



High-Content Screening - big image data meets big pharma

*Predictive drug discovery using quantitative image analysis,
organotypic cell systems and phenotypic screening*

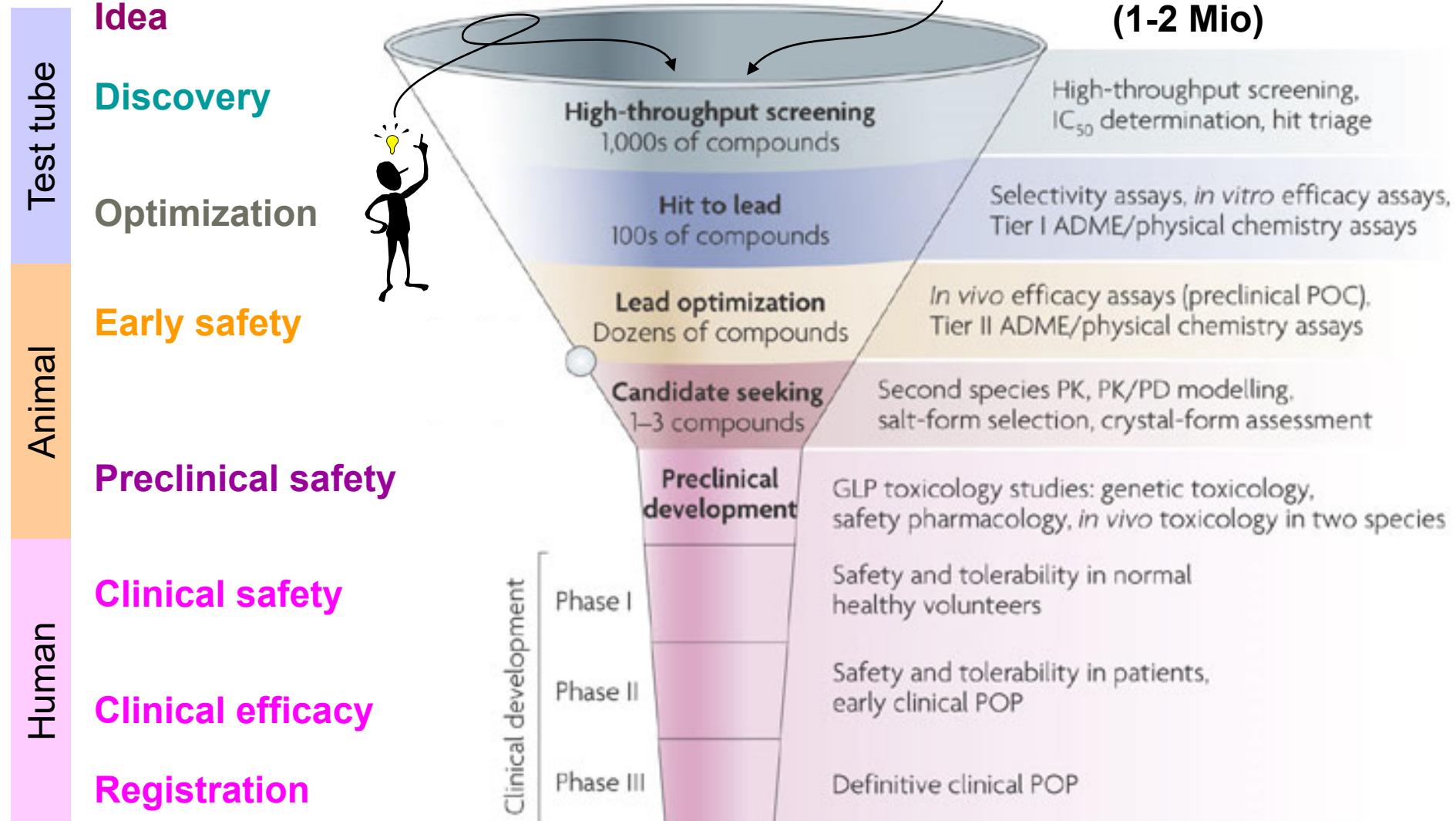
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2007-2015: Senior Scientist Discovery Technologies, F. Hoffmann-La Roche Ltd., Basel

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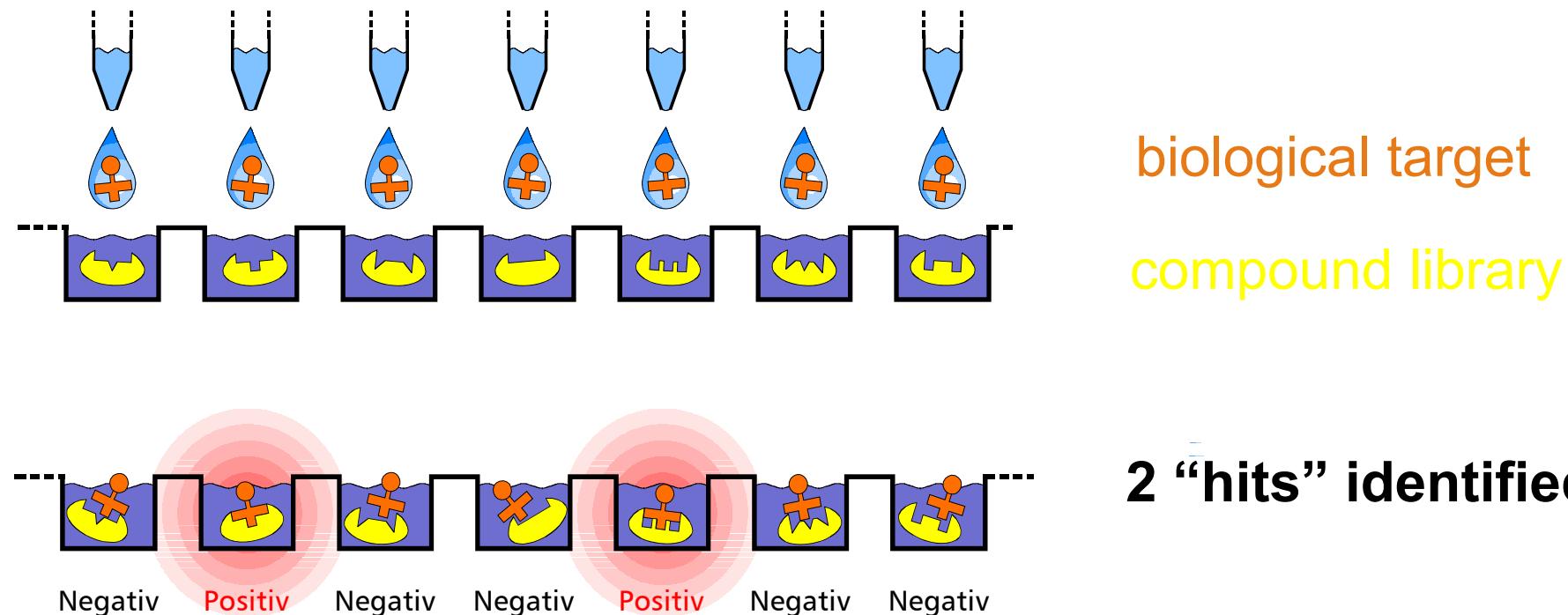
The drug discovery pipeline



What is High Throughput Screening (HTS)?

Well-Well-Well-...

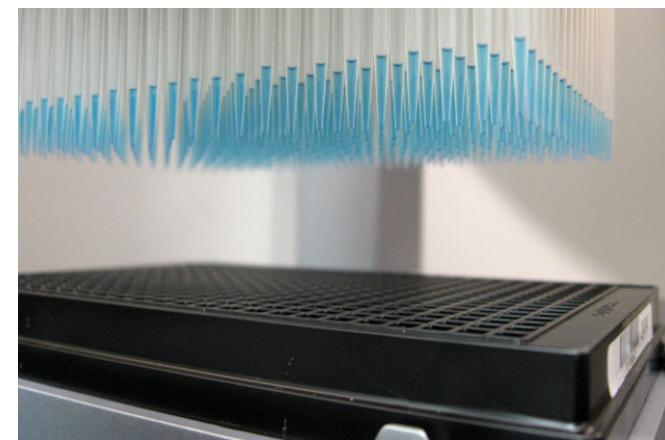
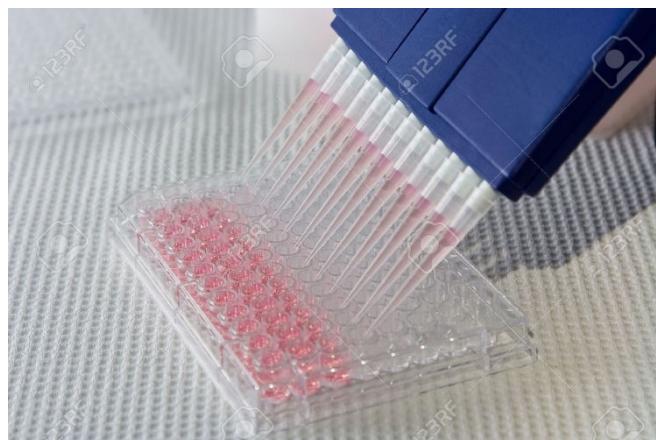
The systematic testing of a compound library against a biological target



High Throughput Screening (HTS): 100'000 or more samples per day

What is an assay?

- An experiment run according to a protocol giving the same result every time and everywhere
 - ... provided the protocol is formulated to the required detail
 - ... provided the protocol is followed strictly

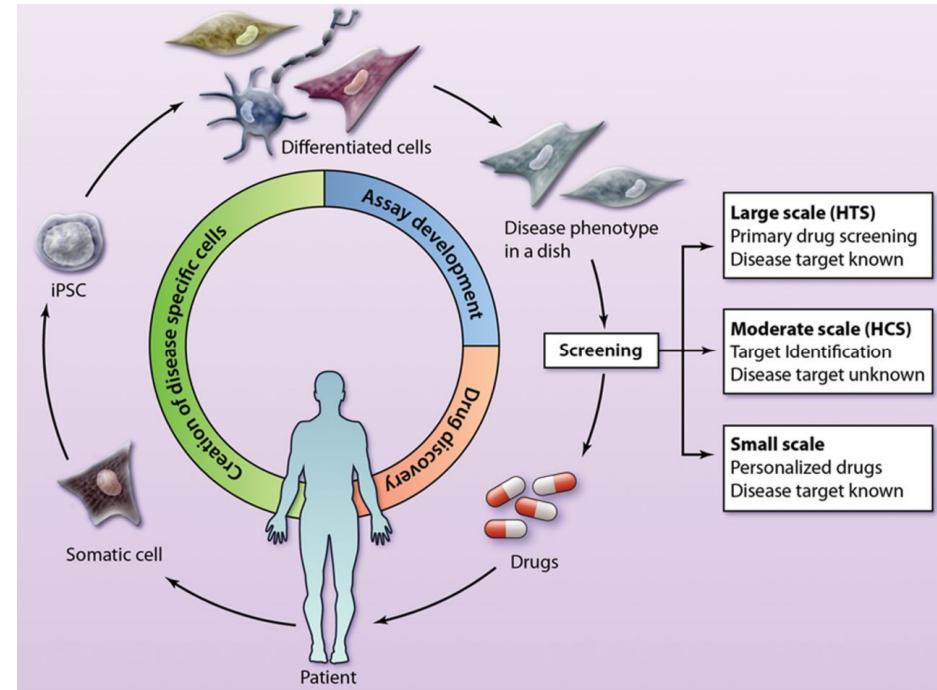


Current bottlenecks in drug discovery

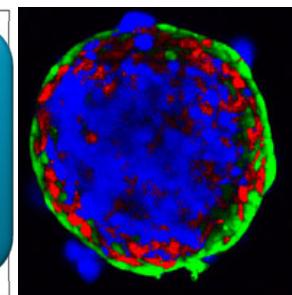
Possible improvements

- **More predictive models**
 - Patient-derived or genome-edited reprogrammed cells instead of overexpressing immortalized cell lines
 - 3D co-culture organotypic systems instead of 2D mono culture

- **More predictive assays**
 - Phenotypic cell-based as opposed to target-based biochemical



The modality of choice:
High-Content Screening

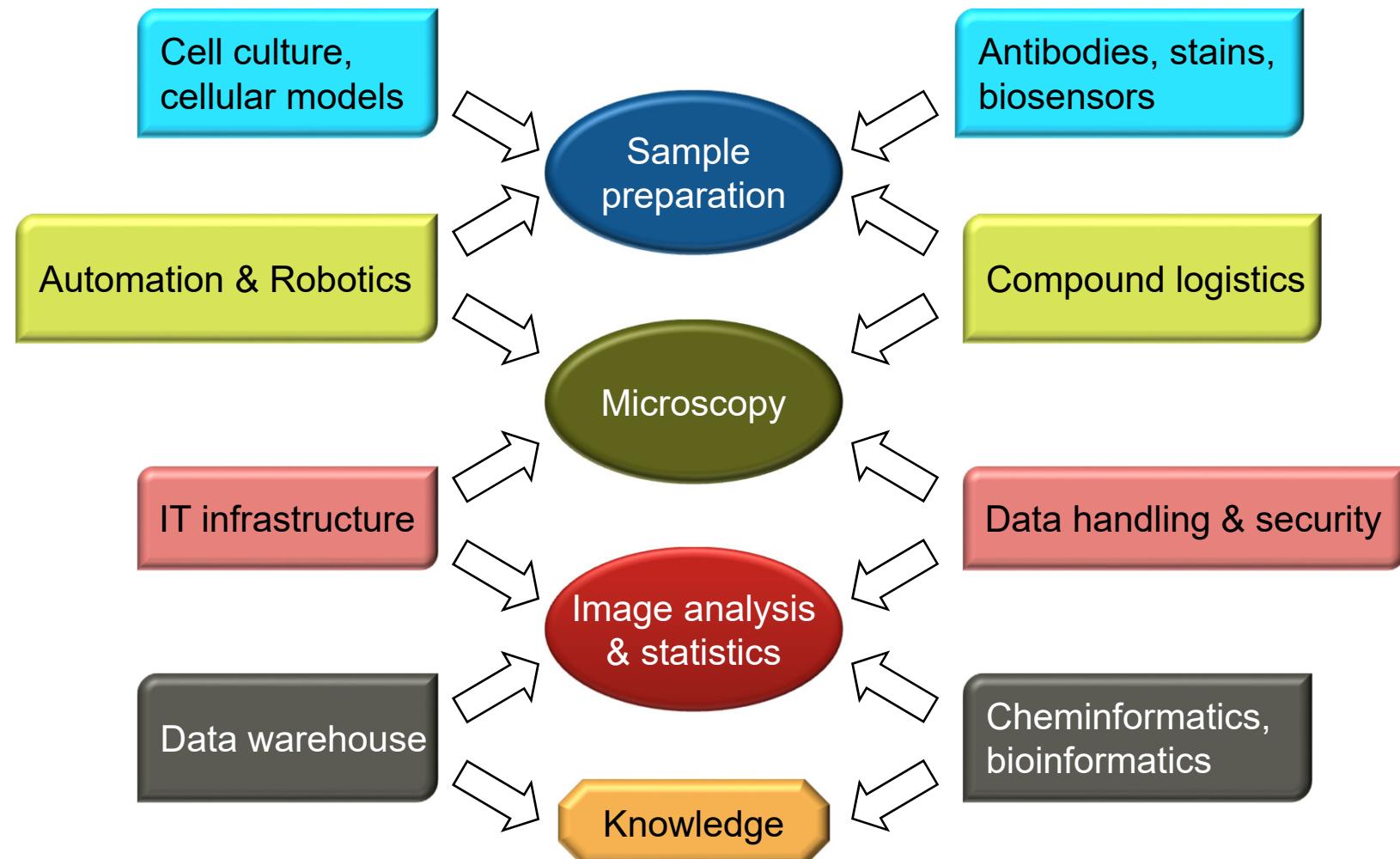


Q: What is High-Content Screening

- A: automated (fluorescence) microscopy (of cultured cells *in vitro*) in combination with automated quantitative image analysis
- Q: what does “high-content” mean?
- A: (literally: result consists of more than 1 value.)
 - Many different readouts (features) derived from an image, such as,
 - * nucleus area, circumference, roundness, length, width, ...
 - * cytoplasm area, circumference, roundness, length, width, ...
 - * intensity of marker 1 in nucleus, in cytoplasm, in spots, ...
 - * intensity of marker 2 in nucleus, in cytoplasm, in spots, ...
 - * number of nuclei per image, average number of spots per cell, ...
 - * ...



The elements of HCS



Industrial HCS facility



F. Hoffmann-La Roche Ltd. 2014

50.000 images in 24h

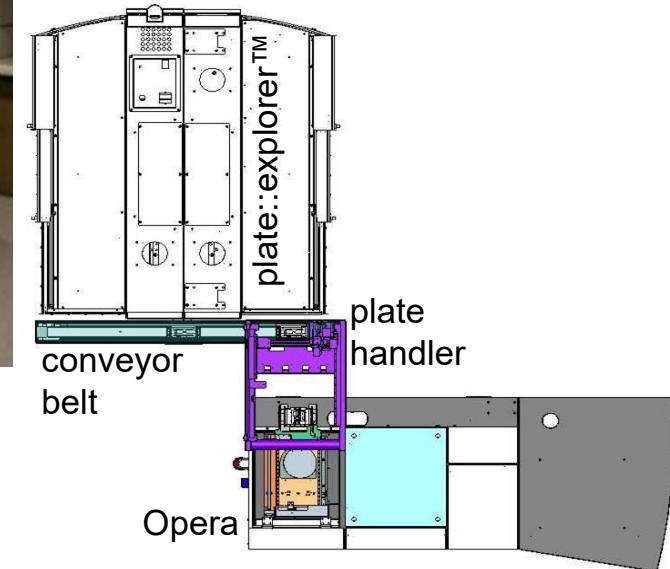
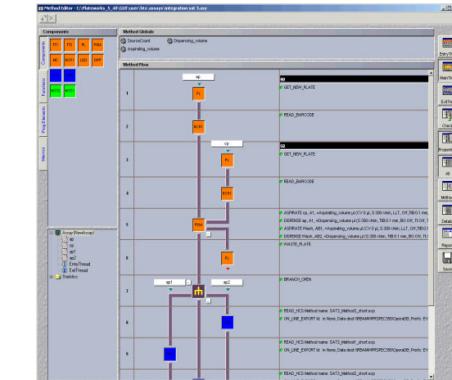


Image analysis in HCS mode

Characteristics

- Typical image type:
 - 2-5 channels, quasi monochrome
 - 2D (95%), 3D (1%),
2D+time (4%), 3D+time (0%)
- Typical image objects:
 - Nucleus, Cytoplasm, Spots, Tubes
- Typical segmentation steps
 - Nucleus: good starting point
 - Cytoplasm: expand nucleus

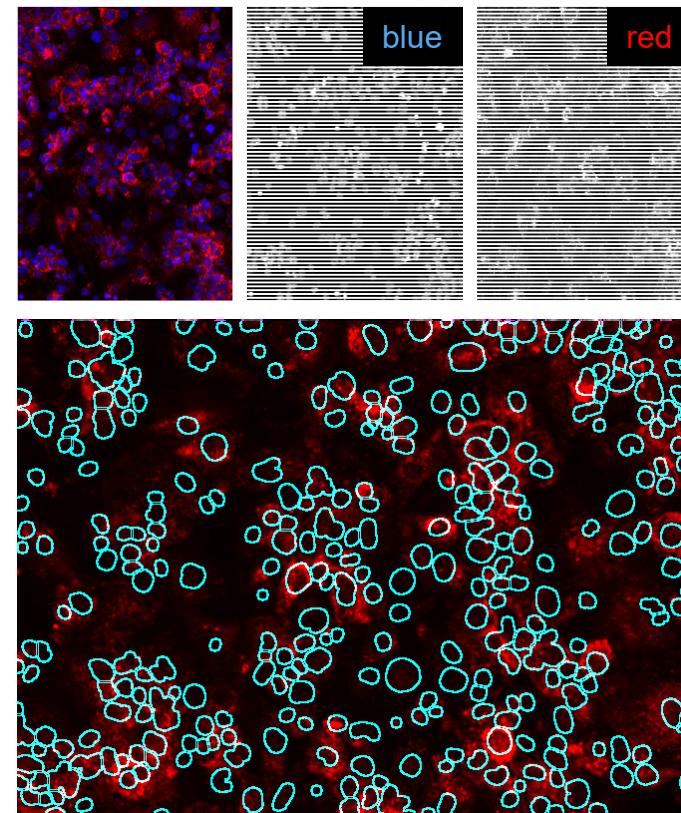


Image analysis in HCS mode

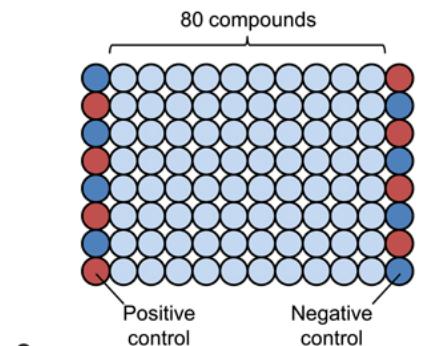
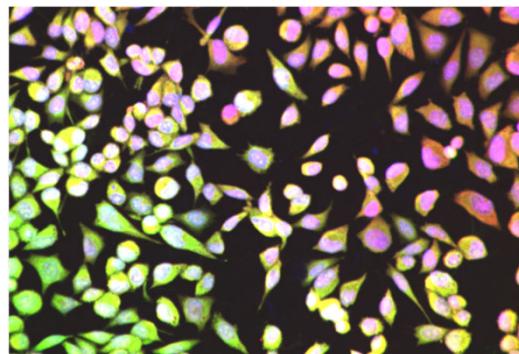
Requirements

- Throughput per image field:
 - 1 s^{-1} for screening (HT)
 - 0.01 s^{-1} for profiling (MT)
- Robust without human intervention:
 - Heterogeneous cell population
 - Training data are never representative
 - Always expect the unexpected
 - Often low signal to noise, variable background

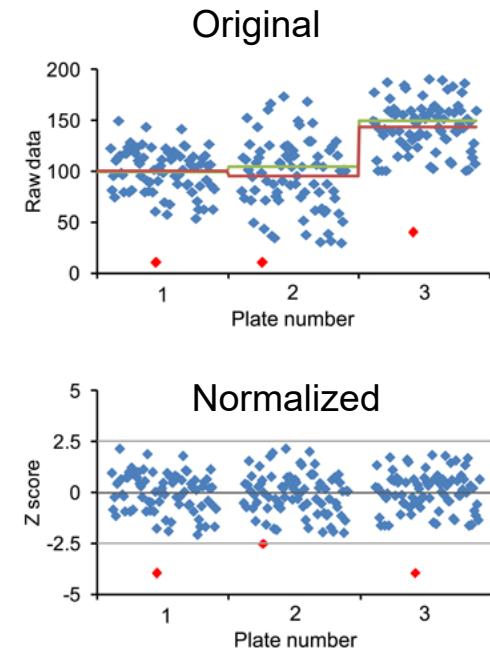
HCS: all is not well

Correction of systematic errors

- Plate to plate variation:
 - biological batch effect
- Well to well variation:
 - geometric patterns, pipetting errors
- Within field variation:
 - inhomogeneous illumination



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	-100	-68	-1	10	31	1	7	3	19	-84	13	17	9	28	7	15	22	20	12	21	22	18	23	29	
B	-97	-99	-32	63	-31	-61	-47	-12	71	6	-18	-42	-51	-50	-14	-42	15	-9	-64	-48	2	0	-20	-15	
C	-101	-101	41	27	5	14	20	16	9	10	16	25	14	3	10	16	10	40	22	19	38	18	33	25	
D	-99	-103	-47	-22	76	-52	-44	-32	-10	-48	-59	-27	77	69	-10	-8	-13	-24	-59	-9	-41	-5	31	-45	
E	-104	-68	13	20	10	13	11	15	2	14	14	5	14	16	16	15	1	23	30	39	44	22	-5	30	
F	-99	-100	-59	-44	-49	-79	-30	-11	-30	51	-9	-65	-9	-67	-12	-12	-50	-21	-2	-14	-52	-21	58	-50	
G	-101	-100	3	14	4	3	15	21	14	11	26	-16	23	16	23	12	14	19	-7	32	6	11	35	16	
H	-99	-99	-62	-45	-51	-18	-23	-43	-52	54	-69	-65	-55	-69	58	-33	-102	-48	-21	-20	-28	-111	38	-67	
I	-101	-103	-5	11	7	10	2	9	5	19	-13	16	15	29	7	15	21	14	20	30	7	26	21	14	
J	-104	-97	-51	-37	-42	-73	-33	-66	-61	-72	-8	-29	-81	-49	-6	-59	-102	-75	-76	-54	-26	-31	8	-38	
K	-97	-101	35	10	-11	9	11	11	19	11	12	8	11	20	5	25	5	15	15	21	15	28	33	-26	
L	-104	-100	1	38	-18	-37	-51	-39	-58	-24	-22	-35	-54	-47	-5	-38	-95	-46	-44	-43	-23	-6	-42	-81	
M	-99	-101	-5	2	-3	5	5	11	9	9	2	9	8	10	18	9	1	12	95	15	57	30	11	19	11
N	-100	-99	-50	-69	-54	-17	-79	-56	-51	-8	-21	-29	-84	-90	-46	-30	-96	-57	-80	-9	-78	-59	-31	-31	
O	-99	-103	5	37	3	-3	8	5	3	8	-5	10	6	-3	15	5	24	23	26	5	12	11	9		
P	-102	-100	4	-71	-18	-70	-37	-44	-80	-16	-47	-67	-52	-15	-36	-60	4	-81	-38	-72	-23	-78	-34	-59	



Typical HCS assay repertoire

- Micronucleus formation
- Internalization
- Trafficking
- Translocation
- Subcellular localization
- Calcium flux
- Apoptosis
- Proliferation
- Migration
- Sarcomere structure
- Neurite outgrowth
- Synapse formation
- Endothelial tube formation
- 3D spheroid microtissue

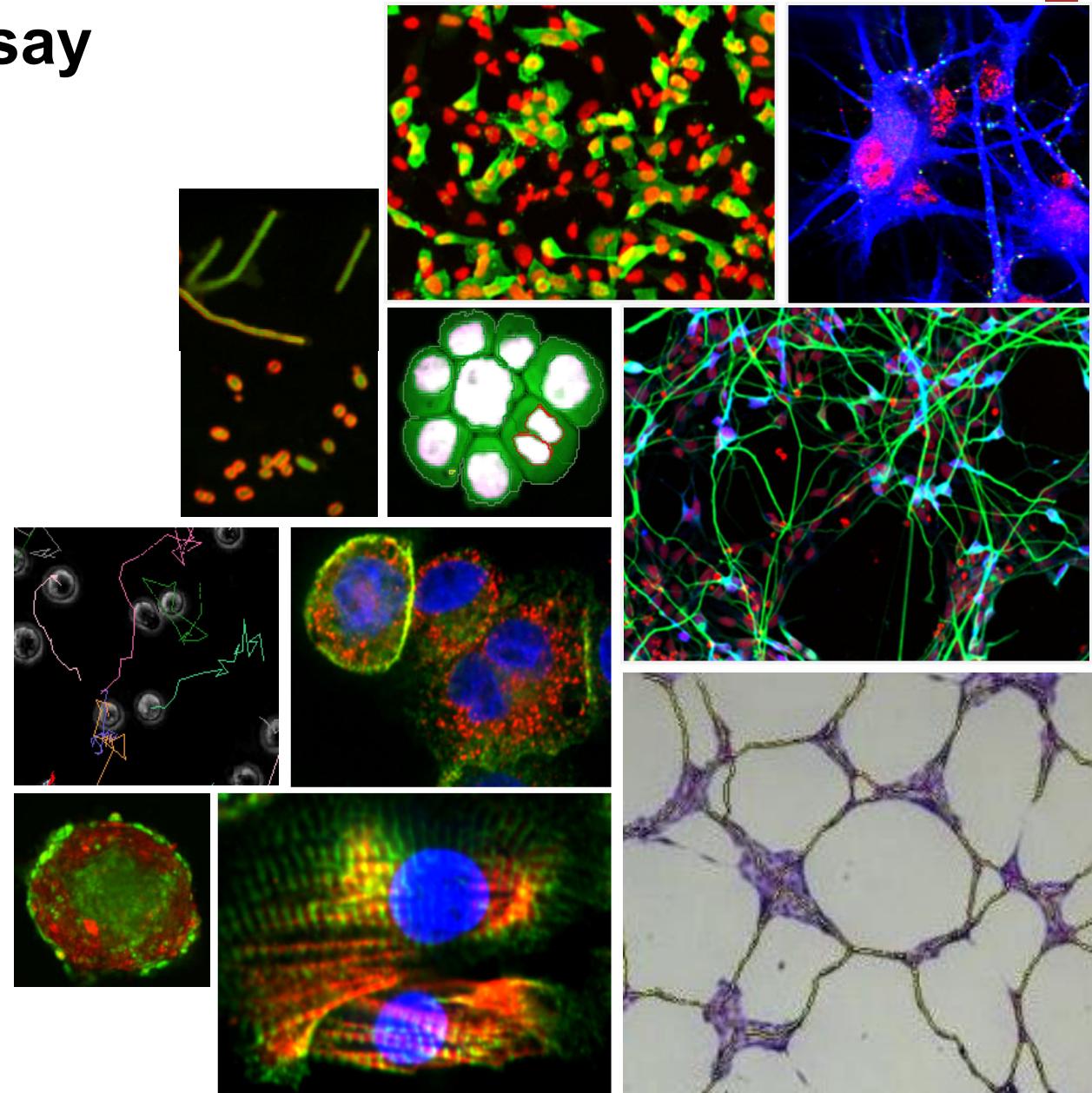


Image analysis in HCS: a case study

OPEN
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Cell Reports
Report

Disease Modeling and Phenotypic Drug Screening for Diabetic Cardiomyopathy using Human Induced Pluripotent Stem Cells

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Cell Chemical Biology
Resource

CellPress

Molecular Phenotyping Combines Molecular Information, Biological Relevance, and Patient Data to Improve Productivity of Early Drug Discovery

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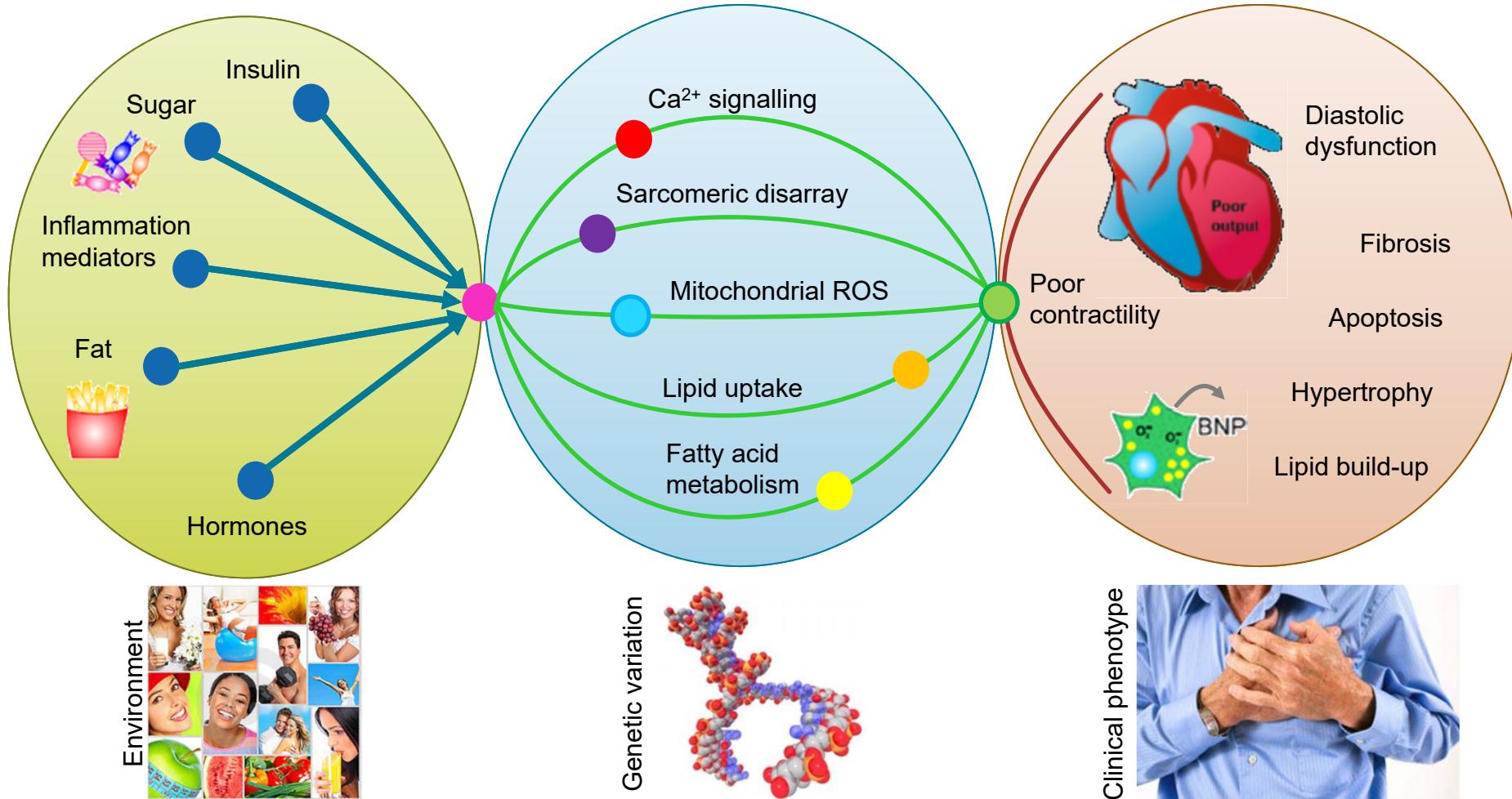
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<http://dx.doi.org/10.1016/j.chembiol.2017.03.016>

Diabetic cardiomyopathy

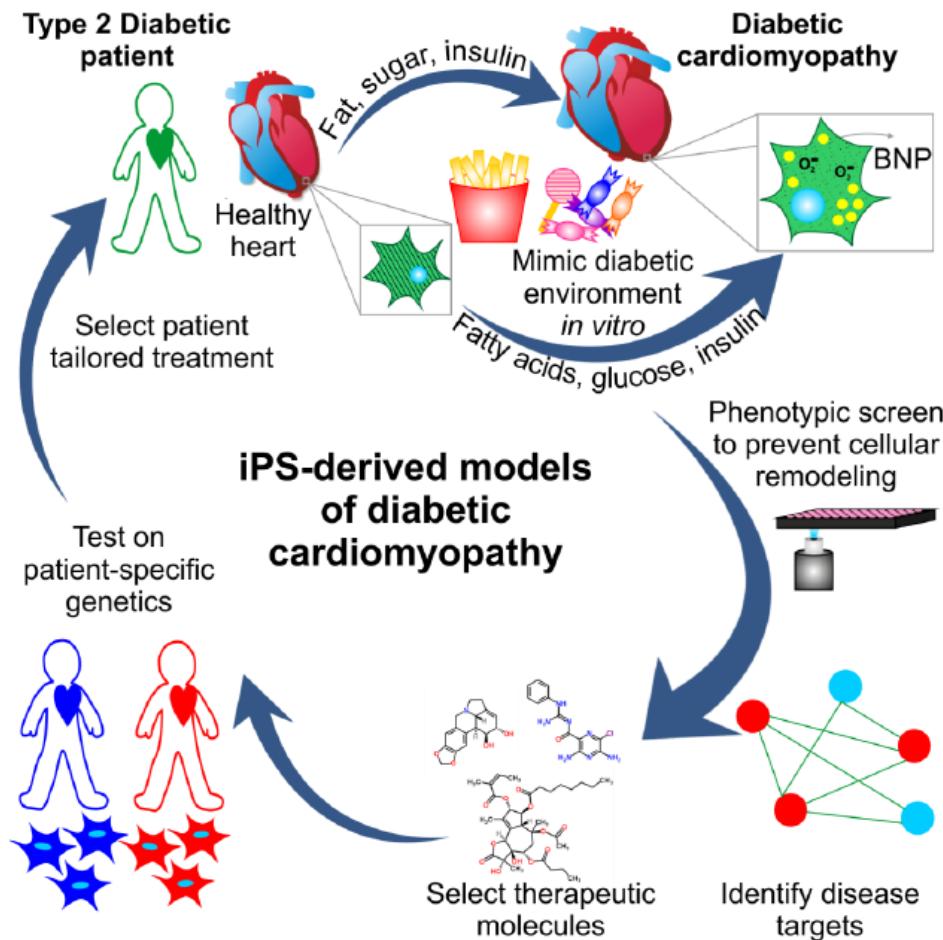
Multiple inputs, multiple outputs



* BNP – brain natriuretic peptide

Diabetic cardiomyopathy modeling

«Patient in a dish»



Find compounds that protect cardiomyocytes against glucose-induced stress

The sarcomere integrity assay

Principle

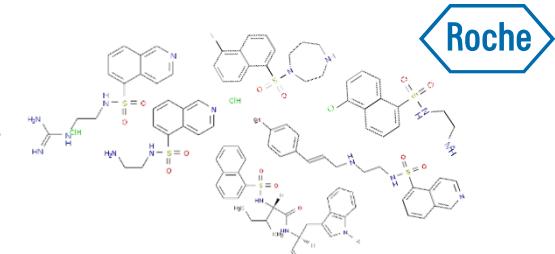
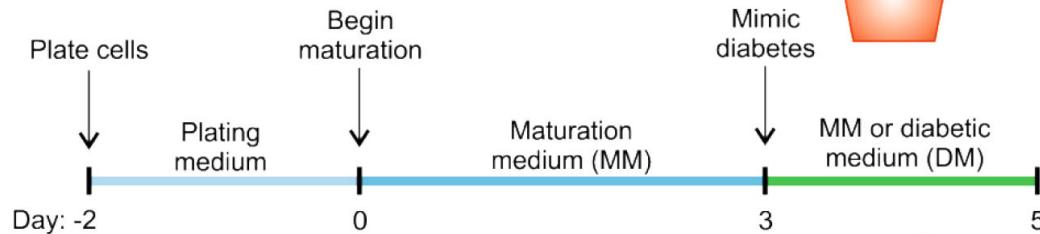


Plate the cells

Mature the cells

Apply the library



No Glucose
Insulin
Fatty acids



Glucose
Insulin
Fatty acids
Cortisol
ET-1

Assay readouts:

- BNP secretion
- Cell area
- Sarcomere integrity

Mimik the diabetic microenvironment

Sarcomere structure in cardiomyocytes

Fluorescence staining

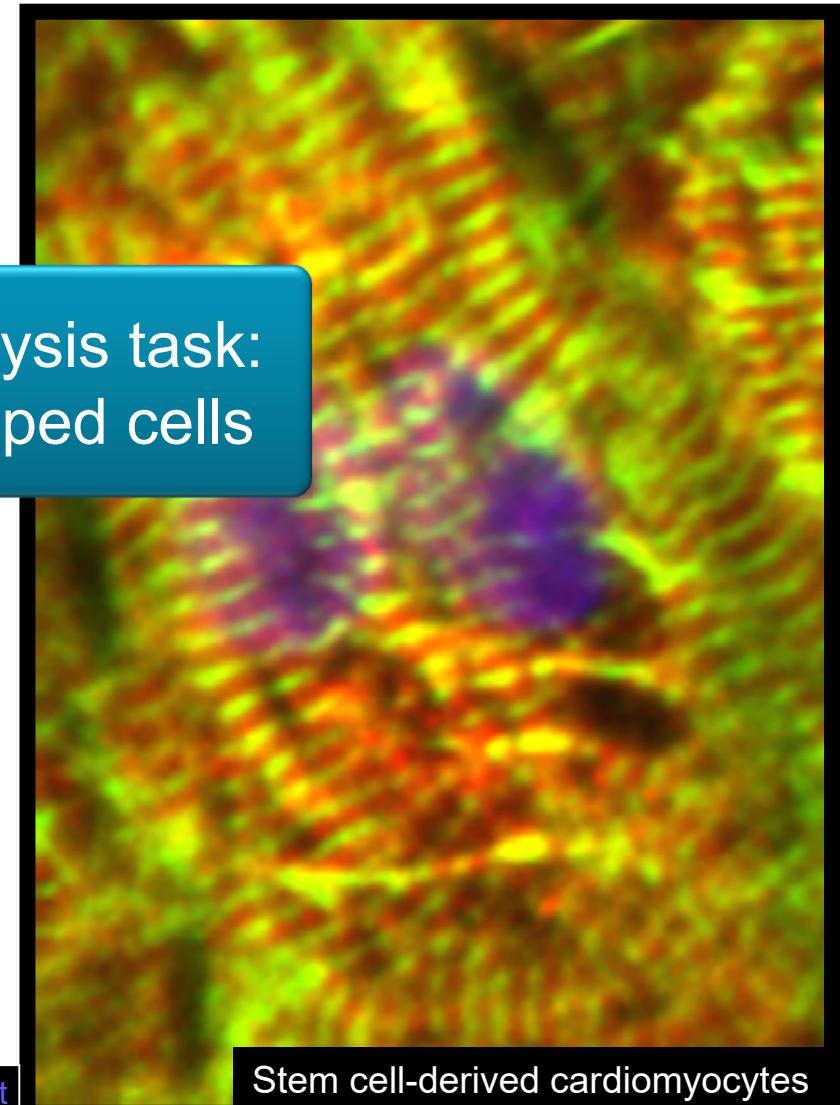
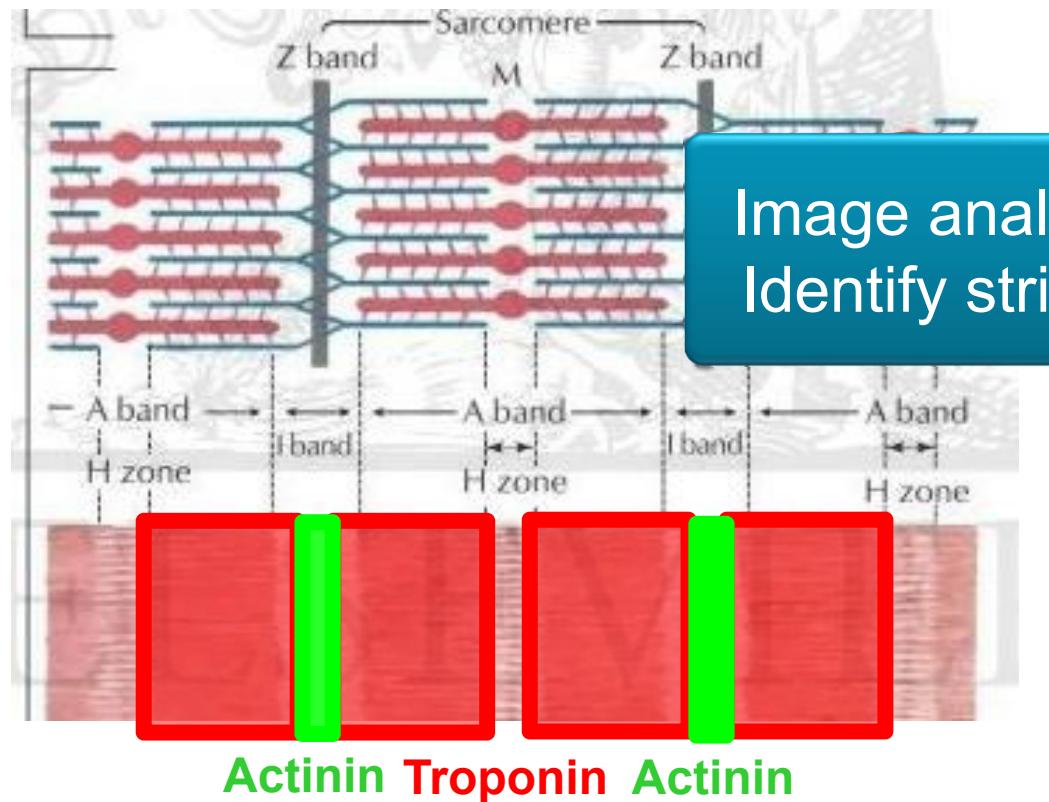
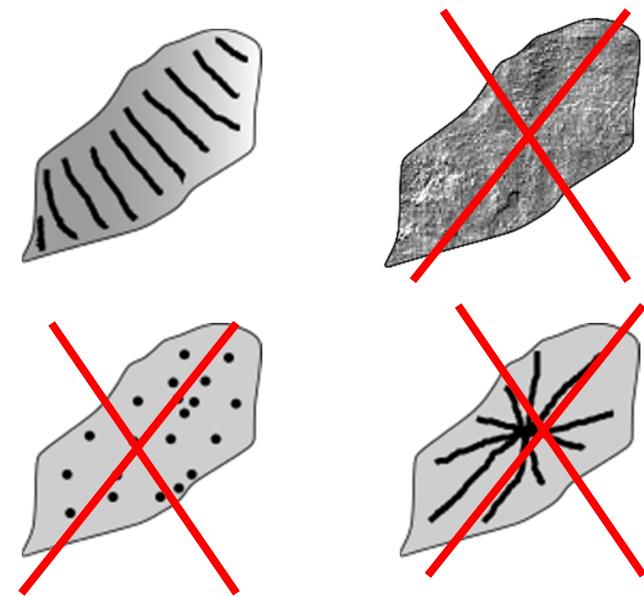


Image analysis: identify myocyte sarcomers

Concept

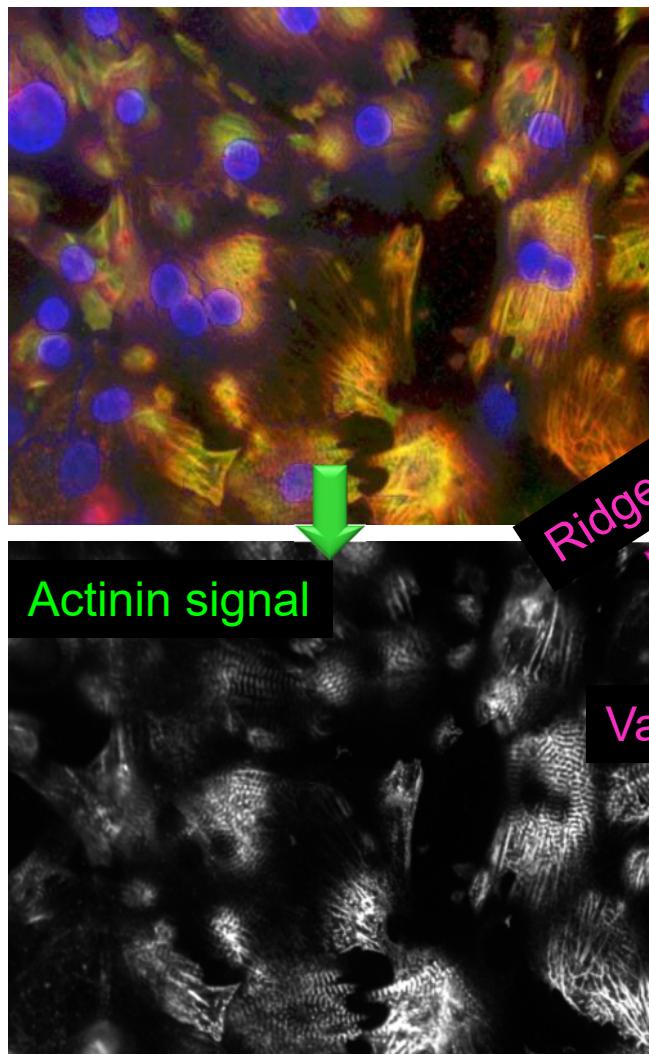
- Striped cells:
 - extended patches of parallel equidistant linear features
 - Not: homogeneous stain, spotted stain, star-shaped stain
- Object-based approach:
 - Stripe object lengths
 - Stripe object orientations
 - Stripe object distances
- Problem:
 - Very difficult and calculation intensive
 - Big bullet for «small» target



- Solution:
 - Linear features: texture filtering
 - Equidistant parallel features: Image auto-correlation / Fourier Transform

Sarcomere integrity image analysis

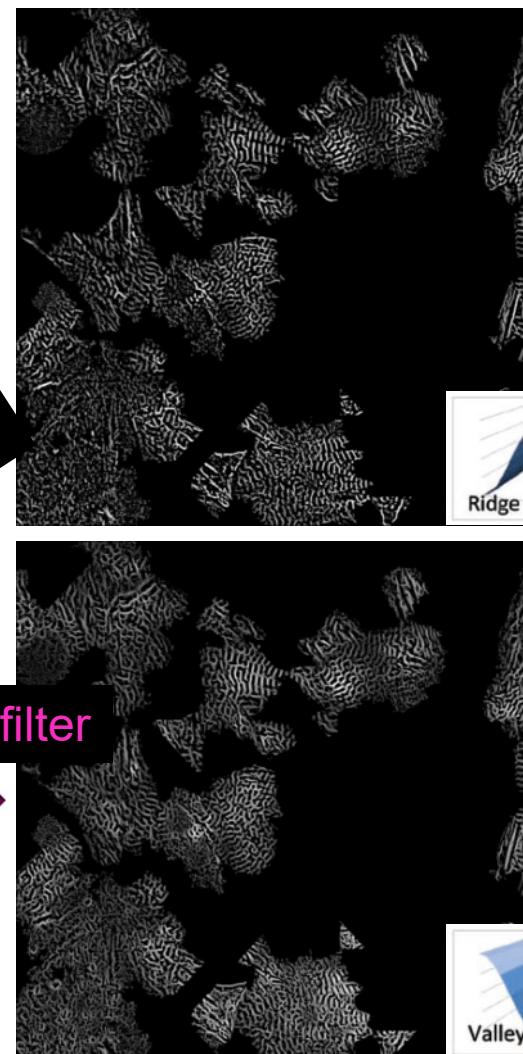
Ridge-Valley filtering



Ridge filter

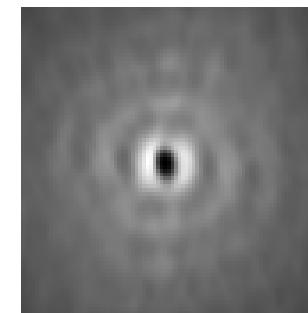
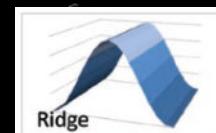
Actinin signal

Valley filter



Ridge-valley cross correlation features:

- Modulation amplitude: sarcomere strength
- Modulation frequency: sarcomere length



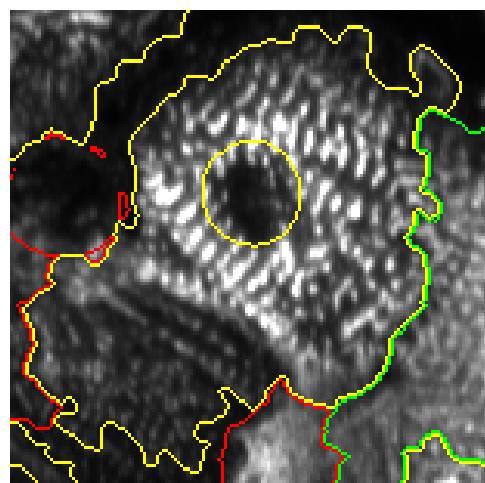
Whole image

- No cell classification
- No correlation with other cellular features

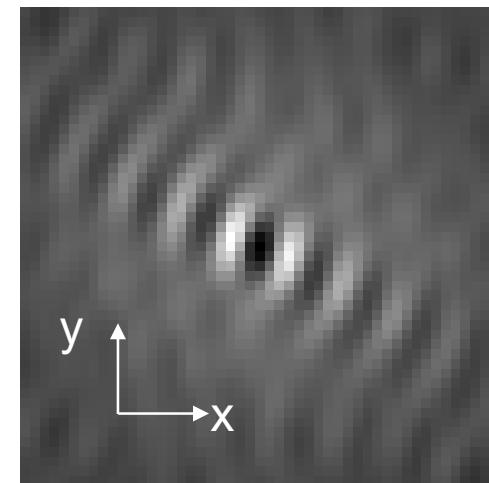


Sarcomere ICCS analysis: per individual cell

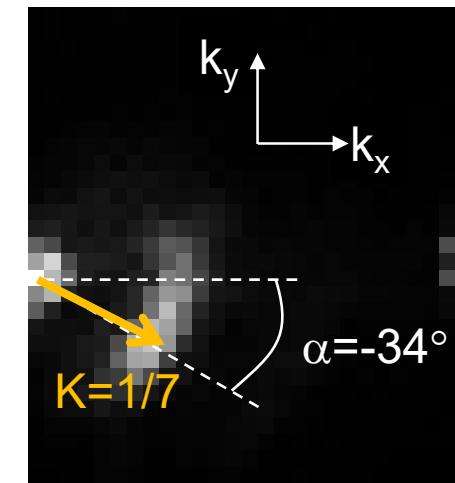
Actinin image



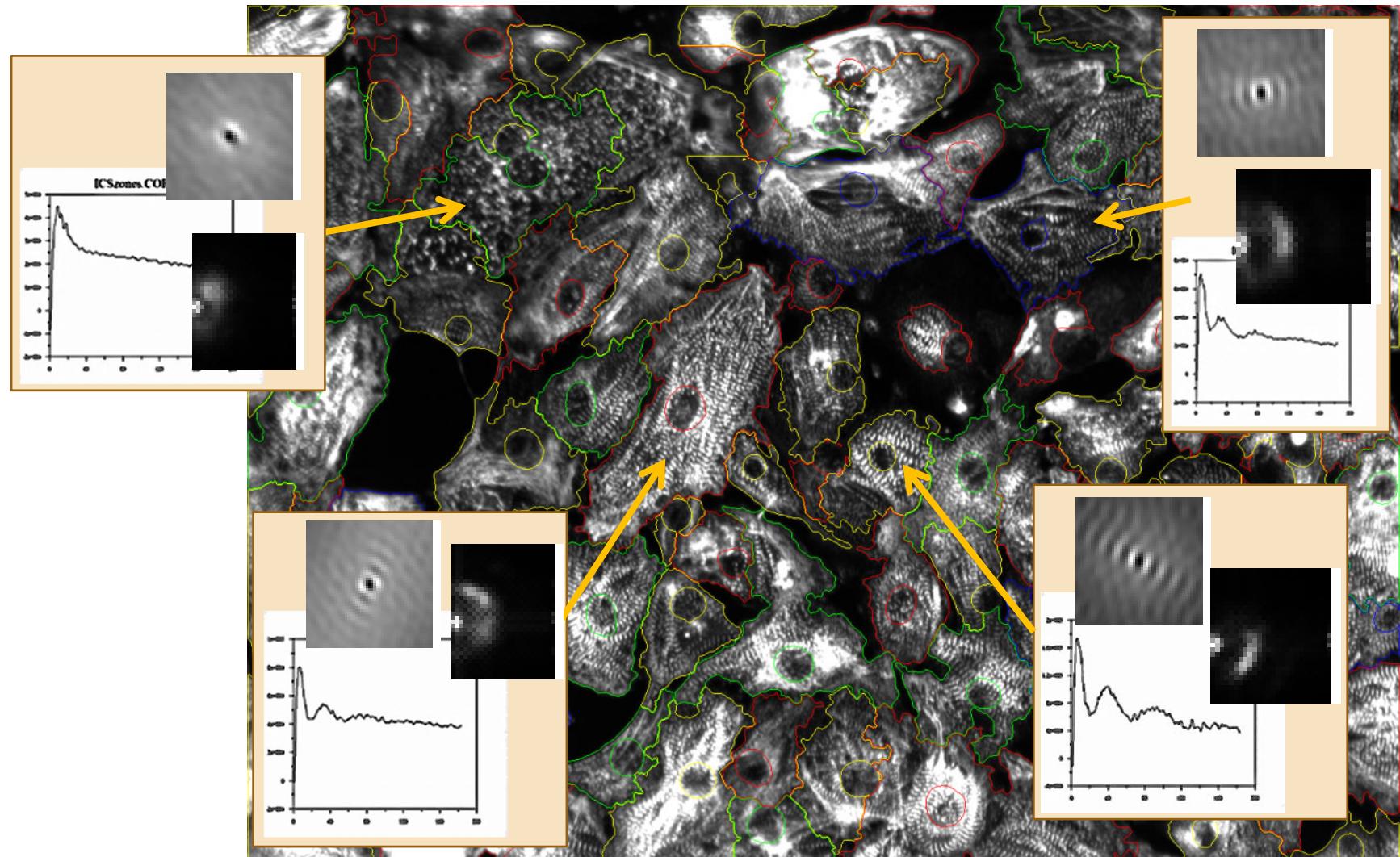
R-V cross correlogram



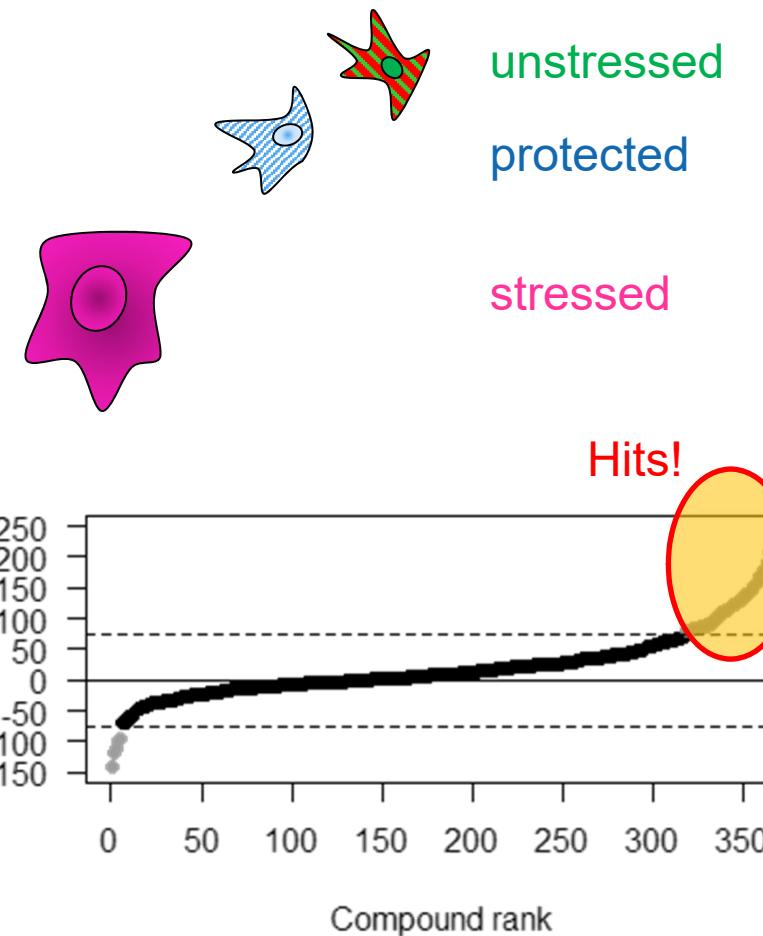
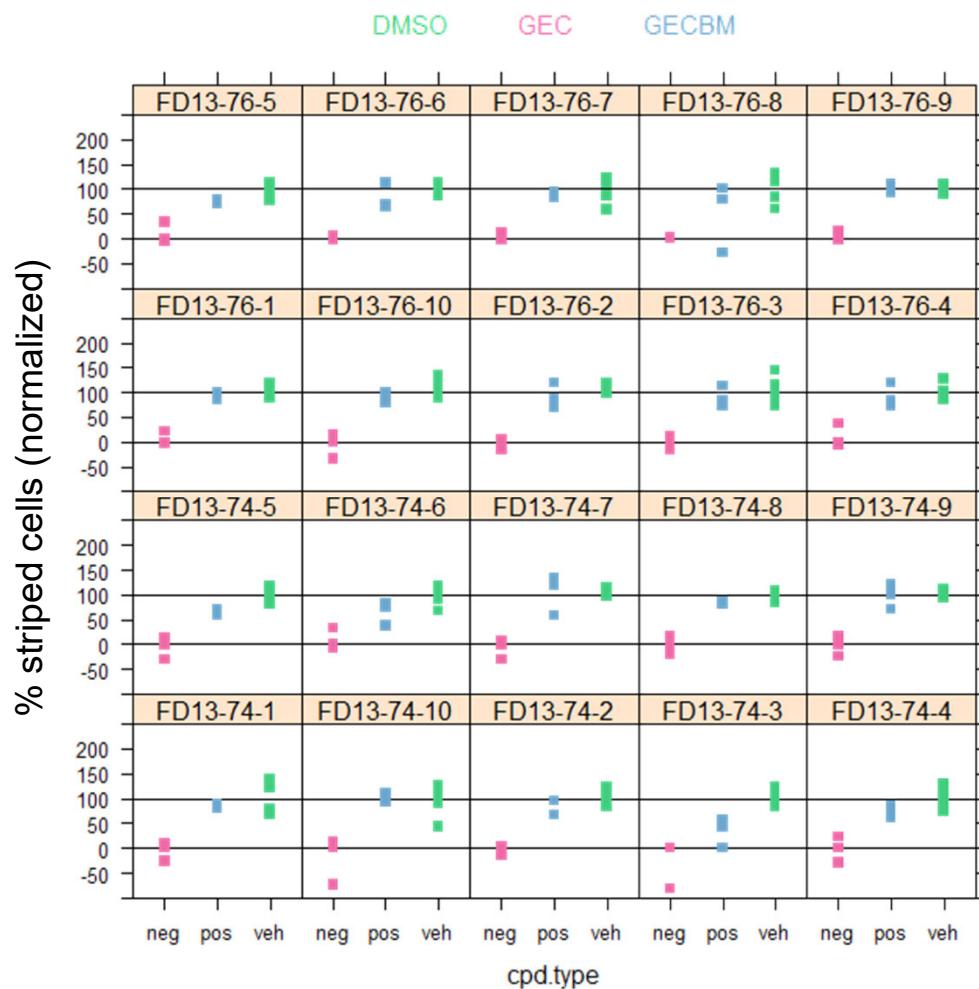
R-V cross spectrogram



Sarcomere ICCS analysis: per individual cell



Sarcomere integrity (SARITY) screen summary



Summary

- Introduction to image-based screening (HCS) in drug discovery
- Requirements for image analysis in screening
- Prototypic HCS campaign:
 - Modern cellular disease model
 - Phenotypic assay development
 - Tailored image analysis for feature generation
 - Quality control of HCS results