Flow Post-its
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Under Pressure

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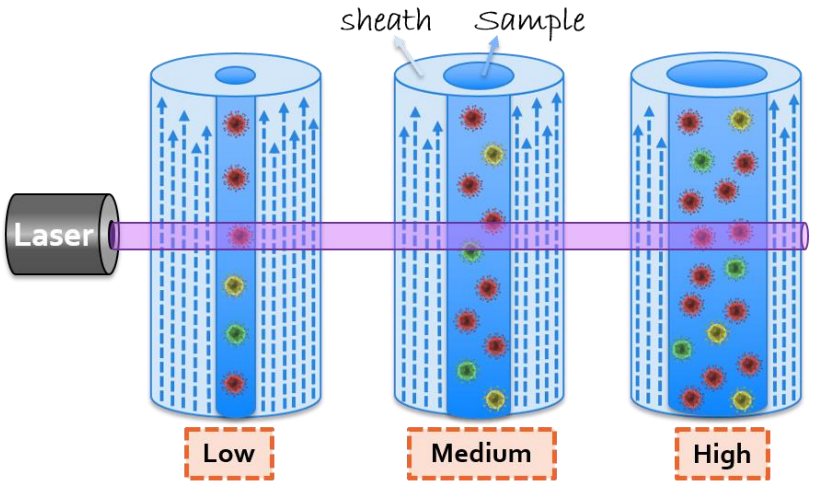
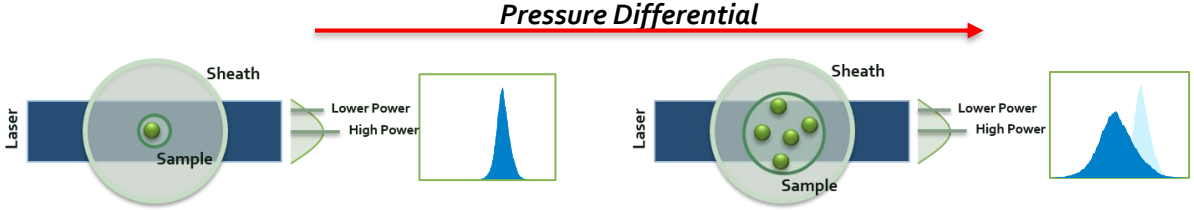
Flow cytometers rely on a stable **fluidics** setup for a successful experiment. **Sheath fluid,** typically PBS or H_2O, provides a **vehicle** in which a sample is directed through one or more laser light sources. Understanding sample delivery and the pressure differences between sheath and sample is critical when working on flow cytometers.





Flow Rate & Resolution

- Due to the gaussian distribution of power for most lasers, widening the core stream can result in cells being sub-optimally excited by the "edges" of the laser.
- When this occurs, **MFI** can decrease and **rSD** can increase.

If increasing flow rate:

- Has no impact on population distribution: *Use high flow rate*.
- Changes population distribution: *Use low flow rate and increase cellular concentration.*

- **Sheath pressure** remains *constant* during your experiment. Analyzers typically run at a set pressure, whereas sorters can operate at a range of pressures, depending on nozzle size and applications.
- **Sample pressure** is *variable* and can be adjusted to increase or decrease event rate.
- Samples are injected at a higher pressure, allowing for a core sample stream to form.
- **Hydrodynamic focusing** occurs due to the differential pressure between the sample and the sheath, allowing for the layers to stay separate. Poor sample preparation and high aggregates can disrupt the separation of layers.
- Increasing sample pressure results in a *wider core stream*, which can lead to multiple events passing a laser at one time, leading to **electronic aborts**.
- Best practice is to **concentrate your samples appropriately** and **run at a lower** flow rate where cells are best focused.

Memorial Sloan Kettering Cancer Center
Flow Cytometry Core Facility
https://fccf.mskcc.org
Whitehead Institute