Single Molecule Imaging of Axonal Transport in Live Neurons

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Abstract: We report a single molecular imaging method that tracks axonal transport in live neurons, and a super-resolution method, dynamic object tracking that resolves individual microtubules in live neurons below the diffraction barrier.

OCIS Codes: (180.2520) Fluorescence microscopy; (110.2960) Image analysis

Summary:
Neurons are morphologically polarized cells with their axons extending to lengths several orders of magnitude greater than the size of their cell bodies. Hence, the proper function of neurons relies heavily on active transport of materials between the cell bodies and axon termini. To gain insight into the mechanisms governing axonal transport process, we used quantum dot-labeled nerve growth factor (QD-NGF) to track the axonal transport of individual endosomes. Transport of NGF-containing endosomes was imaged using a pseudo total-internal-reflection-fluorescence microscopy. The single molecular sensitivity in live neurons was achieved by designing a microfluidic device that effectively isolates chemical environment of distal axons from that of the cell bodies. With single molecule imaging, we have observed complex phenomena of axonal transport: active movements, frequent pauses and infrequent reversal of direction. Interestingly, quantitative analysis at the single molecular level revealed that the majority of endosomes contained only a single NGF dimer, which has made single molecule detection an essential approach for NGF studies.

Virtually all axonal endosomes are carried to their destination by molecular motors travelling along microtubules, which are the primary structure scaffold of axons and also serve as cytoskeletal "tracks" for axonal transportation. Therefore, it is of great interest to understand the spatial organization of microtubules in live axons. Light diffraction prevents conventional optical microscopy from resolving individual axonal microtubules as there are 10-20 microtubules packed in a mammalian axon of ~1 μm diameter. Using single molecule imaging, we report a super-resolution imaging method, dynamic object tracking (DOT) that resolves individual microtubules in live neurons below the diffraction barrier. DOT imaging uses dynamic trajectories of moving endosomes to accurately depict densely-packed microtubules that are otherwise un-resolvable using conventional optical imaging. We applied DOT imaging to resolve more than six axonal microtubules packed in an axon of 1 μm diameter.