

MetaMorph NX



MetaMorph NX

An Introduction to version 2.0

Agenda

- General overview of the MetaMorph NX
- 4D Viewer
- How to use some features in MetaMorph NX

435.3 Zoom To Fit Space 100% Zoom

Multi Wave Time Stage 0

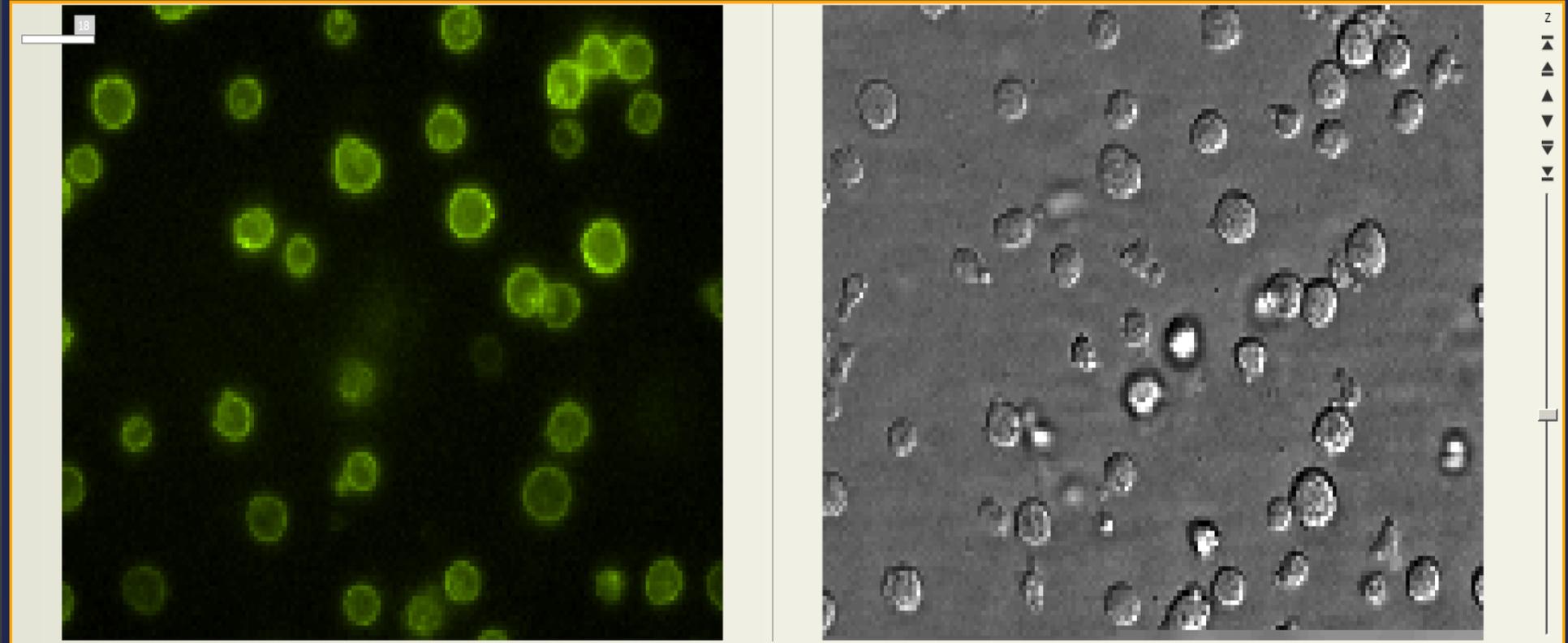
Single View View Mode Open in 4D Viewer Export to PowerPoint

Channel Display Channel Display Reset All Scaling GFP DIC

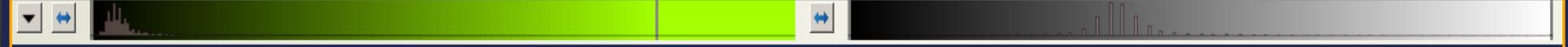
Reset Layout Layout

Object Overlay Threshold Overlay Zoom Preview Regions Calibration Bar Overlay

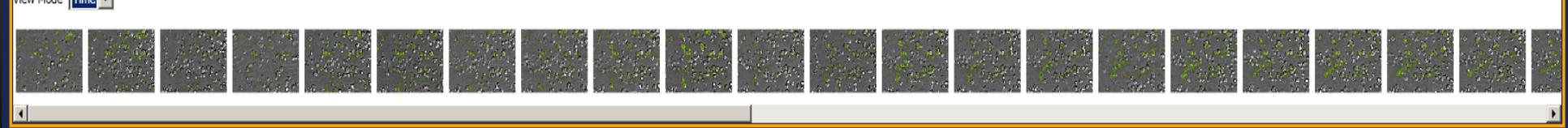
Multi Wave Time Z

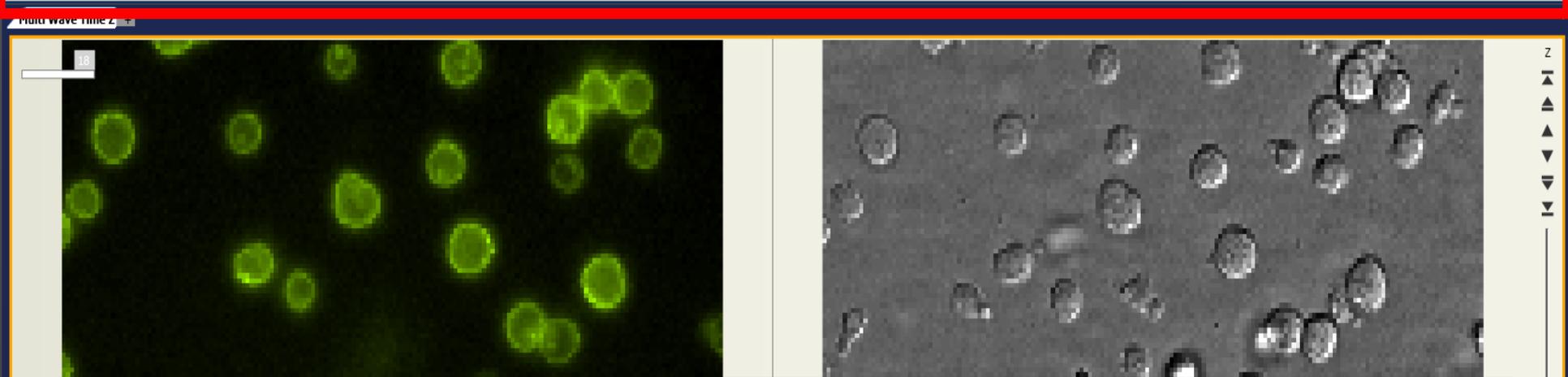


Time 0 of 0-44 Z 10 of 0-20 57, 58 13



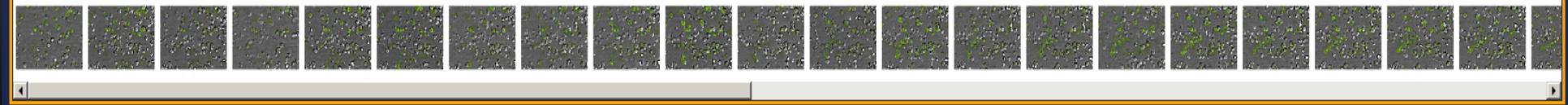
Filmstrip View Mode Time





The Ribbon Interface

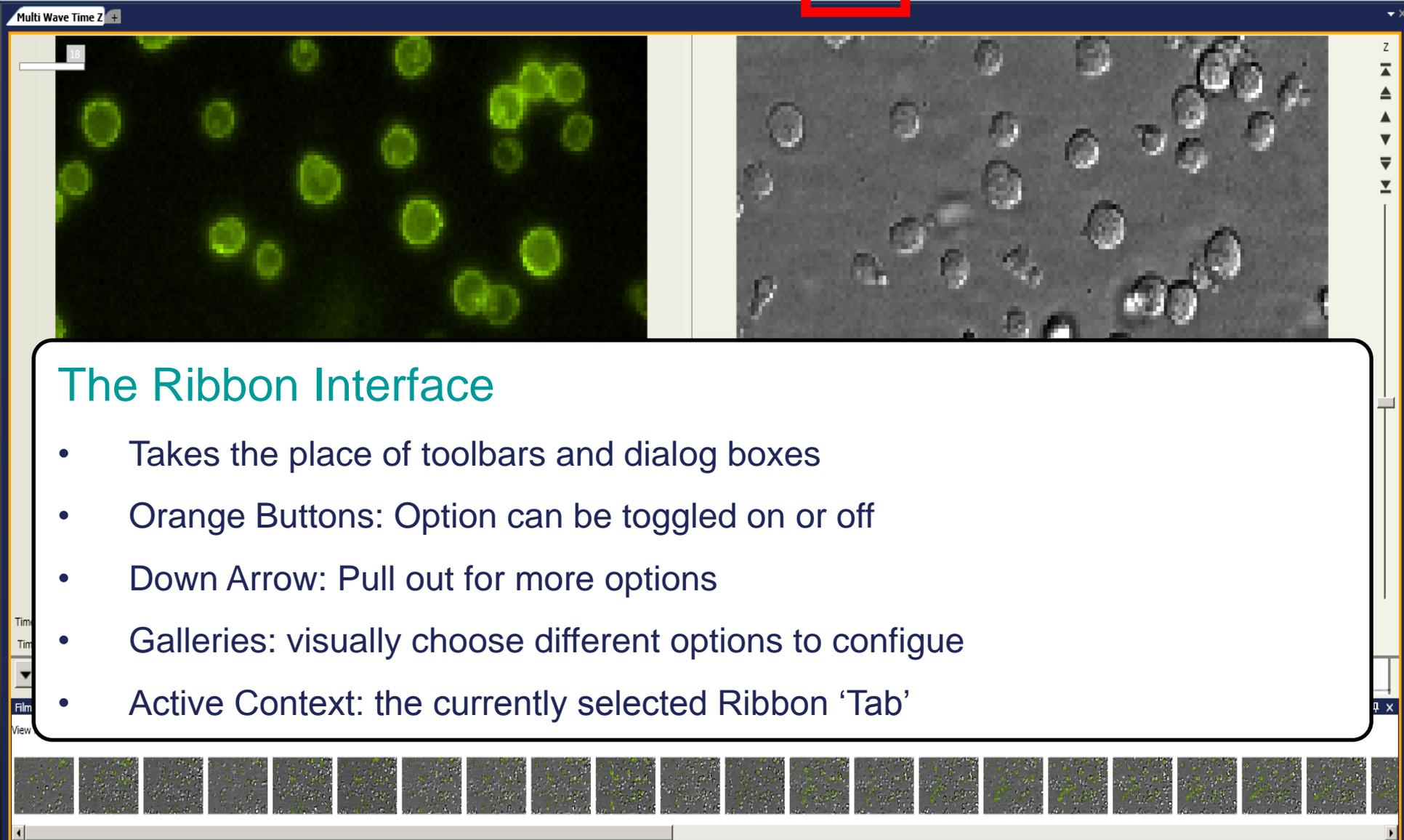
- Takes the place of toolbars and dialog boxes
- Orange Buttons: Option can be toggled on or off
- Down Arrow: Pull out for more options
- Galleries: visually choose different options to configure
- Active Context: the currently selected Ribbon 'Tab'





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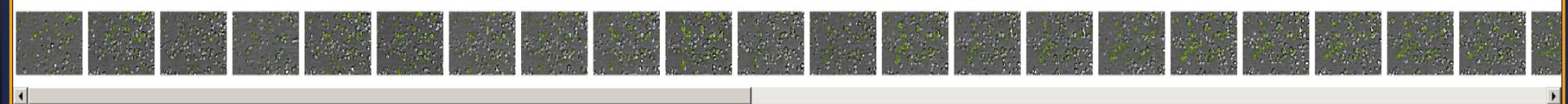
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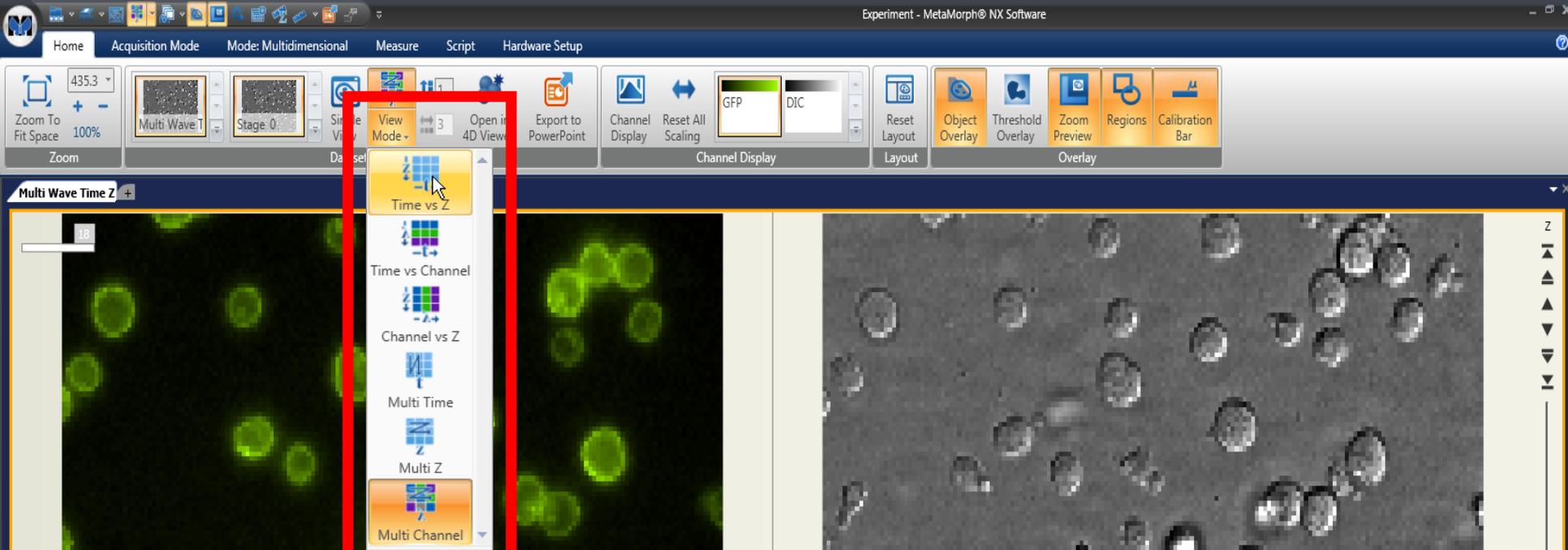




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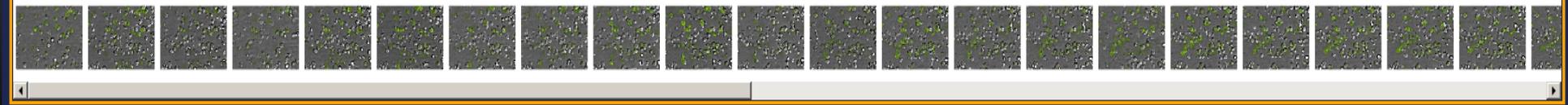
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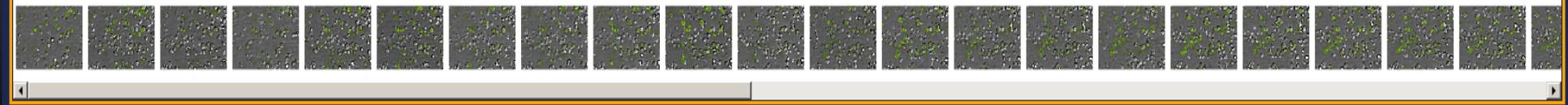
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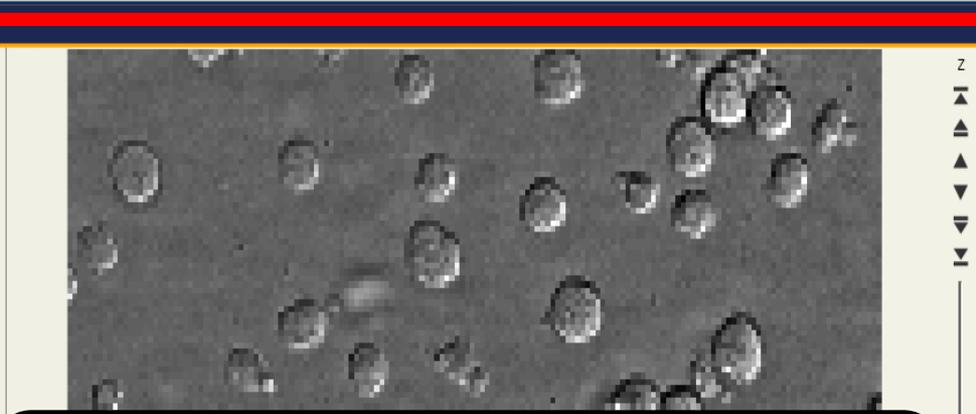
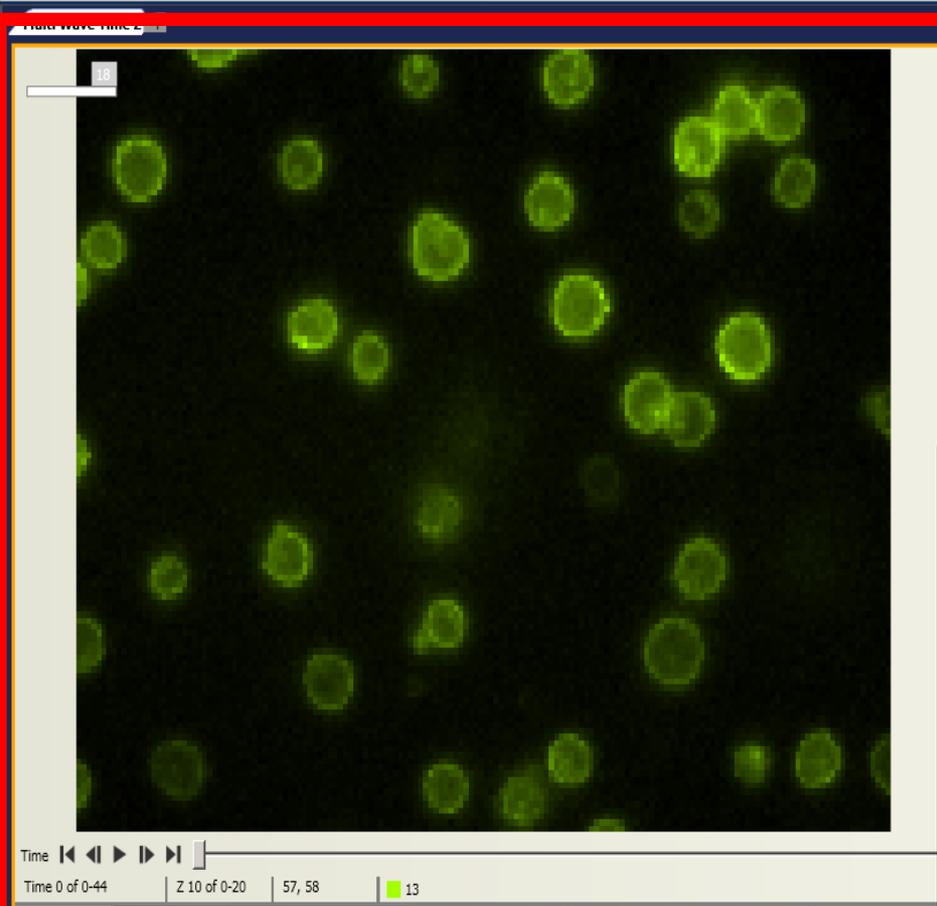




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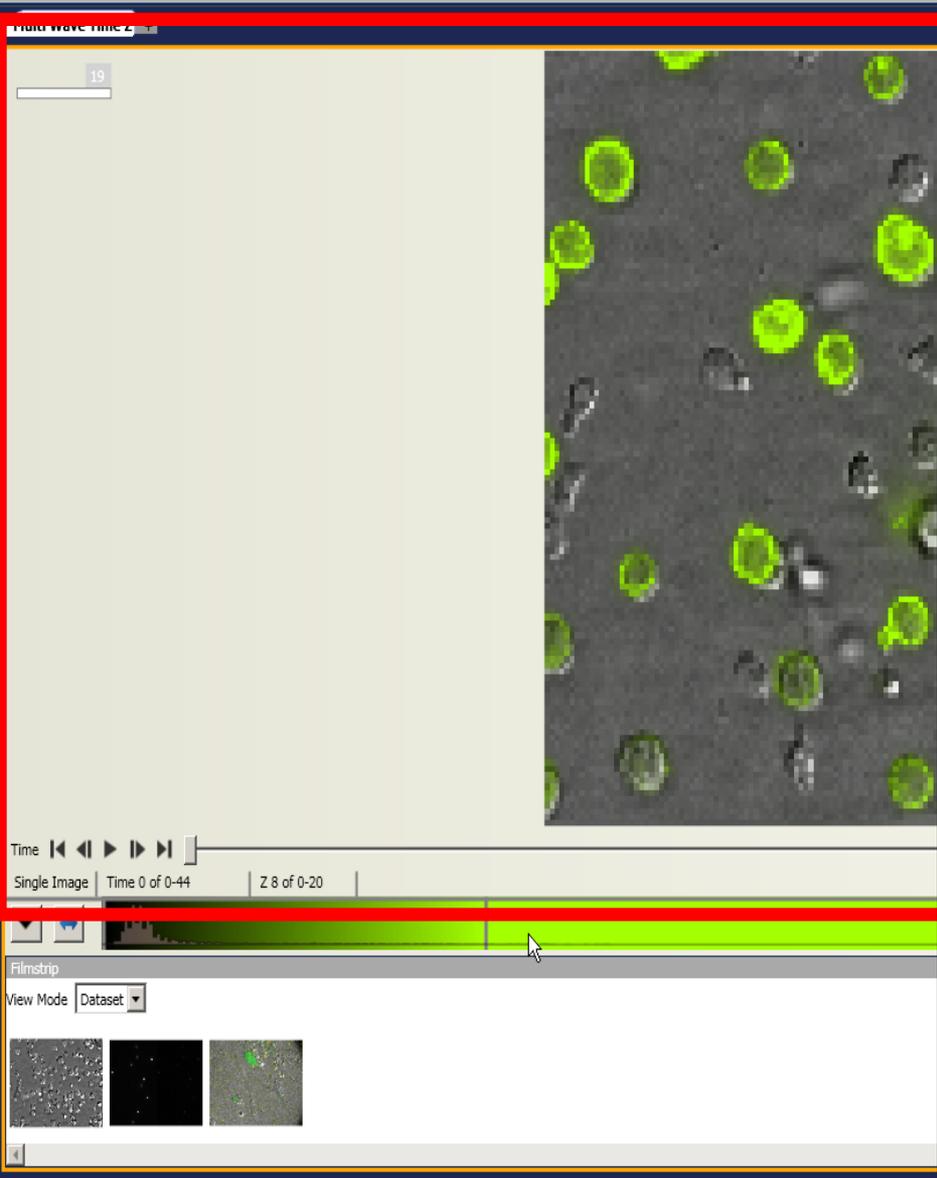
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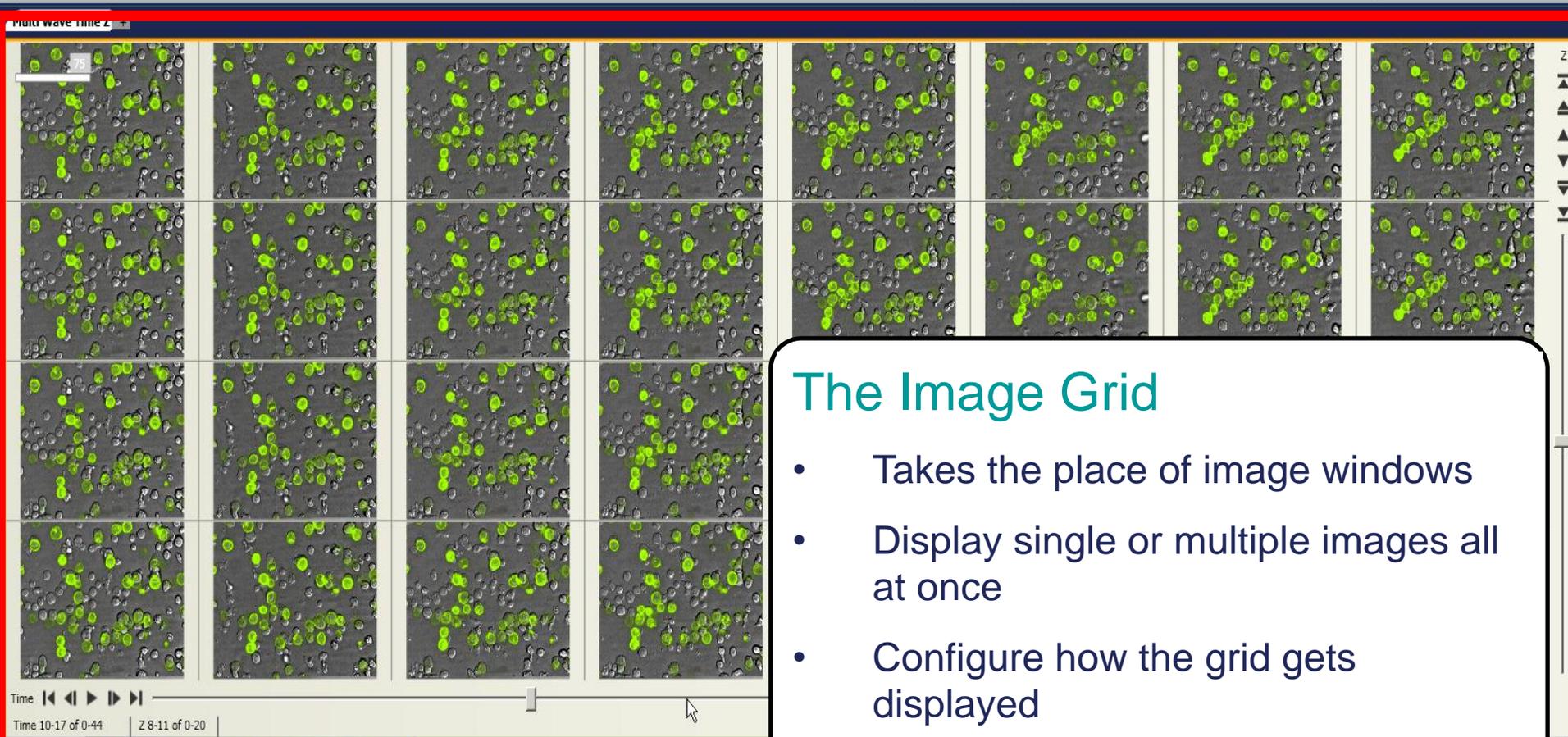
- ## The Image Grid
- Takes the place of image windows
 - Display single or multiple images all at once
 - Configure how the grid gets displayed
 - Choose the current Z or T being viewed using sliders
 - Displays which T, Z, Stage, being displayed





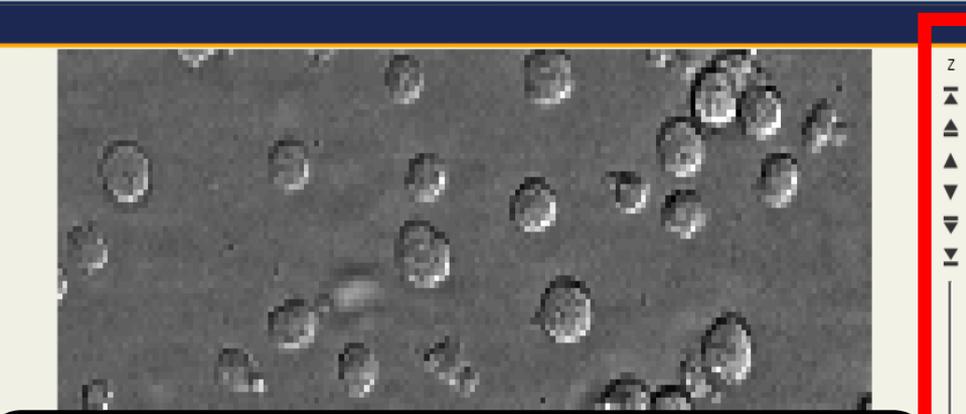
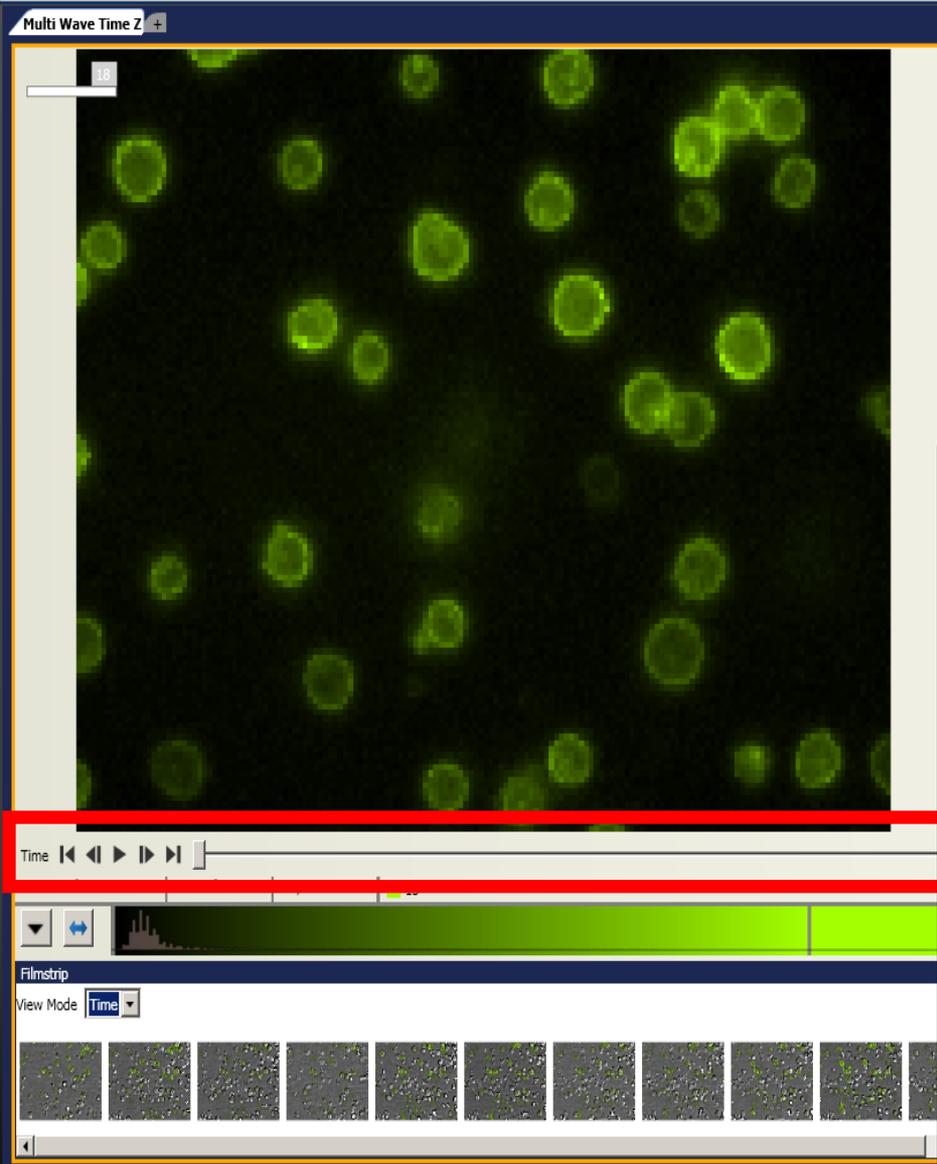
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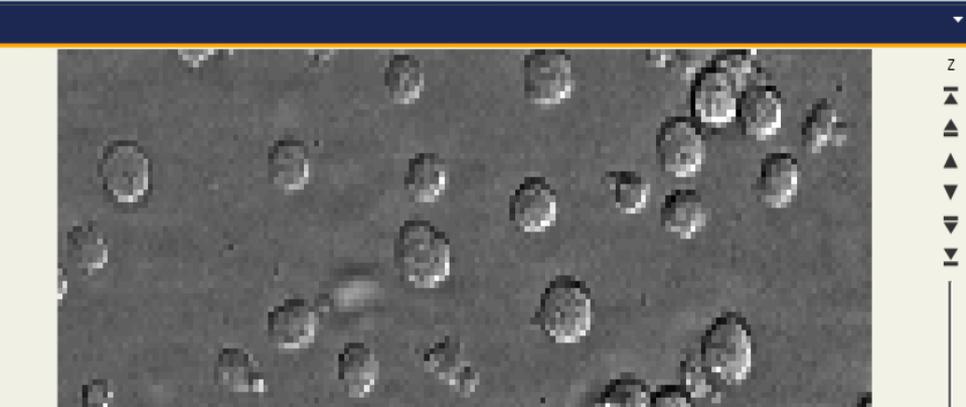
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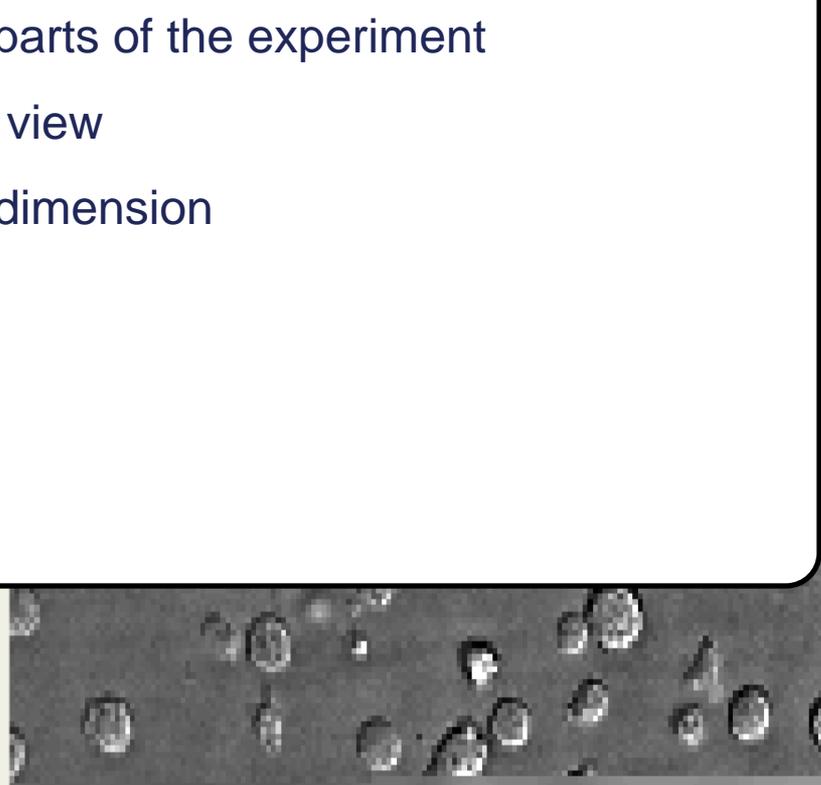
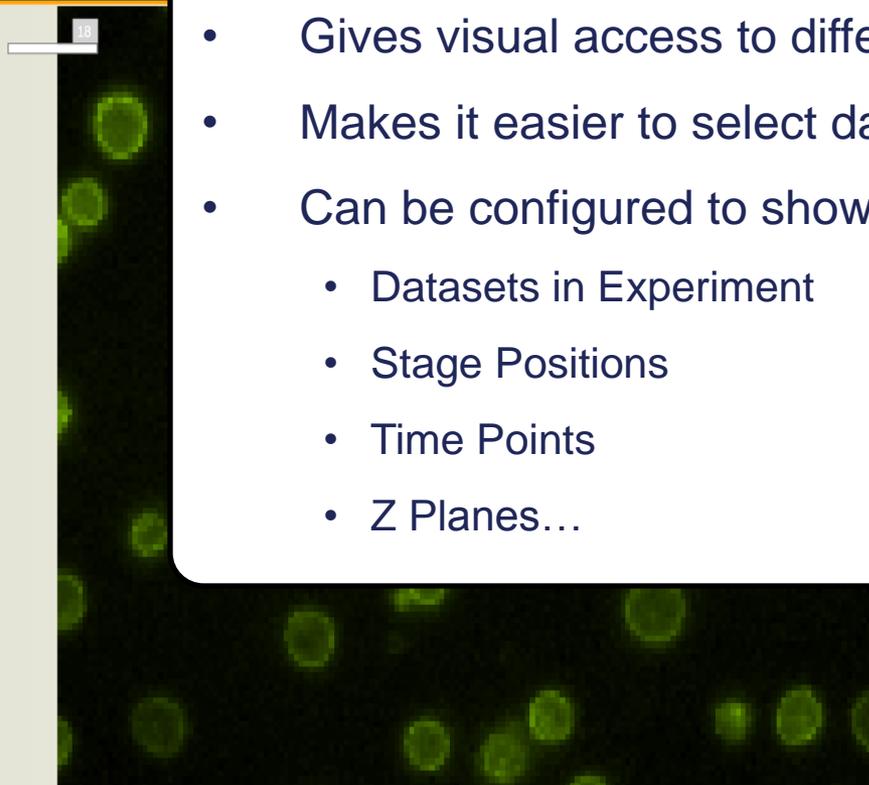
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435.3
Zoom To Fit Space 100%
Zoom

Multi Wave Time Z

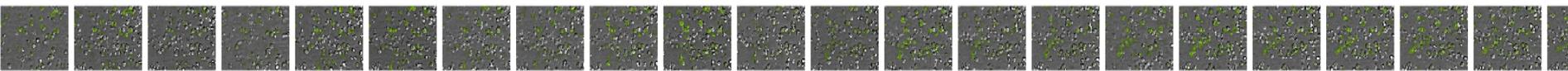


Time [play] [stop] [rewind] [fast forward]

Time 0 of 0-44 | Z 10 of 0-20 | 57, 58 | 13

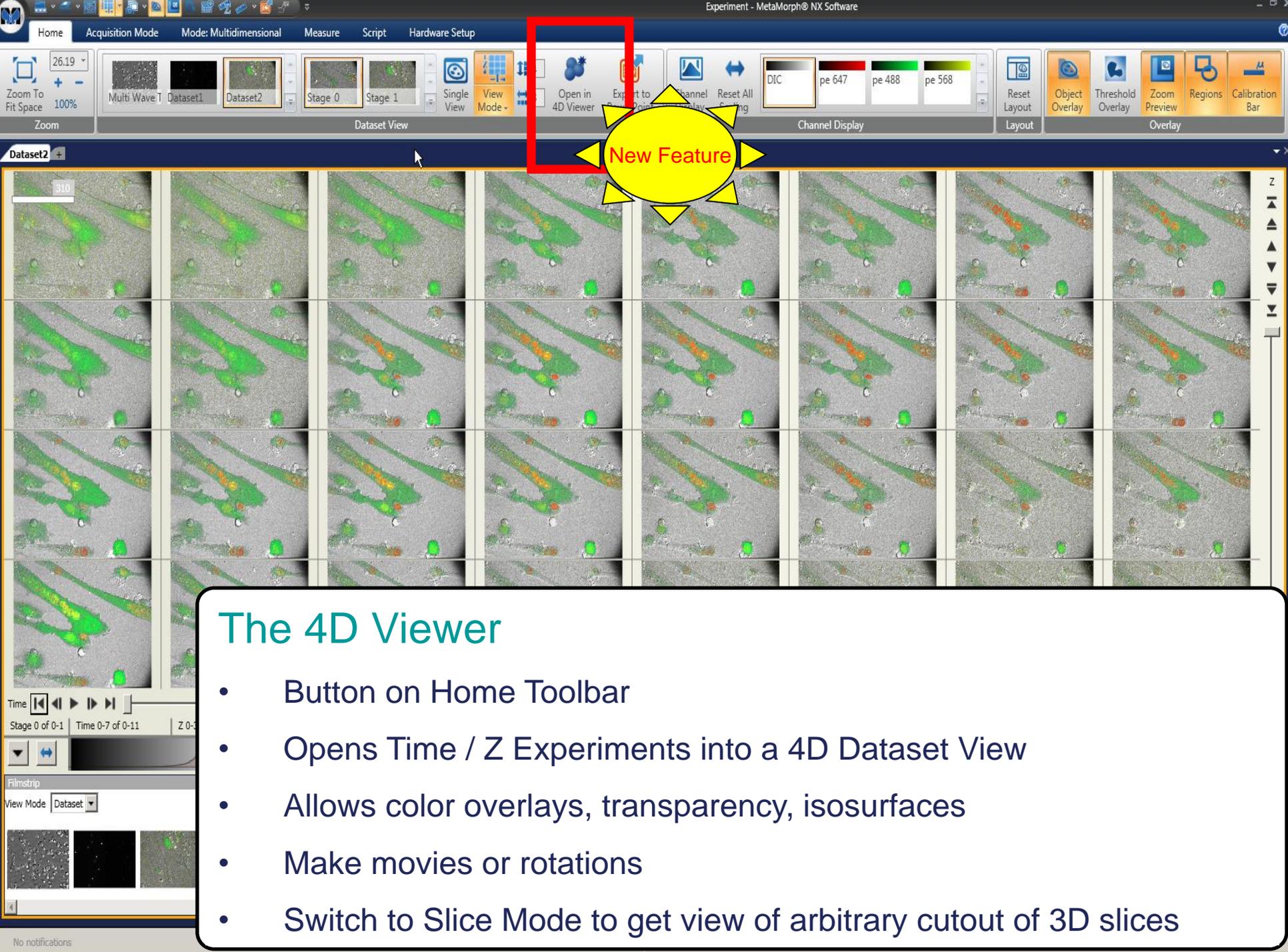
Filmstrip

View Mode Time



The Filmstrip

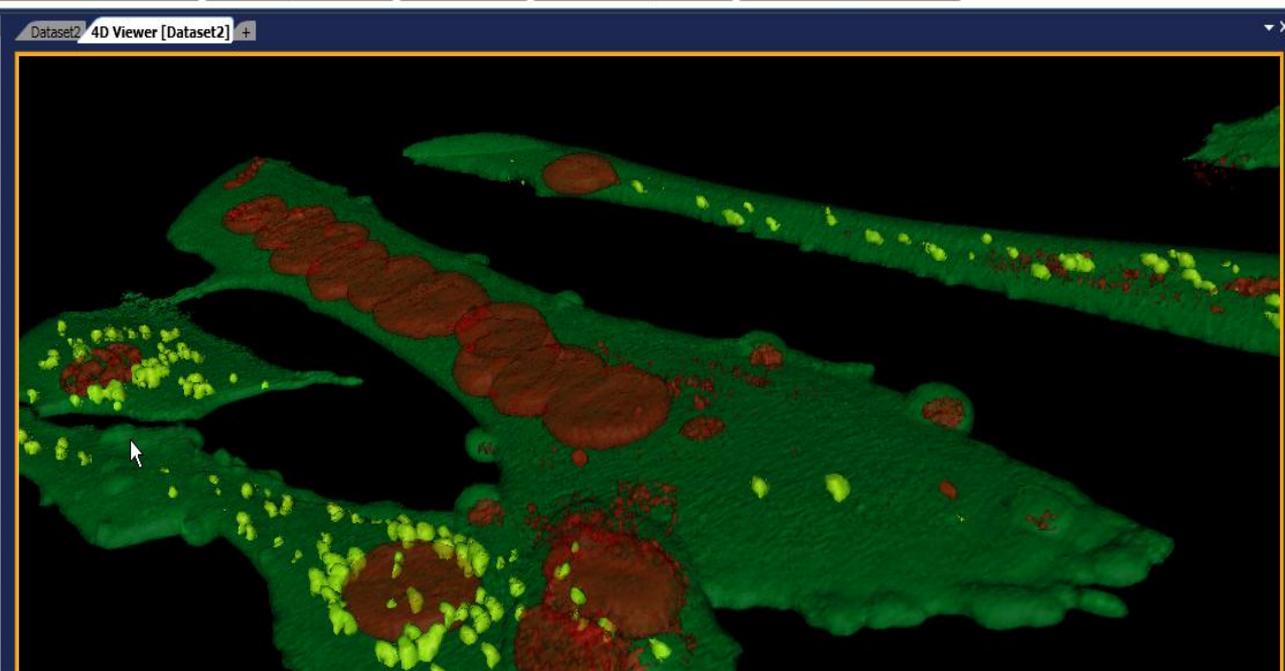
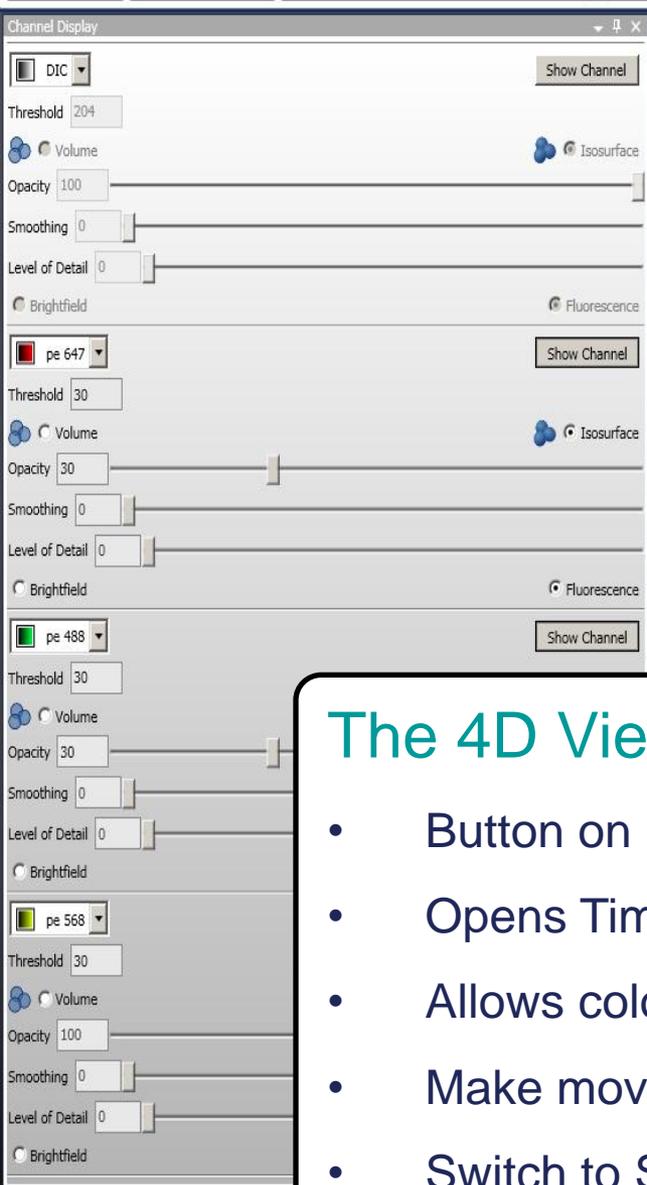
- Gives visual access to different parts of the experiment
- Makes it easier to select data to view
- Can be configured to show any dimension
 - Datasets in Experiment
 - Stage Positions
 - Time Points
 - Z Planes...



New Feature

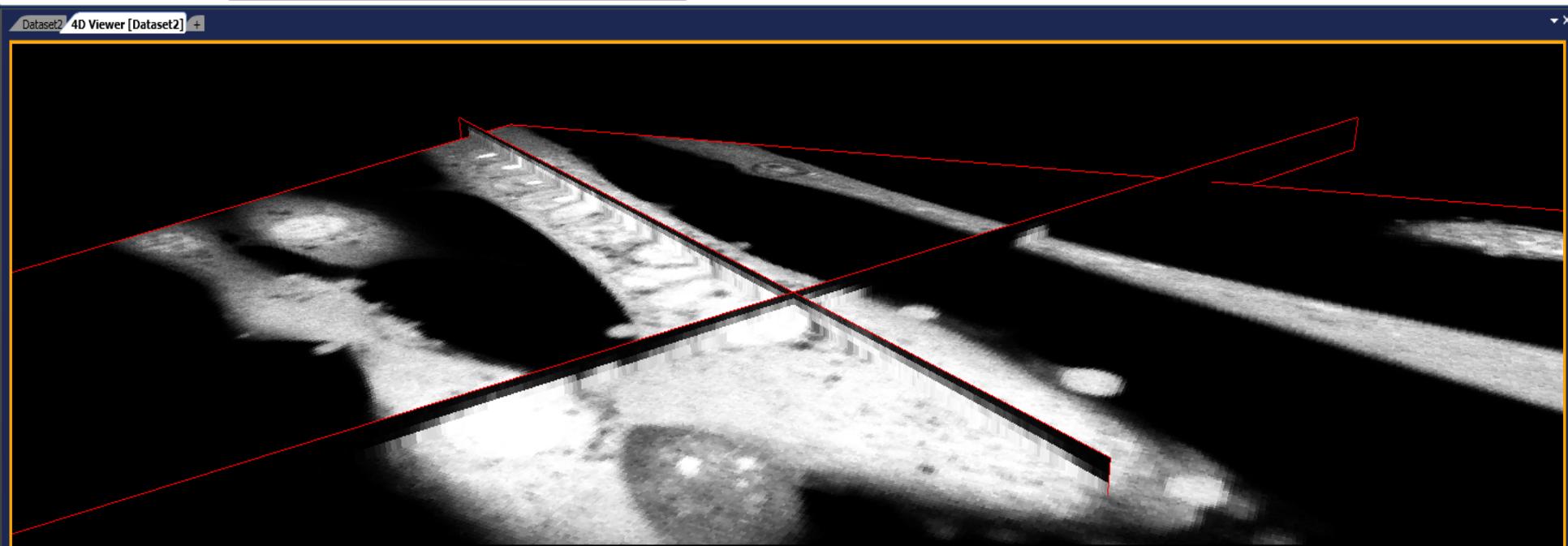
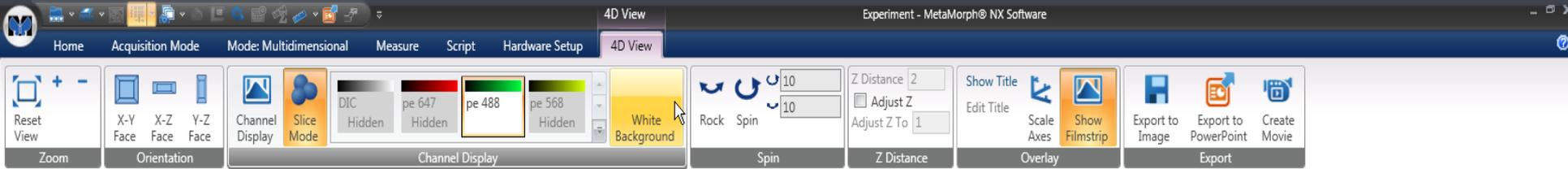
The 4D Viewer

- Button on Home Toolbar
- Opens Time / Z Experiments into a 4D Dataset View
- Allows color overlays, transparency, isosurfaces
- Make movies or rotations
- Switch to Slice Mode to get view of arbitrary cutout of 3D slices



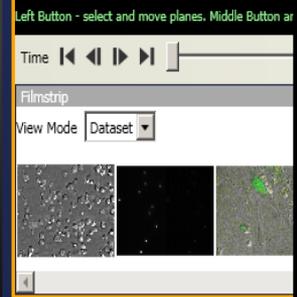
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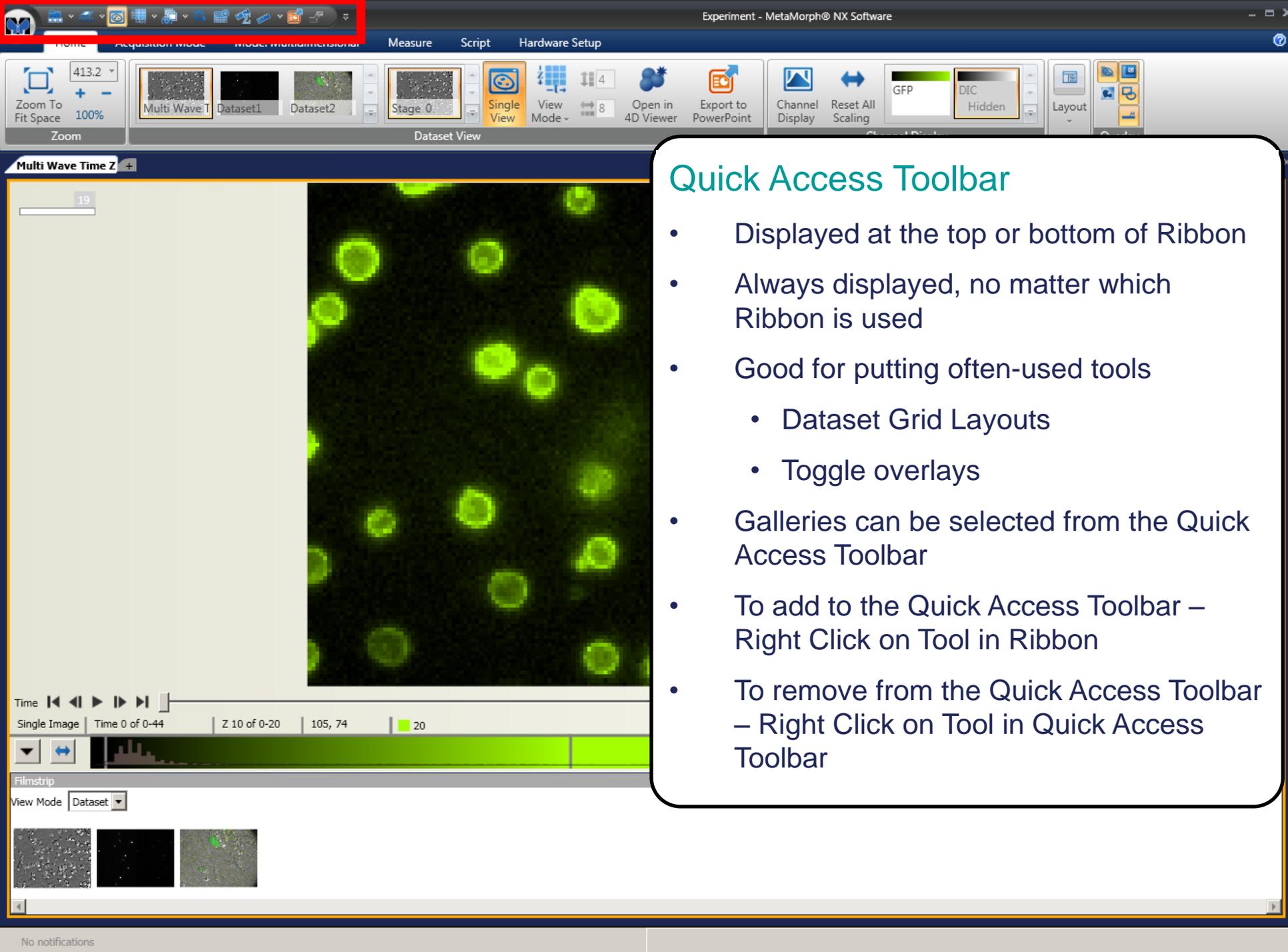
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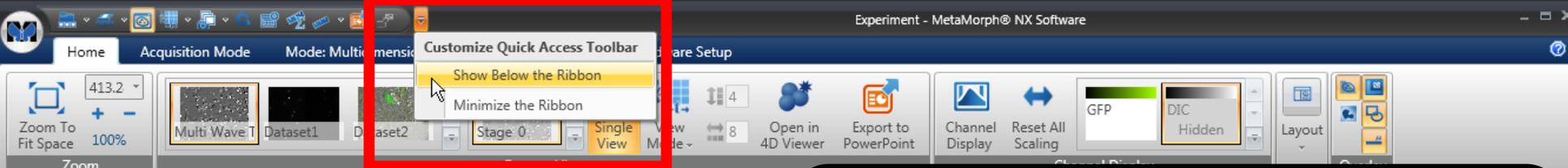
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Quick Access Toolbar

- Displayed at the top or bottom of Ribbon
- Always displayed, no matter which Ribbon is used
- Good for putting often-used tools
 - Dataset Grid Layouts
 - Toggle overlays
- Galleries can be selected from the Quick Access Toolbar
- To add to the Quick Access Toolbar – Right Click on Tool in Ribbon
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The screenshot shows the MetaMorph NX Software interface. At the top, there is a ribbon with tabs for Home, Acquisition Mode, Mode: Multidimensional, Measure, Script, and Hardware Setup. Below the ribbon is the Quick Access Toolbar, which is highlighted with a red box. This toolbar contains icons for zooming, viewing, and dataset management. The main window displays a 3D view of a cell with green fluorescence. The bottom of the interface shows a time slider, a filmstrip, and a view mode dropdown set to 'Dataset'.

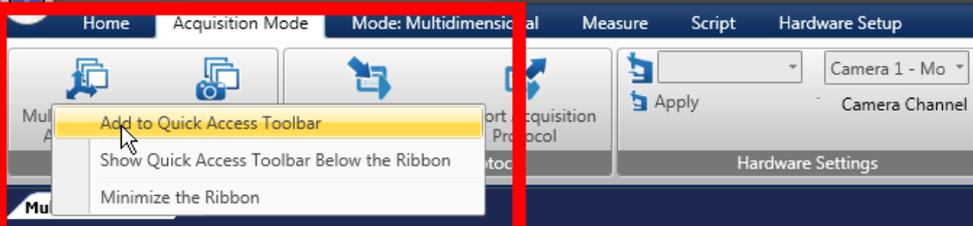
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The screenshot shows the MetaMorph NX Software interface. The top ribbon includes tabs for Home, Multidimensional, Measure, Script, and Hardware Setup. A Quick Access Toolbar is located at the top left, containing icons for Zoom, Fit, and Space. A dropdown menu is open, listing view modes: Time vs Z, Time vs Channel, Channel vs Z, Multi Time, Multi Z, and Multi Channel. The main window displays a 3D visualization of a dataset with green fluorescent spots. The bottom of the interface features a time slider, a filmstrip, and a view mode selector set to 'Dataset'.

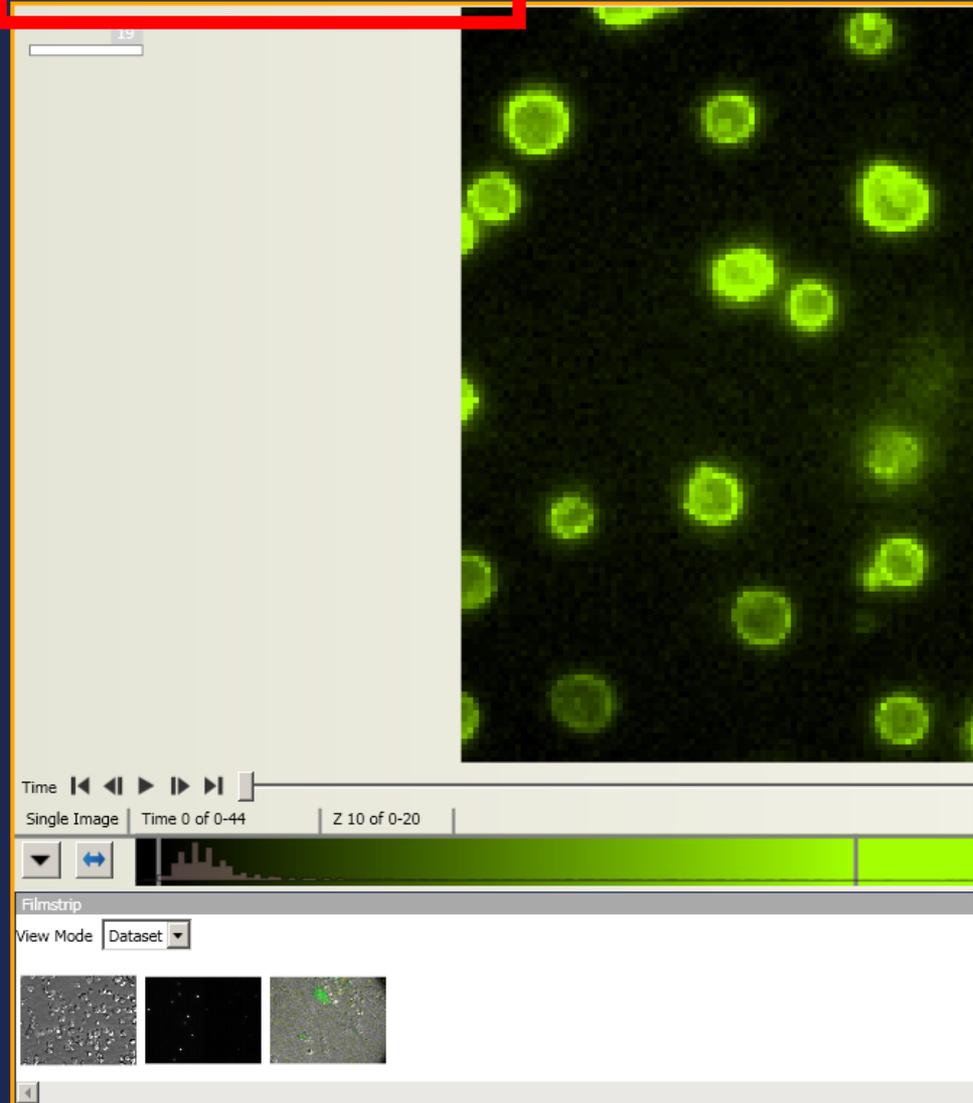
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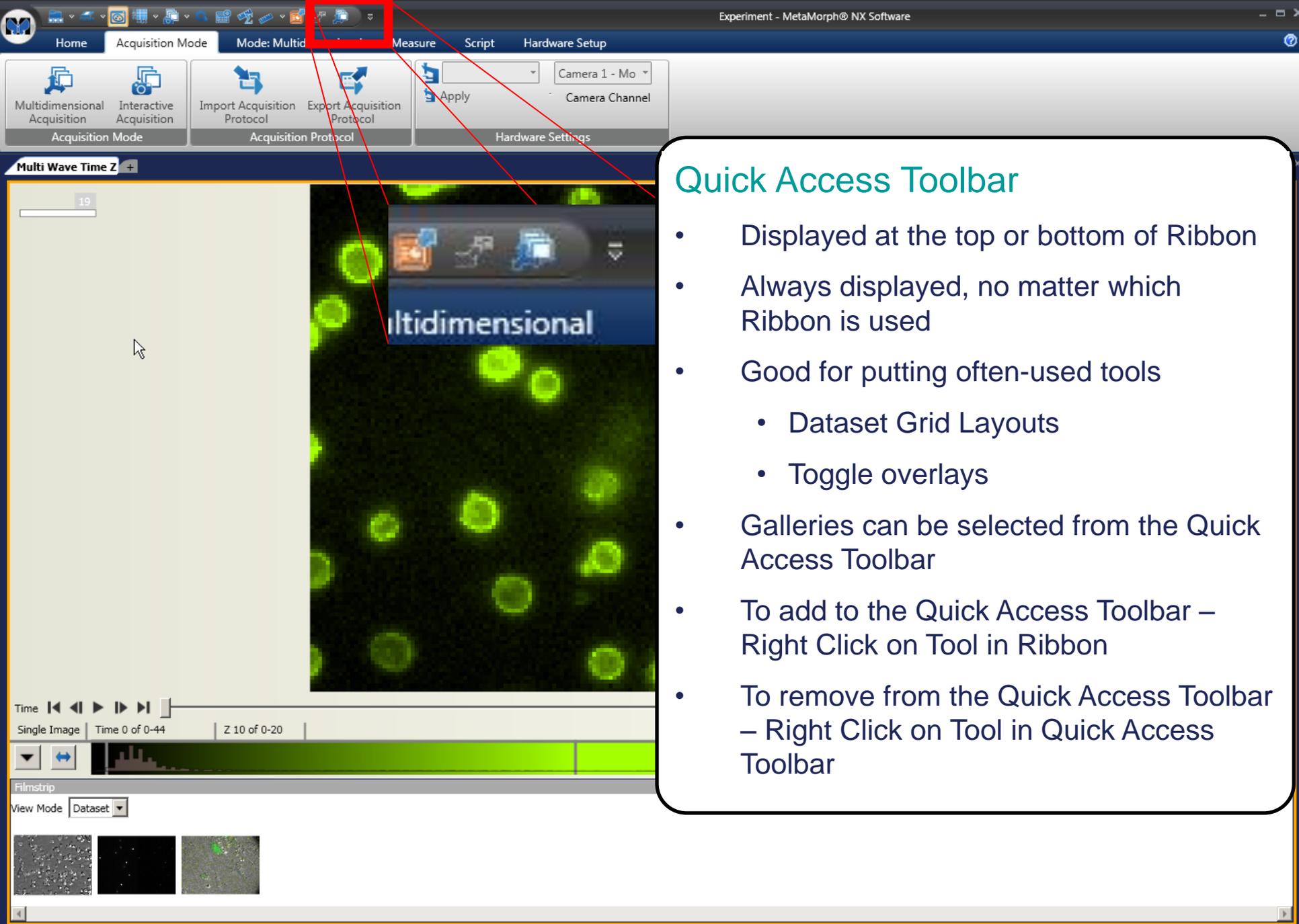
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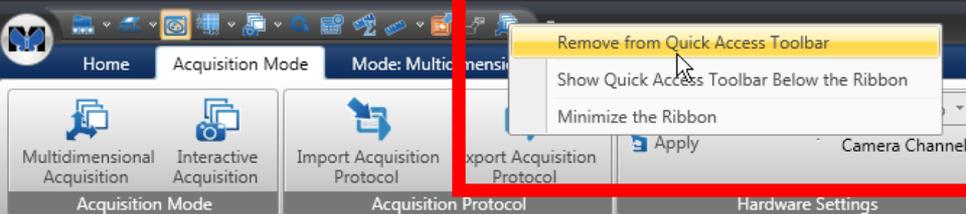
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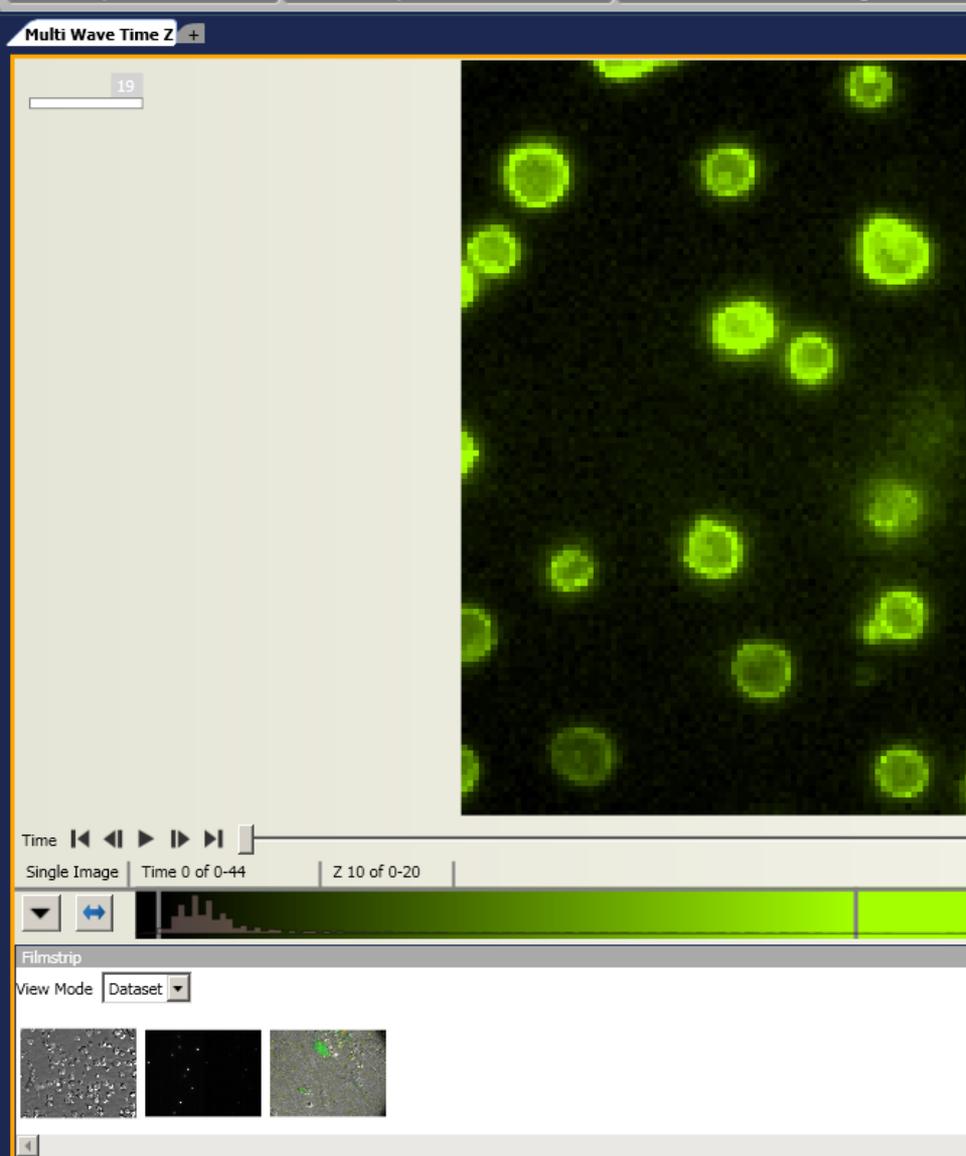
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MetaMorph NX



Using MetaMorph NX

Features of the software to make it easier to use

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Zoom To Fit Space 100% Zoom

Multi Wave 1 Dataset1 Dataset2 Stage 0

Single View View Mode 4 8 Open in 4D Viewer Export to PowerPoint

Channel Display Reset All Scaling

GFP DIC Hidden

Layout

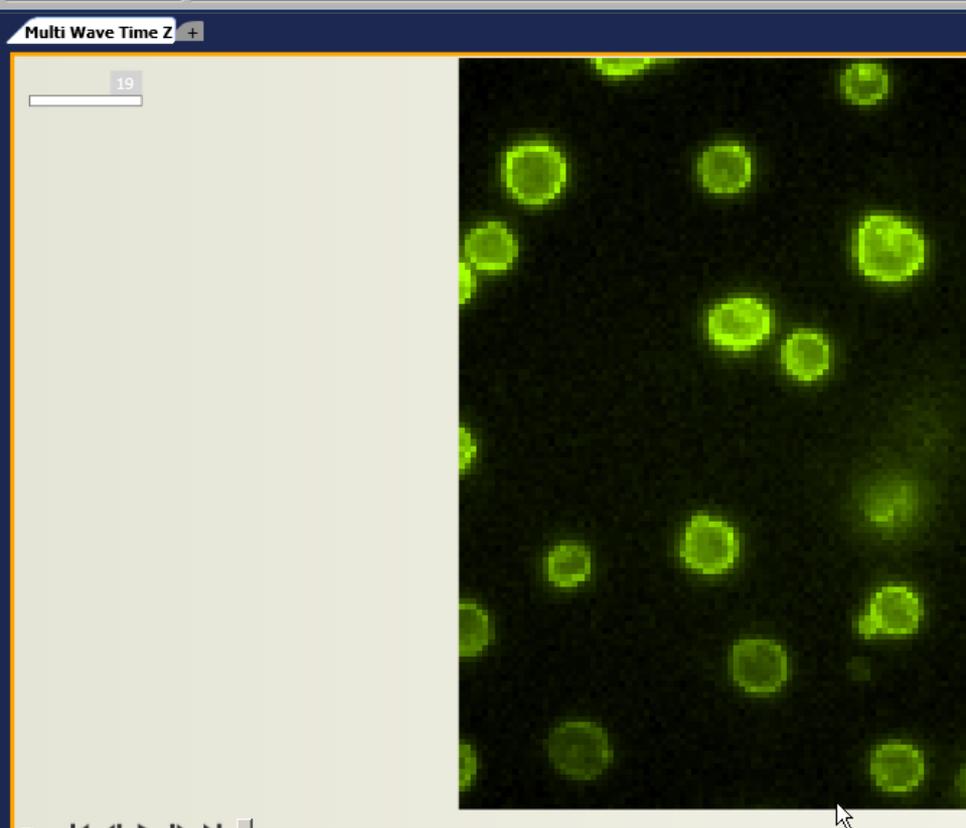


Image Scaling

- Displayed under Image Grid
- One Scale Bar for each channel
- Histogram displayed inside Scale Bar
- Options for:
 - Fixed: Absolute min/max intensity
 - Normalized: % of pixels for min/max
 - Camera: Full scale of camera used to acquire image
- Calipers for setting min/max
- Can also use Channel Display

Single Image Time 0 of 0-44 Z 9 of 0-20 63, 126 7

Filmstrip

View Mode Dataset

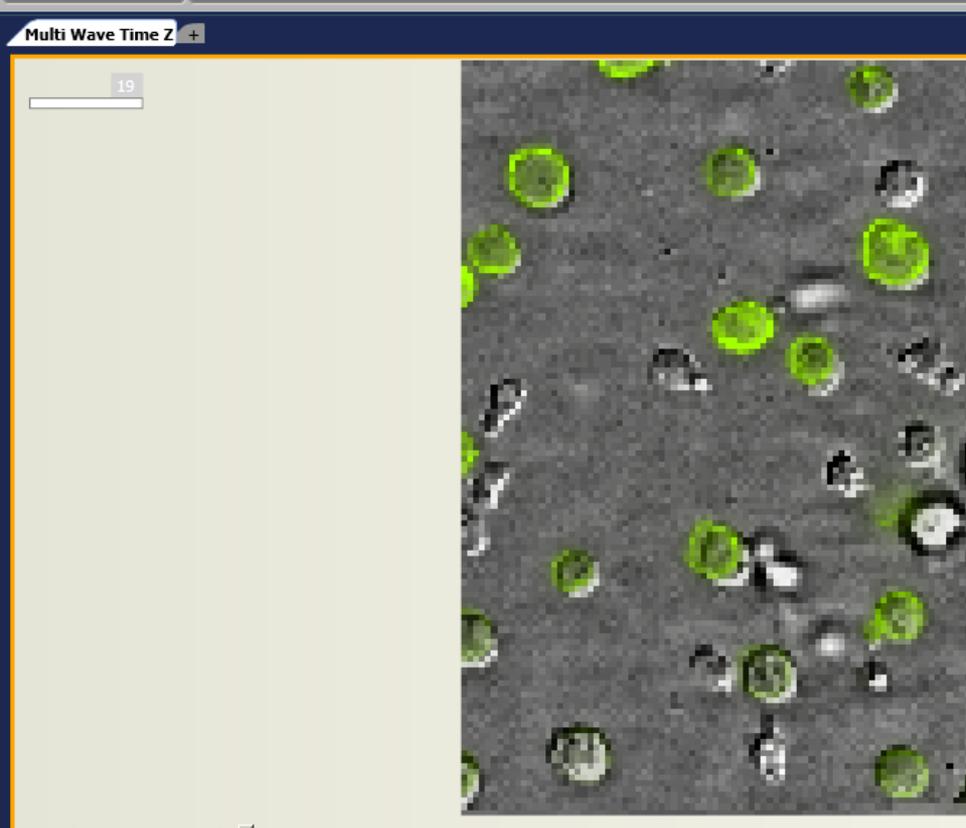
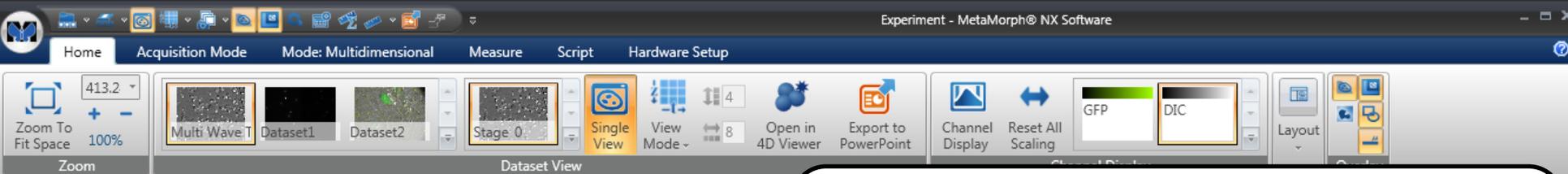
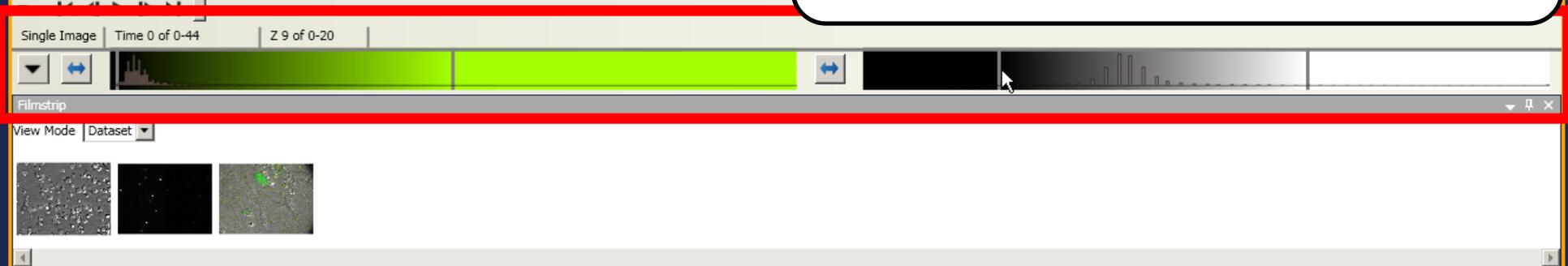


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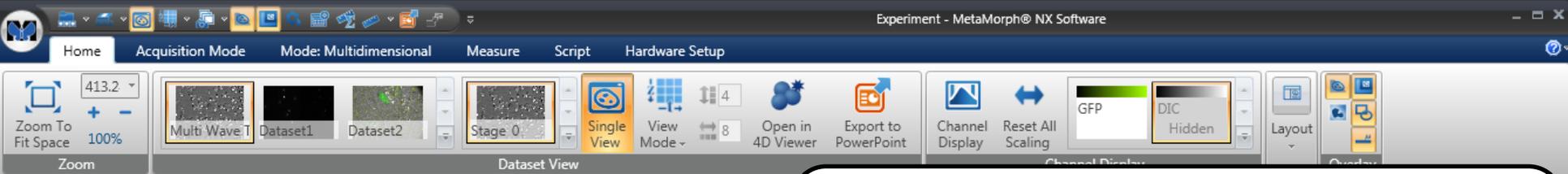


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Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Zoom To Fit Space 100% Zoom

Multi Wave 1 Dataset1 Dataset2 Stage 0

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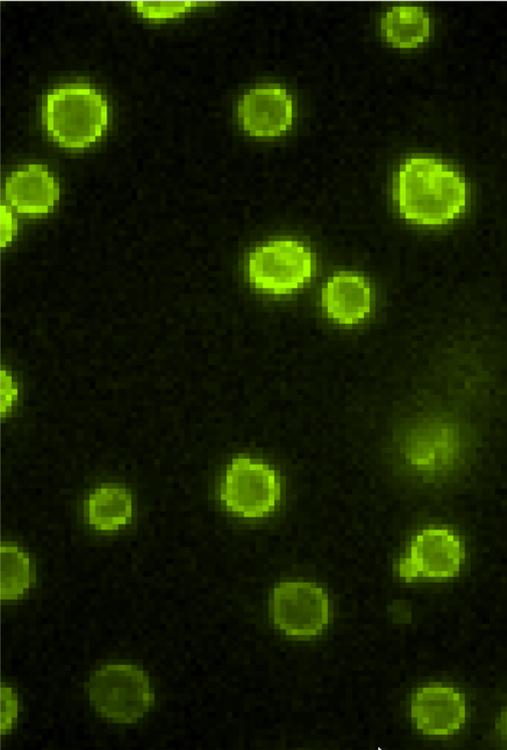
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Layout

Multi Wave Time Z +

19



Time [playback controls]

Single Image Time 0 of 0-44 Z 9 of 0-20 63, 126 7

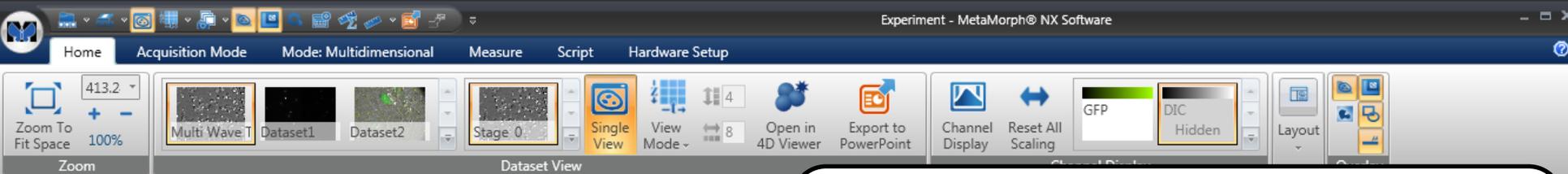
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Multi Wave Time Z +

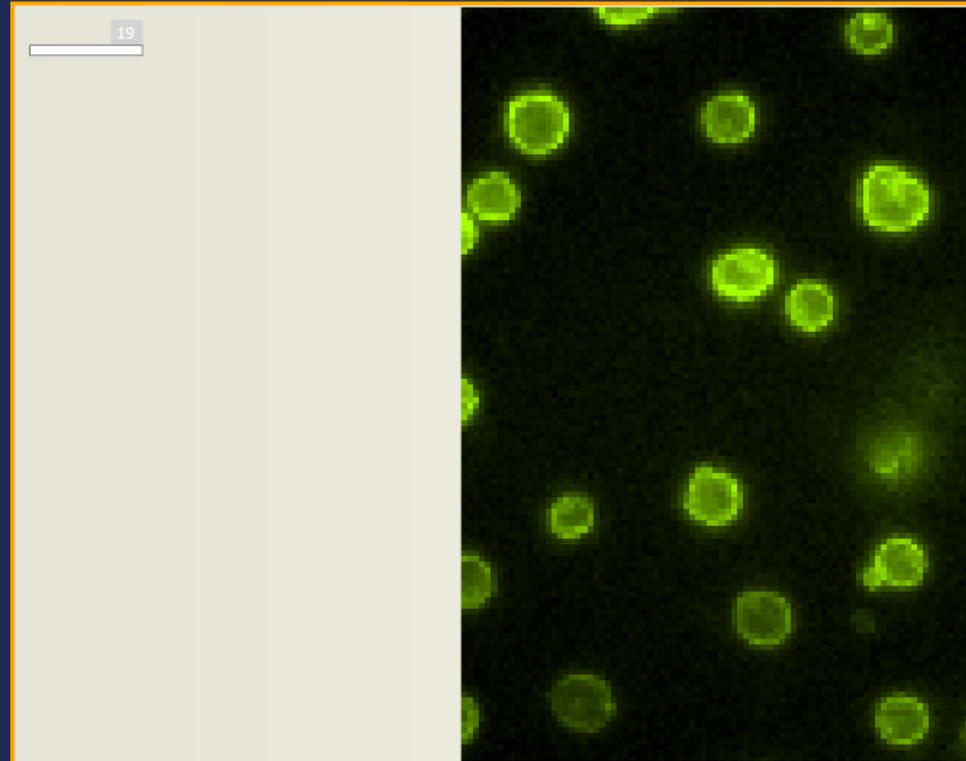
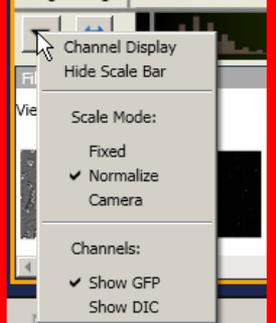


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Single Image | Time 0 of 0-44 | Z 9 of 0-20



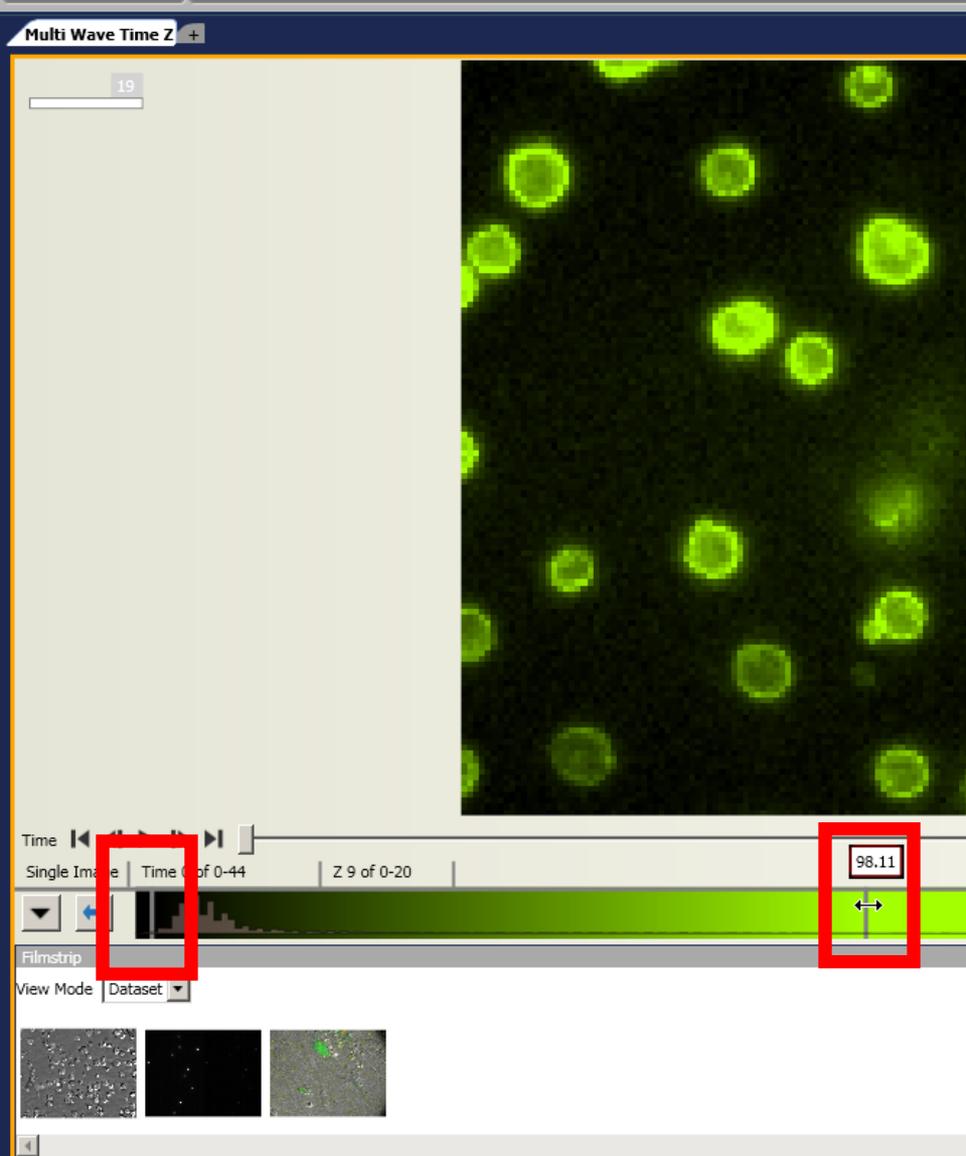
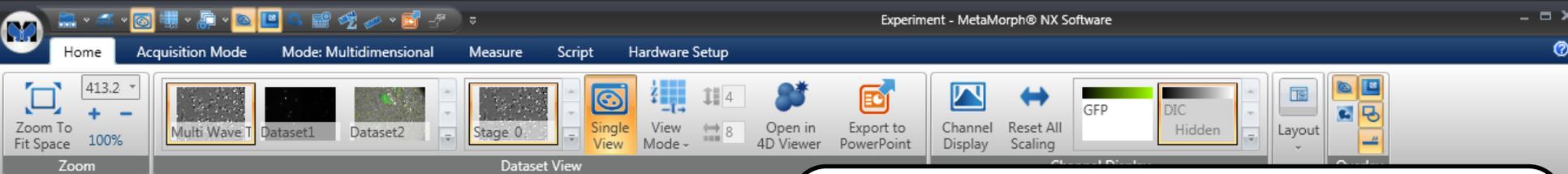


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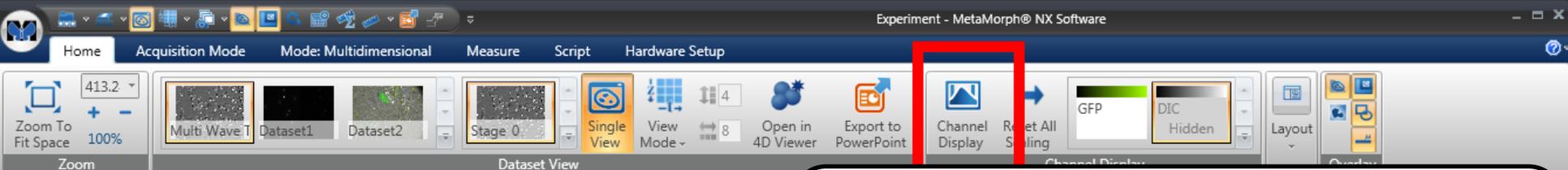
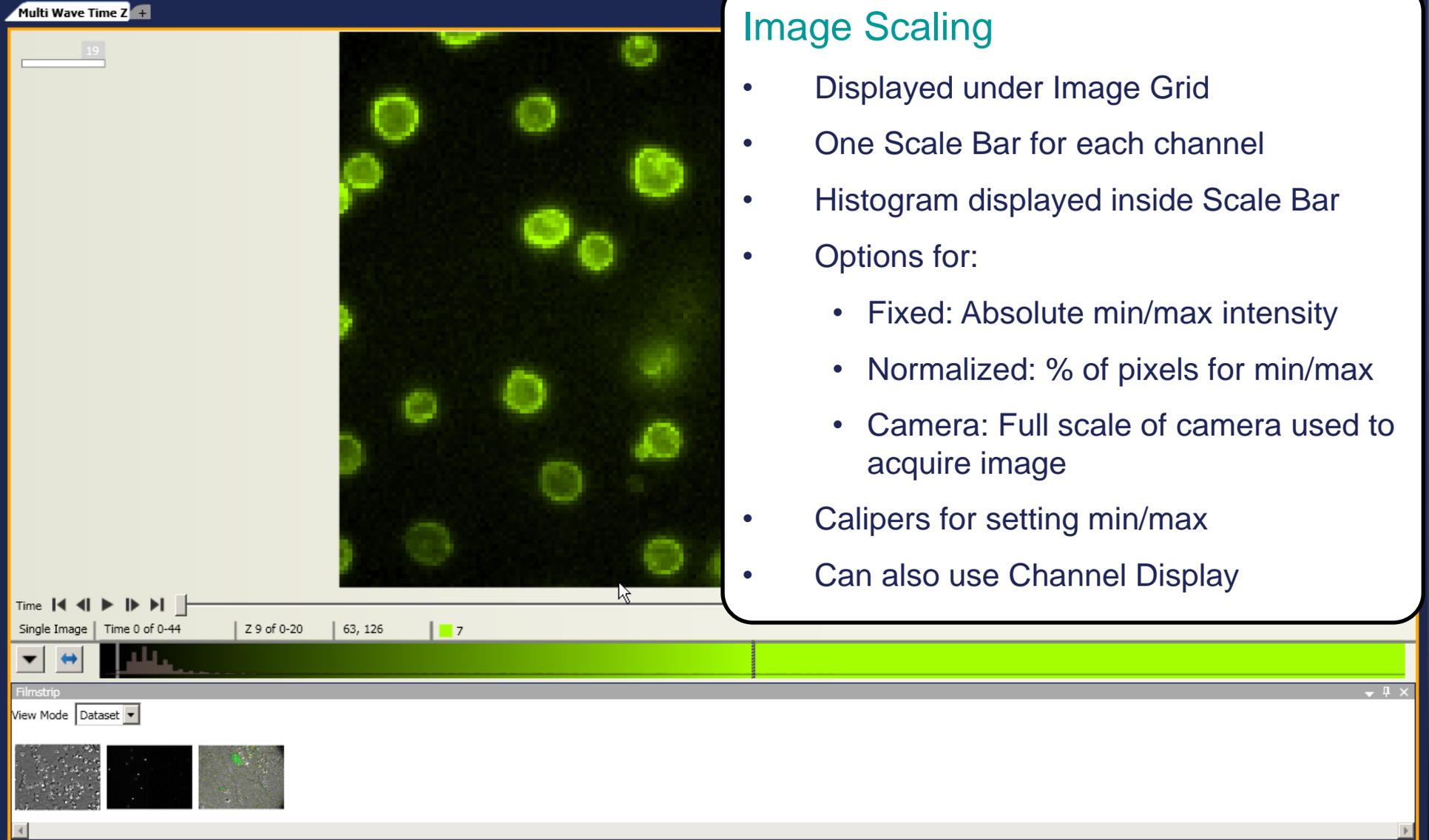


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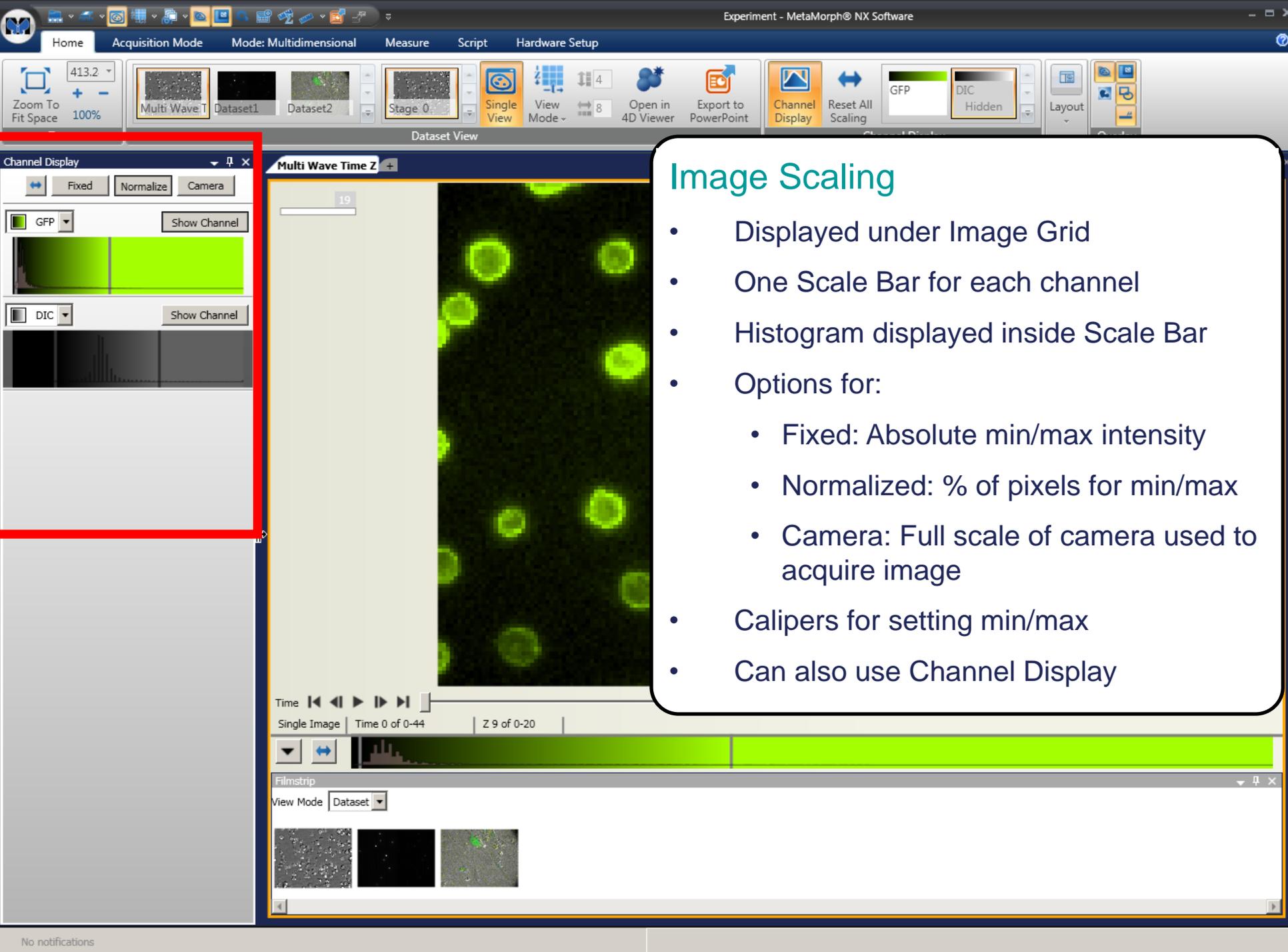


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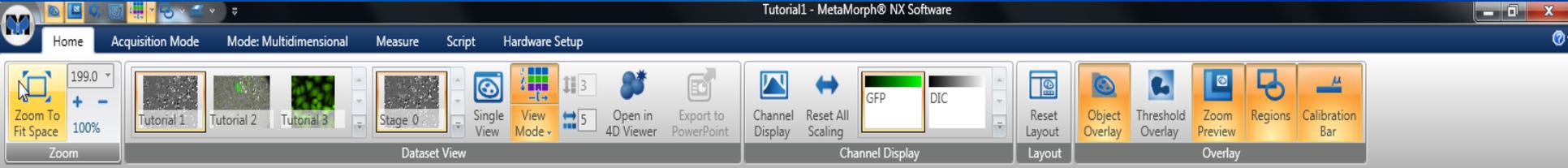
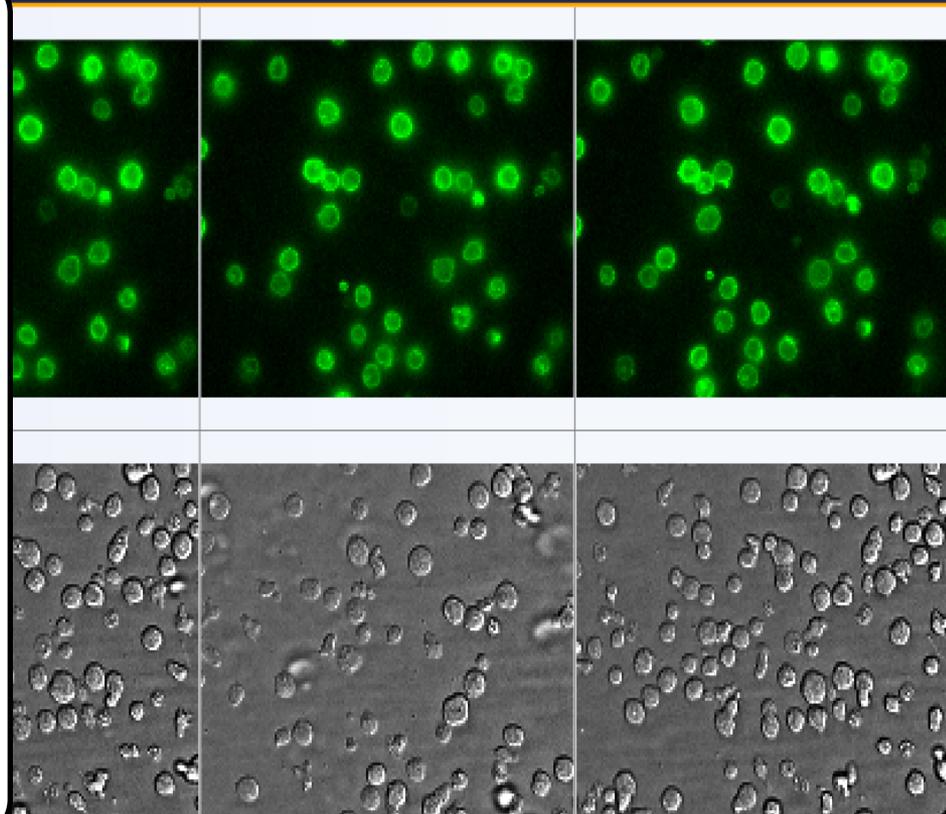


Image Zoom

- Change Zoom via Mouse
 - Put mouse over image and scroll using mouse wheel
- Home ribbon also has options for zooming
- Zoom overlay appears when the entire image can't be seen in the grid.
- Move the zoomed view window around image using clear part of the zoom overlay
- Zoom overlay can be toggled on and off.



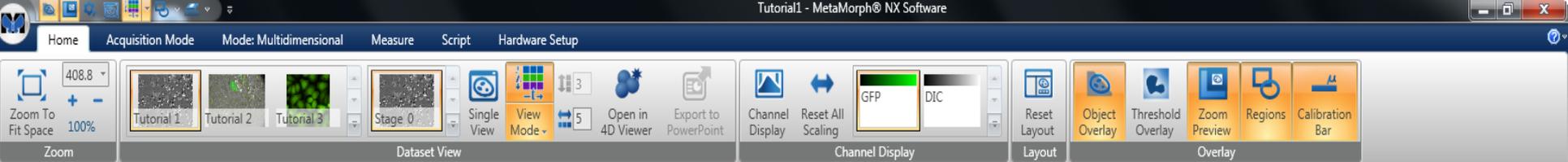
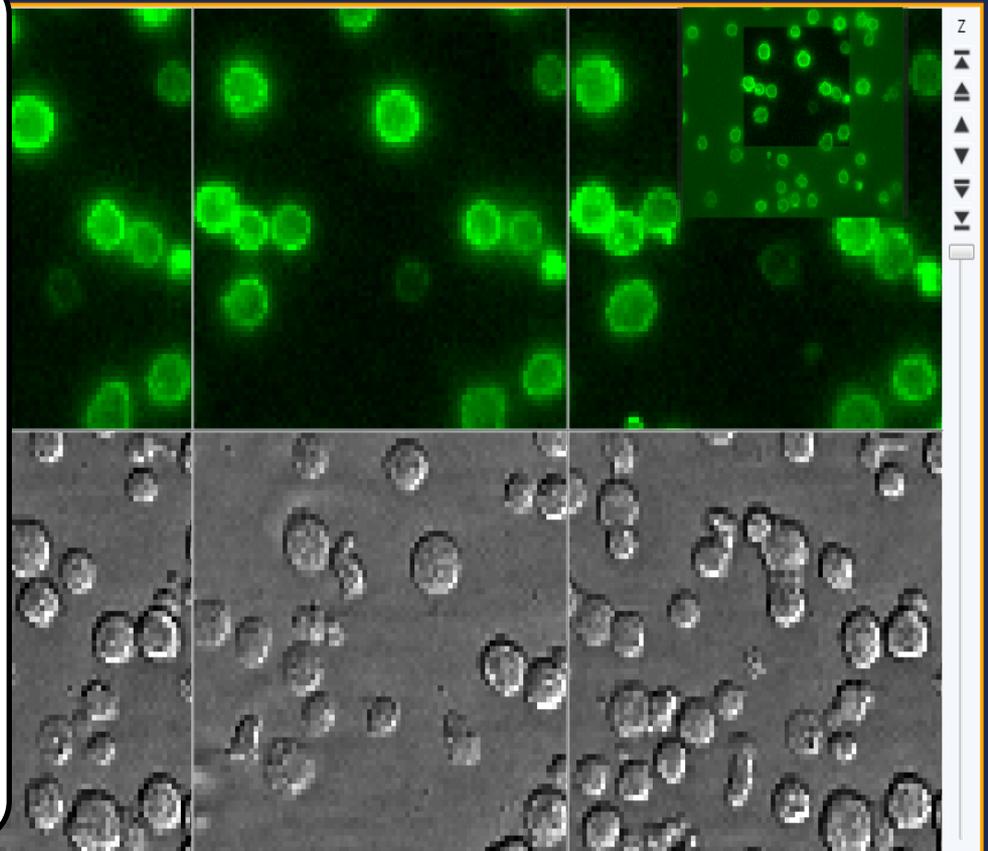


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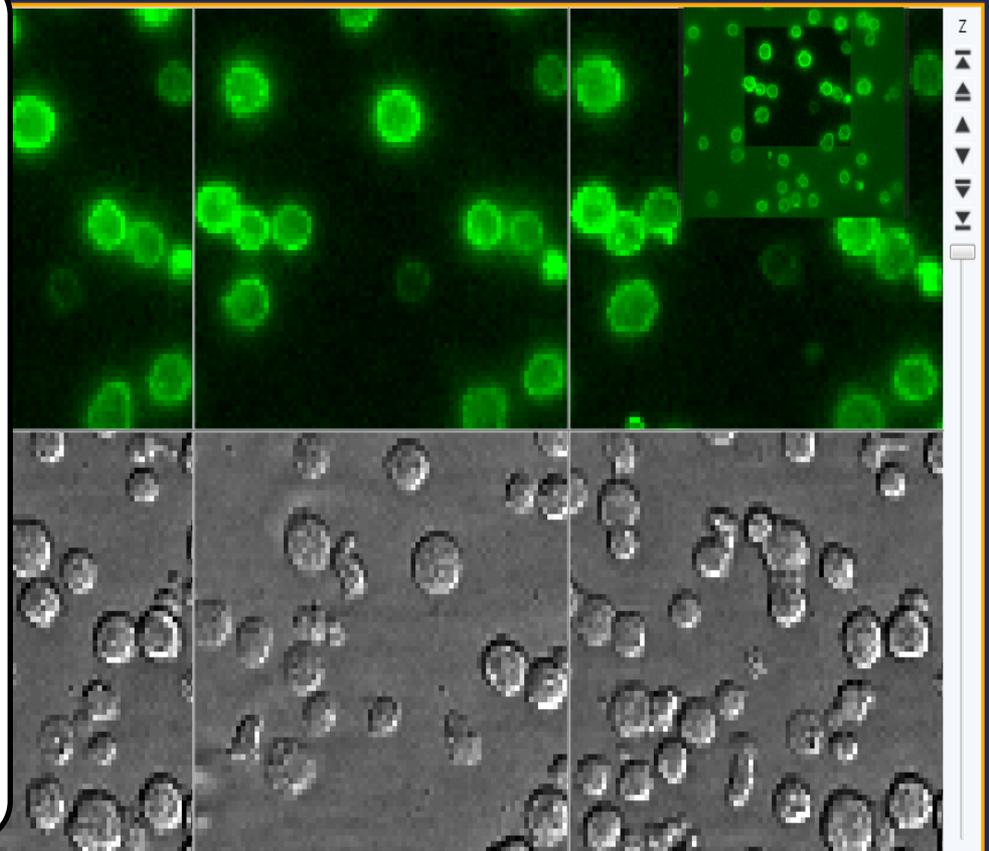
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The screenshot shows the software's top ribbon with various toolbars. The 'Zoom To Fit Space' button, located in the 'Zoom' group, is highlighted with a red rectangular box. Other visible buttons include 'Zoom', 'Single View', 'View Mode', 'Open in 4D Viewer', 'Export to PowerPoint', 'Channel Display', 'Reset All Scaling', 'Channel Display', 'Reset Layout', 'Object Overlay', 'Threshold Overlay', 'Zoom Preview', 'Regions', and 'Calibration Bar'.

Image Zoom

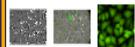
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Time Time 0-4 of 0-14 | Z 0 of 0-4

Filmstrip

View Mode Dataset



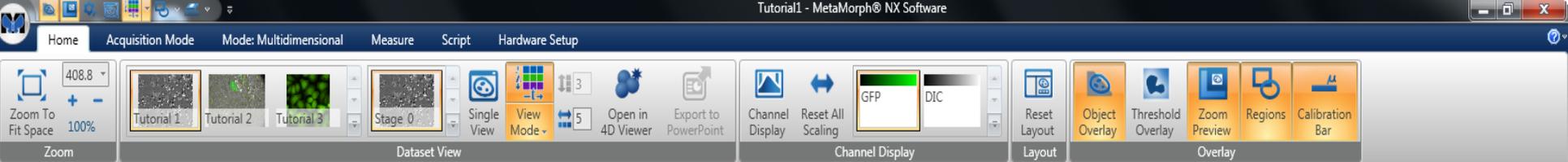
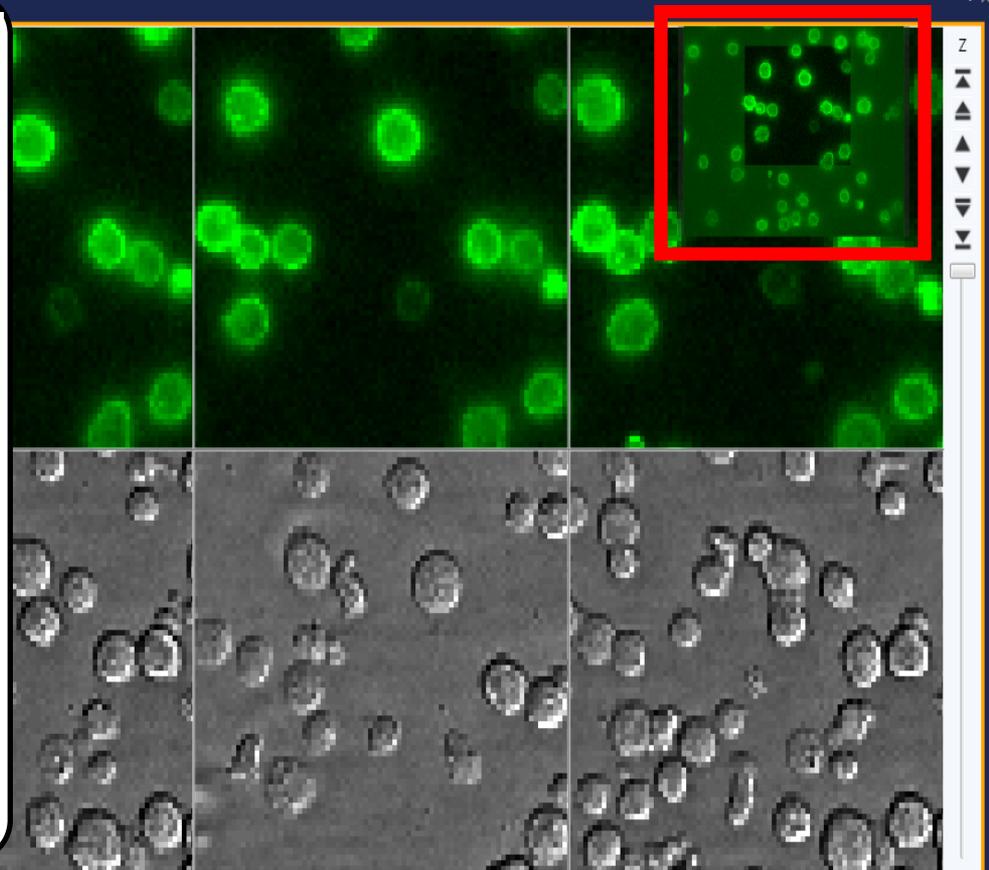


Image Zoom

- Change Zoom via Mouse
 - Put mouse over image and scroll using mouse wheel
- Home ribbon also has options for zooming
- Zoom overlay appears when the entire image can't be seen in the grid.
- Move the zoomed view window around image using clear part of the zoom overlay
- Zoom overlay can be toggled on and off.



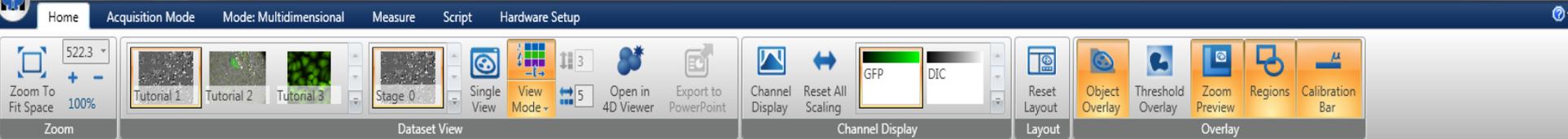
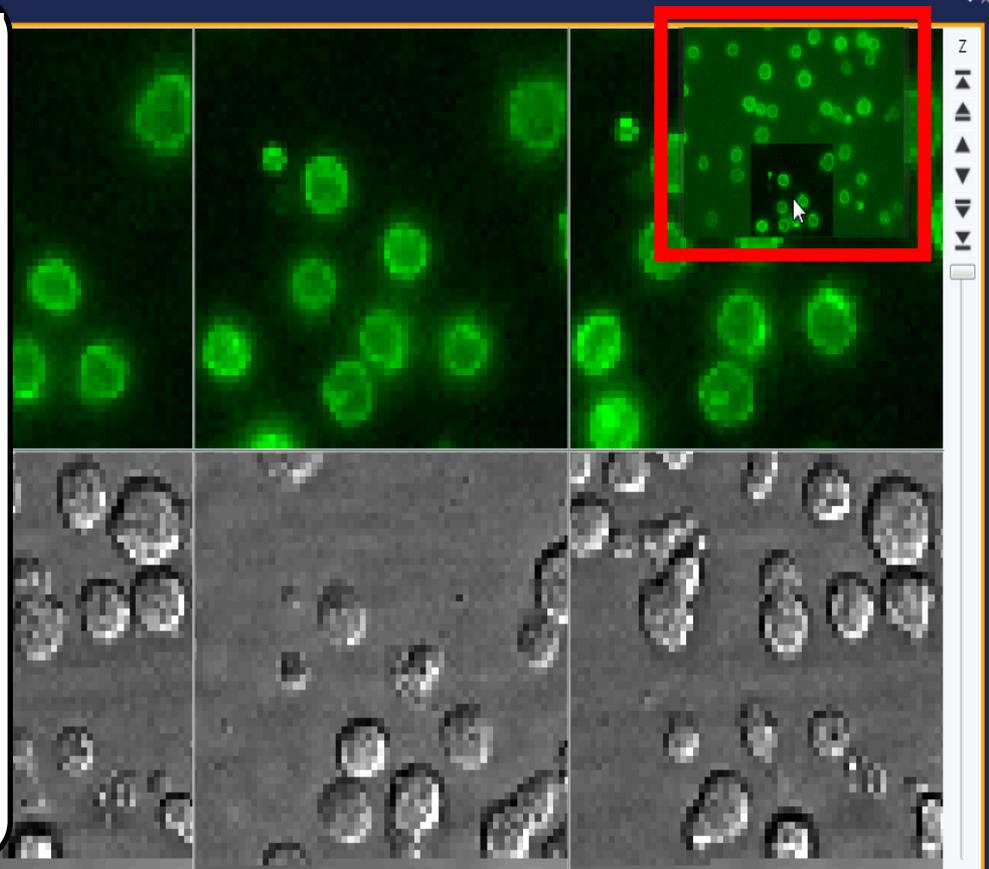


Image Zoom

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 - Put mouse over image and scroll using mouse wheel
- Home ribbon also has options for zooming
- Zoom overlay appears when the entire image can't be seen in the grid.
- Move the zoomed view window around image using clear part of the zoom overlay
- Zoom overlay can be toggled on and off.



Time Time 0-4 of 0-14 | Z 0 of 0-4



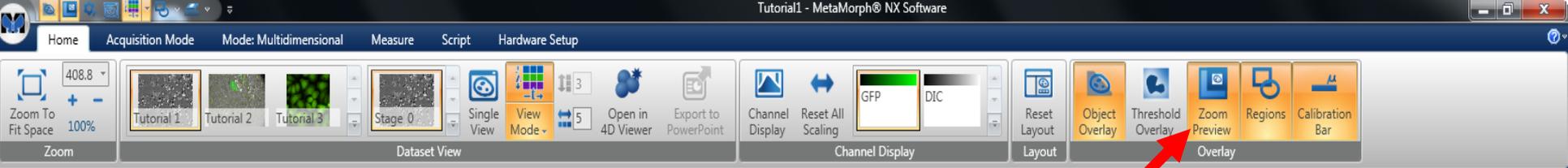
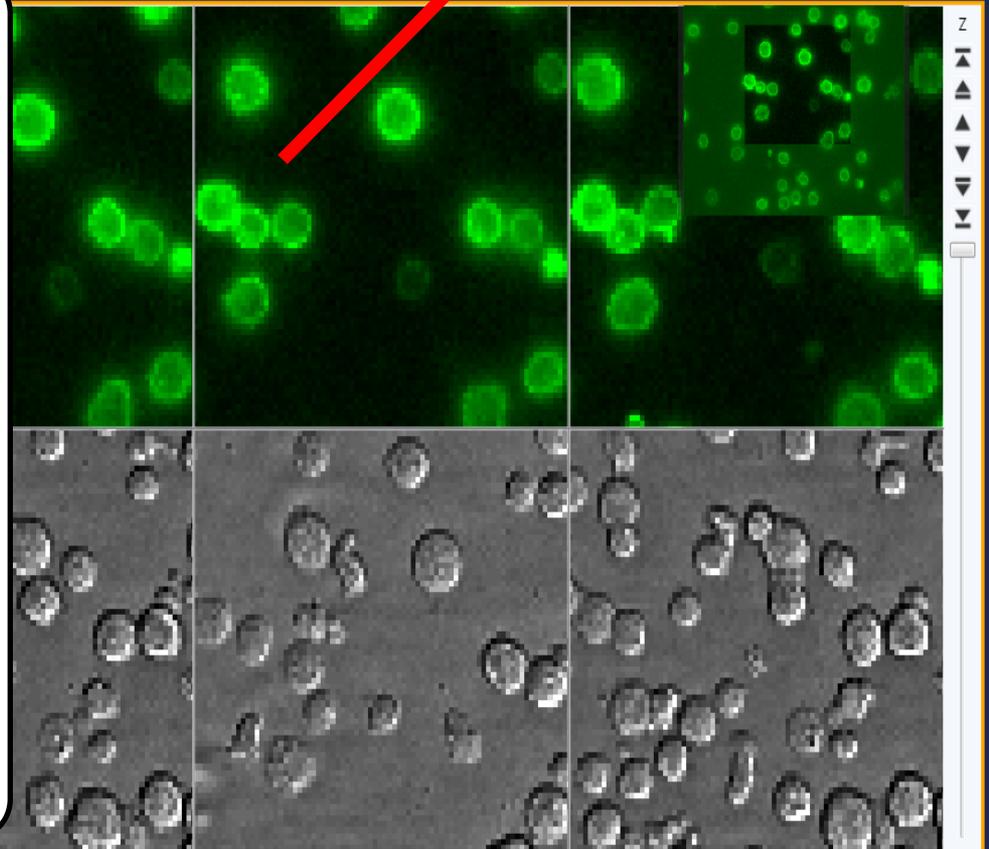
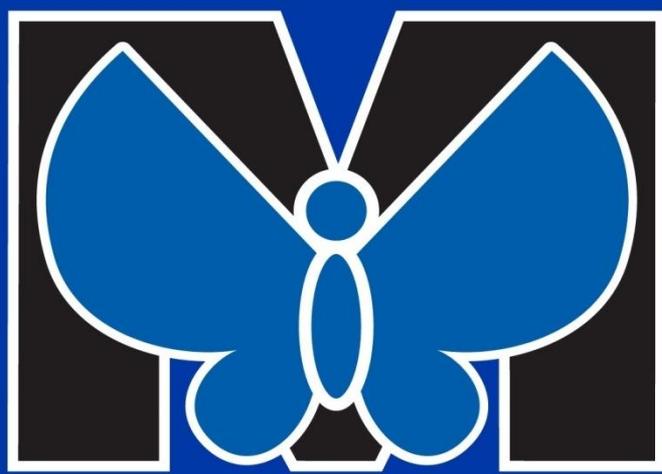


Image Zoom

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- Zoom overlay can be toggled on and off.





MetaMorph NX



Opening Images in MetaMorph NX

Opening, creating Experiments from external data

The screenshot shows the MetaMorph NX software interface. At the top, there is a menu bar with options like 'Home', 'Equation mode', 'MODE: multidimensional', 'Measure', 'Script', and 'Hardware Setup'. Below the menu bar is a toolbar with various icons for zooming, viewing, and exporting. A red box highlights the 'Multi Wave Time Z' dataset in the toolbar. The main window displays a large image of green fluorescent cells. Below the main image is a filmstrip showing a sequence of frames. The bottom status bar shows 'Time 0 of 0-44' and 'Z 10 of 0-20'.

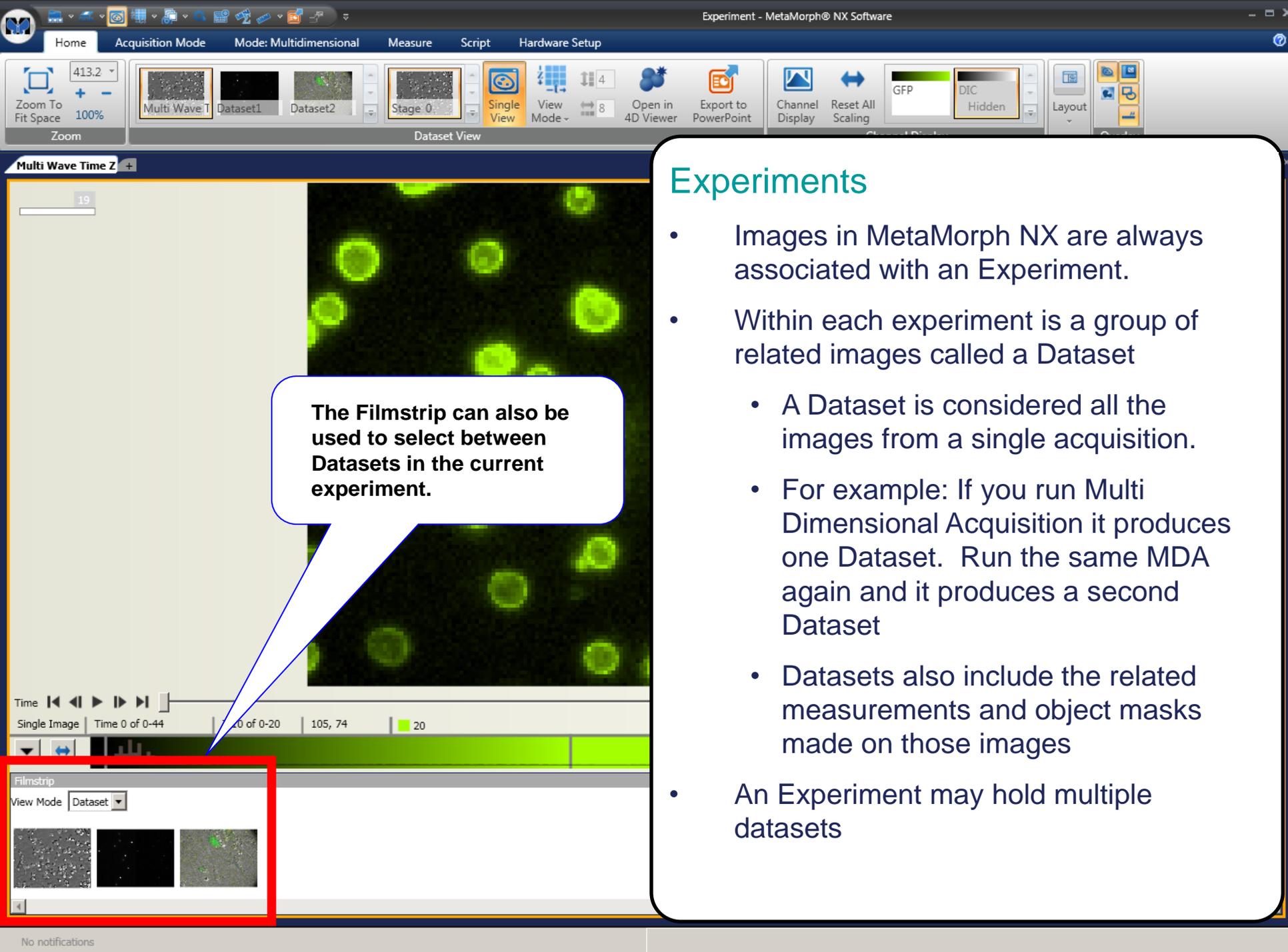
Experiments

- Images in MetaMorph NX are always associated with an Experiment.
- Within each experiment is a group of related images called a Dataset
 - A Dataset is considered all the images from a single acquisition.
 - For example: If you run Multi Dimensional Acquisition it produces one Dataset. Run the same MDA again and it produces a second Dataset
 - Datasets also include the related measurements and object masks made on those images
- An Experiment may hold multiple datasets

The Home Ribbon has a Dataset Gallery to select different datasets in the experiment.

Experiments

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 - A Dataset is considered all the images from a single acquisition.
 - For example: If you run Multi Dimensional Acquisition it produces one Dataset. Run the same MDA again and it produces a second Dataset
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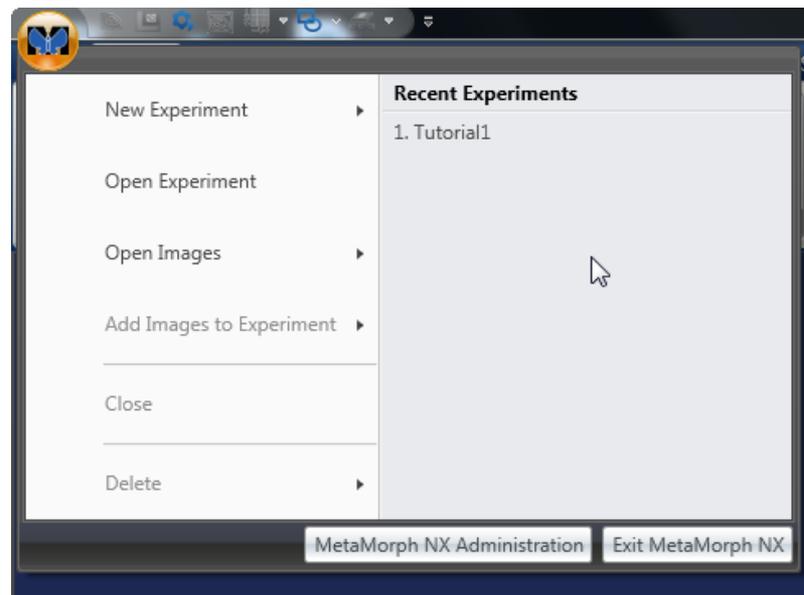
Experiments

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 - A Dataset is considered all the images from a single acquisition.
 - For example: If you run Multi Dimensional Acquisition it produces one Dataset. Run the same MDA again and it produces a second Dataset
 - Datasets also include the related measurements and object masks made on those images
- An Experiment may hold multiple datasets

The Filmstrip can also be used to select between Datasets in the current experiment.

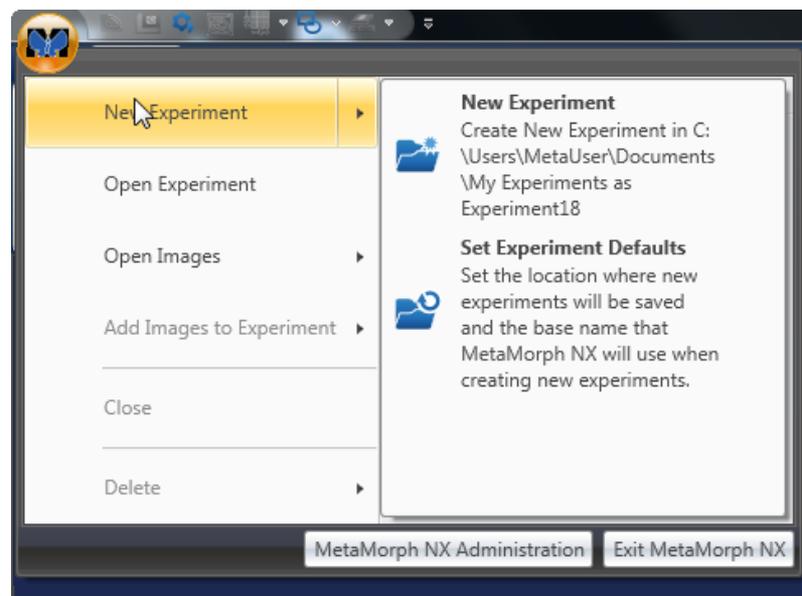
Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- New Experiment
 - Create a new experiment
 - Gives details where the experiment will be created
 - Set Experiment Defaults
 - Change the location on disk where experiments are saved
 - Change names given to experiments and datasets



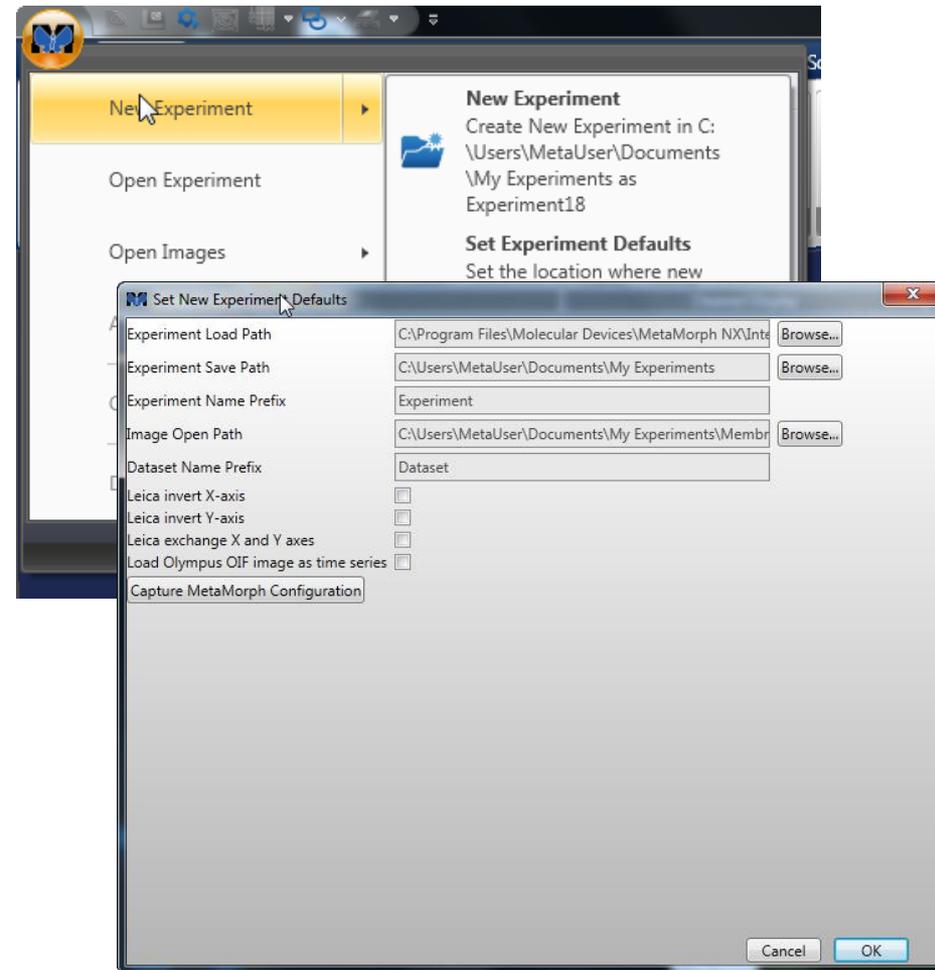
Working with experiments

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 - Set Experiment Defaults
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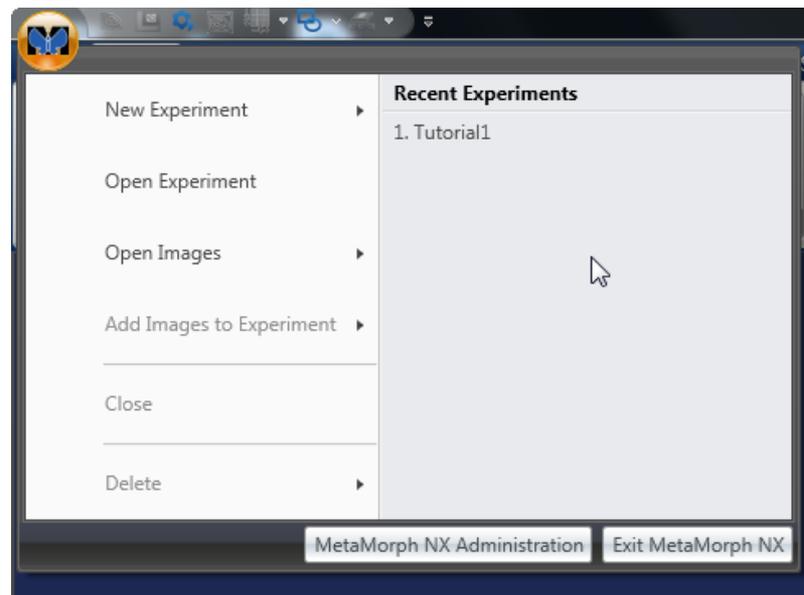
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 - Change names given to experiments and datasets



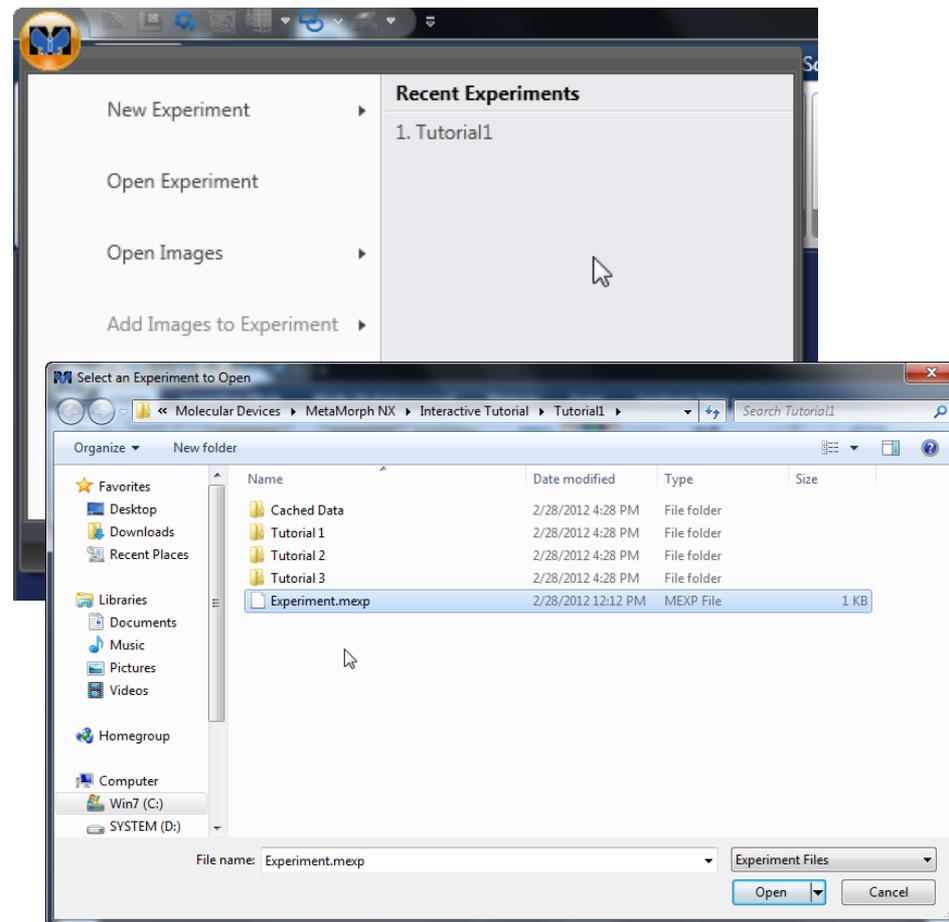
Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- Open Experiment
 - Loads a previously saved experiment into MetaMorph NX
 - Experiments are saved into a folder with their name on it
 - Always open the 'Experiment.mexp' file.



Working with experiments

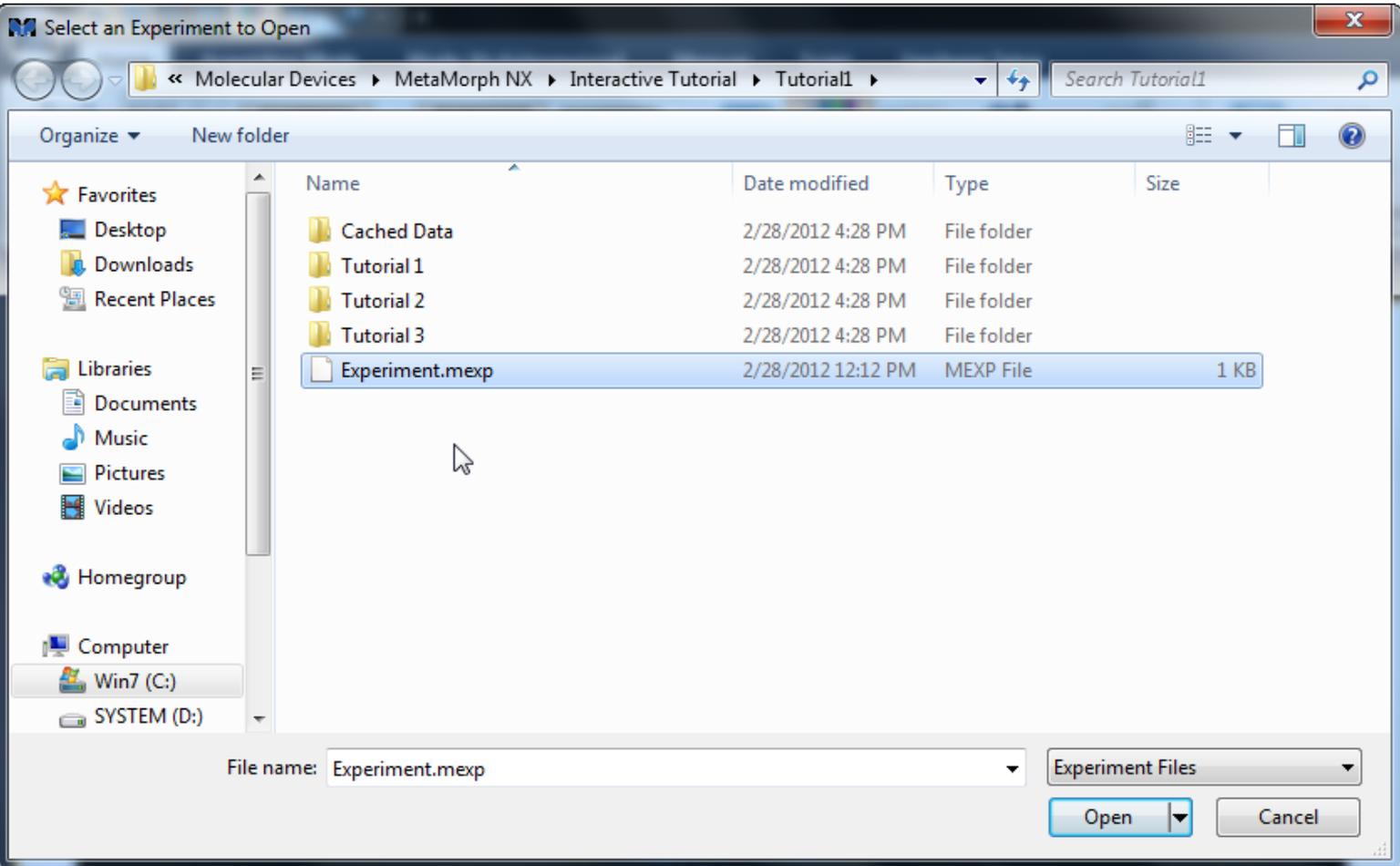
- Application Menu
 - Holds tools for working with the current experiment files
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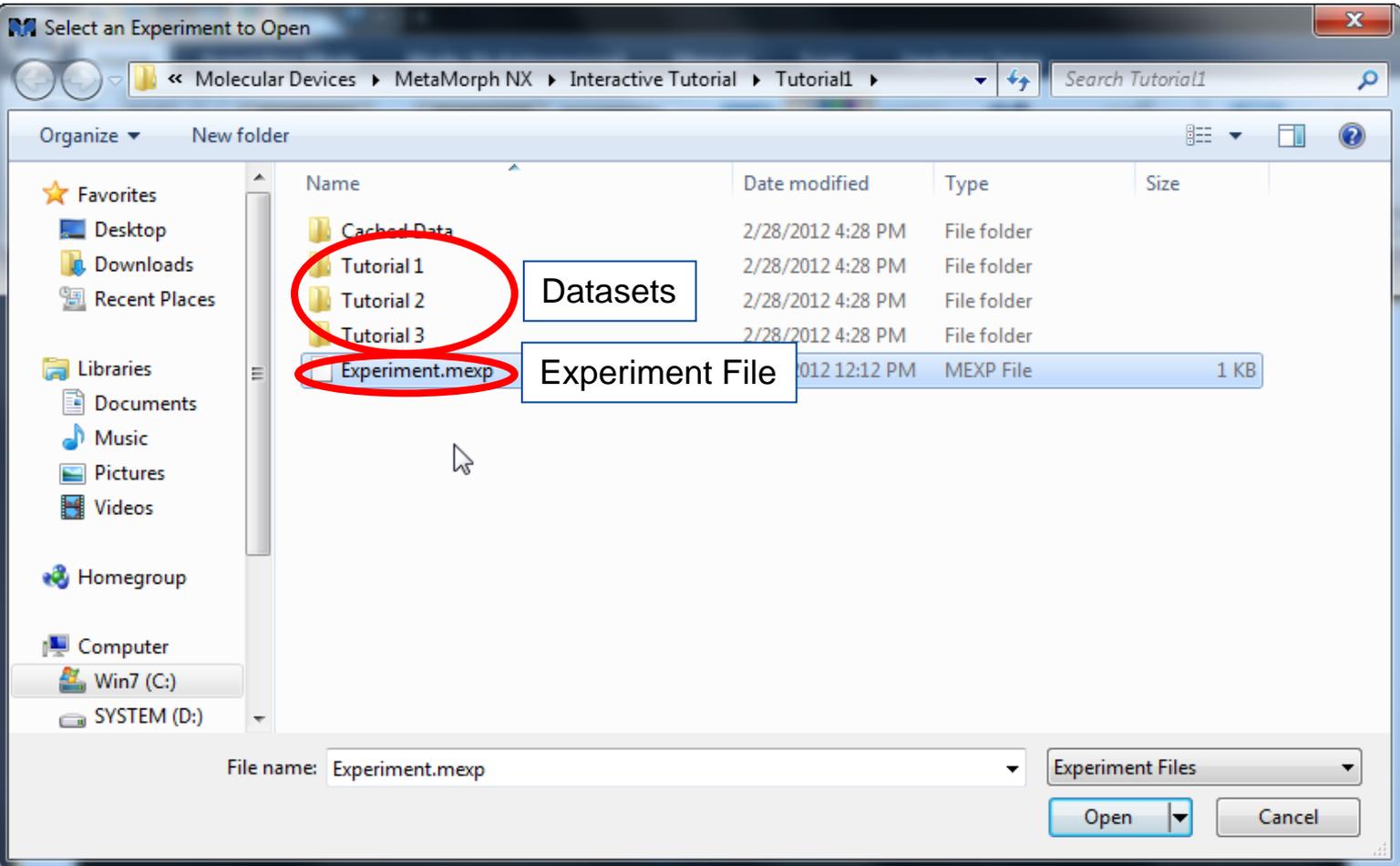
Experiment File Structure

Experiment Path

Experiment Name

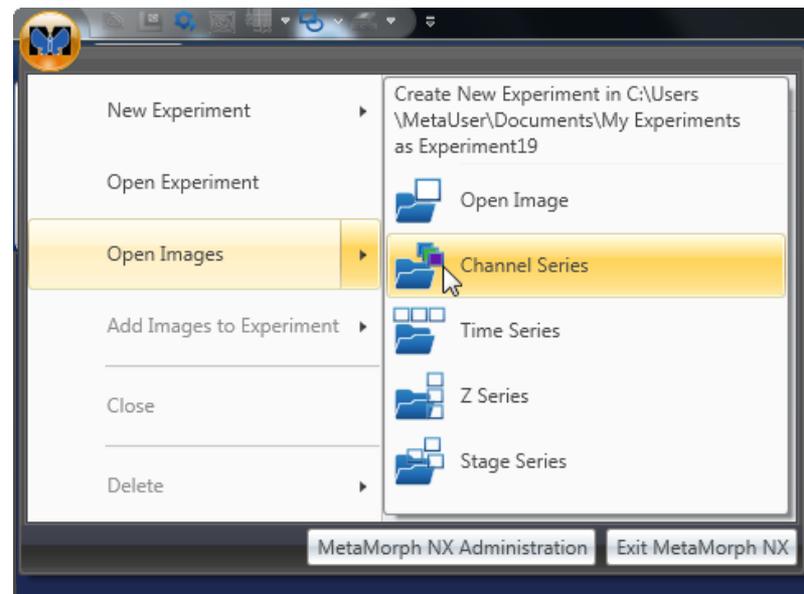


Experiment File Structure



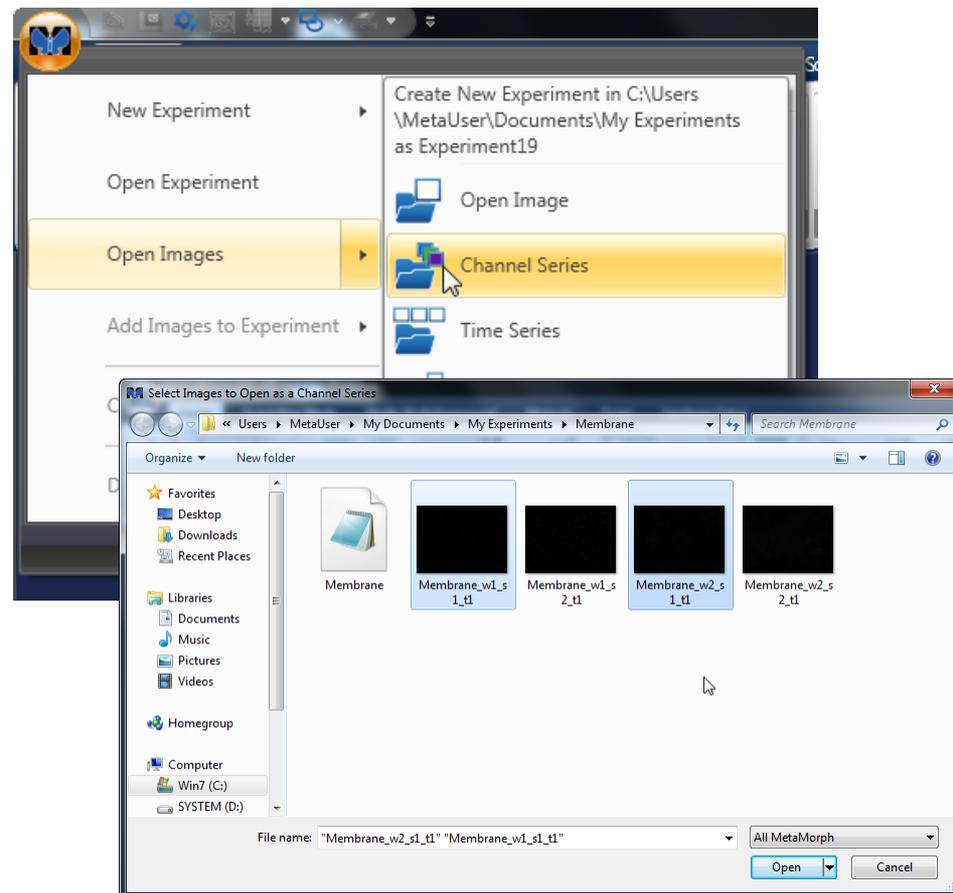
Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- Open Images
 - Opens images acquired outside of MetaMorph NX into a **New Experiment**
 - Open Image
 - Select a single image, or import a .ND file from MetaMorph
 - Channel / Time / Z Series
 - Import multiple images or stage is a series of the given dimension
 - Stage Series
 - Opens multiple images – each image is considered a new stage location.



Working with experiments

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Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Zoom To Fit Space 90.68 100% Zoom

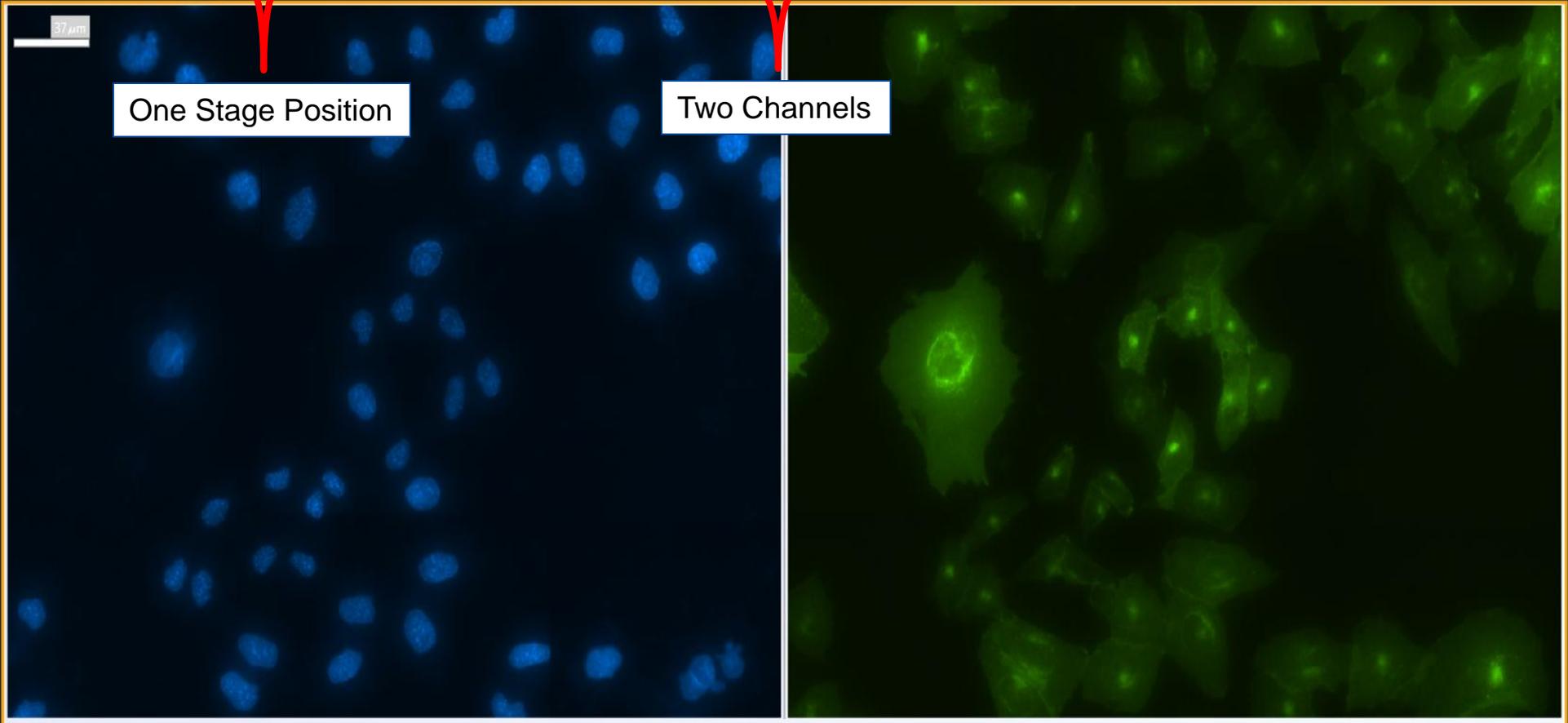
Dataset1 Stage 0 Single View View Mode 1 5 Open in 4D Viewer Export to PowerPoint

Channel Display Reset All Scaling Channel Display Channel 1 Channel 2 Reset Layout Layout

Object Overlay Threshold Overlay Zoom Preview Regions Calibration Bar Overlay

One Stage Position

Two Channels

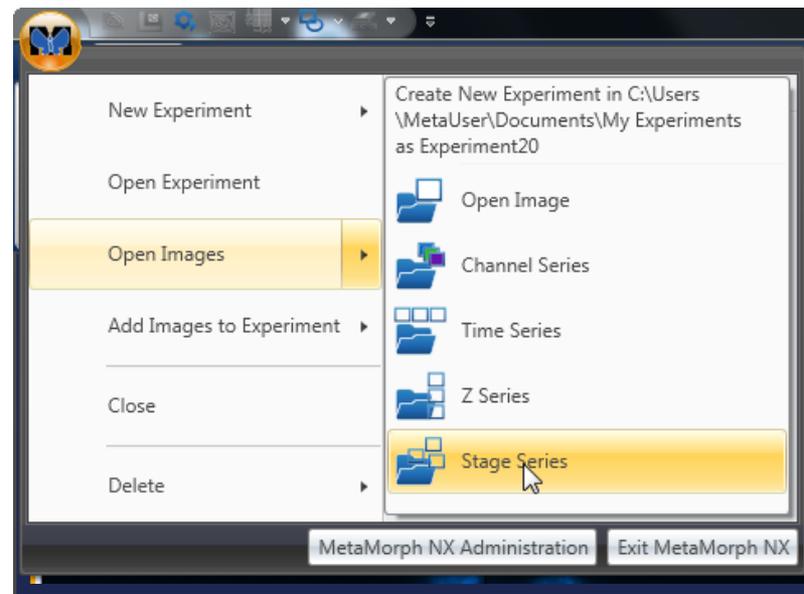


Filmstrip

View Mode Dataset

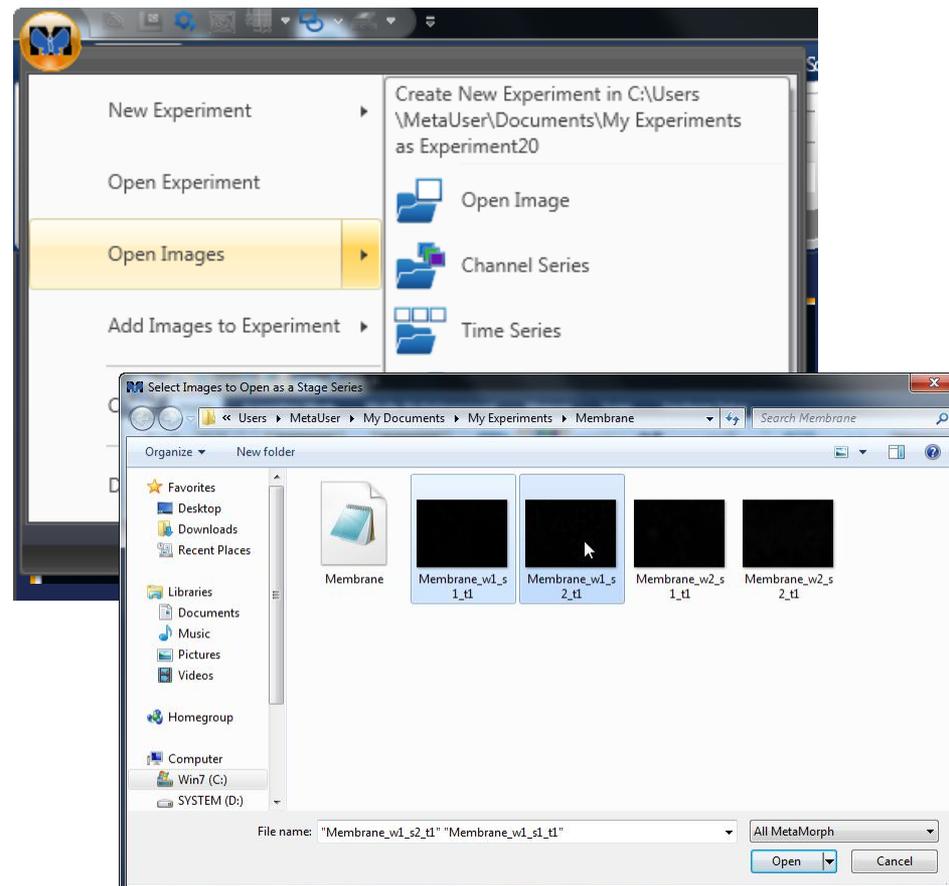
Working with experiments

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- Open Images
 - Opens images acquired outside of MetaMorph NX into a **New Experiment**
 - Open Image
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Working with experiments

- Application Menu
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 - Opens images acquired outside of MetaMorph NX into a **New Experiment**
 - Open Image
 - Select a single image, or import a .ND file from MetaMorph
 - Channel / Time / Z Series
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 - Stage Series
 - Opens multiple images – each image is considered a new stage location.



Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Zoom To Fit Space 83.82 Zoom 100%

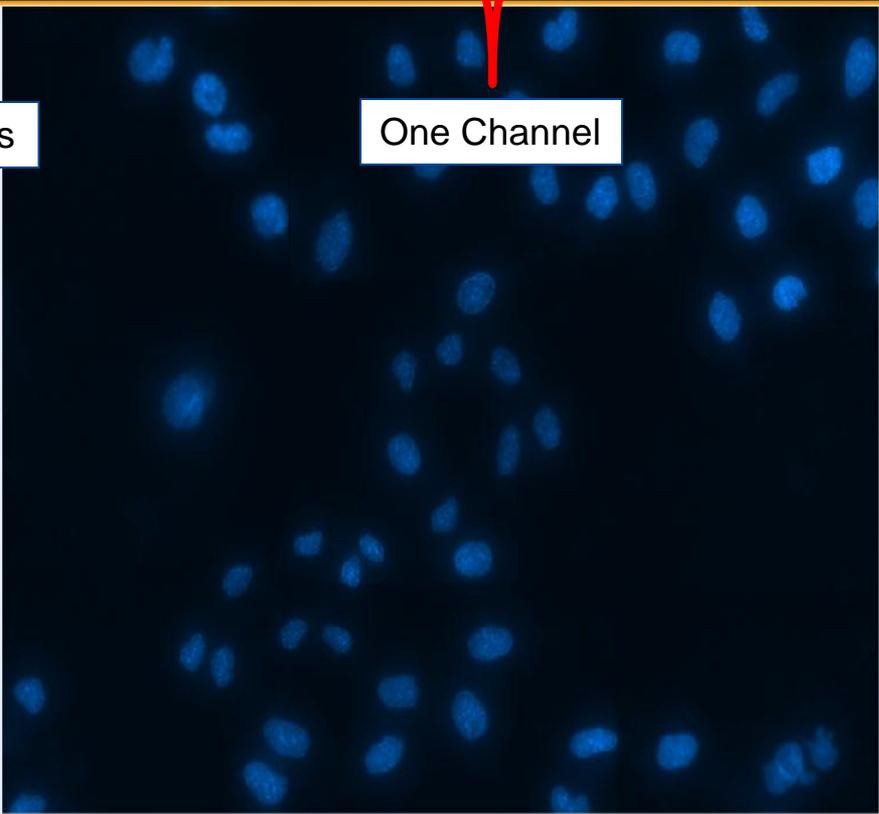
Dataset1 Stage 0 Stage 1 Single View View Mode 5 Open in 4D Viewer Export to PowerPoint

Channel Display Reset All Channel Scaling Channel Display Reset Layout Layout

Object Overlay Threshold Overlay Zoom Preview Regions Calibration Bar

Two Stage Positions

One Channel



Stage 0 of 0-1

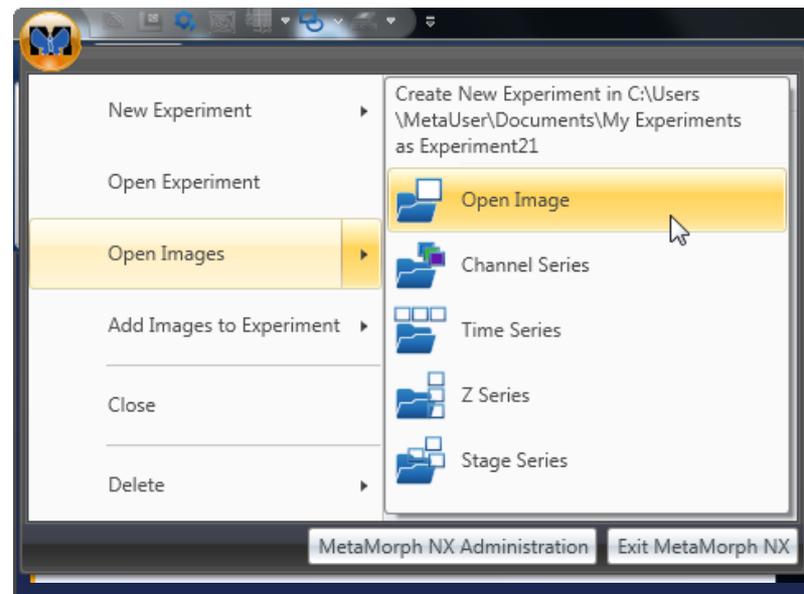
Filmstrip

View Mode Stage

No notifications

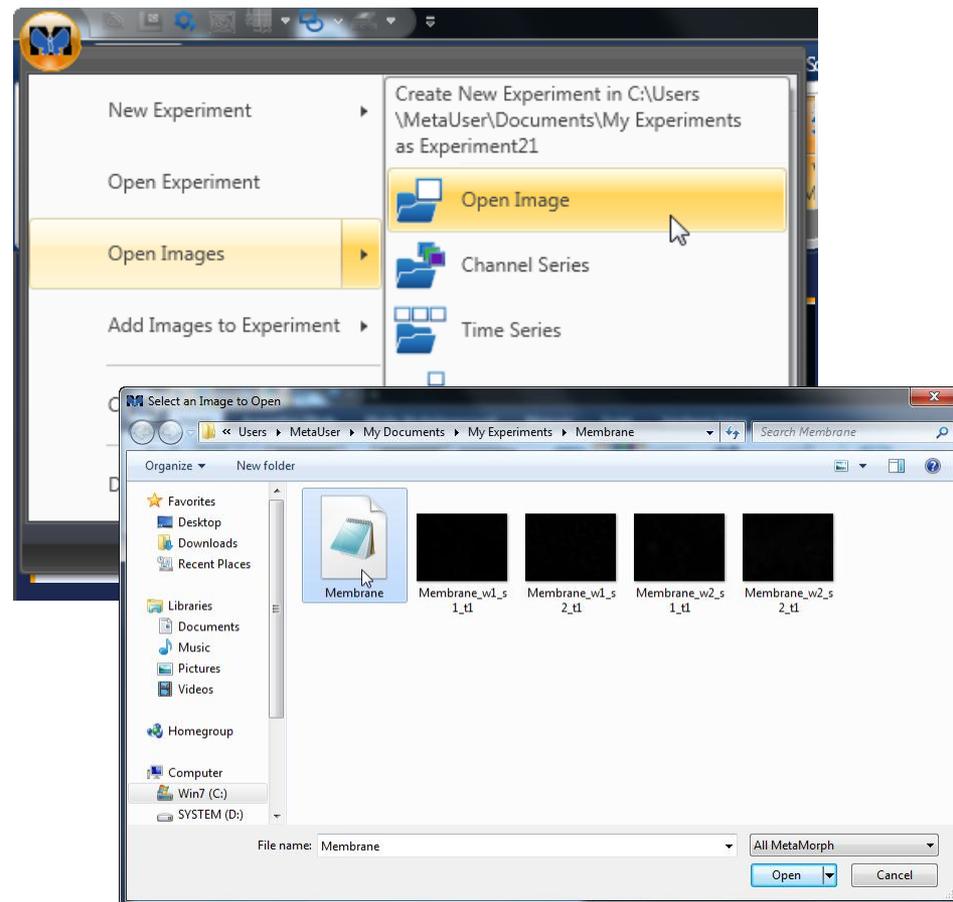
Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- Open Images
 - Opens images acquired outside of MetaMorph NX into a **New Experiment**
 - Open Image
 - Select a single image, or import a .ND file from MetaMorph
 - Channel / Time / Z Series
 - Import multiple images or stage is a series of the given dimension
 - Stage Series
 - Opens multiple images – each image is considered a new stage location.



Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- Open Images
 - Opens images acquired outside of MetaMorph NX into a **New Experiment**
 - Open Image
 - Select a single image, or import a .ND file from MetaMorph
 - Channel / Time / Z Series
 - Import multiple images or stage is a series of the given dimension
 - Stage Series
 - Opens multiple images – each image is considered a new stage location.



Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

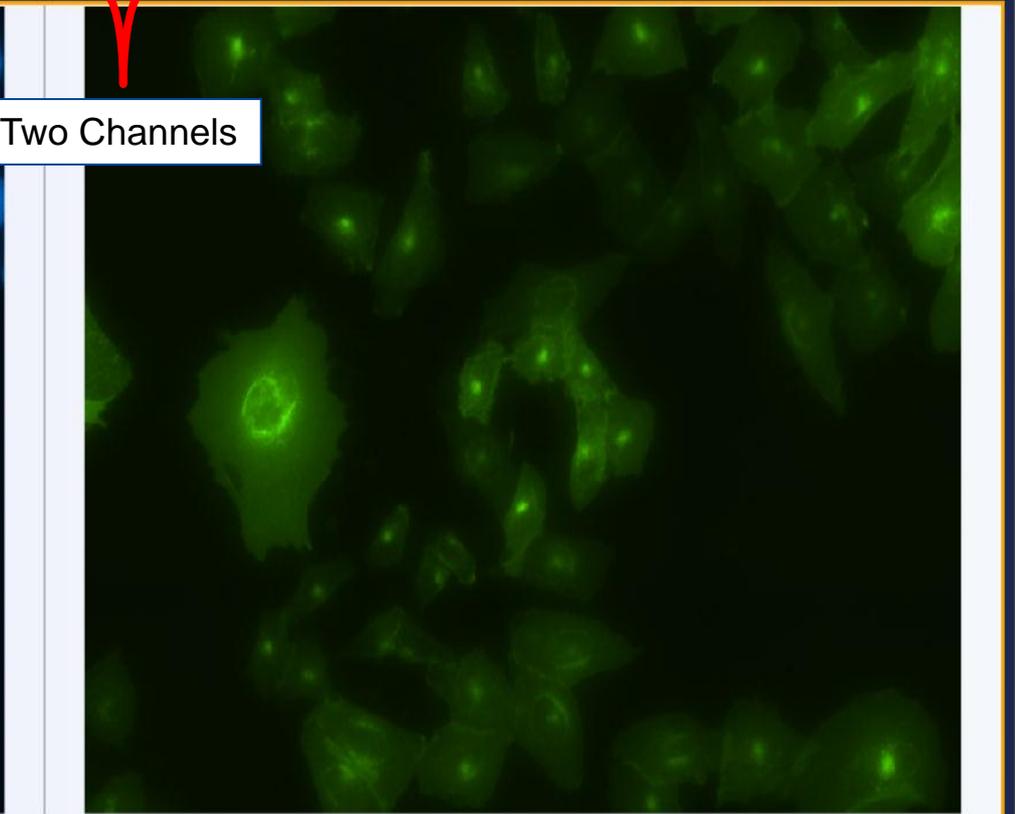
Zoom To Fit Space 83.82 Zoom 100%

Dataset1 Stage 0 Stage 1 Single View View Mode 5 Open in 4D Viewer Export to PowerPoint

Channel Display Reset All Scaling Channel Display Reset Layout Layout

Object Overlay Threshold Overlay Zoom Preview Regions Calibration Bar

Dataset1



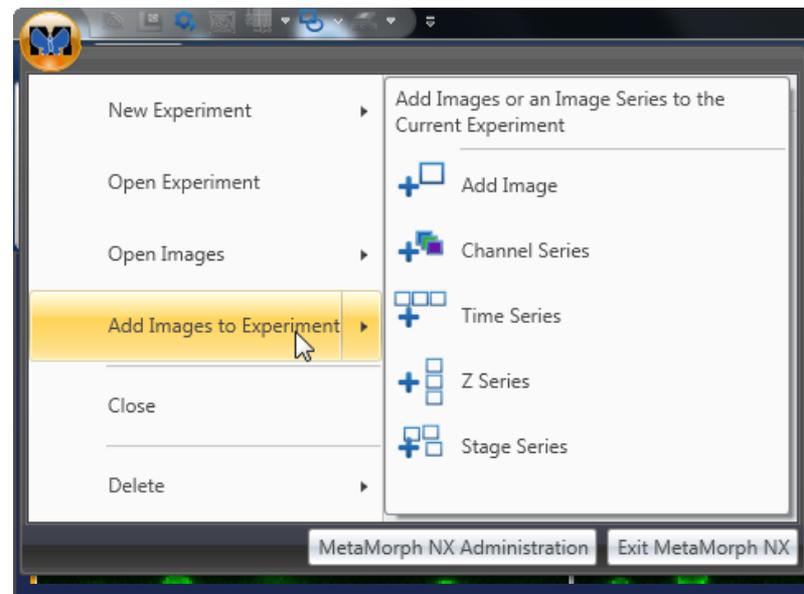
Stage 0 of 0-1 Time 0 of 0-0 Z 0 of 0-0 532, 413 149

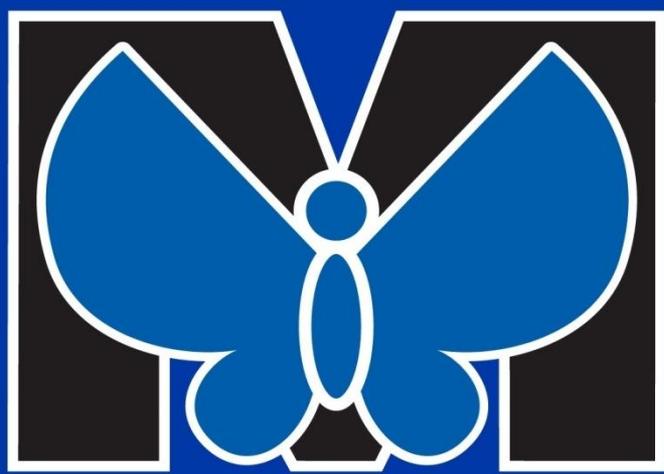
Filmstrip

View Mode Stage

Working with experiments

- Application Menu
 - Holds tools for working with the current experiment files
- Also tools for MetaMorph NX Administration.
- Add Images to Experiment
 - Opens images acquired outside of MetaMorph NX adding them to the **Current Experiment** as a new Dataset
 - Open Image
 - Select a single image, or import a .ND file from MetaMorph
 - Channel / Time / Z Series
 - Import multiple images or stage is a series of the given dimension
 - Stage Series
 - Opens multiple images – each image is considered a new stage location.





MetaMorph NX



Measurements

Basic Measurement Tools in MM NX

The New Measure Ribbon

Experiment1 - MetaMorph® NX Software

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Regions Measure Regions - Measure Threshold - Threshold Overlay Convert to Regions - Measure

Measure Range All Time All Z All Stage

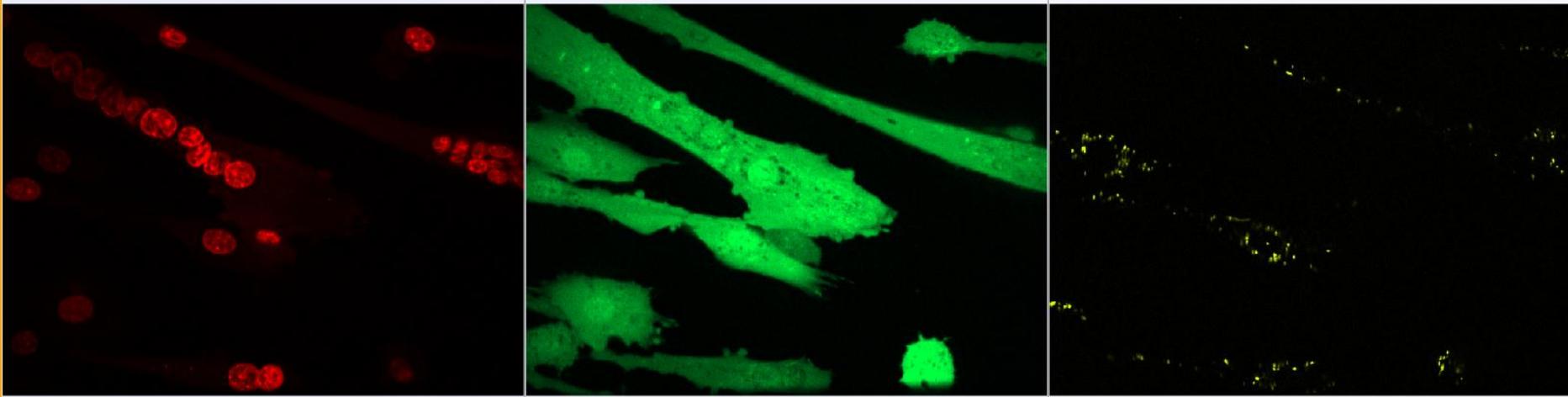
Measurement Set Plane Information Convert to Regions Configure Results

Show Table Show Summary Add Line Graph Add Scatter Plot

Export to Excel Export to File Export Table

Dataset4

100



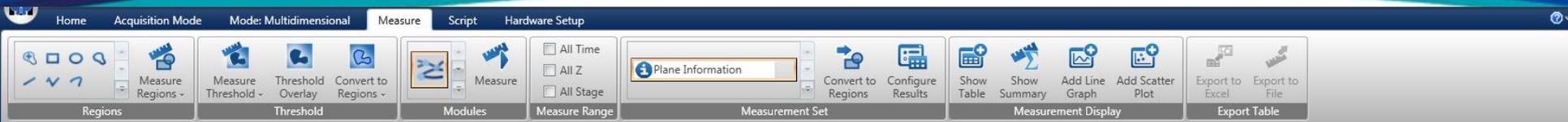
Time  Stage 0 of 0-1 Time 0 of 0-11 | Z 2 of 0-6

Filmstrip View Mode Dataset



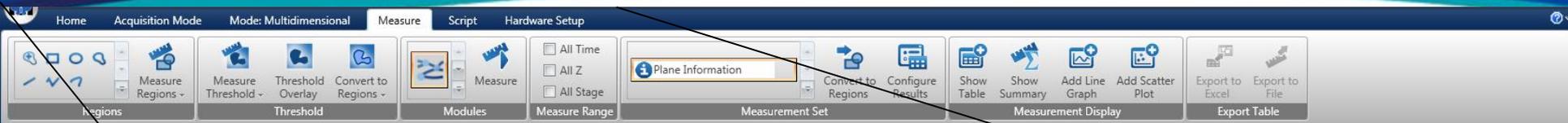
No notifications

The New Measure Ribbon



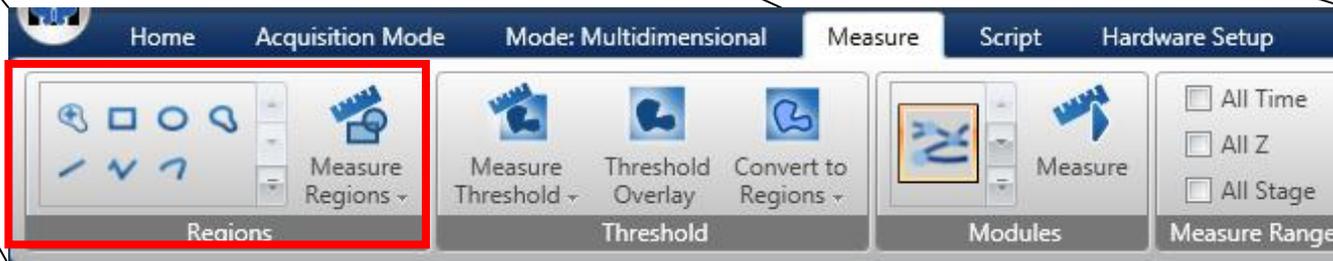
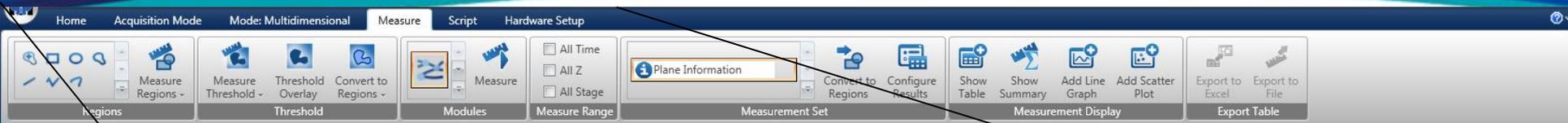
- MetaMorph 2.0.1 has re-designed the measure ribbon
 - Reorganized tools like Regions, Thresholding, and Modules
 - Added space for the Application Modules
 - Added tools for exporting data
 - Added tools for turning thresholds and Module masks to Regions of Interest.

Measurement Tools



- MetaMorph 2.0.1 has re-designed the measure ribbon
 - The Left side of the Ribbon contains tools for making measurements.

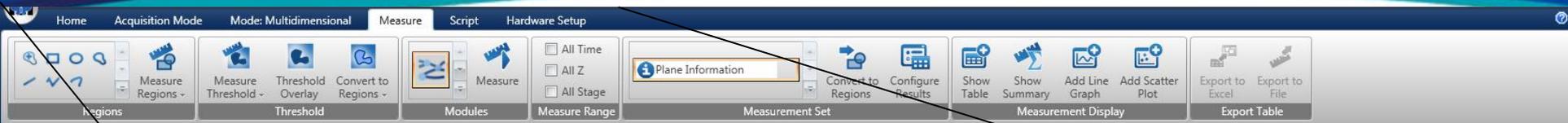
Region Tools



- **Regions**

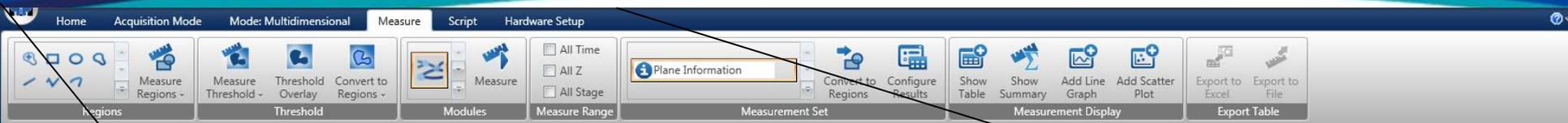
- The first section is for Regions of Interest.
- There is a gallery for choosing the type of Region to draw.
- There is a drop down menu for configuring Region Measurements
- There is a button to Measure Regions

Region Tools



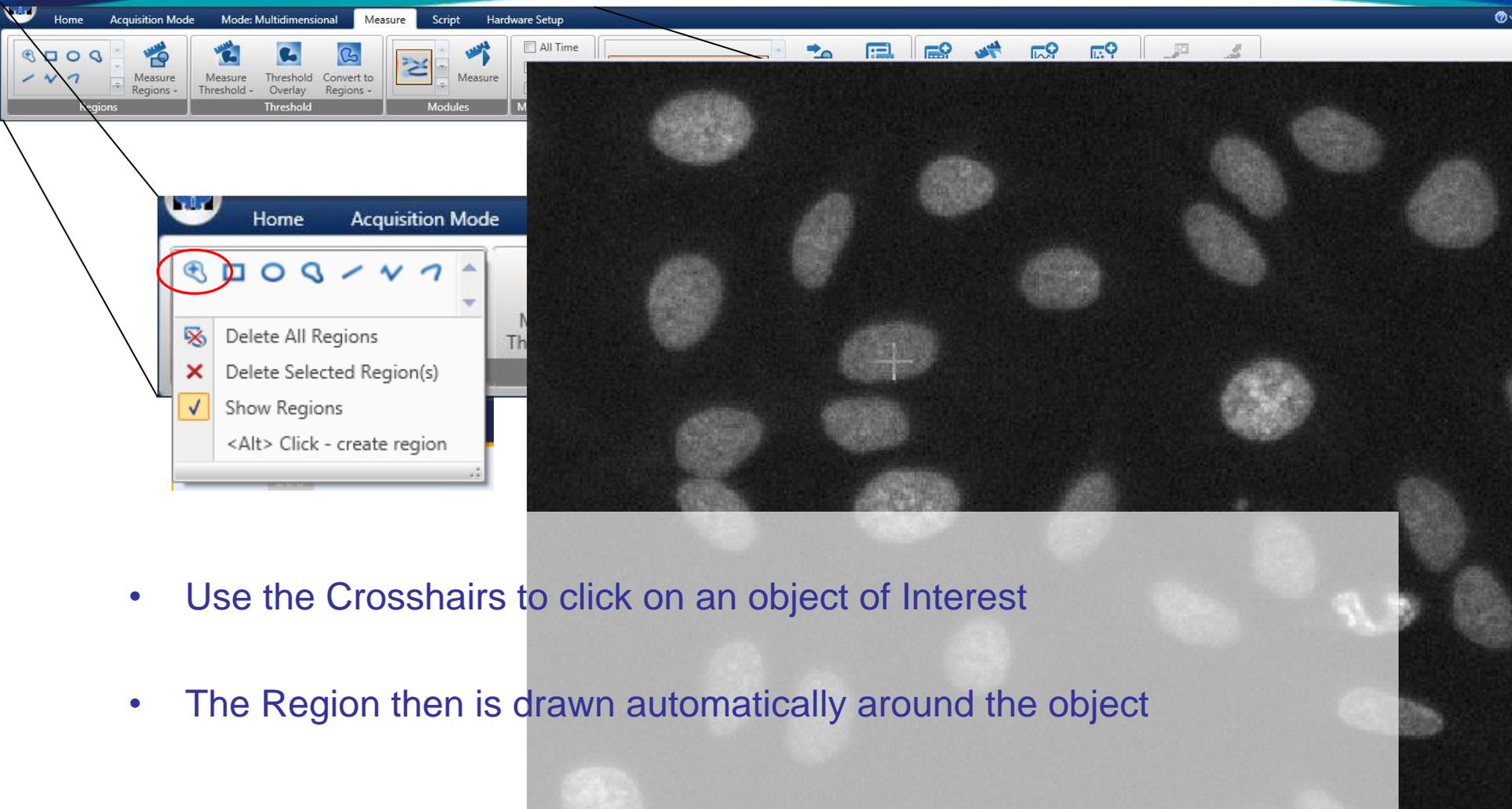
- The first section is for Regions of Interest.
- There is a gallery for choosing the type of Region to draw.
- There is a drop down menu for configuring Region Measurements
- There is a button to Measure Regions

Region Tools



- The first section is for Regions of Interest.
- New Auto Find Tool helps place Regions by clicking on an object of Interest. There is a gallery for choosing the type of Region to draw.
- There is a drop down menu for configuring Region Measurements
- The Region then is drawn automatically around the object
- There is a button to Measure Regions

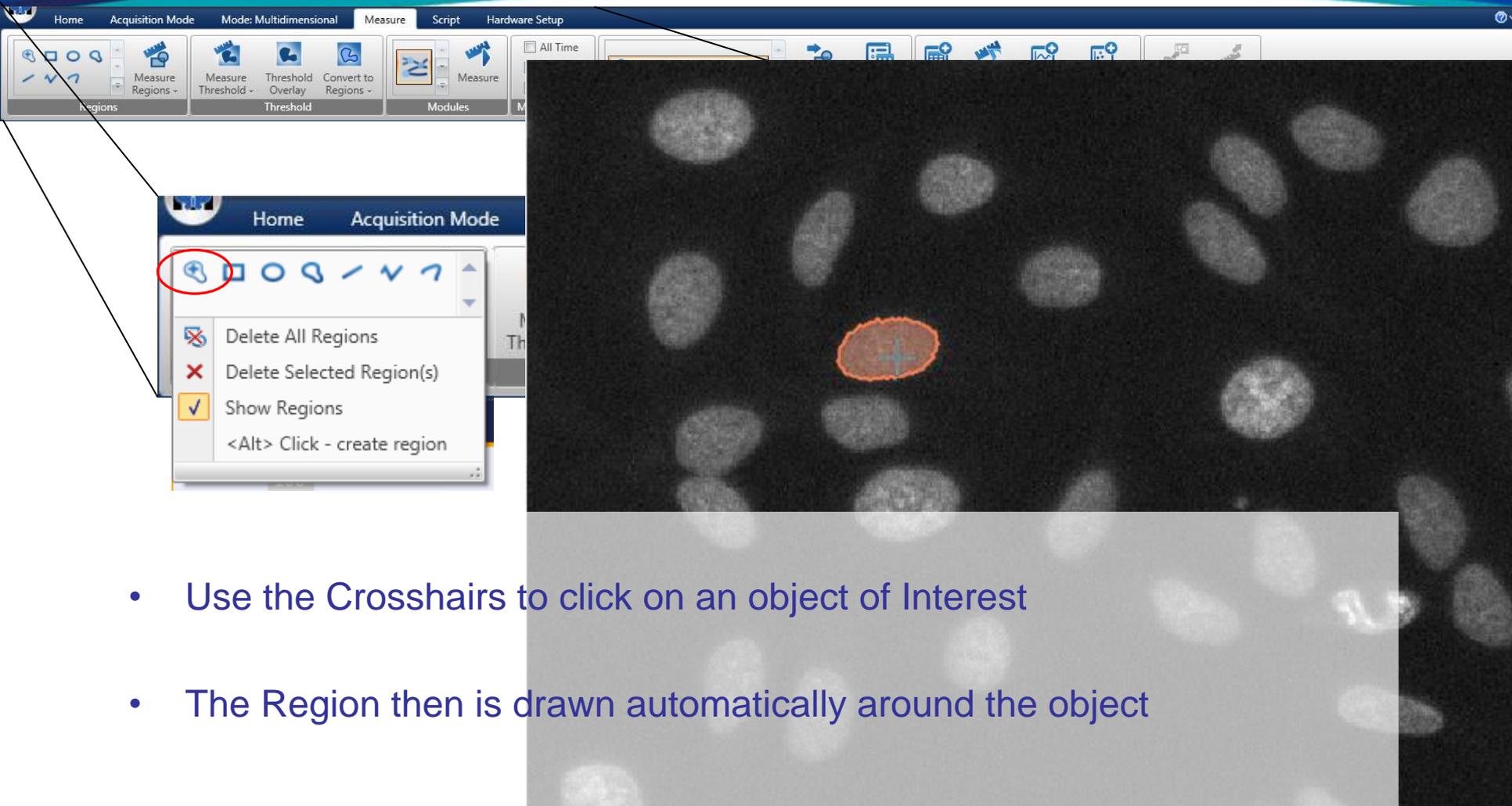
Region Tools



The screenshot displays the software interface with the 'Home' and 'Acquisition Mode' tabs. The 'Regions' toolbar is visible, and a context menu is open, showing options: 'Delete All Regions', 'Delete Selected Region(s)', 'Show Regions', and '<Alt> Click - create region'. The 'Show Regions' option is checked. The background image shows a grayscale microscopy view of cells with a white crosshair centered on one of them.

- Use the Crosshairs to click on an object of Interest
- The Region then is drawn automatically around the object

Region Tools



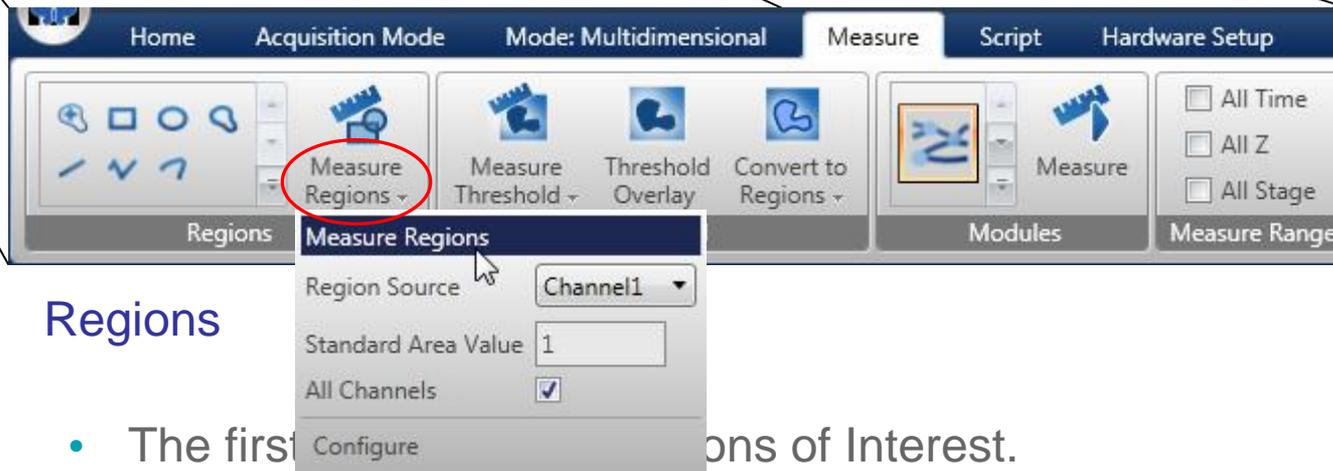
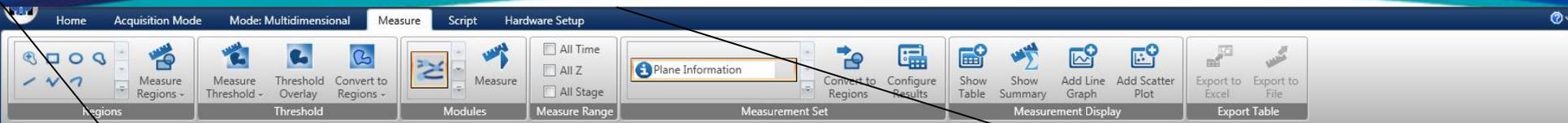
The screenshot displays the software interface with the 'Home' tab selected. The 'Regions' group in the ribbon is expanded, showing a dropdown menu. The 'Crosshairs' icon is circled in red. The dropdown menu includes the following options:

- Delete All Regions
- Delete Selected Region(s)
- Show Regions
- <Alt> Click - create region

The main image shows a grayscale microscopy view of cells. A red crosshair is positioned over one cell, and a red region is automatically drawn around it. A semi-transparent gray box is overlaid on the bottom portion of the image.

- Use the Crosshairs to click on an object of Interest
- The Region then is drawn automatically around the object

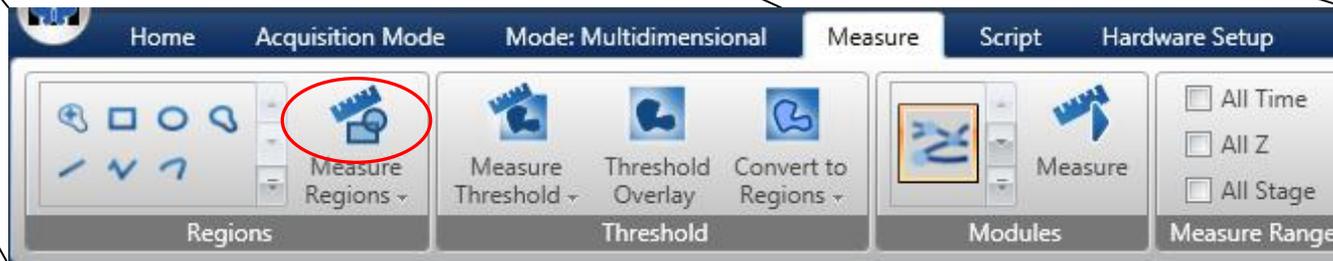
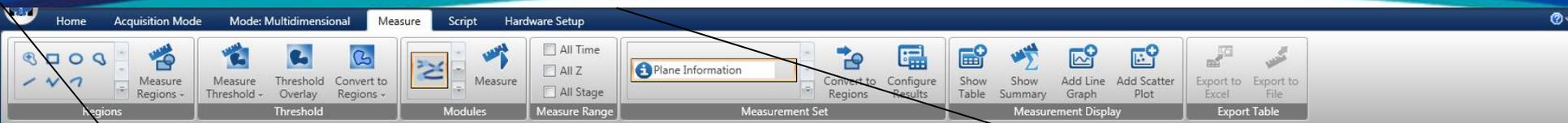
Region Tools



- Regions

- The first step is to define Regions of Interest.
- There is a gallery for choosing the type of Region to draw.
- There is a drop down menu for configuring Region Measurements
- There is a button to Measure Regions

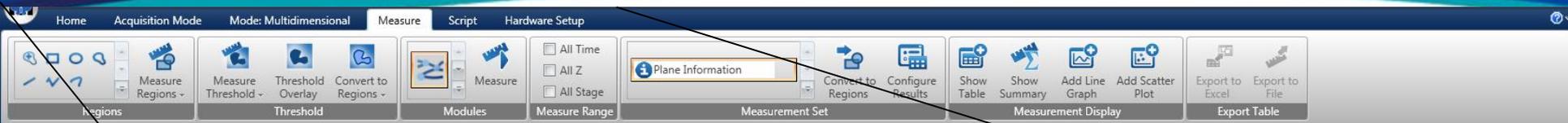
Region Tools



- **Regions**

- The first section is for Regions of Interest.
- There is a gallery for choosing the type of Region to draw.
- There is a drop down menu for configuring Region Measurements
- There is a button to Measure Regions

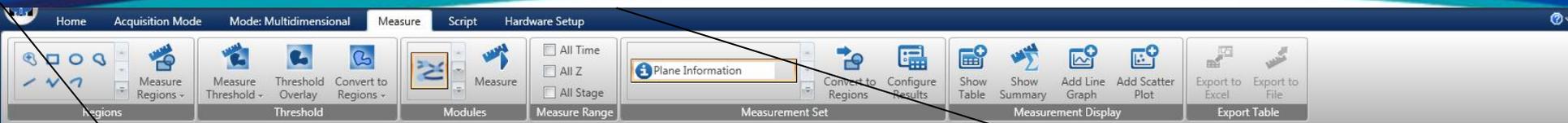
Region Tools



- TIP

- Press the ALT key and click on the image to continue to place Regions of the same type

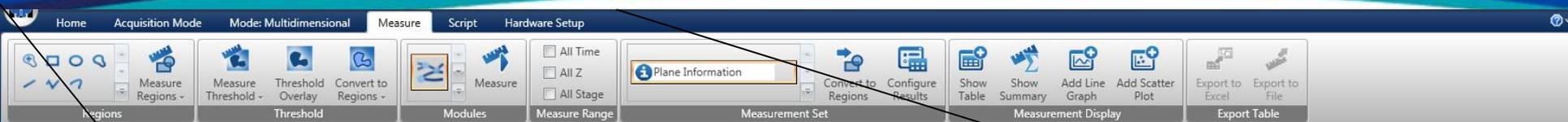
Threshold Tools



- **Threshold**

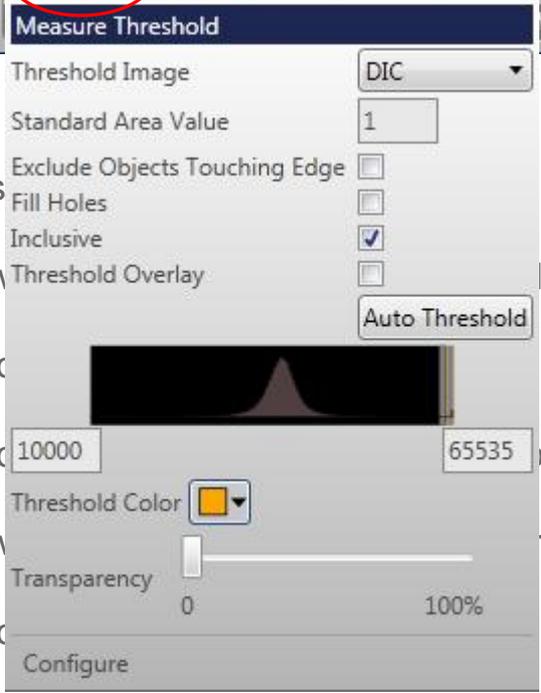
- The next section is for Thresholding.
- There is a drop down menu for setting the Threshold
- There is a button to Measure Thresholded Areas
- There is a button to turn the Threshold overlay on or off
- There is a drop down menu for configuring how Thresholds are turned to Regions
- There is a button to turn Thresholds into Regions

Threshold Tools



- **Threshold**

- The next section is
 - There is a drop down
 - There is a button to
 - There is a button to
 - There is a drop down
 - There is a button to
- Thresholds are turned to Regions



Regions

Measure Regions

Measure Threshold

Threshold Image: GFP

Standard Area Value: 1

Exclude Objects Touching Edge:

Fill Holes:

Inclusive:

Threshold Overlay:

Auto Threshold

639 1704

Threshold Color:

Transparency: 0 100%

Configure

Plane Information

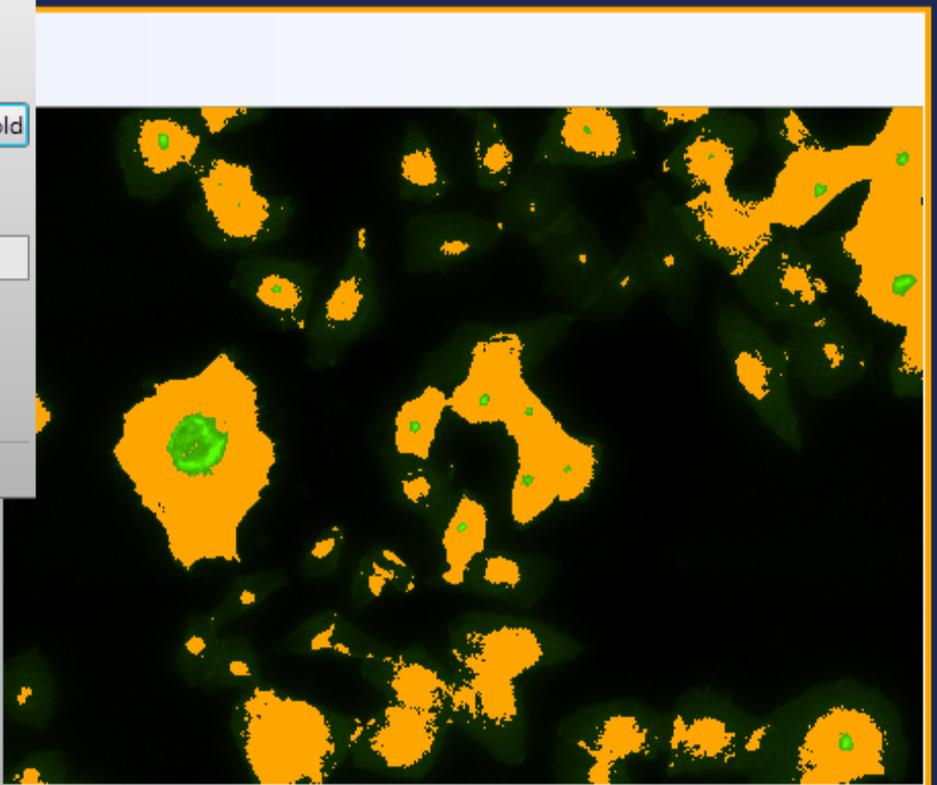
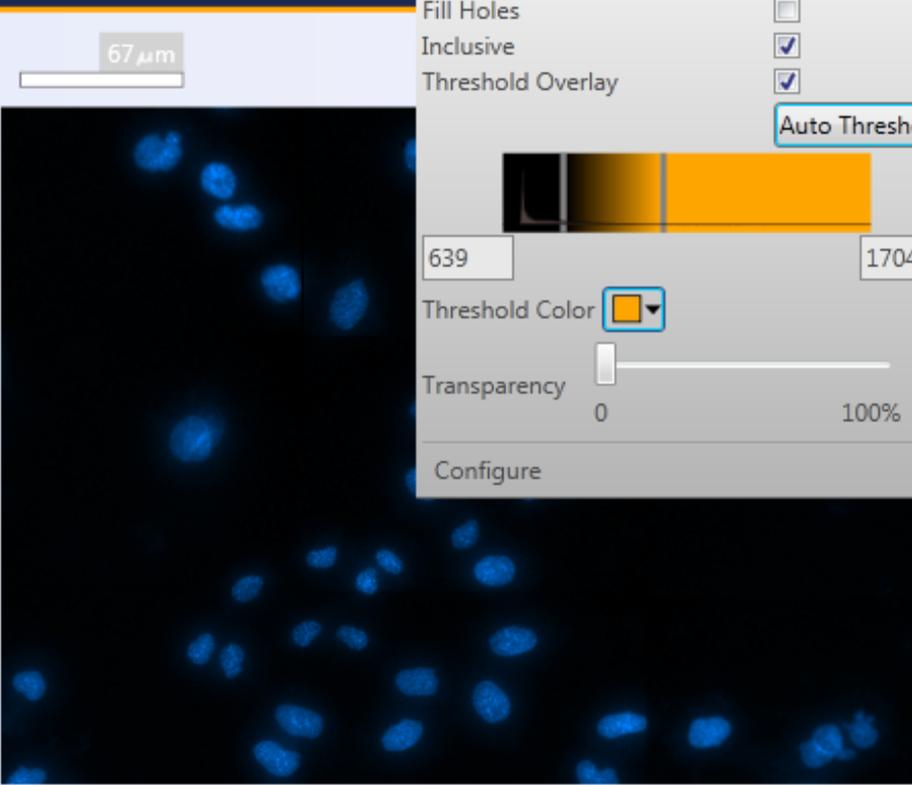
Measurement Set

Convert to Regions

Configure Results

Meas... Exp...

Dataset1



Stage 0 of 0-1

Navigation and zoom controls

Regions

Measure Regions

Measure Threshold

Threshold Image: GFP

Standard Area Value: 1

Exclude Objects Touching Edge:

Fill Holes:

Inclusive:

Threshold Overlay:

Auto Threshold

639 1704

Threshold Color: [Color Picker]

0 100%

Configure

Plane Information

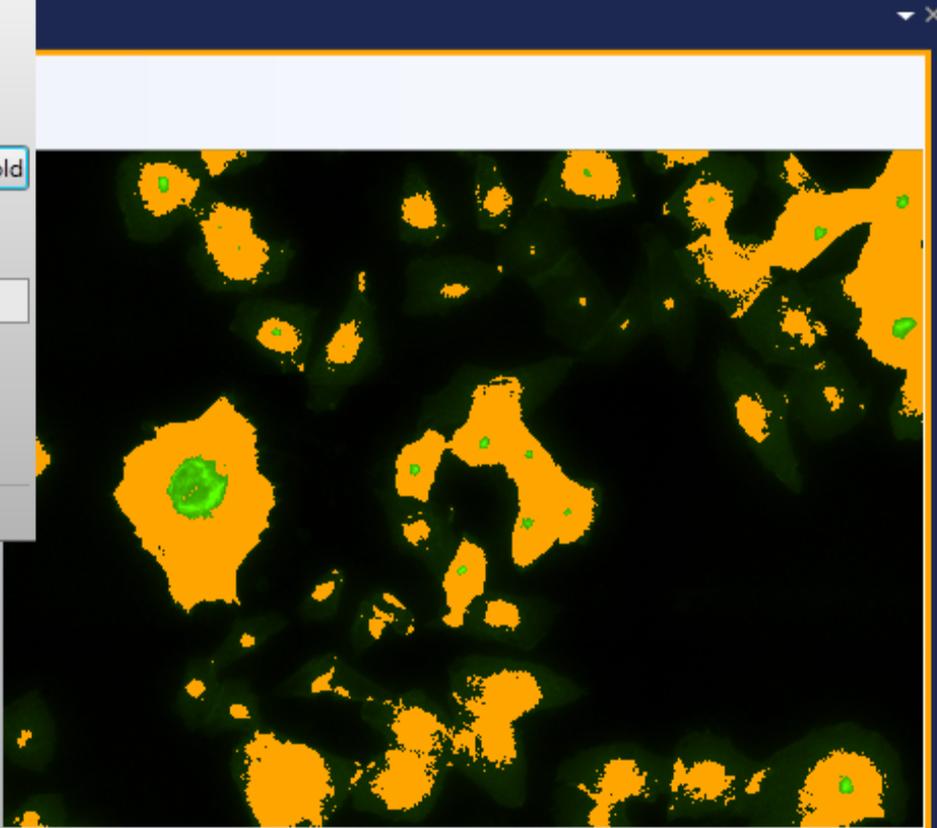
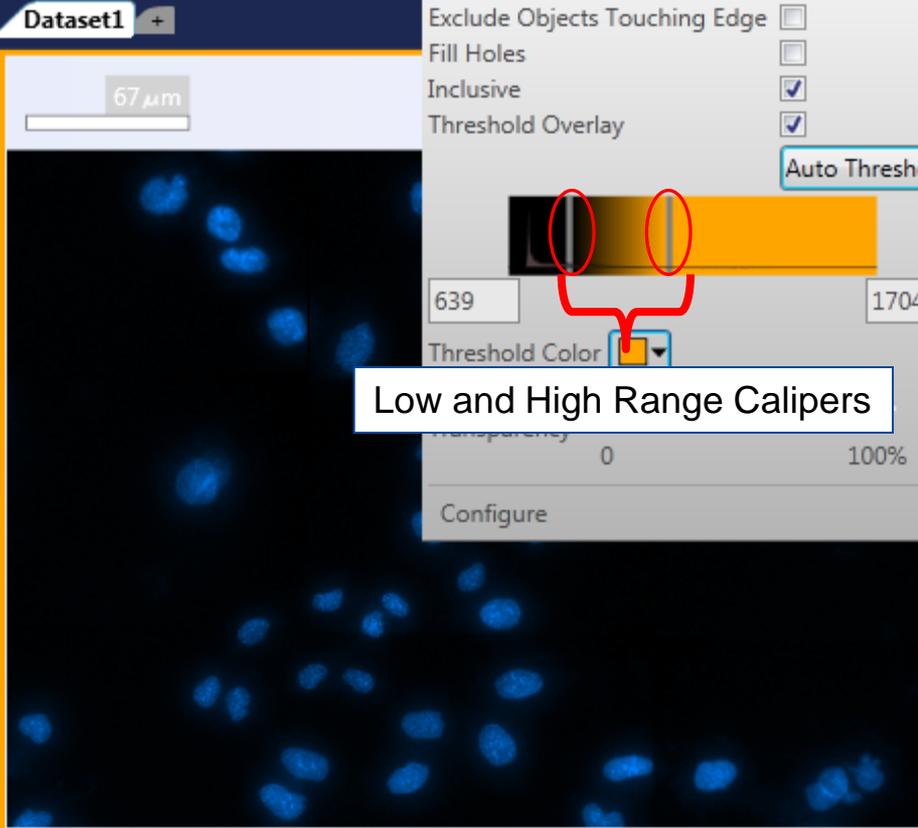
Measure Using Threshold

Convert to Regions

Configure Results

Measurement Set

Meas... Exp...



Low and High Range Calipers

Stage 0 of 0-1

Navigation and status bar with arrows and colored segments.



Measure Regions

Regions

Measure Threshold

Threshold Image GFP

Standard Area Value 1

Exclude Objects Touching Edge

Fill Holes

Inclusive

Threshold Overlay

Auto Threshold



639

1704

Threshold Color

Transparency 0 100%

Configure

Threshold Mask Cleanup

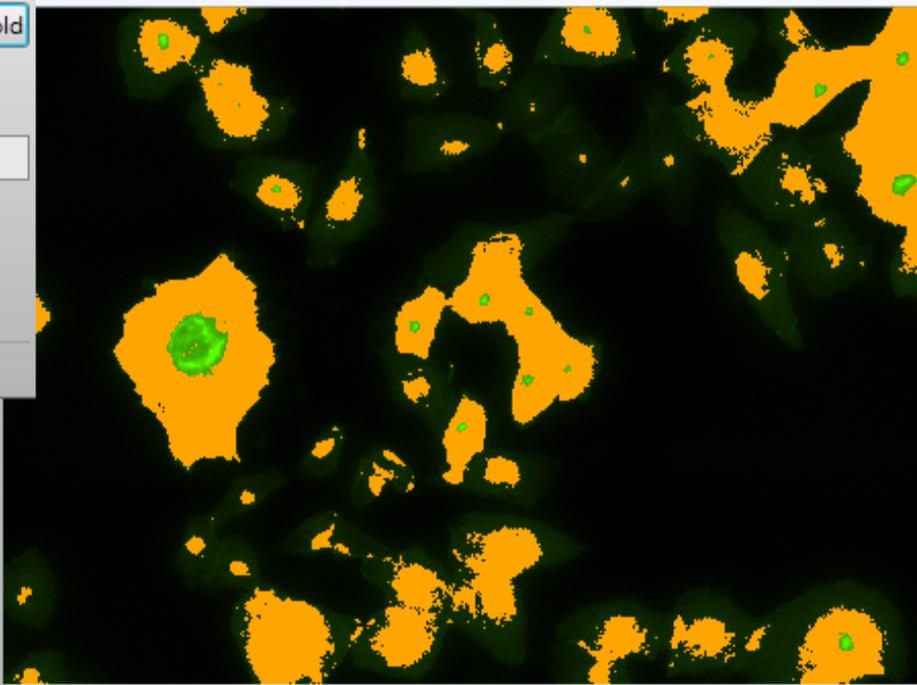
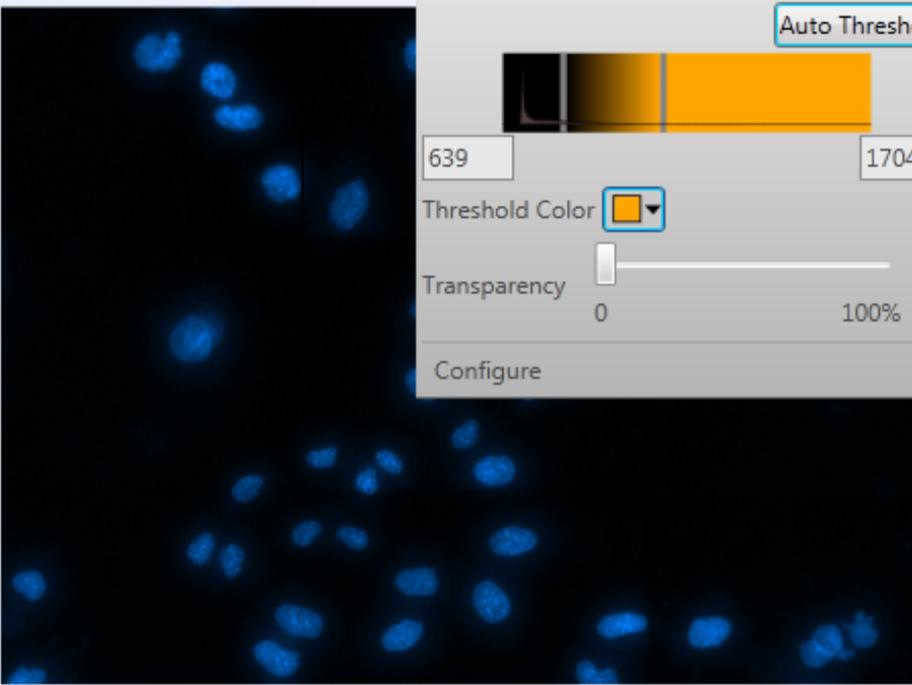
Plane Information
Measure Using Threshold

Convert to Regions
Configure Results

Meas... Exp...

Dataset1

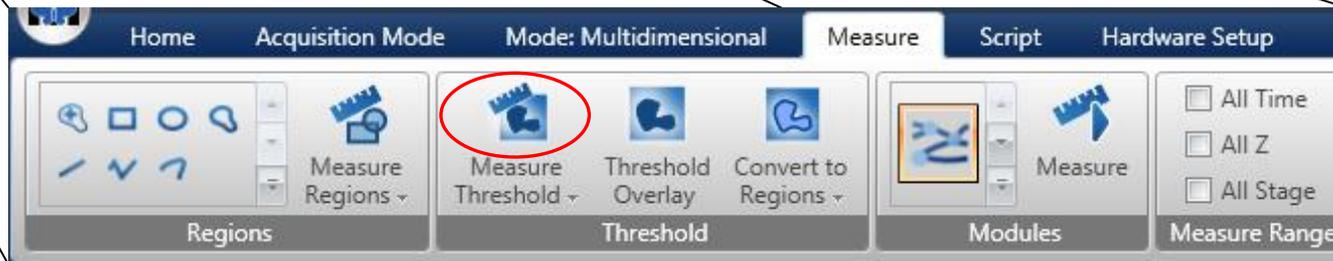
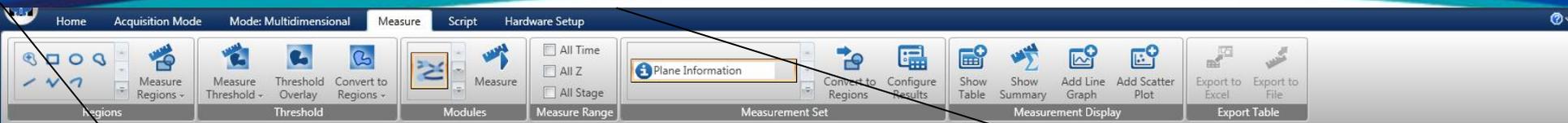
67 μm



Stage 0 of 0-1



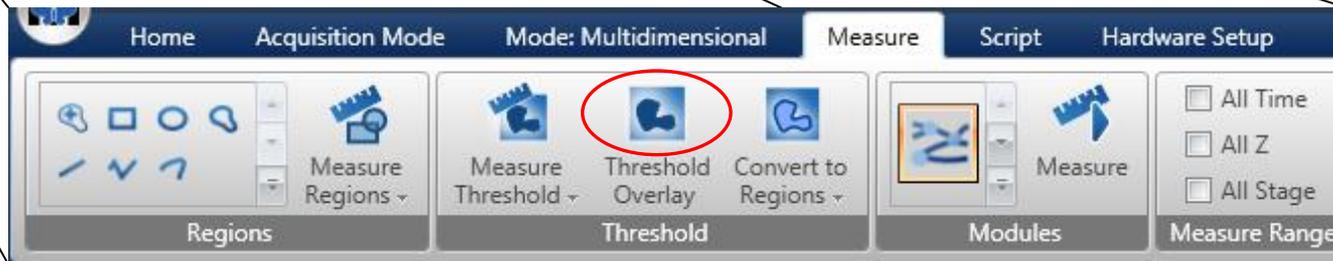
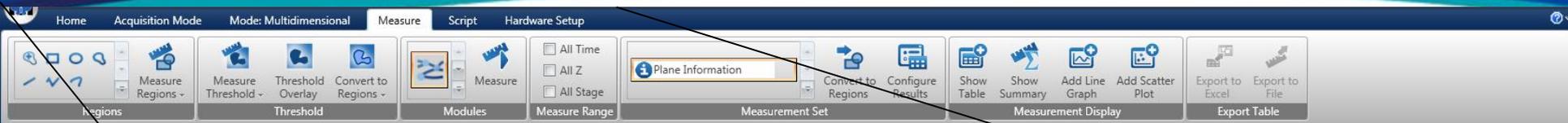
Threshold Tools



- **Threshold**

- The next section is for Thresholding.
- There is a drop down menu for setting the Threshold
- There is a button to Measure Thresholded areas
- There is a button to turn the Threshold overlay on or off
- There is a drop down menu for configuring how Thresholds are turned to Regions
- There is a button to turn Thresholds into Regions

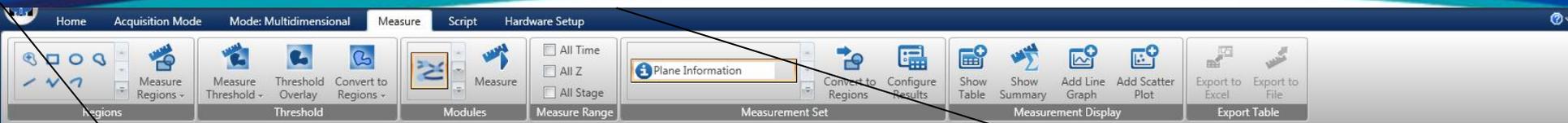
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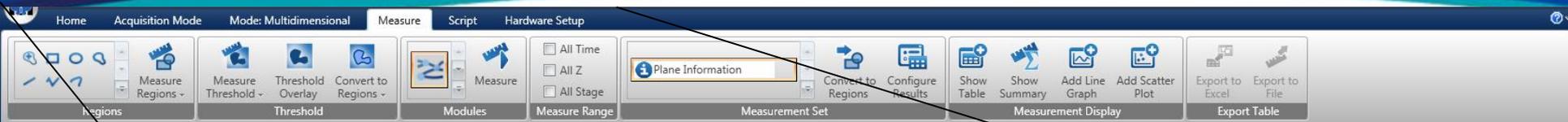
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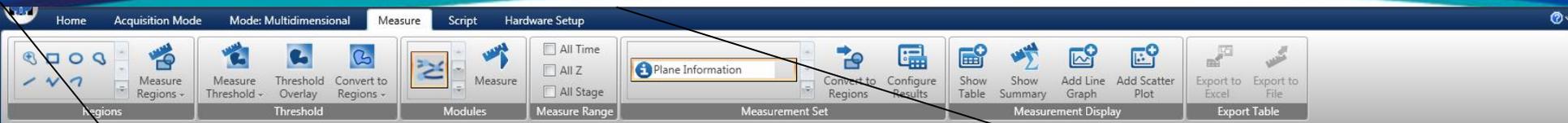
Threshold Tools



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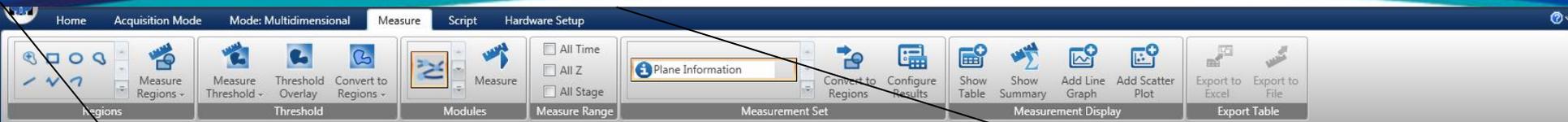
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Modules



- Module
 - The next section is for Modules.
 - There is a gallery for selecting which Application or Custom Module to run
 - You can click on the Module to configure it
 - Custom Modules can be edited or deleted
 - Use Create Module to make a new Custom Module
 - Press Measure to run the selected Module

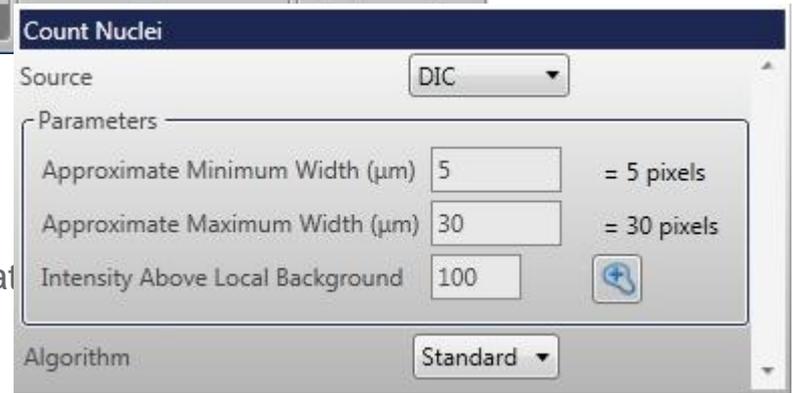
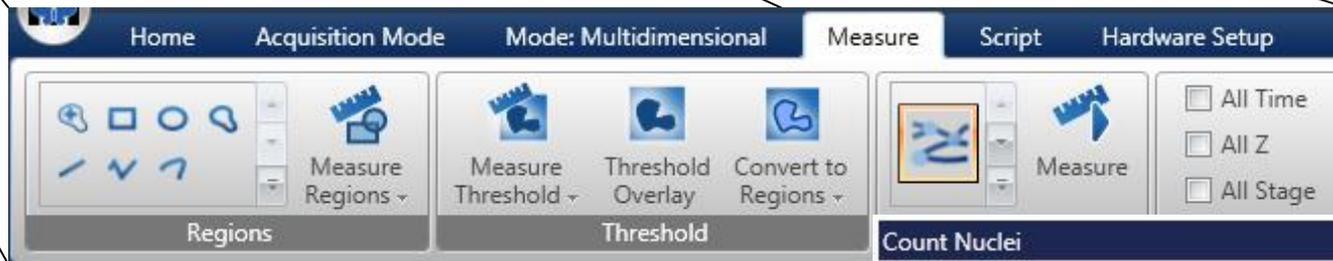
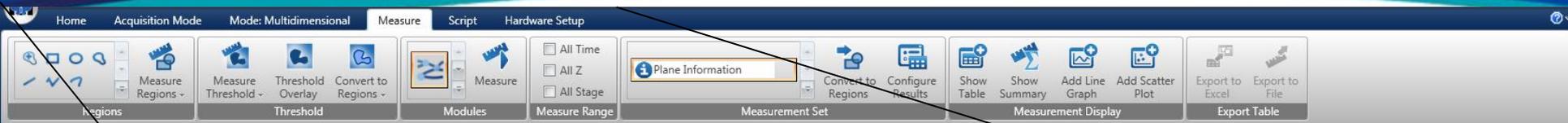
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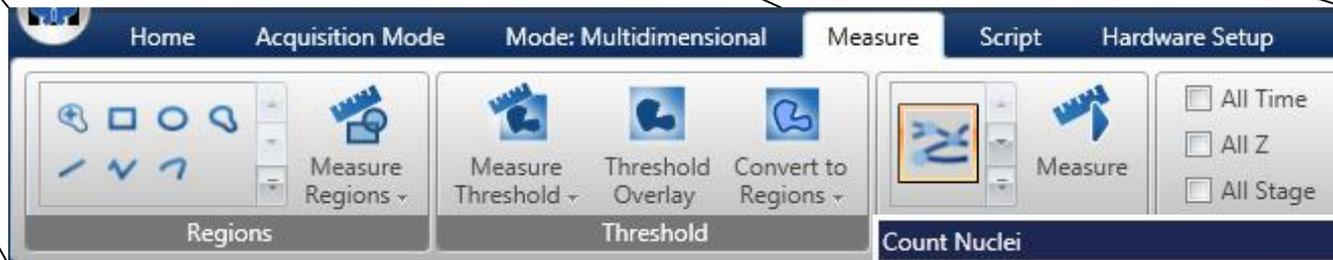
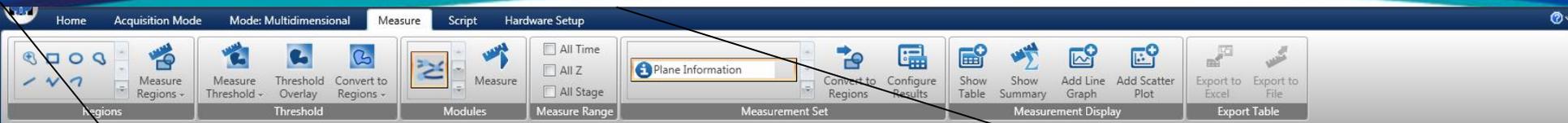
Modules



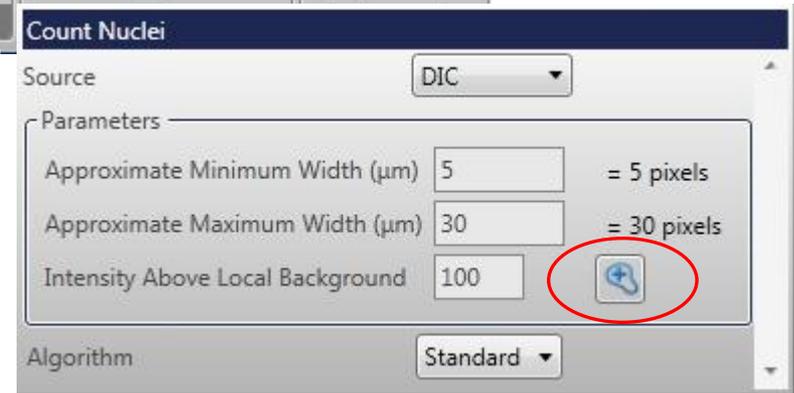
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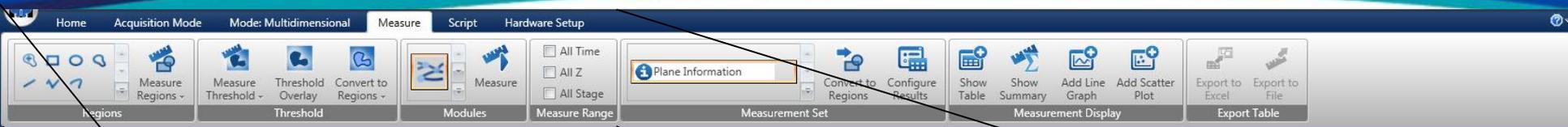
Modules



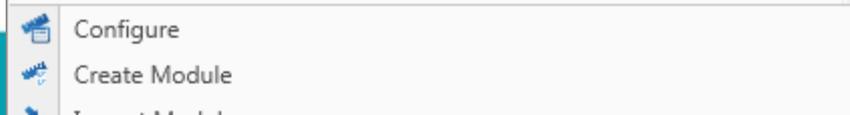
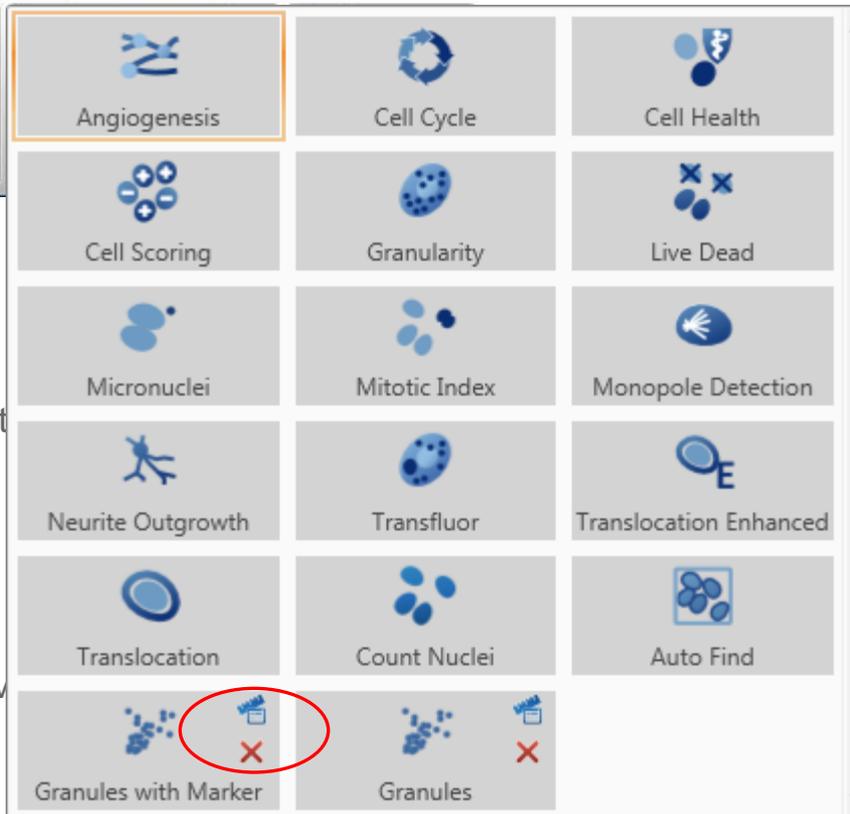
- Many of the Application Modules also have the Click To Find feature
 - Modules are easier to setup – no measuring intensities or sizes needed
- Modules also have Fast Algorithm options
 - Perform similar to Standard algorithm, but run 2x faster (or more)



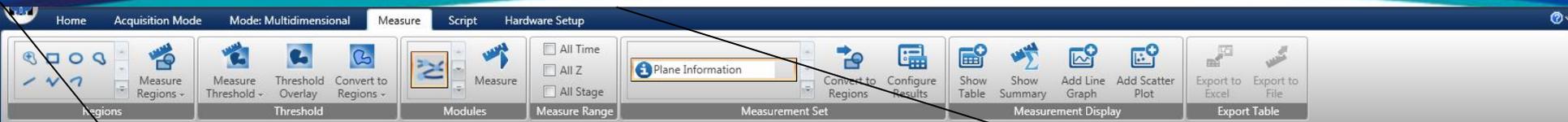
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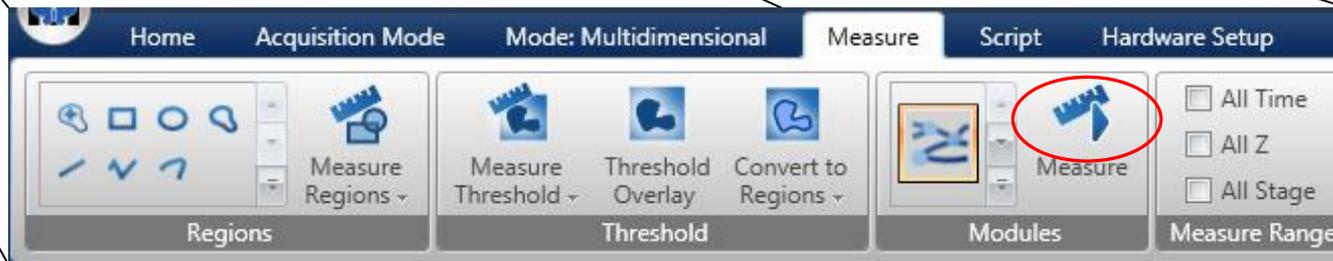
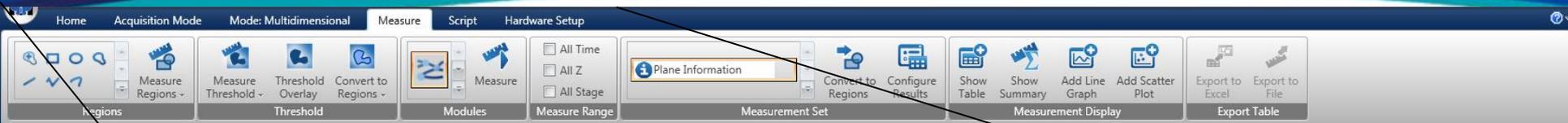
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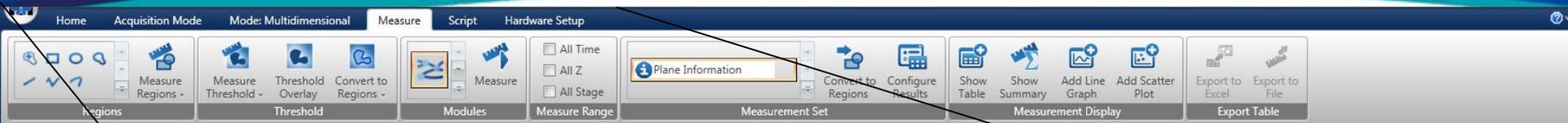


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Modules



- Measured Images

- Measure Range defines what Images to measure using any of the 3 Measure buttons.
- If no options are turned on, only the Visible images are measured
- Turn on All Time, All Z, and All Stage to measure your entire dataset.

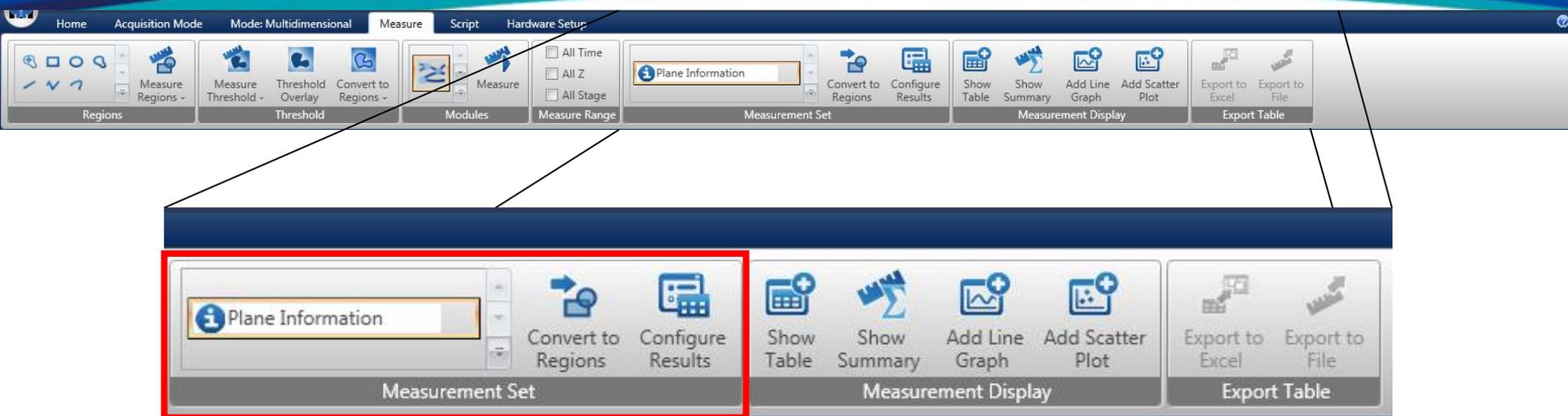
Measurement Tools



- **Measurements**

- The Right side of the Ribbon contains tools for after you make measurements.
- Such as selecting or displaying data, and converting Masks to Regions.

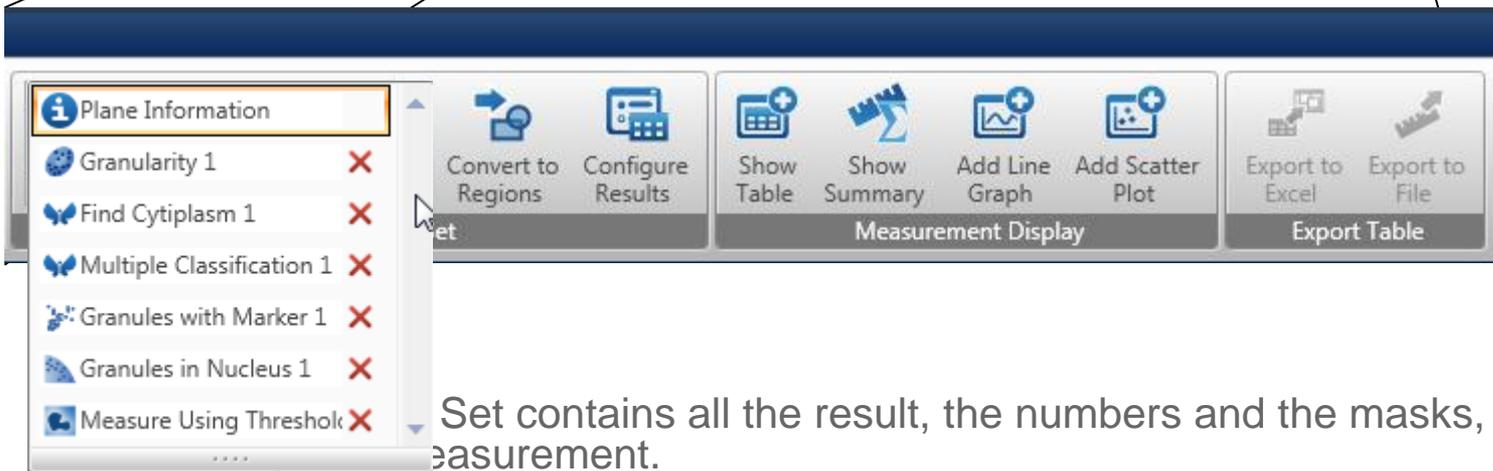
Measurement Tools



- **Measurement Set**

- A Measurement Set contains all the result, the numbers and the masks, from any one measurement.
- There is a gallery to choose which Measurement Set to work with.
- There is a button to turn the masks associated with the active Measurement Set into Regions
- There is a button to configure what Objects to keep in for Display.

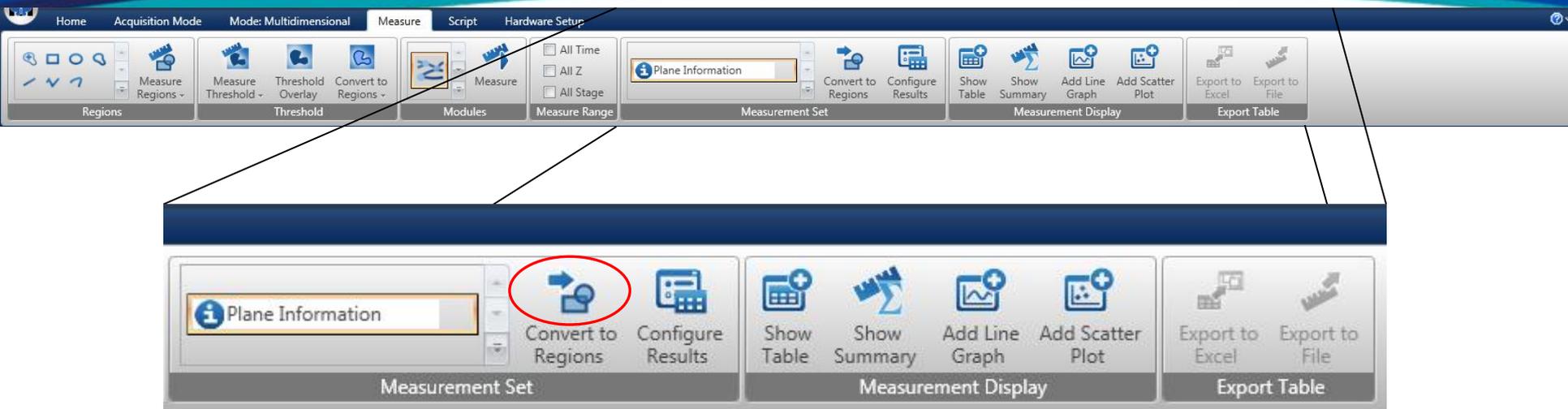
Measurement Tools



Set contains all the result, the numbers and the masks, measurement.

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Measurement Tools



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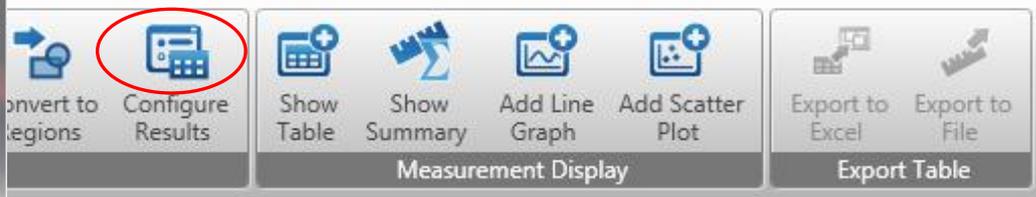
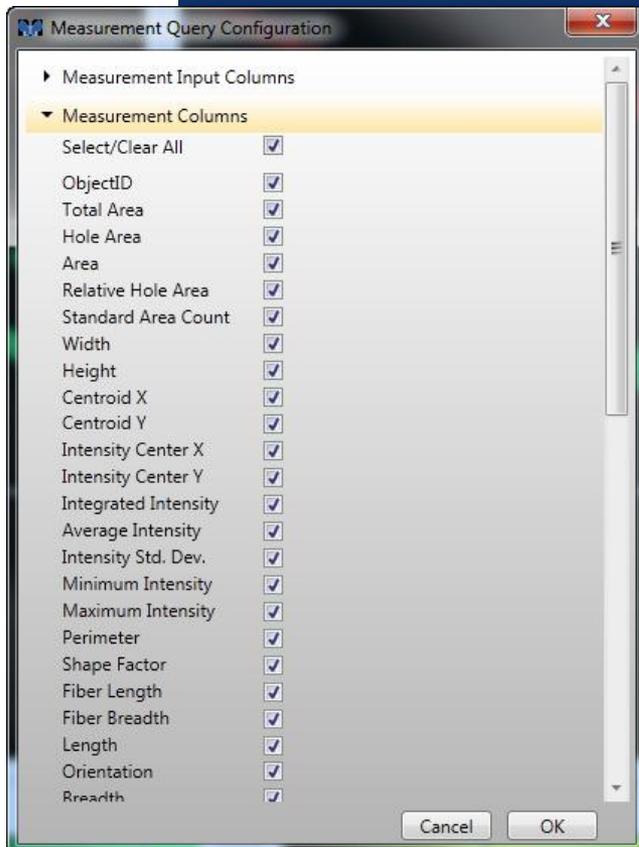
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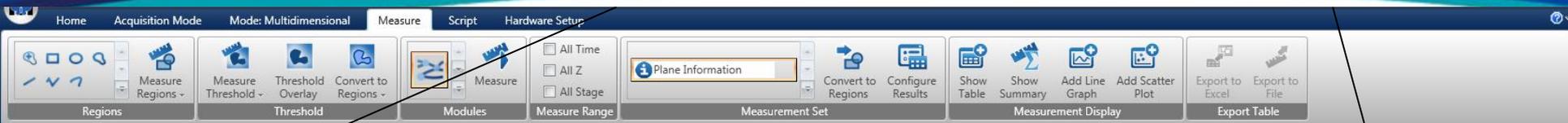
Measurement Tools



- Measurement Set Configuration

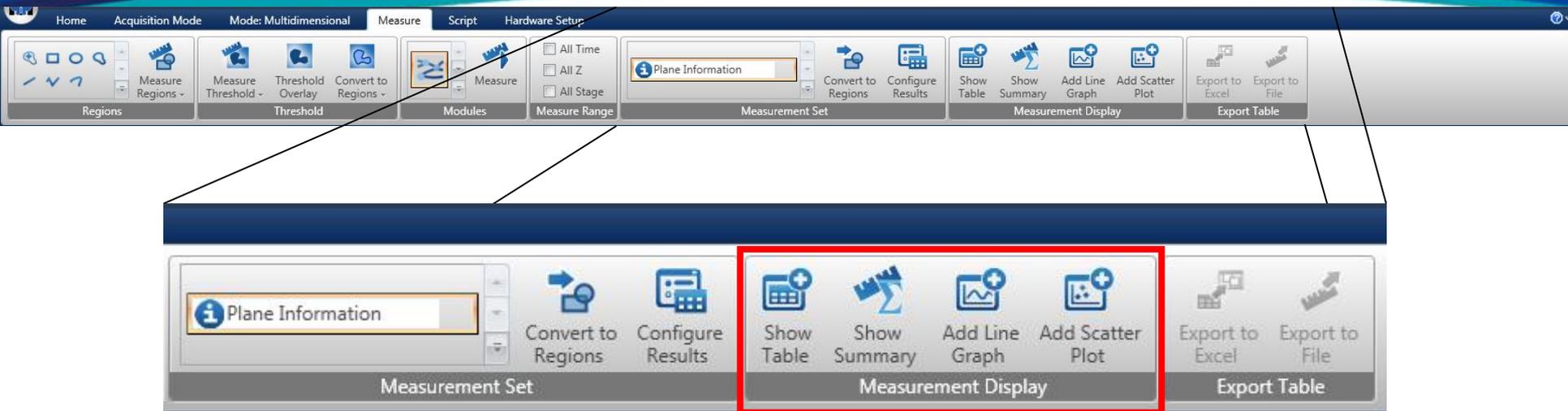
- Choose which Measurement Columns will be displayed.
- Filter un-desired Objects out.

Measurement Tools



- Measurement Set Configuration
 - Choose which Measurement Columns will be displayed.
 - Filter un-desired Objects out.

Measurement Tools



- Measurement Display

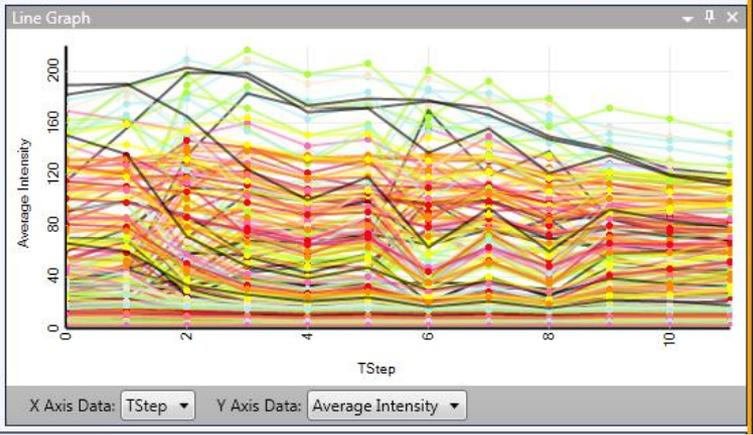
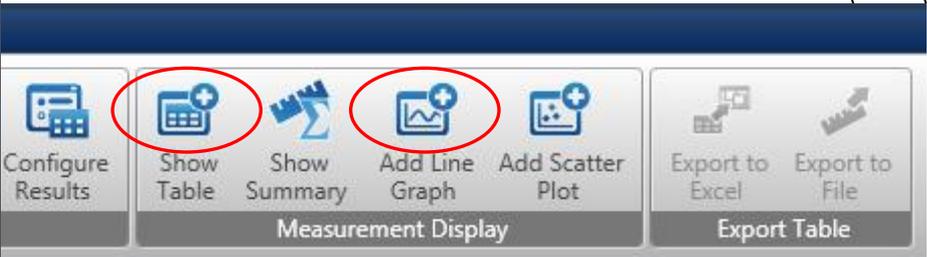
- The next section controls different ways of displaying the Data
- Show Table will display a table with all the Object data on it.
- Show Summary will show a table with statistical summary information
- Add Line Graph and Add Scatter Plot will plot the data

Measurement Tools



Data Table

Row ID	Orientation	Breadth	Ell. Form Factor
1	30.27	-82.41	13.17
2	40.16	-18.89	31.06
3	42.19	-31.43	31.75
4	35.11	-70.02	30.88
5	28.23	-67.07	15.54
6	45.19	-65.14	39.59
7	31.38	-30.65	23.28
8	44.60	-19.65	25.14
9	42.76	79.22	38.16



is different ways of displaying the Data

a table with all the Object data on it.

w a table with statistical summary information

d Scatter Plot will plot the data

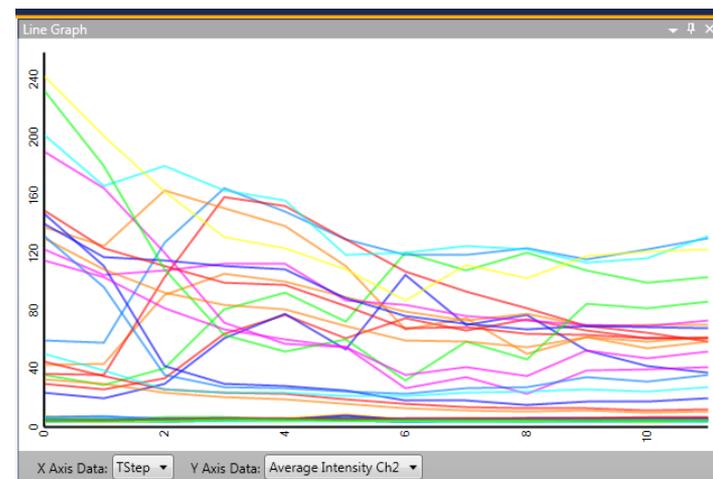


The Graph Ribbon



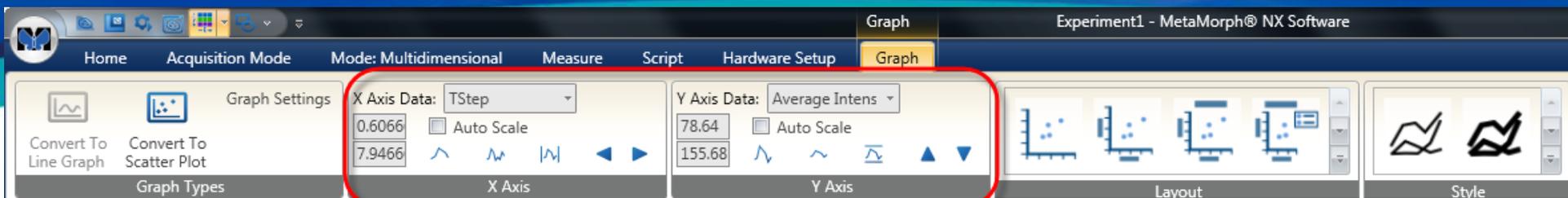
- When a Line Graph or Scatter Plot is shown, the Graph Ribbon becomes available

- The Graph Ribbon allows you to resize and move the displayed area of the graph
- The Graph can have different Layouts, controlling what labels and text get displayed

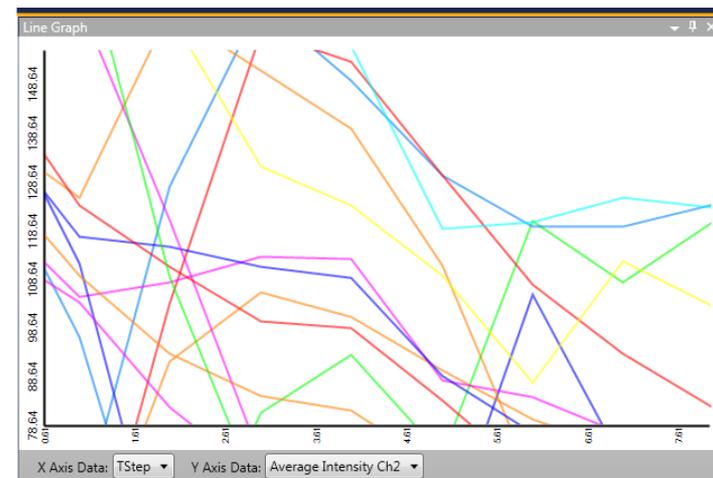


- Graphs can have different styles, controlling colors, line thicknesses and data points
- Graph Settings can be used to further modify the style.

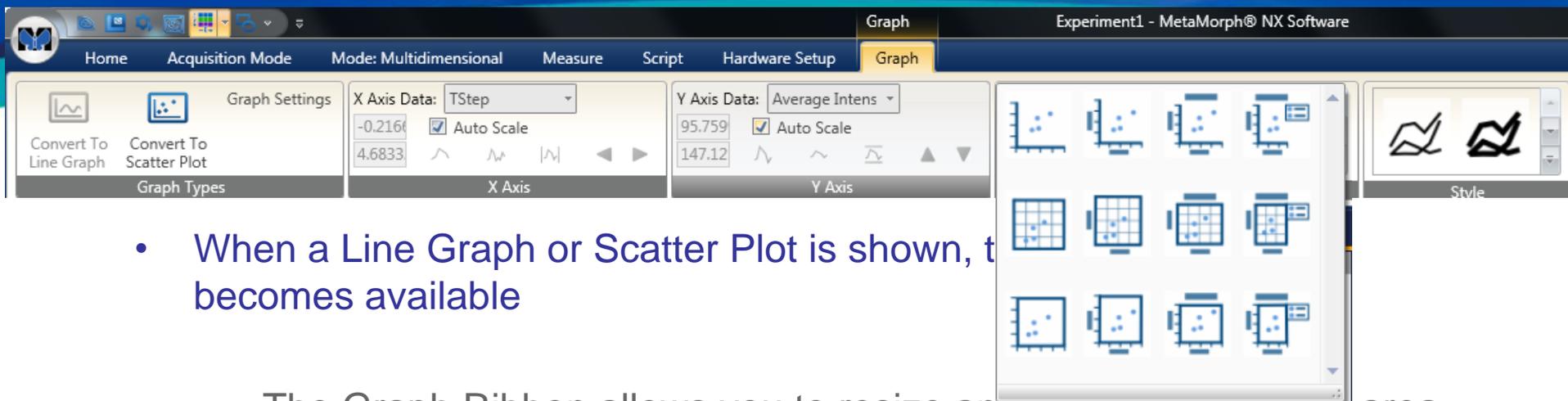
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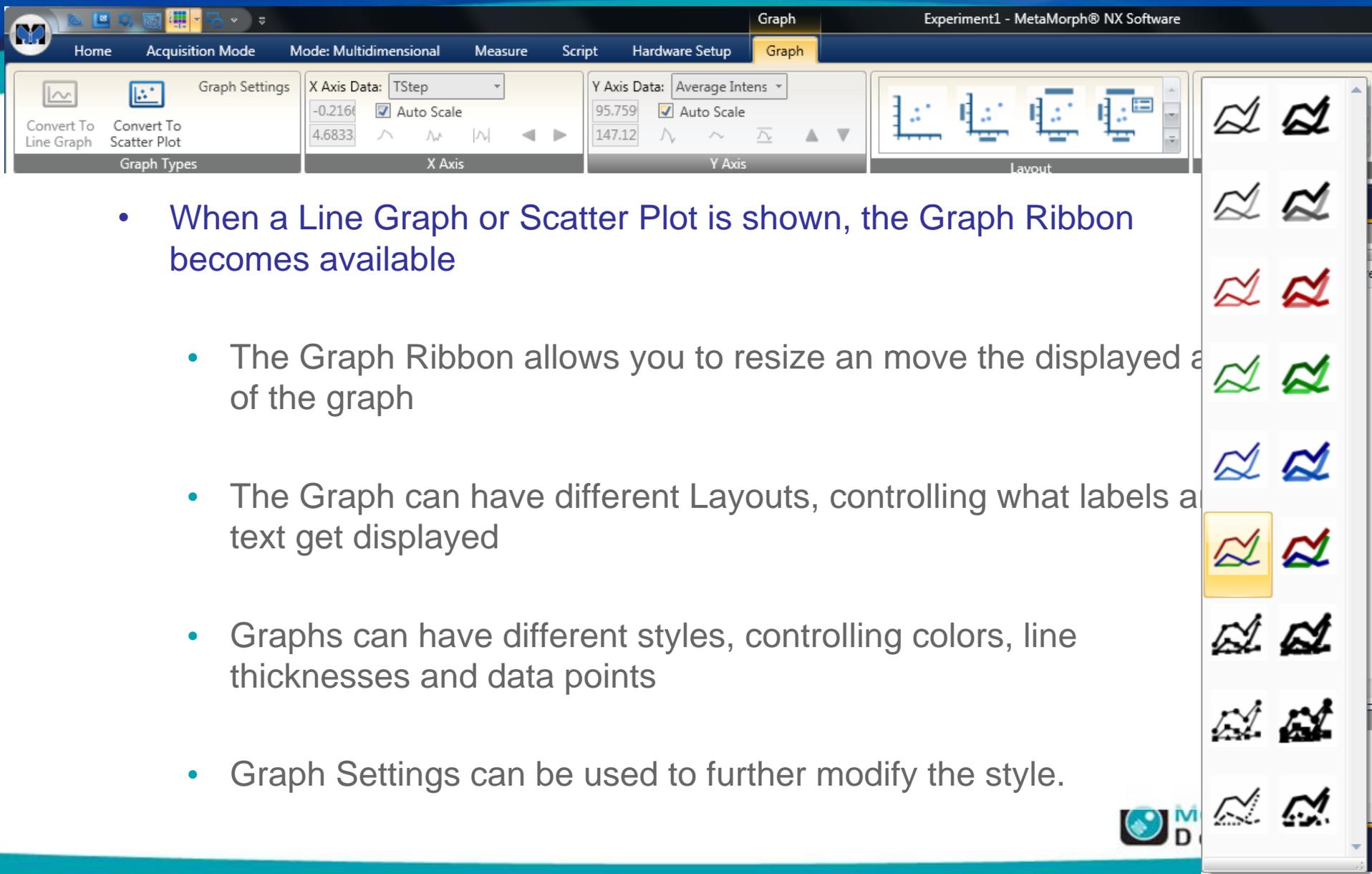


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The Graph Ribbon



Experiment1 - MetaMorph® NX Software

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup **Graph**

Graph Settings

Convert To Line Graph Convert To Scatter Plot

Graph Types

X Axis Data: TStep

-0.2164 4.6833

Auto Scale

X Axis

Y Axis Data: Average Intens

95.759 147.12

Auto Scale

Y Axis

Layout

- When a Line Graph or Scatter Plot is shown, the Graph Ribbon becomes available
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The Graph Ribbon



- V
b

shown, the Graph Ribbon

size and move the displayed area

outs, controlling what labels and

controlling colors, line

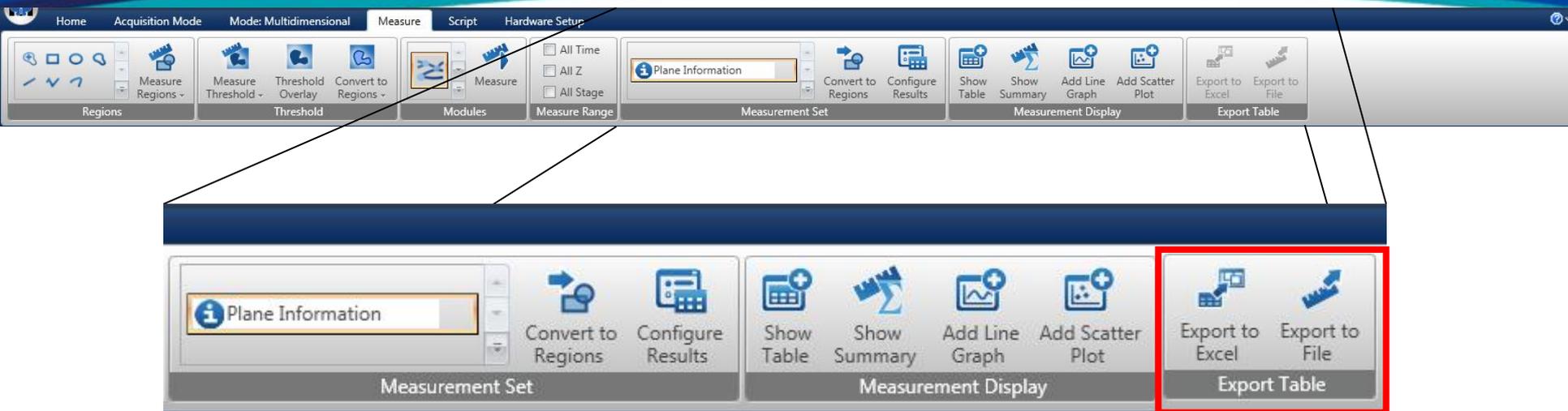
then modify the style.

Measurement Tools

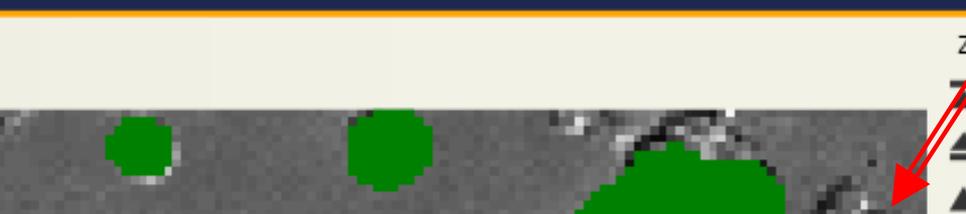


- Working with data logs
 - Measurements made in MetaMorph NX are always maintained in a 'database' – you do not need to open a log file to record measurement values.
 - Measurements are stored with the Dataset and maintained between MetaMorph NX sessions (i.e. if you close NX and re-open it the measurements are still there).
 - You can export a **displayed data table** to Excel or to a file
 - Displayed Data Table: You export the table as displayed – including the column and object filtering performed in the 'Configure Results' panel.

Measurement Tools



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Data Table

Row ID	Intensity	Maximum Intensity	Perimeter	Shape Factor	Fiber Length
1	100.00	131.00	24.49	0.61	9.03
2	100.00	128.00	30.73	1.00	7.68
3	100.00	100.00	22.49	1.00	5.62
4	100.00	172.00	98.81	0.44	41.53
5	100.00	136.00	32.97	1.00	8.24
6	100.00	100.00	26.73	1.00	6.68
7	100.00	83.00	26.73	1.00	6.68
8	100.00	163.00	29.90	1.00	7.47
9	100.00	141.00	36.97	1.00	9.24
10	100.00	155.00	37.56	0.74	11.66
11	100.00	146.00	36.14	1.00	9.04
12	100.00	190.00	32.97	1.00	8.24
13	100.00	169.00	37.56	1.00	9.39
14	100.00	122.00	28.14	1.00	7.04
15	100.00	144.00	50.28	0.78	13.59
16	100.00	69.00	15.66	1.00	3.91
17	100.00	117.00	17.66	0.81	4.41
18	100.00	53.00	14.49	0.96	3.62

Microsoft Excel - Book1

File Edit View Insert Format Tools Data Window Help

T17 = 1

	A	R	S	T	U
1	Row ID	Maximum Intensity	Perimeter	Shape Factor	Fiber Length
2	1	131.00	24.49	0.61	9.03
3	2	128.00	30.73	1.00	7.68
4	3	100.00	22.49	1.00	5.62
5	4	172.00	98.81	0.44	41.53
6	5	136.00	32.97	1.00	8.24
7	6	100.00	26.73	1.00	6.68
8	7	83.00	26.73	1.00	6.68
9	8	163.00	29.90	1.00	7.47
10	9	141.00	36.97	1.00	9.24
11	10	155.00	37.56	0.74	11.66
12	11	146.00	36.14	1.00	9.04
13	12	190.00	32.97	1.00	8.24
14	13	169.00	37.56	1.00	9.39
15	14	122.00	28.14	1.00	7.04

Ready NUM

MetaMorph NX can directly export to Excel 2003, 2007, or 2010 if it is installed on the system.

Convert to Regions
 Configure Results
 Show Table
 Show Summary
 Add Line Graph
 Add Scatter Plot
 Export to Excel
 Export to File

Set Measurement Display Export Table

Z
 ▲
 ▲
 ▲
 ▼
 ▼
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Row ID	Area	Maximum Intensity	Perimeter	Shape Factor	Fiber Length
1	100.00	131.00	24.49	0.61	9.03
2	100.00	128.00	30.73	1.00	7.68
3	100.00	100.00	22.49	1.00	5.62
4	100.00	172.00	98.81	0.44	41.53
5	100.00	136.00	32.97	1.00	8.24
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9	100.00	141.00	36.97	1.00	9.24
10	100.00	100.00	37.56	0.74	11.66

Export to File

File Path: C:\Program Files\Molecular Devices\MetaMorph NX\Interactiv Browse...

File Type: Excel

Worksheet: Measure Using Threshold 1

Excel
Csv

Cancel OK

Or MetaMorph NX can write data to an XLS file or CSV file if Excel is not present on the computer.

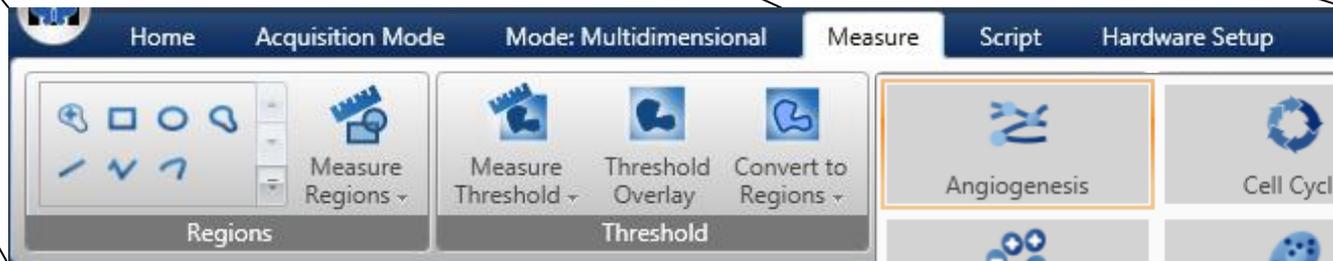
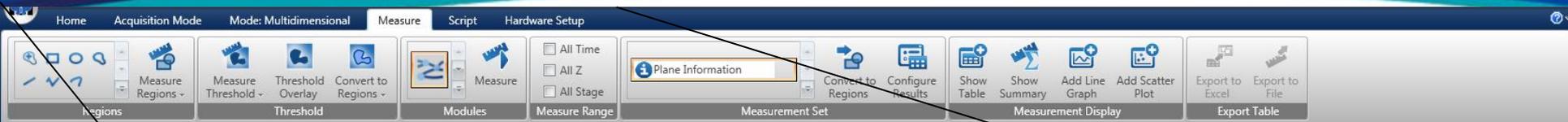
Using the Analysis Builder

Custom Modules

Why Custom Modules? (aka the analysis builder)

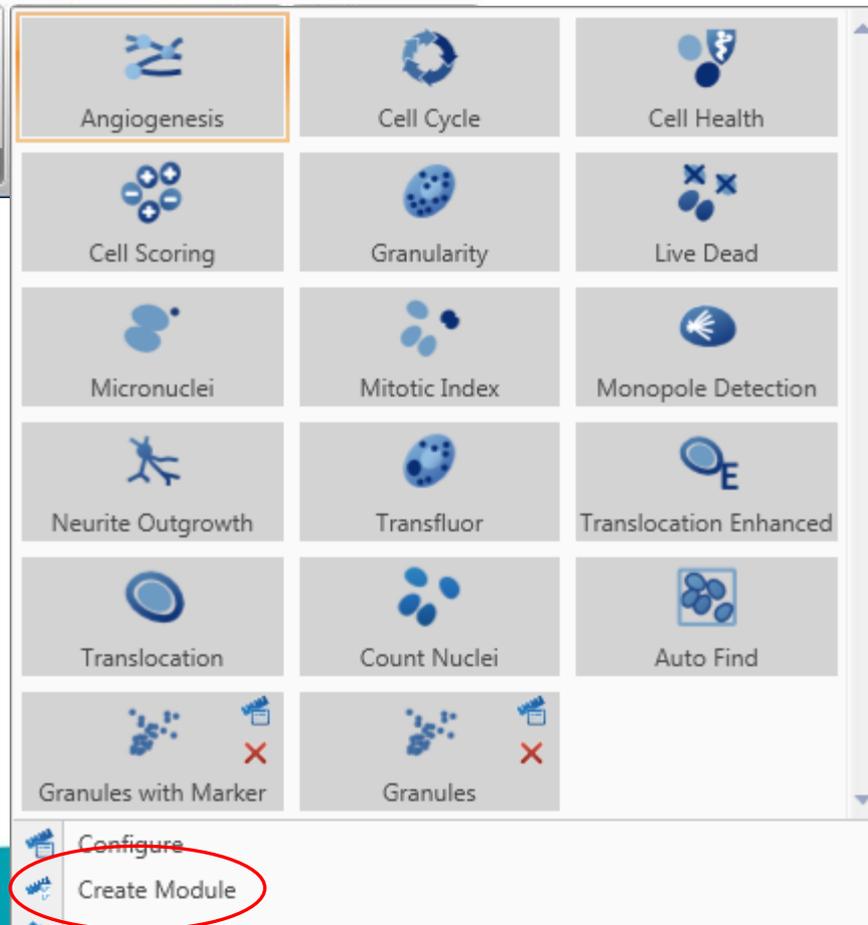
- Application Modules are great but...
 - Many times customers need to 'add to' what App Modules provide
 - So many things to measure – not all application modules
- Not Journals!
 - Easier to use than journals
 - Most segmentation tools available to journals are present
 - UI makes it easier to find the tool, test options, without getting lost in menus or minutia
- Custom Modules are Modules
 - Can be run exactly like application modules
 - Measurements are logged the same as application modules

Modules



- Custom Modules

- Use the Analysis Builder to make custom modules
- Open the Analysis Builder using the Create Module on the Modules palette



The Analysis Builder

Custom Module Experiment1 - MetaMorph® NX Software

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup Custom Module

Find Objects Modify Objects Add Import Module Export Import/Export Side by Side Split View Result View

Dataset2 Localized Granularity

Create Custom Module Segment Measure

1 Setup

Example Image 1 Cells and Puncta
Example Image 2 Nucleus
▶ Test Images

2 Auto Find Blobs

Source Nucleus
Automatic
Approximate Minimum Width (µm) 6.5
Approximate Maximum Width (µm) 22.72
Shading Correction
Result Nuclear Mask

Apply

3 Simple Threshold

Source Cells and Puncta
Threshold Low 485
Threshold High 65535
Inclusive
Result All Cells

Apply

4 Grow Objects Without Touching

Source Nuclear Mask

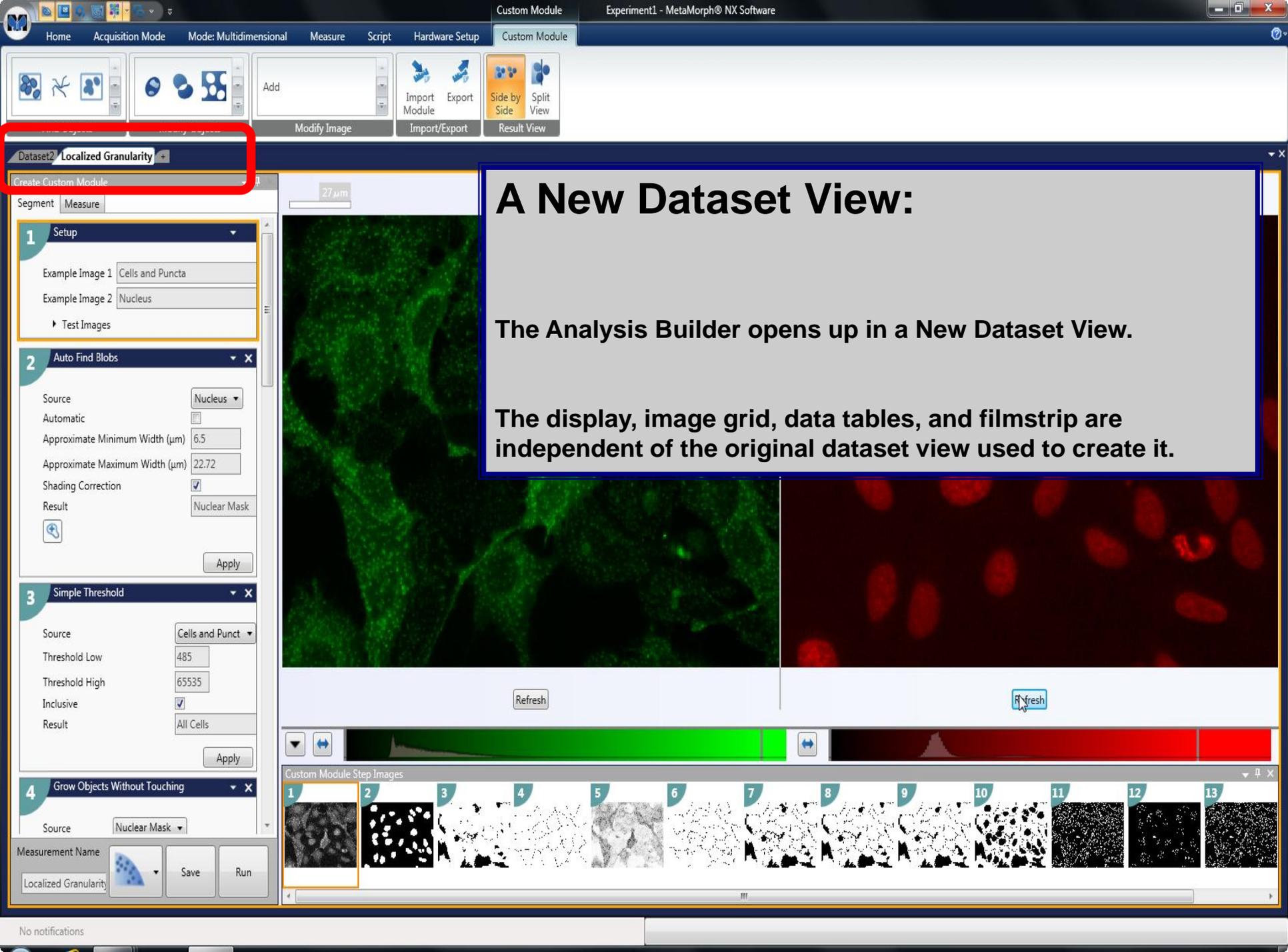
Measurement Name Localized Granularity Save Run

Channel 0 27 µm Channel 1

Refresh Refresh

Custom Module Step Images

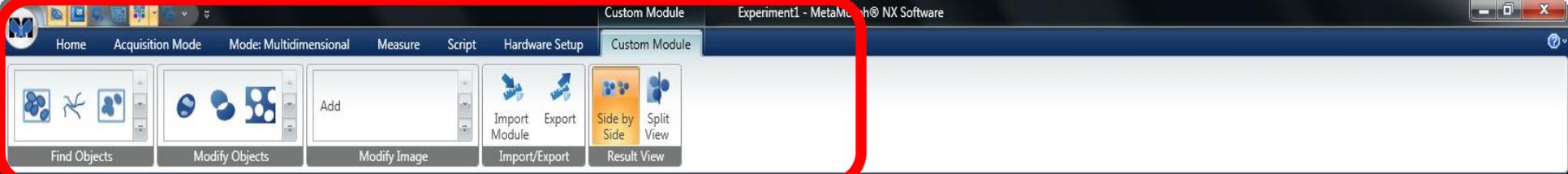
1 2 3 4 5 6 7 8 9 10 11 12 13



A New Dataset View:

The Analysis Builder opens up in a New Dataset View.

The display, image grid, data tables, and filmstrip are independent of the original dataset view used to create it.



The Ribbon:

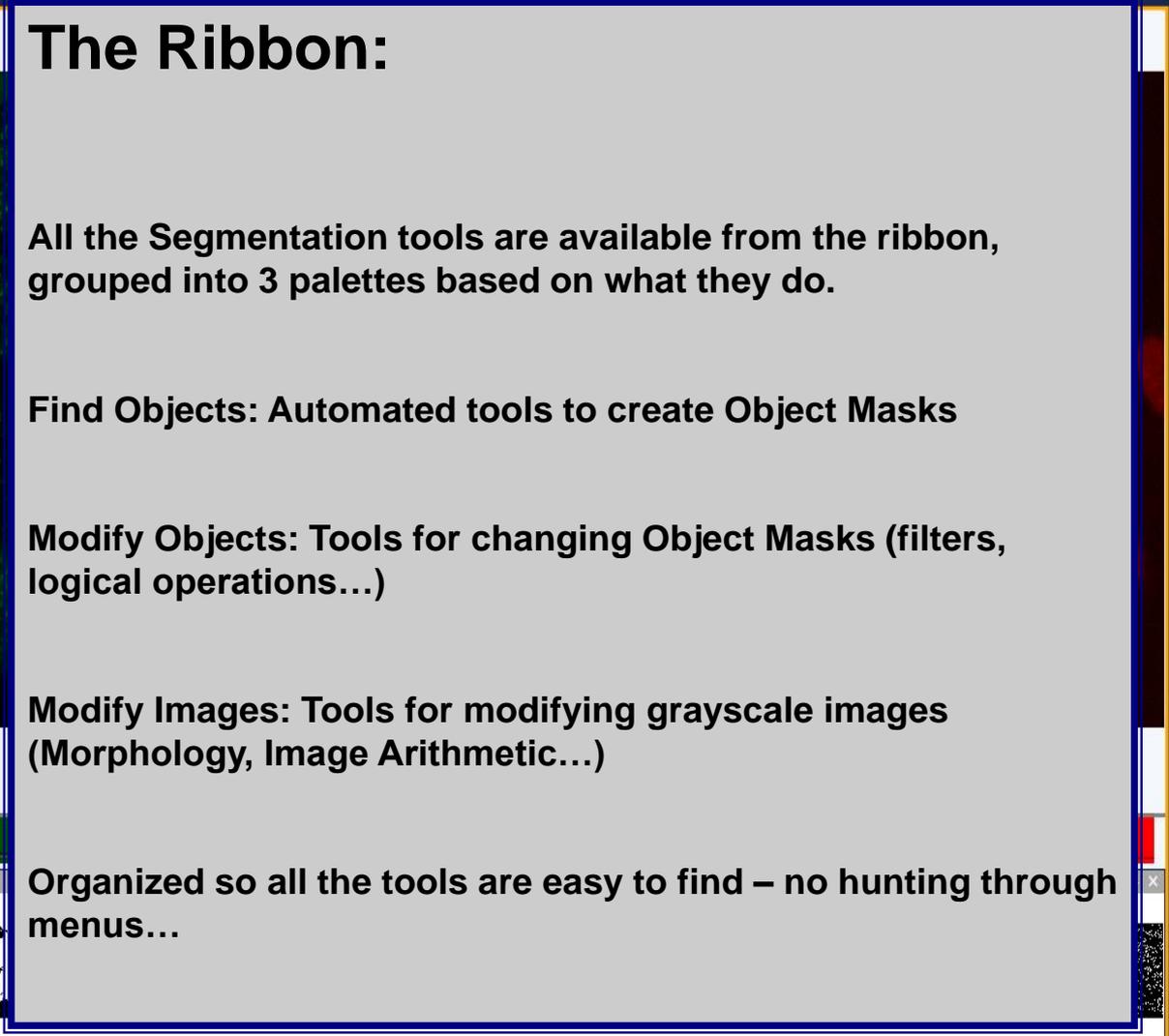
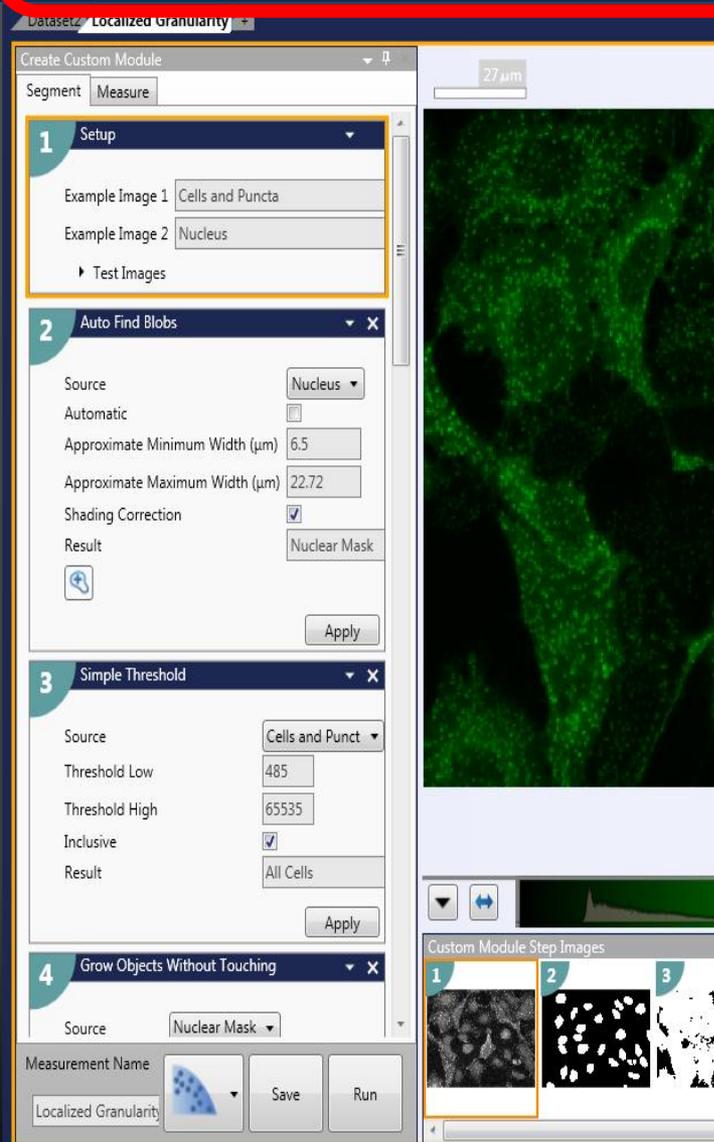
All the Segmentation tools are available from the ribbon, grouped into 3 palettes based on what they do.

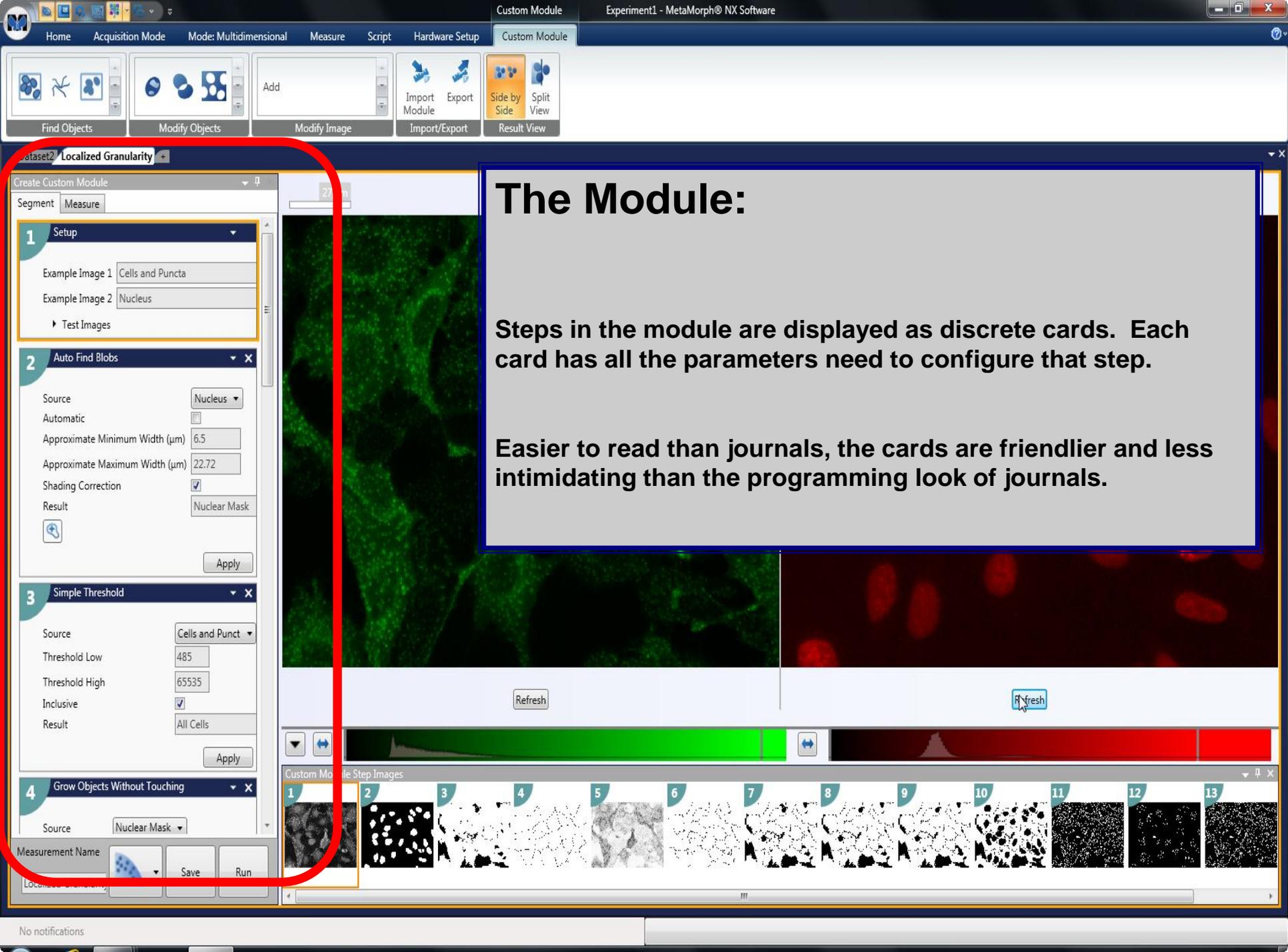
Find Objects: Automated tools to create Object Masks

Modify Objects: Tools for changing Object Masks (filters, logical operations...)

Modify Images: Tools for modifying grayscale images (Morphology, Image Arithmetic...)

Organized so all the tools are easy to find – no hunting through menus...





The Module:

Steps in the module are displayed as discrete cards. Each card has all the parameters need to configure that step.

Easier to read than journals, the cards are friendlier and less intimidating than the programming look of journals.

Find Objects Modify Objects Modify Image Import/Export Result View

Dataset2 Localized Granularity

Create Custom Module

Segment Measure

14 Measure Mask

Measurement Inputs
Standard Area Value: 1

Objects to Measure
Mask of Objects: Cell Mask
Image to Measure: Cells and Puncta

Features within Each Object
Mask of Features: Nuclear Mask
Image to Measure: Nucleus

Features within Each Object
Mask of Features: Nuclear Granules
Image to Measure: Cells and Puncta

Features within Each Object
Mask of Features: Cytoplasmic Granules
Image to Measure: Cells and Puncta

Measurement Name: Localized Granularity

Save Run

Single Image

The Filmstrip:

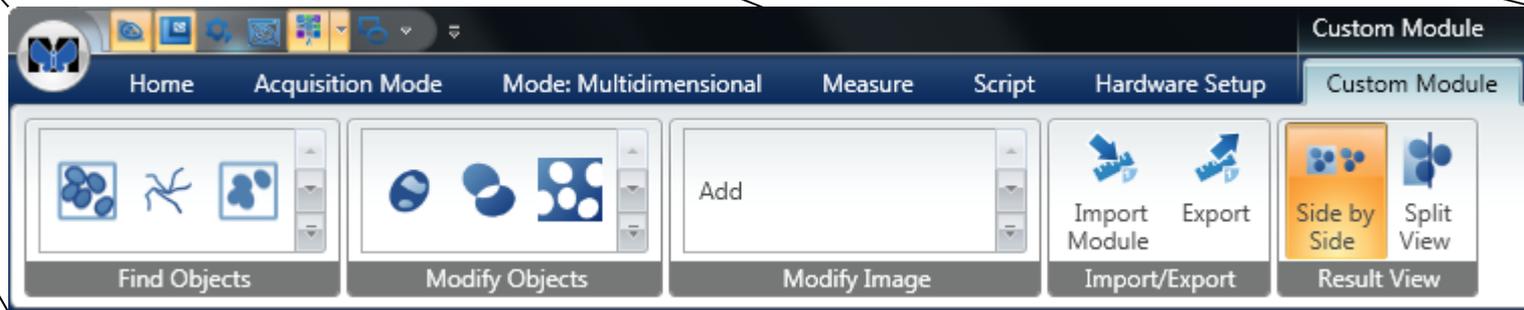
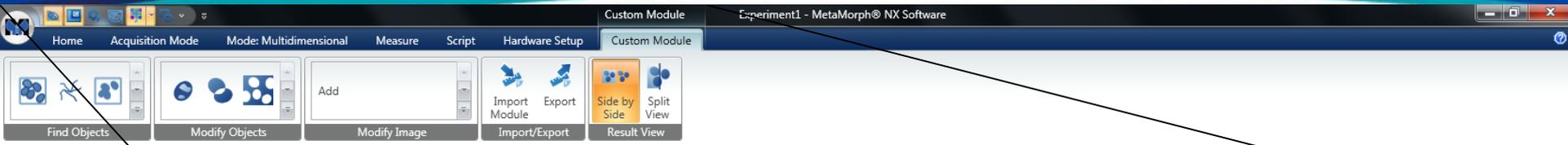
Each step in the module has its own thumbnail result in the filmstrip.

Easier to identify what each step does, and if it is working.

Easier to navigate back to a step which may be causing a problem – just click the offending thumbnail.

18	25.00	1.66
19	22.00	1.18
20	26.00	1.20
21	21.00	1.95

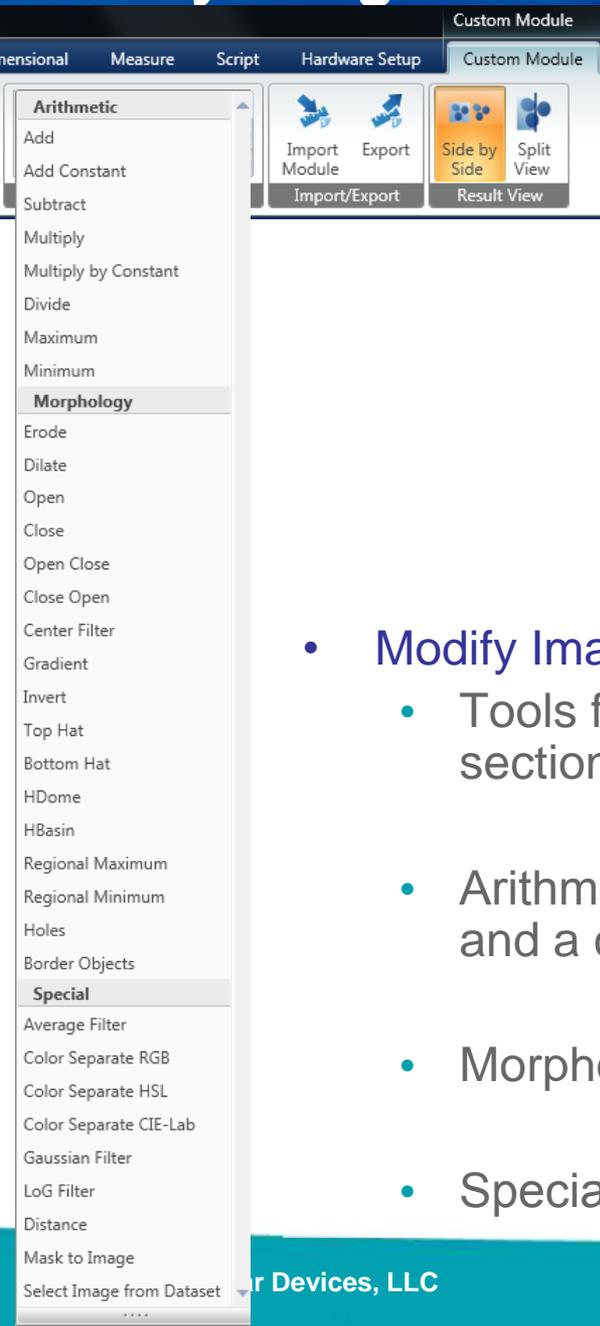
Custom Modules



- The Custom Module Ribbon

- Modify Image: Tools for processing grayscale images
- Find Objects: Tools for creating masks and detecting objects from grayscale images
- Modify Objects: Tools for changing or selecting masks.

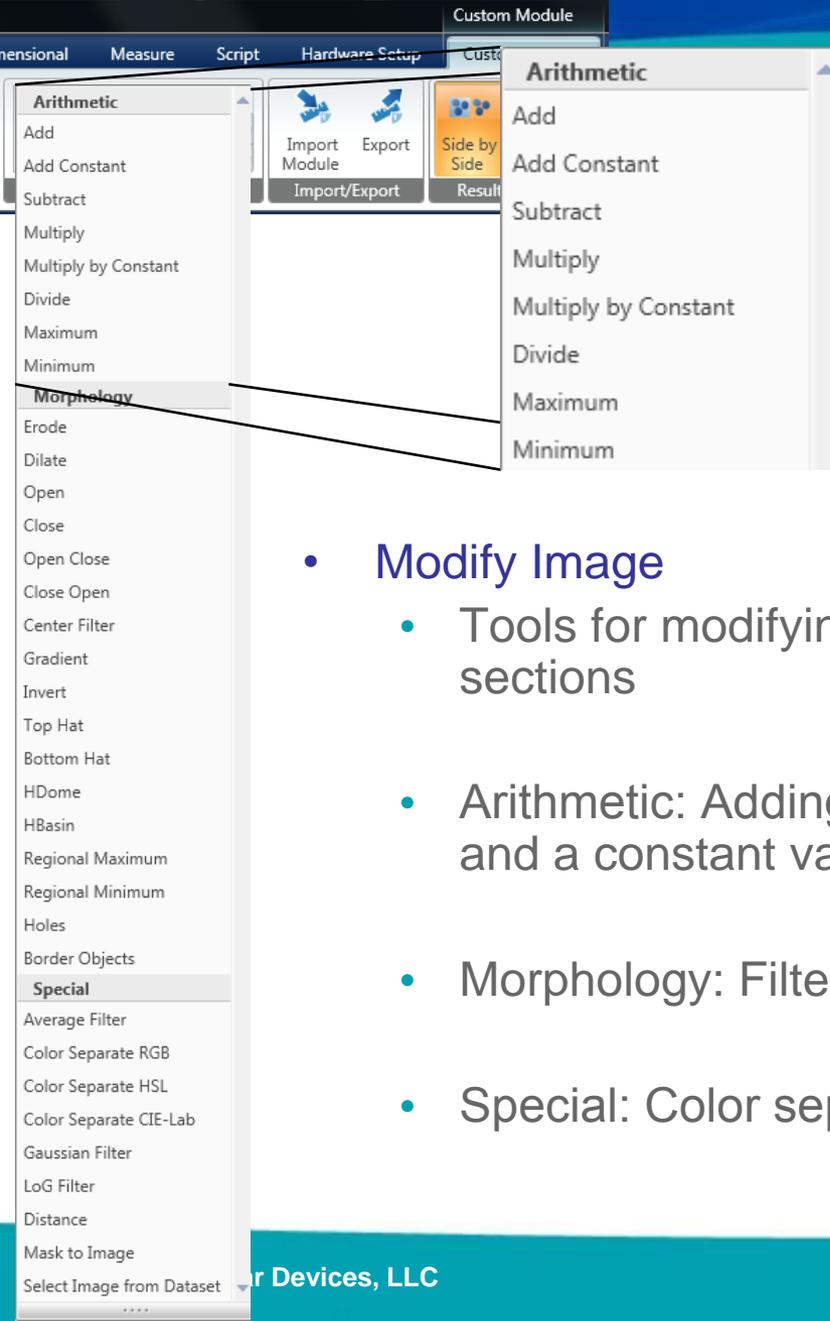
Modify Images



- **Modify Image**

- Tools for modifying real image data, grouped into functional sections
- Arithmetic: Adding, Subtracting, etc... two images or an image and a constant value
- Morphology: Filters based on shape, size and relative intensities
- Special: Color separation techniques, basic filters...

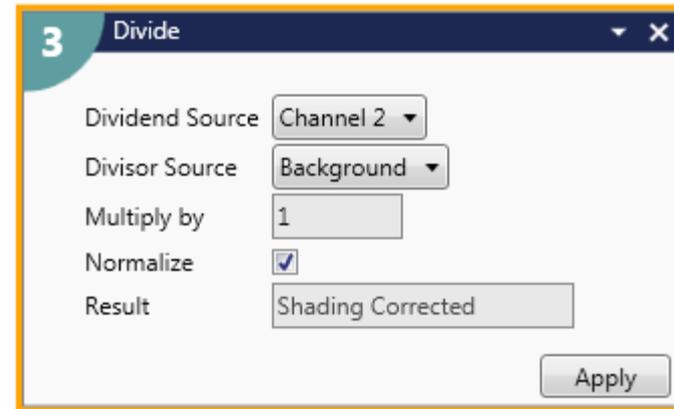
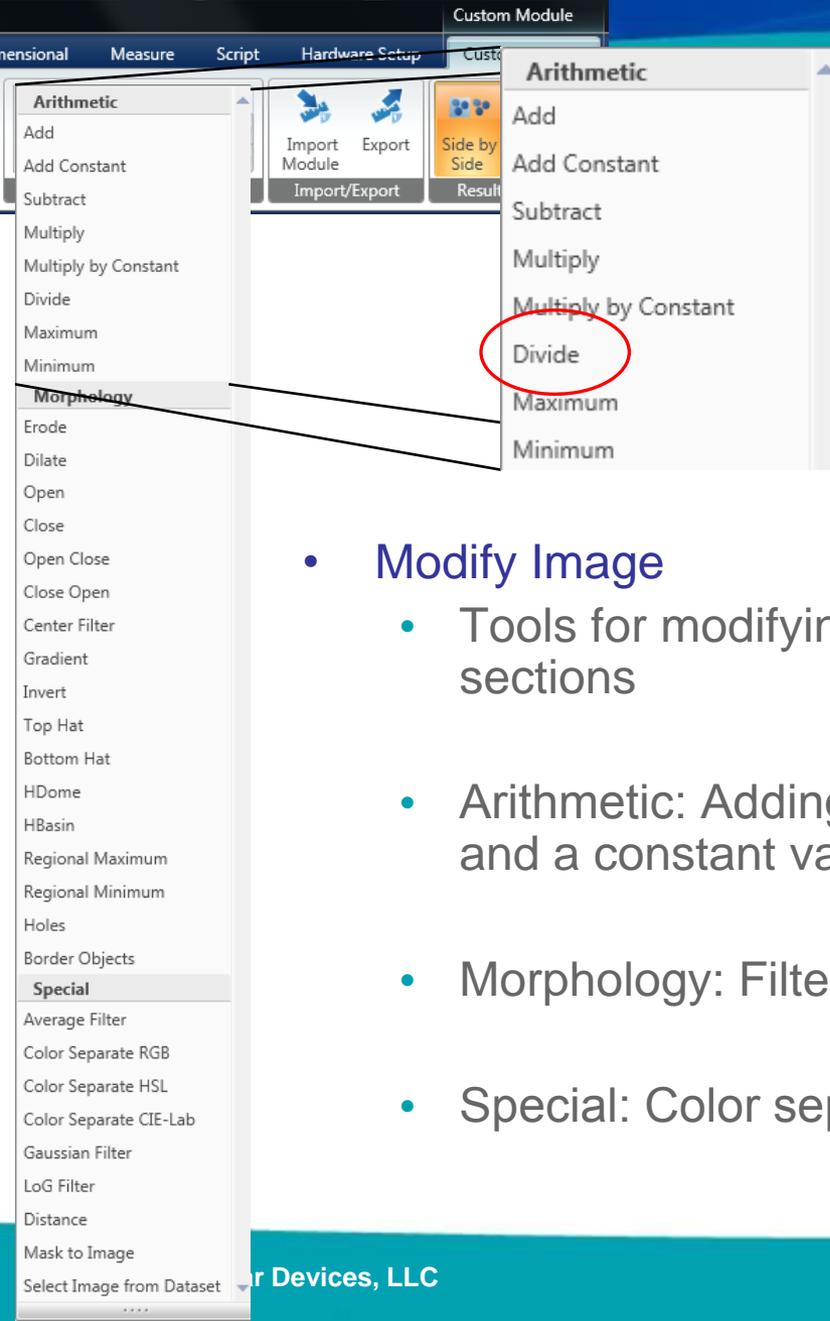
Modify Images



- **Modify Image**

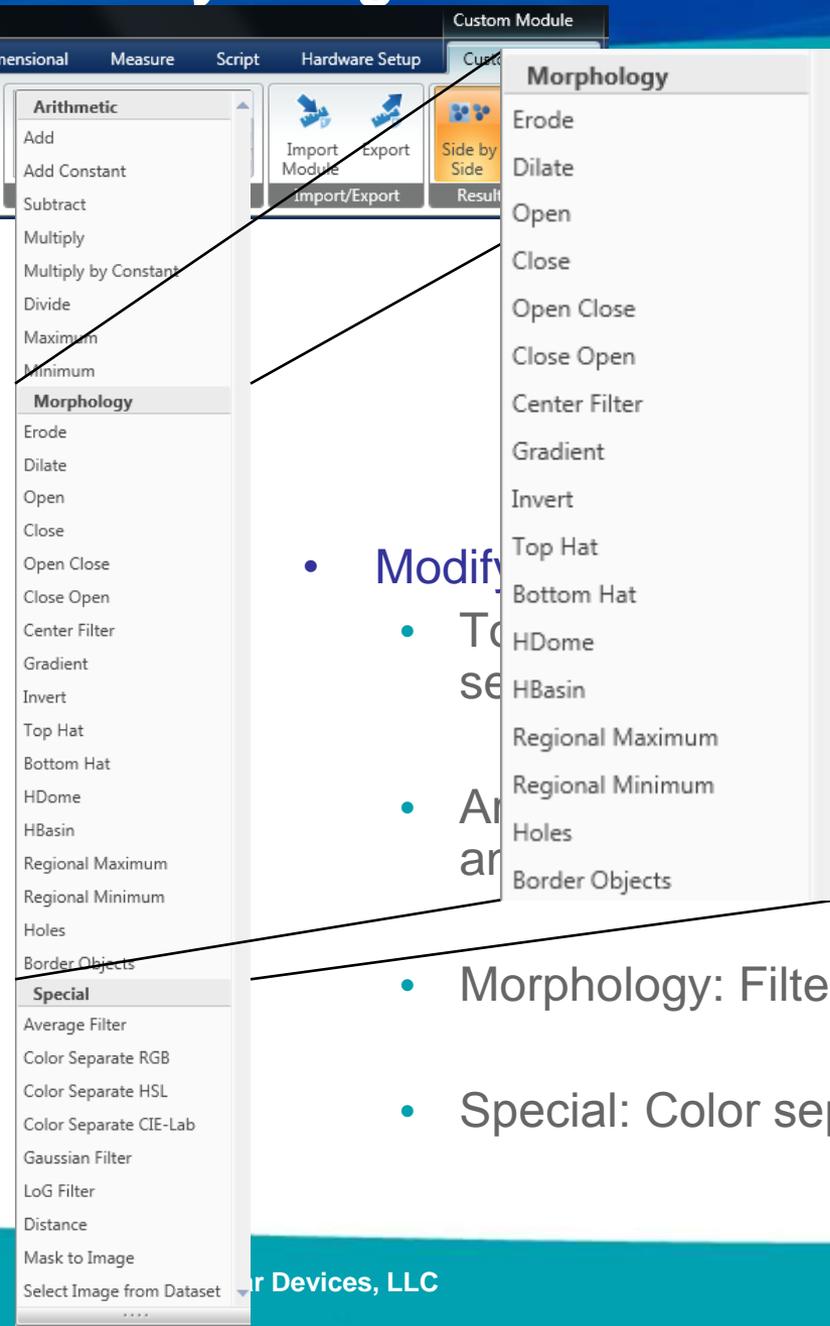
- Tools for modifying real image data, grouped into functional sections
- Arithmetic: Adding, Subtracting, etc... two images or an image and a constant value
- Morphology: Filters based on shape, size and relative intensities
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 - Special: Color separation techniques, basic filters...

Modify Images



- Modify
 - To
 - An

g real image data, grouped into functional

, Subtracting, etc... two images or an image
value

- Morphology: Filters based on shape, size and relative intensities
- Special: Color separation techniques, basic filters...



Modify Images

Custom Module

Dimensional Measure Script Hardware Setup Custom

Arithmetic

- Add
- Add Constant
- Subtract
- Multiply
- Multiply by Constant
- Divide
- Maximum
- Minimum

Morphology

- Erode
- Dilate
- Open
- Close
- Open Close
- Close Open**
- Center Filter
- Gradient
- Invert
- Top Hat
- Bottom Hat
- HDome
- HBasin
- Regional Maximum
- Regional Minimum
- Holes
- Border Objects

Special

- Average Filter
- Color Separate RGB
- Color Separate HSL
- Color Separate CIE-Lab
- Gaussian Filter
- LoG Filter
- Distance
- Mask to Image
- Select Image from Dataset

- Modify
- To
- SE
- An
- ar

2 Close Open

Source Channel 3

Size (pixels) 13

Filter Shape Circle

Grayscale Reconstruction

Sequential Filters

Result Close Open

Apply

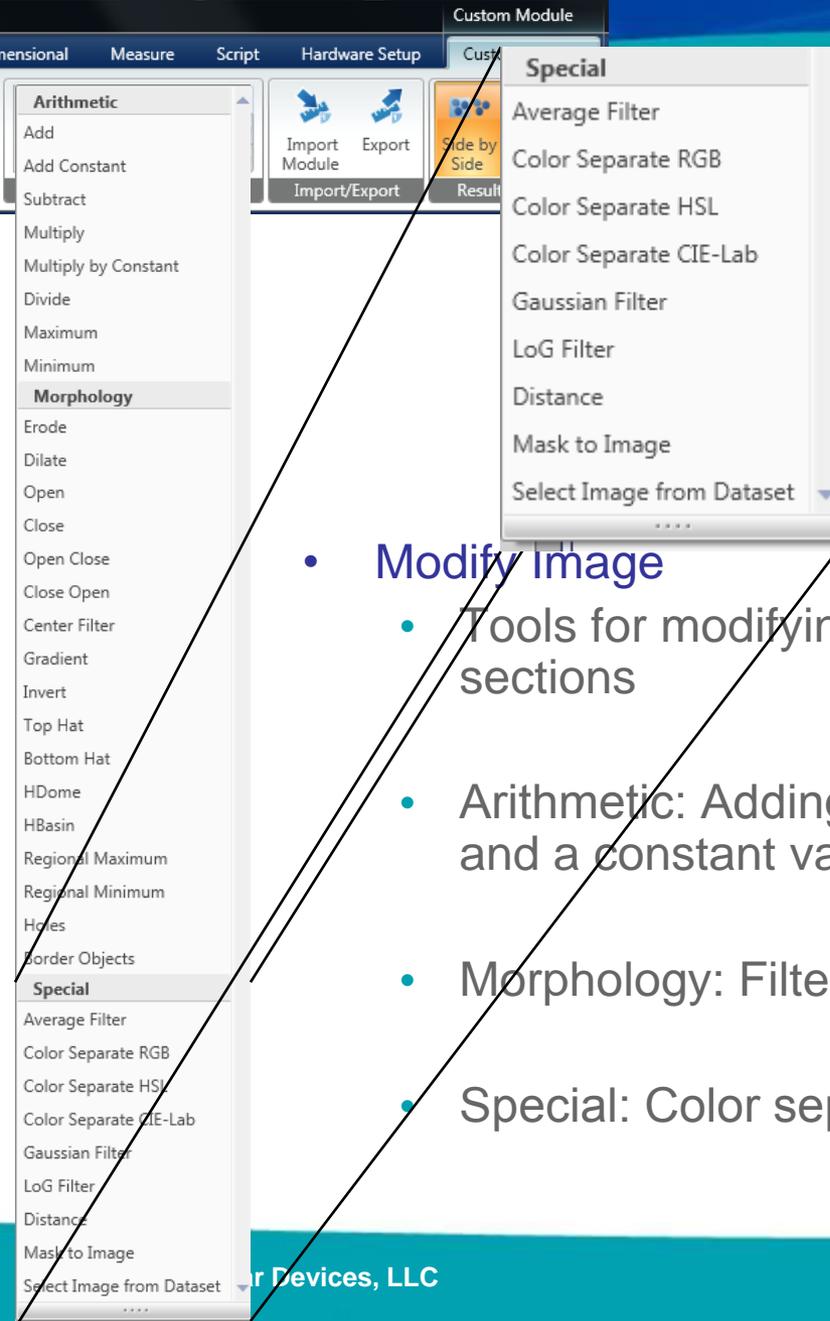
g real image data, grouped into functional

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lue

- Morphology: Filters based on shape, size and relative intensities
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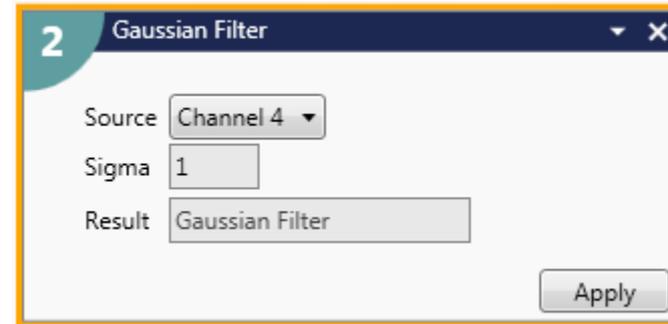
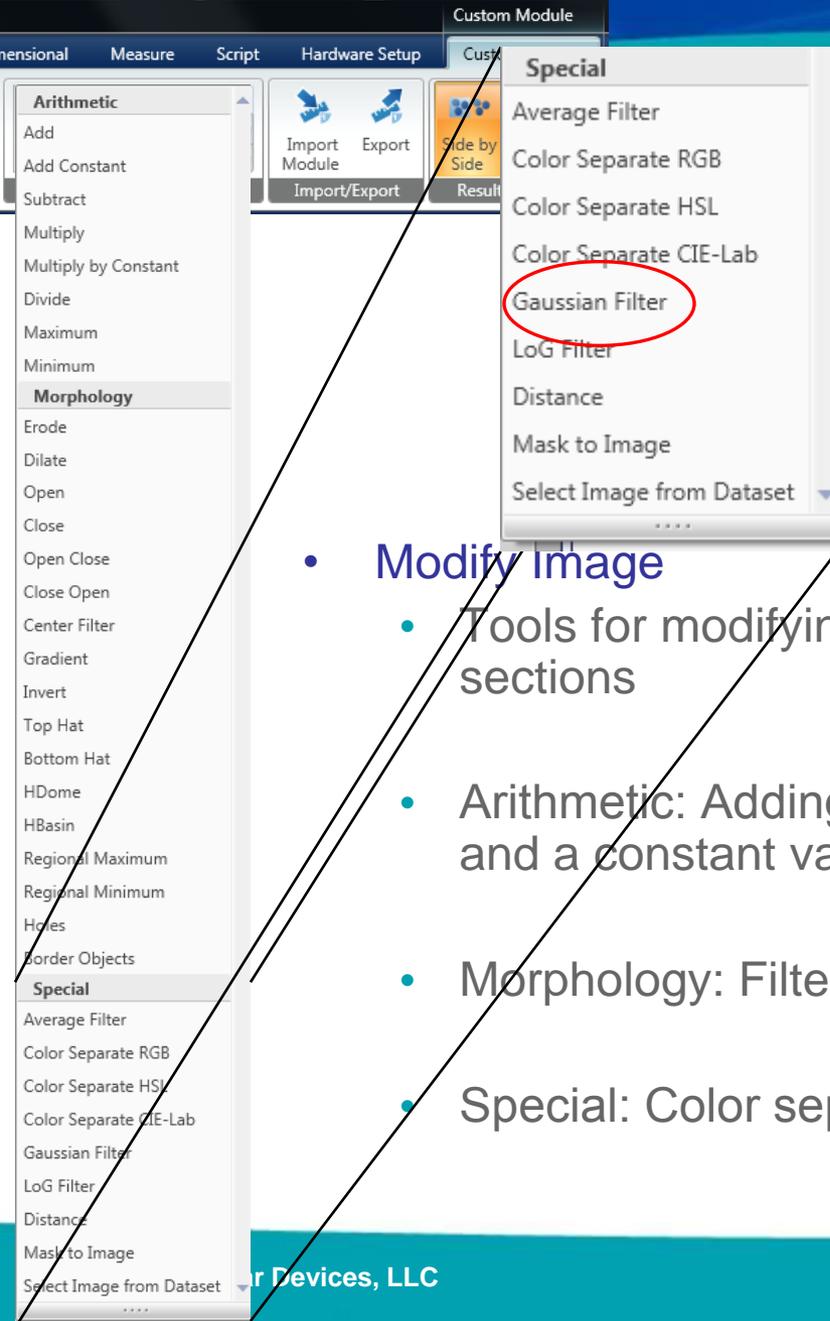
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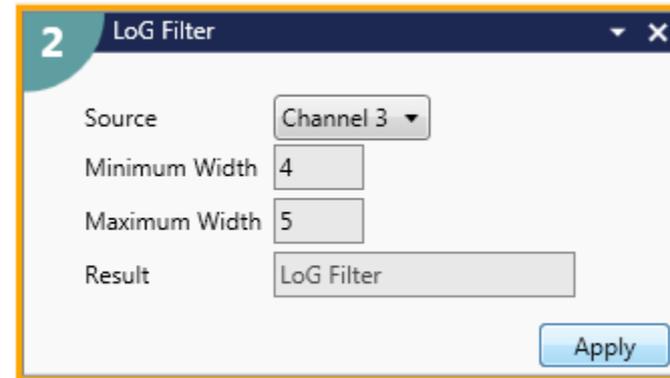
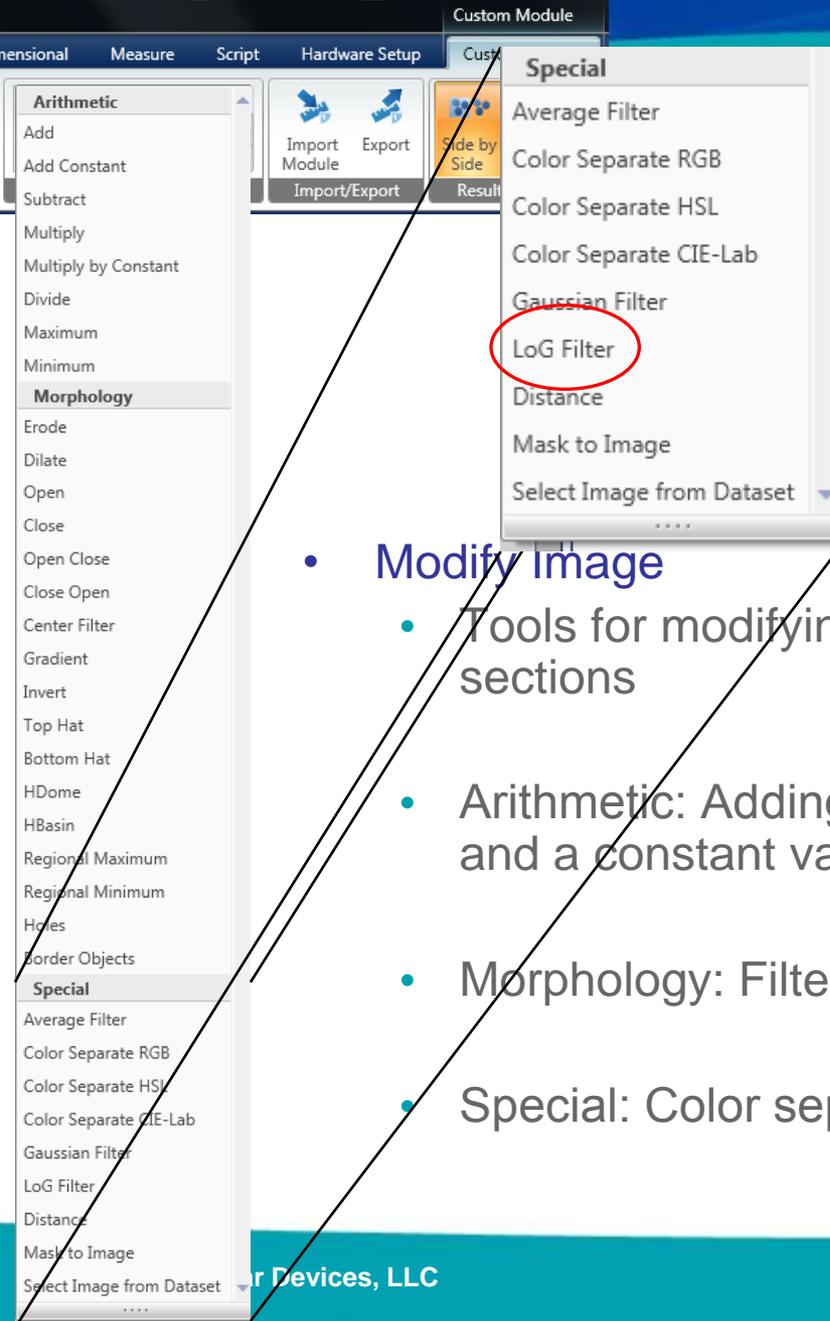
Modify Images



- **Modify Image**

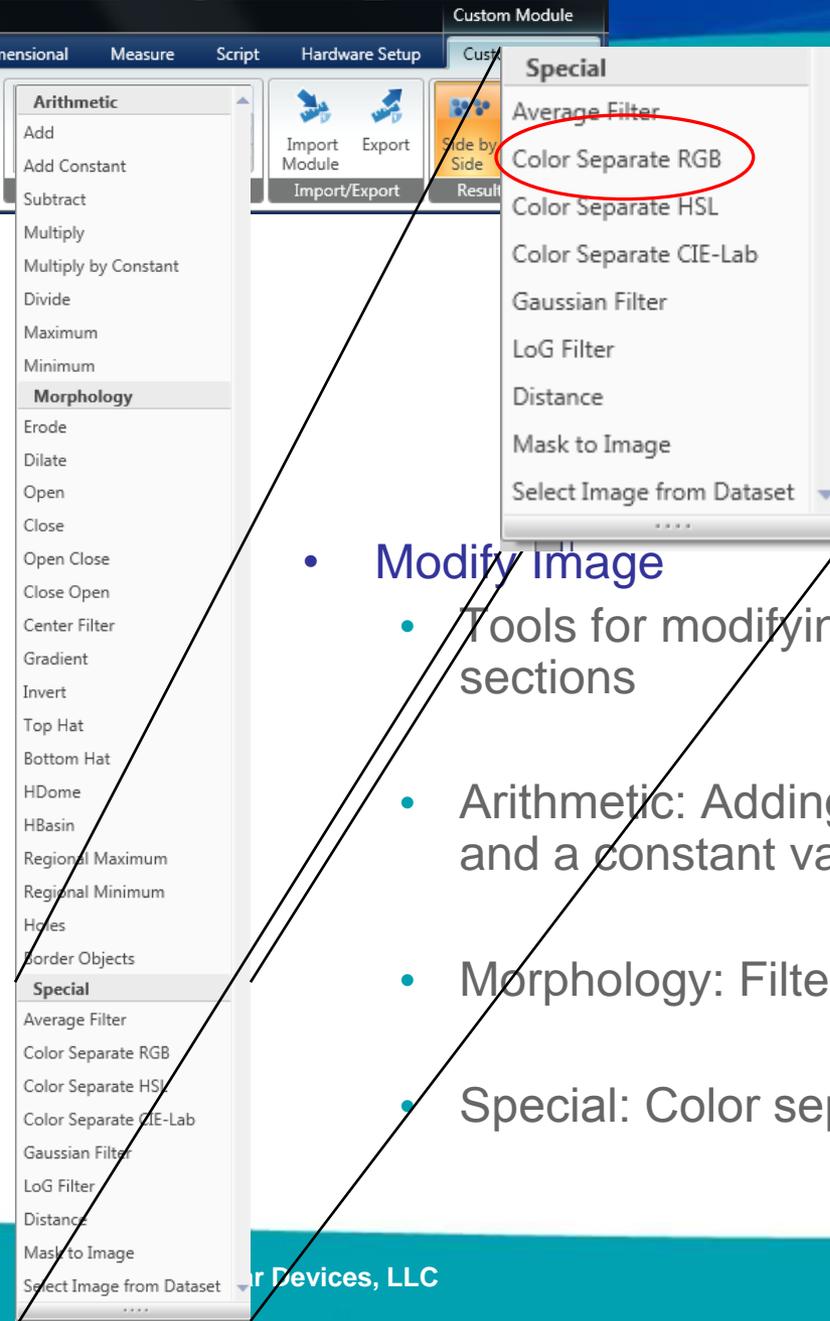
- Tools for modifying real image data, grouped into functional sections
- Arithmetic: Adding, Subtracting, etc... two images or an image and a constant value
- Morphology: Filters based on shape, size and relative intensities
- Special: Color separation techniques, basic filters...

Modify Images



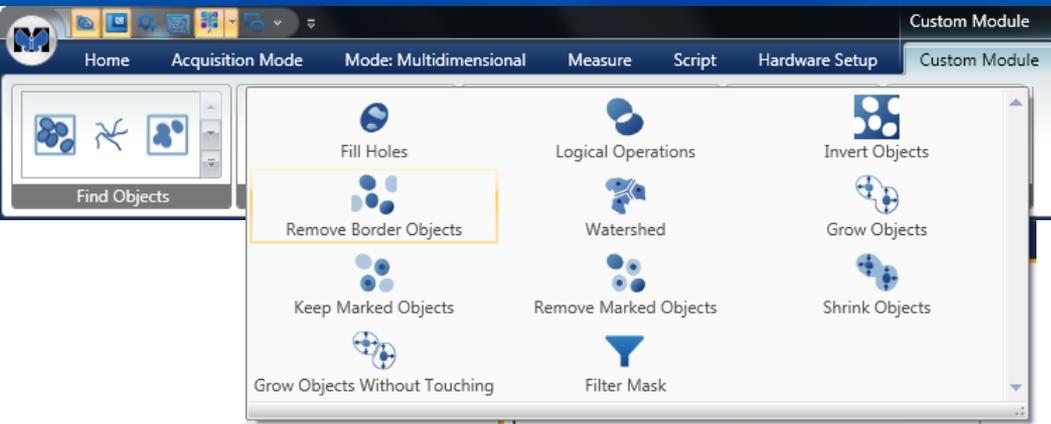
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Modify Images



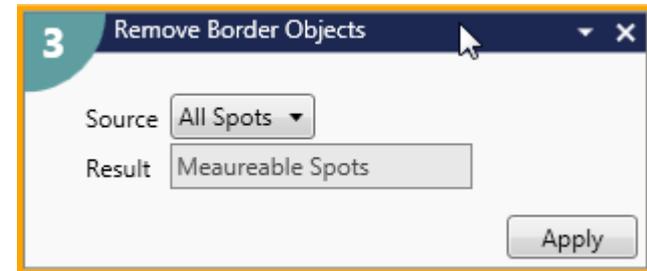
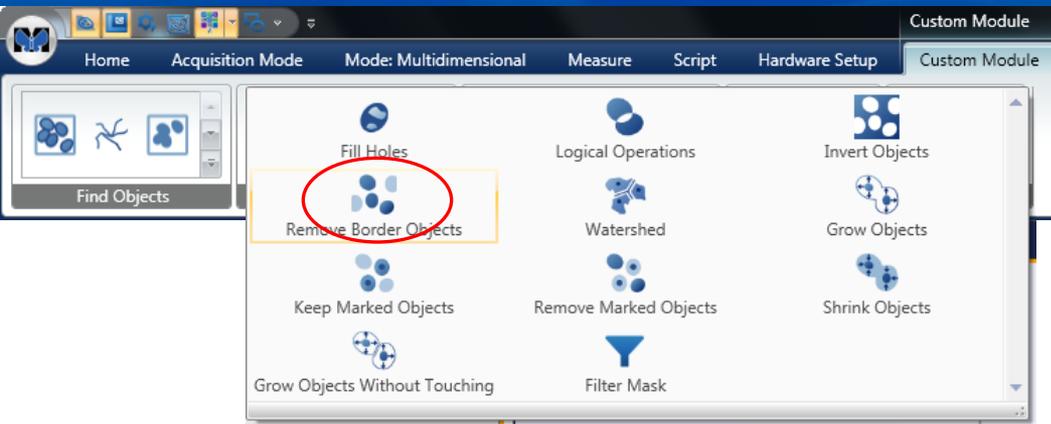
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Modify Objects



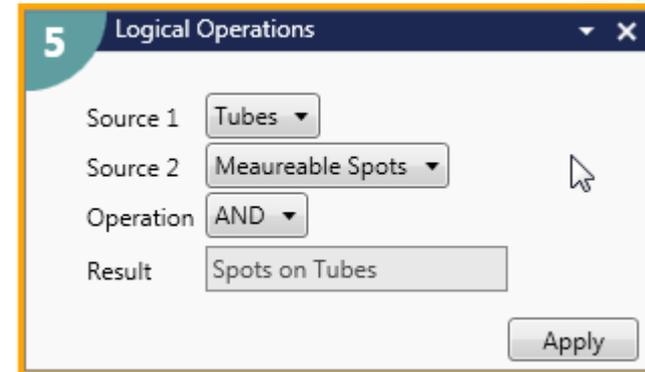
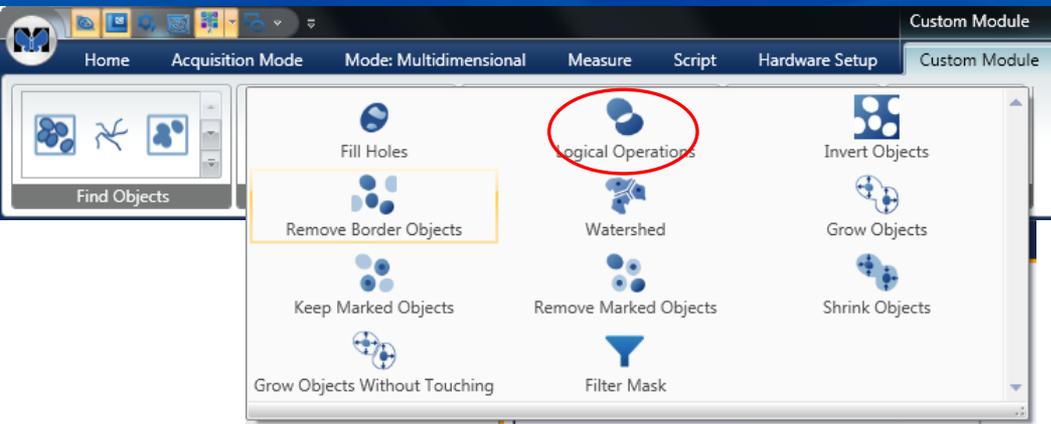
- **Modify Objects**
 - Tools for resizing, selecting, or reshaping objects on a mask
 - Example object selecting tools:
 - Logical Operations
 - Keep/Remove Marked Objects
 - Filter Mask
 - Example object resizing tools:
 - Grow Objects
 - Grow Objects Without Touching
 - Shrink Objects
 - Example reshaping tools:
 - Invert Object
 - Watershed
 - Fill Holes

Modify Objects



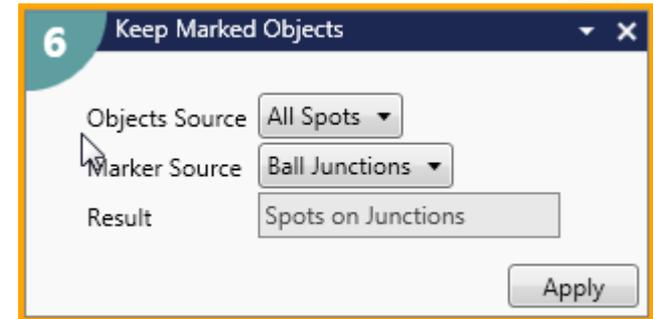
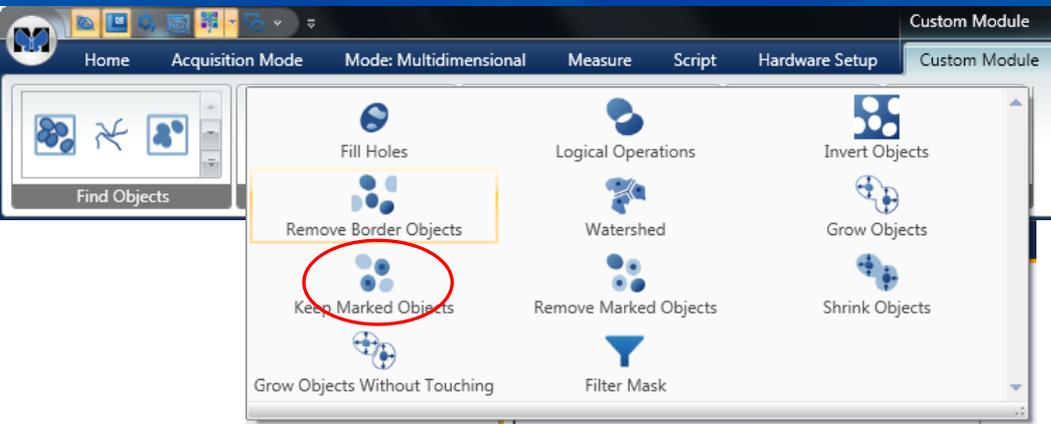
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Modify Objects



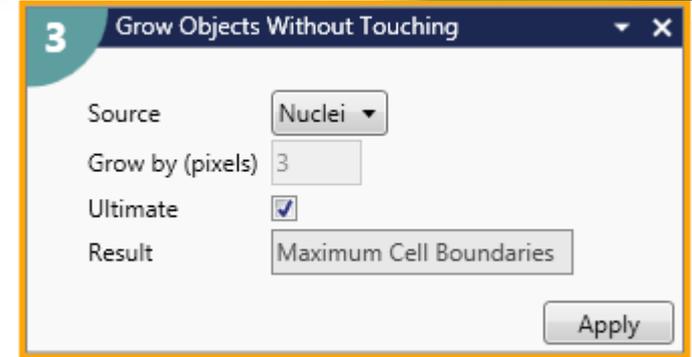
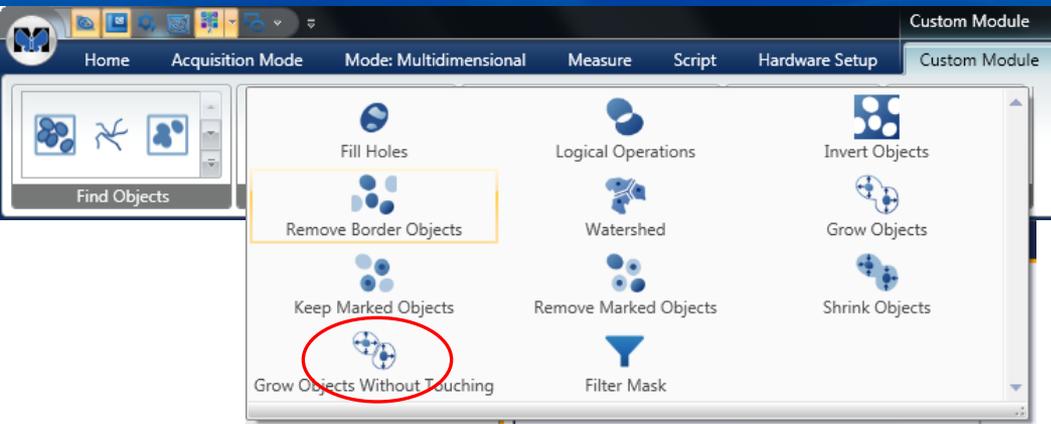
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Modify Objects



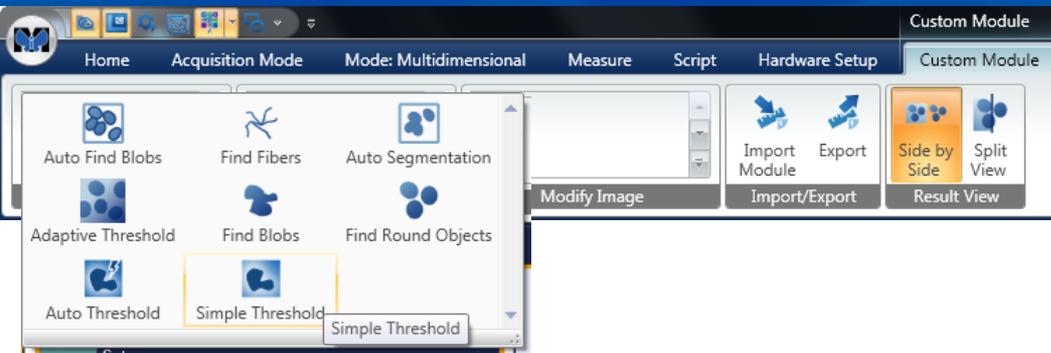
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Modify Objects



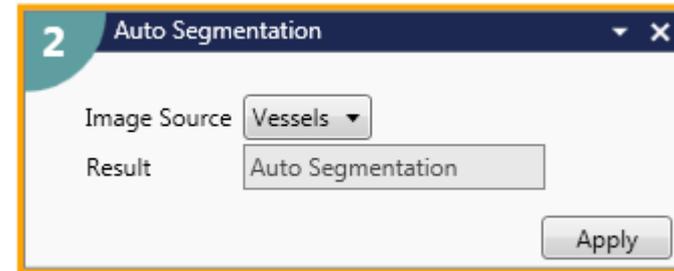
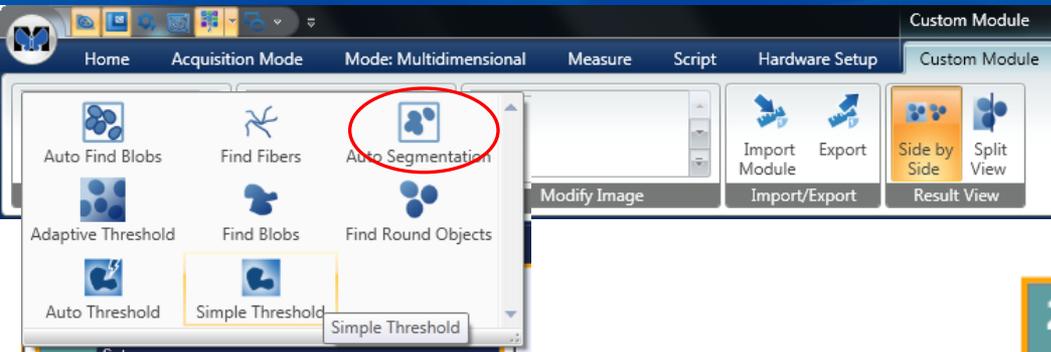
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Find Objects



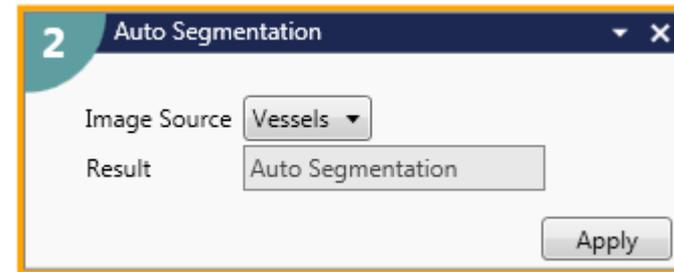
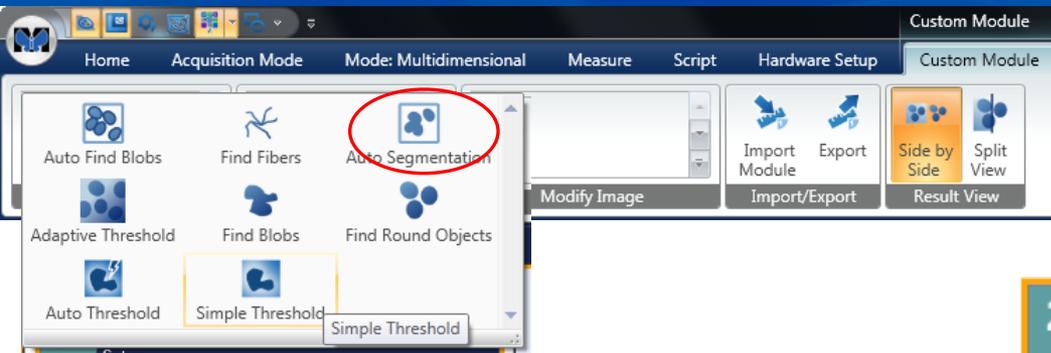
- Find Objects
 - Tools for finding objects in grayscale images and making masks for them.
 - Simple to use tools like:
 - Auto Segmentation: No Configuration Needed
 - Auto Threshold: Like auto thresholding in MM – for Bright or Dark objects
 - Simple Threshold: Set Min / Max intensity of objects
 - Tools like the Application Modules use:
 - Auto Find Blobs for finding things like nuclei
 - Find Blobs for finding things like cytoplasm
 - And a few more tools:
 - Find Fibers for tube or process detection
 - Find Round Objects for truly round things (like puncta)

Find Objects



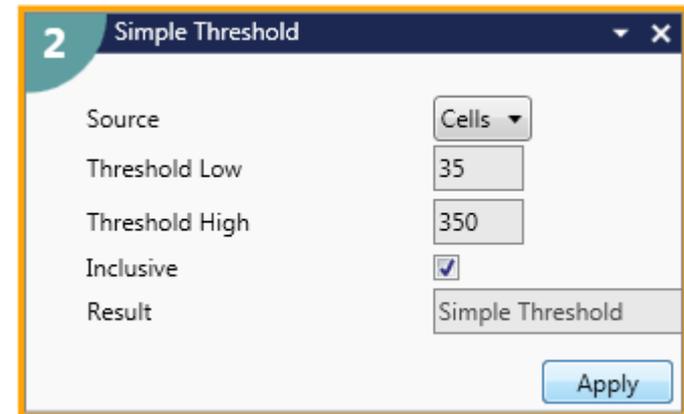
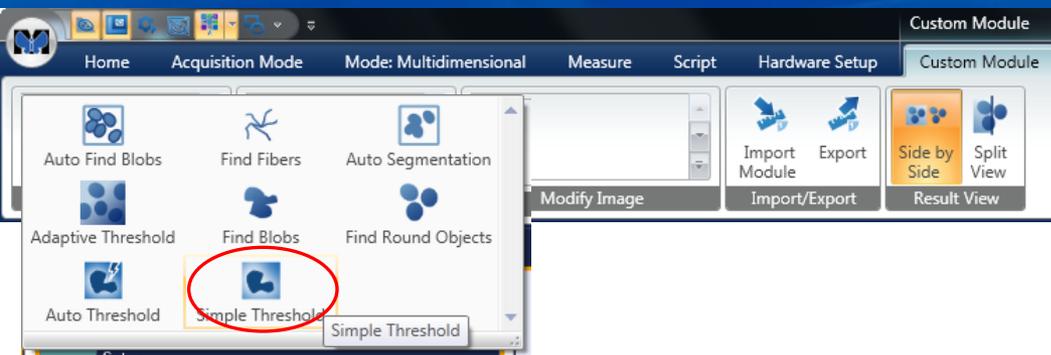
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Find Objects



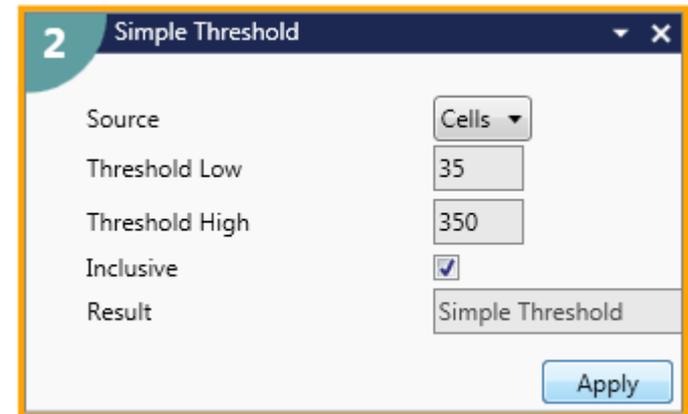
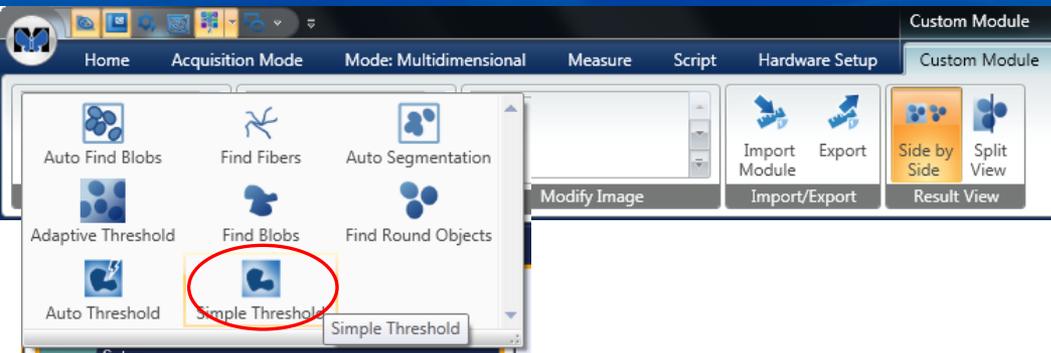
- Auto Segmentation
 - Find all kinds of Objects with NO values to configure.
 - Great for objects of all sizes and shapes
 - Does not split touching objects
 - May need to filter small objects out

Find Objects



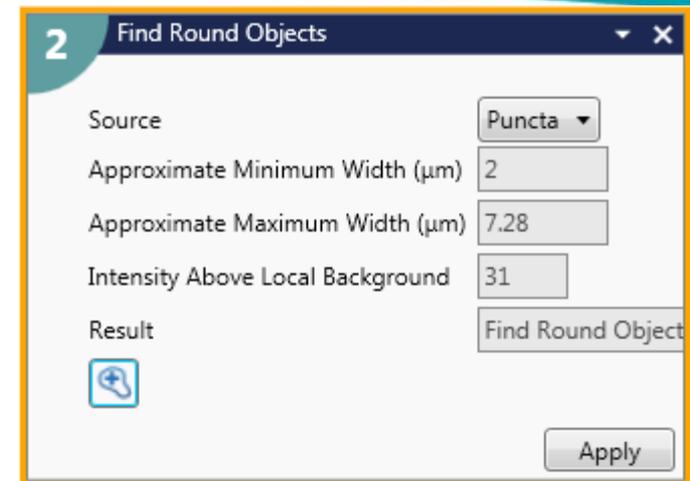
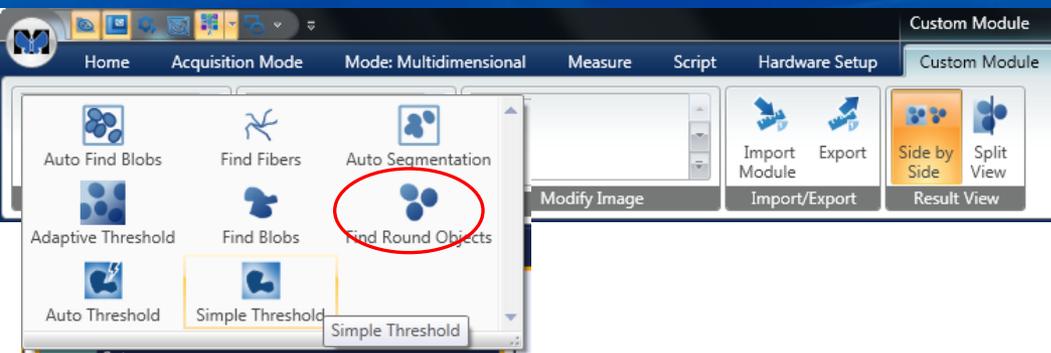
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 - Simple Threshold: Set Min / Max intensity of objects
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Find Objects



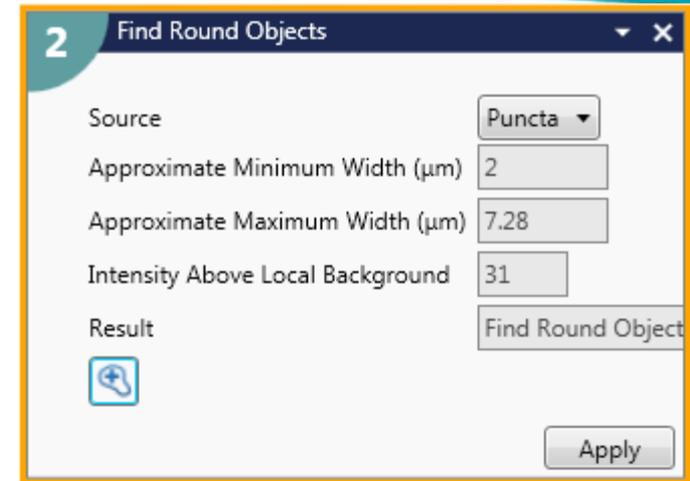
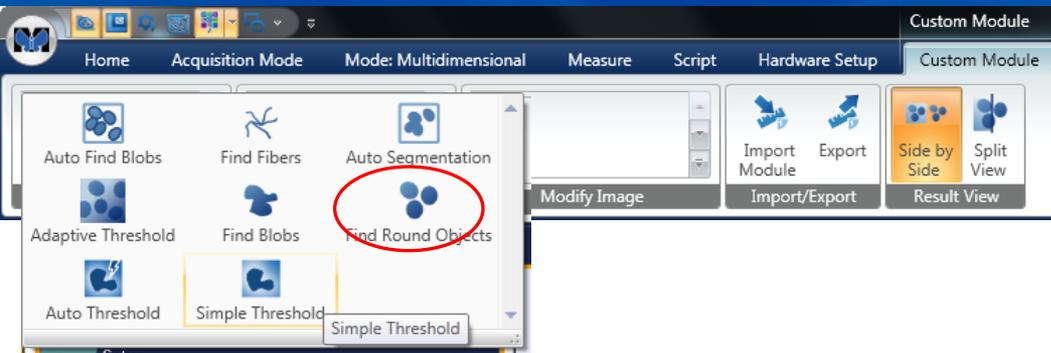
- Simple Threshold
 - Identify objects based on their absolute intensity
 - Set a Low and High Threshold Value
 - Inclusive On = Target intensities are between the Low and High values
 - Inclusive Off = Target intensities are those values NOT between the Low and High

Find Objects



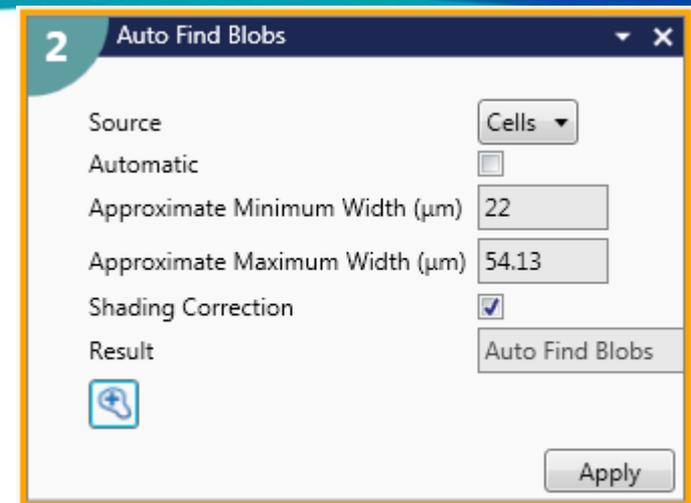
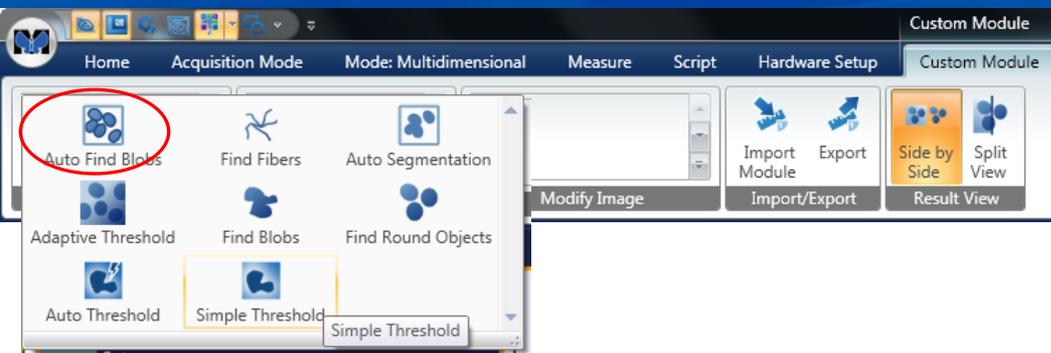
- Find Objects
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 - Simple to use tools like:
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Find Objects



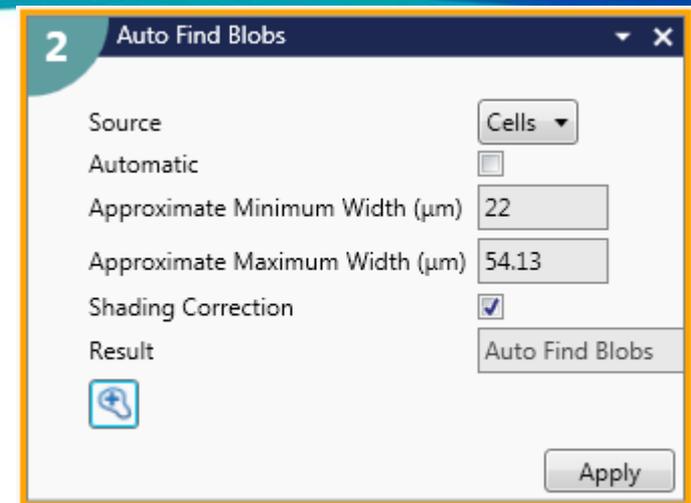
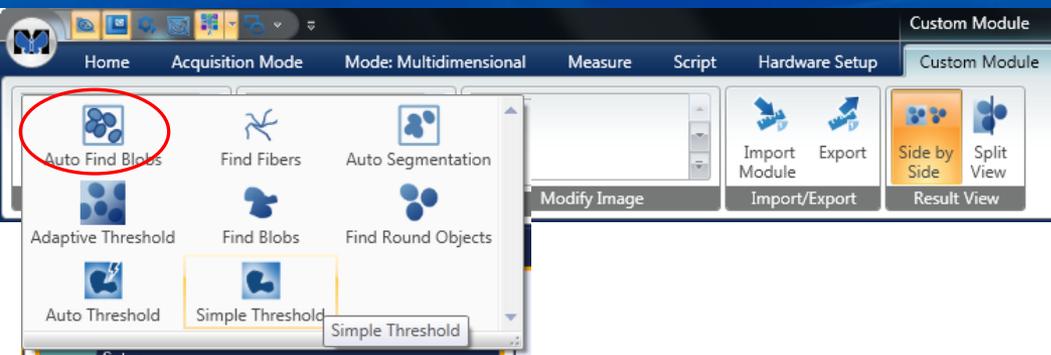
- Find Round Objects
 - Great at finding round things
 - Splits touching objects
 - Best for small objects
 - Not best option for nuclei – nuclei can be more cigar shaped ...

Find Objects



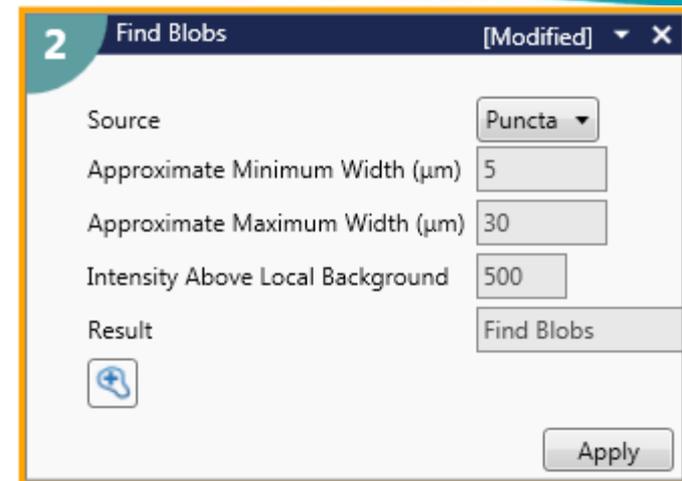
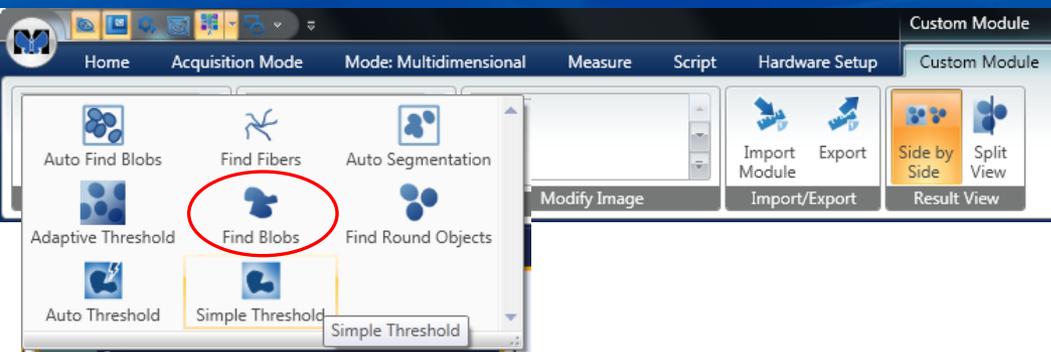
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 - Simple to use tools like:
 - Auto Segmentation: No Configuration Needed
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 - Tools like the Application Modules use:
 - Auto Find Blobs for finding things like nuclei
 - Find Blobs for finding things like cytoplasm
 - And a few more tools:
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Find Objects



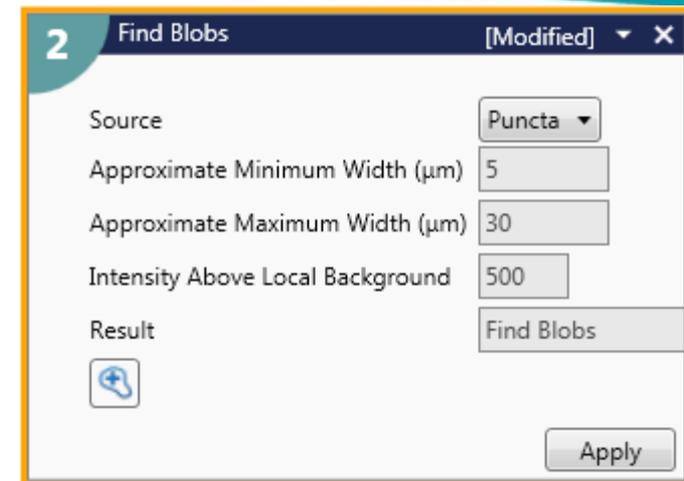
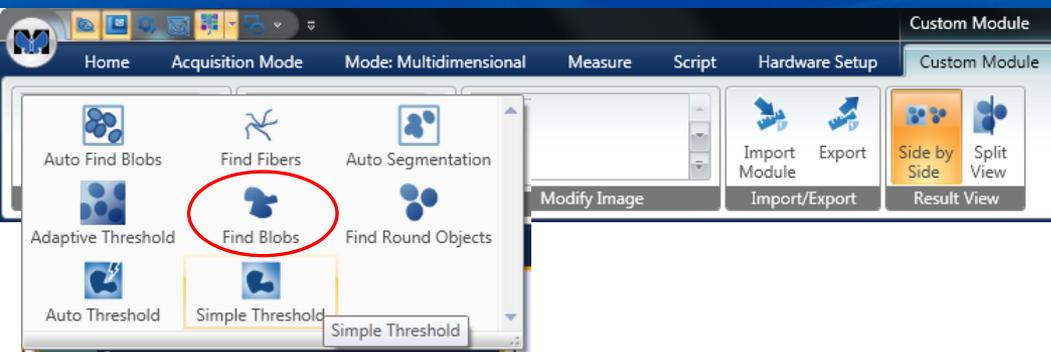
- Auto Find Blobs
 - Great at finding objects with little or no configuration
 - With 'Automatic' option on – Zero configuration setup, just apply and go
 - Splits objects
 - may find smaller objects than you want
 - Can add Filter Mask to get rid of the small stuff
 - Splits touching objects
 - **Best tool for Nuclei**, or other relatively uniform objects

Find Objects



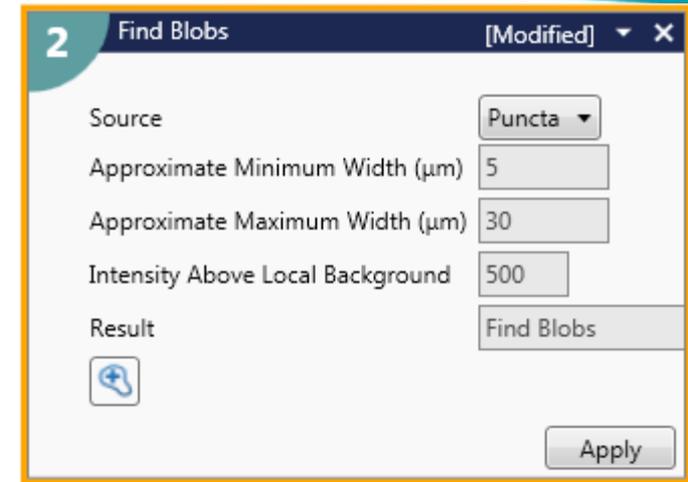
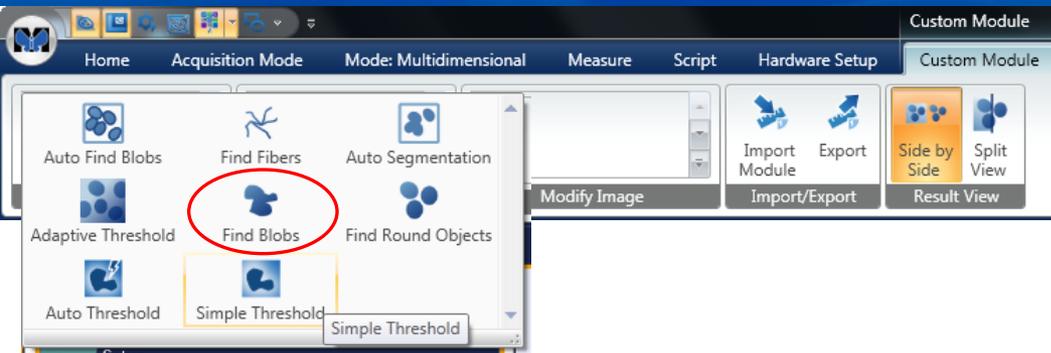
- Find Objects
 - Tools for finding objects in grayscale images and making masks for them.
 - Simple to use tools like:
 - Auto Segmentation: No Configuration Needed
 - Auto Threshold: Like auto thresholding in MM – for Bright or Dark objects
 - Simple Threshold: Set Min / Max intensity of objects
 - Tools like the Application Modules use:
 - Auto Find Blobs for finding things like nuclei
 - Find Blobs for finding things like cytoplasm
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 - Find Round Objects for truly round things (like puncta)

Find Objects



- Find Blobs
 - Finds arbitrary shaped objects, like Cytoplasm
 - Finds borders of objects based on Local Intensity differences
 - Min / Max Width define the details of the object borders, not necessarily how big the objects are
 - Small Minimum Width shows all the blebs and formations on the edge of a cytoplasm
 - Large Minimum Width shows mostly the same area, but the smaller details will be clipped.
 - Will not split touching objects

Find Objects



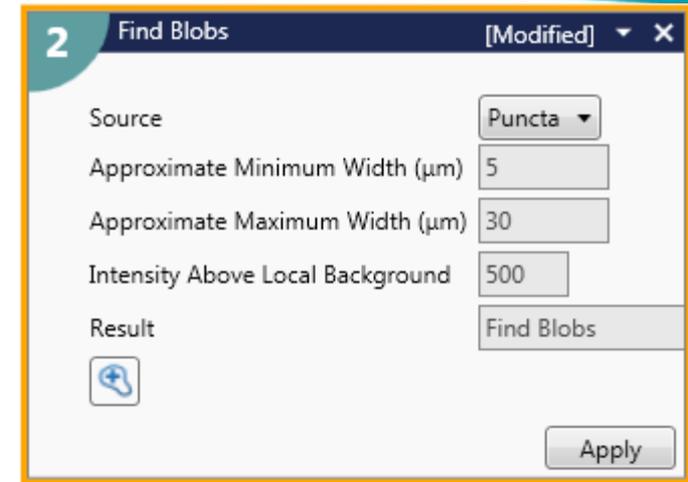
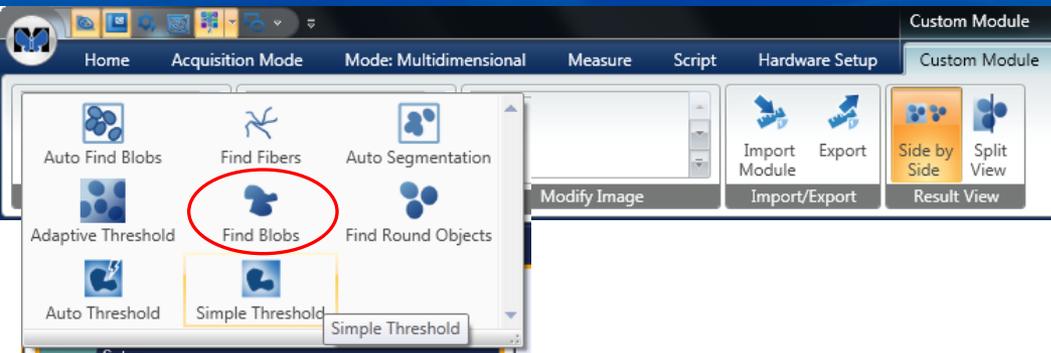
Find objects, like Cytoplasm

Objects based on Local Intensity differences

Approximate Minimum Width: 5 defines the details of the object borders, not necessarily how big the objects are

- Small Minimum Width shows all the blebs and formations on the edge of a cytoplasm
 - Large Minimum Width shows mostly the same area, but the smaller details will be clipped.
- Will not split touching objects

Find Objects



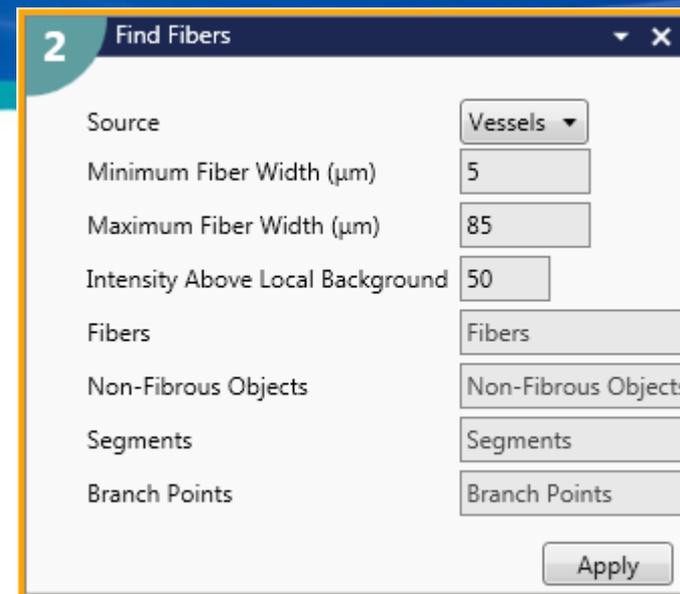
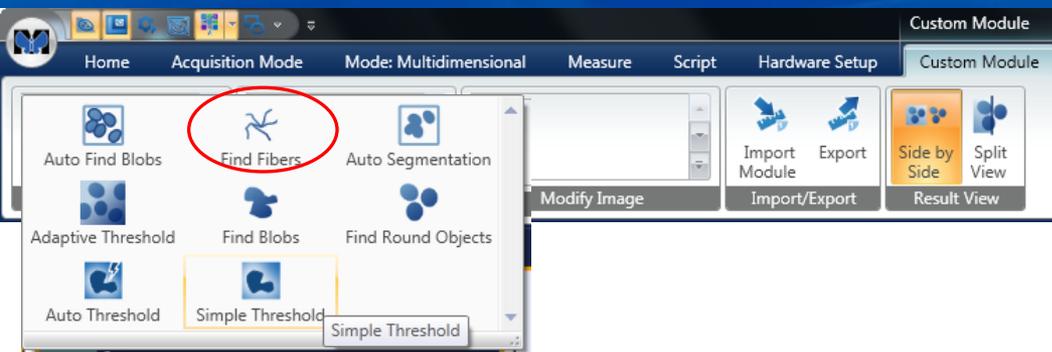
Find objects, like Cytoplasm

Objects based on Local Intensity differences

Approximate Minimum Width: 20 defines the details of the object borders, not necessarily how big the objects are

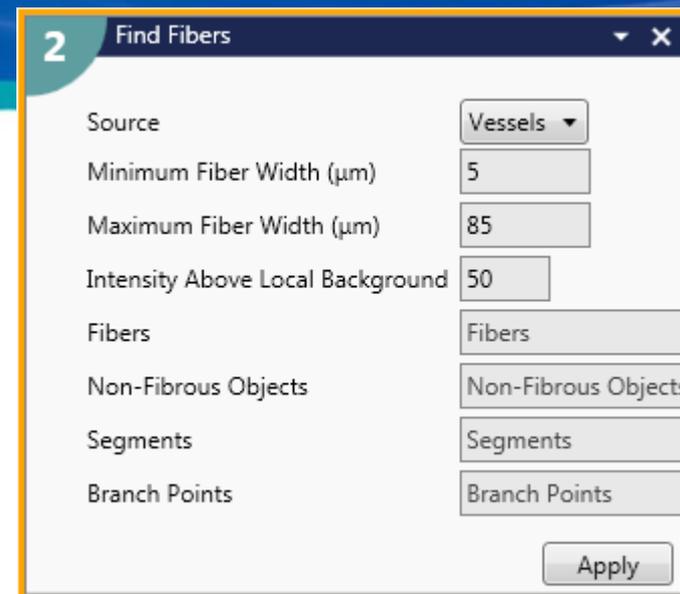
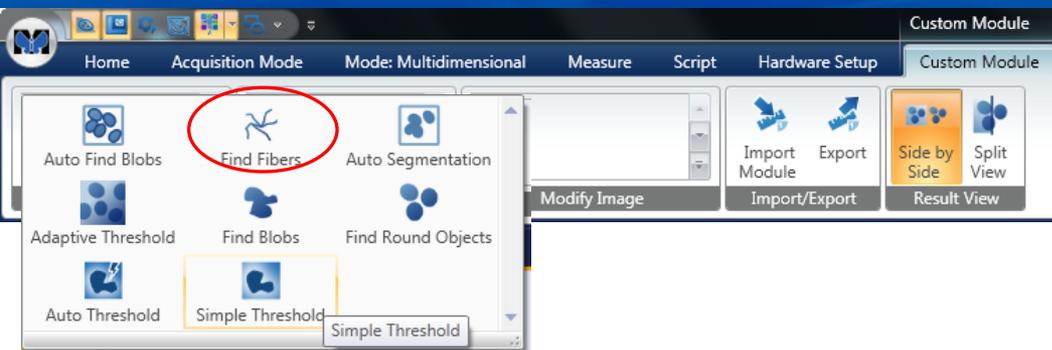
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 - Large Minimum Width shows mostly the same area, but the smaller details will be clipped.
- Will not split touching objects

Find Objects



- Find Objects
 - Tools for finding objects in grayscale images and making masks for them.
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 - And a few more tools:
 - Find Fibers for tube or process detection
 - Find Round Objects for truly round things (like puncta)

Find Objects



- Find Fibers
 - Find long tubes or fibers, their branches, and objects too large to be fibers.
 - Four masks produced
 - Fibers: Objects with Widths between the Min Fiber Width and Max Fiber Width
 - Non-Fibrous Objects: Objects with Widths > Max Fiber Width
 - Segments: Skeleton of Fibers – good for measuring length of Fibers
 - Branch Points: Single points where segments meet each other.
 - Segments + Branch Points = total Fiber system

Finding Granules In the Nucleus

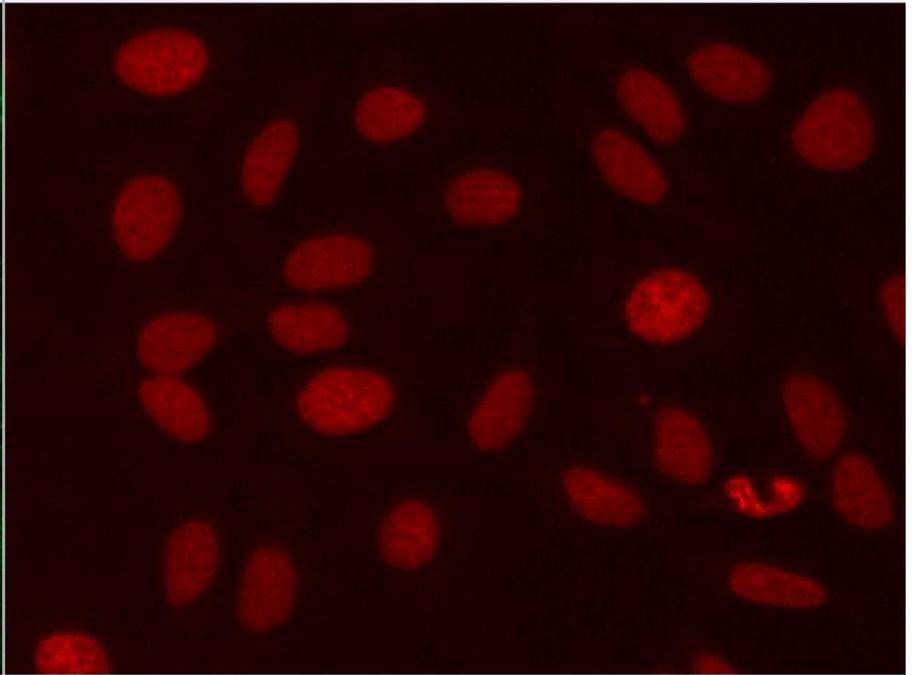
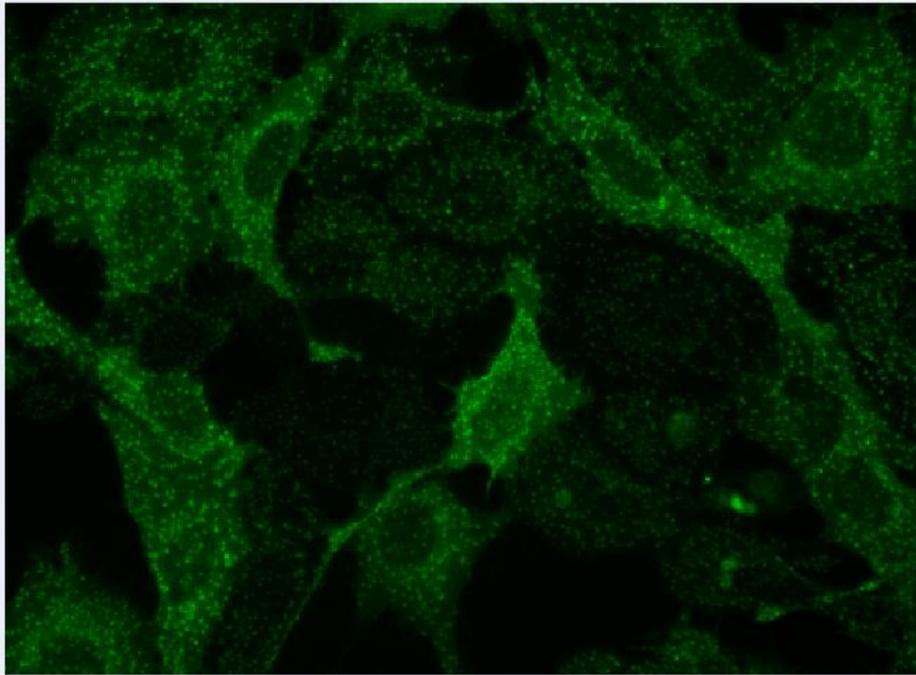
An Example Custom Module

Granules in Nucleus

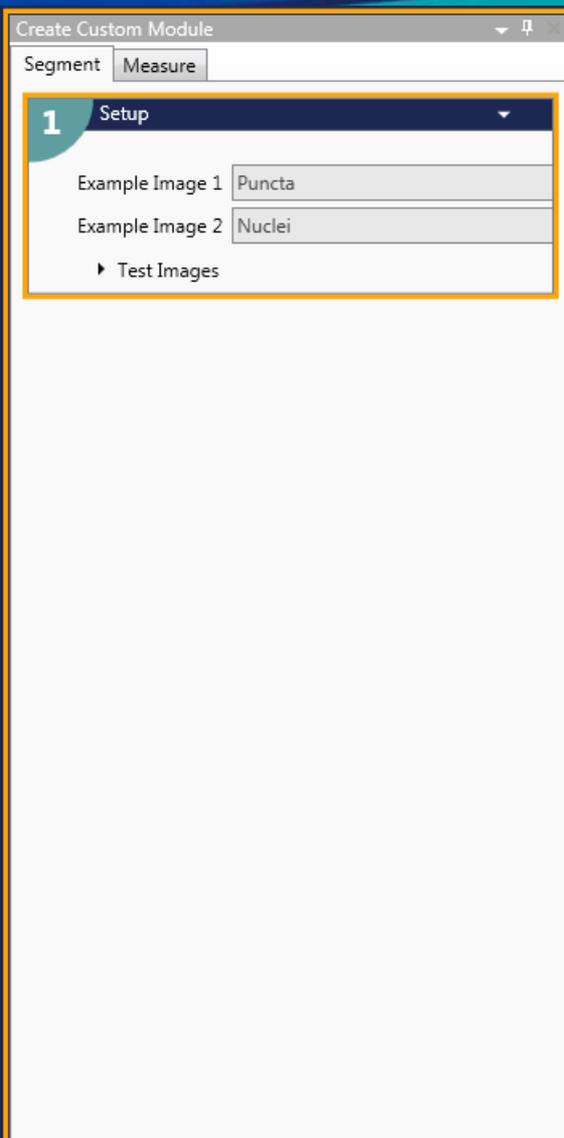
- **Goals:**
 - Count the number of Granules in a GFP stain that are localized to the Nucleus
- **Measurements to make:**
 - Nuclear Area and Intensity
 - Granule Count
 - Total Granule Area and Intensity
 - Average Granule Area
- **Inputs:**
 - DRAQ5 Nuclear Stain
 - GFP Stain localize to granules (in and out of nucleus)

Granules in Nucleus

- Example Images



Granules In Nucleus



- Setup:
 - Assign real names to be used for the input images
 - These names will be used throughout the Custom Module when referring to the 'Input' images
 - These names will be used when running the Custom Module from the Measurement Ribbon to assign the 'Input' images

Granules In Nucleus

Create Custom Module

Segment Measure

1 Setup

Example Image 1 Puncta

Example Image 2 Nuclei

▶ Test Images

2 Auto Find Blobs [Modified] X

Source Nuclei

Automatic

Approximate Minimum Width (μm) 5

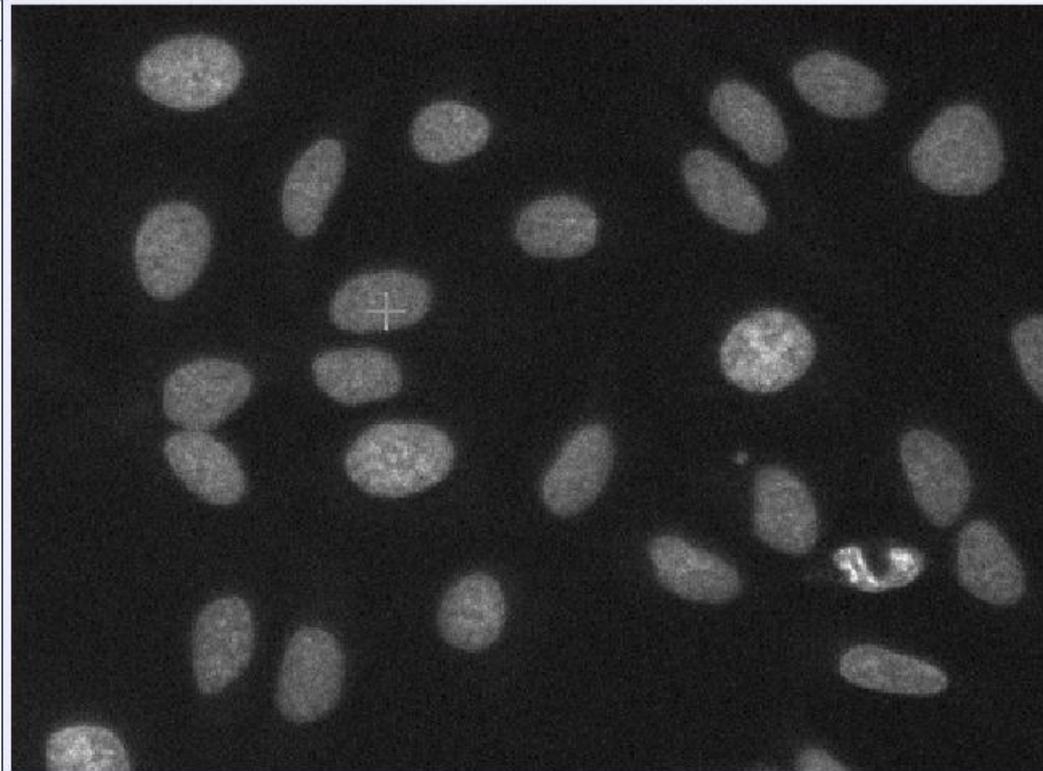
Approximate Maximum Width (μm) 30

Shading Correction

Result Auto Find Blobs



Apply



- Finding the Nuclei
 - Auto Find Blobs using Click to Find
 - Turn Automatic Off
 - Click on a few Nuclei
 - Hit Apply to find all nuclei

Granules In Nucleus

Create Custom Module

Segment Measure

1 Setup

Example Image 1 Puncta

Example Image 2 Nuclei

▶ Test Images

2 Auto Find Blobs [Modified] X

Source Nuclei

Automatic

Approximate Minimum Width (μm) 5

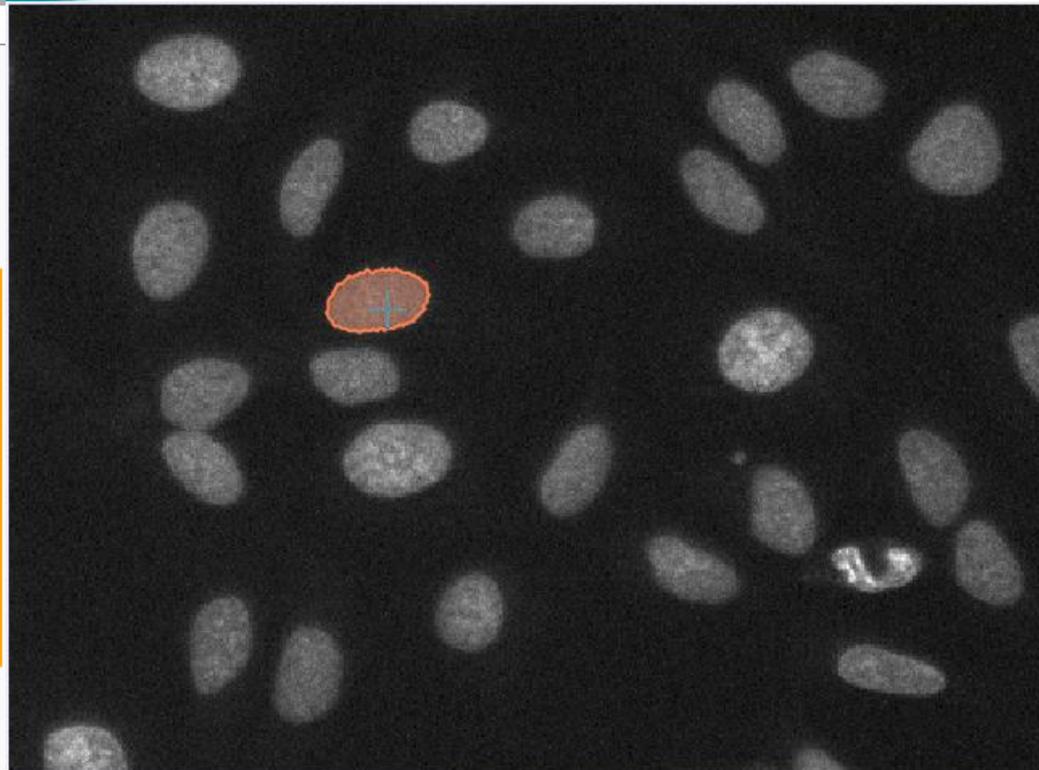
Approximate Maximum Width (μm) 30

Shading Correction

Result Auto Find Blobs

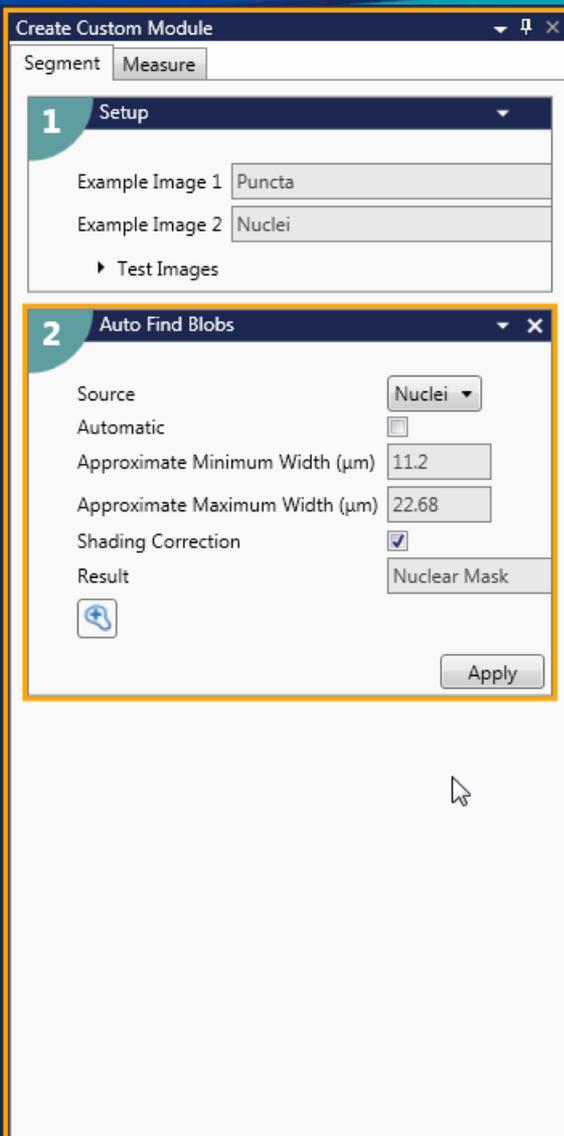


Apply



- Finding the Nuclei
 - Auto Find Blobs using Click to Find
 - Turn Automatic Off
 - Click on a few Nuclei
 - Hit Apply to find all nuclei

Granules In Nucleus



- Finding the Nuclei
 - Auto Find Blobs using Click to Find
 - Turn Automatic Off
 - Click on a few Nuclei
 - Hit Apply to find all nuclei

Find Objects Modify Objects Modify Image Import/Export Result View

Dataset2 Granules in Nucleus

Create Custom Module

Segment Measure

1 Setup

Example Image 1 Puncta

Example Image 2 Nuclei

Test Images

2 Auto Find Blobs

Source Nuclei

Automatic

Approximate Minimum Width (µm) 11.2

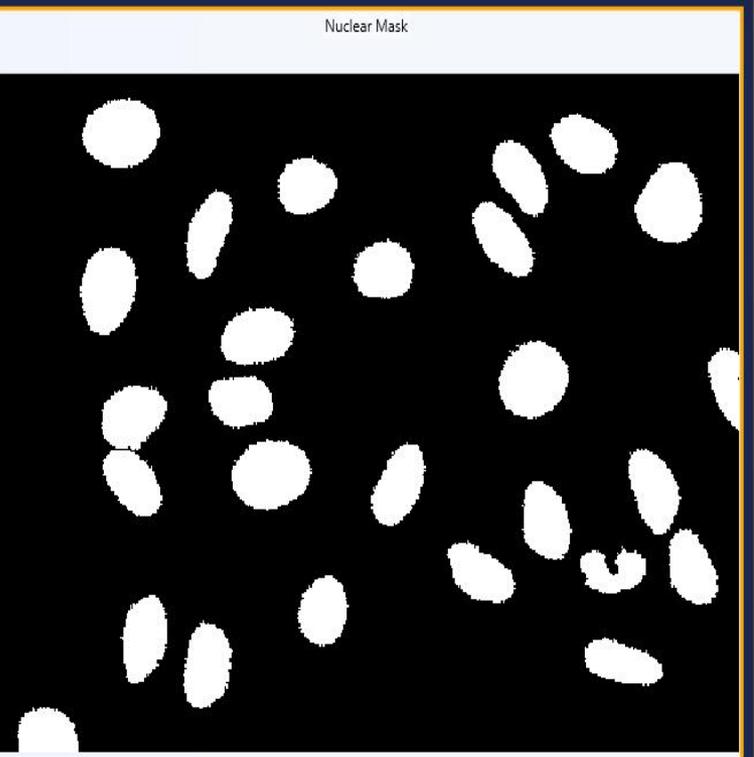
Approximate Maximum Width (µm) 22.68

Shading Correction

Result Nuclear Mask

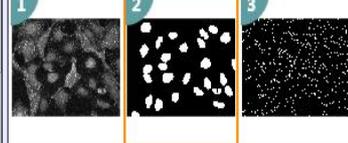
Apply

Measurement Name Granules in Nucleus Save Run

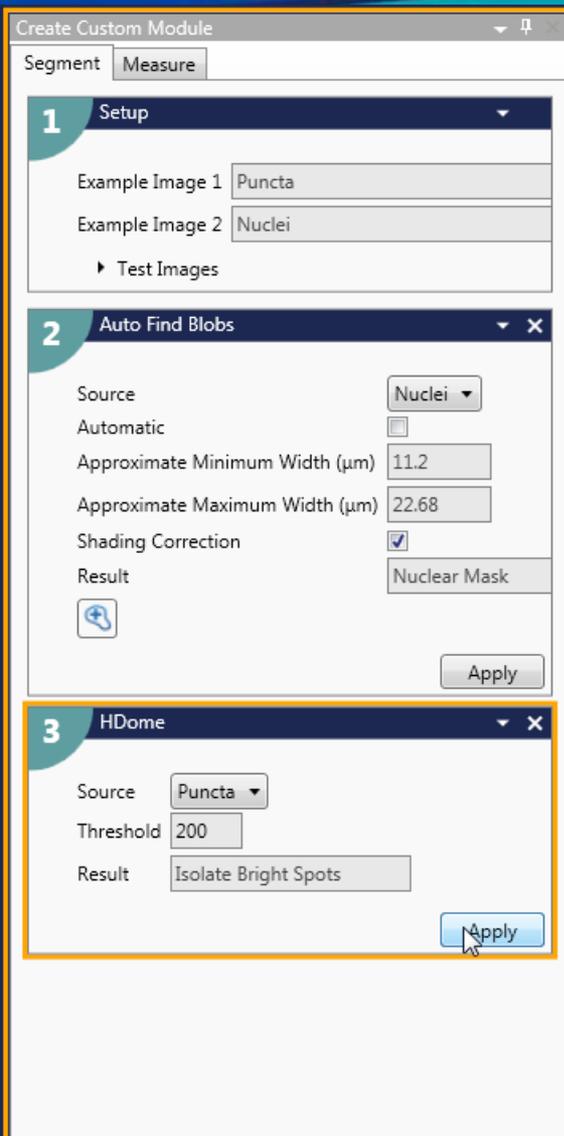


Custom Module Step Images

1 2 3



Granules In Nucleus



- Finding Granules

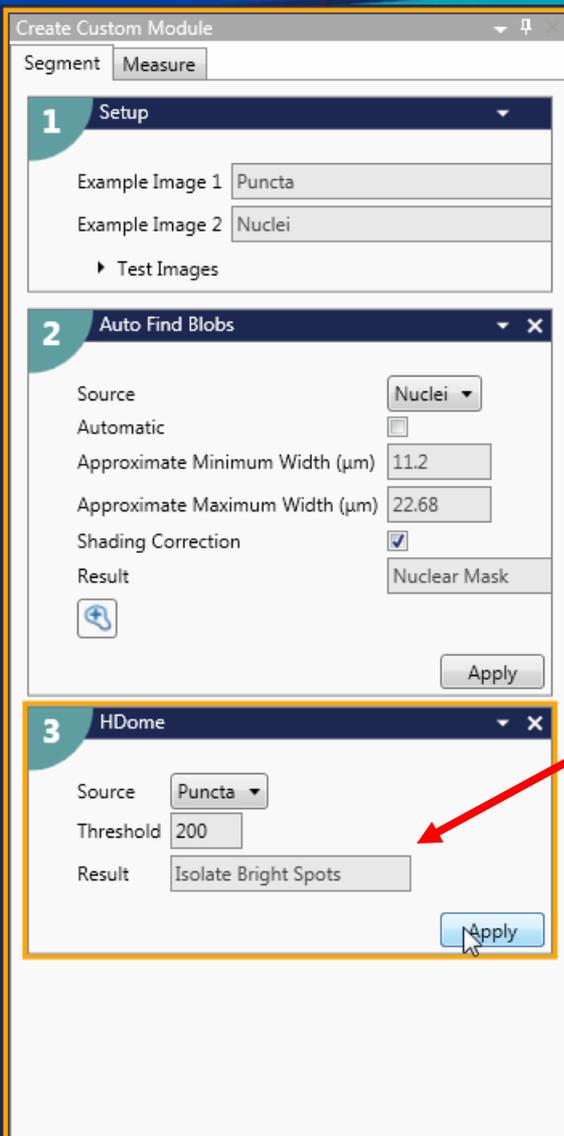
- Separate Granules from background using Modify Images > Morphology > HDome

- HDome uses a Relative Intensity Threshold

- Don't forget to provide meaningful names to the Result Images

- After HDome, use Find Objects > Auto Segmentation to create a Mask of All Puncta

Granules In Nucleus



- Finding Granules

- Separate Granules from background using Modify Images > Morphology > HDome

- HDome uses a Relative Intensity Threshold

- Don't forget to provide meaningful names to the Result Images

- After HDome, use Find Objects > Auto Segmentation to create a Mask of All Puncta

Find Objects Modify Objects Modify Image Import/Export Result View

Dataset2 Granules in Nucleus

Create Custom Module

Segment Measure

1 Setup

Example Image 1 Puncta

Example Image 2 Nuclei

Test Images

2 Auto Find Blobs

Source Nuclei

Automatic

Approximate Minimum Width (µm) 11.2

Approximate Maximum Width (µm) 22.68

Shading Correction

Result Nuclear Mask

Apply

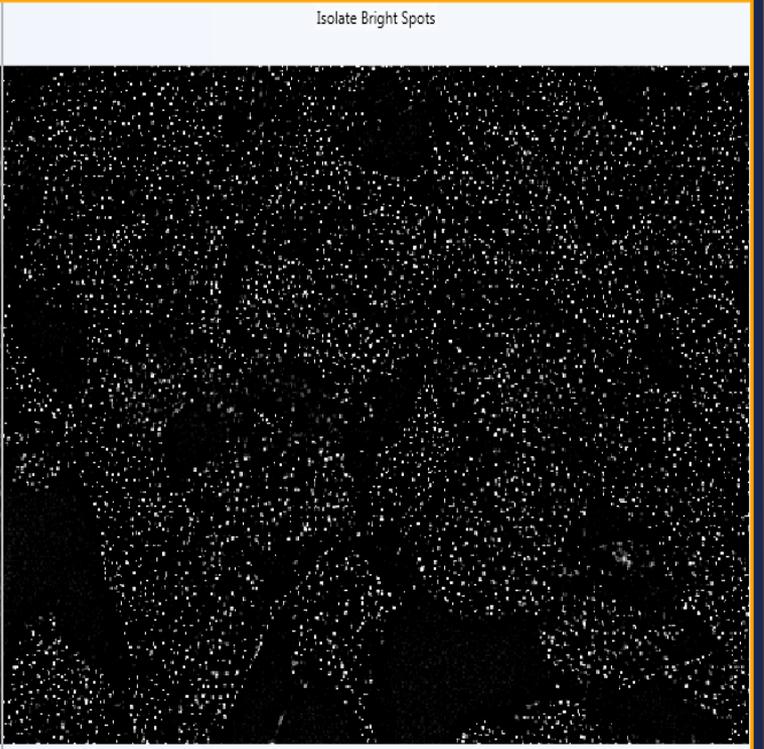
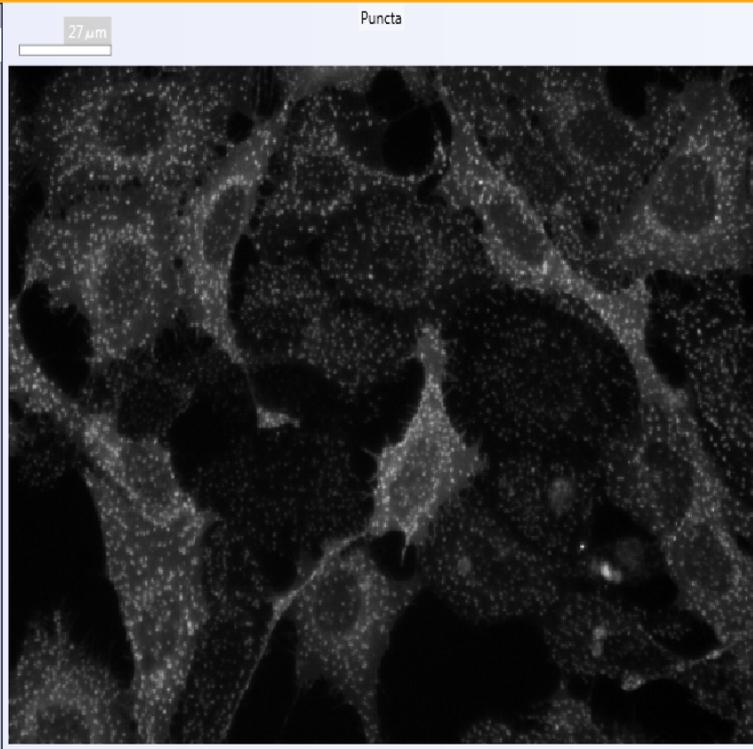
3 HDome

Source Puncta

Threshold 200

Result Isolate Bright Spots

Apply



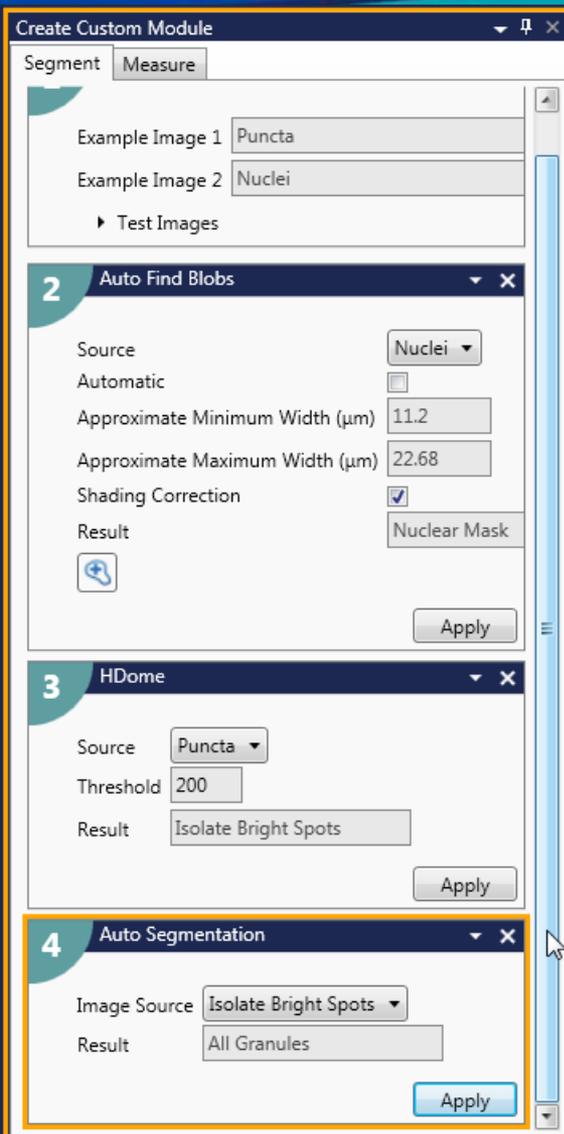
Navigation icons: back, forward, zoom in, zoom out

Custom Module Step Images

1 2 3 4

Measurement Name Granules in Nucleus Save Run

Granules In Nucleus



- Finding Granules

- Separate Granules from background using Modify Images > Morphology > HDome
- HDome uses a Relative Intensity Threshold
- Don't forget to provide meaningful names to the Result Images
- After HDome, use Find Objects > Auto Segmentation to create a Mask of All Puncta

Find Objects Modify Objects Modify Image Import/Export Result View

Dataset2 Granules in Nucleus

Create Custom Module

Segment Measure

Example Image 1 Puncta
Example Image 2 Nuclei
Test Images

2 Auto Find Blobs

Source Nuclei
Automatic
Approximate Minimum Width (µm) 11.2
Approximate Maximum Width (µm) 22.68
Shading Correction
Result Nuclear Mask

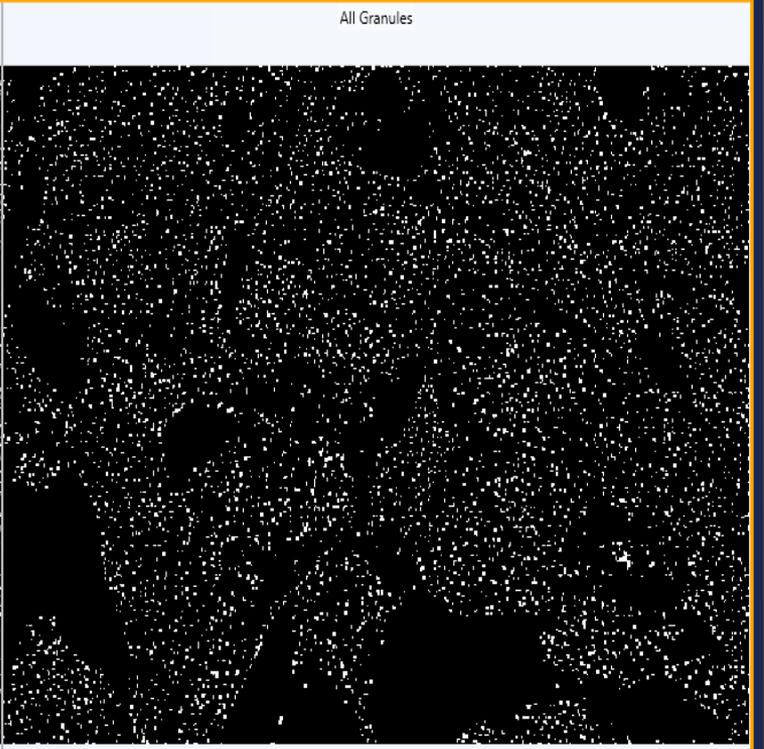
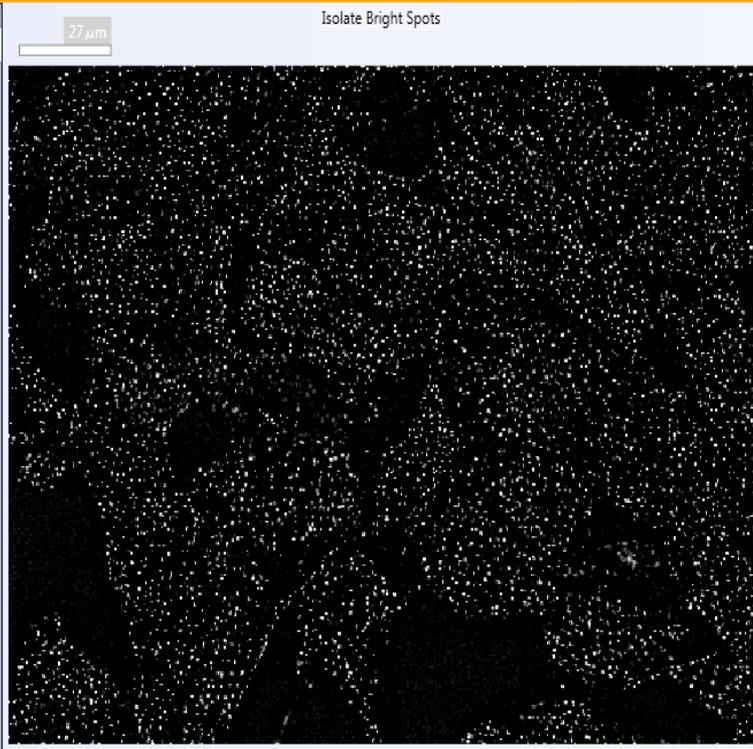
3 HDome

Source Puncta
Threshold 200
Result Isolate Bright Spots

4 Auto Segmentation

Image Source Isolate Bright Spots
Result All Granules

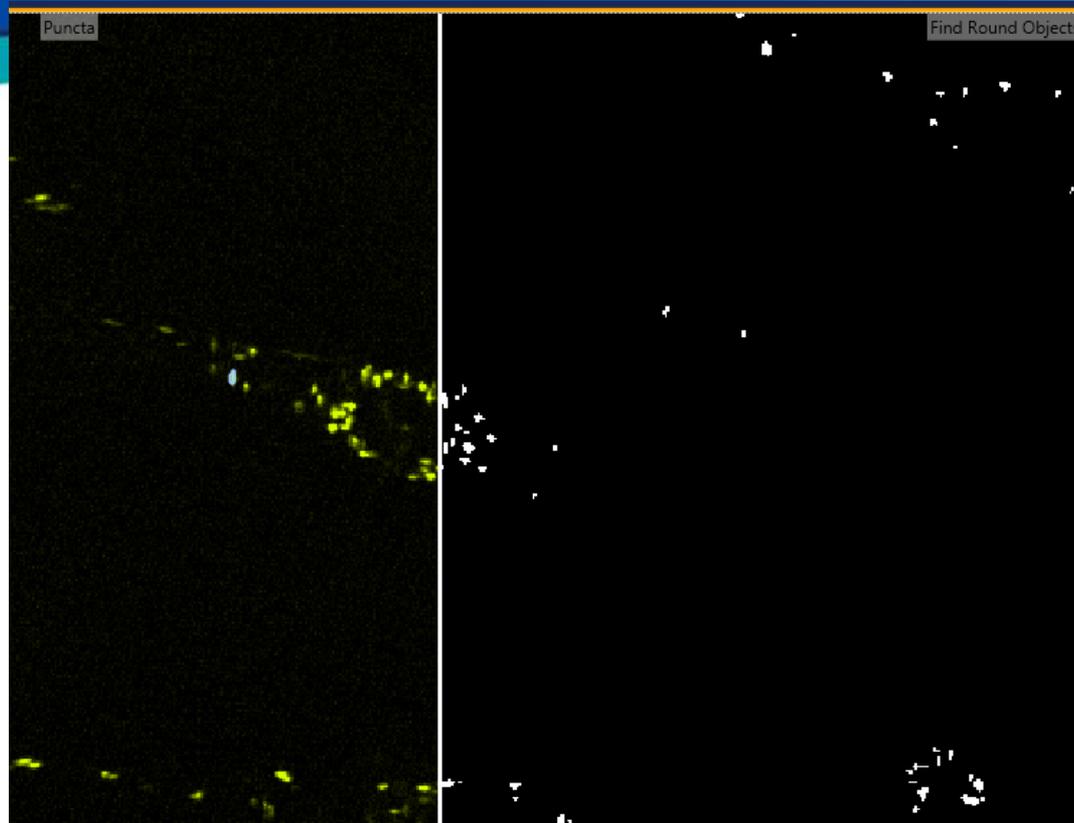
Measurement Name Granules in Nucleus Save Run



Custom Module Step Images

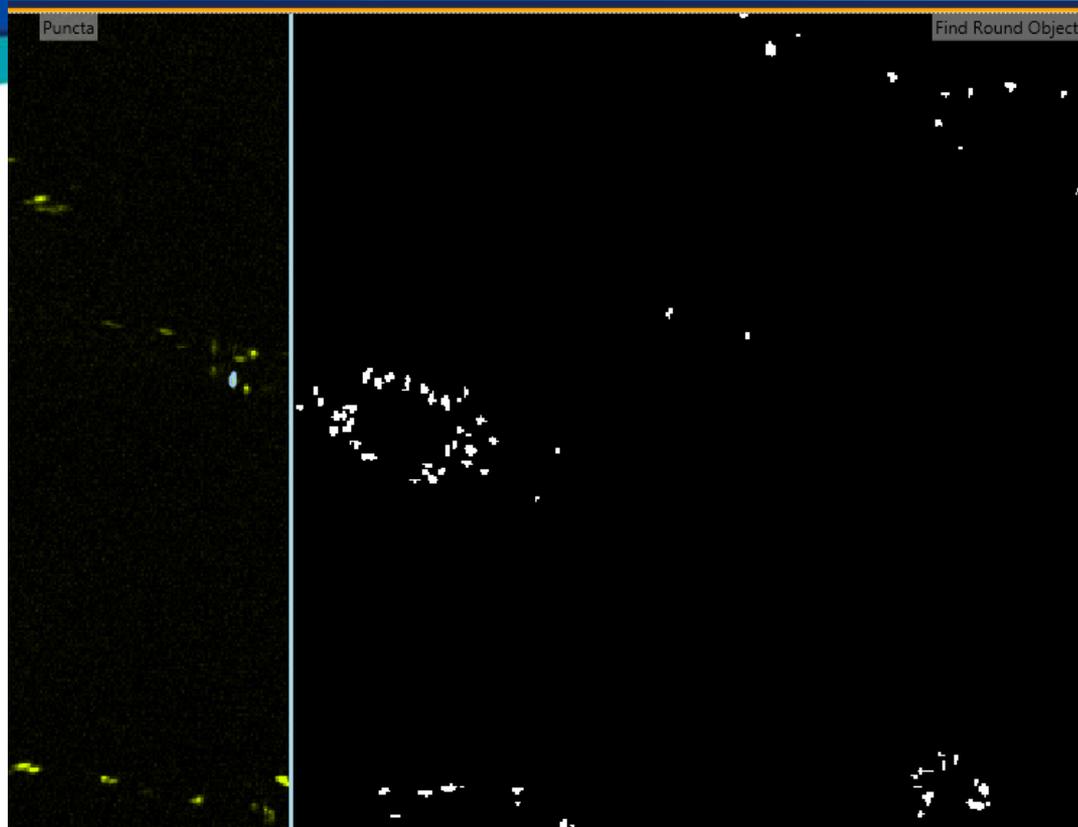
1 2 3 4 5

Granules In Nucleus



- How Good Did Segmentation Do
 - Use Side By Side view to see!
 - Slide the Bar left and right to see which spots (on the Left) were turned into objects (on the Right)

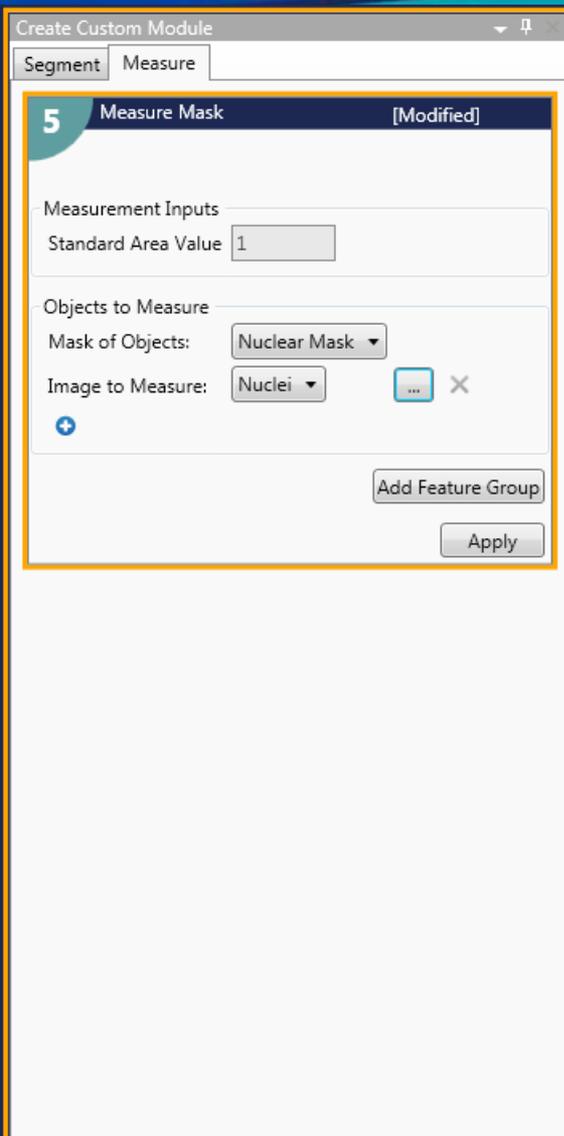
Granules In Nucleus



- How Good Did Segmentation Do
 - Use Side By Side view to see!
 - Slide the Bar left and right to see which spots (on the Left) were turned into objects (on the Right)

Granules In Nucleus

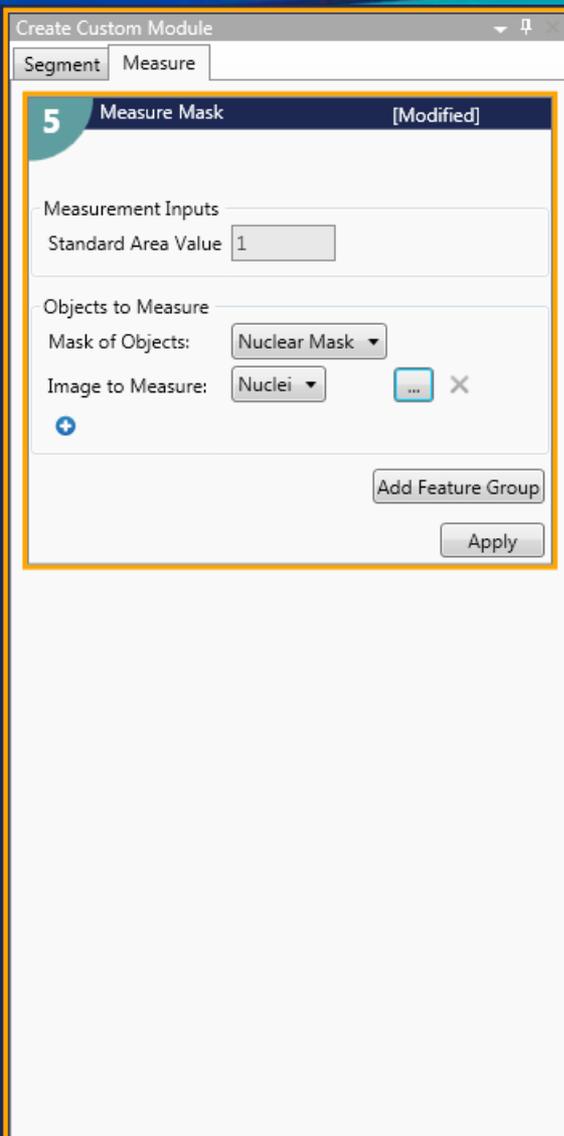
- Making Measurements: Measure Tab
 - Custom Modules are designed to measure 'Features within Objects'
 - For Example 'Granules within Nuclei'
 - The first step is to measure the outer-borders: the Objects of Interest
 - In this case, the Nuclei
 - In the Object to Measure section:
 - Mask of Objects = Nuclear Mask
 - Define the widest area to measure in
 - Image to Measure = Nuclei
 - Grayscale image to get Intensities from
 - You can measure more than One image using the  icon.



Granules In Nucleus

- Making Nuclear Measurements

- Press the ... button to configure what values gets measured
- Turn off the Right column of values. They are not needed for the 'Object' measurement.
- Select which rows to measure from the Left column of values
 - Area, Integrated Intensity, Average Intensity
 - Can re-name the values to be more meaningful



Granules In Nucleus

- Making Nuclear Measurements

Measurement Selection Configuration

Measurement Name	Average	Column Label	Sum	Column Label
Total Area	<input type="checkbox"/>	Total Area_Average	<input type="checkbox"/>	Total Area_Sum
Hole Area	<input type="checkbox"/>	Hole Area_Average	<input type="checkbox"/>	Hole Area_Sum
Area	<input checked="" type="checkbox"/>	Nucleus Area	<input type="checkbox"/>	Area_Sum
Relative Hole Area	<input type="checkbox"/>	Relative Hole Area_Average	<input type="checkbox"/>	Relative Hole Area_Sum
Standard Area Count	<input type="checkbox"/>	Standard Area Count_Avera	<input type="checkbox"/>	Standard Area Count_Sum
Width	<input type="checkbox"/>	Width_Average	<input type="checkbox"/>	Width_Sum
Height	<input type="checkbox"/>	Height_Average	<input type="checkbox"/>	Height_Sum
Centroid X	<input type="checkbox"/>	Centroid X_Average	<input type="checkbox"/>	Centroid X_Sum
Centroid Y	<input type="checkbox"/>	Centroid Y_Average	<input type="checkbox"/>	Centroid Y_Sum
Intensity Center X	<input type="checkbox"/>	Intensity Center X_Average	<input type="checkbox"/>	Intensity Center X_Sum
Intensity Center Y	<input type="checkbox"/>	Intensity Center Y_Average	<input type="checkbox"/>	Intensity Center Y_Sum
Integrated Intensity	<input checked="" type="checkbox"/>	Nucleus Total Intensity	<input type="checkbox"/>	Integrated Intensity_Sum
Average Intensity	<input checked="" type="checkbox"/>	Nucleus Average Intensity	<input type="checkbox"/>	Average Intensity_Sum
Intensity Std. Dev.	<input type="checkbox"/>	Intensity Std. Dev._Average	<input type="checkbox"/>	Intensity Std. Dev._Sum
Minimum Intensity	<input type="checkbox"/>	Minimum Intensity_Average	<input type="checkbox"/>	Minimum Intensity_Sum
Maximum Intensity	<input type="checkbox"/>	Maximum Intensity_Average	<input type="checkbox"/>	Maximum Intensity_Sum
Perimeter	<input type="checkbox"/>	Perimeter_Average	<input type="checkbox"/>	Perimeter_Sum
Shape Factor	<input type="checkbox"/>	Shape Factor_Average	<input type="checkbox"/>	Shape Factor_Sum
Fiber Length	<input type="checkbox"/>	Fiber Length_Average	<input type="checkbox"/>	Fiber Length_Sum
Fiber Breadth	<input type="checkbox"/>	Fiber Breadth_Average	<input type="checkbox"/>	Fiber Breadth_Sum
Length	<input type="checkbox"/>	Length_Average	<input type="checkbox"/>	Length_Sum
Orientation	<input type="checkbox"/>	Orientation_Average	<input type="checkbox"/>	Orientation_Sum
Breadth	<input type="checkbox"/>	Breadth_Average	<input type="checkbox"/>	Breadth_Sum
Ell. Form Factor	<input type="checkbox"/>	Ell. Form Factor_Average	<input type="checkbox"/>	Ell. Form Factor_Sum
Dual Centroid X	<input type="checkbox"/>	Dual Centroid X_Average	<input type="checkbox"/>	Dual Centroid X_Sum

Cancel OK

Press the ... button to configure what values gets measured

Turn off the Right column of values. They are not needed for the 'Object' measurement.

Select which rows to measure from the Left column of values

- Area, Integrated Intensity, Average Intensity
- Can re-name the values to be more meaningful

Granules In Nucleus

- Making Granule Measurements

- The Granules are 'Features' found inside the Nucleus.
- Click on the 'Add Feature Group' button to add an new section for measuring the Granules.
- Mask of Features = All Granules
- Image to Measure = Puncta
- Press ... to select which measurements to make on Granules

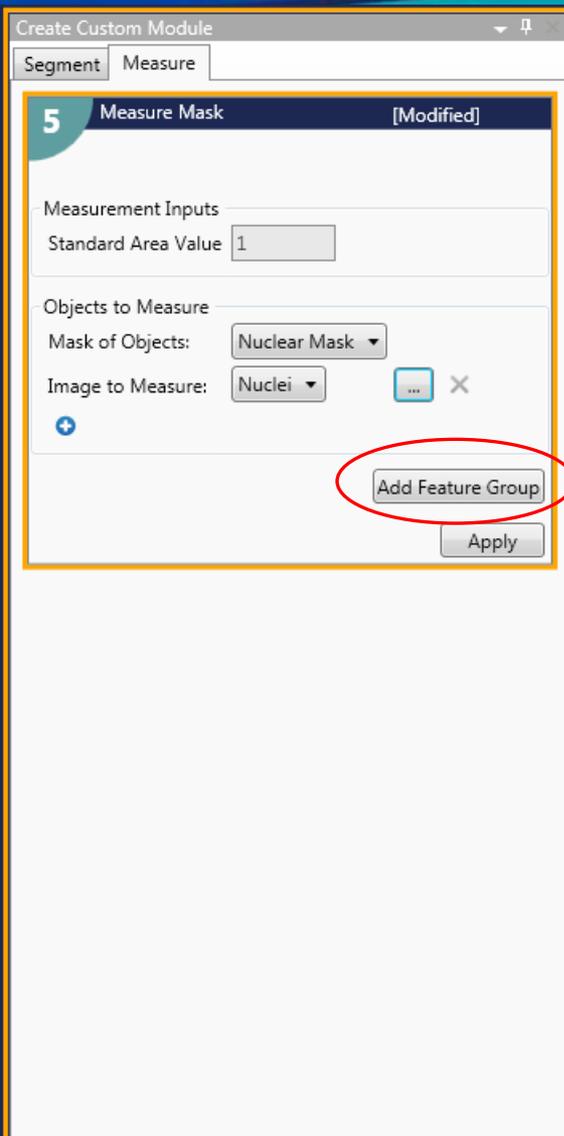
The screenshot shows a software interface for creating a custom module. The title bar reads 'Create Custom Module'. There are two tabs: 'Segment' and 'Measure', with 'Measure' selected. The main area is titled '5 Measure Mask [Modified]'. It contains the following fields and controls:

- Measurement Inputs:** A text box labeled 'Standard Area Value' containing the number '1'.
- Objects to Measure:**
 - 'Mask of Objects:' dropdown menu set to 'Nuclear Mask'.
 - 'Image to Measure:' dropdown menu set to 'Nuclei', followed by a blue ellipsis button and a close button (X).
 - A blue plus sign (+) button below the 'Image to Measure' dropdown.
- 'Add Feature Group' button.
- 'Apply' button.

Granules In Nucleus

- Making Granule Measurements

- The Granules are 'Features' found inside the Nucleus.
- Click on the 'Add Feature Group' button to add an new section for measuring the Granules.
- Mask of Features = All Granules
- Image to Measure = Puncta
- Press ... to select which measurements to make on Granules



Granules In Nucleus

- Making Granule Measurements

- The Granules are 'Features' found inside the Nucleus.
- Click on the 'Add Feature Group' button to add an new section for measuring the Granules.
- Mask of Features = All Granules
- Image to Measure = Puncta
- Press ... to select which measurements to make on Granules

The screenshot shows a software interface for creating a custom module. The window title is 'Create Custom Module' and it has two tabs: 'Segment' and 'Measure'. The 'Measure' tab is active, showing a configuration panel for 'Measure Mask' (indicated by a '5' in a blue circle and '[Modified]').

The configuration is organized into sections:

- Measurement Inputs:** A text field for 'Standard Area Value' containing the number '1'.
- Objects to Measure:** A dropdown menu for 'Mask of Objects' set to 'Nuclear Mask'. Below it, 'Image to Measure' is set to 'Nuclei', with a blue '+' button to the left and a blue '...' button with an 'X' to the right.
- Features within Each Object:** A dropdown menu for 'Mask of Features' set to 'All Granules'. Below it, 'Image to Measure' is set to 'Puncta', with a blue '+' button to the left and a blue '...' button with an 'X' to the right.

At the bottom of the configuration area, there are three buttons: 'Remove Feature Group', 'Add Feature Group' (highlighted with a blue border), and 'Apply'.

Granules In Nucleus

- Making Granule Measurements

Measurement	Selected	Measurement	Selected
Area	<input checked="" type="checkbox"/>	Average Granule Area	<input checked="" type="checkbox"/>
Relative Hole Area	<input type="checkbox"/>	Relative Hole Area_Average	<input type="checkbox"/>
Standard Area Count	<input type="checkbox"/>	Standard Area Count_Avera	<input type="checkbox"/>
Width	<input type="checkbox"/>	Width_Average	<input type="checkbox"/>
Height	<input type="checkbox"/>	Height_Average	<input type="checkbox"/>
Centroid X	<input type="checkbox"/>	Centroid X_Average	<input type="checkbox"/>
Centroid Y	<input type="checkbox"/>	Centroid Y_Average	<input type="checkbox"/>
Intensity Center X	<input type="checkbox"/>	Intensity Center X_Average	<input type="checkbox"/>
Intensity Center Y	<input type="checkbox"/>	Intensity Center Y_Average	<input type="checkbox"/>
Integrated Intensity	<input type="checkbox"/>	Integrated Intensity_Averag	<input checked="" type="checkbox"/>
Average Intensity	<input checked="" type="checkbox"/>	Average Granule Intensity	<input type="checkbox"/>
Intensity Std. Dev.	<input type="checkbox"/>	Intensity Std. Dev._Average	<input type="checkbox"/>
Minimum Intensity	<input type="checkbox"/>	Minimum Intensity_Average	<input type="checkbox"/>
Maximum Intensity	<input type="checkbox"/>	Maximum Intensity_Average	<input type="checkbox"/>
Perimeter	<input type="checkbox"/>	Perimeter_Average	<input type="checkbox"/>
Shape Factor	<input type="checkbox"/>	Shape Factor_Average	<input type="checkbox"/>
Fiber Length	<input type="checkbox"/>	Fiber Length_Average	<input type="checkbox"/>
Fiber Breadth	<input type="checkbox"/>	Fiber Breadth_Average	<input type="checkbox"/>
Length	<input type="checkbox"/>	Length_Average	<input type="checkbox"/>
Orientation	<input type="checkbox"/>	Orientation_Average	<input type="checkbox"/>
Breadth	<input type="checkbox"/>	Breadth_Average	<input type="checkbox"/>
Ell. Form Factor	<input type="checkbox"/>	Ell. Form Factor_Average	<input type="checkbox"/>
Pixel Centroid X	<input type="checkbox"/>	Pixel Centroid X_Average	<input type="checkbox"/>
Pixel Centroid Y	<input type="checkbox"/>	Pixel Centroid Y_Average	<input type="checkbox"/>
Line Length	<input type="checkbox"/>	Line Length_Average	<input type="checkbox"/>
Features Count	<input type="checkbox"/>	Granule Count Per Cell	<input checked="" type="checkbox"/>

Press ... to select which measurements to make on Granules

- The Left Column is the Average of all Features per Object
- The Right Column is the Sum of all Features per Object
 - Area on Left Column = Average Granule Area
 - Area on Right Column = Total Granule Area
- The last measurement on the Right Column is the Count of Features
- Measure:
 - Average / Total Granule Area
 - Average / Total Granule Intensity
 - Total Granule Count

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup Custom Module

Find Objects Modify Objects Modify Image Import/Export Result View

Dataset2 Granules in Nucleus

Create Custom Module

Segment Measure

5 Measure Mask

Measurement Inputs
Standard Area Value: 1

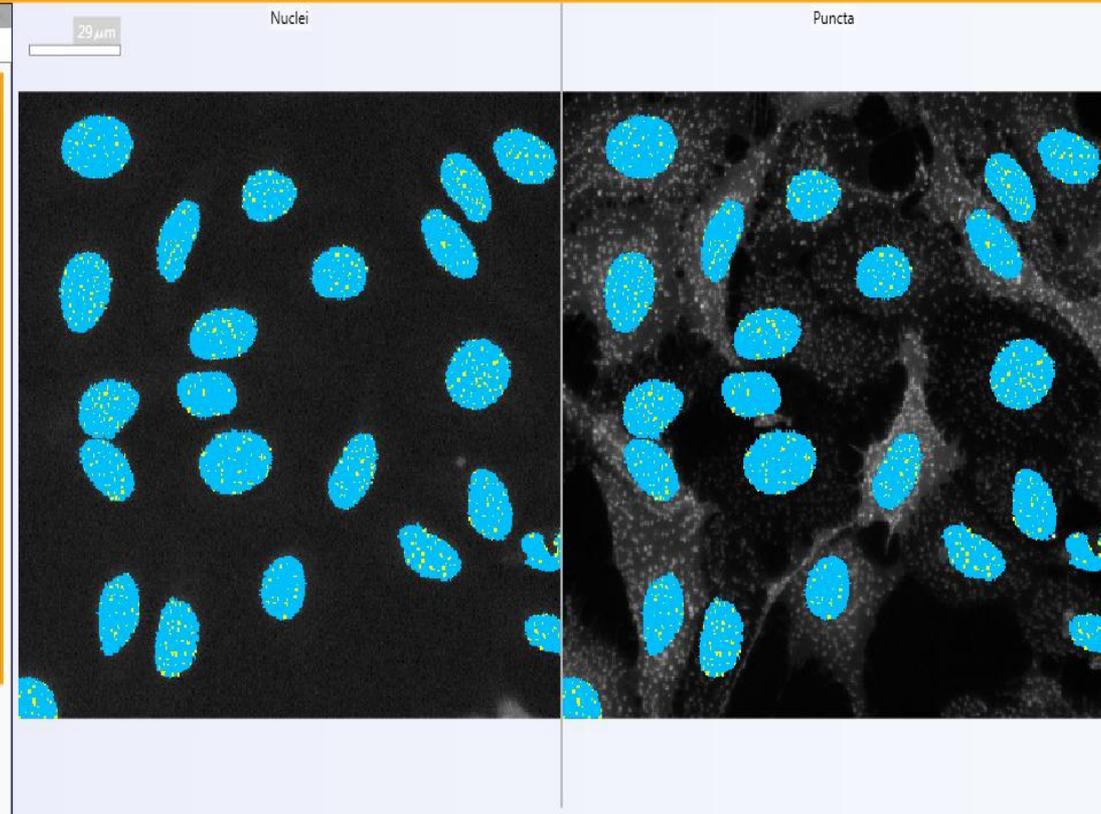
Objects to Measure
Mask of Objects: Nuclear Mask
Image to Measure: Nuclei

Features within Each Object
Mask of Features: All Granules
Image to Measure: Puncta

Remove Feature Group
Add Feature Group
Apply

Measurement Name: Granules in Nucleus

Save Run



Data Table

Row ID	Granule Int	Granule Count Per C
1	1,416.29	42.00
2	1,020.74	34.00
3	999.72	41.00
4	1,272.08	41.00
5	1,339.72	47.00
6	1,455.44	32.00
7	1,517.56	32.00
8	1,082.56	33.00
9	1,491.83	58.00
10	939.00	40.00
11	809.00	51.00
12	948.20	26.00
13	882.18	19.00
14	750.24	46.00
15	758.19	37.00
16	1,845.74	42.00
17	1,136.60	29.00
18	1,510.95	34.00
19	975.01	31.00
20	1,190.66	35.00
21	942.39	29.00



Custom Module Step Images

1 2 3 4 5

Granules In Nucleus

Create Custom Module

Segment Measure

5 Measure Mask

Measurement Inputs

Standard Area Value 1

Objects to Measure

Mask of Objects: Nuclear Mask

Image to Measure: Nuclei

Features within Each Object:

Mask of Features: All Granules

Image to Measure: Puncta

Remove Feature Group

Add Feature Group

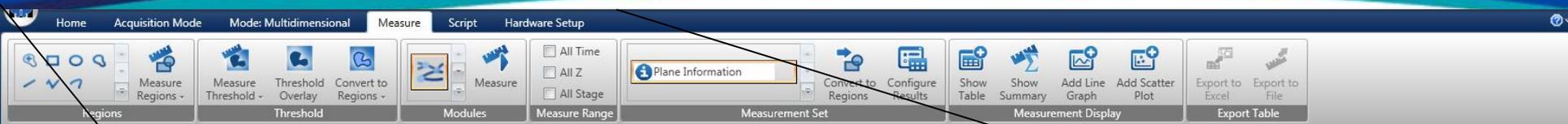
Apply

Measurement Name Granules in Nucleus

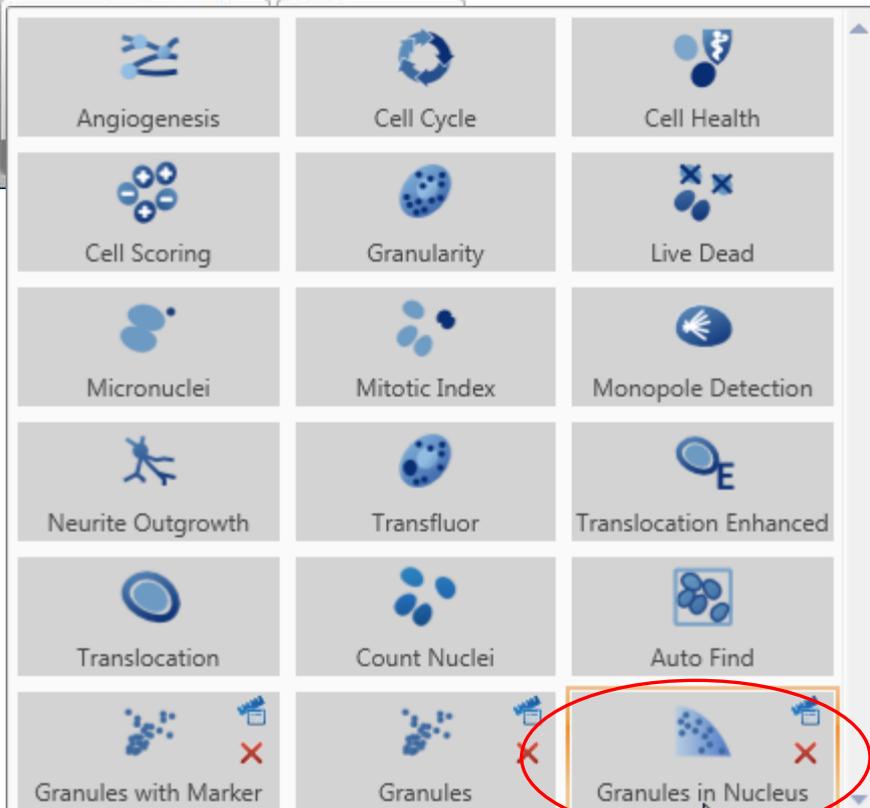
Run

- Saving the Custom Module
 - At the bottom of the Module, provide a Measurement Name
 - Select from a list of icons to display for the measurement
 - Save the measurement to add it to the list of Modules
 - Then close the Custom Module dataset view

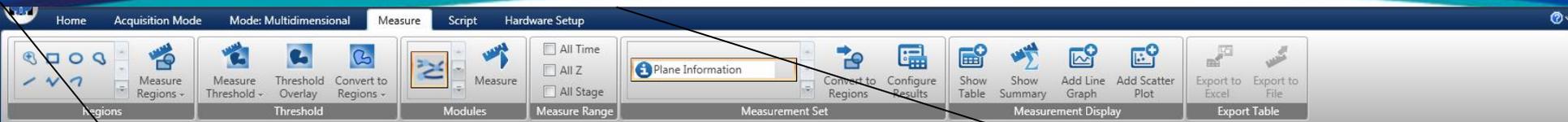
Modules



- Running a Custom Module
 - Select the Module you made from the same list as the Application Modules
 - Click on it in the Ribbon
 - Assign Images
 - Run Measurement

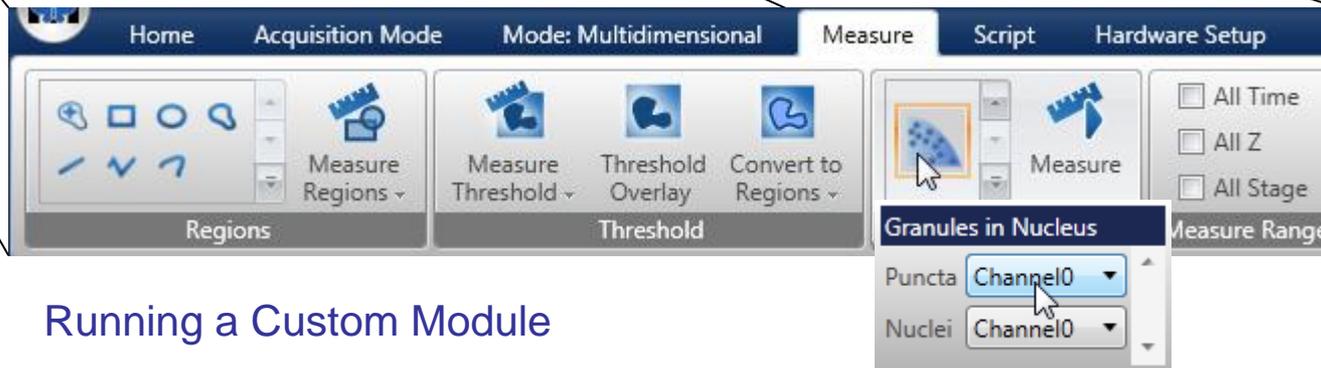
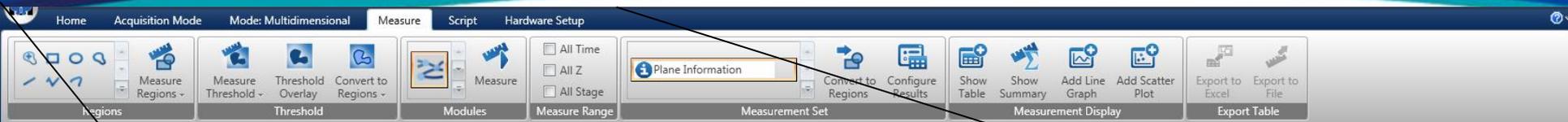


Modules



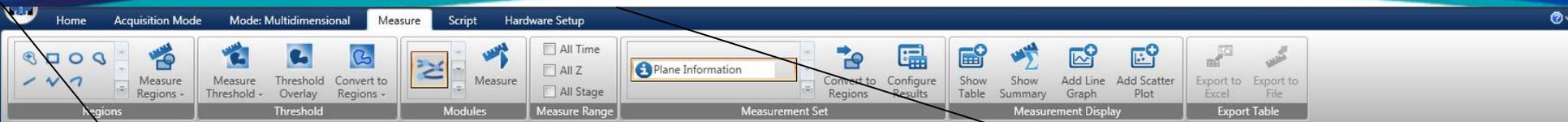
- Running a Custom Module
 - Select the Module you made from the same list as the Application Modules
 - Click on it in the Ribbon
 - Assign Images
 - Run Measurement

Modules



- Running a Custom Module
 - Select the Module you made from the same list as the Application Modules
 - Click on it in the Ribbon
 - Assign Images
 - Run Measurement

Modules



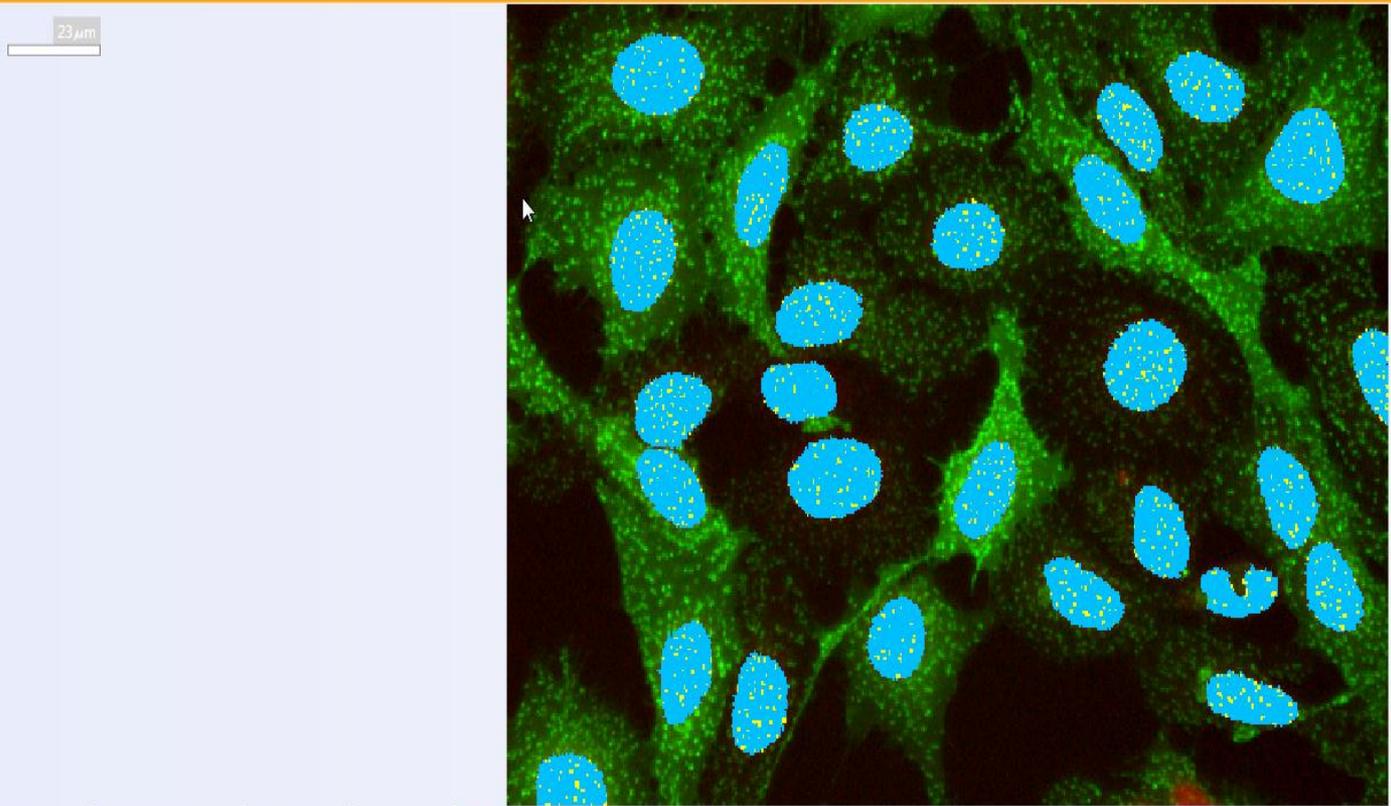
- Running a Custom Module
 - Select the Module you made from the same list as the Application Modules
 - Click on it in the Ribbon
 - Assign Images
 - Run Measurement

Home Acquisition Mode Mode: Multidimensional Measure Script Hardware Setup

Regions Measure Regions - Measure Threshold - Threshold Overlay - Convert to Regions - Measure - Measure Range - Measurement Set - Convert to Regions - Configure Results - Show Table - Show Summary - Add Line Graph - Add Scatter Plot - Export to Excel - Export to File

Granules in Nucleus 2 X
Granules in Nucleus 3 X

Dataset2



Single Image Time 0 of 0-0 | Z 0 of 0-0 | 7, 83 | 453 | 129



Filmstrip View Mode Dataset

Thumbnail images showing different views or stages of the dataset, including a full view of the granules and a zoomed-in view of a single nucleus.

This part of the presentation
will be handled in software

Process images based on shapes and sizes

Morphology Filters