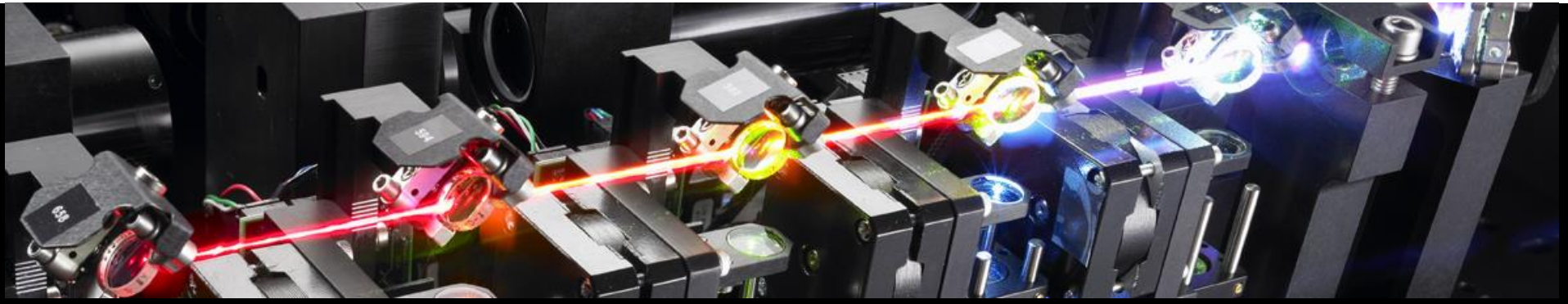
LS&E
Infrastructure Unit

הטכניון
מכון טכנולוגי
לישראל
Technion
Israel Institute
of Technology



Imaging Flow Cytometry (IFC)

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Flow Cytometry Application specialist
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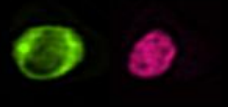
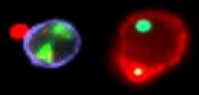
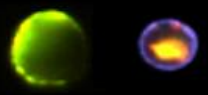
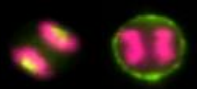
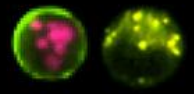
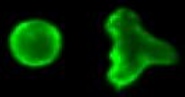
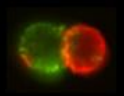
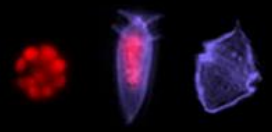
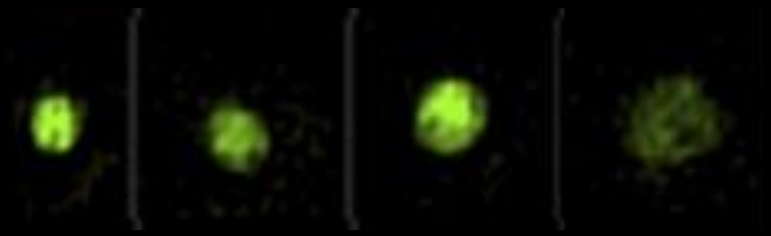
Tel: 04-8293676
LS&E site: https://isu.technion.ac.il/

Imaging Flow Cytometry - Outline

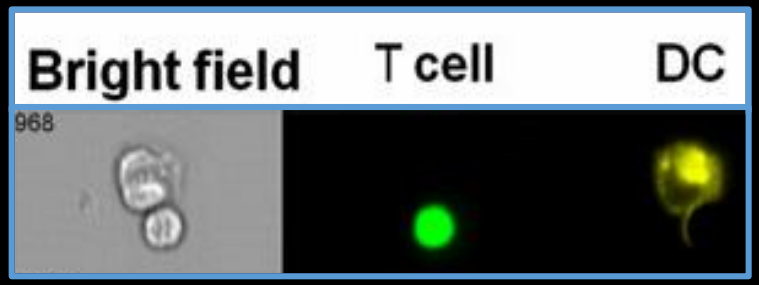
- How it works
- Applications:
  - Cell signaling 
  - Internalization 
  - Co-localization 
  - Microbiology 
  - Cell cycle 
  - Apoptosis 
  - Shape change 
  - Cell-cell interactions 
  - Oceanography 
- Planning your experiment

Imaging Flow Cytometry

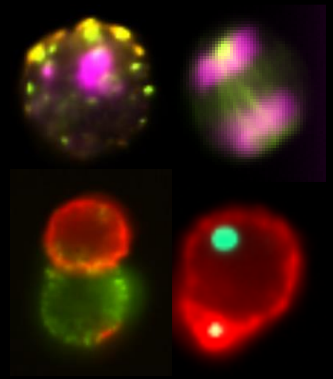
Intensity



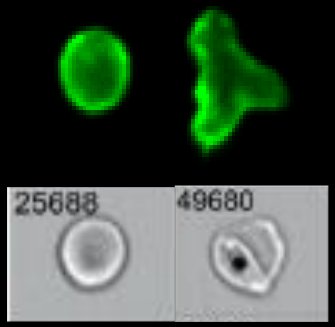
Size



Texture



Shape



ImageStream X mark II – Amnis®

Lasers:

405, 488, 561, 642

6 Channels:

435-505 nm
505-560 nm
560-595 nm
595-642 nm
642-745 nm
745-780 nm

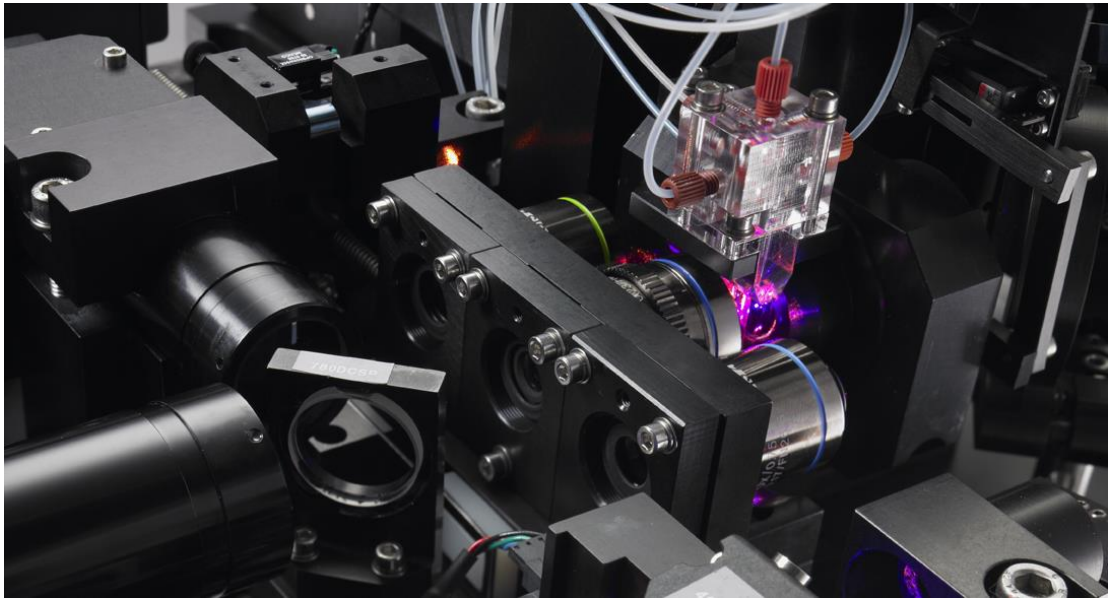
Operation formats:

Eppendorf tubes

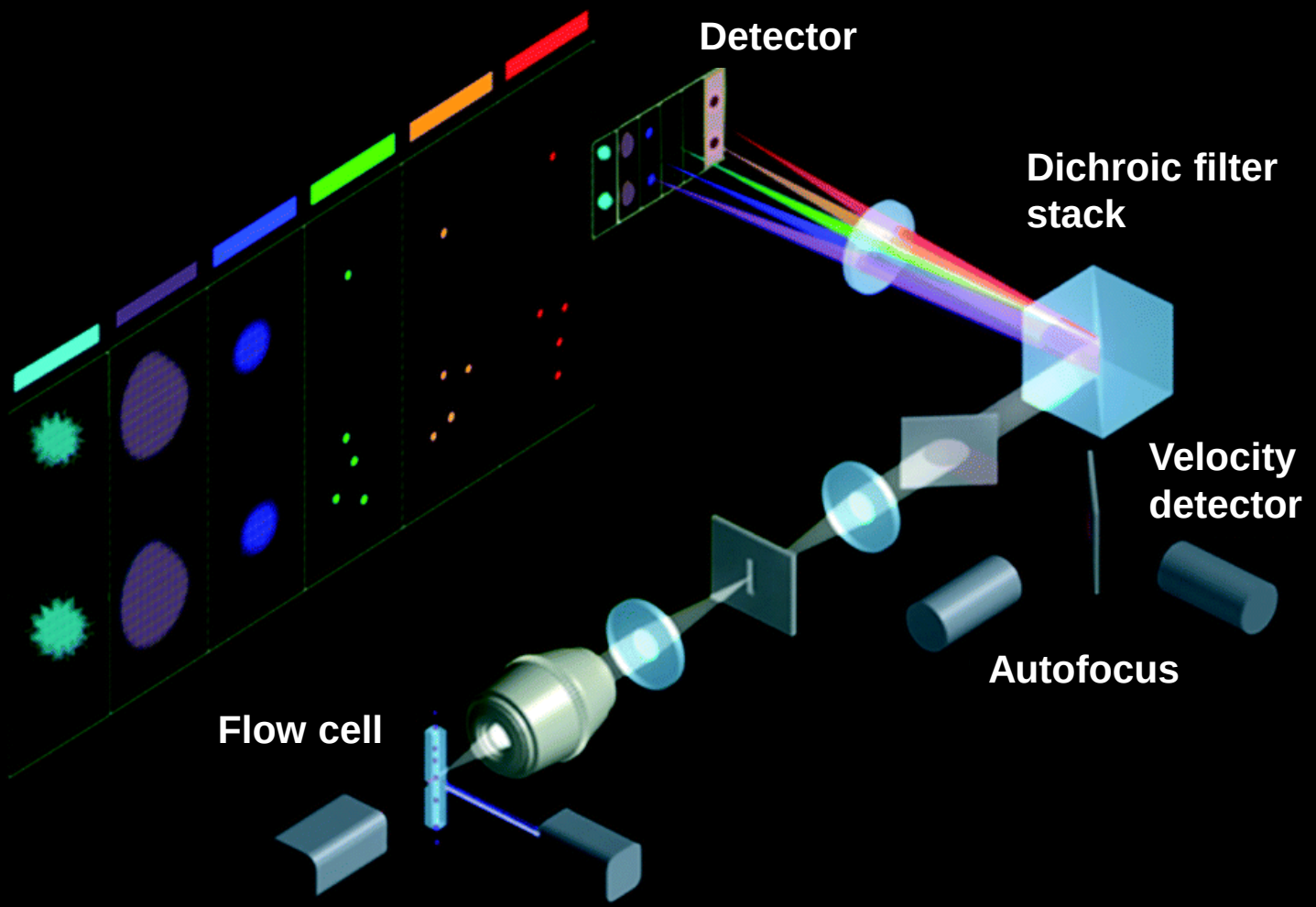
Automated - 96 well plates

ImageStream X mark II – Amnis®

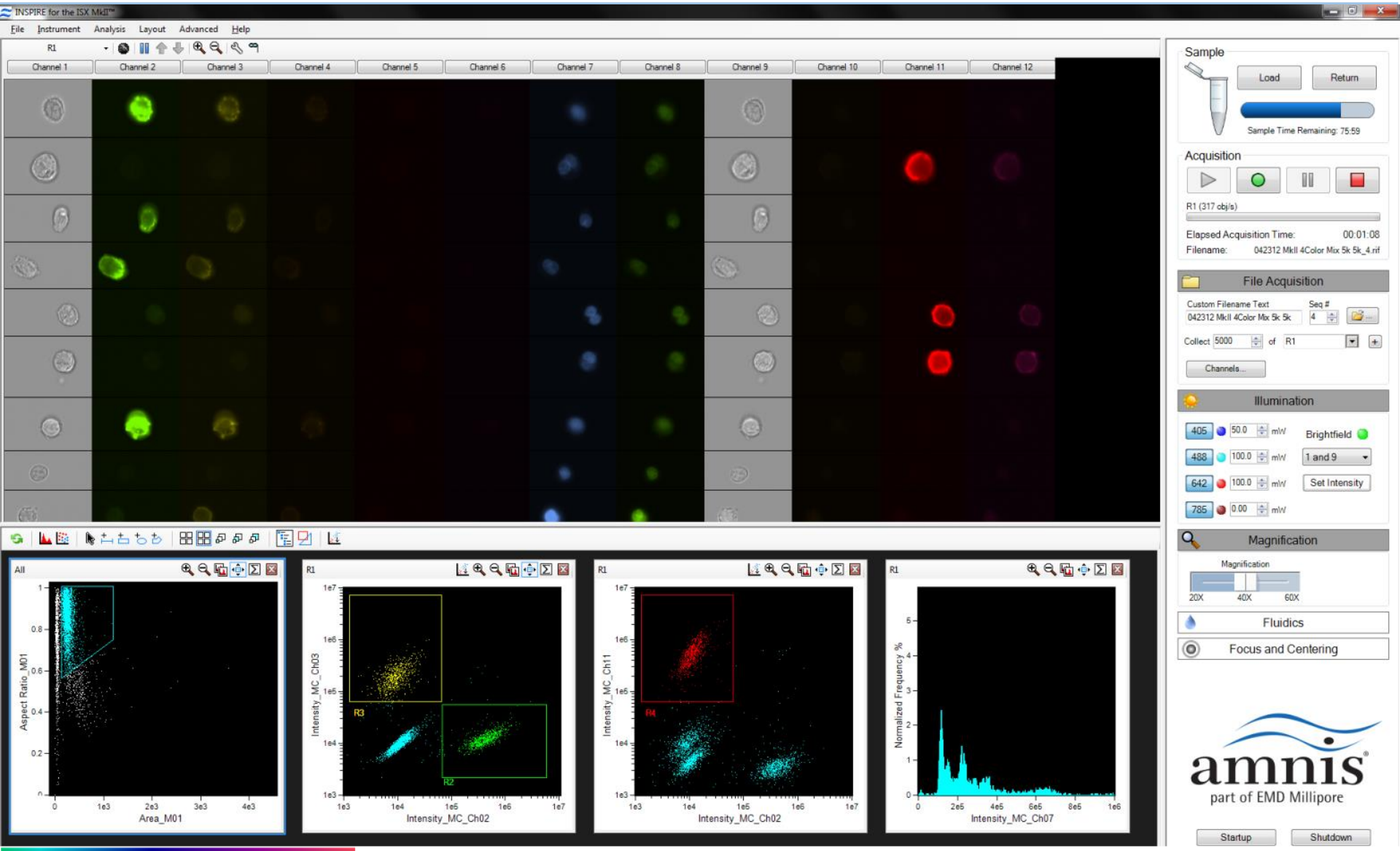
- **40x objective;**
  - 60um field of view
  - 0.5um per pixel
  - 4um depth of field
  - 0.75 NA.
- **20x objective;**
  - 120um field of view
  - 1um per pixel
  - 8um depth of field
  - 0.75NA
- **60x objective;**
  - 40um field of view
  - 0.33um per pixel
  - 2.5um depth of field
  - 0.9NA.



ImageStream – Operation layout

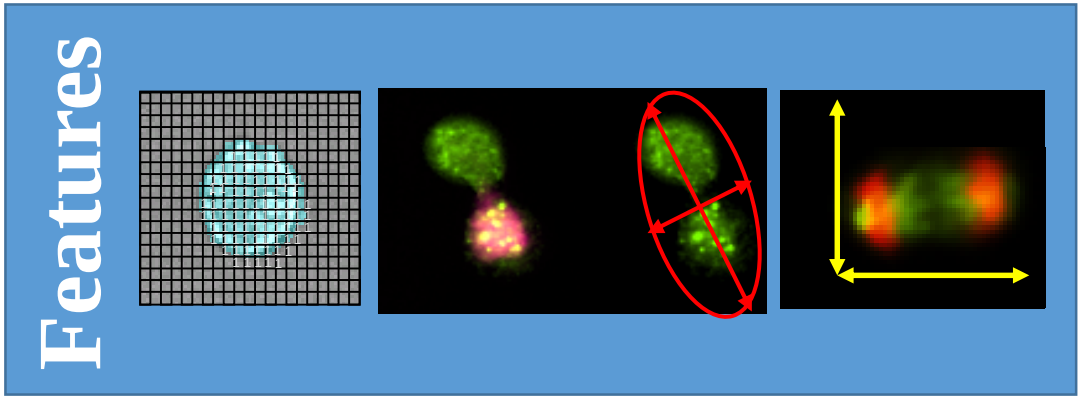


INSPIRE: Instrument Controller



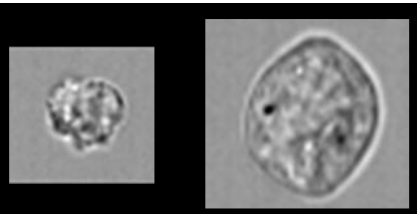
IDEAS® Analysis Software

- High content **morphometric analysis** of tens of thousands of images
- **“Automated feature finder”** quickly finds the best feature for analysis
- **User defined features** for advanced image-based discrimination of cells
- **Application wizards** for validated protocols



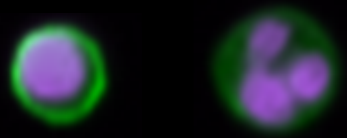
Feature Categories

Size features



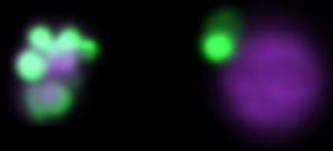
Area; Diameter; Major Axis; Minor Axis; Major Axis Intensity; Minor Axis Intensity; Perimeter; Thickness Max and Min; Spot Area Min; Width; Height; Length

Shape features



Aspect Ratio; Aspect Ratio Intensity; Circularity; Compactness; Elongatedness; Lobe Count; Shape Ratio; Symmetry 2,3,4

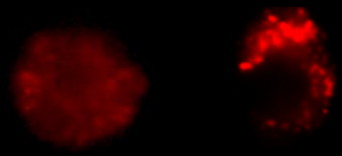
Location features



Angle; Angle Intensity; Centroid X; Centroid Y; Centroid X/Y Intensity; Δ Centroid X; Δ Centroid Y; Δ Centroid XY; Max Contour position; Spot Distance Min; Valley X and Valley Y

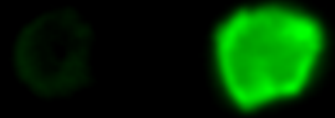
Feature Categories

Texture features



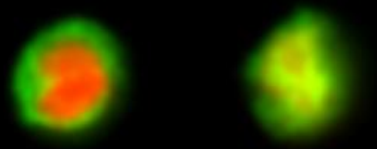
Bright R3; Bright Detail Intensity R7; Contrast; Gradient Max; Gradient RMS; Modulation; Spot Count; StDetail Intensity d thDev; and e Haralick (H) texture features H-Contrast; H-Correlation; H-Energy; H-Entropy; H-Homogeneity; and H-Variance

Signal Strength



Bkgd Mean; Bkgd StdDev; Intensity; Raw Intensity; Raw Max Pixel; Raw Min Pixel; Raw Mean Pixel; Raw Median Pixel; Max Pixel; Min Pixel; Mean Pixel; Median Pixel; Saturation Count; Saturation Percent; Spot Intensity Min

Comparison features



Intensity Concentration Ratio; Internalization; Similarity; Bright Detail Similarity R3

Combined features

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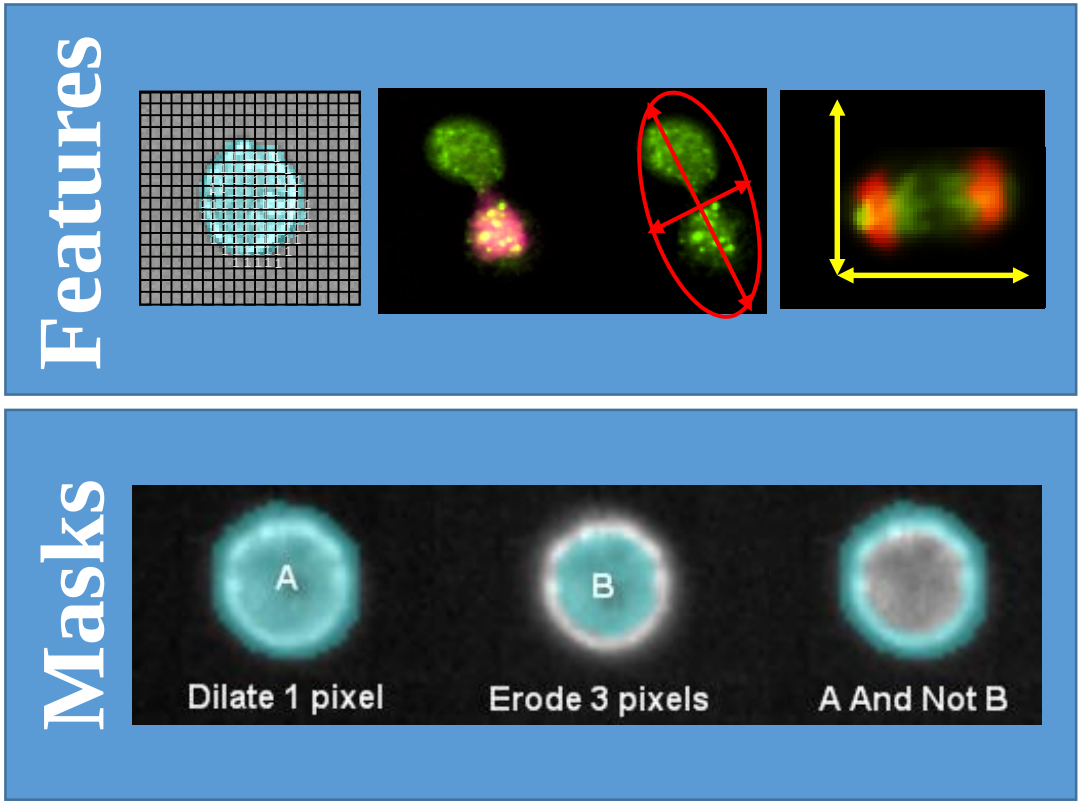
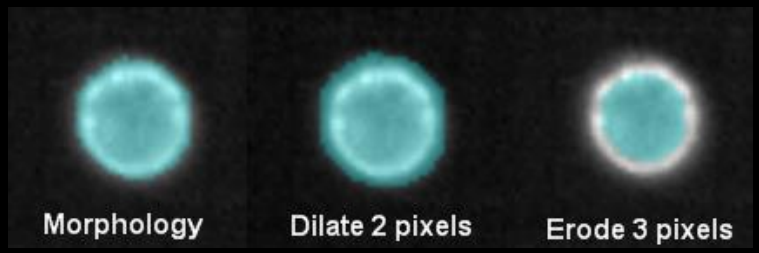
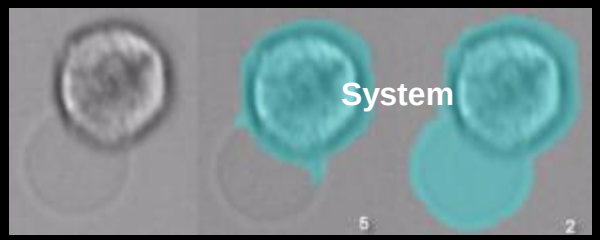
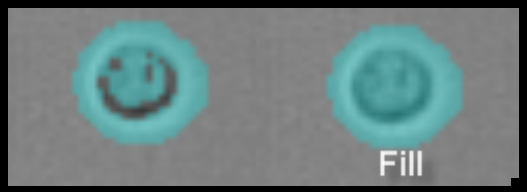
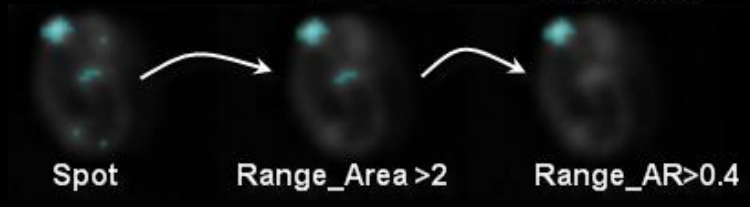


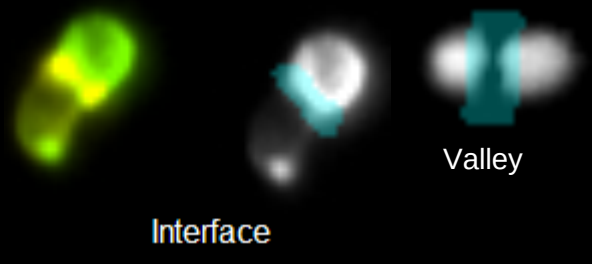
Masks Define a Region of Interest











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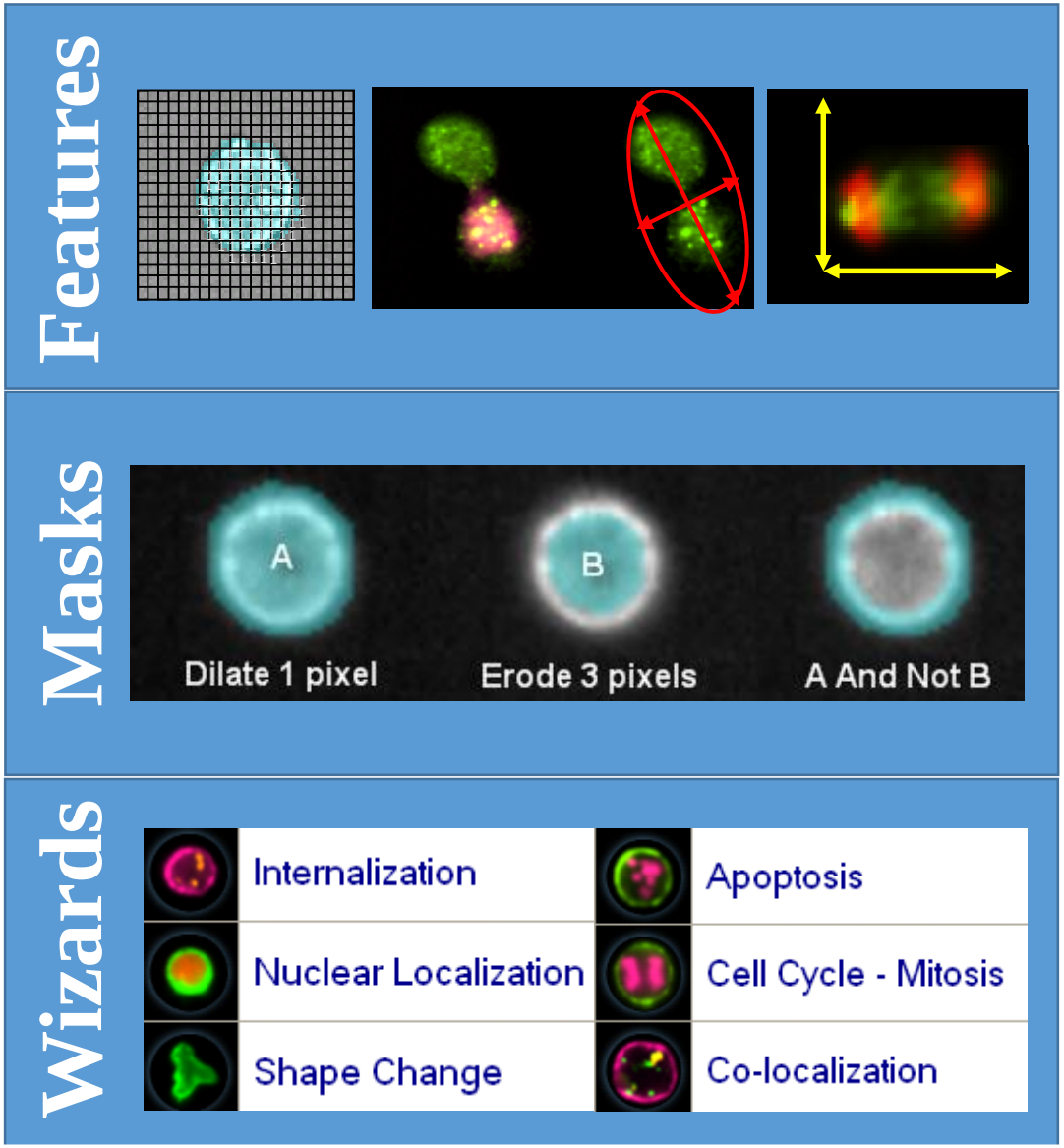
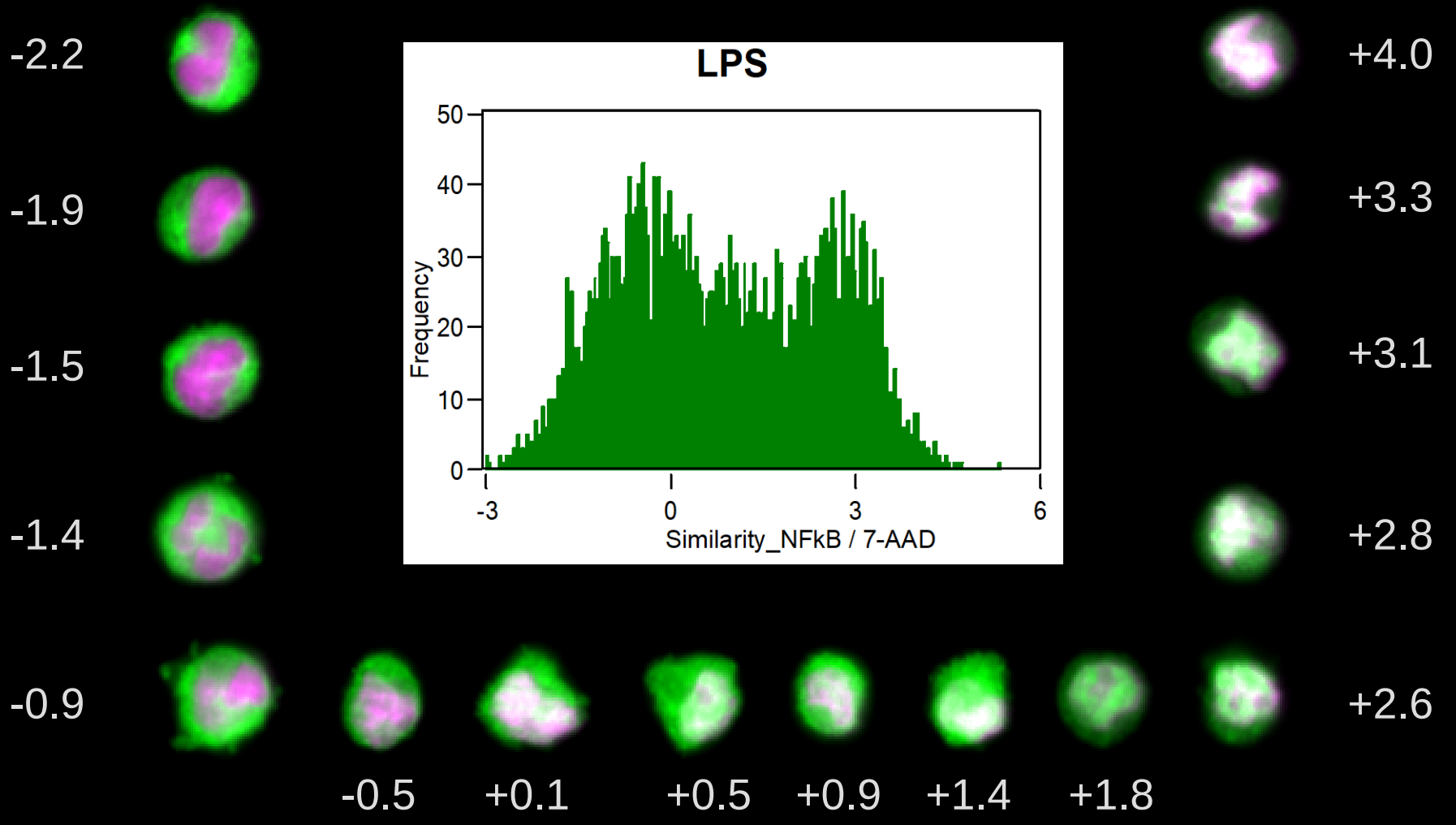


ImageStream Quantitation: How do I measure what I see?



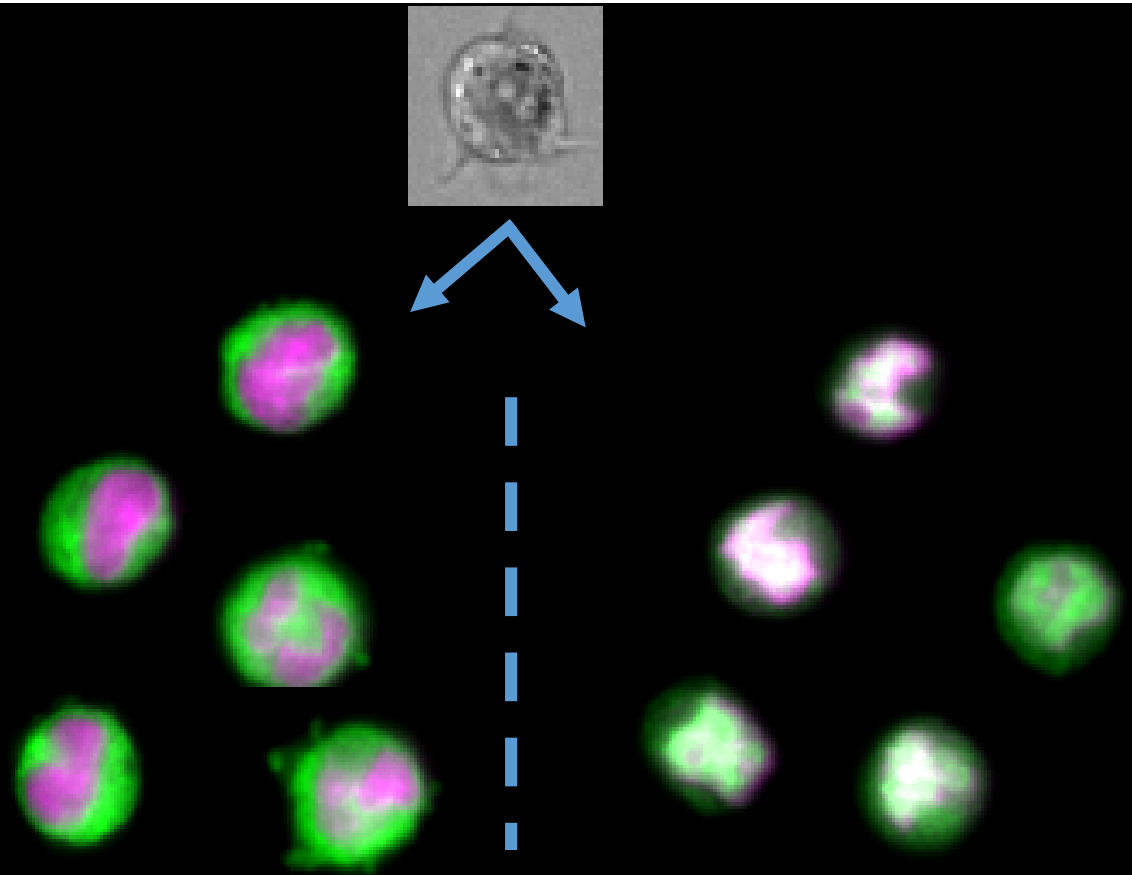
Finding the best translocation analysis

Incubate cells with / without LPS for 1 hr

Fix and perm cells. Label NFkB with FITC and stain nuclei with DRAQ5

Collect large event image files on the ImageStream

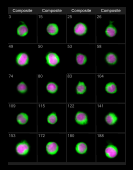
Quantify nuclear translocation of NFkB



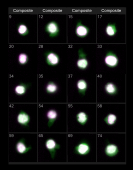
Which feature is best at distinguishing the samples?

Truth sets: untreated vs LPS-treated cells

Untreated



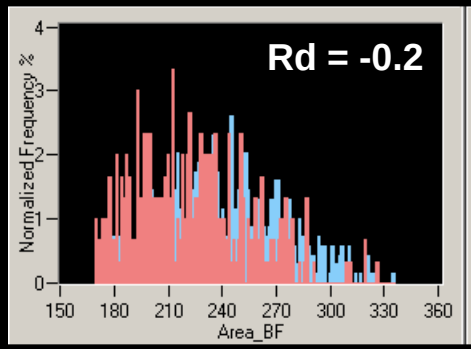
LPS-Treated



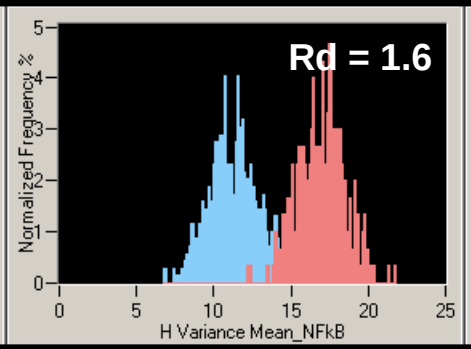
Finding the best translocation analysis

Feature finder wizard helps you find the features for distinguishing the populations.

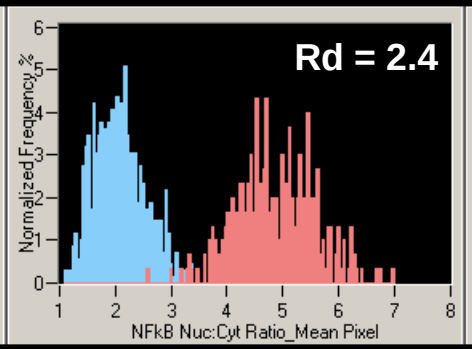
Area_BF



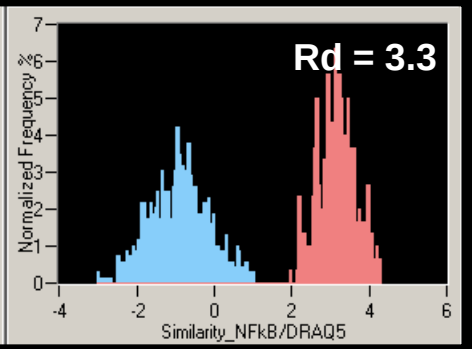
Variance_NFkB



Nuc:Cyt NFkB



Similarity



Feature		Untreated		Positive Control	
Name	Category	Mean	Std. Dev.	Mean	Std. Dev.
Area_Brightfield	Size	237.7	35.0	223.7	32.9
H Variance Mean_NFkB	Texture	11.5	1.8	17.0	1.5
NFkB Nuc:Cyt Ratio_Mean Pixel	Comparison	2.1	0.5	4.9	0.7
Similarity_NFkB/DRAQ5	Comparison	-0.9	0.8	3.1	0.5

Rd - statistical measure of discrimination

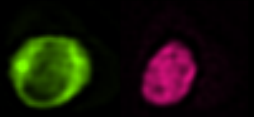
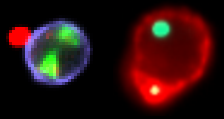
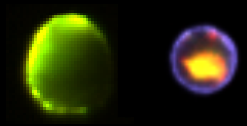
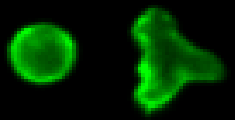
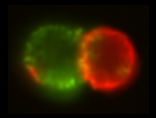
Fisher's Discriminant ratio (Rd)

- Measure of the statistical separation a feature provides between two populations (1&2) using their means and standard deviations:

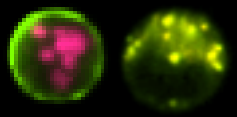
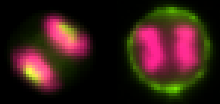
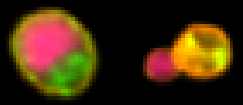
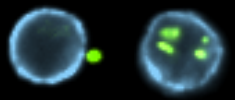
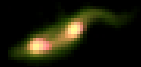
$$Rd = (Mean_1 - Mean_2) / (StdDev_1 + StdDev_2)$$

- Populations 1&2 can be:
  - Different samples
  - Different hand-picked truth sets
  - Same set of cells analyzed with different features

ImageStream – Applications

Field of Study	Example from Imagestream
 Cell signaling	NFkB Translocation, HIV induced NFAT, FoxP3 localization
 Internalization	CpGB, phagocytosis of Bacteria by monocytes
 Co-localization	Ligand colocalization to lysosomes
 Shape change	MCP-1 activation of monocytes, Differentiation of FDCP cells
 Cell-cell interaction	Immune synapse formation, NFkB activation from T-cell APC conjugation

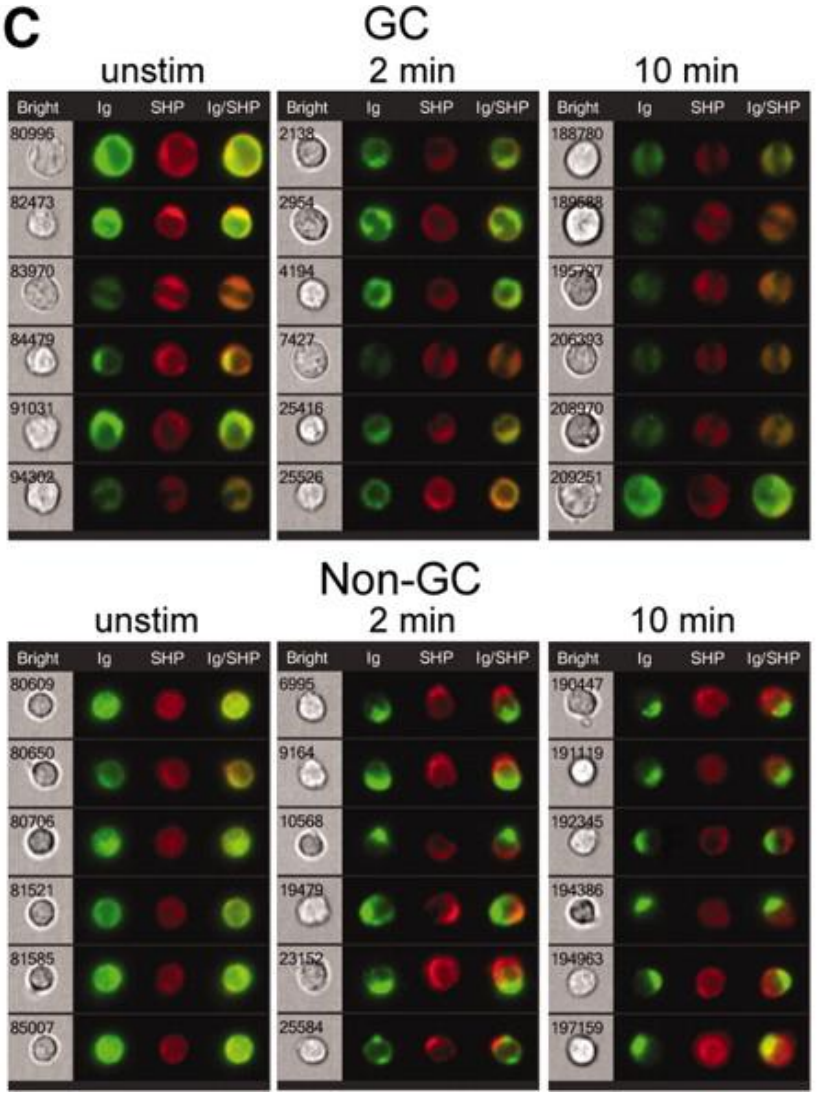
ImageStream – Applications

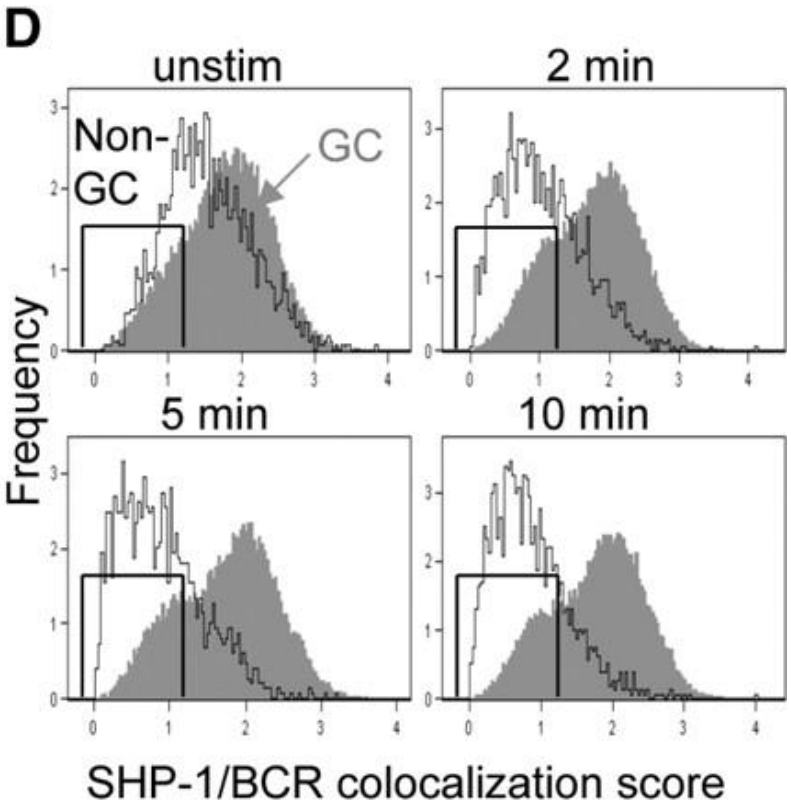
Field of Study	Example from Imagestream
 Cell death & autophagy	Apoptosis, nuclear fragmentation, caspase3 activation
 Cell cycle & mitosis	Morphological classification of mitosis
 Stem cell biology	Erythroid differentiation
 Microbiology	Bacterial phagocytosis in PBMC
 Parasitology	Babesia infection in RBCs

Cell Signaling

LS&E Infrastructure Unit

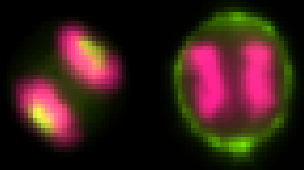
B cell receptor signal transduction





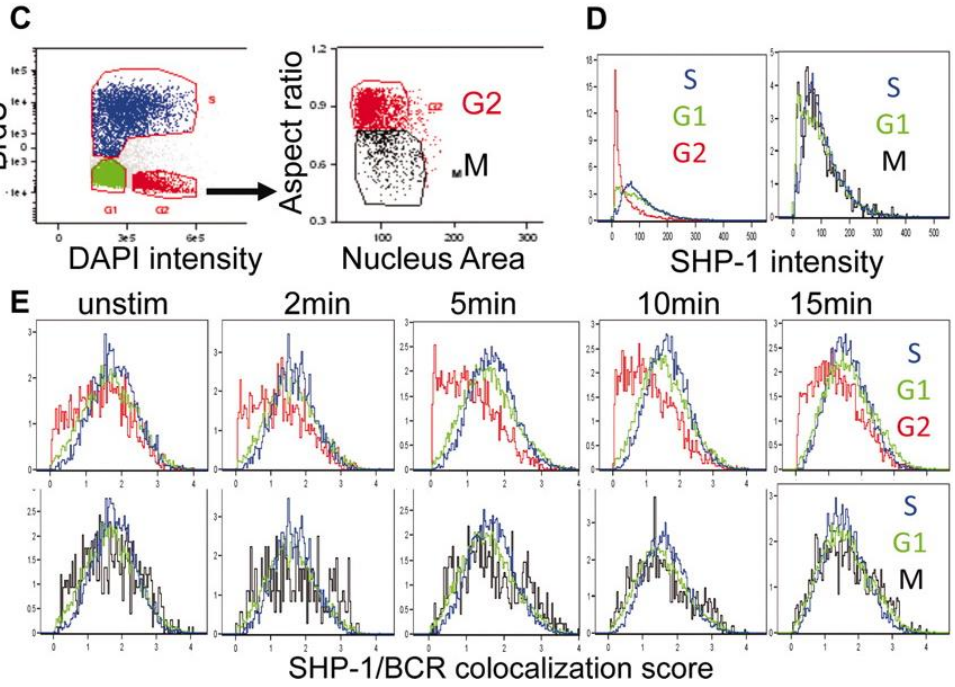
Khaliol A.M. (2012), *Science*

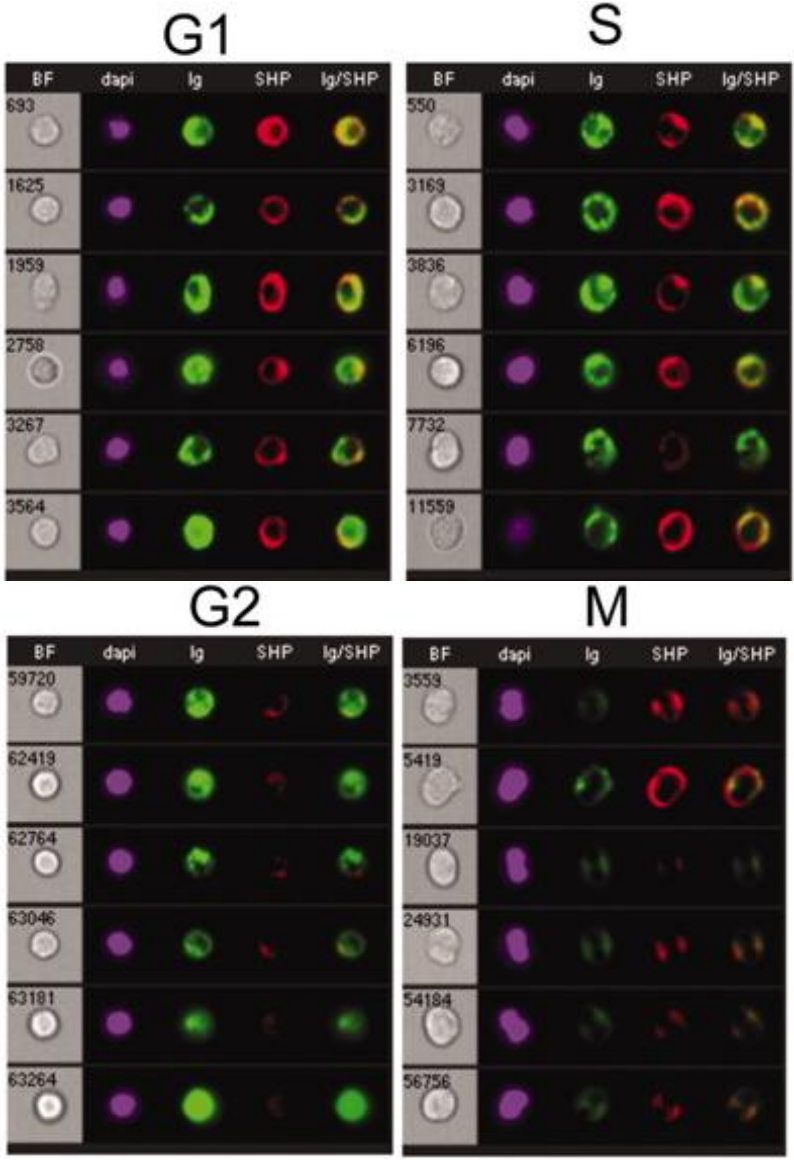
Cell Cycle



LS&E Infrastructure Unit

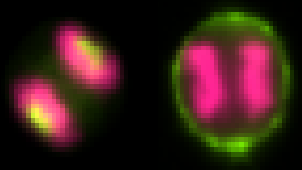
B cell receptor signal transduction





Khaliol A.M. (2012), *Science*

Cell Cycle



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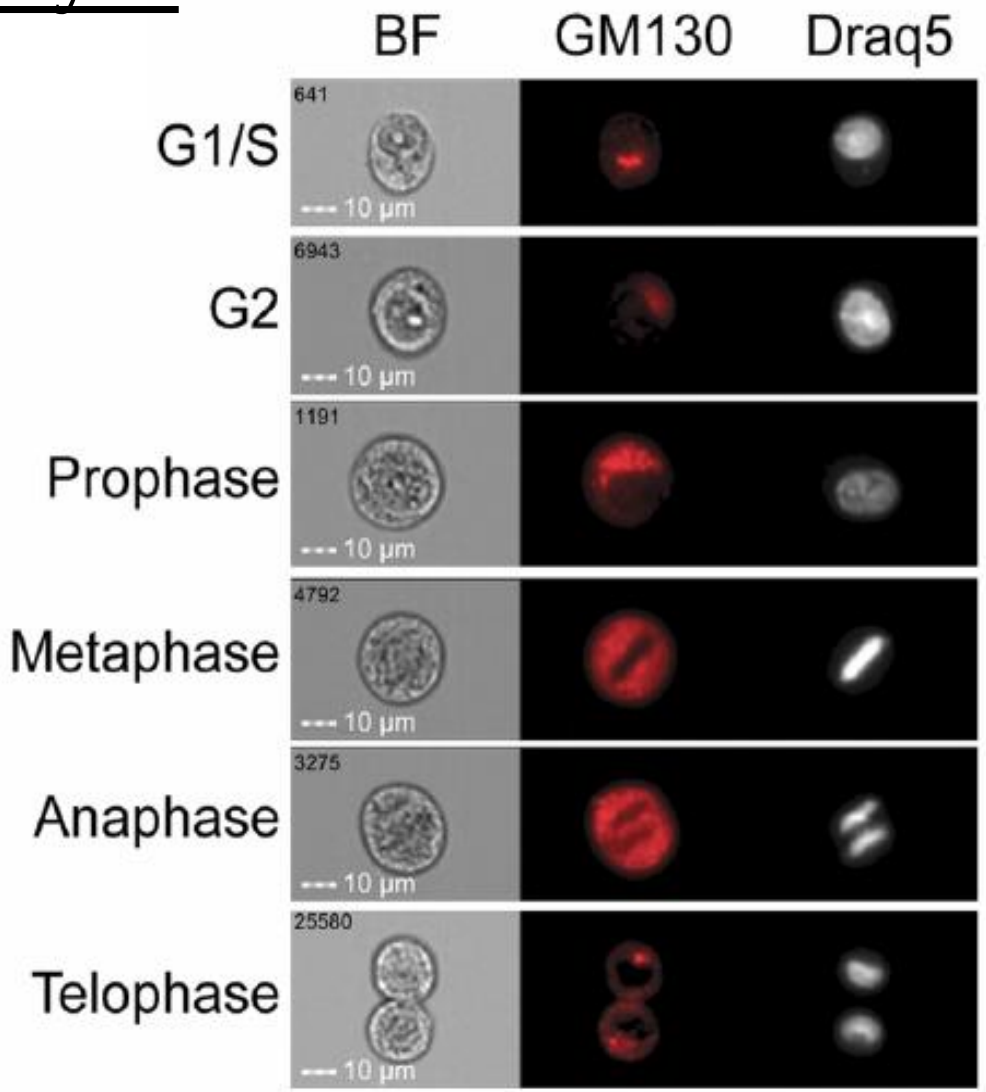
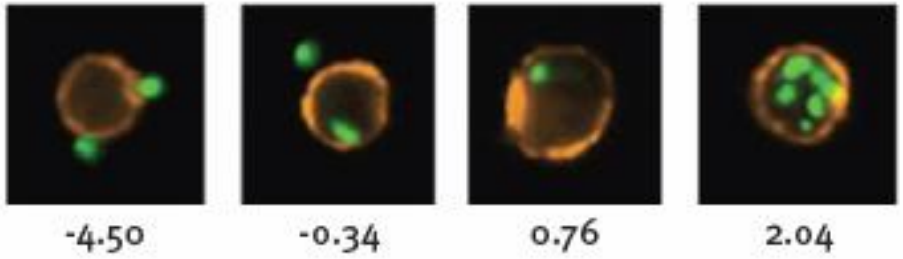
Golgi structure during cell cycle



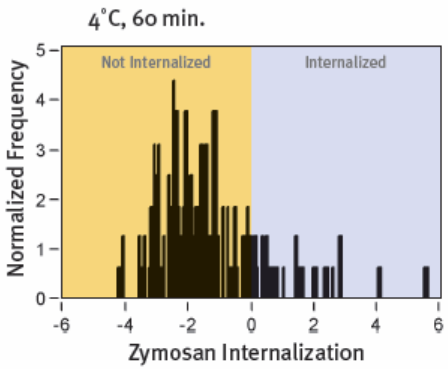
Wortzel I. (2017), *Sci. Rep.*

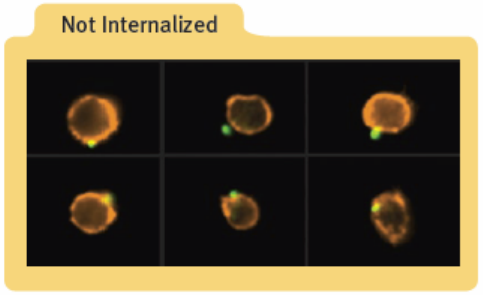
Internalization

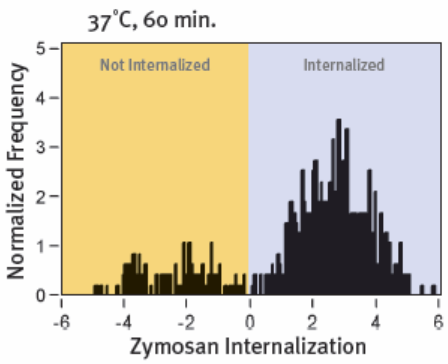


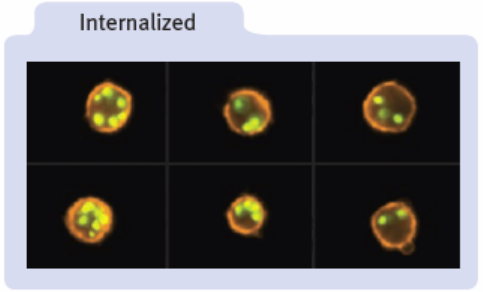
Internalization score

Phagocytosis by murine macrophages



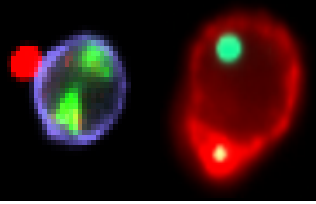




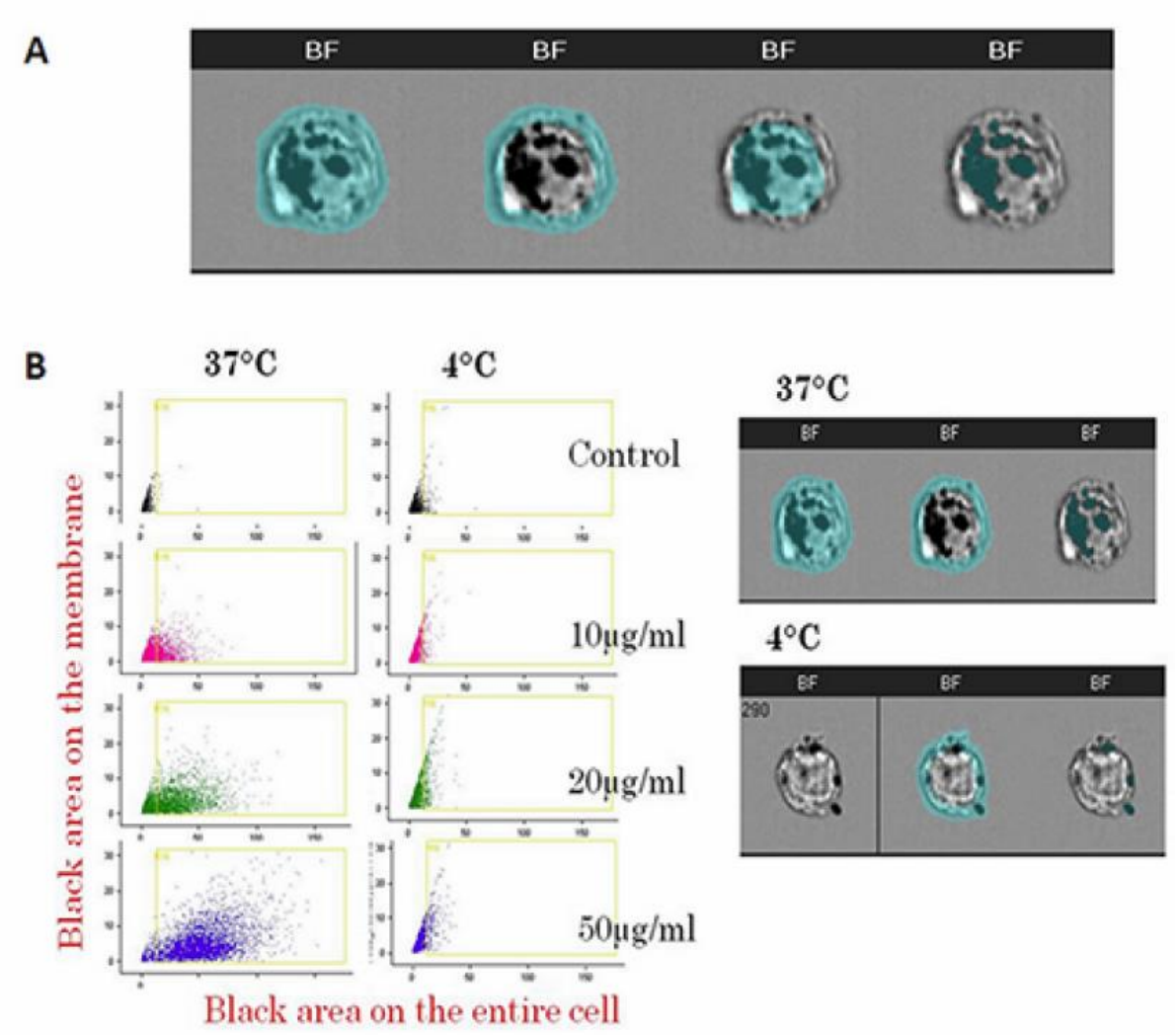


Phagocytosis of FITC-labeled Zymosan particles (green) by RAW cells (orange)

Internalization

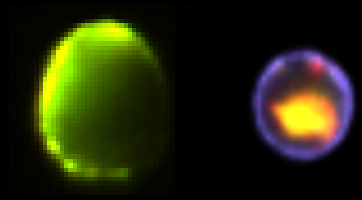


Carbon nanotubes uptake



Marangon I. (2013), *JOVE*

Co-localization



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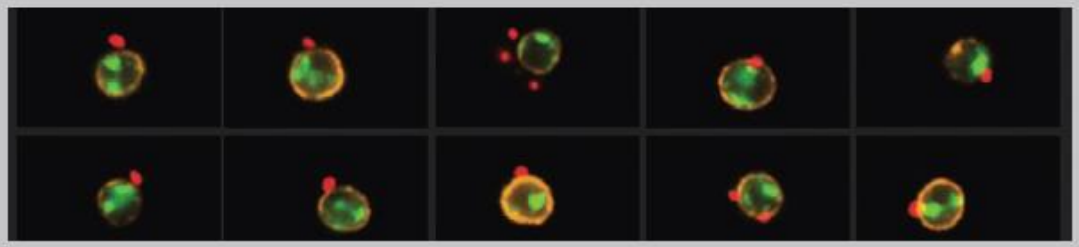
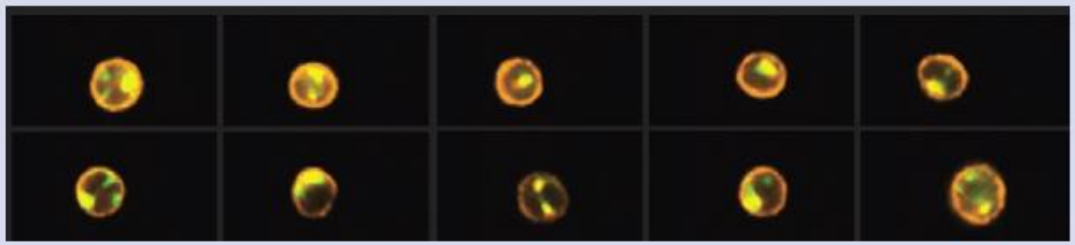
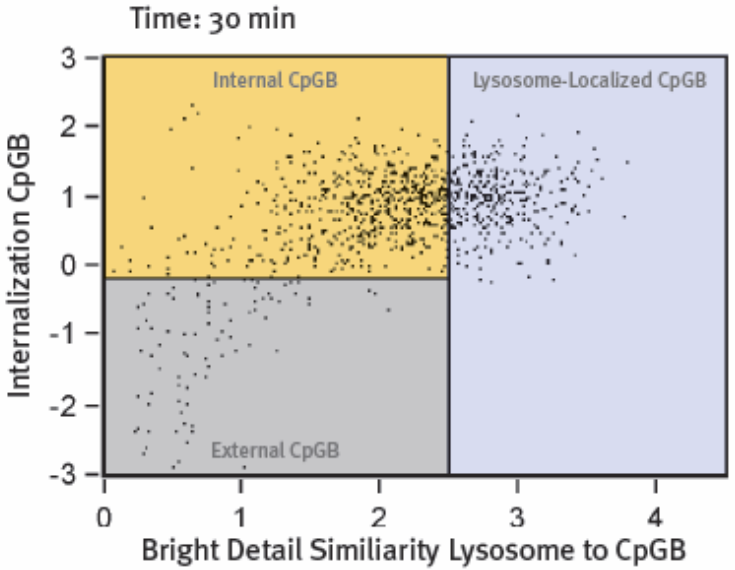
External CpGB



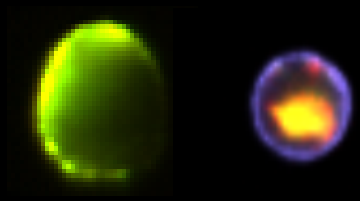
Internal CpGB

Lysosome-Localized CpGB



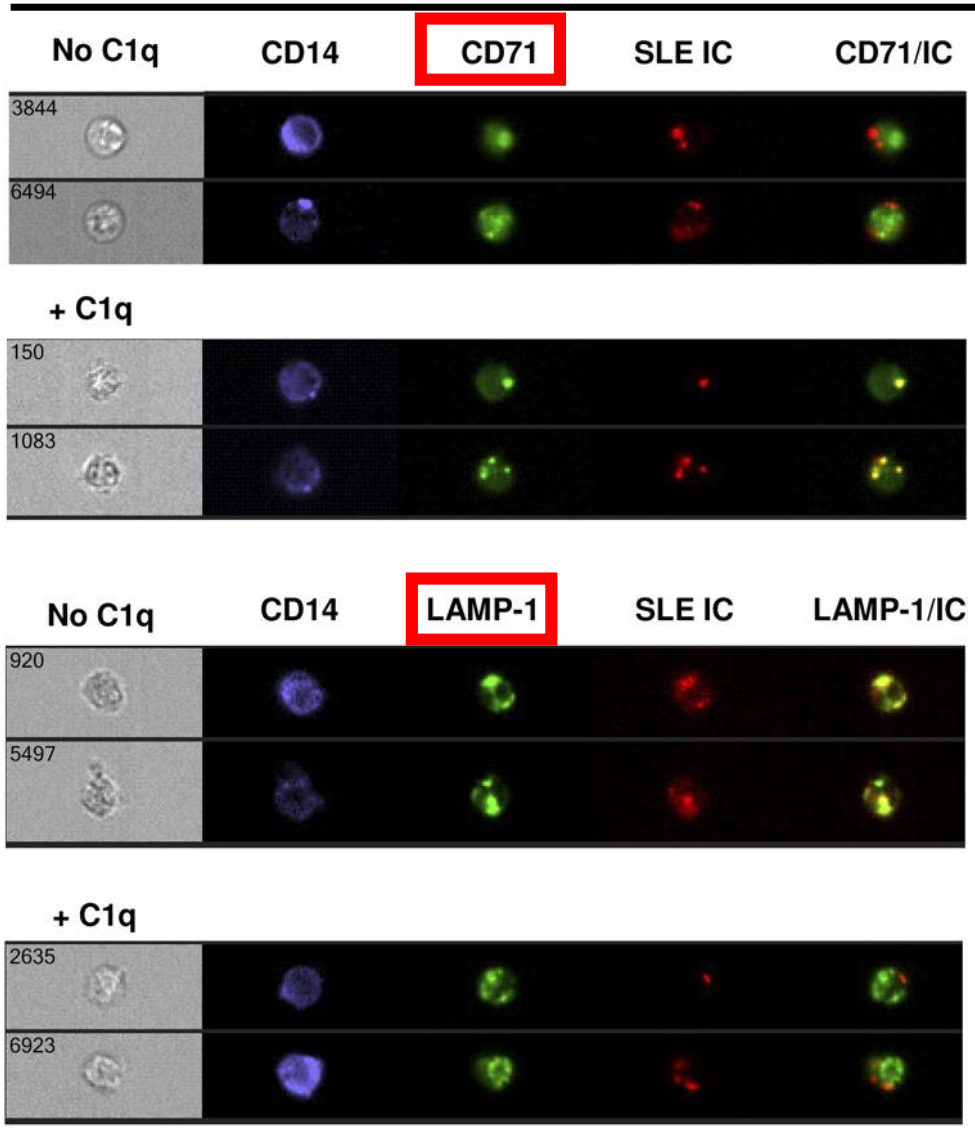


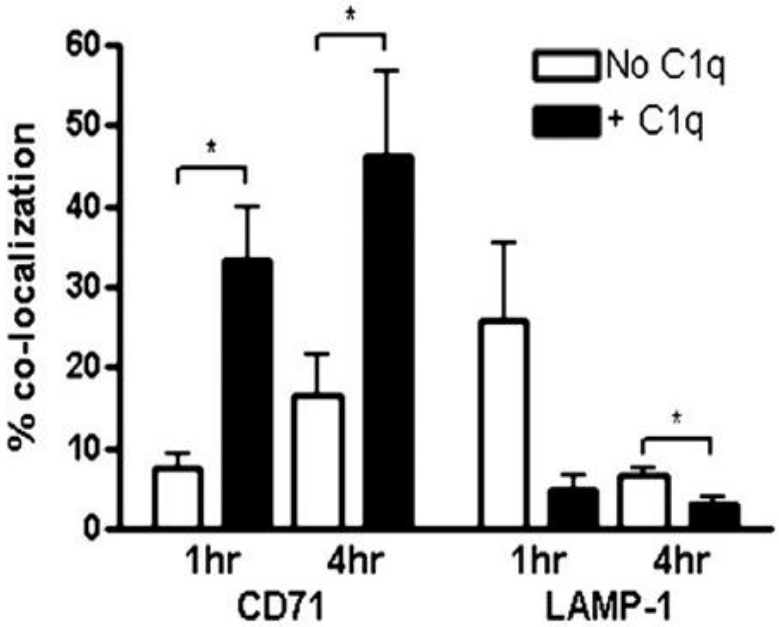
Co-localization



LS&E Infrastructure Unit

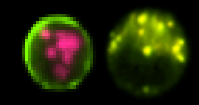
Immune cluster localization within monocytes





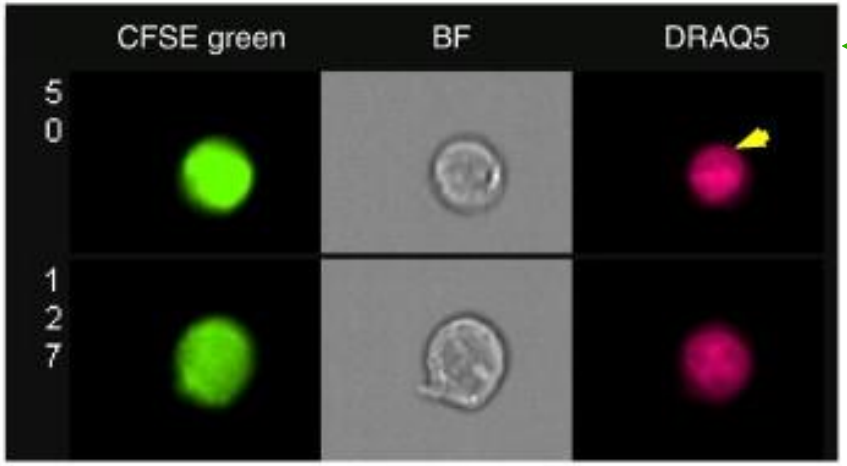
Santer D.M. (2010), *The J. of Immunology*

Cell death & Autophagy

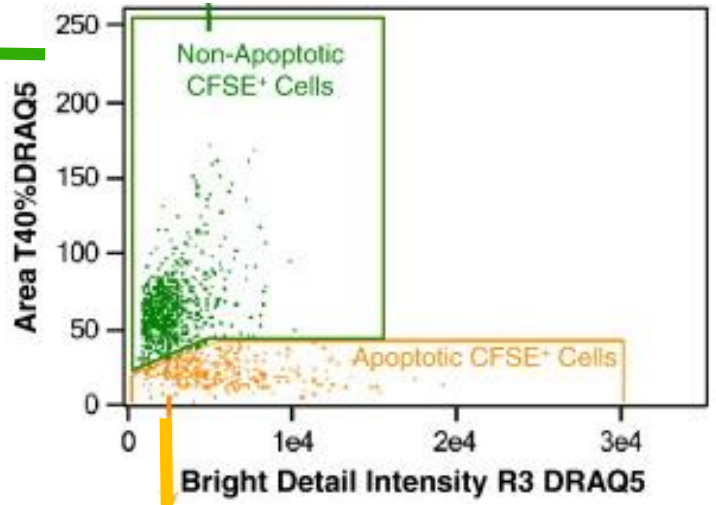


LS&E Infrastructure Unit

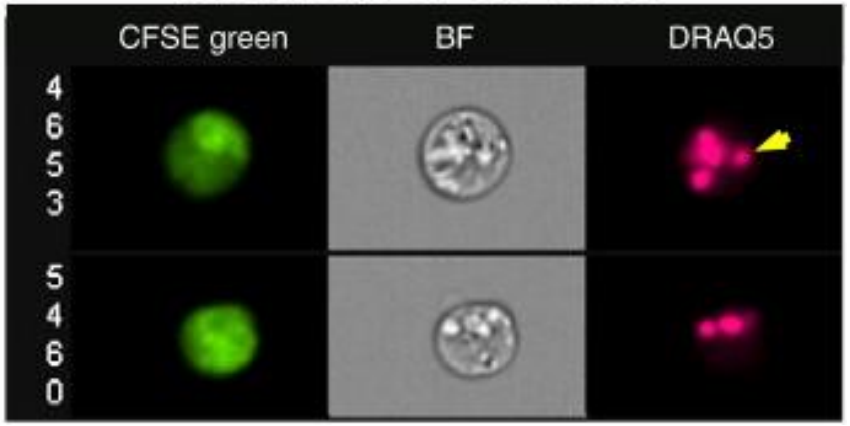
Ab Dependent Cell mediated Cytotoxicity (ADCC) activity



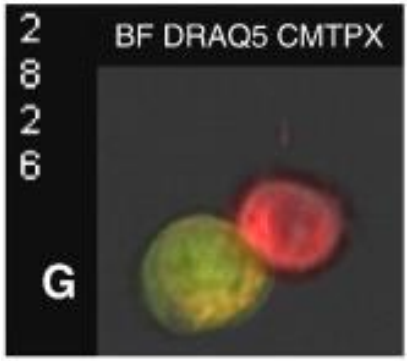
Non-Apoptotic CFSE⁺ Cells



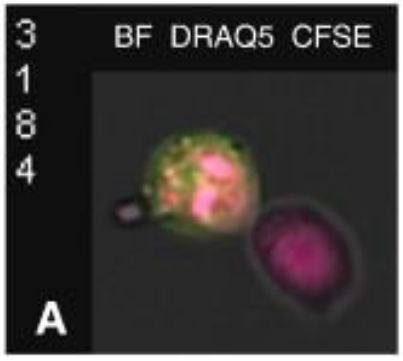
Apoptotic CFSE⁺ Cells



Non-apoptotic Target Cell

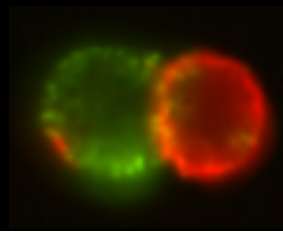


Apoptotic Target Cell



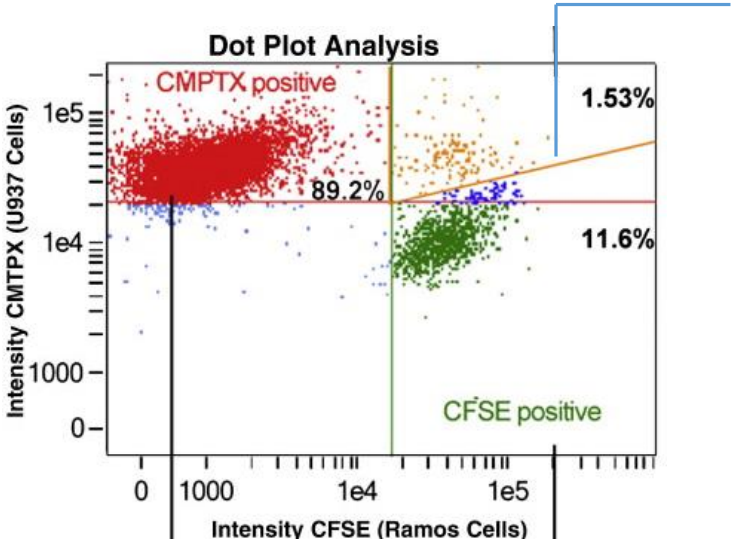
Helguera G. (2011), *J. of Immuno Methods*

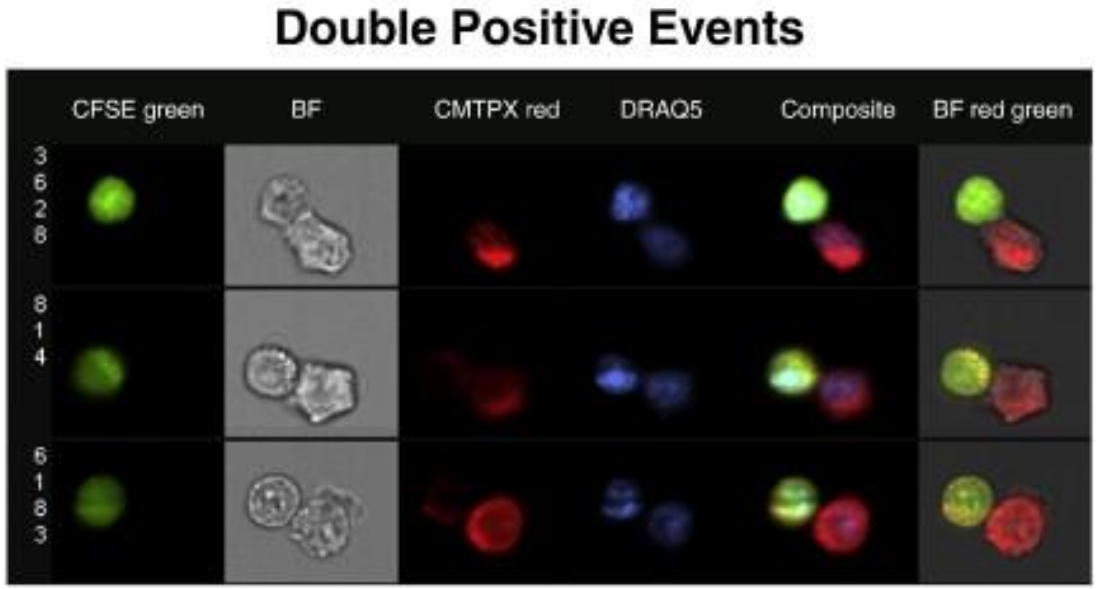
Cell-cell interaction

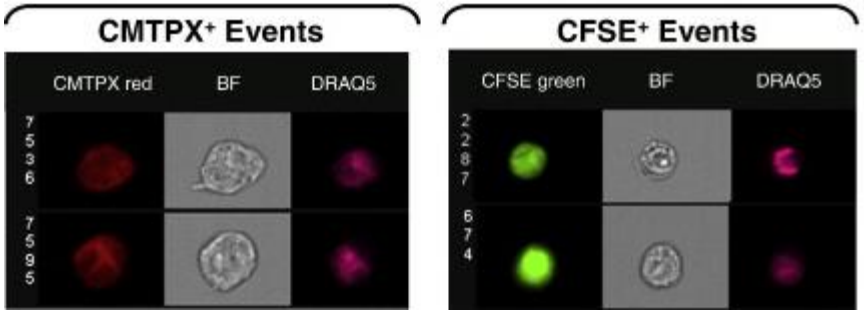


LS&E Infrastructure Unit

Ab Dependent Cell mediated Cytotoxicity (ADCC) activity

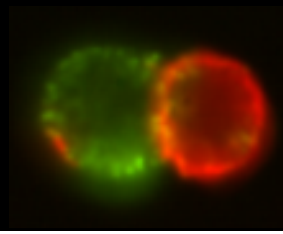






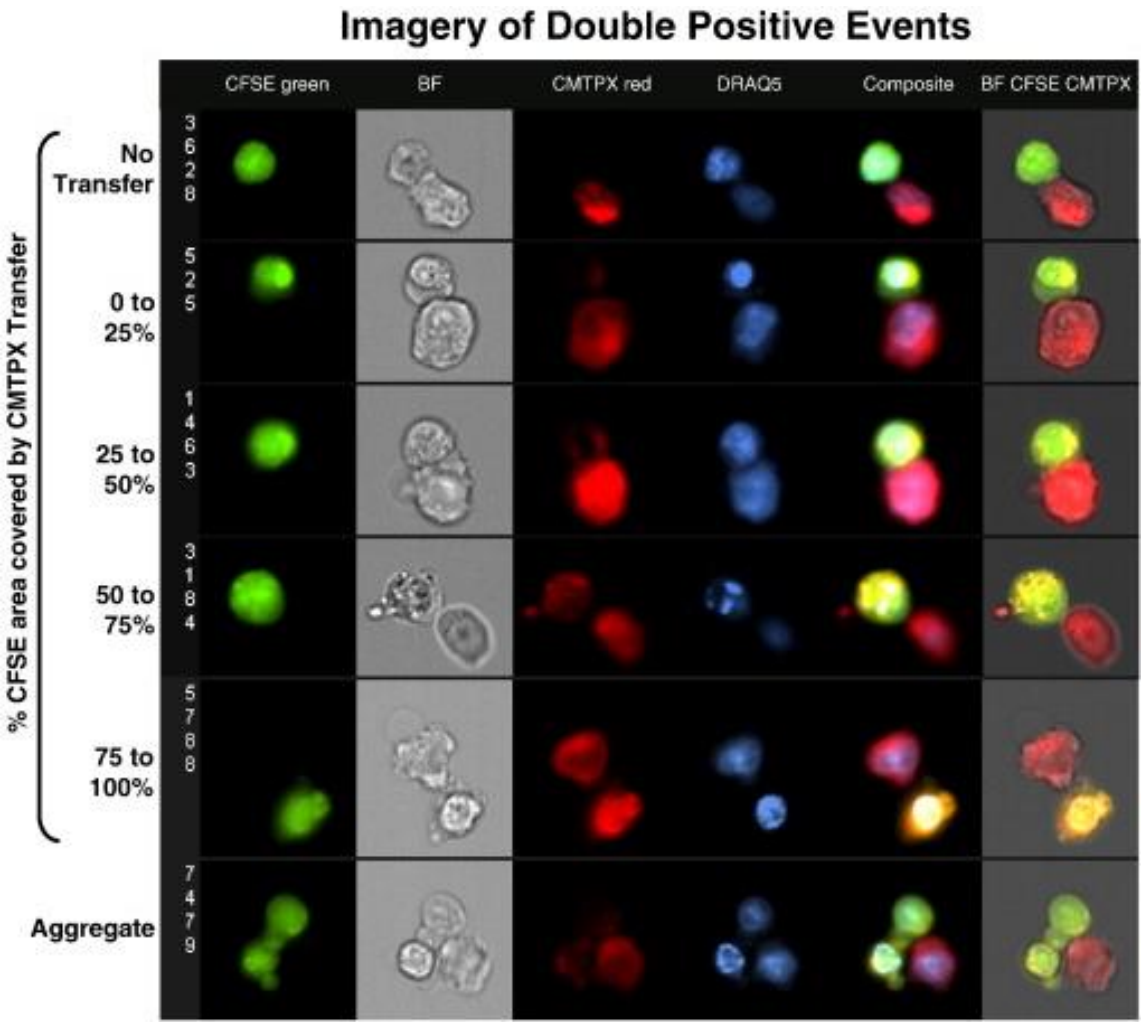
Helguera G. (2011), *J. of Immuno Methods*

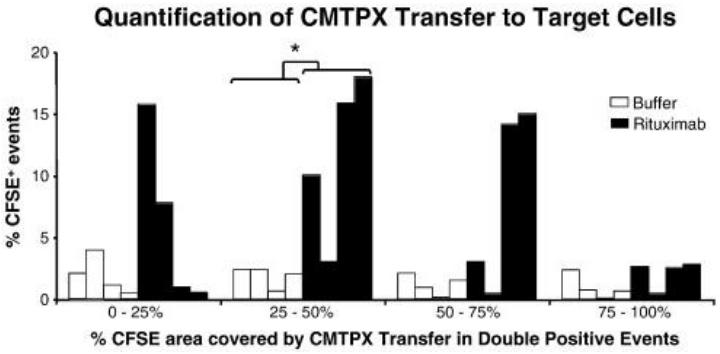
Cell-cell interaction



LS&E Infrastructure Unit

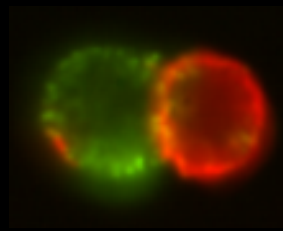
Ab Dependent Cell mediated Cytotoxicity (ADCC) activity





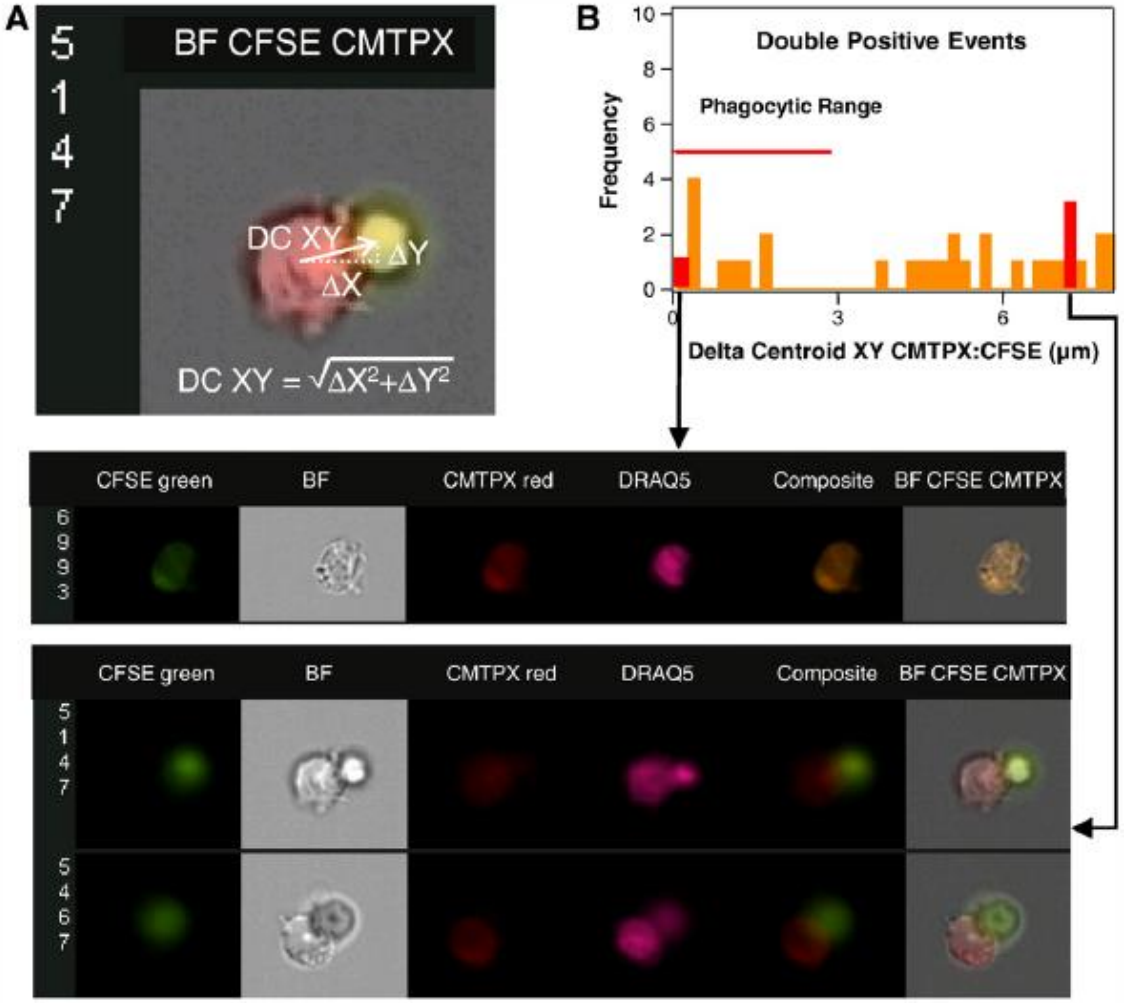
Helguera G. (2011), *J. of Immuno Methods*

Cell-cell interaction



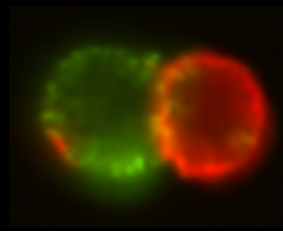
LS&E Infrastructure Unit

Ab Dependent Cell mediated Cytotoxicity (ADCC) activity



Helguera G. (2011), *J. of Immuno Methods*

Cell-cell interaction



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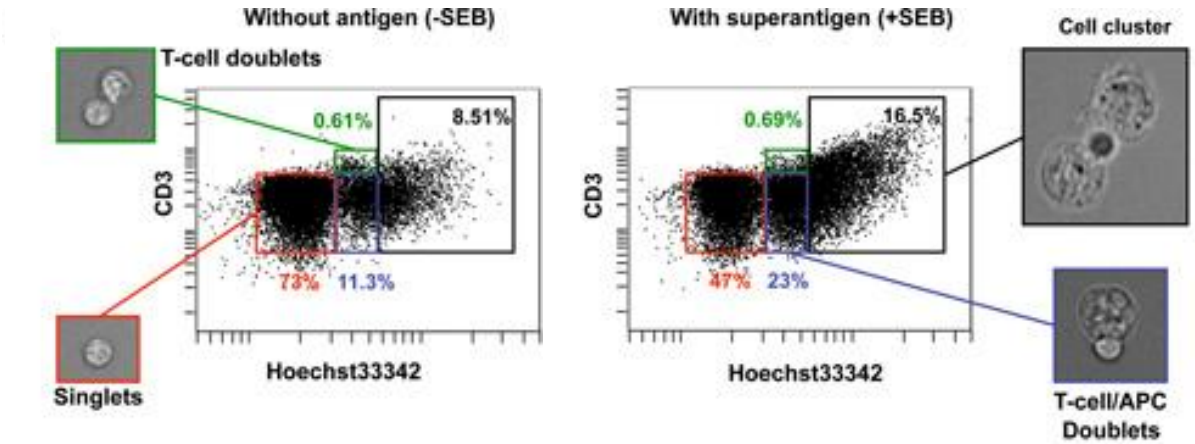
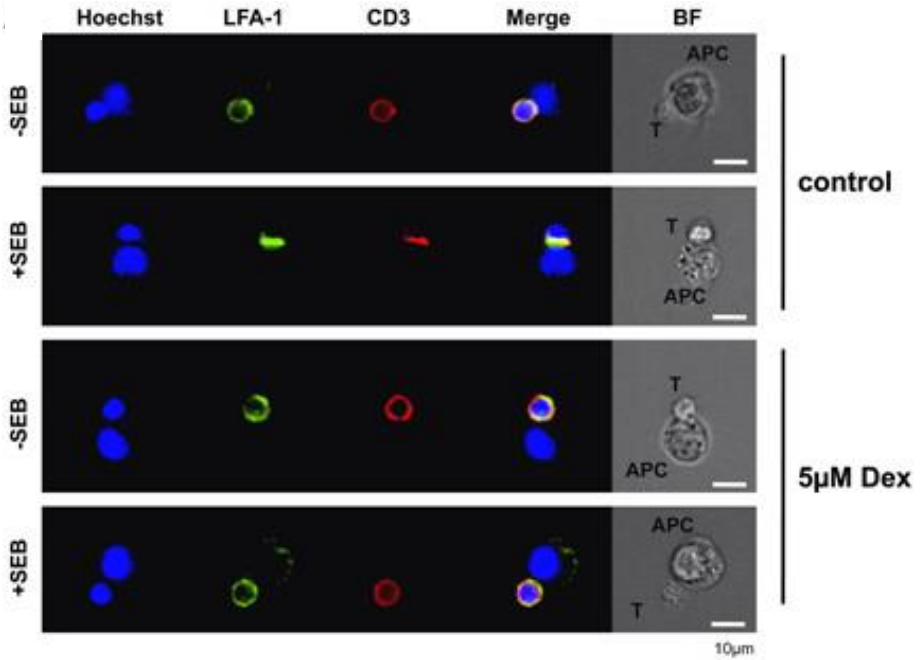
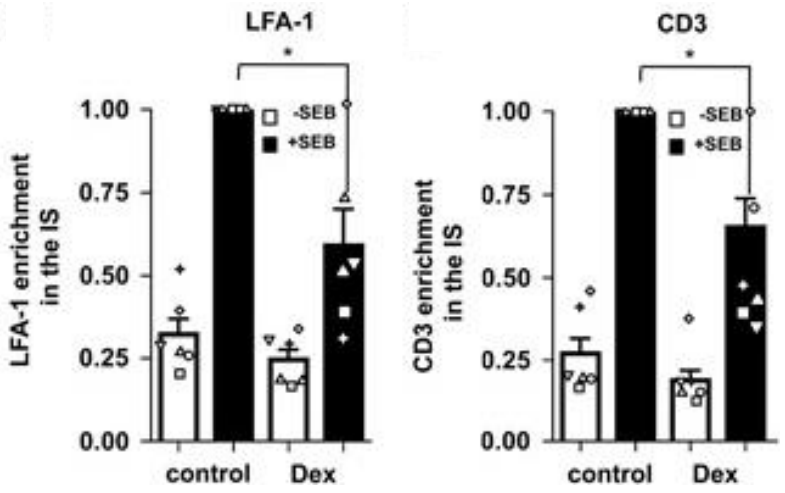
Immunosuppressive dexamethasone in primary T cells

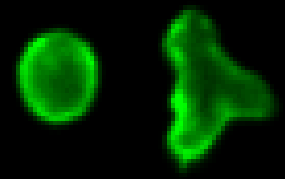


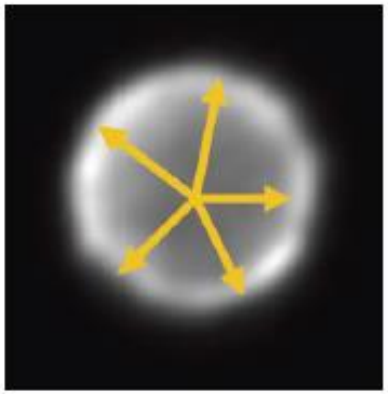




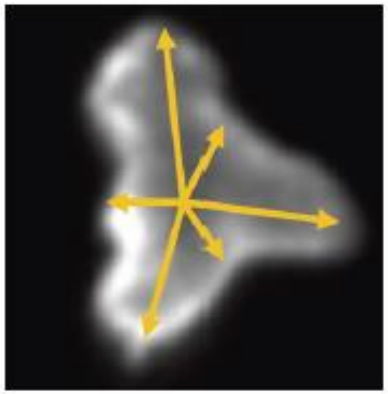
Wabnitz G.H. (2011), *Eur. J. of Immunol*

Shape change





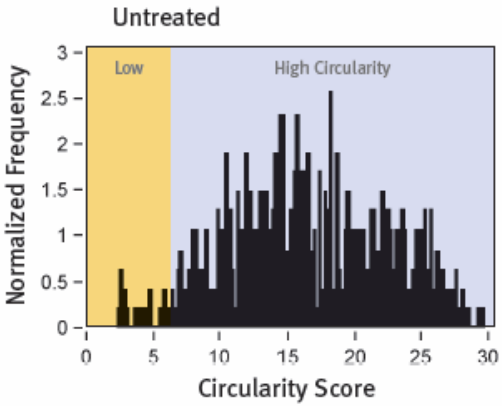
23.8

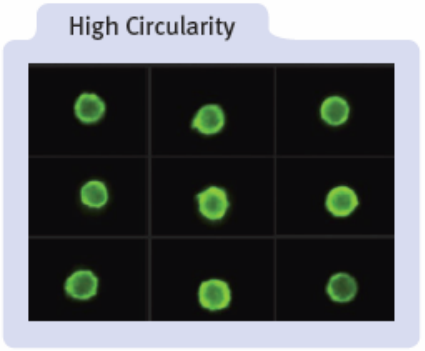


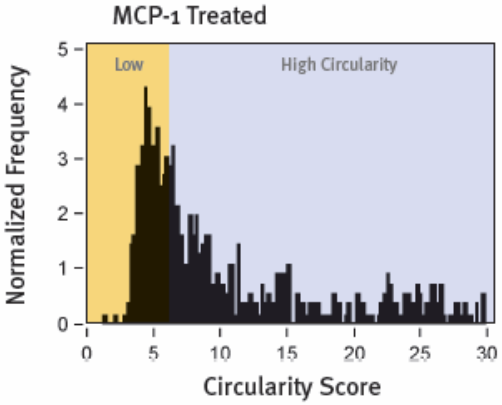
4.6

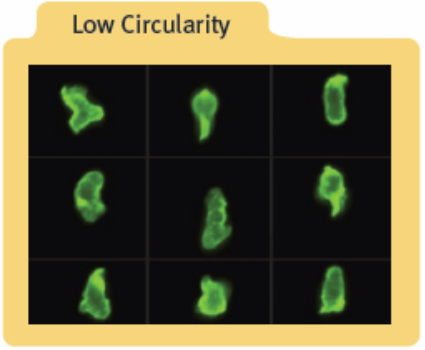
Circularity score

Shape Change in Primary Monocytes







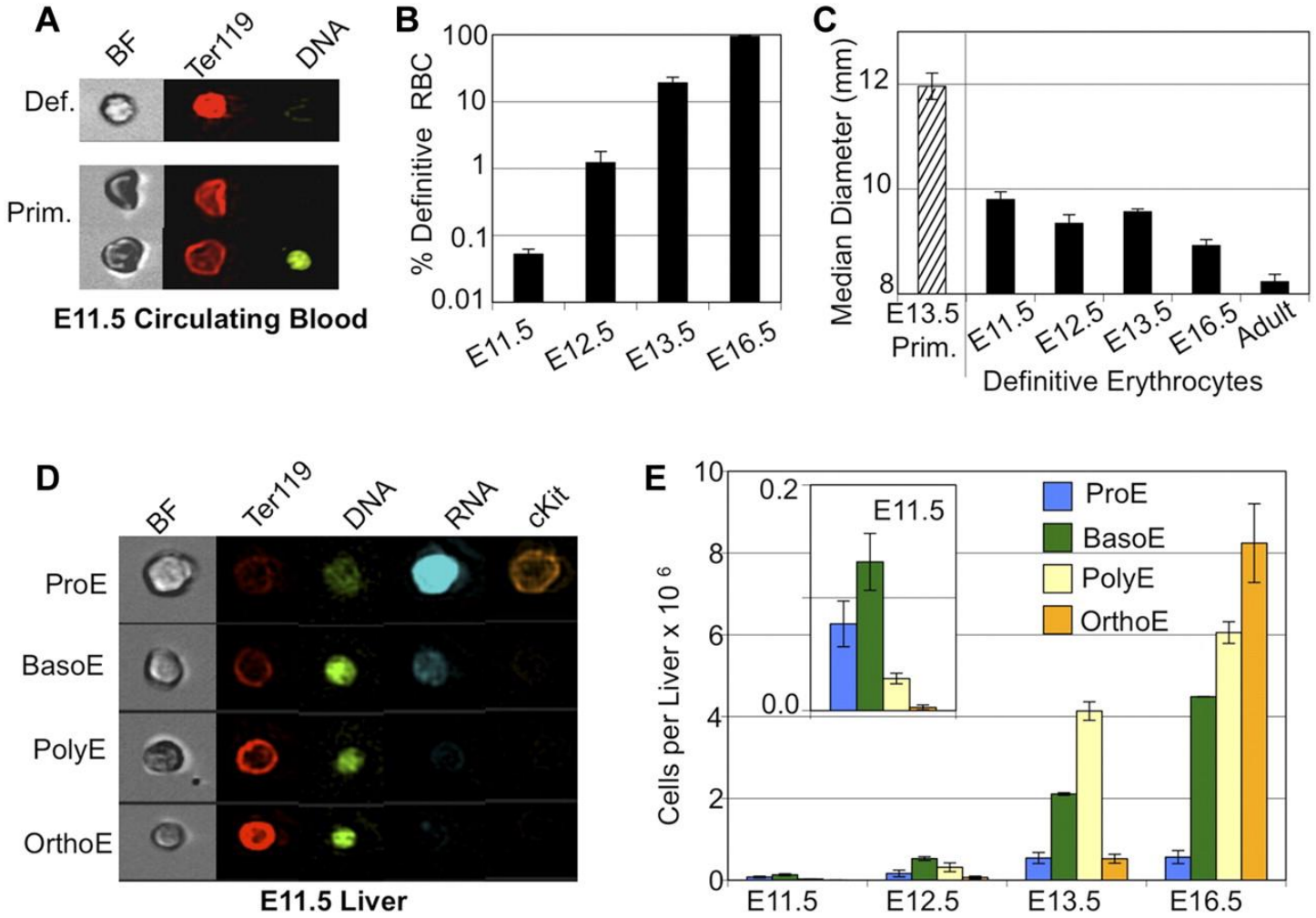


Chemoattractant MCP-1 induces monocyte shape change and migration to sites of inflammation

Stem cell biology

LS&E Infrastructure Unit

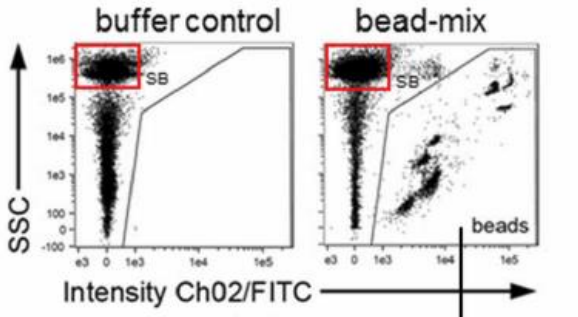
Erythroid differentiation



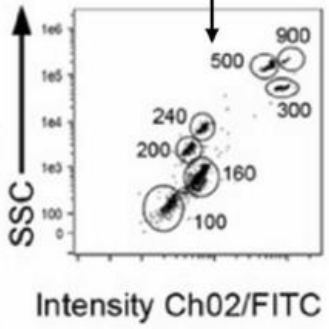
McGarth KE. (2011), *Blood*

Extracellular vesicles

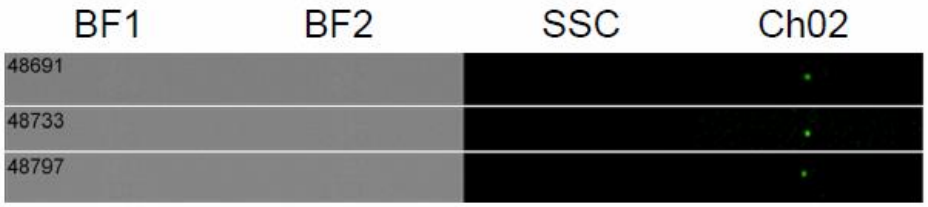
Submicron-Sized Polystyrene Beads as Calibrators

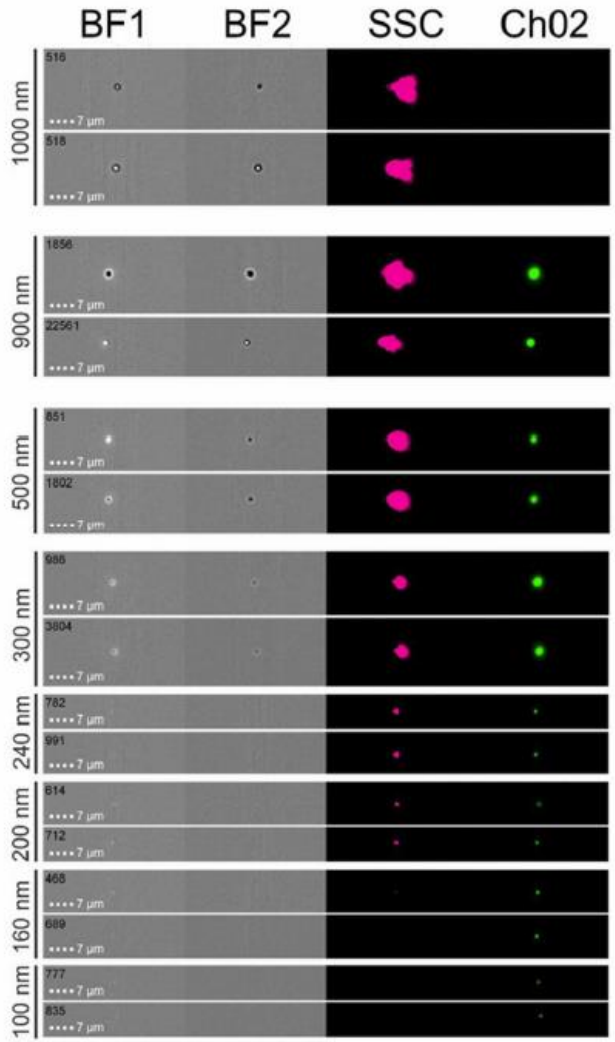


Amnis ISX MkII



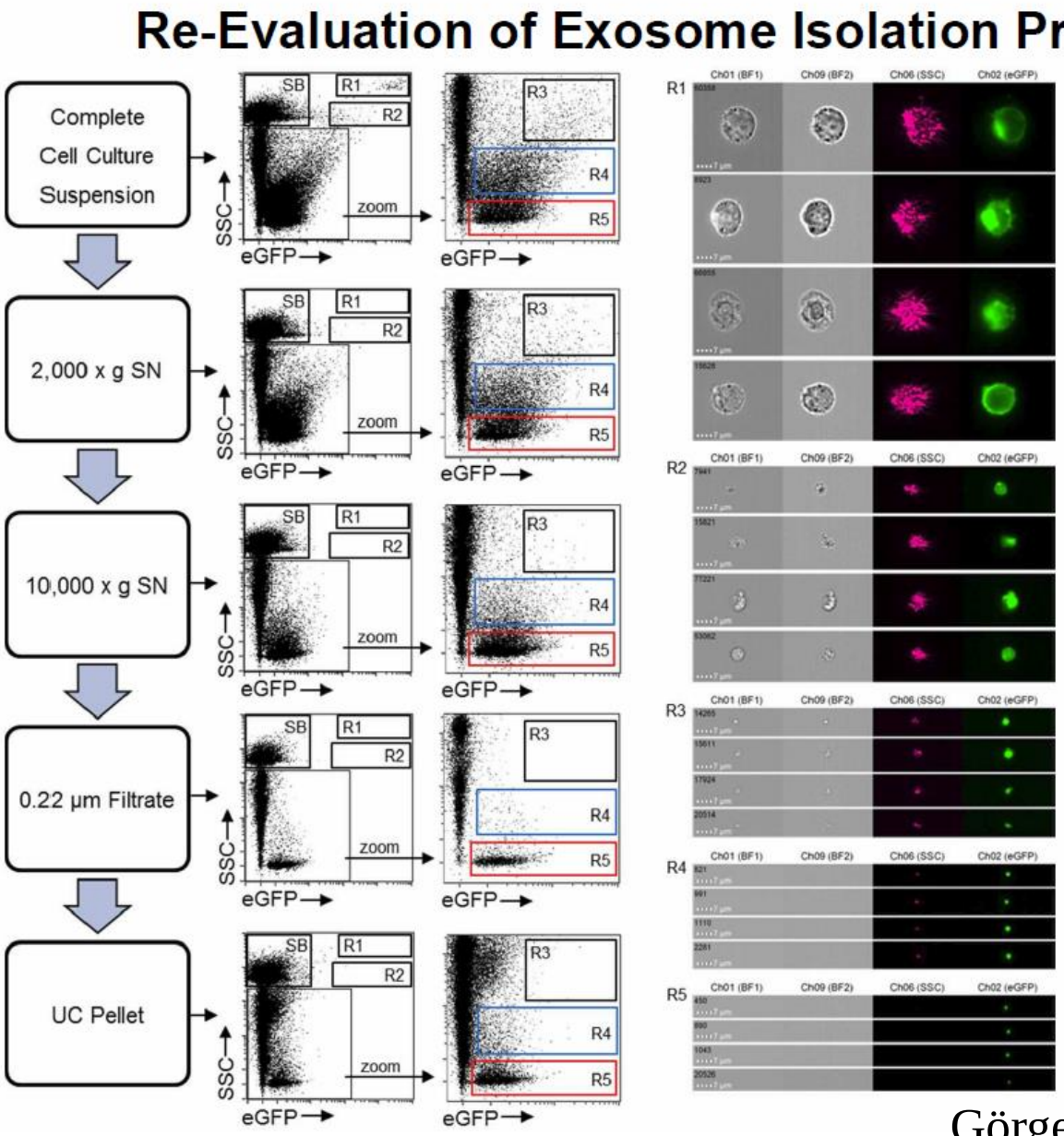
24 nm beads (Thermo Scientific)





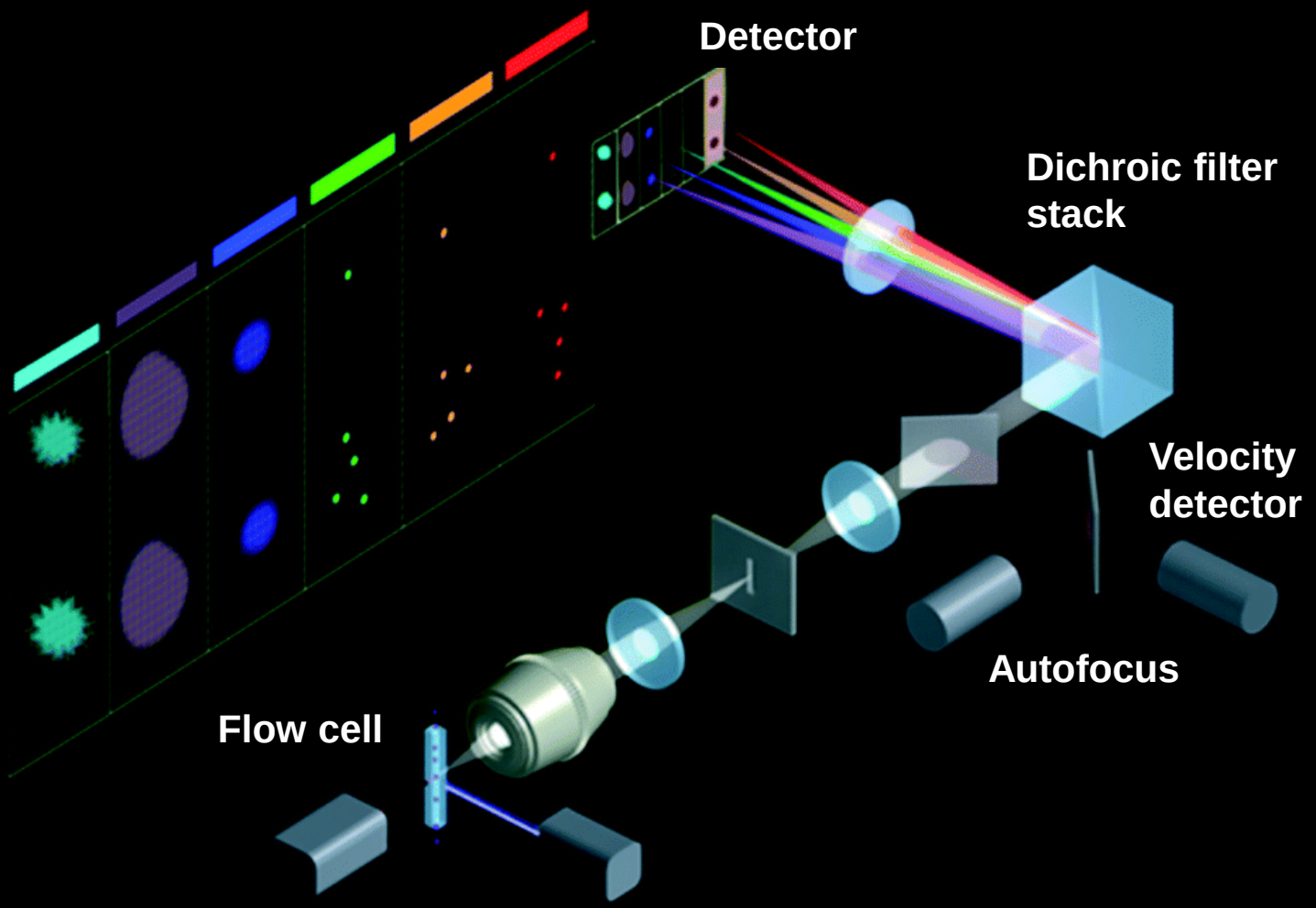
Görgens A. (2016), *Science* (webinar)

Extracellular vesicles



Görgens A. (2016), *Science* (webinar)

Planning your experiment



Planning your experiment

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		Excitation laser (nm)				
Ch	**Band**	**405**	**488**	**561**	**642**	**785**
1	**435-505**	DAPI, BV421, Hoechst, PacBlue, CascadeBlue, eFluor450, DyLight405, CFP, LIVE/DEAD Violet				
2	**505-560**	BV510, PacOrange, Cascade Yellow, AF430, eFluor525, QD525	FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, LysoTrackerGreen, MitoTrackerGreen			
3	**560-595**	QD565, QD585, eFluor565	PE, PKH26, DSRed, mOrange, Sytox Orange, Cy3	PE, AF546, Cy3, DyLight550, PKH25, DSRed		
4	**595-642**	QD625, eFluor625, BV605	PE-TexRed, PI, RFP, QD625, eFluor625	AF568, Cy3, PE-TexRed, TexRed, AF610, RFP, mCherry, PI		
5	**642-745**	QD705, eFluor700, BV711	PE-Cy5, PE-AF647, 7AAD, PerCP, PerCP-Cy5.5, DRAQ5, QD705	PE-Cy5, PE-AF647, DRAQ5, 7AAD	APC, AF647, AF660, AF680, APC, Cy5, DyLight649, PE-AF647, PE-Cy5, DRAQ5, PerCP, , PerCP-Cy5.5	
6	**745-780**	QD800, BV786	PE-Cy7, PE-AF750, QD800	PE-Cy7, PE-AF750	APC-Cy7, APC-AF750, APC-H7, Cy7, AF750, PE-Cy7, PE-AF750	SSC

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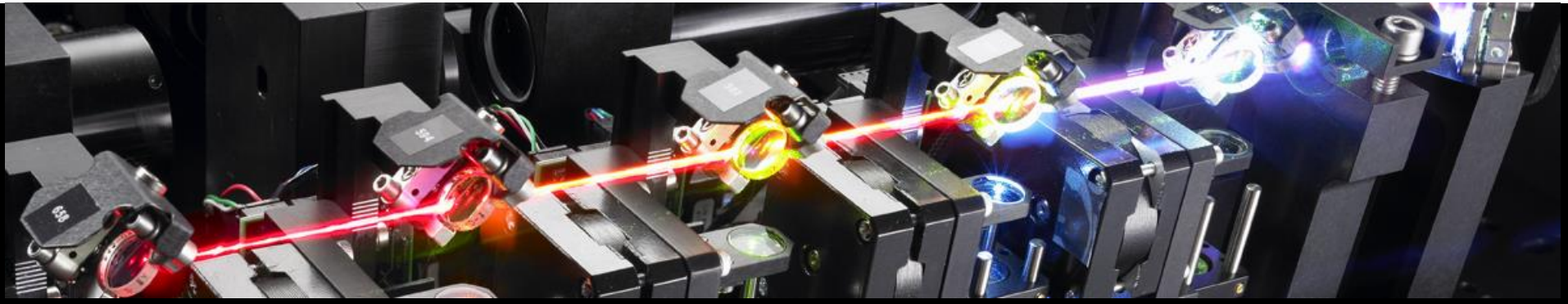

- *ImageStream free lab first trail*
- *FCS Express 6*

Thank You!!!

Dr. Yael Lupu-Haber
e-mail: yaelupu@technion.ac.il **Tel: 04-8293676**
LS&E site: https://isu.technion.ac.il/