

Automated Change Analysis of Registered Angiogenic Vasculature Images from *In Vivo* Multiphoton Time Series Images

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Biological changes induced by tumor growth on preexisting host vasculature bed [1] was monitored *in vivo* using a combination of chronic animal windows and multi-photon microscopy (Figure 1), and quantified using highly automated 3-D algorithms for tracing vasculature. For the purpose of noninvasive monitoring, host vasculature bed on a living specimen was prepared with a chronic window with a tumor pre-grown at its midst is highlighted by fluorescent dextrans injected intravenously [2]. The immunodeficient specimen, while undergoing angiogenesis, is imaged on approximately the same volume every 24 hours for 7 days using a multiphoton laser-scanning microscope that captures structural changes that are induced by the presence of tumor on the host vasculature bed.

The image series were pre-processed to eliminate some artifacts, and then the vasculature was traced by an automatic 3-D algorithm. Originally developed for tracing dye-injected neurons [3], this algorithm was modified extensively to robustly detect vasculature center and boundaries in presence of structural irregularities of angiogenic vasculature and illumination artifacts. These are caused by extravasated dye and non-uniform dye distribution due to blood detoxification by the specimen's liver, requiring the dye to be injected repeatedly prior to each image capture. From the traced centerlines and borderlines, morphometric data (e.g., lengths, counts, and widths) are computed and serve as the basis for change analysis. The tracing algorithm is accurate, highly adaptive, and fast, taking less than a minute to trace one $768 \times 512 \times 32$, 8 bit/pixel 3-D image stack on a Dell Pentium III 1 GHz computer. Manual validation of the tracing algorithm on 6 images indicated average spatial error of 2 pixels and average concordance of 81% between manual and automated traces. The subjectivity and tedium of manual tracing was eliminated by use of this algorithm.

Two forms of change analysis were investigated. One method is illustrated in Figure 3A. It is based on automatic registration of successive images using the traced centerlines as landmarks. After registration, all images in the time series share a common coordinate system, and only data collected within the common volume are used for change analysis. Plots of morphometric data can be readily generated over this common region as a function of time, as exemplified by Figure 2B. A second method is to directly display and quantify the changes in the vessel segments between the two images, as illustrated in Figure 2A. Due to its speed and objectivity, this framework of change analysis is at most suitable for angiogenesis assays and hypothesis testing studies.

References

- [1] R. K. Jain et al., *Nat. Rev. Cancer* 2 (4) (2002) 266.
- [2] E. B. Brown et al., *Nature Med.* 7 (3) (2001) 864.
- [3] K. A. Al-Kofahi et al., *IEEE Trans. Inform. Technol. Biomed.* 6 (2) (2002) 171.
- [4] This research was supported by the NSF Center for Subsurface Sensing and Imaging Systems (EEC-9986821), MARA of Malaysia, and by NCI grants (P01CA80124 and R24CA85140).

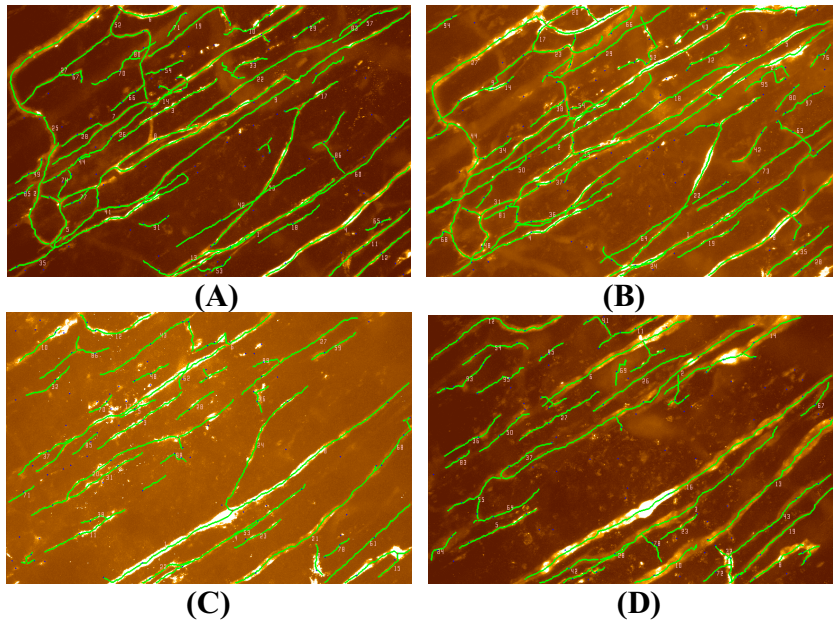
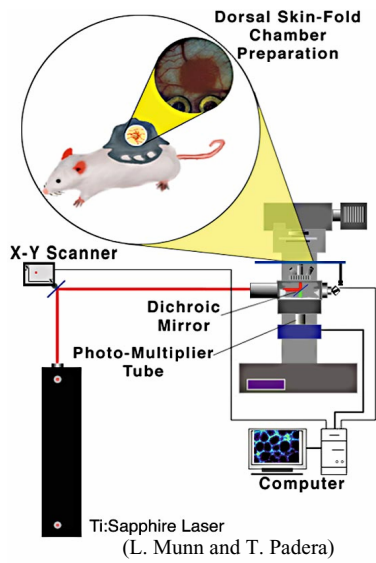


FIG 1: Illustrating the set-up of the multiphoton laser-scanning microscope, showing a specimen prepared with a window and cancer cells grown at its midst.

FIG 2: Maximum-intensity projection *in vivo* image of a host angiogenic vasculature bed, taken 24 hours apart shown in (A) through (D). Also shown are automatically traced vasculature centerlines. Changes of these centerlines are shown in Fig. 3a.

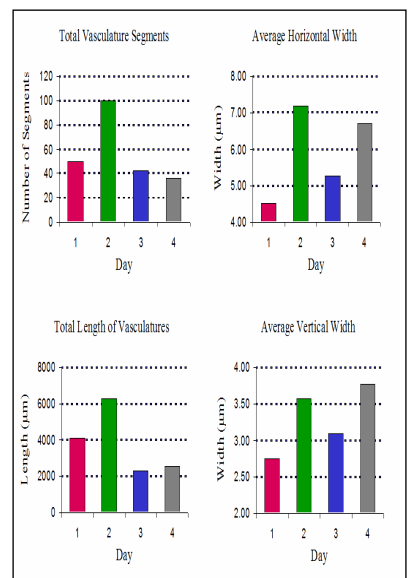
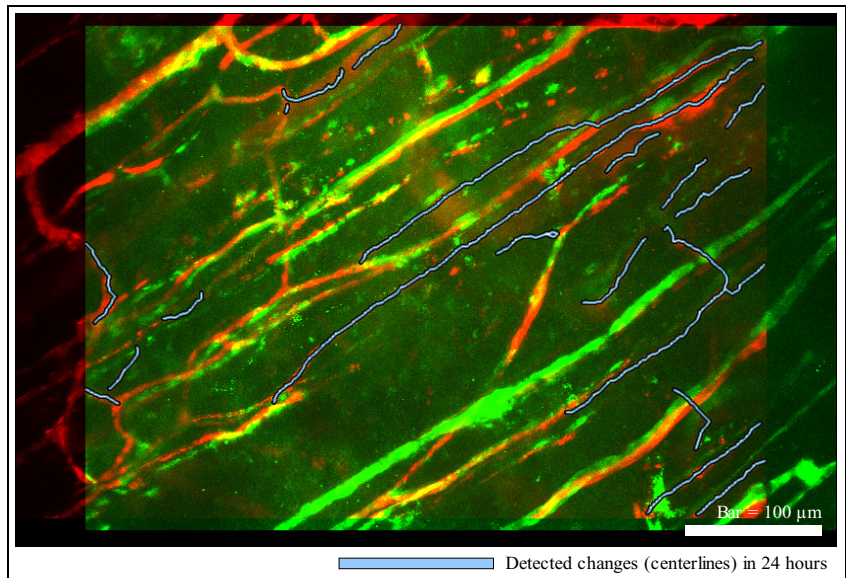


FIG 3: (A) The images in Fig. 2, are registered to a common coordinate system and displayed here in red and green color, respectively. The overlaid traced in blue are detected as the changed vessels by the algorithm. Some of the detected changes could be due to imaging limitations. (B) A plot of the statistics gathered by the tracing algorithm for images of the first 4 days in the time series dataset.