Pipeline for multiplex immunohistochemistry-based image cytometry

Overview

This pipeline enables single cell-based segmentation and quantification of staining intensity in serially digitized and co-registered multiplex immunohistochemistry images. Measurements of chromogenic signal intensity with 26 different shape-size measurements in the images are extracted and recorded as a file format compatible with flow and image cytometry data analysis software, FCS Express 5 Image Cytometry Version 5.01.0029 (De Novo Software).

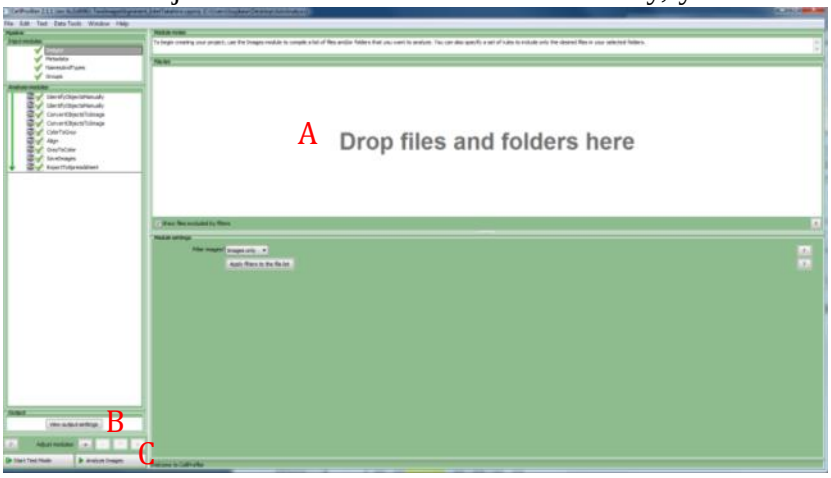
Download and Install

We have tested our modules on Windows and Mac OS X, but they should also run on Linux.

- Install CellProfiler version 2.1.1.
- Download " CellID_FlowCyt - 6.9.15.cpproj".
- Install latest ImageJ/Fiji.
- Download macro "AEC extraction".

Step-by-Step Tutorial

1. Prepare a series of post-coregistration TIF files from multiplex immunohistochemistry. File names of the images should contain “One” “Two” “Three”…”Twelve” (Case sensitive). A hematoxylin image should be "One". If the number of images is less than 12, create dummy files and prepare a total of 12 files including “One” to “Twelve”.
   e.g. XXX_Nuclei_One.tif
   YYY_PD-1_Two.tif
   ZZZ_CD3_Three.tif
2. Open a series of 11 AEC-based tif images except nuclei (hematoxylin) image in ImageJ/Fiji, and run the macro "AEC extraction". Following "Stack to images", save the 11 images to your favorite folder. Copy hematoxylin image to this folder. The folder will have 12 images including 11 AEC and one hematoxylin images.
3. Run CellProfiler with "CellID_FlowCyt - 6.9.15.cpproj" pipeline. Drop 12 images from Step2 to "A". Select your favorite directory for output folder (B). Run the pipeline (C). If needed, memory size for JAVA can be adjusted via File>Preferences. Generally, you can set up memory consumption up to 70% of RAM.



5. After run, you will see three files in your selected output directory ("Image.cptoc", "NucleiGreen.cpout", and "Temp-.tif"). Move these three files to the directory containing images from Step 2.
6. Start FCS Express 5 and build up your own gating strategy and quantification.