Cytometer Setup Controls
The daily quality check you might want to do

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Cytometers have no brain!

- Cytometers can’t clean themselves
- Cytometers don’t tell you what is in them
- Cytometers are just as good as the users knowledge
- No cytometer of the same brand and build is the same

But how can I be sure that I’m working with a truly good or at least acceptable instrument?

A few simple things to observe to protect your experiment…

1. The proper art of cleaning your instruments before, while and after you've finished
2. Testing your instrument to see if it is working correctly
    1. Test your instrument with 8 peak standard beads
    2. Test your instrument with your own specific controls
    3. Trust in manufacturer test protocols…not the worst option
3. Check your instruments filter configuration and your filters
4. Be sensible about your input pressure as it can cost you precious resolution and money

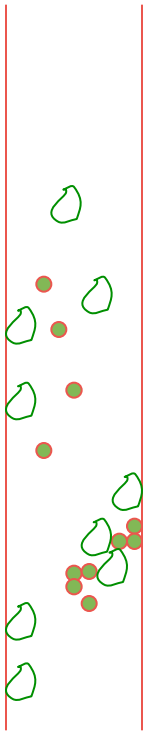
The proper art of cleaning

The injection needle into a cytometer is typically 140-170um in diameter.

Cells and DNA/Protein debris will make a very sticky team

Dirt usually builds up continuously in an instrument and can be a silent culprit!

→ Invest 5-10min for cleaning before you start your samples



Instrument sclerosis!

The proper art of cleaning

With what should I clean my instrument?:

FACS Clean (1% bleach)

FACS Rinse (Detergent)

ddH2O

NEVER USE DMSO or other strong solvents! It will destroy the flow cell.

EtOH is not a cleaning reagent, it is a fixative!!!

How do I clean properly?:

Before you start run 5min Rinse, Clean, ddH20 each in this order

→ ddH20 is improtant, Clean can bleach your fluorescent dyes

During your run, clean the instrument with Rinse once in a while for 2min

→ Especially useful for sticky samples and organ preparations

After your run, repeat the 5min Rinse, Clean, ddH20

→ If you ran DAPI, Hoechst, Indo, PI, 7AAD or similar things, use Clean a bit longer!

Testing your instrument: Daily!

Flow Cytometers are very robust machines if they are cleaned and well treated…

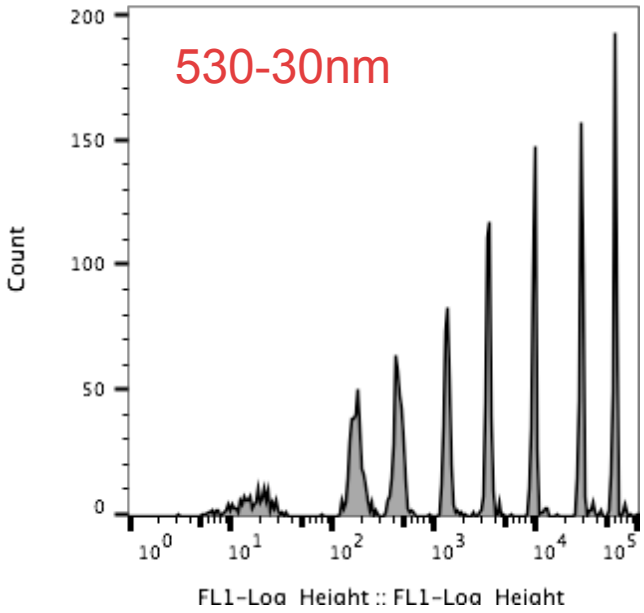
…however, even the best instruments should be checked before you run your expensive or time consuming experimental samples.

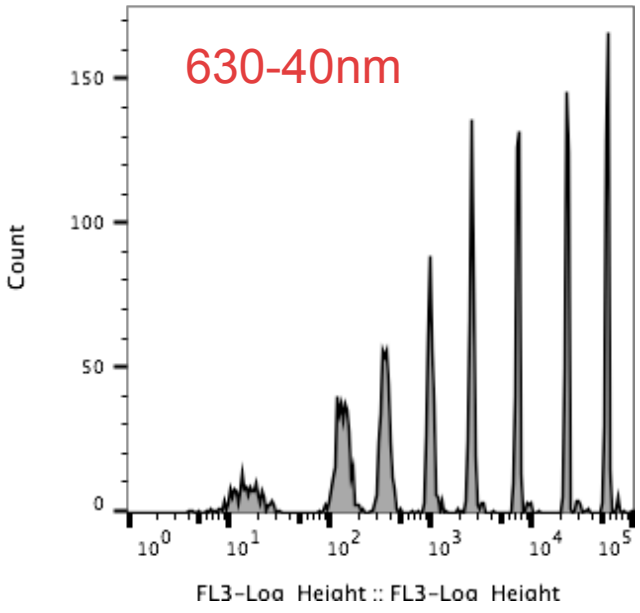
What do we use for testing instruments:

- SpheroTech 8-peak beads
- BD CST beads
- Beckman Coulter Flow Check Pro
- Antibody stained compensation beads (spectral overlap check)

Testing your instrument: 8peak beads

Spherotech 8peak beads are very precisely manufactured beads with 8 different fluorescence intensities and very broad excitation/emission spectrum.

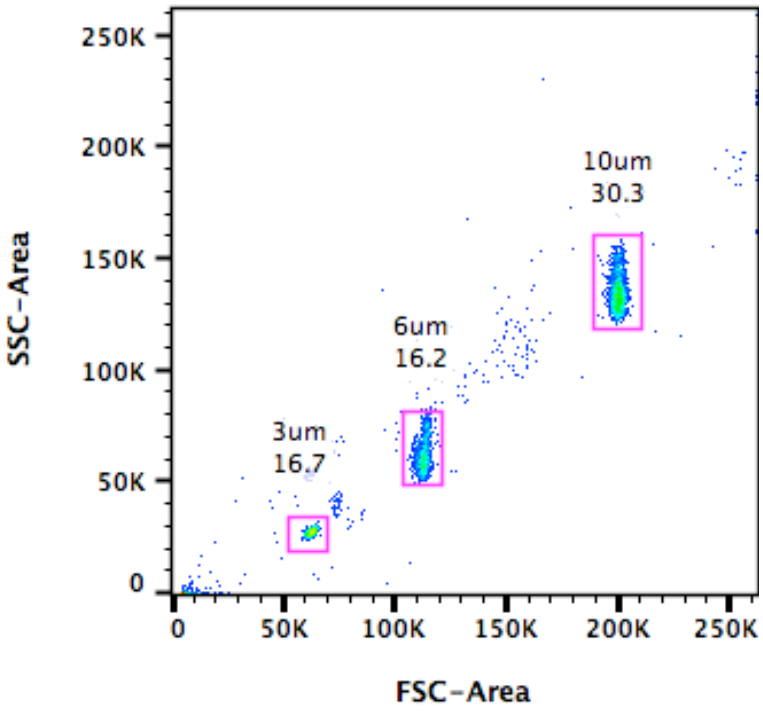


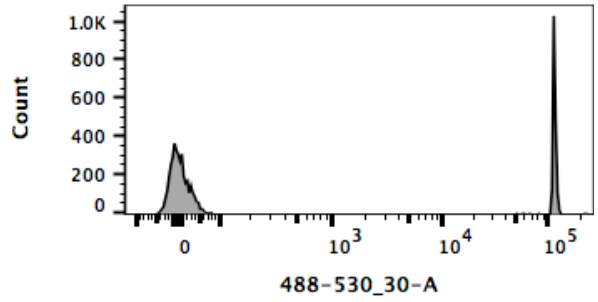


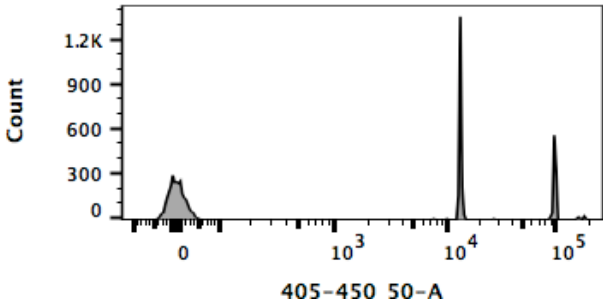
8peaks are small (3.5um) and very homogeneous: a well aligned instrument has CVs approx. 2% on the bright peak

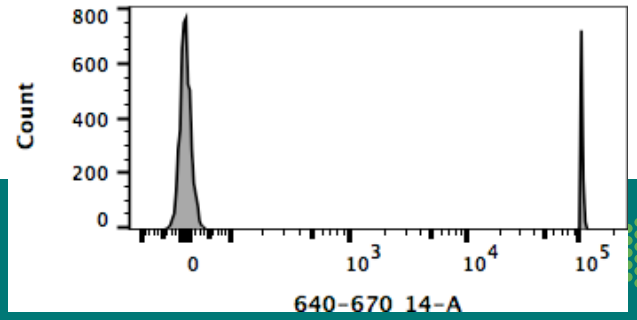
Testing your instrument: Flow Check Pro

FlowCheck Pro beads are super good beads when it comes to check FSC and SSC properties of your flow cytometer. They actually have 3 discreet populations&colours excitable by 405/488/640 lasers and give very good CVs.



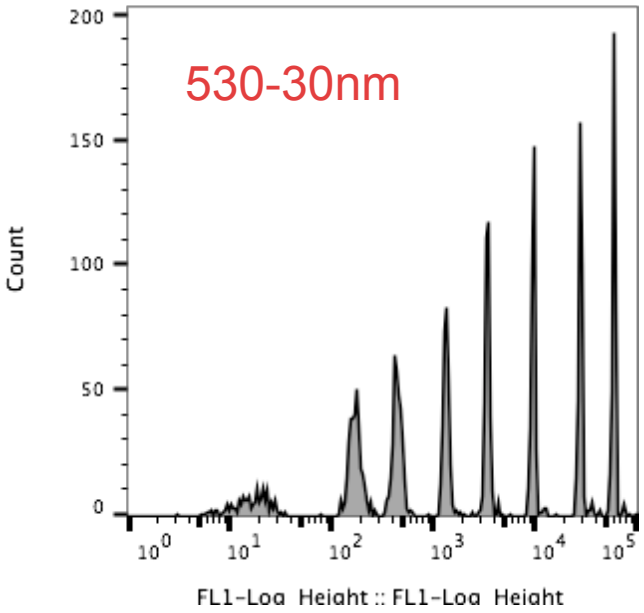


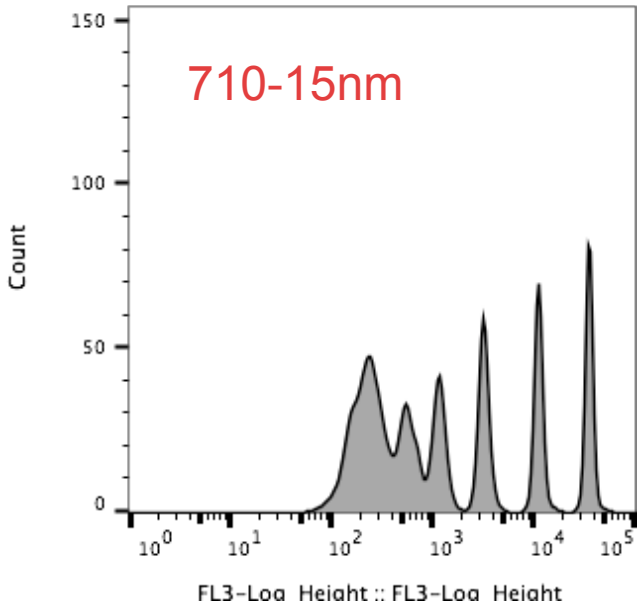




Testing your instrument: 8peak beads

It is of very great use to test all your channels with 8peaks to see which channels (PMT/Filter combos) are your most dynamic!

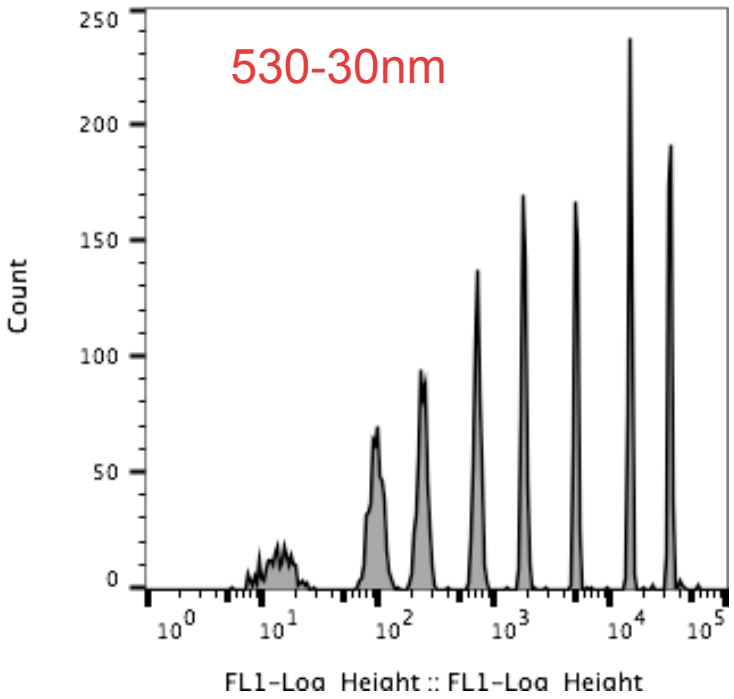


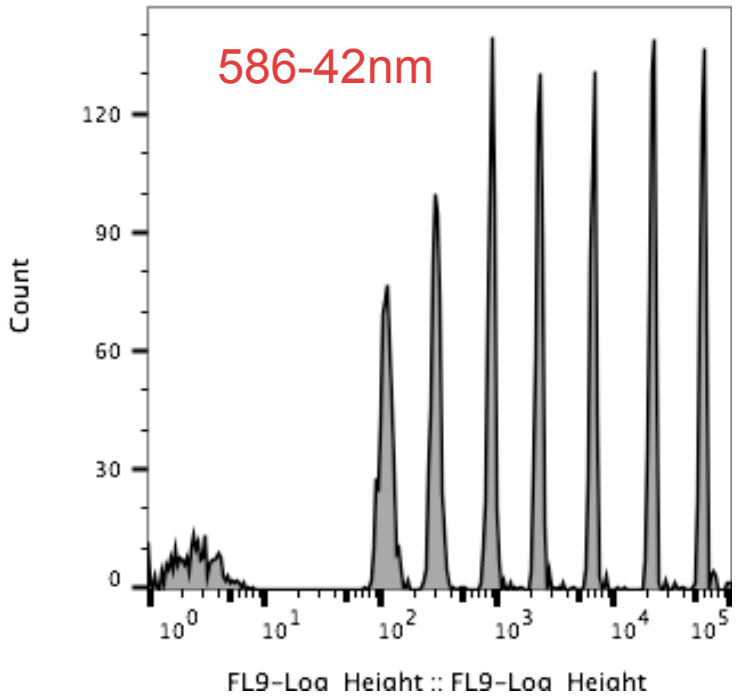


Just see if you can resolve all 8 bead populations in all your channels. Mark those with reduced ability!

Testing your instrument: 8peak beads

Dynamic range is good, but resolution is of even bigger importance!
Check the separation of your dimmest populations.





If possible, have your most important marker on your most dynamic, best resolved and brightest channel.

Testing your instrument: 8peak beads

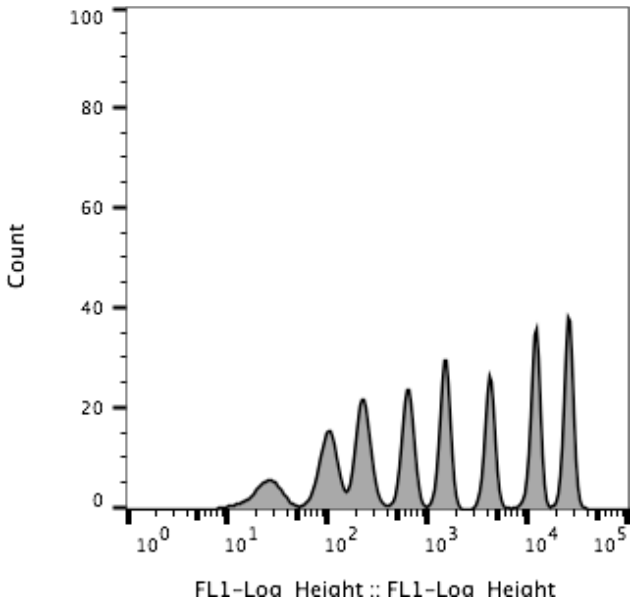
My cytometer has very low resolution and dynamic range is reduced in a few channels. What can I do?

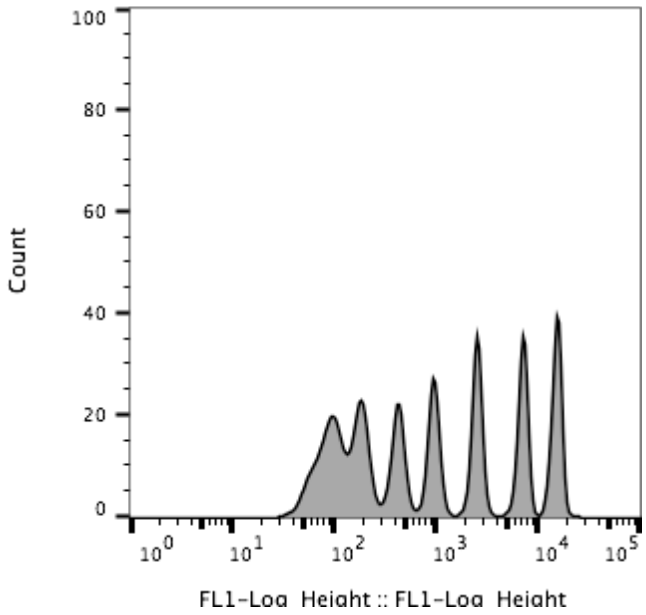
1. Clean your instrument! 90% of all issues can be solved this way
2. Align your lasers or get help to do this (CFs, engineers, etc…)
3. Check if your low resolution channel is close to a laser line
   → Some filters are not very steep in their blocking efficency and this can result in lower sensitivity
3. Try out different filters or beam splitters to see if your PMT is working properly
4. Longpass and Shortpass filters have an angle of incidence and if this is not perfectly aligned, then you will lose resolution and performance. Instruments can be sensitive to vibrations! Better have no centrifuges close to your cytometers…

Testing your instrument: 8peak beads

My cytometer has very low resolution and dynamic range is reduced in a few channels. What can I do?

Example: Suboptimal band pass filter & laser spillage

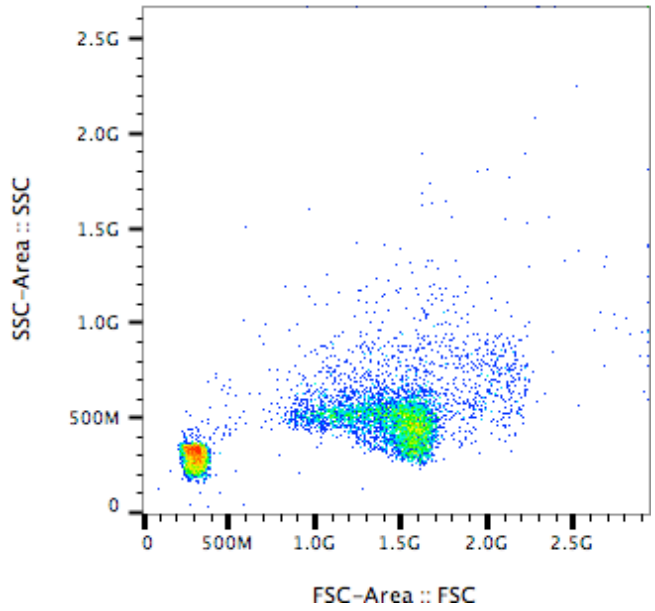


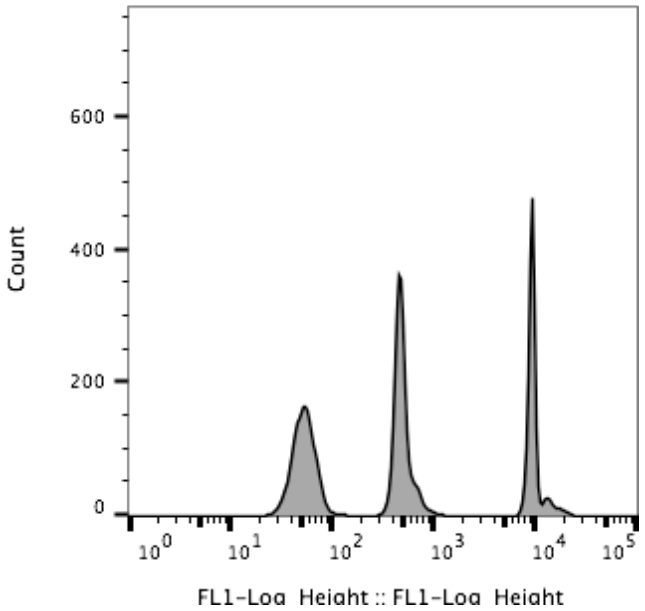


Testing your instrument: CST beads

Cytometer Setup and Tracking (CST) beads is the answer to simplified daily setup control from BD.

CST beads are basically a 3peak bead system with the dimmest bead being smaller that the two fluorescence carrying beads.





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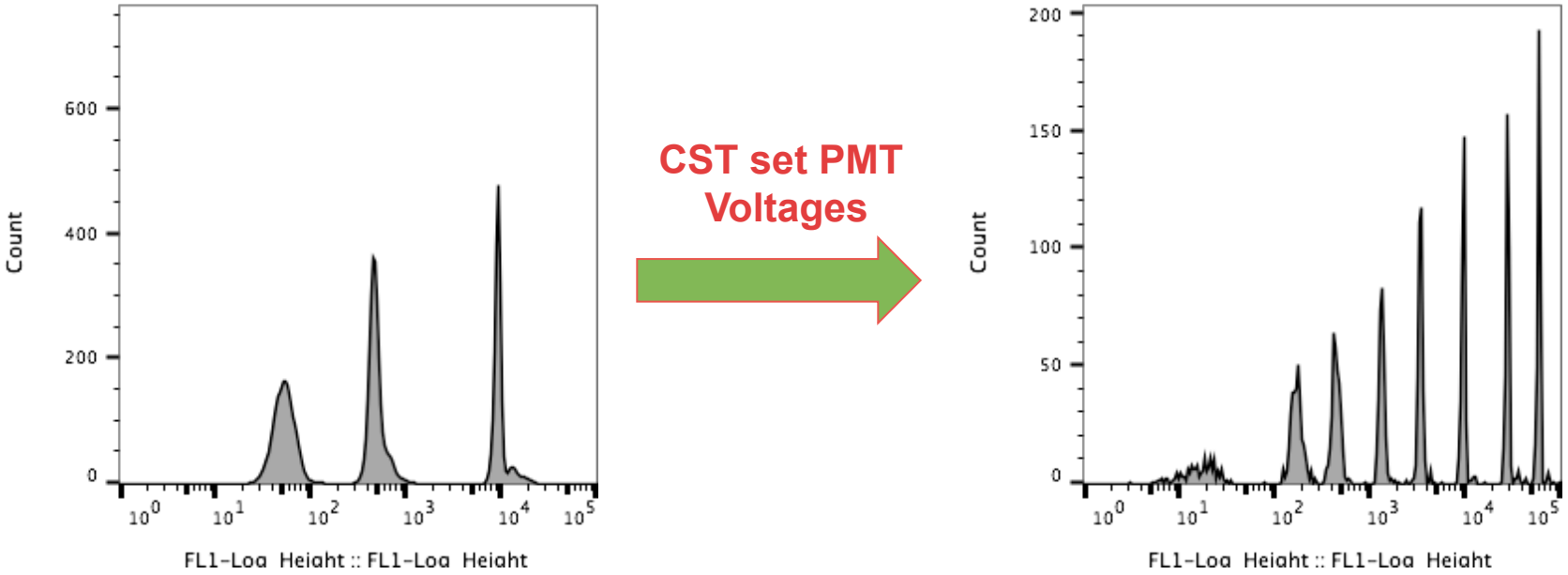
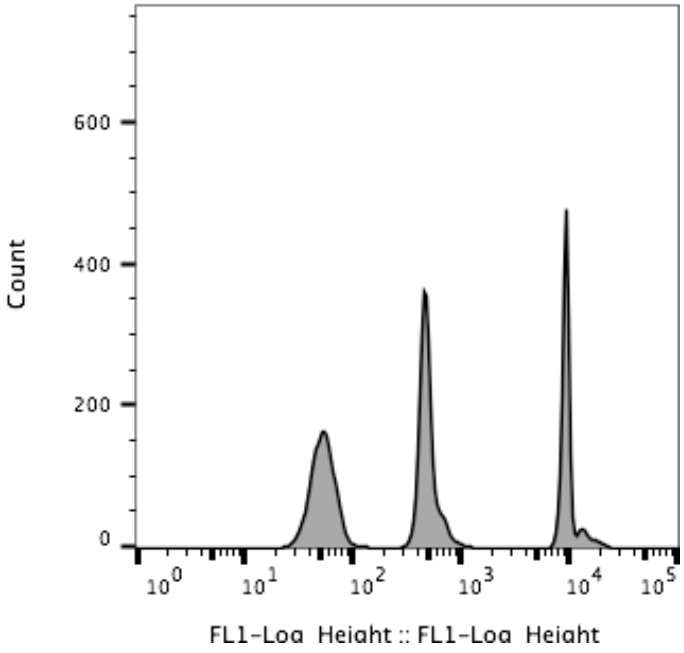
Testing your instrument: CST beads

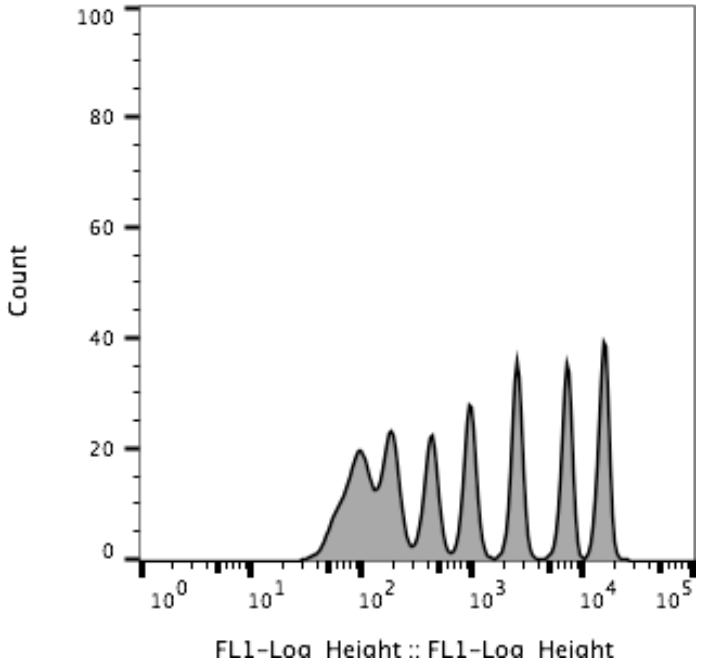
CST beads will help you to identify initial PMT voltage settings for your BD instrument that will enable you to resolve dimly fluorescent “populations” from non stained.



Testing your instrument: CST beads

CST will try to optimise the resolution of dim and no stained beads even with a non-perfect filter/PMT combo. It's sole purpose is to tell you "I'm matching acceptable company standards"





CST is a great tool as long as you've checked your instrument prior to falling back on CST for daily use!

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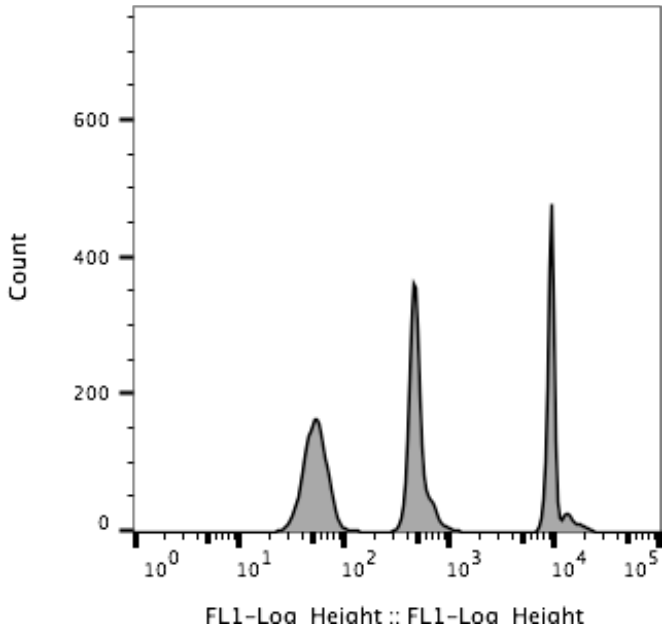

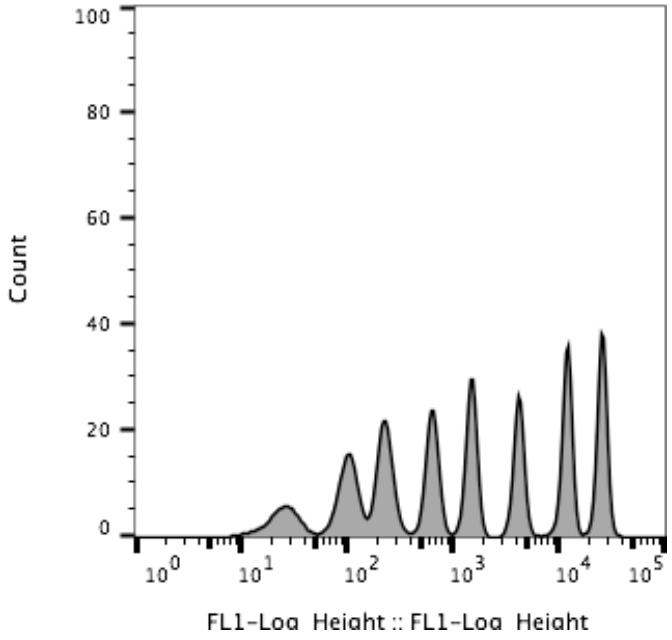

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Testing your instrument: CST beads

If CST has its caveats, why should I still use for my analyser?

1) A even imperfect daily QC method is better than none!
2) It will assist you with time delay setup
3) CST will still tell you if your instrument is in acceptable shape on the day you want to measure as it is able to evaluate
   → Fluidic stability
   → Noisy data due to imperfect cleaning
   → A faulty laser or a faulty PMT

There are many QC methods in use and I would still grant CST a big plus for keeping FACS standardised and more comparable if they are used properly.

Check the filter configuration!

Cytometers are so damn simple to adept to specific scientific needs…but they will not tell you right away that they were fiddled with!

Changing filters for PE!!!

582/42 and 586/15 can be a massive difference, especially for compensation.

Check the filter configuration!

It is very simple to check if the instrument has the correct filter configuration:

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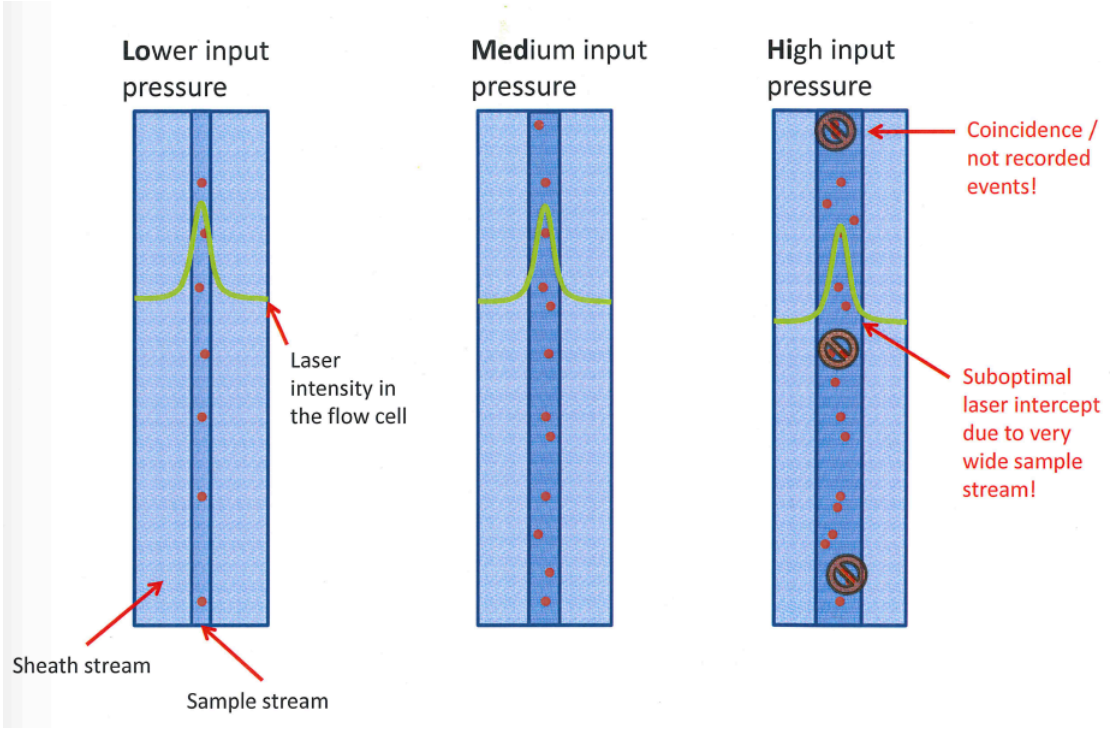
Keep your input pressure under control

"Speed" or number of events passing through a cytometer within a second are set by the difference between internal sheath pressure and sample pressure.



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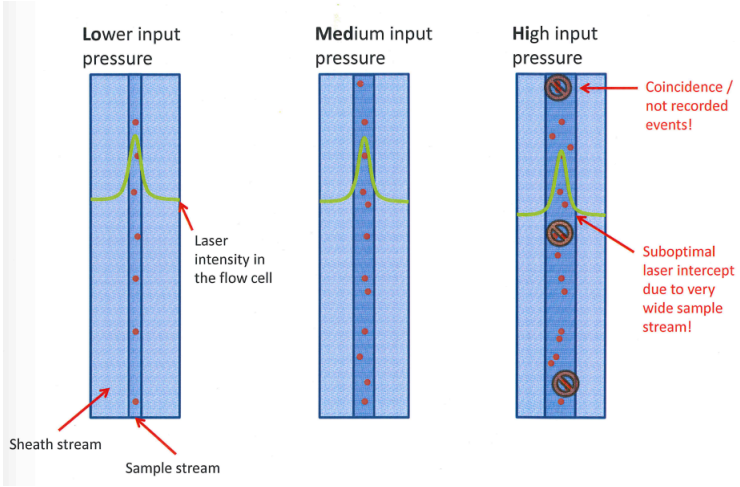
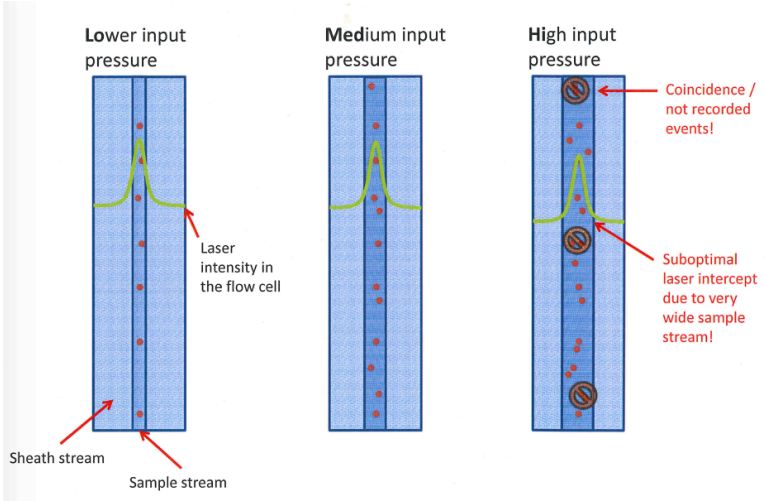


Pictures of increasing input pressure on data quality from MoFlo as example

Keep your input pressure under control

What can I do to avoid spending too much time at the instrument and wasting my precious samples by applying high input pressure?!



Tips that work!:

1. Concentrate your sample and use 1ml inlay tubes
2. Avoid FCS as part of your buffer if possible and include EDTA
3. Include a cleaning step between your samples
4. Use the instruments ability to analyse >10.000 events/sec

Daily Checklist:

1. Clean your cytometer thoroughly with solvent and water (Rinse, Clean, H2O)
2. Check the last QC performed on the instrument (CST if BD instrument)
3. Check the instrument and your necessary parameters if 8peak beads to see if the peaks are in the same position
4. Run your samples on low or max medium input pressure
5. Clean the instrument at strict intervals during your measurements
6. Live happily ever after your flow sessions and don't worry about instrument quality if you follow advice above