MBP1405H – Introduction to Bio-Microscopy
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Written Assignment

I. Introduction

In the past decades, fluorescence and phosphorescence probes, based on particle absorption and emission of light, are the conventional toolkits to label and observe the molecules of interest in the application of chemical, biology and material science. However, there are some limitations of fluorescence bringing inconvenience to the research and one is about the exploration on the small biomolecules. In the paper I reviewed, the author proposed a technique to overcome this challenge which is called *Bioorthogonal Chemical Imaging* platform. This platform integrates the stimulated Raman scattering (SRS) microscopy (a nonlinear Raman microscopy technique) with a small Raman-compatible probe to detect the tiny, live biomolecules with high sensitivity, specificity and biocompatibility [1]. Apart from the new technique, the paper provided detailed explanation on the choice of Raman-active probe and its application in medicine and biology, indicating the significant potentials in other relevant fields. In the following contents, I will present my thoughts on the strength and weakness of the paper and how I learnt from this technique.

II. Innovation and Strength

Before the invention of this new technique, several Ramen microscopies has been applied to probe small molecules. Spontaneous or conventional Raman microscopy uses the vibrational spectroscopy instead of fluorescence sources to detect molecules [1]. However, this technology not only consumes longer acquisition time, but also has low sensitivity and biocompatibility issues. While the Surface Enhanced Raman Scattering (SERS), Coherent Anti-stokes Raman Scattering (CARS) and SRS (Fig. 1) are developed to overcome former drawbacks, there are more considerable problems generated during the application such as complexity and limited performance.

As far as I am thinking, the platform utilizes the strength of SRS and combines with the concept of vibrational probe deriving from vibrational spectroscopy. The discovered Raman active probes such as alkynes (C≡C) moieties [2, 3] and deuterium isotope [4] provide positive properties like high-contrast vibrational signal and small, simple structure. In the spectroscopy, the particles are used to detect local electrostatics and vibrational dynamics through the Stark effect [5]. When combining with SRS integrated in the platform, the energy gap of two lasers from Raman microscopy is uniquely resonant with the vibration level of the probe and thus the peak signal from either Stokes or pump beams are located in a region without any other molecule signals (Fig 2.). Therefore, it is rational to improve the specificity and contrast of probing small molecules.

Additionally, such probes, have small and simple structure, act as observer that neither does not interact with cell molecules nor participates in any chemical or biological reactions inside the cell. In other words, the probe is a special small-molecular-marker with unique vibrational dynamics, which can discover and tag targets through contactless and zero-energy-exchange effect. In summary, SRS with these probes, as known as *Bioorthogonal Chemical Imaging* platform, has the opportunity to record the dynamic changes without generating spectroscopical and biochemical modifications in the live cell with acceptable spatial and temporal resolutions.

Furthermore, the imaging speed is faster than other Raman microscopies with probe and without probe. According to Yamakoshi et.al [3], the average imaging speed of a conventional Raman microscopy with the alkynes probe is approximately 10 mins. In the platform, the SRS uses two spatially and temporally synchronized picoseconds laser pulses that jointly accelerate the vibrational transition or Raman scattering of the photons by 10^8 times [1] and thus reduce the image acquisition time, which shows the capability of capture dynamics in the live cell within acceptable range.

III. Technical and Practical Limitations

Every coin has two sides. Even though the platform solves major issues in probing small molecules, besides the weakness mentioned in the paper, there are still two main deficiencies happening in the experiment.

Firstly, the technique's complexity and the requirement for specialized equipment may limit accessibility for some research labs. The SRS process of the platform is dependent on the spontaneous Raman cross-section, the spectral linewidth, the path length of the light-field-matter interaction etc. Besides the conventional Raman microscopy setup, it commonly requires an external laser source modified to the Stokes frequency interacting with the pump beam to generate the desired effect (Fig. 3). The monetary and time costs of SRS construction are already approached the limits of many small laboratories. Apart from the SRS, the researchers need to set up and operate the probe successfully that including the probe does not have any contamination, the probe penetrate the target cell uniformly and efficiently. The incorrect or unexpected operation in each step stands for the failure and should restart experiments. These consumed probe materials are also a significant expense and probably become the burden of the lab. Therefore, in the future, if the platform can be developed to reduce the operation complexity (considerable factors or experiment steps) or integrate the probe into the SRS, that will be the one of the most innovative technologies.

Furthermore, the potential for vibrational probes to interfere with native molecular functions warrants further investigation to ensure the technique's broad applicability and reliability. In the paper, the platform believes that the probe like (C≡C) has approximately no interaction with the molecules in the cell. However, they did not mention in which experiments they find above situation and whether the property of probe is applicable for all molecules. That would be better if the author can provide a table of molecules which have no or inert reaction with each probe or corresponding experiment setup for other scientists to reproduce.

Last but not least, temporal resolution of this work may be a problem when tracking intracellular chemical composition in the ultrafast dynamics such as DNA, protein, and lipid. Since the Raman microscopy has low conversion efficiency, it is difficult to capture the ultrafast movement of intracellular components with a high frame rate compared to the quantitative phase microscopy (QPM). Also, combining with probe, it takes longer time for SRS to collect signal from probe to keep spatial resolution and thus the temporal resolution will be given up. The balance between spatial and temporal resolution is considerable in the future.

IV. Advancements in the Field

The introduction of bioorthogonal chemical imaging via stimulated Raman scattering (SRS) microscopy, as presented by Lu Wei et al., marks a transformative advancement in the realm of live-cell imaging. This innovation particularly benefits the study of small biomolecules, a domain where traditional imaging modalities have faced significant challenges. The technique's ability to provide high-resolution, real-time visualization of biomolecular processes without the need for fluorescent tagging not only circumvents the limitations associated with fluorescence-based methods but also minimizes potential perturbations to cellular function. This breakthrough enhances our capability to explore intricate cellular metabolism pathways, understand drug molecule interactions within their native cellular environments, and uncover the underlying mechanisms of various diseases at a molecular level. By enabling researchers to observe and analyze these processes with unprecedented clarity and specificity, the technology paves the way for ground-breaking discoveries in cellular and molecular biology. Its application extends beyond mere observation, offering the potential to elucidate complex biological mechanisms, evaluate therapeutic interventions in real-time, and accelerate the development of novel treatments. This method represents not just an incremental improvement but a significant leap forward, opening new avenues for scientific exploration that were previously deemed challenging or impossible to navigate with existing imaging technologies.

V. Implication for Other Investigation

The implications of this technology extend across various fields of biomedical research, offering new methodologies for investigating the molecular underpinnings of diseases, the cellular targets of drugs, and the intricate mechanisms of cellular metabolism (e.g. evaluating the pharmacokinetics of Terbinafine Hydrochloride (TH)). Its application could revolutionize our approach to understanding disease development and pharmacodynamics, leading to more effective therapeutic strategies. Oncology research stands to benefit from the ability to track tumor metabolism and microenvironment changes, potentially leading to new cancer diagnostics and treatments. For example, we can design the probe to specify and tag the immune cells around the prostate tumor and capture the factors that affects the tumor derivation from live cell and its grow up.

In addition, this technique provides a new idea about how to break through physical and biological limitations for the field of microscopy or spectroscopy. Due to the limitations of conventional Raman microscopy, scientists cannot tag the small biomolecules. Instead of continuing the method on discovering the chemical bond of entire cell, researchers switch to find the most common and basic chemical bond and thus detect and tag the cell through the signal

difference of the probe. It is reasonable to consider exploration in the intersection field of physics and biology which will creates many amazing innovations.

VI. Insight on My Research

Although my work is about Magnetic Resonance Imaging (MRI) instead microscopy or spectroscopy, the innovative idea behind this technique is potential to insight my research. The mechanism of Raman microscopy cannot probe the small biomolecules directly and thus scientists utilize the special marker that can tag the small molecules indirectly. Usually, MRI research is abstract and cannot visually inspect the changes (protons, quark) during the experiment. To fully understand the mechanism, converting the quantum model to a classic macro-model is vital and thus we can observe the particle interactions from human perspectives. In my future research, when solving the problem directly is not feasible, trying to investigate a middle outcome that connects both inputs and targets can also achieve the expectations.

VII. Potential for Future Work

Future research directions include the expansion of the vibrational probe library, enabling the study of a wider range of biomolecules and cellular processes. Expanding the library of vibrational probes would allow for the visualization and study of a wider range of biomolecules, thereby enhancing our understanding of complex biological processes. Integrating SRS microscopy with other imaging techniques, such as fluorescence microscopy or electron microscopy, could provide complementary data, offering a more holistic view of cellular mechanisms. These advancements could lead to significant breakthroughs in diagnosing and treating diseases, understanding cellular responses to therapies, and designing new drugs with enhanced efficacy.

Additionally, refining imaging agents for greater biocompatibility, expanding the technique's application in translational medicine, and exploring its potential in understanding complex diseases are good directions. Ongoing collaborations across disciplines are essential to realize these goals, underscoring the collaborative nature of scientific discovery.

Moreover, with the high speed of SRS, there are a huge number of generated data and require fast and accurate analysis to extract essential information. To speed-up the image acquisition and data collection, it is rational to combine with the machine learning technology. Orringer et.al [7] have proved that machine learning (ML) method such as U-Net can segment the brain tumor within a few seconds and thus improve the working efficiency of Raman microscopy.

VIII. Conclusion

The development of live-cell *Bioorthogonal Chemical Imaging* using SRS microscopy and vibrational probes by Lu Wei et al. is a significant advancement in the field of chemical imaging. While it presents certain limitations, its strengths and potential for future development herald a new era in the study of cellular processes at the molecular level. This technique's ability to provide detailed, real-time insights into the dynamics of small biomolecules in live cells has profound implications for biomedical research and beyond. As we stand on the cusp of new

discoveries, this technique not only enhances our current understanding but also lights the path toward future scientific breakthroughs.

Reference

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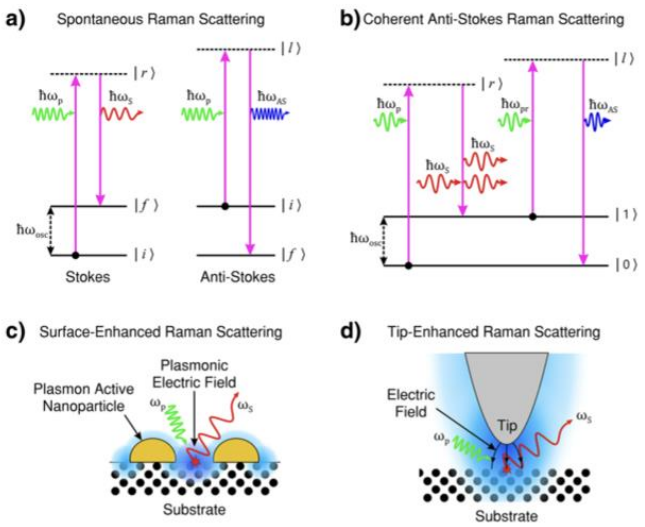

Fig. 1 Energy transfer process in each Raman scattering process: a) Spontaneous Raman Scattering. b) Coherent Anti-Strokes Raman Scattering. c) Surface-Enhanced Raman Scattering. d) Tip-Enhanced Raman Scattering [2]

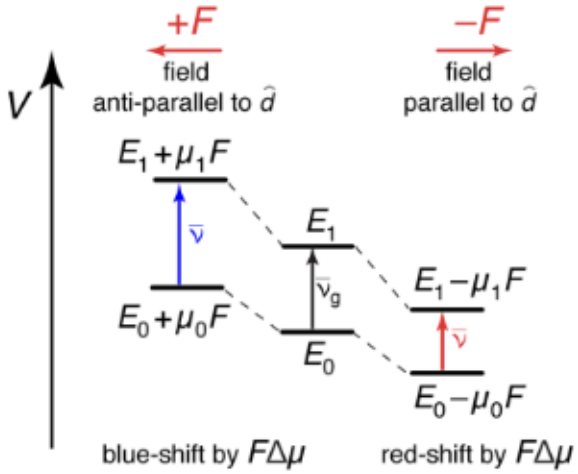

Fig. 2 Vibrational frequency happens in the Stark effect [6]

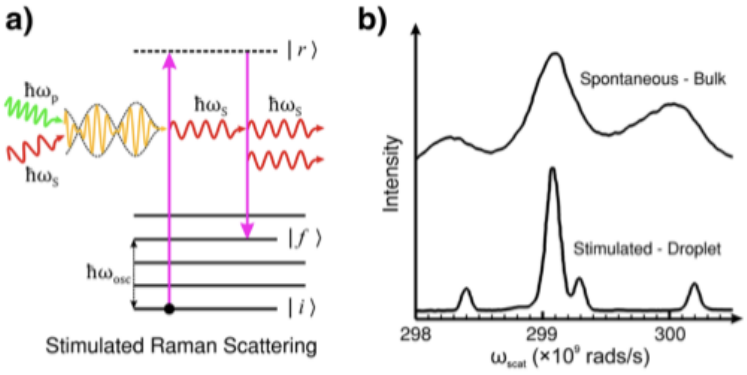

Fig. 3 Energy level diagram of simulated Raman scattering (SRS) [2]