Lighting Every Corner

Wide Field Illumination for Fluorescence Instrumentation

IDEX HEALTH & SCIENCE

Intelligent Solutions for Life™
Fluidics | Optics | Consumables | Assemblies

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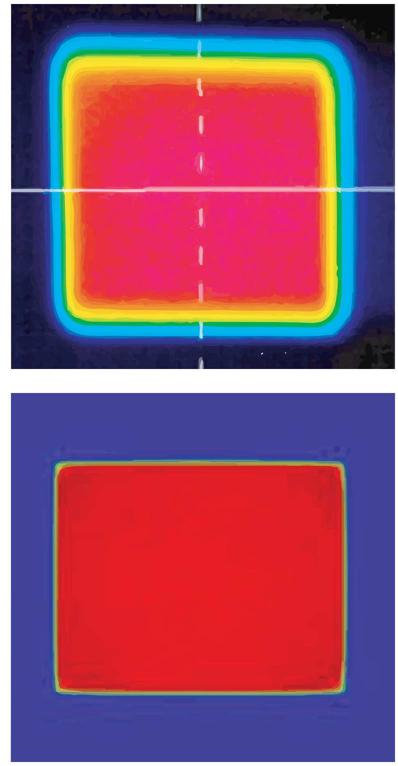
Overview

Many fluorescence-based instruments need to analyze or image large areas quickly — from flow cells and multi-well plates, to the entire field of view of a microscope. The throughput of these instruments, however, is often limited by their ability to uniformly illuminate the full field of view with sufficient excitation light of the right wavelengths. As molecular diagnostics and sequencing find increasing application in clinical treatment, throughput is a significant cost barrier to widespread adoption.

One approach to scanning large areas is to illuminate a small area at a time with the desired intensity, stitching together multiple measurements to cover the entire sample area. This increases the time needed for a single sample measurement, but offers the benefit of good fluorescence signal strength. Alternatively, the same beam can be expanded to cover the full field of view to eliminate scanning, but with a corresponding loss in signal.

The ideal solution, however, is an illumination source that can provide the needed intensity over a sufficiently large field of view to reduce scanning. In response, IDEX Health & Science has developed a Wide Field Illumination Module capable of delivering uniform, high-intensity output over a large field of view at multiple wavelengths. Designed for drop-in OEM integration, the module may be customized with up to five LED and/or laser sources to provide fields of view of up to several millimeters. Using proprietary beam-shaping technology, the module can redistribute even the most non-uniform source beam distributions to have better than 10% beam uniformity.



Above: Square and rectangular flat top illumination beam profiles created by the Wide Field Illumination Module.

Traditional Limitations in Beam Uniformity and Intensity

Design of illumination optics for a fluorescence based system has traditionally required balancing excitation wavelength and intensity against the size and spatial uniformity of the beam created. A small illumination beam can provide high intensity, but requires scanning in increments across the full field of view (Figure 1). Expanding to a larger beam size allows the sample to be scanned more quickly, but at a sacrifice to intensity and thus, sensitivity (Figure 2). The irradiance or power density of the source must then be increased to compensate. Additionally, the beam is seldom uniform across its diameter, further reducing sensitivity at the beam periphery and limiting the effective scan rate.

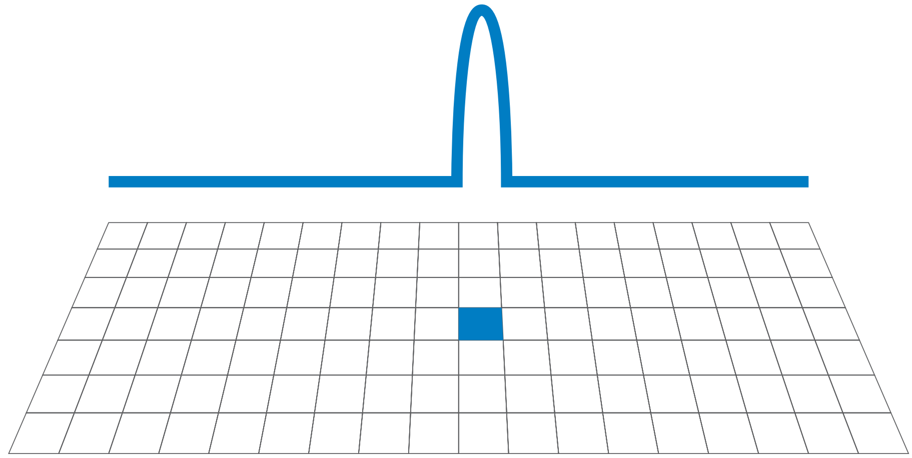


Figure 1

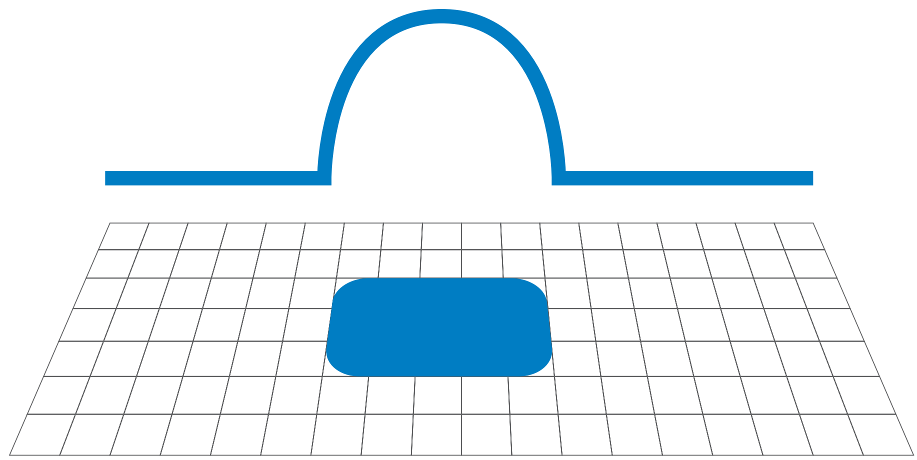


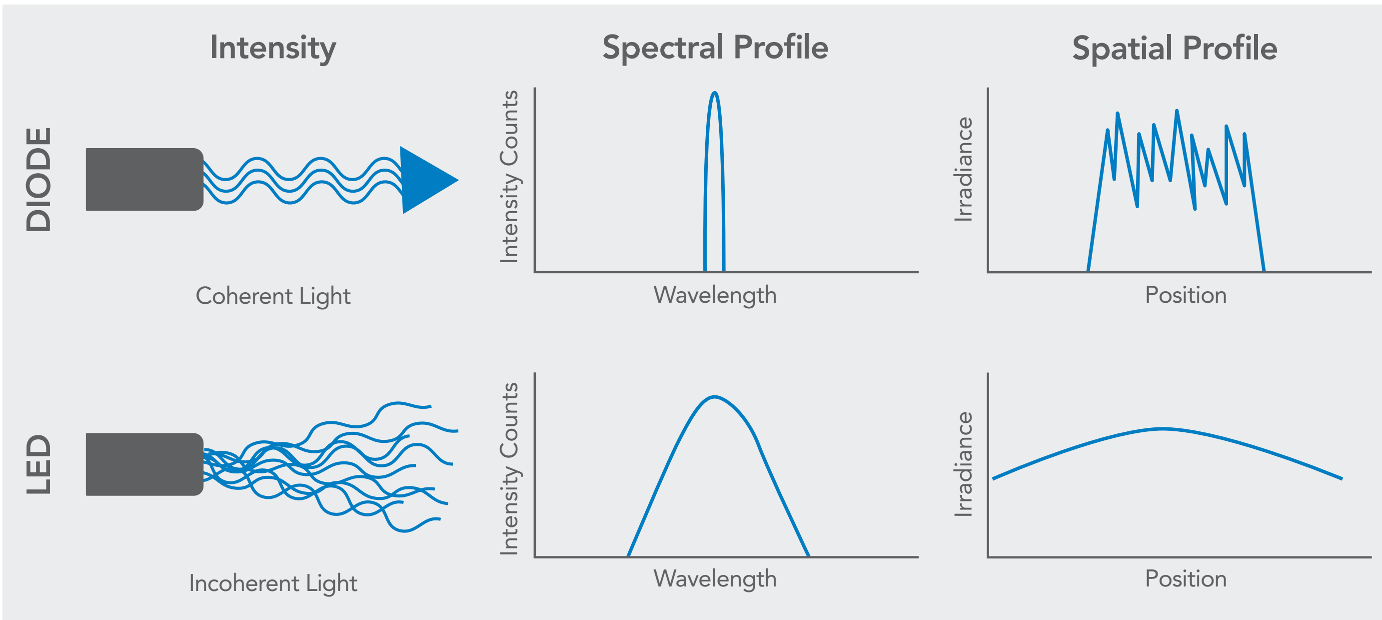
Figure 2

An additional level of complexity is added when wavelength is considered. In an ideal world, high intensity, fully tunable coherent light would come in a small package. In reality, however, the optical designer must mix-and-match between diode lasers and LEDs to create the best excitation source for their fluorophore(s) of interest.

Diode Laser Sources

Diode lasers provide intense light at a single wavelength, allowing more photons to reach the imaging plane and decreasing exposure time. The collimated nature of the light and its spectral purity facilitates beam combining using simple filters in a compact package with high efficiency. Unfortunately high-power diode laser wavelength availability is limited to those favored by the major commercial laser display applications, and may not overlap well with the target fluorophore(s).

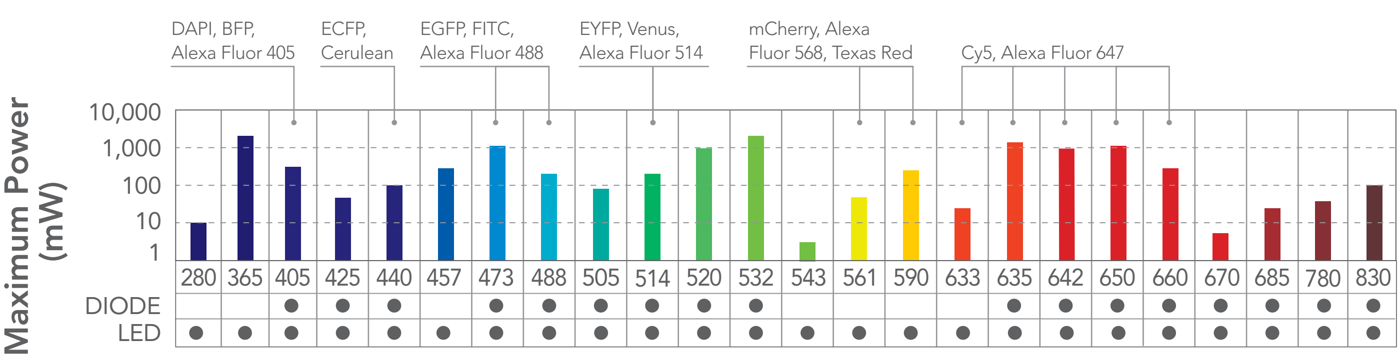
Spatial uniformity is also an issue, as lower cost high-power laser diodes exhibit variable speckle and interference. This results in significant non-uniformity at the imaging plane, which can impact image quality and accuracy of quantitative or relative measurements.



LED Sources

LEDs, in contrast, are available at many wavelengths through the UV and visible due to their use in a variety of commercial applications. This enables the optical designer to optimize excitation of their detection dyes for best efficiency by wavelength. The spatial beam profile of an LED varies smoothly, with a predictable roll off that can easily be normalized in software to simplify imaging at the flow cell plane.

The light provided, however, exhibits etendue effects that limit how much light can be captured, as well as how it can be shaped. The result of the lower intensity generated is that exposure times must be greater. Complex dichroic filters or even a filter switcher may be required to combine LED wavelengths, reducing light path efficiency even further. While this can be mitigated with higher power, onboard cooling must then be added, increasing the overall module size and power draw.



Available Illumination Wavelengths

The Benefits of Beam Shaping

Beam shaping is a natural solution for these issues, but has traditionally been either difficult or expensive to implement in commercial instrumentation for multi-wavelength sources. Many beam-shaping techniques are unique to a specific input intensity distributions or source type, and are not universally applicable.

A good example of this is Powell lens systems, which can be used to reshape a Gaussian beam into a flat top. While initial performance can be very good, Powell lens systems are very sensitive to misalignment — even 1 µm change in the input beam alignment can cause the flat top spatial profile to be lost. Deviations from a Gaussian input or changes in input beam size versus the design parameters can also degrade the flat top profile over time or temperature changes, limiting use to lab conditions. They are also incompatible with multi-mode lasers (which are not Gaussian in shape); if higher power is needed from the illumination source, Powell lenses are not an option for beam shaping. Considering these factors in sum, Powell lenses deliver limited value given their cost.

Diffractive optics avoid the dependence on input beam size and format, but are highly wavelength dependent and low in efficiency (typically <70%). They also exhibit an undesirable degree of ripple across the beam profile, and offer a limited working distance.

In developing the Wide Field Illumination Module, IDEX Health & Science strove to create a flexible OEM platform capable of homogenizing almost any input source profile, with minimal dependence on input alignment and beam shape for robust, long-term performance. This allows us to leverage both laser and LED wavelength availability by combining the two source types into one module without impact to the uniformity and intensity of the output flat top beam profile. The resulting compact module is capable of generating a high-intensity, rectangular or square-top beam profile composed of up to five LED and/or laser wavelengths, each of which can be power adjusted or turned on and off at will.

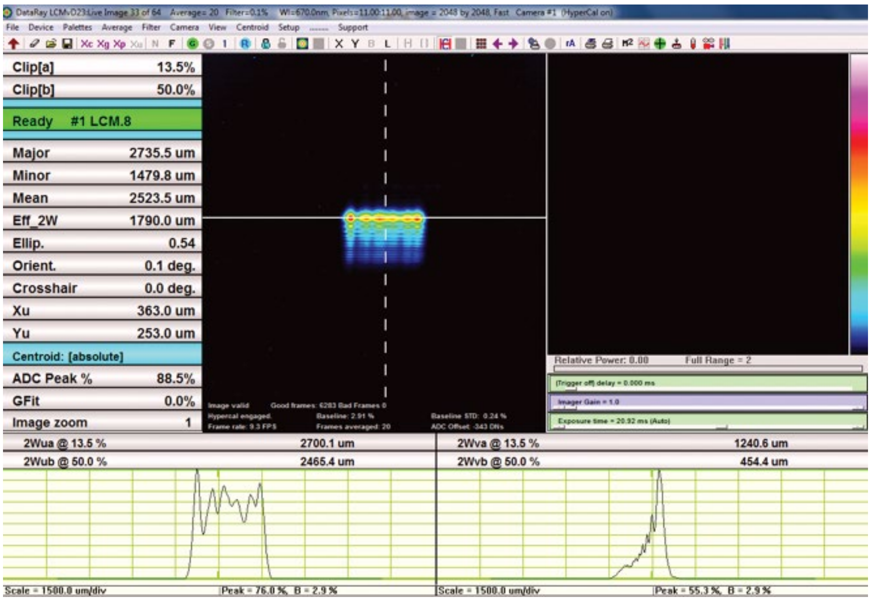
Module Performance

While each module design is unique to the customer, >80% efficiency can be expected for all integrated light sources. Spatial variation of the customized illumination beam is typically <10% prior to input into the instrument's optical system, regardless of the input beam profiles. Modulation of up to 100% in the input beam can be accommodated and remapped with less than 10% variation across the field of view. The output optics of the module are designed to image at the focal plane of a lens, and may be specified for field of view dimensions from 200 µm to several mm in square, rectangular, or other shapes.

As can be seen in these beam analytics, it is possible to start with a highly skewed, non-uniform beam such as the multi-mode red laser diode shown. In this case, the intensity of the input beam varied from 40% to 100% along the x-axis of the peak intensity, and from 0% to 100% along the central y-axis. In viewing the output beam, intensity variation was corrected to within 10% along the central x- and y-axes of a rectangular beam. This demonstrates the ability of the Wide Field Illumination Module to homogenize even the most distorted beam profiles, including hotspots and significant asymmetry.

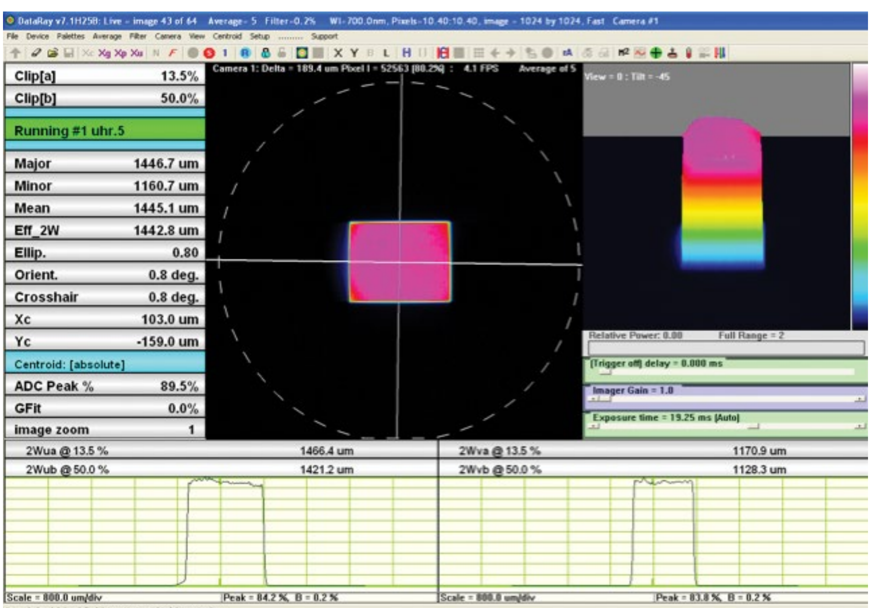
Module Performance (Continued)...

Multi-mode Red Laser Diode After Collimation



Near-field pattern of a multi-mode red diode laser after collimation, prior to beam shaping.

Red Laser After Beam Shaping



Same source as viewed at the sample, after beam shaping by the Wide Field Illumination Module and focusing through an objective.

Module Design

Each module is designed as a drop-in illumination subassembly for an OEM customer's instrument, including sources, drive and monitoring electronics, thermal management, and power for multiple diode lasers and/or LEDs. The exact number and choice of wavelengths can be customized to the needs of each application, depending on the fluorophore(s) being used and the excitation intensity desired at each wavelength.

High-performance thin film filters are used to efficiently combine the various sources into a single optical path, with an option for additional filtering to narrow LED outputs. This allows a band of wavelengths to be built up, each of which can be controlled independently via electronics.

As compared to rotating a filter wheel to select the excitation wavelengths, this approach is faster, more dependable, and introduces less vibration into the final instrument. Also, as the illumination sources are typically modulated on and off to read different fluorophores, less heat is generated overall. Sources may be operated in CW mode if needed, and at powers up to 1 W.

To address the challenge of beam uniformity, IDEX Health & Science has developed proprietary laser beam shaping technology capable of eliminating speckle and interference while expanding the beam to achieve

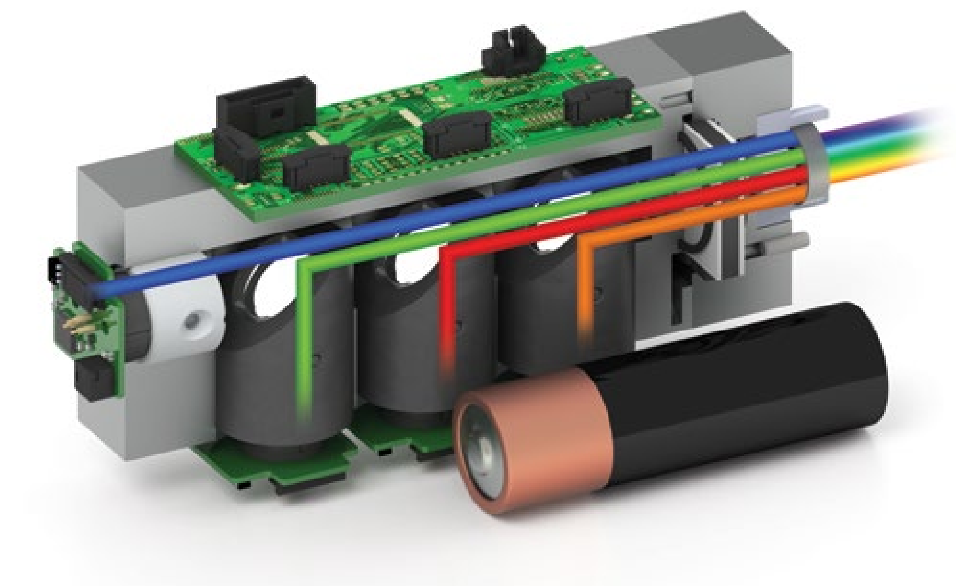
Module Design (Continued)...

a uniform high intensity across a wide field of view. These optics act to redistribute the power of each laser or LED in the module evenly across the full field of view with minimal loss of power, correcting for extreme spatial variations due to hotspots, multimode beam profiles, dark zones, and more.

The resulting flat top beam provides wide field illumination that is homogeneous with respect to the various color inputs, as well as overall. Uniformity across the field of view is typically better than 10%, even for up to 10:1 modulation in input intensity. The beam geometry may also be customized to create square, rectangular, and other illumination areas, as required, giving the customer ultimate flexibility.

To create a complete solution, the light sources, beam routing, and beam shaping optics are integrated into a single, highly efficient module with > 80% throughput for each source. These modules are designed to be extremely compact, lightweight, and low power to reduce instrument size and complexity. A dual-wavelength module, for example, can be as small as 50 mm x 60 mm x 25 mm.

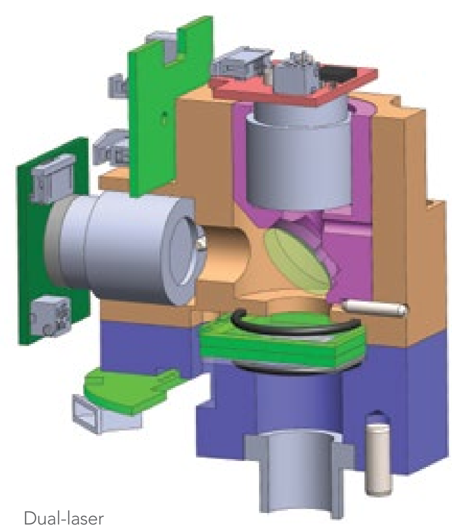
These pre-aligned, drop-in modules can save considerable time in alignment and assembly of OEM instruments for flow cytometry, sequencing, and molecular diagnostics. Cost and performance benefits can also be gained by leveraging IDEX Health & Science's vertically integrated supply chain, with access to best-in-class light sources, optics, and filters.



Module operation illustration and actual size comparison.
LED-laser combiner dimensions: 103mm x 26mm.

Specifying the illumination portion of the system at the module level reduces risk and overhead in instrument manufacture, and benefits manufacturing tolerances. The only required inputs to the module are DC power and source control, which can be provided as a TTL signal or analog/digital modulation of each light source. Modules include onboard monitoring of each light source for intensity feedback control at the instrument level.

Each wide field illumination module is designed specifically to the needs of the OEM customer. The application, fluorophores of interest, sample format, imaging area, and sensitivity needs are considered in combination with power budget and instrument layout to create a fully optimized solution that delivers the needed spectral profile and intensity uniformly across the desired illumination field.



Dual-laser beam combiner.

Module Reliability

As discussed previously, alternative beam shaping technologies are sensitive to small changes in input beam profile or alignment, which can degrade long-term performance and limit operating conditions. The Wide Field Illumination Module, in contrast, has been designed to be spatially invariant — even significant changes in the input beam as light sources age will not compromise the output beam shape or uniformity. This makes it a significantly better solution for commercial and field applications. Resistant to shock and vibration, these modules offer a typical operating temperature range of 10 – 40 °C.

To validate field performance and reliability, a series of environmental tests were performed in-house, comparing spatial beam profiles and operation before and after. Tests included temperature cycling, shock, and vibration at conditions appropriate to the biomedical instrumentation market. In all cases, the modules maintained <10% variation in output intensity across the spatial beam profile.

Specifications	
Temperature, Operating	+15 °C to +40 °C
Temperature, Non-Operating	-20 °C to +60 °C
Relative Humidity, Non-Condensing	20% to 80%
Shock, Operating	< 1 g; < 11 msec
Shock, Non-Operating	< 25 g; < 11 msec
Vibration, Operating	< 0.3 g; 5 Hz to 500 Hz sinusoidal, 0.25 octave/min < 1.0 g; 5 Hz to 500 Hz sinusoidal, 0.25 octave/min
Vibration, Non-Operating	< 2.0 g; 5 Hz to 500 Hz sinusoidal, 0.25 octave/min

Conclusion

Whether scanning across a full sample cell or array or quickly imaging a portion thereof, the custom Wide Field Illumination Module from IDEX Health & Science offers instrument designers a high-performance solution to cover more imaging area, consistently. Delivering up to five light sources directly to your sample with a rectangular or square-top spatial profile at high intensity, these modules allow you to optimize both performance and cost while reducing your instrument's size and complexity. Improve your throughput and reduce analyzing time with a compact, versatile wide field illumination module, available only from IDEX Health & Science.

For ordering, technical support, and contact information please visit www.idex-hs.com

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