AIM Core Newsletter

September 2020

In a nutshell. . . (scroll down to learn more about)

Tomorrow's Zoom Training for the CX7

Hate spending hours scanning your plate or slide looking for your sample? Learn how eureka scan combines automated sample finding with High Content Analysis.

Plans to get your next 96Seahorse Experiment Going

Don't know how to make use of the powerful Metabolism Analyzer that's at your finger tips? We're here to help!

Flow Baby Flow! Guava and Amnis updates

Hours of recorded training from Luminex and Guava are available to help you with High Content imaging flow analysis (Amnis) or simpler flow (Guava)

CX7 EurekaScan Training & New User Training: Th 9/10/20 @ 1-5pm on Zoom

Come 1) learn about the Eureka Scan feature on the Cellomics CX7 &
2) get a New User intro training
with Bret Samelson
Training will be recorded for future access

Join Zoom Meeting
https://hsc-unm.zoom.us/j/93009591105

Save time and effort with improved high-content screening

What is EurekaScan™ Finder?

- Thermo Scientific EurekaScan™ Finder is a new feature for HCS Studio software that significantly reduces your instrument runtime (CellInsight CX7 LED and CellInsight CX7 LZR) and analysis efforts by automating the detection and subsequent capture of events at higher magnification.

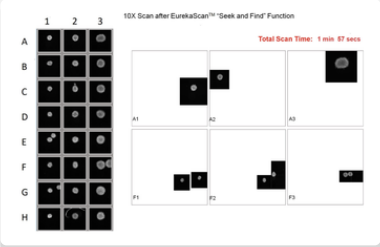
Why is there a need for EurekaScan™ for high-content screening?

- Without EurekaScan™ Finder, if the well is sparsely populated or if you need to image rare events at high magnification, scan times are significantly enhanced due to the instrument performing autofocus and imaging on the blank fields.
- Also, typical analysis times are prolonged as you need to review and discard a large number of irrelevant images.

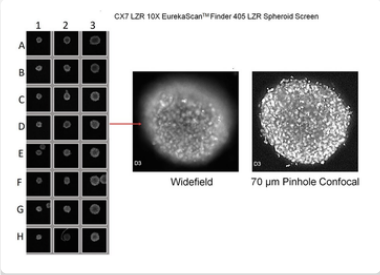
The solution is EurekaScan™ Finder

- With EurekaScan™ Finder objects/events recognized at lower magnification during the first scan are automatically used to drive the scan area of the high magnification pass(es), thus saving time and effort.
- EurekaScan™ Finder can also handle more than two passes (double-pass high-content screening) for those scenarios that could benefit from three or more passes, such as identifying non-sparse fields at low magnification, finding rare events at higher magnification, and then evaluation of rare events at same or higher magnification.

It's time consuming to manually search for rare events



Cut an average of 28 minutes of manual searching to 1 minute

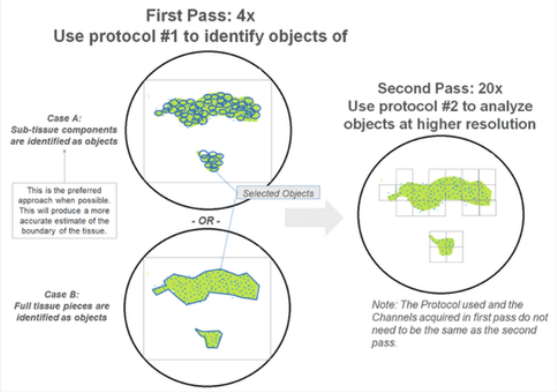


Low level scanning, then high level confocal imaging

Flexible with various sample types

EurekaScan™ Finder offers two different approaches based on the type of sample and user-stipulated criteria that will inform high-content screening:

Scenario I: when studying tissues

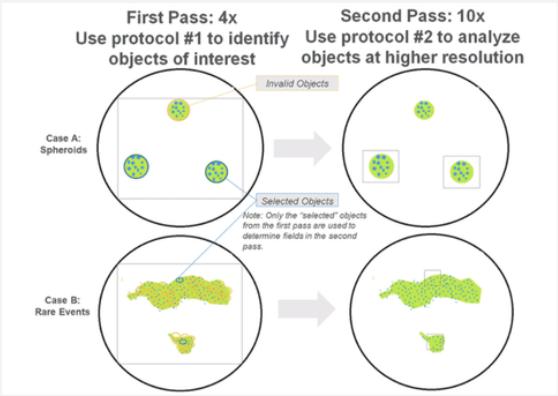


Click image to enlarge

Scenario I: In this scenario (tissue), the selected objects in first pass are bigger than the second pass's field of view (case A), or multiple objects appear in the second pass's field of view (case B).

In which case, the second pass will utilize a uniformly spaced lattice pattern to define the second pass's field locations.

Scenario II: when studying spheroids, rare-events



Click image to enlarge

Scenario II: In this scenario (spheroids, rare events), the selected objects in the first pass are smaller than the second pass's field of view and are spaced apart such that multiple objects are unlikely to be in the same field of view.

In which case, the second pass will center the field of views around each object.

Note: The software will automatically choose whether to "center-on-object" or use "lattice pattern" based upon each the selected objects average size and density, on a per-well basis.

The real story behind Archimedes' Eureka! - Armand D'Angour

xFe 96 Seahorse really wants to be useful

Our newest metabolic analyzer, (Moose), the xFe 96 Seahorse can answer a lot of different questions about your cells! Interested in designing an experiment but not quite sure where to start or what you CAN ask of your cells? Contact Jay Dunn: Jay.Dunn@agilent.com and Craig Smith: Craig.Smith@Agilent.com to discuss your pilot experiment and overall experimental goals. When we have several investigators who are willing to take the next step, we will schedule training (part 2) around running and analyzing your pilot experiment.

Click here to watch the first Seahorse training (Part A)

Click here to watch the first Seahorse training (Part B)

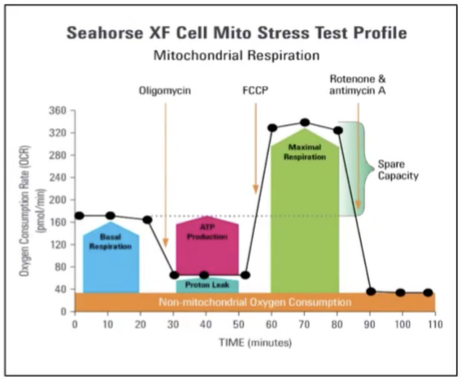
Click here to watch the Intro and Basics tutorial

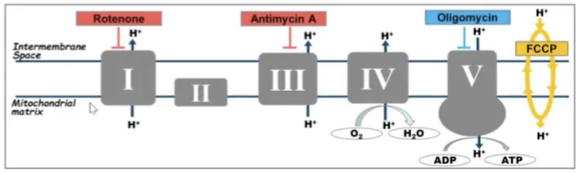
Below are some examples of the types of questions you can tackle with this technology.

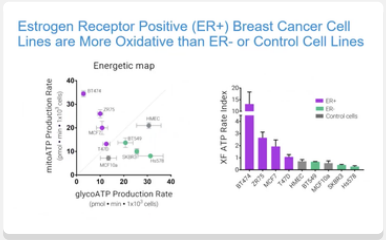
What Assay Provides the Most Comprehensive View of Mitochondrial Function?

Agilent XF Cell Mito Stress Test delivers critical information beyond basal respiration

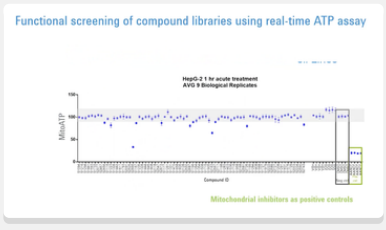
- Provides a comprehensive picture of mitochondrial function:
  - ✓ Basal Respiration
  - ✓ ATP Linked Respiration
  - ✓ Maximal Respiration
  - ✓ Spare Respiratory Capacity
  - ✓ Proton Leak Linked Respiration
- Assess mitochondrial liabilities due to genetic modification or drug intervention



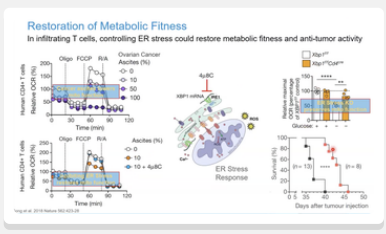




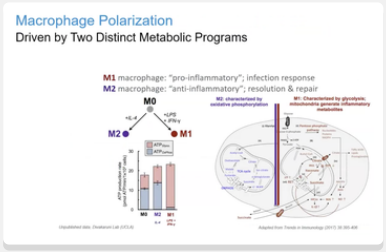
Differences in estrogen receptor + and - cell lines



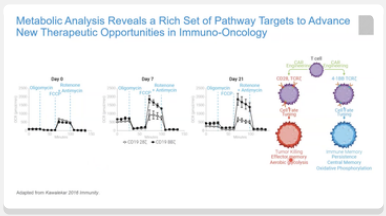
Compound screening



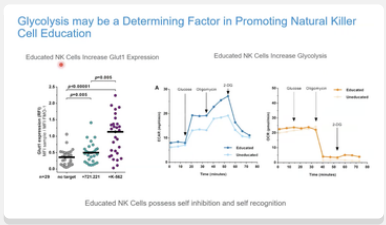
Infiltrating T cells in the PC restored anti-tumor activity



Macrophage Polarization



Finding targets for Immuno-Oncology Therapeutics



NK Cell Education

Guava Flow Cytometer

Our MK(II) has a Violet and a Blue laser. It has 11 channels (including FSC and SSC) and can process individual screw cap centrifuge tubes or 96 well U bottom plates.

The fluorochrome chart below lists example fluorochromes in text colors that correspond to the excitation laser (so only look at the violet and blue text). The table just below lists our 11 channel capability: Color describes bandpass filters for detection and "-V" or "-B" describes the laser of excitation.

FSC Intensity	SSC Intensity	BLU-V MFI	GRN-V MFI	YEL-V MFI	RED-V MFI	NIR-V MFI	GRN-B MFI	YEL-B MFI	RED-B MFI	NIR-B MFI

Detection channel (peak/range):

Blue (450/45 nm)	Green (525/30 nm) (512/18 nm)*	Yellow (583/26 nm) (575/25 nm)*	Orange (620/52 nm) (609/30 nm)*	Red (664/20 nm)	Red (695/50 nm)	NIR (785/70)
AlexaFluor® 405	Alexa Fluor® 430	BV 570®	BV 605®	BV 650®	BV 711®	BV 785®
BV 421®	BV 510®	Cascade Yellow	QD 625	eFluor 650	QD 705	eFluor 750
Cascade Blue	Cascade Yellow	Pacific Orange	Ethidium bromide	QD 655	7-AAD	QD 800
DAPI	Pacific Green	QD 565	PE-Dazzle™594	7-AAD	PE-AlexaFluor® 647	PE-AlexaFluor® 750
DyLight™ 405	Pacific Orange	QD 585	PE-Texas Red	Acridine Orange (RNA)	PE-AlexaFluor® 700	PE-Cy7
eFluor 450	QD 525	DsRED	7-AAD	Ethidium bromide	PE-Cy5.5	FM® 5-95
Hoescht 33258	QD 545	Ethidium bromide	Alexa Fluor® 568	PE-Cy5	PerCP	PE-Cy7
LIVE/DEAD Violet	Zombie Aqua	JC-1 (aggregate)	EthD-1, EthD-2	Propidium Iodide (PI)	PerCP-Cy5.5	APC-H7
Marina Blue	Acridine Orange (DNA)	R-PE	Ethidium bromide	7-AAD	7-AAD	APC-Alexa Fluor® 750
Pacific Blue	Alexa Fluor® 488	RFP	mCherry	Ethidium bromide	PE-Cy5.5	APC-Cy7
	BODIPY-FL	Alexa Fluor® 532	MitoTracker®Red	Nile Red	APC-Cy5.5	
	Calcein	Alexa Fluor® 555	Nile Red	PE-Cy5	DRAQ5	
	CFSE	Cy3	PE-Dazzle™594	Propidium Iodide (PI)	DyeCycle™ Ruby	
	CF™ 488	DiI	PE-eFluor 610	Alexa Fluor® 647		
	Cy2	DsRED	PE-Texas Red	Alexa Fluor® 660		
	DyLight™ 488	dTomato	Propidium Iodide (PI)	APC		
	FAM	DyLight®550	Rhodamine Red-X	BODIPY 650/665		
	FITC	Ethidium bromide		Cy5		
	GFP/eGFP	mOrange		DiIC1(5)		
	JC-1 (monomer)	PO-PRO™-3 Iodide		DyLight™ 650		
	Oregon Green	R-PE		eFluor® 660		
	Rhodamine 110 & 123	RFP		MitoSense Red		
	SYBR Gold	SYTOX® Orange		Sytox® Red		
	SYBR Green			TO-PRO® 3		
	SYTOX Green			TOTO®-3		
	Thiazole Orange					
	TO-PRO-1					
	YFP/eYFP					
	YO-PRO-1					
	YOYO-1					

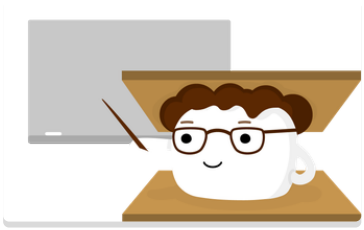
Excitation:
Violet laser (405 nm)
Blue laser (488 nm)
Green laser (532 nm)
Red laser (642 nm)

Happy Data and Help Tips Contests!

Email me a pic of your happiest data with an explanation of why it makes you able to sleep with joy in your heart!

Did you learn something helpful in the Core that might help someone else? Email me your helpful tip & if others find that it IS helpful then you get a prize!

All entries will be compiled and we'll vote at the end of September for the best "happy" data and most helpful tip! Prizes and bragging rights to be had!



Here to help!

I'm not always physically in the core, but if you need help or want to schedule a meeting, just email me. I can support /communicate via Zoom anytime. If you have trouble with the kiosk, just send me an email of when/what equipment you actually used and I can adjust the records. Lab: 505-272-7102. Google Voice: 505-216-6882.

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Thesis Defense