

## A System for Automated Acquisition of 3D Biomedical Images Using Confocal Microscopy

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Keywords: automated acquisition, confocal microscopy, image stitching, light attenuation

The described system for the coarse-to-fine positioning of XY motorized microscope stage allows us interactive control of image acquisition in confocal microscopy. An image captured through a low-resolution objective serves as navigation map where the user points to positions or areas of interest in which a high-resolution image will be captured. Our contribution presents an extension of a two-level coarse/fine algorithm to a multi-level environment.

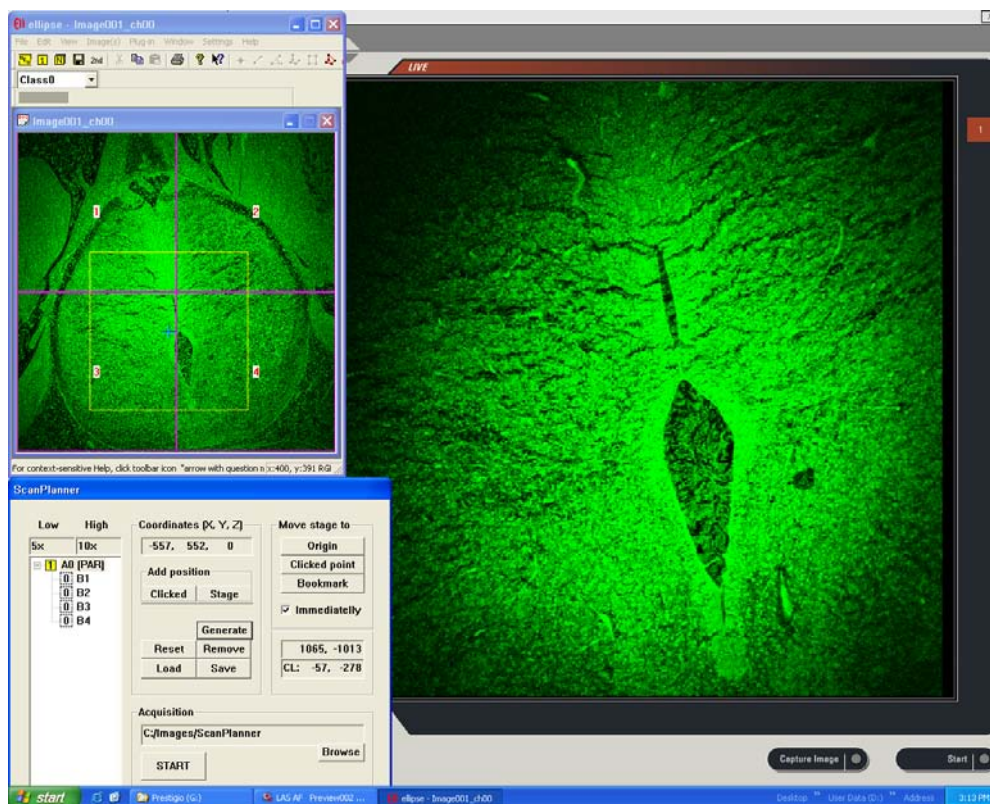
A microscope itself represents a multi-resolution device because it is supplied with a set of objectives with different magnification (~resolution). The system must be first calibrated to determine the correspondence between the screen coordinates and the real units required to control the motorized stage. Our navigation algorithms allow various applications: A) Time lapse experiments: From positions marked in a low-resolution image, high-resolution images are acquired periodically. Then an animation can be created, representing, e.g. a “movie” of dividing cells. B) Generation of stereological probes: Instead of a point of interest, an area of interest can be outlined in the low-resolution image. Stereological probes (e.g. “counting frames”) are subsequently generated inside this area, respecting specific stereological rules where position is random, systematic random or given by a probabilistic function. C) Image tiling and stitching: The user often needs to have a high-resolution image of the whole area of interest larger than the field of view provided by a high-resolution objective. Then the resulting image can be assembled from neighbouring positions, completely covering the user-defined area of interest. The algorithm generates stage compatible coordinates of neighbouring positions, captures the corresponding images (overlapped if necessary) and creates a new image composed from these images.

The whole system is controlled by the main dialog with a tree-like control. At the beginning, the tree control contains only the root node labeled “A0” (level A, region 0) and represents the initial image captured by the lowest resolution objective, see Fig. 1. A virtual objective can be used instead of the real one and then the program generates a white image for “blind navigation” (like using a white paper instead of a map). A manually entered (clicked) position on the left image can be saved which adds a new node to a child level of a tree control (B2-B4 in Fig. 1). After entering the child nodes, the SCAN process moves the stage to saved positions and captures corresponding images. Any node can be defined as a new parent and the whole DEFINE/SCAN process can continue on the next level acquiring images at higher magnification. It is up to operator’s decision what parents and how deep in the tree structure he continues. Some nodes evidently represent regions that are not interesting and where the higher resolution image acquisition makes no sense. Automatically

generated positions are an alternative to manually entered ones. This option is very useful in applications like image tiling, stitching, generation of stereological probes, etc. An example of image stitching which creates a new large image is shown in Fig. 1. An important feature of our software consists in easy visualization of any captured image just by clicking the node on the tree control. Both corresponding image and its position on lower resolution parent are displayed. The software was developed in C++ as a plug-in module of the Ellipse image processing software package ([www.vidito.com](http://www.vidito.com)).

The described system is accompanied by algorithms that compensate both uneven illumination of neighbouring captured images (neighbouring fields of view) and light attenuation of confocal 3D images with depth, which are effects observed in confocal microscopy. By applying these algorithms resulting images of high quality are obtained. The system also enables us acquisition of confocal data sets for performing 3D reconstructions of large biological specimens [1].

1. Čapek, M. - Brůža, P. - Janáček, J. - Karen, P. - Kubínová, L. - et al.: Volume Reconstruction of Large Tissue Specimens from Serial Physical Sections Using Confocal Microscopy and Correction of Cutting Deformations by Elastic Registration. *Microscopy Research and Technique*. 2009, vol. 72, no. 2, p. 110-119. ISSN 1059-910X.
2. Supported by grants of MŠMT ČR MSM6840770012, LC06063, KONTAKT MEB080831, GAČR 102/08/0691, 304/09/0733, AVČR A100110502, VEGA-2-0164-07.



**Figure 1.** Software for multi-resolution control of a confocal laser scanning microscope: A low-resolution image (upper left) serves as a map, a high-resolution image (right) represents a live image from the current position. The frame in the center of the left image outlines the area which is visible in the right image. Labels 1,2,3,4 represent generated centers of neighboring images proper for stitching. Lower left part shows the control dialog containing the tree control with two levels which can be extended to a multi-level structure.