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Fluctuation Analysis with the Spinning Disk Confocal Microscope

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Analysis of the fluctuations in time and space of confocal images has the potential to provide information about molecular diffusion and molecular interactions directly in live cells. Fluctuation image analysis has been commonly done in the laser scanning microscope. In the slow regime, when the fluctuations are slower than the frame rate, the time correlation between the same pixel in different frames of an image stack provides all the information about diffusion and brightness. In the fast regime, for example for molecules diffusing in the cytoplasm, the frame rate is too slow to follow the fluctuations due to diffusion. In the raster scan confocal microscope, these fluctuations are detectable because of the correlation of the intensity with the next pixel in the same line or in the next line. In fluctuation spectroscopy an important parameters is the sampling time that must be shorter that the time of the decay of the fluctuation. In the spinning disk confocal microscope, the sampling time at each pixel is very short. However, in the normal data acquisition protocol of the spinning disk microscope the intensity at one pixel is averaged with the intensity at the same pixel after the disk has performed several rotations. In this work we triggered the camera acquisition so that each pixel is visited only once per frame acquired. While we are observing fluctuations due to fast moving bright particles, the fluctuations due to dim particles seem to be buried in the noise of the system. We are investigating the origin of this extra noise and developing methods to characterize it so that it can be properly subtracted.

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