

## TECHNICAL NOTE

Flow Cytometers Using Optical Waveguides in Place of Lenses for Specimen Illumination and Light Collection<sup>1</sup>

Howard M. Shapiro and Michael Hercher

Howard M. Shapiro, M.D., P.C., West Newton, Massachusetts 02165 (H.M.S.), and Optra, Peabody, Massachusetts 01960 (M.H.)

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Optical waveguides represent alternatives to lenses for delivery of light to, and collection of light from, small regions of space such as the observation point(s) of a flow cytometer. The flow chamber and directly associated illumination and collection optics of a flow cytometer can be built using fiber optic waveguides in fixed positions or as an integrated optical assembly incorporating deposited or implanted waveguides, providing illumination and collection geometries and efficiencies comparable to conventional designs, with the advantage that the optical waveguide design, once assembled, never again requires alignment of the optics. A prototype apparatus in which fiber optics were

used to illuminate and collect light from a point inside a thin-walled, round quartz capillary was observed to measure fluorescence of polystyrene spheres with precision close to that obtained from a conventional flow cytometer using the same laser source. The design of the fiber optic system is readily adaptable to construction of multiple illumination beam instruments; other optical waveguide flow cytometer designs that dispense with the capillary share this advantage and can also provide orifices for electronic volume sensing and for jetting prior to droplet sorting.

**Key terms:** Flow cytometry, fiber optics, integrated optics

In optical flow cytometry, it is necessary to focus illuminating beams at the selected wavelength(s) to small spots on the sample stream, to efficiently collect light from the sample, and to divert light in selected spectral regions to detectors while minimizing interference from stray light. Conventional flow cytometers use lenses for illumination and collection; these optical components require adjustable mounts, as does the flow chamber, and performance of the instruments is seriously degraded by the misalignment of optical components, which occurs more frequently as the number of measurement parameters and illuminating beams increases. While it is possible, to some extent, to manipulate optical design to make performance less sensitive to small changes in optical alignment, this generally requires sacrifices of precision, sensitivity, or both.

Recent advances in optics, principally related to the development of fiber optic systems for telecommunications, have provided simple, economical, and efficient alternatives to microscope objectives and other lenses for the collection of light from, and the delivery of light to, small regions of space. These are optical waveguides, of which the most conspicuous examples are fiber optics. We have recently designed, built, and tested a flow cytometer in which fiber optics are used in place of lenses to illuminate and collect light from the sample stream.

## MATERIALS AND METHODS

Fiber optics for illumination and collection were fitted to a flow chamber of the type used in the "Cytomutt" series of flow cytometers, in which cells are observed during transit in sheath flow through a quartz capillary approximately 150  $\mu\text{m}$  in inside diameter and 250  $\mu\text{m}$  in outside diameter. This flow chamber design has been extensively described in previous publications (5,6). The fiber optics used for illumination and collection were positioned along two axes orthogonal to one another and to the direction of sample flow by gluing them into an aluminum plate 1 cm square by 1 mm thick with perpendicular grooves milled into the surface and a 250- $\mu\text{m}$ -diameter hole drilled through the plate at the point of intersection of the grooves. A diagram of the assembly is shown in Figure 1.

Laser illumination was delivered to the observation region through a single-mode N. A. 0.11 fiber with a 5- $\mu\text{m}$  core diameter and a 125- $\mu\text{m}$  cladding diameter (Light-wave Technologies, Inc., Van Nuys, CA). Axial and or-

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Address reprint requests to Howard M. Shapiro, M.D., 283 Highland Avenue, West Newton, MA 02165.

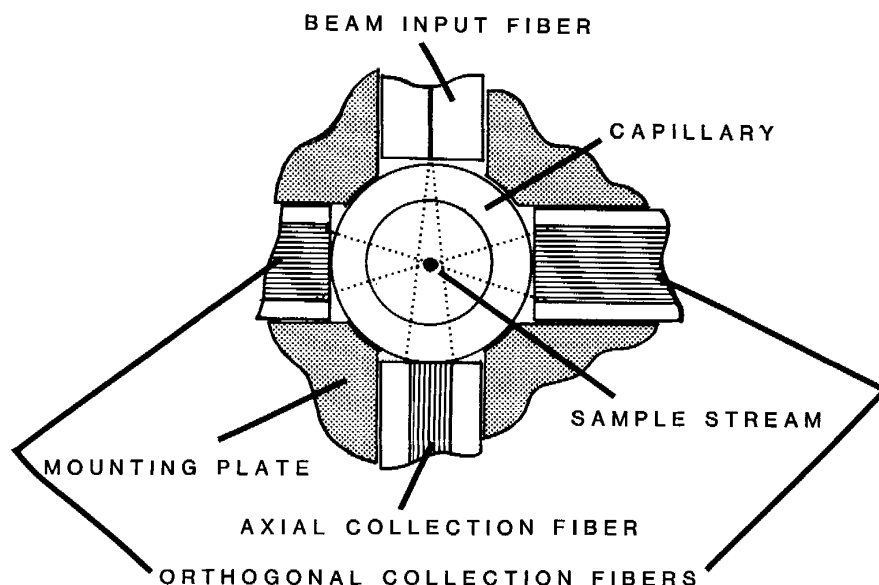


FIG. 1. Diagram of the fiber optic waveguide flow cytometer looking along the axis of sample flow. The dotted lines illustrate the divergence of the illuminating beam and the angles over which light from the sample stream is collected by the axial and orthogonal collecting fibers. The hatched regions shown in the fibers correspond to the core

dimensions; the clear regions represent the external cladding of the fibers. The illuminating fiber end in the system described was placed farther away from the capillary than is shown in the figure to obtain a larger spot size at the observation point. Spaces shown between the fiber ends and the capillary contain optically transparent adhesive.

thogonal light collection were accomplished using silica multimode fibers (Newport Corp., Fountain Valley, CA), the axial fiber (N. A. 0.2) having 50  $\mu\text{m}$  core and 125  $\mu\text{m}$  cladding diameters and the orthogonal fibers (N. A. 0.3) having 100  $\mu\text{m}$  core and 140  $\mu\text{m}$  cladding diameters. The fibers were fitted to the mounting plate and positioned while being observed through a binocular microscope; a light-cured, optically transparent adhesive (Norland Products, Inc., New Brunswick, NJ) was then used to secure the fibers in place. When assembly was completed, the adhesive filled the space between the fiber ends and the outer wall of the capillary.

Emission from a He-Cd laser (Model 4240, Liconix, Sunnyvale, CA) at 441 nm was coupled into the single-mode fiber using a Newport Model F-915 coupler. Lengths of the illuminating and collecting fibers were approximately 1 m each; both ends of each fiber were cleaved but not polished. Light from an orthogonal collecting fiber was directed to a photomultiplier tube (Model 931B, RCA, Lancaster, PA) fitted with 520-nm long-pass dielectric and 530-nm long-pass color glass filters (Ditric Optics, Hudson, MA).

The photomultiplier anode signal was amplified using a baseline-restoring preamplifier and the amplified signal was routed to a microprocessor-based data analysis system via peak detector and front-end circuitry described elsewhere in detail (6). Fluorescence intensity measurements were made of 3.7- $\mu\text{m}$  polystyrene spheres

(Polysciences, Warrington, PA, no longer available). The beam input coupler and the core stream position were adjusted to maximize signal amplitude and histograms of fluorescence intensity were accumulated.

## RESULTS AND DISCUSSION

When both the input beam coupler adjustment and core position were optimized, measurements of polystyrene spheres yielded a distribution containing a symmetric singlet peak with a coefficient of variation (CV) of 2.4%. This precision compares favorably with that of our conventional flow cytometer design using the same laser source and a similar flow chamber; the lowest CV we have observed using that apparatus for measurements of the 3.7- $\mu\text{m}$  spheres is 1.6%. The signal amplitudes we observed using the fiber system were quite low; the photomultiplier gain used, estimated from applied voltage, was several hundred times the gain required to produce equivalent pulse amplitudes in the conventional flow cytometer, and it is therefore likely that photon statistics contributed significantly to the variance of the fluorescence intensity distributions.

We believe that the low signal amplitudes were due primarily to inefficient coupling of the He-Cd laser beam into the illuminating fiber. We were unable to measure beam power at the end of the illuminating fiber nearest the capillary because the fiber had been glued into place beforehand. However, subsequent measurements made

using a calibrated power meter (Liconix) and another length of single-mode fiber indicate that at most a few hundred microwatts of the 35-mW input power could have reached the capillary. While some of the coupling inefficiency was the product of our inexperience, the fiber characteristics clearly played a role. The fiber we used was intended for single-mode transmission of a 633-nm (He-Ne) laser beam; a smaller core diameter would be necessary for optimal single-mode operation at the shorter wavelength of 441 nm. With experience, we were able to obtain 4.7 mW output from the end of the single-mode fiber with 6.2 mW input from a He-Ne laser at 633 nm, an efficiency of better than 75%, while, at 441 nm, we never achieved a coupling efficiency above 10%, i.e., 3 mW out for 30 mW in. We would expect to be able to match the figure of 75% efficiency using a single-mode fiber of appropriate dimensions (4). Light collection could also be improved by using multimode fibers of higher N. A. at the orthogonal positions; plastic fibers with N. A. above 0.5, which are readily available, would collect approximately as much light as do the lenses used in most conventional laser source instruments.

The use of a single-mode fiber to introduce the illuminating beam into the system was essentially mandated by our observation that, in accordance with theory, a laser beam transmitted through a multimode fiber produces very uneven illumination, while transmission through a single-mode fiber preserves the Gaussian intensity profile of a Gaussian input beam. In order to achieve single-mode operation, it is necessary to keep core diameters on the order of a few micrometers and to operate at relatively low numerical apertures. The diameter of the spot formed at a distance  $X$  by a Gaussian beam emerging from a single-mode fiber with a numerical aperture of 0.11 is approximately  $2X(\tan 6^\circ)$ . Thus, if the end of the single mode fiber were placed against the outer wall of the capillary, approximately 125  $\mu\text{m}$  from the sample stream, the spot diameter at the observation point would be about 25  $\mu\text{m}$ . A larger spot size at the observation point could be achieved by leaving space between the end of the fiber and the capillary; we actually positioned the fiber end about 125  $\mu\text{m}$  from the capillary wall in hopes of obtaining a spot approximately 50  $\mu\text{m}$  in diameter.

The choice of fibers for fluorescence collection is not affected by any requirement that the fibers preserve beam mode structure; it is thus possible to take advantage of the higher N. A. of multimode fibers for this purpose. In order to make use of the full aperture of a collecting fiber, it is only necessary that the fiber end be close enough to the observation point for the fiber to subtend an angle equal to or larger than the cone corre-

sponding to its numerical aperture. The angles over which light is collected by the axial and orthogonal fibers, as well as the angle at which the illuminating beam diverges, are shown in Figure 1.

Although fiber optics have been used for light transmission in flow cytometers, e.g., the Ortho (Ortho Diagnostics Systems, Westwood, MA) System 30 and 50 series, and others (1,2; L. Kamensky, personal communication) have proposed instruments in which fibers and/or other waveguides would replace lenses for illumination and collection, we are not aware of any published reports of successful construction of such apparatus. Our own efforts have benefited from the continuing advances in fiber optics resulting from the application of this technology to telecommunications; the improvement in transmission and decrease in size of fibers achieved in recent years now make flow cytometer designs such as the one we have described practical as well as feasible.

A multistation flow cytometer based on our fiber optic design could be built by placing one or more additional fiber holders downstream along the capillary. It would also be possible to eliminate the capillary from the design, instead using the intersection of four or more fibers of similar outside diameter to define a polygonal orifice that could be used for jetting prior to droplet sorting or for electronic volume measurement. Finally, the emerging technology of integrated optics (3,7) could be used to implant or deposit optical waveguides with specific characteristics on a substrate containing an orifice or flow channel; this method of fabrication would permit other optical elements, such as gratings and lenses, and conceivably also solid state laser or diode sources, detectors, and associated electronics to be included in a "flow cytometer on a chip." Even the smaller scale of integration represented by the fiber optic system, however, appears to offer some advantages over conventional designs in terms of compactness and freedom from adjustments.

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