We maken **onbewust** interpretatie fouten, wanneer we gebruik maken van **"conventionele"** flow cytometrie.

Hoe kan de **ImageStream** ons helpen dit te voorkomen

Erik Mul (Core facility Manager)

Bloed is leven

Sanquin

Intro:

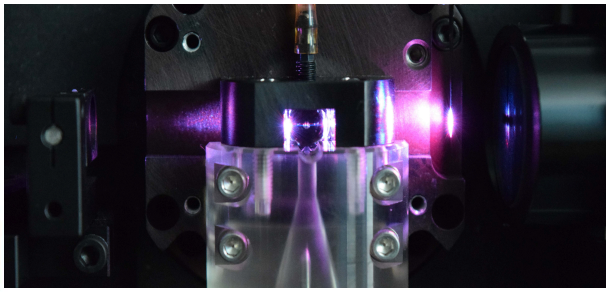
- Werking conventionele flow cytometer
- Werking Imagestream
- Vergelijking flow vs. imaging

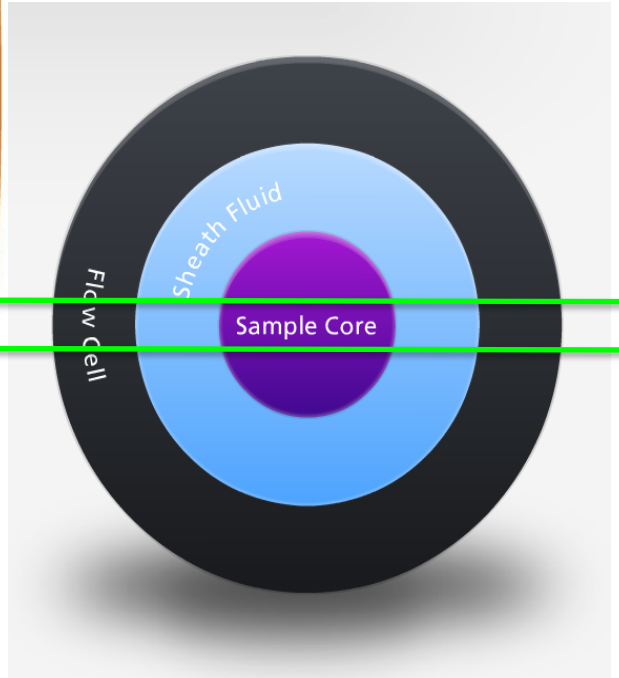
Voorbeelden ter vergelijking:

- Identificatie van circulerende tumor cellen (CTCs)
- Analyseren van multinucleated giant cells (MGCs)
- Fagocytose
- Analyseren “CRISPR-Tat” transfectie
- Small particles
- Extracellulaire Vesicles (EVs)
- Analyseren van bijv. kolommateriaal (kwaliteits controle)

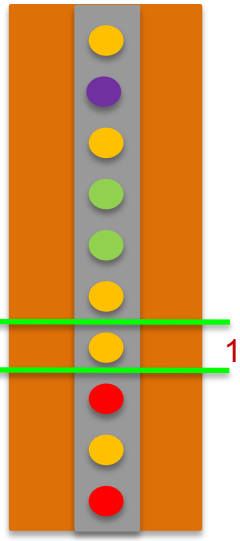
Conclusie / Vragen:

Werking van een conventionele flow cytometer

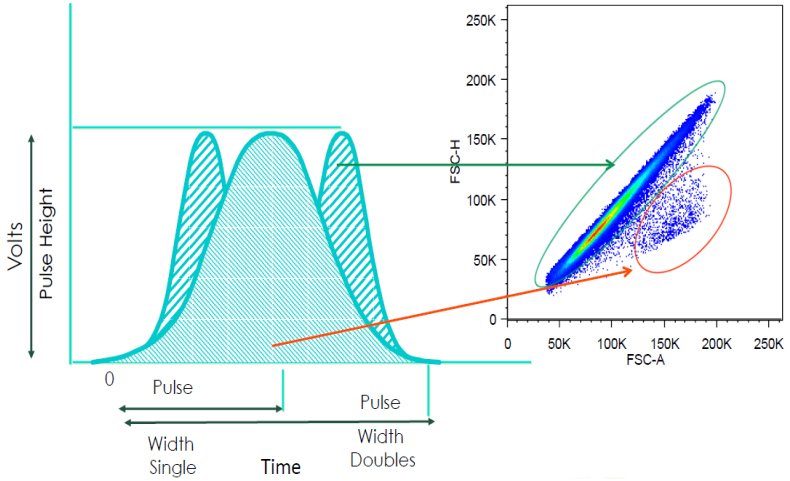




Flow-cell



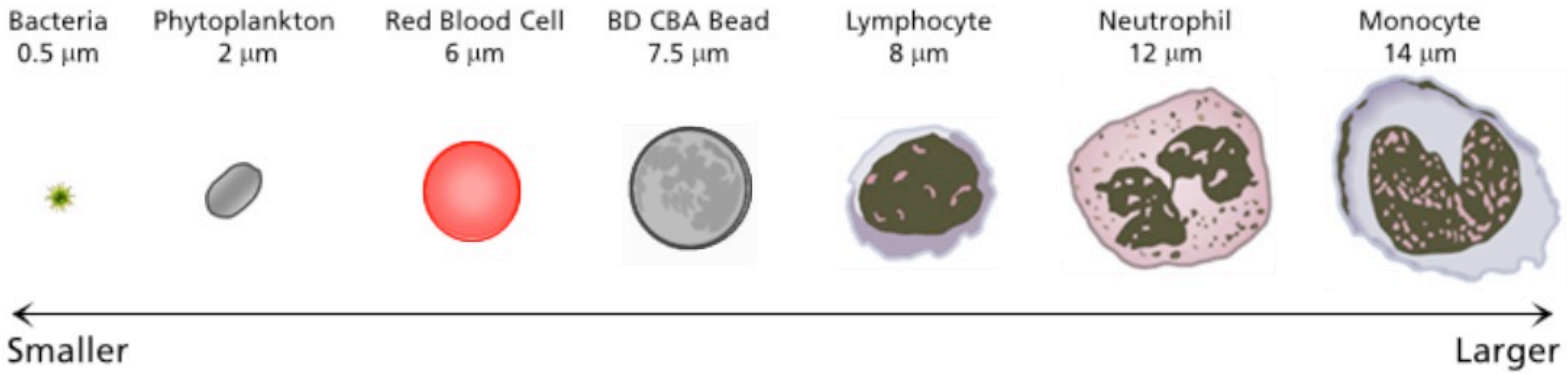
10 um Laserbeam

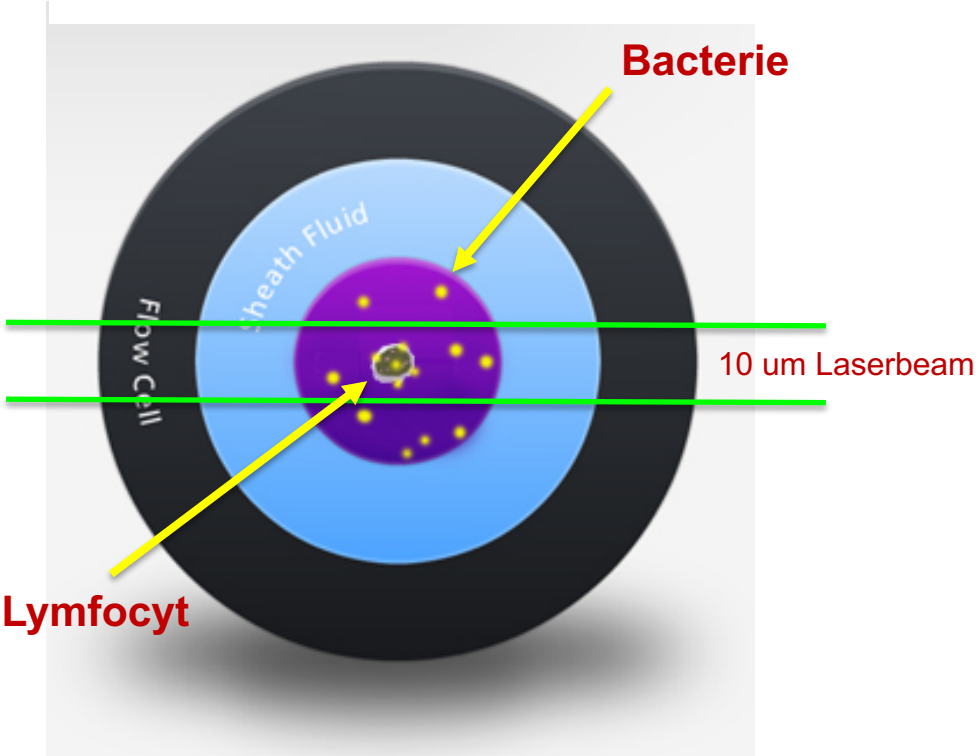


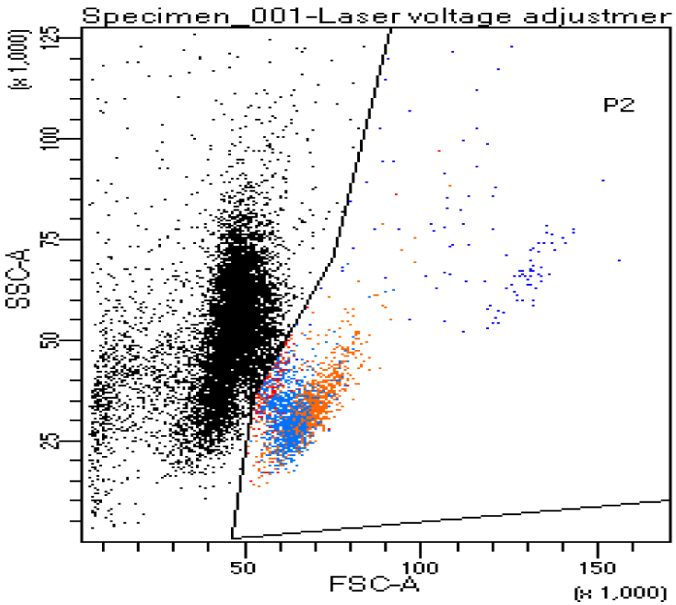
Signalen : Height, Width en Area

Doublet discriminatie mogelijk

Object grootte t.o.v. laserbeam





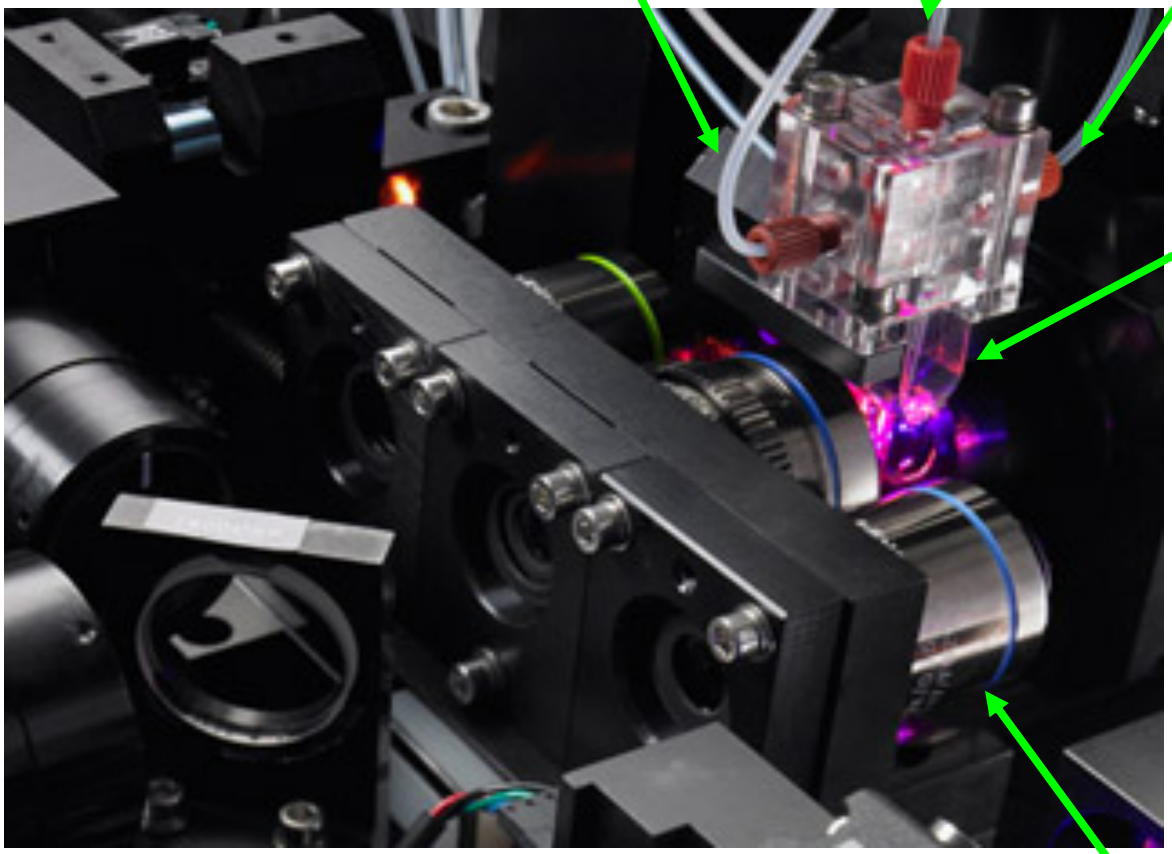


Werking ImageStream

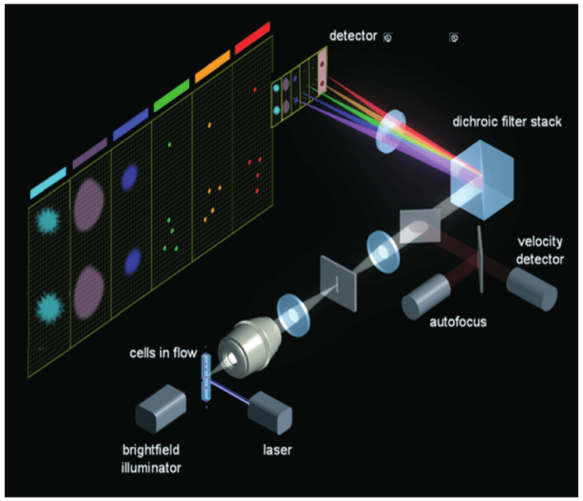
PBS

Speedbeads (1 um)

Monster



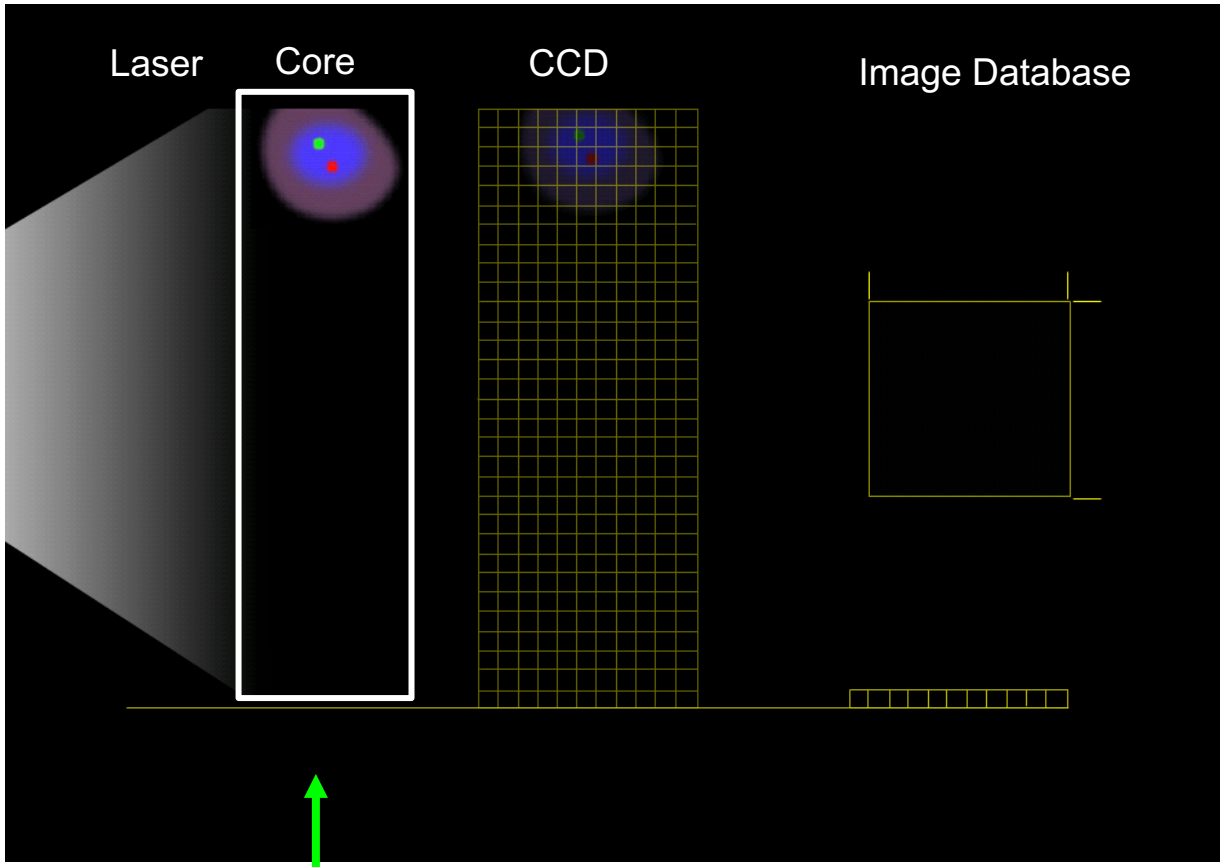
Flow-cell



Objectieven (20x, 40x en 60x)

Hoe analyseert de Imagestream ?

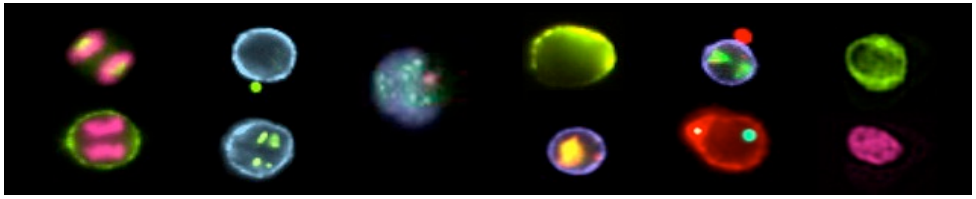
Time Delay Integration (TDI)



- 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 a high resolution.

Vergelijking flow vs. imaging

	flow cytometer	Imagestream
Parameters:	FSC, SSC, Fluorescentie, Tijd	FSC, SSC, Fluorescentie, Tijd
Meet volume:	Minimaal 12 ul / min (Low)	+/- 1 ul / min
Snelheid door flow-cel	3 meter / sec	4,4 cm / sec
Aantal cellen / sec	+/- 20.000	+/- 500
Dimensie:	1 pixel	65.536 pixel (256x256 image)
Kenmerken: (vorm, verdeling, lokalisatie)	**4** (Height, Width, Area en tijd)	**> 85**



Informatie overdracht !!

Een kind met blond haar tot op de schouders met een witte trui aan heeft beide handen gestrekt tegen de ogen gedrukt zodat het niets kan zien.

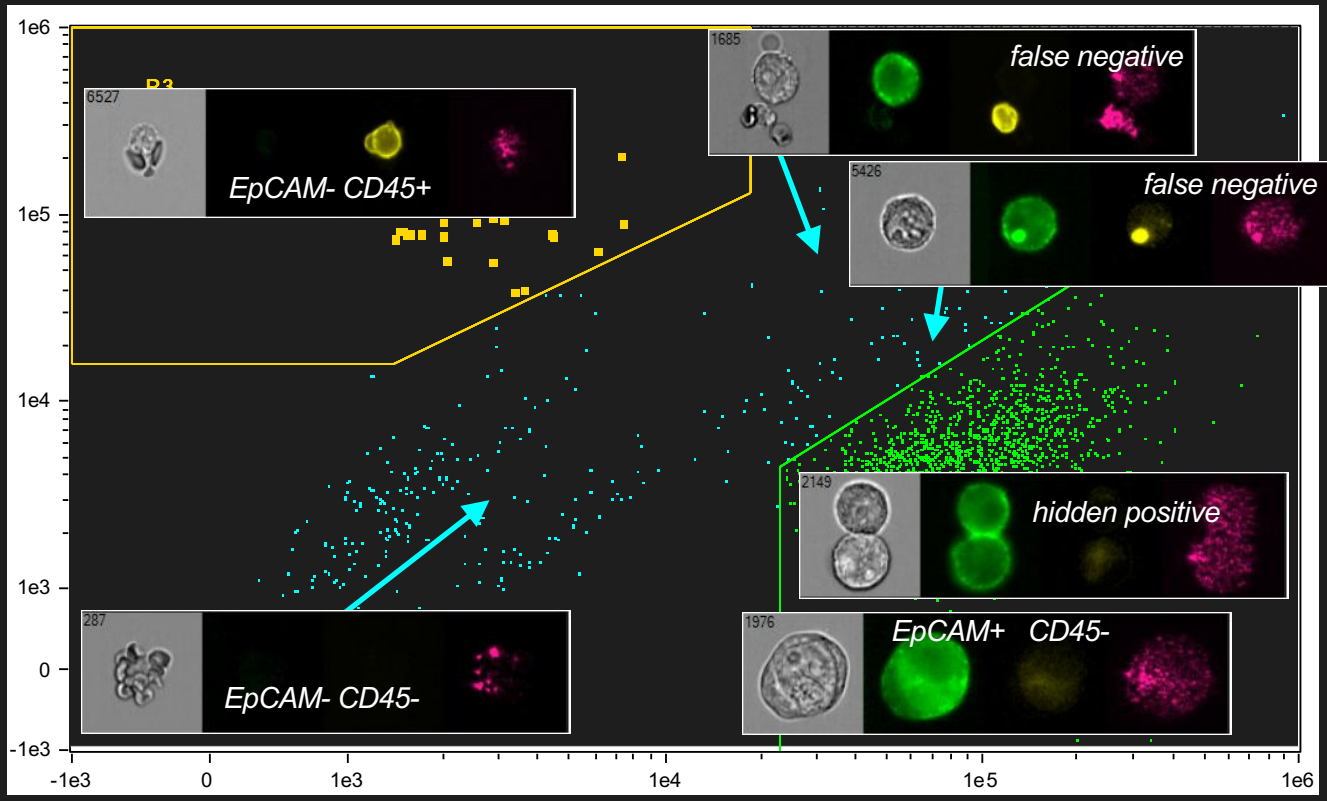
Beelden worden 60.000x sneller verwerkt dan tekst

7 VOORBEELDEN TER VERGELIJKING:

Identificatie van circulerende tumor cellen (CTCs)

Sanquin

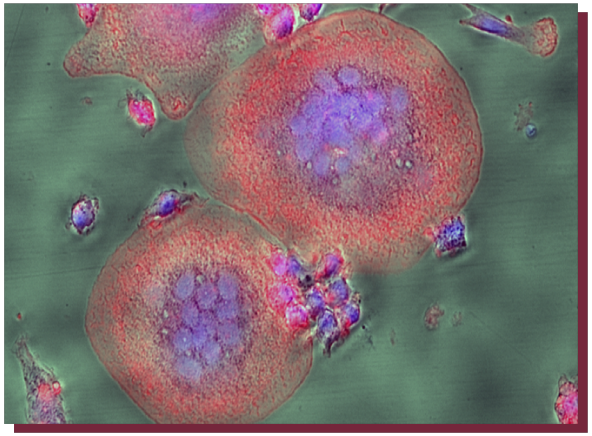
(EpCAM) can be used for CTC enrichment as it has little or no expression on leukocytes and is expressed by the majority of epithelial derived cancers



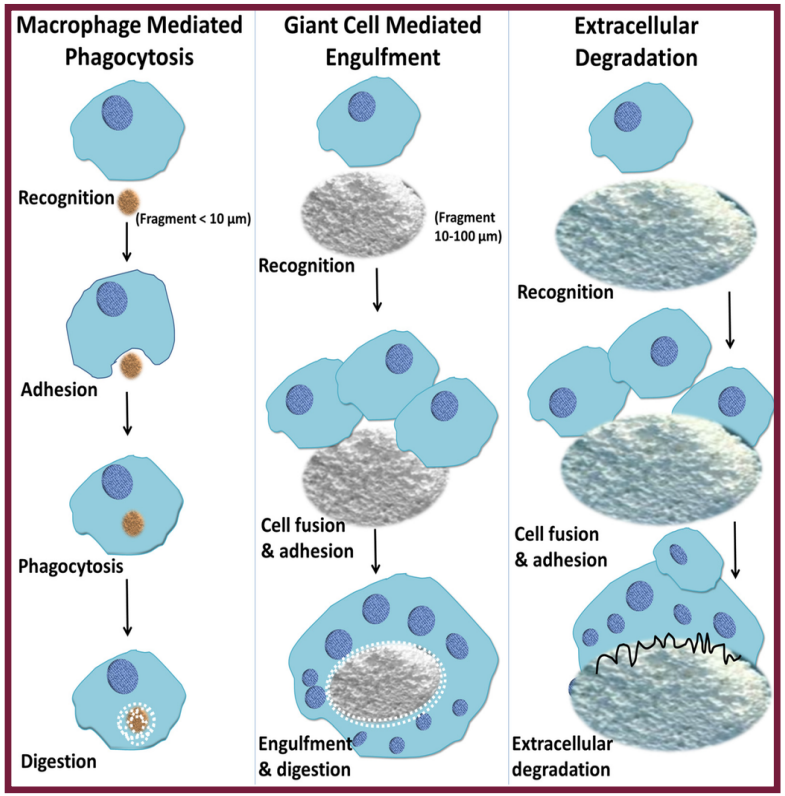
The Benefits of Imaging Cytometry for Rare Sub-Populations:

- Gating with confidence (false negative & false positive detection)

Multinucleated giant cells (MGCs)



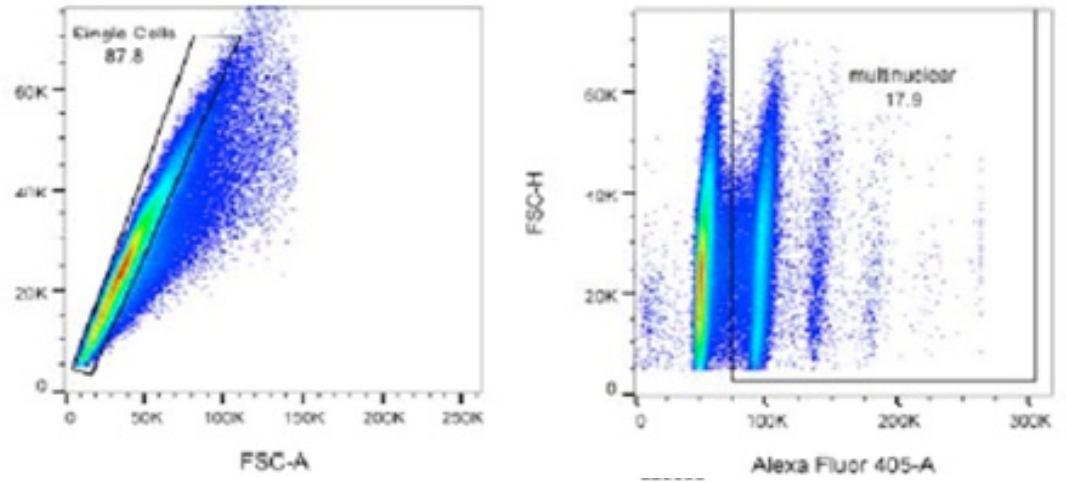
- Gefuseerde macrofagen, worden gevonden in chronische ontstekingen (Tuberculose, ziekte van Crohn)
- In botten (osteoclasten)



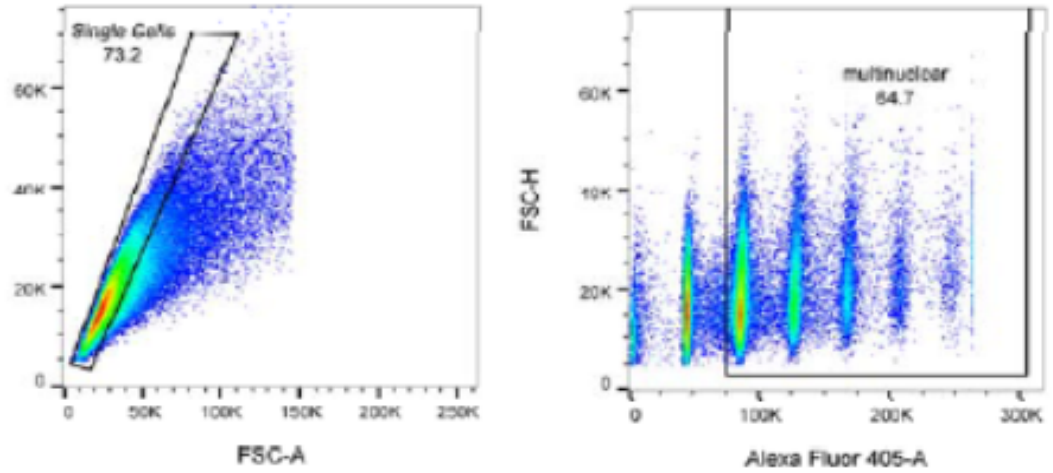
Dr. S.E.M. Heinsbroek PhD,
(Tytgat Institute for Liver and Intestinal Research, AMC)

FACS analyse van multinucleated Giant cell (gefuseerde macrofagen) vorming

Niet gestimuleerd



“Curdlan 5 ug/ml”, LPS of IFNg gestimuleerd

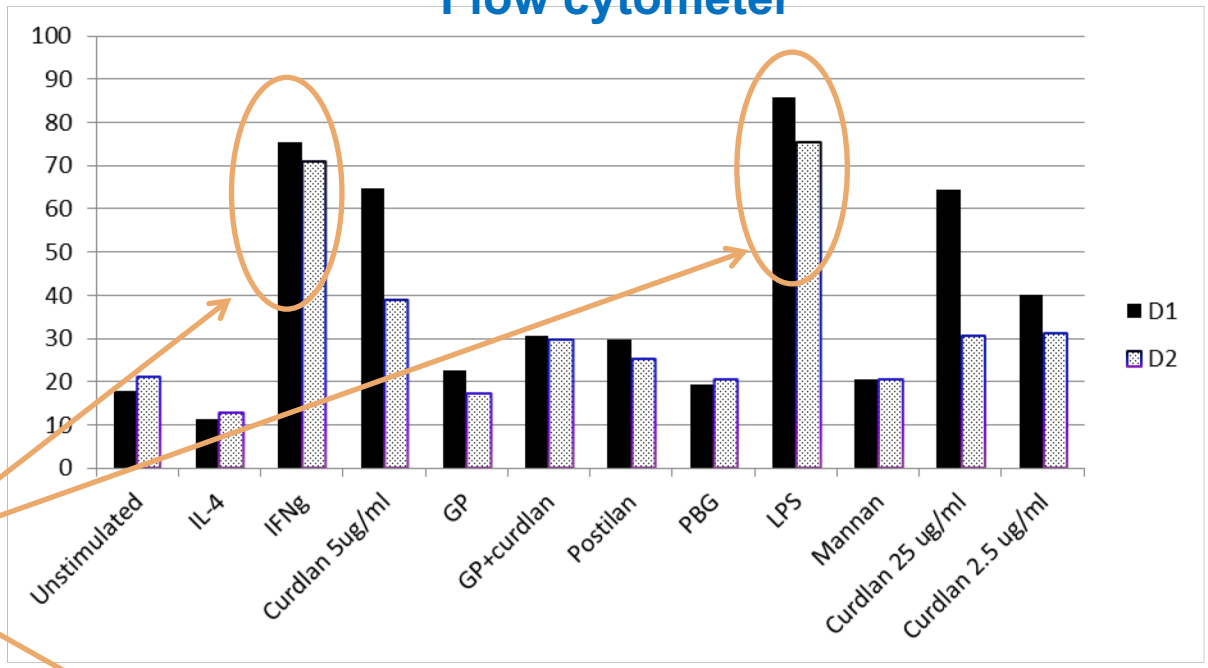


“Curdlan 5 ug/ml” is hierin een positieve controle

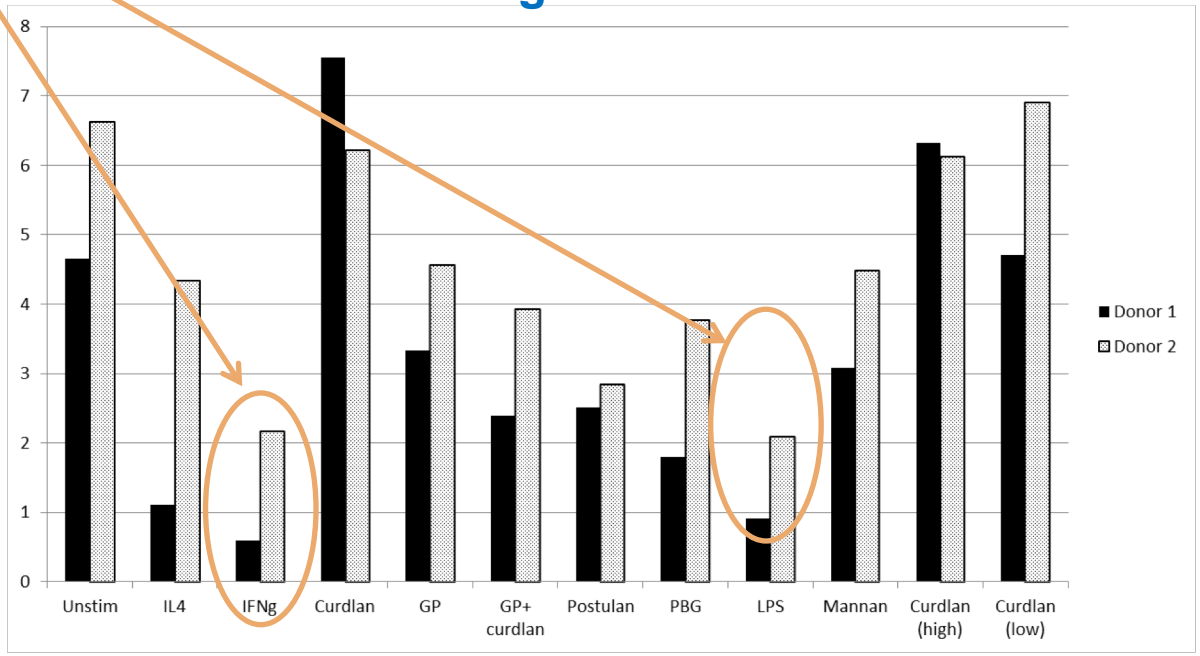
Sanquin

Groot verschil tussen resultaten verkregen met de FACS of met ImagestreamX !

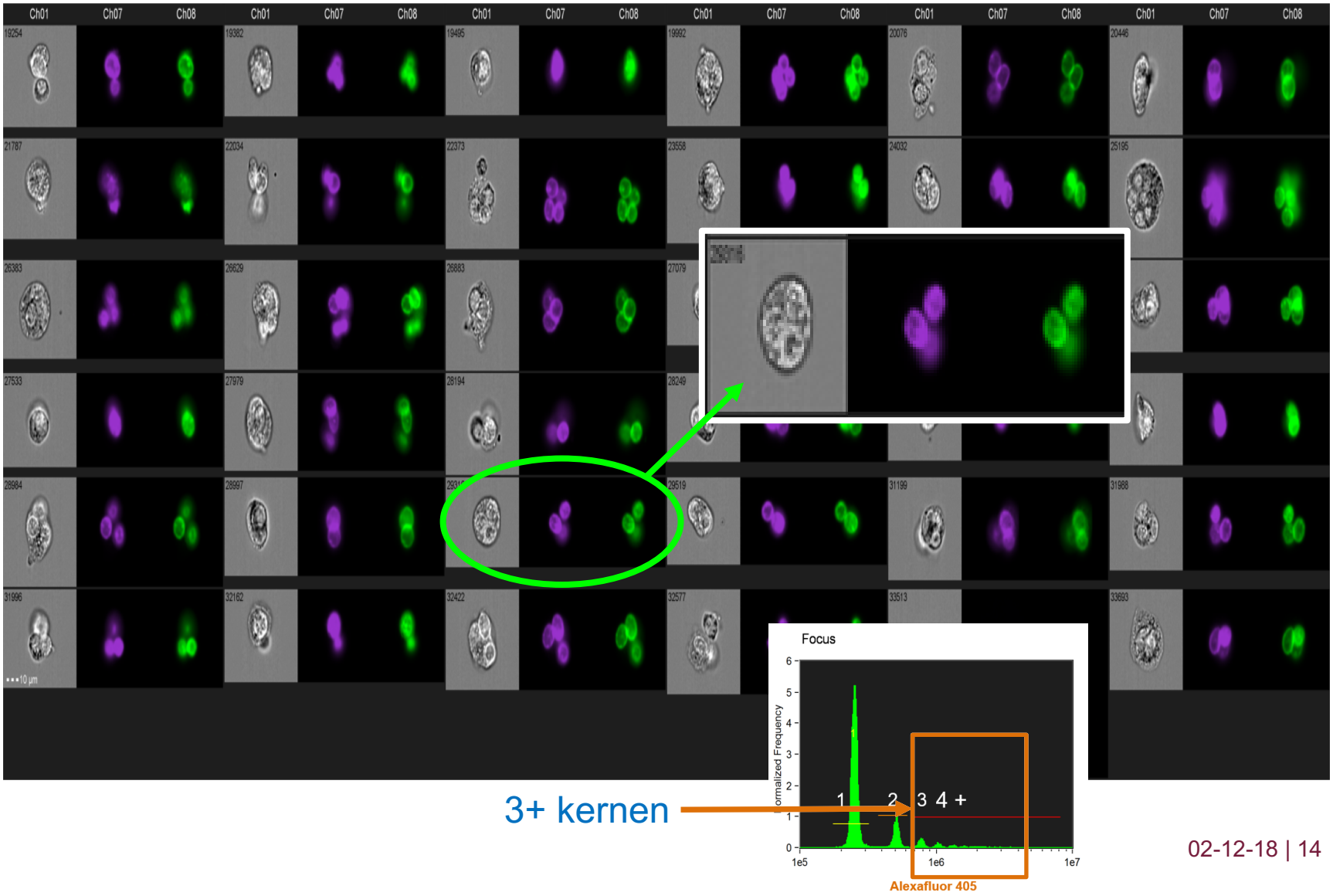
Flow cytometer



ImagestreamX

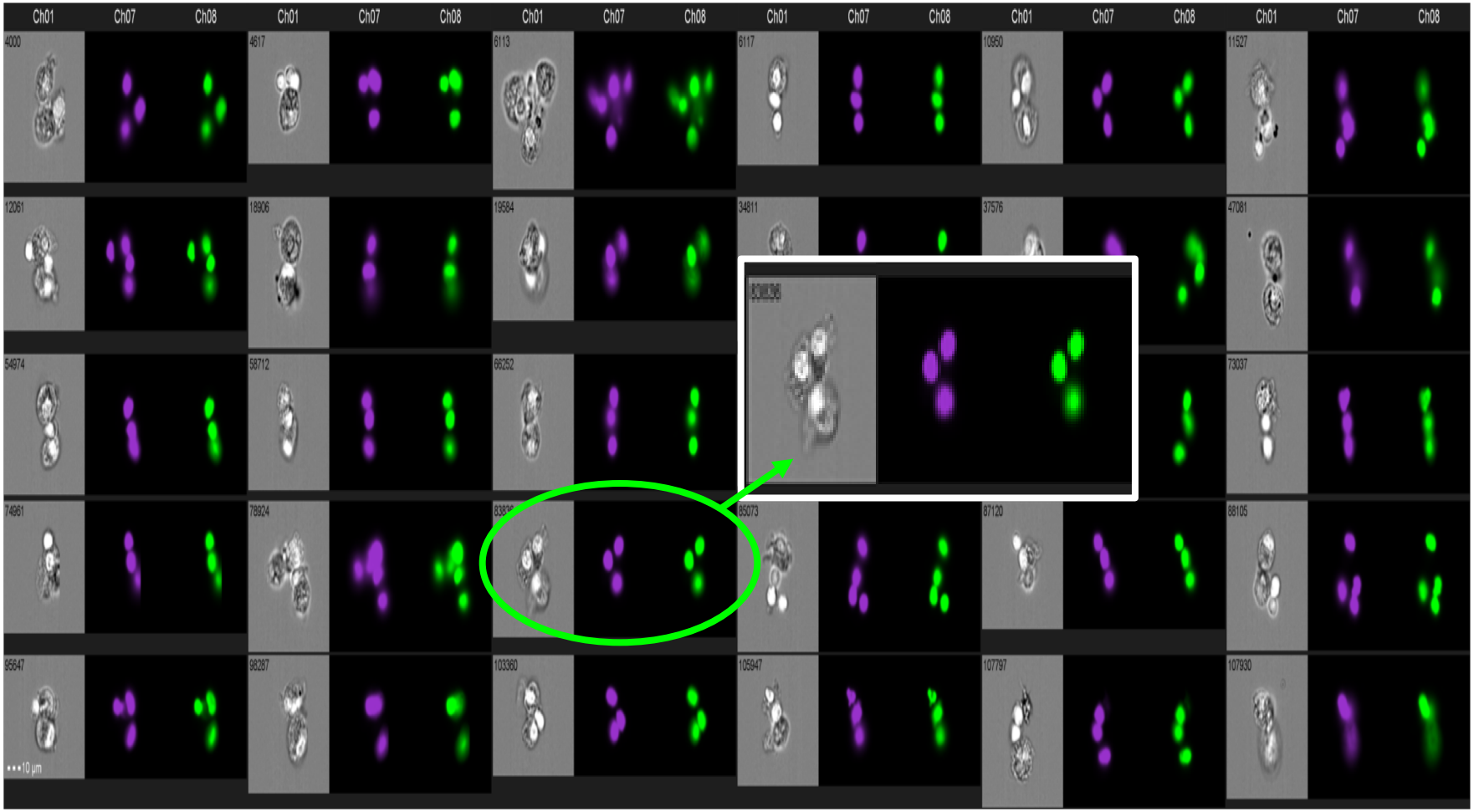


“Curdlan 5 ug/ml” geïnduceerde Giant cell vorming (>2 kernen)



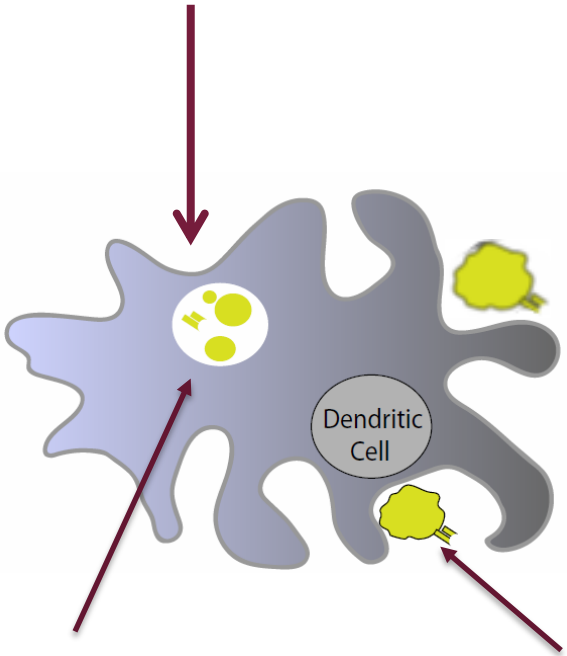
3+ kernen

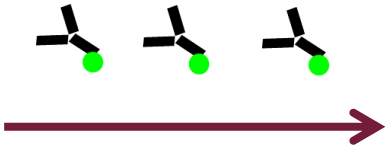
LPS of IFNg gestimuleerd hoofdzakelijk (>95%) vals positieve events (aggregaten in plaats van fusie !).



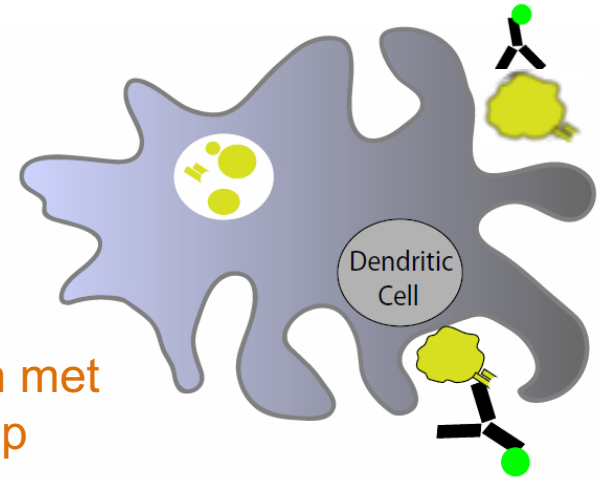
Gebonden of geïnternaliseerde trombocyten door dendritische cellen

1e stap: PKH gekleurde trombocyten geïncubeerd met dendritische cellen





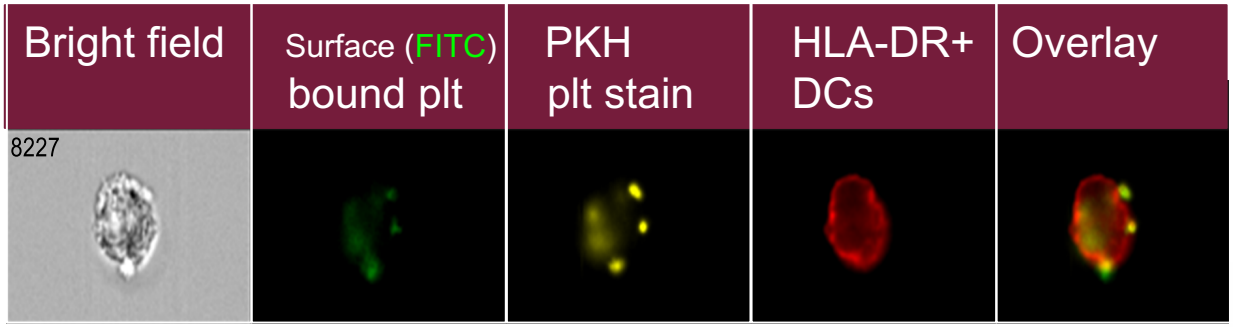
2e stap: Aankleuren met membraan marker op trombocyten (FITC)



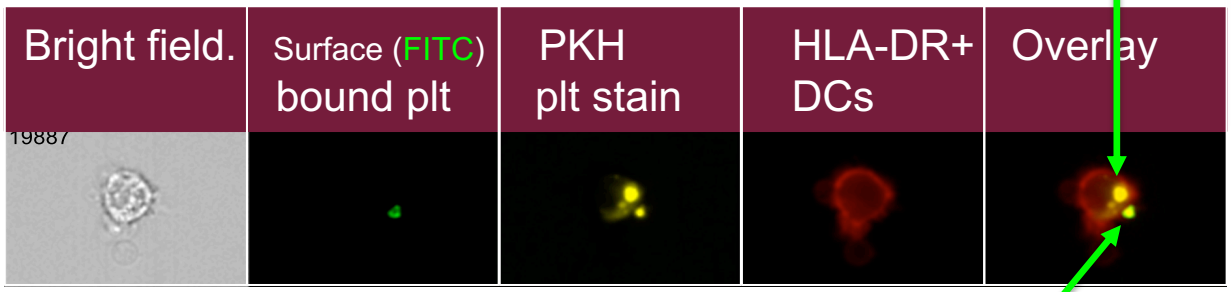
Anno Saris, PhD

Gebonden of geïnternaliseerde plaatjes door dendritische cellen

External

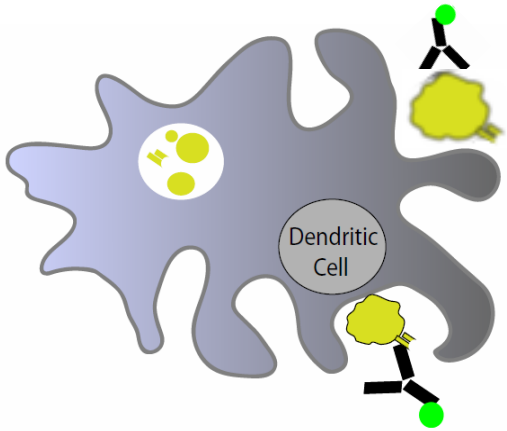


External en internal



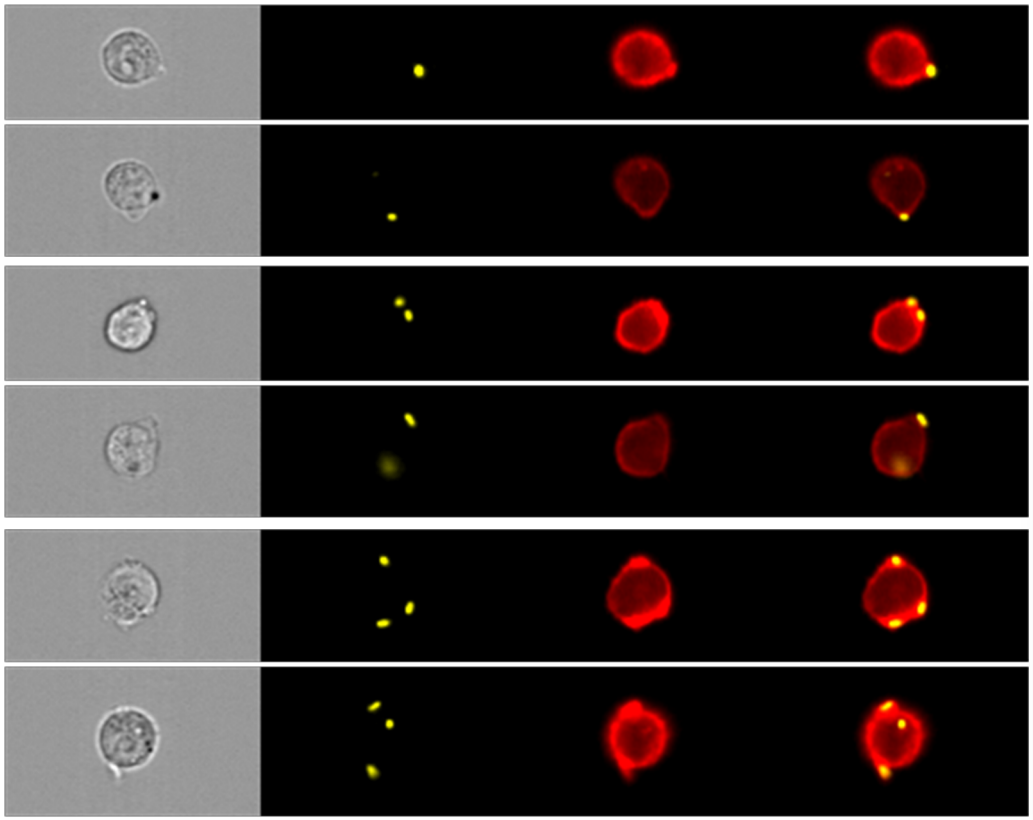
Internal

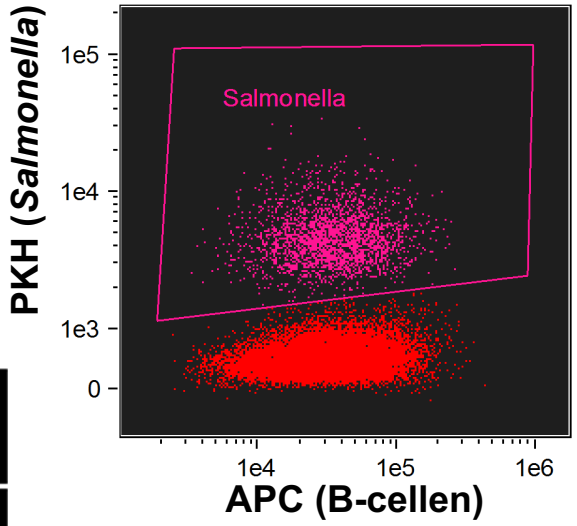
External

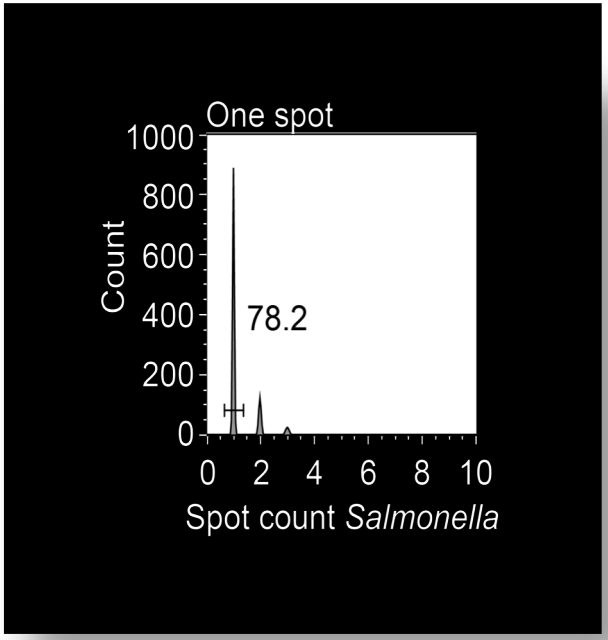


Anno Saris, PhD

Salmonella "spot" counting

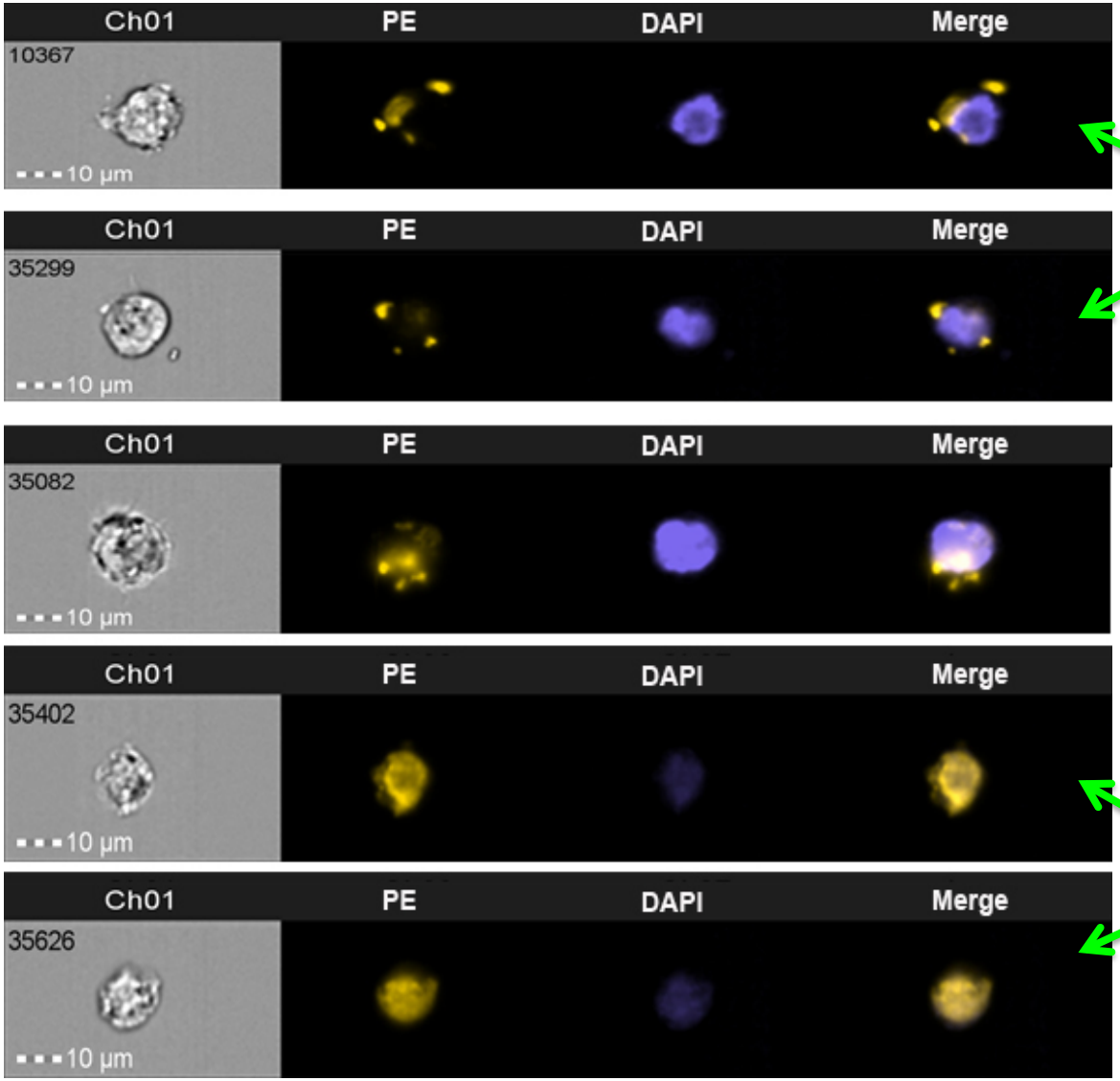






Niels Versteegen, (PhD candidate)

B-cel, Crispr-TAT transfectie, eiwit aangekleurd met “PE”



Verkeerde localisatie !

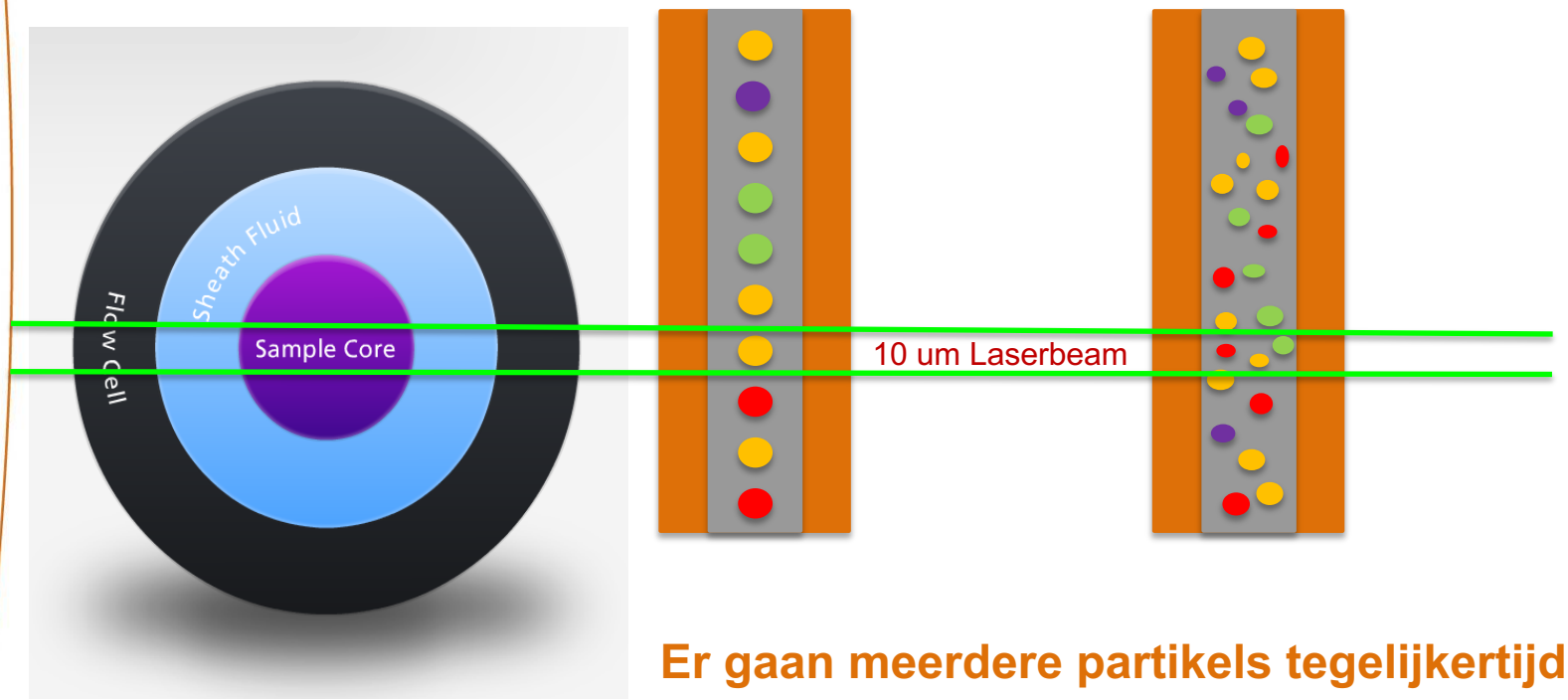
Na analyse van de samples bleek niet **>65% (FACS)** maar slechts **<3.5% (ImageStreamX)** goed gelocaliseerd

Juiste localisatie !

Werking van een conventionele flow cytometer (EVs ??)

Lymfocyt monster
(600 events/sec)

EV monster
(>8000 events / sec)

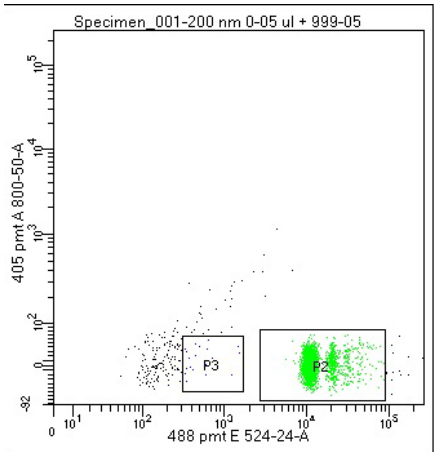


Er gaan meerdere partikels tegelijkertijd door de laserbeam (swarm effect).

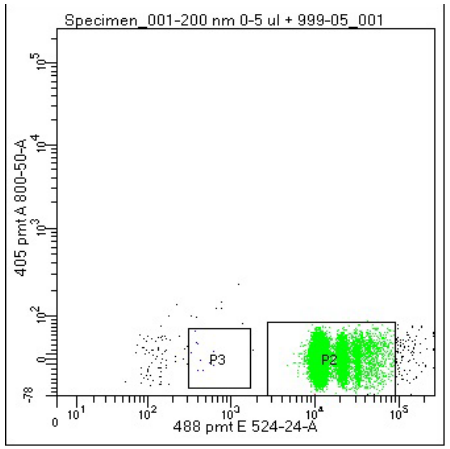
Small particles op een flow cytometer

Gemeten aantal: ???

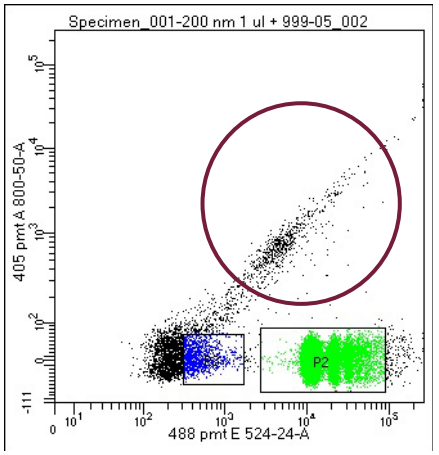
200 nm beads (CFSE)



1000x verdund



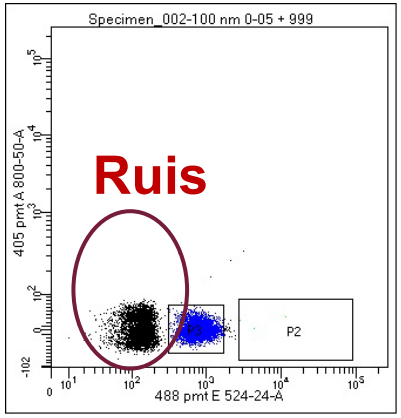
100x verdund

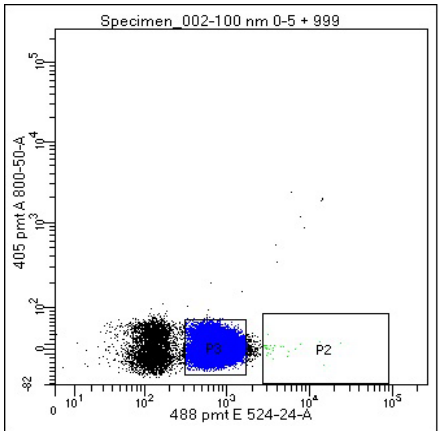


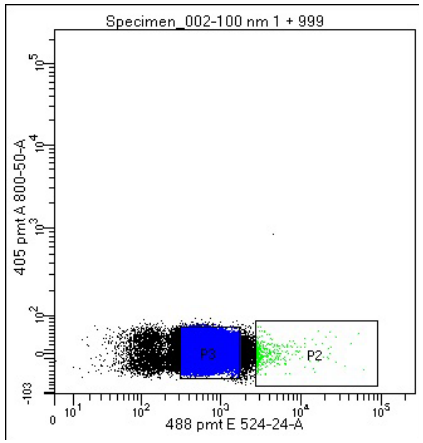
Swarm effect

10x verdund

100 nm beads (CFSE)

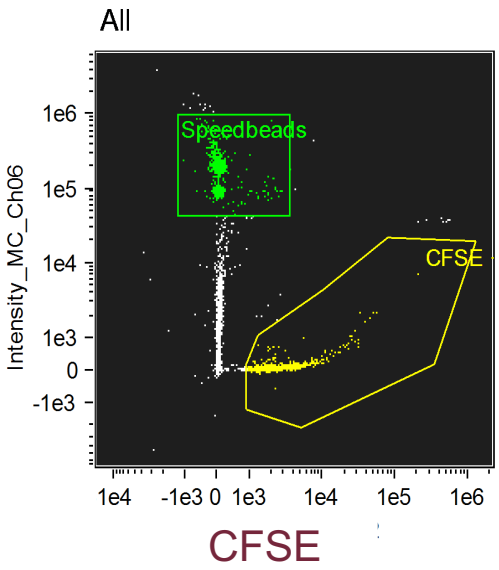




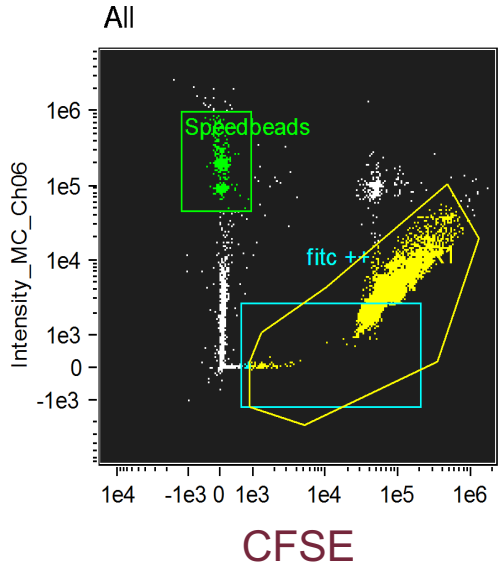


Meten van aantal CFSE / FITC beads op een Imagestream

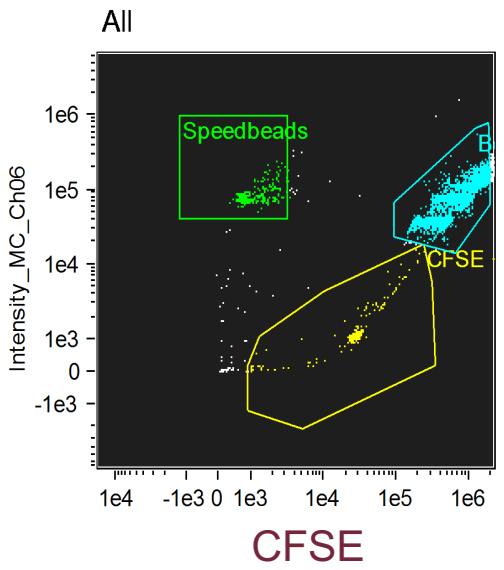
100 nm



200 nm



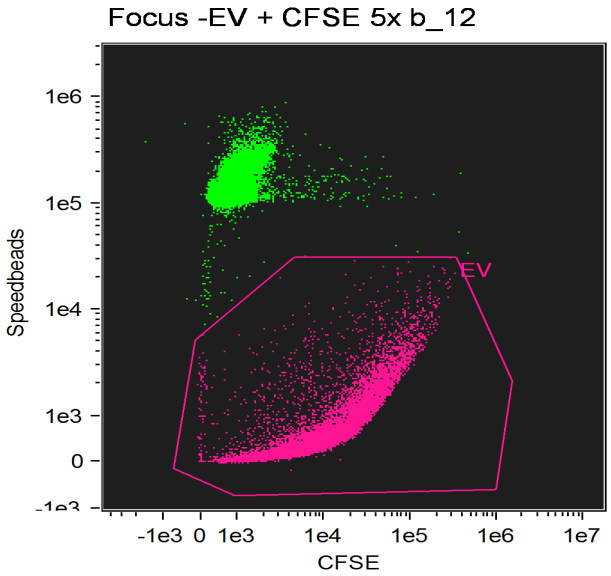
500 nm

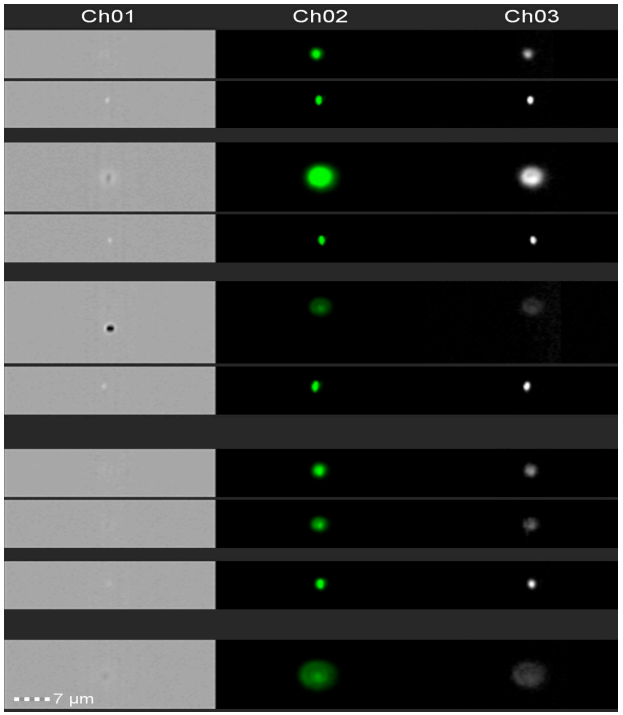


Gemeten aantallen / ul :

Verdunnig	100 nm	Aantal / ul		200 nm	Aantal / ul
1000x	25 / 4,233 ul	6		335 /6,42 ul	52
100x	344 / 8,147 ul	42		5078/ 12,393 ul	410
10x	5233 / 11,401 ul	459		12590 / 3,76 ul	3345

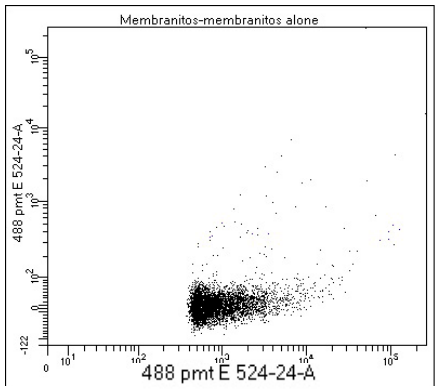
Meten van aantal CFSE EV's op de ImageStream wel mogelijk



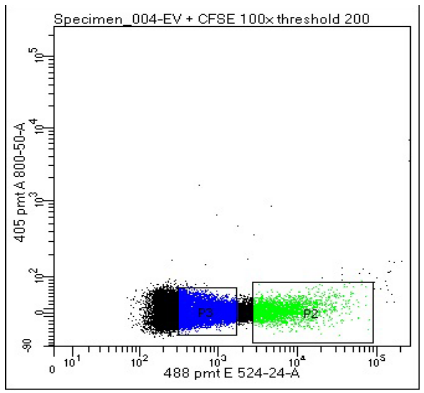


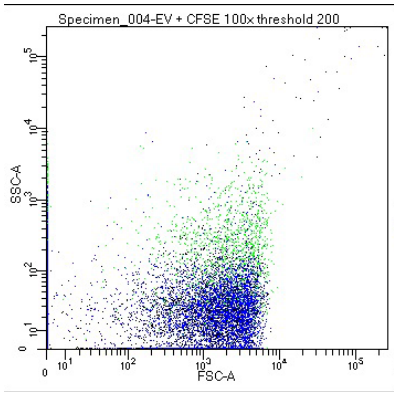
Symphony A5 meting:

CFSE medium

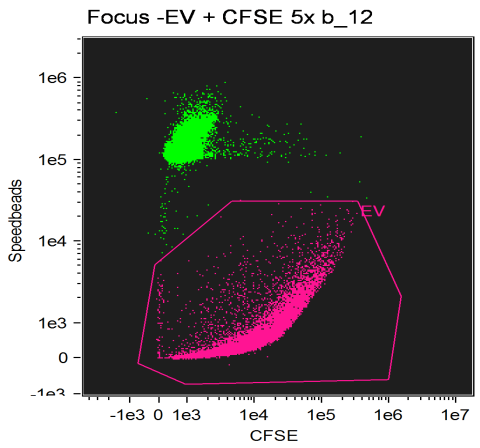


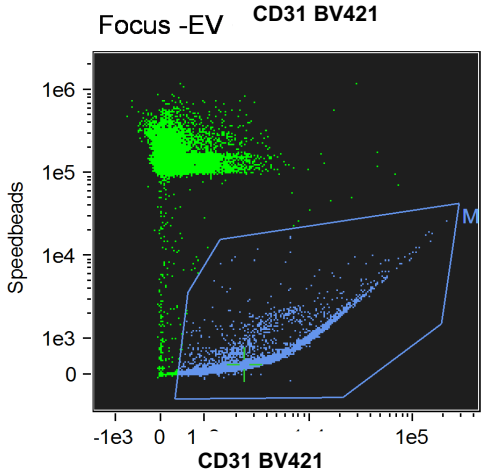
CFSE EV





Meten van aantal EVs in plasma op een Imagestream





CFSE als 100% populatie

CD31 BV 421 als sub-populatie

Gemeten aantallen / ml :

Monster	CFSE + / ml	CD31 BV421 + /ml	flow cytometer
PBS	51.944	22.678	142.335
Donor 1	1.652.851	111.490	Swarm effect
Donor 2	5.206.069	1.930.944	Swarm effect
Donor 3	4.058.597	1.445.077	Swarm effect

Maar ook voor analyseren van bijv. kolom materiaal

Kwaliteitscontrole kolom materiaal (Sepharose).

Conclusie / Vragen ??

Erik Mul (Core Facility manager)
Simon Tol
Mark Hoogenboezem
Tom Ebbes
Kim Falize

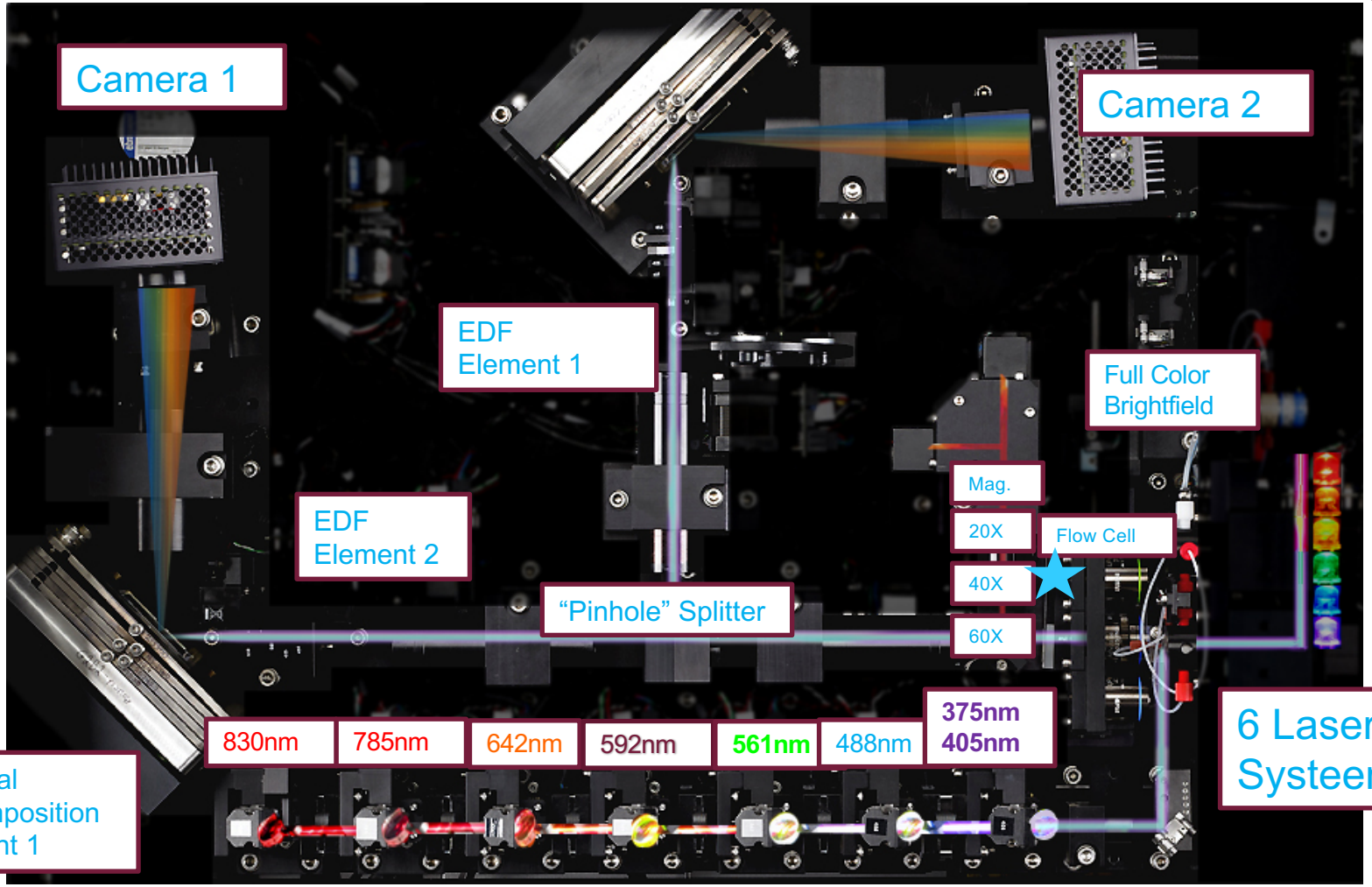
Is er al een Cell-sorter gebaseerd op imaging ??

Sanquin

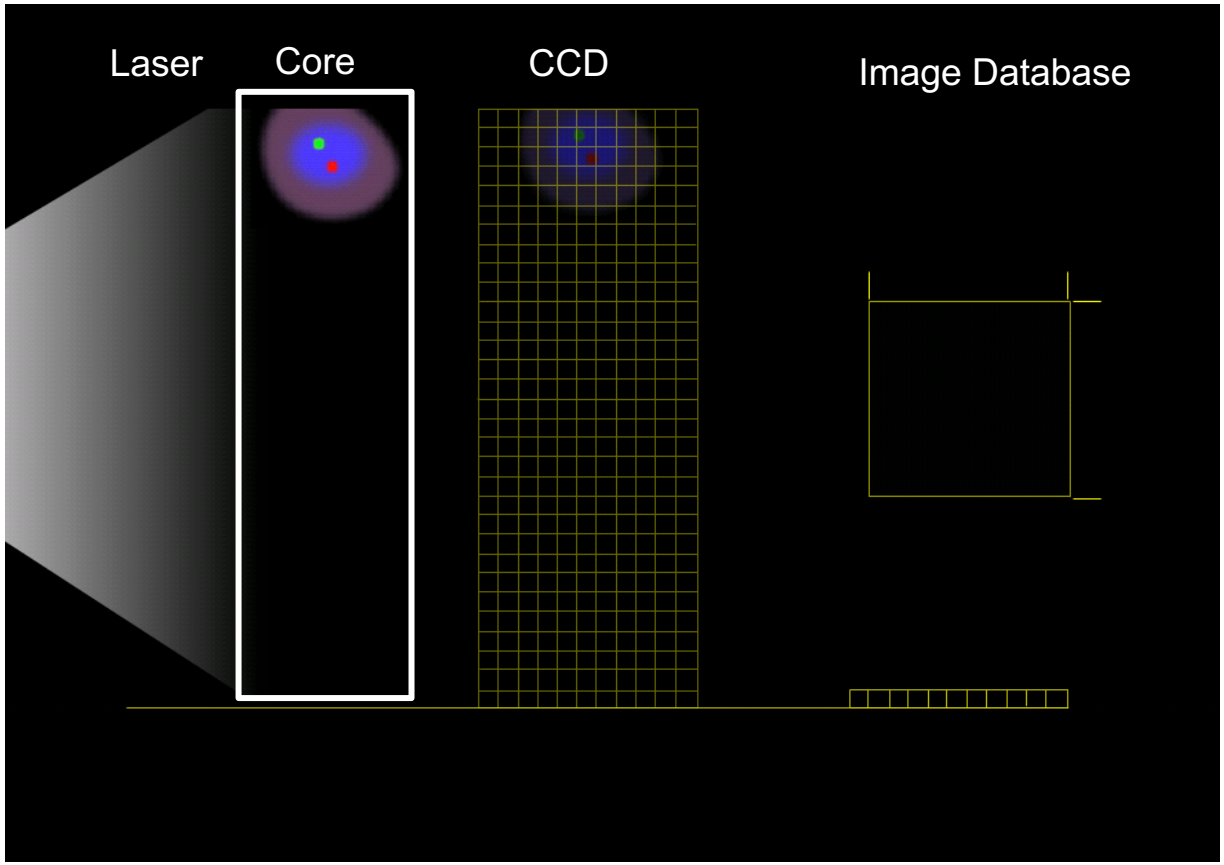
UNION BIOMETRICA

Large Particle Analysis & Sorting

ImageStreamX Mark II (van binnen)



Time Delay Integration (TDI)



- 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 a high resolution.