

**Molecular
Devices**

Together through life sciences.

MetaXpress® Software: *Analysis Training*

Together through life sciences.

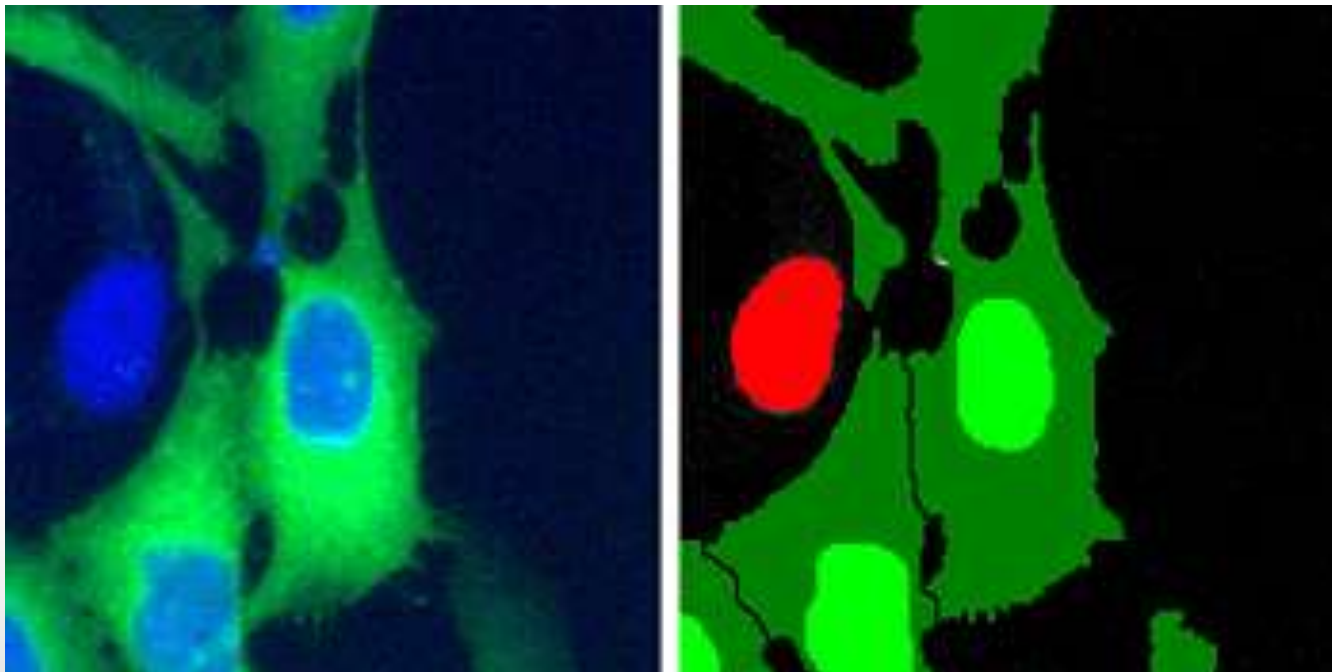
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**Molecular
Devices**

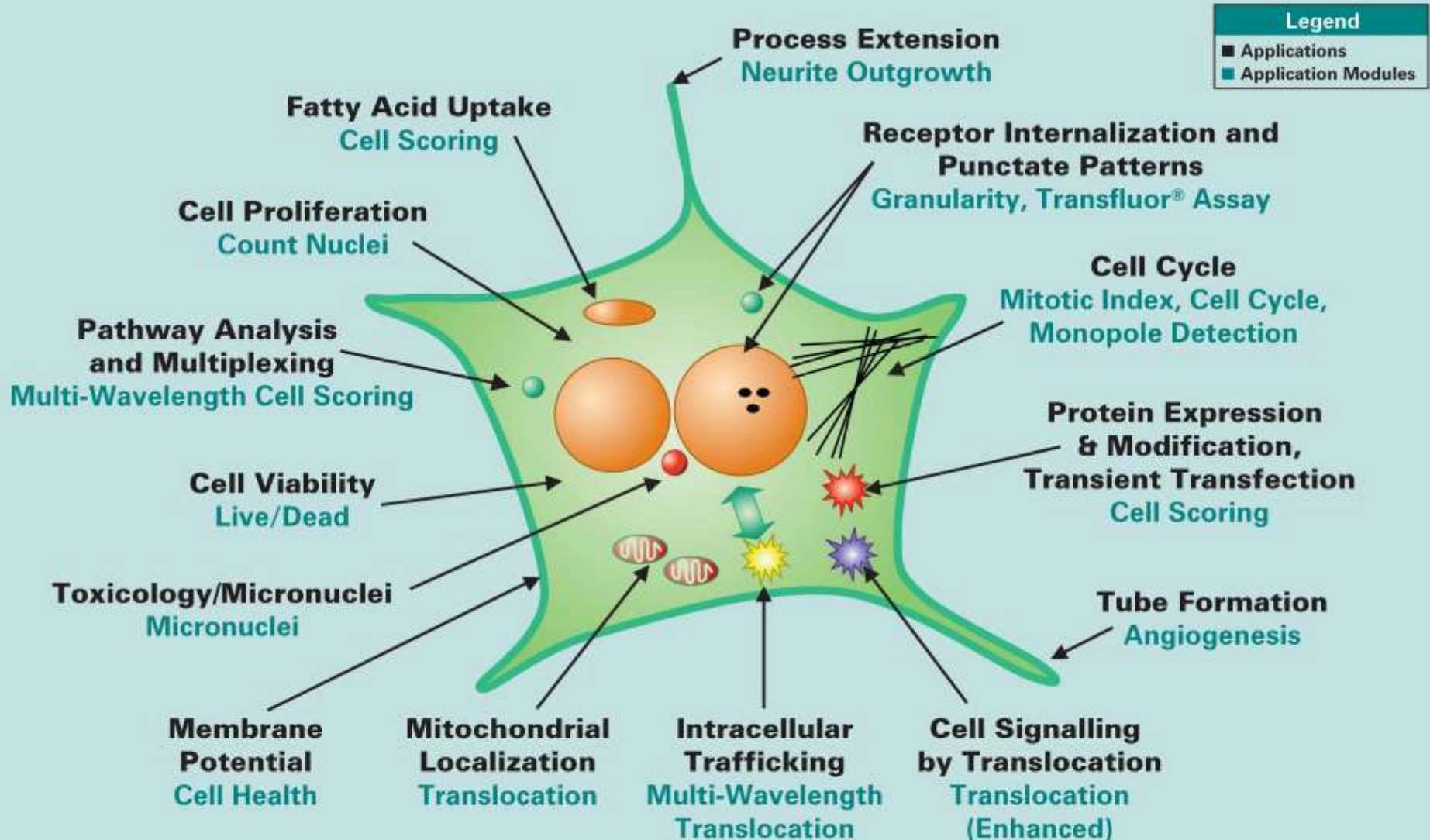
MetaXpress: Application Modules

- “Canned,” walk-away automation
- Advanced segmentation, feature detection, and measurement
- Site-by-site and cell-by-cell data
- Validated results
- Can be incorporated into a journal for increased customization



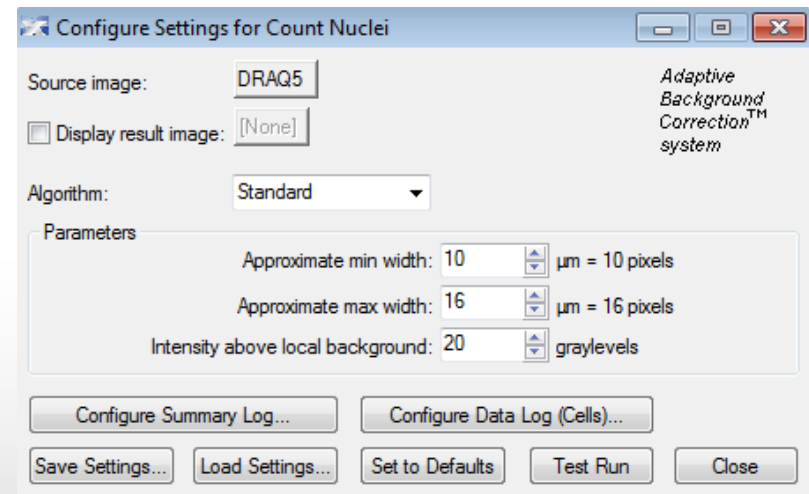
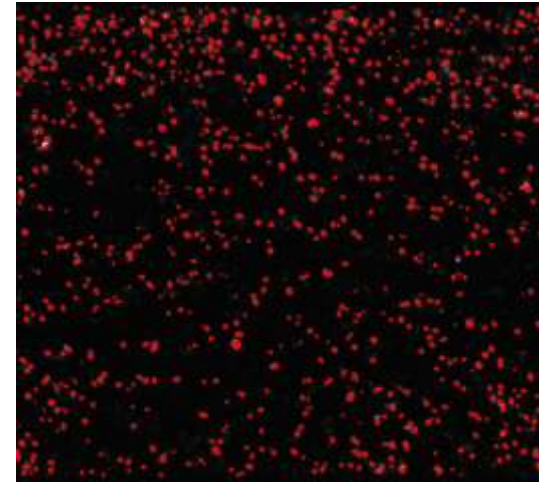
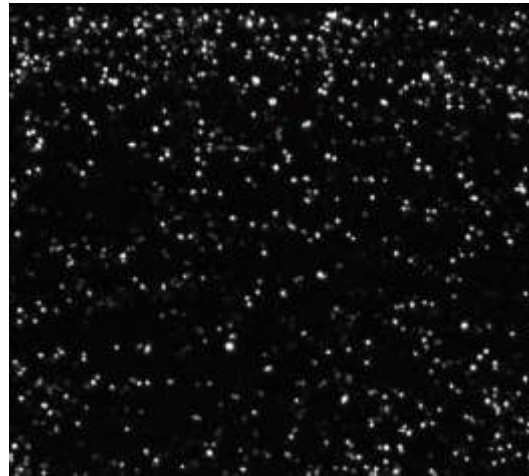
Application Modules for Hundreds of Assays

Easy Custom Analysis with Journaling



MetaXpress: Application Modules

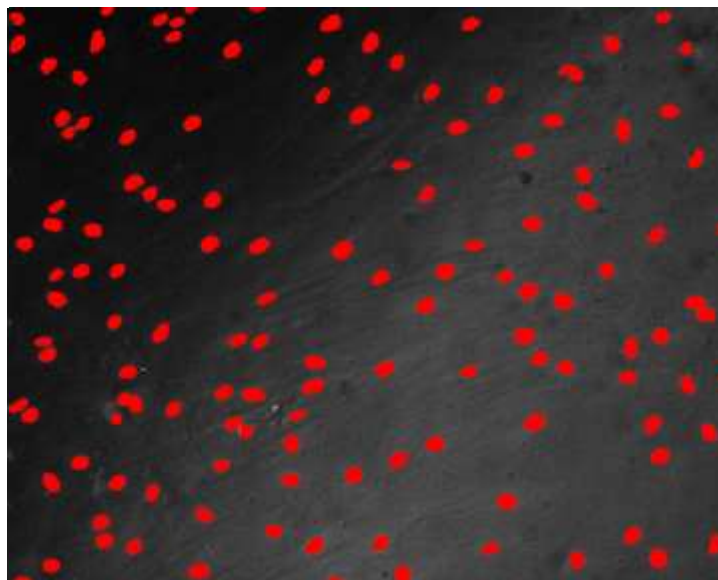
- **All** Application Modules share the same **basic controls**
- **Simple configuration**
 - Select wavelength
 - Set size range of objects
 - Set intensity above local background
- Test and save settings
- The module will **automatically split** touching cells



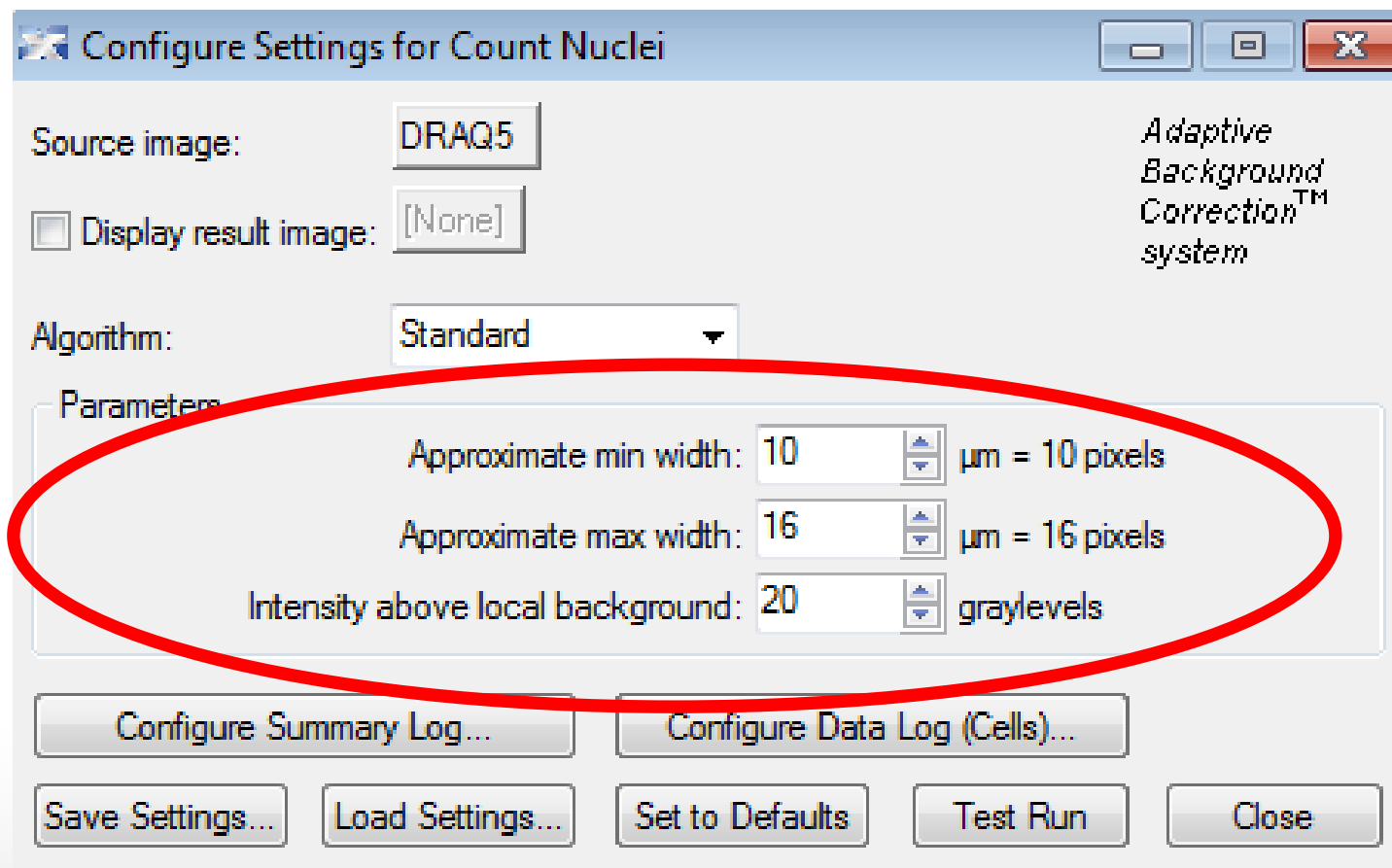
Adaptive Background Correction

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

A screenshot of a software dialog box titled "Configure Settings for Count Nuclei". The dialog has a standard Windows-style title bar with minimize, maximize, and close buttons. The main area contains several settings: "Source image:" with a button labeled "DRAQ5"; a checkbox "Display result image:" which is unchecked, followed by a button labeled "[None]"; "Algorithm:" with a dropdown menu showing "Standard"; and a "Parameters" section containing three settings: "Approximate min width:" with a value of 10 and a unit of $\mu\text{m} = 10$ pixels; "Approximate max width:" with a value of 16 and a unit of $\mu\text{m} = 16$ pixels; and "Intensity above local background:" with a value of 20 and a unit of graylevels. A red oval is drawn around the "Parameters" section. At the bottom, there are five buttons: "Configure Summary Log...", "Configure Data Log (Cells)...", "Save Settings...", "Load Settings...", "Set to Defaults", "Test Run", and "Close".

Source image:

☐ Display result image:

Algorithm:

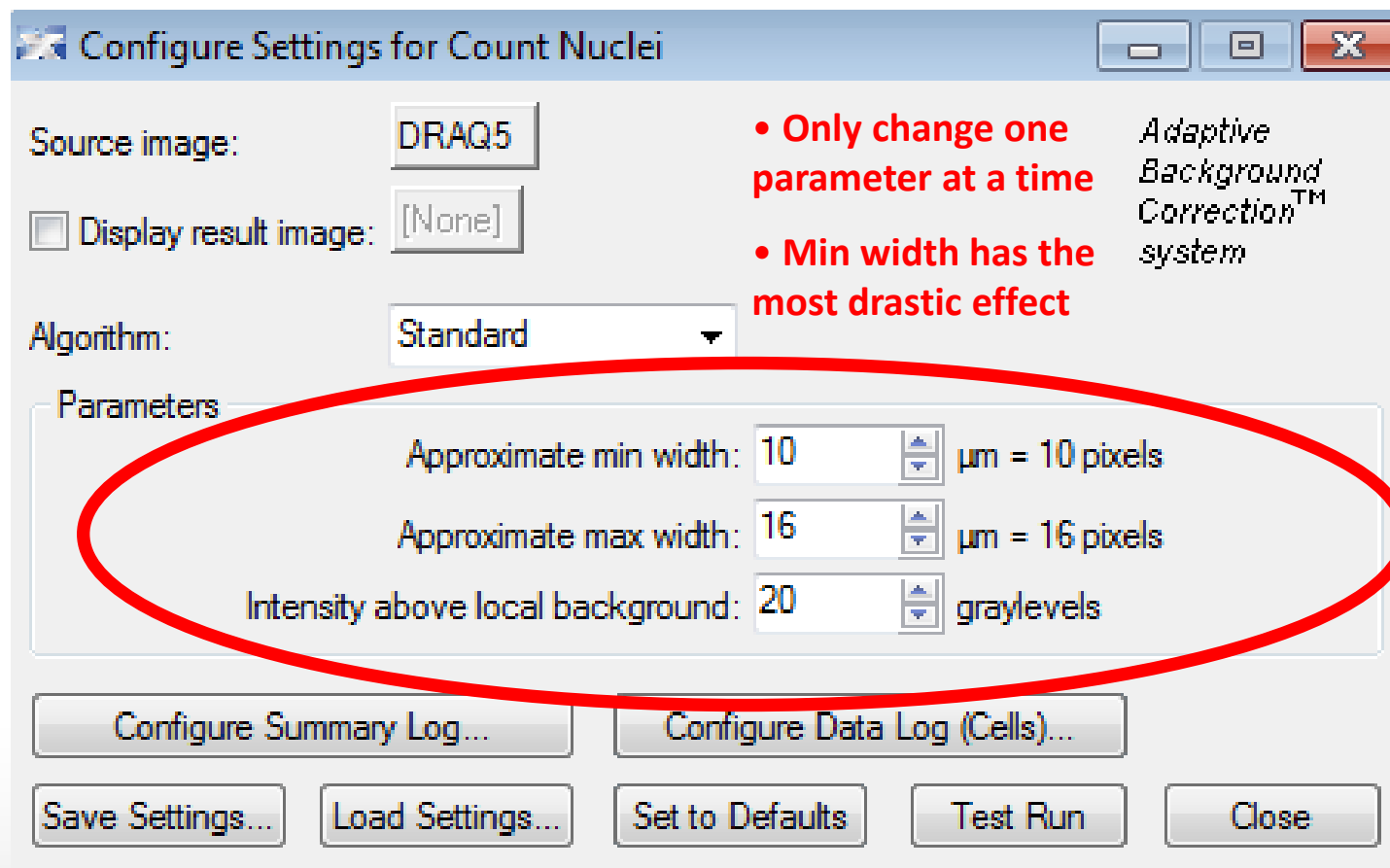
Parameters

Approximate min width: $\mu\text{m} = 10$ pixels

Approximate max width: $\mu\text{m} = 16$ pixels

Intensity above local background: graylevels

Optimizing settings

A screenshot of a software dialog box titled "Configure Settings for Count Nuclei". The dialog has a standard Windows-style title bar with minimize, maximize, and close buttons. Inside, there are several configuration options. A red oval highlights the "Parameters" section, which contains three spinners for "Approximate min width", "Approximate max width", and "Intensity above local background". To the right of the dialog, there are two red bullet points and the text "Adaptive Background Correction™ system". At the bottom, there are five buttons: "Configure Summary Log...", "Configure Data Log (Cells)...", "Save Settings...", "Load Settings...", "Set to Defaults", "Test Run", and "Close".

Source image:

☐ Display result image:

Algorithm:

Parameters

Approximate min width: $\mu\text{m} = 10$ pixels

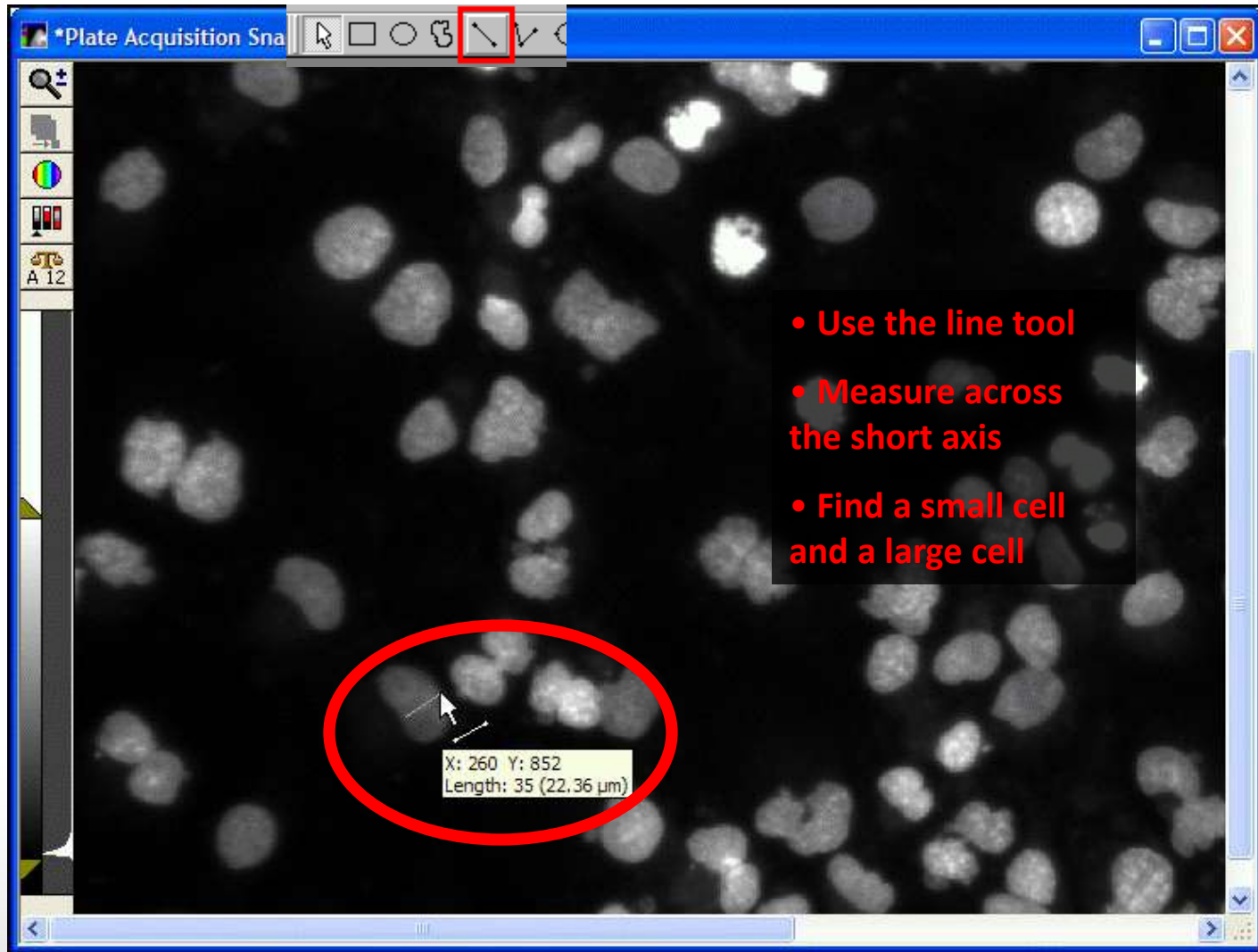
Approximate max width: $\mu\text{m} = 16$ pixels

Intensity above local background: graylevels

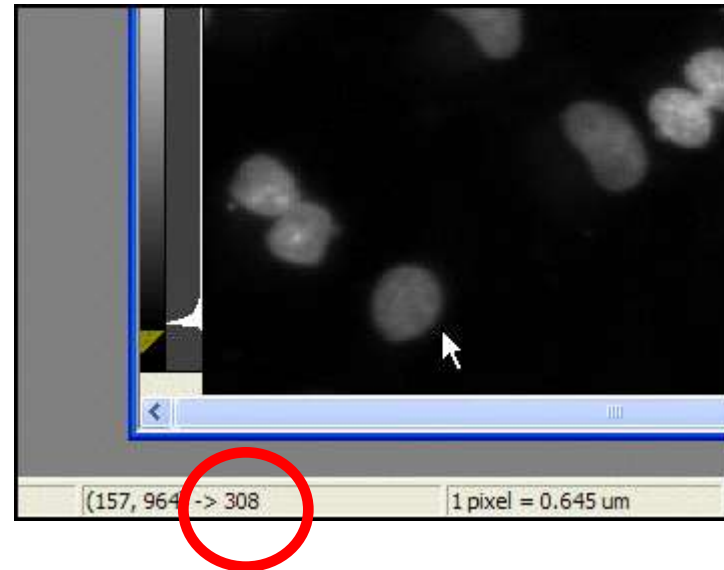
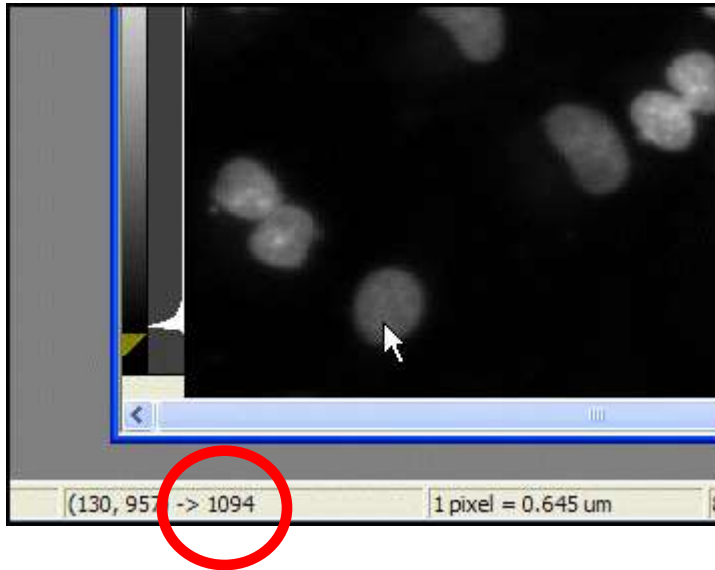
- Only change one parameter at a time
- Min width has the most drastic effect

Adaptive Background Correction™ system

Measuring width



Measuring Intensity above Local Background



1. Find a dim cell
2. Measure intensity just inside and outside the cell
3. Subtract to find the difference ($1094 - 308 = 786$)
4. "Pad" the value by about 100 gray values ($786 - 100 = 686$).
Note: For FAST algorithm, cut this value in half.

Selecting measurements

Configure Settings for Count Nuclei

Source image:

☐ Display result image:

Algorithm:

Parameters

Approximate min width: $\mu\text{m} = 10$ pixels

Approximate max width: $\mu\text{m} = 16$ pixels

Intensity above local background: graylevels

• Summary log = "Site by Site" data *Adaptive Background Correction™ system*

• Total cells, intensity, average area, etc.

Selecting measurements

Configure Settings for Count Nuclei

Source image:

☐ Display result image:

Algorithm:

Parameters

Approximate min width: $\mu\text{m} = 10$ pixels

Approximate max width: $\mu\text{m} = 16$ pixels

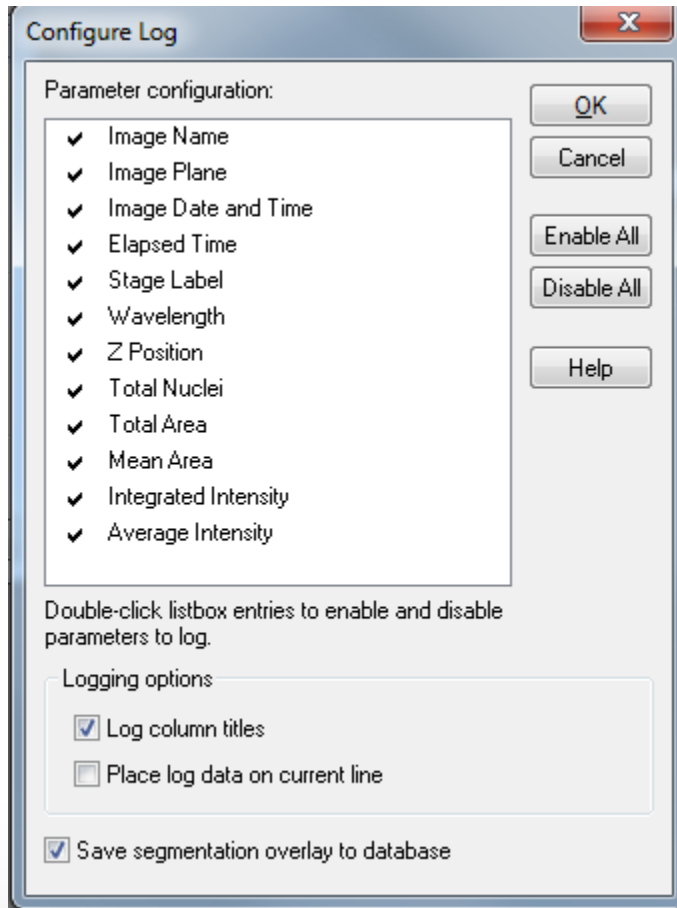
Intensity above local background: graylevels

• Data log = "Cell by cell" data

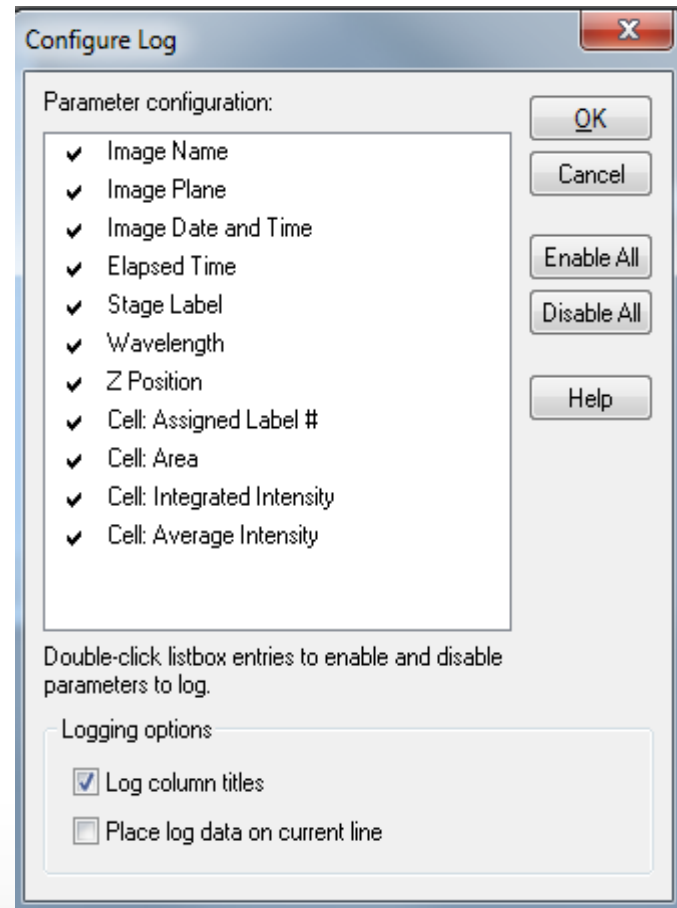
• Area, intensity, etc. for each individual cell

Adaptive Background Correction™ system

Selecting measurements

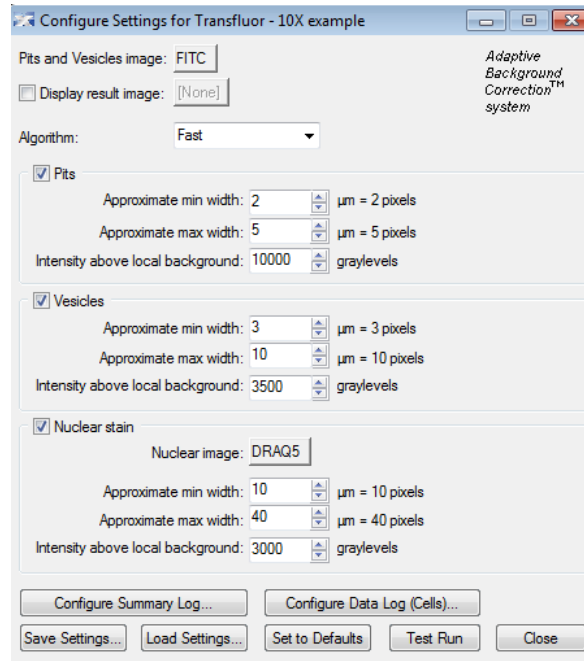


Configure Summary Log



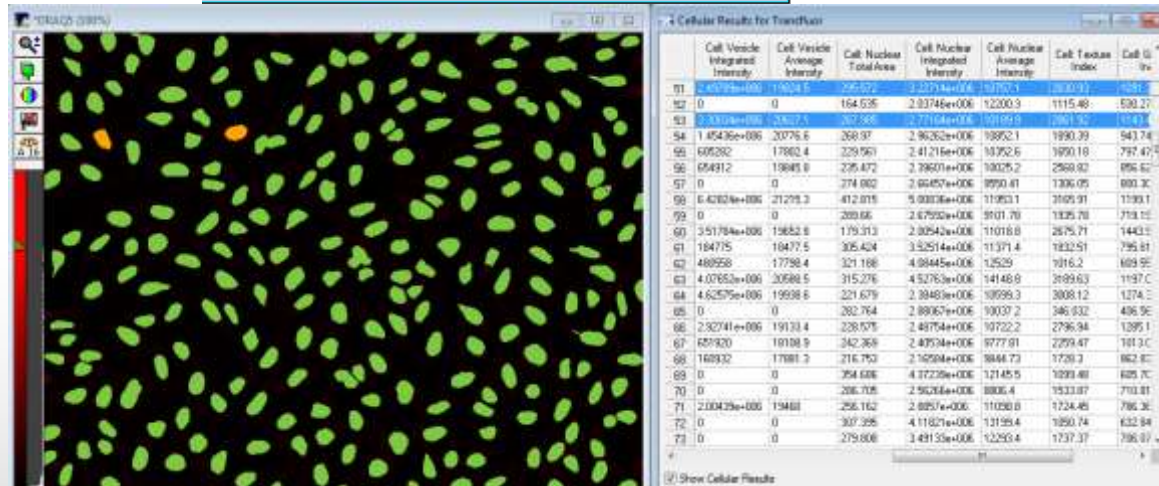
Configure Data Log

Module – interactive feedback



Interactive optimization of analysis parameters

Immediate graphic feedback on detection results



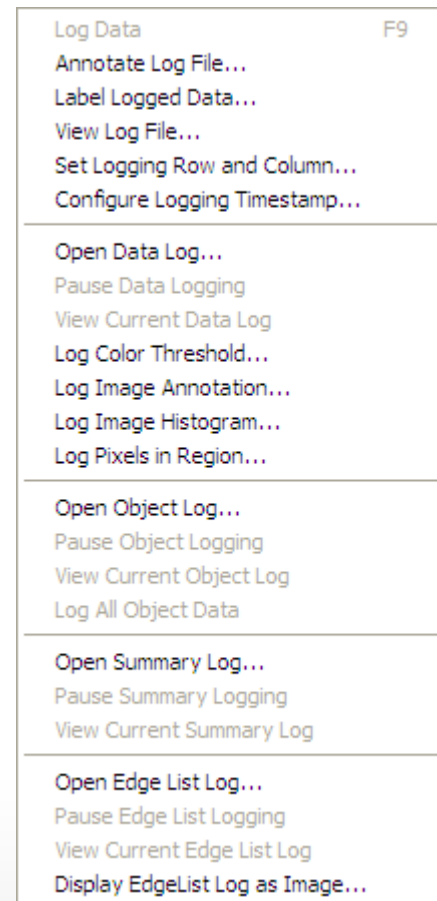
Links numerical results to image overlays

Logging out your data

- Four types of logs in MetaXpress
 - **Data Log:** Opens an existing or new data log for storing measurements other than morphometric measurements
 - **Summary Log:** Opens an existing or new summary log for storing summaries of morphometry statistics
 - **Object Log:** Opens an existing or new object log for storing per-object morphometric measurement data
 - **Edge List Log:** Opens an existing or new edgelist log for storing each object's centroid and vertex X,Y-coordinate data in an image
- Log to a text file or to Microsoft Excel using DDE (Dynamic Data Exchange)

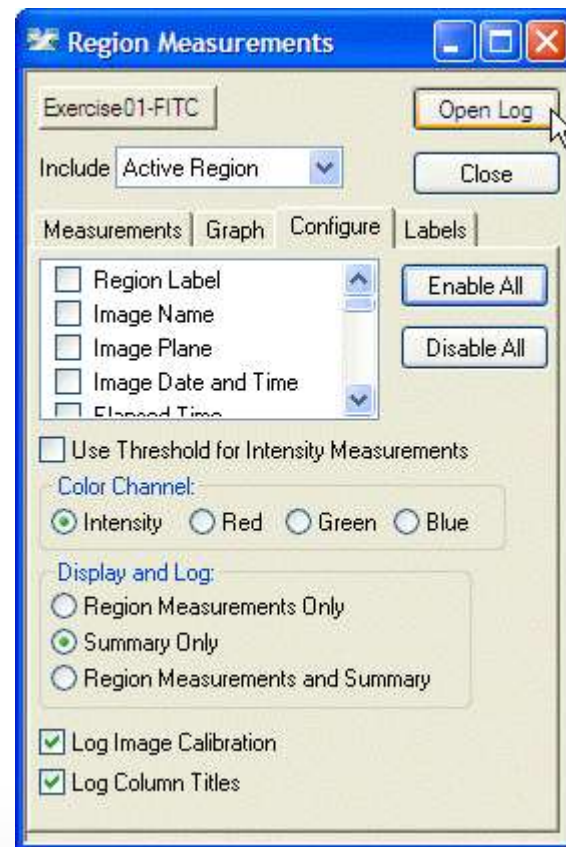
Opening and closing logs

- Log menu
 - Open
 - Close
 - Pause / Resume (useful in journals)



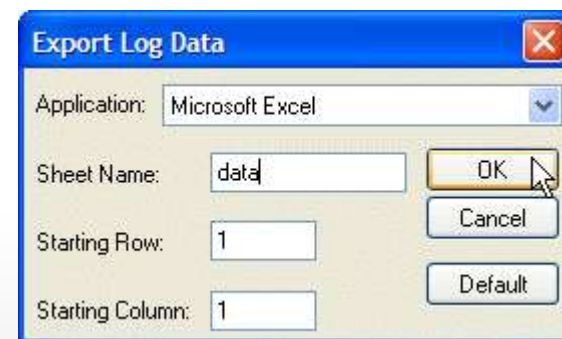
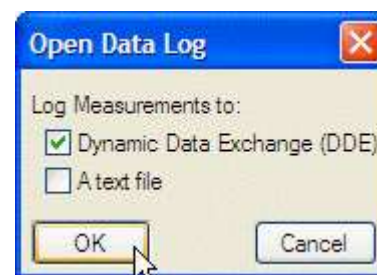
Opening and closing logs

- Log menu
 - Open
 - Close
 - Pause / Resume (useful in journals)
- Specific measurement dialogs
 - “Open Log” button turns into “Log Data”



Opening and closing logs

- Log menu
 - Open
 - Close
 - Pause / Resume (useful in journals)
- Specific measurement dialogs
 - “Open Log” button turns into “Log Data”
- For MS Excel, select Dynamic Data Exchange
 - Specify sheet name
 - If sheet not open, new sheet will be created in currently open workbook
 - If no workbook open, new one will be created



Screening > Review Plate Data

Select plate
for review

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

☒ Show Values ☐ Intensity Profile ☐ Color Composite

Image Overlay: Show cell segmentation Col: Cyan

Source R: <None> G: <None> B: DRAQ5

Selections [In Green]

Load Images ◀ ▶ Clear

Reset Image Displays Cellular Results... Close

Select plate for review

Select Plate for Review

Plates

- System Administrator [Creator Name - Plate Info]
 - 11/14/06 [Date Created - Plate Info]
 - 11/15/06 [Date Created - Plate Info]
 - 12/22/06 [Date Created - Plate Info]

| Name [Plate Info] | Acquisition Name [Plate Info] | Barcode [...] | Creator... |
|--|-------------------------------|---------------|------------|
| Transfluor Agonist A_PRICKERT-UCLT1_16 | Transfluor Agonist A | <NULL> | System # |
| Transfluor Agonist B_PRICKERT-UCLT1_17 | Transfluor Agonist B | <NULL> | System # |
| Transfluor Agonist C_PRICKERT-UCLT1_18 | Transfluor Agonist C | <NULL> | System # |
| Transfluor Agonist D_PRICKERT-UCLT1_20 | Transfluor Agonist D | <NULL> | System # |

Plate Statistics

| Plate Name | Site Count | Well Count | Series Count | Compound Count | Controls Count | Control Statistic | Datasets | Measurement Sets |
|------------|------------|------------|--------------|----------------|----------------|-------------------|----------|------------------|
|------------|------------|------------|--------------|----------------|----------------|-------------------|----------|------------------|

Select Cancel

Configure Branches

Available

- Acquisition Name [Plate Info]
- Barcode [Plate Info]
- Date Annotated [Plate Info]
- Date\Time Annotated [Plate Info]
- Date\Time Created [Plate Info]
- Description [Plate Info]
- Experiment Set [Plate Property]
- Global ID [Plate Info]
- Name [Plate Info]
- Unique ID [Plate Info]
- X Wells [Plate Info]
- Y Wells [Plate Info]

Selected

- Creator Name [Plate Info]
- Date Created [Plate Info]

OK Cancel

Configure branches

Review Plate Data: Display Tab

Review Plate Data - Transfluor Agonist D_PRICKERT-UCLT1_20

Select Plate...

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: **Data view**
Well arrangement
Time vs Well
Measurement vs Well

| | 04 |
|---|-------------------------------------|
| A | 17221.56 |
| B | 17298.99 19113.79 19815.42 16205.88 |
| C | 18780.35 18054.89 18765.95 17130.41 |

Configure thumbnail montage area

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

☒ Show Values ☐ Intensity Profile ☐ Color Composite

Image Overlay: Show cell segmentation Col: Cyan

Source R: <None> G: <None> B: DRAQ5

Selections [In Green]

Load Images

Reset Image Displays Cellular Results...

Close

*HTS - FITC (100%)

| A01 | A02 | A03 | A04 |
|---------|----------|----------|---------|
| 8680.69 | 10877.14 | 10148.65 | 9552.04 |
| B01 | B02 | B03 | B04 |
| 8154.13 | 9189.68 | 9217.99 | 9696.76 |
| C01 | C02 | C03 | C04 |
| 9381.02 | 9437.31 | 9820.45 | 9436.88 |

*HTS - DRAQ5 (100%)

| A01 | A02 | A03 | A04 |
|---------|----------|----------|---------|
| 8680.69 | 10877.14 | 10148.65 | 9552.04 |
| B01 | B02 | B03 | B04 |
| 8154.13 | 9189.68 | 9217.99 | 9696.76 |
| C01 | C02 | C03 | C04 |
| 9381.02 | 9437.31 | 9820.45 | 9436.88 |

Wavelength selection

Color overlay option

Review Plate Data: Run Analysis Tab

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Montage: 1 x 2 Time point: 1 of 1

Display **Run Analysis** Measurements Graph

Analysis: <Count Nuclei>
NuclearSpots

Settings: <Angiogenesis Tube Formation>
<Cell Cycle>
<Cell Health>
<Cell Proliferation HT>
<Cell Scoring>
☒ Log int <Count Nuclei>
<Granularity>
<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>
NuclearSpots

Configure Settings...
Run Analysis for All Positions
Run Analysis for Selections
Run Analysis for Site
Create Custom Module

Clear
Close

Create Custom Module appears if you have the Custom Module Editor option on key.

↑ Options missing "<>" are custom assays.

Review Plate Data: Run Analysis Tab

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Montage: 1 x 2 Time point: 1 of 1

Display **Run Analysis** Measurements Graph

Analysis: <Count Nuclei> Configure Settings...

Settings: 10X Edit List...

Counts nuclei using a single nuclear stain.

☒ Log into the database

Run Analysis for All Positions

Run Analysis for Selections

Run Analysis for Site

Create Custom Module

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

Configure module settings

Run analysis for all positions, a subset of marked selections, or current image displayed.

Right click on wells to mark as selection. Turn off heat map to see marked selections (in green).

Review Plate Data: Measurements Tab

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Select analysis & measurement parameter to view in data table

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transfluor: Transfluor Example ☒ Show Heat Map Heat Map...

Measurement: Cell: Pit Average Intensity (Tran) 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

Review Plate Data: Measurements Tab

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Show heat map. Toggle on/off.

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transfluor: Transfluor Example ☒ Show Heat Map Heat Map...

Measurement: Cell: Pit Average Intensity (Trar 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

Review Analysis Results Within MetaXpress

Wavelengths: ☒ DAPI ☒ FITC ☒ Cy3

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 | 94.0 | 94.4 | 94.8 | 92.6 | 93.8 | 92.7 | 94.1 | 92.1 | 93.6 | 93.8 | 94.1 | 92.4 | 93.7 | 93.4 | 93.5 | 93.6 | 94.0 | 92.5 | 93.8 | 94.3 | 93.3 | 93.8 | 92.4 | 94.2 |
| B | 88.3 | 88.7 | 88.8 | 89.0 | 86.3 | 88.5 | 88.2 | 87.4 | 88.2 | 90.4 | 85.6 | 86.2 | 87.0 | 85.9 | 88.3 | 86.1 | 85.5 | 84.6 | 87.5 | 85.1 | 86.3 | 85.9 | 89.5 | 89.3 |
| C | 88.1 | 84.9 | 85.9 | 84.8 | 78.5 | 76.1 | 79.2 | 74.5 | 81.2 | 72.9 | 79.8 | 76.2 | 81.8 | 77.0 | 86.4 | 90.3 | 80.3 | 77.6 | 91.4 | 91.7 | 21.5 | 18.3 | 19.2 | 23.5 |
| D | 88.2 | 86.5 | 86.2 | 86.5 | 78.0 | 81.2 | 76.7 | 78.3 | 77.4 | 81.5 | 76.3 | 78.3 | 77.6 | 78.4 | 85.7 | 81.0 | 76.0 | 84.0 | 92.9 | 93.1 | 17.0 | 18.1 | 18.9 | 24.8 |
| E | 91.9 | 87.5 | 88.0 | 87.1 | 83.8 | 81.5 | 81.4 | 81.3 | 81.3 | 81.2 | 79.5 | 78.6 | 78.4 | 84.3 | 86.2 | 88.4 | 83.8 | 89.5 | 96.2 | 96.7 | 18.5 | 19.7 | 23.6 | 32.7 |
| F | 89.9 | 85.3 | 87.4 | 86.5 | 79.7 | 78.9 | 81.2 | 81.6 | 84.9 | 78.3 | 80.1 | 72.8 | 80.5 | 78.0 | 87.6 | 85.1 | 86.1 | 80.5 | 97.3 | 95.9 | 27.9 | 19.7 | 22.1 | 22.9 |
| G | 88.4 | 88.8 | 86.6 | 86.7 | 91.5 | 91.0 | 92.7 | 93.4 | 95.0 | 93.7 | 93.5 | 92.8 | 95.4 | 94.9 | 96.7 | 95.8 | 95.1 | 96.9 | 89.2 | 90.6 | 42.8 | 49.6 | 65.3 | 68.7 |
| H | 90.9 | 87.2 | 88.9 | 86.5 | 92.4 | 91.3 | 92.3 | 93.6 | 94.5 | 93.3 | 94.0 | 93.7 | 95.9 | 94.7 | 97.2 | 96.4 | 97.2 | 96.9 | 92.1 | 91.9 | 50.8 | 55.1 | 66.8 | 67.8 |
| I | 91.2 | 88.7 | 88.3 | 86.2 | 91.1 | 91.8 | 92.9 | 92.9 | 93.4 | 93.8 | 94.3 | 93.5 | 95.8 | 94.6 | 96.2 | 96.6 | 96.4 | 96.0 | 92.5 | 89.4 | 47.6 | 51.2 | 68.7 | 58.7 |
| J | 90.5 | 87.7 | 89.2 | 85.7 | 91.1 | 90.8 | 93.4 | 91.8 | 93.6 | 93.3 | 94.7 | 93.7 | 95.9 | 94.7 | 97.1 | 97.4 | 96.1 | 97.0 | 91.2 | 94.4 | 51.4 | 47.5 | 72.8 | 64.5 |
| K | 91.4 | 89.2 | 88.9 | 86.9 | 81.4 | 81.7 | 82.1 | 82.1 | 82.6 | 83.9 | 81.2 | 83.8 | 82.3 | 82.9 | 86.1 | 89.7 | 85.7 | 84.1 | 96.5 | 97.3 | 33.4 | 33.0 | 21.3 | 24.6 |
| L | 93.0 | 90.2 | 89.2 | 88.0 | 83.3 | 83.6 | 84.1 | 83.0 | 85.3 | 84.2 | 86.6 | 86.3 | 87.7 | 86.0 | 94.1 | 91.1 | 93.4 | 88.7 | 97.7 | 96.0 | 37.5 | 38.5 | 36.7 | 34.9 |
| M | 92.4 | 92.2 | 90.4 | 89.3 | 86.0 | 84.6 | 85.0 | 86.1 | 87.5 | 86.9 | 87.5 | 89.2 | 86.5 | 89.4 | 88.6 | 92.7 | 91.4 | 91.6 | 96.6 | 97.4 | 34.6 | 39.9 | 25.4 | 27.8 |
| N | 90.7 | 89.9 | 88.5 | 87.4 | 83.5 | 84.2 | 83.0 | 85.2 | 84.0 | 84.6 | 81.8 | 86.3 | 81.9 | 85.1 | 88.4 | 89.1 | 83.7 | 87.7 | 95.1 | 95.5 | 39.3 | 33.6 | 22.3 | 27.5 |
| O | 93.1 | 93.0 | 91.0 | 91.0 | 90.1 | 91.4 | 51.9 | 53.0 | 20.3 | 17.2 | 8.4 | 11.3 | 5.2 | 6.3 | 6.5 | 7.9 | 2.9 | 9.8 | 6.9 | 10.9 | 6.1 | 13.1 | 40.5 | 38.0 |
| P | 94.5 | 92.8 | 92.8 | 91.3 | 92.8 | 91.0 | 46.2 | 43.7 | 16.8 | 23.9 | 13.5 | 12.5 | 6.2 | 6.1 | 11.5 | 9.2 | 7.9 | 4.6 | 12.8 | 12.1 | 10.1 | 11.5 | 37.0 | 44.0 |

Montage: 24 x 16 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Multi Wavelength Cell Scoring ☒ Show Heat Map Heat Map...

Measurement: % Positive W3 (MultiWaveSco) 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

Quickly view analysis data values and heat map

Review Plate Data: Graph Tab

Review Plate Data -

Select Plate... Transfluor Agonist D_PRICKERT-UCLT1_20

Wavelengths: ☒ DRAQ5 ☒ FITC

Data view: Well arrangement Print Table

| | 01 | 02 | 03 | 04 |
|---|----------|----------|----------|----------|
| A | 15829.65 | 23395.25 | 18103.91 | 17221.56 |
| B | 17298.99 | 19113.79 | 19815.42 | 16205.88 |
| C | 18780.35 | 18054.89 | 18765.95 | 17130.41 |

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Analysis: Transfluor: Transfluor Example

Graph view: ☒ Plate ☐ Multiple graphs of displayed wells ☐ Single Well

Graph type: Histogram
Histogram
Measurement vs Well Column
Measurement vs Well Row
Measurement vs Well Number
Scatter Plot

Measurement: Cell: Pit Average Intensity

Number of bins: 6 ☒ Auto scale

Scale min: 0 Max: 100

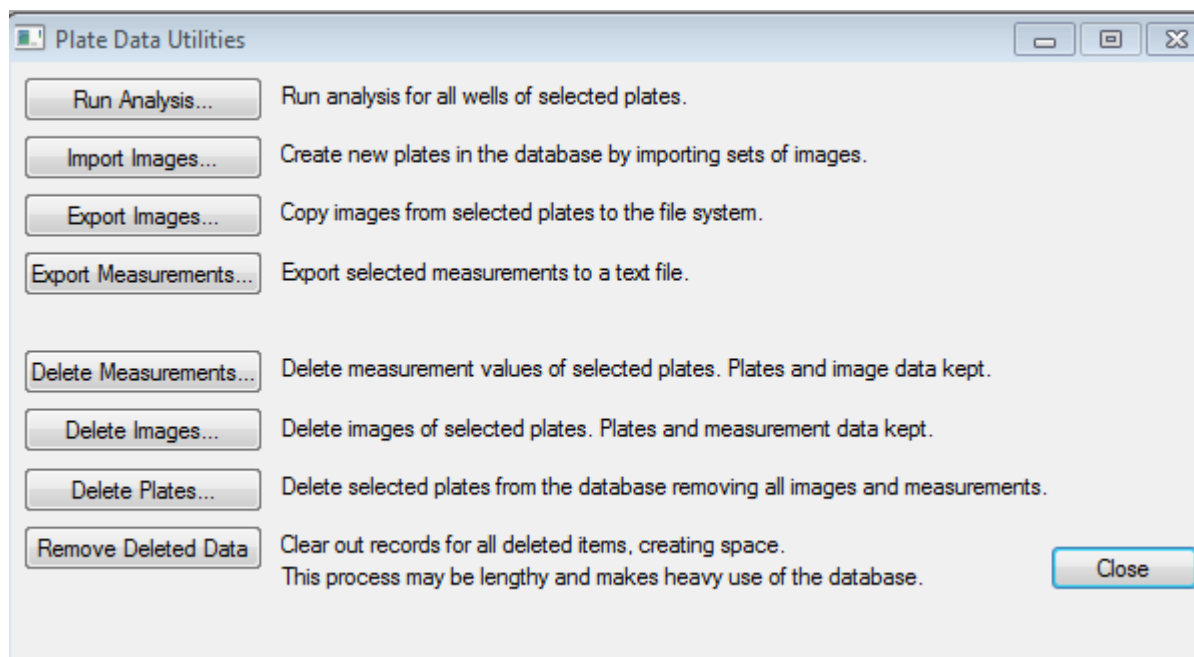
Set Display to Default Show Graph

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

Screening > Plate Data Utilities



Screening > Plate Data Utilities > Export Measurements

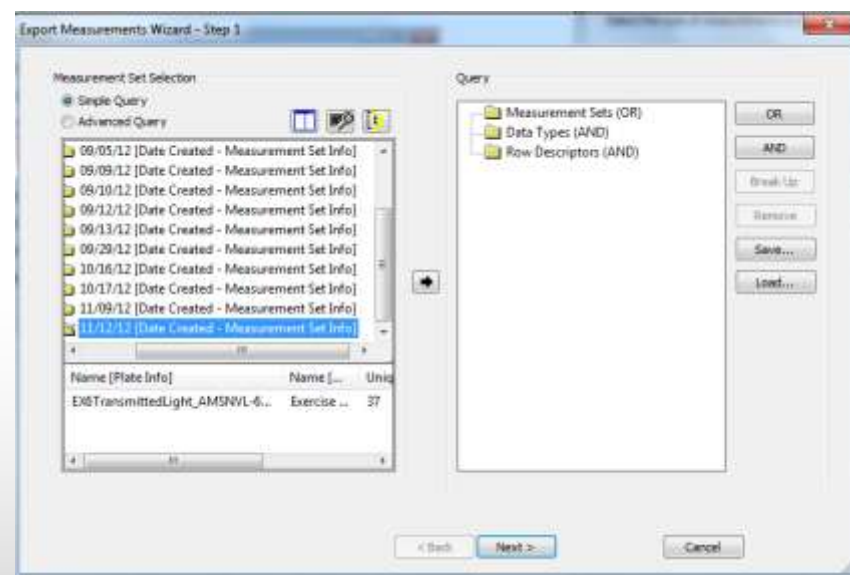
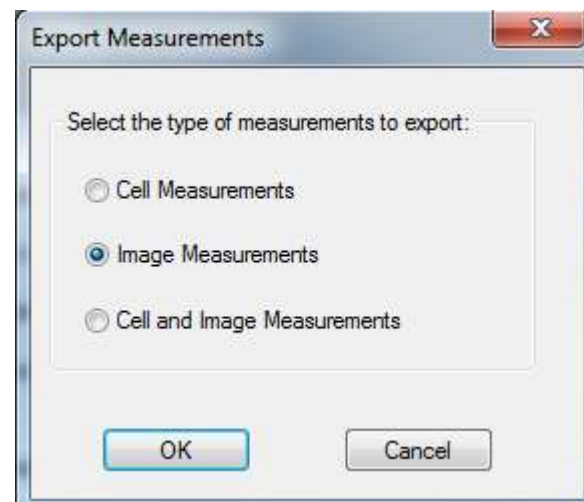
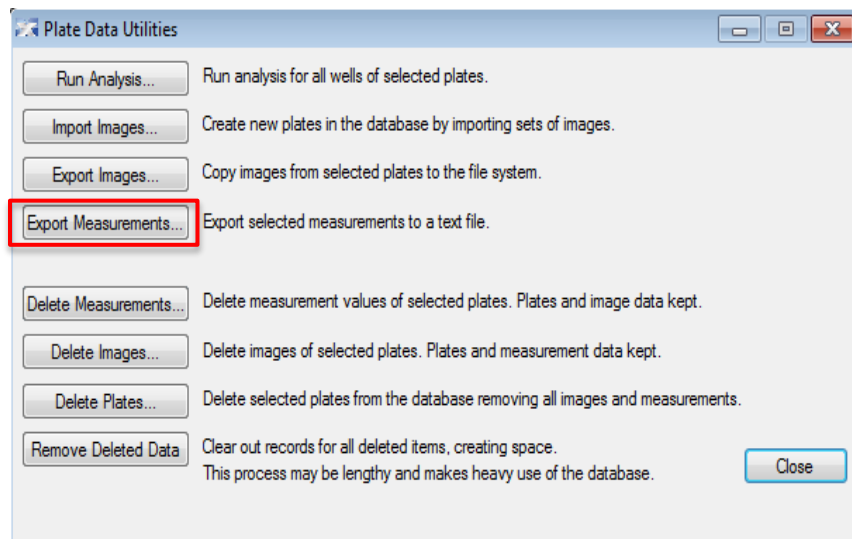
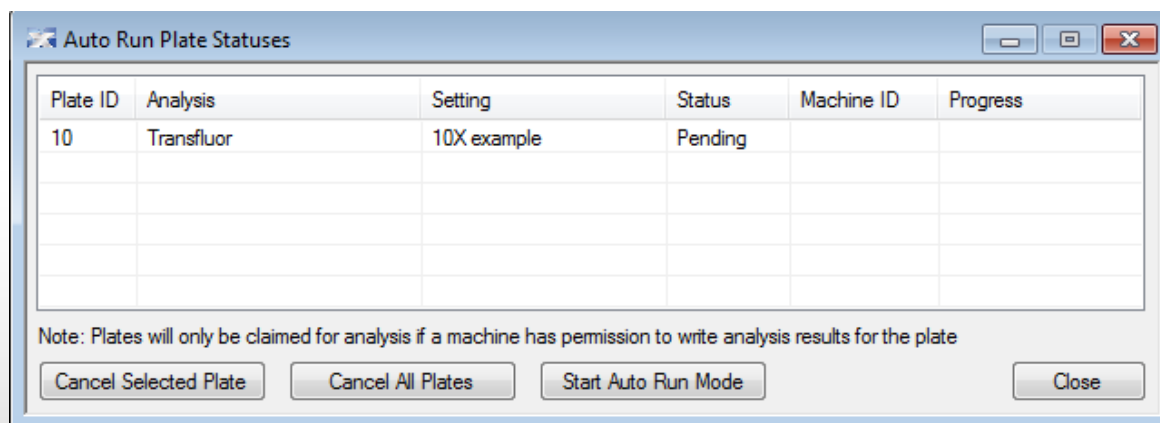
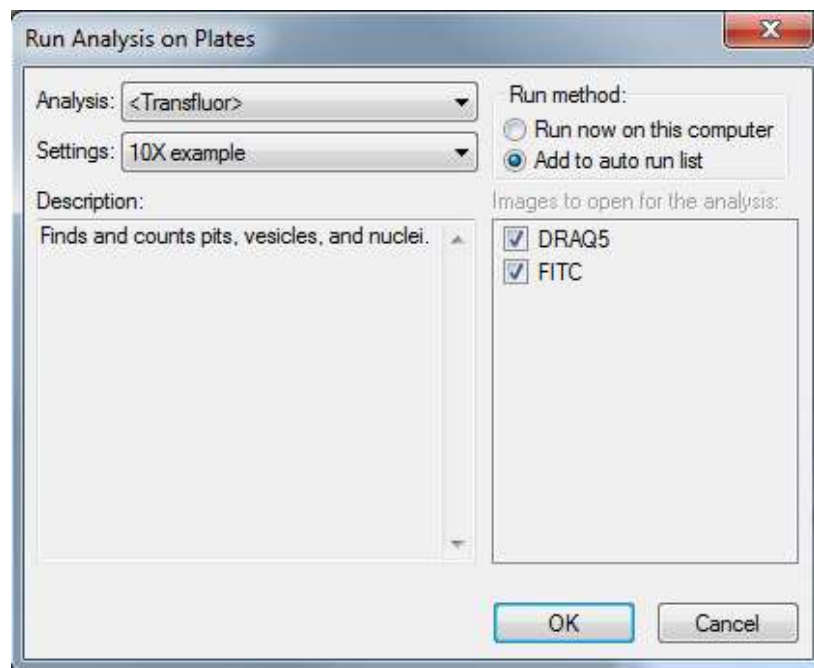
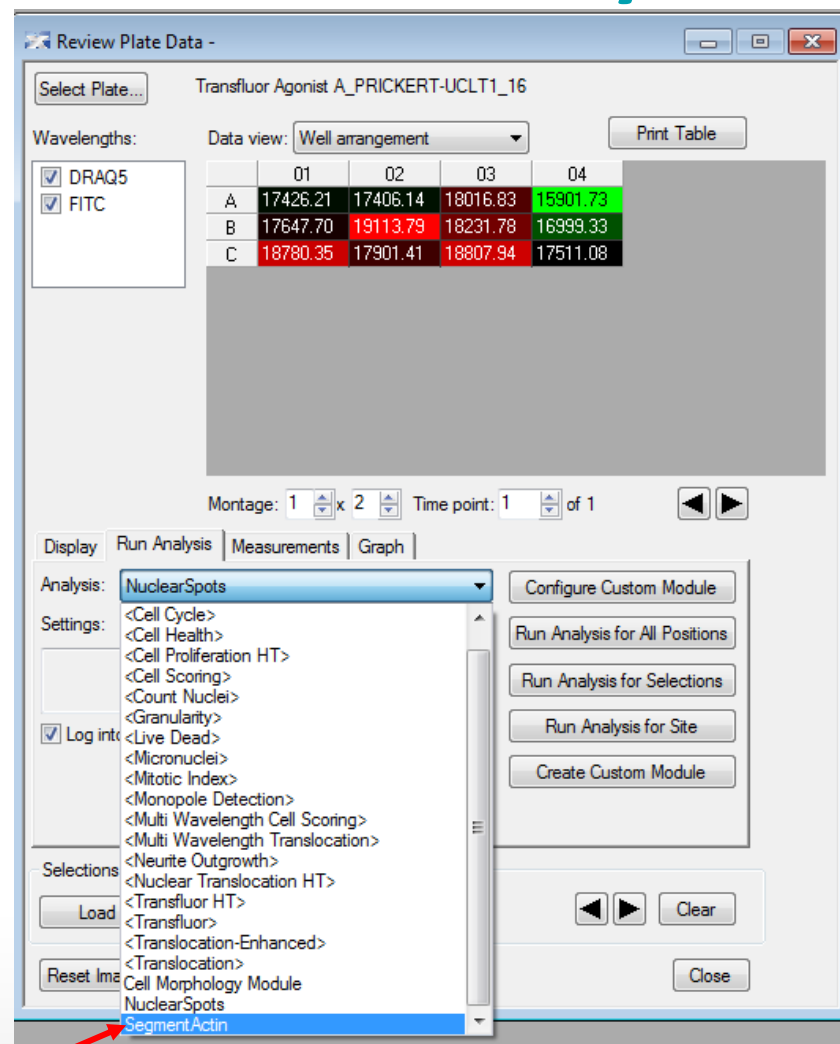
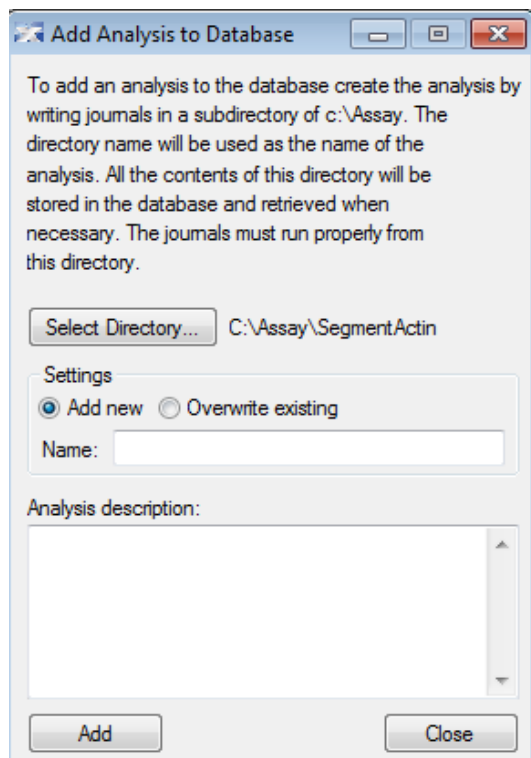


Plate Data Utilities: Run Analysis for Batch Analysis

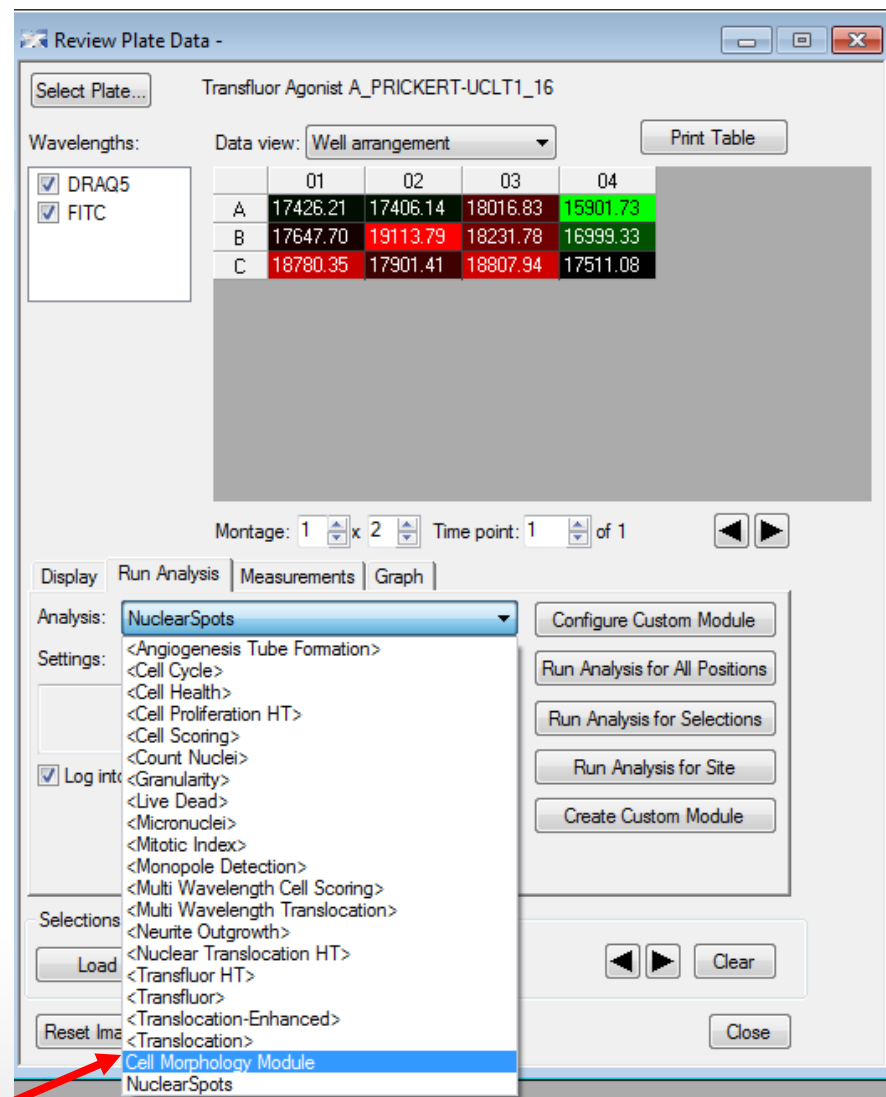
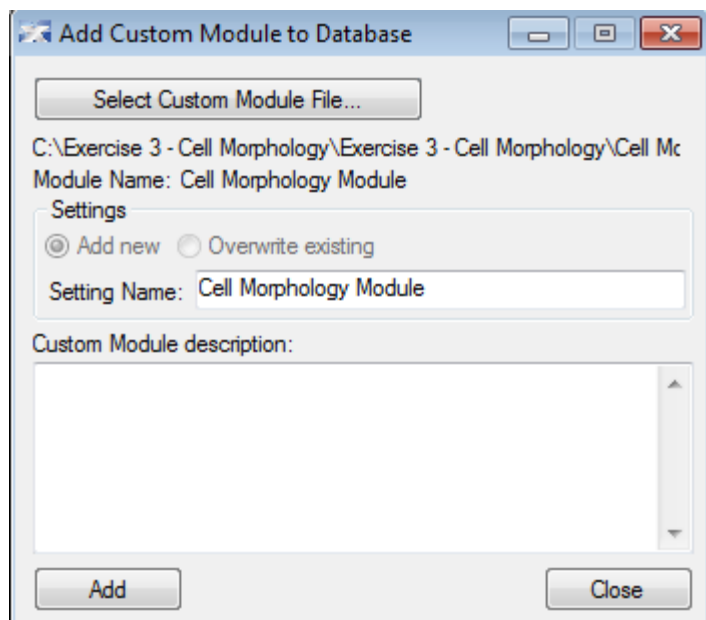


Add Analysis to Database: Custom Analyses



Assay will appear in Run Analysis tab. **Add Analysis to Database** is only used if you create your own custom analysis with a journal (macro).

Add Analysis to Database: Custom Module



Custom Module will appear in Run Analysis tab.



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