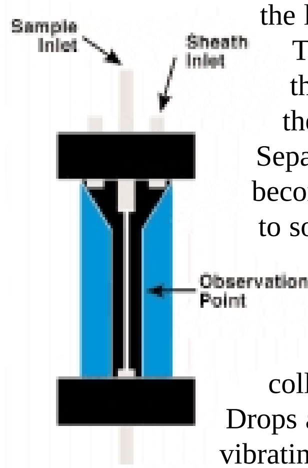
signal sent by red blood cells. This allows the machine to detect other types of cells (of different size) that are less abundant such as lymphocytes, monocytes, or neutrophils.

The Eye of the Machine. The Flow Chamber is the observation point and is similar to a small test tube (cuvet). The cuvet contains the sample stream that is surrounded by a second liquid called ***sheath fluid***. Fluid mechanics tells us that, if one smoothly flowing stream of fluid (the core stream) is put into the center of another smoothly flowing stream of fluid (the sheath stream), the two streams will not mix much; this is a condition called ***laminar flow***. Changes in how fast the fluids flow may be desirable at times. A slower rate means the sample will spend more time in the illumination chamber, and will scatter and emit more light.



On the Same Wavelength? Most fluorescent materials send out signals over a fairly broad range of wavelengths. For example, fluorescein isothiocyanate (FITC, pronounced FIT-SEE) is described as green fluorescent, but that is just what your eye is telling you—if you look at it under a fluorescence microscope, you see green. If you measure the spectrum of FITC in a spectrofluorometer, the emission maximum is in the green spectral region. To measure FITC fluorescence on a flow cytometer, a detector is fitted with a green band pass filter that identifies signals between 510 nm - 540 nm. However, the emission spectrum of FITC isn't a steady signal registering between 510 nm and 540 nm. The FITC signal resembles a bell curve that extends above and below the 510-540 nm detection range of the green filter. When using multiple dyes there is a spectral overlap. What that means is that the signals from a green dye will overlap with signals from a red dye as each of their signals spread beyond the range of their respective filters. The values that overlap with another dye are subtracted to determine accurately the true level of fluorescence emitted by each dye; this process is called ***compensation***.

One at a time, please. Sometimes cells in a sample stick together, or **aggregate**. When this happens, they appear larger and generally have a different shape than single cells. This causes an increase in both forward scatter and side angle scatter because there is a larger object from which the light will reflect. Depending on the size of the aggregate, the light will reflect at different angles. Therefore it is fairly easy to discriminate these cells with a scatter plot and exclude them from the desired cell population. Separating aggregates from single cells becomes even more important when it comes to sorting.

Sorting depends on looking at individual drops containing cells, and not just analyzing them, but collecting them based on their attributes. Drops are formed from the sample stream by vibrating the flow nozzle at a high frequency. Drops and cells both have a charge associated with them, and as the drops pass between two highly charged plates, they are moved to one side or the other of the main sample stream, based on their electric charge. The charge will determine whether the cell will be kept or sent into waste. ***Doublets***, cells pairing together as they travel through the detection point, cause confusion to the processor about whether the drop should be collected or not. Usually, both cells are sent to the waste receptacle to preserve the purity of the sorted cells. Since cells don't march through in single-file, it is not likely to have 100% recovery in cell sorting.

Apply the Knowledge. Flow Cytometry has a multitude of uses. Cell number, cell type, binding capacity and specificity, are all very easy to measure using Flow. It is considered the most efficient method to determine cell cycle stages. The most important thing to remember in preparing to do Flow Cytometry is the need for the cell sample to be in a monodisperse suspension—in other words, no aggregates. As a result of the physical properties of the machine itself, it is clear that the more separated the cells are within a solution, the better the results will be.