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.

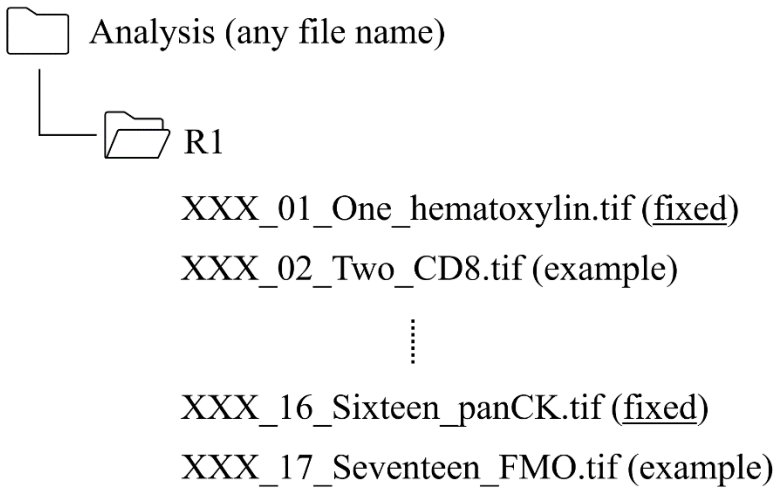
- Install CellProfiler version 2.2.0 (Broad Institute) (install time:2-3 minutes)
- Download "03_17plex_Size9to40_04282022.cpproj"
- Install ImageJ/Fiji version 1.51 (National Institutes of Health) (install time:2-3 minutes)
- Download macros "AEC_extraction" and "Tissue_segmentation"

Step-by-Step Tutorial

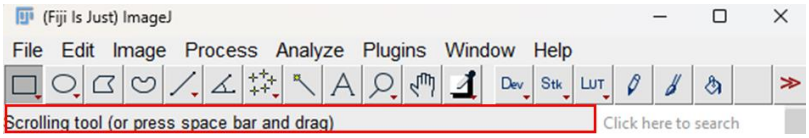
1. First, we need to determine regions of interest (ROIs), of which image structure completely overlap between all staining markers from a single identical section (Coregistration), to enable subsequent multiple image analysis. For this end, raw images acquired by NanoZoomer are processed by the program code developed by SCREEN Holdings Co.,Ltd. (https://www.screen.co.jp/en), which is not currently publicly available pending patent application. Alternatively, the method published at https://github.com/multiplexIHC/12plex-IHC (see "README_Coregistration.pdf") can be used for this Coregistration. During this Coregistration, select up to four ROIs. These ROIs are saved as TIF files, and store these TIF files in folders named RX (for example, R1 for 1st ROIs but R4 for 4th ROIs), and place them in a parent folder of any name given (for the demo files, only the R1 folder is included to reduce overall file size). The file names of the TIF images should contain "01_One" "02_Two" "03_Three" …. "17_Seventeen" (Case sensitive). A hematoxylin and a pan-cytokeratin should be "01_One" and "16_Sixteen", respectively. If the number

of images is less than 17 as performed in the demo files, create dummy files to ensure a total of 17 files including “One” to “Seventeen”.

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

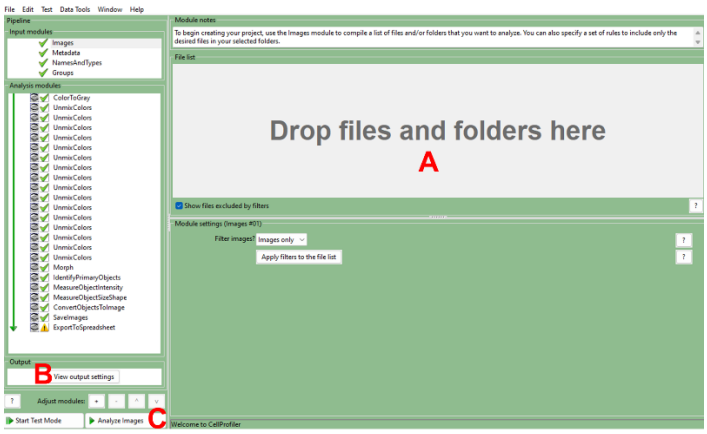


3. Run ImageJ/Fiji with the “AEC extraction” macro (drag and drop the macro file into the area enclosed by a red line in the figure below, and Press the “Run”). Select the parent folder of the one containing the 17 files (labeled “Analysis” in the figure above). A “Processed” folder will then be created, following the same hierarchy as the 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”. You need “01_noblank”, “02_tumornest”, and “03_stroma” for subsequent analyses. You can see summarized results in “Segmentation” to see how well the tissue segmentation was done. If tissue segmentation was not well performed (e.g. tumor nest and stromal regions are not well separated), you need to modify the x and/or y values of “setMinAndMax(x, y)” and/or the z values of “iterations=z” in the “Tissue segmentation” macro . Do “try&see” to figure out the best values.

6. Open CellProfiler and use “03_17plex_Size9to40_04282022.cpproj” pipeline. To do this, just double click the "03_17plex_Size9to40_04282022.cpproj" application. 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) (for example, the directory containing images from Step 5). 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, if you did not do so at the step 6.
8. Start FCS Express 7 and build up your own gating strategy and quantification.