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NOVEL OPTICAL DEVICES FOR BIOMEDICAL IMAGING AND SPECTROSCOPY

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ABSTRACT

With the growing demand for better medical care worldwide, biomedical imaging and spectroscopy which provide critical information for disease diagnosis and personal health condition have become a focal point. In order to output high quality imaging and spectroscopy results, which is critical for diagnosis accuracy, optical devices have been extensively explored. In this dissertation, I present several novel optical devices for biomedical imaging and spectroscopy. They are all capable of promoting the performance of existing imaging and spectroscopy applications or enabling new applications.

In Chapter two, I present a laser based light source which is ultrafast, multi-line, dual polarization, broadly tunable, and power scalable. This light source could be an ideal source for nonlinear optical imaging. We combine soliton self-frequency shift and pulse division method together to achieve these features. Specifically, power scalable is achieved through pulse division and recombination, which provides a general solution for conventional soliton self-frequency shift source whose pulse energy is limited. Also, benefited from the pulse division, divided pulses can be shifted individually, which enables multi-wavelength and dual polarization capability. Simultaneous dual-polarization second-harmonic generation imaging is demonstrated based on these features.

In Chapter three, I present a biodegradable step-index optical fiber, which is suitable for light delivery under in vivo condition. The fiber has desirable mechanical and biological properties which are superior to traditional silica optical fiber. This is attributed to the programmable citrate-based material platform we use to fabricate the fiber. The fiber has a 0.4dB/cm loss, with a 1/e length longer than 10cm. By using this fiber, successful light delivery in tissue as well as fluorescence excitation and collection are accomplished, which prove the
fiber’s usefulness for \textit{in vivo} applications. Further, successful image delivery verifies the fiber’s ability for image transmission, which can potentially enable endoscopy applications.

In Chapter four, I demonstrate two smartphone based detection devices, suitable for spectroscopy applications. Both of them benefit from the smartphone, which is ideal for point-of-care applications. Specifically, we developed a G-Fresnel smartphone based spectrometer. Successful protein concentration measurement using Bradford reagents demonstrates its ability for general spectroscopy applications. In addition to the smartphone spectrometer, we also developed a smartphone chloridometer based on a citrate chloride sensor. Through clinical verification, the device shows high accuracy and broad measurement range, which can potential serve as a device for cystic fibrosis diagnosis.
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Chapter 1

Introduction

Biomedical imaging and spectroscopy are frequently used tools in today’s medical diagnosis and treatment. Despite that they derive from different principles, both of these methods have helped doctors and researchers to obtain more information about patients’ condition.

Specifically, biomedical imaging is a process to visualize tissue’s microscopic view or body’s inner structure, which presents doctors with the target objects. These captured images could reveal clues and evidence of disease, which is critical for medical diagnosis. For example, optical imaging is recommended by World Health Organization for prompt parasite-based diagnosis[1]. Commonly used biomedical imaging techniques include optical coherence tomography, bright field microscopy, dark field microscopy, endoscopy, photoacoustic imaging and so on. Each of them has their own strengths and weaknesses, which are usually selected based on the requirements of applications. For example, imaging single-celled organism using bright field microscope suffers from strong background, which decreases the signal to noise ratio. This could be resolved by using dark field microscopy[2]. Spectroscopy, on the other hand, studies light matter interaction, which is generally used for qualitative and quantitative analysis of the samples’ chemical composition. It is currently one of the most widely used methods in physical testing of body fluid, which covers urine, saliva, sweat etc. These measurements generally require assistance from professionally developed chemical assays. In short, assays that are mixed with body waste sample tend to exhibit change in light absorbance, fluorescence emission, and luminescence, which could be picked up by plate reader. By this means, certain indicators for patients’ health condition can be obtained[3]. For example, thiocyanate
concentration in saliva, which is indicator for cigarette smoking, can be quantified through measuring its infrared absorbance[3], [4]. Furthermore, spectroscopy is usually combined with imaging to form spectral imaging. Spectral imaging techniques provide richer information to researchers. Taken together, biomedical imaging and spectroscopy serve as the major tools to provide evidence for doctors and researchers to perform clinical analysis and medical intervention.

As the Chinese saying goes, “One who wishes to do his work well must first sharpen his tools.” Therefore, in order to achieve higher quality biomedical imaging and spectroscopy, it is important to develop advanced optical devices. At the same time, the advancement of optical devices propels progression of biomedical imaging and spectroscopy. Specifically, the evolution of excitation source, light transmission tool, and detection device have not only improved existing bio-techniques, but also enabled innovative methods to emerge. For example, the invention of the ultrafast laser has enabled the two-photon microscopy for imaging tissues and cells[5], [6], which is superior in suppressing background, increasing penetration depth, and reducing phototoxicity. As such, my dissertation incorporates several optics designs of excitation source, light transmission tool, and detection devices respectively with the attempt to enhance biomedical imaging and spectroscopy.

Laser based source is a widely used excitation source in today’s biomedical imaging and spectroscopy. The key difference between laser source and other light sources is that laser emits light coherently. Coherence includes spatial coherence and temporal coherence. Spatial coherence refers to cross correlations between two spatial locations in a wave for all times. Good spatial coherence allows light to be focused into a diffraction limited spot. This tiny focal spot can produce high irradiance, which is ideal for weak processes such as multi-photon fluorescence and harmonic generation. Temporal coherence refers to correlation between a wave and itself after some time delay, which can be reflected by spectrum width of the emitted light. High temporal coherence light has a narrow width, which is close to single frequency. Therefore, output light
with high temporal coherence has great phase correlation, which is ideal for certain imaging applications. The world’s first laser was built in 1960 by Theodore H. Maiman at Hughes Research Laboratories, based on theoretical work proposed by Charles Hard Townes and Arthur Leonard Schawlow. This laser utilized flash lamp as pump source and ruby crystal as gain media to produce a pulse red lasing wavelength at 694nm. Later, by using Helium and neon gas, Ali Javan, and William R. Bennett, and Donald Herriott constructed the world first continuous operation laser. After half a century’s dedication, laser sources with different wavelengths covering from deep ultraviolet light to far infrared, operation condition including pulsed and CW have been developed. Yet, demanded by the requirements from increasingly complicated biomedical imaging and spectroscopy experiments, more research is needed for developing new laser-based sources so as to improve current laser sources performance.

In chapter 2, I will present a versatile laser-based light source we developed, which is suitable for nonlinear optical imaging with biological samples. Specifically, we combine pulse division method to soliton self-frequency shift tunable laser source and demonstrate multi-line, dual polarization and power-scalable Ultrafast Source. The power scalable feature can be attractive, as it provides a simple solution towards the power limitations of conventional soliton self-frequency shift based light sources (output pulse energy is fixed at each wavelength). Also, the system’s dual polarization and multiple individual tunable lines properties are ideal for nonlinear imaging, since it is capable to conduct multiple resonant-frequency excitation. In general, this light source can be an ideal device for novel nonlinear imaging technique.

Optical fiber is the core light transmission tool used in biomedical imaging and spectroscopy. Based on total internal reflection at the interface between core and cladding layer, optical fiber can achieve ultra-low loss over long distance propagation. Moreover, due to the cladding layer, light is confined inside optical fiber’s core, which is free from the influence of the external environment. The world’s first optical fiber with a cladding for image transmission was
demonstrated by Bram van Heel in 1953. Later, optical fiber’s development was primarily focused on enabling data transmission and reducing propagation loss as it is mostly used in telecommunications. However, though commercial silica optical fiber has ultra-low loss over the visible and near-infrared region which is desirable for telecommunication, it is fragile and brittle in nature, which is not ideal for biomedical applications which usually require working under *in vivo* conditions. Therefore, it is important to develop optical fiber with optimum optical, mechanical and biological properties.

In chapter 3, I will present a biopolymer optical fiber which is capable of serving as a light transmission tool in biomedical imaging and spectroscopy. As stressed previously, the fundamental challenge of this area is the hitherto lack of waveguide with desirable optical, mechanical and biological functionality. To combat this, by using citrate based polymer, we fabricate a biocompatible and biodegradable step-index fiber with desirable mechanical property. Due to the step-index structure, the fiber achieves high transmission efficiency (low loss), which is ideal for light delivery. Further, through using the fabricated step-index fiber, we demonstrate preliminary multimode fiber imaging, *in vivo* light delivery and *in vivo* fluorescence sensing which validate the feasibility for deep tissue implantation and continuous monitoring. The results indicate that this citrate biopolymer fiber can be an ideal light transmission tool for performing biomedical imaging and spectroscopy inside body.

With the advent of the digital age, image sensors have become one of the most important detection devices, especially for biomedical imaging and spectroscopy applications. Though early analog sensors for visible light were video camera tubes, it was replaced by semiconductor charged coupled device (CCD) in the 1970s. Currently, CCD and active pixel sensors in complementary metal-oxide-semiconductor (CMOS) are the most popular image sensors. Compared with CMOS, CCD sensors have larger effective area, which leads to better light sensitivities. This makes it the best fit for applications seeking for high light sensitivities such as
astronomy. However, CMOS sensors have a much lower cost and power consumption, which are ideal for the majority of applications in daily life. Therefore, current digital cameras use CMOS sensor as their image sensors, including smartphone cameras. The availability of image sensors on smartphones enables smartphones to be used as detection devices in biomedical imaging and spectroscopy applications.

In chapter 4, I will present designs of two smartphone-based devices, which utilize smartphone cameras as detectors, for spectroscopy applications. Specifically, we developed a smartphone spectrometer and a smartphone chloridometer. The smartphone spectrometer, based on a unique diffraction device G-Fresnel, is able to achieve a compact size (~23cm³) and high performance (~1.5nm resolution) simultaneously. In virtue of the wide application of optical spectroscopy, the G-Fresnel smartphone spectrometer can provide a detection platform for biological/biomedical and environmental sensing applications. On the other hand, as evidenced in the accurate chloride measurement of quality control samples and cystic fibrosis patients’ samples, the smartphone chloridometer demonstrates desirable performance with the assistance of a chloride sensor. The integration of such low-cost smartphone based detection device and novel sensor material offers a powerful analytical solution in response to the challenges of point-of-care chloride sensing such as prohibitive costs as well as a lack of viable sensor materials. Both of these smartphone devices are ideal detection devices in biomedical spectroscopy applications.
Chapter 2

Divide pulse soliton self-frequency shift

2.1 Motivation for ultrafast broadly tunable optical source

Nonlinear optical microscopies utilize nonlinear light interactions between light and matter to generate signal contrast and therefore achieve imaging. Compared with traditional microscopy which measures the first order susceptibility of the sample, nonlinear light interactions, or in other words, nonlinear optical processes measure higher order susceptibilities of the sample, which usually possesses unique properties, and therefore can be utilized in microscopy to improve signal to noise ratio, enable label free detection, extract three dimensional information etc.[7] Currently, various nonlinear optical processes have been explored by scientists to develop novel microscopy methods for biomedical imaging. For example, second-harmonic generation, whose signal can only be generated from non-centrosymmetric structure, was employed to study collagen structure in tissue[8], [9]. Here, I would divide nonlinear processes used for imaging into two categories based on its nonlinear susceptibility order. First one is second-order nonlinear process, which includes SHG and sum-frequency generation (SFG). The other one is third-order nonlinear process, which includes Coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), third harmonic generation (THG), and two-photon excited fluorescence (TPEF). As mentioned before, each individual nonlinear process has its own properties, which could enable specific applications. For example, CARS microscopy is widely used for labeling free noninvasive imaging as it offers chemical selectivity[10].

To effectively use these nonlinear processes for imaging, there are two requirements on the pump source, high intensity and broad tunability. First, nonlinear susceptibility is small,
which makes high pump intensity a necessity. Therefore, ultrafast lasers, which offer high peak power, is the ideal tool for nonlinear imaging. Ultrafast laser is able to produce electromagnetic pulses that are equal or less than the order of picosecond ($10^{-12}$) on the time scale. In comparison with typical laser systems which are continuous wave (CW), these pulsed lasers are able to generate orders of magnitude higher peak intensity. For example, a picosecond laser with 1ps pulse duration and 100MHz repetition rate has a duty cycle of $10^{-4}$. This corresponds to $10^{4}$ peak power differences between this laser and a CW laser with the same average power. This small duty cycle has the same effect as focusing the light spatially using objective lens. The only difference is ultrafast concentration light in time. In addition to high intensity, the other crucial feature needed from nonlinear optical microscopy is broad tunability. For example, in Coherent anti-Stokes Raman spectroscopy, in order to locate the resonantly enhanced vibrational frequency for different Raman makers (example: DNA backbone 830 and 1093 cm$^{-1}$; S-H stretch 2573 cm$^{-1}$)[11], the stoke beam needs to have a wide tunable range (e.g. over 200nm range) to reach different Raman peaks. Thus, there is no doubt a versatile ultrafast tunable laser system is crucial for nonlinear optical microscopy.

Currently, the most popular ultrafast tunable laser systems are passive mode-locked Ti: Sapphire laser system and fiber laser system. Ti: Sapphire mode-locked laser system is the most popular laser system used in nonlinear optics. Initially invented in mid 1980s, Ti: Sapphire laser system which generates a large tuning range from 660 nm – 980nm, was a CW tunable source. After the late 1980s, Ti: Sapphire mode-locked laser was developed based on kerr lens mode locking mechanism, expanding the capability of the system. Currently, Ti: Sapphire mode-locked laser based OPO/OPA can be tuned from 630nm to 2600nm, satisfying many requirements of nonlinear microscopy. In comparison, ultrafast tunable fiber laser system has a much shorter history, but its robustness, low cost, and potential portability have quickly made it an attractive source for nonlinear microscopy. Although first fiber based source that is able to output similar
power as Ti: Sapphire was demonstrated in 2009[12], enabling broad tunability and high power simultaneously still remains as the major challenge today.

In this chapter, I will present a versatile ultrafast broad tunable light source, which is multi-line, dual polarization, and power scalable[13]. We name it divided pulse soliton self-frequency shift (DPS) because it is achieved through combining the techniques of soliton self-frequency shift and pulse division. The method could be the ideal solution for nonlinear microscopy as it offers high power and broad tunability simultaneously. I will first give a brief introduction of soliton self-frequency shift and pulse division technique. Afterwards, the details of the divided pulse soliton self-frequency shift method and its application for simultaneous dual polarization SHG imaging will be presented.

2.2 Introduction to Soliton self-frequency shift and pulse division

2.2.1 Soliton self-Frequency shift

Soliton self-frequency shift (SSFS) is a process that happens during soliton pulse propagation inside optical waveguide. It causes the center frequency of the soliton pulse to redshift into higher wavelength. The amount of center wavelength shift is controlled by the input pulses’ peak power and the optical fiber length. The principle of this process is intra-pulse stimulated Raman scattering, as soliton continuously transfers energy from higher frequency to lower frequency. Initially, the phenomenon was discovered by Mitschke and Mollenauer in 1986[14]. They reported a redshift of the center frequency of a sub-picosecond soliton pulse after propagation in a single-mode, polarization maintaining fiber and named this phenomenon soliton self-frequency shift. Subsequently, the theoretical basis of SSFS was derived by Gordon[15]. Based on his result, SSFS should be only significant for sub-picosecond pulses, which is
primarily why SSFS by ps-scale pulses was not observed. In his derivation, SSFS wavelength shift was solved through considering Raman effect as a delayed response in NLSE. The dependence of wavelength shift (in terahertz per kilometer) on pulse width is estimated by:

\[ \frac{dv}{dz} \propto \frac{1}{\tau^4}. \]

Currently, theoretical estimation of SSFS is usually completed through solving the higher order nonlinear Schrödinger equation (NLSE) listed below numerically using split-step Fourier method [16].

\[ i \frac{\partial u}{\partial z} + \frac{1}{2} \frac{\partial^2 u}{\partial t^2} + |u|^2 u = i \delta \frac{\partial^3 u}{\partial t^3} - i s \frac{\partial}{\partial t} (|u|^2 u) + \tau \mu \frac{\partial |u|^2}{\partial t} \]

On the left hand side, the second term represents Group velocity dispersion (GVD) and the third term represents self-phase modulation (SPM). The terms on the right hand side of the equation represent third order dispersion, self-steepening, and intrapulse Raman scattering respectively. The numerical solution to the higher order NLSE is generally accurate in predicting SSFS behavior.

Though SSFS was developed three decades ago, it remains as an active research topic due to its attractive properties. However, its limitation has not been overcome yet, hindering its commercial possibility. In order to fully understand this, I will start with a brief explanation of optical soliton, which is the foundation of SSFS. Soliton refers to a wave packet that does not change its shape during propagation. In optics, solitons can be divided into two categories, spatial solitons and temporal solitons. Spatial solitons refer to a balance between nonlinear Kerr effect (\( \chi^3 \) process) and optical diffraction. Practically, it acts similarly to a graded index waveguide which confines the spatial mode of the beam. Temporal solitons also utilize nonlinear Kerr effect, however instead of confining the spatial profile, it helps maintain the temporal profile of the optical pulse by balancing the linear dispersion. In this work, solitons which propagate inside an
optical fiber are temporal soliton. In the following section, I will provide a more detailed description of the physical picture of temporal solitons. When a pulse propagates inside an optical fiber, it experiences two effects which change its pulse shape based on the assumption that the pulse energy is limited so that no other nonlinear effects will happen. These two effects are group velocity dispersion and nonlinear Kerr effect. Group velocity, different from phase velocity which defines the phase speed of a specific frequency component of a pulse, is the velocity that a wave packet propagates at. As group velocity is frequency dependent, different frequency components of a pulse walk off and spread in time domain. For example, inside a waveguide with anomalous dispersion, $D > 0$, a pulse’s higher frequency components propagate faster and lower frequency components propagate slower. Therefore, the pulse will get chirped and broaden in time. On the other hand, pulse will also experience optical Kerr effect. To be more specific, the phase of the pulse can be expressed using $\phi(t) = \omega_0 t - k_0 z = \omega_0 t - k_0 z[n + n_2 I(t)]$, where $n_2$ is the nonlinear refractive index coefficient. Therefore, the frequency of the field can be obtained by taking the time derivative of the phase $\omega(t) = \omega_0 - k_0 z n_2 \frac{\partial I(t)}{\partial t}$. Consider a single pulse profile in time domain, derivative of the intensity is initially positive and reaches zero at its peak value. It further goes negative as the intensity starts to drop. It is obvious that the frequency of the leading edge of the pulse is smaller than $\omega_0$ and the trailing edge of the pulse has a frequency larger than $\omega_0$. Therefore, an optical pulse influenced by the optical Kerr effect will have lower frequency part in the front and higher frequency part in the back. Actually, an optical pulse will experience these two effects simultaneously. When they balance out each other, a temporal soliton will be formed and the pulse is able to propagate without changing its shape.

As we now understand what temporal soliton is, the key requirement and limitation for SSFS are delineated. The waveguide must be anomalously dispersed for the target spectral range to support soliton formation. Thus, up till the invention of microstructured optical fibers, which
expand the anomalous dispersion range to the near infrared, SSFS has only been demonstrated from 1.3μm to 2μm, which is the anomalous dispersion range for traditional silica fiber. For example, in 1987, Beaud showed wavelength shift from 1.36μm to 1.54μm using dye laser and single mode fiber[17]. In 2001, by using passively mode-locked Er-doped fiber laser and 220m long polarization maintaining fiber, Nishizawa demonstrated wide tuning from 1.5μm to 2μm[18]. But with the advancement of fiber fabrication method, limitations pertaining to anomalous dispersion range of optical fiber were addressed. Specifically, fibers with anomalous dispersion covering the near infrared and even mid infrared were developed and could serve as ideal platforms for SSFS. By using nonlinear photonic crystal fiber, SSFS wavelength tuning from 0.78μm to 1.3μm was demonstrated[19]–[23]. Further, in 2016, Tang demonstrated a 100fs soliton source tunable from 2 to 4.3μm using fluoride fiber, expanding the range to mid infrared[24].

From optical soliton background, we have learned the limitation for the wavelength tuning range of SSFS, but there is another limitation for this technique which is limited output power. As addressed previously, the wavelength shift of SSFS is controlled by varying the input pulse peak power. Therefore, for all SSFS systems, output pulses’ peak power at each wavelength were fixed, which significantly limits SSFS to be used for applications requires high peak power. Therefore, in order to increase the pulse energy, Chris Xu et al. have demonstrated SSFS using large mode area (LMA) fiber, the pulse energy of soliton can be successfully increased from 1nJ to 45nJ over 1.55μm to 2.13μm[25]–[27]. However, there is still no method to increase the pulse energy other than changing fibers.
2.2.2 Pulse division

In ultrafast optics, pulse division is a pulse shaping method used to split a single ultrashort pulse into multiple ones. Currently, two methods are primary used for pulse division. First is birefringent crystal sets[28][29]. The birefringent crystal has two axes, the ordinary (O) and the extraordinary (E) which have different refractive indices, $n_o$ and $n_e$ respectively. Hence, light with two different polarizations would experience different group velocity. By carefully selecting the crystal thickness $x$, the temporal delay $\tau$ between the two polarizations can be introduced and is shown in the following equation, where $V_o$ is the group velocity of ordinary polarization and $V_e$ is the group velocity of extraordinary polarization.

$$\tau = \Delta t = x \left( \frac{1}{V_o} - \frac{1}{V_e} \right)$$

An input single pulse would be separated into two pulses with equal intensity through adjusting the polarization of the pulse $45^\circ$ to the ordinary axis and extraordinary axis. The system diagram is shown in Figure 2-1.
Figure 2-1: Systematic diagram of pulse division using birefringent crystal pairs. A single high power pulse is divided into four pulses by using two crystals

The other method is using polarizing Beamsplitter for pulse splitting[30]. Polarizing Beamsplitter is a polarizer. It reflects vertically polarized light while transmits horizontally polarized light. Therefore, two polarizing Beamsplitter can be used to divide a single pulse into two sub pulses. Different from crystal set whose time delay is determined by the crystal thickness, pulse separation $\tau$ of polarizing beamsplitter set is determined by the length of the delay line $l_d$:

$$\tau = \frac{2l_d}{c}.$$  A basic system for generating a pulse train using polarizing beamsplitter is shown by Figure 2-2. Input pulse at 45 degree polarization is coupled into the polarizing beamsplitter set. The horizontally polarized component of the input pulse transmits through the polarizing beamsplitter while the vertical component gets reflected. The vertically polarized pulse is sent through a delay line which introduces a time delay to it compared with the horizontally polarized pulse. The second polarizing beamsplitter combines the two pulses into a single beam, which successfully achieves pulse division.
Figure 2-2: schematic diagram of polarizing beamsplitter based pulse division system. A delay is introduced between the separated pulses with orthogonal polarizations.

Pulse division method has been used to decrease the peak power of a pulse, which could mitigate nonlinear effect during ultrashort pulse propagation and enable pulse energy amplification. This method was initially demonstrated in 2007, as Zhou implemented pulse division and coherent recombination using birefringent crystal set for ultrafast pulse amplification[31]. Compared with classic chirped pulse amplification (CPA) method, key advantage of the divided pulse amplification method is its effectiveness for dealing with picosecond (ps) pulses. In practice, ps pulses are hard to maintain high fidelity during stretch and compress. Based on this method, divided-pulse laser which enables 16 times scaling of a ps ytterbium-doped fiber laser was demonstrated. As an extension, this method was combined with CPA to allow further power scaling of the ultrafast system[32], [33].
2.3 Working principle of divided pulse SSFS and experimental setup

The key idea of DPS is to separate a single pulse into multiple seed pulses and shift them independently[13]. By using the pulse division method to divide a high energy pulse into N number of seed pulses and coupling all of them subsequently into an optical fiber that supports soliton formation and is able to sustain it, pulse copies will shift individually according to its peak powers through SSFS[34]. Through this mean, by initially generating a train of pulses with different peak powers and utilizing the one-to-one mapping of input peak power to output wavelength, our system is able to fast output multiple color pulses from a single origin of pulse (controlled by the number of pulse divisions N), as well as to achieve the ability to broadly tune the wavelengths of each of these pulses (controlled by adjusting the power splitting ratio for each pulse divider) (Figure2-3).

Figure 2-3: Schematic diagram of divided pulse soliton self-frequency shift, pseudocolor is used in the figure.
In addition to multi-color output, the DPS source also provides the unique ability for power scaling pulses undergoing intraband Raman scattering. Generally, fixed experimental parameters like the peak power and spectral width of an input pulse, as well as the length of fiber available in an experiment, will create a fixed relationship between the achievable output power and wavelength of a SSFS pulse. The DPS uses pulse division and recombination techniques to effectively decouple the achievable wavelength shift from the final average and peak power of the SSFS pulse. Specifically, if N pulses are passed through an appropriate length of optical fiber that can sustain soliton formation, the N input pulses can generate N output pulse copies that are each shifted in wavelength by the same amount. Recombination of these N pulse copies will theoretically result in a single pulse at the same wavelength with an average and peak power N times greater than otherwise achievable with a single pulse passed through the same length of optical fiber. In this way, pulse division used in conjunction with the soliton self-frequency shift decouples the inherent and oftentimes limiting the correlation between power (average and peak) and wavelength shift, allowing for power scalable broadly tunable pulse generation.

Figure 2-4: Schematic of the divided pulse soliton self-frequency shift architecture. HWP, half waveplate; PBS, polarizing beam splitter; OL Objective lens; PCF, photonic crystal fiber
Figure 2-5: Photo of the experiment setup including prism pairs for dispersion compensation, two delay stages, and fiber coupling stage.

The schematic diagram of the DPS experimental architecture is depicted in Figure 2-4 and a photo of the experiment setup is shown in Figure 2-5. During the experiment, we used a Ti:Sapphire laser (KMLabs, 30 fs, central wavelength ~845 nm, average output power ~ 670 mW, repetition rate 87 MHz) as the pump source for the DPS system. In order to compensate the group velocity dispersion introduced by the series of broad-band coated (from 800-1550 nm) polarizing beam splitter (PBS) cubes arranged in a Mach-Zender like configuration for pulse divisions, a pair of SF11 prisms was introduced. The effect of each division stage was to create two pulse copies for each single pulse input. The two newly generated pulses possessed orthogonal polarization states and their differences in time were defined by the path difference.
between the interferometric arms of the division stages. By changing the orientation of the half-wave plate before the division stage, relative power between divided pulses could be controlled. In this system, we chose PBS cubes instead of birefringent crystals for pulse division and recombination as they allow the overall temporal separation between pulses to be determined by the path length through air (rather than a dielectric material), ensuring that the path length between pulses was largely wavelength independent. Note that these division stages are also capable of performing recombination of the divided pulses through input from the reverse direction, provided that the polarization of each pulse is rotated by 90 degrees (i.e., the alternating linear polarization states are exchanged) before re-entrance. Detailed description for recombination will be discussed later.

An exemplary pulse divider for generating four pulse copies is illustrated in Figure 2-4. The difference in path length between the first division stage was 100 mm, creating two pulse copies with orthogonal polarization states separated by 333 ps. In Figure 2-4, pulses with vertical or out of plane polarization are depicted by dots, pulses with horizontal or in plane polarization are depicted with vertical arrows, and pulses with a 45 degree polarization state relative to these two, are represented by tilted arrows.

A preceding half-wave plate controlled the power ratio between the first two pulses while the following half-wave plate rotated the polarization states of each of these two pulses before entering the second division stage, which introduced a 50mm path length difference. As a result of passing through the entire division system, four pulse copies separated in time by 167 ps with alternating vertical and horizontal polarization states were generated. The energy of the individual pulses could be controlled through proper adjustment of the tandem half-wave plates. The system is scalable and further pulse copies can be added by incorporating additional PBS cube pairs. The resultant pulse train was subsequently coupled into a 1.8 meter long highly nonlinear photonic
crystal fiber (NL-PM-750 with a zero-dispersion wavelength of 750 nm) for SSFS with a 40X objective. A similar strength objective was used for collimating the fiber output.

![Diagram of pulse recombination setup](image)

**Figure 2-6:** Schematic of pulse recombination setup, HWP, half waveplate; PBS, polarizing beam splitter; PCF, photonic crystal fiber

The ability to generate a larger number of pulses is practically limited only by the available power of the pump source. Although the division technique is itself theoretically lossless, the availability of photonic crystal fiber with net anomalous dispersion near 800 nm necessitates a smaller fiber core to provide large waveguide dispersion which translates to coupling efficiencies on the order of 30%. For this reason, along with available pump power, recombination studies were restricted to a two pulse DPS system in order to best demonstrate source capabilities with the expectation that results are scalable. A schematic of the recombination stage is shown in Figure 2-6. Due to the birefringence in the polarization-maintaining PCF, an additional stage that allowed for compensation of the temporal delay induced by the birefringence of the PCF was used for pulse recombination. Initially, the polarization states of the pulses were rotated with a half waveplate so that they were orthogonal to their polarization states in the division stage. Next, through scanning the delay time between the pulses in the recombination stage while monitoring autocorrelation traces, it was possible to temporally recombine the two pulses. In order to generalize this approach for more than two
pulses, a train of SSFS pulses can be recombined by passing them reversely through the division stage in Figure 2-4 after the temporal delay induced by fiber birefringence has been compensated for. In this case, the recombined pulses would exit the system at the first PBS cube in Figure 2-4 with an orthogonal polarization state to the input.

2.4 Performance and application

Figure 2-7: a) Soliton center wavelength shifting with changing input pulse peak power b) Wavelength tuning of two pulse copies while changing half-wave plate angle to modify power ratio between two pulse copies. c) Red line: two pulse copies with different wavelength tuning. Blue line: two pulse copies with identical wavelength. d) Four pulse copies with different wavelength.
To characterize the performance of the system, we tested the SSFS system’s tunability by coupling single pulse into a PCF, thereby demonstrating the expected relationship between single pulse soliton energy and the frequency shift of the resultant output pulse from past literature. An Andor spectrometer (SR-500i-A-R) with an InGaAs IR camera (iDus InGaAs 490A-1.7) was used to measure the output pulse spectrum. A wavelength shift from 850 nm to 1250 nm was achieved (shown by Figure 2-7a) by varying the input pulse energy and, as expected, the longest shifted wavelength pulse at 1250 nm is limited by the 2nd zero dispersion wavelength (~1270 nm) of the photonic crystal fiber. Beyond this point, the fiber dispersion becomes normal and no longer supports soliton propagation. The polarization of the SSFS output pulses was maintained due to the birefringence in the polarization-maintaining PCF. The average power of the first order soliton at 1100 nm with 28nm bandwidth (corresponding to a 40 fs pulse) was measured to be ~3 mW. Similar to what was addressed previously, the one-to-one mapping between the input pulse energy and the output wavelength limits the achievable peak power and average power at a particular wavelength for conventional SSFS architectures.

For the DPS architecture, we first generated two pulses by blocking one arm of the division system shown in Figure 2-4. A broadly tunable two-color output was achieved by varying the input pulse energy by rotating the waveplate controlling the ratio of pulse splitting in the second pair of PBS cubes. The result is shown in Figure 2-7b, which plots the center wavelengths of the two pulse copies as a function of the waveplate rotation angle. As can be seen, most of the power was initially confined to pulses with a horizontal polarization state, leading to significant wavelength shift. As the waveplate was rotated, power was gradually transferred to the vertical polarized beam, resulting in a continuous tuning of the wavelength in opposite directions over a range of 300 nm spanning from 850 nm to 1150 nm. Independent tuning of each pulse
wavelength is also possible (e.g. with a variable density neutral density filter in each polarization arm).

A typical spectrum of the two-color DPS source is shown in Figure 2-7c, depicting two ultrashort pulses centered at 975 nm and 1030 nm, respectively. In addition, the input pulse energy can be tuned (by adjusting the waveplate) so that the two pulse copies have identical center wavelengths (shown in Figure2-7c). In this case, the achievable average power at the output wavelength is twice as much as can be obtained through single pulse SSFS.

Four pulses were also generated by adjusting the splitting ratios in the first and second division stages. Figure 2-7d shows representative pulse spectra obtained in the multi-color DPS source with shifted center wavelengths of 905 nm, 932 nm, 974 nm and 1004 nm. Tuning of center wavelengths beyond 1 nm for the four pulse system is limited by the power of the pump laser. It is expected that a DPS source constructed from a higher power pump source with a longer center wavelength (enabling the use of PCF with a larger mode area) will extend the DPS source capabilities demonstrated here even further (e.g. higher average power, four or more colors, even broader tunability).
Figure 2-8: a) Autocorrelation and spectrum of recombined pulse. b) Autocorrelation and spectrum of individual pulse. c) Spectrum of recombined pulse and individual pulse.
Next, we demonstrated recombination of two pulses. The two pulses were shifted to a center wavelength of 1100 nm, while a 1050 nm long pass filter was employed to block higher order solitons, supercontinuum generation, and residual pump light. An autocorrelation of the recombined pulse is shown in Figure 2-8a and the corresponding spectrum is shown in Figure 2-8a and Figure 2-8c. These figures show that the pulse is slightly chirped and the pulse width is approximately 75 fs (assuming a hyperbolic secant temporal profile), which correlates well with the single pulse autocorrelation and spectrum depicted in Figure 2-8b and Figure 2-8c. Figure 2-8 thus demonstrates successful recombination of the two shifted pulses and shows the capability of the DPS system for power scalability.

![Figure 2-9: SHG images of BaTiO3 crystal domain at (a) 454 and (b) 485 nm. Images are 36 by 36 pixels with each pixel separated by 1.7 μm. The SHG signals were integrated over the respective spectra with the background subtracted.](image)

As mentioned previously, the dual polarization, multi-color, tunable characteristics of the DPS source is an ideal pump source for nonlinear microscopy. Here, we applied it for polarization multiplexed imaging, in which the different polarization states are encoded by two different wavelengths. This is the first demonstration of simultaneous dual-polarization second harmonic generation (SHG) microscopy to our knowledge. It is anticipated that this method may be useful for imaging of polarization sensitive dynamic processes. SHG imaging probes the
spatial distribution of the second order nonlinear susceptibility tensor $\chi^2$[35], [36]. Polarized SHG imaging is often necessary to reveal the rich tensorial properties of $\chi^2$, which contain useful information about molecular orientation or domain structure[37], [38]. As an example, a tetragonal barium titanate (BaTiO$_3$) crystal consisting of alternating domains was imaged[39]. A two-color DPS excitation source was used, which was comprised of pulses at center wavelengths of 908 nm and 970 nm, and with orthogonal polarization states. Dual-polarization SHG imaging was performed by raster scanning of the sample using a focused excitation beam. By examining the SHG signal at 454 nm and 485 nm, polarized SHG images at both polarization states was obtained simultaneously in one scan as shown in Figure 2-9a and Figure 2-9b. As expected, the results reveal that the observation of a particular domain is dependent on the polarization state of the excitation beam.

Extension of simultaneous dual-polarization second harmonic generation microscopy to biological samples is also possible. Samples that are polarization sensitive such as starch can benefit from this light source.

### 2.5 Conclusion

In summary, this chapter discuss an optical architecture of a multi-color, broadly tunable ultrafast optical source called DPS. The multi-line and broadly tunable properties make it an appealing source for nonlinear imaging techniques such as coherent anti-stoke Raman scattering (CARS), stimulated Raman scattering (SRS) and sum frequency generation (SFG). Further, the dual polarization facilitates imaging of polarization sensitive dynamic samples. Moreover, the power scalable design of the DPS source decouples the center wavelength and the output pulse power, which can be a general solution for existing SSFS based sources, making SSFS based source an appealing excitation source for many applications and, specifically, for nonlinear
optical imaging methodologies. The ability of the optical imaging instrumentation to penetrate deeper into biological tissue and to provide greater sample selectivity offer two capabilities that have enabled significant growth in the biological and biomedical imaging fields. The DPS architecture provides a source option for meeting these aims by generating multi-color and broadly tunable pulses in a way that inherently lends itself to an ease of alignment, tunability, design, and power scalability.
Chapter 3

Biodegradable step index fiber and its applications

3.1 Motivation for biopolymer waveguides

Optical technology has a significant impact on biomedical research and health care. To be specific, optical microscopy and optical treatment play a critical role in disease diagnosis and medical intervention. Optical microscopy refers to imaging of objects that cannot be seen by naked eye. This generally involves but no limited to using microscope as a tool. In optical microscopy, the contrasts of image are created by optical signal absorbed, generated or scattered from the sample. Typically, optical microscopy helps gain understanding of tissue structure and constituent biological materials, which has become a necessity in today’s biomedical research and healthcare. Despite the fact that biopsies are already one of the most commonly used method for cancer diagnosis and distinction[40], optical microscopy has not unleashed its full power in disease diagnosis. First, not only is optical microscopy able to achieve structure imaging of target region, it can also accomplish imaging under in vivo condition. For example, methods such as confocal microscopy are able to perform 3-dimentional imaging of sub-surface tissue structure at sub-micron axial and lateral resolution[41]. Furthermore, optical microscopy has the ability to permit selective imaging. For instance, fluorescence microscopy using nontoxic biological dye or nanoparticles to label target tissue or cells is already used for imaging dynamic process in tissue with high spatial resolution[42]. In addition, label free techniques are also available. Nonlinear optical microscopy techniques, such as CARS and SFG, are able to selectively image a specific type of material, as different materials possess different resonance frequencies[10], [43], [44].

Apart from optical microscopy which is useful for diagnosis, treatments using optical methods also prove to be effective. For example, photothermal therapy (PTT) is a treatment that
utilizes electromagnetic radiation to deal with various medical conditions[45]. Specifically, the photosensitizer can kill cells in the target region through heat releasing after it is delivered into target regions and excited by light at a specific band. This method has been shown as an efficient way to kill cancer tissue. In addition, photodynamic therapy (PDT), which also uses phototoxicity to kill target tissues, proves to be effective in killing microbial cells, including bacteria, fungi and viruses.

Although these methods demonstrate their effectiveness on medical diagnosis and treatment, there is a fundamental challenge to efficiently implementing these methods under in vivo condition, which is tissue turbidity. The scattering and absorption nature of the tissue cells prevent either the excitation light or the signal from passing through them with desirable intensity and wavefront shape. Specifically, tissue cells contain chromophores that include multiple types of hemoglobin, water, melanin, fat inside tissue cells, which lead to absorption in the visible region and near infrared region. The mismatch of refractive indices in different tissue components leads to strong scattering. For example, in tissue cells, both cell nuclei and mitochondria are strong scatters[46], [47]. This scattering is even enough to generate random lasing[48]. The absorption and scattering natures of tissue not only restrict the visible light’s 1/e penetration depth to be only around 1mm to 2mm, but also set constraint for near-infrared light propagation (~3mm), despite that it exhibits significantly less absorption & scattering compared to visible light. Therefore, it is obvious that tissue turbidity is the major obstacle for in vivo optical microscopy and treatment, heavily hindering us from taking advantages of these techniques to monitor post-surgical healing of tissues or organs, performing highly targeted light-based therapy, and optogenetic stimulation, to name a few examples.

Therefore, it is important to find a means to allow us to efficiently “communicate” with imbedded devices, or materials underneath tissue cells. Efforts have been attempted to explore methods such as wavefront shaping[49], speckle correlation[50] to overcome this problem; but
these methods all have limitation and implantable waveguides remains one of the most effective methods to address the challenge. However, traditional silica fibers are fragile and brittle in nature, presenting a significant limitation as an implantable device. Therefore, having a biopolymer waveguide with desirable mechanical and biological properties is crucial.

In this chapter, I will present my work on the development of a biocompatible, biodegradable step-index optical fiber, which is suitable for light delivery under in vivo conditions. By taking advantage of citrated based material platform and developing a specific fabrication method, we fabricate a fiber with biodegradable, step-index, flexible, and low loss properties. First off, I will give a brief introduction to optical waveguide and biopolymer waveguides, which include their working principle and related literature review. Then I will introduce the material platform we used for developing the biodegradable step-index optical fiber, including detailed material characterization. Subsequently, fiber fabrication and initial characterization of the fiber will be described. Finally, in vivo fluorescence collection, multimode fiber imaging based on our fiber will be presented, demonstrating the capability of our fiber.

3.2 Introduction to waveguide and biopolymer waveguides

An optical waveguide is a physical structure that is used for guiding optical waves propagation between its end points. Similar to traditional electromagnetic waveguide (e.g. for radio waves), optical waveguide structure sets a restricted spatial region for optical wave propagation. Typical optical waveguides are made of dielectric materials and the optical wave restriction is achieved through total internal reflection. To better illustrate its working principle, I will use planar waveguide as an example in the following paragraph.
Planar waveguide is the simplest optical waveguide and its structure is shown in Figure 3-1. As can be seen from the figure, region II with higher refractive index is sandwiched by region I and region III with lower refractive index from the top and the bottom. Thus, light initially propagating inside region II will experience a refractive index contrast from high to low at the two interfaces, resulting in total internal reflection for a certain angle of incidence (angle determined by the refractive index ratio). This can be easily understood by using Snell’s law. For light incident from high refractive index to low refractive index, propagation direction is governed by Snell’s law: \( n_1 \sin \theta_i = n_2 \sin \theta_t \). As \( n_1 \) > \( n_2 \), \( \sin \theta_t \) will reach its maximum 1 when \( n_1 \sin \theta_i = n_2 \). At this point, there will be (evanescent wave in the low refractive index medium), and this is called total internal reflection. Therefore, Light with these incident angles \( \theta_i \geq \arcsin \left( \frac{n_2}{n_1} \right) \) is confined inside region II. Further, as light is 100% reflected from the interface, the process is loss free, which enables long distance propagation. As an extension, applying this refractive index step into one of the other two dimensions, light propagation can be restricted into
a single dimension. This waveguide can deliver light between its two end points. Optical fibers and majority of on chip waveguides are single dimensional waveguides.

Figure 3-2: Cross section of single material optical fiber and step index optical fiber. \( n_2 \) is the core material refractive index, \( n_1 \) is the cladding material refractive index, \( n_0 \) is the refractive index of surrounding environment. \( n_2 > n_1 > n_0 \).

Since our biodegradable polymer optical fiber is used for point to point light delivery, more discussion will be provided on single dimensional waveguide. To be more specific, single dimensional optical waveguide is divided into two different categories namely single material and step index. Notably, single material waveguide does not mean the waveguide effect is achieved through one refractive index value (does not meet the requirement of total internal reflection). It refers to waveguide made of single material, which utilizes surrounding media (e.g. air) as the lower refractive index layer to achieve total internal reflection. Step index waveguides, on the contrary, are made of at least two different materials. The higher index material forms the core of the waveguide while the lower index material surrounds the core, forming the cladding. Total internal reflection is accomplished at interfaces between the core and the cladding with no field leaking beyond the cladding layer. For better illustration, cross section for single index optical fiber and step index optical fiber are shown in Figure 3-2. The step material fiber has an extra
cladding layer with refractive index $n_1$. This extra layer confines light inside the core area with refractive index $n_2$. Major benefit for this extra layer design is maintaining the fiber transmission regardless of outer environment. For example, single material optical fiber’s transmission loss increases dramatically if outer surface contacts with nonuniform surrounding medium, which can ruin the refractive index contrast. For step index fiber, as total internal reflection happens between the core and the cladding layers, as long as the cladding layer is thick enough to avoid evanescent wave reaching the cladding outer layer, low transmission loss stays unaffected. This is the reason why telecommunication optical fibers are all step index optical waveguides. More detailed benefits from step index structure for our fiber will be discussed later in this chapter.

Below, I give a brief literature review of existing research on biopolymer waveguides. Telecommunication fibers are made of doped glass (silica), which is low cost, low loss, and easy to fabricate. But due to the emergence of using optical waveguide, especially optical fiber, as implantable devices focusing on applications such as sensing, imaging and optogenetic, silica optical fiber’s undesired mechanical property has been a limitation. Therefore, biopolymers that are flexible, optically, biocompatible, and even biodegradable become ideal material candidates for fabricating optical fibers. In some of the early work for biopolymer optical fiber, waveguides made from single biocompatible materials, such as poly(ethylene glycol) (PEG) [51], silk[52], agarose gel[53], and poly(L-lactic acid) (PLA)[54] have been reported. Although these efforts have shown that biopolymer waveguide with favorable biological properties can have low transmission loss, they still face significant challenges for light delivery and collection under deep tissue condition in practical applications, due to the single index optical waveguide drawback we discussed previously. Therefore, driven by the need from deep tissue applications, two types of step material optical waveguides with good mechanical, optical, and biological performance have been developed based on silk and hydrogel materials, significantly propelling biopolymer optical fiber towards real-world applications. However, substantial work is still needed.
3.3 Citrate-based polymeric elastomers

3.3.1 Introduction to citrate-based polymeric elastomers

Citric acid, a Krebs cycle intermediate is a key component used in the citrate methodology, through which various crosslinkable elastomeric polymers can be synthesized by reacting the multifunctional citric acid with different diols and/or amino acids via a facile polycondensation reaction[55]–[58]. Unlike natural materials (e.g., silk) or traditional synthetic polymers (e.g., poly lactic-co-glycolic acid (PLGA)) that usually lack tunability for key mechanical, and/or degradation properties, the family of citrate-based biodegradable elastomers possesses tunable mechanical strengths (from tens of Pascal to mega Pascal), programmable degradation rates (from a few days to over a year), reactive nature between citrate-based polymers, and multi-functionalities (e.g., adhesive, fluorescent) [59]. Citrate-based elastomers have been used as implant materials for applications such as soft tissue engineering (blood vessel, nerve, and skin) [60]–[62], wound healing and bioadhesives[63]–[67], and biosensing[68]. Therefore, citrate-based elastomers can serve as an ideal material platform for the fabrication of fully biodegradable and seamlessly integrated step-index optical fibers for diverse in vivo applications if they also have desirable optical properties.
Figure 3-3: a) Schematic of a flexible core/cladding step-index optical fiber and the chemical structures of the core (POMC) and cladding (POC) materials. b) FTIR spectra of POMC and POC. c) Refractive indices and d) material attenuations of POMC and POC.
3.3.2 Material characterization

To develop a low-loss step-index bio-optical fiber, the core material requires a higher refractive index than the cladding and their mechanical properties (e.g., tensile strength and modulus) should be matched. Traditional materials, as we mentioned above, lack tunability, making it hard to meet the aforementioned requirements. However, due to the advantage of citrated-based polymer’s programmability, both requirements can be accomplished by tailoring the chemistry of citrate-based polymers. Specifically, we prepared two exemplary citrate-based elastomers, namely poly (octamethylene citrate) (POC) and poly (octamethylene maleate citrate) (POMC) with nearly identical polymer backbones but slightly different chemical structures (see Figure.3-3a), arising from the presence of extra maleate groups in POMC by replacing part of citric acid with maleic anhydride during the synthesis process. In detail, to prepare POC pre-polymer, citric acid (CA) and 1, 8-octanediol (OD) with a mole ratio of 1:1 were added to a round-bottom flask, and the mixture melted within 20 minutes by stirring the contents in the flask at 160 °C. Once the constituents melted, the temperature was changed to 140 °C and the reaction was allowed to progress for an additional 1.5 hours to produce the POC pre-polymer. For the preparation of POMC pre-polymer, CA, maleic anhydride (MAn), and OD, with a feeding molar ratio of 0.4: 0.6: 1, were mixed and reacted based the same procedure as POC pre-polymer synthesis. The difference between these two polymers is supported by the appearance of a C=C stretch peak at 1643cm⁻¹ of POMC in the attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra (Figure.3-3b). Refractive indices of POMC and POC were measured with an ellipsometer (J A Woollam M2000-U). Measurement was performed at three angles, 55°, 65°, and 75° to ensure the accuracy. Testing samples were prepared by spin-coating 20% (w/v) pre-polymer solutions on cover slip at the speed of 1000 rpm for 60s, and then followed by a thermal
crosslinking. POC was crosslinked at 70 °C for 7 days and 80 °C for 3 days, while POMC was crosslinked at 70 °C for 3 days followed by crosslinking at 80 °C for 3 more days. Raw ellipsometer data is shown in Figure 3-4, and Cauchy model was used to fit the measurement result and to extract refractive index from it. The result has good accuracy because both fitting mean square error (<5) and light depolarization (<1%) are low. Although there is only little difference in chemical structure between POC and POMC, POMC possesses a higher refractive index than POC within a broad range of wavelength from 300 nm to 1000 nm (Figure 3-3c), with an index difference of ~0.003 corresponding to a numerical aperture of approximately 0.1. Other than refractive index, we also characterized the other important optical property, i.e. material attenuation. Crosslinked POC and POMC cubes inside silica cuvette with a side length of 10 mm were prepared. The crosslinked cubes were placed in a Plate Reader for testing under the scanning range from 325 nm to 1000 nm to obtain the absorption results. By subtracting the loss that came from the cuvettes, material attenuation was measured. Result is presented in Figure 3-3d. It indicates that both POC and POMC have relatively low absorption (<0.13dB/cm) at visible and near-infrared wavelengths, which enable potential organ scale light propagation.
Figure 3-4: a) Ellipsometry results for POMC film spin coated on silica slide, b) Ellipsometry results for POC film spin coated on silica slide
Mechanical tests were conducted according to the ASTM D412a standard on an Instron 5966 machine equipped with a 500 N load cell. Tests were performed on polymer films (3cm in length, and 0.5cm in width). Samples were pulled until failure at a rate of 100 mm/min to obtain the stress–strain curves. The initial slope (0–10%) of the curve was used to determine the initial modulus of the samples. POC and POMC exhibit elastomeric nature with matched stress-strain curves when the external strain is less than 20% (Figure 3-7a).

Last but not the least, degradation tests were conducted. The percent mass remaining was calculated based on the following equation:

\[
\text{Mass Remaining} \, (%) = \frac{W_t}{W_0} \times 100\% 
\]

In accelerated degradation study, POC degraded slightly faster than POMC in 0.05M NaOH solution (Figure 3-5a). Under this condition, POC completely degraded in 12 hours, while it took 16 hours for POMC to finish the process. The degradation profiles of POC and POMC in PBS (pH 7.4) are presented in Figure 3-5b. During the first 4 weeks, POC and POMC had matched degradation profiles, and there were no significant differences even after 12 weeks. Finally, in vitro cyto-compatibility of POC and POMC degradation products and films were tested on 3T3 fibroblast cells using a well-known polymer poly lactic-co-glycolic acid (PLGA5050) used in many Food and Drug Administration (FDA)-approved medical devices as a control. The polymer films and their degradation products showed minimal cyto-toxicity, and the polymer films also effectively supported cell proliferation. Given the above material characteristics, flexible biodegradable step-index optical fibers may be prepared by the use of POMC as the core material and POC as the cladding material (Figure 3-3a).
Figure 3-5: a) In vitro accelerated degradation studies of crosslinked POMC and POC films in 0.05M NaOH solution. b) In vitro degradation studies of crosslinked POMC and POC films in PBS (pH 7.4)
3.4 Biodegradable step index optical fiber

3.4.1 Fiber fabrication

Due to the material property, a special two-step fabrication method was developed to achieve the core-cladding bilayer structure. The schematic diagram of the fabrication process is shown in Figure 3-6. In Step 1, the cladding layer was prepared by using a surface-polished stainless steel wire with a diameter of 500 μm as the mold. We coated liquid POC pre-polymer on the surface of the metal wire and then thermally crosslinked the polymer at 70 °C for 4 days. In order to detach the POC cladding tube from the wire, the polymer-coated wire was immersed in 30% ethanol solution overnight, and the POC tube was then removed from the metal wire due to slight swelling in ethanol. In Step 2, for the preparation of fiber core an air pressure pump was used to infiltrate POMC pre-polymer into the fabricated cladding tube. After thermal crosslinking at 70 °C for 3 days followed by 3 days at 80 °C, the POC cladding/POMC core were seamlessly grown together and a step-index polymer fiber was obtained.
3.4.2 Fiber characterization

The fabricated polymer fiber was mechanically flexible and the fiber can be easily twisted around a glass tube as shown in Figure 3-8a. Their favorable elastomeric properties were supported by the classical stress-strain characteristics of elastomeric materials with an initial modulus of $3.39 \pm 0.31$ MPa, a tensile strength of $1.31 \pm 0.25$ MPa, and an elongation of $61.49 \pm 5.81\%$, which are consistent with the mechanical properties of individual POC and POMC polymer films (Figure 3-7a). Therefore, the mechanical flexibility of the optical fiber makes it
suitable for \textit{in vivo} biomedical applications. The side view of a fiber under an optical microscope (Nikon, Eclipse, Ti-U) is presented in Figure 3-8b, showing the core/cladding structure. Additionally, fibers can be simply cut using normal razor blades; the resulting cross section captured by scanning electron microscopy (SEM) in Figure 3-8c shows a smooth facet amicable for optical coupling.

![Graph](image)

**Figure 3-7:** a) Tensile stress-strain curves of crosslinked POMC film, optical fiber and crosslinked POC film. b) Light transmission changes with fiber degradation.

In order to examine the optical performance of the fiber, we coupled a 633nm HeNe laser light into the citrate-based fiber to test the wave guiding effect. Figure 3-8d shows successful light delivery, demonstrating the light guiding property of the fiber. The total transmission of the fiber was measured not only at dry condition, but also at wet condition that the fiber may experience \textit{in vivo}. Under both conditions, the tested 3-cm-long fiber was able to yield ~58% transmission. The side view of the fiber with the laser light on is shown in Figure 3-8e; the output field mode of the polymer fiber is shown in Figure 3-8f. Both figures clearly suggest that the laser light was confined inside the core region. During the measurement, the fiber was physically surrounded by plasticine. Yet, light transmission was not affected in spite of the contact between the fiber and the plasticine, supporting the concept that a step index fiber may achieve efficient
light transmission in the fiber core due to the intrinsic cladding layer that shields the optical field from the surrounding tissues when used for in vivo applications. The propagation loss of the fiber was determined using a cut-back method. An average propagation loss of 0.4db/cm was measured. The additional loss compared with the material attenuation is likely introduced by the surface roughness of the metal wire mold and fabrication defects. The 1/e penetration depth of our fiber is over 10cm, and is suitable for in vivo experiments.

We also performed in vitro degradation studies on the fibers in phosphate-buffered saline (PBS), which showed that the fibers gradually degraded and reached a weight loss of 9.5% after one month (Figure 3-7b). During the period, optical transmissions were also monitored. Light transmission was reduced from 60% to 25% during the one month degradation process. The decrease of light transmission might be resulted from the defects formed at the core/cladding interface during degradation.
Figure 3-8: a) A photograph of a citrate-based fiber twisted around a glass tube, b) side-view image of a citrate-based fiber under a microscope, c) Scanning electron microscopy of a cleaved fiber facet, d) A photograph showing light guidance, e) side view image of light delivery using a citrate-based fiber, and f) output field mode of a citrate-based fiber coupled with a 633nm HeNe laser light.
3.5 Multimode fiber imaging and in vivo testing

3.5.1 Multimode fiber imaging

The unique optical and mechanical characteristics coupled with programmable degradation capability of our fibers lend themselves to both *in vitro* and *in vivo* bio-sensing and imaging applications. In recent years, multi-mode fibers have drawn extensive interest for delivery of ultrashort laser pulses and endoscopic imaging[69]–[71]. Here we show the proof of concept of using the citrate-based fiber for image transmission, which indicates the tantalizing potential for implantation inside human body for an extended period of time to allow long-term monitoring and imaging. The schematic multimode fiber imaging illustrating concept is shown in Figure 3-9. Due to the multi-modal propagation, the output of the fiber contained random speckle patterns when coherent light is used, which did not resemble the input image at all. In order to retrieve the images, least square retrieval algorithm and pre-calibrated impulse response of the fiber were used to reconstruct the input spatial pattern. We designed an image projection system
to perform this experiment. The schematic diagram is shown in Figure 3-10. A digital micromirror array (DMD) (Texas instruments Discovery 1100) illuminated by a spatially filtered He-Ne laser beam was used to project spatial patterns onto the proximal end of a fiber through an imaging system. A 92:8 (T: R) pellicle Beamsplitter was used to monitor the image projection quality at the input end. The corresponding output pattern at the distal end of the fiber was recorded by using a charge-coupled device (CCD) camera.

To perform imaging using the citrate-based fiber, calibration of the system response is needed. Individual pixels were projected at the proximal end of the fiber and the corresponding output patterns at the distal end were captured, yielding the intensity impulse response matrix $H = [h_1, h_2, \ldots, h_y]$ of the system, where the $i^{th}$ column vector ($\tilde{h}_i$) of $H$ represents the corresponding output pattern, or the impulse response, of the $i^{th}$ input pixel.

For a given input image $\tilde{x}$, its output pattern is given by $\tilde{m} = H\tilde{x} + \tilde{n}$, where $\tilde{m}$ is the measured pattern and $\tilde{n}$ is the coherent noise (speckle) due to interference among the output fields produced by different pixels of the input. This equation can be approximately inverted using the least square method $\tilde{x} \approx (H^tH)^{-1}H^t\tilde{m}$. Experimentally, each input pixel (hereafter called super pixel) was comprised of 100 physical pixels (10×10) of the DMD. Since the DMD had a pixel size of 13.68μm, the actual super pixel size at the fiber end after the telescope imaging system was approximately 9 × 9 μm². A total of 64 super pixels (8 × 8) were used during the experiment to generate input images. Output images at the distal end were recorded by a charge-coupled device camera (480 by 720 pixels), with only the core area of the fiber retained in all recorded images. The impulse response $M$ of the system was a 90000 by 64 matrix, where 90000 is the number of pixels in an output pattern and 64 is the number of available super pixels at the input end. Once the impulse response matrix was measured, a projected input can be reconstructed from its output image. The experimental result is shown by Figure 3-11a-b. The
three letters (P, S, and U), initially projected at the proximal end of the fiber, and the corresponding output random speckle patterns at the distal end were shown. By using the pre-recorded impulse responses which are shown in Figure 3-11d, we were able to retrieve the input pattern (Figure 3-11c), demonstrating the capability of the citrate-based fiber to deliver spatial images.

Figure 3-10: Schematic diagram of the experiment setup of the citrate-based polymeric fiber imaging

Current system’s retrieval result quality is primarily limited by two factors. First is the stability of the system, as change in system parameter will result in a change in impulse response of the fiber. Therefore, for large perturbation stored impulse response can no longer be used for inversely reconstructing the input image. Second is the correlation between nearby pixel’s impulse response. If two nearby pixels are too close to each other their output mode patterns are almost the same, preventing faithful retrieval. Therefore, individual pixels have to maintain a
certain distance between each other to enable a large enough difference of the output patterns. In the future, phase recording will be included as it could mitigate the noise term $\vec{n}$ during the retrieval process. With phase information, this method can enable true high resolution large field imaging using our citrate based fiber.
Figure 3-11: a) The projection image at fiber’s proximal end, b) output speckle patterns of each input images, c) reconstructed images, and d) Recorded impulse response images of the fiber.
Figure 3-12: a) Blue light (473 nm) guidance through a citrate-based polymeric fiber under a thin porcine tissue slice at bending angles of (1, 2) 0°, (3, 4) 30° and (5, 6) 90°. b) Red light (633 nm) delivery through fiber under a thin porcine tissue slice at bending angles of (1, 2) 0°, (3, 4) 30° and (5, 6) 90°.
3.5.2 In vivo testing

To confirm the practicality for light guiding in tissues, a fiber was placed under a piece of thin porcine tissue slice with a thickness of ~2mm for study (Figure 3-12a, b). In Figure 3-12a, a 473nm diode-pumped solid-state laser was coupled into the fiber, and the blue light transmitted efficiently along the fiber under different bending angles of 0°, 30° and 90°, which can be verified by the observation of a bright spot at the distal end of the fiber. The testing based on a 633nm HeNe laser was also conducted on the fiber as shown in Figure 3-12b, and the fiber showed decent light guiding effects for the red light as well. The above studies suggest that the citrate-based polymeric fiber has the ability to guide lights under different wavelengths in tissues, which enables in vivo detection and sensing.

To study the feasibility of using citrate-based fibers for in vivo deep tissue detection and bio-sensing, we performed fluorescence detecting experiments with an animal study on a Sprague Dawley (SD) rat and tested our fiber’s capability to collect signal underneath deep tissues. The experimental procedures are described in Figure 3-13a. An agar gel doped with Rhodamine B was placed deep into the belly area of the rat, and two fibers with a length of 7cm for excitation light delivery and fluorescence collection were then inserted. As shown in Figure 3-13c, the excitation laser light at a wavelength of 532 nm was delivered by the delivery fiber A to illuminate the dyed gel. Emitted red fluorescent light was then detected at the output end of the collection fiber B; a long-pass filter (Chroma ET542lp) was used to block the scattered excitation light. The fluorescence spectrum (Figure 3-13b) captured by using an optical spectrometer (Ocean Optics Flame) accurately matched the fluorescence spectrum of the original Rhodamine B gel. The in vivo study confirmed efficient organ scale detection capability of the proposed fiber, and demonstrated its mechanical flexibility and feasibility to be implanted inside body without damaging surrounding tissues.
Figure 3-13: a) Schematic of in vivo deep tissue fluorescence detecting with fibers. b) The fluorescence spectrum collected from the end of the light collection fiber B. c) A photograph of the experiment procedures for in vivo deep tissue fluorescence detection with fibers.
3.6 Conclusion

In summary, in this chapter I presented a flexible biodegradable step index fiber using designable citrate-based elastomeric polymers through a two-step fabrication method. The obtained step index fiber presented favorable biodegradability and mechanical flexibility. A 0.4dB/cm loss allowed us to perform both *in vitro* and *in vivo* studies inside deep tissue, which showed efficient light transmission and optical signal detection abilities. Preliminary imaging experiment also showed the feasibility of using the fiber for deep tissue implantation and continuous monitoring. Using our method, the diameters of core materials and the lengths of fibers can be varied by changing the size of the wire mold. The refractive indices and mechanical properties of core and cladding materials can be further tailored by modifying chemical structures of the citrate-based platform polymers, while maintains seamless integration of the core and the cladding. In addition, benefited from the preferred biological properties (nontoxic and biodegradable), the fiber could be left inside the body without the need of a secondary surgery to remove it. This not only reduces cost and pain for patients, but may also improve treatment efficacy, as studies have shown that continuous treatment is more effective over single-time treatment[72]. We believe our citrate-based step index fiber could be the ideal vehicle for optical applications including imaging, sensing, and treatment.
Chapter 4

Smartphone spectroscopy

4.1 Motivation for smartphone based sensing devices

Point of care (POC) testing attracts significant interest today in both industry and academia as there is a growing requirement for it. Technically, POC refers to medical diagnostic tests performed at the time of patient care[73], which is different from traditional tests that are conducted inside medical labs and hospitals. The number of available POC tests has increased from fewer than 10 in 1995 to over 110 in the market today[74], benefiting from both the advancement of biomedical research and novel device system design. Also, according to the reports prepared by research companies, market size for POC has reached around 8 billion dollars in 2013. All the evidences have pointed toward that the POC is probably the future focus in healthcare. The major benefit for POC is providing instant rapid diagnosis for patients, which can help improve patients’ medical conditions and clinical outcome. Therefore, simplifying the operation difficulty for end users, decreasing the device cost, and providing accurate tests’ result are major requirements for POC. Smartphone-based sensing device for POC, which emerges in the past decade, naturally becomes attractive.

Smartphone-based sensing devices refer to the scientific detection instrument developed on smartphones. They are able to perform measurements based on existing scientific method but using smartphone as a detector. Compared with traditional cellphones that only focus on communication functionality, smartphone is an integration of computer, camera, and cellphone, which makes it capable of performing POC tests. Smartphone camera serves as a good detector for measurement. Also, smartphone has good computational ability and communication ability
which can process and transfer data. What is more, smartphone is widely available today. In fact, because of smartphone’s general abilities and the continuous decrease of prices, smartphones have become a must-have tool in daily life. According to the report in 2016, the number of smartphone end users in the world had increased to 2.1 billion[75]. It is worth noting that more than half of the smartphone end users are located in developing countries, which makes smartphone based devices extra valuable as those regions have limited access to standard lab resources. Considering the popularity and functionality of smartphones, there is no doubt that smartphone based devices have great potential.

Smartphone-based detection devices are great for POC, since they are able to take advantages of all the benefits brought by the current advanced smartphone. First, smartphone-based devices for POC in general are cheaper in comparison with other POC tools with similar functionality, dimension, and performance. For example, smartphone-based devices directly use the high quality complementary metal-oxide semiconductor (CMOS) camera on smartphone for detection and smartphone screen for display results, which saves the electronics cost from building a standalone device. In addition to cost, wireless internet communication and fast processors in smartphones are also important. Smartphone-based POC are able to collect, compute, and analyze data at high speed and share result instantly with users. This allows for potential quicker feedback from the doctors, which is crucial for many medical & health conditions, such as post-surgery monitoring. Last but not the least, the wide availability of today’s smartphone lower the economic barrier for people to take advantage of these POC tests. Especially, this is beneficial for people in developing countries where hospital and lab resources are limited, causing difficulties for people to get timely diagnosis. Also, it can help people in developed region as these smartphone-based POC tests allow easy monitoring of important health conditions. In general, smartphone POC could be the main trend of POC in the future.
In this chapter, I will discuss two smartphone-based detection devices that I designed, fabricated, and tested. Both are based on optical sensing method and targeted POC tests. At the beginning, I will give a brief review of current available smartphone-based detection devices for POC. After these discussions, I will introduce my work on the G-Fresnel smartphone spectrometer and the citrated-acid based smartphone chloridometer. Detailed working principle, design, characterization, and applications for these devices will be included. Finally, future work on developing next generation devices will be covered.

4.2 Review of smartphone based devices as detectors

The use of smartphone as detector benefits from the introduction of camera to smartphones. Here, I give a review of the current available devices. They are divided into two categories by the optical method used: microscopy and spectroscopy.

First, I will discuss smartphone based microscope. The basic principle of all the smartphone based microscopes is to use the smartphone camera for output recording. As a pioneer, Breslauer et al. introduced a design of smartphone microscope which is able to perform bright field imaging and fluorescence imaging[76]. The smartphone microscope has a relatively compact size comparing with benchtop microscope. Specifically, the setup uses an optical tubing to integrate all the optical components, including excitation light source (including filter and LED), condenser lens set, sample holder, and objective lens together to achieve reduction in size. Smartphone camera serves as the imaging detector, which is attached to the setup at the end. The bright field microscope and fluorescence microscope can be easily switched by moving filters in and out. Both the bright field microscope and fluorescence microscope are capable of outputting high resolution image of medical samples, proving the effectiveness of this design. Later in 2010, a method of microscopy which uses in-line holography was reported by Tseng et al[77]. The
method used an LED light source to illuminate a sample. Scattered light from sample interferes with the unscattered light and forms an in-line hologram. The real sample image is retrieved from the recorded hologram. Compared with previous methods which transfer a microscope setup into a compact form, this method is lens-free and only requires an LED in the setup, which significantly decreases the dimension of the device. As a next step, updated prototype for this smartphone microscope was presented with machine learning algorithms, which increases the efficiency and reduces the complexity at the user end[78]. To briefly summarize devices are all capable of performing cost efficient POC tests, especially for resource-limited area.

On the other hand, optical spectroscopy based POC method has also received a lot of attention. Compared with smartphone microscope for imaging based POC, which requires well-trained technician or machine learning algorithm for result analysis (microscope image cannot directly provide quantitative result), spectroscopy data can provide quantitative information. In general, optical spectroscopy measures light intensity as a function of wavelength, which could probe targeting property value both qualitatively and quantitatively. For simplicity, here I would further separate optical spectroscopy based smartphone POC method into three different categories: absorbance detection devices, optical spectrometer, and fluorescence detection devices.

I would like to start from absorbance detection method first. Ozcan’s group[79] presented their work by integrating commercial colorimetric assay kit with smartphone for allergen test. The setup utilizes two identical light sources to measure the transmission efficiency of the target sample and the control sample, which can be translated to absorbance of the target sample. As the commercial assay links absorbance of the sample to allergen contamination, the device is capable of performing multiple types of allergen test in food through using colorimetric assay kits. As the device design follows standard absorption spectroscopy working principle and use smartphone camera as a single channel detector, other biomarkers targeting different sensing applications
have been implemented on similar smartphone based devices. Further, newer versions of devices were developed, aiming for better measurement efficiency and accuracy. For example, Berg et al. presented their design of multi-channel absorption measurement setup for enzyme-linked immunosorbent assay (ELISA) measurement[80] which was able to measure 96 samples simultaneously. In their setup, multiple fibers were used to collect individual samples’ signal and to deliver to camera together, which achieved multichannel measurement. Successful clinical measurement of mumps IgG, measles IgG, and herpes simplex virus IgG were demonstrated with an accuracy of ~99%, proving its usability.

Compared with absorbance method, smartphone spectrometer based POC is also a popular method as it can provide more detailed information. However, more functionality means more complex system. In order to get the spectral information, smartphone spectrometers require the use of dispersive optics, optical filters or other wavelength identification methods, which increase the system complexity compared with intensity only detection. In 2013, Dustin et al. reported using smartphone spectrometer and photonic crystal for label free biodetection[81]. In this work, they designed a smartphone spectrometer which contained lens pair, polarizer, sample holder, and diffraction grating inside. The output spectrum produced (by diffraction grating) from spectrometer was directly coupled into the smartphone camera and displayed on phone screen. The resonance frequency of the photonic crystal would shift with different biomolecule applied on top of it. This is because different molecules have different refractive indices. The shift was detected up by the smartphone spectrometer and used for distinguishing different antibodies. This label free diagnosis of molecular species, which can be used for bio-detection, owes to smartphone spectrometer’s color recognition capability, demonstrating the necessity to have detailed optical spectral information. Similar to absorption measurement, multi-channel spectrometer which boosts the measurement efficiency, was developed by Wang et al[82]. In their design, a prism arrays was used to enlarge the field of view of the system, which increased
the number of samples that could be measured at the same time. A demonstration of cancer
detection using ELISA method was conducted, which verified the system’s capability.

Last but not the least, fluorescence-based methods are heavily used in smartphone-based
POC. In 2013, Coskun et al. demonstrated a smartphone accessory that can perform albumin test
in urine through fluorescence-based sensing method[83]. The whole setup weighed only 148
grams which included built-in laser diode source, sample holder, filter, and battery. Laser diode
excited both the sample, which were mixture of sample urine and fluorescence assay, and control
group simultaneously and the signals generated were collected by the smartphone camera. By
comparing the fluorescence intensity between sample and control group, albumin concentration in
urine was measured with a detection limit around 5-10μg/mL. Targeting applications for this
device include early diagnosis of kidney disease and routine monitoring of patients suffering from
diabetes, hypertension, or cardiovascular diseases. Similar to absorption device which could
expand applications by switching assays, this fluorescence-based device can also expand
applications by changing fluorescence assays. Further, Yu et al. used smartphone spectrometer for
fluorescence detection which expands the detection from fluorescence intensity only to spectrum
of fluorescence[84]. This makes the device capable for general fluorescence detection usage as it
detects broad wavelengths range which gives tolerance for emission wavelength shift. Apart from
device advancement, developing new fluorescence assays for new sensing applications is the
other approach. For example, Algar et al. developed semiconductor quantum dot which was
suitable for fluorescence-based bioanalysis and demonstrated its feasibility on smartphone
device[85].
4.3 G-Fresnel smartphone spectrometer

In the current section, I elaborate on my work toward developing a G-Fresnel smartphone spectrometer with a few nanometer resolution covering the visible range[86]. The spectrometer utilizes a G-Fresnel device for focusing and dispersion, which enables miniaturization while maintaining performance. Proof of principle application using Bradford assay for protein concentration measurement demonstrates its capability in chemical sensing. The G-Fresnel smartphone spectrometer could be an ideal platform for POC applications.

4.3.1 Introduction to G-Fresnel

G-Fresnel is a hybrid diffraction element which was demonstrated by Yang et al. in 2010[87][88]. The key idea of the G-Fresnel is to combine multiple optical elements into one, thus achieving focus and dispersion dual functionality using a single device. Here I would like to give a brief overview of the G-Fresnel’s working principle.

Fundamentally, to achieve the dual functionality focus and dispersion of the transfer function of the thin film G-Fresnel should be a product of the transfer function of lens and grating, which can be shown in the following equation:

\[ \tau(x, y) \propto e^{-j \frac{\pi}{\lambda F} (x^2 + y^2)} e^{j \frac{2\pi}{\Lambda} y} \]

where \( \lambda \) is the wavelength of the light, \( F \) is the focal length of the lens and \( \Lambda \) is the grating period. Dispersion and focusing effects of this device can be theoretically estimated by calculating the transfer function’s free space propagation result under Fresnel approximation. The result can be shown in the following equation:
\[ f(x, y, z) \propto \int\int e^{j\frac{2\pi}{\lambda}(\frac{1}{d-x}+\frac{1}{d+y})(x^2+y^2)} p(x', y')e^{-j2\pi\left[\frac{x_0}{\lambda_0} - \frac{x}{\lambda_0} + \frac{x}{\lambda_0} + \frac{y}{\lambda_0}\right]} dxdy' \]

where \( p \) is the pupil function of the G-Fresnel, \((x_0, y_0, d)\) is the input point source position.

Further, since the G-Fresnel uses Fresnel lens for focusing, whose focal length is wavelength dependent \((\lambda F = \lambda_0 F_0, \lambda_0 \text{ and } F_0 \text{ are the design wavelength and focal length for Fresnel lens})\), the above equation can be modified to get its final form. From the equation, the focusing points of different wavelengths lay on the line defined by the following equation:

\[ L = \frac{\Lambda d}{\lambda_0 F_0 - x_0 \Lambda} x_i - \frac{\lambda F_0 d}{\lambda_0 F_0 - x_0 \Lambda} \]

where \( L \) is the distance away from the G-Fresnel, \( x_i \) is an axis parallel to the G-Fresnel plane. This is the ideal position for the spectrometer detector which offers the best resolution across the detection range.

Experimentally, three different types of G-Fresnels were proposed and were distinguished based on their fabrication methods[87], [88].

PDMS soft lithography is the first method that was proposed and demonstrated to achieve this dual functionality by using a single flat thin device. Briefly, PDMS (Dow Corning Sylgard 184 Silicone) pre-polymer is poured onto the surface of a Fresnel lens. After baking the PDMS for 12 hours at 60 °C, the PDMS is cured completely and a negative Fresnel lens mold is obtained. The same method is used to fabricate a negative mold of a diffraction grating. A G-Fresnel can be fabricated by sandwiching PDMS pre-polymer between the grating and the negative Fresnel lens molds (with minimum alignment requirement) followed by curing. By this means, the fabricated device (schematic diagram shown in Figure 4-1a) has Fresnel lens pattern and diffraction grating on either side, which makes it dual functional.
Figure 4-1: a) Double side G-Fresnel, b) Single side G-Fresnel with grating pattern embedded in Fresnel lens c) Holographic G-Fresnel recording process
Compared with the G-Fresnel fabricated by using the PDMS soft lithography method, other types of G-Fresnels have been proposed to be made by Electron-beam lithography method and thin film holographic method. A major advantage of these two types of G-Fresnels comparing with the first one is that they are single side dual functional devices which are suitable for low-cost volume production.

One of the two proposed single side G-Fresnels utilizes the similar principle as the double side G-Fresnel. But instead of two sides, this proposed device embeds a grating height profile on top of a Fresnel lens height profile, which achieves the dual functionality (Figure 4-1b). But since the pattern is a grey scale pattern which requires electron-beam lithography method for fabrication, both the cost and fabrication difficulty are high.

The other proposed single side G-Fresnel is holographic G-Fresnel that does not have these problems. It is easy to customize, cost efficient and has good diffraction efficiency. To be specific, the holographic G-Fresnel is the hologram formed between a plane wave and a diverging spherical wave coming at an angle (Figure 4-1c). The intensity profile of the hologram plane can be described by the following equation:

\[
I(x, y) = \left| a + b e^{\frac{j\pi}{\lambda F}[(x-d)^2 + y^2]} \right|^2 = |a|^2 + |b|^2 + ab^* e^{-\frac{j\pi}{\lambda F}[(x-d)^2 + y^2]} + \text{c. c.}
\]

where \( F \) is the distance between the diverging spherical wave’s focusing point to the hologram plane, \( d \) is the distance between the diverging spherical wave’s focusing point to the center of the plane wave. The hologram term can be identified as a G-Fresnel transfer function:

\[
e^{\frac{-j\pi}{\lambda F}[(x-d)^2 + y^2]} \propto e^{j2\pi\left(\frac{d}{\lambda F}\right)x} e^{-\frac{j\pi}{\lambda F}(x^2 + y^2)}
\]
where the grating period is $\frac{\lambda F}{d}$ and $F$ is the focal length. Therefore, the recorded hologram is able to serve as a G-Fresnel and its parameters can be modified through changing the recording setup.

Simulation was performed to verify the device performance. In general, we first calculated the hologram parameters by simulating interference pattern of the signal and reference beams. Then, a point source was placed at a specific position to simulate light passing through the hologram. By checking the output light propagation, we could check the performance of the hologram, which could serve as guidance for experimentation. In specific, the recorded device parameter was determined by transferring the spatial angle $\theta$ between the signal beam and the reference beam to grating period $\Lambda$ at different locations on the hologram.

$$\Lambda = \frac{\lambda}{2 \sin \left( \frac{\theta}{2} \right)}$$

The thickness of the hologram device was ignored in this case (treated as a thin hologram). After the calculation was done, a point source was placed at the designated location to serve as a virtual slit. Geometric ray tracing was then performed. Light from point source was considered as individual ray with its own input angle, which could be transferred to propagation vector $k$ with $k_\perp$ (vertical component of propagation vector) and $k_\parallel$ horisontal component of propagation vector. By bringing in the hologram which introduces $\kappa = \frac{2\pi}{\Lambda}$ to the input beam’s $k_\perp$, output propagation angle $\beta$ from different locations of the hologram were determined by the following equation.

$$\beta = \arctan \left( \frac{k_\perp - \kappa}{\sqrt{k_\parallel^2 - (k_\perp - \kappa)^2}} \right)$$
Results are shown in Figure 4-2. The holographic G-Fresnel with 4mm diameter was placed along the x axis. Specifically, the simulated G-Fresnel was recorded using a 405nm laser light, with a focal length F set to 5.7mm and d set to 4mm. Input light is designed to come from 45 degree which locates 25mm away from the device. From the result, we can find that although suffering from strong spherical aberration, dispersion and focusing effects are confirmed from this result.

4.3.2 Design of G-Fresnel smartphone spectrometer

In developing the G-Fresnel smartphone spectrometer, I used double side G-Fresnel as a demonstration. In order to understand how the G-Fresnel smartphone spectrometer works, we
need to first take a look at traditional optical spectrometer’s working principle. Figure 4-3 shows the optical lay out for a diffraction grating based spectrometer, which is the most widely used type. The whole process includes collimation, dispersion and focusing, which is illustrated in the Figure.

Figure 4-3: Diffraction grating optical spectrometer’s inner alignment

For the G-Fresnel spectrometer (Figure 4-4), we directly placed the G-Fresnel in front of the smartphone camera with the Fresnel lens side facing out. The light coming through the slit is initially collimated by the Fresnel lens side, and then dispersed by the grating side. Further, collimated and dispersed light will be focused by the smartphone camera’s lens onto the CMOS detector. As can be easily seen, the design of the G-Fresnel smartphone spectrometer and traditional diffraction grating based optical spectrometer follow the same principle, but the G-Fresnel spectrometer’s design offers reduced in alignment difficulty & increased in stability, and can shrink in size which are desired characteristics of a compact spectrometer.
Figure 4-4: Systematic diagram of the G-Fresnel smartphone spectrometer.

Figure 4-5: a) photo of a transmission G-Fresnel device compared to a US quarter dollar coin b) a prototype G-Fresnel smartphone spectrometer
A G-Fresnel smartphone spectrometer’s prototype was fabricated to verify the performance. The G-Fresnel used in this prototype was fabricated by using a 1 inch focal length Fresnel lens (Edmund Optics 2.0” x 2.0”, 1.0” FL, Aspheric Fresnel Lens) and a 1200 lines/mm diffraction grating (Thorlabs Vis Trans Grating, 1200 Grooves/mm, 36.9° Blaze Angle, 12.7 mm x 12.7 mm). The prototype enclosure was fabricated using a 3D printer. Slots were designed to hold the G-Fresnel and the slit at pre-aligned positions. The device was then secured to a smartphone case, which could be attached to the smartphone as a single accessory. The smartphone used in our experiments is an HTC One (M8) with Android OS, v4.4.2. Actual G-Fresnel and the spectrometer photo are shown in Figure 4-5. The spectrometer box has a dimension of 1.8” x 0.8” x 0.9”, which is easy to carry around. Additionally, the system is designed to use either an optical fiber or free space for light delivery.

4.3.3 Device calibration and characterization

The calibration of the spectrometer was divided into two steps (i.e., wavelength calibration and intensity calibration). A mercury and argon calibration source (Ocean optics HG-1 Mercury Argon Calibration Source) with several emission lines in the visible range were used for wavelength calibration. A typical spectrum is shown in Figure 4-6a. By comparing the spectrum with the emission wavelengths of the calibration source, the pixel-wavelength relationship was obtained as plotted in Figure 4-6b. The linear relationship enables easy calibration for the device. To perform the intensity calibration, we compared the measurements of a common optical signal obtained by using the G-Fresnel smartphone spectrometer and a commercial spectrometer. To this end, a fiber probe (Ocean Optics R400-7-SR) consisting of 6 surrounding fibers for light delivery and a central fiber for light collection was placed above an optical diffuser (Ocean Optics WS-1 Reflectance Standards). The diffuse reflectance spectrum was measured by using the G-Fresnel
spectrometer and an Ocean Optics USB4000 spectrometer, respectively. Let us denote the reference spectrum (measured by the Ocean Optics spectrometer) as $I(\lambda)$. Note that colored imaging sensors used by today’s smartphones are usually Bayer sensors, which are 2D arrays of detector pixels with red, green, and blue color filters arranged in a Bayer pattern. The red, green, and blue filter response functions $r(\lambda)$, $g(\lambda)$ and $b(\lambda)$ can be calibrated by comparing the measurement results $R(\lambda)$, $G(\lambda)$, and $B(\lambda)$ (i.e., the red, green, and blue pixel values) from the smartphone spectrometer with $I(\lambda)$:

$$R(\lambda) = r(\lambda)i(\lambda), \quad G(\lambda) = g(\lambda)i(\lambda), \quad B(\lambda) = b(\lambda)i(\lambda).$$

When another spectral measurement was made, results $R'(\lambda)$, $G'(\lambda)$ and $B'(\lambda)$ from the smartphone spectrometer could be converted to a calibrated spectrum $I'(\lambda)$ in the least square sense:

$$I'(\lambda) = \frac{r(\lambda)R'(\lambda) + g(\lambda)G'(\lambda) + b(\lambda)B'(\lambda)}{r(\lambda)^2 + g(\lambda)^2 + b(\lambda)^2}.$$ 

It should be pointed out that both the focusing (at infinity) and white balance of the smartphone camera need to be fixed during the whole process in order to ensure calibration and measurement consistency.

Figure 4-6: Wavelength calibration a) Measured spectrum of a mercury argon calibration source b) Linear relationship between the pixel number and the calibrated wavelength
Figure 4-7: Schematic diagram of the spectral holography setup for characterizing the wavelength resolution of the cellphone camera. A supercontinuum source generated by propagating a 1064nm pulsed laser (JDS Uniphase NanolaseTM NP-10620-100 laser) in a 20-meter long nonlinear photonic crystal fiber (NKT Photonics Highly Nonlinear PCF SC-5.0-1040) was directed to a Michelson interferometer. One arm of the interferometer can be adjusted by a linear translational stage. The resulted spectral interference pattern is measured by using the smartphone spectrometer.

Due to the built-in sophisticate post image-processing in smartphones, the retrieved spectra may still suffer from these artifacts. A better approach is to directly use the raw data from the smartphone camera, which is free from image processing.

The spectral resolution was experimentally studied by using spectral holography. A supercontinuum source generated by coupling a 1064 nm pulsed laser (JDS Uniphase NanolaseTM NP-10620-100 laser) in a 20-meter long nonlinear photonic crystal fiber (Blaze Photonics SC-5.0-1040) was directed to a Michelson interferometer (shown by Figure 4-7). The slight optical path length difference between the two arms of the Michelson interferometer resulted in a spectral interference pattern as shown in Figure 4-8a measured by using the G-Fresnel smartphone spectrometer. The interference pattern was clearly resolved by the G-Fresnel spectrometer, yielding a spectral resolution of ~ 1.6 nm at 595 nm. The spectral holography
measurement also demonstrated the viability of applying the G-Fresnel spectrometer to coherence measurement.

Figure 4-8: a) Spectral holography characterization b) Calculated spectral resolution

The theoretical spectral resolution of the device was also calculated as shown in Figure 4-8b. A geometric ray tracing method was used to estimate the image size $D(\lambda)$ of the slit (5$\mu$m width) on the smartphone CMOS sensor. The spectral resolution, defined as the wavelength difference such that the slit images at two wavelengths are separated by $D(\lambda)$, can be obtained by $\Delta \lambda = \frac{D(\lambda)}{F \frac{\partial \theta}{\partial \lambda}}$, where $F$ is the focal length of the smartphone camera lens and $\frac{\partial \theta}{\partial \lambda}$ is the angular dispersion of the G-Fresnel. Our calculation also took into account of the wavelength dependent focal length of the G-Fresnel. As shown in Figure 4-8b, a resolution of 1.3 nm to 2.1 nm can be potentially achieved across the wavelength measurement range of 400 nm-650 nm, where the longer wavelengths are limited by the built-in infrared filter in the smartphone camera. We note that the spectral resolution at the long wavelength end agrees well with the calculated result, but spectral resolution at the short wavelength end (full width at half maximum ~ 5nm near 400 nm, Figure.
4-6a) is worse than the theoretical estimate, which is likely due to non-optimized system alignment and possible performance degradation of the Fresnel side at shorter wavelengths.

4.3.4 Application of the G-Fresnel smartphone spectrometer

One promising application of the G-Fresnel smartphone spectrometer is its ability to perform quantitative colorimetric analysis and thus provide a portable platform for chemical assay. In particular, determining the concentration of proteins in solutions is an important and regular step in many laboratory workflows that involve protein extraction and analysis in biological research, biomedical assay, food science, biocatalytic industry and environmental monitoring\cite{89}, \cite{90}. Thus, here we conducted measurement of protein concentration measurement using the G-Fresnel smartphone spectrometer with the assist of Bradford assay. Bradford assay, as a typical colorimetric protein assay, is a fast and one of the most widely used spectroscopic analytical procedure. It analyzes the red shift of the absorbance spectrum of a reagent solution when binding to protein occurs.
Figure 4-9: a) Schematic diagram of the measurement scheme. Source light (Ocean Optics tungsten halogen light source HL-2000-HP) is delivered through an optical fiber to a specimen placed in a sample holder. The transmitted light is collected by another optical fiber and sent to the cellphone spectrometer for analysis. b) Transmission spectrum images obtained by the cellphone spectrometer for deionized water and specimens with 1 μM – 10 μM Rhodamine 6G (R6G), respectively. c) Absorbance spectra of dye solutions with different R6G concentrations (1 μM – 10 μM). Each measurement was repeated three times to ensure the accuracy.
Figure 4-10: Absorbance at 524nm and 480nm as a function of the concentrations of R6G. The linear regression curves have $R^2$ values of 0.9995 (524 nm) and 0.9971 (480 nm) respectively, indicating an excellent linear response of the device.

To measure the absorbance of the sample, a sample holder (1” × 1” × 2”) was fabricated by using a 3D printer. The whole setup is shown by Figure 4-9a. Standard polystyrene cuvettes containing a specimen solution can be placed inside the sample holder. The sample holder also has two pre-aligned through-holes, which are used to attach a source fiber that is connected to a tungsten halogen light source (HL-2000-HP) and a detection fiber that couples the transmitted light to the G-Fresnel spectrometer. It is worth mentioning that an optical fiber can potentially be used to direct the built-in smartphone flashlight to a sample, or alternatively, the tungsten halogen light source may be replaced with a white LED. The transmission spectrum of specimen is normalized against a reference spectrum (i.e., transmission spectrum of the solvent, e.g. water) to obtain the absorbance $A=-\log_{10}\left(\frac{T_{\text{specimen}}}{T_{\text{water}}}\right)$. To characterize the system linearity, the absorbance spectrums of Rhodamine 6G (R6G) dye solutions with concentrations of 1μM, 2μM, 4μM, 6μM, 8μM, and 10μM were measured (Figure 4-9b-c). By plotting the absorbance at two exemplary
wavelengths (480nm, 524nm) as a function of concentration, the linear regression curves have $R^2$ values of 0.9995 (524 nm) and 0.9971 (480 nm) respectively, indicating an excellent linear response of the device ideally suitable for performing quantitative concentration measurement (Figure 4-10). For protein concentration measurement, commercial Bradford reagent (SIGMA-ALDRICH B6916, Linear concentration range: 0.1–1.4 mg/ml) was used to measure the concentration of bovine serum albumin (BSA). Initially, 0.01ml, 0.02ml, 0.04ml, 0.06ml, 0.08ml, 0.1ml BSA solution with a concentration of 0.1mg/ml was added into cuvettes. Then all the cuvettes were filled to 0.1ml with de-ionized water (DI water). After that, 5ml Bradford reagent was added to each cuvette. After mixing and resting the solutions for 10 minutes, the transmission spectrums were measured by the smartphone spectrometer. The captured spectrum images of DI water and Bradford reagent with 0.1mg/ml and 1mg/ml BSA are shown in Figure 4-11a. The absorption of the blue part of the spectrum can be clearly observed. A series of spectra with the BSA concentrations varied from 0.1 mg/ml to 1 mg/ml are shown in Figure 4-11b. There clearly exists an isosbestic point near 525nm and the absorbance across the isosbestic point exhibit opposite correlation to the protein concentration. In other words, as the BSA concentration increases, the absorbance spectrum experiences a red shift and an absorbance peak appears at around 580 nm (expected to be 595nm). The wavelength discrepancy is mainly attributed to detector’s RGB filter response. The green and red band edge causes the apparent dramatic increase in absorbance at 580nm as also previously noted. Nevertheless, this error does not seem to affect the concentration measurement. We plotted the absorbance at 595nm as a function of the concentration of BSA (Figure 4-11c) and obtained a linear relation with $R^2=0.998$, demonstrating the capability of our smartphone spectrometer for quantitative measurement of protein concentration. It should be noted that in this study the sensitivity is primarily limited by the Bradford assay itself. With the rapid development of biotechnology, increasingly colorimetric
methods and reagents with high sensitivity have been reported. Our G-Fresnel smartphone spectrometer can also serve as a platform for these assays with high sensitivity.

![Image of transmission spectrum images for DI water, Bradford reagent with 0.1mg/ml and 1mg/ml BSA, absorbance spectra of Bradford reagent with different concentrations of BSA, and linear relation between the BSA concentration and the absorbance at 595nm.]

Figure 4-11: a) Transmission spectrum images for DI water, Bradford reagent with 0.1mg/ml and 1mg/ml BSA b) Absorbance spectra of Bradford reagent with different concentrations of BSA c) Linear relation between the BSA concentration and the absorbance at 595nm

4.3.5 Conclusion

We developed a compact (1.8” x 0.8” x 0.9”) smartphone optical spectrometer in the visible wavelength range by using a G-Fresnel diffractive optical element. The wavelength-pixel mapping relationship was calibrated by using a mercury and argon calibration source with discrete emission lines. The intensity of the spectrometer was calibrated against a commercial optical spectrometer. We also demonstrated spectral holographic measurement by using the G-Fresnel smartphone spectrometer and carried out theoretical analysis of the spectral resolution, which indicated that the system could achieve a wavelength resolution of few nanometers. Several factors, including the grating groove density, chromatic aberration and performance of the G-Fresnel, and slit size, limit the resolution of our G-Fresnel spectrometer. By optimizing the fabrication of the G-Fresnel and its performance at the shorter wavelengths as well as compensating for the chromatic dispersion using a tilted detector array, spectral resolution of 1
nm can be potentially achieved as indicated by previous theoretical analysis. The G-Fresnel device combines the functions of collimation, dispersion and collection in a single thin-film element. It can have a low f-number, leading to a compact system. The G-Fresnel thus opens a promising new avenue towards spectrometer miniaturization. We also demonstrated smartphone based Bradford assay, a powerful method for determining protein concentrations crucial for a variety of applications ranging from disease diagnosis to fundamental biomedical research. We performed proof-of-concept measurement of the BSA concentration. As the concentration of BSA increased, an absorption redshift was observed. A linear relationship between the absorbance and the protein concentration with $R^2 > 0.99$ was demonstrated. Notably, as we provide a general strategy for portable and convenient quantitative colorimetric analysis, it is expected that this platform can also be extended to other spectroscopic applications, such as, for food safety and POC tests.

### 4.4 Smartphone chloridometer

As mentioned at the end of the previous section, smartphone spectrometer can be a platform for POC testing as it is a generic tool. Yet, some applications which require high signal to noise ratio to obtain high sensitivity, still need additional specialized tool for it. In this section, a smartphone-based fluorometer that we developed particularly for chloride sensing application is presented[91]. We implemented a recently developed citrate-derived fluorescence chloride sensor, which offers high sensitivity and broad linear range. This is the first clinical study of a smartphone-based chloride sensor, paving the way for point-of-care diagnostic systems for cystic fibrosis (CF).
4.4.1 Motivation for smartphone chloridometer

Chloride is an essential electrolyte that maintains homeostasis within the body. Thus, evidence of various conditions and diseases can be presented in the chloride levels of biological fluids. For example, cystic fibrosis (CF) is a genetic multi-organ disease caused by a defective transmembrane ion regulator gene presented not only in the lungs and pancreas, but also in sweat glands such that elevated sweat chloride (> 60 mM) is the primary diagnostic criteria for CF\[92\][93][94]. Likewise, chloride levels in urine and serum are respectively used for the screening of metabolic alkalosis and Addison’s disease\[92\]. Hence, diagnostics provided at the point-of-care would facilitate early detection of such diseases and enable timely treatments\[93\]. Early diagnosis of CF, for instance, has been shown to prevent serious malnutrition and promote long-term growth of affected infants. Moreover, there is a significant demand for low-cost chloridometers not only for diagnostic purposes, but also for routine personal health monitoring\[94\][95]. For instance, sweat chloride is a predictive pharmacodynamic biomarker of pulmonary improvement in the treatment of cystic fibrosis, enabling tracking of systemic response and patient compliance through routine monitoring of sweat chloride\[95\]. Regular monitoring of sweat chloride can also assess dehydration in athletes\[94\].

Despite the clinical importance of sweat chloride as a diagnostic marker, the transition of chloride sensors from laboratories to the point-of-care has been hindered by prohibitive costs. Chloridometers based on ion-selective electrodes (ISE) typically cost several thousand dollars and suffer from significant interference from nitrates and bicarbonates, while automated analyzers based on coulometry or colorimetry can cost in the tens of thousands\[96\]. Consequently, a recent wide-scale survey of about 400 clinical labs revealed that the determination of sweat chloride for the diagnosis of CF was performed with manual titration in almost 70% of labs, while automated analyzers were utilized in less than 7%\[97\]. Manual titration with mercuric nitrate is time
consuming and prone to technical error, leading to rates of misdiagnosis as high as 15%[98]. Thus, there is an urgent need for new point-of-care diagnostic tools for sweat chloride that can be readily translated into clinical settings as well as for routine personal health monitoring.

4.4.2 Citrate-derived chloride sensors

In clinical measurements, sensor materials are expected to perform well in many different biological fluids and are often exposed to a broad analytical range. Chloride sensing is generally challenging in this respect, given that physiological chloride levels vary from 20 mM (sweat), 100 mM (CF sweat, serum), to over 200 mM (urine), among the highest for biomolecules. To address this issue, a citrate-derived synthesis platform for the design of new fluorescence chloride sensors based on a facile and low-cost reaction of citrate and a primary were reported[68]. The resulting family of chloride sensors possessed versatile sensing properties that can be tailored to suit the mean and range of various clinical tests such as sweat, urine, and serum analyses. Briefly, analyte recognition and signal transduction occur via fluorescence quenching mechanisms, in which the presence of chloride in a solution of CA-Cysteine (Figure 4-12a) leads to non-radiative relaxation of the excited fluorophore, resulting in visible attenuation of fluorescence. It has advantages including low cost, high chloride selectivity (Figure 4-12c) and impressive linearity from 0.8mM to 200mM (Figure 4-12b). Here, we implemented this powerful cost-efficient chloride sensor in our smartphone chloridometer.

4.4.3 Smartphone based chloridometer

We designed a smartphone operated fluorometer equipped with an ultraviolet LED to generate fluorescence signals from CA-Cysteine, which is then captured by the smartphone
camera. As mentioned previously, CA-Cysteine’s fluorescence emission is linearly dependent on the chloride concentration. Therefore, this combination of smartphone fluorometer and CA-Cysteine sensor is a smartphone chloridometer.

Figure 4-12: a) Synthesis of a citrate-derived chloride sensor, CA-Cysteine. b) Linear range of chloride detection from quenching of CA-Cysteine fluorescence intensity, N=3. c) CA-Cysteine quenching efficiency, showing chloride selectivity against common ions (0.1 M for all).
A schematic diagram and photo of the chloridometer system are shown in Figures 4-13a-b. In detail, the chloridometer works as a smartphone accessory that is equipped with an ultraviolet (UV) light emitting diode (LED) light source to generate fluorescence from our sensor material, which is collected and measured by the smartphone camera for quantitative determination of chloride from prepared samples. The UV-LED excitation light source (365 nm excitation, maximum 1 W output power) was soldered onto a printed circuit board, adjoined to an aluminum block for heat dissipation, and powered by a 9V battery. To maintain a stable operating temperature and reliable device performance, a 7-ohm high power resistor was used to control the voltage and current that applied to the LED, limiting the applied voltage to 4.1V and current to 700 mA as specified by the manufacturer. An HTC One M9 smartphone was used for all experiments, with its camera optimized with an exposure time of 10 ms, ISO of 100 and focused at near field to capture a DNG raw image of the cuvette sample holder placed very close to the camera. Our device design accommodates most types of smartphones given that the camera parameters (e.g. white balance, gain level, exposure time, and focal lengths) are fixed during the calibration curve and sample measurement. The fluorescence intensity of each measurement was determined by the summation of the total pixel values of the captured fluorescence pattern. This blue fluorescence was collected by the smartphone camera from a direction orthogonal to that of the excitation light to reduce UV light breakthrough. In addition, a 441.6 nm band-pass filter was used (L441.6-10 Ø1” Laser Line Filter) to remove the excitation light from fluorescence since the fluorescence emission wavelength of CA-Cysteine is maximal at 441 nm. The device was 3D printed (via Solidoodle) out of black acrylonitrile-butadiene-styrene plastic.
Figure 4-13: a-b) Photo and schematic diagram of the chloridometer system. c) Calibration curve obtained by the Stern-Volmer relation to linearize fluorescence quenching rates (I₀/I) over chloride concentration to determine the KSV (slope). d) Raw images of CA-Cysteine fluorescence captured by a smartphone camera in the presence of increasing chloride concentrations.

Signal processing of captured fluorescence images was performed by summation of the total pixel values of the captured fluorescence image to quantify the fluorescence intensity of
each measurement (Figure 4-13d). A calibration curve (Figure 4-13c) was then established by linearizing fluorescence quenching effects according to the Stern Volmer equation:

\[
\frac{I_0}{I} = K_{SV}[Cl^-] + 1
\]

where \(I_0\) is the unquenched fluorescence intensity of CA-Cysteine, \(I\) is the quenched intensity at [Cl\(^-\)], and \(K_{SV}\) represents the chloride sensitivity of the sensor. Relative standard deviations in 0, 5, 10, 15, 20, and 25 mM Cl\(^-\) measured in triplicates were 0.93%, 0.37%, 0.29%, 0.54%, 0.03%, and 0.66% respectively. Finally, patient sweat samples were prepared in our sensor solution, and the degree of fluorescence quenching was compared to the calibration curve to determine sweat chloride levels. As sweat is composed of numerous electrolytes that may interfere with chloride readings, we also performed a systematic investigation among common mono- and polyatomic ions and found that the environment sensitivity of CA-Cysteine fluorescence was limited to heavy halides, of which only chloride was deemed clinically relevant since physiological bromide and iodide are typically less than 40 µM and 1.6 µM respectively.

### 4.4.4 Clinical tests

To evaluate our smartphone-based device as a sweat diagnostics system for cystic fibrosis, we established quantitative metrics to define success in two aspects: (1) analytical validation with sweat controls according to College of American Pathologists guidelines, and (2) clinical validation with sweat from 10 individuals with or without CF using a Bland & Altman approach.

We performed analytical validation for sweat diagnostics using artificial sweat controls. Sweat controls (Quantimetrix, USA) simulate the human sweat at three levels, where QC-1 represents sweat of healthy individuals, while QC-2 and QC-3 represent the lower and upper ends of CF sweat respectively. Three replicates of each sweat control were prepared and analyzed
based on the above protocol. QC-1 was measured at 21 ± 5.6 mM, QC-2 at 55 ± 3.6 mM, and QC-3 at 103 ± 7.4 mM, compared to the respective reference values of 23 mM, 51 mM, and 106 mM as obtained by Quantimetrix. The measured results agreed well with the reference values, obtaining ranges well within the evaluation criteria of ± 10.0 mM/L or 15% (whichever is greater) set by the College of American Pathologists (CAP) Laboratory Accreditation Program, and thus validating our smartphone-based chloridometer as a new sweat test method for the diagnosis of CF. Sources of error contributing to measurement variability include fluctuations in the excitation light source, which may be resolved in future prototypes with a photodiode or by implementing as self-reference to calibrate the excitation power.
Figure 4-14: Clinical validation of our smartphone-based chloridometer for CF diagnosis was implemented via a) intraclass correlation coefficient measured by a regression plot and b) agreement measured by a Bland & Altman plot against the clinical reference (standard mercuric nitrate titration).
Lastly, we performed clinical validation of our device with sweat from 5 healthy and 5 CF individuals to evaluate performance in the detection of cystic fibrosis. Sweat from the right arm was analyzed by mercuric nitrate titration (reference method) by clinical technicians and sweat from the left arm was analyzed using our smartphone-based device. There was excellent correlation between the two methods, as evidenced by an intraclass correlation coefficient (ICC) of 0.972 with a 95% confidence interval of (0.882, 0.9935) (Figure 4-14a), which is considered a good-to-excellent level of reliability. The regression plot shows that CF and non-CF populations could be statistically identified with either method, even such that all CF individuals presented chloride above 60 mM while all non-CF individuals below 40 mM as per diagnostic criteria, meaning any disagreement between the two methods would not affect diagnostic results. The Bland & Altman plot showed a mean difference of -4.39 mM with a standard deviation of 14.6 mM, along with differences between methods within 29.2 mM at a 95% limit of agreement (Figure 4-14b). This is an acceptable level of agreement between the two methods given that the typical range for sweat chloride measurements in different arms are ±15 mM. We speculate that the negative bias (-4.39 mM) and the variability (±14.6 mM) in clinical samples compared to sweat controls may be attributed to exfoliated particles absorbing incidental light.

4.4.5 Conclusion

In conclusion, we have achieved a sweat diagnostics system for cystic fibrosis by developing a low-cost smartphone based chloridometer equipped with a novel citrate-derived fluorescence sensor. The sensor device exhibited a broad linear range of 0.8-200 mM chloride, maintaining relative standard deviations below 1% throughout the detection range. Next, we have performed analytical and clinical validation of our device as a reliable and convenient optical sweat test method for the diagnosis of CF, which may significantly improve diagnostic accuracy
by reducing technical complexity and human error in fast-paced clinical settings, and may have broader impact on the implementation of diagnostic tests in resource-scarce settings with limited access to sophisticated instrumentation.

4.5 Future direction

4.5.1 Developing holographic G-Fresnel

Although the current design of the G-Fresnel smartphone spectrometer already achieved compact, high performance, and low cost, there is still potential for improvement. As mentioned in the G-Fresnel introduction, current double side G-Fresnel cannot be easily replicated by surface patterning method which limits its potential to be massively produced. Single side G-Fresnel which embeds grating pattern on top of Fresnel lens is expensive to fabricate (electron beam lithography is slow and costly). Therefore, holographic G-Fresnel, which is single side, easy to fabricate and flexible to change parameters, becomes an ideal alternative fabrication method for the current G-Fresnel.

Figure 4-15: Hologram recording setup
The experiment setup for recording holographic G-Fresnel is shown in Figure 4-15. An Ondax 405nm diode laser with 1m coherent length was used as the light source. The laser beam was sent through a polarizing Beamsplitter to divide into signal beam and reference beam. Two half waveplates were used in the setup. One was placed before the polarizing Beamsplitter to adjust the power between the signal beam and the reference beam. The other one was placed after the signal beam, which was used to match the signal beam’s polarization with that of the reference beam. Before recording, both beams were spatially filtered to generate the best quality hologram. As addressed previously, the parameters of the holographic G-Fresnel are determined by the lens position and angle between the two beams. For example, 20-degree angle equals 1.16µm grating period (857lines/mm).

The holographic G-Fresnel was recorded by using g-line photoresist (Shirpley 1805) which was spun coated on a silicon or silica wafer. After exposures, 351 developer which was mixed with DI water at 1: 5 ratio was used to develop the hologram and a holographic G-Fresnel on photoresist was made. During the process, exposure time and developing time of the device can be varied which would influence the surface shape of the G-Fresnel. Figure 4-16a shows macroscopic view of the device and microscopy image is shown in Figure 4-16b. Exact spatial pattern of the hologram was measured by atomic force microscopy (AFM) and the result is shown in Figure 4-16c.
Figure 4-16: a) Macroscopic view of the holographic G-Fresnel, b) Microscope image of the holographic G-Fresnel, c) AFM scan of the hologram pattern on photoresist, d) AFM scan of the hologram pattern transferred to silicon
Although the holographic G-Fresnel’s pattern appears great on photoresist, the duration, strength, and fabrication cost of the device prevent it from being massively produced and used. To combat this, we developed a pattern transfer method, which effectively addressed the issue. Initially, in order to achieve this goal, we used reactive ion etching to transfer the pattern from the photoresist to silicon[99]. The reactive ion etching (RIE) is an anisotropic etching process which can transfer the exact spatial profile from the photoresist to silicon. The basic principle is shown in Figure 4-17. Experimentally, we used PlasmaTherm Versalock etcher to etch the sample with SF$_6$ and O$_2$ gas. Etching rate, selectivity (etching speed ratio between silicon and photoresist) can be controlled by modifying the etching parameters including gas pressure, chuck power and gas ratio. Not only is this etching process able to successfully pattern transfer the hologram from soft resist material to hard mold, it also provides us the ability to control hologram grating depth (through modify etching process’s selectivity). Figure 4-16d shows the AFM image of the holographic G-Fresnel after etching, with its depth deeper than the original pattern on photoresist.
Currently, hologram recording, pattern formation on photoresist and etching recipe of the pattern transfer have been investigated. As the next step, surface relief method would be used to duplicate the single side G-Fresnel by using polycarbonate. Specifically, diffraction efficiency of the device would to be optimized through varying the grating depth. Estimation based on theoretical calculation of thin holographic grating would be used as a guidance for depth search. We believe, with the new single side holographic G-Fresnel, smartphone spectrometer can be fabricated at low cost, significantly improving the accessibility of the device and expanding its applications power.

4.5.2 Self-calibrated smartphone chloridometer

The major drawback of the current smartphone chloridometer comes from LED’s fluctuation. To resolve this issue, we proposed a multichannel chloridometer which can measure multiple samples simultaneously and perform self-calibration. Proposed design of multi-channel-smartphone chloridometer is shown in Figure 4-18, which measures fluorescence from four cuvettes with a single LED source. In specific, the illumination source, UV LED, is placed at the bottom of the device to simultaneously illuminate four tilted cuvettes simultaneously. The emission from samples are collected by the smartphone from top. A longpass filter is placed before the smartphone camera to filter away the emission residue. Three out of four cuvettes contain sensor and samples with known concentrations which are used to produce the calibration curve covering the region of interest. Afterwards, by using this curve and the fluorescence measurement from the forth unknown sample, chloride measurement of the unknown sample is achieved. As all four cuvettes are illuminated by a single LED light source, time-dependent fluctuations from LED output is removed. Spatial-dependence of the LED light source can be pre-determined by placing same sample in four cuvettes and measuring their individual output.
This factor will be stored and taken into account in all newly performed measurements. It is expected that this method is able to effectively reduce the error caused by LED intensity fluctuation. In addition, it also enables chloride concentration measurement in a single shot, which greatly improves measurement speed.

Figure 4-18: Schematic of proposed smartphone based chloridometer for simultaneous multi-capture.
Chapter 5

Conclusion

This dissertation presents my work on developing new optical devices for biomedical optical sensing (such as imaging and spectroscopy). In particular, these devices can be divided into three categories, excitation source, light transmission device, and detection device.

Soliton self-frequency shift, a phenomenon caused by intra-pulse Raman scattering is employed as a convenient way to produce tunable ultrafast light sources. But the fundamental limitation of these sources is that they have limited pulse energy, which hinders their usage in biomedical imaging and spectroscopy applications. Previous research work on SSFS based sources has been primarily focused on finding new types of fiber with larger mode area or different anomalous dispersion regions, which, unfortunately, cannot fundamentally overcome the problem. In Chapter 2, I describe a technique that we developed, called divided pulse soliton self-frequency shift, which provides a general solution to the problem. The method initially splits a high energy pulse to multiple-pulses with lower energy, then shifts their wavelength individually. Finally, these wavelength shifted pulses can be coherently recombined, thus achieving power scalable for SSFS based source. Two-pulse case was demonstrated for the proof of concept. The number of pulses could be extended to a larger number, which will enable a much higher energy pulse. Furthermore, multi-line dual polarization was obtained, which is useful for nonlinear microscopy. As a proof of concept, dual polarization second harmonic generation imaging of BaTiO$_3$ crystal was performed. This ultrafast light source could be applied to many nonlinear microscopy techniques.
Biopolymer waveguides are a desired tool for delivering light into body under *in vivo* conditions. Not only are these waveguides able to deliver light into body to fulfill applications such as imaging and sensing similar to silica fibers, but they also have desirable biological, mechanical properties which make them more attractive over silica fibers in biological applications. In Chapter 3, I present a biodegradable step-index polymer fiber which is fabricated using citrate based materials. The programmable nature of citrate based material permits tunable refractive index, which enables us to fabricate step-index structure. Step-index structure and low material attenuation endows the fiber with outstanding optical properties, which is suitable for organ scale light guiding. Further, preliminary imaging experiment confirmed the capability for this fiber to be used for image delivery and subsequently verified its imaging and sensing potential. Additionally, results confirm that the whole fiber is biodegradable and nontoxic, which indicates the fiber is free from secondary removal surgery after being used in body. This saves the cost and reduces pain for patients and also enables continuous monitoring and treatment. This fiber is a promising light transmission tool for biomedical imaging and spectroscopy.

Smartphone based detection devices, which have been developed based on smartphone, are promising for point of care applications, as they have high quality imaging sensor, powerful computational, and communicational capability. The wide availability of smartphones lowers the barrier for everyone to get access to POC tests. In chapter 4, I demonstrated two smartphone based detection devices that are capable of performing POC tests. First, by using dual functionality G-Fresnel inside the spectrometer, a compact G-Fresnel smartphone spectrometer which has high resolution over the visible range while maintaining compact dimensions was developed. Successful protein concentration measurement by using Bradford reagent demonstrates the device’s capabilities and potential for performing POC tests. Second, a smartphone chloridometer which is able to accurately measure sweat chloride concentration was developed. Clinical tests on both patients suffering from cystic fibrosis and health people were
performed to verify the effectiveness. Accurate measurements and successful differentiation between patients and healthy people confirms the feasibility of the device. Both smartphone-based detection devices are cost efficient and easy to operate, making them more accessible to users.
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