

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

Disclaimer

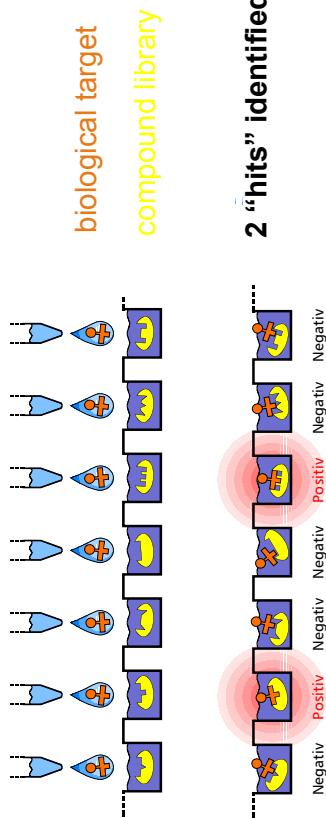
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What is High Throughput Screening (HTS)?

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

Q: What is High-Content Screening

- A: automated (fluorescence) microscopy (of cultured cells *in vitro*) in combination with automated quantitative image analysis

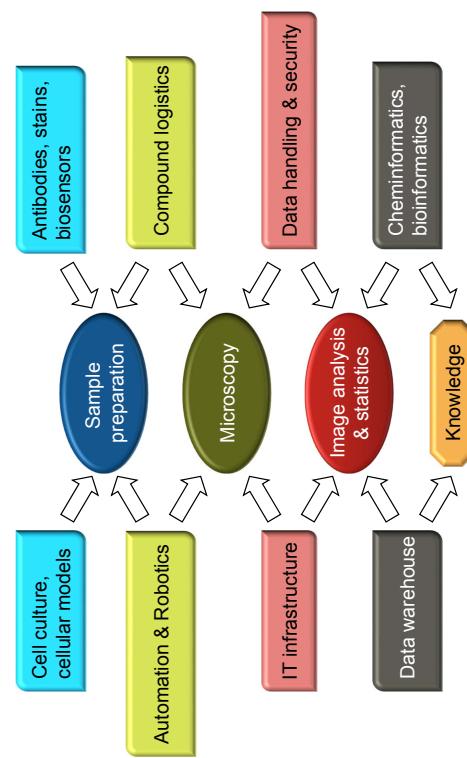
▪ Q: what does "high-content" mean?

▪ 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

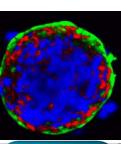
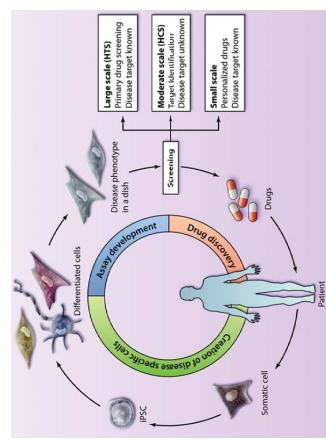


Q: What is an assay?

- 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



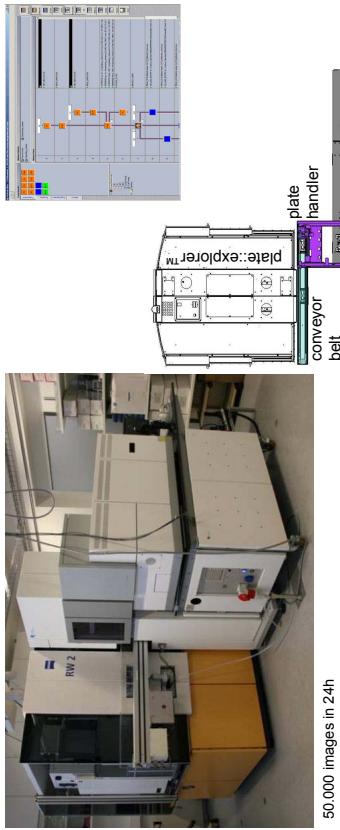
The modality of choice:
High-Content Screening

Image analysis in HCS mode Requirements

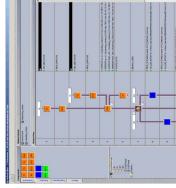
- Throughput per image field:
 - 1 s^{-1} for screening (HT)
 - 0.01 s^{-1} for profiling (MT)
- Robust without human intervention:
 - Heterogeneous cell population
 - Training data are never representative
 - Always expect the unexpected
 - Often low signal to noise, variable background

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The HCS facility at Roche Basel

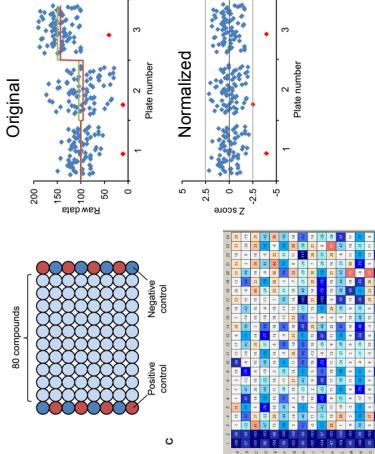
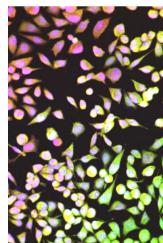


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HCS: all is not well

Correction of systematic errors

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



Original

Normalized

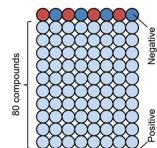
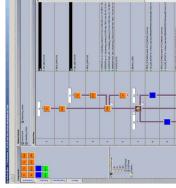


Image analysis in HCS mode

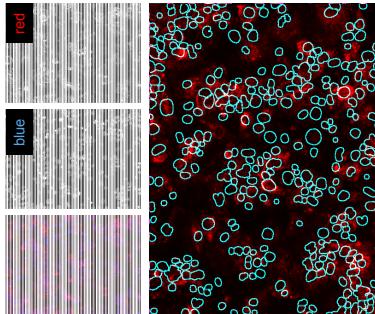


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Image analysis in HCS mode

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

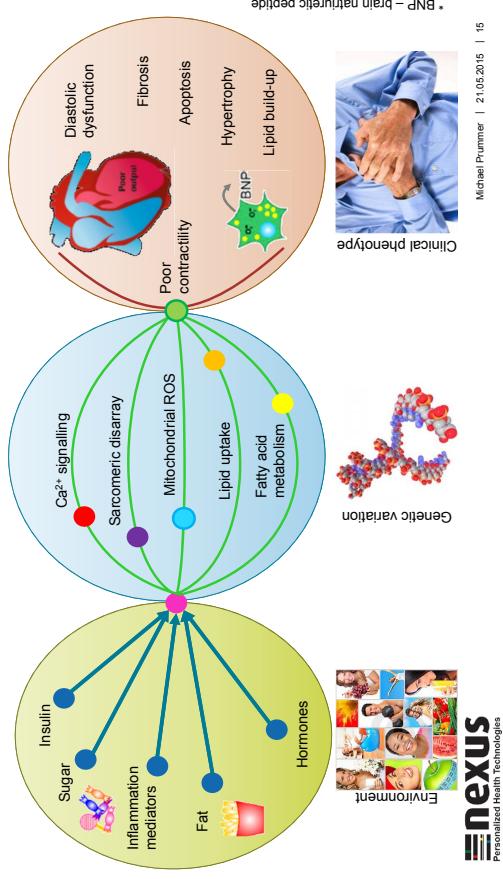


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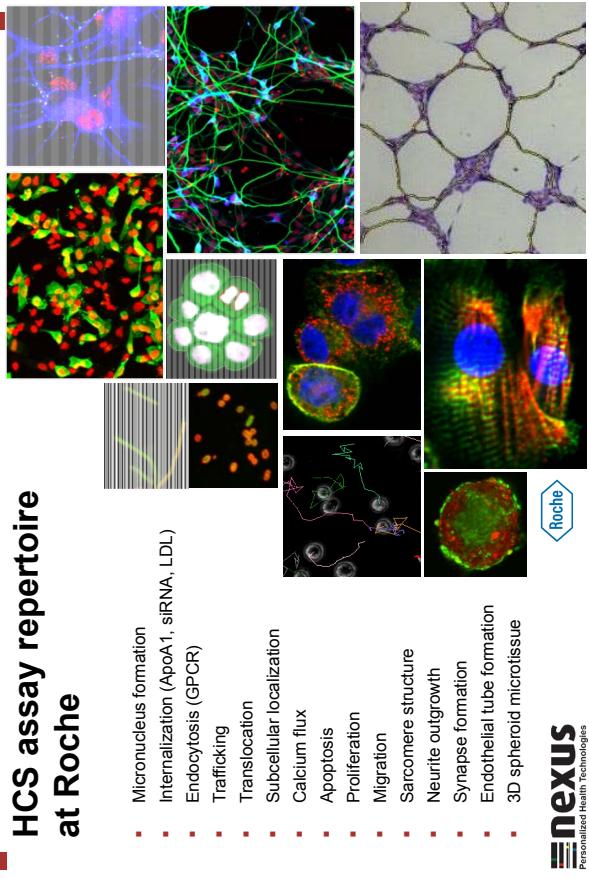
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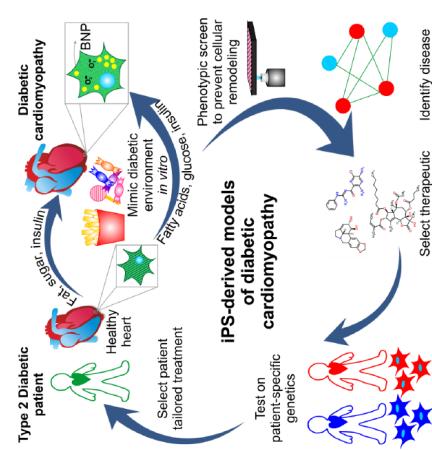
Diabetic cardiomyopathy Multiple inputs, multiple outputs



HCS assay repertoire at Roche



Diabetic cardiomyopathy modeling «Patient in a dish»



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

Cell Reports 9, 810–820, November 6, 2014
OPEN ACCESS
Cell Reports

Fay M, Drawnel S, Boccardo M¹, Michael Prummer¹, Frédéric Belobal¹, Alexandra Graft¹, Michael Weber¹, Elisa Reginato¹, Barbara L. Brune¹, Daniel J. D’Amico¹, Sean J. Murphy¹, Mark B. Tuck¹, Mark A. Kotilinek¹, Gianni Cicali¹, Gianluca Martorana¹, Barbara Bozzi¹, Henning Stahlberg¹, Benjamin J.J. Hall¹, Maria Chiara Magrone¹, Kylie Kohli¹, Kenneth H.C. Chien¹, Jacques Bailey¹, and Roberto Racine^{1*}.
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²Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA
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<http://dx.doi.org/10.1016/j.celrep.2014.09.055>

Image analysis in HCS: a case study

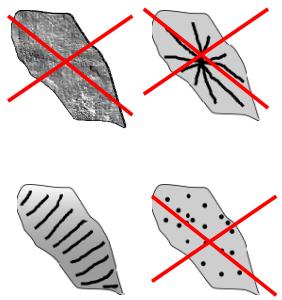


Cell Reports
Report

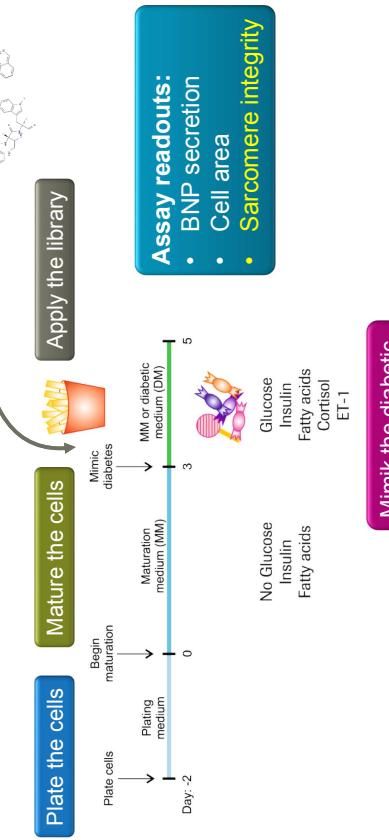
Image analysis: identify myocyte sarcomeres Concept

- 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

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The sarcomere integrity assay Principle



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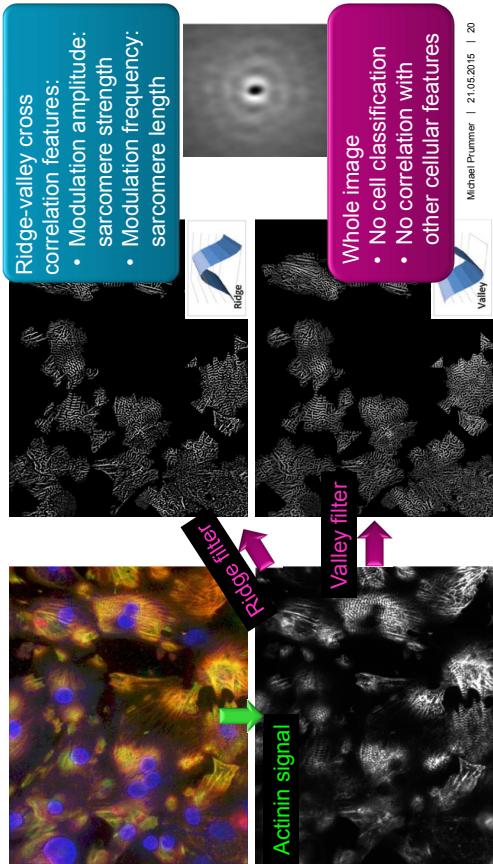


Assay readouts:

- BNP secretion
- Cell area
- Sarcomere integrity

Stem cell-derived cardiomyocytes

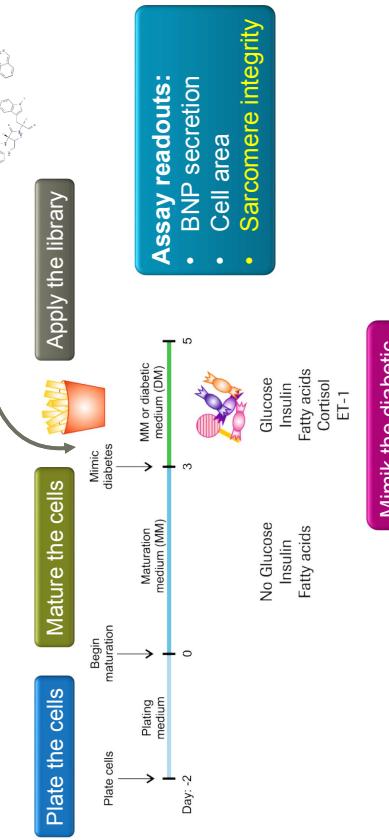
Sarcomere integrity image analysis Ridge-Valley filtering



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Stem cell-derived cardiomyocytes

The sarcomere integrity assay Principle



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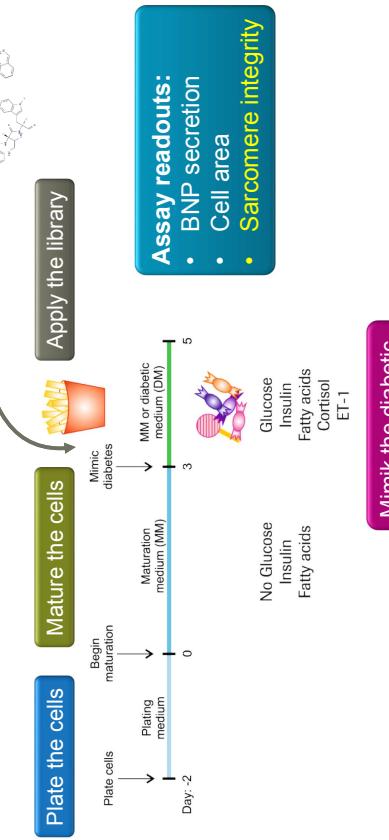


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Stem cell-derived cardiomyocytes

Sarcomere integrity (SARITY) screen summary

