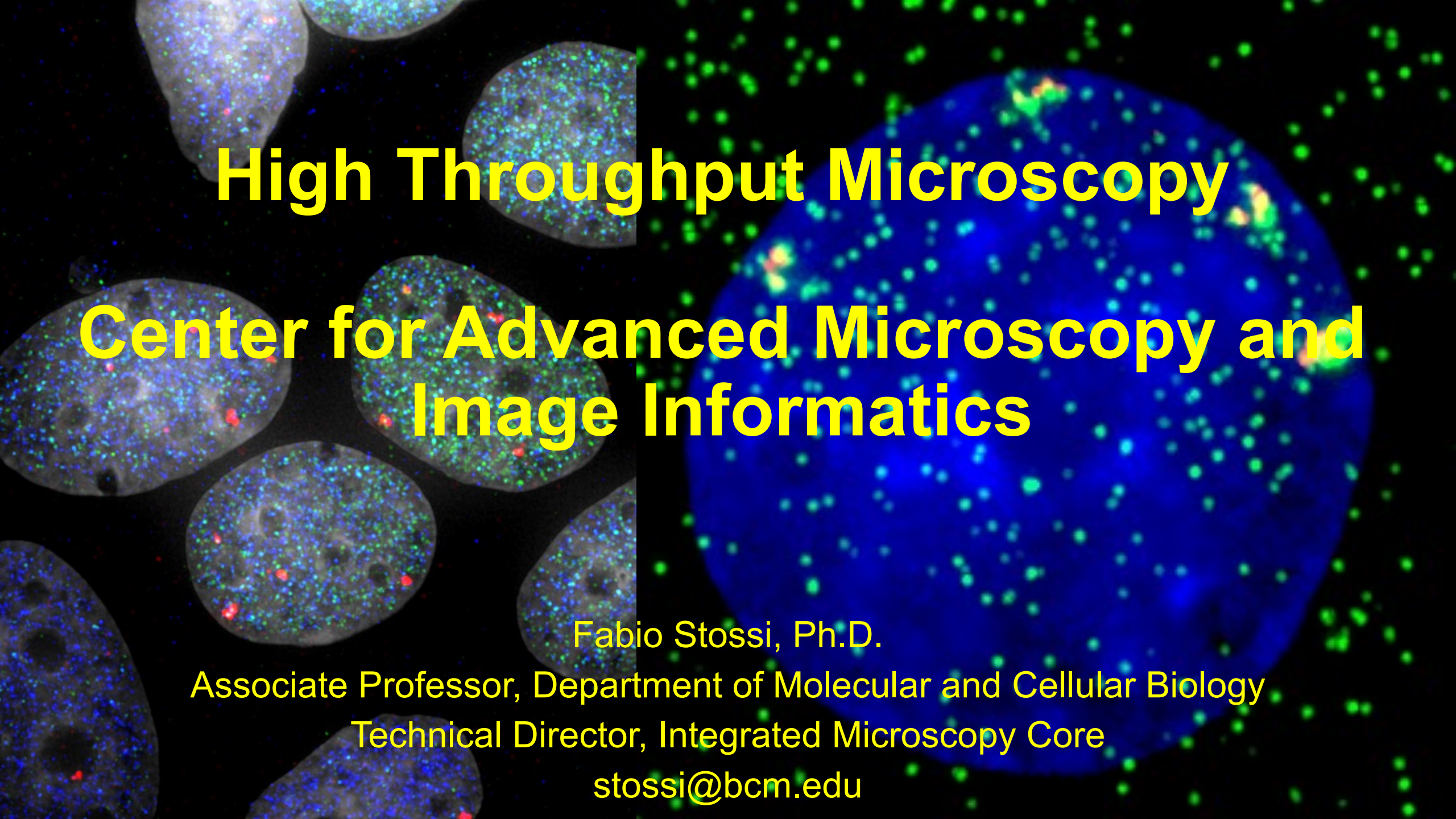

High Throughput Microscopy

Center for Advanced Microscopy and Image Informatics

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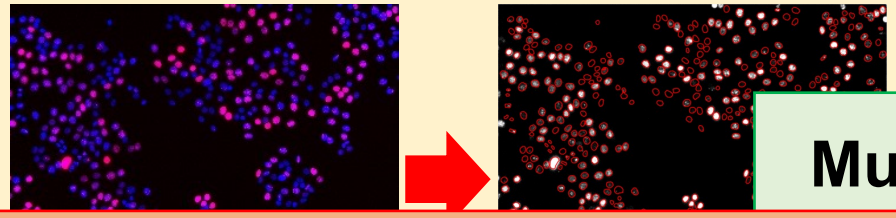
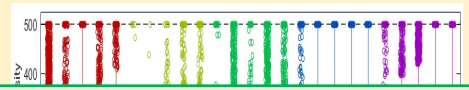
Why microscopy?

Take a pretty picture!

Extract Quantitative Measurements

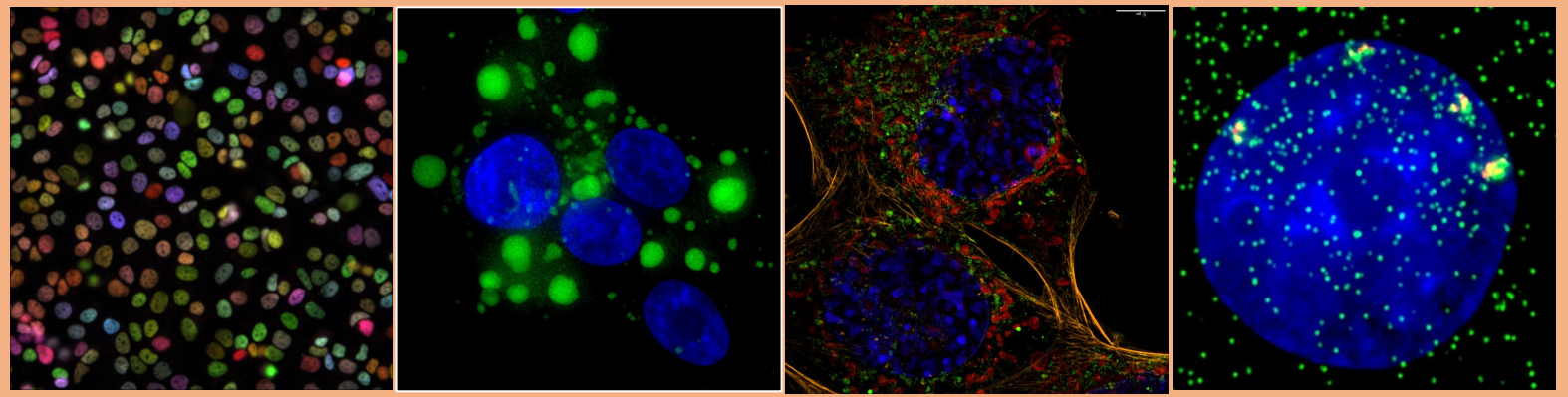




Multiplex, Miniaturize, Throughput

ftware + Servers

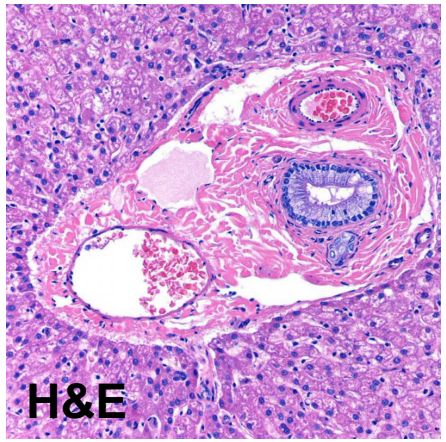
Single Cell Omics

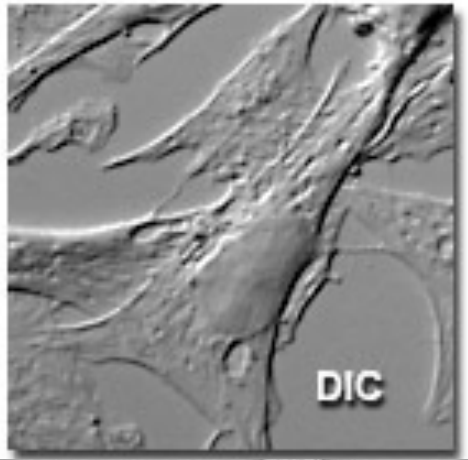


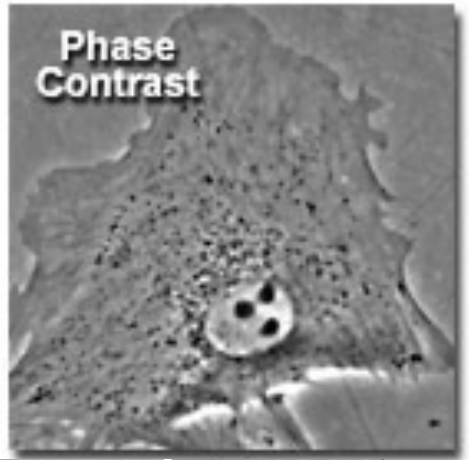
Protein Metabolism Structures RNA/DNA

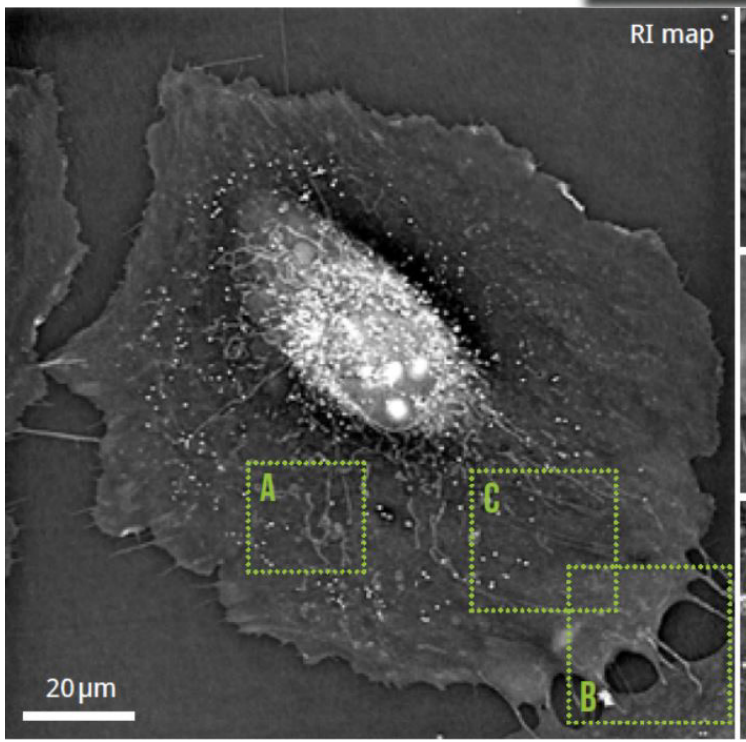
What Types of Microscopy can you do/think about?

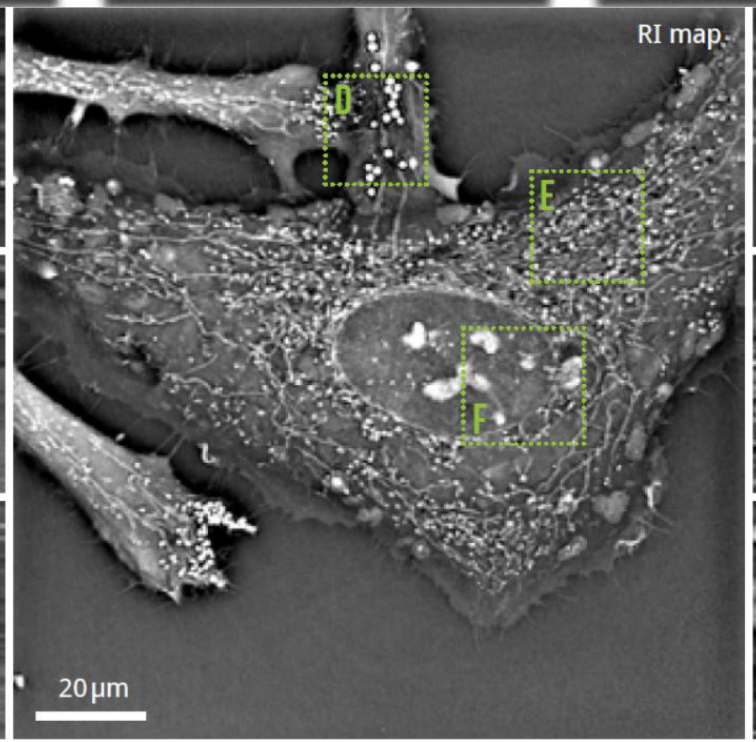
Brightfield (color) or label free (“transparent”) methods (DIC, phase contrast, ptychography, qpi)

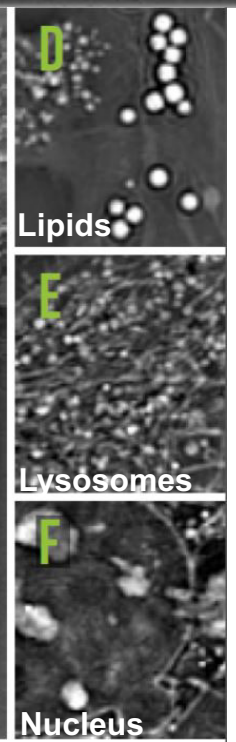






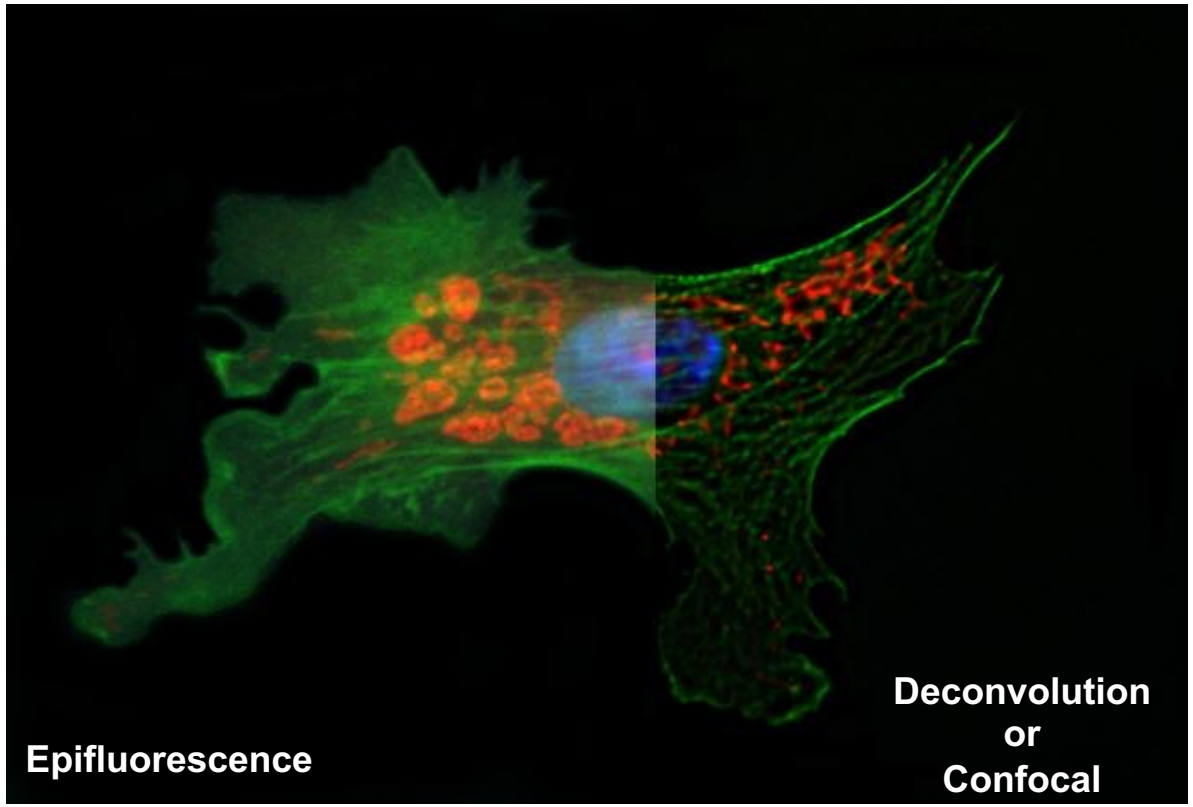


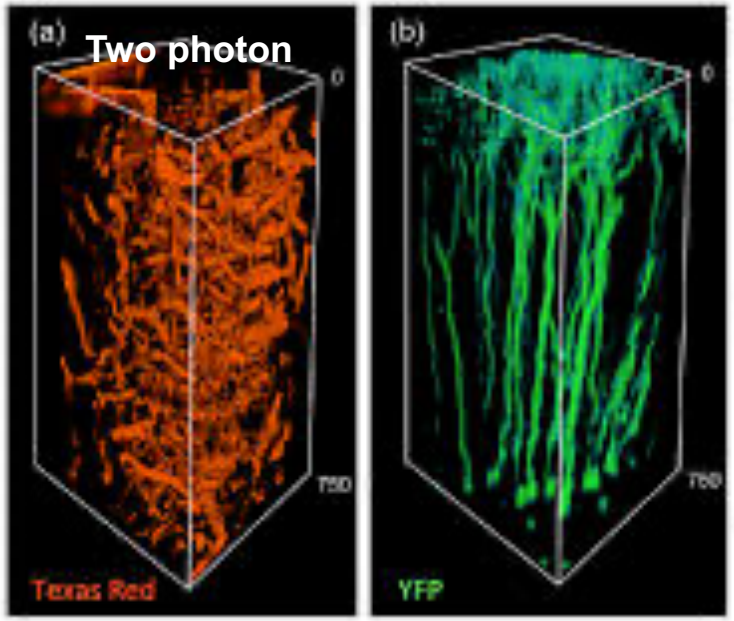


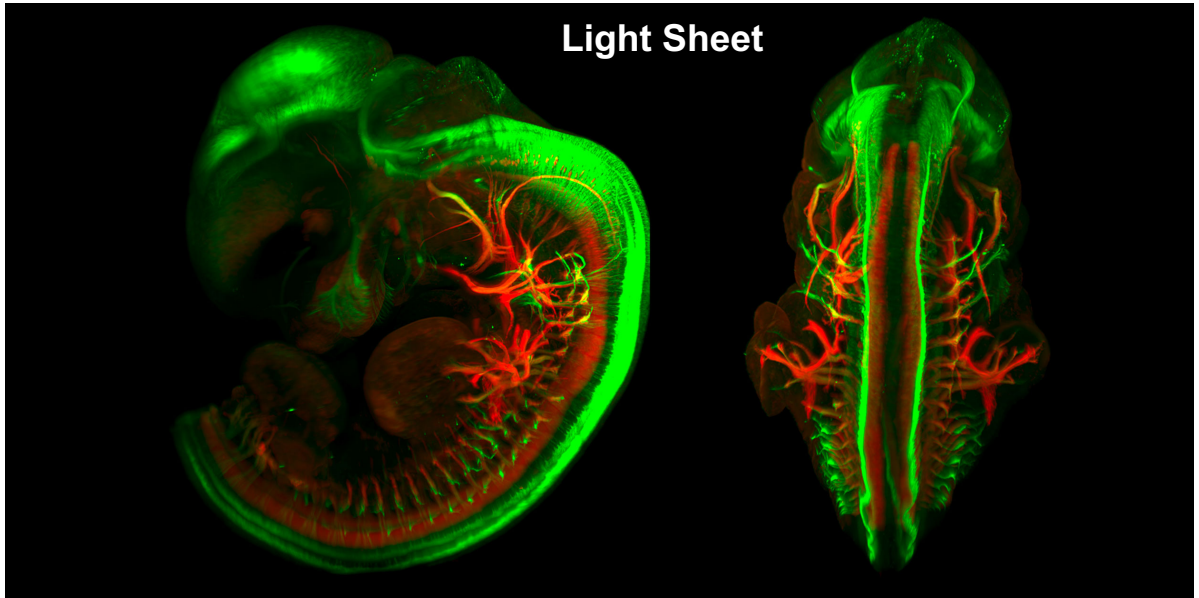


What Types of Microscopy can you do/think about?

Fluorescence-based: epifluorescence, deconvolution and confocal (spinning disk+laser scanning), multiphoton, light sheet, etc.



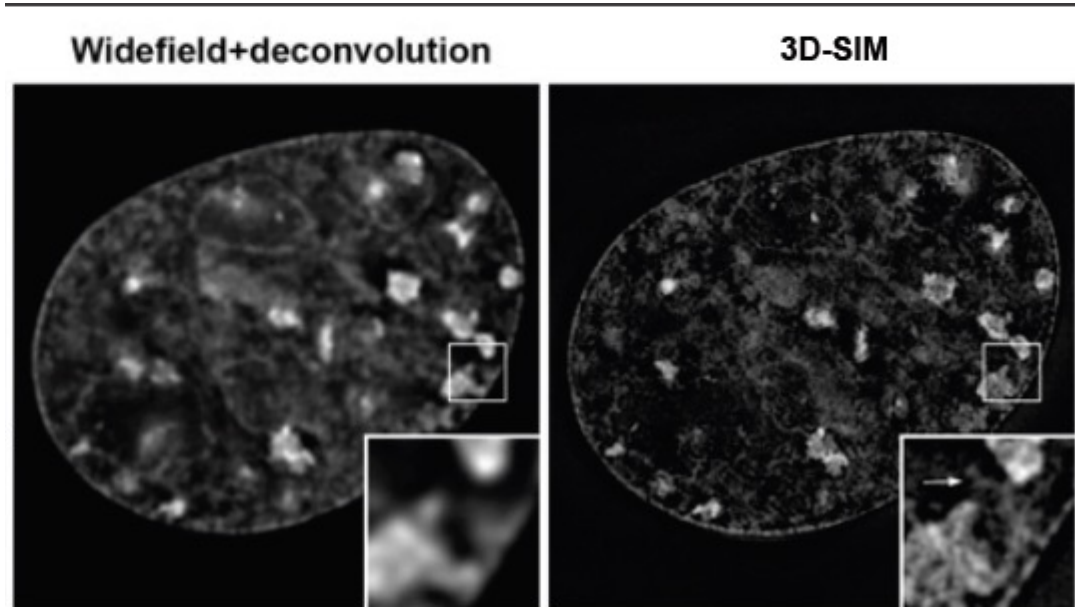




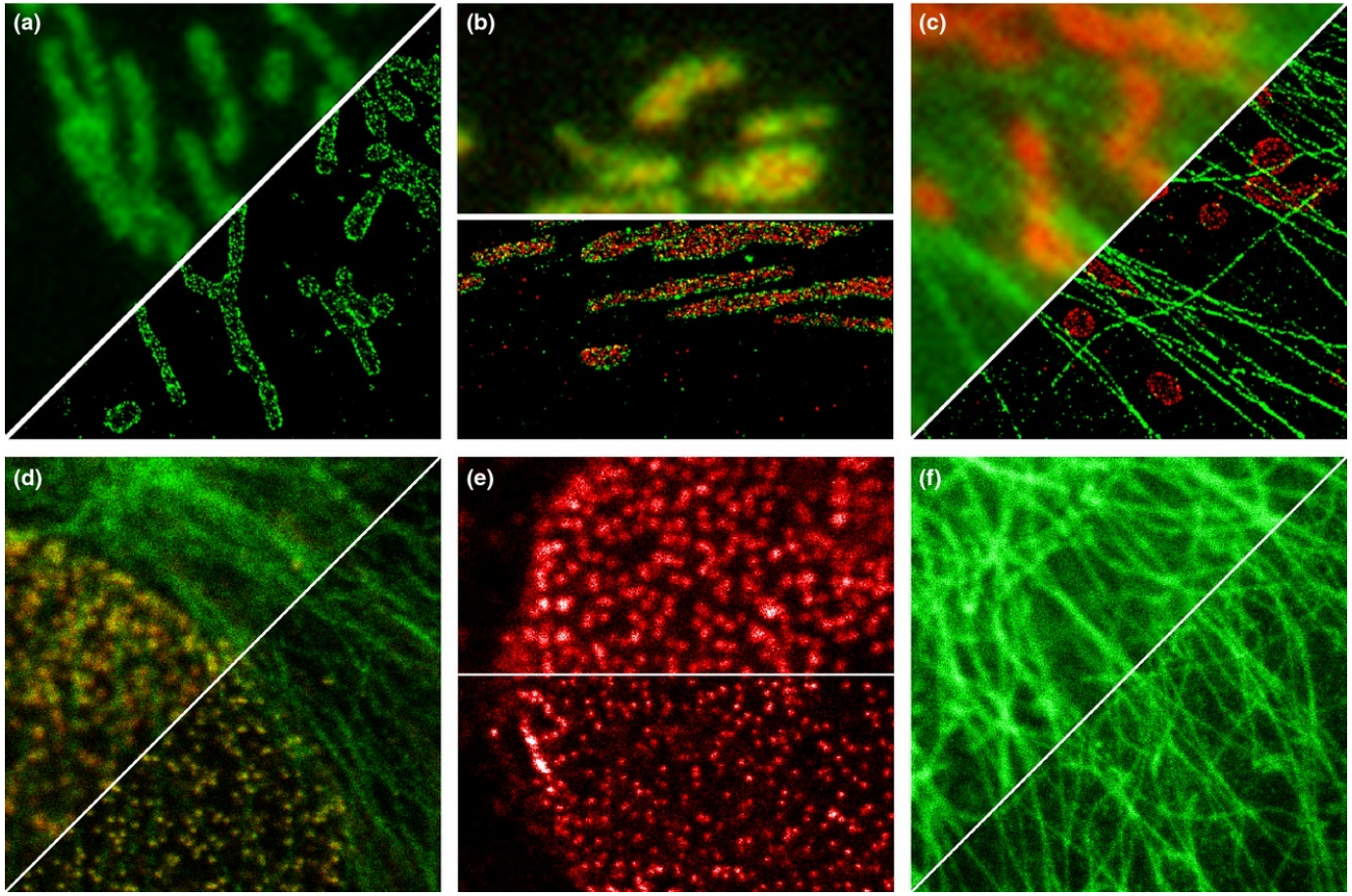
Multiplexing
Target specific
Higher sensitivity
3D (from cells to organs to model organisms)

What Types of Microscopy can you do/think about?

Super-resolution: structured illumination (SIM), stimulated emission/depletion (STED), localization microscopy (STORM/PALM)



STORM

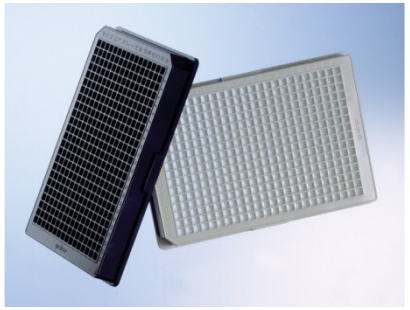


STED

- **<u>Why</u>** do a HTM experiment?
  - Multiplexing (i.e., 4 fluorescent markers + brightfield + other?)
  - Cost and time
  - Number of replicates/conditions per experiment
  - Single cell end points/subpopulation analysis
  - Identify outlier responses and heterogeneity
  - Couple with **High Content Analysis (HCA)**: extract 100s of features per cell/big data

High Throughput Microscopy Workflow

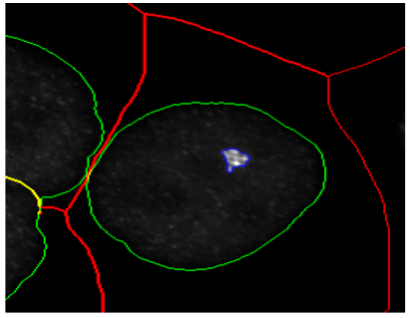
Plate cells on optical glass plate



Robotic Processing

High Throughput Imaging

Segmentation and Image Processing



Extract Features & Measurements

Study Phenotype/Function at the Single Cell Level: Strategies

- Knock-down: siRNA, CRISPRi, CRISPRko, shRNA
- Over-expression: cDNA Libraries (BACs, plasmids)
- Chemical Libraries (drugs, natural products, synthetic moieties)

- Reporter Cell Lines
- Antibody based
- Label-free

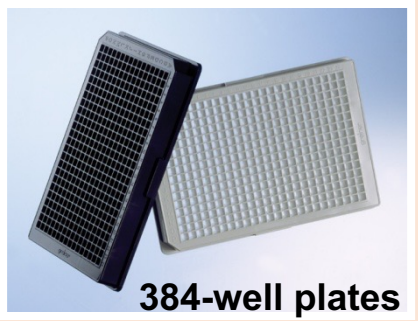
- Live vs. Fixed – time dynamics

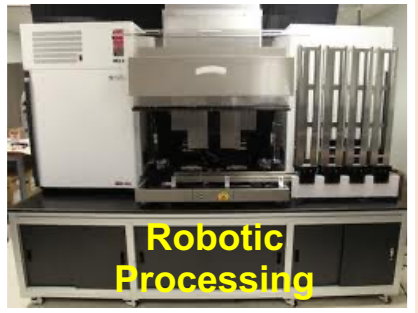
- Microscopy: label-free, epi, decon, confocal, FRET (in vitro). Coming/non-commercial: FRAP, light sheet, two-photon, TIRF, FLIM-FRET

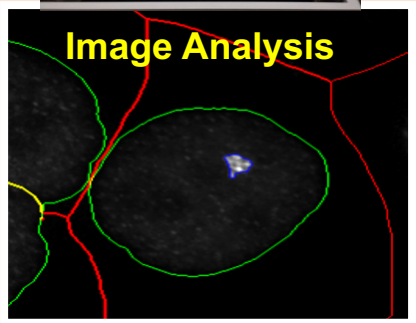
Assay of Interest:

- time-course
- dose-response
- antibody testing
- RNAi screening
- drug screening
- live imaging
- fluorescent proteins
- multiplex IF
- RNA/DNA FISH
- tox/EDC screens
- ……

HIGH THROUGHPUT WORKFLOW







SINGLE CELL (or bulk) END POINTS (100s features/cell – high dimension data)