



Example data set: MitoVolumeFraction1.zip

Pipelines used: Mitochondria:cell volume fractionator (basic)

Protocol used: http://help.imageanalyst.net/protocols_MitochondrialVolumeFractionator_Zen_v3.html

QUICK START GUIDE

1. Unzip contents of MitoVolumeFraction1.zip onto your hard drive.
2. Open the recording using the File/"Open Image Series/Measurement" or the  toolbar button. In the Open Image/Measurement dialog select Zeiss Multi Time Lapse LSM (*_Sum.lsm) as file type. Select "All_volumefraction01_Sum1_Subset.lsm". (Don't press Open).
3. Select the "Mitochondria:cell volume fractionator (basic)" pipeline in the Pipelines/"Morphological Measurements"/Applications main menu point.
4. The default parameters of the pipeline have been configured to work with this particular recording.
5. Press the  button on the main toolbar or in the bottom of the Multi-Dimensional Open dialog.
6. The pipeline will automatically load a template spreadsheet, fill in the data and calculate the volume fraction.
7. Save the contents of the Excel Data Window using the File/Save Excel Data main menu.

THE EXAMPLE DATA SET

Sample: Human non-diabetic primary β -cells in a coverglass bottomed 96-well plate (Corning 4580). Published in Endocrinology 2015 doi:10.1210/en.2015-1552.

Fluorophores:

- Channel (1): calcein AM (0.75 μ M for 30 min) (488 nm excitation)
- Channel (2): MitoTracker Red CMX (0.75 nM for 30 min) (561 nm excitation)

Microscope: Zeiss LSM780 with 488nm Ar laser and 561 diode laser, spectral detector, Definite focus

Experimental paradigm: http://help.imageanalyst.net/protocols_MitochondrialVolumeFractionator_Zen_v3.html, using the "Automated image acquisition at manually selected coordinates for sparse and small cells" section

THE PIPELINE: MITOCHONDRIA:CELL VOLUME FRACTIONATOR (BASIC)

Analysis of Mitochondria:cell volume fraction from confocal microscopic recordings of a cytosolic or cellular stain (e.g. Calcein-AM) and a mitochondrial stain (MitoTracker Red).

The algorithm has been calibrated to electron microscopy, if using a red fluorescent probe for mitochondria (e.g. MitoTracker Red CMX), the pixel size is ~44nm and the pinhole was set to ~ 1 Airy unit for recording the images. Smaller resolution will result overestimation of the volume fraction. For image acquisition please follow protocols given at <http://help.imageanalyst.net/protocols.html>.

The volume fraction is given by the 2/3 times the ratio of the sum of mitochondrial pixels over the sum of all cellular (including mitochondrial) pixels, where the sum is calculated for all recorded images. The sum and ratio calculation takes place in an Excel template.

The algorithm is a simplified version of the one described in J Physiol. 2012 590:12 2845-71. Handling of saturation and spectral unmixing has been removed compared to the original version (Mitochondria:cell volume fractionator (original)).

Note: The "Sensitivity scaling for the cytosolic stain (percentile)" may need to be adjusted. A lower value (slightly below 100) increases sensitivity. And estimates bigger cell size. Cells aggregating the probe in lysosomes need a lower percentile value to select cells and not lysosomes.

Keywords: volume fraction, volume density, mitochondrial biogenesis, mitochondrial abundance, mitochondrial mass, amounts of mitochondria, confocal microscopic stereology, mitochondrial membrane potential measurement