Pipeline for multiplex immunohistochemistry-based image cytometry

Overview

This pipeline facilitates the segmentation and quantification of staining intensity on a single-cell basis in serially digitized and co-registered multiplex immune histochemistry images, enabling the analysis of both tumor cell nests and intratumoral stroma areas within tumor regions separately. Measurements of chromogenic signal intensity are extracted and recorded as a file format compatible with image cytometry data analysis software, FCS express 7 Image Cytometry v.7.04.0020 (De Novo Software).

Download and Install

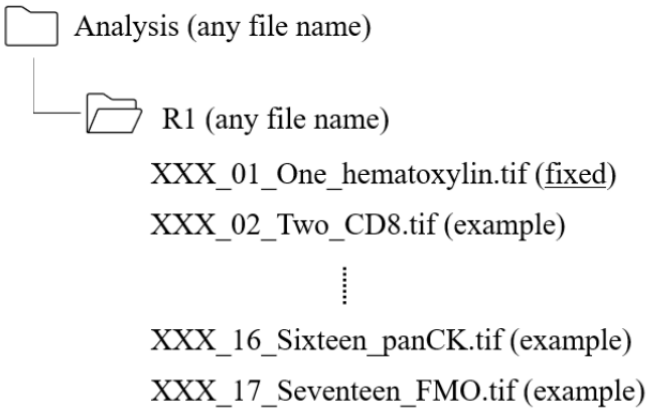
We have tested our modules on Windows 10.

- Install CellProfiler version 2.2.0 (Broad Institute) (install time:2-3 minutes)
- Download "03_17plex_Size9to40_04282022.cpproj"
- Install ImageJ/Fiji version 1.51 (National Institutes of Health) (install time:2-3 minutes)
- Download macros "AEC_extraction" and "Tissue_segmentation"

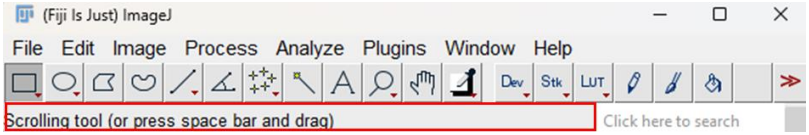
Step-by-Step Tutorial

1. Prepare a series of post-coregistration TIF files from multiplex immunohistochemistry. File names of the images should contain "01_One" "02_Two" "03_Three" …. "17_Seventeen" (Case sensitive). A hematoxylin image should be "01_One". If the number of images is less than 17, create dummy files and prepare a total of 17 files including "One" to "Seventeen".

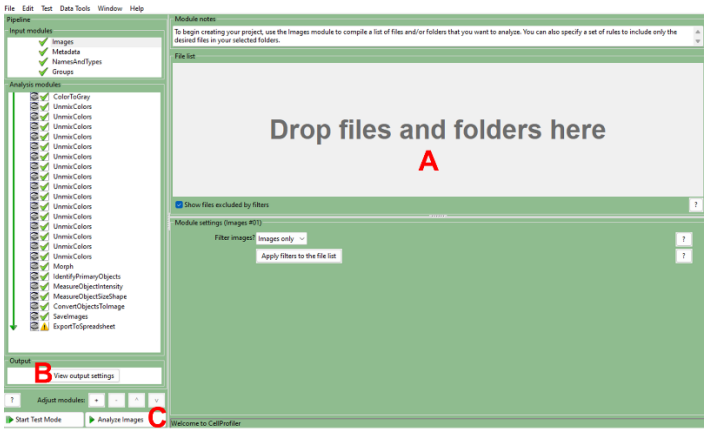
2. Organize those 17 files into a folder hierarchy as shown in the figure below.



3. Run ImageJ/Fiji with the "AEC extraction" macro (drag and drop the macro file into the area enclosed by a red line in the figure below). Select the parent folder of the one containing the 17 files (labeled "Analysis" in the figure above). A "Processed" folder will then be created, following the same hierarchy as parent folder (run time:3 minutes).



4. Drag and drop the "Tissue segmentation" macro into ImageJ/Fiji. Then, drop pan-cytokeratin image in the "Processed/R1" folder to ImageJ/Fiji, then click Run button (run time: 30 seconds).
5. After run, you will see four folders under the names "01_noblank", "02_tumornest", "03_stroma", and "Segmentation".
6. Open CellProfiler and use "03_17plex_Size9to40_04282022.cpproj" pipeline. Drop 17 images from the folders created in Step 5 that you want to analyze into (A). Select your favorite directory for output folder (B). Run the pipeline (C) (run time: 3 minutes).



7. 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 5.
8. Start FCS Express 7 and build up your own gating strategy and quantification.