MEDICAL IMAGING

Fiber delivery systems enhance flow cytometry designs

FIONA EVANS

Analytical instruments used in genomics increasingly rely on flow cytometry systems that are fast, rugged, stable, and compact with excellent image resolution—qualities enabled by singlemode optical fiber systems that deliver laser beams to the sample.

Advanced research in gene expression and the growing market for high-throughput clinical and diagnostic screening are driving step changes in biophotonics technology. These advances enable the applications to leave the arena of academic research and be used in high-volume industrial-scale research, with the ultimate aim of bringing personalized genomics and thereby personalized medicine ever closer.

Many of the screening instruments use high-specification lasers, advanced optical systems, and detectors to achieve reliable, high-speed, high-resolution analysis of very small target samples in microfluidics or microarrays. Analytical processes such as DNA and blood analysis—both enabled by flow cytometry techniques—rely upon optical system designs, which in turn are based on free-space optics or optical fiber delivery systems.

DNA and blood analysis

DNA analysis is, after immunofluorescence, the second most important application of flow cytometry and it has driven advances in flow cytometry instrumentation. The first mapping of the human genome took ten years to complete; now it takes less than three days, although the data analysis takes much longer. Information can also now be gained more quickly from shorter partial DNA and RNA read

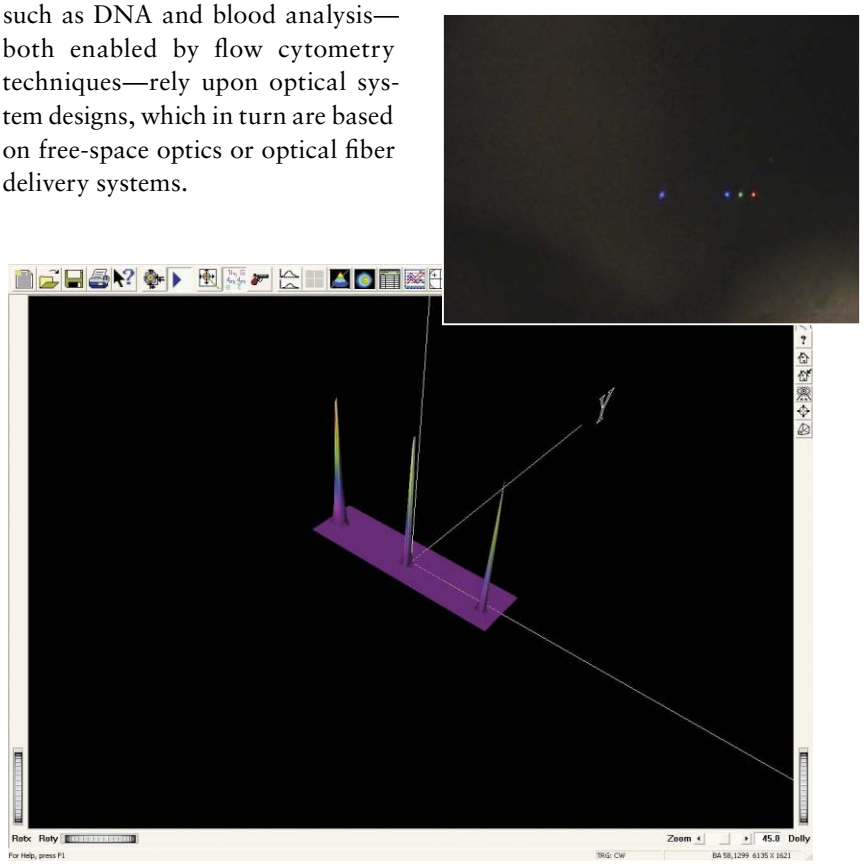

FIGURE 1. 3D beam profile image is taken directly from the output of a three-line kineFLEX-Hydra fiber delivery system. The singlemode fiber channels in this example are actively aligned so that the polarization axes are parallel with each other. These beams are intrinsically highly Gaussian and perform well with integrated beam-shaping optics, such as focusing lenses or lenslet arrays. The integrated optics create a closed system that will repeatedly deliver perfectly sized and spaced spots in a flow channel or microarray. Inset: Parallel, Gaussian, and multicolored beam spots are projected onto a black card from a three-line kineFLEX-Hydra.

lengths to investigate target sections of the DNA strand in order to answer specific queries, such as those related to hereditary diseases or cell and gene mutations as cancer indicators.

Working with what are often irreplaceable samples, researchers in this field need instrumentation that can reliably gather data in a short time, and in a manner that is repeatable between samples and over the long term. The stability and resolution of the instrument is critical and any changes will have a significant impact on the quality of the data and the results.

In a similar fashion, blood analysis for diagnostic and immunology purposes is a large and rapidly expanding market. Advances in genetic research are now leading to new diagnostic tests and test equipment.

The design of all these analytical instruments is as much about reducing sources of error as it is about improving performance, and optical fiber delivery of the lasers is a useful tool that addresses both concerns. Optical fiber delivers the additional benefit of allowing the instrument designer to place the lasers in an easily accessible location for field servicing.

Analysis techniques

The instruments used in these new fields of gene expression and pharmacogenomics are based on the principles of microscopy and flow cytometry. Speed and throughput are key drivers in the design of these instruments, which operate on a busy daily schedule in core facilities to accommodate a large and growing demand for analysis.

Some of these facilities are unable to increase output without building additional laboratory space. Consequently, there is a need for smaller-footprint instruments so the core facilities can fit more instruments into existing laboratories.

In flow cytometry, each excitation laser source usually is dedicated to one probe on the sample per run. One way to speed up the analysis is to run parallel processes by using multiple illumination wavelengths fired in series on the sample. The primary challenge is to focus the light onto a moving sample in the flow stream, which is usually less than 100 µm wide.

To accrue meaningful data from a moving target, both the detector and illumination source need to be as stable and stationary as possible; otherwise, movement from either one will cause image jitter and reduce resolution. The second physical challenge is often positioning the beams from multiple light sources in sufficient proximity to generate parallel illumination spots focused in the flow cell, but also to sufficiently separate these spots to prevent crosstalk in the detection channels, which may collect emitted and fluorescent light simultaneously.

In addition, in genetic analysis the instruments are working with much smaller sample sizes and some of them now image at the single-molecule level. This means that the challenges originally faced by flow cytometry have increased.

Free-space optical systems

Flow cytometers have traditionally used an optical system of lenses, filters, and microscope objectives to shape the beam and deliver it to the flow cell. The principles are well understood, although this often results in a long optical beam train, making it more susceptible to the effects of laser jitter. An open beam path with optics mounted at various locations is also more susceptible to thermal temperature differences that will cause beam movement on the sample.

Careful alignment of the optics is necessary as small movements can cause large changes in the beam pointing on the sample. As a result, instruments that use free-space optics can be susceptible to physical knocks and changes in environmental conditions, including heat generated by the laser.

Nevertheless, free-space optics are still widely used in routine flow cytometer applications. The best designs have been refined to keep the optical path as short as possible, and to use additional cooling methods to regulate the temperature inside the housing.

Fiber-optic delivery

For demanding applications such as microbial screening and gene expression, the use of singlemode fiber to deliver the

FIGURE 2. The two-line kineFLEX-Hydra fiber delivery system includes the integrated output beam-shaping optics that shape parallel and collinear beams. The system includes two kineMATIX manipulators, one at each input end for aligning and attaching the fibers to the individual lasers. By choosing to decouple the laser from the fiber at the laser side, the fiber can remain *in situ* and the output end can remain buried within the instrument when the laser is replaced at end of life. This then allows the laser to be located in a more convenient location for servicing and heat dissipation.

laser beam to the sample offers several advantages in functionality, from increased laser stability and image resolution to reduced instrument size and greater ruggedness.

Using singlemode fiber dramatically reduces the effect of laser beam-pointing instability (or jitter) regardless of the laser technology employed. In the best systems, the improvement can range from 30 µrad/°C to better than 1 µrad/°C. The fiber also acts as a spatial filter and eliminates beam astigmatism to create a near-perfect Gaussian profile. The resulting beam is much more stable over time than those in free-space systems, and is without accumulated errors from multiple optical interfaces, which ensures a more reliable and more stable measurement (see Fig. 1).

Enclosed in the fiber, the beam is much less sensitive to movement and thermal temperature changes, so this reduces the need for instrument alignment service visits and creates a more robust instrument design. Fiber also allows the laser source, which generates heat, to be located away from the flow cell, which means more flexibility for instrument layout, including the option of mounting the laser externally to the instrument head. It also makes servicing considerably easier, since the optical alignment of the instrument is fixed and independent of the laser, so there is no need to perform a lengthy re-alignment process when the laser is serviced.

Using singlemode fiber reduces the effect of laser beam-pointing instability (or jitter) regardless of the laser technology employed, potentially improving between 30 µrad/°C and better than 1 µrad/°C.

To meet the need for a smaller beam path to reduce instrument footprint and still deliver multiple illumination spots focused in a flow cell, Qioptiq created a singlemode polarization-aligned fiber array, the kineFLEX-Hydra, which delivers two or more spatially separated and parallel beams to the flow cell (see Fig. 2).

Each fiber core is actively aligned with submicron precision to achieve the required beam-polarization axis parallelism. Beam-shaping optics and filters can also be integrated into the fiber array to further reduce the optical train. In collaboration with one instrument

Fiber Delivery Systems continued on page 60