In-depth molecular targeted confocal imaging with both excitation and emission wavelength in NIR-II window

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Abstract: Achieving deep penetration depth while maintaining cellular or molecular resolution is one of the main objectives and challenges in bioimaging. The NIR-II (1000-1500nm) fluorescence imaging has drawn much attention because it provides several advantages over the visible and NIR-I region (300-900nm): low absorption of biological tissue, negligible autofluorescence and reduced scattering. Accredit to the emerging versatile nanomaterials with NIR-II emission (quantum dots, lanthanide-based nanocrystals, single-walled carbon nanotubes, etc.) and newly developed NIR-II sensitive InGaAs detectors, the new NIR-II optical window has been intensively investigated for non-invasive deep tissue imaging in recent years [1].

Until nowadays, however, most of the NIR-II imaging applications are whole-body animal imaging experiments performed in wide field for the visualization of vascular structure or organs with relatively low resolution and no molecular targeting ability [2]. In order to further apply NIR-II window to deep tissue imaging with cellular or sub-cellular resolution, confocal microscopy or other advanced imaging techniques must be modified to accommodate the NIR-II wavelength. To our knowledge, confocal microscopy in the NIR-II window, which can enable 3D deep tissue imaging with sectioning ability, is not commercially available yet.

To fill this gap, we develop NIR-II confocal microscopy adapted from a Nikon C1 microscope. A 1064nm pulsed laser source is combined with the gated detection scheme of an InGaAs single-photon detection module. PbS quantum dots with 1300nm peak emission are selected as NIR-II to NIR-II probes because of its high brightness, broad excitation spectra, and biocompatible functionalization for specific biological imaging applications. β-tubulin, a cytoskeleton structure, in HeLa cells are labelled by immunofluorescence with goat anti-mouse immunoglobulin (IgG) secondary antibody tagged PbS QDs for NIR-II to NIR-II confocal imaging. Similarly, β-tubulin in fixed mouse brain tissue are labelled. And the 3D deep tissue imaging capability are further investigated with the tissue sample.

Reference: