

Resource Summary Report

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Preparation of human Red Blood Cells for confocal imaging

DOI:10.17504/protocols.io.yjyfupw

Type: Protocol

Proper Citation

Marie Anne Balanant 2019. Preparation of human Red Blood Cells for confocal imaging. protocols.io dx.doi.org/10.17504/protocols.io.yjyfupw

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.yjyfupw>

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Summary: This protocol is used to label red blood cells (RBC) for confocal imaging. Fluorescent beads are used to calibrate voxel size on the confocal microscope. The imaging protocol was optimised to obtain high quality data, by mounting the cells in a high refractive index and optimising acquisition parameters. Spherical aberration results in a decrease in image intensity when the imaging plan gets further away from the coverslip. It is caused by difference in refractive indices between the different components present over the beam pathway. In order to reduce spherical aberration, the cells are fixed in glutaraldehyde after staining so they can be resuspended in a medium with a high glycerol content and maintain their shape. Glycerol increases the reflective index of the medium, bringing it closer to the reflective indices of the coverslip and the immersion oil. Acquisition parameters are first chosen using the system optimisation function, then adjusted. The z-axis step size is chosen as close as possible to the lateral resolution to avoid under- or over-sampling. The pinhole is reduced to 50 μm to increase resolution and reduce out-of-focus light. A 2-step line averaging is selected during scanning to remove noise. Data is analysed and quantitative volume and surface area values were extracted using a home-written matlab code (not included in this protocol).

Associated Publications: Geekiyanage NM, Balanant MA, Sauret E, Saha S, Flower R, Lim CT, Gu Y (2019) A coarse-grained red blood cell membrane model to study stomatocyte-discocyte-echinocyte morphologies. PLoS ONE 14(4): e0215447. doi: [10.1371/journal.pone.0215447](https://doi.org/10.1371/journal.pone.0215447)

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