CELLS SETTLE DOWN FOR STAINING

Cultured cells must be attached to a surface—usually glass or plastic—prior to immunofluorescent staining. Adherent cell lines can be attached by growing them on these surfaces, but suspension cell lines need to be deliberately adhered, either by coating the surface with a charged macromolecule such as poly-L-lysine to induce cell attachment or by cytocentrifugation, where the cells are centrifuged onto the surface of a slide attached to a special apparatus. However, these approaches incur extra time and cost, and cytocentrifugation can also cause cell deformations. In this issue of *BioTechniques*, a team led by Ivan V. Litvinov at McGill University describe a simple and gentle method for adhering suspension cells to glass coverslips or plastic plate well bottoms. The cells are suspended in PBS and added to the wells of a 12-well plate—with or without a glass coverslip at the bottom of each well—then left for 30 min to allow settling and attachment of the cells by gravity sedimentation onto the coverslip or well bottom, after which they can be immunofluorescently stained without detaching from the surface.

See “Protocol for adhesion and immunostaining of lymphocytes and other non-adherent cells in culture” on page 230.

SPHEROID VOLUME DETERMINATION

3-D multicellular cancer spheroids are now preferred over 2-D cell cultures for cancer drug screening, since they better reflect the in vivo physiology of tumors. A direct indicator of the anticancer effect of a candidate drug is a reduction in the size of the cancer spheroid. Given that most spheroids are not perfectly spherical, size is best measured using a spheroid's volume rather than its diameter. For volume determination, confocal and light sheet fluorescence microscopes can be used to image the cancer spheroids and produce a z-stack of optical sections. ReViMS, a new software tool described in this month's issue of *BioTechniques* by Filippo Piccinini and his colleagues, then uses this z-stack to estimate the volume of the spheroid. Unlike other existing programs with similar functionality, ReViMS is user-friendly and freely available. Written in MATLAB, the program segments each fluorescent image in the z-stack and then reconstructs the volume of the spheroid. The method demonstrated high accuracy and precision when tested on cancer spheroids that had each been imaged from three different angles.

See “ReViMS: Software tool for estimating the volumes of 3-D multicellular spheroids imaged using a light sheet fluorescence microscope” on page 227.

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FOLLOW THE BLINKING LIGHT

Single-molecule fluorescence in situ hybridization (smFISH) is used for imaging RNA transcripts in single cells. The technique requires the use of multiple fluorescent probes targeting a single RNA molecule to enhance the signal-to-noise ratio and identify true positive signals from background fluorescence. Differentiating true signals from background, however, remains challenging in smFISH experiments. To solve this problem, Cui et al. suggest an alternative to smFISH that uses fluctuations in photoswitchable dyes to set quantitative thresholds for determining true from false signals. The technique, dubbed fluctuation localization imaging-based FISH (fliFISH,) relies on identifying the on-time (blinking) fraction of each probe. As more probes bind to a specific RNA molecule, the ensemble on-time fraction increases in a predictable way that is different from autofluorescence or unbound probes. Using fliFISH, the authors successfully assessed the gene expression pattern of a transcription factor in mouse pancreatic islets.

Cui, Y. et al. 2017. Fluctuation localization imaging-based fluorescence in situ hybridization (fliFISH) for accurate detection and counting of RNA copies in single cells. *Nucleic Acids Res.* doi: 10.1093/nar/gkx874. [Epub ahead of print]

ATTRACTIVE PROTEIN PURIFICATION

From restriction enzymes to CRISPR/Cas9, methods developers often repurpose naturally occurring molecules as molecular tools. Jiang et al. follow this trend by adapting a recently discovered magnetic protein for use in a one-step protein purification protocol. While the magnetic protein in question may form the molecular basis of magnetoreception in animals, in the hands of Jiang and co-authors, fusion of this protein to a specific protein of interest allows for isolation and purification using iron beads. Following expression of a magnetic protein/target protein fusion in cells, the fusion protein can be absorbed by the iron beads, which can be separated from the solution. The magnetic/target fusion protein is then detached from the bead, and the target protein is cleaved away from magnetic protein. To demonstrate the robustness of this magnetic protein purification scheme, the authors isolated GFP as well as three other enzymes with differing molecular sizes that had all been fused to the magnetic protein for purification.

Jiang, M. et al. 2017. Novel application of magnetic protein: Convenient one-step purification and immobilization of proteins. *Sci Rep.* 7:13329.

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

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