Fortune-telling with Images: Cell Painting for discovering drugs

Shantanu Singh
Sr. Group Leader
Imaging Platform

@snhantau
Images are a treasure trove of information

Mouse testis stained for Sertoli cells

The role of cadherins, which are cell adhesion proteins, in cancer has been one of the most studied and debated topics in cancer biology, specifically in breast cancer. Multiple studies have shown that losing E-cadherin promotes breast cancer invasion and metastatic behavior. The thought is that breast cancer cells lose E-cadherin, the molecular glue holding the cells together, in order to move away and travel somewhere else. However, most breast cancer metastases in human patient samples retain E-cadherin expression. A recent paper by the Ewald group at Johns Hopkins University published in Nature in 2019 showed that E-cadherin is actually required for breast cancer survival. While loss of E-cadherin in cell culture models promotes invasive behaviors, deletion of E-cadherin decreased the number of breast cancer metastases in mice. This is because cancer cells without E-cadherin are more sensitive to damage and die more easily. This is an important study in finally laying to rest the role of E-cadherin in breast cancer.

Shown here are endothelial cells stained for VE-cadherin, another type of cell adhesion protein that is more specific for the vascular system.

Taxol is a cancer drug that stabilizes microtubules and disrupts cell division, but is also used as a reagent by scientists in studying microtubules dynamics. Microtubules are part of the cell "skeleton." They're important in maintaining cell structure and changing cell shape, as happens during cell division when a cell splits in two. Disrupting this process with drugs prevents proper cell division and can cause the cell to die. Cancer cells divide quickly, which is why they are especially affected by these drugs.

Shown here are taxol treated cells stained for myosin (blue), tubulin (green), and DAPI (red).

Fibrosis, or tissue scarring, occurs after tissue damage as a compensatory means to maintain tissue structure and integrity. Fibrosis occurs in almost all organs such as the heart, kidneys, lungs, liver, and more and is typically the result of lack of blood supply. Heart attacks occur due to vascular occlusion of the coronary arteries, resulting in lack of blood flow to the heart muscle. Without blood and oxygen, the muscle dies. Immune cells clear out the dead tissue, leaving an empty space that fibroblasts fill with collagen. This collagen is scar tissue, and unlike live heart muscle, it is unable to contract and impedes the heart's ability to pump blood.

Recently, researchers at Penn have actually designed special cells to attack this fibrotic collagen to reduce scarring and improve heart function after a heart attack. Research like this can delay or prevent heart failure and improve quality of life for patients.

Imaged with a Zeiss LSM 880 Microscope.

Image credit: @immunofluorescence (Derek Sung)
Cell Painting

Jennings, et al.
Cell Painting assay
Maximize information for morphological profiling

6 stains, 5 channels imaged, revealing 8 constituents/organelles:

Gustafsdottir, et al. PLOS ONE 2013
Exploring relationships among perturbations

Correlations among compounds within 155 MOAs

Clustering of 110 genes / alleles

Natoli et al. (In progress)

Rohban, et al. eLife 2017
Building predictive models from imaging data

Virtual screening of large compound libraries

**Input**
- Chemical structure (CP)
  - Chemprop
- Images (MO)
  - Cell Painting assay
- mRNA (GE)
  - L1000 assay

**Feedforward neural net**

**Assay readout predictions for 378 different assays**

**Performance of Different Feature Sets**

- GE+MO+CP: 55
- MO+CP: 53
- GE+MO: 49
- GE+CP: 17
- MO: 63
- CP: 41
- GE: 14

In progress, Based on work by Simm, et al. Cell Chem Bio 2018

Predicting many cell health assays from Cell Painting

github.com/broadinstitute/cell-health

Open Science Project in progress
Finding phenotypes of rare disorders

(... and drugs that revert them)

- 7,000 rare diseases
- 4,550 are known monogenic
- Only 6% have FDA approved treatments
- Tag, overexpress, and image 3,584 disease variants (& 1,202 wild-type)
- Identify changes in protein localization & cell morphology

Project in progress

Marzieh Haghighi
Mikko Taipale
Jessica Lacoste
Scaling up profiling of genes using Pooled Cell Painting

*In situ* sequencing allows assignment of perturbations

Pooled perturbation library (shRNA, CRISPR, cDNA) → Collect image-based single cell profiles (Cell Painting) → Assign perturbations to cells (in situ barcode sequencing) → Group perturbations based on morphological similarities

Feldman, et al. 2019 Cell

Julia Bauman, Gregory Way, Avtar Singh, Beth Cimini, Celeste Diaz, Erin Weisbart, Hillary Tsang, Sanam Kavari, Varsha Prakash, Marzieh Haghighi, Maria Lozada, Shantanu Singh, Anne Carpenter, Paul Blainey, JT Neal

*Funding: Calico Life Sciences, LLC.*
Predicting drug response and resistance

Determine drug susceptibility for individual patients’ tumor cells

Treat patient tumor cells with various combinations of drugs

Morphological changes inherent, prior to treatment, that indicate susceptibility or resistance?

Morphological changes within 24 hours?

Cell death at 2 weeks

github.com/broadinstitute/profiling-resistance-mechanisms

Project in progress
Linking gene expression and cell morphology

Morphology $\rightarrow$ Gene expression

Synthesized images showing transition of phenotype

Lafarge, et al., MIDL 2019

Marzieh Haghighi
Gratitude

Broad Imaging Platform

Anne Carpenter (Director)  
Alice Lucas  
Allen Goodman  
Becki Ledford  
Beth Cimini  
David Stirling  
Erin Weisbart  
Pearl Ryder  
Nasim Jamali  

Profiling group  
Shantanu Singh (Grp. Leader)  
Adeniyi Adeboye  
Greg Way  
Hamdah Abbasi  
Hillary Tsang  
Marzieh Haghighi  
Niranj Chandrasekaran  
Saketh Mynampati  
Yuen Ler Chow

Many thanks to our many biology collaborators!

Recent major funding for this work provided by:

- NIH NIGMS: R01 GM089652
- NIH NIGMS: MIRA R35 GM122547
- NSF CAREER: DBI 1148823
- NSF/BBSRC: DBI 1458626
- Chan Zuckerberg Initiative DAF
- Starr Cancer Consortium

@snhantau  
shsingh@broadinstitute.org