Automated Segmentation of Fluorescence Microscopy Images for 3D Cell Detection in human-derived Cardiospheres

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Supplementary Information

CellProfiler pipeline

The pipeline implemented in CellProfiler is the same one used for cell segmentation in a previous work (Buggenthin et al, *An automatic method for robust and fast cell detection in bright field images from high-throughput microscopy*, BMC bioinformatics, 2013, 14.1: 297). The following modules were sequentially called for each image: Correct Illumination (Gaussian filter, Average object size: 60 px), Apply threshold (Otsu global), Identify primary objects (Typical diameter of cells: 5 to 50, splitting method: Intensity, method to draw dividing lines: Shape), Convert objects to image (saved binary mask).

Fiji algorithm

The semi-automatic pipeline implemented in Fiji consisted of: (i) conversion of RGB image into grayscale, (ii) manual intensity thresholding, (iii) hole filling and (iv) small particles removal (all objects with area lower than 10 μm^2 are deleted). For the nuclei segmentation, here an additional step was included in the analysis: (v) automatic cell separation based on watershed transform (Beucher and Mayer, *The morphological approach to segmentation: the watershed transformation*, Optical Engineering-New York-Marcel Dekker Incorporated, 1992, 34: 433-433).