

MAPPING THE MOTION OF PROTEINS *in vivo*

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Various microscopic techniques are used to monitor the dynamics of fluorescent molecules in living organisms. On the fast time scales (sub-ms), fluorescence correlation spectroscopy is often applied to characterize the motion of single molecules. The result of this technique represents an averaged behaviour of molecules at the single probed location, or along the scanned path in the case of scanning fluorescence correlation spectroscopy [1]. Alternatively, particle tracking, which generally requires brighter particles and has a lower temporal resolution, yields the full particle trajectory and preserves the individuality of the tracked particle. If the motion of a large number of particles in an image is correlated, a meaningful flow maps can be reconstructed [2].

Here we are, however, interested in mapping the flow of fluorescent structures where individual particles cannot be clearly discerned. In this case, the flow mapping in a sequence of images is based on the analysis of the temporal changes in the fluorescence intensity pattern. One way to produce the flow map is to divide the image into smaller segments, and to determine the most probable displacement of each segment between subsequent frames by image correlation [3, 2]. This procedure requires sufficiently high signal-to-noise ratio, or averaging over several consecutive frames. The flow vector direction thus obtained is independent in each individual image segment.

Our goal is to find an alternative approach applicable to images with weak signals. It is assumed that the changes of the fluorescence pattern from one frame to another can be described by a smooth transformation expressed analytically and dependent on only a small number of parameters. Since the transformation is continuous across the whole image and has a limited number of degrees of freedom, the flow vectors in neighbouring locations are not independent, and an increase in robustness compared to the correlation techniques is expected. The data were acquired with an imaging system which combines two-photon excitation and photon-counting detection. This results in high sensitivity, low background signal, and allows us to take an advantage of the known well-defined noise described by the Poisson distribution. The method, implemented for one-dimensional data (kymographs) and also for two-dimensional images, was applied to GFP-labelled protein motion on the cortex of living cells.

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