

ImageXpress[®] Micro and MetaXpress[®] 5.3 Review Images and Analysis Guide

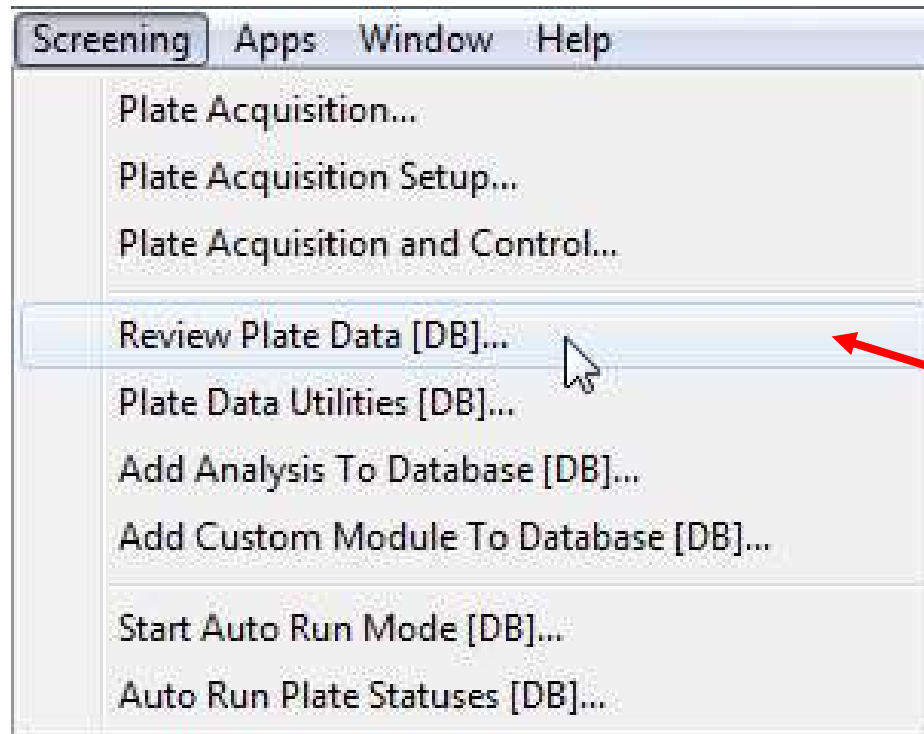


Revision A

MetaXpress® 5.3 Software

Review Images

Reviewing Images



**Click on Review
Plate Data under the
Screening menu**

Review Plate Data

Select Plate
for review

Review Plate Data -

Select Plate... Transflour Glass Plate-20x PA_AMSNVL-BNTXBS1_33

Wavelengths: Data view: Well arrangement Print Table

	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22
A															
B															
C											.	.			
D											.	.			
E											.	.			
F											.	.			
G											.	.			
H											.	.			
I											.	.			
J											.	.			
K											.	.			
L											.	.			
M											.	.			
N											.	.			

Montage: 2 x 12 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Show Values Image Overlay: Show cell segmentation Col: Cyan

Intensity Profile

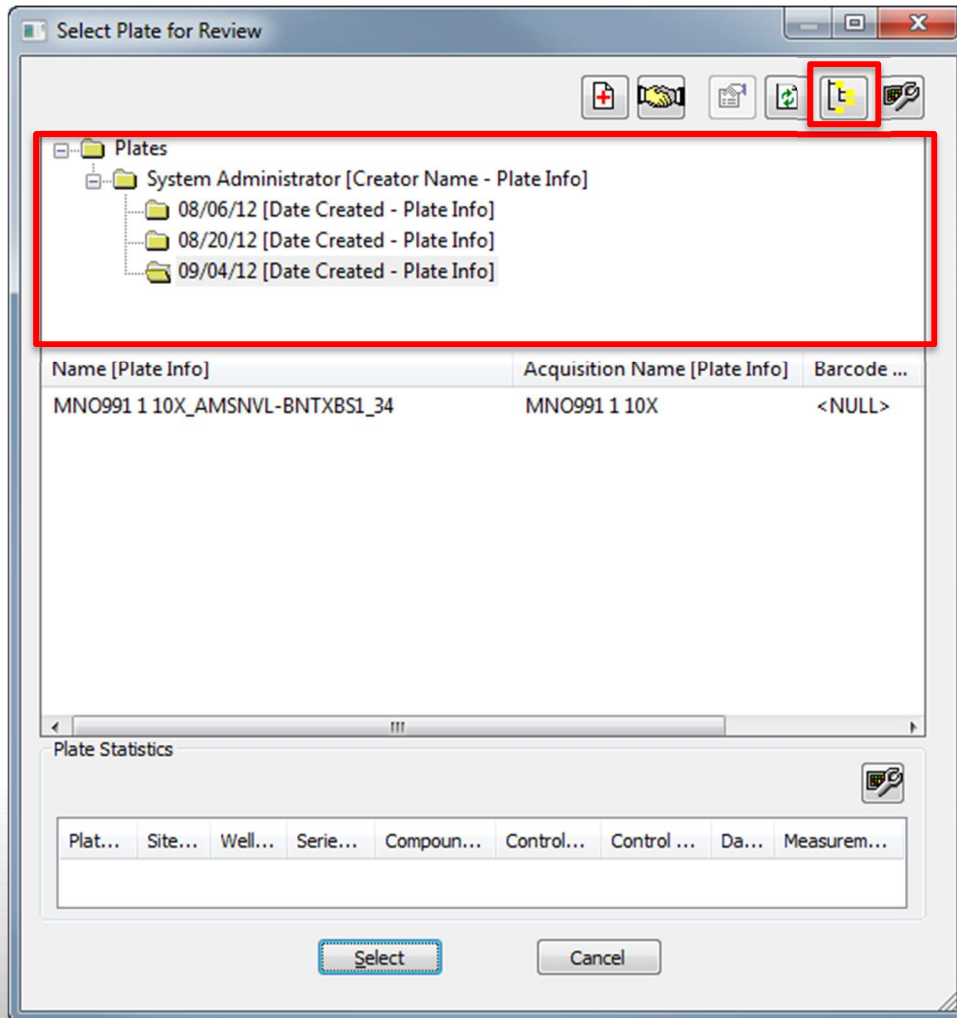
Color Composite Source R: <None> G: <None> B: <None>

Selections [In Green]

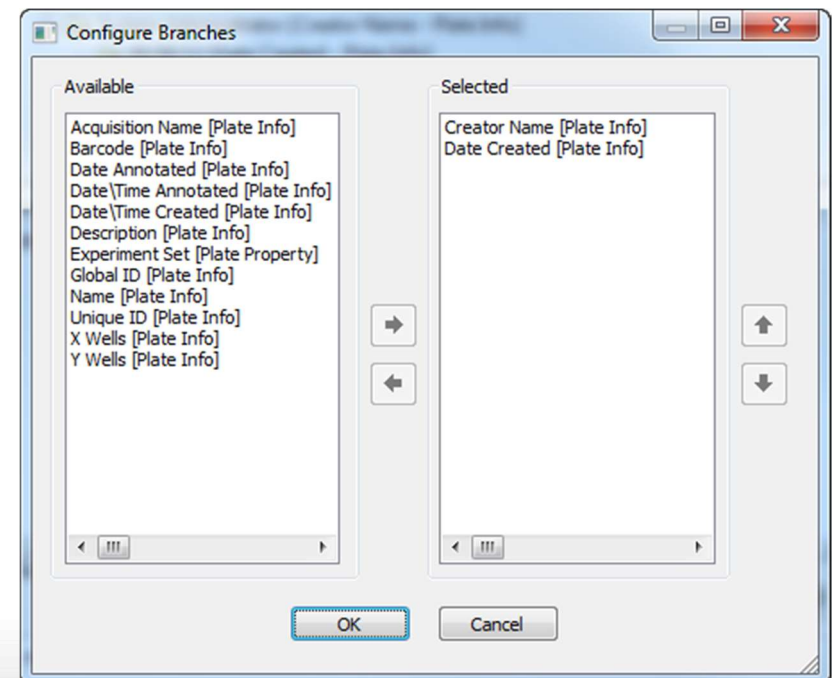
Load Images Clear

Reset Image Displays Cellular Results... Close

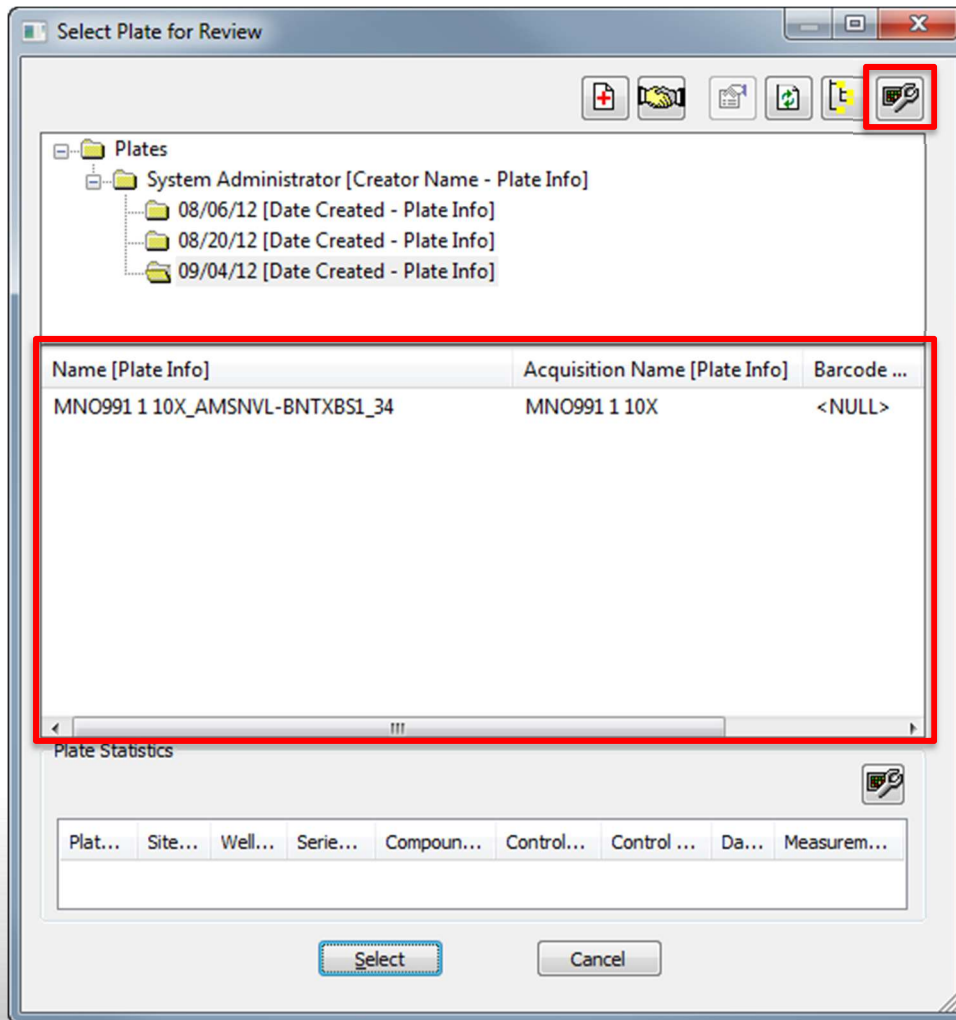
Select Plate for Review



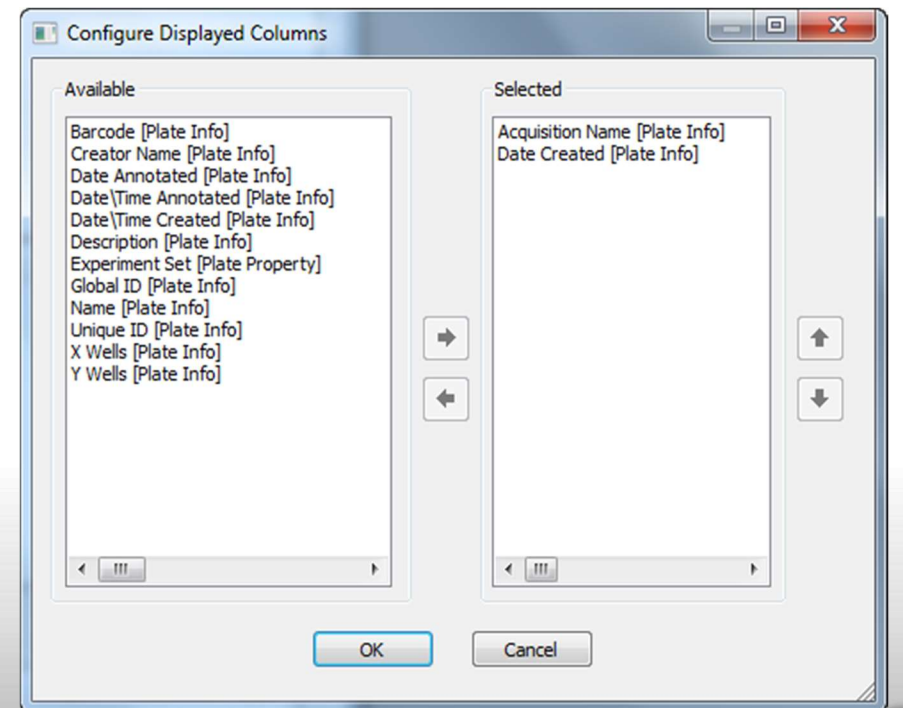
Configure Branches
allows you to determine
how plates are organized



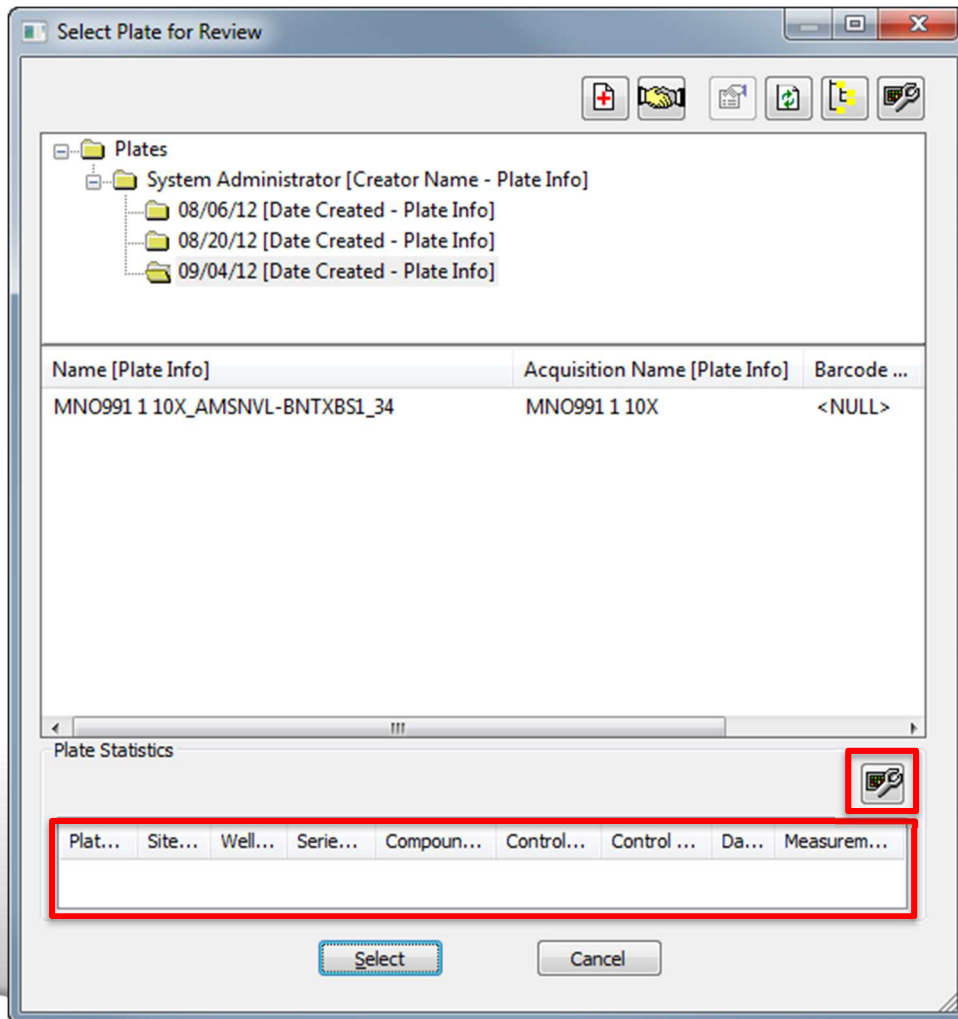
Select Plate for Review



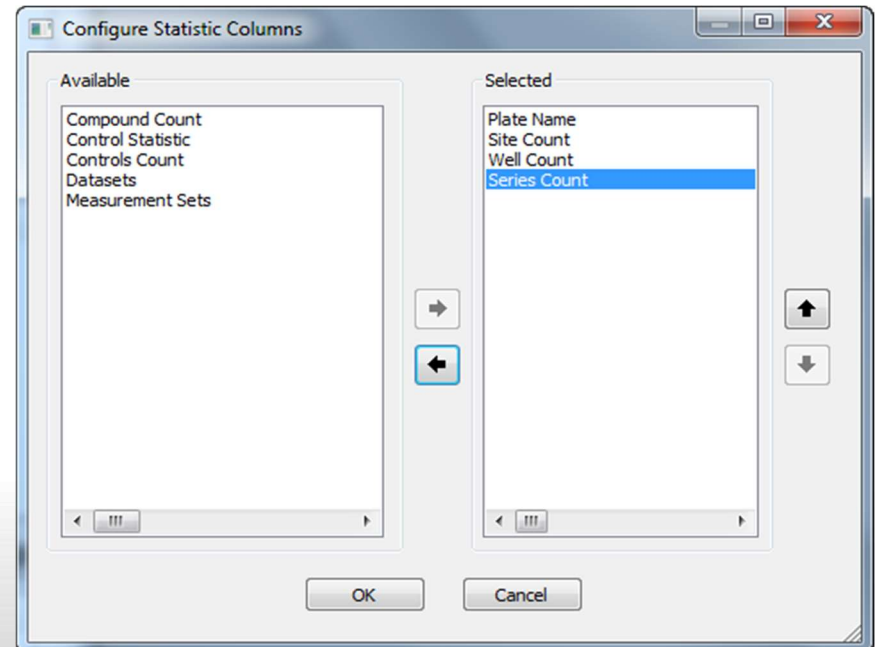
Configure Displayed Columns allows you to choose what information is displayed about the plates in the folders



Select Plate for Review



Configure Statistic Columns allows you to choose what information is displayed about the plate selected



Review Plate Data: Display Tab

Data table view

Wavelength selection

Configure thumbnail montage area

Color overlay option

The screenshot displays the 'Review Plate Data' software interface. The main window shows a data table with columns for well positions (01-24) and rows for plate positions (A-K). A dropdown menu is open over the table, showing options: 'Well arrangement', 'Time vs Well', and 'Measurement vs Well'. The 'Well arrangement' option is selected. To the left of the table, there are checkboxes for 'Wavelengths: DAPI' and 'FITC', both of which are checked. Below the table, there are controls for 'Montage: 3 x 3' and 'Time point: 1 of 1'. At the bottom of the main window, there are tabs for 'Display', 'Run Analysis', 'Measurements', and 'Graph'. Under the 'Display' tab, there are checkboxes for 'Show Values' (checked), 'Intensity Profile', and 'Color Composite'. There are also dropdown menus for 'Image Overlay: Show well information', 'Col: Cyan', 'Source R: <None>', 'G: FITC', and 'B: DAPI'. To the right of the main window, there are two thumbnail montage windows. The top one is titled '*HTS - FITC (100%)' and shows a 4x4 grid of green fluorescence images with numerical values overlaid. The bottom one is titled '*HTS - DAPI (100%)' and shows a 4x4 grid of red fluorescence images with the same numerical values overlaid. The numerical values in both montages are: 8680.69, 10877.14, 10148.65, 9552.04 for the top row; 8154.13, 9189.68, 9217.99, 9696.76 for the second row; 9381.02, 9437.31, 9820.45, 9436.88 for the bottom row.

Review Plate Data: Montage Display

**Configure
thumbnail montage
area**

OR

**Click and drag to
highlight a
selection of wells in
the plate grid**

The screenshot shows the 'Review Plate Data' software interface. The main window displays a plate grid with columns 01-24 and rows A-K. A red selection box highlights a vertical column of wells from row C to row I. Below the grid, the 'Montage' settings are set to '1 x 7' and 'Time point: 1 of 1'. Two thumbnail montage displays are shown on the right, each labeled '*HTS...' and 'A 16'. The left thumbnail shows a red fluorescence image, and the right thumbnail shows a green fluorescence image. The interface includes various controls such as 'Wavelengths', 'Data view', 'Print Table', 'Show Values', 'Intensity Profile', 'Color Composite', and 'Image Overlay'.

Review Plate Data: Site Display

Select a single site to view in the montage or click the *All Sites* button to view all sites at once

CountNuclei-20X-Bin2-4Sites_AMSNVL-C0H8KV1_14

Wavelengths: DAPI

Data view: Well arrangement Print Table

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	-	-																						
B	-	-																						
C	-	-																						
D	-	-																						
E	-	-																						
F	-	-																						
G	-	-																						
H	-	-																						
I	-	-																						
J	-	-																						
K	-	-																						

Sites All Sites

1	2
1	-
2	-

Montage: 1 x 6 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Show Values Intensity Profile Color Composite

Image Overlay: Show well information Col: Yellow

Source R: <None> G: <None> B: DAPI

Selections [In Green]

Load Images Clear

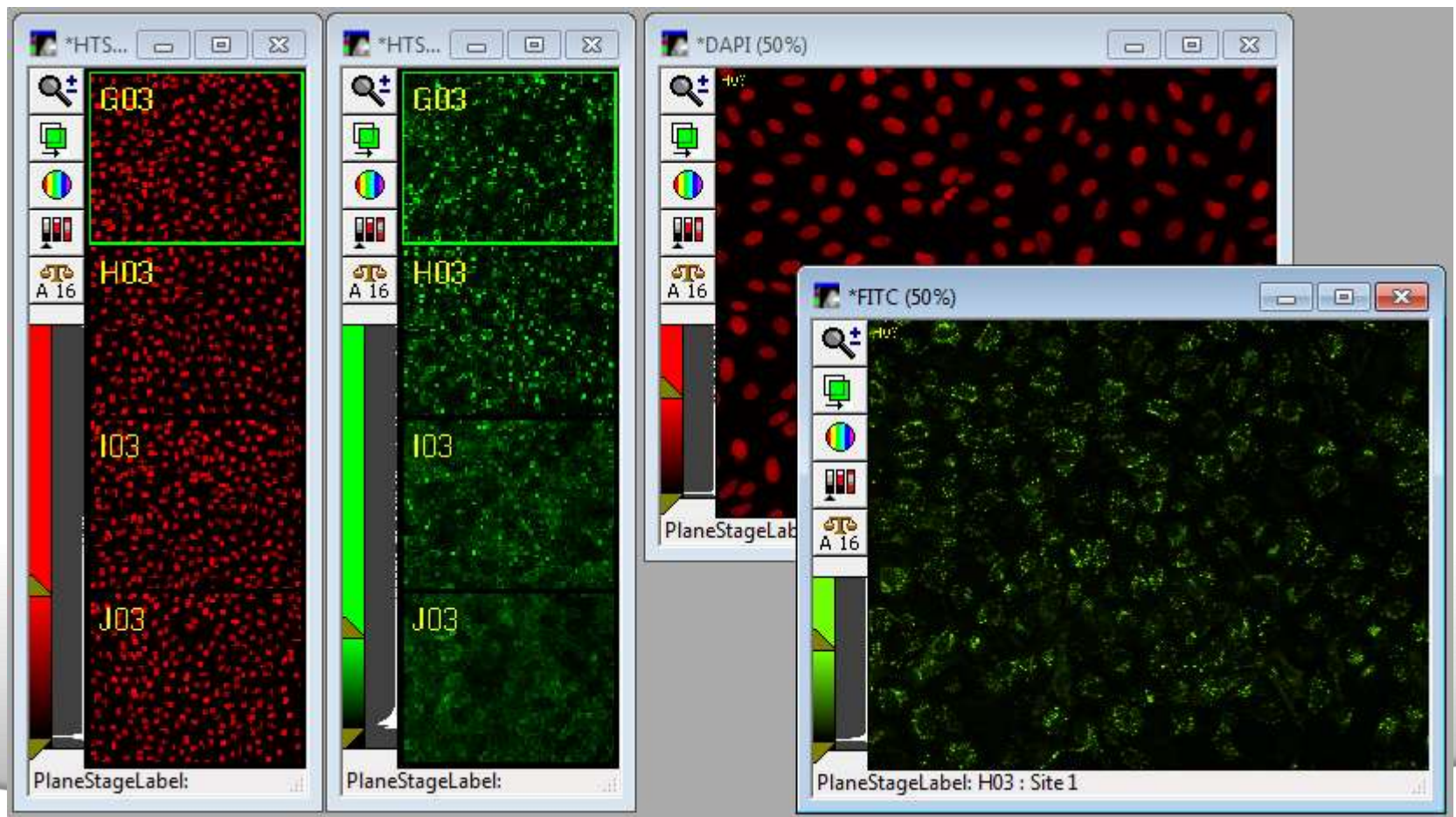
Reset Image Displays Cellular Results... Close

MetaXpress® 5.3 Software

Viewing and Adjusting Image Windows

High Resolution Images

Click on a well in the montage window to view high resolution images



Together through life sciences.

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 **Molecular
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Image Scaling: Adjusting contrast

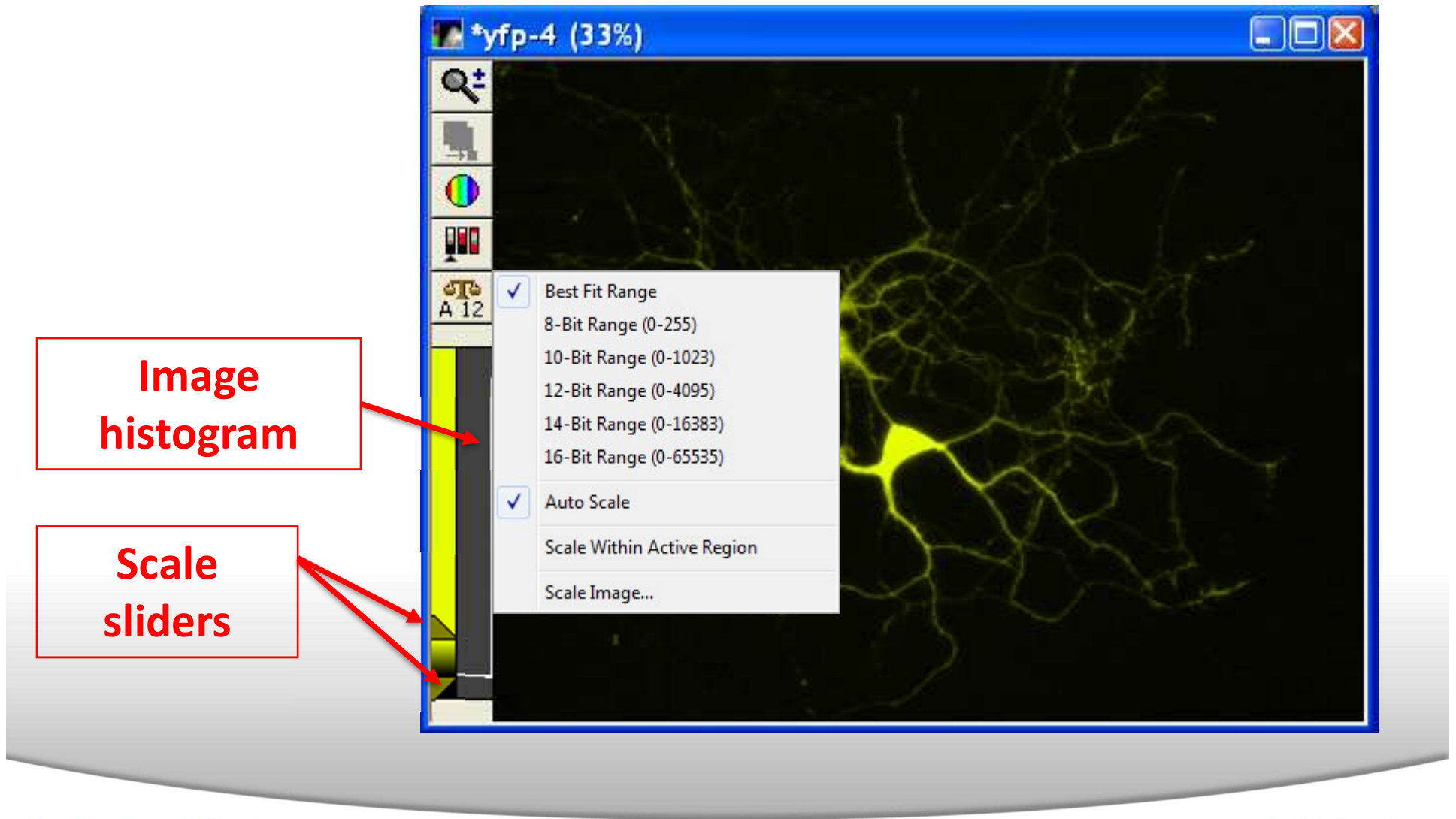


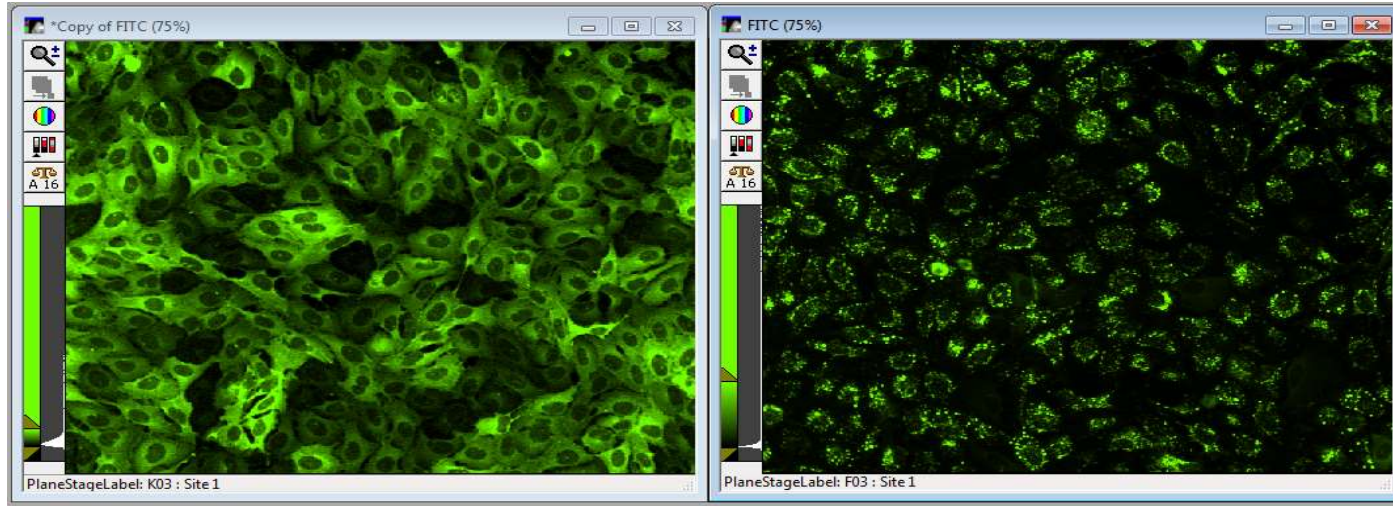
Image histogram

Scale sliders

Image Scaling: Auto Scale

Auto scale automatically adjusts the scaling based on the minimum and maximum intensities in each image

Auto Scale On



Auto Scale Off

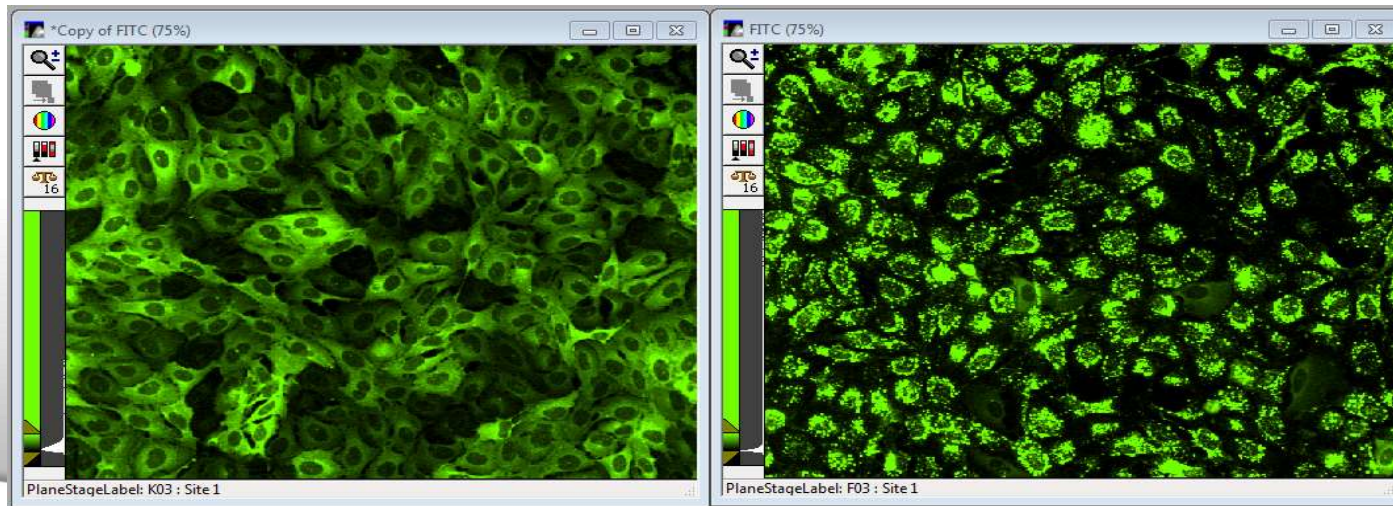


Image Scaling

In this example, pixels in the upper 1% of the intensity range will be assigned the maximum possible intensity (usually 65,535) and pixels in the lower 1% of the intensity range will be assigned the minimum possible value (0, or black)

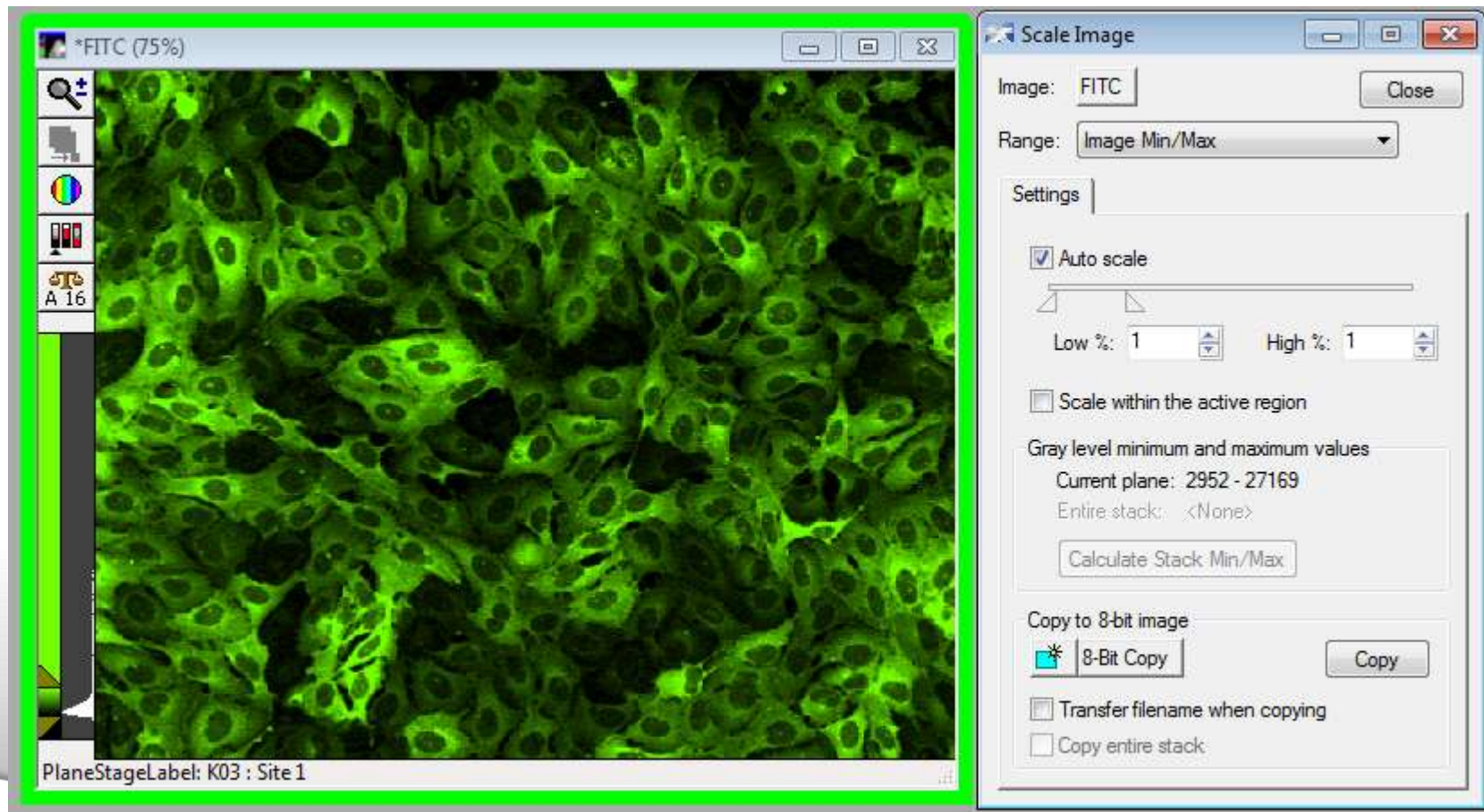
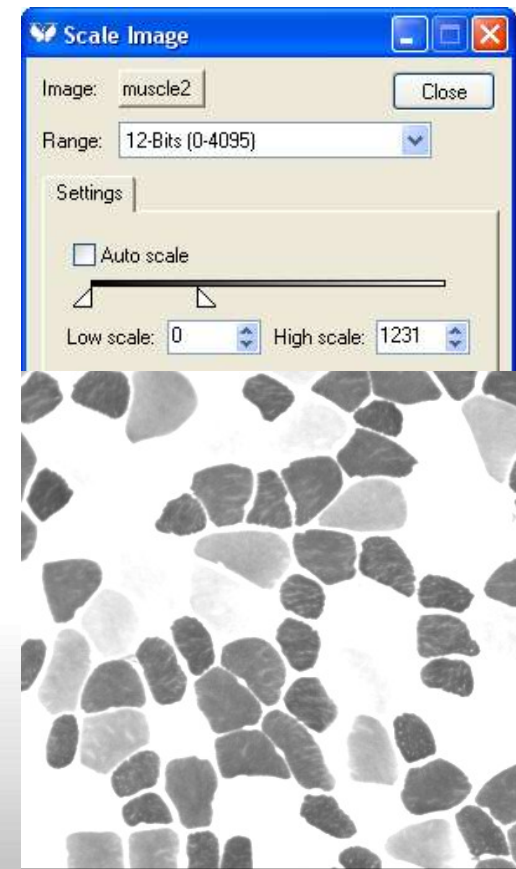
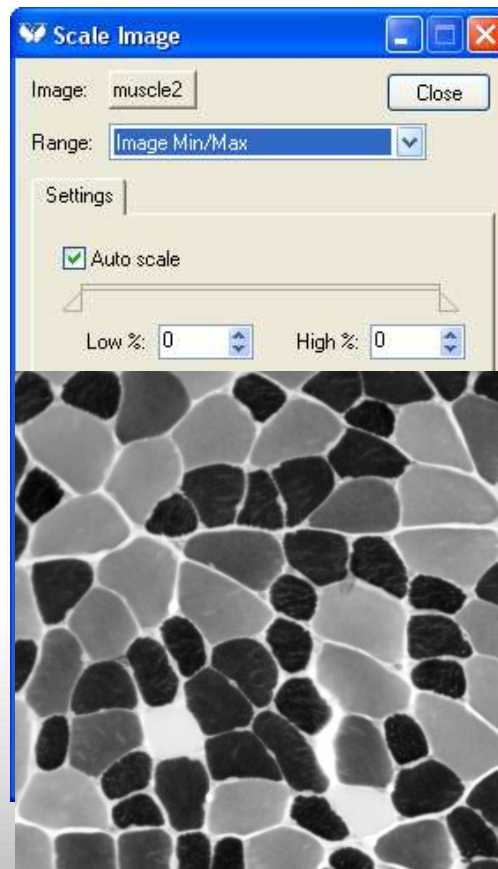
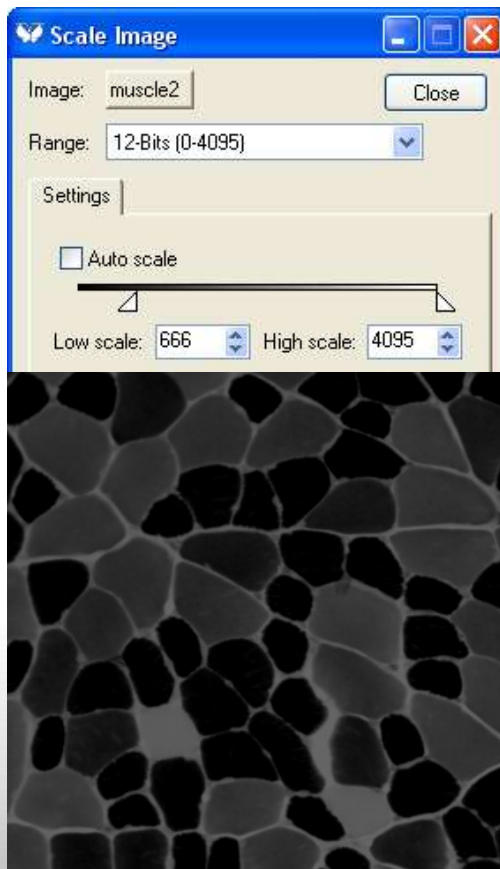


Image Scaling

Changing the scaling does not change the raw image data.

It only changes the image display.



Pseudocolor and Look Up Tables (LUT)

Maps intensity values in image to a color

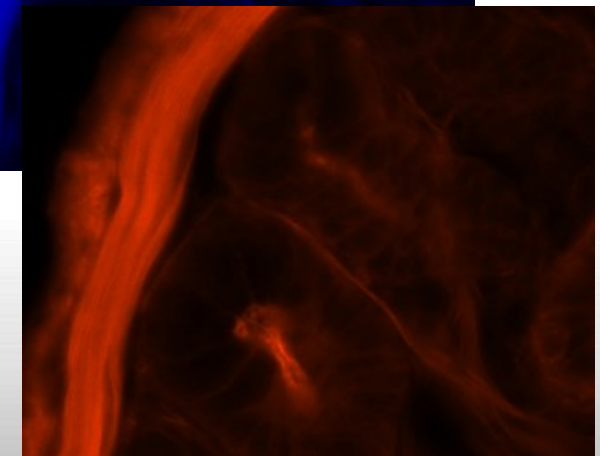
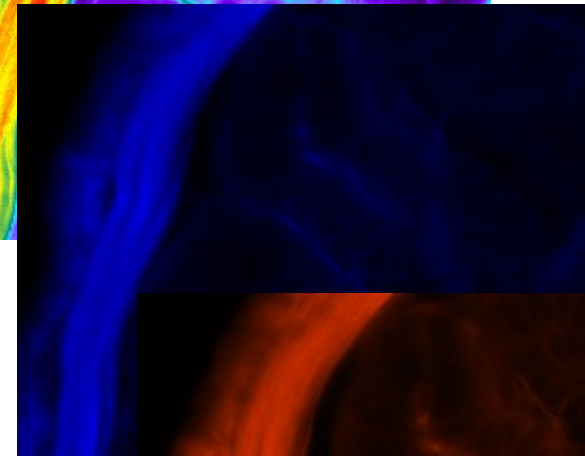
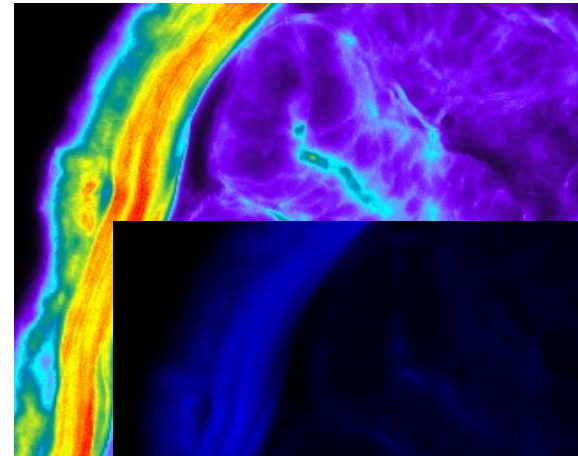

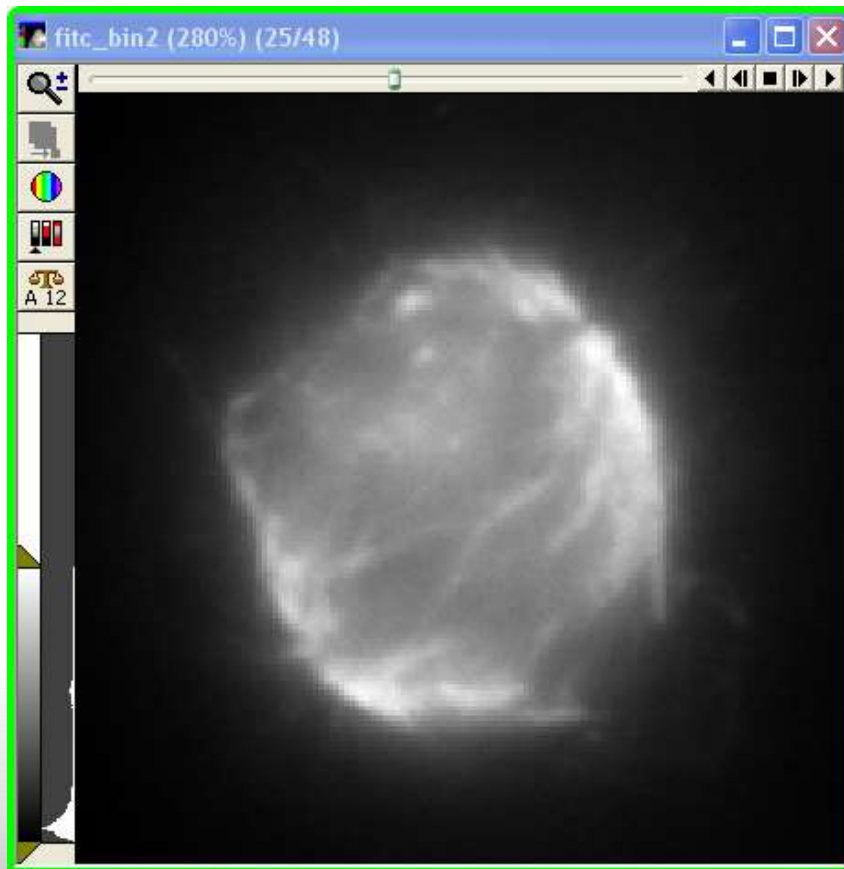


Image Information

Edit → Image Info or **Alt-I** or  icon



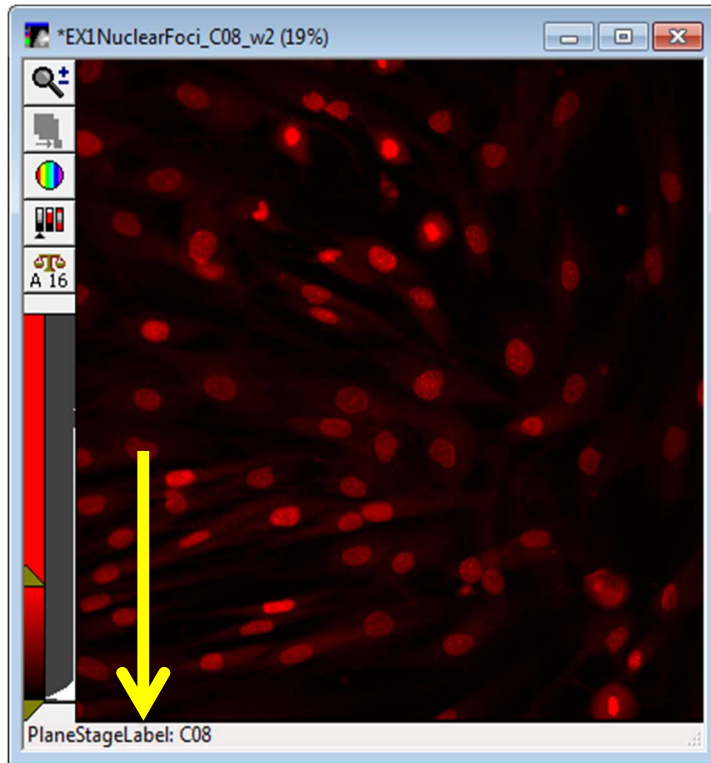
Property Name	Property Value
Location on Disk	C:\Presentation Resources\Images\fitc_bin2.st
File Type	Metamorph Stack File Format
Creation Timestamp	Tue Feb 19 15:48:46:821 2002
Last Saved Timestamp	Tue Aug 1 09:36:17:465 2006
Lookup Table Model	Monochrome
Storage Requirement(Megabytes)	2.06 MB
Image Width	150
Image Height	150
Image Depth (bits)	16
Image X Calibration (pixel/pixel)	2
Image Y Calibration (pixel/pixel)	2
Number of Planes	48
Plane Stage Label	
Plane Stage Position X	

Plane Number: 25 Show Annotation >>

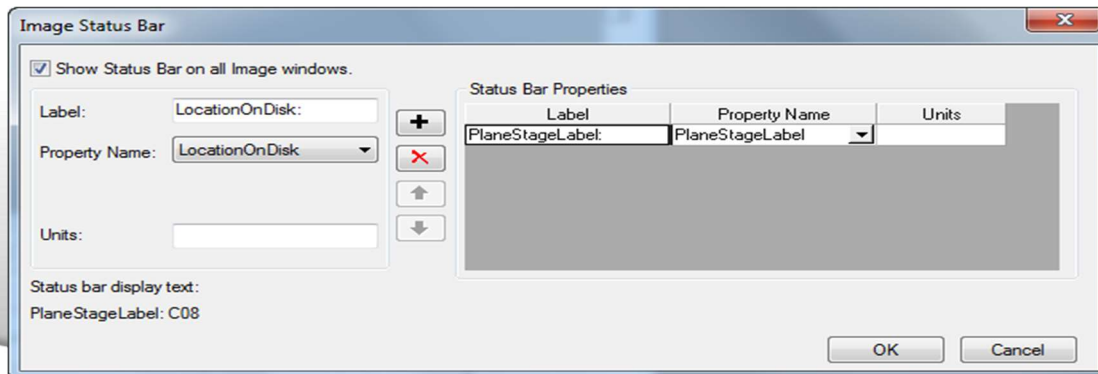
Open Log Configure Log... Image Status Bar... Print... Close

Data Log Not Open

Add Image Information to Bottom of Image Window



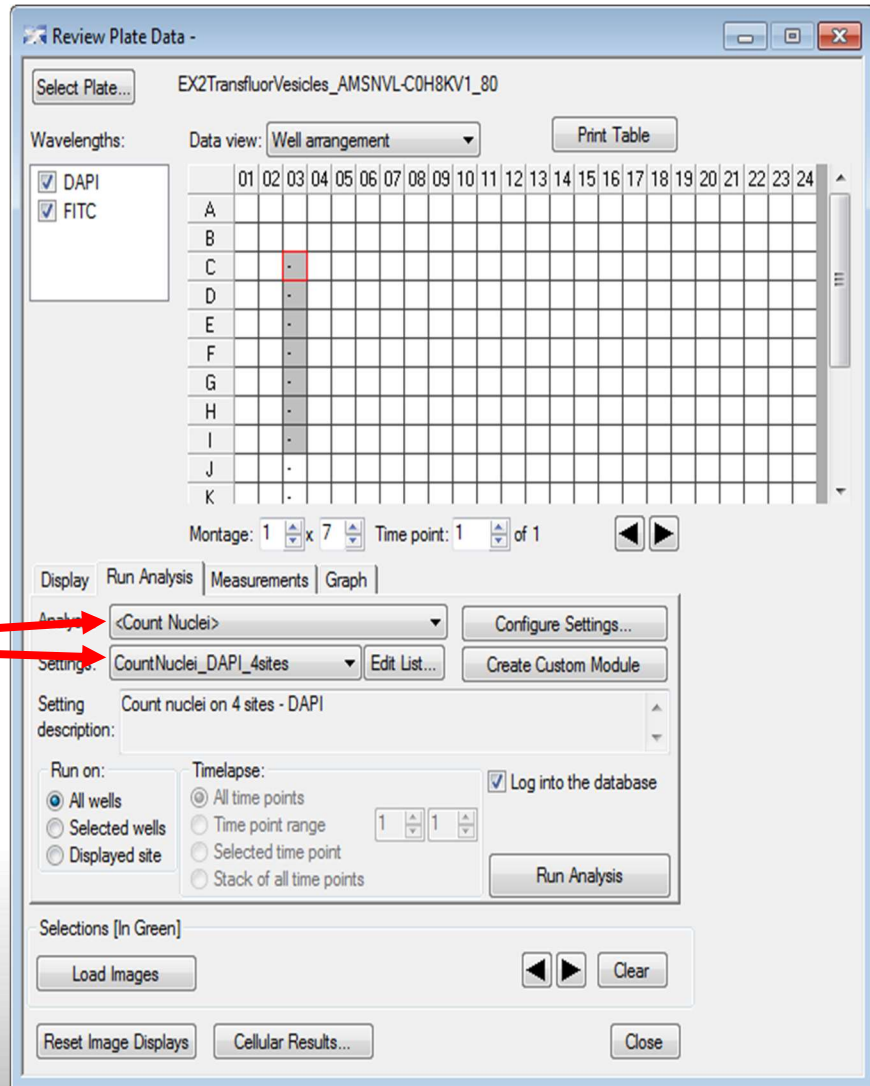
- Under the Edit menu, select **Image Status Bar**
OR
- On the Image Info screen, click on **Image Status Bar ...** button
- Choose the information you would like to see under the Property Name drop-down menu and click the + button



MetaXpress® 5.3 Software

Running Analysis

Review Plate Data: Run Analysis Tab



Select Analysis method
and Settings file to run
on plate

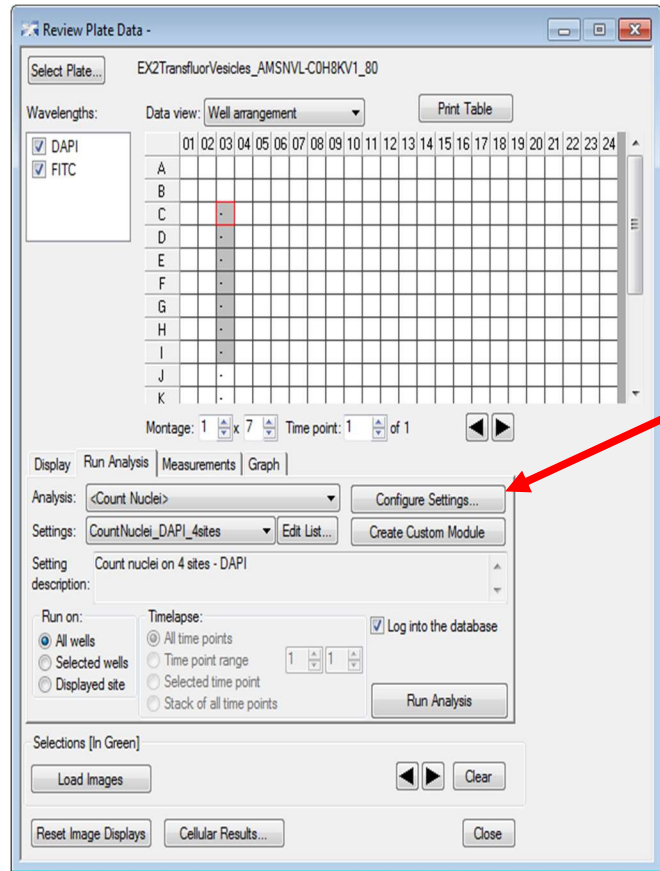
Review Plate Data: Run Analysis Tab

**Options missing "<>"
are Custom Modules or
Journals**

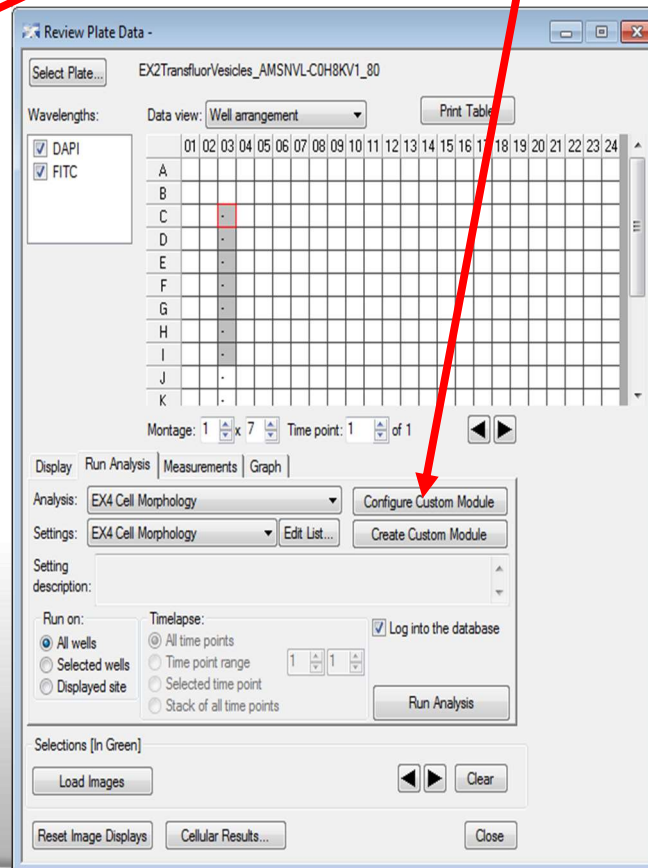
The screenshot shows the 'Review Plate Data' window with the following details:

- Window Title: Review Plate Data -
- Select Plate: EX2TransfluorVesicles_AMSNVL-C0H8KV1_80
- Wavelengths: DAPI, FITC
- Data view: Well arrangement
- Print Table button
- Plate Grid: 12 columns (01-12), 12 rows (A-K). A red box highlights the cell at row C, column 03.
- Montage: 1 x 7, Time point: 1 of 1
- Display: Run Analysis (selected), Measurements, Graph
- Analysis: <Count Nuclei>
- Settings: <Live Dead>, <Micronuclei>, <Mitotic Index>, <Monopole Detection>, <Multi Wavelength Cell Scoring>, <Multi Wavelength Translocation>, <Neurite Outgrowth>, <Nuclear Translocation HT>, <Transfluor HT>, <Transfluor>, <Translocation-Enhanced>, <Translocation>
- Run on: All wells (selected), Select, Display
- Log into the database: checked
- Run Analysis button
- Clear button
- Close button
- Module List (bottom): EX1 Nuclear Foci, EX2 Nuclear Foci, EX2 Transfluor Vesicles (highlighted by red arrow), EX3 Three CH Cell Scoring, EX4 Cell Morphology, EX4 Cell Morphology-WAppModule, EX5 Puncta on Neurons, EX6 Transmitted Light

Review Plate Data: Run Analysis Tab



**Configure
module settings**



Together through life sciences.

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Review Plate Data: Selecting Wells

Select a subset of wells by right-clicking on the well

OR

Shift/Ctrl + Left-Click and Drag to highlight a section of wells

The screenshot shows the 'Review Plate Data' window for a plate named 'EX2TransfluorVesicles_AMSNVL-C0H8KV1_74'. The 'Wavelengths' section has 'DAPI' and 'FITC' checked. The 'Data view' is set to 'Well arrangement'. A grid of wells (A-K, 01-24) is displayed, with a 4-well selection (rows D-F, column 03) highlighted in green. Below the grid, the 'Montage' is set to 1 x 4 and 'Time point' is 1 of 1. The 'Analysis' section is set to 'EX2 Nuclear Foci'. The 'Run on' section has 'All wells' selected. The 'Timelapse' section has 'All time points' selected. The 'Log into the database' checkbox is checked. The 'Run Analysis' button is visible. At the bottom, there are buttons for 'Load Images', 'Clear', 'Reset Image Displays', 'Cellular Results...', and 'Close'.

Review Plate Data: Run Analysis Tab

Run module on:

- **All Wells:** module is run on all wells acquired
- **Selected Wells:** module is run on selected wells (highlighted in green)
- **Displayed Site:** module is run on the displayed site

Review Plate Data - EX2TransfluorVesicles_AMSNVL-C0H8KV1_80

Select Plate... Wavelengths: Data view: Well arrangement Print Table

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								

Montage: 1 x 7 Time point: 1 of 1

Display Run Analysis Measurements Graph

Analysis: EX4 Cell Morphology Configure Custom Module

Settings: EX4 Cell Morphology Edit List... Create Custom Module

Setting description:

Run on:
 All wells
 Selected wells
 Displayed site

Timelapse:
 All time points
 Time point range 1 1
 Selected time point
 Stack of all time points

Log into the database

Run Analysis

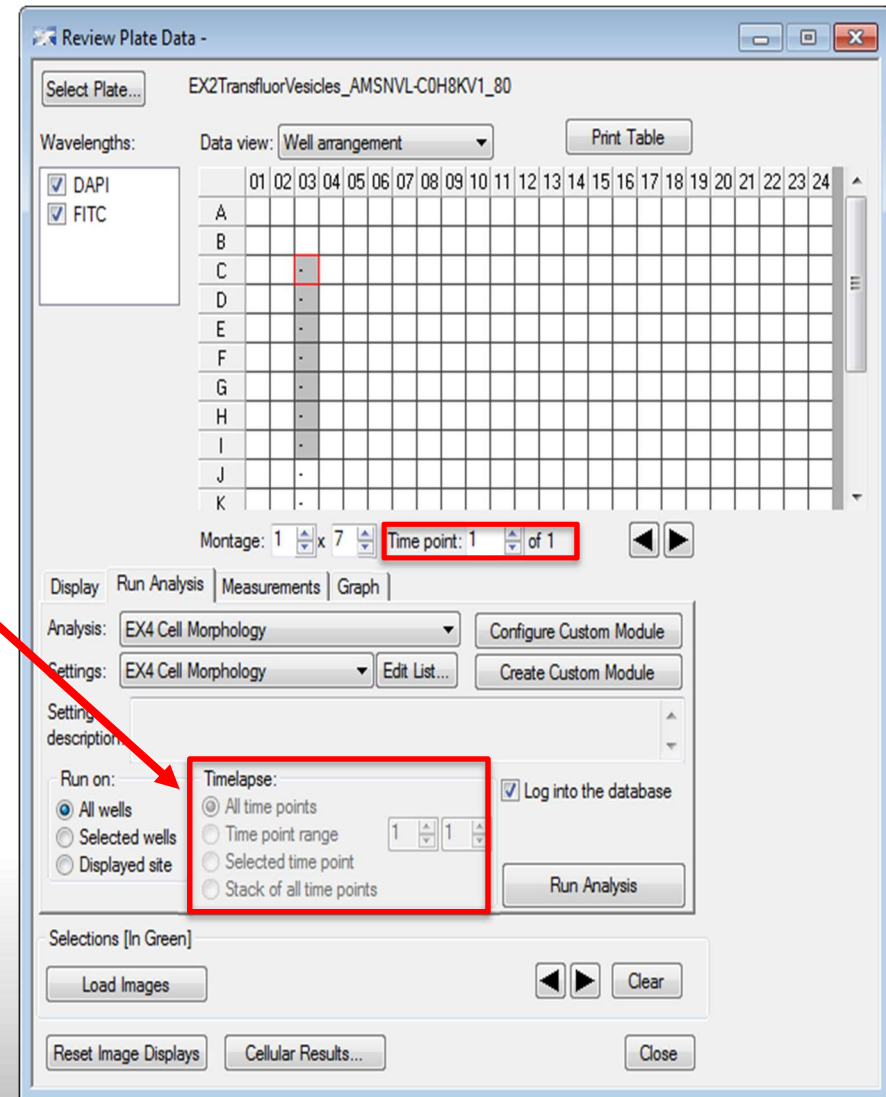
Selections [In Green]
Load Images Clear

Reset Image Displays Cellular Results... Close

Review Plate Data: Run Analysis Tab

Run module on:

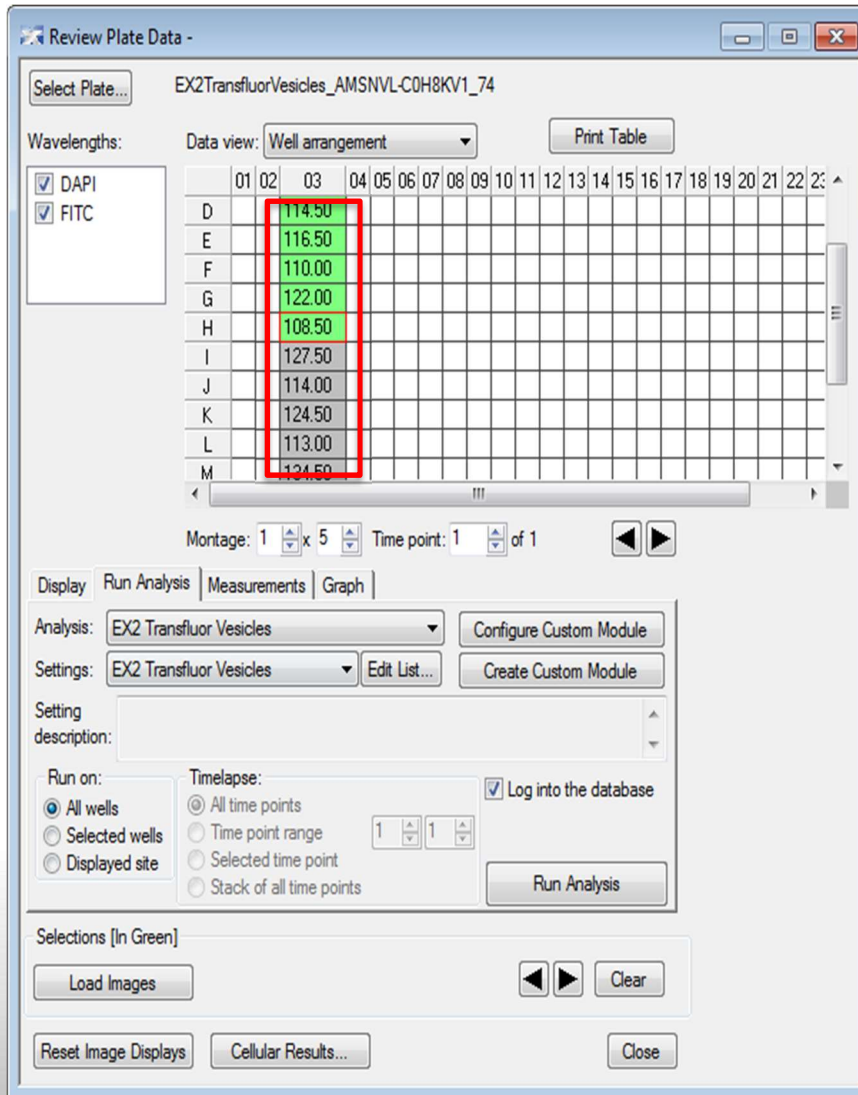
- **All time points:** module will be run on all time points acquired
- **Time point range:** module will run on the specified range of time points (time point range must be consecutive)
- **Selected time point:** module will run on one selected time point
- **Stack of time points:** select this option if running a legacy journal which analyzes the planes in a stack as separate time points



MetaXpress® 5.3 Software

Review Data

Review Plate Data: Plate Grid Colors



Green Wells: wells selected by user

Grey Wells: wells displayed in montage view

Red-Outline Well: well selected in montage view and displayed in high resolution

Review Plate Data: Measurements Tab

Review Plate Data -

Select Plate... EX2TransfluorVesicles_AMSNVL-C0H8KV1_80

Wavelengths: DAPI FITC

Data view: Well arrangement

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22
A																						
B																						
C		260.00																				
D		228.00																				
E		233.00																				
F		214.00																				
G		244.00																				
H		216.00																				
I		251.00																				
J		229.00																				

Montage: 1 x 1 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: EX2 Nuclear Foci: EX2 Nuclea

Measurement: Total # of Nuclei.Total (EX2 Ni)

Select Wells Based On Variable Range

Value is: Between 0 and 100

Data Log Not Open

Selections [In Green]

Select Analysis and Measurement from the drop-down menus to view in data table

Review Plate Data: Measurements Tab

Review Plate Data -

Select Plate... EX2TransfluorVesicles_AMSNVL-COH8KV1_80

Wavelengths: DAPI FITC

Data view: Well arrangement Print Table

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23
E			430.00																				
F			402.00																				
G			430.00																				
H			428.00																				
I			147.00																				
J			6.00																				
K			2.00																				
L			7.00																				
M			0.00																				
N			3.00																				

Montage: 1 x 1 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: EX2 Nuclear Foci: EX2 Nuclea Show Heat Map Heat Map...

Measurement: Total # of Foci.Total (EX2 Nuc) Display Format: ###

Select Wells Based On Variable Range

Value is: Between 0 and 100 Select

Data Log Not Open Configure Log... Open Log

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

View data with a heat map overlay

Review Plate Data: Graph Tab

The screenshot shows the 'Review Plate Data' window with the following data table:

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22
E			430.00																			
F			402.00																			
G			430.00																			
H			428.00																			
I			147.00																			
J			6.00																			
K			2.00																			
L			7.00																			
M			0.00																			
N			3.00																			

The 'Graph' tab is active, showing the following configuration:

- Analysis: EX2 Nuclear Foci: EX2 Nuclei
- Graph view: Plate
- Graph type: Scatter Plot (selected from a dropdown menu)
- Measurement: Total # of Nuclei.Total (E)
- Measurement2: Total # of Foci.Total (EX)

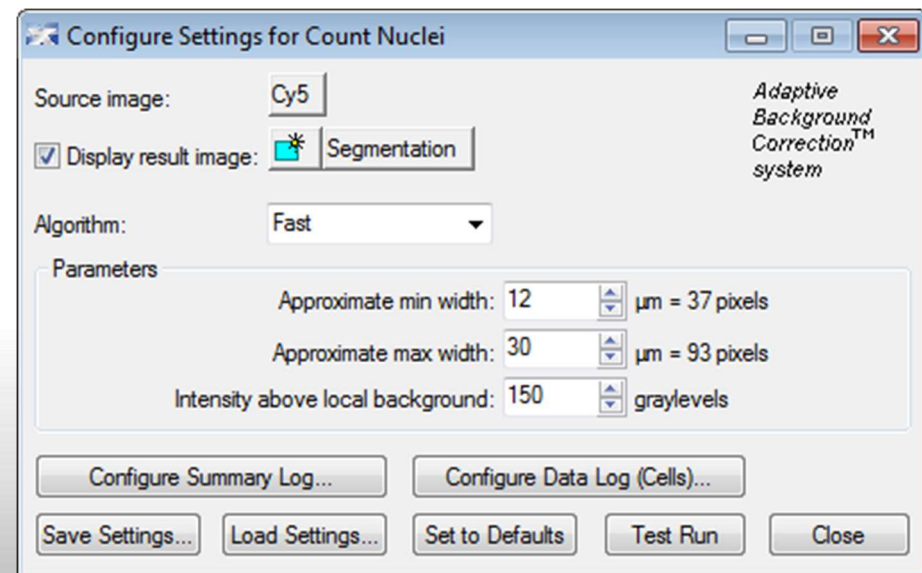
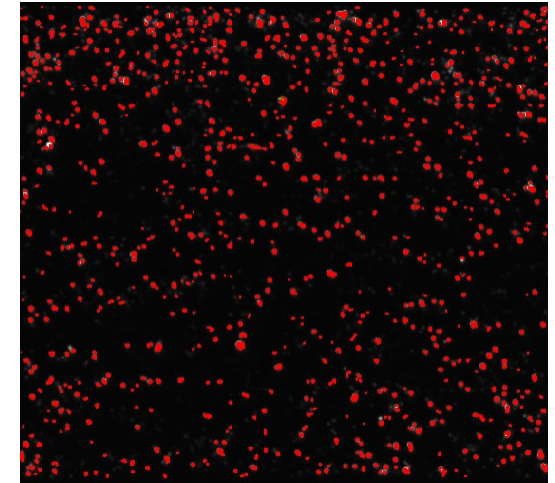
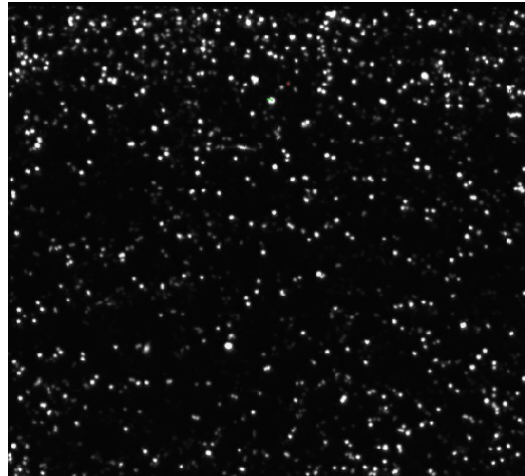
Select graph type and configure axes

MetaXpress® 5.3 Software

Setting up an Application Module

MetaXpress Application Modules

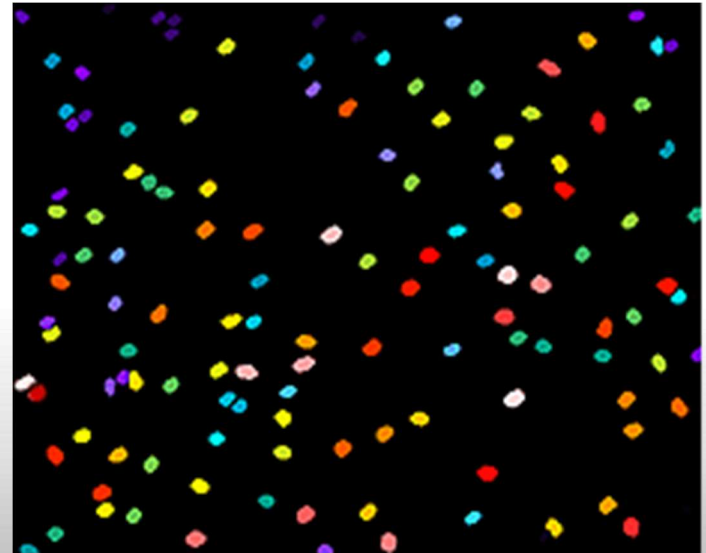
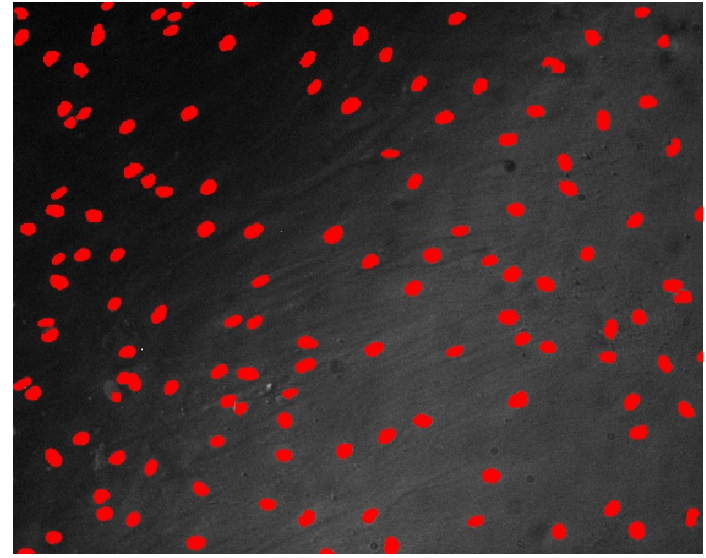
- **All** Application Modules share the same **basic controls**
- **Simple configuration**
 - Select wavelength
 - Set size range of objects
 - Set intensity above local background
 - Test and save settings
- The module will **automatically split** touching cells



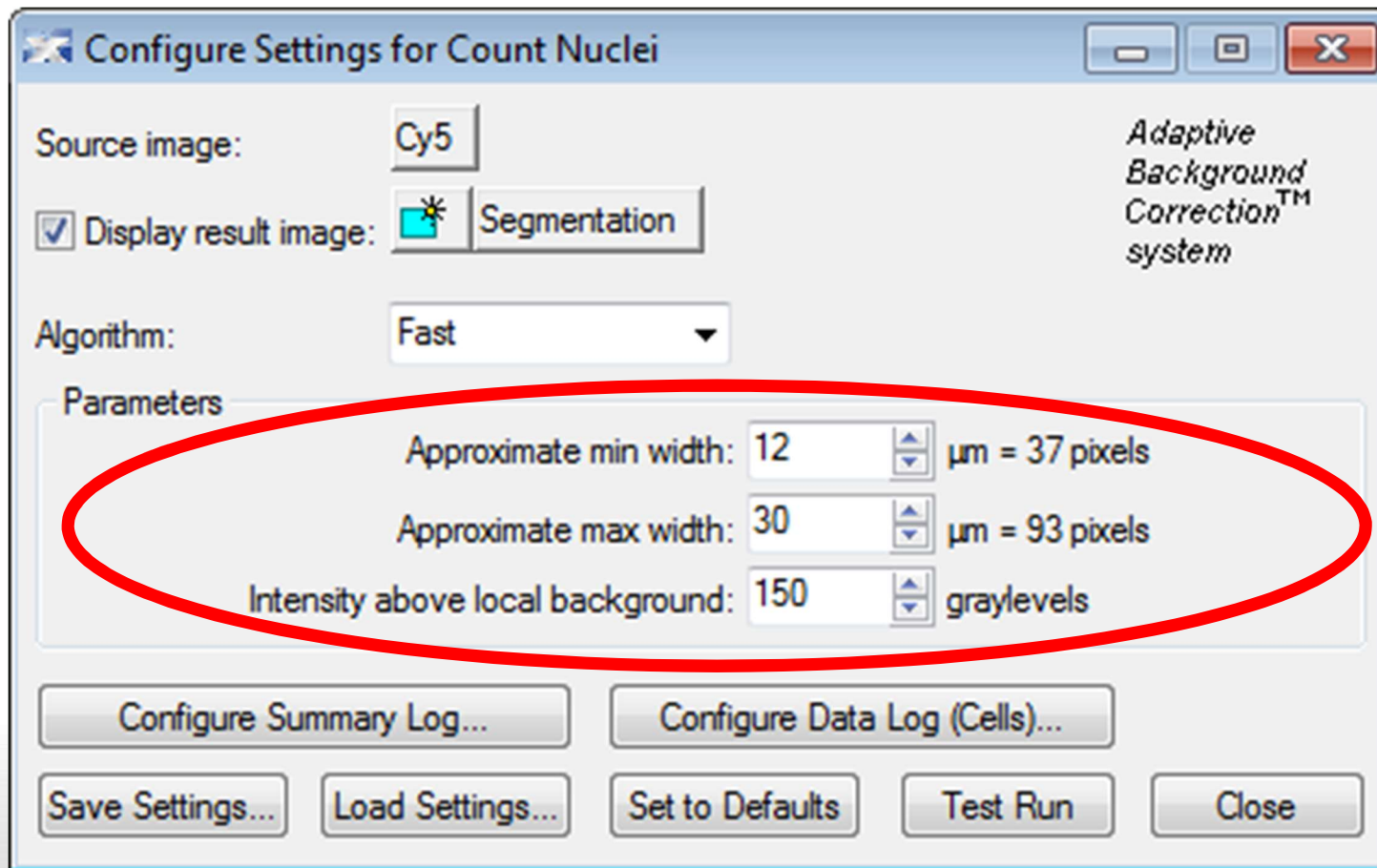
Adaptive Background Correction

Built in background management

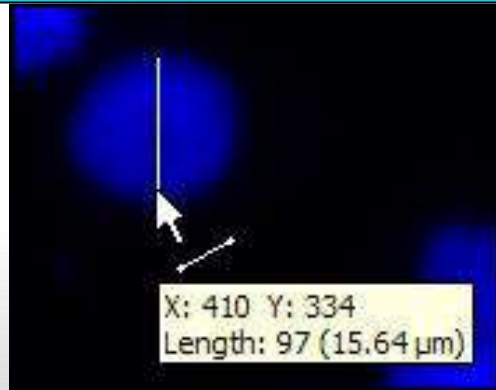
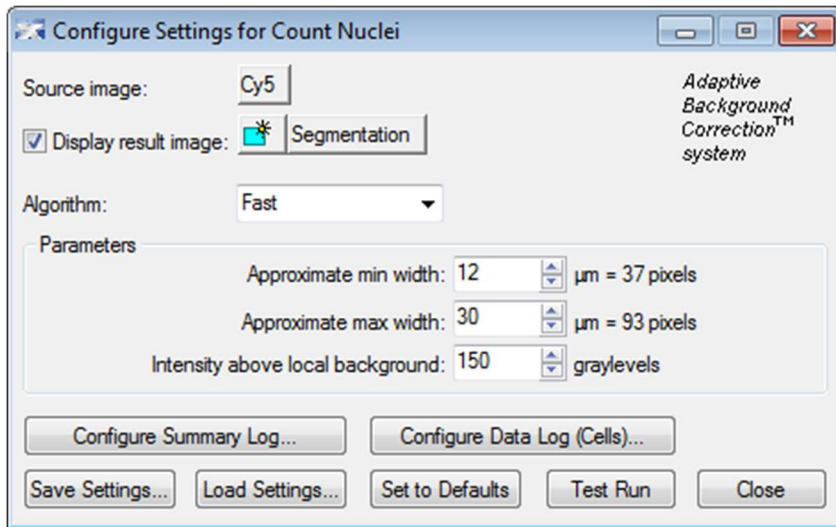
- ***Adaptive Background Correction*** is automatically performed by each application module
- **Detection even in noisy** and poorly stained images
- **Splits touching cells**
- **Consistent** performance across multiple plates



Configuring Settings – The Basics



Module Settings – Measurement Parameters

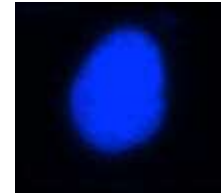


- **Set the Approximate min width and Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in μm)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

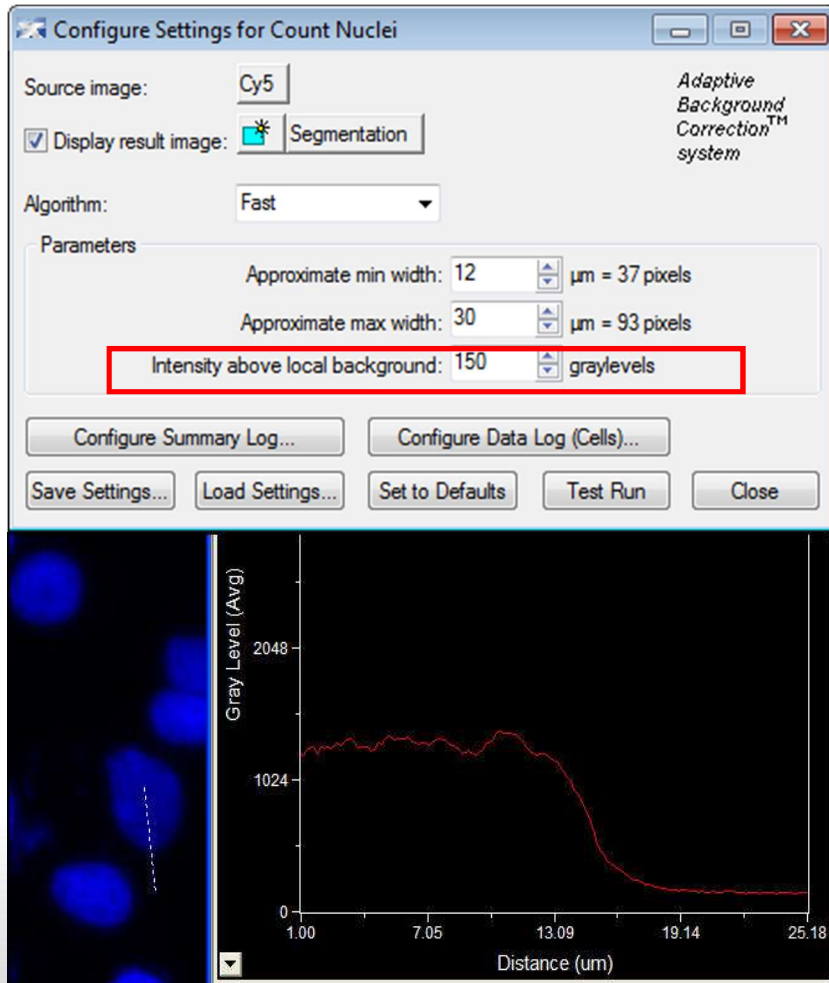
Module Settings – Measurement Parameters

Effects of varying width settings

- Min width too small: splits nuclei
- Min width too large: omits smaller nuclei
- Max width too small: may shrink nuclear boundaries
- Max width too large: may slightly enlarge nuclear boundaries



Module Settings – Measurement Parameters



The **intensity above local background** is used for finding the nuclei

- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background
- Draw a line across a cell into the background and use **Measure > Linescan** to determine intensity values; or simply mouse over the cell and the background and view the intensity values

Test Run Settings With an Interactive Preview

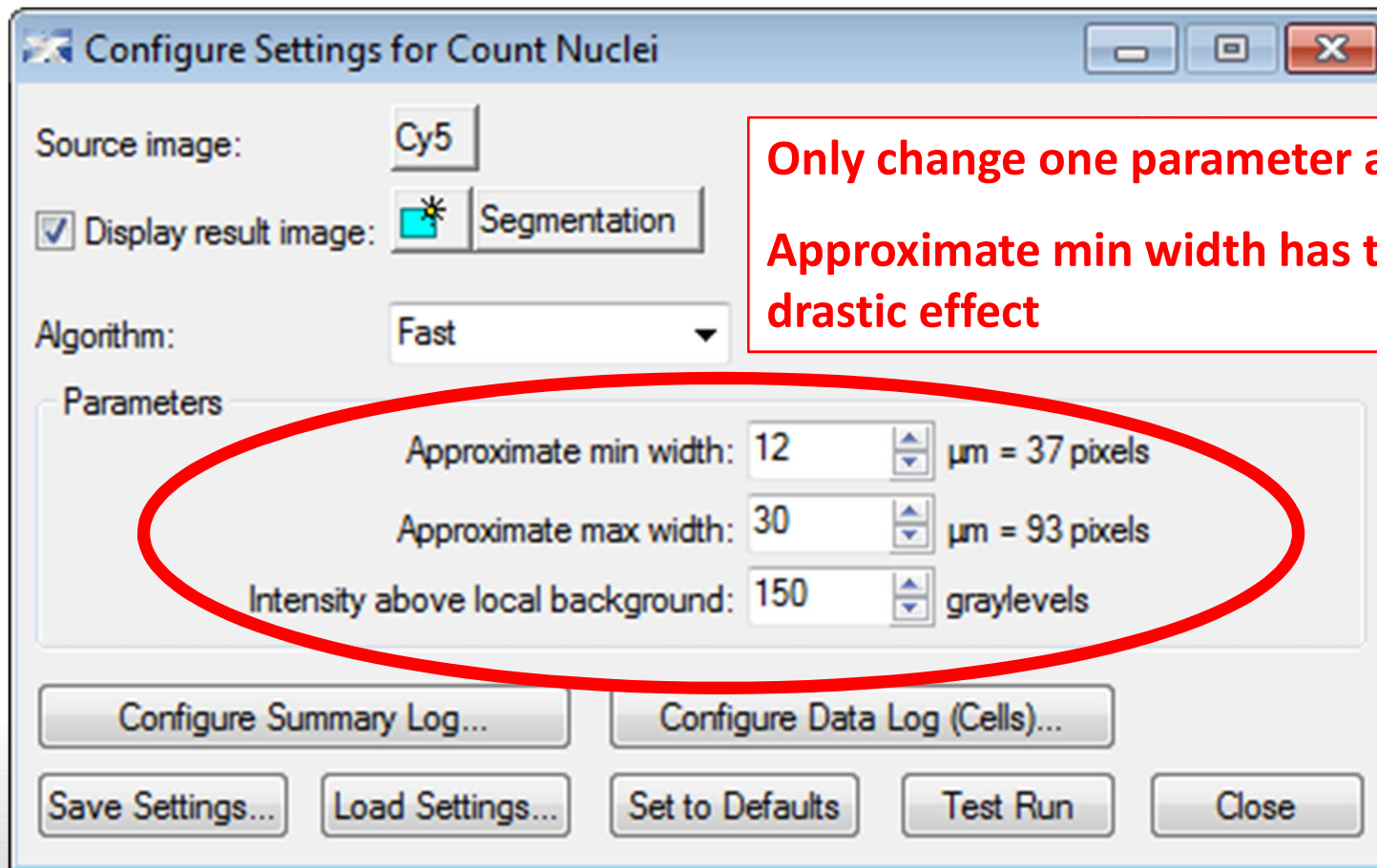
Useful for setting cutoffs for Positive / Negative cells (e.g. Cell Scoring, Translocation)

Cell-by-cell analysis results linked to image

The screenshot shows the MetaXpress software interface. The main window displays a fluorescence image of cells with green and orange spots. A red arrow points from the text 'Cell-by-cell analysis results linked to image' to the image. To the right, the 'Configure Settings for Transfluo - Transfluo Example' panel is visible, showing settings for Pits and Vesicles, and Nuclear stain. Below the image is a table of cellular results.

Cell Assigned Label #	Cell Pit Count	Cell Pit Total Area	Cell Pit Integrated Intensity	Cell Pit Average Intensity	Cell Vesicle Count	Cell Vesicle Total Area	Cell Vesicle Integrated Intensity	Cell Vesicle Average Intensity	Cell Nuclear Total Area	Cell Nuclear Integrated Intensity	Cell Nuclear Average Intensity	Cell Texture Index	Cell Gradient Index	Cell Laplacian Index
50	7	13.6248	334114	2550.49	0	0	0	0	241.711	4.59102e+006	1975.48	293.099	261.2	124.791
51	22	46.1788	1.17485e+006	2646.06	0	0	0	0	229.438	4.4639e+006	2023.53	454.328	397.24	208.377
52	25	45.8668	1.22161e+006	2770.09	2	20.0732	660387	3421.69	366.518	8.58825e+006	2437.07	532.658	408.072	187.149
53	17	32.659	921829	2935.76	1	24.6495	922779	3893.58	226.59	4.63456e+006	2136.73	667.025	502.959	245.091
54	31	59.1796	1.55976e+006	2741.22	1	9.56858	304833	3313.4	331.26	7.08414e+006	2224.22	512.776	406.944	207.207
55	19	35.3621	935863	2752.54	0	0	0	0	231.31	4.51086e+006	2028.26	464.98	426.992	207.646
56	29	91.6295	2.6668e+006	3027.02	0	0	0	0	292.778	7.18508e+006	2552.43	623.956	452.657	241.169
57	21	39.7304	1.06987e+006	2800.7	2	26.0016	919048	3676.19	213.629	5.24932e+006	2555.66	731.004	611.568	308.254
58	28	60.2196	1.60986e+006	2780.42	0	0	0	0	295.482	5.84355e+006	2056.86	434.692	349.411	161.253
59	11	21.3213	621592	3032.16	1	20.2812	766261	3929.54	232.87	5.43761e+006	2428.59	689.804	421.955	207.684
60	20	47.4269	1.22523e+006	2696.91	0	0	0	0	284.249	5.41963e+006	1993.03	391.567	306.188	156.876
61	4	8.73653	245367	2921.04	1	8.42451	219137	2705.4	57.3074	1.12874e+006	2048.52	644.314	444.689	207.335
62	28	55.8514	1.47684e+006	2750.17	0	0	0	0	234.014	5.07012e+006	2253.39	452.939	330.231	160.36
63	29	60.6356	1.49768e+006	2568.91	0	0	0	0	347.693	7.25064e+006	2168.9	470.77	407.006	204.388
64	20	35.2581	946261	2797.23	1	11.3367	347980	3192.48	201.668	4.57022e+006	2357	562.464	416.778	214.865
65	38	71.9723	2.12067e+006	3064.55	1	8.52851	303603	3702.48	217.581	4.68951e+006	2241.64	733.26	645.833	342.629
66	23	52.8352	1.51808e+006	2988.34	1	11.9607	434406	3777.44	154.345	3.58856e+006	2418.17	595.357	524.042	274.486
67	22	43.9446	1.16645e+006	2757.56	2	18.9291	658068	3615.76	217.893	4.50757e+006	2151.58	651.938	496.96	262.275
68	15	27.6657	719458	2704.73	0	0	0	0	343.013	7.84852e+006	2379.78	394.932	304.985	150.382

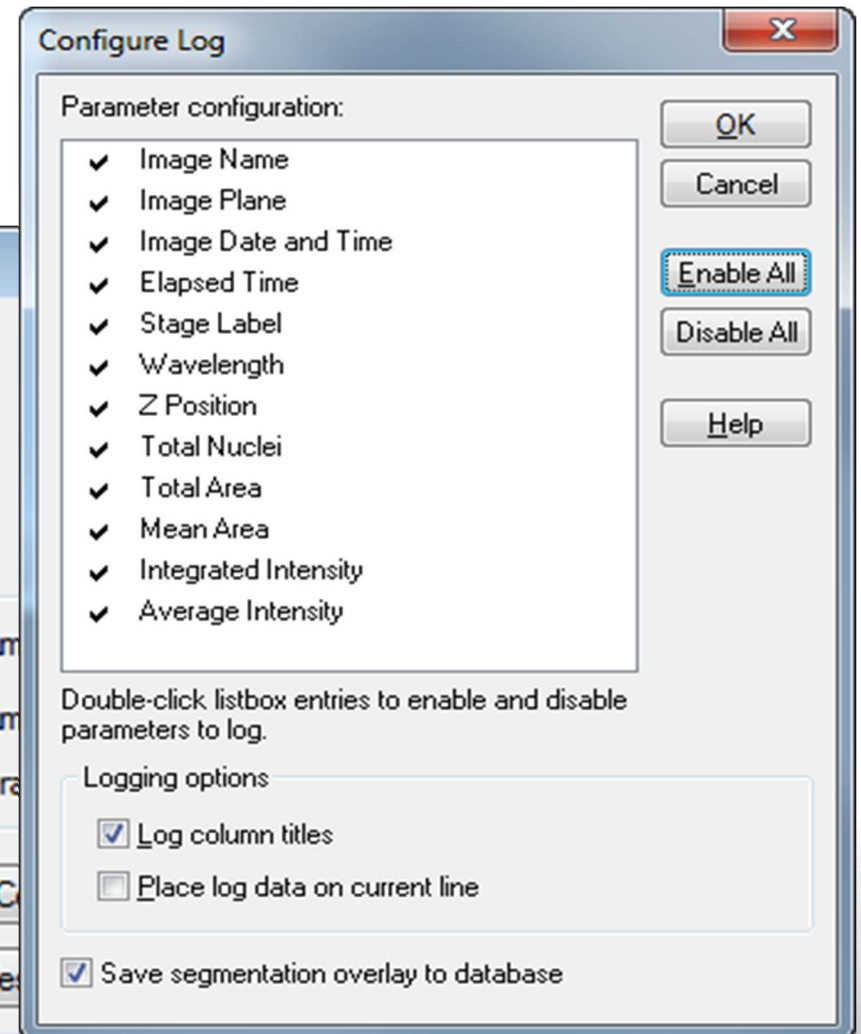
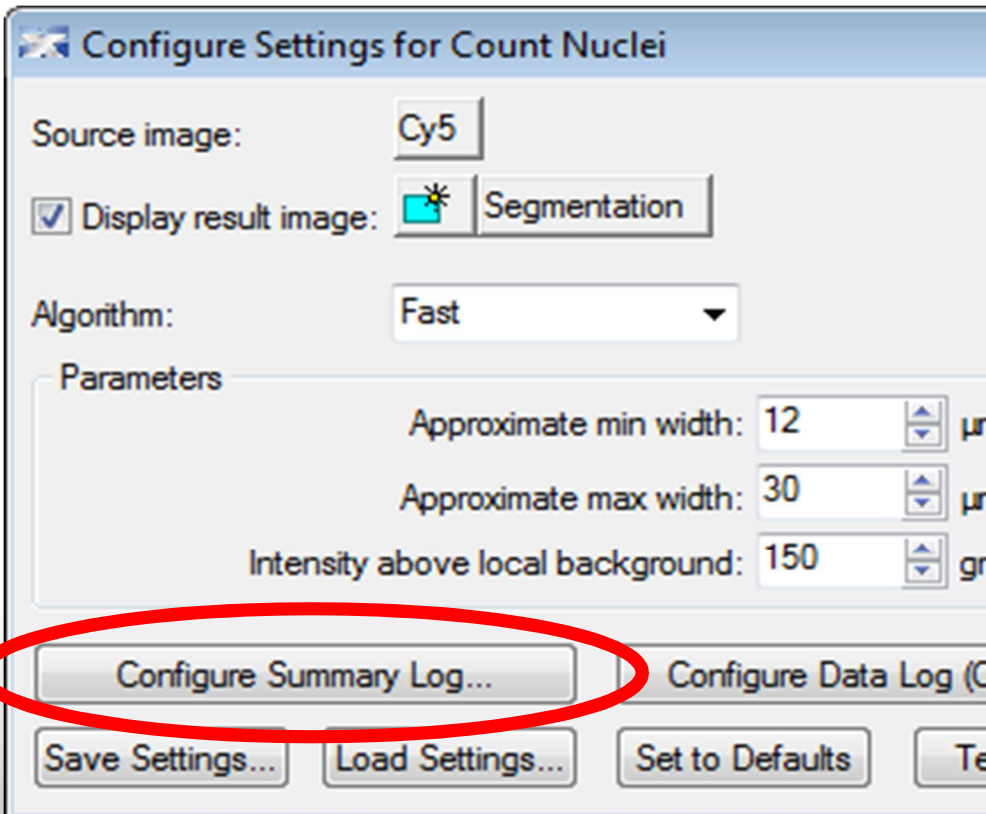
Optimizing Settings



Selecting Measurements

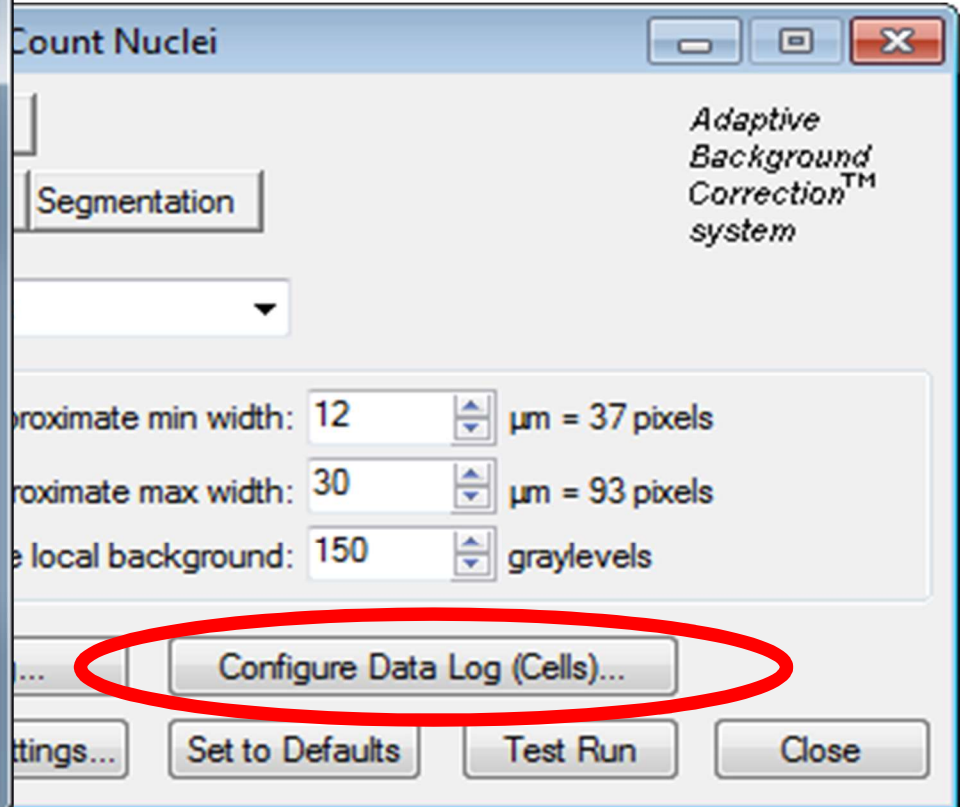
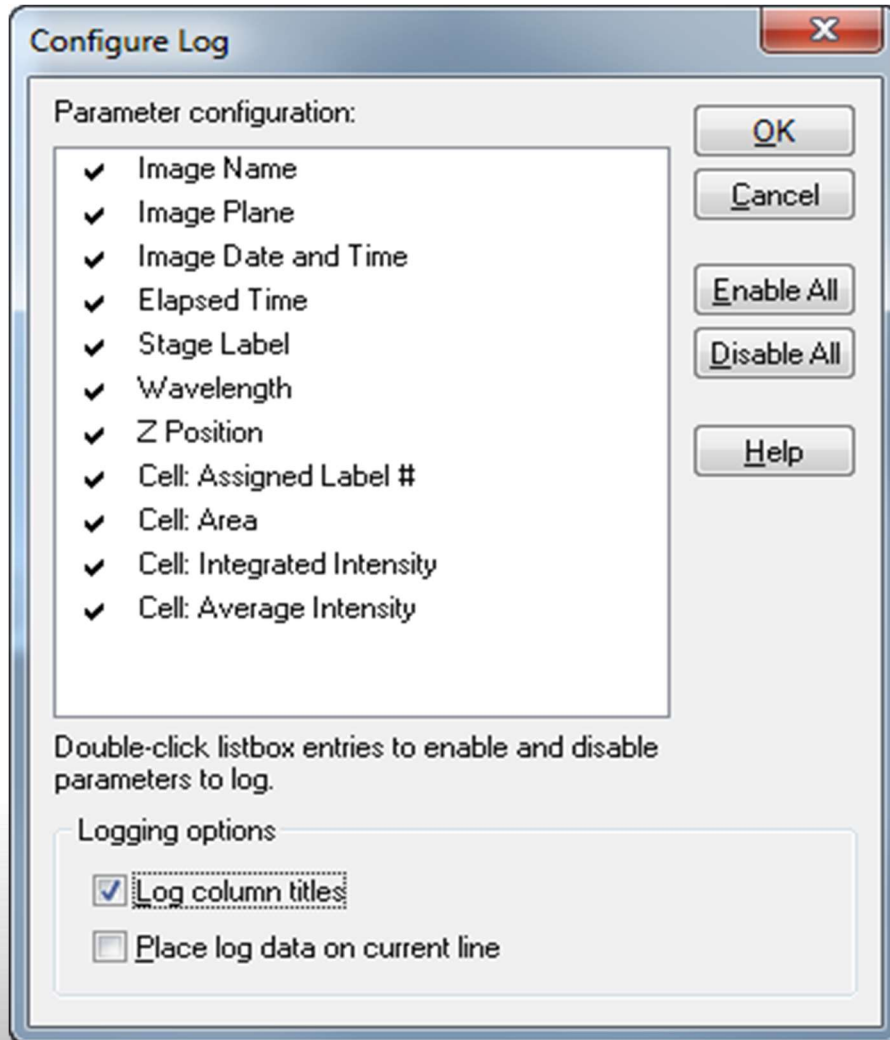
Summary log = Site by Site (well) data

Total cells, percentage positive, average area, integrated intensity, etc.

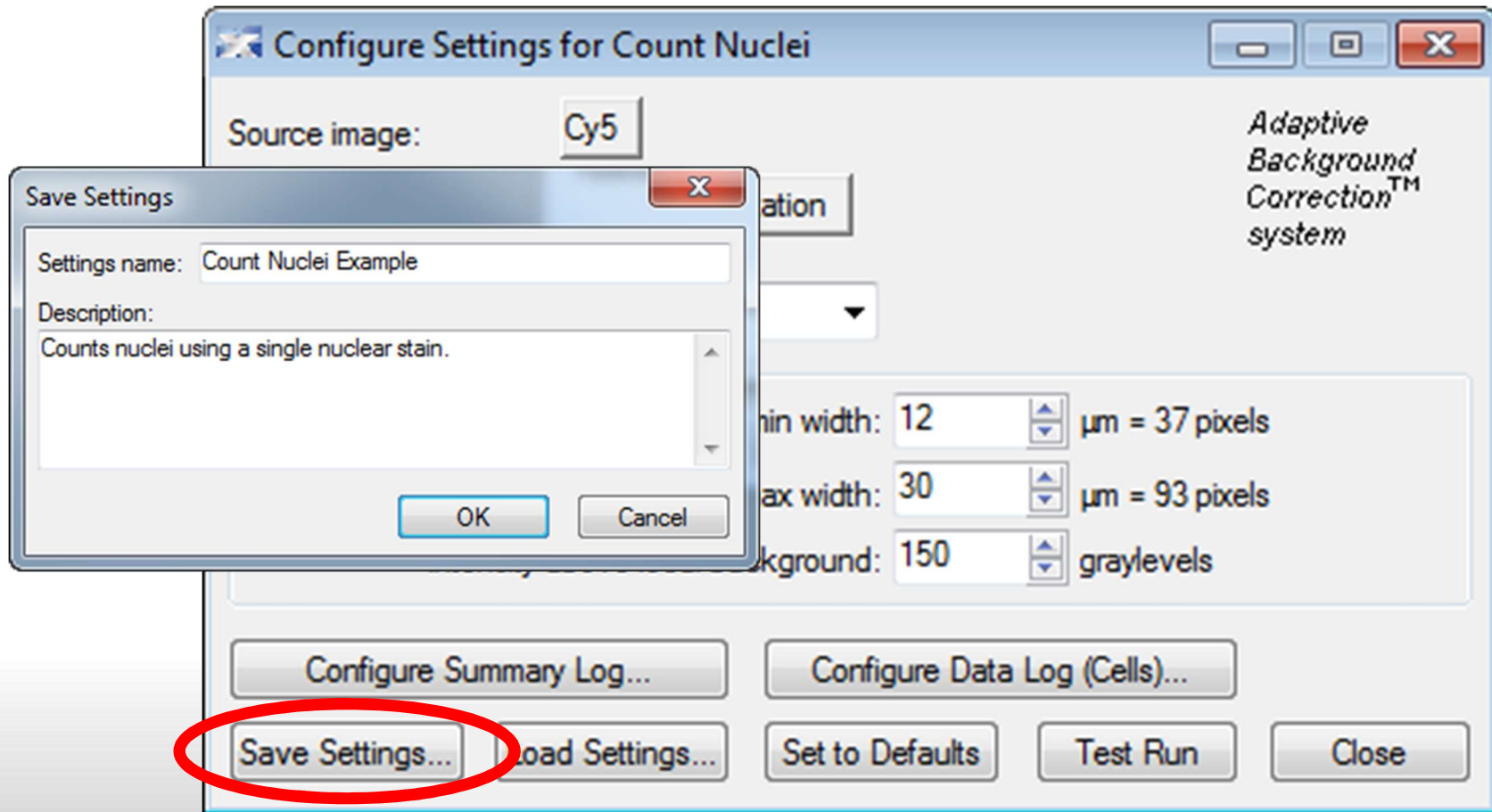


Selecting Measurements

Data log gives you cell-by-cell data
i.e. area, intensity, etc. for each
individual cell



Save Settings to the database

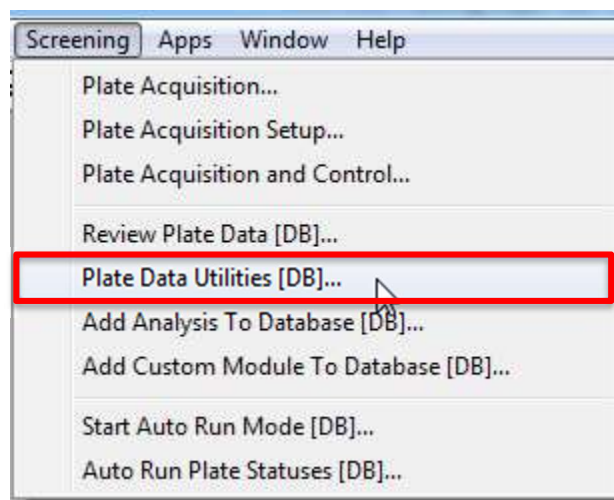


MetaXpress® 5.3 Software

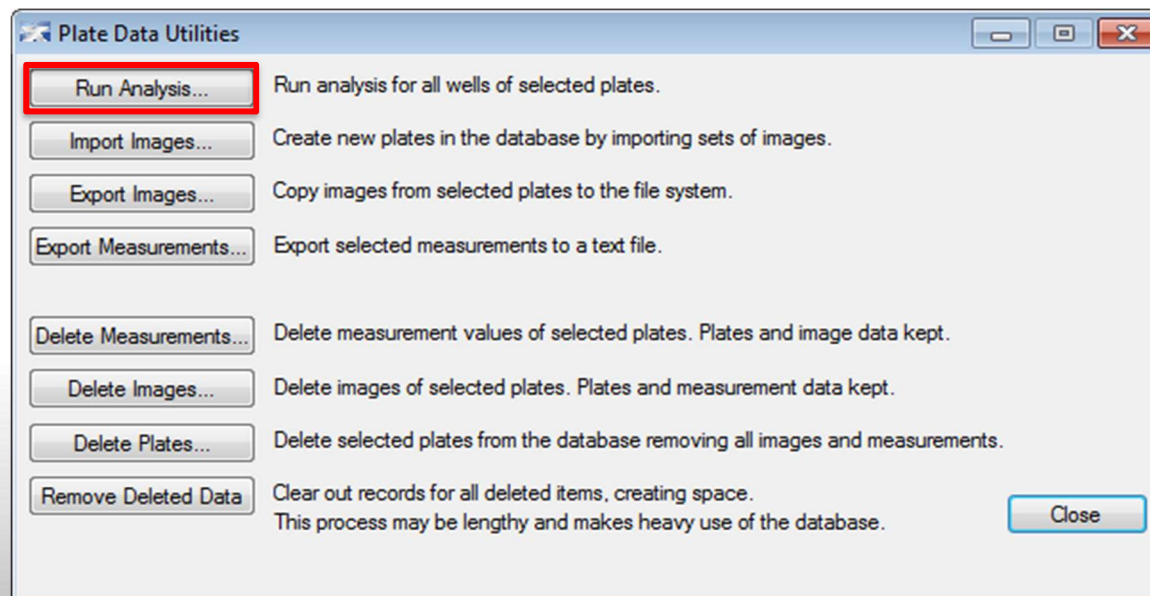
Batch Analysis

Batch Analysis

Under the **Screening** menu,
select **Plate Data Utilities**

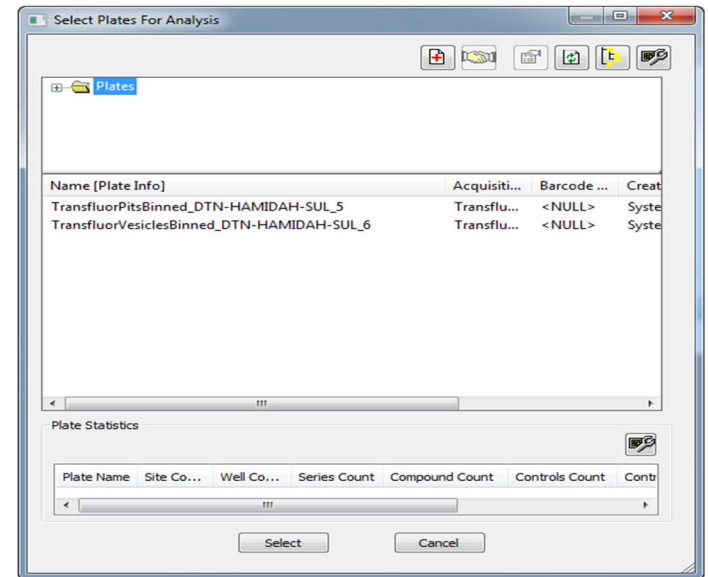


Click on **Run Analysis**
button



Batch Analysis

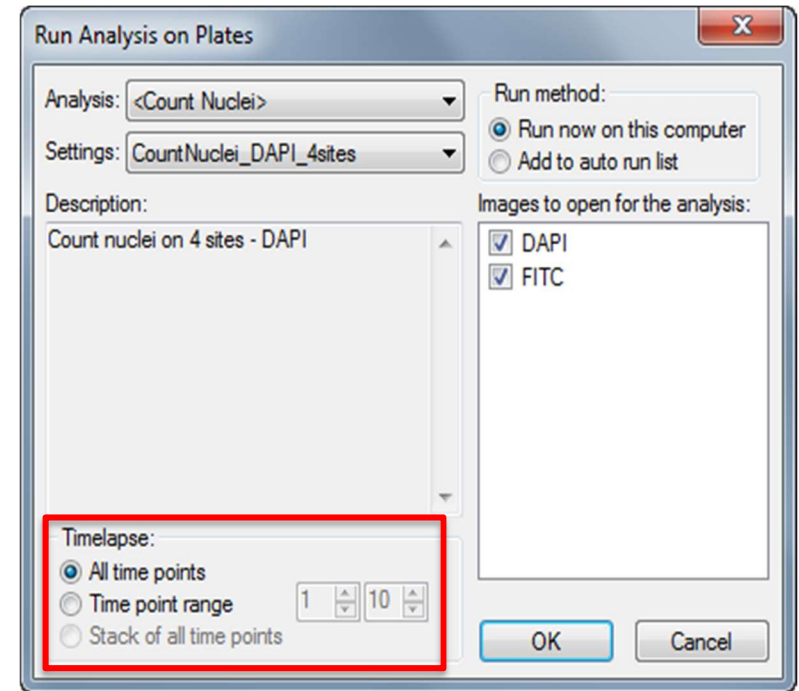
In the Select Plates for Analysis dialog box, highlight the plates you want to run the analysis on and click the **Select** button



Batch Analysis

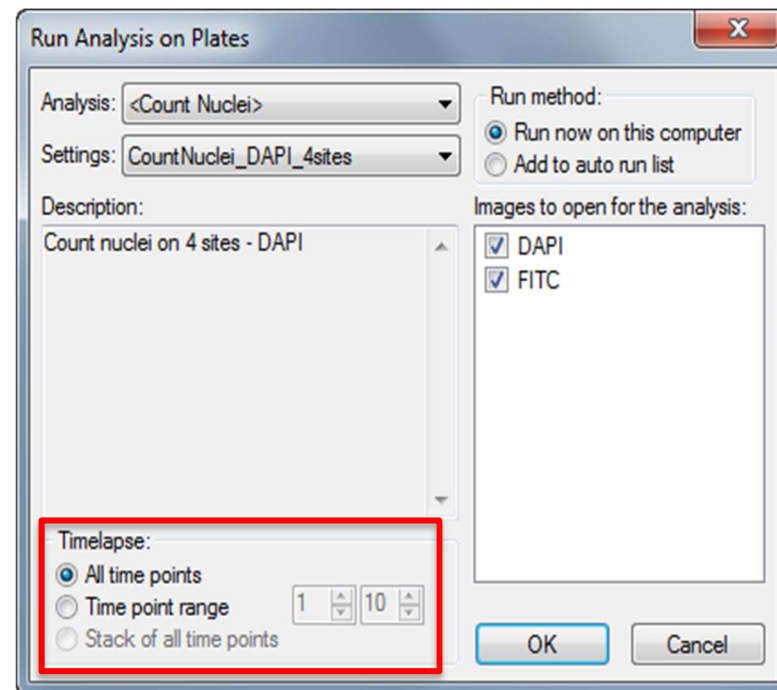
Select the analysis and settings from the drop-down menu

- If the dataset contains Timelapse data, select time points for analysis:
 - **All time points**: all time points will be analyzed
 - **Time point range**: select a single time point or a consecutive range to analyze
 - **Stack of all time points**: select this option if running a legacy journal that uses a timelapse journal which analyzes the planes in a stack as separate time points



Batch Analysis

- Choose the Run Method:
 - **Run now on this computer**
 - **Add to auto run list:** if running analysis on another computer or using MetaXpress PowerCore™ Software
- Under the **Screening** menu, select **Auto Run Plate Statuses**, and click **Start Auto Run Mode** to start the analysis



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Exporting Data

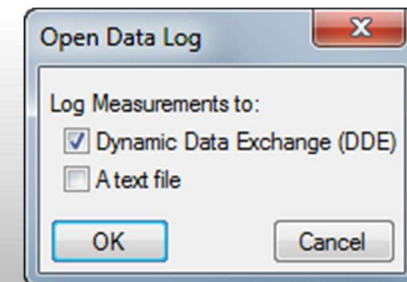
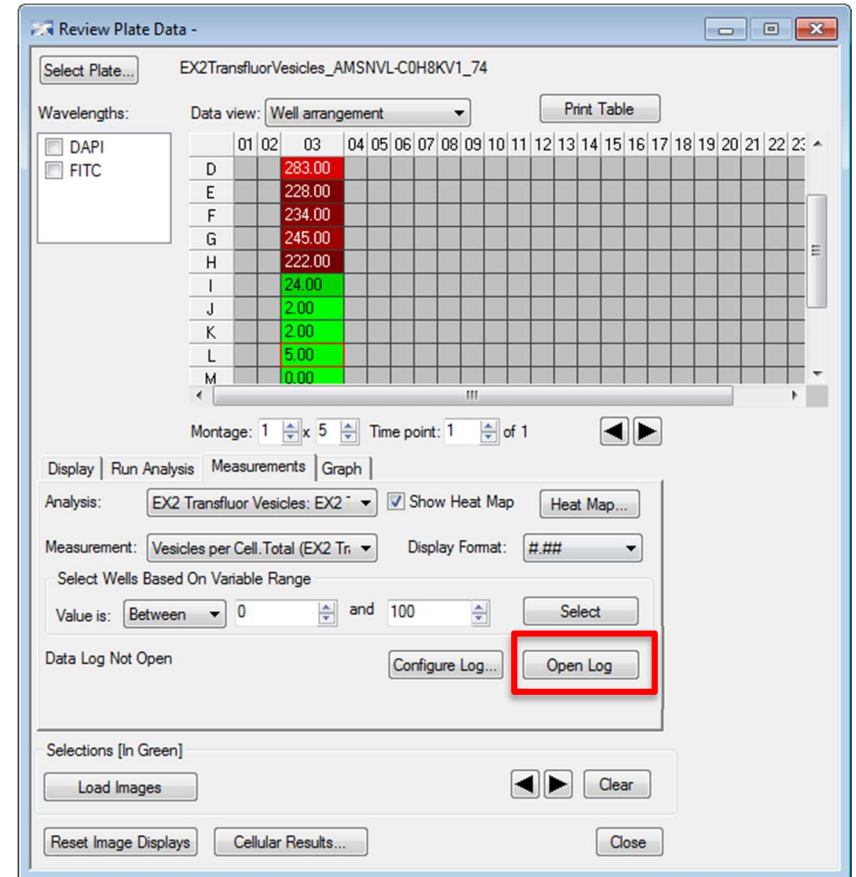
Review Plate Data: Opening a Data Log

Method 1

- In **Review Plate Data** under the **Measurement** tab, click on the **Open Log** button

Method 2

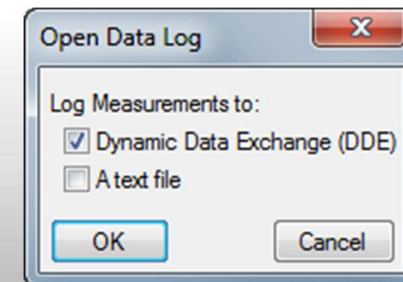
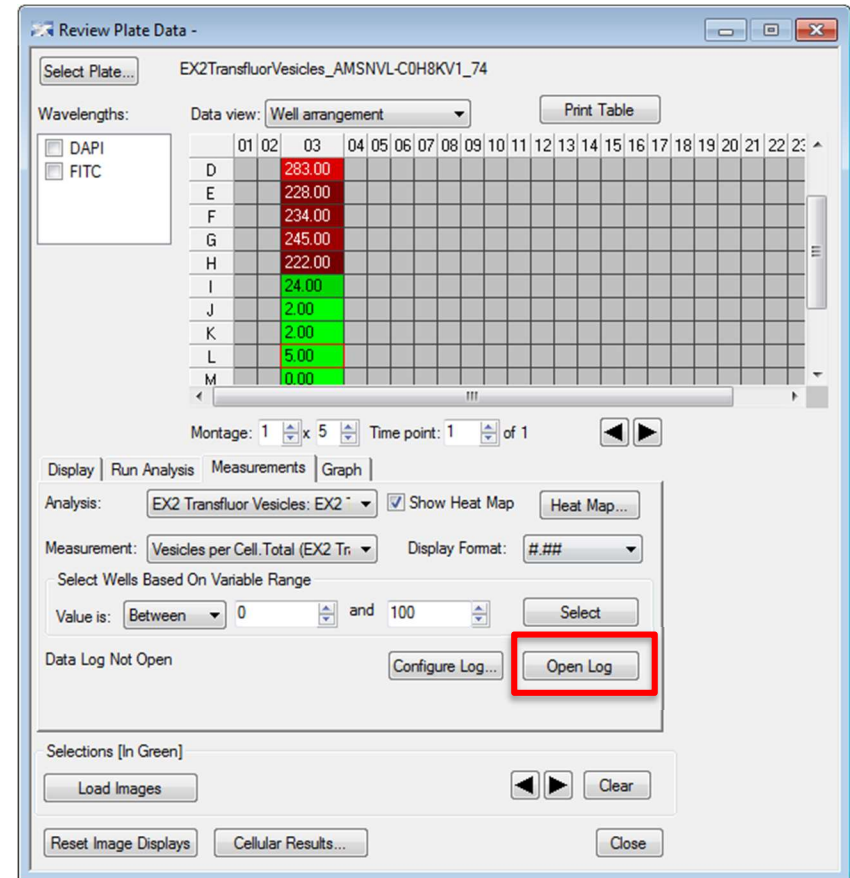
- Under the **Log** menu, select **Open Data Log**



Review Plate Data: Export to Excel / Text File

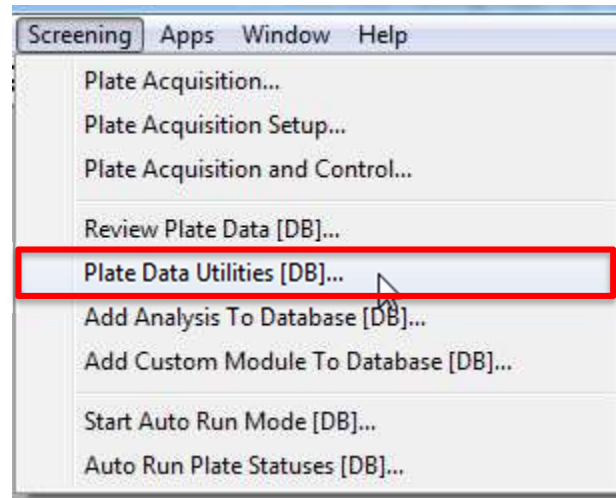
Select

- **Dynamic Data Exchange** to open an Excel file
- **A text file** to save to a text file
- Select the measurement you want to export from the **Measurement** drop-down menu and click **Log Data**
- To export all the data at once (in column format), change the data view to **Measurement vs Well** and click **Log Data**

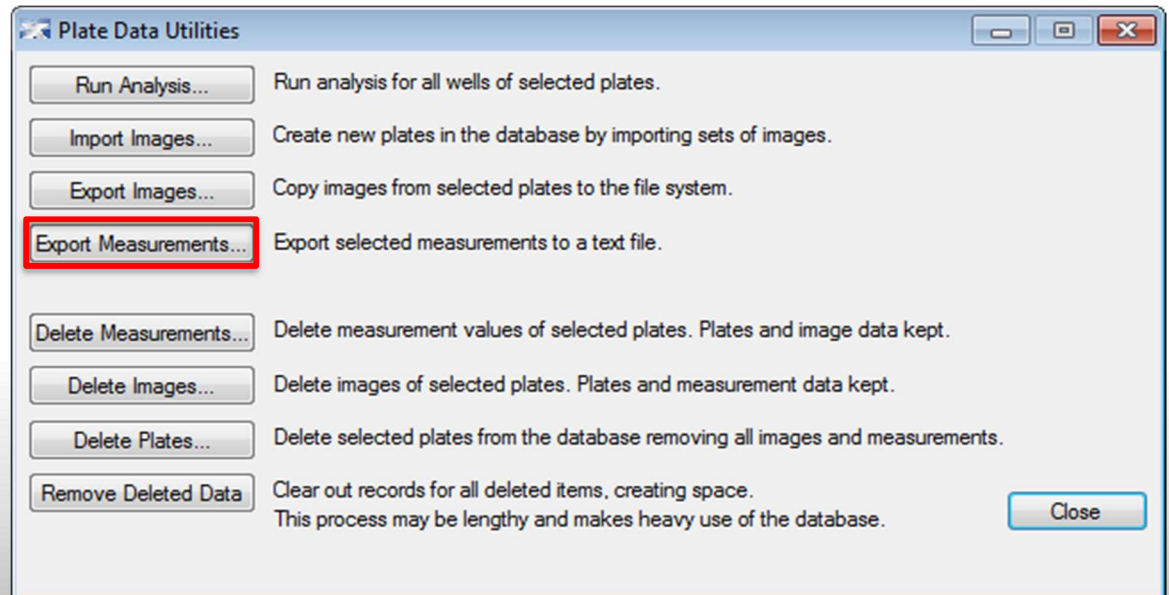


Batch Export Data

Under the **Screening** menu,
select **Plate Data Utilities**



Click on **Export
Measurements...** button



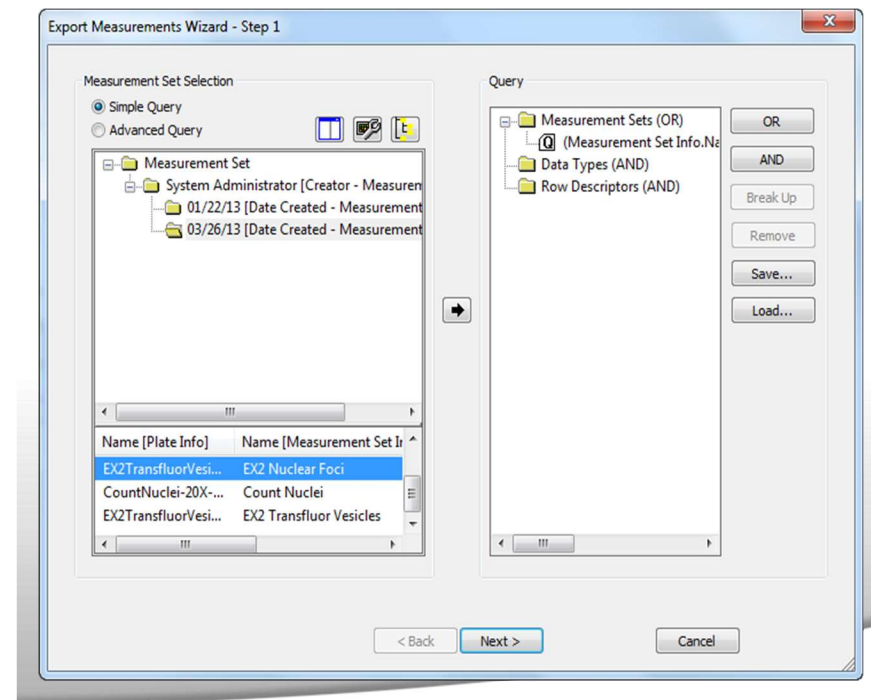
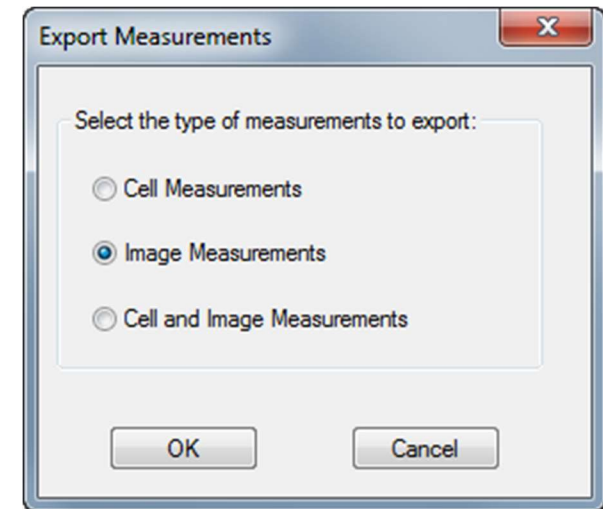
Batch Export Data: Selecting Measurement Type and Plates

Type of Measurements to export

- **Cell Measurements:** to export cell-by-cell data
- **Image Measurements:** to export image summary data
- **Cell and Image Measurements:** to export both cell-by-cell and image summary data

Export Measurements Wizard – Step 1: Plates

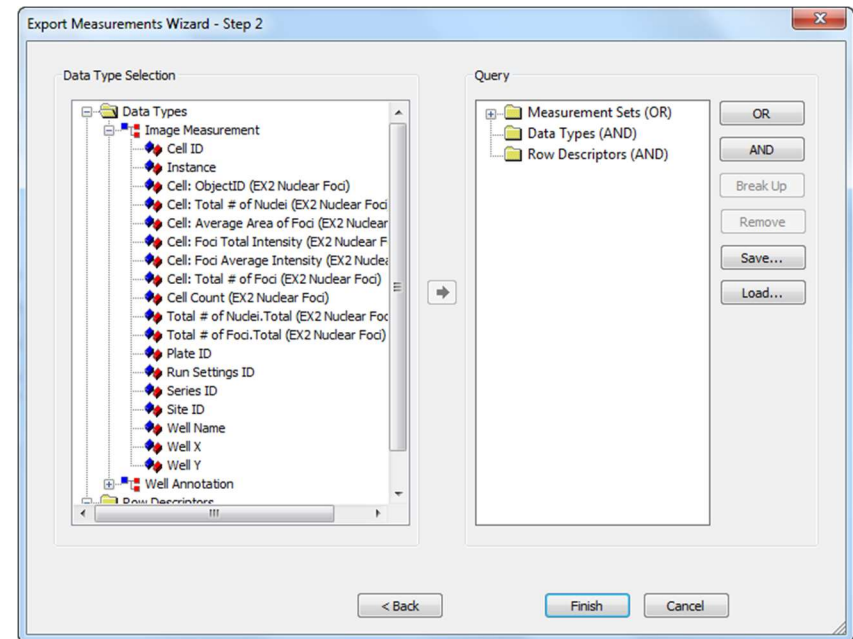
- Browse for the measurement set(s) to be exported
- Highlight multiple using Shft/Ctrl
- Press the black arrow to move the measurements to the **Query** section
- Click **Next**



Batch Export Data: Query Data

Export Measurements Wizard – Step 2

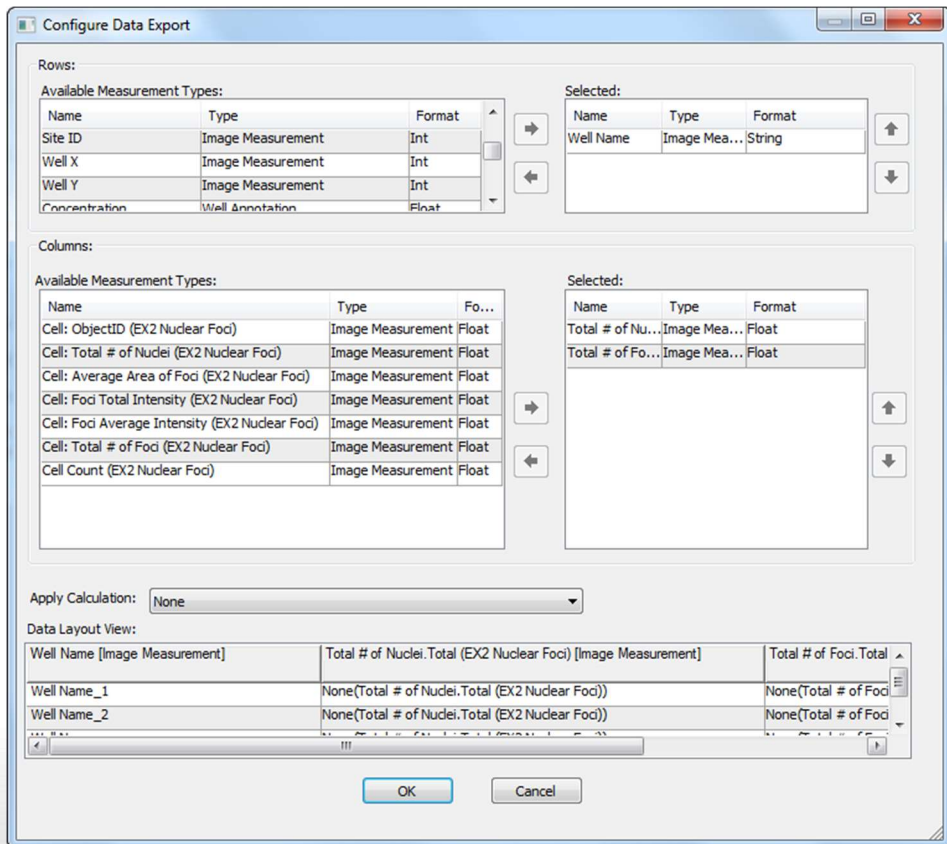
- This section is used to filter the measurements according to either measurement values or plate metadata. Only measurements that meet the criteria will be shown.
- Click **Finish**



Batch Export Data: Selecting Measurements

Customize export

- Under the **Row** section, select the parameters to define how the data will be organized (Plate ID, Well Name, Cell ID)
- Under the **Column** section, select the measurements you want to export
- **Apply calculation:** math functions can be applied (to combine measurements for multiple sites, cells, etc.)
- Click **OK**



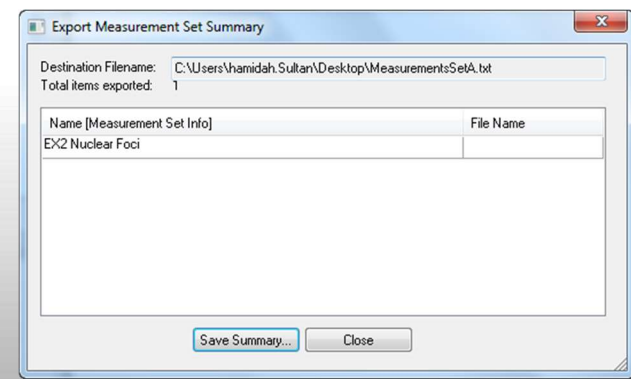
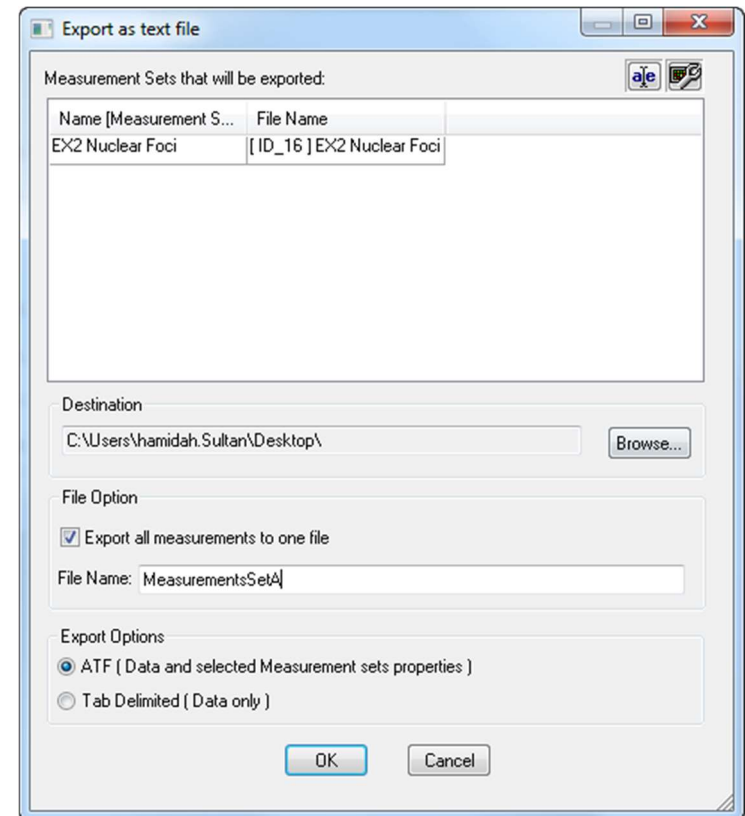
Batch Export Data: Save File

Saving the file

- **Browse...** for the directory where you would like to save the text file.
- Click the **Export all measurements to one file** to save all data to one file
- Enter a file name
- Click **OK**

Summary file

- Click **Save Summary** to save a text file with summary information
- Click **Close**



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: <http://mdc.custhelp.com/app/home>
- Email support@moldev.com
- Technical Support can also be reached by telephone: 1-800-635-5577, select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments.



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