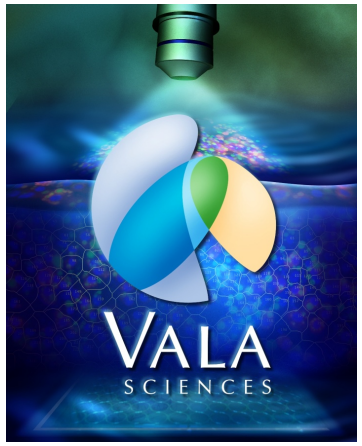


Providing innovative strategies for high content imaging and analysis

Patrick M. McDonough, Ph.D.
VP of Biology, Vala Sciences Inc
Computational Toxicology Communities of Practice webinar
Nov. 17, 2011

A brief history of Vala Sciences Inc:

The Vala Sciences Inc was founded in 2003. The biology and engineering team previously founded Q3DM (Quantitative 3-Dimensional Microscopy), a San Diego based company that developed the EIDAQ/IC-100 one of the first High Content Assay workstations.

Jeffrey Price, MD. Ph.D., the CEO of Vala, and cofounder of both Q3DM and Vala is a pioneer in the field of High Content Analysis (HCA, typically cell-based assays which feature high resolution, multi-channel fluorescence imaging, with automated image acquistion and image analysis software).

Vala Sciences offers instrumentation, software, reagents, and assays for HCA for both cells and tissue, relevant to high throughput screening, preclinical research, and digital pathology applications.

Recent funded grants and contracts to Vala Sciences

Grants:

2006 NIH SBIR 1R43DK074333-01 Title: *HT Image Assay of Lipid Droplet Formation in Human Adipocytes*. Phase I = $142,606, Phase II = $1,503,000. PI: McDonough

2006 NIH STTR 1R41DK076510-01 Title: *Development automated assay-regulators insulin synthesis*. Phase I = $200,371. PI: McDonough

2006 NIH FastTrack STTR 1R42HL086076-01 Proposal Title: Live cell and HCS assays to quantify production of cardiomyocytes from stem cells. Phase I = $249,866, Phase II = $1,305,000. PI: McDonough

2009 NIH 1R03MH082378-01 Proposal Title: High Throughput Imaging Assay for Beta-Catenin. $25,000 (NIH Network Molecular Libraries Probe Production Centers). PI: McDonough

2009 NIH 1R03DA026213-01 Proposal Title: A High Throughput Imaging Assay for Hepatic Lipid Droplet Formation. $25,000 (NIH Network Molecular Libraries Probe Production Centers. PI: McDonough

2009 CIRM Title *Differentiation of ventricular, atrial, and pacemaker type cardiomyocytes from stem cells*. 2 years, $900,000. PI: McDonough

2010 NIH SBIR phase I Title: *High content analysis of mitochondrial replication* Phase I = $270,000 PI: Whittaker

2010 NIH STTR 1R41DK082087-01 Title *Automated quantification of lipid droplets in fatty liver tissue sections*. Phase I = $298,466. Phase II budget = $1,464,024. PI: McDonough

2010 NIH STTR 1R41AR055604-01A2 Title *Automated analysis of skeletal muscle fiber crossectional area and metabolic type*. Phase I = $153,853. Phase II budget = $1,200,000. PI: McDonough

2011 (nearly approved) NIH STTR FastTrack Title: *Optogenetic Multiparametric Assay for HT Cardiotoxicity Testing.* PI: Cerignoli.

Contracts:

2009 NIH FastTrack SBIR contract Title: *Drug Safety Assessment in iPS Derived Cardiomyocytes*. Phase I = $119,000 Phase II = $840,000. PI: Cerignoli

2010 NIH SBIR FastTrack contract Title: *Hapten and Qdot based assay for breast cancer biomarkers*. Phase I = $150,000 Phase II = $1,700,000. PI: McDonough

2011 NIH SBIR contract Title: *Automated karyometry as a companion diagnostic for chemoprevention of breast cancer* Phase I = $204,000 PI: McDonough

Overall, Vala has received approx. $12,500,000 in funding from grants and contracts (the NIH has been the primary source of grant/contract support).

Additional recent contracts include:

Johnson & Johnson - project to develop Kinetic Image Cytometry (KIC) methods for simultaneous measurement of intracellular calcium and voltage in cardiac myocytes.

Sanofi Aventis - project to test candidate pharmaceuticals for effects on voltage-dependent channels in cardiac myocytes using KIC

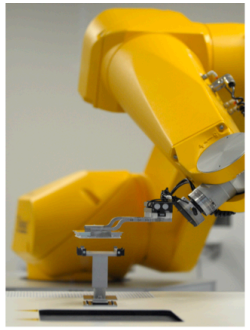
Vala has also recently been approved for a $1,500,000 loan from the SBA (Silvergate Bank, San Diego, CA).

Vala Collaborators:

<u>Sanford-Burnham Medical Research Institute</u>. Sanford-Burnham has previously collaborated with Vala Sciences Inc on the following grants and contracts including:

STTR Fast-Track 1 R42 HL086076-01 “Live cell and HCS assays to quantify production of cardiomyocytes from stem cells” and SBIR contract HHSN268200900044C “Drug Safety Assessment in IPS Derived Cardiomyocytes”).

Two of Vala’s MLSCN/MLPCN assays (to identify activators of beta-catenin (R03 MH082378), and inhibitors of hepatic lipid droplet formation (R03 MH083261) were screened on large chemical compound libraries (approx. 200,000 compounds) by the Conrad Prebys Center for Chemical Genomics at Sanford-Burnham.



Sanford|Burnham
Medical Research Institute
From Research, The Power To Cure

Conrad Prebys Center Chemical Genomics

Conrad Prebys Center for Chemical Genomics

One of the most advanced infrastructure for small molecule drug discovery in non-profit world.

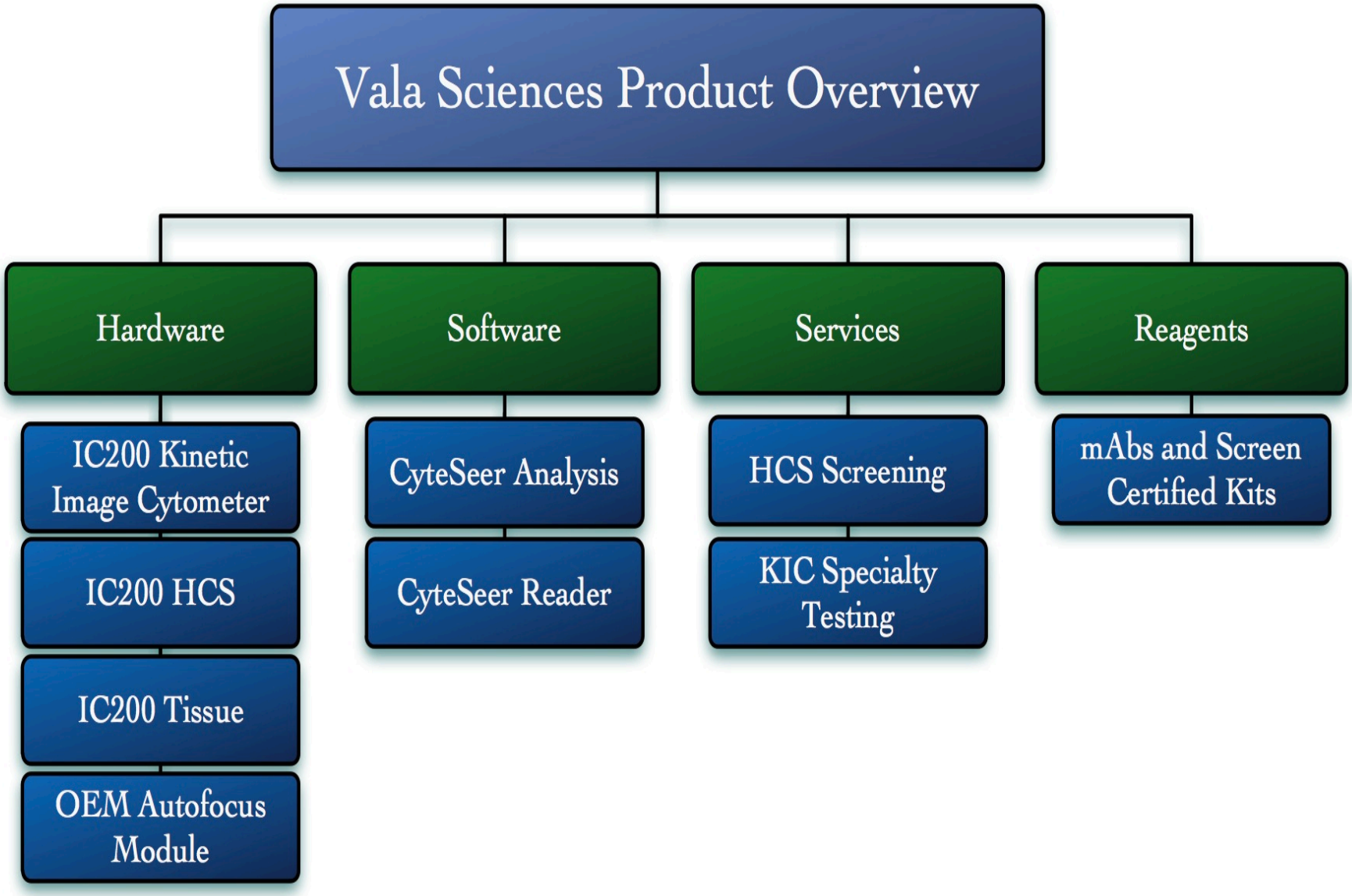
- Staffed by ~80 professionals, most with pharmaceutical company experience
- Functional Units include:
  - Assay Development (including advanced HCS for phenotypic screens)
  - HTS & Compound library management (including ultra-HTS robotics and total libraries > 900K compounds)
  - Chemical fragment screening by NMR
  - Affinity Selection-Mass Spectrometry (ASMS) screening
  - Cheminformatics
  - Medicinal Chemistry
  - Pharmacology
  - Structure-based drug optimization (robotic protein crystallography) (NMR)
- Current throughput is ~40 HTS campaigns per year, current capacity to undertake ~50/year.

World Class HTS Capabilities

5 Fully Integrated Robotic Liquid Handling Systems
(HRE unipod and tripod /1536 format), acoustic liquid dispensing
(BC Biomek 2-bridge FX 8/384 format)
Multiple plate readers, tip box & plate hotels, CO2 Incubators, etc.

4 Fully integrated HCS Systems
Robotics arms, tip wash & cell dispese, plate hotel & incubator
IN Cell housed in sterile room

Off-line Instruments & Workstation
6 Plate readers, 2 Plate washers, 3 Bulk reagent dispensers
Plate Workstation: Plate sealer, barcode labeler, 384/1536 liquid handler
Liquid Handler Workstation 96/384/1536
High Content Microscope
GPRC - Hamamatsu FDSS-7000


Vala Sciences Product Overview
Hardware
Software
Services
Reagents
IC200 Kinetic Image Cytometer
IC200 HCS
IC200 Tissue
OEM Autofocus Module
CyteSeer Analysis
CyteSeer Reader
HCS Screening
KIC Specialty Testing
mAbs and Screen Certified Kits

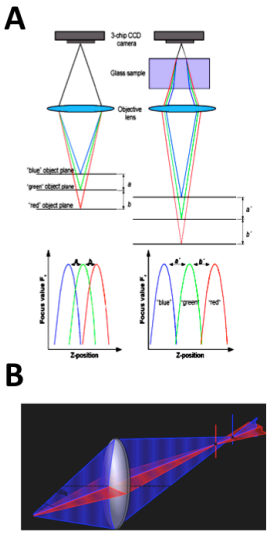
Instrumentation:

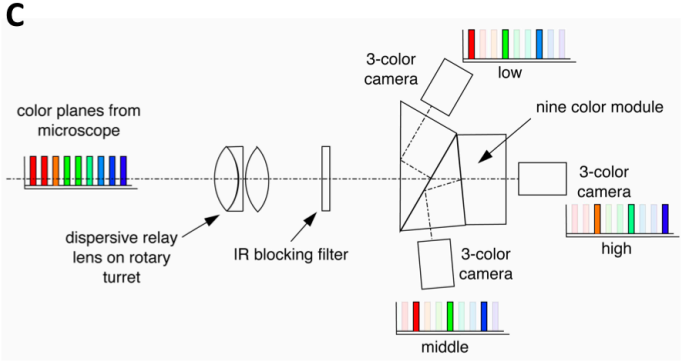
Jeffrey H. Price, M.D., Ph.D.

2003 Q3DM Eidaq IC100 (also marketed by Beckman Coutler) (rapid autofocus, multichannel fluorescence, microtiter plates, slides)

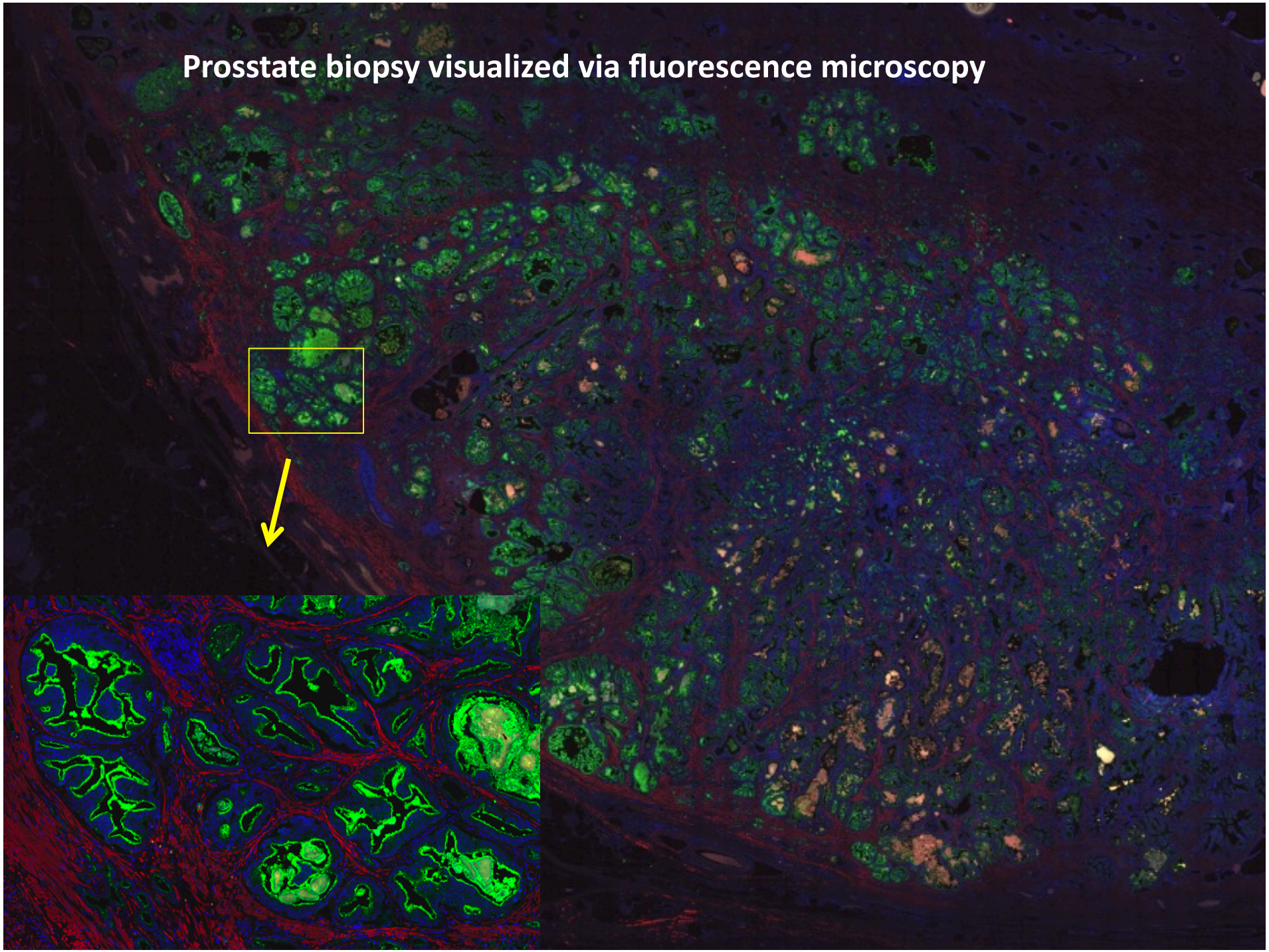
Present: IC200 and IC300 product line. Even faster autofocus, continuous scanning, large format cameras, Kinetic Image Cytometry

Recent customers include the Sanford Burnham Medical Research Institute, University of Houston, Baylor College of Medicine, Genomics Institute of the Novartis Research Foundation.





Autofocus based upon chromatic abberation


Prosstate biopsy visualized via fluorescence microscopy

Software: CyteSeer®

CyteSeer 2.7.10.16

Navigation:

Source Folder: /Volumes/vala_public/Andrew/Experiments/AH68b_20111027 Browse...

Results Folder: /Volumes/vala_public/Andrew/Experiments/AH68b_20111027/CyteSeerResults_2011-10-27_15-40-14 Browse...

Image Naming Convention: IC 100 Plate Batch

Define Algorithm:

Algorithm To Run: Colocalization New... Edit... Copy... Delete

Channel Name	Channel Folder	Analyze?	Prefilter	Filtering Radius	Thresholding	Sensitivity %	Size Parameter	Size Value	Loader	Configure
Nucleus	channel_0	☑	None	1	SavitskyGolay	100	Minimum Size	5	Single Slice	
Lipid	channel_1	☑	None	1	SavitskyGolay	50		0	Single Slice	
Protein	channel_2	☑	None	1	SavitskyGolay	50		0	Single Slice	

Wells to Run Algorithm On:

Well	Run?
F01	☑
F02	☑
F03	☑
F04	☑
F05	☑
F06	☑
F07	☑
F08	☑
F09	☑
F10	☑

Clear All Select All Configure... Run... Cancel

Images Within Well:

Images Across: 3

Images Down: 2

Computational Cluster Size:

Images Across: 3

Images Down: 2

Gates

Name	Source DataTable	Result DataTable	Enabled?

Add Move Up Remove Move Down Edit...

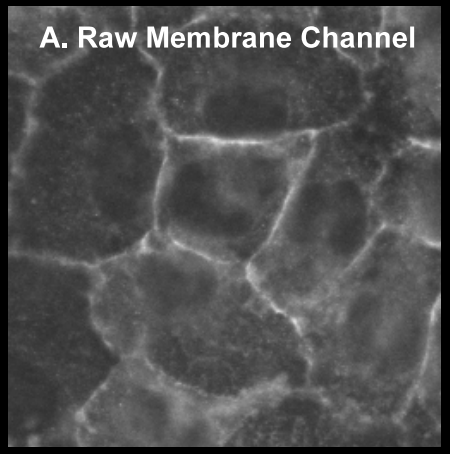
Randall Ingermanson

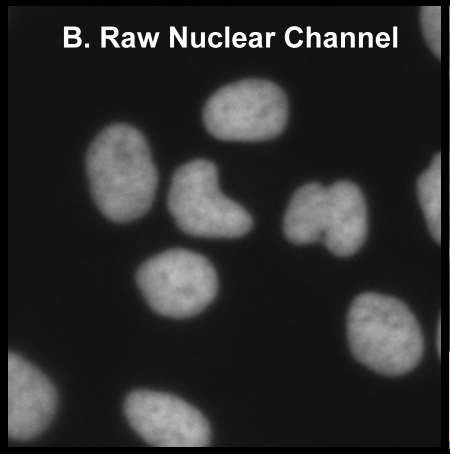
Jeff Hilton

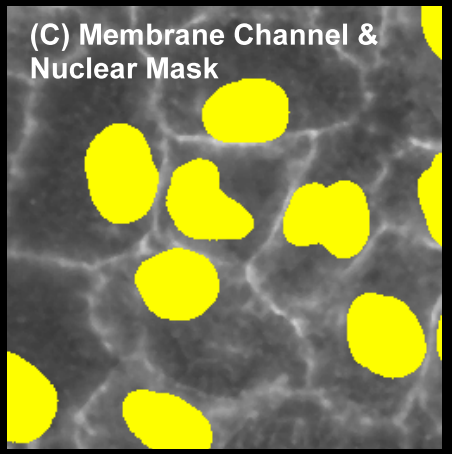
Features of CyteSeer®

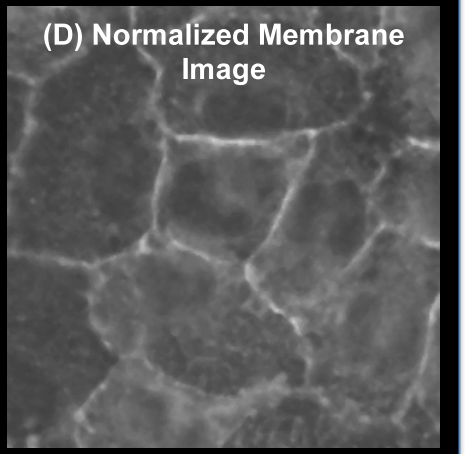
- Coded in Java, cross-platform compatible
- True cell-by-cell cytometry
- Plug-in and pipe-line architecture.
- Easily modified and optimized for specific applications
- Can be downloaded and tested for free from our website

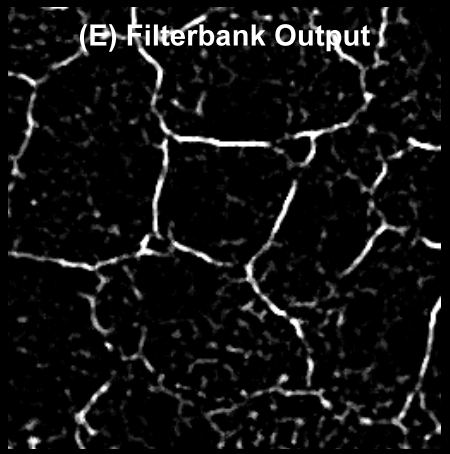
http://www.valasciences.com

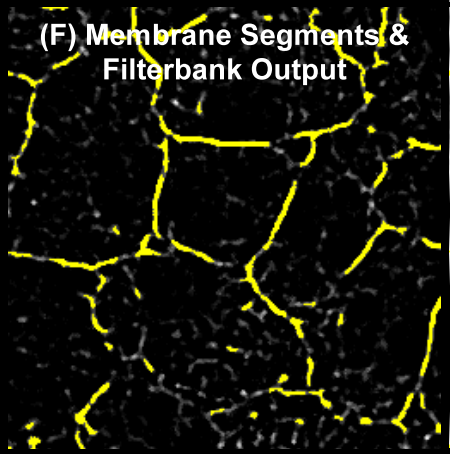


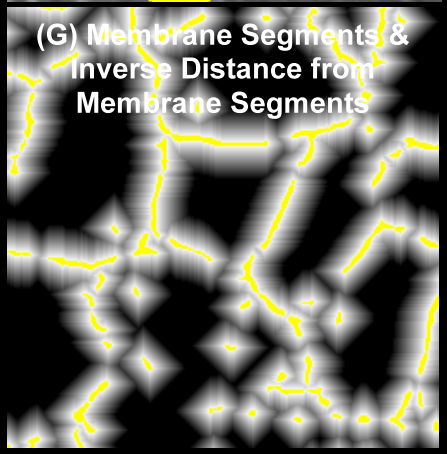


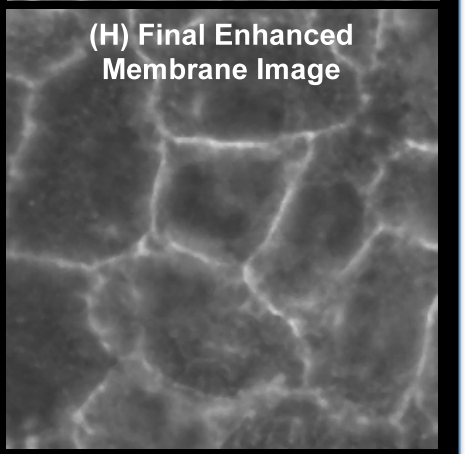


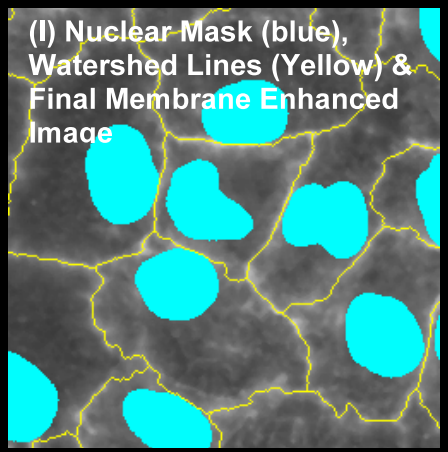


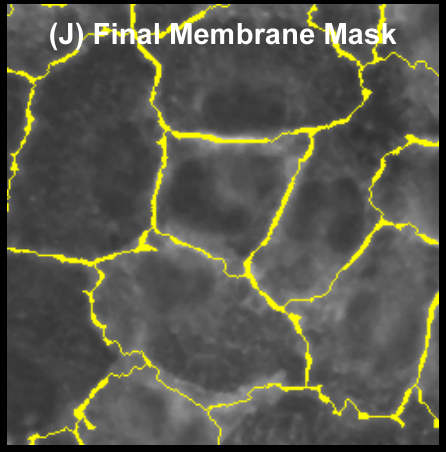




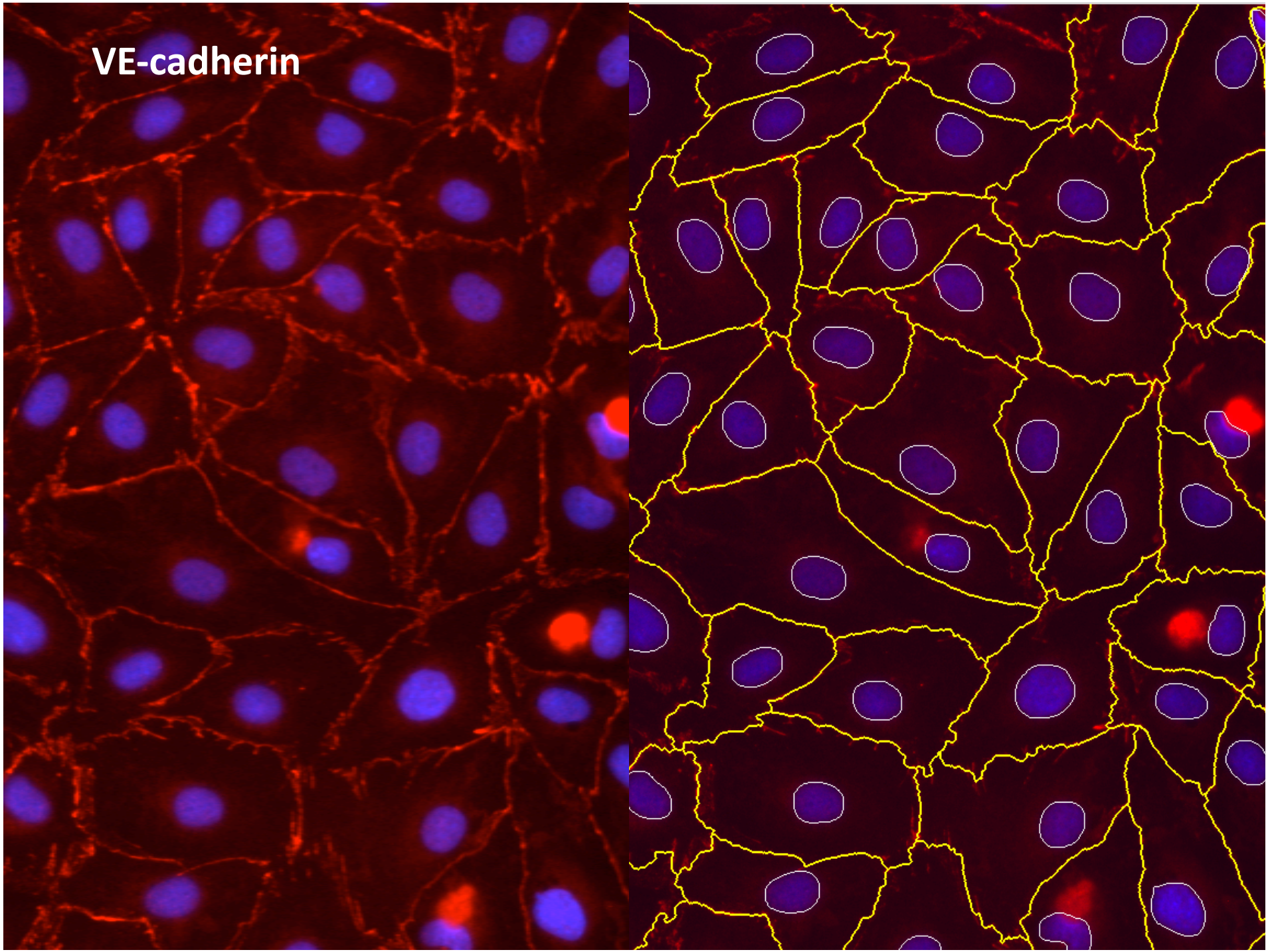






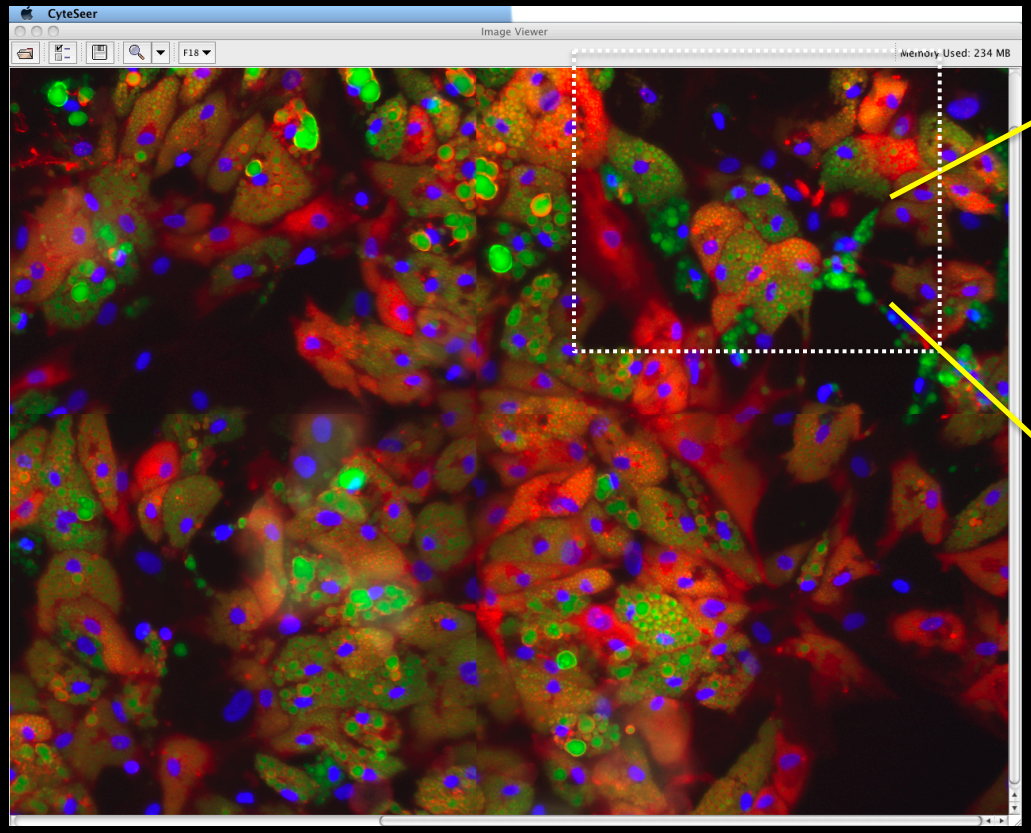


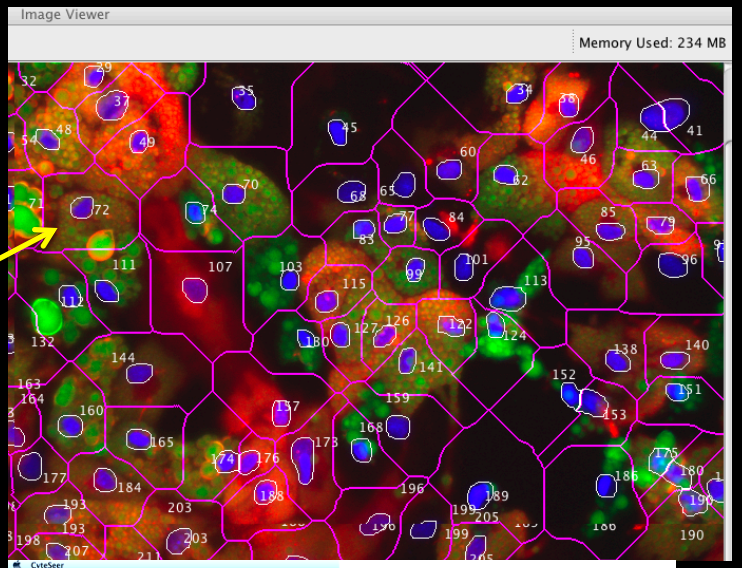
The Membrane Analysis Algorithm:

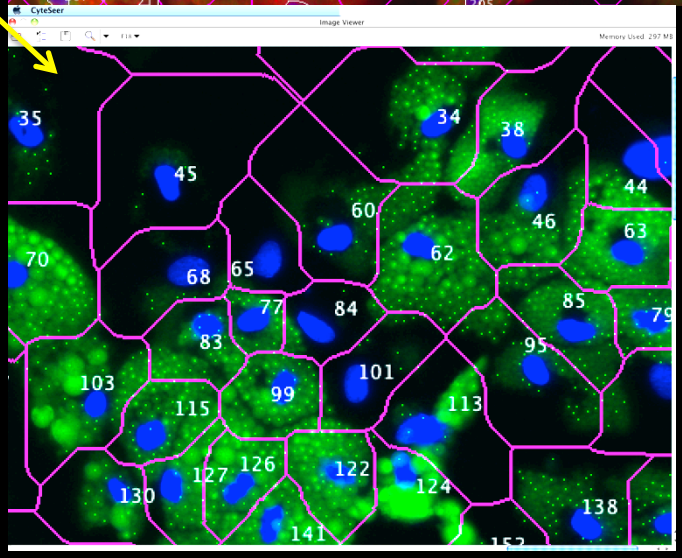

VE-cadherin

The Lipid Droplet/Colocalization Algorithm:

(human adipocytes, lipolysis assay (nuclei, lipid droplets, phosphoperilipin)



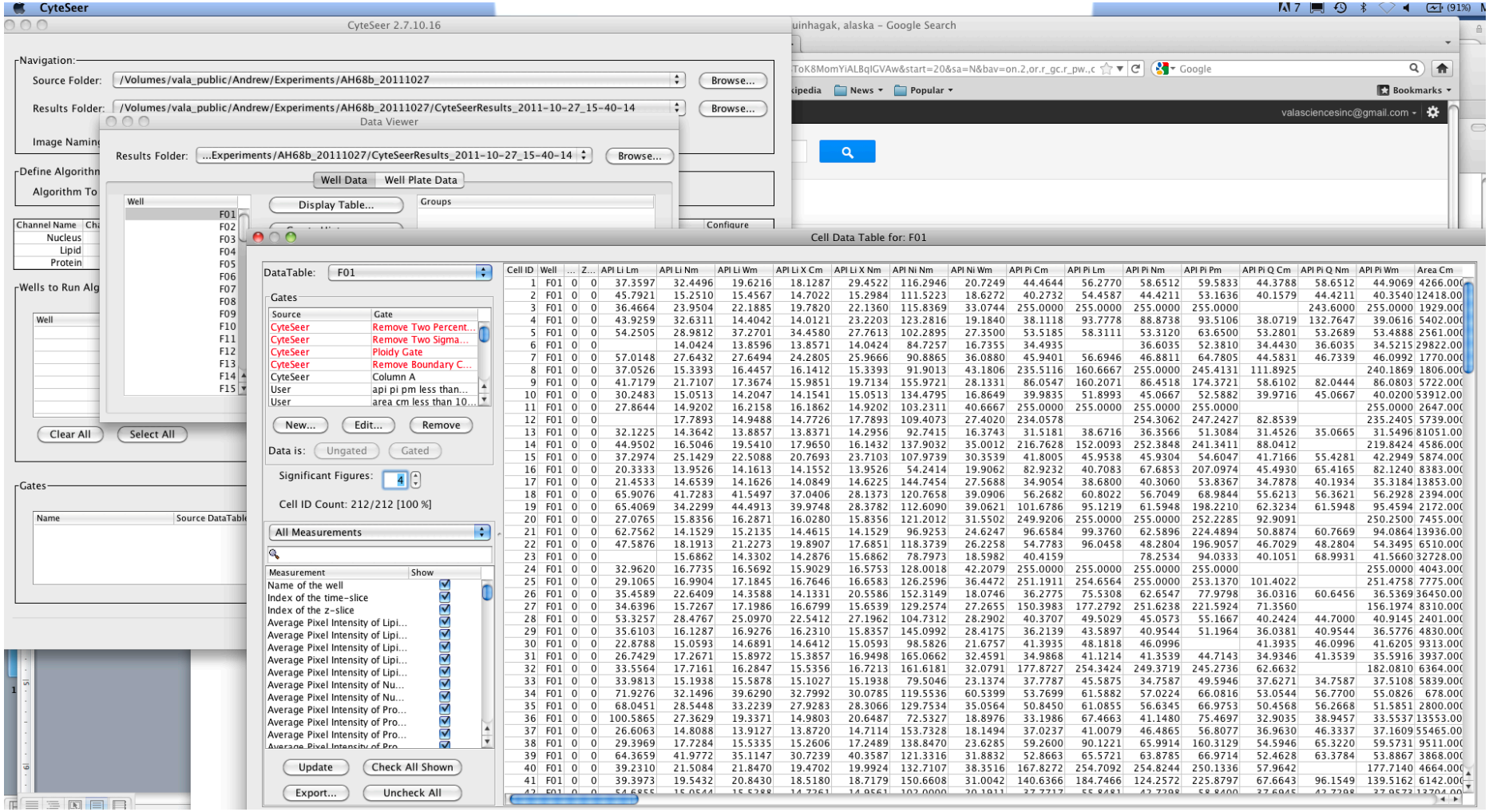




A rich set of data parameters are derived by CyteSeer for each cell.

Membrane algorithm (94)

Lipid droplet/colocalization algorithm (98)



Data parameters related to intensity of different cellular compartments in different image channels

TII Mi Nm	Total Integrated Intensity of Membrane Image on Nucleus Mask
API Mi Nm	Average Pixel Intensity of Membrane Image on Nucleus Mask
MPI Mi Nm	Median Pixel Intensity of Membrane Image on Nucleus Mask
SPI Mi Nm	Standard Deviation of Pixel Intensity of Membrane Image on Nucleus Mask
TII Mi Mm	Total Integrated Intensity of Membrane Image on Membrane Mask
API Mi Mm	Average Pixel Intensity of Membrane Image on Membrane Mask
MPI Mi Mm	Median Pixel Intensity of Membrane Image on Membrane Mask
SPI Mi Mm	Standard Deviation of Pixel Intensity of Membrane Image on Membrane Mask
TII Mi Cm	Total Integrated Intensity of Membrane Image on Cytoplasm Mask
API Mi Cm	Average Pixel Intensity of Membrane Image on Cytoplasm Mask
MPI Mi Cm	Median Pixel Intensity of Membrane Image on Cytoplasm Mask
SPI Mi Cm	Standard Deviation of Pixel Intensity of Membrane Image on Cytoplasm Mask
TII Mi Wm	Total Integrated Intensity of Membrane Image on Whole-Cell Mask
API Mi Wm	Average Pixel Intensity of Membrane Image on Whole-Cell Mask
MPI Mi Wm	Median Pixel Intensity of Membrane Image on Whole-Cell Mask
SPI Mi Wm	Standard Deviation of Pixel Intensity of Membrane Image on Whole-Cell Mask

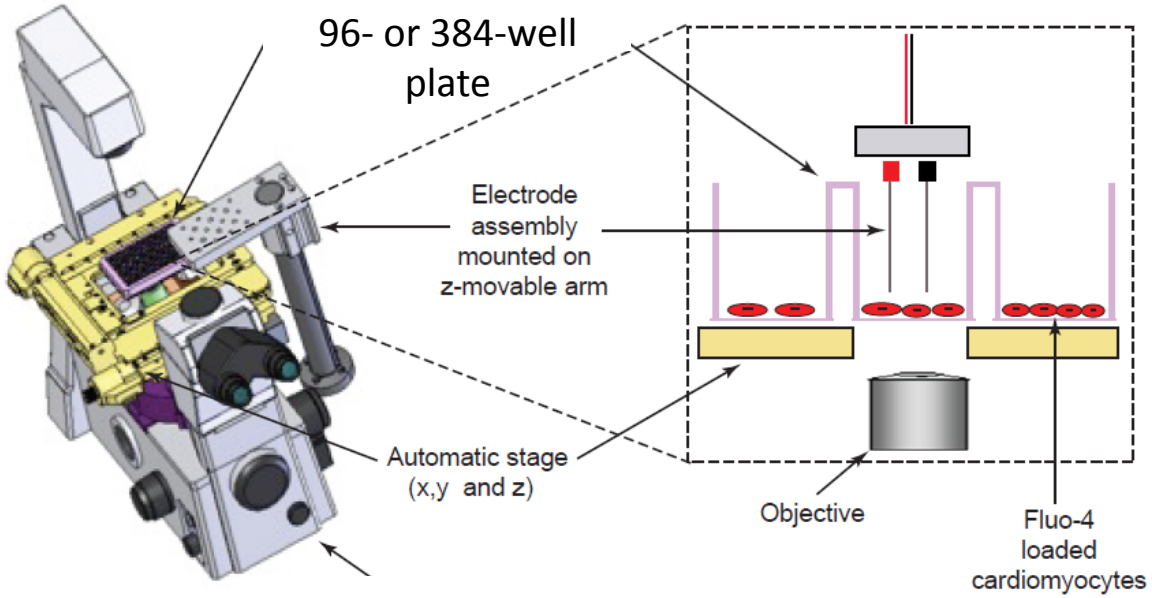
Colocalization of labels between differerent image channels for different cellular compartments

PCC Ni Mi Wm	Pearson Correlation Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
K1 Ni Mi Wm	Manders K1 Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
K2 Ni Mi Wm	Manders K2 Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
MOC Ni Mi Wm	Manders Overlap Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
M1 Ni Mi Wm	Manders M1 Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
M2 Ni Mi Wm	Manders M2 Coefficient of Nucleus Image vs Membrane Image on Whole-Cell Mask
Masked PCC Ni Mi Nm	Masked Pearson Correlation Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask
Masked K1 Ni Mi Nm	Masked Manders K1 Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask
Masked K2 Ni Mi Nm	Masked Manders K2 Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask
Masked MOC Ni Mi Nm	Masked Manders Overlap Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask
Masked M1 Ni Mi Nm	Masked Manders M1 Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask
Masked M2 Ni Mi Nm	Masked Manders M2 Coefficient of Nucleus Image vs Membrane Image on Nucleus Mask

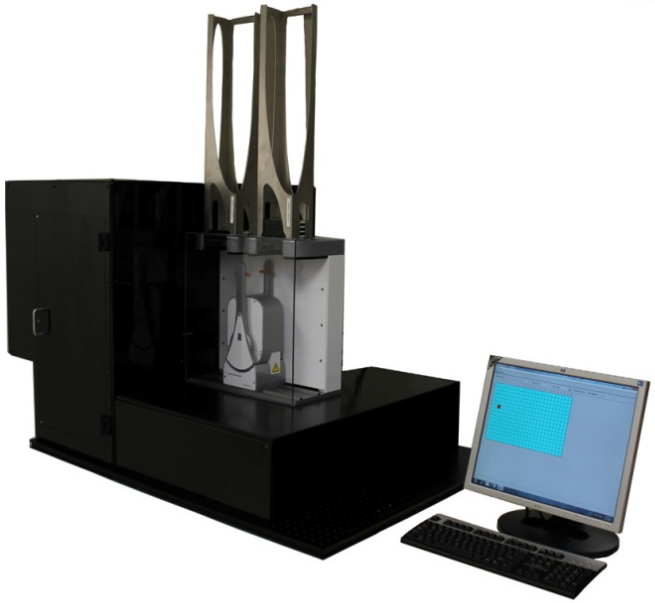
Kinetic Image Cytometer (KIC)

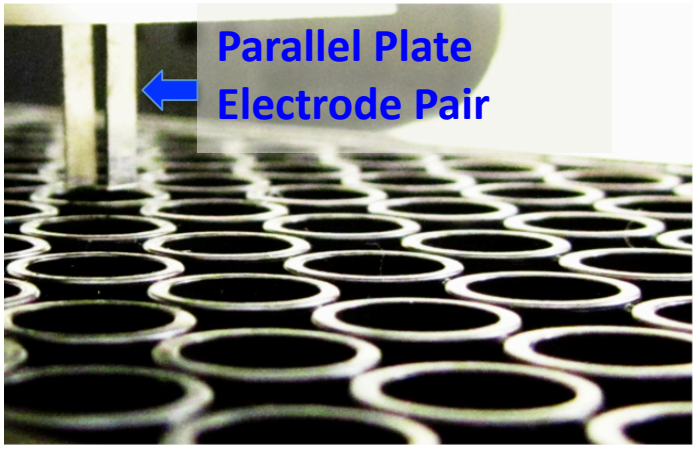
Fabio Cerignoli

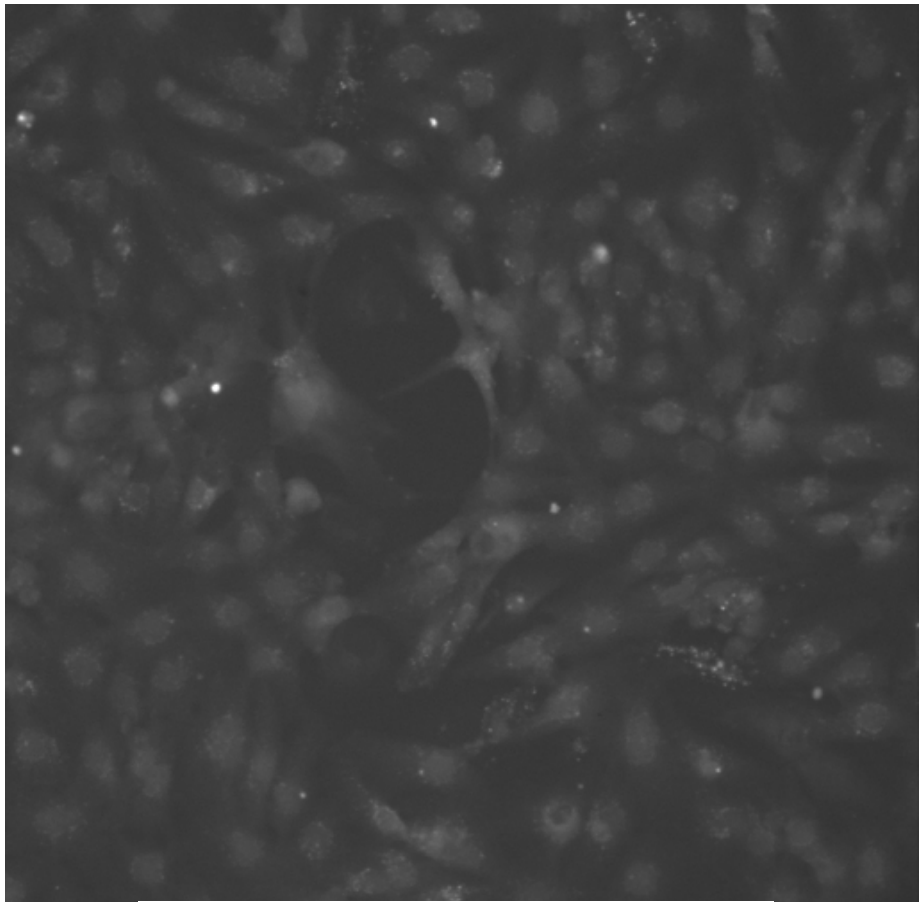
Ross Whittaker

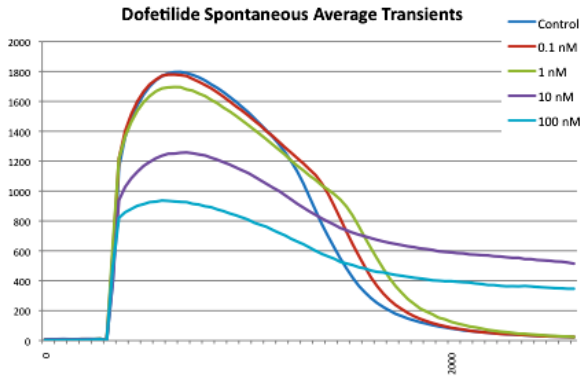


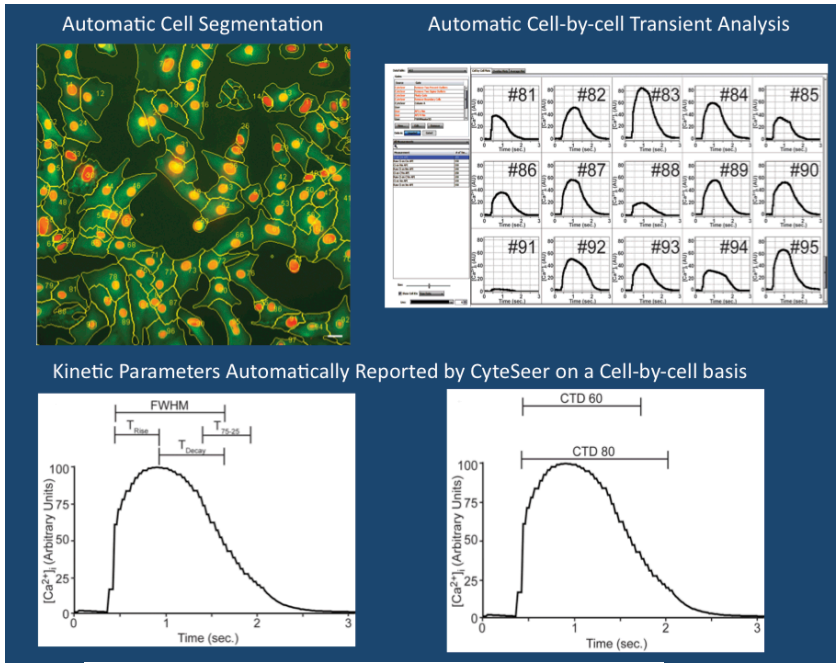
Nikon Ti-E Microscope

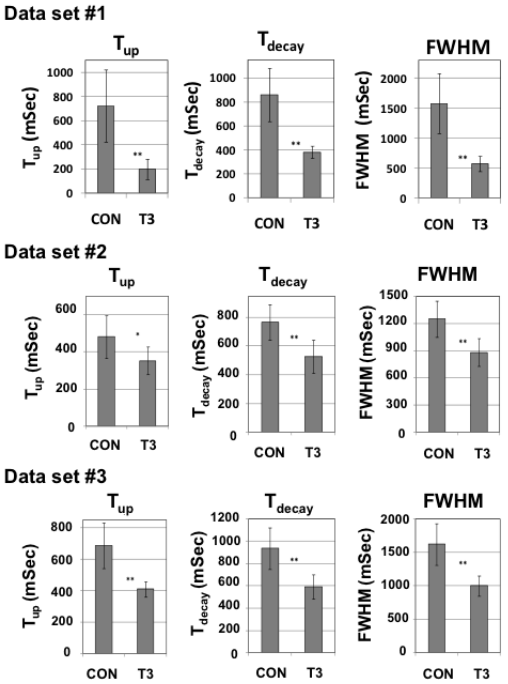







Dofetilide Spontaneous Average Transients
Control
0.1 nM
1 nM
10 nM
100 nM
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
2000


Automatic Cell Segmentation
Automatic Cell-by-cell Transient Analysis
#81
#82
#83
#84
#85
#86
#87
#88
#89
#90
#91
#92
#93
#94
#95
Kinetic Parameters Automatically Reported by CyteSeer on a Cell-by-cell basis
FWHM
T Rise
T 75-25
T Decay
100
75
50
25
0
[Ca2+]i (Arbitrary Units)
0
1
2
3
Time (sec.)
CTD 60
CTD 80
100
75
50
25
0
[Ca2+]i (Arbitrary Units)
0
1
2
3
Time (sec.)


Data set #1
Tup
Tdecay
FWHM
Tup (mSec)
Tdecay (mSec)
FWHM (mSec)
1000
800
600
400
200
0
2000
1500
1000
500
0
**
CON
T3
Data set #2
Tup
Tdecay
FWHM
Tup (mSec)
Tdecay (mSec)
FWHM (mSec)
600
400
200
0
800
1500
1200
900
300
*
**
CON
T3
Data set #3
Tup
Tdecay
FWHM
Tup (mSec)
Tdecay (mSec)
FWHM (mSec)
800
600
400
200
0
1200
1000
2000
1500
1000
500
**
CON
T3

Vala is offering the following assays related to KIC-Cardiac Myocyte:

CD1, Cardiac differentiation , human cardiac myocytes (long term effects on iPS-derived cardiac myocytes)

CT1, Cardiac toxicity, human cardiac myocytes (acute effects on iPS-derived cardiac myocytes)

Adipogenesis

Excess adipogenesis, leads to obesity. Abnormally low adipogenesis may contribute to cachexia.

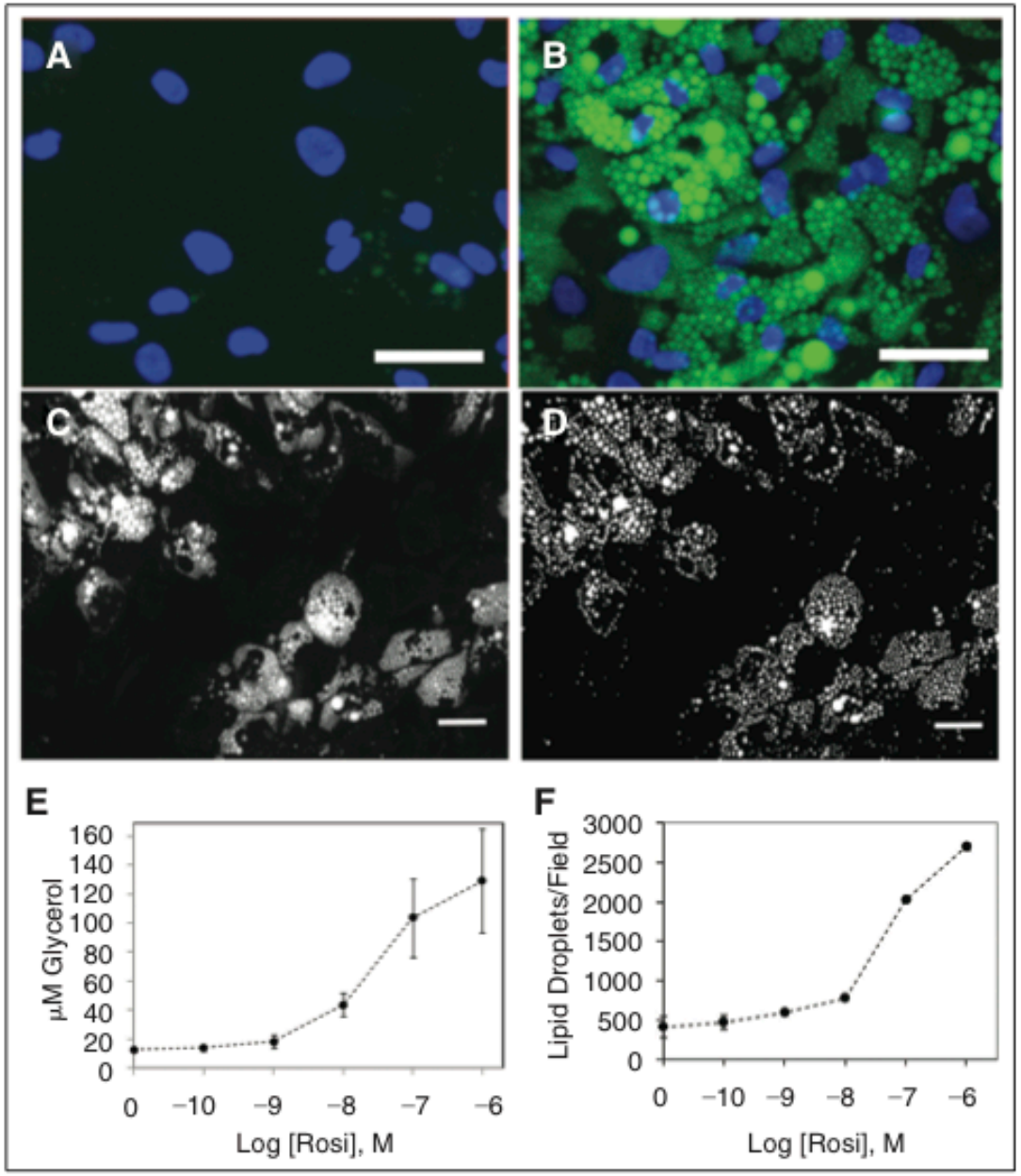
2006 NIH SBIR 1R43DK074333-01 Title: *HT Image Assay of Lipid Droplet Formation in Human Adipocytes*.

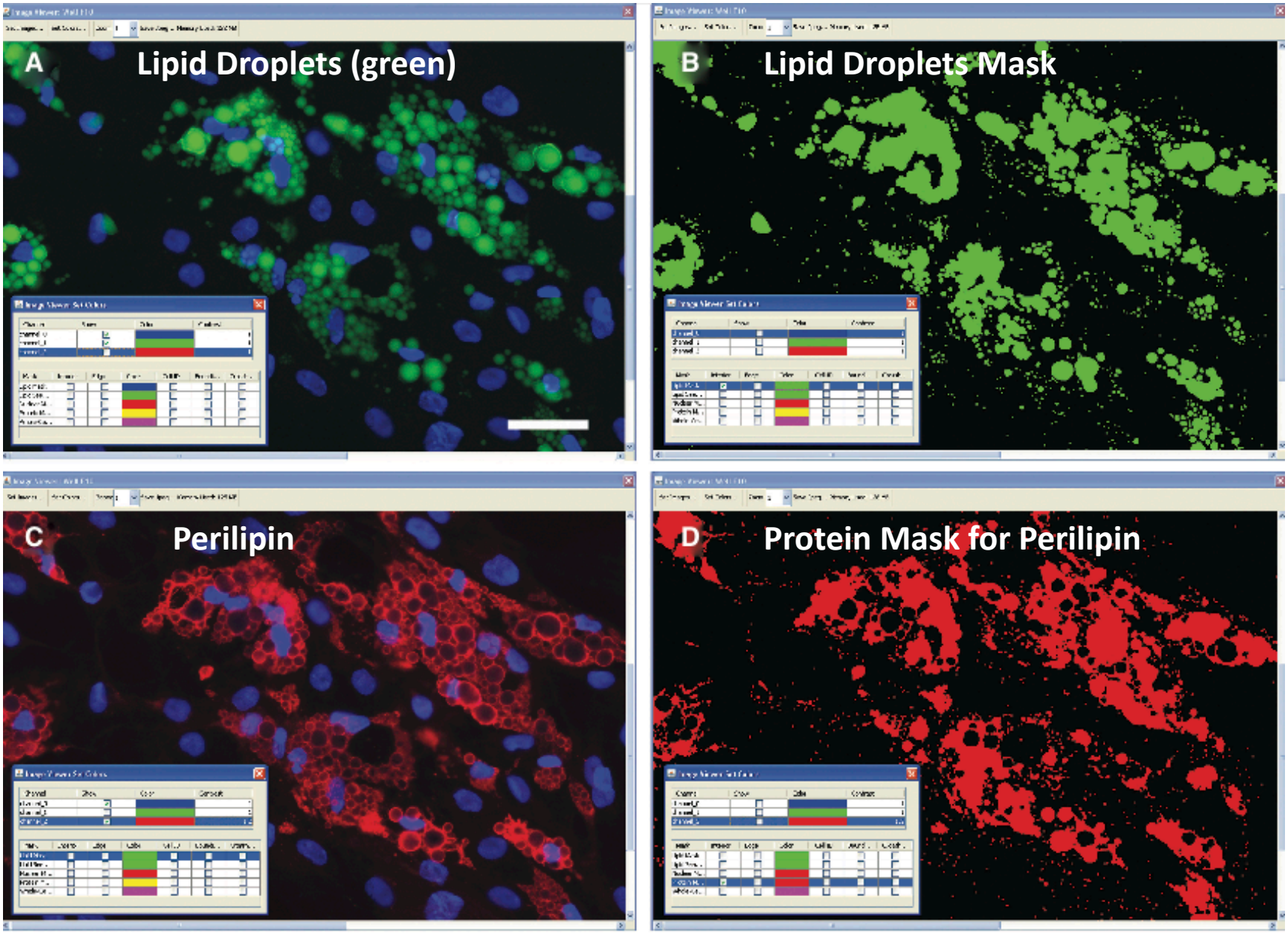
Collaborators: Zen-Bio Inc (suppliers of primary adipocytes and other cell types), Baylor College of Medicine (development of custom antibodies)

The basics of assay development were published in a peer-reviewed paper:

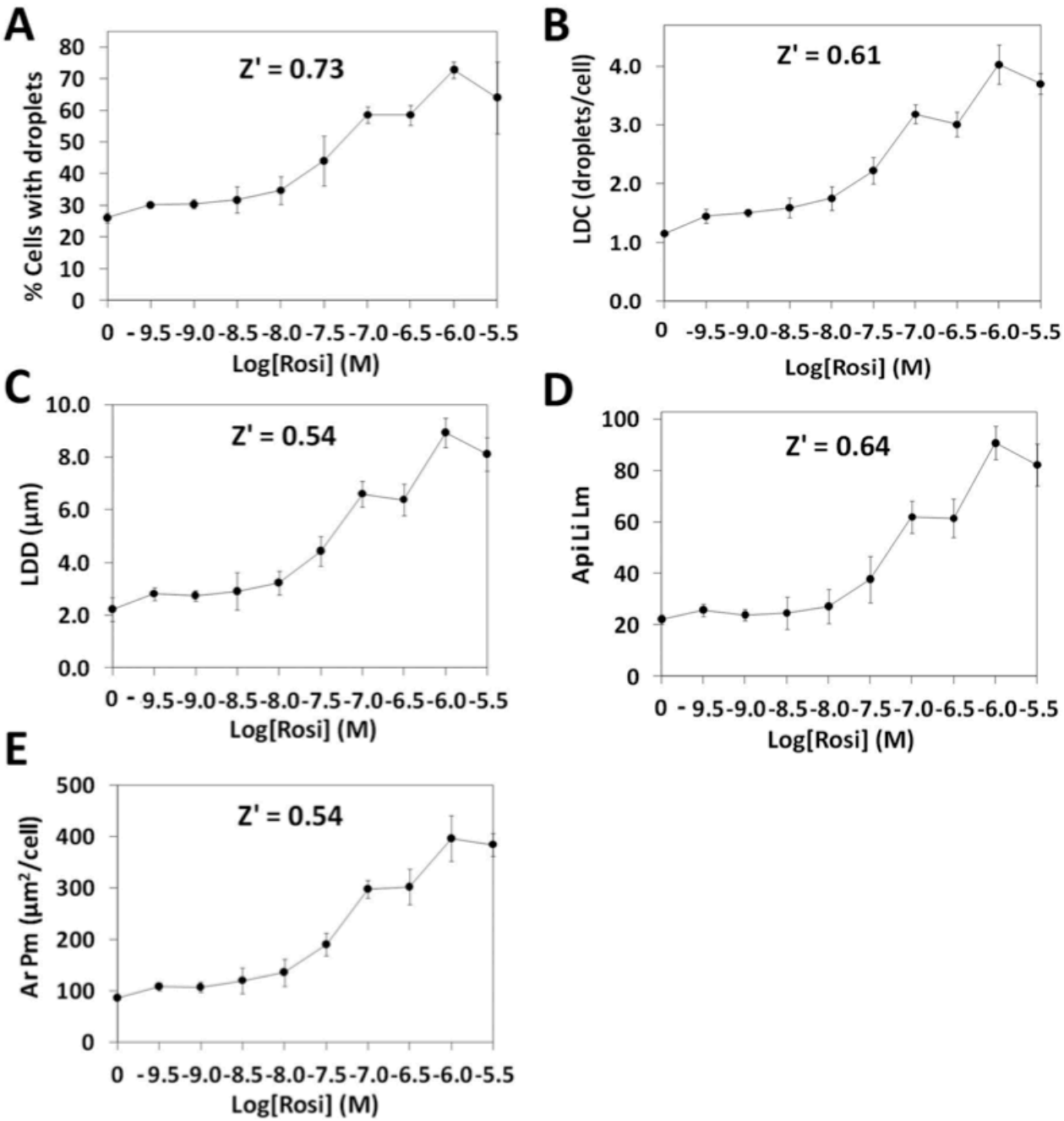
McDonough, P. M., Agustin, R. M., Ingermanson, R. S., Loy, P. A., Buehrer, B. M., Nicoll, J. B., Prigozhina, N. L., Mikic, I., Price, J. H. 2009. Quantification of lipid droplets and associated proteins in cellular models of obesity via high content/ high throughput microscopy and automated image analysis. *ASSAY and Drug Development Technologies, 7:440-460*. NIHMSID#194280

Early experiment demonstrating the effect of rosiglitazone on differentiation of preadipocytes (A) to adipocytes (B), lipid droplets as a gray scale image (C), creation of the Lipid Droplet Mask (D), and the correlation between biochemical analysis of triglycerides (E) and lipid droplets as assayed via microscopy (F).




A
Lipid Droplets (green)
B
Lipid Droplets Mask
C
Perilipin
D
Protein Mask for Perilipin

Data from the adipogenesis assay



Vala is offering the following assays related to Adipogenesis:

A1, Adipogenesis, Human adipocytes, agonists

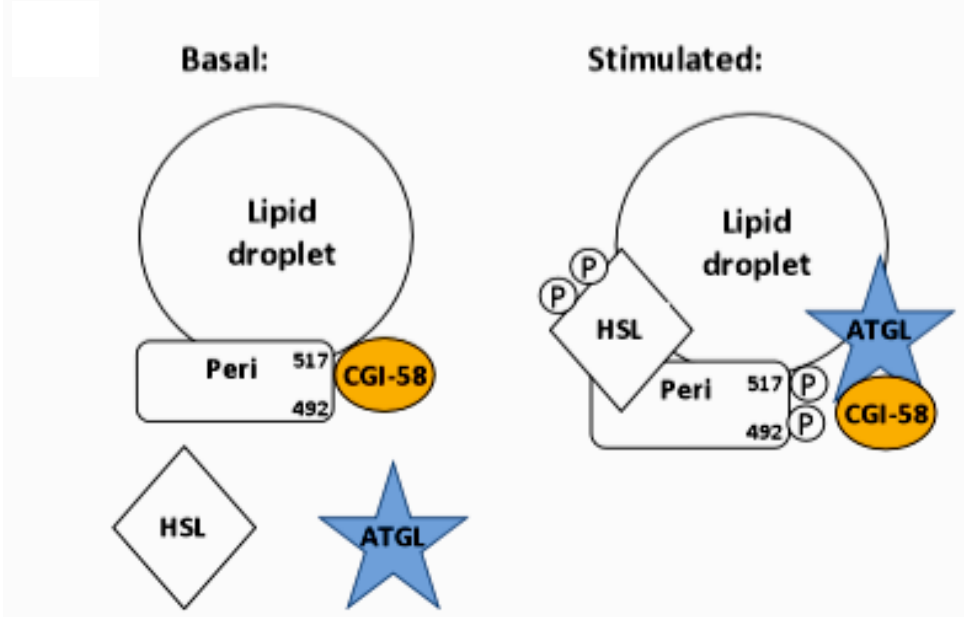
A2, Adipogenesis, Human adipocytes, antagonists

Related assay:

LD2, Lipid droplet formation, Huh-7 cells (hepatocytes). This is related to fatty liver disease.

Lipolysis

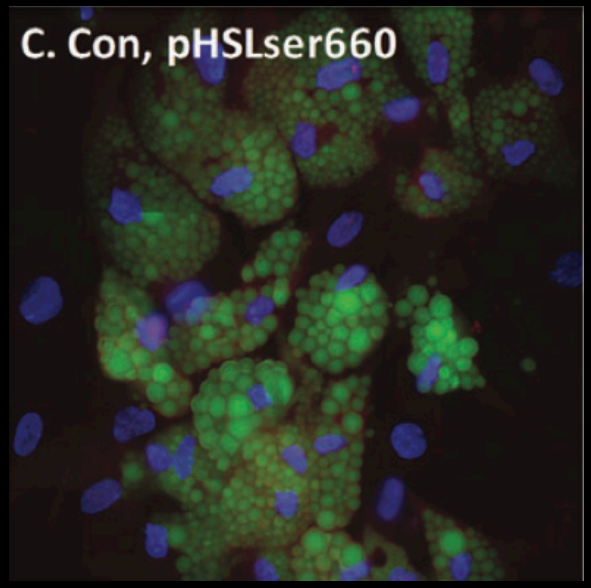
McDonough, P. M., Ingermanson, R. S., Loy, P.A., Koon, E. D., Whittaker, R., Laris, C. A., Hilton, J. M., Nicoll, J. B., Buehrer, B. M., Price, J. H. (2010). Quantification of Hormone Sensitive Lipase Phosphorylation and Colocalization with Lipid Droplets in Murine 3T3L1 and Human Subcutaneous Adipocytes via Automated Digital Microscopy and High-Content Analysis. Assay Drug Dev Technol. 2010 Dec 27.

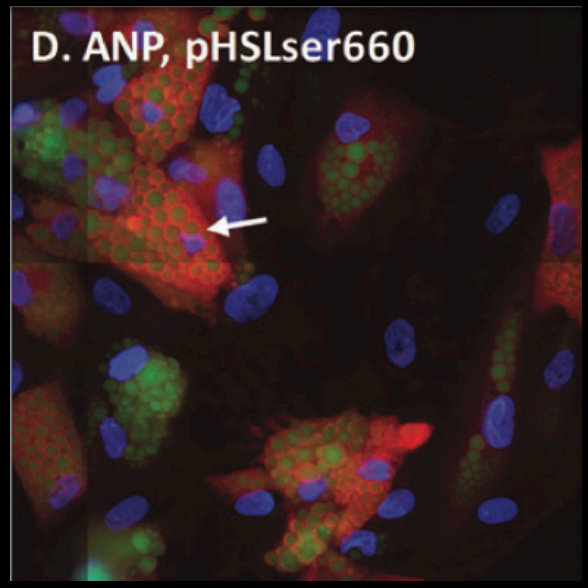


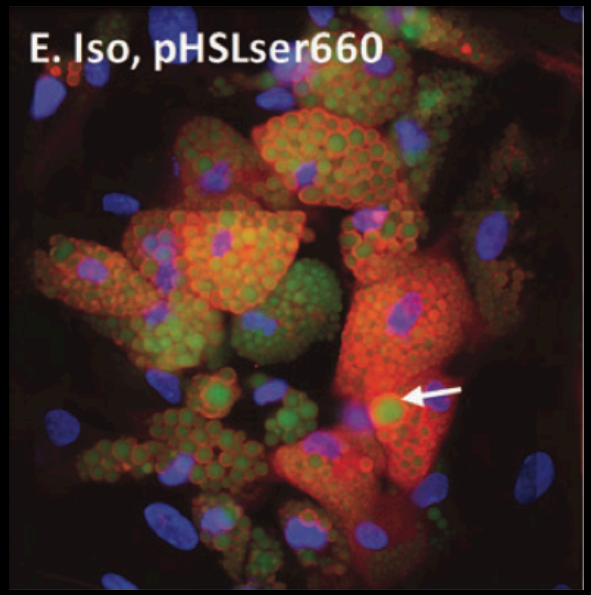
For lipid droplets to be metabolized, the process of lipolysis must be initiated.

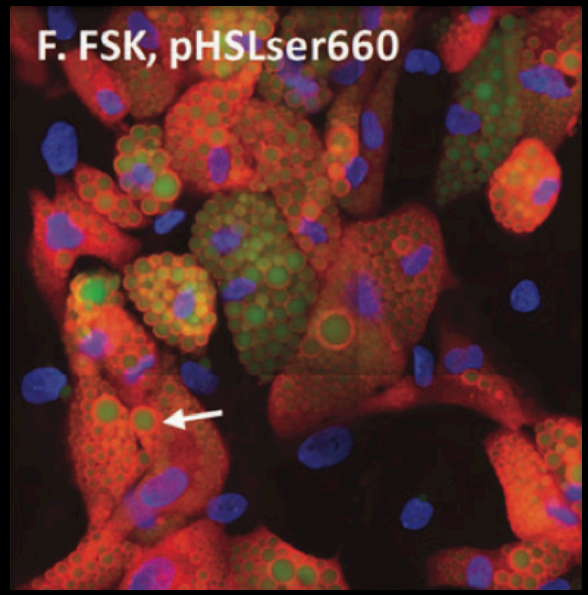
The activation of lipolysis is associated with phosphorylation of perilipin (Peri), and Hormone Sensitive Lipase (HSL). Adipocyte triglyceride lipase (ATGL) is also critically important. Phosphorylation of perilipin attracts HSL and ATGL to the lipid droplets.

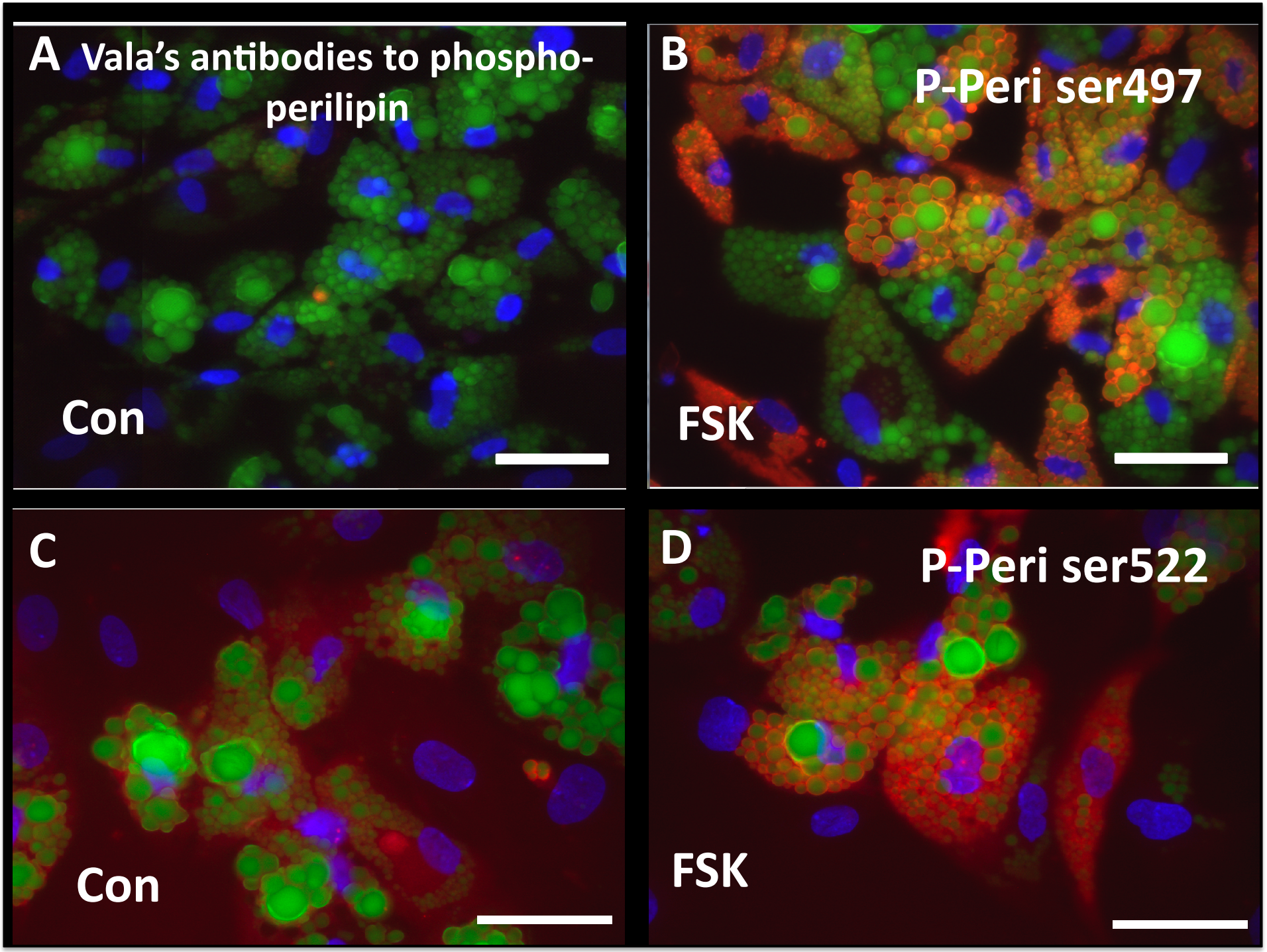
HSL and perilipin are substrates for cAMP activated protein kinase (PKA), and cGMP-activated protein kinase (PKG)



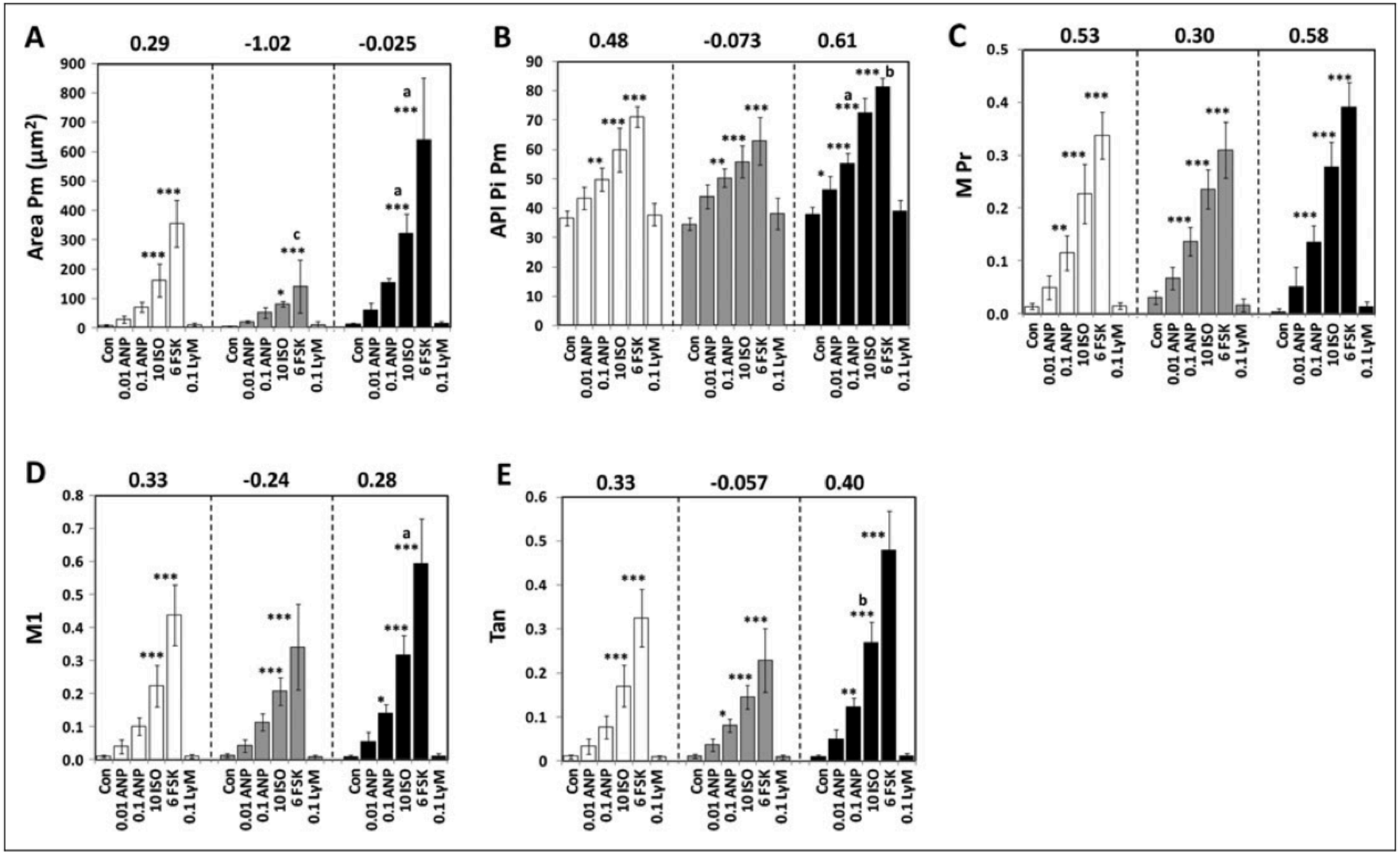







A
Vala's antibodies to phospho-perilipin
Con
B
P-Peri ser497
FSK
C
Con
D
P-Peri ser522
FSK

Results from Vala's HCA-based lipolysis assay:



Vala is offering the following assays related to Lipolysis:

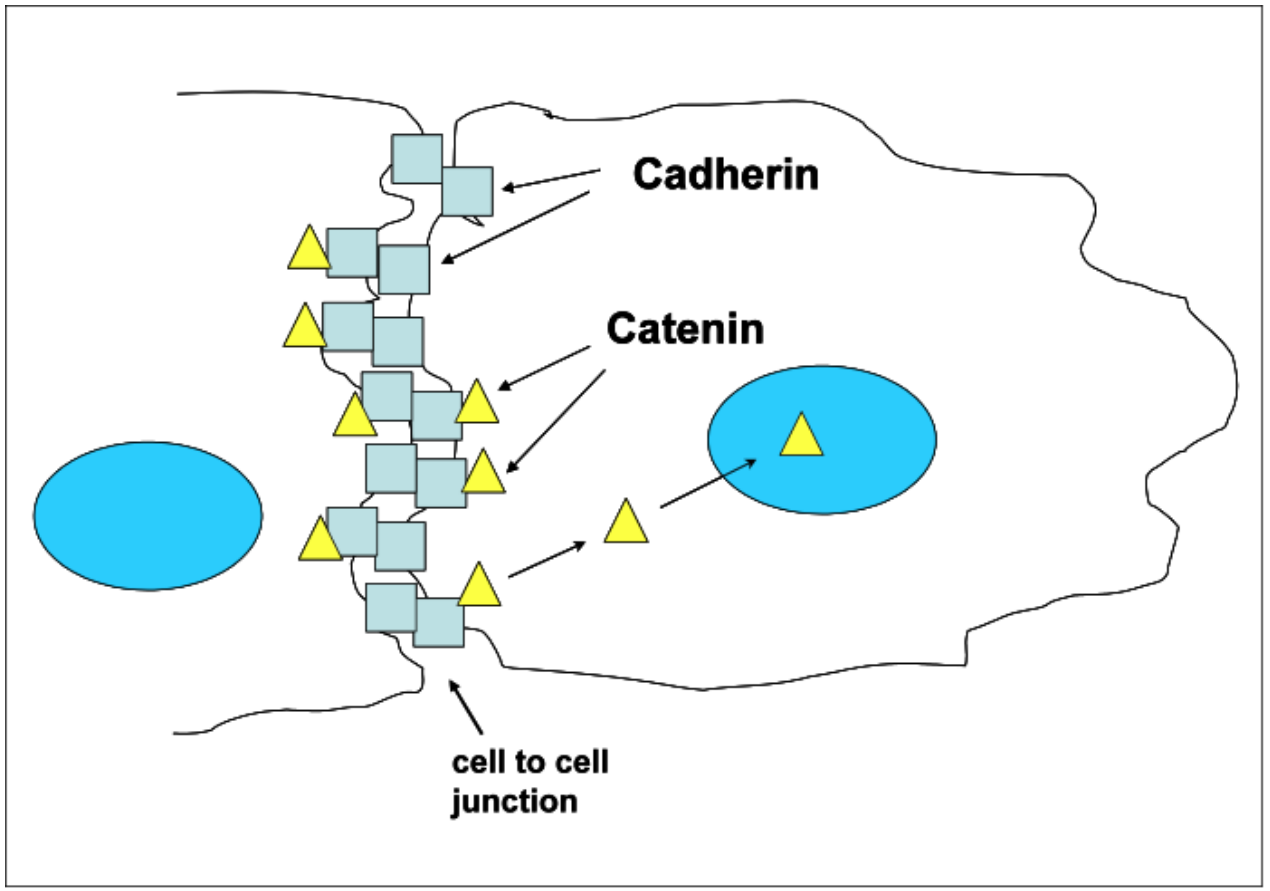
L1, Lipolysis, Human adipocytes, agonists

L2, Lipolysis, Human adipocytes, antagonists

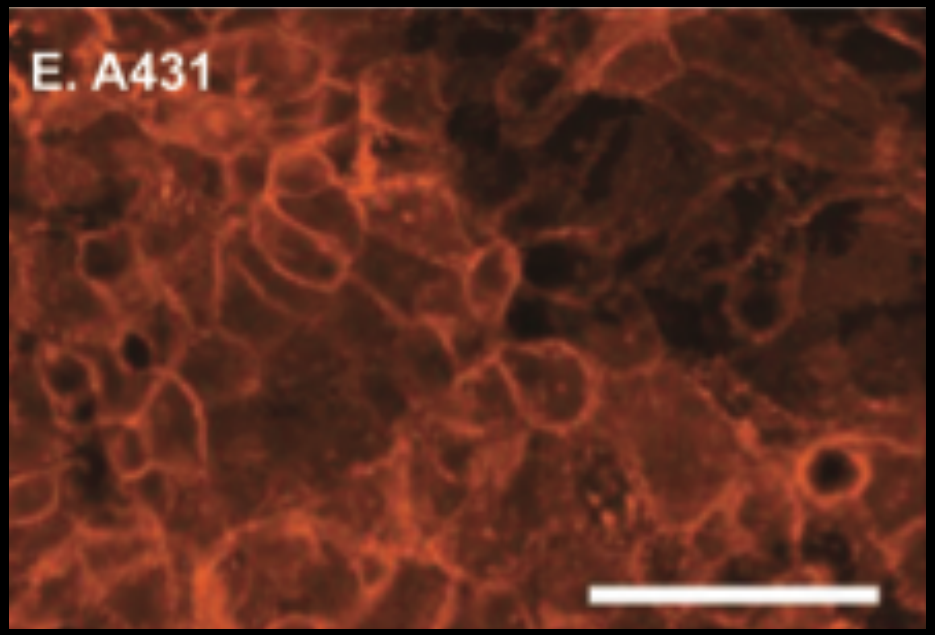
L3, Lipolysis, murine 3T3L1 adipocytes, agonists

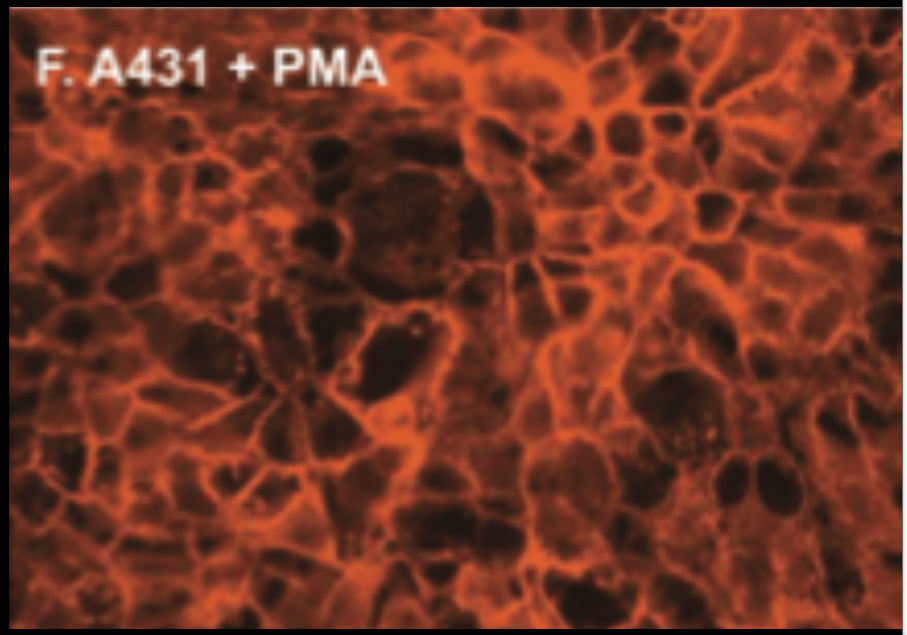
Cadherins and beta-catenin

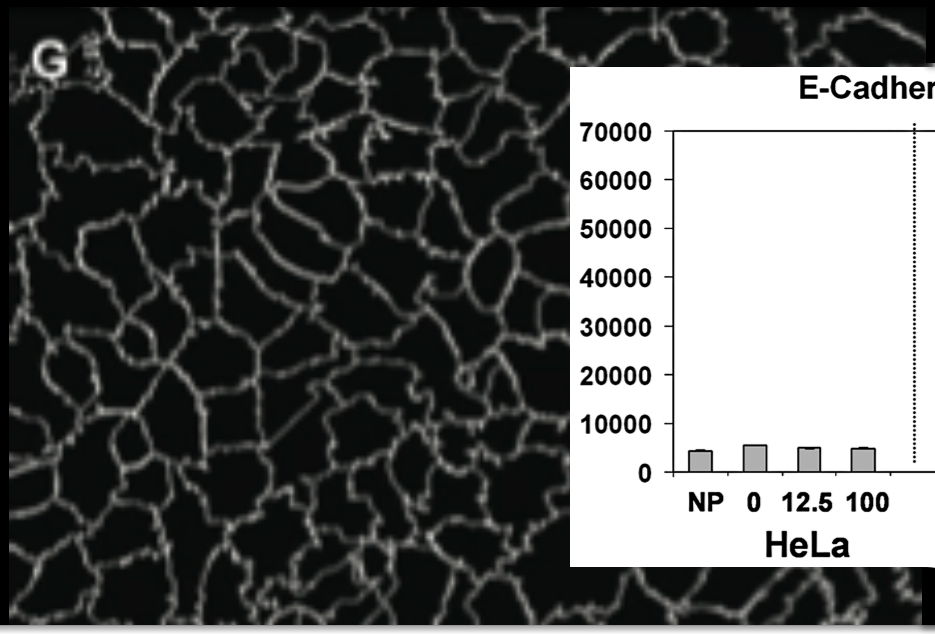
Prigozhina, N. L., Zhong, L., Hunter, E. A., Mikic, I., Callaway, S., Roop, D. R., Mancini, M. A., Zacharias, D., Price, J. H., McDonough, P. M. (2007) Plasma membrane assays and three-compartment image cytometry for high content screening. ASSAY and Drug Development Technologies, 5:29-48

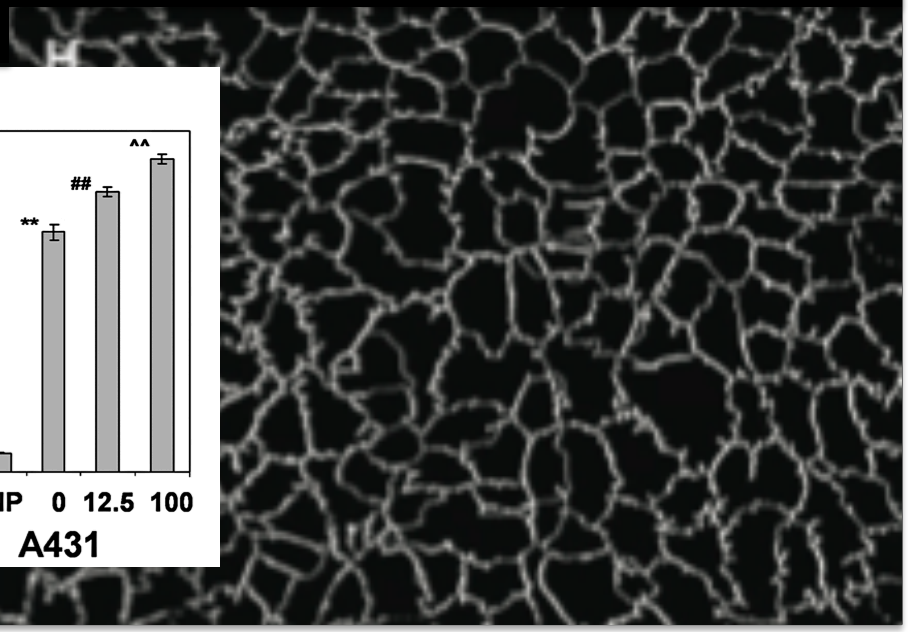


E-Cadherin


E. A431

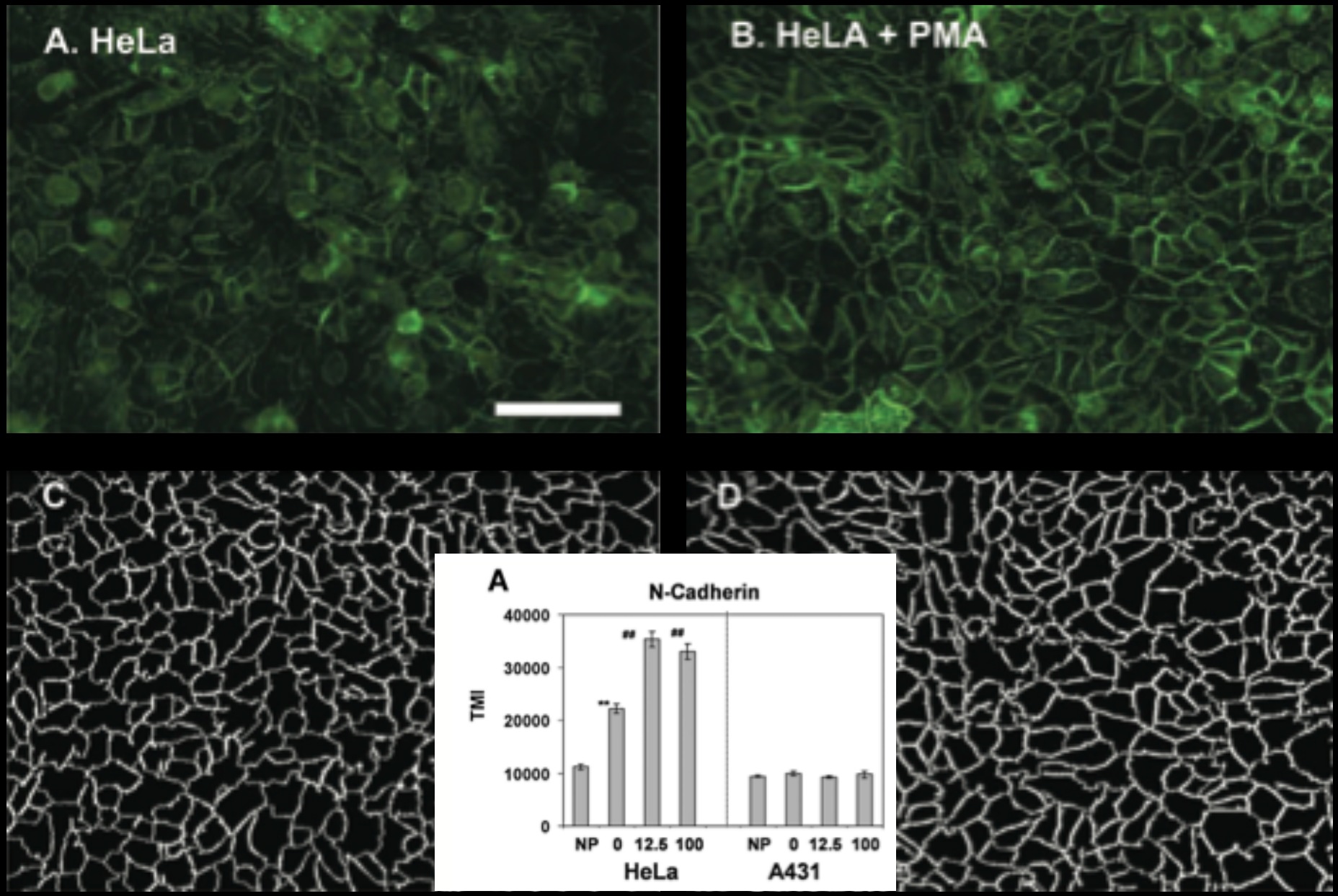

F. A431 + PMA


G

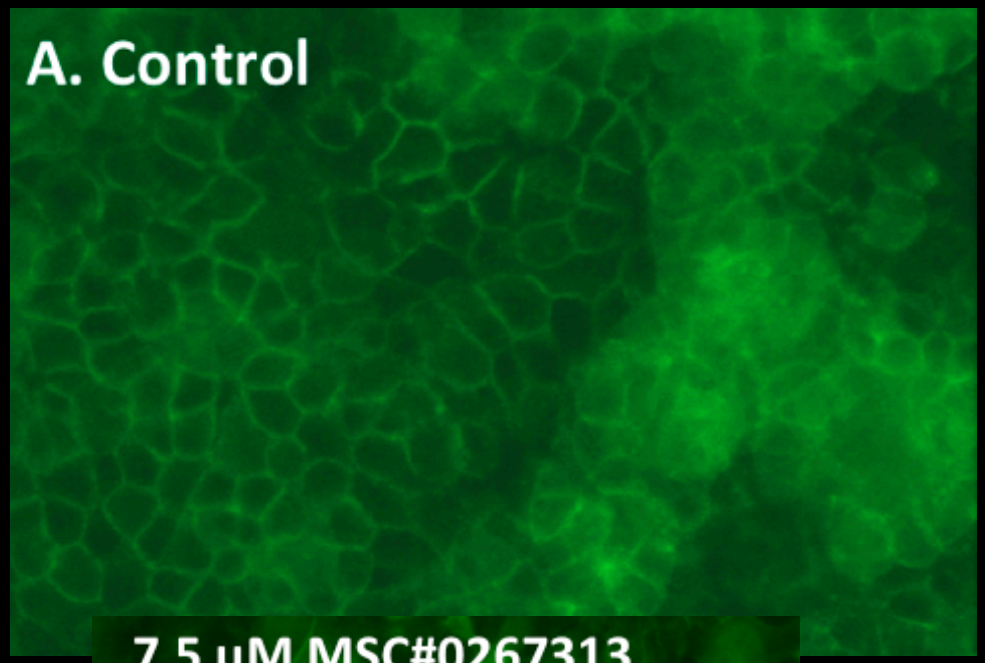

H

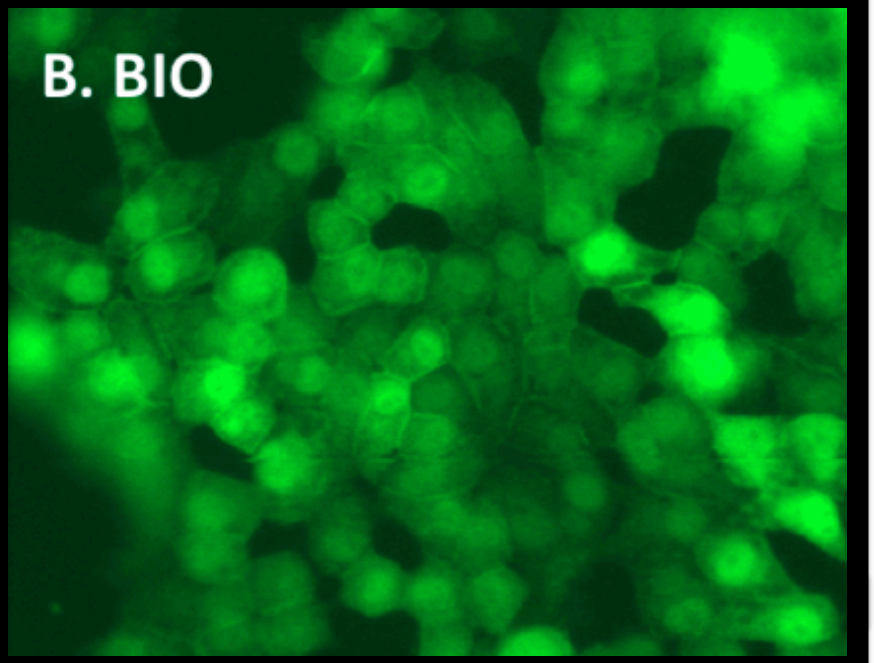
E-Cadherin
70000
60000
50000
40000
30000
20000
10000
0
**
##
^^
NP
0
12.5
100
NP
0
12.5
100
HeLa
A431

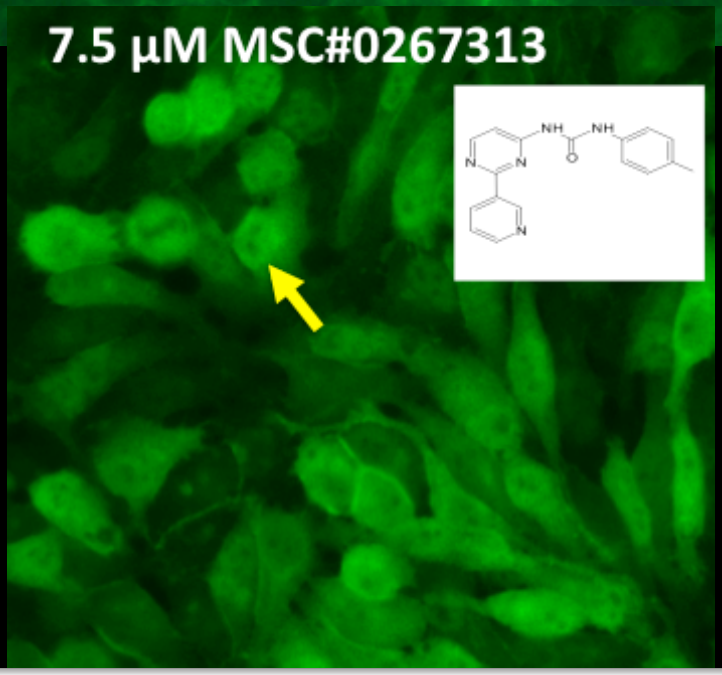
N-Cadherin


A. HeLa
B. HeLA + PMA
C
D
A
N-Cadherin
TMI
40000
30000
20000
10000
0
**
##
##
NP
0
12.5
100
NP
0
12.5
100
HeLa
A431

Beta-Catenin







Vala is offering the following assays for Cadherins and Beta-Catenin:

ECAD1, E-Cadherin, A431 cells, agonists

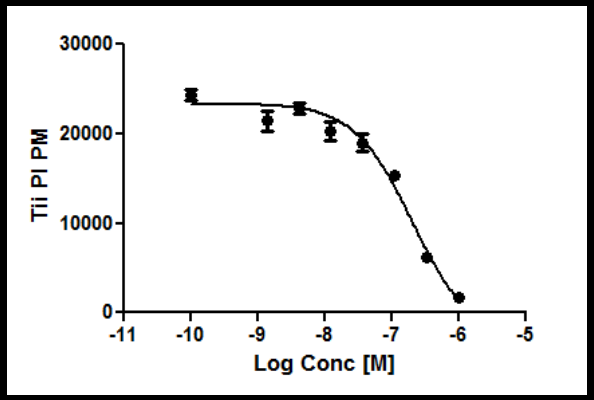
NCAD1, N-Cadherin, HeLa cells, agonists

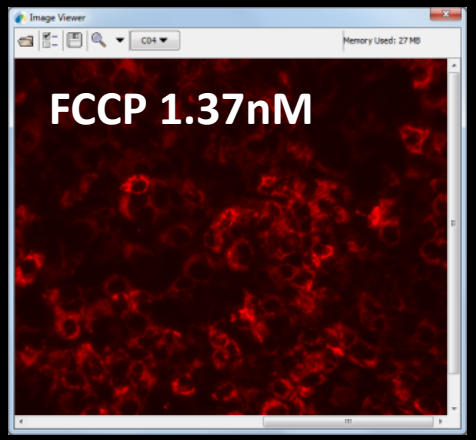
VCAD1, VE-Cadherin, Human vascular endothelial cells, agonists

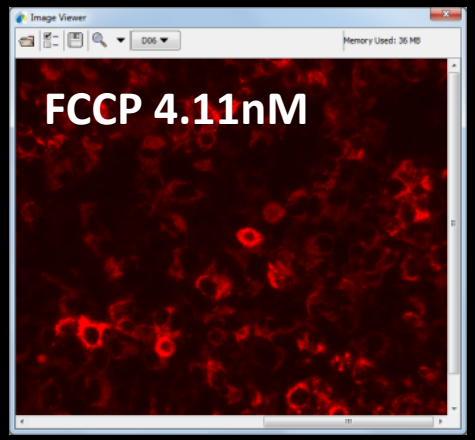
BC1, Beta-Catenin, HeLa, agonists

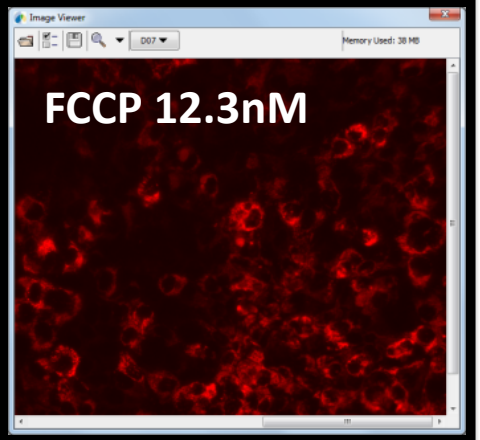
Ross Whittaker

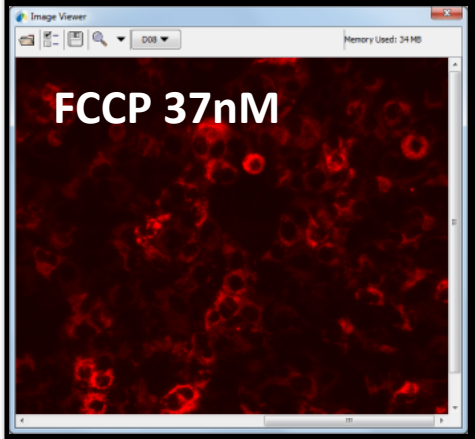
Assessing mitochondrial membrane potential ($\Delta\psi_M$) using HCA

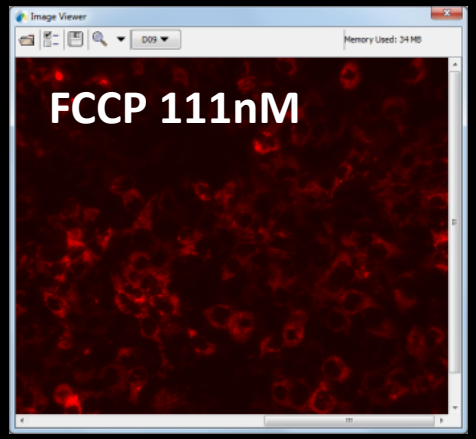


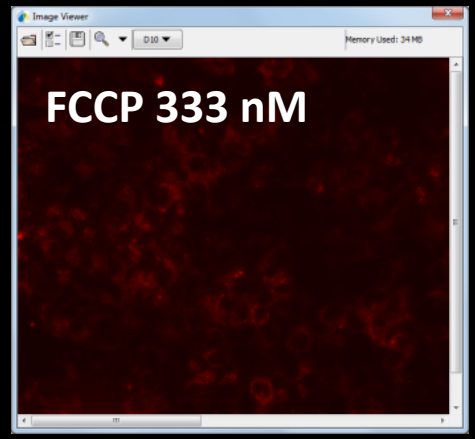


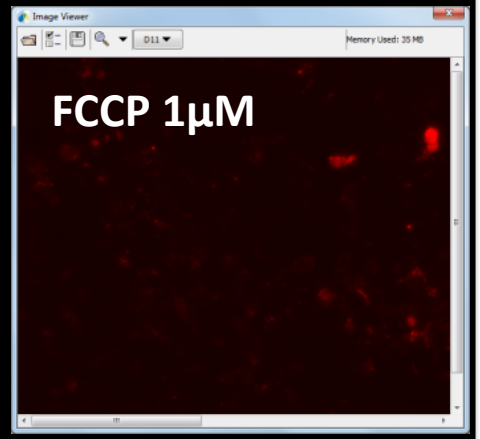












Vala is offering the following assays related to Mitochondrial Function

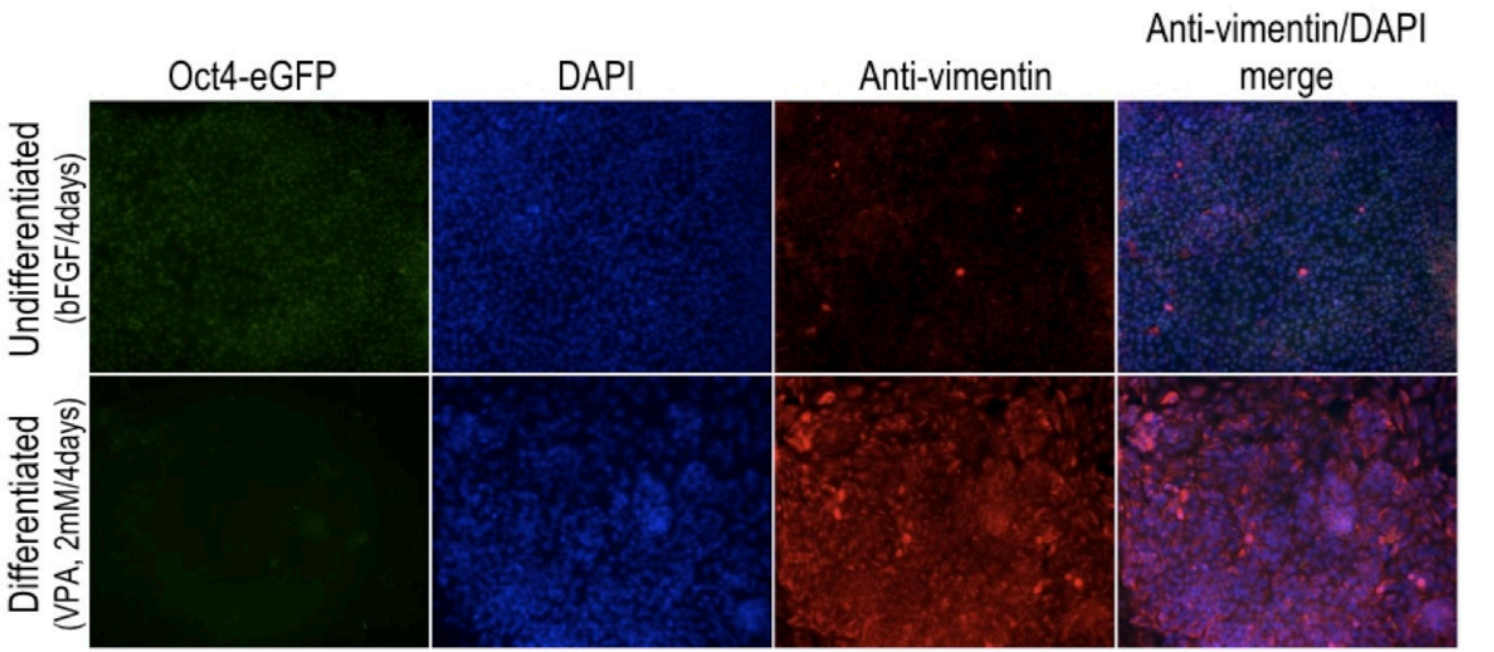
MP1, Mitochondrial membrane potential, Huh-7 cells, acute

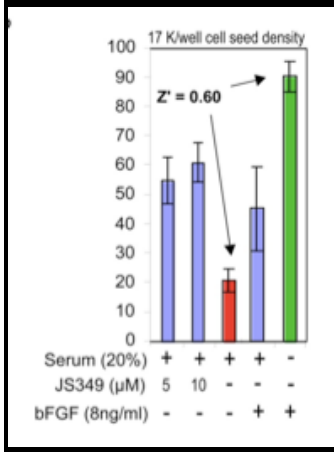
MP2 Mitochondrial membrane potential, Huh-7 cells, long term

Mark Mercola

Stem cell pluripotency

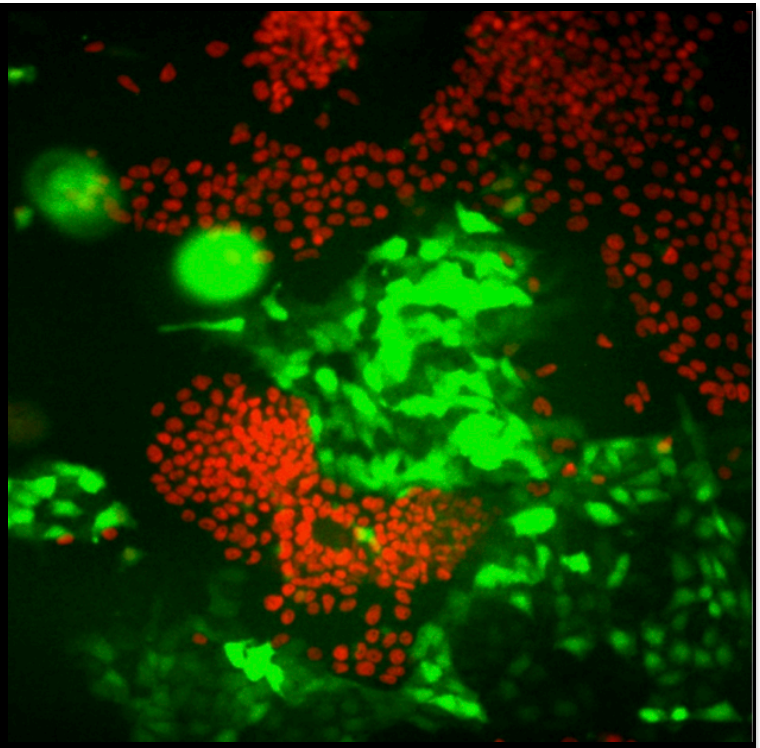
Assay SCP1, Stem cell pluripotency:
Compounds that reduce pluripotency reduce GFP expression in human ES cells expressing GFP from an Oct4 promoter





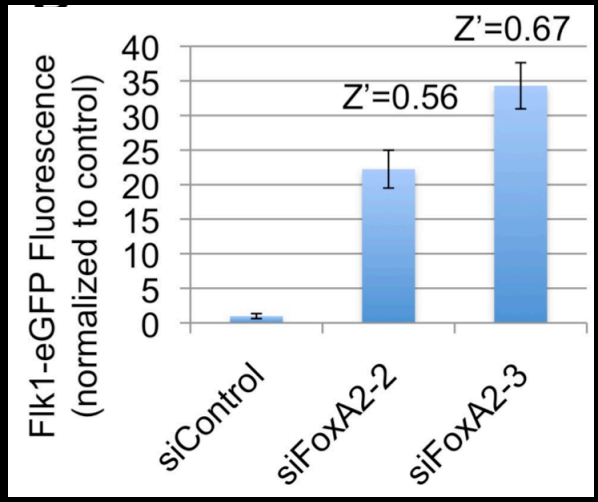
Mark Mercola

Mesoderm-endoderm formation



Assay MEF1, Mesoderm endoderm formation, mouse embryonic stem cells

mESCs engineered to express eGFP under control of the vascular growth factor receptor-2. Cells are treated with compounds and assayed for both GFP (indicates mesoderm formation), and FoxA2 (indicates endoderm formation.



Mark Mercola

Heart cell formation

Assay HCF1: Heart cell formation, mouse embryonic stem cells

mESCs engineered to express eGFP under control of the alpha-myosin heavy chain promoter. Cells are subjected to a differentiation protocol in the presence of test compounds. Increases in GFP indicated increased formation of cardiac myocytes.

A related assay was recently published:

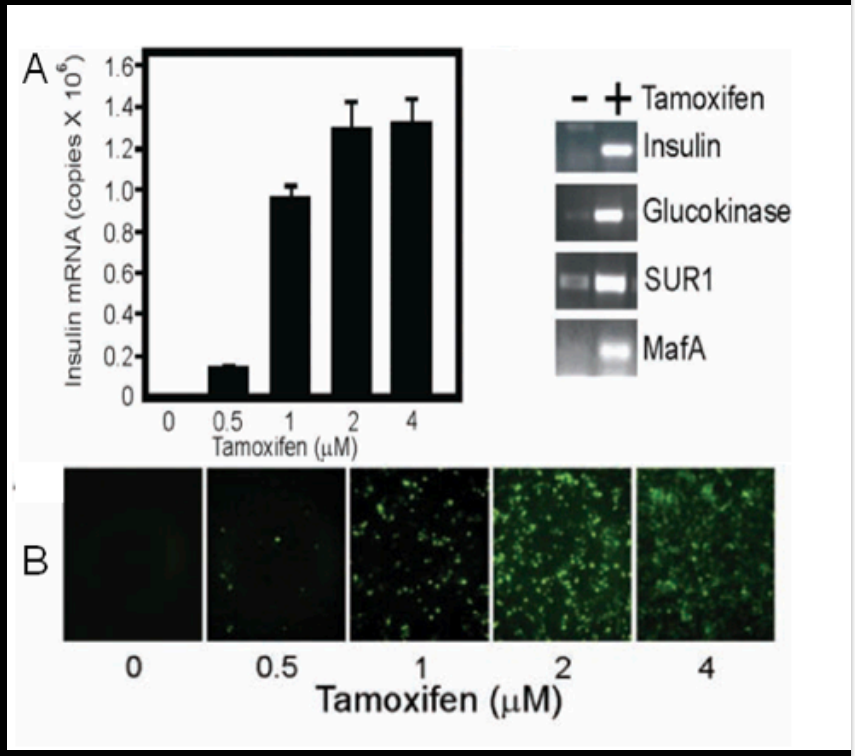
Willems, E., et al. 2011. Small-molecule inhibitors of the Wnt pathway potently promote cardiomyocytes from human embrynoic stem cell-derived mesoderm. Circulation Ressarch 109:360-3641, Mesoderm endoderm formation, mouse embryonic stem cells

Pam Itkin-Ansari

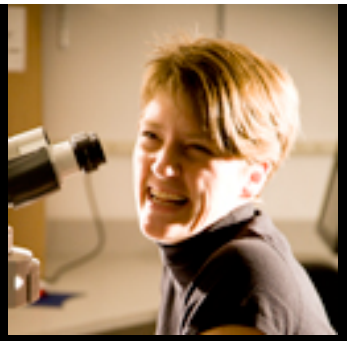
Pancreatic beta cell differentiation/ maturation

Assay PBCD1: Pancreatic Beta Cell Differentiation

TPNE cells (derived from human fetal islets) express GFP downstream from the human insulin promoter. Compounds that promote differentiation of this pancreatic beta cell precursor towards the mature beta cell increase GFP expression.



Kiselyuk, A., et al. Phenothiazine neuroleptics signal to the human insulin promoter as revealed by a novel high-throughput screen. J Biomol Screen 15, 663-670 (2010).



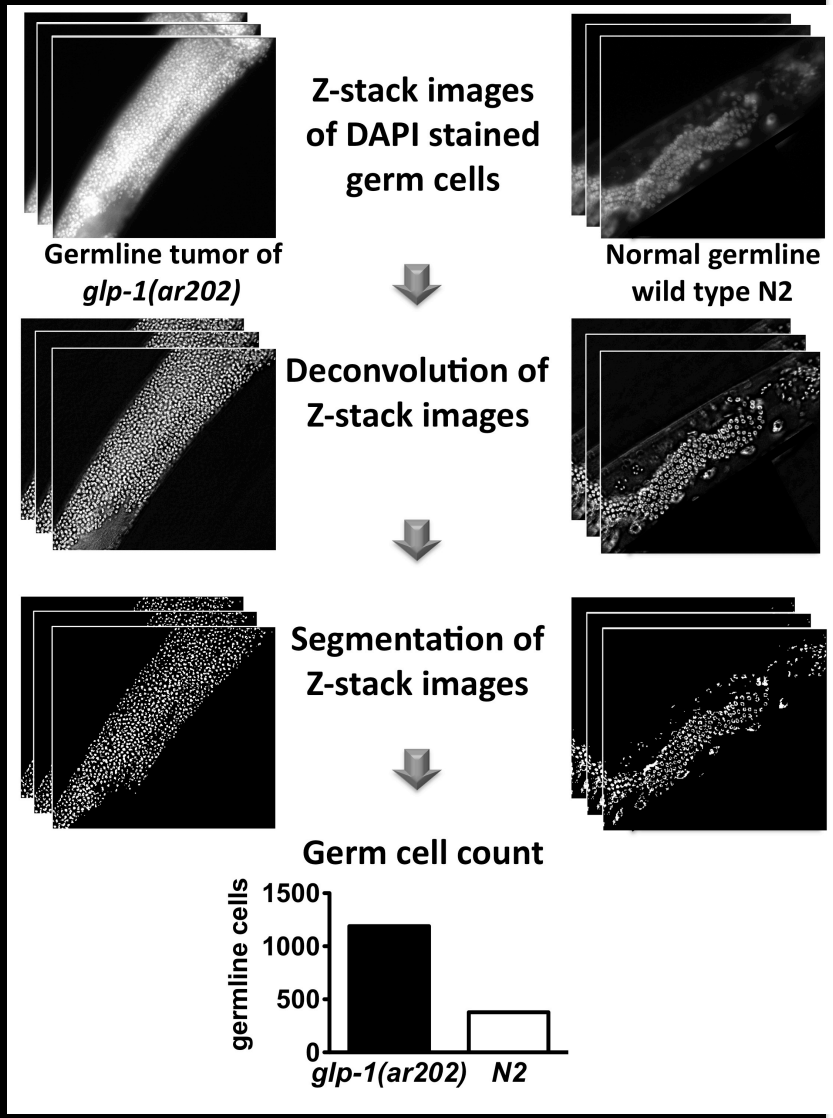
Malene Hansen

The Notch pathway

The appearance of germ cells in C. elegans is dependent Notch activity and germ cells can be quantified via high content microscopy.

Assay CE1: C. elegans, Notch antagonists. Test for the ability of compounds to inhibit worms with a gain of function mutation in Notch.

Assay CE2: C. elegans, Notch agonists. Tests for the ability of compounds to increase germ cells in wild-type worms.



Alexi Terskikh

Assay NO1: Optogenetic-based assay of synaptic connectivity of human neurons

iPS- or neuronal-precursor-derived neurons are engineered to express channel rhodopsin, and loaded with fluo-4 to monitor calcium with KIC. Elevations in calcium in post-synaptic cells correspond to neurotransmission.

Assay MCDO1: Multiple choice differentiation outcomes

Utilizes pluripotent human neural crest stem cells. Quantifies the effects of compounds to influence differentiation to neurons, smooth muscle, glia, and melanocytes.

Vala people:

Andrew Heisel

Fabio Cerignoli

Ramses Agustin

Claire Weston

Robyn Garcia

James Evans

Ross Whittaker

Piyush Gehalot

Mike Markoudakis

Emily Arsenault

Constance Allison

Jeff Hilton