

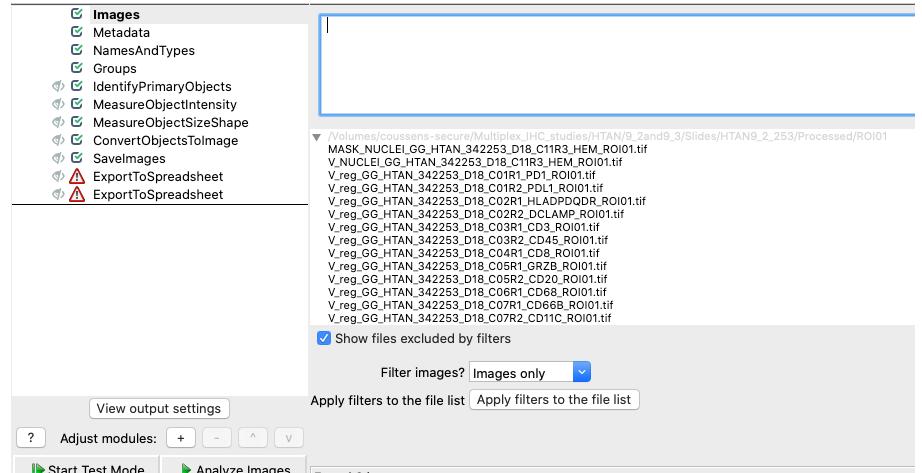
# Cell Profiler Instructions for use in FCS Express for miHHC Image Cytometry

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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.

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.

## 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.

The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.

Assign a name to Images matching rules

Process as 3D? Yes No

Select the rule criteria File Does Contain C11R3  
File Does Start with V\_NUCLEI

to assign these images A10\_DNA

Select the image type Grayscale image

Set intensity range from Image metadata

Select the rule criteria File Does Contain C01R2

to assign these images A18\_PDL1

Select the image type Grayscale image

	A01_CD11c	A02_CD163
1	V_reg_GG_HTAN_342253_D18_C07R2_CD11C_ROI01.tif	V_reg_GG_HTAN_342253_D18_C08R1_CD163-001_ROI01.tif
2	V_reg_GG_HTAN_342253_D18_C07R2_CD11C_ROI02.tif	V_reg_GG_HTAN_342253_D18_C08R1_CD163-001_ROI02.tif

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.

## 4

## 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.

The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.

Do you want to group your images? Yes No

Metadata category: Slide

Metadata category: ROI

Add another metadata item

Grouping list	Group: Slide	Group: ROI	Count
1	HTAN9_2_253	ROI01	1
2	HTAN9_2_253	ROI02	1

	Group number	Group index	Group: Slide	Group: R
1	1	1	HTAN9_2_253	ROI01
2	2	1	HTAN9_2_253	ROI02

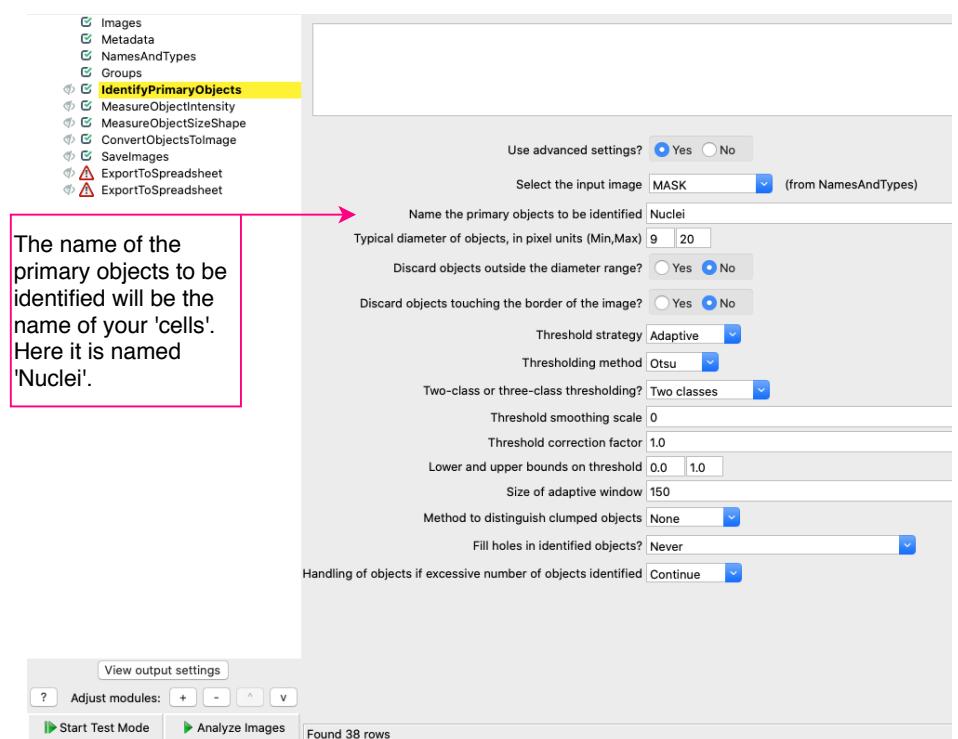
Image sets

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## IdentifyPrimaryObjects

Primary objects are the nuclei objects identified in the binary mask during processing. Use the binary nuclei mask as the input image.

Use the advanced settings to make sure all settings are set to NOT modify the input binary mask.

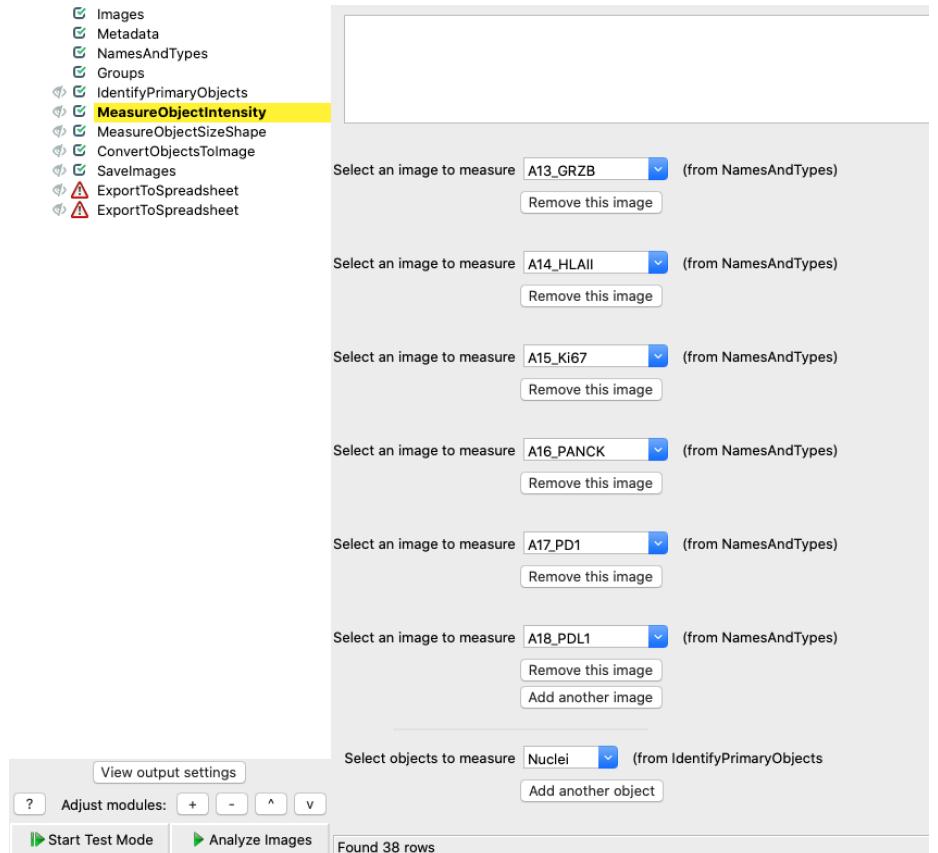


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## MeasureObjectIntensity

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

Each image selected will be have intensity measurements taken for every object (Nuclei, from IdentifyPrimaryObjects).



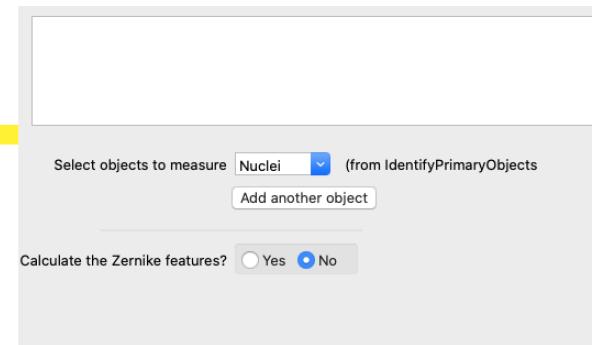
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## MeasureObjectSizeShape

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

Zernike features are optional.

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



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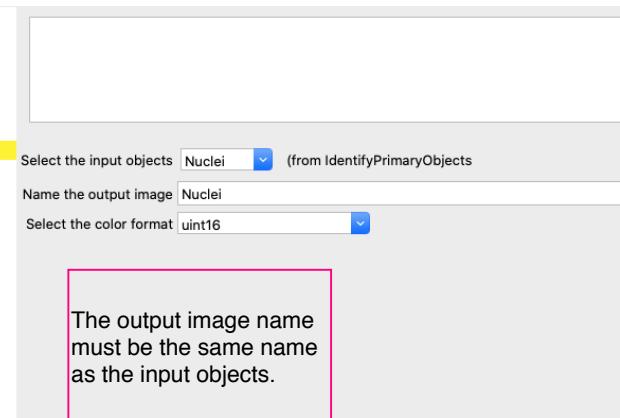
## ConvertObjectsToImage

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
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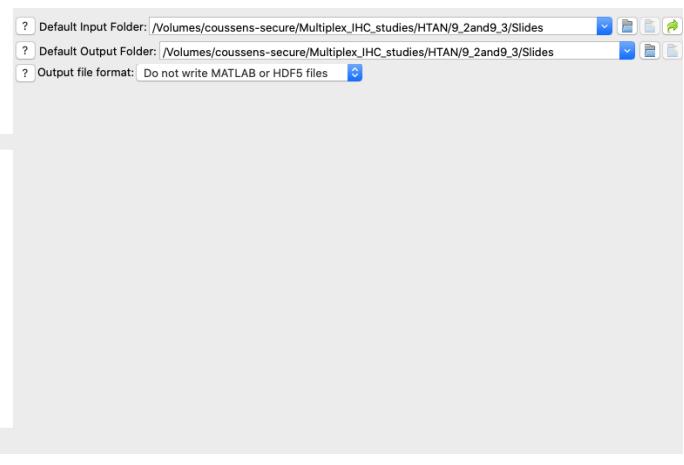
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## Output Settings

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.

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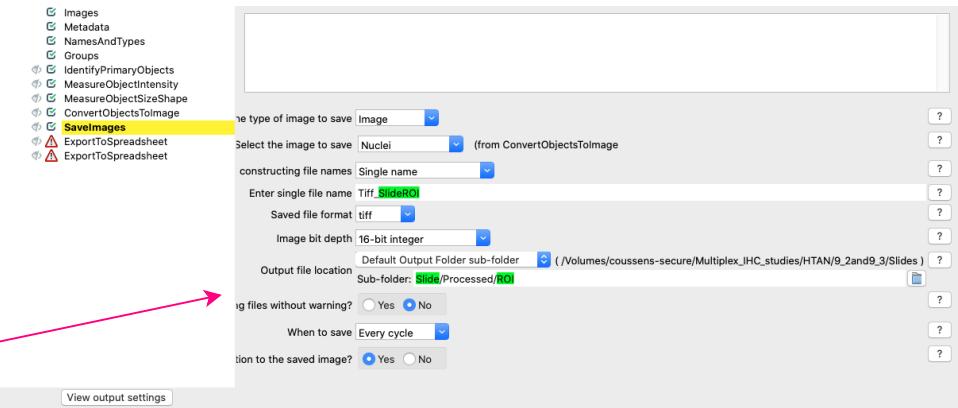


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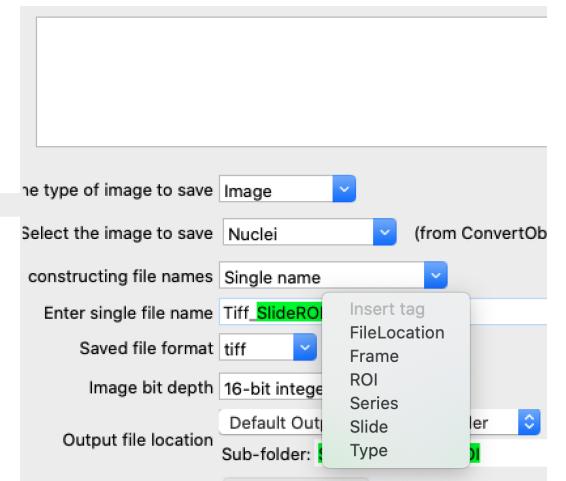
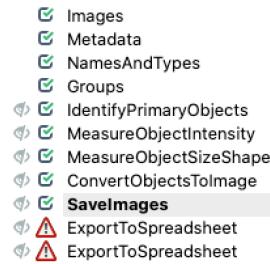
**SavelImages**

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.



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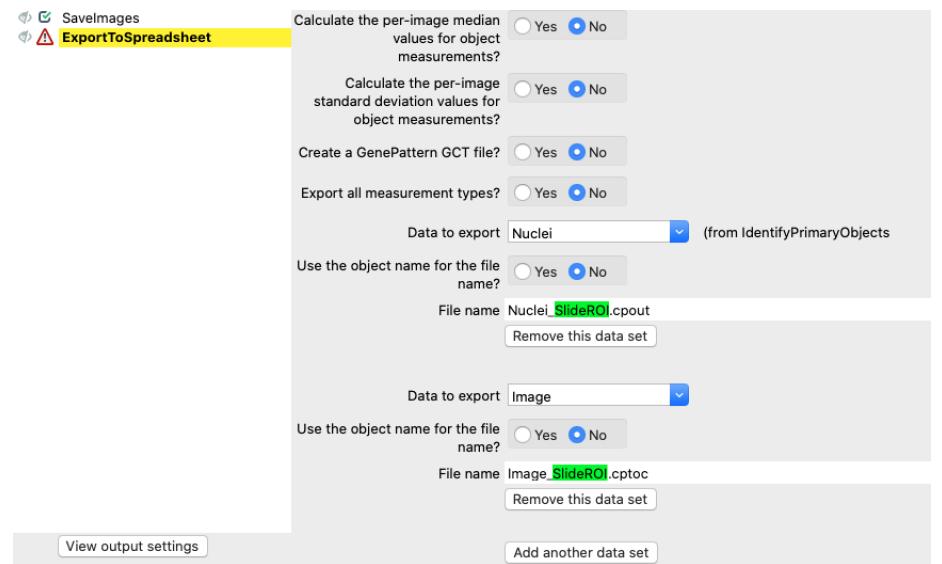
**ExportToSpreadsheet**

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

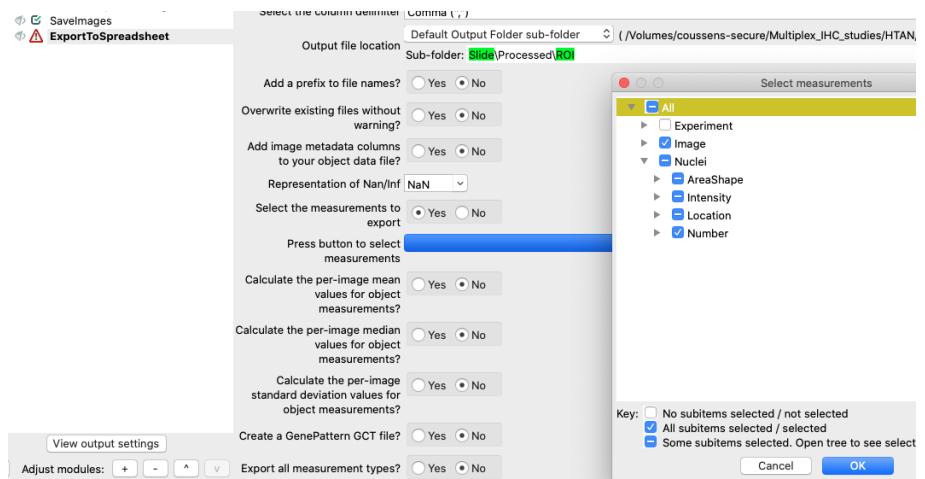
- Mean Intensity (all markers)

Location:

- Center X

- Center Y

Number



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## Create Batch Files

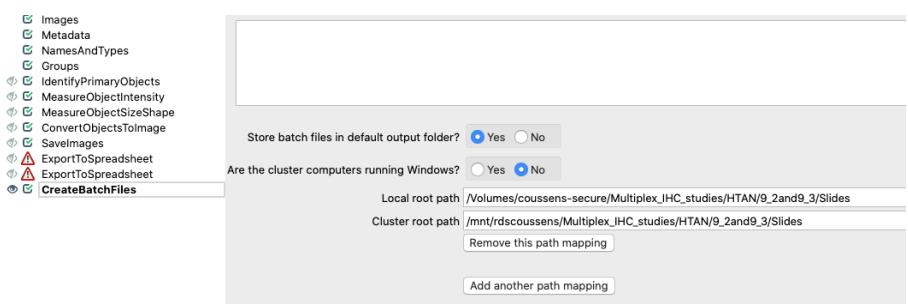
### For command line run only

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
```