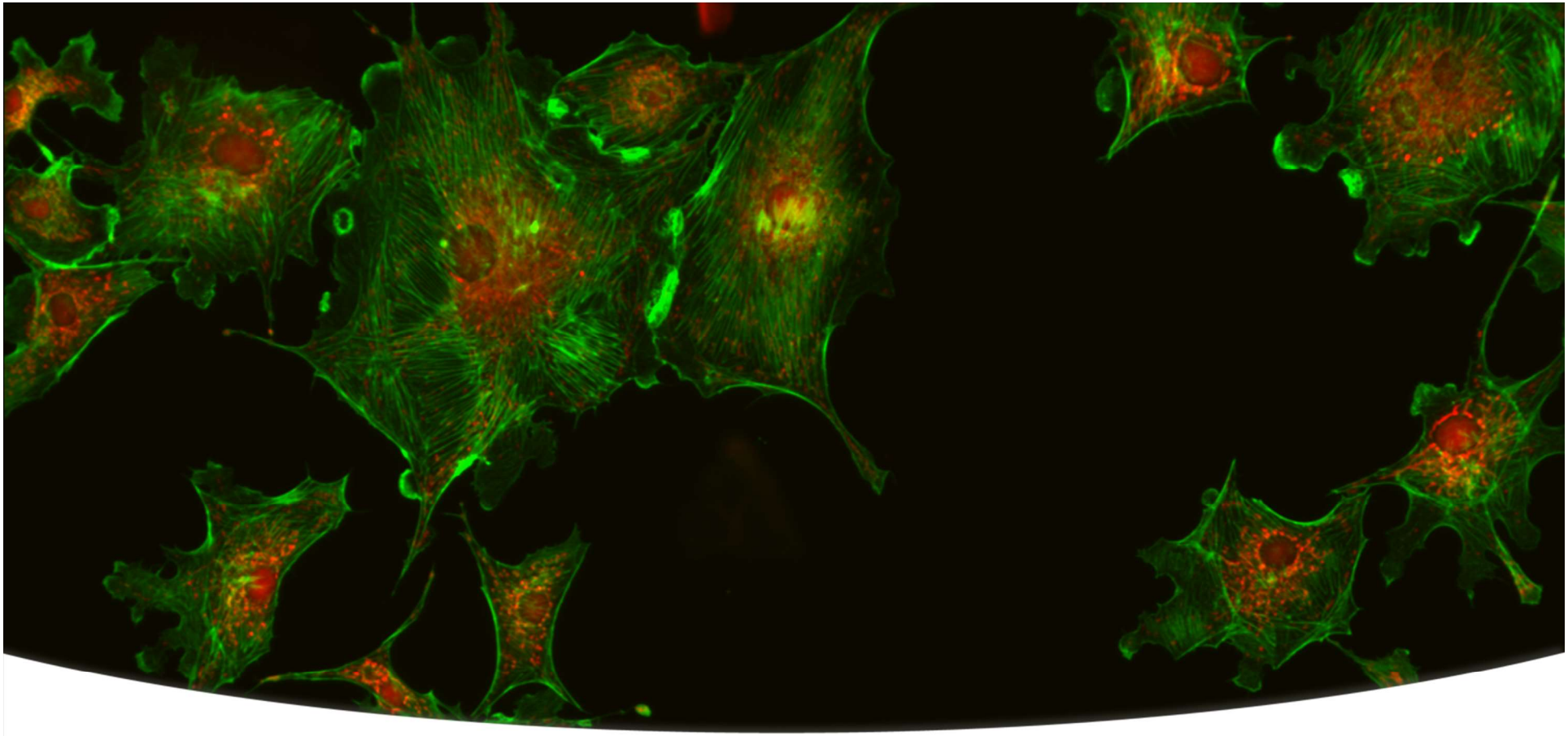


ImageXpress® Micro and MetaXpress® 5.3
Review Images and Analysis Guide

Molecular Devices

Revision A

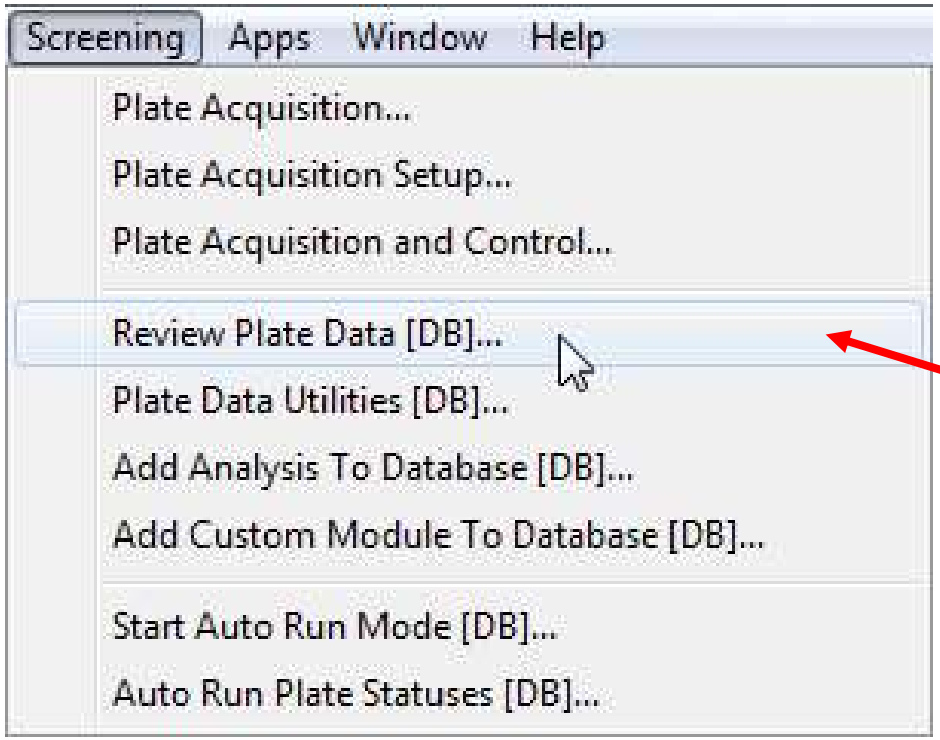
MetaXpress® 5.3 Software

Review Images

Together through life sciences.

Molecular Devices

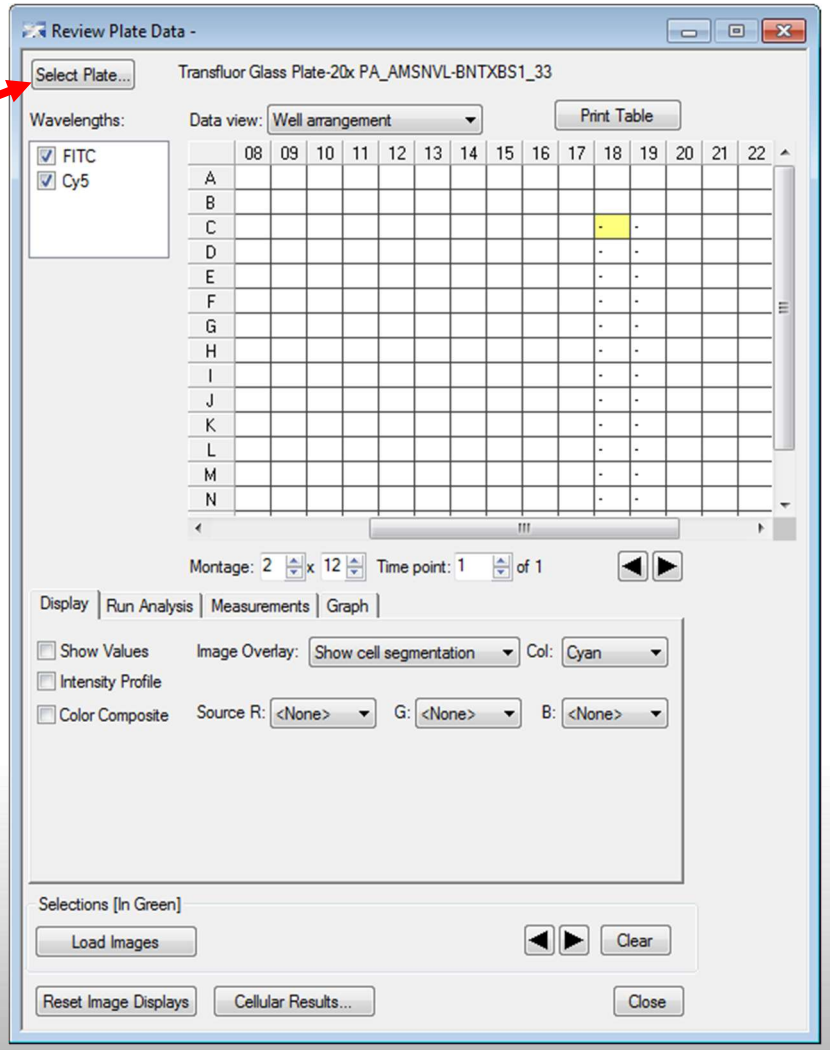
Reviewing Images



Click on Review Plate Data under the Screening menu

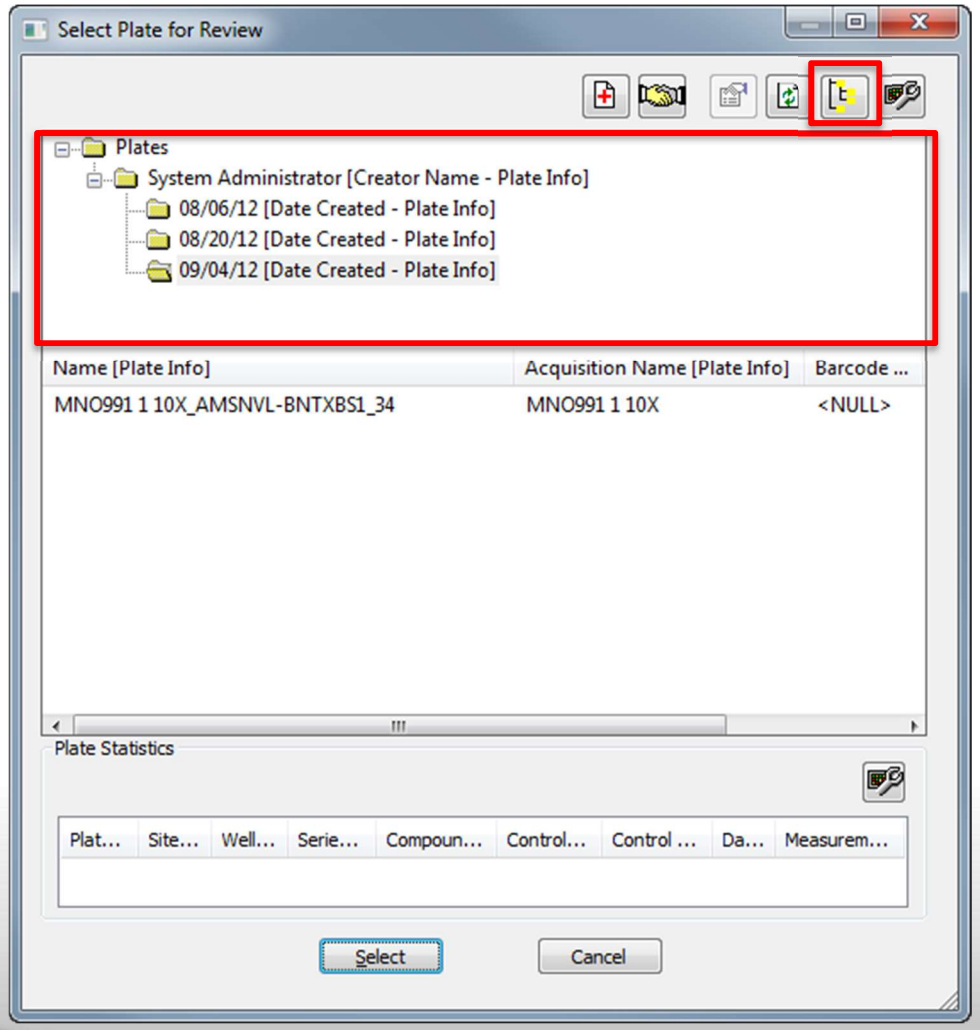
Review Plate Data

Select Plate for review

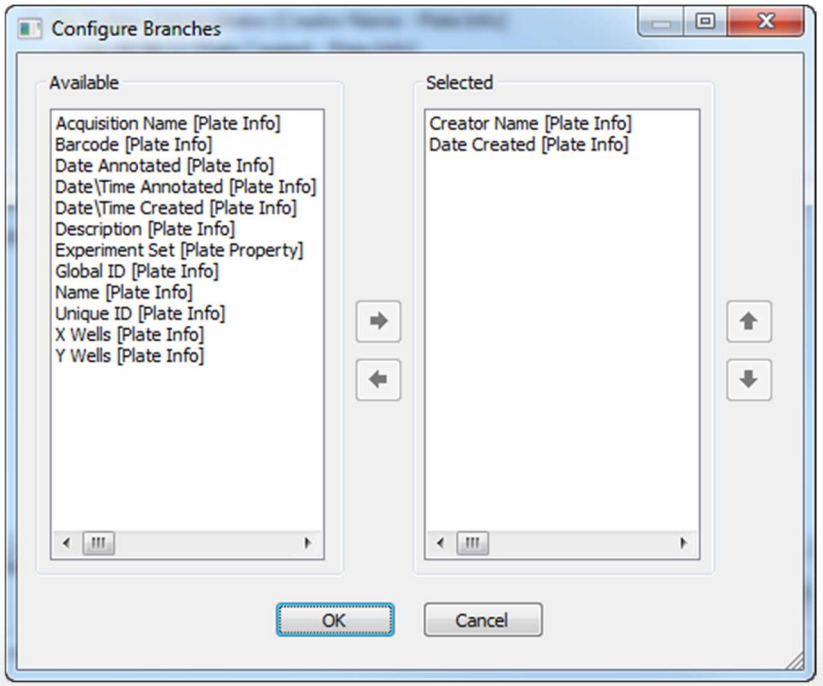


Molecular Devices

Select Plate for Review

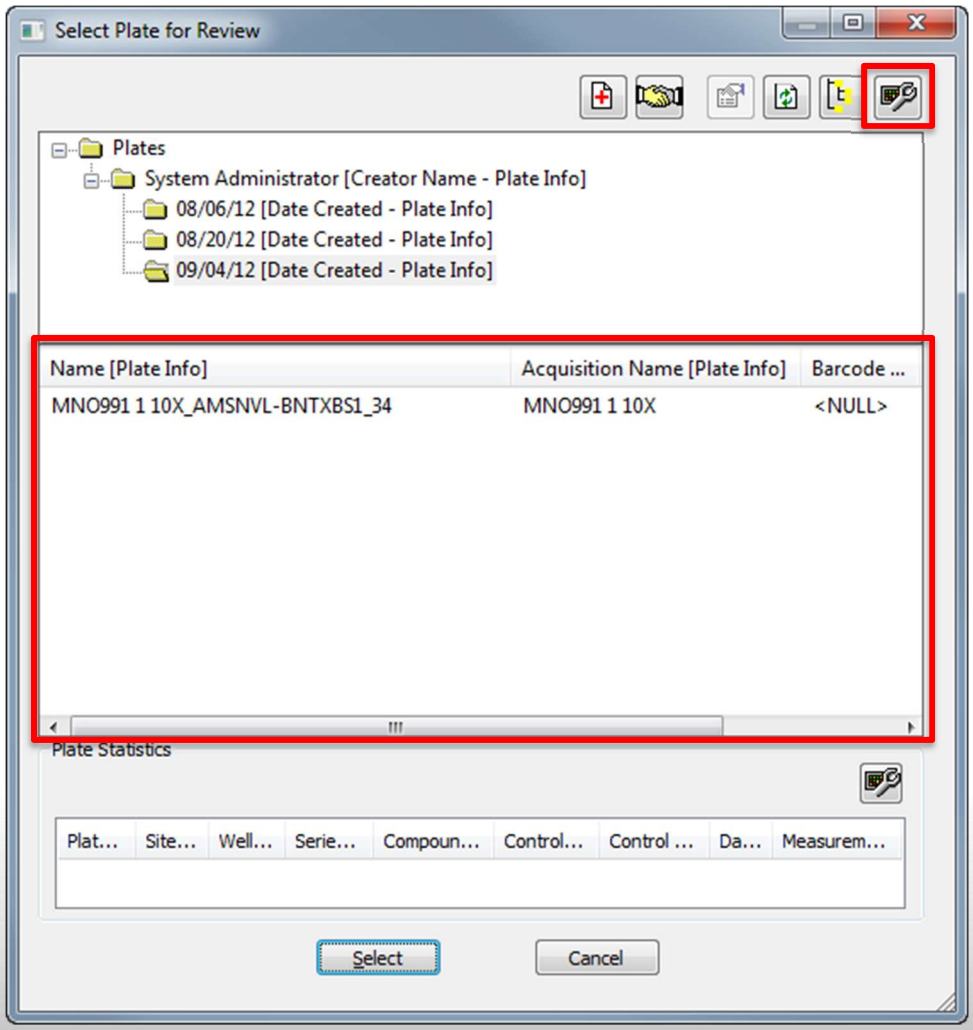


Configure Branches allows you to determine how plates are organized

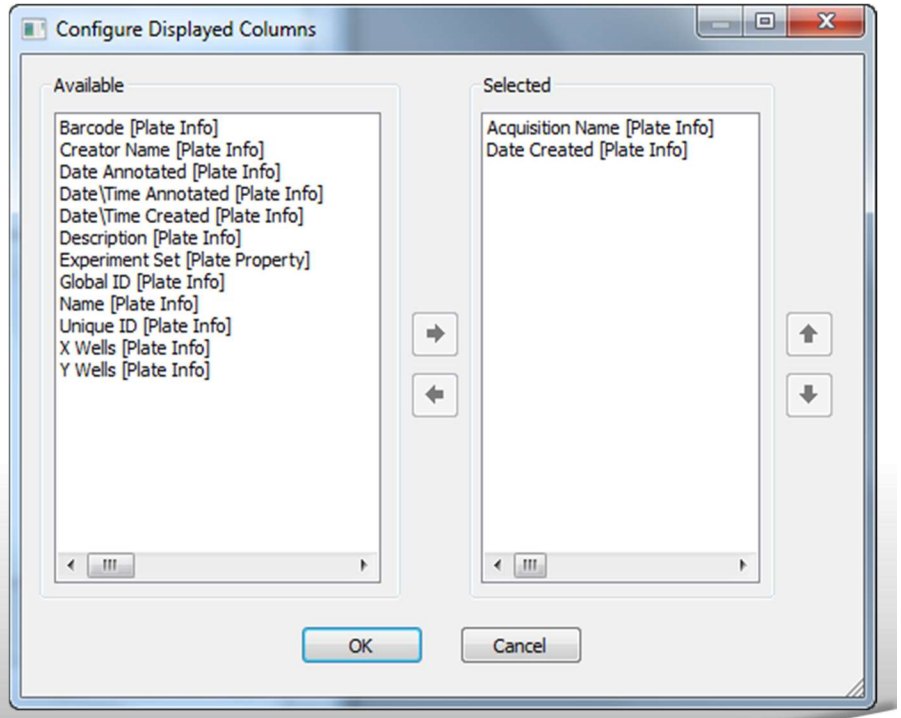


Molecular Devices

Select Plate for Review

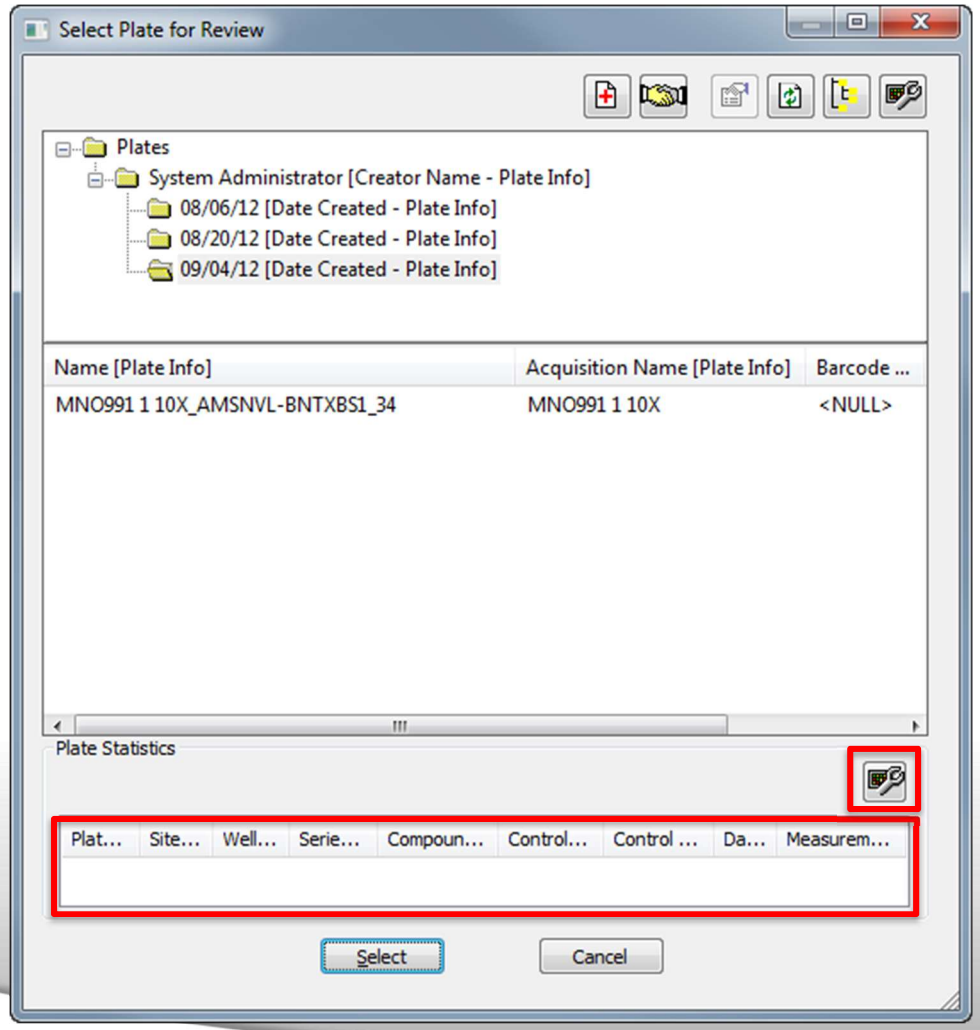


Configure Displayed Columns allows you to choose what information is displayed about the plates in the folders

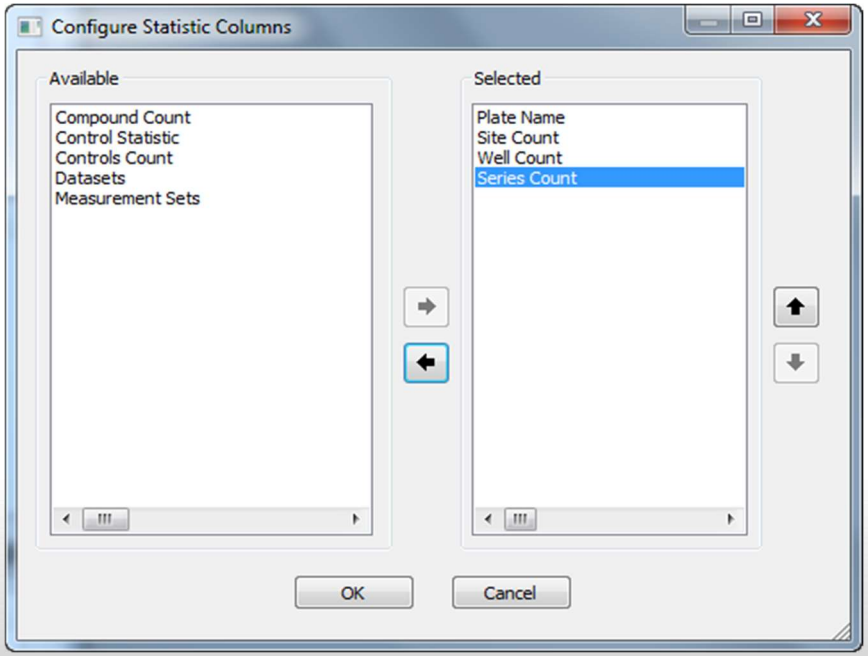


Molecular Devices

Select Plate for Review

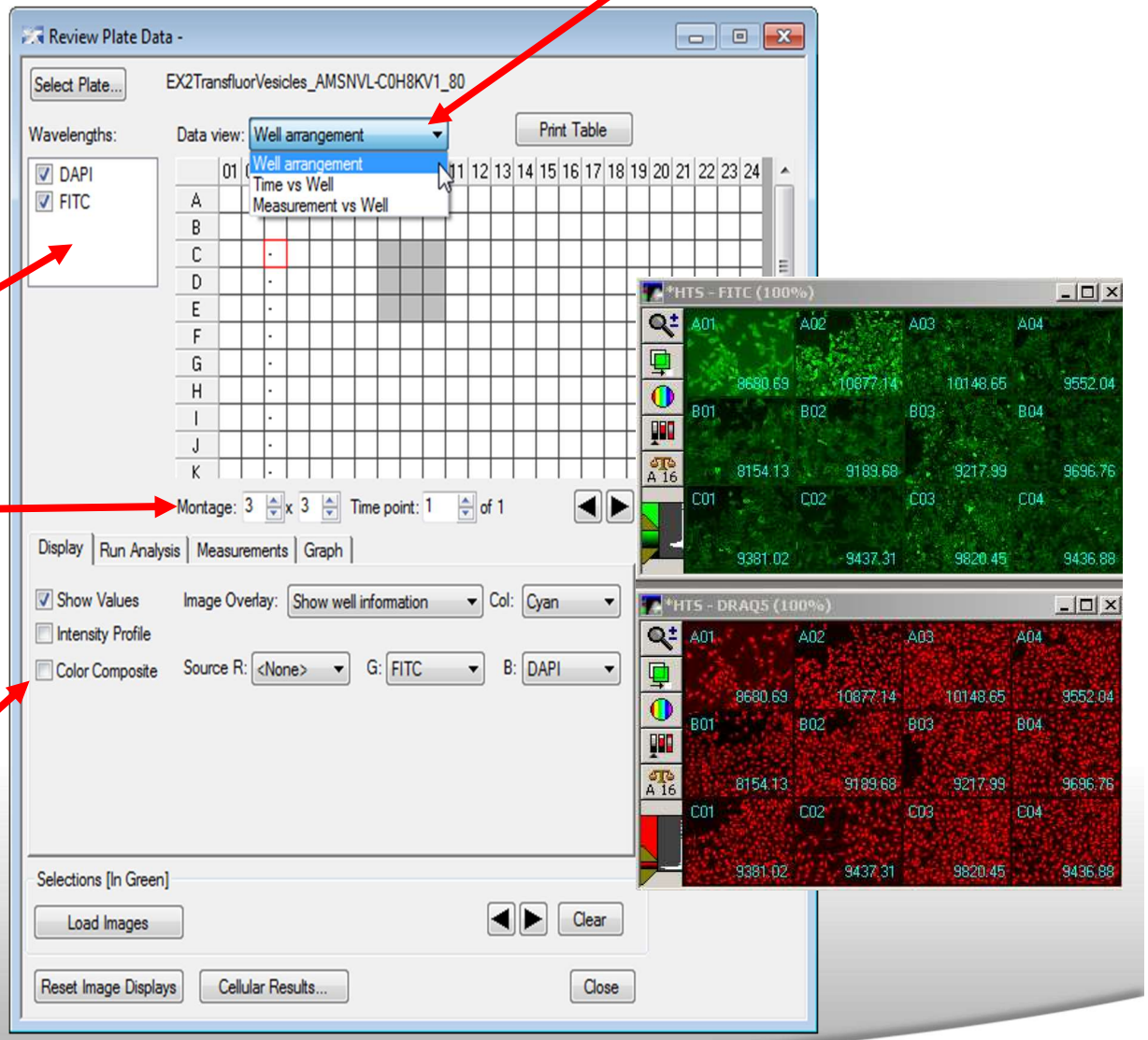


Configure Statistic Columns allows you to choose what information is displayed about the plate selected



Molecular Devices

Review Plate Data: Display Tab



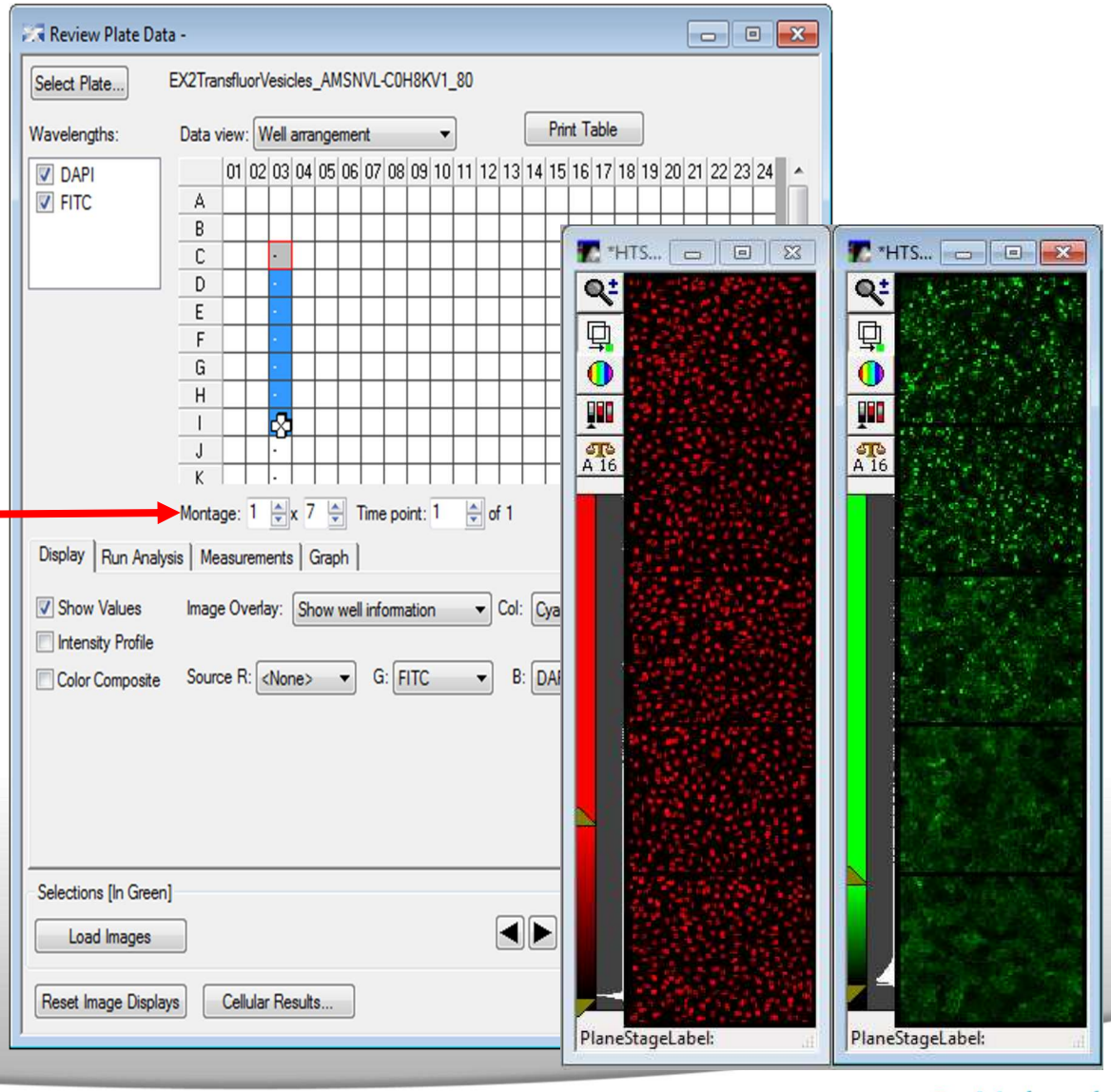
Molecular Devices

Review Plate Data: Montage Display

Configure thumbnail montage area

OR

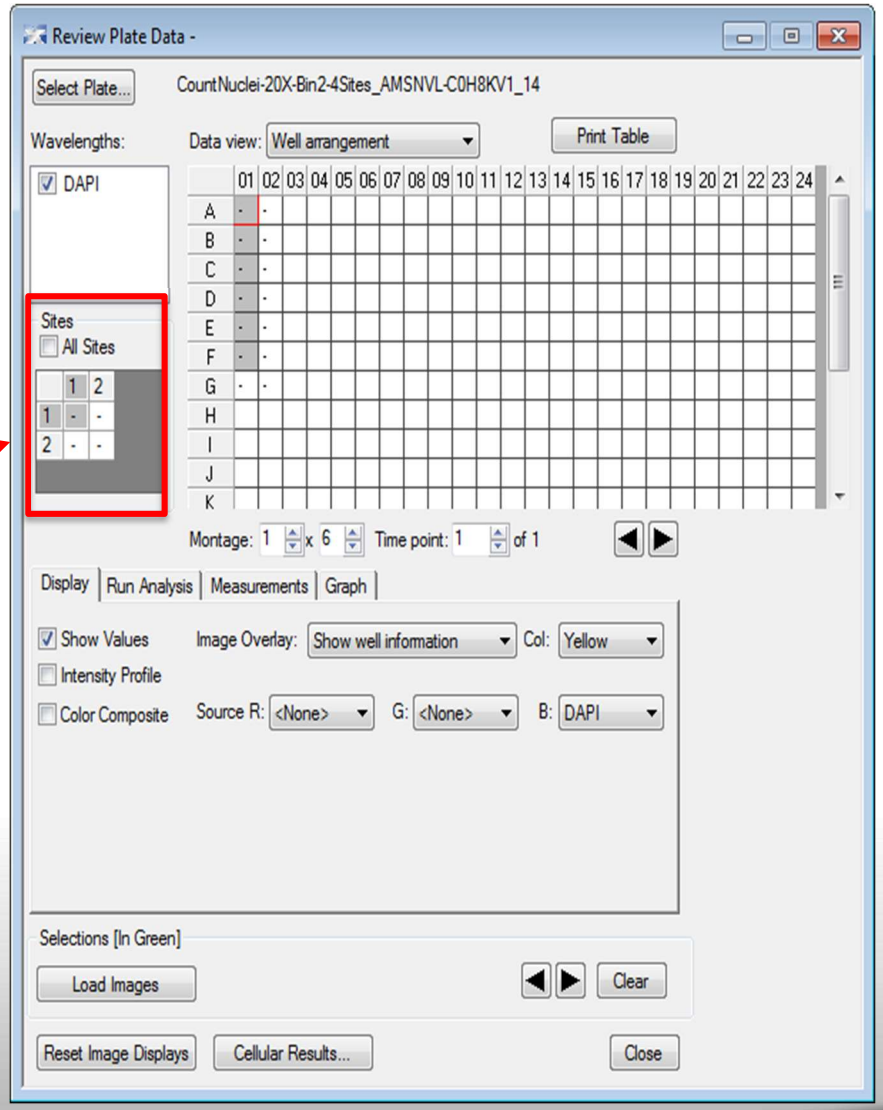
Click and drag to highlight a selection of wells in the plate grid



Molecular Devices

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



Molecular Devices

MetaXpress® 5.3 Software

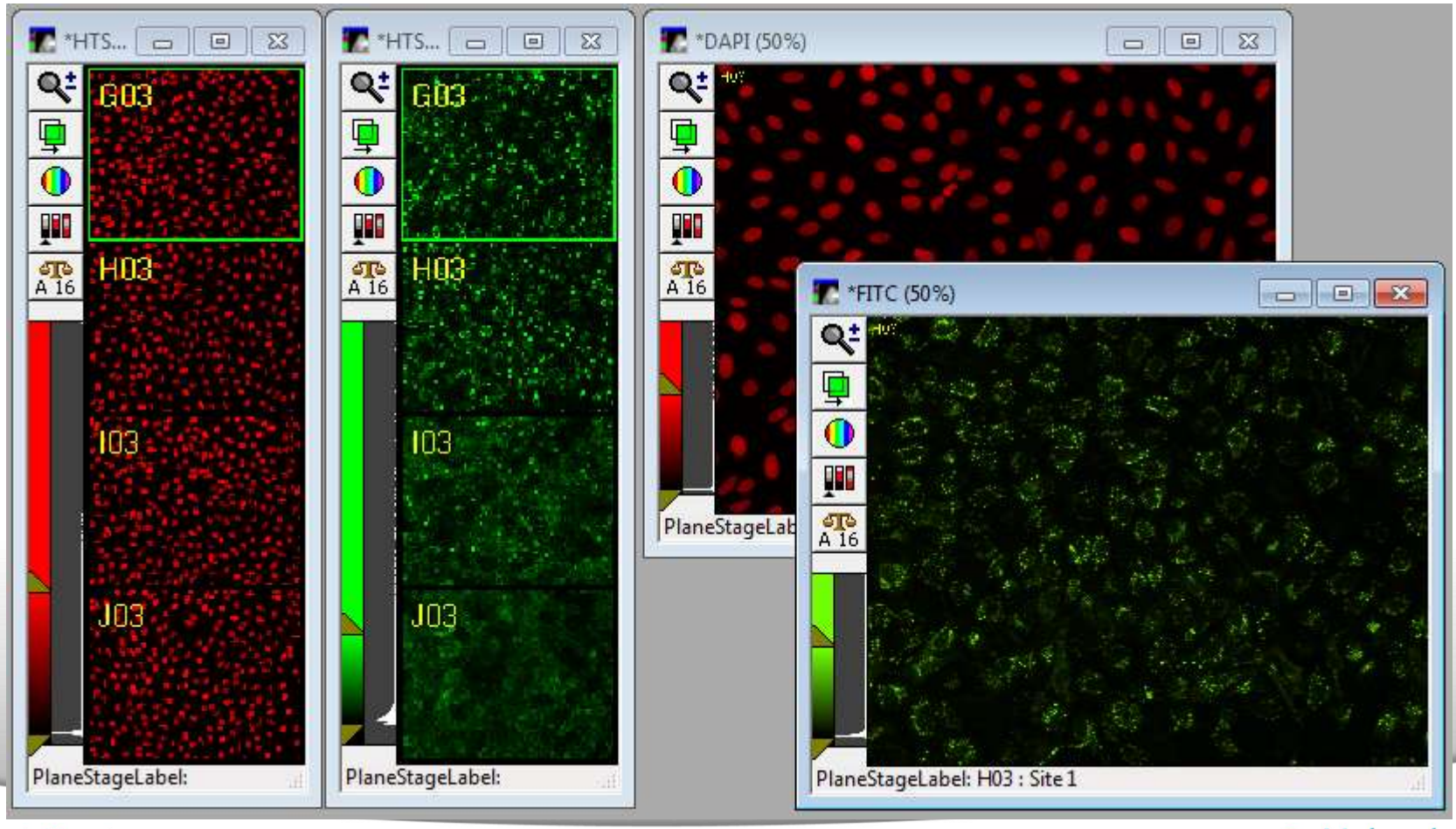
Viewing and Adjusting Image Windows

Together through life sciences.

Molecular Devices

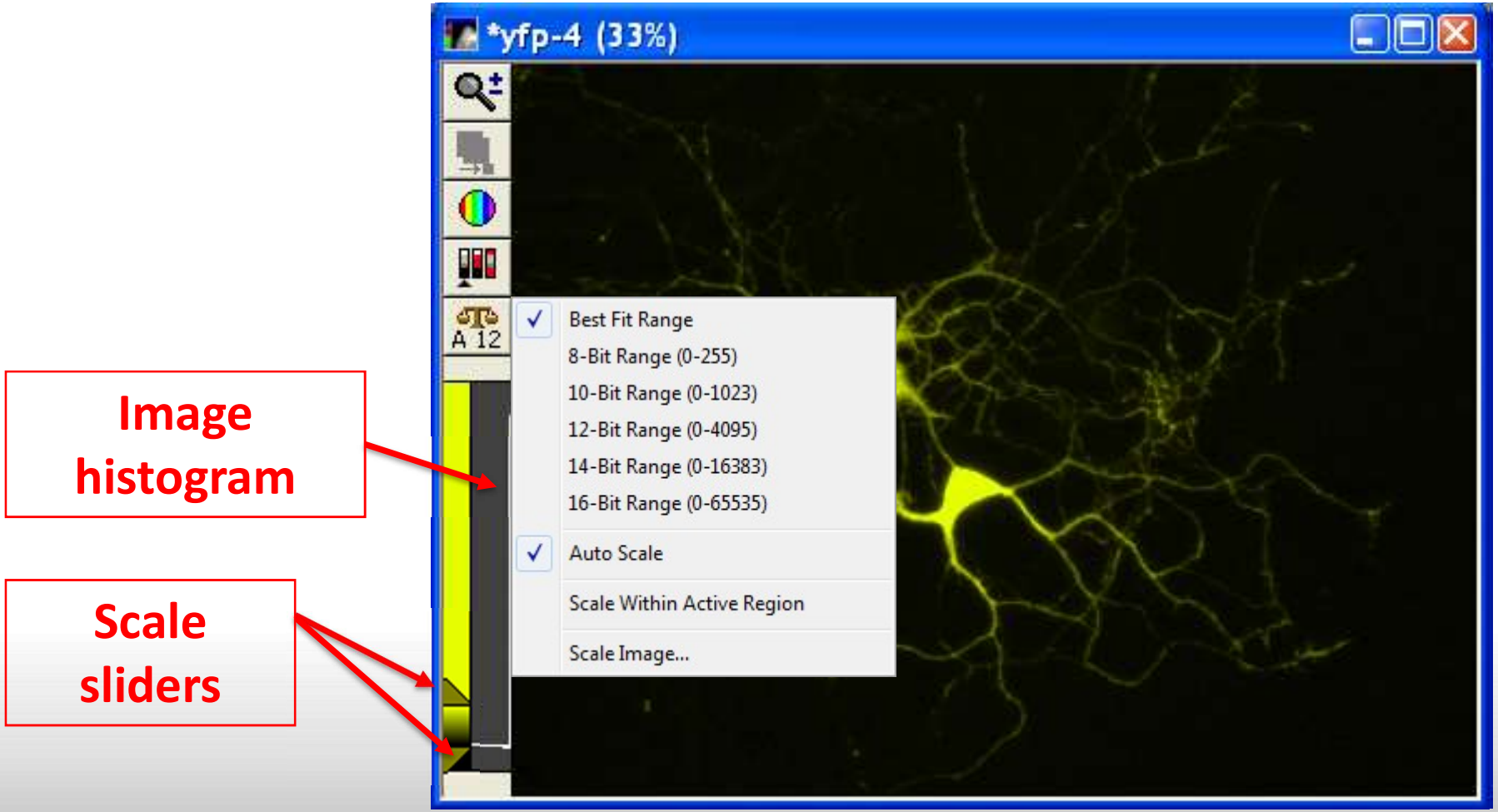
High Resolution Images

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



Molecular Devices

Image Scaling: Adjusting contrast

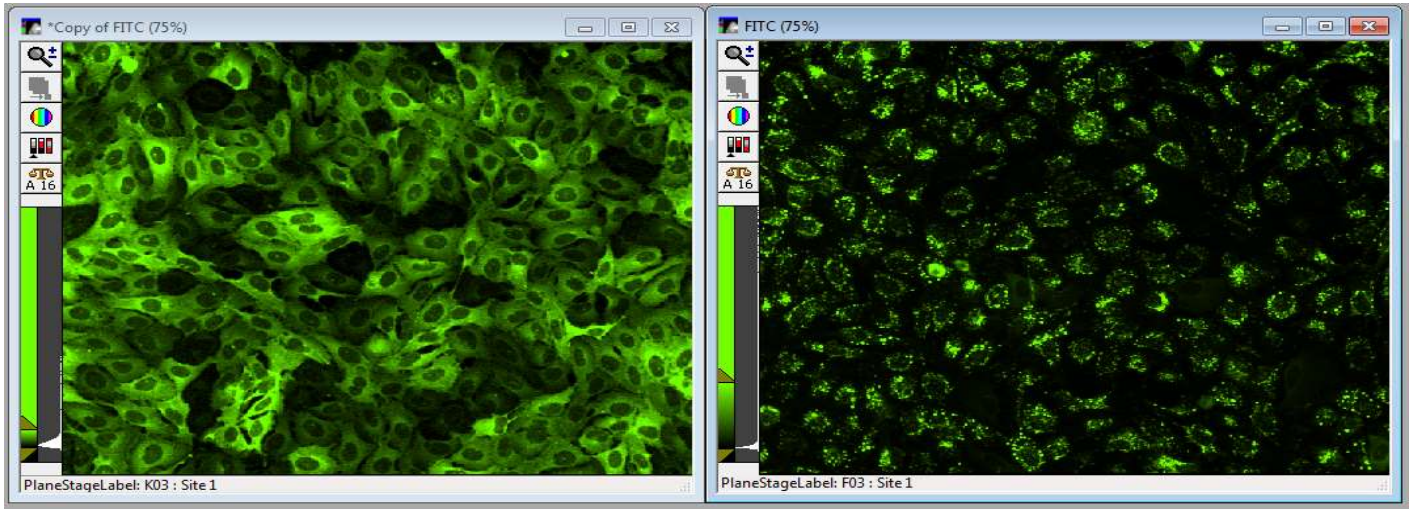


Molecular Devices

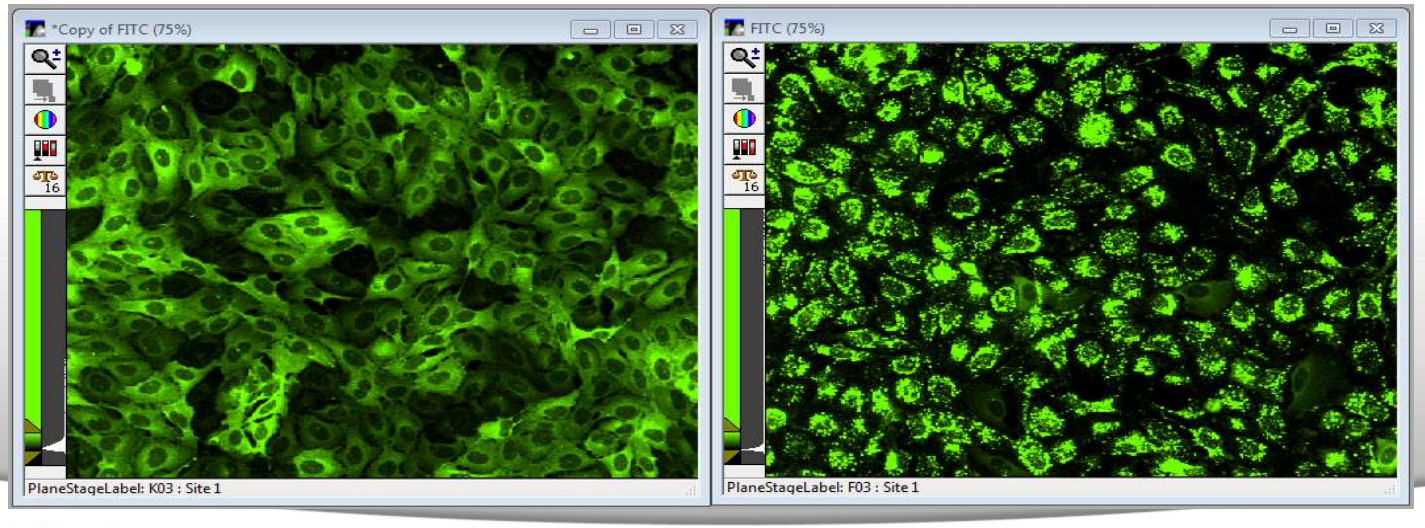
Image Scaling: Auto Scale

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

Auto Scale On



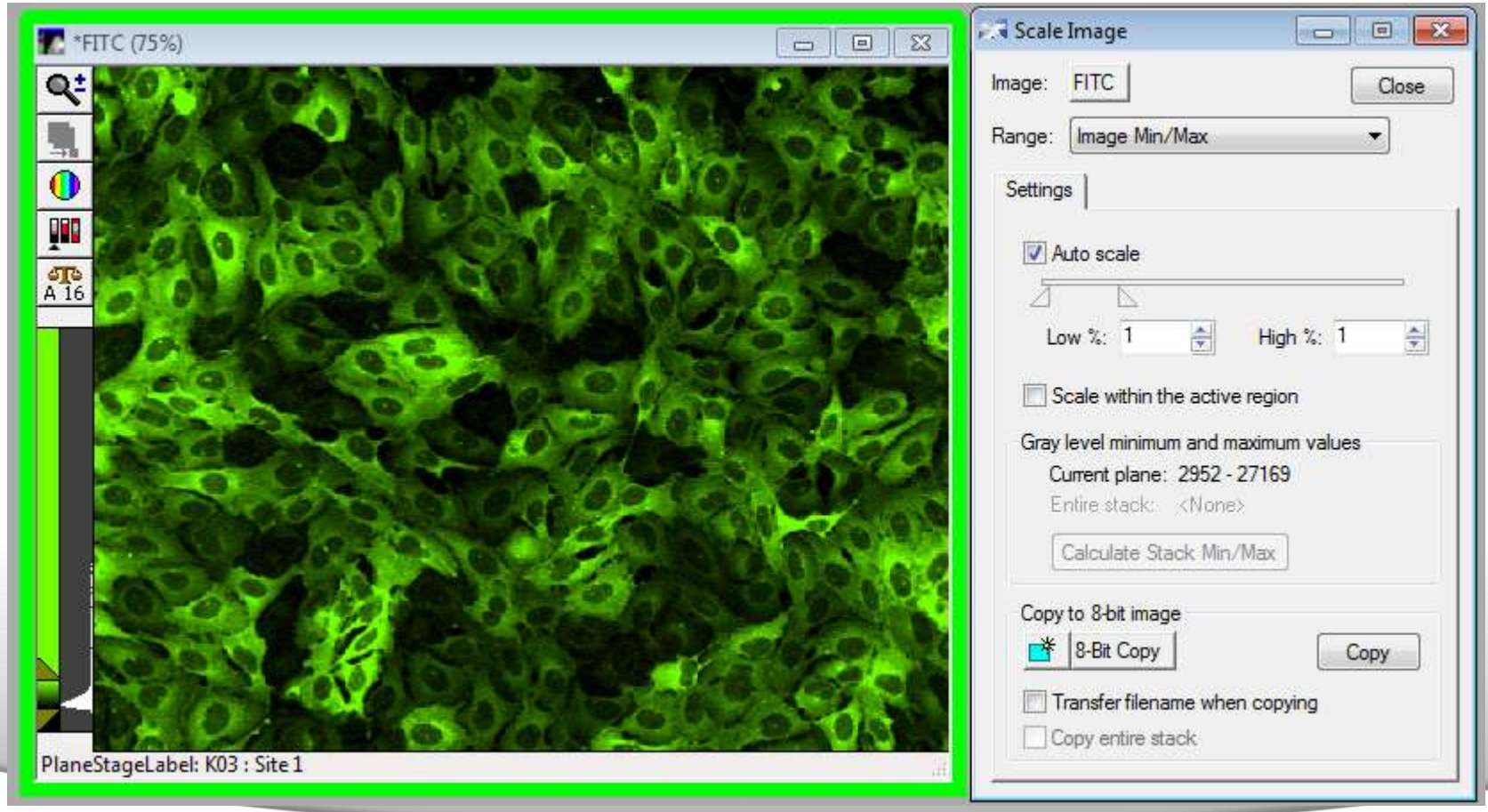
Auto Scale Off



Molecular Devices

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)

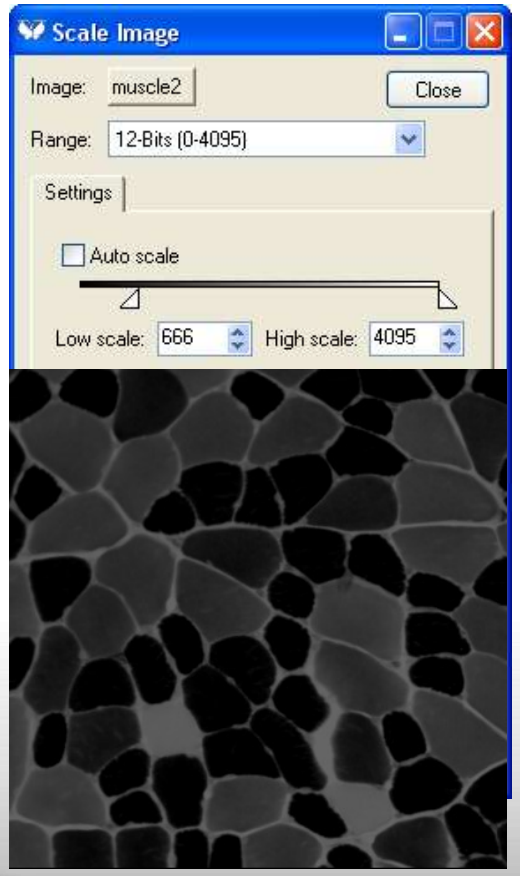


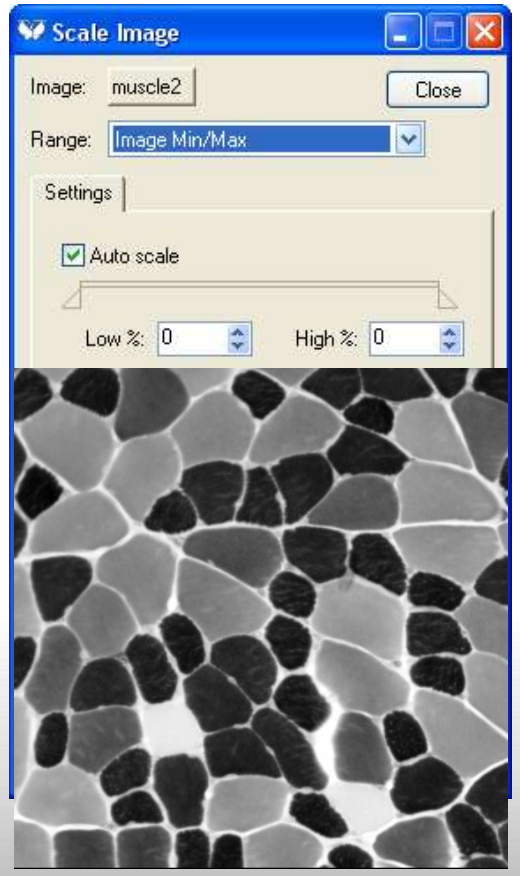
Molecular Devices

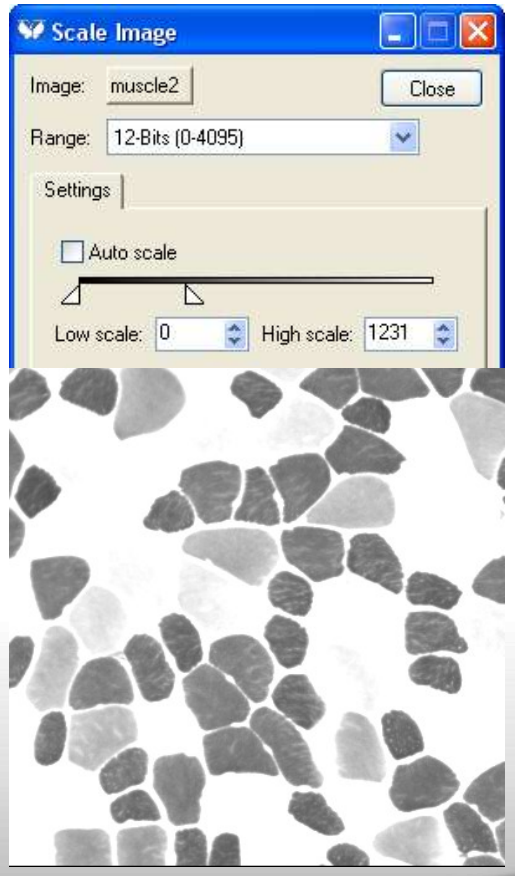
Image Scaling

Changing the scaling does not change the raw image data.

It only changes the image display.



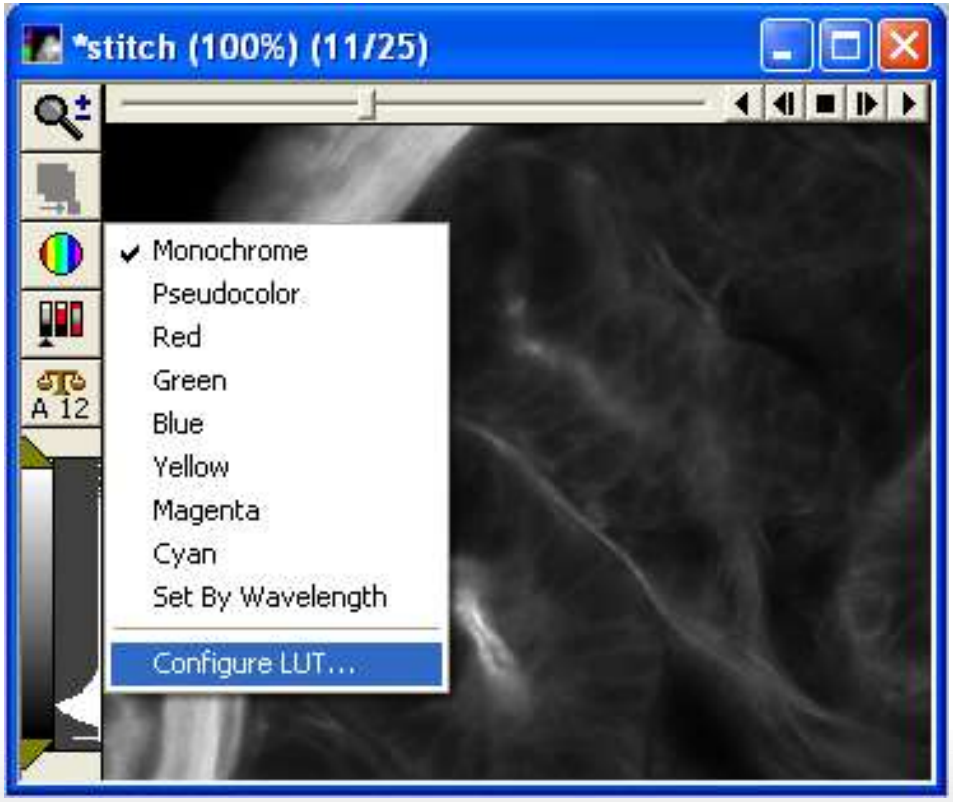




Molecular Devices

Pseudocolor and Look Up Tables (LUT)

Maps intensity values in image to a color



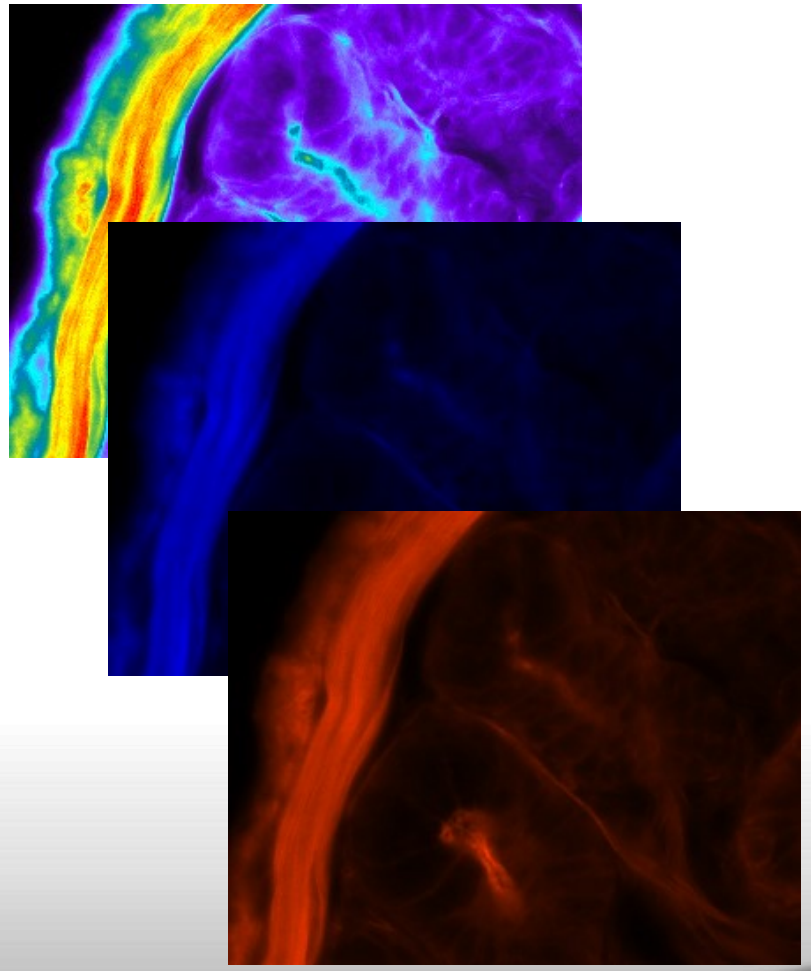
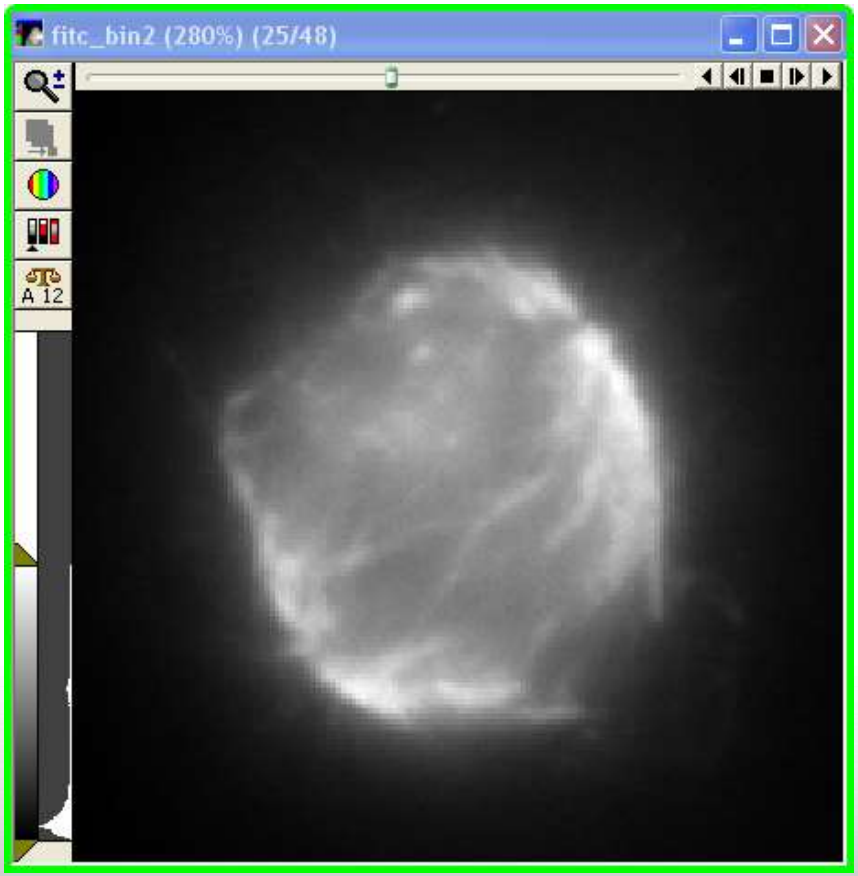
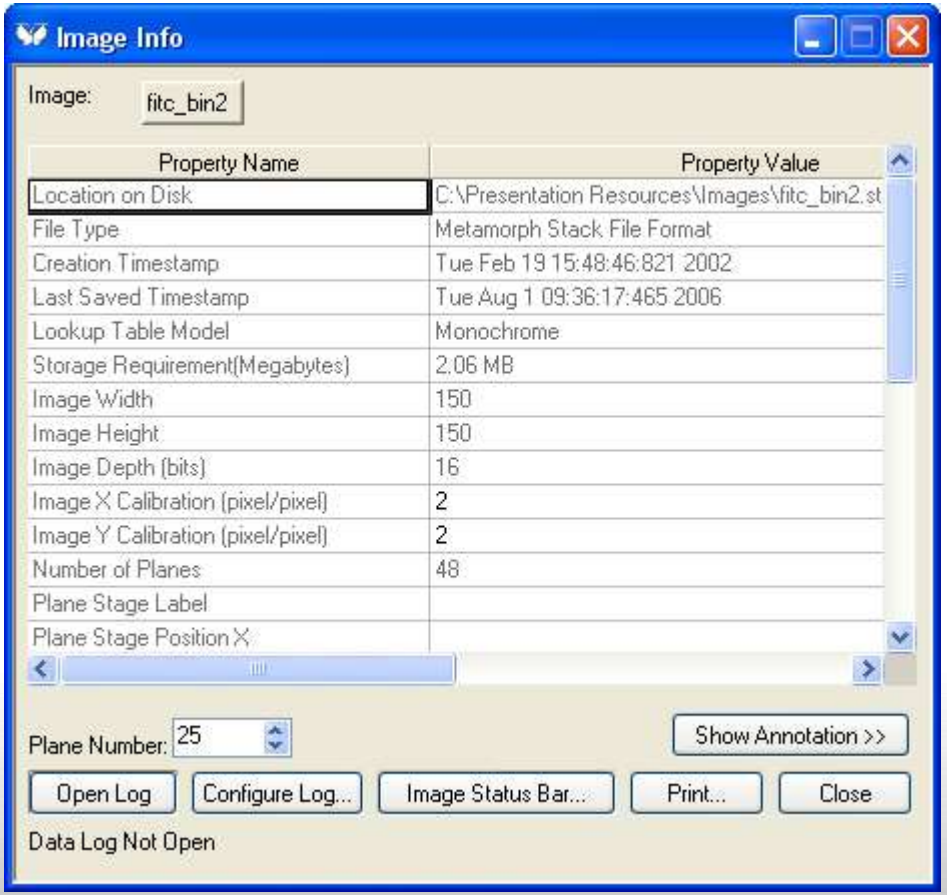


Image Information

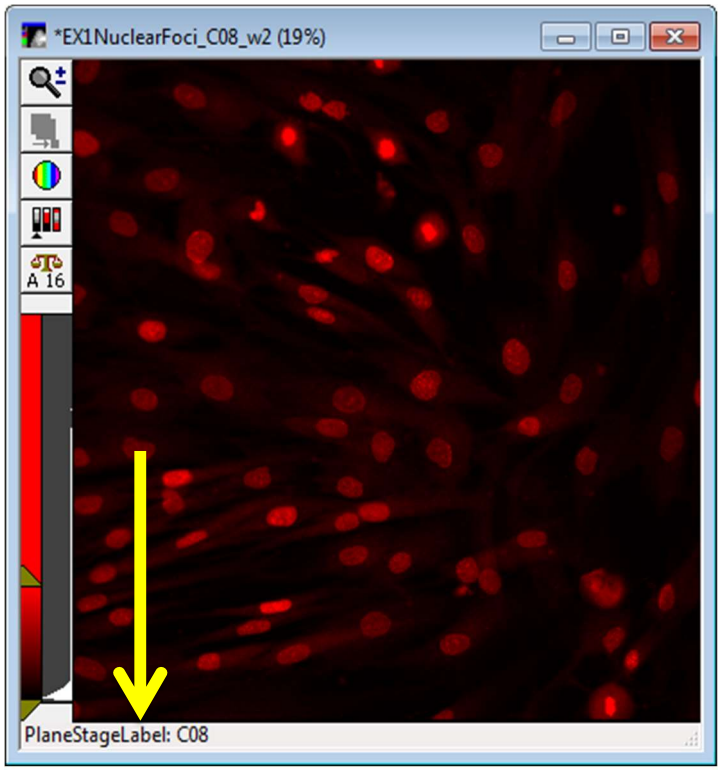
Edit → Image Info or **Alt-I** or  icon





Molecular Devices

Add Image Information to Bottom of Image Window

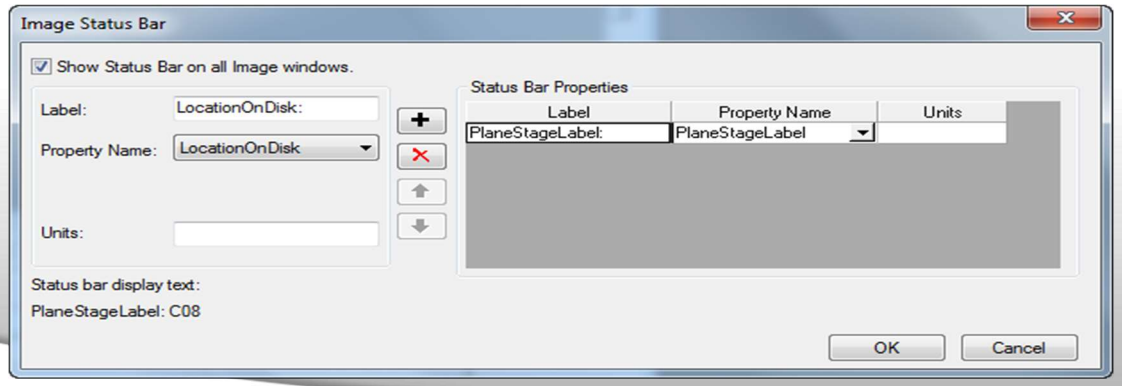


- Under the Edit menu, select **Image Status Bar**

  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



Molecular Devices

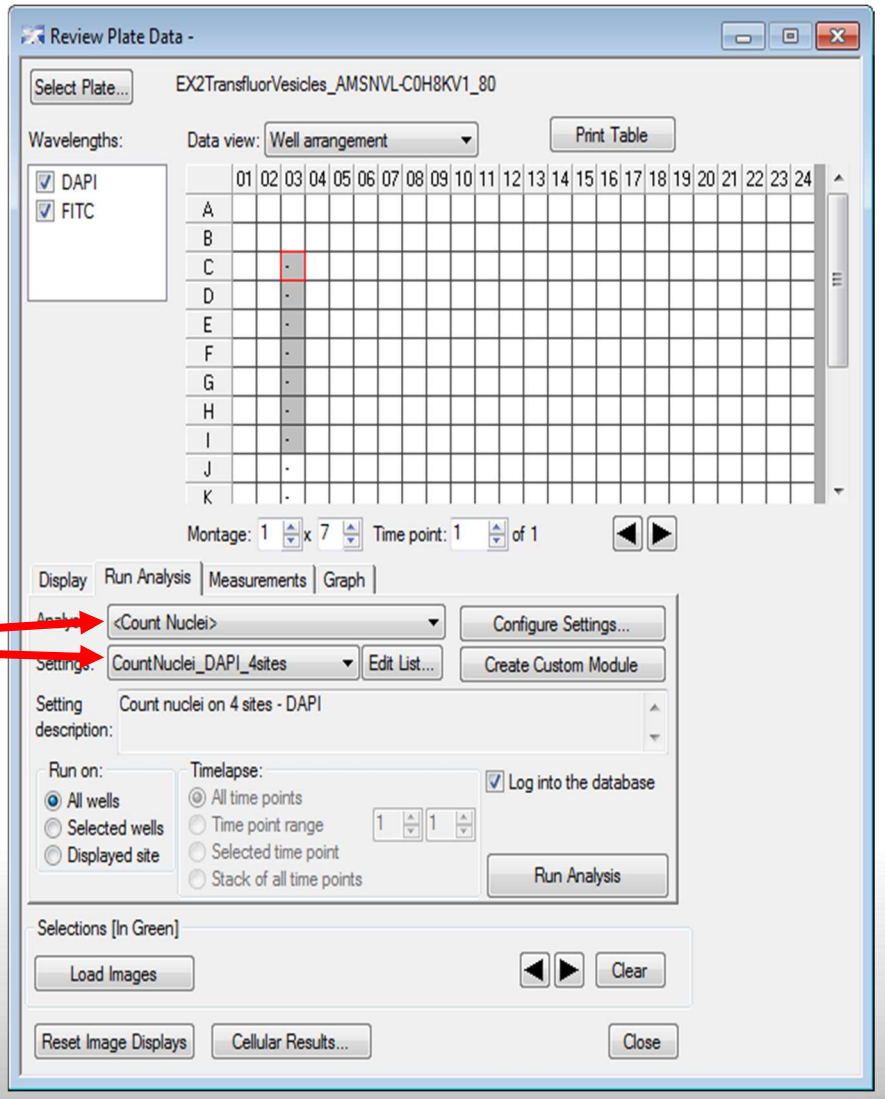
MetaXpress® 5.3 Software

Running Analysis

Molecular Devices

Review Plate Data: Run Analysis Tab

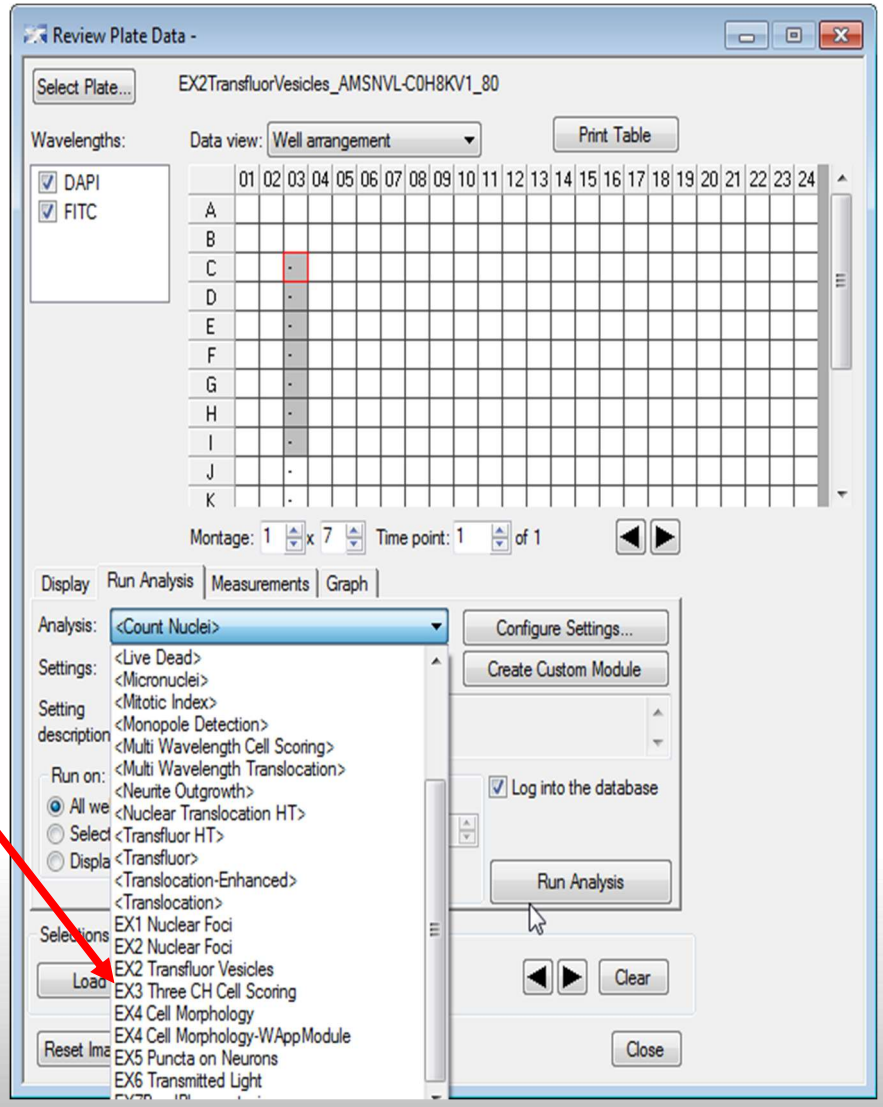
Select *Analysis* method and *Settings file* to run on plate



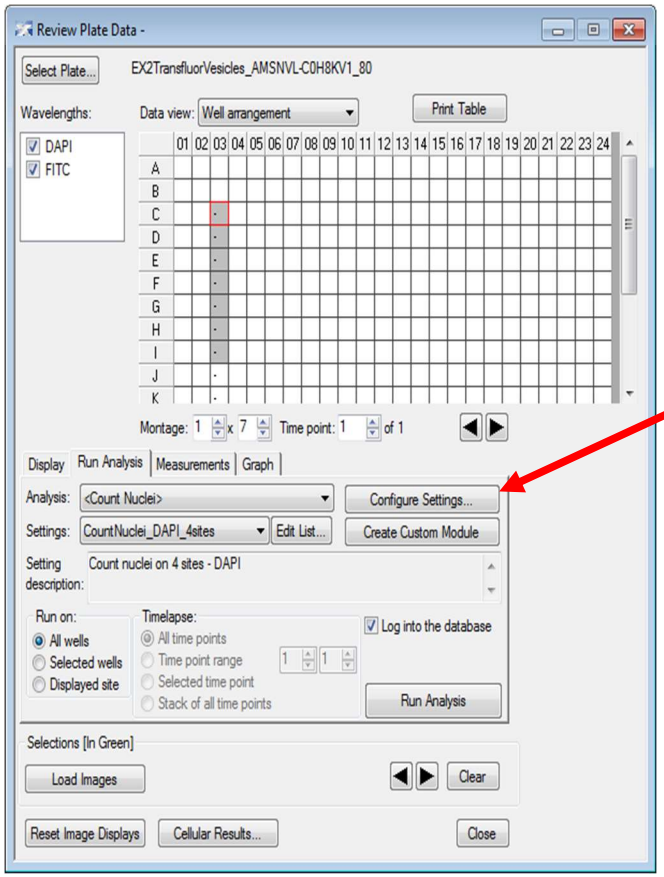
Molecular Devices

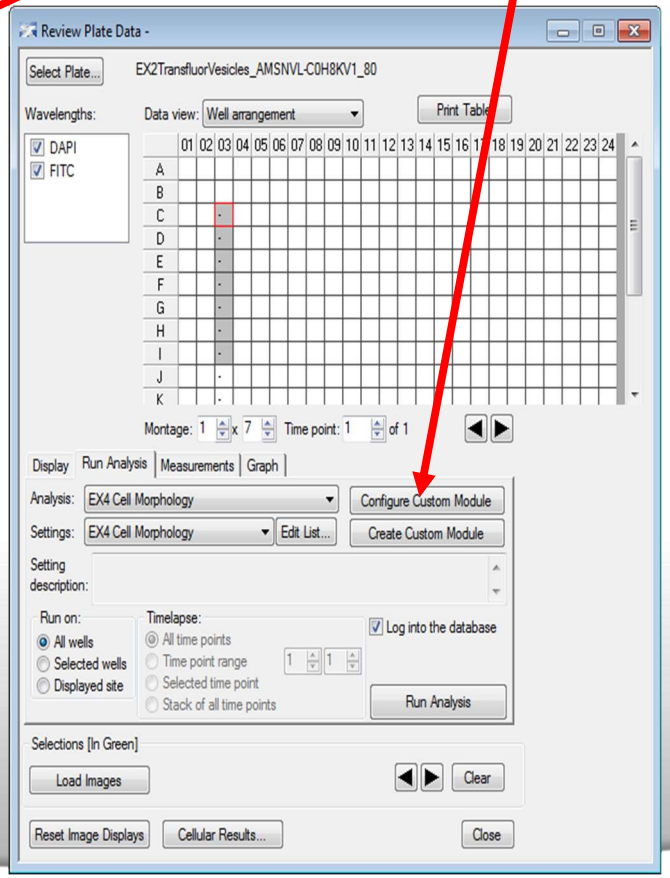
Review Plate Data: Run Analysis Tab

Options missing “<>” are Custom Modules or Journals



Review Plate Data: Run Analysis Tab





Configure module settings

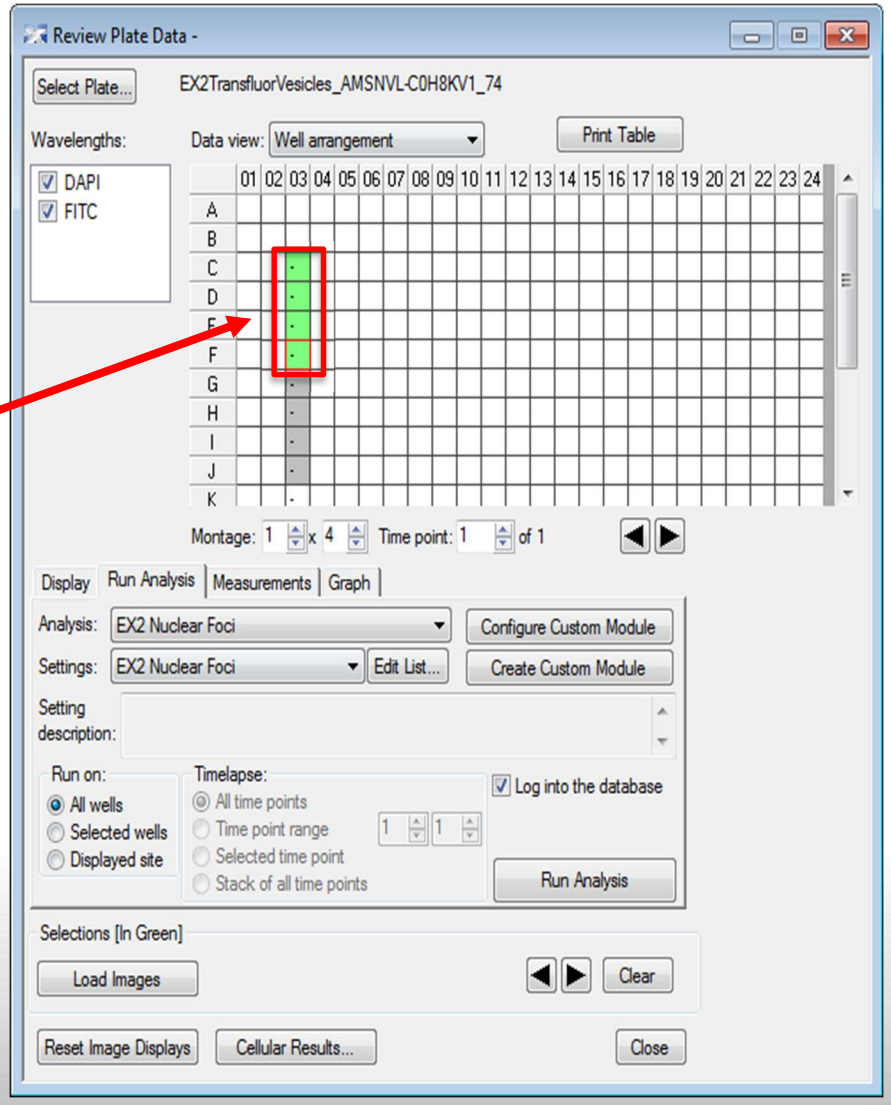
Molecular Devices

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

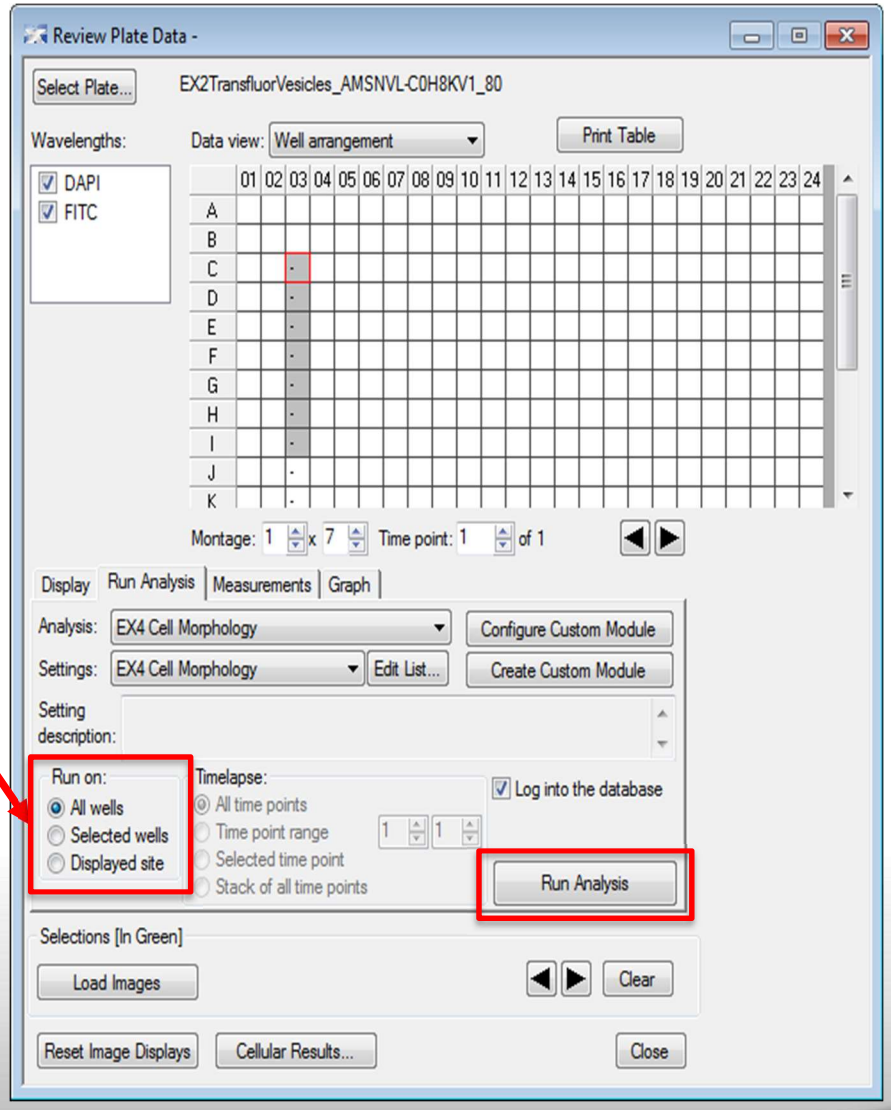


Molecular Devices

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

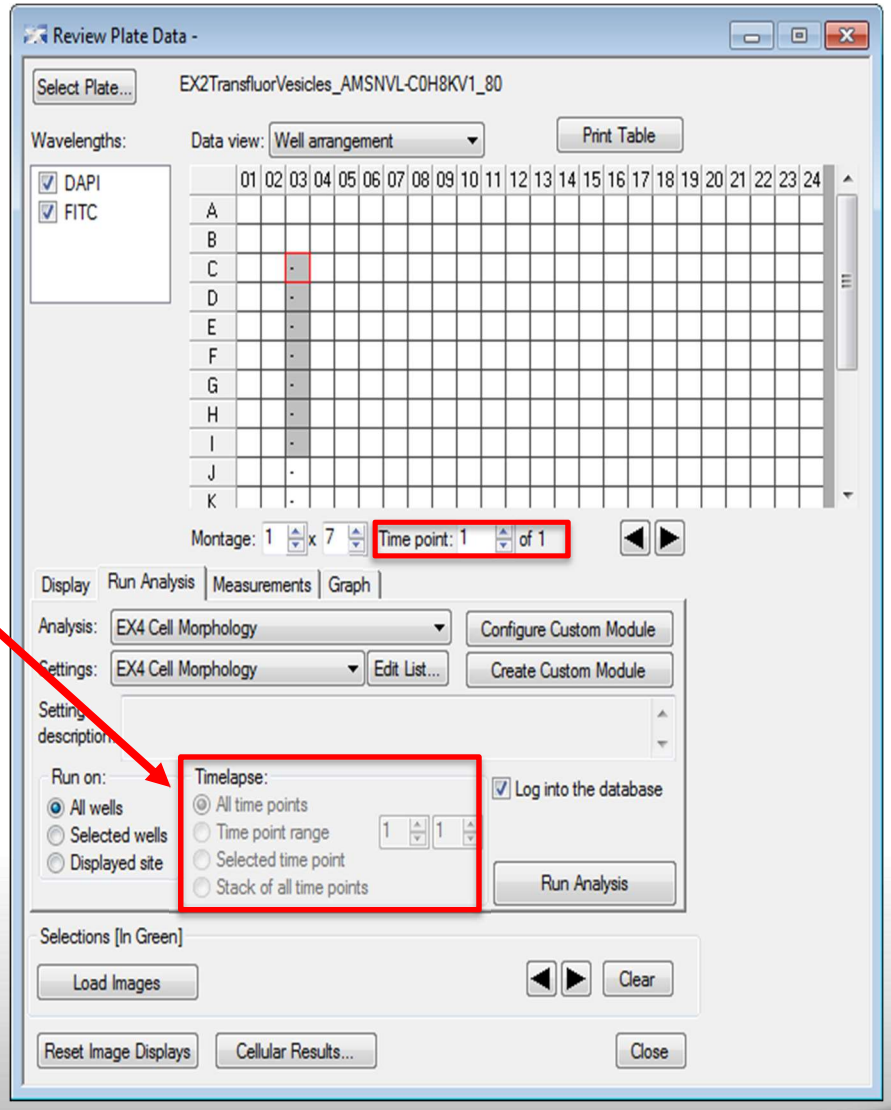


Molecular Devices

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



Molecular Devices

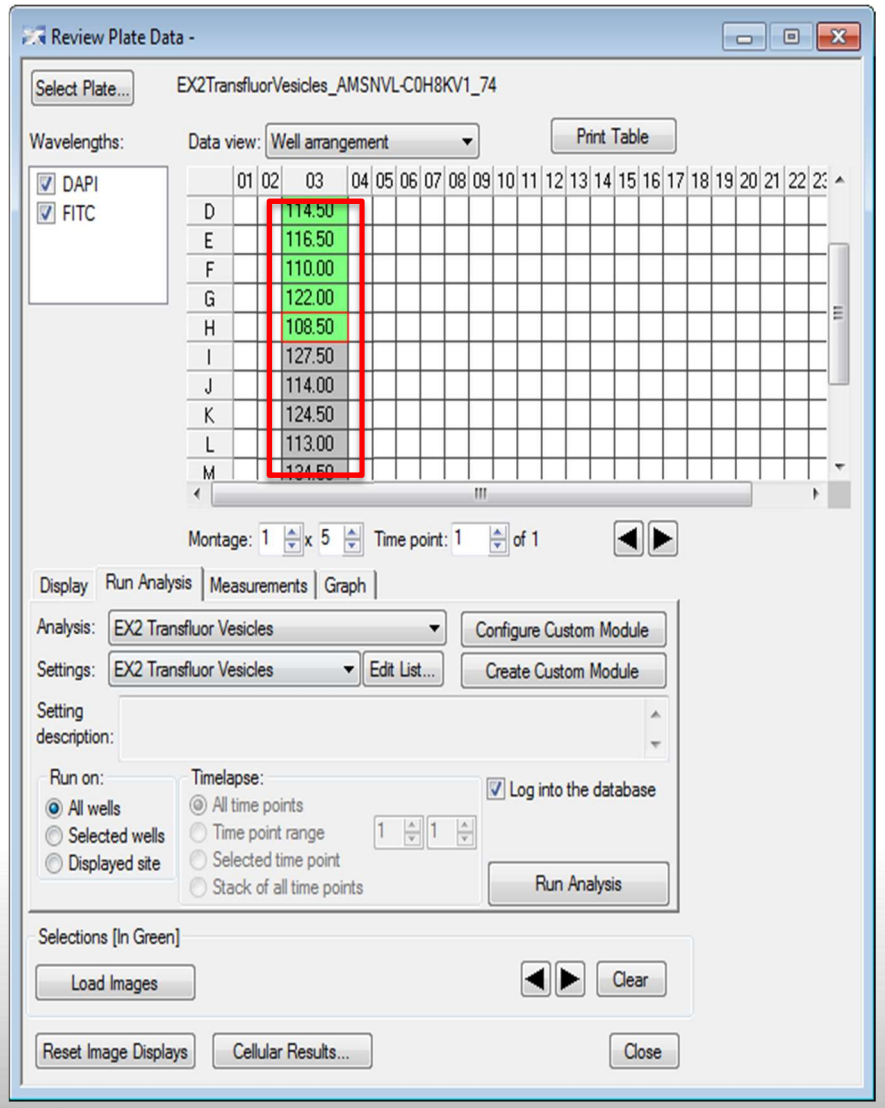
MetaXpress® 5.3 Software

Review Data

Together through life sciences.

Molecular Devices

Review Plate Data: Plate Grid Colors



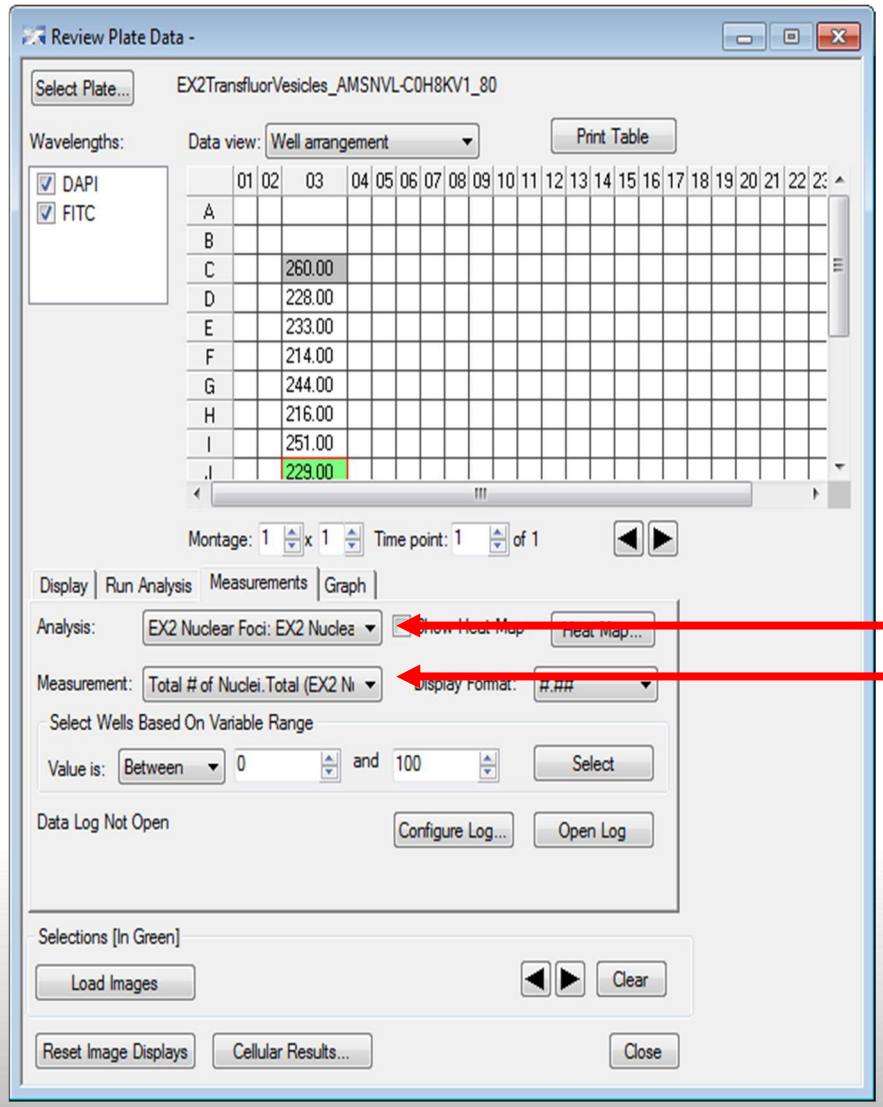
Green Wells: wells selected by user

Grey Wells: wells displayed in montage view

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

Molecular Devices

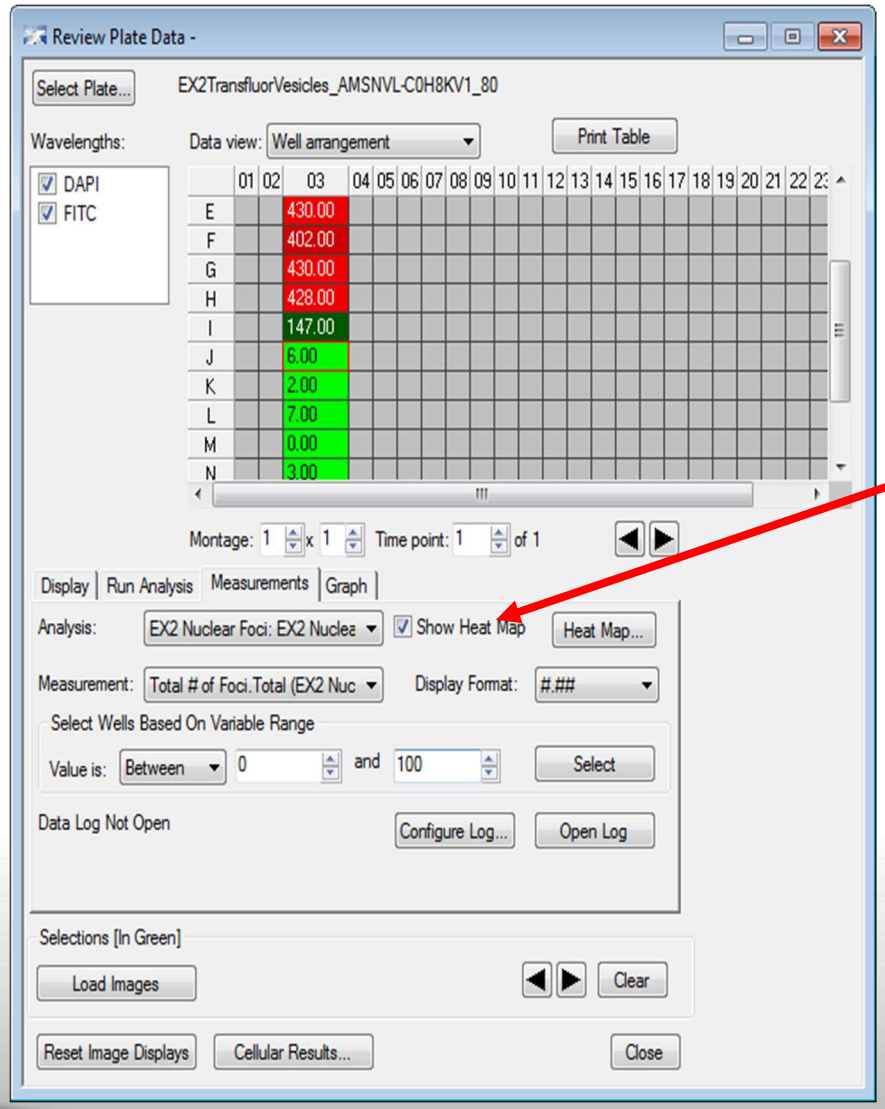
Review Plate Data: Measurements Tab



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

Molecular Devices

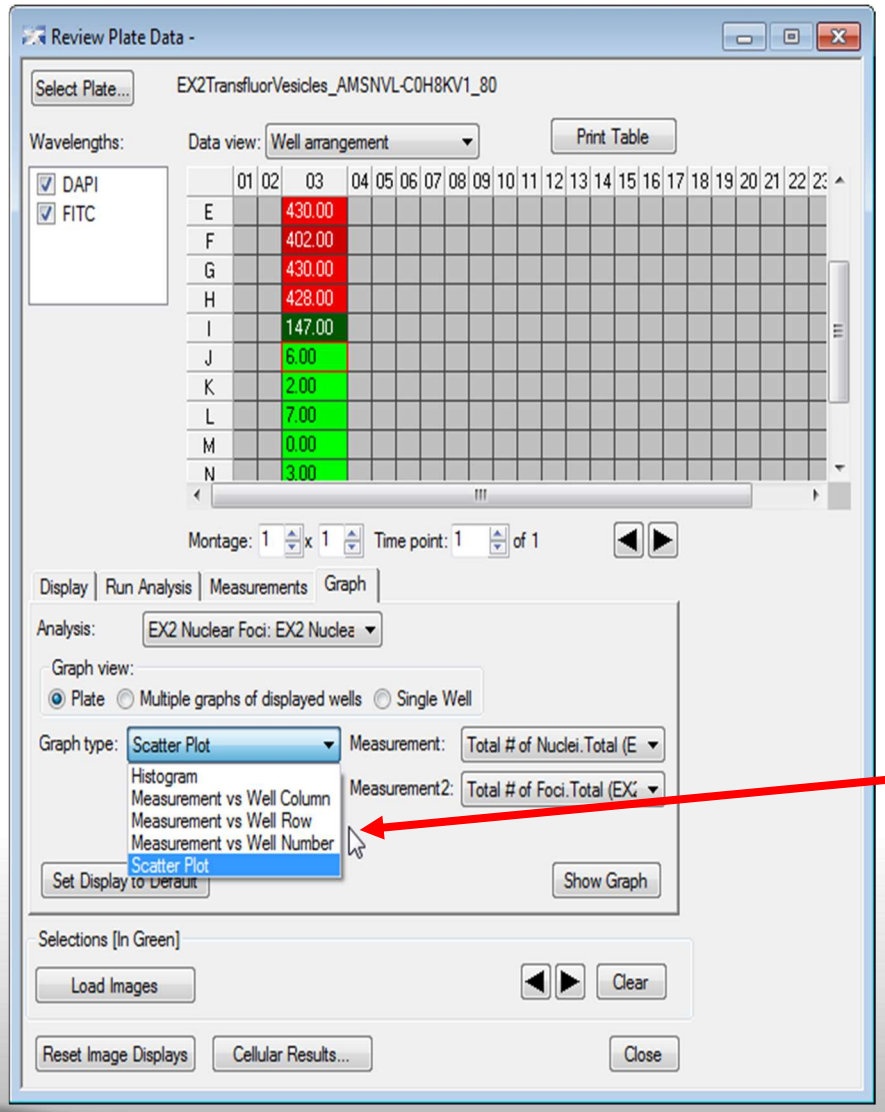
Review Plate Data: Measurements Tab



View data with a heat map overlay

Molecular Devices

Review Plate Data: Graph Tab



Select graph type and configure axes

Molecular Devices

MetaXpress® 5.3 Software

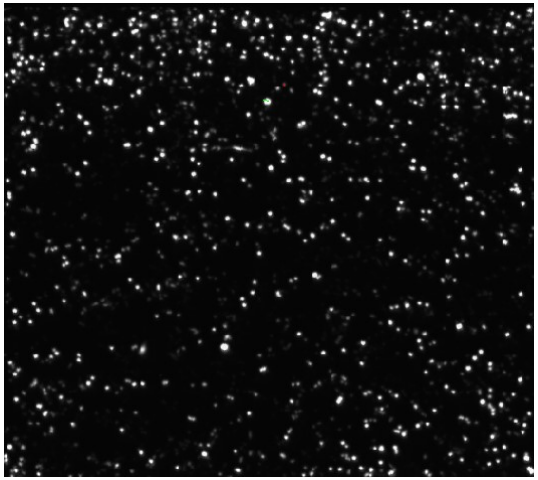
Setting up an Application Module

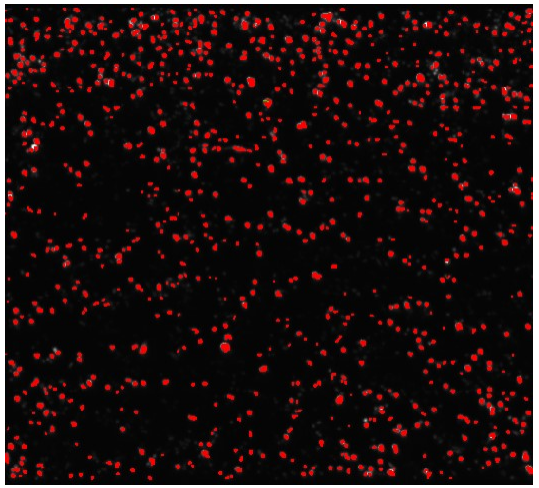
Together through life sciences.

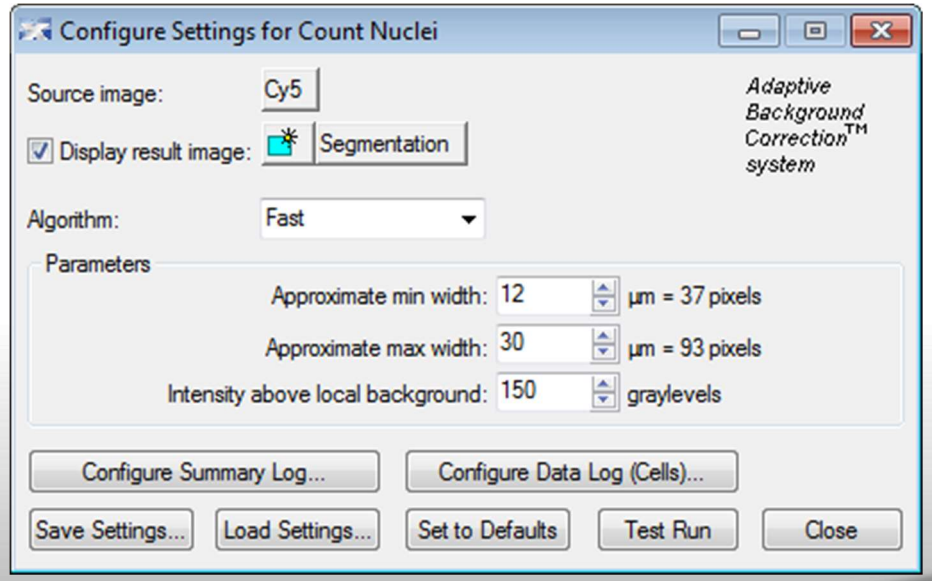
Molecular Devices

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





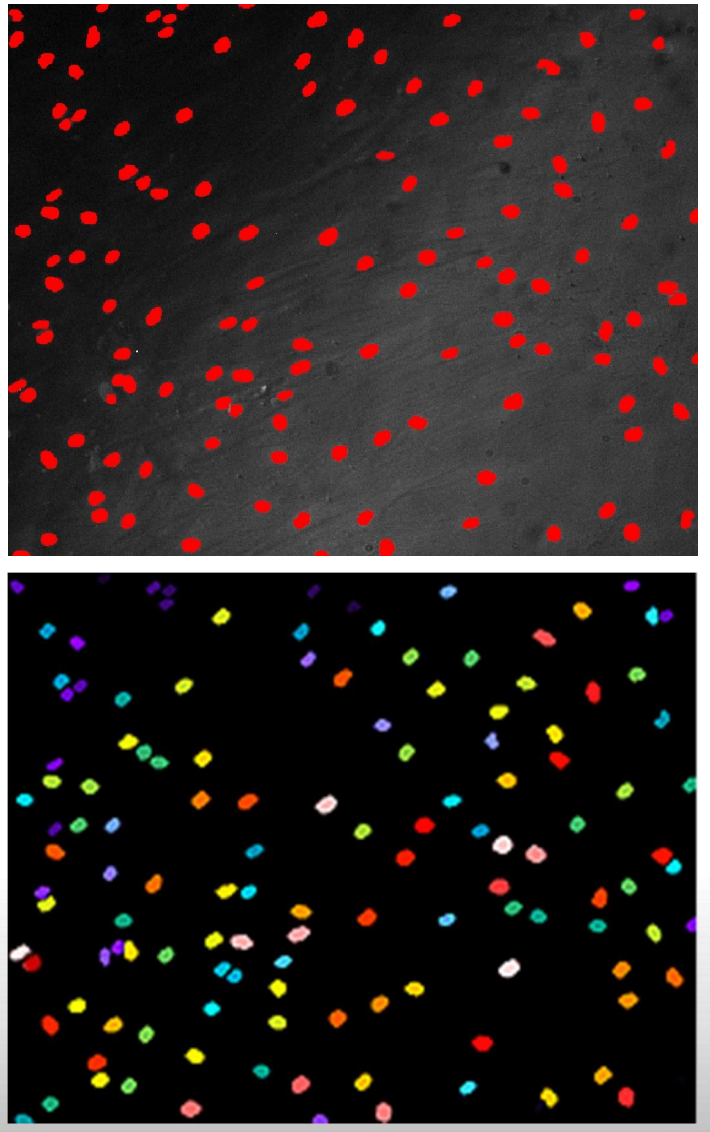


Molecular Devices

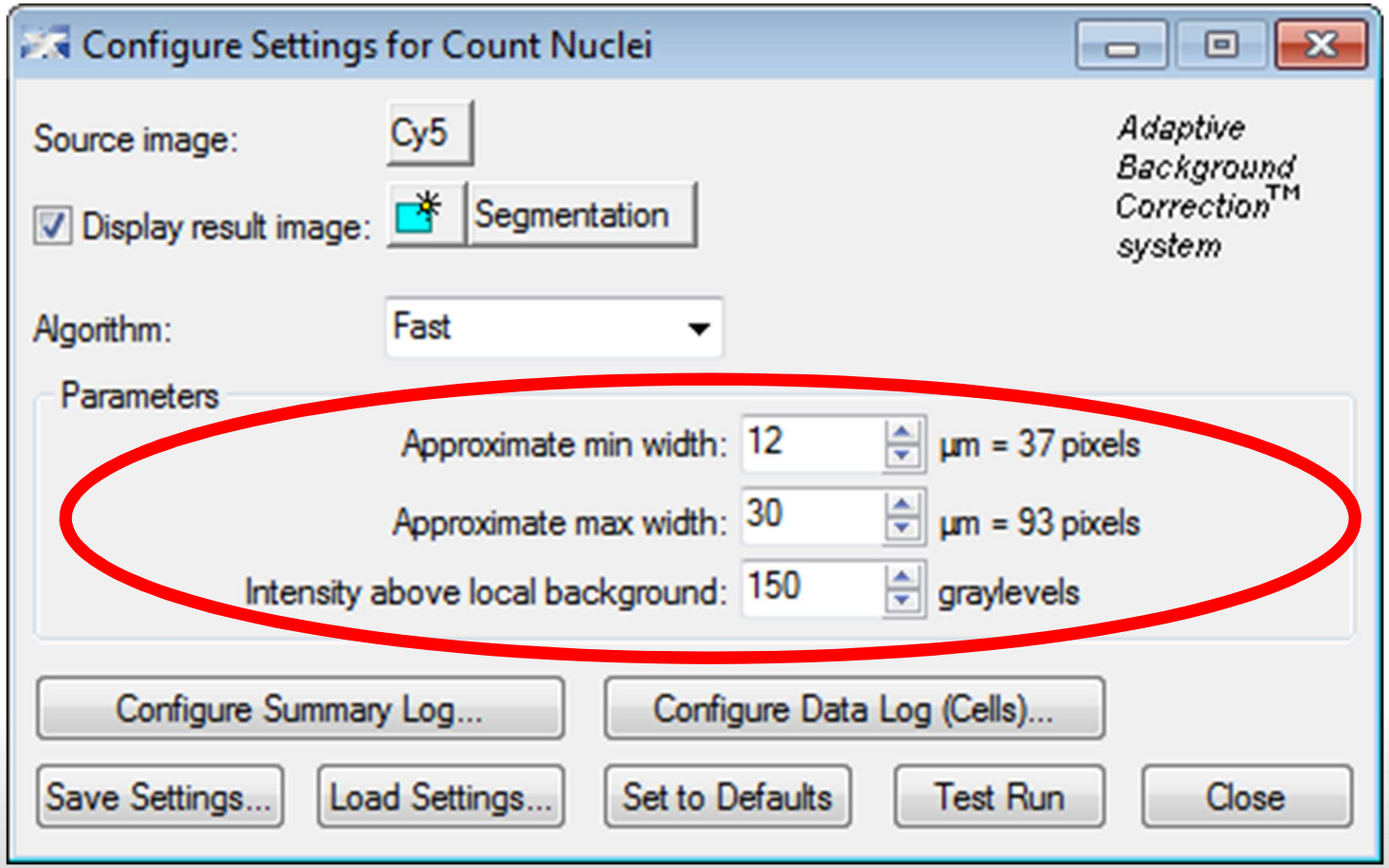
Adaptive Background Correction

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

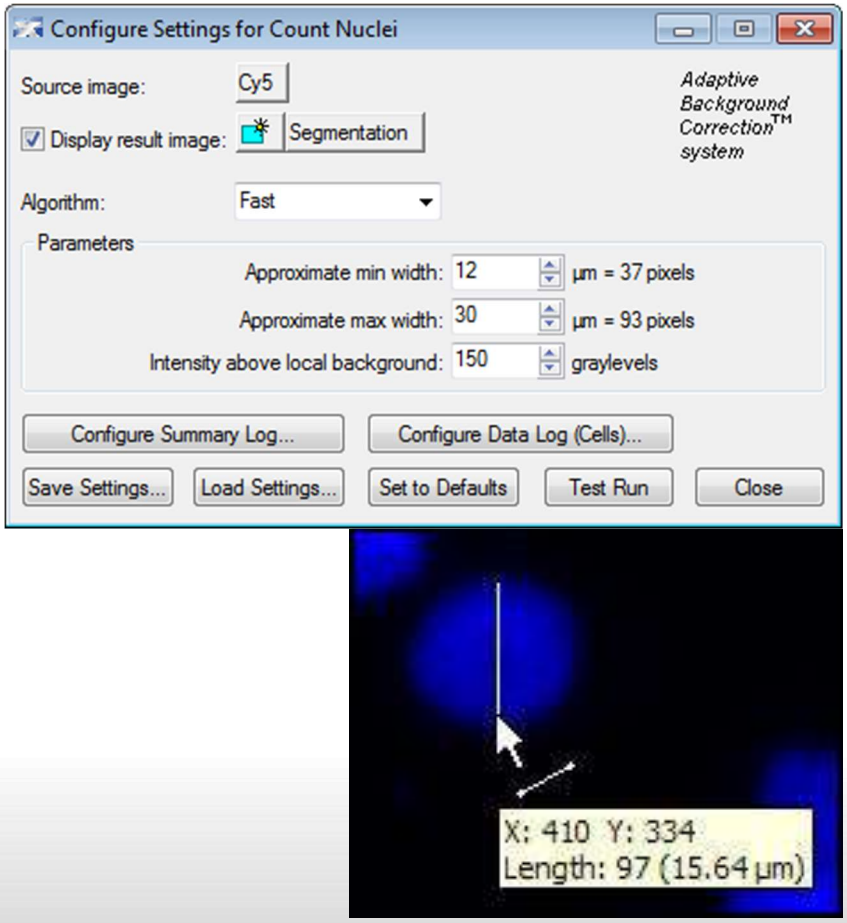


Configuring Settings – The Basics



Molecular Devices

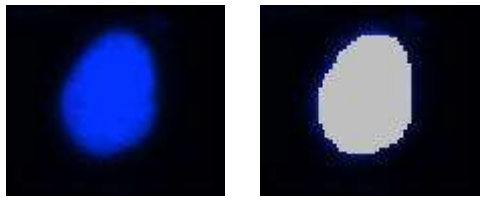
Module Settings – Measurement Parameters



- **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 um)
- 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



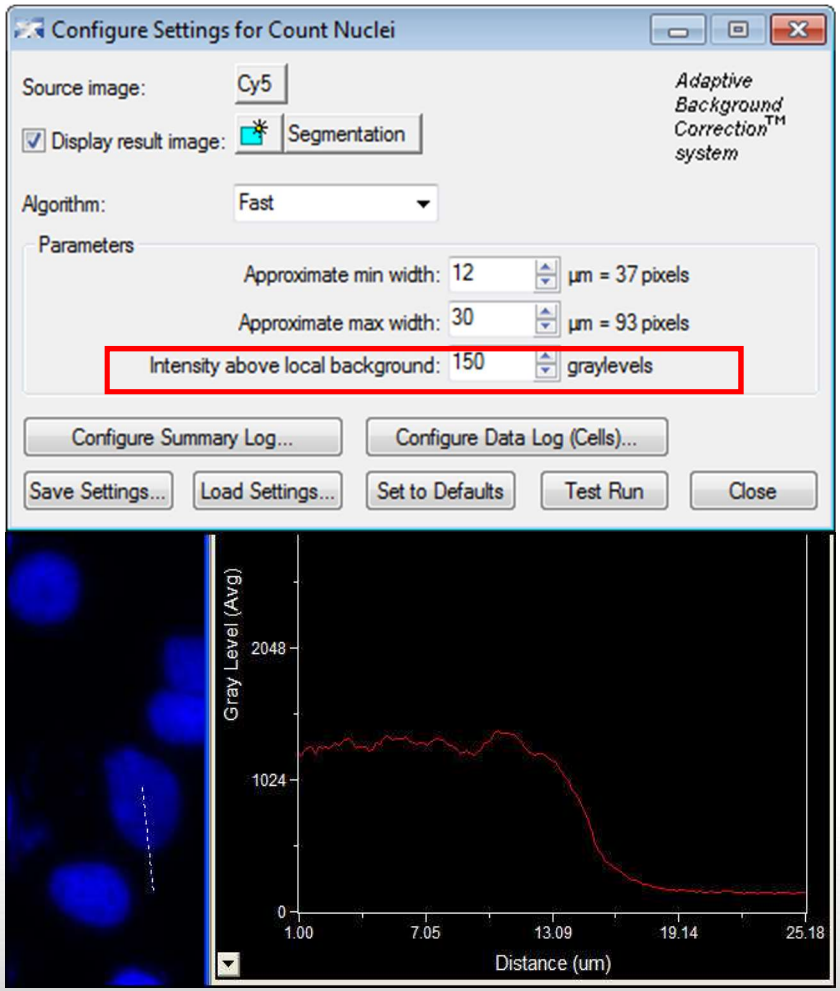
- Min width too small: splits nuclei

- 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

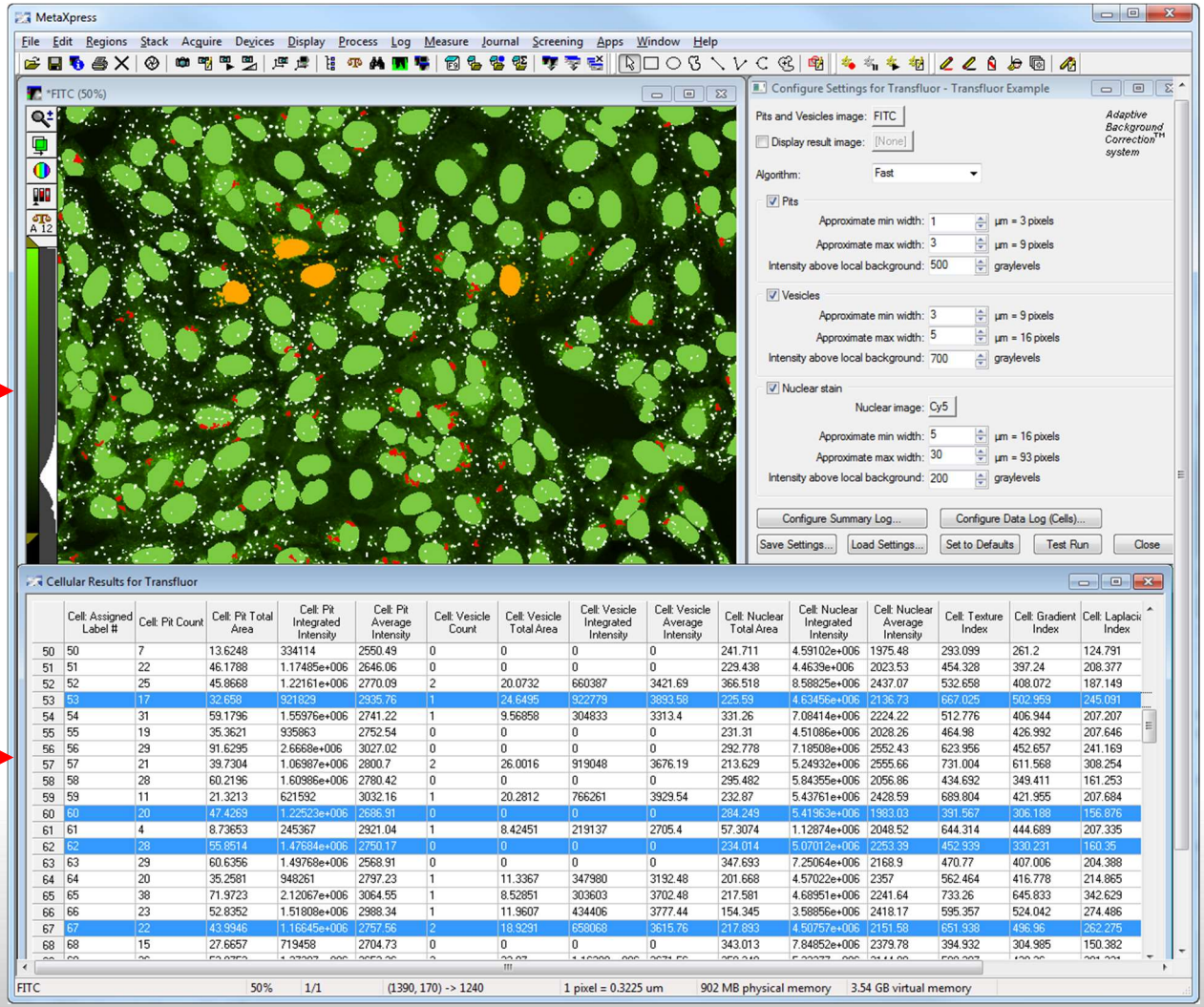
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background

- 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)

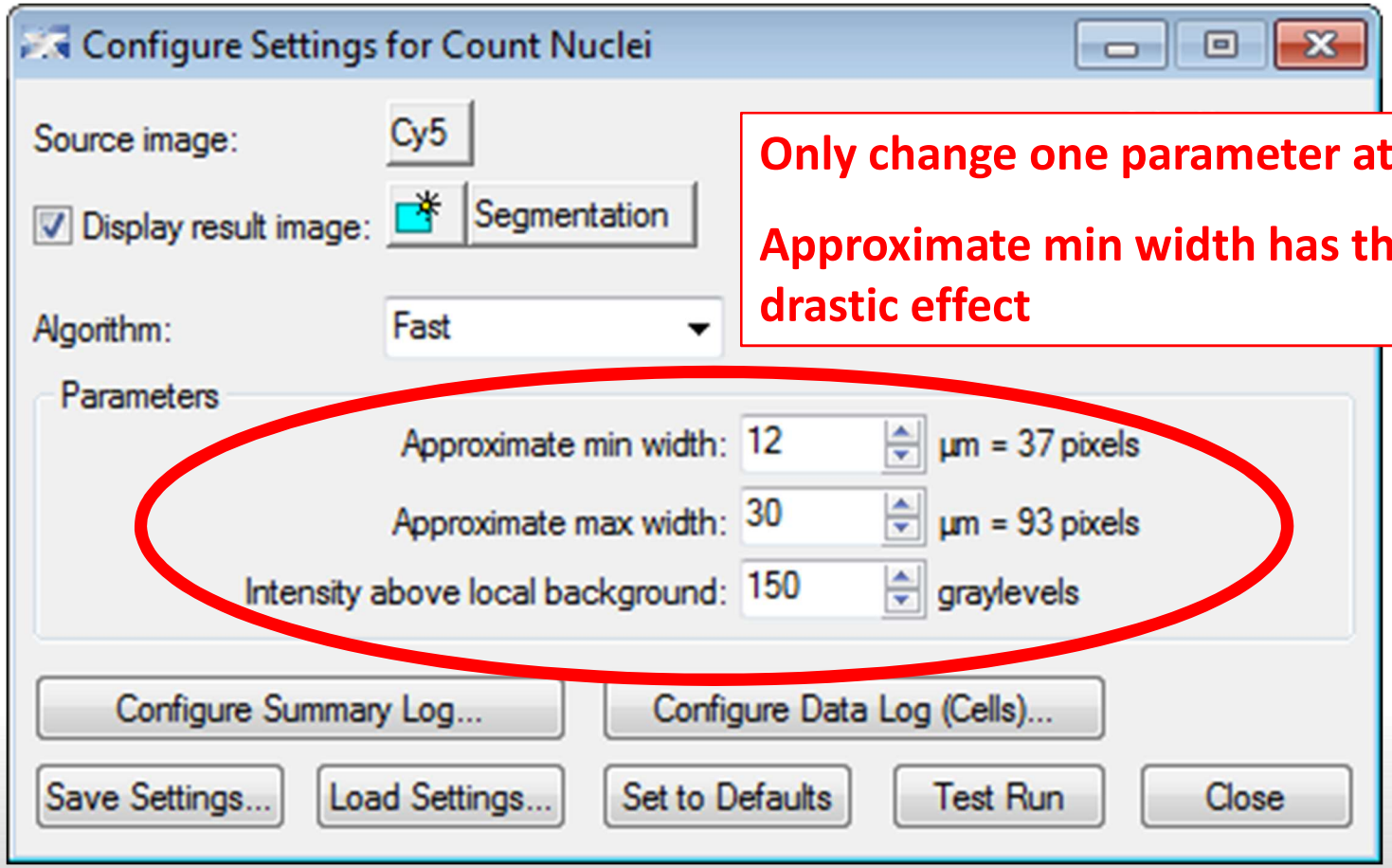
Cell-by-cell analysis results linked to image



	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: Laplaci Index
50	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	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	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	53	17	32.658	921829	2935.76	1	24.6495	922779	3893.58	225.59	4.63456e+006	2136.73	667.025	502.959	245.091
54	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	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	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	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	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	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	60	20	47.4269	1.22523e+006	2686.91	0	0	0	0	284.249	5.41963e+006	1983.03	391.567	306.188	156.876
61	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	62	28	55.8514	1.47684e+006	2750.17	0	0	0	0	234.014	5.07012e+006	2253.39	452.939	330.231	160.35
63	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	64	20	35.2581	948261	2797.23	1	11.3367	347980	3192.48	201.668	4.57022e+006	2357	562.464	416.778	214.865
65	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	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	67	22	43.9946	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	68	15	27.6657	719458	2704.73	0	0	0	0	343.013	7.84852e+006	2379.78	394.932	304.985	150.382

Molecular Devices

Optimizing Settings



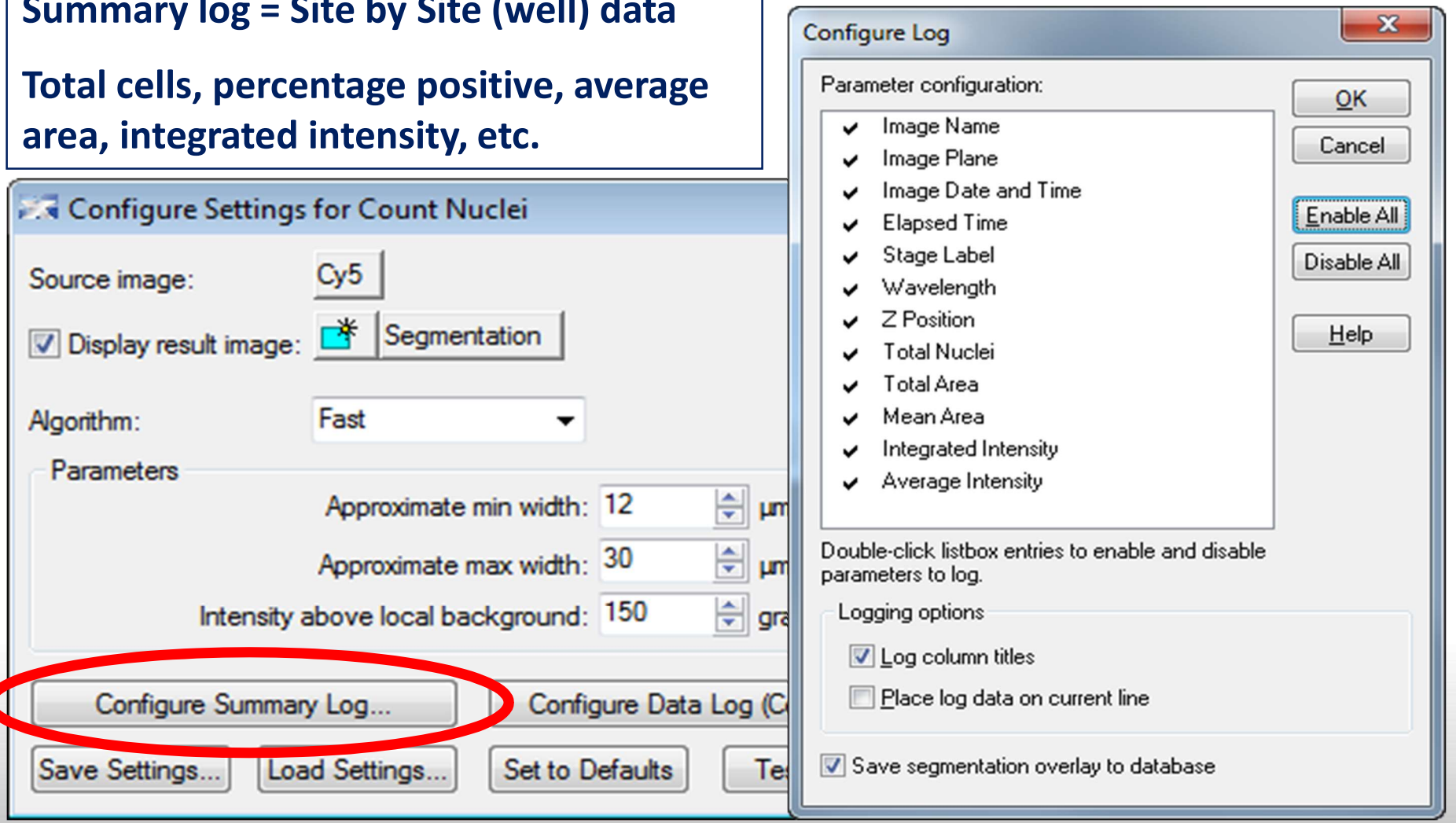
Only change one parameter at a time

Approximate min width has the most drastic effect

Selecting Measurements

Summary log = Site by Site (well) data

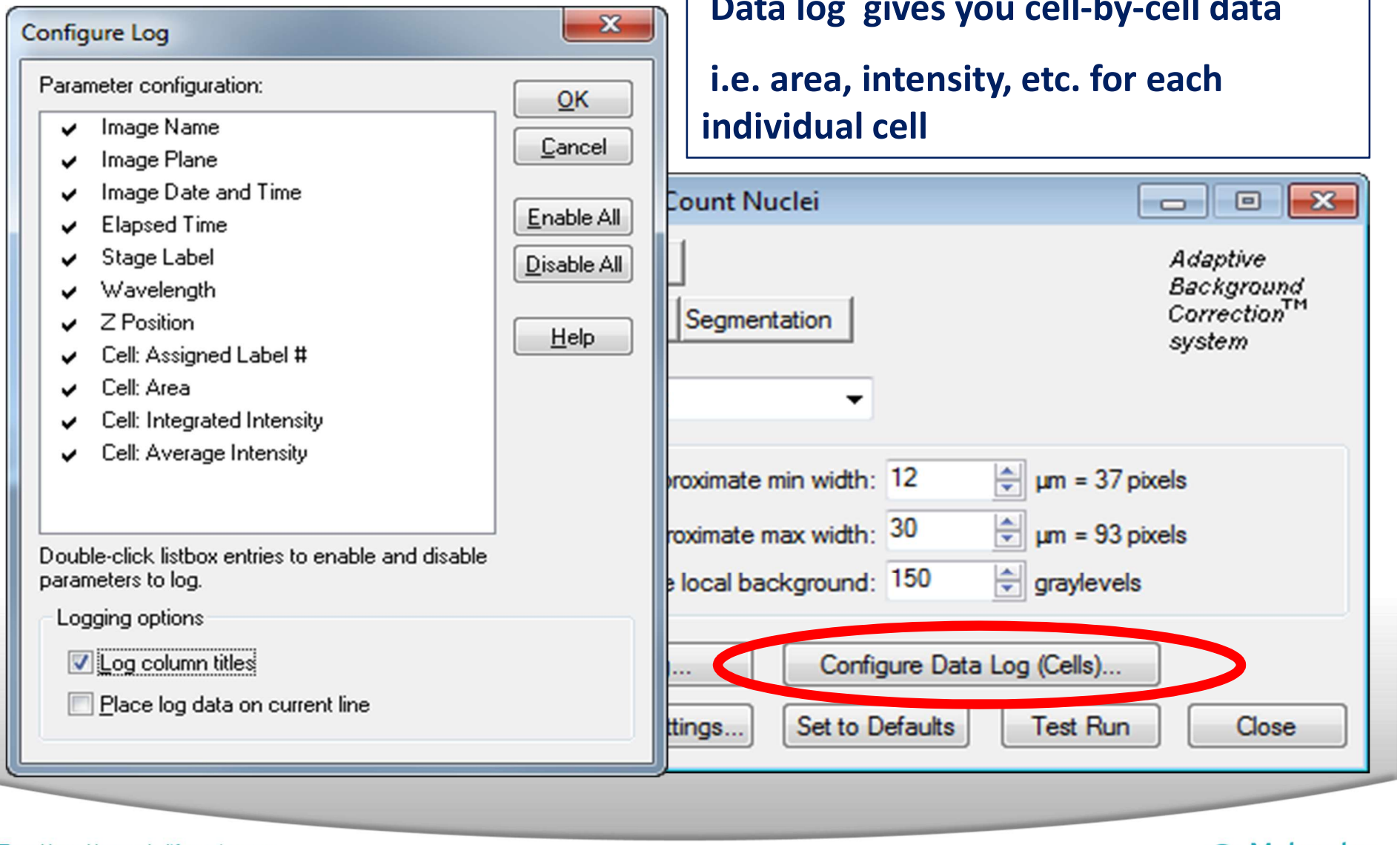
Total cells, percentage positive, average area, integrated intensity, etc.



Selecting Measurements

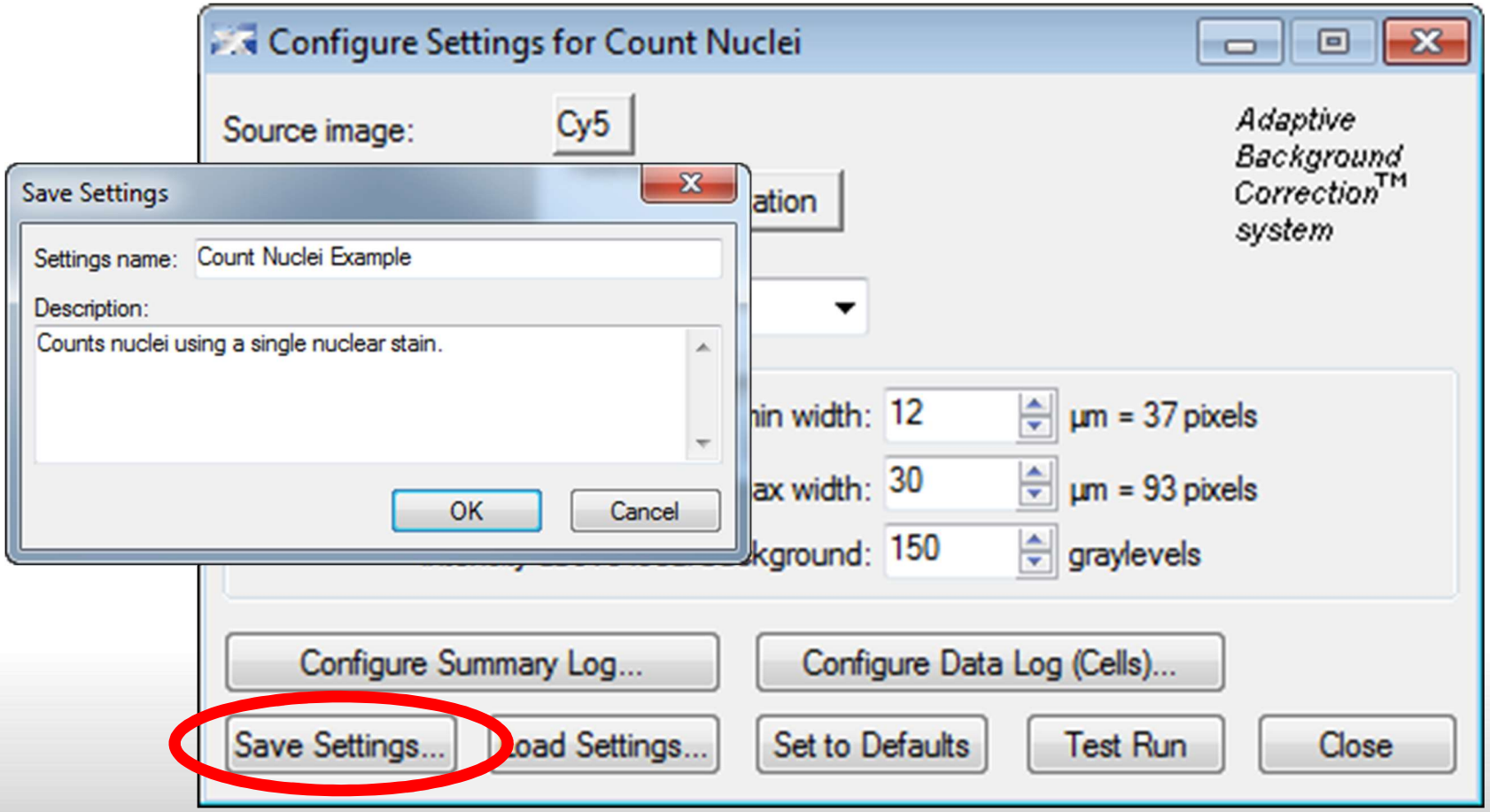
Data log gives you cell-by-cell data

i.e. area, intensity, etc. for each individual cell



Molecular Devices

Save Settings to the database



MetaXpress® 5.3 Software

Batch Analysis

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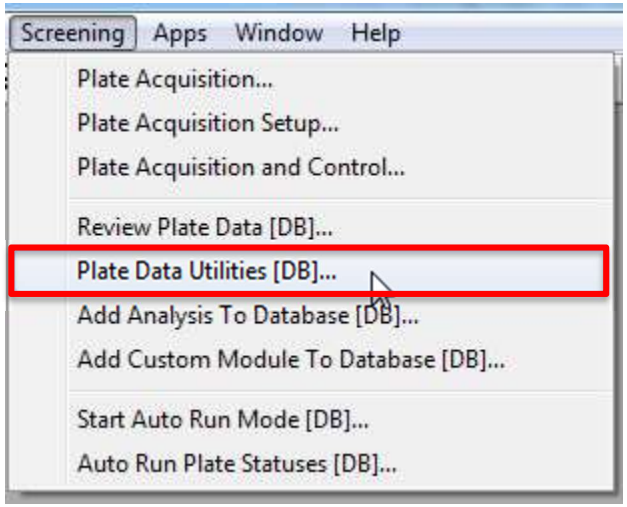
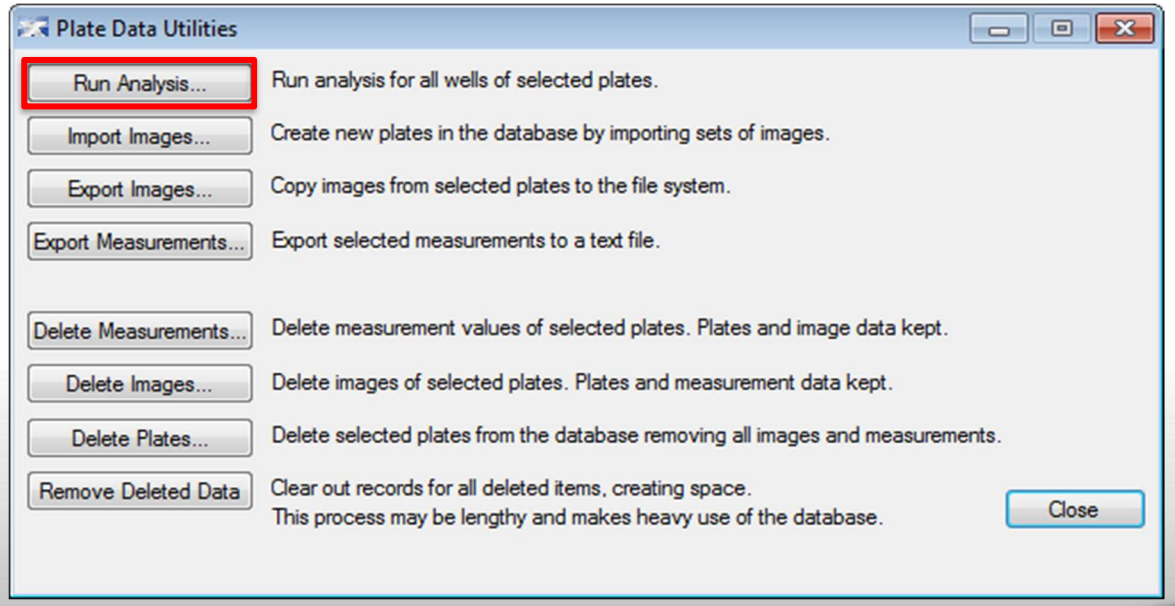
Molecular Devices

Batch Analysis

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



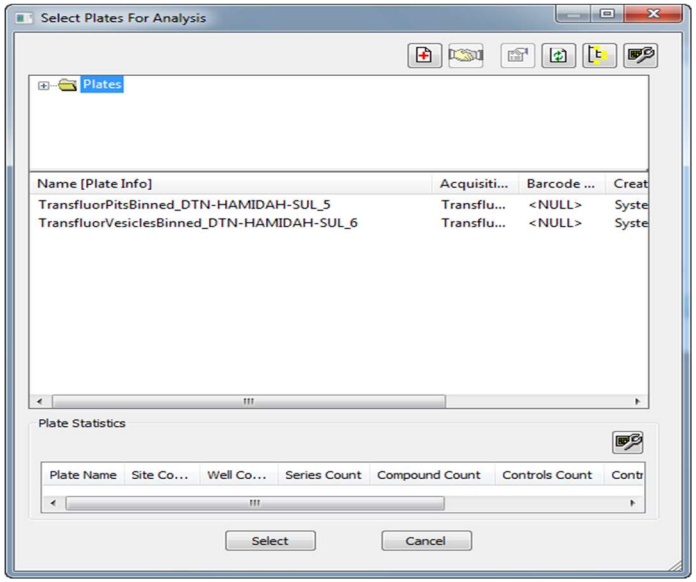
Click on **Run Analysis** button



Molecular Devices

Batch Analysis

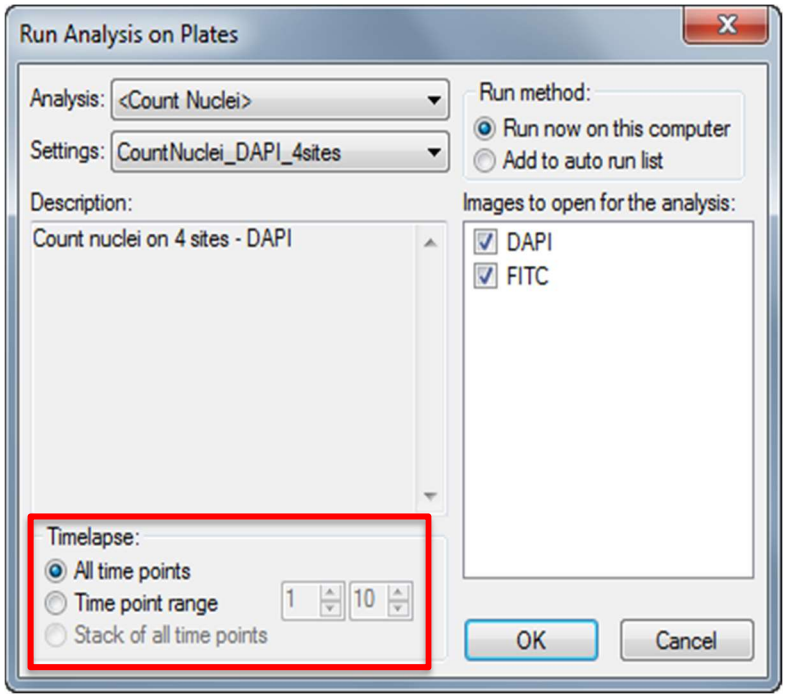
In the Select Plates for Analysis dialog box, highlight the plates you want to run the analysis on and click the **Select** button



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



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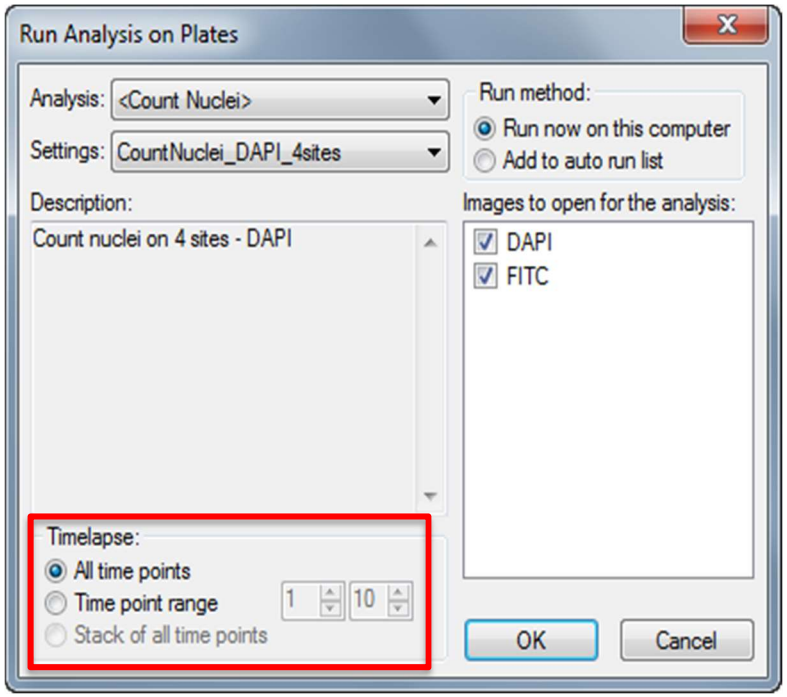
Batch Analysis

- 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



Molecular Devices

MetaXpress® 5.3 Software

Exporting Data

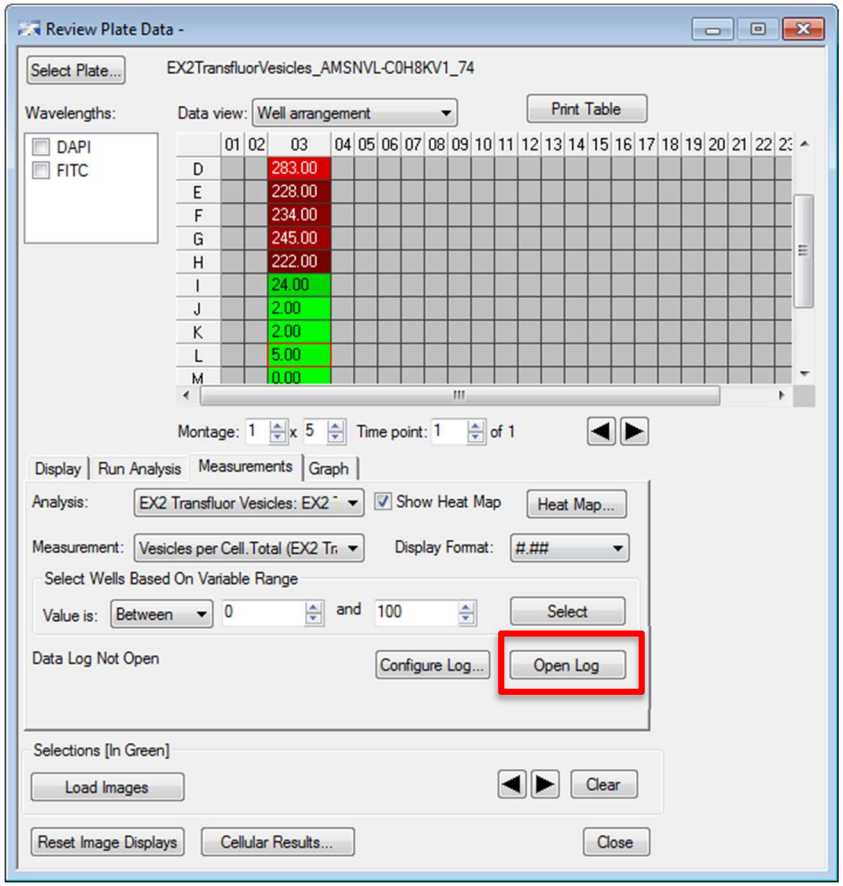
Review Plate Data: Opening a Data Log

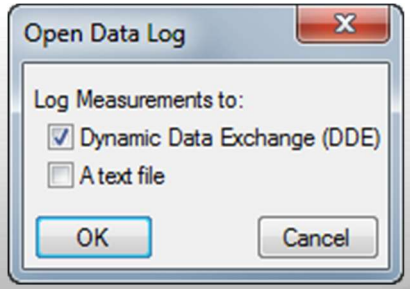
Method 1

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

Method 2

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





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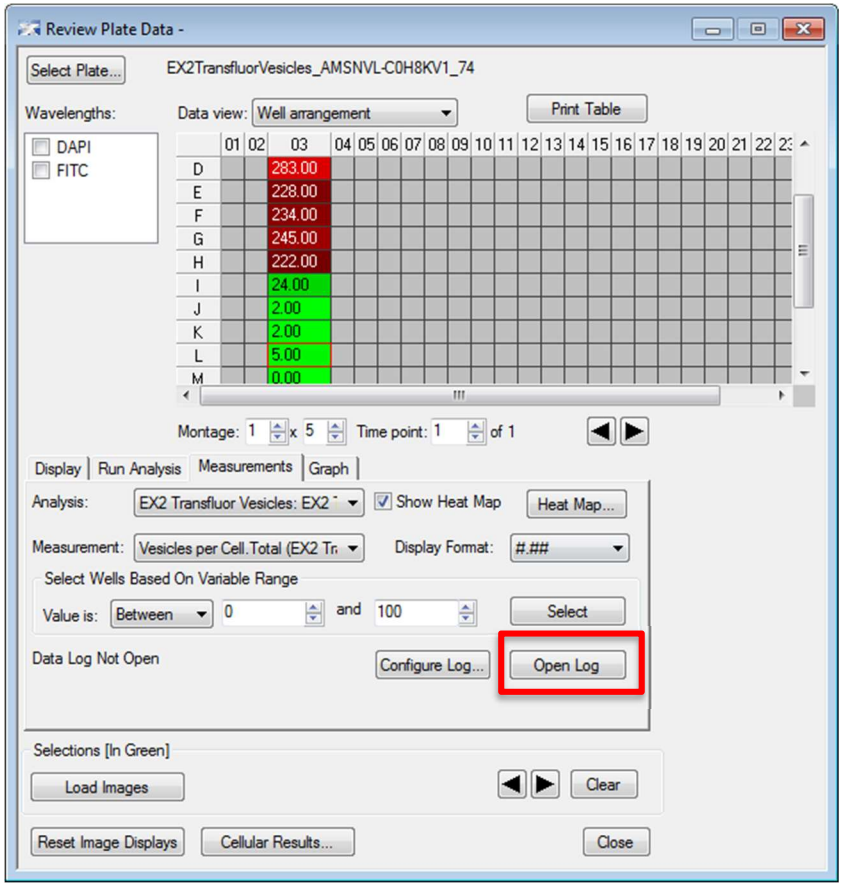
Review Plate Data: Export to Excel / Text File

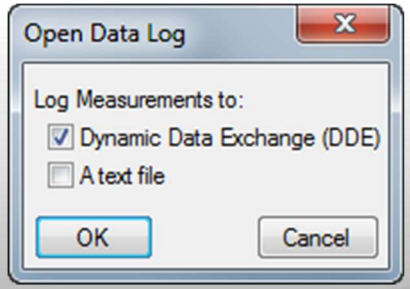
Select

- **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**





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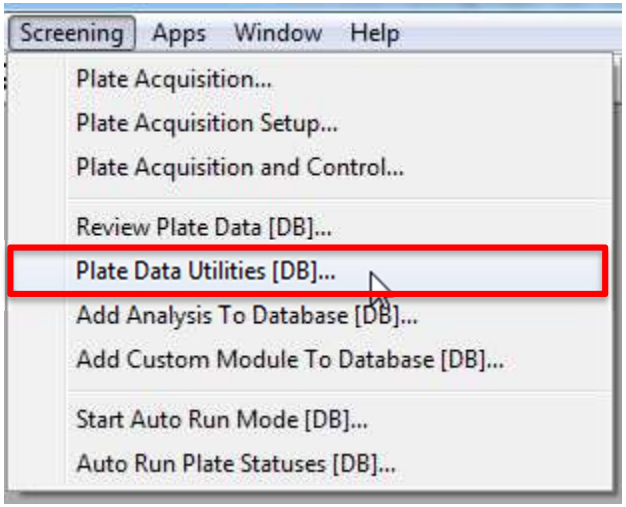
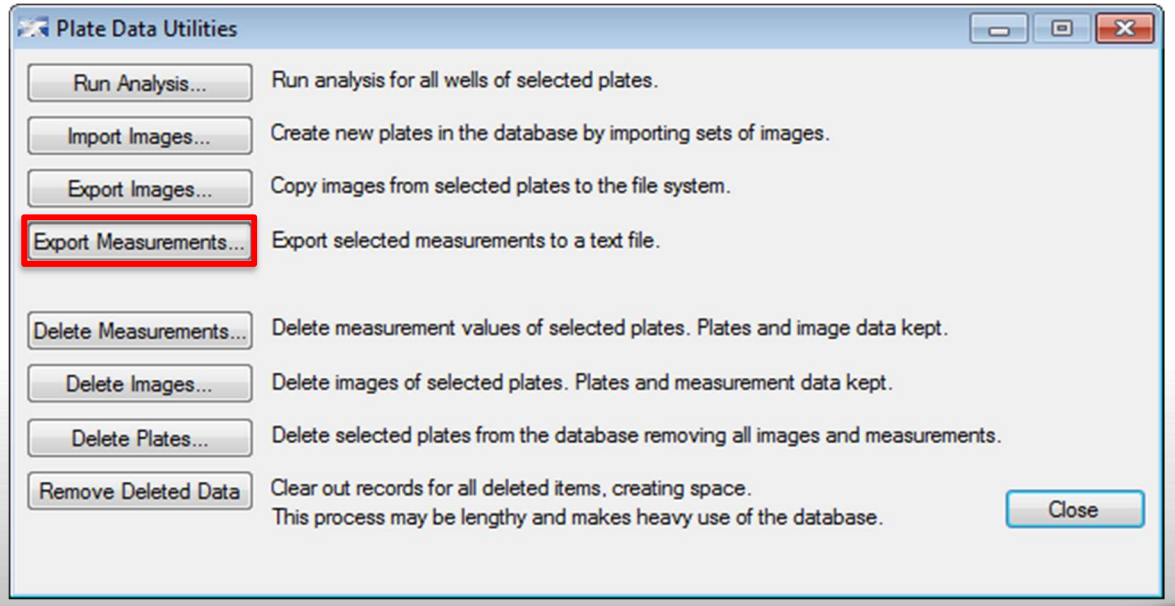
Batch Export Data

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



Click on **Export Measurements…** button

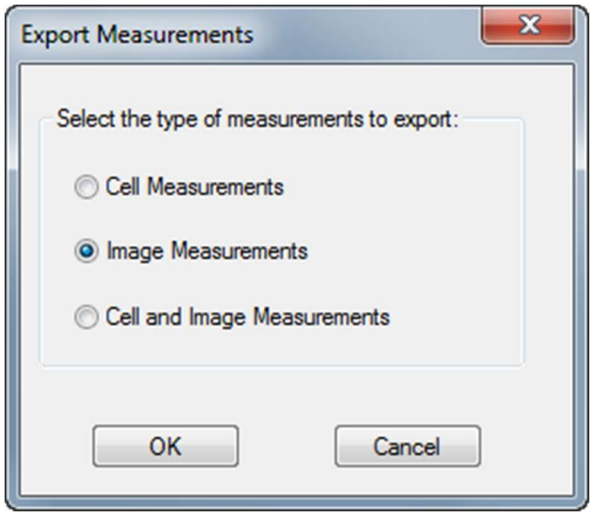


Molecular Devices

Batch Export Data: Selecting Measurement Type and Plates

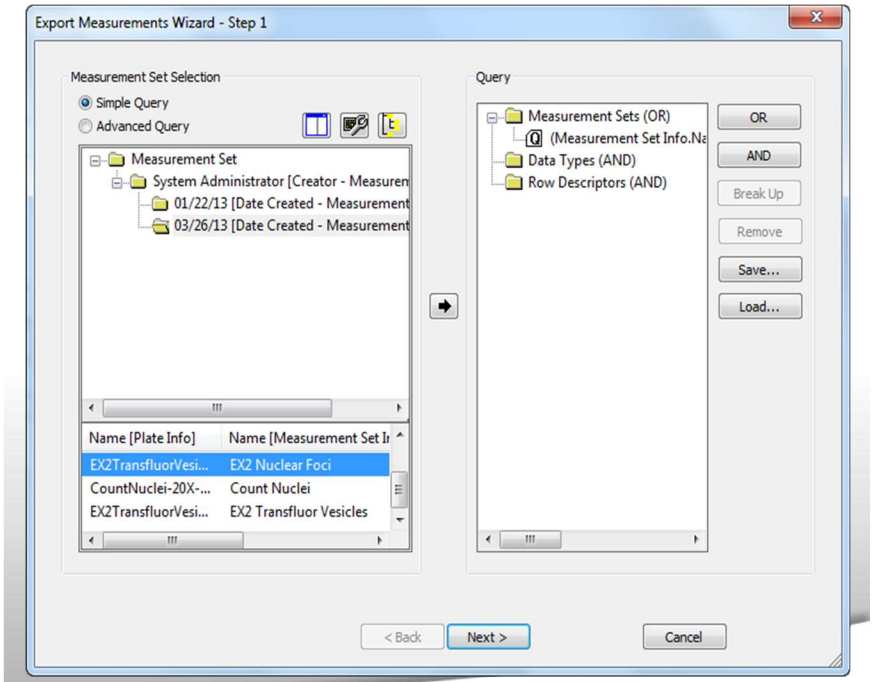
Type of Measurements to export

- **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**



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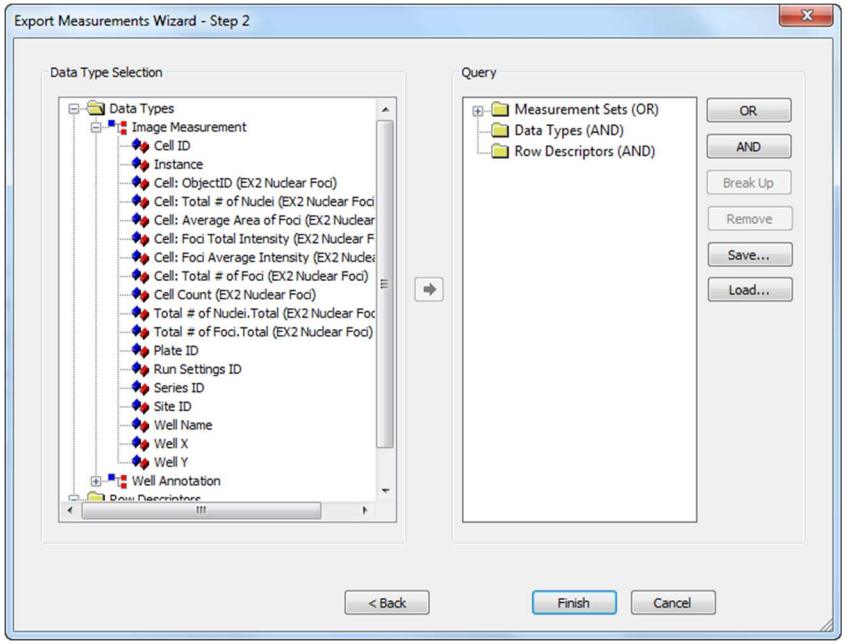
Batch Export Data: Query Data

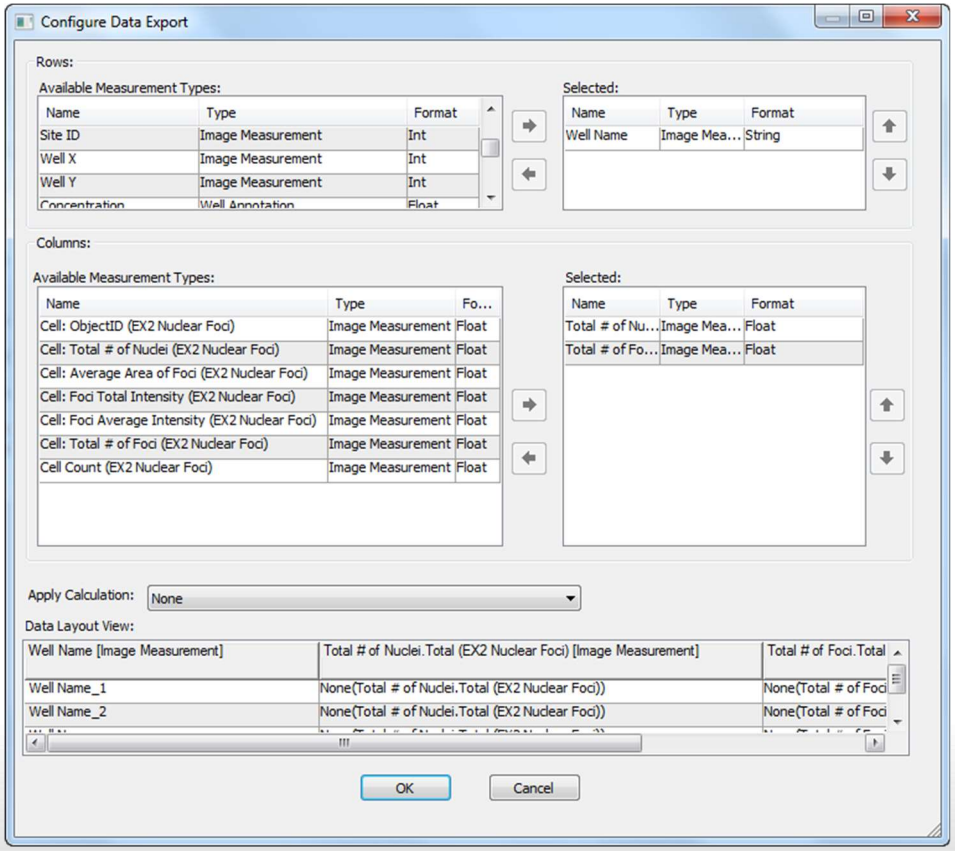
Export Measurements Wizard – Step 2

- 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**

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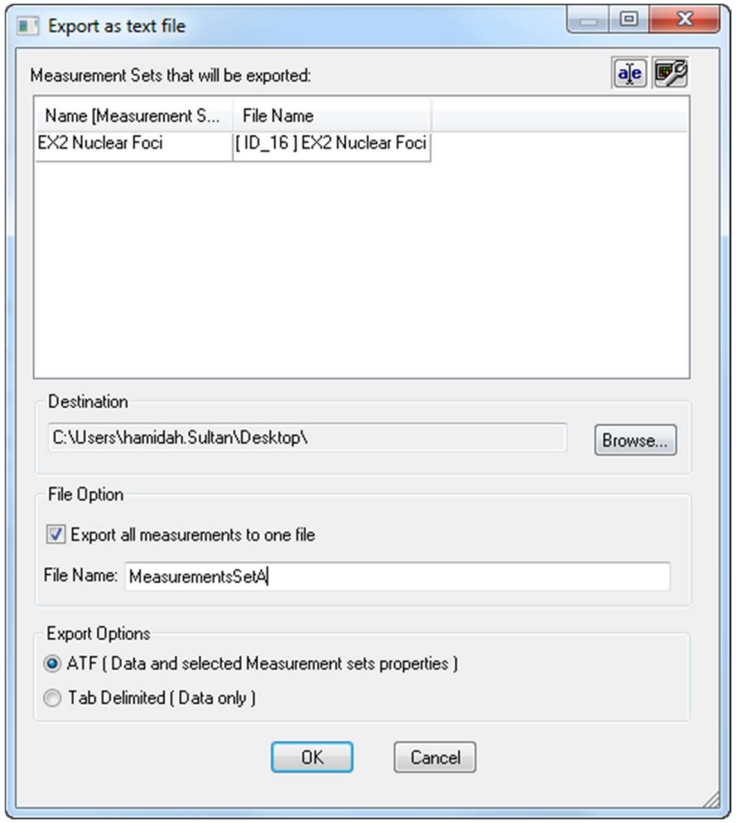
Batch Export Data: Save File

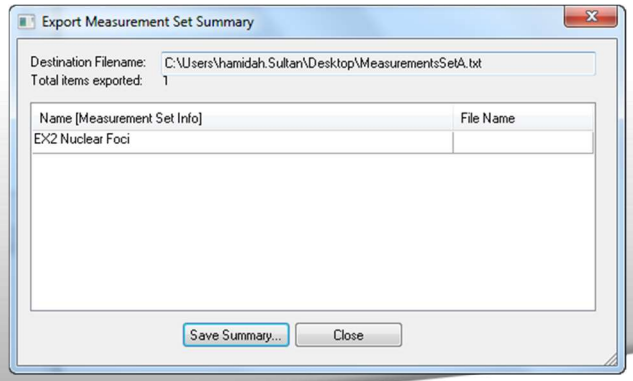
Saving the file

- **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**



Molecular Devices

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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