Validation of Histology Image Registration

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ABSTRACT

The aim of this paper is to validate an image registration pipeline used for histology image alignment. In this work a set of histology images are registered to their correspondent optical blockface images to make a histology volume. Then multi-modality fiducial markers are used to validate the alignment of histology images. The fiducial markers are catheters perfused with a mixture of cuttlefish ink and flour. Based on our previous investigations this fiducial marker is visible in medical images, optical blockface images and it can also be localized in histology images. The properties of this fiducial marker make it suitable for validation of the registration techniques used for histology image alignment. This paper reports on the accuracy of a histology image registration approach by calculation of target registration error using these fiducial markers.

Keywords: Histology volume, validation, fiducial marker, multimodality contrast agent, image registration, image correlation, histopathology

1. INTRODUCTION

3D histology volume has the potential to provide useful information for quantitative analysis and assessment of pathologic findings in volumetric medical images. Higher precision in histology volume reconstruction provides more accurate information. There are some distortions that might occur during the preparation of the histological sections, such as shrinkage, expansion, tears, and folds. These distortions make it difficult to register the serial microscopic histology images into a 3D stack in order to reconstruct a histology volume. They reduce the similarities between the consecutive sections and consequently make the registration more complicated.

Different methods have been proposed to register the histological sections and to create a histology volume. Some techniques involve intensity variations [1], and some others are based on the shape of the sections [2], [3]. For some specimens the anatomical structures can be used as landmarks [4], [5] along with landmark-based registration methods [6],[7]. But these internal structures might not be detectable through the whole volume and for some specimens, such as a mouse tumour, no reliable anatomical structures can be identified.

Our work aims to validate a point-based registration approach [2], which utilizes a set of optical blockface images as reference. Registering the histology sections to one another without reference images propagates the registration error and changes the actual shape of the histology volume, which is referred to as banana effect. The reference images are used to eliminate the propagation of registration error and reconstruct the histology volume as accurately as possible. This approach is validated by measuring the Target Registration Error (TRE) using a multi-modality fiducial marker, which is detectable both in histology and optical images of the paraffin block face [8]. A marker with these characteristics can be reliably used to validate the registration techniques which are intensity-based shape-based, or based on intrinsic landmarks

This paper reports on some initial results of the experiments that we have accomplished to validate a histology image registration pipeline as follows. In the next section we explain the type of the sample used for this experiment along with a brief description of the registration pipeline. Section 3 is dedicated to the results and the paper is summarized and concluded in section 4.

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2. MATERIALS AND METHODS

The goal of this work is to register histology images to reconstruct a histology volume and then to validate the technique. In order to eliminate the propagation of registration error in the z direction to the other sections, we use blockface images as references. Each histology image is registered to its corresponding blockface image using edge-based registration techniques. Since blockface images are obtained from the surface of the embedded tissue in paraffin block they are not deformed relative to each other. Therefore, they can reliably be used as references to rebuild histology volume. There are two sets of fiducial markers used in this work. One set is used to align the blockface images and the other set, which is implanted in the tumour, is used for validation of the registration technique. Detailed description of each set is provided in the following.

2.1 Sample

A xenograft of human breast cancer cell line (MDA) in the hip of a SKID mouse was used to prepare the histology and blockface images with fiducial markers. This tumour was excised and fixed in 10% Neutral Buffered Formalin (NBF). After preparing histology images, 20 pairs of H&E and correspondent blockface images were used to evaluate the proposed image registration method.

2.2 Histology

Since the tumour was fixed before, we implanted the fiducial markers in the fixed tissue. However, the localization of the fiducial markers will be more accurate when they are implanted in the fresh tissue. After implanting the fiducial markers in the tumour, the tissue was processed with an automatic tissue processor and embedded in paraffin. Sections with 5µm thickness and 20µm separation were cut from the paraffin block and stained with Hematoxylin and Eosin (H&E) as shown in figure 1-b. The sections were digitized by an Aperio ScanScope scanner with a resolution of 0.2µm and then downsampled to 20µm resolution resulting in an image size of 8400 x 5700 pixels. Before each section is cut, an optical blockface image was also obtained from the surface of the block (figure 1-a). Since the tissue is fixed in its place in paraffin block and has not been deformed through histology processes, the blockface images are reliable reference images. If each histology image is registered to its correspondent blockface image, the registered histology images will be aligned to each other too. These optical images are used for image registration and as it is shown in figure 1-a, the fiducial markers in the tumour are also detectable on these images; therefore, it is feasible to use them for validation.

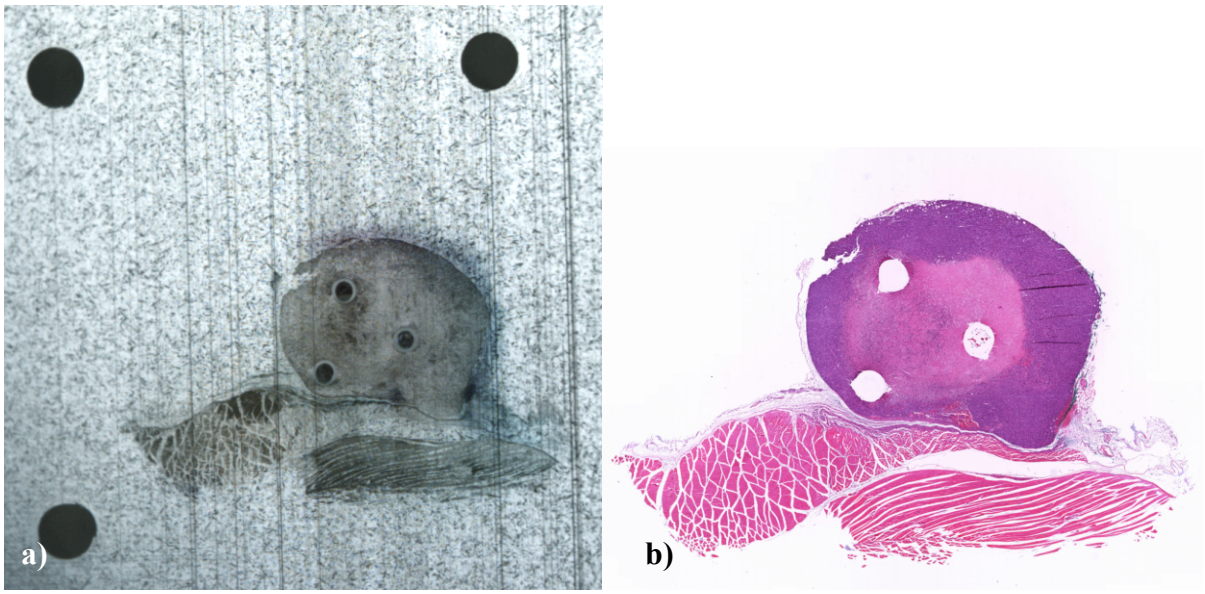

Figure 1: Fiducial markers in the tissue and in the paraffin block, a) Blockface image, b) correspondent histology image

2.3 Blockface Images

Blockface images are taken from the paraffin blocks of the tissue, mounted on the microtome, before each section is cut. These optical images are obtained by a telecentric lens to eliminate the barrel distortion, which usually occurs when using regular lenses. The images are taken at an oblique angle to use the reflection of the surface of the block for contrast enhancement between the tissue and paraffin surface and to eliminate the shadow of the tissue in depth, under the paraffin surface. A photographic filter is also used to polarize the light coming from the block surface and the tissue to balance the contrast. To correct for the displacement of the block on the rotary microtome, three holes are drilled in the three corners of the block, which are easily detectable in the blockface images (fig. 1-a). The centroids of these holes are used along with landmark-based rigid registration to align the blockface images.

2.4 Fiducial Markers for Validation

In this section we describe the fiducial markers which we use for validation of our image registration pipeline. Since we employ an edge-based registration approach, we need to be able to validate the alignment of the internal structures. We have accomplished this by implanting fiducial markers in the tumour. Our experiments with many types of markers have shown that 26 Gauge catheters perfused with a mixture of cuttlefish ink, flour, and water can be very effective landmarks for histology images [8]. The iron contained in cuttlefish ink [9] makes it detectable in several imaging modalities such as MRI and CT and the color of the ink also makes it detectable in optical images. Because of the organic nature of the injected dough, it stays on the positively charged microscope slides along with the histology sections. We have shown in [8] that the dough stays on about 50% of the slides and on the remainder of the slides the cross section of the catheter can be detected and used for validation. These landmarks are suitable to assess the alignment of the internal structures of the tissue, which are usually more of interest than the boundaries of the tissue. Since the fixed tissue is fragile and the boundaries of the cross section of fiducial markers are more accurate when they are implanted in fresh tissue.

2.5 Image Registration

Since there is not much similarity between the intensity values of the H&E and blockface images (Fig. 1), we are using edge-based registration techniques. The boundaries of the sections are first segmented and edge points are selected. An initial alignment is achieved using Fourier descriptors and then the rigid registration is refined by Iterative Closest Points (ICP). Deformable registration is also performed to correct the deformations of the histology images. Each step is explained in the following.

2.5.1 Edge Segmentation

Histology edge segmentation was done on the blue channel, where the contrast between different tissue types was higher. To detect the edges of H&E sections, the sections were segmented from the background using the Otsu thresholding technique, which is a nonparametric and unsupervised method of automatic threshold selection [10] [11].

The one-pixel wide edges of the binary image were then extracted by using sobel filtering followed by a thinning operation. The longest connected edge was then retained for further processing (figure 2).

For this work, the boundaries of the optical blockface images are assumed as fixed boundaries and segmented manually to minimize the registration error caused by automatic segmentation error (figure 2).

For both fixed and moving images, the biggest object in the image is selected. The closed boundaries are then represented by a sequence of piecewise linear fits using chain code [12]. These edge points are used for initial alignment using Fourier Descriptors.

The high curvature edge sections are then removed from the histology contour using a rolling ball filter [2] and random edge points are selected from the remained boundary points using uniform distribution. These random edge points are used with Iterative Closest Points algorithm [16] to refine the alignment. More details come in the following sections.

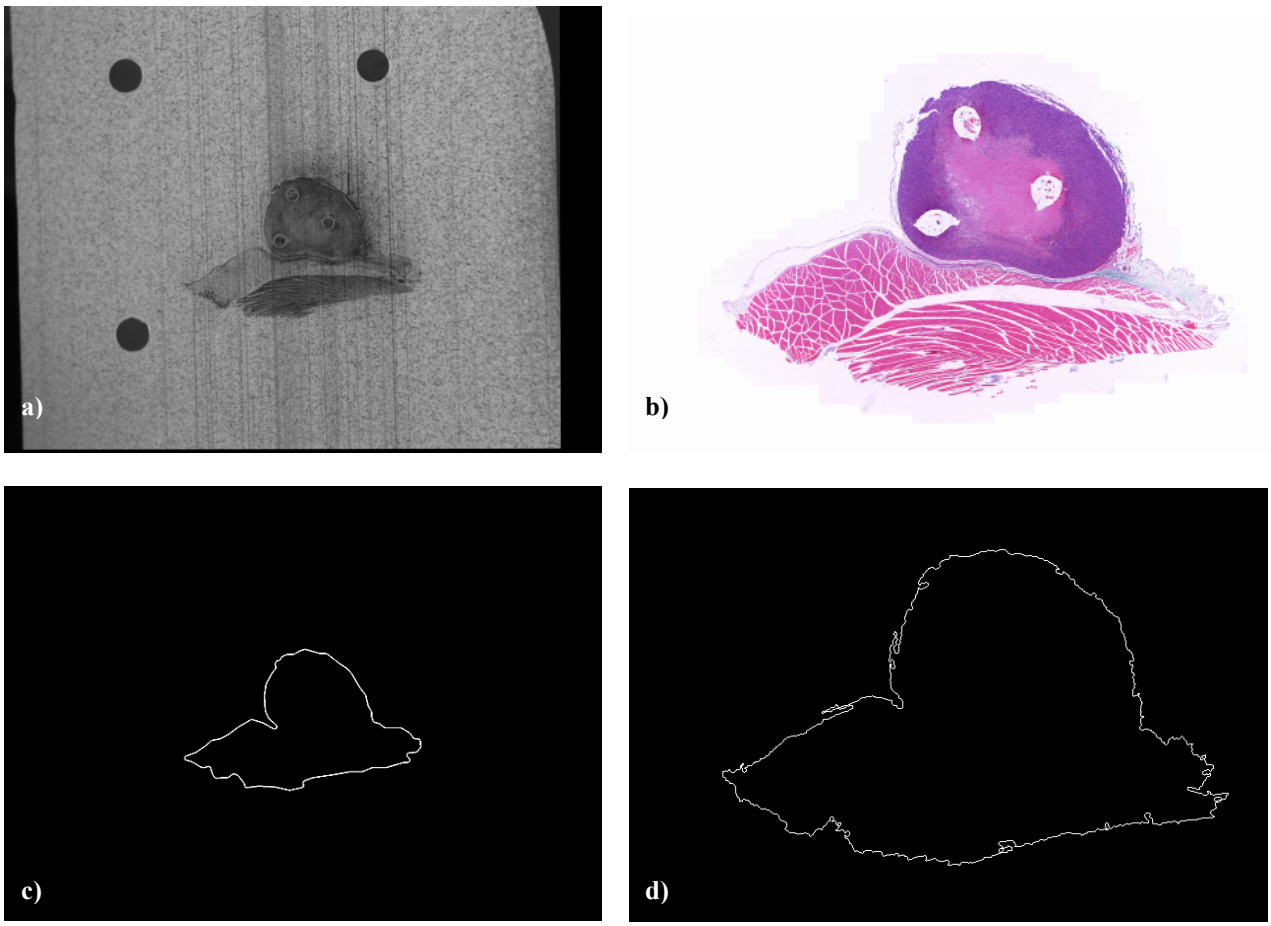

Figure 2: : a) Blockface image, b) correspondent histology image
c) Blockface edge image, d) Histology edge image

2.5.2 Fourier Descriptors (FDs)

Since the size of the histology images is different from the blockface images we need to have an initial rigid registration to achieve an estimate of the translation and rotation along with the scale factor. To obtain this initial transformation we use normalized Fourier Descriptors which are invariant to rotation and translation of the boundary points and also independent of the starting point. Fourier descriptors are first utilized by Giardina and Kuhl [13] to approximate a closed contour in two dimensions by harmonically related rotating vectors with elliptical loci [13]. Further more in [14] they have shown that the orientation of the 1st harmonic or basic ellipse of a closed contour determines the direction of the closed contour elongation. They have also shown that normalizing the Fourier descriptors of a closed contour makes the coefficients rotation, scale and translation invariant [14]. Inspired by Duan *et al*'s work [15], we employed normalized Fourier descriptors to find the rotation angel and translation values as well as the scale factor for initial alignment of the boundaries of the blockface and the histology images.

2.5.3 Iterative Closest Points (ICP)

To refine the alignment we use Iterative Closest Points (ICP) algorithm [2][16]. This technique uses point sets to find the transformation parameters [16]. Similar to what we explained in [2], random edge points on the moving image (histology) and the whole set of edge points on the fixed image (blockface) are selected. After initial alignment by Fourier Descriptors, these random points are also transformed and then used as moving point set for this step. ICP finds the transformation which minimizes the average distance between the moving edge points and their closest points on the fixed point set and transforms the moving points. This iterates till the average distance is minimized or is less than a maximum error. The accumulated transform provides the rigid registration transform (figure3).

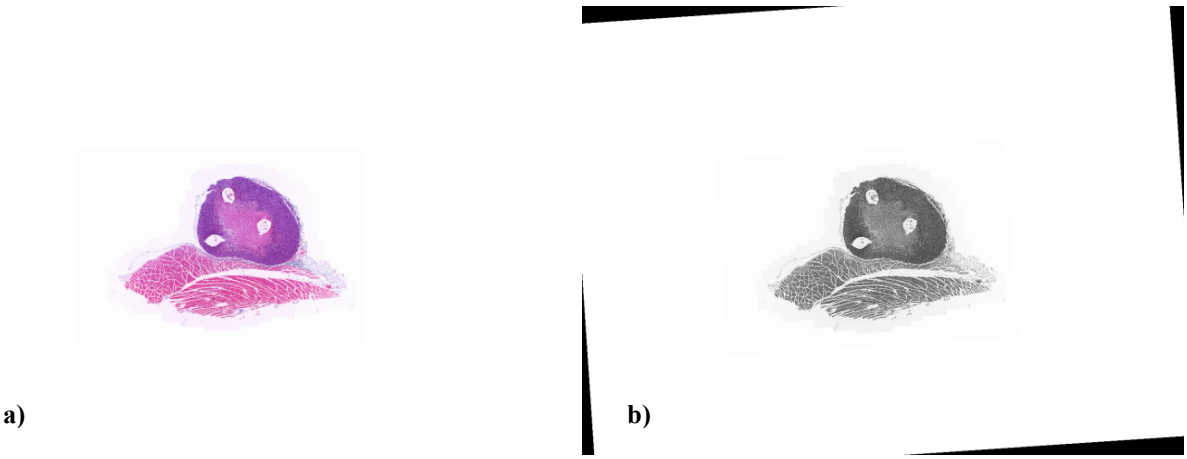

Figure 3: a) Scaled Histology image, b) Histology image after rigid registration.

2.5.4 Thin-plate Splines (TPS)

In order to compensate for the deformation of the sections that might occur during histology processes a deformable registration is necessary. For non-rigid registration we have used Thin-Plate Splines (TPS) [2][17]. This technique estimates the displacement by minimizing the bending energy of the physical analogy of a thin sheet of metal over a fixed set of control points [17].

Following the rigid registration, the closest points on blockface edges to the rigidly aligned histology edge points are extracted as control points and used to deform the histology images [2]. Thin-Plate Spline interpolation was first used by Bookstein as a point-based elastic registration technique to register medical images [17]. The original Thin-Plate Spline registration method assumes that the positions of the control points are exactly known. Sprengel *et al* [18] modified the original Thin-Plate Spline method from interpolation to approximation to consider the localization error of the control points. They have regularized the approximation by the stiffness parameter which determines the relative weight between the approximation and the smoothness of the transformation [18]. With the stiffness of zero the transformation is and interpolation and the source control points are exactly mapped on target control points. With higher values for stiffness the transformation is an approximation with different smoothness of transformation and the control points are not exactly mapped to the target points.

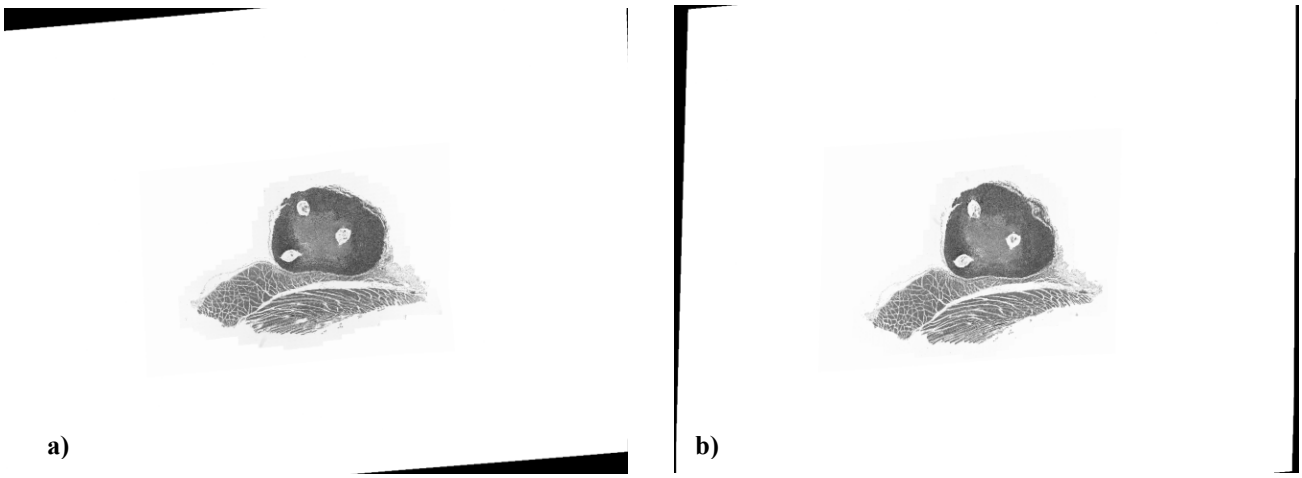

Figure 4: Deformed histology image a) with the stiffness of 0.0, b) with the stiffness of 1.0.

In this work, as it is shown in figure 4, we have deformed the images with two different stiffness values and measured the registration error.

3. RESULTS

In this work 10 pairs of H&E and correspondent blockface images were used to assess the proposed pipeline. We registered each histology section to its correspondent blockface image using their boundaries. After each alignment the average target registration error (TRE) is measured based on the centroids of the fiducial markers. In order to reduce the error the centroids are manually selected.

Before alignment, the 'average' Euclidian distance between the centroids of the fiducial markers in the histology images and their correspondent blockface images was measured as 19.00 mm. We have calculated TRE after initial alignment using Fourier Descriptors as 141 μm (standard deviation = 203). The calculated TRE after the refinement of the rigid alignment using ICP is 73 μm (standard deviation = 89 μm). The deformable registration has been done with two values for stiffness parameter in Thin-Plate Spline approximation method. With the stiffness of zero the TRE after deformable registration is calculated as 107 μm (standard deviation = 73) and 141 μm (standard deviation = 86) with the stiffness of 1.00. As it is shown, with the stiffness of zero the global deformation is less comparing to the image deformation with the stiffness of 1.0. As it is shown by the values of TRE, the deformable registration using the boundary points degrades the accuracy of the global alignment. Thin-Plate Spline method assumes that the tissue is homogeneous and assigns one stiffness value to the whole object. Whereas the tumour is more rigid than the surrounding muscle and connective tissue and the deformation mostly occurs in more deformable tissue. Consequently the more local the deformable registration is the more accurate the global registration is.

While these are the preliminary results, they are encouraging and show that the proposed rigid registration approach is promising, but for deformable registration the internal features should also be considered.

4. CONCLUSION AND FUTURE WORKS

This paper reports on the preliminary results of validation of the proposed histology image registration, which uses boundary points of the histology and blockface images. The edge-based registration pipeline is composed of the Fourier Descriptors method for initial alignment, the Iterative Closest Points algorithm for refining the aligment, and Thin-Plate Splines technique for non-rigid registration. In our previous work [8], we presented the development of a multimodality fiducial marker, which is suitable for validation. After each level of alignment, the target registration error has been measured and as it is shown in the previous section this pipeline is a promising. The deformable registration needs to improve by considering more internal features in addition to the boundary points.

We will continue this work by registering the whole set of histology sections of the tumour and reconstruction of the tumour histology volume. The 3D rendering of the fiducial markers will also be done as a visual confirmation of the correct registration.

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