

**Molecular
Devices**

Together through life sciences.

MetaXpress® Software: *Analysis Training*

Together through life sciences.

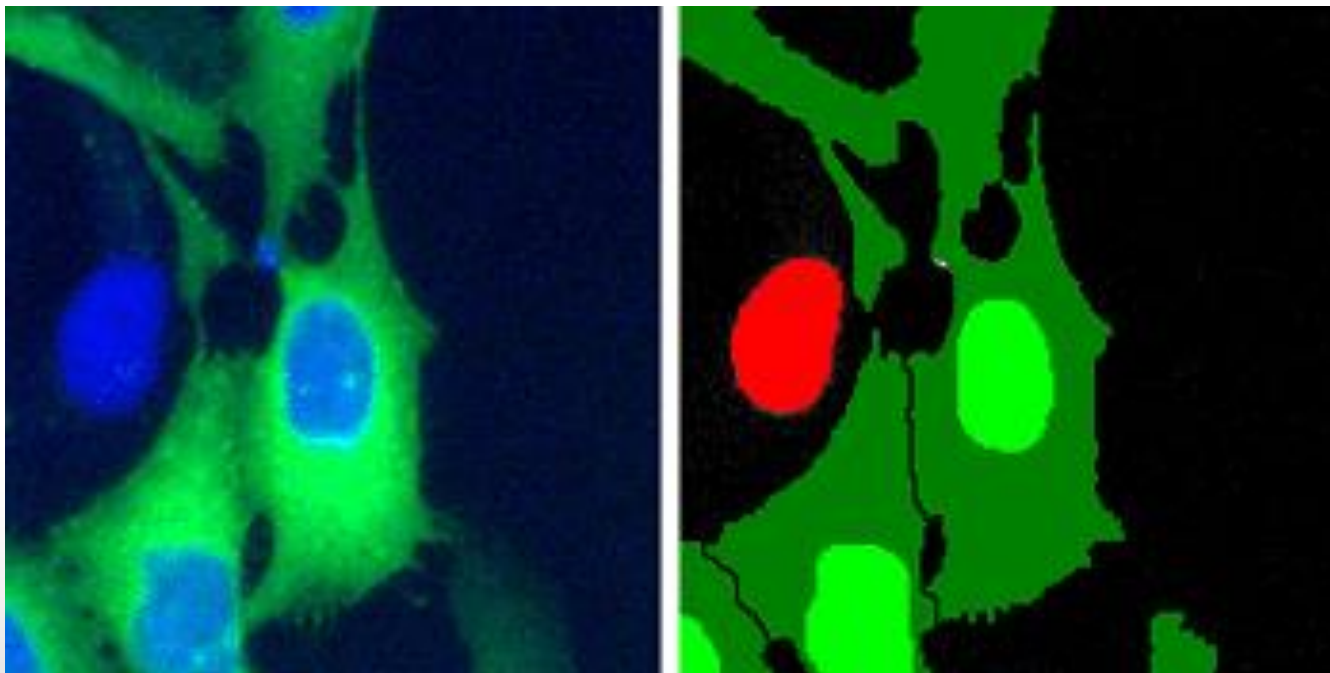
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**Molecular
Devices**

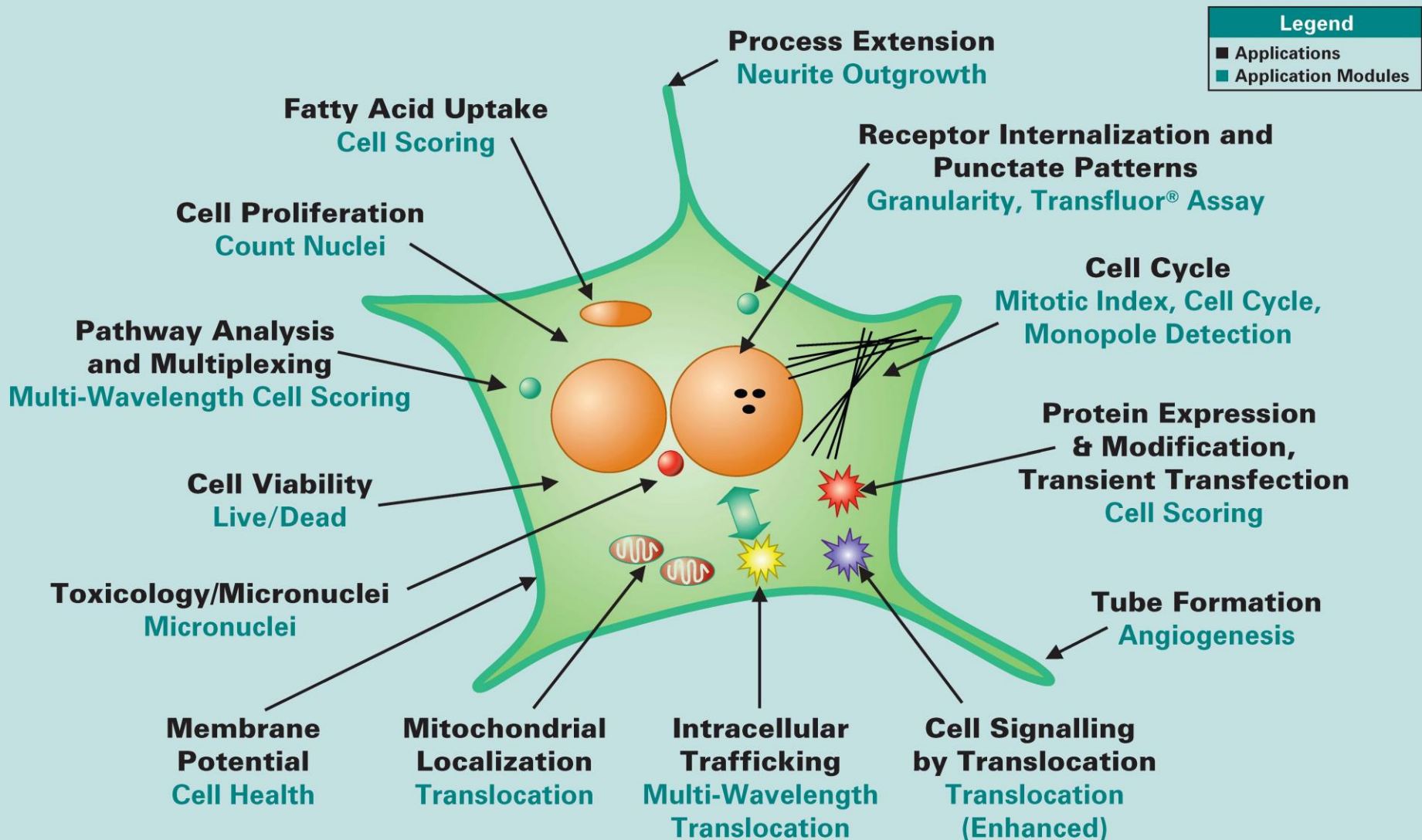
MetaXpress: Application Modules

- “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



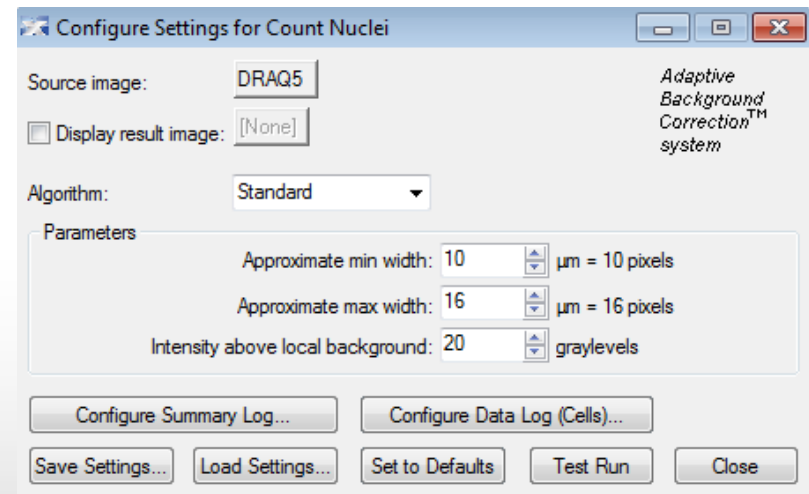
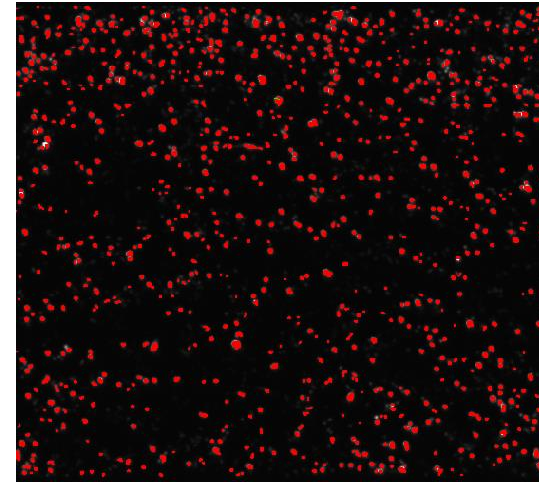
Application Modules for Hundreds of Assays

Easy Custom Analysis with Journaling



MetaXpress: Application Modules

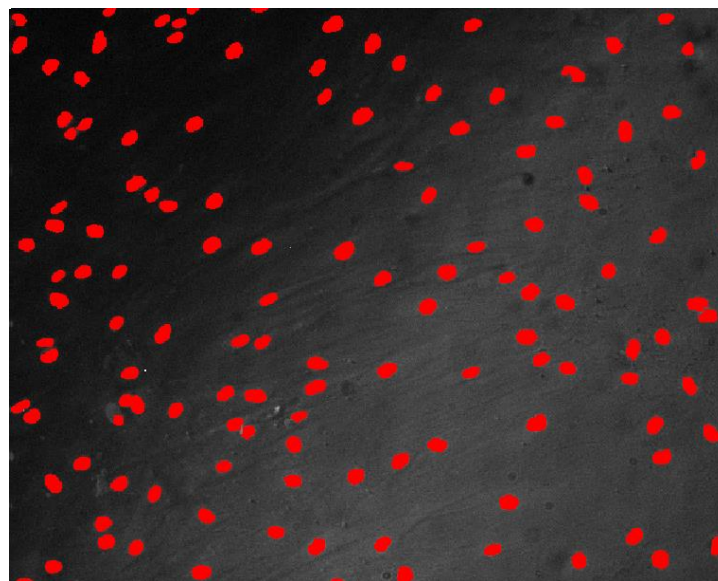
- **All** Application Modules share the same **basic controls**
- **Simple configuration**
 - Select wavelength
 - Set size range of objects
 - Set intensity above local background
- Test and save settings
- The module will **automatically split** touching cells



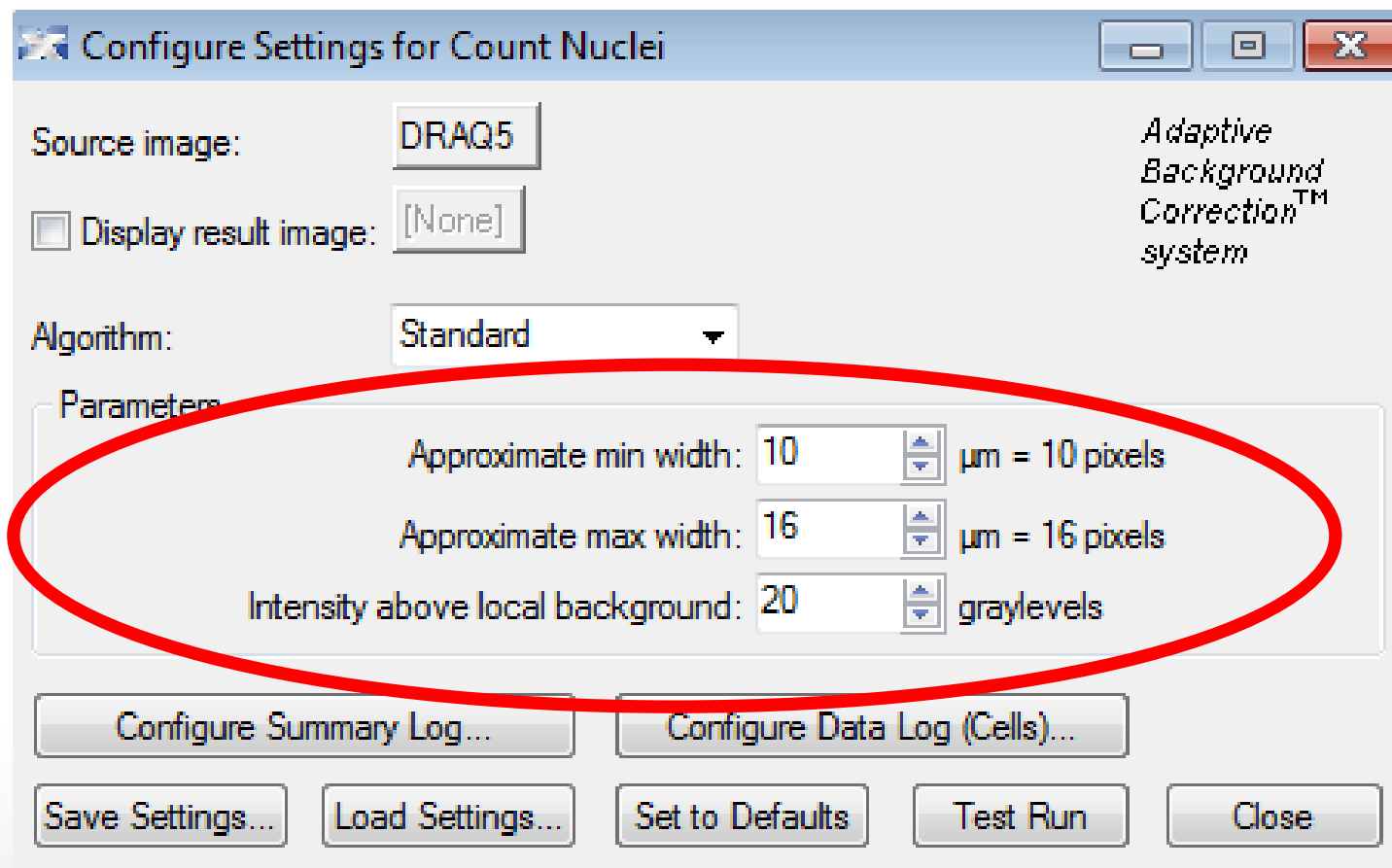
Adaptive Background Correction

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



Optimizing settings

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

• Only change one parameter at a time

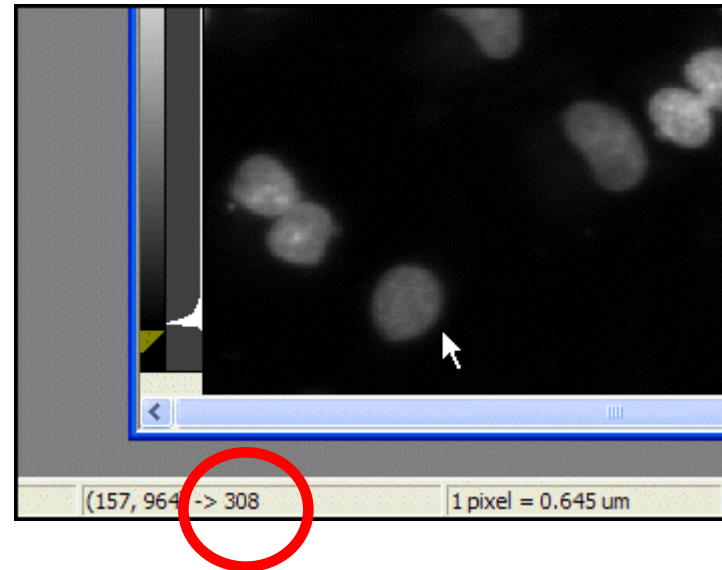
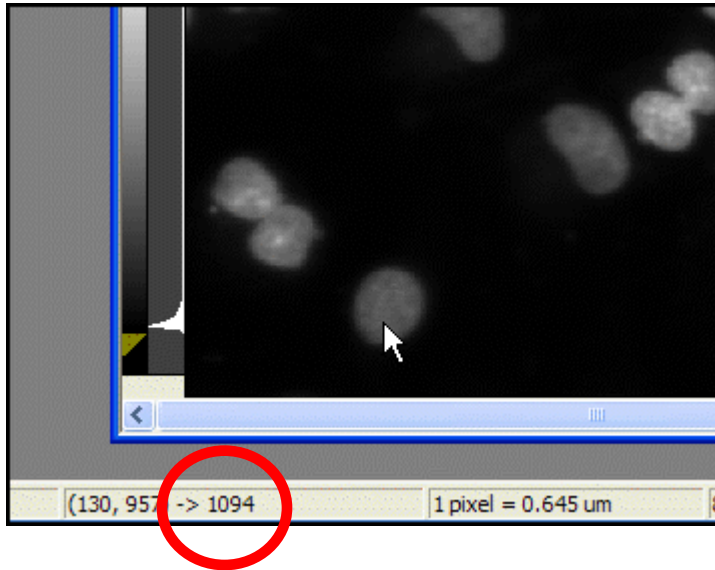
• Min width has the most drastic effect

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

• Total cells, intensity, average area, etc.

Adaptive Background Correction™ system

Selecting measurements

Configure Settings for Count Nuclei

Source image: DRAQ5

Display result image: [None]

Algorithm: Standard

Parameters

Approximate min width: 10 μm = 10 pixels

Approximate max width: 16 μm = 16 pixels

Intensity above local background: 20 graylevels

• Data log = "Cell by cell" data

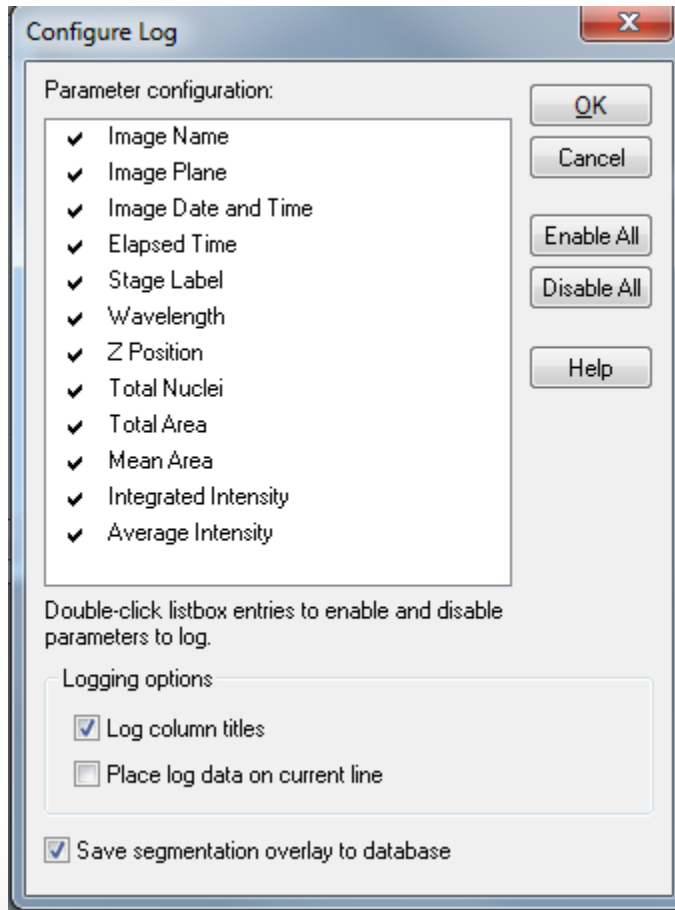
• Area, intensity, etc. for each individual cell

Adaptive Background Correction™ system

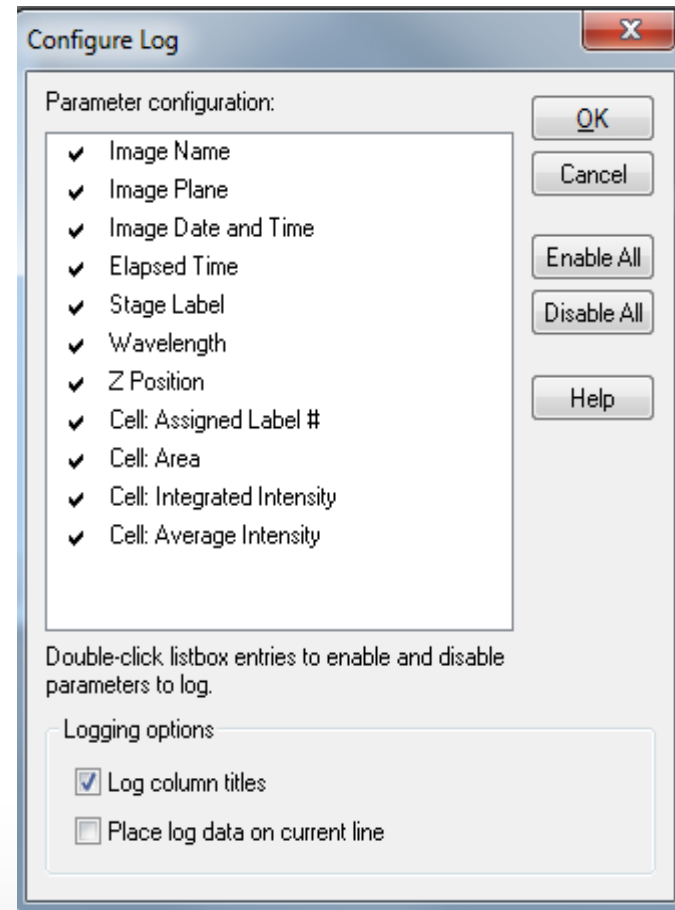
Configure Summary Log... **Configure Data Log (Cells)...**

Save Settings... Load Settings... Set to Defaults Test Run Close

Selecting measurements

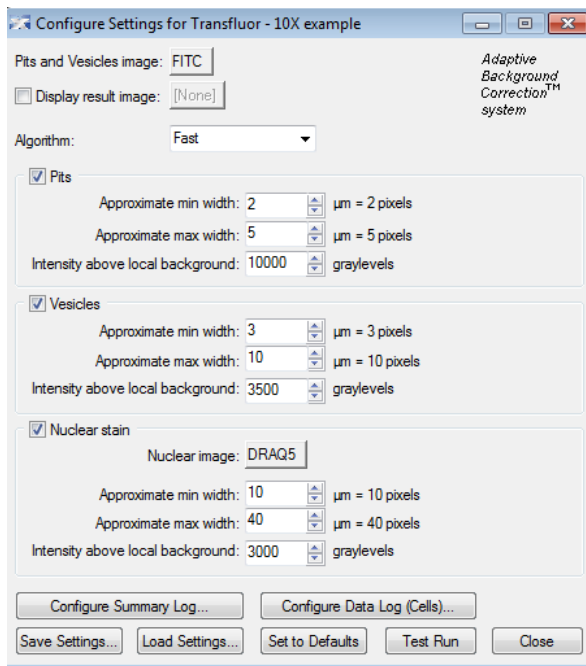


Configure Summary Log

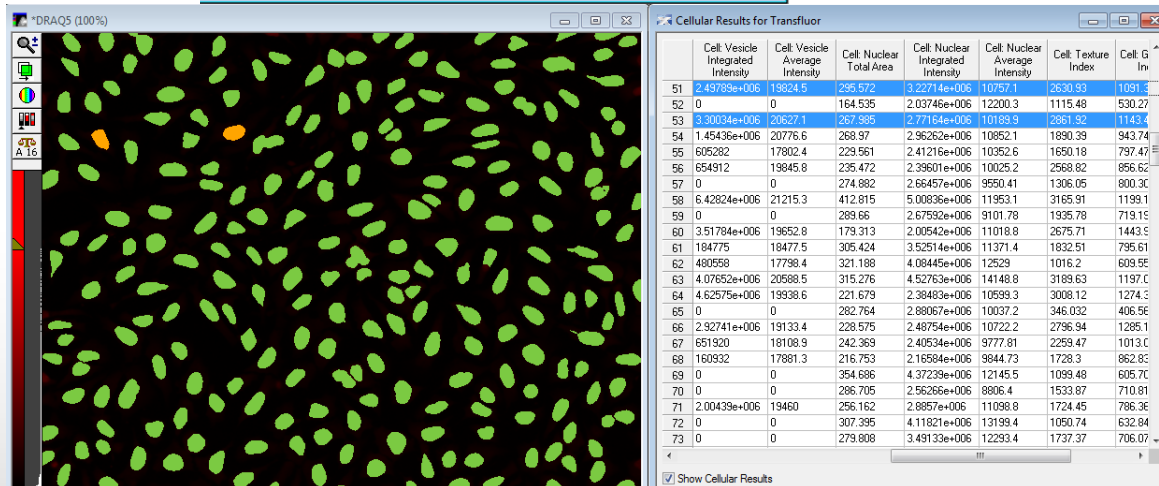


Configure Data Log

Module – interactive feedback



Interactive optimization of analysis parameters

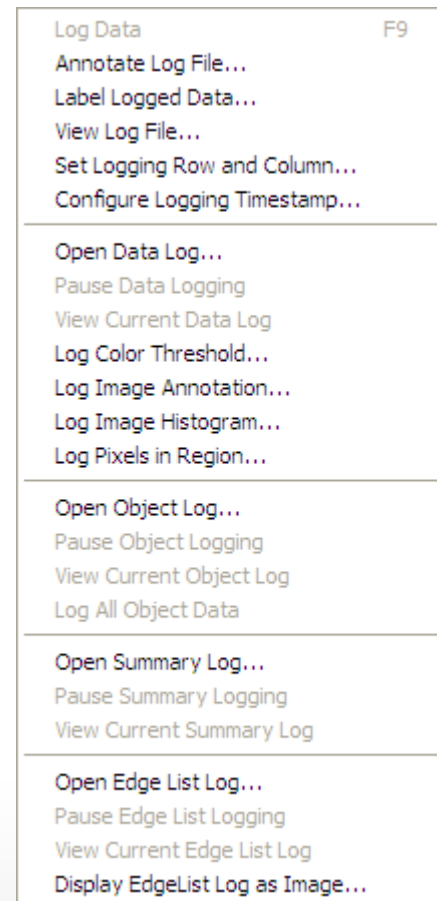


Logging out your data

- 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)

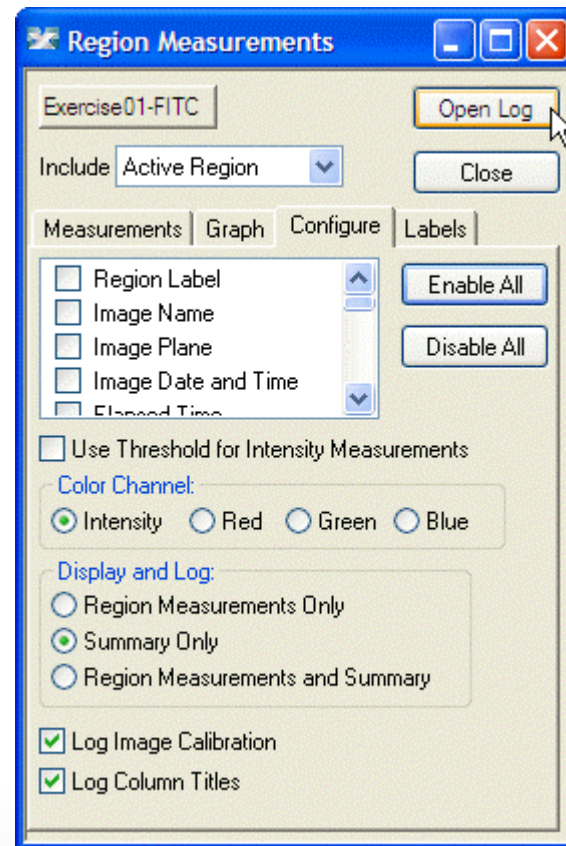
Opening and closing logs

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



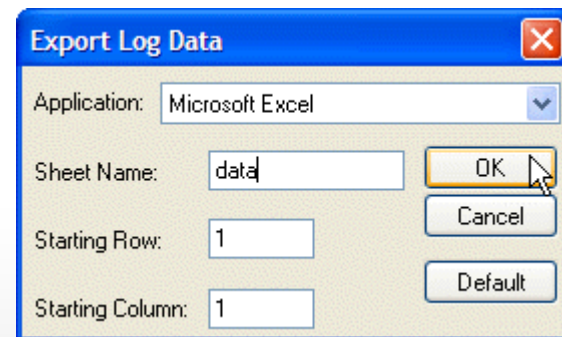
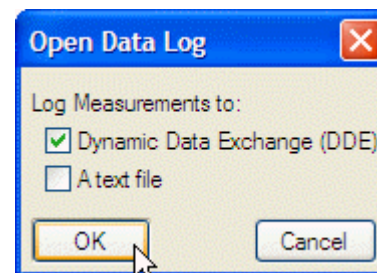
Opening and closing logs

- 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... Transflour 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 Image Overlay: Show cell segmentation Col: Cyan

Intensity Profile

Color Composite 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 A
Transfluor Agonist B_PRICKERT-UCLT1_17	Transfluor Agonist B	<NULL>	System A
Transfluor Agonist C_PRICKERT-UCLT1_18	Transfluor Agonist C	<NULL>	System A
Transfluor Agonist D_PRICKERT-UCLT1_20	Transfluor Agonist D	<NULL>	System A

Plate Statistics

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

Select Cancel

Configure branches

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

Review Plate Data: Display Tab

Wavelength selection

Color overlay option

Review Plate Data - Transfluor Agonist D_PRICKERT-UCLT1_20

Select Plate... Wavelengths: DRAQ5 FITC

Data view: Well arrangement

	Well arrangement	Time vs Well	Measurement vs Well	
A	158		17221.56	04
B	17298.99	19113.79	19815.42	16205.88
C	18780.35	18054.89	18765.95	17130.41

Print Table

Configure thumbnail montage area

Montage: 1 x 2 Time point: 1 of 1

Display | Run Analysis | Measurements | Graph

Show Values Image Overlay: Show cell segmentation Col: Cyan

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

Color Composite

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

Review Plate Data: Run Analysis Tab

Review Plate Data -

Select Plate... Transflur 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>

Counts nu: <Cell Cycle>

Log int: <Count Nuclei>

Selections: <Granularity> <Live Dead> <Micronuclei> <Mitotic Index> <Monopole Detection> <Multi Wavelength Cell Scoring> <Multi Wavelength Translocation> <Neurite Outgrowth> <Nuclear Translocation HT> <Transflur HT> <Transflur> <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

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

- Window Title: Review Plate Data -
- Select Plate...: Transfluor Agonist D_PRICKERT-UCLT1_20
- Wavelengths: DRAQ5, FITC
- Data view: Well arrangement
- Print Table button
- Data 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>

Settings: 10X

Counts nuclei using a single nuclear stain.

Log into the database

Selections [In Green]

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 - Transflur Agonist D_PRICKERT-UCLT1_20

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

DRAQ5
 FITC

	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: Transflur: Transflur 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 Plate Data: Measurements Tab

Review Plate Data - Transflur Agonist D_PRICKERT-UCLT1_20

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

DRAQ5
 FITC

	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: Transflur: Transflur 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... Transflur 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: Transflur: Transflur Example

Graph view: Plate Multiple graphs of displayed wells Single Well

Graph type: Histogram
Measurement: Cell: Pit Average Intensity

Number of bins: 6 Auto scale

Scale min: 0 Max: 100

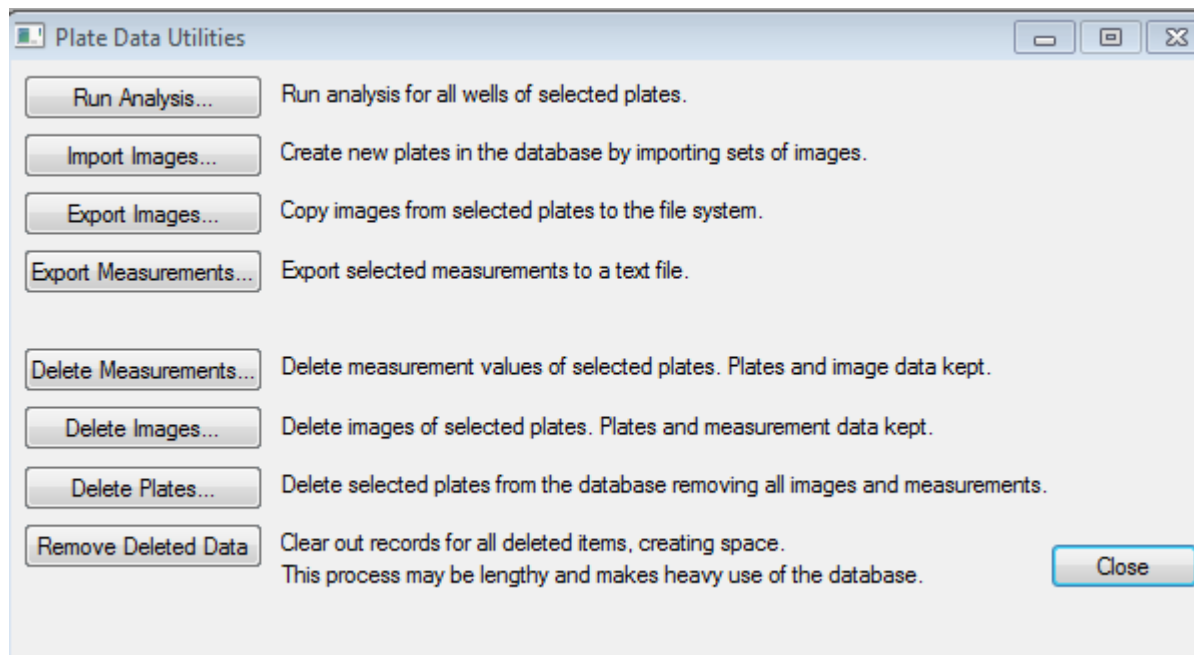
Set Display to Default Show Graph

Selections [In Green]

Load Images Clear

Reset Image Displays Cellular Results... Close

Screening > Plate Data Utilities



Screening > Plate Data Utilities > Export Measurements

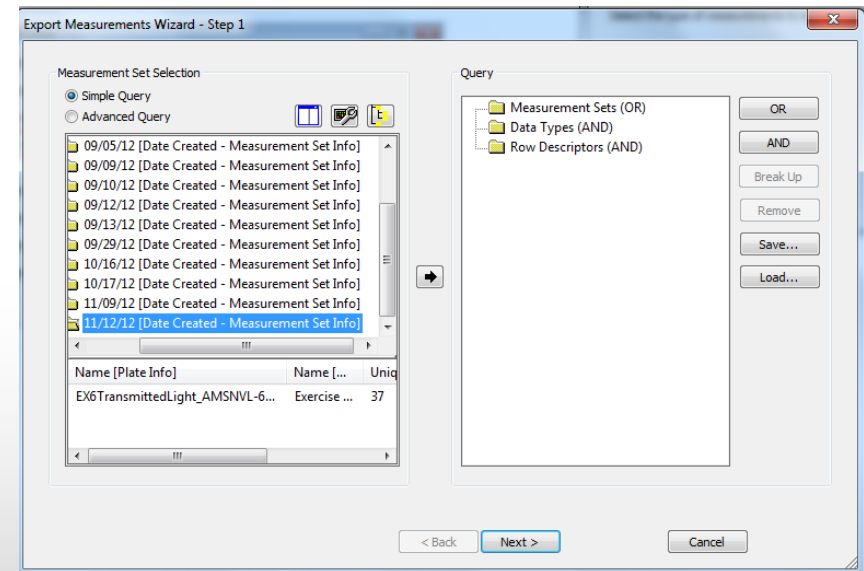
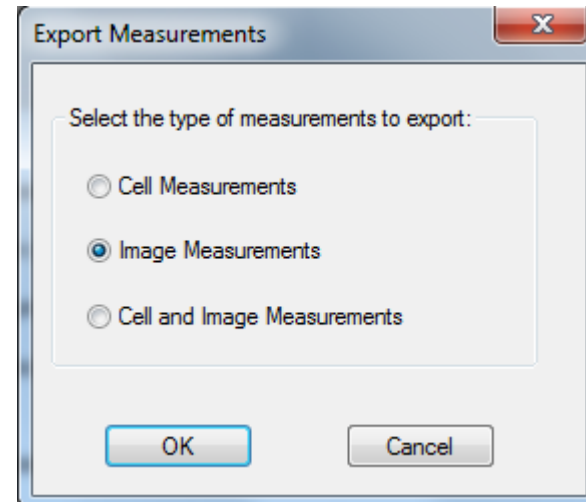
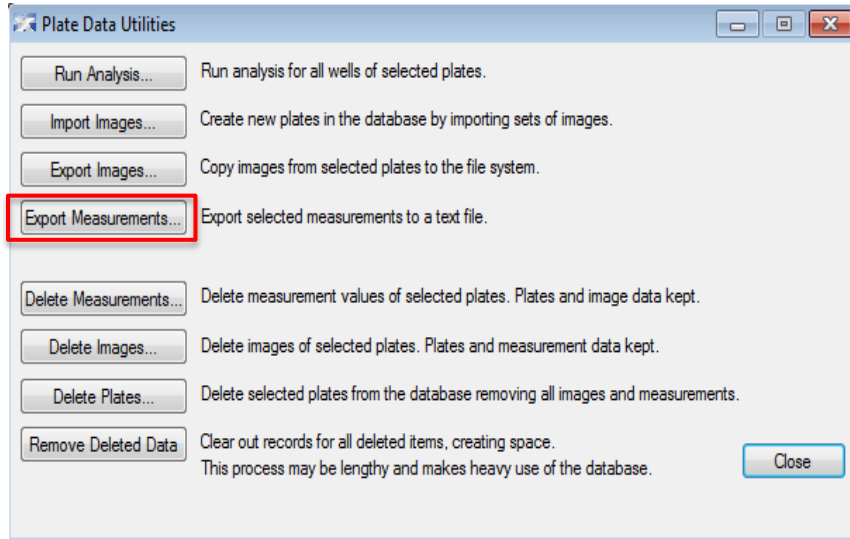


Plate Data Utilities: Run Analysis for Batch Analysis

Run Analysis on Plates

Analysis: <Transfluoer>

Settings: 10X example

Run method:

Run now on this computer

Add to auto run list

Description:

Finds and counts pits, vesicles, and nuclei.

Images to open for the analysis:

DRAQ5

FITC

OK Cancel

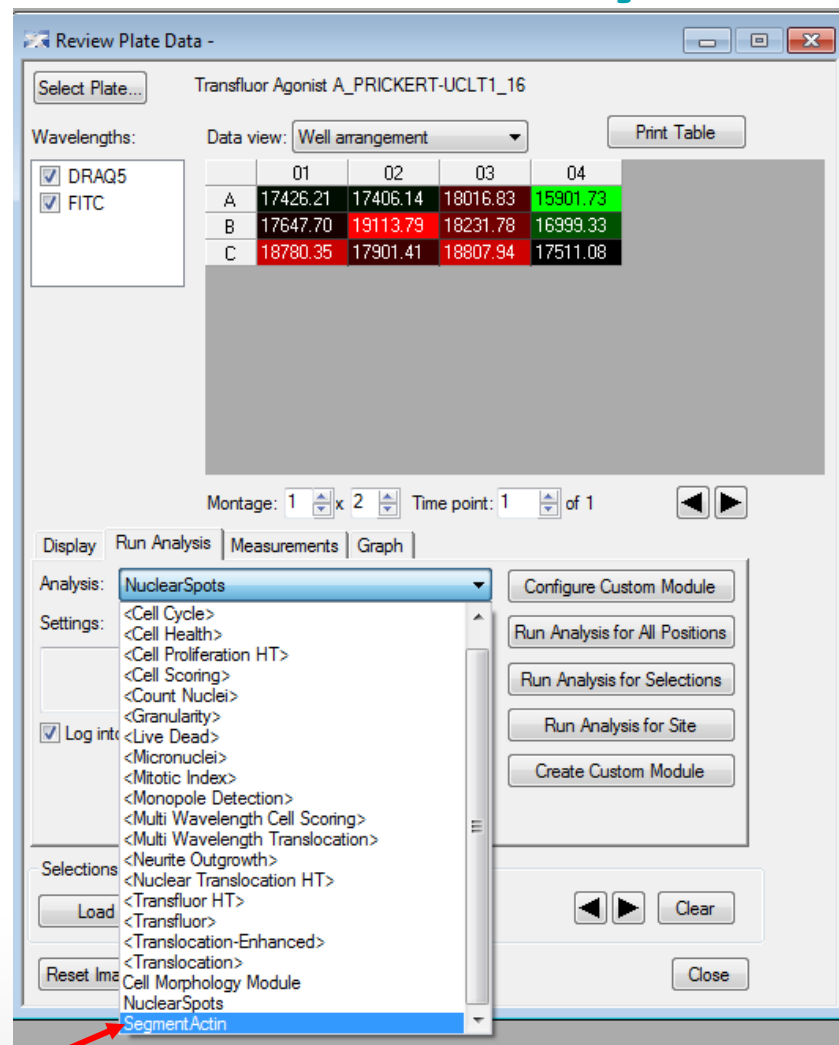
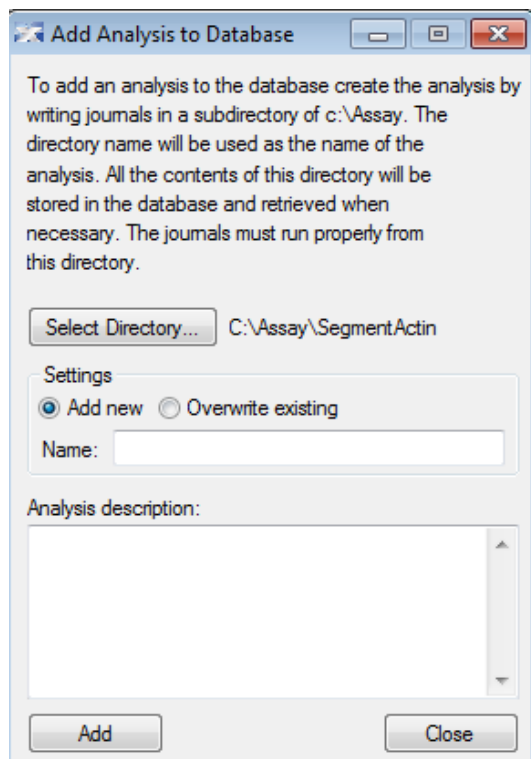
Auto Run Plate Statuses

Plate ID	Analysis	Setting	Status	Machine ID	Progress
10	Transfluoer	10X example	Pending		

Note: Plates will only be claimed for analysis if a machine has permission to write analysis results for the plate

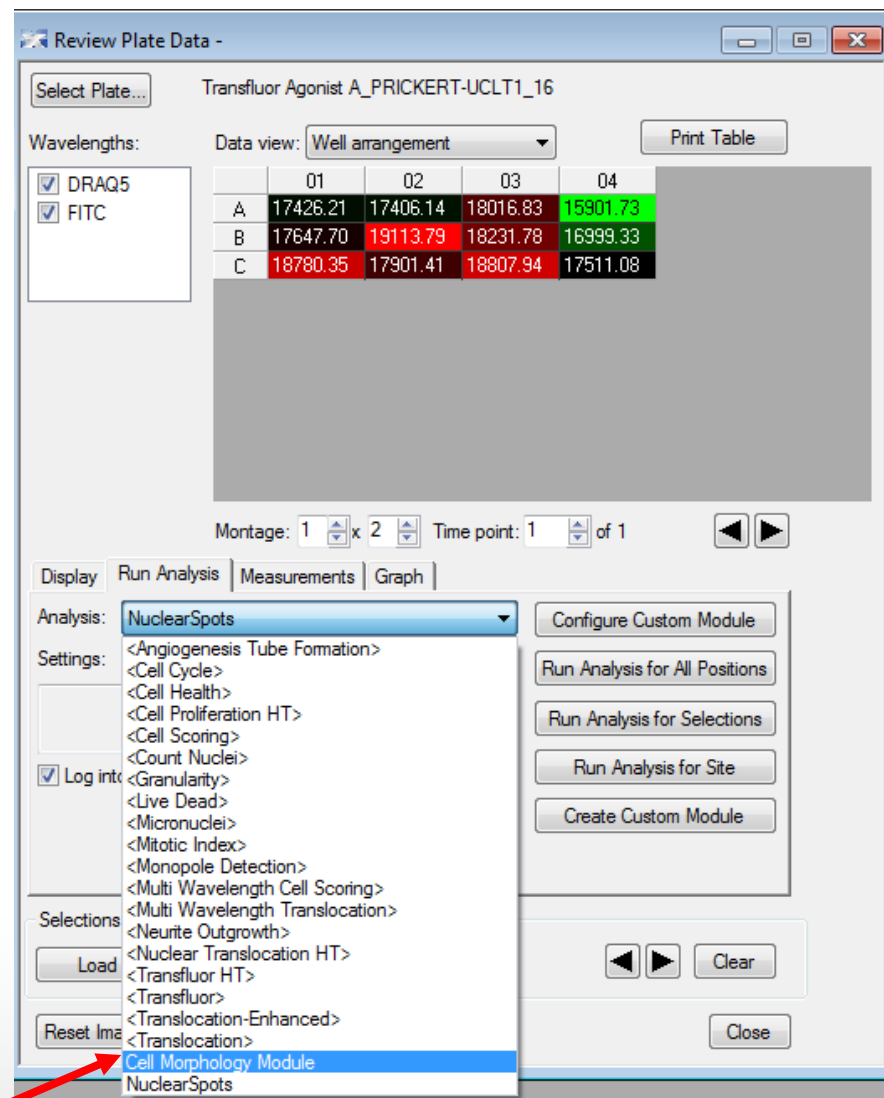
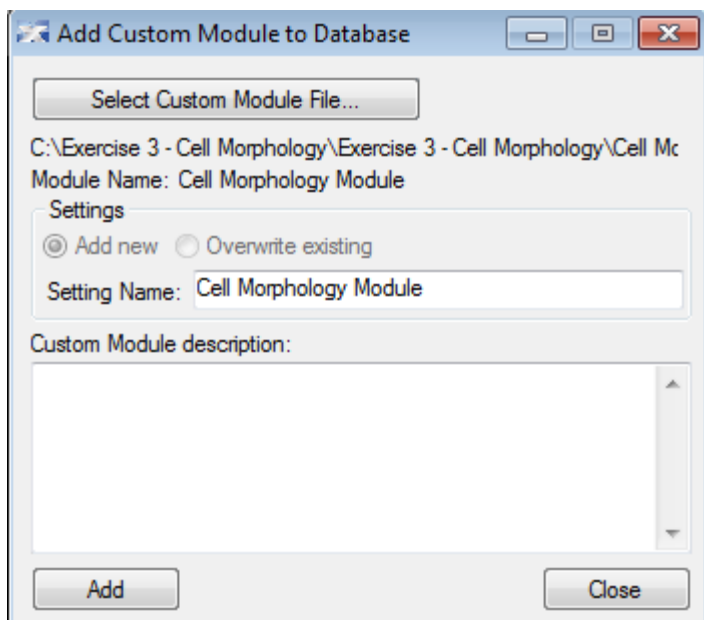
Cancel Selected Plate Cancel All Plates Start Auto Run Mode Close

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.



Together through life sciences.

www.moleculardevices.com