Large-as-life imaging

Despite the push to image biological specimens and processes at ever-higher resolution, there is also a need to image very large samples at conventional resolutions. Such methods would allow easier investigation of larger scale relationships and variations in organisms. For example, the structure of neuronal pathways in the nervous system could be determined as well as the effects of disease or mutation on the organization of these or other large-scale fine structures. Two papers in this issue describe different advances that expand these possibilities. Stelzer and colleagues describe a way to improve the resolution of single-plane illumination microscopy (SPIM), whereas Dodt and colleagues combine a tissue-clearing procedure with another version of light sheet–based microscopy called ultramicroscopy. Both permit cellular- and subcellular-resolution imaging of very large samples that cannot be imaged by conventional fluorescence microscopy.

Brief Communication p311, Article p331, News & Views p307

Playing tag with \(\alpha\)-synuclein

Several degenerative neurological diseases are characterized by the accumulation of amyloid protein aggregates. Despite the detailed information that fluorescence imaging can provide on such dynamic processes in living cells, tagging the proteins involved without affecting the processes under study has proven to be difficult. Jovin and colleagues report that tetracysteine labeling of \(\alpha\)-synuclein, the major component of amyloid fibrils and plaques observed in Parkinson disease, does not affect protein function. They show that tetracysteine-mediated labeling of \(\alpha\)-synuclein with FlAsH and ReAsH allows fluorescence imaging of the dynamics of aggregation in living cells.

Article p345

esiRNAs go to the big screen

Genome wide screens with small interfering RNAs (siRNAs) yield a plethora of biological data, but their interpretation is often complicated by issues of specificity and efficacy, not to mention the high cost of synthetically generated siRNAs for a large-scale RNAi screen. Buchholz and colleagues now show that more cost-effective siRNAs, enzymatically generated from cDNA (esiRNAs), also perform well in large screens. To ensure specificity, they developed software to identify the region in the cDNA most likely to yield esiRNAs with high knockdown capabilities and fewer off-target effects. They determined these regions for the protein-coding genes of human, mouse and rat, and showed that when the methodological pieces of target selection and esiRNA generation are put together, the resulting large-scale screen is on par with screens performed with synthetically generated siRNAs—at a fraction of the cost.

Article p337, News & Views p308

Single-molecule measurements pore in

Single-molecule measurements provide detailed mechanistic information about protein function that is often hidden in ensemble measurements. Unfortunately, single-molecule measurements often take a great deal of time to complete and are not amenable to scaling. This makes replicate measurements time consuming. Akeson and colleagues now show how nanopores can be used to obtain many simultaneous single-molecule measurements. In particular they examine DNA-protein interactions by threading individual ssDNA-protein complexes through protein nanopores in a lipid bilayer and measuring the force required to dissociate the complex when a voltage is applied across the membrane.

Brief Communication p315

Culturing cells in 3D

There is nothing like the natural environment for cells to thrive and maintain phenotypic and signaling integrity. When scientists study cells grown in two dimensional cultures, important cues are lost and the information gleaned is at best diminished and at worst artifactual. To introduce a robust 3D culturing method, which mimics the natural milieu of cells, Bissell and colleagues present a step-by-step protocol to culture malignant and normal breast epithelial cells in a 3D model of laminin-rich extracellular matrix. The authors offer several options for culturing cells in three dimensions, such as an embedded assay or an ‘on-top’ assay, so that end users will be able to choose the method that best meets their experimental needs.

Protocol p359