Quality Controls:

Get your instruments under control!

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Arbitrary units?

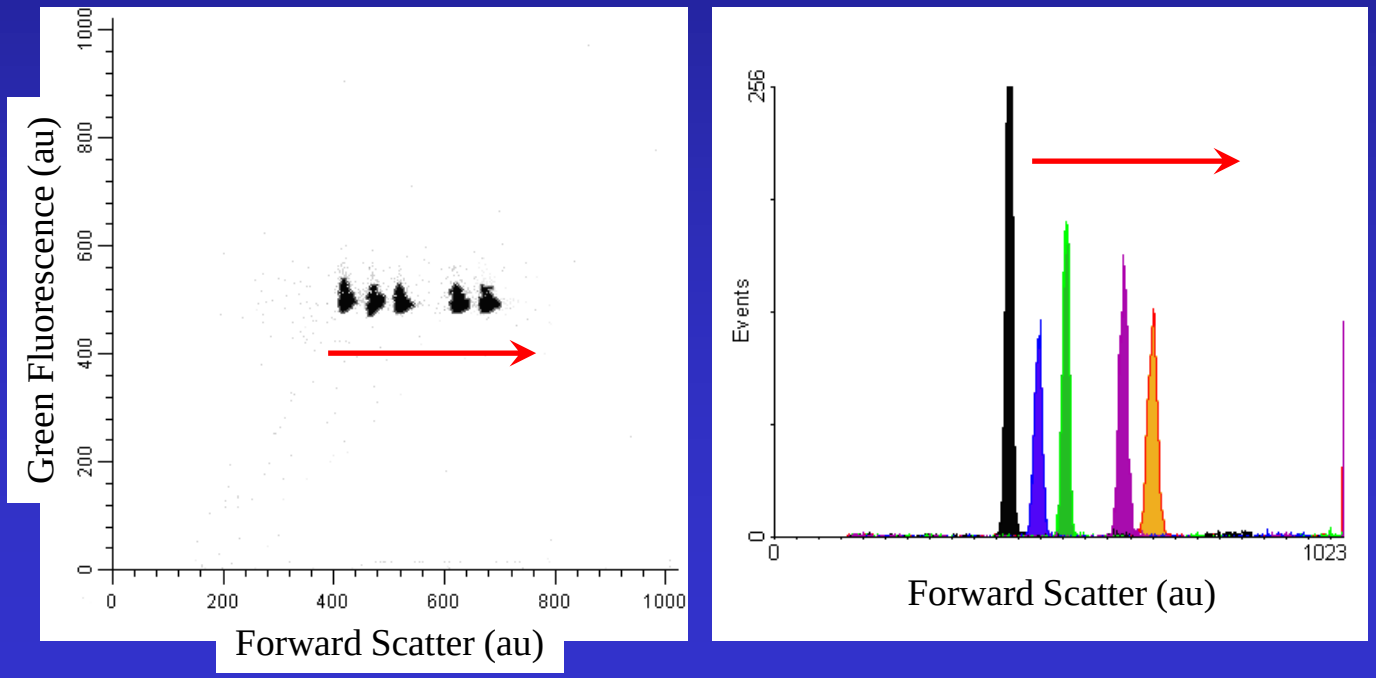
In flow cytometry ➔ scatter and fluorescence scales are in arbitrary units

Scatter and Fluorescence values = function of set up (Voltage and gain of PMT)

Example : beads 10 µm

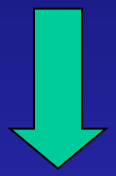
FS-PMT Voltage or gain

\- +



Quality Control Tests

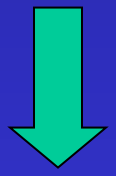
Daily basis



Confidence in the instrument performance

&

Confidence in the results



Use of a Standard

What's a Standard?

In theory :

- A standard = a **reference** (defined by a user, a laboratory, or any aknowledged authority)
- Properties **accurately known** (i.e., provided by the manufacturer)

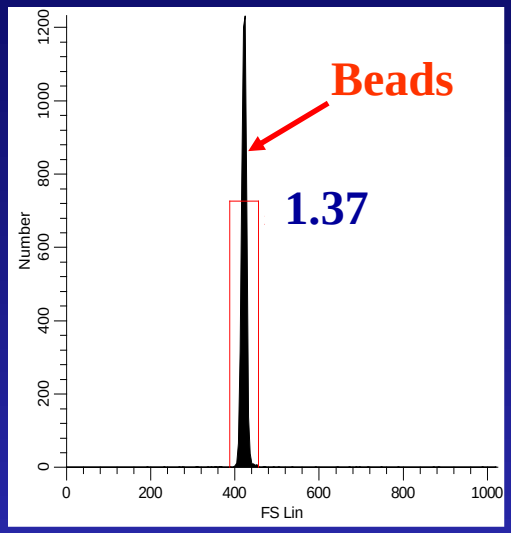
In practice :

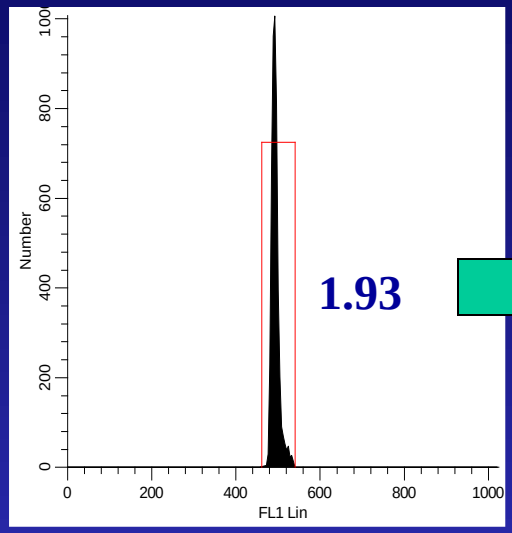
- A **manufactured particle** (fluorescent beads: several sizes and excitation or emission wavelengths)
- A **biological particle** (i.e., chicken and trout erythrocytes → DNA measurements)
- Used as an absolute reference for qualitative and quantitative comparisons

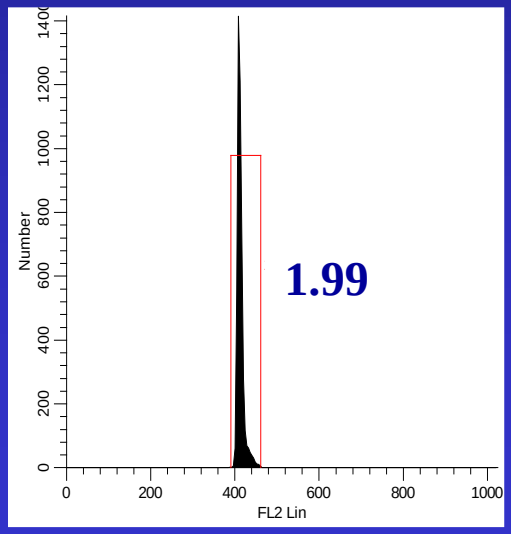
Quality Control of Instruments

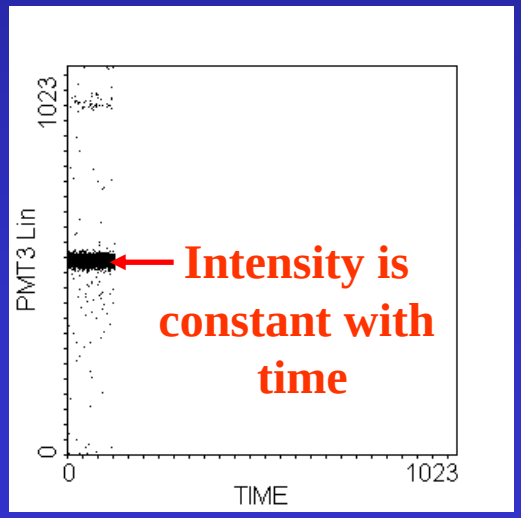
- Check the alignment of the optical pathway
- Check the stability of the machine (Quality Control)
- Test the capacities of the cytometer (flow rate)
- Set up the Sorting (define the delay)

To check the alignment of the cytometer









Number of events

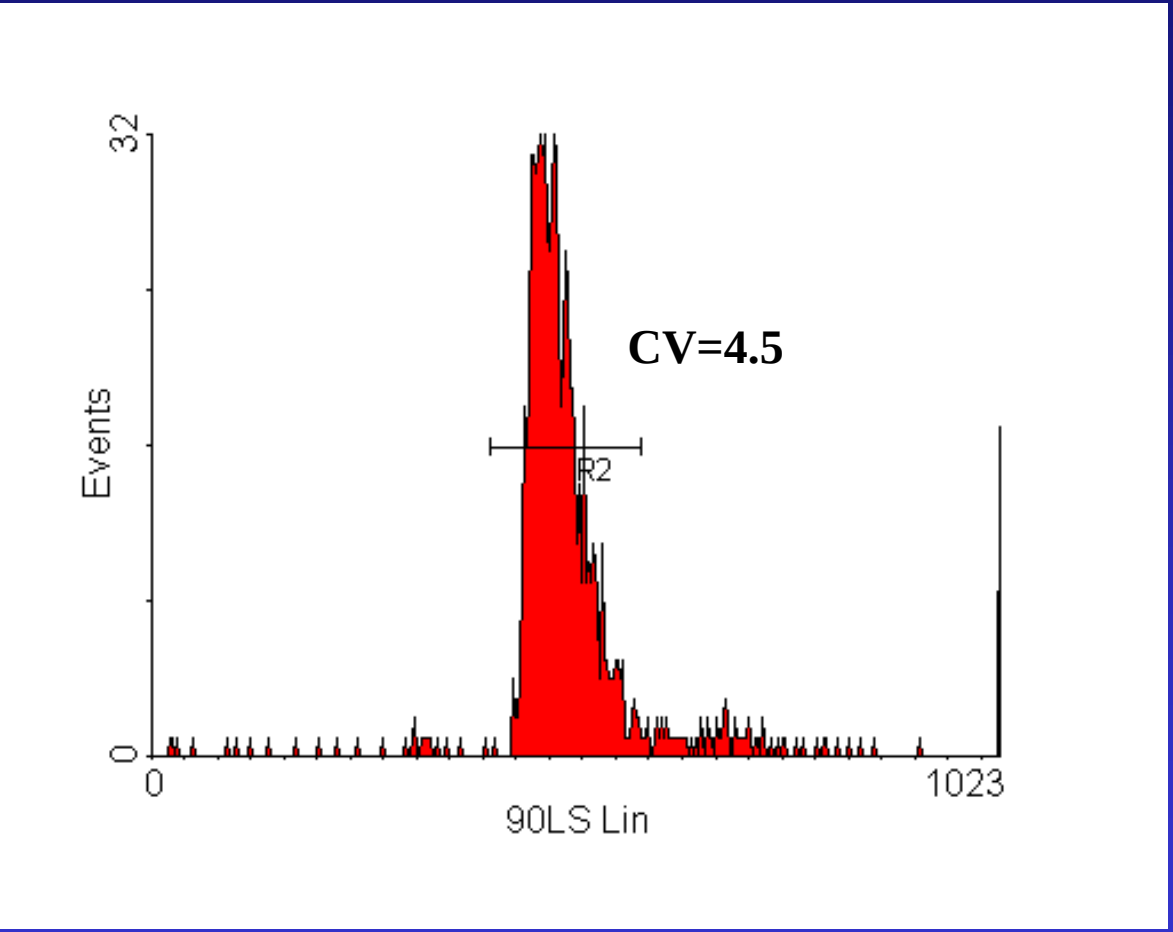
Parameter intensity (au)

CVs < 2% expected for beads (1-10 µm)

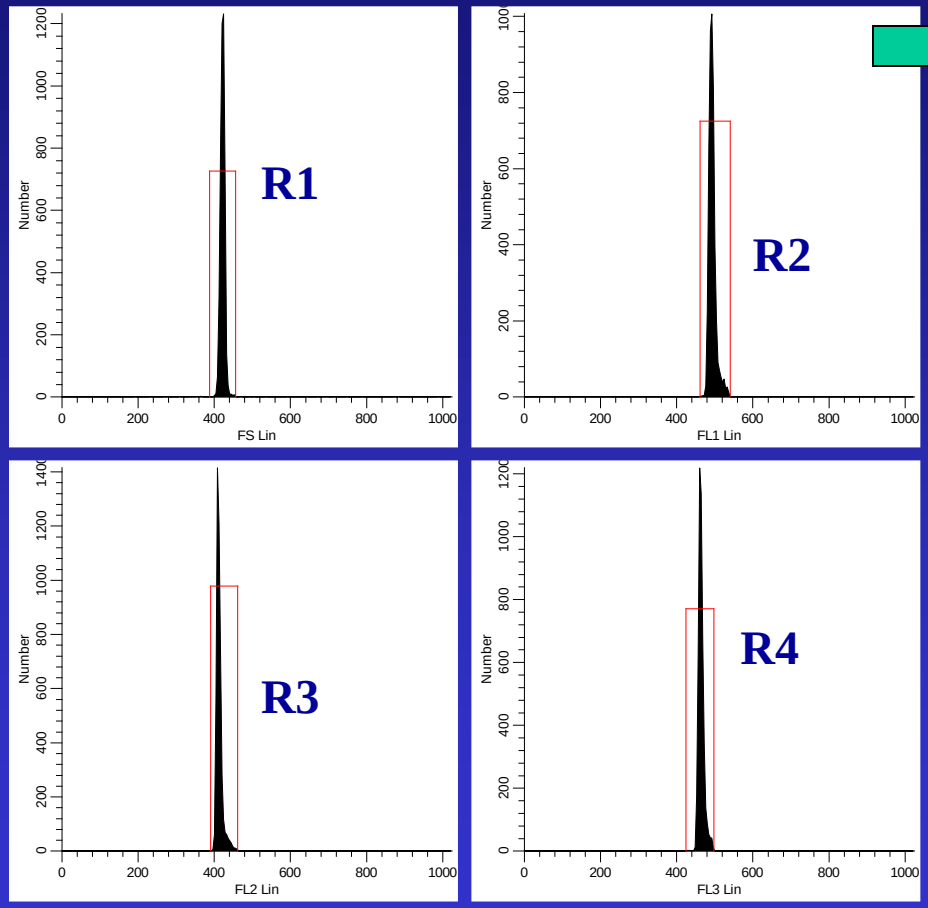
If CVs > 2% ➔ check the alignment (flow cell or laser position, dichroic mirrors)

Very useful on <u>sorters</u>

Example of bad alignment



Check the stability of the cytometer over time



Once a protocol is defined for a particular standard bead solution

Beads must always be plotted in the same regions

Internal quality control

Daily quality control

Problem of stability of the cytometer?

Many possible problems :

- **Leak in the fluidics → modified flow rates; instability**
- **Check for bubbles**
- **Dirty flow cell**
- **Laser dying → beam intensity decreases**
- **Bead solution too old or damaged → photobleaching**

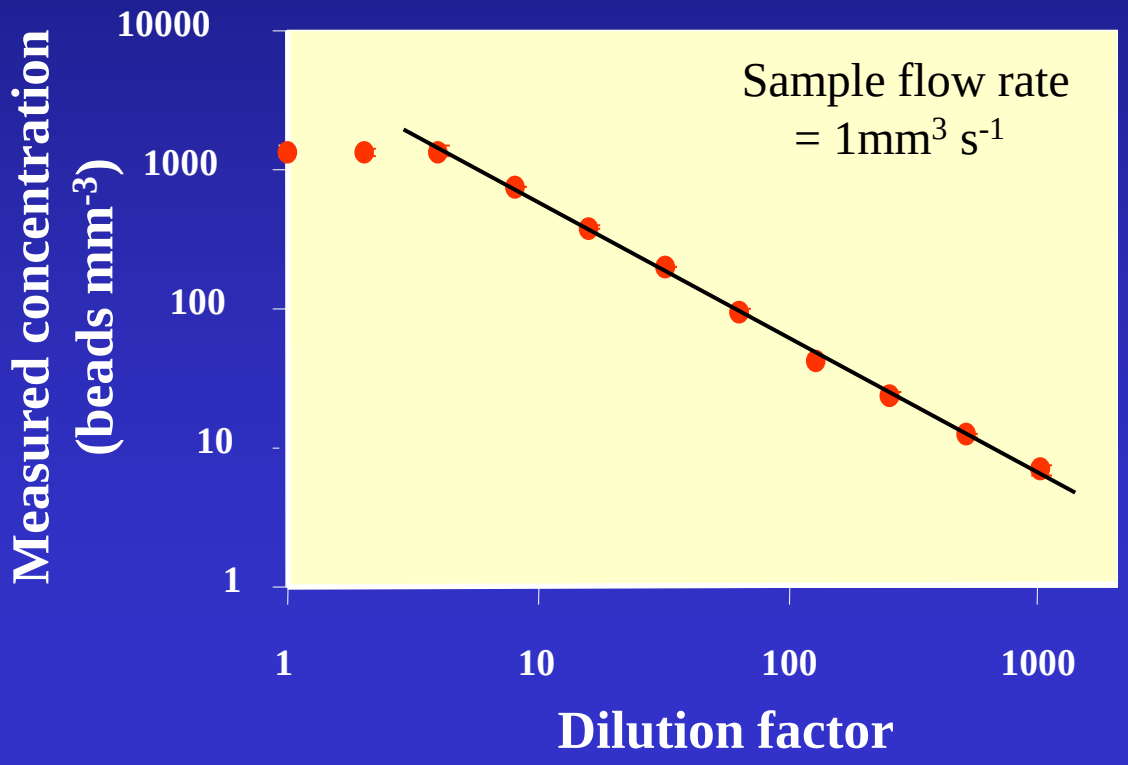
Test the capacities of the cytometer

For example :

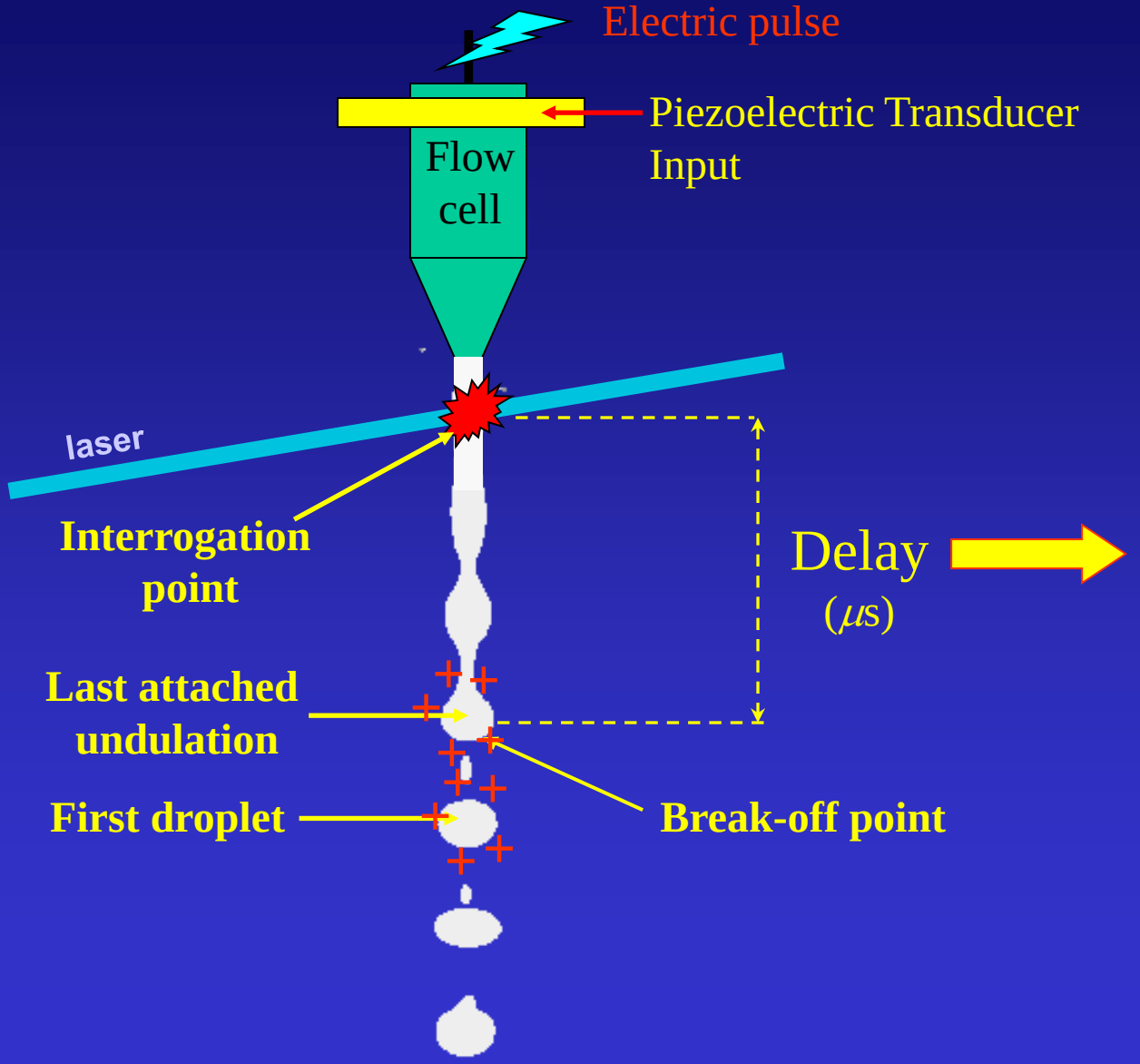
Determination of the “dead time” of a Cytoron Absolute (Ortho Diagnostic Systems)

→ Value furnished by the manufacturer = 2000 events s^{-1}

Analysis of 1 µm fluorescent bead solutions (dilutions in cascade)

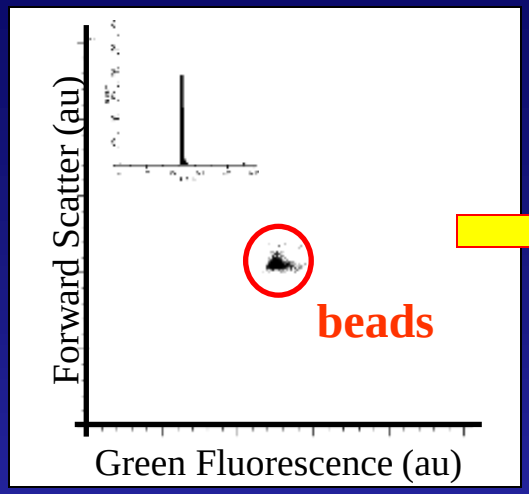


Droplet formation and timing

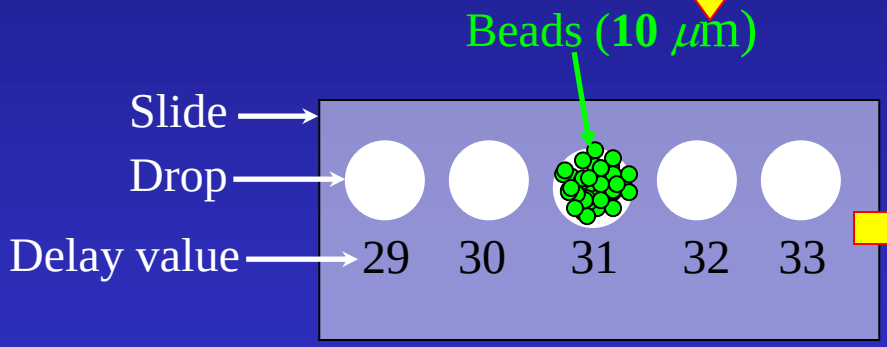


Delay determined using fluorescent beads

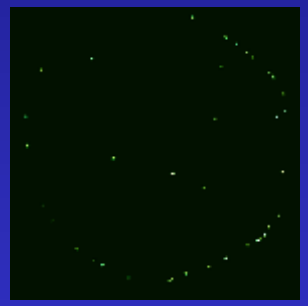
Set up the delay with fluorescent beads



Sort 50 beads on a slide with different delays (ex : from 29 to 35)



Count beads (epifluorescent Microscope)



- **If you achieve 50 out of 50 beads, set the delay to that setting (ex: 31).**
- **If beads are split between 2 drops, adjust the flow cell vertically**

Sorting process on the Altra (Coulter, USA)

Calibration

The necessary step to convert arbitrary units into absolute physical values

Calibration = adjustment of an instrument in order to express the results in some accurate physical measure.

Calibrator = a material known to have accurate measured values for one or several characteristics

Example :

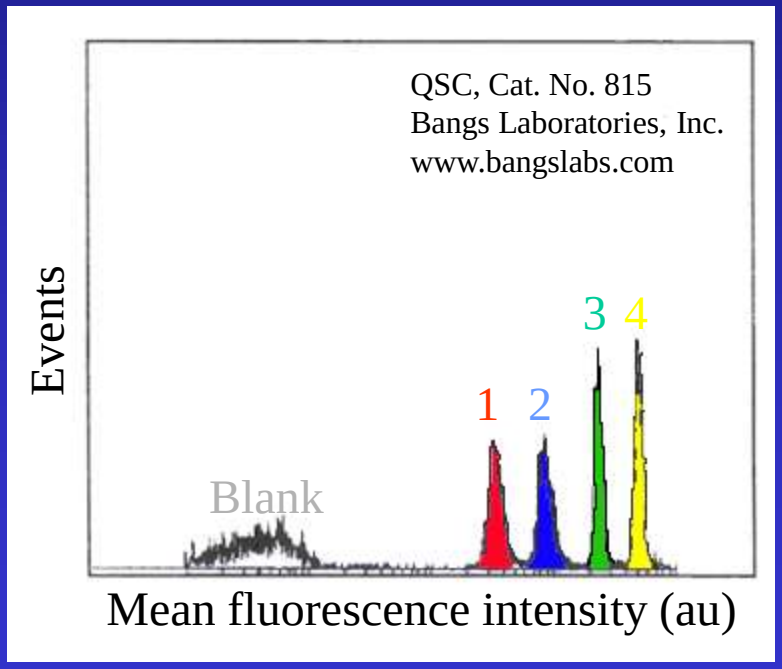
Quantitation of antibody binding capacity of cell populations by flow cytometry

→ *Quantum Simply Cellular (QSC)*

QSC Beads (Quantum Simply Cellular)

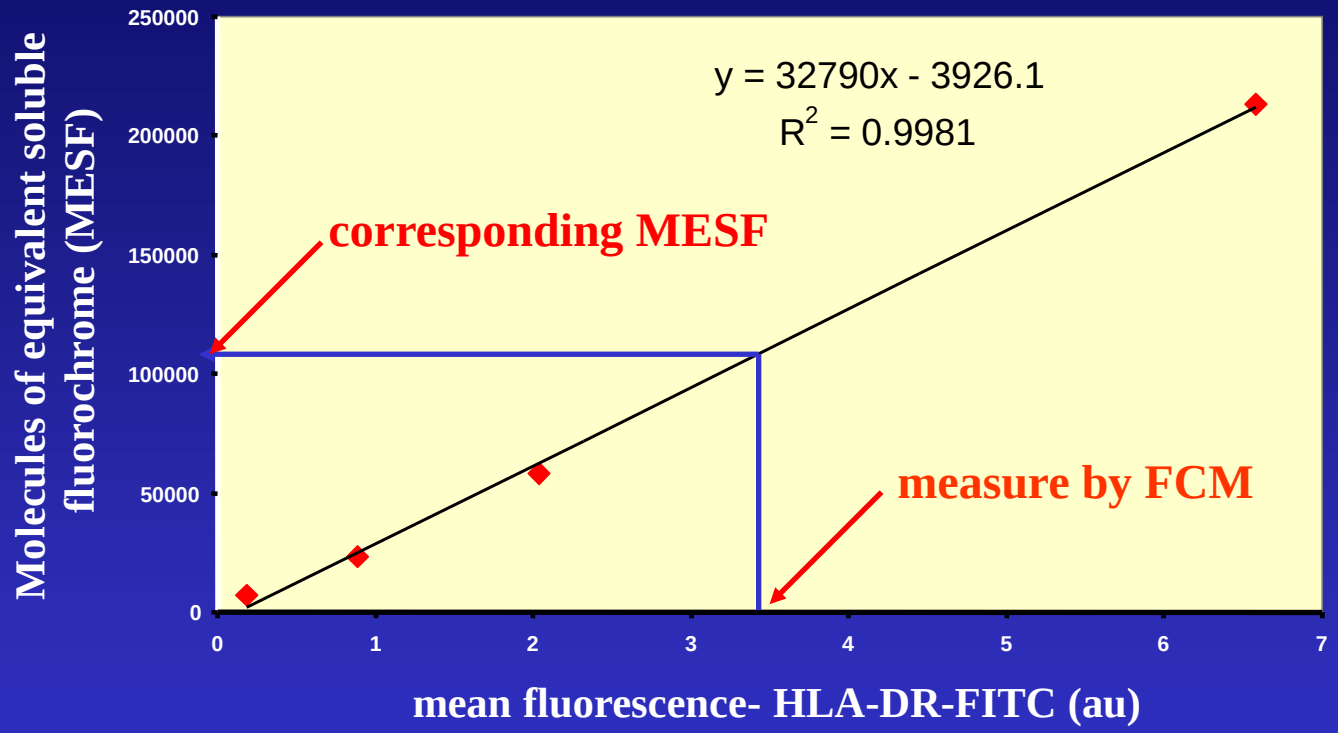
Identical microbeads with various calibrated binding capacities of goat-anti-mouse IgG on their surface :

Antibody binding capacity (ABC) provided by the manufacturer :



MESF=Molecules of equivalent soluble fluorochrome

Quantum Simply Cellular Beads
Calibration Curve



Binding capacity
of cells in your sample

Courtesy of K. Rhageb

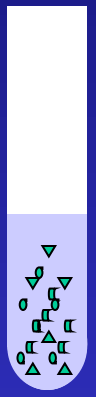
Absolute Counts

Determination of cell concentration by flow cytometry

Get the control of the volume analyzed

Four different methods

I. Direct Absolute Count



Sample

CYTORON ABSOLUTE
(Ortho Diagnostic Systems)

Cell concentration
(# of events / volume)

To analyze a bead solution of known concentration
➔ Control of the fluidic

II. Weigh a Sample

Sample

Weigh the sample before analysis (Weight W_0)

Analysis (Flow Cytometer)

cell number (N)

Weigh the sample after analysis (Weight W_1)

Volume analyzed $V = (W_0 - W_1)/\rho$

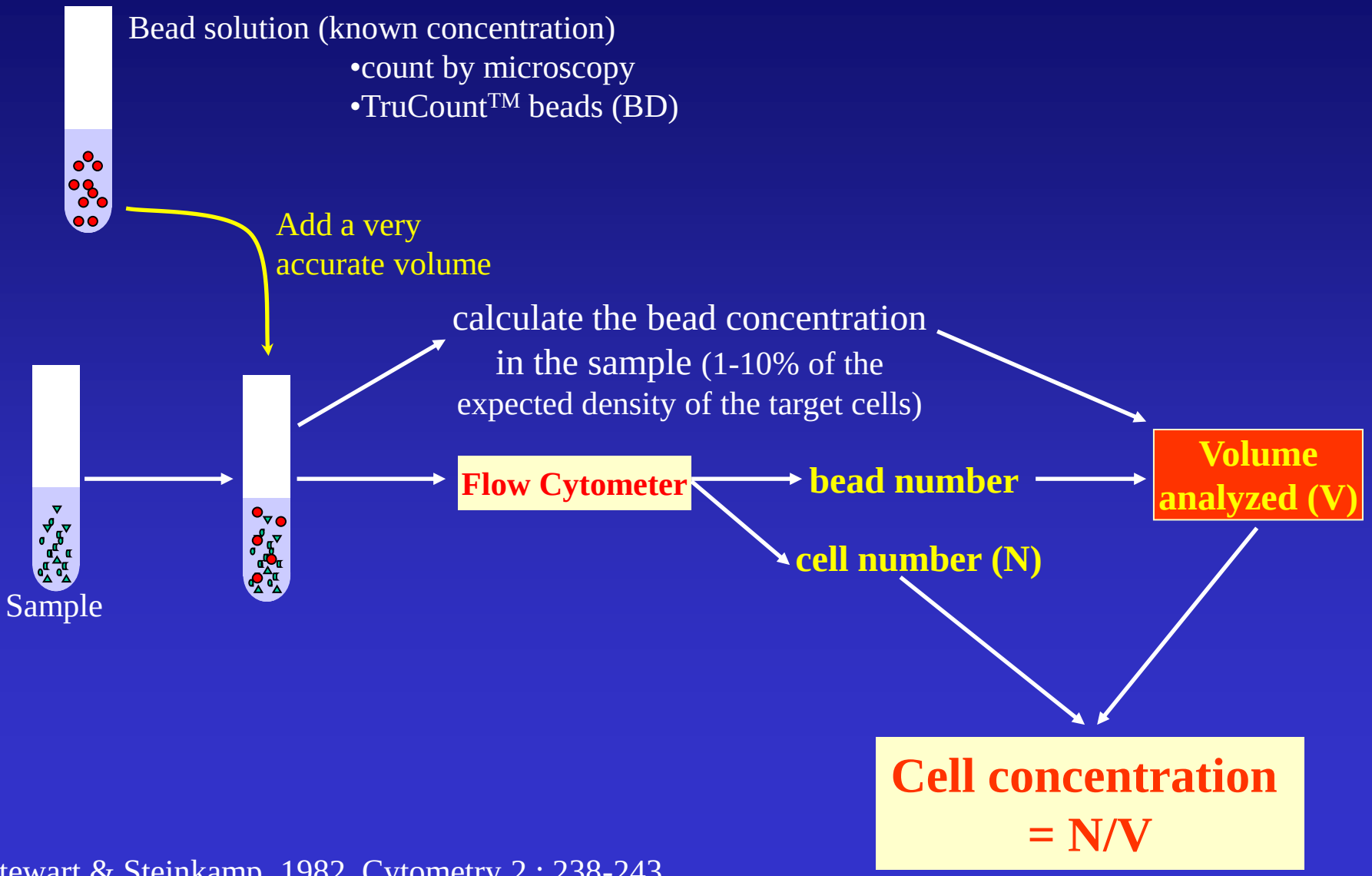
density

Cell concentration = N/V

Disadvantages

- Time consuming
- Less accurate (back flow of sheath fluid in the sample)
- Analysis in one run

III. Add Beads in the Sample



Stewart & Steinkamp, 1982, Cytometry 2 : 238-243

IV. Determination of the Flow Rate

Hypothesis: flow rate (µl/s) through a flow cytometer = constant

Bergeron *et al*, 2003, Cytometry 52B:37-39

Conclusion: volume (V) analyzed in a fixed time = constant

No need to add beads in the samples.

If N events are analyzed by the cytometer in the fixed time

then cell concentration = N/V (cells/µl)

Hint!

- *Analyzes must be done with the same flow rate*
- *Volume accurately determined (microscopy,* TruCount™ beads) *and controlled*
- *Beads not necessary in the sample, but can be used as internal standard*

Conclusion

Quality Control Tests are mandatory …

- **➔ To assess the alignment**
- **➔ To assess and control instrument performance**

for quantitative and reproducible applications on any flow cytometer.

References

R.A. Hoffman, Current Protocols in Cytometry, 1997 : 1.3.1-1.3.19
J.C.S. Wood, Current Protocols in Cytometry, 1997 : 1.4.1-1.4.12
Cytometry, Volume 33, Number 2, 1998 :