Amnis Imaging Flow Cytometry Systems

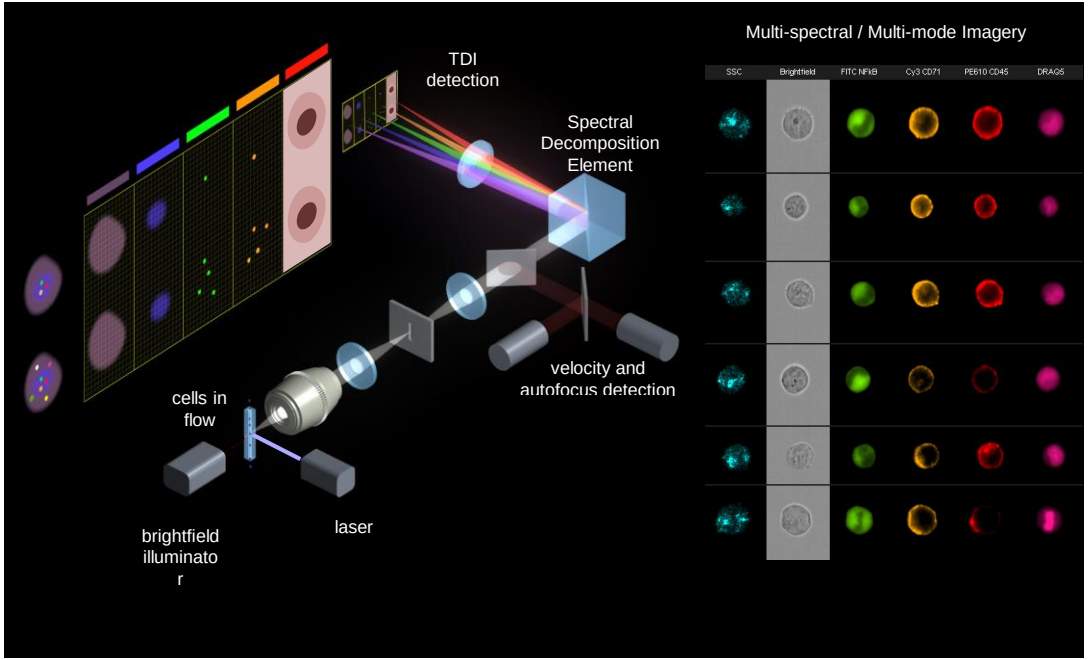
Carlo Raviolo

System overview and benefits

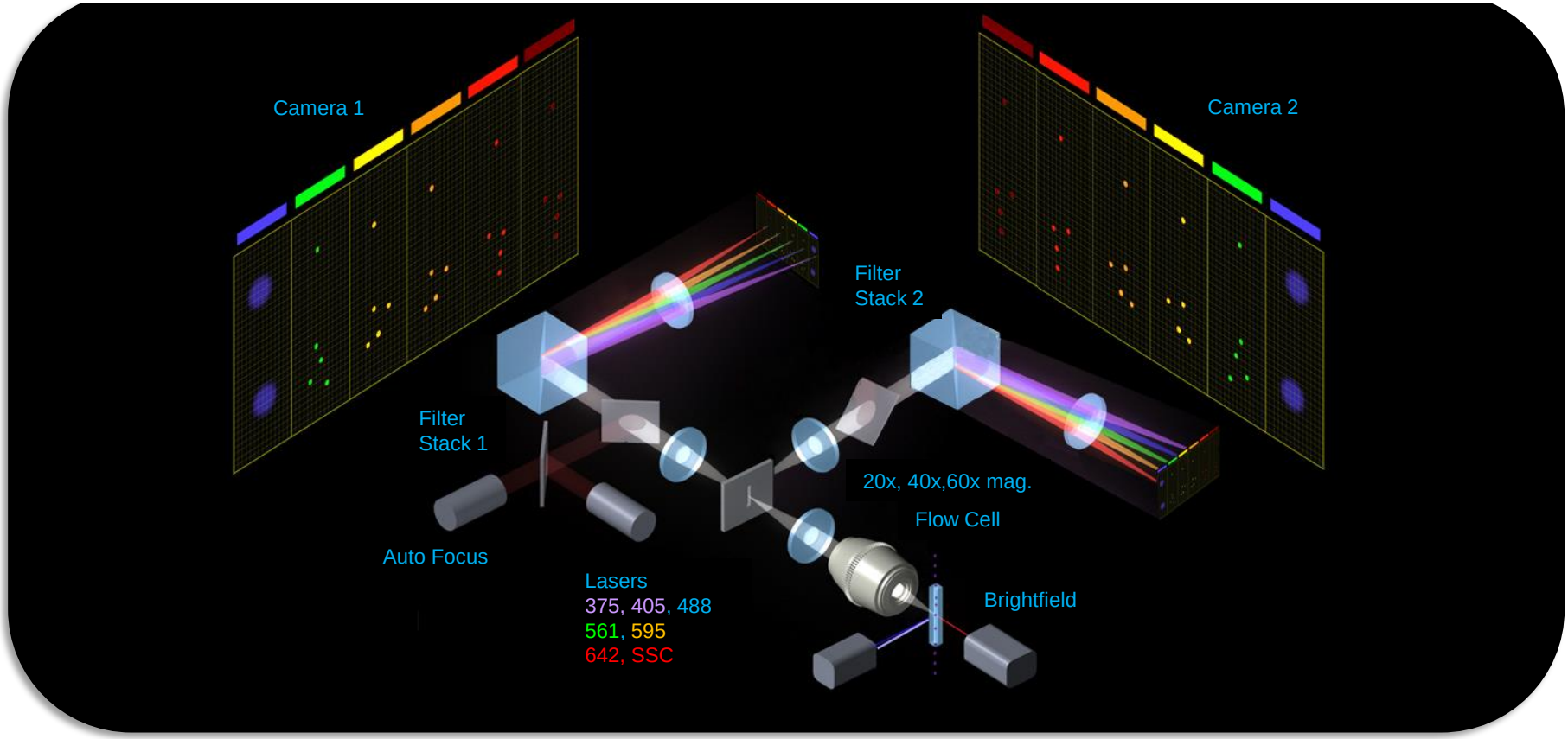
Amnis combines some of the best features of Flow Cytometry and Microscopy

	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



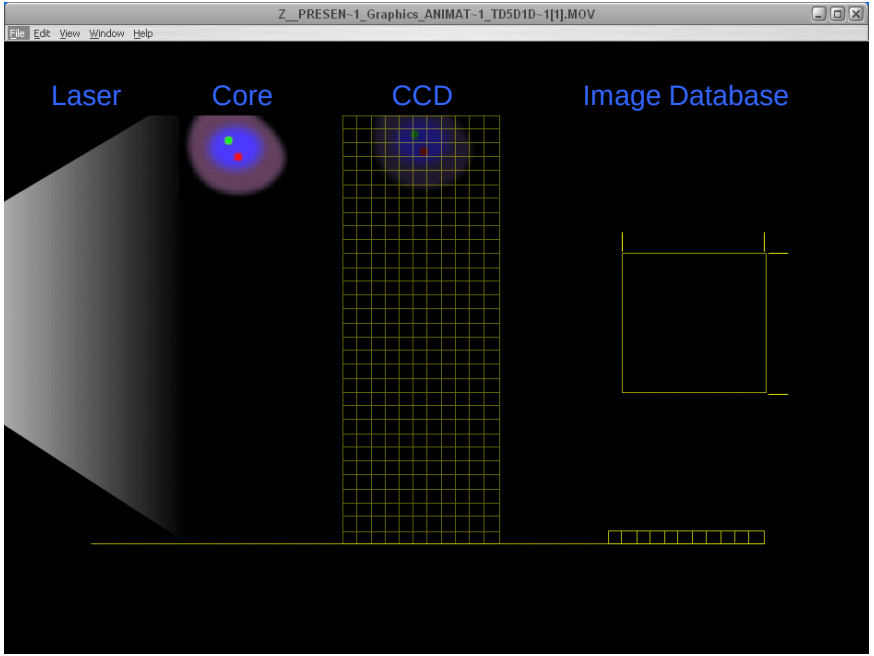
Three different light sources by defualt

- 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

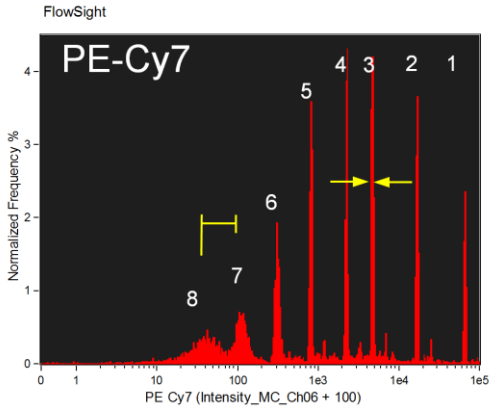


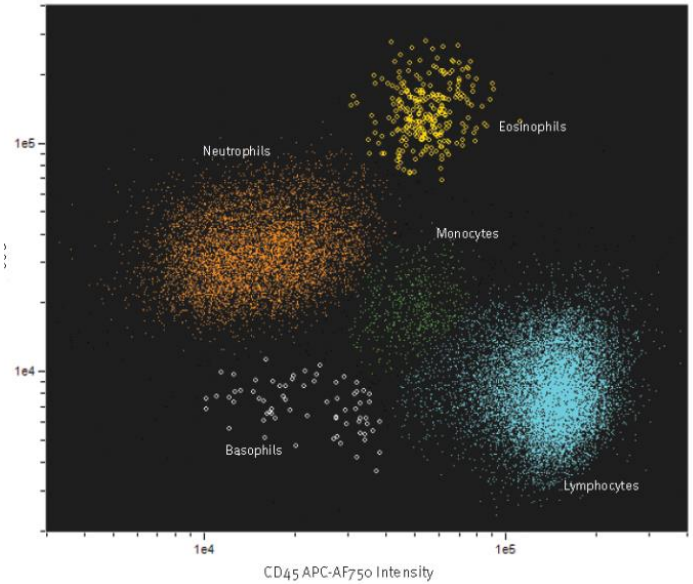
TDI CCD

- 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.

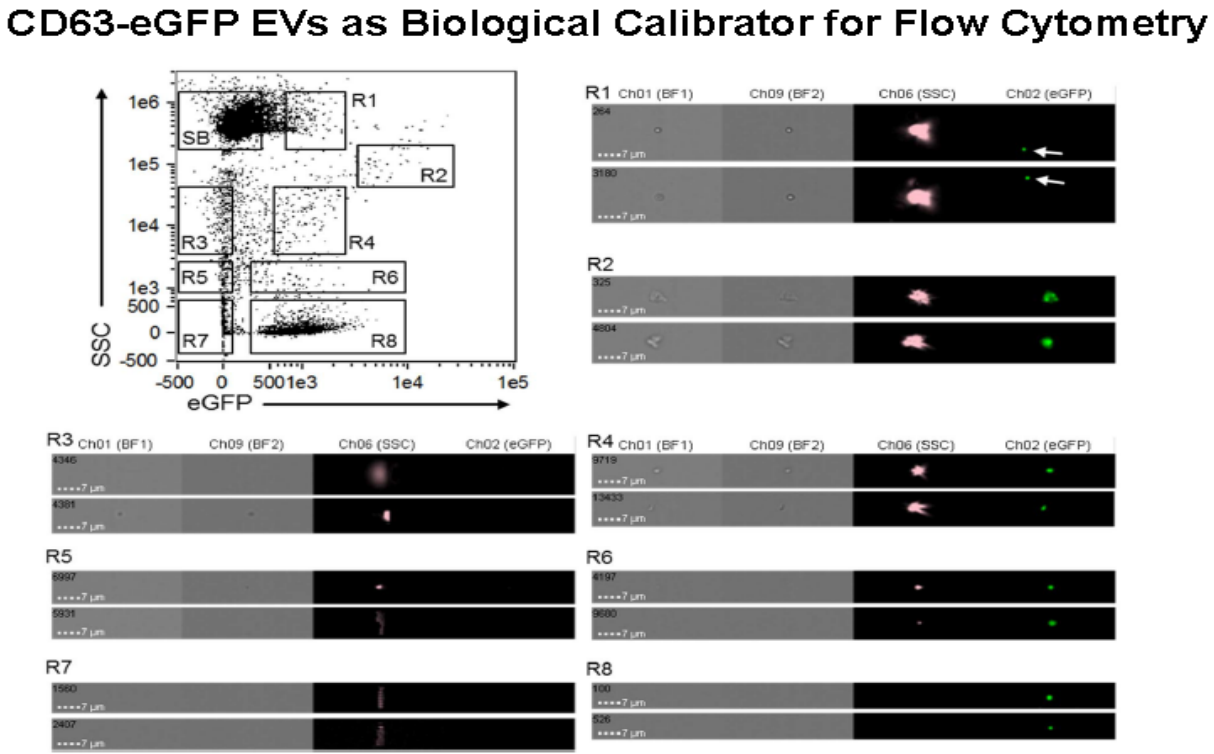
Advantages of Imaging Flow Cytometry

- **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



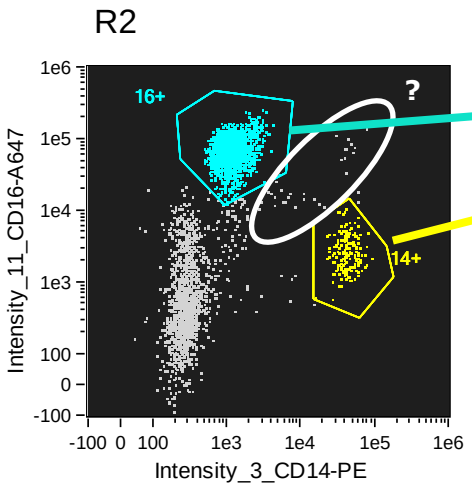


Higher sensitivity allows detection and identification of microvescicles and exosomes much better than conventional cytometry

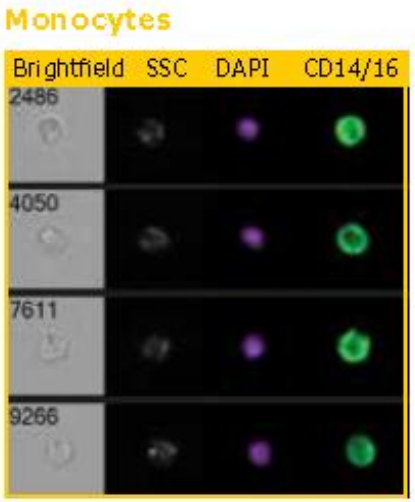


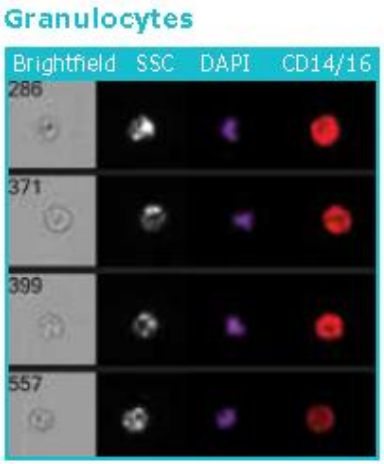
André Gorgens, Essen University Hospital, webinar series, June 22°, 2016

Gating Without Guesswork



CD14+ and CD16+ populations





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

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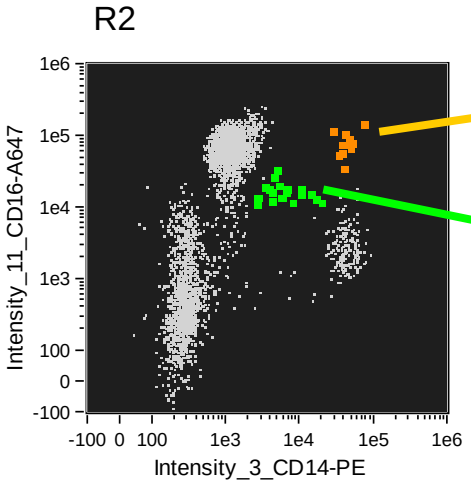
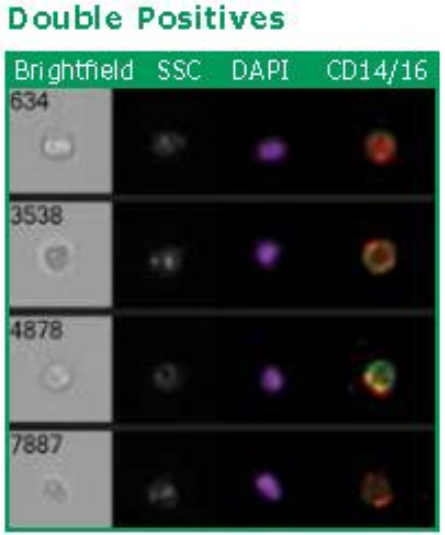
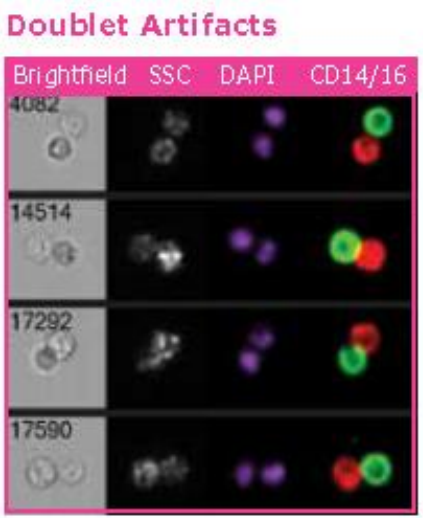
No More “Mystery Populations”



CD14+ CD16+ populations





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

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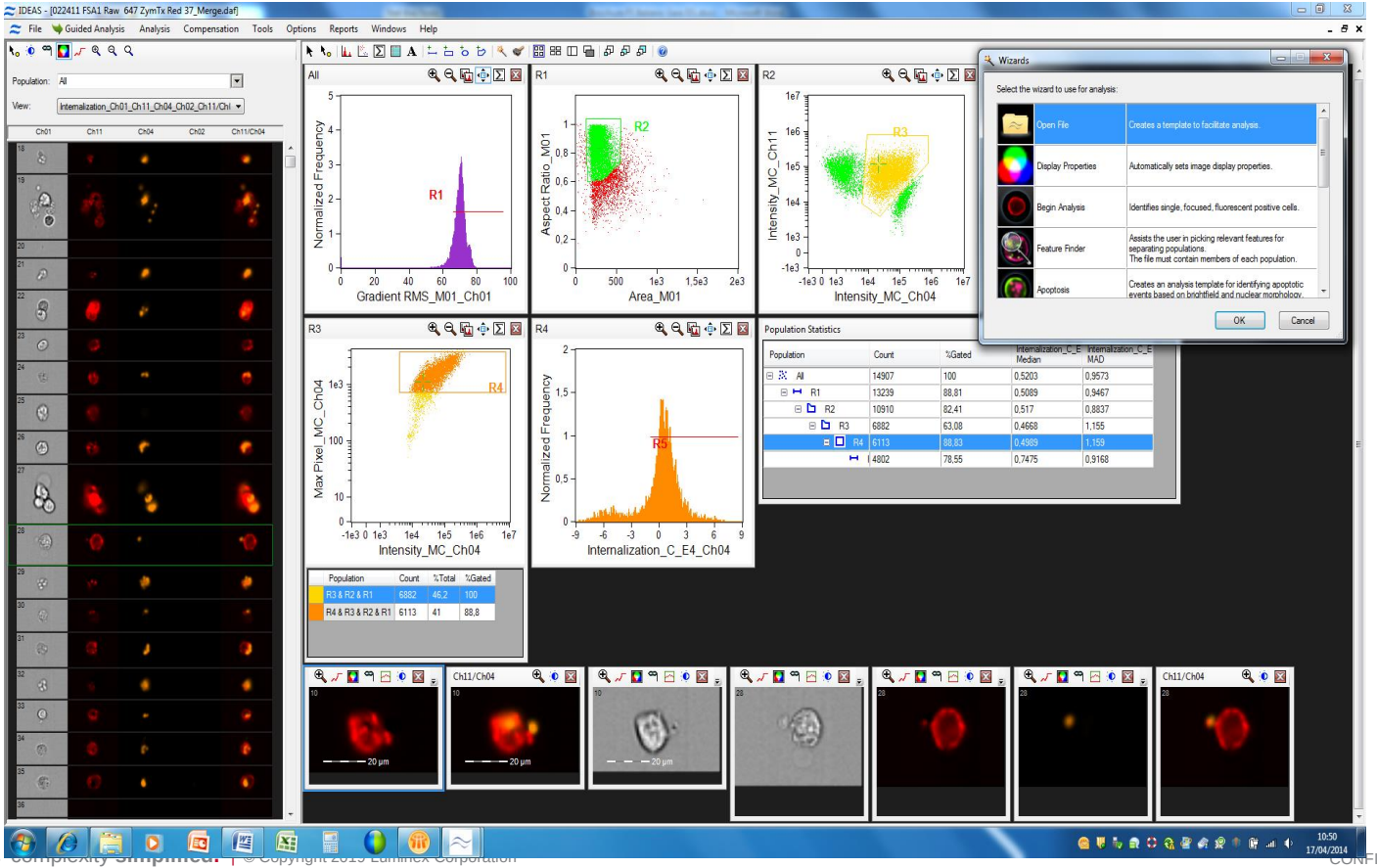
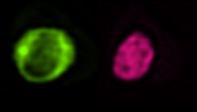
IDEAS SOFTWARE

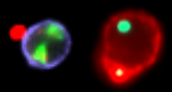


Quantitative Imaging Applications



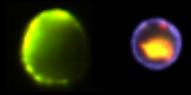
Cell signaling:

NFkB Translocation, HIV induced NFAT, FoxP3 localization



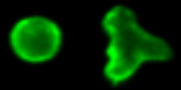
Internalization & phagocytosis

CpGB, Internalization, phagocytosis of Bacteria by monocytes



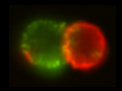
Intracellular co-localization

Ligand colocalization to lysosomes



Shape change & chemotaxis

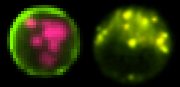
MCP-1 activation of monocytes, Differentiation of FDCP cells



Cell-cell interaction

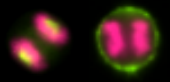
Immune synapse formation, T-cell APC conjugation

Quantitative Imaging Applications



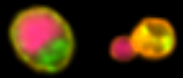
Cell death & autophagy

Apoptosis, nuclear fragmentation, caspase3 activation, LC3 clustering



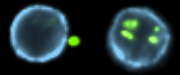
Cell cycle & mitosis

Morphological classification of mitosis



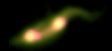
Stem cell biology

Eryithroid differentiation, hematopoiesis



Microbiology

Bacterial phagocytosis in PBMC



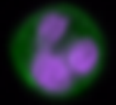
Parasitology

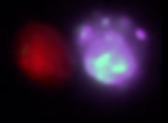
Babesia infection in RBCs, Trypanosomiasis

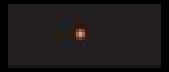
Emerging Applications


RBC Morphology Sickle cell


Sperm Analysis


AML ALL classification

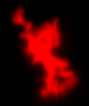

Asymmetric Cell Division


Microparticle quantification


Water Quality Testing / Algae

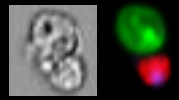

Clinical diagnostics: HPV


Micro-nuclei assay

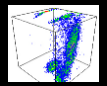

Protein Aggregation


Circulating tumor cell

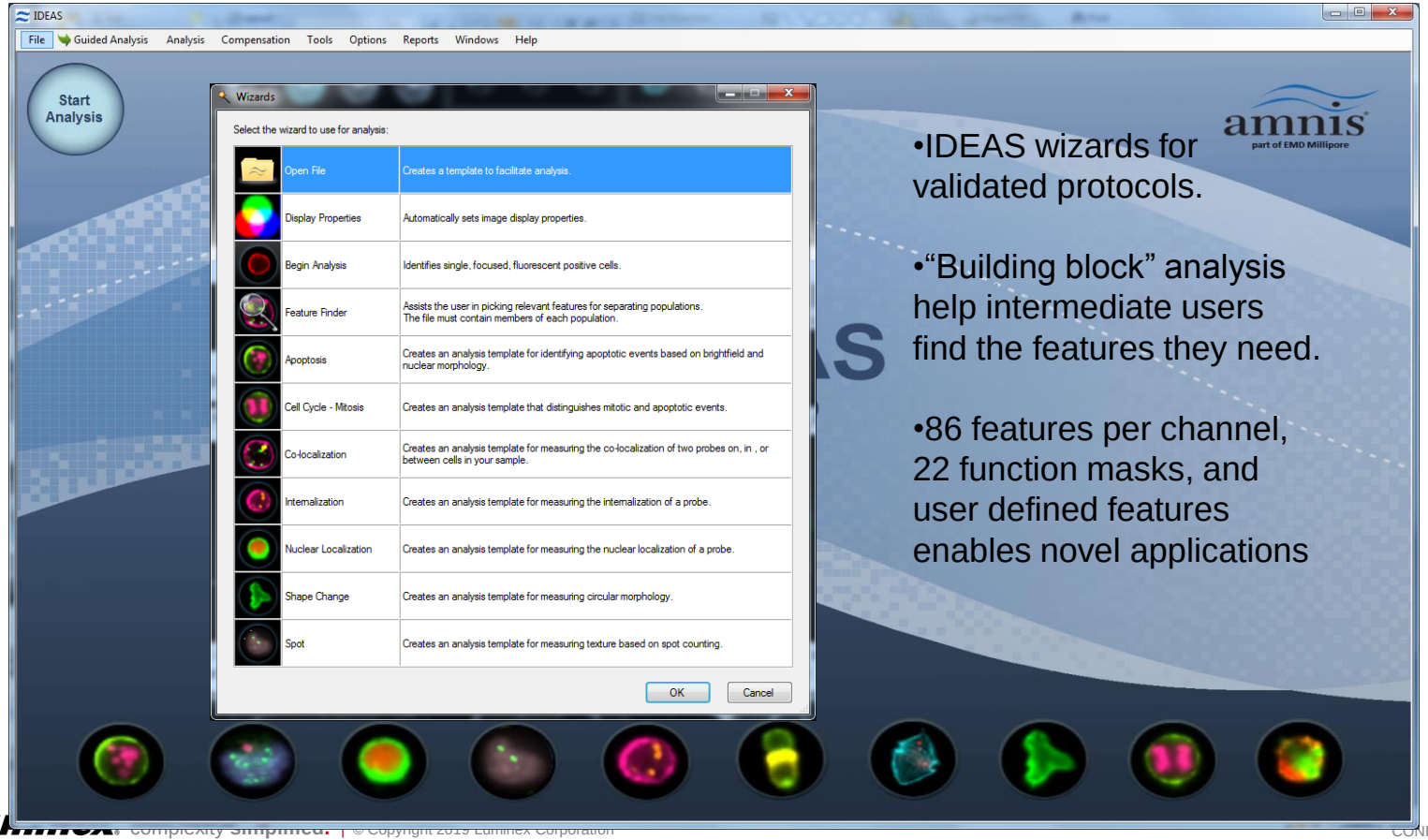

Exosome Internalization


NK Cell Granzime killing


Netosis


CRISPER gene corrections

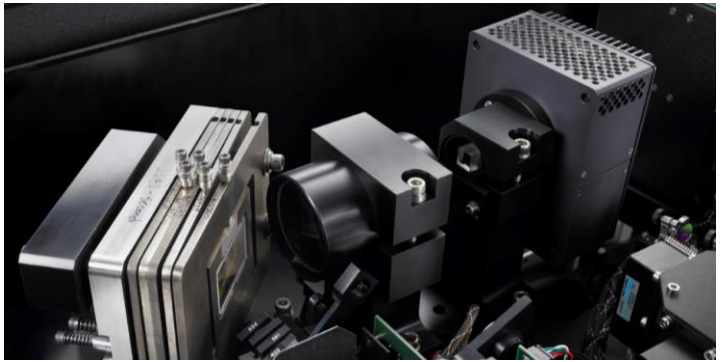
QI Applications overview



- 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.



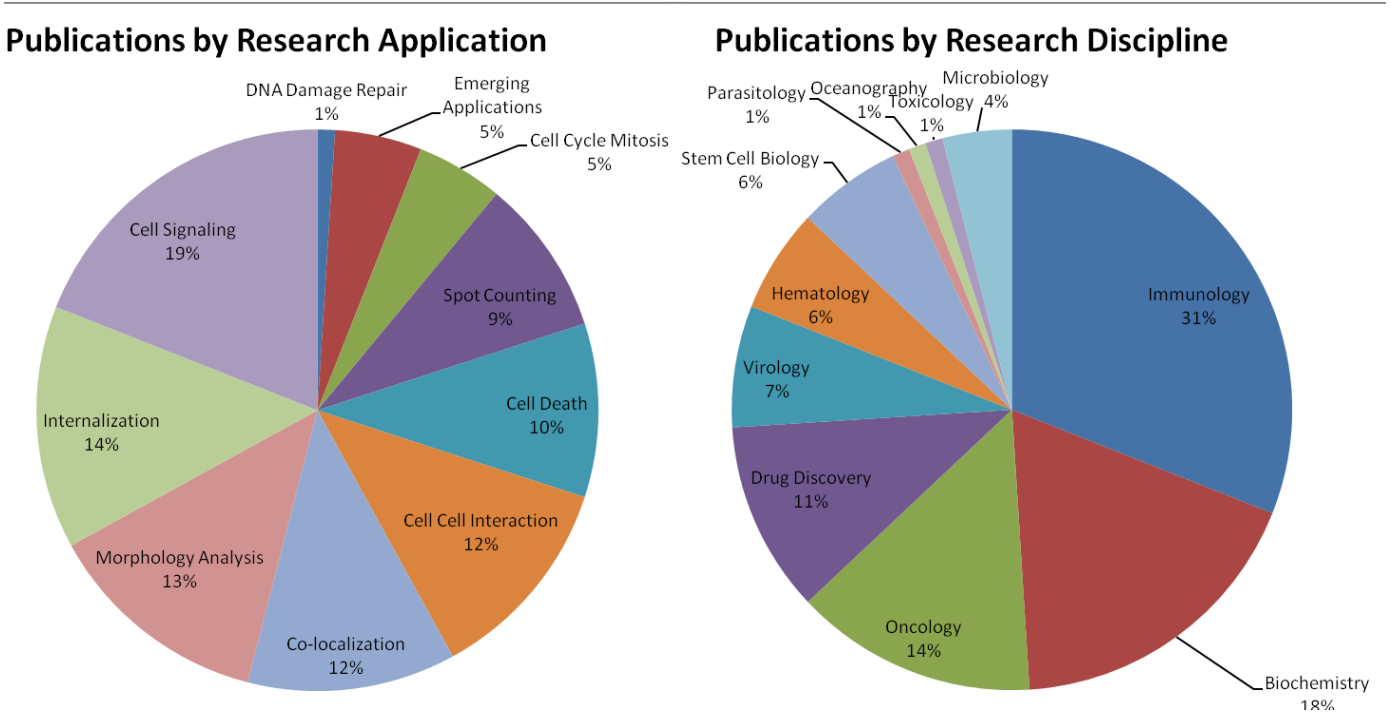
Camera 1, ex.405, 488, 560, 785

Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6
468/76	532/56	578/36	628/64	690/60	760/80
Brightfield	FITC	PE	ECD	PerCP	SSC
	AF488	Cy-3	PE-TxR	Draq5	PE-Cy7
	GFP	AF555	PI	PerCp5	PE-750
	YFP	DS-Red	7AAD	PE-647	SSC
	Syto		PE-610	PE-Cy5	
	SpecGrn			PE-680	

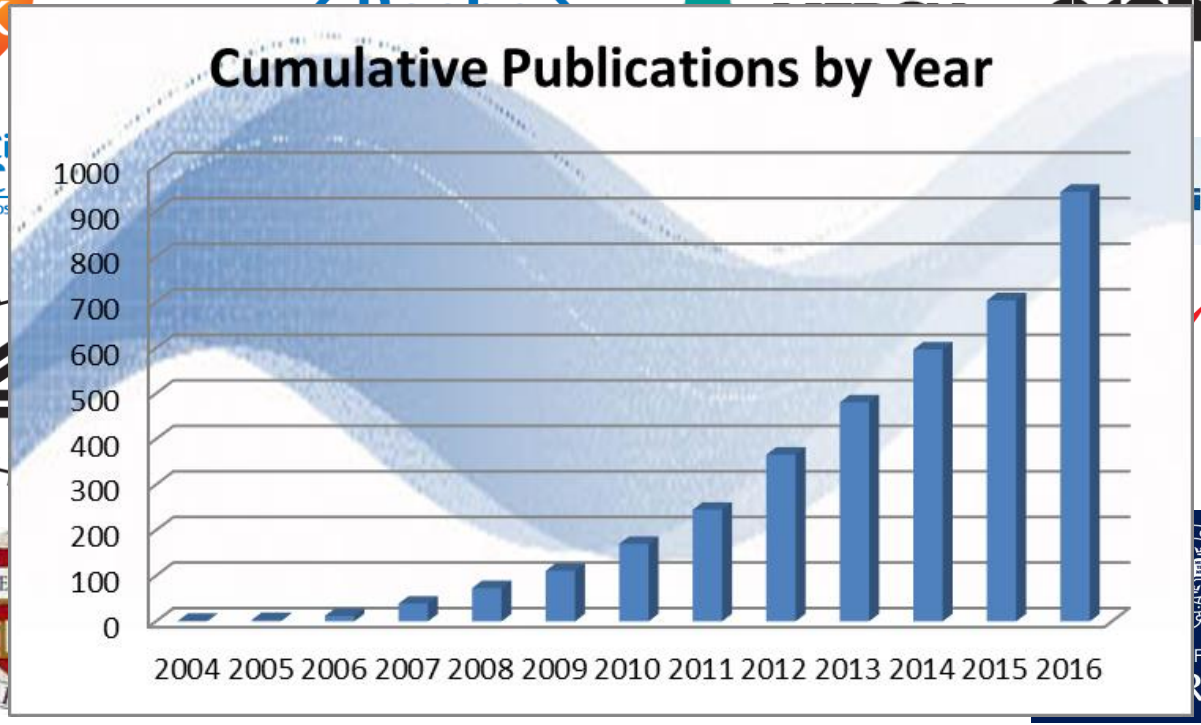
Camera 2, ex 375, 592, 642

Channel 7	Channel 8	Channel 9	Channel 10	Channel11	Channel 12
468/76	532/56	578/36	628/64	690/60	760/80
DAPI	PacOrng	Brightfield	TxR	AF647	APC Cy7
PacBlu	AF430		AF594	AF660	APC AF750
MarBlu			AF568	Cy5	
Hoechest			AF610	APC	
				APC-Cy5.5	

Publications by Research Discipline



Rapid Increase in Publications



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 tedios 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.