Super-resolution Microscopy in the study of Peripheral Nerve Regeneration

Iván Coto Hernández, PhD[1]; Suresh Mohan, MD[1]; Nate Jowett, MD[1]

[1]Surgical Photonics & Engineering Laboratory, Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA.

ABSTRACT

INTRODUCTION

Histologic study of peripheral nerve regeneration is primarily based on conventional light microscopy, having a diffraction-limited resolution limit of ~200 nm. While visualization of nano-scale neural appendages is possible using electron microscopy, this approach is tedious and not compatible with intravital techniques. Over the past two decades, several new optical microscopy techniques have allowed imaging of subcellular nanostructures with fluorescent labels at effective resolutions below 40 nm using visible light, minimizing tissue phototoxicity and photodamage, and unleashing a vast potential for live cell imaging. Herein, we demonstrate the utility of super-resolution microscopy in the study of peripheral nerves.

METHODS

Sciatic nerve sections from GFP-variant expressing mice, and primary Schwann cell cultures labelled with mCherry were imaged with super-resolution fluorescence techniques like super-resolution radial fluctuation microscopy (SRRF).

RESULTS

Stain-free super-resolution imaging of axial cryosections of sciatic nerves demonstrated robust visualization of myelinated and unmyelinated axons. Super-resolution imaging of primary Schwann cell cultures demonstrated considerably enhanced resolution of cytoplasmic components.

CONCLUSIONS

The remarkable increase in contrast and structural clarity achievable by means of super-resolution techniques provide the novel capacity for imaging of unmyelinated axon and neuronal appendage morphology by means of stain-free light microscopy.

CONTACT

Iván Coto Hernández, PhD
Massachusetts Eye and Ear Infirmary
Harvard Medical School, Boston, MA, USA
Email: ivan_cotohernandez@meei.harvard.edu

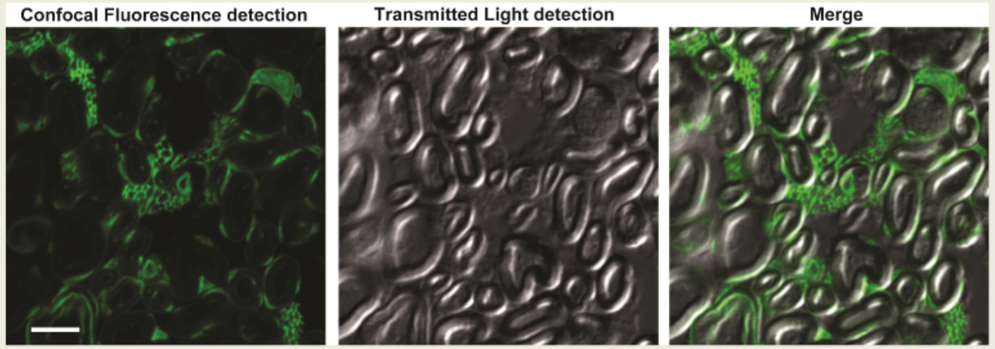

Fig 1. Combination of epifluorescence and transmitted light detection (DIC) on unstained sciatic nerve from Sox10-Venus mice. The pseudo three-dimensional effect enables robust visualization of unstained tissue.

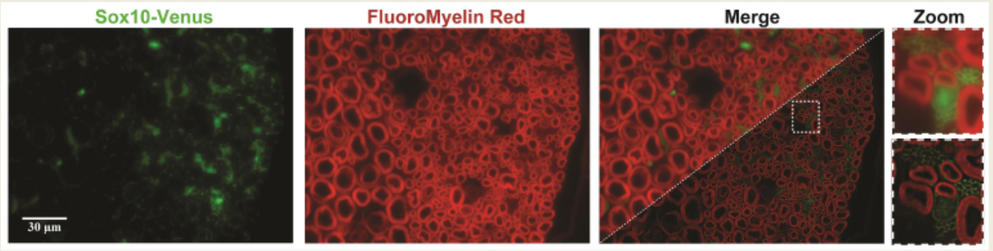

Fig 2. Concurrent visualization of myelinated and unmyelinated fibers of peripheral nerve in Sox10-Venus mice by widefield fluorescence microscopy. Deconvolution enhances image quality (lower right half of *Merge* and lower figure of *Zoom*).

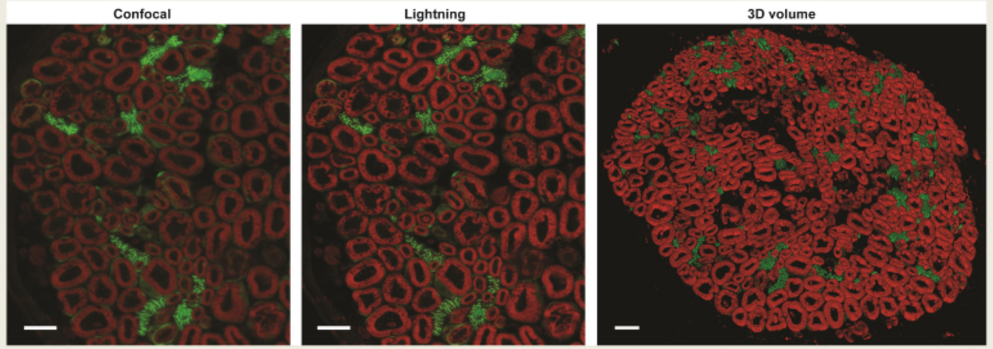

Fig 3. Lightning microscopy enhances contrast, effective resolution and image quality in real-time, allowing high quality volume rendering.

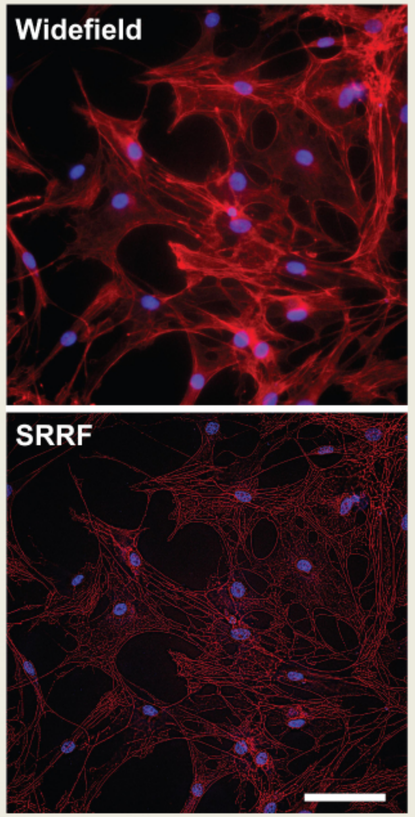

Fig 4. Comparison between conventional and super-resolution imaging of rat Schwann cells expressing cytoplasmic mCherry with a DAPI nuclear stain. Scale bar: 1 µm.

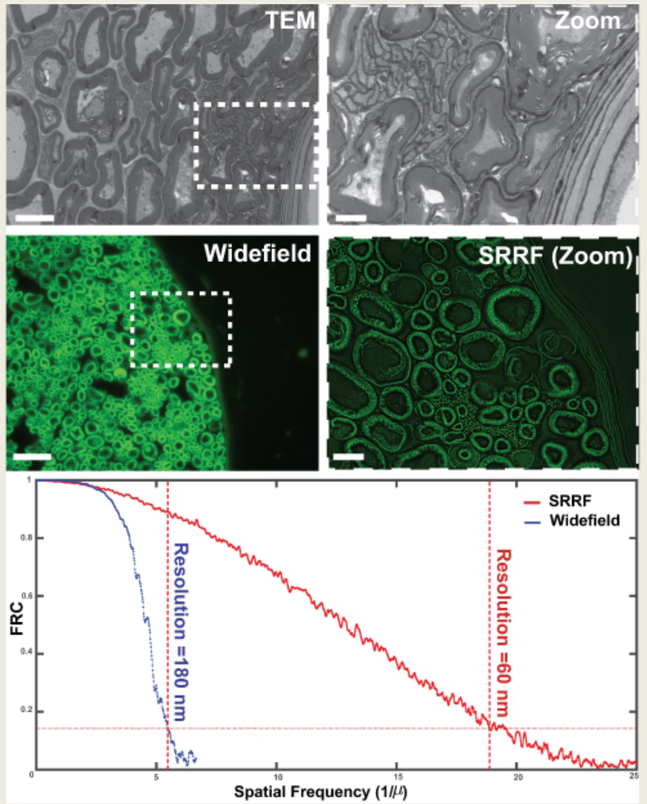

Figure 5. Imaging of sciatic nerves of Sox10 Venus mice using light and electron microscopy (EM). Unmyelinated fibers are better visualized using conventional EM or super-resolution radial fluctuation microscopy (SRRF). Resolution estimation was performed using a Fourier Ring Correlation (FRC).

REFERENCES

1. Shibata S, Yasuda A, Renault-Mihara F, et al. Sox10-Venus mice: a new tool for real-time labeling of neural crest lineage cells and oligodendrocytes. Mol Brain 2010;3:31.
2. Wang W, Kang S, Coto Hernández I, et al. A Rapid Protocol for Intraoperative Assessment of Peripheral Nerve Myelinated Axon Count and its Application to Cross-Facial Nerve Grafting. Plast Reconstr Surg 2018;(in press).
3. N. Gustafsson, S. Culley, G. Ashdown, D. M. Owen, P. M. Pereira, and R. Henriques, "Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations," Nat. Commun., vol. 7, p. 12471, 2016.