Advantages of Monolithic Laser Combiner Technology in Confocal Microscopy Systems

White Paper

Abstract

Fluorescence microscopy techniques require a reliable light source at the desired wavelength or wavelengths, with minimal downtime for maintenance and alignment. Lasers are a popular light source, although the alignment and upkeep of laser combiners is a time-consuming prospect for many users. This paper describes a new combiner based on a complex monolithic optic design paradigm that provides improved performance over existing laser-based technologies.

Introduction

Fluorescence techniques are widely used in a variety of biological research applications, pharmaceutical discovery processes, and clinical diagnostics. As use of these techniques increased, the development of fluorescent reagents, illumination sources, and imaging optics increased in parallel. The majority of the measurements using fluorophores are designed around identifying and localizing single molecules and identifying structures within single cells in applications such as cell biology, biophysics, neuroscience, and systems biology.

Some of the most common microscopy techniques require sophisticated, high-resolution imaging schemes that use multiple fluorophores to carefully identify and locate biochemical structures, molecules, and molecular interactions. While a full discussion is beyond the scope of this paper, there is a rich literature base discussing techniques such as total-internal-reflection fluorescence (TIRF), fluorescence resonant-energy transfer (FRET), fluorescence lifetime imaging (FLIM), fluorescence recovery after photobleaching (FRAP), and fluorescence in situ hybridization (FISH). All these approaches use multi-wavelength illumination to provide high specificity to a mixture of fluorophores used in various assays.

The widespread availability of solid-state lasers enabled the evolution of illumination sources from monochromator/filter-based illumination to laser-based instrumentation that provides higher power in a narrower line width. At the same time, out-of-bandwidth leakage is eliminated. These devices integrate discrete optics and multiple lasers, but are expensive, cumbersome, and difficult to maintain.

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Optical Designs

Today’s optical designs have evolved to allow complex optical functionality, yet this added functionality requires the manufacturer or researcher to insert and align a variety of discrete optical components, mounts and tuning hardware in the optical path. This time-consuming and expensive process adds complexity to the optical path and decreases alignment stability while increasing the path length and the number of beam-to-surface interfaces.

The next generation of microscopy optimization simplifies and stabilizes the illumination source, the beam-combining and -splitting optics, and the beam-directing optical assemblies. Simplified optical integration reduces alignment time, shrinks bench-top footprint, and enables the use of microscopy instruments in realistic laboratory environments where repeated alignment and contamination of optical surfaces by condensation of volatile organics and other contaminants is an ongoing concern.

Laser-based options for achieving the multiple wavelengths of light required for many microscopy applications include: breadboard, optical bench, and complex monolithic optical design (CMO).

Breadboard

Breadboards were an early laser-based solution for illuminating biological samples with multiple wavelengths of light. They offer easy integration and flexible use of multiple lasers, and provide a wide range of wavelengths for high-resolution imaging. The simplest wavelength combination architecture is an assembly of discrete optics in separate optomechanical mounts. With precision mounts, breadboard assemblies can have a high degree of accuracy achieved through manual alignment of each precision mount relative to the others. However, the degrees of freedom afforded by each assembly also create a highly random assembly process and require time-consuming realignments, and result in a relatively high manufacturing cost.

Optical Bench

Optical benches (integrated optomechanical assemblies) can eliminate several degrees of freedom required for a breadboard assembly, making them more robust and easier to use in laboratory settings. Further, changing lasers connected to the optical bench is relatively straightforward. However, the alignment challenges remain comparable to those of the breadboard as both present a challenge in keeping the optics clean and protected from environmental factors such as dust, solvents, and even temperature changes.

Complex Monolithic Optic (CMO) Design

CMO-based laser combiners, the newest of the three technologies described in this paper, offer significant improvements over both breadboard and optical bench assemblies. CMO design integrates optical building blocks – such as those used for chromatic separation and combination, for beam steering, and for phase and amplitude control – into a single optical assembly. The optical surfaces are environmentally isolated because they are immersed in glass, which protects the coatings from environmental degradation.

CMO Technology

CMO technology is unique, from fabrication through use in the laboratory. In this section, we discuss the distinct differences throughout the life cycle beginning with fabrication, assembly and installation through to ease-of-use, reliability, and expandability.

Assembly

CMO design uses external and reusable fabrication and assembly tooling to ensure the precision dimensions and coatings of the individual optical surfaces and guarantee that assembly tolerances meet performance requirements (Figure 1). The tooling enables the tolerances to be controlled precisely during the optical fabrication so that all alignment tolerances are built into the optical components. Each optical surface within the assembly is secured to its surrounding optics using highly stable optical bonding techniques that achieve better than 25-µrad parallelism while maintaining a 20-µm beam centering, an accuracy that can be held over a wide variety of shipping, storage and operational conditions.

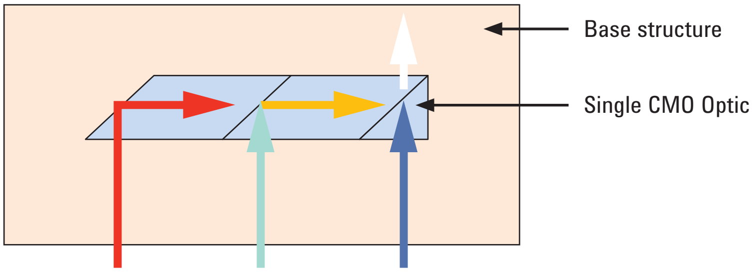

Figure 1. In a complex monolithic optic (CMO) design for three-wavelength combination, each optic component within the assembly is secured to its surrounding optics using highly stable bonding techniques.

CMO Technology (continued)

For comparative purposes, Figure 2 shows a typical three-wavelength breadboard combiner assembly that uses precision optical mounts to steer the beam and combine the three discrete wavelengths. Most microscopy applications favor consistency, and Figure 3 illustrates a common optical bench configuration for reducing manual alignment in standard combiners.

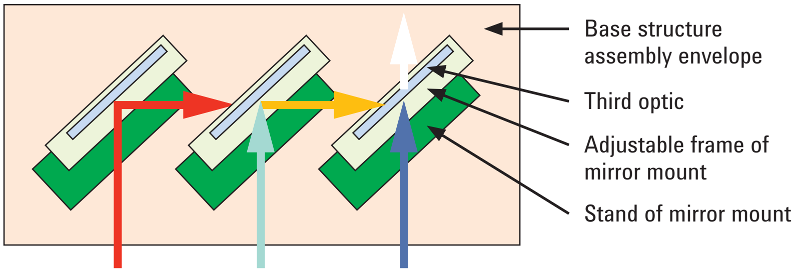

Figure 2. A typical assembly for combining three wavelengths, using mirror mounts, beam splitters, and reflection optics.

Incorporating the optical mounts into the base of the housing structure (Figure 3) replaces the manual alignment precision with precision that is machined into the base structure. This approach restricts the degrees of freedom (for instance, once a mount is incorporated directly onto the base structure, its ability to move in the X- and Y-directions is no longer limitless) and thus reduces the randomness of the assembly.

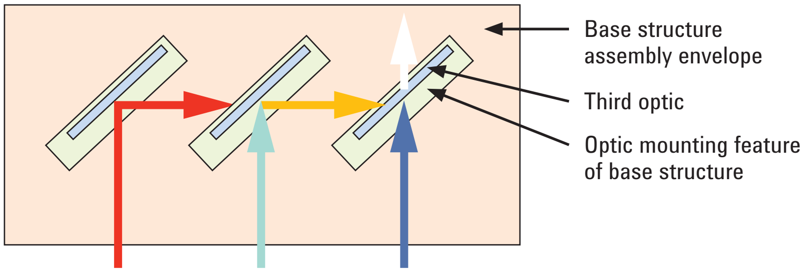

Figure 3. An integrated optomechanical design for three-wavelength combination has complex and precise mounting features fabricated into the base structure.

However, the high-precision fabrication of the base structure can be more costly than manual alignment of individual optical mounts. In this scheme, although the degrees of freedom in the assembly are reduced, the assembly maintains an undesirable level of randomness for the most demanding alignment requirements.

Installation

Compared with breadboard and optical bench combiners, monolithic laser combiners offer a substantial reduction in time needed for installation as well as a smaller bench top footprint. CMO design, proven to have a significantly higher reliability along with a 50% reduction in volume and weight in the semiconductor lithography market, does not require the optical surfaces to be mounted discretely, so the optical surfaces must be only negligibly larger than the clear aperture. This simplifies optical paths and allows tight beam spacing and folded beam paths that are not practical with the optomechanical designs. In turn, the overall assembly is smaller and lighter. In contrast, the optomechanical mounts used in the breadboard and integrated optomechanical assemblies require the optical components to be as much as 25 percent larger than the clear aperture so that the optic can be mounted without degrading optical performance.

The initial alignment of a system based on breadboard or optical bench design frequently takes up to a day of work by a trained technician or field service engineer. If the microscope needs to be moved at some point in the future, this process may be repeated. Even mundane incidents such as jostling or vibration can cause the laser and optics to become misaligned, stalling lab productivity until it is restored. One of the key features of the CMO design is built-in alignment. This is particularly advantageous during installations and moves. Typical setup and installation of a monolithic laser combiner takes only a few minutes, as the only two steps needed are connecting the instrument to the microscope and to a power source.

Maintenance and ease-of-use

Once a laser microscopy system is installed, its productivity is partly dependent on the amount of maintenance required to keep it operating effectively. The two factors with the greatest impact on maintenance are:

1. number of mechanical interfaces
2. number of beam-surface interactions

In the three-wavelength setup shown in Figure 2, the breadboard assembly requires nine mechanical interfaces (three interfaces per optic — the optic to the frame, the frame to the stand and the stand to the base plate), the integrated optomechanical design requires three mechanical interfaces (each optic to the enclosing structure), and the complex monolithic optic requires only one mechanical surface (the optic assembly to the enclosing structure which is fixed prior to shipment); the beam steering function still needs to be integrated in the CMO architecture, but can be done by methods far less sensitive to misalignment than those shown in figure 2 and 3. The more flexibility is built in to the beam alignment architecture, the more precisely performance can be driven for a single instant in time. Unfortunately, alignment flexibility is inversely proportional to alignment stability. So, increased flexibility results in greater performance degradation as a function of time.

CMO Technology (continued)

The degree of randomness in an assembly is proportional to the number of interfaces between the optical interfaces, so the probability of optical system failure from alignment drift scales with the number of mechanical interfaces. This scaling also applies to the challenges of realignment after deliberate changes such as moving the instrument, or accidental changes such as jolting or even vibration to the work surface. Thus, as the number of laser lines, and the complexity of an optical system increases, the CMO design advantage increases.

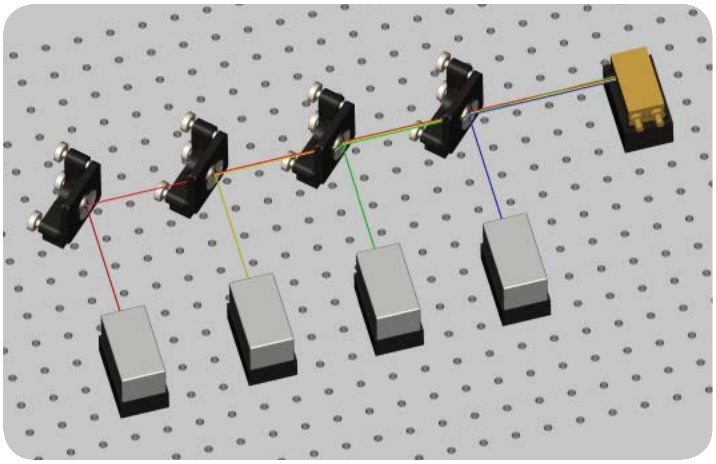

Figure 4. More points for adjustment mean more time adjusting – for both random and deliberately introduced changes.

The probability of an optical system failure also scales with the number of beam-to-surface interactions. Breadboard and integrated optomechanical assemblies are susceptible to alignment instabilities and environmental changes requiring repeated cleaning, adjustment, and realignment for optimal performance. For example, an individual mirror or dichroic filter mounted in the high-precision optomechanical mount of a breadboard assembly initially can achieve 1-µrad parallelism and 200-µm beam centering. Over time, the interfaces of the optic with the adjustable frame and with the mount to the base plate are subject to thermal cycles and internal stresses that can degrade the beam parallelism to as much as 500 µrad. The integrated optomechanical design initially can achieve 500-µrad parallelism with 100-µm beam centering; however, as with its breadboard assembly counterpart, the performance can degrade significantly over time and with environmental changes, resulting in decreased illumination power delivered to the image plane.

Nearly all optical surfaces of a complex monolithic optic used for beam combination are immersed in the glass substrate, protecting the interface from environmental degradation caused by particulates or by other surface contamination. Using this type of optic design is not advantageous for the simplest wavelength combination design (two-channel), but the advantage scales quickly with increasing complexity. For the three-channel combiner, using a complex monolithic optic yields a 25 percent reduction in the number of beam-to-surface interactions; for a seven-channel combiner, it yields almost a 70 percent reduction in the number of beam-to-surface interactions (again, the CMO combiner requires a beam delivery architecture that can be chosen to be more robust than those chosen for simultaneous beam-combination and beam-steering functions as in figures 2 and 3).

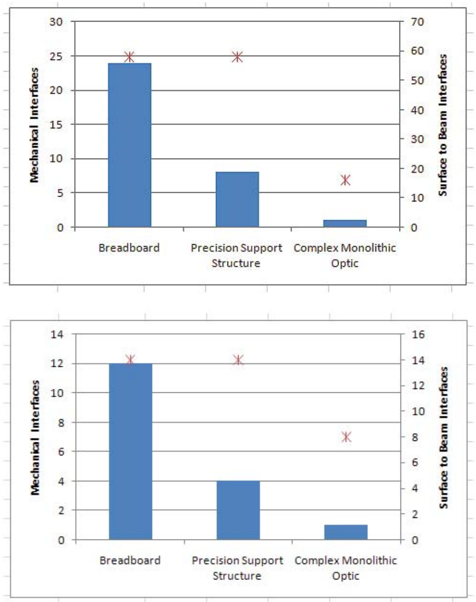

Figure 5. Reduced probability for optical system failures through reduced beam-surface interactions. 5a shows four-channel complexity, and 5b shows eight channels.

Expandability

Laser microscopy continues to be an evolving field, and researchers desire an ever-expanding roster of wavelengths for their investigations. It is also interesting to note that reagent development has largely followed the development of illumination sources, with absorbance spectra carefully tuned to available laser wavelengths. Initially these were designed around argon-ion and HeNe laser lines, which were the most convenient sources available during the early phase of this development. When researchers gain the ability to work with additional wavelengths without compromising alignment stability, the potential for new reagents and new scientific insight is enabled. This makes expandability an imperative, rather than an option.

Standard combiners accommodate expansion by adding another laser to the breadboard, with all the accompanying alignment challenges. Optical benches reduce alignment challenges somewhat, but are still time-consuming to work with. Further, expanding to include additional wavelengths typically requires a full round of realignment. However, CMO technology offers novel potential for expandability because of its cost-effective mechanical scalability. With no interfaces for alignment, the practical limitations on multiple wavelengths are removed.

Business Benefits

Cost-effective

The average lifespan for fluorescence microscopes is approximately ten years. During this time, most users upgrade the microscope illumination and imaging optics two to three times. An upgrade to a CMO-based monolithic laser combiner with its built-in ease-of-use, gives the user more uptime, resulting in greater productivity.

Embedding the optical surfaces into the glass substrate and building the alignment tolerances into the optical fabrication process enable a cost-effective alternative to high-precision optomechanical assemblies. Taking these measures decreases labor needed for system alignment and eliminates optical mounts and other hardware (screws, pins) required for breadboard and integrated optomechanical assemblies.

Additional savings are realized through the time saved on realignments. Calculating potential savings through elimination of planned realignments is simple and typically varies according to the number of wavelengths used. Factors may include optical technician’s time, training time for a biologist to learn how to do the alignment, and experimental downtime while alignment is being performed. Recently, users have reported that they spend four to eight hours per week aligning current systems. Unplanned realignments can be especially costly for labs using techniques that require hours of data collection, such as PALM, or for labs where using samples in a timely manner is critical.

CMO technology is proven technology that is already in use in industries ranging from aerospace and defense to semiconductors. In fact, the transition of optical microscopy platforms to designs that integrate new optical capabilities mirrors the evolution of displacement interferometers in the semiconductor lithography market a few years ago. Today’s microscopy researchers desire an easy-to-use, stable microscope platform that requires minimal setup, adjustment, cleaning, or alignment. Monolithic laser combiners based on CMO design offer all these things and the additional benefits of small footprint and reasonable price.

Summary

Many microscopy applications have a fundamental requirement of combining multiple wavelength sources. Three laser-based options for achieving this are: breadboard, optical bench, and CMO-based monolithic laser combiners. The alignment and upkeep of standard laser combiners is a time-consuming and unattractive prospect for users. CMO technology offers an advanced solution that is easy to use and enables more uptime for fluorescence microscopy labs.

Breadboard and optical bench designs are inherently limited by alignment instabilities due to mechanical drift and environmental changes. Maintaining peak performance requires cleaning, adjustment, and realignment. As the number of wavelengths included in these systems is increased, alignment needs also scale – exponentially. The number of surfaces and interfaces scales likewise, presenting a real challenge to productivity.

Traditional laser combiner and optical bench systems with discrete optics that are individually mounted and manually aligned in optomechanical assemblies work well in some laboratory setups. When time is not a factor, and there are trained technicians to maintain alignment and adjust for environmental factors, these systems with myriad points of adjustment may be suitable. However, most microscopy implementations require reliable performance and long-term system stability, and the traditional architecture is more of an encumbrance.

Complex monolithic optics offers a stable, intrinsically aligned solution for microscopists. By enclosing critical optical surfaces in a monolithic structure, CMO technology reliably provides light at the needed wavelengths, without needing to be cleaned or realigned, even in demanding environments. The small bench top footprint and built-in alignment enable functionality in workspaces where movement or lack of space might otherwise be prohibitive.

CMO technology is a cost-effective, user-friendly alternative to standard laser combiners and optical benches that offers higher reliability, accuracy and also overcomes the scalability limitations of these systems.

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