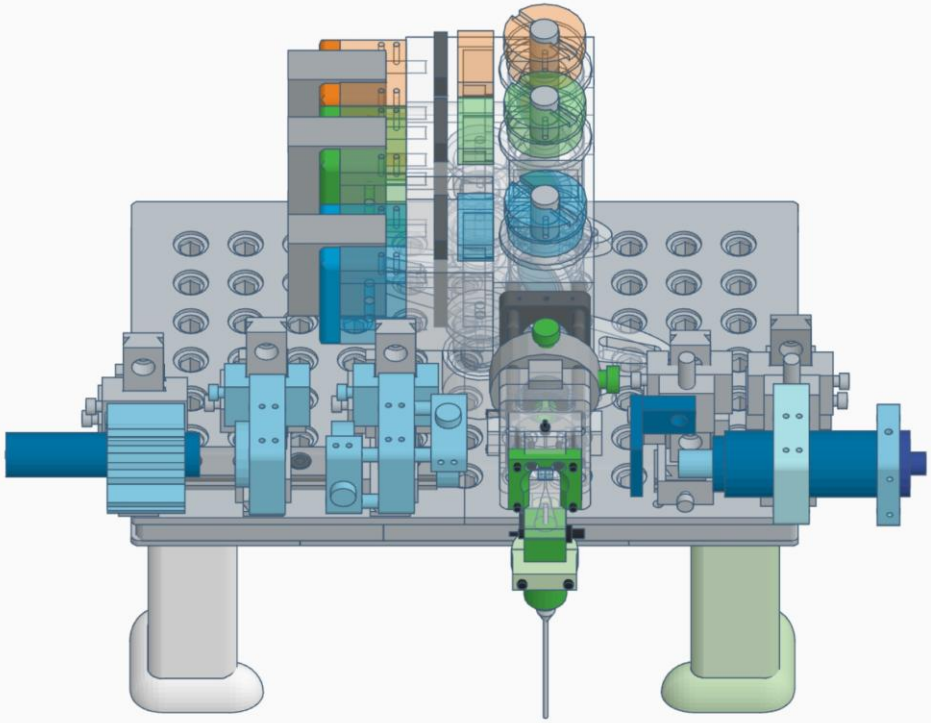
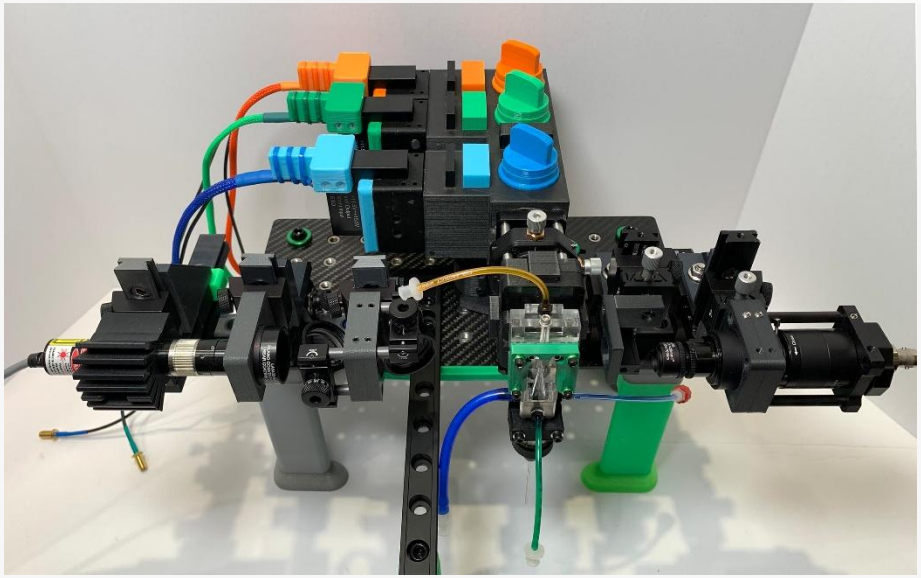
# The Make Your Own Flow Cytometer Laboratory

Bill Telford

NCI Flow Cytometry Laboratory
National Cancer Institute
National Institutes of Health

www.cytometryworks.com





NIH National Institutes of Health
*Turning Discovery Into Health*

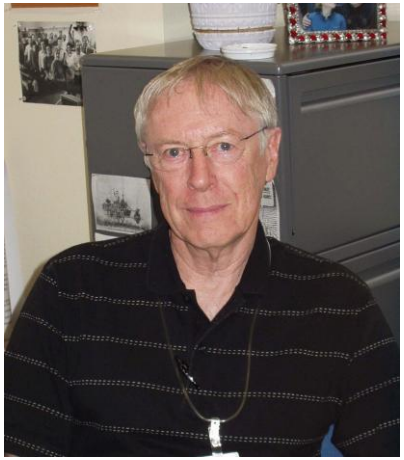
ISAC
INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

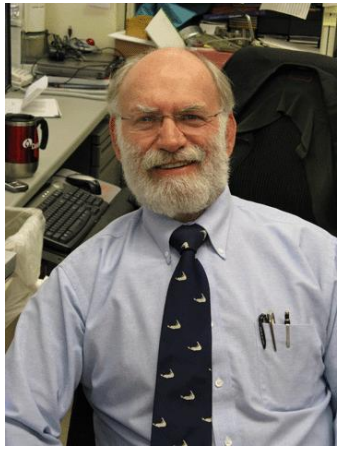
Los Alamos NATIONAL LABORATORY

National Flow Cytometry Resource

The original Build Your Own Flow Cytometer Lab (now the Build Lab) was developed by **James Jett, John Martin and Mark Wilder** at the National Flow Cytometry Resource, Los Alamos National Labs.

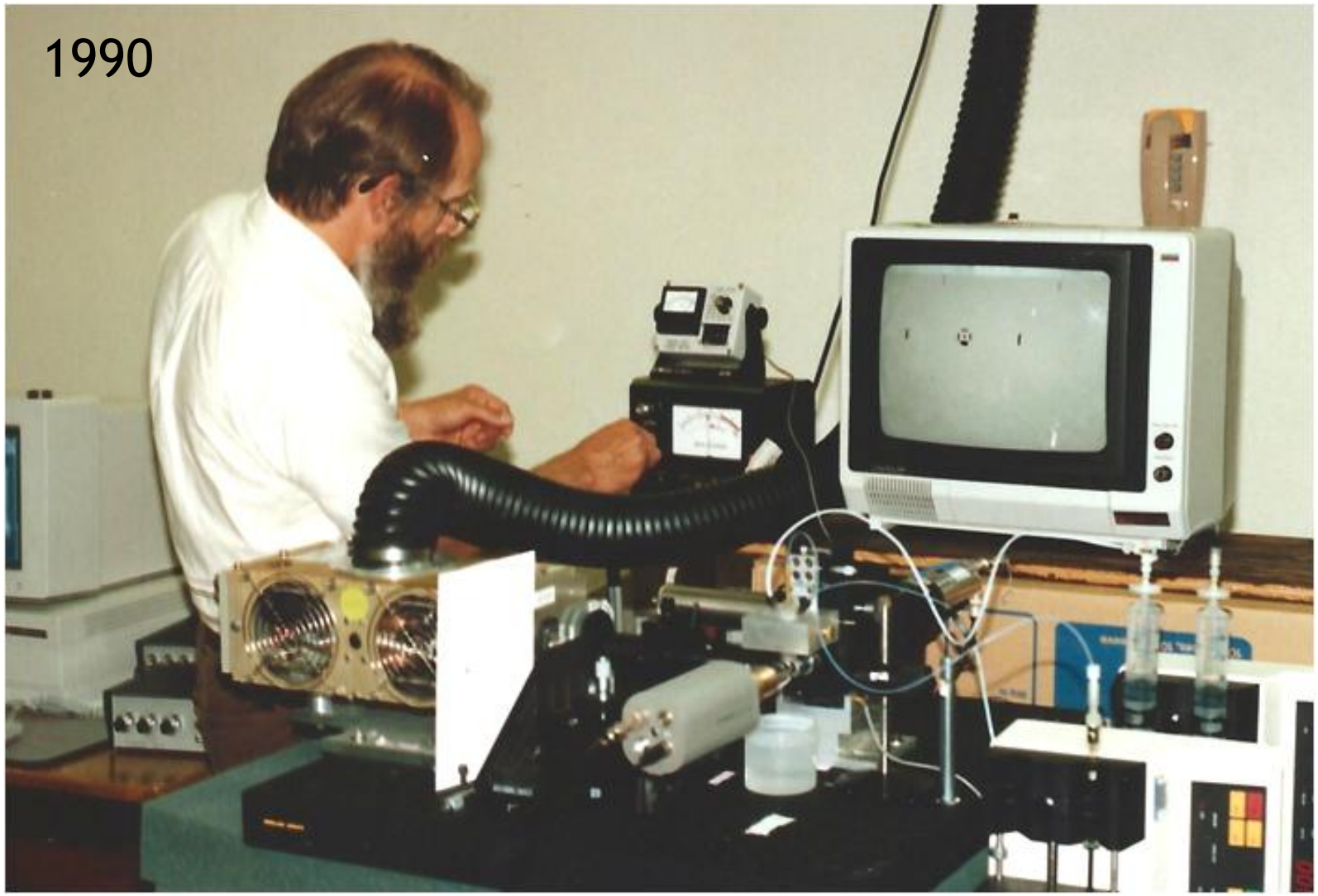
It was first presented at the Annual Workshop in Flow Cytometry at Bowdoin College, Maine, USA (the first one held there) in 1990.





# The Original Build Your Own Cytometer Lab (The Build Lab)

James Jett, John Martin, Mark Wilder
Los Alamos National Laboratory



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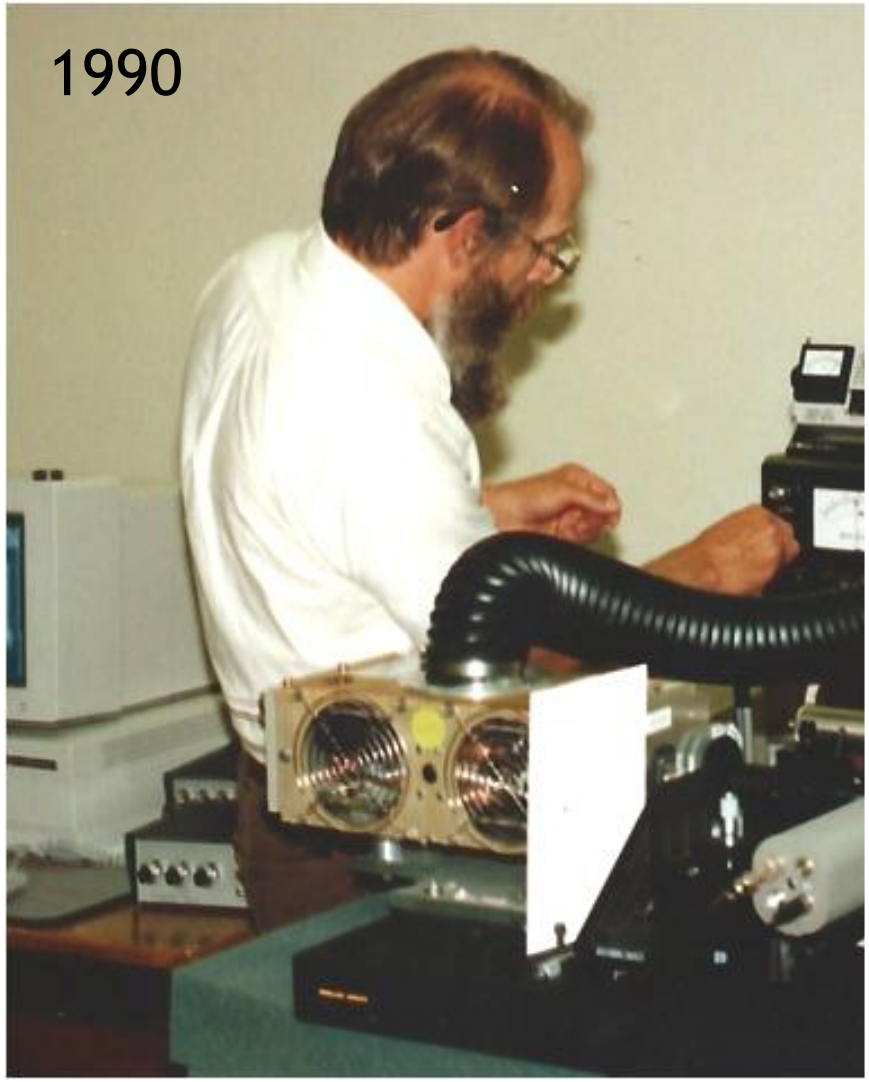


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# The Original Build Your Own Cytometer Lab (The Build Lab)

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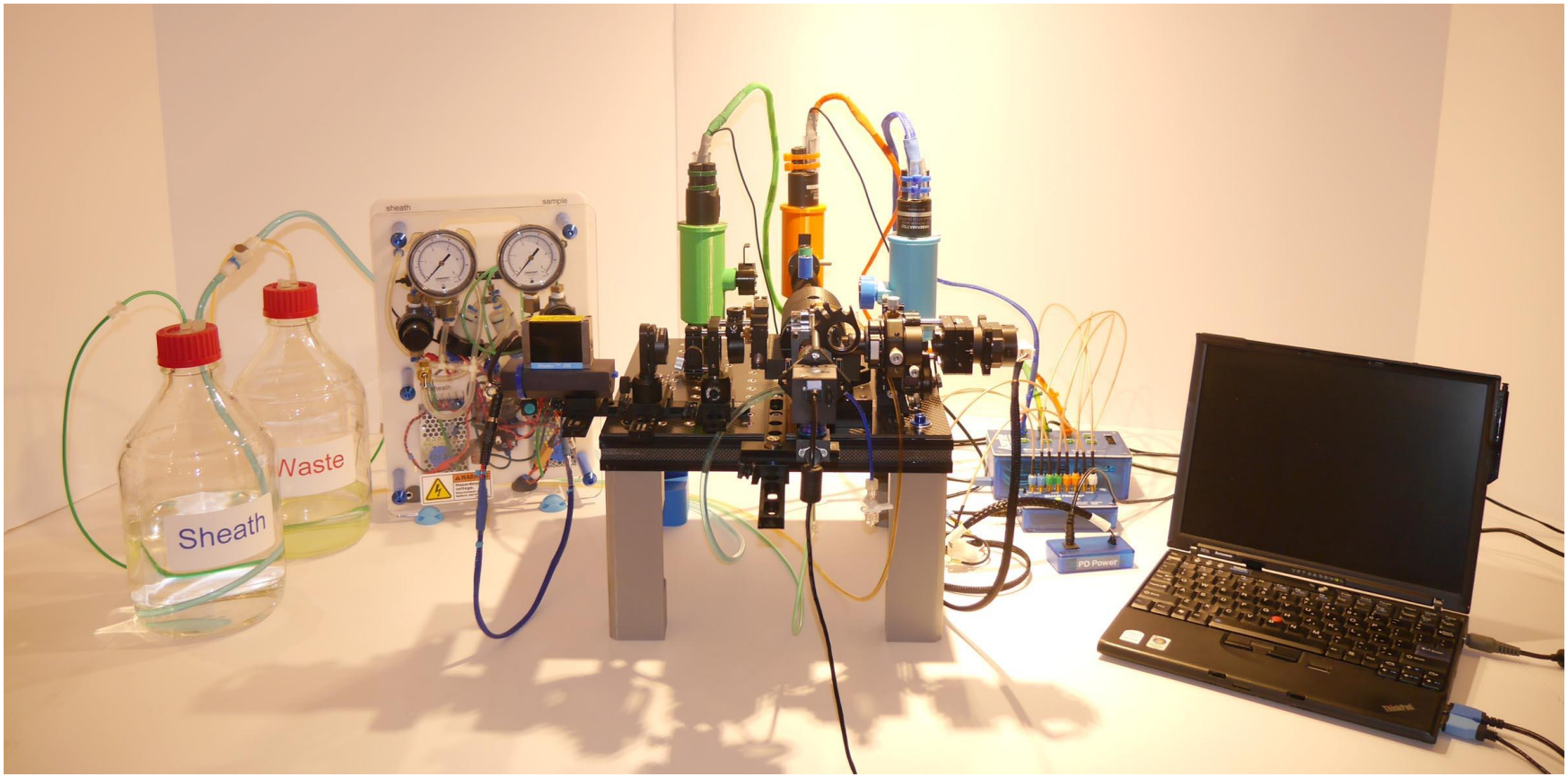
Los Alamos National Laboratory

# The Original Make Your Own Cytometer Lab (The Flow Cytometry Maker Lab)



# The 3D Printed Make Your Own Cytometer Lab (The Flow Cytometry Maker Lab)

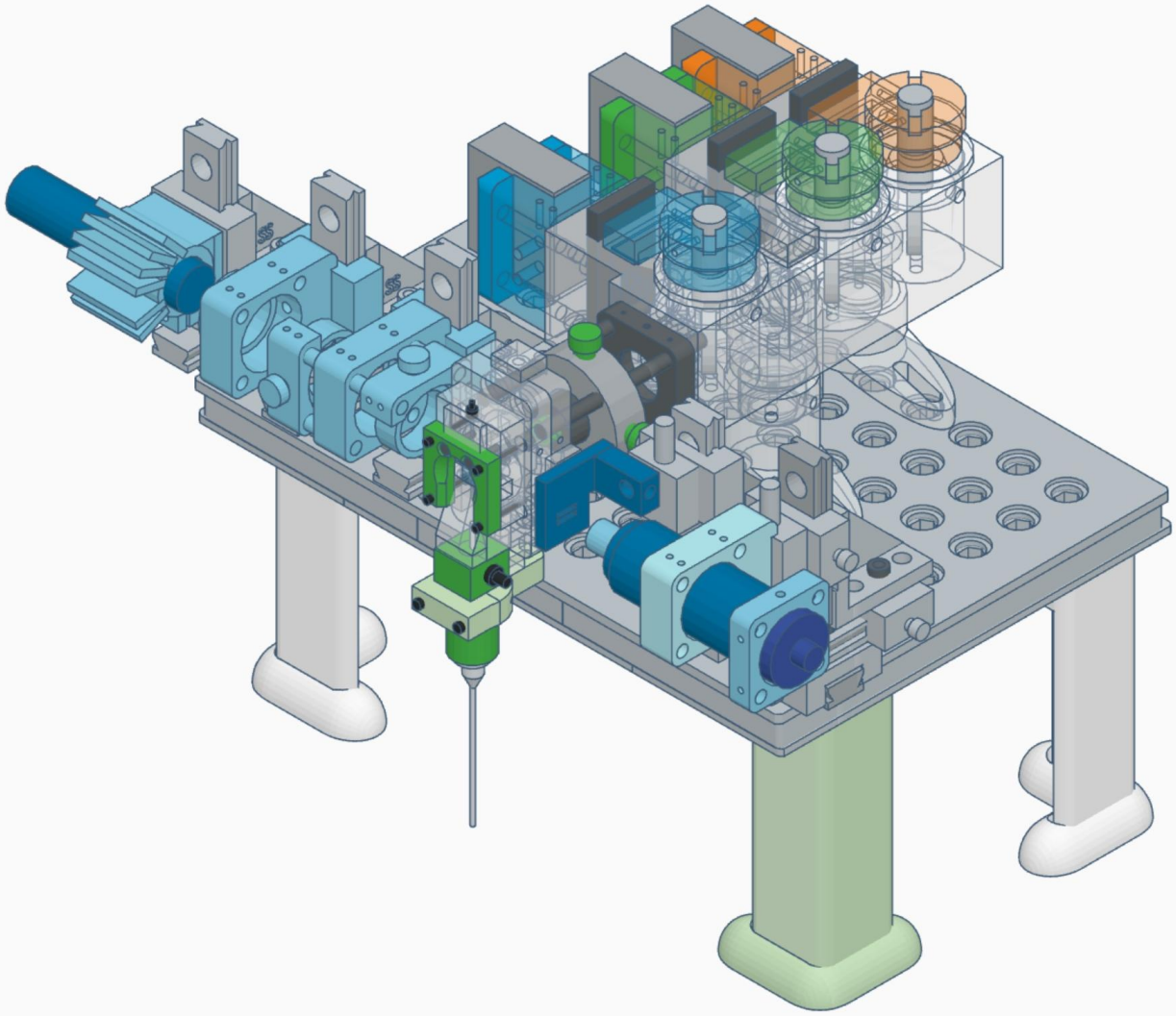
Industrial aluminum parts replaced with 3D printed plastics.

We are no longer constrained by commercial parts. We can build the instrument we want to build.

Much lighter in weight.

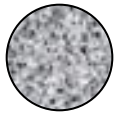
Other laboratories can build their own system (although you still need a laser, flow cell, detectors and electronics).

We are aiming for ~90% of the system built by additive manufacturing.

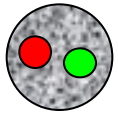


# What goes into a flow cytometer?

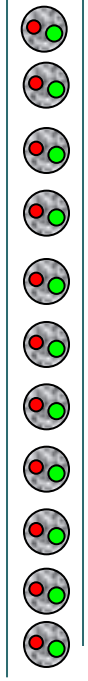
First, you need a **biological sample**…



…usually labeled with a **fluorescent marker**



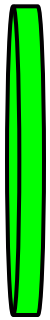
You need to **move** these cells in a linear stream through a focused light source.

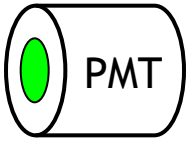


You need a **light source** to excite the fluorescent molecular on or in the cell.

Usually a **laser**!

You need **optics** that only admits light from the fluorescent probe.

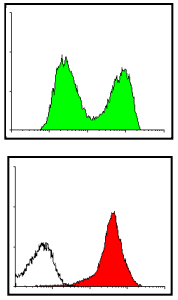





You need a **sensitive light detector** (usually a **photomultiplier tube**).

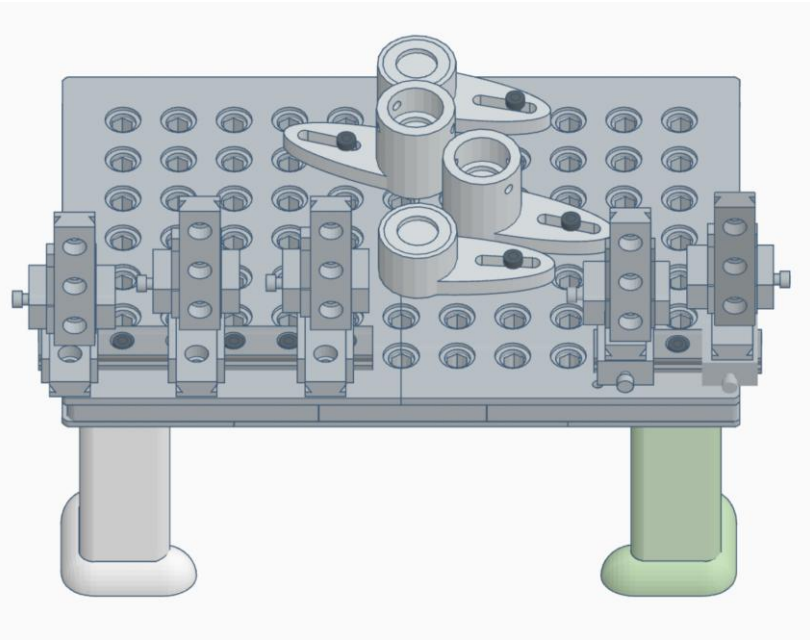
You need electronics that can convert analog fluorescent signals to digital ones.

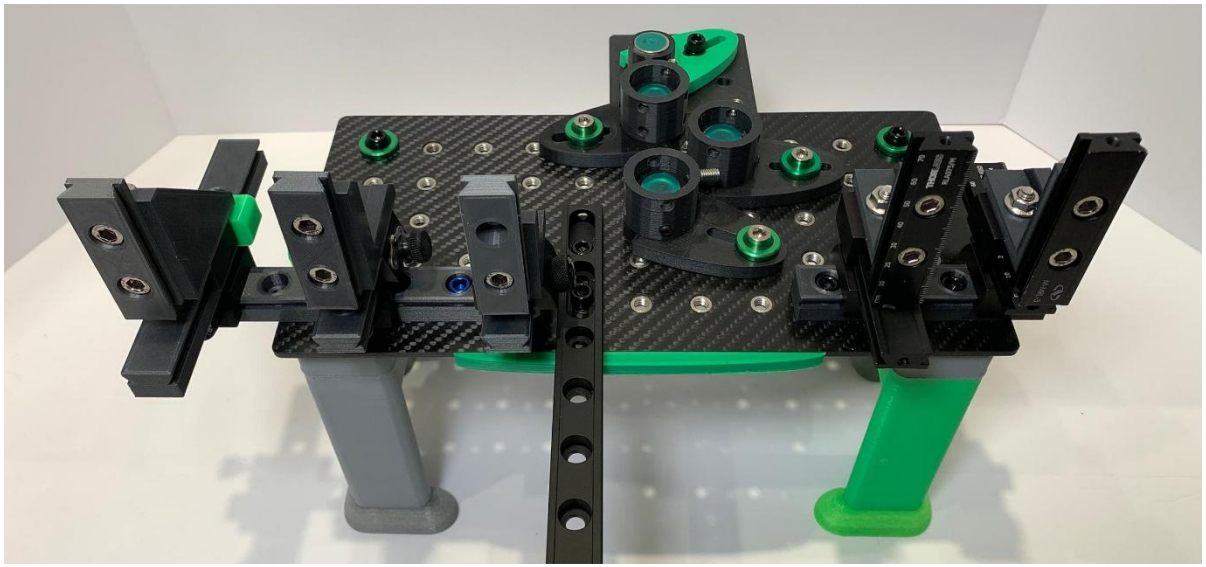
Finally, you need a computer to process the digital signals and display the data



# Baseplate

We start with the baseplate. All the cytometer components will be mounted here.



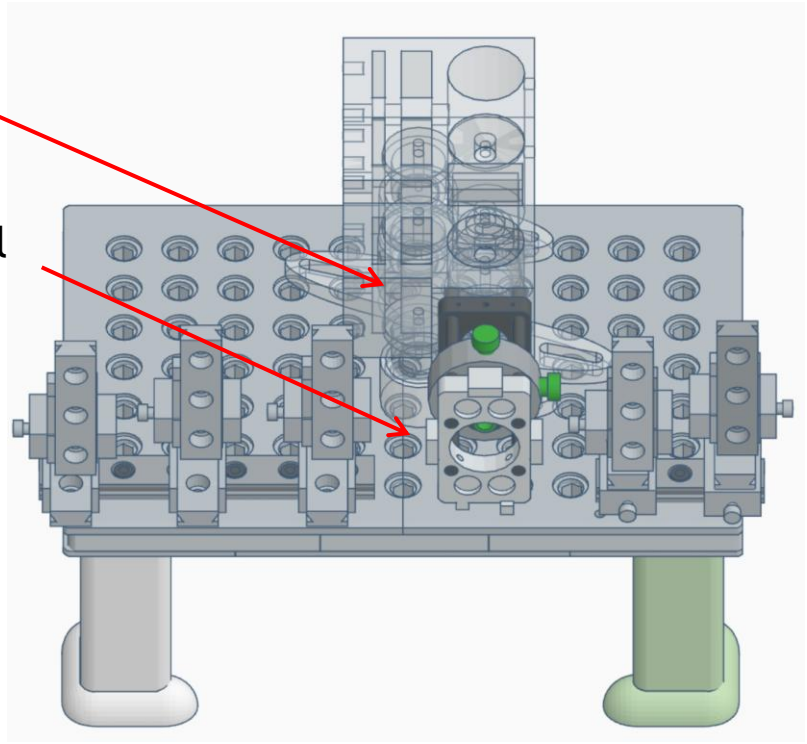


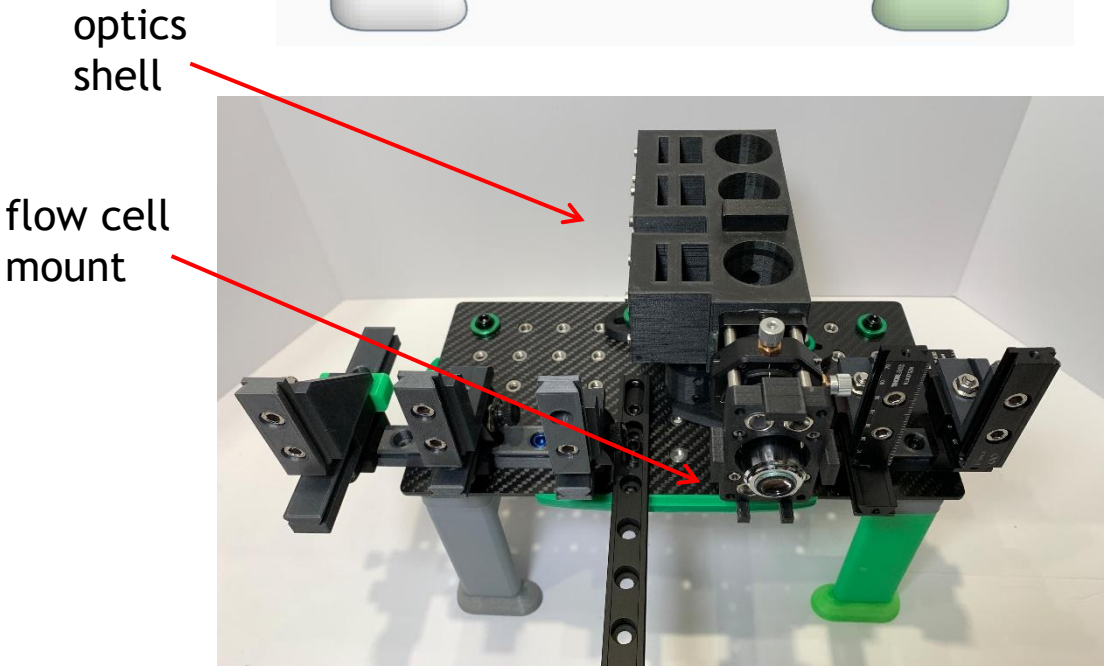
# Installing the optics shell and flow cell mount

We first install the flow cell mount and the optics shell.

The flow cell mount will hold the flow cell assembly and allow it to be moved in X-Y directions to align it to the detectors. It also holds the signal collection optics.

The optics shell will hold the dichroics, filters, pinholes and PMTs.



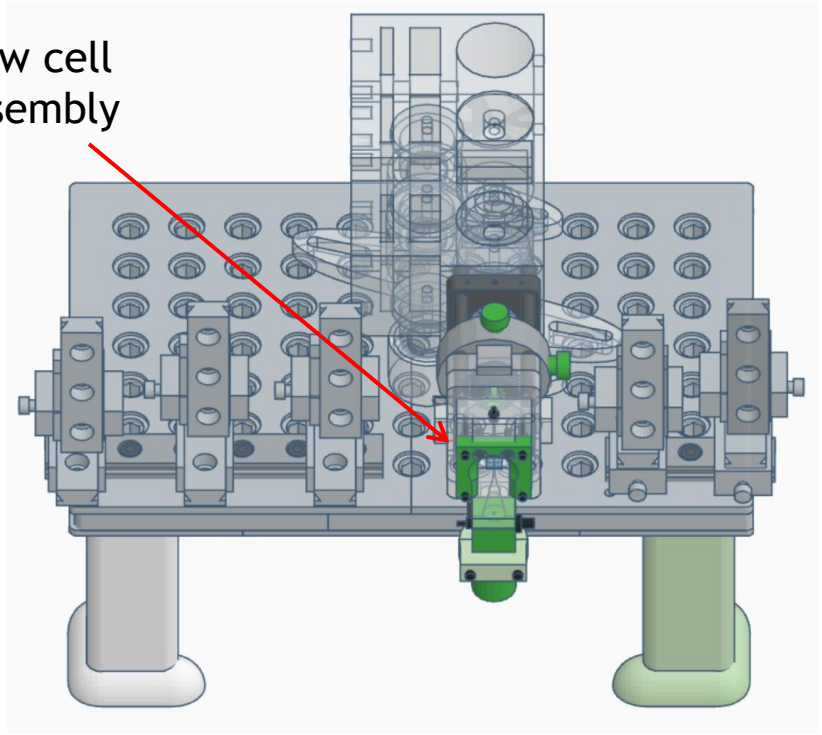



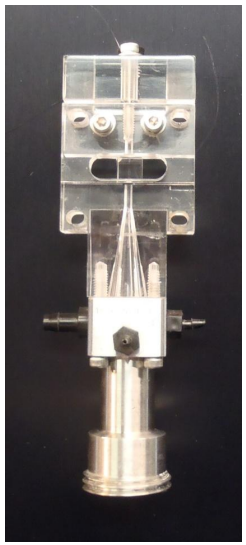
# Installing the flow cell

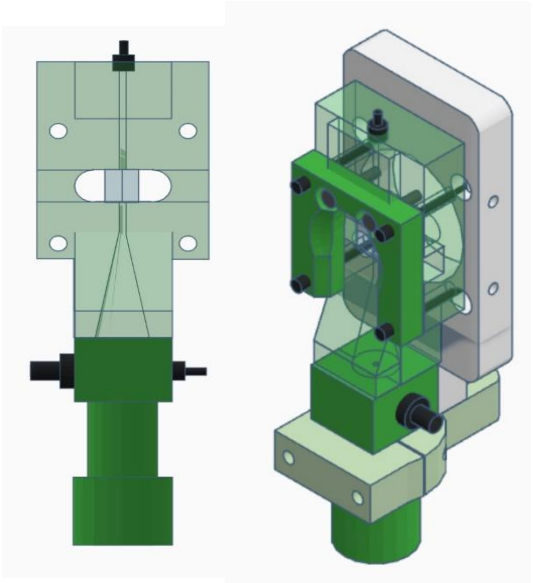
We pass our cells through a quartz flow cell, where they are illuminated with a laser.

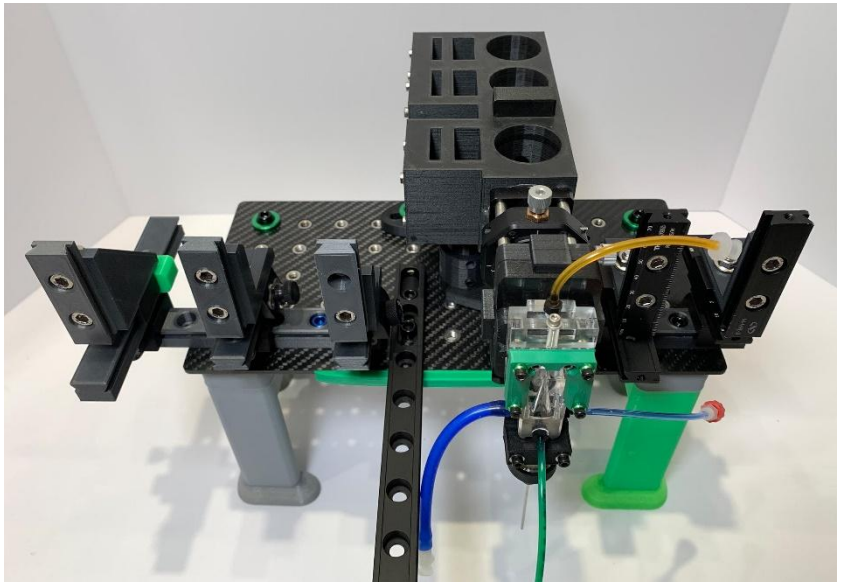
We use sheath buffer to focus the cell stream into the center of the flow cell and the laser beam.

Hydrodynamic focusing maximizes sensitivity of the flow cytometer.



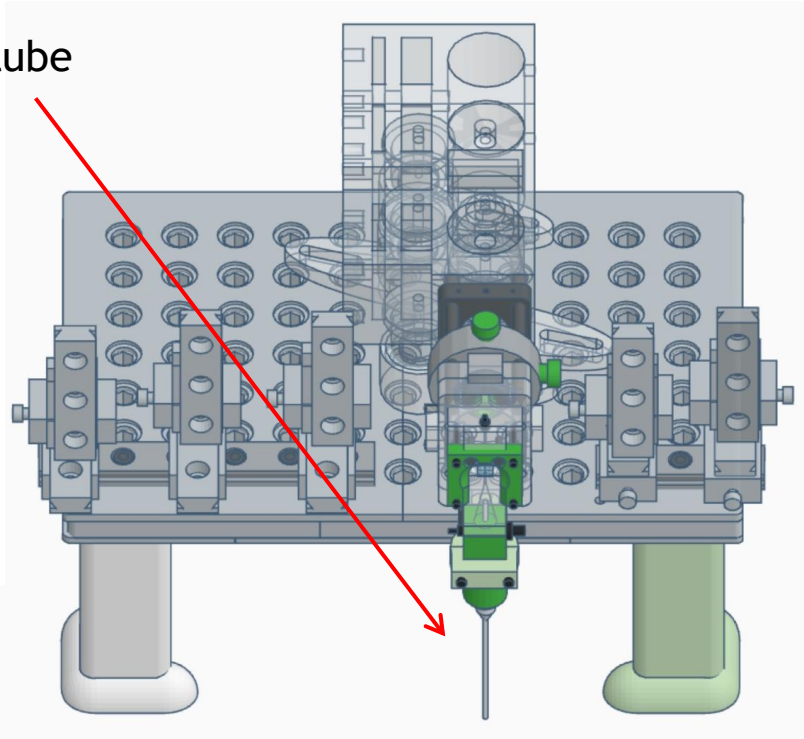


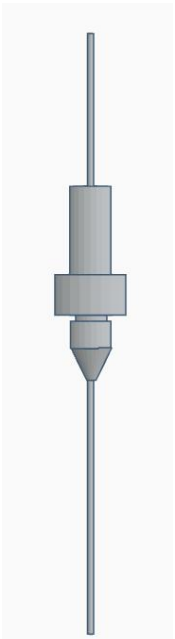


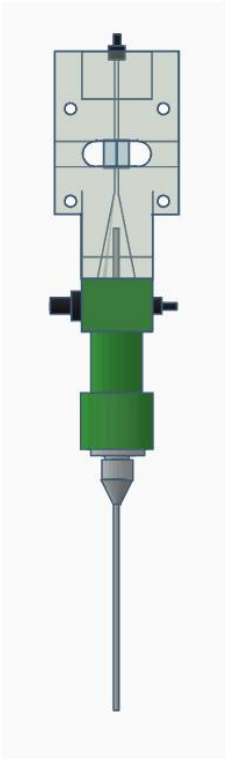
# Installing the SIP tube

We now insert the SIP tube (sample injection port) into the flow cell.

The SIP tube injects sample into the conical sheath reservoir and the flow cell. It has a port to allow the sample tube to be pressurized.



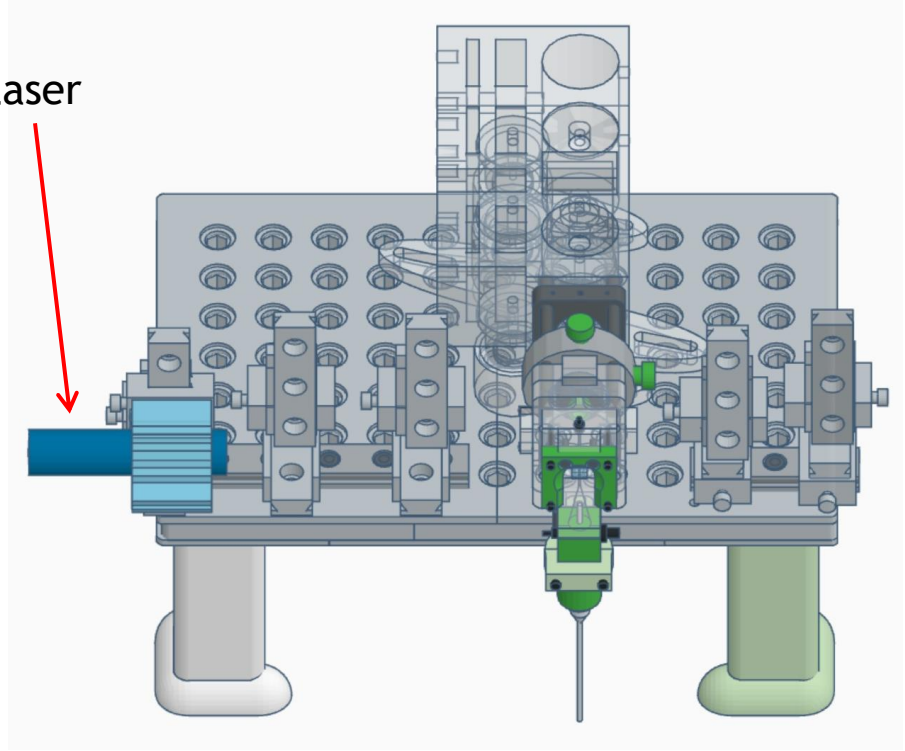


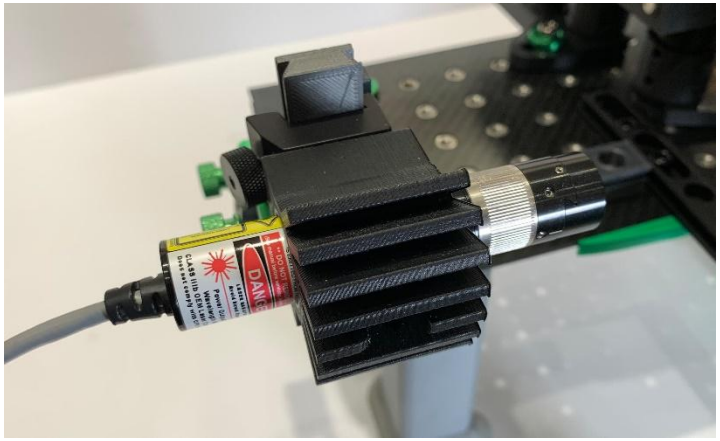
A special O-ring (the BAL seal) seals the sample tube to the SIP tube. This cytometer is a positive pressure system.

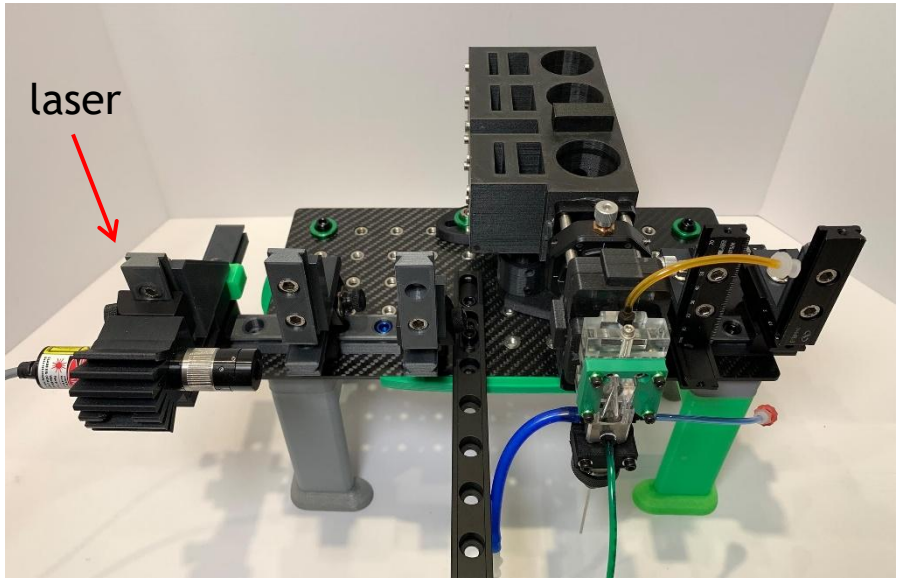
# Installing the laser

Next, we will install our blue-green 488 nm laser aligned to the flow cell. It illuminates the sample stream and allows measurement of light scattering (cell size and optical density). It will also excite fluorescent probes attached to our cells.

Our cytometer has only one laser wavelength. Modern cytometers can have many.

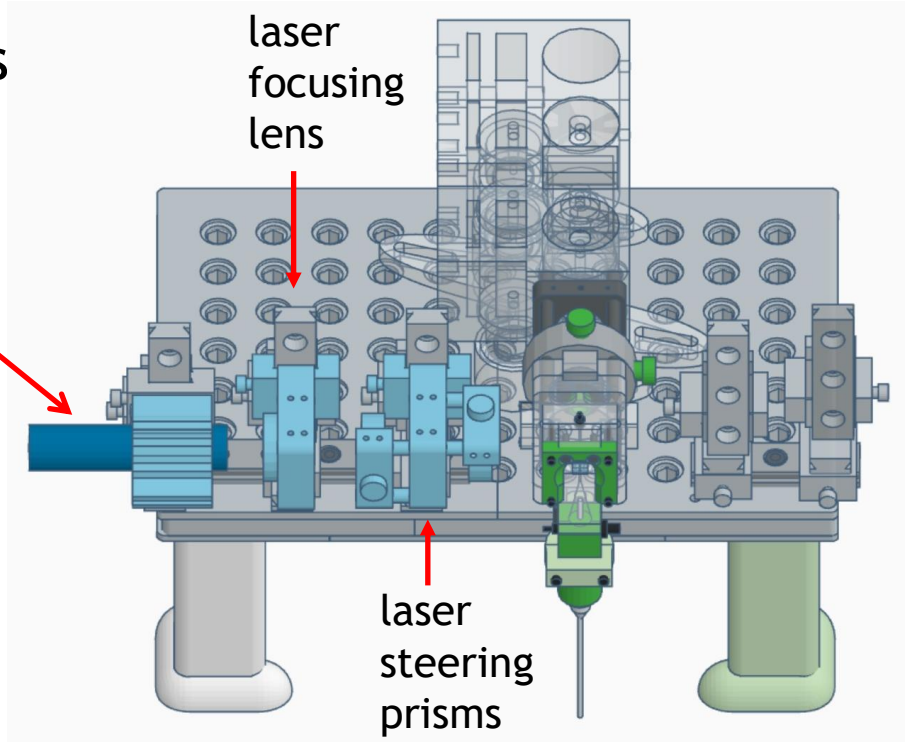


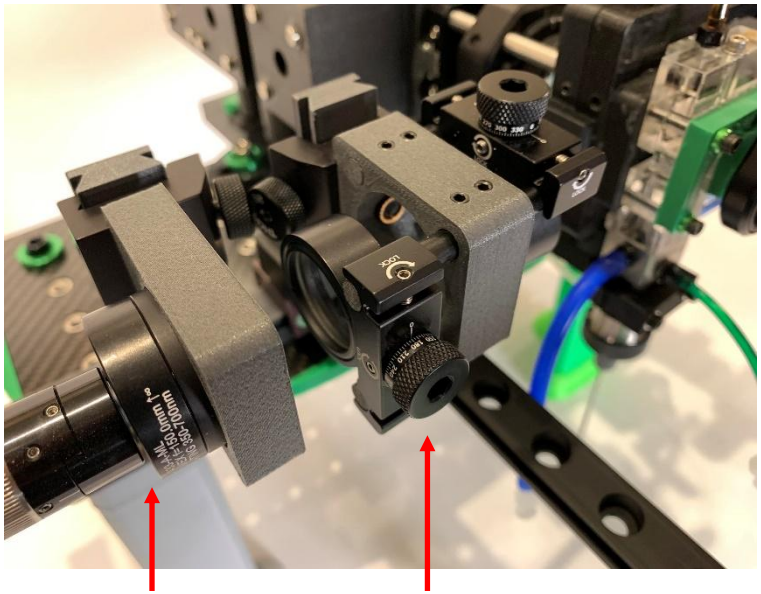



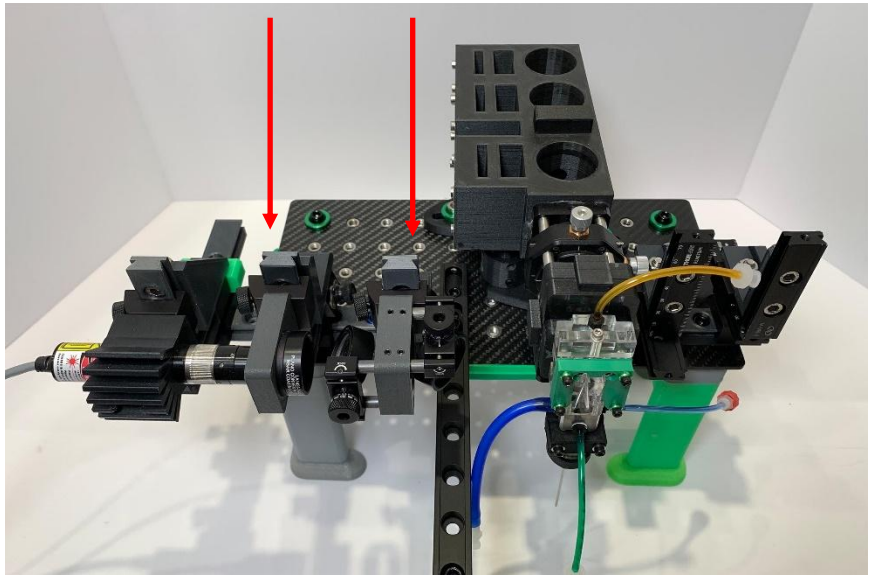


# Installing the laser, focusing lens and steering prism

We use a focusing lens and prisms in the laser path to focus the laser beam on the flow cell and steer the beam to the cell stream.



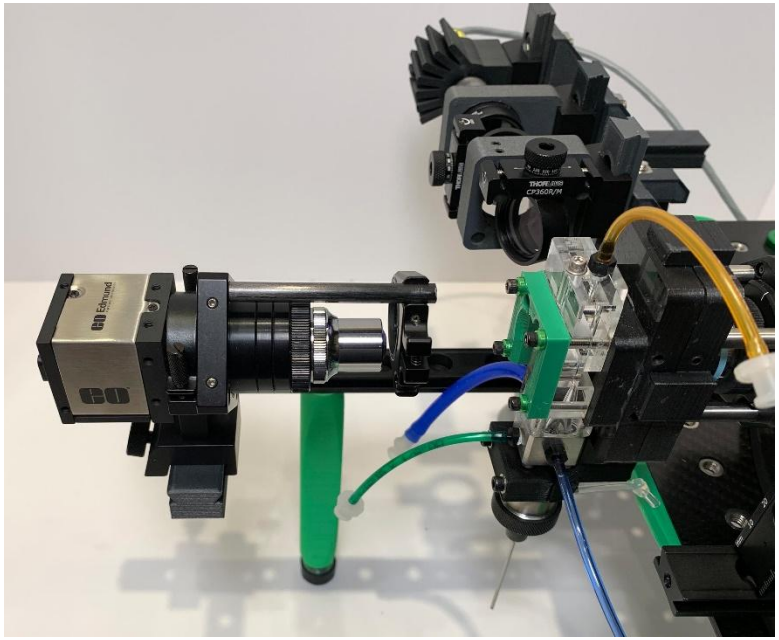



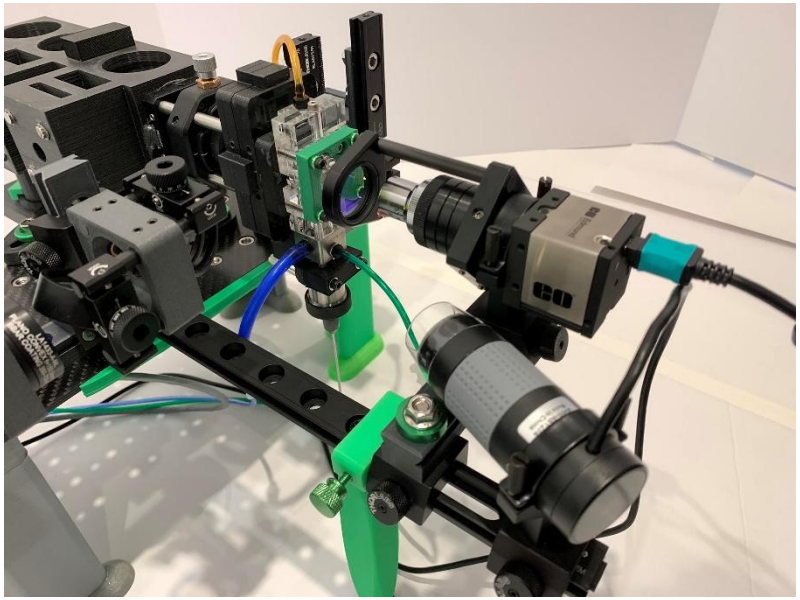



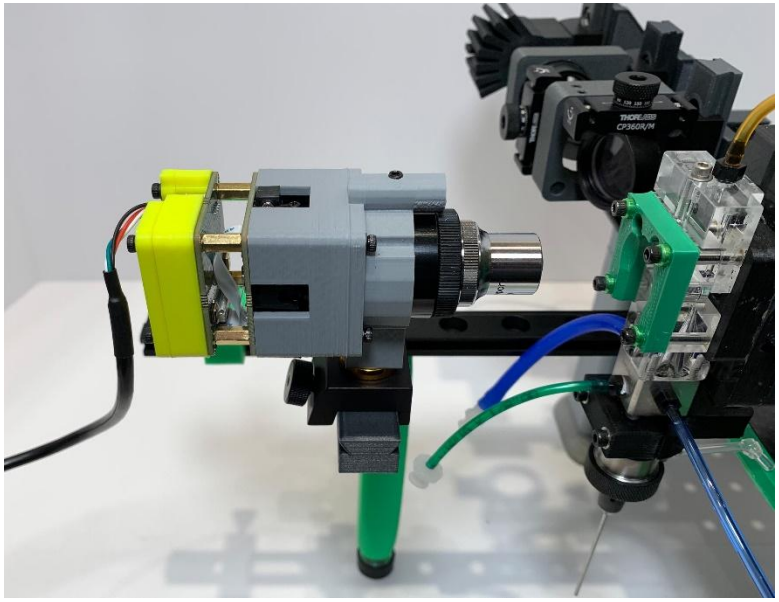
# Installing the camera

A camera installed in front of the flow cell allows us to see the laser beam and align it to the flow cell. We can also visualize fluorescent beads intercepted by the laser.

We may also use a second camera that will view the sample stream as it enters the flow cell.

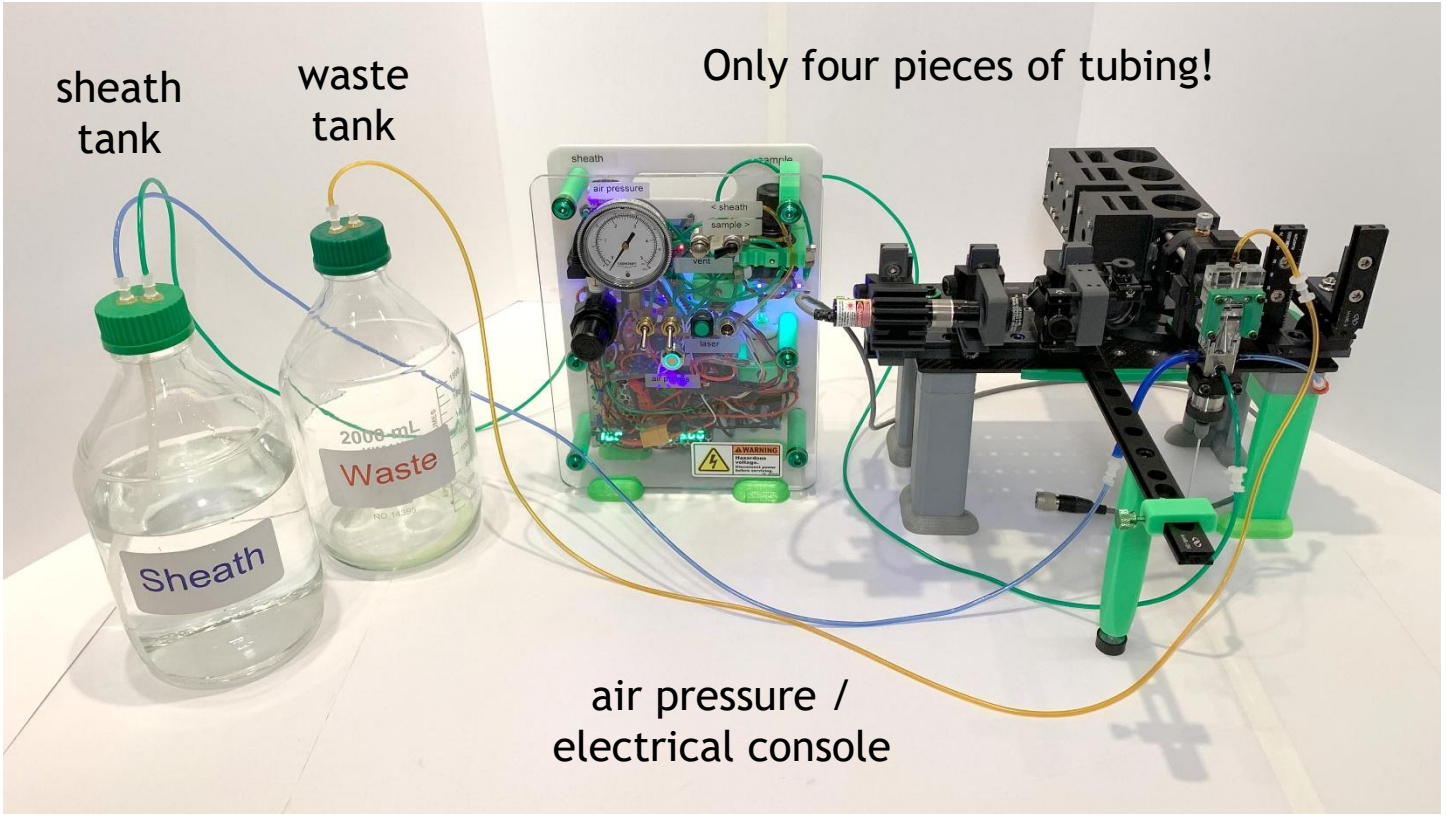






# Instrument fluidics

We will then connect the fluidics (or “plumbing”) of the cytometer. The fluidics console provides air pressure for the sheath buffer (which focuses the sample stream) and the cell sample.



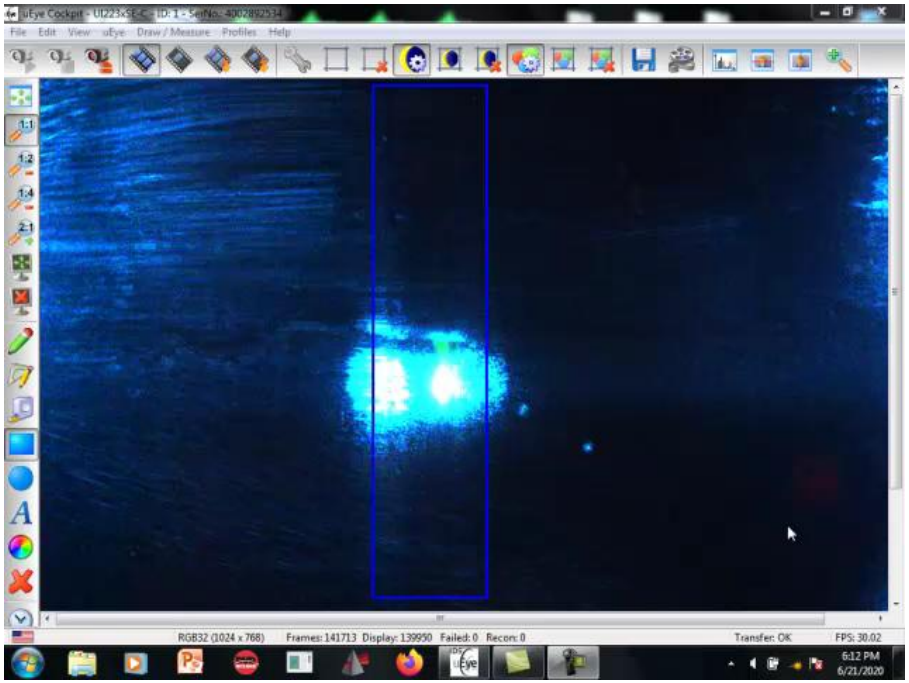

Air lines are green

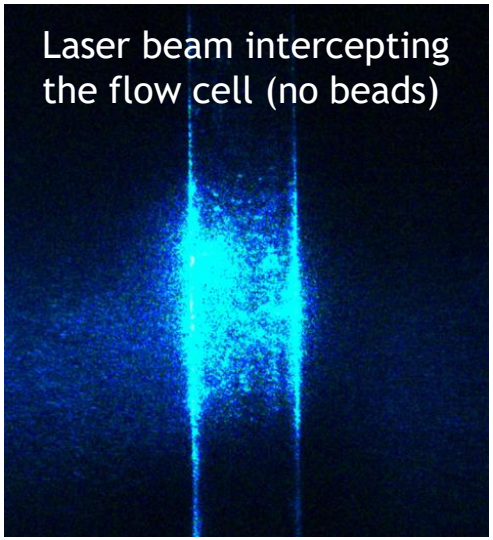
Sheath lines are blue.

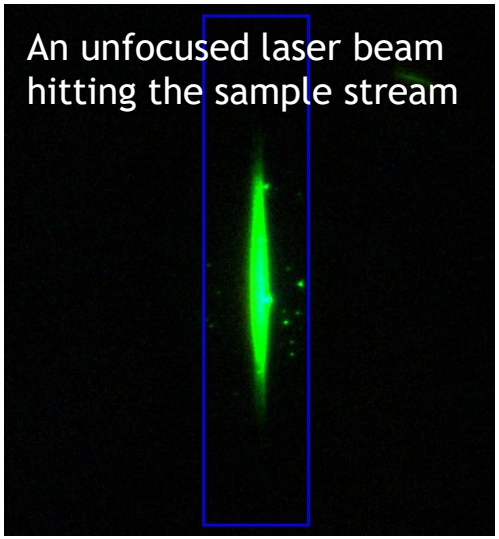
Waste lines are orange

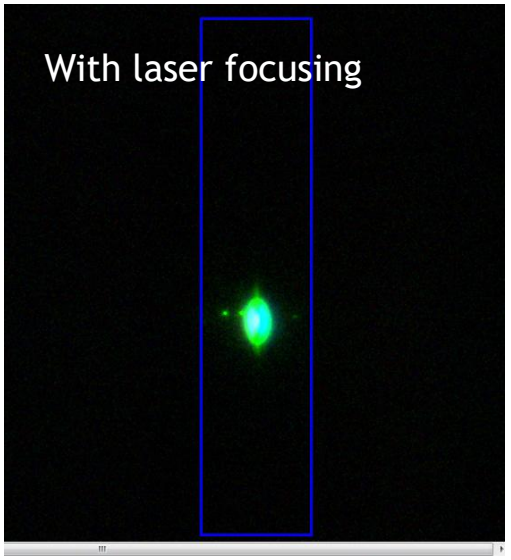
# Focusing the laser beam

We will then turn on the laser and run some fluorescent particles on our pressured cytometer. We will use the camera to visualize the beam, align it to the flow cell capillary, and visualize the laser-excited fluorescent bead stream and spot.

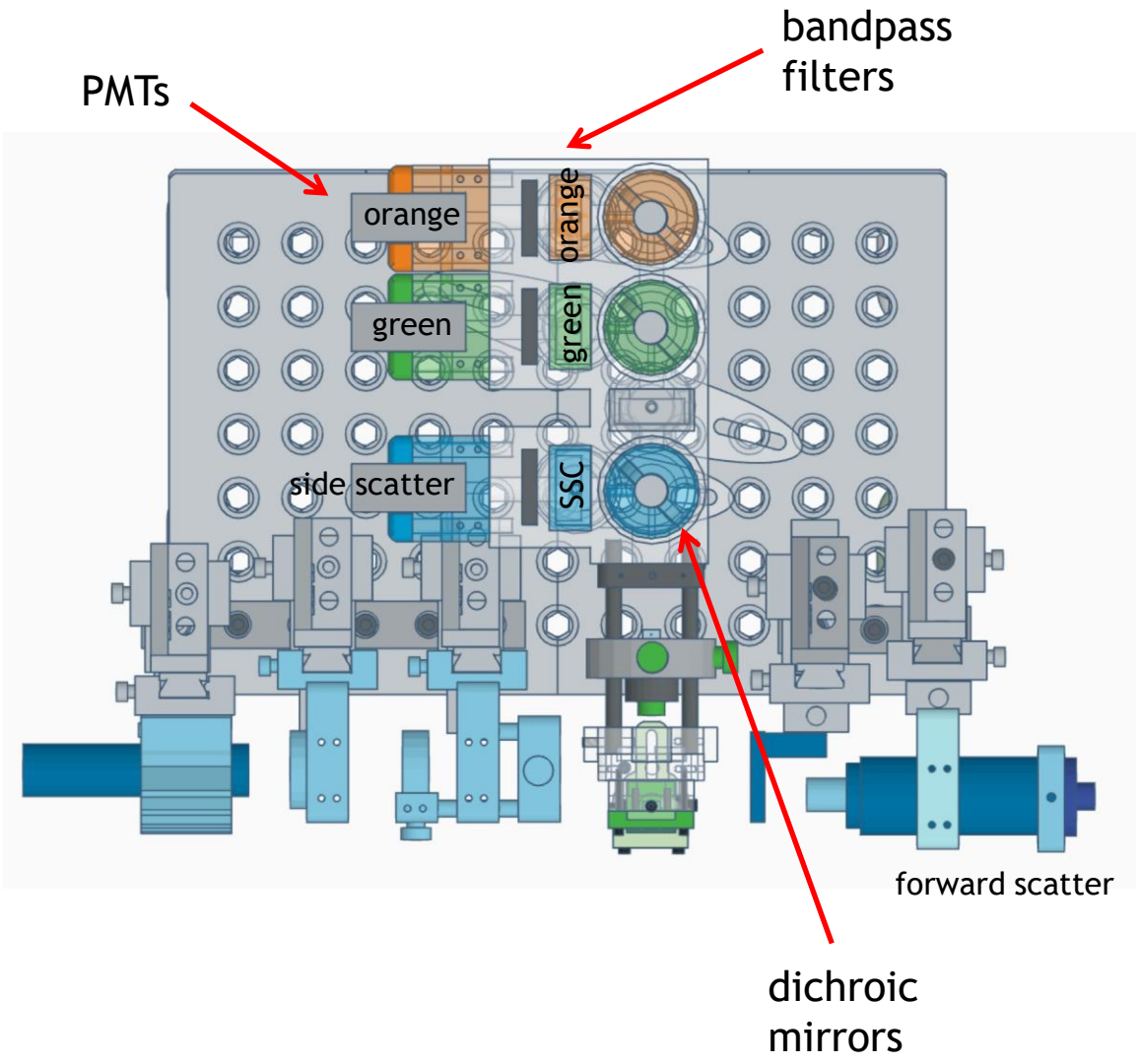




# Instrument optics




We will then install the instrument detectors and optics.

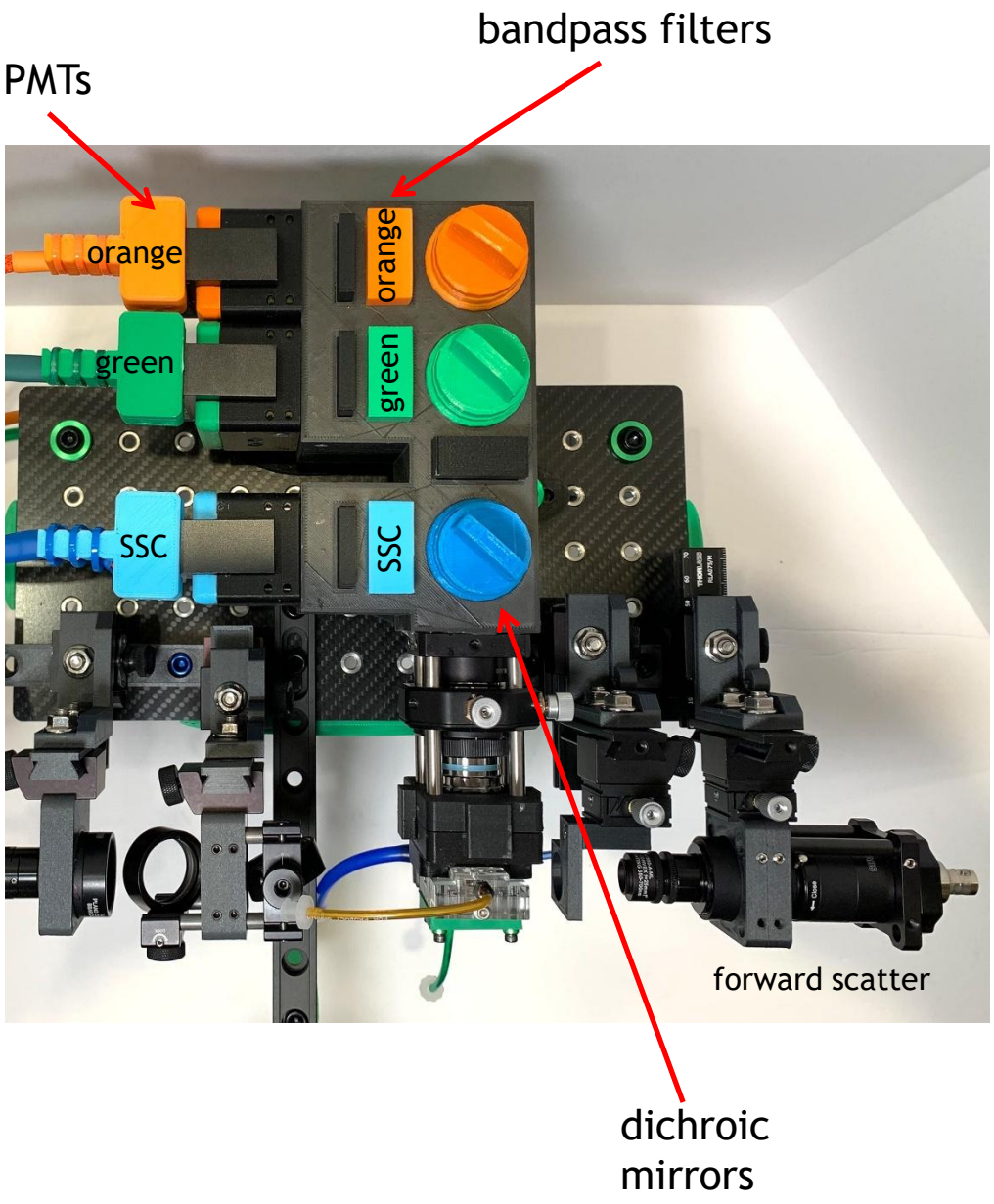
We use photomultiplier tubes (PMTs) to detect side scatter snd cellular fluorescence. PMTs are extremely sensitive photon detectors.

We use dichroic mirrors to separate fluorescent signals of different colors, and bandpass filters to further purify the fluorescent signals.

We use a photodiode to detect forward scatter.

Modern flow cytometers can detect many fluorescent markers simultaneously. Our simple machine can only separate a few.

# Instrument optics

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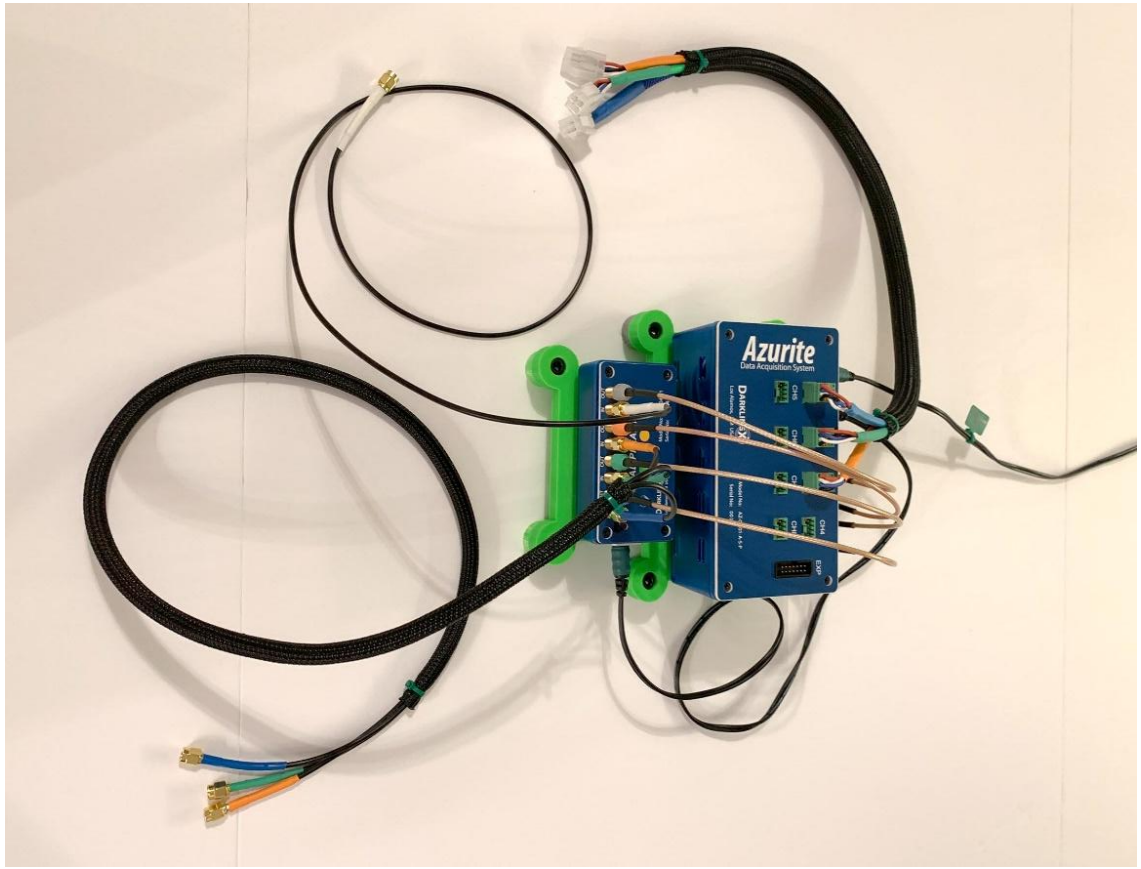
# Acquisition electronics

We then connect our acquisition electronics to the system.

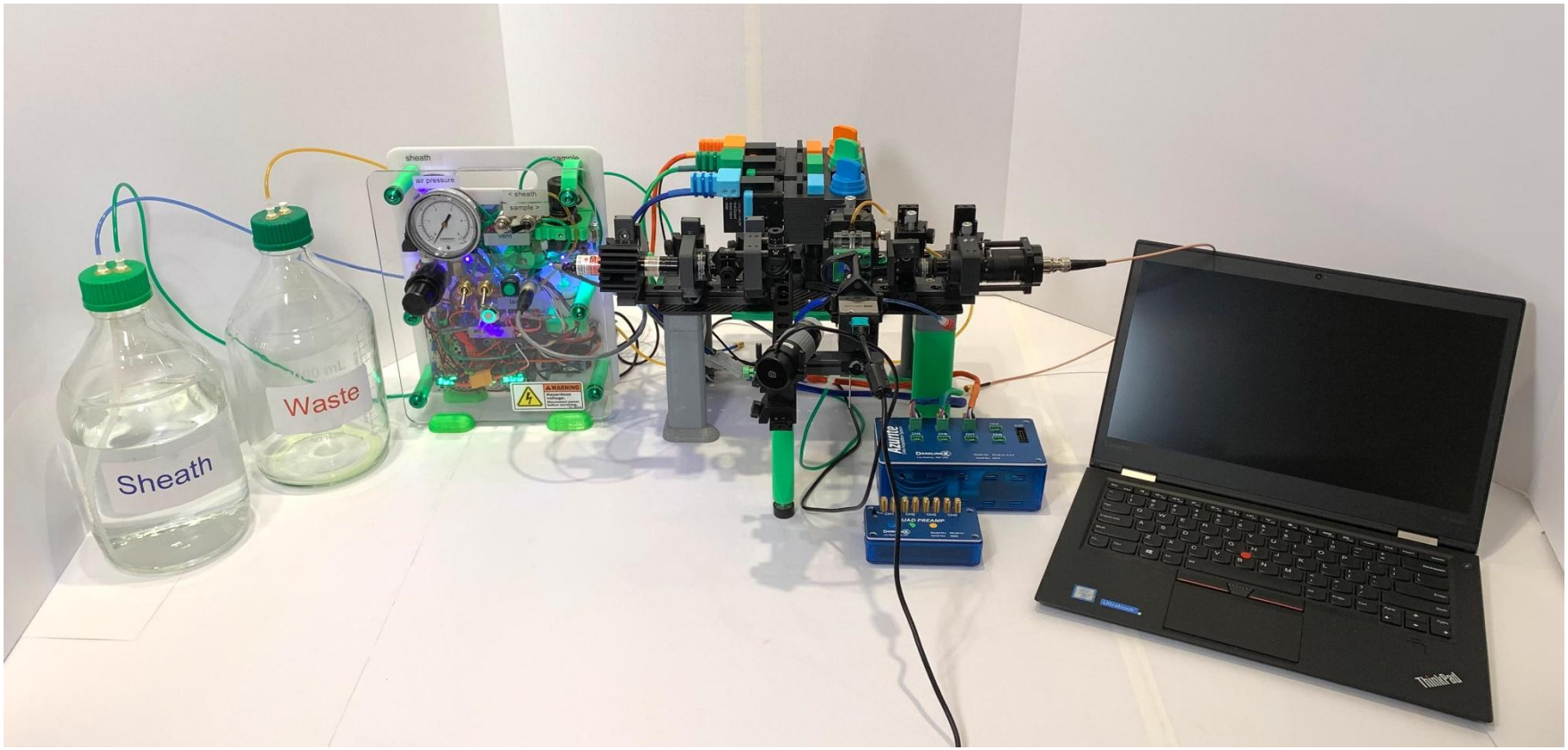
Preamplifiers, amplifiers and digitizers.

Voltage controls for the PMTs.

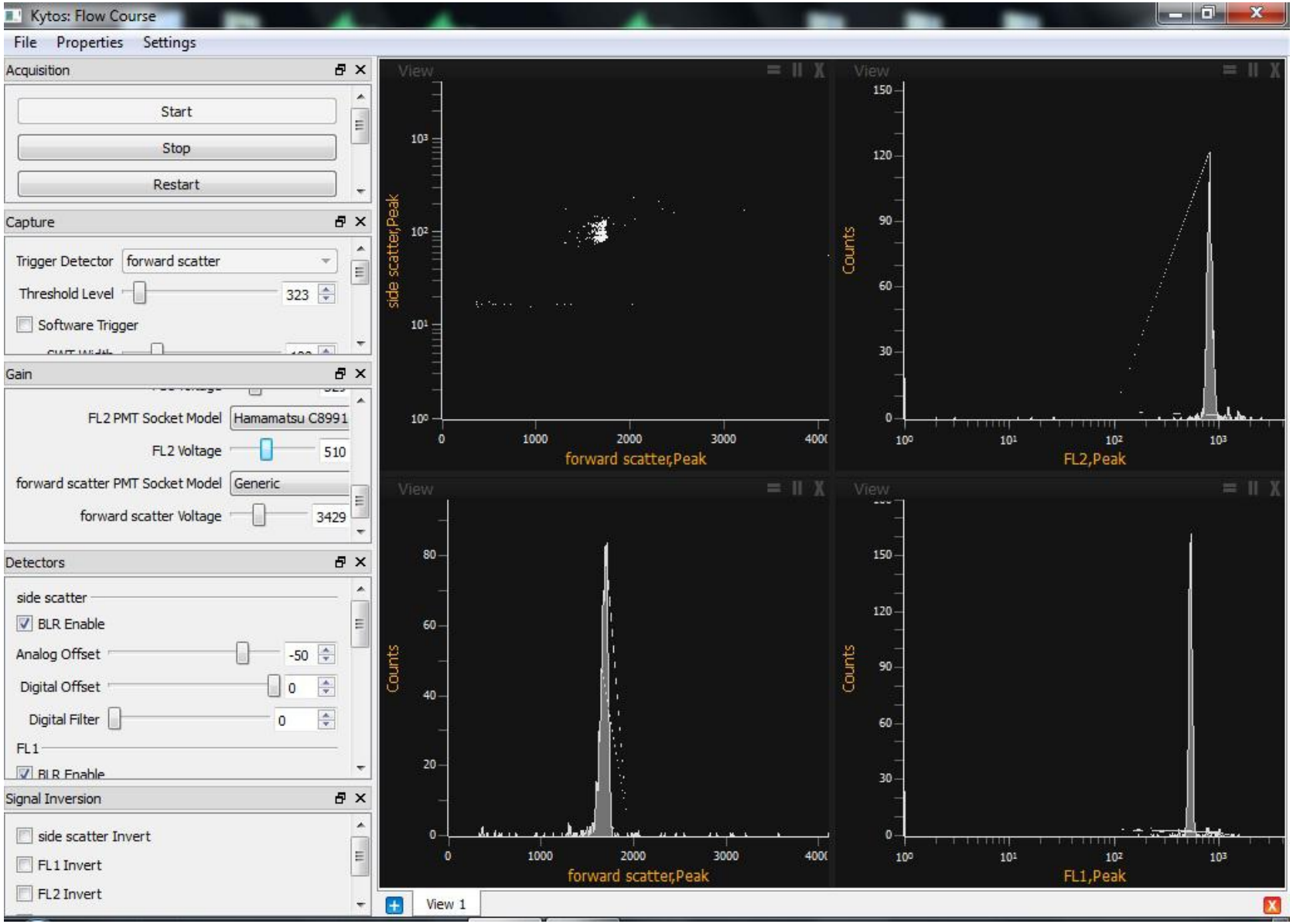
The electronics are then connected to a computer with acquisition software.



# Instrument fully assembled!



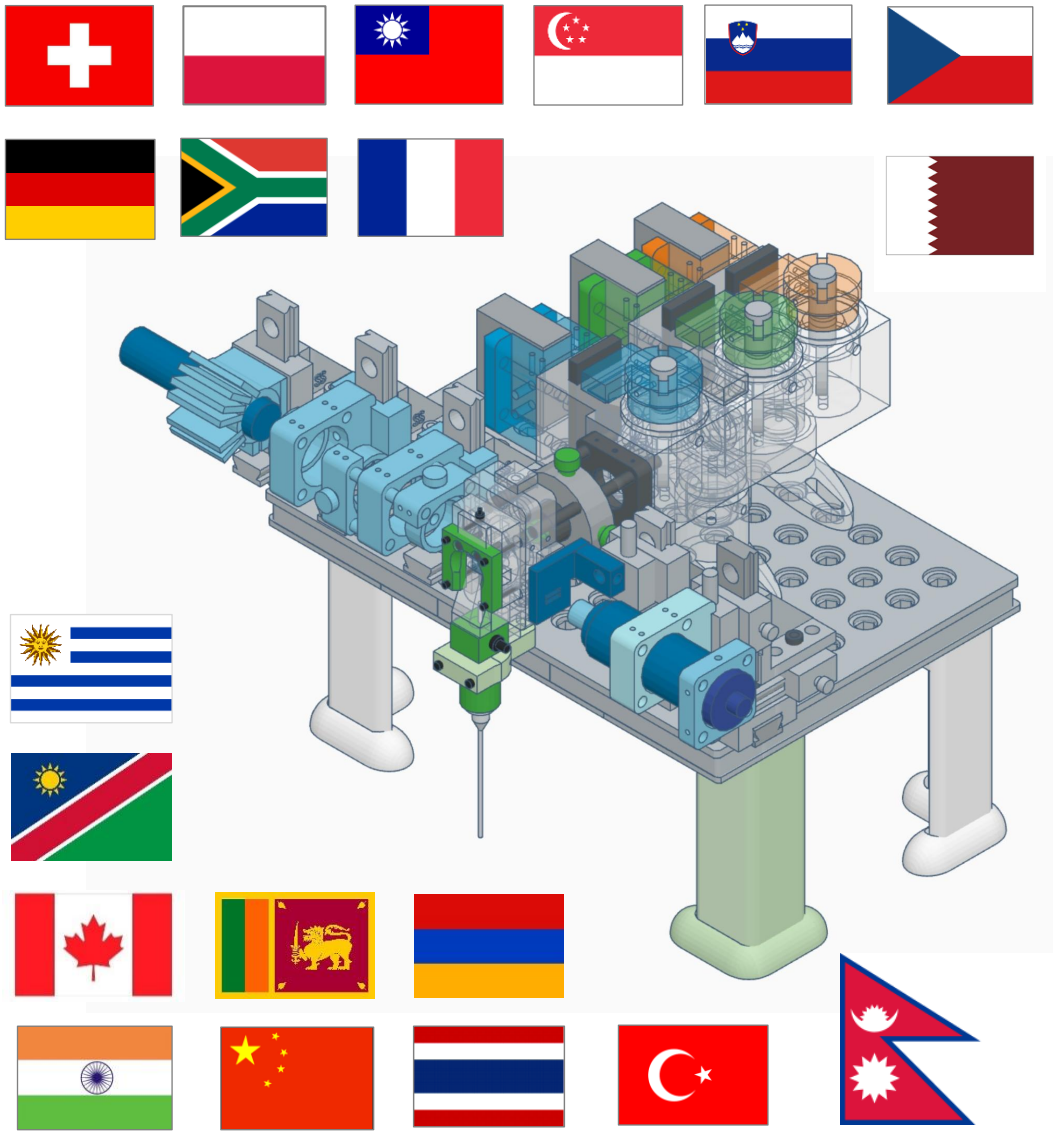
# Kytos acquisition software



# The Make Your Own Flow Cytometer Lab

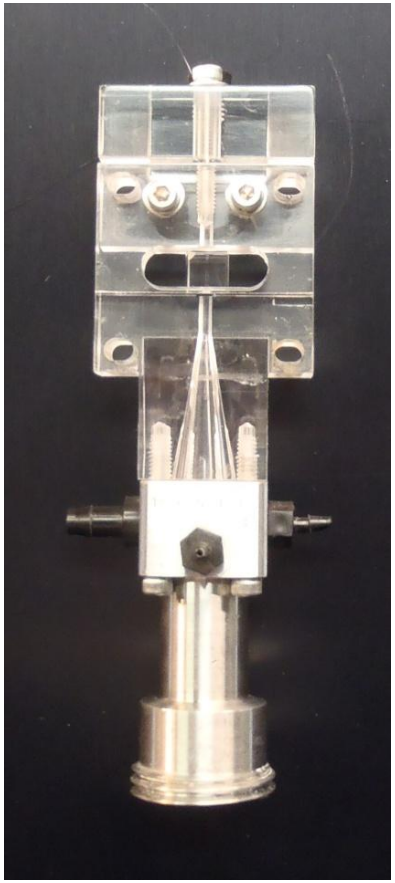
The MYO Lab has been conducted in 20 countries over the last twelve years.

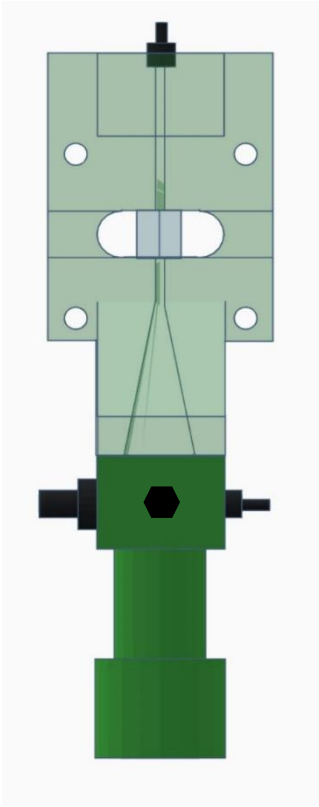
We went virtual during the pandemic but are now live again!

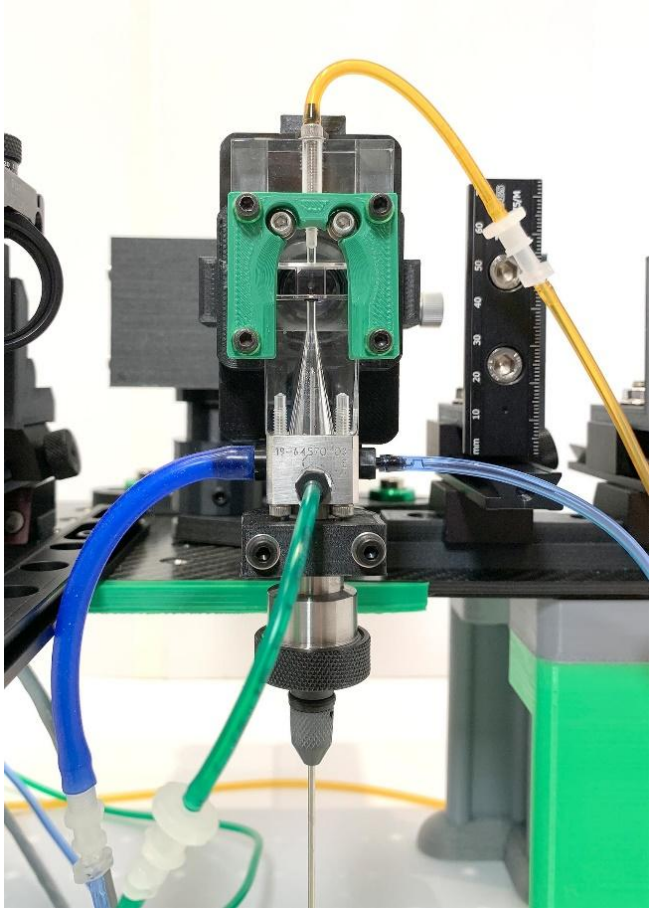


# The flow cell

The flow cell is a 200-micron quartz capillary in a plastic mount. Ports connect the flow cell to sample air pressure, sheath buffer, waste and a vent. A SIP (sample injection port) tube is inserted into the flow cell

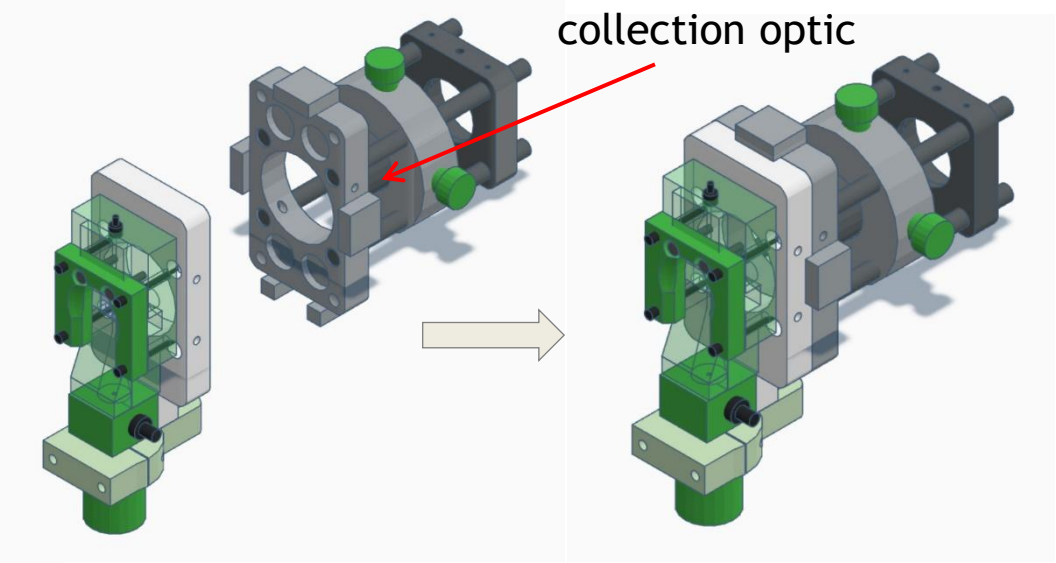


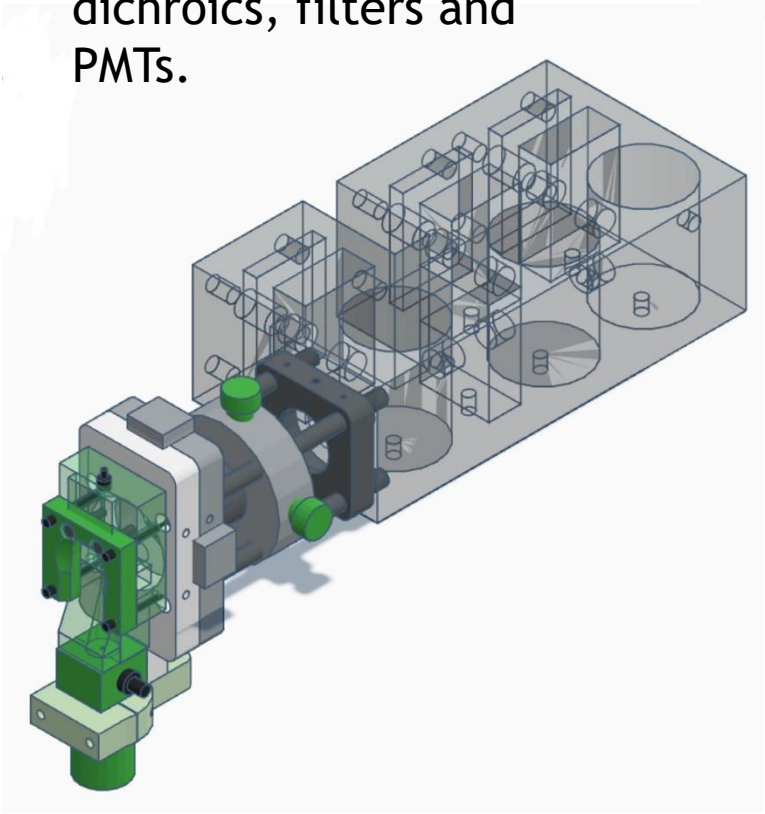


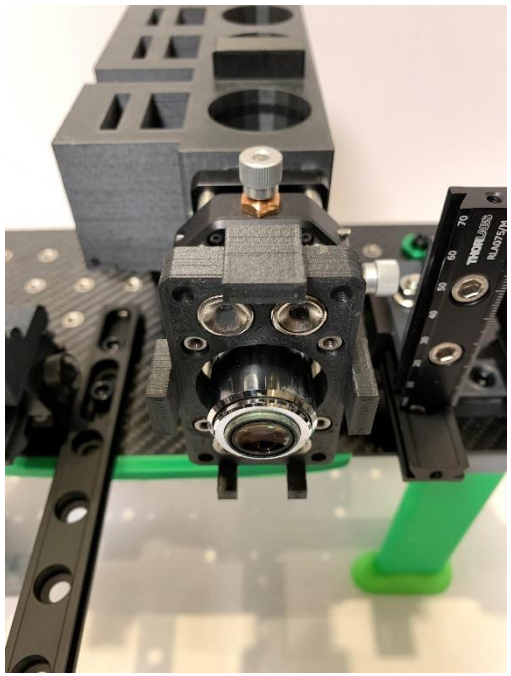


# Installing the flow cell

The flow cell is installed in front of a collection optic or objective that collects side scatter and fluorescence signals and focuses them onto the dichroics, filters and PMTs.



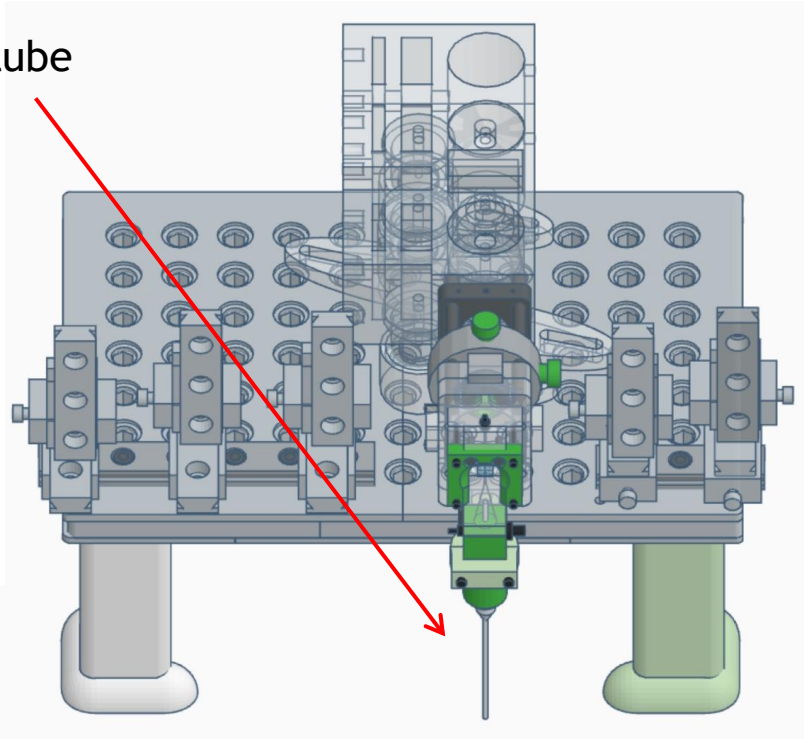



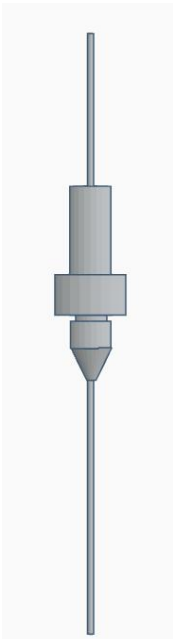


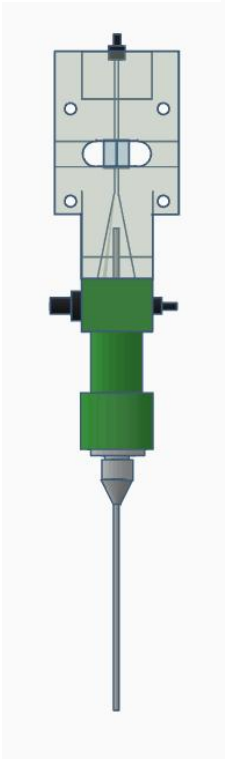
# Installing the SIP tube

We now insert the SIP tube (sample injection port) into the flow cell.

The SIP tube injects sample into the conical sheath reservoir and the flow cell. It has a port to allow the sample tube to be pressurized.





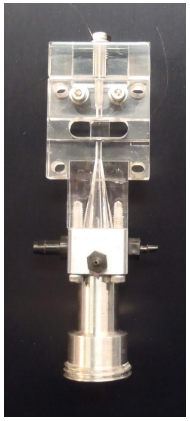
A special O-ring (the BAL seal) seals the sample tube to the SIP tube. This cytometer is a positive pressure system.

# The flow cell

When a sample is placed on the SIP tube, the tube is pressured, and sample is pushed up into the flow cell.

Sheath buffer flows in the conical reservoir below the flow cell and encircles the sample stream.

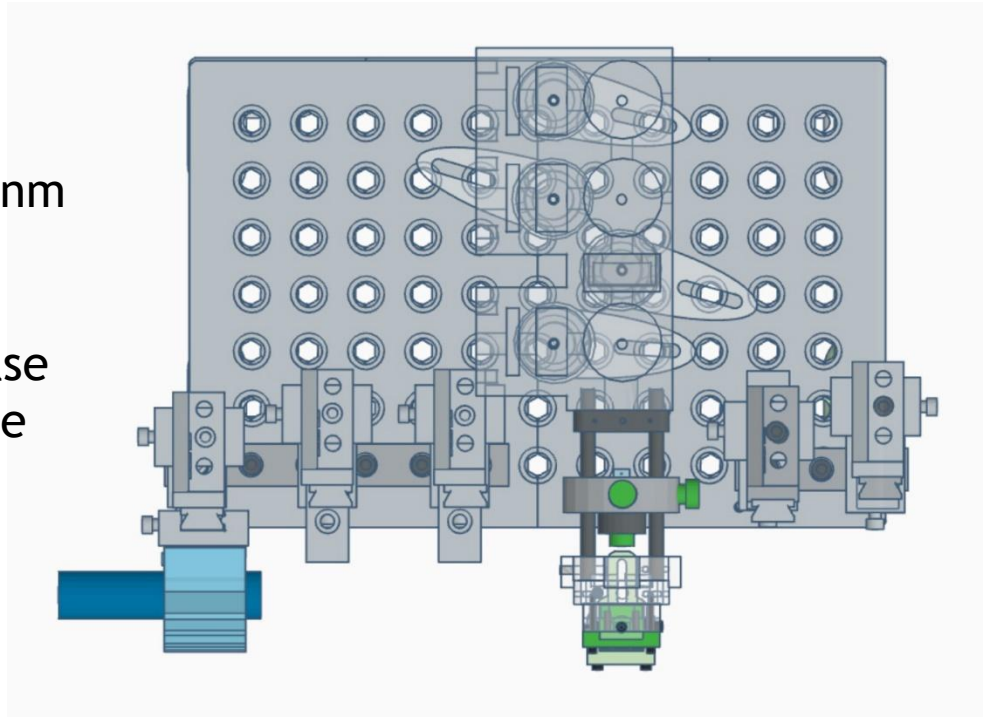
The sheath flow hydrodynamically focuses the sample stream into the center of the flow cell.

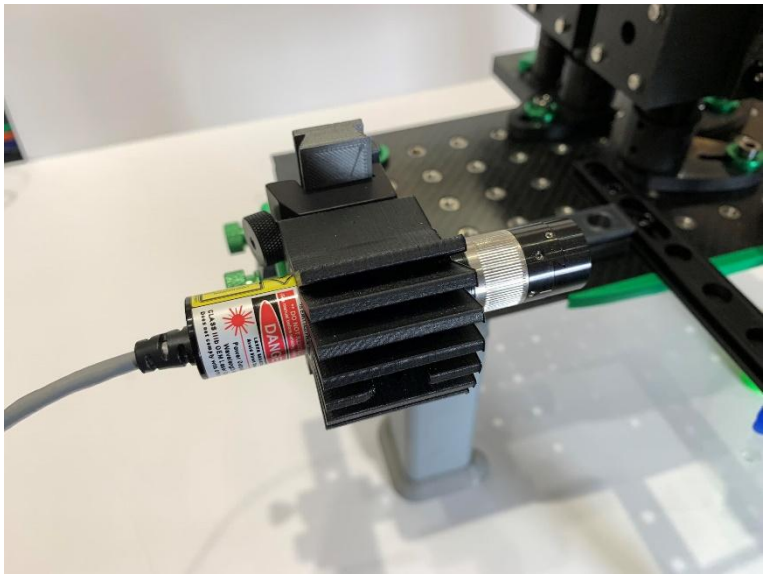


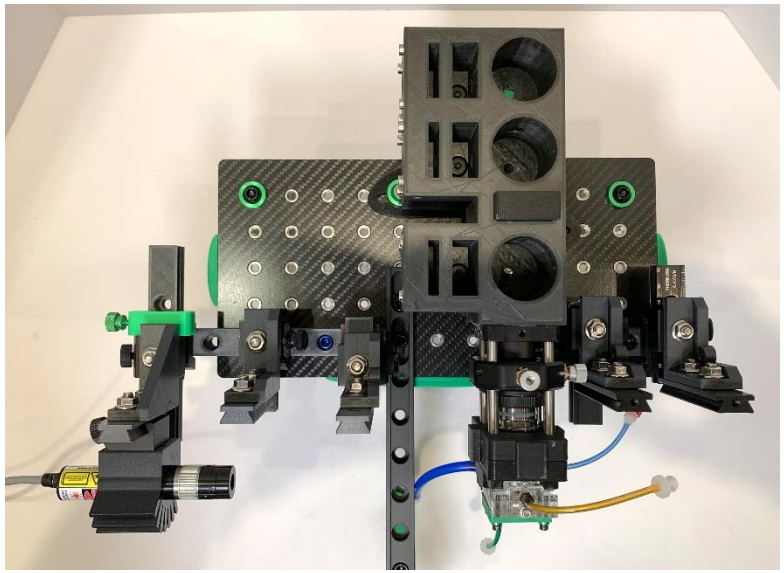
# Installing the laser

The laser is a direct diode 488 nm emitting at 5 mW.

The laser is mounted on the base plate and roughly aligned to the flow cell.



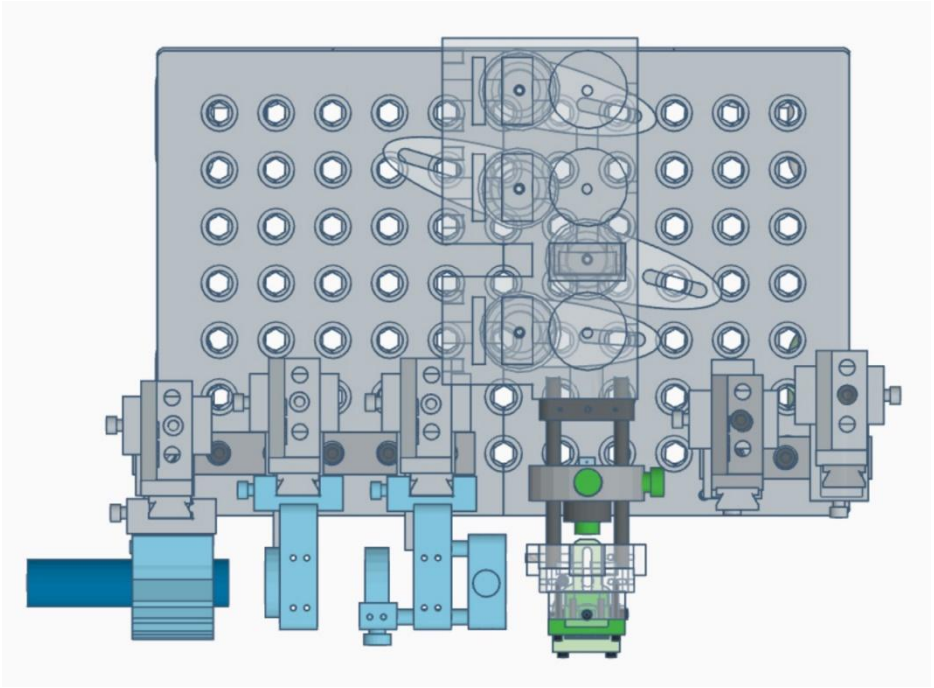


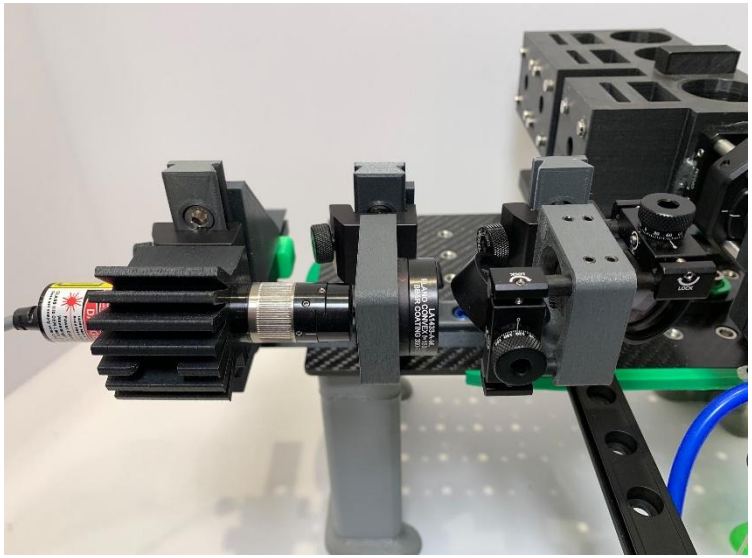


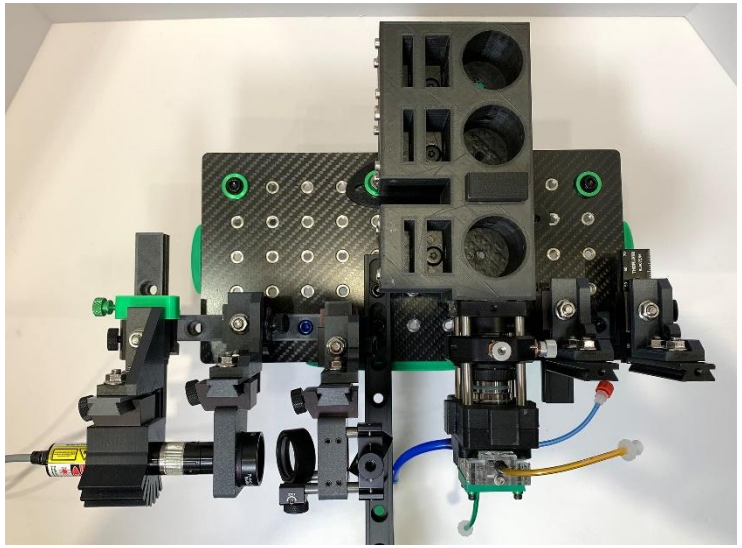
# Laser focusing lens and steering prisms

We use a plano-convex focusing lens to focus the laser beam onto the flow call capillary.  A multi-laser cytometer would use an achromatic laser to focus multiple wavelengths.

We use two flat prisms than rotate up/down or left/right to diffract and translate the laser beam and align it to the flow cell.

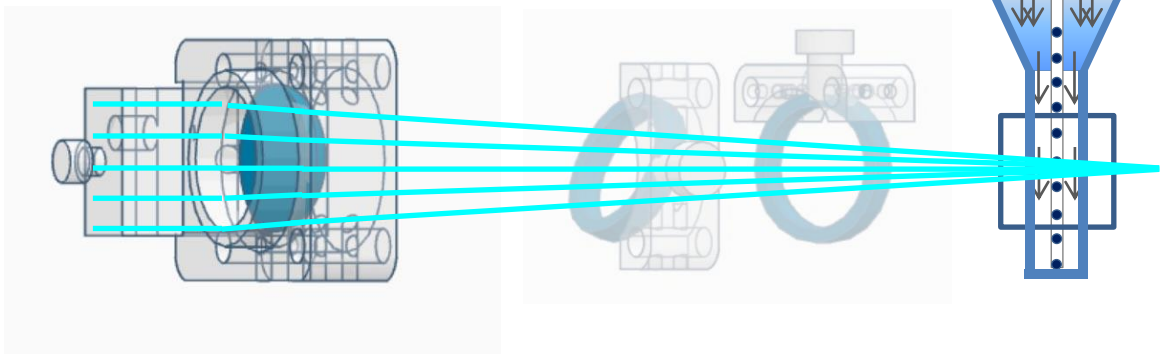


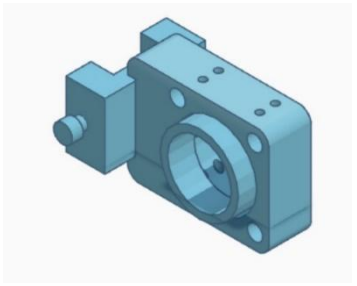


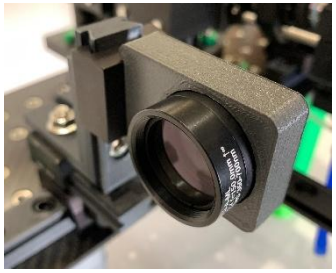


# Laser focusing lens and steering prism

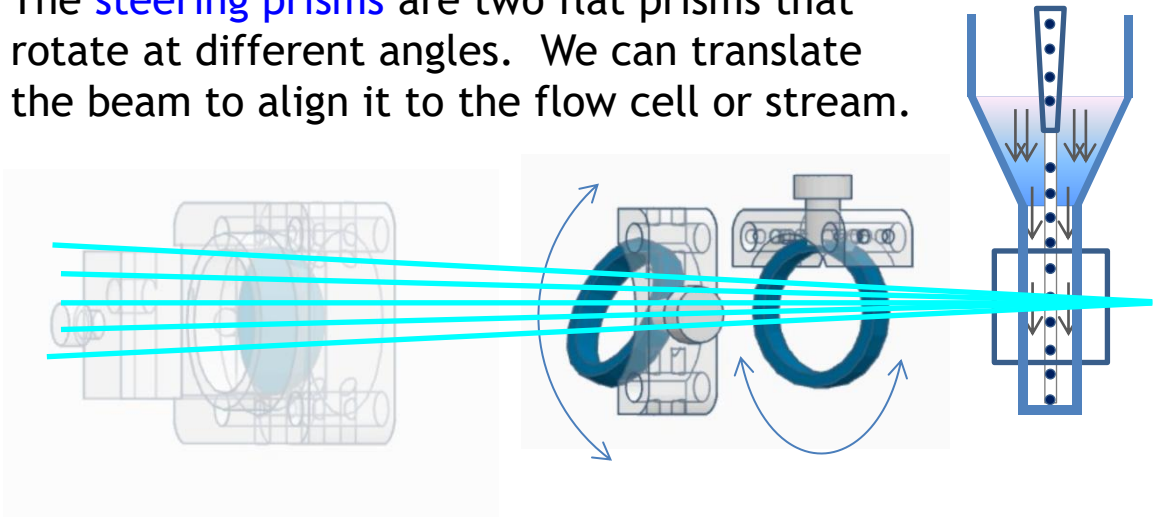
The focusing lens focuses the laser beam to a point indicated by the focal length.

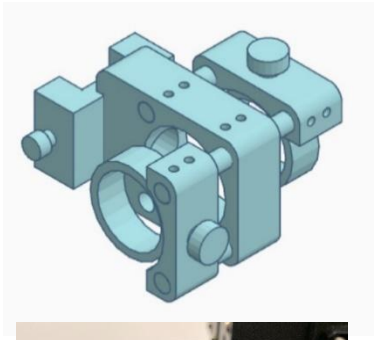


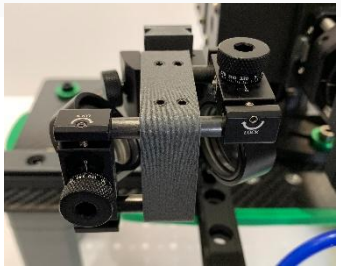




The steering prisms are two flat prisms that rotate at different angles. We can translate the beam to align it to the flow cell or stream.





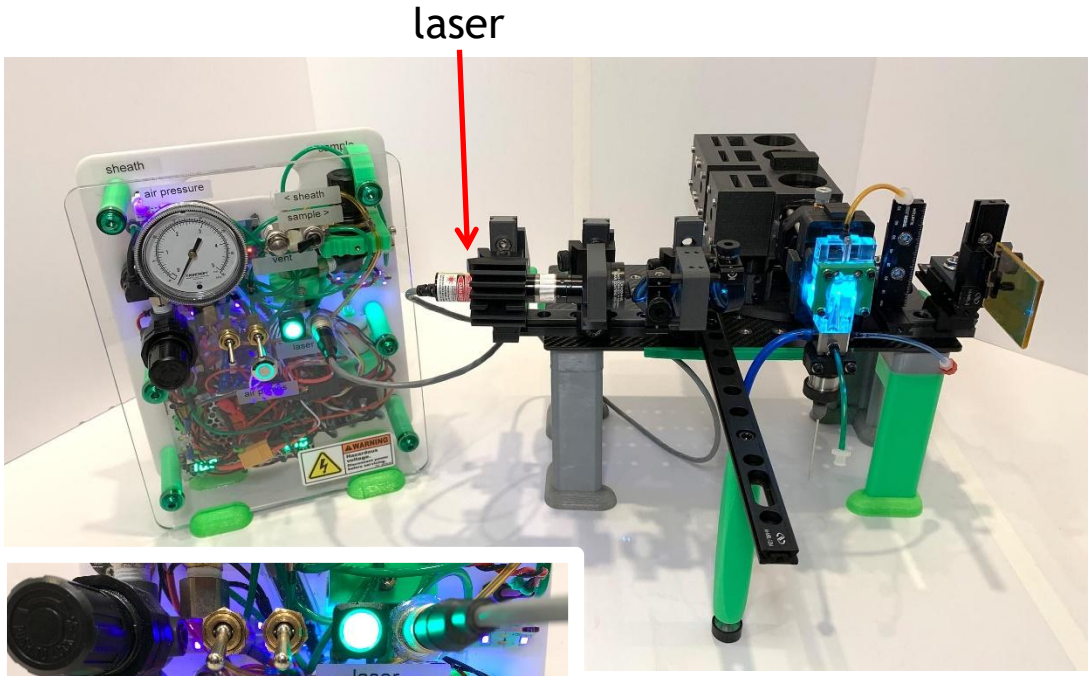


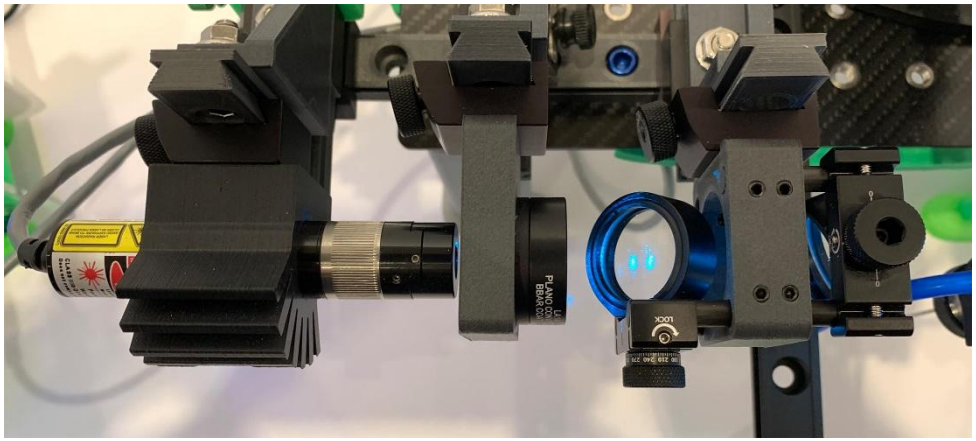
# Turning on the laser

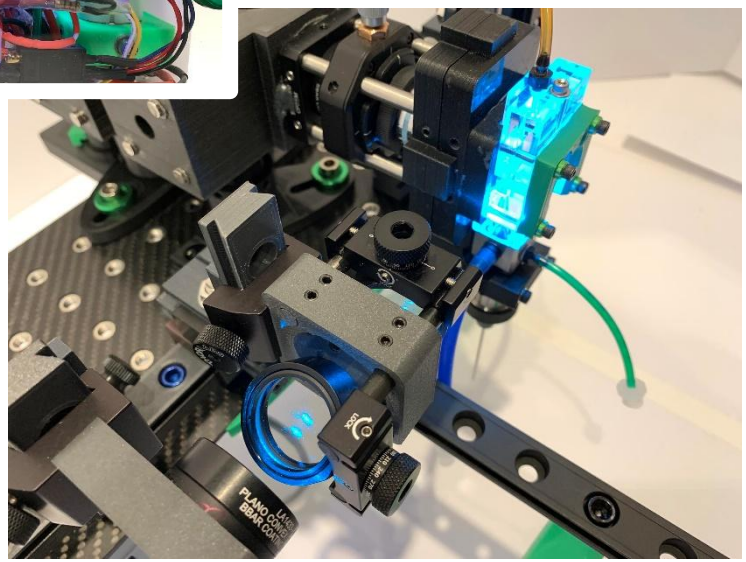
The laser is powered using the fluidics console.

The beam passes through the focusing optic, steering prisms and to the flow cell.

A beam shield is mounted on the other side of the flow cell to “catch” the beam until the FCS detector is installed.

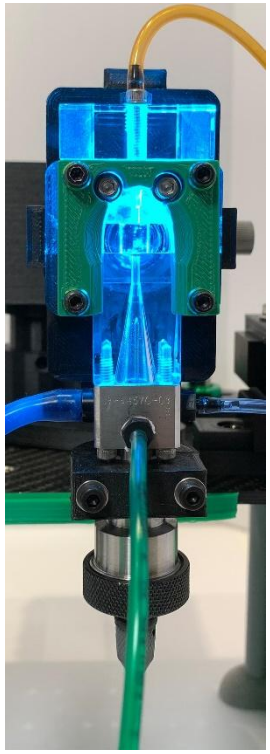


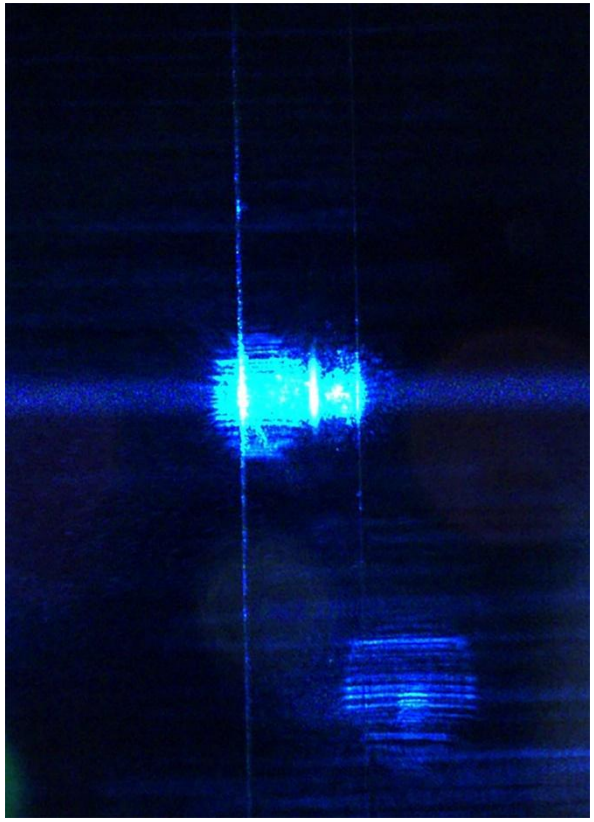



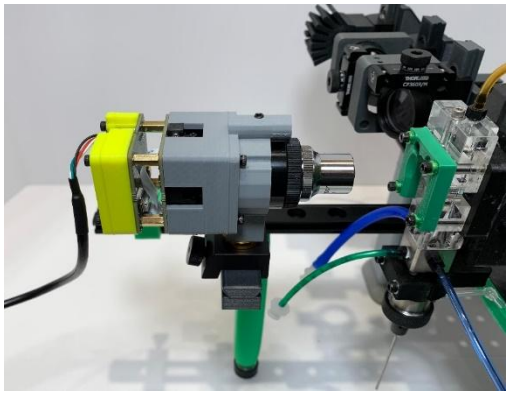


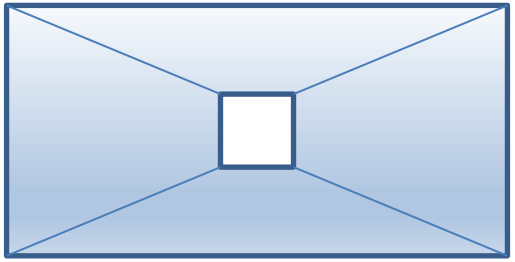
# Visualizing the flow cell

We then use the camera to roughly align the laser to the flow cell.







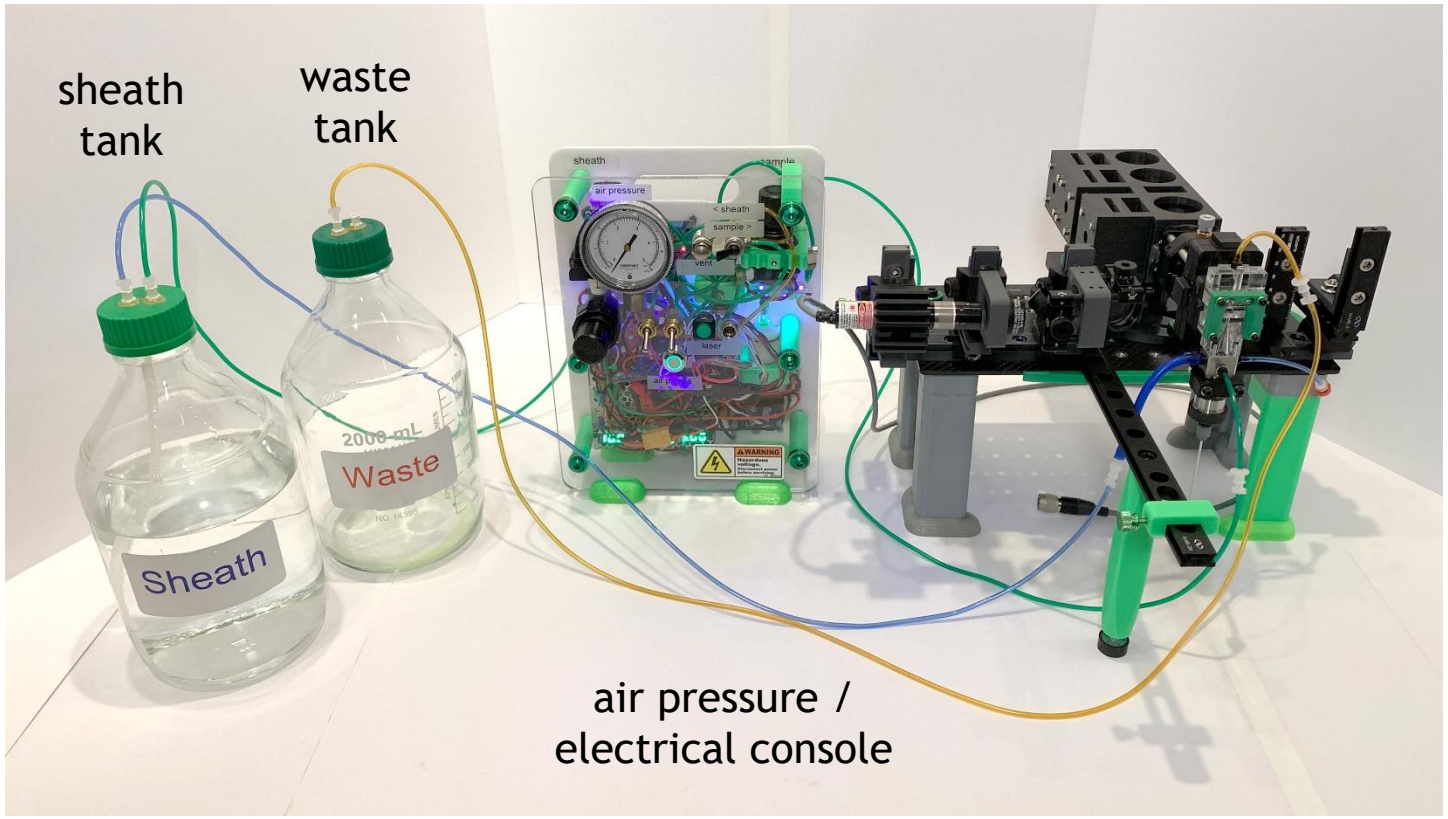


This flow cell is rectangular or square, ~200 µm across.

Most cell types can pass through this space.

# Instrument fluidics

We will then connect the fluidics (or “plumbing”) of the cytometer. The fluidics console provides air pressure for the sheath buffer (which focuses the sample stream) and the cell sample.

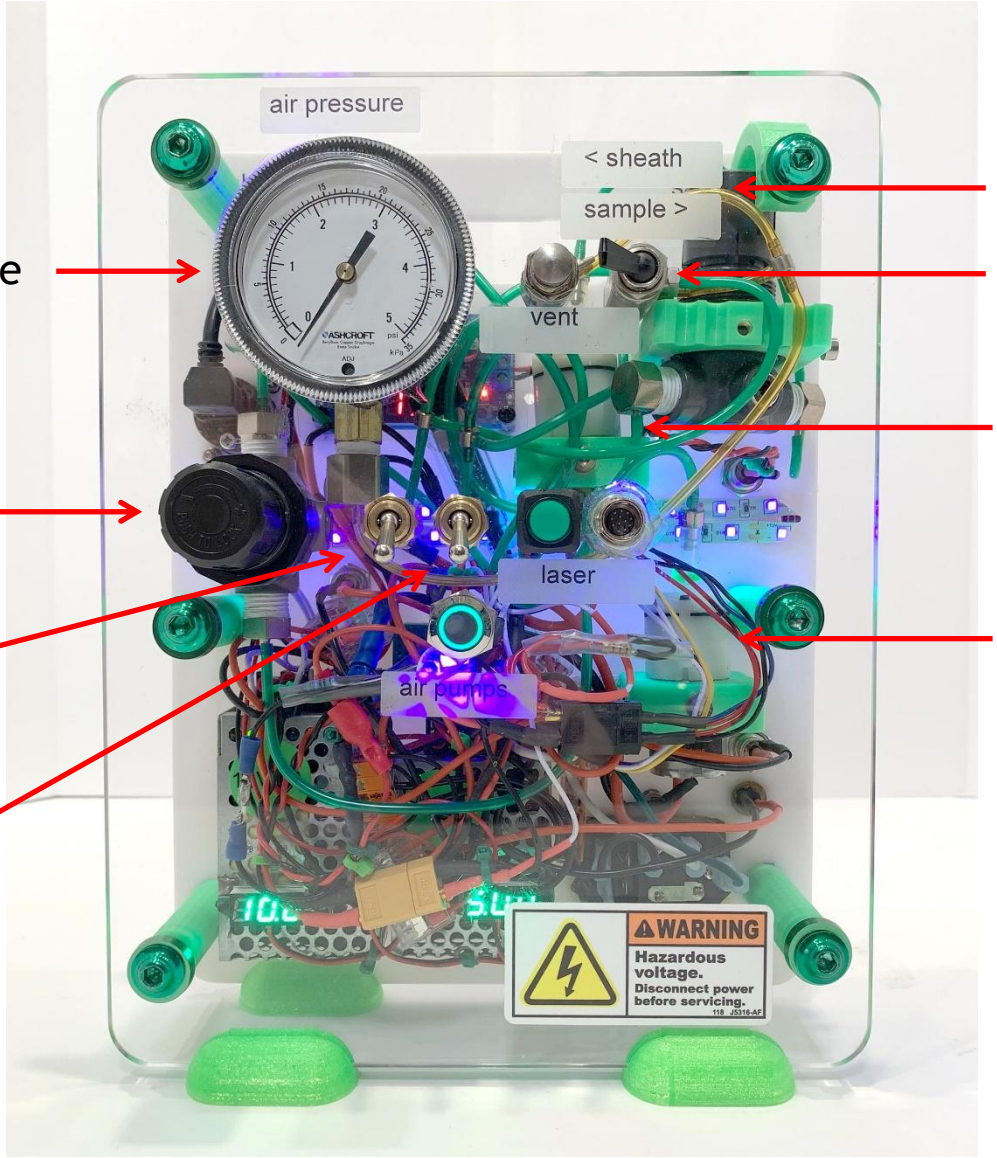



Air lines are green

Sheath lines are blue.

Waste lines are orange

# Instrument fluidics




sheath / sample pressure gauge

sample pressure regulator

sheath ON/ OFF valve

sample ON/ OFF valve

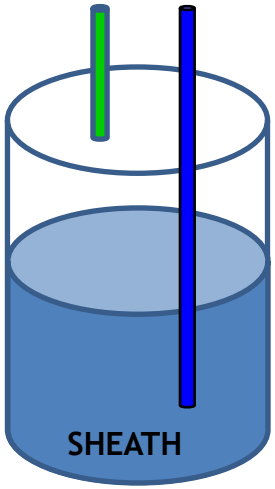
sheath pressure regulator

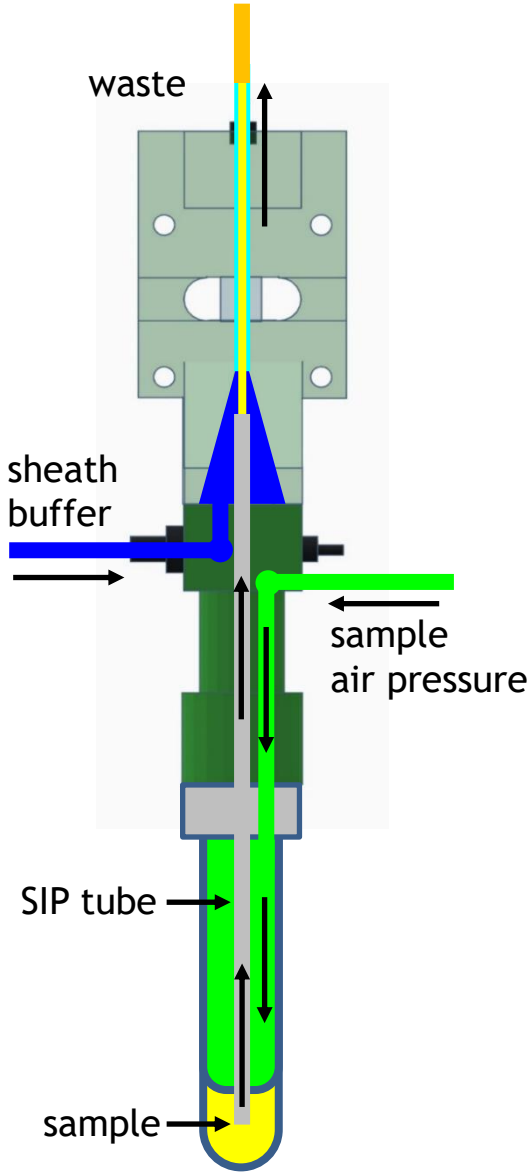
sheath / sample gauge switch

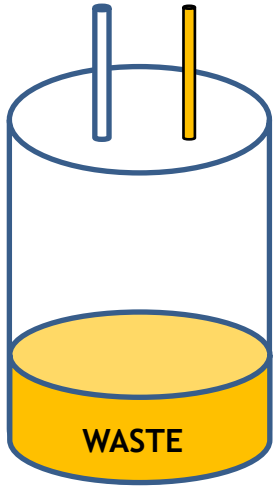
sheath air pump

sample air pump

# Instrument fluidics



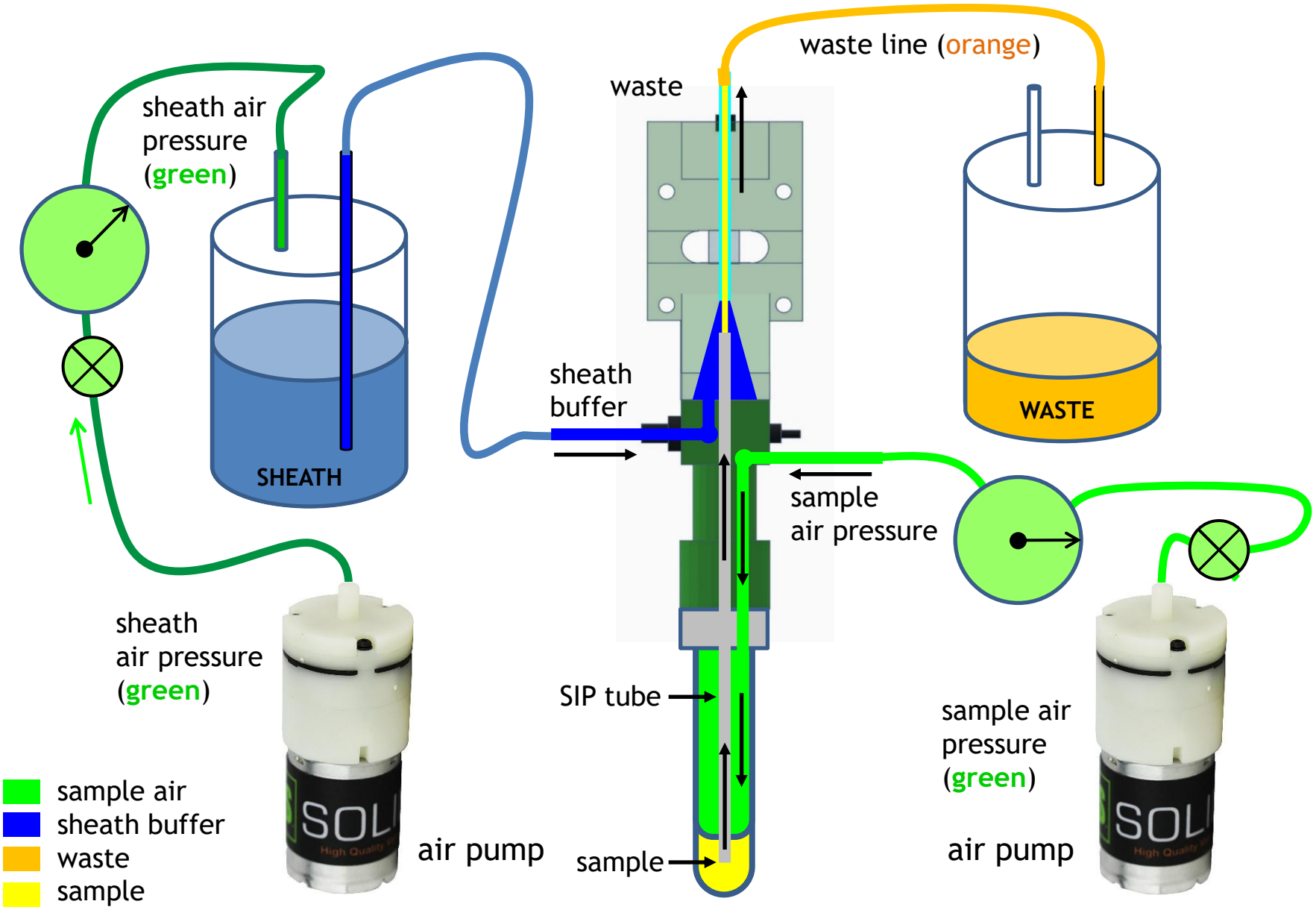




air pump

air pump

sample air
sheath buffer
waste
sample

# Instrument fluidics



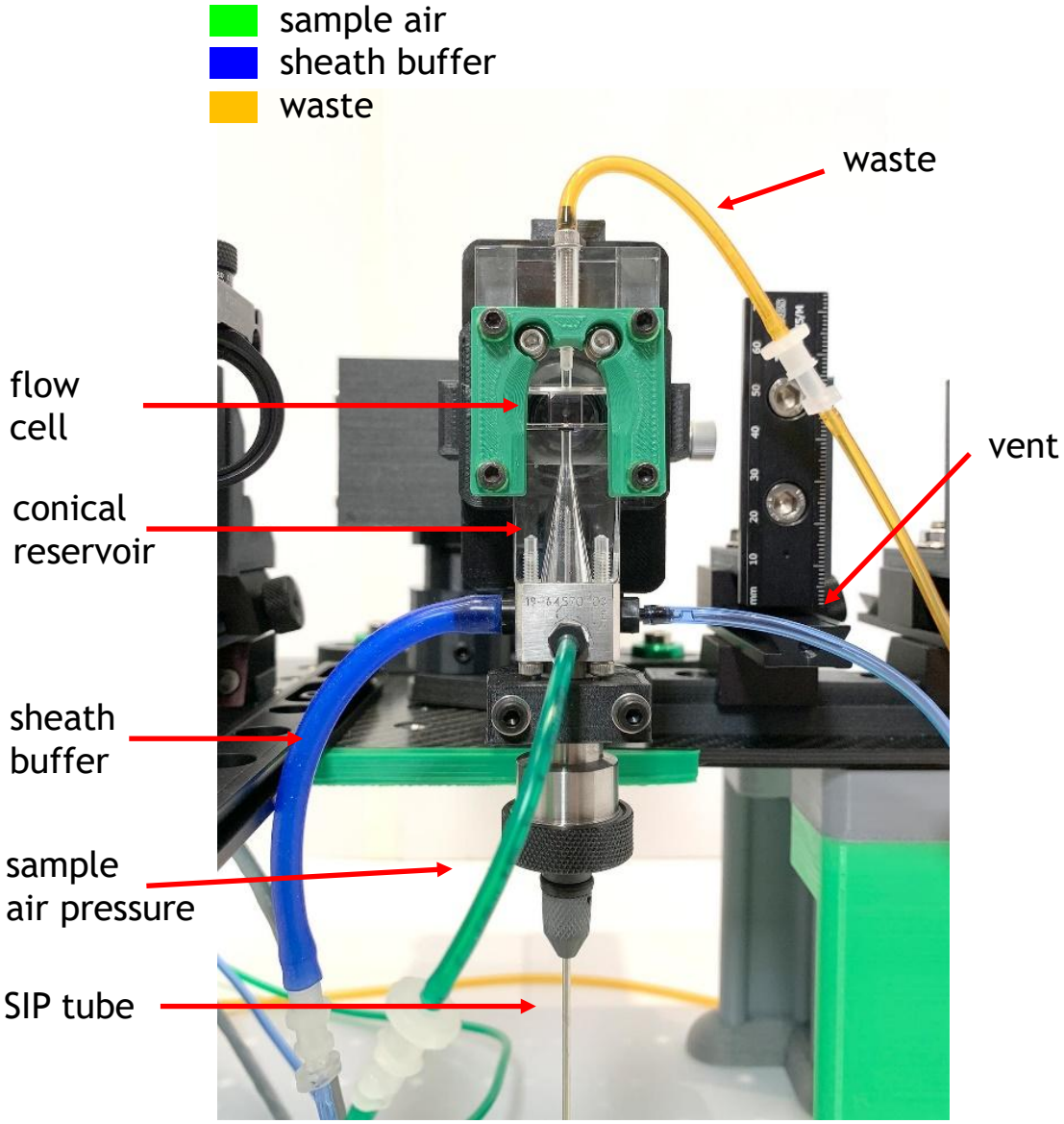
# Fluidics and the flow cell

When a sample is placed on the SIP tube, the tube is pressured, and sample is pushed up into the flow cell.

Sheath buffer flows in the conical reservoir below the flow cell and encircles the sample stream.

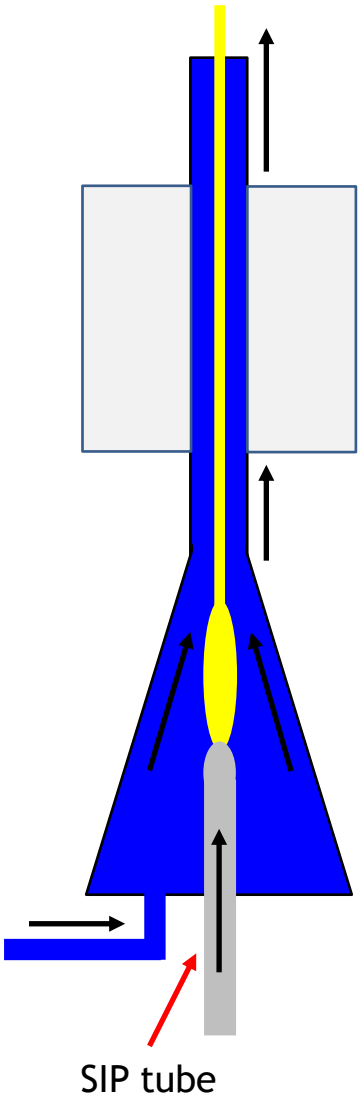
The sheath flow hydrodynamically focuses the sample stream into the center of the flow cell.

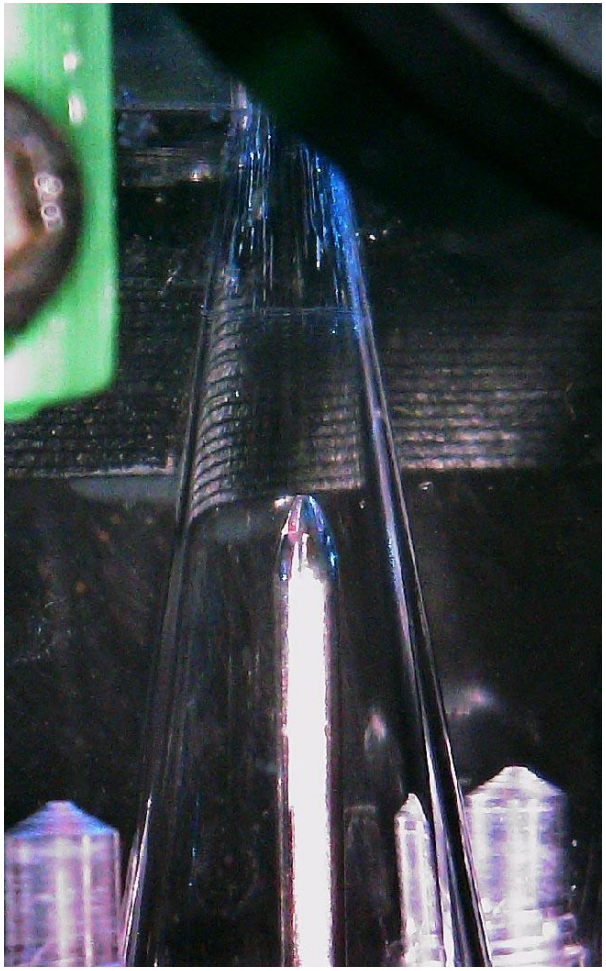
Sample pressure is always slightly higher than sheath pressure.



# Injecting sample stream into sheath flow

Using a special camera aimed at the conical reservoir, can see the sample stream containing fluorescent beads leaving the SIP tube and being injected into the sheath flow. The stream gets narrower and narrower until it is too thin to see. It then enters the flow cell *hydrodynamically focused*.



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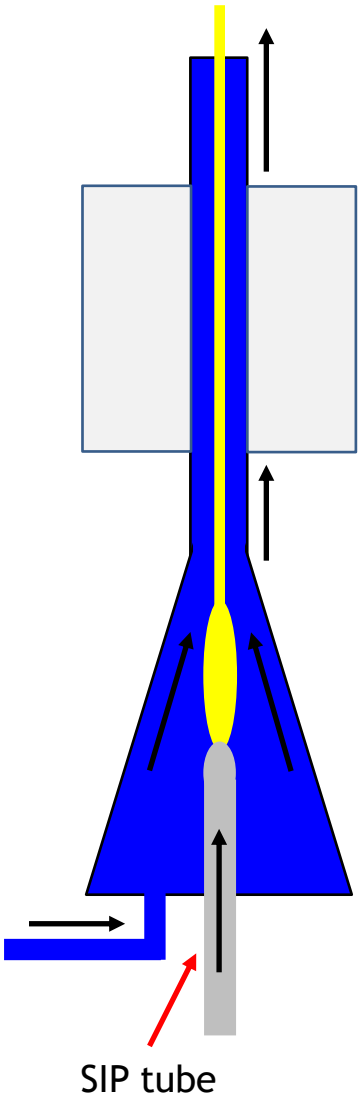
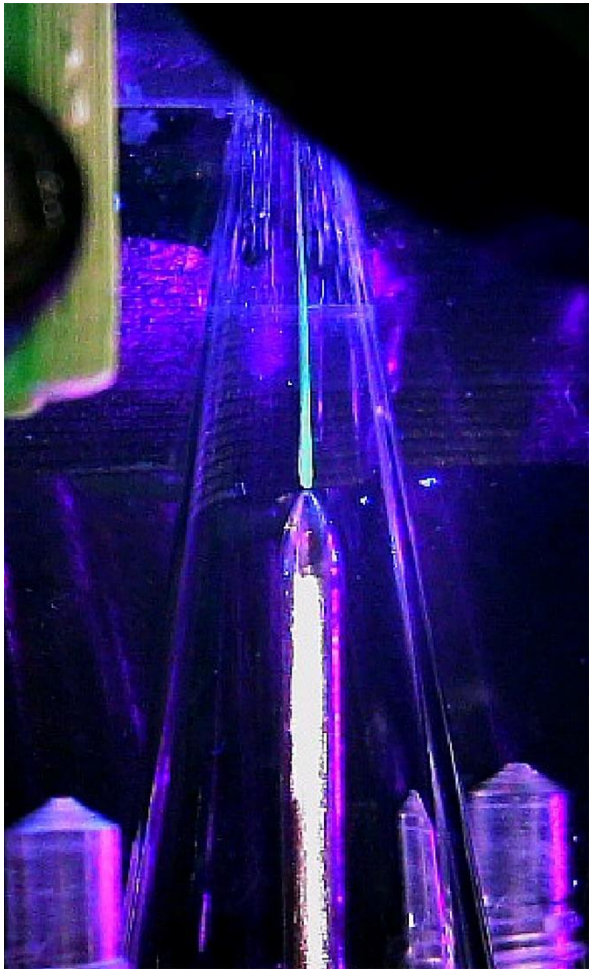
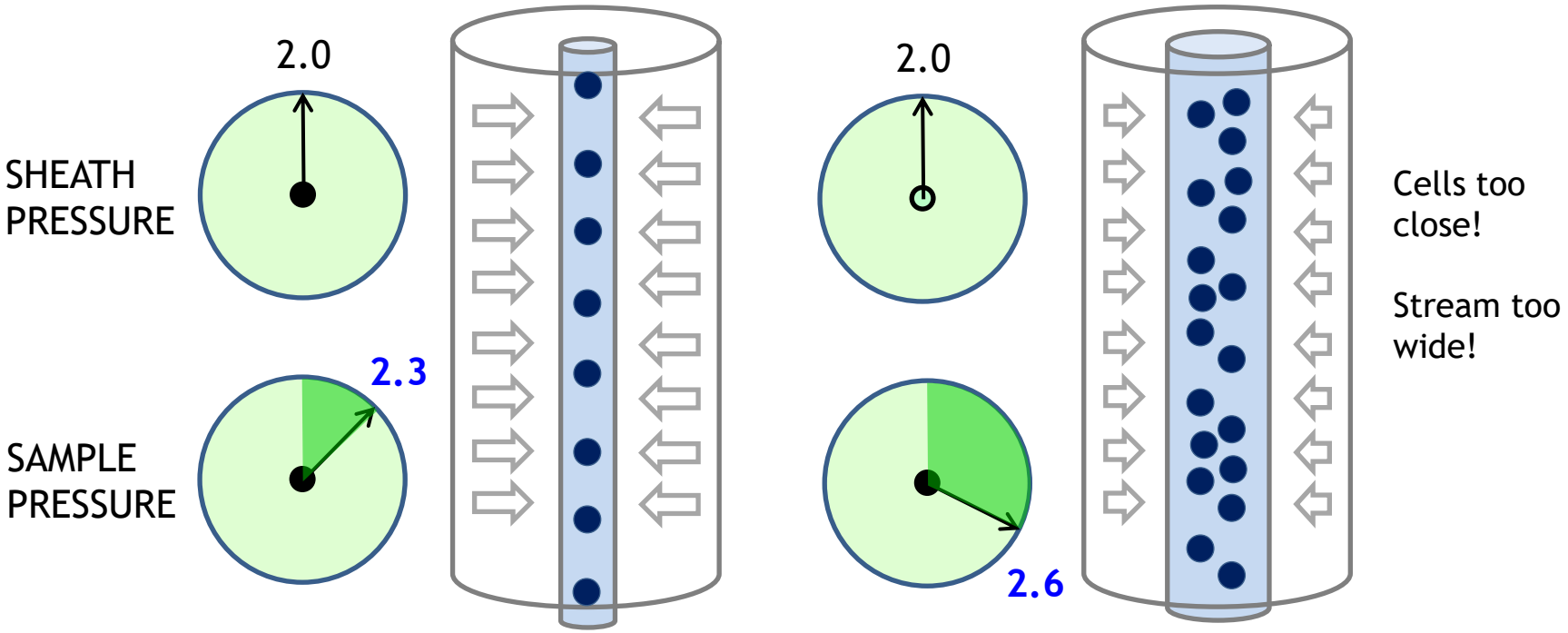


# Sheath versus sample pressure

Normally, we keep the sheath pressure FIXED, and adjust the sample pressure (sometimes called the sample differential). Sample pressure should be HIGHER than sheath pressure, or the sample won't inject into the sheath flow.

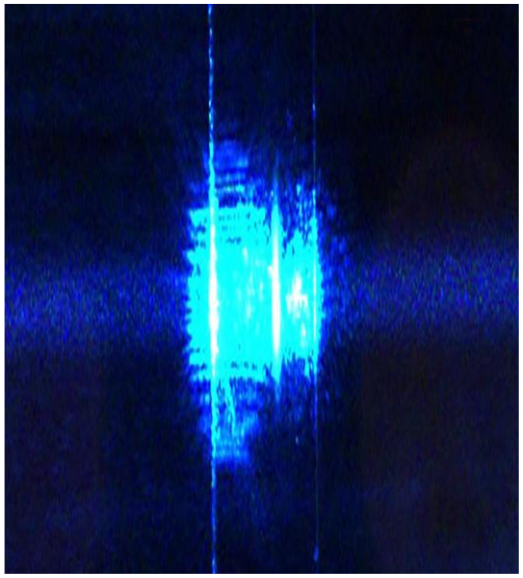



BUT we try to keep the sample pressure as low as possible. This keeps the sample stream narrow, so it is completely intercepted by the laser beam. It also minimizes the possibility of more than one cell from passing through the laser at once (coincidence).

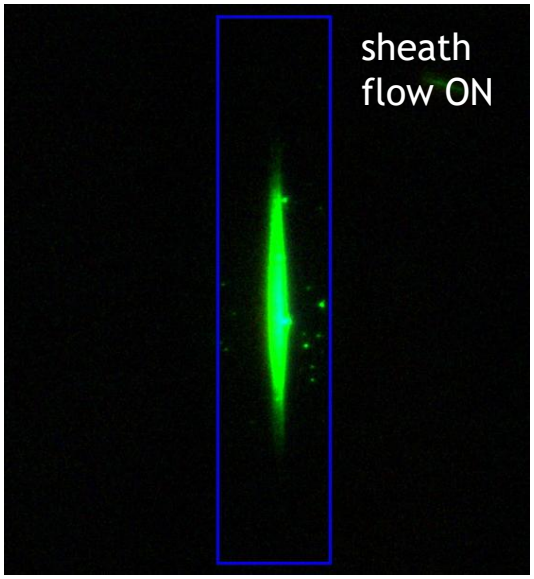
# The importance of sheath pressure

We use fluorescent beads as cell substitutes to test our instruments and align them. We also use them for daily instrument quality control.

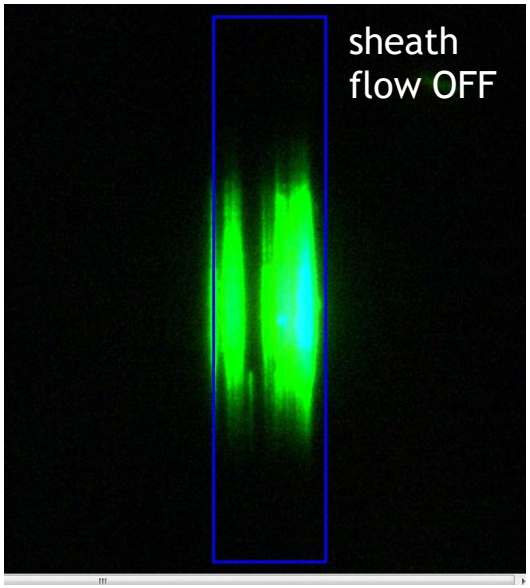
This is the upper camera image of the flow cell cuvette illuminated with the laser.



We now run some green fluorescent beads with sheath buffer ON. They remain confined to the center of the flow cell.




If we turn the sheath pressure OFF, the beads are no longer confined, but fill the entire flow cell.

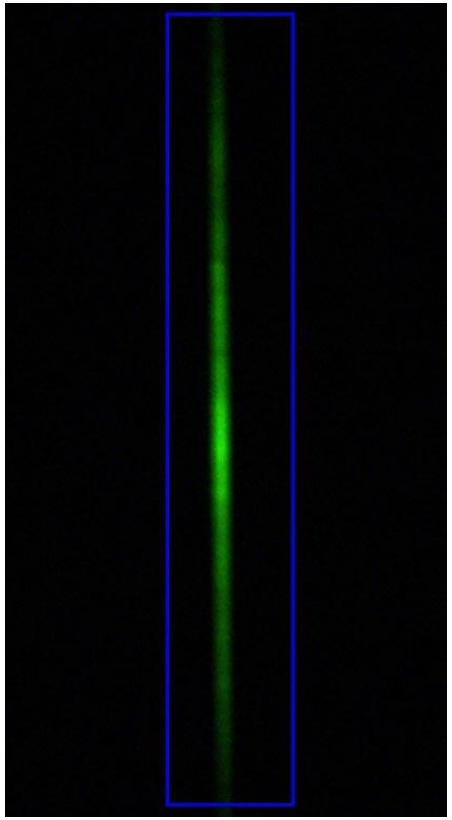



Sheath flow keeps the cells right in the laser beam, allowing accurate scatter and fluorescent measurements. Almost all flow cytometers use a sheath system.

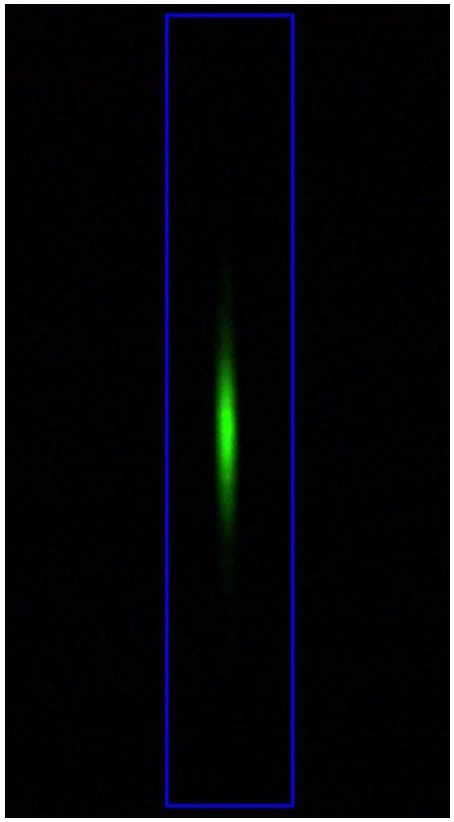
# Focusing the laser beaam

The focusing optic is used to focus the laser beam to a tight spot intercepting the stream. Without focusing, we do cannot concentrate enough laser energy onto the sample stream or resolve single cells.

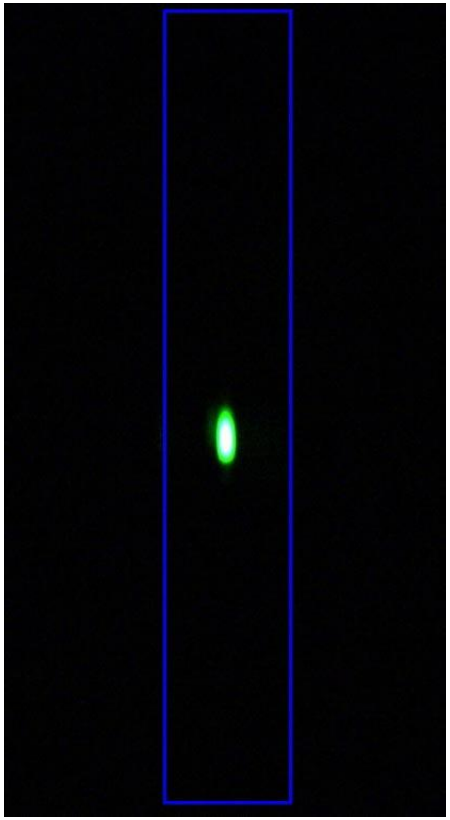
No focusing optic



With focusing optic but beam not focused

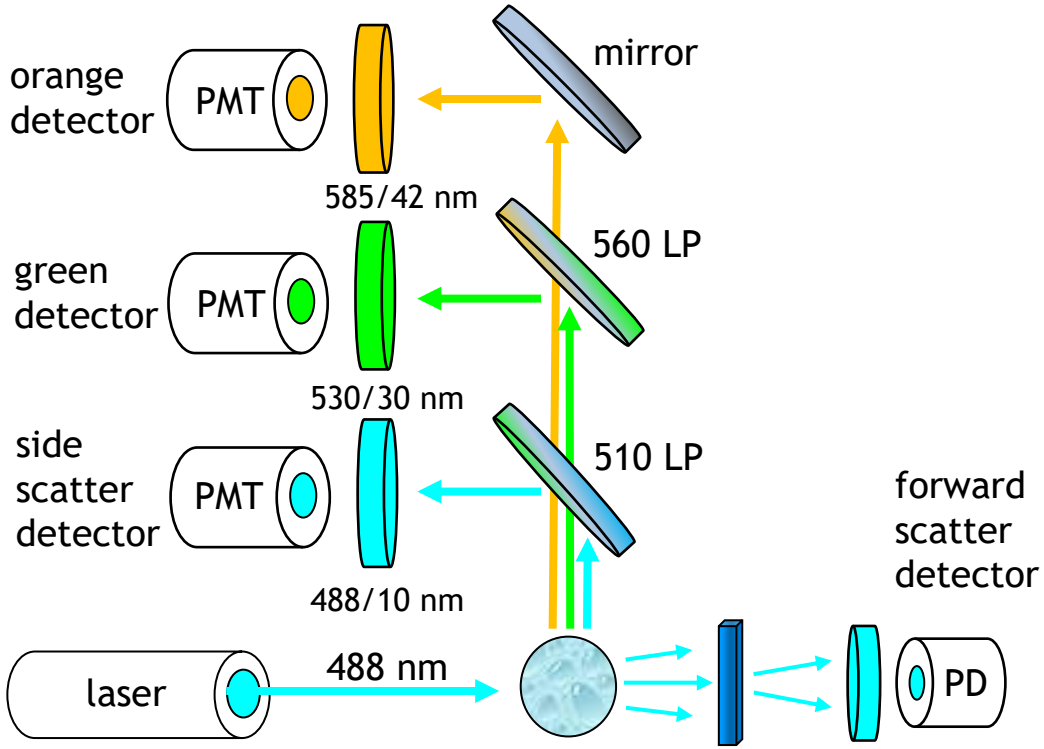


Laser beam properly focused



# Optical layout

This optical layout allows the detection of four signals… forward scatter, side scatter, green fluorescence and orange fluorescence.



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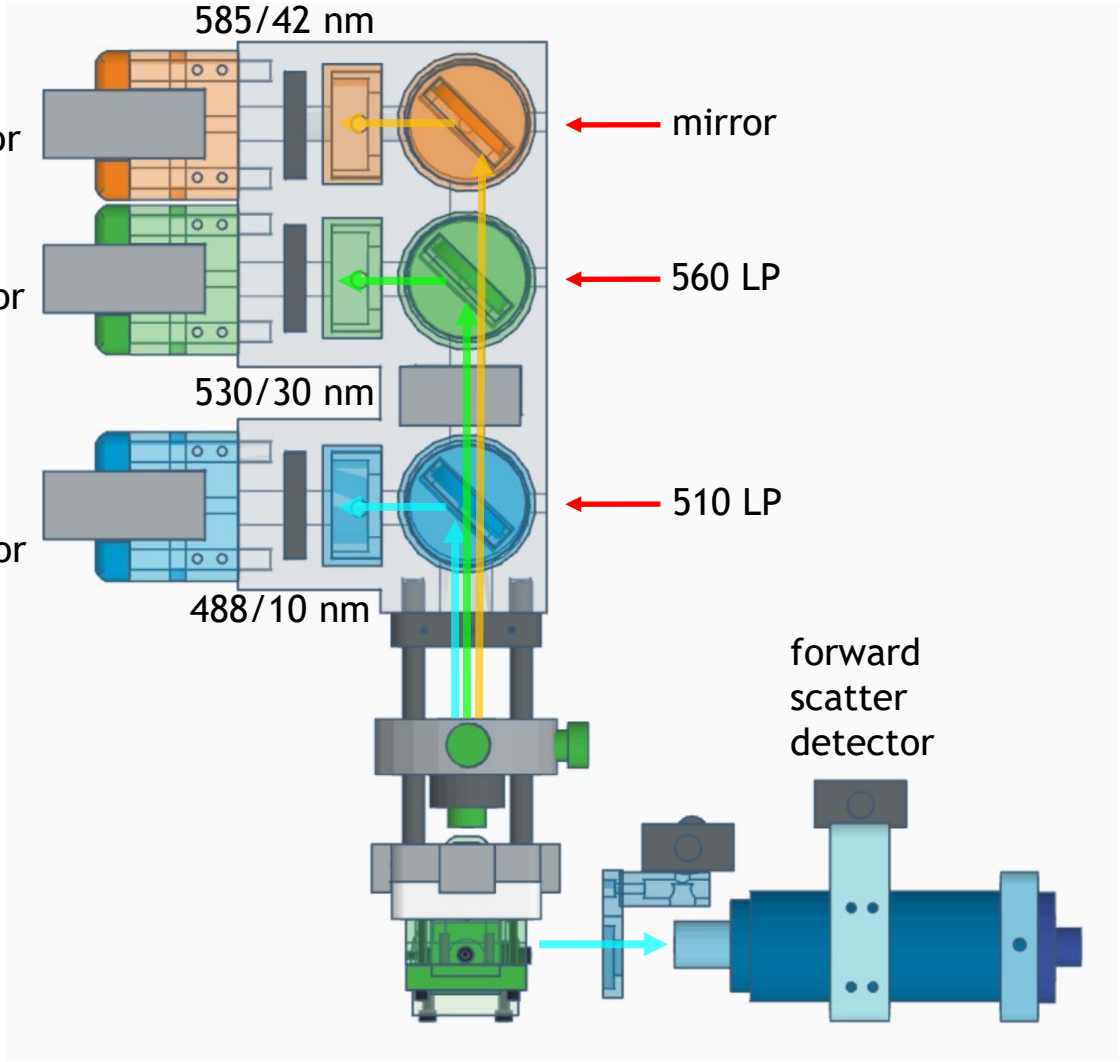
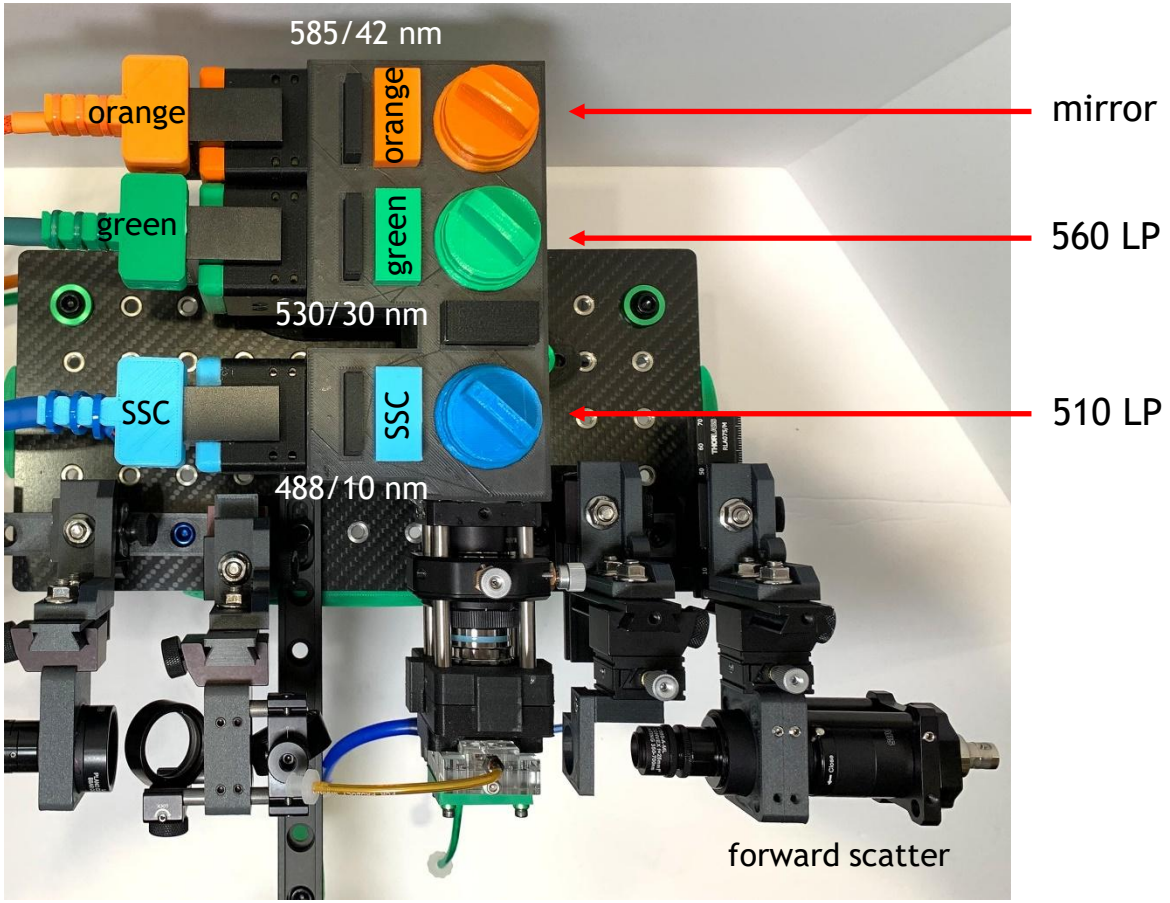


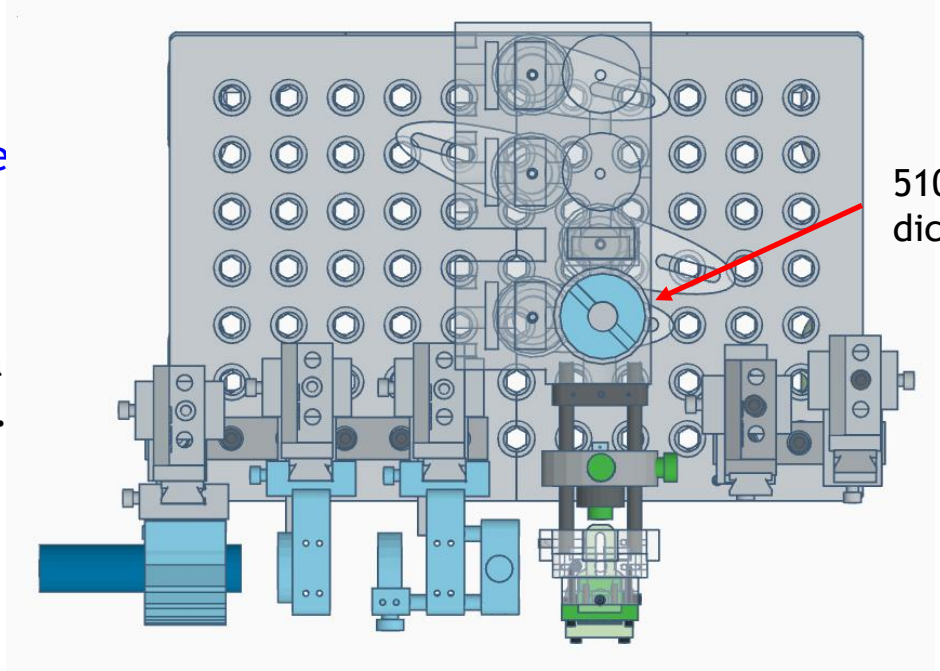
# Optical layout

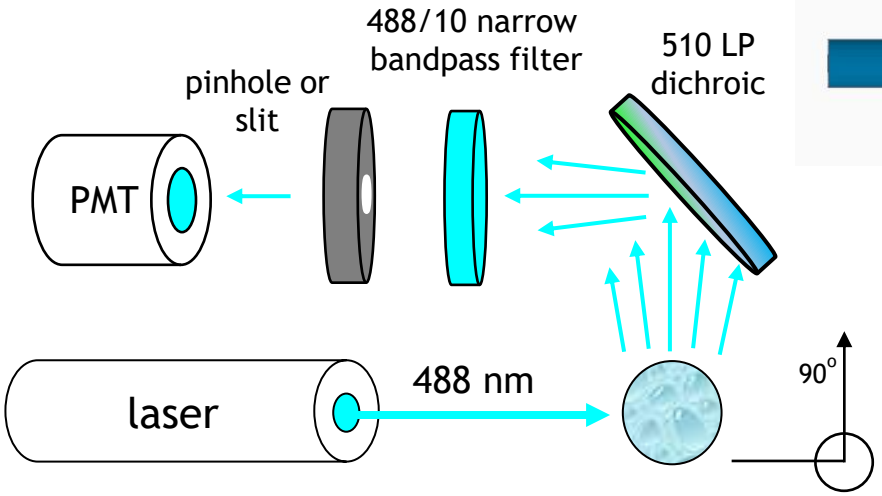
This optical layout allows the detection of four signals… forward scatter, side scatter, green fluorescence and orange fluorescence.

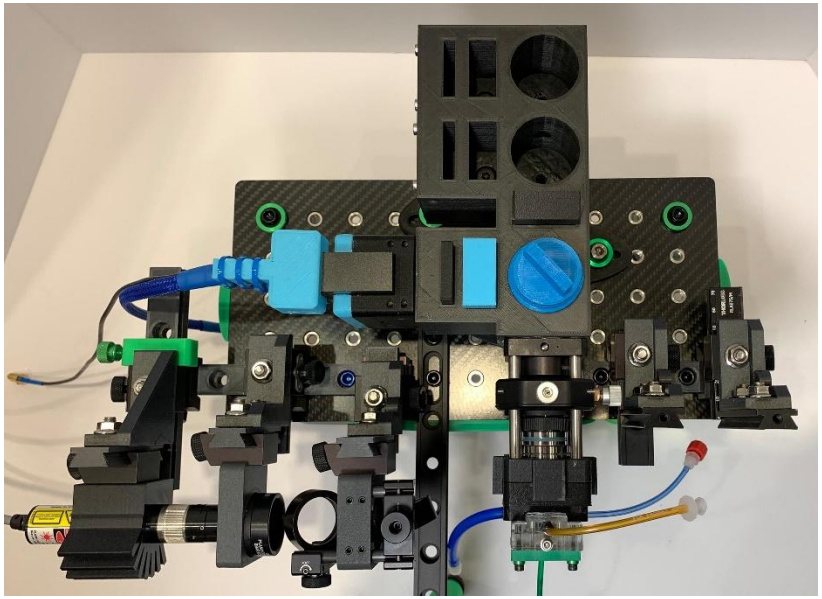


# Side scatter detector

We first install our first dichroic for the side scatter position. Since we are detecting 488 nm laser light, we use a 510 nm long pass dichroic mirror to reflect the signal to the detector and a 488/10 nm filter to clean up the signal.



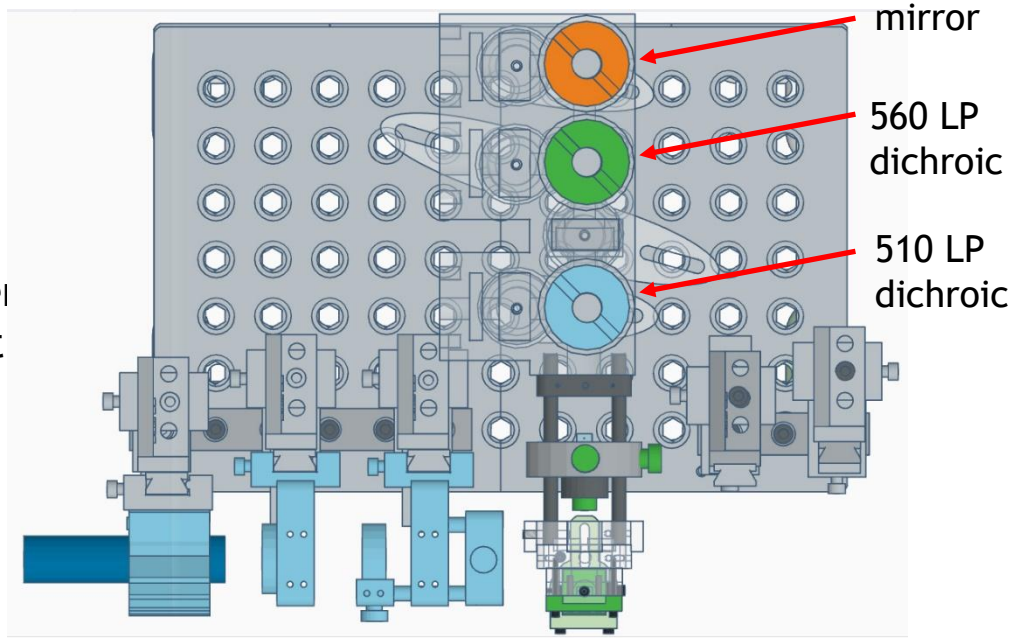



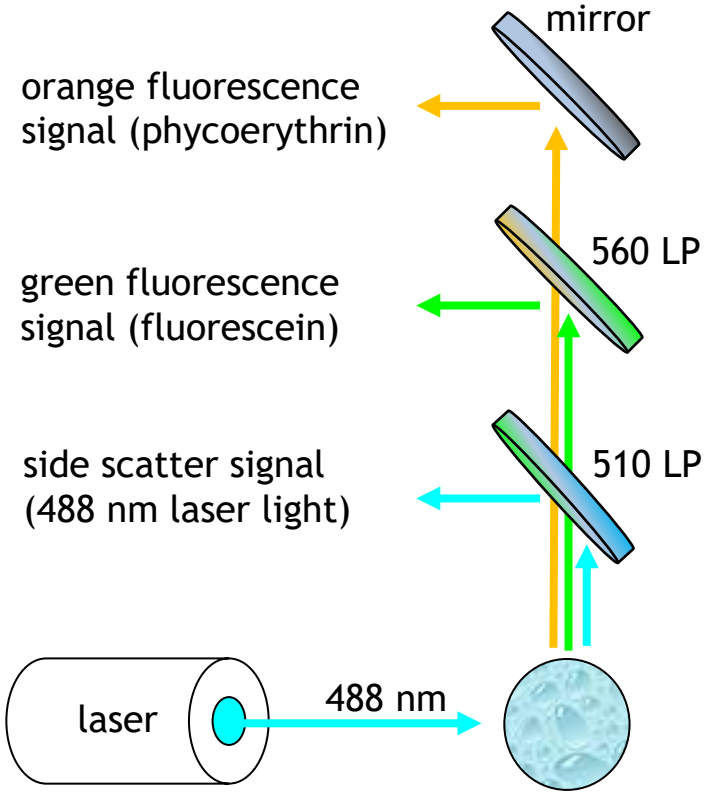



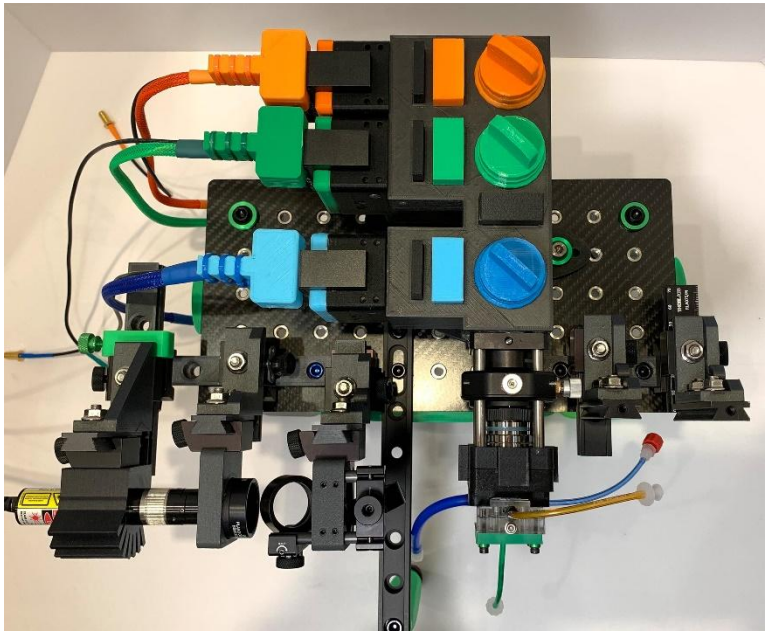
Remember that **side scatter** (SSC) is laser light scattered at 90 degrees to the direction of the incoming laser. It is roughly a measure of cellular optical density. It is usually detected with a special low sensitivity PMT.

# Dichroics

We then install our second dichroic for the green fluorescence detector and a mirror for our orange detector. A 560 nm long pass dichroic reflects the green signal, and a 100% mirror reflects what is left (orange and above).

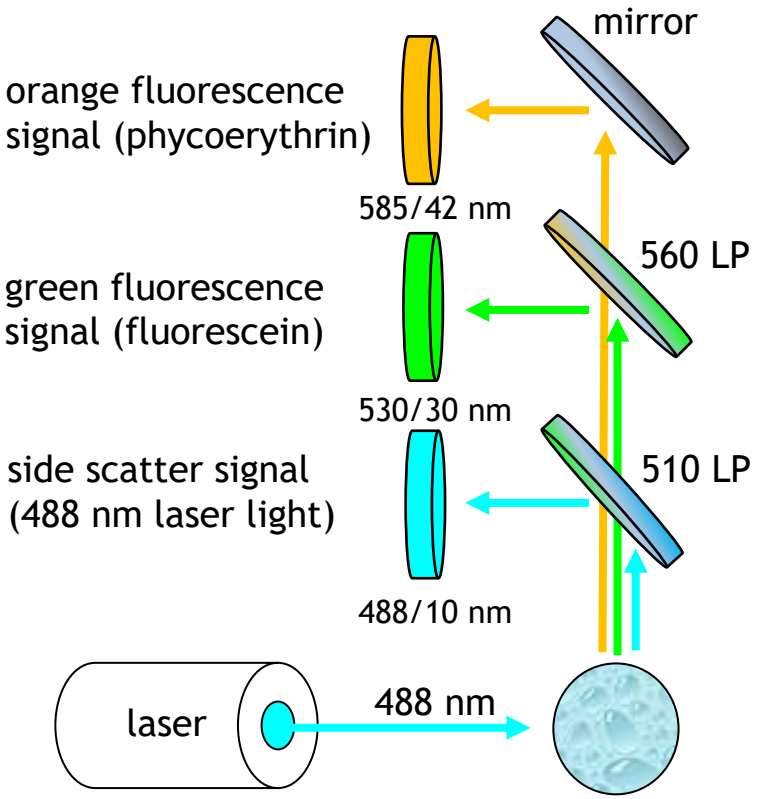


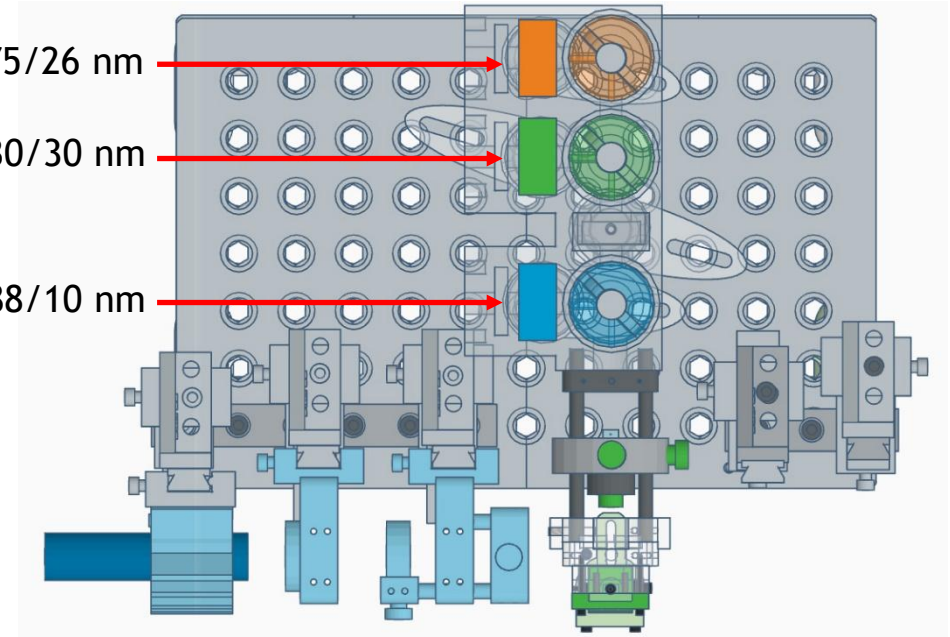



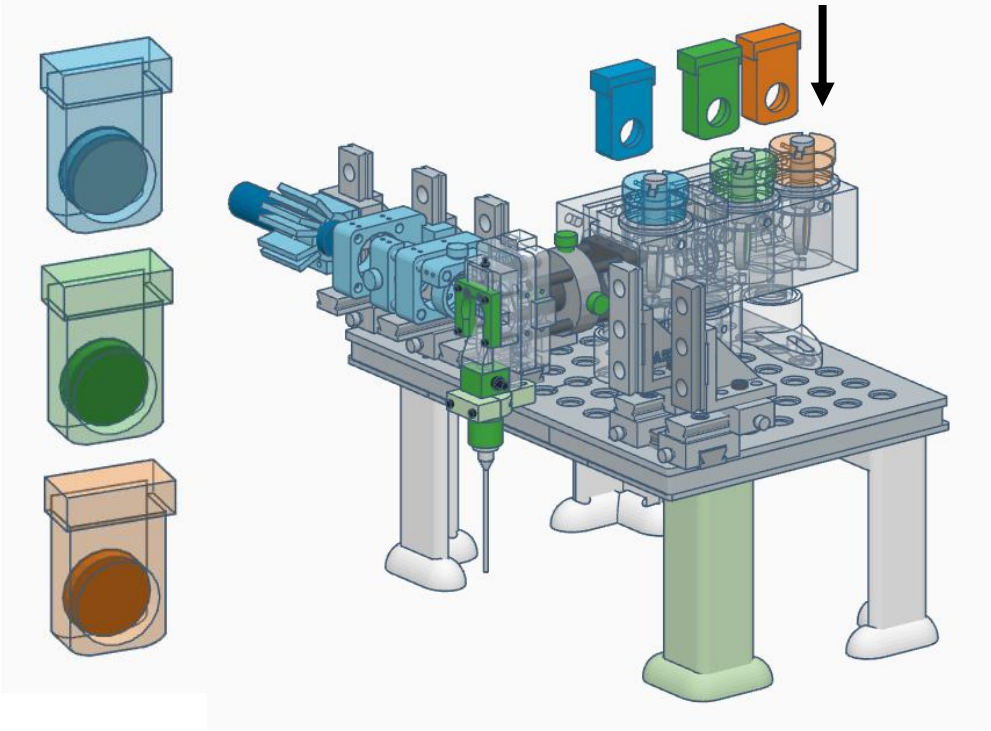



# Bandpass filters

We then install bandpass filters for side scatter, green and orange fluorescence. Side scatter is a 488/10 nm (or nothing), green fluorescence is 530/30 nm, and orange fluorescence is 572/26 nm.



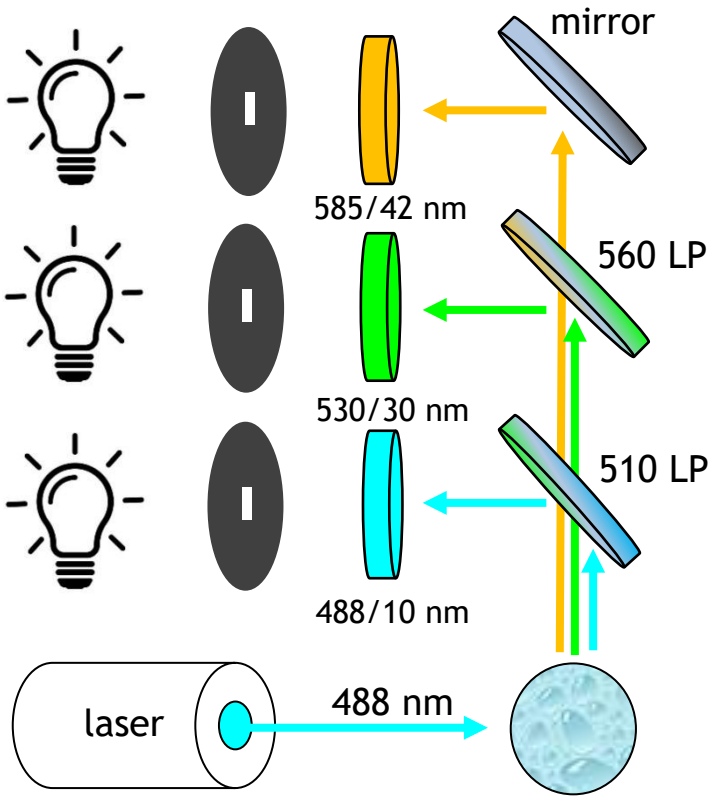



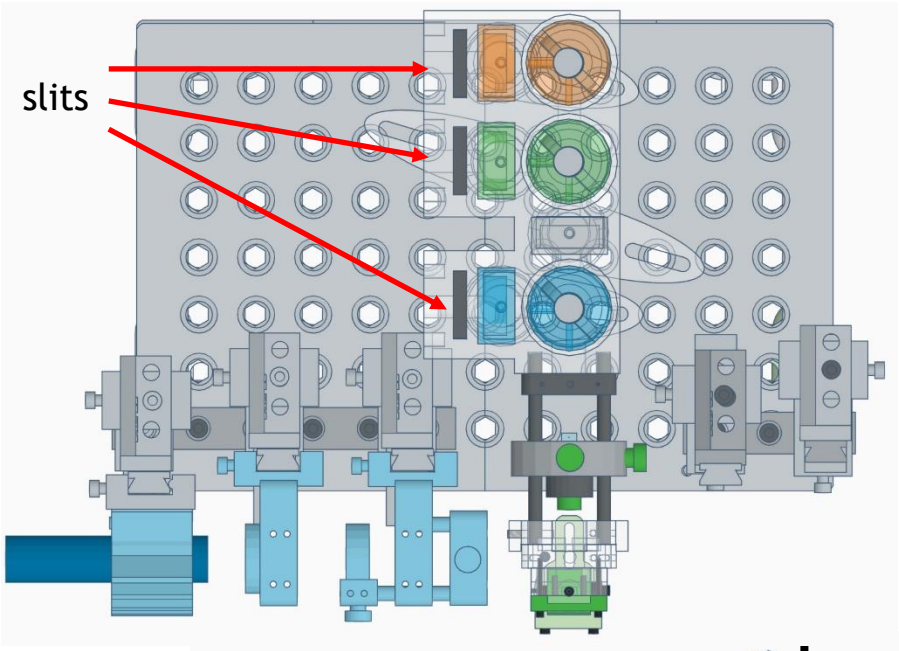



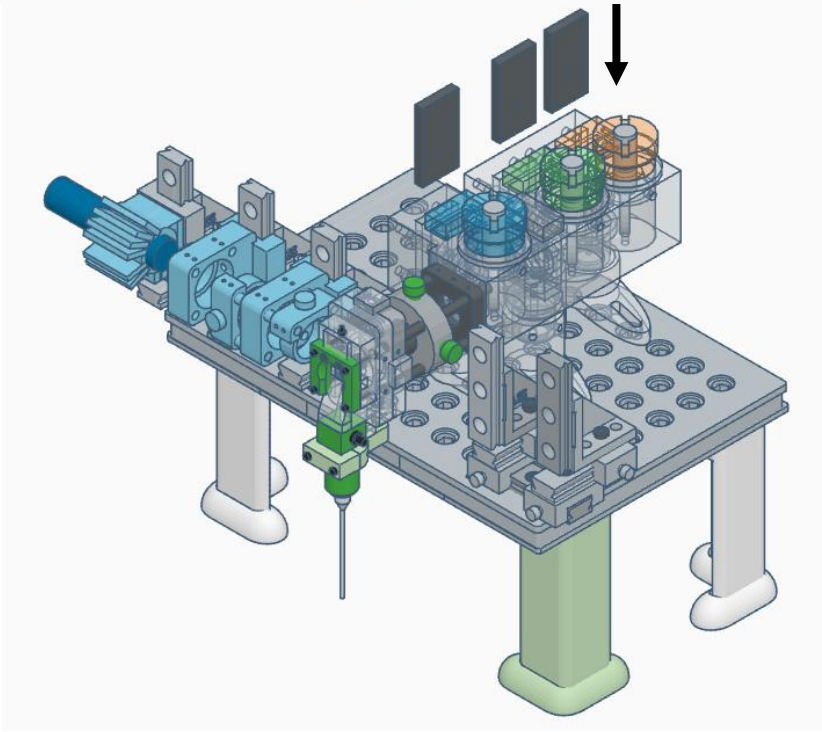
# Backlighting and alignment

We then install narrow slits behind the filters to limit the light entering the detectors and allow alignment.

We backlight the optical paths to align the laser intercept to the detector paths.

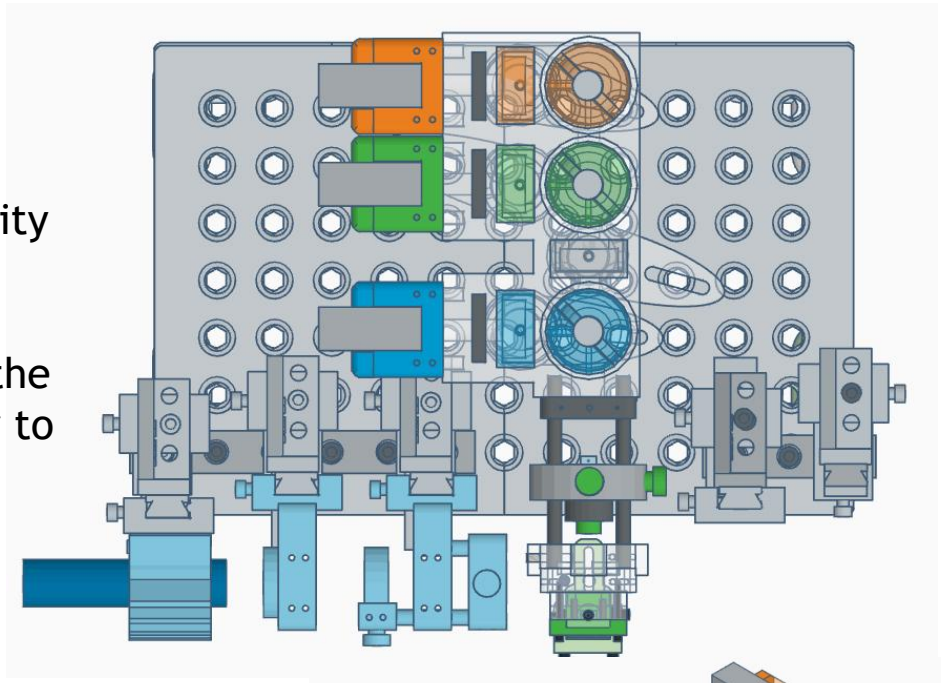


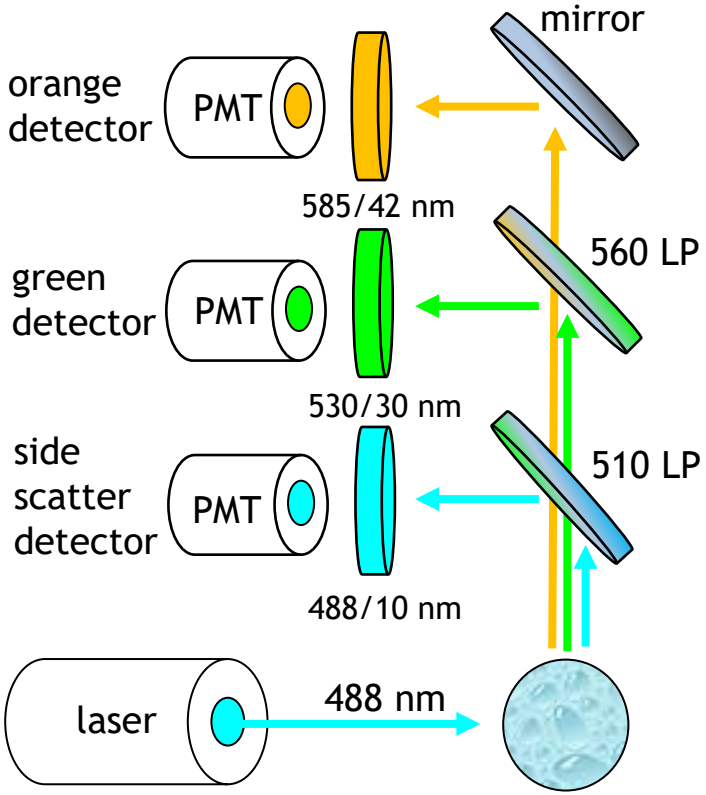



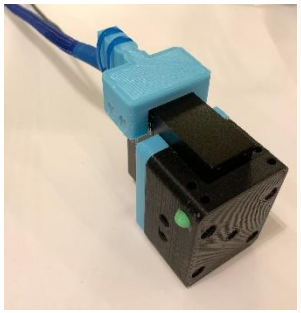



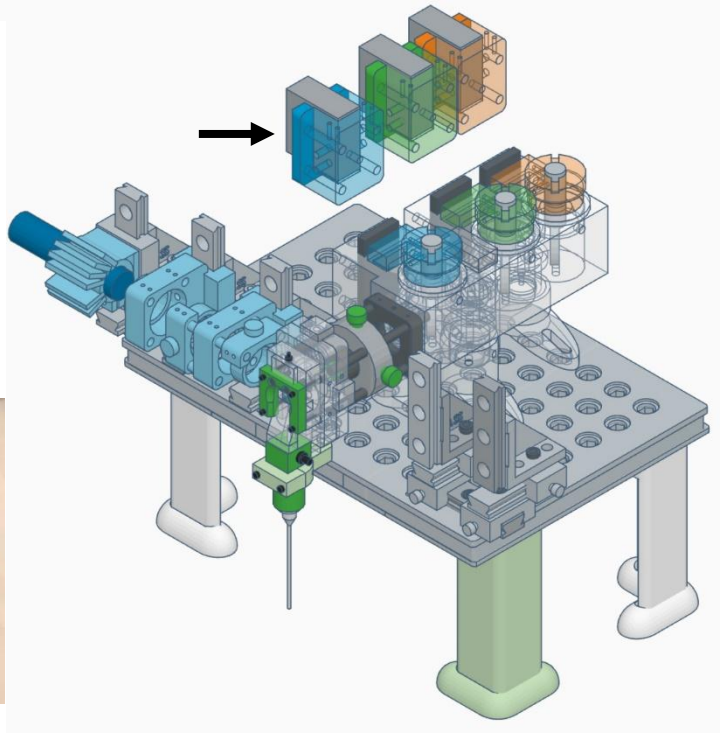
# Installing the PMTs

We then install the photomultiplier tubes (PMTs). These are high-sensitivity light detectors for detection of side scatter, fluorescein and PE detection. They have two cables, one to power the photocathode and dynodes, the other to send signals to the amplifiers.







# Instrument alignment

We can adjust the alignment of side scatter and fluorescence signals by…

Adjusting the laser alignment using the steering prisms (coarse) or moving the focusing optic (fine).

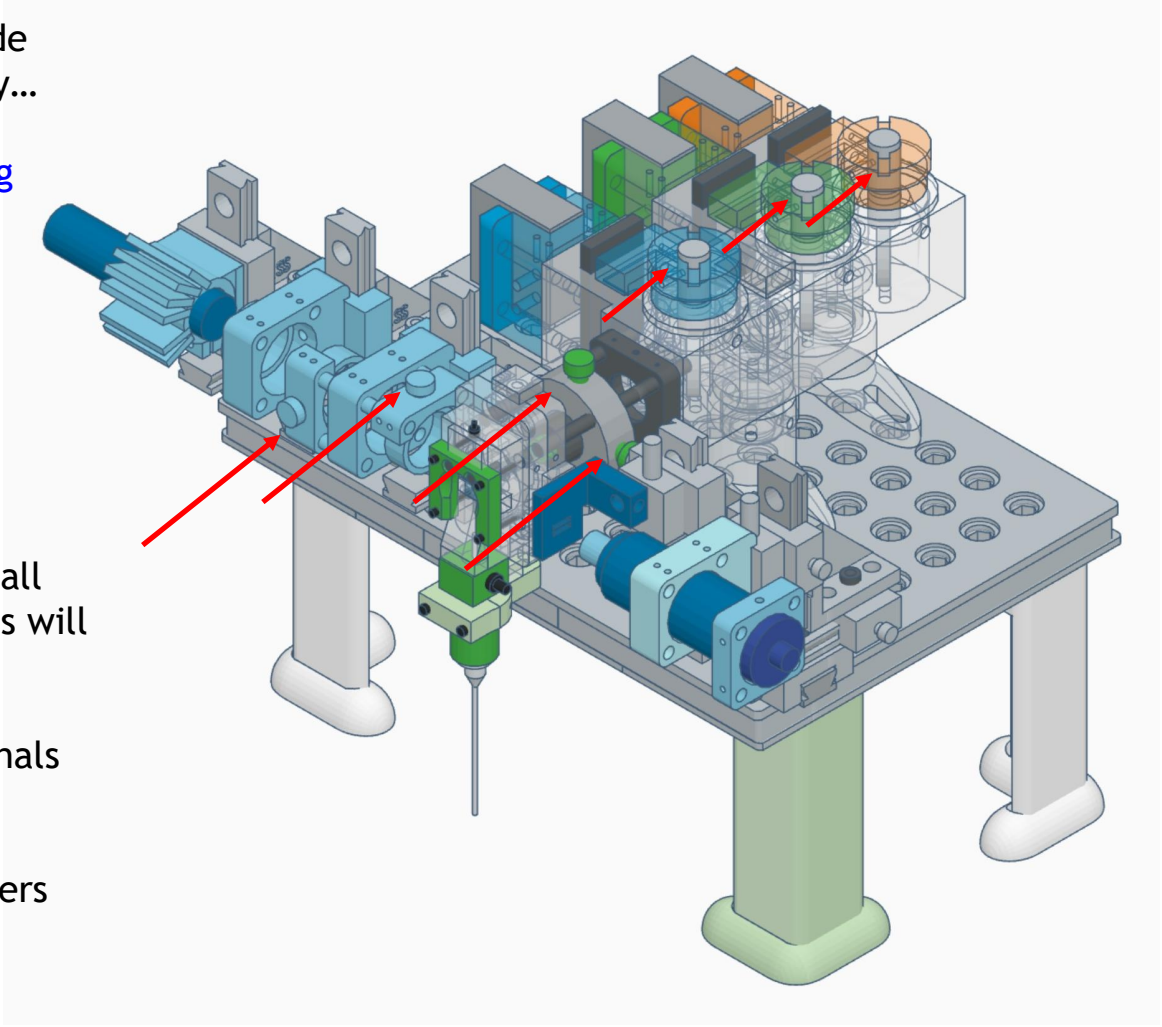
Adjusting the collection optic X-Y position.

Turning the dichroic mounts.

Some of these adjustments affect all parameters (laser alignment). This will also affect forward scatter.

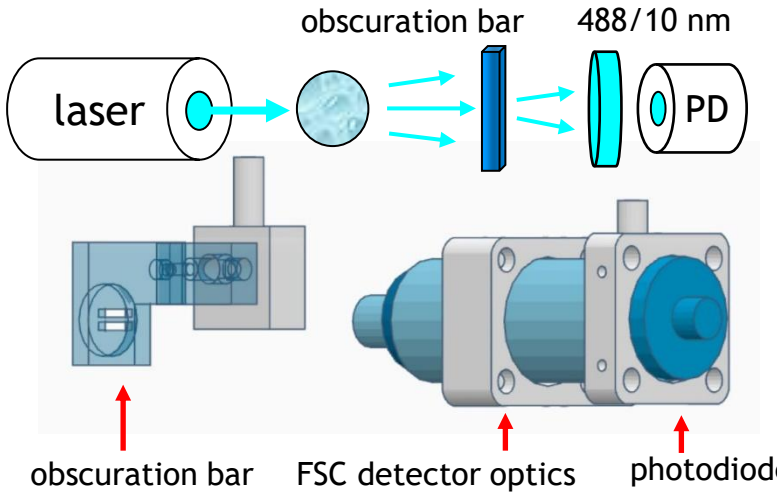
Some affect all 90-degree PMT signals (collection optic position).

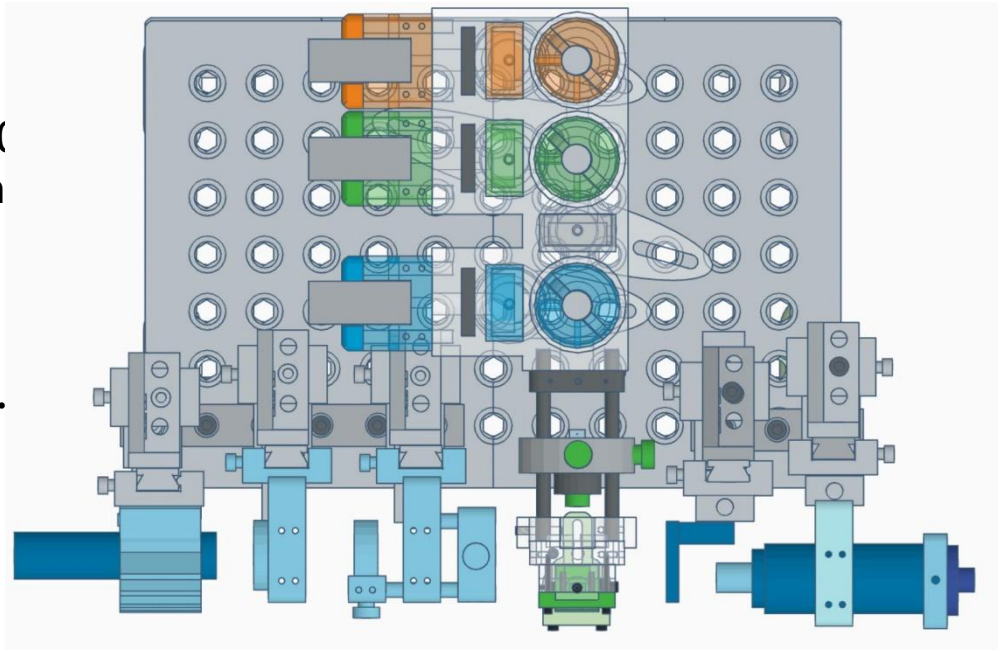
Some effect only specific parameters (dichroics and FSC detector).



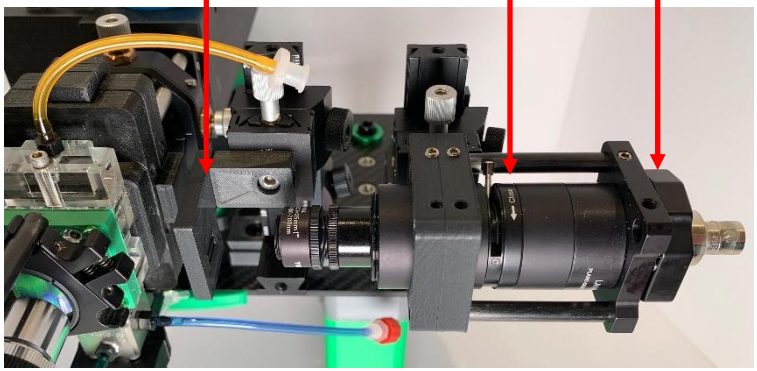
# Forward light scatter detector

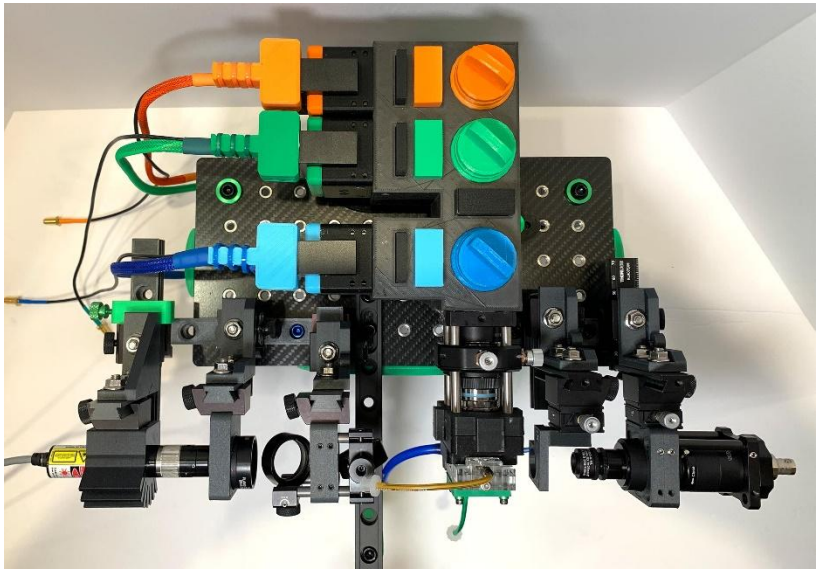
Laser light scattered by the cell at a 18( degree angle is forward scatter, a rough measure of cell size. We use an obscuration bar to block direct non-scattered laser light, allowing only scattered light to enter the photodiode.



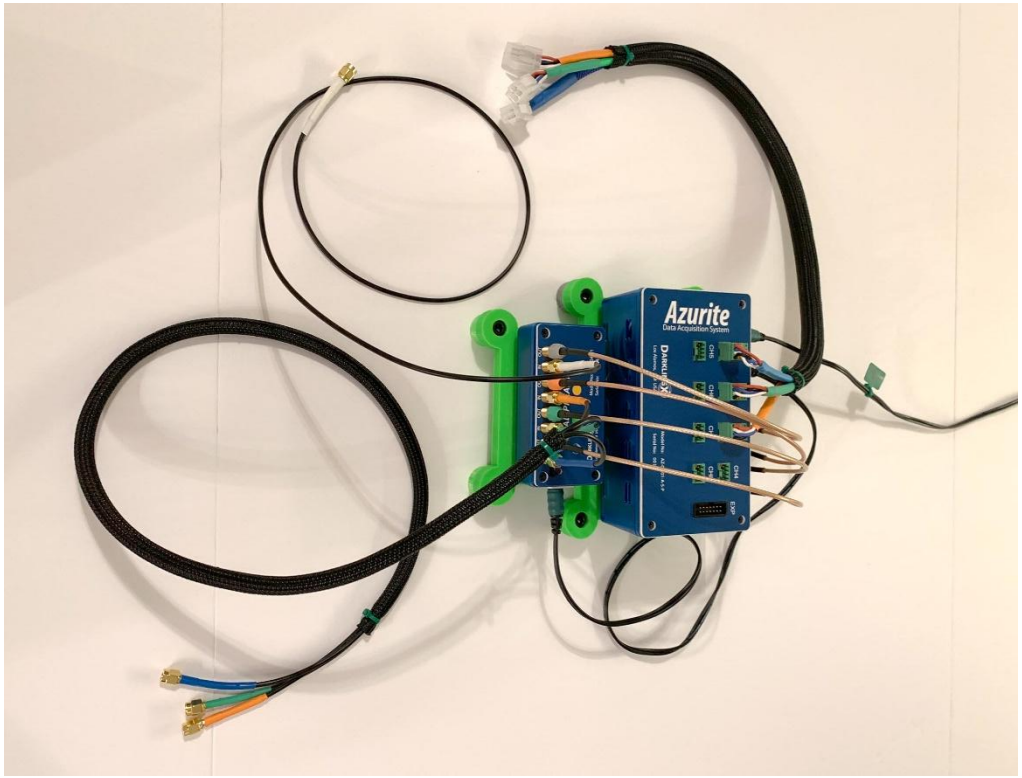



FSC detector optics





# Acquisition electronics




We use a two-stage electronic system to acquire signals from our detectors.

The detectors are connected to a **preamplifier** the little black box) amplifies the signals from the detectors.

**Amplifiers and analog-to-digital converters** (ADCs, in the blue box) amplify the signals again, and convert analog pulses into digital data the computer can display.

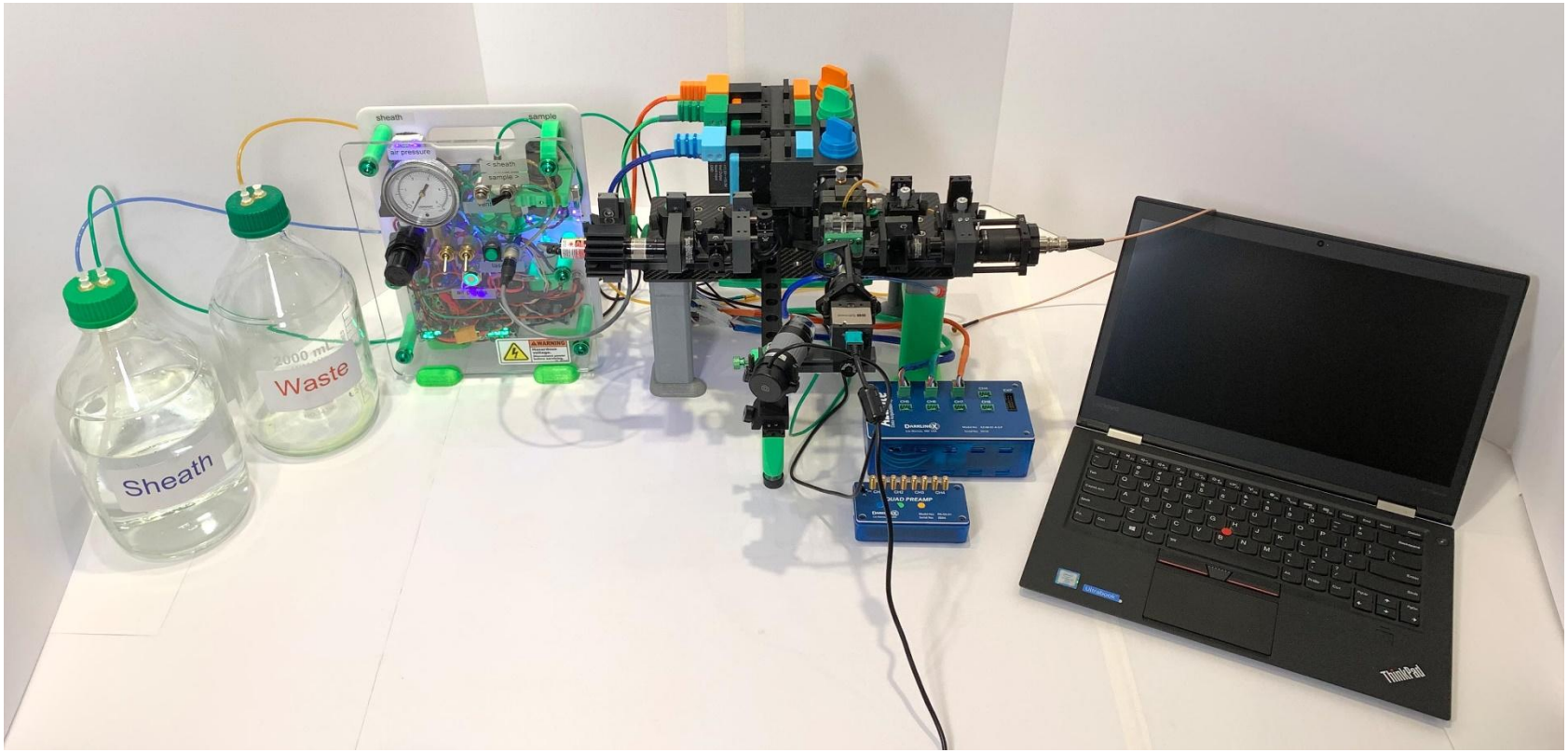
The data is then sent to a **computer** for processing and display.

The colored cables supply power to the PMTs.

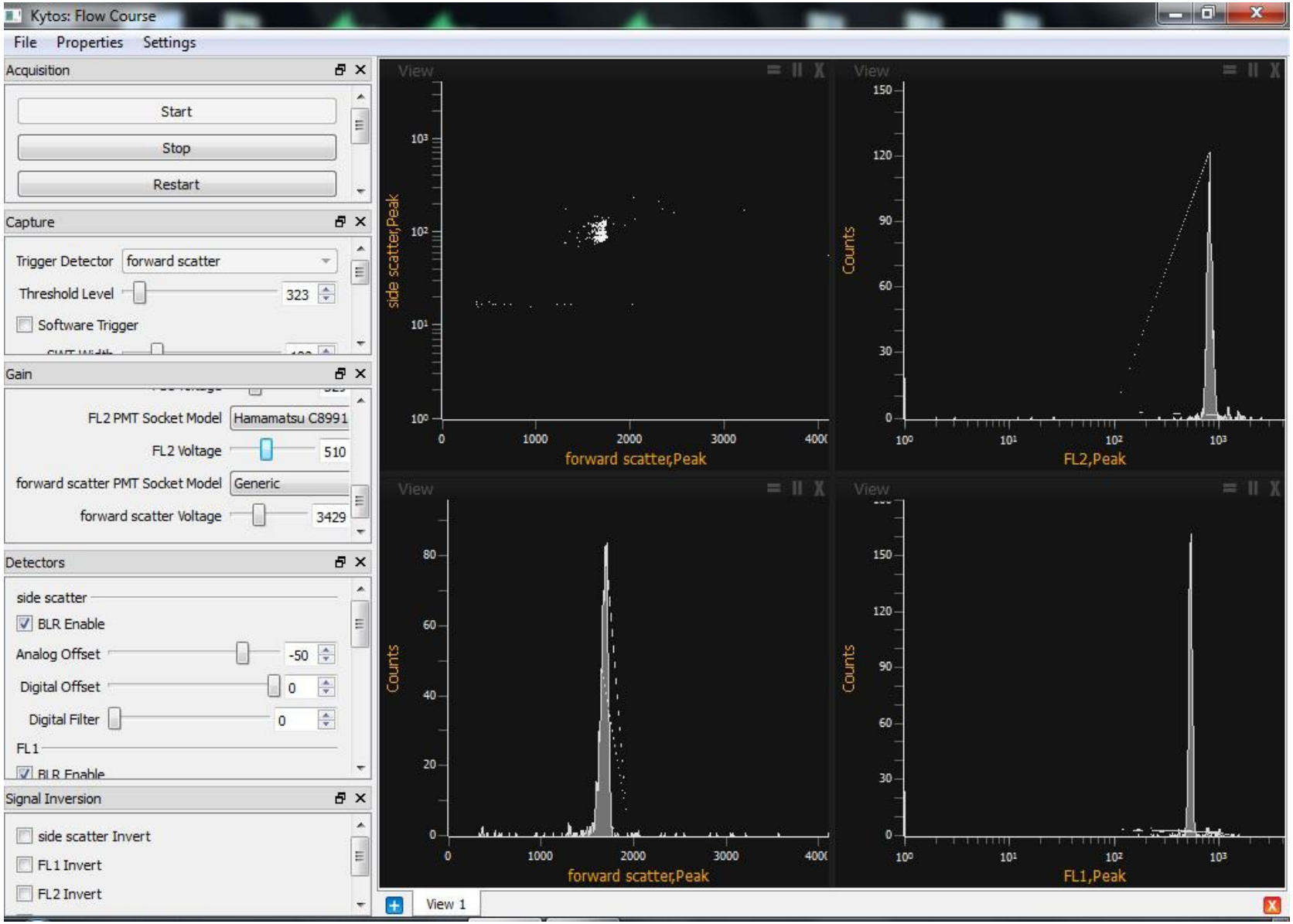
Mark Naivar

DARKLINGX

# Finished!


sheath
sample
air pressure
< sheath
sample >
2000 mL
Waste
Sheath
QUAD PREAMP
ThinkPad

# Kytos acquisition software



Make Your Own Cytometer link:

https://www.cytometryworks.com

https://www.cytometryworks.com/MYO_Cytometer_Virtual_2020.html