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Instructions for CellProfiler™ Cell Image Analysis Software* to Analyze Data from Radius™ Cell Migration Assays ***Calcein AM***

The following instructions and figures are provided as a guide to aid in Radius™ Cell Migration Assay data quantitation. They are designed to analyze single, 10X magnification images that have been stained with the Calcein AM provided in the Radius™ Cell Migration Assay Kit. For each image to be analyzed the image file name must be entered, and the manual threshold value must be optimized, which may require some trial and error. Please refer to the CellProfiler™ manual for more detailed instructions in its use and customization at http://cellprofiler.org. Please contact Cell Biolabs with any questions at tech@cellbiolabs.com.

1. Create and name a new folder (e.g. Radius™ Calcein AM) and copy over the files you downloaded along with these instructions (*3 Sample Calcein AM image files and the Radius Assay Calcein AM.cp file*).
2. Download and install version 2.1.1 of the CellProfiler™ Cell Image Analysis Software: https://cellprofiler.org/previous-releases.
3. Launch the CellProfiler™ software.
4. Load the custom pipeline file by doing one of the following:
   - Click ***File > Import > Pipeline from File...*** Browse to the file *Radius Assay Calcein AM.cp* in the folder created in step 1, then click "Open".
5. A window will open prompting to Convert Legacy Pipeline. Select "Don't Convert".
6. Click the View Output Settings button (lower left corner of the main window) to set both the Default Input and Output Folder paths to the folder containing the images you want to analyze (for sample images refer to the folder created in step 1).
7. Select the LoadImages Module (upper left under Analysis Modules)
   - In the right settings window, set the "*Input Image File Location"* to Default Input Folder (*containing the 3 Sample Calcein AM image files).*
   - Also in the right settings window, enter the image name into the field "*Text that these images have in common"*. Enter the file name to be analyzed exactly as it appears in the window, including extension (e.g. Sample Calcein AM 1.jpg, Sample Calcein AM 2.jpg, Sample Calcein AM 3.jpg). You will need to type it, as the CellProfiler program does not have a browse feature for this step.
     Note: This step will need to be done for each image to be analyzed.
8. Select the IdentifyPrimaryObjects Module (upper left under Analysis Modules)
   - In the right settings window, enter the threshold value into the "*Manual threshold"* field.
     Note: This will need to be optimized for each image. Values may range from 0-1 and require some manipulation to determine the best fit. For the Sample Calcein AM Images 1, 2 and 3 we provided, use a manual threshold of 0.96, 0.85 and 0.96 respectively.
9. Select the Analyze Images button from the bottom left. An analysis summary window will appear.
   Note: The image part of the analysis summary may be saved as an image file by clicking *File > Save > Save as .png File.* The table part of the analysis summary may be saved as an Excel file by clicking *File > Save Table > Save as .csv File.*

*CellProfiler™ cell image analysis software is a free program owned and offered by the Broad Institute. There is no relationship between Cell Biolabs, Inc and the Broad Institute. Cell Biolabs offers these instructions as a courtesy to our customers who wish to analyze data obtained using our Radius™ Cell Migration Assays.

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Examples of Results

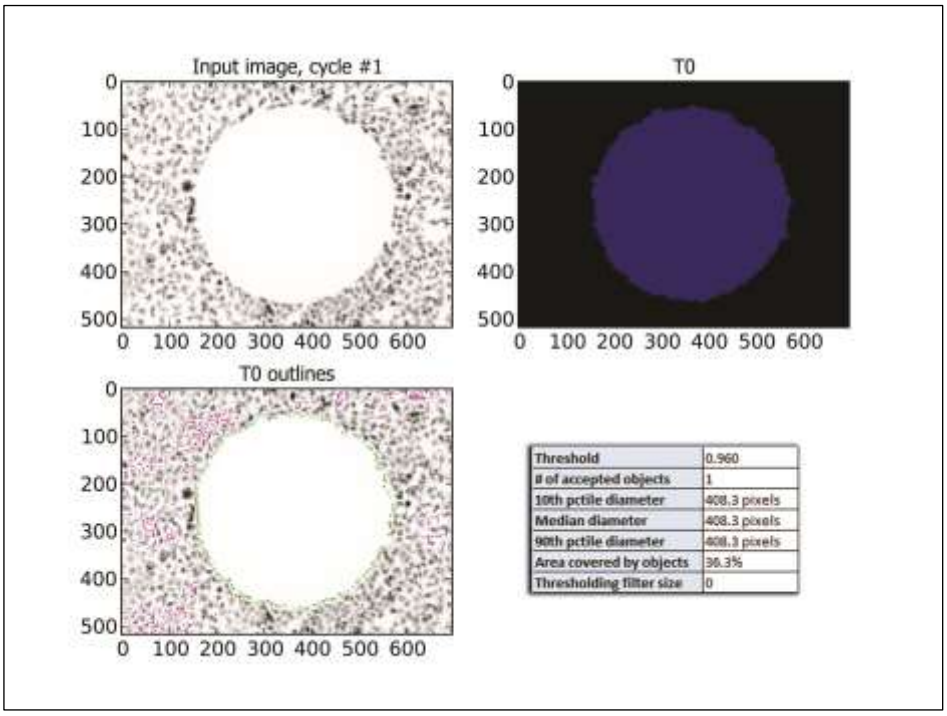

Figure 1: Sample Calcein AM 1.jpg analyzed with manual threshold value of 0.96.

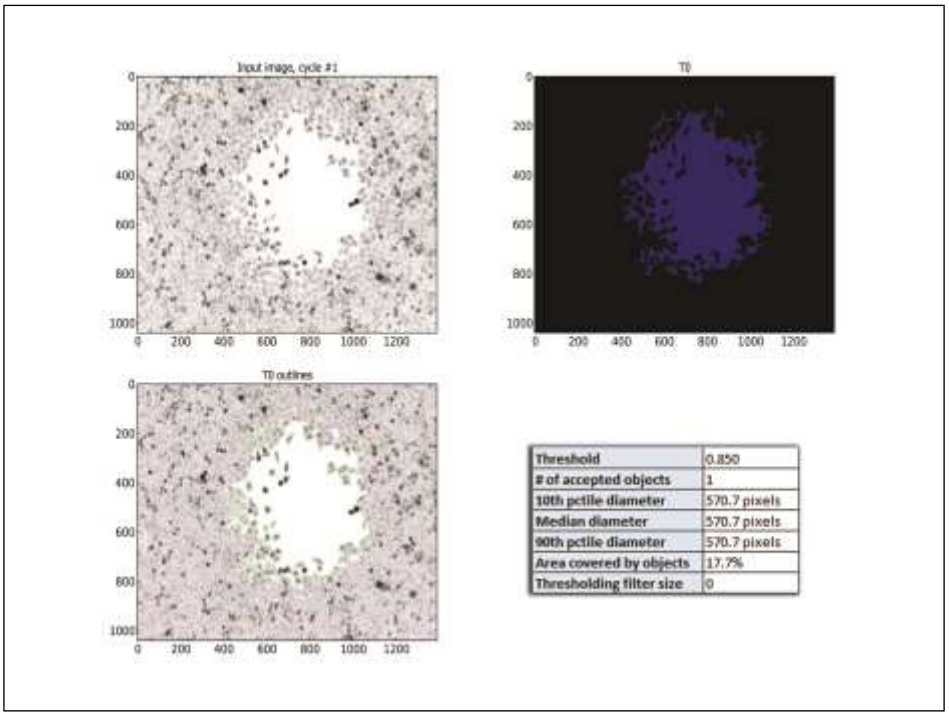

Figure 2: Sample Calcein AM 2.jpg analyzed with manual threshold value of 0.85.

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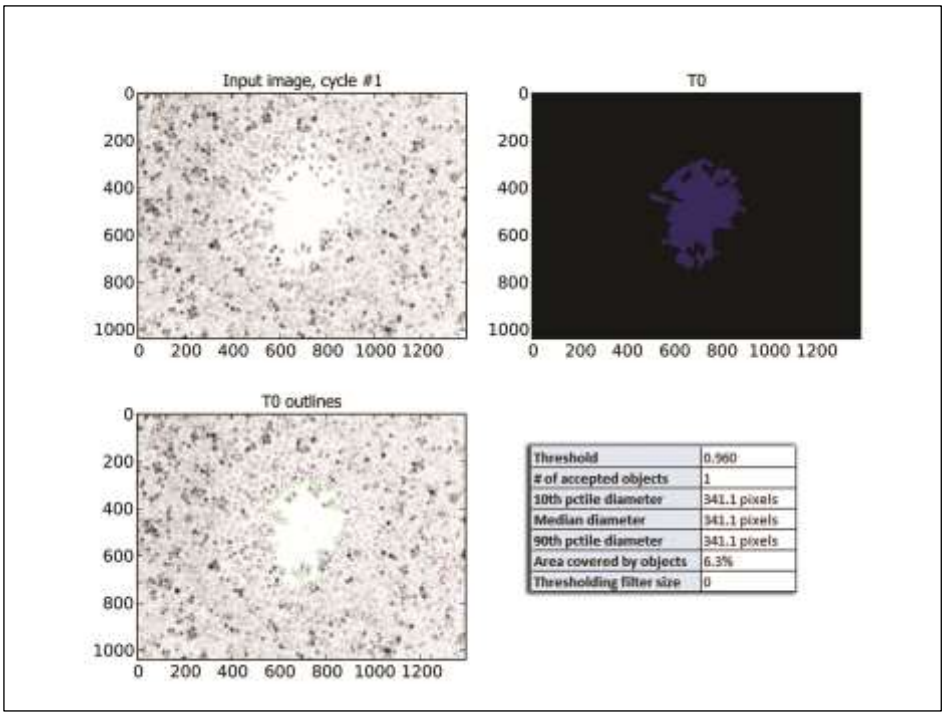

Figure 3: Sample Calcein AM 3.jpg analyzed with manual threshold value of 0.65.

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