Descanned Point Detection Of Single Fluorophore Fluorescence

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An inverted microscope system combined with laser scanning functionality is used for standard wide field fluorescence detection with an EMCCD camera, and descanned point detection of single fluorophores with one or two avalanche photo diode[s].

The system consists of a standard microscope frame with an integrated dual color emission module used for wide field imaging (FRET). Furthermore the microscope is equipped with a galvanometer based laser scan head (Yanus Scanhead), and a galvanometer based light source mode switch (Polytrope II).

This system setup provides fast real-time live modes, and real time protocol operation using an arc lamp based, or laser based fluorescence excitation light sources. Possible applications within this system configuration are (2D)TIRF, Structured Illumination or FRAP / photo activation for example.

For descanned point detection of single fluorophores a parallel beam path (port II) is separated by a dichroic mirror (with long pass characteristics) from the standard laser scan head condenser optics beam path (port I). The dichroic filter reflects fluorescence excitation light into the sample. On the other hand emission light passes the dichroic filter, and is aligned on the entrance aperture / core of a multimode fiber. The diameter of the fiber core and the magnification of the objective lens define the detection volume of the descanned point scanning confocal setup.

The multi mode fiber then guides fluorescence photons to a photon counting unit consisting of one or two avalanche photo diodes. Additional photon counting electronics, and PC software tools allow data processing in order to monitor time traces of photons or photon correlation characteristics of single fluorophores.

The seamless integration of the point scanning descanned fluorescence detection into the inverted microscope frame setup provides an inherent robust feature compared to traditional confocal microscopes.

In the presentation the optical setup and first experimental results of the setup will be presented.