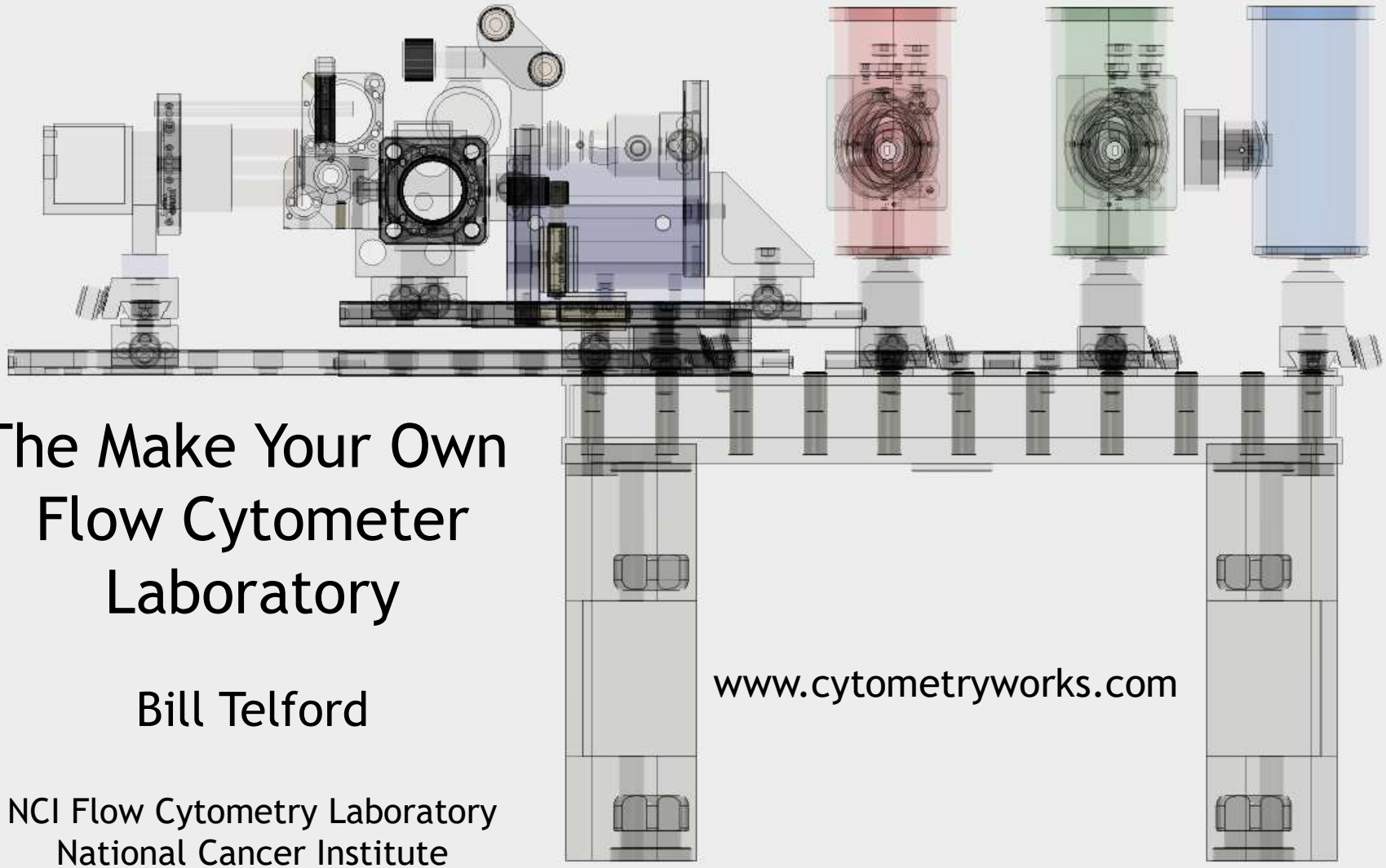


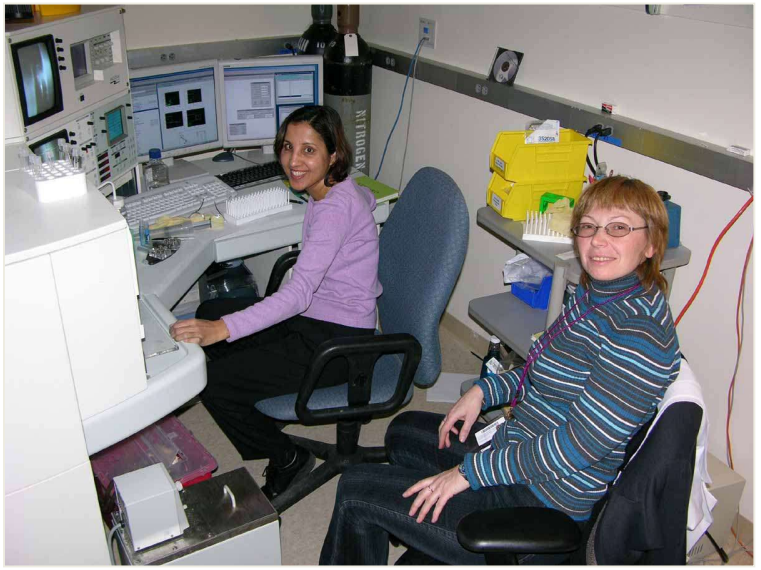
The Make Your Own Flow Cytometer Laboratory

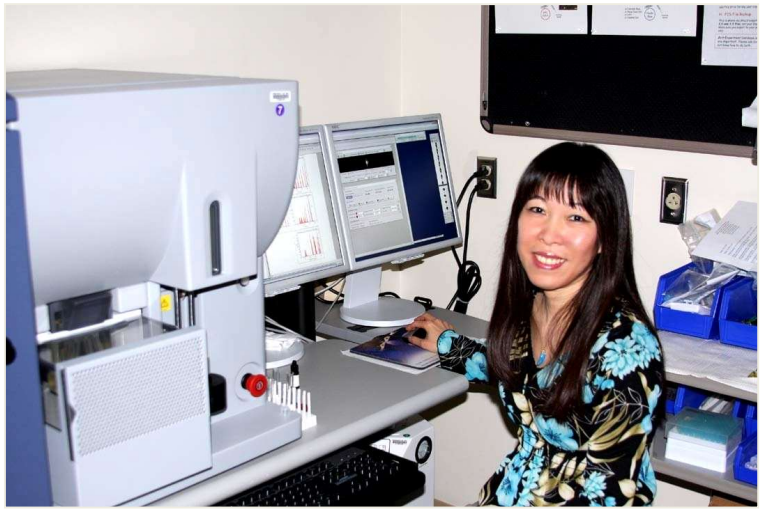
Bill Telford

NCI Flow Cytometry Laboratory
National Cancer Institute
National Institutes of Health

www.cytometryworks.com

NCI ETIB Flow Cytometry Core Laboratory





National Institutes of Health

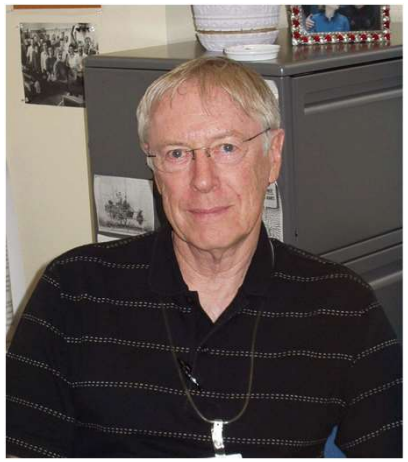
NATIONAL CANCER INSTITUTE®

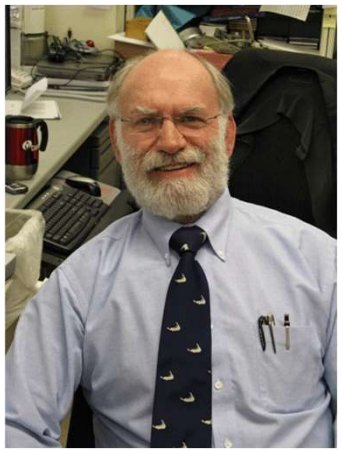
Los Alamos
NATIONAL LABORATORY

National Flow
Cytometry Resource

The original Build Your Own Flow Cytometer Lab (now the Build Lab) was developed by **James Jett, John Martin and Rob 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.



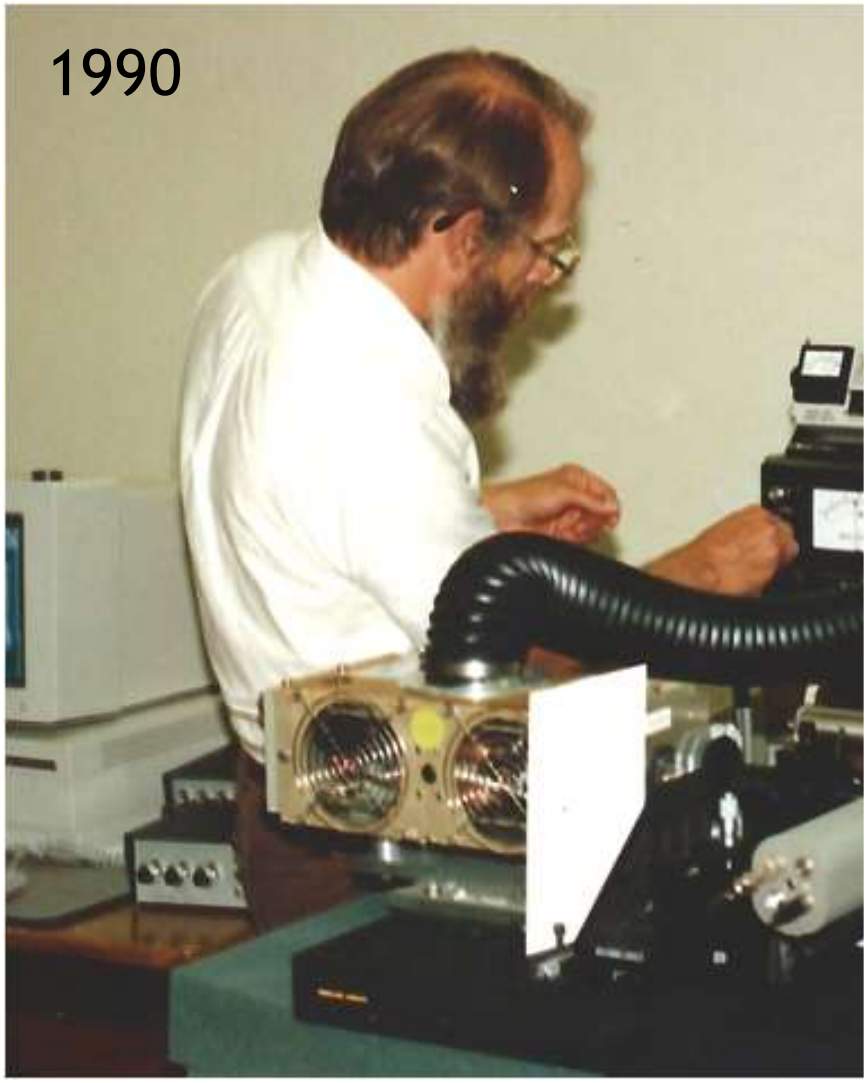


The Original Build Your Own Cytometer Lab (The Build Lab)

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

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

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



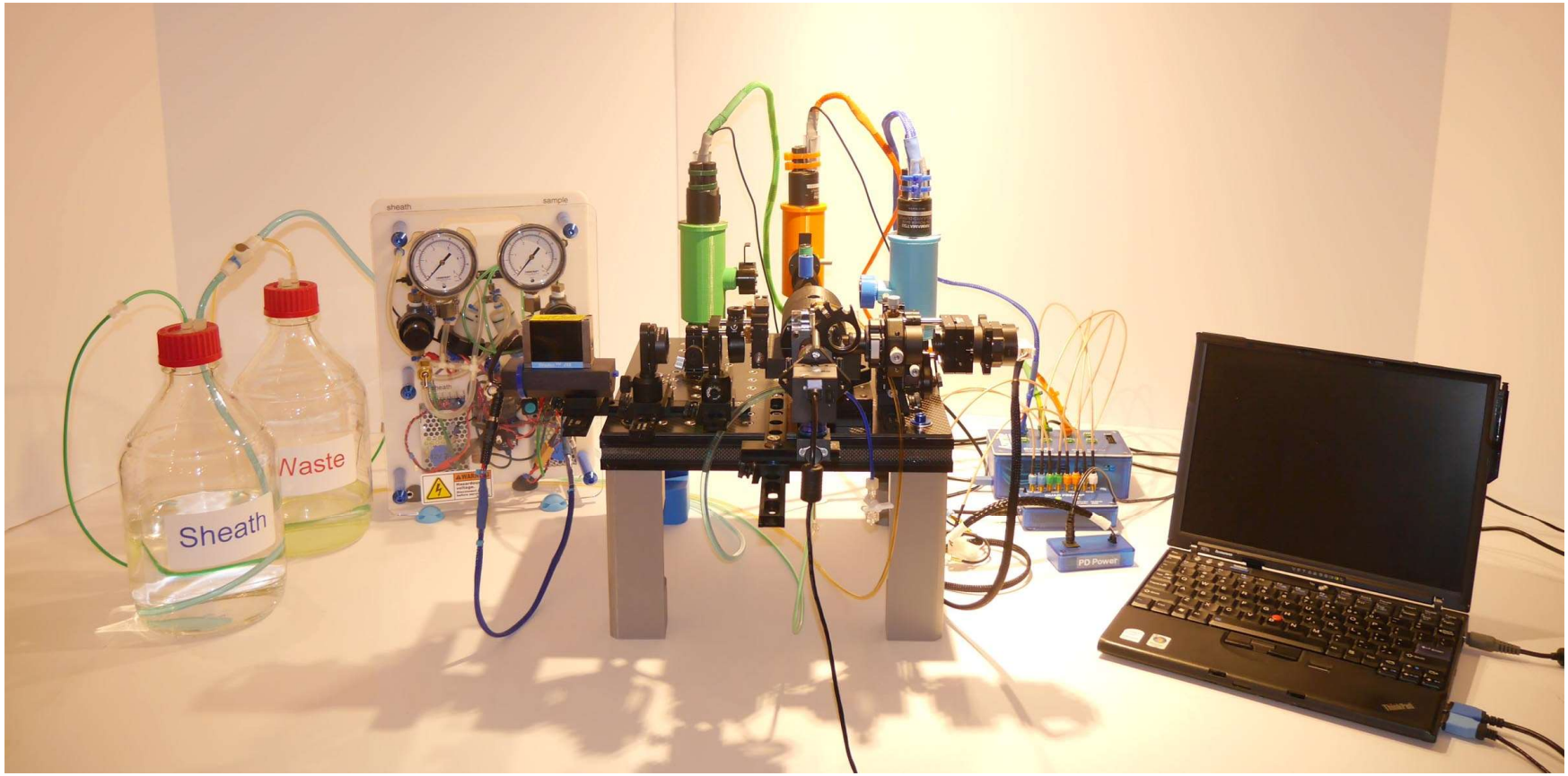
The Original Build Your Own Cytometer Lab (The Build Lab)

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

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

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

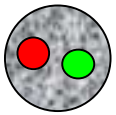
The Make Your Own Cytometer Lab (The Flow Cytometry Maker Lab)



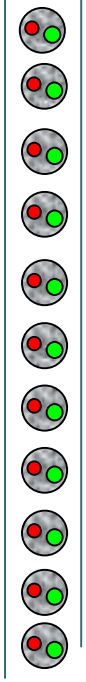
What goes into a flow cytometer?

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

…usually labeled with a **fluorescent marker**



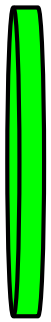
You need to **move** these cells in a linear stream through a focused light source.

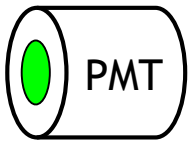


You need a **light source** to excite the fluorescent molecular on or in the cell.

Usually a **laser**!

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

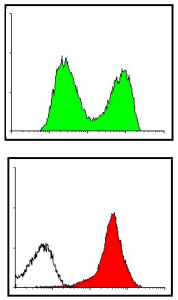




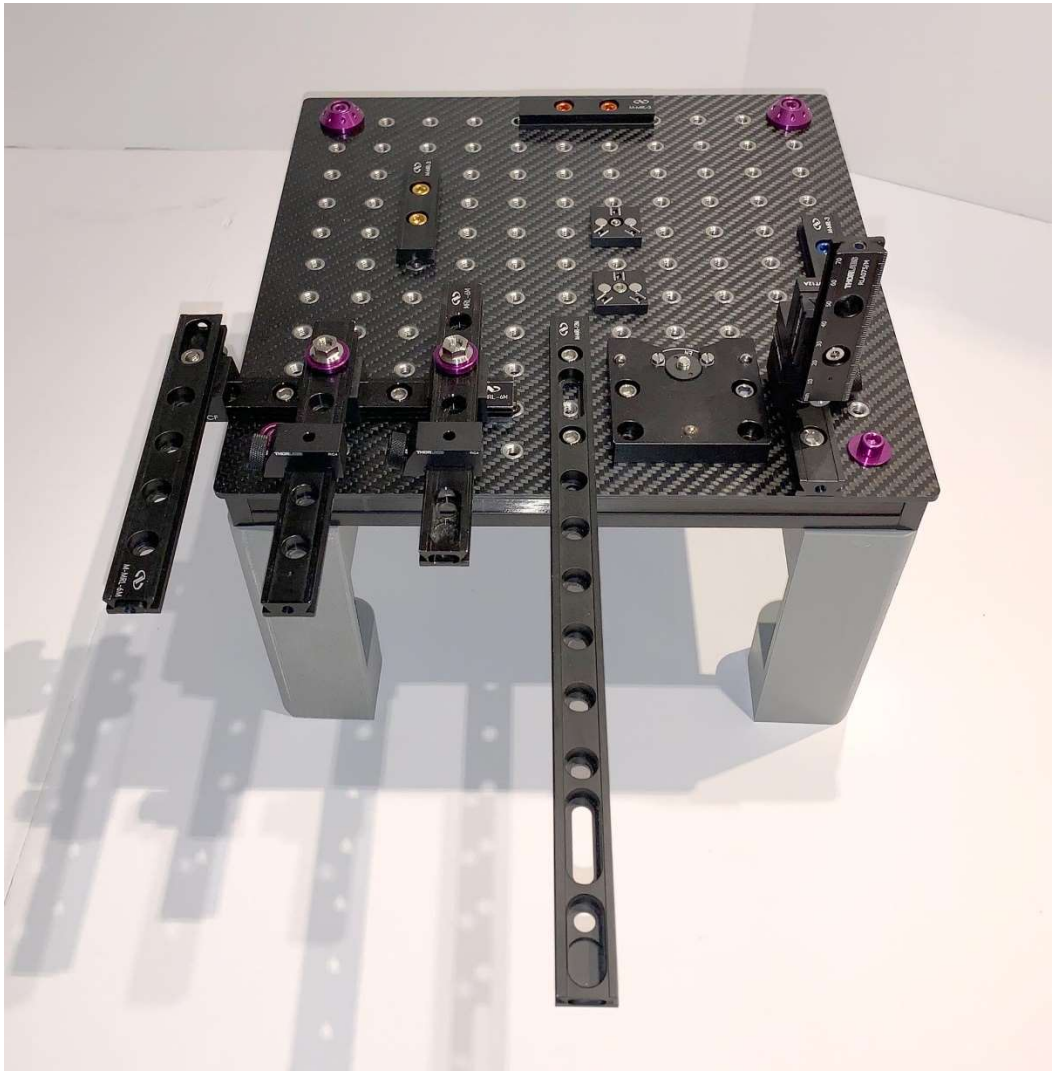
You need a **sensitive light detector** (usually a **photomultiplier tube**).

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

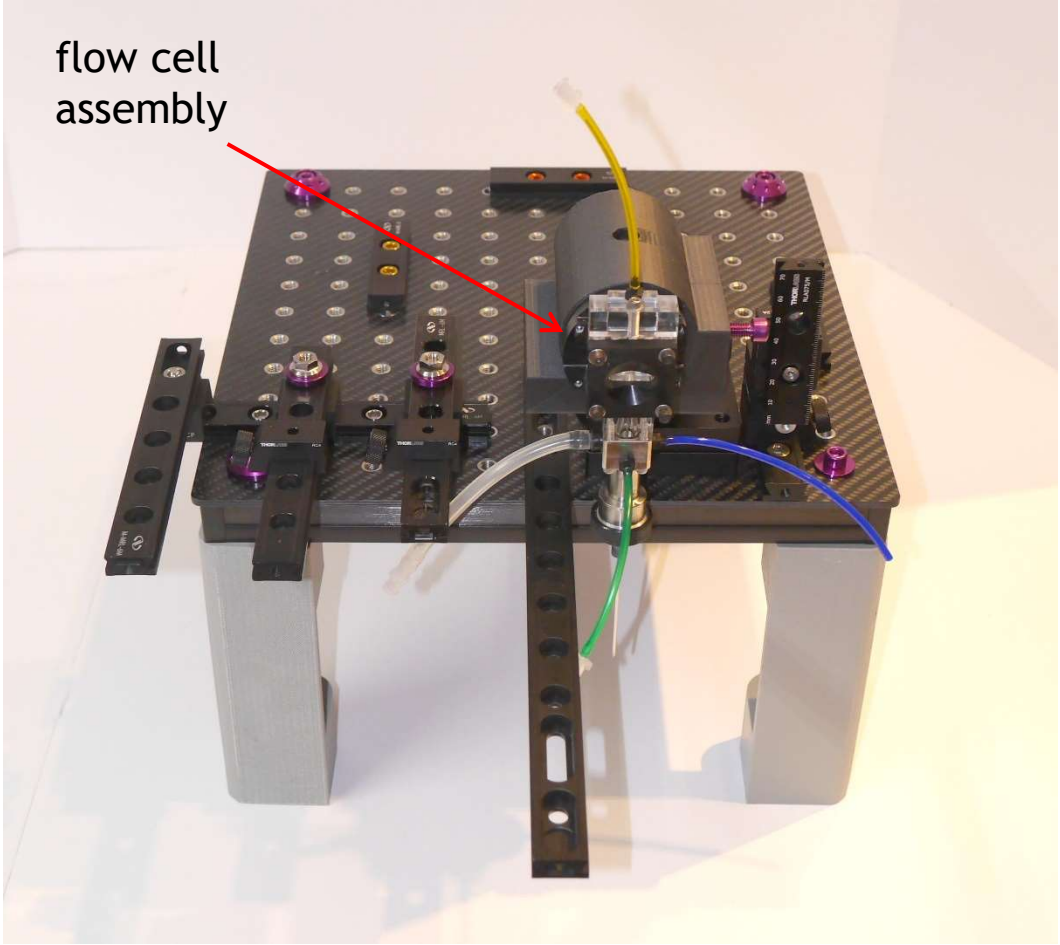
Finally, you need a computer to process the digital signals and display the data



Baseplate



Installing the flow cell



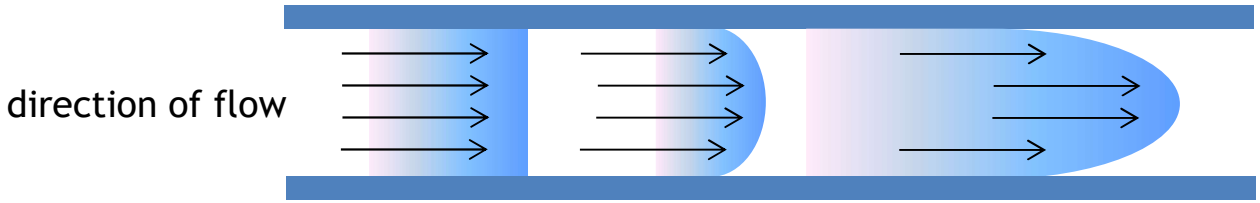
We pass our cells through a quartz flow cell, where they are illuminated with a laser.

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

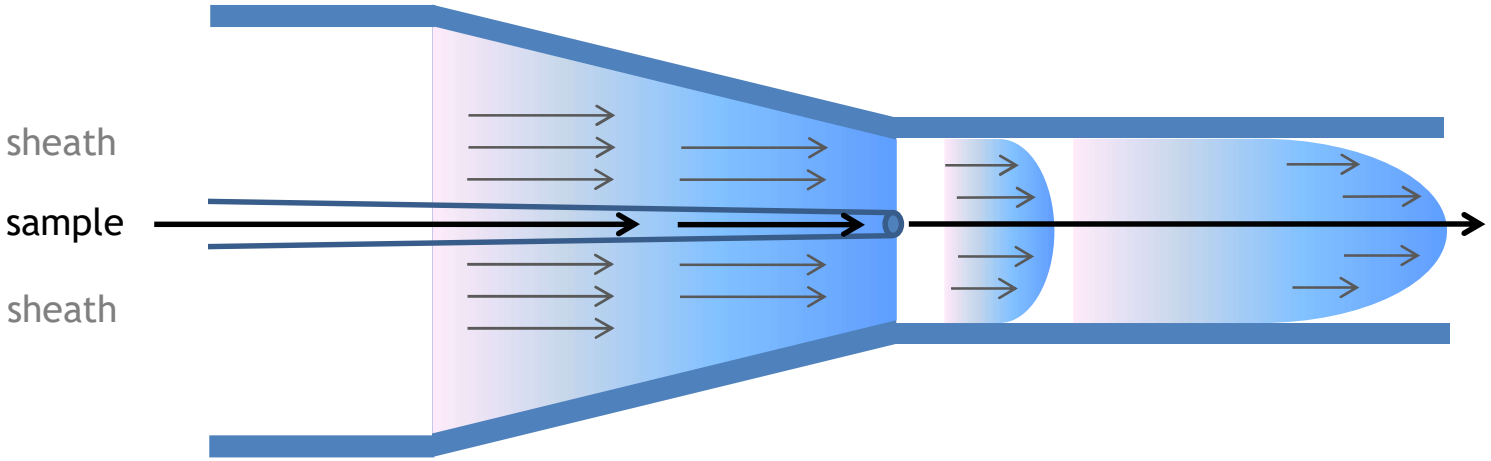
Hydrodynamic focusing maximizes sensitivity of the flow cytometer.

Sheath and sample flow

A sample stream in a simple capillary tube will not “focus” the cells in a narrow stream. Cells at the edge will move more slowly than those in the center.



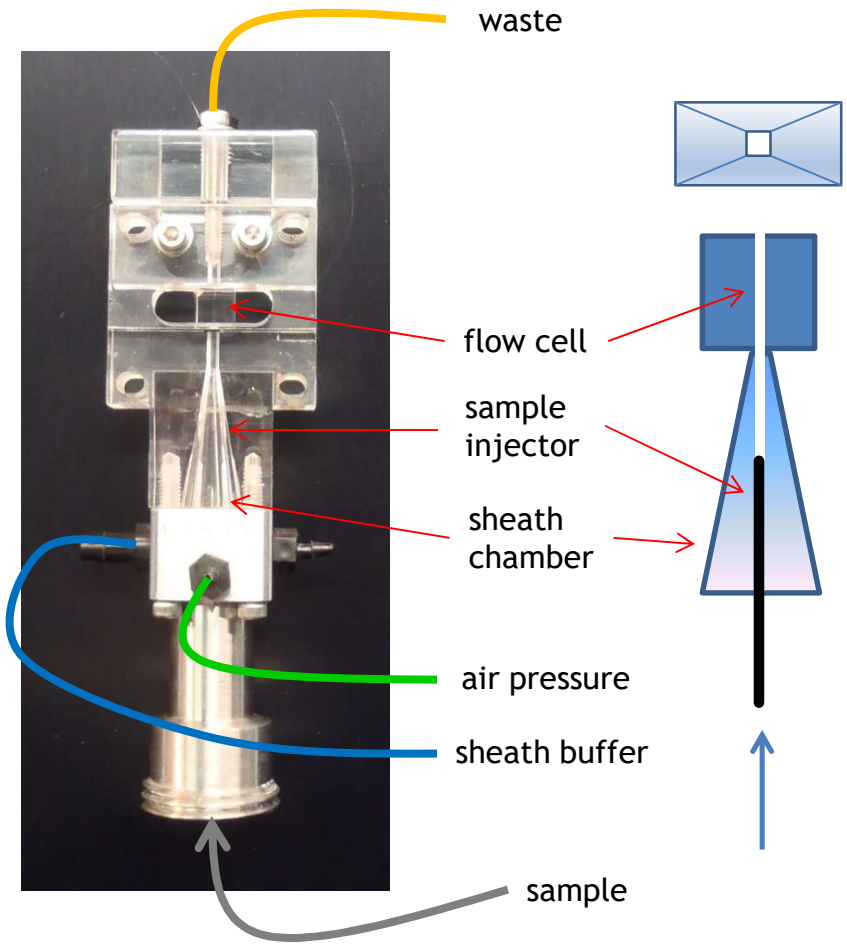
A separate **sheath** stream surrounding a **sample** stream **hydrodynamically focuses** the cells in a narrow path. The sheath stream also regulates sample flow.

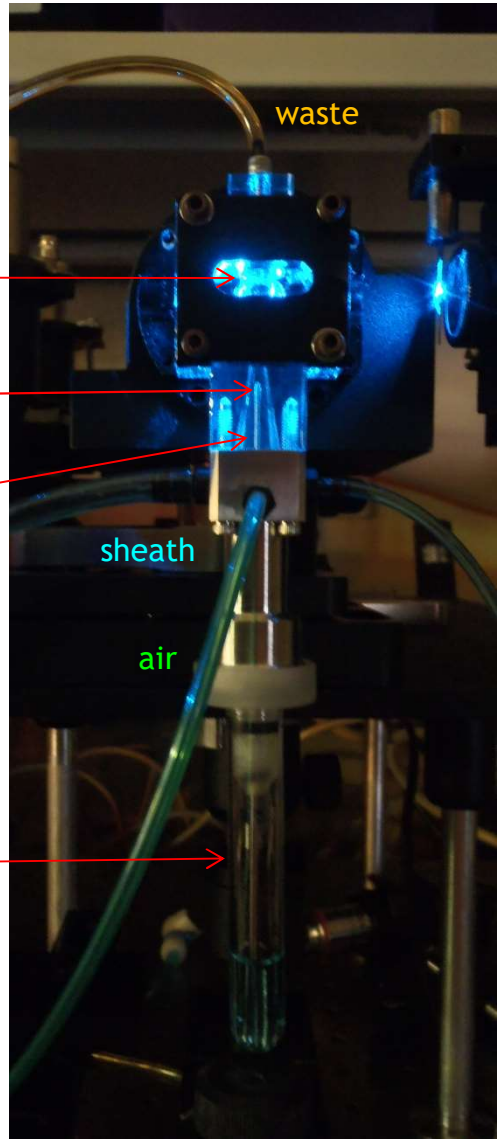


Hydrodynamic focusing using a sheath system is critical for flow cytometry.

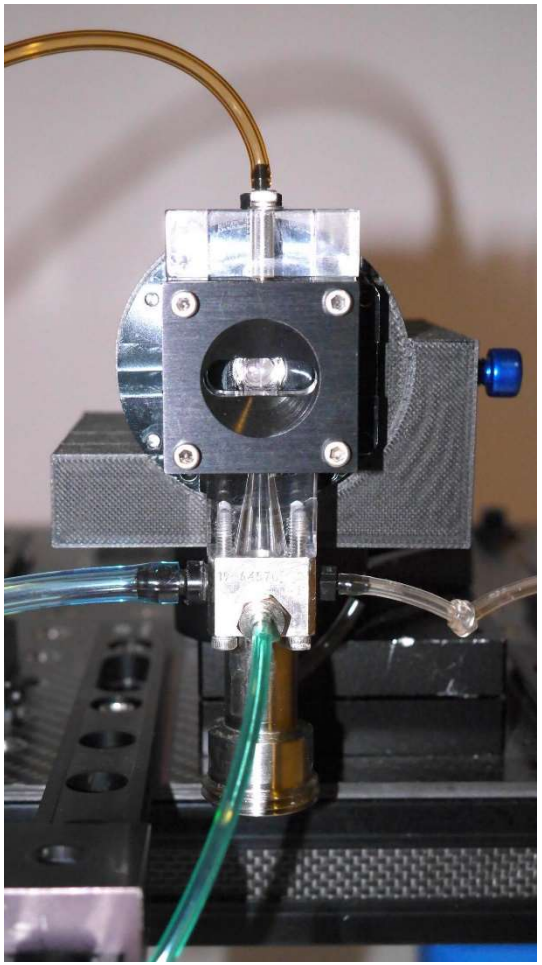
Our cuvette flow cell is borrowed from a BD FACSCalibur

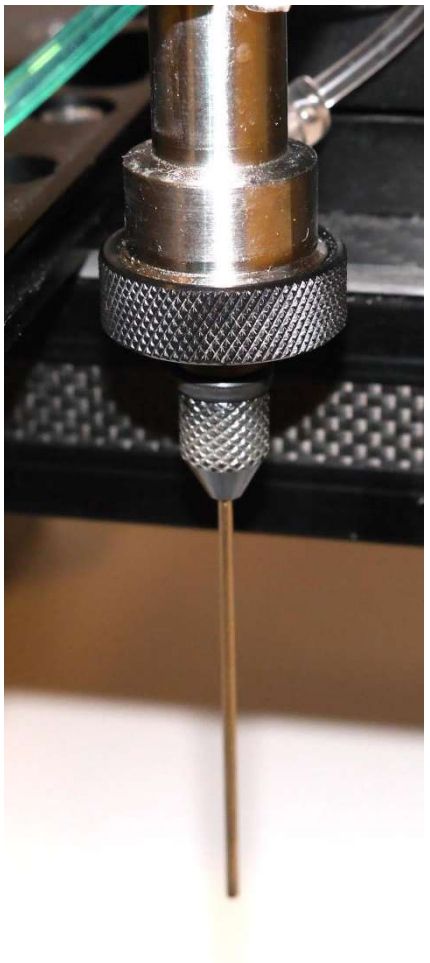
BD FACScan / FACSort / FACSCalibur
A classic cuvette design (inverted, though!).



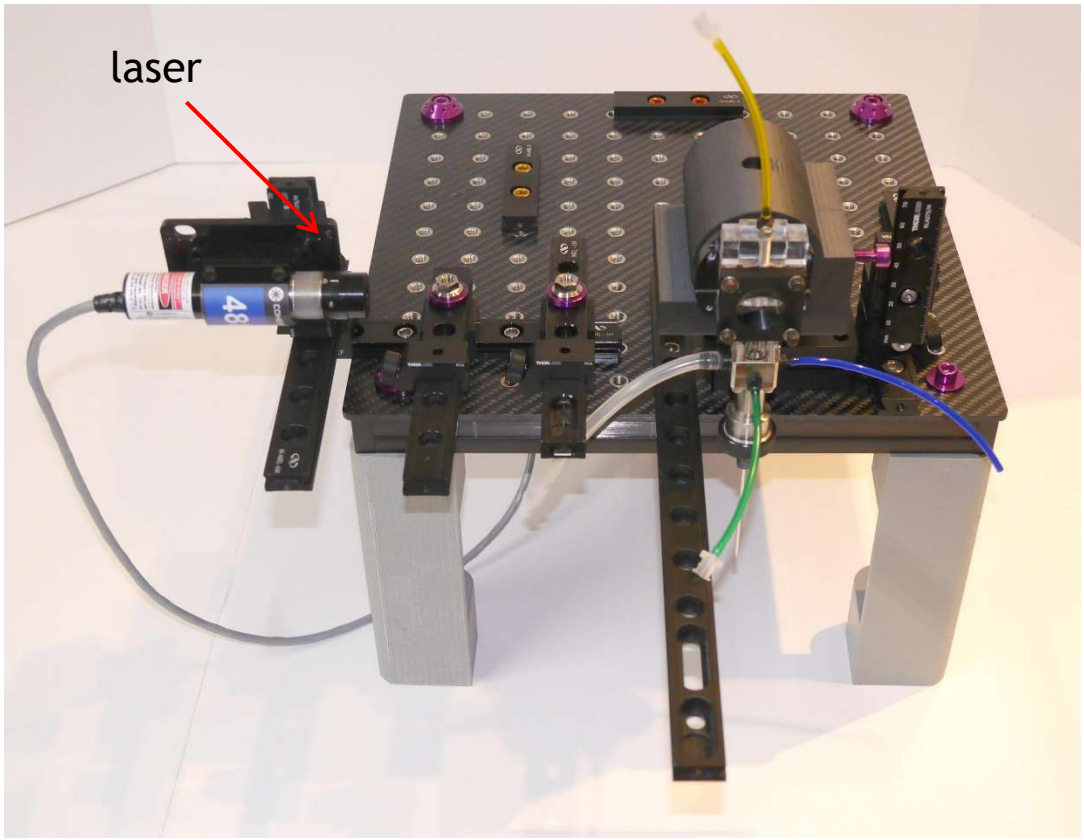


Cuvette flow cell





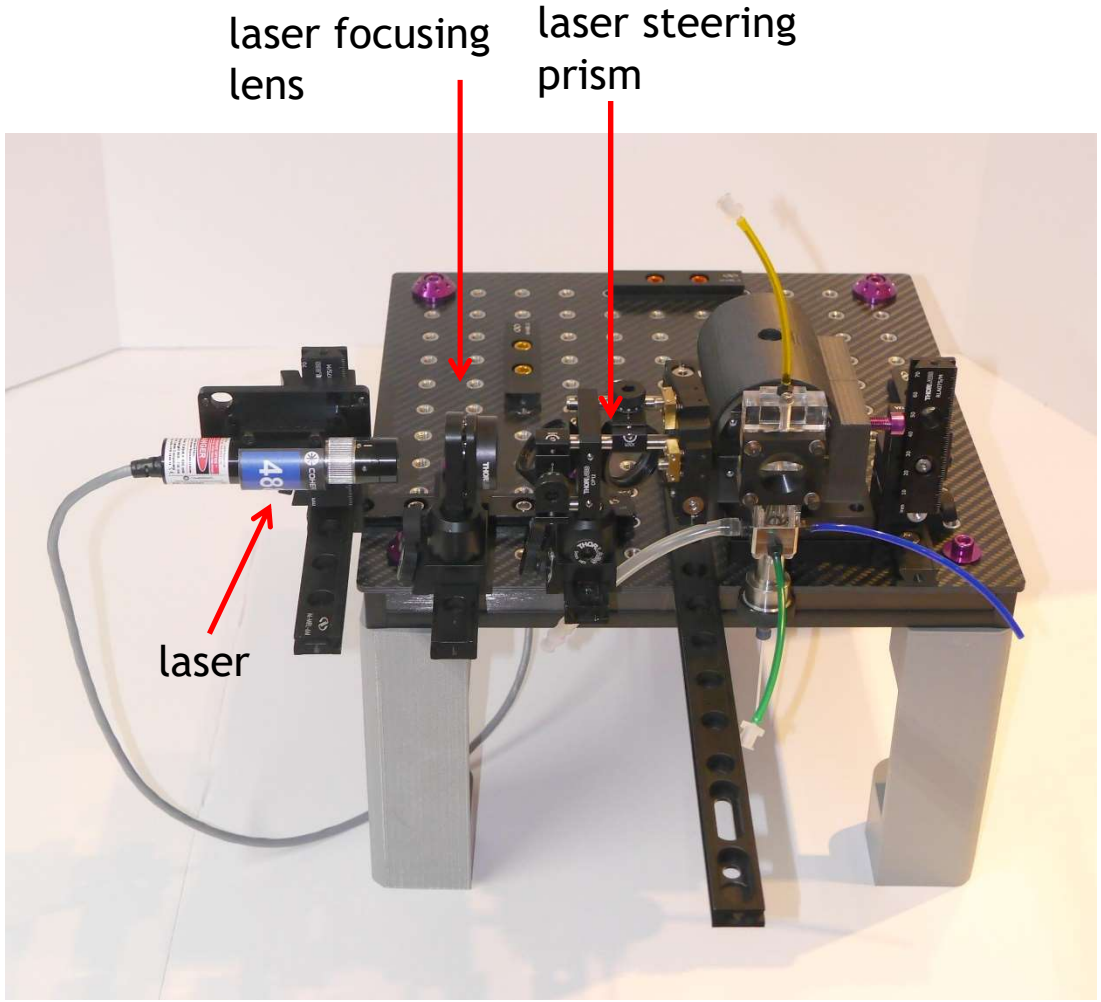
Installing the laser



A blue-green 488 nm laser is aligned to the flow cell. It illuminates the sample stream and allows measurement of light scattering (cell size and optical density).

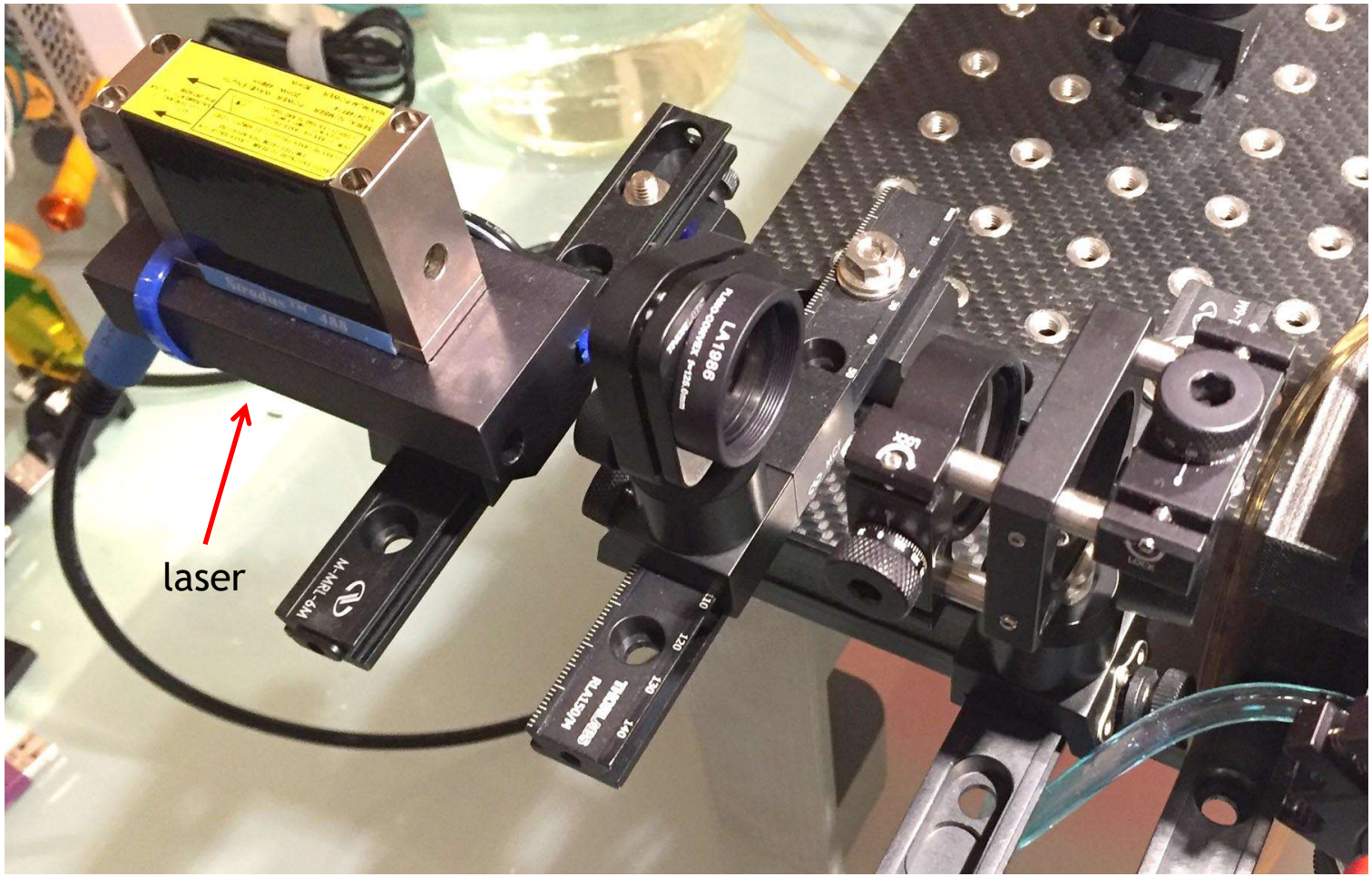
We can label our cells with fluorescent probes. The laser also excites these probes, allowing measurement of many cell characteristics.

Installing the laser, focusing lens and steering prism



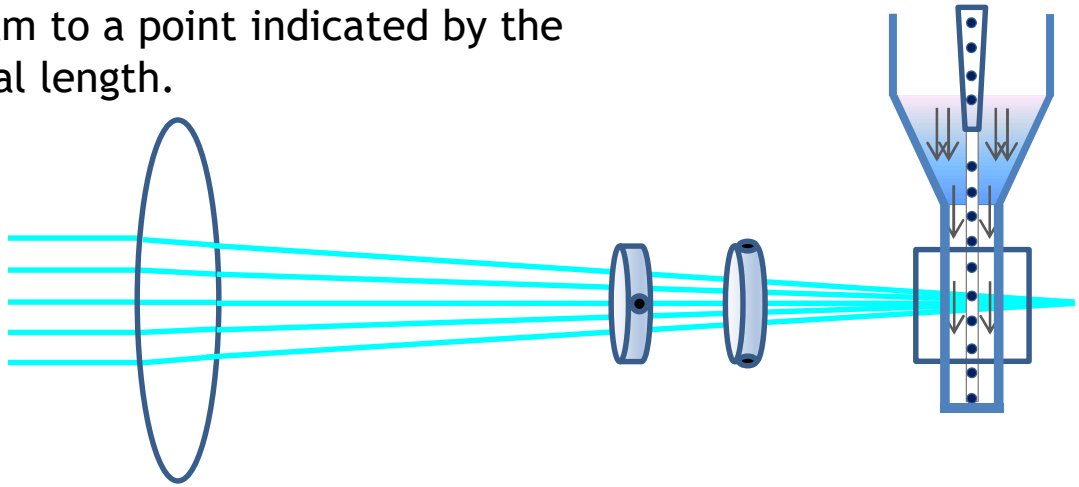
We use lenses and prisms in the laser path to focus the laser beam on the flow cell and steer the beam to the cell stream.

Installing the laser, focusing lens and steering prism

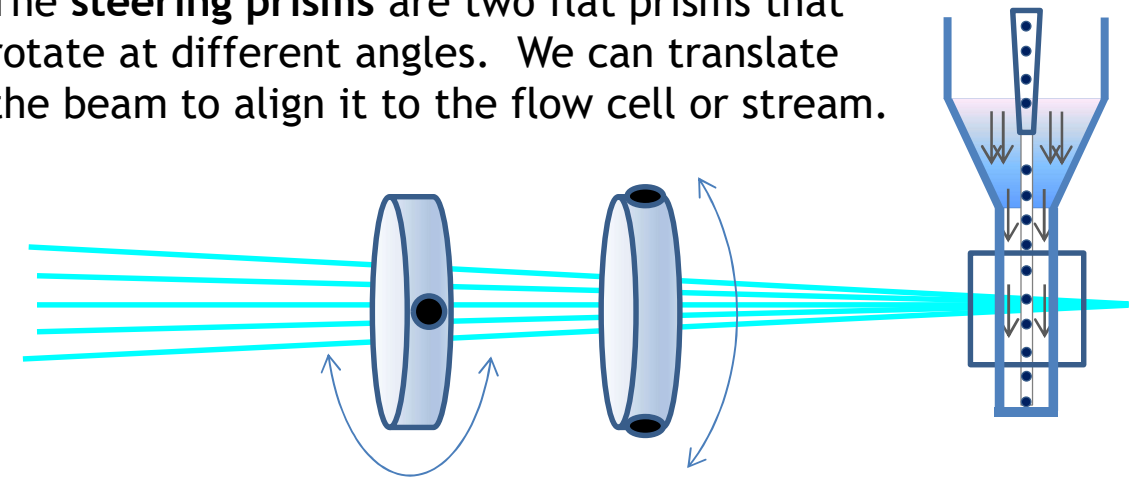


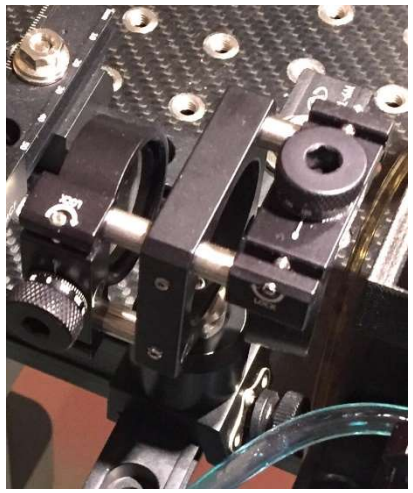
Installing the laser focusing lens and steering prism

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

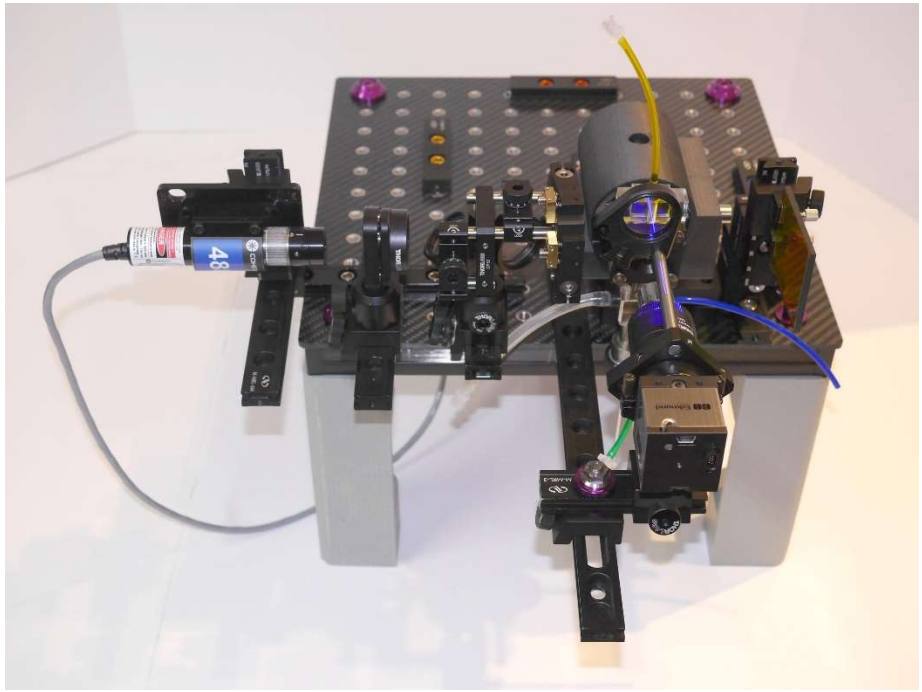


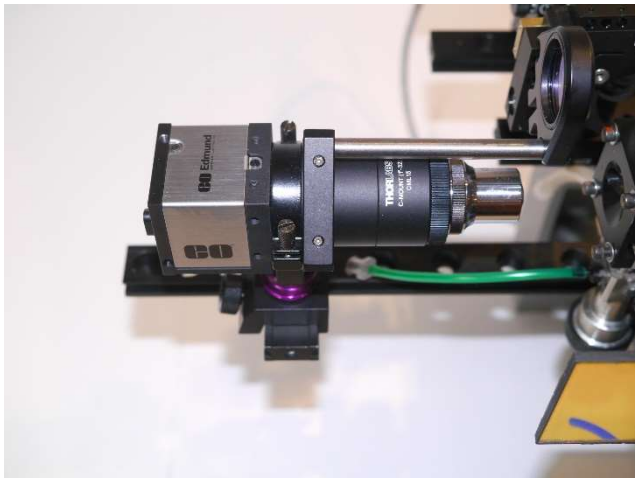
The **steering prisms** are two flat prisms that rotate at different angles. We can translate the beam to align it to the flow cell or stream.





Installing the camera

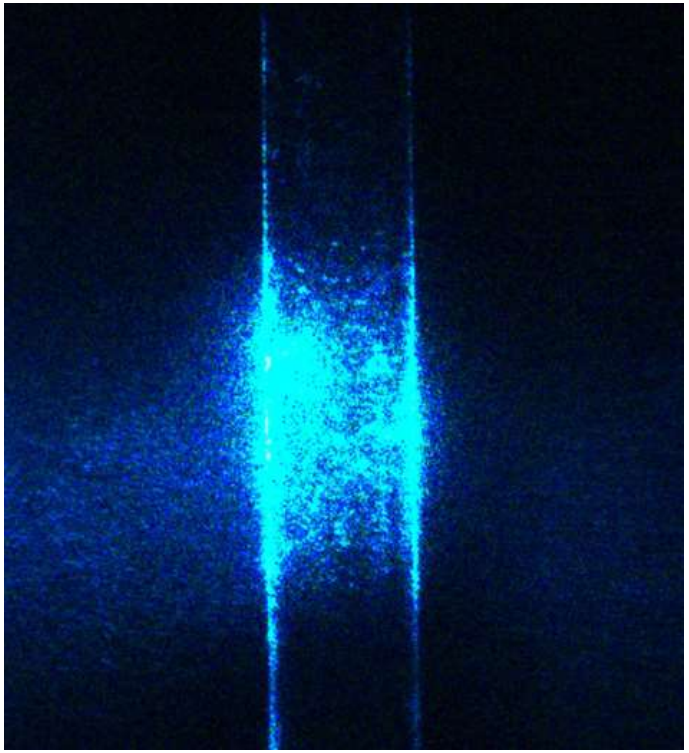


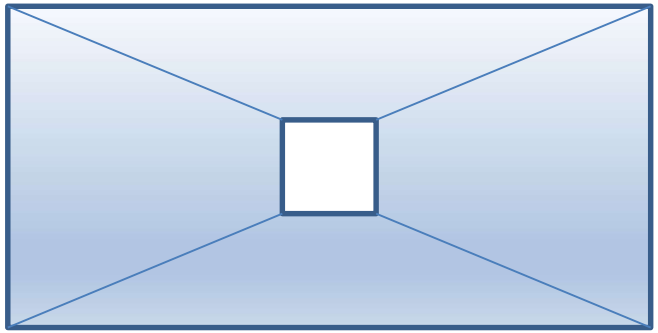


A camera installed in front of the flow cell allows us to see the laser beam and align it to the flow cell.

Visualizing the flow cell

We turn on the laser and camera and focus on the flow cell. We then steer the laser beam to intercept the flow cell.



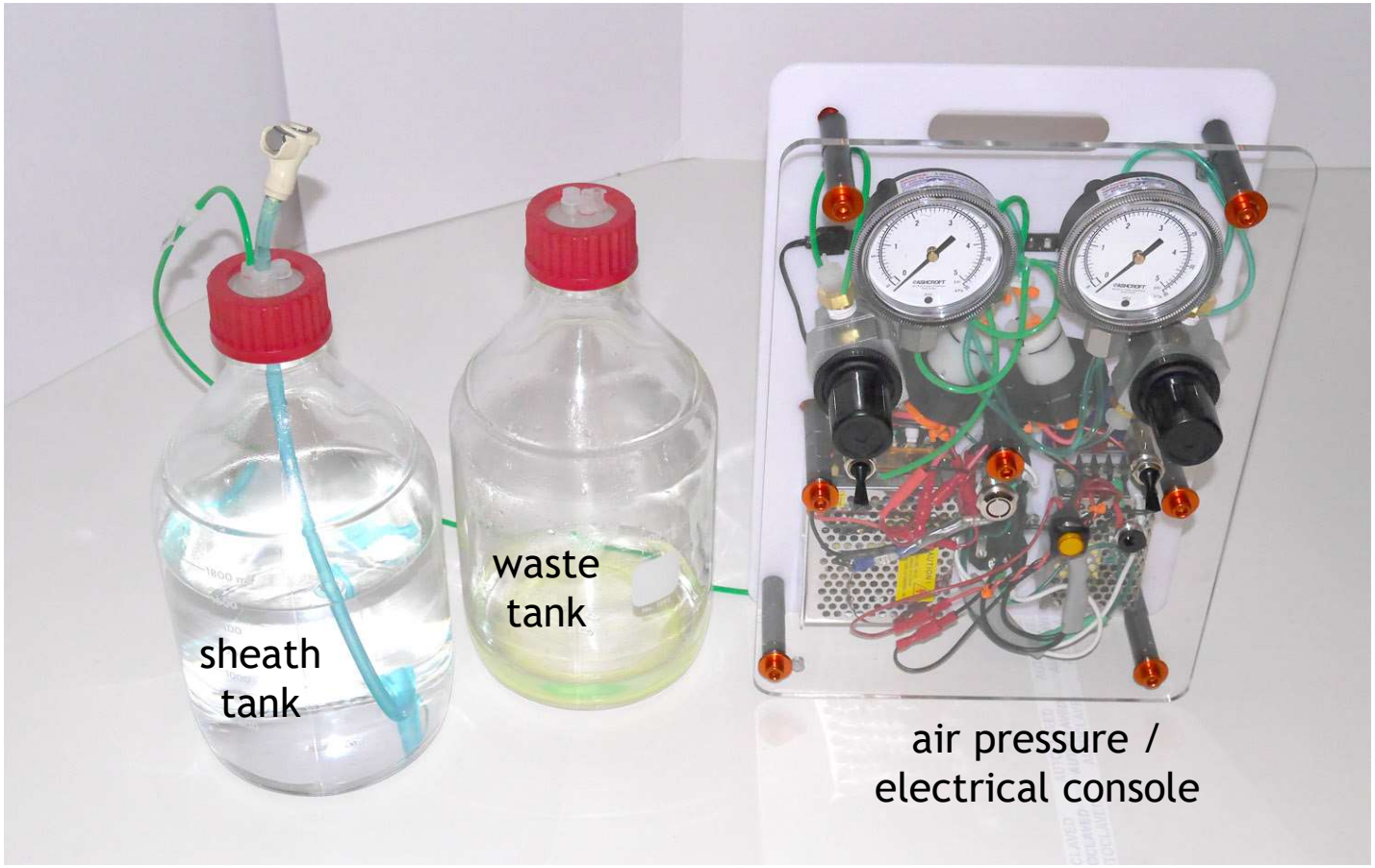


This flow cell is square, ~200 μm across.

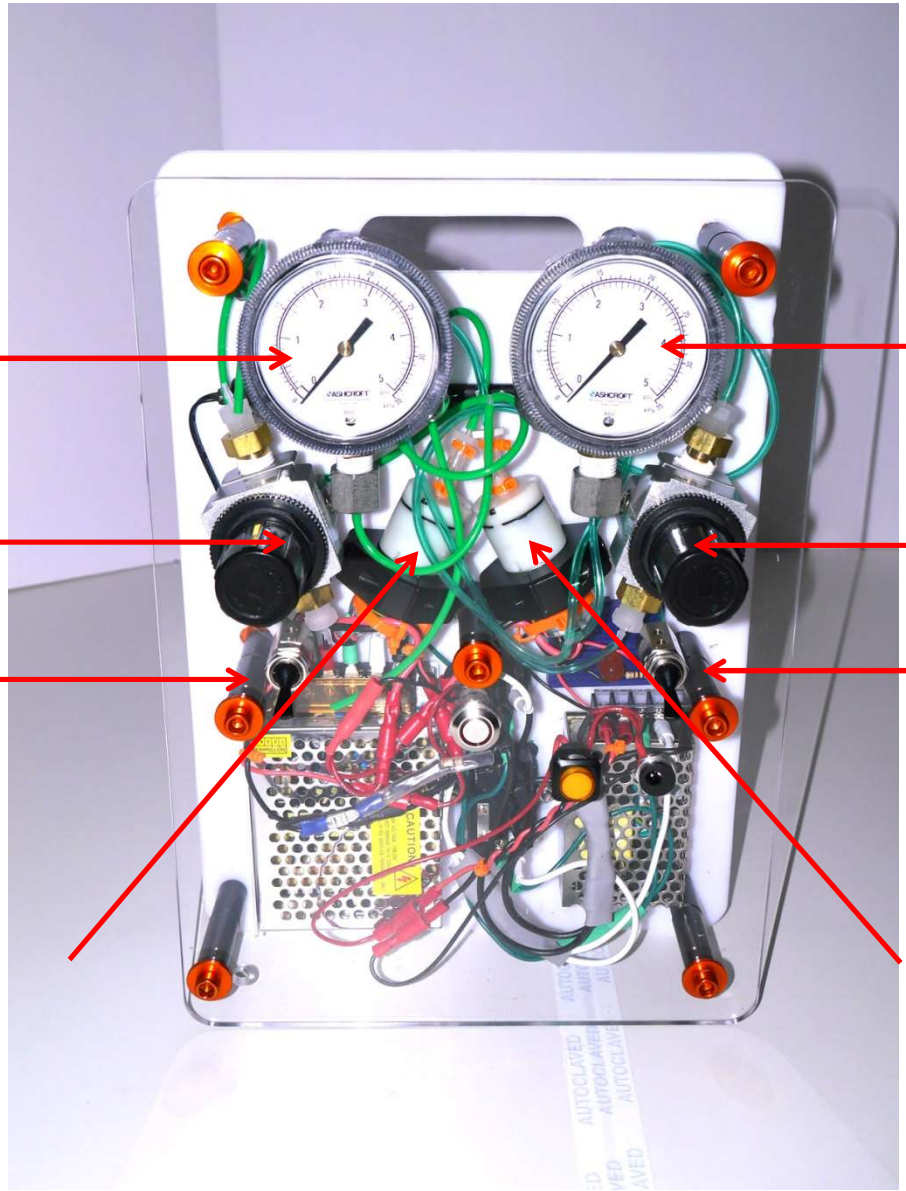
Most cell types can pass through this flow cell.

Instrument fluidics

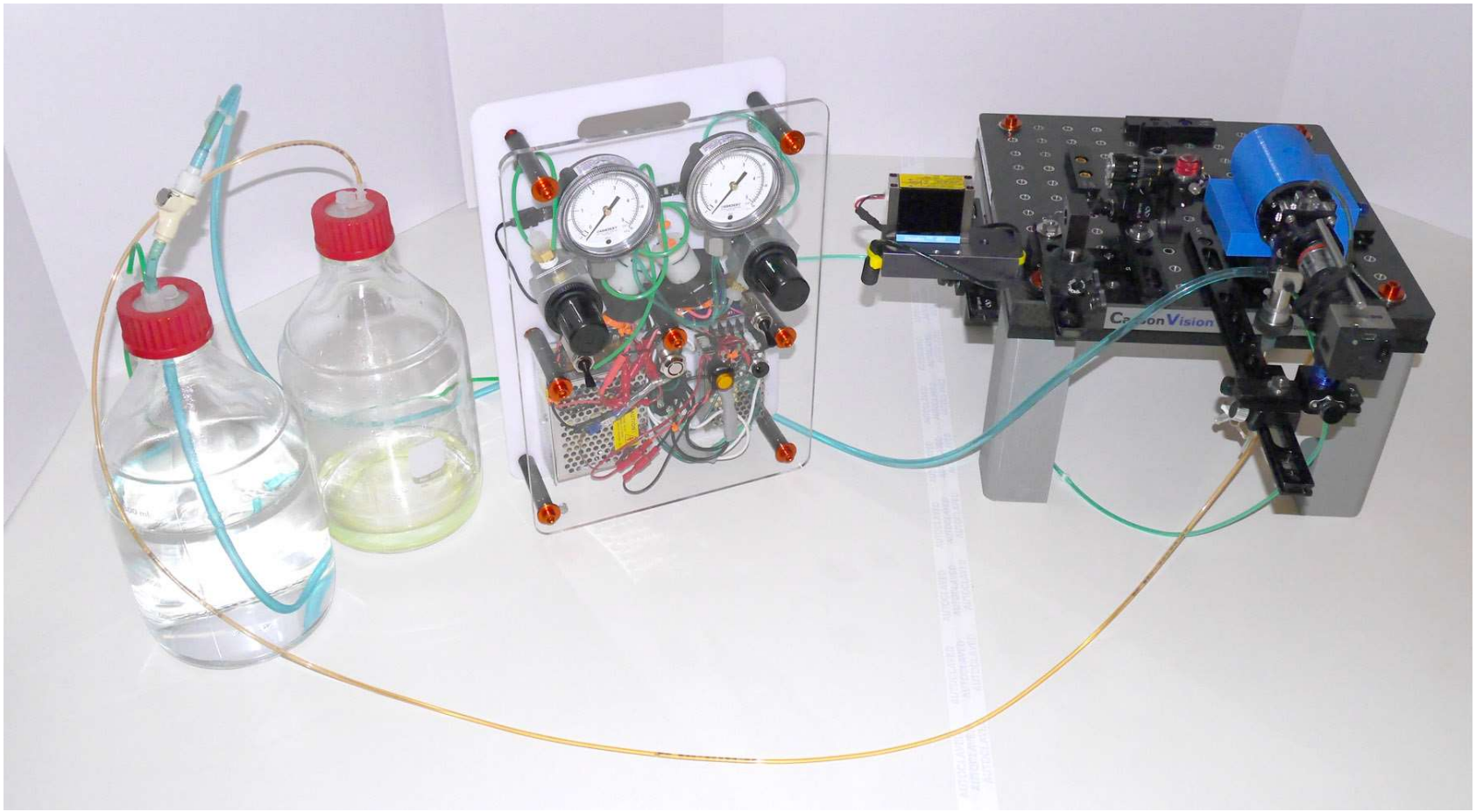
We now need to connect the fluidics (or “plumbing”) of the cytometer. This console provides air pressure for the sheath buffer (which focuses the sample stream) and the cell sample.



Instrument fluidics



Instrument fluidics

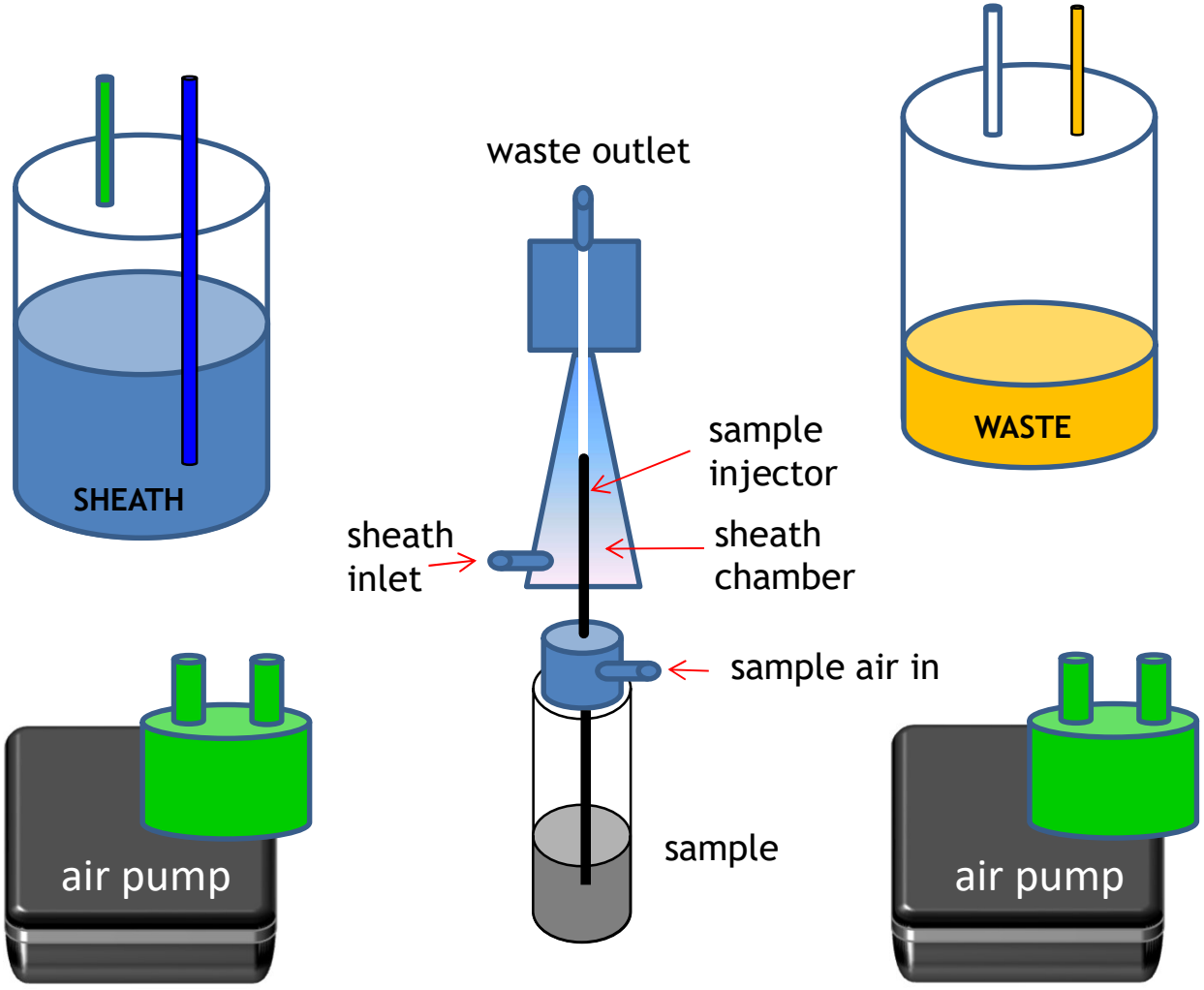


Air lines are green

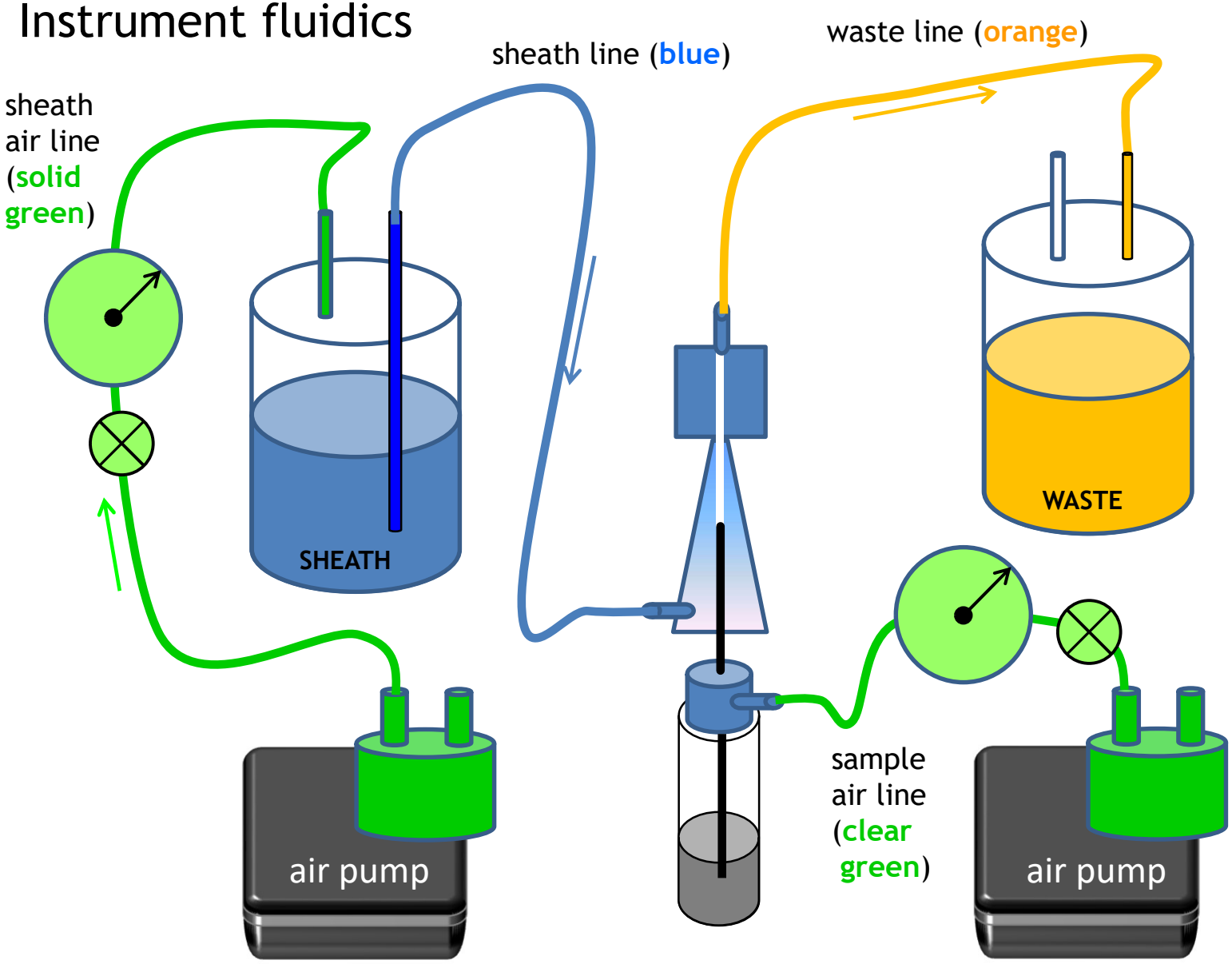
Sheath lines are blue.

Waste lines are orange

Instrument fluidics



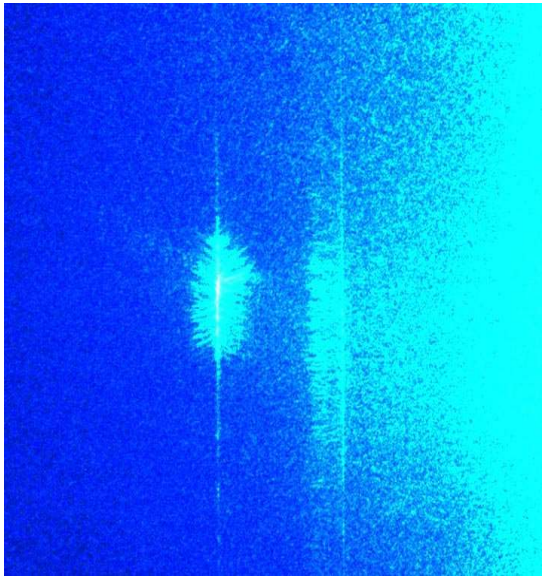
Instrument fluidics



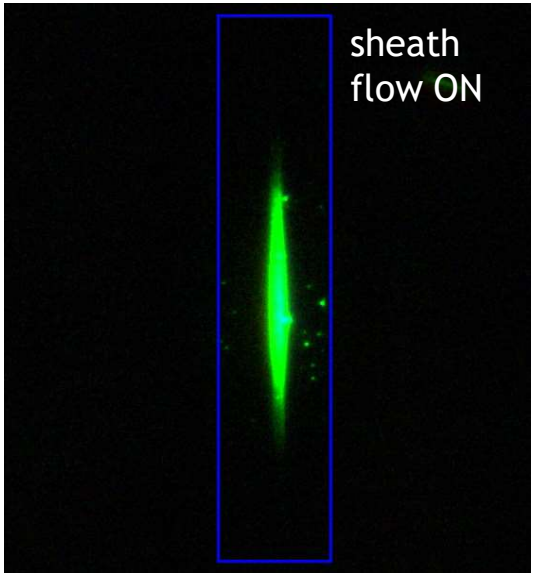
The importance of sheath pressure

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

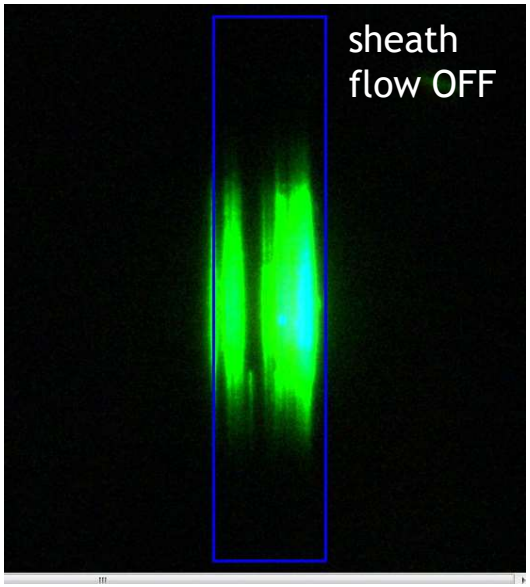
This is a camera image of a flow cell, illuminated with the laser.



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



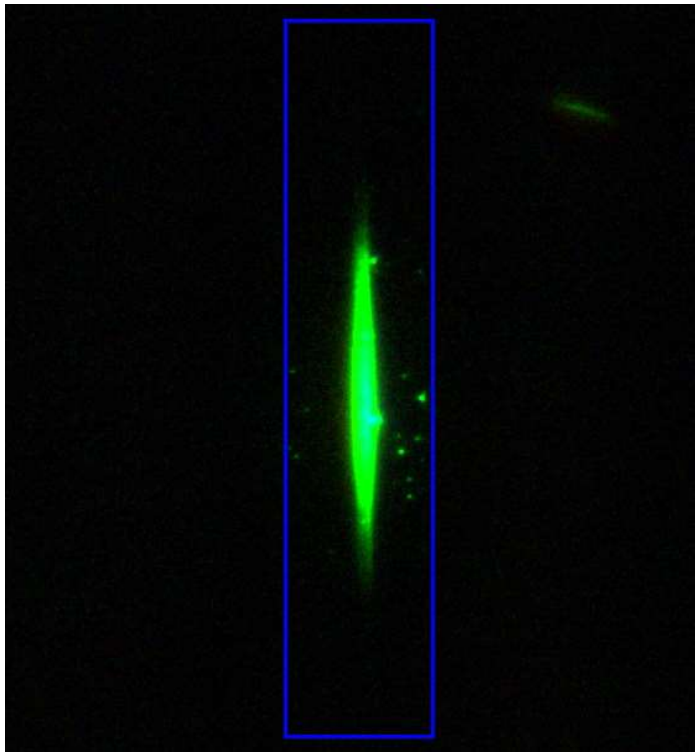
If we turn the sheath pressure OFF, the beads are no longer confined, but fill the entire flow cell.



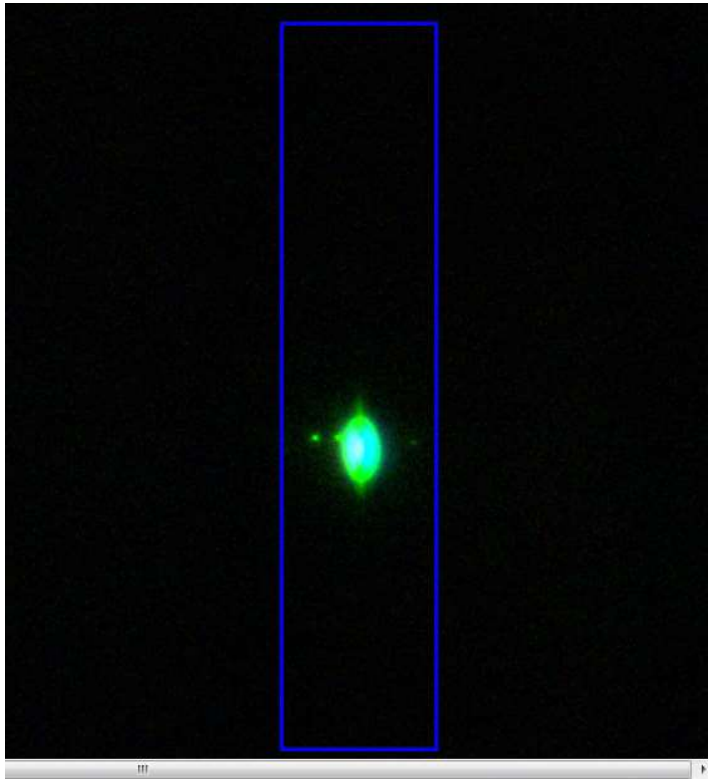
Sheath flow keeps the cells right in the laser beam, allowing accurate scatter and fluorescent measurements. Almost all flow cytometers use a sheath system.

Focusing the laser beam

An unfocused laser beam hitting the sample stream

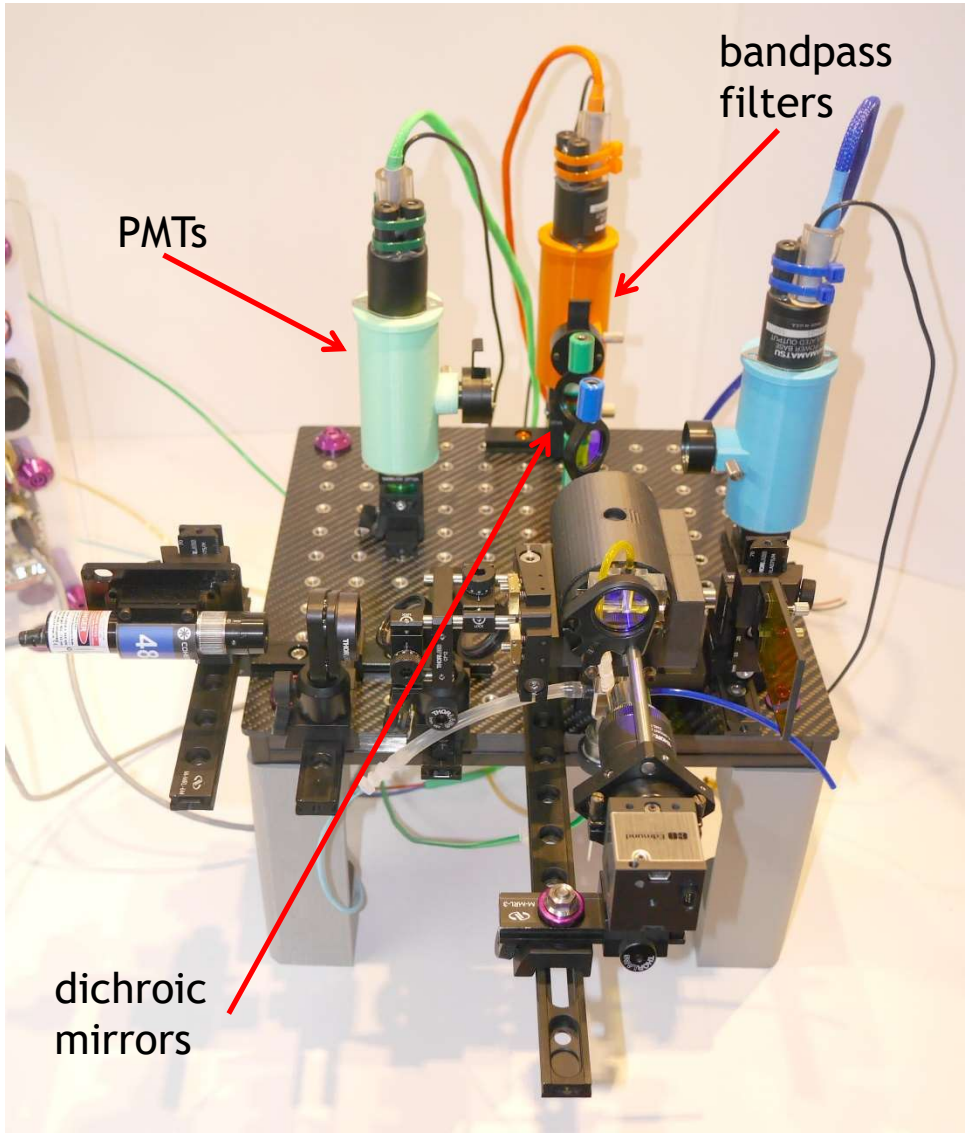


With laser focusing



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

Side scatter and fluorescence PMTs



We now install the instrument detectors and optics.

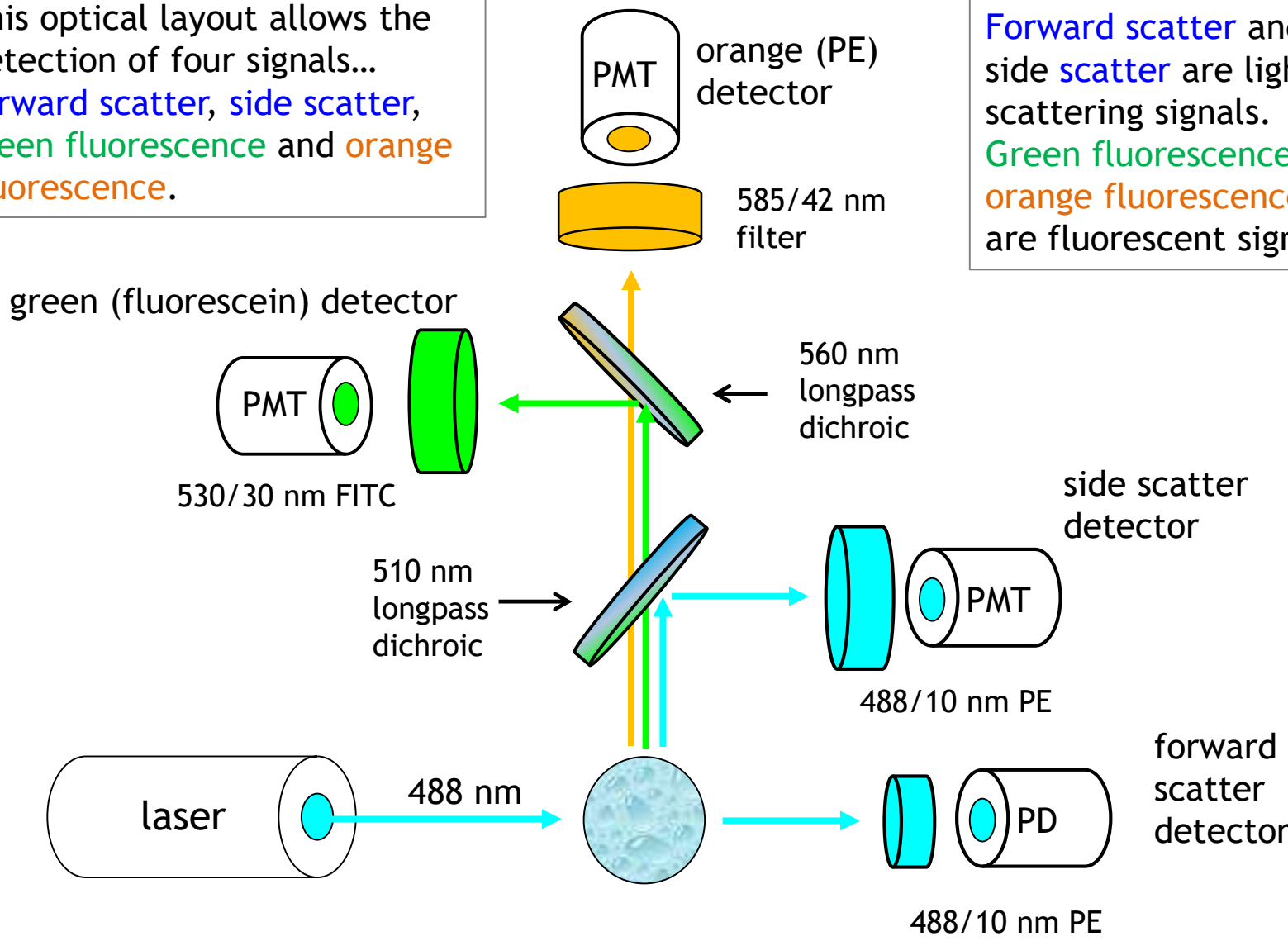
We use photomultiplier tubes (PMTs) to detect cellular fluorescence. PMTs are extremely sensitive photon detectors.

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

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

Optical layout

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



Forward scatter and side scatter are light scattering signals. Green fluorescence and orange fluorescence are fluorescent signals.

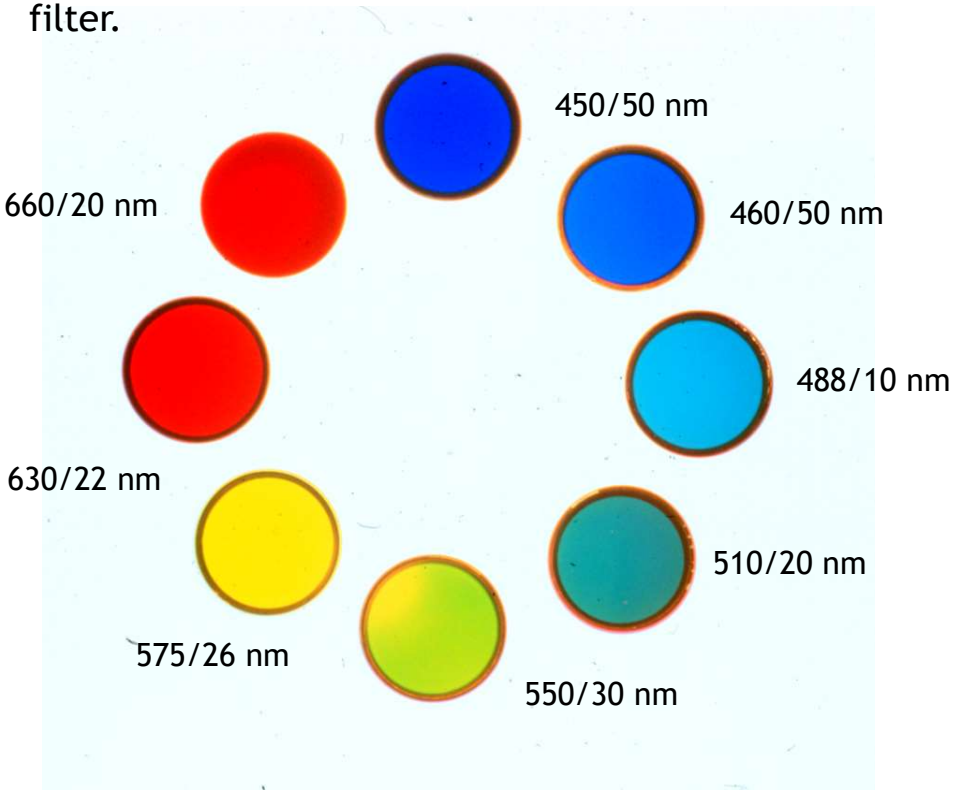
Bandpass filters

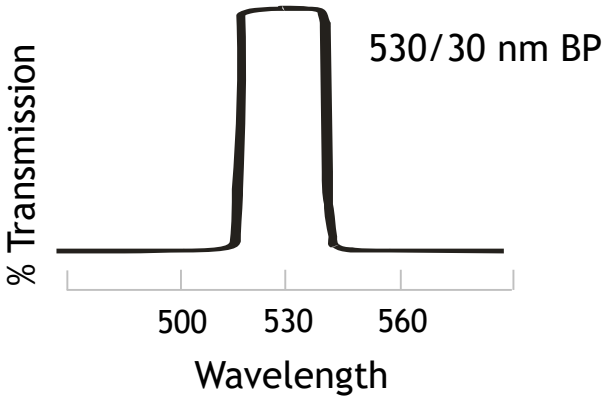
Bandpass filters only allow a certain range of light to pass through. They are used as the final filter step for fluorochrome detection.

Bandpass → BP 530/30 nm

The first number refers to the center wavelength of the filter.

The second number refers to the size of the filter window.





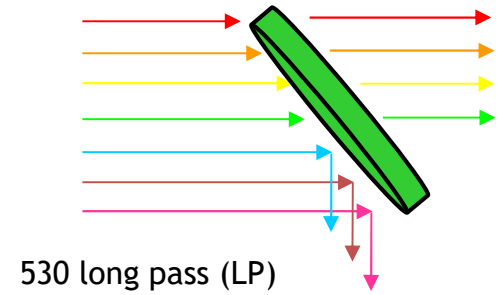
Bandpass filters are now made in a wide of center wavelengths and windows or bandwidths. Their specifications are critical when designing optical detection systems.

Filters and dichroics

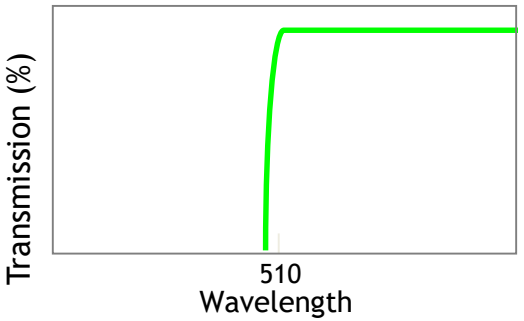
Long pass dichroics (LP) transmit light **longer** than the indicated value and reflects light shorter than that value. **Short pass dichroics (SP)** transmit light **shorter** than the indicated value and reflect light longer than that value. They separate the fluorescent signals of multiple fluorochromes.

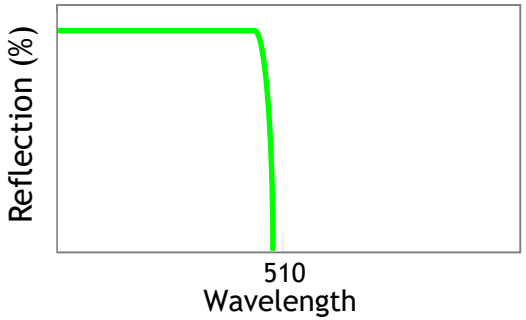
A **long pass dichroic (LP)** transmits light ***longer*** than the indicated wavelength, and reflects light ***shorter*** than that wavelength.

We use two longpass dichroics to separate the side scatter, FITC and PE signals on the MYO system.



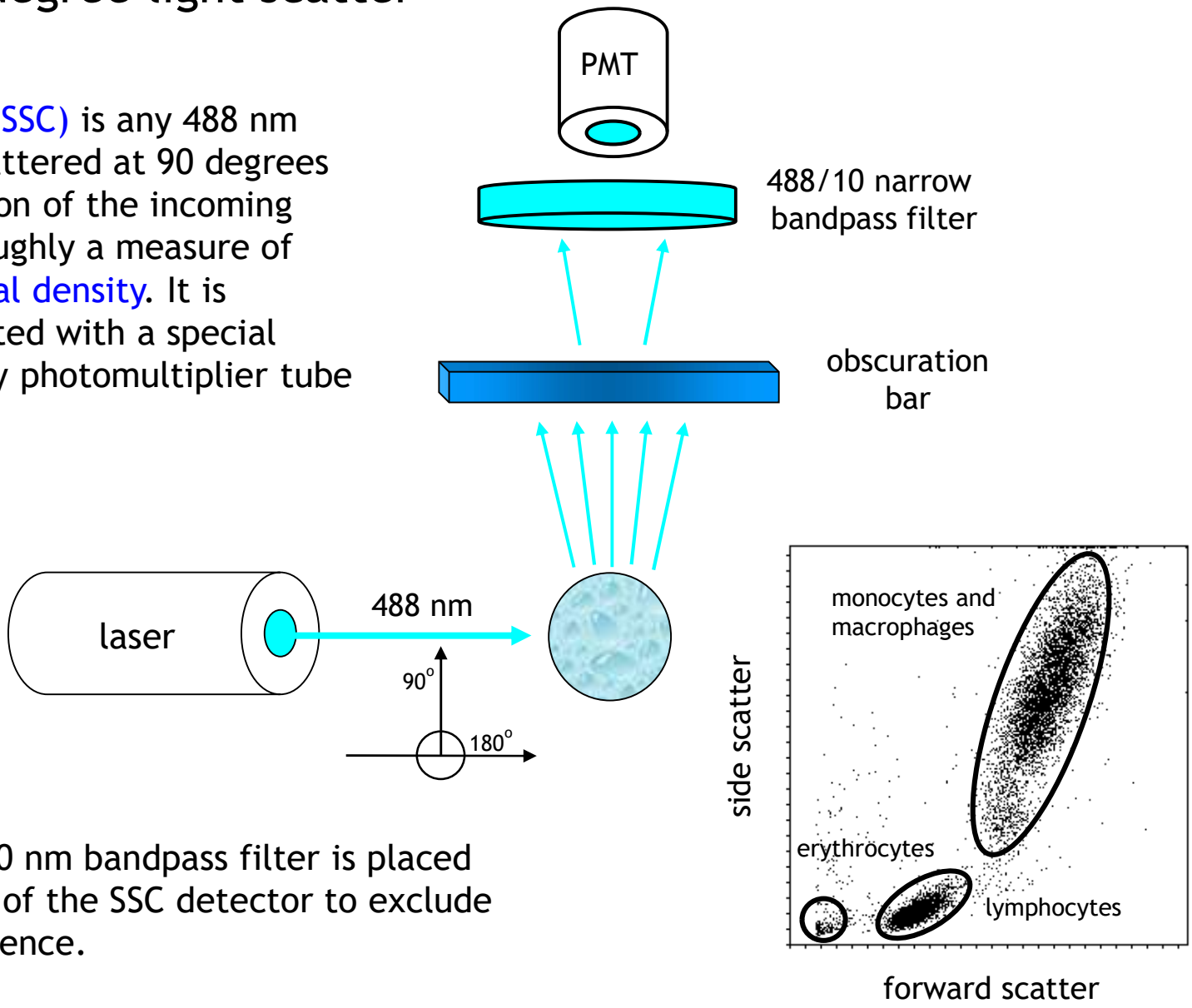
530 long pass (LP)
dichroic mirror (DM)





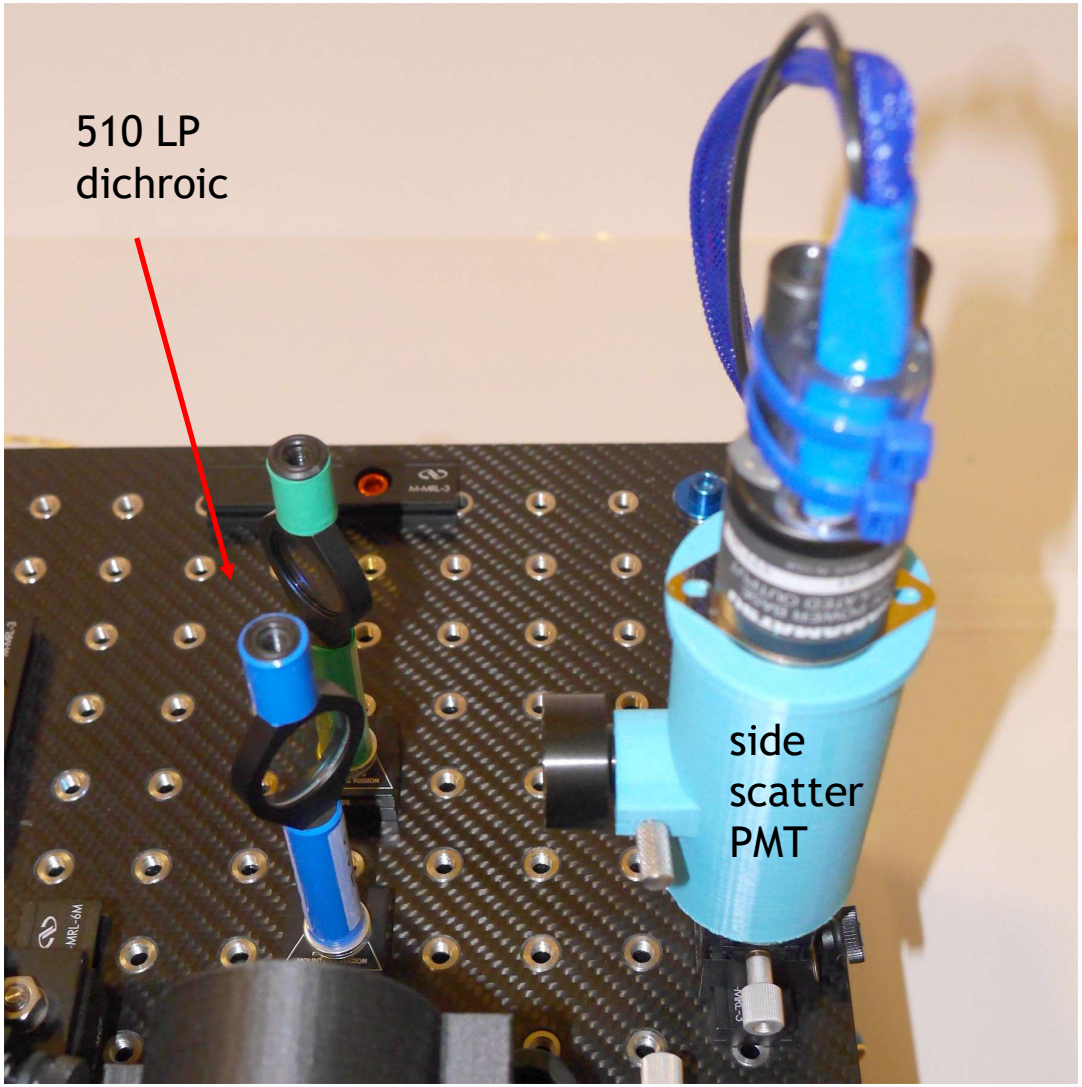
Side or 90 degree light scatter

Side scatter (SSC) is any 488 nm 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 photomultiplier tube (PMT).



A 488/10 nm bandpass filter is placed in front of the SSC detector to exclude fluorescence.

Side scatter



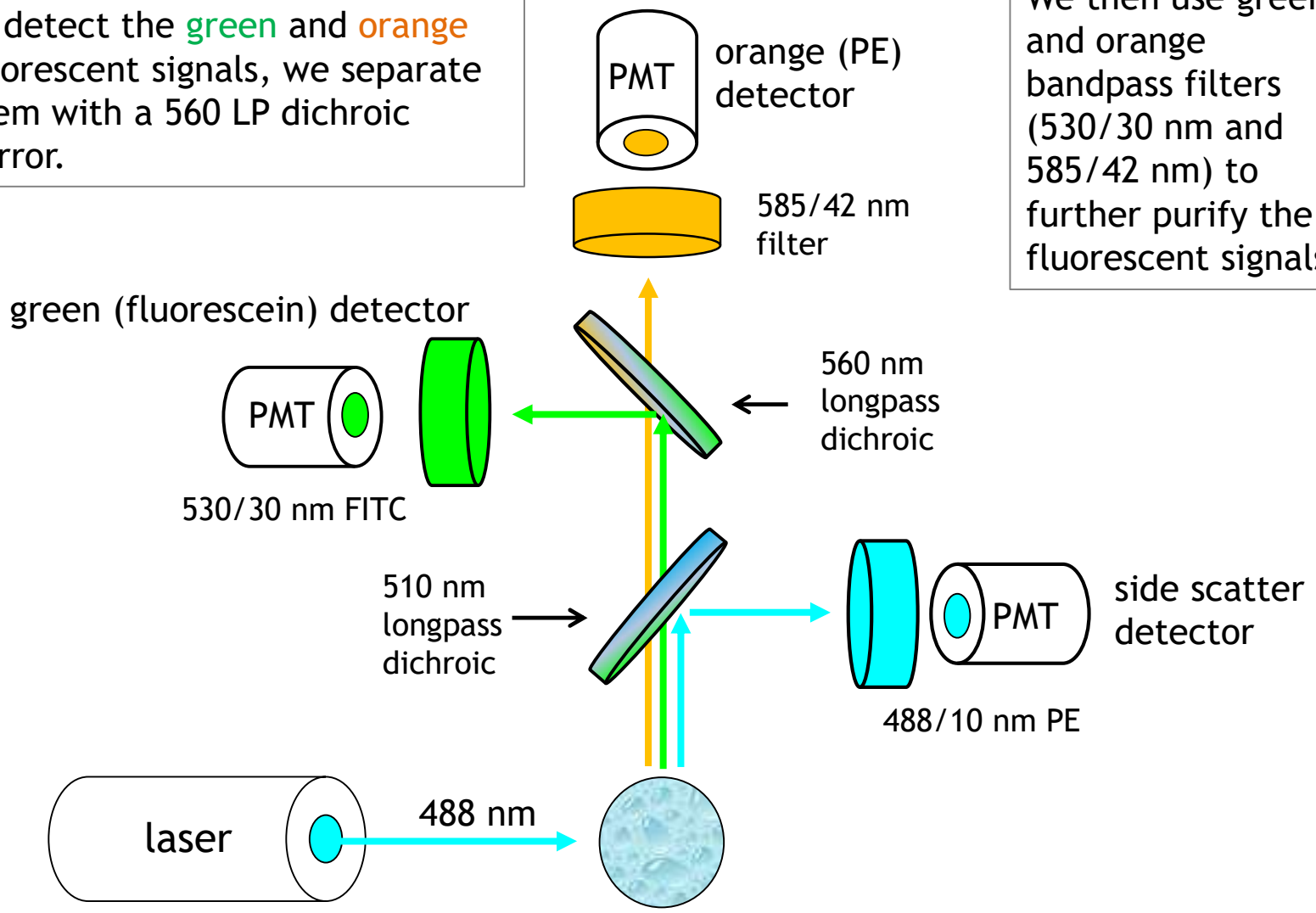
We first install our side scatter detector. Since we are detecting 488 nm laser light, we use a 510 nm longpass dichroic mirror to reflect the signal to the detector.

The longer green and orange fluorescent signals will pass through the dichroic mirror to reach their detectors (not yet installed).

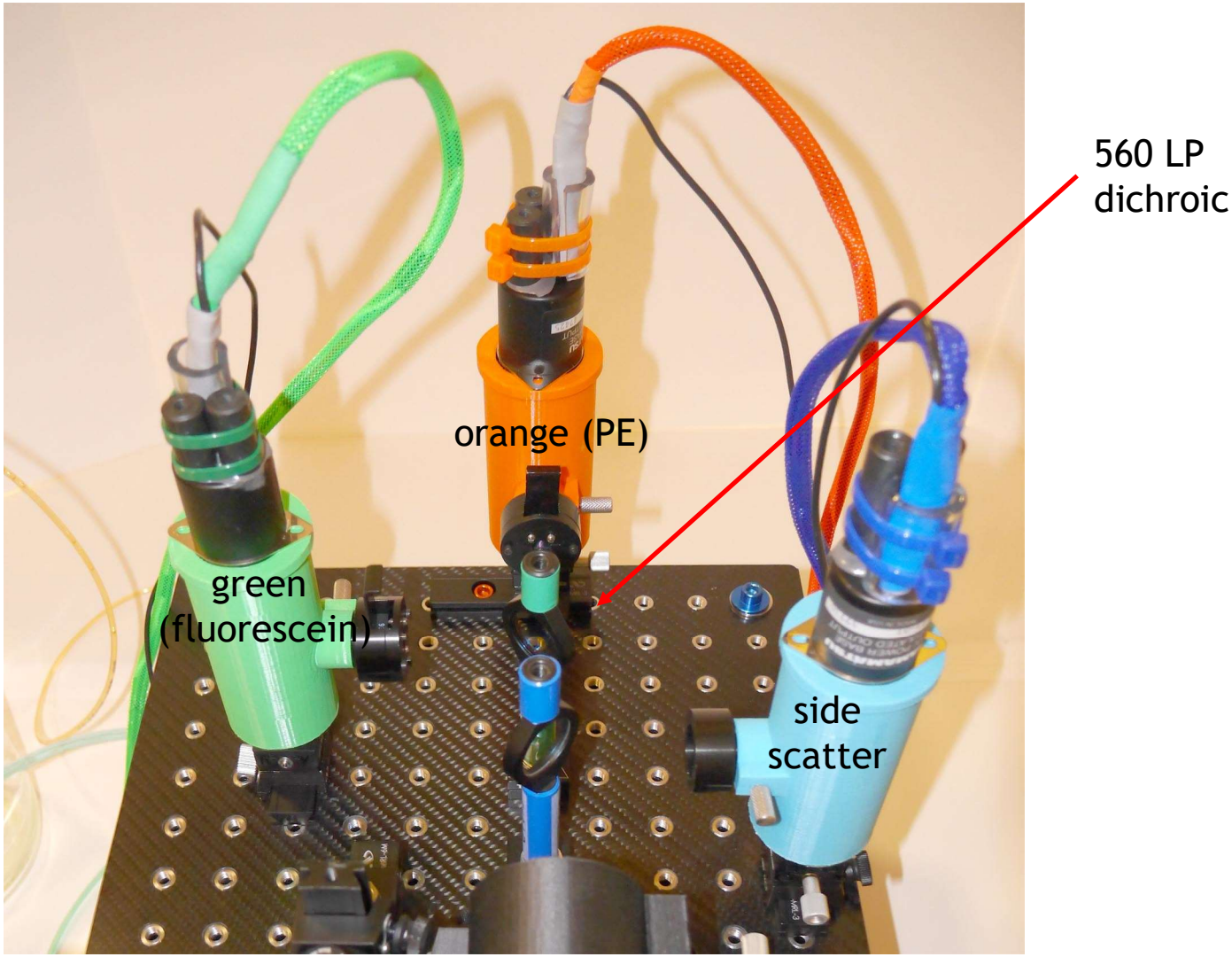
Fluorescence detection

To detect the green and orange fluorescent signals, we separate them with a 560 LP dichroic mirror.

We then use green and orange bandpass filters (530/30 nm and 585/42 nm) to further purify the fluorescent signals.

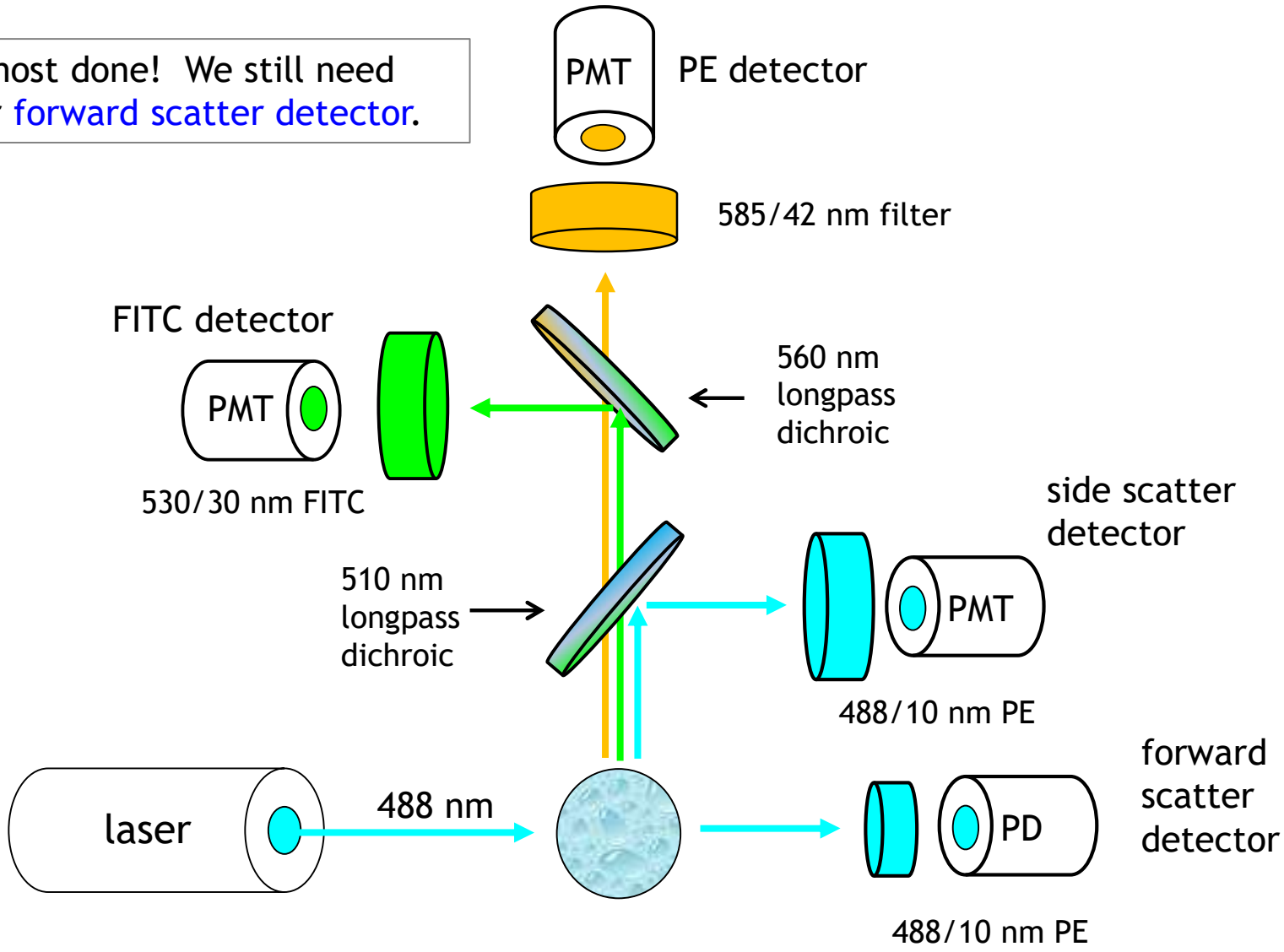


Side scatter and fluorescence PMTs



Final optical layout

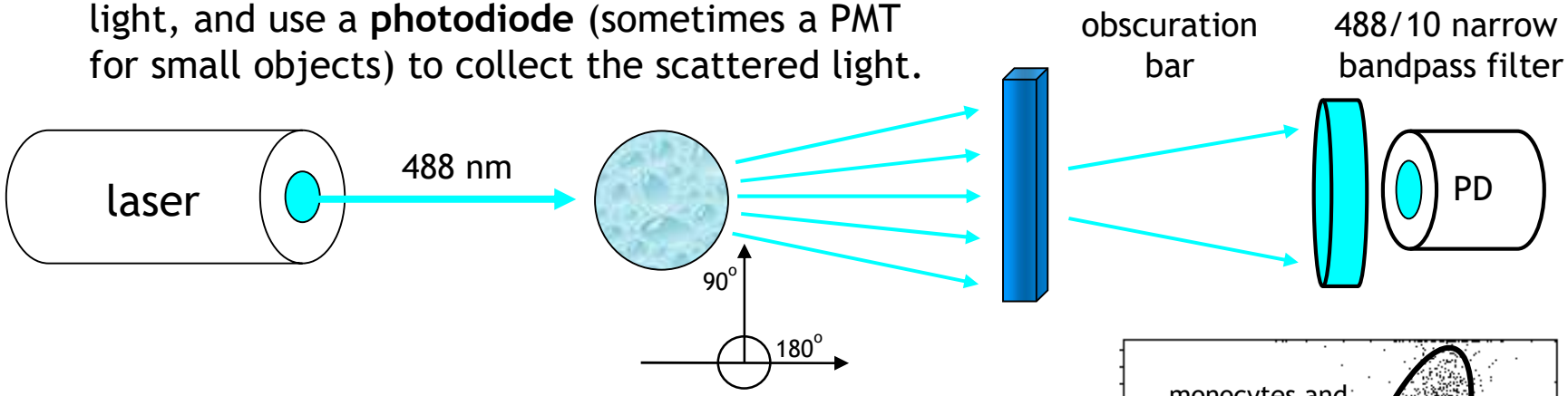
Almost done! We still need our forward scatter detector.

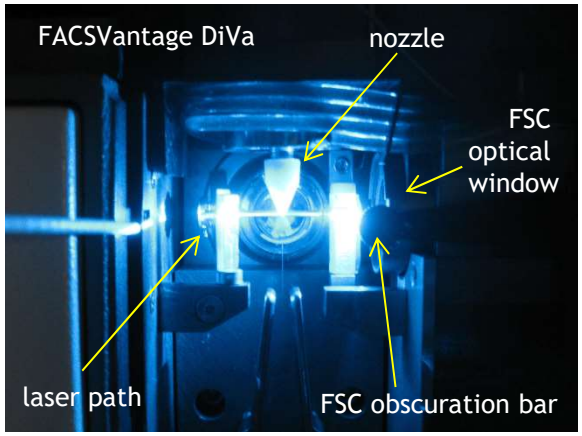


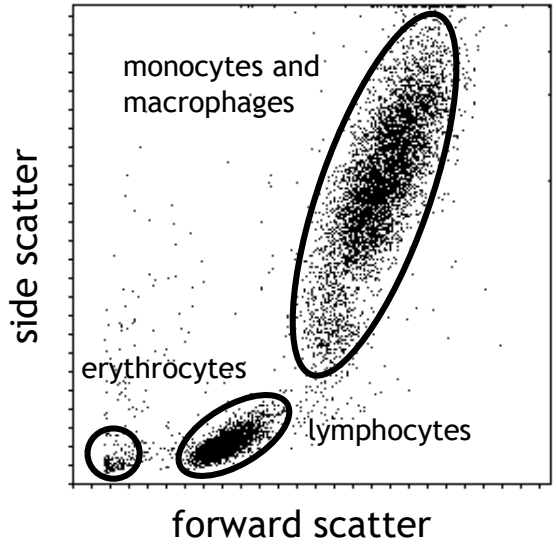
Forward light scatter

We use the 488 nm laser light scattered by the cell at a 180 degree angle to measure **forward scatter**, a rough measure of **cell size.**

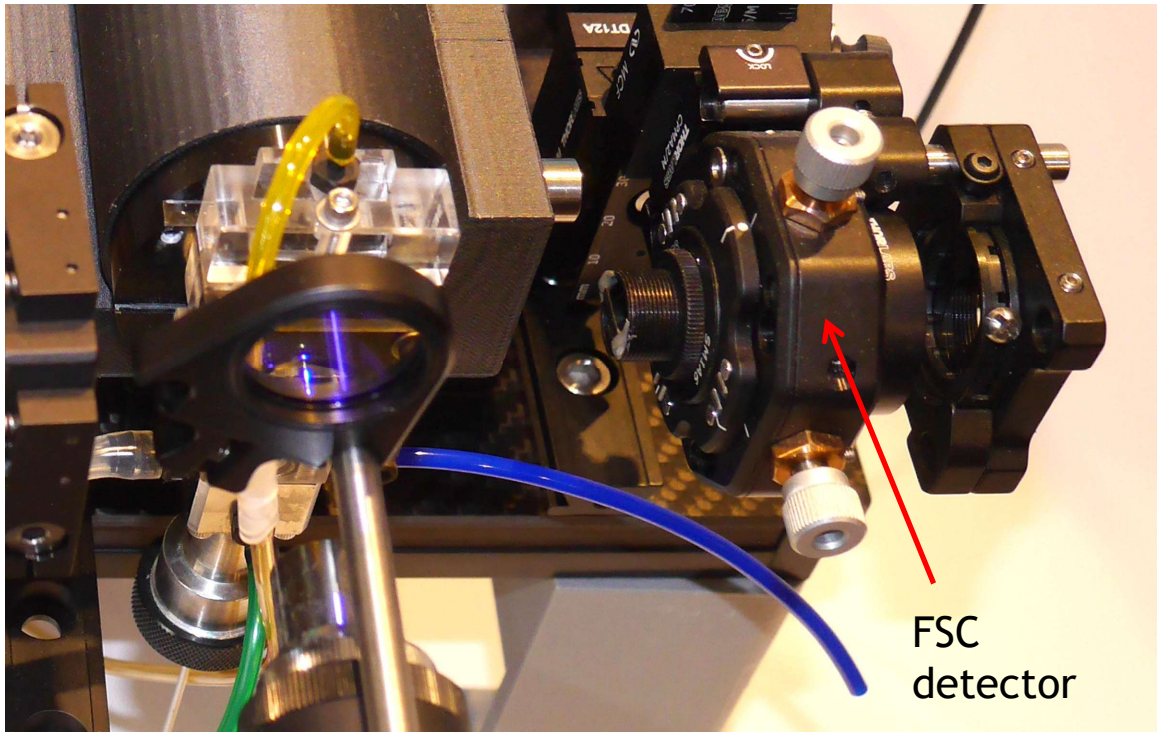
Once the laser light strikes the cell, we use a narrow metal bar (called an obscuration bar) to block unscattered laser light, and use a **photodiode** (sometimes a PMT for small objects) to collect the scattered light.







Forward scatter detector

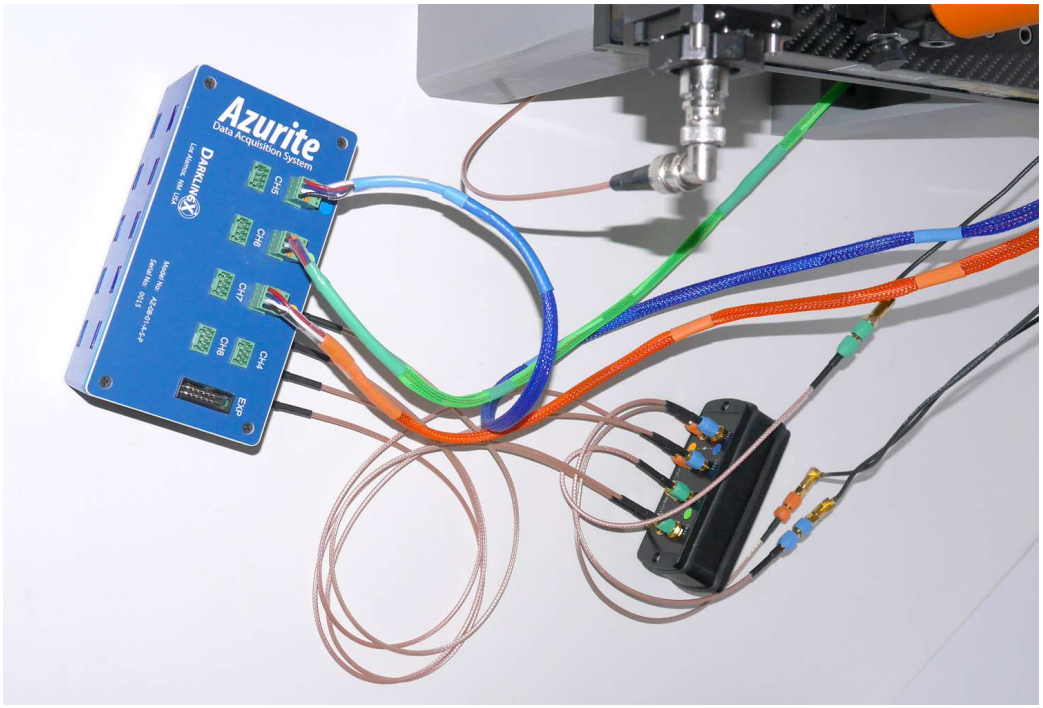


Finally we install our forward scatter detector. This is a photodiode (not a PMT), since the signal is very strong.

It is installed at a 18-degree angle to the incoming laser beam to detect scattered laser light (cell size).

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.

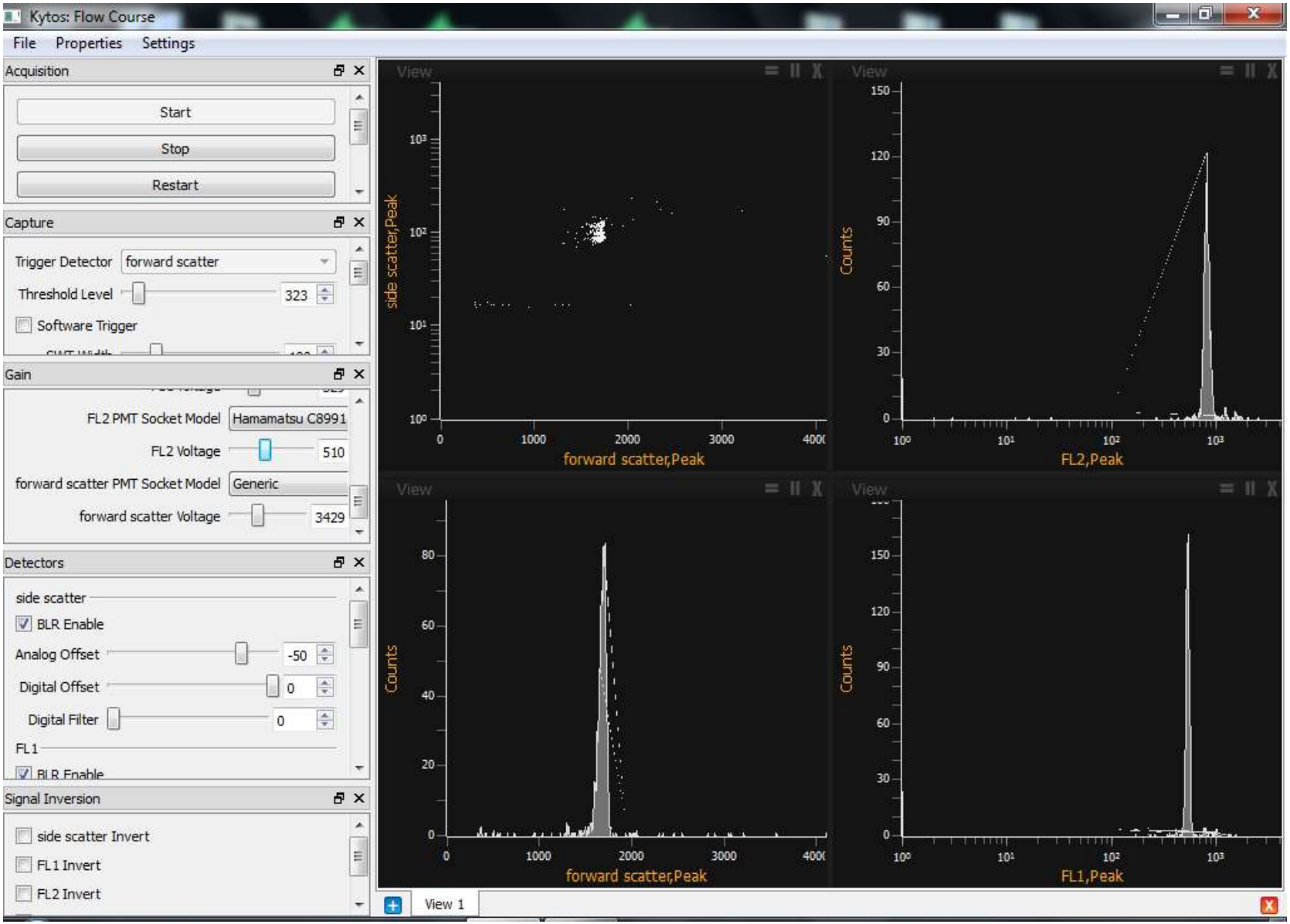
The data is then sent to a **computer** for processing and display.

The colored cables supply power to the PMTs.

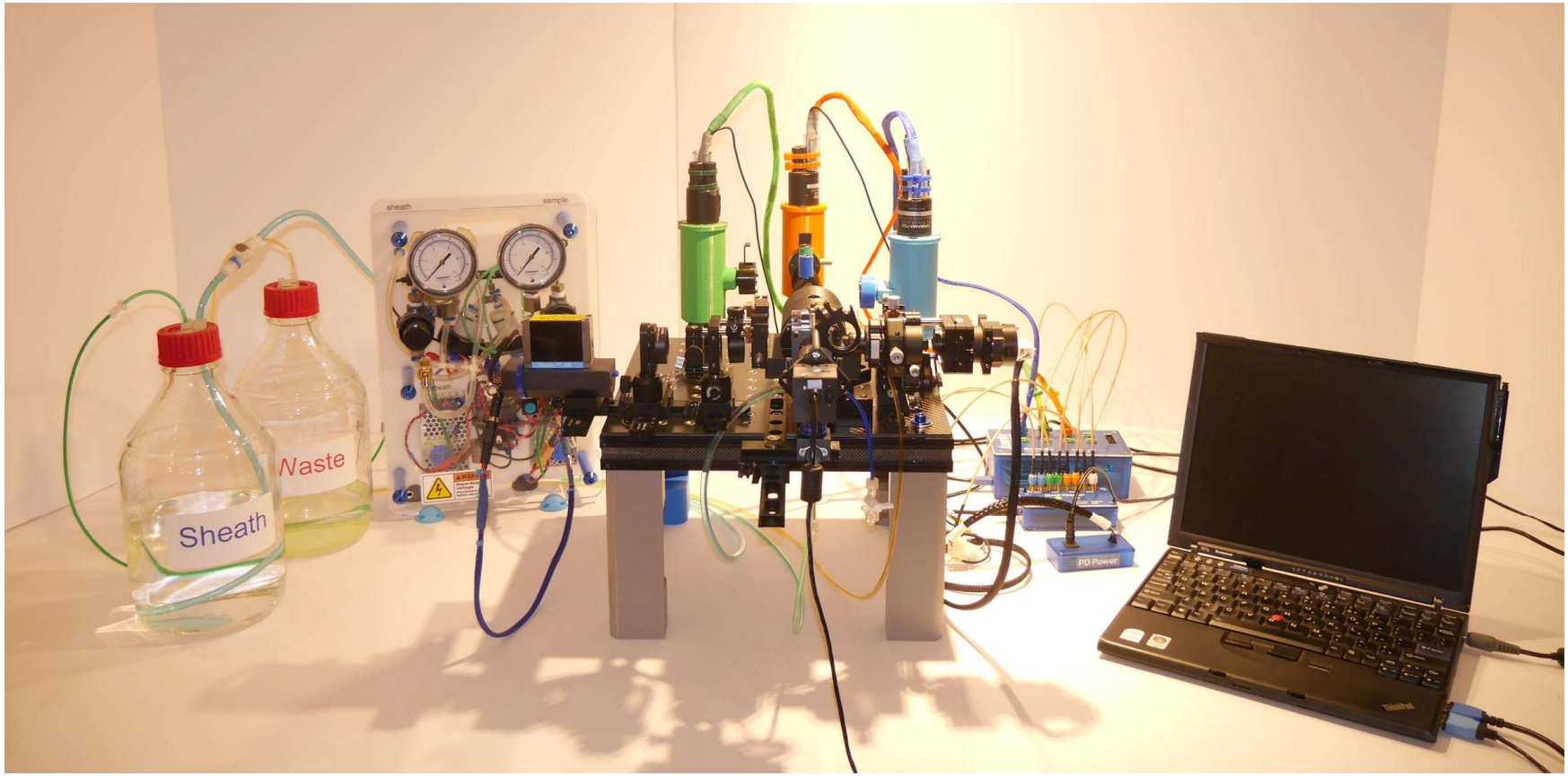
Mark Naivar

DARKLINGX

Kytos acquisition software



Finished!



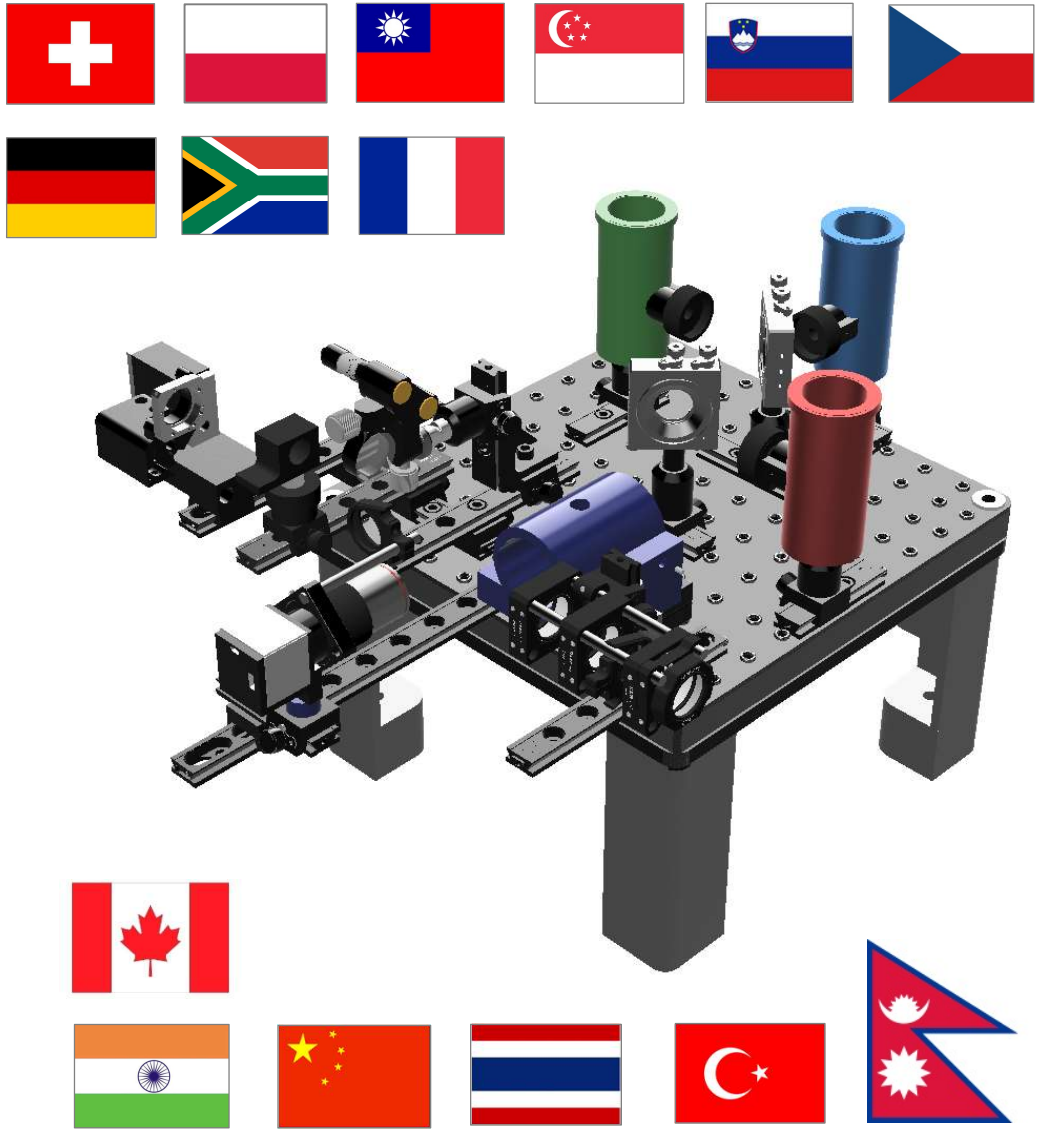
The Make Your Own Flow Cytometer Lab

The MYO lab has been conducted in 13 countries over the last six years.

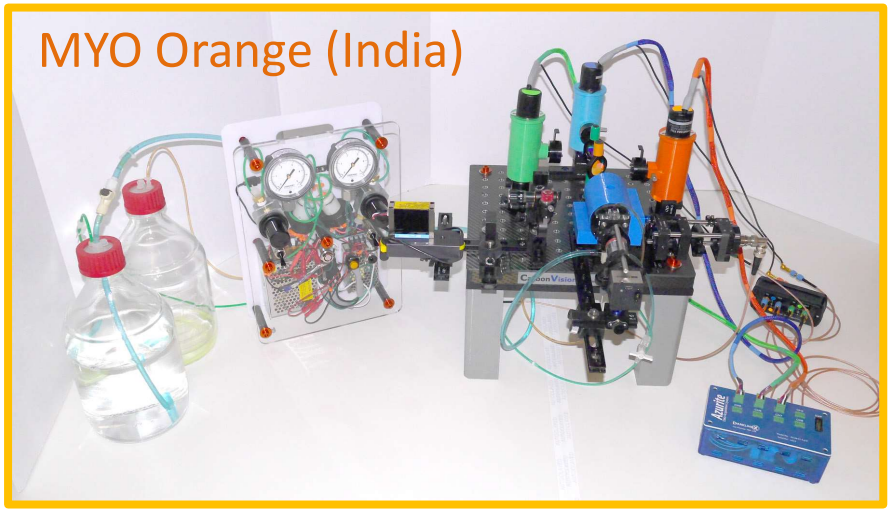
Like the original BYO Build Lab, it is a hands-on experience … something we can't do right now.

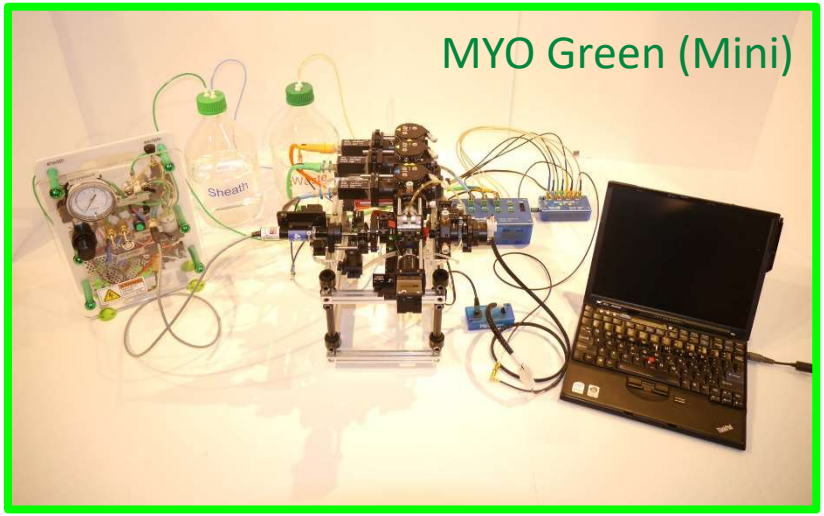
We've put together a video presentation of the lab with live commentary.

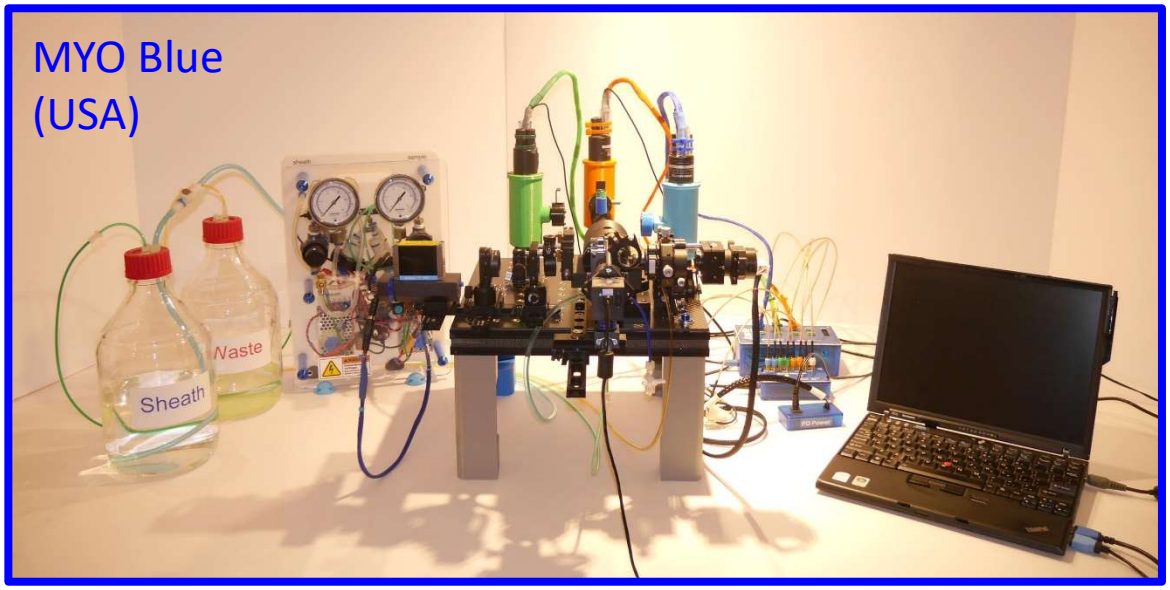
And hopefully we will bring the system for your university, institution or workshop very soon!

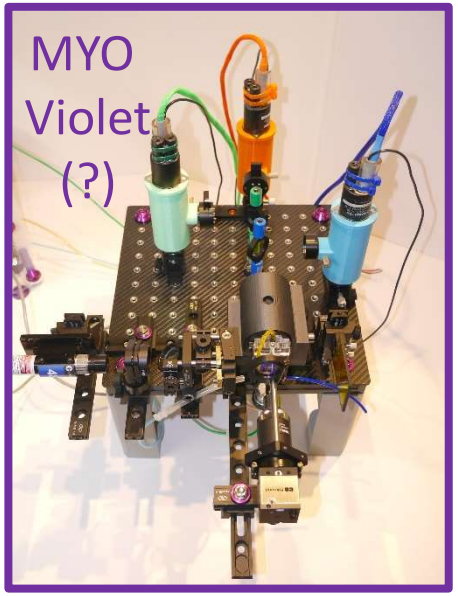


The MYO family…










Alan Saluk
Lucknow India 2017


Bill Telford
Nepal 2019


Hemant Agrawal
Uttara Chakraborty
MIMR, BLR INDIA

Make Your Own Cytometer link:

http://www.cytometryworks.com

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

International Society for the Advancement of Cytometry (ISAC)

http://www.isac-net.org