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THE TEAM



Laboratory Technicians

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Hind Azegrouz
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PhD students

Carmen Muñoz-Agudo

THE CELLOMICS UNIT

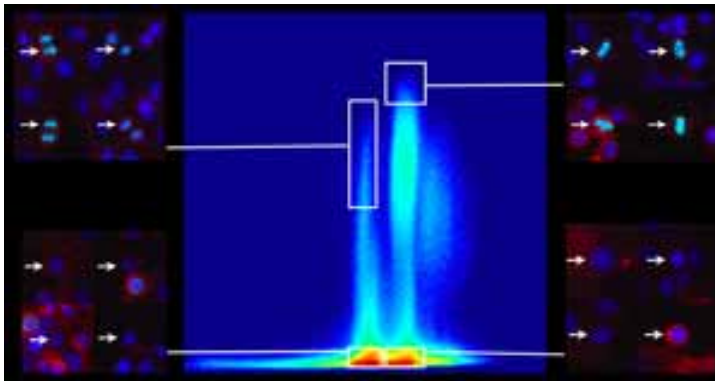
Who are we and what do we do:

- The Cellomics Unit is a core facility at CNIC
- Provides the CNIC with the two principal cell analytical techniques; flow cytometry and high content screening (HCS)
- Supports quantitative image-based research.
 - Flow Cytometry
 - High Content Screening
 - Image Analysis.
 - HCS
 - Biomedical Imaging

WHAT WE DO

High Content Imaging

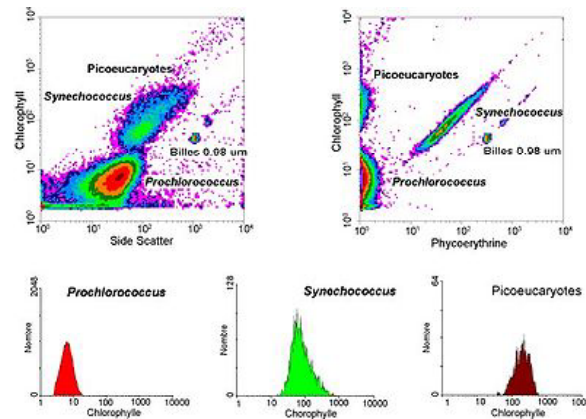
- Adherent/suspension Cells



- Quantitable
- Automatable
- Medium-large throughput
- Cell population studies
- Spatial Information (subcell Localization)

Flow Cytometry

- Cells in suspension



- Quantitable
- Automatable
- Medium-large throughput
- Cell population studies
- Fast

GENOMIC (siRNA) SCREENING

Loss of function genomic screening: Identify gene function through inactivation of a gene or its corresponding mRNA

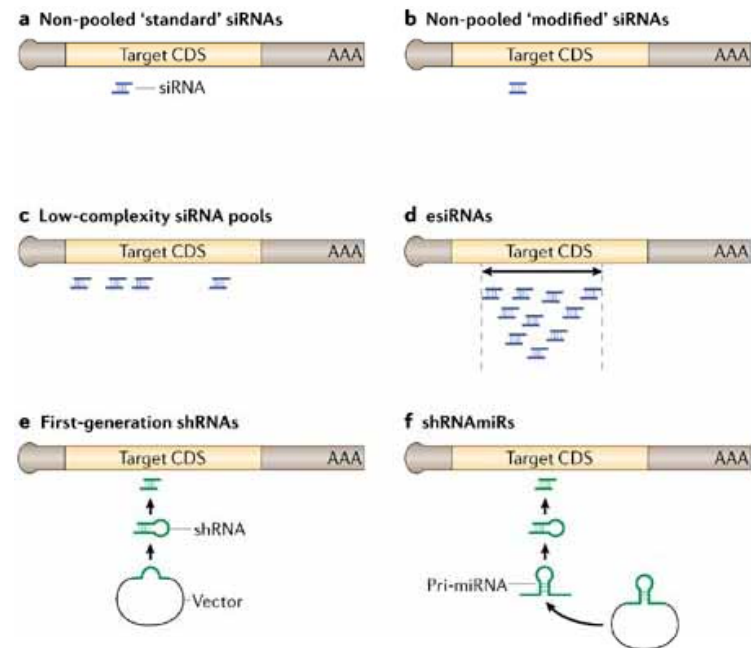
Genome Wide siRNA libraries; Large scale screens

- Human On target Plus Genome Wide Collection (sets of 4 individual siRNAs/gene)

- Mouse siGenome wide collection (sets of 4 individual siRNAs/gene)

(Dharmacon)

Approaches to RNAi mediated gene knock down in mammalian cells

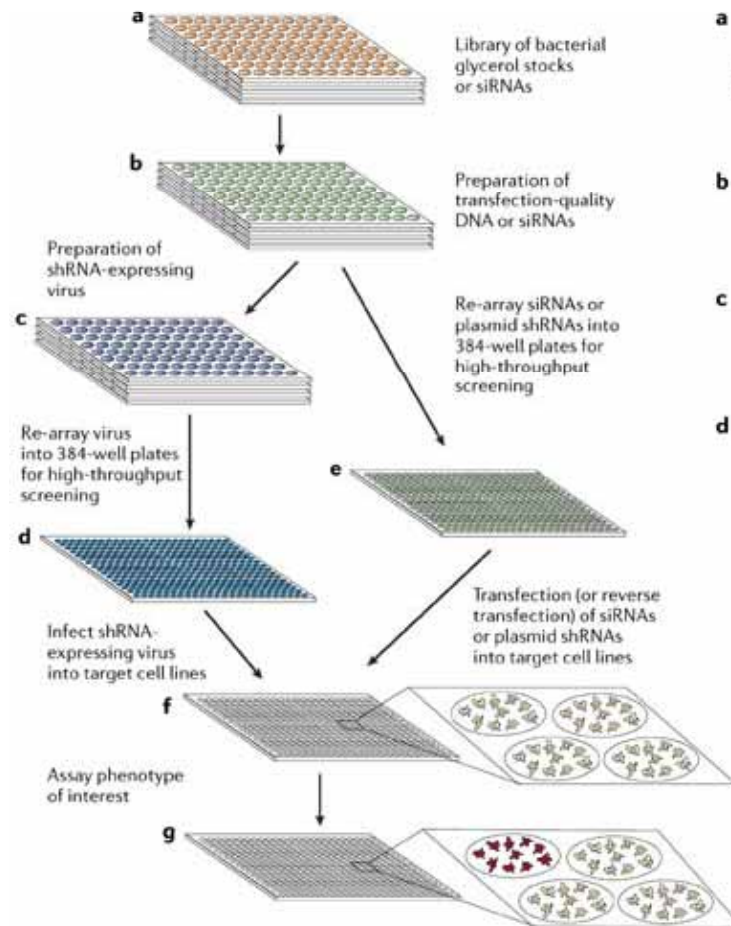


Copyright © 2006 Nature Publishing Group

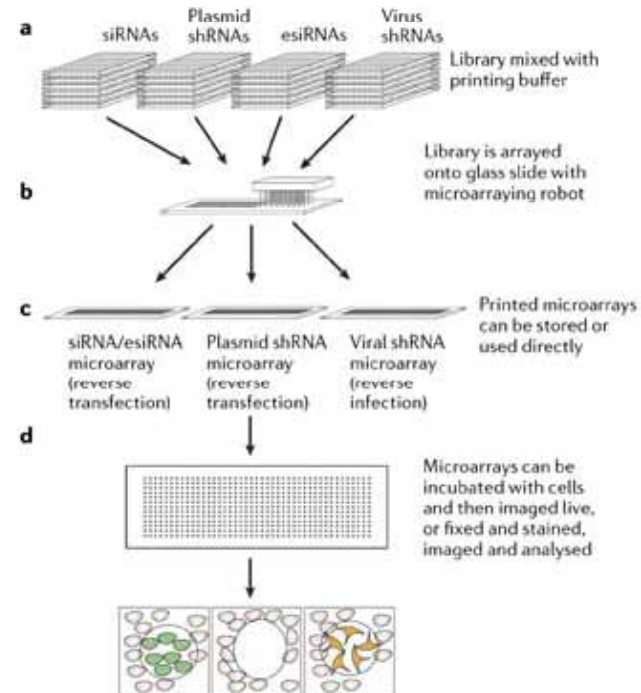
Nature Reviews Genetics
Echeverri & Perrimon. *Nature Reviews Genetics*,
published online 11 April 2006 | doi:10.1038/nri1836

GENOMIC (siRNA) SCREENING

A Multi-well-plate-based RNAi



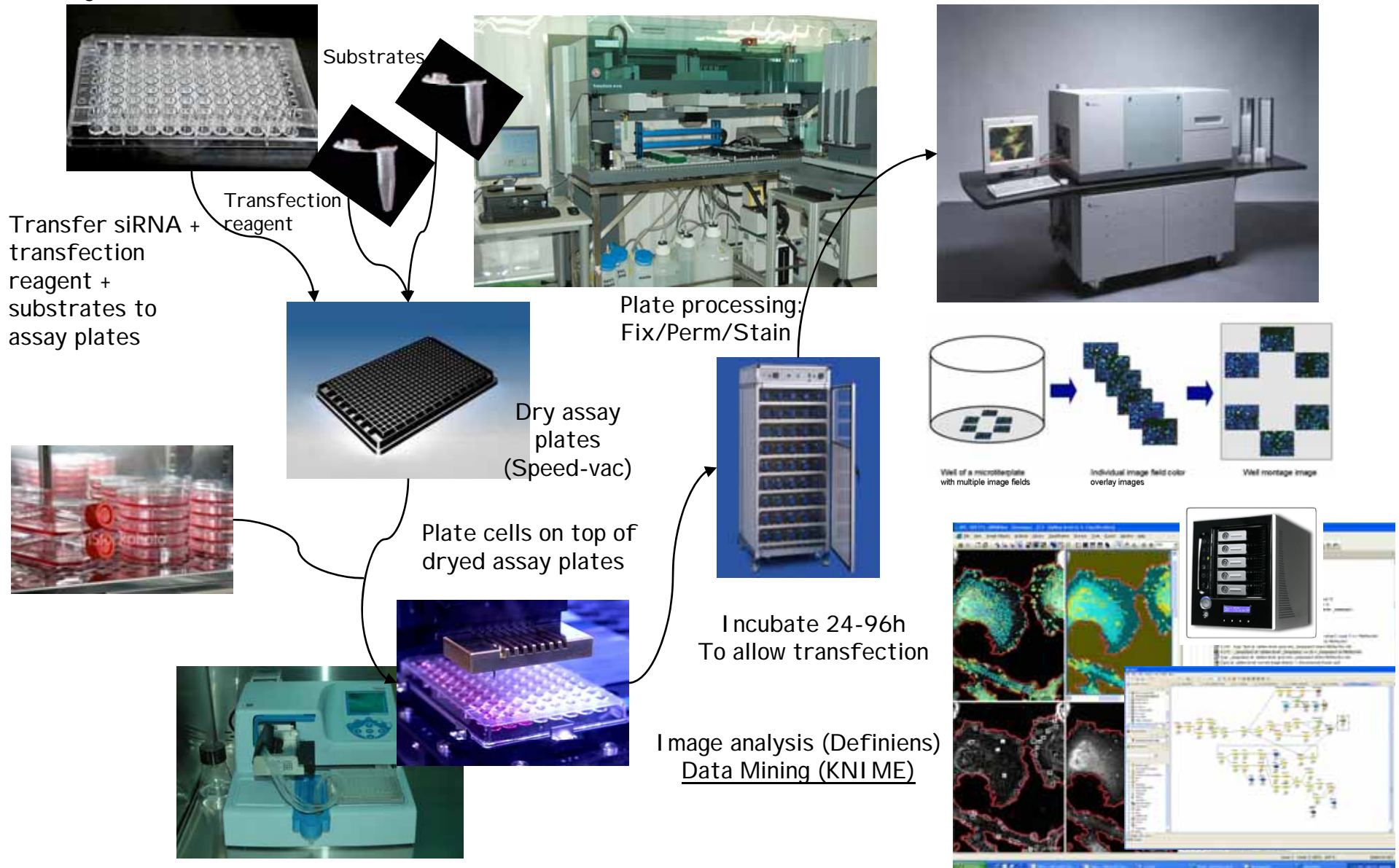
B RNAi-based cell microarrays



High Content Screening of siRNAs collections

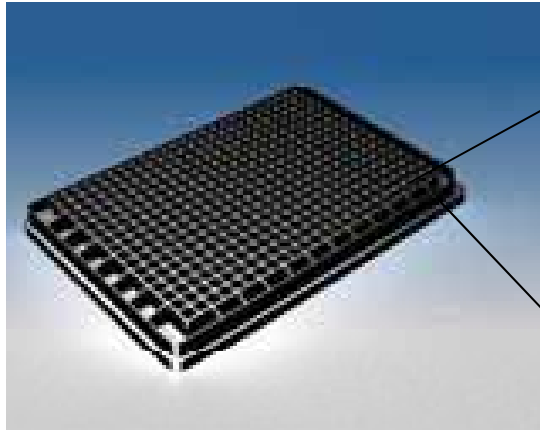
siRNA genome wide collection (Human/mouse)
18300 genes (sets of 4 siRNAs Dharmacon)

High content Imaging

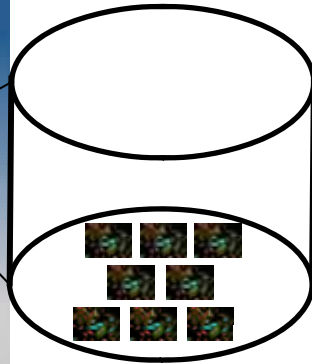


CELL LEVEL ANALYSIS

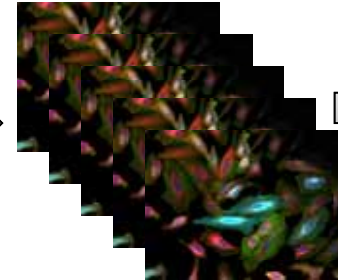
Plate



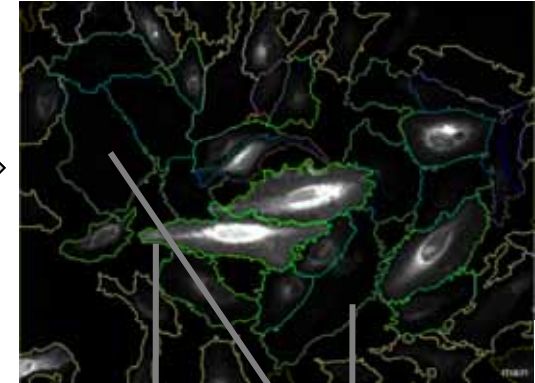
Well 384 X



Field 10 X



Cell 10-20 X



100 X

Feature extraction: Phenotypic descriptors

Cell level analysis

GFP+Cell

GFP-Cell

Cell classification & Evaluation

Row ID	Group	Group Name	Class Name	Dx	Dy	Dz	Dx_2D	Dy_2D	Dz_Max	Dz_Min	
119	HT pep8	1.21.1.21	Cell	247	228	0.123	223.939	193.299	215.685	26.878	28.141
120	HT pep8	1.21.1.21	Cell	219	213	0.178	187.611	166.607	181.008	26.371	29.408
124	HT pep8	1.21.1.21	Cell	203	204	0.142	145.307	126.249	132.637	18.555	19.844
125	HT pep8	1.21.1.21	Cell	360	282	0.081	308.546	217.178	258.682	38.179	44.217
126	HT pep8	1.21.1.21	Cell	163	103	0.091	167.87	109.896	142.939	23.728	26.574
128	HT pep8	1.21.1.21	Cell	203	188	0.15	178.588	146.511	129.375	29.839	32.707
129	HT pep8	1.21.1.21	Cell	277	226	0.198	225.528	146.542	166.486	35.12	41.221
130	HT pep8	1.21.1.21	Cell	272	208	0.201	215.207	174.189	168.624	27.271	27.461
131	HT pep8	1.21.1.21	Cell	228	175	0.192	173.31	139.542	136.766	24.368	25.636
132	HT pep8	1.21.1.21	Cell	195	241	0.142	186.978	126.509	118.729	26.981	27.28
136	HT pep8	1.21.1.21	GFPPositiveCell	268	265	0.132	277.41	162.267	143.001	26.773	21.811
137	HT pep8	1.21.1.21	Cell	129	124	0.153	115.815	116.411	101.283	18.658	18.241
138	HT pep8	1.21.1.21	Cell	258	278	0.248	254.061	289.645	198.12	36.252	39.809
139	HT pep8	1.21.1.21	Cell	187	181	0.102	171.725	156.41	110.073	21.58	21.864
140	HT pep8	1.21.1.21	Cell	190	173	0.222	287.129	285.535	229.938	27.465	29.238
141	HT pep8	1.21.1.21	Cell	311	310	0.15	283.066	268.799	242.378	41.307	44.888
142	HT pep8	1.21.1.21	GFPPositiveCell	908	844	0.294	615.789	850.846	463.072	53.294	49.248
143	HT pep8	1.21.1.21	GFPPositiveCell	112	182	0.097	175.666	230.814	133.282	23.697	39.244
144	HT pep8	1.21.1.21	GFPPositiveCell	180	153	0.104	186.781	247.983	114.512	25.434	37.445
180	HT pep8	1.21.1.21	Cell	273	217	0.134	275.826	394.73	254.919	44.826	83.201
181	HT pep8	1.21.1.21	Cell	172	149	0.194	166.87	138.832	171.842	17.207	16.804
182	HT pep8	1.21.1.21	Cell	167	170	0.122	164.9	104.907	118.462	48.378	71.729
183	HT pep8	1.21.1.21	Cell	103	147	0.203	102.723	196.243	146.452	64.049	61.754
185	HT pep8	1.21.1.21	Cell	268	324	0.223	141.371	197.06	156.806	48.004	47.554
187	HT pep8	1.21.1.21	Cell	211	197	0.086	201.252	234.528	176.835	33.678	36.742
188	HT pep8	1.21.1.21	Cell	632	808	0.129	490.189	560.784	424.126	58.588	75.998

Row ID	Group	Group Name	Class Name	Dx	Dy	Dz	Dx_2D	Dy_2D	Dz_Max	Dz_Min	
189	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
190	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
191	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
192	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
193	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
194	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
195	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
196	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
197	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
198	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
199	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123
200	HT pep8	1.21.1.21	Cell	215	215	0.123	215.123	195.123	215.123	26.123	28.123

data volume per plate 6×10^6

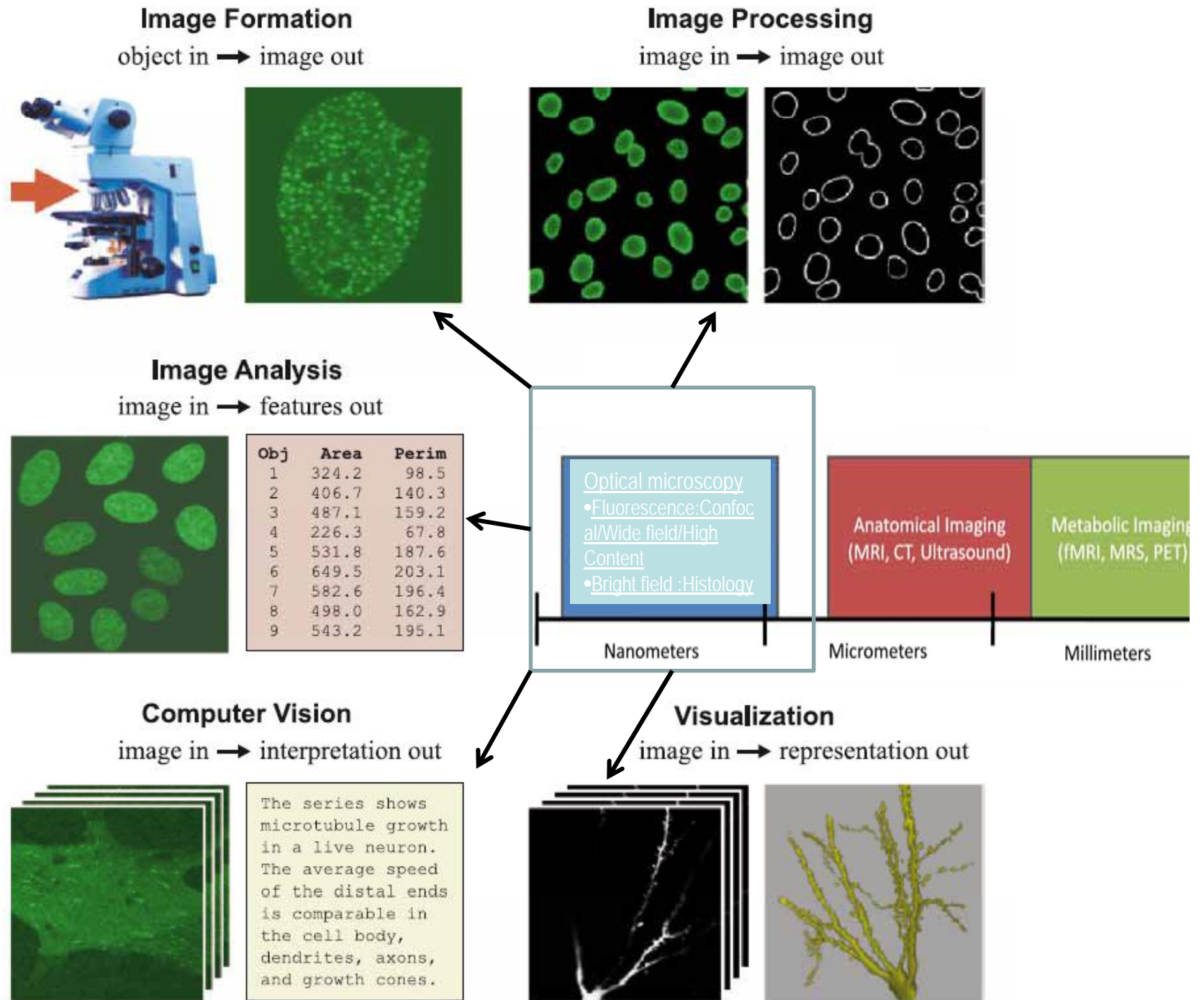
IMAGE ANALYSIS AT CELLOMICS

Established in 2011 with the aim of providing analysis solutions for image-based scientific applications by developing computational techniques that extract information from biological images.

- Group of Image Analysts based at the Cellomics Unit
 - María Montoya Coordinator
 - Hind Azegrouz
 - Gopal Karemore
 - Microscopy Unit (Antonio Manuel Santos)

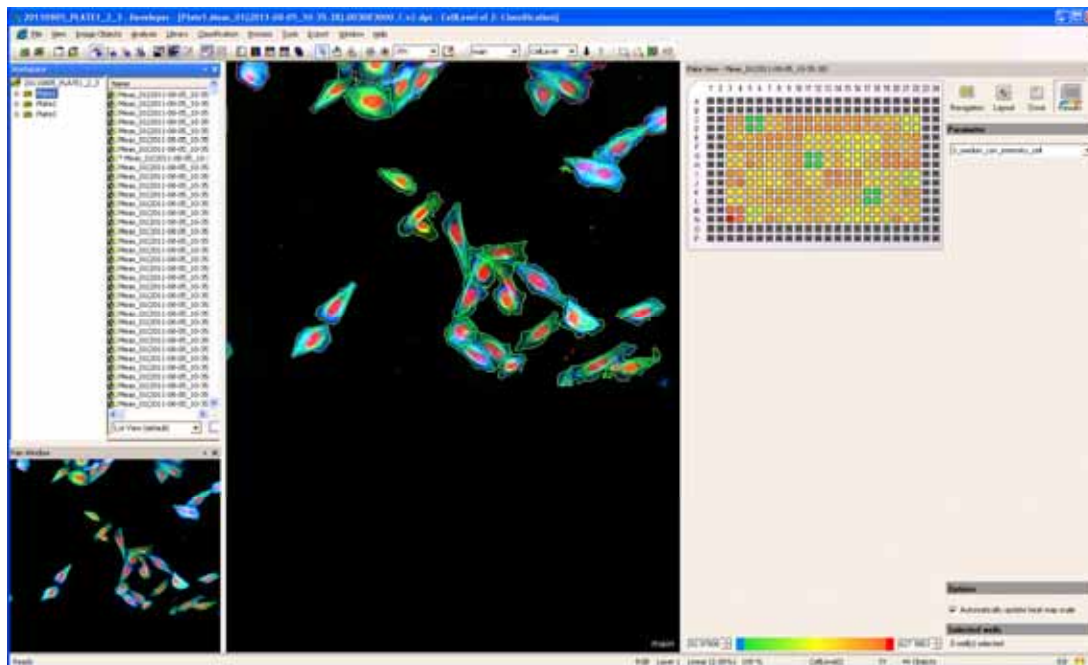
IMAGE ANALYSIS AT CELLOMICS

- Support scientific projects that require image analysis
 - Image acquisition requirements
 - Use of standards/controls...
- Train and support use of image analysis software available
 - Metamorph
 - Volocity
 - Imaris
 - Definiens
- Develop image analysis tools using different programming environments
 - Opensource software (Image J/Cell profiler)
 - Specific programming environments (Definiens, Acapella, Matlab, Amira)



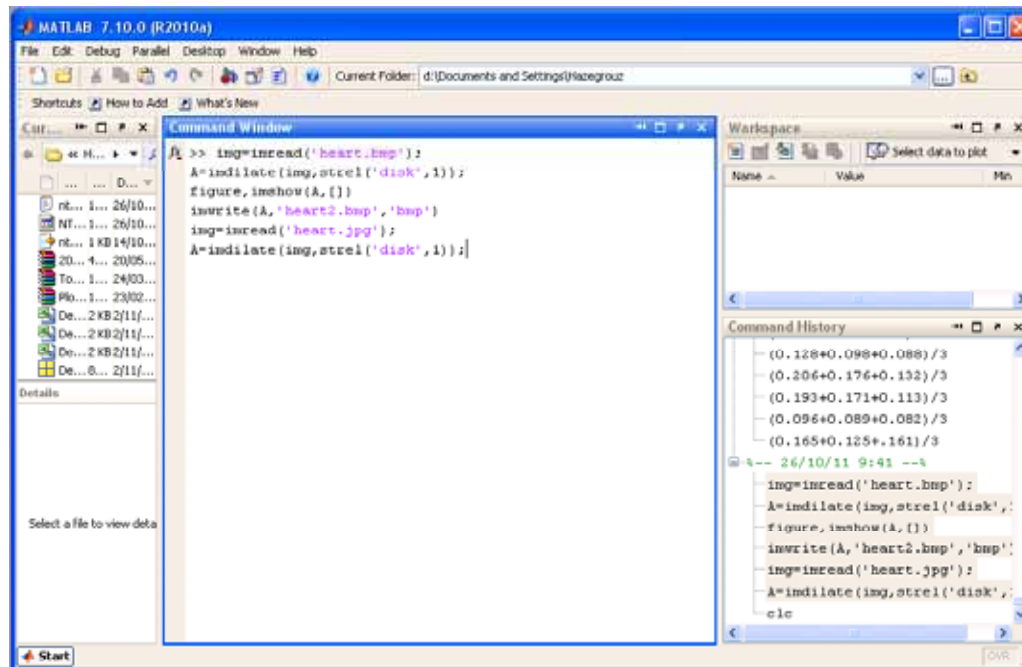
COMPUTATIONAL TOOLS FOR IMAGE ANALYSIS

- **Image processing**
 - We use Definiens: The output data in csv files make it easy to process afterwards
 - It has several algorithms that allows fast processing of the images



COMPUTATIONAL TOOLS FOR IMAGE ANALYSIS

- **Image processing**
 - We also use Matlab: It allows for fast prototyping, deployment of GUI in different workstations



COMPUTATIONAL TOOLS FOR IMAGE ANALYSIS

- **Data mining**
 - Mainly performed using Knime open-source software
 - It allows simple data handling, machine learning, clustering, visualization, encompasses use of other languages: R, matlab, java ...
 - At CNIC, we developed in house nodes to handle specific data from HCS

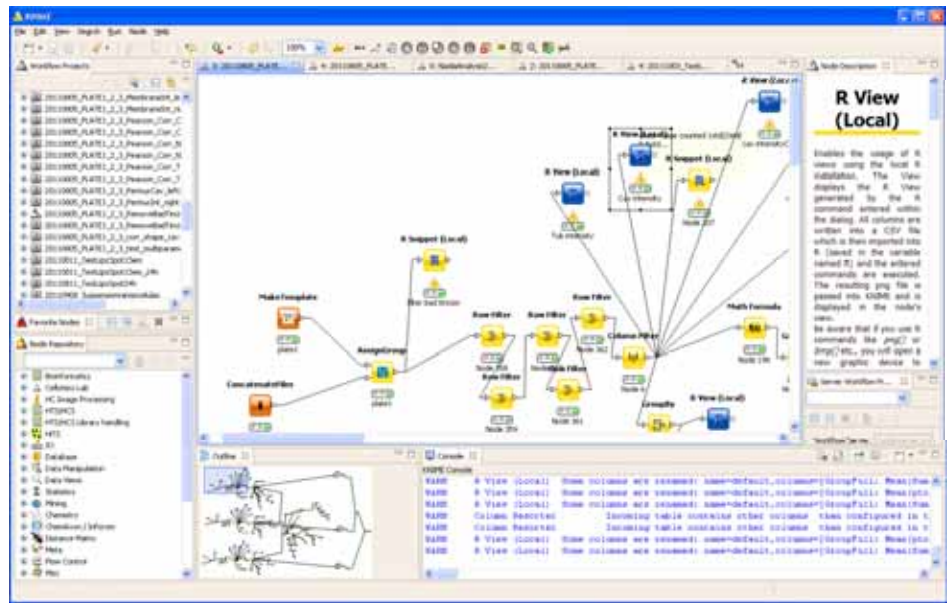
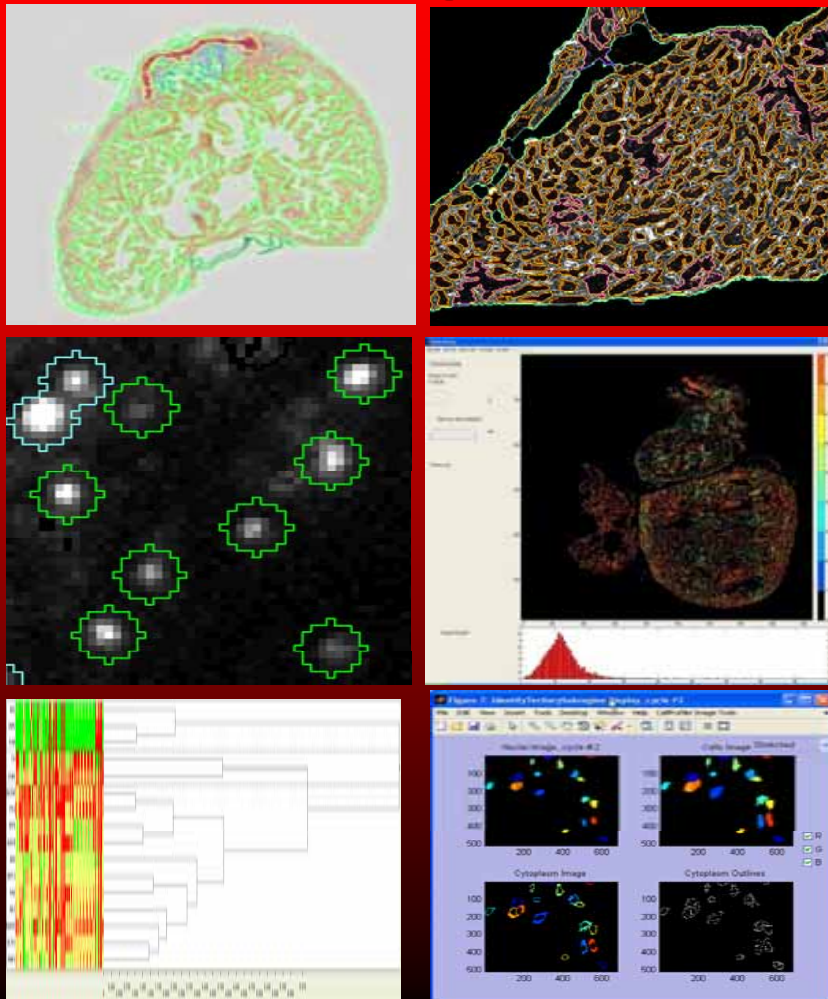
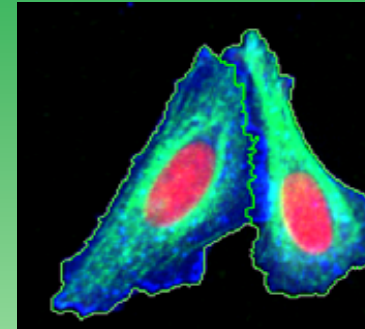


IMAGE PROCESSING-CELLOMICS UNIT

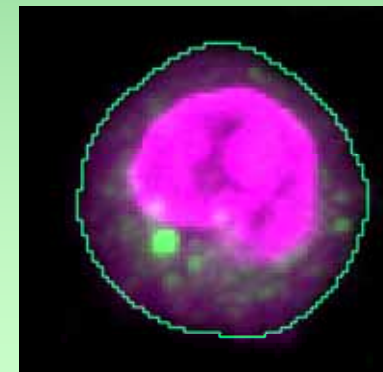
- **Biomedical image processing**



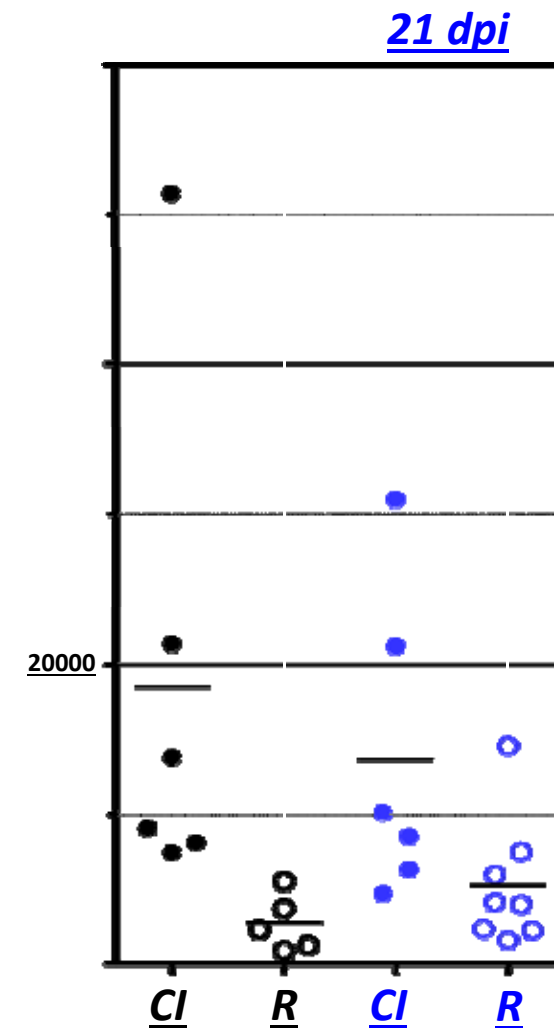
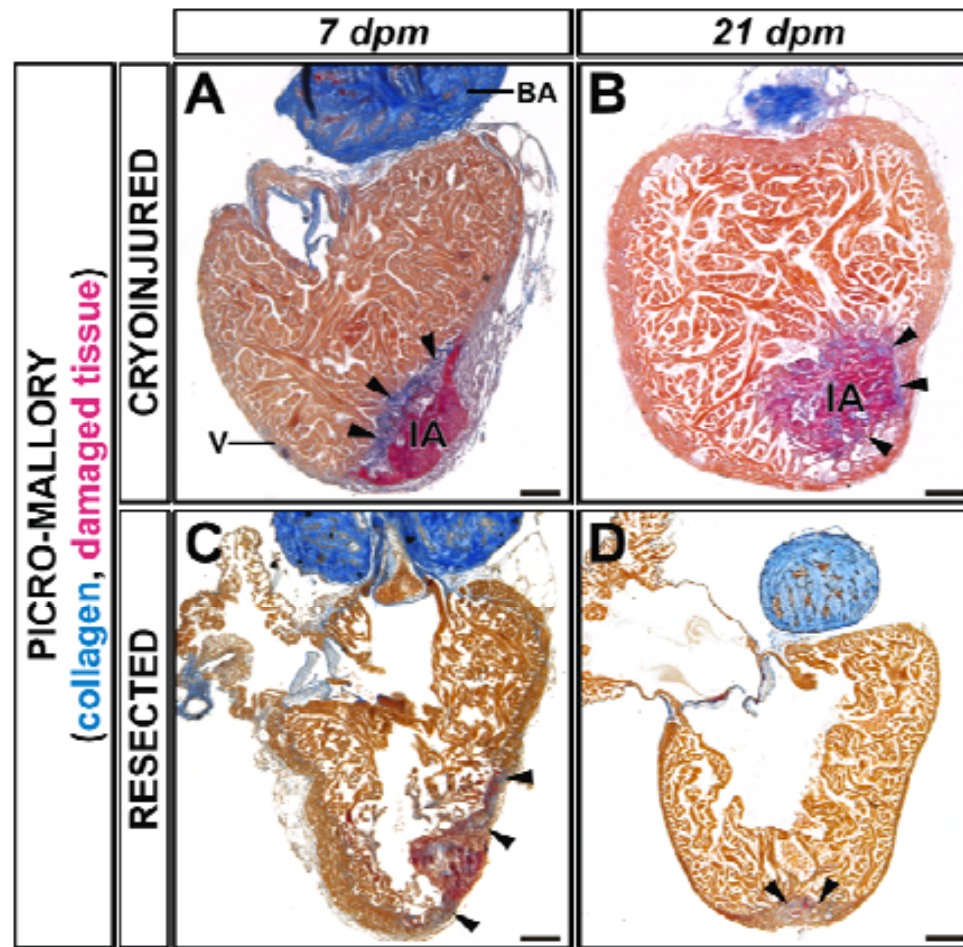
- **High Content Screening**
- Rc regulation



- Rc internalization



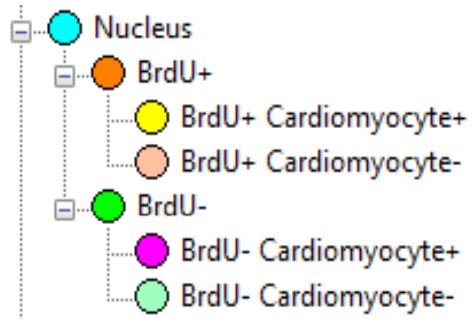
Zebrafish as a model in cardiovascular research



Cryoinjured hearts reveal increased collagen deposition compared to resected ones.

Telomere length

- Different nucleus classes

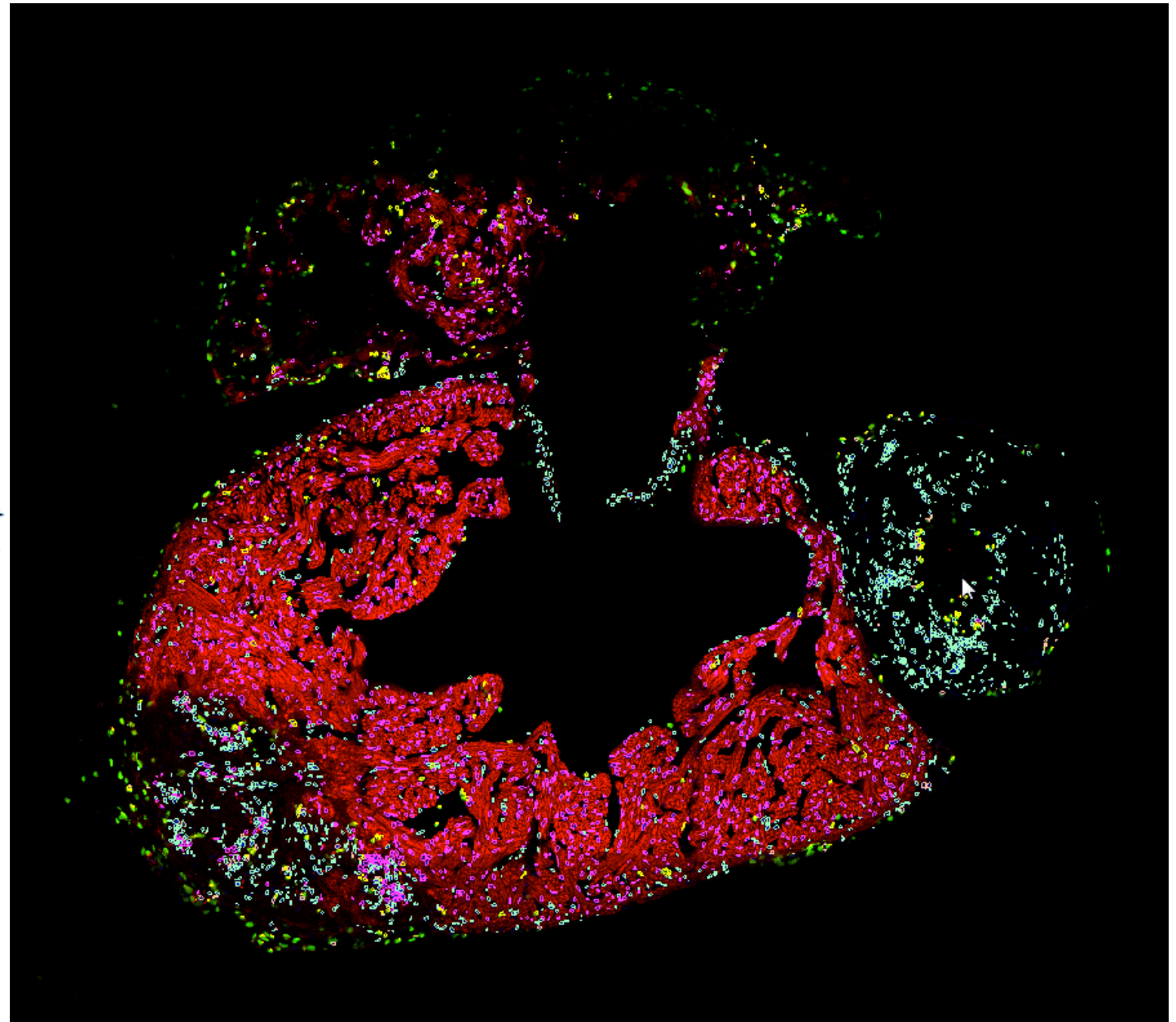


DAPI

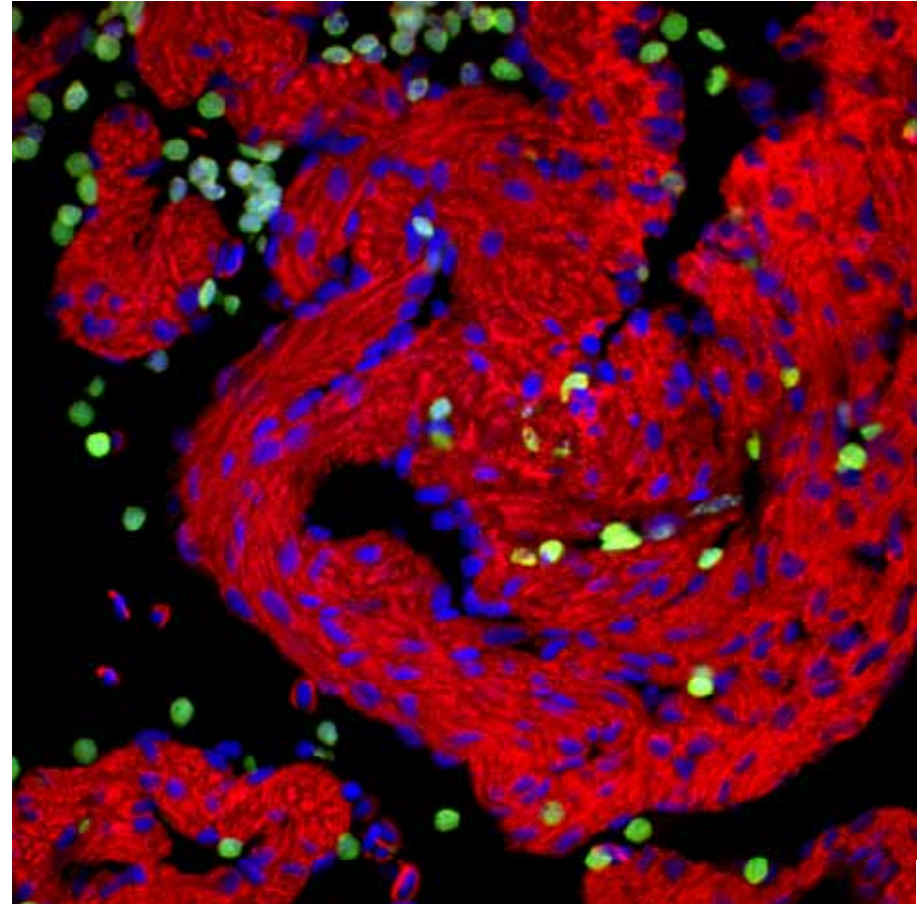
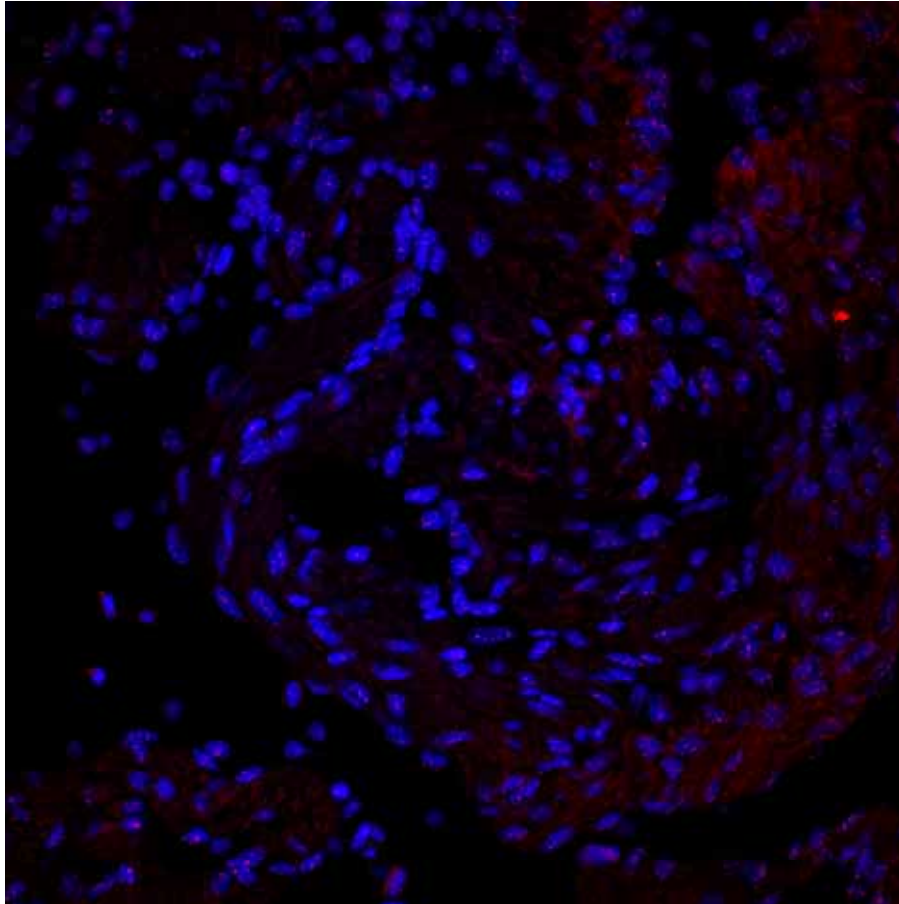
Telomeres

Myocardium

BrdU (+)



Telomere length



DAPI

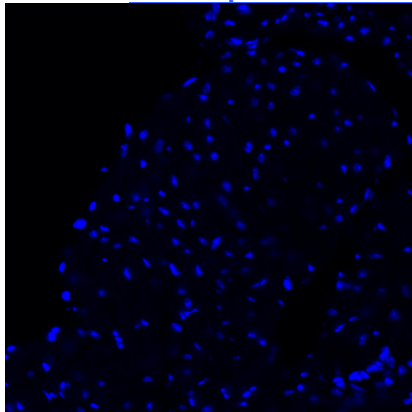
Telomeres

Myocardium

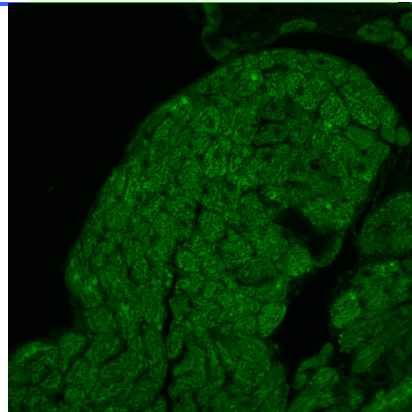
BrdU (+)

Analysis of gene expression in cardiac myocytes

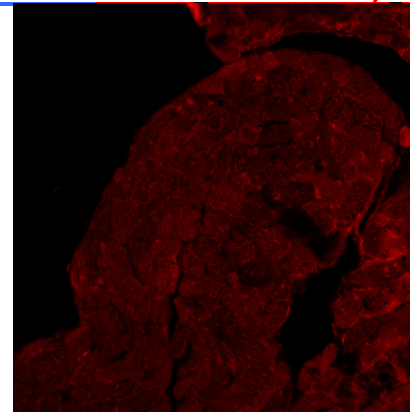
Dapi



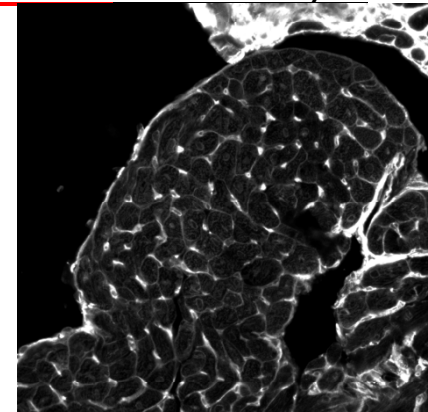
Autofluorescence



GFP antibody



WGA+ Cy3



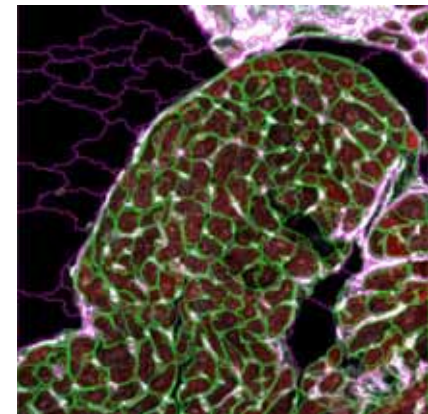
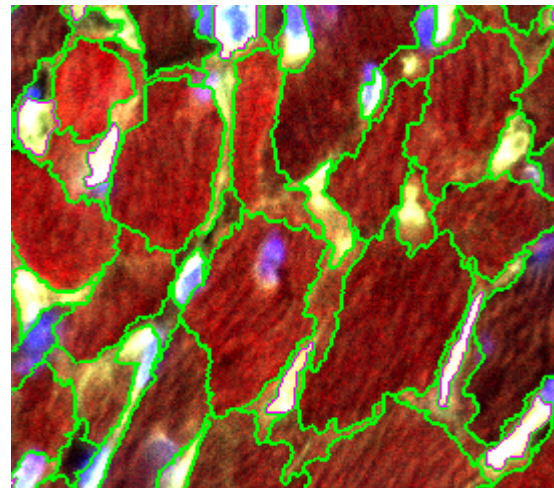
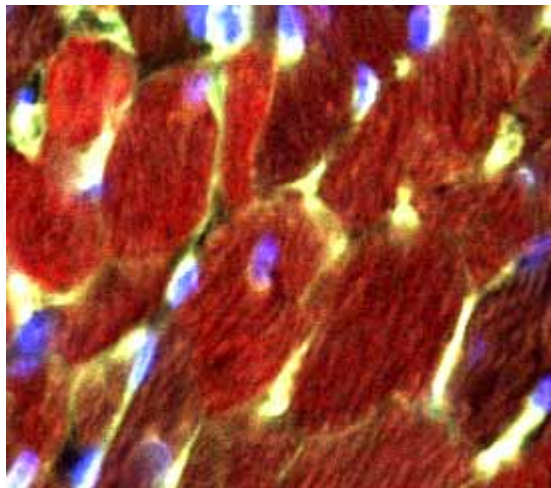
Cell type: cardiac myocytes; adult heart cryosections

Image capture: Nikon A1R Confocal microscope with the 40x magnification

Image Analysis: Definiens

Project background

- see whether the cells have recombined one or two of the nuclei by analyzing the intensity of the fluorescence, as the induction is random in cardiac myocytes.



Readout: Intensity and size of the different cells

Cristina Villa Del Campo, Miguel Torres

Quantification of MCSF chemotactic factor effect on monocytes

Phalloidin-FITC

Control

Treatment

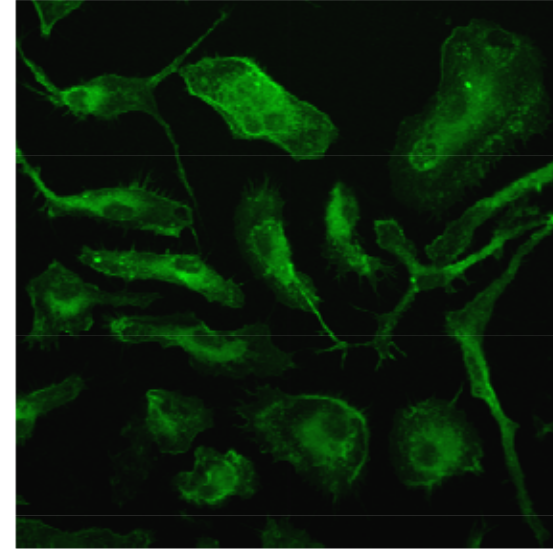
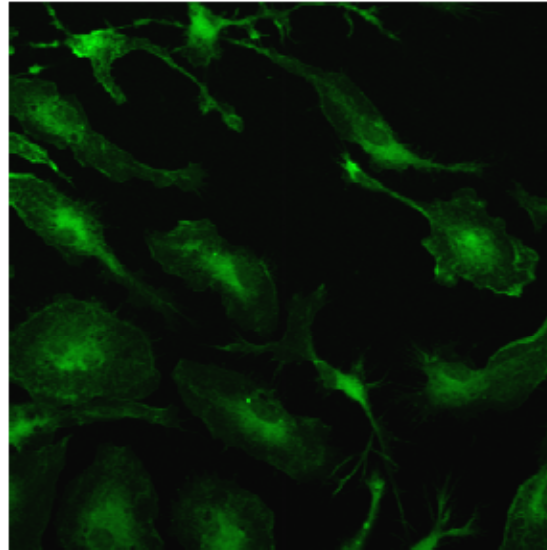
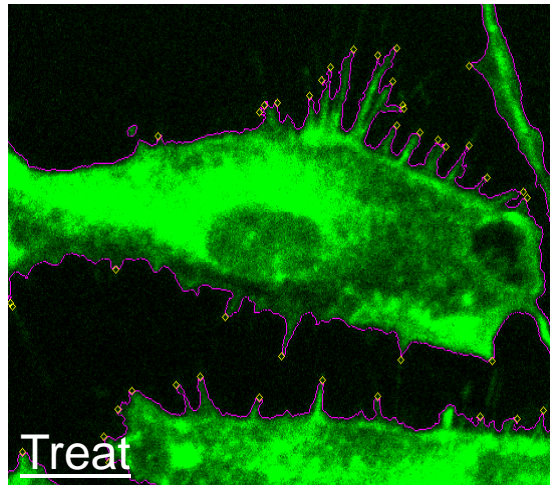
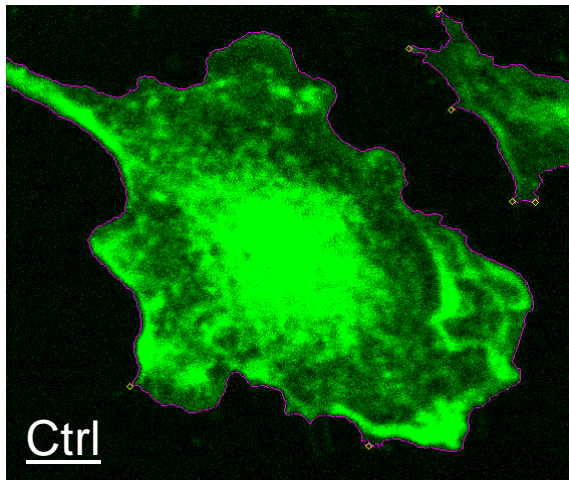


Image Analysis: Definiens

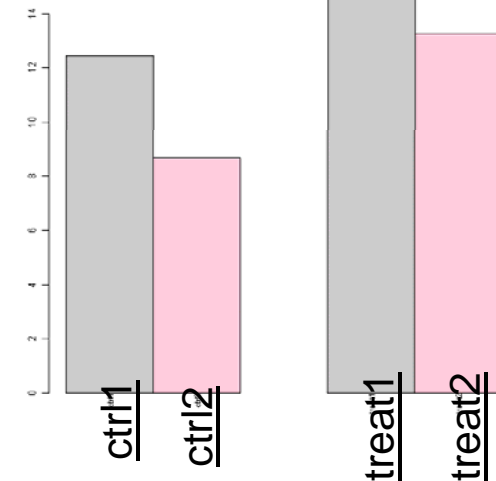
Project background

- MCSF, monocyte colony stimulating factor, 15 min treatment

Readout: Number of filopodia per cell
in Control versus treatment cells

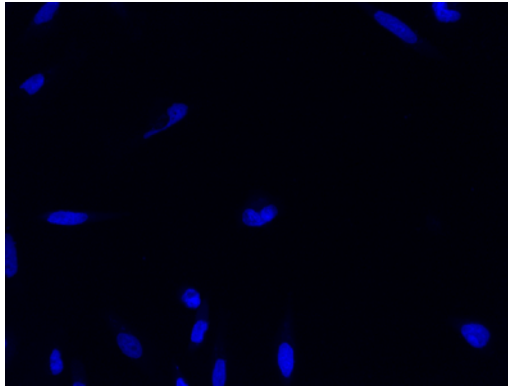


Tamas Roszer, Mercedes Ricote

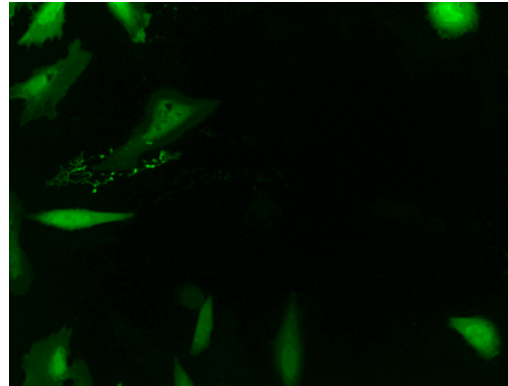


Assay development siRNA screening for genes that promote progerin production by activating aberrant LMNA splicing

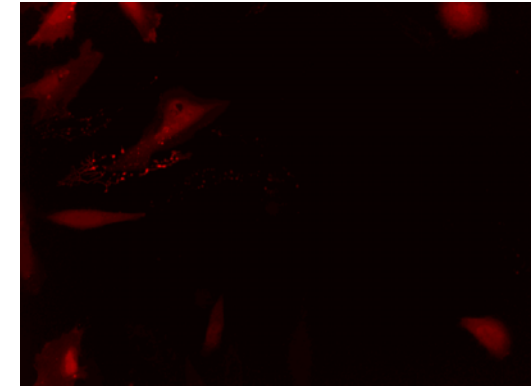
Hoechst



GFP



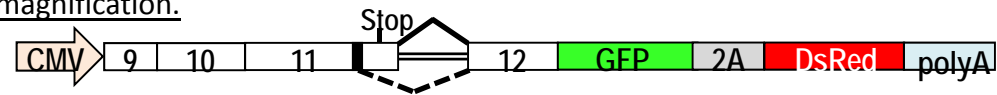
DsRed



Cellular Assay: HeLa cells expressing progerin splicing reporter (GFP-RFP)

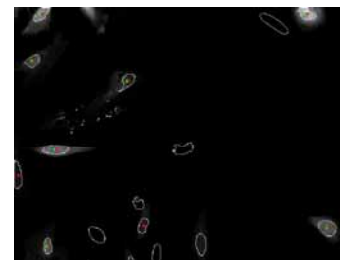
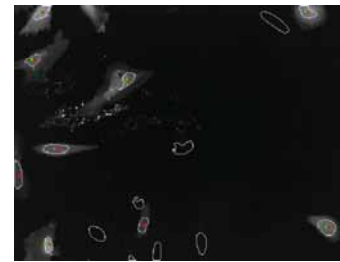
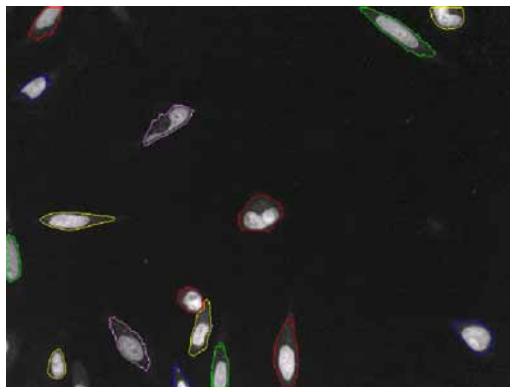
Image capture: Opera automated confocal microscope. 20X magnification.

Image Analysis: Acapella

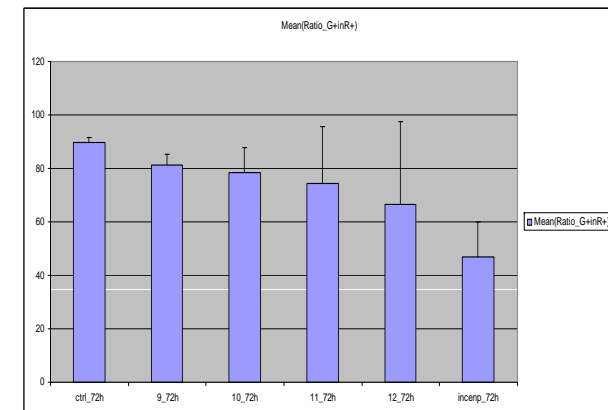


Red

Green

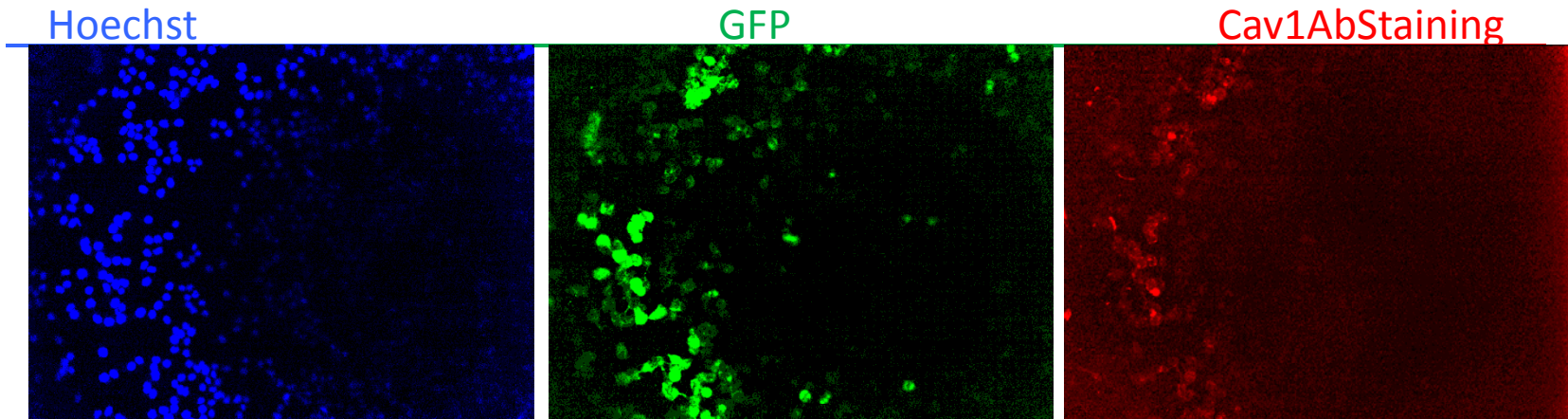


1^{ary} Readout: proportion of green+ in red+



Magda Zajac, José Rivera, Vicente Andres

Assay development: siRNA HCS for stromal genes in tumor cell invasion



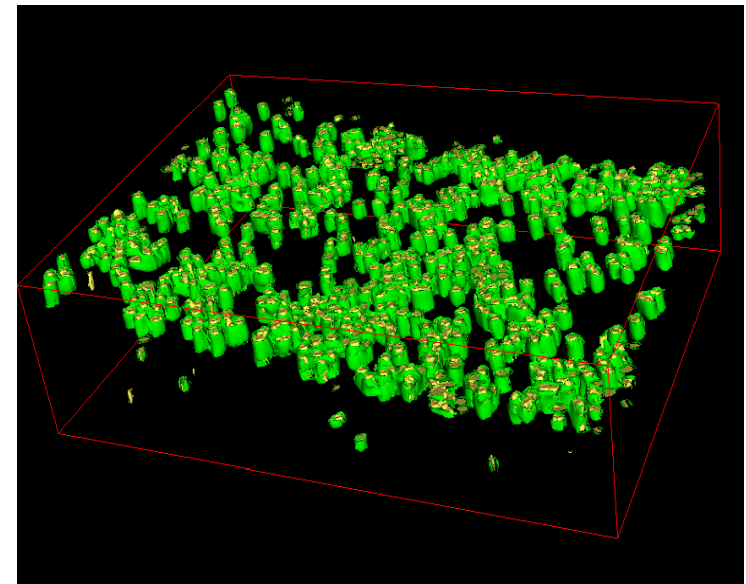
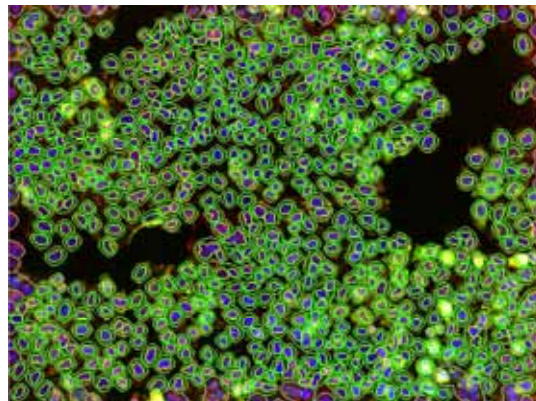
Cellular assay: Hela

Image capture: Opera automated confocal microscope. 10X magnification. More than 60 z-layers captured

Image Analysis: Definiens

Biological background

- Quantify tumor cell invasion in 3D collagen matrices
- Assess transfection (posCtrl(Cav1)-siRNA) efficiency in 3D.



Silvia Fernández Soriano, MA Del Pozo

TRAINING ORGANIZATION

EXTERNAL

- 2010. Definiens symposium
- 2011. HCS workshop (Perkin Elmer)

INTERNAL

- 2011. Image cytometry course

IMAGE CYTOMETRY WORKSHOP: MICROSCOPY IMAGE-BASED CELL AND SUBCELLULAR STRUCTURE IDENTIFICATION AND QUANTITATION.

María Montoya. [Quantitative cell Imaging](#).

Hind Azegrouz and Antonio M Santos-Beneit. Open source software for quantitative cellular image analysis ([Cellprofiler/Image J](#)).

Antonio M. Santos-Beneit. [Imaris](#) applications for cell identification and analysis.

Hind Azegrouz. Custom development of automated cell image segmentation and analysis routines with [Definiens](#).

The collage features the following elements:

- DEFINIENS** logo with the tagline "Understanding Images".
- CellProfiler** logo with the tagline "cell image analysis software".
- BITPLANE** logo with the tagline "SCIENTIFIC SOLUTIONS".
- A screenshot of the CellProfiler software interface showing a workflow tree and analysis parameters.
- A 3D visualization of cells in a grid, with blue and red spheres representing different cell components.
- The **ImageJ/FIJI** logo.
- A screenshot of the ImageJ/ Fiji software interface showing a microscope icon and a cell image.
- A screenshot of the ImageJ/ Fiji software interface showing a 3D visualization of cells in a grid.

NETWORKING

- REMOA (Spanish advanced light microscopy network)
- SDDN (Spanish drug discovery network)
- EUROBIOIMAGING
- EU-OPENSREEN