

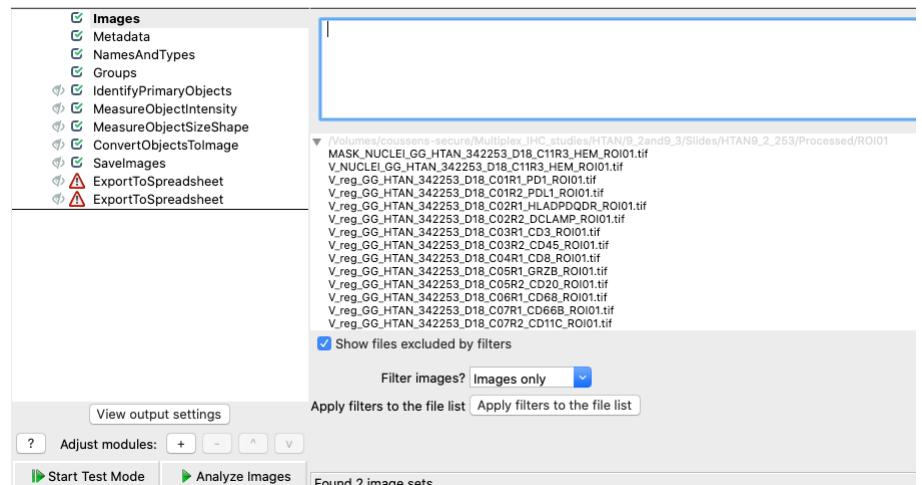
# Cell Profiler Instructions for use in FCS Express for mIHC Image Cytometry

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## Images Module

Drag and drop image into the main window.

Load all 'Processed' folders from the cohort slides into the Images Module if you are running on command line. Otherwise, load just those from slides you wish to process



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## Metadata Module

There are 2 required metadata that need to be captured. These should be ROI from filenames and Slide name from Folder name. Additional metadata can be optionally captured.

Update	I	L	Series	Frame	FileLocation	ROI	Slide	Type
1	M_ROI01.tif	0	0	0	file:/Volumes...HEM_ROI01.tif	ROI01	HTAN9_2_253	MASK
2	M_ROI01.tif	0	0	0	file:/Volumes...HEM_ROI01.tif	ROI01	HTAN9_2_253	V_
3	1_ROI01.tif	0	0	0	file:/Volumes...PD1_ROI01.tif	ROI01	HTAN9_2_253	V_
4	1_ROI01.tif	0	0	0	file:/Volumes...DL1_ROI01.tif	ROI01	HTAN9_2_253	V_
5	R_ROI01.tif	0	0	0	file:/Volumes...QDR_ROI01.tif	ROI01	HTAN9_2_253	V_
6	IP_ROI01.tif	0	0	0	file:/Volumes...AMP_ROI01.tif	ROI01	HTAN9_2_253	V_
7	3_ROI01.tif	0	0	0	file:/Volumes...CD3_ROI01.tif	ROI01	HTAN9_2_253	V_
8	5_ROI01.tif	0	0	0	file:/Volumes...D45_ROI01.tif	ROI01	HTAN9_2_253	V_
9	8_ROI01.tif	0	0	0	file:/Volumes...CD8_ROI01.tif	ROI01	HTAN9_2_253	V_

The slide names should have a naming convention that can be identified by regular expression.

The ROI metadata will have a standard convention generated by the pipeline.

Update	I	L	Series	Frame	FileLocation	ROI	Slide	Type
1	M_ROI01.tif	0	0	0	file:/Volumes...HEM_ROI01.tif	ROI01	HTAN9_2_253	MASK
2	M_ROI01.tif	0	0	0	file:/Volumes...HEM_ROI01.tif	ROI01	HTAN9_2_253	V_
3	1_ROI01.tif	0	0	0	file:/Volumes...PD1_ROI01.tif	ROI01	HTAN9_2_253	V_
4	1_ROI01.tif	0	0	0	file:/Volumes...DL1_ROI01.tif	ROI01	HTAN9_2_253	V_
5	R_ROI01.tif	0	0	0	file:/Volumes...QDR_ROI01.tif	ROI01	HTAN9_2_253	V_

## 3

## Names and Types

This important module tells Cell Profiler how to identify and name each image file as it goes through the Cell Profiler pipeline.

We use the cycle/round identifier in the image filename to uniquely identify each file. The name assigned to each image is used in processing for downstream modules.

We use the marker name and an alphanumeric key prepended to maintain column order in the output file.

Hit 'Update' to see all files identified. If there are any incorrect or inconsistent filenames, the update will not work and a warning will be issued. All names should be fixed before proceeding.

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## Groups Module

In the Groups module, each image set (ROI per slide) is grouped such that each ROI from every slide is listed as a single group.

The number of Image sets should be the number of total ROIs for the batch of slides. Keep track of this number for the command line run.

If there are any issues, such as missing files or inconsistent file/folder names, then there will be Na/Nan values in the grouping list. This issue should be identified and resolved before going forward.

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## MeasureObjectIntensity

Select each image by the assigned name from the Names and Types module.

- Images
- Metadata
- NamesAndTypes
- Groups
- IdentifyPrimaryObjects
- MeasureObjectIntensity **MeasureObjectIntensity**
- MeasureObjectSizeShape
- ConvertObjectsToImage
- SaveImages
- ExportToSpreadsheet
- ExportToSpreadsheet

Select an image to measure A13\_GRZB (from NamesAndTypes)  
Remove this image

Select an image to measure A14\_HLAll (from NamesAndTypes)  
Remove this image

Select an image to measure A15\_Ki67 (from NamesAndTypes)  
Remove this image

Select an image to measure A16\_PANCK (from NamesAndTypes)  
Remove this image

Select an image to measure A17\_PD1 (from NamesAndTypes)  
Remove this image

Select an image to measure A18\_PDL1 (from NamesAndTypes)  
Remove this image  
Add another image

Select objects to measure Nuclei (from IdentifyPrimaryObjects)  
Add another object

View output settings  
Adjust modules: + - ^ v  
Start Test Mode Analyze Images Found 38 rows

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## MeasureObjectSizeShape

Select the Nuclei (Primary Objects) to measure area of each nuclei cell object.

Zernike features are optional.

- Images
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- NamesAndTypes
- Groups
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- MeasureObjectSizeShape **MeasureObjectSizeShape**
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Select objects to measure Nuclei (from IdentifyPrimaryObjects)  
Add another object

Calculate the Zernike features?  Yes  No

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## ConvertObjectsToImage

This module converts the binary mask with identified primary objects to a labeled mask.

Selecting uint16 allows for a maximum of  $2^{16}$  labeled nuclei objects per image.

The downstream gating in FCS Express Image Cytometry is limited to 16 bit labeled image to visualize object masking, however if this is not utilized, uint32 could be selected to process larger regions.

- Images
- Metadata
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- ConvertObjectsToImage **ConvertObjectsToImage**
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Select the input objects Nuclei (from IdentifyPrimaryObjects)

Name the output image Nuclei

Select the color format uint16

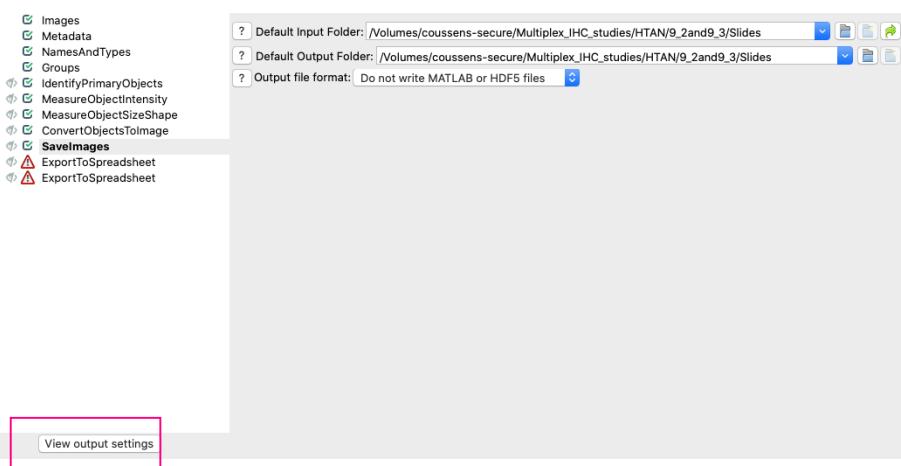
The output image name must be the same name as the input objects.

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## Output Settings

Output settings can be found on the bottom of the modules list.

The default input folder should be the path location of the input files: the parent study folder location. The default output folder should be the same parent folder.

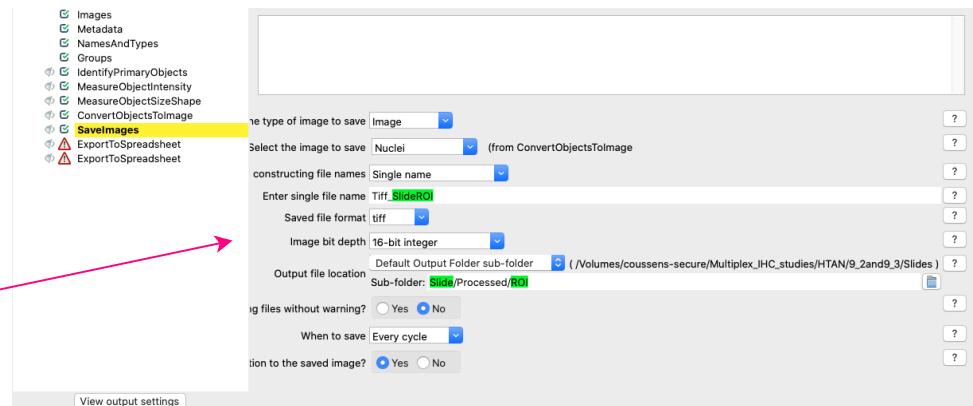


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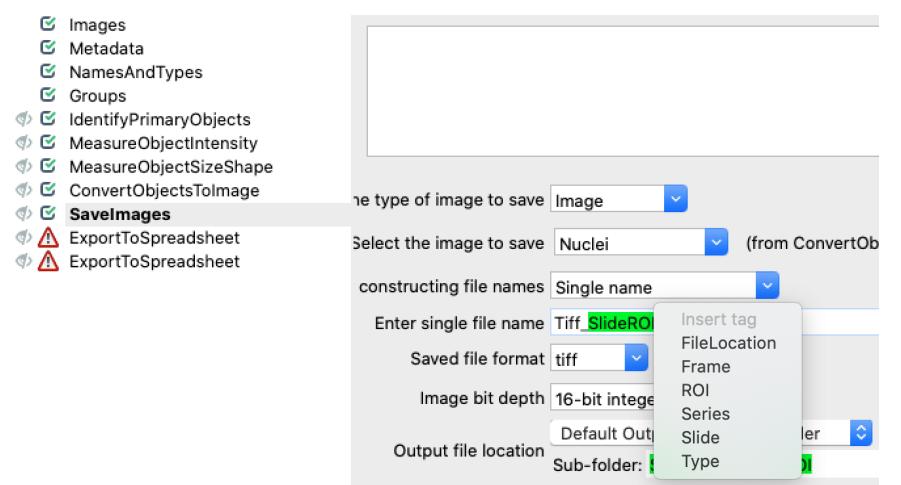
## SaveImages

This module saves the labeled image (converted objects to image) as an image such that each individual nuclei object appears indexed in grayscale.

For the output file location, the default output folder should be the parent folder, followed by the metadata Slide tag, then Processed folder, and the metadata ROI



The green highlighted parts of the name are linked to metadata. Right click in the naming bar to get a list of metadata items to include a metadata tag in the filename.

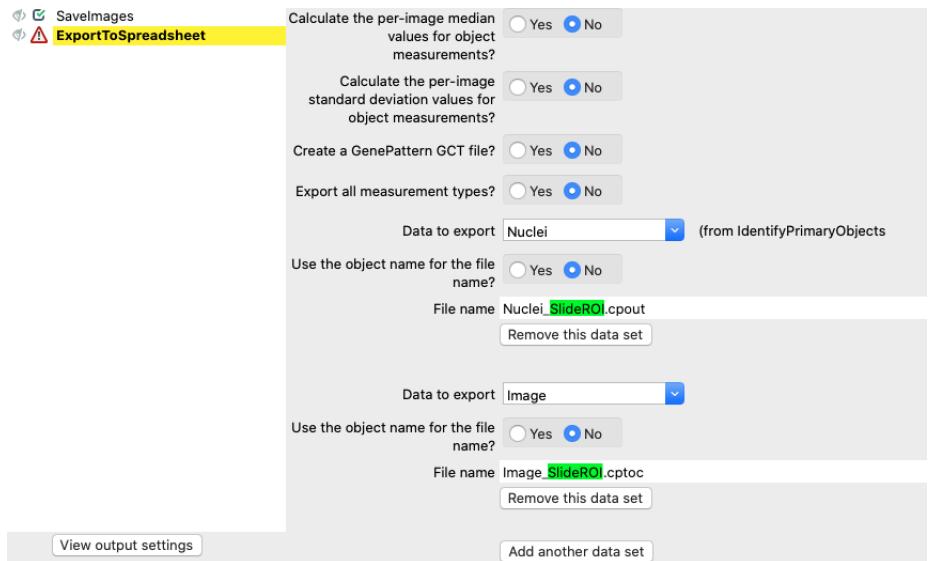


## ExportToSpreadsheet

Export to spreadsheet module allows you to select which data you would like to export. There are two main data to export for image cytometry in FCS Express: measurements from the Nuclei (primary objects) measurements, and Image data.

For naming these data exports, the data must be in the exact format of:  
`<[data]\_SlideROI.cfout>  
`<Image\_SlideROI.cptoc>

You can also optionally export a regular CSV.



Select measurements to export by clicking the button and selecting all data within 'Image' and the following for Nuclei:

AreaShape:

- Area

Intensity

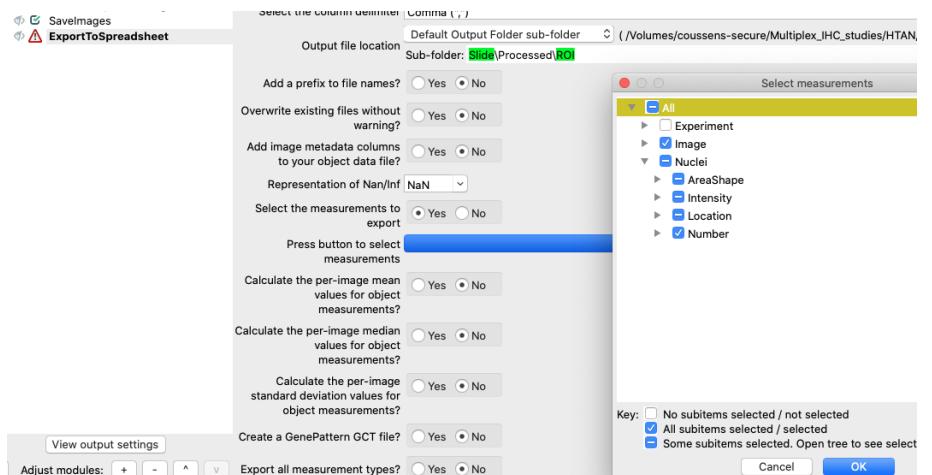
- Mean Intensity (all markers)

Location:

- Center X

- Center Y

Number



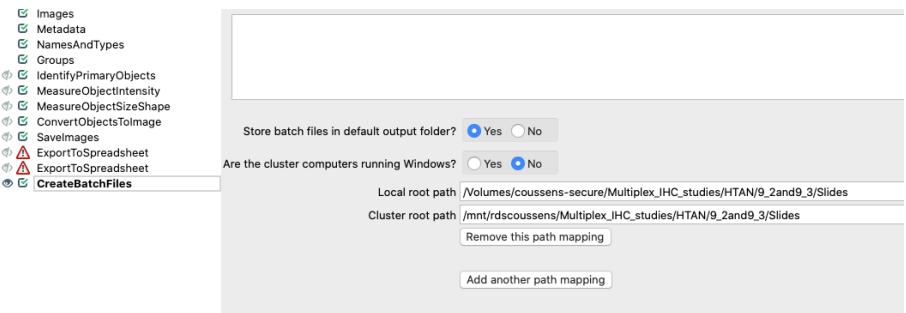
## For command line run only

If you are running CP in command line, you have loaded ALL processed folders from the entire cohort.

The local root path should be the same as the parent input folder of the images i.e. the parent directory of the cohort.

The cluster root path is how the path is read from the cluster. If you are running in batch locally, the cluster and local root path are the same.

With CreateBatchFiles module checked on, upon clicking Analyze, CP will generate a Batch\_data.H5 file that can be used to run all image sets in one command.



### Cell Profiler command line run:

```
cellprofiler -p </your/cluster/root/path/to/Batch_data.h5> -c -r -f <first image index> -l <last image set>
```

Example:

```
cellprofiler -p /mnt/rdscoussens/Multiplex_IHC_studies/HTAN/9_2and9_3/Slides/Batch_data.h5 -c -r -f 1 -l 150
```