2P, 3P multimodal microscopy applied to liver cancer and neurophotonics

Laboratoire Photonique Numérique et Nanosciences, Université de Bordeaux, 33400 Talence, France

AUTHORS Nasim Akhtar **,** Stéphane Bancelin **,** Laurent Cognet

Multiphoton microscopy has emerged as a powerful tool for visualizing the morphology and function of tissues and cellular circuits in the intact mammalian brain and liver and dynamics with high resolution and minimal invasiveness. This technique has significantly enhanced our understanding of brain and liver function and organization. Multiphoton techniques like 2PM and 3PM address this, using longer wavelengths for improved depth. Tissue absorption becomes significant beyond 1300 nm, influencing the optimal spectral window. Balancing tissue scattering and absorption, the optimal penetration wavelength is around 1700 nm. At 1700 nm, three-photon excitation enables the use of red fluorescent dyes. This makes 3PM particularly effective for imaging deep in brain tissue. Beyond the application 3PM , the 1700 nm spectral window could be use for 2PM but very few groups have attempted that, because of the lack of dyes and effective fluorescence detectors for near-infrared emissions. Our research focuses on using single-walled carbon nanotubes (SWNTs) as new fluorophores for 2PM at 1700 nm. SWNTs, known for their exceptional optical properties, have been extensively studied and applied in advanced microscopy techniques, including super-resolution microscopy. Specifically, our investigation focuses on 2PM and 3PM fluorescence microscopy at the same excitation wavelength to determine which enables deeper imaging. For 2P we will use as novel fluorophores Single-Walled carbon NanoTubes (SWNTs) and for 3P use red fluorophore.

SWNTs can emit light at different wavelengths, and we can control these properties during sample preparation. To identify the best fluorophore for 2PM imaging within our laser's range (1628–1700 nm), we analyzed several SWNTs using spectroscopy. After testing multiple options, we identified the one that produced the strongest fluorescence, with an optimal excitation peak at 1675±5 nm. This makes it the best choice for our experiments. In microscopy, using a 1670 nm laser, for testing our NIR-PMT we successfully detected carbon nanotubes with a near-infrared photomultiplier tube (NIR-PMT) and also we validated that this signal is 2P photon signal.

Our next step is to conduct 2P and 3P imaging experiments on mouse brains to compare which technique is more effective for deep imaging. Additionally, I also started to on the comparison between 2P and 3P signal, considering both signal generation and collection, using simulation.

Figure caption

a) Comparison of 2P emission spectra for SWNTs at different excitation wavelengths b) SWNTs 2PM image(Scale bar 1µm).