

Flow Cytometry and the Machines (continued)

The Cytometry Research machines each have an argon laser that emits photons of a 488 nm wavelength. Dyes used to prepare cells for a flow cytometry experiment on our machine must be able to be excited by 488 nm photons. Some commonly used dyes are FITC, PE, GFP, 7AAD, and Propidium Iodide.

Not a Straight Shot from Point A to Point B.

When traveling through a medium such as air, there are superficial interactions between the photons and particles in the medium. When light passes from one medium (such as air) and encounters a denser one (such as a cell), there can be a change of direction. The light that “bounces off” is described as **reflection**. The light that travels into the medium is described as **refraction**. The bigger the difference between the media (for example, between air and a cell) at the point of contact, the more likely it is that light will be scattered.

Monitoring the Light Paths. The parts of the Flow Cytometer that actually detect the differences in the light paths and collect the photons are called **photodiodes**. Photodiodes are silicon solid-state devices that take photons in and put electrons out. A **photomultiplier tube** is used to measure the weaker side angle scatter and fluorescence levels.

The Difference Between Red and Blue. In order to detect color emissions of the variety of fluorescent markers available, there needs to be a way to separate the different wavelengths in the spectrum.

Mirrors with dichroic coatings transmit certain wavelengths of light, while reflecting others.

Dichroics separate the different colors of light based on wavelengths. With a long reflect setting, shorter wavelengths pass through. With a short reflect setting, longer wavelengths pass through. The picture below illustrates how this works. As light passes through each of the dichroics, a color is reflected toward a detector rather than being transmitted through to the next dichroic. At the end of the path the only color left is the original blue light from the argon laser, and that is detected as side scatter.

Adjusting the Controls. There is no cytometry (measurement of physical and/or chemical characteristics of cells) to go along with the flow if the detectors are not set properly. The machine has to be set to differentiate between cells, debris, and air. Cells arrive at random times, rather than fixed intervals. In order to make any useful measurement, there must be a way to determine whether or not there is a cell present in the measurement region. In other words, the signal from one of the sensors must be significantly different when a cell is present than when one is not. This signal is called **the triggering signal**. When the triggering signal is above a threshold level set by the user, a measurement is taken. The threshold level is usually linked to particle size and direction of travel (forward scatter). The level is not linked to fluorescence. For example, to analyze a sample of whole blood, we could set the threshold level above the level of the triggering

