M³ a New Software to Quantify Blood Vessels in Three Dimensions Examined in the SEM / ESEM which also Allows to Render 3D Models of Vascular Trees

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Scanning electron microscopes (SEMs) are potential tools for morphological research. They have a great resolution and a high depth of focus, which gives SEM micrographs pseudo three-dimensionality. Adversely, the high depth of focus prevents accurate dimensional or spatial measurements of imaged microstructures from either the SEM video-display, printed micrographs or from photo-negatives. Macroscopic objects are viewed close up using binocular vision. Binocular vision is also used in microscopy where stereophotogrammetry and related techniques applying stereo paired images, and a variety of hardware tools calculate the third dimension (z-coordinate) using the parallax.

A method (3D-morphometry, *ComServ*, Austria) for dimensional and angular measurements of microstructures imaged in the SEM was developed in our lab in 1999 [1]. It uses digitized stereo paired images frame-grabbed (slow scan) directly from the SEM's photo-display, vector equation-based algorithms for calculation of spatial coordinates and derived distance- as well as angular measurements, and offers dynamic data exchange (DDE) together with on-line graphs of frequency distributions of measured variables. Formulas for central perspective depth computation allow the overall error to be less than 1.0%.

Meanwhile (2009), the method was further improved and the new java based software M³ is suitable to be used with Windows Vista as well. A modern graphical user interface (GUI) (Fig. 1), a new data interface (open office) is supported and an improved usability facilitates measurement processes [2].

This technique is currently used to analyze a) the geometry of microvascular trees in terms of vascular parameters (i.e. diameters, interbranching distances, branching angles and intervascular distances) and b) to determine bifurcation indices and area ratios followed by a calculation of optimality principles which are realized within single arterial bifurcations respectively venous merging [3-4]. Moreover the program allows to generate anaglyphic 3D images (Fig.2) and to build up automated rendering models of vascular trees (Fig.3).

References

[1]   Minnich et al. *J. Microsc.,* 195 (1999) 23-33.
[2]   Minnich et al. *Proc. XX. Int. Symposium on Morphological Sciences, Timisoara*, (2008) 170.
[3]   M. LaBabera, *Science*, 249 (1990) 992-1000.
[4]   C.D. Murray, *J. Gen. Physiol.*, 9 (1926) 835-841.
[5]   This work was supported by *ComServ,* Austria and the University of Salzburg. Further information on the program can be gained at http://comserv.at/index.php/software.
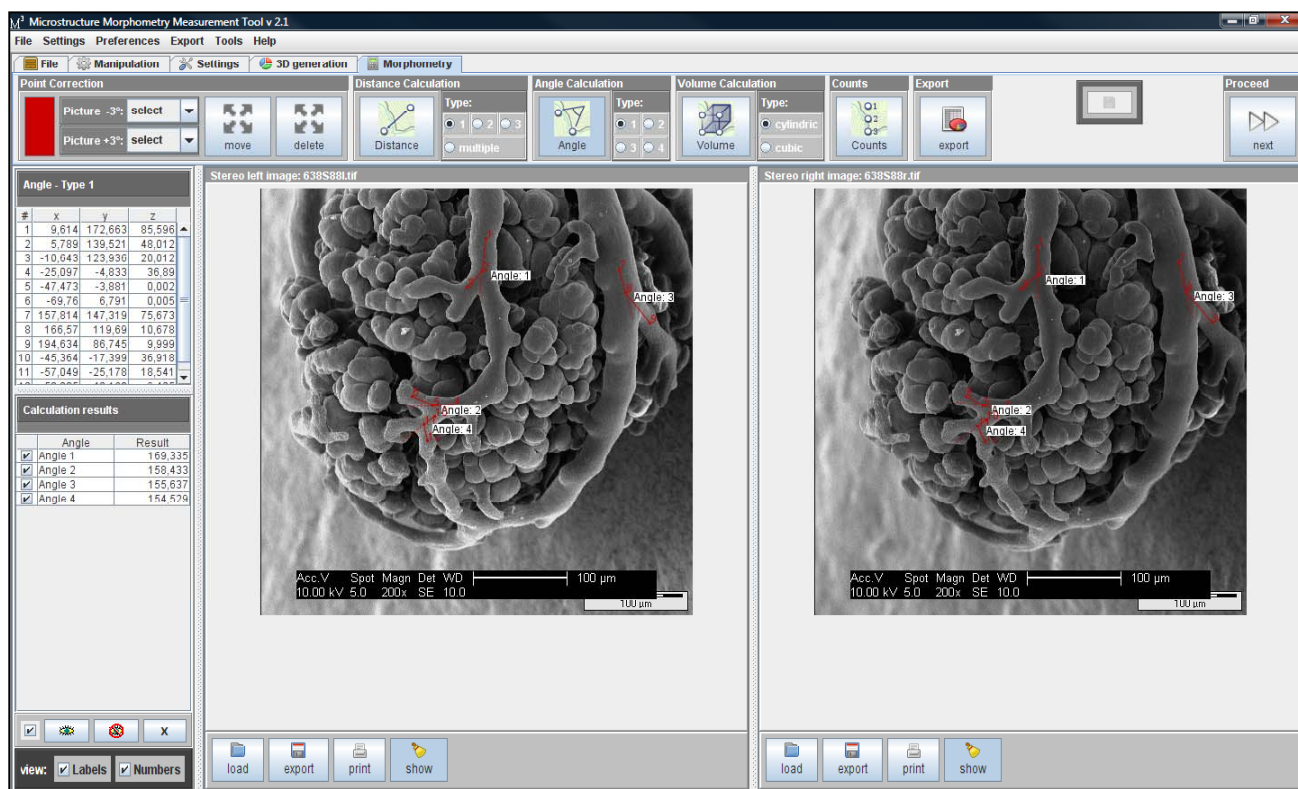
FIG. 1. The graphical user interface (GUI) showing the morphometry dialog while measuring branching angles of feeding arteries of the spleen of the adult *Xenopus laevis*.
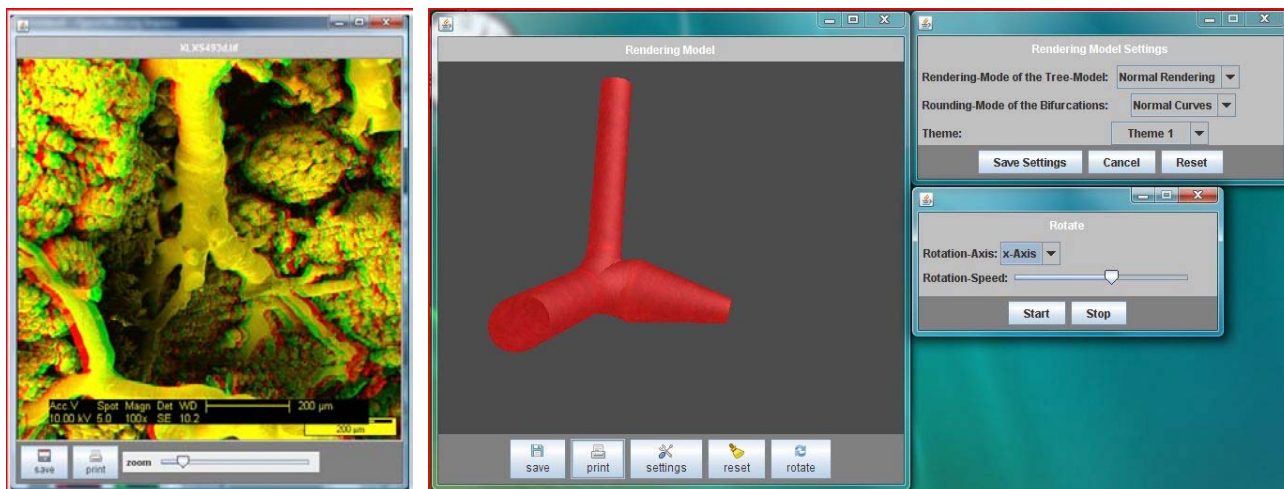

FIG. 2. Three dimensional insight into the spleen of *X.l.* using the anaglyphic method obtained from stereo paired SEM images.

FIG. 3. 3D rendering model of a selected bifurcation which can either be automatically animated or manipulated by hand using the pointing device (move, zoom, rotation).