INTRODUCTION
Real-time imaging of whole cells in their native environment proves to be a significant challenge in biomedical research. The introduction of fluorescence microscopy has allowed for the imaging of proteins and antibodies without perturbing their function or activity. In the case of small molecules, fluorescent probes can be bulky and can disrupt the observation of its cellular process or mechanism of action. A method to image small molecules without large perturbations to their structure would greatly aid in elucidating their mechanism of action and localization in cells. Stimulated Raman Scattering (SRS) uses the distinct bond vibrational frequency in small molecules with minimal to no change to the chemical structure. In SRS, the introduction of a second laser, Stokes beam, is used to tune the frequency difference with another incident beam, pump beam, to match the molecular vibration that stimulates excitation of the Raman-active mode. Excitation of the molecule results in a loss and gain in intensity of the pump and Stokes beams respectively, where the change in intensity is processed to produce an image (Fig. 1). Use of Stimulated Raman Imaging (SRI) for small-molecule metabolite localization in cells or characterization of disease phenotypes would greatly aid diagnostics.

“CELL SILENT” TAGS FOR STIMULATED RAMAN IMAGING
Identifying the disease state, localization and uptake of drug candidates or small-molecule metabolites, and other phenotypic markers of drug action would bolster the development of candidate molecules. Better crop protection is essential to ensure food security with the global population increasing, and protection will come with advancements in basic plant science and agrochemical research. A major challenge in this field is the quantifying heterogeneity of chemical composition in plants at the subcellular level. Manfield and coworkers demonstrated the use of SRI for the agrochemical uptake of fungicides and distinct subcellular structures in maize leafs. The nitrile functional groups on the commercial and industrial scale fungicides azoxystrobin and chlorothalonil respectively were able to be selectively imaged using stimulated Raman. Nitrile, azide, alkyne, and isonitrile functional groups have molecular vibration frequencies that do not overlap to any Raman signal produced from cells enabling them to be powerful probes for SRI.

Isotopologues differ only from their parent structure in their isotopic composition and can serve as an alternative tagging strategy as they have no perturbation in the chemical structure of the parent compound. Incorporation of deuterium (2H) or 13C may provide favorable Raman characteristics, as the
C–D peak falls within the cell “silent” region. Current methods to monitor protein synthesis require the fixation of cells or may not provide spatial information of new protein. SRI can provide spatiotemporal imaging with no sample preparation using isotopically labeled amino acids. Manen and coworkers were able to observe the incorporation of deuterated phenylalanine, tyrosine, and methionine incubated with their respective unlabeled counterpart in live cells. For methionine and tyrosine, a better signal to noise ratio was achieved using the C–D peaks while quantification for phenylalanine was obtained through the ring breathing band. Additionally, Shen and coworkers were able to monitor protein degradation using $^{13}$C labeled phenylalanine in living cells with subcellular resolution. Tagging provides one avenue for imaging small molecules, but disease markers in cancer such as vascular proliferation can be identified without any tags.

**STIMULATED RAMAN IMAGING IN BIOMEDICINE**

An advantage to imaging biomolecules within cells using SRS, is no additional sample preparation or staining. This would be especially useful to identify the histopathology of a tissue sample in real-time from surgery to determine cancerous or non-cancerous tissues. Lu and coworkers have reported the use of SRI in comparison with the traditional standard of identifying the histopathologic classification of tumors, haemotoxylin and eosin (H&E) staining, of 41 specimens from 12 patients. Using the CH$_2$ and CH$_3$ molecular vibrations of lipid and protein respectively, they demonstrated that SRI could detect many of the characteristics associated with brain tumor formation and classification, like vascular proliferation, as well as the visualization of structures not available to traditional H&E staining. SRI revealed previously unobserved lipid droplets in glioma cells and the small bulbous protrusions in myelinated fibers. The biomedical application of SRS imaging can not only be used to identify cancerous tissue samples, but can potentially be used to monitor the progression of disease.

Amyotrophic lateral sclerosis (ALS), is responsible to the neural degeneration of nerve cells resulting in the loss of voluntary movement in muscle cells. Due to the high degree of variability in monitoring disease progression in rodent models, the need for a reliable and precise method to track ALS disease states will improve development for ALS drug candidates. Tian and coworkers have reported the use SRI and electromyography (EMG) to monitor neural degradation in several mouse models and human post mortem tissue. The structural information that SRS was able to provide in tandem with EMG may allow for the earliest *in vivo* detection of sciatic nerve degeneration in mouse models. SRI detected the presence of lipid ovoids in some disease models that showed no active degeneration by EMG and was used to evaluate a drug candidate that was able to slow the progression of peripheral neural degradation.

**OUTLOOK AND CHALLENGES**

Stimulated Raman Imaging is a promising technique that allows for the selective imaging of lipids, protein degradation, DNA synthesis, and histopathological features of disease states with no staining or additional sample preparation. One of the most prominent challenges of using SRI in identifying small molecule localization, especially drug candidates, are the above physiological concentrations of analyte required for detection, 200 µM to 2 mM. The advance of more sensitive tags like aryl-conjugated alkynes, or the further development of the technology that is used for SRI would address this major hurdle.

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