DIGITAL SLIDE ORGANIZER

Tools for enhancing reproducibility in life science experiments are much in demand at the moment. When it comes to microscopy imaging, organizing and analyzing the data generated during experiments can be a daunting task, even for smaller data sets. In this issue of *BioTechniques*, Benjamin Nanes describes his development of a new software solution called Slide Set. This new tool enables batch processing and analysis of microscopy images while also allowing interactive region-of-interest segmentation, which is more effective for many small- to medium-sized data sets with higher signal-to-noise ratios than the automated segmentation used in existing high-throughput workflows. By organizing imaging data into tables, where image files can be associated with metadata, and then enabling analysis commands to be repeated across different data sets, Slide Set creates an automated solution for image analysis at any scale that can be employed in most labs. Slide Set also allows for the integration of other ImageJ-based plugins, creating an effective, reproducible software solution for your batch image processing needs.

See “Slide Set: Reproducible image analysis and batch processing with ImageJ” on page 269.

SQUASHING IMAGE QUANTIFICATION PROBLEMS

When it comes to quantifying subcellular objects and their localization, there are a number of software tools that have been described in recent years. One of these is Squassh, a software solution that enables automated detection, segmentation, and quantification of subcellular structures as well as analysis of co-localization patterns. In this issue of *BioTechniques*, the developers of the original Squassh tool describe two enhanced modules for this analysis platform: Squash3C and SquashAnalyst. Squash3C extends the functionality of the first Squassh program by enabling analysis of three-channel fluorescence data sets as well as live-cell movies. But the bigger advance presented in this article could be in SquashAnalyst, which provides users an interactive web interface for analysis of their Squassh data sets. From segmentation and figure generation to object filtering and statistical significance testing, SquashAnalyst offers a clean, accessible tool that simplifies the task of quantifying subcellular objects in microscopy images.

See “Subcellular object quantification with Squash3C and SquashAnalyst” on page 309.

Selected and written by Nathan S. Blow, Ph.D.

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INFORMATIVE MICROBIAL COMMUNITY ANALYSIS

High-throughput sequencing has yielded rich taxonomic profiles of microbial communities in a culture-independent manner. Sequencing can also be used for functional profiling by identifying the presence of particular genes, but current methods to link phylogeny and function are unwieldy. In response, Spencer et al. offer “epicPCR” (emulsion, paired isolation, and concatenation PCR). Bacterial cells are emulsified in oil in the presence of acrylamide, which is polymerized to produce beads that contain no more than one cell each. When embedded cells are lysed, the genomes remain sequestered within the bead. The amplification step, also performed in an emulsion, is a fusion PCR combining phylogenetically informative 16S rRNA sequences and target functional sequences into one amplicon. The sequence readout thus reveals who in the microbial community is doing what. The authors show the utility of epicPCR by identifying sulfate-reducing prokaryotes in anoxic lakewater samples. They also propose a strategy that could be further optimized to provide quantitative data.

S.J. Spencer et al. Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers. ISME J. [Epub ahead of print, September 22, 2015; doi:10.1038/ismej.2015.124].

LIGHTING UP NUCLEOCYTOPLASMIC LOCALIZATION

Red light triggers phytochrome B (PhyB) to associate with phytochrome interacting factor (PIF), while far-red illumination reverses the interaction. The lesser toxicity and deeper tissue penetration of these longer wavelengths make PhyB-PIF attractive for optogenetics. Now, Beyer et al. have adapted this system to reversibly control nucleocytoplasmic localization. First, the authors confirmed in metazoan cells that the interaction of these *Arabidopsis* proteins leads to PIF-mediated nuclear import. Next they showed that if PhyB is expressed as a fusion protein with the protein of interest (e.g., a transcription factor) and a nuclear export signal, switching from red to far-red light leads to nuclear import and then exit (a cycle that can occur repeatedly). While the system requires an exogenous cofactor, this has the advantage of giving an additional option for temporal control. The authors conclude by showing that PhyB-PIF works in zebrafish, making it the first plant phytochrome-based optogenetics tool for vertebrates.

H.M. Beyer et al. 2015. Red light-regulated reversible nuclear localization of proteins in mammalian cells and zebrafish. ACS Synth Biol. 2015 Sep 18;4(9):951-8.

Selected and written by Nijsje Dorman, Ph.D.

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