

Simultaneous fast scanning TIRF and targeted laser illumination

TIRF microscopy offers a unique opportunity to observe cellular events within a very well defined plane ($\leq 300\text{nm}$) adjacent to the coverslip to which a sample is adhering. The technique can provide high contrast quantitative fluorescence images, but conventional approaches to achieve the required illumination rely on laser excitation being focussed to a single point. TIRF images resulting from such point illumination are prone to artefacts including shadowing and interference patterns which can limit the usefulness of the acquired data. An alternative to the single point approach is to scan the illumination point around the objective so the sample is evenly illuminated from multiple directions. Here we demonstrate uniform TIRF imaging using a fast scanning mirror system, which allows the sample to be completely scanned during a single image capture. The system also allows precise, calibrated penetration depth control that can be dynamically altered between image frames. A commercially available implementation of this technique will be described, together with a dual laser illuminator that allows real time multiple angle TIRF microscopy and simultaneous targeted laser action.