Center for Cellular Construction

Project 5 – Cell State Inference Engine

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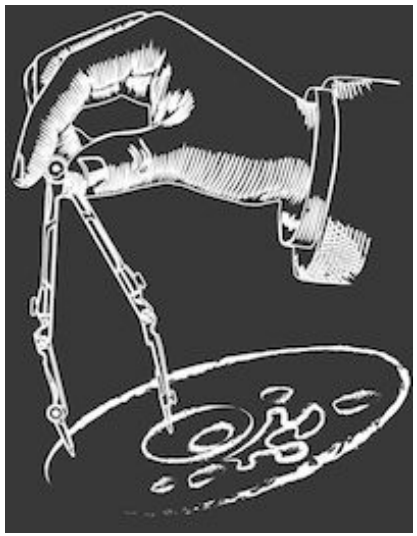
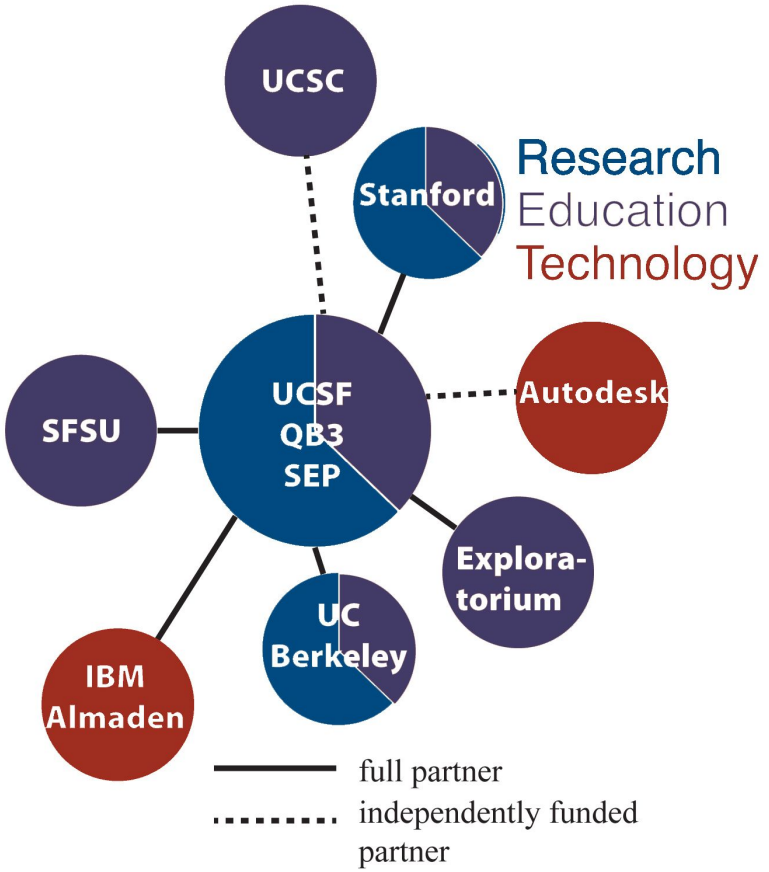




CellCAD (project 2) vs Inference Engine (project 5): Two ways to leverage predictive models of cell organization

Forward problem: Build predictive models from hypothetical control mechanisms (Project 2)

- Dynamical systems based approach
- Large parameter space sampling
- Noise and control
- "Local to global" model extrapolation

Inverse problem: Start from data to infer relevant mechanisms and parameters (Project 5)

Complementary, synergistic approaches to predict cell type, state and function!

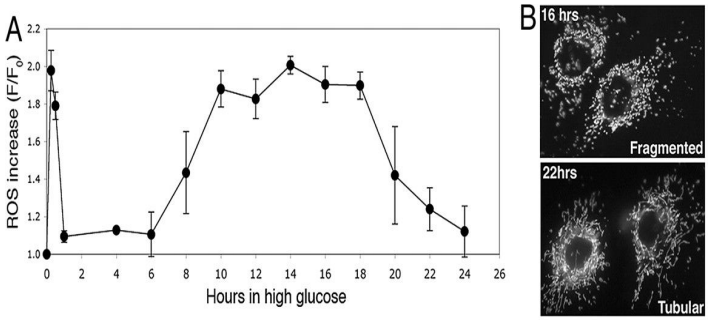
The cell sensing machinery

Cells are bristling with an array of sensitive and specific biosensors

Cell interior is full of signaling pathways that response to environmental and internal metabolic state.

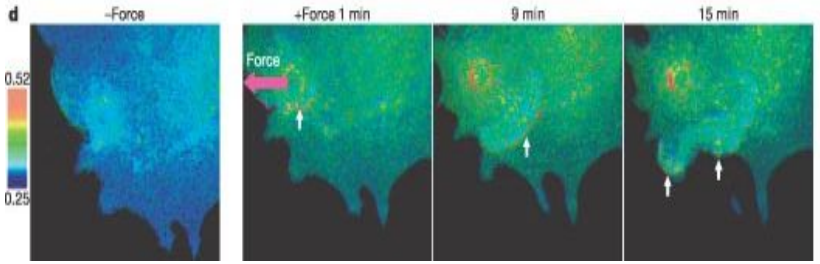
Examples:

Chemical state
Sugar, Oxygen alter mitochondrial structure



Yu, et al, PNAS 103 (2005)

Mechanical state
Extracellular stress alters focal adhesions



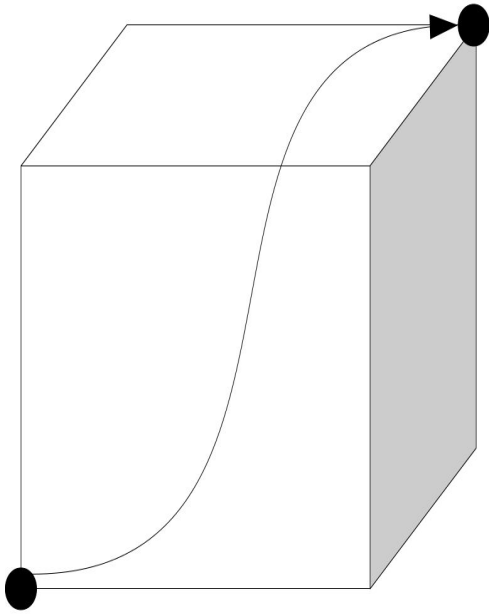
Wang, et al, Nature 434 (2005)

Organelle morphology:
A multi-mode reporter of cell state and environment

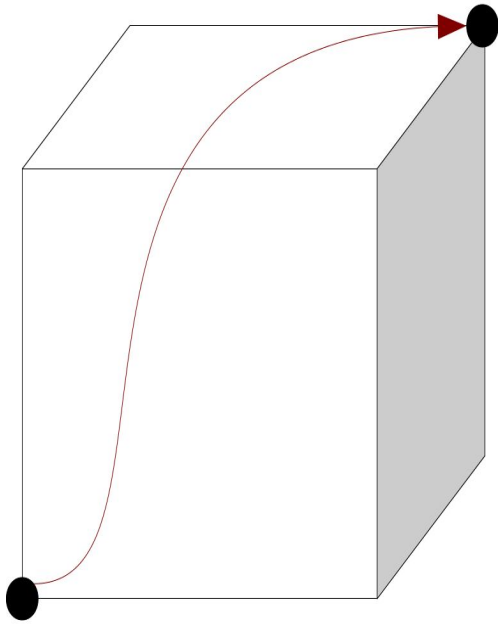
- Organelles as "containers" of chemical reactions
- Organelles' morphology connected to how cells sense (i.e. state and function)
- Little mechanistic understanding of how cells modulate their morphology in response to specific environmental stresses
- Overall cell morphology as a proxy for cell state and type:
    - Modifications of single organelle morphology
    - Modifications of organelles morphology wrt one another
    - Modifications of whole cell architecture

State-space description of cells

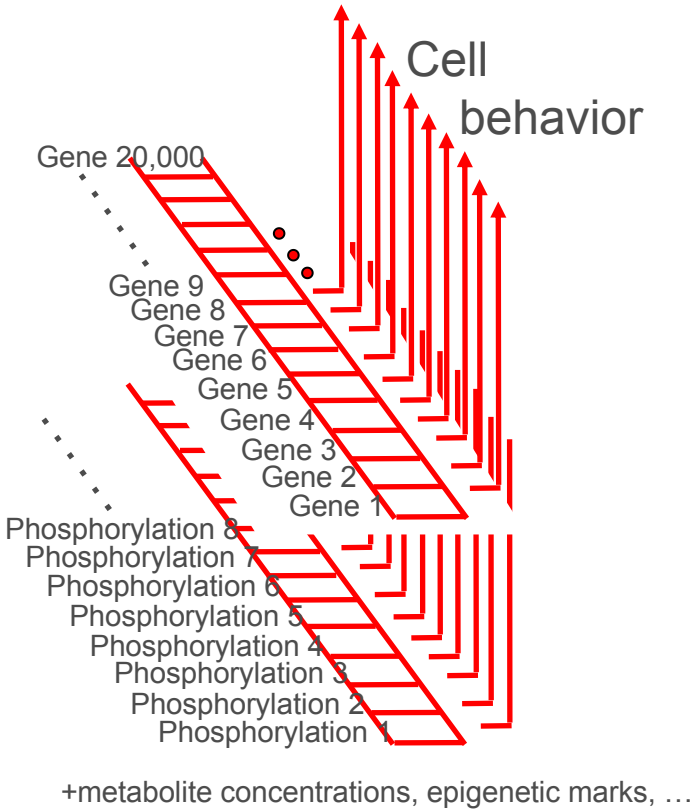
Molecular phase space



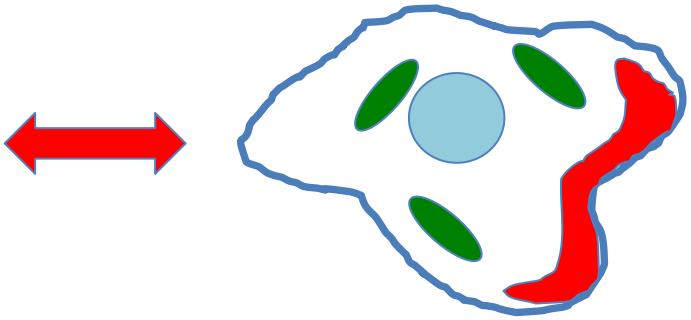
Morphology phase space



Organelle morphology as a reduced dimensionality state space



Microscopic State Variables



Organelle-level State Description

No technology to measure all microscopic state variables at once

Technology to measure organelle-level state already exists = microscopy

A dynamical systems view

Map cell state from data onto a multidimensional "morphology" phase space

Track morphology vectors

- -in time
- -as function of targeted perturbations (broad molecular screens)
- -as function of control initial states (single, double deletions)
- etc.

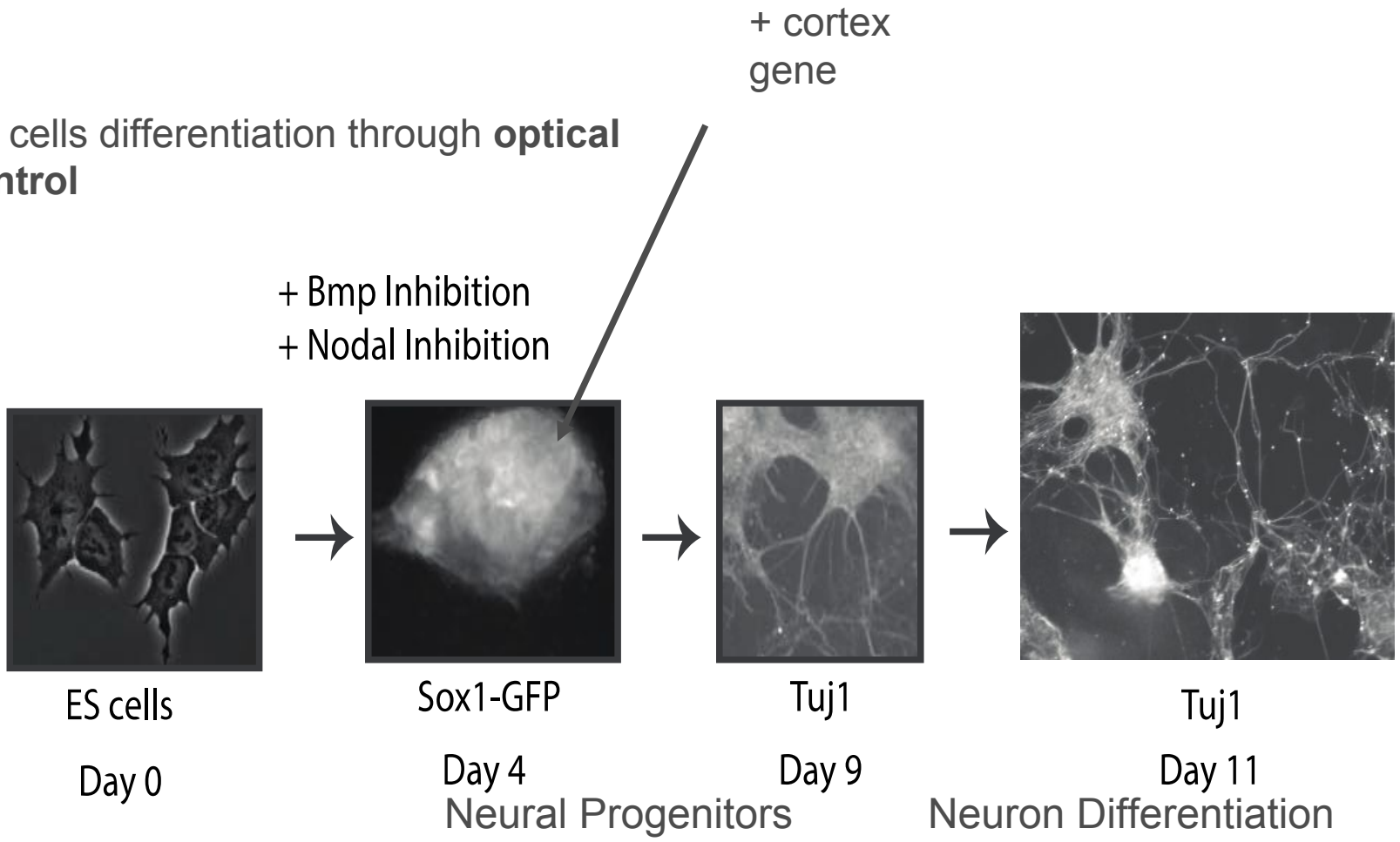
Identify crucial parameters (and combinations thereof) from data

- -accuracy
- -sensitivity
- -control

Generate minimal basis set of identifiable cell structures

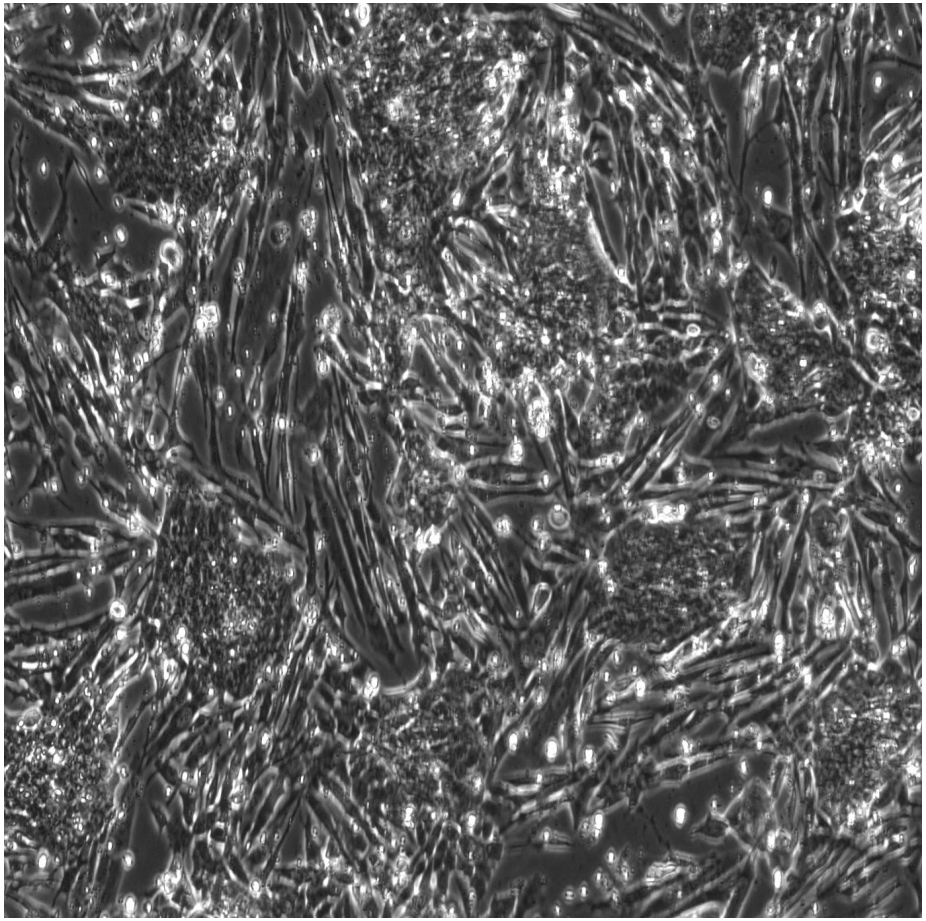
Predicting cell type from organelle morphology

ES cells differentiation through **optical control**



Matt Thomson

Induce muscle differentiation



- ES cells change dramatically during differentiation
- Fine tuned controls at hand
- Time and space controllable system
- Morphology could indicate cell type
- Predictive algorithm from data

Matt Thomson

Technical requirements

Render organelles visible
- Antibody stains – specific but costly
- Chemical stains – less specific but lower cost, often require live-cell imaging
- Engineered cells expressed fluorescent tagged constructs

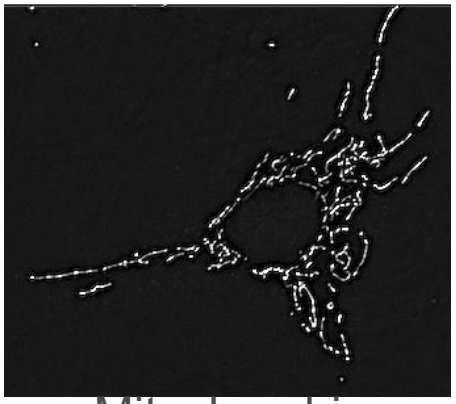
Imaging
- Live-cell fluorescence (OMX)
- High-throughput fluorescence of fixed cells (InCell 2200)
- Super-resolution 3D (OMX)

Extract numerical descriptors of organelle size and shape
- Custom image analysis workflows
- GE InCell image analysis software

Estimation of cell state parameters from organelle data
- Model inversion
- Big data strategy (Cellular Facebook)

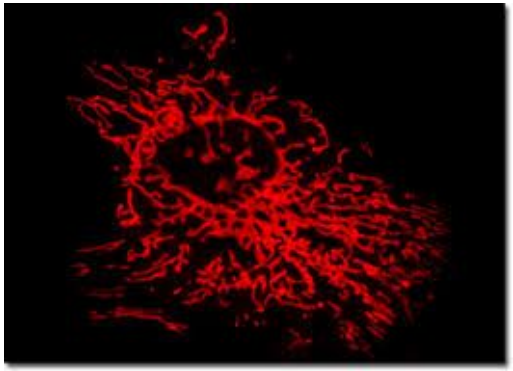
Examples of organelle-specific stains

Antibodies



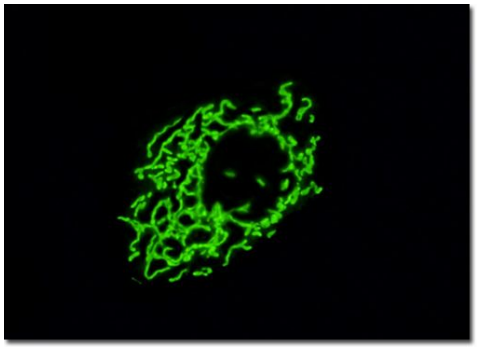
Mitochondria
anti-mtHSP70

Dyes

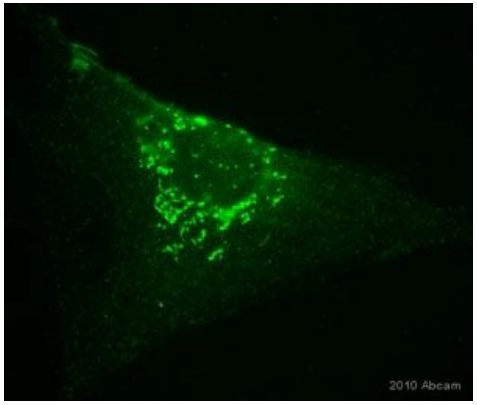


Mitochondria
mitotracker

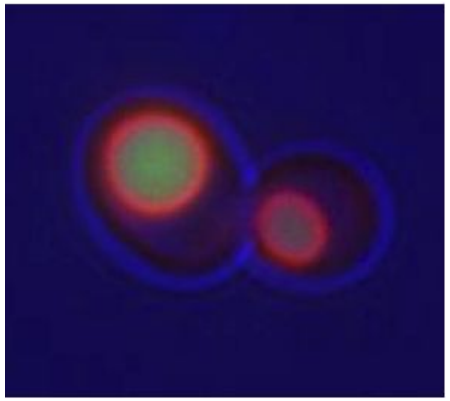
Fluorescent protein



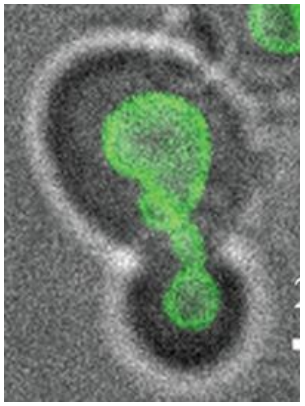
Mitochondria
Mito-targeted eGFP



Golgi:
Anti-GM130



Vacuole:
FM4-64

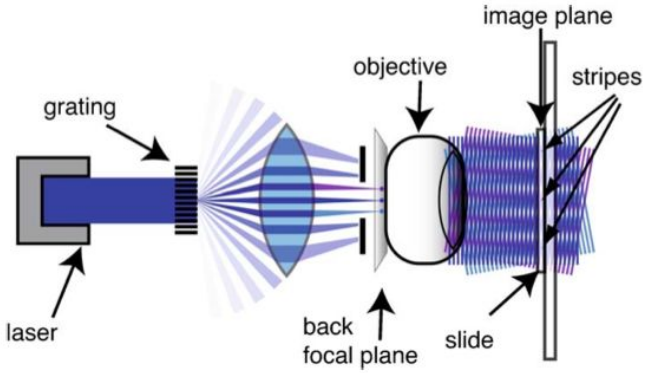


Vacuole:
VPH1-GFP

InCell 2200:
High throughput automated imaging

OMX:
Live-cell and super-resolution 3D imaging

Structured Illumination Microscopy



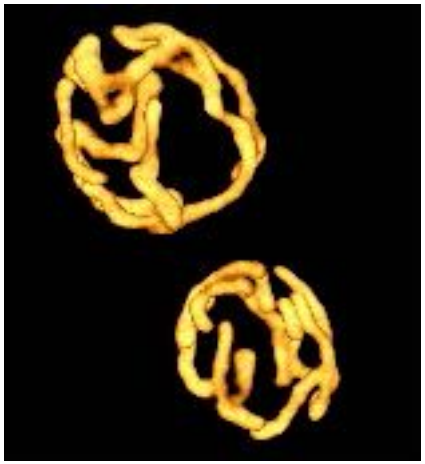
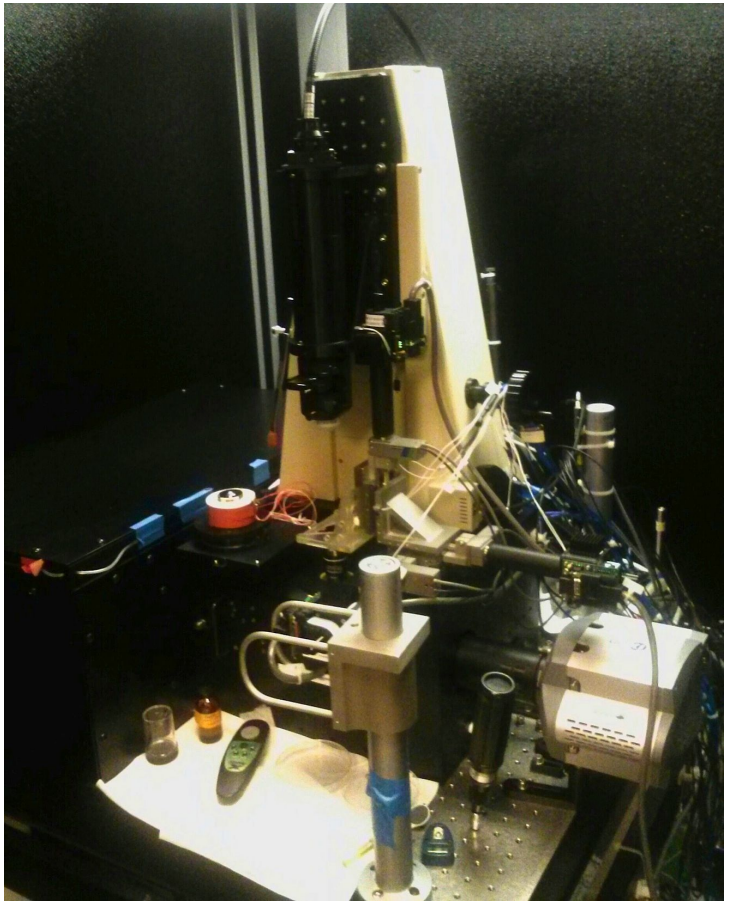
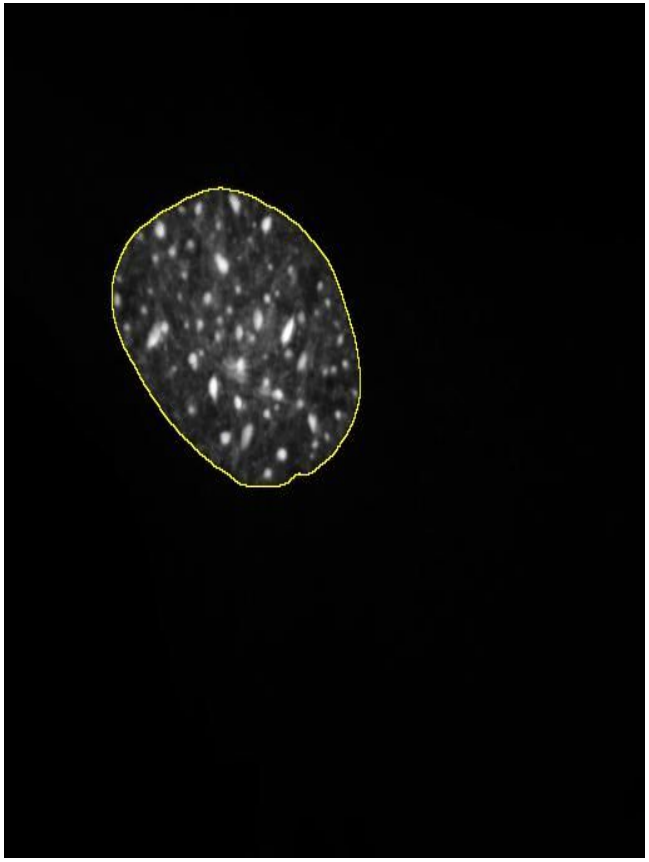


Image from Susanne Rafelski, Marshall Lab



Jennifer Fung

Quantitative metric through automatic segmentation algorithms

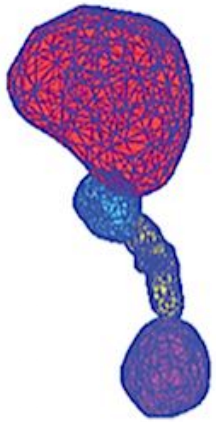


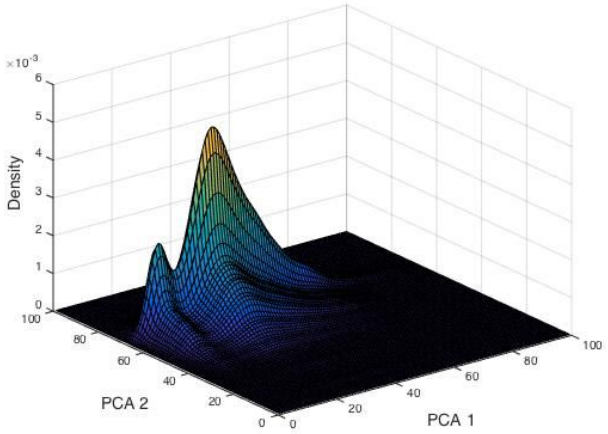
2D-3D Segmentation algorithms can provide precise quantitative information about organelle morphology

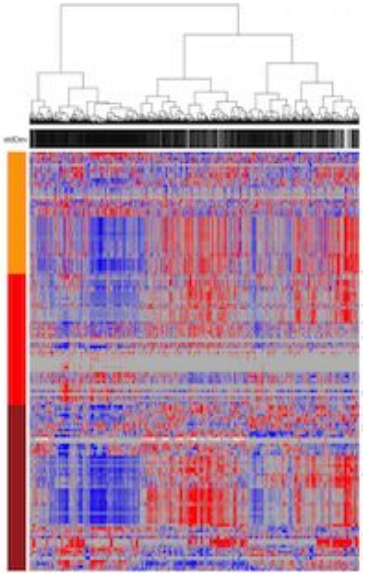
~ 100s measurements per organelle (many not independent)

- Size [volume, surface, feret diameter,…]
- Shape [solidity, eccentricity, branches,…]
- Ratios relative to other organelles

From morphology vectors to cell state





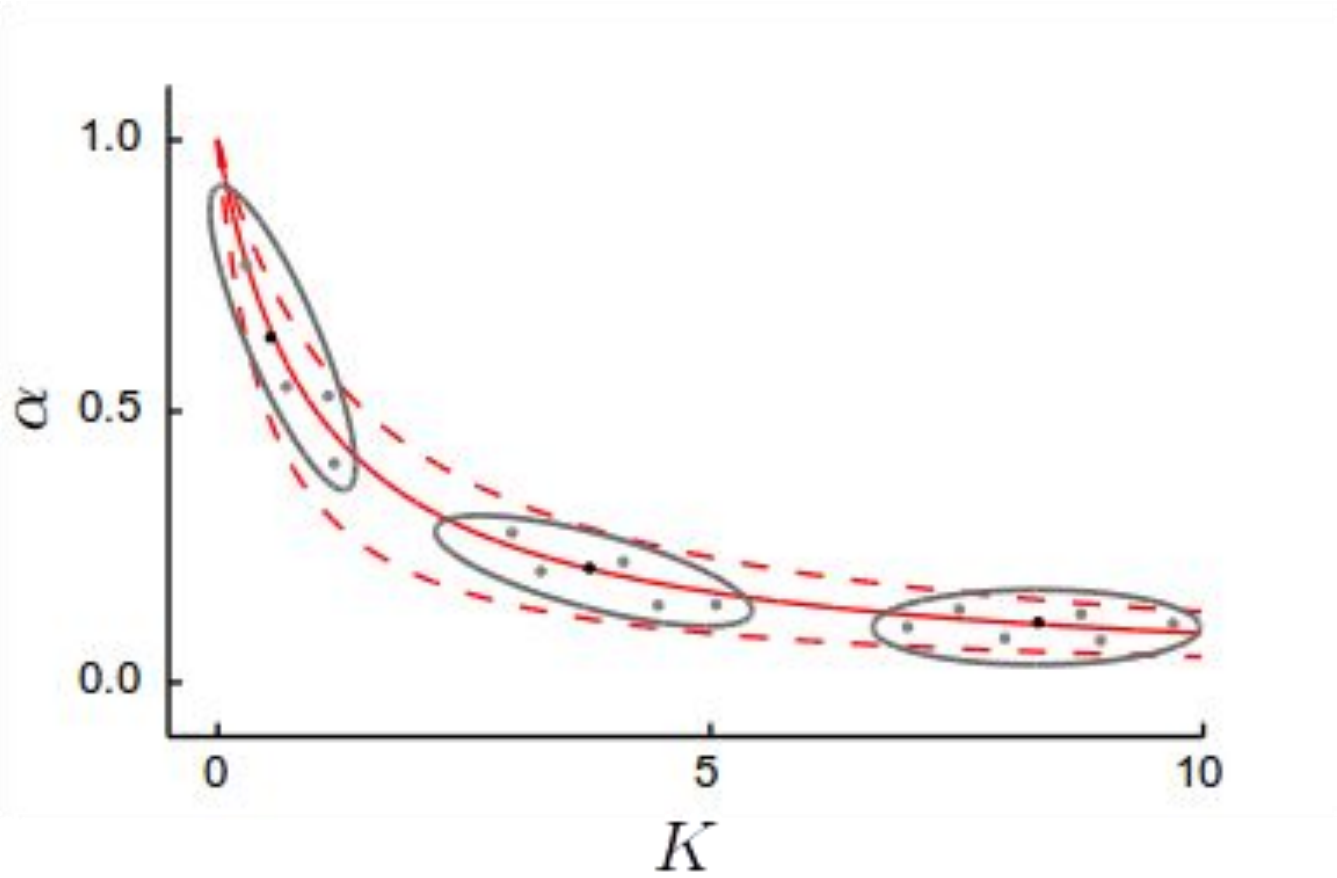


Compute n-dimensional description of each cell from image data

Dimensionality reduction (PCA, LDA, etc)

Define states by clustering, machine learning

Model-inversion approach to infer cell state "think globally, act locally"



See Project 2 - identify manifold of parameter-space points consistent with data

Relate model parameters to metabolic and environmental indicators

Big data approach to infer cell state "Cellular Facebook"

Generate large number of cell states, types, functions

"Periodic table" of cell states and types to be used for **classification**

Orthogonal integration of other datasets generate complete ontology

- genetics
- molecular
- chemical-bioproducts
- environmental conditions

Trained algorithms to provide new analytic capabilities beyond grant scope

Real World applications

- New reporters in bioreactors during process development
- Monitoring bioremediation (pollutant disposal)
- Response to threats by sensing toxins (spirotox, alteration of morphology through metabolism)
- Sentinel cells in food (e.g. melamine in milk)
- Potential applications in tele-cytopathology / digital medicine

Cellular Sentinel Workflow

Collect samples, fix on-site

Send to Central facility

Automated staining

Automated microscopy

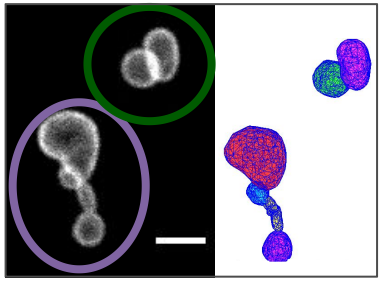
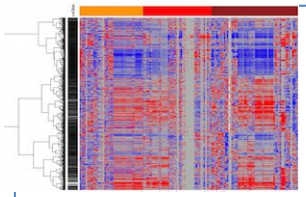
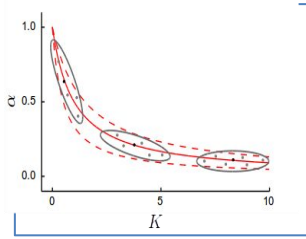


Image analysis

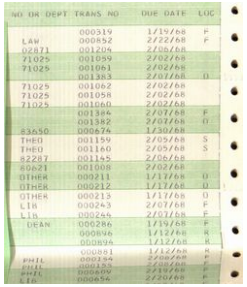
Inference engine



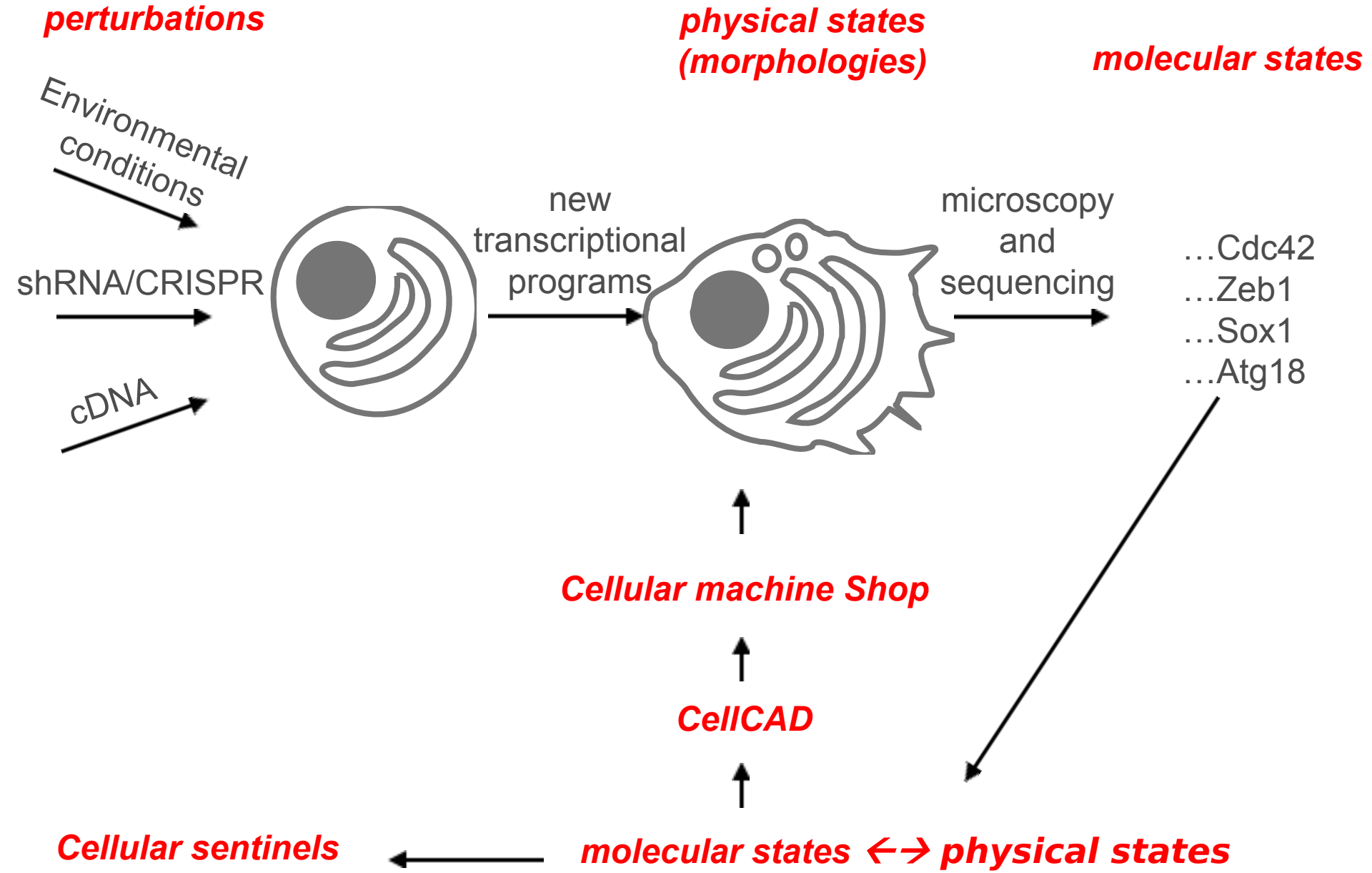
Big data (facebook)



Model inversion



Generate report for each sample, correlate cell state and morphology (eg. potential toxins, growth conditions, etc)


perturbations
physical states (morphologies)
molecular states
Environmental conditions
shRNA/CRISPR
cDNA
new transcriptional programs
microscopy and sequencing
…Cdc42
…Zeb1
…Sox1
…Atg18
Cellular machine Shop
CellCAD
Cellular sentinels
molecular states ←→ physical states

Cell-State Inference Engine

Wallace Marshall, Project Lead
Organelle image analysis and systems biology

Research Team
Simone Bianco, Inference algorithms from big data, linking cell data to genomics
Jennifer Fung, Live-cell and super-resolution microscopy
Matt Thomson, Information theory
Hana El-Samad, Network inference algorithms
Mark Chan, Image analysis, yeast cell biology
Sophie Dumont, Mechanbiosensors
Blake Riggs, Organelles during cell division
Sindy Tang, Microfluidics