

# High Content Screening of Combinatorial Library for Identifying Biomarkers

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# Outline

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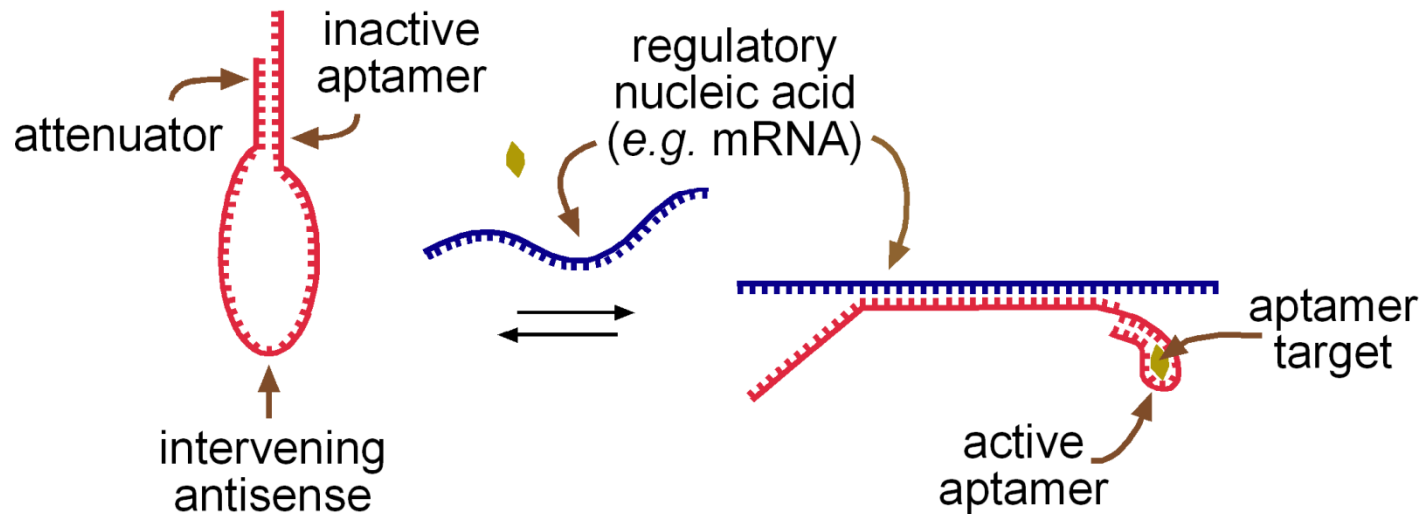


- Motivation
  - Issues
  - Approach
    - Model systems
    - Ligand library
    - Chemoinformatics
  - Results
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# Motivation

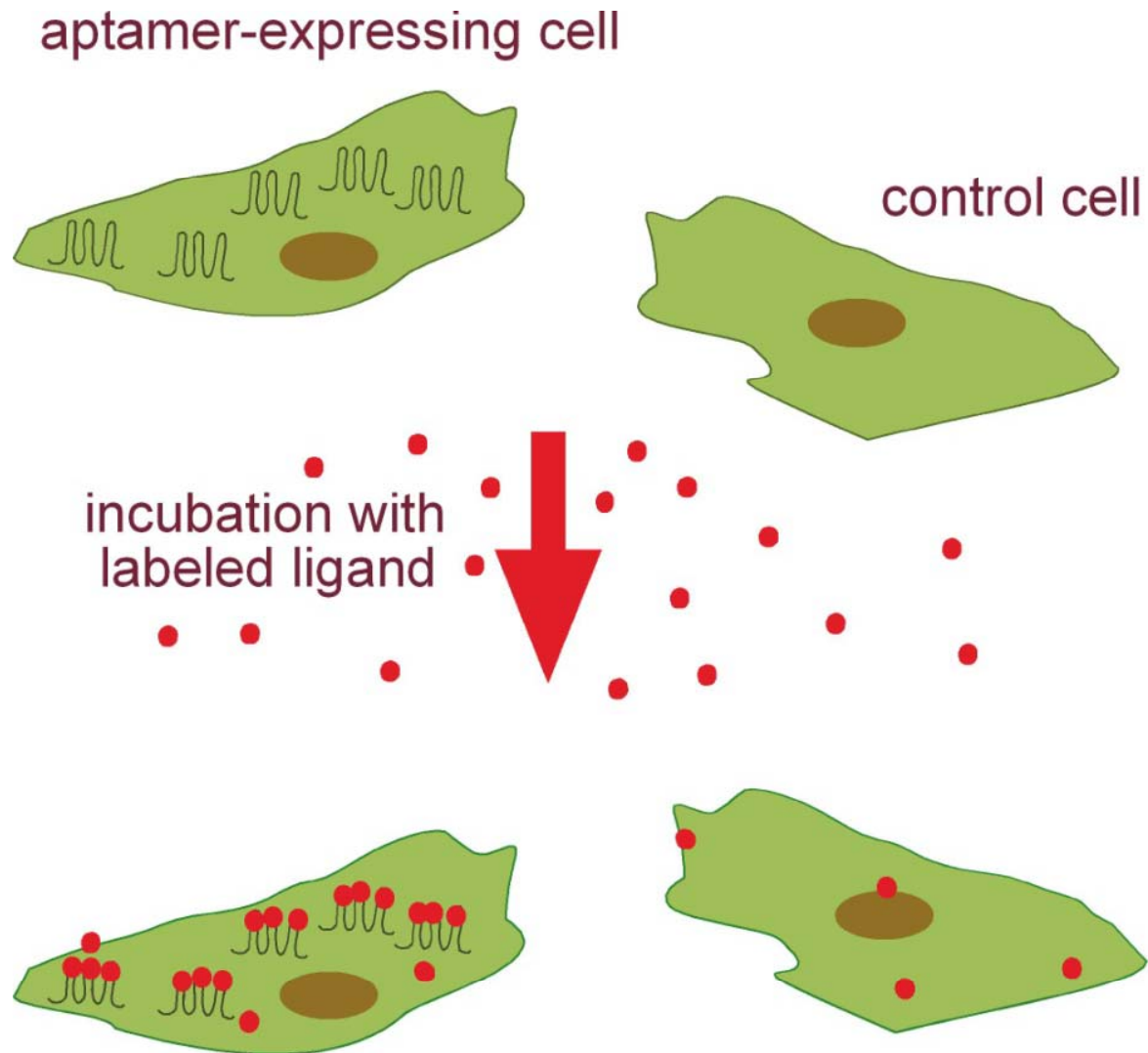


- Develop technology for *in vivo* imaging of gene expression
  - Mammalian as well as plant species
- Molecular Methodology



**Marit Nilsen-Hamilton,  
Iowa State University**

# Experimental Design



# Issues



- Identify improved ligands with
  - Lower background
    - Improved in- and out- fluxes
  - Lower toxicity
  - High signal to noise ratio
- Design new aptamer constructs that bind to ligand

# Approach

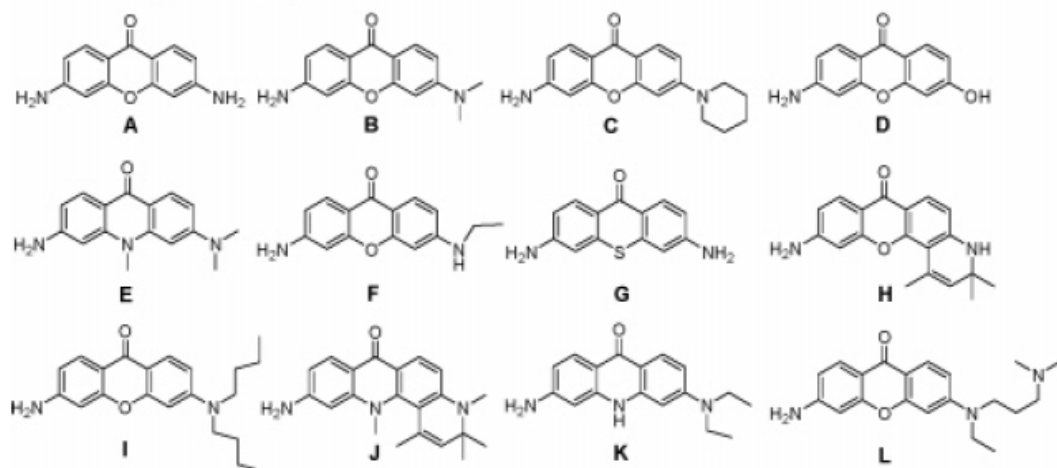


- Use a combinatorial fluorescent library of small molecules
- Develop a screening protocol against 3 mammalian cell lines
  - Chemoinformatics system
- Screen *candidate ligands* against the model system
  - Plant species
  - Higher level mammalian model system
- Improve properties of screened ligand by local perturbation

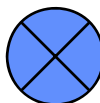
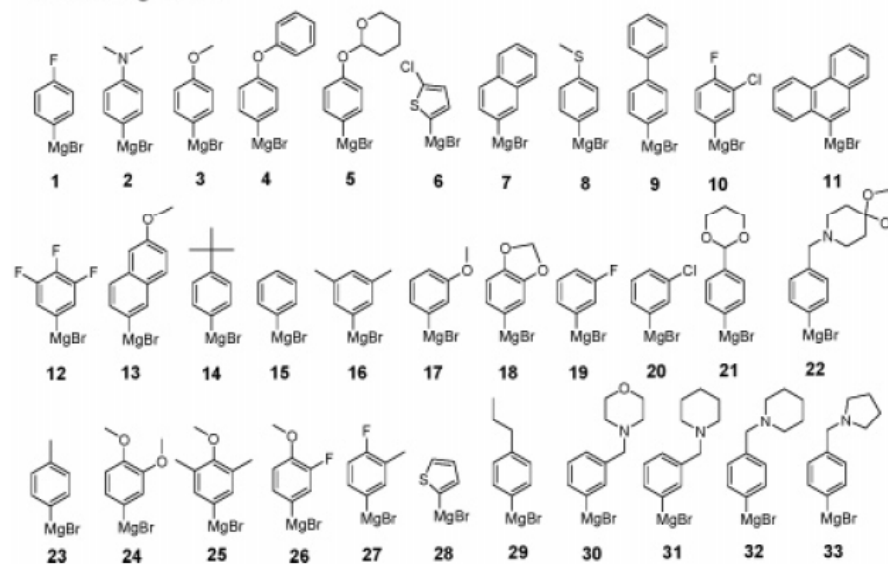
# Combinatorial library



## R<sup>1</sup> Building Block (S5):



## R<sup>2</sup> Building Block:



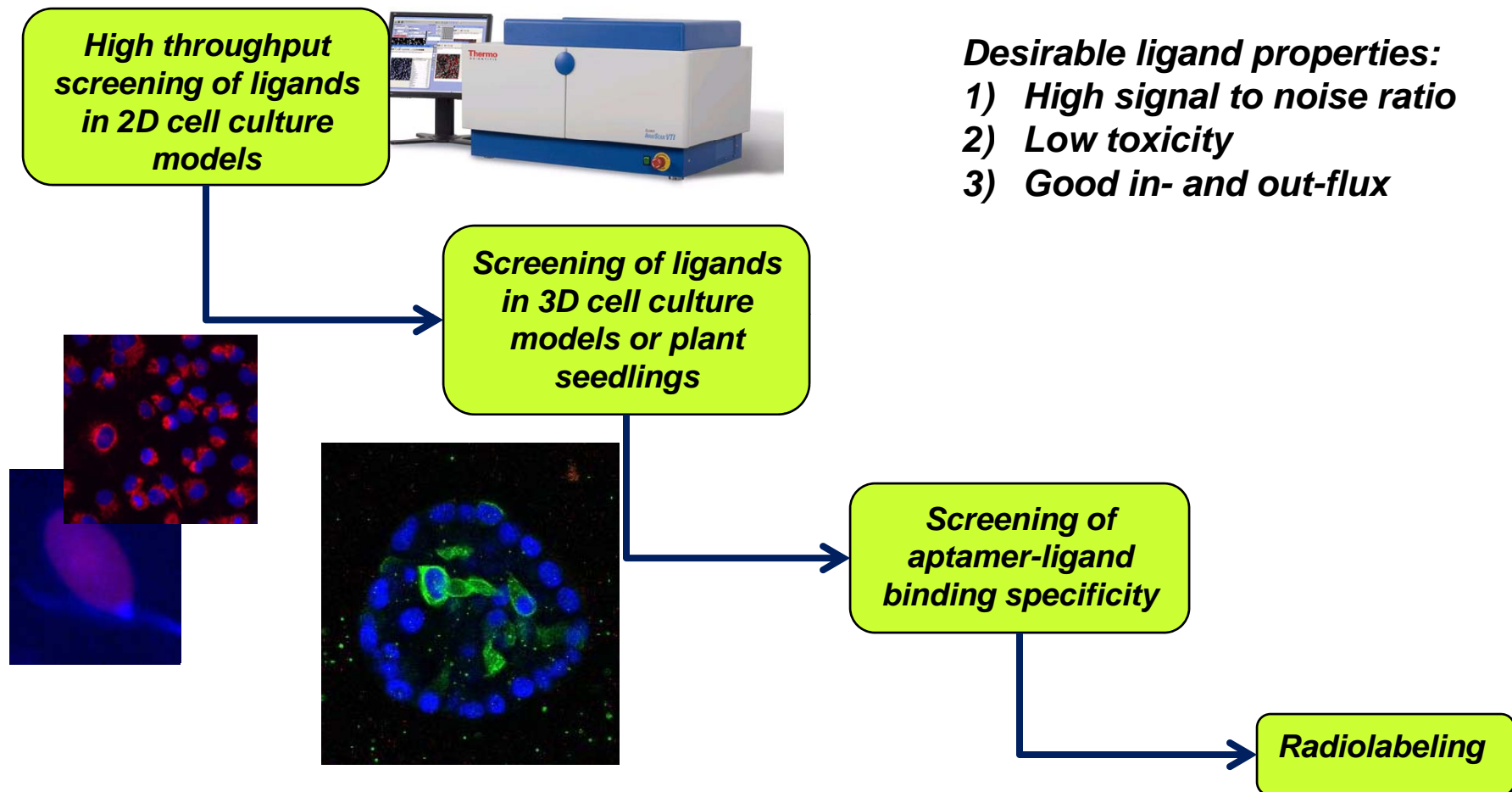
# Protocol



- Live cell imaging on a well-by-well basis
  - Nuclear is labeled with *vibrant red ruby*
  - Two time points
    - Immediately after incubation
    - 60 minutes after washout
  - Approximately 1000 wells
  - Cellomic instrument
- Quantitative analysis on a cell-by-cell basis
  - segmentation
  - Registration
- Tight integration with the BioSig extension on Chemoinformatics
- Validation with confocal microscopy



# Pipeline for screening ligands



**Optimizing of ligand properties for nuclear imaging of aptamer RNA**

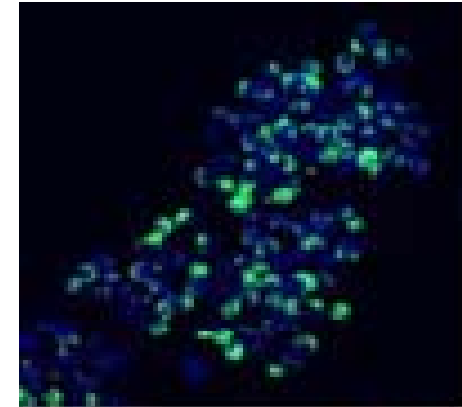
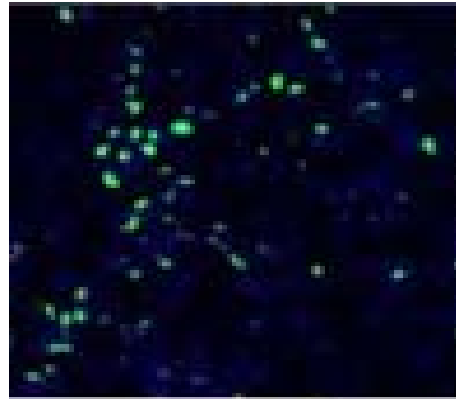
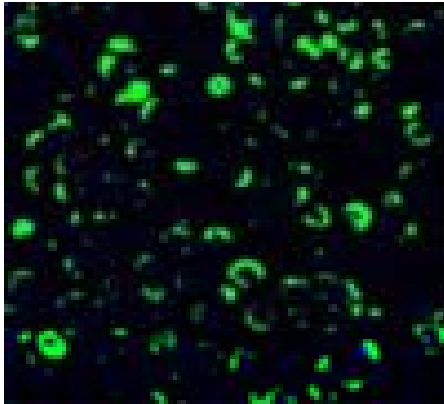


A thick red arrow pointing from left to right, indicating the direction of optimization.

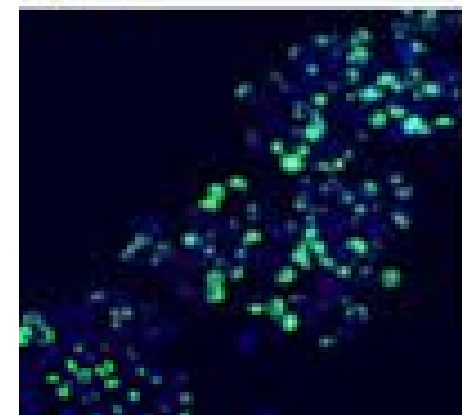
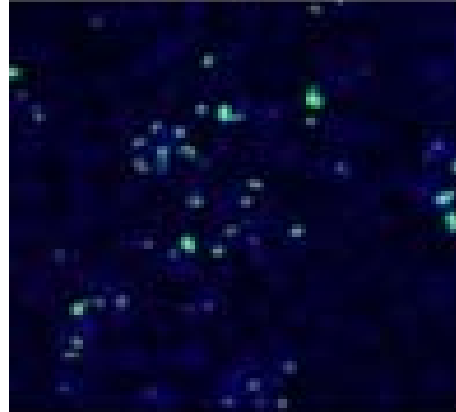
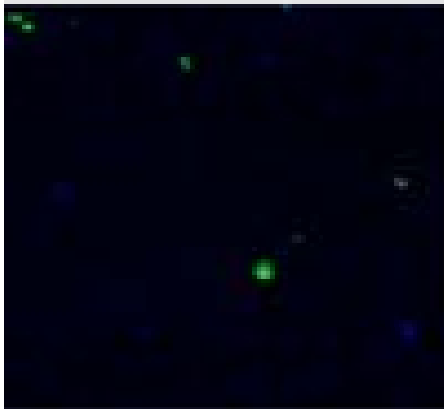
# Ligand response is heterogeneous



*Time = 0*



*Time = 60 minutes*

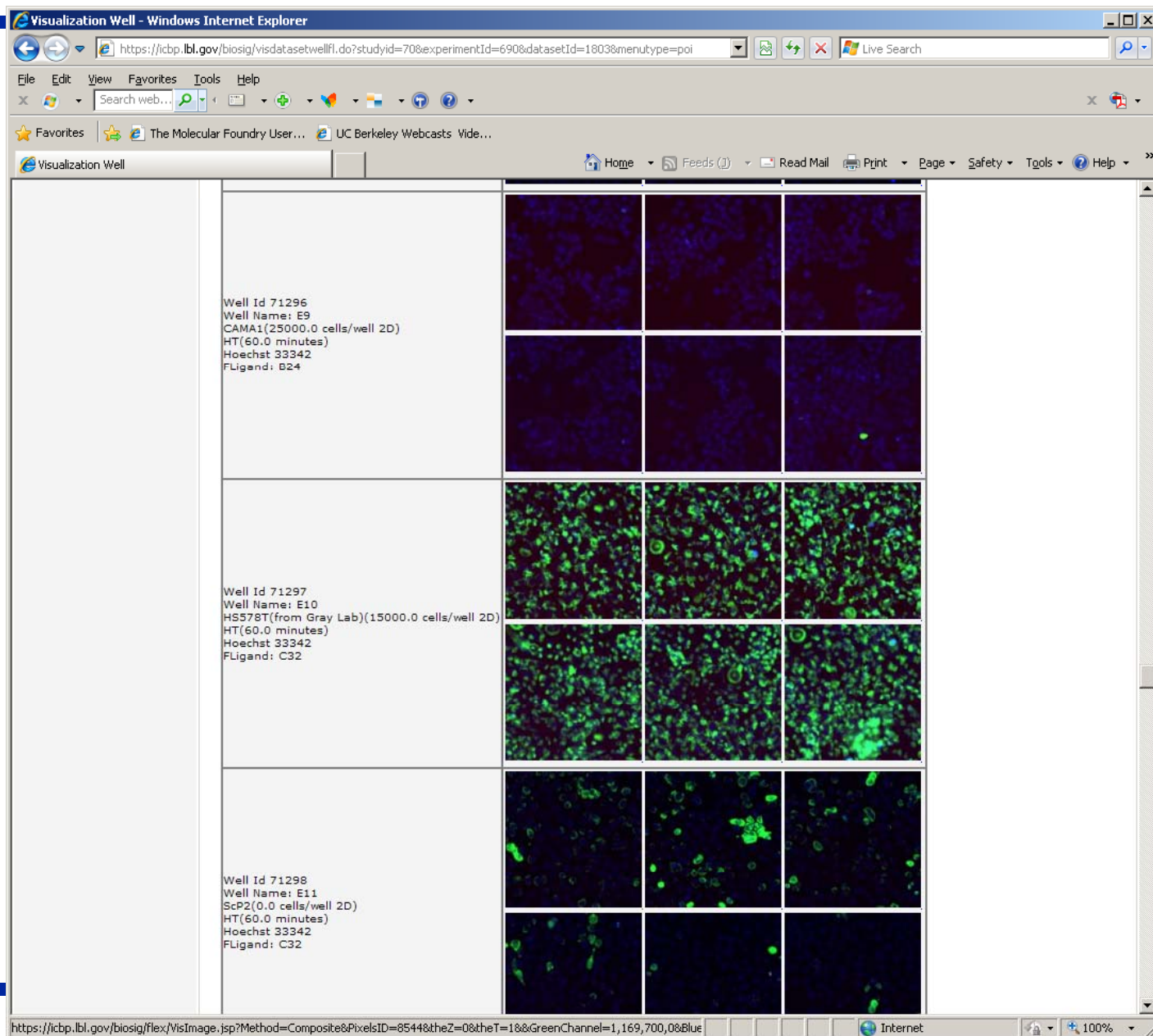


**ScP2**

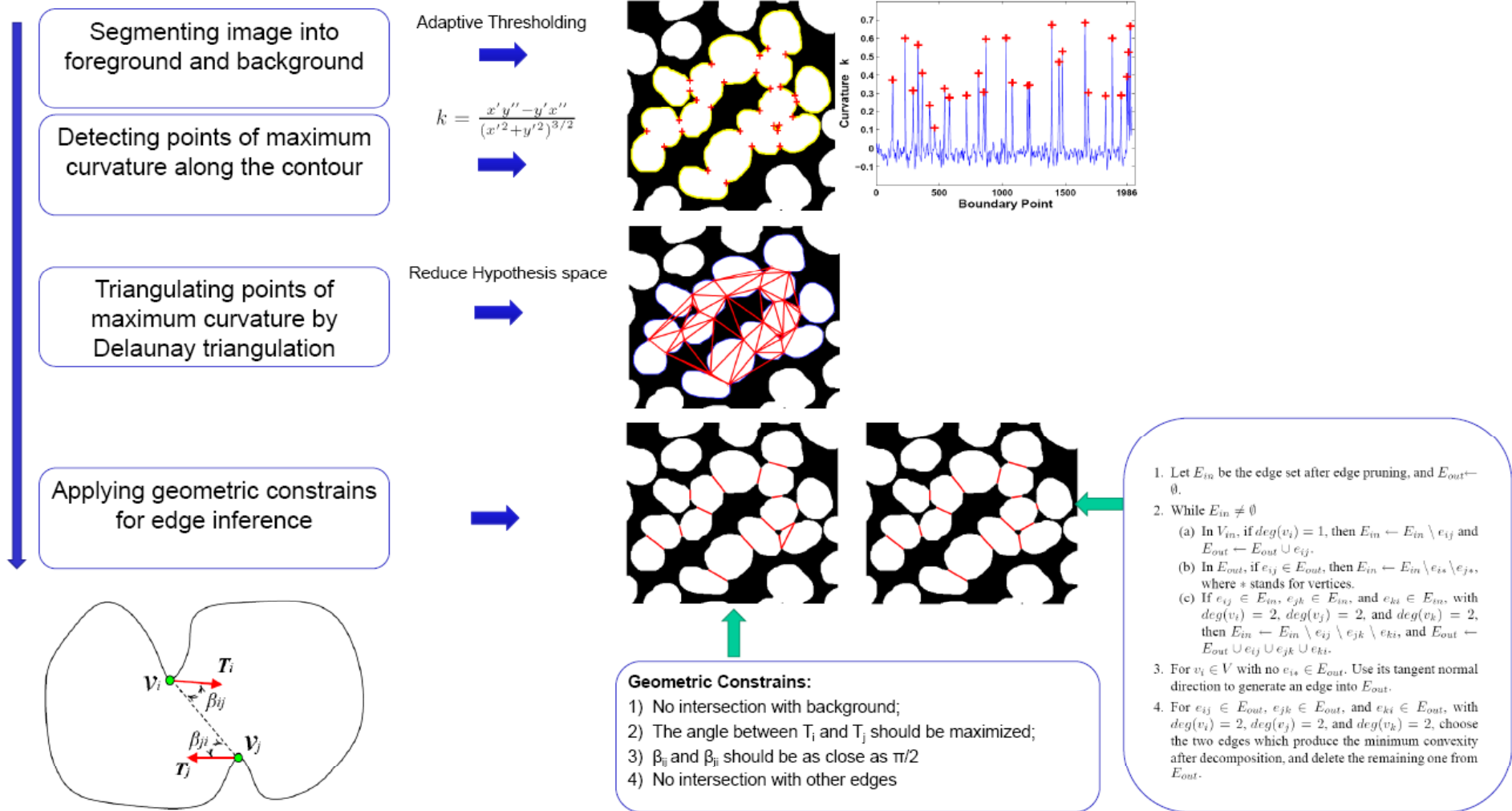
**HS578**

**CAMA1**

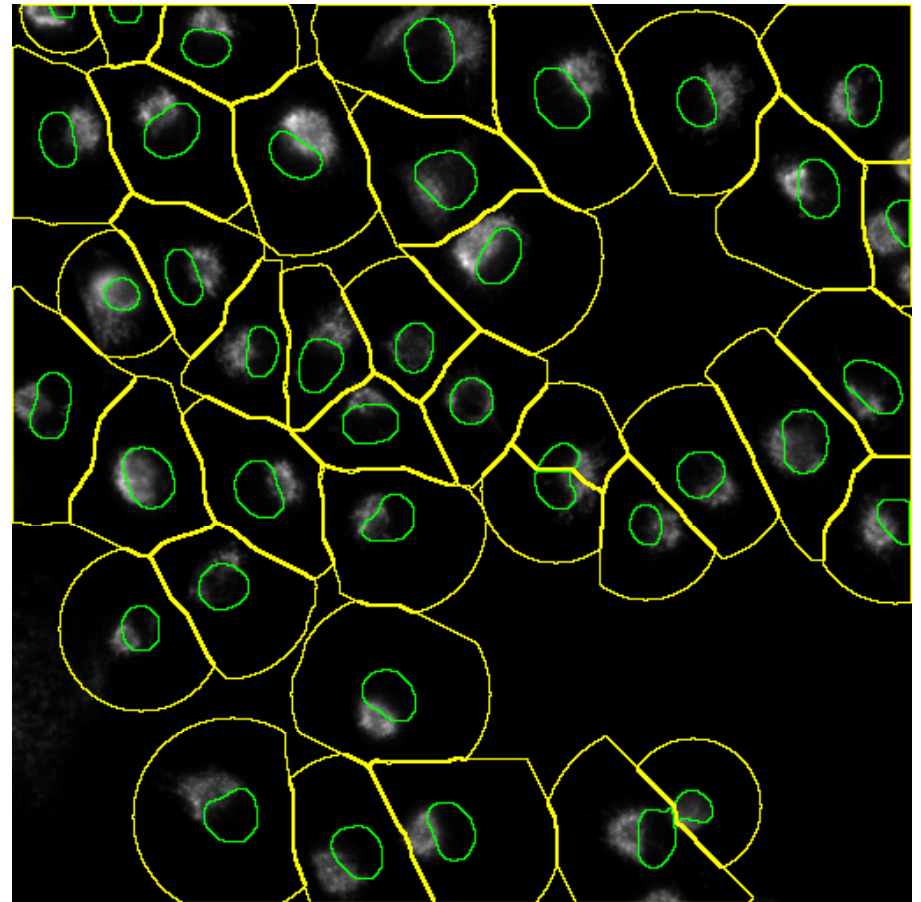
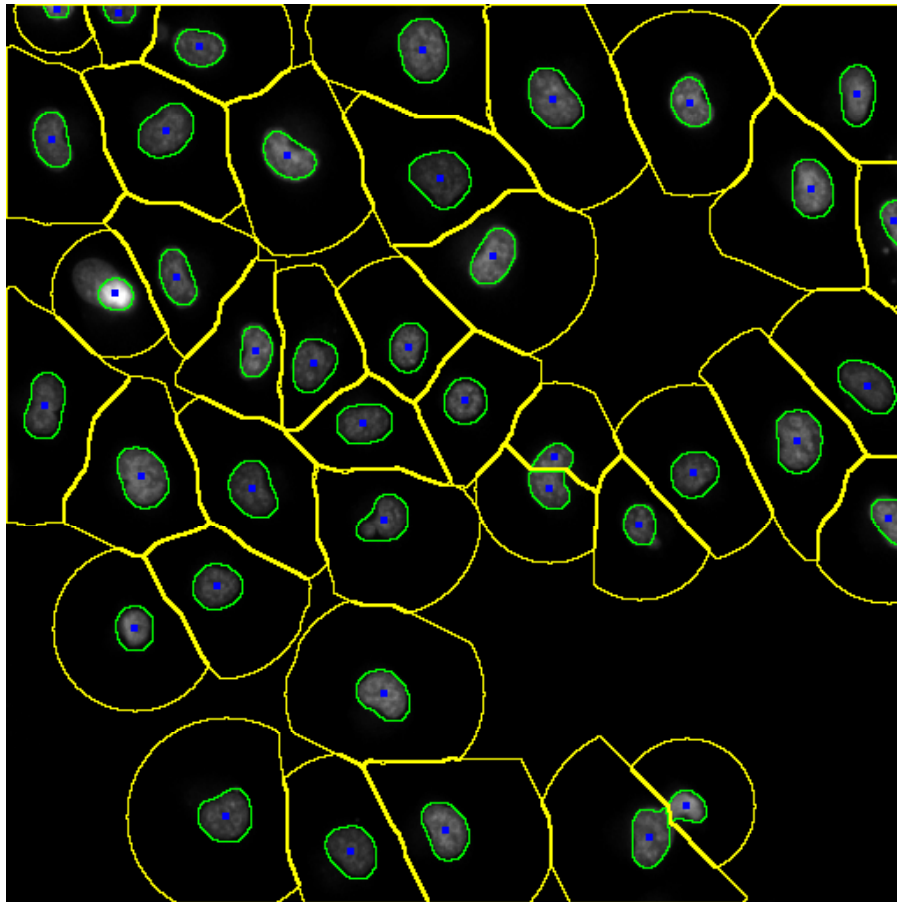
# Data access through BioSig



# Segmentation



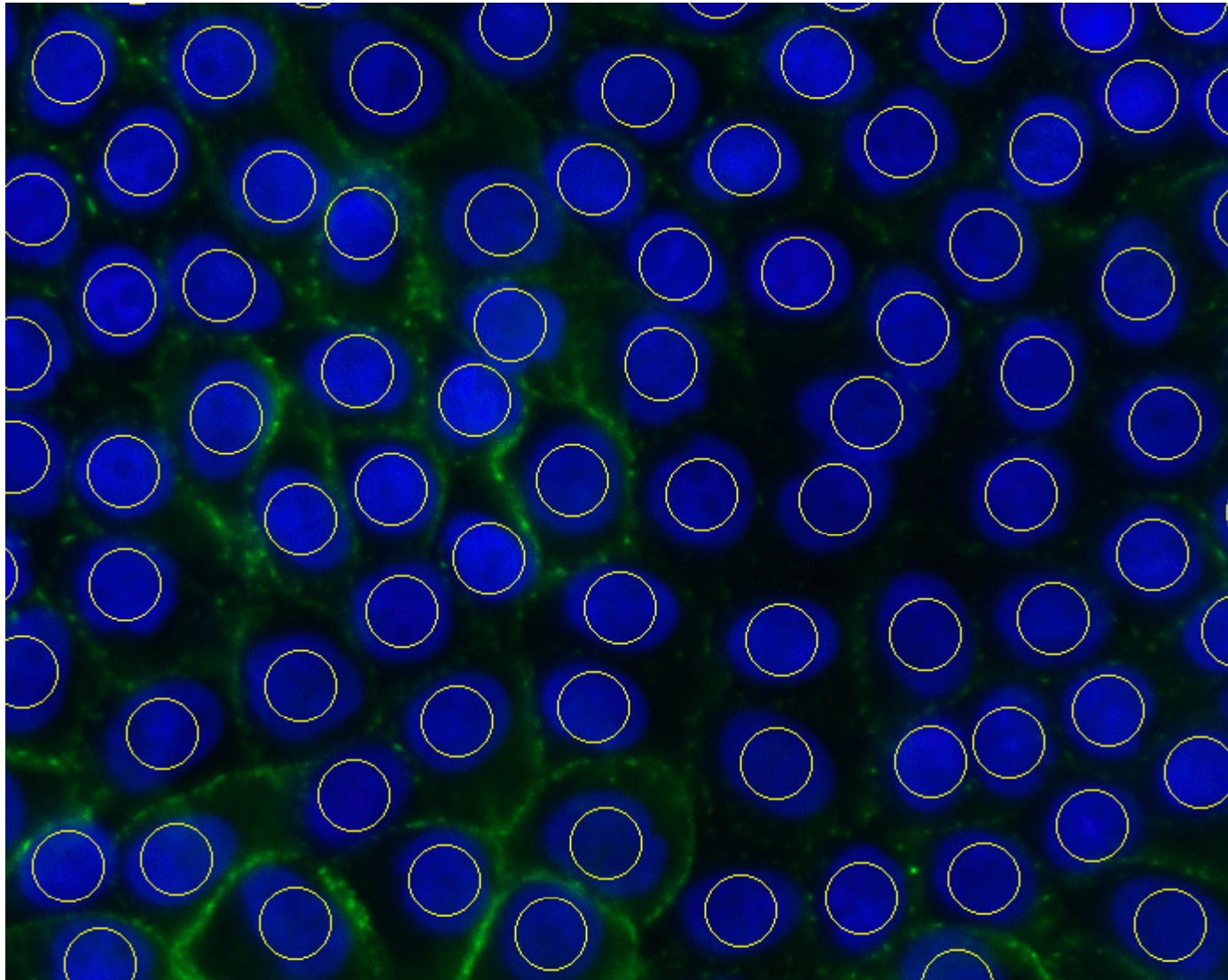
# Fluorescent signal measurement





# Evolving Fronts for Local Neighborhood Establishment

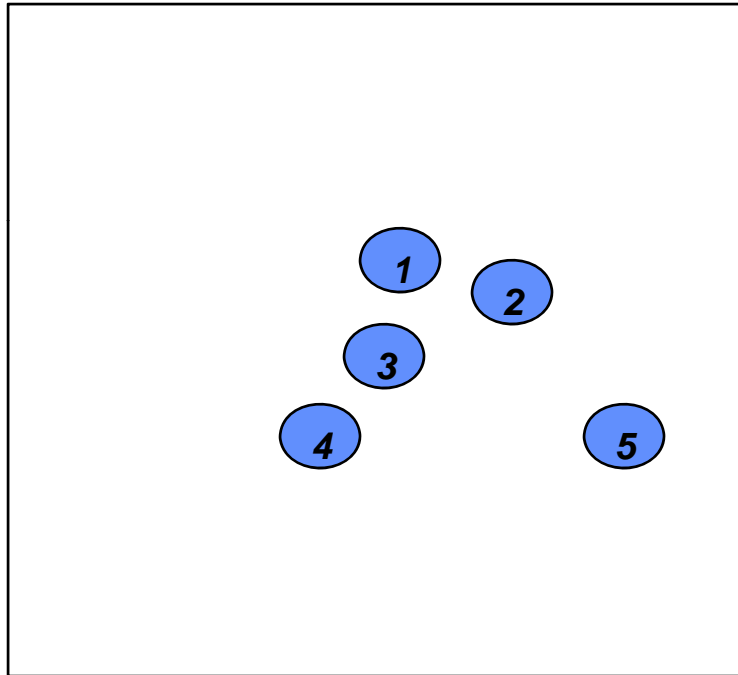
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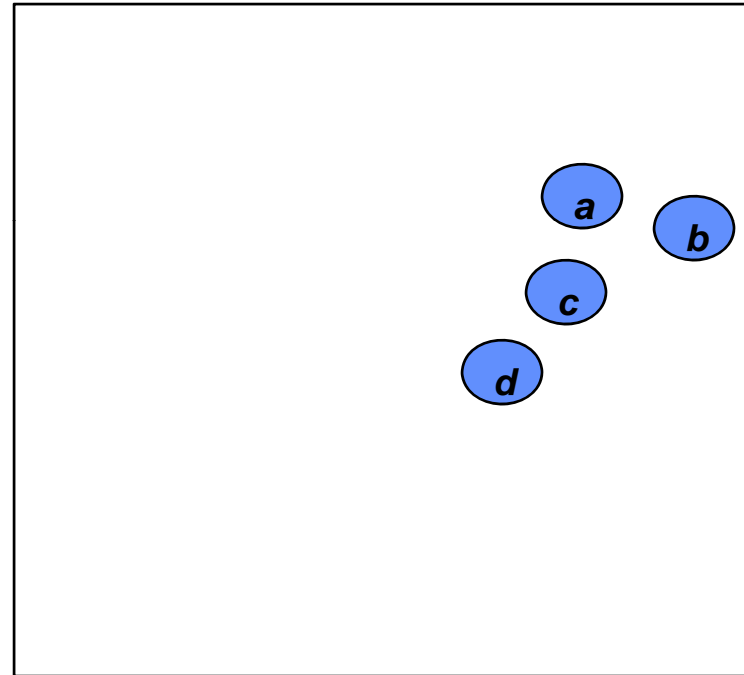
# Registration



$T_0$



$T_1$



$a = 1; b = 2; c = 3; d = 4$

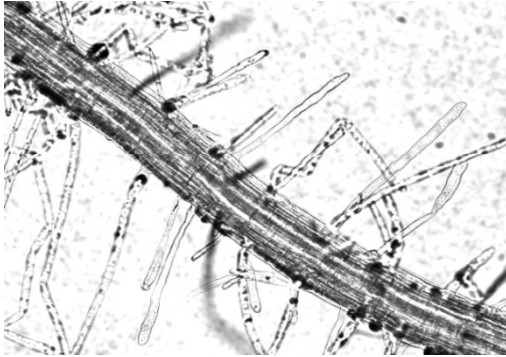
## CAMA1



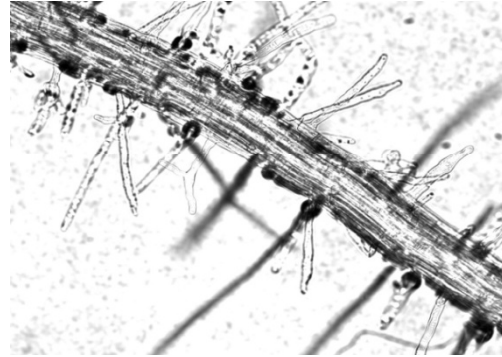
# Identify candidate ligands and validate



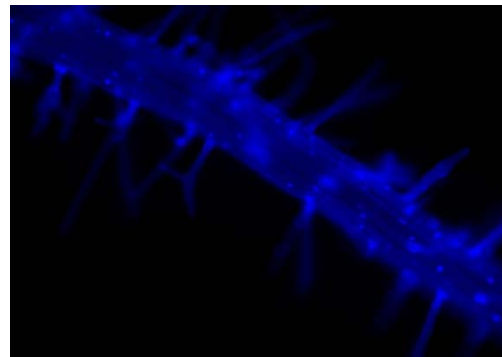
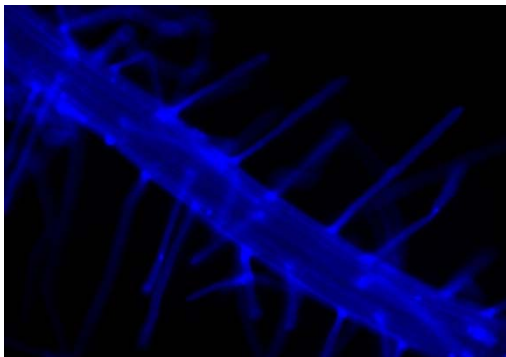
*0 min*



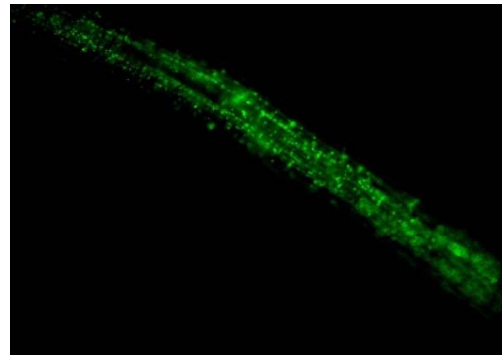
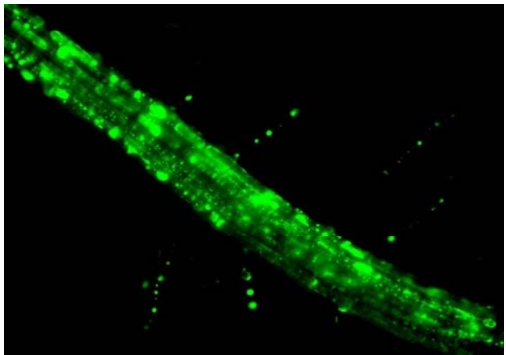
*60 min*



*Bright  
field*



*Hoechst*



*Ligand  
E14*

# Results



- A number of ligands with desirable properties have been identified
- Multiple mammalian cell lines are crucial for screening ligands
- Mammalian lines can serve as a proxy for other model system (e.g., plant species)
- BioSig extension for Chemoinformatics analysis has expedited throughput

# Acknowledgement

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## **LBNL**

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- Kai Zhang

## **Iowa State Univesity**

- Marit Nilsen-Hamilton