

# Clonogenic Assay Tool

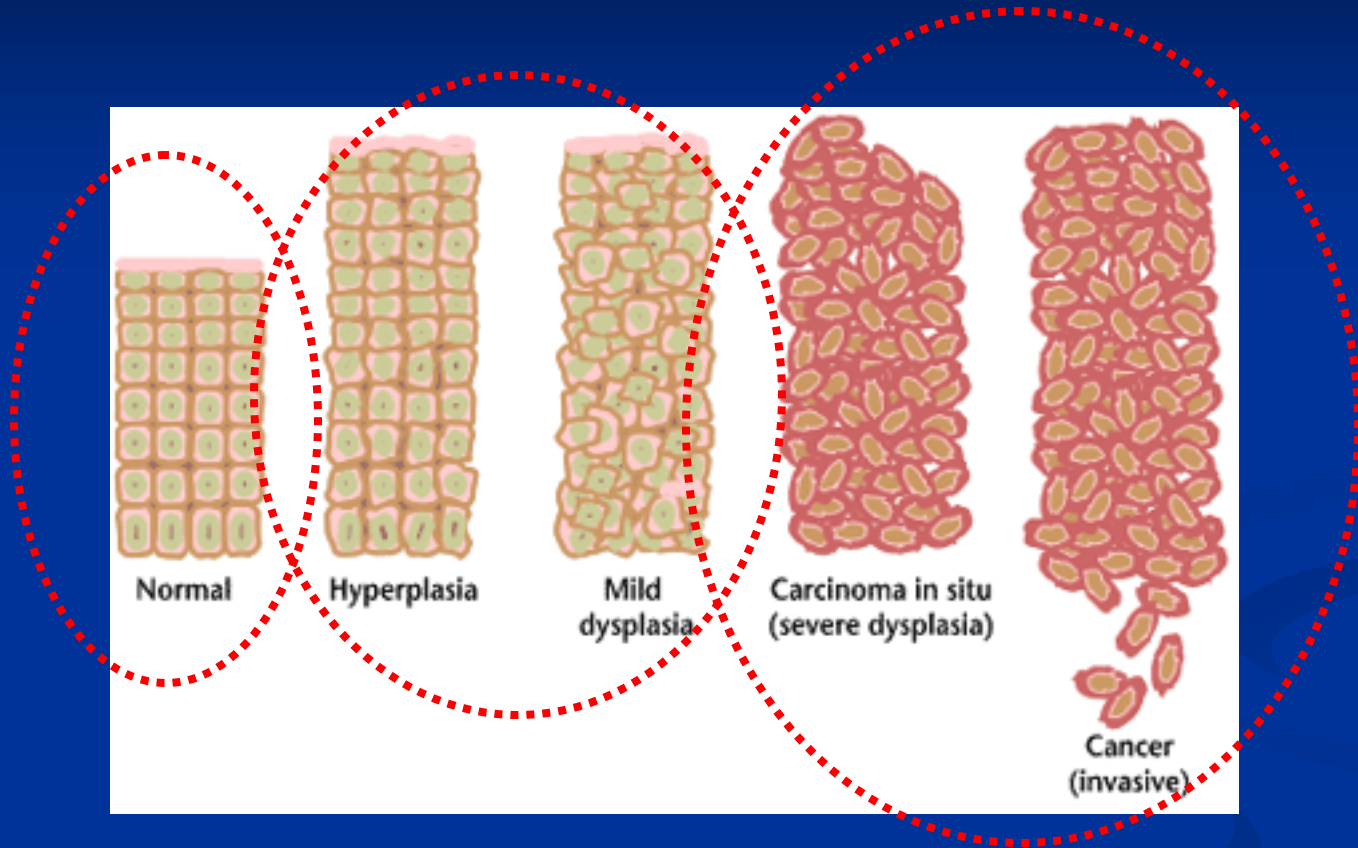
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# Cancer Progression/Stages



Source: wikipedia.org

Healthy  
Cells

Pre-Cancerous  
Cells

Cancerous Cells

# Rationale/Goals

- Many different methods to study drug effects on cancerous cells ability to proliferate...
  - Microtubule Analysis, Mitotic Arrest, Clonogenic Potential, and many others.
- Clonogenics— ability of cells to generate clones.
- Goal – develop a new tool that will help us analyze the results of clonogenic assay, examining the quantity and quality of colonies being formed (number, size (area), density, and possibly other parameters of colonies).
- “Aren’t there already programs out there for this?”

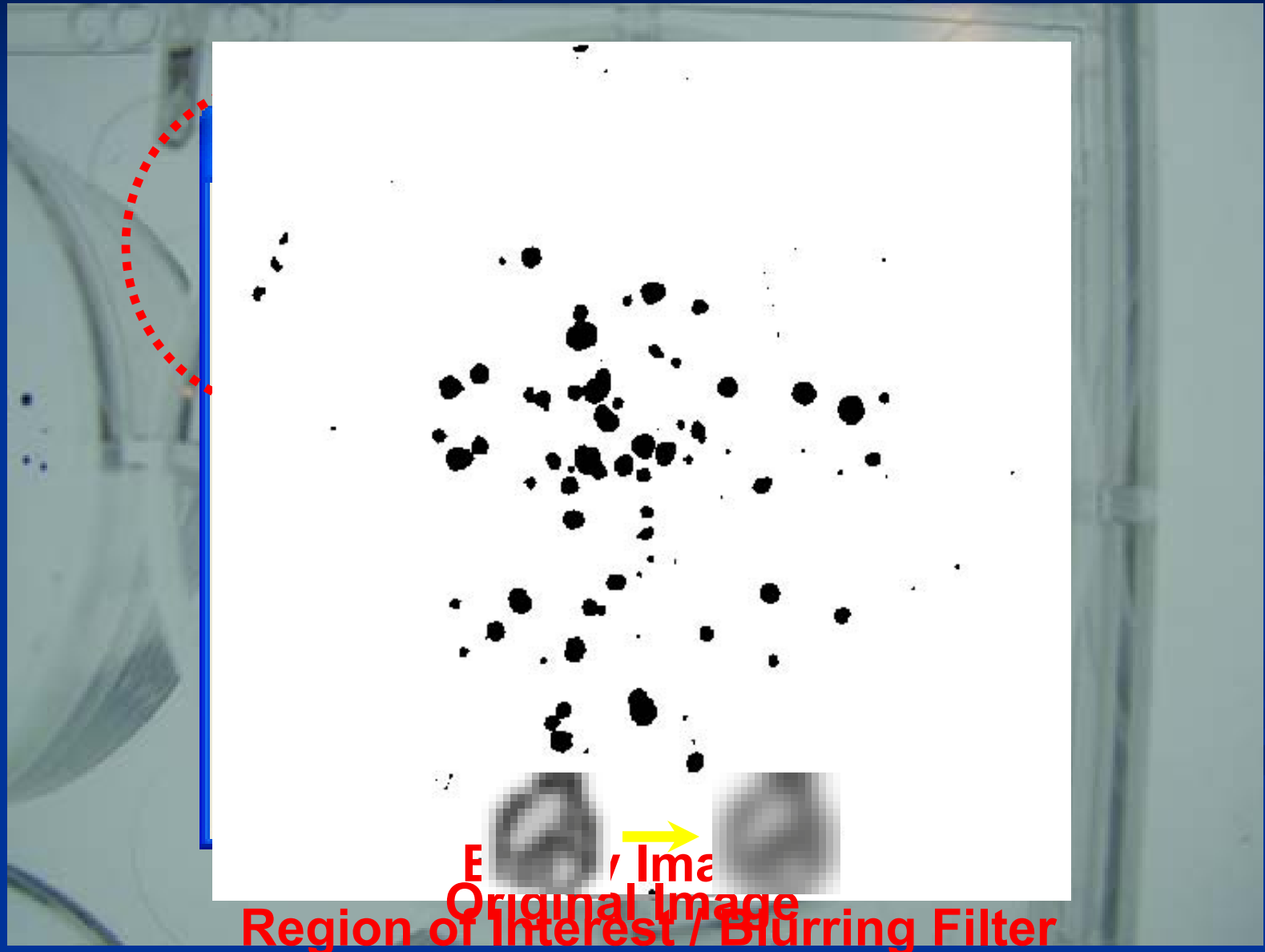
# Objectives

- Learn the **Igor Pro** programming language and software environment.
- Explore different capabilities which exist in the Igor Pro language which will help us achieve our goal.
- Develop the colony analysis tool.

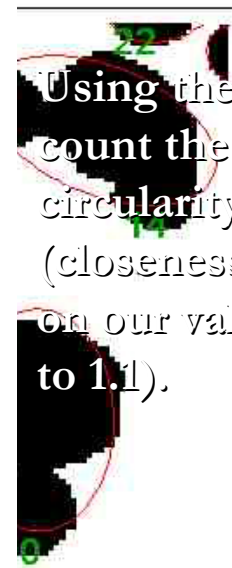
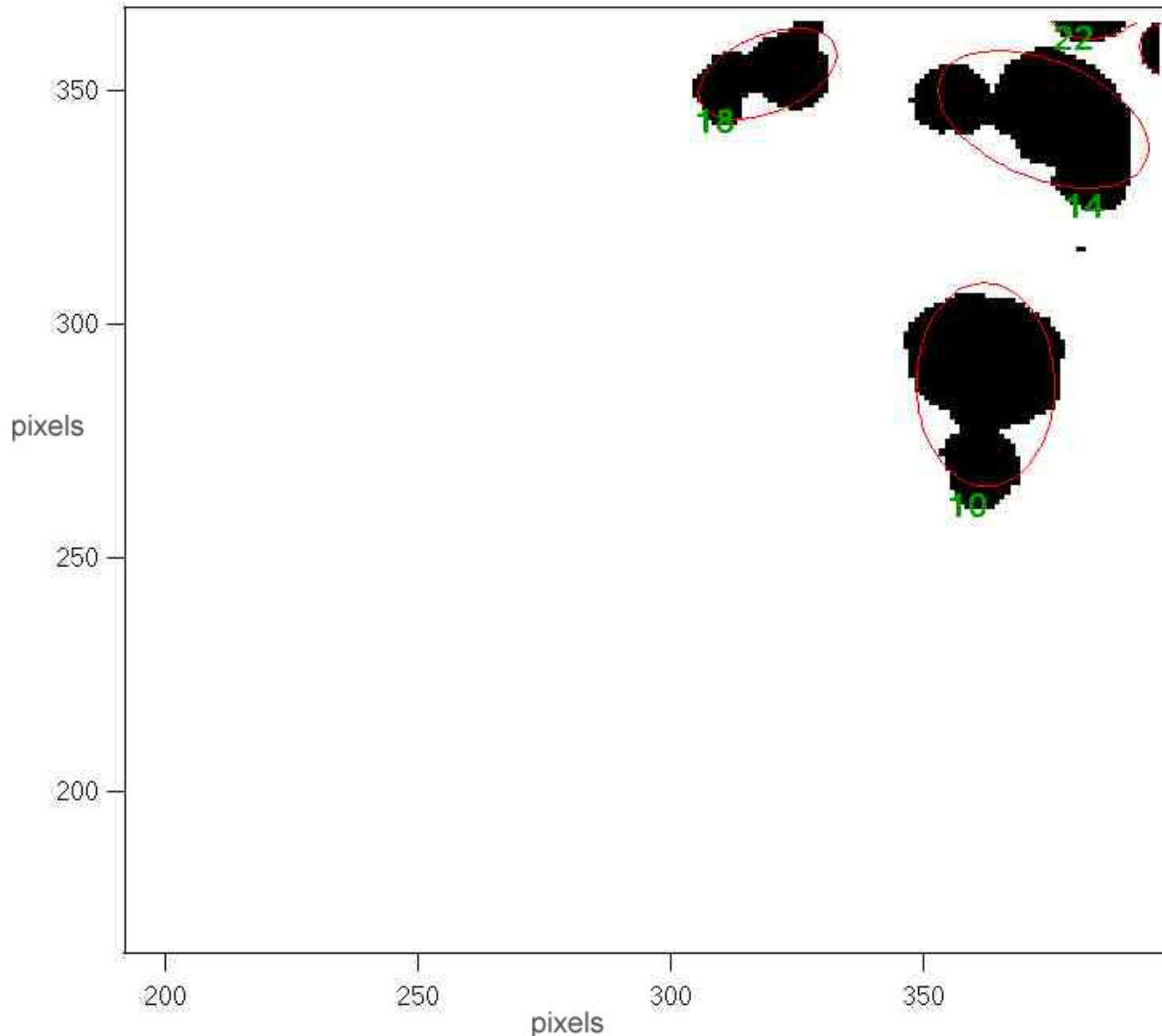
# Flow of Clonogenic Assay Tool

- Identify Region of Interest (ROI)
- Separate the ROI from the rest of the image.
- Condition the image to make it optimal, if needed.
- Using thresholding, separate background from colonies and generate binary image.
- Identify colonies as separate objects, analyze objects and separate into single colonies and complex colonies (multiple colonies joined together).
- Reprocess complex colonies in order to separate single colonies in each of them.
- Analyze and record all necessary information from the colonies.

# Image Filtering



# Colony Counting



Using the circularity, we count the ones whose circularity is close to 1 (closeness being based on our values, usually 0.9 to 1.1).

For the remaining elliptical ones, we pass it back to be re-evaluated to determine if its 2 colonies, or more than 2 colonies – using reverse thresholding to determine. The “circular”

# Accomplishments

- Learned how to perform Image Processing and Analysis.
- Programmed a panel/tool in Igor Pro.
- Experimented with numerous different filters and modules, implementing only the most effective ones.
  - Performed a colony count.



# Acknowledgements

Samantha Freeman, INSET Coordinator

Dr. Evelyn Hu

Dr. Nick Arnold

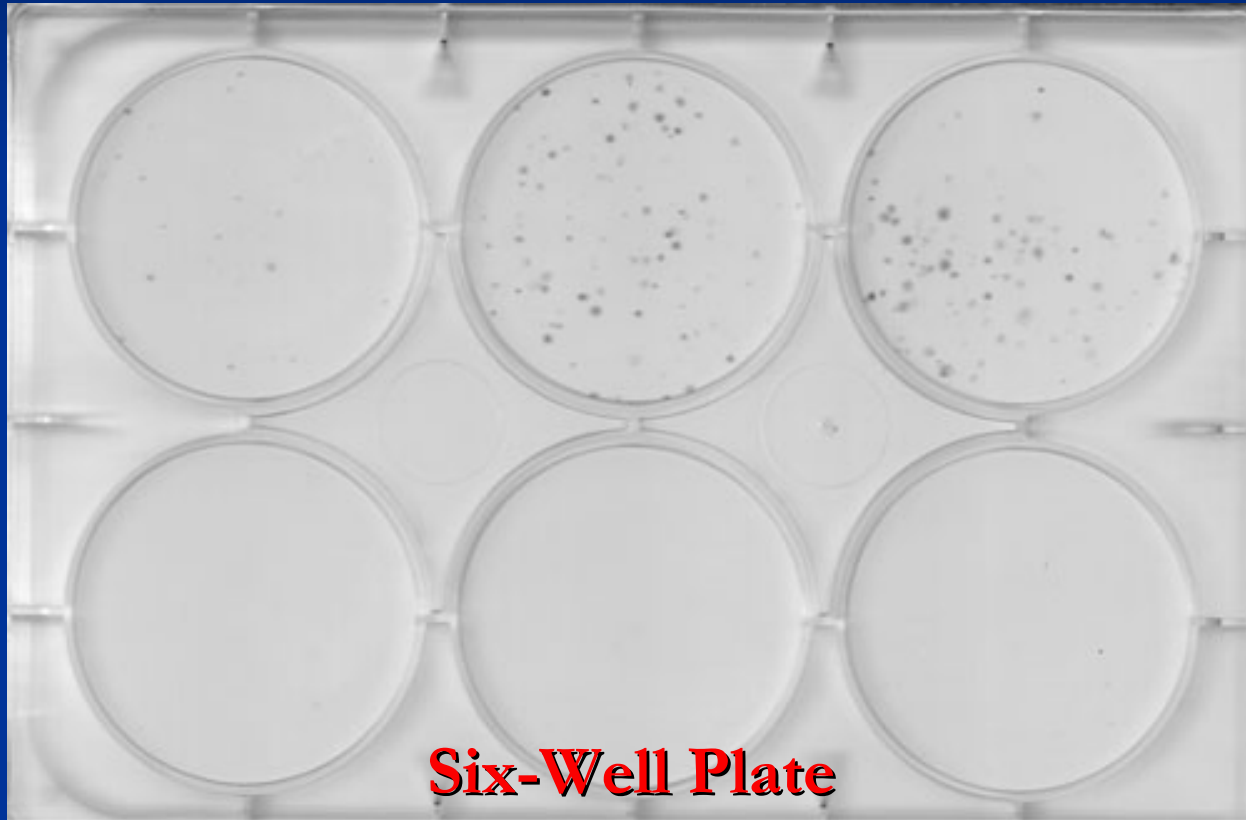
Liu-Yen Kramer

Luke Bawazer

And to everyone else who helped us... Thank you!



Questions?



**Six-Well Plate**

- We use matrix multiplication to cancel the background, therefore leaving only our region of interest in tact.
- In this image, a blurring filter has also been applied