Wide-field Structured Illumination Microscopy with Compressive Optimization Approach

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In a wide-field microscope, the out-of-focus blur and associated noise are of primary concern in obtaining high resolution images from thick samples. Structured illumination microscopy is used to improve lateral resolution and remove the out-of-focus blur from the images.

In structured illumination microscopy (SIM) [1,2], incoherent low frequency patterned illumination is used to achieve better optical sectioning while, coherent patterned illumination is used to obtain improved lateral resolution with the acquisition of multiple raw images that are numerically processed to reconstruct the final image. SIM has become a standard tool for imaging in a wide field optical microscopy applications of thick samples [3].

A structured illumination optical system is proposed to record the wide field images and an algorithm based on compressive method is used to reconstruct the 3D image from a pair of images recorded with uniform and structured illumination. This illumination configuration has the benefit of simplicity in design to be able to incorporate in existing optical microscopes. The numerical computations can be performed on standard desktop machines in reasonable time for visualization of the recorded data.

In the case of thick samples, there remains a problem of effectively removing the out-of-focus blur from images at greater depths within the sample. By recording a pair of uniform illumination image of the sample along with a sinusoidal patterned illumination, we perform a compressive reconstruction of the data based on the total variation (TV) criterion that expresses very well the sparsity of biological samples. Results shown in the Fig.1 demonstrate the effectiveness of our method.

Fig.1. Uniform (a) and structured (b) illuminated images of a mouse kidney sample in a customized wide filed microscope and reconstruction of the final image from a set of 100 images of the pair with the proposed method in (c).

Reference: