L12 Visualization of cytoskeletal filaments with super-resolution structured illumination microscopy (SIM)

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Duration of the experiment: 60 minutes
Max. number of participants: 4
Location: Cell Culture Laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). No prior experience with laboratory work is required. Basic skills in using chemical/biological lab equipment (e.g., pipettes, analytical balances) are helpful but not mandatory.

The aim of this laboratory practice is to perform structured illumination microscopy (SIM) of cytoskeletal filaments and determine the resolution of acquired images.

THEORETICAL BACKGROUND

Structured illumination microscopy (SIM) is a method of super-resolution microscopy that bypasses the diffraction limit, a physical barrier that restricts the optical resolution to roughly 250 nm laterally and 500–800 nm axially. SIM achieves super-resolution by illuminating the sample with an interference pattern which interacts with sample high frequency features (otherwise non-resolvable details) and produces larger-scale interferences or Moire fringes. Mathematical reconstruction of high-frequency information from a series of images taken at different angles results in a wavelength dependent resolution of 100–130 nm laterally and 300–400 nm axially [1–3]. In comparison with other super-resolution microscopy techniques, SIM imaging is fast and uses low light intensity producing little phototoxicity. SIM can also image multiple colors and uses conventional fluorophores [2].
Cytoskeleton is a network of protein filaments that give cells shape and supports their function. The filaments are attached to the cell membrane and stretch across the cell. Application of short, high-voltage electric pulses to cells results in structural and chemical changes which can also induce cytoskeleton disorganization [4,5]. Not all cytoskeletal filaments are equally affected by electric pulses. The intermediate filaments (keratin, vimentin) are more resistant to pulses, while thin filaments (actin) and microtubules are prone to fragmenting, buckling, and depolymerizing [5,6].

EXPERIMENT

Visualization of cytoskeletal filaments will be performed on selected samples labelled with antibodies and/or fluorescent proteins. The labeling of cytoskeletal filaments and mounting of cell samples are a time-consuming procedure and will be therefore performed ahead of the workshop. The labeling protocol will be available on the spot.
The cytoskeletal filaments will be imaged on Elyra 7 (Zeiss) in Lattice SIM configuration. After taking raw images, each pair of participants will analyze the images on computers in Cell lab 1, using ZEN black software (Zeiss).

Protocol for image analysis:
Open ZEN black software and load raw images. Reconstruct SIM images by adjusting SIM Method parameters to Standard and clicking Apply. Evaluation of resolution will be performed in two ways. First,

choose a thin single filament. Open Profile tab and draw a line across the filament. Measure the full width at half maximum (FWHM) of the filament by aligning the markers to left and right half maximum intensity of the filament signal. Write down the measured distance. Second, find two neighboring filaments. As in the preceding task, draw a profile line. Align the markers in the center of filament intensities and write down the measured distance. Compare the two measured values.

REFERENCES:

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[4] Kotnik T., Rems L., Tarek M., Miklavčič D. Membrane Electroporation and electropermeabilization: mechanisms and models. *Annu Rev Biophys*, 48:63-91, 2019.

[5] Graybill P.M., Davalos R.V. Cytoskeletal disruption after electroporation and its significance to pulsed electric field therapies. *Cancers*, 12:1132, 2020.

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EXPECTED RESULTS

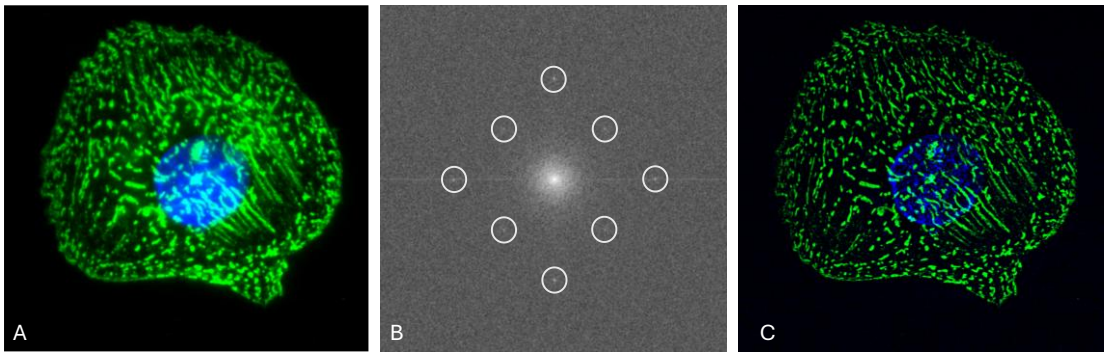

Figure 1: SIM reconstruction of fluorescently labeled α-actinin (green) and nuclei (blue) in neonatal cardiomyocytes in culture. (A) Maximum intensity projection of raw images taken in LatticeSIM configuration (63x objective, NA 1.4). (B) Fourier transform of one of the channels from image A. Note eight dots (encircled) surrounding the central point. These are the high-frequency details. (C) SIM reconstruction of image A resulting in high resolution image.

NOTES & RESULTS