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High-Content Screening

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High Content Screening (HCS)

- Using an automated imaging instrument to identify **conditions** causing a specific cellular **behavior**
 - **Conditions** are usually presence of small molecules compounds (potential drugs) or inhibitory RNAs
 - **Behavior** is usually a change in expression or subcellular distribution of one or more fluorescent probes (an assay)
- Primarily used to find possible drugs that affect a desired “target”



Example HCS Instruments





High Content Screening

- Referred to as “High Content” to distinguish it from “High Throughput” techniques (HTS)
 - rate at which *samples* are analyzed typically lower
 - but collected images have *greater information content* than readouts from enzyme or binding assays
- Term “High Throughput Microscopy” also used



Automated Analysis

- HCS implies online, automated image analysis to provide rapid determination of whether a given compound was “positive” or “negative” for a given assay (or the extent to which it was positive)
 - Typically integrated into the instrument



Robotics for HCS

- Sample handling robots used to store, dissolve and distribute small molecule compounds (Compound Management)
- Liquid handling robotics used to add desired dilution of compound to cells
- Robots often used to move and store plates





Assays

- In principle, an assay can take advantage of anything detectable by imaging
- In practice, typically use fluorescent probes to detect specific molecules or structures
 - Exogenous (added to the cells during assay)
 - Stains
 - Antibodies
 - Endogenous (present in the cell line being used)
 - Fluorescent proteins

HCS Image Analysis Flowchart

STEPS

After preprocessing (to normalize plates), segment to get single cell region. Orient cells by registration (if required).

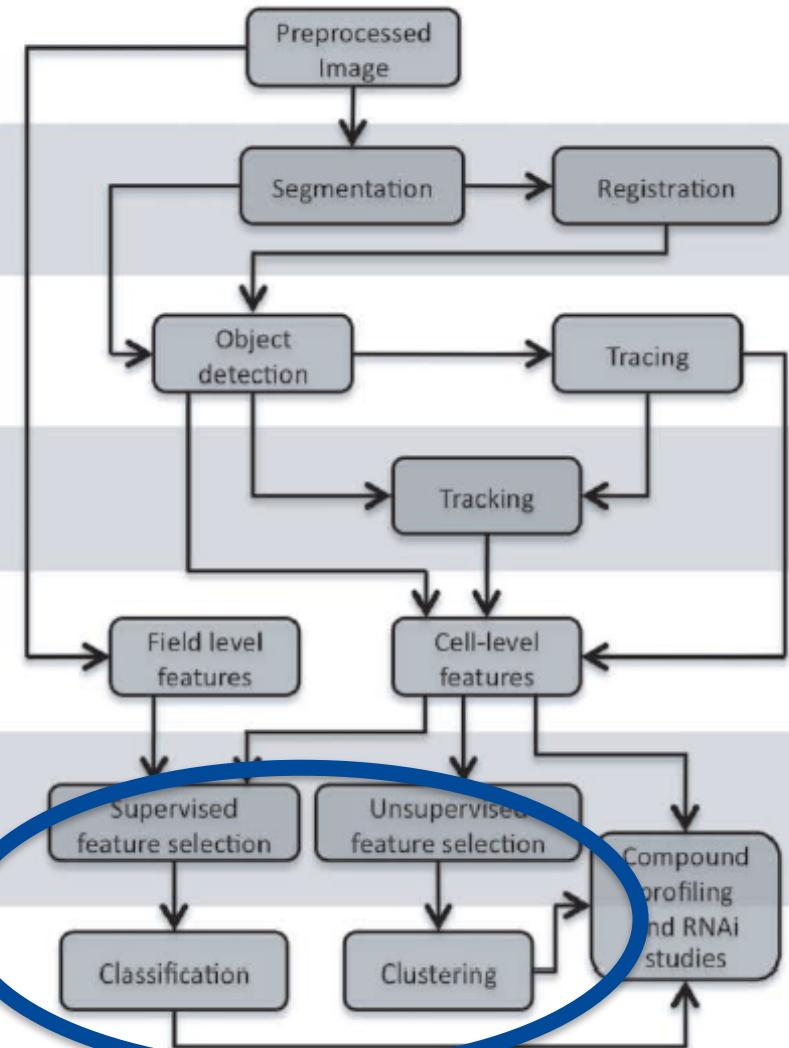
Use object detection to find organelles or to get nuclear shapes. If filamentous structures, use tracing.

If time series data, track to get location of each object or filament tip in subsequent frames.

Represent every image as a set of "Features", that can be computed on every cell or directly on the entire field image.

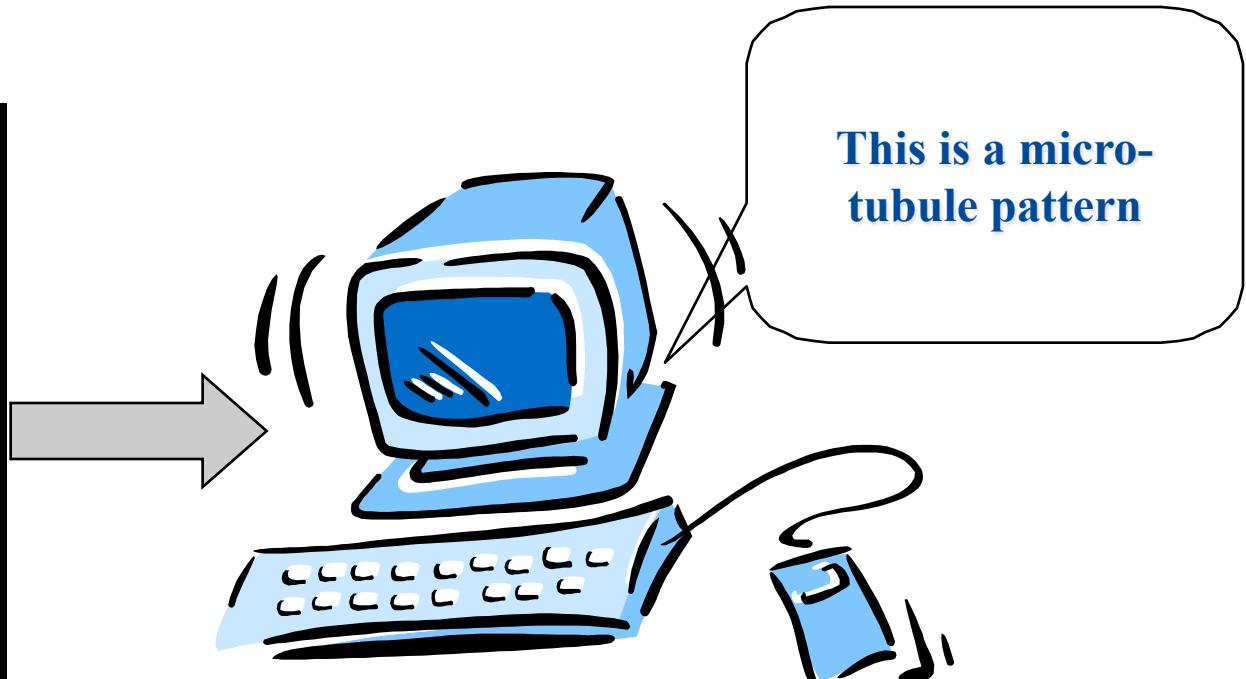
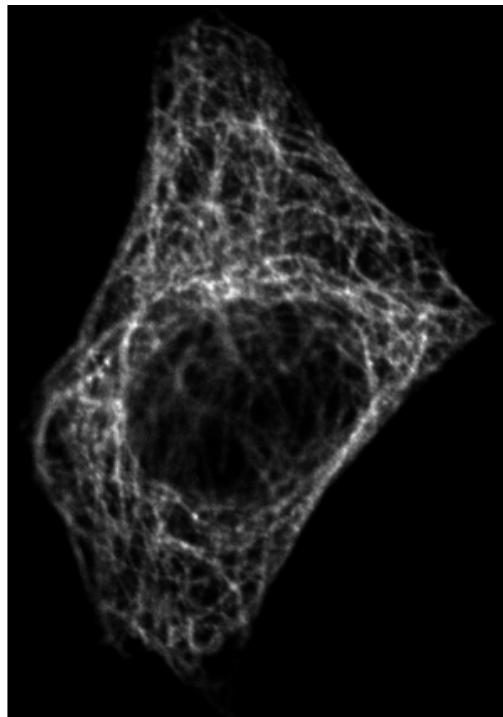
Using feature selection may improve classification accuracy on test images. Unsupervised feature selection can be used to remove redundant features and may provide a better grouping of phenotypes.

Automated learning: classification if phenotypic label or class (e.g. a cell pattern or cell state) is known. Cluster if looking for novel phenotypes.





What is Classification?



Automatically recognize major subcellular patterns in microscope images



Classification

- We have a set of **training data with known labels**
- We learn a model (classifier) from the training data
- We use the model to predict the label of unlabeled data (**test data**)



Clustering vs. Classification

- In clustering the labels of the data are not known beforehand
- Images of similar features are grouped together to form clusters

Example: Single Linkage Clustering

Data: 1 5 13 20 25

Cluster: 1 2 3 4 5

Merge Pair: [1, 5]

Cluster: 1 1 3 4 5

Merge Pair: [20, 25]

Cluster: 1 1 3 4 4

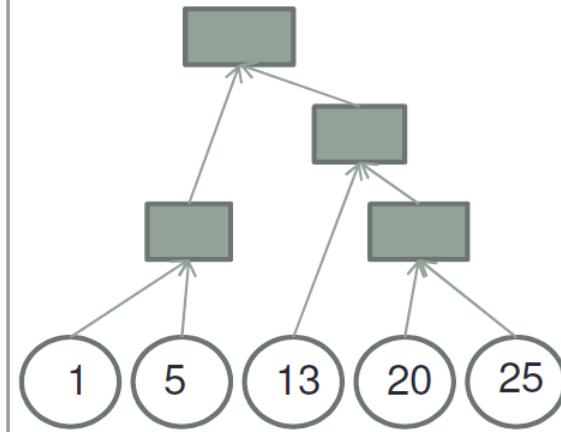
Merge Pair: [13, 20]

Cluster: 1 1 3 3 3

Merge Pair: [5, 13]

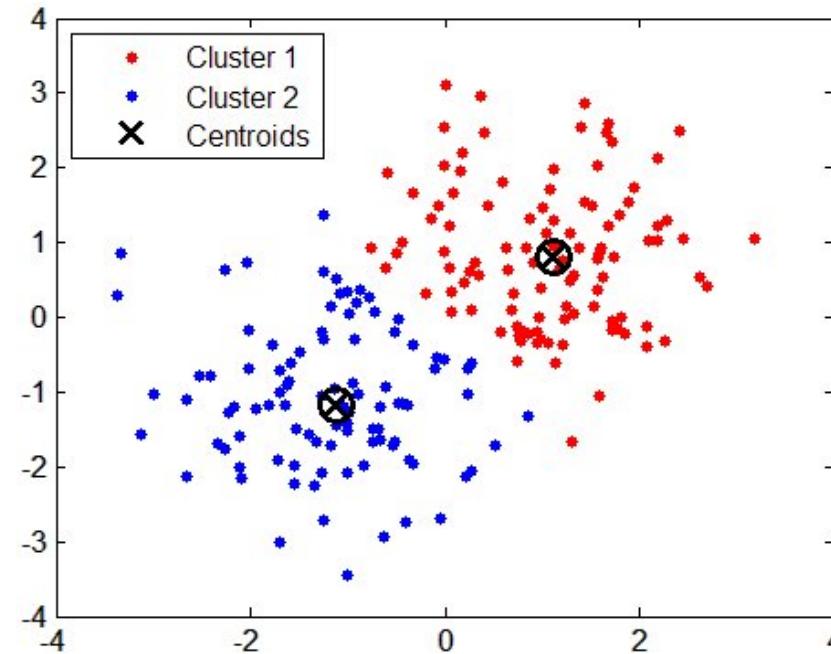
Cluster: 1 1 1 1 1

	Distance matrix					
	1	5	13	20	25	
1	-	4	12	19	24	
5	4	-	8	15	20	
13	12	8	-	7	12	
20	19	15	7	-	5	
25	24	20	12	5	-	



K-means Clustering

- Initialize K-means
 - Pick k random means
- Iterate until Convergence
 - assign points to closest mean
 - update means



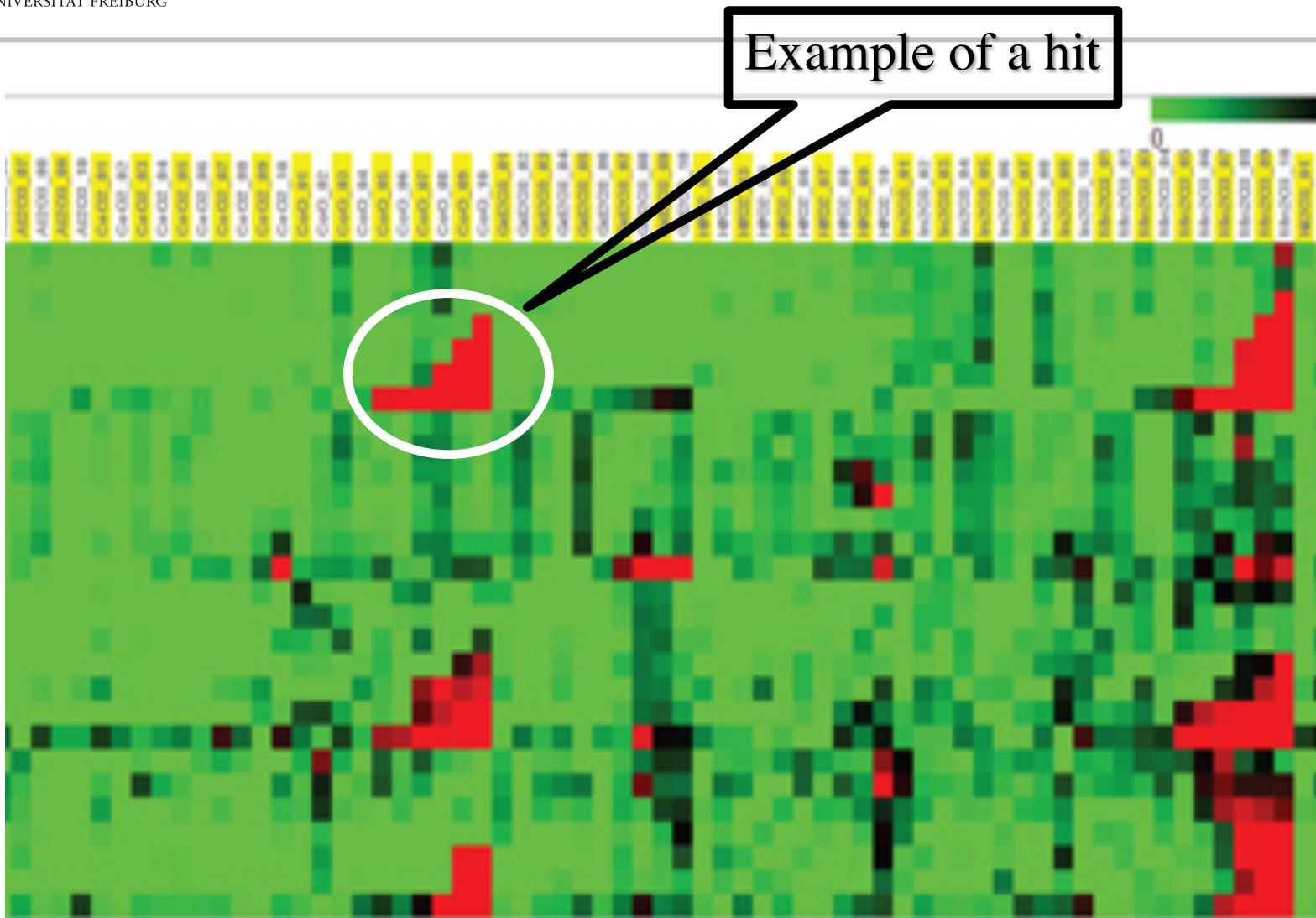


Evaluation of HCS assays

- Evaluate classifiers by confusion matrices, precision/recall, F measure
- Results typically expressed as heat maps showing positives and negatives



HCS Heat Map





Example of HCS Applications

- Learn which genes affect cell morphology

A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes

Zheng Yin^{1,2,6}, Amine Sadok^{3,6}, Heba Sailem³, Afshan McCarthy³, Xiaofeng Xia^{1,2}, Fuhai Li^{1,2}, Mar Arias Garcia³, Louise Evans³, Alexis R. Barr³, Norbert Perrimon⁴, Christopher J. Marshall³, Stephen T. C. Wong^{1,2,5,7} and Chris Bakal^{3,7}



Example of HCS Applications

- The way in which cells adopt different morphologies is not fully understood.
- Cell shape could be a continuous variable or restricted to a set of discrete forms.
- Yin et al. developed quantitative methods to describe cell shape and show that Drosophila haemocytes in culture are a heterogeneous mixture of **5 discrete morphologies**.





Example of HCS Applications

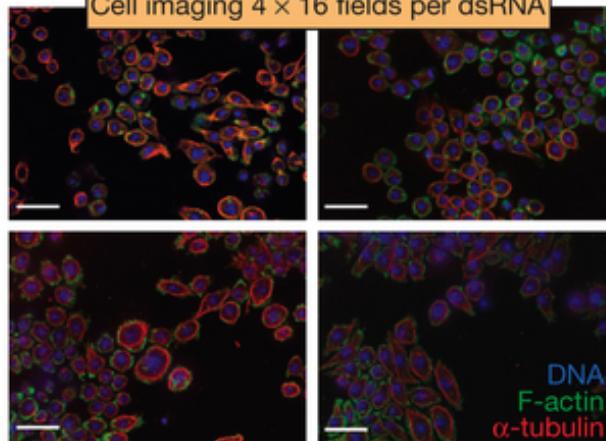
- HCS screen type:
 - First five discrete cell shape clusters were identified using clustering
 - RNAi screen of genes affecting the morphological complexity of heterogeneous cell populations
 - Isolation of a conserved gene network that regulates contractility and protrusion in *Drosophila haemocytes*
 - Verification of the gene network on human and mouse melanoma cells



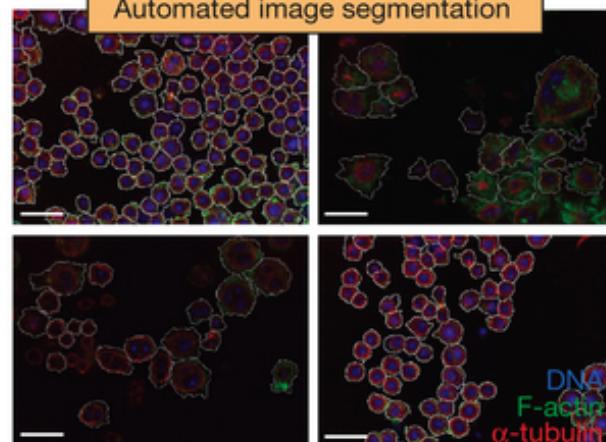
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899 dsRNAs targeting *Drosophila*
kinases and phosphatases

Cell imaging 4 × 16 fields per dsRNA



Automated image segmentation



Feature extraction

Plate	Rep	Well	Site	Cell	f1	f2	f3	...	f211
401	1	A1	1	1	34.5	-19.7	0.2	...	
401	1	A1	1	2	27.5	-12.3	0.1	...	
...

Yin et al (2013) Nature Cell Biology

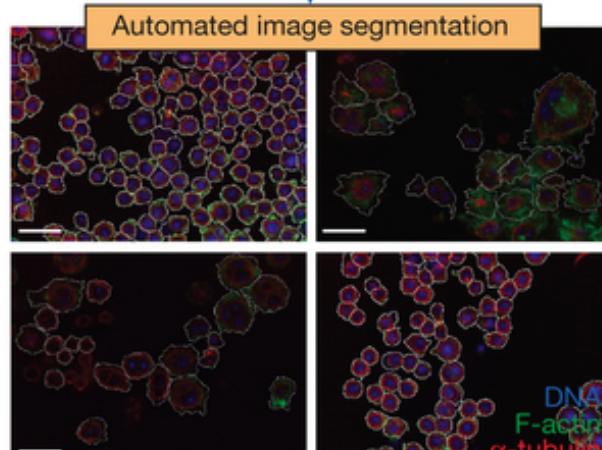
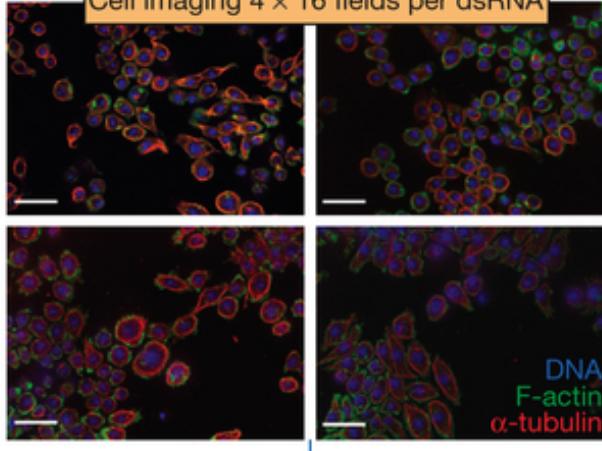
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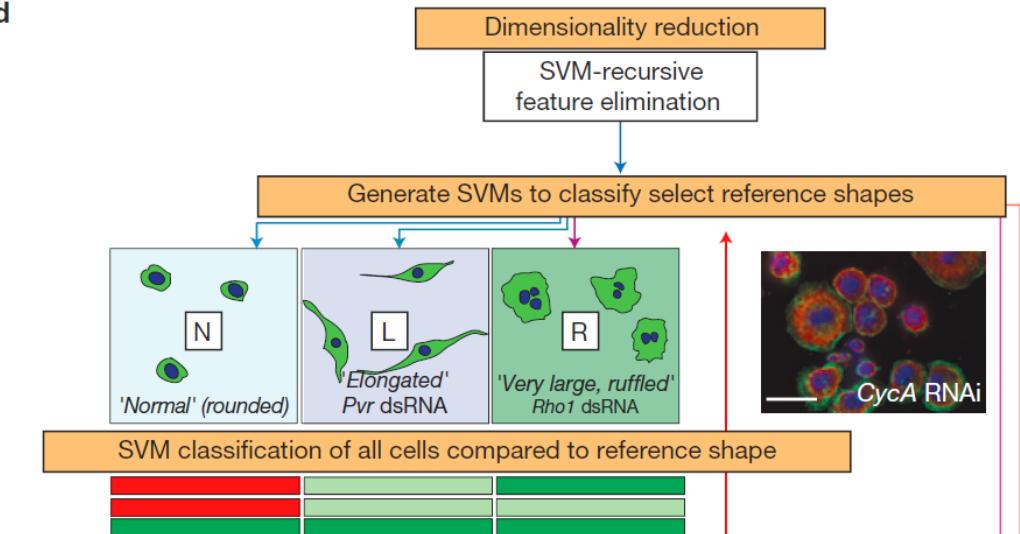
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Automated image segmentation

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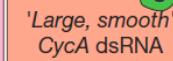
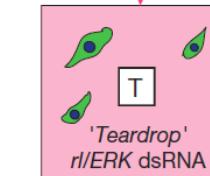
d



e

SVM classification of all cells compared to reference shape

Online shape detection



f

Unfiltered QMSs for single cells/dsRNAs

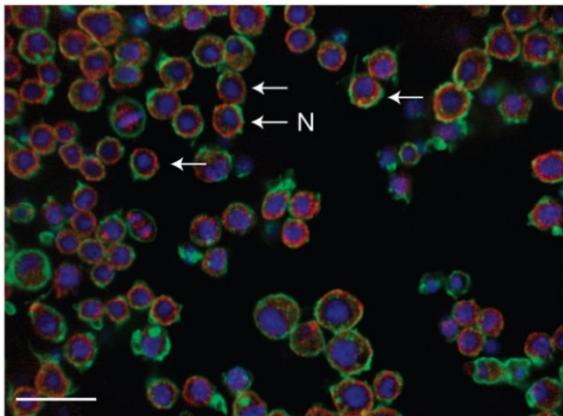
Reclassification of all cells compared to reference shapes

Yin et al (2013) Nature Cell Biology

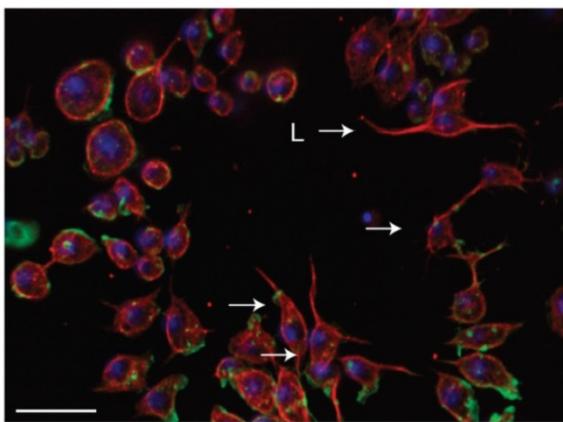


Clustering cells by morphology

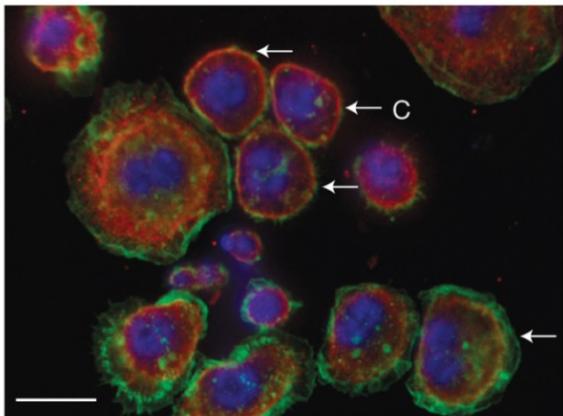
- Use feature vectors for all of the control cells to learn morphology phenotypes
- And the probability that a cell will show one of those phenotypes



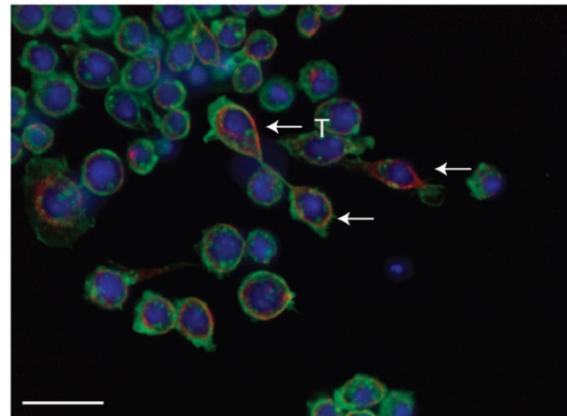
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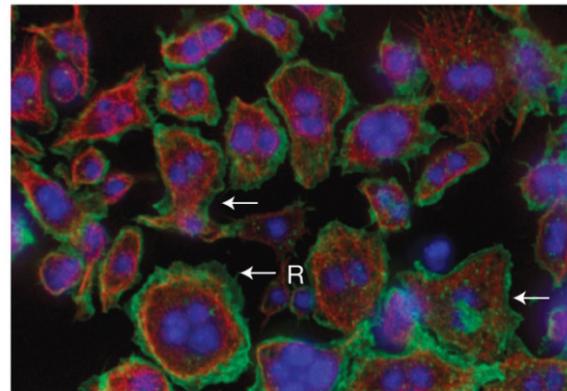
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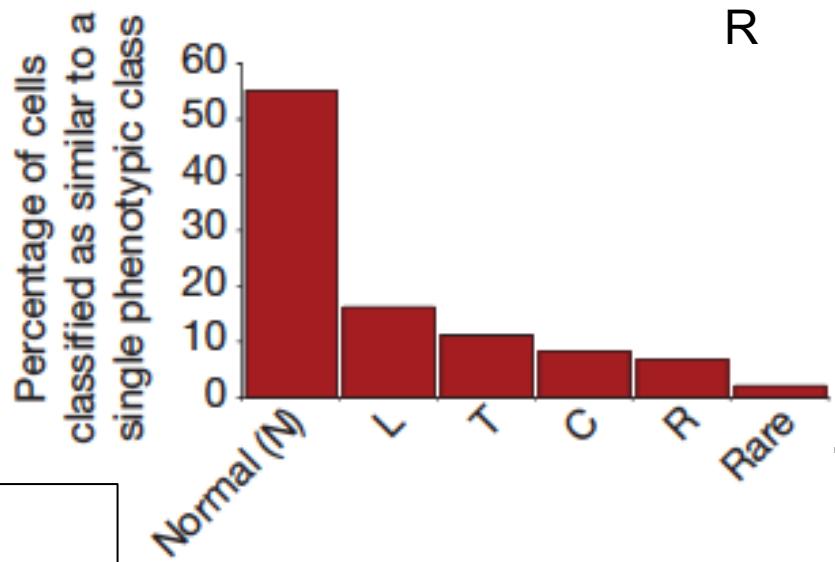
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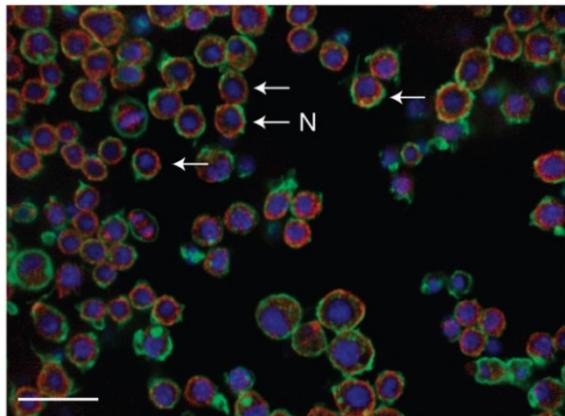


T

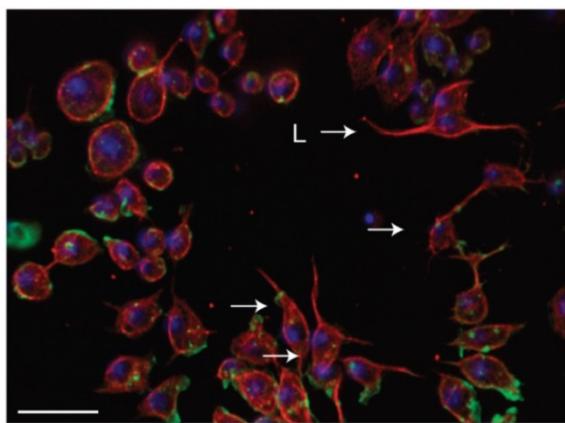


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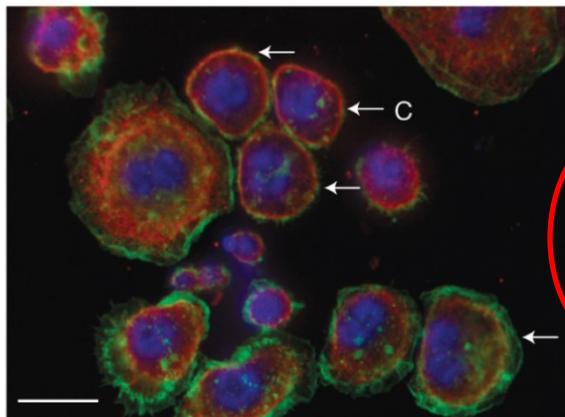




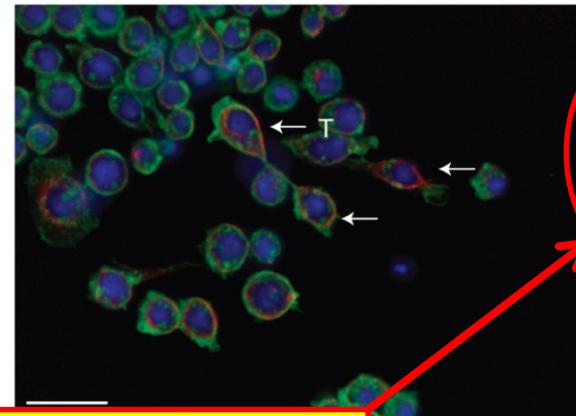
N



L



C



Identified by
Humans

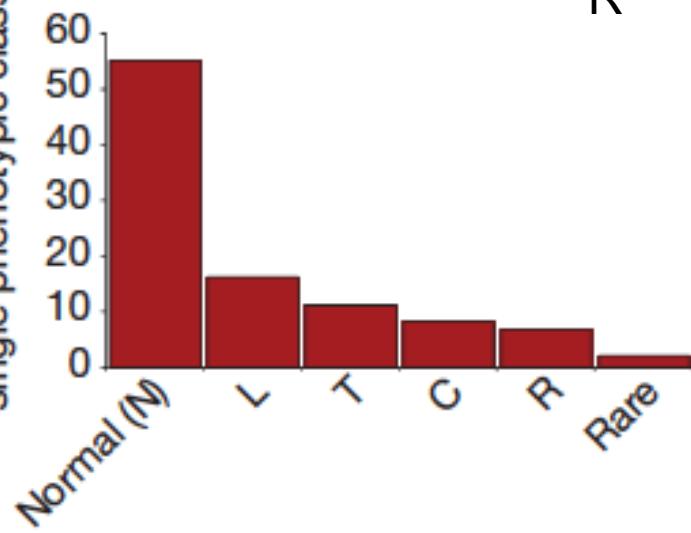


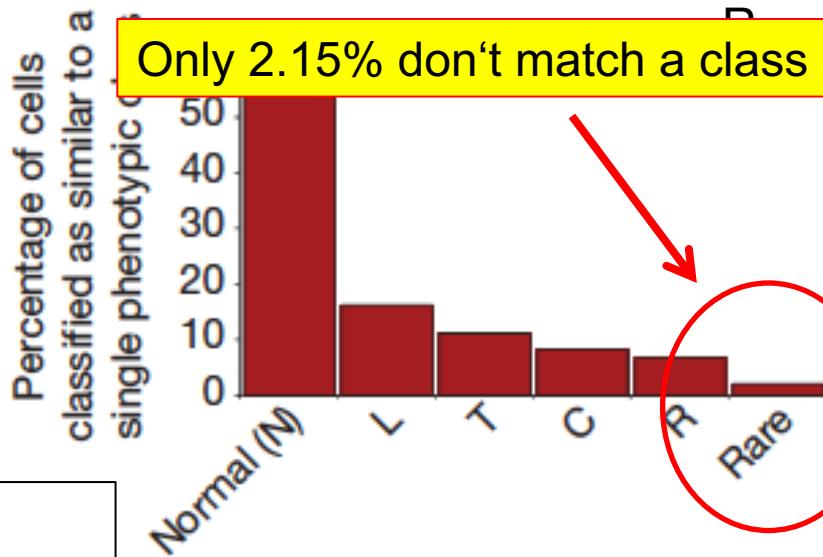
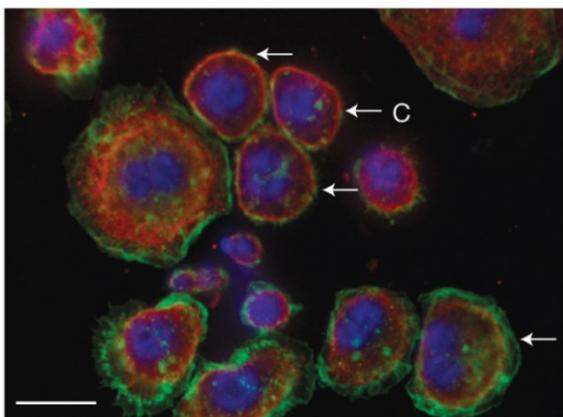
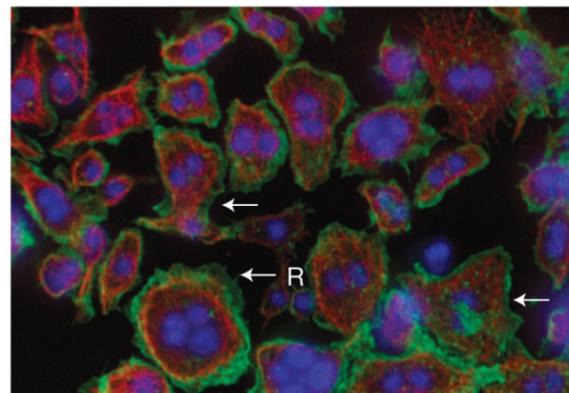
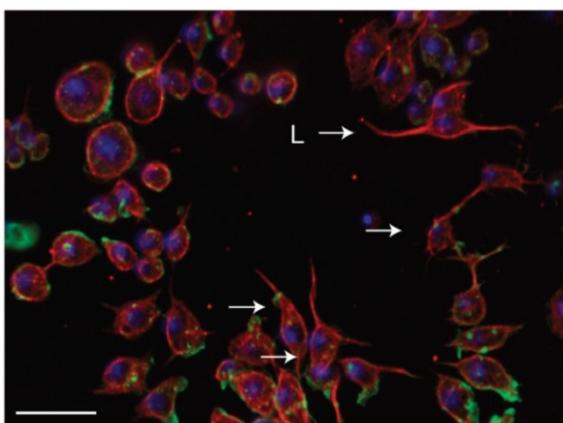
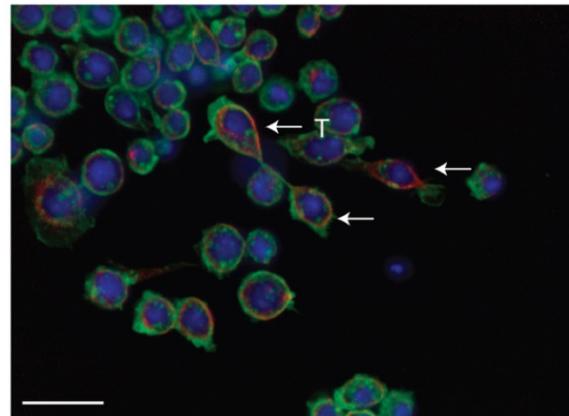
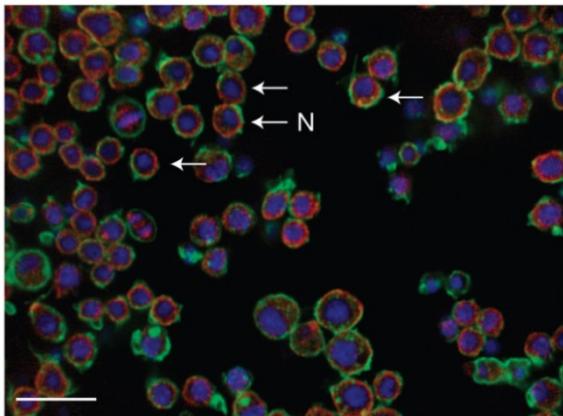
T



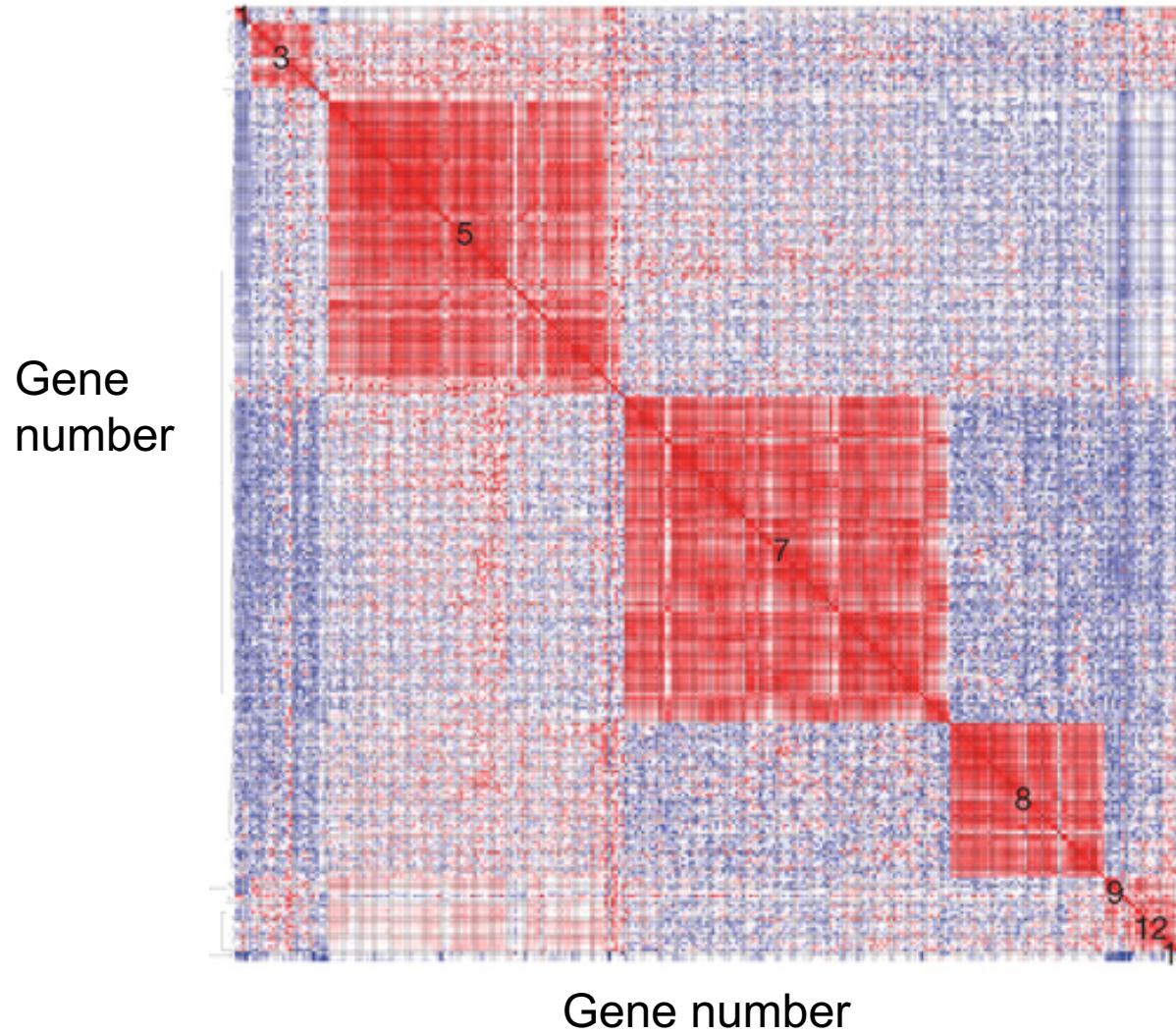
R

Percentage of cells
classified as similar to a
single phenotypic class

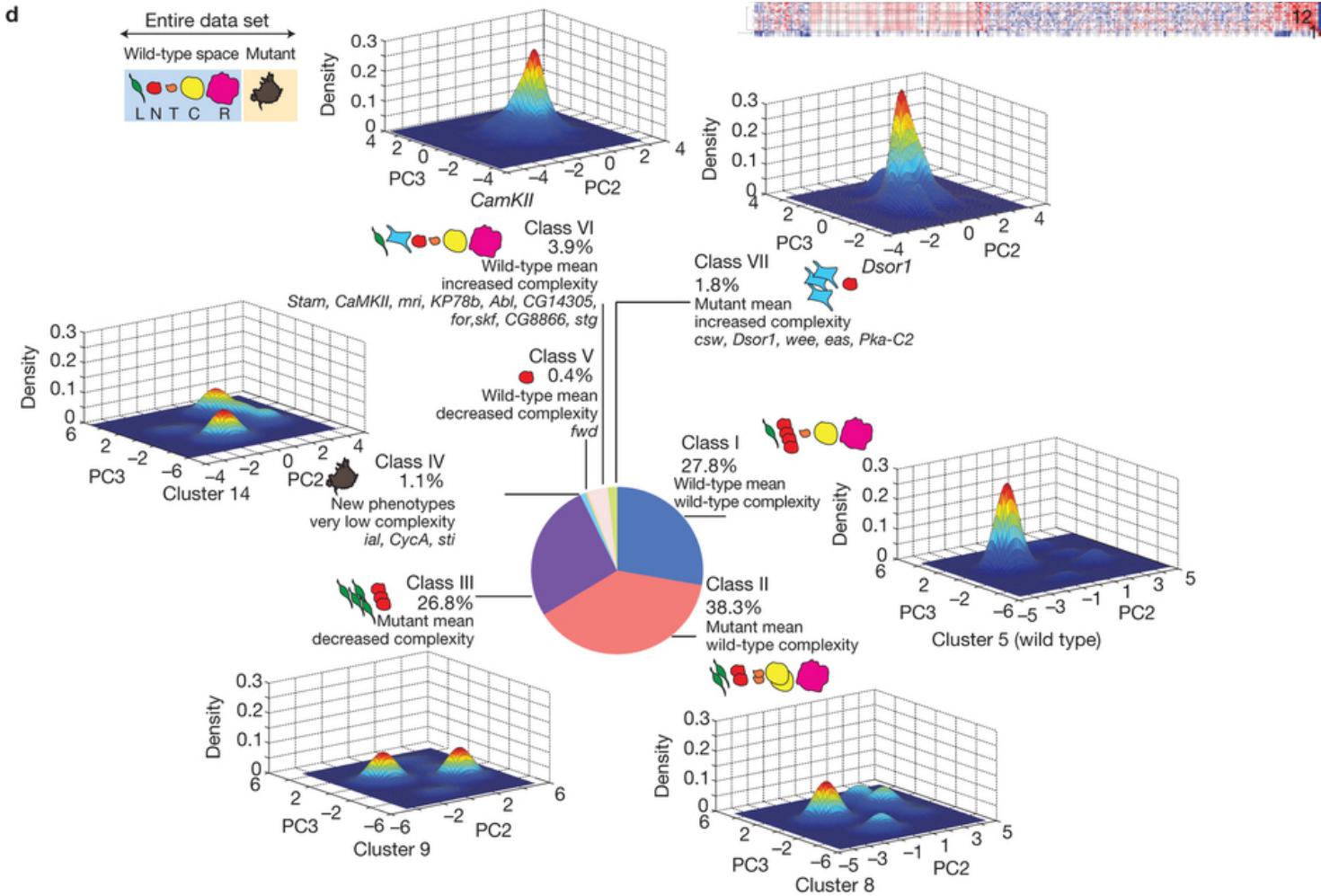




Clustering by similarity in features after siRNA treatment



Inhibiting genes shifts balance between morphology phenotypes





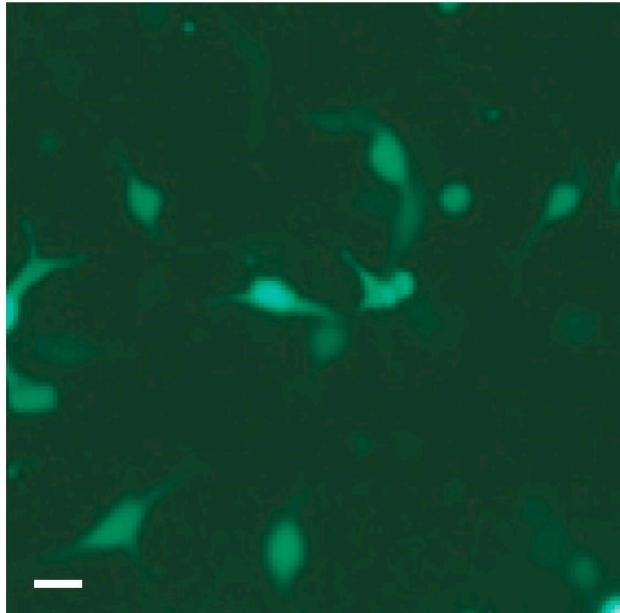
Transfer the Result to Melanoma Cells

- Two observations:
 - the shape of WM266.4 cells, which do not express PTEN, phenocopies that of PTEN-deficiency in Drosophila (high ratio of elongated to rounded)
 - hop/JAK -deficient Kc populations are also heavily enriched in elongated cells at the expense of other shapes
- PTEN and hop/JAK RNAi results in the seventh and eighth highest L scores respectively in the entire Kc data set
- PTEN loss induces elongation cells in tissue culture and *in vivo*

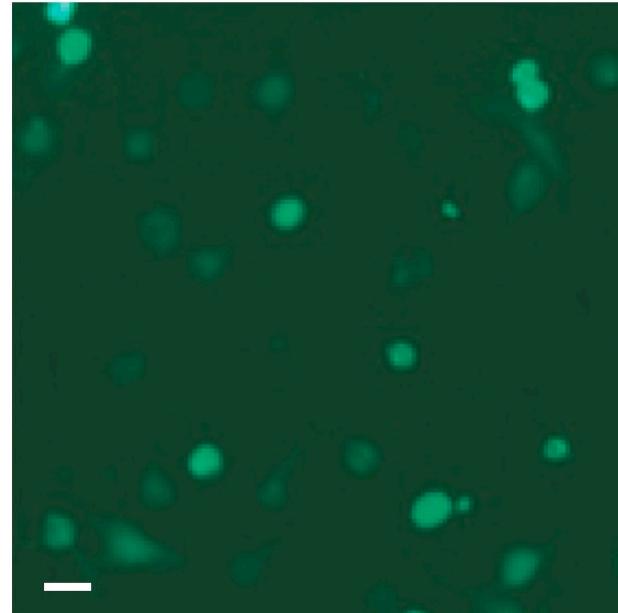
A conserved class of genes that promote cell rounding

- RNAi-mediated knockdown of 12/15 genes in mouse and 7/15 genes human cells results in significant increases in elongation that phenocopy their depletion in Drosophila

Empty-EGFP



PTEN-EGFP





Summary

- HTS is a fast method to screen large drug librarys
- HCS can provide deep insights into biological processes