

innovations

from The University of Vermont

TITLE: FOCUS FEEDBACK SYSTEM FOR MICROSCOPY

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DESCRIPTION: There has been a recent surge in the use of totally internally reflected (TIR) microscopy for high spatial resolution imaging of objects at a glass-liquid interface. Most often TIR microscopy is used in fluorescence imaging and thus referred to as TIR fluorescence microscopy (TIRFM). For “through the objective” TIRFM to work properly, a focused laser beam enters the back of the objective close to the outer edge of its diameter. The light leaves the objective into the sample at the required critical angle for TIR. The light is then TIR at the glass – liquid interface where it creates an evanescent field propagating into the liquid from the glass surface. This invention takes advantage of the returning TIR light, which is adjusted such that the reflected beam returns back into the objective lens when the image plane is coincident with the evanescent field. When the objective is slightly out of focus, the returning light impinges on a different area of the objective lens, causing the light refracted by the lens to exit the objective at a different angle. Also, if the objective is greatly out of focus, or the coupling oil between the objective and glass has an air bubble, no light will return through the objective. Using this phenomenon, our invention provides an accurate means of establishing the critical distance between the high NA objective and the specimen plane where TIR is desired, and confirms the quality of TIR. This invention uses the return TIR beam as a signal source which can have multiple applications including focus control and TIR quality indication in a TIRFM system.

ADVANTAGES: At present there are no automated methods for determining whether or not total internal reflectance (TIR) has been obtained in a sample slide on a microscope stage. This invention provides a rapid and extremely accurate method for confirming the existence of TIR. In addition, the same signal used to confirm TIR can be used to drive a feedback-based auto focusing system. This would provide significant advantages over any existing focusing system by being rapid, extremely sensitive, and accurate.

PATENT STATUS: Patent Pending

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