3D BIOPSY IMAGING BY COMBINED BRIGHT FIELD AND FLUORESCENT MICROSCOPY

M. Asselman, M. Boamfa, D. Clout, N.A. Larsen[*)], A. Pierik, R. Vulders, P.J. van der Zaag
Philips Research Laboratories
High Tech Campus 11, 5656 AE Eindhoven, The Netherlands
[*)] Philips BioCell
Gydevang 42, 3150 Lillerød, Denmark
E-mail: p.j.van.der.zaag@philips.com

KEY WORDS: Tissue biopsy imaging, 3D imaging, tissue clearing, pathology, bright field and fluorescence imaging, imaging speed

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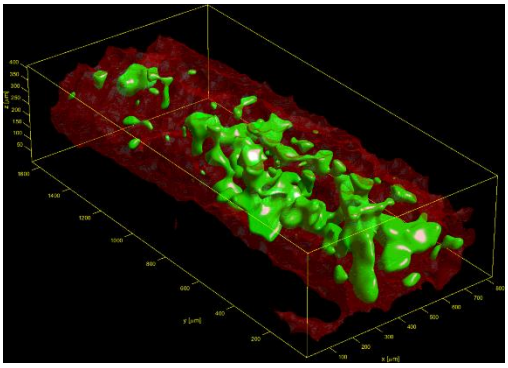
In pathology the diagnosis of the disease state is typically done on 4-5 µm thin tissue 2D slides. Particularly in prostate cancer, this is known to be difficult from such thin slides, as the cancer growth is three dimensional. This has led to 3D studies of intact prostate biopsies with the aim of getting a more reliable assessment of the disease state as cancer growth patterns can be imaged [1]. Typically these studies are done with confocal microscopy [1] or light-sheet microscopy [2]. We will show that an interesting alternative approach is the use of the Fluidscope™ technology which provides a volumetric image of an intact 3D biopsy up to 1 mm thick using a *combination* of bright-field (BF) and fluorescence (FL) imaging. Using the oblique optical sectioning method of this system, a cleared tissue sample can be imaged relatively quickly in approx. 3 minutes. Fig. 1 shows results obtained using only bright-filed imaging of a 500 µm thick tissue sample, highlighting the tubuli, lined by epithelial cells, the point at which cancer-growth typically starts. We will show that the combination of BF and FL enables visualization of the overall tissue architecture using BF and specifically stained cells (such as cancer cells) within the biopsy using FL. Such a combination is not possible for confocal and light-sheet microscopy, as both are fluorescent techniques.



We have also investigated whether the tissue clearing needed in our 3D tissue imaging is compatible with the typical next steps in conventional 2D pathology such as H&E and antibody staining. This appears to be the case, which opens the potential of using this approach to assess where cancer occurs in tissue biopsies in order to select the optimal biopsy and the relevant section within it, for better conventional 2D pathology analysis.

Fig. 1: Geometrical reconstruction of a cleared tissue sample

[1] M.E. van Royen E.I. Verhoef C.F. Kweldam, et al., "Three-dimensional microscopy analysis of clinical prostate specimens," *Histopathology*, **69,** 985-992 (2016).
[2] P.A. Santi, S.B. Johnson, M. Hillenbrand, et al., "Thin-sheet laser imaging microscopy for optical sectioning of thick tissues," *BioTechniques.* 0736-6205 **46**, 287–294 (2009).