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Generation and Deconvolution of 3-D Voxel Cubes with Leica DM and Optimas

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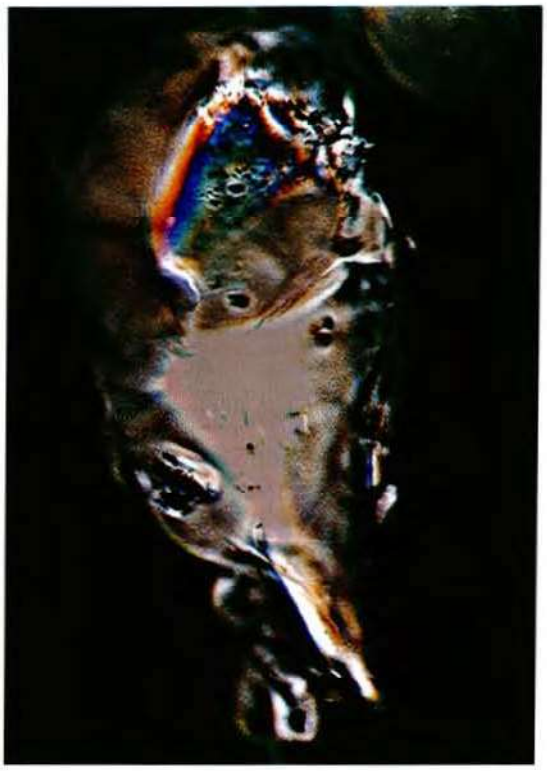
The new generation of research light microscopes opens the way for an easy interaction with PC-based commercial image processing systems. The DM microscope from Leica used by these work is one of such microscopes with many function controlled via RS232C. In these work z-axis position, light intensity and zoom are used only. The z-axis has a very high precision of 0.1 µm per step. The light intensity is controlled by the lamp voltage in the range from 5 to 12 V DC in 256 steps. The zoom can be changed by PC from 0.9x up to 2.5x with a resolution by 0.1x. So it is possible to work with a very high magnification. The camera is a SONY 3chip 1/2" color DXC930P. It is possible to control all camera parameters and functions by PC applicating the 930Control kit. It is also possible to work in the low light field applicating the long time integration mode. Up to 256 normal frames can be integrated directly on the CCD-chips, so that the camera is up to 256 times more sensitive then before. It is possible to acquire color fluorescence images particularly FISH (fluorescence *in situ* hybridization) in a very high quality. A cooling kit with Peltier-element and head sink reduced the noise for image calculation and long time integration. A special image processing filter -called "starkiller"- eliminates really the last pixel defects. Parameter set for special probes and color light temperatures can be saved or loaded, so an optimal adjustment is possible. A color frame grabber CFG from Imaging Technology Inc. digitise the RGB-signals of the CCD camera with a resolution of 768x 512 pixel and 3x 8bit. A normal ISA-bus PC 486/50 was extended in the RAM to 32 MByte to opens the field of application with larger voxel cubes without swapping to the hard disc drive. The systems works under MS-Windows for work groups version 3.11. The image processing software is OPTIMAS from Optimas Inc. version 4.1 extended by Macros written with the ALI- language from OPTIMAS. Functions being critical in time are written as fast C-functions by MS-Visular C++ version 1.5 as Dynamic Link Libraries and are included into ALI. OPTIMAS is an open System with an interface -the Register() and Call() functions- to include fast owner functions. These functions creates voxel cubes in an optimal structure for the i486 processing unit. Using these special structure is possible to the CPU to do a lot of image calculation work directly with dates in the very fast 1. level cache on the CPU. A macro generates multiple layers grabs and fills the voxel cube V with the information from the 2-D image fields (dimensions: sizeX, sizeY) automatically up to sizeZ steps. The first operation is a deconvolution like:

$$Va(x,y,z)=V(x,y,z)+A*V'(x,y,z+1)+B*V'(x,y,z-1)$$

$$V'(x,y,z) =\Sigma V(x+i,y+j,z+k) \quad -1<=i,j,k<=1 \quad 0<=x<sizeX,\ 0<=y<sizeY,\ 0<=z<sizeZ$$

A and B are parameter to describe a simple Point Spread Function with 0>A,B>=-5/90. The results can larger the 8bit and small values are also important to, so it is necessary to scale with a large factor and a non-linear gamma transformation is also necessary to a good D2- field on the display monitor. These operations will be done in some seconds for the full cube. The global result is a new color voxel cube with more details and a broad contrast. The new voxel can be displayed as a sequence of differed cuts ore different projections can be calculated. Also the way for 3-D segmentation is open. The experience from these will be included into a now more powerful version based on fast 300MHz DEC-Alpha RISC processing units with high speed PCI- frame grabber, up to 256MByte RAM and larger 2. level cache based on Windows- NT. The problem of visible digitising noise after the calculation will be reduced by frame integration behind



the ADCs and an extension to 10bit input frames ore by cooling the ADCs. This work could be done by the friendly help and support due to the Institute of Molecular Biology of the University Jena especially to Mr. Schwertner.