

# Amnis Imaging Flow Cytometry Systems

Carlo Raviolo

System overview and benefits

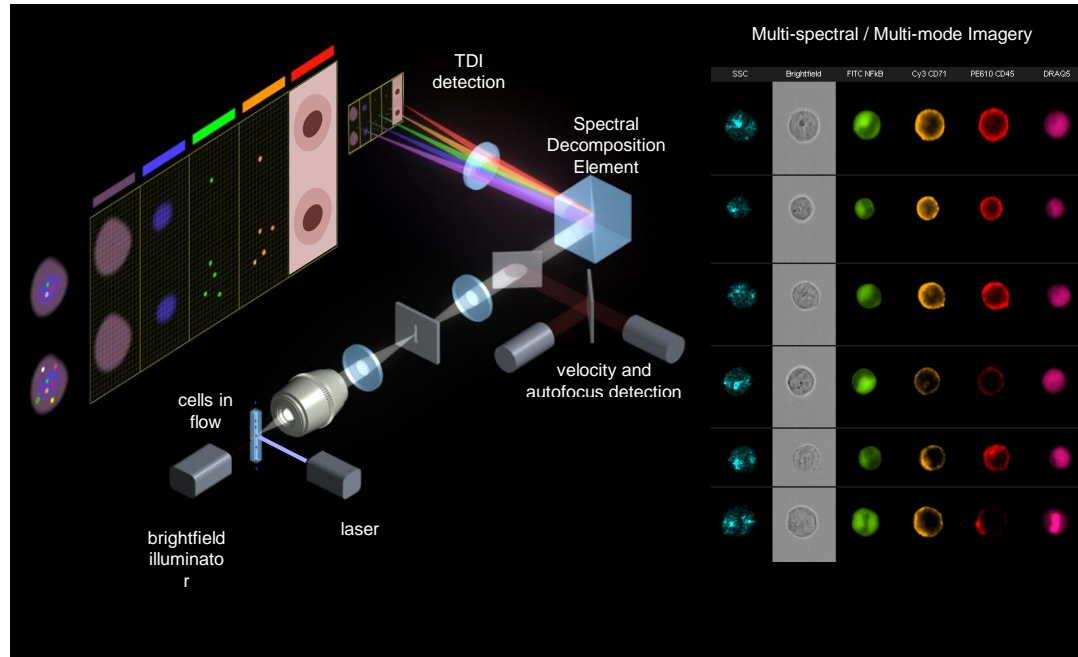
CONFIDENTIAL

# Amnis combines some of the best features of Flow Cytometry and Microscopy

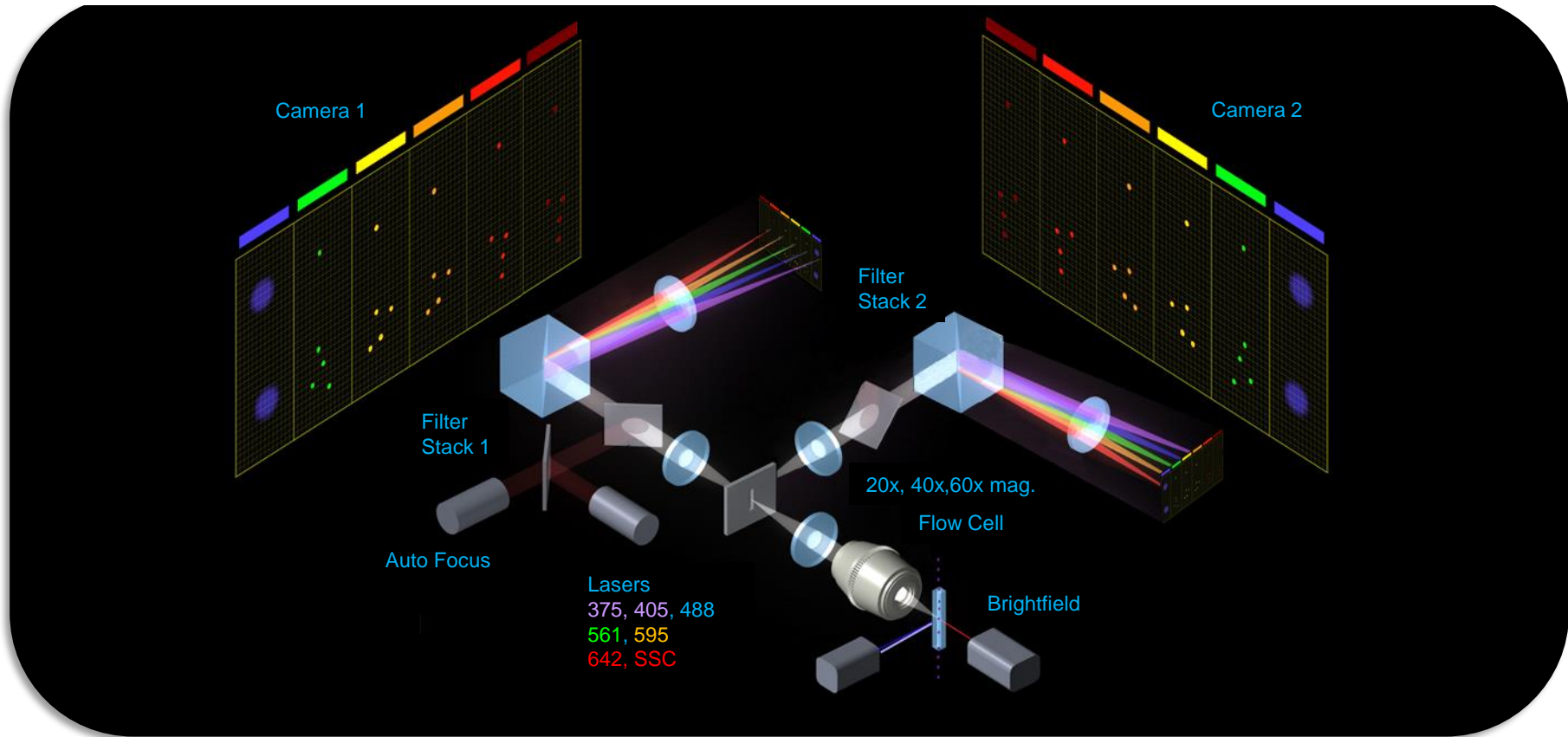


	Flow Cytometry	Microscopy	Amnis
High speed	+	-	+
Statistical power	+		+
Imaging	-	+	+
Information content		+	+
Research benefit	Objective, statistical discrimination of cells based on intensity	Discrimination of cells based on appearance	Objective, statistical discrimination of cells based on appearance

# Amnis technology – FS Optical layout



# ImageStreamX MKII Optical Layout with 2 CCD camera



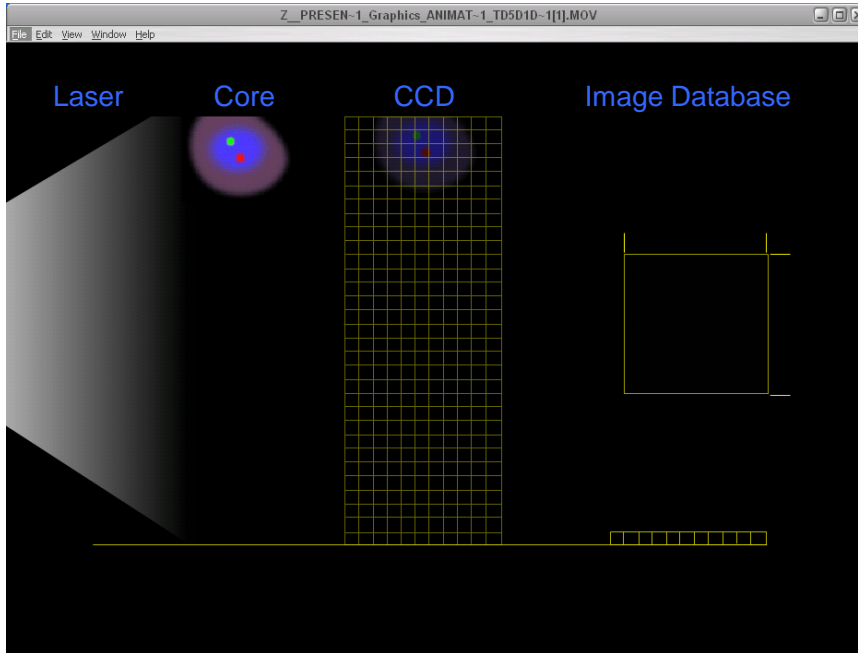
## Three different light sources by default

- 488 nm blue laser, dedicated exclusively to the emitted fluorescences
- Bright field, dedicated to assess the size and shape of the cells, much more precise than the conventional FSC, since measures the actual area of the cells.
- 785 nm dedicated laser for the SSC, which improves the resolution

# Advantages of Imaging Cytometry over conventional cytometry

- Highest sensitivity and resolution
- Visual verification of the events acquired
- Quantitative Imaging Applications

# Higher sensitivity thanks to the Time Delay Integration

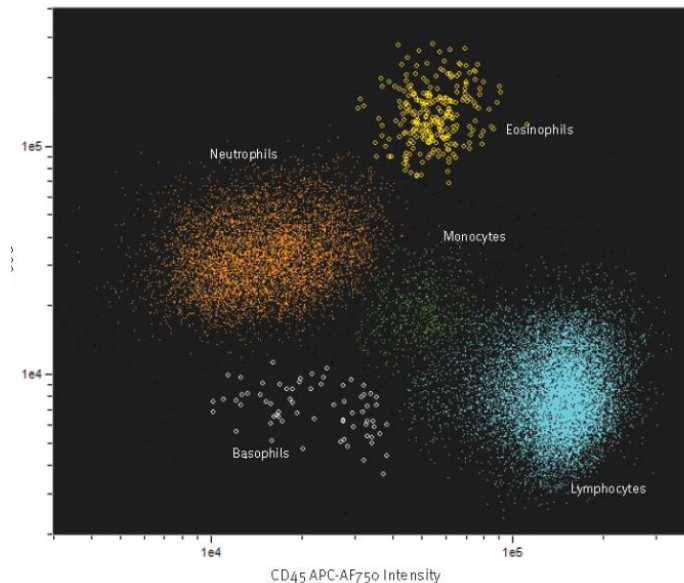
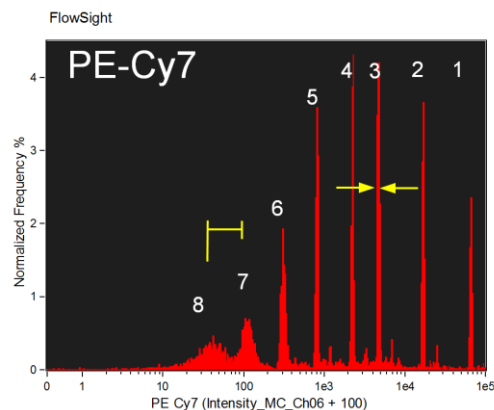


## TDI CCD

- Excite fluorescence over the entire height of the detector
- Light is detected in the first pixel row and transferred to the pixel below in exact synchrony with the velocity of the cell as it goes streaming by.
- Light is integrated over the entire height of the detector to achieve high photonic sensitivity
- Images don't streak or blur and maintain 0.5um per pixel resolution.

# Advantages of Imaging Flow Cytometry

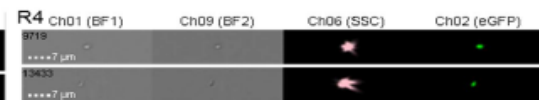
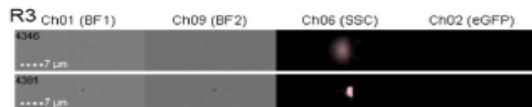
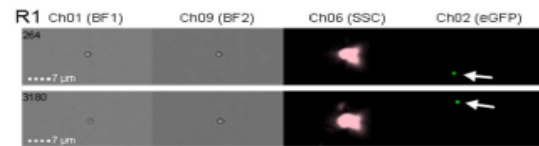
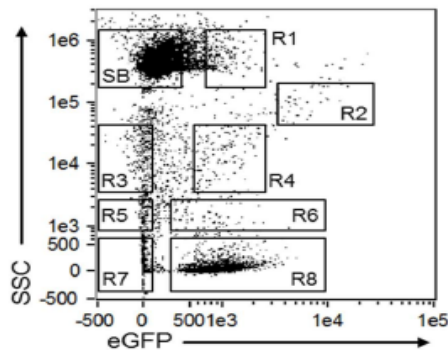
- **High fluorescence sensitivity:**
- The fluorescence sensitivity of imaging flow cytometers exceeds that of most conventional cytometers
  - Higher absolute sensitivity allows detection of dim cell markers
  - Uniform sensitivity eases panel design (red dyes + dim markers)
  - Antibodies can be titered down to lower operating costs
  - Mixed cell populations can be resolved due to lower CVs





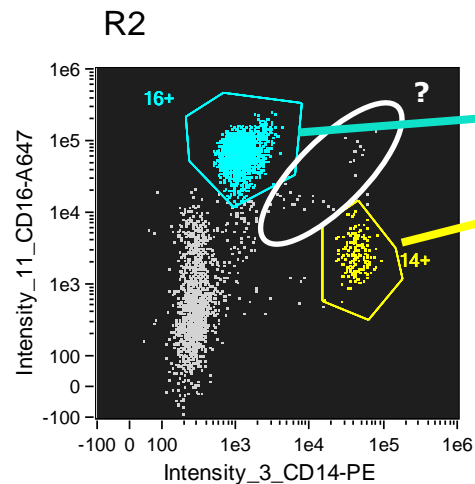
# Higher sensitivity allows detection and identification of microvesicles and exosomes much better than conventional cytometry

## CD63-eGFP EVs as Biological Calibrator for Flow Cytometry



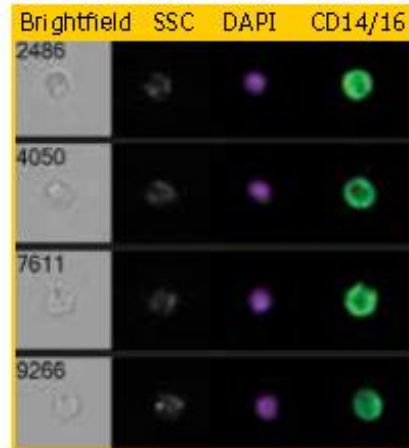
André Gorgens,  
Essen University  
Hospital, webinar  
series, June 22°,  
2016

# Gating Without Guesswork

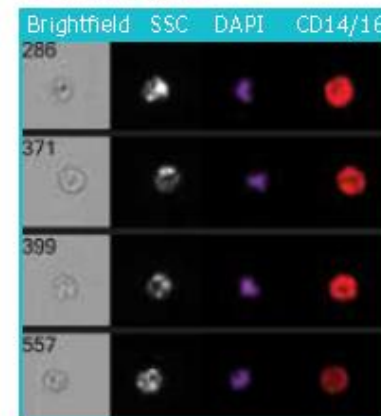


CD14+ and CD16+ populations

## Monocytes

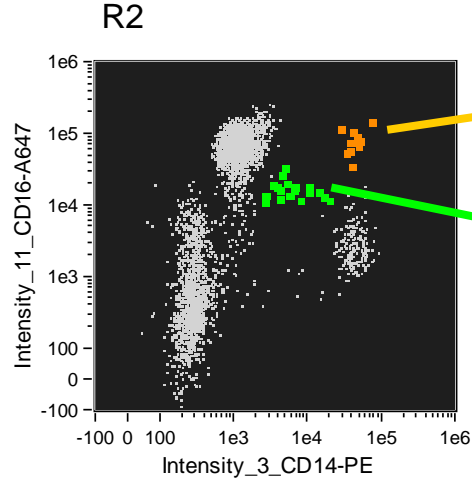


## Granulocytes



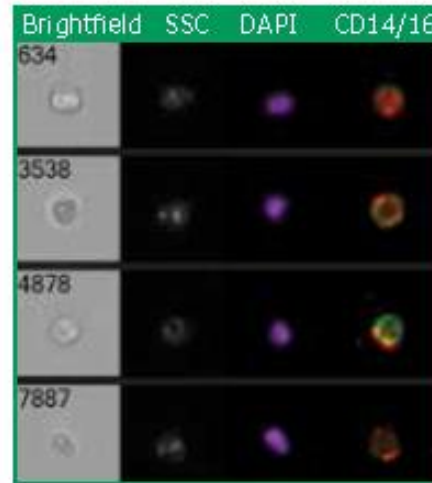
- Visually inspect any population to confirm location/shape of gate
- Click on any dot to verify its identity

# No More “Mystery Populations”

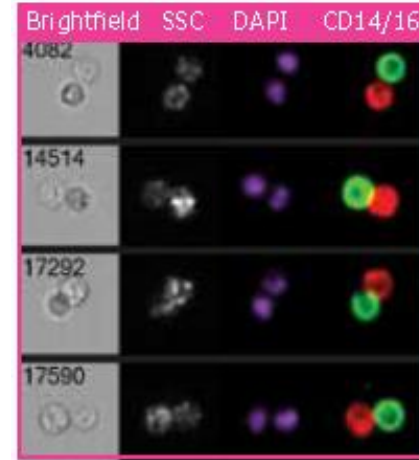


CD14+ CD16+ populations

## Double Positives



## Doublet Artifacts



- Artefactual populations are readily identified and eliminated
- Unexpected results can be investigated and exploited

# IDEAS SOFTWARE

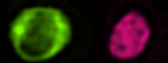
The screenshot displays the IDEAS software interface for flow cytometry analysis. The main window is titled "IDEAS - [022411 FSA1 Raw 647 2ymTx Red 37\_Merge.daf]". The interface includes a menu bar (File, Guided Analysis, Analysis, Compensation, Tools, Reports, Windows, Help), a toolbar, and a central workspace with several panels:

- Population:** All
- View:** Internalization\_Ch01\_Ch11\_Ch04\_Ch02\_Ch11/Ch1
- Channel Selection:** CH01, CH11, CH04, CH02, CH11/CH04
- Image Stack:** A vertical list of images from frame 18 to 36, showing cell morphology and fluorescence.
- Flow Cytometry Plots:**
  - R1:** Histogram of Normalized Frequency vs Gradient RMS\_M01\_Ch01.
  - R2:** Scatter plot of Aspect Ratio\_M01 vs Area\_M01 with gates R1 (green) and R2 (red).
  - R3:** Scatter plot of Intensity\_MC\_Ch11 vs Intensity\_MC\_Ch04 with gate R3 (yellow).
  - R4:** Scatter plot of Max Pixel\_Intensity\_MC\_Ch04 vs Intensity\_MC\_Ch04 with gate R4 (orange).
  - R5:** Histogram of Normalized Frequency vs Internalization\_C\_E4\_Ch04.
- Population Statistics:**

Population	Count	%Gated	Internalization_C_E Median	Internalization_C_E MAD
All	14907	100	0.5203	0.9573
R1	13239	88.81	0.5089	0.9467
R2	10910	82.41	0.517	0.8837
R3	6882	63.08	0.4668	1.155
R4	6113	39.83	0.4969	1.159
R5	4802	78.55	0.7475	0.9168
- Summary Table:**

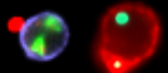
Population	Count	%Total	%Gated
R3 & R2 & R1	6882	46.2	100
R4 & R3 & R2 & R1	6113	41	88.8
- Wizards Dialog:** A dialog box titled "Wizards" is open, listing analysis templates:
  - Open File: Creates a template to facilitate analysis.
  - Display Properties: Automatically sets image display properties.
  - Begin Analysis: Identifies single, focused, fluorescent positive cells.
  - Feature Finder: Assists the user in picking relevant features for separating populations. The file must contain members of each population.
  - Apoptosis: Creates an analysis template for identifying apoptotic events based on brightfield and nuclear morphology.
- Image Panels:** A row of seven image panels at the bottom showing individual cell images with 20 µm scale bars.

# Quantitative Imaging Applications



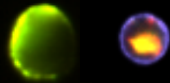
Cell signaling:

NFkB Translocation, HIV induced NFAT, FoxP3 localization



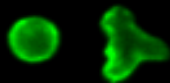
Internalization & phagocytosis

CpGB, Internalization, phagocytosis of Bacteria by monocytes



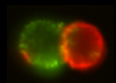
Intracellular co-localization

Ligand colocalization to lysosomes



Shape change & chemotaxis

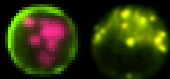
MCP-1 activation of monocytes, Differentiation of FDCP cells



Cell-cell interaction

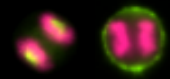
Immune synapse formation, T-cell APC conjugation

# Quantitative Imaging Applications



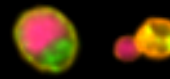
Cell death & autophagy

Apoptosis, nuclear fragmentation,  
caspase3 activation, LC3 clustering



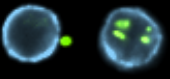
Cell cycle & mitosis

Morphological classification of mitosis



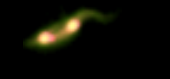
Stem cell biology

Erythroid differentiation, hematopoiesis



Microbiology

Bacterial phagocytosis in PBMC



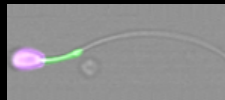
Parasitology

Babesia infection in RBCs,  
Trypanosomiasis

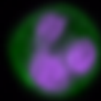
# Emerging Applications



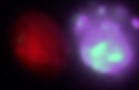
*RBC Morphology Sickle cell*



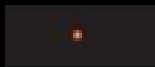
*Sperm Analysis*



*AML ALL classification*



*Asymmetric Cell Division*



*Microparticle quantification*



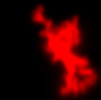
*Water Quality Testing / Algae*



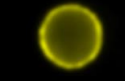
*Clinical diagnostics: HPV*



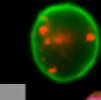
*Micro-nuclei assay*



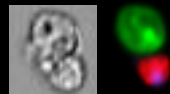
*Protein Aggregation*



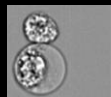
*Circulating tumor cell*



*Exosome Internalization*



*NK Cell Granzyme killing*

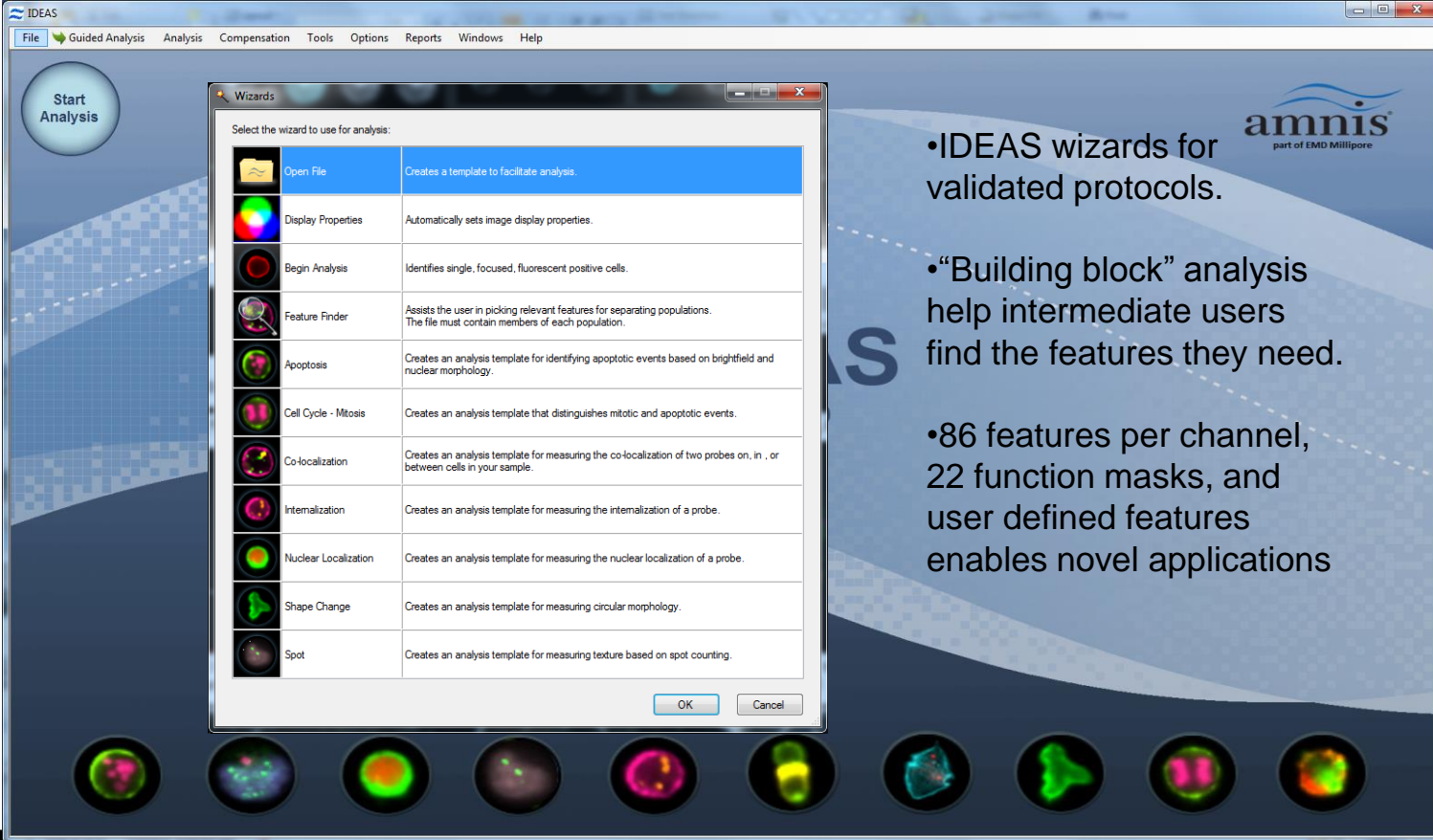


*Netosis*



*CRISPR gene corrections*

# QI Applications overview














IDEAS

File Guided Analysis Analysis Compensation Tools Options Reports Windows Help

Start Analysis

Wizards

Select the wizard to use for analysis:

	Open File	Creates a template to facilitate analysis.
	Display Properties	Automatically sets image display properties.
	Begin Analysis	Identifies single, focused, fluorescent positive cells.
	Feature Finder	Assists the user in picking relevant features for separating populations. The file must contain members of each population.
	Apoptosis	Creates an analysis template for identifying apoptotic events based on brightfield and nuclear morphology.
	Cell Cycle - Mitosis	Creates an analysis template that distinguishes mitotic and apoptotic events.
	Co-localization	Creates an analysis template for measuring the co-localization of two probes on, in, or between cells in your sample.
	Internalization	Creates an analysis template for measuring the internalization of a probe.
	Nuclear Localization	Creates an analysis template for measuring the nuclear localization of a probe.
	Shape Change	Creates an analysis template for measuring circular morphology.
	Spot	Creates an analysis template for measuring texture based on spot counting.

OK Cancel

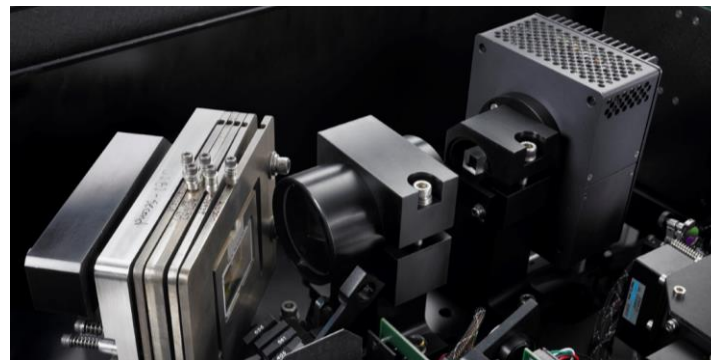
amis part of EMD Millipore

- IDEAS wizards for validated protocols.
- “Building block” analysis help intermediate users find the features they need.
- 86 features per channel, 22 function masks, and user defined features enables novel applications



# ImageStream - 12 Channel - Two Detector System

- 12 channel data, two brightfield, SSC and up to 9 colors of fluorescence.



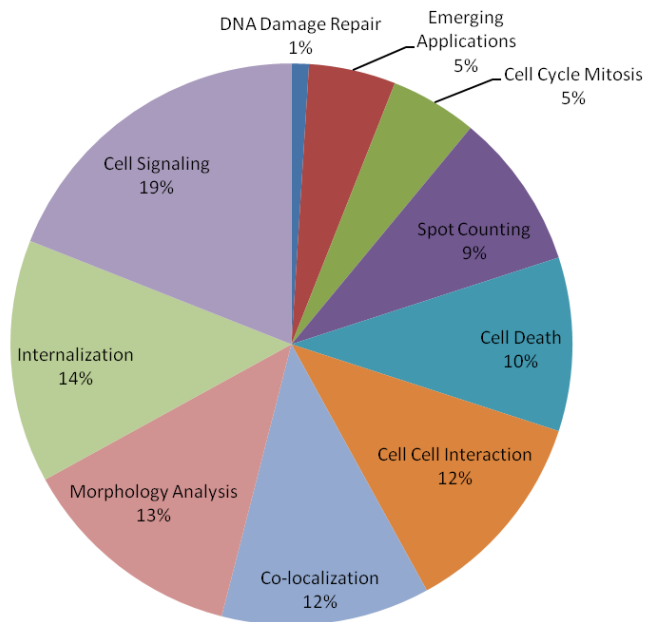
Camera 1, ex.405, 488, 560, 785

Camera 2, ex 375, 592, 642

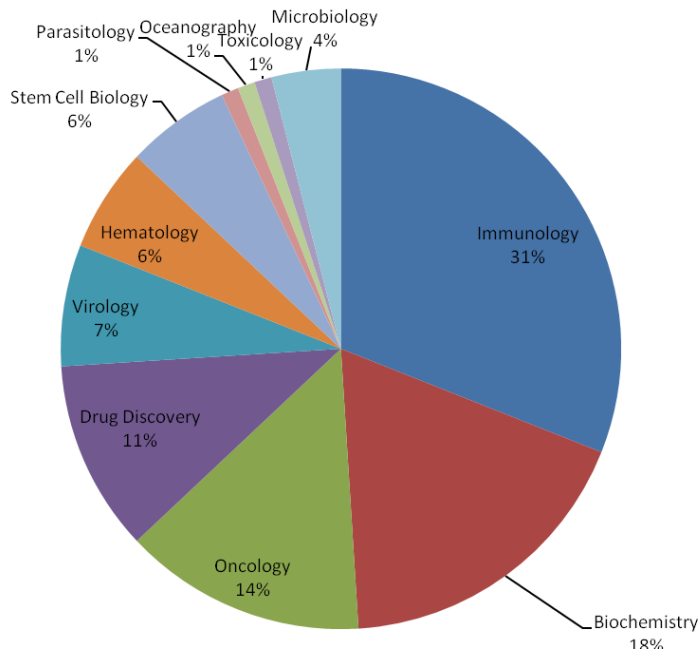
Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6	Channel 7	Channel 8	Channel 9	Channel 10	Channel 11	Channel 12
468/76	532/56	578/36	628/64	690/60	760/80	468/76	532/56	578/36	628/64	690/60	760/80
Brightfield	FITC	PE	ECD	PerCP	SSC	DAPI	PacOrng	Brightfield	TxR	AF647	APC Cy7
	AF488	Cy-3	PE-TxR	Draq5	PE-Cy7	PacBlu	AF430		AF594	AF660	APC AF750
	GFP	AF555	PI	PerCp5	PE-750	MarBlu			AF568	Cy5	
	YFP	DS-Red	7AAD	PE-647	SSC	Hoechst			AF610	APC	
	Syto		PE-610	PE-Cy5						APC-Cy5.5	
	SpecGrn			PE-680							

# Publications by Research Discipline

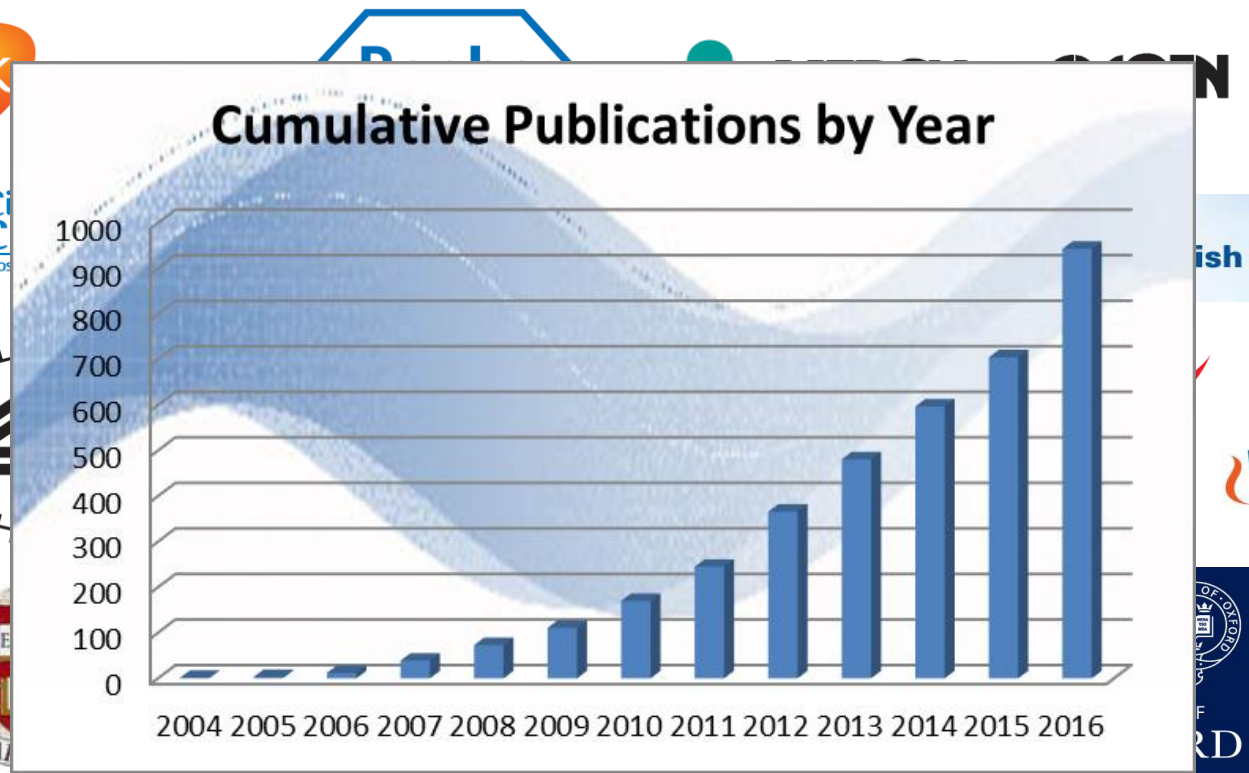
## Publications by Research Application



## Publications by Research Discipline



# Rapid Increase in Publications



THE UNIVERSITY OF TEXAS  
MD Anderson  
Cancer Center  
Making Cancer History®

NATIONAL  
OF H



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NOVARTIS



# Summary

- Amnis Imaging Cytometry is an innovative and exclusive technology from Merck that overcomes the traditional limitations of flow cytometry and FL microscopy;
- It provides higher sensitivity and resolution, compared to conventional cytometry, and in addition it provides visual verification of the events, and a series of imaging applications that usually require the use of different and more tedious and time consuming techniques;
- Thanks to this technology, users are offered a tool that allows them to work as a conventional cytometry on one side, and ideas for new experiments on the other side.