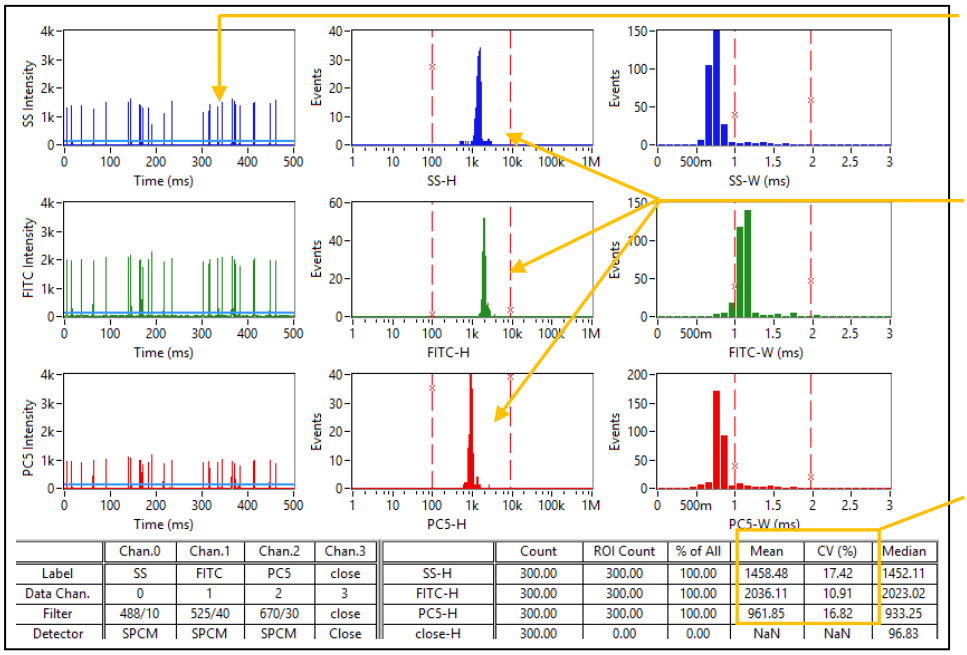
Motivation for this SOP

Aligning the optics of the NanoFCM can be tricky and should be done with care. A misaligned instrument will affect the following users and may take a long time to correct.

Assessment of the current instrument state

Check the current state by running QC beads. You are aiming for a picture similar to this…



	Chan.0	Chan.1	Chan.2	Chan.3
Label	SS	FITC	PC5	close
Data Chan.	0	1	2	3
Filter	488/10	525/40	670/30	close
Detector	SPCM	SPCM	SPCM	Close

	Count	ROI Count	% of All	Mean	CV (%)	Median
SS-H	300.00	300.00	100.00	1458.48	17.42	1452.11
FITC-H	300.00	300.00	100.00	2036.11	10.91	2023.02
PC5-H	300.00	300.00	100.00	961.85	16.82	933.25
close-H	300.00	0.00	0.00	NaN	NaN	96.83

Single events are recorded.

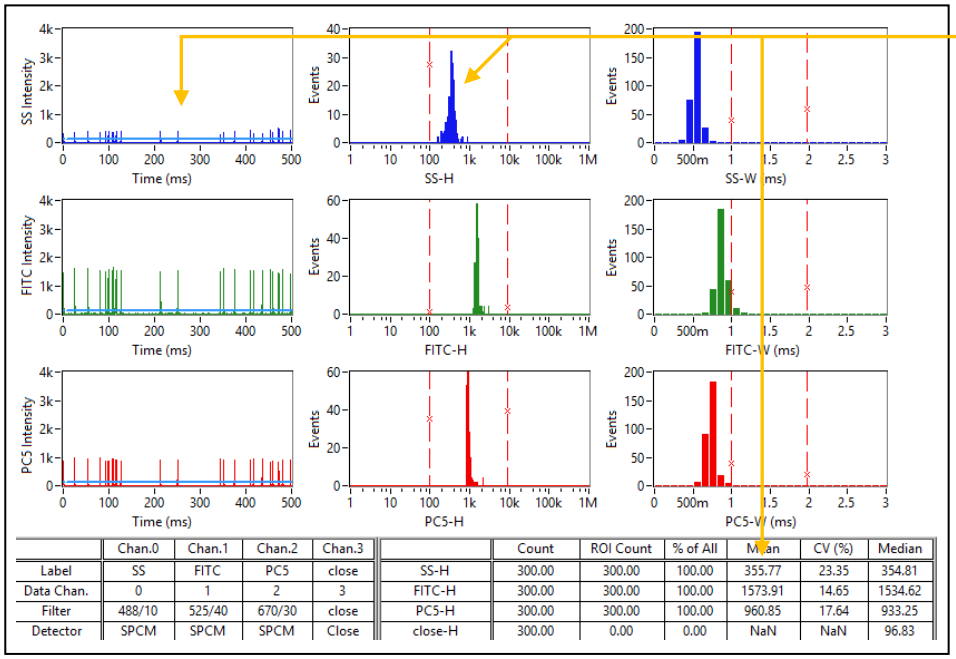
The three peaks are narrow

The Mean values are close to the target values in the Feedback Form and the CVs are below 20%.

Usually, you won't find the instrument performing like this right from start. In the following we want to assess the two most common scenarios…

1) Mix of good and bad channel performance

The overall situation is not too bad, but some adjustments are needed.



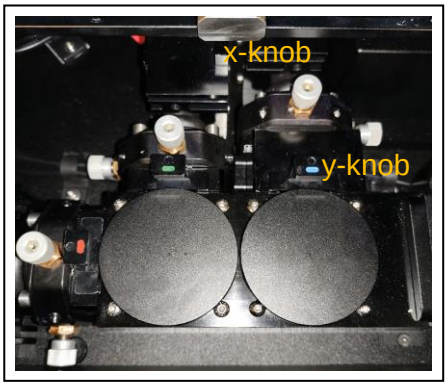
	Chan.0	Chan.1	Chan.2	Chan.3
Label	SS	FITC	PC5	close
Data Chan.	0	1	2	3
Filter	488/10	525/40	670/30	close
Detector	SPCM	SPCM	SPCM	Close

	Count	ROI Count	% of All	Mean	CV (%)	Median
SS-H	300.00	300.00	100.00	355.77	23.35	354.81
FITC-H	300.00	300.00	100.00	1573.91	14.65	1534.62
PC5-H	300.00	300.00	100.00	960.85	17.64	933.25
close-H	300.00	0.00	0.00	NaN	NaN	96.83

SS channel is off.
Note: low event intensity, broad histogram peak and low Mean values.

The shape of the histogram for the FITC channel looks fine but it may be possible to reach a slightly higher Mean value.

PC5 channel is already optimal or very close to it.



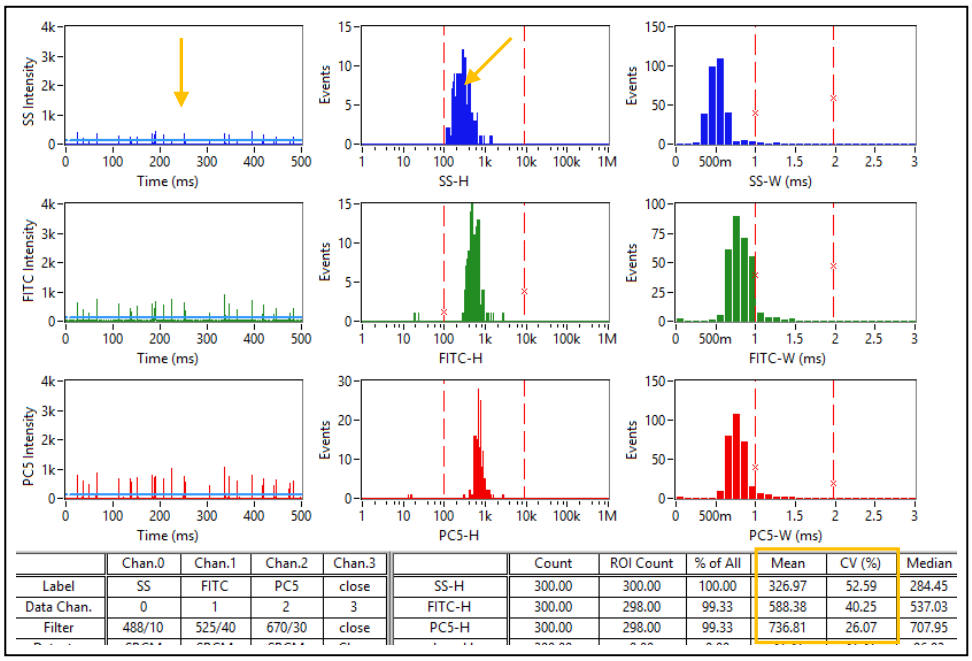
Alignment workflow for the SS detector (or any other)

a) Note down the current SS Mean value and start turning the x-knob slowly in one direction. There is a delay of ~2 sec until the Mean value reflects the current change (be patient!). If the Mean value increases keep turning the x-knob in the same direction. Otherwise turn in the other direction.
b) To reach the point where you get the highest Mean value: Keep turning the x-knob until the Mean value no longer increases but start to decrease. Then turn the knob back to the point where you got the highest Mean value.
c) Check the peak width in the histogram. If the peak is too broad (CV >20%), repeat the same procedure that you did for the x-knob for the y-knob. The y-knob typically needs much less adjustment than the x-knob!).
d) Double check the x-knob optimum after you are done with the y-knob adjustment.
e) If you are still far away from the target values after those adjustments, it is likely that you will need to fine tune the laser focusing lens as described below.

In the example above, the FITC channel only needed adjustment of the x-knob to reach the optimum.

2) All channels appear misaligned

Often the situation looks worse than it actually is.

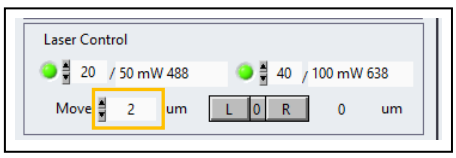


	Chan.0	Chan.1	Chan.2	Chan.3		Count	ROI Count	% of All	Mean	CV (%)	Median
Label	SS	FITC	PC5	close	SS-H	300.00	300.00	100.00	326.97	52.59	284.45
Data Chan.	0	1	2	3	FITC-H	300.00	298.00	99.33	588.38	40.25	537.03
Filter	488/10	525/40	670/30	close	PC5-H	300.00	298.00	99.33	736.81	26.07	707.95

Low event intensities in the time trace.

All peaks in the histograms are broad and the Mean values are low.

Alignment workflow for laser focusing lens & detectors



a) When all channels show broad histogram peaks, you usually have to readjust the focusing lens. Start by changing the “Move” parameter from the default 10 to 2. Next, click on the L or R button to adjust the lens. The histogram peaks should become narrower and the Mean values should increase. If not change direction.
b) Once you found the right direction keep on moving the lens slowly (there should be at least 2 sec between each click) while keeping track of the Mean values. At some point the numbers will start to decrease again. Now return to the highest Mean value by moving the lens back in the opposite direction. Note that the stepping motor is not very precise and the displayed um values are not reliable. Instead, orient yourself on the Mean values of the QC beads.
c) Next, adjust the individual detector alignment as described under 1).