COLONIC CYCLORAMAS FOR QUANTIFICATION OF 3D CRYPT MORPHOLOGY IN MICE AT PROGRESSIVE STAGES OF COLORECTAL CANCER

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Keywords: Automated volume segmentation, colonic cyclorama, synchrotron X-ray phase contrast tomography, bioimaging, soft tissue, colorectal cancer.

Summary: Colorectal cancer (CRC) is the third deadliest cancer worldwide. We describe a protocol to non-destructively image murine colons with synchrotron X-ray phase contrast micro-computed tomography and reconstruct the mucosal layers in 3D. Our approach allows processing of murine colons in their native shape, enabling precise quantitative morphometry of the colonic structure in health and progressive stages of CRC.

1. INTRODUCTION

Colorectal cancer (CRC) is the third most lethal cancer worldwide [1]. Early alterations in the colonic crypt (elements that constitute the colon) structure are of predominant importance as they demonstrate the disease onset [2, 3]. Standard practice in biomedical research involves the use of animal models, where colons are typically cut open and laid on a flat surface to assess the pathology macroscopically [4]. They are then chemically fixed (e.g. using formalin), embedded in paraffin wax, cut as planar slides, and stained using standard histology dyes such as haematoxylin and eosin (H&E) [5]. This process yields two dimensional cross-sections of colonic crypts that are examined using an optical microscope for morphological and histological abnormalities at a certain plane. Although morphological deformations are widely observed, they correlate poorly with dysplasia [6], which involves several other planes above or below the point of pathology, and bears increased risk for CRC development [7]. In the following, we describe a new protocol to non-destructively image murine colons with in-line synchrotron X-ray phase contrast micro-computed tomography (μCT) and quantify the crypt shape in three dimensions (3D). By fixing and embedding the colons in their native shape we alleviate geometrical deformations inherent in the traditional workflow of serial sectioning for light microscopy-based histology. Instead, we digitally unroll the μCT volume of the colons using a novel image processing technique (colonic cycloramas) that we developed to identify and segment the crypt lumens in 3D. Our approach provides complete reconstruction of the gut mucosa in space, thus enabling precise quantitative morphometric studies of the colonic structure in health and progressive stages of colorectal cancer.

2. EXPERIMENTAL METHODS

An azoxymethane and dextran sodium sulphate (AOM/DSS) model [8, 9] has been employed to induce CRC to male C57BL/6J mice. Their colons were excised at progressive stages of the disease, inflated with 10% neutral buffered formaldehyde (formalin) and left to fix for 24 hours before they were embedded in cylindrical paraffin wax blocks. In-line phase contrast X-ray μCT was performed at beamline *I13-2* at Diamond Light Source, Didcot, UK, at an energy equal to 25 keV and pixel size of 2.2 μm. Paganin phase retrieval [10] and standard filtered CT backprojection were employed for CT reconstruction. The 3D volumes were then digitally unrolled using our colonic cyclorama technique, producing stacks of cycloramas (panoramic views of the colon). These have the unique property that they are locally perpendicular to the colonic crypts, thus portraying consistent cross-sections of the crypts (see Figure 1 – left). As crypt lumens appear darker than the surrounding tissue (crypt epithelium), we performed automatic segmentation by thresholding the grayscale values on the cycloramas and rolled them back to their initial shape (see Figure 1 – right). The resulting 3D reconstruction allows quantitative characterisation of the crypt lumens' shape in health and progressive stages of CRC.

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3. RESULTS

Our novel colonic cyclorama technique enables visualisation and quantification of murine colons in their native shape. This crucial advancement alleviates the need to physically unroll the colons that inevitably introduces deformations to the colonic crypts. We employed our novel image processing method to digitally unroll and segment the crypt lumens in healthy and diseased (with early stage colorectal cancer) murine colons to obtain their 3D reconstructions such as those shown in Figure 1. We will now proceed to quantify the 3D morphology (volume, surface area, sphericity) of the crypt lumens. This workflow demonstrates how X-ray μCT can be used to introduce 3D quantification of the colonic structure, enabling the study of the early stages of colorectal cancer in an animal model.

References

[1] Globocan 2012. "Epidemiology of colorectal cancer worldwide", 21 June 2017; http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

[2] A. K. Gupta, T. P. Pretlow, and R. E. Schoen, "Aberrant crypt foci: What we know and what we need to know", *Clinical Gastroenterology and Hepatology,* vol. 5, pp. 526-533, 2007.

[3] M. J. Wargovich, V. R. Brown, and J. Morris, "Aberrant crypt foci: the case for inclusion as a biomarker for colon cancer", *Cancers (Basel),* vol. 2, pp. 1705-16, 2010.

[4] R. Suzuki, H. Kohno, S. Sugie *et al.*, "Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon carcinogenesis in mice", *Carcinogenesis,* vol. 27, pp. 162-9, 2006.

[5] D. S. Levine, and R. C. Haggitt, "Normal Histology of the Colon", *American Journal of Surgical Pathology,* vol. 13, pp. 966-984, 1989.

[6] A. K. Gupta, P. Pinsky, C. Rall *et al.*, "Reliability and accuracy of the endoscopic appearance in the identification of aberrant crypt foci", *Gastrointestinal Endoscopy,* vol. 70, pp. 322-330, 2009.

[7] D. Lam-Himlin, F. Bhaijee, and C. Arnold, "Classification and diagnosis of colorectal dysplasia in inflammatory bowel disease", *Diagnostic Histopathology,* vol. 21, pp. 283-289, 2015.

[8] C. Neufert, C. Becker, and M. F. Neurath, "An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression", *Nat Protoc,* vol. 2, pp. 1998-2004, 2007.

[9] B. Parang, C. W. Barrett, and C. S. Williams, "AOM/DSS model of colitis-associated cancer", *Methods Mol Biol,* vol. 1422, pp. 297-307, 2016.

[10] D. Paganin, S. C. Mayo, T. E. Gureyev *et al.*, "Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object", *Journal of Microscopy,* vol. 206, pp. 33-40, 2002.

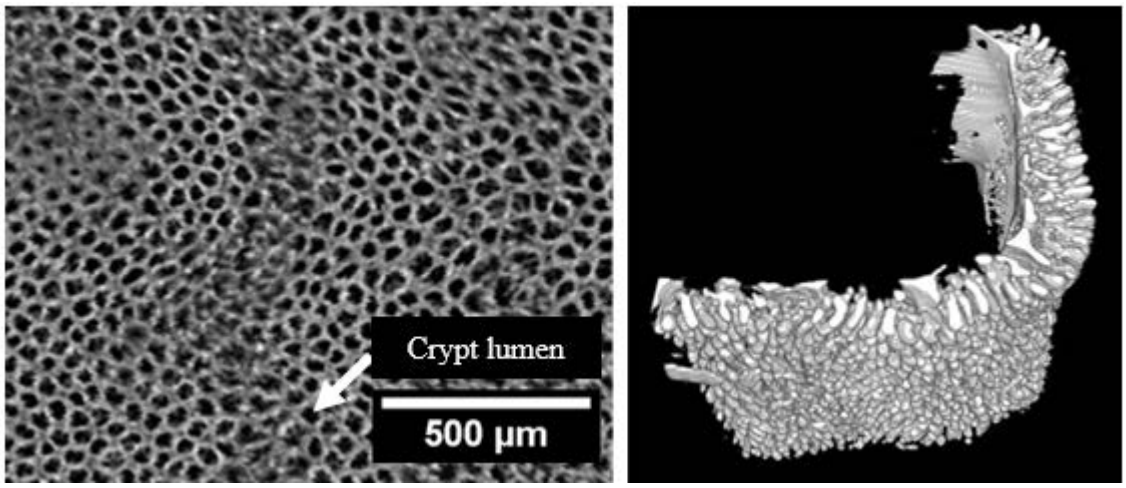

Figure 1: Colonic cycloramas used to segment the crypts and reconstruct their 3D shape. (Left) Colonic cyclorama of a healthy murine colon imaged using synchrotron X-ray phase contrast imaging at beamline *I13-2* at Diamond Light Source. Crypt lumens appear darker circular profiles, thus enabling automatic segmentation. (Right) Digitally rolling the segmented crypt lumens back to their initial shape yields a 3D reconstruction of the crypts in 3D, which enables the quantification of their morphology.