

King's College London : Wohl Cellular Imaging Centre

NIS-Elements General Analysis Help Guide

Automated Cell Counting

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4-6-2020

In this example, I want to count the number, the area and the fluorescent intensity of DAPI stained nucleus in my image.

To do this we use a technique called thresholding, this allow you to define parameters such as signal intensity, shape and size, so you can “teach” the software what to count as DAPI and what to exclude.

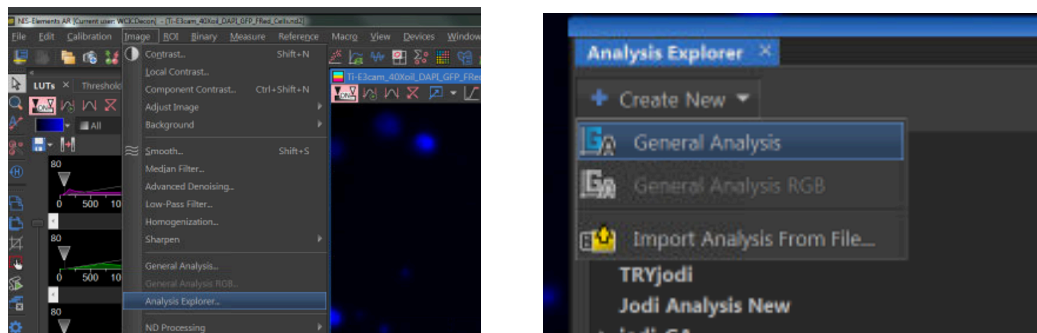
If you have multiple images you want to compare, you should use the same thresholding parameters on all of them, this eliminates bias.

STEP 1 : Setting up General Analysis

Move your images onto the local computer and open your image in NIS-elements.

Go to Image > open Analysis Explorer...

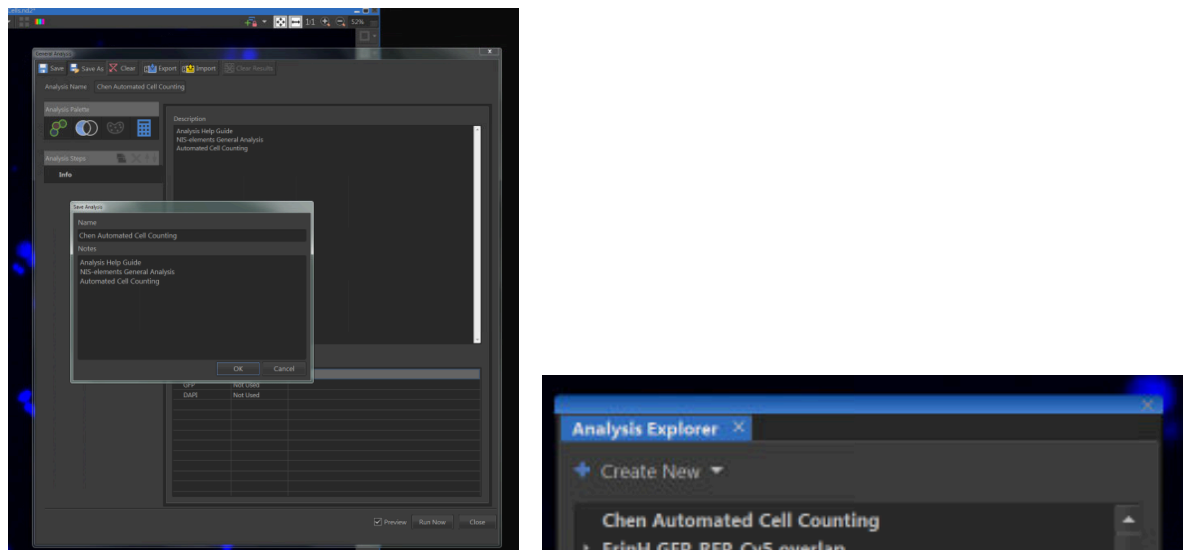
In Analysis Explorer > create new > General Analysis



You are now in General Analysis

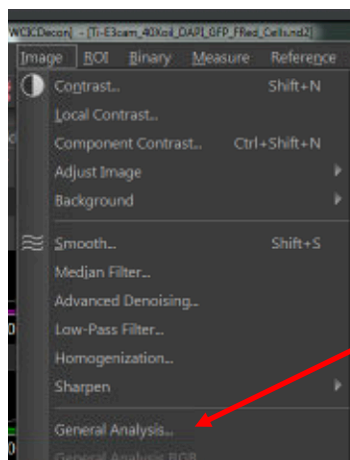
“Save As” and keep saving as you make changes to this General Analysis, so you can reuse these settings on comparable images.

You can find your saved settings in Analysis Explorer

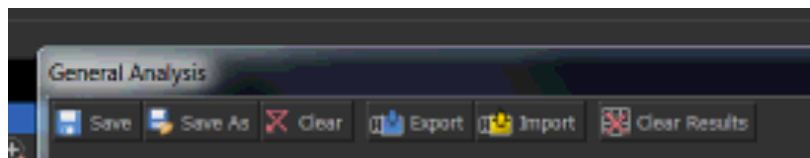


STEP 2 : Save As and Export General Analysis

Some workstations do not have Analysis Explorer, you will need to click on General Analysis instead.



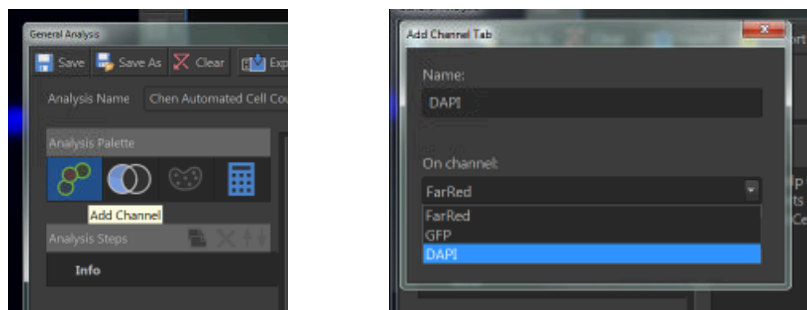
To use this General Analysis again, you need to... **“SAVE AS”** and **“EXPORT”**



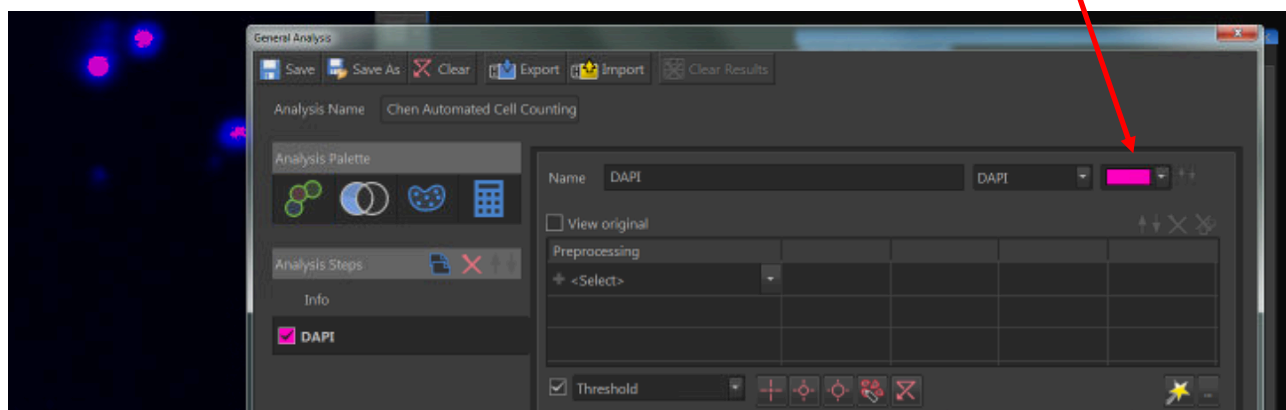
- 1) Make changes to General Analysis parameters as demonstrated in this guide.
- 2) **BEFORE clicking on “Run Now” ... Click on “SAVE AS”**
- 3) Give your General Analysis a name and description.
- 4) **Click on “Export”**, now export your saved General Analysis into a suitable folder.
- 5) You can move it to one of your folders in the WCIC shared network drive, if you want access to this General Analysis on multiple workstations.
- 6) If you make any changes and use the “save” button, just remember to export again.

STEP 3 : Add channel

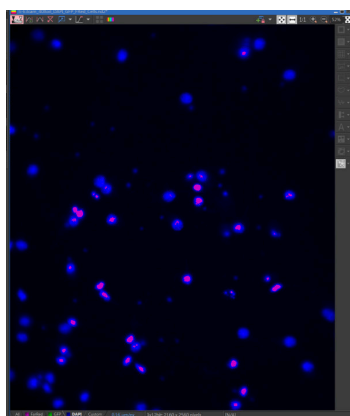
I want to count the number of nucleus in my image stained with DAPI, so I need to add the DAPI channel.



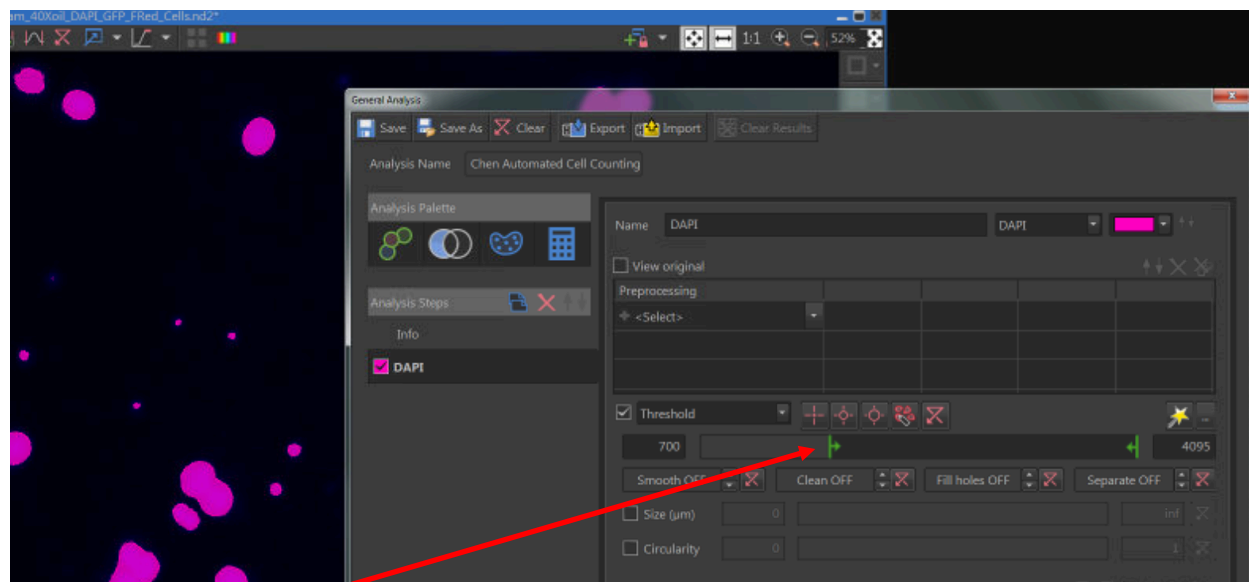
Once DAPI channel is added, this different colour represents what the software considers DAPI depending on thresholding parameters. You can change this colour here.



STEP 4 : Thresholding - define signal intensity to include all DAPI

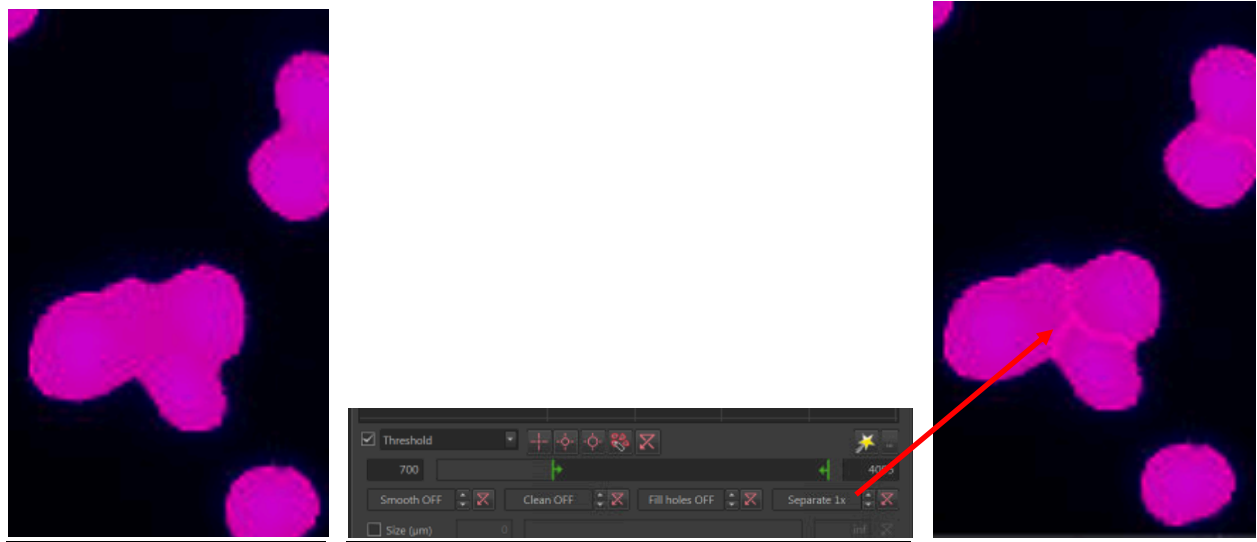


Here not all DAPI have been included in the threshold.



You can drag these bars to define the minimum and maximum DAPI signal intensity, I have dropped my min intensity to 700 to include all the DAPI signal, I am able to do this because I have enough signal intensity difference between my minimal DAPI signal and my background intensity (around 200).

STEP 5 : Thresholding – Smooth / Clean / Fill holes / Separate

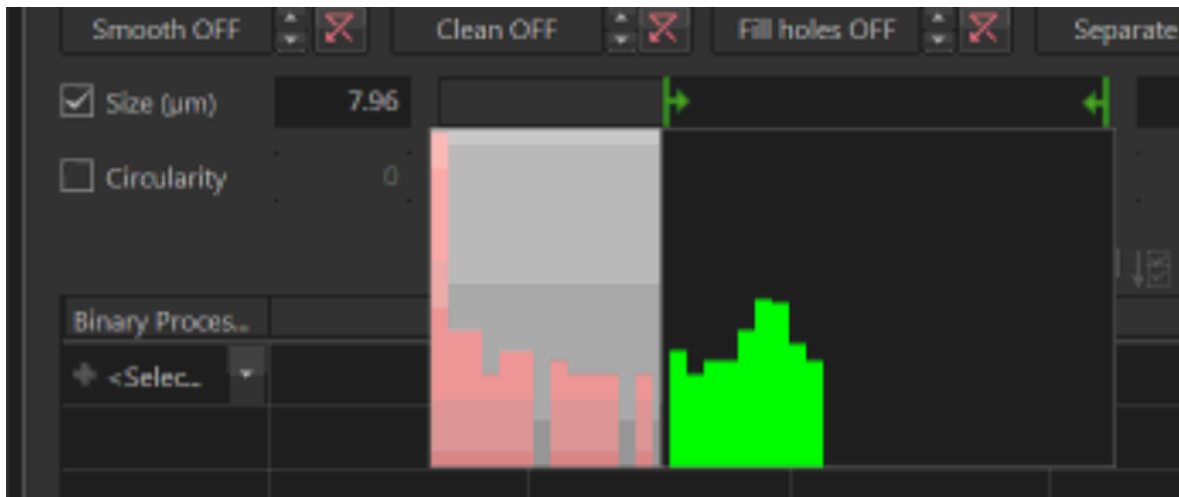
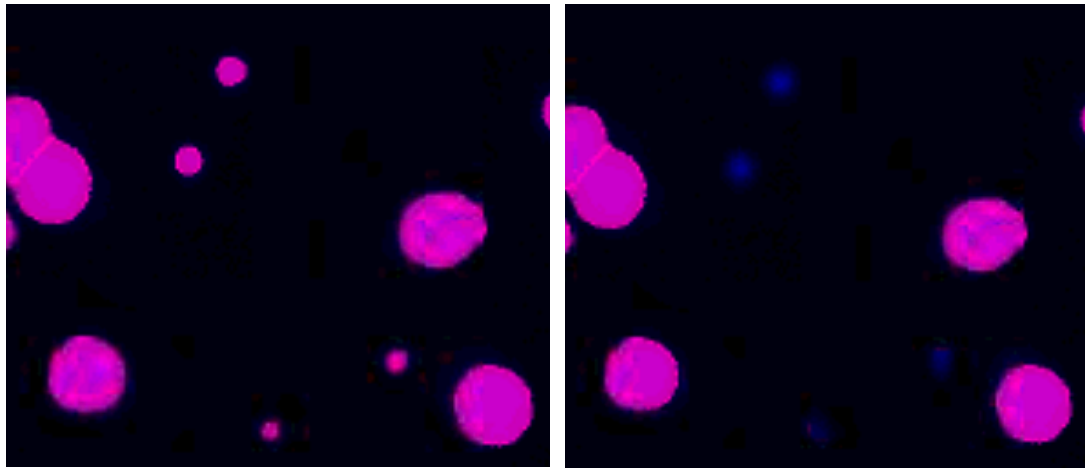


In this example, some of my DAPI are too close to each other and I need to separate them, so they are identified as individual nucleus.

There are a variety of tools in threshold to make your counting more accurate. Here I'm going to use separate, try different versions of separate by clicking on the arrows, find the best one suited to your needs.

You can use smooth and clean to better define your object edges, if you have porous or gaps between objects which you would like to ignore, please use fill holes.

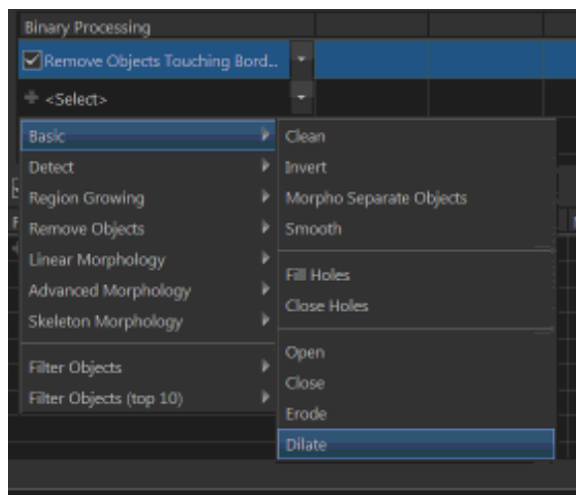
STEP 6 : Thresholding – Size / Circularity



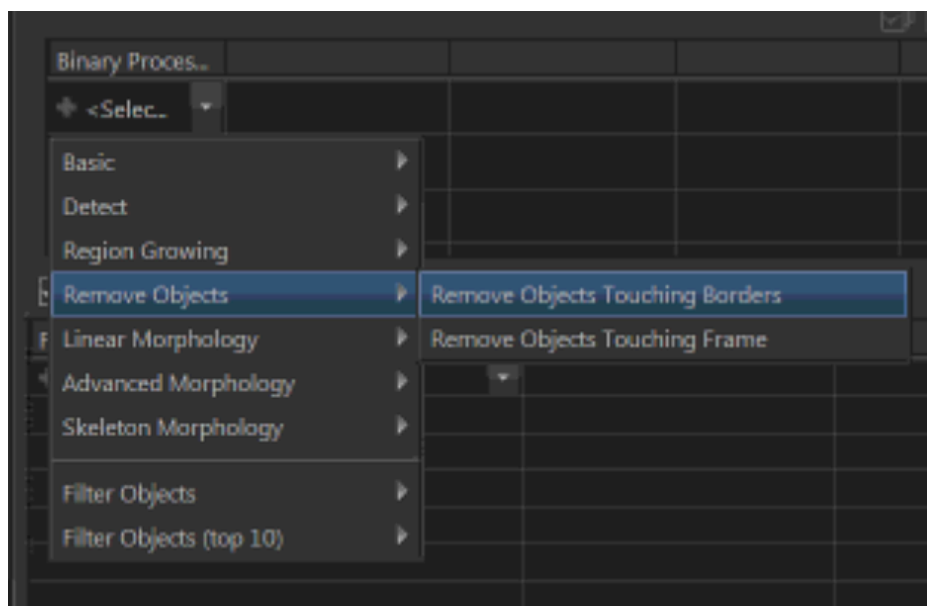
In this example, I have some debris in my field of view also stained with DAPI, I can exclude these using size thresholding.

You can adjust circularity, to exclude objects depending on how circular they are in shape.

STEP 7 : Binary Processing



Binary processing contains further thresholding parameter to include more signal (Open, Dilate) or exclude signal (Close, Erode).

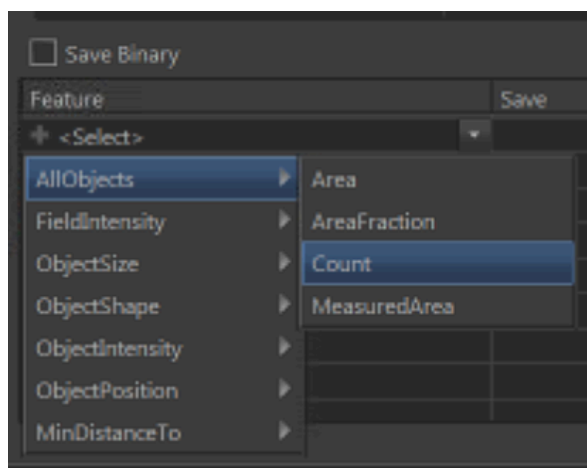


Other parameters can be applied to make your measurements more accurate. In this example, I want to remove objects touching borders because any nucleus touching the boarder may misrepresent the true area of the nucleus.

STEP 8 : Feature Selection

In “Feature” you can select different measurements you want to take depending on the purple coloured binary layer you just created. You DO NOT need to select “Save Binary” to make any measurements.

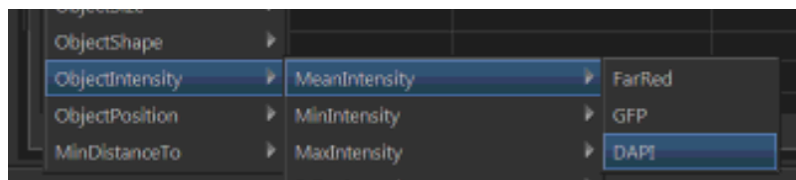
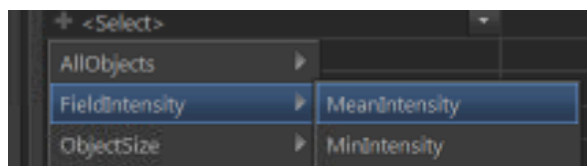
I recommend not ticking the “Save Binary” box, this will allow you more flexibility, such as to make changes to the analysis when you optimise batch processing.



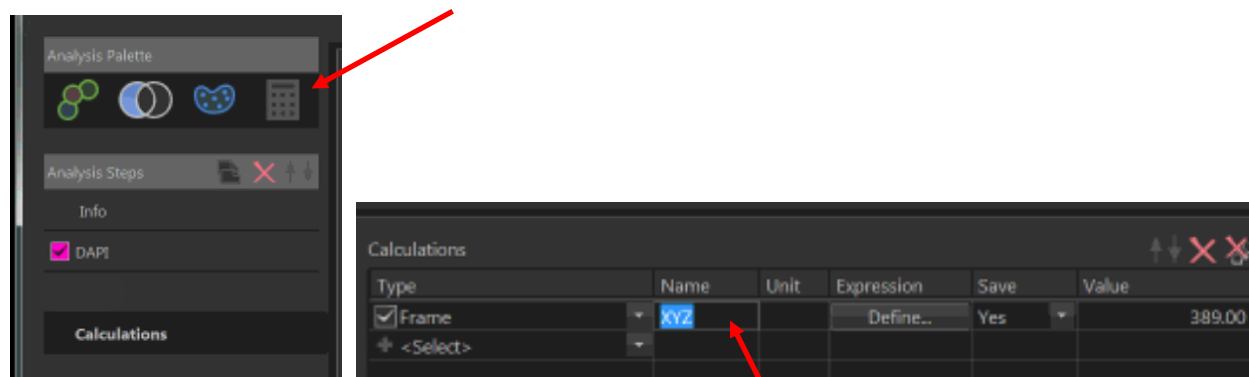
What to measure?

The drop-down list will provide a number of possible measurements, if you want the overall mean intensity, you can select “FieldIntensity > MeanIntensity”. However, if you want the mean intensity of each individual DAPI / object in a list, then select “ObjectIntensity > MeanIntensity > DAPI”

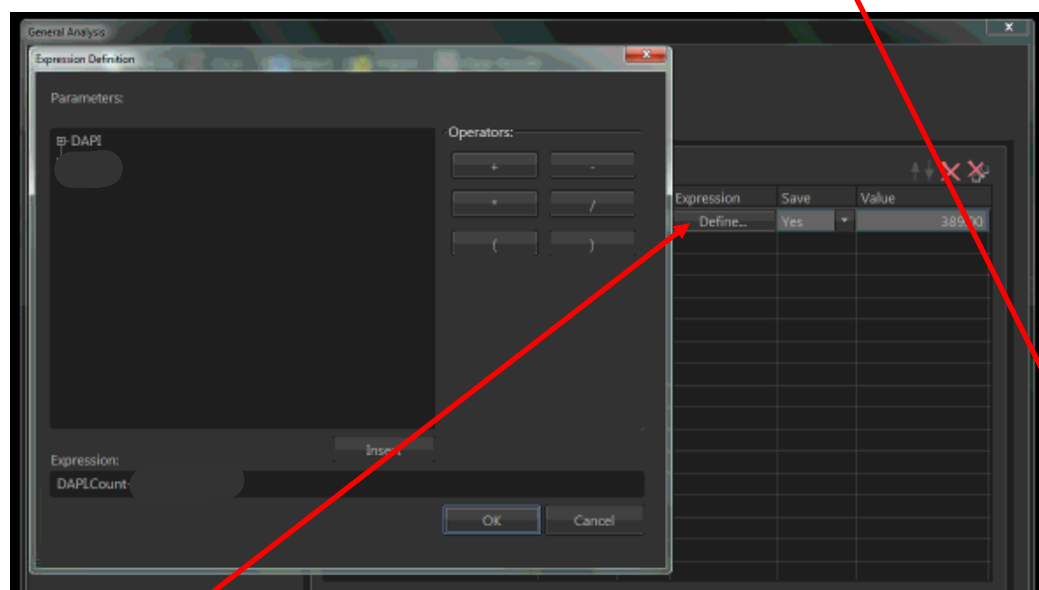
Similar principles apply to other measurements such as area, shape, distance, etc.



STEP 9 : The Calculations Tab (Optional)

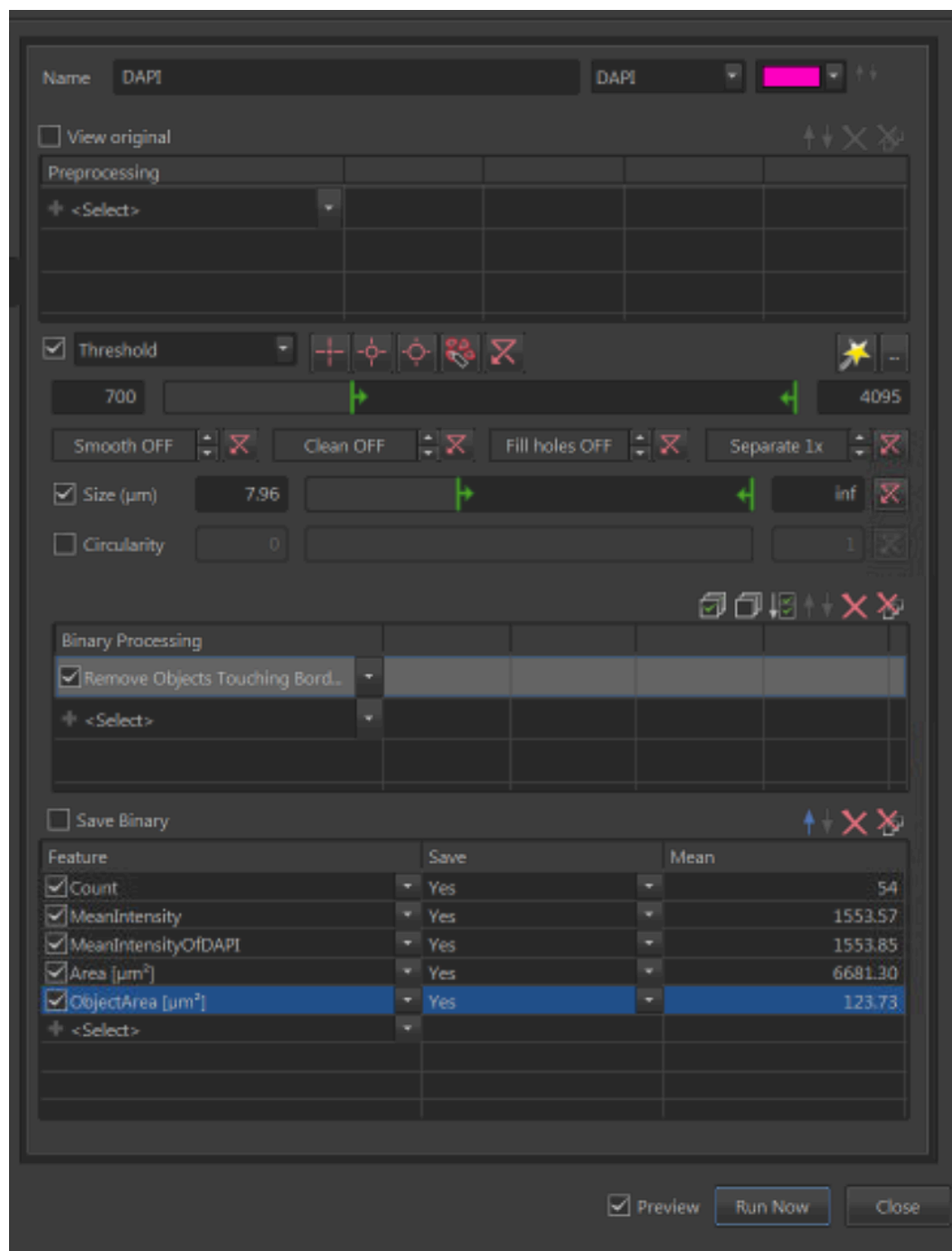


The Calculations Tab is a good tool for labelling measurements so it is easier to find in the list of results the software will give you after you run the general analysis.



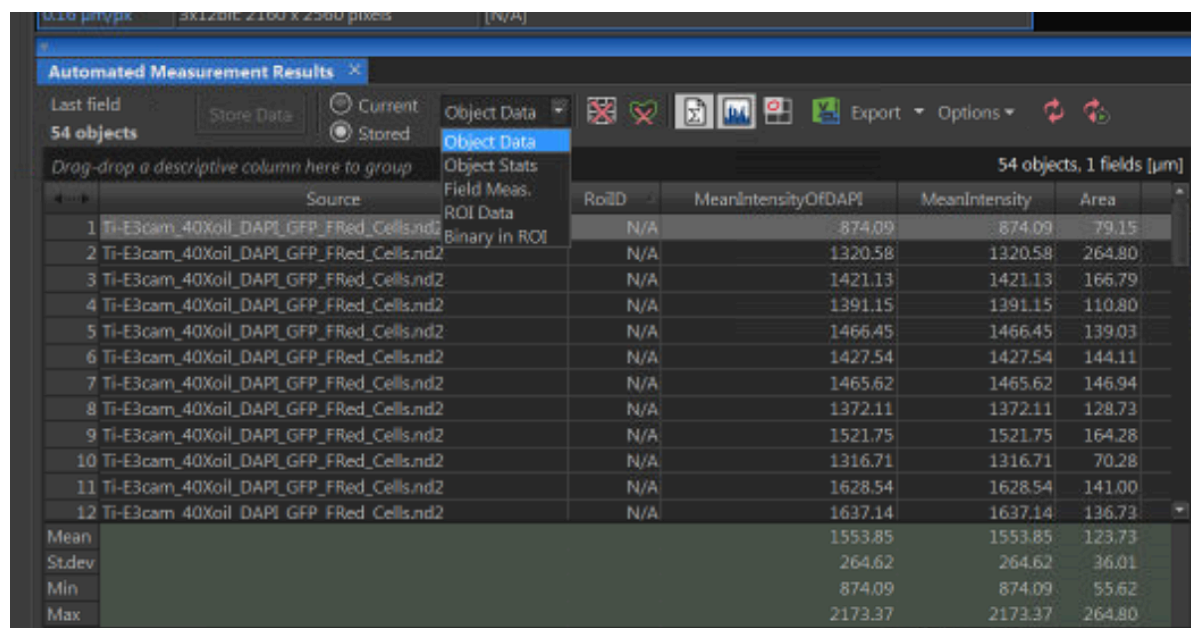
Define what particular measurements you want to take from any of the ticked feature selections you've made in the last step. Then give your defined measurement a name.

STEP 10 : Run Now (“Save As” and “Export” before “Run Now”)



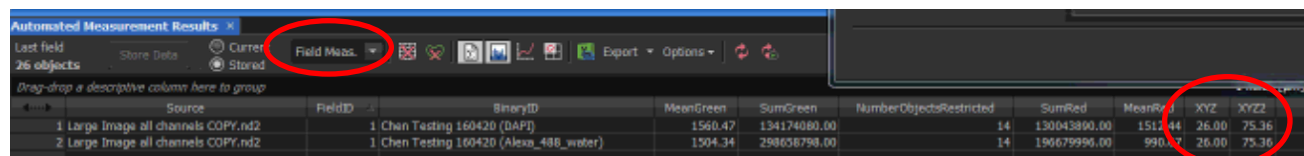
Clicking on “Run Now” will measure the features you’ve selected.

STEP 11 : Automated Measurement Results



The screenshot shows the 'Automated Measurement Results' window with the 'Object Data' tab selected. The window displays a table of measurements for 54 objects. The table has columns for Source, FieldID, MeanIntensityOfDAPI, MeanIntensity, and Area. The data is summarized in a table below.

| Source | FieldID | MeanIntensityOfDAPI | MeanIntensity | Area |
|--|---------|---------------------|---------------|--------|
| 1 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 874.09 | 874.09 | 79.15 |
| 2 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1320.58 | 1320.58 | 264.80 |
| 3 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1421.13 | 1421.13 | 166.79 |
| 4 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1391.15 | 1391.15 | 110.80 |
| 5 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1466.45 | 1466.45 | 139.03 |
| 6 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1427.54 | 1427.54 | 144.11 |
| 7 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1465.62 | 1465.62 | 146.94 |
| 8 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1372.11 | 1372.11 | 128.73 |
| 9 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1521.75 | 1521.75 | 164.28 |
| 10 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1316.71 | 1316.71 | 70.28 |
| 11 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1628.54 | 1628.54 | 141.00 |
| 12 Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1637.14 | 1637.14 | 136.73 |
| Mean | | 1553.85 | 1553.85 | 123.73 |
| Std.dev | | 264.62 | 264.62 | 36.01 |
| Min | | 874.09 | 874.09 | 55.62 |
| Max | | 2173.37 | 2173.37 | 264.80 |



The screenshot shows the 'Automated Measurement Results' window with the 'Field Meas.' tab selected. The window displays a table of measurements for 26 objects. The table has columns for Source, FieldID, BinaryID, MeanGreen, SumGreen, NumberObjectsRestricted, SumRed, MeanRed, XYZ, and XYZ2. The data is summarized in a table below.

| Source | FieldID | BinaryID | MeanGreen | SumGreen | NumberObjectsRestricted | SumRed | MeanRed | XYZ | XYZ2 |
|-------------------------------------|---------|--|-----------|--------------|-------------------------|--------------|---------|-------|-------|
| 1 Large Image all channels COPY.nd2 | 1 | Chen Testing 160420 (DAPI) | 1568.47 | 134174080.00 | 14 | 130043890.00 | 1511.44 | 26.00 | 75.36 |
| 2 Large Image all channels COPY.nd2 | 1 | Chen Testing 160420 (Alexa_488_waterr) | 1504.34 | 298636798.00 | 14 | 190679996.00 | 990.17 | 26.00 | 75.36 |

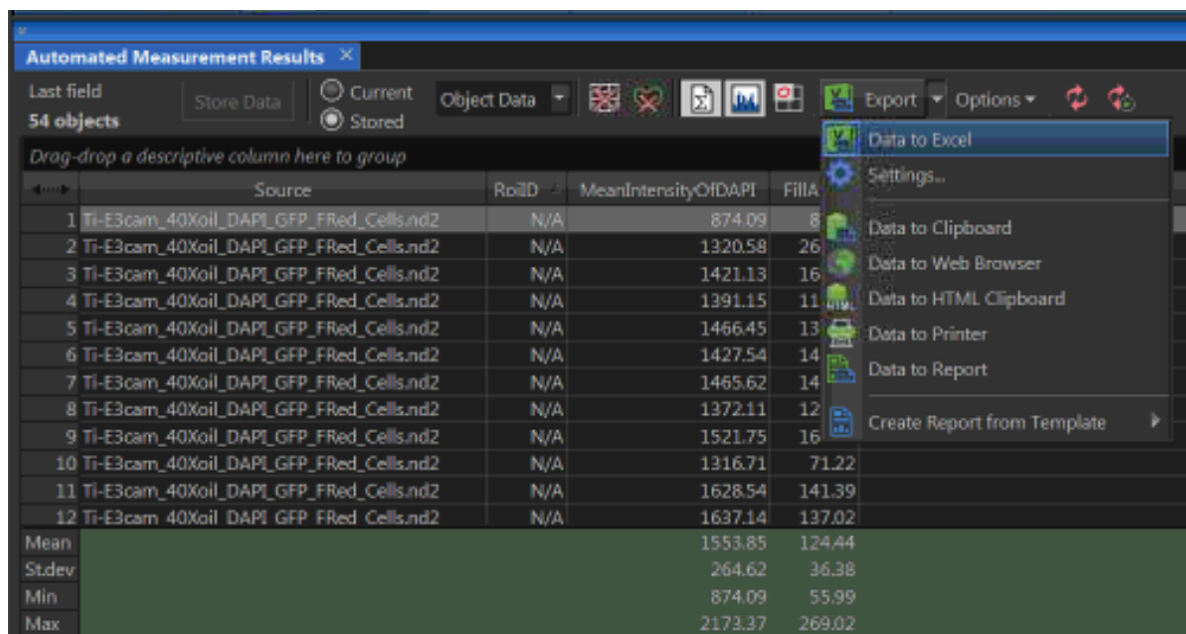
Results will be displayed in the automated measurement result tab.

Select which data set you would like to display. Object Data will provide measurements of each individual DAPI whereas Field Measurement will provide overall mean measurements.

Field Measurements also displays the measurements you defined and renamed in the Calculations Tab.

Whichever drop-down list you select now, will be what you export in the next step.

STEP 12 : Export



Automated Measurement Results

Last field: 54 objects

Store Data: Current (selected), Stored

Object Data

Export Options

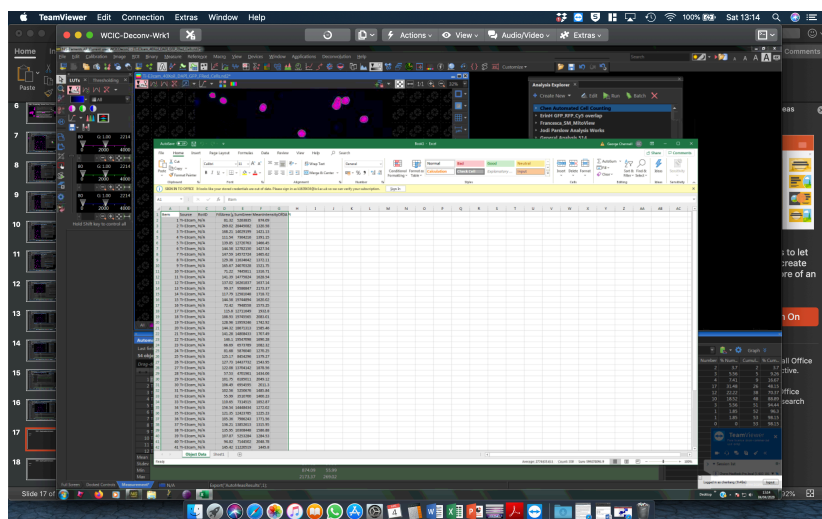
Drag-drop a descriptive column here to group

| | Source | ROIID | MeanIntensityOfDAPI | FILL |
|--------|---|-------|---------------------|--------|
| 1 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 874.09 | 8 |
| 2 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1320.58 | 26 |
| 3 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1421.13 | 16 |
| 4 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1391.15 | 11 |
| 5 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1466.45 | 13 |
| 6 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1427.54 | 14 |
| 7 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1465.62 | 14 |
| 8 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1372.11 | 12 |
| 9 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1521.75 | 16 |
| 10 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1316.71 | 71.22 |
| 11 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1628.54 | 141.39 |
| 12 | Ti-E3cam_40Xoil_DAPI_GFP_FRed_Cells.nd2 | N/A | 1637.14 | 137.02 |
| Mean | | | 1553.85 | 124.44 |
| Stddev | | | 264.62 | 36.38 |
| Min | | | 874.09 | 55.99 |
| Max | | | 2173.37 | 269.02 |

Export Options:

- Data to Excel
- Settings...
- Data to Clipboard
- Data to Web Browser
- Data to HTML Clipboard
- Data to Printer
- Data to Report
- Create Report from Template

Clicking on the drop down button to select the format you want to export your measurements, then click on the "Export" button.



TeamViewer

WIC-Decon-Wk1

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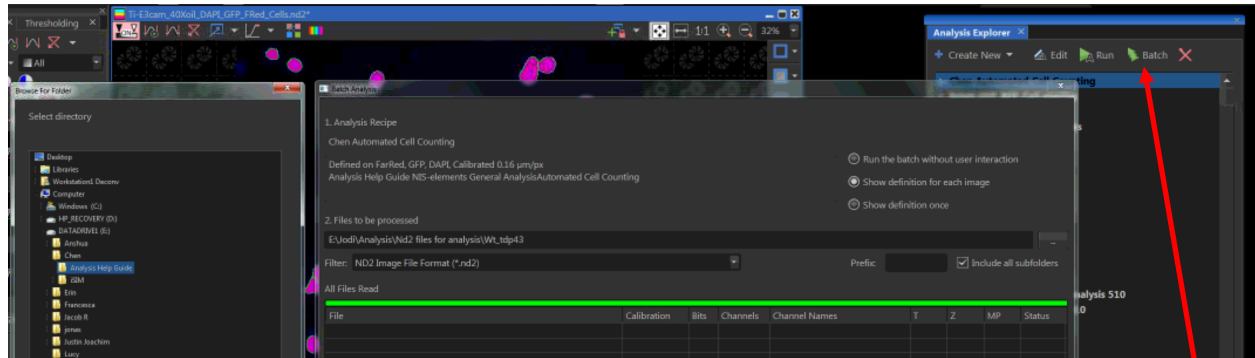
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Export to Excel

Exported to excel

STEP 13 : Batch Analysis



The final step is to apply this analysis to all the images you want to compare, using BATCH ANALYSIS.

For fast processing, please make sure the images are saved on the local computer (DATADRIIVE1).