STUDYING SLOW MEMBRANE DYNAMICS WITH SCANNING FCS

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Here we demonstrate the capacity of scanning fluorescence correlation spectroscopy for accurate measurements of diffusion and binding parameters on membranes. The high flexibility, reduction of photo bleaching and a remarkable signal to noise ratio combined with an intrinsic robustness against instabilities extend the use of FCS to study extremely slow diffusion as found in yeast cell membranes and permit the application of several FCS-related techniques on membranes. Two focus spatial cross correlation measures diffusion without the need for calibrating the detection volume and therefore greatly enhances the accuracy compared to traditional FCS. Dual color cross correlation with pulsed interleaved excitation (PIE) permits binding studies without the risk of false positive results due to membrane movements or spectral cross talk. The simple implementation in a commercial laser scanning microscope should help establishing scanning FCS as a standard method for membrane studies.