FIBER-BASED WAVELENGTH-SWEPT SPONTANEOUS RAMAN SPECTROSCOPY FOR BRAIN TISSUE CLASSIFICATION

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Raman scattering has been used for brain tissue identification and characterization. Raman spectroscopy is a technique that provides information about the molecular composition of a sample by measuring the scattering of laser light. It is based on the inelastic scattering of photons, known as Raman scattering, which occurs when light interacts with the vibrational modes of molecules. In the context of brain tissue, Raman spectroscopy can be used to analyze the molecular composition such as protein, lipids, and other biomolecules. Raman spectroscopy has been shown to be a powerful tool for tissue identification and it has the advantage of being non-destructive and minimally interfering with the ongoing process inside the tissue. The conventional setup includes a monochromatic light source for excitation and a spectrometer for collection suffers from extremely low efficiency due to light scattering in tissue. We present a *wavelength-swept* Raman spectroscopy strategy that overcomes this limitation by using a laser that can sweep over a significant wavelength range to excite tissue with different wavelength over time, and a photodetector with a fixed narrow-bandpass filter to collect the Raman signal at the given wavelength and therefore a varying Raman frequency. The most important gain of this strategy is the large optical invariant of the setup that advantageously compares to that of a spectrometer. Indeed, the diameter of the detector is 1 mm which is 20 to 100 times larger than the typical entrance slit of a spectrometer with a resolution of 1 nm for an approximately equal acceptance cone.
Theoretical comparison of the detection efficiency between swept-source Raman spectroscopy (SSRS) and spectrometer-based Raman spectroscopy has been done using the Raytracing Python module [1]. For the experimental data acquisition, the swept-source is a Ti:Sapphire that automatically sweeps the excitation wavelengths from 800 nm to 820 nm. A bifurcated fiber bundle is used to deliver the laser light to the sample and to collect the light from scattered back from the sample. An ultra-narrow bandpass filter 1064/1 nm and an InGaAs femtowatt photoreceiver are employed for signal collection, allowing to cover the Raman shift region from 2800 to 3100 cm^{-1}. Finally, the photoreceiver is connected to a lock-in amplifier to enable phase-sensitive detection, thus increasing even more the SNR. The setup was tested on non-human primate fixed brain tissue of 1 mm thickness. We acquired 140 spectra, 20 from each region including white matter (WM) and regions with gray matter (GM) such as subthalamic nucleus (STN), the internal (GPi) and external (GPe) globus pallidus, substantia nigra (SN)and striatum (putamen and caudate nucleus).

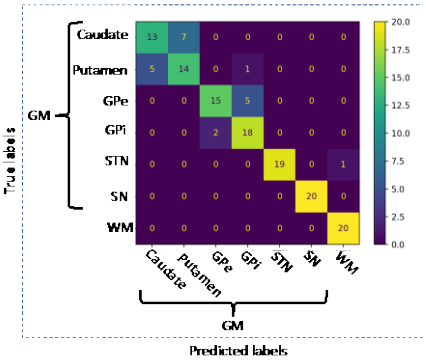

Figure 1 – The confusion matrix of classification using 3 first principal components with 98.5% accuracy.

Fiber collection efficiency and coupling efficiency have been calculated with simulations for several diameter and NA of fiber. The collection efficiency increases as we use a larger fiber with a higher NA. When *collection* fiber diameter and NA are increased, the coupling efficiency into the spectrometer decreases significantly while it is not the case in SSRS, meaning that using a large area detector result in a better efficiency. With a spectrometer there is a tradeoff between the collection efficiency and the coupling efficiency. Additionally, the signal-to-noise ratio (SNR) is improved significantly with SSRS setup. Using principal component analysis (PCA) with a k-nearest neighbor (KNN) classifier and leave-one-out cross validation, we can observe the the first principal component (PC) has a very high explained variance ratio and it can be used to separate WM and GM in two classes with 100% accuracy. If we take the three first PCs into account and perform the classification on 5 different regions including striatum (caudate and putamen), GP (GPi and GPe), STN, SN, and WM, we still have 98.5% accuracy for classification (Fig 1).

Reference:
[1] Pineau Noël V, Masoumi S, Parham E, Genest G, Bégin L, Vigneault M-A, et al. Tools and tutorial on practical ray tracing for microscopy. Neurophotonics. 2021;8: 010801.