SIMULTANEOUS MULTI-PLANE FLUORESCENCE CORRELATION SPECTROSCOPY

Shubin Li[1], Zhengyun Zhang[1], Zhi Chen[1], Thorsten Wohland[2], and George Barbastathis[1,3]

[1]Singapore-MIT Alliance for Research and Technology, BioSyM IRG
1 CREATE Way, #04-13/14 Enterprise Wing, Singapore 138602, Singapore
[2]National University of Singapore, Department of Biological Sciences and Chemistry
14 Science Drive 4, Singapore 117557
[3]Massachusetts Institute of Technology, Department of Mechanical Engineering
77 Massachusetts Avenue, Cambridge, MA 02139, USA
E-mail : shubin@smart.mit.edu

KEY WORDS: Fluorescence correlation spectroscopy (FCS), light sheet, volume holography

Fluorescence correlation spectroscopy (FCS) enables studying aggregate dynamics of particles, which in turn reveals biochemical and biophysical information [1]. While imaging FCS improved on traditional FCS by enabling simultaneous 2D capture, scanning is still required for volumetric data. We propose a multi-plane FCS technique using a multiplexed volume hologram (MVH) [2] placed in the infinity section of a microscope to simultaneously capture images at multiple planes, each illuminated by its own light sheet. We note that for *any* simultaneous multi-plane FCS technique, cross-talk between planes cannot be immediately ignored—*i.e.,* the image of one plane will contain out-of-focus contributions from other light sheets. For a two-plane imaging system, the resulting autocorrelation function has two additional terms: the autocorrelation for the out-of-focus plane, and the cross-correlation between the two planes:

$$g(\tau) = \alpha^2 g_1(\tau) + (1-\alpha)^2 g_2(\tau) + 2\alpha(1-\alpha) g_{12}(\tau)$$

where g_1 and g_2 are the autocorrelation functions for the two planes, g_{12} is the cross correlation, and α is the first light sheet's fractional power. The additional terms, owing to the extent of the defocus blur, have a tendency to increase the width of $g(\tau)$ and hence affect retrieved parameters, *e.g.,* the diffusion coefficient D will be underestimated. Experiments using 200 nm fluorescent beads yield a mean diffusion coefficient of 1.8, undershooting the theoretical value of 2.15 as expected. An improved fitting model leveraging the multi-plane correlation function can yield more accurate results, applicable for *any* simultaneous multi-plane FCS technique.

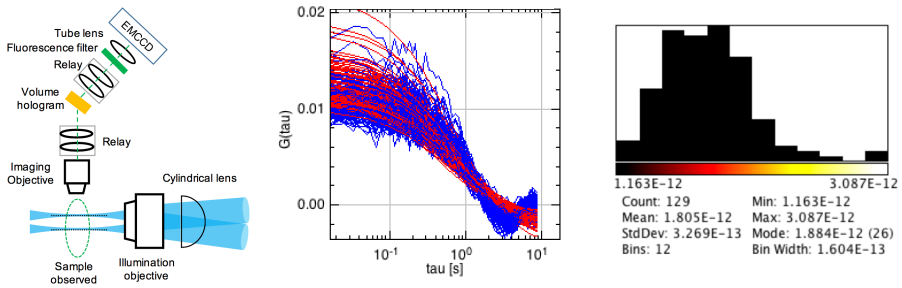

Figure 1: Optical setup is shown on left, with per-pixel $g(\tau)$ shown in centre and histogram of D shown on right.

[1] T. Wohland, X. Shi, J. Sankaran, and E. H. Stelzer, "Single plane illumination fluorescence correlation spectroscopy (spim-fcs) probes inhomogeneous three-dimensional environments," *Opt. Express*, **18(10)**, 10627–10641 (May 2010).

[2] Y. Luo, P. J. Gelsinger, J. K. Barton, G. Barbastathis, and R. K. Kostuk, "Optimization of multiplexed holographic gratings in pq-pmma for spectral-spatial imaging filters," *Opt. Lett.*, **33(6)**, 566–568 (Mar 2008).