FLOW CYTOMETRY

PROBLEM

Flow cytometry measures cell properties one cell at a time. As such, the technique works with extremely weak optical signals that can be easily overwhelmed by background noise. High signal to noise ratio (SNR) and high temporal resolution in flow cytometry signals are thus critical requirements for successful measurements by this technique. Achieving these signal characteristics places extreme demands on the quality of the optical, electrical, and mechanical components in flow cytometry systems. For example, laser light sources, optomechanical components, and optical components must have exceptional accuracy and stability, while narrowband filters must be spectrally stable and have high optical density (OD) to minimize background interference. It is often problematic to source and assemble compatible optical/optomechanical components into a flow cytometry system with adequate SNR and temporal resolution.

BACKGROUND

Flow Cytometry characterizes, sorts, and collects different cells from a heterogeneous mixture. The method measures the properties of individual cells or particles as they pass through a beam of light, typically a laser. The laser and/or light source is scattered and excites biomarkers or labels on the cells in a sample stream and counts and sorts the number of cells having specific scattering or fluorescent responses. The properties that can be determined from the scattered light and fluorescent emissions include relative particle size, relative granularity or internal complexity and relative fluorescence intensity. Cells can be counted and sorted, according to their light scattering or fluorescence properties. The advantage of flow cytometer over other forms of cell sorting is that a number of measurable parameters can be detected simultaneously and used as counting and sorting parameters.

Flow cytometry is used to determine the presence of antigens either on the surface or within cells, for the analysis of DNA or RNA content, and for functional studies on cells. Flow cytometers find use in:

- Medicine
  - Hematology
  - Oncology
  - Immunology
- Genetic testing
- Biochemistry and molecular biology (e.g. proteomics, glycomics)
- Marine Science
- Biosynthesis
- Cell health and biology (including stem cells)
- Screening
- Cell cycle analysis
- BioProcess

A flow cytometer consists of three major systems. The first is the fluidics system that focuses and transports cells to create a narrow, single particle wide stream (the size of a single cell or particle is typically ~30 um diameter) that can be analyzed by a laser beam. The

second is the optical system composed of lasers or light sources that illuminate the cells in the sample stream along with optical mirrors, filters and beamsplitters that direct the light signals from the sample to optical detectors for counting and processing. Dichroic mirrors/filters with precision coatings are needed for their narrow transmission/reflection bands and for their high optical density for out-of-band rejection. All photonics components require ultra-precise and stable motion/position control to achieve alignments that must have µm resolution. The third system is the electronics signal processing, and data handling equipment that converts the detected optical signals to usable information for processing and analysis.

The Fluidics System

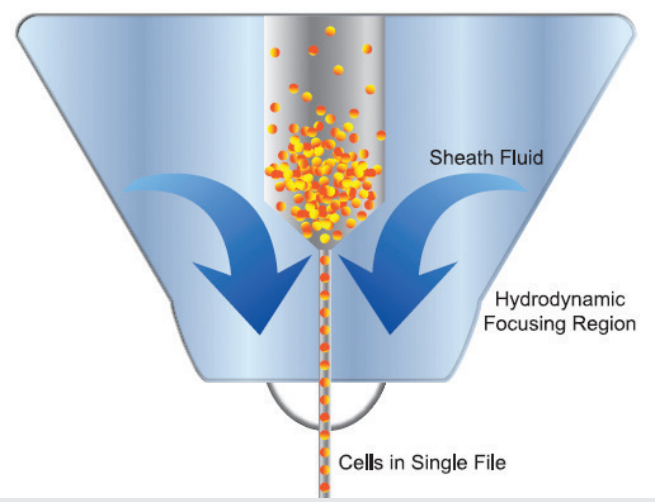

Figure 1 - Hydrodynamic focusing produces a single stream.

The fluidics system creates a stream of single particles that can be interrogated individually by the instrument's laser detection system. This is done by hydrodynamically focusing the sample stream of mixed fluid/particles using the Bernoulli effect (Figure 1). Hydrodynamic focusing can be induced when two streams of fluid having different flow rates are fed concentrically through a flow cell. The outer, sheath fluid stream has a lower flow rate than the central, sample fluid stream and, under optimal conditions (laminar flow), there is no mixing of the central fluid stream. The degree of focusing of the sample stream is determined by the shape and size of the flow cell and by the pressure difference between the sample and sheath fluid flows. Since the shape of the flow cell is fixed, the degree of focusing of the central stream is usually varied by manipulating the pressure difference between the sheath and sample fluid streams. Properly focused, the sample stream has a cross-sectional area similar to that of the cells being analyzed; within this stream the cells move in single file. This gives the flow cytometry system single-cell resolution, allowing each cell to be separately interrogated, counted, and sorted.

Optics and Detection

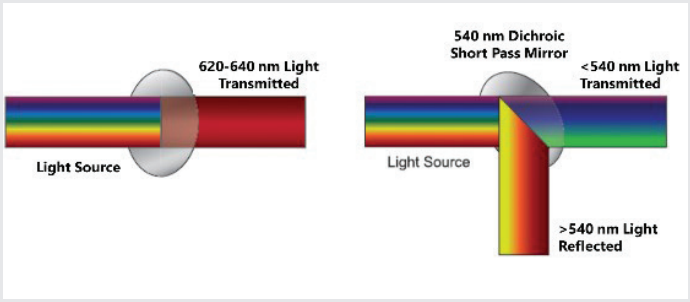

Figure 2 - Dichroic optical filters acting as either a band pass filter or a short pass mirrors.

When the sample stream is interrogated by (i.e. passes through) a focused laser beam, the entrained cells within the stream scatter the laser light in either forward and sidewise directions and this light can be measured and used to count, characterize, and sort the cells. Forward Scattered (FSC) light provides information on cell particle size while Side Scattered (SSC) light provides information on the relative granularity and internal complexity of the cells. Cell populations can be separated based on FSC and SSC, but more often separation is accomplished through the detection of a specific protein attached to a cell using fluorescence. A fluorophore that emits

light after laser excitation is used to stain the protein of interest and cells containing this protein fluoresce, allowing them to be detected and separated in the cytometry analysis. By combining the FSC and SSC information with fluorescence labelling, it is possible to differentiate and collect different types of cells in a heterogeneous population such as blood.

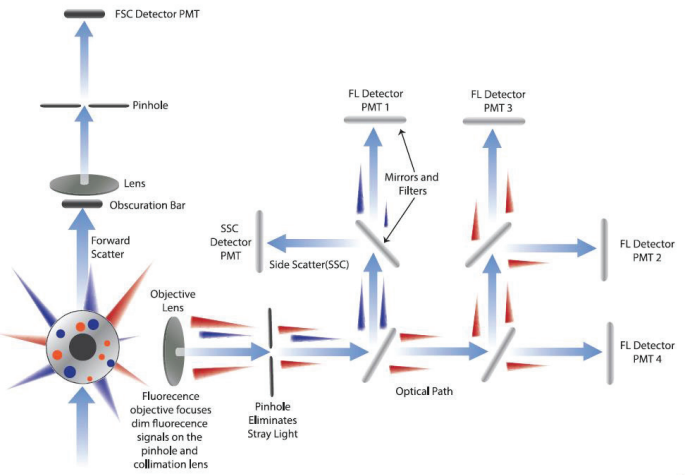

Figure 3 - Schematic overview of the optical configuration of a typical flow cytometer setup.

The optical detectors in flow cytometers are usually PMTs with the specificity of detection controlled by optical filters and mirrors. There are three major filter types: long pass filters that transmit light above a cutoff wavelength; short pass filters that transmit light below a cutoff wavelength; and band pass filters that transmit light within a narrow band of wavelengths. Dichroic filters that block light by phased reflection, allowing only certain wavelengths of light to pass through while interfering with other wavelengths are used in cytometers (Figure 2). When placed at an angle to the oncoming light a dichroic filter acts as a mirror allowing it to perform two functions: transmitting specific wavelengths in the forward direction and reflecting light at a 90° angle. Figure 3 shows how these filters are used to direct light of selected wavelengths to different PMT detectors in a flow cytometer.

Flow cytometers require several photonics components, including:

- Lasers and other light sources with small, tightly focused beams that can illuminate a single cell or particle. Laser light with a specific wavelength is needed to excite specific labels or fluorophores on a cell.
- Dichroic mirrors/filters with precision coatings are needed for narrow reflection/transmission bands and high optical density for out of band rejection.
- Manual or motorized positioners are needed to precisely align and control all photonics components with micron spatial resolution.
- A vibration isolation system is required to insure environmental stability.

Signal and Pulse Processing

Every time a cell passes through the interrogation point, it produces scattered light or a fluorescence signal that can be detected by one of the detectors shown in Figure 3, depending on the wavelength of the signal. These signals are digitally processed and mapped and, for some instruments equipped with a sorting feature, the electronics system can initiate sorting actions that charge and deflect cells.

III. SOLUTION

MKS Instruments' Equipment for Flow Cytometry

The choice of lasers and optical components is critical to the precision and accuracy of the flow cytometer. A poor quality or malfunctioning laser in a cytometer used for medical applications can compromise data integrity and lead to bad clinical decisions that could directly affect disease diagnosis. Similarly, low performance optical components can cause a reduction in signal-to-noise ratio that leads to untrustworthy results. Meticulously designed optical systems minimize these risks and keep the instruments running reliably for a long time.

MKS Instruments provides components, sub-assemblies, and fully integrated systems that meet the exacting requirements of flow cytometry applications. Some of the high quality MKS optomechanical components that are employed in flow cytometers include vibration isolation, tables, mirror mounts, motion stages, motion controllers and software, laser power sensors, laser power meters and laser beam profilers (Figure 4). MKS Spectra-Physics DPSS and direct diode lasers are well suited to flow cytometry applications in terms of their overall performance envelope, compactness, ruggedness, reliability, long-term stability, and common footprint that enables interchangeability. MKS Vanguard and Explorer One XP high-power lasers are employed in high-power, high-throughput cytometry applications. MKS Excelsior fiber coupled (visible CW) and free space lasers with multiple wavelength options accommodate many different fluorophore choices.

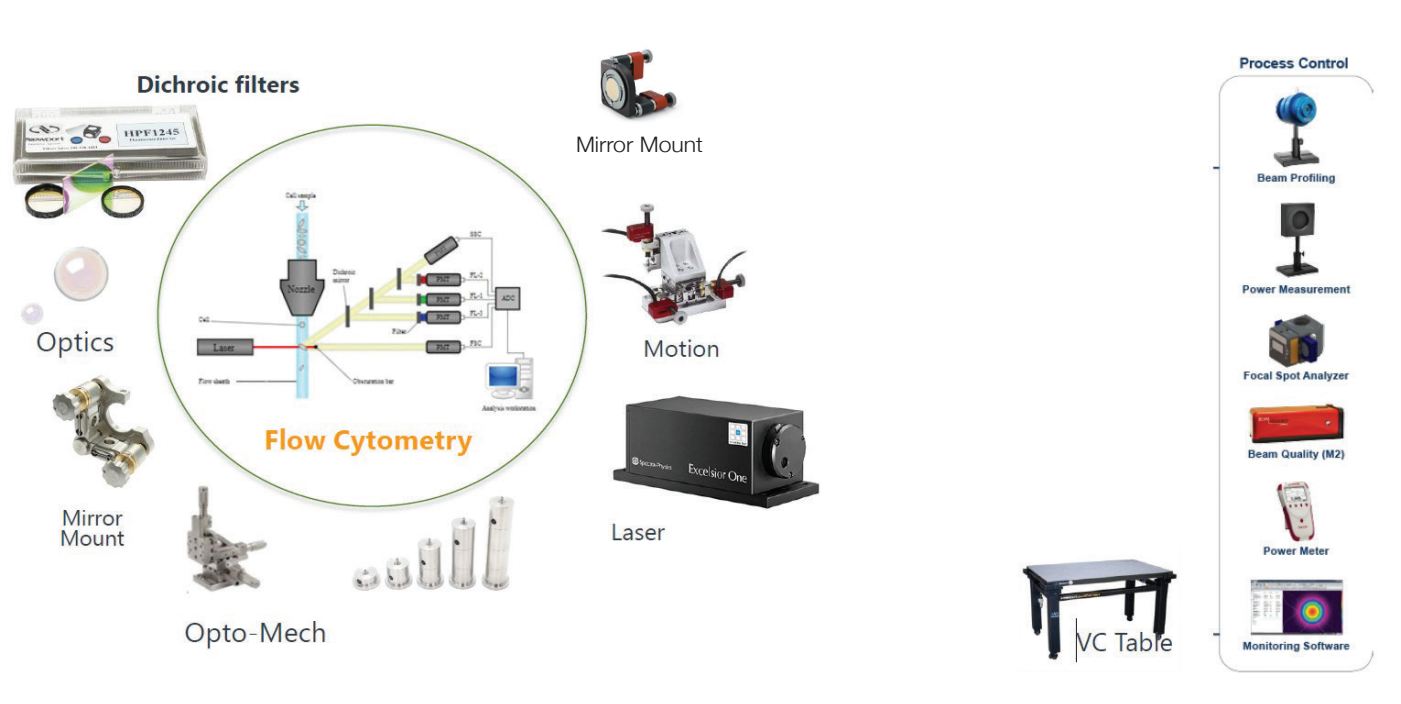

Figure 4 - MKS products employed in flow cytometry systems.

MKS Instruments also provides a full suite of optomechanical and electromechanical design and manufacturing services. Figure 5 shows a flow cytometry system designed and manufactured by MKS' Integrated Systems Business. There can be up to 10 laser sources and up to 40 detectors in a flow cytometer such as the one depicted in Figure 5. MKS Instruments' lasers provide narrow linewidth illumination enhancing the

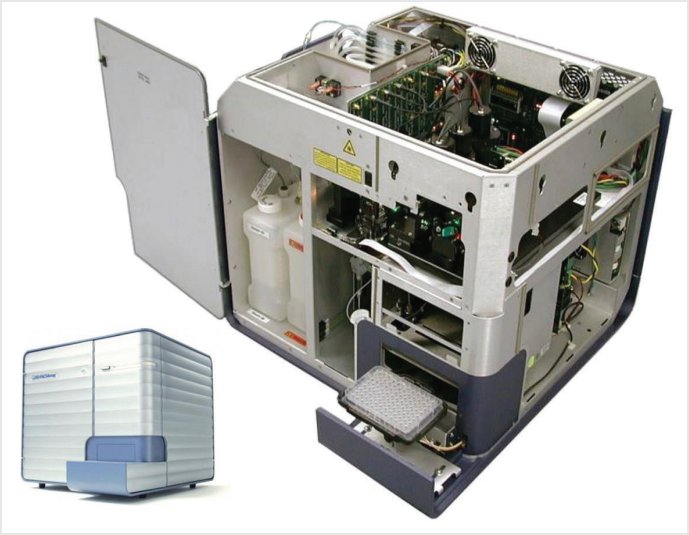

Figure 5 - Flow cytometer system.

excitation of fluorophores and increasing the intensity of fluorescence emissions for improved signal detection and maximal accuracy in analyses. Where required, aspheric lenses are used to focus the beam upon the cells within the central fluid stream.

MKS component solutions also include top adjust mounting hardware. The mechanical design and stainless-steel materials of construction ensure that Suprema® mirror and filter optic locking mounts are stable exhibit super long-term stability and stiffness both during shipment and in operation. The ZeroDrift™ version of the Suprema mounts offers additional long-term stability, compensating for steel's thermal expansion and minimizing any long-term thermal drift. Suprema mounts fulfill the critical accuracy requirements in adjusting the laser alignment with the sample stream. Both manual and motorized stages along with dichroic mirrors are used to align the flow-cell or microfluidic chip to the beam path. MKS Instruments also supplies optical measuring instruments such as beam profilers, power meters and detectors.

Flow Cytometry Measurements

Every cell in a flow cytometry experiment that is detectable by FSC, SSC, and fluorescence is counted as a distinct event and the cells can be sorted and collected according to pre-defined parameters. Cell count data can be visualized in a variety of ways, including as univariate histograms, bivariate dot plots, density plots and contour diagrams. Details of the data analyses in flow cytometry experiments is beyond the scope of this Note, however, we will provide a brief discussion of density and contour plots for illustrative purposes.

Dot and contour plots compare two or three cytometry response parameters simultaneously using a plot in which each event is represented by a single dot. This allows the plot to characterize inter-relationships between multiple variables using, for instance, a plot of cell count vs. simultaneous FSC and SSC values. Figure 6 shows one such plot of a population of bone marrow aspirate cells that illustrates how different cell types are characterized by their different FSC and SSC responses. In the plot, detection events for similar cells tend to cluster in distinct areas of the scattered light experimental space. By further analyzing the flow cytometry data from such cell mixtures important hematological diagnoses can be determined. This capability has caused flow cytometry to become a critical analytical technique for hematological diagnosis of illnesses such as leukemia and other immunodeficiency diseases. This diagnostic ability has also made flow cytometry an essential technique for the determination of the efficacy of cancer chemotherapies. Numerous other applications of flow cytometry in medical and biological sciences have been documented.

IV. CONCLUSION

MKS Instruments supplies many of the critical, high precision components needed in a flow cytometry instrument. MKS lasers have exceptional stability over the widest temperature range in the industry and the best beam pointing stability. Fine pitch position adjusters ensure the alignment is dialed in and maintained for the duration of a cytometry experiment. Tuned mass damping of MKS vibration control solutions effectively isolates cytometers from outside vibration, minimizing resonances, while honeycomb design in these solutions minimizes deflection under load. Beam profilers, along with power meters and detectors are used in signal processing. MKS Instruments provides fully integrated flow cytometry systems through its MKS Optical Solutions Business.

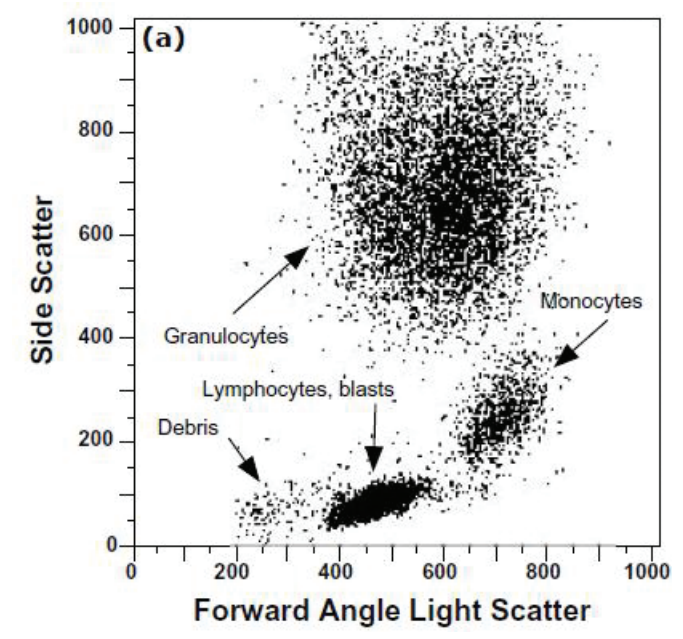

Figure 6 - Dot plot of flow cytometry data (from "Principles and Applications of Flow Cytometry", R. S. Riley and M. Idowu, http://www.flowlab-childrens-harvard.com/yahoo_site_admin/assets/docs/PRINCIPLESANDAPPLICATION.29464931.pdf).

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