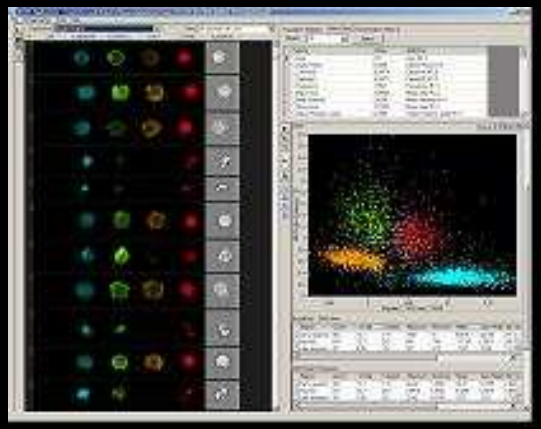
The ImageStream® System for Imaging Cells in Flow:

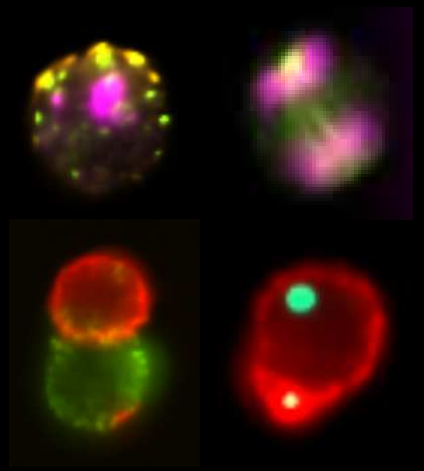
A New Tool for New Applications

www.amnis.com

The ImageStream® System

- ImageStream 100 Flow Imaging Instrument
  - Brightfield, darkfield, and 4 fluorescent images at 5000 cells per minute
- IDEAS® Statistical Image Analysis Software
  - Quantitative cellular image analysis and population statistics
- Novel Applications
  - Translocation, co-localization, cell classification, cell cycle, FISH-IS, etc.

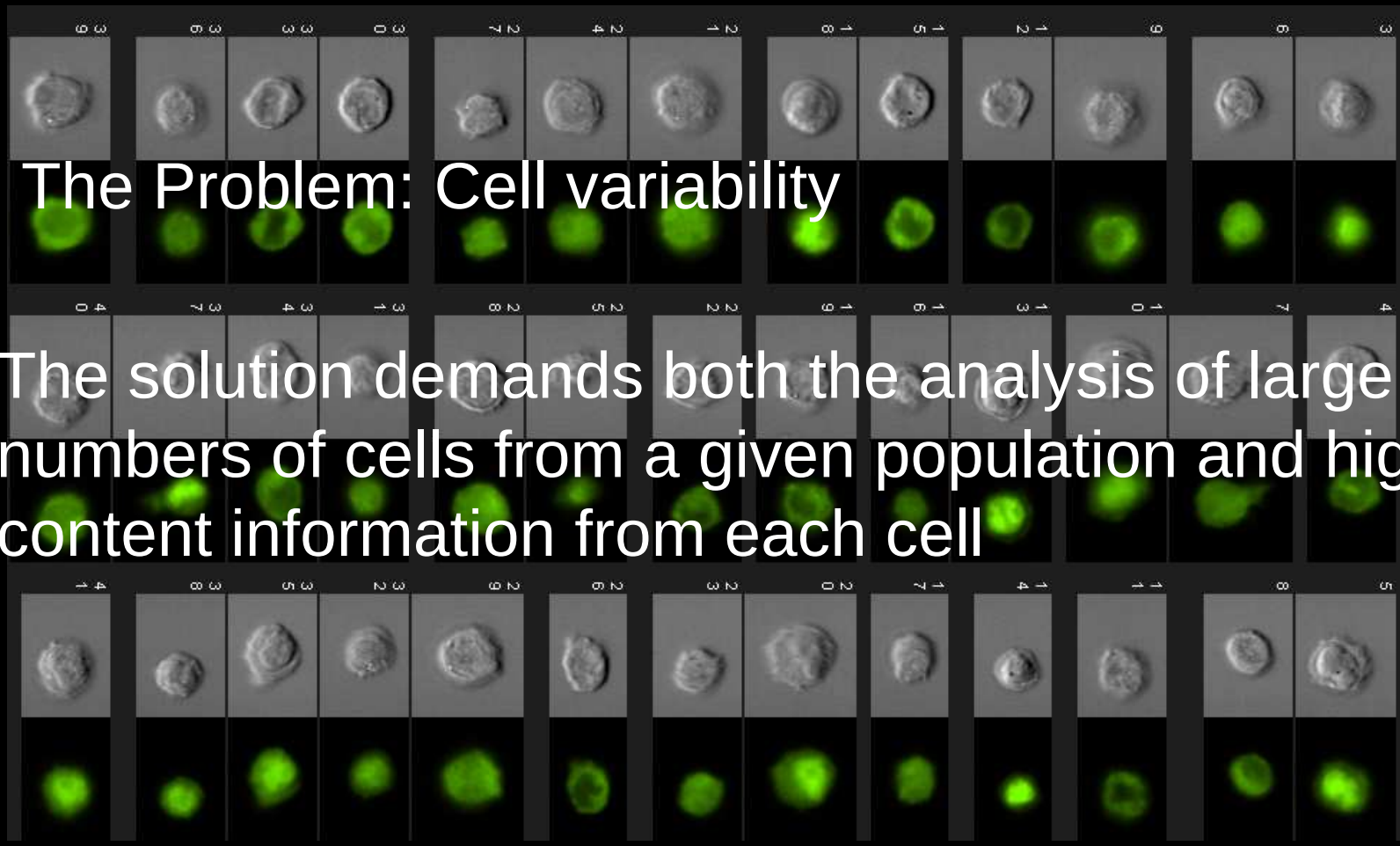




Issues in Quantitative Cell Biology

amnis®

The Goal: Statistically robust assays with predictive power



The Problem: Cell variability

The solution demands both the analysis of large numbers of cells from a given population and high content information from each cell

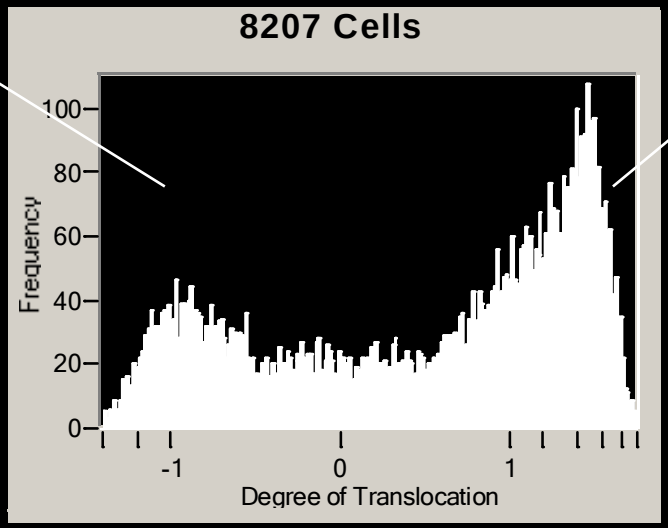
Illustration: 78 images of 39 cells...Find the outliers.

Question: How Many Cells Do You Need?

Example: NF-κB translocation assay in monocytes

LPS-stimulated cell, low translocation

LPS-stimulated cell, high translocation



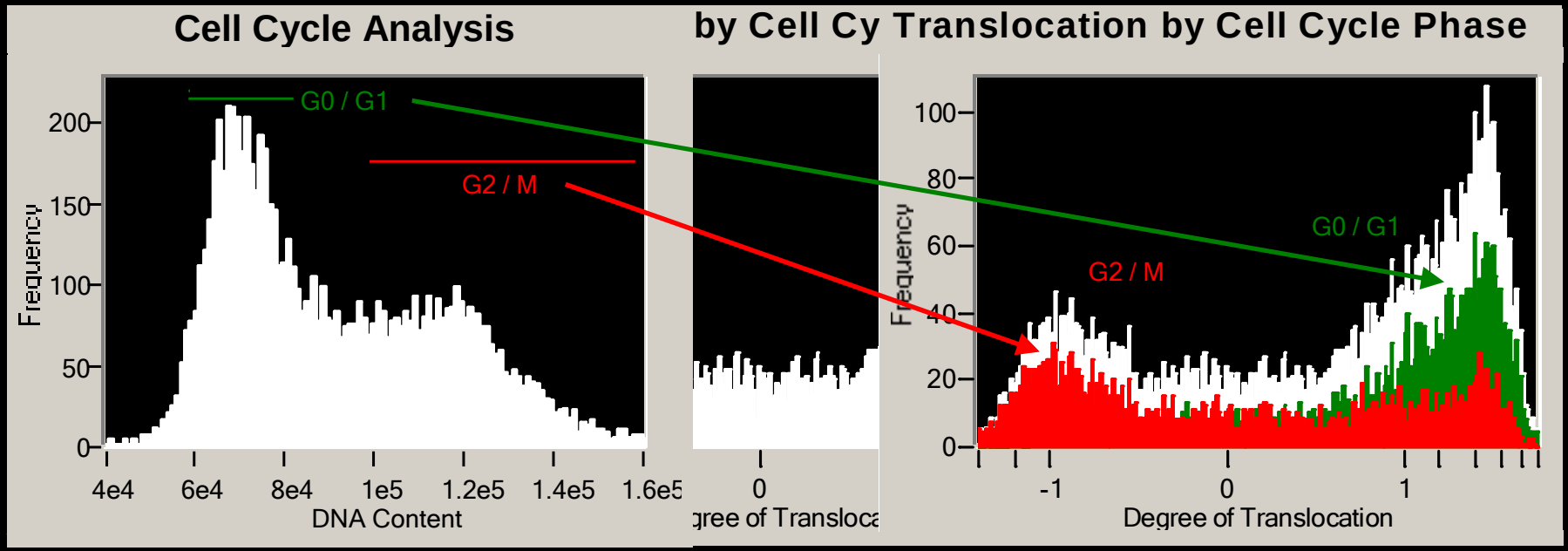
Answer:

Robust image-based assays can require measuring thousands of cells.

How Much Information per Cell is Required?

Example:

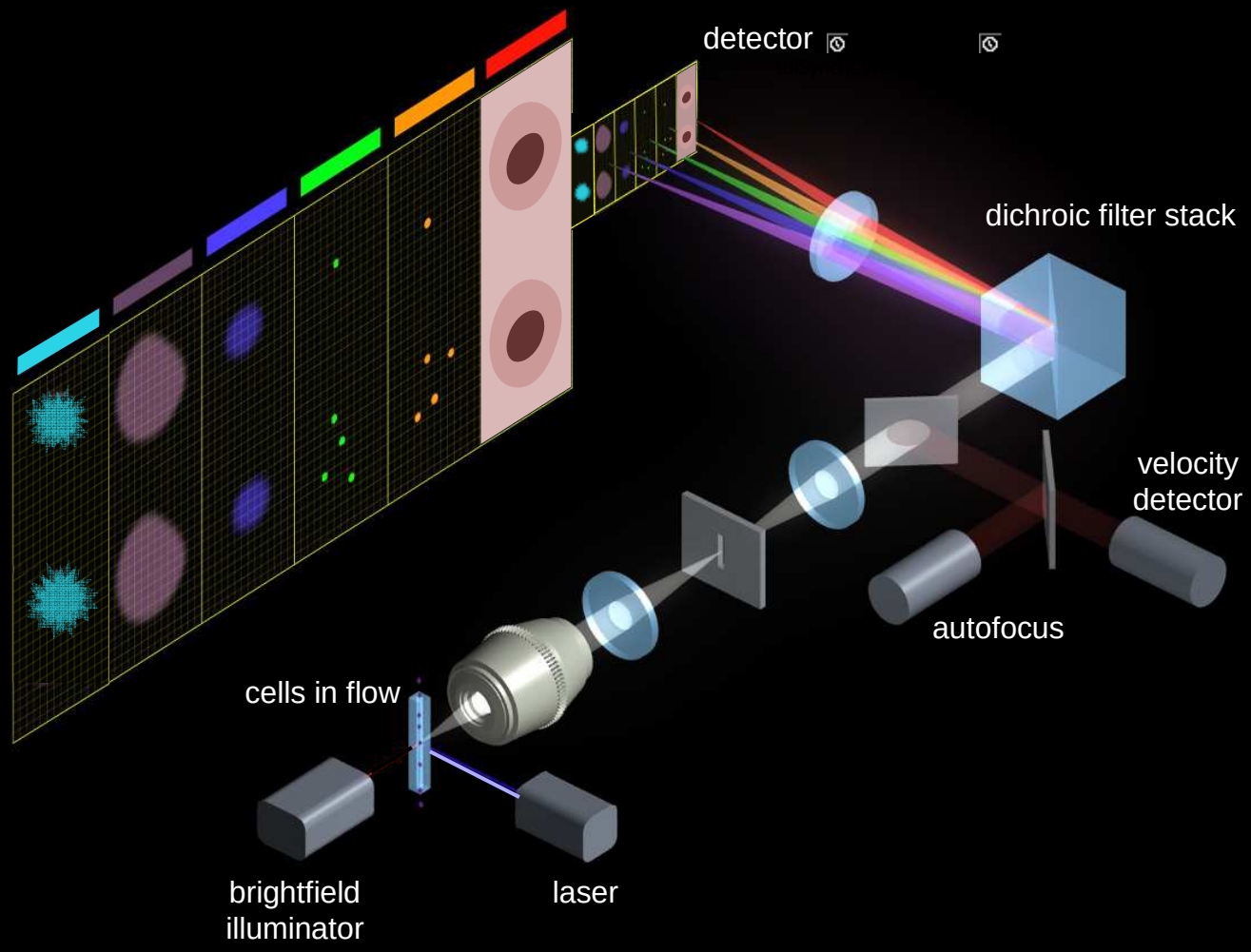
Incomplete NF-κB translocation in LPS-stimulated monocytes



Conclusion:

The more you measure, the more you can understand.

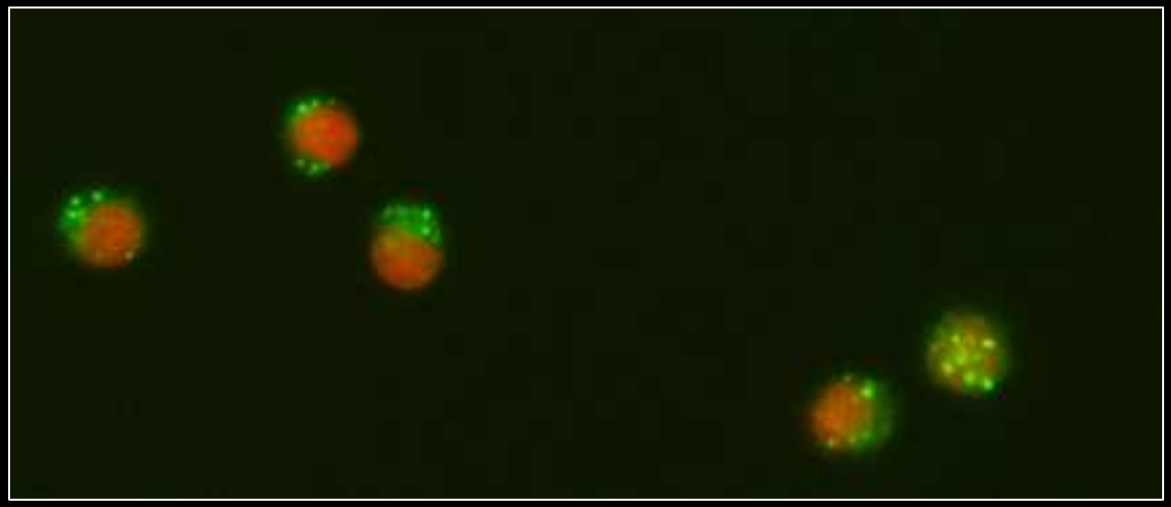
ImageStream 100 Optical Layout



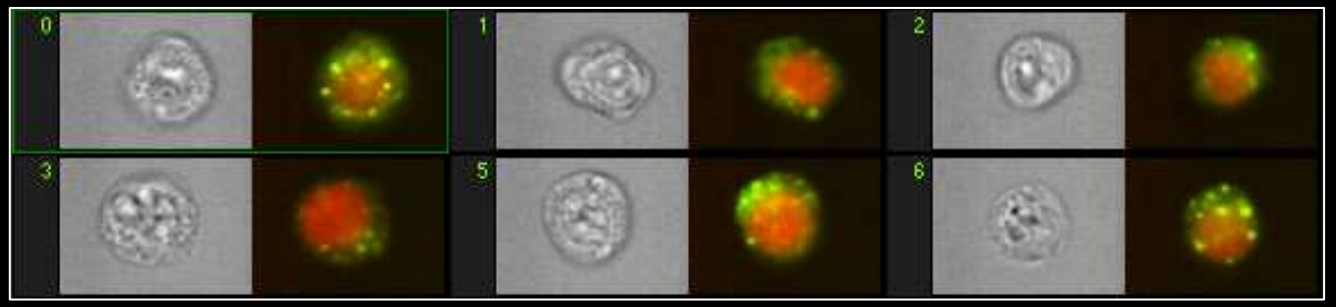
Resolution Comparable to Microscopy

Intracellular Identification of Early Endosomes: EEA1-Alexa Fluor® 488 + 7-AAD

fluorescence microscopy:
(40X mag)



ImageStream 100:
(36X mag)



IDEAS™ Software

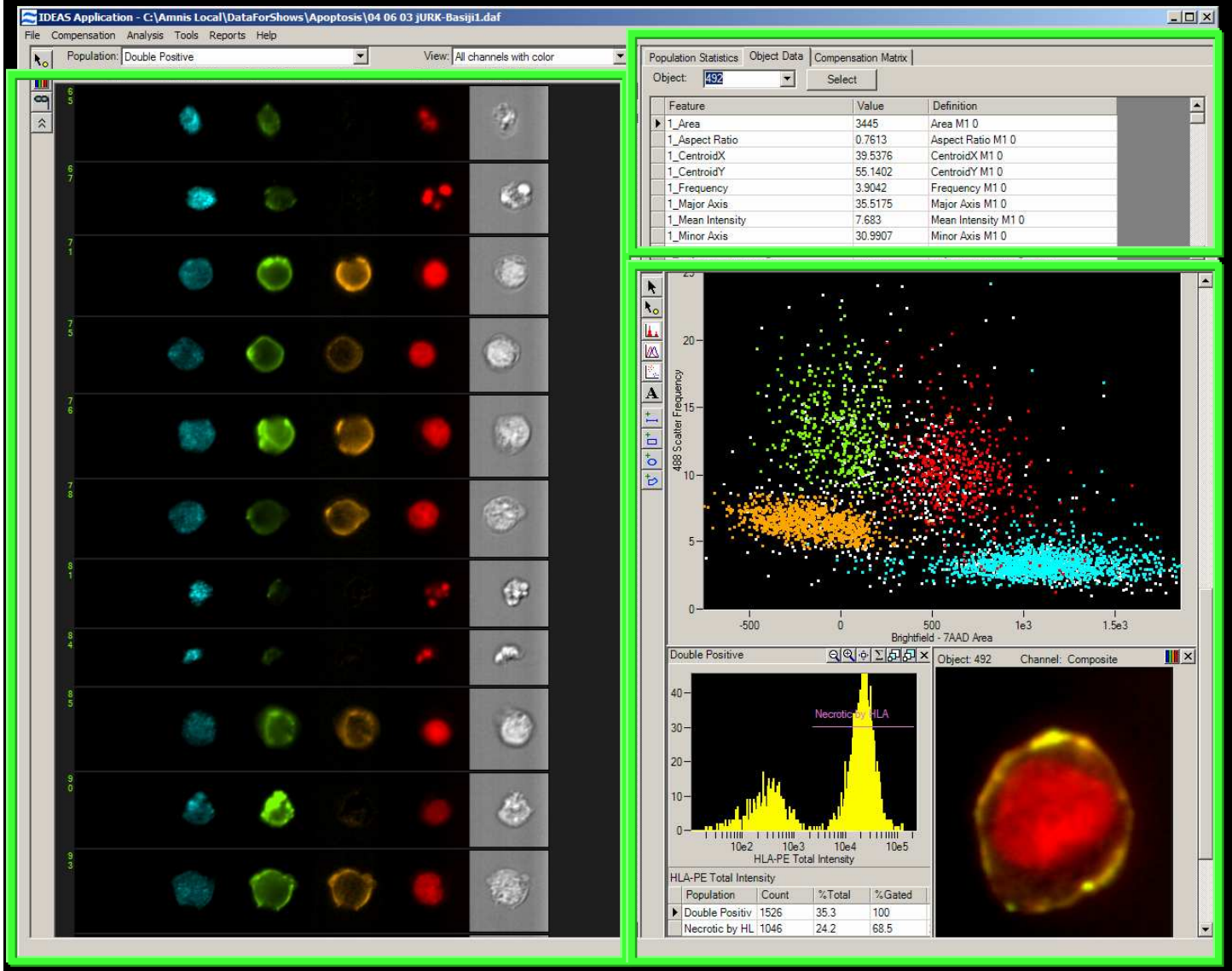


Image Gallery

- see every cell
- flexible viewing
- enhance & color
- tag populations
- virtual cell sort

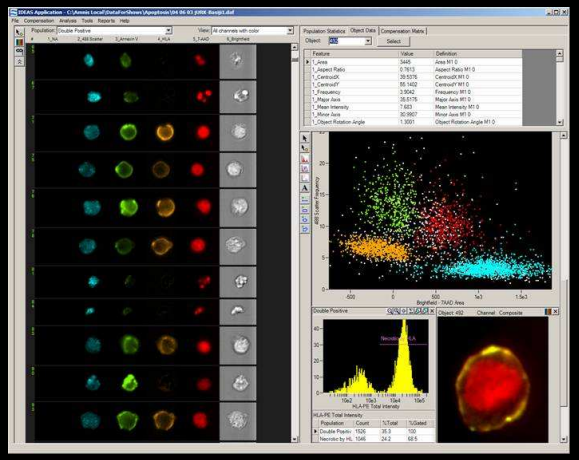
Tabular Data

- 200+ params/cell
- population statistics
- object values

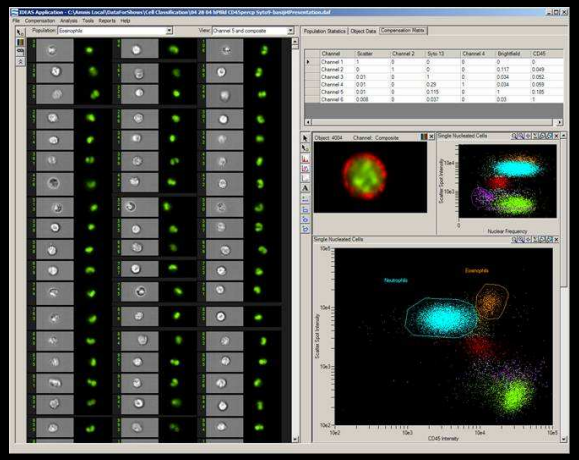
Workspace

- uni + bivariates
- flexible gating
- click dot to view cell
- custom parameters

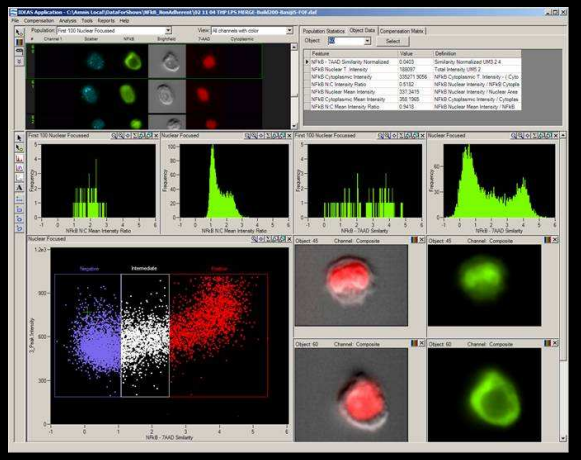
Example Applications



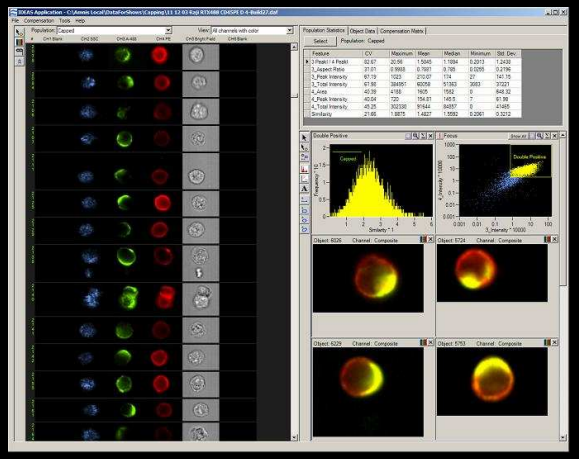
Apoptosis / Necrosis



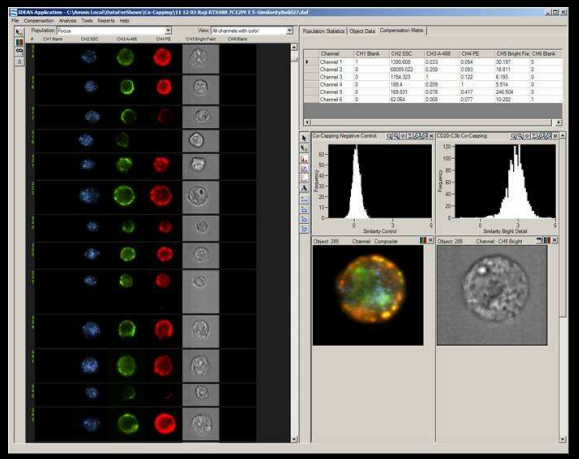
Cell Classification



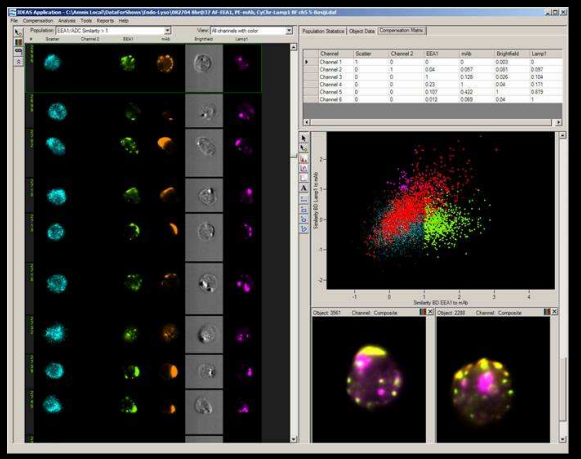
NF-κB Translocation



Marker Cap Quantitation

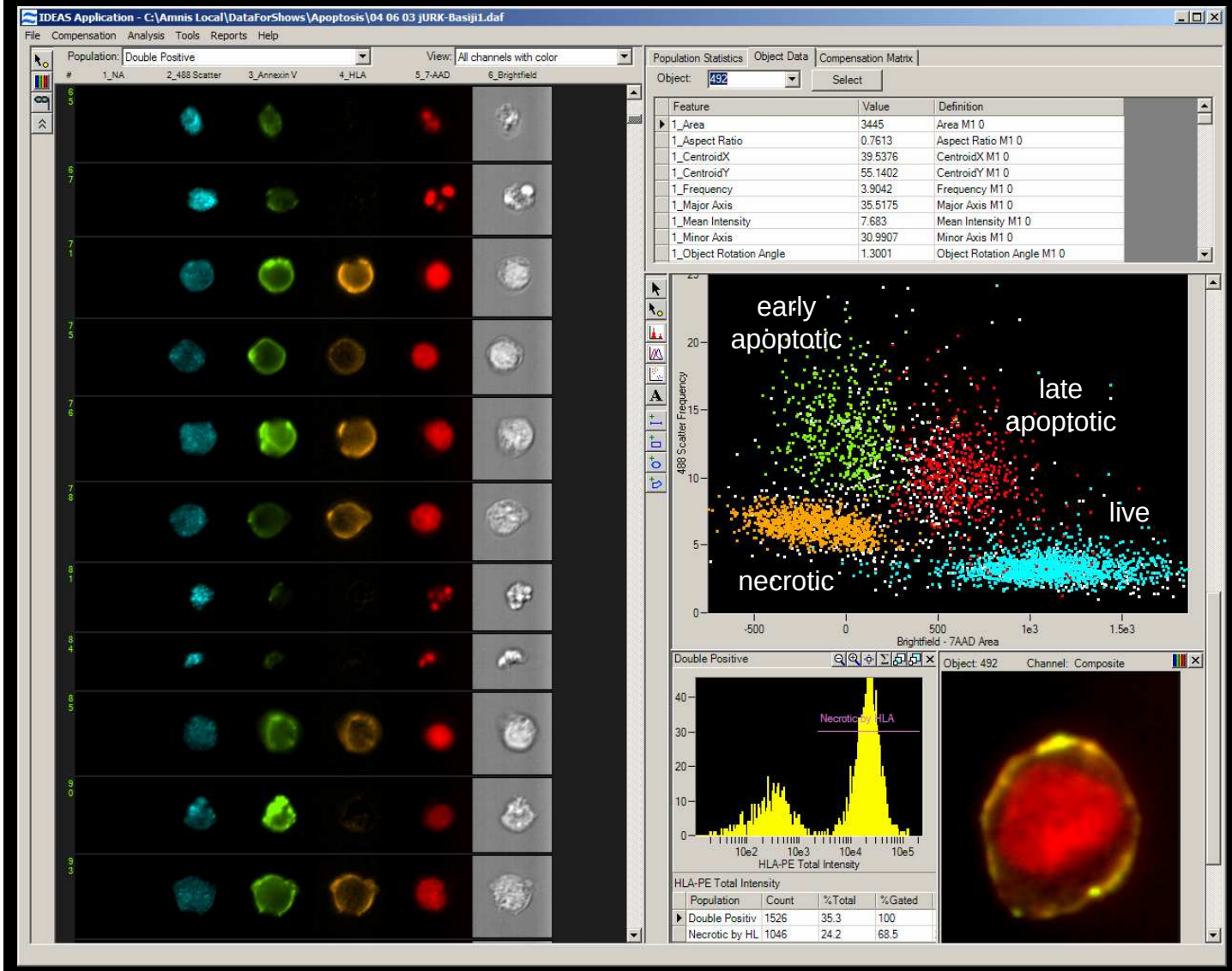


Marker Co-Localization



Molecular Trafficking

Mechanisms and Stages of Cell Death



Jurkat cells

Treat with peroxide or camptothecin

Imagery (L-R):
darkfield
Annexin V-AF488
α-HLA PE
nucleus (7-AAD)
brightfield

Use morphology to discriminate live cells from early apoptotic, late apoptotic, and necrotic cells.

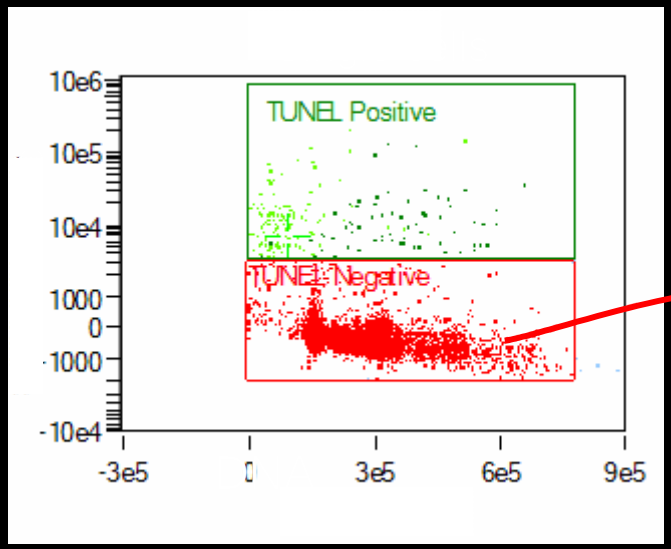
Apoptosis:
Accurate cell death measurements

amnis®

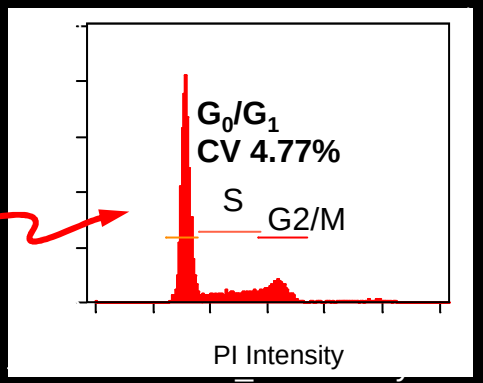
Negative control

Population Statistics

Population	Count	%Gated
Single	19155	100
TUNEL Positive & Single	180	0.94
TUNEL Negative & Single	18969	99
TUNEL True Positive & Single	113	0.59



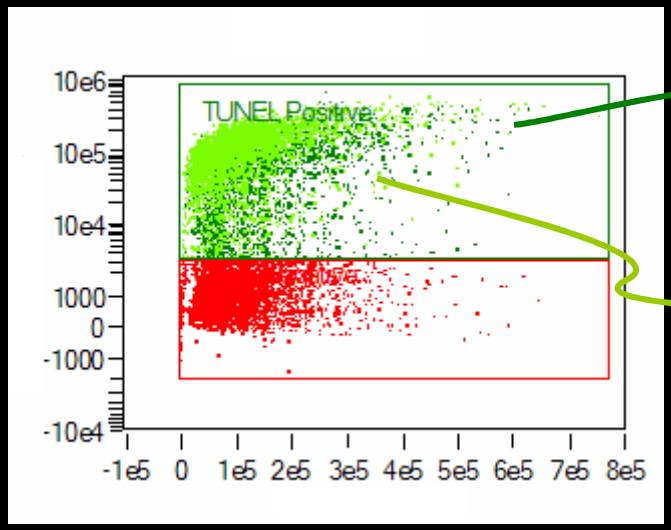
DNA Distribution



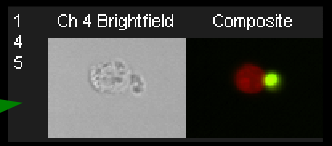
Apoptosis-induced

Population Statistics

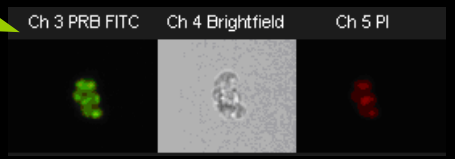
Population	Count	%Gated
Single	20613	100
TUNEL Positive & Single	6521	31.6
TUNEL Negative & Single	14092	68.4
TUNEL True Positive & Single	4310	20.9



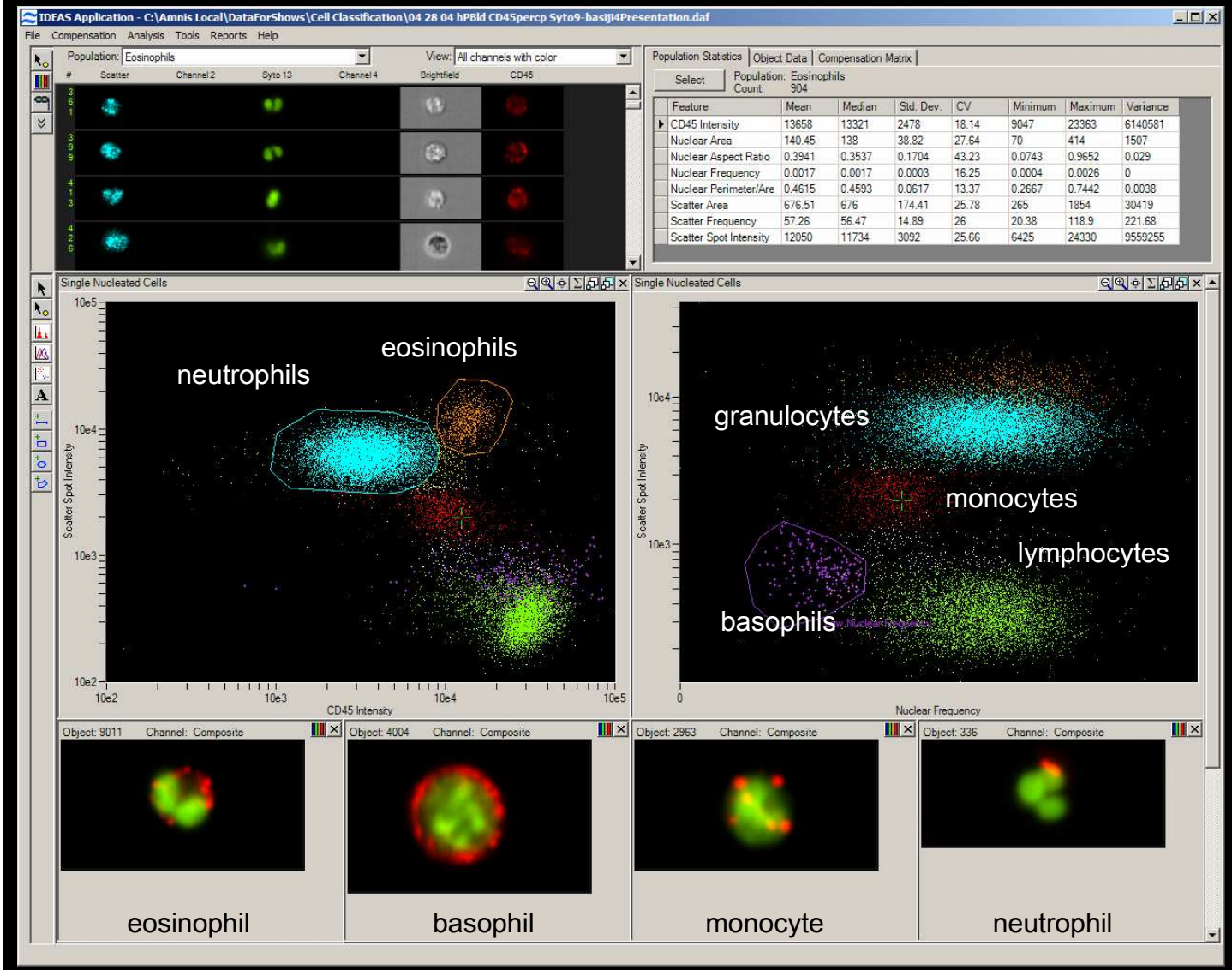
False positive **33%**



True positive



Morphologic Cell Classification



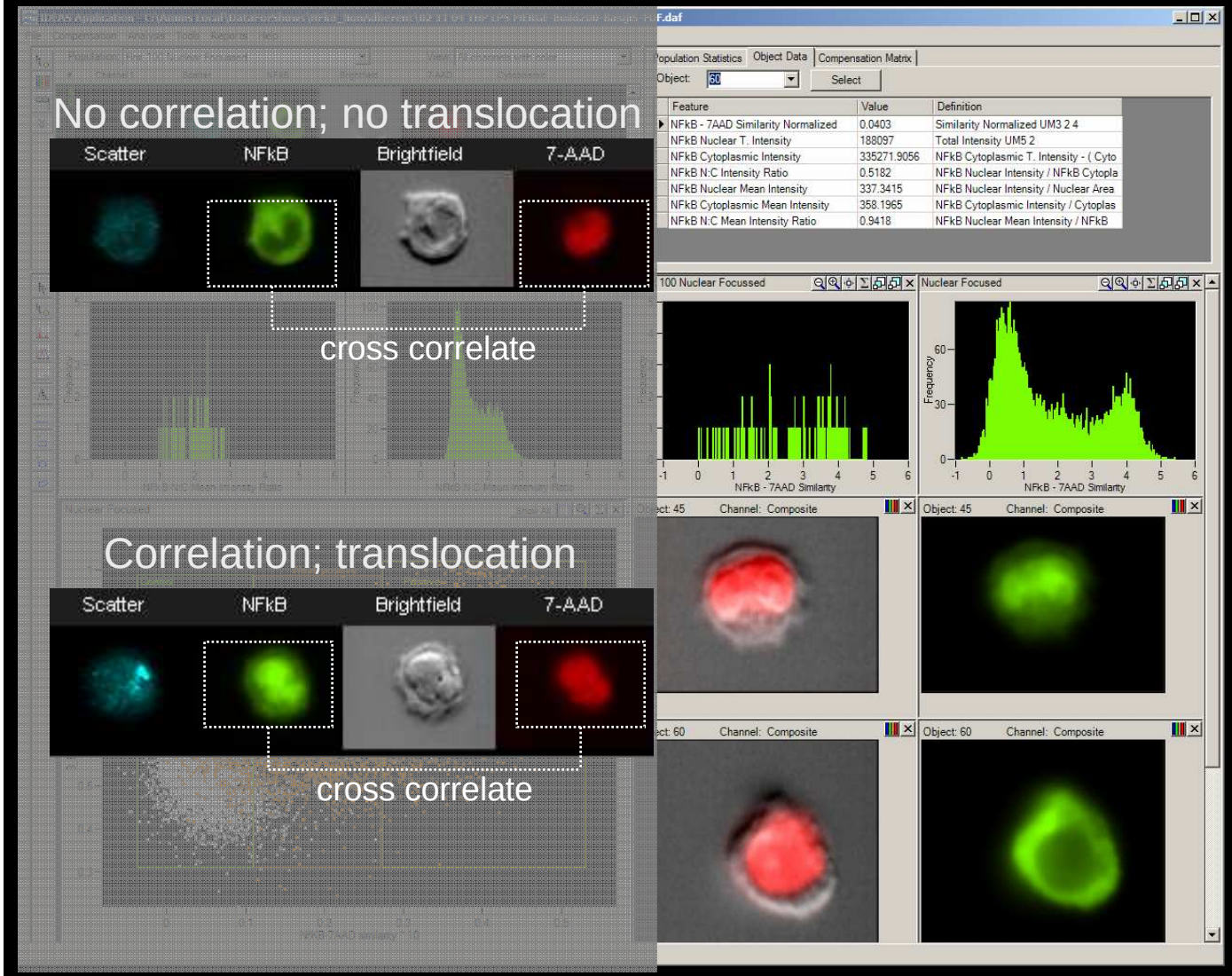
Human blood

Lyse erythrocytes

Imagery:
darkfield
SYTO-DNA
α-CD45-PerCP
brightfield

Build classifiers using correlation of stained populations with morphologic parameters.

NF-κB Translocation in Monocytes



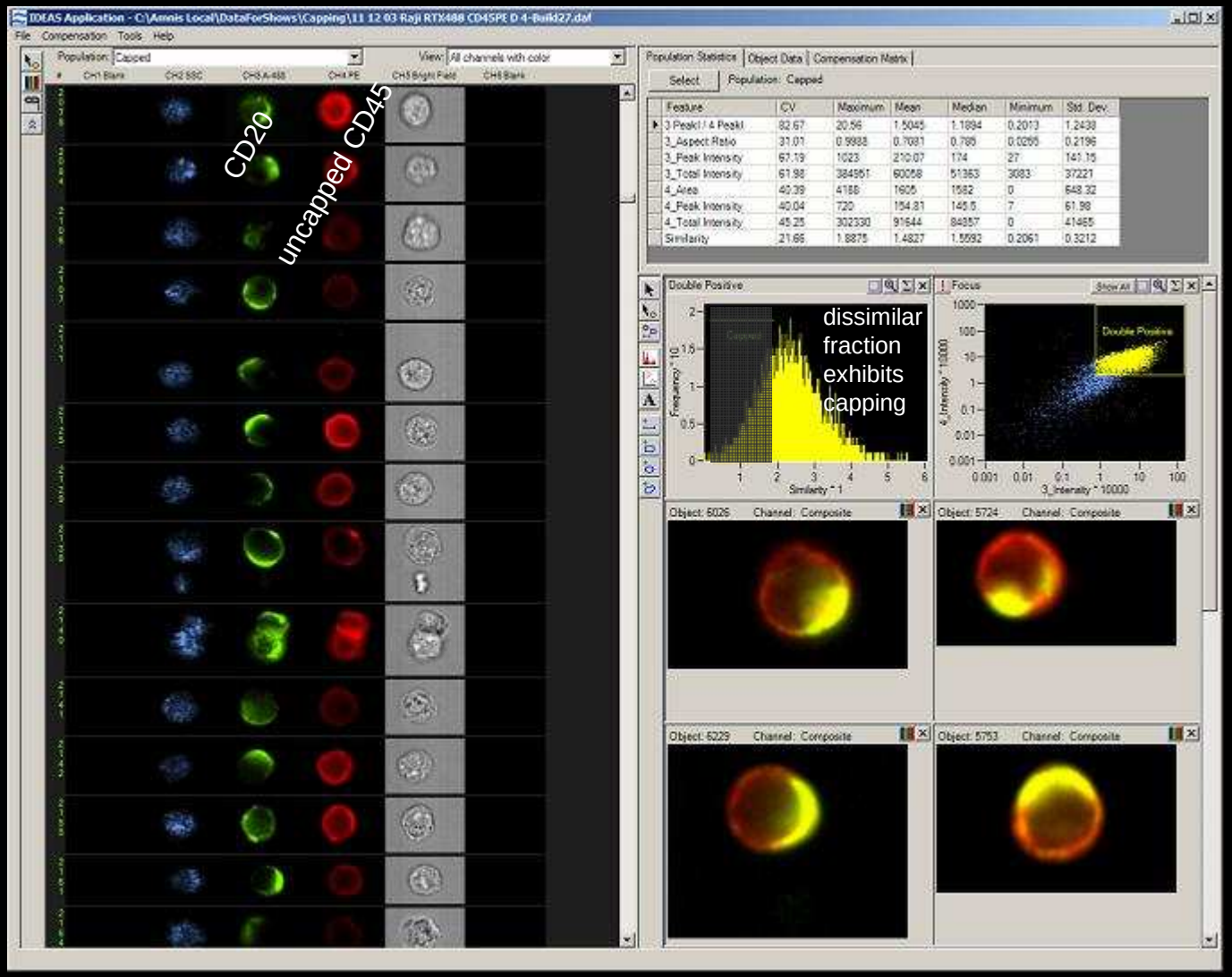
THP-1 cells

Treat with LPS

Images (L-R)
- darkfield
- α-NFκB-FITC
- brightfield
- nucleus (7-AAD)

Measure NFκB to 7-AAD similarity to quantify degree of translocation

Quantitation of Marker Capping



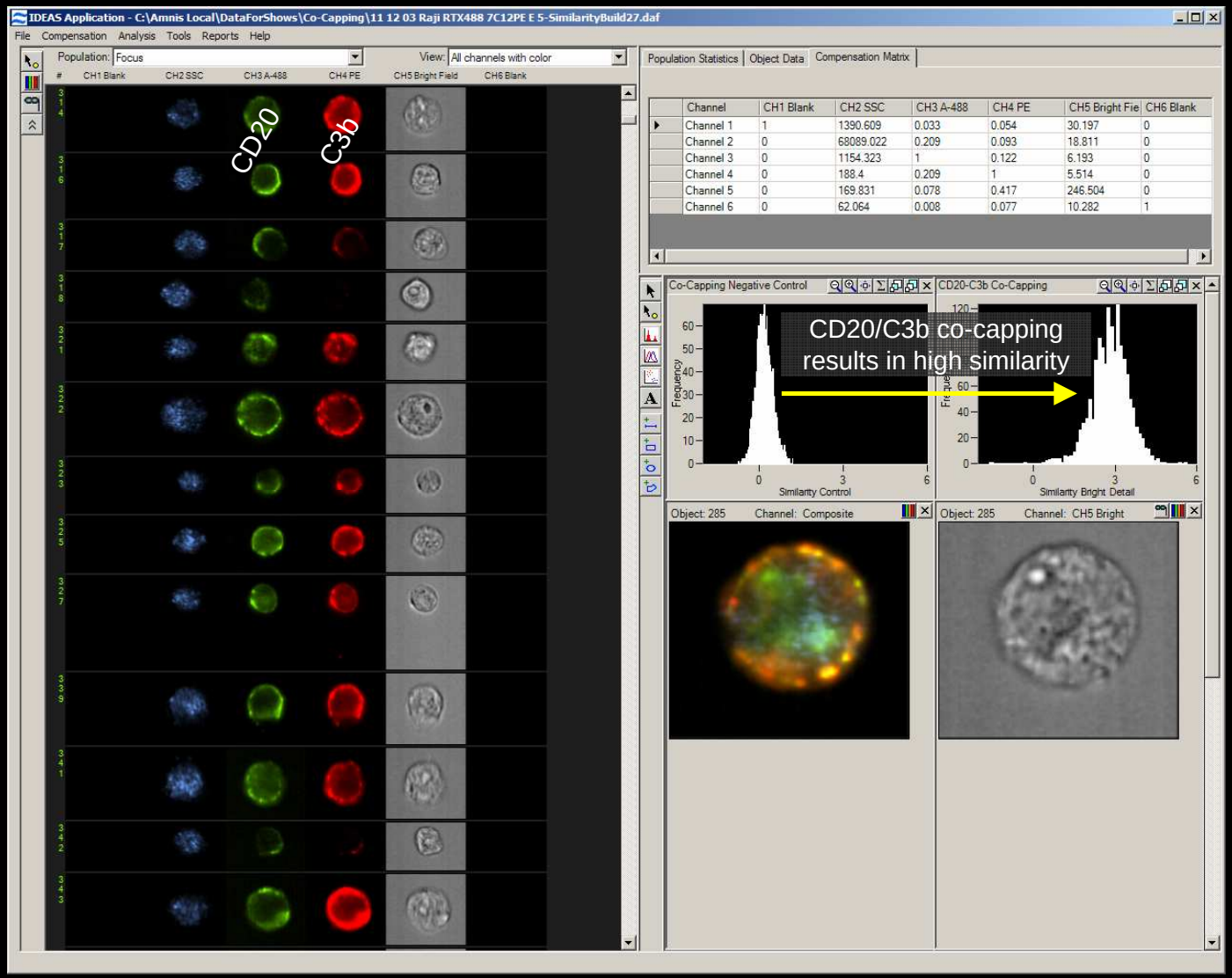
Raji cells

Imagery (L-R):
darkfield
rituximab - AF488
α-CD45 - PE
brightfield

Measure similarity between fluorescent images

Data produced in collaboration with Dr. Paul Beum and Dr. Ronald Taylor, University of Virginia School of Medicine

Marker Co-Localization



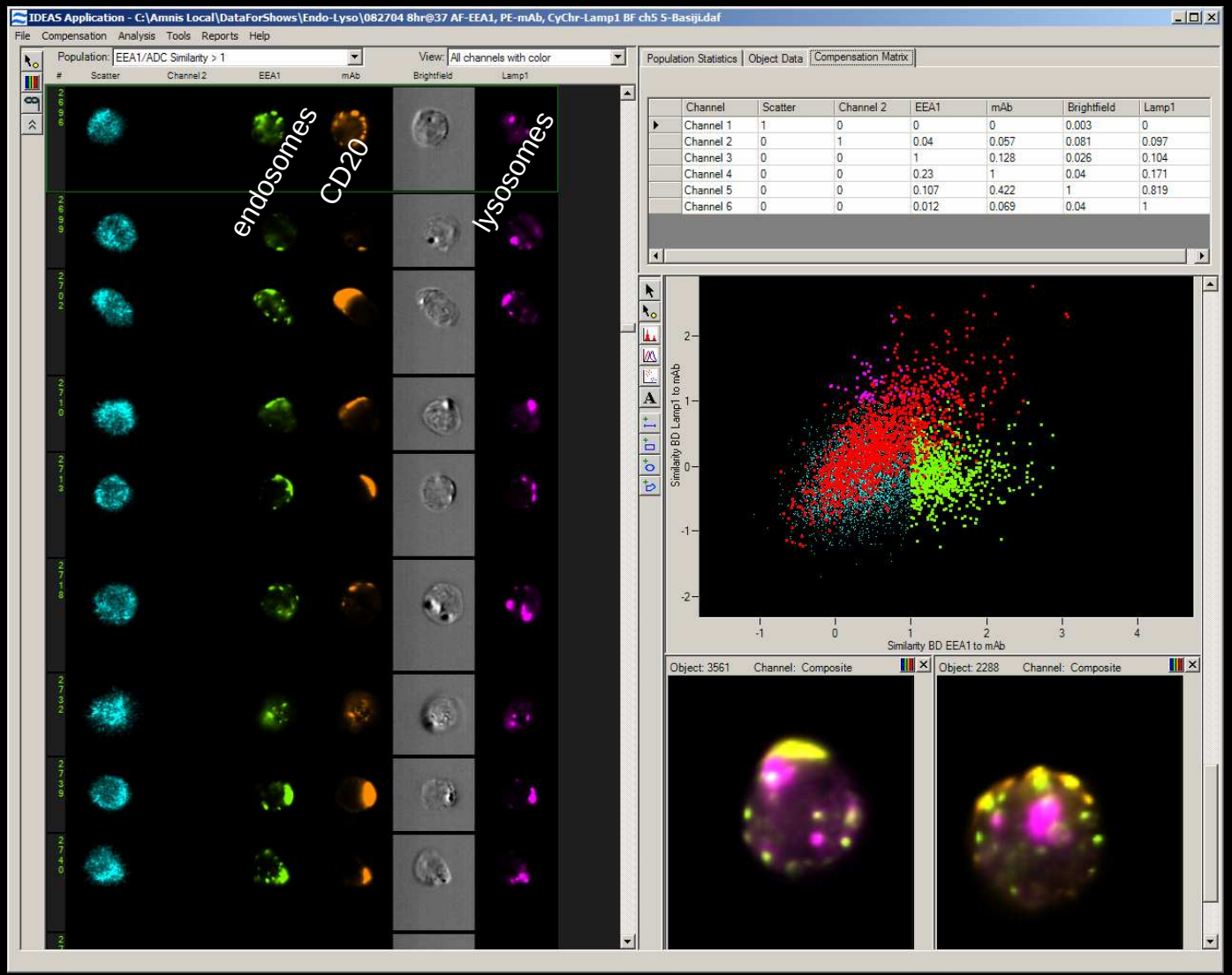
Raji cells

Imagery (L-R):
darkfield
rituximab - AF488
α-C3b(i) - PE
brightfield

Measure similarity between fluorescent images and compare to control.

Data produced in collaboration with Dr. Paul Beum and Dr. Ronald Taylor, University of Virginia School of Medicine

Molecular Trafficking



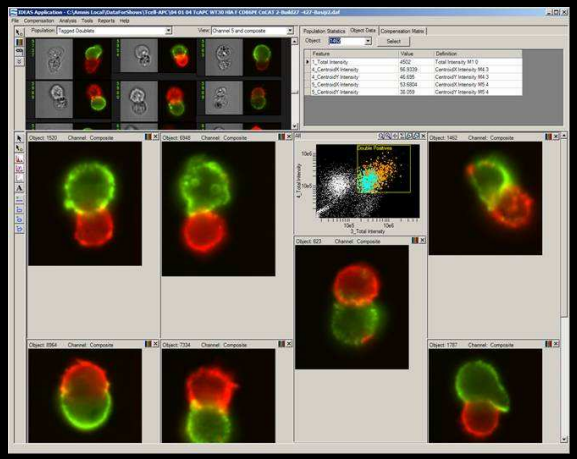
Ramos cells

Imagery (L-R):
darkfield
EEA1 - AF488
α-CD20 - PE
brightfield
Lamp1 - CyChrome

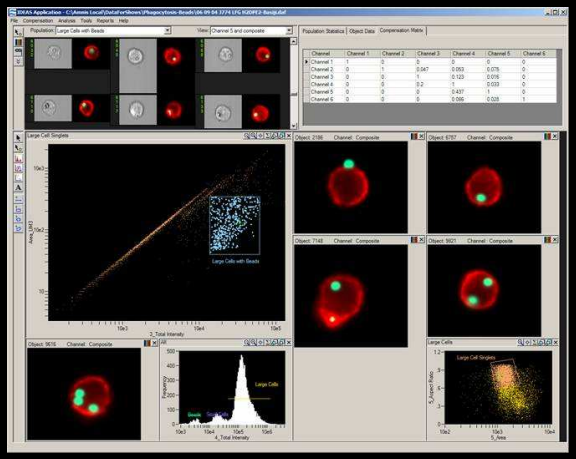
Incubate at 37 ºC for 0.5, 1, 2, 4, 8 hours. Measure similarity between mAb and endo / lyso images.

Monitor time-course of mAb-endo and mAb-lyso association.

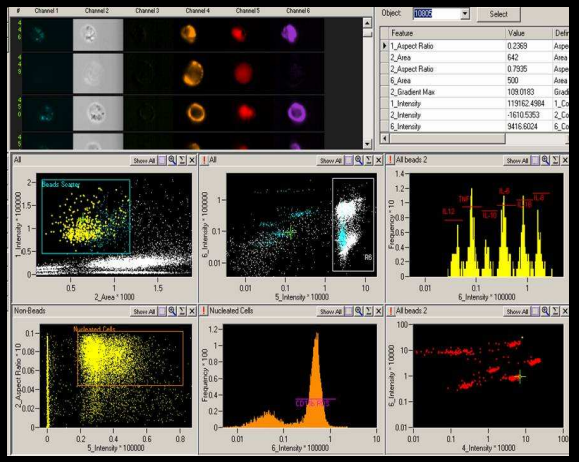
Additional Applications



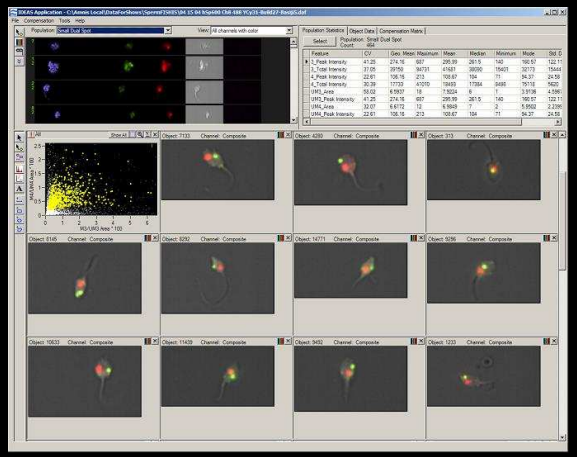
T Cell / APC Conjugates



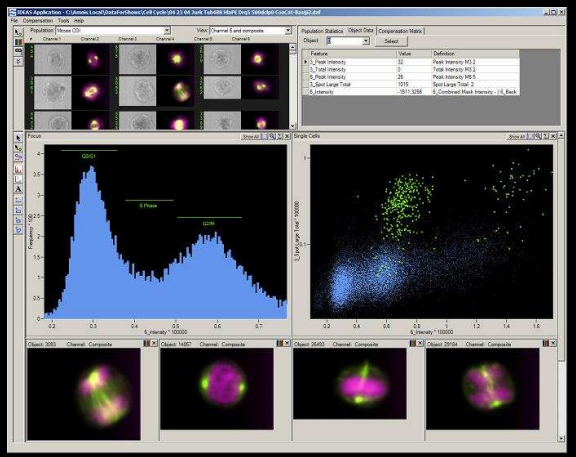
Phagocytosis



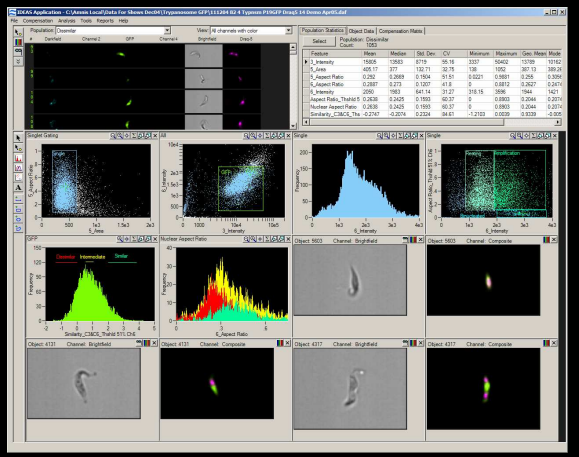
Multiplexing



High Throughput FISH-IS

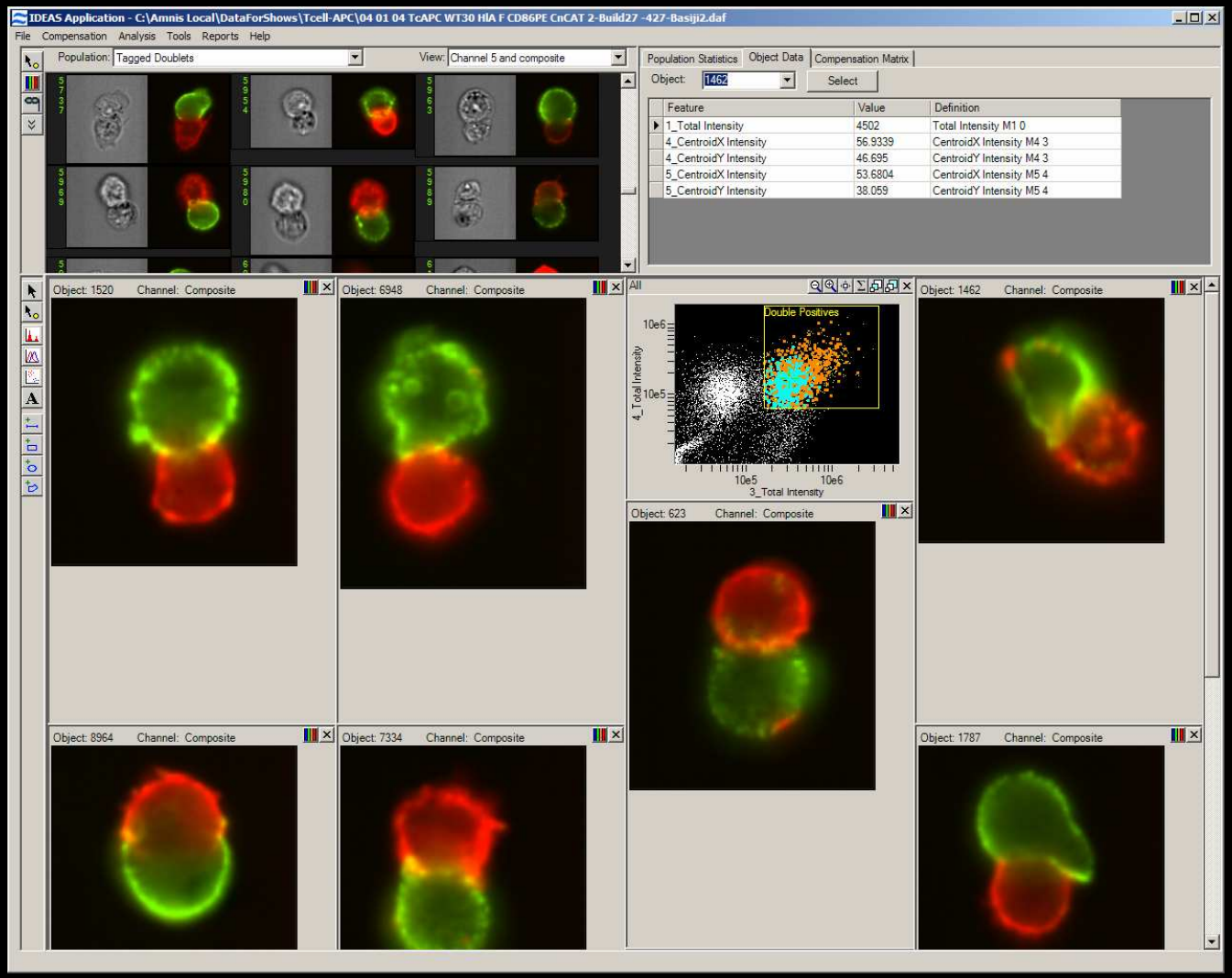


Cell Cycle and Mitosis



Infectious disease

T-cell / APC Interactions



Murine cells

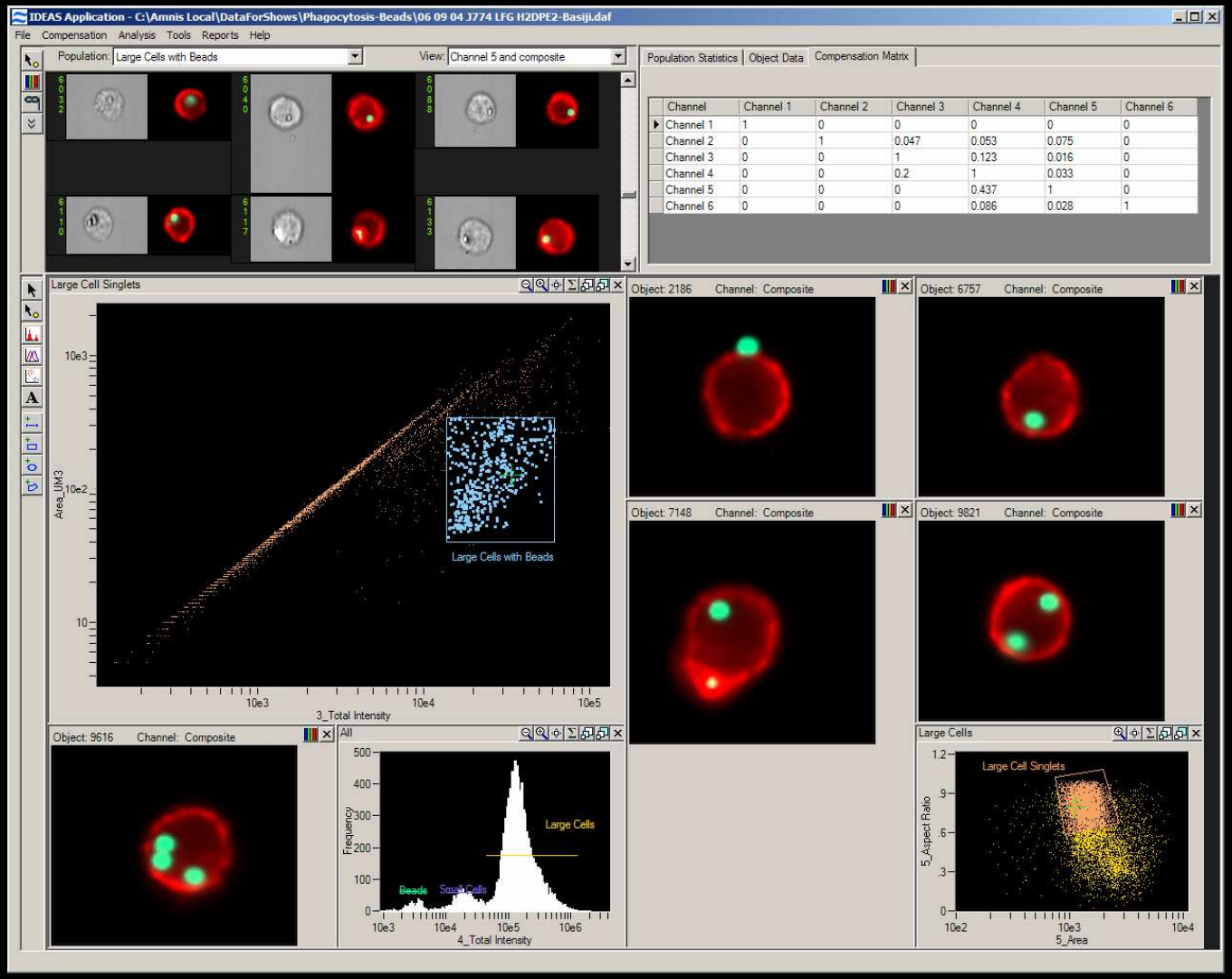
Imagery (L-R):
brightfield
HLA-FITC + CD86-PE

Define contact area using logical AND of FITC and PE masks.

Measure mean CD86 intensity at synapse vs. remaining APC area.

Data produced in collaboration with
Dr. Rafick-Pierre Sekaly,
University of Montreal

Phagocytosis



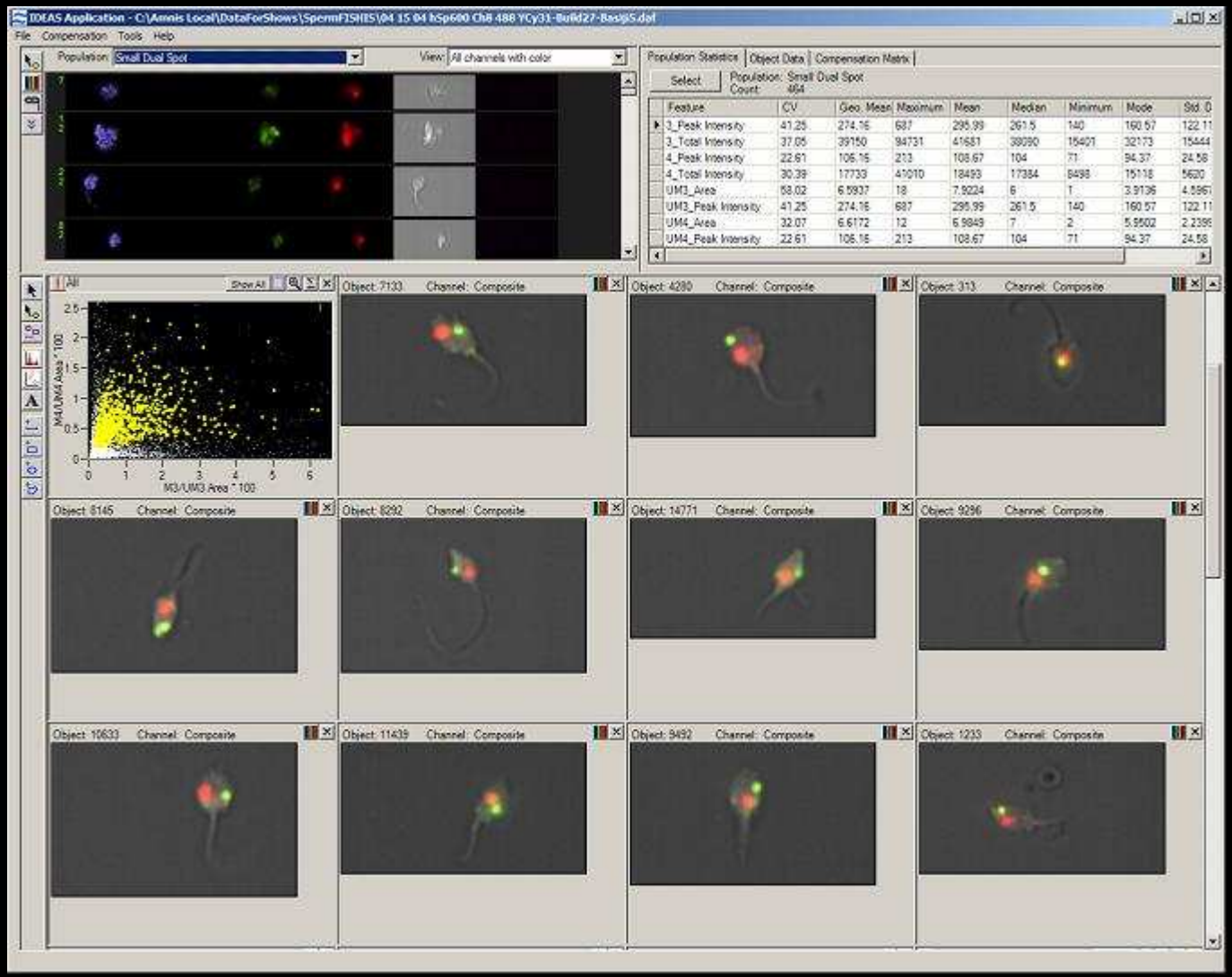
Murine J774a cells

Mix with 2.5um fluorescent beads at equal concentration, shake 2 hr @ 37º C
Wash, fix, run.

Imagery (L-R):
brightfield
FITC beads + H-2D-PE

Find H-2D positives, define cell singlets using brightfield area and aspect ratio, find phagocytic 5% using FITC intensity.

High Throughput FISH-IS™



Human Sperm

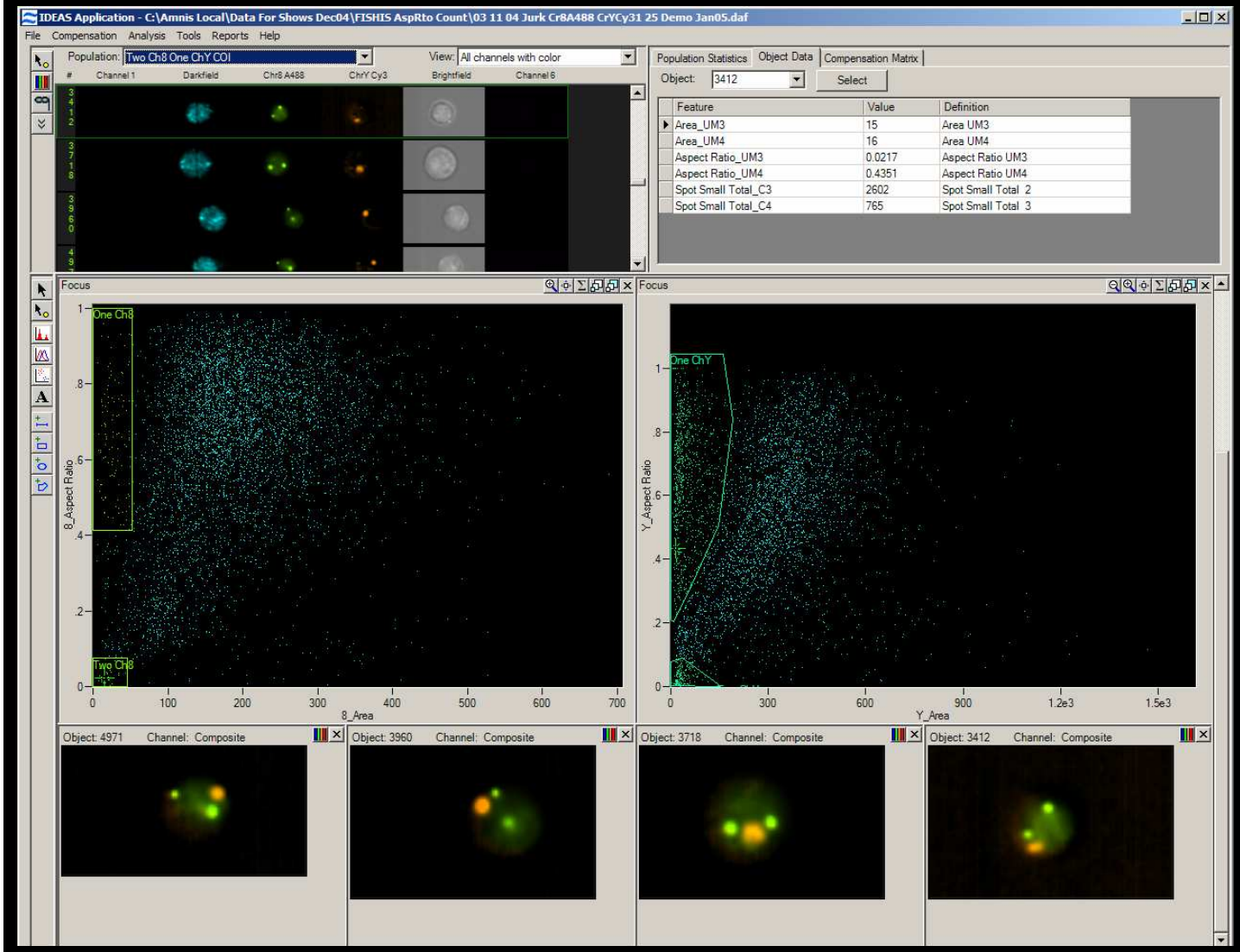
FISH-IS with
Chr. 8-FITC probe
Chr. Y-Cy3 probe

Imagery (L-R):
darkfield
chromosome 8-FITC
chromosome Y-Cy3
brightfield

Identify bright FISH with fluorescent mask area, count spots, quantitate intensity

Work supported in part by NIEHS SBIR N43-ES-35507.

High Throughput FISH-IS™



Jurkat cells

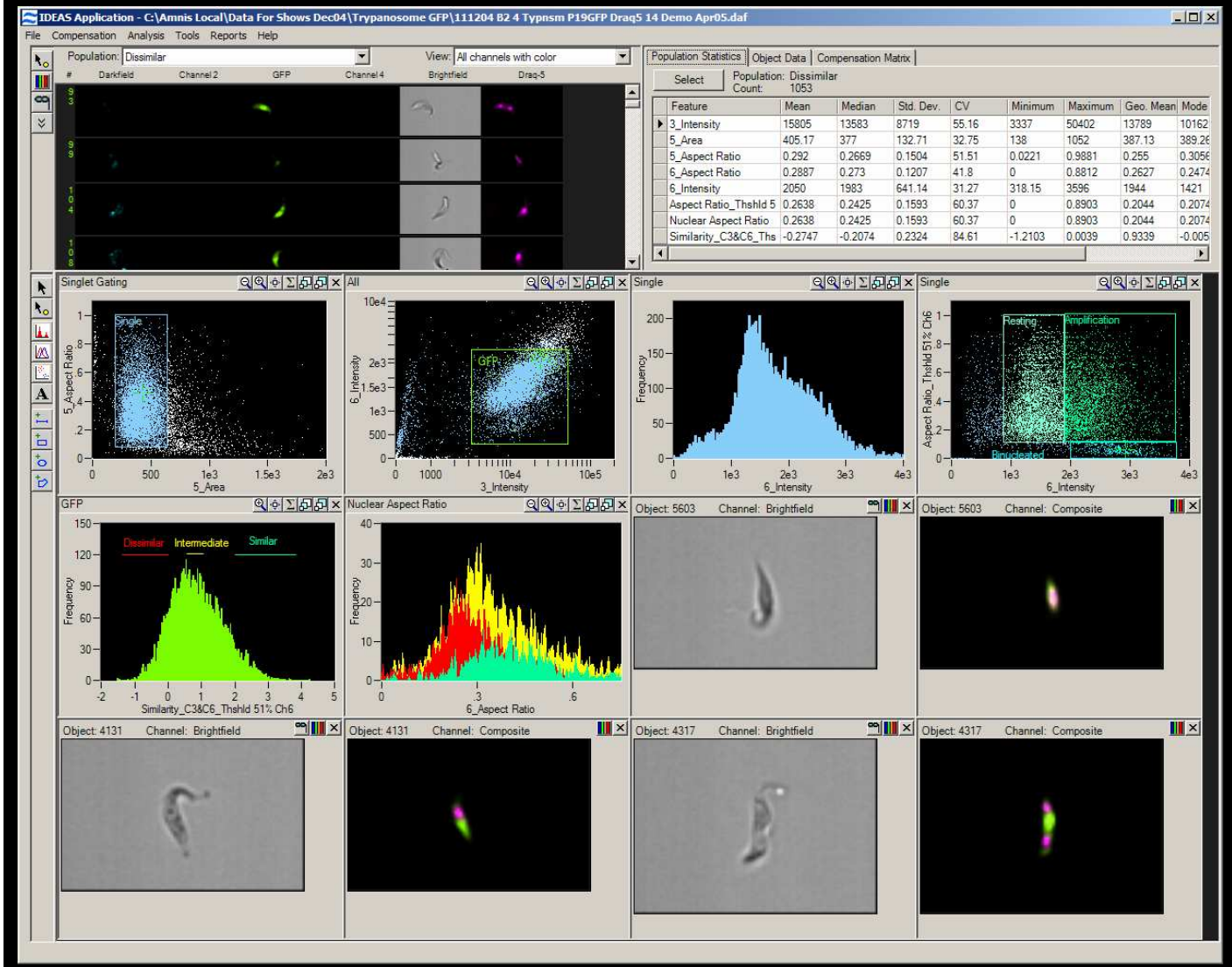
FISH-IS with
Chr. 8-AF488 probe
Chr. Y-Cy3 probe

Imagery (L-R):
darkfield
chromosome 8
chromosome Y
brightfield

Identify bright FISH with fluorescent mask area, count spots, quantitate intensity

Protocol development assistance kindly provided by Dr. Farideh Bischoff.

Infectious Disease



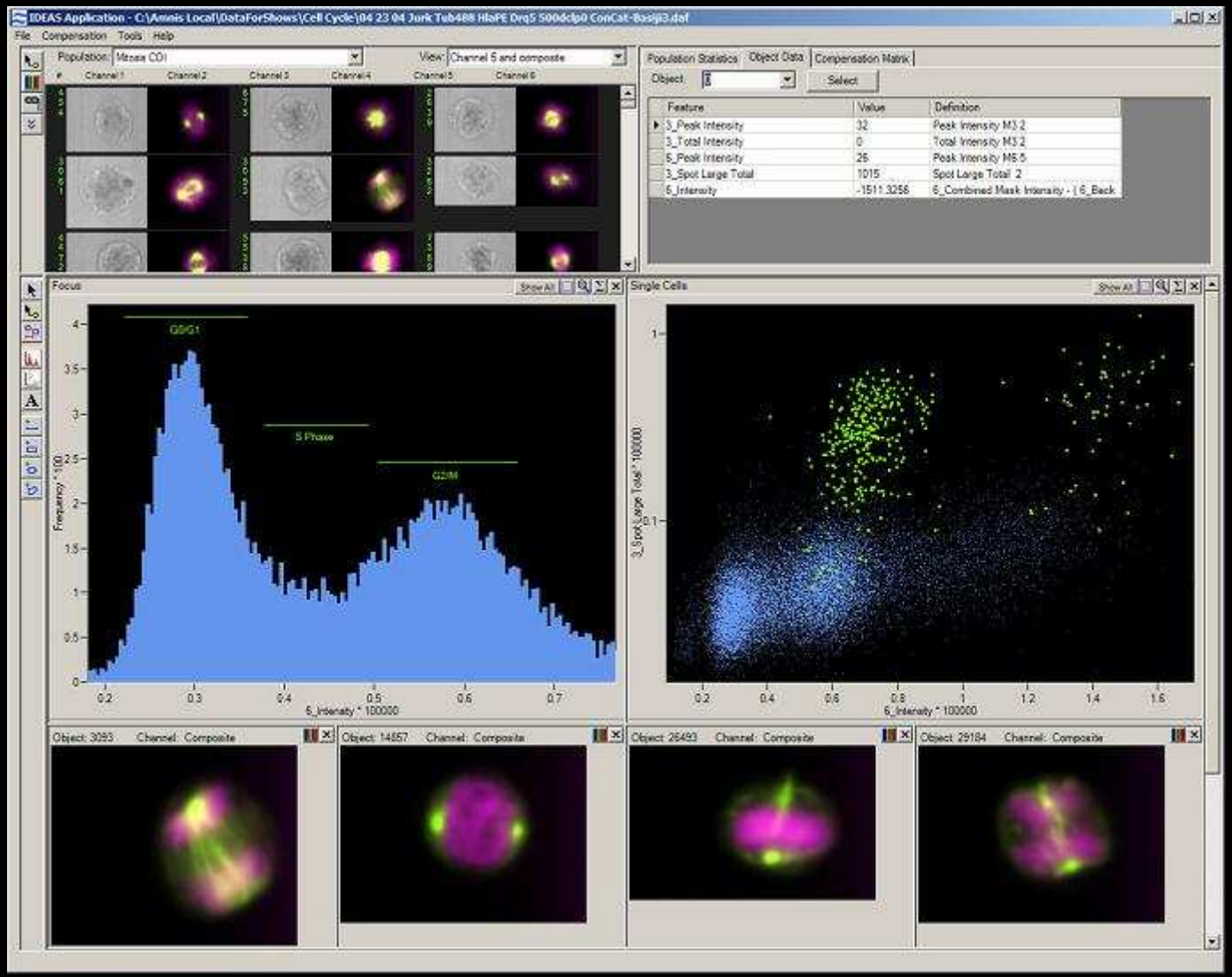
GFP-transfected
Trypanosome brucei

Imagery (L-R):
darkfield
GFP-NP19
brightfield
DRAQ5™ (DNA)

Measure similarity of GFP to DRAQ5 to quantify degree of translocation

Data produced in collaboration with Dr. Marilyn Parsons, Seattle Biomed. Res. Inst.

Cell Cycle and Mitosis Analysis



Jurkat cells

Stain with DRAQ5™, α-tubulin-AF488, α-HLA-PE

Imagery:
brightfield
DRAQ5™ + tubulin

Quantitate cell cycle using total intensity, identify mitotic cells using peak intensity of tubulin and/or DRAQ5™.

Multiplexed Cytokine/Hematology

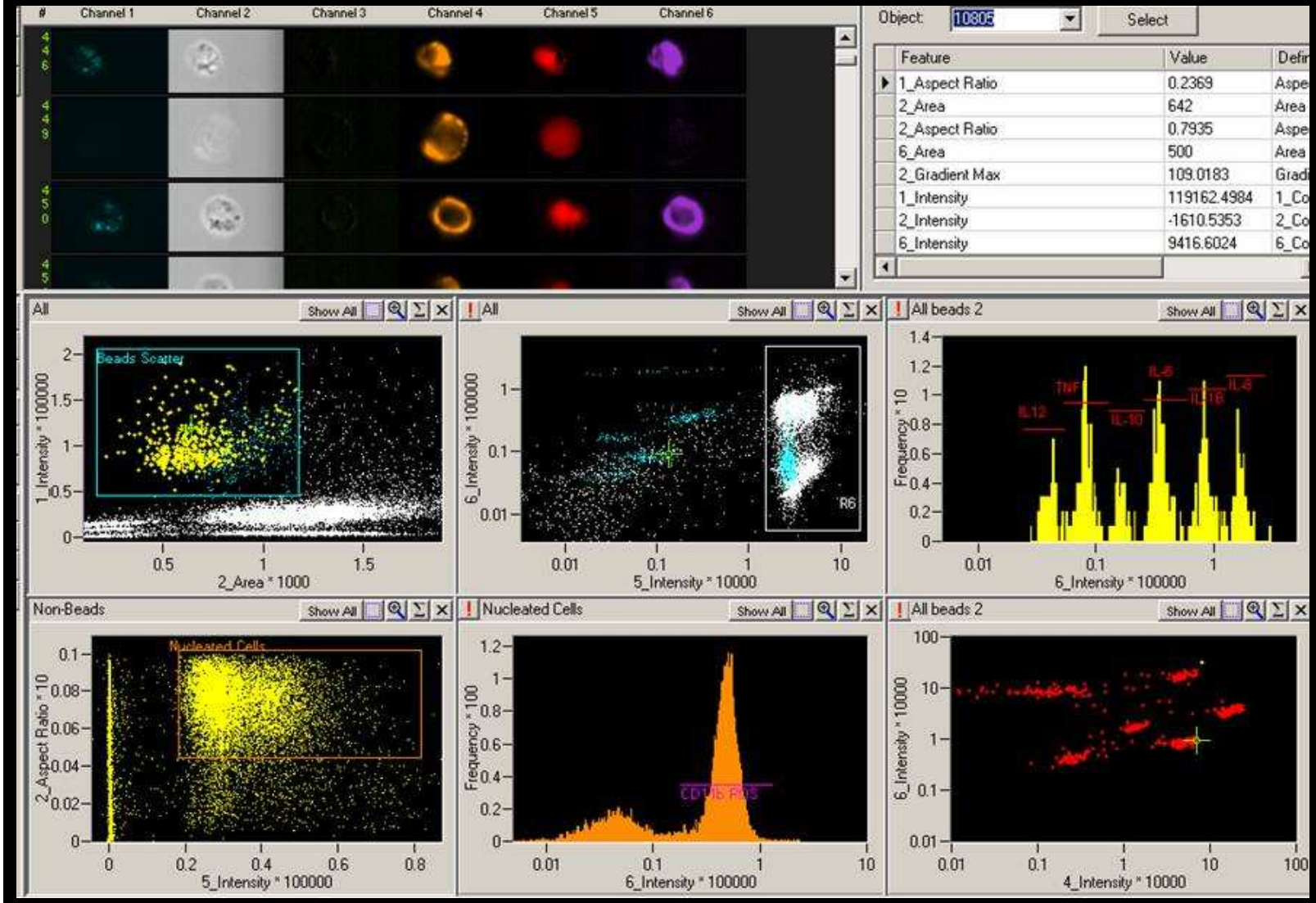


Figure kindly provided by Gary Elliott, Ph.D. Amgen, Inc.

Customer Driven Applications

- Human bone marrow
- Nuclear translocation in multiple cell types
- Phagocytosis in rare human blood cells
- DNA repair
- Shape change assays
- Bead – Cell conjugates
- Biomarker identification

Summary

ImageStream System Delivers Quantitative Cell Biology:

- Easily measure many cells to describe population structures
- Replace biomarkers with quantitative morphology
- Apply rigorous assays with analytical flexibility
- Get results that are objective and verifiable

Clarity from Complexity