

Using high-throughput microscopy to study single cell pharmacology.

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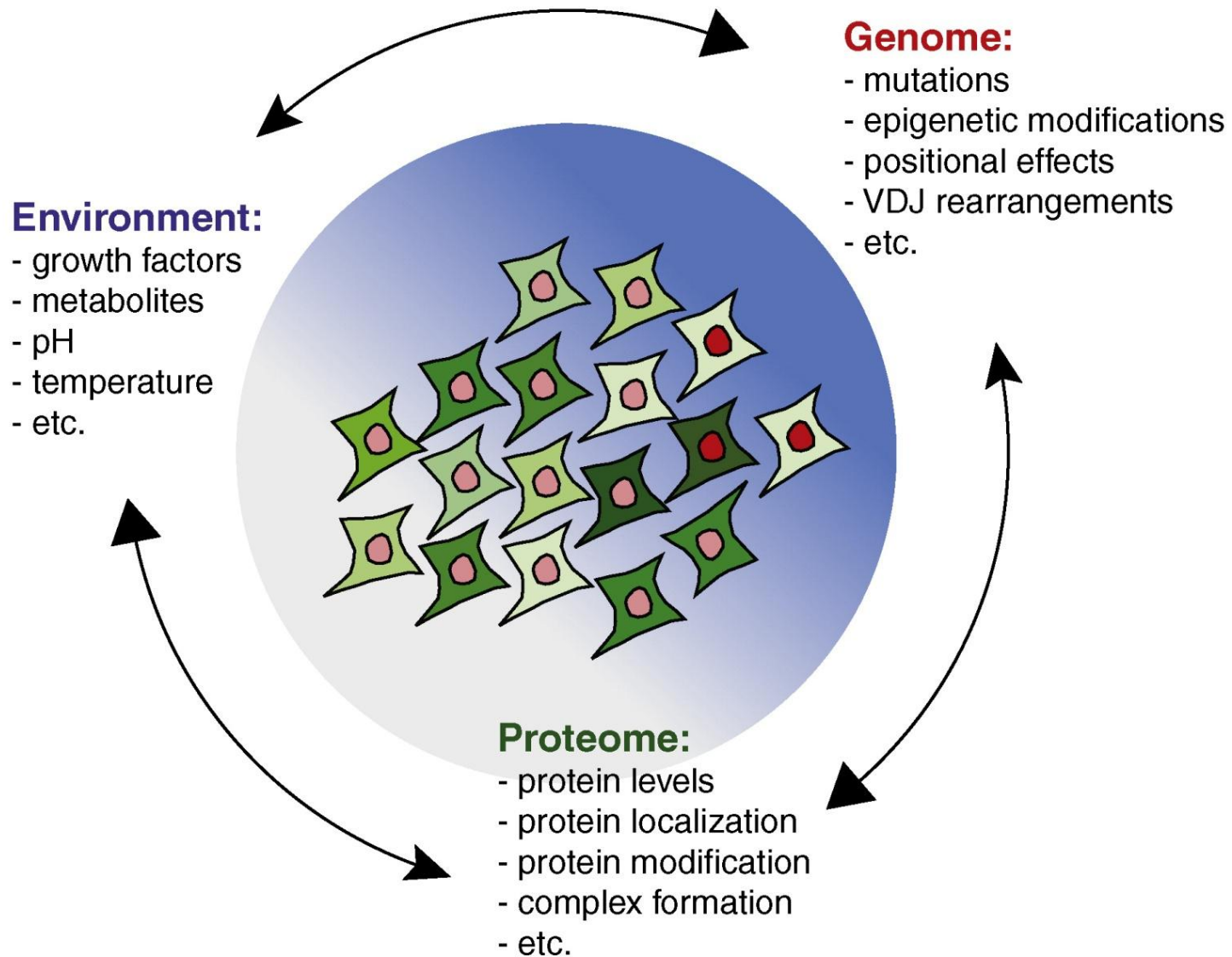
Why do we care about single cell variability?

- **ErbB1-L858R mutation is associated with sensitivity to gefinib and erlotinib**
Pao et al. PNAS. 101:13306
- **subpopulations of cells with MET amplification in NSCLC**
Turke et al. Cancer Cell. 17:77
- **proteomic variance causes cell-to-cell variability in resistance to TRAIL**
Spencer et al. Nature. 459:428
- **epigenetic changes causing transient drug tolerance in cancer subpopulations**
Sharma et al. Cell. 141:69
- **identification of tumorigenic CD44(+)CD24(-/low) cells in breast cancer**
Al-Hajj et al. PNAS. 100:3983
- **Epithelial-Mesenchymal Transition can generate cells with stem cell properties**
Mani et al. Cell. 133: 704

Key questions we would like to answer.

- **What is the extent of variability?**
- **How common is it?**
- **How does it affect the sensitivity to drugs?**
- **What is the connection to EMT/CSC?**
- **What are the sources of heterogeneity?**

What are the origins on single cell variability?



High-Throughput Microscopy - Workflow

- perturb cells in 96 well plate with ligand/inhibitor
- fix at desired time point and stain with antibodies
- image with automated high throughput microscope
- extract quantitative data/couple with computable metadata

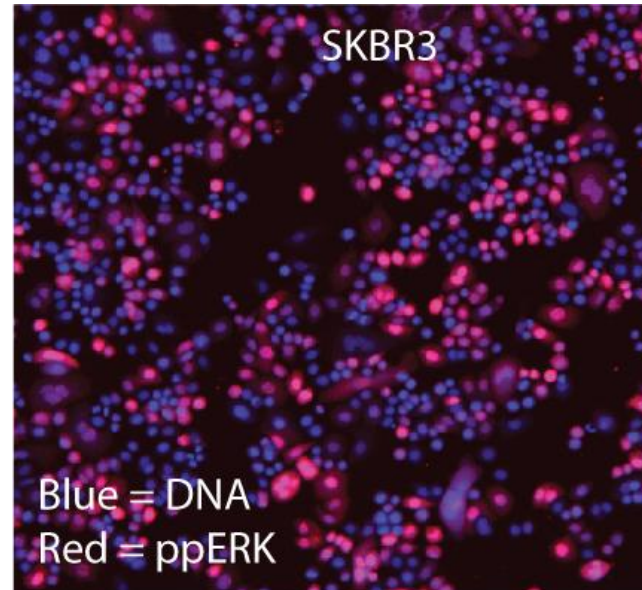
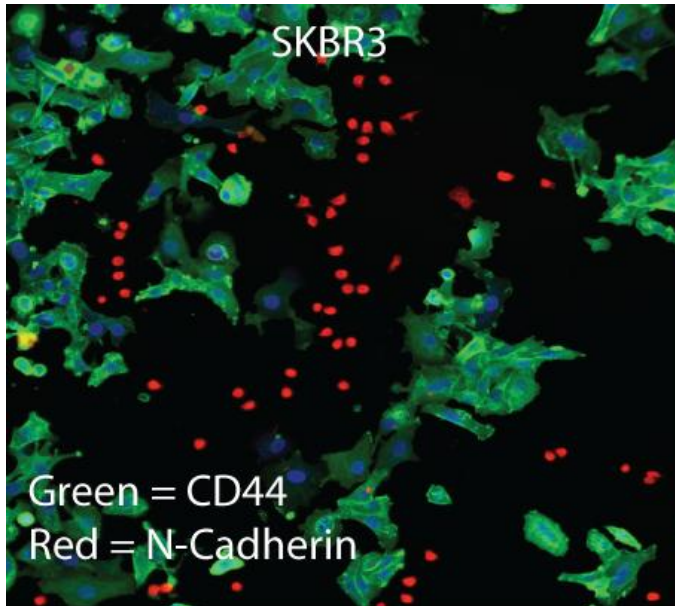
Advantages:

fast, easy, cheap, reliable – single cells

Disadvantages:

antibody dependency – fixed-cell assay

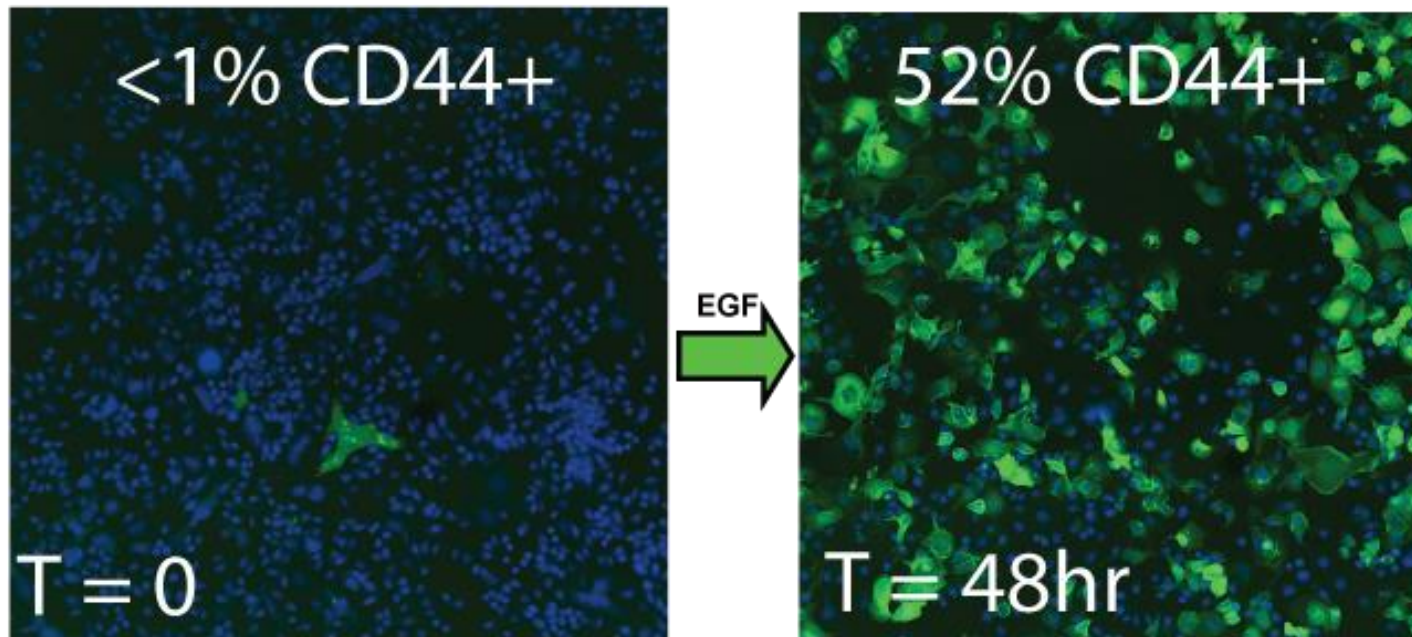
Breast cancer cell lines as model system.



Single cell variability is ubiquitous in ‘homogeneous’ cell line.

Does not require complex environmental cues, mutations, ...

Breast cancer cell lines as model system.

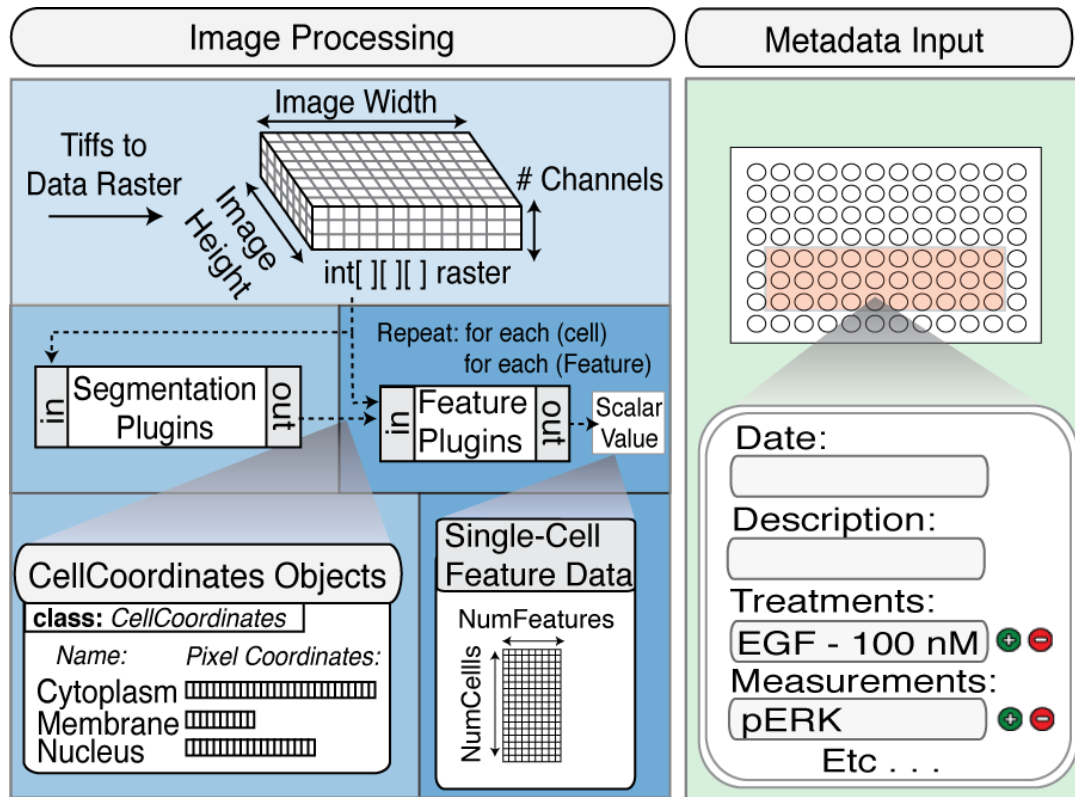


Heterogeneity can be induced by relatively simple treatments.

Not all cells respond equally to the same cue. (short term)

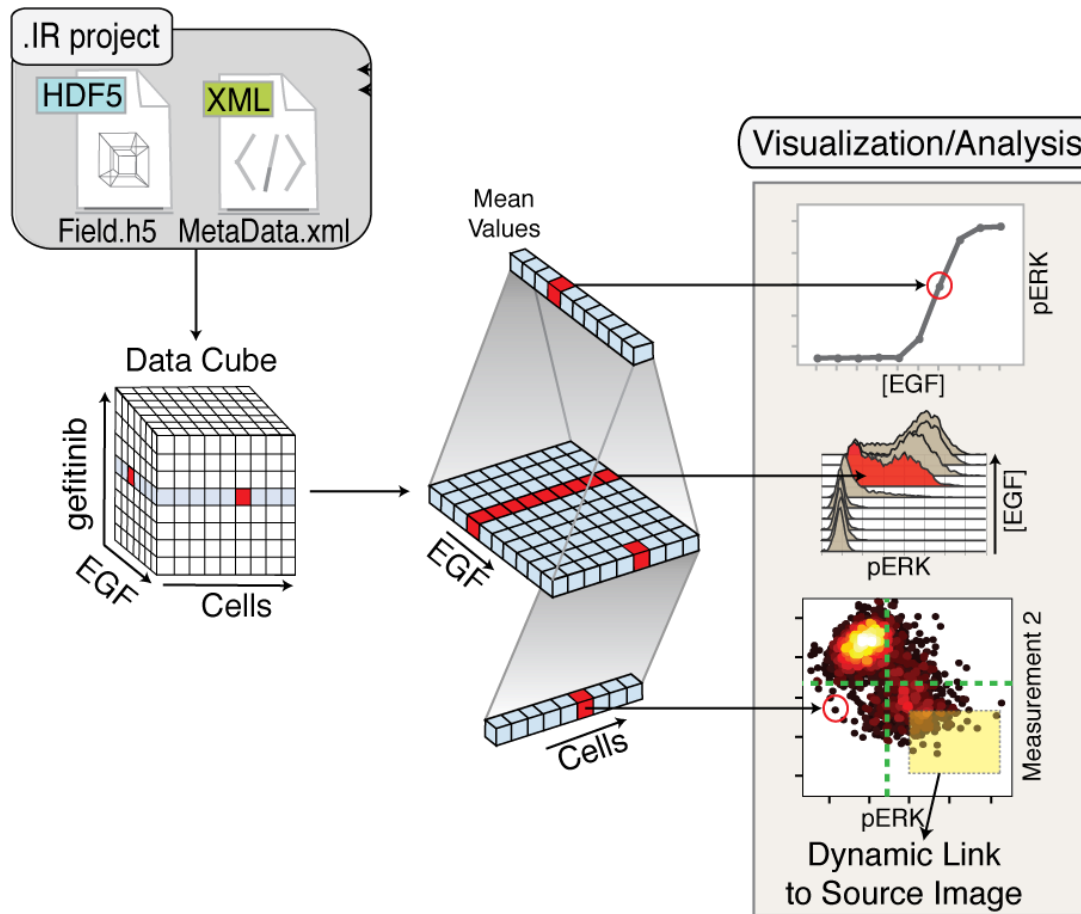
Not all cells have equal phenotypes after receiving the same cue. (long term)

HDF5/XML couples extracted data to metadata



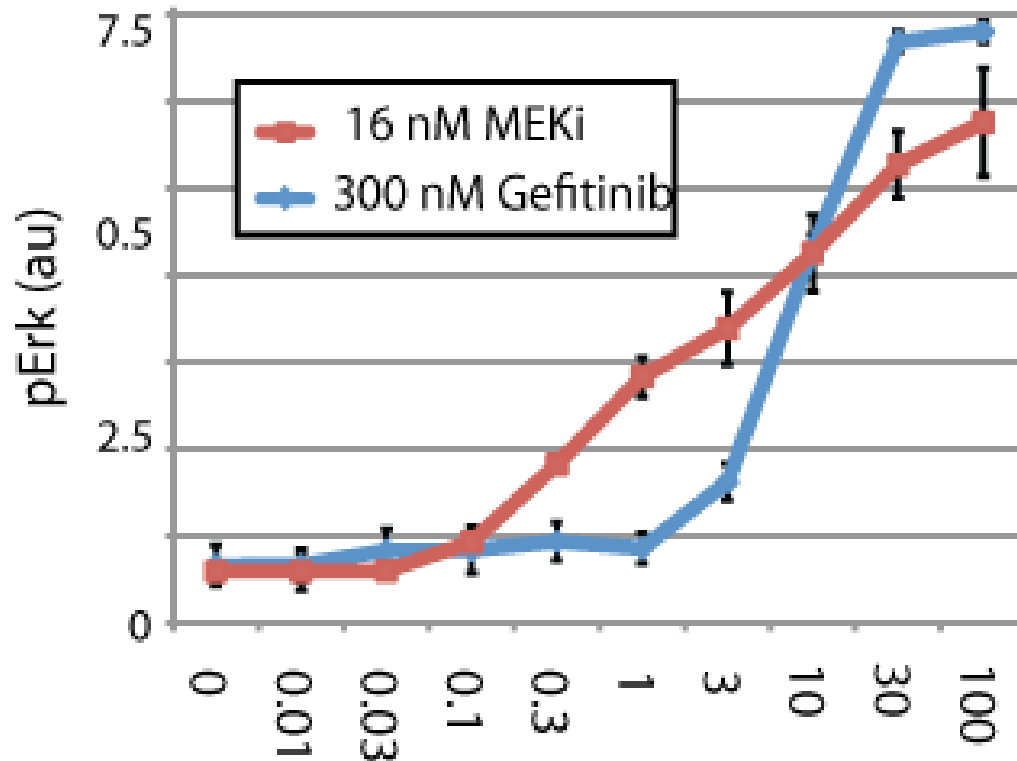
- Data stored in multidimensional hypercube.
- All population and single cell data accessible.
- Metadata for each data point is stored and computable.
- Raw images and data are stored together and linked.

HDF5/XML facilitates rapid visualization of data



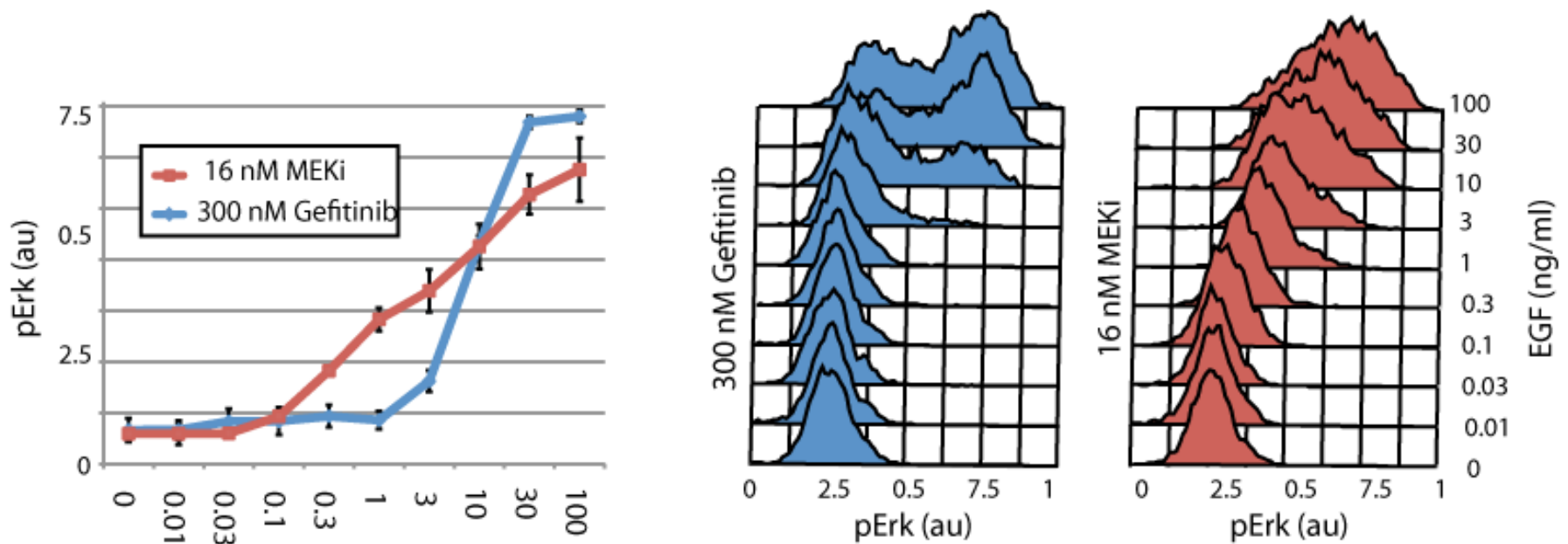
- Data can be visualized as population averages.
- Underlying single cell distributions can be shown.
- Correlation of multiple features in single cells.

Single cell distribution of population averages.



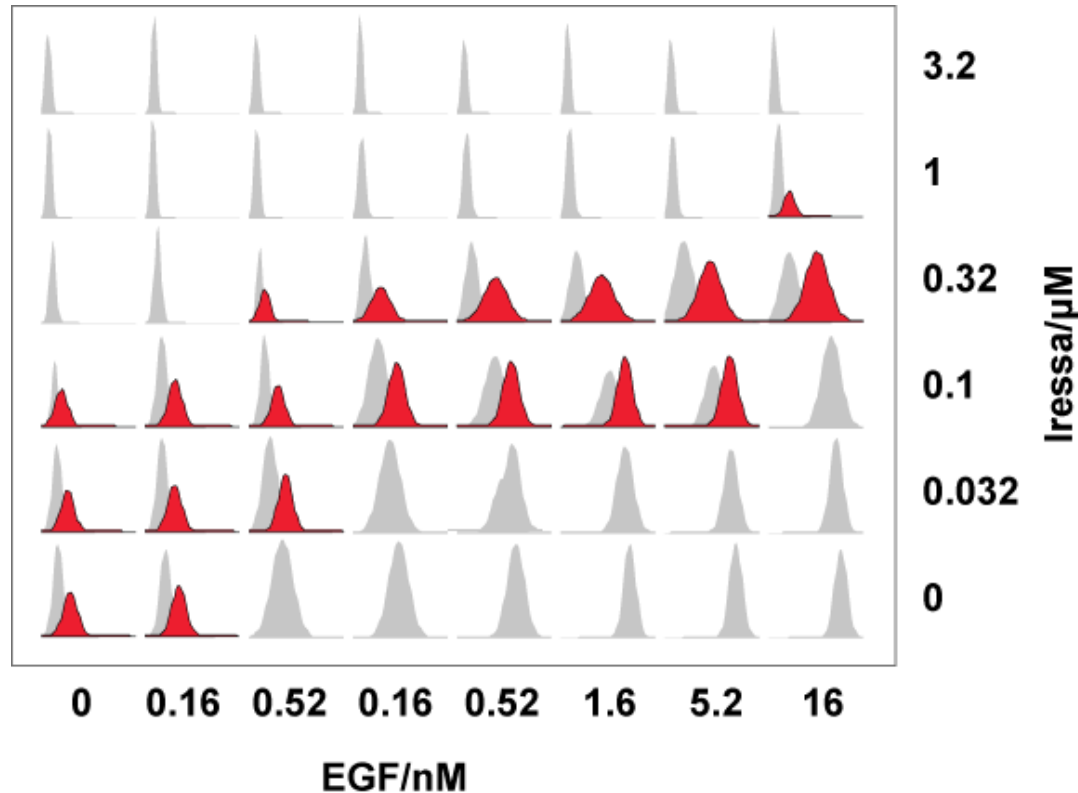
- Two drugs affecting Erk phosphorylation following EGF stimulation.

Single cell distribution of population averages.



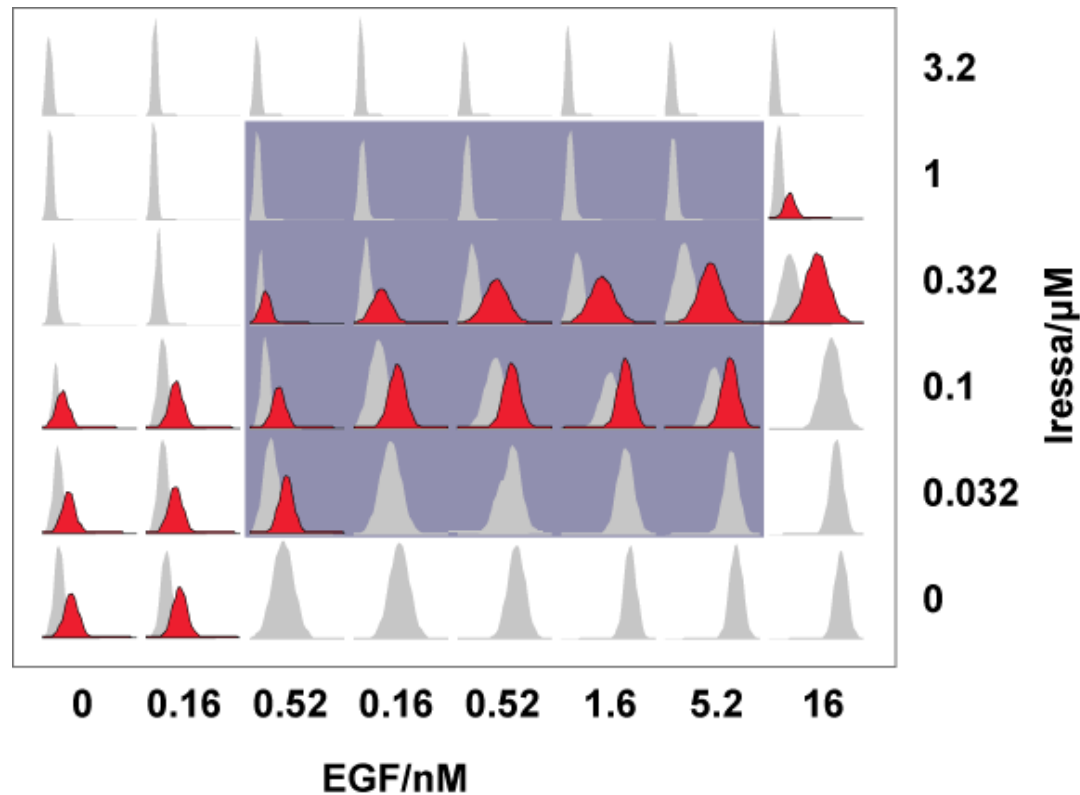
- Two drugs affecting Erk phosphorylation following EGF stimulation.
- HDF5/XML allows for rapid comparison of population and single-cell data.

High heterogeneity of Erk phosphorylation.



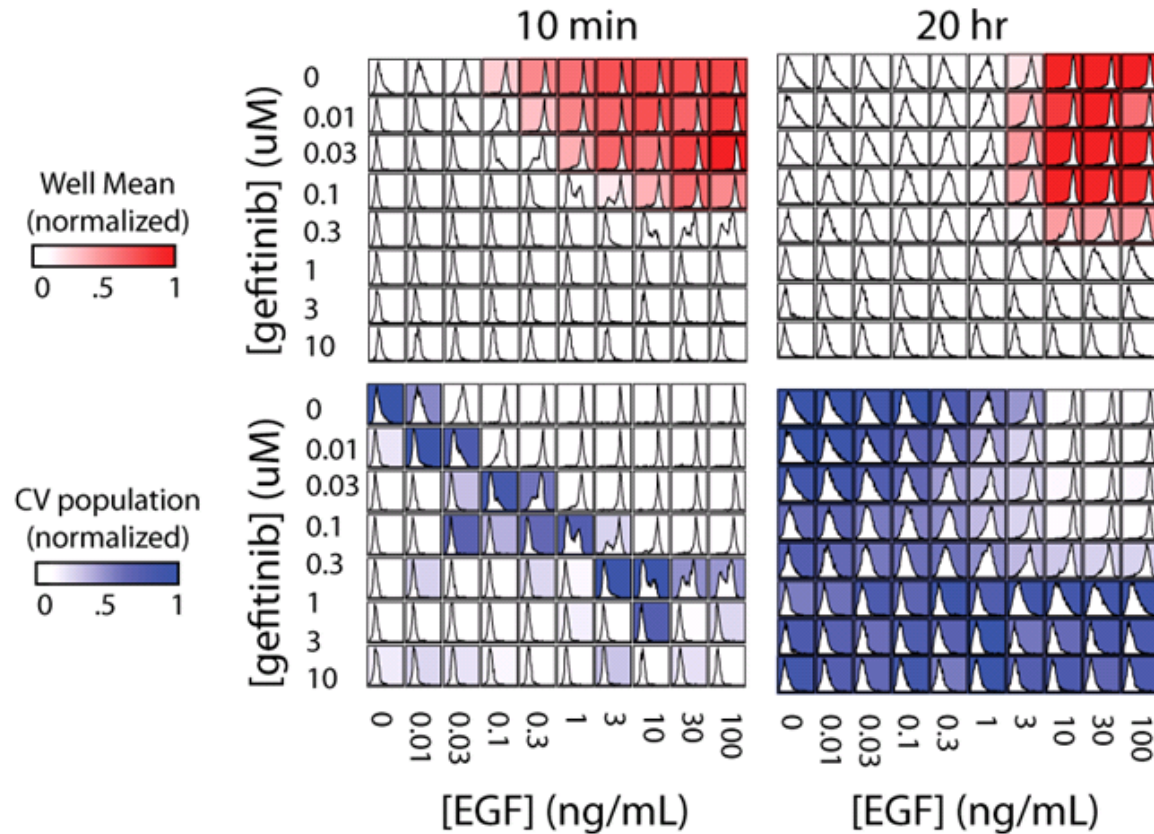
- representation of treatment with various concentrations of EGF and Iressa
- fixed after 5 minute incubation
- data extracted via ImageRail
- bimodal responses indicated in red

Heterogeneity under physiological conditions.



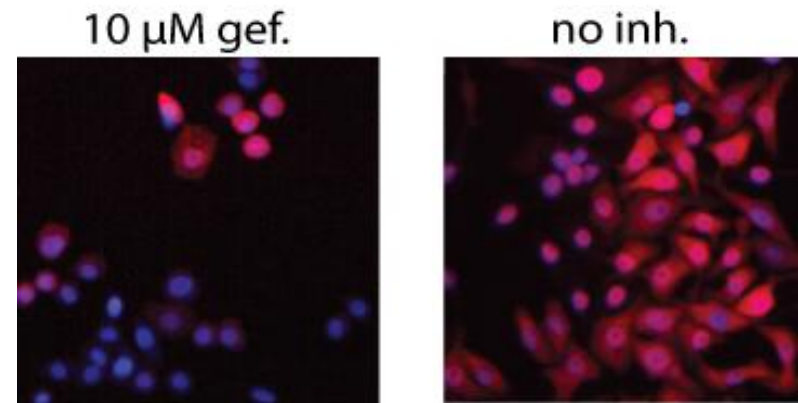
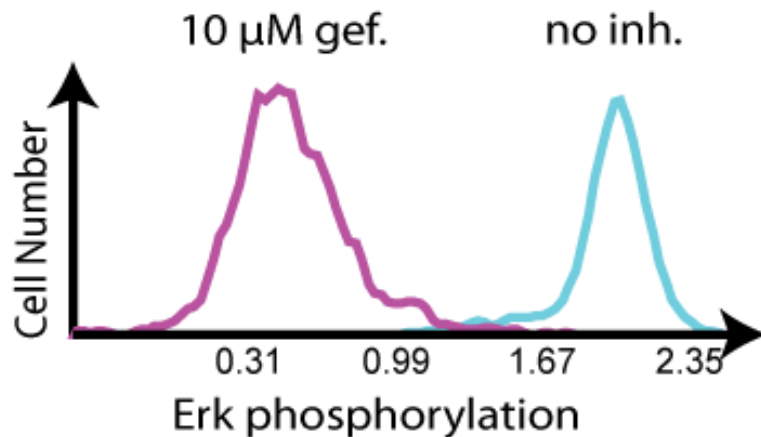
- shaded area represents physiologically relevant concentrations
- significant heterogeneity in the phosphorylation of Erk

Heterogeneity changes over time.



- Heterogeneity in pErk signal remains over time.
- Level of heterogeneity changes over time.
- Even at very high concentrations there is fairly high heterogeneity.

Some cells apparently are insensitive to drugs.

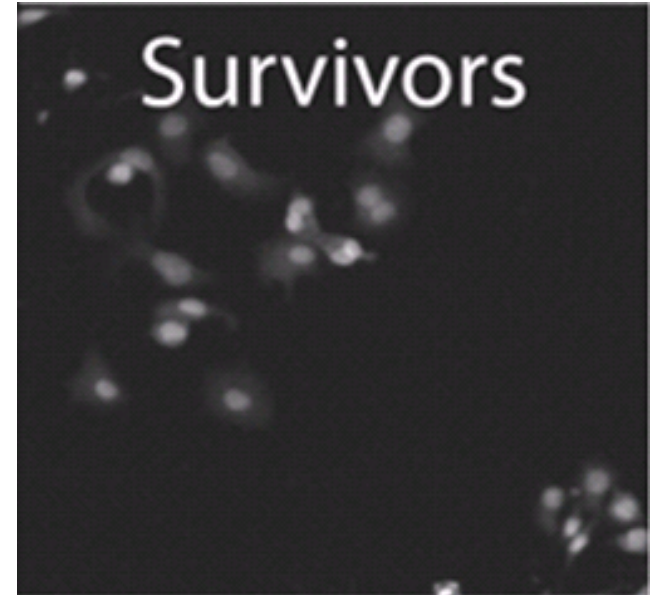
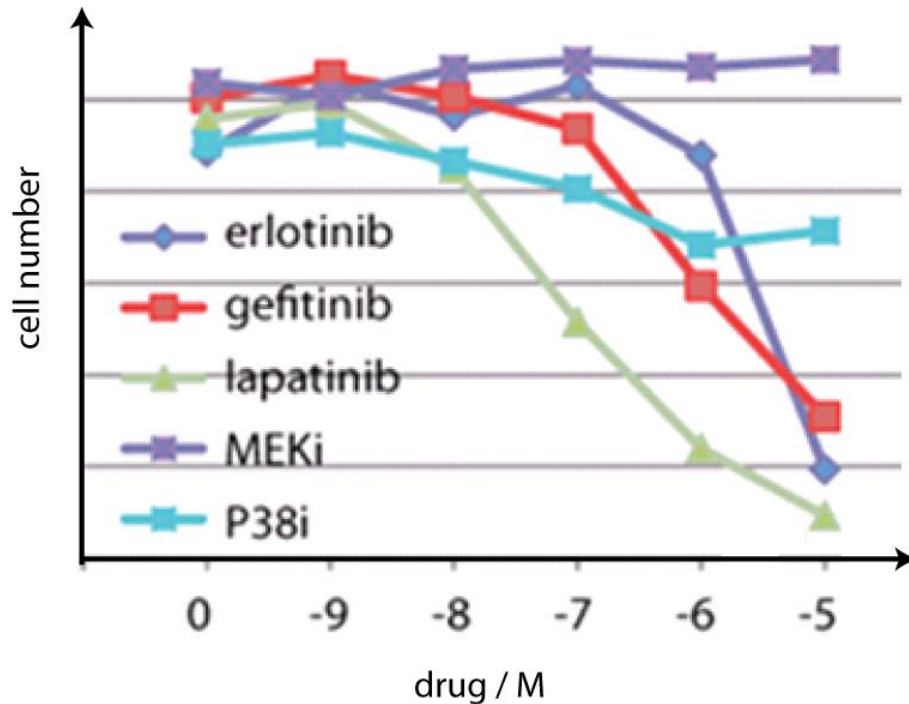


- after 20 hours the majority of cells with high Iressa have no detectable pErk
- virtually all cells with no inhibitor have high pErk levels.
- small subpopulation of cells treated with 10μM Iressa also has high pErk

→ Is this a small population of Iressa resistant cells?

Subpopulation of cells survives drugs treatment.

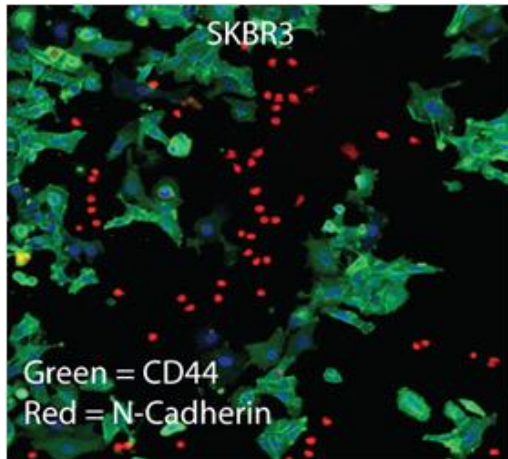
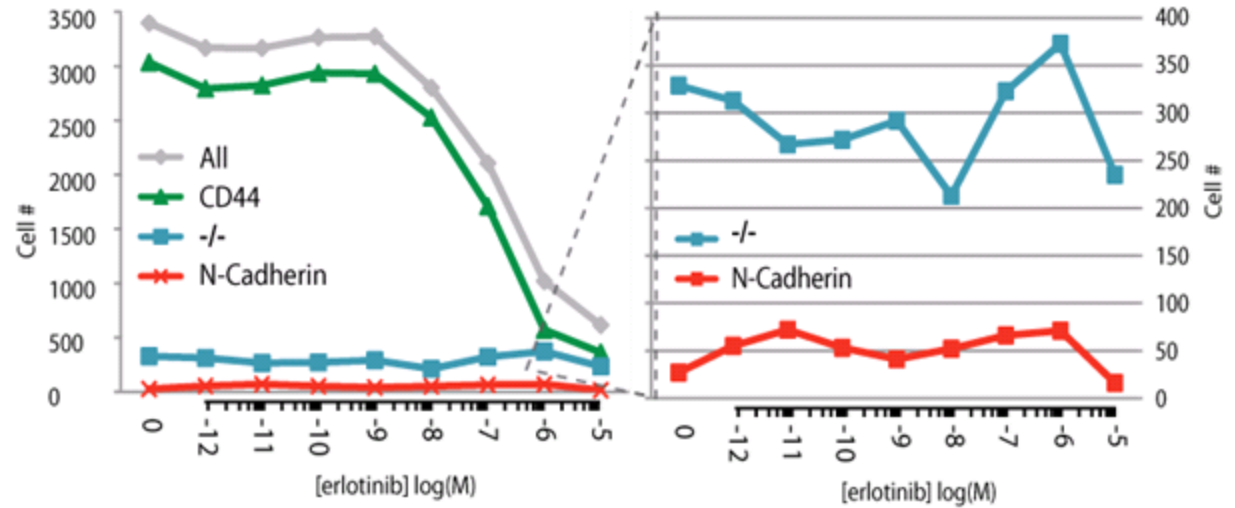
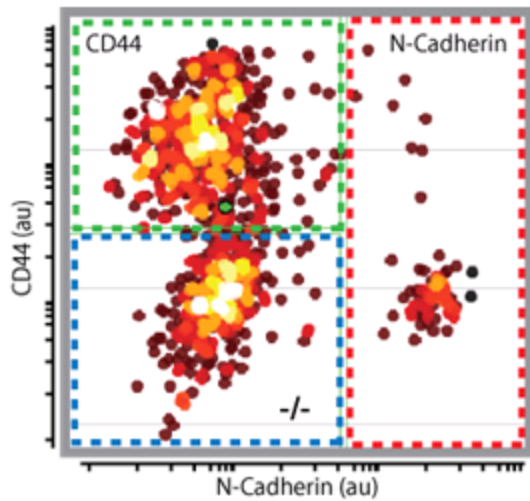
Treat homogeneous cells for 74 hours with drugs targeting ErbB pathway.



• After 4 days of drug treatments, there are small surviving populations.

→ How do these survivors differ from the original population?

Sensitivity is correlated with EMT markers.



Subpopulation of N-Cad+ /CD44 and -/- cells survive.

CD44 expression correlates with drug sensitivity.

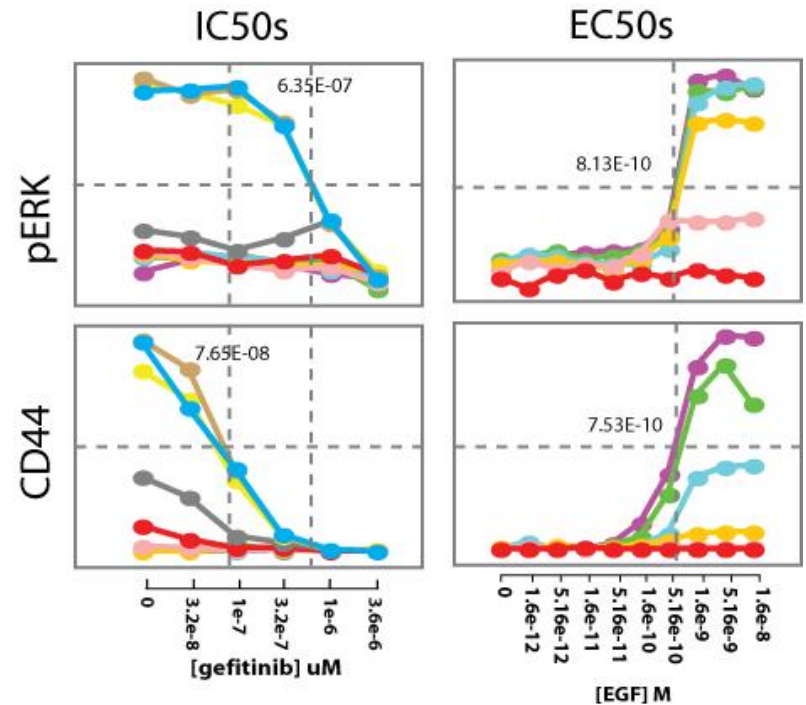
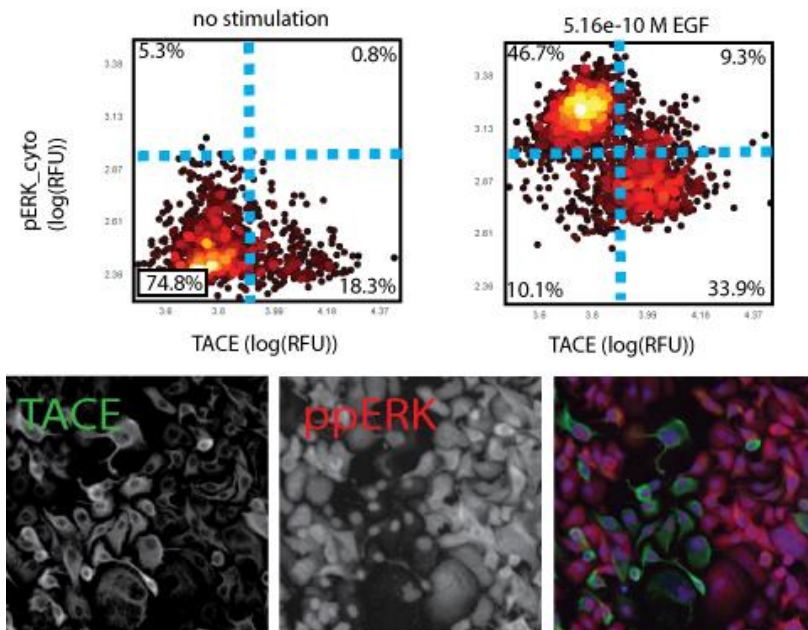
Take-home messages.

- 1. High degree of functionally relevant heterogeneity in cell lines.**
- 2. Heterogeneity in both signaling responses and drug sensitivities.**
- 3. Single-cell assays and data handling are crucial.**

Ongoing single-cell projects.

Characterize properties of surviving populations.

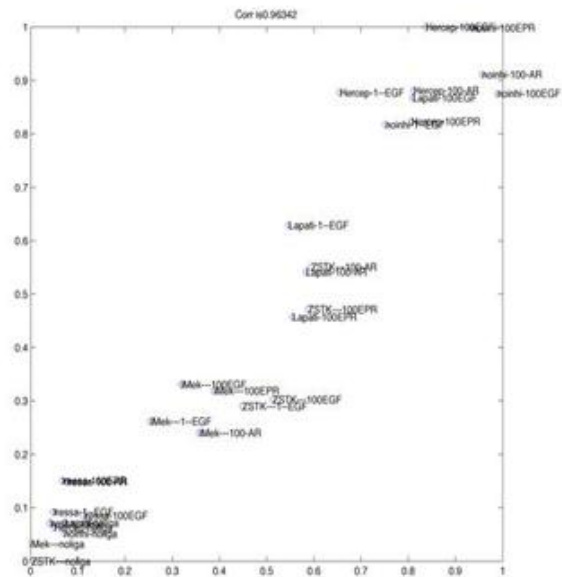
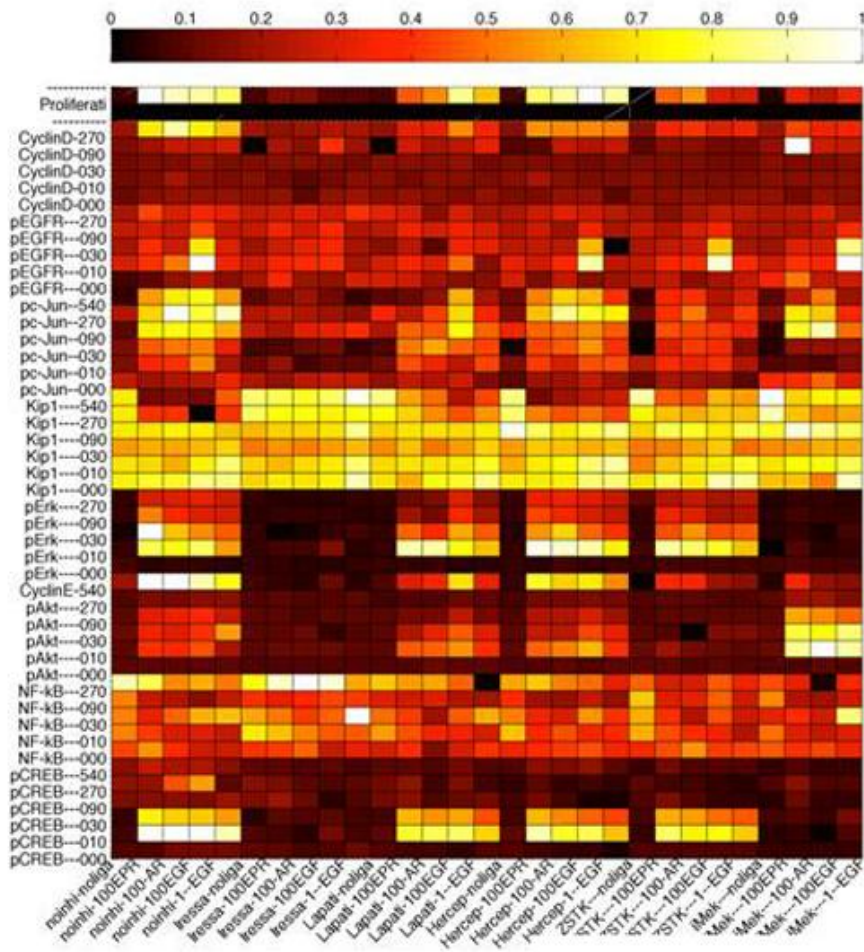
Bjorn Millard, Mario Niepel



Ongoing single-cell projects.

Study EGF induced mitosis.

William Chen, Daniela Robles



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imagerail

datarail



Systems Biology Pipeline



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