Transport of Intensity Microscopy for Distinguishing Single and Bundled Microtubules

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Microtubules are filamentous protein polymers about 25 nm in diameter, ubiquitous in eukaryotes and providing key functionality in cellular biology. Although microtubules may span many microns in length, their slender width places them well below the diffraction limit. Unlabeled microtubules lack any meaningful absorption of a passing wavefront, and are therefore sub-resolution phase objects.

Fluorescent labeling of tubulin, the building blocks of microtubules, is convenient and specific but fluorescence imaging can lead to photo-toxicity and photo-bleaching [1], and may alter native activity of the polymer. For *in vitro* biophysics experiments, for example in conjunction with optical tweezers, general require extended exposure times and live imaging. Label-free microscopy methods are preferable for such experiments, and a number of these have been demonstrated over the years for imaging microtubules. Video-Enhanced Differential Interference Contrast (VE-DIC) was one of the first label-free microscopy modes demonstrated for visualizing dynamic microtubules and remains in common usage [2].

Investigating a reduced system of microtubules and one or more associated proteins introduces another challenge: proteins that are known to interact with microtubules may understandably interact with more than one filament at once, causing bundling that could confound native activity (see for example Fig. 3 in [3]). If the effect is subtle, an effect due to an increased microtubule surface area availability may be mistaken for a synergistic increase in the microtubule interactions of one or more proteins.

In VE-DIC images, a gold standard for label-free microtubule visualization *in vitro*, it is notoriously difficult to distinguish between filaments containing single or multiple microtubules [1], and in practice the judgement will rely on operator expertise and experience.

By solving the Transport of Intensity Equation (TIE) for image intensities at several closely spaced focal planes, we provide an alternative for visualizing microtubules and detecting multiple microtubule bundles. Fig. 1a is a TIE image of microtubule filaments after several days of co-incubation with Dam1, a microtubule associated protein involved in cell division. In Fig. 1b we see the peak-to-peak TIE signals vary by a factor of about 4, while we saw no such increase for microtubules incubated under the same conditions but without Dam1.

$$k_0 \frac{\partial I}{\partial z} = \nabla(I \nabla(\phi)) \qquad \textbf{(Transport of Intensity Equation)}$$

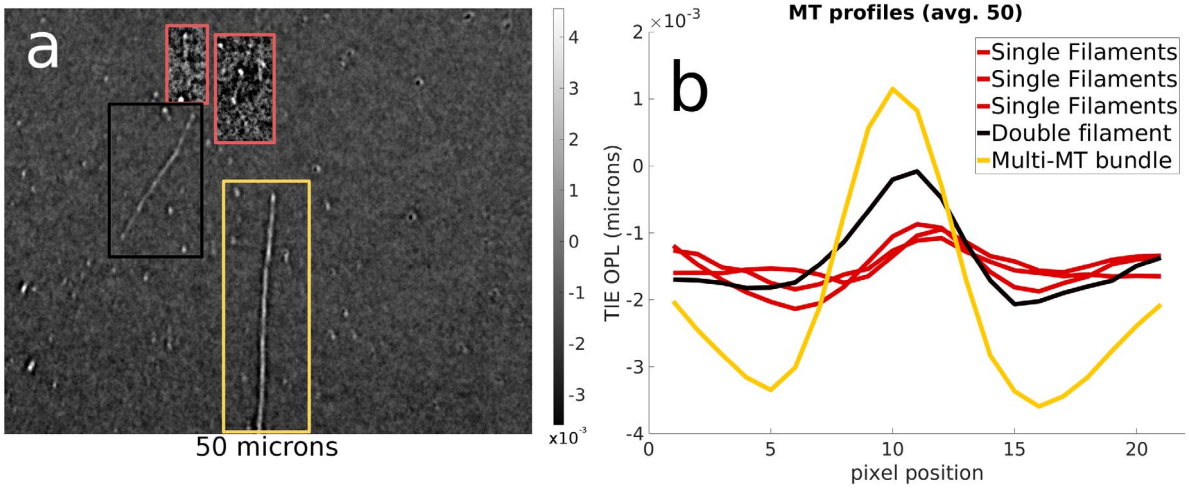

Fig. 1. Stabilized microtubules incubated with Dam1 show discernbile bundling in TIE **i**mages. **a.)** TIE image of microtubules, color bar: microns optical path length **b.)** Profile plots of microtubules in a, averaged over 50 profiles. The pixel values for the single filaments (in red boxes) have been stretched.

References

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