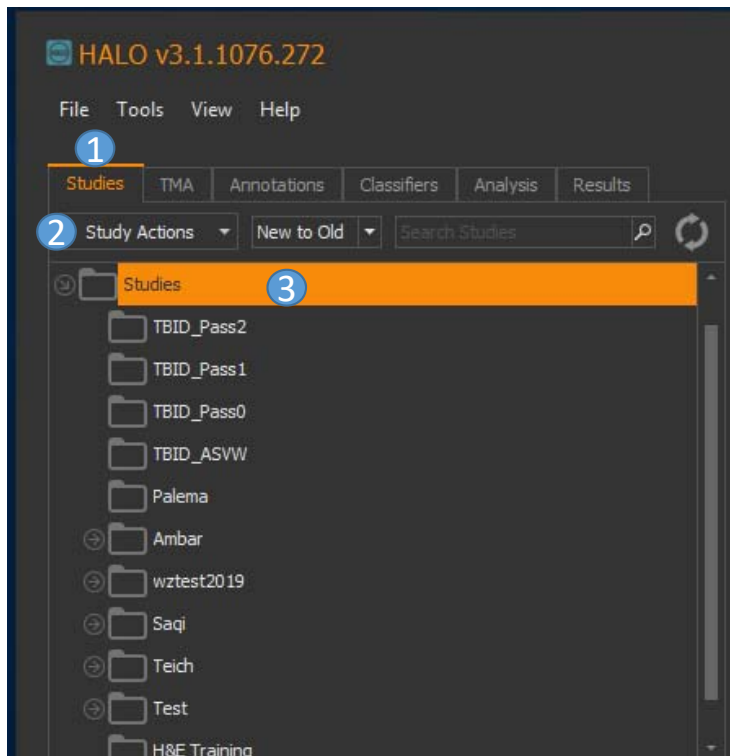# Halo Introduction

DCPL

2022
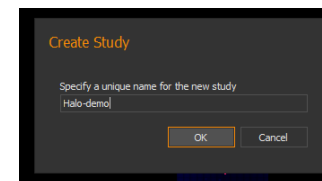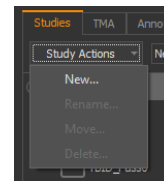
Halo-Studies Tab

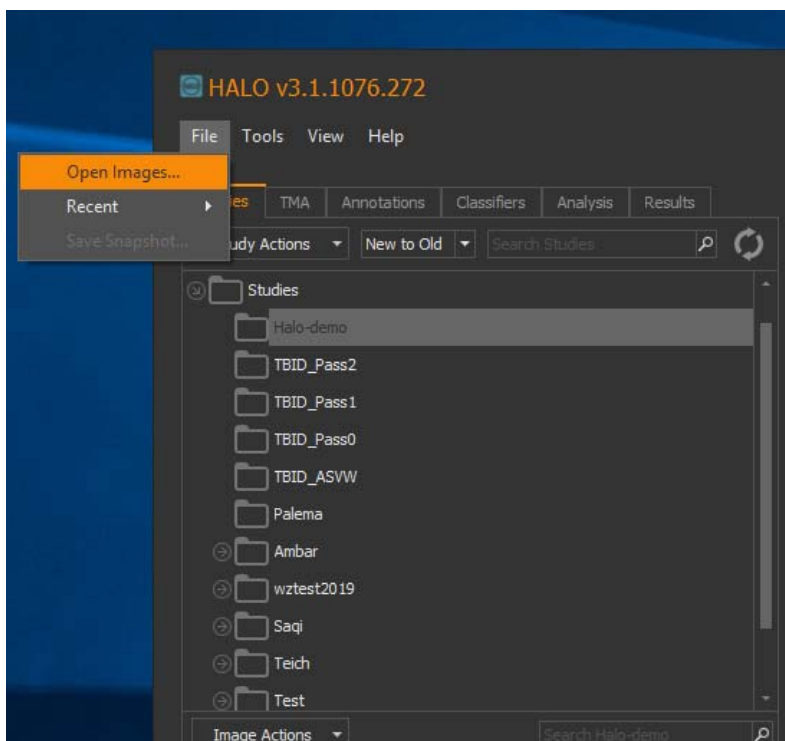In Halo platform, we use **Studies Tab** to organize / manage multiple images.

① Studies Tab

② Study Actions – a pull-down menu where you can create
New/Rename/Move/Delete studies

③ List of studies

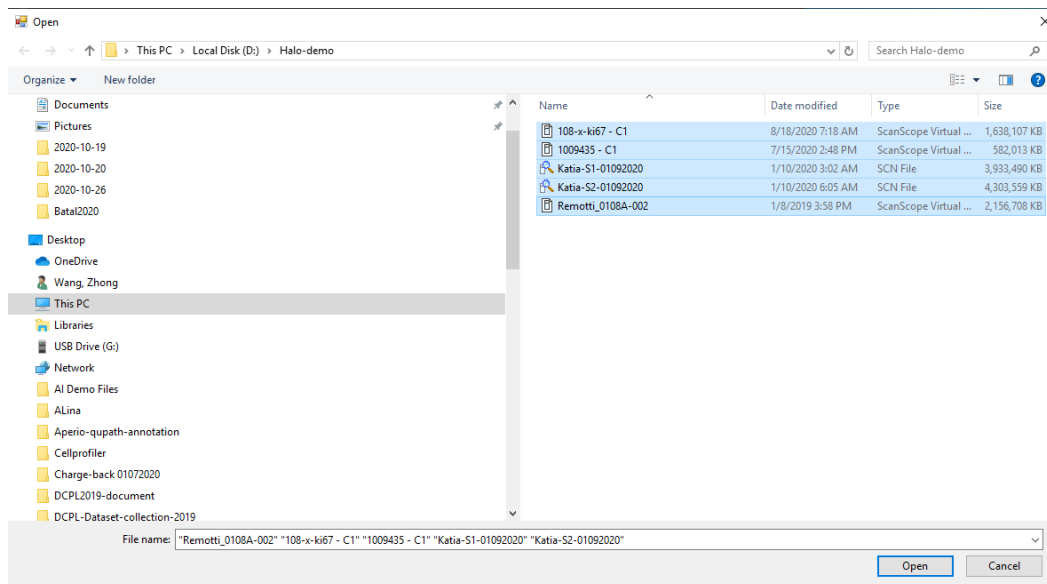Example: three steps to create a new study named as Halo-demo

Halo-Studies Tab- add images to study (Four steps)

Step 1: File menu -> Open Images

Step 2: From your PC, select the folder and your files, then click **open**

Halo-Studies Tab- add images to study

Step 3: Select Study window will appear, choose the proper study name, in our case: Halo-demo

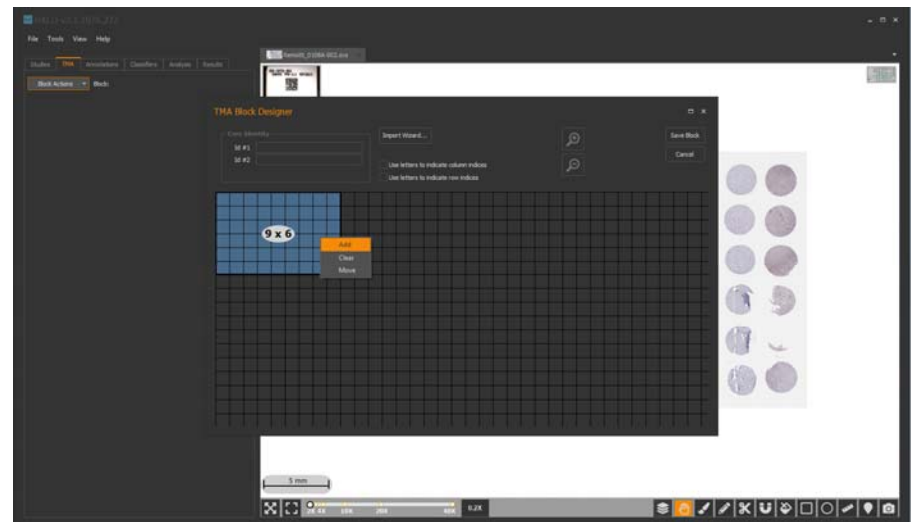Step 4: added images are listed in region ① , double click you can open images accordingly

Halo-TMA Tab

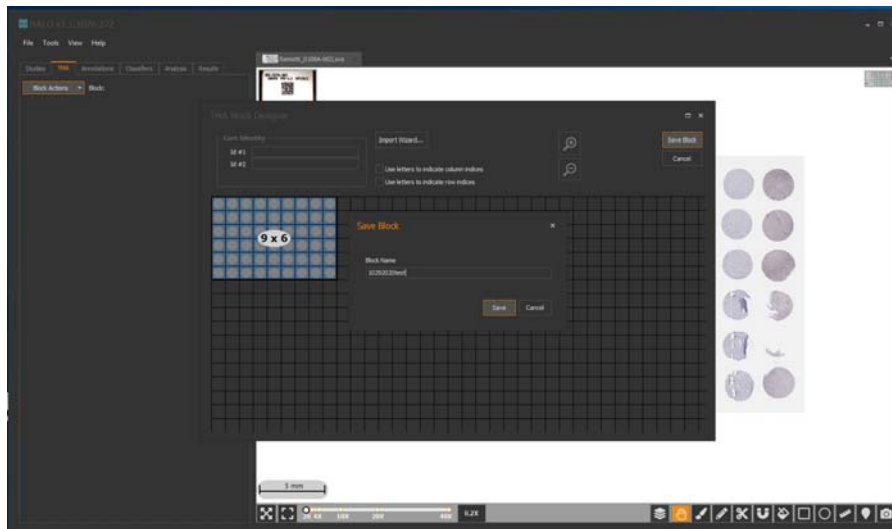Step 1: For TMA slide, go to **TMA Tab,** click **Block Action -> New**
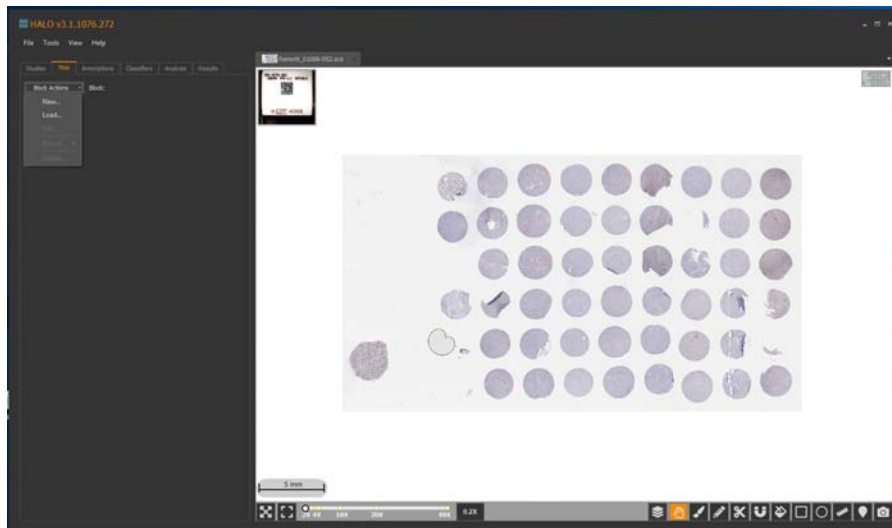
Step 2: you will see TMA Block Designer , drag and draw the TMA layout (9 by 6 in this example), right click mouse and click **Add**
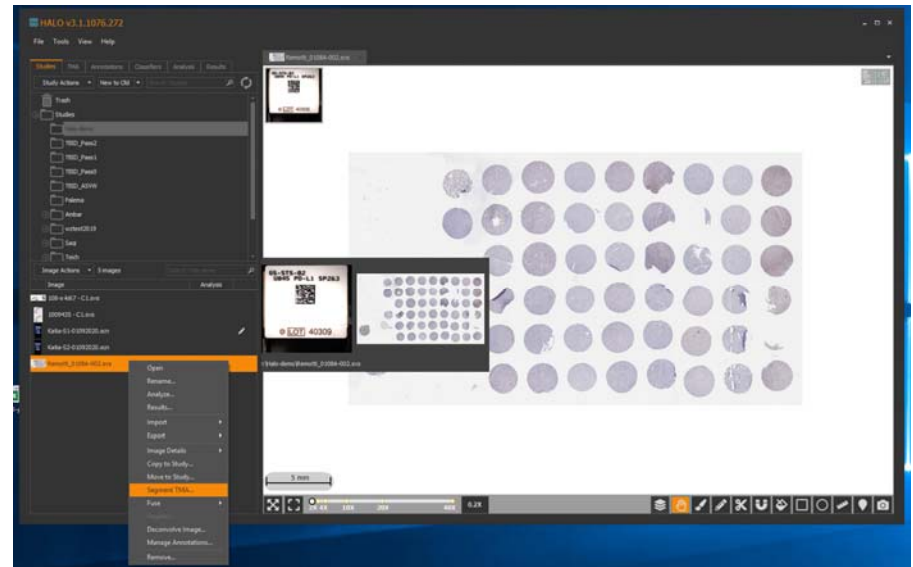
Halo-TMA Tab

Step 3: after click **Add** (step 2), the layout will have grey spots, then you can click Save Block (top right) and give current block a name, say 10292020test, then click Save

Step 4 : Go back to **Study Tab**, find the TMA image from summary list and right click mouse and choose **Segment TMA**

Halo-TMA Tab

Step 5: a **Select Block** window will appear and select the block just created: 10292020test, then click **Select**

Step 6: a **TMA Segmentation Alignment** window will appear, at this step, you can drag each red circle to align your tissue spot, spot size can be adjusted at button ①, when you finish the alignment, click **Save** button at bottom right

Halo-TMA Tab

Step 7: at this step, you can select/ click individual tissue spot from spot summary region ①, spot image can be viewed at viewer ②, spot segmentation can be further tuned from **Slide Actions** menu ③ ;
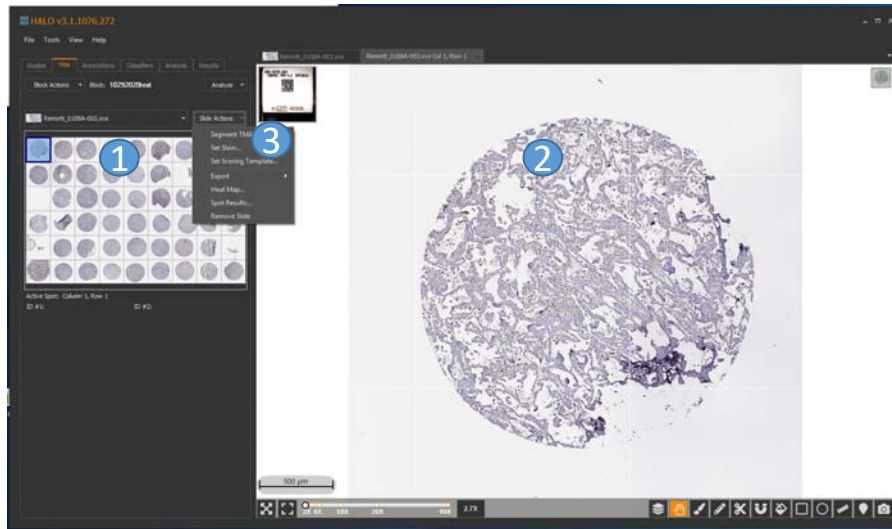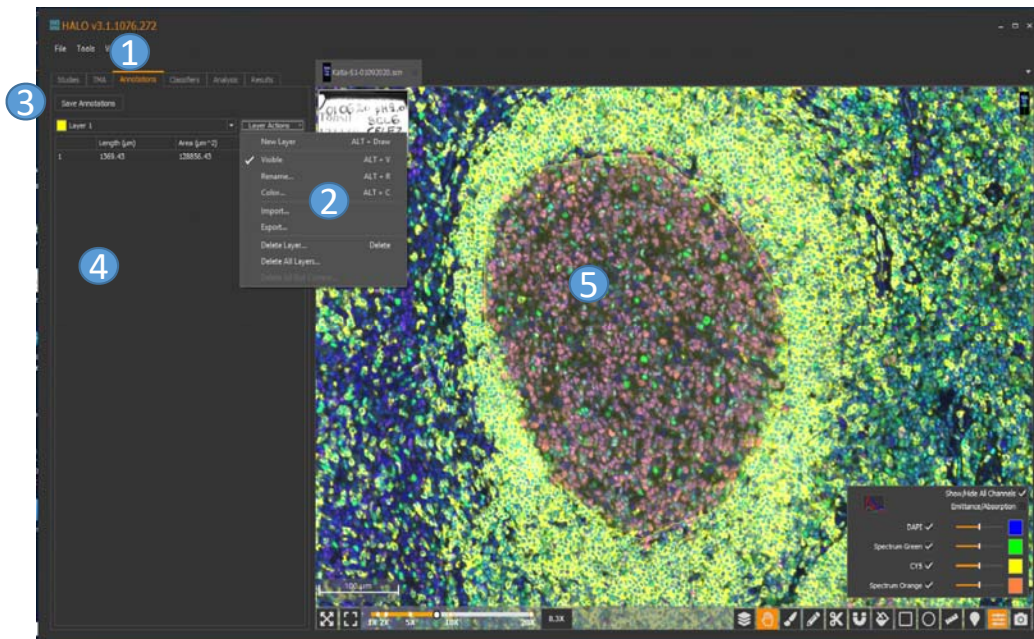
Step 8: now tissue spots are segmented properly, right click individual spot from spot summary region, you can further manipulate / tune the specific spot, for example: **Analyze Spot** (apply a analysis module to that spot)
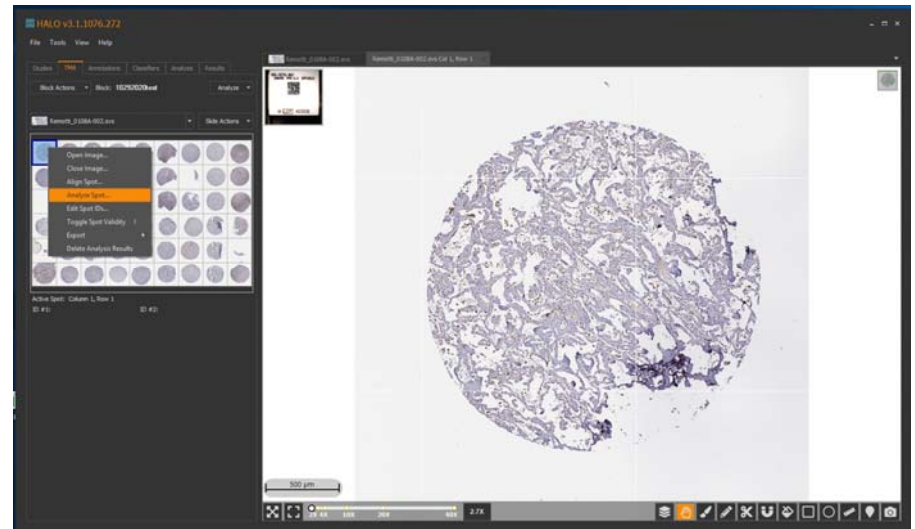
Halo- Annotation Tab

Annotation is used when you want to highlight a region of interest (ROI) for further processing.

①   Annotation Tab

②   Layer action- a pull-down menu containing commands to manipulate/manage different annotation layers;

③   Save Annotation- when you complete the annotations for one image, remember to save them;

④   Summary list for annotations;

⑤   Annotated region in image viewer;

 : move image to check different regions;

 : annotation tools

Halo- Analysis Tab

Step 1: To start analysis, go to Analysis Tab, Use **Settings Actions** pull-down menu to select **Load**
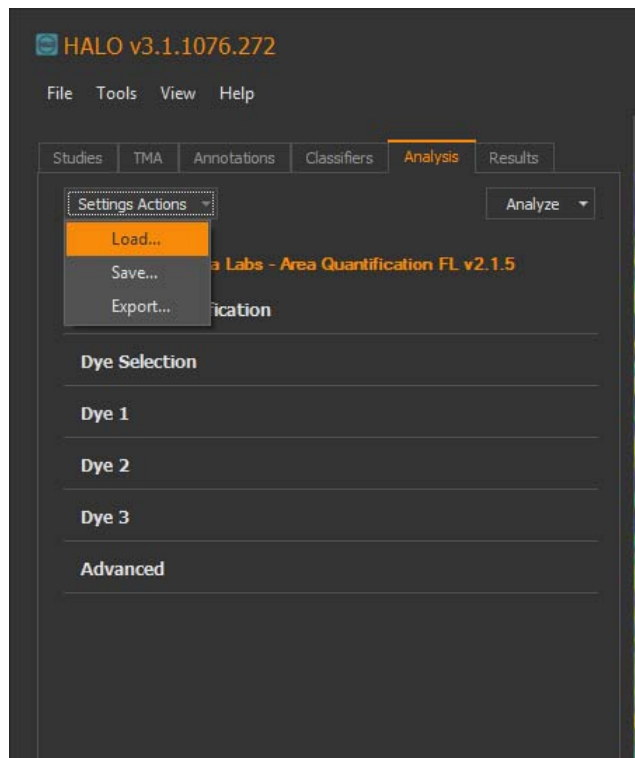

Step 2: **Select Algorithm Settings** window will appear, check **Show all installed module versions** ①, then all analysis modules and all module versions will show up as a list. We will use HighPlex FL v3.2.1 ② to process a FL image as an example, click **Select** ③ to confirm the algorithm module selection.
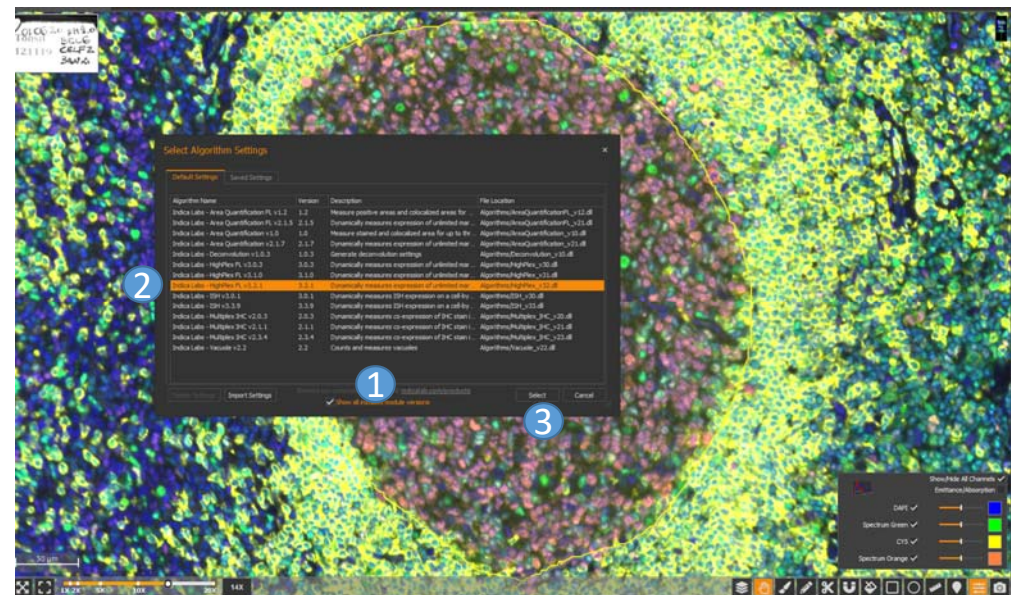
Note: check the **Description** to understand the algorithm's function. Algorithm names with letter FL are used to process FL images, without letter FL are used to process BF images.

Halo- Analysis Tab
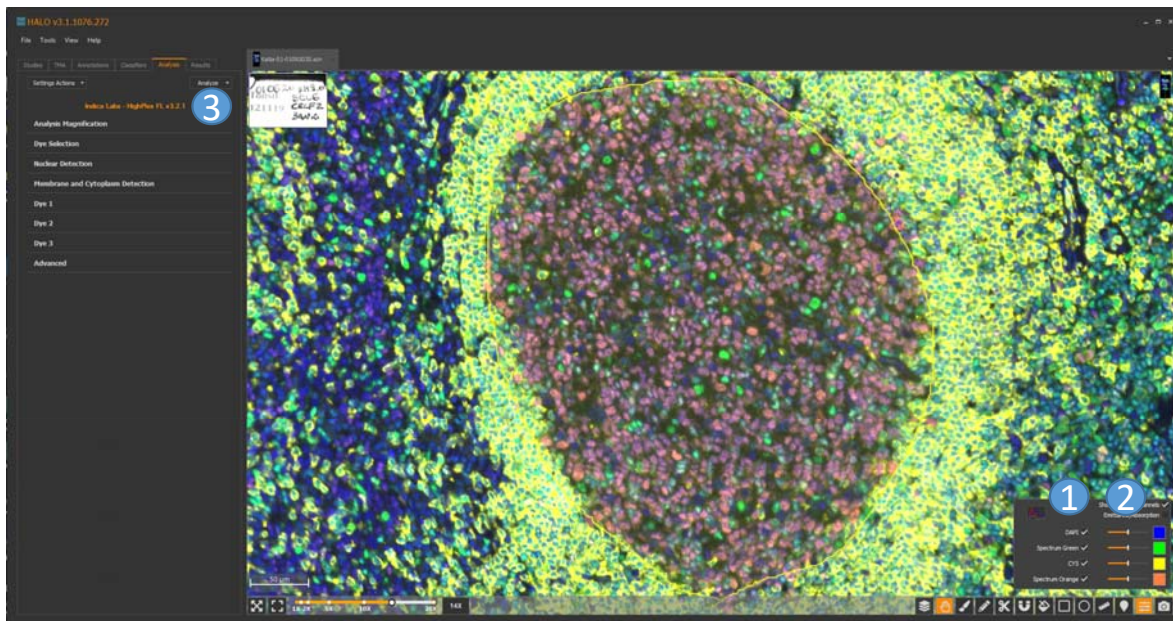
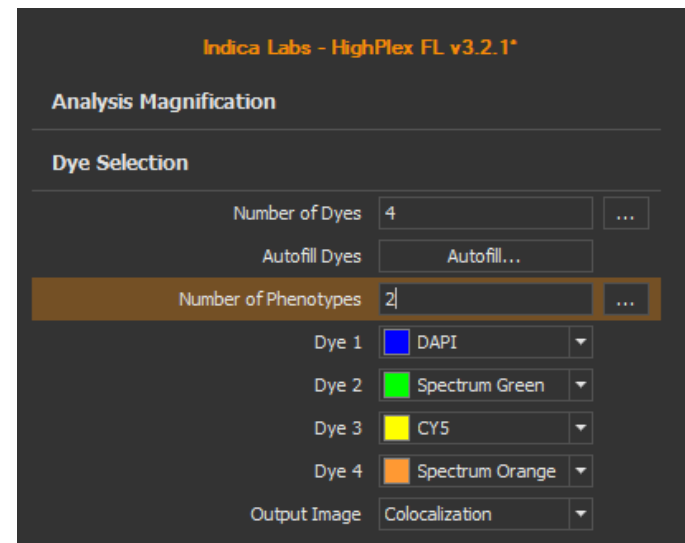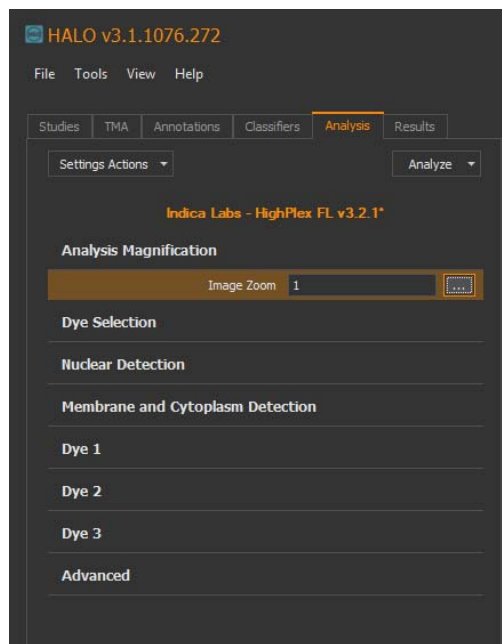Step 3: understand the image data and algorithm module structure

Let's revisit previous FL image. This is 4-channel FL image containing four stainings: DAPI/Green/Cy5/Orange(Cy3).

1. You can turn on/off channel by clicking check/uncheck.

2. You can drag this control bar to adjust the display for specific channel.

3. Algorithm module has a step-by-step processing structure. Specifically, for this HighPlex module, we can determine cell phenotypes based on different stainings / staining combinations.

Halo- Analysis Tab- HighPlex module details

Step 4 : **Analysis Magnification** - zoom level used for analysis, 1 corresponds to full image magnification, typically just use this default setting

Step 5 : **Dye Selection** – load dye/channel definitions (four dyes in this case); assuming we want to determine two cell phenotypes as an example

Halo- Analysis Tab- HighPlex module details

Step 6 : **Nuclear Detection**

This step is used to determine nuclear objects. If you select specific parameter (1),
its definition is described at the bottom region (2).

Nuclear Contrast Threshold: contrast between nuclear intensity and background

Minimum Nuclear Intensity: minimum value of nuclear intensity , range 0-1

Maximum Image Brightness: maximum value of nuclear intensity , range 0-1

Nuclear segmentation Aggressiveness: range 0-1, larger value more nuclear objects

Fill Nuclear Holes: logic value 0 or 1, if you want a solid nuclear object choose 1

Nuclear Size: range of nuclear area, in this example: 11.3-571.7 (micron squared)

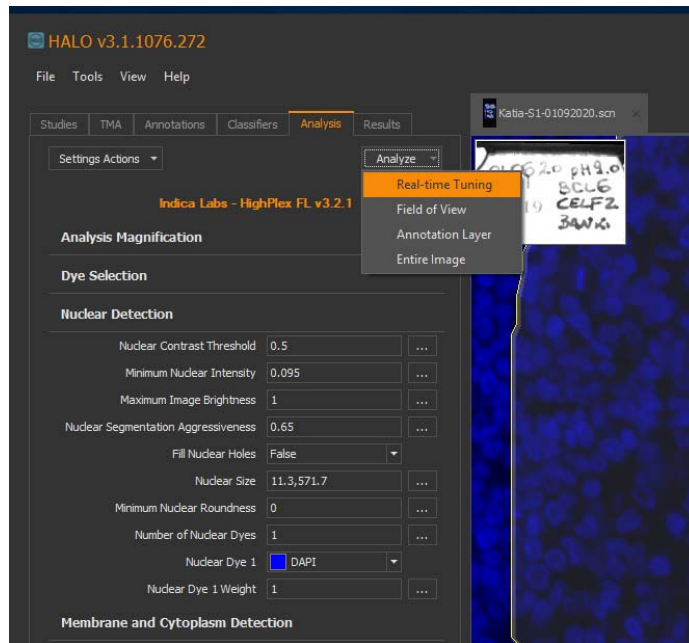Minimum Nuclear Roundness: minimum roundness of nuclear objects

Number of Nuclear Dyes: number of dyes to use for nucleus detection

Nuclear Dye 1: staining channel used to determine nuclear object

Nuclear Dye 1 Weight: how heavily this dye should influence nuclear detection

Halo- Analysis Tab- HighPlex module details

Step 8 : **Real-time tuning**– a tuning (analysis markup) window will appear and highlight the nuclear objects. In this example, we use DAPI channel to determine the nuclear objects, other color channels can be turned off temporarily. To switch between analysis markup image and original image , you can use the Toggle Action button

Step 7: **Analyze**- you can start with default module parameters to run your initial analysis, choose **Analyze** pull-down menu then click **Real-time tuning**.

Analysis markup
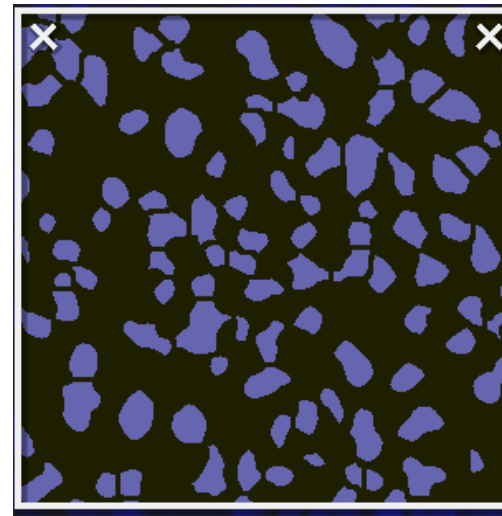
Original image
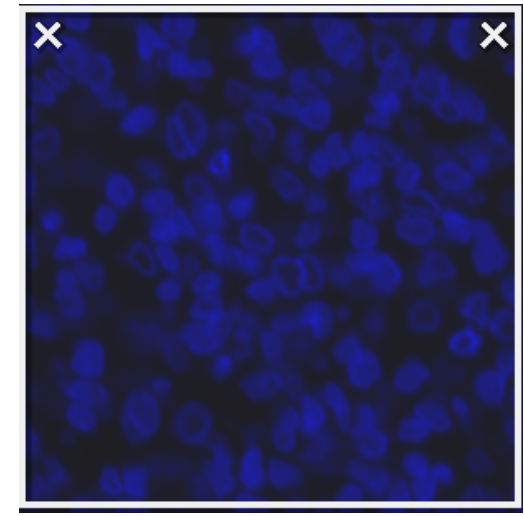
By comparing the markup image and original image, you can adjust nuclear detection parameters accordingly to optimize the result. Then you can further apply updated parameters to Field of View /Annotation Layer /Entire Image to confirm.

Halo- Analysis Tab- HighPlex module details

Step 9 : **Membrane and Cytoplasm Detection**
After nuclear objects are determined, we can further define the cell objects
by following parameters:
Maximum Cytoplasm Radius: max cytoplasmic radius around each nucleus
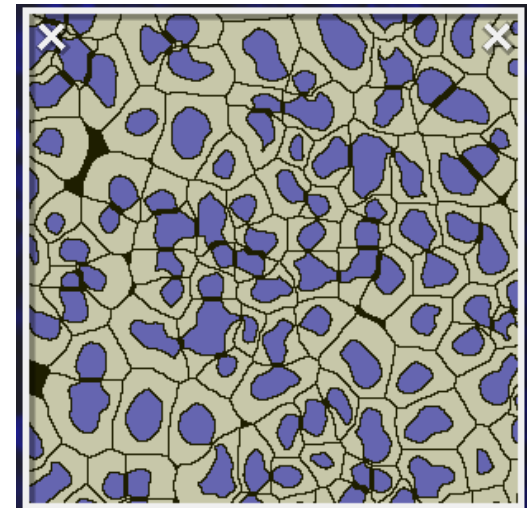Membrane Segmentation Aggressiveness: how aggressively connected cells
should be separated
Cell Size: cell area range, should be larger than Nuclear Size
Number of Membrane Dyes: number of dyes to use for membrane detection

Current Real-Time Tuning window
Detected cell objects

Halo- Analysis Tab- HighPlex module details

Current Real-Time Tuning window

Step 10 : Determine positive DAPI signal threshold and staining locations (nucleus/cytoplasmic)

DAPI Nucleus Positive Threshold : three-level thresholding technique can be applied to represent Weak/Medium/Strong DAPI signals. In most cases, we just need a single threshold to determine positive valid signal, for example, (**20,255,255**) means DAPI intensity above 20 is considered as valid signal (positive DAPI);

DAPI Nucleus % Completeness Threshold: percent of nucleus that must be positive for the dye (DAPI) in order for the nucleus to be considered positive as a whole; range : 0-100;

DAPI Cytoplasm Positive Threshold: DAPI in this case is not a cytoplasmic dye. (255,255,255)

is used to block this definition;
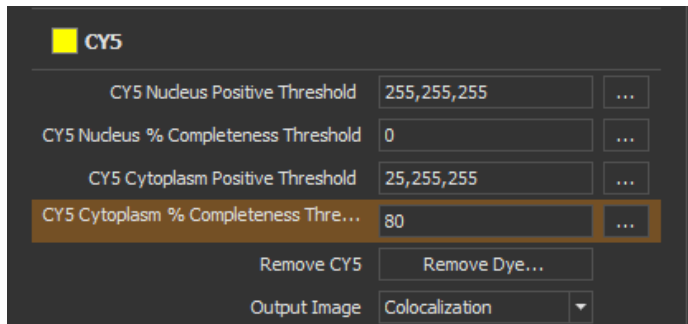
DAPI Cytoplasm % Completeness Threshold: N/A

Now the current tuning window is shown on left side: nuclear objects with positive DAPI signal (>20) are shown in blue; DAPI is not a cytoplasmic dye (blocked from definition) , so cytoplasmic regions are shown in grey.
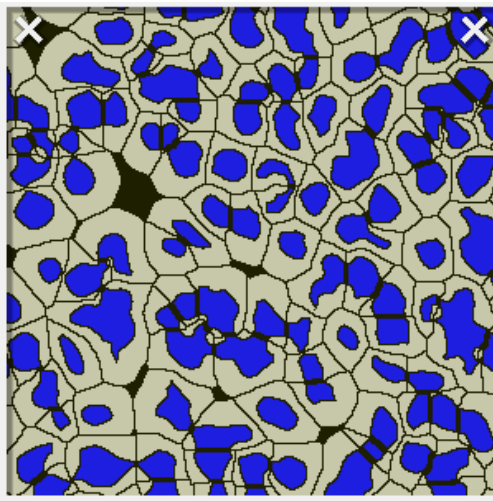
Halo- Analysis Tab- HighPlex module details

Current Real-Time Tuning window

Step 11 : Determine positive CY5 signal threshold and staining locations (nucleus/cytoplasmic)

Apply the same concept described in Step 10 to CY5 channel. CY5 is used as a cytoplasmic dye in this example, so parameters can be updated as follows:
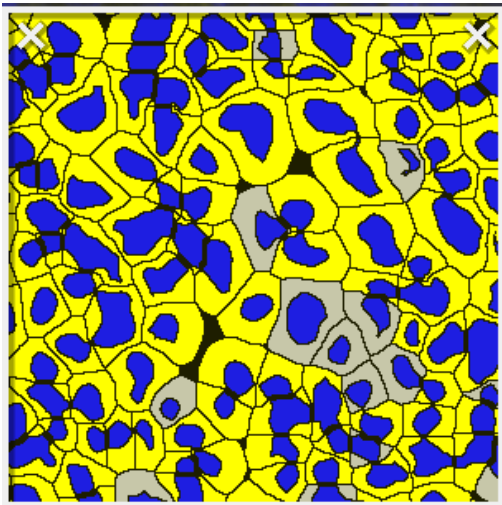
CY5 Nucleus Positive Threshold : (255,255,255) is used to block this definition;

CY5 Nucleus % Completeness Threshold: N/A

CY5 Cytoplasm Positive Threshold: (25,255,255) is used to determine valid CY5 signal;
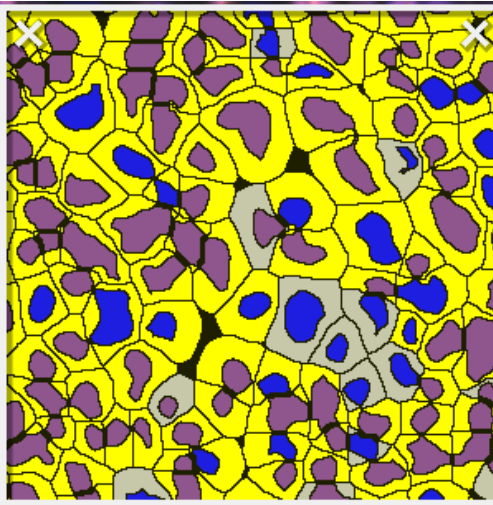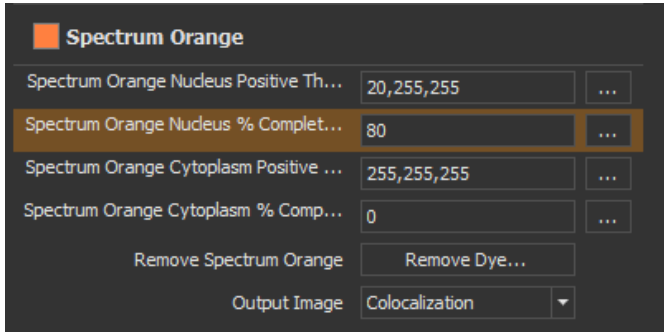
CY5 Cytoplasm % Completeness Threshold: 80 is used here as an example

Now the current tuning window is shown on left side: cytoplasmic regions with positive CY5 signal (>25) and CY5 cytoplasm completeness >80% are shown in yellow; otherwise cytoplasmic regions are shown in grey.

Halo- Analysis Tab- HighPlex module details

Spectrum Orange			
Spectrum Orange Nucleus Positive Th...	20,255,255		...
Spectrum Orange Nucleus % Complet...	80		...
Spectrum Orange Cytoplasm Positive ...	255,255,255		...
Spectrum Orange Cytoplasm % Comp...	0		...
Remove Spectrum Orange	Remove Dye...		
Output Image	Colocalization	▼	

Current Real-Time Tuning window

Step 13 : Determine positive Orange(CY3) signal threshold and staining locations (nucleus/cytoplasmic)

Similarly, we apply the same concept to orange channel (CY3). Orange (CY3) is another nuclear dye , only expressed in a specific cell type so parameters can be updated as follows:

Orange Nucleus Positive Threshold : (20,255,255) is used to determine valid orange signal;

Orange Nucleus % Completeness Threshold: 80 is used here as an example

Orange Cytoplasm Positive Threshold: (255,255,255) is used to block this definition;

Orange Cytoplasm % Completeness Threshold: N/A

Now the current tuning window is shown on left side: nuclear objects with positive orange signal (>25) and orange nucleus completeness >80% are shown in brown; otherwise nuclear objects are remained in blue color. Up to now, cell objects have different color patterns. Different color patterns represent different cell phenotypes.

Halo- Analysis Tab- HighPlex module details

Phenotype 1

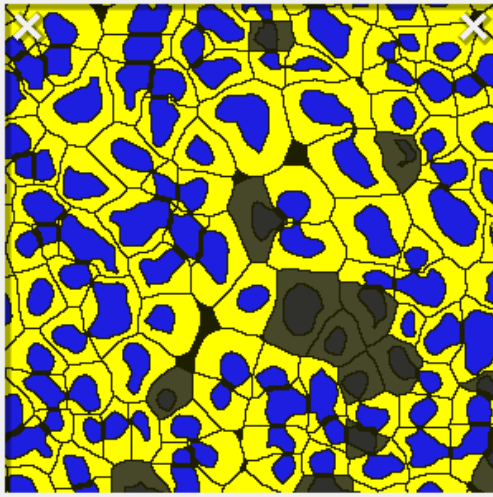|	
Phenotype 1 Name	Phenotype 1
Phenotype 1 Number of Criteria	2
Phenotype 1 Criteria 1 Channel	■ DAPI
Phenotype 1 Criteria 1 Filter	AND Positive
Phenotype 1 Criteria 2 Channel	■ CY5
Phenotype 1 Criteria 2 Filter	AND Positive
Output Image	Phenotype 1

Current Real-Time Tuning window

Step 14: **Define Phenotype 1**

To determine / define phenotype 1, following parameters are used:

Phenotype 1 Name: cell subtype name;

Phenotype 1 Number of Criteria: number of phenotype criteria;

Phenotype 1 Criteria 1 Channel: DAPI channel is considered;

Phenotype 1 Criteria 1 Filter: **AND Positive** means positive DAPI signal located at nuclear region;

Phenotype 1 Criteria 2 Channel : CY5 channel is considered;

Phenotype 1 Criteria 2 Filter: **AND Positive** means positive CY5 signal located at cytoplasmic region

Output Image: Phenotype 1 objects

Phenotype 1 cells: blue nuclear regions and yellow cytoplasmic regions

Not Phenotype 1 cells: grey cell objects

Halo- Analysis Tab- HighPlex module details
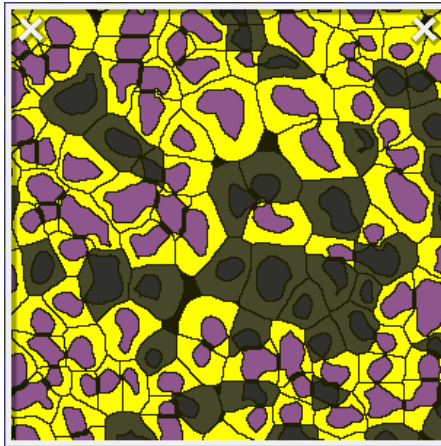

Current Real-Time Tuning window

Step 15: **Define Phenotype 2**

Similarly, we can define Phenotype 2. Now, following three criteria are used to define phenotype 2:

Positive DAPI signal located at nuclear region

Positive CY5 signal located at cytoplasmic region

Positive Orange (CY3) signal located at nuclear region

Phenotype 2 cells: brown nuclear regions and yellow cytoplasmic regions

Not Phenotype 2 cells: grey cell objects

Compared to previous page, because we applied one more criterion here, so more grey cell objects appear in the same tuning window.

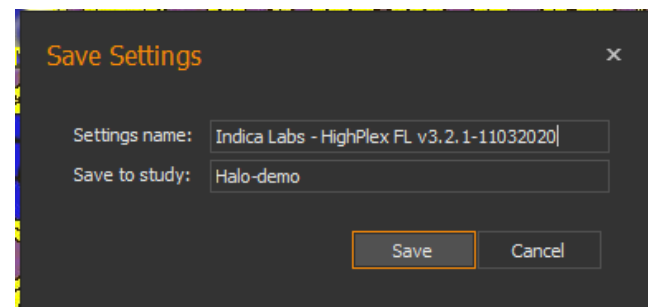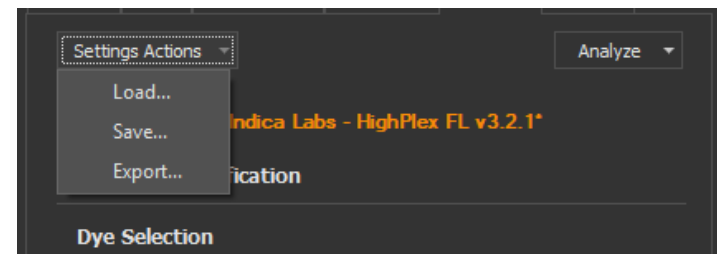# Halo- Analysis Tab- HighPlex module details

Step 16: Advanced
Store Object Data : select **True** if you want to have cell object data
Classifier/Class list/Classifier Output Type: you can combine with
classifier to identify cell phenotypes within specific tissue structure
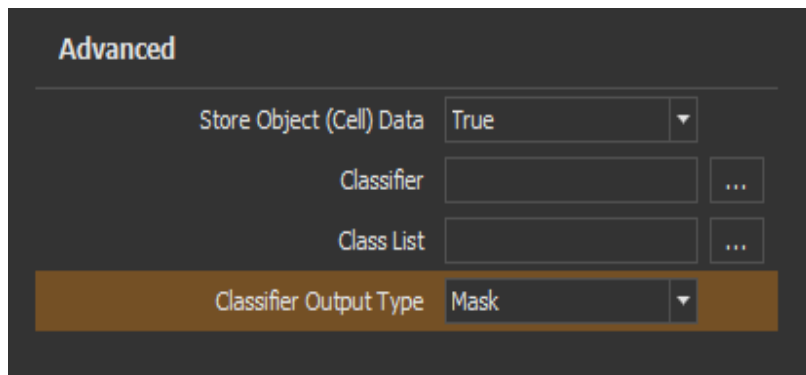
Step 17: save your algorithm module
After parameter optimization, click **Save** from **Settings Action** menu. Give your
algorithm settings a name and save it to your study, for example: Halo-demo. Then
you can reapply this saved algorithm setting to similar images in the future.

Halo- Analysis Tab- HighPlex module details
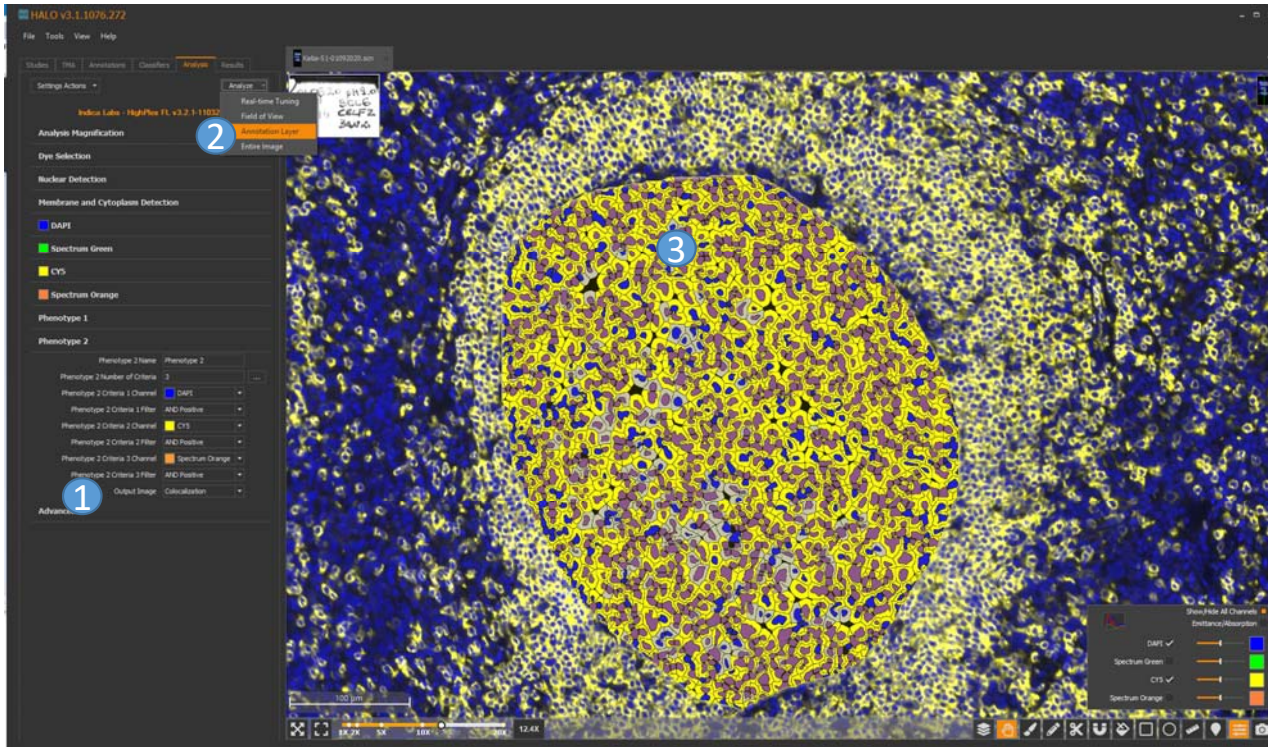
An analysis example for an annotated ROI

Check list:

① Output Image : Colocalization mode

② Analyze menu: Annotation Layer

③ Image Viewer: result image

Halo- Results Tab

Results Tab

① : cell object summary results

② : detailed cell object data

All these results can be either copy/paste or export to txt or Excel format.