis, we first used medical practice Paraformaldehyde (PFA) to fix the cell. As well known, PFA is usually used to form covalent chemical bonds (or cross-links) between the proteins in the cytoplasm,¹⁸⁵ and usually possesses the highest preservation capability. Using the image sequence collected by the object on the bottom in Fig. 8.5, we calculated the individual cell activity and plotted it before and after the fixation below in Fig. 8.5.

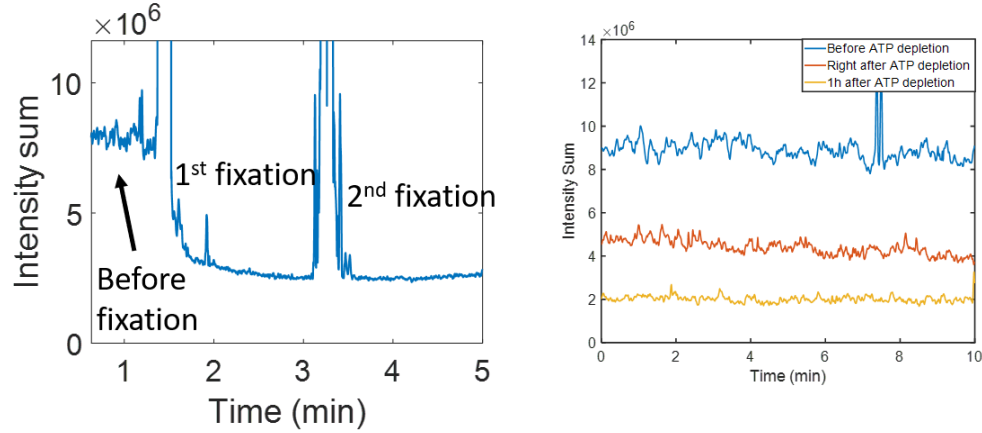


Fig. 7.5 HeLa Cell metabolism measurement demonstration. (a) Before, after 1st and 2nd cell fixation via Paraformaldehyde (PFA); (b) Before, right after and after 1h ATP depletion via 10 mM Sodium Azide + 10 mM 2-deoxyglucose in PBS solution.

Clearly shown as the high intensity sum value before the PFA fixation, the cell was still maintained at a relatively high level of activity at this time point. Interestingly, almost instantaneously, after the 1st dose of PFA, the cell activity dropped from 7.5×10^6 to 2.5×10^6 , with a three fold decreasing trend. To further determine that the cell fixation was done, we added a second dose of PFA into the same cell containing reservoir and we find that the cell activity did not change too much as can be seen in the figure where the intensity sum did not change before and after the second dose of paraformaldehyde.

Given the capability to identify the cell fixation process via our measurement setup and calculation, we would also like to discover the possibility of identifying a more gentle treatment, the ATP depletion. Therefore, 10 mM Sodium Azide + 10 mM 2-deoxyglucose in PBS solution was chosen to act as the ATP depletor. After the treatments, we found that there is a big distinction among the three treatment effects, where the blue curve in Fig. 8.5b is the cell activity before the treatment, and the orange curve represents the cell activity right after the drug dose (within 30s) and the

bottom yellow curve represents the cell activity level after 1 h of the ATP depletion. One may suspect that the distinction between the orange and yellow curve may not 100% come from the ATP depletion drug (cell may have an intrinsic decreasing trend), but note that the distinction before treatment and right after treatment is already sufficient to support the conclusion that our metabolic screening method is capable of capturing the transitional cell metabolic levels (ATP level will restore to its original level after the depletion drug has been catabolized).

7.3 Cell metabolism heterogeneities and long-duration cell-cell variation

Unlike predictable physical system, individual heterogeneity is an intrinsic behavior of most biological systems, especially in carcinoma cells and tissues,^{186, 187} and this predominantly pattern determines the trend that more and more single cell analysis techniques/tools are being developed.^{77, 188-190} Therefore, in order to provide a comprehensive picture of the HeLa cancer cell metabolism, we chose to screen the variations among different cells and we find that the cell activity levels among different cells can be distinguished both spatially and temporally (Fig. 8.6).

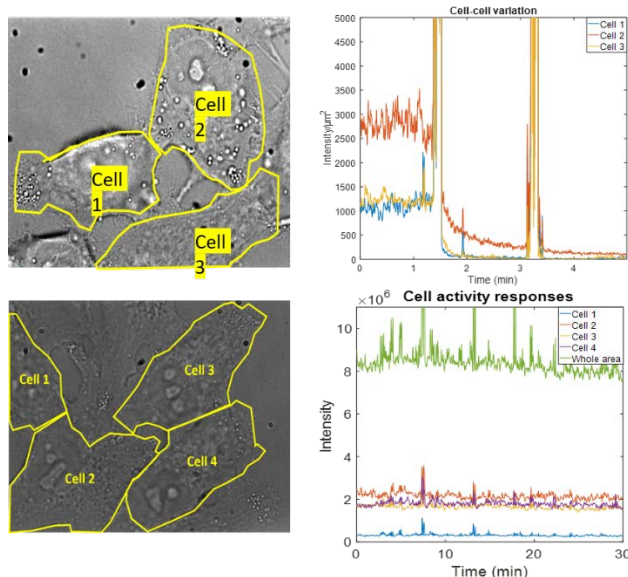


Fig. 7.6 Cell metabolism heterogeneities and long-duration cell-cell variation. (a) Three individual cells contoured in yellow; (b) Corresponding cell activities before and after the PFA fixation; (c) Four more cells for long duration analysis; (d) Corresponding normalized cell activities for the four cells + the whole chip region.

It can be seen from the above figure that three different cells in the Fig. 8.6a with different shapes suggests much different cell activity levels before and after the Paraformaldehyde fixation, with cell 1 carries the lowest activity level while cell 2 carries the highest activity level within the recorded time period. This phenomenon can also be demonstrated directly from the images, as in the cell 1, most organelles have been focused on the ends of the cell suggesting an apoptosis shrinkage behavior, while in the cell 2, the organelles are more spread out over the entire cell. Usually, a more spread out cell organelle distribution suggests that the cell is still undergoing high metabolic activity. That is to say, our demonstration proves that the capability of identifying different cell stages regarding the metabolic pathway.

On the other hand, our screening method should also be able to detect the cell activity distinction over longer periods. Therefore, we chose another conjunction of four cells in Fig. 8.6c to record the long-time response from our screening method. As

can be seen from the Fig. 8.6d, we find that even after 30 min, the chosen four cells' activity levels remained relatively constant except for some decreasing trend and crossings between cells. The distinction between the four cells chosen was also identified clearly as the four colors of the cell activities. These observations further demonstrated our screening method's capability to discriminate between different individual cells' activities, which helps in the most current single cell research trend.¹⁹¹

7.4 Capturing the metabolic pathway transitional switch

Since the discovery of this effect, people have always believed that the OXYPHOS is kind of deactivated. However, recently so many evidence have started to challenge this hypothesis¹⁹² and in the year of 2012, it was proven to be switchable from glycolysis, where OXYPHOS and glycolysis is cooperative and competitive.¹⁹³ Therefore, in this subchapter, active metabolic pathway switch upon the strike of GLUT1 by STF-31 is going to be discovered and proved.

Capable of precision monitor and analysis of cell activities without drug treatment, drug screening capability is explored herein. For RCC (Renal carcinoma cancer cells), the cell viability experienced a sharp decrease on the fourth day and the remaining cells are monotonically dying, with increased concentration and time of period. 1 μ M of STF-31 is already triggering the cell death starting from the 4th day, in this case, we used 20 μ M of STF-31 to make the cell activity response more overwhelmingly and quickly. We diluted the desired amount of STF-31 powder into complete culture medium, to provide a control environment for the cells to activate without losing viability too soon. Thereafter, for treatment of the drug, the whole culture medium

inside the cell chip was removed completely and replaced with the drug dosed culture medium (with 20 μM of STF-31). Cell response over 14 hours are captured by our algorithm and plotted in Fig 8.7.

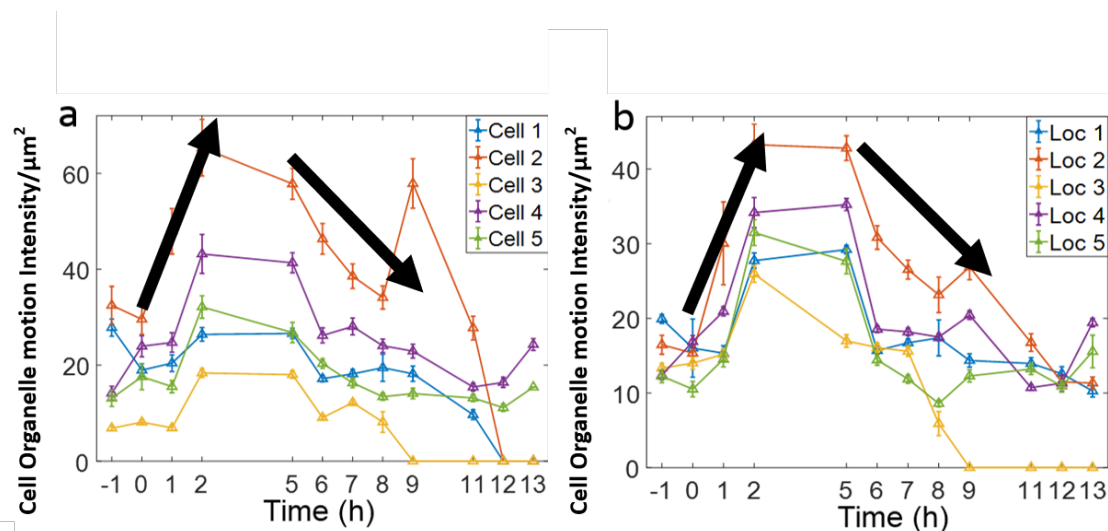


Fig.7.7 Cell activity representation of HeLa metabolic pathway switch. (a) Five individual cell activities over the sample period. (b) Five total regions activities over the sample period.

Unlike normal proliferating cells, most cancer cells utilize aerobic glycolysis as fuel supply for survival, which is known as the Warburg effect.^{174, 194} Therefore, people have discovered multiple ways to target this specific pathway as a cure, such as using a small molecule STF-31 to inhibit the glucose transport into the cytoplasm.^{177, 195, 196} This series of drug (STF-31, 3PO and WZB-117) has been proven to be effective towards inhibition of GLUT1 to $\sim 50\%$. Notably, our label free screening methodology successfully detected the chemical lethality induced by effective inhibition of GLUT1 via 20 μM STF-31 (Fig. 8.7a and Fig. 8.7b), as after approximately 12 h, all five locations and five individual cells showed a nearly diminished activity level eventually. We believe that this eventual diminished cell activity level is caused by the GLUT1 inhibition from our 20 μM STF-31 in complete medium, given that between each time point, the cells were kept under standard

incubation conditions and five individual locations containing multiple cells were chosen to eliminate the possibility that the chosen five cells were intrinsically lethal (selection bias).

Interestingly, we observed in the above Fig. 8.7 that before the eventual diminishing of the cell activity level, there exists an initial increasing trend of activity at ~3 h. We propose that it is because of the cut off glucose supply, the OXYPHOS process were triggered which induces the mitochondrial motion increase initially, as discussed above for the metabolic pathway switch. But since it is still lack of enough nutrition, the ultimate fate of the cell metabolism is going to be decreased. Note that within the five cells, the decreasing trend is showing a non-homogeneous distribution, like the third cell is already totally dead at 9th hour while the rest of the cell samples are still showing a hidden trafficking of organelles.

However, we only found that this metabolic pathway was switchable because of the intactness of mitochondrial function,^{193, 197} and further experimental proof which measures the true level of mitochondrial respiration should be supplemented to demonstrate our claim.

7.5 Conclusion

Taken together, we conclude from our label free screening that STF-31 may function as a GLUT1 inhibitor, whereby its action as cytotoxicity represents an efficient way of killing cancer cells. Low expression levels of GLUT1 may trigger in conjunction with high expression levels of mitochondrial activity (OXPHOS), and this may temporarily increase a cell's activity towards the survival to uptake the surrounding lactate, free fatty acids and ketones released by surrounding catabolic

cells.¹⁹⁸ However, eventually the cell viability would decrease to a much lower level caused by the lack of sufficient glucose transported in the supporting cell culture medium because either metabolic pathway would utilize the glucose to supply the TCA cycle for energy consumption. Our facile and label free cell activity screening system proves to be effective towards identifying the drug efficiency of glycolysis, and it can also be applied to more cell line metabolic activity screenings.¹⁹⁹

VIII. MEASURING ELECTROCHEMICAL IMPEDANCE OF SINGLE CANCER CELLS

8.1 Introduction to single cell impedance analysis and cell-substrate adhesion

8.1.1 Single cell analysis

Unlike predictable physics or engineering, in the field of biology and biophysical science, individual heterogeneity always exists in different cells, tissues and organism.²⁰⁰ And especially cell heterogeneity lies in cell cycle phases, complex response to microenvironment changes and cell proliferation rates.²⁰¹ All these differences will ultimately affect the individual cell and even tissues and organism's biological performance and viability. Therefore, the implementation of single cell analysis, concerning the protein activities, metabolism pathways, cell-cell informational interactions must be addressed to gain comprehensive understanding of events happening intra-cell or inter-cell.²⁰² However, since the size of cells are usually small (less than 30 μ m in diameter), the existence of interference of large concentration on target molecules and dynamic behaviors differ greatly and complicatedly,²⁰³ in order to capture the individual response of single cells to controlled experimental conditions, approaches with enough time resolution as well as precision are usually in high demand. Fortunately, many systems and methods have been developed to meet these requirements. Some of the current popular single cell analysis tools will be discussed in section 8.2.

8.1.2 Cell adhesion analysis

In the behavioral studies of cell-cell activities in multicellular organisms, the in vivo cell-cell adhesion and in vitro cell-substrate attachment should be understood thoroughly to fully comprehend the cell differentiation, cell cycle, cell migration and motility, and cell survival.²⁰⁴ In general, while most mammalian cells tend to attach firmly to the substrate provided,²⁰⁵ the affinity of cells to substrate is a crucial consideration in biomaterial design and developments, and changes in cell adhesion can be the signature event in a wide range of diseases including arthritis cancer, osteoporosis, and atherosclerosis.²⁰⁶

In the present project, we employed the proposed imaging protocol to provide high throughput cell-substrate attachment screening. We mainly focused on observing the increased regional impedance caused by cell attachment on the conductive polymer scaffolds. Demonstration of our method was conducted by manually patterning the substrate with scaffold material and measure the direct optical response change induced by sinusoidal wave voltage application. After direct seeding of well and healthy HeLa cancer cells on uniformly distributed CP scaffolds and provided moisture and nutrition to let them grow, the intrinsic ion doping/dedoping dependent absorbance change property of the CP scaffold facilitated the capability of localized cell impedance imaging, where firmly attached cell would block the ion flow inside the solution on top and loosely attached cell contributes much less in the overall impedance construction.

Compared to traditional ECIS (Electrochemical Impedance Spectroscopy) method to study the total impedance response due to attachment, our implementation of in-situ

imaging gave us the ability to screen multiple scattered/networked cells simultaneously and thus acquire the local electrical impedance quantitatively. Our method is better than what Byoung *et al*²⁰⁷ proposed for mapping local impedance of working electrode, because we don't require the AFM tip to conduct the topography, which usually damages the cells and interfered by a lot of concentrated components in the electrolyte.

8.2 Popular single cell analysis tools

IHC based biopsy diagnosis. For modern cancer diagnosis, combined with the gold standard biopsy technology, immunohistochemistry (IHC) has been effective towards breast cancer diagnosis²⁰⁸ and usually they use pre labelled antibodies to bind and test the remaining amount of the antibody on the cancer cell surface after incubation for a certain time. But there is a problem where different pathologist use different and arbitrary methods to distinguish, and biopsy results may be completely opposite when tissue is removed from different part of the tumor. Ultimately, IHC takes advantage of the labelling antibodies which specifically binds to the target cells, which induces interference from the surroundings as well as damage to the viability of the cells.

Microfluidic devices. As a very hot topic recently, a PDMS channel based microfluidic device has become capable of measuring the single HeLa cell impedance by trapping single cells through flow cytometry.¹⁸⁰ But the probability of cell trapping is only 10% with a very high chance of trapping cell debris and fabrication of the device is relatively complicated and time-consuming. There are indeed already some cell trapping improvements on microfluidic device for single cell imaging, like

integrated valve, microwells, U shaped channel,¹⁸¹ among which the droplets based method has recently been reported and is current state of the art. However, they still require delicate single cell manipulation, such as specially diluted cell suspension or controlled air flows and sorting protocols which may not be as fast and easy enough. At the same time, the trade-off between high temporal resolution and enough details exists, since manipulation of single cells is not always an easy task.

Fluorescence microscopy. One of the most adorable and most dynamic optical technique to investigate the cell behaviors is fluorescence microscopy, and for the past two decades, it started being combined with super resolution techniques, like SIM, STORM/PALM and STED, subcellular level of cell membranes and proteins came up to people's eyes, reaching a spatial resolution as high as 20 nm.^{209, 210} They are profoundly proven to be very effective methods to get biomolecules and target cellular protein information out of bunch cells, so that biophysical properties of the current cell being studies could be obtained. However, this ultra-method has its own limitations, because it is basically a manipulation of the excitation of the fluorophores (dyes), whether from the illumination end or the object end. This usually requires the cells to be fixed and stained accordingly, making the technique not quite applicable for live cell imaging let alone monitoring the dynamics of live cell interactions with the substrate.

Electrical Cell-substrate Impedance Spectroscopy. Electrochemical impedance Spectroscopy (EIS)¹⁹ have been proved to be an effective method to analyze the global impedance across the electrochemical system electrode for almost a century.^{207, 211, 212} Initially it was invented for analysis of impedance across interfaces and bulk materials,

and usually employs an equivalent circuit model to fit out the impedance parameter. For single cell analysis perspective, Electric Cell-substrate Impedance Sensing (ECIS), was first introduced by Ivar Giaever and Charles R. Keese in the year of 1993.²¹³

What it does is quite similar with EIS and tries to extract the cell impedance data out of the global impedance parameters. Practically, ECIS provides high throughput screening for cell monitoring when fixed or sinusoidal wave potentials are being applied to the whole cell system. It measures the total impedance from a large population of cells located on the substrate. The increase of the impedance obtained from the entire electrode surface could be caused by the increase of cell coverage confluency and/or by the increase of the cell attachment strength. However, within one round of experiment, only one set of impedance parameters can be fitted out by the combination of global impedance contributions, which does not give insights about how well each localized individual cell performs and behaves. It also only provides the accumulated signal from the entire electrode surface, where the heterogeneous responses from single cells are averaged out, making it not ideal for single cell analysis.

Surface plasma resonance imaging. As a label free optical sensing technique, surface plasma resonance (SPR) has been extensively applied into the subcellular level cell imaging on sensitive substrates.²¹⁴ 3D images of multiple cell lines with a near diffraction limit spatial resolution were obtained and readily translated by Alexander W Peterson *et al.*²¹⁵ Due to the surface sensitive property of the technique, it is advantageous when it comes to cell attachment analysis and membrane movement analysis. Signals from the imaging setup are basically reflection images and the

corresponding cell behaviors can be translated accordingly. However, the system requires a polarized light illumination and usually takes longer time to calibrate the appropriate incident angle to trigger the surface plasma resonance. The cell attachment on the plasmon surface is another issue that SPR should address before solid conclusion could be drawn, as the intrinsic cell attachment on gold/silver substrate is low.^{216, 217}

Raman micro-spectroscopy. Upon easily obtained data transformation and interpretation, raman spectroscopy can be implemented into single cell imaging and biomolecule sensing too. Basically, the obtained Raman spectrum contains the fingerprint database and reflect the signal induced peak shift of the single cell components, including the cell membrane proteins and intra-cell molecules, lipid metabolism, lipid droplets, cell growth and proliferation, drug intake effects and cell death/apoptosis behaviors.²¹⁰ They have been reviewed extensively and proven to be effective method regarding the single cell cellular molecule sensing. However, the signal of Raman is typically very weak which demands highly optimized imaging setup and the interference inside the cells is usually large compared to target molecules. Local heating generated by the imaging laser is also introducing stress on the live cells which may lead to unwanted results.

Therefore, in this last chapter, a facile methodology which obtains the live dynamics of the live cells' metabolism is going to be discussed. The principal theory and experimental setup are going to be discussed in detail.

8.3 Theory

Organic electronic materials, such as the conductive polymer, have unique electrical, electrochemical, and optical properties, among which the most important properties is the electrochromism property.²¹⁸ When a potential is applied onto the conductive polymer, the surrounding ions will be either injected into or extracted from inside of polymer based on the direction of potential applied. This ion moving processes will reorganize the electronic structure of the polymer, resulting in reduced or increased energy gap for the covalent bonds transition, and thus change the optical absorbance of the conductive polymers. Therefore, utilizing this tunable optical transparency property, we could obtain the localized impedance information which induces the local transparencies.

For the detailed analysis of AC impedance of the cells, typically a sinusoidal wave potential is applied. In this case, while the electrode potential is applied positive, the conductive polymer (CP) turns into oxidative status and thus become more transparent and while the electrode potential reverses, the CP turns into more reductive status and become less transparent. This property gives us capability to perform a similar cyclic voltammetry and thus analyze the polymer response caused by different voltages, as shown in the below Fig. 8.1.

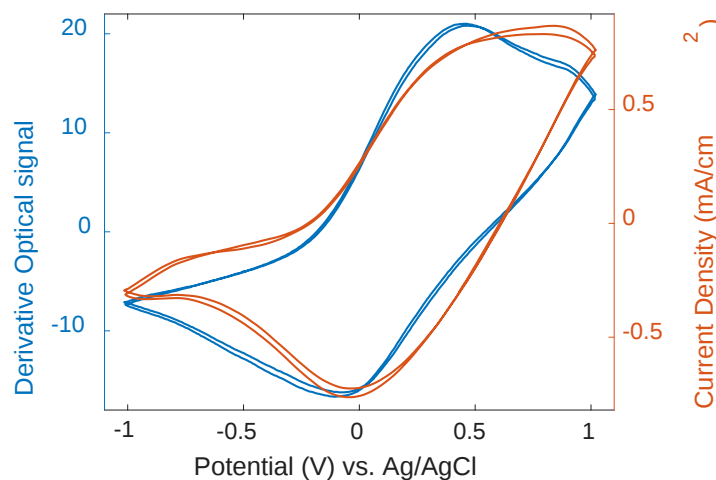


Figure 8.1. $D(\Delta I)$ (intensity signal derivative) vs. potential, and current density vs. potential.

To demonstrate our imaging capability to capture the transitional electrochemical current, we performed the popular cyclic voltammetry runs on a uniformly distributed CP and plotted the derivative optical signal vs. potential to compare with the real CV as shown in orange curve. It is clear that these two curves show a similar trend response to potential, both in amplitude and frequency. That is, when the potential was increased to positive range, ions in the electrolyte start to dope into the CP and thus induces a similar increment of optical signal. On the contrary, when the polymer film is reduced by applying the potential to more negative ranges, the ions start to reversibly de-dope out of the CP and thus causes a decrease in the optical signal.

Therefore, we were able to establish a direct connection between the measured optical signal and the current density of the working electrode. Crudely speaking, higher current density means larger amount of ions dope into the polymer and should induce higher optical response. Actually, the relation between the optical response and the current follows the integration law as

$$\Delta I(x,y) = \alpha \int i(x,y,t) dt = \frac{2A\alpha}{\omega} = \frac{\alpha i_{amp}}{\omega} \quad (12)$$

where $\Delta I(x,y)$ is the optical intensity changes at this location (x,y) , α is the linear dependence coefficient, i is the current amplitude and ω is the angular frequency of potential applied. Usually, the current from across the working electrode surface is obtained, but we managed to use this equation to give the current a hint on how to relate to the localized optical response. Note that similar with the current, the optical response is also frequency dependent, so a calibration between these two signals at certain fixed frequency is required.

By performing temporal Fourier transform on the optical (orange curve in Fig. 8.2a), we were able to extract the exact optical response for each amplitude at 2 Hz. Therefore, a linear dependence of optical response vs. the current amplitude can be obtained (Fig. 8.2c), and for each imaging scaffold, the slope α can be fitted out and further applied to local intensity extraction calculations and eventually the local impedance extraction, according to the Eq (2)

$$Z_{local} = \frac{\Delta v}{\Delta i} = \frac{\alpha \Delta v}{\omega \Delta I} \quad (13)$$

where α is the slope of the calibration, ω the angular frequency, Δv the applied potential amplitude Δi the current amplitude and ΔI (%) the intensity change percentage.

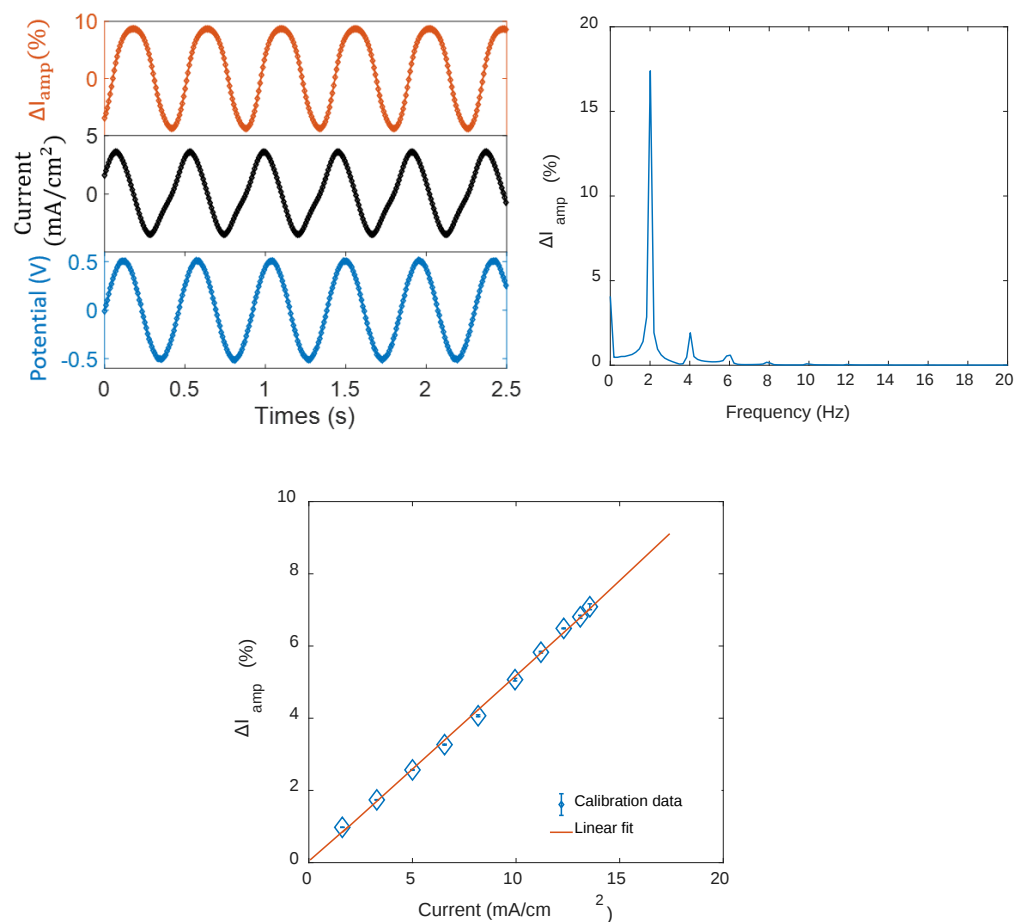


Fig. 8.2. Measurement principle demonstration. (a) Optical responses, current and applied potential vs. time. A 2 Hz sinusoidal potential is applied to system, and the corresponding potential, current, and the images were recorded and synchronized. (b) FFT amplitude of optical signal. (c) Dependence of optical intensity vs. current. From the curve, we can extract the value of $\alpha = 0.0109$.

In this way, for a fixed point of optical intensity, a fixed point of impedance can be calculated and implemented to the whole chip area to obtain the map.

Equivalent Circuit Simulation. To further understand our experiment system, the equivalent circuits have been established and verified with the experimental results. Fig. 8.3a shows the equivalent circuit with (right) and without (left) cell attachment in the system. We have also performed the traditional EIS measurements on a PEDOT:PSS substrate in phosphate buffer and fitted with the circuit model (Fig. 8.3b).

The results show excellent agreement, which further proves that our sensing system can measure the system impedance.

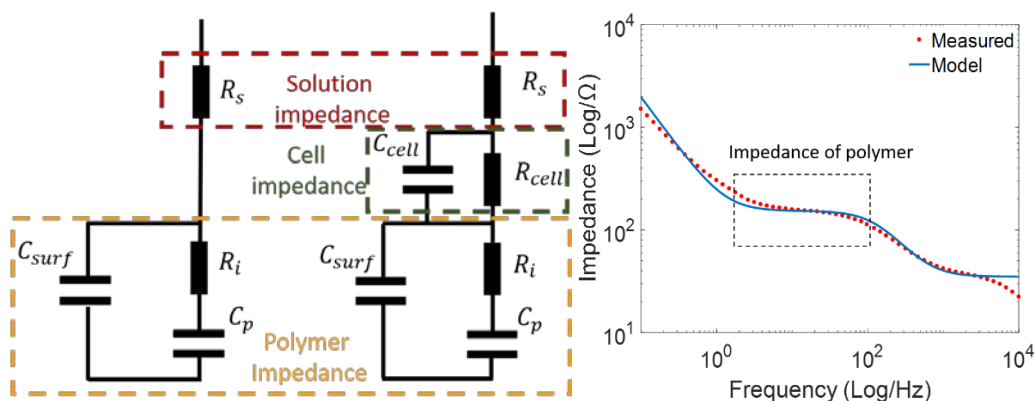


Fig. 8.3. Electrical impedance modelling of the single cell impedance measurement. (a) equivalent circuits with (right) and without cell (left); (b) Impedance amplitude vs frequency on a sensing surface without cells. The red circles are the experimental results, while the blue curve is the fitted result from the equivalent model (a) left. The fitting parameters are $R_s = 35 \, \Omega$, $R_i = 120 \, \Omega$, $C_p = 8 \times 10^{-4} \, \text{F}$, $C_{surf} = 1 \times 10^{-5} \, \text{F}$.

Note that the equivalent circuit provided us the capability to decompose the overall impedance into multiple parts, thus calculating the individual cell signal obtained from the imaging setup which is going to be discussed next.

8.4 Experiment and setup

Sensing Substrate Fabrication. Clevios PEDOT:PSS conductive polymer (purchased from Heraeus Group) was chosen as the imaging scaffold and spin-cast (500–2000 rpm for 2 min) on the ITO ($22 \times 22 \, \text{mm}$, Thickness #1, 8–12 Ohms Resistivity, from SPI supplies) glass coverslip substrate, which serves as the working electrode. Hard baking ($140 \, ^\circ\text{C}$ for 30 min) is then followed to ensure good contact and electrical conductivity of the scaffold as well as moisture removal. Patterned PEDOT:PSS substrate was fabricated by drop-casting silica beads on the as-prepared

substrate. Exposed PEDOT:PSS areas were etched off using enough power of oxygen plasma for 2 min, while preserving the pristine ITO surface at the same time. The residual silica beads were then sonicated off in deionized water with a duration of 1 min.

Electrochemical Impedance Measurements. An electrochemical cell was made of PDMS using a silver wire as a quasi-reference electrode and a platinum wire as a counter electrode. The potential of the scaffold was controlled using a bipotentiostat (Pine AFCBP1), and a sinusoidal potential modulation was applied through a function generator (Agilent 33120A). The modulation frequency ranged from 1 to 26 Hz at an amplitude up to 500 mVpp. Electrochemical impedance spectroscopic measurements were performed with the bipotentiostat from CH Instrument (660E).

Optical Imaging and Imaging Processing. Utilizing the electrochromism property of the conductive polymer, we placed the imaging scaffold on the bottom of the reservoir and constructed the transmitted imaging setup based on the widely used bright field inverted microscope (Fig. 8.4), where the light intensity travels from the source on the top to the bottom pane of the sample and reaches the camera via the objective. Postprocessing of the image sequence was accomplished using a program written in MATLAB.

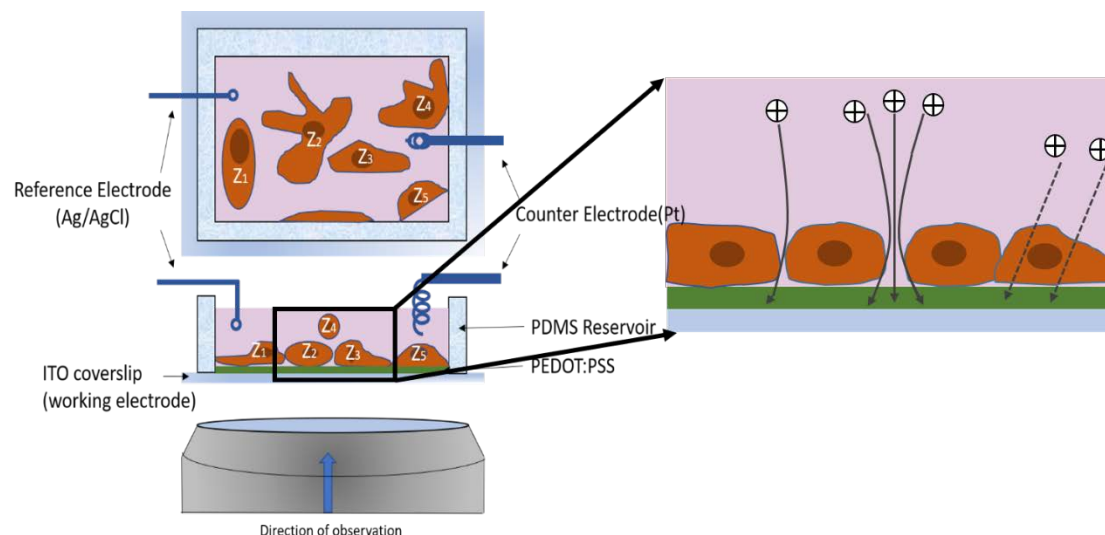


Fig. 8.4 Schematic of cell impedance imaging setup. Inset: detailed representation of the ion conducting/blocking principle.

Cell Culture. HeLa (ATCC CCL2) cells were cultured in a humidified atmosphere at 37 °C with 5% CO₂ and 70% moderate relative humidity. The culture medium consisted of DMEM, 1× (Dulbecco's Modification of Eagle's Medium) with 4.5 g/L glucose, L-glutamine, and sodium pyruvate + 10% fetal bovine serum and 1% penicillin as the antibiotic. Cells were regularly passaged with 0.05% trypsin and 1 × phosphate-buffered saline (PBS) when they reached a confluency of ~75%.

We employed the same system above and used it to image cell impedance. The only difference lies in that instead of seeding cells on glass coverslip for metabolism analysis in the previous chapter, cells being studied for attachment and cell-cell communication is seeded on a fabricated conductive polymer chip.

8.5 Localized electrochemical reactions and single cell impedances

The applied potential oscillates between -0.5 to 0.5 V vs Ag/AgCl, and the resulted ionic doping and dedoping processes will generate sinusoidal optical responses (red curve in Fig. 8.2a). Comparing with the electrical current signal (black curve in Fig

8.2a), the optical responses has a 90° phase shift. This is expected since the optical signal is proportional to the integration of current according to Eq (1). According to the experimental discussion, since a sinusoidal potential is applied on the electrode surface, after the calculation of the integration, the current density $i_{amp}(x,y)$ can be connected to the optical intensity oscillation amplitude $\Delta I_{amp}(x, y)$ using

$$\Delta I_{amp}(x, y) = \frac{\alpha}{2\sqrt{2}} \times \frac{i_{amp}(x, y)}{f} \quad (14)$$

where f is the frequency of the potential being applied to the sensing surface. Note that the thickness of the film will not affect the coefficient α , and we have carefully chosen the film thickness in the range of 100-500 nm range.

To demonstrate our imaging scaffold's capability to capture the localized surface impedance, a patterned PEDOT:PSS substrate was fabricated by etching the polymer film with the oxygen plasma. The silica beads are randomly cast onto the polymer film and used as the mask to form the pattern. 2 Hz sinusoidal potential was applied, and the corresponding optical images were recorded with the camera. A MATLAB code was used to perform the FFT on each pixel, and the optical oscillation amplitudes of those pixels at 2 Hz were extracted and plotted in Fig. 8.5b. The result shows large variations in different polymer regions, which is due to the thickness fluctuation introduced by the etching process. Using Eq (3), the localized current density (Fig. 8.5c) can be calculated from the optical oscillation amplitude (Fig. 8.5b) with the coefficient constant α (the value is obtained in Fig. 8.2c). Fig. 8.5d shows the phase responses of the entire region. Although there are relatively big amplitude variations, the phase values in different polymer regions are consistent with each other. This

proves that our optical signals directly reflect the electrochemical reactions.

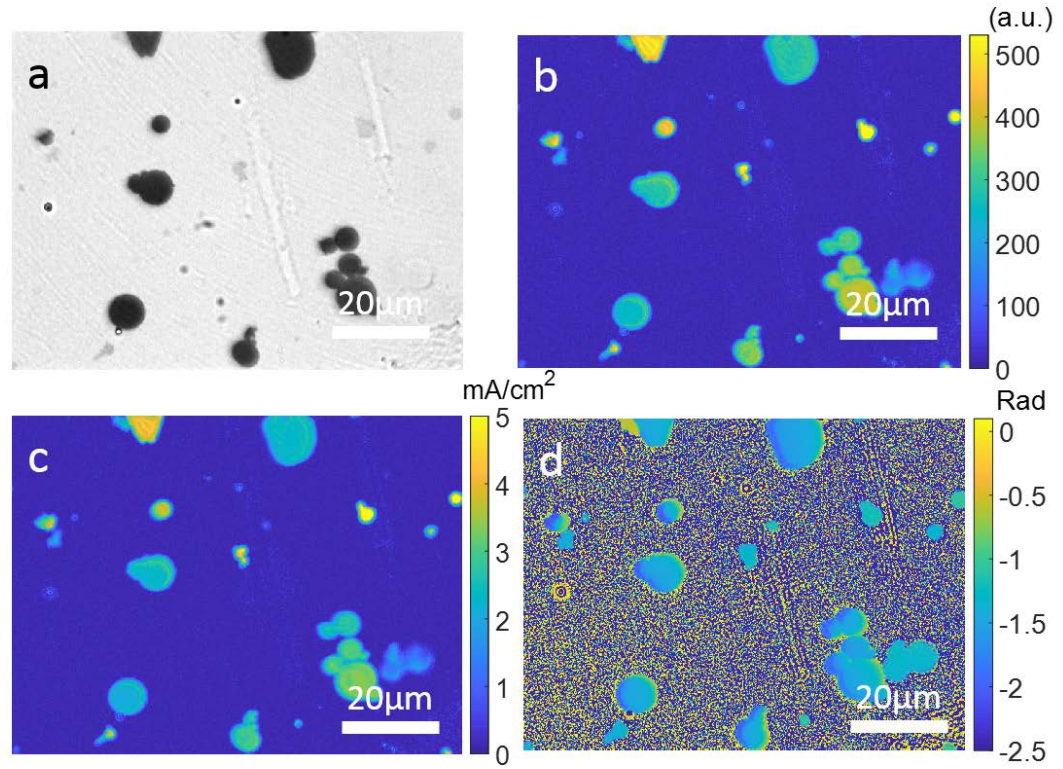


Fig. 8.5 Conductive polymer-based impedance measurement principle demonstration. (a) Transmitted image of the patterned PEDOT:PSS surface. (b) Optical amplitude image of the patterned PEDOT:PSS surface. (c) Current density of the same measurement. (d) Phase map of the current density responses. The phase is randomly distributed in the ITO region because the oscillation signal is zero.

Given the capability of measuring the localized substrate impedance, we are ready to implement the Eq (10) to derive the localized impedance. Firstly, the total localized impedance can be given by

$$Z(x, y) = \frac{v_{pp}}{i_{amp}(x, y) \times A} \quad (15)$$

where v_{pp} and A are the peak-to-peak amplitude of the applied potential and the area per pixel, respectively. In our measurement, $A = 13.6 \times 10^{-10} \text{ cm}^2$, we have

demonstrated that the optical signal generated by the doping and de-doping process is directly related. Taking the Eq (10) into Eq (15), we obtain

$$Z(x, y) = \frac{\alpha v_{pp}}{2\sqrt{2}Af} \times \frac{1}{\Delta I_{amp}(x, y)}. \quad (16)$$

Using this equation, we could obtain the localized impedance throughout the whole imaging surface area, thus deriving the difficult-to-measure small single cell impedance. As before, we applied sinusoidal wave potential with a frequency of 2 Hz and we recorded the intensity values at each pixel and calculated the pixelized impedance according to Eq (12).

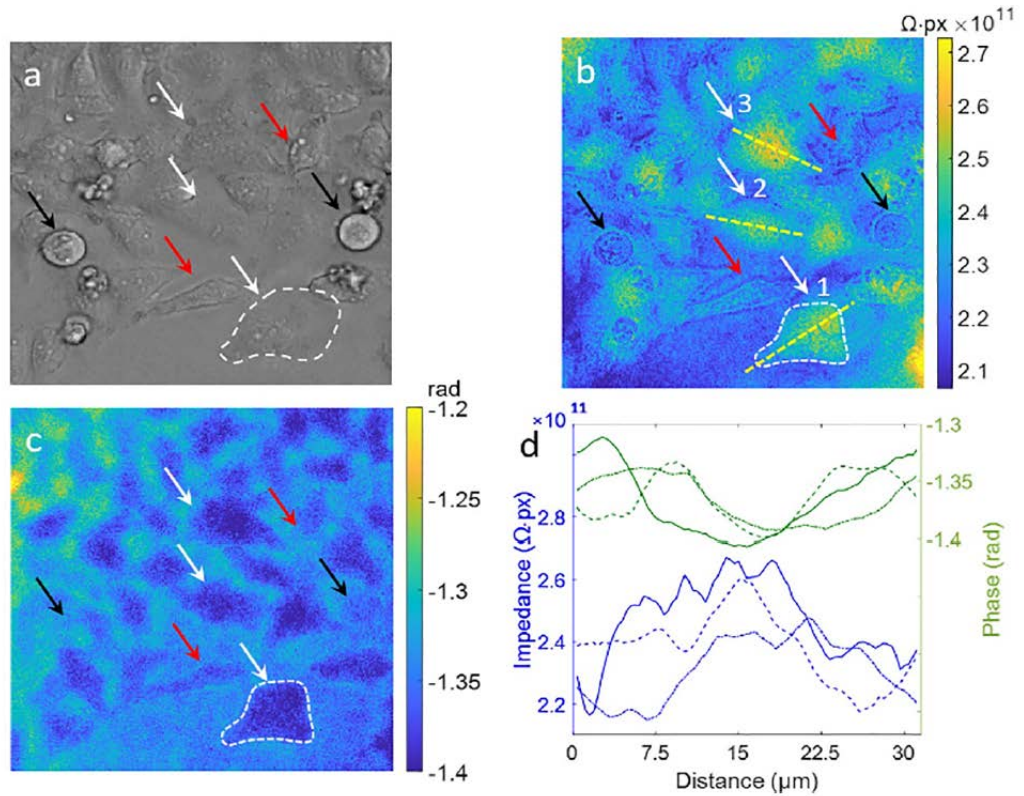


Fig. 8.6 Single-cell impedance images. (a) Transmitted image of the cells. (b) Impedance amplitudes of the single cells. (c) Phase image of the cells. (d) Impedance (left axis, blue curve) and phase (right axis, green curve) distribution of single cells. The distributions were plotted along the longest axis of the cells (yellow dashed lines in (b) on cells #1–#3). The solid, dashed, and dotted curves are obtained from cells #1, #2, and #3 (labeled in b), respectively.

As can be seen from the Fig.8.6 a-c, the transmitted images of cultured cells and the corresponding impedance amplitude and phase images were plotted side by side to provide sufficient direct visualization of the different individual cells. These figures show that different cells will result in different impedance. For the cells of which the viability is low (cells pointed out with the black arrows in Fig. 8.6a–c), they do not show obvious differences in impedance amplitude and phase signals compared with adjacent bare polymer regions. On the contrary, if the cells are not dead, meaning maintaining some level of viability, these cells tend to spread out but not fully attached to the surface (cells pointed out with the red arrows in Fig. 8.6a–c), the impedance amplitude and phase of those cells do show some contrast to the surrounding bare polymer regions. Lastly, when the cells are healthy and viable enough to fully spread out and attached well to the substrate (cells pointed out with the white arrows in Fig. 8.6a–c), both impedance amplitude and phase show the most significant contrast to their surrounding polymer areas.

We have successfully demonstrated that our method can image the individual cell impedance and show that different cells have different impedance amplitudes and phases, reflecting the heterogeneity of cell–substrate interactions. We also found that even for the individual cell, the impedance distribution is not uniform across the cell surface area. To illustrate this phenomenon, we have identified 3 well-attached cells labeled with the numbers 1-3 in Fig 8.6b. These cells all have a bigger impedance in the center regions than the edge regions. We hypothesize that this is due to the thickness of the cells. The cell center normally hosts the cell nucleus, and the

thickness is much bigger than the edge regions. To verify our hypothesis, we have performed the finite element simulation using COMSOL Multiphysics (see Supporting Information for detailed model establishment) to calculate the impedance responses of the cells.

8.6 Comsol simulation result

The results (Fig. 8.7 below) show that the cell center regions have much higher impedance due to the cell nucleus.

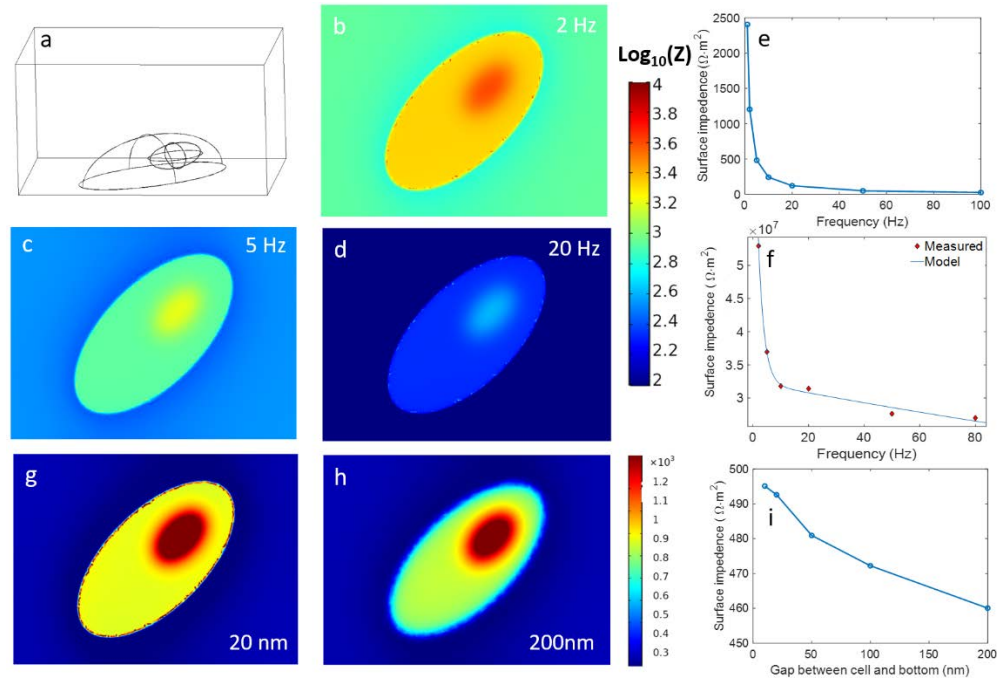


Fig. 8.7. Frequency and cell-substrate distance effects. (a,b) Impedance amplitude images at 2 and 20 Hz. (c,d) Simulated cell impedance image at 20 and 200 nm cell-substrate distances. (e) 3D drawing of the COMSOL simulation model. The model is built with the RF module in COMSOL Multiphysics. (f) Simulated average cell impedance vs frequency. (g) Measured impedance frequency responses of cells. (h) Impedance response at different cell-substrate distances. (i) Impedance response at different cell-substrate distances.

We have also plotted the cell impedance amplitudes and phases of these three cells (cells #1–#3 in Fig. 8.6b) across their longest axis in Fig. 8.6d, and the result shows the bigger impedance amplitude in the cell center. The boundaries of the cells are

more apparent in the phase image than the amplitude image. This is because the phase of the cells will suddenly change at the cell edge. In addition, cell #3 does not show any obvious contrast in the transmitted image (white dashed circle in Fig. 8.6a). However, the amplitude and especially the phase images show a clear subregion, circled by the white dashed line in Fig. 8.6b-c, which has a much bigger impedance amplitude and phase. Note that the white dashed region in Fig. 8.6b-c is much smaller than the cell boundary (white dashed circle in Fig. 8.6a). This shows that the cell attachment strength could be highly localized and heterogeneous within one cell.

8.7 Cell-substrate interaction dynamics and bulk impedance statistics

To verify our hypothesis that the different impedance in Fig. 9.6b is caused by cell attachment strength variation, we simulated the impedance response of a single cell at different cell-substrate distances with the 3D COMSOL model. Fig. 9.7g-h shows the cell impedance at 20 and 200 nm cell-substrate distances, and Fig. 8.7i shows the impedance response versus cell-substrate distance. The results show that when the cell is closer to the substrate, the impedance will be bigger, which proves that our hypothesis is solid and sound.

Therefore, we collected different impedance images at different stages of the cell attachment below, where cell images and impedance images after 1 day, 3 days, and 5 days of cell culture on the substrate were plotted side by side. After 1 day of cell culture, most of the cells have spread out. However, the impedance does not show strong contrast comparing with the surrounding bare polymer regions. This indicates that the cells have not formed a good contact with the substrate. After 3 days of culture, the impedance of cell regions has an obvious contrast comparing with the bare

polymer regions. This shows that the cells have established a strong attachment with the substrate. On the other hand, we cannot see any difference from the transmitted image. This demonstrates the sensitivity of the method to study the cell–substrate interactions. After 5 days of culture, there are multilayers of cells formed on the substrate (Fig. 8.8e). Although the cell–substrate interaction may not increase, thicker cell thickness will lead to a much bigger impedance.

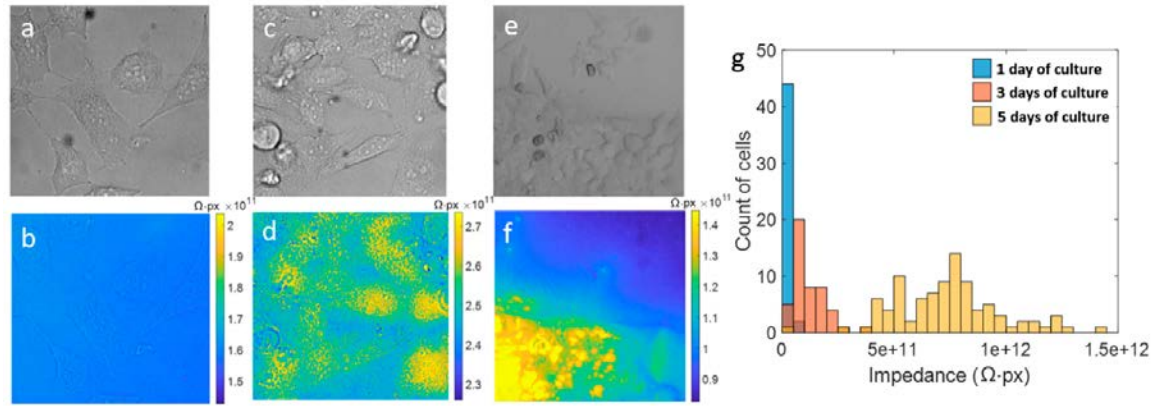


Fig. 8.8 Study of cell–substrate interaction dynamics. (a–f) Transmitted images (the images in the top row) and the corresponding impedance amplitude images (images in the bottom row) after 1 day, 3 days, and 5 days of the cell culture. (g) Histogram of single-cell impedance at different culture times.

Given the sequence of these cell impedance amplitude, we utilized the equivalent circuit model in Fig. 8.3 right to decompose the total impedance to three parts according to

$$Z = Z_{poly} + Z_{cell} + Z_{sol} \quad (17)$$

where Z_{poly} , Z_{cell} , and Z_{sol} are the impedance of the polymer layer, the impedance of cells, and the impedance of the solution, respectively. Therefore, to calculate the cell impedance, we only need to subtract the impedance of the cell regions with that of the regions without cells and integrate with the cell area. We have calculated each cell's

impedance and plotted them in a histogram (Fig. 8.8g). From the figure, we can see that the longer culture time will result in a bigger impedance and stronger cell–substrate interactions.

8.8 Conclusions

In this chapter, we proposed and demonstrated a new imaging-based method that transforms the collective electrochemical impedance signals to the responses that could be imaged with a simple optical microscope. Our impedance benchmarking method uses a thin PEDOT:PSS layer as the sensing/optical imaging interface. Using this scaffold, we have successfully imaged the single cell impedance images and observed different responses from different cells. Within a single cell, the impedance response also varies: the center of the cell tends to have bigger impedance which is due to the cell nucleus. This is verified with the 3D COMSOL simulation. Different days of cell culture on the imaging scaffold also leads to different cell-substrate adhesion stages and this result was verified in a histogram.

IX. CONCLUSIONS AND PERSPECTIVE

9.1 Conclusions

As demonstrated in this dissertation, surface plasmon resonance imaging (SPRI) technique is very useful and can be applied to different applications. As demonstrated, SPRI has been applied to the in-situ label free characterization of lithium metal battery systems. SEI formation dynamics and corresponding lithium plating/nucleation has been characterized in nano scale. Applications of this technique into more advanced electrolytes as well as effective surface plasmon generation via in situ plating of lithium metal itself was achieved. Multiple stages of lithium SEI formation and nucleation was labelled both in the form of a representative curve and spatial resolved images. By using an indirect measurement technique, the localized distribution of the electrochemical current can be mapped.

Besides the application of plasmonic based imaging in the lithium metal battery, more discussion has been implemented into the field of oxygen evolution reaction catalyst screening as well as cell metabolism and cell impedance measurements. Given the capability of imaging the localized reactions and electrochemical currents, and by virtue of some facile methods of algorithm, we were able to establish the whole picture of advanced imaging in the application of bio and energy related fields. All results are accompanied with direct benchmarking results with simulation as well as spatial resolved local properties of these systems.

9.2 Perspective

One of the challenges of SPRI method is how to increase the optical signal beyond the detection range ~ 200 nm. The optical signal (refractive index) change of some of the reaction is not very big because the molecule may only lose or get an electron and it will not change the optical properties of the molecule a lot. However, during the lithium plating process, most of the time the grown lithium metal will penetrate beyond this limit. Our group has been developing a 3D microscopy to gain the z-axis information of the lithium plating, yet the correlation between the 3D images and the 2D surface plasmon images has been established. Therefore, a comprehensive system which can measure the lithium plating and the lithium nucleation around $1\text{ }\mu\text{m}$ range will benefit the process of lithium metal anode characterization.

Another aspect of the potential improvement on the characterization results is that we should be able to further enhance the Raman detection signal to noise ratio to prove that our initial layer is comprised of the proposed components, including LiF and Li_2O . We could also gain the information about the exact amount of these components on the Cu substrate.

I believe more and more battery and bio-related applications will begin by applying and improving SPRI technique and the transmission microscope described in this dissertation. For example, more advanced electrolytes and more cell lines to be measured in the application of the technique.

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