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A Compact Multi-channel Laser Scanning Confocal Optical Microscope

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INTRODUCTION

Confocal scanning optical microscopes have become important imaging tools for, engineers and scientists. However, their high cost, difficulty to use and lack of portability greatly limit their aplications. We have design a laser point scanning confocal optical microscope based on a new optical design to overcome some of the drawbacks of the existing confocal microscopes.

OPTICS DESIGN

The most important feature of our confocal microscope is its unique arrangement of the illumination pinhole and detection pinhole. Instead of placing the two pinholes at two conjugate planes, as is usually done in conventional confocal microscopes, our design combines the two pinholes together, thus completely eliminating the need of aligning the two pinholes to each other. This design significantly simplifies the alignment procedure of the microscope. It makes it possible to change the excitation filters, barriner filters and even the primary dichroic mirror and pinholes without need of re-alignment. The user can easily check the alignment condition of the microscope by simply inspecting the output laser beam from an empty objective port.

Another important feature of this microscope is its compactness. The portion of optics that has to be directly mounted to the microscope contains only two scanning mirrors, filters, beamspliters, confocal pinholes and a scanning lens, whereas the more bulky components, such as photomultiplier tubes (PMT), laser sources and electronics are mounted outside the optics module. The optical signal from the sample is collected through high transmission multi-mode optical fiber to the PMTs, and the excitation light is introduced through a single-mode optical fiber from one or multiple remote laser sources. The multi-mode fibers have a measured transmission effciency of more than 91% without coatings at the ends, and with anti-reflection coatings, the transmission ef-ficency can be easily raised to 98%. the laser coupling optics averages a 70% overall efficiency, depending greatly upon the beam quality of the lase source.

ELECTRONICS DESIGN

The electronics of the system can be divided into four major parts; scanner control electronics, light detection electronics, digitizing electronics and focus control electronics. The scanner control electronics includes a closed-loop scanner driver and a programmable raster scan generator. The raster scan generator generates the driving signals for the two scanning mirrors, and the scanner driver moves the mirrors to the specified positions. The closed-loop feeback circuity of the scanner driver automatically compensates the inertia effect and other nonlinear effects of the scanning mirror assemblies.

The light detection eectronics consists of a programmable high voltage power supply to the PMT, a matched PMT pre-amplifier, a low noise integration amplifer with a protection circuitry to shut off the PMT voltage when the input light is too intense. The selection of either the integration amplifier or the track/hold amplifier, the setting of PMT voltage and the adjustment of image black level can all be carried out through software controls. The amplified signal from the light detection electronics is then digitized by a high performance multichannel digitizing board. The digitized data can be processed, stored in the computer, or displayed as an image. The digitizing board has an EISA bus interface, which can support a data transfer rate of more than 10 MByte/sec. between the digitizing board and the host computer.

The focus control electronics drives a DC servo motor with a 10,000 count/revolution rotary encoder which is directly coupled to the fine focus shaft of the microscope. For a typical 100 micron/revolution fine focus mechanism, the theoretical resolution is 0.1 micron; however, since all focus mechanism has much greater backlash and exhibits uneven friction, the actual focus resolution is between .1 to .5 micron, depending upon the condition of the fine focus mechanism.

The electronics layout is completely modularized: each functional unit is put on a circuit board, self-contained and independent of each other. The electronics chassis can house up to three PMT boards and an optional photo detector board to simultaneously detect three fluorescence Channels and one transmission channel. This modular electronics design makes the system much more flexible and expandable.

SYSTEM TESTS

We have built several systems to test our design. The test systems have two simultaneous fluorescence channels, two sizes of pinholes and three built-in dichroic mirrors and use a green HeNe laser and Agron Ion laser as a source. After having gone through more than half a year of operation and multiple times of packing, shipping and reinstalling at different locations, they all demonstrate good stability. No extra alignment is need. We hae also tested several key opto-mechanical components, especially the pinhole assembly. We built a mechanism to switch between the two pinholes. After 10,000 cycles, the pinholes were still in good alignment. We have manually operated the beamsplitter assembly and fiber-optical coupling mechanism for more than half a year. No additional adjustment is required. With the laser-to-fiber coupling optics pre-aligned and installed to the laser source, we can easily switch between different laser sources without any alignment and still have a coupling efficiency of around 50%. One of the key tests of they system integrity is the vibration control. Since the system is built from a solid metal frame, and has a very low profile, it demonstrates very good vibration-damping capability, even on a relatively weak microscope stand without additional support. For critical imaging applications, we have found that a table top vibration isolation bench is effective against most ambient disturbances.

We have tested the image resolution using different fluorescence samples. Good depth and transverse resolution are obtained with the small pinhole (20 microns), whereas the large pinhole (50 microns) yield much brighter images but with lower resolution. To check the system optical signal to noise ratio, we measured the total internal light scattering. The combination of special optical arangement and filter selection reduces the total system internal light scattering to such a low level that the reflection from the objective lens becomes the dominant source of background light. Further reducing the background light will require careful selection, or special design of the objective lens.

CONCLUSION

We have developed a high performance compact multi-channel laser scanning confocal optical microscope. The system is capable of producing up to three channel simutaneous fluorescence images and one optional transmission channel. The system is very easy to set up, align, and operate. It is ultra compact and has a light weight (the optics module measures 3.5 "X6.5" X8.5" and weighs less than 8 1b). It can be readily fitted on most upright and inverted microscopes.