BD Accuri™ C6 Flow Cytometer

Nil Emre, PhD

BD Biosciences

R&D Manager, Stem Cells and Cell Function

The BD Accuri C6 Flow Cytometer System

- An affordable, full-featured, easy-to-use flow cytometer
- Two lasters and six detectors

The BD Accuri C6 Flow Cytometer

Innovations in all the major components of a flow cytometer

- **Fluidics**: Peristaltic pumps and pulse dampeners allow miniaturization and direct-volume measurement
- **Optics:** Locked-down alignment
- **Signal detection:** Broad dynamic range obviates voltage adjustments
- **Software:** Intuitive

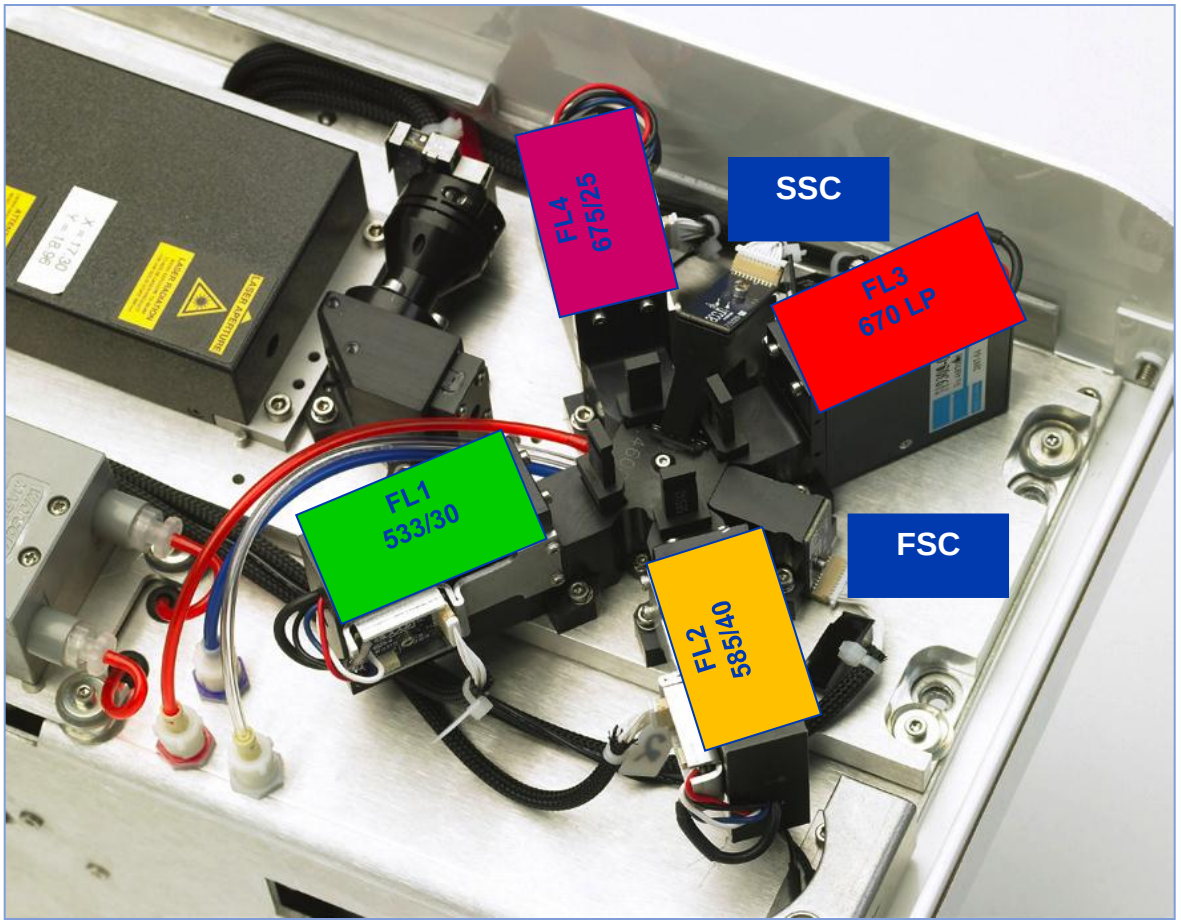
Alignment and Signal Detection are Optimized and Locked Down

488-nm
solid state laser

640-nm diode
laser

PMTs for
fluorescence
detection

Diodes for light
scatter detection



BD

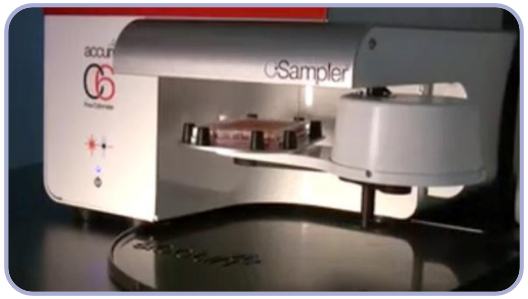
Advantages of Pre-optimized Detector Settings

- Greatly reduces the risk of lost data due to improper setup
  - Saves time and sample
- No specialist training or dedicated operator required
- Predictable, reproducible analysis relative to the sample type and application
- Predictable fluorescence spillover

BD

Enhanced Sample Handling

- Direct volume measurement
- Many types of sample tubes may be used.
  - Flow cytometry tubes
  - Microcentrifuge tubes
  - Ninety-six-well plates with the BD CSampler™ accessory



- Open system conducive to kinetic studies
- BD CSampler™ accessory for automated sample introduction

Intuitive Software

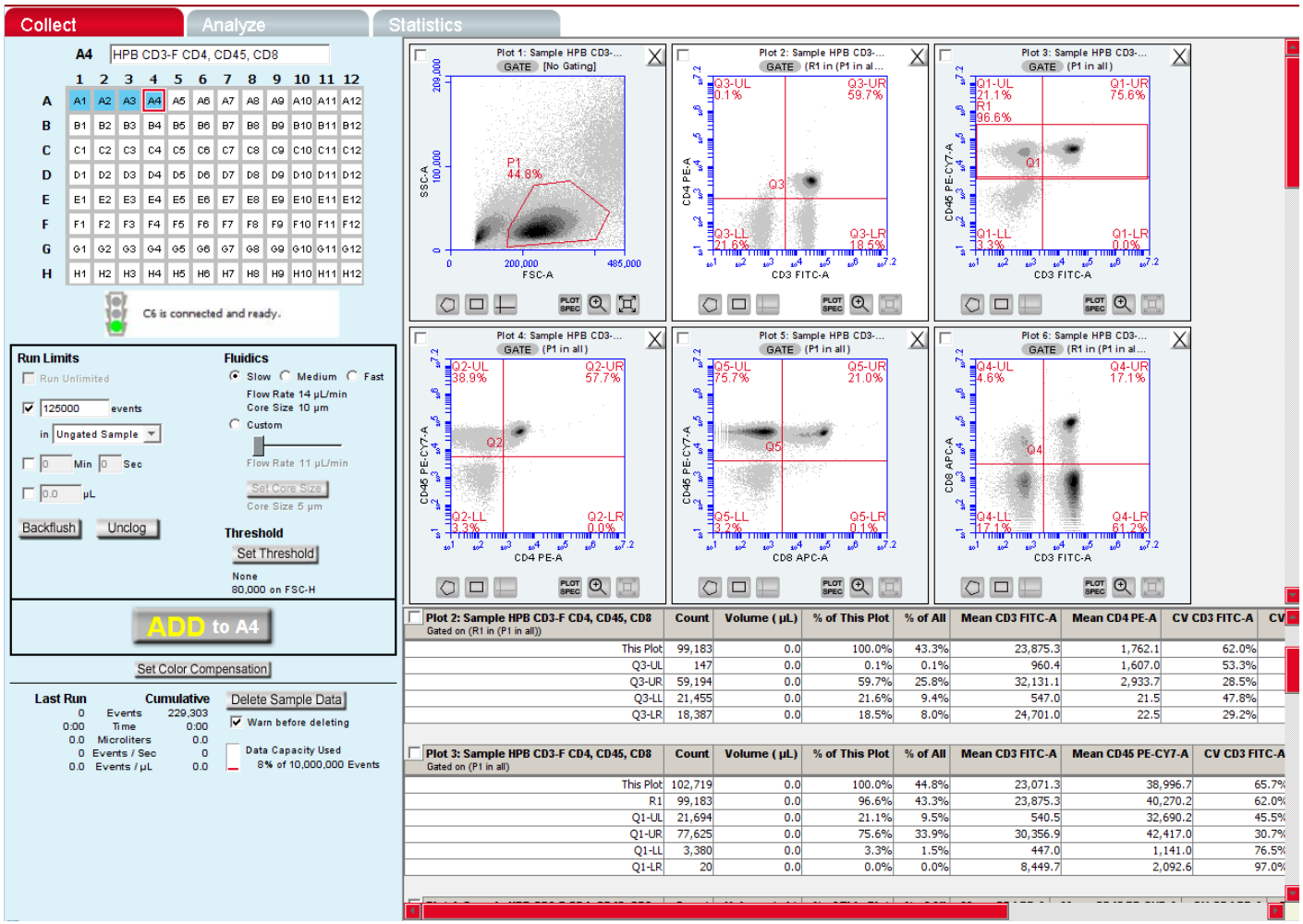
Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates



Histogram, Dot Plot, and Density Plot Display Area

Analysis and Gating Tools

Plot Statistics

Kits and Templates on the BD Accuri C6

- “As Easy as Cell Analysis Is Going to Get” by combining Cell Biology Kits with acquisition and analysis templates.
- Free downloadable BD Accuri C6 Software Templates are matched to each kit at:
  - www.bdbiosciences/go/templates
- Kits and Templates lead to quick and easy set-up and analysis of cell populations.

Kits and Templates on the BD Accuri C6

Available Templates:

Category	Product Information Sheet	Brand	Kit	Cat. No.	Template
Cell Biology	BD Apoptosis Kits and Templates	BD Pharmingen™	Annexin V FITC Apoptosis Detection Kit II	556570	Download
		BD Pharmingen™	Annexin V PE Apoptosis Detection Kit I	559763	Download
		BD™	MitoScreen (JC-1) Kit	551302	Download
		BD Pharmingen™	Caspase-3 PE Assay Kit	550914	Download
		BD Pharmingen™	Caspase-3 FITC Assay Kit	550480	Download
	BD Cell Cycle and DNA Kits and Templates	BD Cycletest™ Plus	DNA Reagent Kit	340242	Download
		BD Pharmingen™	FITC BrdU Flow Kit	559619	Download
		BD Pharmingen™	APC BrdU Flow Kit	552598	Download

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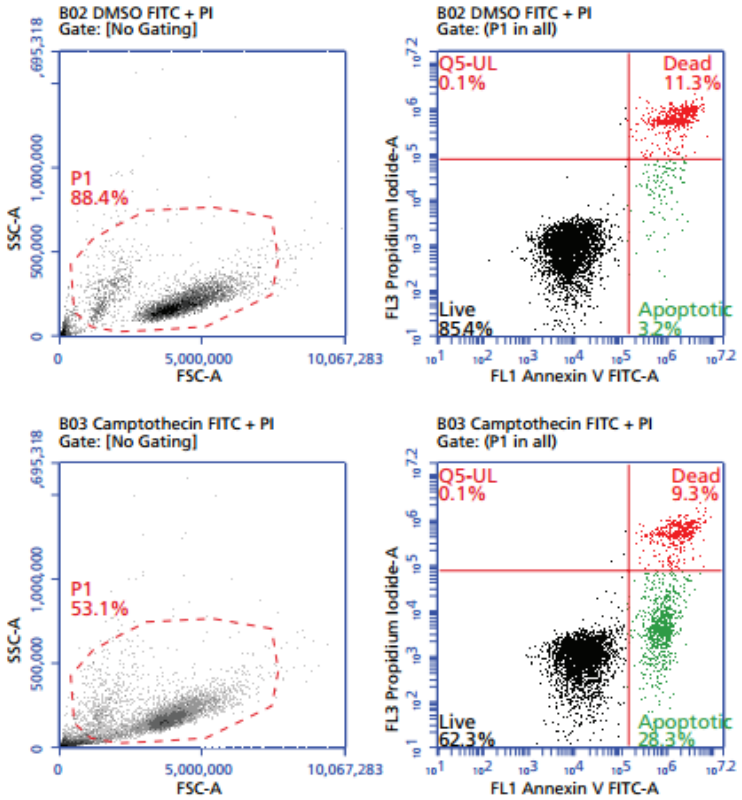
		BD™	MitoScreen (JC-1) Kit	551302	Download
		BD Pharmingen™	Caspase-3 PE Assay Kit	550914	Download
		BD Pharmingen™	Caspase-3 FITC Assay Kit	550480	Download
	BD Cell Cycle and DNA Kits and Templates	BD Cycletest™ Plus	DNA Reagent Kit	340242	Download
		BD Pharmingen™	FITC BrdU Flow Kit	559619	Download
		BD Pharmingen™	APC BrdU Flow Kit	552598	Download

Sample Data:



BD Accuri C6 Promotion

Note:
US Region Only
Promotion Period: Oct 1, 2014 – Dec 31, 2014

For Additional Information…

If you have further questions:

Contact Technical Support (US) at:

877-232-8995, Prompt 3, 2

or email: ResearchApplications@bd.com

Please visit our BD Accuri C6 resources site at:
www.bdbiosciences.com/resources/accuri.

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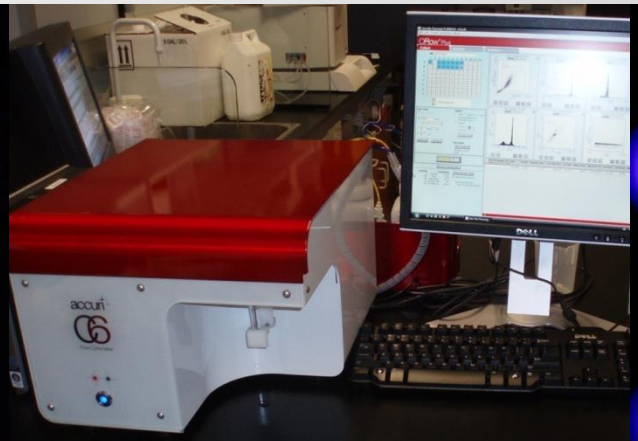
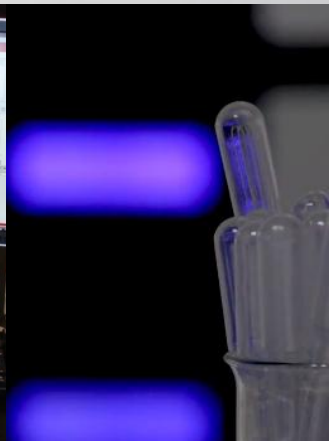
Real Time Flow Cytometry

ISAC International Society for Advancement of Cytometry
ISAC Scholars Program

EXCYTE EXPERT CYTOMETRY

CORE TECHNOLOGIES AT UCD CONWAY INSTITUTE





BD FACS440

Cytometry 9:138–142 (1988)

Flow Cytometric Measurements of Cytoplasmic Calcium Changes in Human Platelets[1]

Theresa A. Davies, Daniel Drotts, Gary J. Weil, and Elizabeth R. Simons[2]

Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

Received for publication June 26, 1987; accepted September 30, 1987

Measurements of cytosolic Ca^{++} in individual cells. Platelets loaded with Indo-1 were diluted to 10×10^6 per ml in HEPES buffer, pH 7.4. Fluorescence measurements were obtained by using a FACS 440 dual laser system (Becton Dickinson) equipped with a Consort 40/PDP-11/23 microcomputer (Digital Corp.). A HEPES

of probe. Thrombin (0.001–0.05 U/ml) was then added directly to the sample and data were collected at the rate of 4,000 cells per time point after stimulation, at approximately 5-s intervals, with the first time point at 10 s. All data were collected in the linear mode to allow

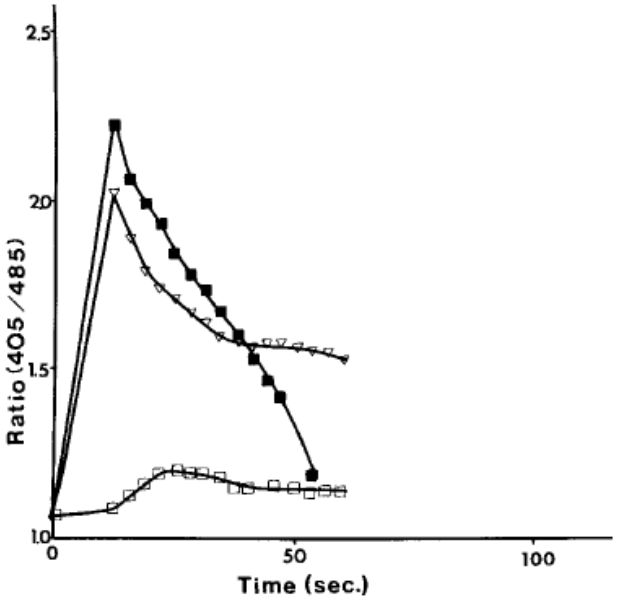


FIG. 2. Time course of the thrombin-induced change in $[Ca^{++}]_{in}$ in responding and nonresponding populations of human platelets. The 405-nm/485-nm ratio of Indo-1 loaded platelets after thrombin stimulation by a 0.005 U/ml (subsaturating) thrombin dose vs. time. The responding population (■) and the nonresponding population (□) were determined by gating above baseline on the FACS. Also plotted is the total platelet response, which illustrates an average of responding and nonresponding populations (▽).

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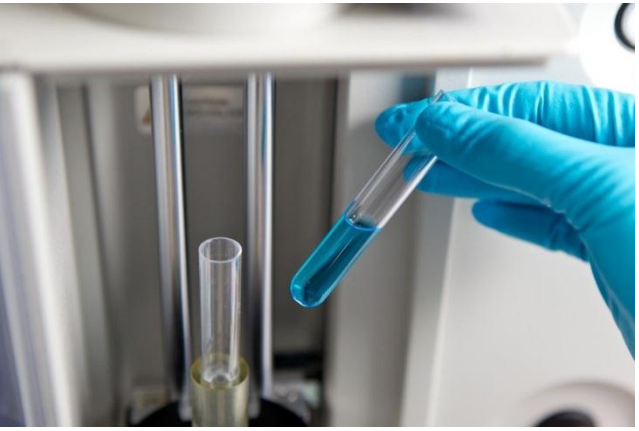
Stop-flow method

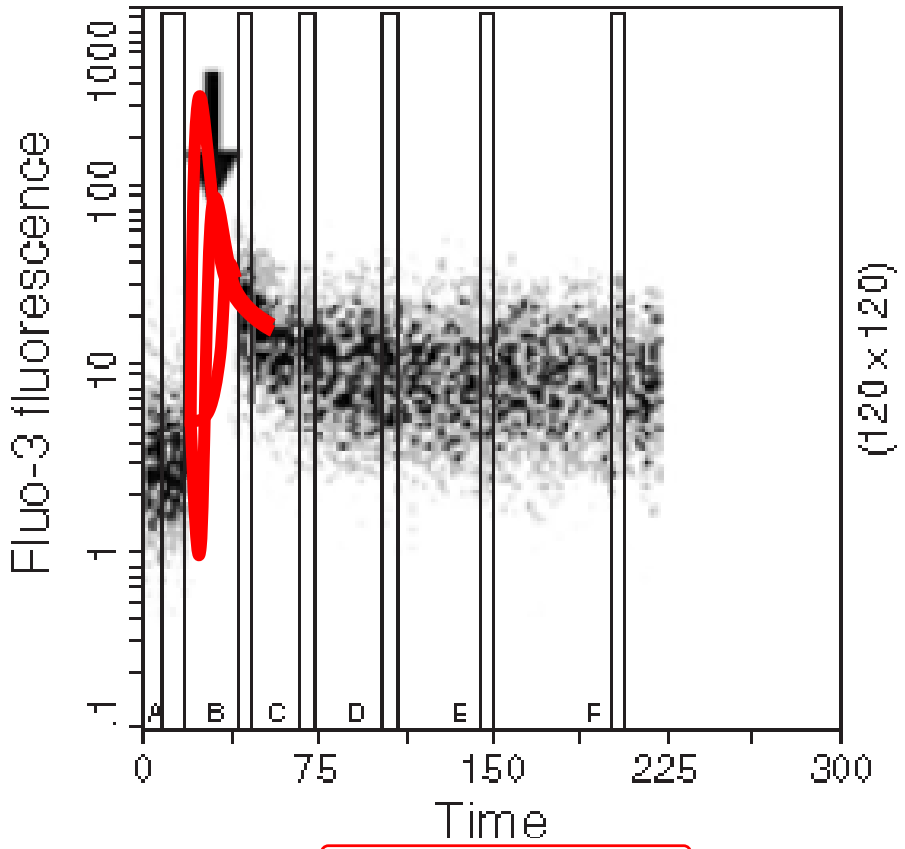


Flow Cytometric Analysis of Calcium Mobilization in Whole-Blood Platelets

Contributed by Maria-do-Céu Monteiro, Maria-José Gonçalves, Filipe Sansonetty, and José-Enrique O'Connor

Current Protocols in Cytometry (2003) 9.20.1-9.20.8

D



Panel D shows the procedure and time course of a typical kinetic assay of intracellular Ca^{2+} mobilization

Continuous measurement

2009

TECHNICAL NOTE

Cytometry PART A
Journal of the International Society for Advancement of Cytometry

A Flow-Cytometric Method for Continuous Measurement of Intracellular Ca^{2+} Concentration

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[1]UCD School of Biomolecular and Biomedical Science, UCD-Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

[2]Flow Cytometry Core Facilities, UCD-Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

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Email: alfonso.blanco@ucd.ie

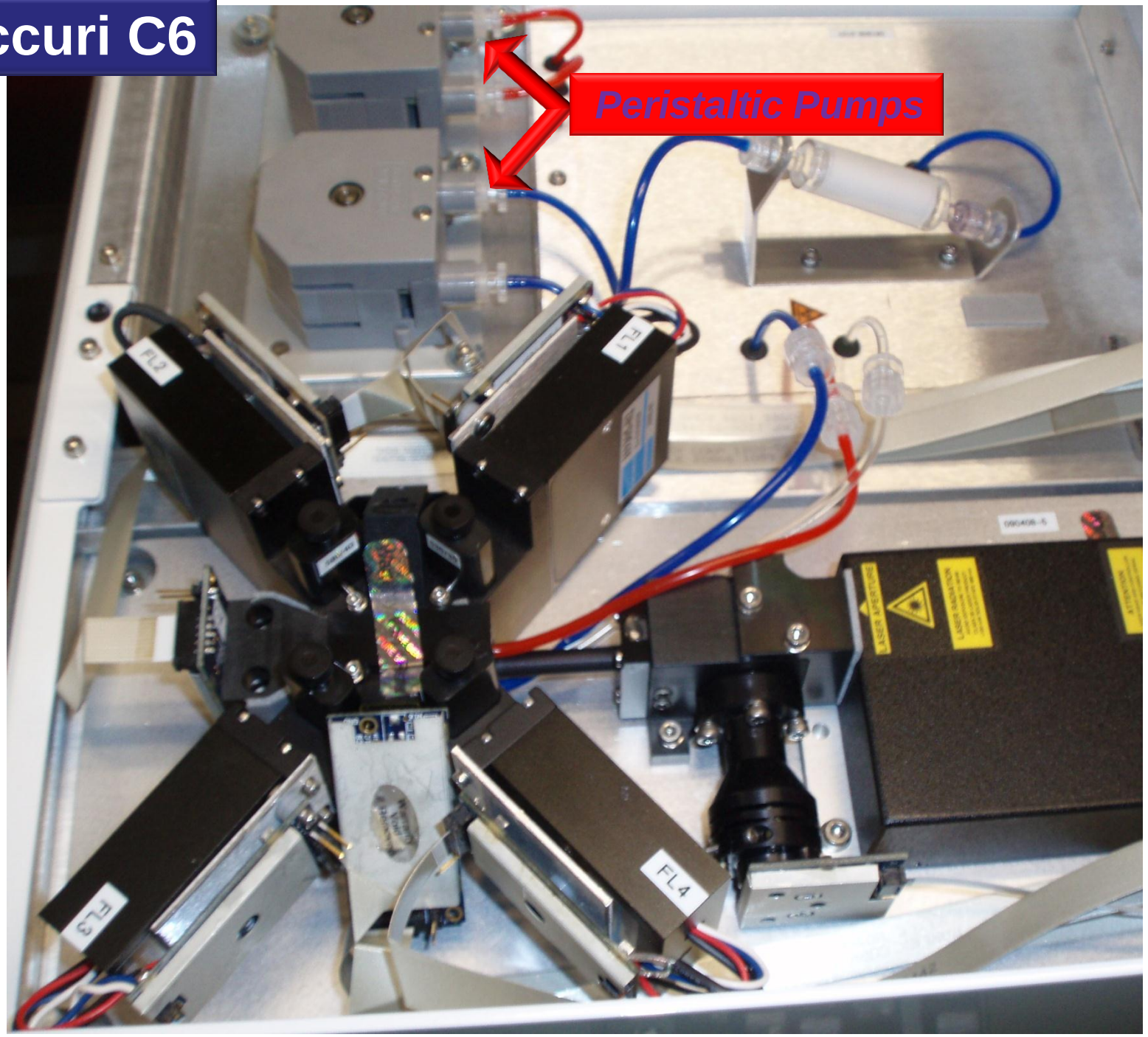
Published online 24 September 2010 in Wiley Online Library

• **Abstract**

Alterations in intracellular Ca^{2+} concentration are amongst the most rapid responses to a variety of stimuli in mammalian cells. In the nervous system in particular, responses occur within nanoseconds. A major challenge in intracellular Ca^{2+} analysis is to achieve measurements within this very fast time frame. To date, the dynamic intracellular Ca^{2+} concentration has been monitored by confocal microscopy, plate-based assays, spectrofluorometry, and flow cytometry, although there are issues with the number of cells analyzed or gaps in recording due to the addition of compounds, with significant loss of detail of a rapid Ca^{2+} response. The new generation of flow cytometers (such as Accuri C6) resolves this problem by allowing the addition of test compounds with continuous monitoring of thousands of cells, providing a method for dynamic Ca^{2+} measurements. This system was tested with commonly used Ca^{2+} modulating agents in C6 glioma cells. Thapsigargin (TG), a blocker of Ca^{2+} uptake into the endoplasmic reticulum (ER), causes a significant increase in the intracellular calcium concentration via ER emptying followed by Ca^{2+} entry via store-operated Ca^{2+} channels (SOCC). This well-established pathway can be partially inhibited by 2-aminoethoxydiphenyl borate (2-APB), a blocker of SOCC. Both the increase with TG alone and the partial increase when coincubated with 2-APB were observed with continuous recording along with calibration curves using an Accuri C6 flow cytometer. With these new cytometers, dynamic Ca^{2+} concentration measurement becomes extremely accessible and accurate, while also providing extensive and valuable data regarding population health and responsiveness.

• **Key terms**

BD Accuri C6



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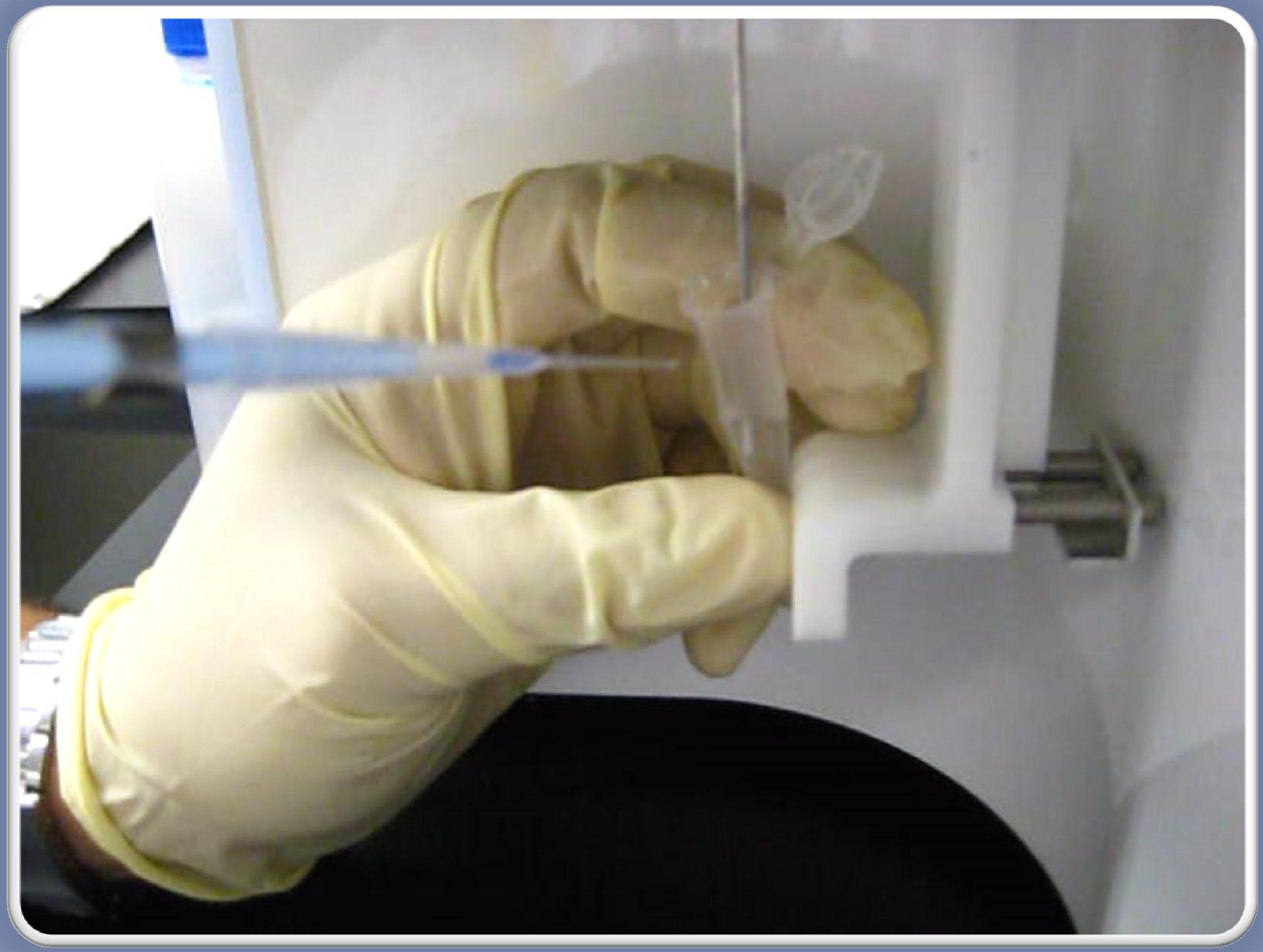
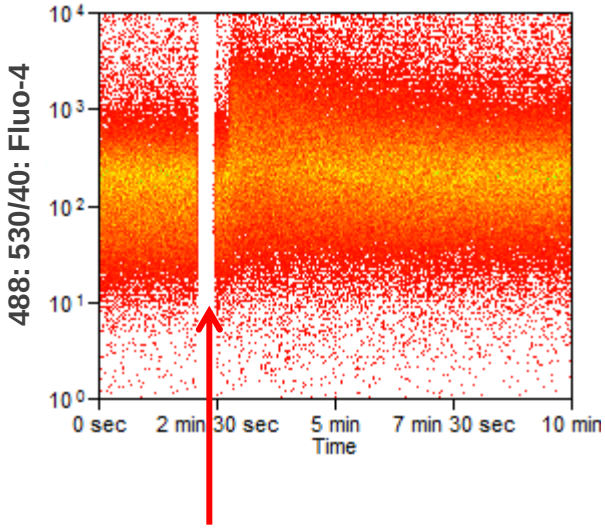
BD Accuri C6

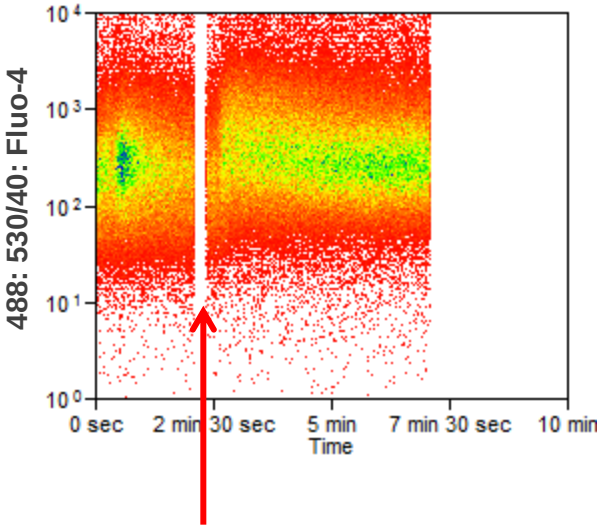
BD Accuri C6



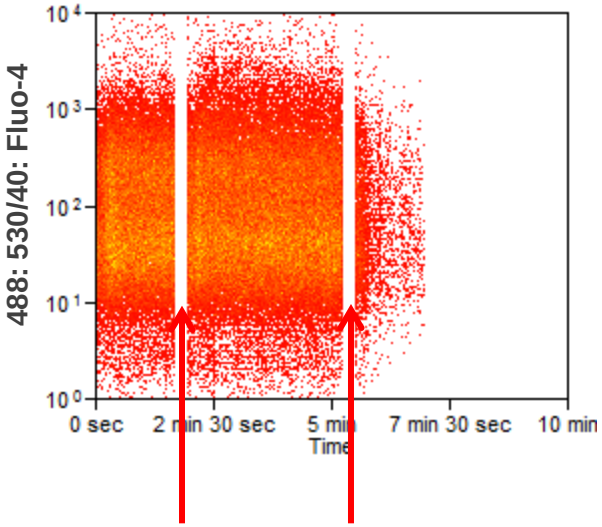
Beckman Coulter Cyan ADP:



A23187

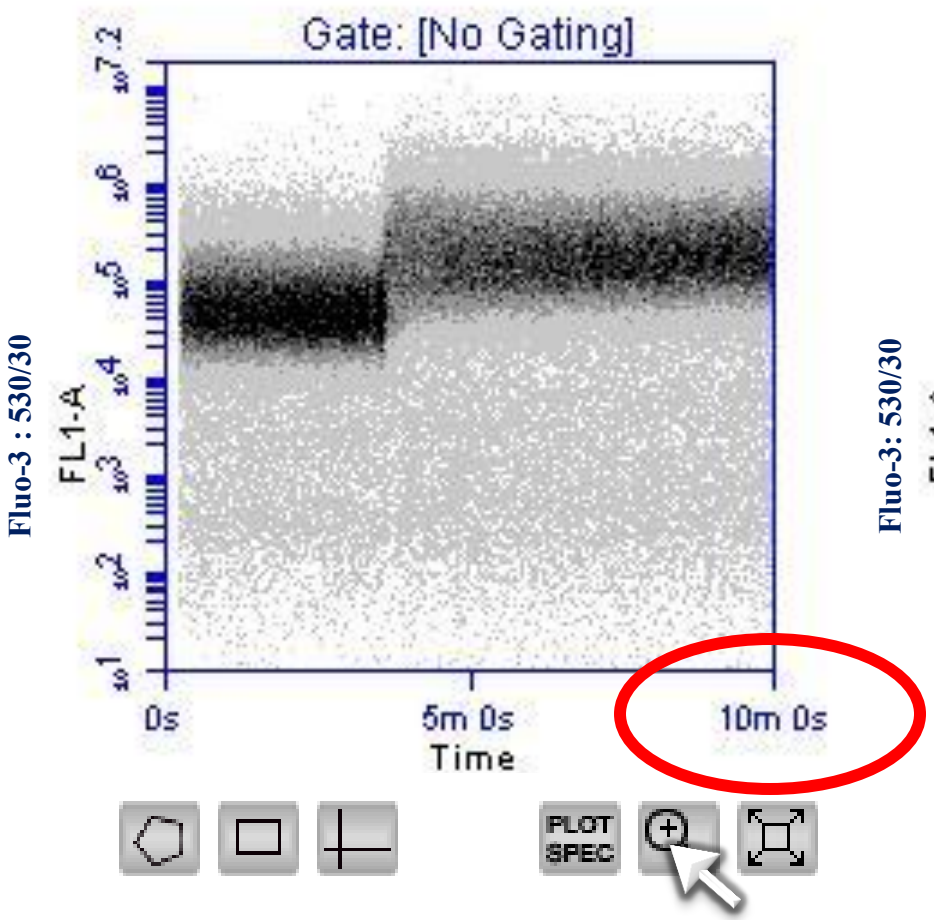


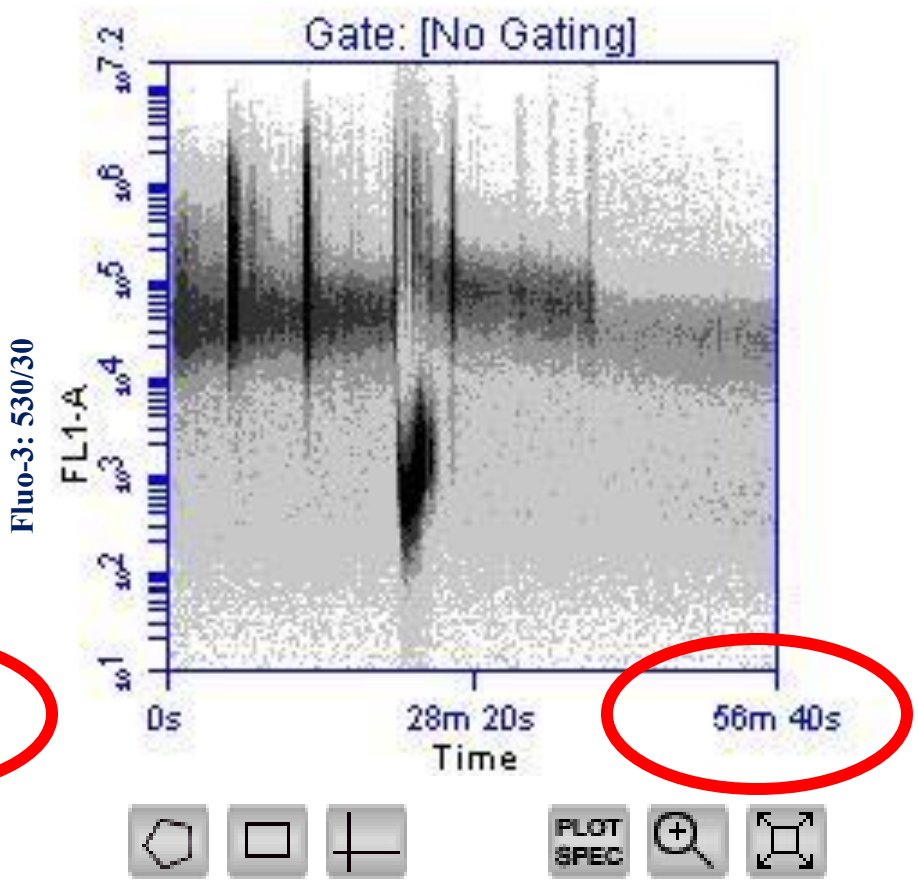
A23187



Thapsigargin A23187

BD Accuri C6. Time limitations?



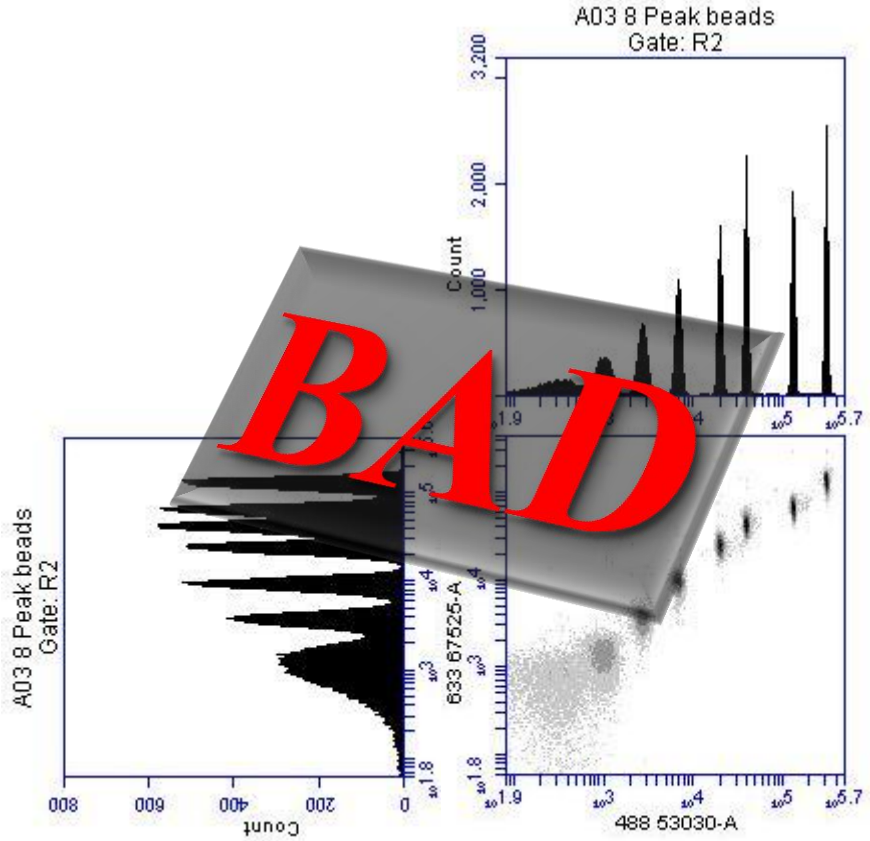


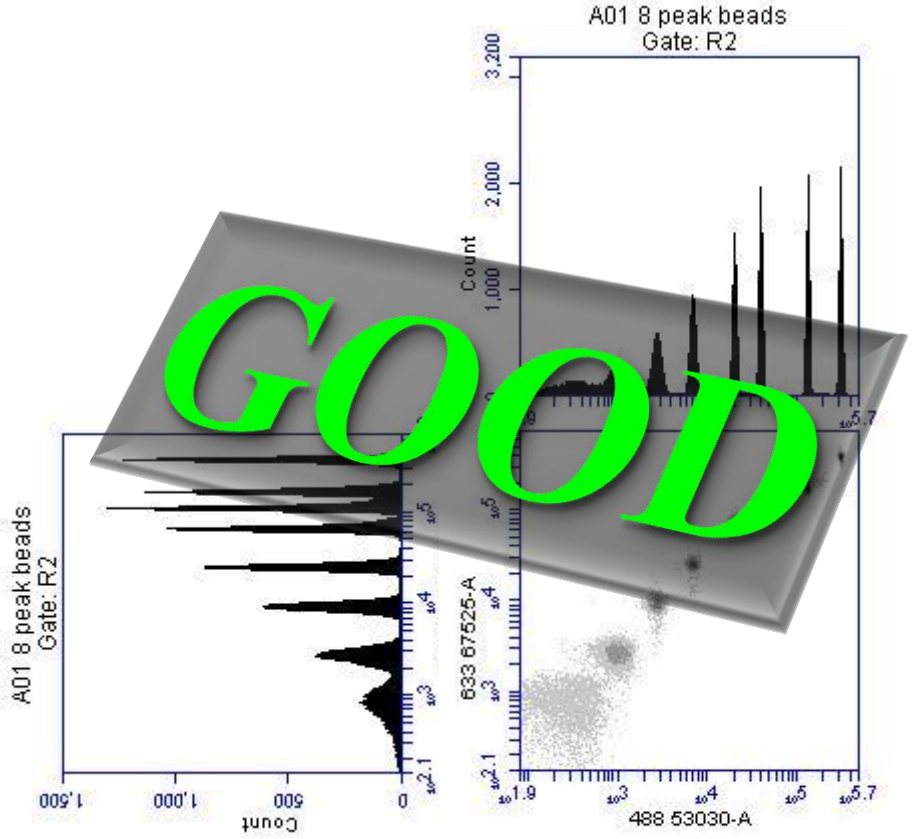
Quality Control

State of the instrument (QC)

Extremely important!!

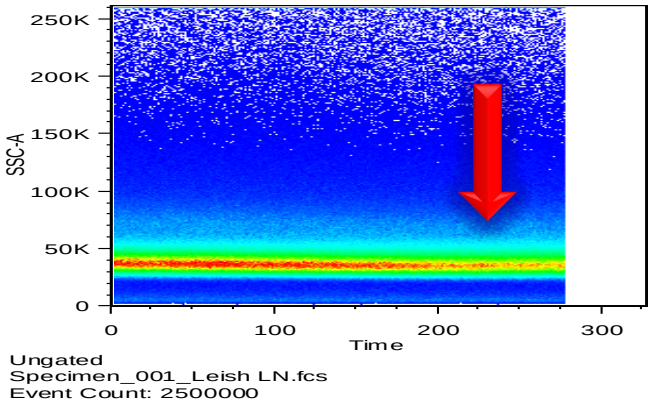
- *Laser alignment,*
- *PMT, laser state*
- *Dirty or clean instrument,...*



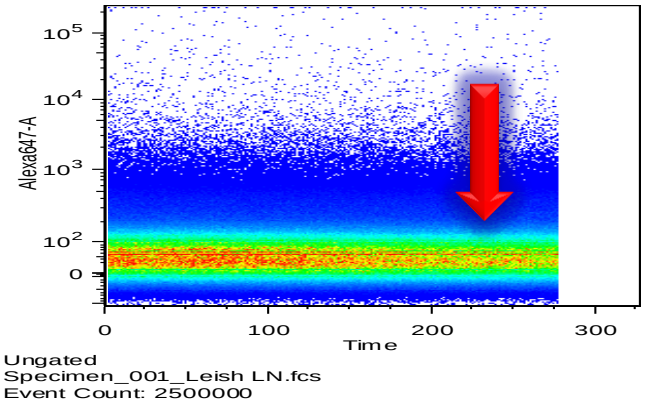


Attention!!

Scatter



Fluorescence

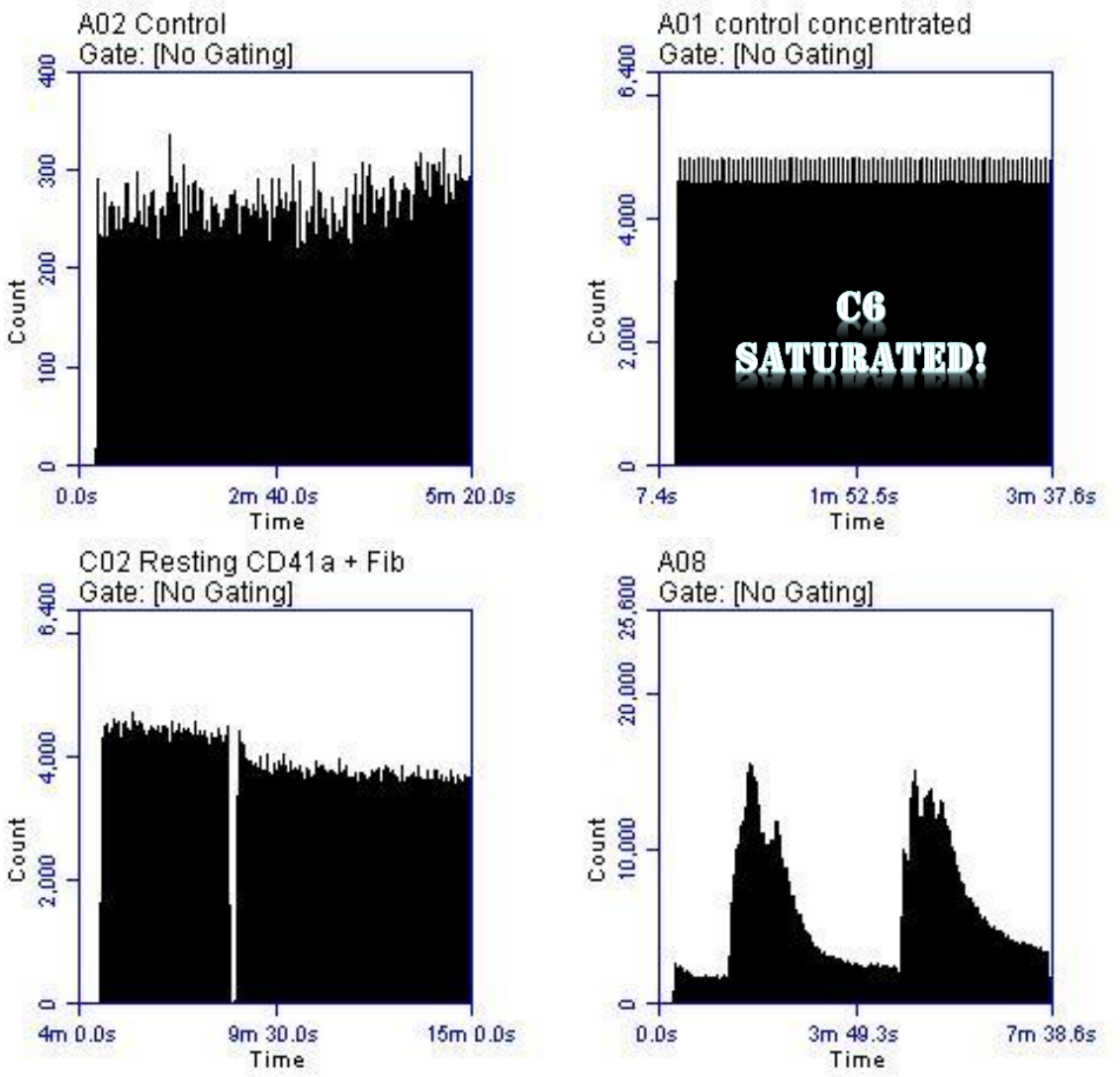


EXCYTE EXPERT CYTOMETRY

UCD DUBLIN

Slide courtesy of *ExCyte. Expert Cytometry*

Attention!!

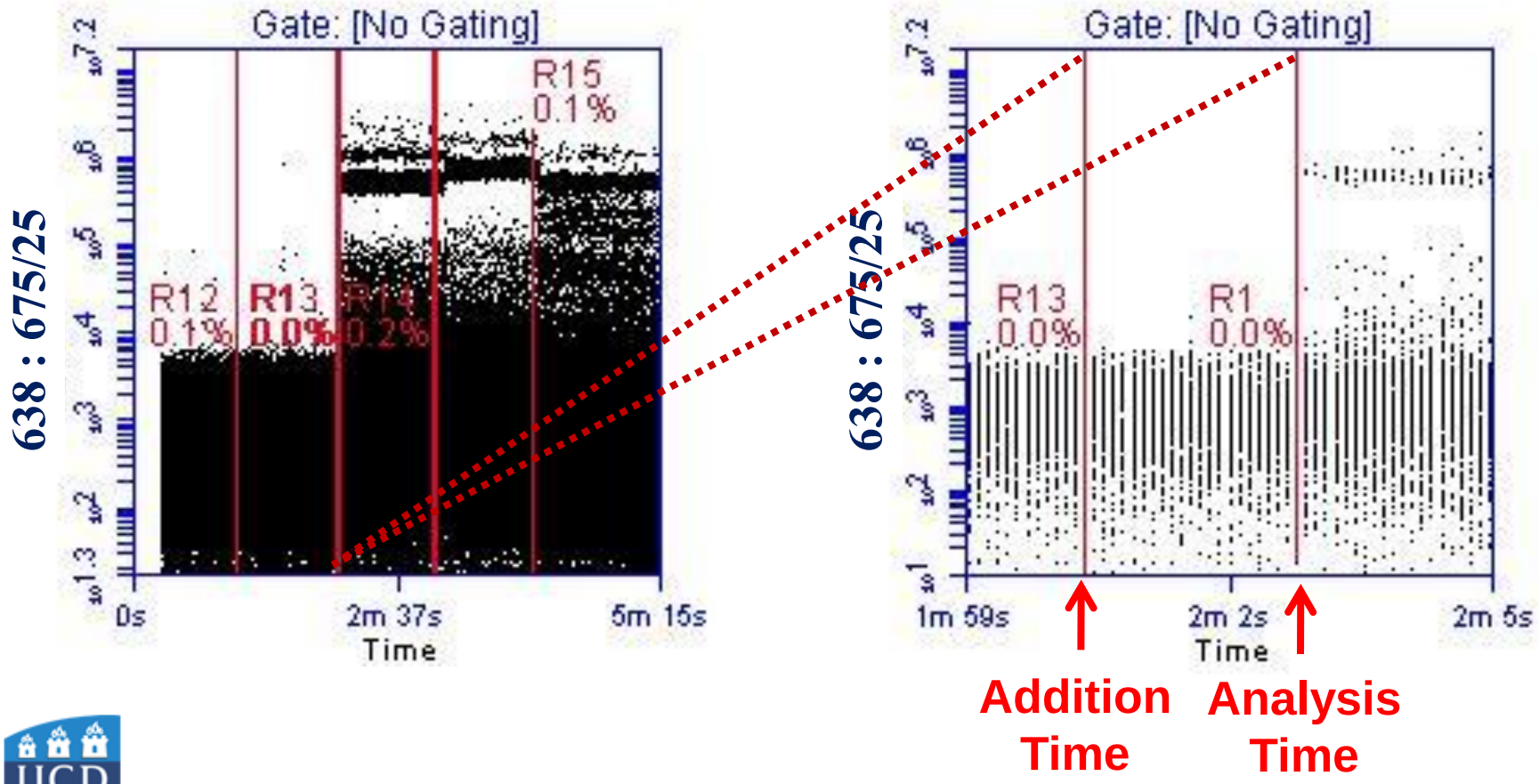


Remember! Maximum Number of Events/Well = 10^6

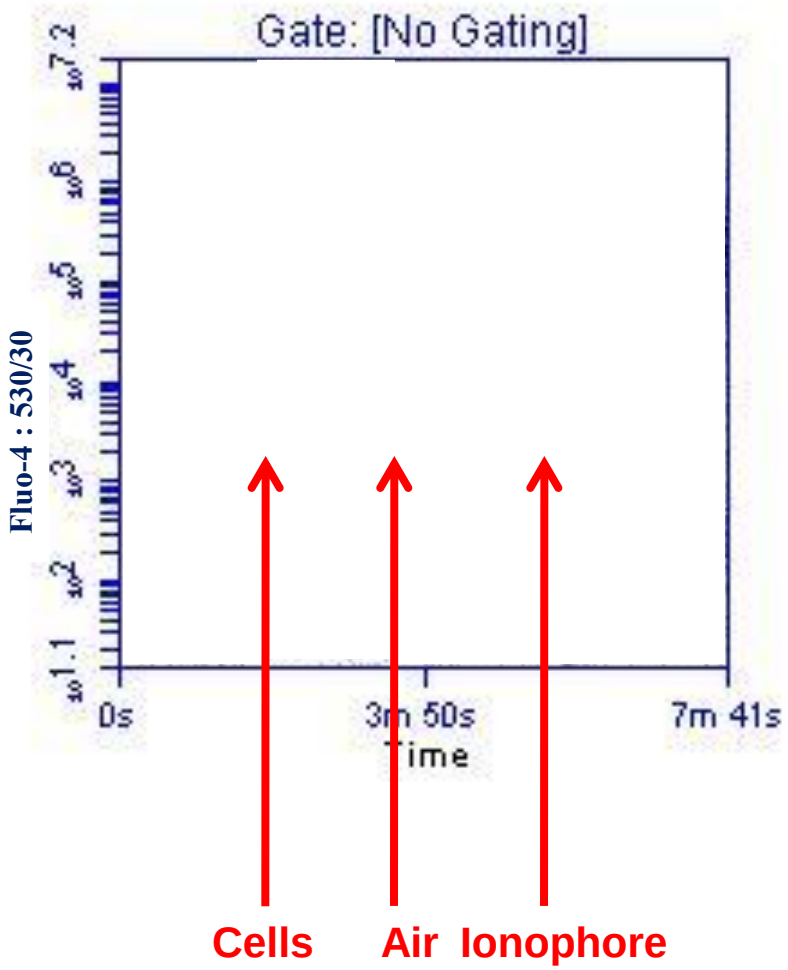
Optimise Number of Events/ sec

based on the time of your experiment

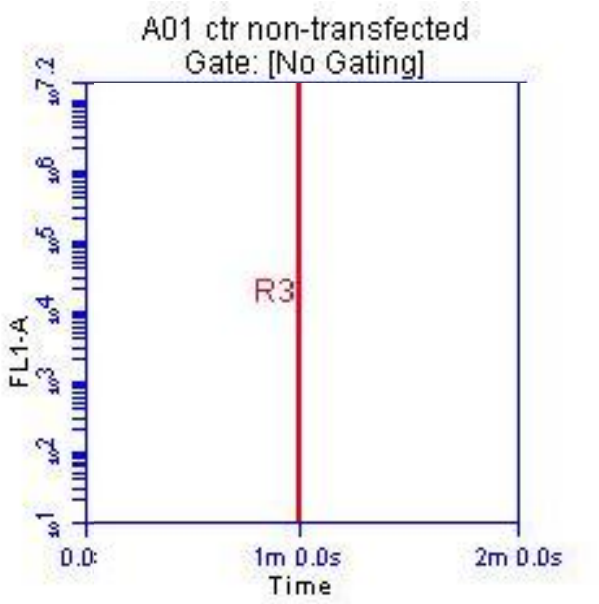
How much time between addition and analysis?

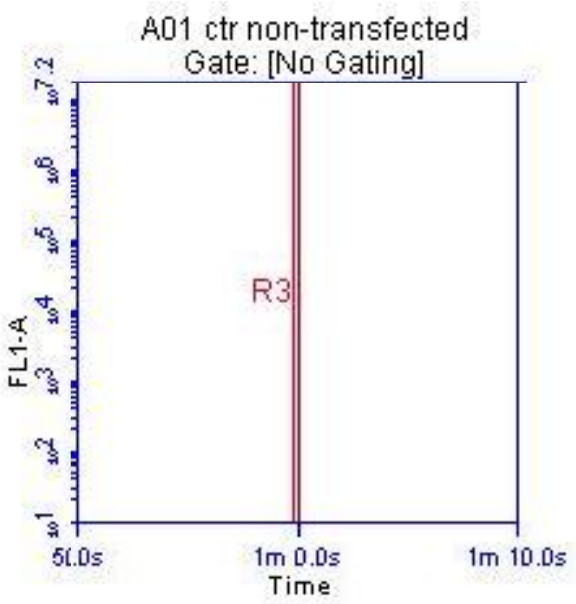


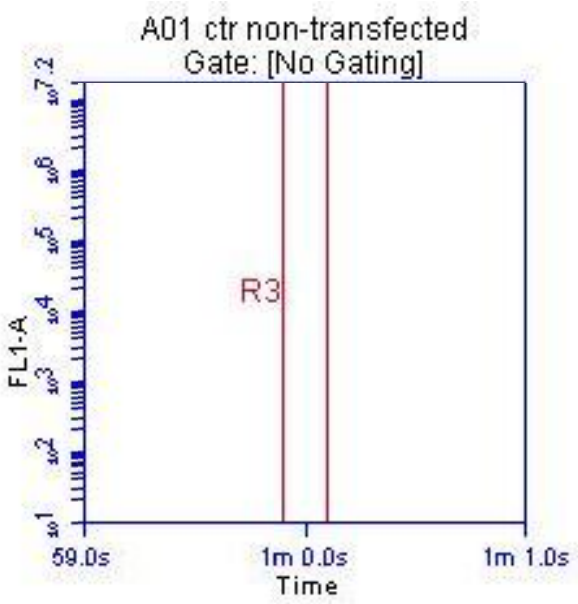
Do air bubbles affect the flow rate?



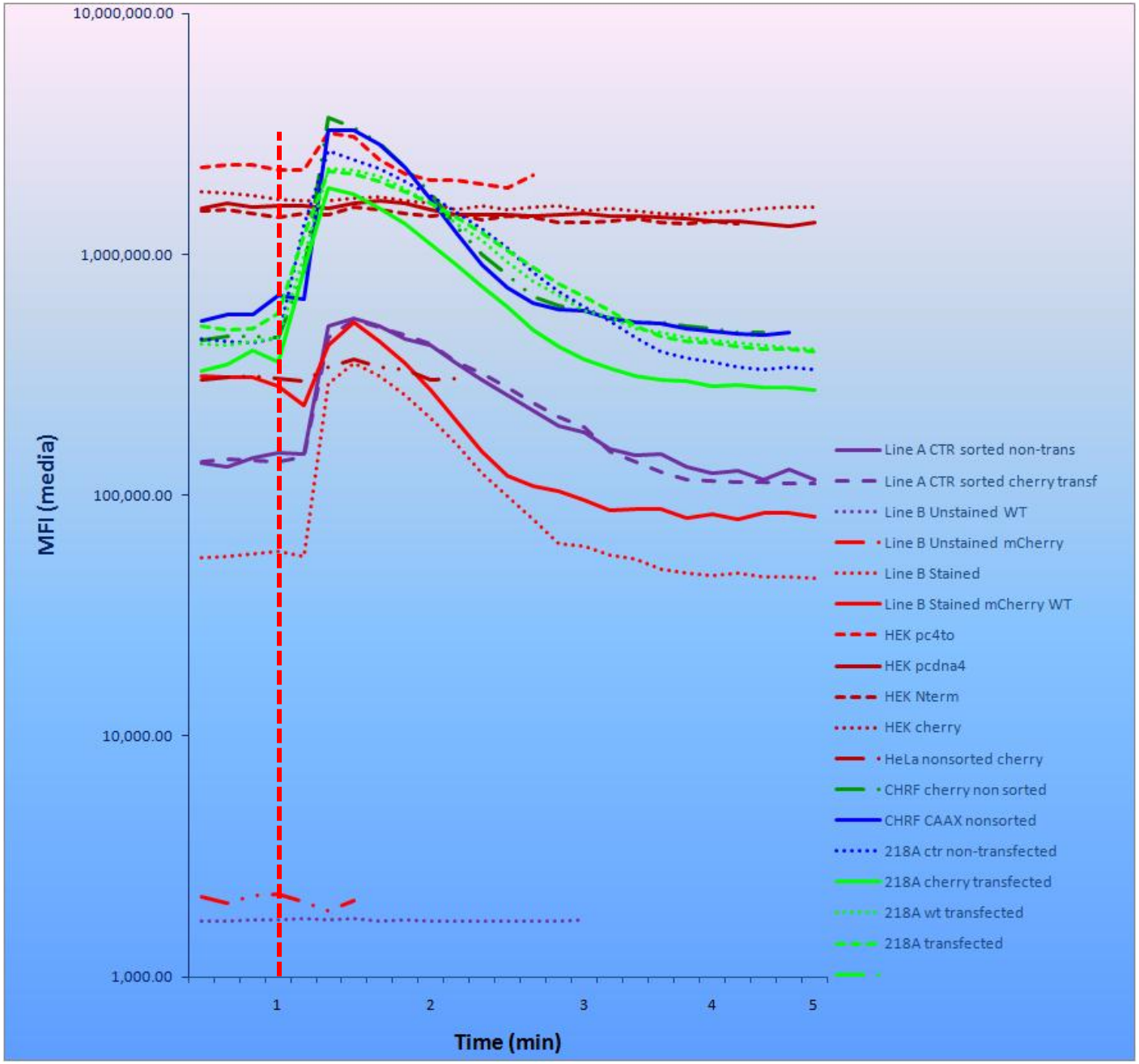
Can you control the addition time?







Calcium flux in some mCherry transfected cell lines



Real Time Applications

Calcium/
other ions

Apoptosis

GFP

Applications

Staining
optimization

Platelet
activation

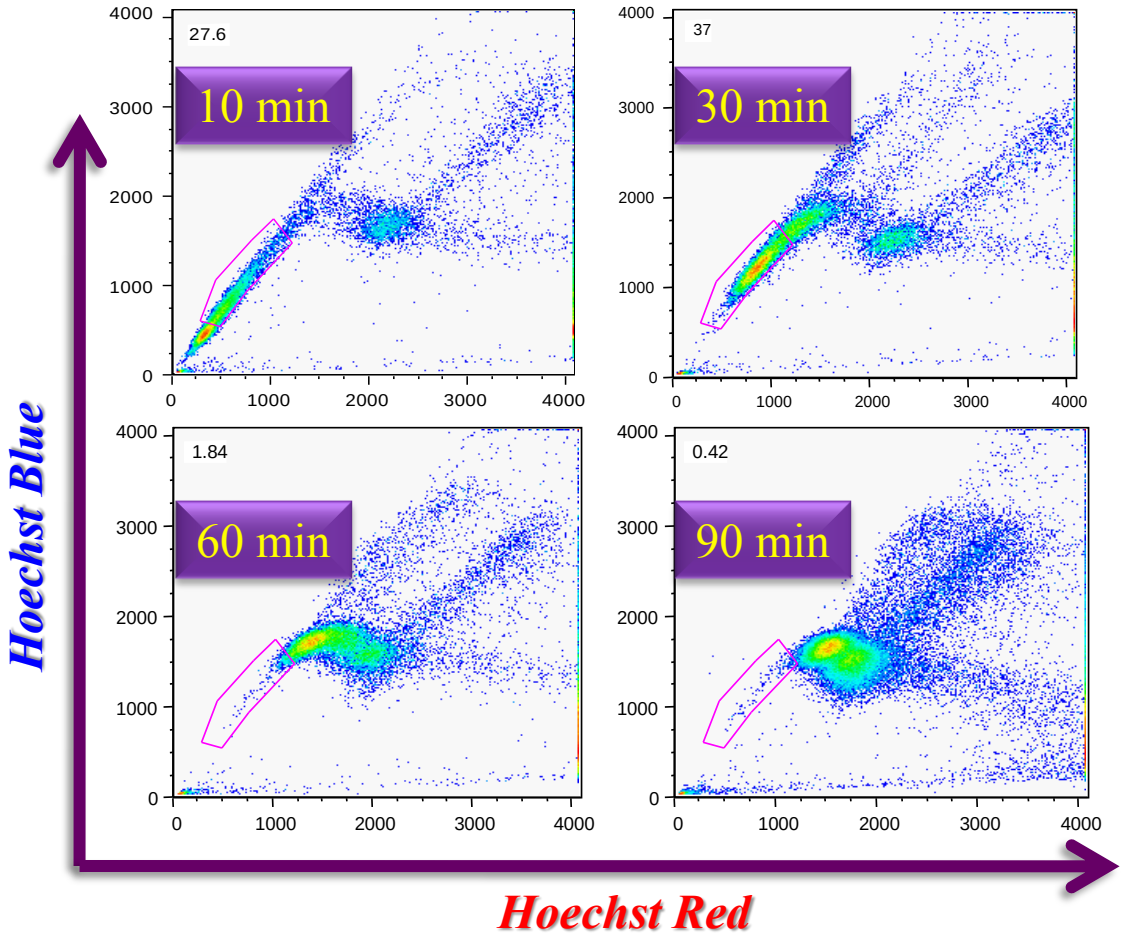
Phagocytosis/
Nanoparticle uptake

UCD
DUBLIN

Optimization

Side Population. Stem Cells

Murine Bone Marrow - 5µg/ml

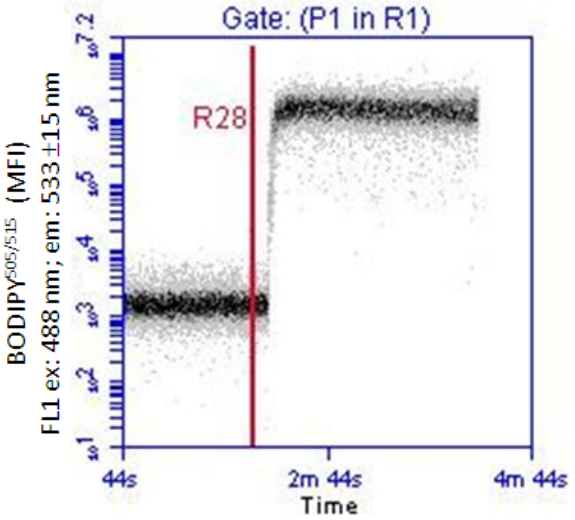


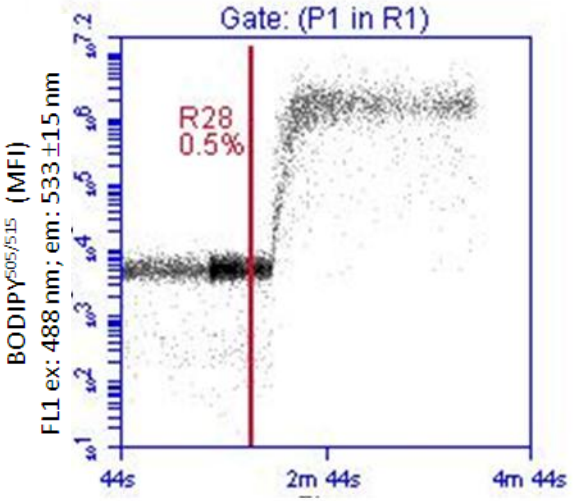
UCD DUBLIN

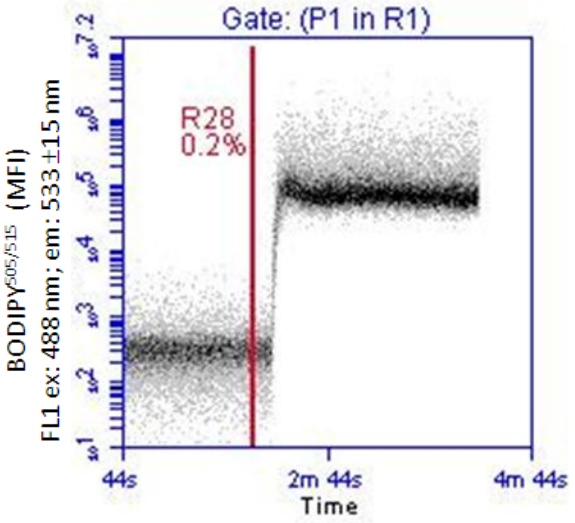
Slide Courtesy of Catherine Simpson

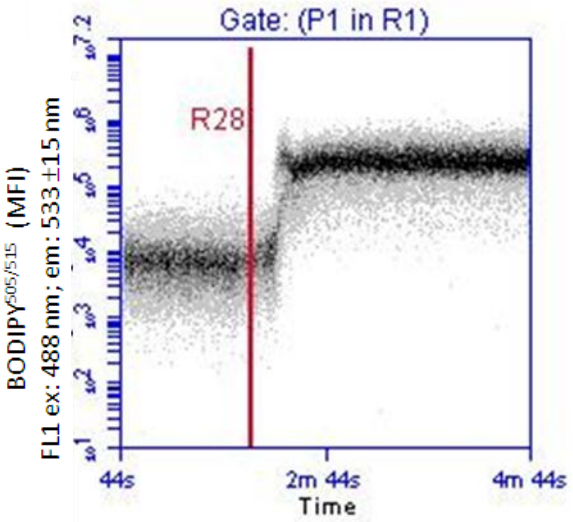
Analysis of Algae physiology

Kinetics of 4 different algae



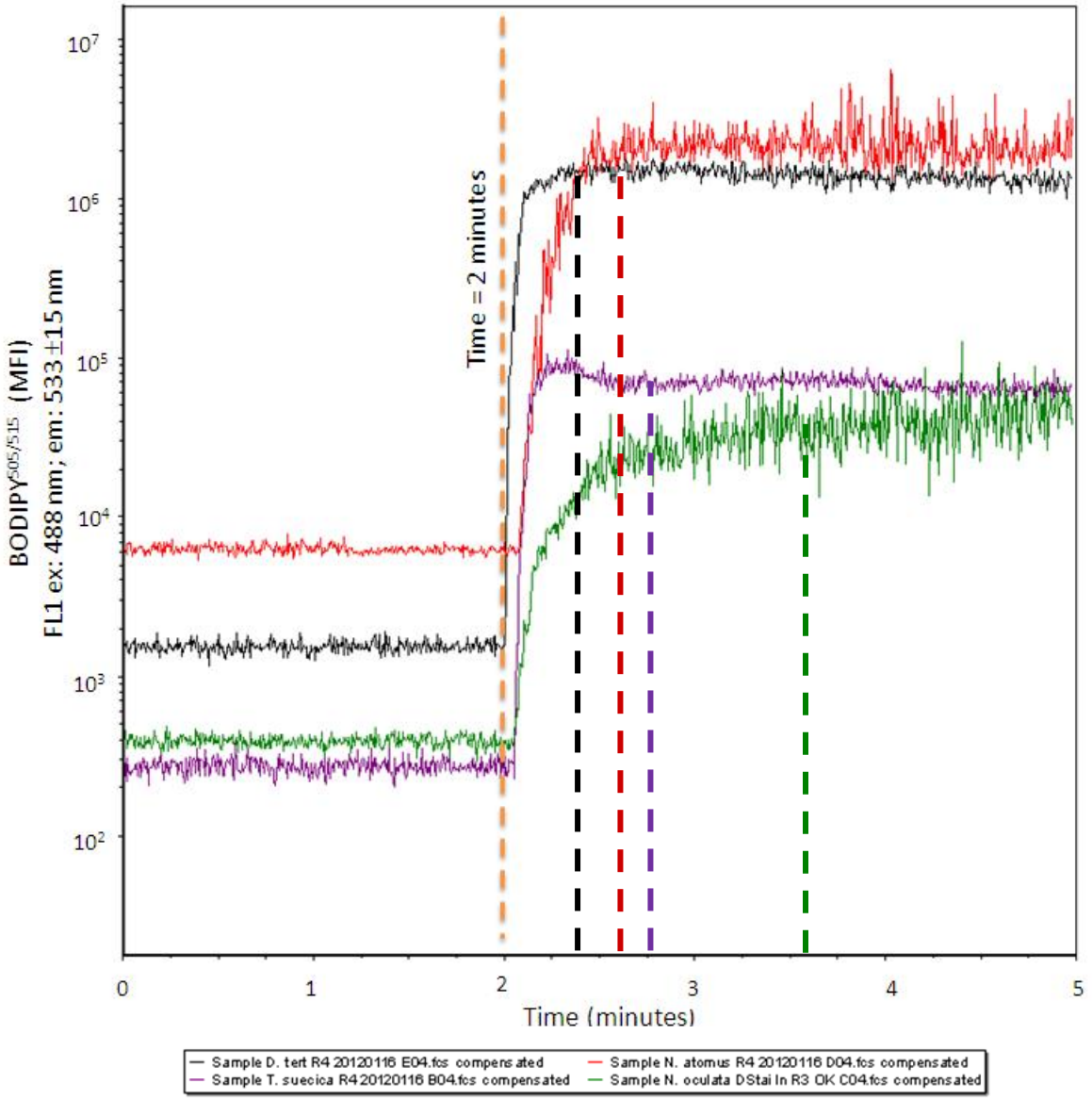




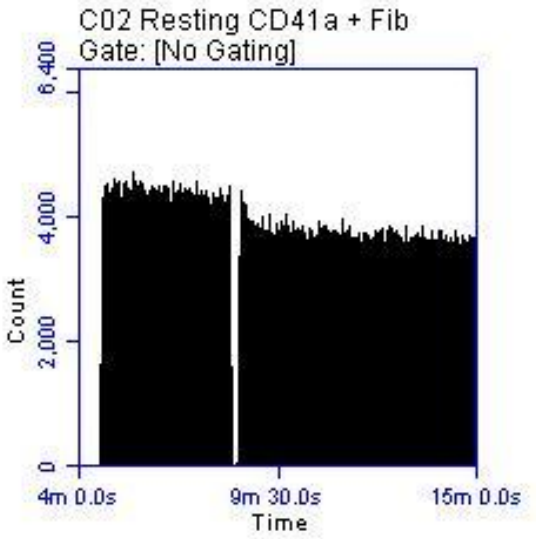


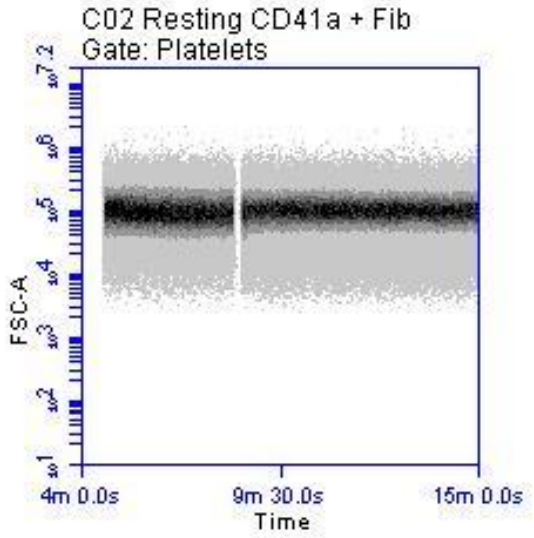
Analysis of Algae physiology

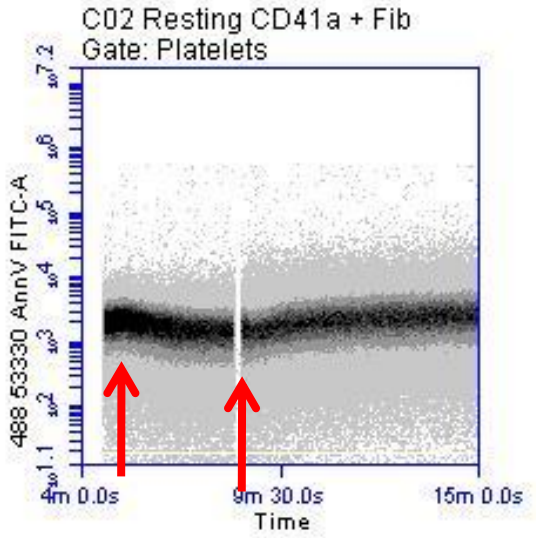
Kinetics of 4 different algae (FCS Express v.4)



Platelets


C02 Resting CD41a + Fib
Gate: [No Gating]
Count
6,400
4,000
2,000
0
4m 0.0s
9m 30.0s
15m 0.0s
Time


C02 Resting CD41a + Fib
Gate: Platelets
FSC-A
4m 0.0s
9m 30.0s
15m 0.0s
Time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Gate: Platelets
488 53330 AnnV FITC-A
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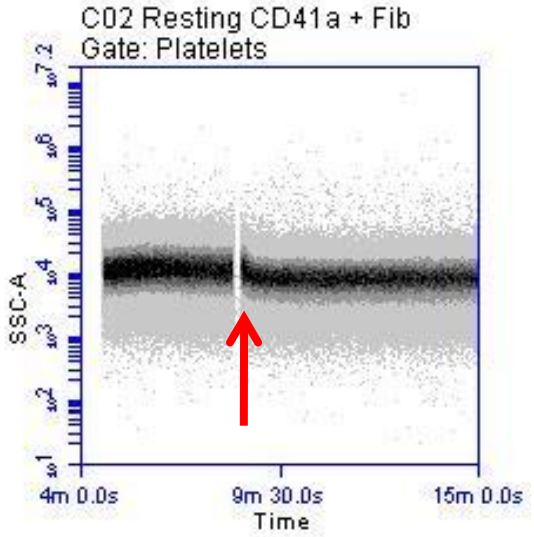
9m 30.0s
15m 0.0s
Time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SSC-A
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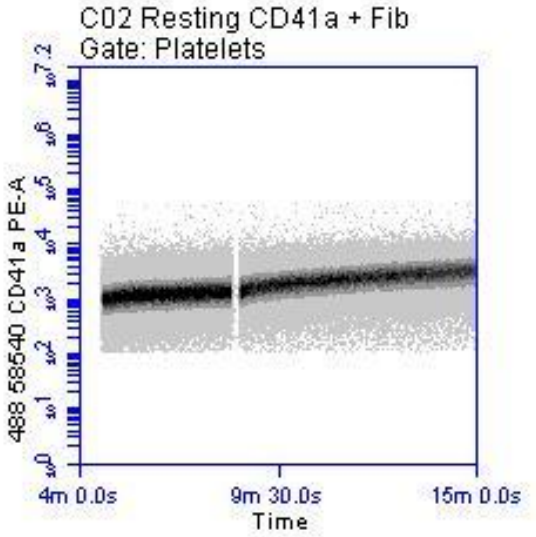
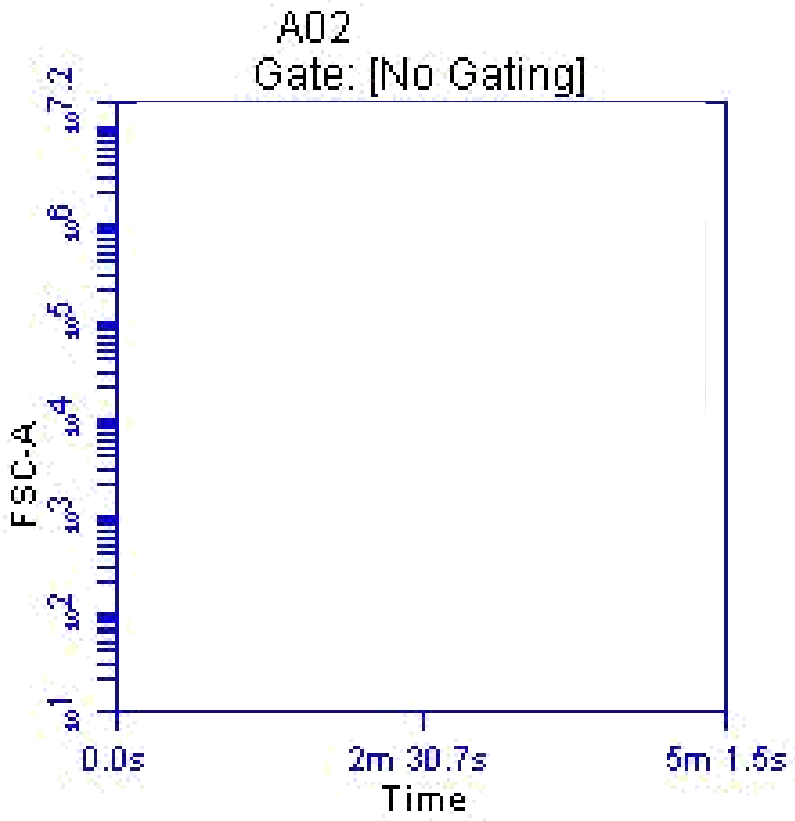
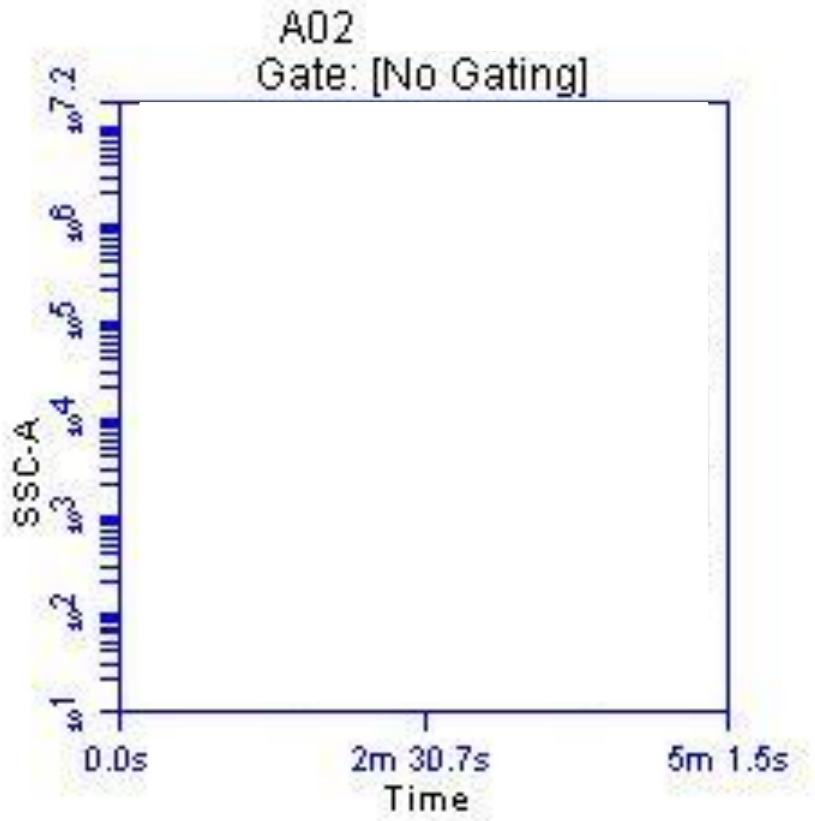
Time

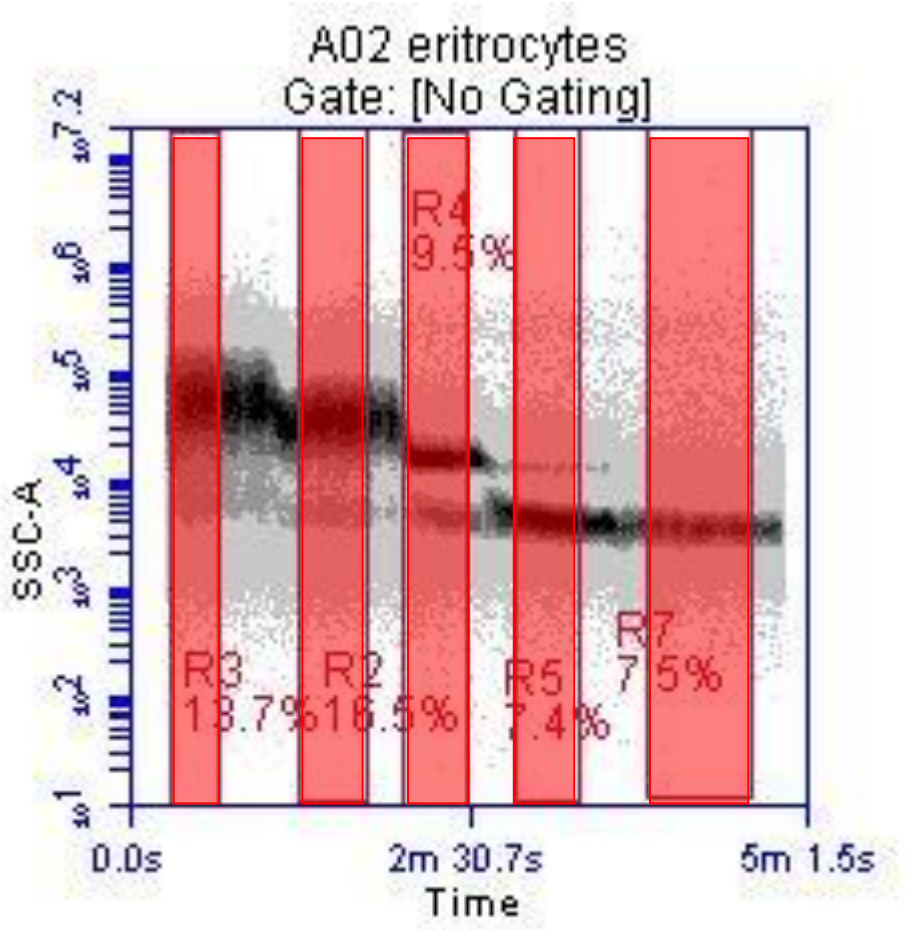

C02 Resting CD41a + Fib
Gate: Platelets
488 58540 CD41a PE-A
4m 0.0s
9m 30.0s
15m 0.0s
Time

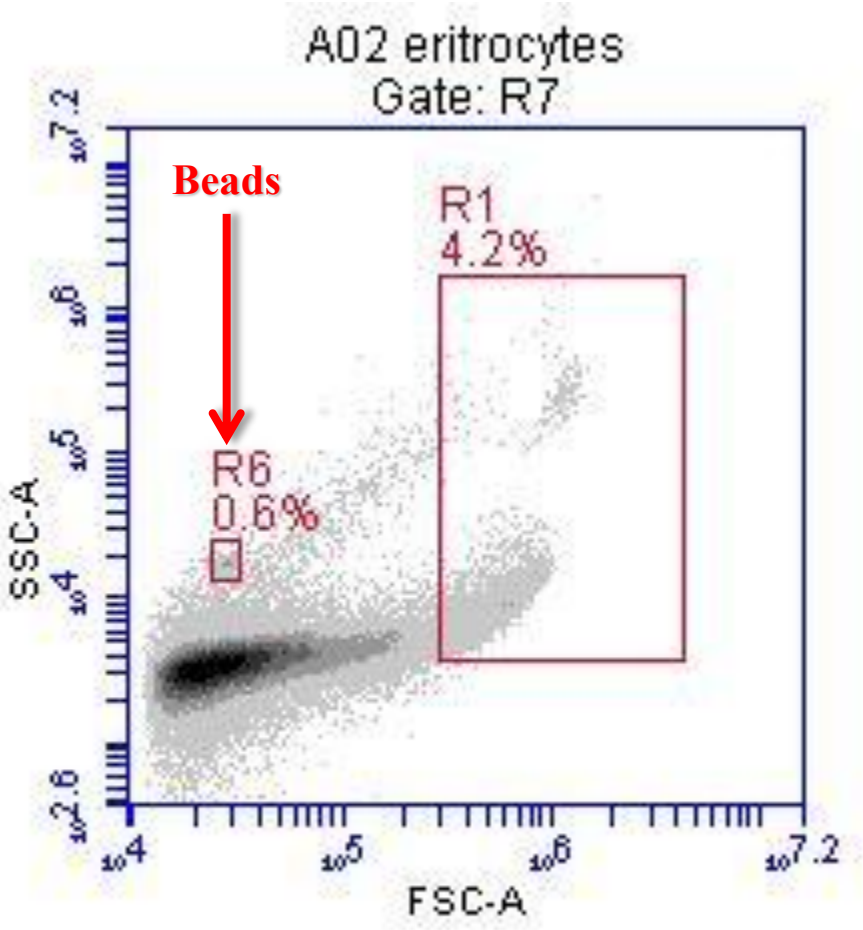
Erythrocytes Lysing



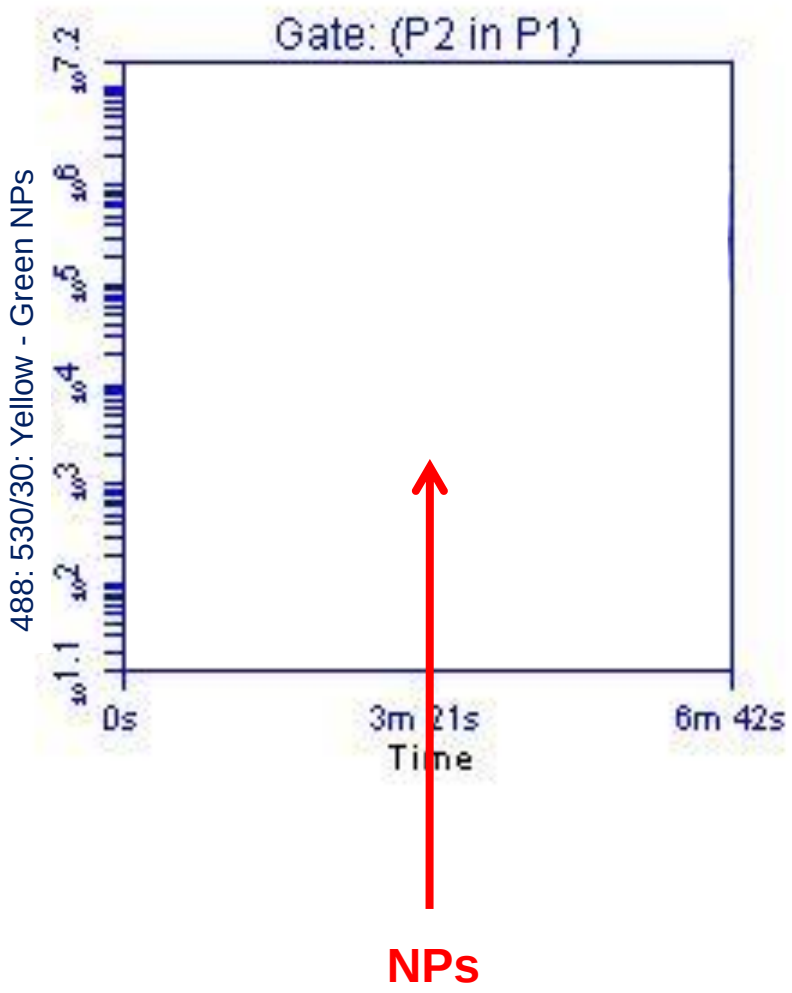


Erythrocytes Lysing

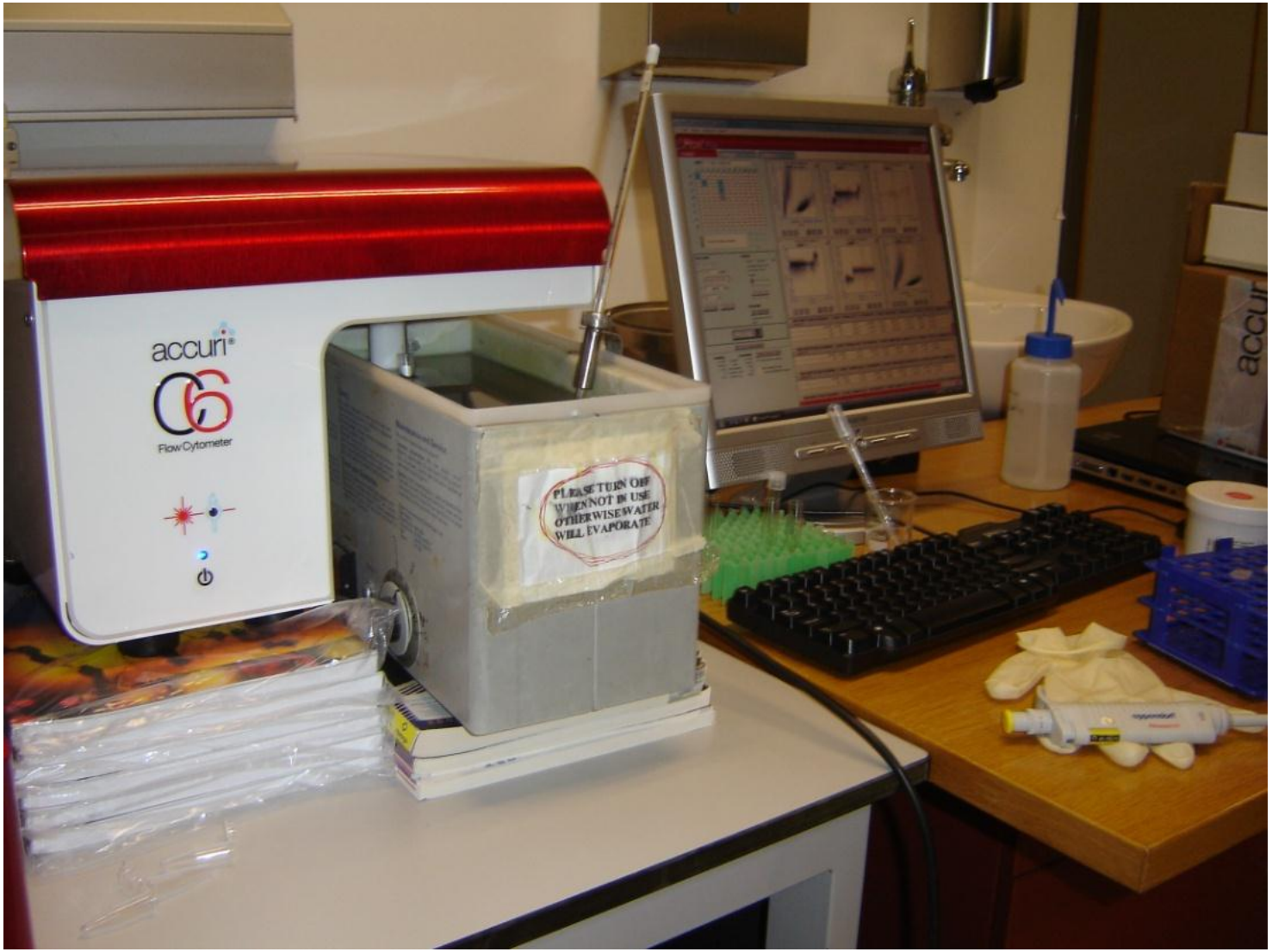




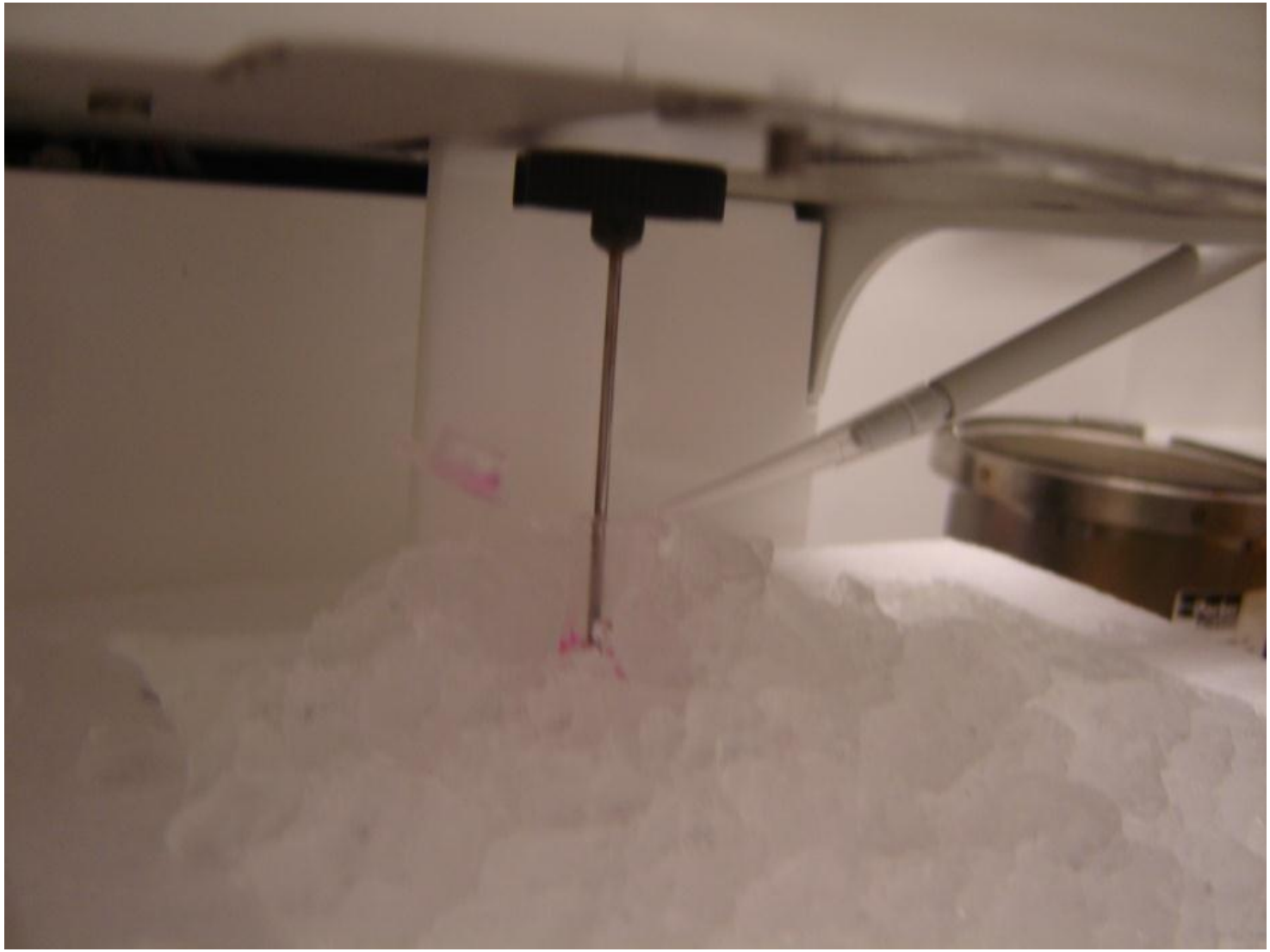
Nanoparticles uptake



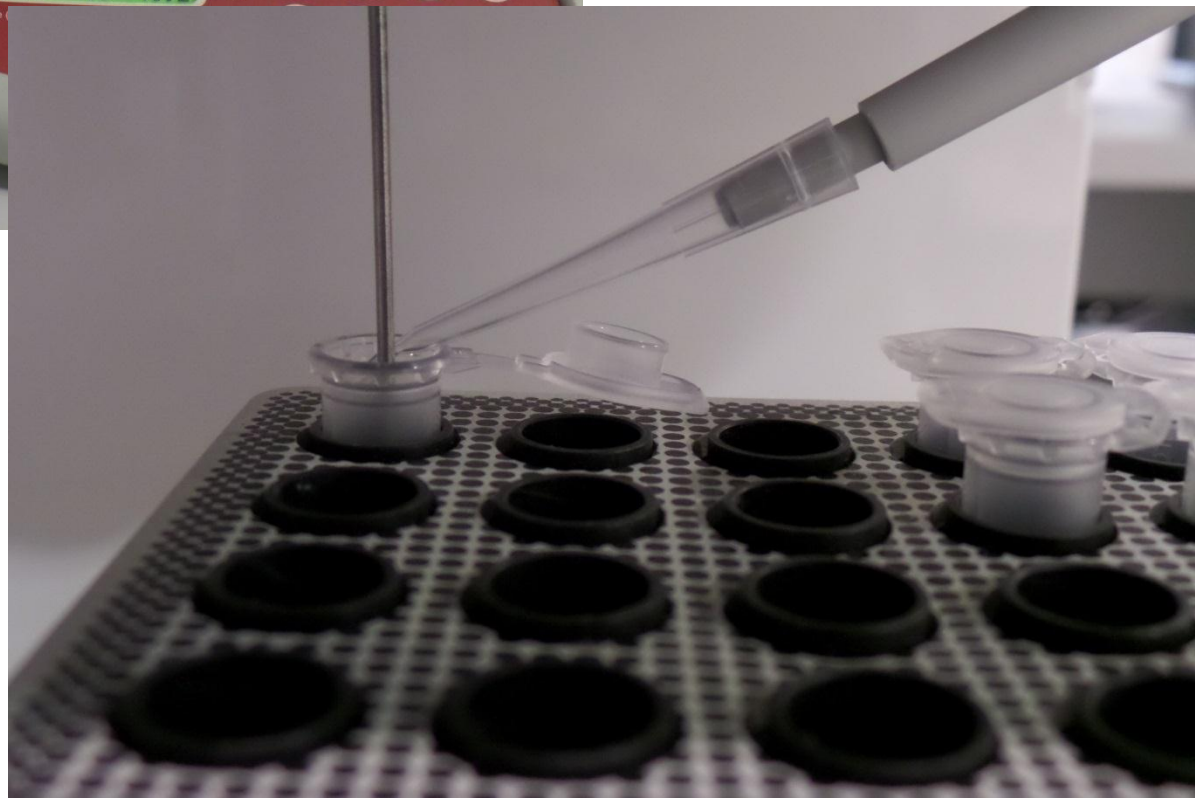
Nanoparticles uptake


accuri
C6
Flow Cytometer
PLEASE TURN OFF
WHEN NOT IN USE
OTHERWISE WATER
WILL EVAPORATE

What if my sample has to be at 4ºC?




BD Accuri™
Flow Cytometer
20 x 1.5 ml
SBI
biometra
a part of Analytik Jena
TS1 ThermoShaker
00:02 420 40.0
00:00 418 40.2
Set
Actual
Shaker



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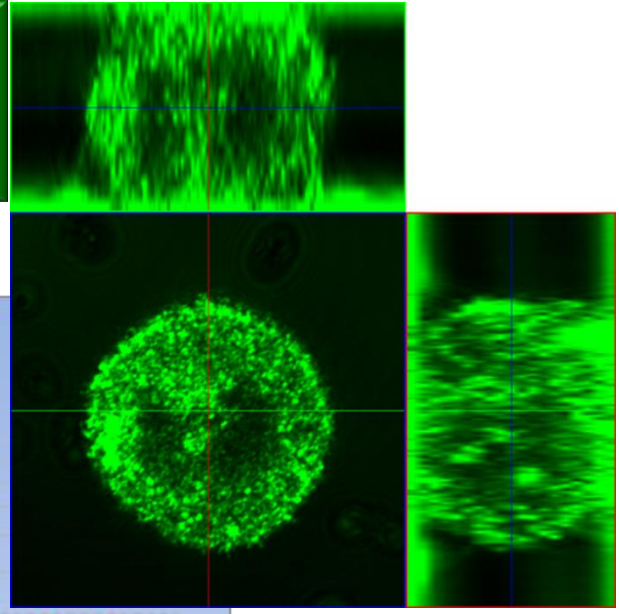
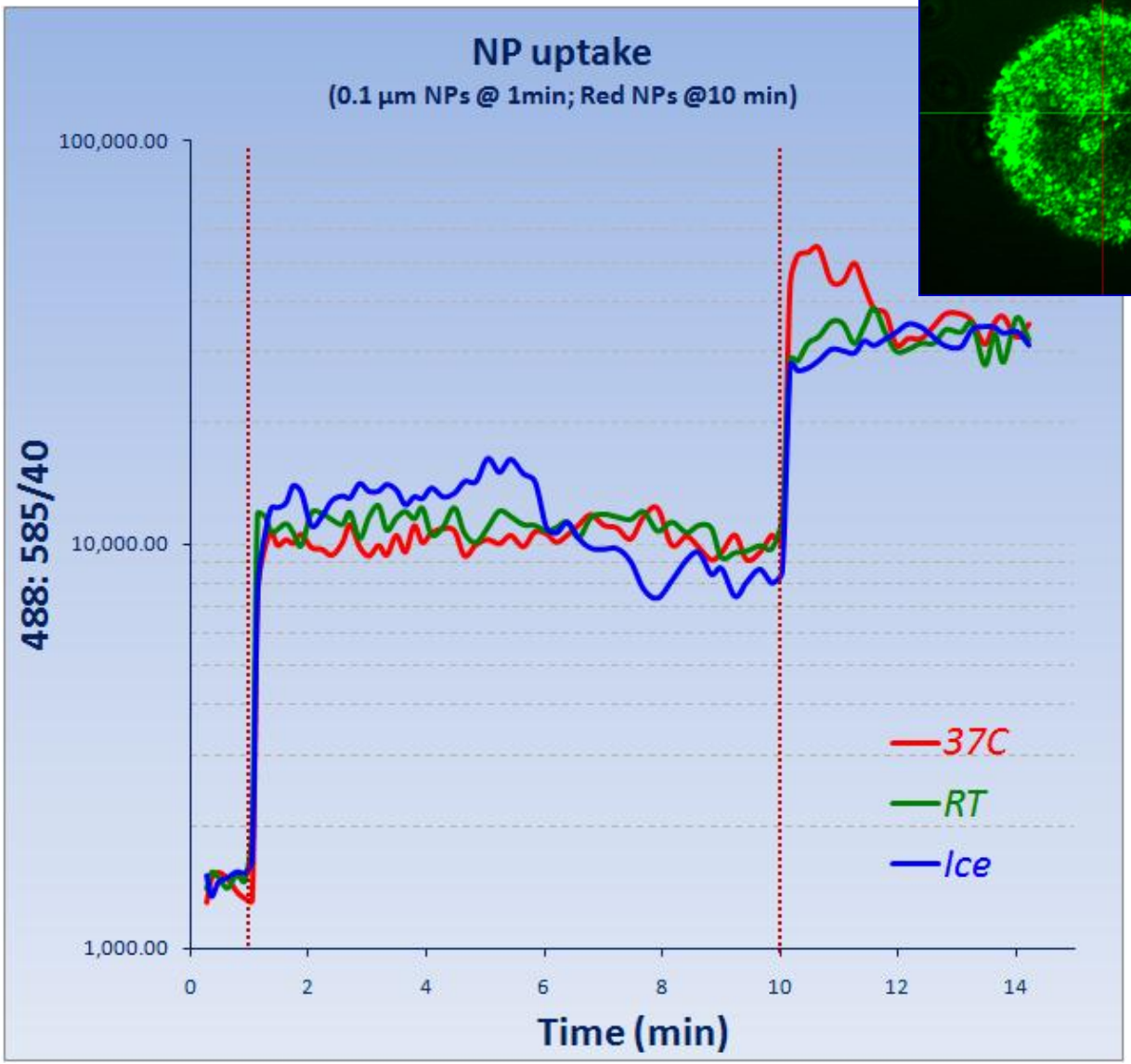
Nanoparticles uptake

Fixed cell in suspension covered by nanoparticles, after incubation at 4°C





Calcium

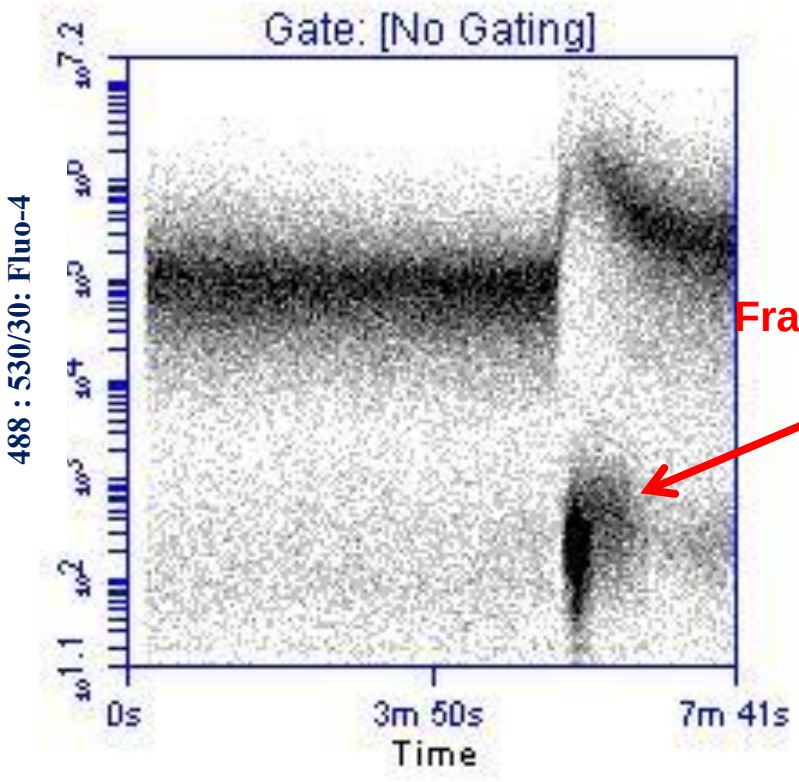
Measurement of Intracellular Calcium Ions by Flow Cytometry

Contributed by Carl H. June, Ryo Abe, and Peter S. Rabinovitch

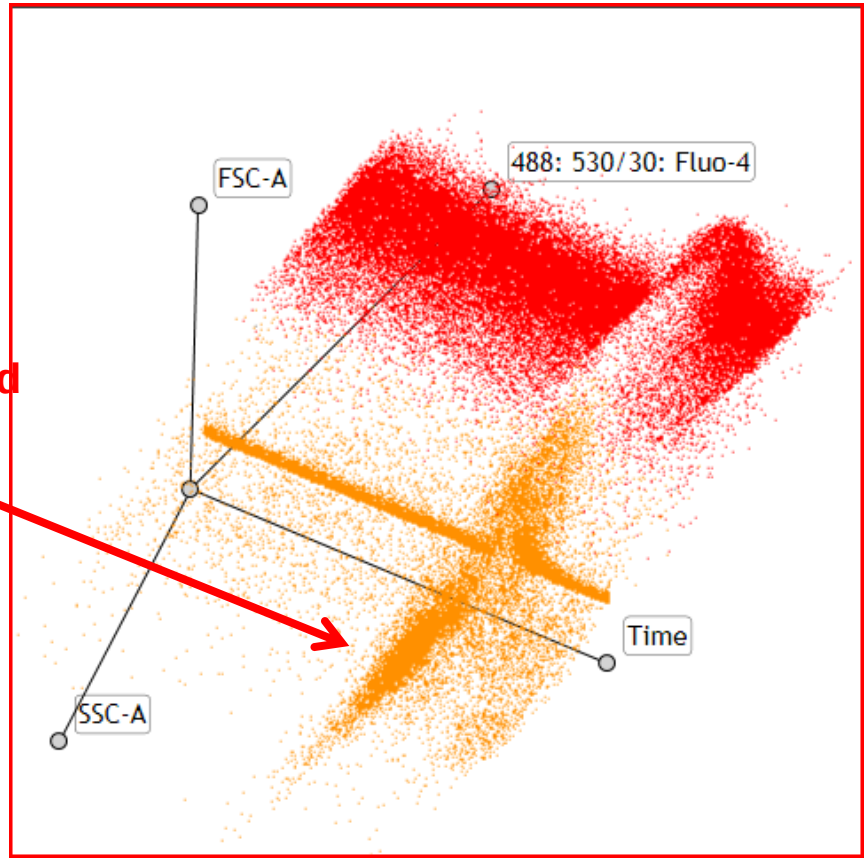
Current Protocols in Cytometry (1997) 9.8.1–9.8.19

Analyze fluo-3-loaded cells

6. Analyze cells by gating on forward and right-angle light scatter. Exclude dead cells by gating out cells without fluo-3 fluorescence.



Fragmented cells



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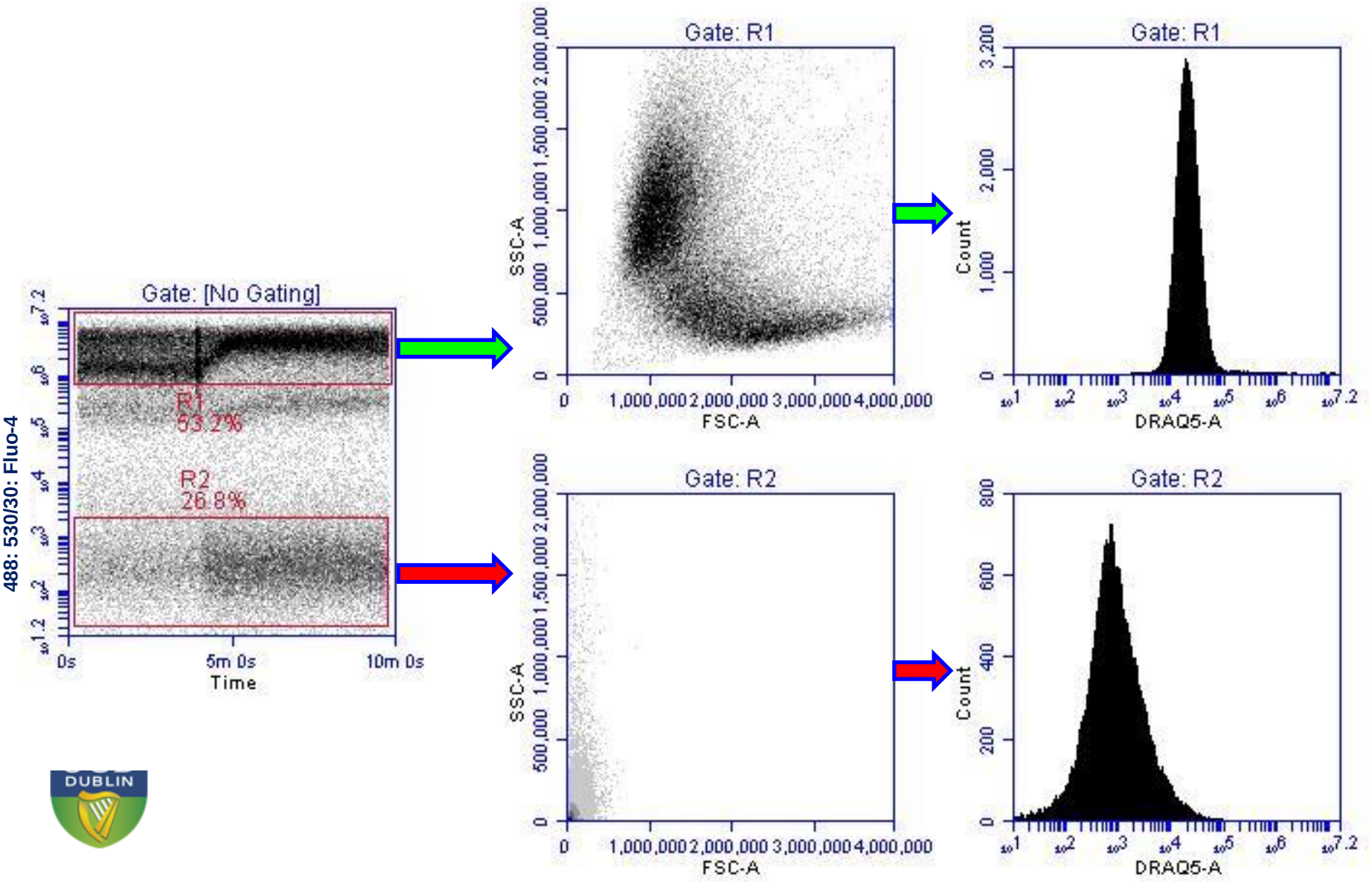
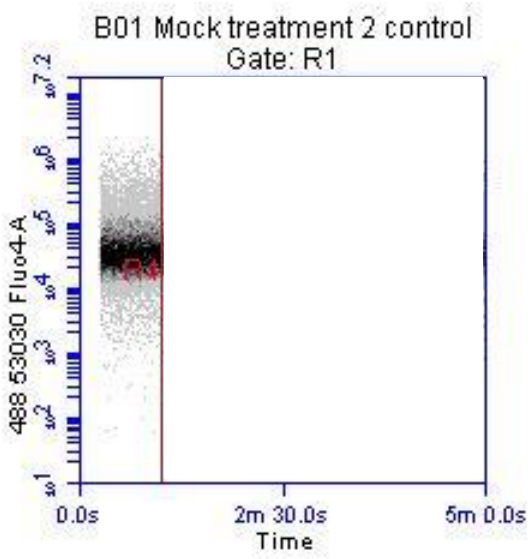
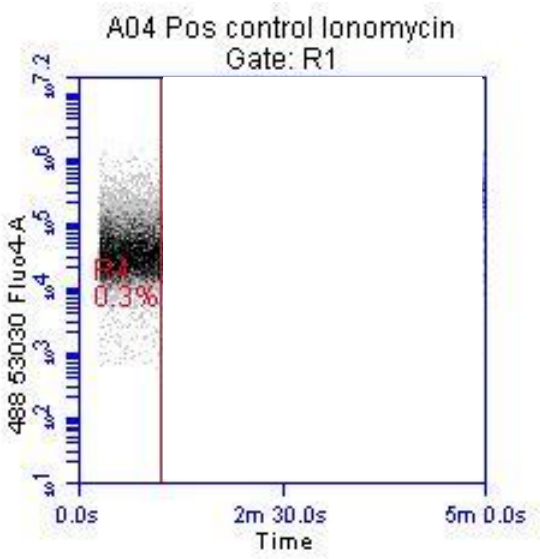
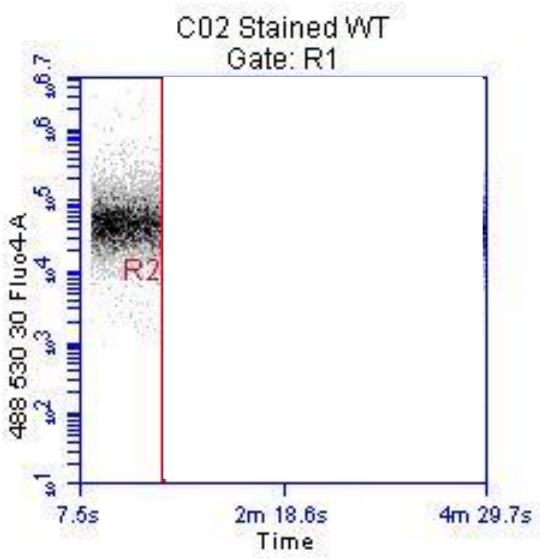
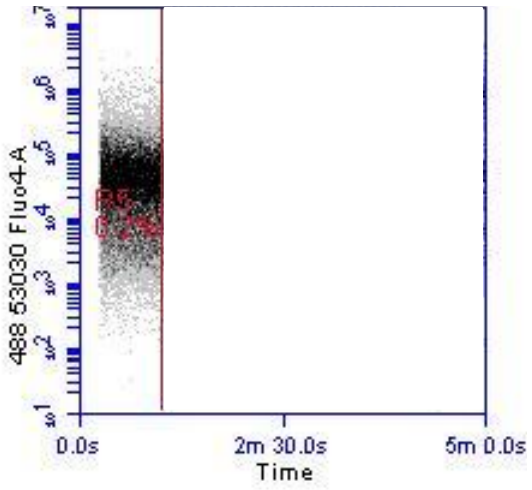


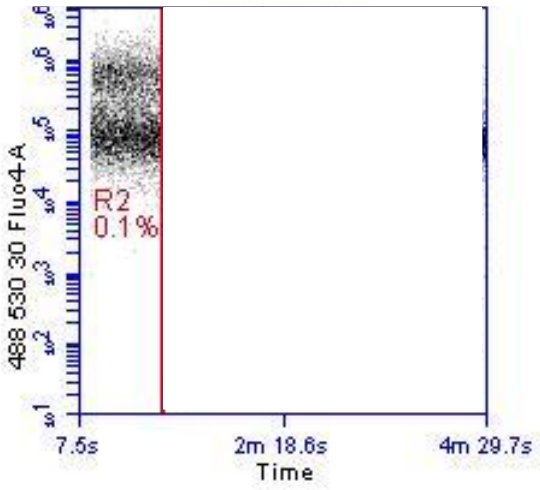
Some Calcium profiles

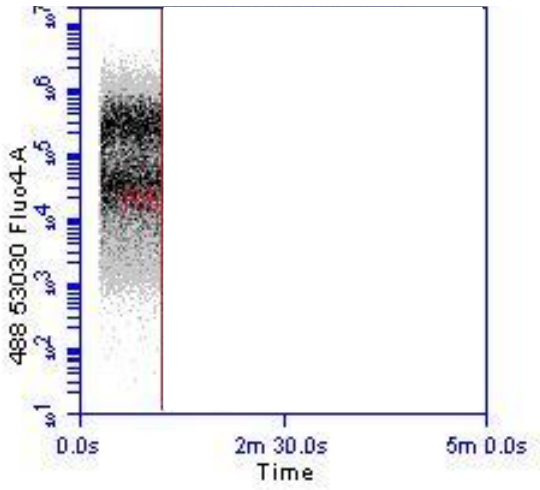






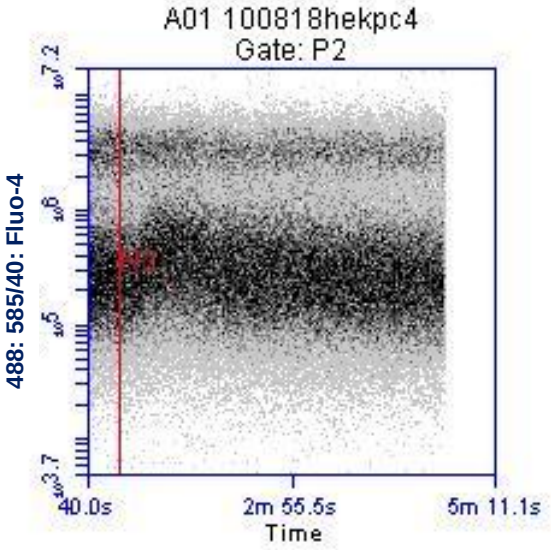


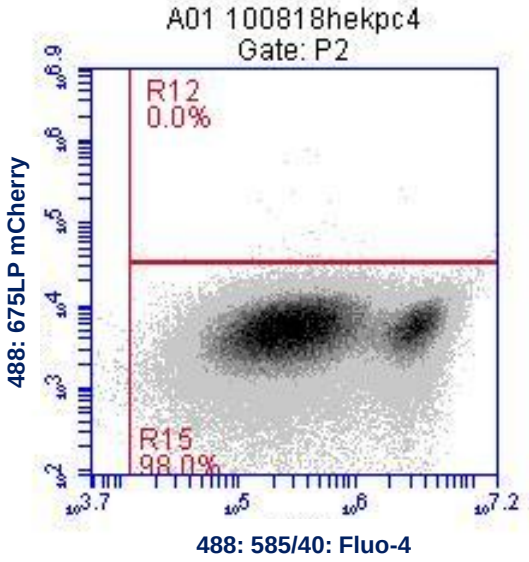


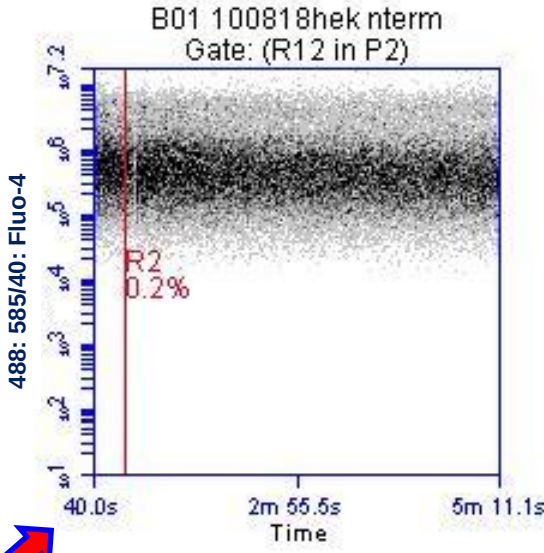


Calcium flux in some mCherry transfected cell lines

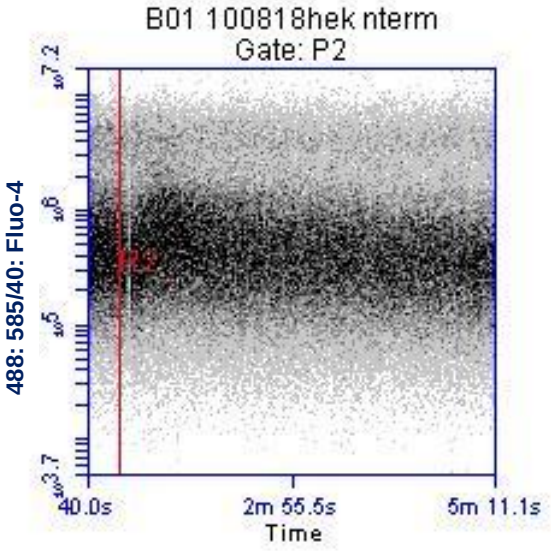
WT

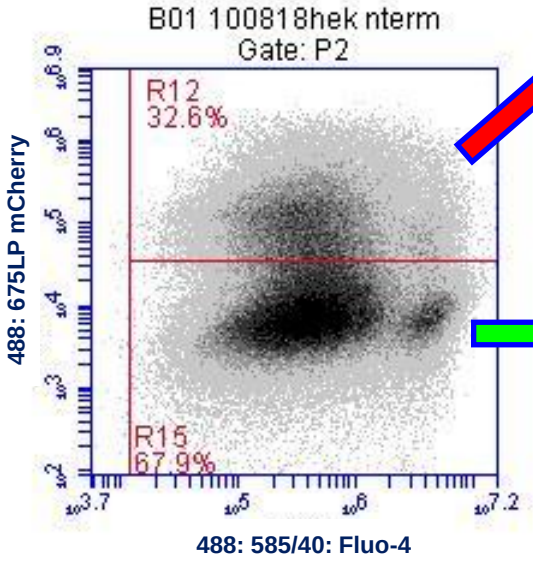


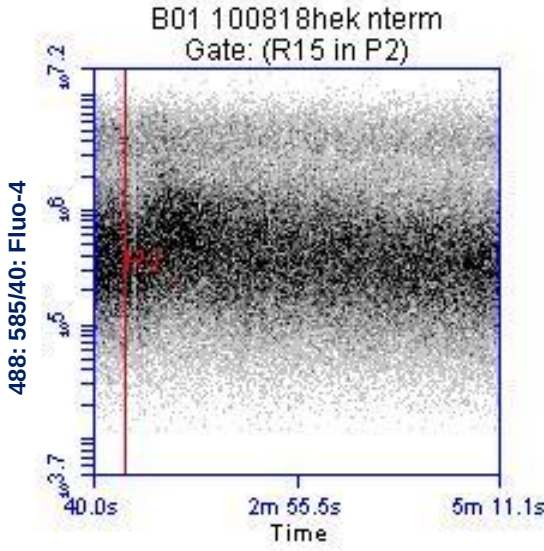




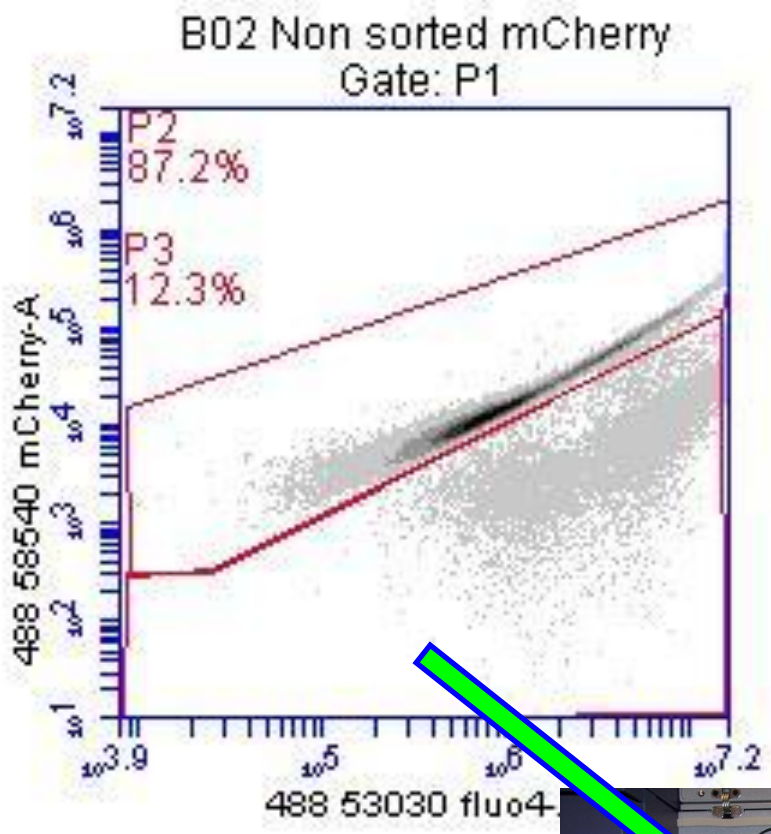
mCherry

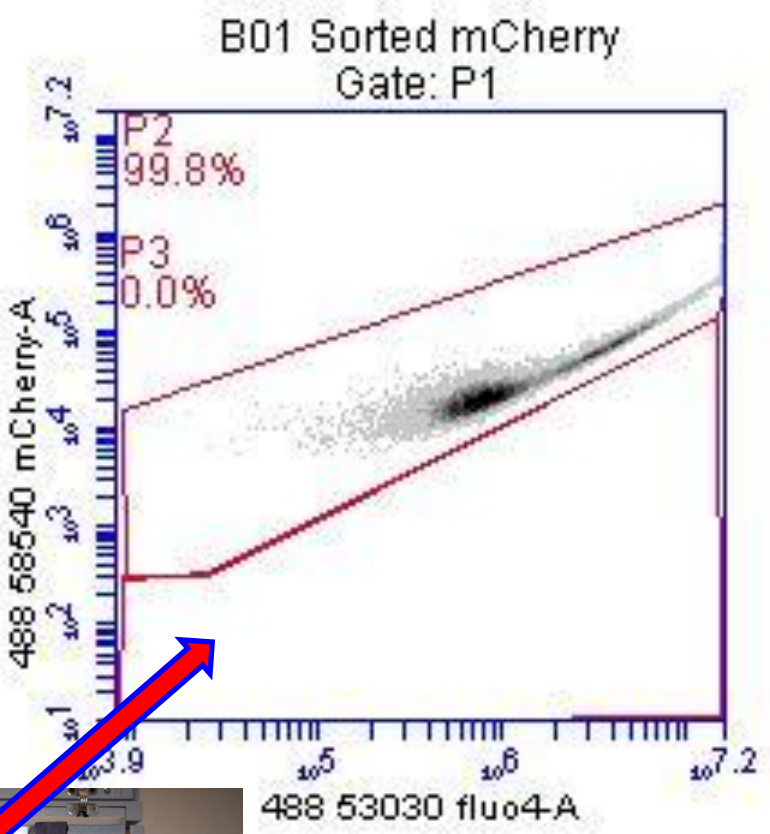


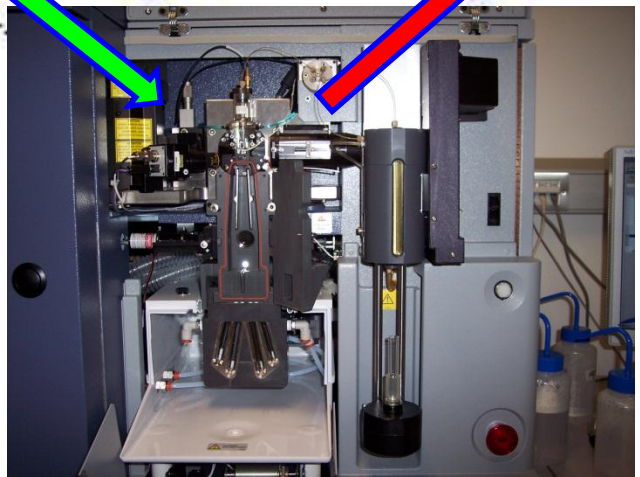




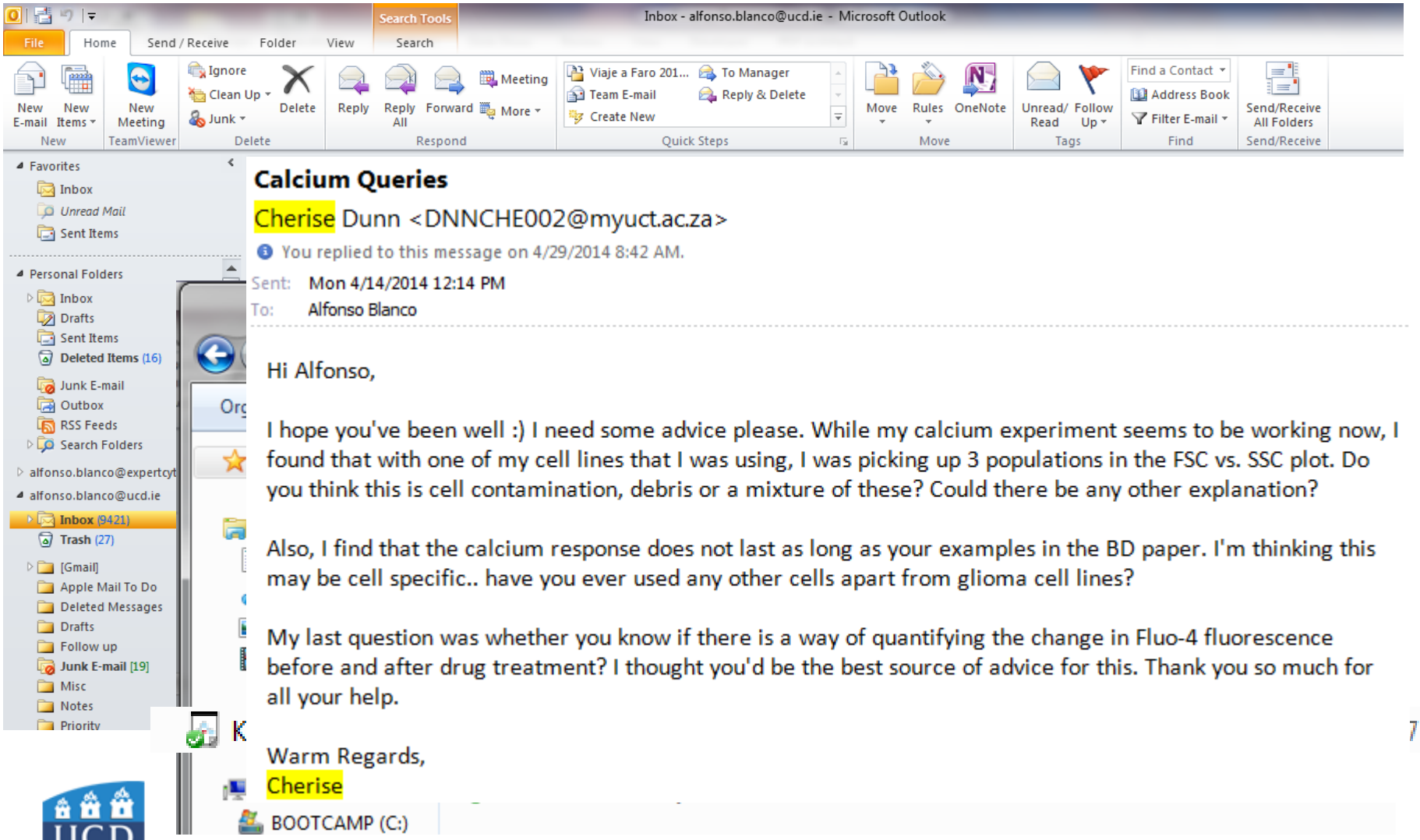
Calcium flux in some mCherry transfected cell lines





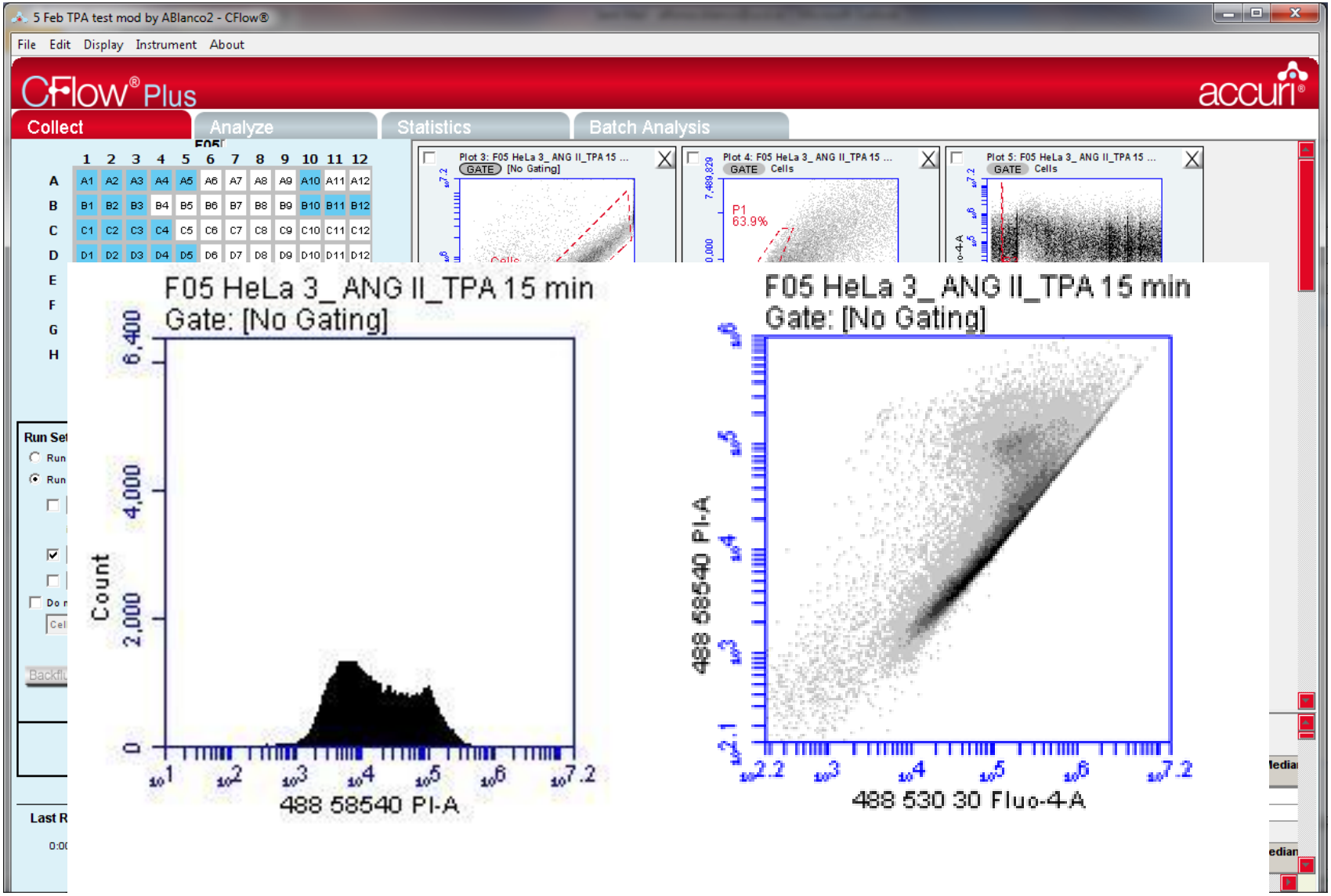


Sharing files and Templates

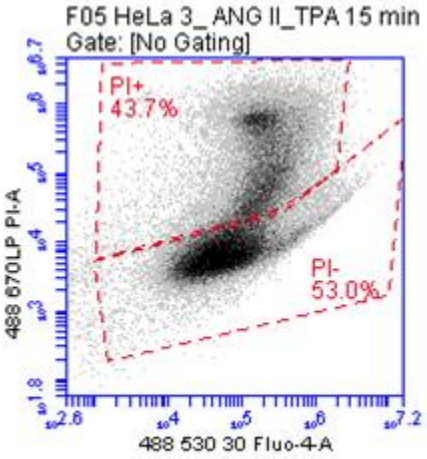


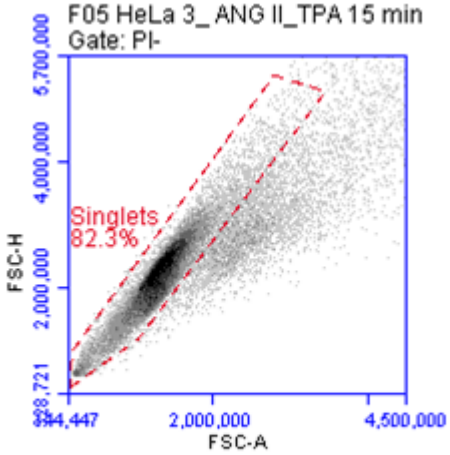
UCD DUBLIN

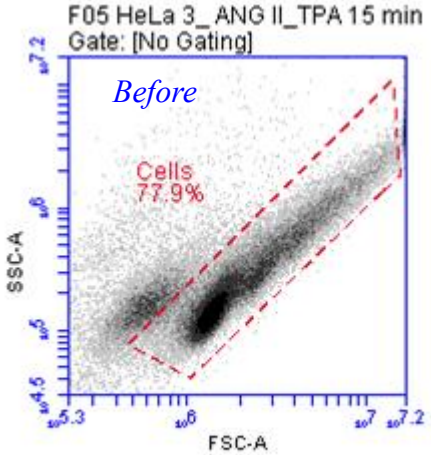
Sharing files and Templates

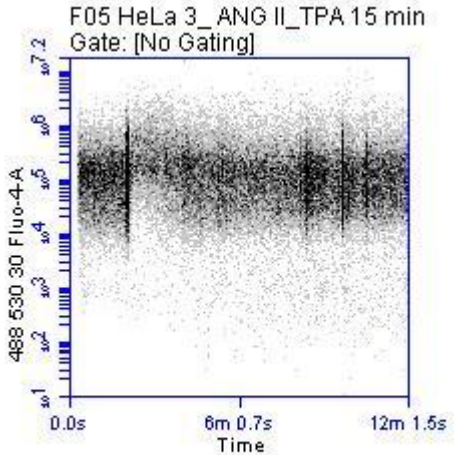


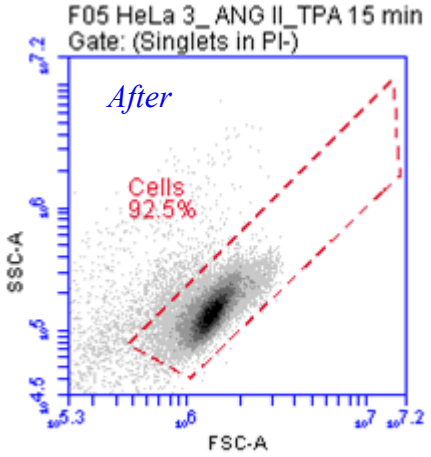
Sharing files and Templates

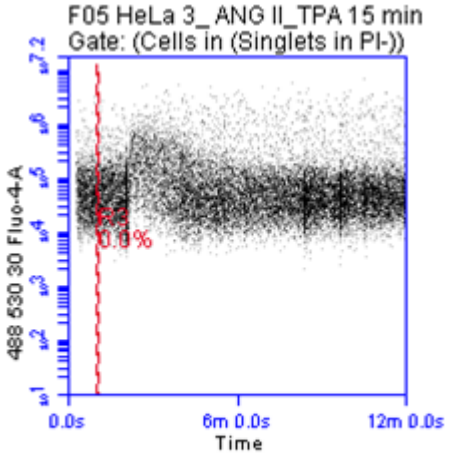












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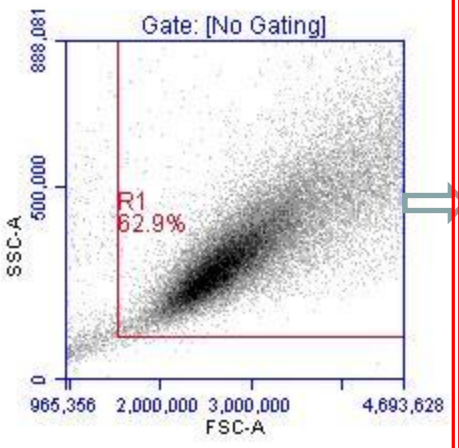
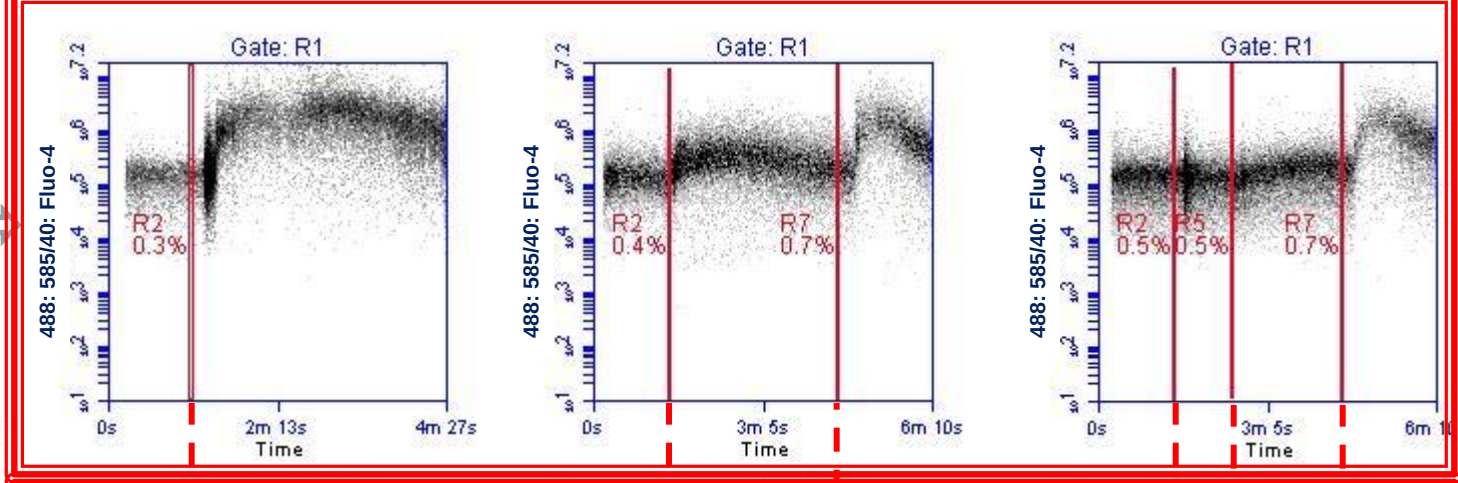
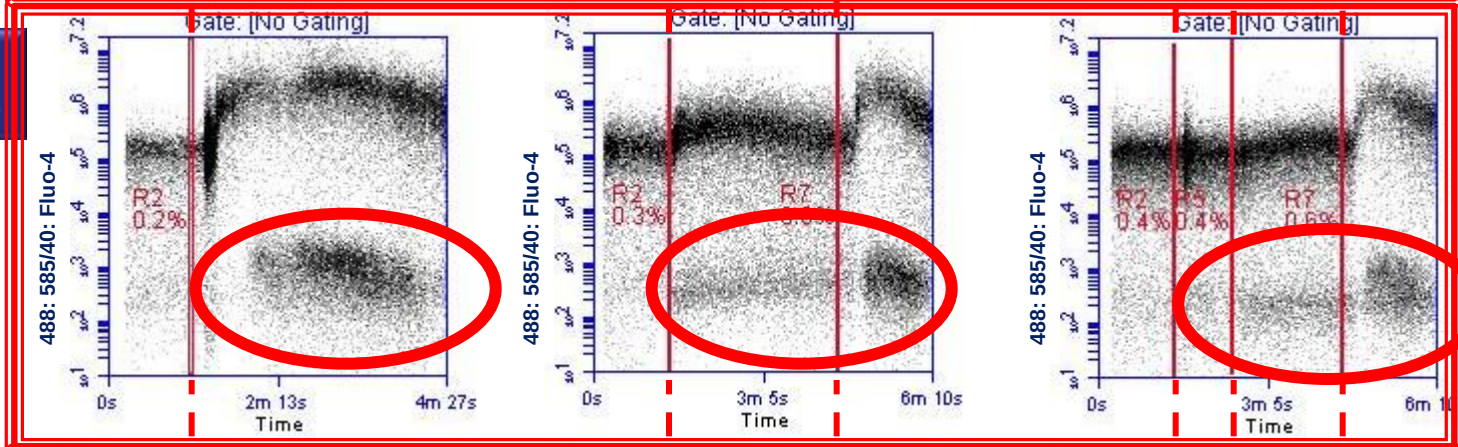
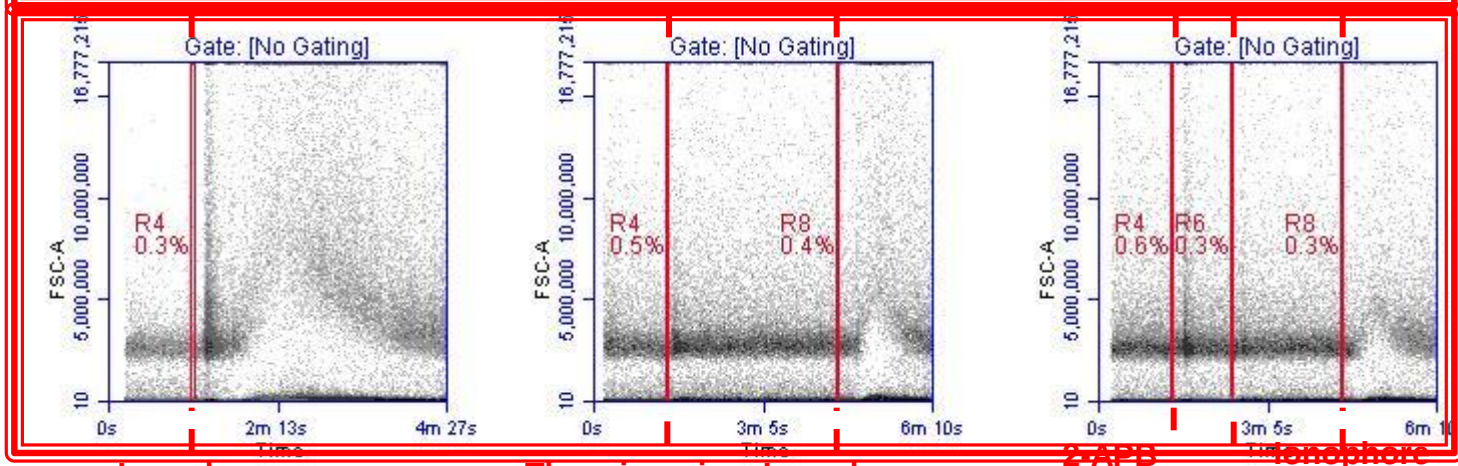
Calcium flux









Ionophore

Thapsigargin

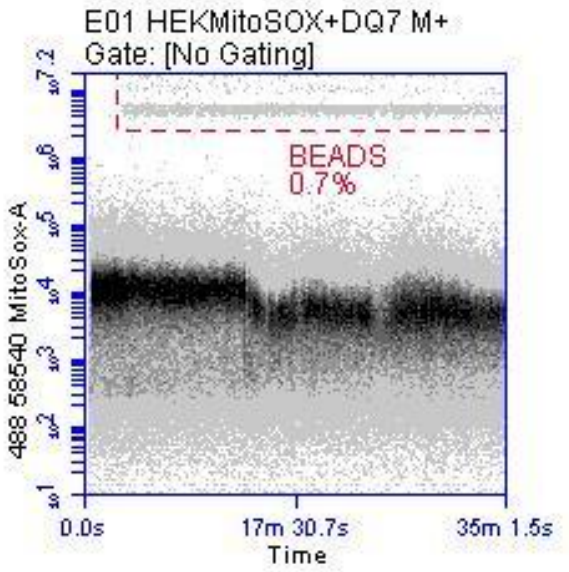
Ionophore

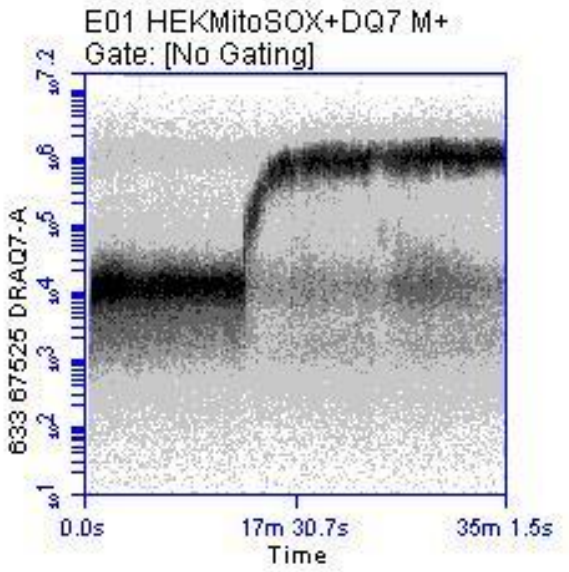
2-APB

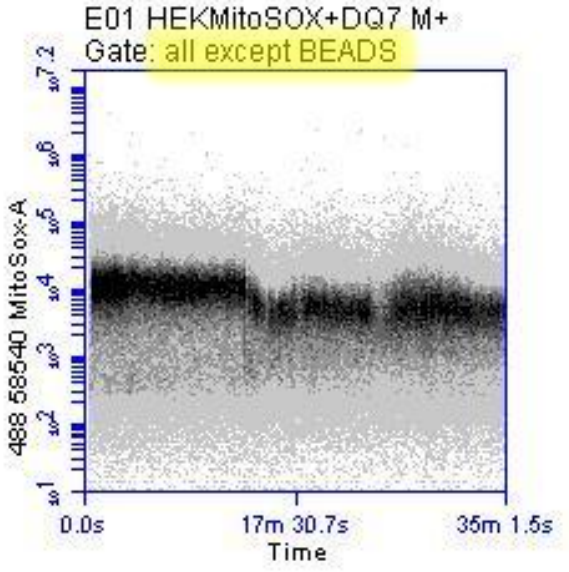
Thapsigargin

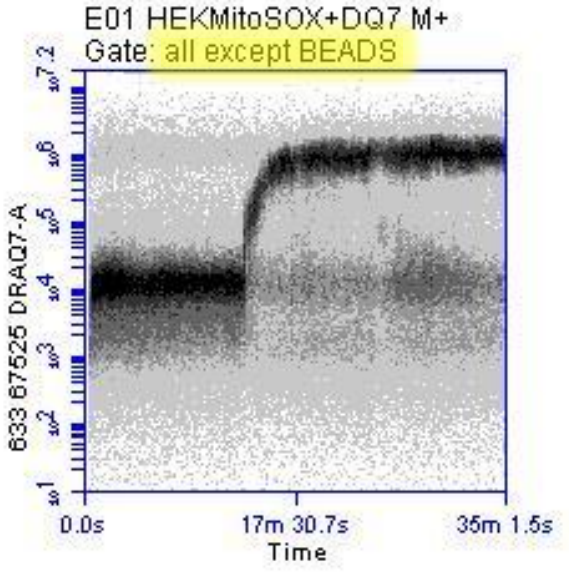
Ionophore

Apoptosis / Viability

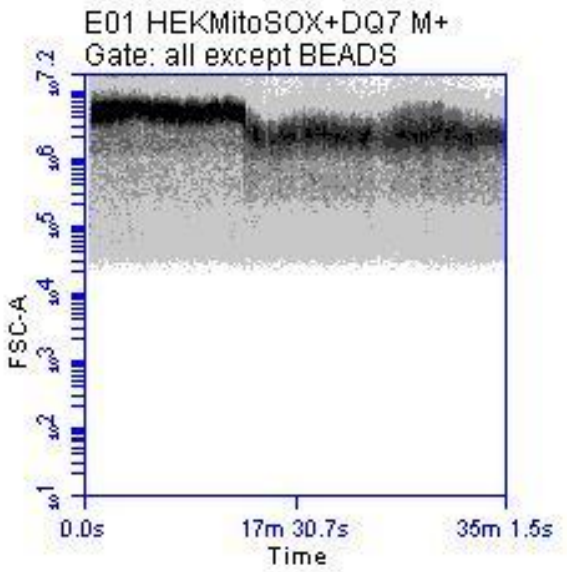


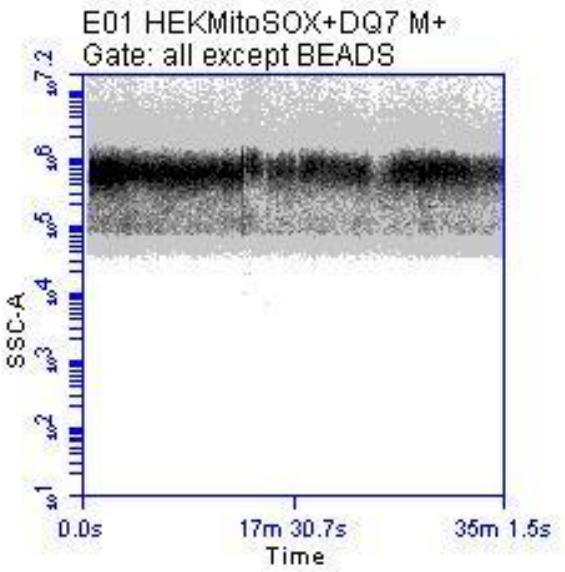


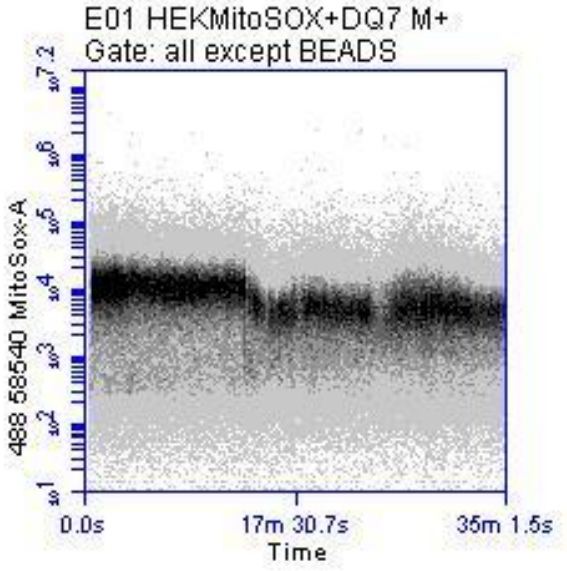


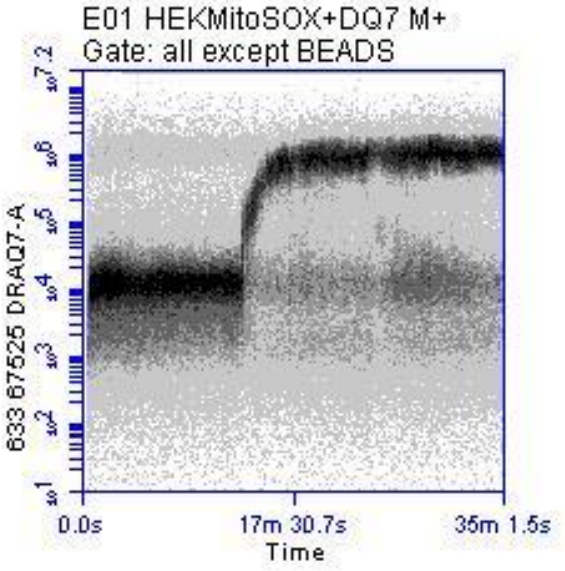


Apoptosis / Viability

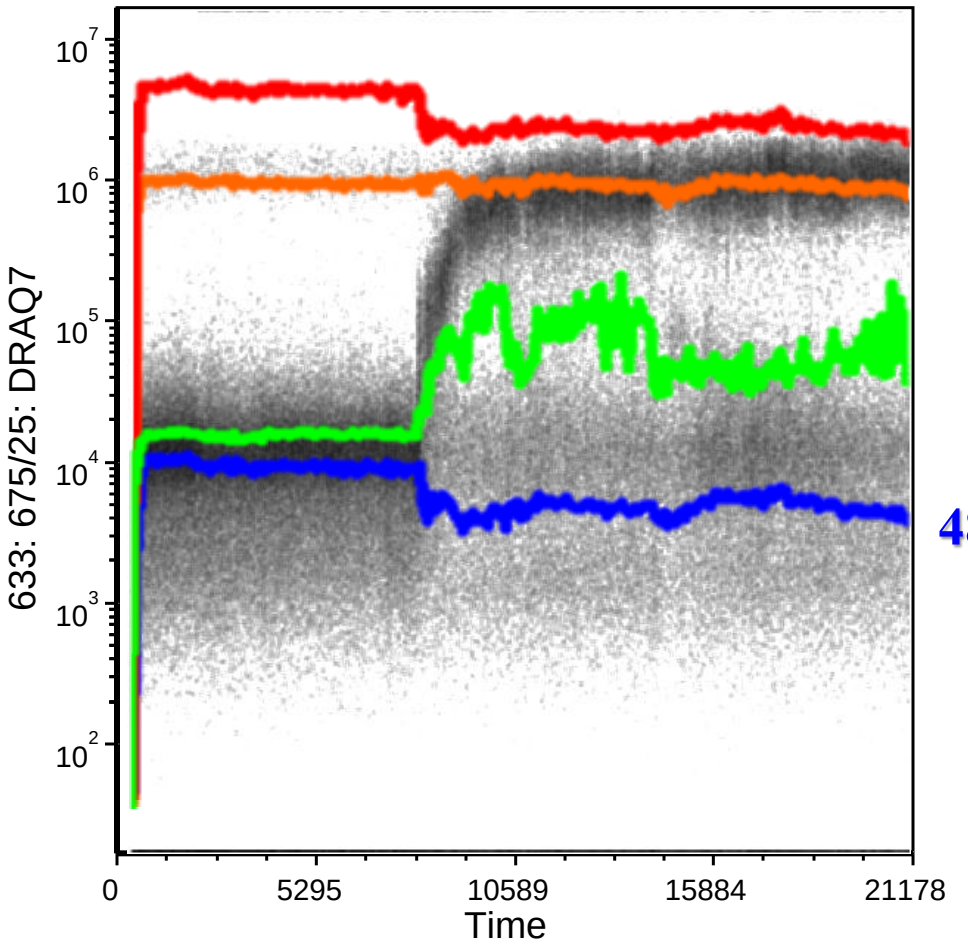








Apoptosis / Viability



FSC
SSC

488: 585/40:
MitoSox

Collaborations

VABREMA biosystems

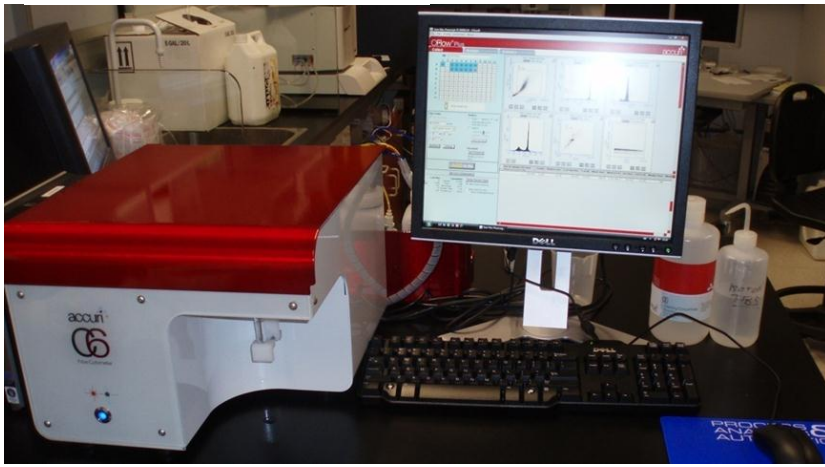
MITOPLICATOR

May 2012

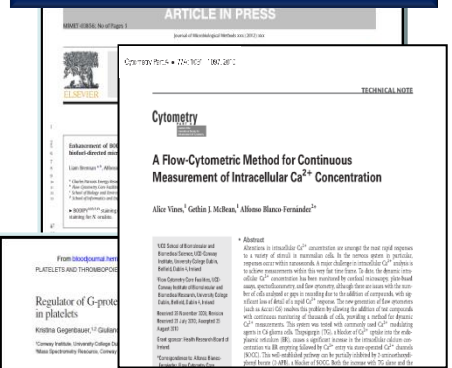
BD accuri® CYTOMETERS

UCD DUBLIN

Conference & Tutorials



Paper per review



White Paper

BD Brochure

Video in Select Science

VABREMA
biosystems

Department of Electrical Engineering

VABREMA
biosystems

MITOPLICATOR

Mitoplicator – Hybridoma

HYBRIDOMA GROWTH | TOTAL AB | AB / CELL

(Data table & experimental settings were taken from the Hycult report, boxes indicate the median, based on which percentages were calculated)

		Total # cells (… * 10^6 alive / dead)								Total µg Ab (IgG2b)		µg Ab / living cell	
Condition:	Treated:	11-11		12-11		15-11		18-11		15-11	18-11	15-11	18-11
1	yes	1.4	0.15	9.0	4.2	12.6	10.6	33.0	36.0	32.6	393.9	2.6	11.9
2	yes	1.4	0.15	10.4	3.8	11.8	14.4	24.0	48.0	33.0	397.4	2.8	16.6
3	yes	1.4	0.15	8.0	0.4	10.6	12.6	24.0	33.0	31.4	397.3	3.0	16.6
4	no	1.4	0.15	9.4	0.6	11.2	12.4	21.0	39.0	31.7	258.1	2.8	12.3
5	no	1.4	0.15	12.0	0.4	8.4	13.8	15.0	30.0	31.2	230.8	3.7	15.4
6	no	1.4	0.15	11.0	2.4	10.4	17.2	9.0	33.0	31.8	256.4	3.1	28.5

Mitoplicator Treatment in Cryotubes
Seeding in T25 (5ml)

Day 1 Count
Transfer to T75 (20ml)

Day 4 Count
Transfer 1/15 in T75 (20ml)
Collect Cells
Collect Supe

Day 7 Count
Collect Cells
Collect Supe

TREATED CELLS VS CONTROLS	Slight increase in dead cells	13% more cells at day 4	60 % more cells at day 7	55 % more Ab at day 7	Ab Production / Cell similar at day 7

"… treatment of this hybridoma seems to have a positive effect on the speed of cell division, no effect on protein production per cell and the protein produced is functional. "

(Results & quote: courtesy of Helma Rutjes, PhD, Head of R&D, HyCult)

UCD DUBLIN

VABREMA biosystems

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Stimulation of Capacitative Calcium Entry in HL-60 Cells by Nanosecond Pulsed Electric Fields*

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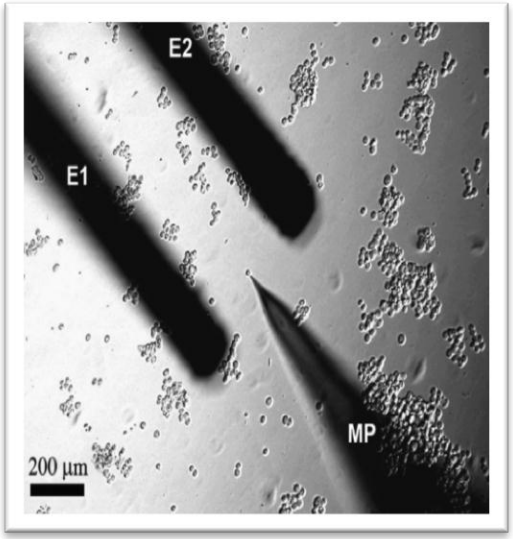
Jody A. White‡, Peter F. Blackmore§, Karl H. Schoenbach¶, and Stephen J. Beebe§¶**

Lipid nanopores can form a stable, ion channel-like conduction pathway in cell membrane

Andrei G. Pakhomov [a,*], Angela M. Bowman [a], Bennett L. Ibey [b], Franck M. Andre [a], Olga N. Pakhomova [a], Karl H. Schoenbach [a]



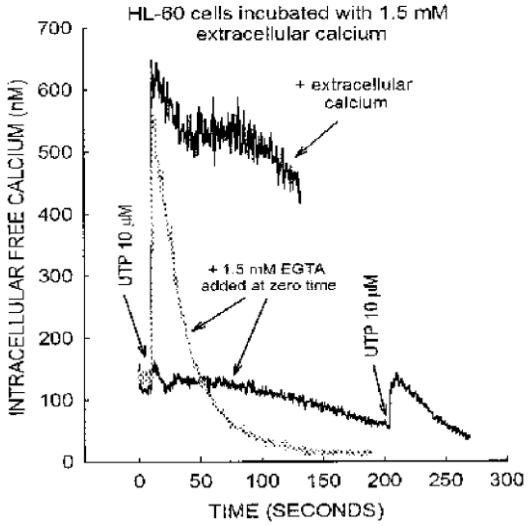
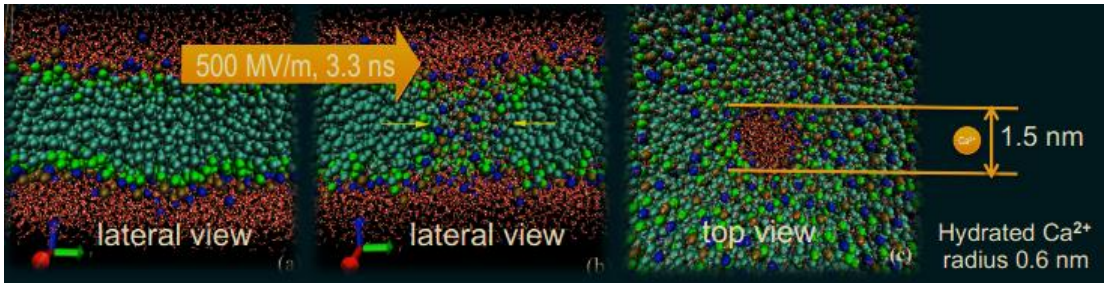
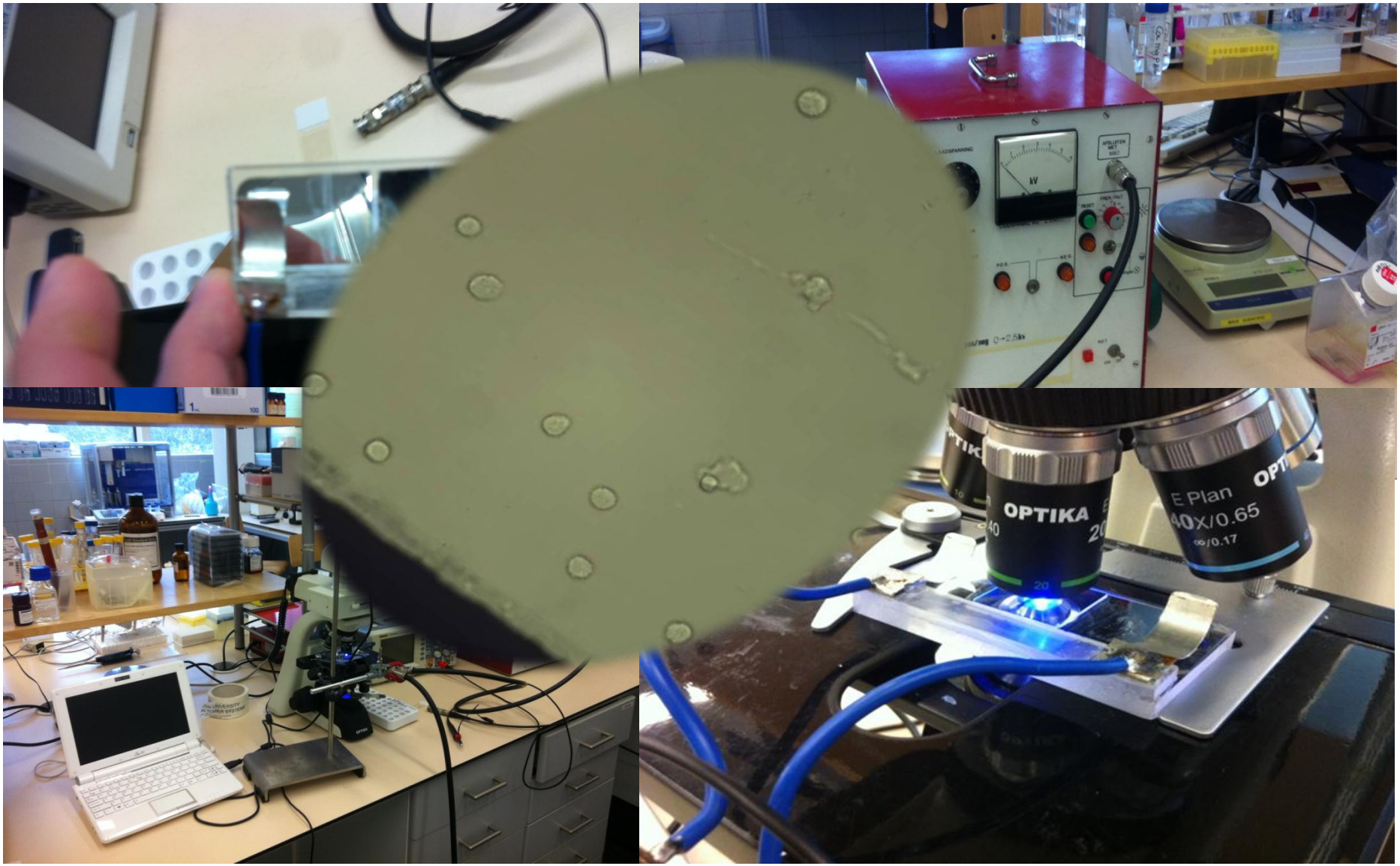


FIG. 2. **HL-60 cells, stimulated by nsPEF, respond by releasing internal calcium stores.** HL-60 cells incubated with extracellular calcium (1.5 mM) respond to nsPEF with a substantial and relatively prolonged release of $[Ca^{2+}]_i$. Cells incubated in calcium-deficient media with added 1.5 mM EGTA respond with similar magnitude; however, the response is quite transient (*gray trace*). This response represents release of internal stores of calcium. HL-60 cells incubated in the same calcium-deficient conditions were allowed to lose some of their calcium before treating with UTP. The gradual loss of calcium before treatment strongly suggests that calcium influx through the plasma membrane is not occurring and that the response seen to UTP is because of internal store release.



VABREMA
biosystems



MITOPLICATOR

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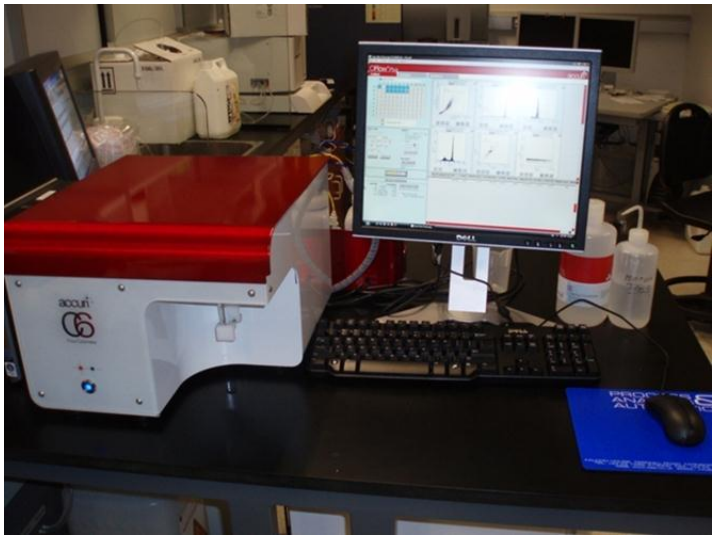
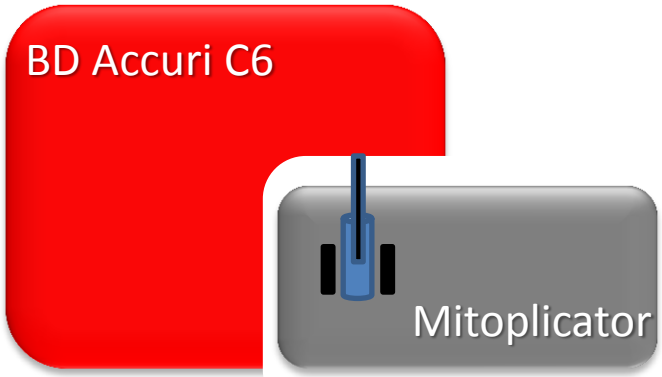


Initial Idea



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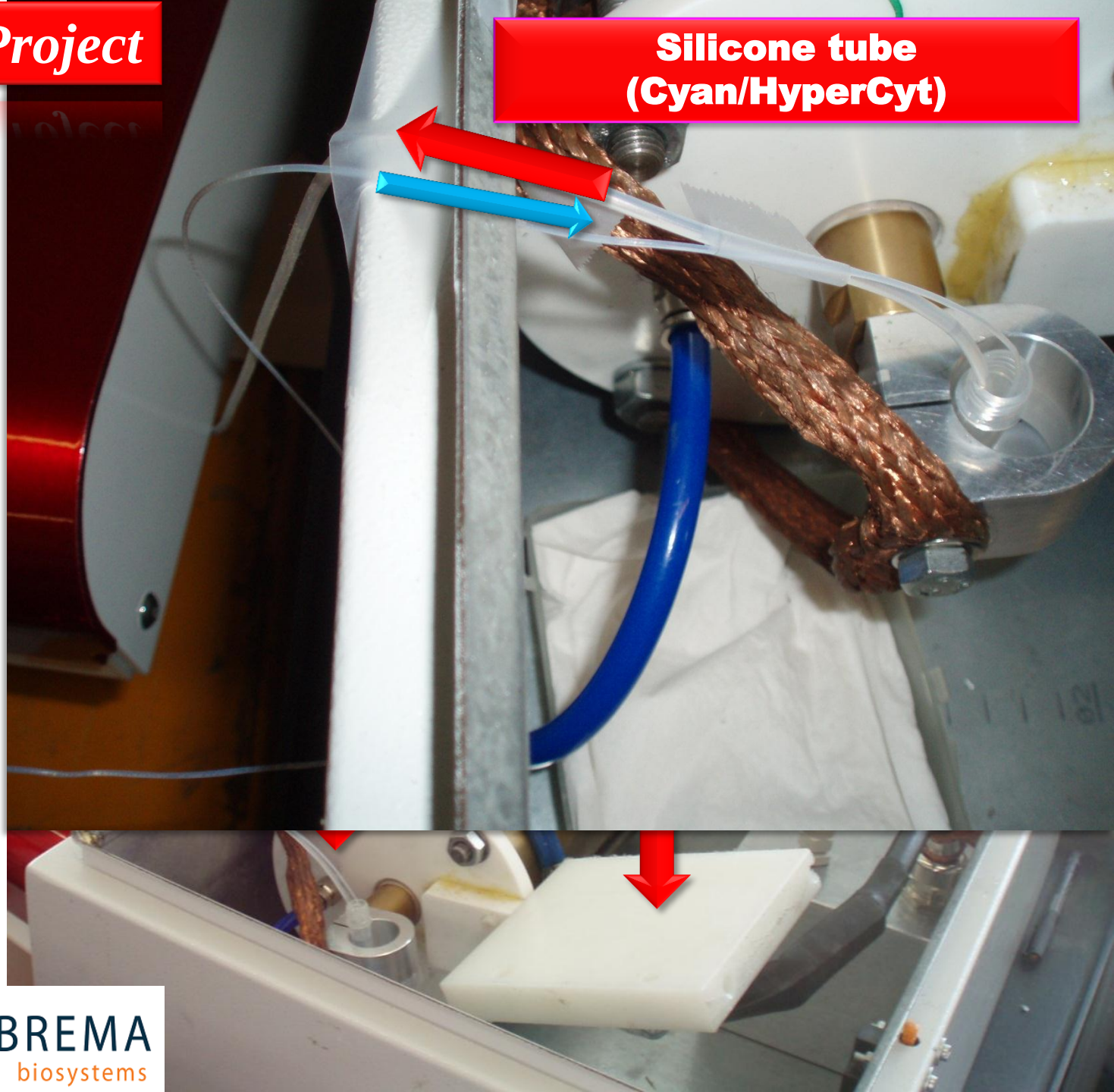
Mitoplicator Project



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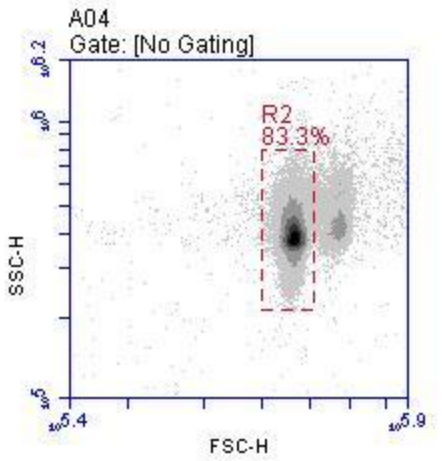
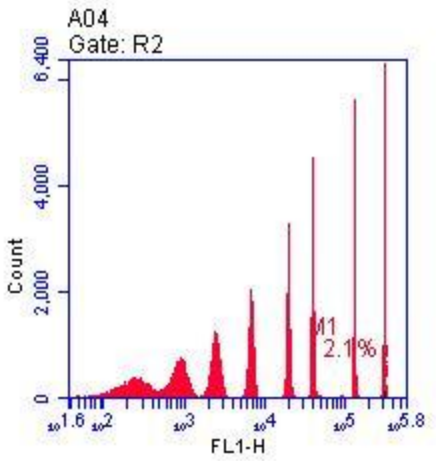
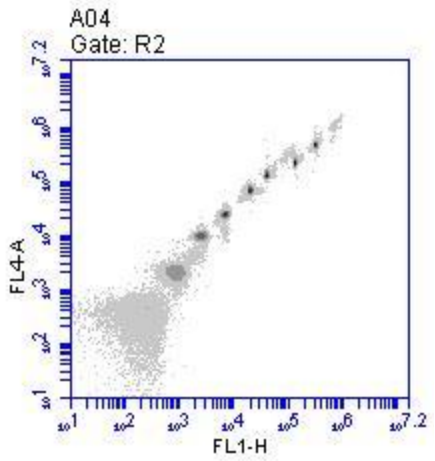
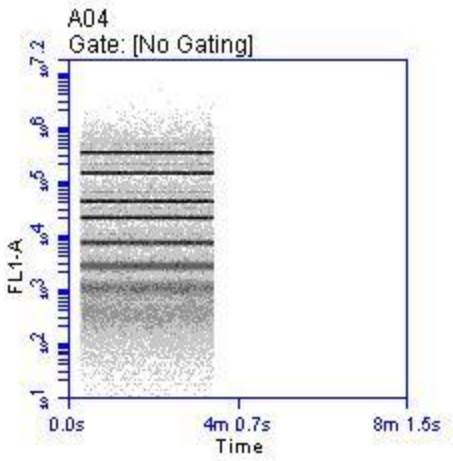
One of the 1st experiments: Stability...

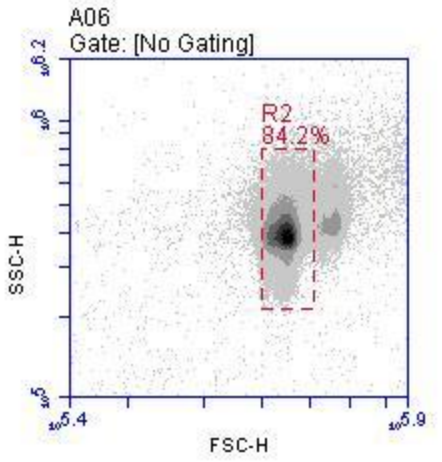


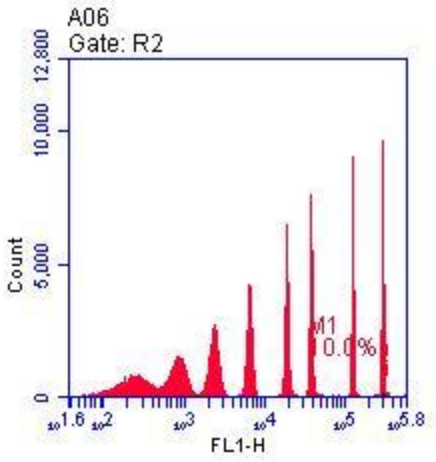


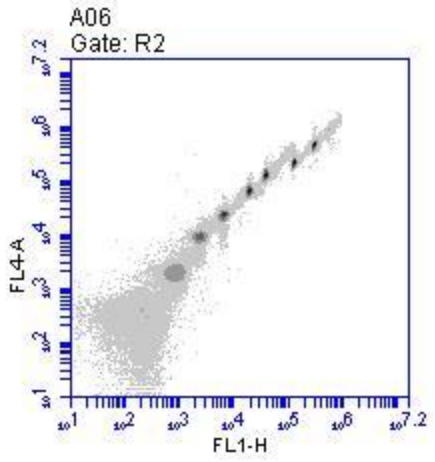


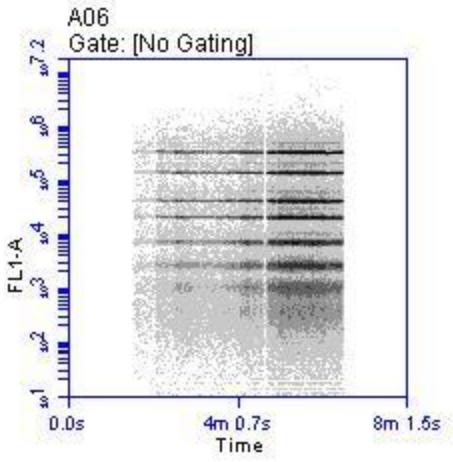


8 Peak beads in tube holder of the Accuri C6









8 Peak beads changing speeds in Mitoplicator

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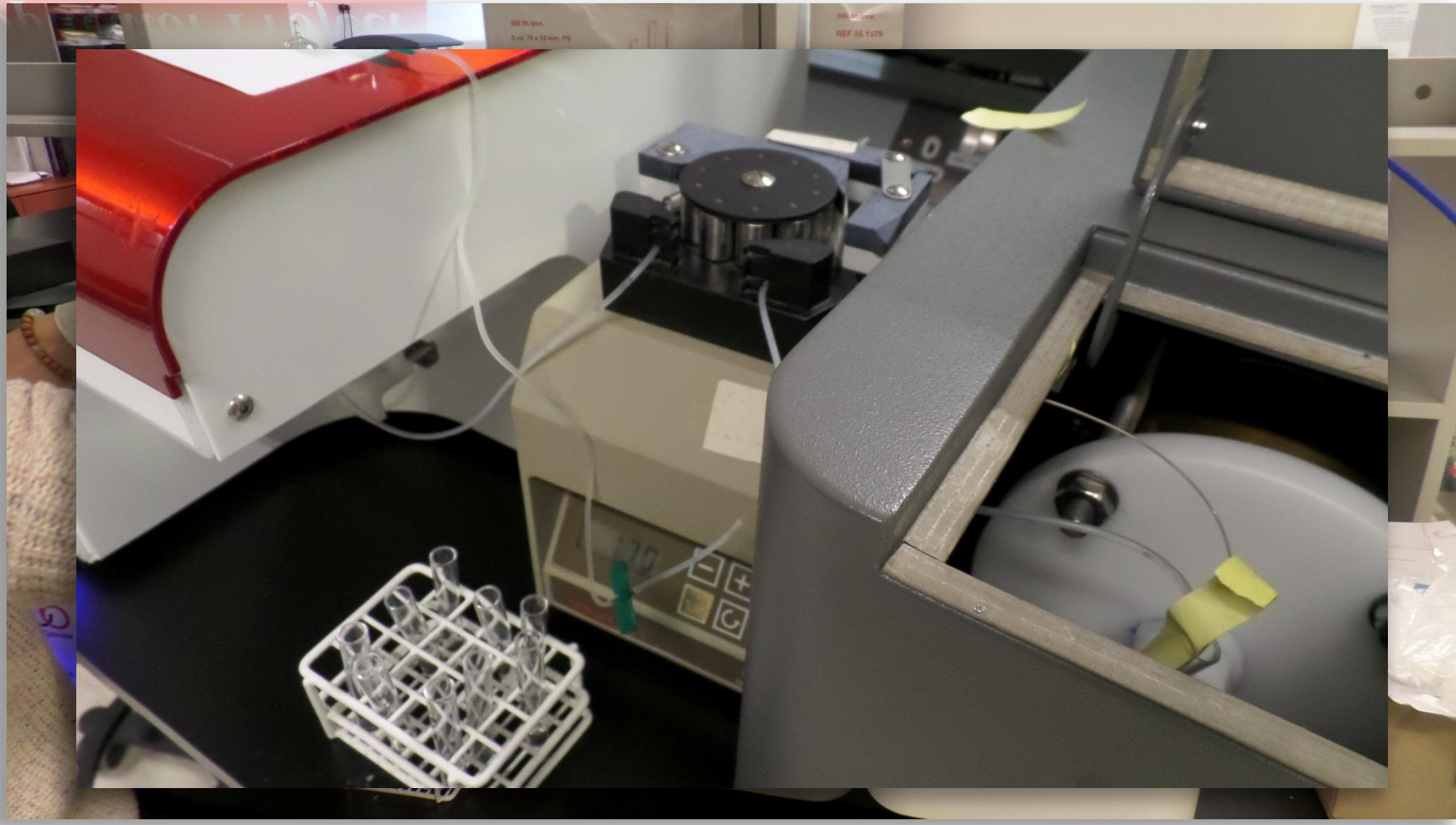
Mitoplicator Project



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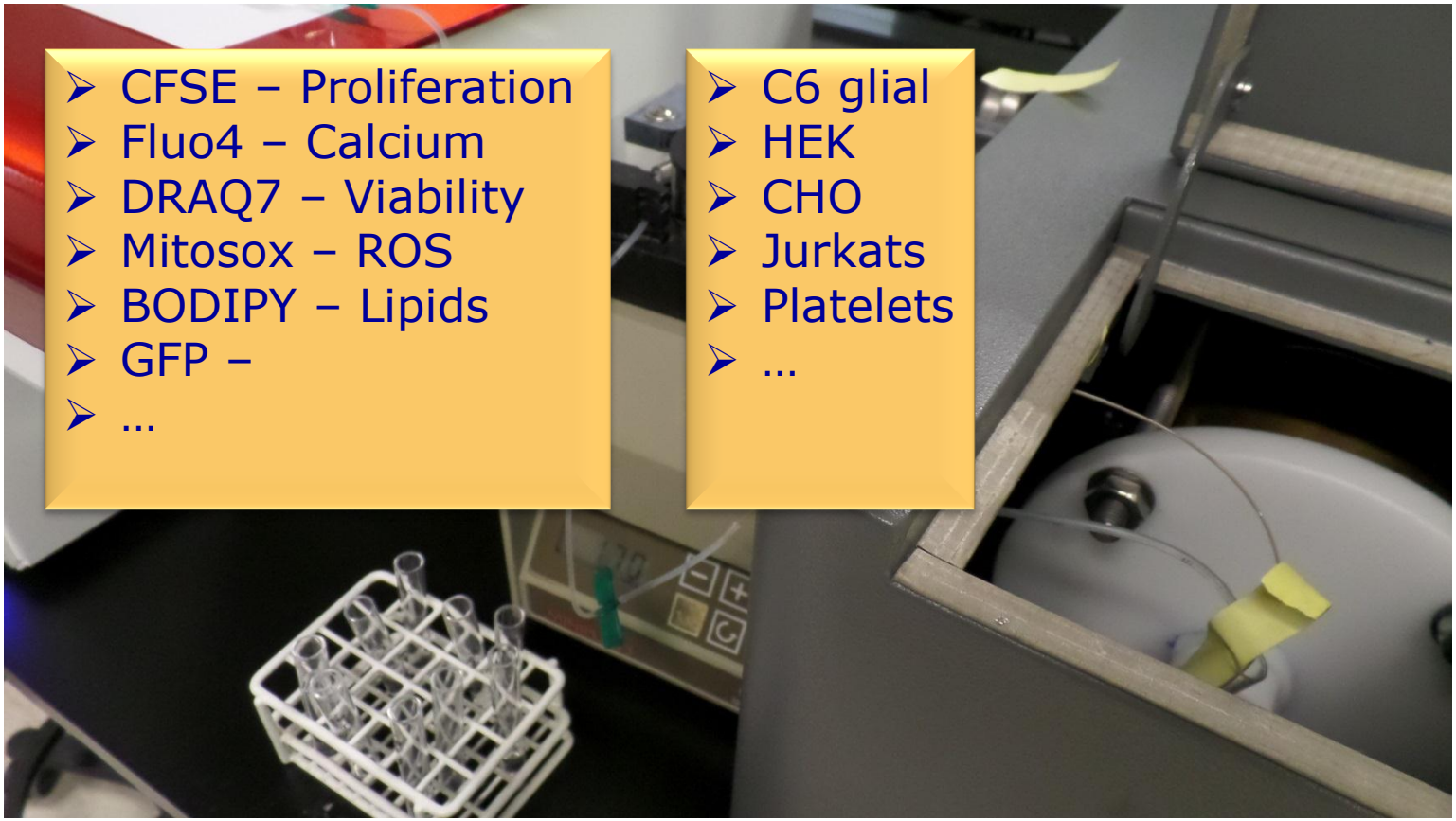
Mitoplicator Project



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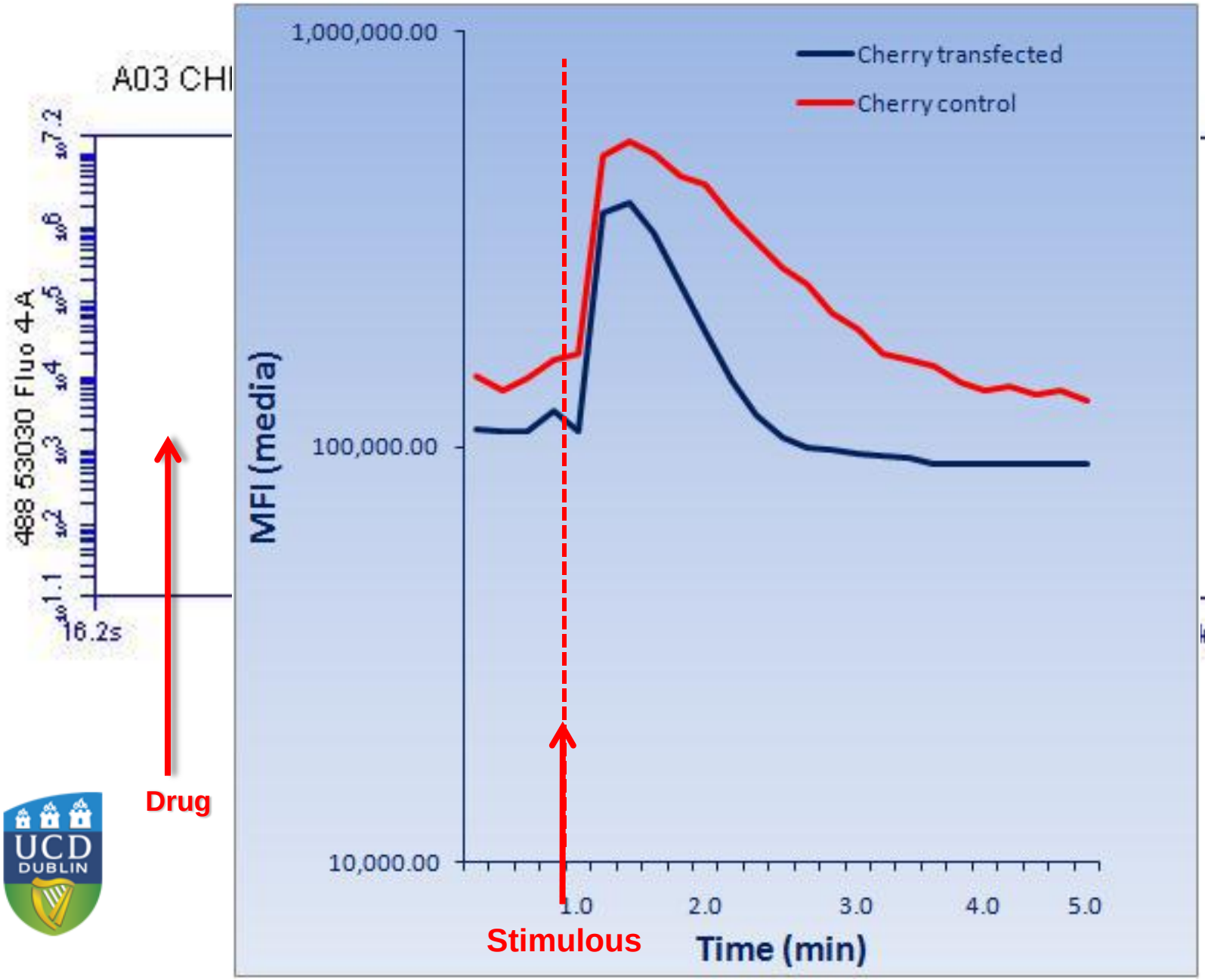
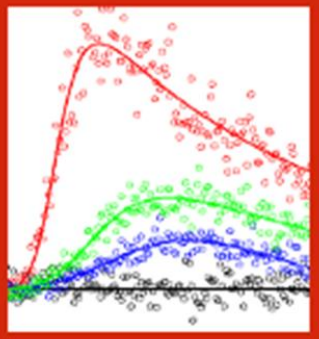
Calcium flux in some mCherry transfected cell lines



Kinetic analysis using FacsKin software



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Kinetic Analysis of Flow Cytometry Data

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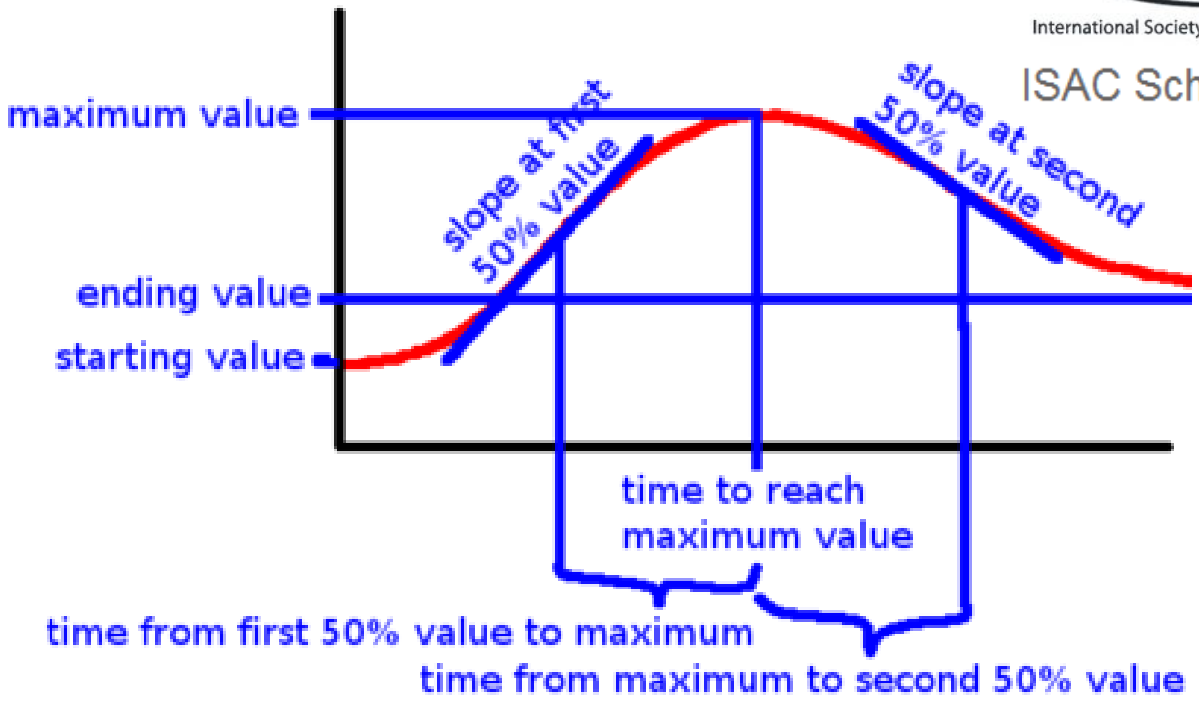

Figure 12. Parameters of dlogist+ function

Kinetic analysis using FacsKin software

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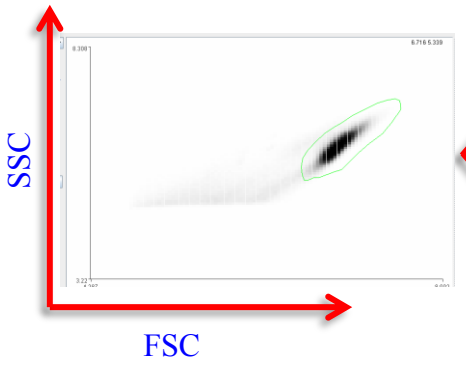
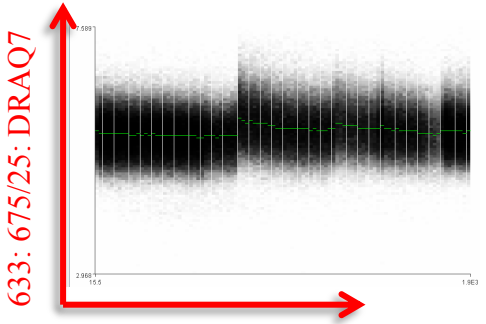
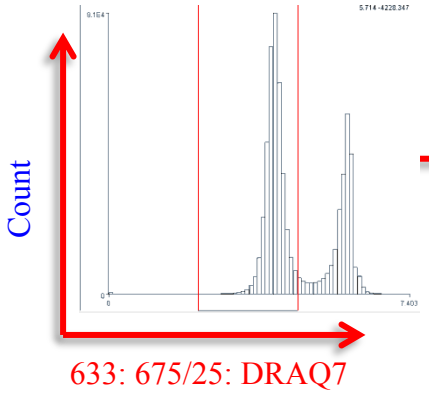


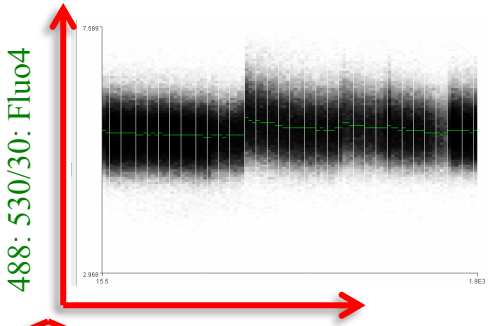


Gate one: Contains 120s baseline and 600s treatment/control (0-720s) (Mitoplicator on/off)

Analysed parameters: DRAQ7

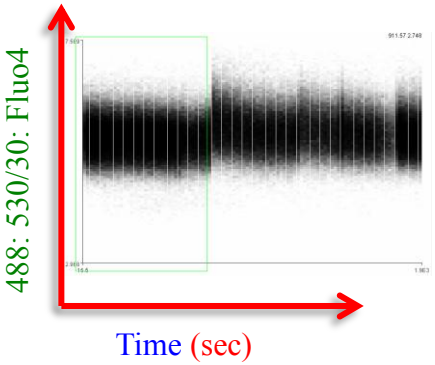


Gate out: DRAQ7+



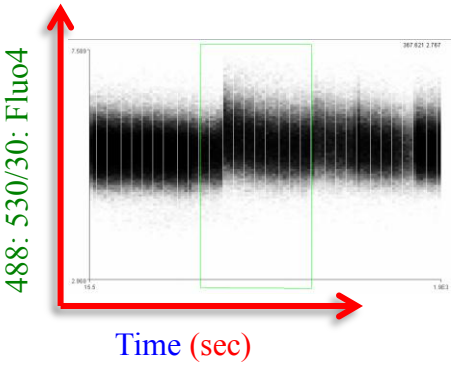
Gate two: Contains 120s baseline and 600s treatment/control (0-720s) (Mitoplicator on/off)

Analysed parameters: FLUO4, FSC SSC



Gate three: Contains 100s baseline and 300s quenching time after the addition of 6ul 1mg/ml ionomycin (600 -1020s)

Analysed parameters: FLUO4, FSC SSC



Kinetic analysis using FacsKin software

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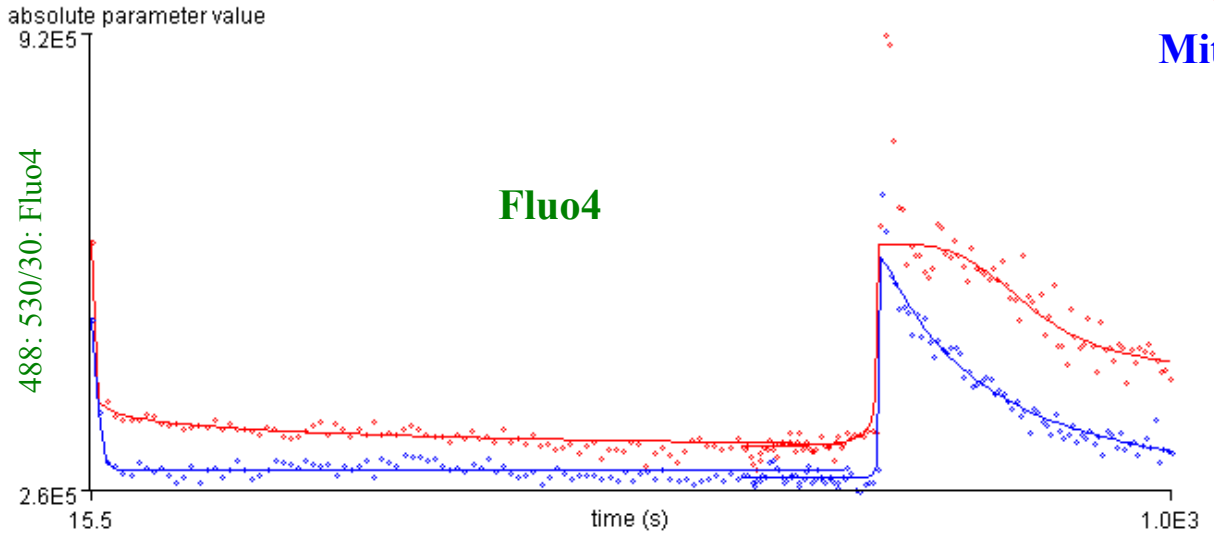
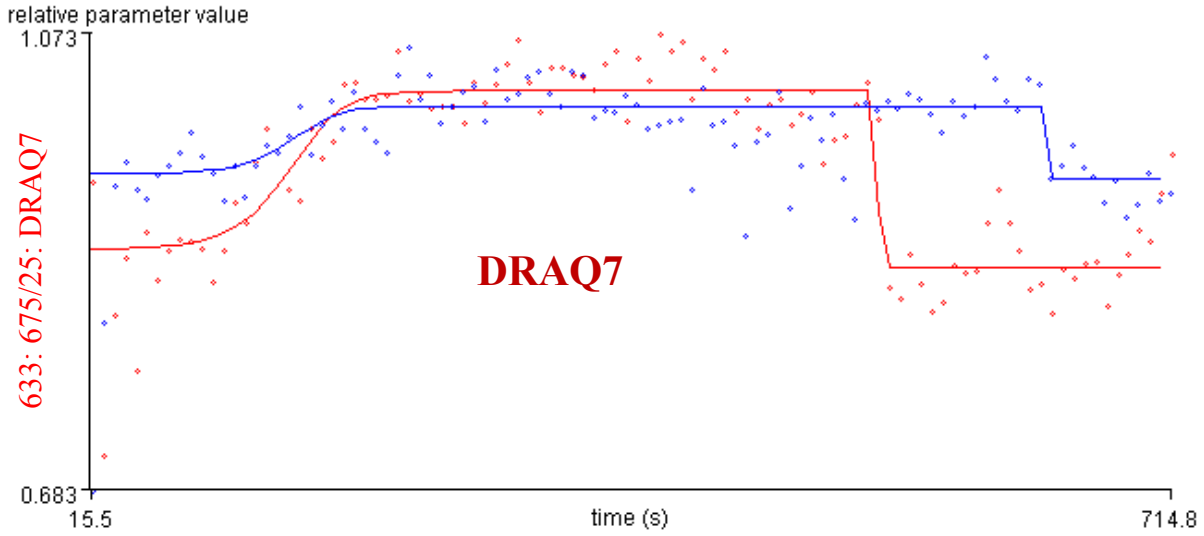
Mitoplicator OFF

Mitoplicator ON





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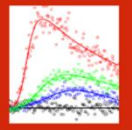
Kinetic analysis using FacsKin software



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Cellular parameter	Mitoplicator treatment		Starting value	Maximum value	Ending value	Time to reach maximum	Time from maximum to second 50% value	Slope at first 50% value	AUC
I.C. calcium (0-720 s)	+		0,99 [0,49-1,07]	1,42 [1,02-1,81]	0,49 [0,00-0,96]	138,64 [0,01-41774,00]	82,24 [17,34-2527,98]	0,11 [0,01-0,69]	608,47 [327,72-613,18]
	-		1,03 [1,02-1,10]	1,37 [1,04-1,76]	0,78 [0,36-1,00]	110,34 [12,08-255,49]	330,91 [9,53-500,67]	0,11 [0,02-0,44]	611,61 [601,92-623,05]
		P=	0,69	0,89	0,49	0,89	0,20	1,00	0,49
I.C. calcium (620-1020 s)	+		1,00 [0,92-1,03]	2,35 [1,98-5,02]	1,72 [0,58-2,68]	751,69 [748,49-771,29]	67,12 [38,06-140,71]	0,67 [0,19-7,49]	599,74 [549,39-621,62]
	-		0,99 [0,97-1,01]	1,89 [1,03-3,78]	0,94 [0,86-41646,00]	752,00 [749,92-754,08]	70,97 [29,35-123,40]	0,74 [0,52-1,46]	591,73 [578,91-607,59]
		P=	0,89	0,49	0,89	1,00	0,89	1,00	0,89
SSC (0-720s)	+		1,01 [0,94-1,03]	1,03 [1,01-1,05]	0,96 [0,88-0,98]	318,24 [0,00-467,47]	166,51 [66,07-265,25]	0,01 [0,00-0,04]	597,72 [592,20-603,35]
	-		1,02 [0,95-1,04]	1,03 [1,02-1,05]	0,91 [0,36-0,95]	203,21 [15,50-451,66]	208,85 [127,30-1895,53]	0,15 [0,00-0,26]	600,47 [599,39-604,29]
		P=	0,69	1,00	0,34	0,89	0,34	0,34	0,49
SSC (620-1020s)	+		1,00 [0,99-1,01]	1,02 [1,01-41646,00]	0,99 [0,92-1,06]	825,72 [710,93-948,71]	145,97 [1,60-17905,04]	0,09 [0,00-0,21]	597,01 [592,13-603,02]
	-		0,99 [0,98-1,00]	1,06 [1,04-1,10]	1,02 [1,00-1,07]	967,99 [841,52-26407,23]	29,84 [1,29-23846,09]	0,02 [0,00-0,08]	593,56 [588,79-598,02]
		P=	0,34	0,34	0,20	0,11	0,69	0,34	0,34
FSC (0-720s)	+		1,01 [0,90-1,02]	1,04 [1,02-41640,00]	0,95 [0,90-41642,00]	340,39 [250,42-552946,52]	129,13 [0,00-19262,83]	0,06 [0,00-0,21]	603,04 [583,39-611,28]
	-		1,01 [0,93-1,02]	1,02 [1,01-1,04]	0,95 [0,94-0,97]	206,13 [0,00-255,22]	325,43 [0,00-487,41]	0,00 [0,00-0,08]	599,99 [599,38-600,77]
		P=	0,69	0,25	1,00	0,06	0,69	0,49	1,00
FSC (620-1020s)	+		0,99 [0,99-1,00]	1,04 [0,99-1641,00]	0,95 [0,92-1,00]	718,60 [0,01-865,07]	562,66 [20,33-41833,00]	0,17 [0,00-0,52]	595,18 [592,25-599,96]
	-		0,99 [0,97-1,00]	1,05 [1,00-41645,00]	0,99 [0,98-1,04]	842,28 [746,04-955,50]	102,79 [37,45-137,67]	0,13 [0,06-,42]	595,46 [580,96-01,10]
		P=	0,89	0,77	0,20	0,34	0,34	0,89	0,89
Draq 7 (0-720s)	+		0,95 [0,89-0,99]	1,02 [1,01-1,03]	0,98 [0,87-1,02]	371,82 [258,03-435,34]	167,58 [126,82-11311,25]	0,00 [0,00-0,00]	597,86 [584,82-602,63]
	-		0,95 [0,86-0,99]	1,02 [1,00-1,04]	0,94 [0,79-0,97]	409,59 [332,14-737,63]	533,43 [170,16-1528,91]	0,00 [0,00-0,00]	595,58 [581,24-601,18]
		P=	0,88	0,89	0,34	0,49	0,49	0,66	0,69

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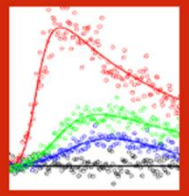
Collaborations



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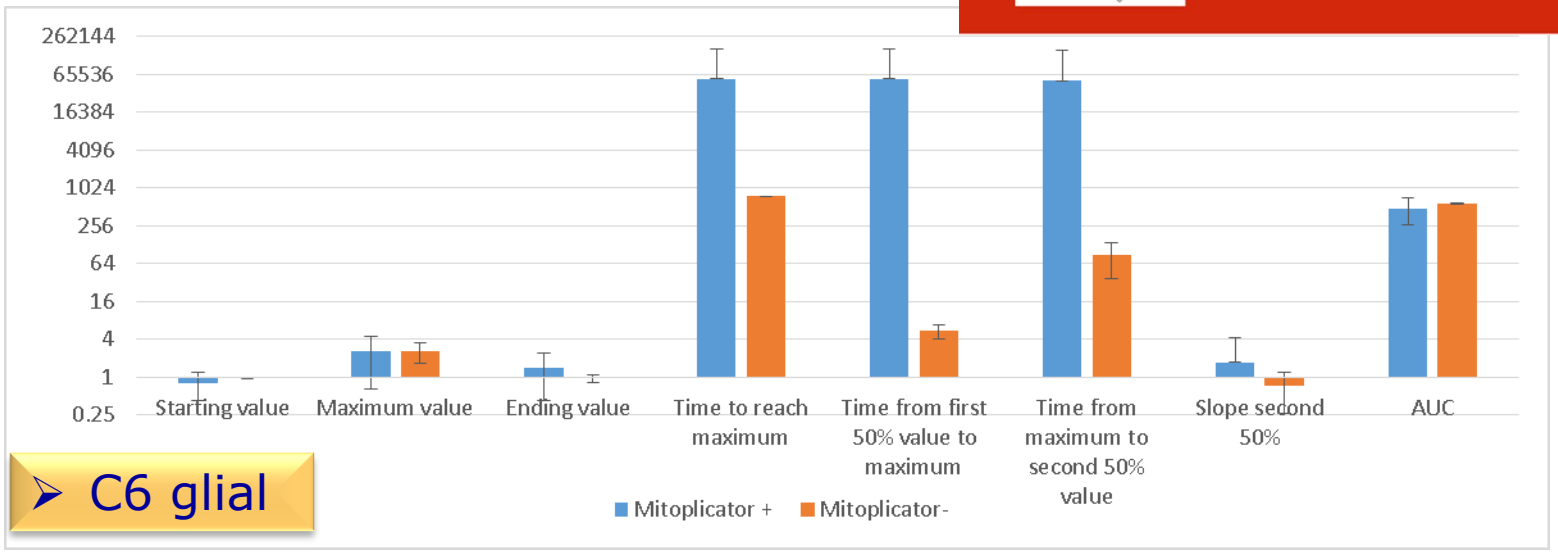
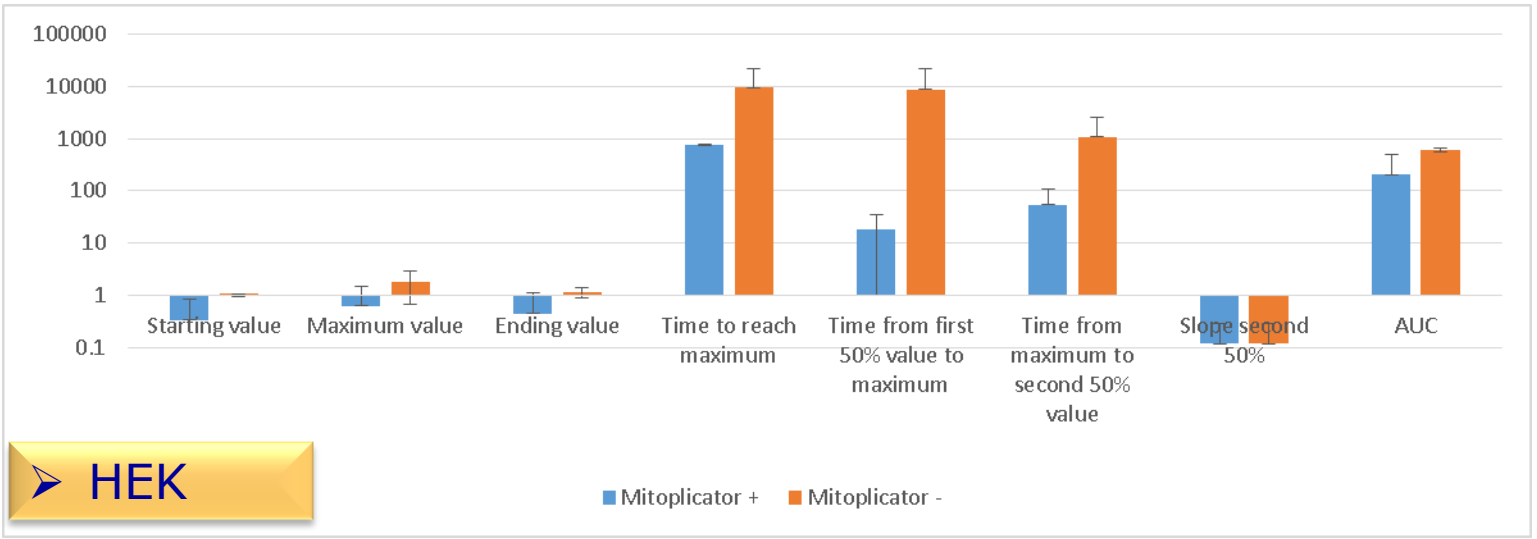


➢ C6 glial



➢ HEK

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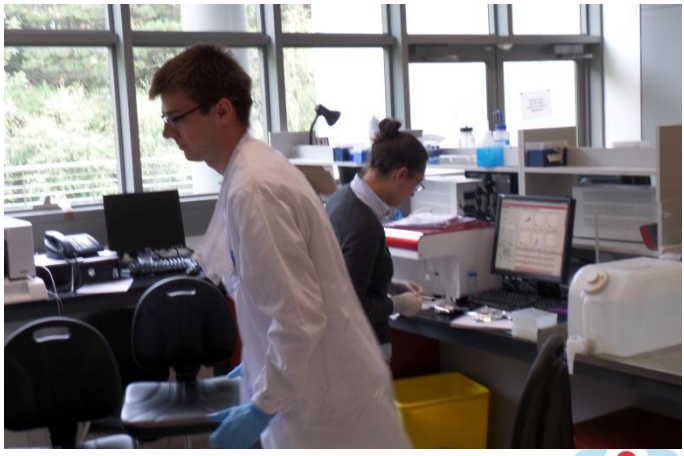

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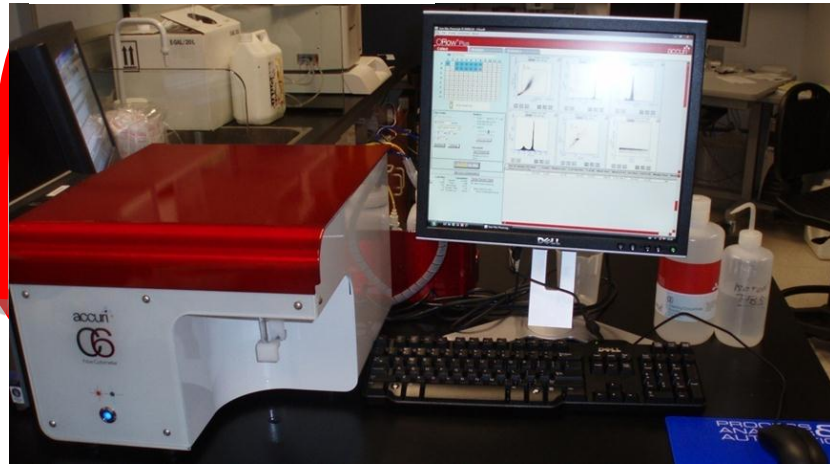

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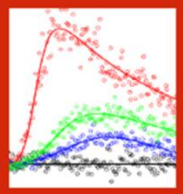

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Acknowledge

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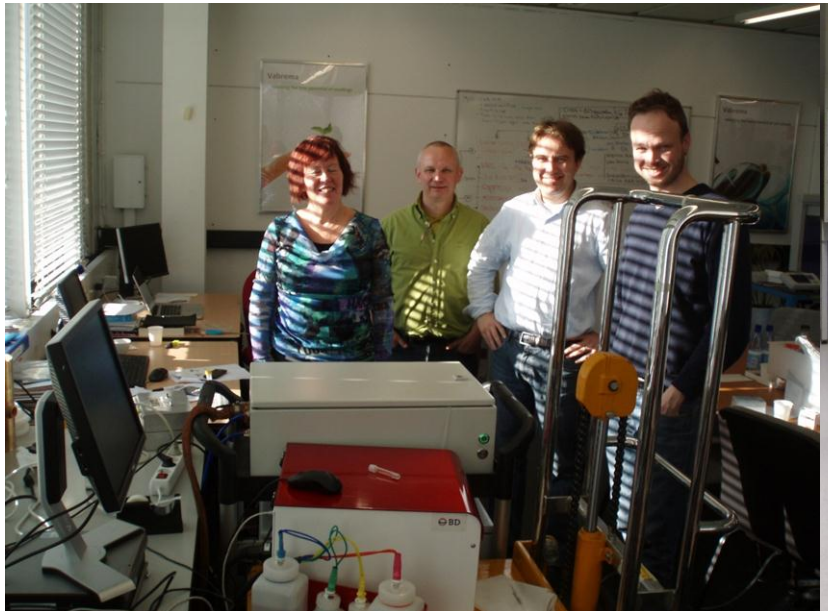


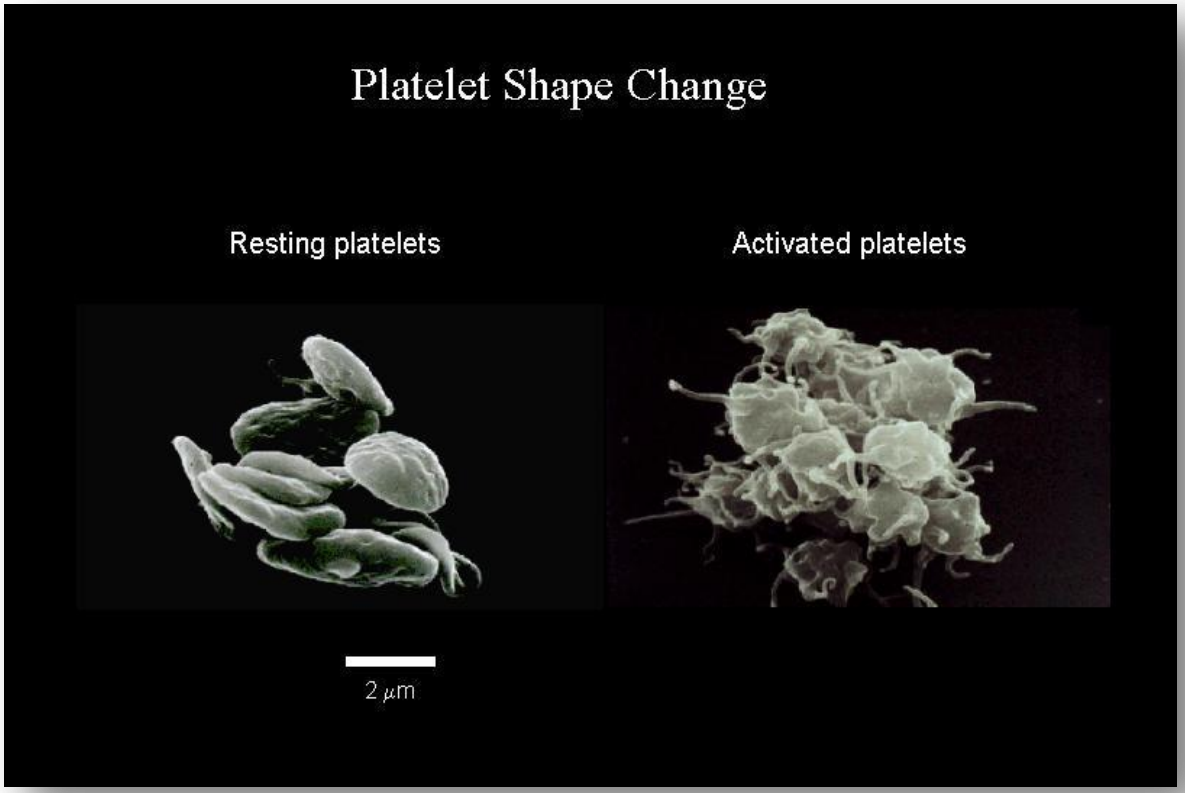
Cell Biology Applications of Real-Time Flow Cytometry Platelets

Chris Jones

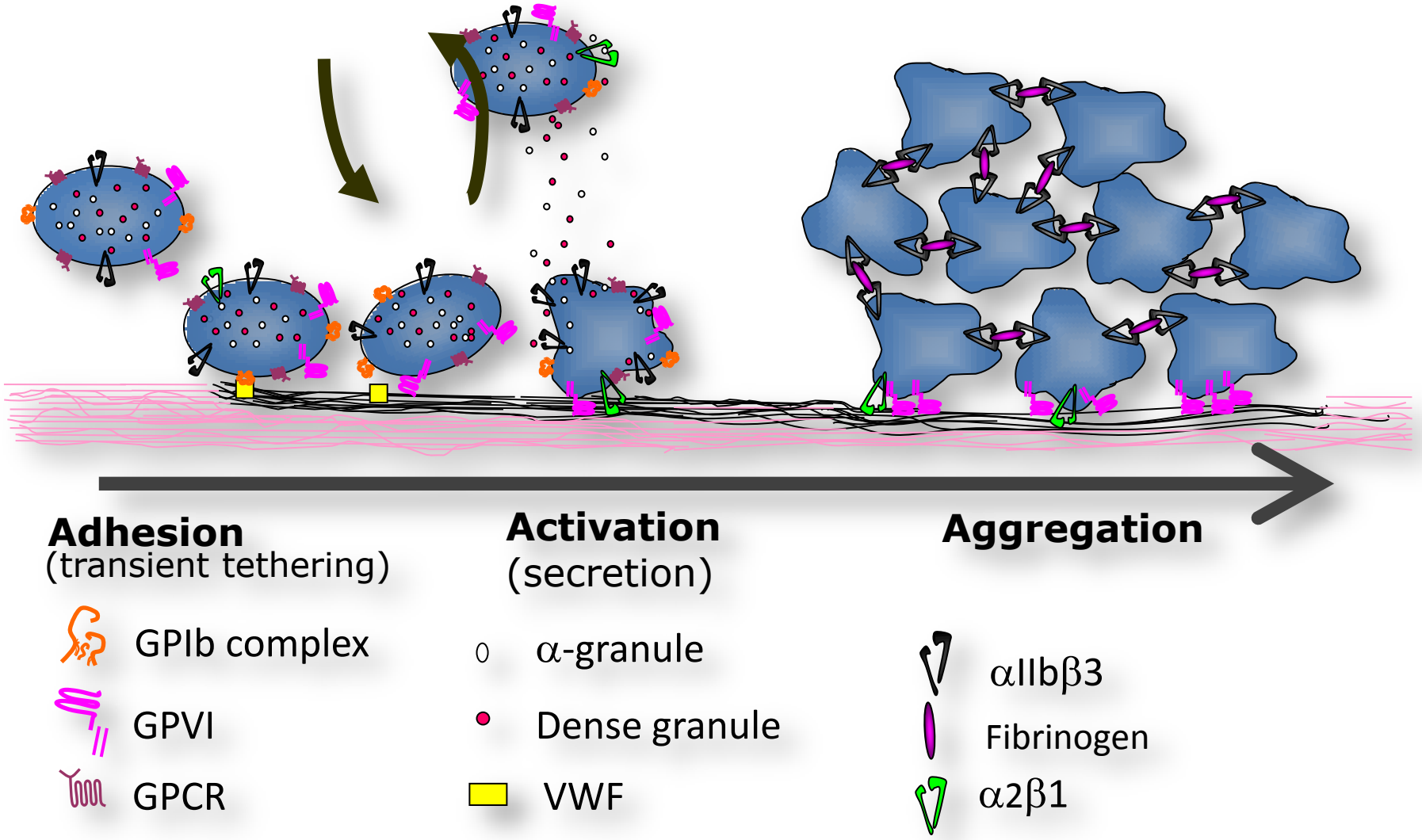
ICMR

University of Reading

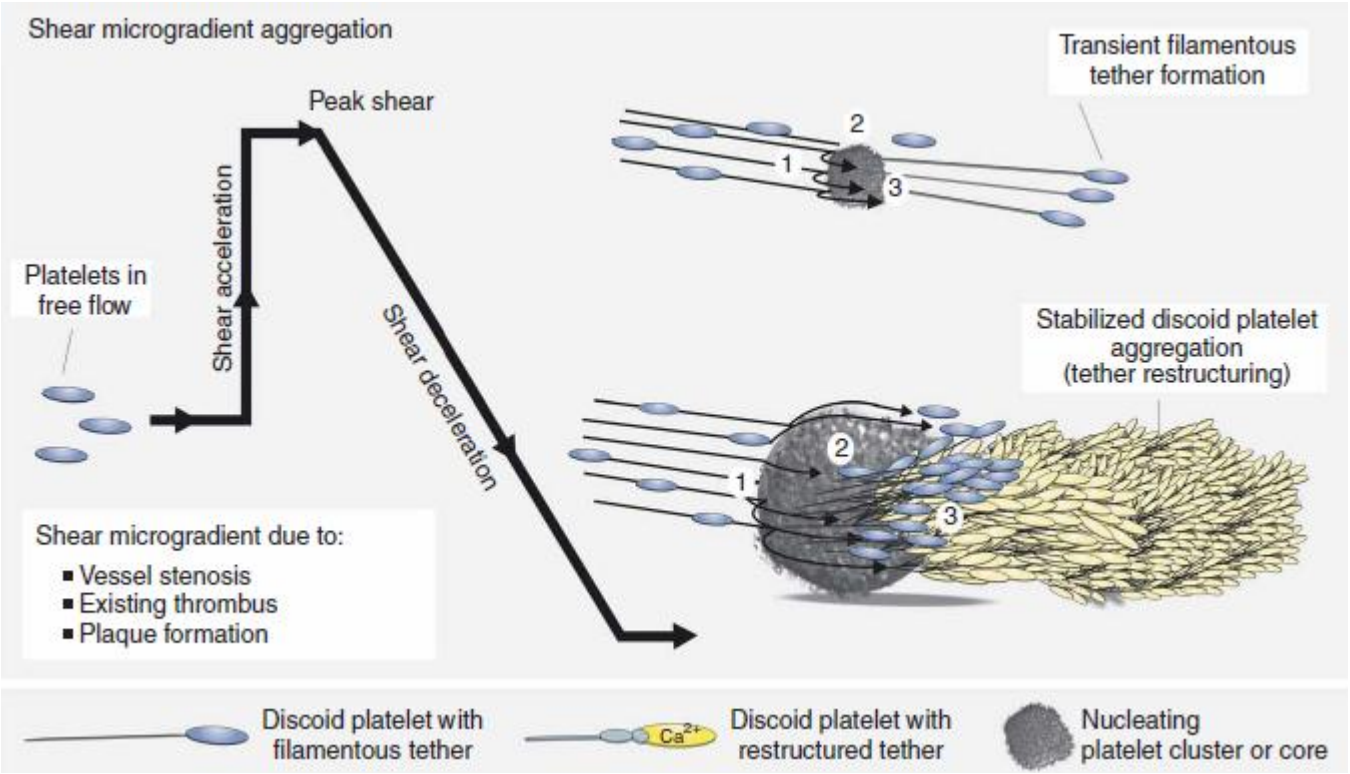
Platelet function



Introduction to platelets



Introduction to platelets

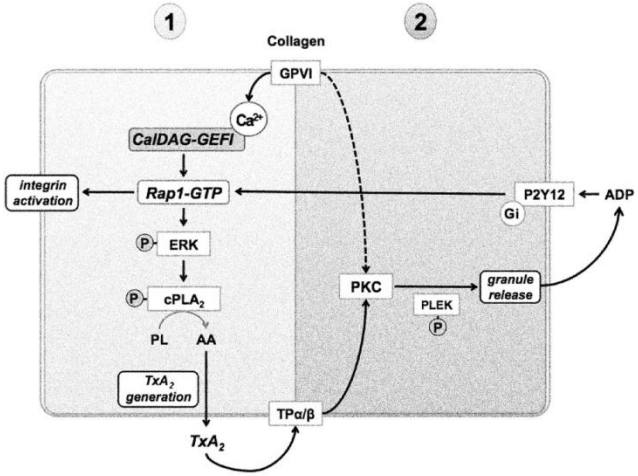


A shear gradient–dependent platelet aggregation mechanism drives thrombus formation

Warwick S Nesbitt[1,5], Erik Westein[1,5], Francisco Javier Tovar-Lopez[2], Elham Tolouei[3], Arnan Mitchell[2], Jia Fu[1], Josie Carberry[3], Andreas Fouras[4] & Shaun P Jackson[1]

NATURE MEDICINE VOLUME 15 | NUMBER 6 | JUNE 2009

The importance of rate



CalDAG-GEFI and protein kinase C represent alternative pathways leading to activation of integrin αIIbβ3 in platelets

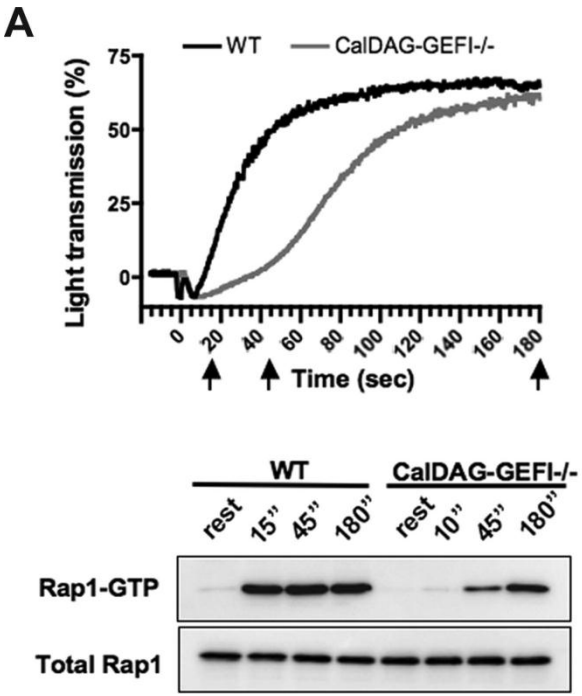
Stephen M. Cifuni, Denisa D. Wagner and Wolfgang Bergmeier
Blood. 2008 Sep 1;112(5):1696-703.

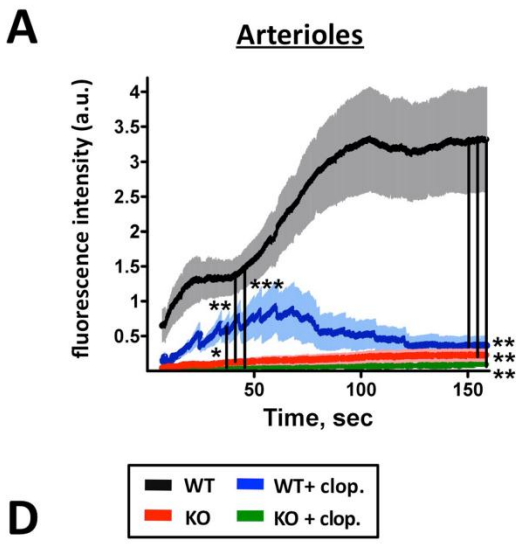
CalDAG-GEFI is at the nexus of calcium-dependent platelet activation

Lucia Stefanini,[1] R. Claire Roden,[1] and Wolfgang Bergmeier[1]
Blood 2009 114:2506-2514

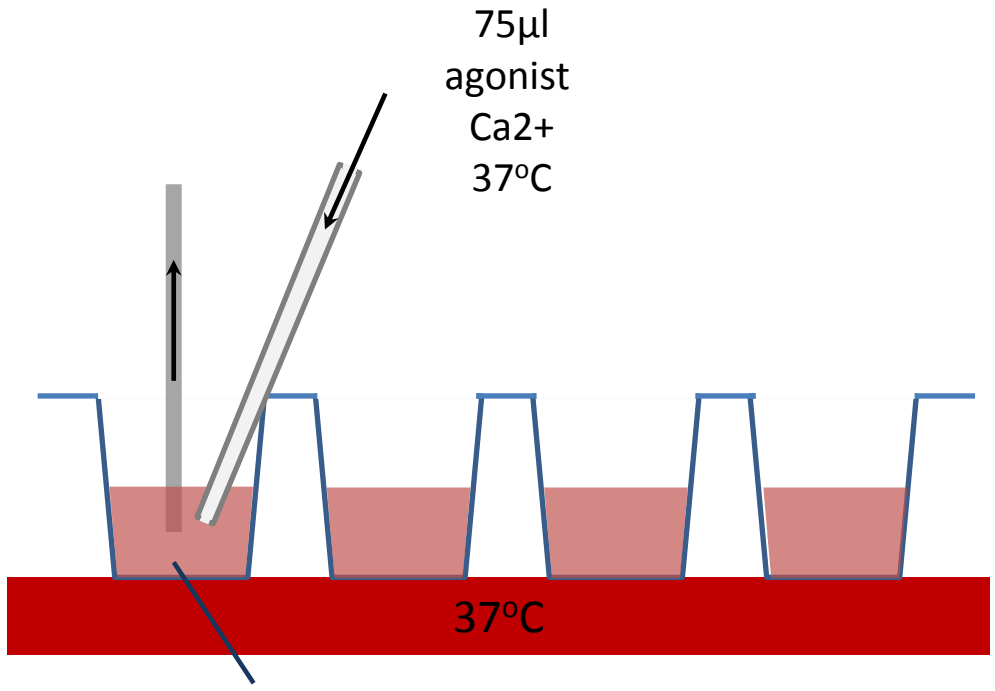
The kinetics of {alpha}IIb{beta}3 activation determines the size and stability of thrombi in mice: implications for antiplatelet therapy

Moritz Stolla, Lucia Stefanini, R. Claire Roden, Massiel Chavez, Jessica Hirsch, Teshell Greene, Timothy D. Ouellette, Sean F. Maloney, Scott L. Diamond, Mortimer Poncz, Donna S. Woulfe and Wolfgang Bergmeier
Blood. 2011 Jan 20;117(3):1005-13

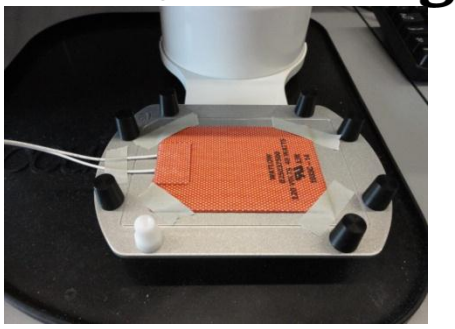


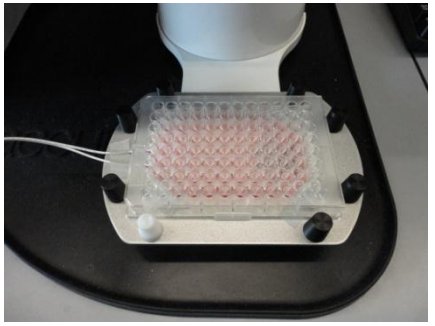


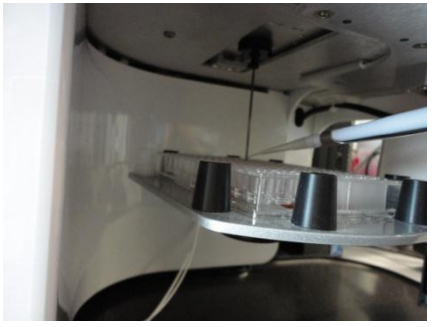
The assay



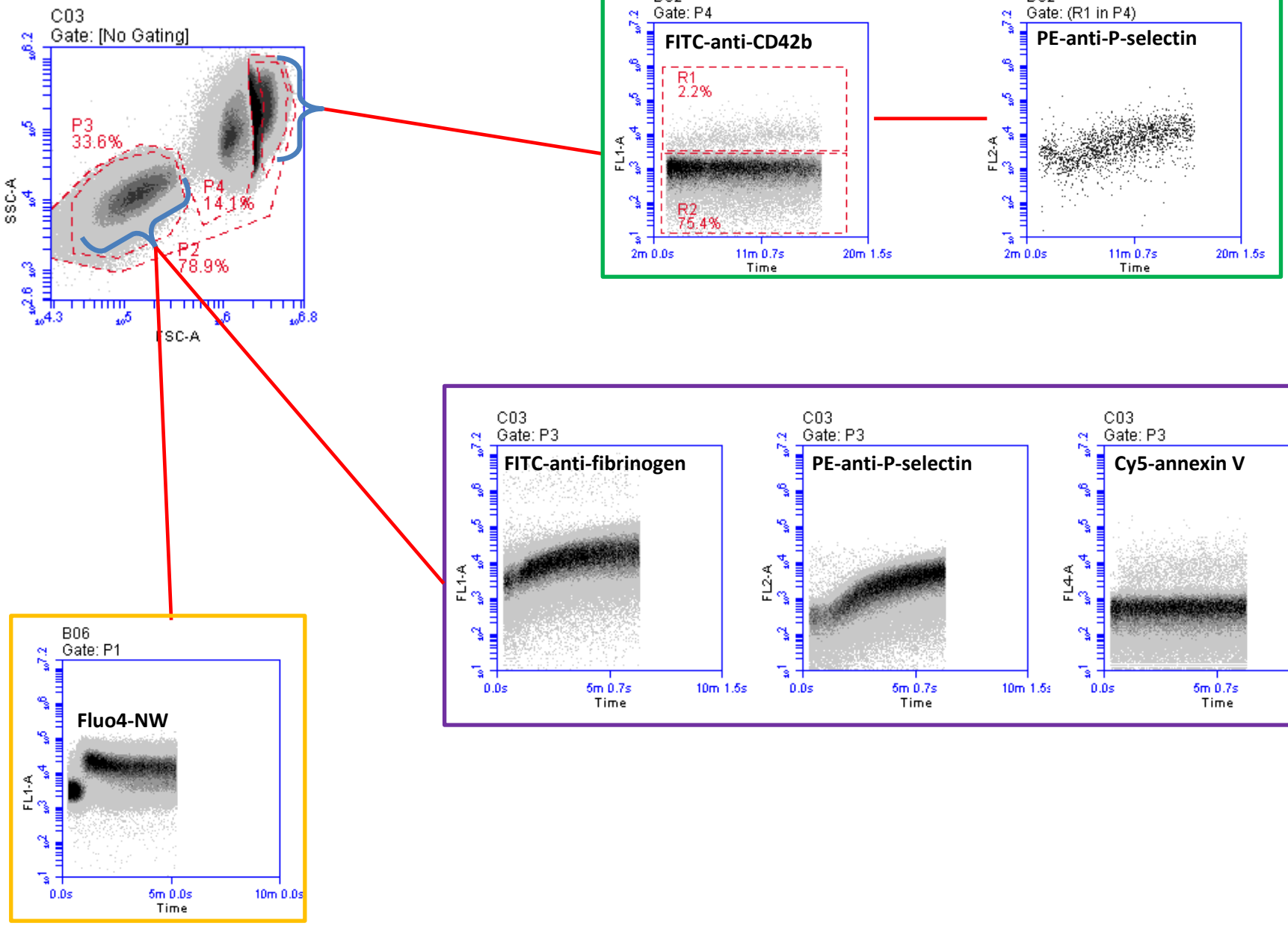
225µl
Citrated Whole blood (1:100)
+
Fluo4-NW — no probenicid
or
FITC-anti-fibrinogen
PE-anti-P-selectin
Cy5-annexin V — dialysed



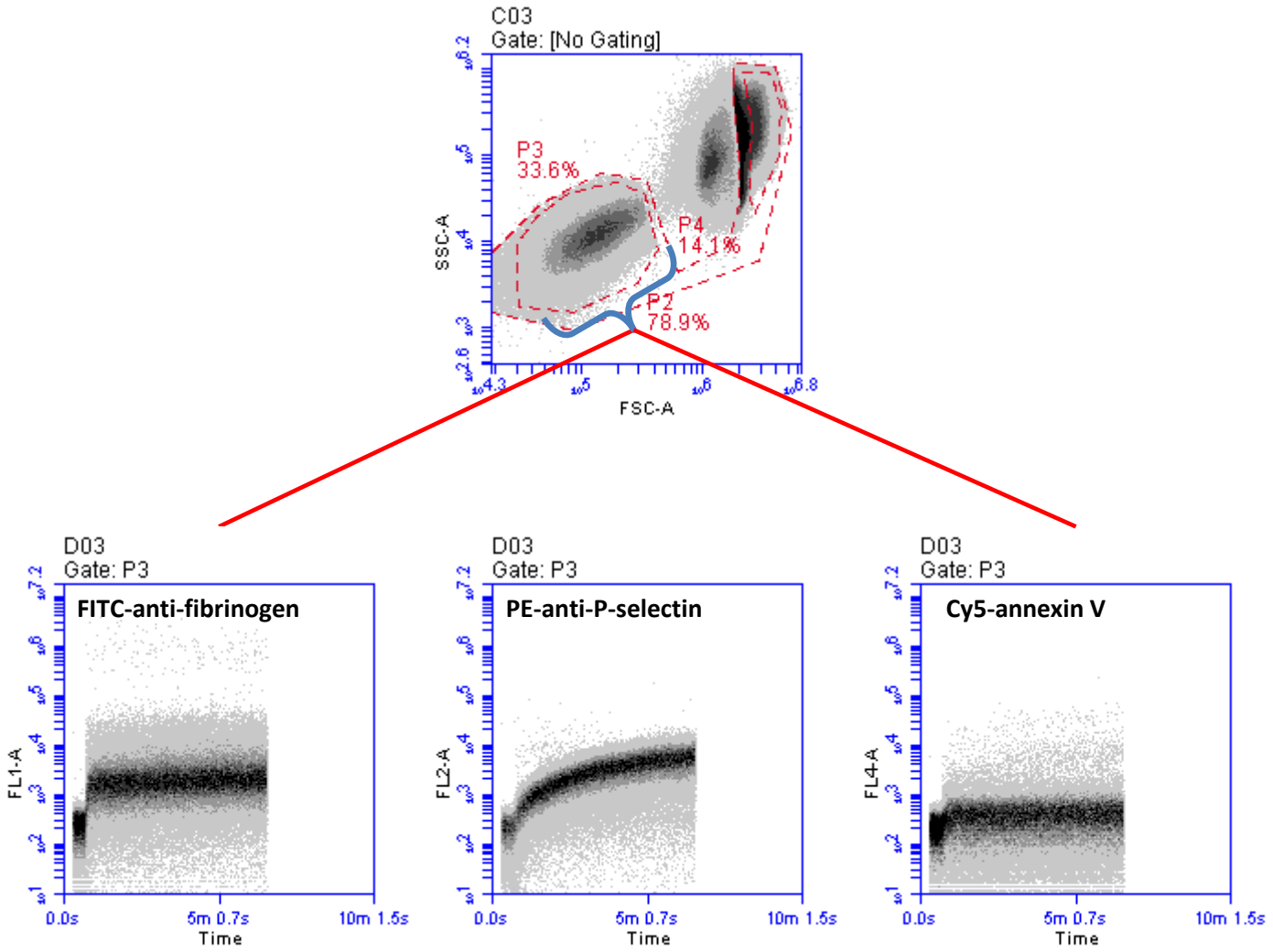




The assay


C03
Gate: [No Gating]
P3
33.6%
P4
14.1%
P2
78.9%
SSC-A
FSC-A
D02
Gate: P4
FITC-anti-CD42b
R1
2.2%
R2
75.4%
FL1-A
Time
D02
Gate: (R1 in P4)
PE-anti-P-selectin
FL2-A
Time
C03
Gate: P3
FITC-anti-fibrinogen
FL1-A
Time
C03
Gate: P3
PE-anti-P-selectin
FL2-A
Time
C03
Gate: P3
Cy5-annexin V
FL4-A
Time
B06
Gate: P1
Fluo4-NW
FL1-A
Time

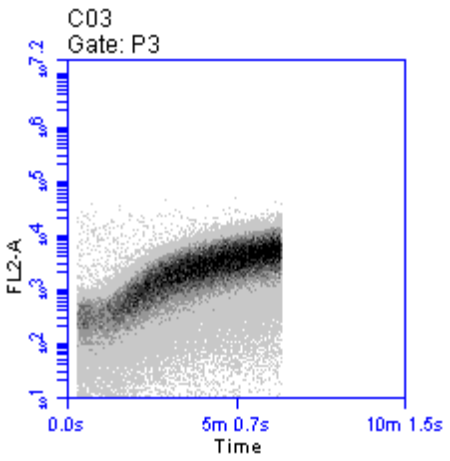
The assay

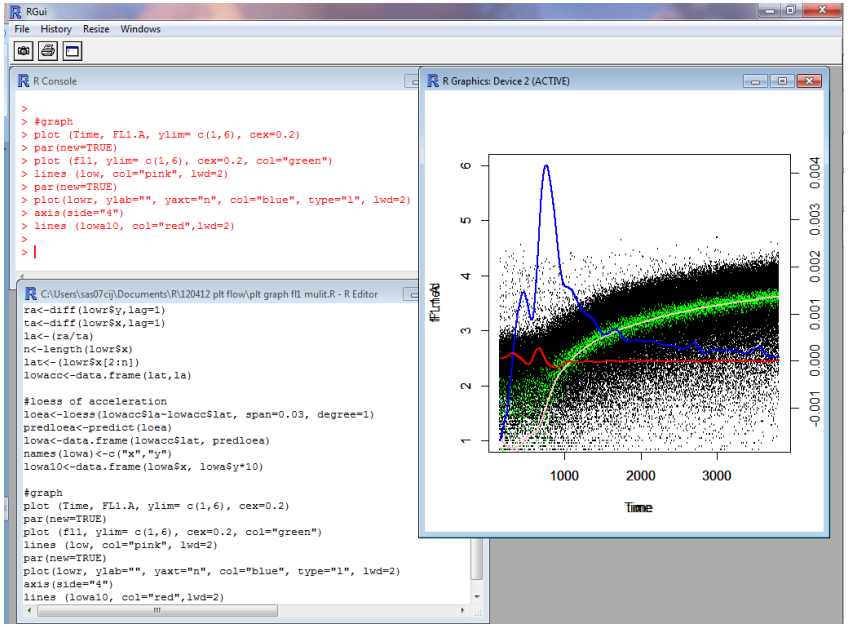

C03
Gate: [No Gating]
P3
33.6%
P4
14.1%
P2
78.9%
SSC-A
FSC-A
D03
Gate: P3
FITC-anti-fibrinogen
FL1-A
D03
Gate: P3
PE-anti-P-selectin
FL2-A
D03
Gate: P3
Cy5-annexin V
FL4-A
0.0s
5m 0.7s
10m 1.5s
Time

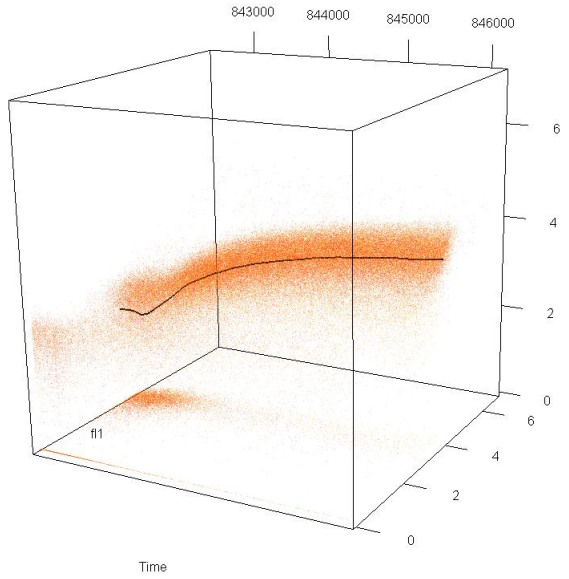
Analysis using R

www.r-project.org

FSC-A	SSC-A	FL1-A	FL2-A	FL3-A	FL4-A	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL4-H	Width	Time
130381	21588	2439	379	386	58	262130	37432	2724	215	263	89	38	142
95998	6941	1046	338	105	307	200804	12846	1084	202	163	324	36	142
138125	7572	3538	472	80	235	259279	11645	3494	335	293	275	39	142
34556	8110	740	355	202	120	82669	16567	787	238	409	49	22	142
205857	14330	2784	397	437	153	396674	25294	3136	270	537	209	42	142
33899	2495	750	164	110	0	76473	5316	605	178	152	62	24	142
195063	13104	1419	340	293	319	392536	21766	1616	246	658	211	40	142
131735	8282	3198	504	394	0	269096	14464	3421	423	417	217	38	142
33757	2425	961	210	31	296	87982	4849	945	178	3	264	23	142
114836	7388	1307	173	0	152	244855	12029	1577	201	111	194	37	142
65290	4829	1608	263	0	0	146061	9801	1709	209	0	45	27	142
186328	22116	1646	138	0	0	388523	39867	1850	110	0	52	41	142
90824	13633	1400	138	110	65	198368	24023	1401	211	278	118	32	142
150220	6475	1388	607	380	11	320760	10668	1507	408	664	285	38	142

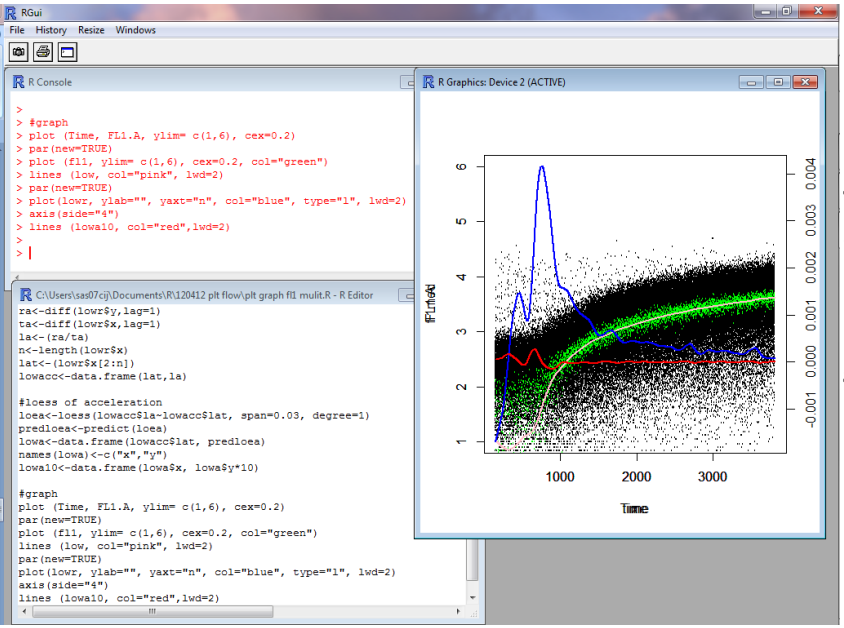


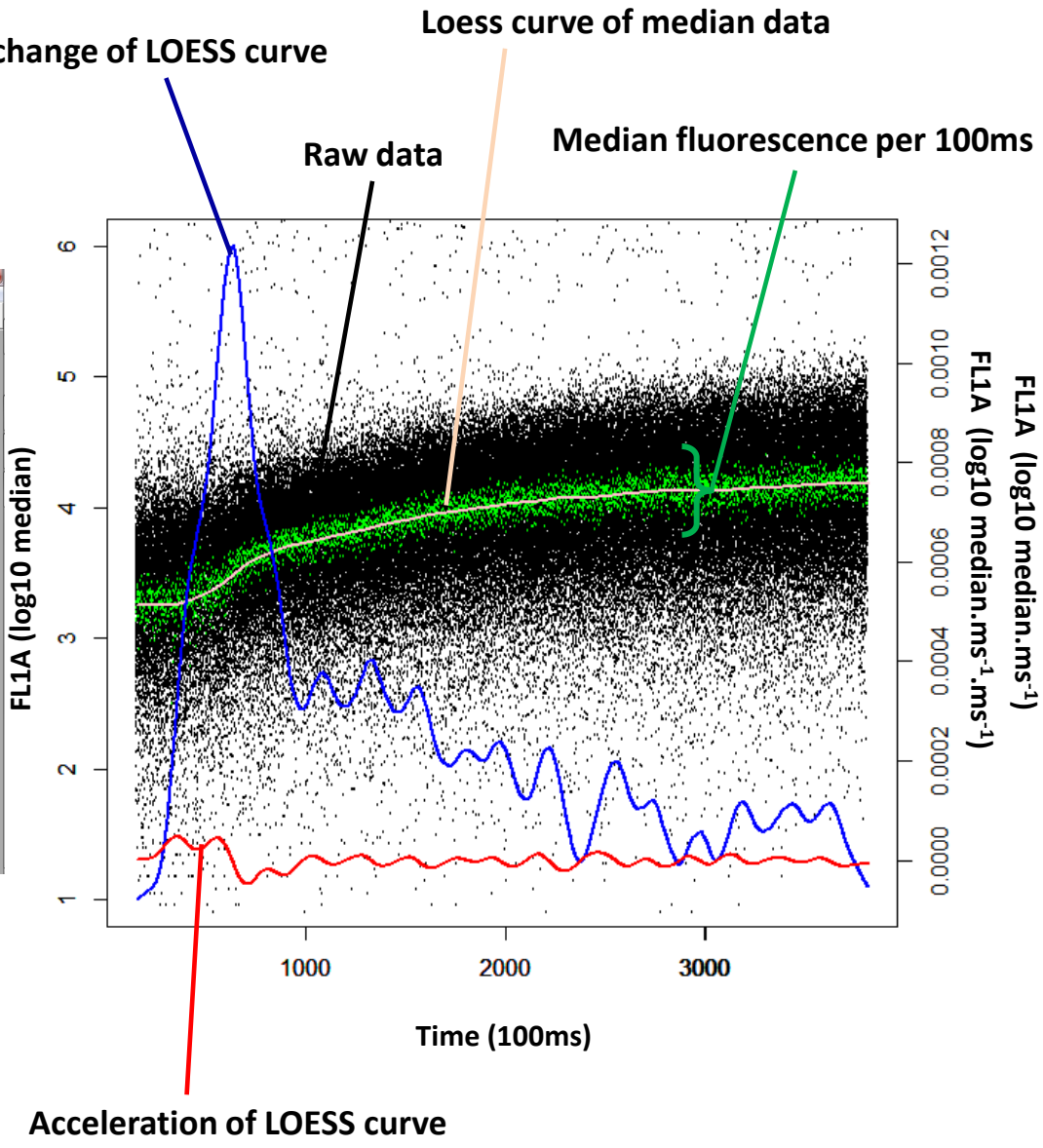




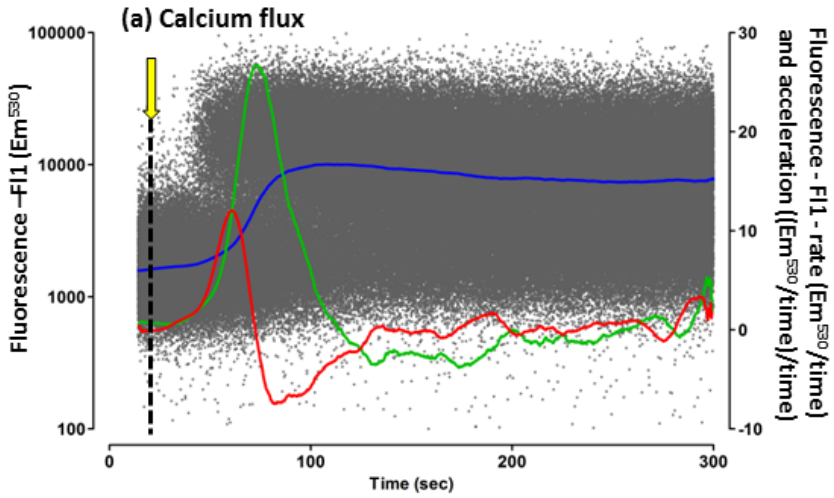
Analysis using R

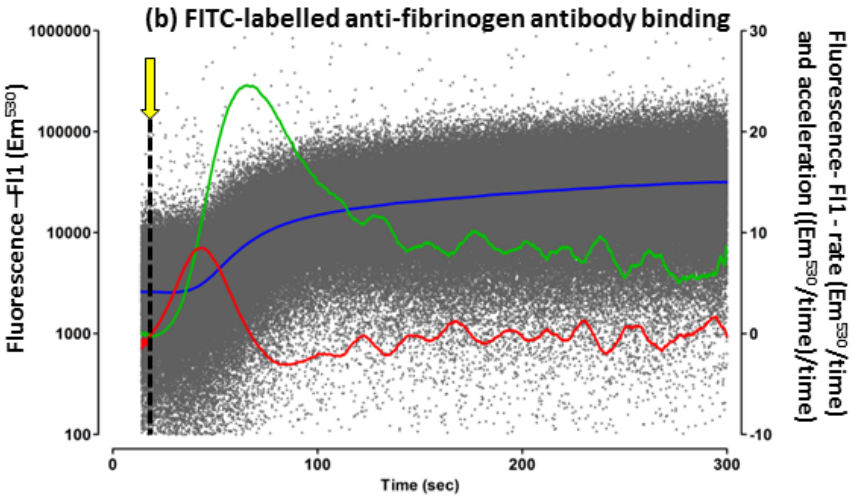
www.r-project.org

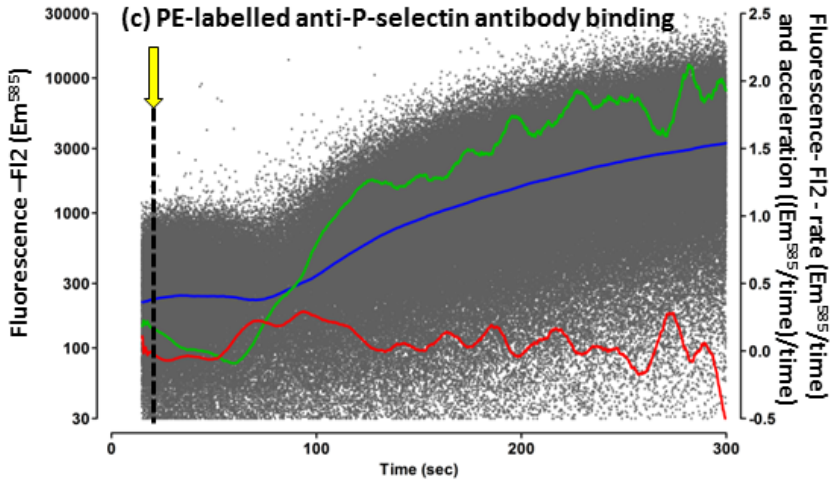


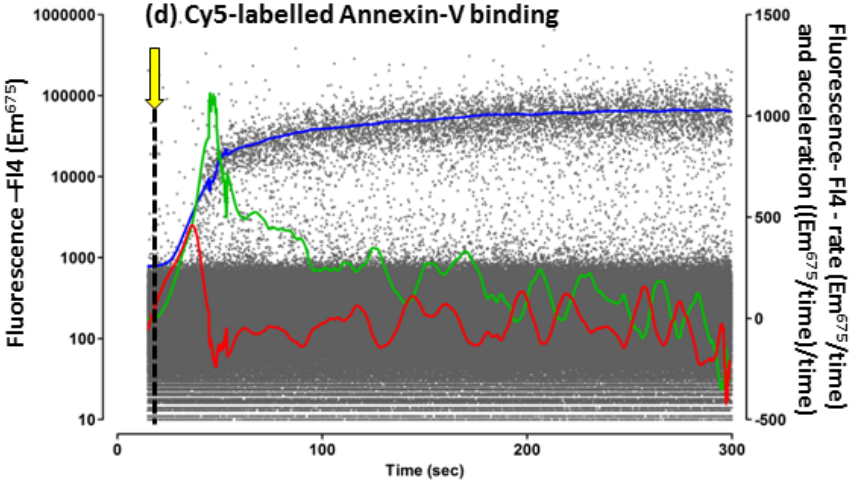


Analysis using R







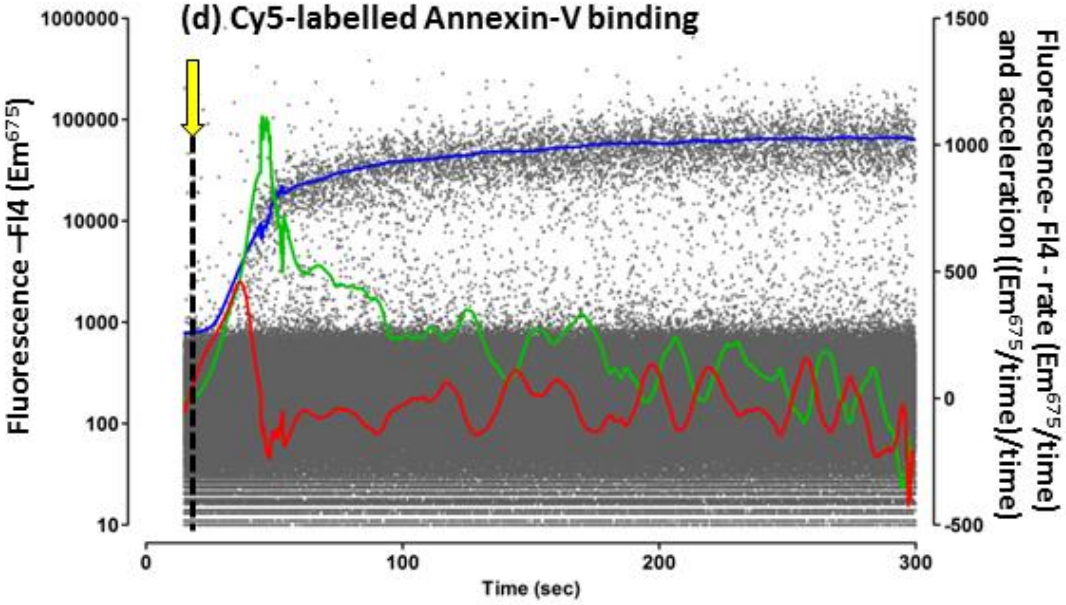


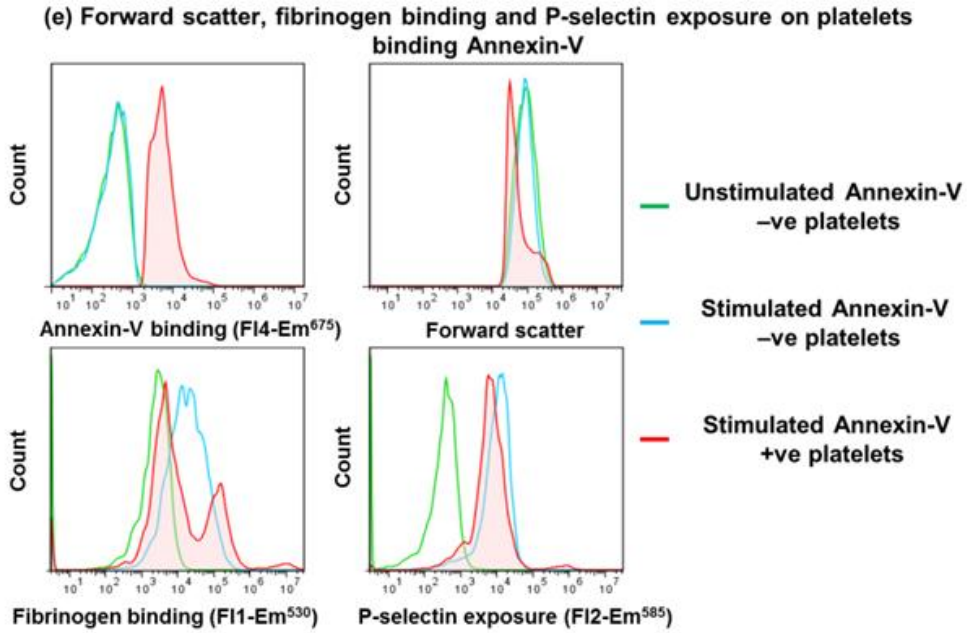
Is the assay robust?

Table 1		CV
Ca^{2+} flux	Max.	0.03
	Max. rate	0.03
	Max. acceleration	0.06
Fibrinogen binding	Max.	0.08
	Max. rate	0.06
	Max. acceleration	0.06
P-selectin exposure	Max.	0.08
	Max. rate	0.06
	Max. acceleration	0.16
Annexin-V binding	Max.	0.06
	Max. rate	0.07
	Max. acceleration	0.09

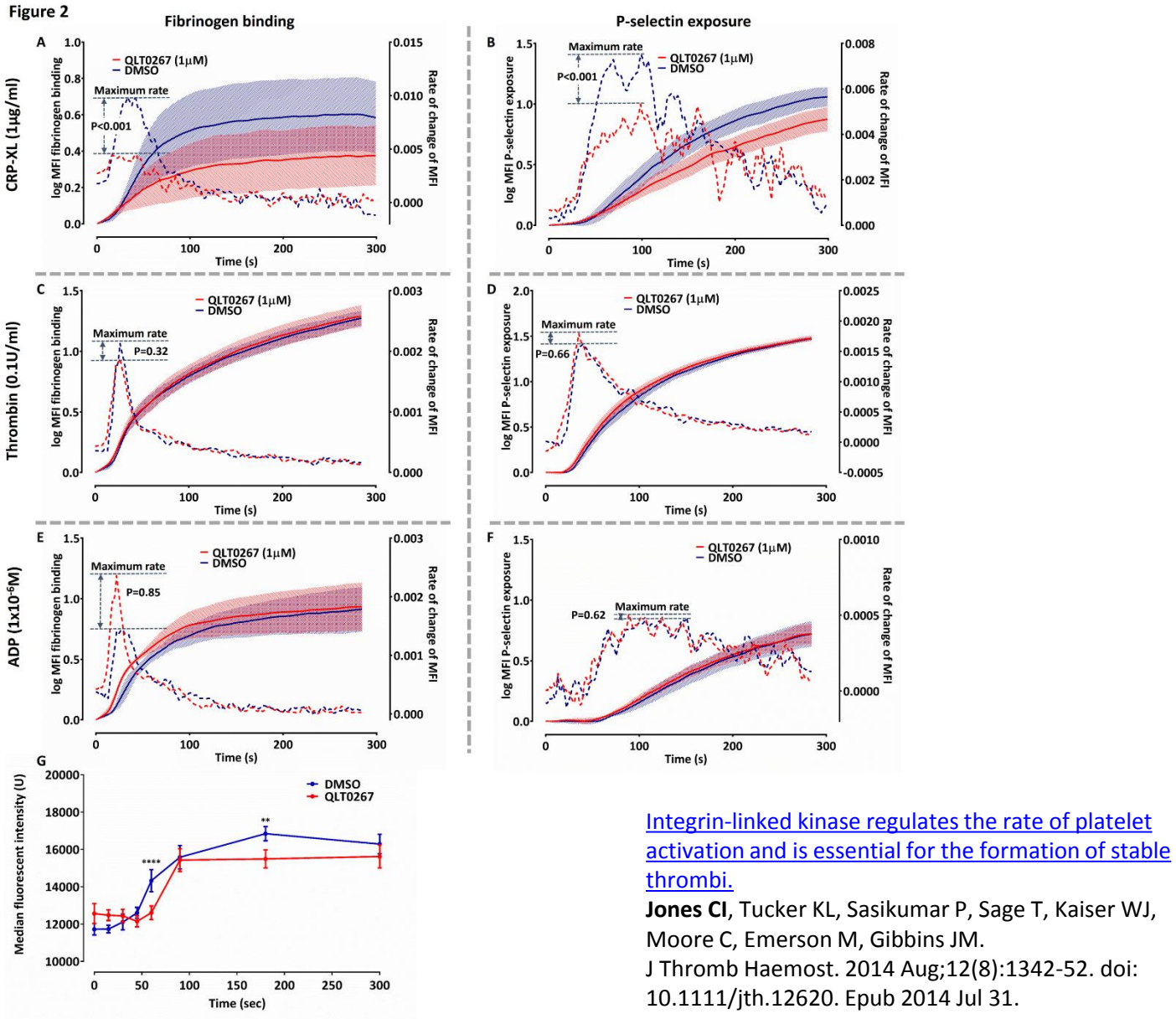
Is rate independent of maximum response?

Table 1		Correlation with maximum	
		r^2	p
Ca^{2+} flux	Max.	.....	.....
	Max. rate	0.004	0.92
	Max. acceleration	0.594	0.13
Fibrinogen binding	Max.	.....	.....
	Max. rate	0.458	0.21
	Max. acceleration	0.260	0.38
P-selectin exposure	Max.	.....	.....
	Max. rate	0.001	0.97
	Max. acceleration	0.009	0.27
Annexin-V binding	Max.	.....	.....
	Max. rate	0.001	0.98
	Max. acceleration	0.434	0.02


(d) Cy5-labelled Annexin-V binding
Fluorescence –Fl4 (Em675)
Fluorescence- Fl4 - rate (Em675/time) and acceleration ([Em675/time]/time)
Time (sec)
1000000
100000
10000
1000
100
10
1500
1000
500
0
-500
0
100
200
300


(e) Forward scatter, fibrinogen binding and P-selectin exposure on platelets binding Annexin-V
Count
Annexin-V binding (Fl4-Em675)
Forward scatter
Fibrinogen binding (Fl1-Em530)
P-selectin exposure (Fl2-Em585)
Unstimulated Annexin-V –ve platelets
Stimulated Annexin-V –ve platelets
Stimulated Annexin-V +ve platelets

Is rate independent of maximum response?



[Integrin-linked kinase regulates the rate of platelet activation and is essential for the formation of stable thrombi.](#)

Jones CI, Tucker KL, Sasikumar P, Sage T, Kaiser WJ, Moore C, Emerson M, Gibbins JM.
J Thromb Haemost. 2014 Aug;12(8):1342-52. doi: 10.1111/jth.12620. Epub 2014 Jul 31.

Summary

- Real time assay for the measurement the calcium flux or fibrinogen binding , P-selectin expression and Annexin V binding of populations of individual platelets

- Analysis methodology capable of efficiently extracting appropriate data for large studies or identifying interesting subpopulations

Acknowledgements

Prof. Jon Gibbins

Steve Garner

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