OLYMPUS

FLUOVIEW® FV1000/FV1200

UPGRADE TO
3D NANOIMAGING
AND SINGLE MOLECULE TRACKING
FOR OLYMPUS FLUOVIEW® FV1000/FV1200

Within the past few years, several methods have been devised in order to obtain images with nanometer resolution of cellular features using an optical microscope (STED, PALM, STORM). Although powerful, these methods are quite inefficient when detecting sparse nano-structures in an image. Also they are inadequate to detect the dynamics of chemical reactions which occur in the sub-second time scale in nanometer-size 3D structures, which are continuously moving and changing shape.

Olympus FluoView® FV1000/FV1200

The Nanoimaging and Single Molecule Tracking Package

With NanoImaging approach to super-resolution, the laser beam does not scan the sample following a predetermined pattern as is the case in raster images. Instead, the laser scanning imaging is based upon a feedback algorithm where the path followed by the laser beam is continuously adjusted and decided during the scan according to the shape of the object to be imaged. The algorithm moves the laser spot at a fixed distance from the object's surface; as the position of the laser spot and its distance from the surface are known parameters, they are utilized to reconstruct the shape of the object. 3D cellular structures can be resolved down to 20-40 nm with a precision of 2 nm in a matter of a few seconds.

The Upgrade Package for the Olympus FV1000/FV1200

Below is a schematic of the NanoImaging units and its connections to the FV1000 confocal microscope. A switch box allows for the user to operate the FV1000 in the standard mode of operations, or to activate the NanoImaging system. When in nanoimaging operation, the galvo-mirrors of the FV1000 are controlled through the electronics provided by ISS. The signal is collected by the internal detectors of the FV1000 and diverted to the ISS photon counting data acquisition unit. Molecules are tracked in the XY plane using the galvo-controlled mirrors of the FV1000 and in the z-axis by a piezo-controlled stage. Instrument control, data acquisition and display are done on a separate computer.

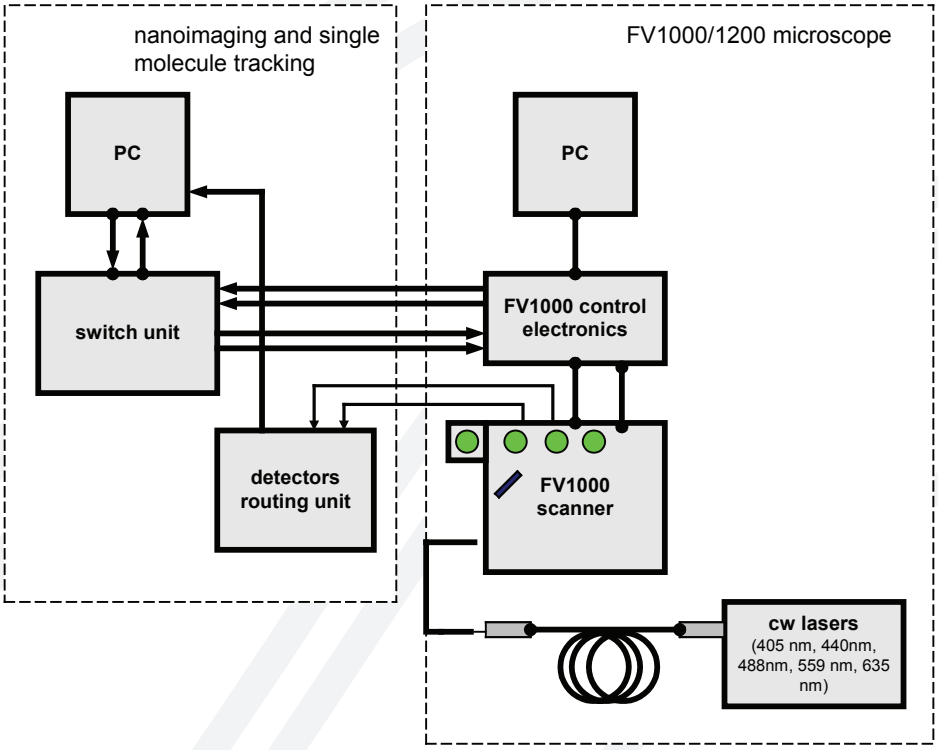

Figure 1. The right section includes the instrument components (PC, control electronics, scanner and laser launcher). The left section of the schematics includes the components provided by ISS with the upgrade package.

Acquisition and Processing Software

Instrument control and analysis software are provided by the SimFCS software (by Globals Unlimited). Once the confocal image has been acquired and the orbit location is selected, the user selects the initial orbit coordinates (radius) and the number of oscillations per orbit. Along an orbit data are acquired at 8-32 oscillations; a linear interpolation at 128 points is used to reconstruct the geometrical shape of the orbit. The operation is repeated at different values of the z-position. Eventually a 3D mesh reconstruction of the object is achieved using the stack of images at different z-planes of the orbit. The final touch is given by covering the mesh with a "texture" given by the specific quantities acquired such as the fluorescence intensity at each point.

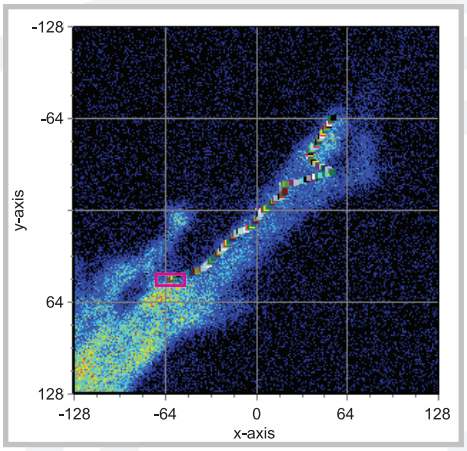

Figure 3. 3D raster scan image of a protrusion of MB231 cell growing in a 3D collagen matrix expressing actin-EGFP ***(courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine).***

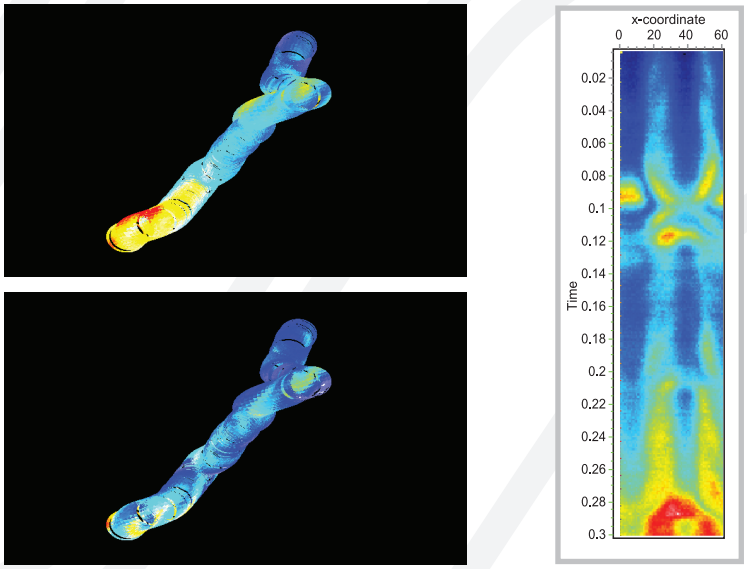

Figure 4. Image of the rectangular portion of the protrusion indicate in Figure 3 where in Channel 1 (top figure) the Actin-EGFP was tracked while in Channel 2 (bottom figure) the SHG signal of collagen was acquired. The diameter of the protrusion changes along the filopodium. The fluorescence is not uniform on the cell surface but clusters at specific direction where contacts are made with the collagen matrix ***(courtesy of Laboratory for Fluorescence Dynamics, University of California at Irvine).***

Principle of Operation: The Modulation Tracking Algorithm

The sequence of operations for using the SMT NanoImaging is straightforward: firstly, a confocal image of the area of interest is acquired; then, the object to be imaged is identified by the user. The SMT NanoImaging is activated through the switch and the laser beam is positioned at a distance of 100-200 nm from the center of the object.

As the laser spot approaches the surface to be imaged, the amount of fluorescence increases. Yet, the increase in fluorescence depends upon the distance as well as upon the concentration of the fluorophores and their respective quantum yield. In order to separate the effect of the distance from the effect due to the concentration, the position of the spot is forced to oscillate perpendicularly to the surface. That is, the intensity of the fluorescence changes during the oscillation ***(Figure 2).***

The beam spot travels in a circular orbit around the object and its distance from the object's surface is varied periodically at a set frequency; typically, for each orbit the number of oscillations is between 8 and 32 depending upon the size of the object. These small oscillations of the radius are used to calculate the modulation function of the orbit,from which the distance of the spot from the surface is determined.

The modulation function is defined as the ratio between the alternating part and the average part due to the local fluorescence of the surface. Practically, the modulation is the ratio between the spatial derivative of the PSF and the intensity. The modulation function increases quasi-linearly as a function of the distance from the surface and this feature allows for its use in determining the distance of the laser spot from the surface along the orbit. In this way, the transversal shape of the object is calculated and reconstructed.

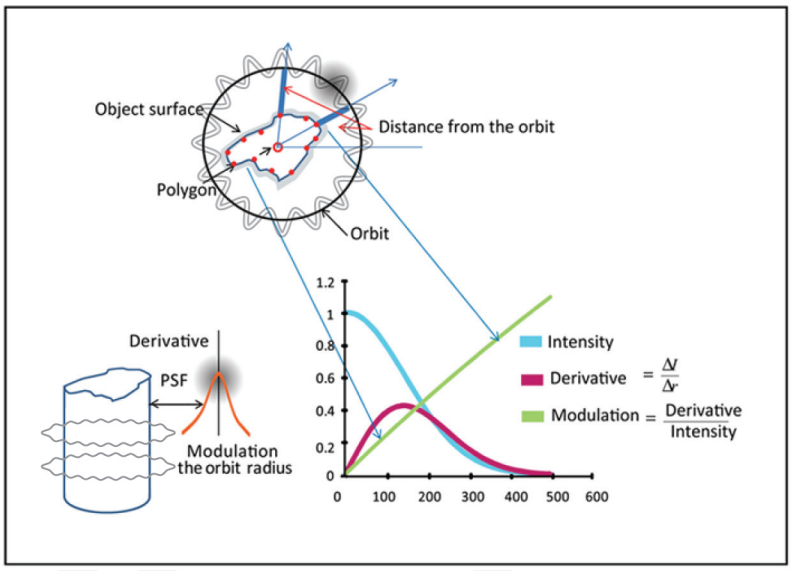

Figure 2. Schematics of the modulation tracking technique.

Specifications

Tracking methodology	XY-axis using galvo-controlled mirrors Z-axis using piezo-controlled stage
Maximum Resolution	20 nm ± 2 nm
Data acquisition frequency	32 to 256 KHz
Circular orbit frequency	2 KHz
Detector Detection Electronics	Internal PMT of FV1000/FV1200 ISS photon counting unit
Computer	3 GHz, 1 GB RAM, 200 GB hard drive and 27" monitor; minimum specifications shown
Acquisition and analysis software	SimFCS by Globals Unlimited

References

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2. ***Measurement of Distance with the Nanoscale Precise Imaging by Rapid Beam Oscillation Method***; Luca Lanzano e Enrico Gratton. Microscopy Research and Techniques 75 (2012) 1253-1264.

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5. ***Distance measurement by circular scanning of the excitation beam in the two-photon microscope***; K. Kis-Petikova, E. Gratton. Microsc Res Tech 63 (2004) 34-49.

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