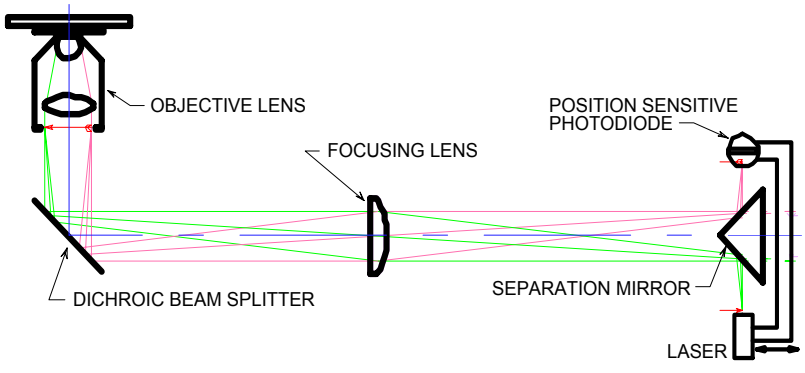
Applied Scientific Instrumentation

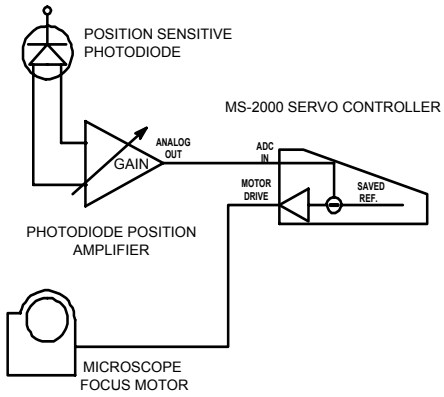
CRIFF Continuous Reflective-Interface Feedback Focus System

- **Closed-loop positioning system with feedback sensing at the sample**
- **Maintain long-term precise nanometer-scale continuous focusing – perfect for TIRF Microscopy**

The CRIFF substantially eliminates focus drift for high power microscopy. The system effectively monitors the distance between the objective lens of the microscope and the cover slip used to mount the sample under examination. Minute changes in the distance between the sample and the objective lens are sensed using an optical detector. This detector provides a feed back signal for the closed-loop positioning system that actively controls the focus of the microscope.

The Z-axis positioning mechanism can be our standard MFC-2000 focus controller system, our 3-axis MS-2000 and MS-4000 XYZ stage systems, or our PZ-2000 piezo-Z top-plate stage systems as well as our manual PZM-2000 version.





We Create Solutions

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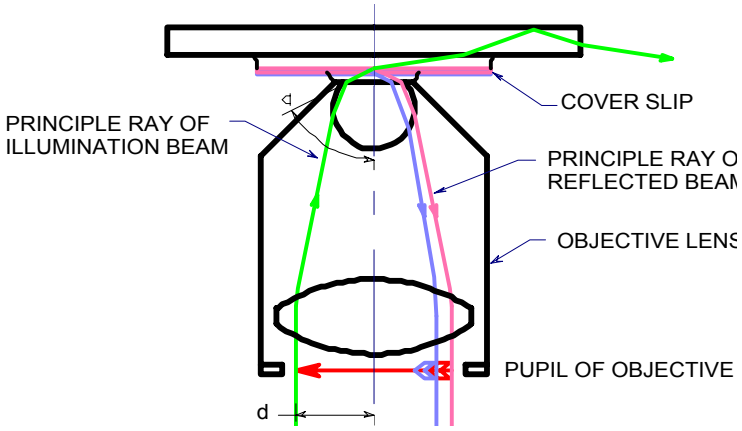
Background

Providing stable focus of a high power microscope over long periods of time can be extremely difficult. Several factors combine to frustrate the microscopist. The very narrow depth of focus of the microscope objective (<300 nm for high NA objectives) means that focus position must be held to less than 100 nm for optimum stability. Focusing mechanisms have inherent mechanical limitations at this scale. Minute rotation of the focus shaft or the compression of grease layers on the gears of the rack and pinion mechanism generates enough motion to defocus the microscope. Equally important are minute dimensional changes in the microscope caused by thermal expansion of the microscope components. Researchers needing to maintain accurate focus for more than a few minutes can be plagued by such focus drifts.

The CRIFF uses a laser with about 1 mW of intensity as the illumination source for the position-sensitive detector. The laser is focused into the back pupil of the objective lens, and introduced parallel to its centerline axis, offset by an amount *d* as shown in the figure on the right. The positioning system will make use of the small geometric shift in the position of the beam that is reflected off the cover slip / sample interface. Ordinary cover slips are used with TIRF objectives, while reflective-coated cover slips are required for objectives with an NA of less than 1.4.



The CRIFF module fits on the camera port of most microscopes. The unit contains a dichroic beam splitter that allows visible light to go to a camera while reflecting the 780 nm laser light. The CRIFF module has provision for a blocking filter in front of the camera.

Specifications

CRIFF-controlled Z-axis Resolution	< 5 nm
CRIFF-controlled Z-axis Drift	< 40 nm per hour
CRIFF Response Time	< 0.5 seconds