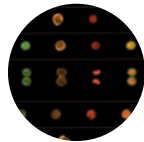


Think outside the dot.



# Insight & Analysis

## Welcome to a Revolution in Cell Analysis: Imaging Flow Cytometry

The ImageStream® system advances your science by combining quantitative cellular imagery with powerful population statistics.

With traditional cell analysis, you've had to choose between visualizing a few cells under a microscope without quantitation or analyzing large cell populations by flow cytometry without imagery. Microscopy gives you detailed fine structure, morphology, and qualitative molecular localization. Flow cytometry gives you robust statistical information and detection of rare sub-populations. No technology has been able to give you all this at the same time and in a single experiment.

Until now.

The ImageStream system from Amnis gives you morphol-

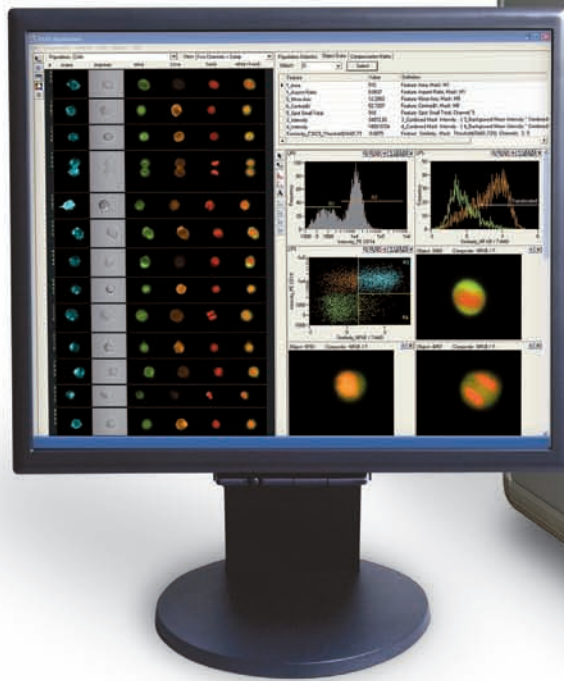
ogy, fluorescence localization, and population statistics for a broad range of applications. Identify and image a rare sub-population. Characterize a heterogeneous sample. Quantitate translocation between cellular compartments. Examine cells in mitosis. Accurately measure an apoptotic sub-population.

And do it all simultaneously.

INNOVATIVE

AMNIS

amis  
ImageStream



# New Technology

## An Entirely New Way to Analyze Cells

It's all about the numbers: 200 quantitative parameters from six simultaneous images per cell; brightfield, darkfield and multiple fluorescence imaging modes; 0.75 NA optics; sub-micron resolution; over 10,000 cells per minute.

### ADVANCED DETECTION TECHNOLOGY DELIVERS HIGH SENSITIVITY WITH HIGH SPEED

A unique six-channel CCD camera and a novel velocity detection system work in concert to collect 1,000 times more light than conventional technology. The technique is called Time Delay Integration. The result is high resolution imagery with fluorescence sensitivity superior to flow cytometry.

### MULTISPECTRAL IMAGING FOR MAXIMUM INFORMATION PER CELL

Illumination in the ImageStream system is provided by a

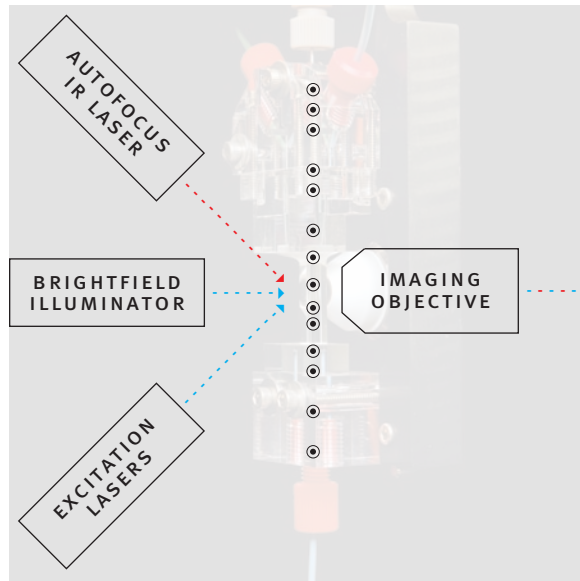
brightfield lamp, a 488 nm laser and up to two additional excitation lasers. Cellular imagery is split into six component images through a unique spectral decomposition element. The result is a brightfield image, a darkfield image, and multiple fluorescence images of every cell.

### SIMPLE OPERATION

ImageStream technology may be complex, but operating the instrument isn't. Highly automated protocols step you through calibration and set up. The complexity of the technology is practically invisible, allowing you to concentrate on your research, not your instrumentation.

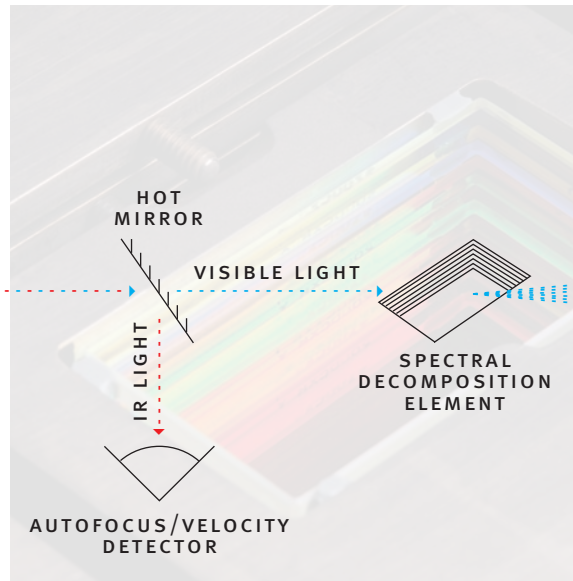
### CELLS IN FLOW

Precisely-controlled fluidics position cells in the plane of focus as they flow through the system smoothly and without tumbling.



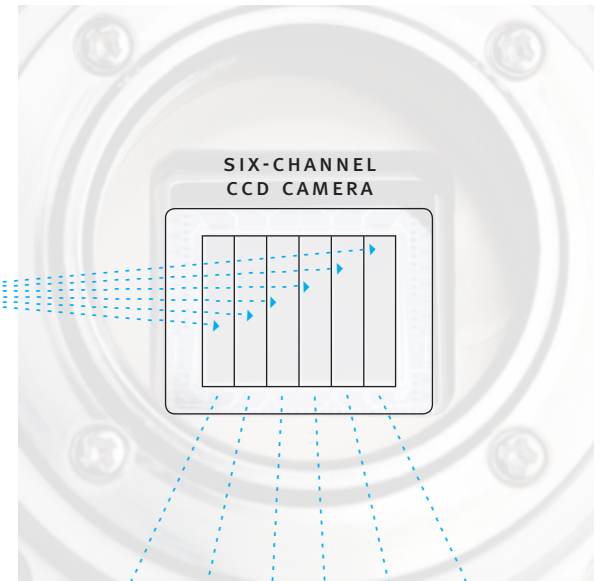
### SPECTRAL DECOMPOSITION ELEMENT

A fan of dichroic mirrors splits the cell imagery into six spectral bands, one for each independent channel of the CCD camera.



### TIME DELAY INTEGRATION

A custom six-channel CCD camera electronically tracks the motion of the cells, increasing the signal 1,000-fold.



### FLUORESCENCE EXCITATION

A solid state 488 nm laser and optional red and violet lasers excite a wide range of dyes used commonly in microscopy and flow cytometry.

### OPTICAL SYSTEM

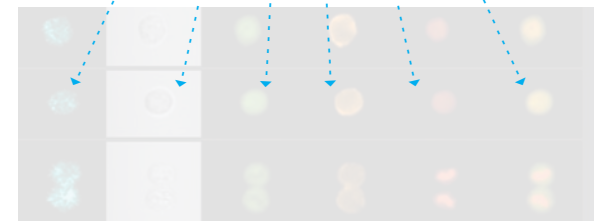
A custom imaging objective with a numeric aperture of 0.75 and high performance optics achieve the image detail of a high quality microscope.

### AUTOFOCUS

A sophisticated autofocus system continually optimizes image quality.

### VELOCITY DETECTION

A patented velocity detection system synchronizes the CCD camera readout with the motion of the cells.

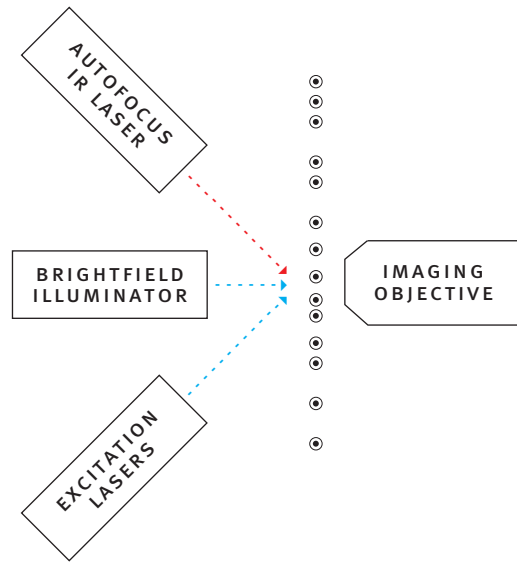


### MULTISPECTRAL IMAGERY

Six images per cell, including brightfield, darkfield, and multiple colors of fluorescence, convey tremendous information.

### CELLS IN FLOW

Precisely-controlled fluidics position cells in the plane of focus as they flow through the system smoothly and without tumbling.



### FLUORESCENCE EXCITATION

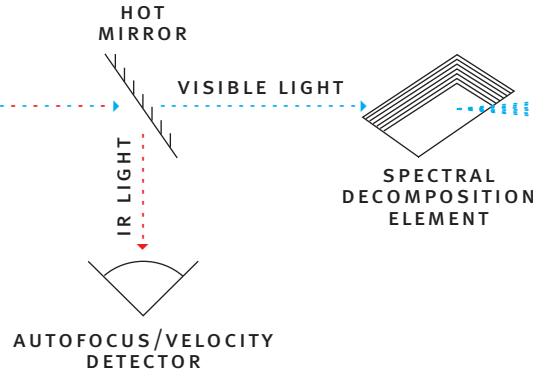
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### AUTOFOCUS

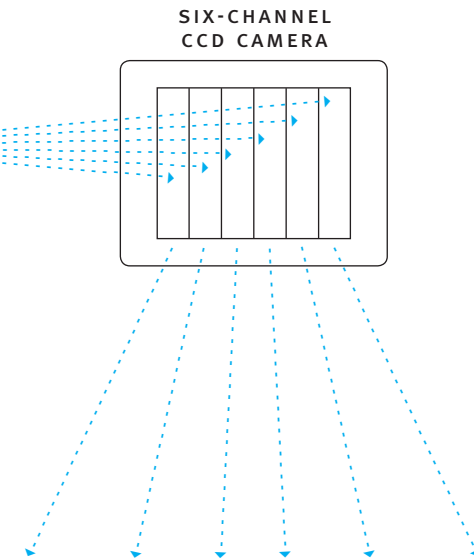
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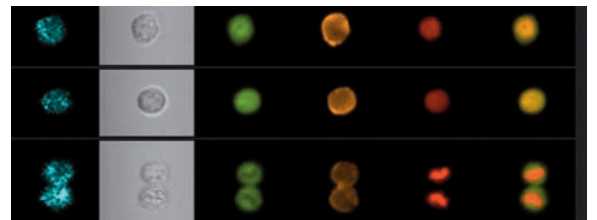
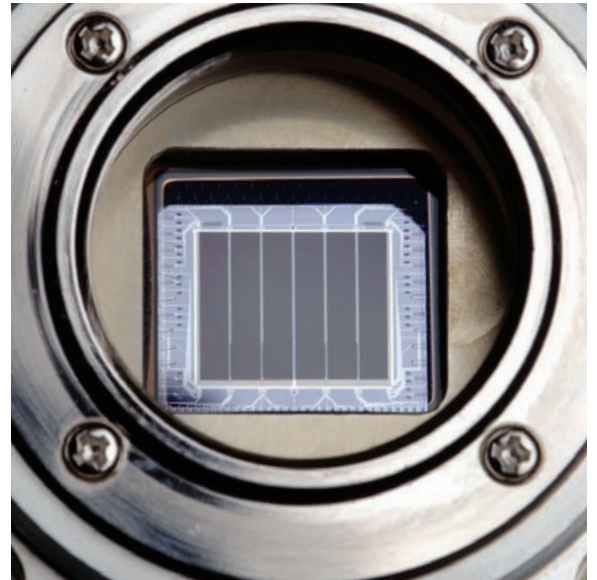
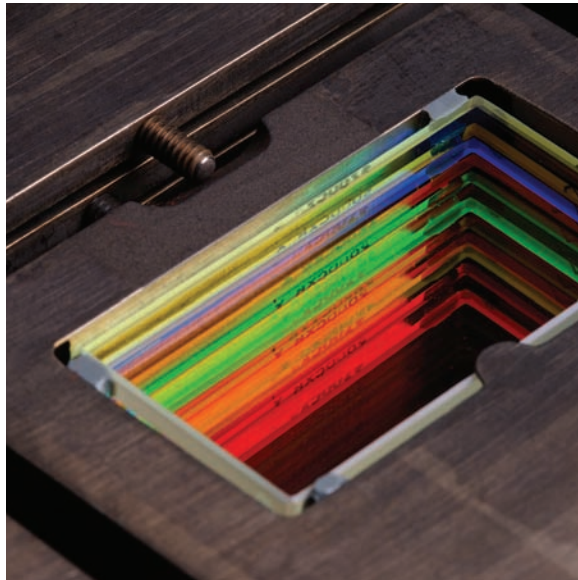
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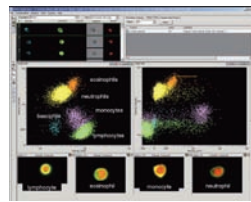


### MULTISPECTRAL IMAGERY

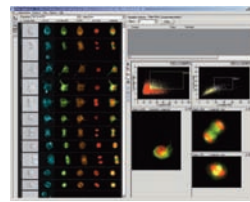
Six images per cell, including brightfield, darkfield, and multiple colors of fluorescence, convey tremendous information.



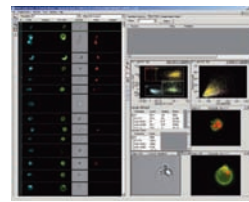
# New Applications



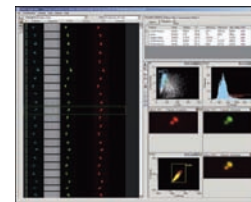
Leukocyte cell classification



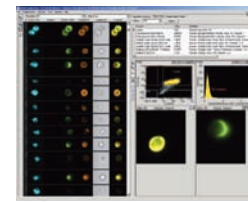
Tubulin stain in adherent cells



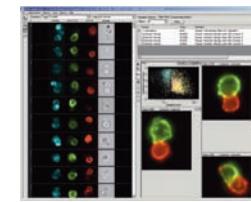
Apoptosis



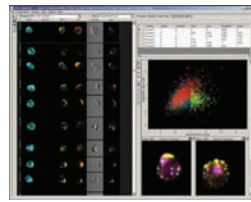
Yeast budding



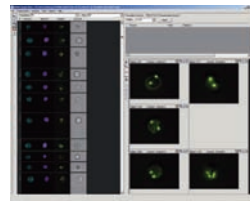
Surface marker capping



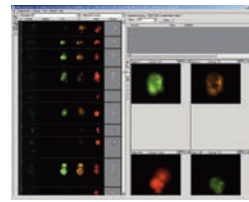
T Cell / APC conjugates



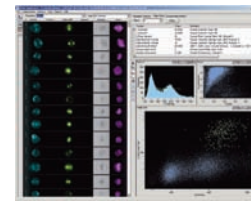
Endocytic processing pathways



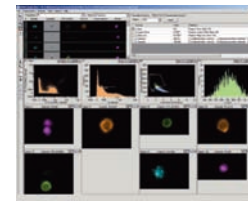
Multicolor FISH in Suspension



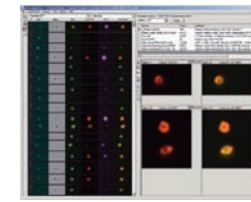
GFP and apoptosis



Cell cycle analysis



Human PBMC



Immune synapse

## One Instrument, Many Applications

VERSATILE

AMNIS

### CELL SIGNALING / PATHWAY ANALYSIS

The ImageStream system brings significant new capabilities to pathway analysis for cells in suspension. The phosphorylation states of key signaling molecules and their locations within the cell can be measured directly. Molecular association with the cell membrane, the cytoplasm, or the nucleus is easily distinguished and quantitated.

### APOPTOSIS

Using only measurements of nuclear morphology, the ImageStream system can directly differentiate apoptotic and necrotic cells, quantify the extent of apoptosis in cell populations, and calculate sub-population frequencies. The need for surrogate markers such as Annexin V or Caspase is reduced or eliminated. Visual analysis significantly reduces the classification errors found in conventional flow cytometric apoptosis assays, reducing false positive and false negative results.

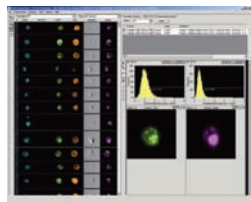
### QUANTITATIVE MORPHOLOGY

Change in cell shape is closely correlated with function in the analysis of lymphocyte or macrophage activation, pseudopod formation, response to drugs, and many other instances. Powerful features in the IDEAS® image analysis software allow you to accurately classify cells based on shape and structure.

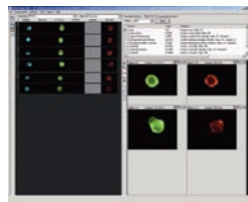
### CELL CLASSIFICATION

Characterization of peripheral blood mononuclear cell populations is a fundamental tool in hematology. The ImageStream system combines classical surface phenotyping with morphologic classification to deliver a full five-part differential analysis with room for the identification of additional sub-populations using fluorescent markers.

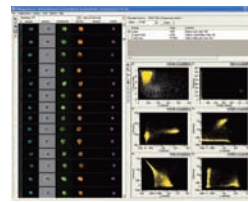




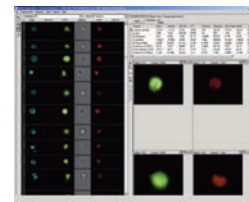
Intracellular co-localization of multiple proteins



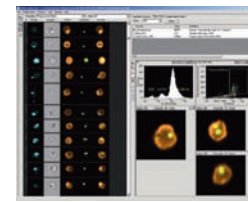
HIV receptor mapping



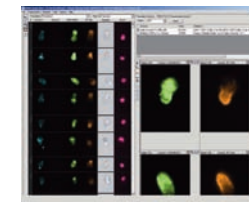
Multiple surface receptors



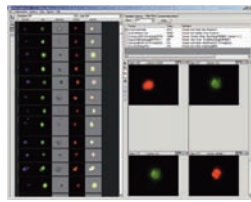
NF-kB translocation to the nucleus



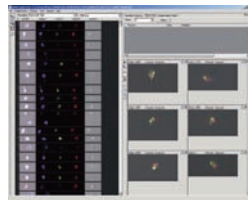
Phagocytosis



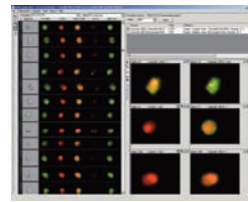
Pseudopod formation



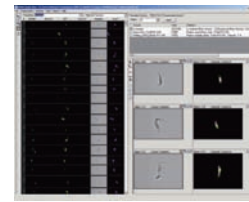
Nuclear translocation of Raf



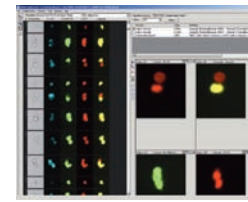
FISH in sperm



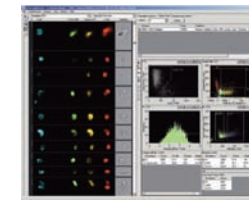
Toxoplasma gondii



Gene expression in trypanosomes



Analysis of TUNEL+ and TUNEL- cells



Caspase and NF-kB in apoptosis

### FLUORESCENCE IN SITU HYBRIDIZATION IN SUSPENSION (FISHIS™)

High throughput FISH is now possible with cells in suspension using the ImageStream system and Amnis protocols. Imagery is acquired rapidly and extended depth of field technology provides exceptionally clear visualization of multicolor chromosome spots in a range of cell types.

### GENE EXPRESSION ANALYSIS

The ImageStream system is well suited to the analysis of Green Fluorescent Protein and other fluorescent markers used in the study of gene expression. The high spatial resolution and sensitivity of the ImageStream system allows quantitation of expression levels and localization of expression to specific regions of the cell and key organelles.

### MOLECULAR DISTRIBUTION

Image similarity algorithms allow you to quantitatively

compare the distribution of multiple signals within single cells for co-localization, co-capping, and similar studies.

### ANALYSIS OF CELL CONJUGATES

Cells communicate through cell membrane-mediated molecular interactions. The ImageStream system not only identifies cell doublets, but also quantitates molecular co-localization at the interface between the interacting cells.

### RECEPTOR MAPPING AND DISTRIBUTION

The ImageStream system not only measures the abundance of important cell surface receptors with exceptional sensitivity and resolution, but can also map their locations and co-localize them with ligands or intracellular organelles. For instance, proteins of interest may be co-localized to endosomal and lysosomal markers to follow intracellular processing and degradation.

# Analytical Power

## Designed by Biologists for Biologists

Powerful, flexible, and extremely easy to learn, the IDEAS statistical image analysis package is integral to the ImageStream system.

### A ROBUST FEATURE SET, EXPANDABLE TO MEET YOUR NEEDS

The IDEAS feature set – the heart of the image analysis package – is extraordinarily robust, providing more than 200 features for every cell. IDEAS also allows you to create almost any new feature you may need from the basic feature set.

### IMAGE DATA AND STATISTICAL DATA ARE FULLY INTEGRATED

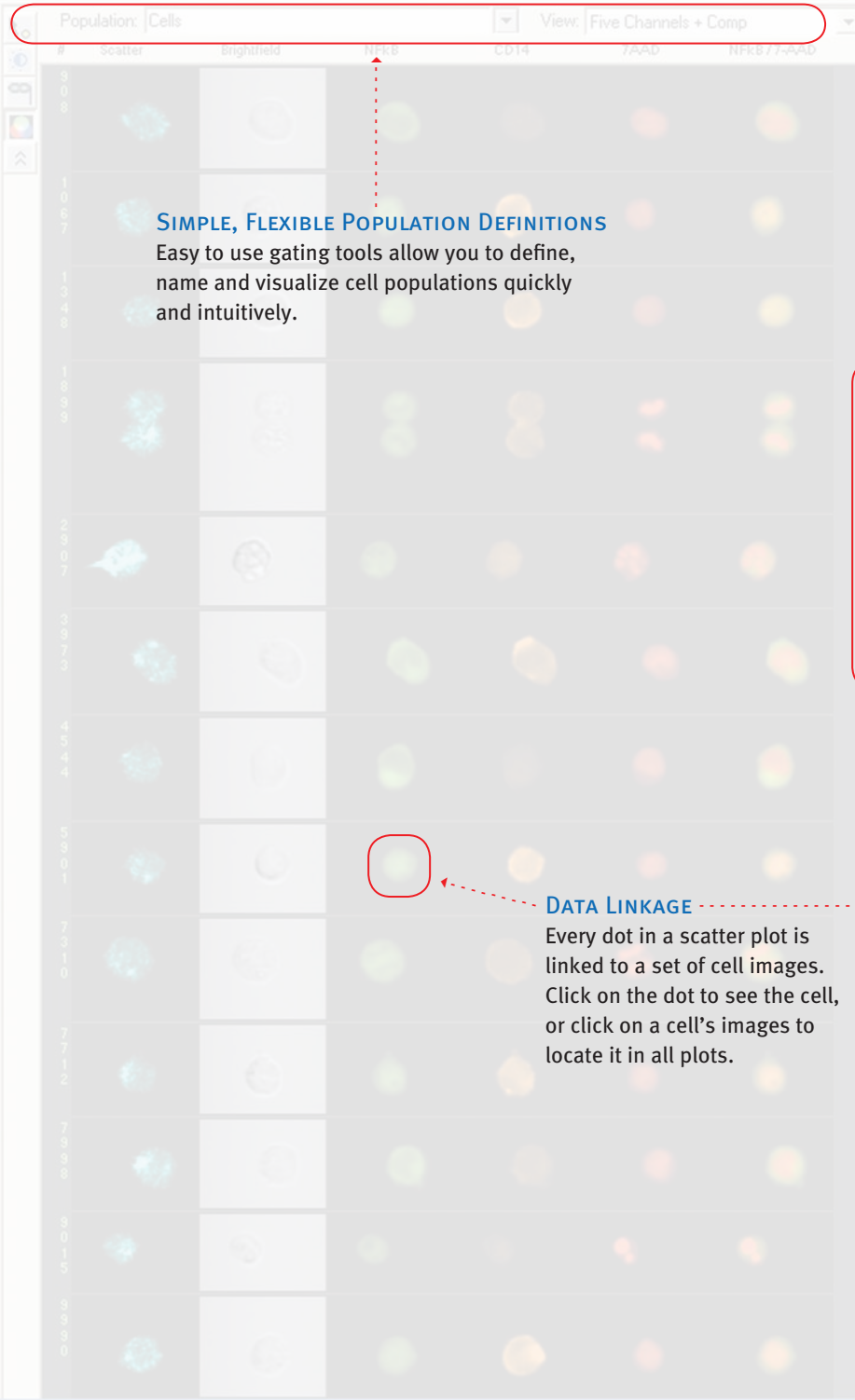
In IDEAS, population statistics and imagery are completely integrated. Every dot on a scatter plot links directly to a cell's images – click on the dot and you'll see the corresponding cell. With its virtual sorting capability, IDEAS will show you all the images of a cell population you define.

### AN EFFICIENT, FLEXIBLE DATA INTERFACE

The IDEAS interface integrates image data, plots, and statistics. The Gallery shows you images of every cell, while the Workspace gives you graphing tools to define and analyze cell populations. The Tabular Data section allows you to view population statistics as well as individual feature values.

### TEMPLATES AND BATCH PROCESSING

Once you've created an analysis scheme in IDEAS, you can save it as a template for batch processing future experiments or to share with your colleagues.



**SIMPLE, FLEXIBLE POPULATION DEFINITIONS**  
 Easy to use gating tools allow you to define, name and visualize cell populations quickly and intuitively.

**DATA LINKAGE**  
 Every dot in a scatter plot is linked to a set of cell images. Click on the dot to see the cell, or click on a cell's images to locate it in all plots.

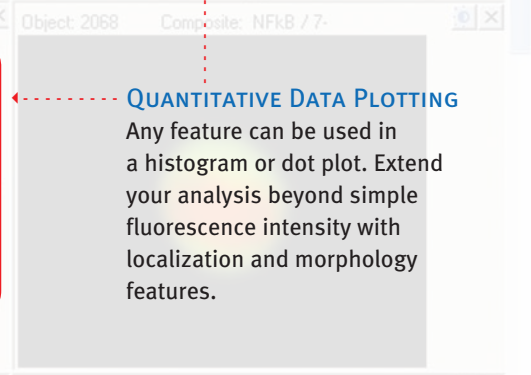
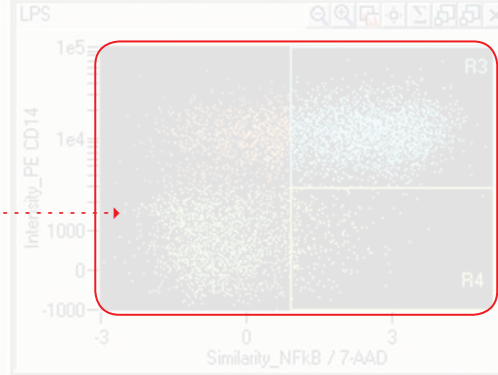
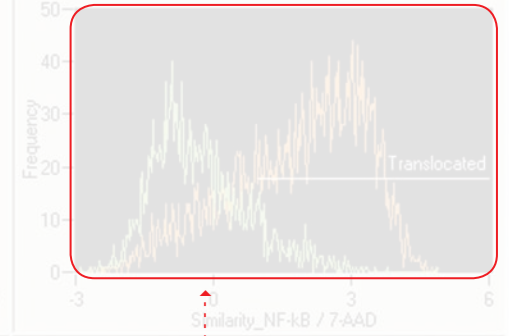
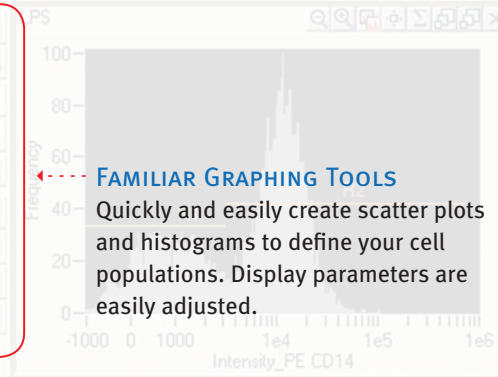
Object: 0 Select

Feature	Value	Definition
1 Area	515	Feature: Area, Mask: M1
2 Aspect Ratio	1.2507	Feature: Aspect Ratio, Mask: M1
3 Minor Axis	2903	Feature: Minor Axis, Mask: M5
4 CentroidX	52.7207	Feature: CentroidX, Mask: M6
5 Spot Small Total	918	Feature: Spot Small Total, Channel: 5
6 Intensity	34072.62	3_Combined Mask Intensity - ( 3_Background Mean Intensity * Combined
7 Intensity	1830.5724	4_Combined Mask Intensity - ( 4_Background Mean Intensity * Combined
8 Similarity	0.6875	Feature: Similarity, Mask: Threshold[7AAD,73%], Channels: 3, 5

**RICH FEATURE SET**  
 IDEAS calculates over 35 features for each image and over 200 features per cell, allowing the discrimination of subtle differences between cell populations.



**FAMILIAR GRAPHING TOOLS**  
 Quickly and easily create scatter plots and histograms to define your cell populations. Display parameters are easily adjusted.



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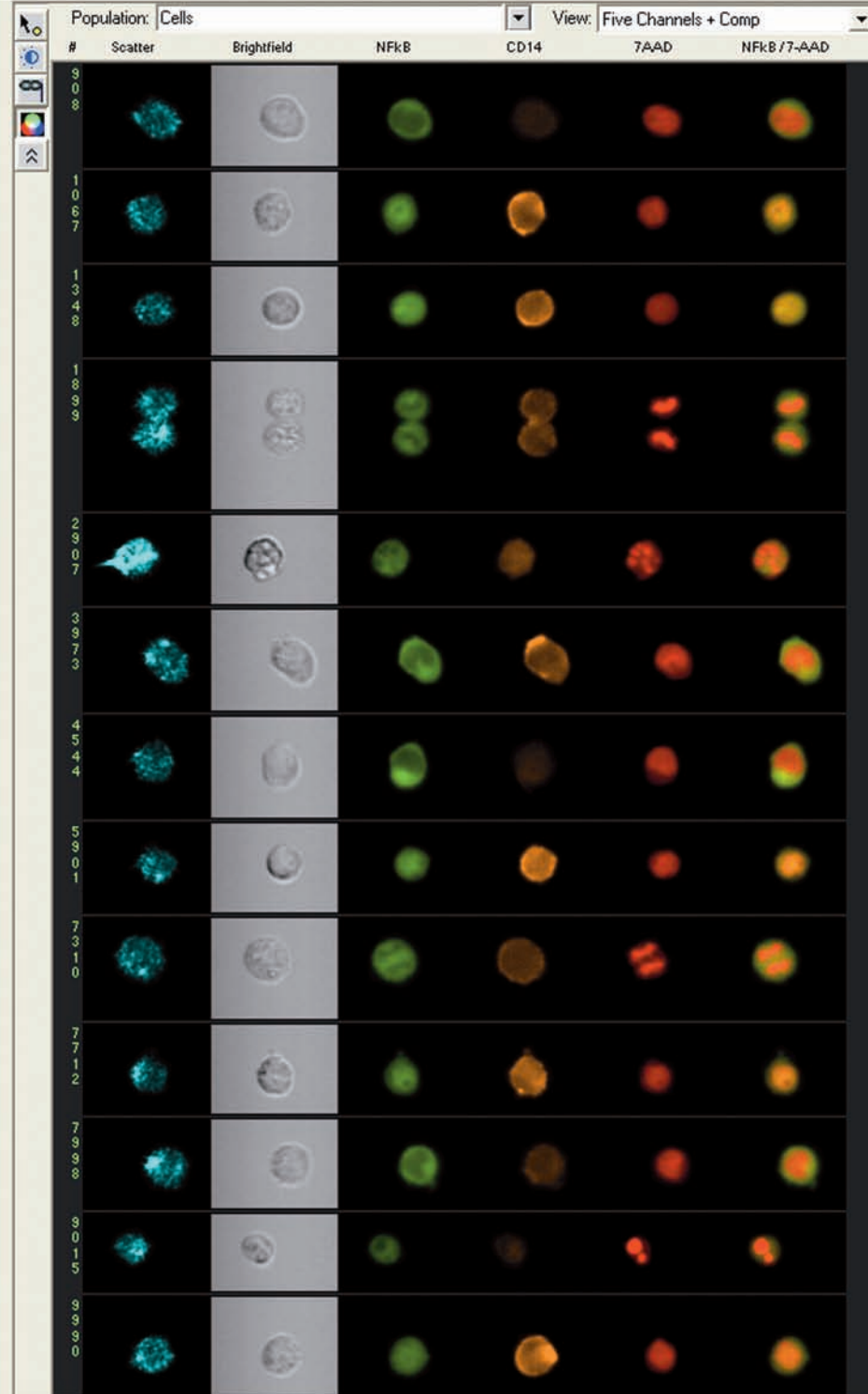
Quickly and easily create scatter plots and histograms to define your cell populations. Display parameters are easily adjusted.

**QUANTITATIVE DATA PLOTTING**

Any feature can be used in a histogram or dot plot. Extend your analysis beyond simple fluorescence intensity with localization and morphology features.

**DATA LINKAGE**

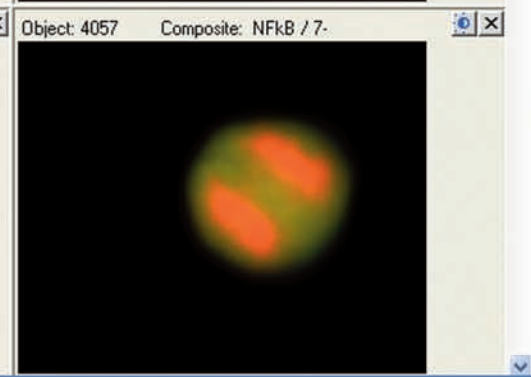
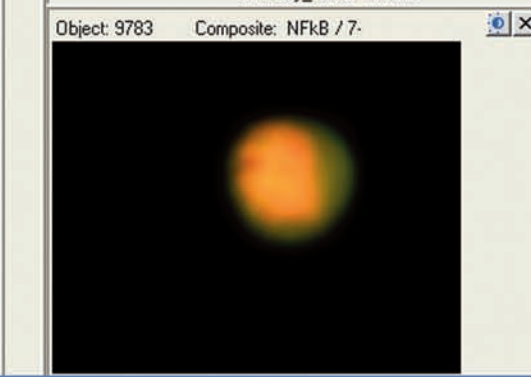
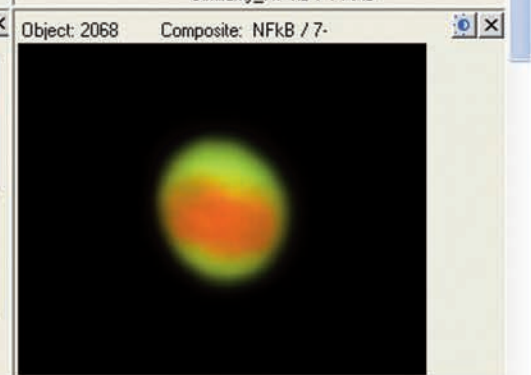
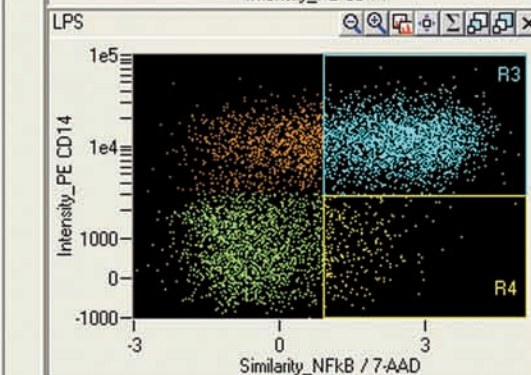
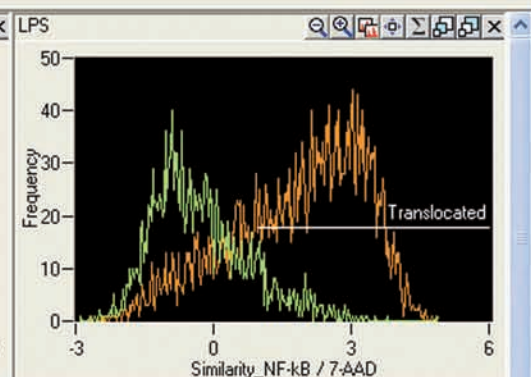
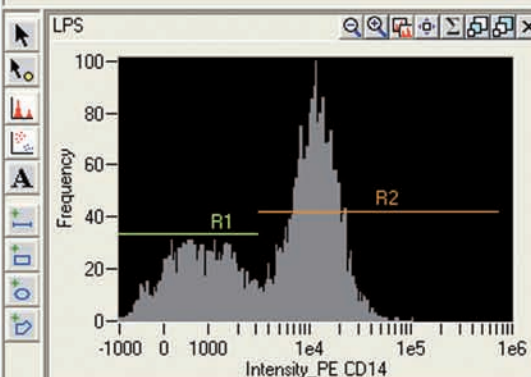
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Population Statistics Object Data Compensation Matrix

Object: **0**

Feature	Value	Definition
1_Area	515	Feature: Area, Mask: M1
1_Aspect Ratio	0.8837	Feature: Aspect Ratio, Mask: M1
5_Minor Axis	12.2903	Feature: Minor Axis, Mask: M5
6_CentroidX	52.7207	Feature: CentroidX, Mask: M6
5_Spot Small Total	918	Feature: Spot Small Total, Channel: 5
3_Intensity	34072.62	3_Combined Mask Intensity - ( 3_Background Mean Intensity * Combined
4_Intensity	1850.5724	4_Combined Mask Intensity - ( 4_Background Mean Intensity * Combined
Similarity_C3&C5_Threshold(7AAD,73	-0.6875	Feature: Similarity, Mask: Threshold(7AAD,73%), Channels: 3, 5



# ImageStream Specifications

PRECISE

## Advanced Engineering Creates Exceptional Performance

AMNIS

### ILLUMINATION SOURCES

SOURCE		WAVELENGTH	MAX POWER
Brightfield Lamp	Standard	430-730 nm	
Blue Laser	Standard	488 nm	200 mW
Ultraviolet Laser	Optional	375 nm	18 mW
Violet Laser	Optional	405 nm	350 mW
Red Laser	Optional	658 nm	80 mW

The ImageStream system includes a solid-state 488 nm laser as the standard excitation source. The new laser options include a red 658 nm laser (80 mW) and your choice of either a high power violet 405 nm laser (350 mW) or a UV 375 nm laser (18 mW). Each of these new laser options is available factory installed or they may be purchased for installation on an existing ImageStream system.

Dyes listed here are examples of the many dyes that may be used on the ImageStream system configured with 405 nm, 488 nm and 658 nm lasers. The ImageStream is a Class 1 laser product.

### DETECTION CHANNELS

CHANNEL 1 488 nm	CHANNEL 2 400-470 nm	CHANNEL 3 500-560 nm	CHANNEL 4 560-595 nm	CHANNEL 5 595-660 nm	CHANNEL 6 660-735 nm
Darkfield	DAPI	Fluorescein	PE	7-AAD	Cy5
	Hoechst 33258	Alexa Fluor 488	Cy3	Alexa Fluor 610	Cy5.5
	Hoechst 33342	Alexa Fluor 500	Alexa Fluor 546	Propidium Iodide	Alexa Fluor 647
	Alexa Fluor 350	Alexa Fluor 514	Alexa Fluor 555	Texas Red	Alexa Fluor 660
	Alexa Fluor 405	Syto Green	YFP	Qdot 605	Alexa Fluor 680
	Alexa Fluor 430	Mitotracker Green	Qdot 565	Qdot 655	Alexa Fluor 700
	Cascade Blue	Spectrum Green	Qdot 585	Brightfield	DRAQ5
	Pacific Blue	CFP	Brightfield		PerCP
	Brightfield	Qdot 525			Qdot 705
		Qdot 545			APC
		Brightfield			Mitotracker Deep Red
					Brightfield

All trademarks are acknowledged.



### PERFORMANCE

- Detection Limit <50 MESF
- Numeric Aperture 0.75
- Pixel Size 0.5 x 0.5 microns
- Field of View 45 microns wide

### SAMPLE VOLUME

- 40-200 microliters

### DATA ANALYSIS

- Automated crosstalk compensation post-acquisition
- Unlimited user-defined image features
- Over 200 standard image features per cell

### INSTRUMENT OPERATION

- Automated sample load, empty, flush, and purge
- Automated focus and core position tracking
- Automated sterilization
- Automated calibration and quality control
- Automated laser alignment

### REQUIREMENTS

- 90-240 VAC, 50-60 Hz
- 100 Mbps ethernet, minimum
- No external air or water required
- 36" w x 24" h x 24" d
- 350 lbs

**AMNIS CORPORATION**

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Suite 210  
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**U.S. TOLL-FREE** +1.800.730.7147

**FAX** +1.206.576.6895

[www.amnis.com](http://www.amnis.com)

