SOCR 3D Cell Morphometry Project

Sample preparation protocols **[1,2]**

1. Fibroblast sample preparation protocol (Michigan)

Fibroblasts (newborn male) were purchased from ATCC (BJ Fibroblasts CRL-2522 normal) and subjected to a G0/G1 Serum Starvation Protocol. They were recovered from cryogenic storage with growth in full media (MEM + 10% FBS Sigma-Aldrich + 1% MEM NEA Gibco Lot 1656019 + 1% Antibiotics Gibco Lot 1523692) for 48 hours. This was followed by a change to serum free media (0.1% FBS) for 4 days, followed by resuspension and growth on coverslips in serum free media for 24hrs. Aliquots were taken for fixation every 2hrs for 12hrs.

2. PC3 sample preparation protocol (JHU)

PC3 EPI /EMT slides were prepared as follows:
1. 40K cells were seeded on slides and grown for 48 hours @ 37C, 5% CO2, and 90% RH
2. slides were washed in PBS @ RT
3. slides were fixed in 4% paraformaldehyde for 30 minutes @ RT
4. slides were washed in PBS @ RT
5. slides were dehydrated in 50% EtOH for 5 minutes @ RT
6. slides were dehydrated in 50% EtOH for 5 minutes @ RT
7. slides were dehydrated in 70% EtOH for 5 minutes @ RT
8. slides were dehydrated in 100% EtOH for 10 minutes @ RT
9. slides were allowed to air dry @ RT.

3. Staining protocol

Before use, cells were rehydrated in descending ethanol concentration washes, 5mins each. Staining proceeded according to the following methods for each stain,with stains for up to five features applied jointly to the same sample in different colors to be imaged on different fluorescent channels:

1. Prolong Gold antifade reagent with DAPI (4’,6-diamidino-2-phenylindole) (In-vitrogen, Lot 168129) was applied to all samples according to manufacturer protocol to image the nucleus as a whole.
2. For EtBr imaging of nucleoli, Ethidium Bromide was applied by application of5ul of EtBr working suspension onto a wet coverslip for 20 seconds, followed by a PBS wash, immediately prior to the application of DAPI.

3. For fibrillarin imaging of the nucleoli, antifibrillarin Alexa 448 label was purchased from ABCAM (EPR10823(B) Lot GR175169-1) and applied according to the manufacturer's protocol prior to the application of DAPI.

References:

[1] Alexandr A. Kalinin, Ari Allyn-Feuer, Alex Ade, Gordon-Victor Fon, Walter Meixner, David Dilworth, Jeffrey R. de Wet, Gerald A. Higgins, Gen Zheng, Amy Creekmore, John W. Wiley, James E. Verdone, Robert W. Veltri, Kenneth J. Pienta, Donald S. Coffey, Brian D. Athey, Ivo D. Dinov (2018) "3D Cell Nuclear Morphology: Microscopy Imaging Dataset and Voxel-Based Morphometry Classification Results". *2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition Workshops*, Salt Lake City, UT, June 18-22, 2018, pp. 2272-2280. url:CVPR 2018 open access

[2] Alexandr A. Kalinin, Ari Allyn-Feuer, Alex Ade, Gordon-Victor Fon, Walter Meixner, David Dilworth, Syed S. Husain, Jeffrey R. de Wet, Gerald A. Higgins, Gen Zheng, Amy Creekmore, John W. Wiley, James E. Verdone, Robert W. Veltri, Kenneth J. Pienta, Donald S. Coffey, Brian D. Athey, Ivo D. Dinov (2018) "3D Shape Modeling for Cell Nuclear Morphological Analysis and Classification". *Scientific reports*, 8. doi:10.1038/s41598-018-31924-2