MICROSCOPY

Mapping molecules on the move

Using a sheet of light to perform fluorescence-correlation analysis, scientists can track protein dynamics in entire cellular 'neighborhoods'.

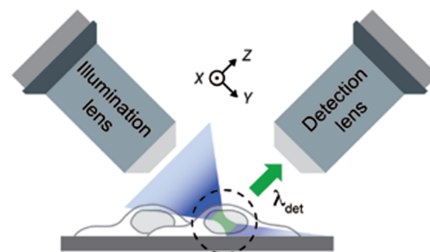
Fluorescence-correlation spectroscopy (FCS) is extremely handy for understanding the comings and goings of proteins or other labeled cellular targets. "Confocal FCS allows you to watch only a tiny area of a cell and observe the fluorescent molecules as they diffuse in and out," explains Michael Knop, of Universität Heidelberg.

However, the confocal platform limits the number of FCS measurements that can be obtained. The single laser illumination spot means that the user is limited to a narrow keyhole perspective rather than getting the 'big picture', and fluorophore bleaching in the vicinity of the illuminated region also constrains the amount of FCS data that can be captured.

Knop, postdoctoral researcher Jérémie Capoulade and Malte Wachsmuth of the European Molecular Biology Laboratory (EMBL) in Heidelberg have now developed an elegant solution that sidesteps these limitations. In their setup, one objective lens projects a narrow light sheet that selectively illuminates only a small section of a sample in a culture dish, thereby restricting the extent of bleaching, while another facilitates fluorescence detection via an extremely sensitive high-speed camera.

The resulting configuration is suitable for high-throughput FCS analysis, generating tens of thousands of frames of pixel-by-pixel data from a 'light pad' with an area of up to 3.8 by 65 square micrometers. "Everybody knows that cells are really complex systems, and that spatial and temporal dimensions matter a lot," says Knop. "Now we can be confident about measurements in a given area because the neighboring values are similar, and we can also map changes across areas."

The researchers gathered valuable functional insights about individual proteins based on the heterogeneities in migration behavior that they observed in different regions of the light pad. In initial tests of the system, they demonstrated the capacity to quantify protein diffusion coefficients and concentrations in cultured cells and even in wing imaginal disks isolated from fruit fly larvae, at depths of up to 30 micrometers.



Schematic of the light-sheet FCS system. A narrow light-sheet from an illumination objective generates a two-dimensional 'light pad' (green region) that is analyzed with a second detection objective attached to a high-speed camera. Image courtesy of J. Capoulade.

They also investigated diffusion of heterochromatin protein 1 (HP1α), a molecule that maintains gene silencing at heterochromatic chromosomal regions, in the nuclei of 3T3 fibroblasts.

Although HP1α is predicted to preferentially associate with heterochromatin, measurements via confocal FCS have revealed a confounding overlap in the apparent range of affinities this protein exhibits for euchromatic versus heterochromatic regions. Light-sheet FCS helped resolve this quandary, revealing that HP1α has distinct behaviors at different euchromatin segments. It appeared to associate strongly with regions neighboring heterochromatin, suggesting that this protein may also be involved in gene regulation at these chromosomal sites.

The motivation to develop this system initially arose from Wachsmuth and Knop's frustrations in attempting to characterize MAP kinase signaling dynamics using confocal FCS, and hopes are high that this platform will take such studies to the next level. "We want to study complex formation among the components of MAP kinase signaling processes in yeast," says Knop. "Not just individual molecules, but the systemic set of all components that govern MAP kinase signaling, and see how they change during the signaling process at a single-cell level and in different cellular areas."

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RESEARCH PAPERS

Capoulade, J. *et al.* Quantitative fluorescence imaging of protein diffusion and interaction in living cells. *Nat. Biotechnol.* **29**, 835–839 (2011).