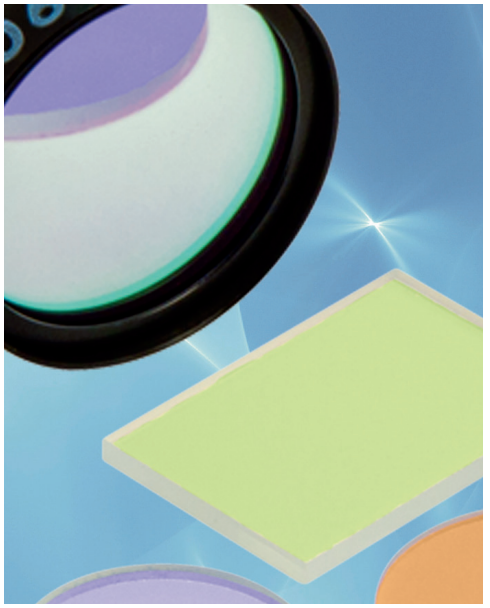
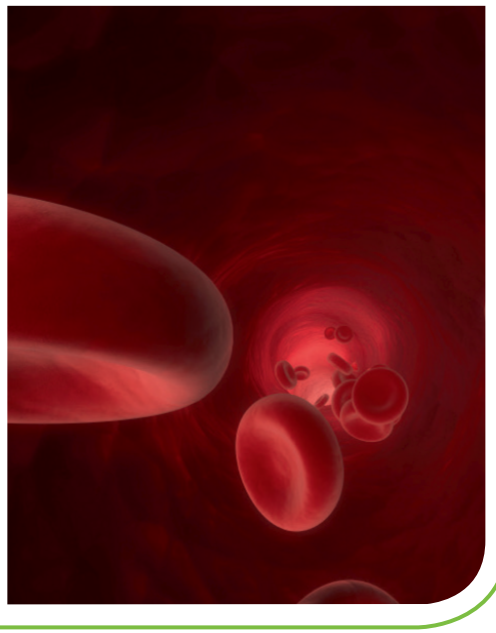
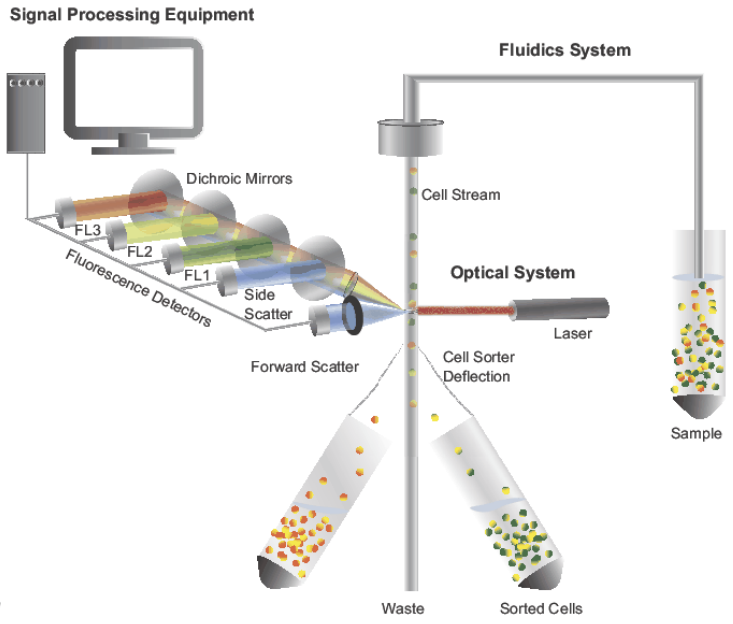
FLOW CYTOMETRY: PERFORMANCE ENHANCEMENT





ADVANCING HEALTHCARE WITH FLOW CYTOMETRY

Flow cytometry is an analytical technique that can rapidly measure the properties of individual cells or particles as they pass through a beam of light, which is typically a laser. A flow cytometer takes a sample of cells, transitions them into a single stream and uses light sources to excite biomarkers or labels on the cells to count the number of relevant constituents. The properties measured include relative particle size, relative granularity or internal complexity, and relative fluorescence intensity.

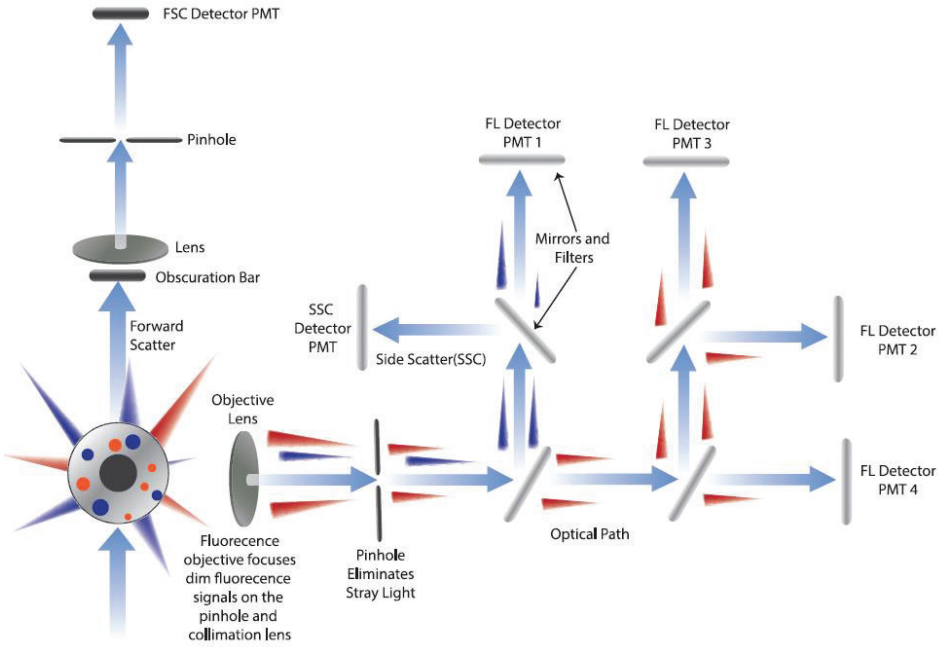


Schematic of a flow cytometry system

The use of flow cytometers will continue to grow as the medical and scientific community addresses important issues such as the high prevalence of targeted diseases (especially cancer and HIV/AIDS), COVID-19 and stem cell research. Additionally, the growing pharmaceutical and biotech industries in emerging economies will also rely on flow cytometry.

Flow Cytometer Design Challenges

Flow cytometry involves working with extremely weak optical signals that can be easily overwhelmed by background noise. Thus, high signal-to-noise-ratio (SNR) and high temporal resolution in flow cytometry signals are critical requirements for successful measurements. High SNR is critical in multiple-wavelength flow cytometers where data sets (dot plots) from multiple wavelengths must be clearly delineated to minimize overlap and increase the statistical confidence level of measurement data. Poor SNR increases probability of



Schematic overview of an optical configuration of a typical flow cytometer setup

misclassification resulting in costly, unplanned outcomes. Achieving these signal characteristics places extreme demands on the quality of a flow cytometer's components. For example, laser-light sources, optomechanical components and optics must have exceptional accuracy and stability, while narrowband optical filters must have high transmission and exceptional out-of-band blocking to minimize background interference.

As flow cytometers become more sophisticated and complex, the number of components increase, which complicates the challenges of aligning the laser beam to the fluidics system, aligning the sample to the laser beam, and aligning the optical components to properly guide the light to the detectors. For illustration purposes only, an optical layout of a typical cytometer with two colors, red and blue, is shown above.

MKS Solutions for Flow Cytometry

It can be very difficult to source and assemble compatible optical/optomechanical components into a flow-cytometry system with adequate SNR and temporal resolution. MKS has a deep understanding of the challenges faced in designing and building flow-cytometry systems and offers many solutions to minimize crosstalk between channels.

Challenges in Flow Cytrometry	MKS Solutions
Maximize SNR	High transmission, high optical density (OD) filters
Maintain alignment between optics and fluidics (including beam-combining optics)	Robust, stable mounts and positioners Mounts with lockable positions Low wavefront distortion mounts
Increase the number of parameters that can be measured	CW lasers with a selection of different wavelengths Optical filters with excellent laser line blocking and steep transition lines

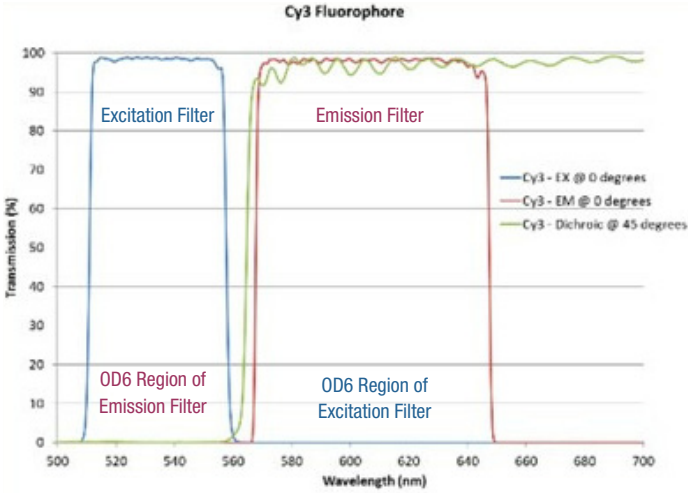
High-Optical-Density Filters with Steep Transitions

For a flow cytometer to operate properly and at its full potential, it is crucial for the detectors in the optical system to receive only the intended signals. Unwanted signals must be blocked as much as possible–at minimum, OD6 filters should be used and, in the case of some high-performance flow cytometers, OD8 at select wavelength ranges.

More specifically, the emission filter should have this high optical density in the excitation band, and the excitation filter should also have this high optical density in the emission band. This drives the need for another critical performance parameter, which is a steep transition slope from the transmission-to-rejection region and vice versa. Even with already a high optical density, if the transition is not steep enough, unwanted optical signals might reach the detectors.

Since as much as possible of the intended signals must pass through the filter, filters with at least 90% passband transmission should be used. In-band transmission levels ≥ 95% are ideal. All of these characteristics are illustrated in the graph of theoretical performance below.

MKS works closely with instrument developers to design optical filters specific to a flow cytometer's complex fluorophore assays and reagents requirements. Through fine-tuning the required transmission bands and rejecting unwanted signals, researchers and scientists can investigate an increasing number of applications.

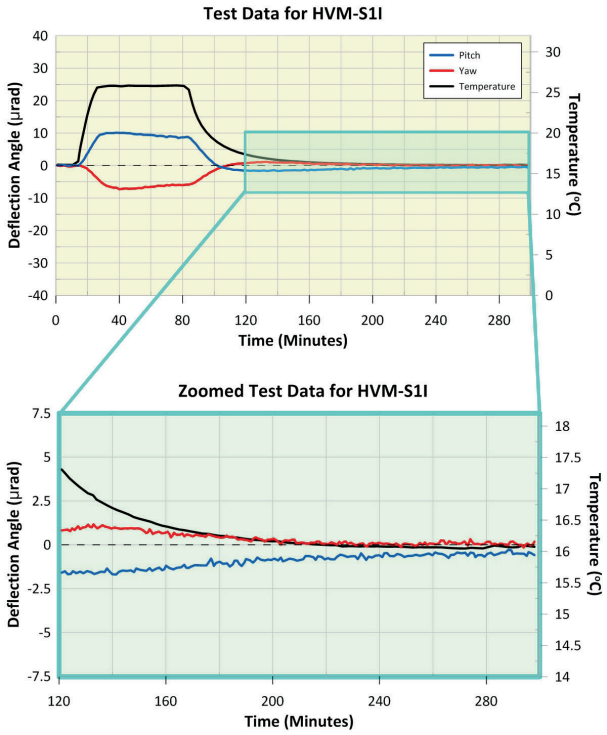


Theoretical performance of MKS excitation, emission and dichroic filter set for Cy3 fluorophore features OD6 blocking, >90% passband transmission and steep transitions.

Stainless-Steel Optical Mounts for Long-Term Stability

It is essential for the optics in a flow cytometer to sustain their initial factory alignment. They should not move, because misalignment can lead to system errors due to degradation of the SNR and possibly system downtime. One source of instability is thermal drift, which can be a problem because lasers may heat the optic and its mount. One way to address thermal drift is to choose appropriate materials for the optical mounts. Stainless-steel mounts, with a lower coefficient of thermal expansion than aluminum, offer the best stability over temperature.

HVM-S1i Stainless Steel Top Adjust Mount



The HVM-S1i's excellent thermal properties include a maximum deflection during peak temperature of 10 µrad in pitch and 7 µrad in yaw and a shift in reflected beam position after temperature cycling of < 1 µrad in pitch and < 1 µrad in yaw.

Low-Wavefront-Distortion Optical Mounts

When a flow cytometer utilizes multiple lasers that are focused on the same point, such as with beam-combining, low-wavefront-distortion optical mounts should be considered. These mounts are designed to hold the optic in a way that will not induce optical distortion and will therefore minimize focal-shift problems.

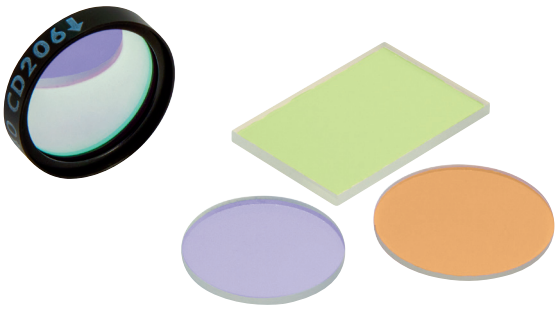
Typical mounts hold optics with set screws that apply pressure to the optic, which can cause wavefront distortion. Our low-wavefront-distortion mounts employ a 3-point axial clamping technique to gently and securely hold the optic to avoid this distortion, which is shown in the picture. Optional remote adjustment mirror mounts can save onsite field service costs.

MKS 3-point axial clamping technique for low wavefront distortion optical mounts

MKS Products for Flow Cytometry

MKS offers many popular and successful products which are broadly utilized in flow cytometers. For more information, please visit www.newport.com or call|
+1 877-835-9620

Fluorescence Filters



MKS designs and manufactures custom excitation-bandpass, emission-bandpass and dichroic filters optimized for the specific fluorophores that will be used in an application such as flow cytometry, DNA sequencing, in-vivo imaging and many others. Our proprietary ODiate™ and Stabilife® coatings are highly-dense thin-film coatings which deliver exceptional stability and durability.

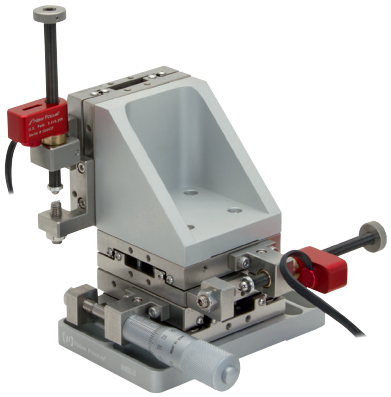
- Full range of performance for spectral ranges from 250 to 2100 nm
- OD6 and OD8 out-of-band blocking with up to 99% peak transmission and 0.5% edge transition
- Superior image quality with transmitted wavefront error up to 0.01λ RMS

Top-Adjust Optical Mounts

The adjustment screws of top-adjust mounts are located above the mirror to enable alignment in a confined space without obscuration of the beam path. Additionally, top-adjust mounts save space in an instrument due to a slimmer profile and smaller footprint than traditional mounts. MKS' top-adjust mounts are designed for "set-and-forget" OEM applications and are available in many configurations.

- Stainless-steel versions
- Low-wavefront-distortion versions
- Lockable adjusters for long-term stability
- Alignment pin holes to enable keying of the mount's position

Stainless-Steel Positioners



Some instruments require a component such as a flow cell, end-detection device or other type of optic to be positioned with high accuracy over extremely small distances. Furthermore, the position of these components must remain very stable. MKS offers a comprehensive set of positioners, including stainless-steel linear positioners for optimal thermal stability.

- Gothic-arch ball-bearing versions for least susceptibility to shock
- 5 mm, 0.5 in. and 1.0 in. travel versions
- Picomotor™ piezo actuator-driven versions for automated movement as small as 30 nm
- 1-, 2- or 3-axis assemblies

Continuous-Wave (CW) Lasers

Available as a free-space or fiber-coupled CW laser, the Excelsior® One™ offers the largest portfolio of up to twelve different wavelength options. Our “It’s in the Box™” design combines the laser head and controller into a single compact package which can be used in applications such as flow cytometry, DNA sequencing and microarray scanning.

Free Space Laser Features:

- 375 to 1064 nm wavelengths
- 10 mW to 500 mW (adjustable) output power
- <0.01 pm to 1.5 nm spectral linewidth
- <6 µrad/°C beam-pointing stability
- <±2% power stability over 8 hours

Motorized Mirror Mounts

Piezo motor-driven mirror mounts allow optical adjustments in much finer increments than are achievable by turning screws with fingers or a hex key. MKS’ motorized mirror mounts offer micron and sub-micron angular resolution. An added benefit of remote adjustment is that operators of flow cytometers can realign optics when needed, reducing the need for in-field service.

- Low-wavefront-distortion versions
- No movement of adjustment actuators when power is off
- 0.5 in., 1 in. and larger diameter versions
- Limit-switch and position sensor versions for repeatability

Piezo Motor Positioners

MKS' compact Agilis™ piezo-motor linear stages are ideal for fast nanometer-scale automated positioning of small components such as beam-routing optics, flow cells and microfluidic chips. Their stainless-steel construction, combined with a holding force which locks the position when idle, result in high stability for "set-and-forget" applications. Remote realignment of optics by technicians can sometimes prevent in-field service of systems.

- Minimum incremental motion of 50 or 100 nm
- 0.5 mm/s speed
- 12 mm and 27 mm travel versions
- Easily attachable for 2- or 3-axis assemblies

Aspheric Condenser Lenses

The use of multiple spherical lenses in the optical system of a flow cytometer may lead to a very long optical path and a larger footprint for the instrument. A solution is to replace several of the spherical lenses with one aspheric lens. Aspheric lenses have a more complex surface profile than spherical lenses, which enables better performance in light-collection, projection, illumination, detection and condensing applications.

- 6.8 to 75 mm diameters, plano-convex and bi-convex
- 5.87 to 49.24 mm effective focal lengths
- Molded Schott B 270® ultra-white glass
- Visible to NIR spectral range

Low-Power Detectors

MKS' wand-style low-power detectors work well with the low power light sources in flow cytometry applications. These photodiode detectors have fast rise times, resulting in faster measurements, and they feature NIST-traceable calibration for the smallest calibration error in the industry.

- Spectral ranges of 200-1100 nm (UV Enhanced Silicon detector) or 400-1100 nm (Silicon detector)
- 20 pW minimum measurable power (without attenuator)
- ±1% to ±4% calibration uncertainty
- ≤3 µs rise time
- Switchable OD3 attenuator attached

Fluorescence Imaging Optical Filter Sets

In addition to our custom designs, MKS offers fluorescence filters as standard catalog products. Made with the same patented Stabilife technology, these filters are available as a set of excitation, emission and dichroic filters designed for a specific fluorophore.

- Close to 50 sets for the most popular standard fluorophores and promising new fluorophores
- ≥90% passband transmission and dichroic reflectance
- ≥OD6 out-of-band blocking
- Steep transitions

WHY MKS?

CRITICAL TECHNOLOGIES

World-class technology and development capabilities for leading-edge processes

PROVEN PARTNER

Recognized leader delivering innovative, reliable solutions for our customers' most complex problems

OPERATIONAL EXCELLENCE

Consistent execution across all aspects of our business

COMPREHENSIVE PORTFOLIO

Largest breadth of product and service solutions for the markets we serve

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Newport is a brand within the MKS Instruments Light & Motion division. The Newport product portfolio consists of a full range of solutions including precision motion control, optical tables and vibration isolation systems, photonic instruments, optics and opto-mechanical components. Our innovative Newport solutions leverage core expertise in vibration isolation and sub-micron positioning systems and opto-mechanical and photonics subsystems, to enhance our customers' capabilities and productivity in the semiconductor, industrial technologies, life and health sciences, research and defense markets.

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