

ANNULAR PUPIL FOCAL MODULATION MICROSCOPY

Ke Si¹, Wei Gong², Nanguang Chen^{2,3}, Colin JR Sheppard^{1,2,4,*}

¹ NUS Graduate School for Integrative Sciences & Engineering, National University of Singapore, Singapore 117456

² Division of Bioengineering, National University of Singapore, Singapore 117576

³ Department of Electrical & Computer Engineering, National University of Singapore, Singapore 117576

⁴ Department of Biological Sciences, National University of Singapore, Singapore 117543

Email: kesi@nus.edu.sg

KEY WORDS: Annular pupil, Phase modulation, Super-resolution, Background rejection.

Confocal fluorescence microscopy has a limited penetration depth because the selective detection mechanism by a pinhole is not so effective when the focal point moves deep into the tissue. Recently, our group has successfully combined the angular gate technique with confocal microscopy, and has developed a focal modulation microscopy (FMM) to effectively reject the background signal [1]. Experimental results for chicken cartilage show that the imaging depth of FMM can be extended to around 600 μm [2]. Besides, the transverse resolution is also improved [3]. However, the prototype FMM system uses two non-overlapped D-shaped apertures, one for normal illumination and the other for phase modified illumination, which destroys the spatial resolution symmetry of the system. In this paper, we apply annular pupils into focal modulation to not only maintain the symmetry of the system, but also improve the axial resolution. The capability of background rejection is also enhanced.

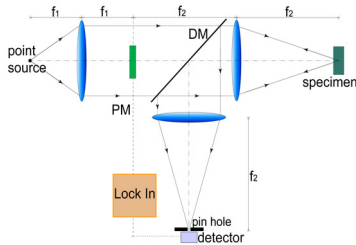


Fig. 1 illustrates a schematic diagram of our prototype experimental setup. Illumination beam is split into two spatially separated beams, and only one half-beam passes through a spatial phase modulator, which causes the two half-beams to have a relative frequency shift. After focusing by the objective lens, the two half-beams interfere to produce an illuminating pattern. The emitted fluorescence signal is processed by lock-in

techniques.

Fig 1: System diagram

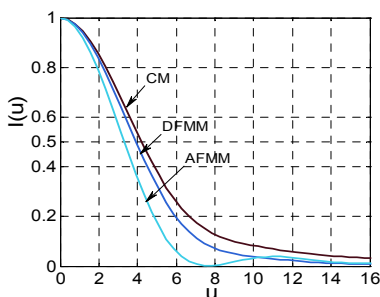


Fig. 2 compares the axial response for a thin fluorescence sheet among confocal microscopy (CM), FMM with D-shaped pupils (DFMM), and FMM with annular pupils (AFMM). It is shown that HWHM of AFMM can be improved by 21.4% and 15.4%, compared with CM and DFMM, respectively.

Reference

- [1]. W. Gong, K. Si, C. J. R. Sheppard, "Improved spatial resolution in fluorescence focal modulation microscopy," *Opt. Lett.* 34. (2009)
- [2]. N.G.Chen, C.H.Wong and C. J. R. Sheppard, "Focal modulation microscopy," *Opt. Exp.* 16, 18764-18769 (2008).
- [3]. K. Si, W. Gong, C. J. R. Sheppard, "Edge enhancement in in-phase focal modulation microscope," *App. Opt.* 48, 6290-6295 (2009).