



Together through life sciences.

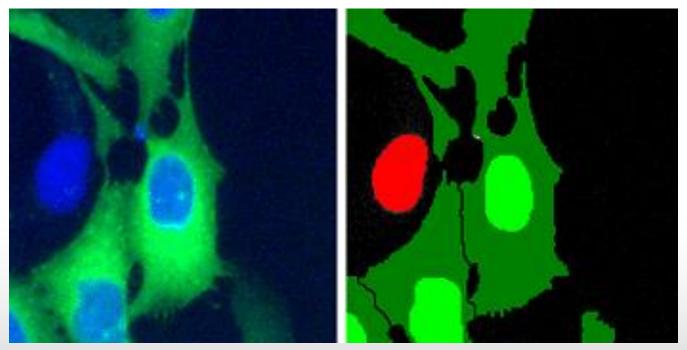
MetaXpress[®] Software: Analysis Training



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MetaXpress: Application Modules

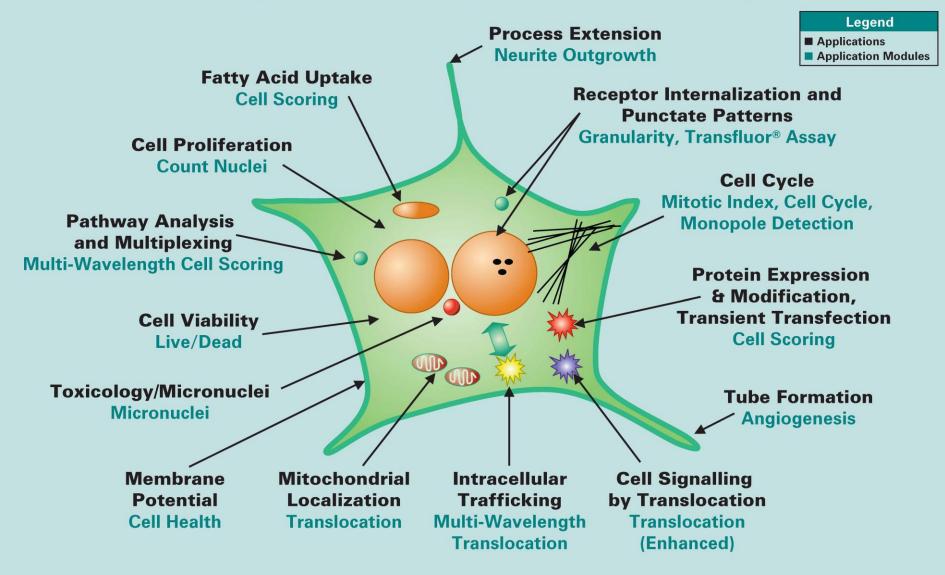
- "Canned," walk-away automation
- Advanced segmentation, feature detection, and measurement
- Site-by-site and cell-by-cell data
- Validated results
- Can be incorporated into a journal for increased customization



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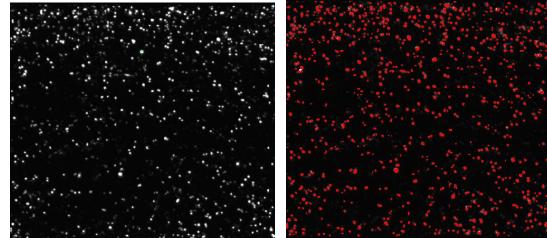


Application Modules for Hundreds of Assays Easy Custom Analysis with Journaling



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



🖂 Configure Settings	for Count Nuclei	- • •
Source image:	DRAQ5 [None]	Adaptive Background Correction™ system
Algorithm:	Standard 🔹	
Parameters	Approximate min width:	10 <u>▲</u> µm = 10 pixels
	Approximate max width:	16 μm = 16 pixels
Intensity a	above local background:	20 graylevels
Configure Summar		gure Data Log (Cells)
Save Settings Loa	d Settings Set to D	Defaults Test Run Close

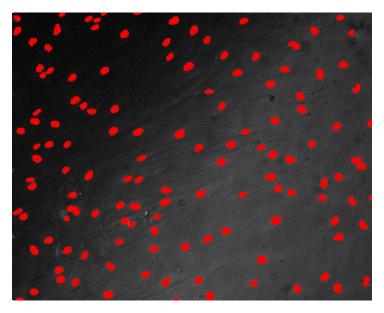


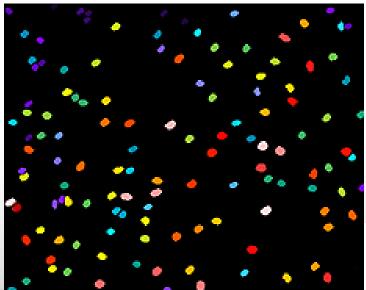
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Adaptive Background Correction

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

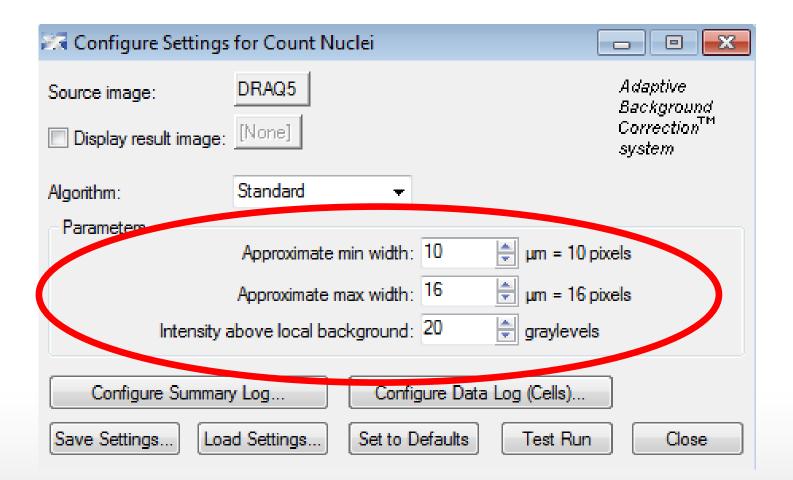






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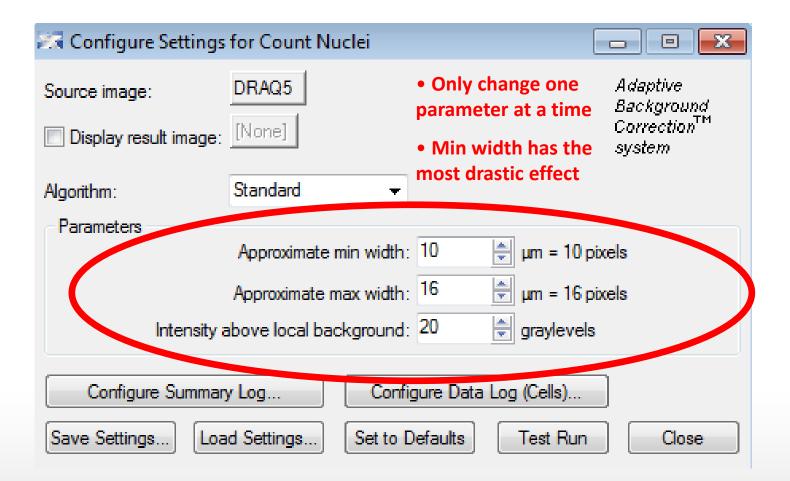
Configuring settings – the basics







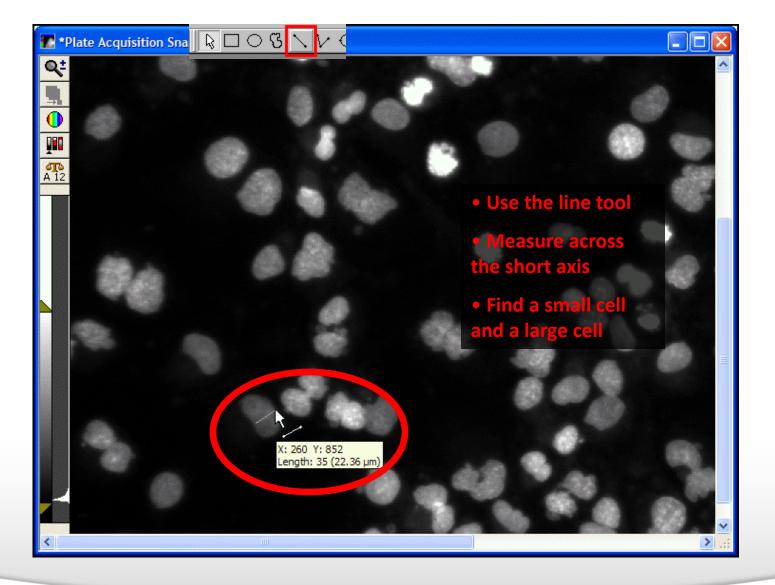
Optimizing settings







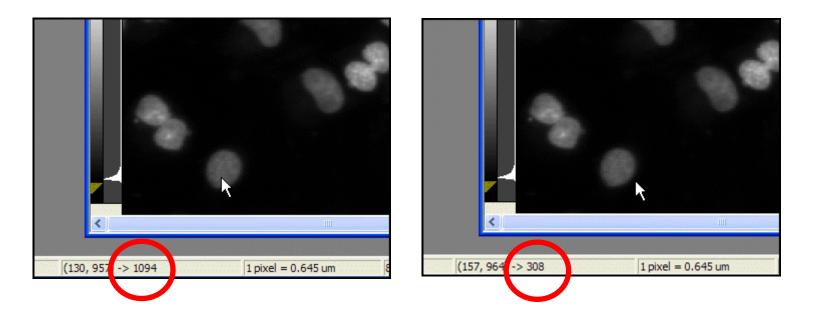
Measuring width







Measuring Intensity above Local Background



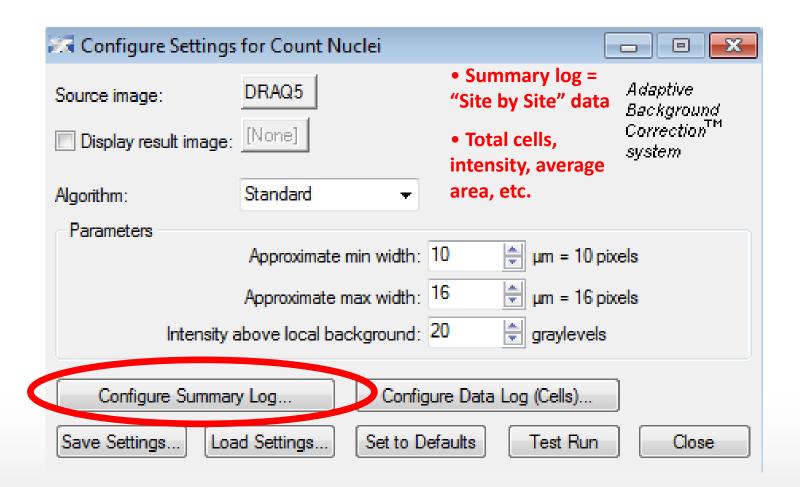
- 1. Find a dim cell
- 2. Measure intensity just inside and outside the cell
- 3. Subtract to find the difference (1094 308 = 786)
- 4. "Pad" the value by about 100 gray values (786 100 = 686).
 Note: For FAST algorithm, cut this value in half.

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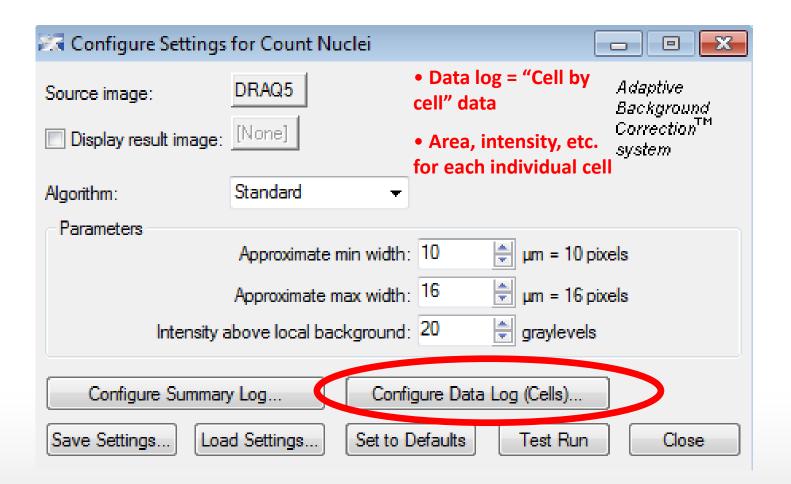
Selecting measurements







Selecting measurements

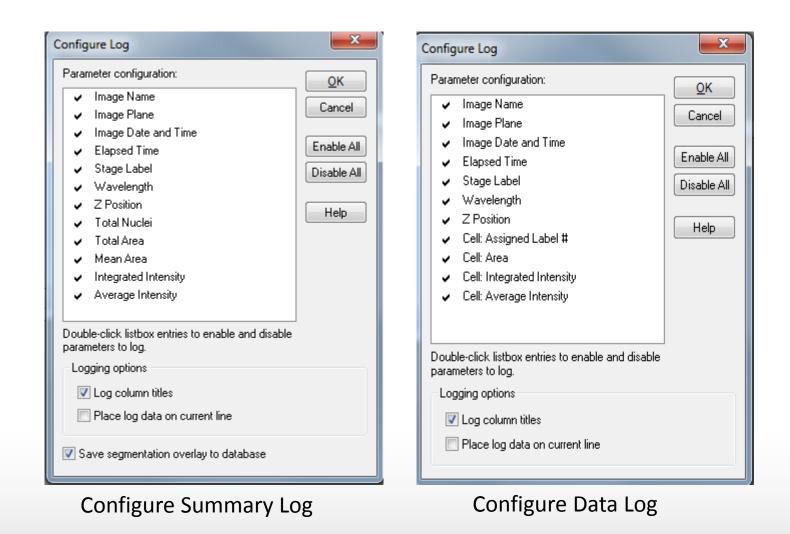




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Selecting measurements





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Module – interactive feedback

Pits and Vesicles image:	FITC			daptive
Display result image:	[None]		c	ackground orrection™ ystem
Algorithm:	Fast	•		
V Pits				
Approxima	te min width: 2	🚔 μm = 2 pix	els	
Approximat	e max width: 5	牵 μm = 5 pix	els	
Intensity above local	background: 1000	0 🍦 graylevels		
Vesicles				
Approxima	te min width: 3	🚔 μm = 3 pix	els	
Approximat	e max width: 10	韋 μm = 10 p	ixels	
Intensity above local	background: 3500	🚔 graylevels		
V Nuclear stain				
Nu	uclear image: DRA	Q5		
Approxima	te min width: 10	🚔 µm = 10 p	ixels	
Approximat	e max width: 40	🗼 μm = 40 p	ixels	
Intensity above local	background: 3000	graylevels		
Configure Summar	v Log	Configure Data Lo	a (Cells)	
		et to Defaults	Test Run	Close
Save Settings	ad Settings			Close
				ellular Results for 1
				Cell: Vesicle

Interactive optimization of analysis parameters

530.27

943.74

797.47

856.62 800.30

1199.1

719.15

1443.5

795.61

609.55

1197.0

1274.3

406.56 1285.1

10130

862.83

605.70

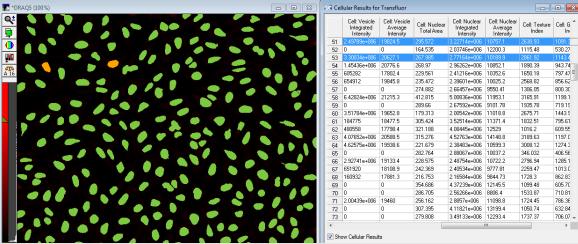
710.81

786.3E

632.84

706.07 🚽

Immediate graphic feedback on detection results



Links numerical results to image overlays



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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)

Log Data

Annotate Log File... Label Logged Data... View Log File... Set Logging Row and Column... Configure Logging Timestamp... F9

Open Data Log... Pause Data Logging

View Current Data Log Log Color Threshold... Log Image Annotation...

Log Image Histogram... Log Pixels in Region...

Open Object Log... Pause Object Logging View Current Object Log Log All Object Data

Open Summary Log... Pause Summary Logging View Current Summary Log

Open Edge List Log... Pause Edge List Logging View Current Edge List Log Display EdgeList Log as Image...



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Opening and closing logs

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

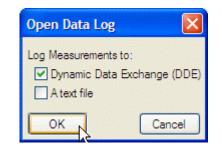
🛣 Region Measurements	
Exercise01-FITC	Open Log
Include Active Region 🛛 👻	Close
Measurements Graph Configure	Labels
Region Label	Enable All
Image Plane	Disable All
Image Date and Time	
Use Threshold for Intensity Measur	ements
 Intensity ○ Red ○ Green (Blue
Display and Log:	
 Region Measurements Only Summary Only 	
Region Measurements and Summ	ary
Log Image Calibration	
🗹 Log Column Titles	



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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



Export Log Da	ita	
Application: M	crosoft Excel	~
Sheet Name:	data	ок 💦
Starting Row:	1	Cancel
Starting Column:	1	Default



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Screening > Review Plate Data

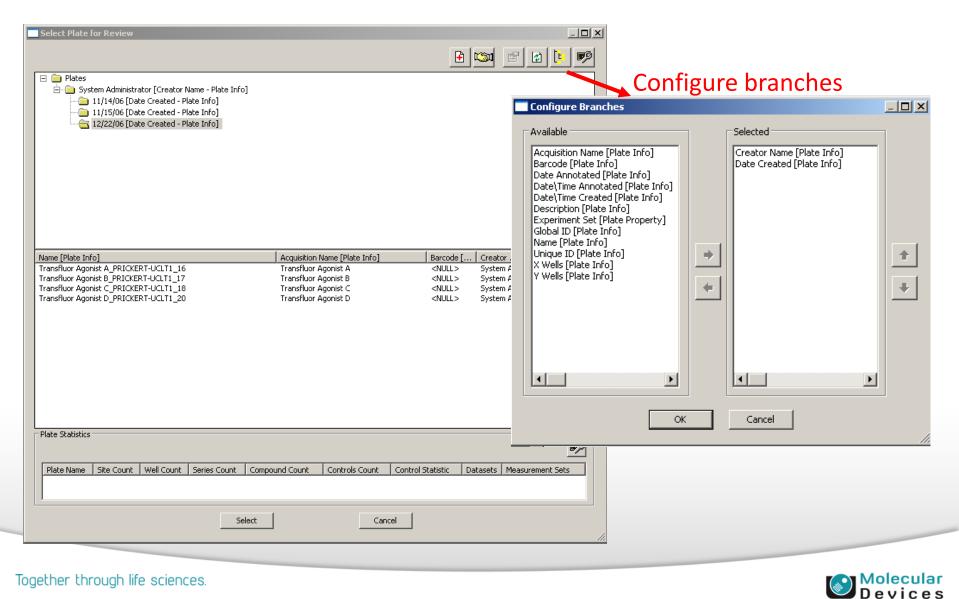


🗟 Review Plate Data	a -					- • ×
Select Plate	Transflu	uor Agonist D	PRICKER1	F-UCLT1_20		
Wavelengths:	Data	view: Well a	rangement	-	ſ	Print Table
_	2010	01	02	03	04	
DRAQ5	A	15829.65	23395.25	18103.91	17221.56	
FITC	B		19113.79	19815.42	16205.88	
		18780.35	18054.89	18765.95	17130.41	
	Monta	ige: 1 🌲 x	2 🌲 Tim	ne point: 1	≑ of 1	
Display Run Analys	sis Me	asurements	Graph			
Show Values	Imag	e Overlay:	Chaw call as	amontation	▼ Col:	Cyan 🔻
	inay	e Ovenay.	Show cell se	gmentation	• 001.	Cyan •
Intensity Profile						
Color Composite	Sour	ce R: <none< td=""><td>⇒ ▼ (</td><td>G: <none></none></td><td>▼ B:</td><td>DRAQ5 🔻</td></none<>	⇒ ▼ (G: <none></none>	▼ B:	DRAQ5 🔻
Selections [In Green]						
Load Images						
Reset Image Display		Cellular Res	ulte			Close
These image Display	•	Cellular Mes	นแร			Close

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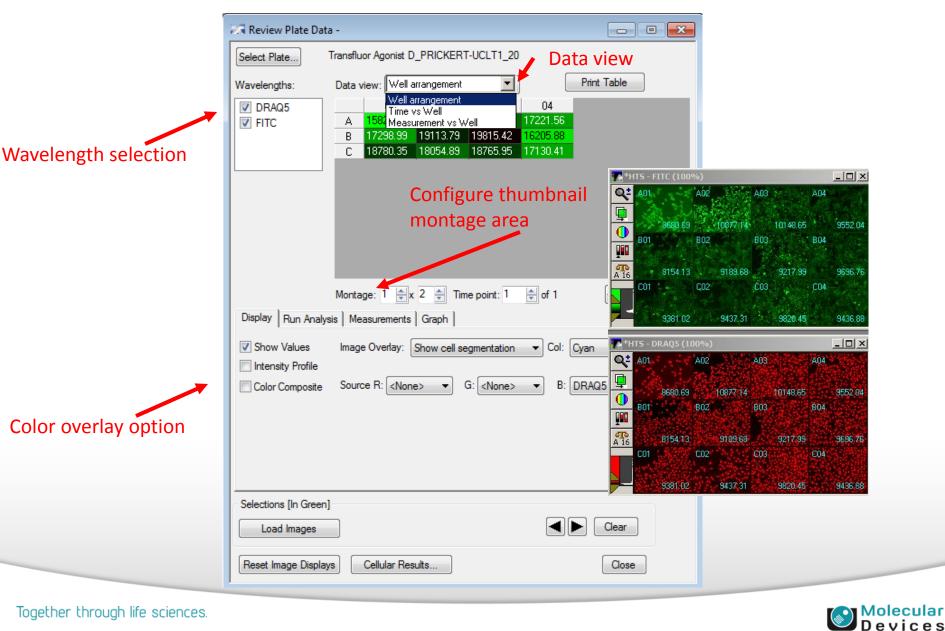


Select plate for review



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Review Plate Data: Display Tab



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Review Plate Data: Run Analysis Tab

Review Plate D	ata -									
Select Plate	Transfluor Agonist D	_PRICKER1	T-UCLT1_20							
Wavelengths:	Data view: Well ar	rrangement	-		Print Table]				
DRAQ5	01	02	03	04						
FITC		23395.25	18103.91	17221.56						
			19815.42 18765.95	16205.88 17130.41						
		10004.00	10100.00	11100.41						
	-									
						_				
	Montage: 1 🚔 x	2 🌲 Tim	ne point: 1	≑ of 1						
Display Run Ana			ne point: 1	🚖 of 1						
	lysis Measurements		ne point: 1)				
Analysis: Count	lysis Measurements Nuclei>		ne point: 1	of 1						
Analysis: Count	lysis Measurements Nuclei> Spots	Graph		Configure	Settings					
Analysis: Count Settings: Angio	lysis Measurements Nuclei> Spots enesis Tube Formation	Graph		Configure						
Analysis: Count Settings: Angio Counts nu Cell C	Ilysis Measurements Nuclei> Spots genesis Tube Formation cle> salth>	Graph		Configure un Analysis fo	Settings					
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Analysis: Settings: Counts nu Counts nu Cell C, Cell H- Cell Pr Cell Pr Cell S, Count Count Cell A- Cell Pr Cell S, Cell S, Count Count Cell S, Count Count Cell S, Count S, Cell S,	Ilysis Measurements Nuclei> Spots genesis Tube Formation cle> sath> oliferation HT> coring> Nuclei> arity> ead> uclei> Index> pole Detection> Vavelength Cell Scoring Vavelength Translocati e Outgrowth> ar Translocation HT> luor HT>	Graph		Configure un Analysis fr Run Analysis f Run Analys	Settings or All Positions or Selections sis for Site		→ C	ppear: Sustom	s if you n Modu	have the

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Review Plate Data: Run Analysis Tab

🕄 Review Plate D	ata -	3
Select Plate	Transfluor Agonist D_PRICKERT-UCLT1_20	
Wavelengths:	Data view: Well arrangement	
 ✓ 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	
	Montage: 1 🔿 x 2 🔿 Time point: 1 🔷 of 1 🚽 🕨	Configure module settings
Settings: 10X Counts nuclei usin	Edit List Run Analysis for All Positions Run Analysis for Selections	Run analysis for all positions, a subset of
V Log into the dat		marked selections, or current image displayed.
Selections [In Gree Load Images Reset Image Disp		Right click on wells to mar as selection. Turn off heat map to see marked selections (in green).



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Review Plate Data: Measurements Tab

🖪 Review Plate Data -
Select Plate Transfluor Agonist D_PRICKERT-UCLT1_20
Wavelengths: Data view: Well arrangement
Image: Constraint of the system 01 02 03 04 Image: Constraint of the system 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 k 2 Time soint: 1 d of 1
Display Run Analysis Measurements Graphy
Analysis: Transfluor: Transfluor Example Image: Share Heat Map Heat Map Measurement: Cell: Pit Average Intensity (Transfluor Example) Display Format: #.### Select Wells Based On Variable Range Value is: Between 0 and 100 Select
Data Log Not Open Log Open Log
Selections [In Green]
Reset Image Displays Cellular Results Close

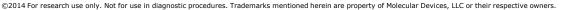
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Review Plate Data: Measurements Tab

🔜 Review Plate Dat	a -		- • -
Select Plate	Transfluor Agonist D_PRICKER	RT-UCLT1_20	
Wavelengths:	Data view: Well arrangement	• • [Print Table
DRAQ5	01 02	03 04	
FITC	A 15829.65 23395.25		
	B 17298.99 19113.79		
	C 18780.35 18054.89	18765.95 17130.41	
	Show heat	t map. Togg	le on/off.
		me point: 1 🚔 of 1	
Display Run Analy		×	1
Analysis: Tran	nsfluor: Transfluor Example 🔻	Show Heat Map	Heat Map
	Pit Average Intensity (Trar 💌	Display Format: #.	## •
Value is: Betwee		100	Select
Data Log Not Open		Configure Log	Open Log
Selections [In Green]		
Load Images			Clear
Reset Image Display	vs Cellular Results		Close

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Review Analysis Results Within MetaXpress

	1	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
DAPI FITC	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
V Cy3	В	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
	н	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
	1	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
	м	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
	P	93.1 94.5	93.0 92.8	91.0 92.8	91.0 91.3	90.1 92.8	91.4 91.0	51.9 46.2	53.0 43.7	20.3	17.2	8.4	11.3	5.2	6.3	6.5	7.9	2.9	9.8	6.9	10.9	10.1	13.1	40.5 37.0	38.0 44.0
	Monta	ge: 24	÷x 1	16 😜	Time p	pint: 1	¢.	of 1			•														
-	lysis Me	asuren	ients	Graph				_			•														
-		asuren	ients	Graph		oint: 1 Show H		_	Heat Ma																
Analysis: M.	lysis Me	asuren ength (ents Cell Sco	Graph ring: •	 -) 🔽 s		eat Ma	_				- -	-												
_	lysis Me ulti Wavek Positive W	asuren ength (/3 (Mu	tiWave	Graph ring: •	 -) 🔽 s	show H	eat Ma	•		ap)		- 	Q	ui	ck	ly	vi	en	/ a	na	ily	sis	5 0	lat	a
Analysis: M. Measurement: 🔀	lysis Me ulti Wavek Positive W ed On Var	asuren ength (/3 (Mu	tiWave	Graph ring: •	 	ihow H Jisplay F	eat Ma	•		ap		-	Q			ly ue									a
Analysis: M. Measurement: 🔀 Select Wells Bas	llysis Me ulti Wavek Positive W ed On Var een ▼	asuren ength (/3 (Mu iable F	tiWave	Graph ring: •	 	ihow H Jisplay F	eat Ma Format:	P [Heat Ma	ap)			Q			ly ue									a
Analysis: Mu Measurement: % Select Wells Base Value is: Betwe	llysis Me ulti Wavele Positive W ed On Var een ▼ n	asuren ength (/3 (Mu iable F	tiWave	Graph ring: •	 	ihow H	eat Ma Format:	P [Heat Mi	ap)			Q												a



Together through life sciences.

Review Plate Data: Graph Tab

🖳 Review Plate	Data -						• X
Select Plate	Transflu	uor Agonist D	_PRICKER	T-UCLT1_20)		
Wavelengths:	Data	view: Well a	rrangement	•) (Print Table	
V DRAQ5		01	02	03	04		
FITC	A	15829.65	23395.25	18103.91	17221.56		
	В	17298.99	19113.79	19815.42	16205.88		
	С	18780.35	18054.89	18765.95	17130.41		
	- 10						
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	- 10						
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		1 🔺	2 🔺 T-		A		
	Monta	age: 1 🌲 x	2 🖃 In	ne point: 1	≑ of 1		
Display Run A	nalysis Me	easurements	Graph				
Analysis:	Transfluor:	Transfluor Ex	ample 🔻				
Graph view:							
💿 Plate 🔘 🛚	Aultiple grap	hs of displaye	ed wells 🔘	Single Well			
Graph type: Hi	stogram		- Measu	urement: C	ell: Pit Avera	ge Intensity 🔻	
Hi	stogram		Numbe	er of bins: 6		Auto scale	
		vs Well Colu vs Well Row	mn				
		vs Well Num	Seele.	min: 0	× N	fax: 100 🚔	
Set Display to	atter Plot				ſ	Show Graph	
Selections [In G	reen]						
Load Image	es l						
Reset Image Di		Cellular Res	ults			Close	

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Screening > Plate Data Utilities

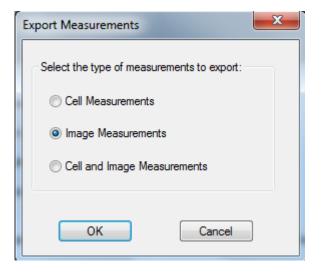
🖳 Plate Data Utilities	
Run Analysis	Run analysis for all wells of selected plates.
Import Images	Create new plates in the database by importing sets of images.
Export Images	Copy images from selected plates to the file system.
Export Measurements	Export selected measurements to a text file.
Delete Measurements	Delete measurement values of selected plates. Plates and image data kept.
Delete Images	Delete images of selected plates. Plates and measurement data kept.
Delete Plates	Delete selected plates from the database removing all images and measurements.
Remove Deleted Data	Clear out records for all deleted items, creating space. This process may be lengthy and makes heavy use of the database.

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Screening > Plate Data Utilities > Export Measurements

🐼 Plate Data Utilities	
Run Analysis	Run analysis for all wells of selected plates.
Import Images	Create new plates in the database by importing sets of images.
Export Images	Copy images from selected plates to the file system.
Export Measurements	Export selected measurements to a text file.
Delete Measurements	Delete measurement values of selected plates. Plates and image data kept.
Delete Images	Delete images of selected plates. Plates and measurement data kept.
Delete Plates	Delete selected plates from the database removing all images and measurements.
Remove Deleted Data	Clear out records for all deleted items, creating space. This process may be lengthy and makes heavy use of the database.



xport Measurements Wizard - Step 1	-	
Measurement Set Selection Simple Query Advanced Query 09/05/12 [Date Created - Measurement Set Info] 09/09/12 [Date Created - Measurement Set Info] 09/12/12 [Date Created - Measurement Set Info] 09/12/12 [Date Created - Measurement Set Info] 09/12/12 [Date Created - Measurement Set Info] 10/16/12 [Date Created - Measurement Set Info] 11/09/12 [Date Create	•	Query CR Data Types (AND) Row Descriptors (AND) Break Up Remove Save Load
(< Back	ck Next > Cancel



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Plate Data Utilities: Run Analysis for Batch Analysis

Run Analysis on Plates	×
Analysis: <transfluor> Settings: 10X example </transfluor>	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

Plate ID	Analysis	Setting	Status	Machine ID	Progress	
10	Transfluor	10X example	Pending			
Note: Plates will only be claimed for analysis if a machine has permission to write analysis results for the plate						

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Add Analysis to Database: Custom Analyses

	Select Plate Transfluor Agonist A_PRICKERT-UCLT1_16
Add Analysis to Database To add an analysis to the database create the analysis by writing journals in a subdirectory of c:\Assay. The directory name will be used as the name of the analysis. All the contents of this directory will be stored in the database and retrieved when necessary. The journals must run properly from this directory.	Wavelengths: Data view: Well arrangement Print Table Image: DRAQ5 01 02 03 04 Image: DRAQ5 01 17406.14 18016.83 15901.73 Image: DRAQ5 01 17406.14 18016.83 15901.73 Image: DRAQ5 01 02 03 04 Image: DRAQ5 01 17406.14 18016.83 15901.73 Image: DRAQ5 01 17405.14 18017.94 16999.33 Image: DRAQ5 01 18780.35 17901.41 18807.94 17511.08
Select Directory C:\Assay\SegmentActin Settings Overwrite existing Name: Image: Im	Montage: 1 1 1 Display Run Analysis Measurements Graph Analysis: NuclearSpots Configure Custom Module Settings: Cell Cycle> Cell Proliferation HT> Cell Proliferation HT> Cell Proliferation HT> Cell Scoring> Run Analysis for Selections Carginularity> Carginularity> Run Analysis for Stee Clargint Clure Dead> Run Analysis for Stee Mutti Wavelength Cell Scoring> Runt Analysis for Stee Create Custom Module Selections Nuclear Translocation > Create Custom Module Value Cransflour HT> Create Custom Module E Carginularity> Create Custom Module E Mutti Wavelength Cell Scoring> Create Custom Module E Selections Clarging Clarging Clarging Clarging Clarging Clarging Clarging Clarging Clarging Value Carging Clarging Clarging Clarging Clarging
Assay will appear in Run Analysis tab.	Add Analysis to Database is only

🖂 Review Plate Data -

used if you create your own custom analysis with a journal (macro).

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- O X

Add Analysis to Database: Custom Module

	💦 Review Plate Data -		
	Select Plate Transfluor Agonist A_PRICKERT-UCLT1_16		
	Wavelengths: Data view: Well arrangement	rint Table	
	Image: Width DRAQ5 01 02 03 04 Image: Width DRAQ5 A 17426.21 17406.14 18016.83 15901.73		
	B 17647.70 19113.79 18231.78 16999.33		
Add Custom Module to Database 📃 💷 💌	C 18780.35 17901.41 18807.94 17511.08		
Select Custom Module File			
C:\Exercise 3 - Cell Morphology\Exercise 3 - Cell Morphology\Cell Mc Module Name: Cell Morphology Module Settings			
Add new Overwrite existing	Montage: 1 卖 x 2 🐳 Time point: 1 🔿 of 1		
Setting Name: Cell Morphology Module	Display Run Analysis Measurements Graph		
Custom Module description:	Analysis: NuclearSpots Configure Custor	m Module	
	Settings: Cell Cycle	NI Positions	
	<cell health=""> <cell ht="" proliferation=""></cell></cell>	Selections	
	<cell scoring=""> <count nuclei=""></count></cell>		
	Log Int (Granulanty)		
	<micronuclei> <mitotic index=""></mitotic></micronuclei>	Module	
Add Close	<pre></pre>		
	Selections		
	Load <nuclear ht="" translocation=""> <transfluor ht=""></transfluor></nuclear>	Clear	
	<transfluor></transfluor>		
	Reset Ima (Translocation> Cell Morphology Module	Close	
	NuclearSpots		

Custom Module will appear in Run Analysis tab.

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