DEVELOPING SCANNING FLUORESCENCE CORRELATION SPECTROSCOPY IN MITOCHONDRIA OF LIVING CELLS

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New methods to quantify dynamics and interactions of intracellular species provide key insights in cell biology. Fluorescence Correlation Spectroscopy (FCS) utilizes temporal autocorrelation of fluorescence fluctuations to study the dynamic properties of labeled molecules. Previous studies characterize diffusion and interaction of proteins in the cytosol and on cell membrane. However, only few deal with tubular organelles like mitochondria [1]. The ability to accurately place the confocal volume in these dynamic organelles limits point FCS in vivo. Originally applied in membranes, scanning FCS (SFCS) addresses some these challenges [2, 3]. SFCS accurately positions the confocal volume by moving it along a linear scanning path. At a scanning orientation perpendicular to the mitochondria, we can also reduce photobleaching due to the brief residence times in the confocal volume. In this study, we looked at the dynamic properties of markers of different mitochondrial compartments.

Figure 1. General scheme of SFCS in mitochondria. (a) The confocal volume is scanned repeatedly perpendicular to a mitochondria. (b) Scan profiles over time produces a pseudo-image (c) Signal from mitochondria is selected and aligned. (d, e) Signals from each scan is used to construct the fluctuation trace, which is correlated and fitted to diffusion models.