

Advances in complex monolithic optics  
for optical tagging in multi-wavelength fluorescence microscopy

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Abstract

Fluorescent tagging techniques for illumination of biological cell structures, organelles, and proteins is a common research tool in many academic and government laboratories. And while the technique is making inroads into commercial laboratories for pharmaceutical discover and clinical diagnostics, the tools necessary to establish a reliable, robust manufacturing process are woefully inadequate for certain applications. In particular, though significant improvement has been made improving microscopy techniques and fluorescent reagents over the last few years, little improvement has been made to the light sources necessary to stimulate the substantial Stokes shifts of the fluorescence that allows the rejection of the illumination with relatively simple optical systems.

Initially, fluorescent lamination was used with HeNe and Ar<sup>+</sup> laser lines. As new reagents were developed, monochromator-based light sources were used to address the diversity of wavelengths; but low-power, off-wavelength light-leakage, and low sensitivity limits the monochromator's usefulness in commercial applications.

Rapid advancement of solid-state laser technology has resulted in a proliferation of wavelengths that largely eliminates the need for monochromator-based illumination instrumentation. Today, a series of discrete optics mounted in optomechanical mounts with precision adjustment enable combination of up to five lasers in a single instrument. However, the alignment freedom of each optomechanical mount that enables such architecture to work in the factory results in a highly-random assembly process that is less robust in the laboratory where the optomechanical assemblies are subject to temporal and environmental instabilities that require frequent realignment and cleaning for optimal performance.

Complex Monolithic Optic (CMO) design uses external assembly tooling to control alignment tolerances that can be locked in place such that internal hysteresis and other instabilities are eliminated. A CMO-designed, multi-wavelength combiner with 25- $\mu$ rad parallelism and 20- $\mu$ m beam centering achieves an accuracy that can be maintained over a wide variety of shipping, storage, and operating conditions. The first product will be offered with up to four common wavelengths (405, 488, 561, and 640 nm) with no ongoing alignment maintenance required. Because CMO-design is a scalable, additional channels can be available to support research needs.