

Enhancement of fluorescence emission difference microscopy using conjugated vortex phase modulation

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We propose and demonstrate an improved method of fluorescence emission difference microscopy (FED). In common sense, subtraction microscopy is regarded as not optimal, mainly because many negative values may be produced in final image, and the higher subtraction factor and the higher resolution, the more artifacts in final image. The proposed method solves this problem to some extent by using a conjugated 0-2 π vortex phase mask to modulate the solid exciting beam in regular FED, to produce an expanded solid spot. The problem of artifact caused by negative value is improved as demonstrated in Fig.1.

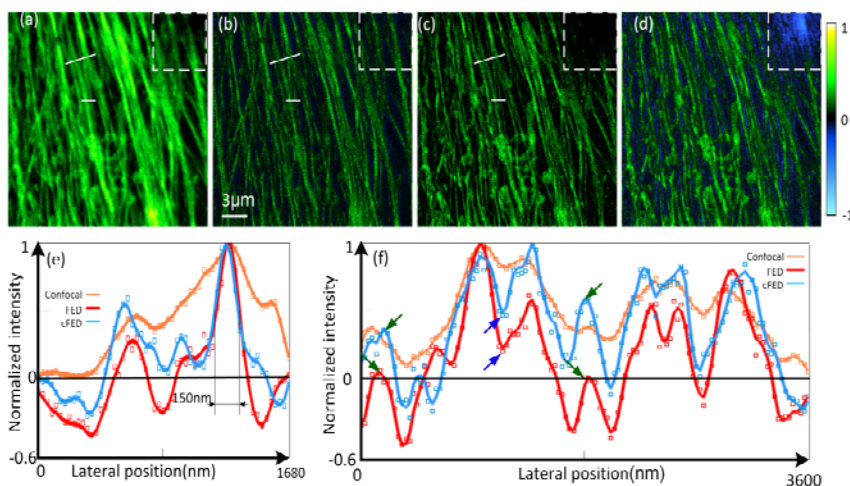


Fig.6 The tubulin experiment results. (a)-(d) Confocal image, cFED image, FED image, FED image with negative value colored, respectively. (e) and (f) the intensity profile of the short and long white line in (a)-(c). All the scale are same shown in (a).

The confocal image, cFED (conjugated vortex FED) image and FED image are shown in Fig. 6. (a), (b) and (c) respectively. Fig. 6. (c) and (d) are both FED images with negative values set to zero in (c) and not set to zero in (d). In cFED image (Fig. 6. (b)) and FED image (Fig. 6. (d)), the negative values are also marked with blue color from dark to light. Comparing the images in Fig. 6, the cFED image may not be as sharp as FED image, however, the artifacts caused by negative

value during subtraction process is much less, especially demonstrated by the dashed white box in the top right corner of images in Fig. 6. In FED image, some sharp but weak signal is removed during the subtraction. As marked by the green arrow in Fig. 6. (f), when a weak signal peak is adjacent to a strong signal peak, it can be weakened greatly after subtraction ($r = 0.7$), which may cause information loss. However, this weak signal peak can be protected and reserved in cFED image (green arrow, blue line, Fig. 6. (f)) even though using a higher r value ($r = 0.85$). Meanwhile, one limitation is that the background cannot be cut down to zero (purple arrow, blue line, Fig. 6. (f)). Under the conditions of $r = 0.7$ in FED and $r = 0.85$ in cFED, the resolution of them are almost the same, as demonstrated by the profile in Fig. 6. (e).