



***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 acquisition 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 crosssectional 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).**

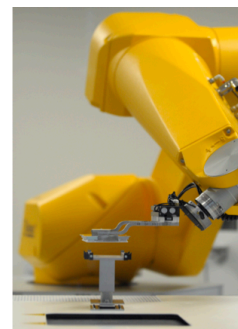
# Vala Collaborators:

## Sanford-Burnham Medical Research Institute.

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 dispense, plate hotel & incubator

IN Cell housed in sterile room

### Off-line Instruments & Workstation

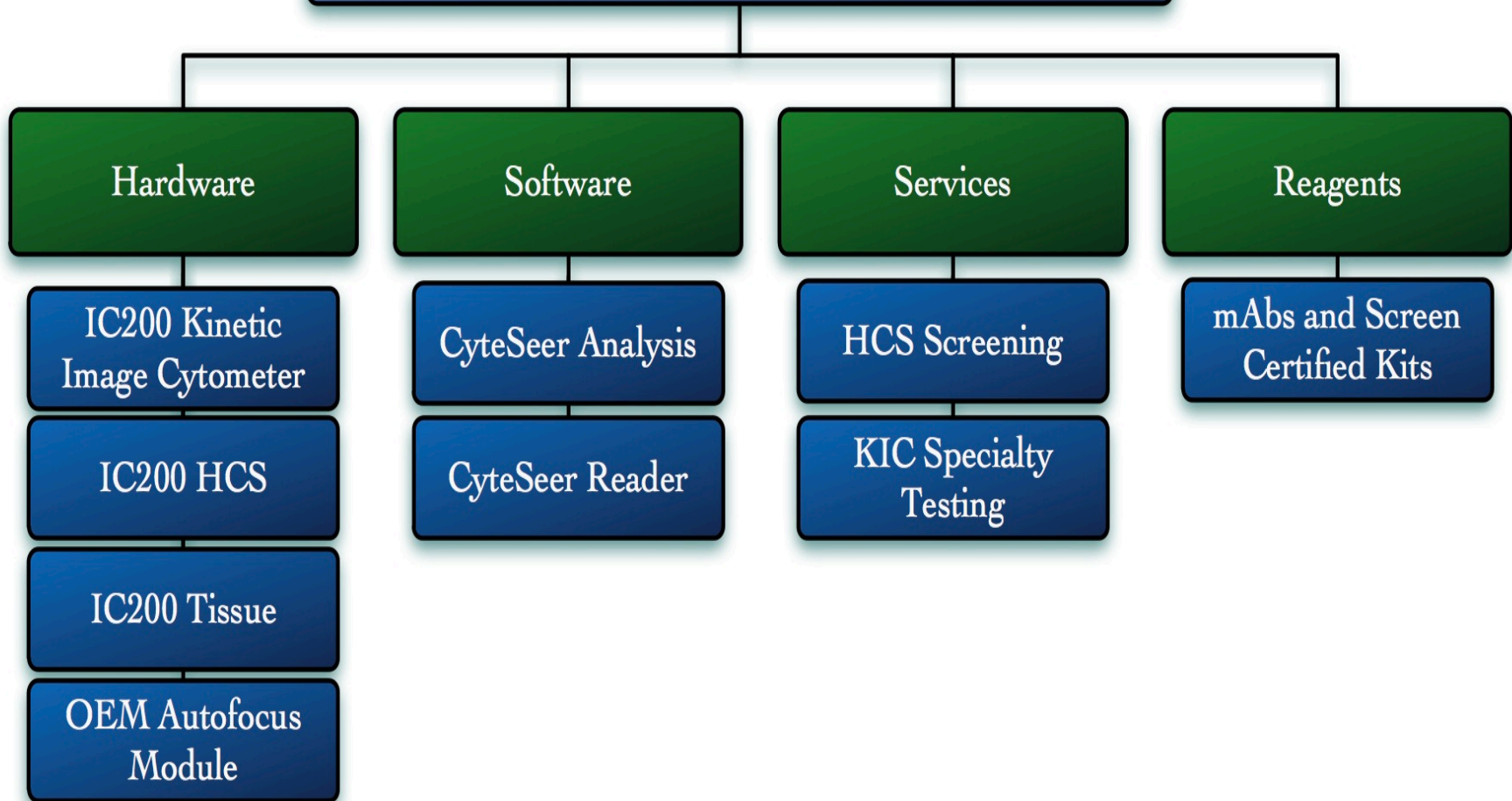
6 Plate readers, 2 Plate washers, 3 Bulk reagent dispensers

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



## *Instrumentation:*

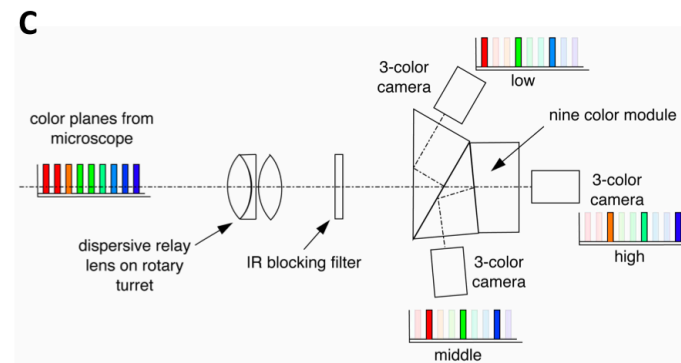
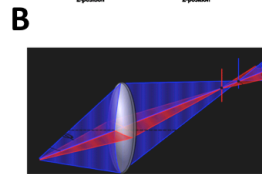
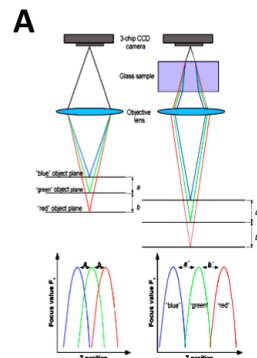
2003 Q3DM Eidaq IC100 (also marketed by Beckman Coutler) (rapid autofocus, multichannel fluorescence, microtiter plates, slides)

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.



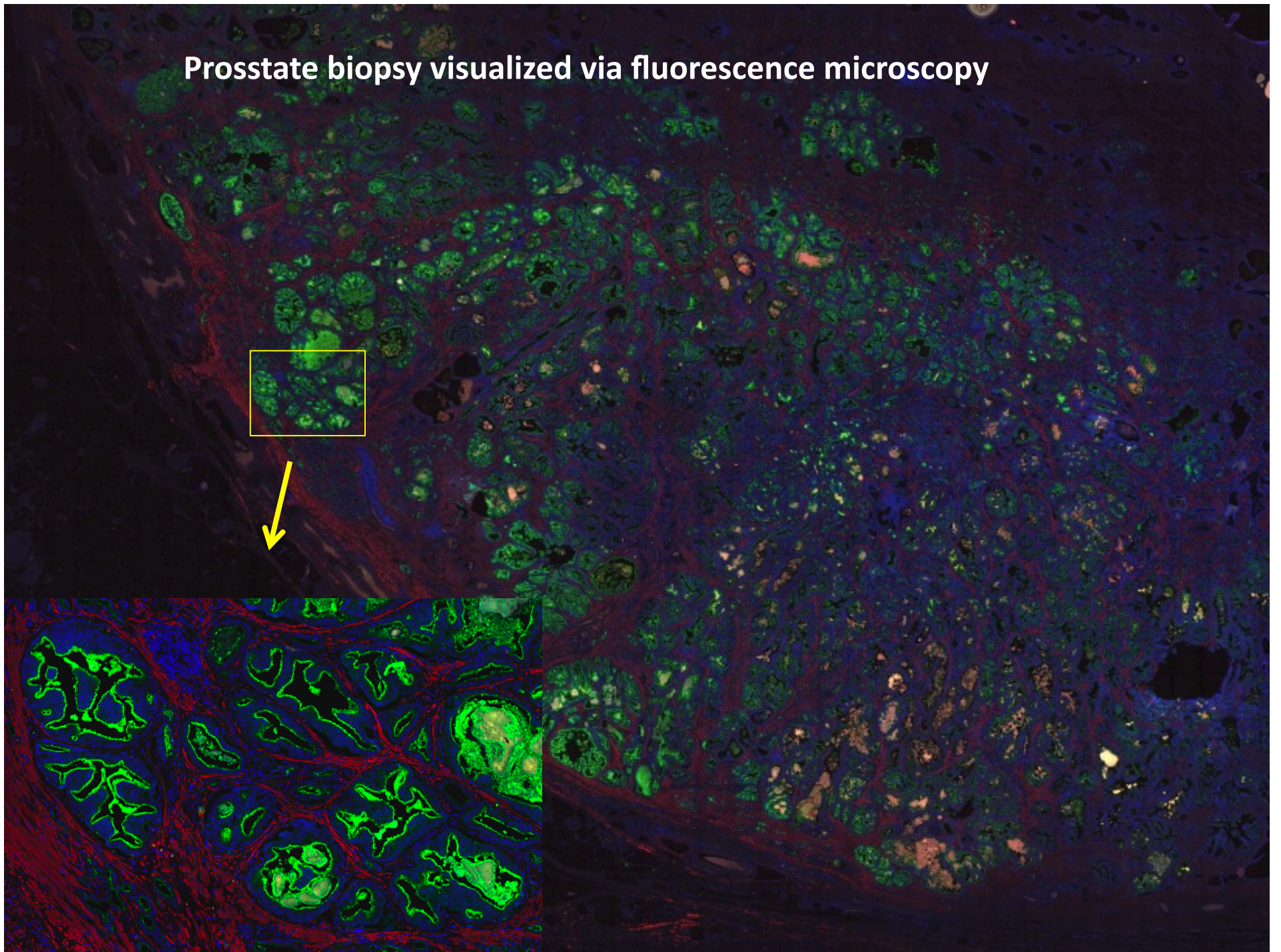
Jeffrey H. Price, M.D., Ph.D.



**Autofocus based upon chromatic aberration**



Prostate biopsy visualized via fluorescence microscopy



# Software: CyteSeer®

CyteSeer 2.7.10.16

Navigation:

Source Folder:

Results Folder:

Image Naming Convention:   Plate  Batch

Define Algorithm:

Algorithm To Run:

| Channel Name | Channel Folder | Analyze?                            | Prefilter | Filtering Radius | Thresholding  | Sensitivity % | Size Parameter | Size Value | Loader         | Configure |
|--------------|----------------|-------------------------------------|-----------|------------------|---------------|---------------|----------------|------------|----------------|-----------|
| Nucleus      | channel_0      | <input checked="" type="checkbox"/> | None      | 1                | SavitskyGolay | 100           | Minimum Size   | 5          | Single Slice   |           |
| Lipid        | channel_1      | <input checked="" type="checkbox"/> | None      | 1                | SavitskyGolay | 50            |                |            | 0 Single Slice |           |
| Protein      | channel_2      | <input checked="" type="checkbox"/> | None      | 1                | SavitskyGolay | 50            |                |            | 0 Single Slice |           |

Wells to Run Algorithm On:

| Well | Run?                                |
|------|-------------------------------------|
| F01  | <input checked="" type="checkbox"/> |
| F02  | <input checked="" type="checkbox"/> |
| F03  | <input checked="" type="checkbox"/> |
| F04  | <input checked="" type="checkbox"/> |
| F05  | <input checked="" type="checkbox"/> |
| F06  | <input checked="" type="checkbox"/> |
| F07  | <input checked="" type="checkbox"/> |
| F08  | <input checked="" type="checkbox"/> |
| F09  | <input checked="" type="checkbox"/> |
| F10  | <input checked="" type="checkbox"/> |

Images Within Well:

Images Across:

Images Down:

Computational Cluster Size:

Images Across:

Images Down:

Gates

| Name | Source DataTable | Result DataTable | Enabled? |
|------|------------------|------------------|----------|
|------|------------------|------------------|----------|



**Randall Ingermanson**



**Jeff Hilton**

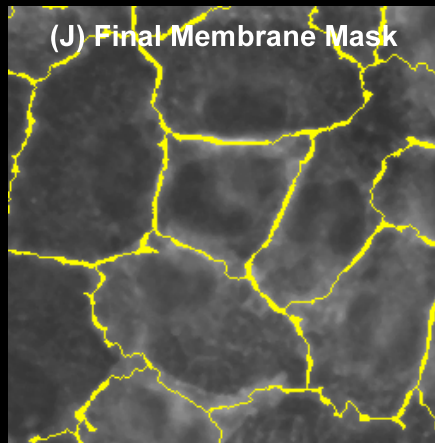
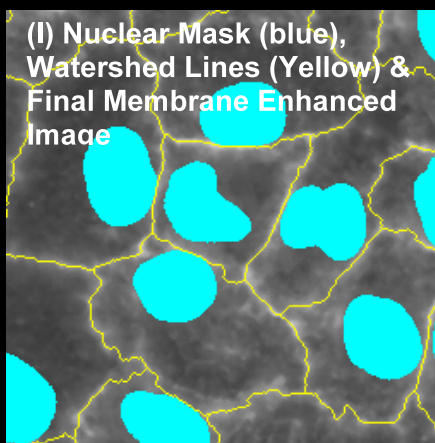
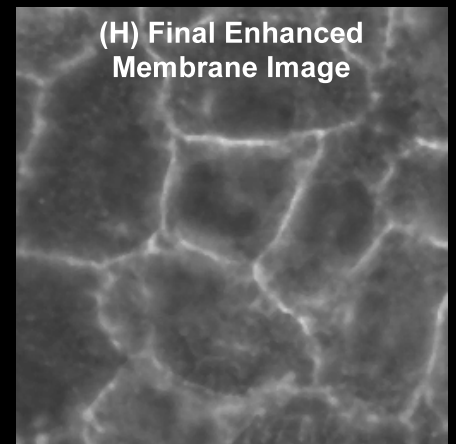
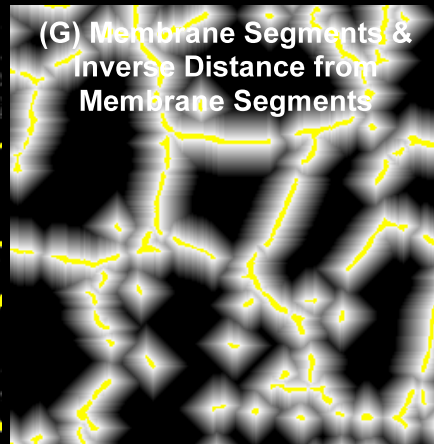
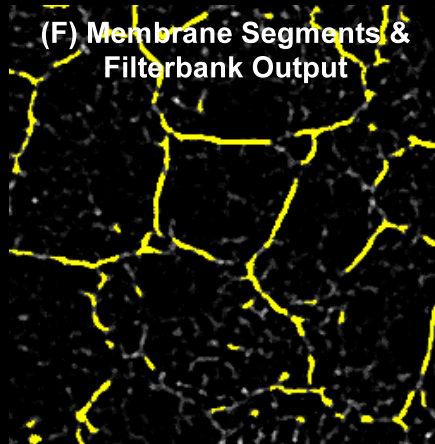
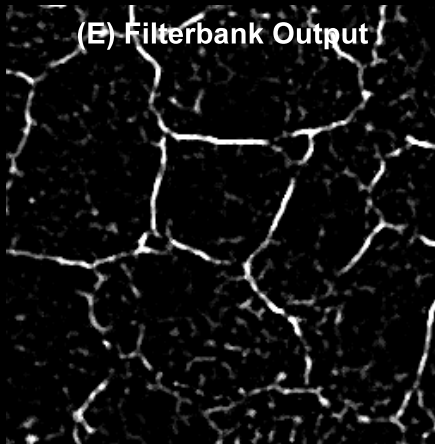
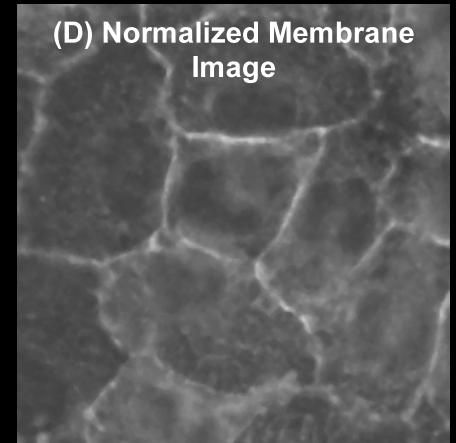
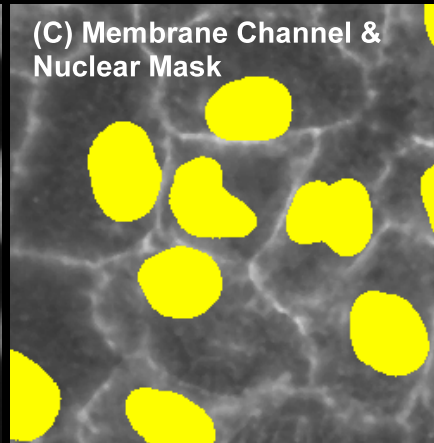
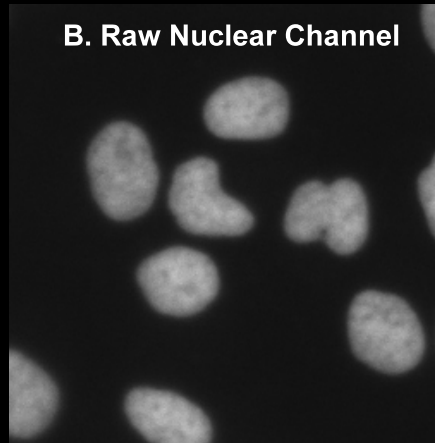
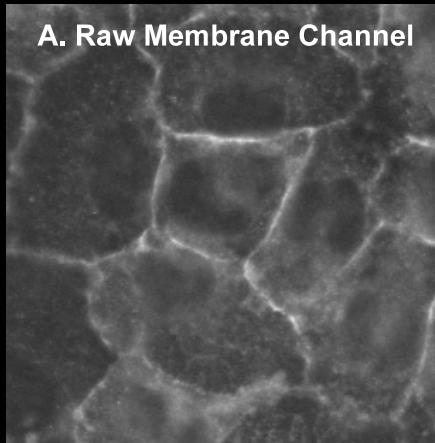


## **Features of CyteSeer®**

- 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**

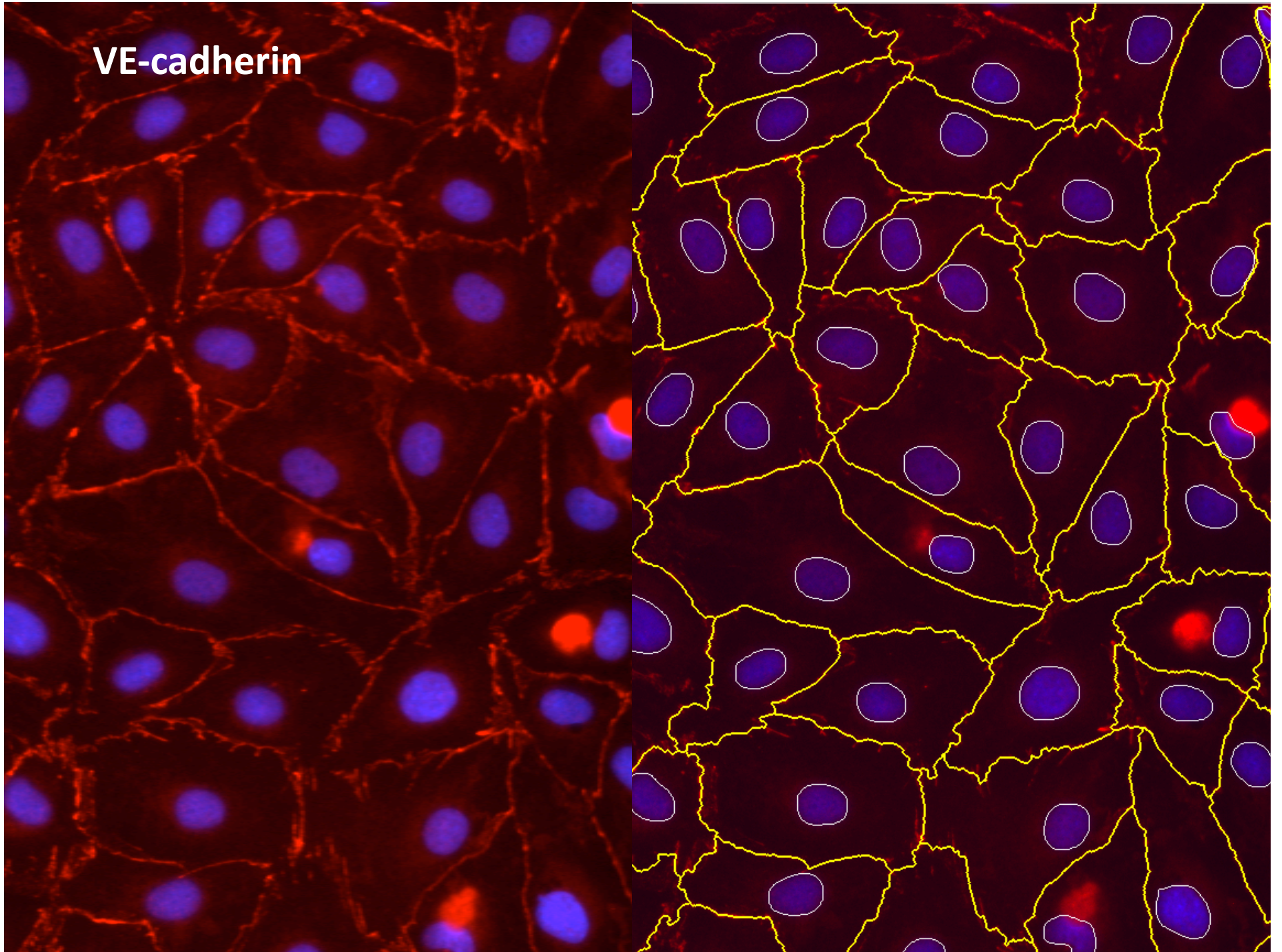
**<http://www.valasciences.com>**





## The Membrane Analysis Algorithm:

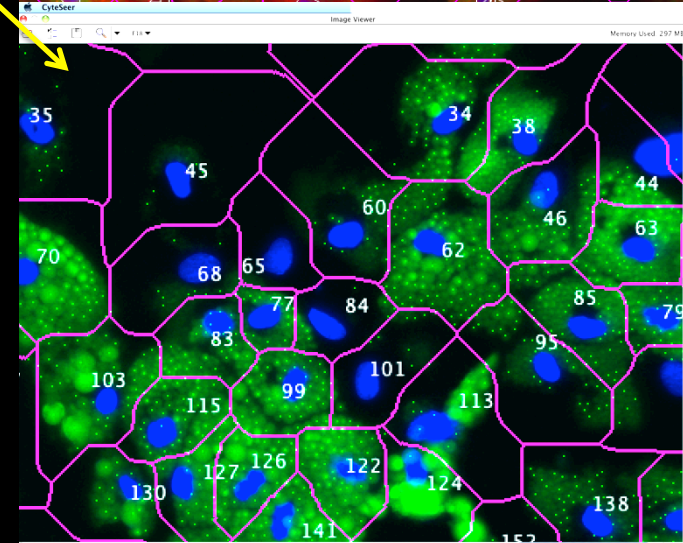
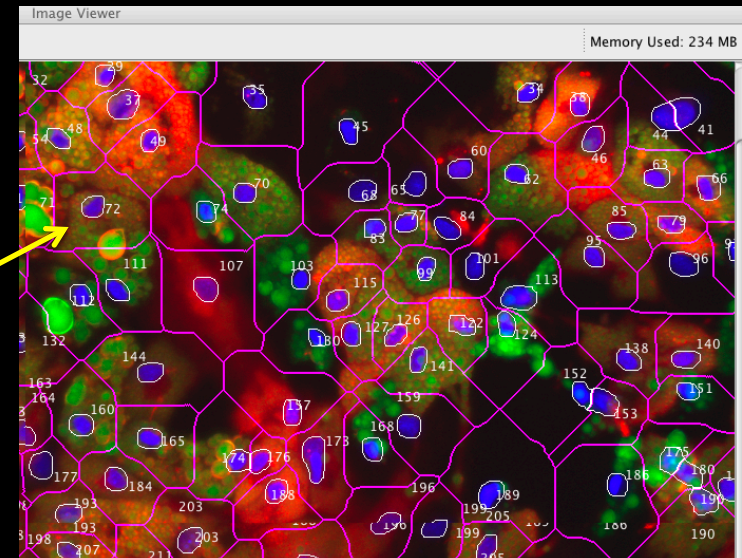
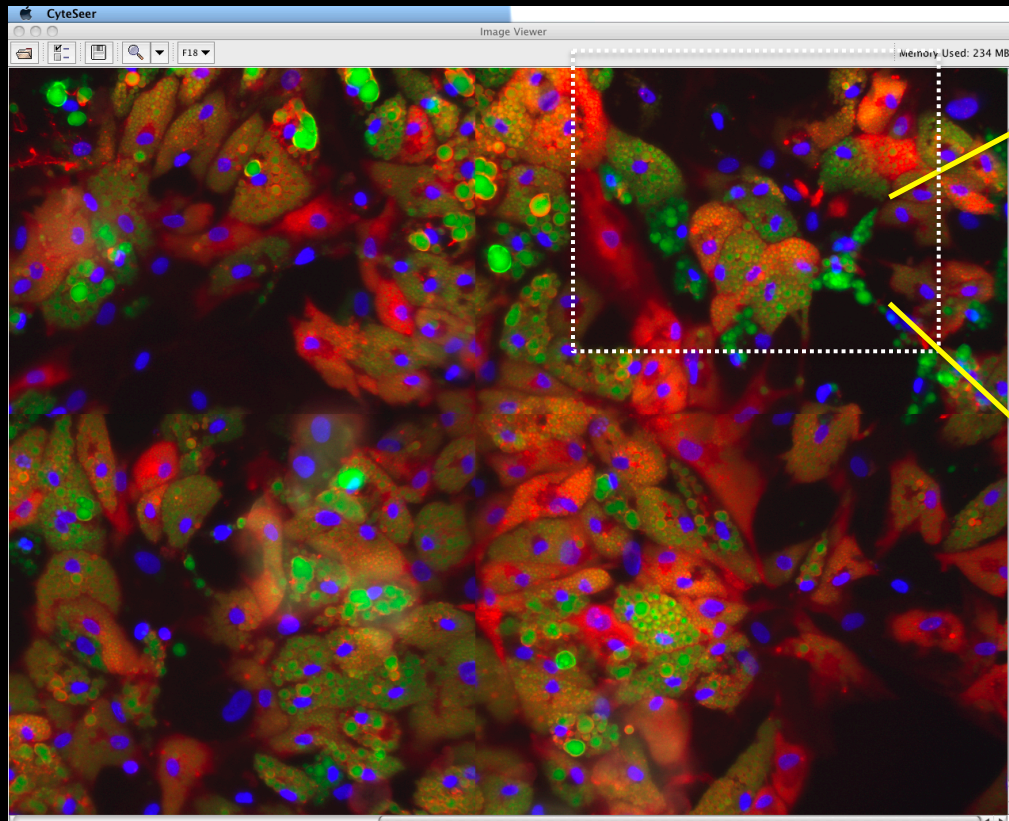
**VE-cadherin**





# 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)

The screenshot displays the CyteSeer 2.7.10.16 software interface. The main window shows a 'Data Viewer' for well 'F01'. The interface includes a navigation pane on the left with options for 'Source Folder', 'Results Folder', and 'Image Naming'. Below this, there are sections for 'Define Algorithm' (with 'Algorithm To' set to 'Membrane') and 'Wells to Run Algorithm' (with 'Well' set to 'F01'). The central area features a 'Data Table' for 'F01' with columns for 'Cell ID', 'Well', 'Z', and various 'API' (Average Pixel Intensity) parameters for different components like 'Lm', 'Nm', 'Wm', 'Cm', 'Pm', 'Qm', and 'Wm'. The table contains 42 rows of data. To the right of the table, there are 'Gates' and 'Significant Figures' sections. The 'Gates' section lists various gates with their sources and actions, such as 'CyteSeer Remove Two Percent...', 'CyteSeer Remove Two Sigma...', 'CyteSeer Ploidy Gate', and 'CyteSeer Remove Boundary C...'. The 'Significant Figures' section shows a value of 4. Below the table, there are buttons for 'Update', 'Check All Show', 'Export...', and 'Uncheck All'. The background shows a web browser window with a search for 'linhagak, alaska' and a search bar.

| Cell ID | Well | Z | API Li Lm | API Li Nm | API Li Wm | API Li X Cm | API Li X Nm | API Ni Nm | API Ni Wm | API Pi Cm | API Pi Lm | API Pi Nm | API Pi Pm | API Pi Qm | API Pi Qm | API Pi Wm | Area Cm  |          |
|---------|------|---|-----------|-----------|-----------|-------------|-------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|----------|
| 1       | F01  | 0 | 37.3597   | 32.4496   | 19.6216   | 18.1287     | 29.4522     | 116.2946  | 20.7249   | 44.4644   | 56.2770   | 58.6512   | 59.5833   | 44.3788   | 58.6512   | 44.9069   | 4266.000 |          |
| 2       | F01  | 0 | 45.7921   | 15.2510   | 15.4567   | 14.7022     | 15.2984     | 111.5223  | 18.6272   | 40.2732   | 54.4587   | 44.4211   | 53.1636   | 40.1579   | 44.4211   | 40.3540   | 12418.00 |          |
| 3       | F01  | 0 | 36.4664   | 23.9504   | 22.1885   | 19.7820     | 22.1360     | 115.8369  | 33.0744   | 255.0000  | 255.0000  | 255.0000  | 255.0000  |           | 243.6000  | 255.0000  | 1929.000 |          |
| 4       | F01  | 0 | 43.9259   | 32.6311   | 14.4042   | 14.0121     | 23.2203     | 123.2816  | 19.1840   | 38.1118   | 93.7778   | 88.8738   | 93.5106   | 38.0719   | 132.7647  | 39.0616   | 5402.000 |          |
| 5       | F01  | 0 | 54.2505   | 28.9812   | 37.2701   | 34.4580     | 27.7613     | 102.2895  | 27.3500   | 53.5185   | 58.3111   | 53.3120   | 63.6500   | 53.2801   | 53.2689   | 53.4888   | 2561.000 |          |
| 6       | F01  | 0 | 14.0424   | 14.0424   | 13.8596   | 13.8571     | 14.0424     | 84.7257   | 16.7355   | 34.4935   | 36.6035   | 52.3810   | 34.4430   | 36.6035   | 34.5215   | 29822.00  |          |          |
| 7       | F01  | 0 | 57.0148   | 27.6432   | 27.6494   | 24.2805     | 25.9666     | 90.8865   | 36.0880   | 45.9401   | 56.6946   | 46.8811   | 64.7805   | 44.5831   | 46.7339   | 46.0992   | 1770.000 |          |
| 8       | F01  | 0 | 37.0526   | 15.3393   | 16.4457   | 16.1412     | 15.3393     | 91.9013   | 43.1806   | 235.5116  | 160.6667  | 255.0000  | 245.4131  | 111.8925  |           |           | 240.1869 | 1806.000 |
| 9       | F01  | 0 | 41.7179   | 21.7107   | 17.3674   | 15.9851     | 19.7134     | 155.9721  | 28.1331   | 86.0547   | 160.2071  | 86.4518   | 174.3721  | 58.6102   | 82.0444   |           | 86.0803  | 5722.000 |
| 10      | F01  | 0 | 30.2483   | 15.0513   | 14.2047   | 14.1541     | 15.0513     | 134.4795  | 16.8649   | 39.9835   | 51.8993   | 45.0667   | 52.5882   | 39.9716   | 45.0667   |           | 40.0200  | 53912.00 |
| 11      | F01  | 0 | 27.8644   | 14.9202   | 16.2158   | 16.1862     | 14.9202     | 103.2311  | 40.6667   | 255.0000  | 255.0000  | 255.0000  |           |           |           |           | 255.0000 | 2647.000 |
| 12      | F01  | 0 |           | 17.7893   | 14.9488   | 14.7726     | 17.7893     | 109.4073  | 27.4020   | 234.0578  |           | 254.3062  | 247.2427  | 82.8539   |           |           | 235.2405 | 5739.000 |
| 13      | F01  | 0 | 32.1225   | 14.3642   | 13.8857   | 13.8371     | 14.2956     | 92.7415   | 16.3743   | 31.5181   | 38.6716   | 36.3566   | 51.3084   | 31.4526   | 35.0665   |           | 31.5496  | 81051.00 |
| 14      | F01  | 0 | 44.9502   | 16.5046   | 19.5410   | 17.9650     | 16.1432     | 137.9032  | 35.0012   | 216.7628  | 152.0093  | 252.3848  | 241.3411  | 88.0412   |           |           | 219.8424 | 4586.000 |
| 15      | F01  | 0 | 37.2974   | 25.1429   | 22.5088   | 20.7693     | 23.7103     | 107.9739  | 30.3539   | 41.8005   | 45.9538   | 45.9304   | 54.6047   | 41.7166   | 55.4281   |           | 42.2949  | 5874.000 |
| 16      | F01  | 0 | 20.3333   | 13.9526   | 14.1613   | 14.1552     | 13.9526     | 54.2414   | 19.9062   | 82.9232   | 40.7083   | 67.6853   | 207.0974  | 45.4930   | 65.4165   |           | 82.1240  | 8383.000 |
| 17      | F01  | 0 | 21.4533   | 14.6539   | 14.1626   | 14.0849     | 14.6225     | 144.7454  | 27.5688   | 34.9054   | 38.6800   | 40.3060   | 53.8367   | 34.7878   | 40.1934   |           | 35.3184  | 13853.00 |
| 18      | F01  | 0 | 65.9076   | 41.7283   | 41.5497   | 37.0406     | 28.1373     | 120.7658  | 39.0906   | 56.2682   | 60.8022   | 56.7049   | 68.9844   | 55.6213   | 56.3621   |           | 56.2928  | 2394.000 |
| 19      | F01  | 0 | 65.4069   | 34.2299   | 44.4913   | 39.9748     | 28.3782     | 112.6090  | 39.0621   | 101.6786  | 95.1219   | 61.5948   | 198.2210  | 62.3234   | 61.5948   |           | 95.4594  | 2172.000 |
| 20      | F01  | 0 | 27.0765   | 15.8356   | 16.2871   | 16.0280     | 15.8356     | 121.2012  | 31.5502   | 249.9206  | 255.0000  | 255.0000  | 252.2285  | 92.9091   |           |           | 250.2500 | 7455.000 |
| 21      | F01  | 0 | 62.7562   | 14.1529   | 15.2135   | 14.4615     | 14.1529     | 96.9253   | 24.6247   | 96.6584   | 99.3760   | 62.5896   | 224.4894  | 50.8874   | 60.7669   |           | 94.0864  | 13936.00 |
| 22      | F01  | 0 | 47.5876   | 18.1913   | 21.2273   | 19.8907     | 17.6851     | 118.3739  | 26.2258   | 54.7783   | 96.0458   | 48.2804   | 196.9057  | 46.7029   | 48.2804   |           | 54.3495  | 6510.000 |
| 23      | F01  | 0 |           | 15.6862   | 14.3302   | 14.2876     | 15.6862     | 78.7973   | 18.5982   | 40.4159   |           | 78.2534   | 94.0333   | 40.1051   | 68.9931   |           | 41.5660  | 32728.00 |
| 24      | F01  | 0 | 32.9620   | 16.7735   | 16.5692   | 15.9029     | 16.5753     | 128.0018  | 42.2079   | 255.0000  | 255.0000  | 255.0000  | 255.0000  |           |           |           | 255.0000 | 4043.000 |
| 25      | F01  | 0 | 29.1065   | 16.9904   | 17.1845   | 16.7646     | 16.6583     | 126.2596  | 36.4472   | 251.1911  | 254.6564  | 255.0000  | 253.1370  | 101.4022  |           |           | 251.4758 | 7775.000 |
| 26      | F01  | 0 | 35.4589   | 22.6409   | 14.3588   | 14.1331     | 20.5586     | 152.3149  | 18.0746   | 36.2775   | 75.5308   | 62.6547   | 77.9798   | 36.0316   | 60.6456   |           | 36.5369  | 36450.00 |
| 27      | F01  | 0 | 34.6396   | 15.7267   | 17.1986   | 16.6799     | 15.6539     | 129.2574  | 27.2655   | 150.3983  | 177.2792  | 251.6238  | 221.5924  | 71.3560   |           |           | 156.1974 | 8310.000 |
| 28      | F01  | 0 | 53.3257   | 28.4767   | 25.0970   | 22.5412     | 27.1962     | 104.7312  | 28.2902   | 40.3709   | 49.5029   | 45.0573   | 55.1667   | 40.2424   | 44.7000   |           | 40.9145  | 2401.000 |
| 29      | F01  | 0 | 35.6103   | 16.1287   | 16.9276   | 16.2310     | 15.8357     | 145.0992  | 28.4175   | 36.2139   | 43.5897   | 40.9544   | 51.1964   | 36.0381   | 40.9544   |           | 36.5776  | 4830.000 |
| 30      | F01  | 0 | 22.8788   | 15.0593   | 14.6891   | 14.6412     | 15.0593     | 98.5826   | 21.6757   | 41.3935   | 48.1818   | 46.0996   |           | 41.3935   |           |           | 46.0996  | 9313.000 |
| 31      | F01  | 0 | 26.7429   | 17.2671   | 15.8972   | 15.3857     | 16.9498     | 165.0662  | 32.4591   | 34.9868   | 41.1214   | 41.3539   | 44.7143   | 34.9346   | 41.3539   |           | 35.5916  | 3937.000 |
| 32      | F01  | 0 | 33.5564   | 17.7161   | 16.2847   | 15.5356     | 16.7213     | 161.6181  | 32.0791   | 177.8727  | 254.3424  | 249.3719  | 245.2736  | 66.6622   |           |           | 182.0810 | 6364.000 |
| 33      | F01  | 0 | 33.9813   | 15.1938   | 15.5878   | 15.1027     | 15.1938     | 79.5046   | 23.1374   | 37.7787   | 45.5875   | 34.7587   | 49.5946   | 37.6271   | 34.7587   |           | 37.5108  | 5839.000 |
| 34      | F01  | 0 | 71.9276   | 32.1496   | 39.6290   | 32.7992     | 30.0785     | 119.5536  | 60.5399   | 53.7699   | 61.5882   | 57.0224   | 66.0816   | 53.0544   | 56.7700   |           | 55.0826  | 678.000  |
| 35      | F01  | 0 | 68.0451   | 28.5448   | 33.2239   | 27.9283     | 28.3066     | 129.7534  | 35.0564   | 50.8450   | 61.0855   | 56.6345   | 66.9753   | 50.4568   | 56.2668   |           | 51.5851  | 2800.000 |
| 36      | F01  | 0 | 100.5865  | 27.3629   | 19.3371   | 14.9803     | 20.6487     | 72.5327   | 18.8976   | 33.1986   | 67.4663   | 41.1480   | 75.4697   | 32.9035   | 38.9457   |           | 33.5537  | 13553.00 |
| 37      | F01  | 0 | 26.6063   | 14.8088   | 13.9127   | 13.8720     | 14.7114     | 153.7328  | 18.1494   | 37.0237   | 41.0079   | 46.4865   | 56.8077   | 36.9030   | 46.3337   |           | 37.1609  | 55465.00 |
| 38      | F01  | 0 | 29.3969   | 17.7284   | 15.5335   | 15.2606     | 17.2489     | 138.8470  | 23.6285   | 59.2600   | 90.1221   | 65.9914   | 160.3129  | 54.5946   | 65.3220   |           | 59.5731  | 9511.000 |
| 39      | F01  | 0 | 64.3659   | 41.9772   | 35.1147   | 30.7239     | 40.3587     | 121.3316  | 31.8832   | 52.8663   | 65.5721   | 63.8785   | 66.9714   | 52.3628   | 63.3784   |           | 53.8867  | 3868.000 |
| 40      | F01  | 0 | 39.2310   | 21.5084   | 21.8470   | 19.4702     | 19.9924     | 132.7107  | 38.3516   | 167.8272  | 254.7092  | 254.8244  | 250.1336  | 57.9642   |           |           | 177.7140 | 4664.000 |
| 41      | F01  | 0 | 39.3973   | 19.5432   | 20.8430   | 18.5180     | 18.7179     | 150.6608  | 31.0042   | 140.6366  | 184.7466  | 124.2572  | 225.8797  | 67.6643   | 96.1549   |           | 139.5162 | 6142.000 |
| 42      | F01  | 0 | 54.6555   | 15.0544   | 15.5288   | 14.7261     | 14.9561     | 102.0000  | 20.1011   | 37.7212   | 55.8481   | 47.7208   | 58.8400   | 27.6048   | 47.7208   |           | 27.6048  | 12704.00 |

## 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 different 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          |



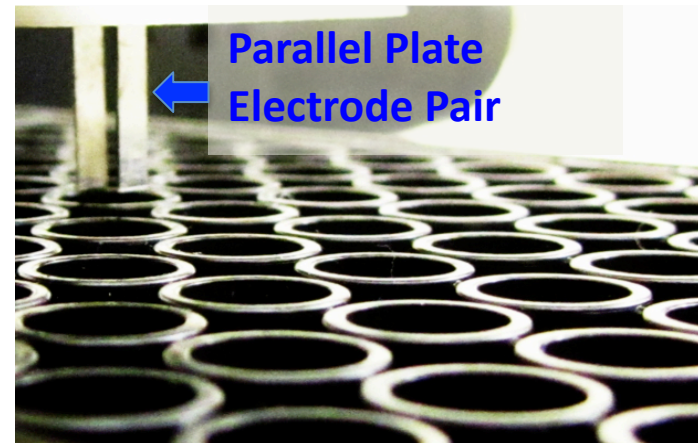
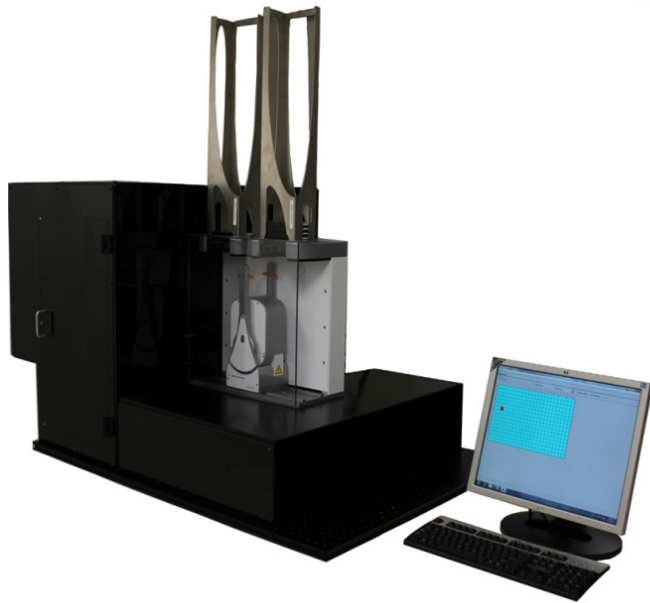
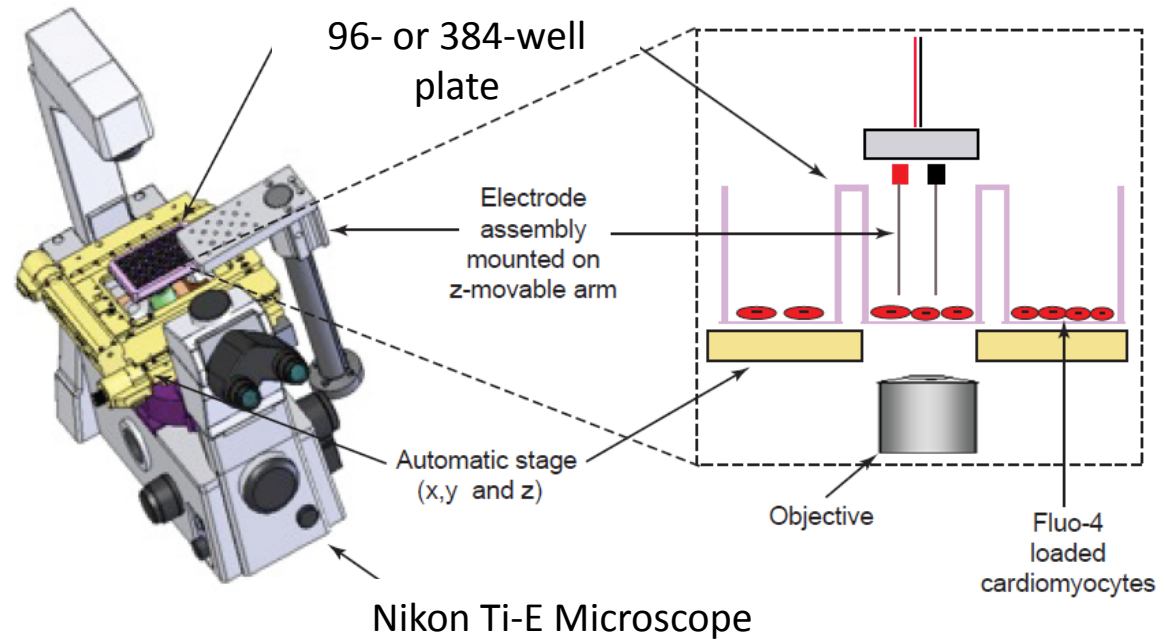
# Kinetic Image Cytometer (KIC)

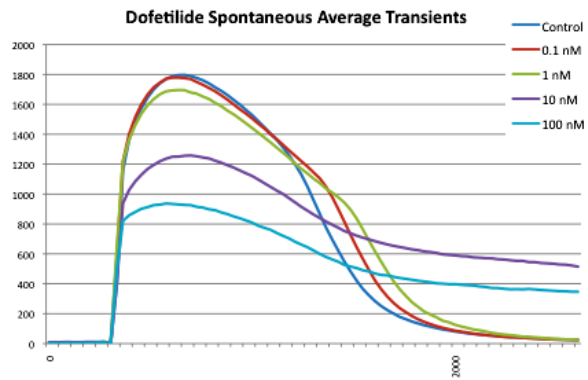
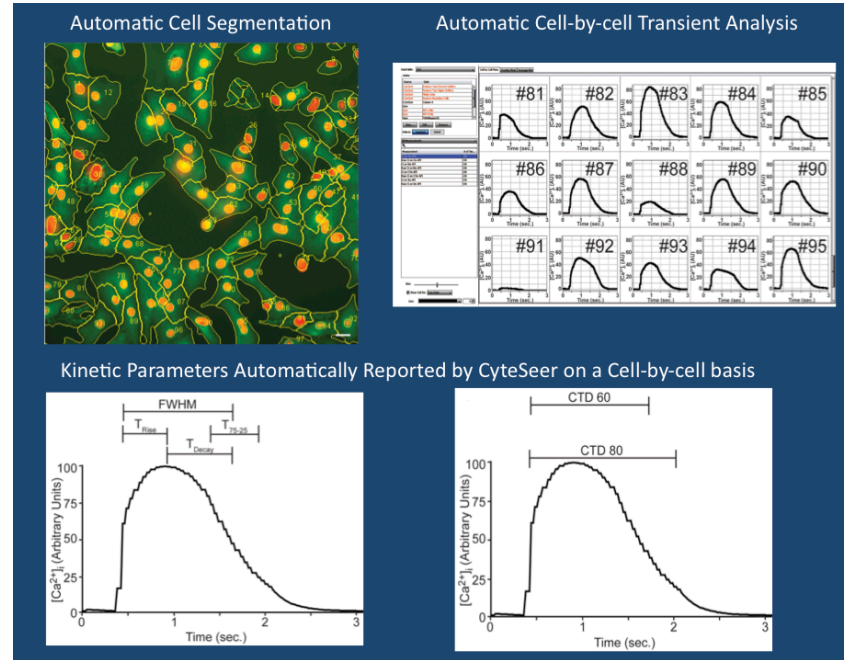
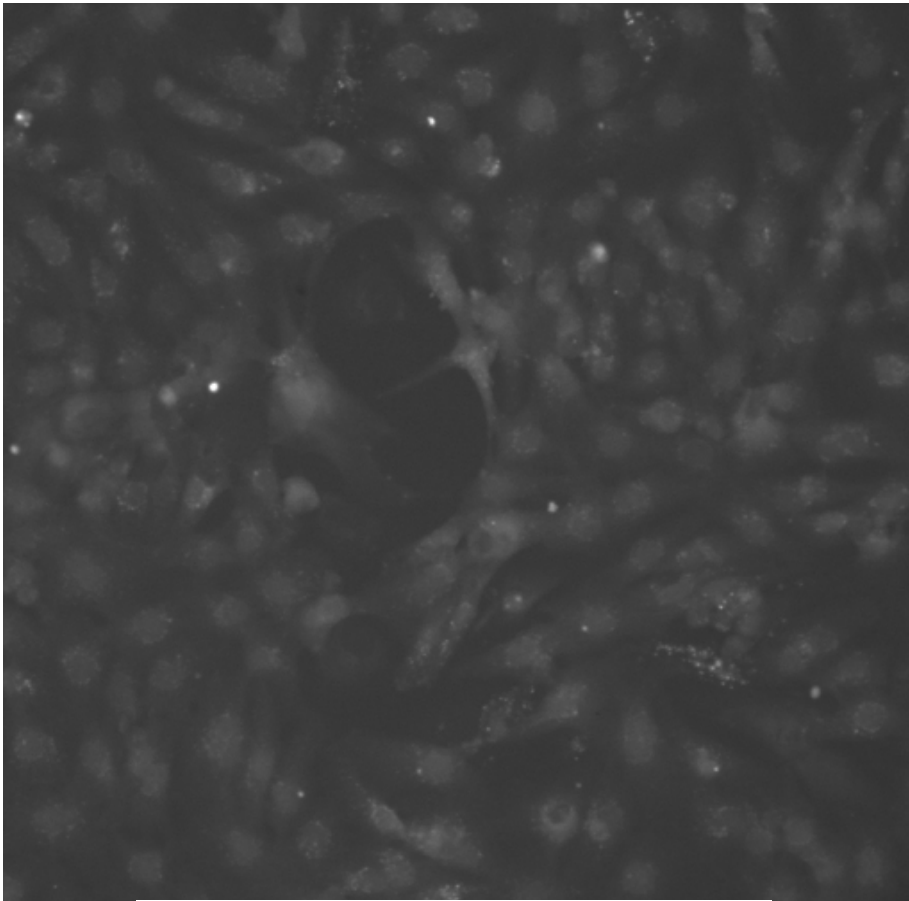


Fabio Cerignoli

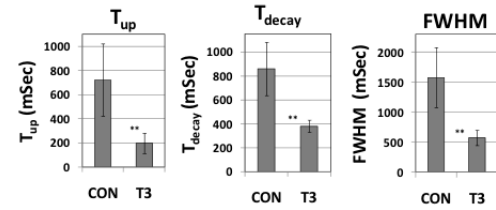


Ross Whittaker

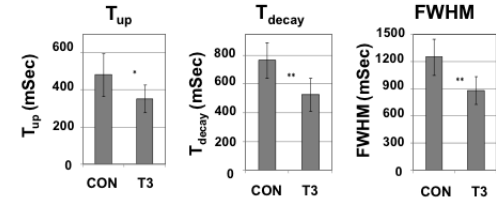




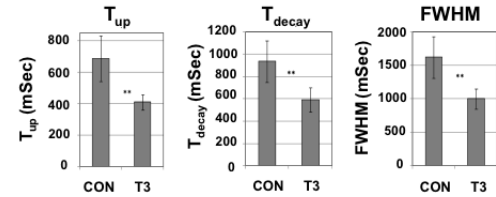
Data set #1



Data set #2



Data set #3



**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.

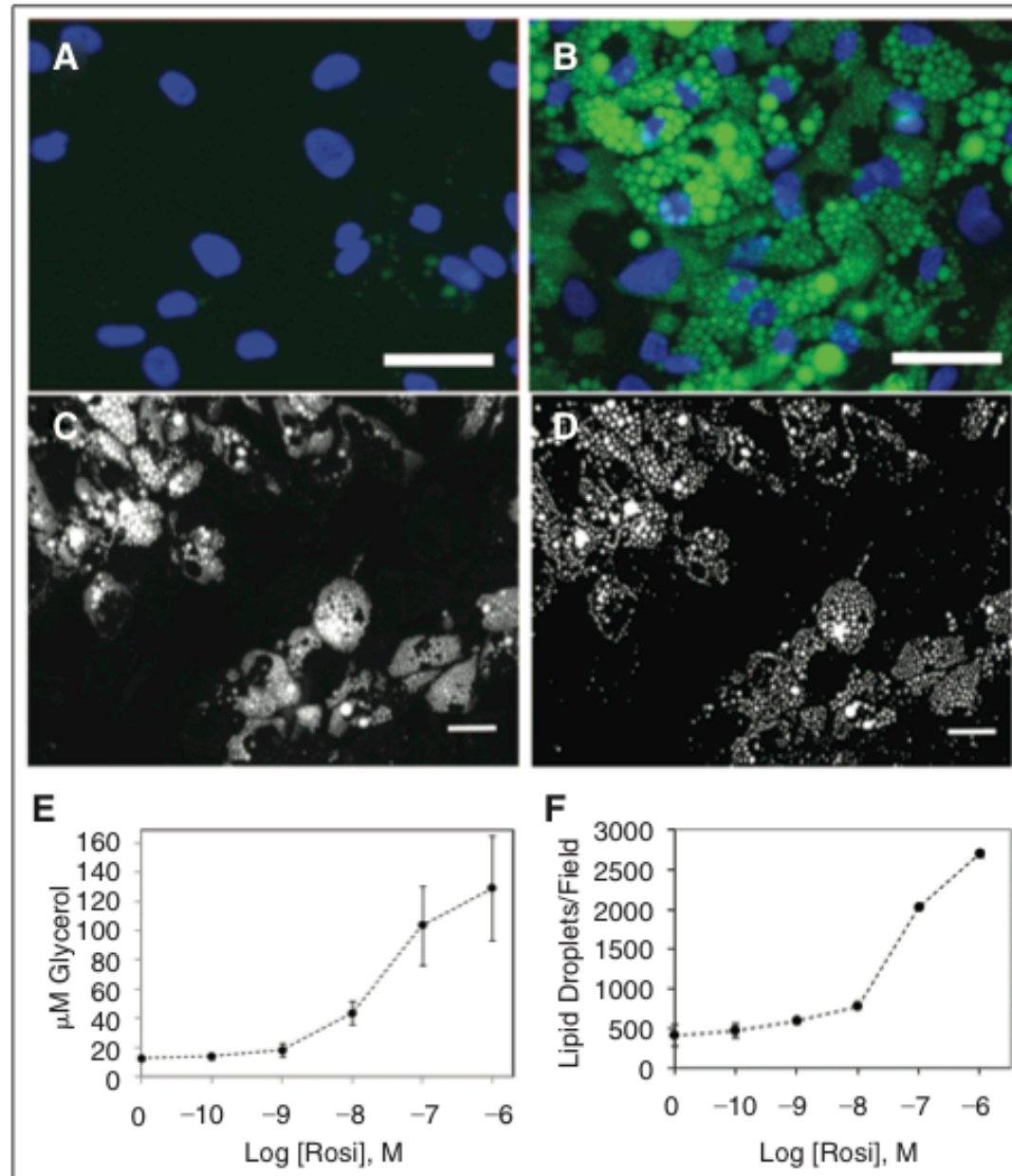
2006 NIH SBIR 1R43DK074333-01 Title: *HT Image Assay of Lipid Droplet Formation in Human Adipocytes.*

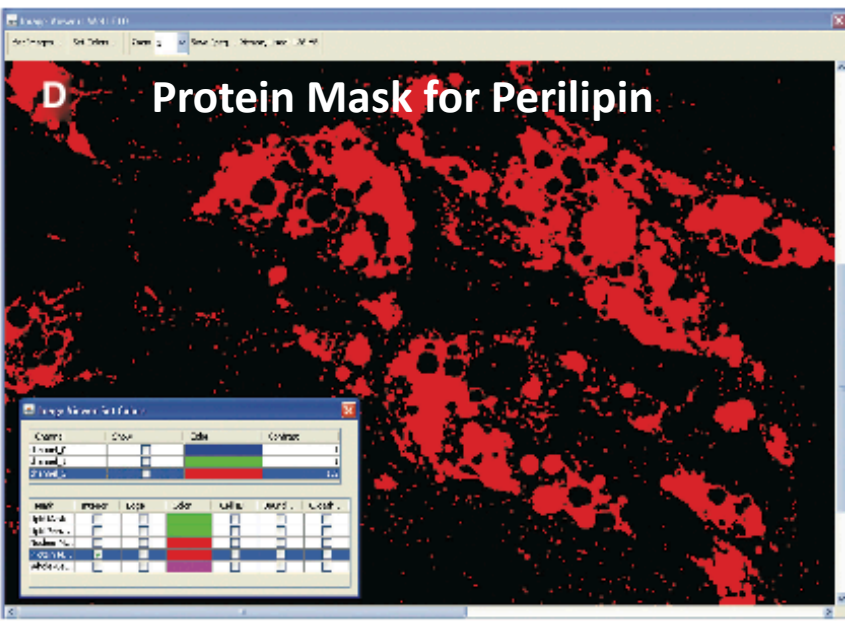
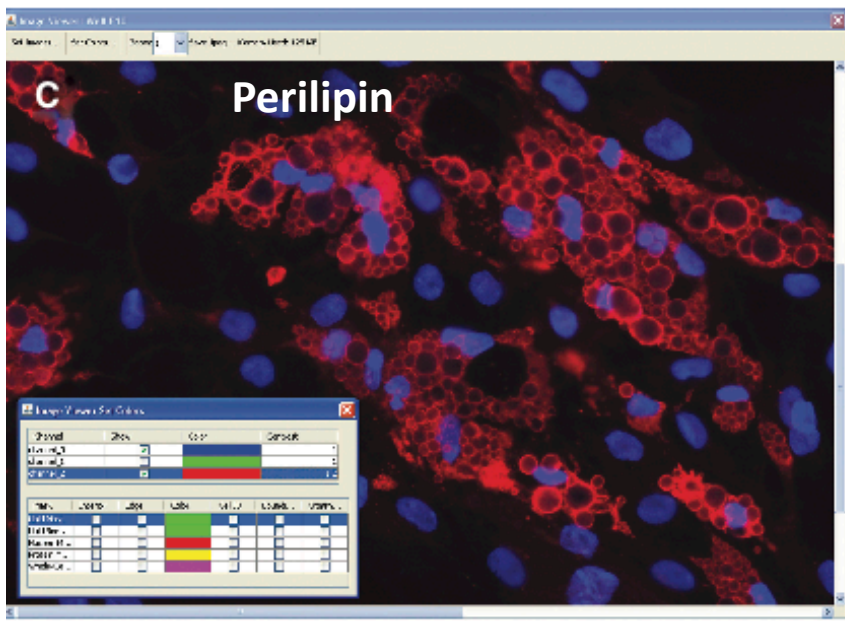
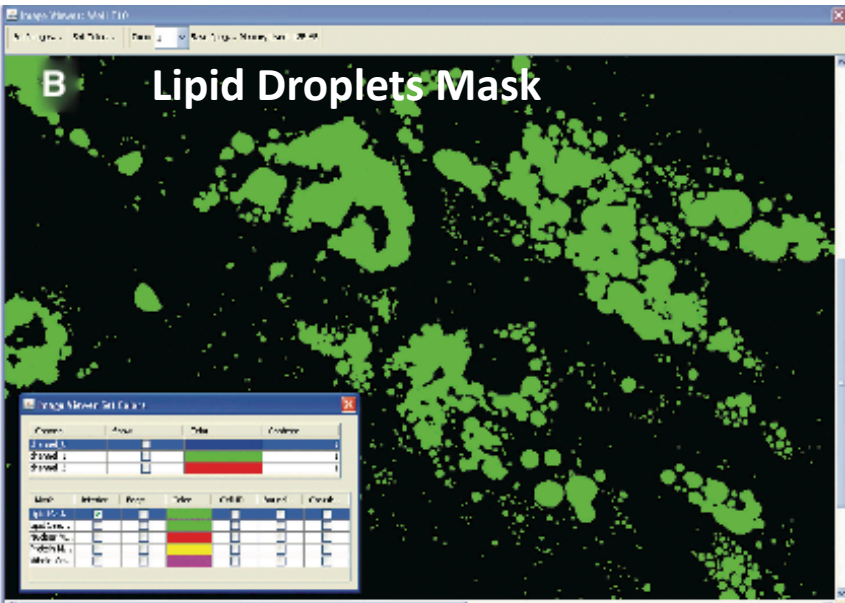
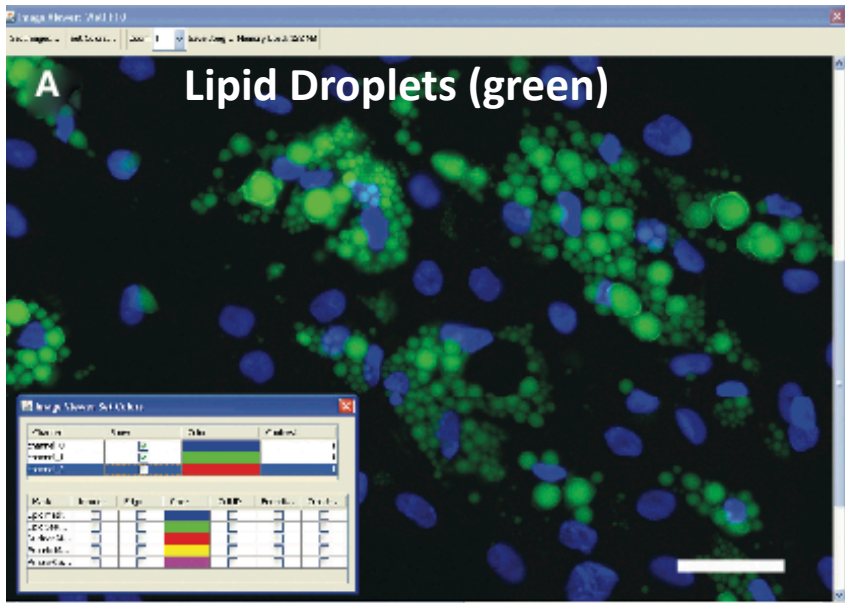
Collaborators: Zen-Bio Inc (suppliers of primary adipocytes and other cell types), Baylor College of Medicine (development of custom antibodies)

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

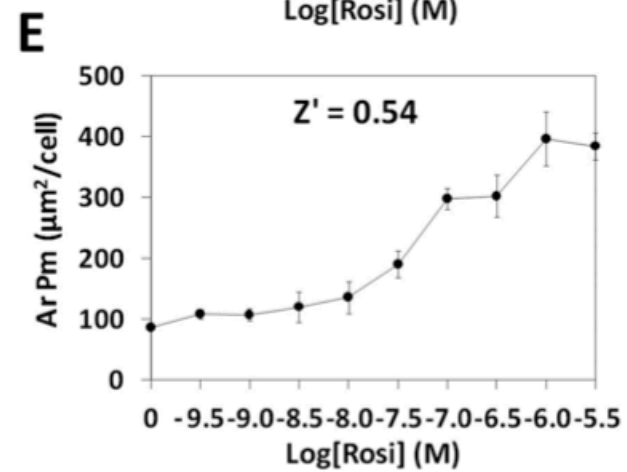
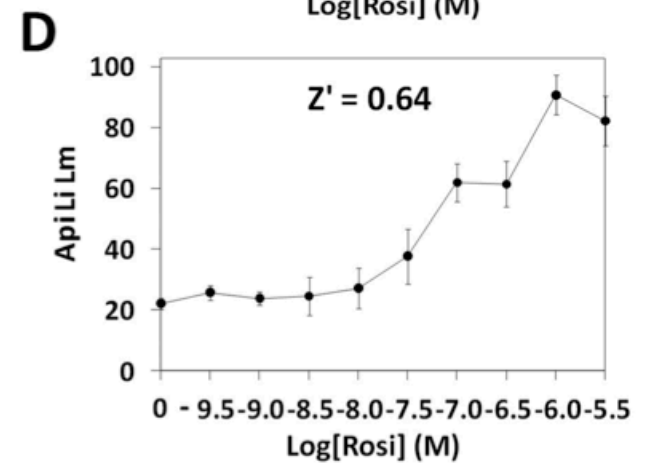
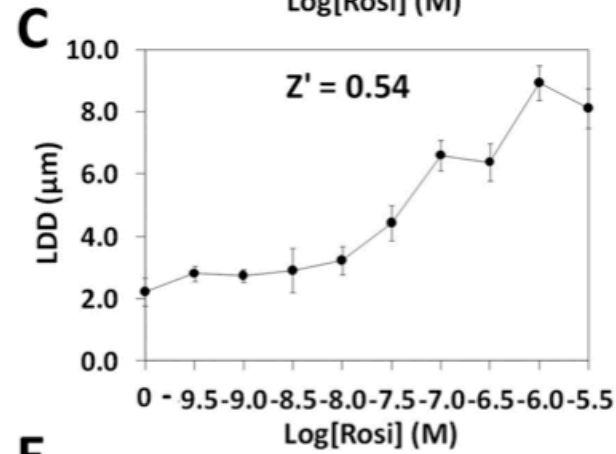
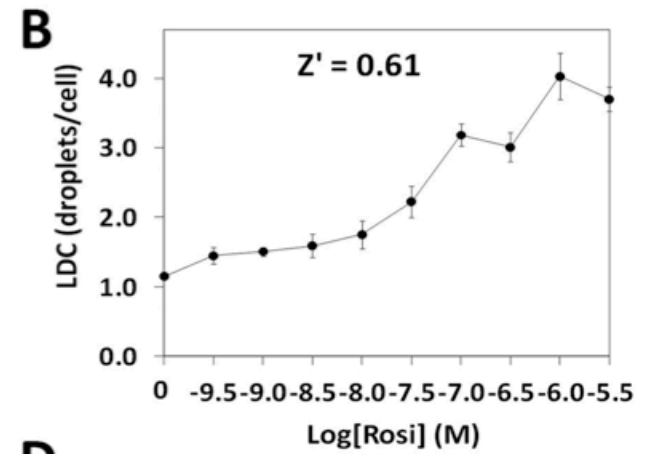
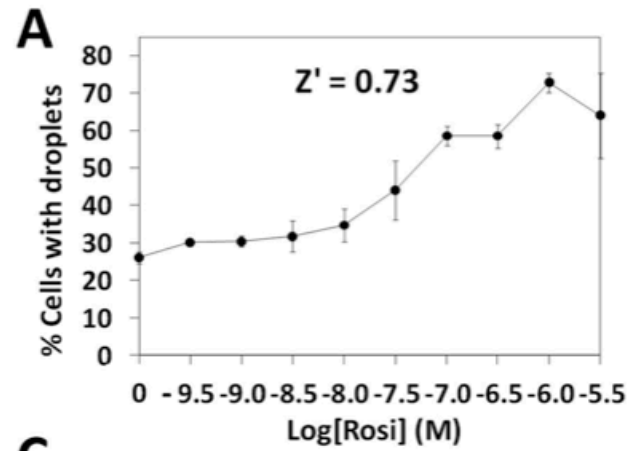
McDonough, P. M., Agustin, R. M., Ingermanson, R. S., Loy, P. A., Buehrer, B. M., Nicoll, J. B., Prigozhina, N. L., Mikic, I., Price, J. H. 2009. Quantification of lipid droplets and associated proteins in cellular models of obesity via high content/high throughput microscopy and automated image analysis. *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).





# Data from the adipogenesis assay



**Vala is offering the following assays related to  
Adipogenesis:**

**A1, Adipogenesis, Human adipocytes, agonists**

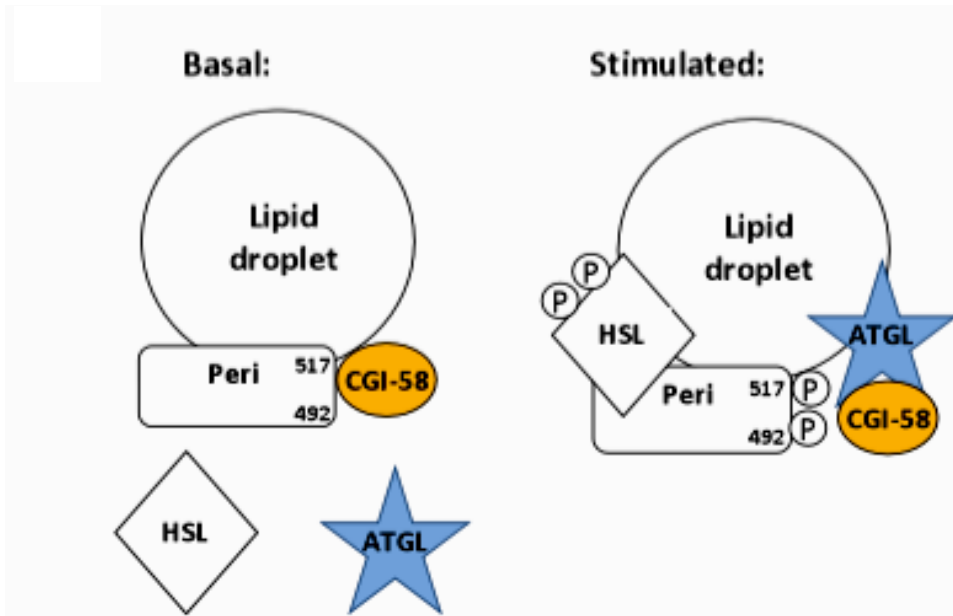
**A2, Adipogenesis, Human adipocytes, antagonists**

**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.

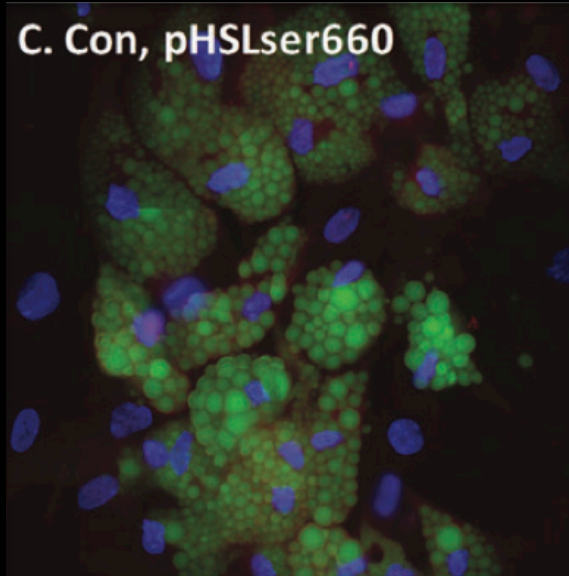
The activation of lipolysis is associated with phosphorylation of perilipin (Peri), and Hormone Sensitive Lipase (HSL). Adipocyte triglyceride lipase (ATGL) is also critically important.

Phosphorylation of perilipin attracts HSL and ATGL to the lipid droplets.

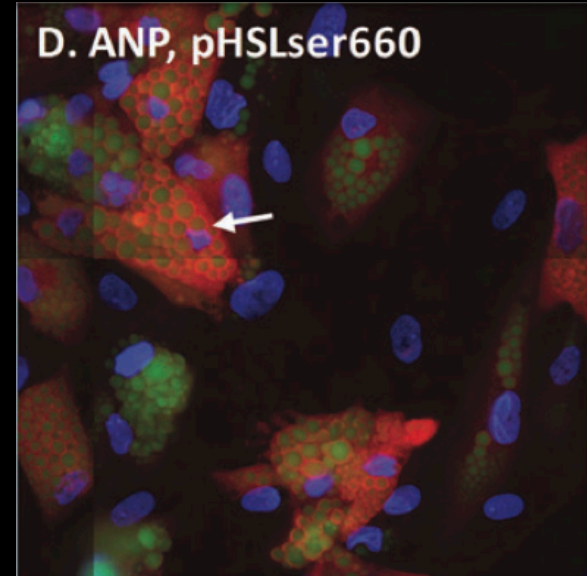


HSL and perilipin are substrates for cAMP activated protein kinase (PKA), and cGMP-activated protein kinase (PKG)

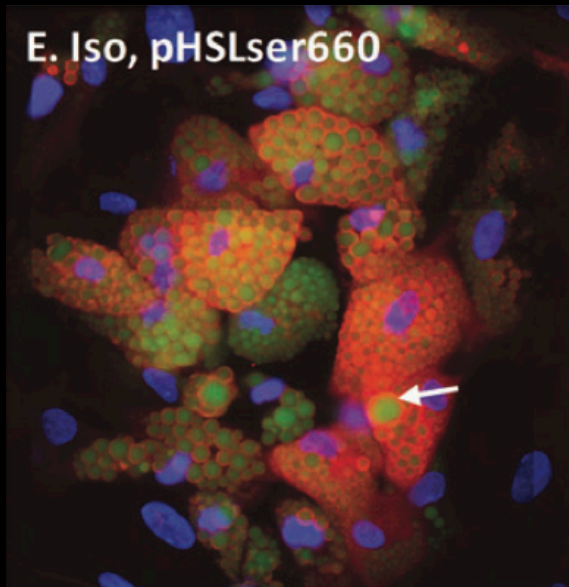
C. Con, pHSLser660



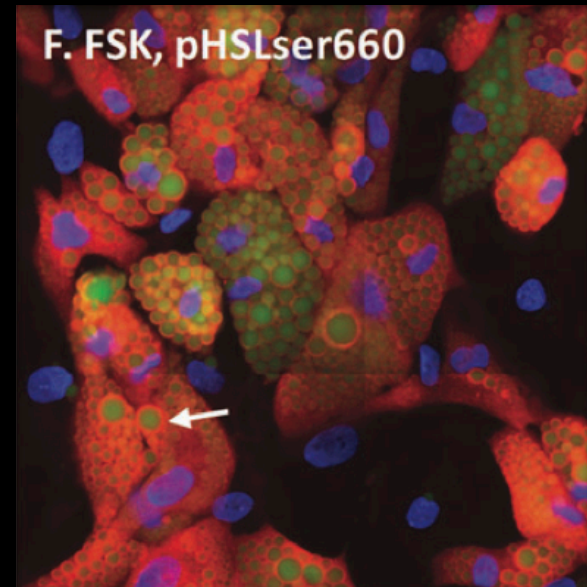
D. ANP, pHSLser660



E. Iso, pHSLser660



F. FSK, pHSLser660





**A** Vala's antibodies to phospho-perilipin

**Con**

**B** P-Peri ser497

**FSK**

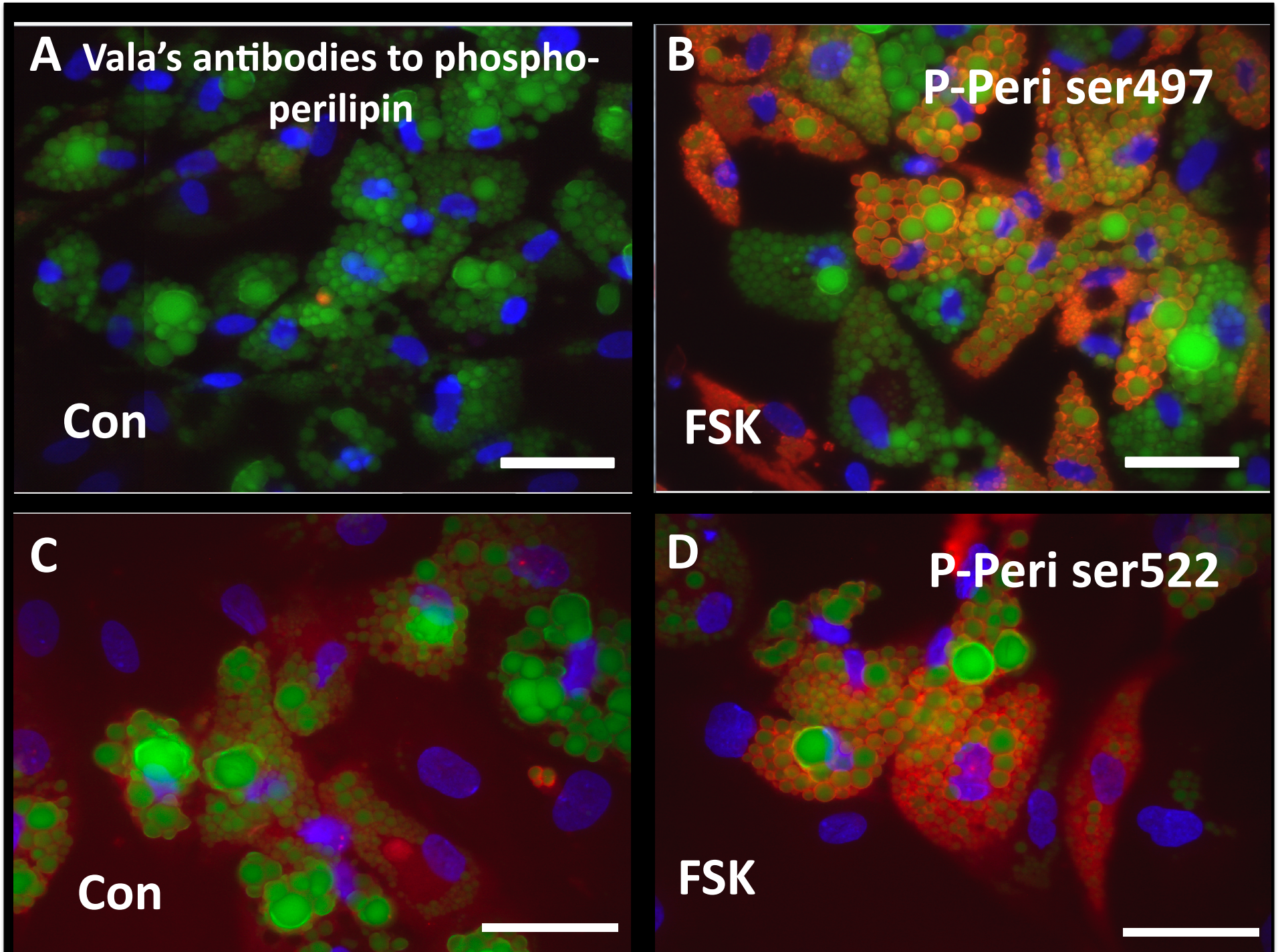
**C**

**Con**

**D**

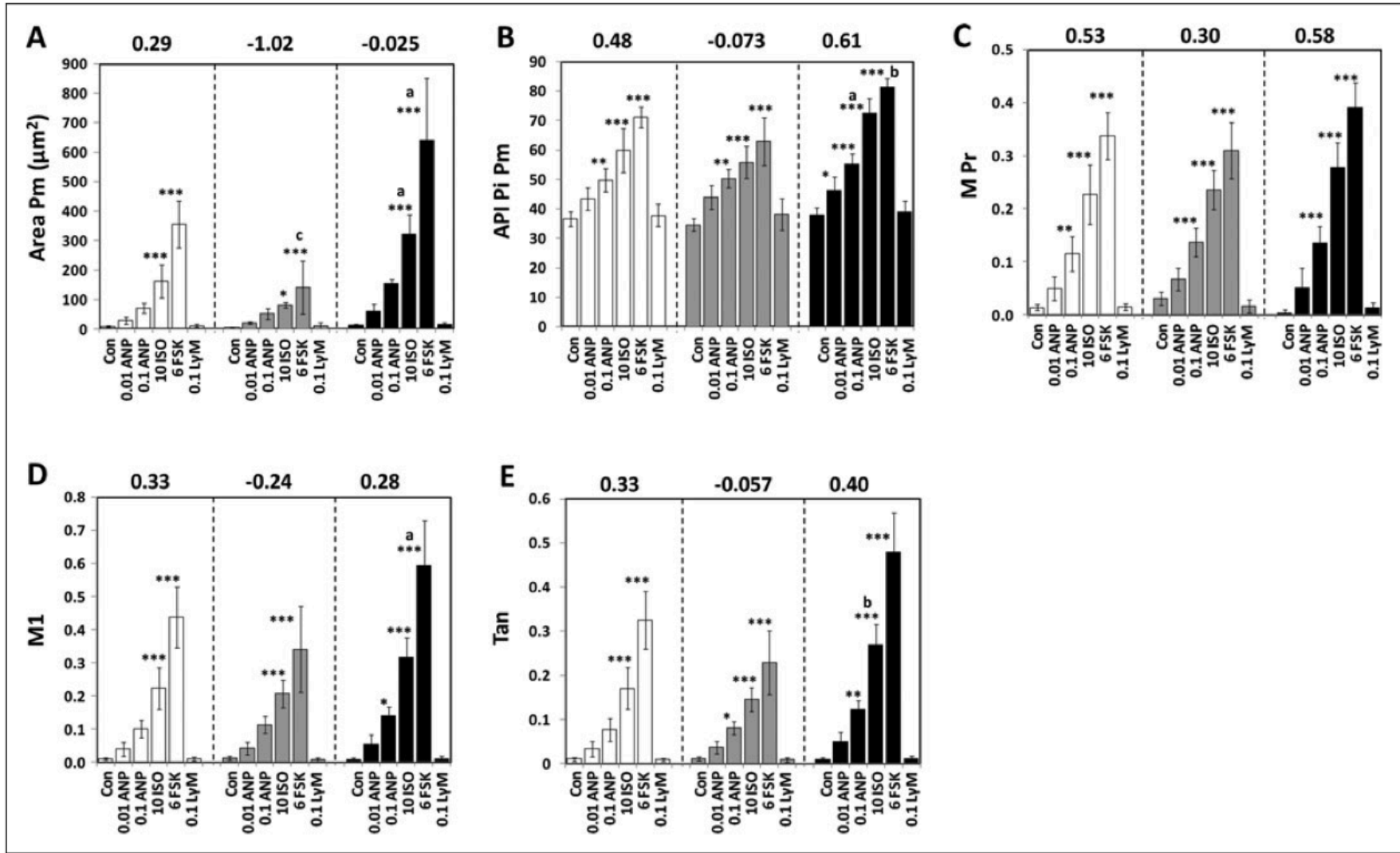
P-Peri ser522

**FSK**





# Results from Vala's HCA-based lipolysis assay:



**Vala is offering the following assays related to  
Lipolysis:**

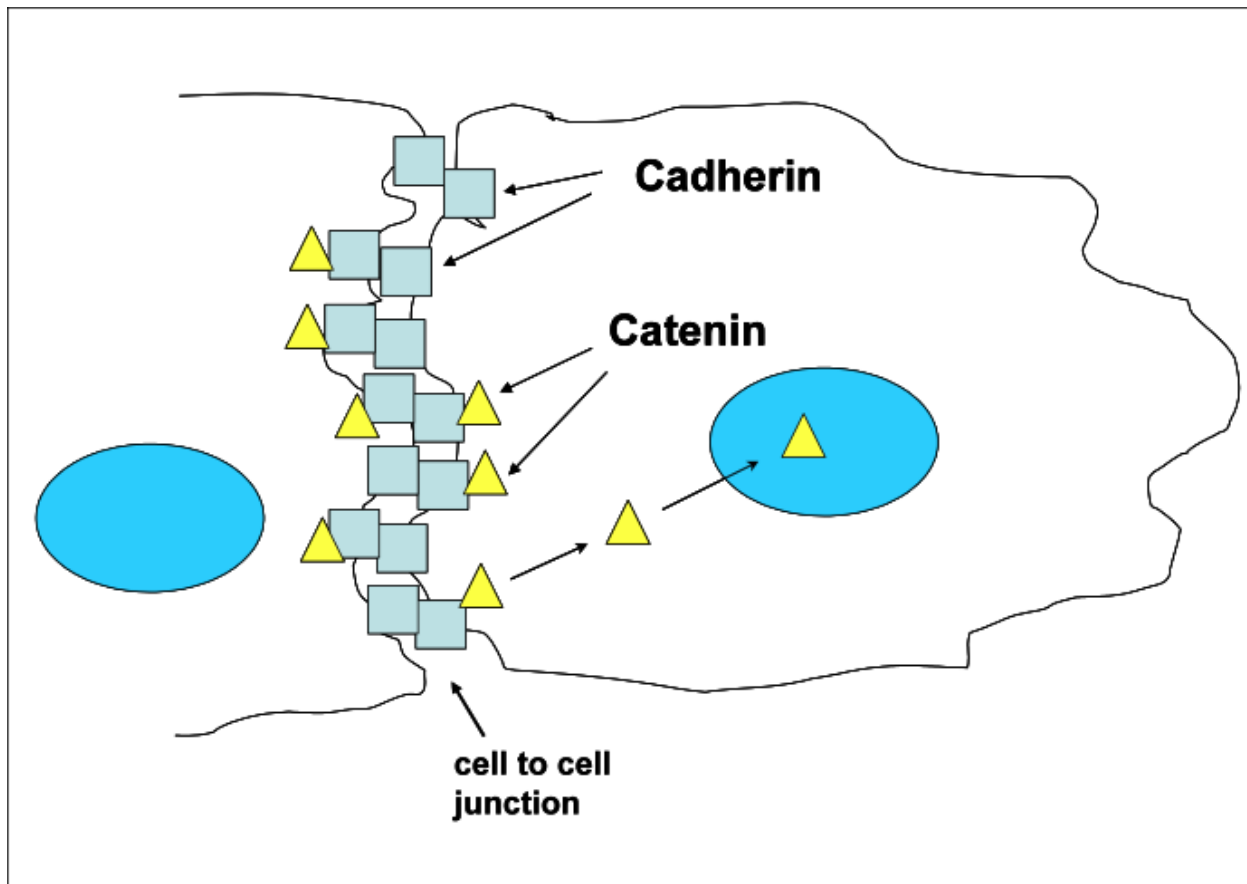
**L1, Lipolysis, Human adipocytes, agonists**

**L2, Lipolysis, Human adipocytes, antagonists**

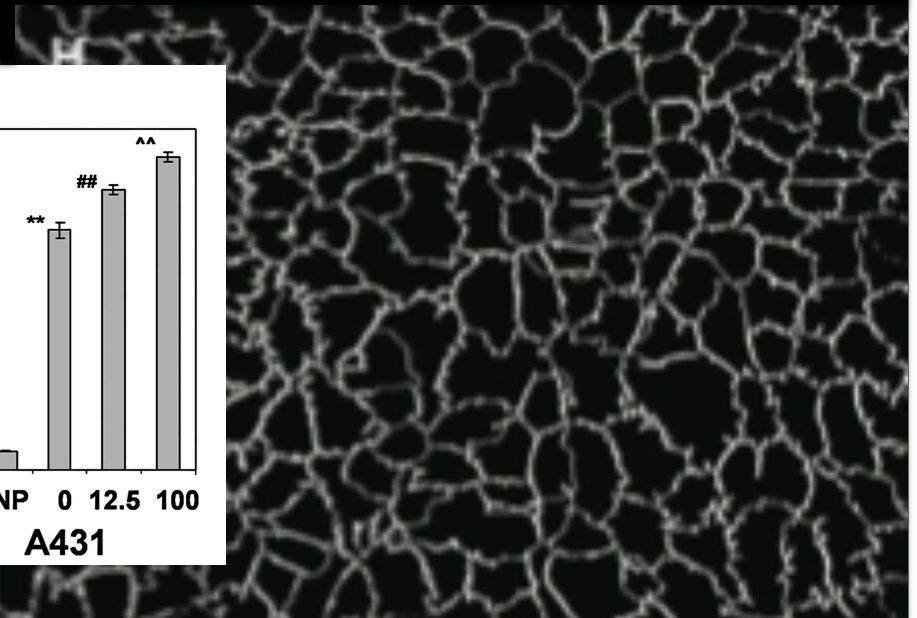
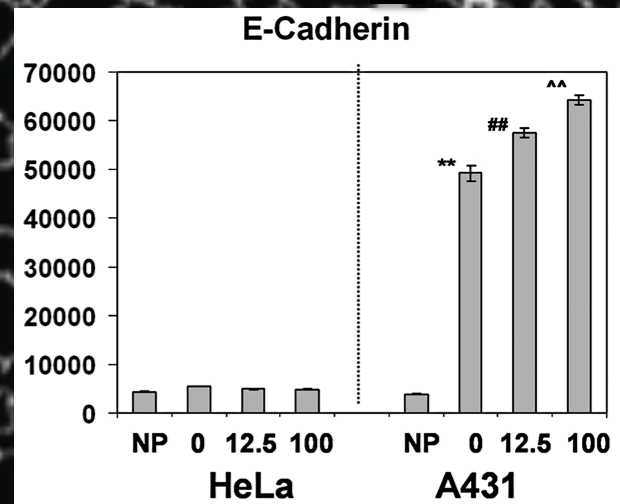
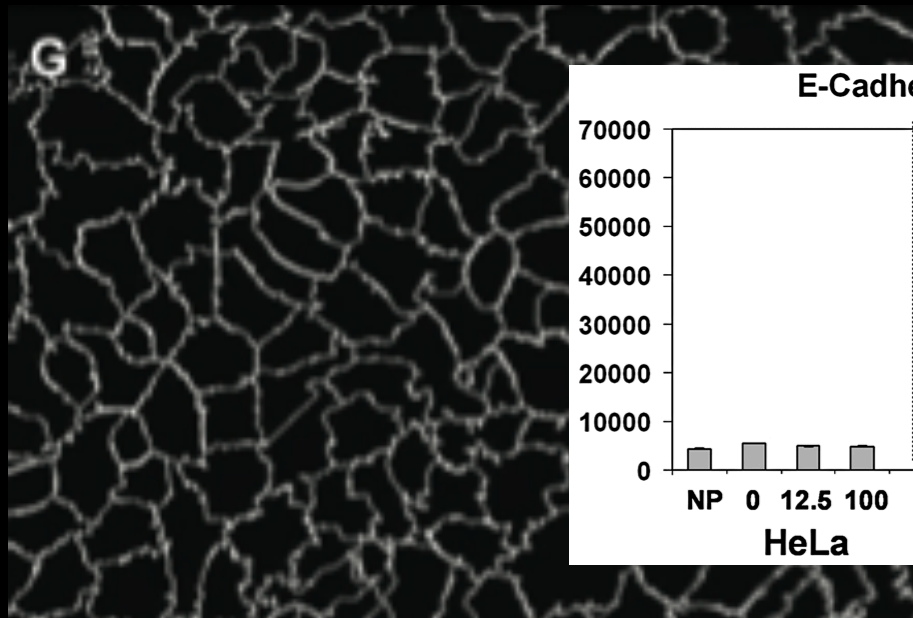
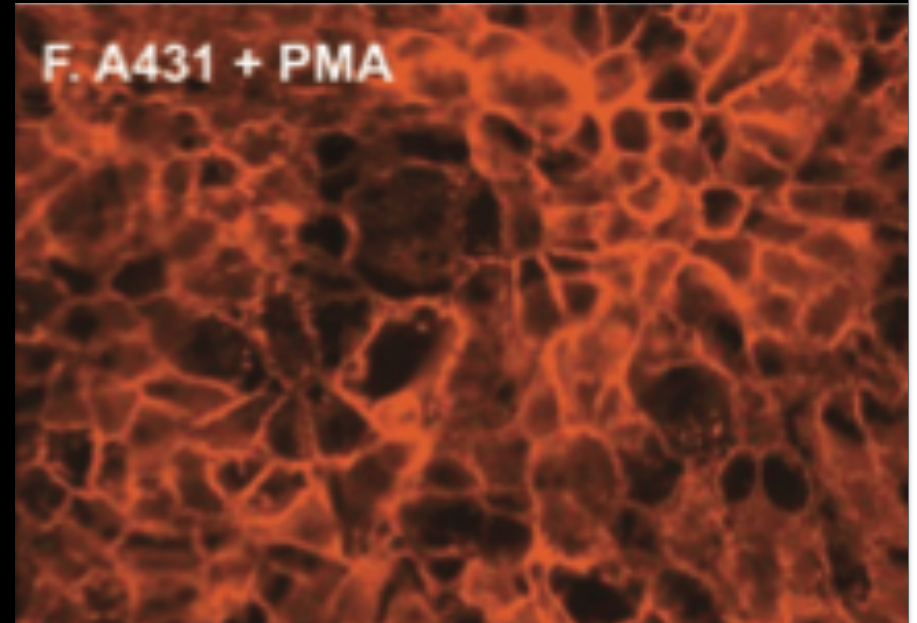
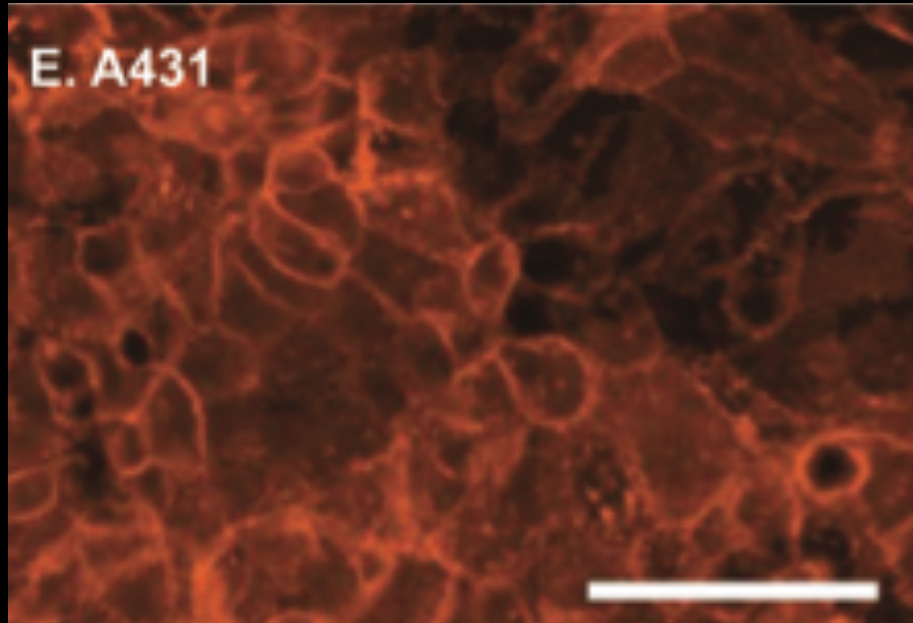
**L3, Lipolysis, murine 3T3L1 adipocytes, agonists**

# 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

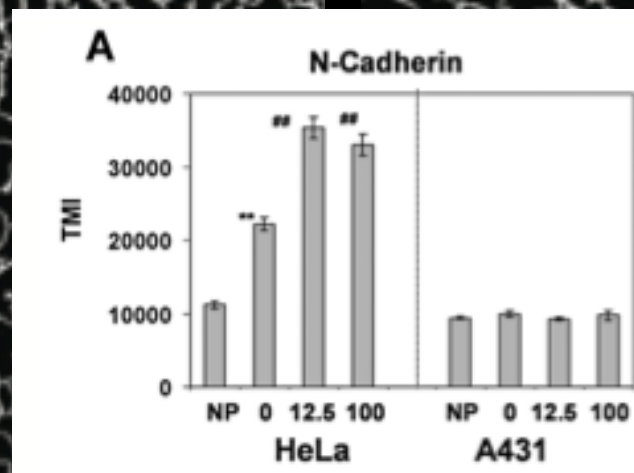
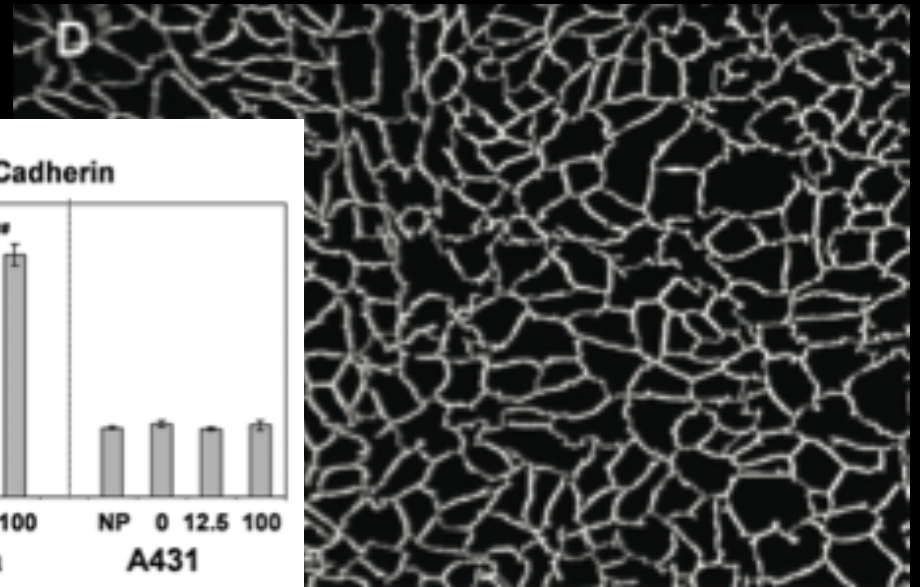
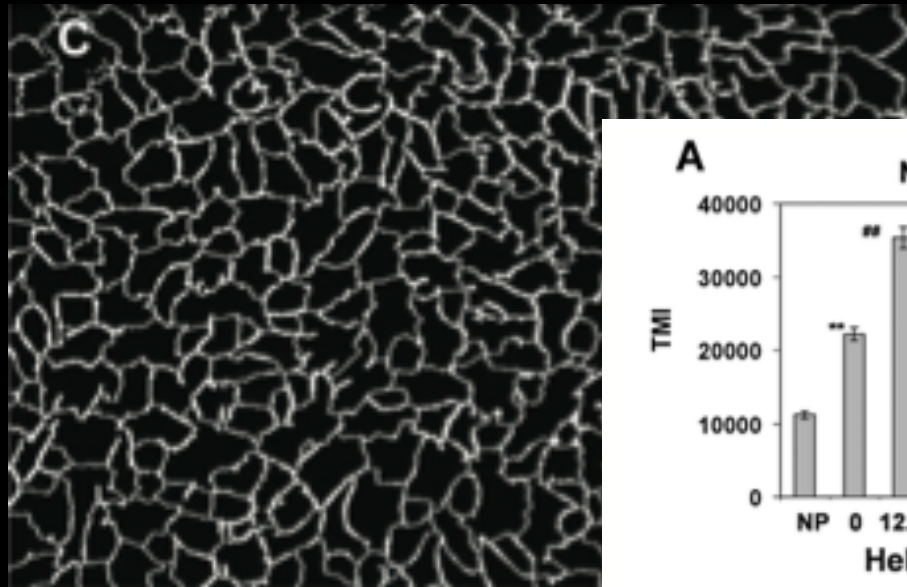
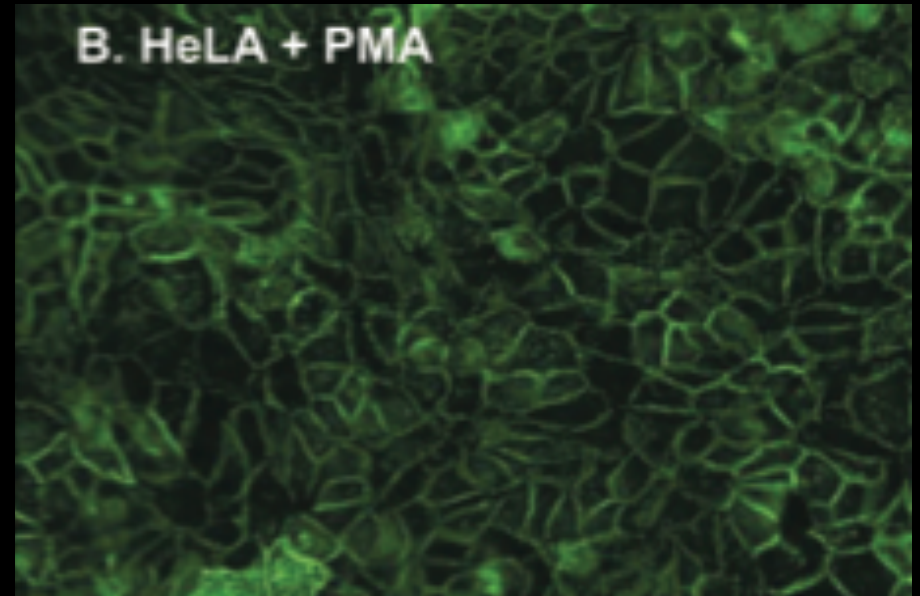
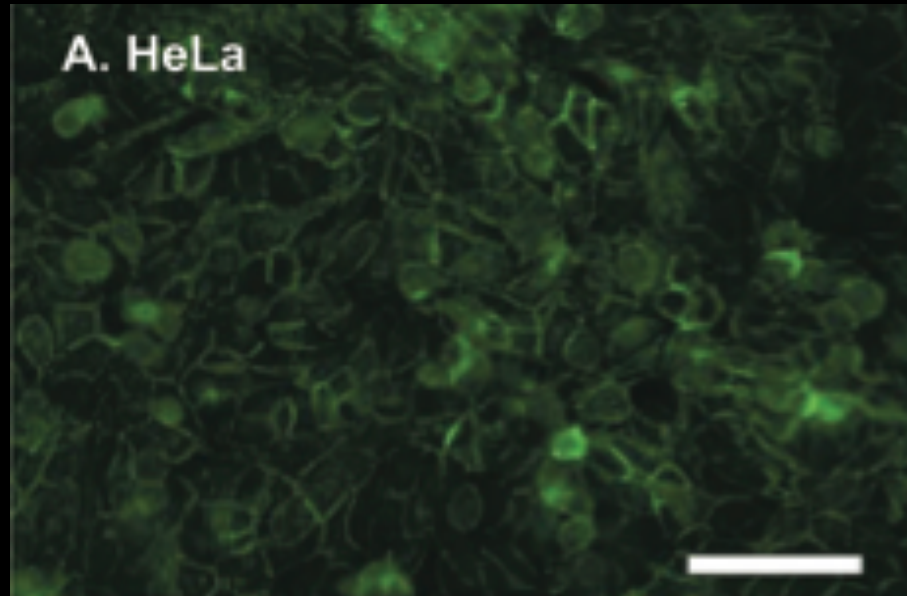


# E-Cadherin



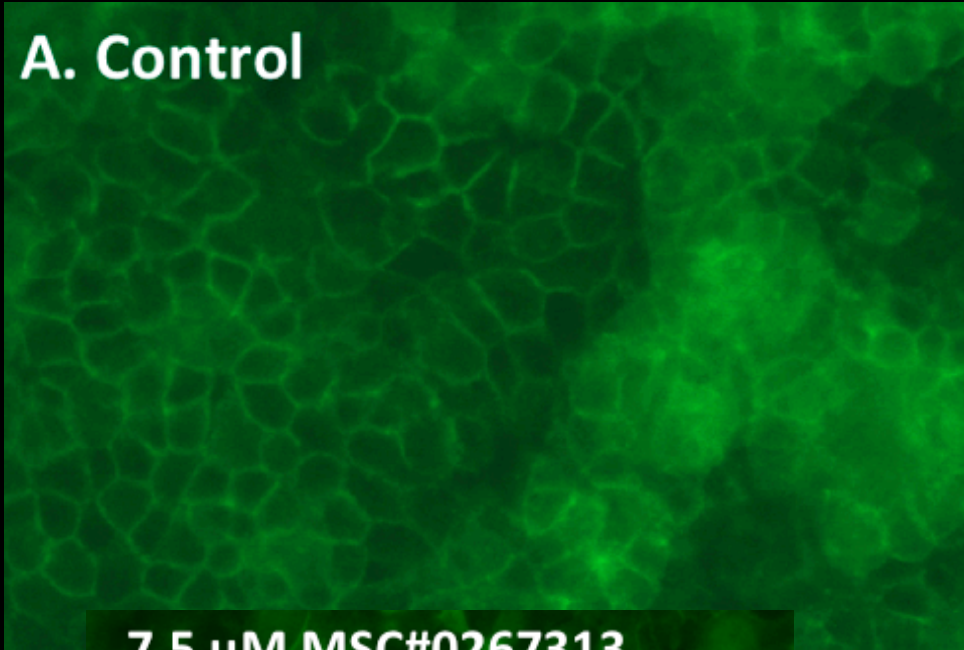


# N-Cadherin

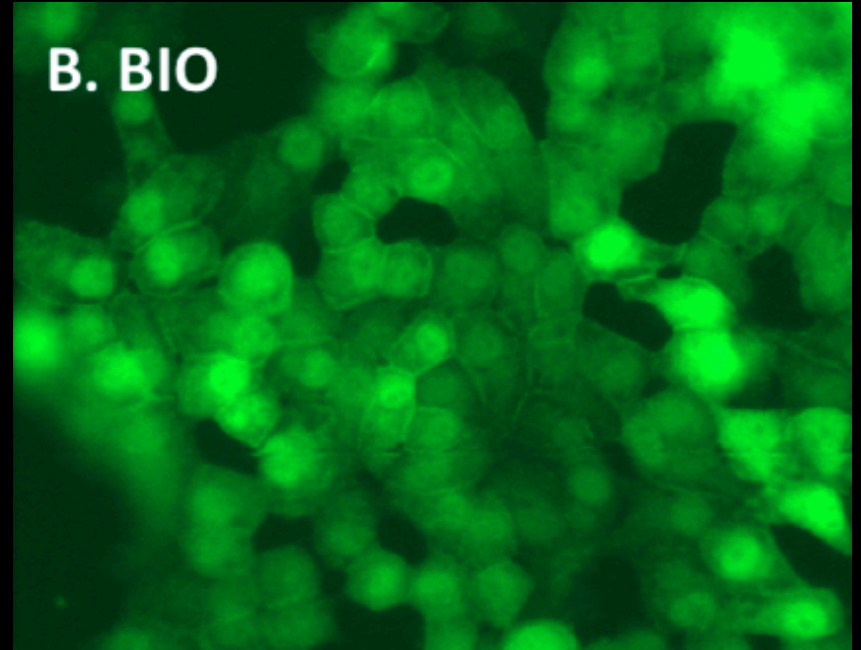


# Beta-Catenin

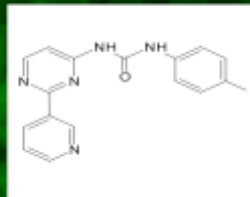
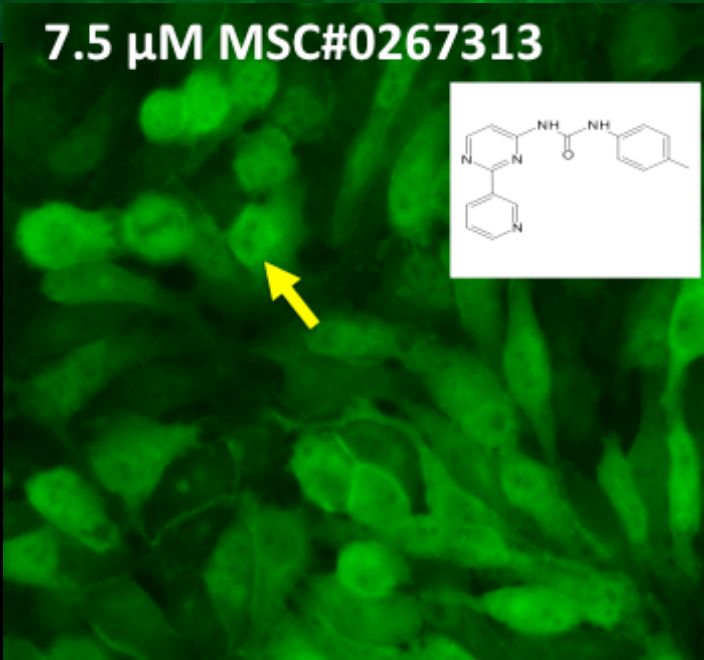
A. Control



B. BIO



7.5  $\mu$ M MSC#0267313



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

**ECAD1, E-Cadherin, A431 cells, agonists**

**NCAD1, N-Cadherin, HeLa cells, agonists**

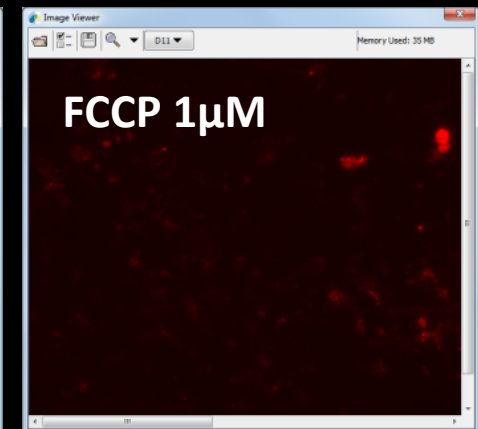
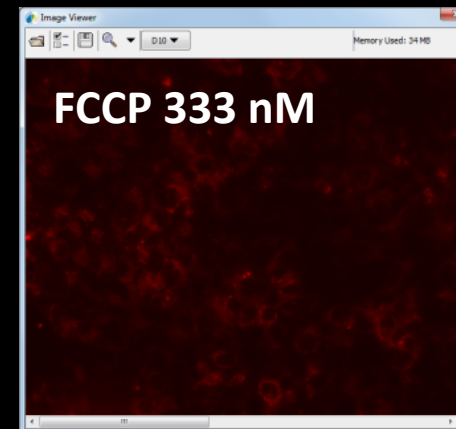
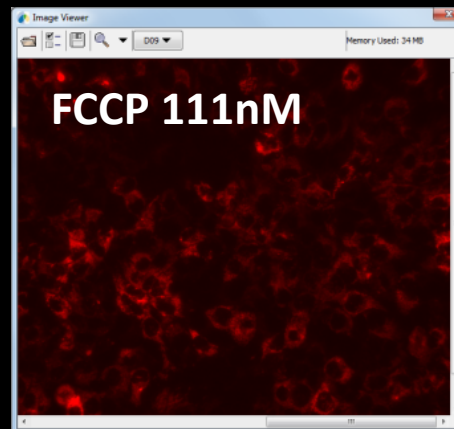
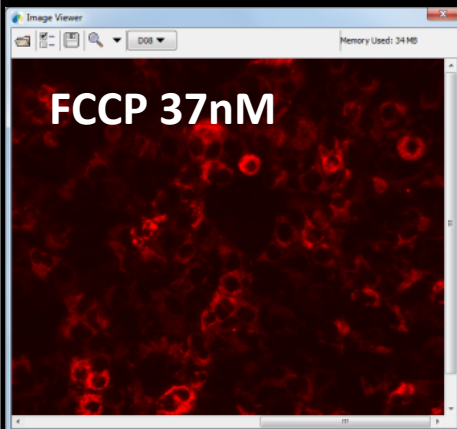
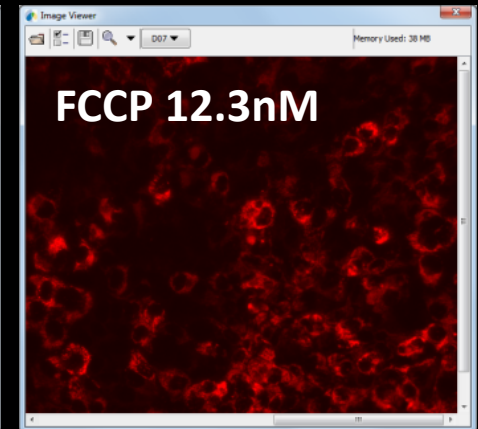
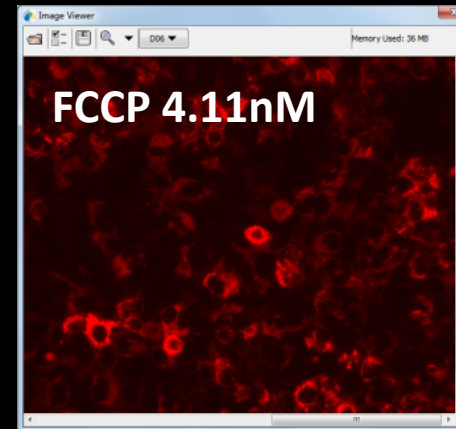
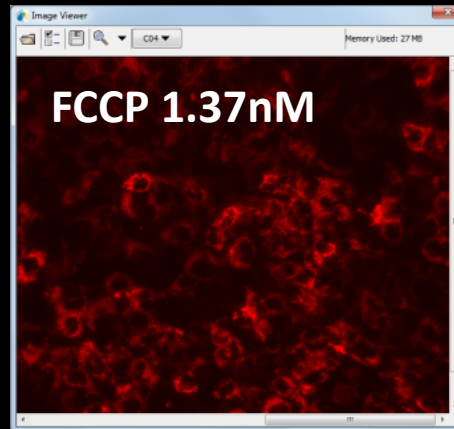
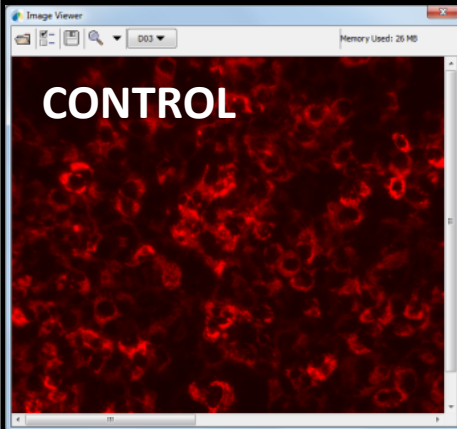
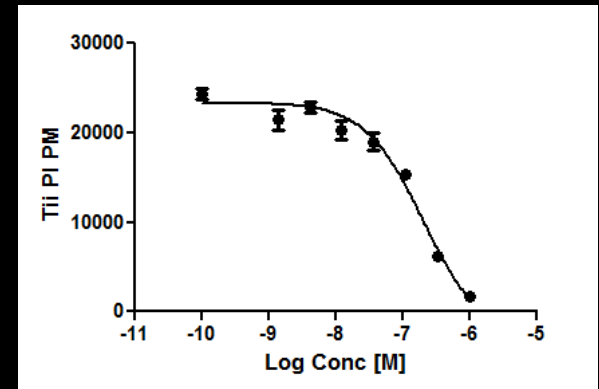
**VCAD1, VE-Cadherin, Human vascular endothelial cells,  
agonists**

**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**

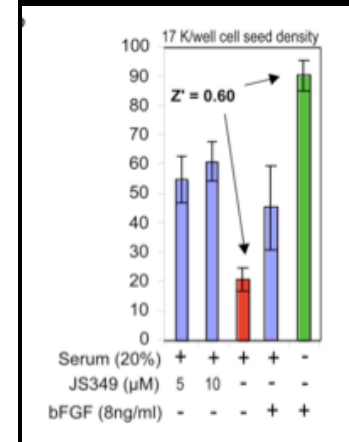
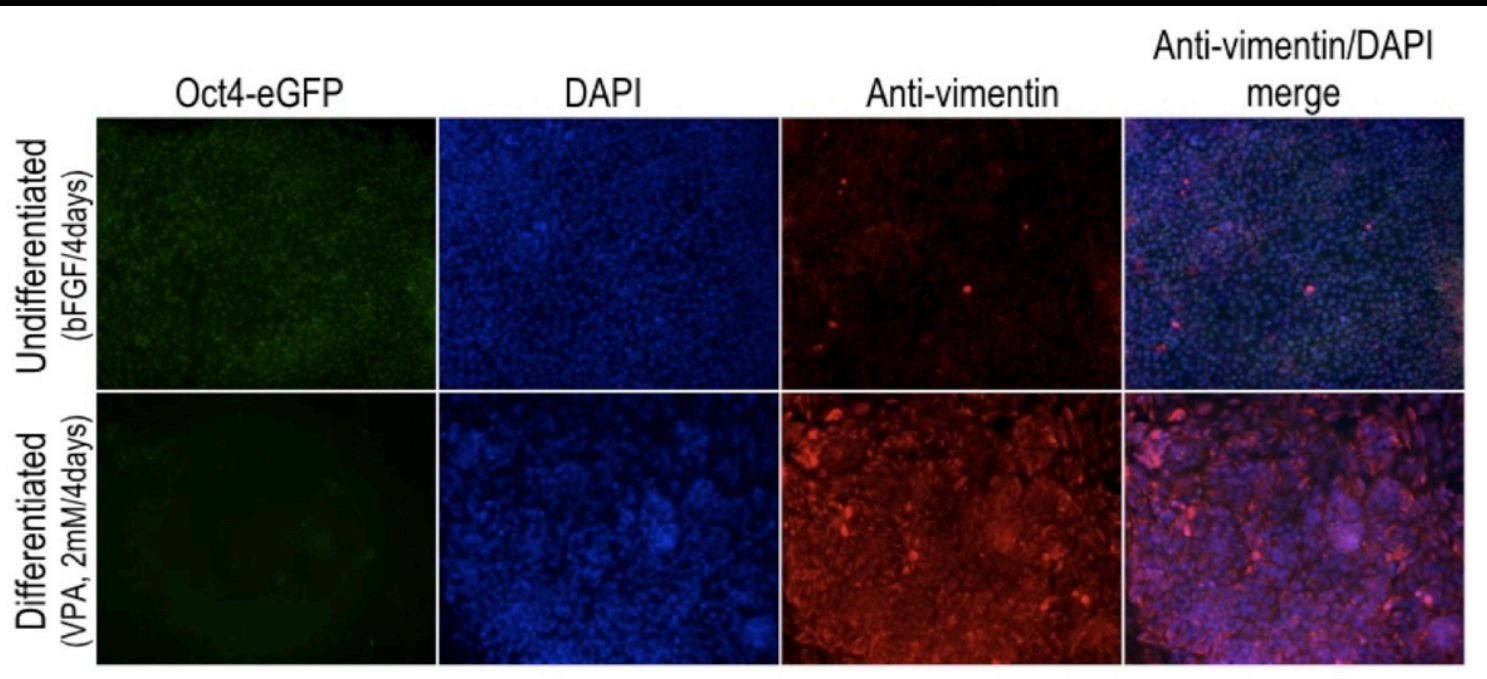
**MP2 Mitochondrial membrane potential, Huh-7  
cells, long term**



# Stem cell pluripotency

Mark Mercola

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



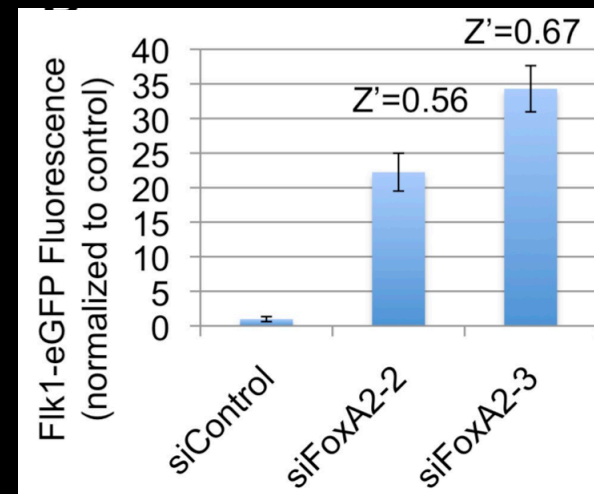
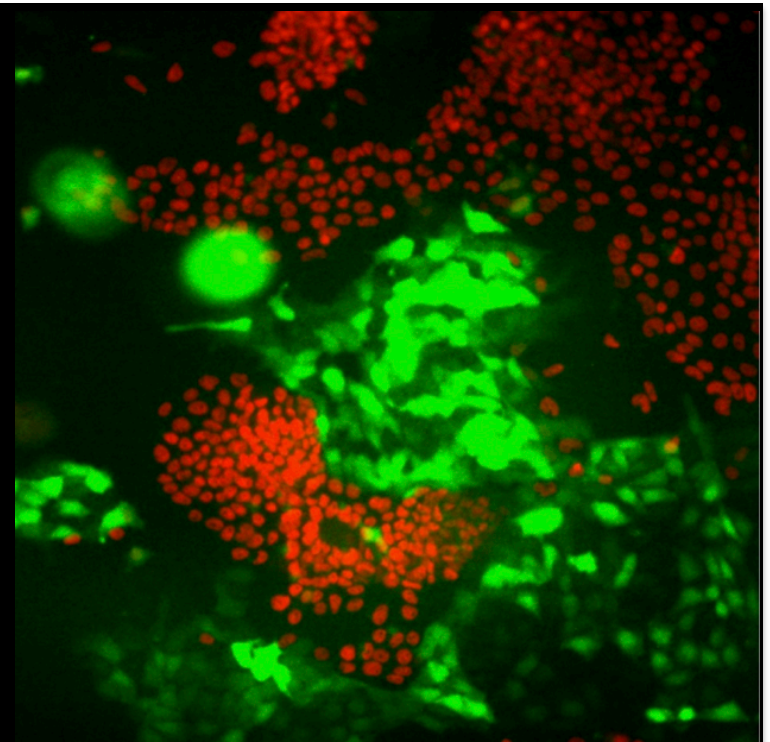


Mark Mercola

# 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 embryonic stem cell-derived mesoderm. Circulation Research 109:360-3641, Mesoderm endoderm formation, mouse embryonic stem cells**





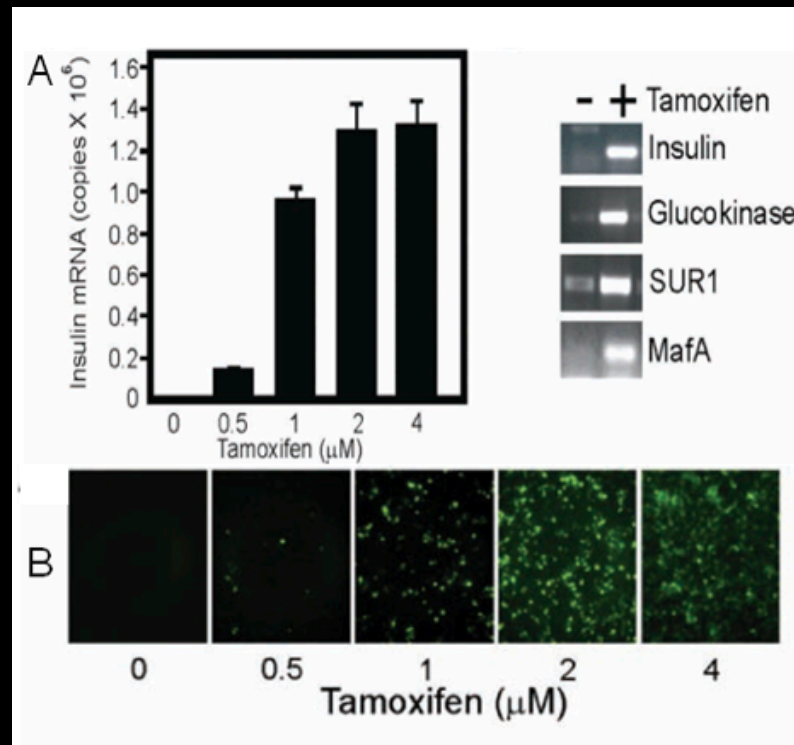
Pam Itkin-Ansari

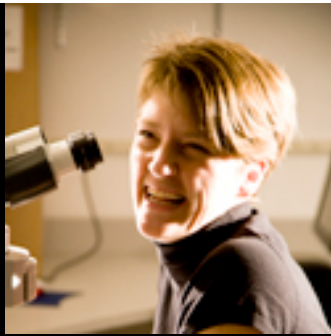
# Pancreatic beta cell differentiation/ maturation

## 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).





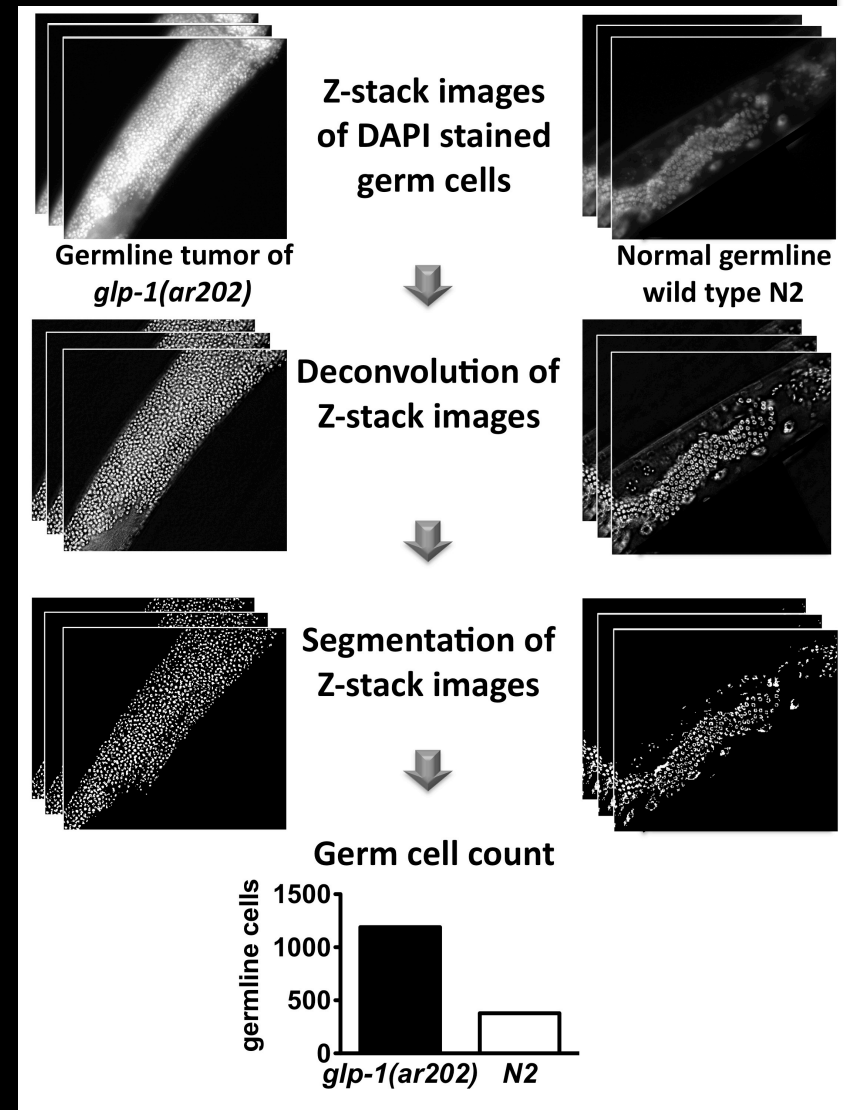
Malene Hansen

# 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**



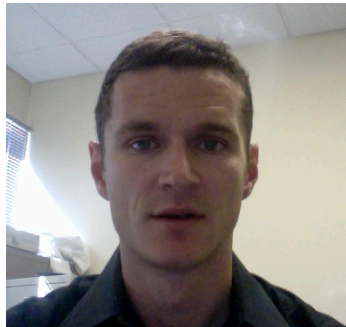
**Ramses Agustin**



**Claire Weston**



**Robyn Garcia**



**James Evans**



**Ross Whittaker**



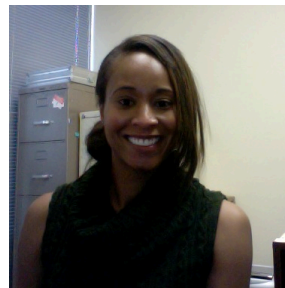
**Piyush Gehalot**



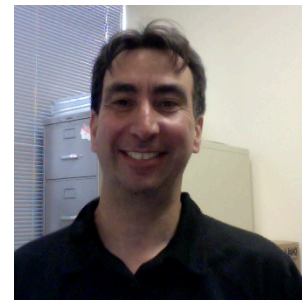
**Mike Markoudakis**



**Emily Arsenault**



**Constance Allison**



**Jeff Hilton**