A MODEL-BASED APPROACH TO EXTENDED DEPTH OF FIELD FOR BRIGHTFIELD MICROSCOPY

François Aguet, Dimitri Van De Ville, and Michael Unser

Biomedical Imaging Group, Ecole Polytechnique Fédérale de Lausanne, Switzerland

INTRODUCTION: Brightfield microscopy suffers from a limited depth of field, often preventing thick specimens from being imaged entirely in-focus. By optically sectioning the specimen, the in-focus regions can be acquired over multiple images. Extended depth of field methods aim at combining the information from these images into a single in-focus image of the texture on the specimen's surface. For the analysis of the in-focus image, knowledge of the specimen's topography can be mandatory. The topography provided by current methods is usually limited to a map of selected pixel positions and is inherently discretized, which limits its use for quantitative evaluation[1-2]. In this work, we present a joint estimation of the in-focus image (texture) and the topography, based on a model of the specimen and the microscope's point spread function (PSF). The method, which can be likened to an object model-based deconvolution, produces a continuous topography.

METHODS: Under the hypothesis that the in-focus information is recorded at the specimen's surface (valid when imaging thick specimens), we propose to model the sample $o(x,y,z)$ as a thin 3-D surface, described by its topography $p(x,y)$, onto which the texture $f(x,y)$ is mapped:

$$o(x,y,z) = f(x,y)\delta(z - p(x,y)). \qquad (1)$$

The image formation and acquisition process is modeled as the convolution between the object and the microscope's 3-D point spread function $h(x,y,z)$, followed by sampling to obtain a stack of images. In order to reduce the computational load, $h(x,y,z)$ is modeled as a Gaussian approximation of a state-of-the-art microscope PSF model[3].

Estimates for the texture and topography are obtained by minimizing the discrete cost function

$$J = \sum_{i,j,k \in S_3} \left(s(i,j,k) - (o * h)(i,j,k) \right)^2, \qquad (2)$$

where S_3 is the 3-D support of the measured image stack s. This is achieved via a two-step optimization approach that alternates between updating the texture and the topography[4]. During each step, the texture (respectively topography) estimation is achieved by performing a steepest descent on J. The estimation is initialized with the topography at a constant level, followed by the first texture estimation step. In general, the method converges rapidly and requires only a few iterations.

RESULTS: We tested our algorithm on phantom data and z-stack acquisitions of biological specimens. In the example shown in Fig. 1, the estimated topography clearly reveals the heap-like structure of the sample.

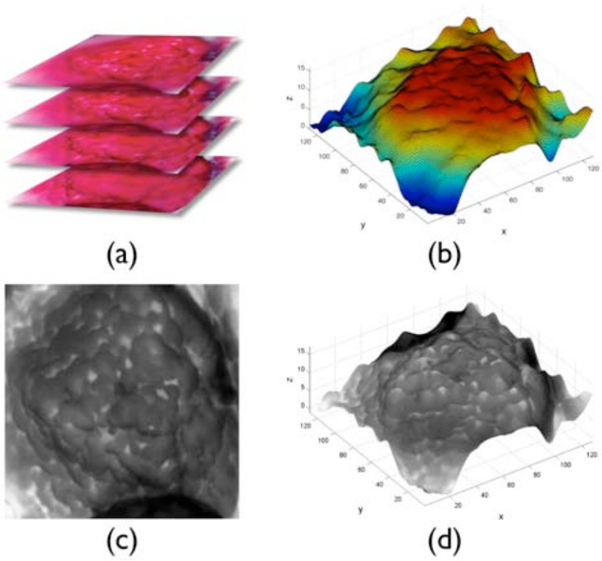

Fig. 1: (a) Partial z-stack acquisition of a murine intestine sample. (b) Estimated topography. (c) Estimated texture. (d) Texture mapped topography.

DISCUSSION & CONCLUSIONS: The joint estimation of the texture and topography of a specimen leads to accurate estimates, devoid of the discretization artifacts that commonly result from existing methods. Promising results from simulated and experimental image stacks demonstrate the efficiency of the method.

REFERENCES: [1]A. G. Valdecasas, D. Marshall, J. M. Becerra, and J. J. Terrero (2001) *Micron* **32**:559–569. [2]B. Forster, D. Van De Ville, J. Berent, D. Sage, and M. Unser (2004) *Microsc Res Tech* **65**:33–42. [3]S. F. Gibson and F. Lanni (1991) *J Opt Soc Am A* **8**:1601–1613. [4]F. Aguet, D. Van De Ville, and M. Unser (2006) *Proc 3rd IEEE Int Symp Biomed Imaging* pp. 778–781.

ACKNOWLEDGEMENTS: This work was supported in part by the Swiss National Science Foundation under Grant 200020-101821.