Real Time super-resolution image acquisition

Recent advances in imaging have allowed identification of structures beyond the Abbe diffraction limit in biological samples, allowing structures as small as 20nm to be localised. Techniques such as PALM or STORM involve capture of fluorescence emission from a limited number of single molecules per camera frame and repeating the process many thousands of times. To achieve the highest quality images with a very low background we make use of a scanning TIRF illuminator at high incident angles to achieve near dark field laser illumination. The resulting thin uniform illumination volume reduces the contribution to the image background of light from out of focus planes giving very high contrast images of the individual fluorescence molecules. Super-resolution images are constructed in real time by calculation of the coordinates of each molecule within the individual images of the sequence using a combination of wavelet filtering and guassian fitting on a GPU-accelerated system, with each point in the resulting image representing detection of fluorescence from a single molecule. We describe a high speed turnkey system for the capture of image sequences and the display of super-resolution images in real-time, limited only by the camera frame rate. The real-time analysis together with automated control of the fluorescence reactivation allows signal capture at each frame to be optimised with the resulting super-resolution image available immediately on completion of the acquisition.