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NEUROGASTROENTEROLOGY

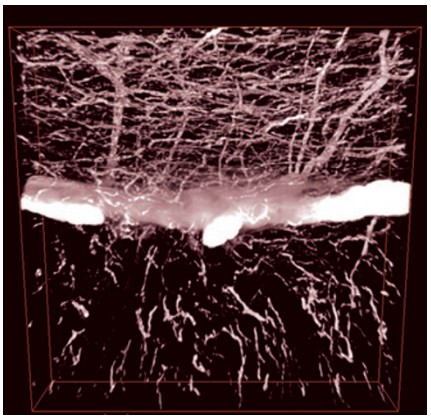
Improving 3D imaging of the enteric nervous system

Imaging the enteric nervous system (ENS)—a complex multidimensional structure comprising dispersed neurites and fibers and structural components—is challenging. Shiue-Cheng Tang and colleagues now show that optical clearing could aid 3D visualization of the ENS.

Conventional microtome-based 2D ENS imaging provides only a limited perspective of the neural network. To generate a comprehensive 3D view, Liu *et al*. imaged the human ileum using confocal microscopy after optical clearing. "Optical clearing reduces light scattering in the specimen, thus promoting photon penetration across the tissue to generate high-definition images," explains Tang.

Human ileal enteric nerve specimens were immunostained with marker PGP9.5 and immersed in optical-clearing solution before deep-tissue confocal microscopy; images were then processed for 3D image rendering and analysis.

Fluorescence signal decay along the focal path in the human ileal wall was reduced in optically-cleared tissues compared with control (saline-immersed) tissues. Indeed, immunostaining signal strength rapidly declined over a penetration depth of 65 μm in control tissue, whereas signal strength slowly decreased in optically-cleared tissue at depths of 65–150 μm. Optical clearing also increased the signal-to-noise ratio (a measure of the ability to distinguish the target from the background). Digital analysis of high signal-to-noise ratio images allowed the identification of nerve fibers and quantification of signal peaks. Stacking these images created a "panoramic 3D view of the gut wall innervations and microstructures including circular and longitudinal muscles and crypts," says Tang.



3D projection of human neural tissue. Courtesy of S.-C. Tang.

The researchers now plan to "develop labeling and imaging methods for observing the gut tissue microstructure, vasculature and innervation networks to study gastrointestinal disease echanisms in animal models ... and to compare healthy and diseased tissues in clinical research."

Katherine Smith

Original article Liu, Y. A. *et al.* Optical clearing improves the imaging depth and signal-to-noise ratio for digital analysis and three-dimensional projection of the human enteric nervous system. *Neurogastroenterol. Motil.* **23**, e446–e457 (2011)