

Simultaneous imaging of multiple focal planes in fluorescence microscopy for the study of cellular dynamics

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The use of fluorescence microscopes in conjunction with highly sensitive CCD cameras has enabled cell biologists to visualize the intracellular dynamics of proteins in real time. The ability to study the protein trafficking pathways within cells, which typically span all three dimensions, holds the promise to provide new insights into the mechanisms of cellular dynamics. However, current microscope design is not well suited for this purpose, since it does not permit the simultaneous imaging of different planes within a cell. Moreover, devices such as piezo positioners that are currently used to collect z-stacks of biological specimen are relatively slow compared to the timescale of cellular dynamics. This means that important events may be missed if they do not occur at the focal plane that is being imaged at a given time.

To overcome these shortcomings, we present a modification of the conventional microscope design that permits the simultaneous imaging of multiple planes within a specimen. In our design, the collected fluorescence signal from the infinity corrected objective lens is split into several channels by beam-splitters (dichroic mirrors). Light in each channel passes through a tube lens and is then collected by a CCD camera, which is located at a plane that is away from the focal plane of the tube lens in that channel. By ensuring that the camera position with respect to the tube lens focal plane is not the same in any two channels, distinct planes within the specimen can be simultaneously imaged.

The proposed design can be used either to image the sample in single color or in multiple colors. To achieve the latter, we have modified the excitation pathway in our microscope in such a way that the sample can be simultaneously illuminated in Total Internal Reflection Fluorescence (TIRF) mode and widefield fluorescence mode in one or more wavelengths. This excitation scheme provides an elegant way to study various types of intra-cellular trafficking events in three dimensions. For instance, TIRF illumination is necessary to study events close to the membrane such as exocytosis, while widefield mode is required for illuminating the interior of cells. This, in combination with multiple labelling of vesicles, tubules or organelles of interest provides a novel methodology to simultaneously study protein dynamics at both the cell surface and the interior of the cell. We validated the above proposed modifications by imaging bead samples and by tracking tubules containing the protein FcRn (neonatal Fc receptor) from the sorting endosome to the plasma membrane in live cells simultaneously across multiple planes.