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19. MAY 2016

Norsk Flowcytometri Forening VIII Nasjonale Konferanse

Crimes against Flow Cytometry

Charlotte Christie Petersen
Aarhus University, Denmark

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AGENDA

- Sample preparation
- Dead cells
- Titration
- Fc receptor blocking
- Acquisition
- Multi-colour FCM

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SAMPLE PREPARATION

› Cells in single-cell-suspensions

› Howard Shapiro's seventh law: No data analysis technique can make good data out of bad data

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SAMPLE PREPARATION

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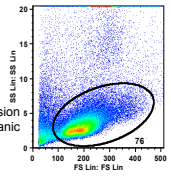
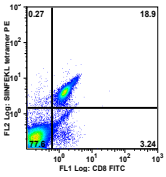
Forward- side scatter

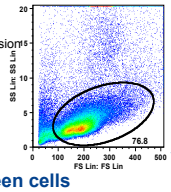
CD8 vs. SIINFEKL tetramer

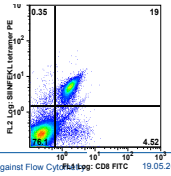
Single cell suspension prepared by mechanic (homogenized) dissociation





Single cell suspension prepared by enzymatic collagenase dissociation





Murine spleen cells

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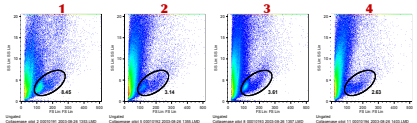
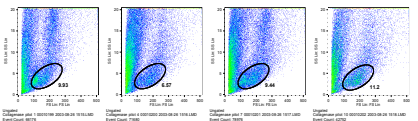
Mouse ID 1 2 3 4

Mechanic (homogenized) dissociation



Enzymatic collagenase dissociation



Murine tumors

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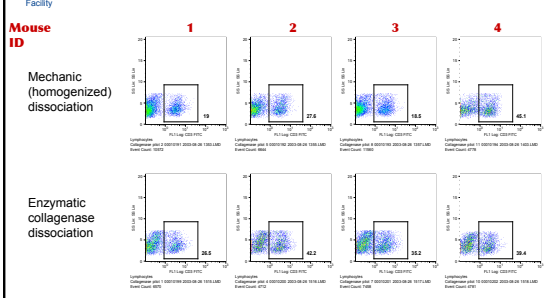

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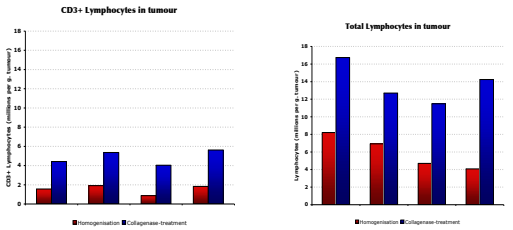

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SAMPLE PREPARATION

› Solid organs: http://www.worthington-biochem.com/tissuedissociation/default.html

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AGENDA

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- Multi-colour FCM

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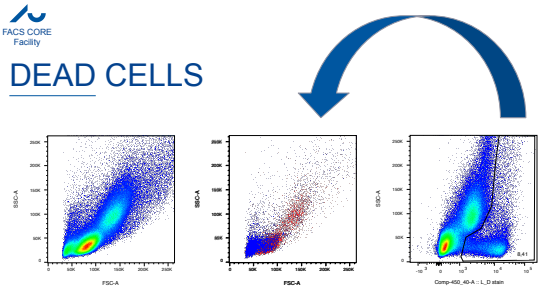
DEAD CELLS



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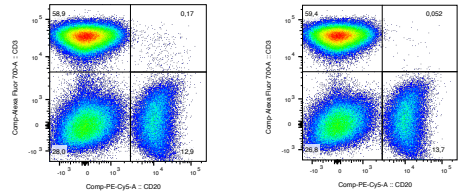
DEAD CELLS



Without dead cell discrimination

With dead cell discrimination

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AGENDA

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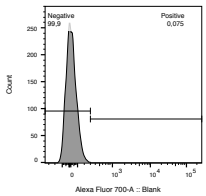
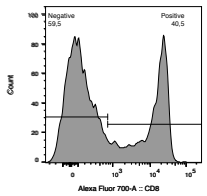
TITRATION





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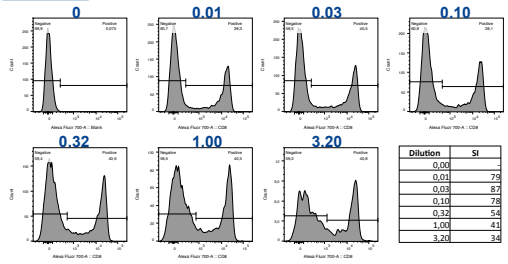
TITRATION

$$\text{Stain Index} = \frac{MFI_{pos} - MFI_{neg}}{2x\ rSD_{neg}}$$



Dilution	SI
0,00	-
0,01	79
0,03	87
0,10	78
0,32	54
1,00	41
3,20	34

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AGENDA

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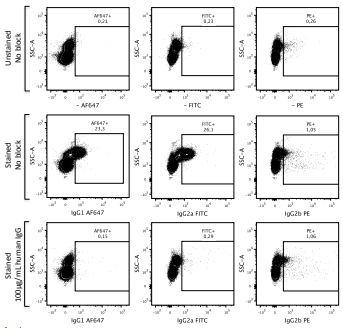
F_C RECEPT



Unpublished data: Morten Nørgaard Andersen
Department of Biomedicine, Aarhus University

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AGENDA

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ACQUISITION

› Correct threshold

› PMT activation and adjustments

› Flow rate

› Time

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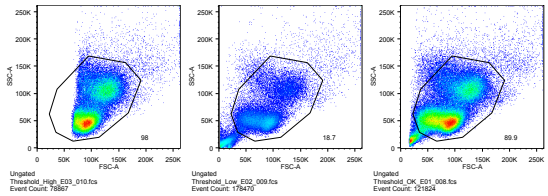
CORRECT THRESHOLD



FSC-H = 60,000 **FSC-H = 200** **FSC-H = 16,000**

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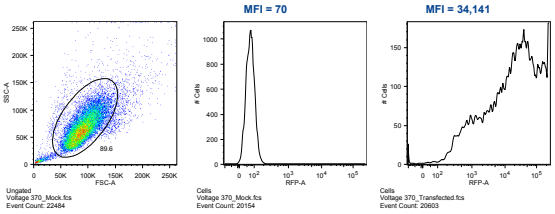
ACQUISITION – PMT ADJUSTMENT



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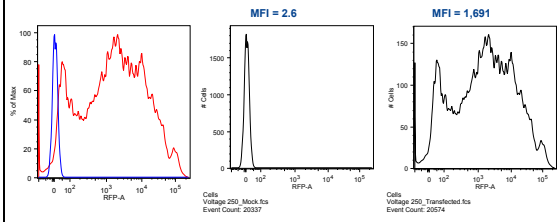
ACQUISITION – PMT ADJUSTMENT



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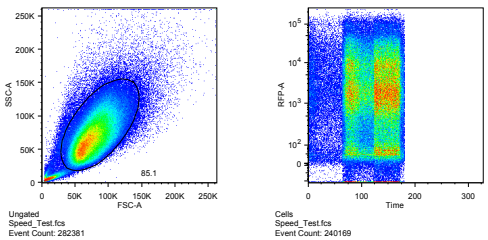
FLOW RATE



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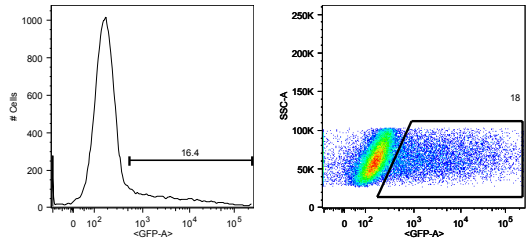
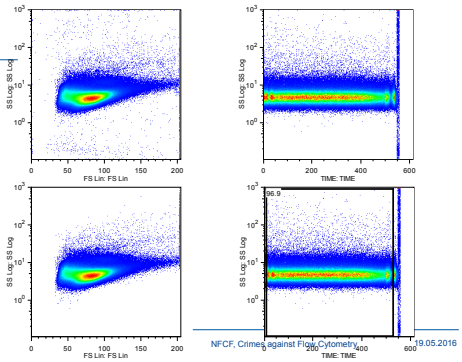
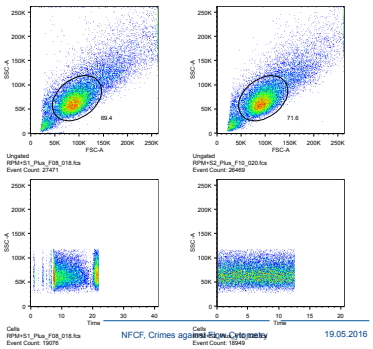
CORRECT GATING



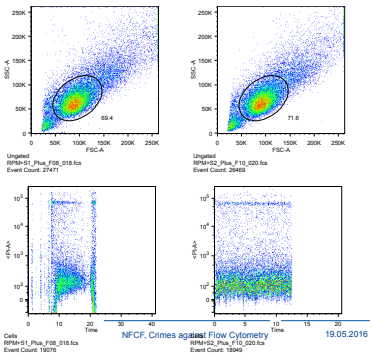
TIME AS A PARAMETER



TIME AS A PARAMETER



TIME AS A PARAMETER



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- Sample preparation
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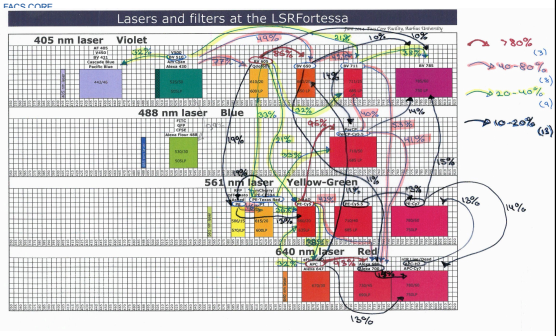

COMPENSATION

A few good and golden rules

1. Controls need to be at least as bright as any sample you will apply the compensation to
2. Background fluorescence should be the same for the positive and negative control populations for any given parameter
3. Your compensation color MUST be the same as your experimental color

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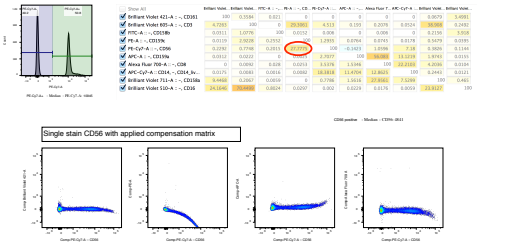
COMPENSATION



Single stain CD56 with applied compensation matrix

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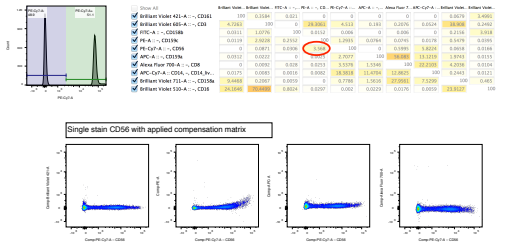
COMPENSATION



Single stain CD56 with applied compensation matrix

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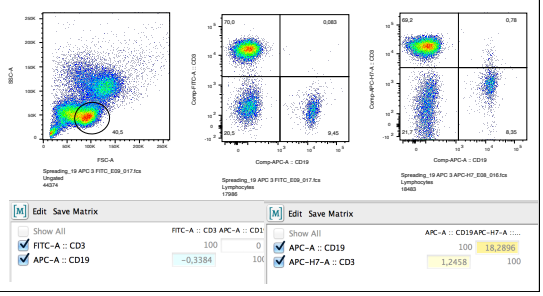
DATA SPREAD



Edit Save Matrix

Show All	FITC-A :: CD3	APC-A :: CD1
FITC-A :: CD3	100	0
APC-A :: CD19	-0,3384	100

Edit Save Matrix

Show All	APC-A :: CD19	APC-H7-A :: ....
APC-A :: CD19	100	18,2896
APC-H7-A :: CD3	1,2458	100

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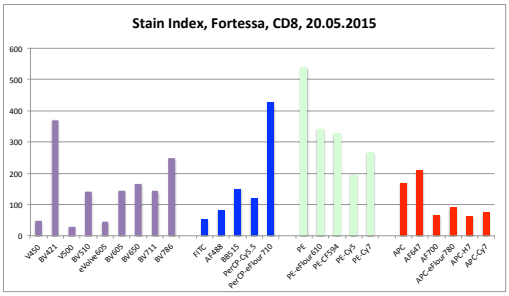

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NOT ALL BAD DATA LOOKS BAD

Data from a 10-color experiment

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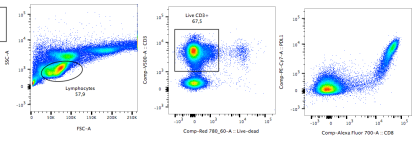
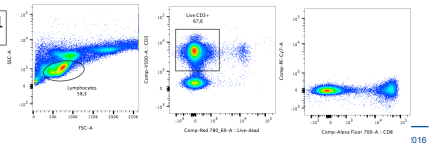
NOT ALL BAD DATA LOOKS BAD

All stains



FMO/PD-L1



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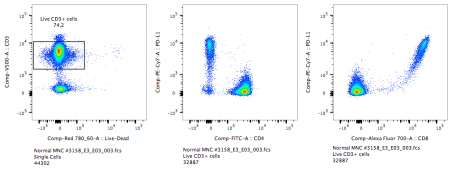
NOT ALL BAD DATA LOOKS BAD

Normal donor and a simple 5-color experiment

Live/Dead nIR, CD3-V500, CD4-FITC, CD8-AF700, PD-L1-PE-Cy7



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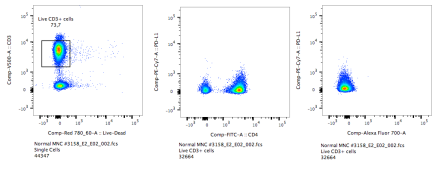
NOT ALL BAD DATA LOOKS BAD

FMO for CD8-AF700

Live/Dead nIR, CD3-V500, CD4-FITC, CD8-AF700, PD-L1-PE-Cy7



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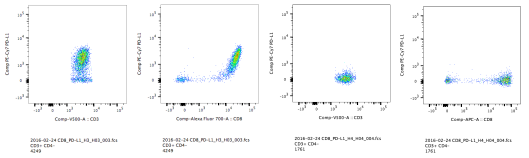
NOT ALL BAD DATA LOOKS BAD

Replacement of CD8-AF700

Gate: CD3+CD4- live lymphocytes



CD8-AF700 CD8-AF647

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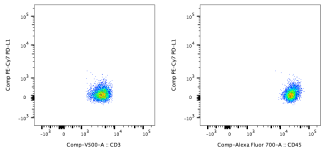
NOT ALL BAD DATA LOOKS BAD

Is it the fluorophore AF700 giving us trouble?

CD8-AF700 is replaced with CD45-AF700



Gate: CD3+CD4- live lymphocytes

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NOT ALL BAD DATA LOOKS BAD

?

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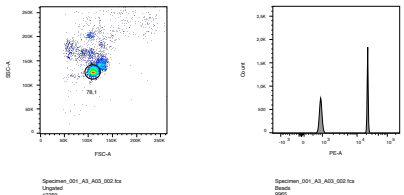
LASER DELAY



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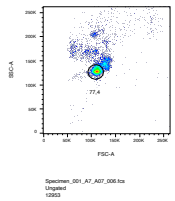
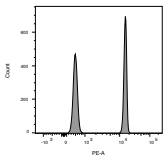
LASER DELAY





Specimen_001_A7_A07_006.fcs
Ungated
12953

Specimen_001_A7_A07_006.fcs
Beads
10030

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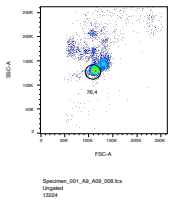
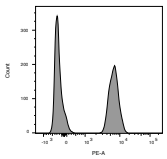
LASER DELAY





Specimen_001_A9_A09_008.fcs
Ungated
13224

Specimen_001_A9_A09_008.fcs
Beads
10099

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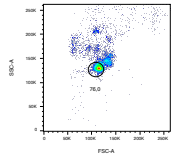
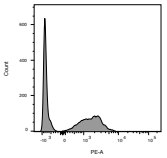
LASER DELAY





Specimen_001_A10_A10_009.fcs
Ungated
13400

Specimen_001_A10_A10_009.fcs
Beads
10187

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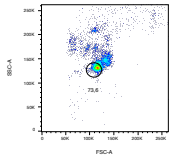
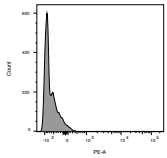
LASER DELAY





Specimen_001_A11_A11_010.fcs
Ungated
13867

Specimen_001_A11_A11_010.fcs
Beads
10284

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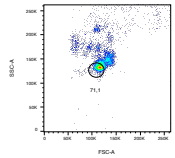
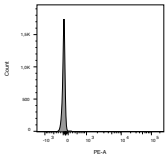
LASER DELAY





Specimen_001_B7_B07_012.fcs
Ungated
14560

Specimen_001_B7_B07_012.fcs
Beads
10346

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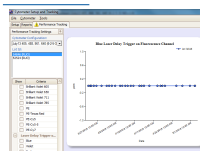
LASER DELAY



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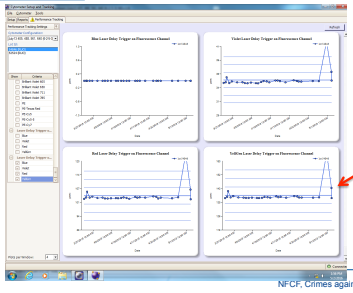
LASER DELAY



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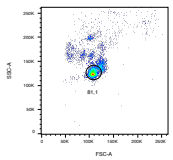
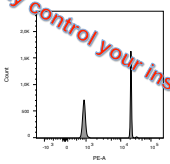
LASER DELAY

Remember to quality control your instrument





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SUMMARY

- Prepare your single cells well
- Block for Fc receptor binding
- Titrate your antibodies
- Remember a viability marker in your panel
- Set up your experiment with thought for threshold and PMT voltages – run your samples as at low flow rate
- Be sceptical if you see something unexpected

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THANK YOU FOR YOUR ATTENTION

Questions?

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