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

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.
Example:
Incomplete NF-κB translocation in LPS-stimulated monocytes

Conclusion:
The more you measure, the more you can understand.
ImageStream 100 Optical Layout

- dichroic filter stack
- detector
- laser
- brightfield illuminator
- cells in flow
- autofocus
- velocity detector
- ImageStream 100 Optical Layout

October 2004
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

October 2004
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

**Negative Control**

- **Population Statistics**
  - Population  | Count | %Gated |
  - Single     | 16155 | 100    |
  - TUNEL Positive & Single | 160  | 0.94   |
  - TUNEL Negative & Single  | 16969 | 99     |
  - TUNEL True Positive & Single | 113  | 0.59   |

**Apoptosis-induced**

- **Population Statistics**
  - Population  | Count | %Gated |
  - Single     | 26813 | 100    |
  - TUNEL Positive & Single | 6621 | 31.6   |
  - TUNEL Negative & Single  | 14092 | 88.4   |
  - TUNEL True Positive & Single | 4510 | 20.9   |

**DNA Distribution**

- **G0/G1**
  - CV 4.77%

**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

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Marker Co-Localization

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 °C 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.
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