

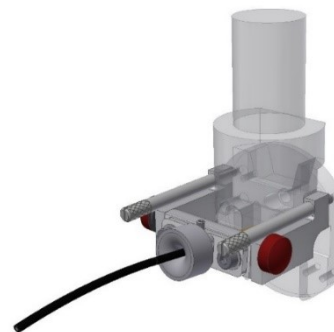
## Introduction

quantiFlash comes with 2 fibers. The fiber ends have to be interfaced with the flow cytometer or to phrase it differently: 2 fibers = 2 points of connection of quantiFlash with the flow cytometer.

### A Mounting the Flow cell adapter

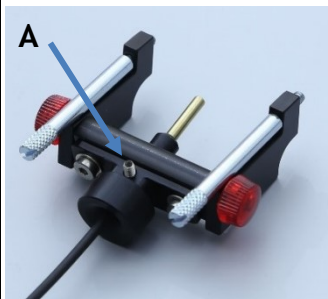
This adapter is an adjustable fiber extension that can be easily mounted on BD instruments with access to the flow cell. These are for example:

- LSR II
- FACSAria
- FACSCanto
- FACSCalibur
- LSRFortessa
- ...



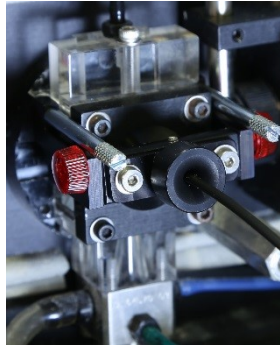
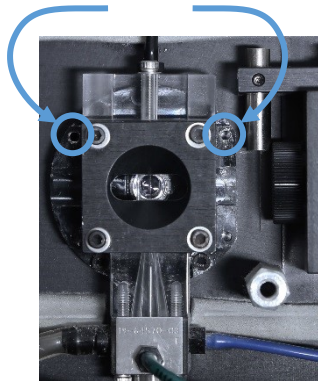
Flow cell adapter mounted to a BD flow cytometer

- **Step 1** Before installing the flow cell adapter please retract the fiber all the way using the Allen keys supplied with the flow cell adapter.
- Loose the screw as indicated in A) and fully retract the fiber as indicated in B)



- Open the hood of your cytometer to get access to the flow cell. Around the flow cell you find 4 thread holes (This is where typically BD service engineers to mount their alignment microscope).
- Mount the adapter to the two upper thread holes. Gently tighten the two screws and take care that nothing is tilted or jammed.

Thread holes

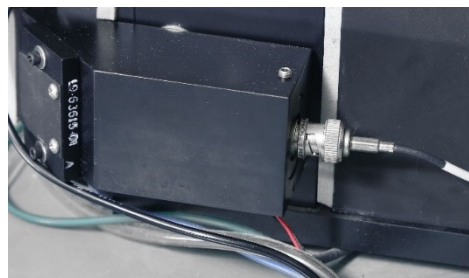


Details on the mounting thread holes and how the mounted flow cell adapter will look like

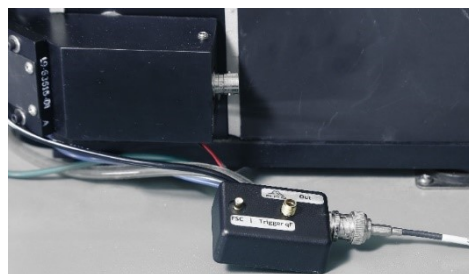
The adapter can stay on the instrument without any interference in the daily operation of the flow cytometer.

## B Mounting the Trigger Switch

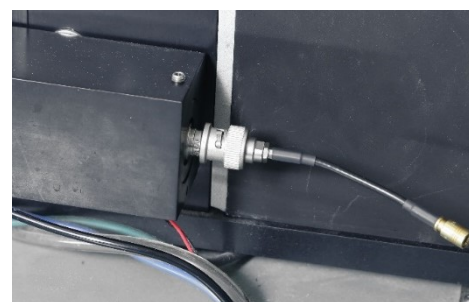
- The next step is making the connection between the quantiFlash Trigger Switch and the BD Instrument.



- Unplug the BNC cable from the FSC diode. (To prevent damage to the FSC diode hold it firmly in place using one hand while gently unlock and pull off the BNC cable from the socket.)
- Take this BNC cable (that you have just pulled off the FSC diode) and connect it to the quantiFlash Trigger Switch.



- Mount the additional short cable (supplied with the quantiFlash Trigger Switch Package) to the FSC diode.



- Finally mount the free end of the supplied cable to the trigger switch.



- To trigger your cytometer with the quantiFlash you have to connect one of the supplied fibers to the “Trigger qF” of the trigger switch.
- Connect the other end to the trigger output of the quantiFlash®



Once installed the trigger switch can stay in place.

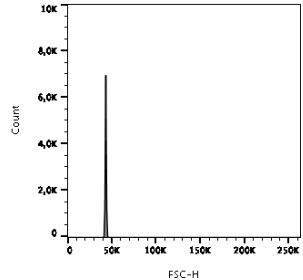
For regular measurements flip the switch on the back side of the quantiFlash Trigger Switch to “FSC” position.

For calibration measurement with the quantiFlash the switch must be turned to “Trigger qF”.

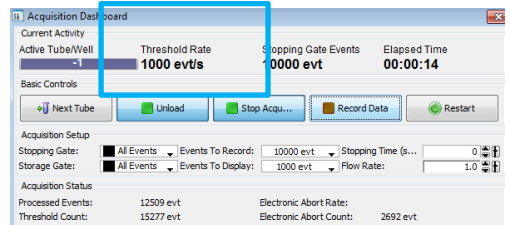
## C Start-Up and Connection Test

<ul style="list-style-type: none"> <li>Switch on the quantiFlash® and choose the following settings</li> </ul>	Count rate: 1000 evt/s Trigger intensity: 50
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- Start a new measurement on the flow cytometer and open a histogram showing the FSC signal.



- To check whether triggering works fine observe the Threshold Rate. The threshold rate should be identical to the count rate set in the quantiFlash® (1000 evt/s).

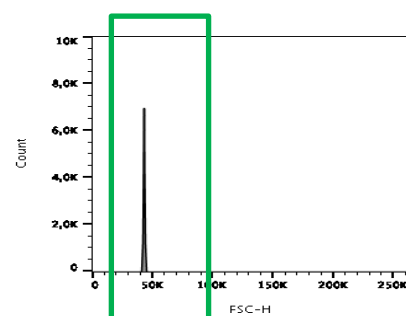


If the threshold rate is higher than 1000 evt/s:

- Lower the FSC Gain until you reach 1000 evt/s.
- If still the Threshold Rate is too high lower the Trigger Intensity of the quantiFlash, e.g. from 50 to 40.

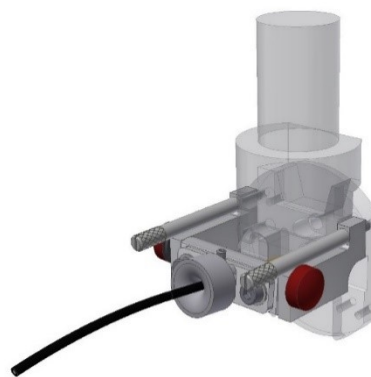
You are done if the Threshold Rate is ok (1000 evt/s) and the FSC Signal is on scale (i.e. the peak is in the first 1/3 of the scale)

If the FSC Signal is off scale lower the Trigger Intensity of the quantiFlash further.



## D Aligning the flow cell adapter

- If using the quantiFlash® on various flow cytometers or if comparing to other groups inevitably there will be some differences in alignment or in coupling efficiency. This difference is not detrimental and will not harm any of the results obtained by the quantiFlash® nor will it alter any of the protocols presented in this manual. So don't worry if a colleague from another lab has achieved an even better alignment - one can easily compensate this by changing the output intensity of the quantiFlash® slightly.



### Follow the next steps as follows

- Start again a new measurement on the flow cytometer and trigger the flow cytometer with the quantiFlash (Trigger Intensity as set during Start-Up and Connection Test, Step 2)

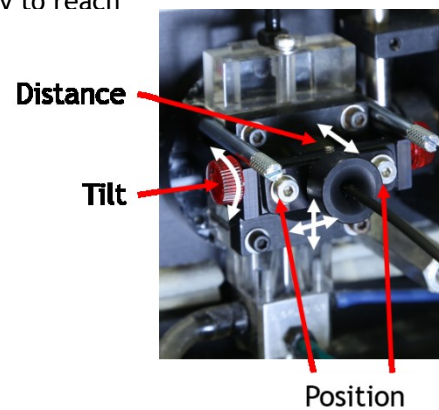
- Turn on the quantiFlash® and chose the following settings:

- Count rate: 3000 events/s
- Pulse duration: 5µs for Analyzer, 2µs for Sorter
- Pulse shape: Gaussian
- Intensity: 0dB

- Prepare a worksheet in the BD FACSDiva software (BD) with a histograms of the fluorescence detection channels with the shortest wavelength (pulse height) and set the events to display to 1000 events. On the 488 nm laser this would be the FITC channels.

- Set the PMT voltage of these channels to 500 V

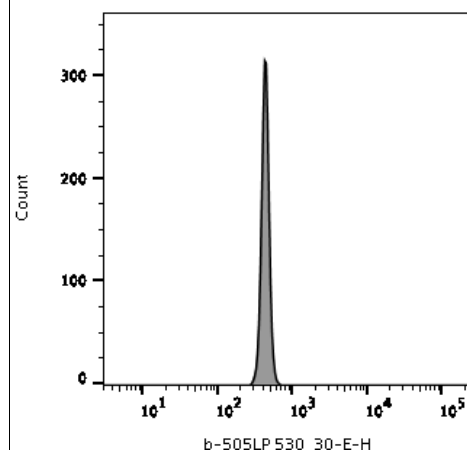
- Observe the signal in your detection channel. Try to reach maximum signal by aligning the tilt of the fiber | loosening the red screws at the side of the flow cell adapter.
- Use the Allen keys supplied with the BD adapter loosen the fiber and gently and carefully push the fiber closer to the flow cell. Do not use force as might damage the surface of the flow cell!
- If you suspect that you have touched the surface of the flow cell retract the fiber a tiny little bit.



If you are using the flow cell adapter on a sorter please make sure that you do not hit the nozzle with the fiber and once again do not use force as this might damage the nozzle.

- To further align the fiber one has to iteratively
- Align the tilt of the fiber
- Align the fiber in the plane perpendicular to the flow cell using the Allen keys supplied
- Carefully and gently try if the fiber can be placed even closer to the flow cell
- If you suspect that you have touched the surface of the flow cell retract the fiber a tiny little bit
- Align the tilt of the fiber
- Two to three iterations are more than enough one will very quickly see, that no further improvements regarding the signal intensity on the histogram. If the signal goes off scale, reduce the output intensity of the *quantiFlash*® by a few dB.

- Add histograms of all fluorescence detection channels of interest (pulse height). The mean channel peak position for all histograms should be somewhere between  $10^2$  and  $10^5$ .
- If the mean channel is  $<10^2$ : re-align the fiber in front of the flow cell by changing the position as well as the tilt angle in relation to the optical axis.
- If the mean channel is  $>10^5$ : lower light input (dB) from *quantiFlash*®



## Additional Information about the Trigger switch

In most BD flow cytometers the forward scatter (FSC) detector is a photo diode connected with a BNC cable to the signal processing electronics of the instrument. The trigger switch is a small box (shown in figure 2) comprising a photo diode and a fiber mount as well as a high quality BNC terminated signal bypass. It is plugged directly in between the FSC detector and the BNC cable delivering the trigger signals to the signal processing electronics. Once it is in place a simple manual switch allows the user to easily decide whether to use the trigger signals from the FSC detector or to trigger the flow cytometer with the *quantiFlash*®.

### Trigger switch

