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HistoStitcher©: An interactive program for accurate and rapid reconstruction of digitized whole histological sections from tissue fragments

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ABSTRACT

We present an interactive program called HistoStitcher© for accurate and rapid reassembly of histology fragments into a pseudo-whole digitized histological section. HistoStitcher© provides both an intuitive graphical interface to assist the operator in performing the stitch of adjacent histology fragments by selecting pairs of anatomical landmarks, and a set of computational routines for determining and applying an optimal linear transformation to generate the stitched image. Reconstruction of whole histological sections from images of slides containing smaller fragments is required in applications where preparation of whole sections of large tissue specimens is not feasible or efficient, and such whole mounts are required to facilitate (a) disease annotation and (b) image registration with radiological images. Unlike manual reassembly of image fragments in a general purpose image editing program (such as Photoshop), HistoStitcher© provides memory efficient operation on high resolution digitized histology images and a highly flexible stitching process capable of producing more accurate results in less time. Further, by parameterizing the series of transformations determined by the stitching process, the stitching parameters can be saved, loaded at a later time, refined, or reapplied to multi-resolution scans, or quickly transmitted to another site. In this paper, we describe in detail the design of HistoStitcher© and the mathematical routines used for calculating the optimal image transformation, and demonstrate its operation for stitching high resolution histology quadrants of a prostate specimen to form a digitally reassembled whole histology section, for 8 different patient studies. To evaluate stitching quality, a 6 point scoring scheme, which assesses the alignment and continuity of anatomical structures important for disease annotation, is employed by three independent expert pathologists. For 6 studies compared with this scheme, reconstructed sections generated via HistoStitcher© scored higher than reconstructions generated by an expert pathologist using Photoshop.

1. Introduction

Histological sections of tissue specimens obtained via biopsy or surgical excision, such as lumpectomy or radical prostatectomy, are used for identifying presence and extent of disease, and if resolution is sufficient, the grade and malignancy of the disease [1–3]. In cases where the patient is scheduled for surgical resection of the diseased tissue or gland, it may be valuable to map disease extent from *ex vivo* histology sections onto *in vivo* radiological imaging, which is commonly acquired prior to excision, to discover signatures for disease on *in vivo* imaging [4–6]. For example, men with prostate cancer confirmed by biopsy and who are scheduled to undergo radical prostatectomy may receive pre-operative MRI of the prostate for identifying the presence of extra capsular spread. By registering these pre-operative MRI with corresponding *ex vivo* histology sections obtained from the radical prostatectomy specimen (see Fig. 1(a) and (b)), pathological regions on histology (purple regions in Fig. 1(a)) are then mapped onto the registered MRI (shown in green in Fig. 1(c)). To achieve the required histology annotation and multi-modal registration steps described above, whole histological sections of entire slices of the specimen rather than fragmented sections of tissue are required.

Obtaining whole-mount histological sections of large specimens, such as an enlarged prostate gland, while maintaining tissue integrity is not always feasible. For large specimens, a single glass slide is often too small to mount the entire contiguous tissue section, necessitating the cutting of the section into smaller fragments and mounting them onto multiple slides. Further, it is difficult to slice large specimens thin enough to achieve the very high spatial resolution required for accurate annotation without the use of specialized procedures and microtomes. For example, obtaining a whole slide for the specimen shown in Fig. 1(b) with a thickness of

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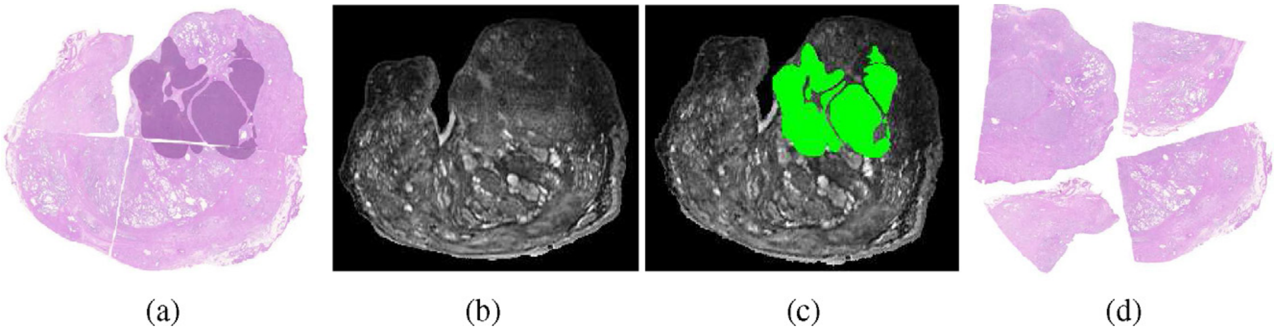

Fig. 1. (a) Reconstructed pseudo-whole-mount histology from a prostate specimen with cancer (delineated in dark purple) may be used to identify signatures for disease on pre-operative *in vivo* imagery, similar to the MRI image shown in (b), by registering the two images. (c) The registered MRI slice that is in alignment with whole histology in (a) allows mapping of cancer onto MRI (green). (d) Quadrants comprising the whole histology section in (a) must be first reconstructed into a pseudowhole-mount section to facilitate improved annotation across cuts and registration with MRI in (b). (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

only 4 μm is difficult, prone to specimen damage, and inefficient, requiring exorbitant amounts of preparation time. As a result of these challenges, it is often preferable to adopt the much simpler approach of cutting large tissue specimens into smaller fragments and preparing multiple slides for separate analysis, such as quadrant sections shown in Fig. 1(d).

Tissue fragments spread across multiple slides presents a significant hindrance for both (1) digital annotation of disease extent by a pathologist, which requires integration of visual cues across disjoint section boundaries, and (2) image processing tasks, such as registration of whole histological sections with *in vivo* imaging modalities. Annotation of multiple slides by a pathologist may be performed separately, however when the targeted pathology of interest crosses the boundary between slides, the process is complicated as the pathologist must repeatedly alternate between adjacent slides. As with any strenuous manual task, the annotations obtained in this manner may be inaccurate or unreliable. In addition to tissue annotation, whole histology slides are valuable in facilitating correlation with *in vivo* imagery by image registration, and much work has been done on registration of whole-mount histology with *in vivo* radiological imaging [7–10]. On the other hand, registration of smaller histologic image fragments to a sub-image or region of the *in vivo* data has not been widely investigated and is likely to be a more challenging registration task. Thus, in order to streamline and improve pathologist slide annotation, and facilitate image processing tasks requiring whole histological sections, it becomes useful to reconstruct a pseudo-whole-mount histological section from multiple individual fragments [6].

With the spread and growing acceptance of digital pathology [1–3], it is feasible for high resolution whole-mount sections to be digitally reconstructed from the images of the smaller fragments. Digitally reconstructed whole-mount histological sections would not only facilitate a variety of image processing tasks, but if pathologists can perform digital annotation of disease extent on high resolution histology images on computer monitors, annotations would be greatly improved compared to manual labeling of slides with felt pen or drawing on standardized examination sheets. Annotation could be improved in terms of both accuracy and efficiency using digital images by allowing labeling at any level of detail and for editing and revision of the markup. For example, in Fig. 1(a) are the digitized histology quadrants of a section from a prostatectomy specimen with cancer. The closest corresponding MRI slice is shown in Fig. 1(b). By digitally combining, or stitching, the quadrants a pseudo-whole-mount section can be generated (see Fig. 1(c)), upon which efficient pathologist annotation can be performed on a high resolution computer monitor. The resulting cancer label established by analysis of the reconstructed pseudo-while-mount section is shown in purple, and can be seen to cross the cut between the original quadrants. Finally, having a reconstructed whole histology section, registration with corresponding *in vivo* imagery, such as MRI, can be performed to achieve mapping of spatial extent of disease (in this case prostate cancer) from the annotated whole histology onto corresponding radiological *in vivo* imaging. For example, the aligned MRI slice obtained in a previous study [4] is shown in Fig. 1(d) with the mapped histopathologic cancer label shown in green. In this paper, we present a software utility called HistoStitcher© for computerized reconstruction of a whole histological section from digital images of the multiple slides of tissue fragments. While we demonstrate the utility of HistoStitcher© in the context of prostate sections in this work, the program is just as readily applicable to other domains and applications such as reassembly of whole histology sections of breast lumpectomy or mastectomy.

The rest of the paper is organized as follows. In Section 2 we discuss the challenges and requirements associated with digital reconstruction of high resolution histopathologic images. In Section 3.1 we present an overview of the HistoStitcher© software, including the interface and workflow. In Section 3.3 we detail the mathematical methods used to determine the optimal image transformation based on user-defined control points. In Section 3.4 we describe the application of the transformation to the full resolution histology and the process of stitching to create a larger image. In Section 3.6 we describe the features of HistoStitcher© that facilitate operation on very large images. In Section 4 we present formalized evaluation criteria and a 6 point scoring scheme for comparing and assessing the quality digitally reconstructed histology sections. In Section 5 we demonstrate the use of HistoStitcher© for reassembly of quadrants of prostate histology sections, and compare the results to manually reassembled (using Photoshop) sections and to block face photographs of the gland taken prior to cutting into quadrants. The reconstruction evaluation scheme is applied to compare the quality of stitches generated using HistoStitcher© to corresponding stitches generated using Photoshop for 6 prostate additional prostate studies. In Section 6 we present concluding remarks and enumerate further applications of HistoStitcher©.

2. Challenges in digital reconstruction of high resolution whole histology

At institutions where high resolution digitization of excised tissue specimens are performed, an imaging software like Photoshop[1] is typically employed to digitally reconstruct a whole histology image when the tissue is fragmented across multiple slides. The

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[1] http://www.adobe.com/photoshop/.