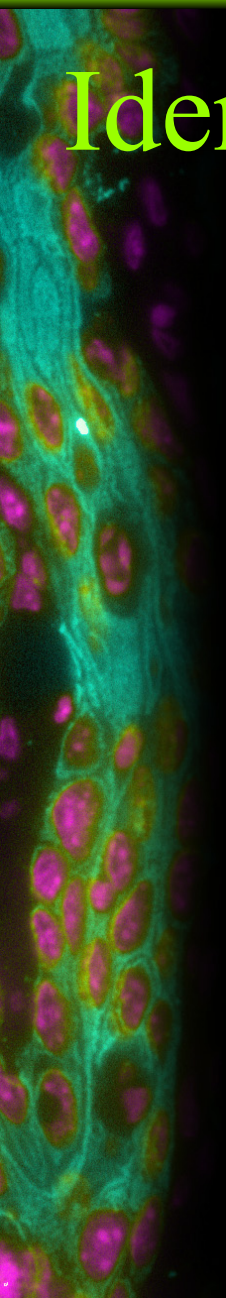
Identification of Intracellular Vesicles

A Pipeline Pilot Imaging Protocol



The ‘best’ image and data analysis for an assay with multi-channel multi-parametric population data is an asymptote:

- Measuring biological state of interest
- Maximizing signal to noise
- Maximizing repeatability

Componentized Image and Data Analysis

There are several common impediments to approaching the 'best' image and data analysis for a given assay.

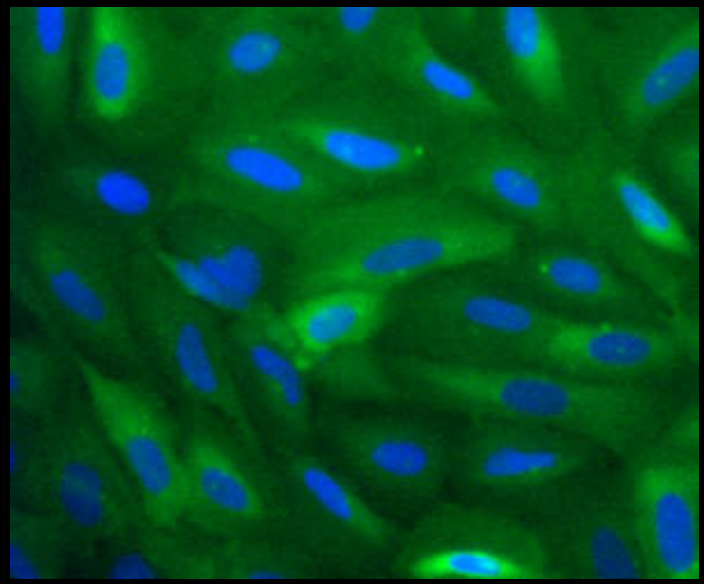
- Tight coupling of acquisition system with analysis system.
- Limitations on flexibility or customization of image and data analysis processes.

One well established solution is to employ componentized analysis in an automated workflow system.

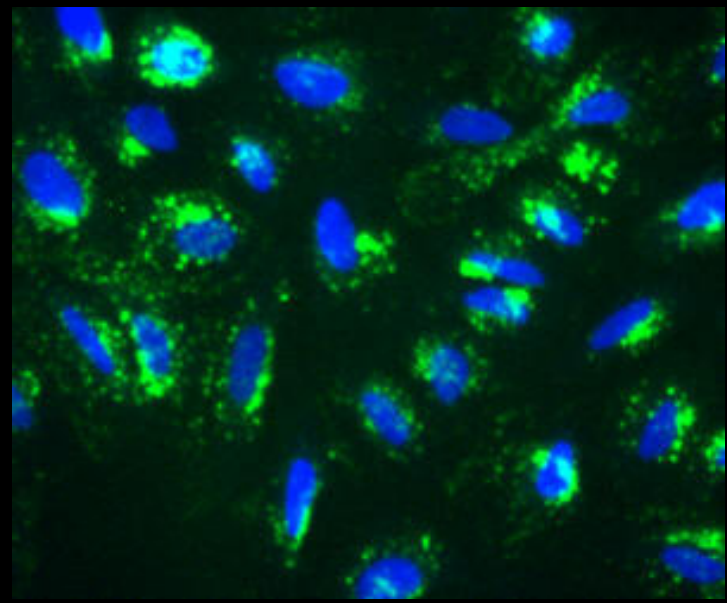
Pipeline Pilot has been widely used for similar tasks in cheminformatics, bioinformatics, statistics & reporting.

The new Imaging Collection brings these capabilities to the image processing arena.

Example: Roche Transfluor GPCR



Negative



Positive

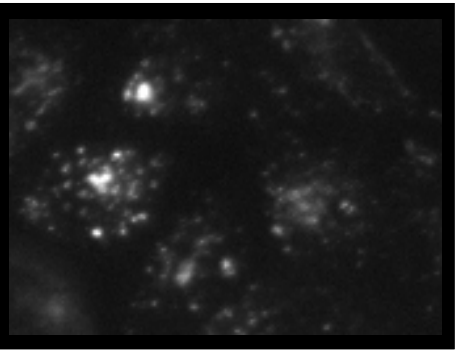
GPCR signal response leads to aggregation of labeled protein into vesicles.

10 Point CRC collected in single row of plate with side by side duplicates.

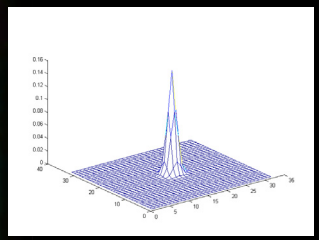
One positive and three negative control wells.

Difference of Gaussians Vesicle Localization Filter

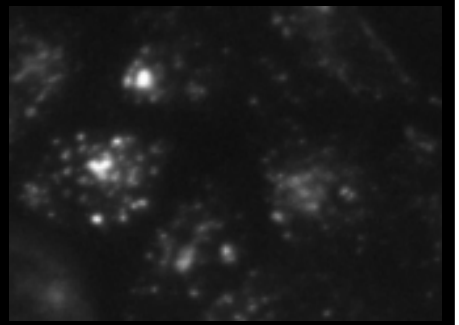
Input Vesicle Image



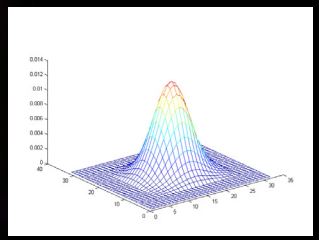
A: Blurred to typical vesicle size



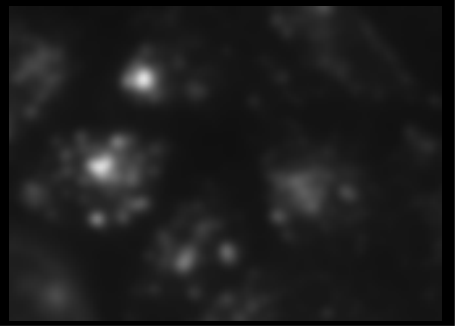
Narrow Gaussian Filter



B: Blurred beyond typical vesicle size

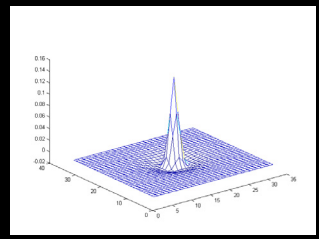


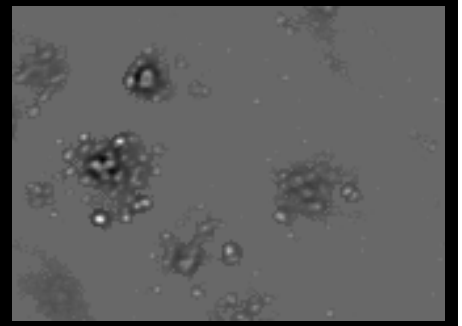
Wide Gaussian Filter



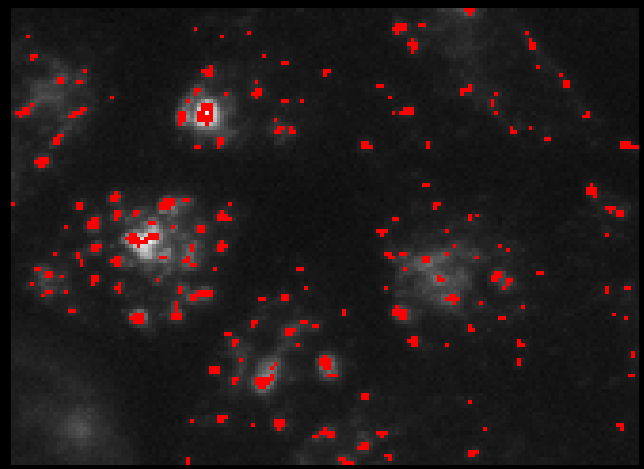
A-B: Difference of Gaussians Response

Sum of Filters



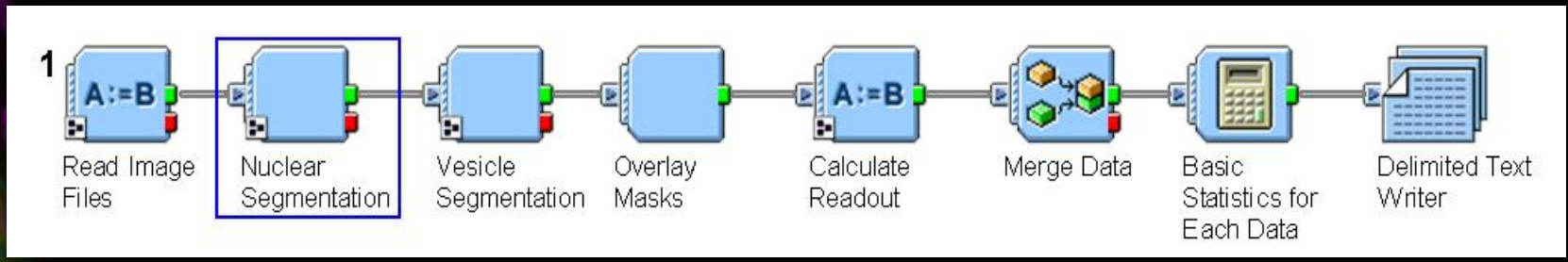


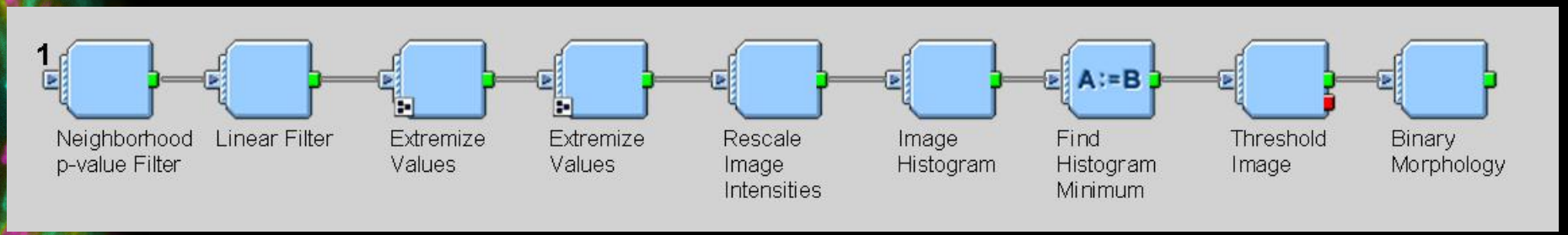
DOG Response > 0 Localizes Vesicles

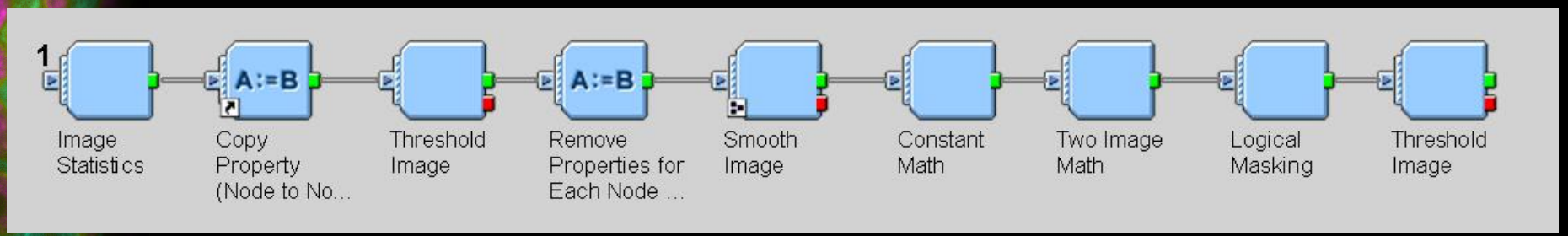


Edward Hunter, PhD Hunter Mikic & Callaway, LLC

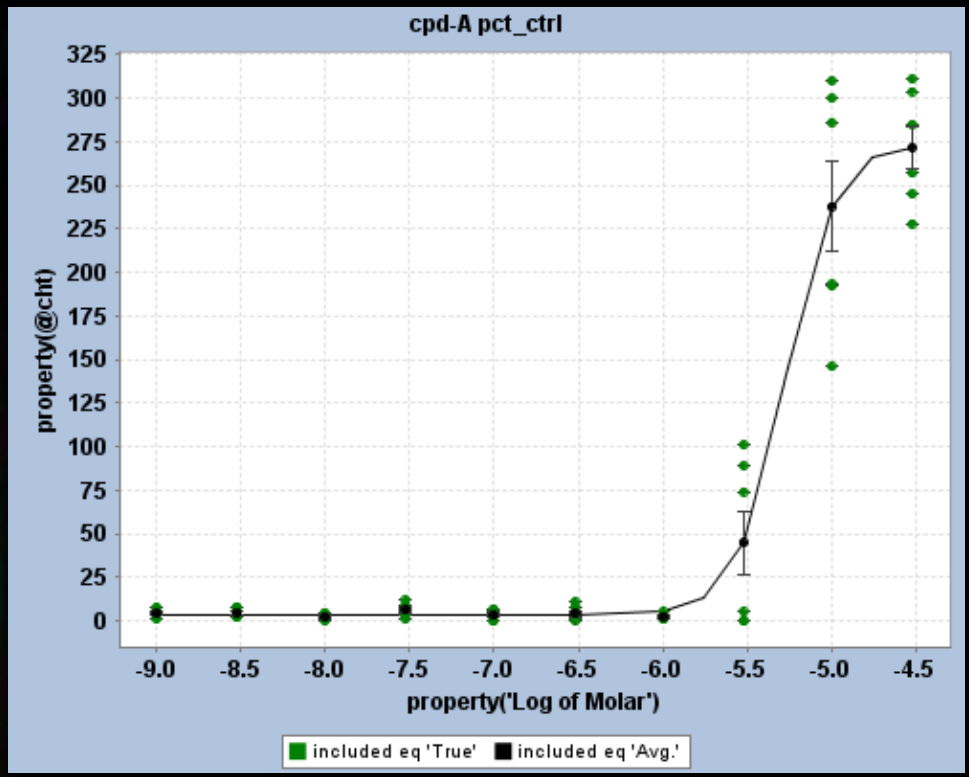
D-O-G Vesicle Localization Protocol

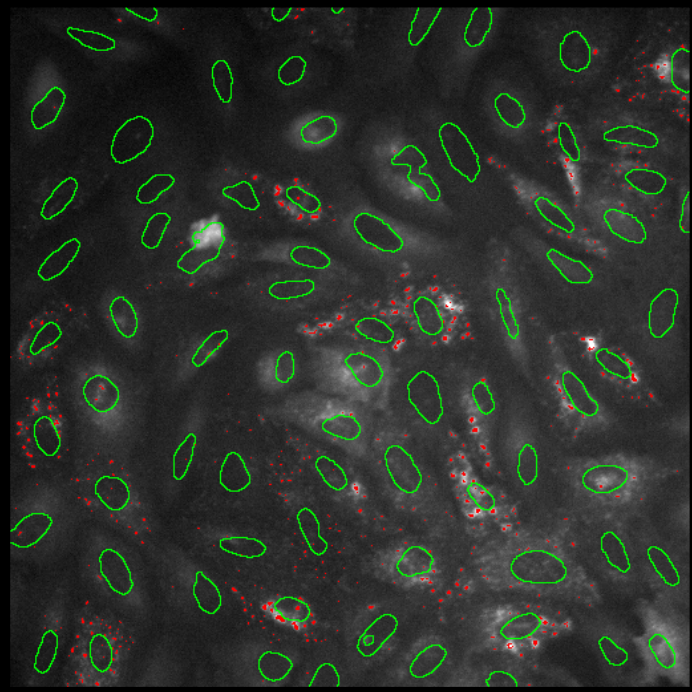


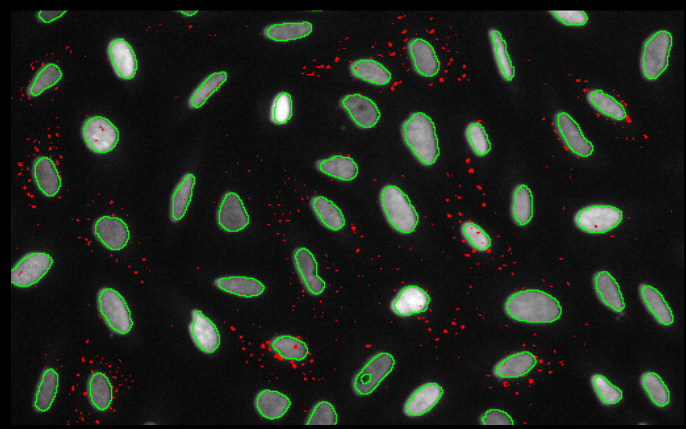




Simple Readout: Count of Vesicles







- Whole protocol executes in ~1 min.
- Other readouts easily obtained by altering or adding to protocol.
- Data feeds seamlessly into reporting collection components.