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Mapping Diffusion in a Living Cell using the Phasor Approach

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The diffusion of a fluorescent protein within the cell has been measured by either using the fluctuation based techniques (FCS, RICS) or through particle tracking. However none of these methods enable us to measure the diffusion of the fluorescent particle at each pixel of the image of the cells. Measurement using the conventional single point FCS at each pixel of the image results in the continuous exposure of the cell to the laser and eventual bleaching of the sample.

To overcome this limitation we developed a new method of scanning while constructing fluorescent image of the cell. In this new method of scanning, the intensity trace at each pixel is collected multiple times before it moves to the next pixel. Alternatively, while acquiring the image, the laser scans each individual line multiple times before moving to the next line. This continues until the complete area is scanned. This is different from the RICS approach where the data is acquired by scanning each line once and scanning the image multiple times. The total time of data acquisition needed for this method is shorter than the time required for the traditional FCS analysis. However, a single pixels the time sequence is relatively small, requiring a non-conventional analysis of the correlation function to extract information about the diffusion and the number of molecules.

These intensity data has been analyzed using the phasor approach that was originally created for the analysis of FLIM data. Analysis using this method results in calculation of diffusion constant of the fluorescent species at each pixel of the acquired image, and thus the diffusion map inside the cell can be created. This enables this new technique to distinguish mobility at every pixel of the image.

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