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Virtual Microscopy Large Slide Automated Acquisition: Error Analysis and Validation

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Abstract

The aim of this work is to assess and analyze the discrepancies introduced in the reconstruction of an entire tumoral bone slice from multiple field acquisitions of a large microscopy slide. The reconstruction tends to preserve the original structural information and its error is estimated by comparing the reconstructed images of eight samples against single pictures of these samples. This comparison is held using the Structural Similarity index. The measurements show that smaller samples yield better results. The detected errors are introduced by the insufficiently corrected optical distortion caused by the camera lens, which tends to accumulate along the sample. Nevertheless, the maximum error encountered does not exceed 0.39 mm, which is smaller than the maximum tolerable error for the intended application, stated in 1mm.

Keywords:

Microscopy; Confocal; XY-table; Stitching; Bone tumor; Histological Techniques; Error evaluation.

Introduction

In a primary bone tumor resection surgery, a surgical specimen is obtained following the guidance of a preoperative plan based on Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) studies [1,2,3]. In certain areas of the MRI images, however, the tumoral region limits cannot be well delineated by simple visual inspection.

Optical bright-field microscopy is a technique used in pathology to view samples and assess the classification of bone tumors [4,5]. The conventional practice in histopathology is to evaluate certain critical portions of the surgical specimen sample. Nevertheless, this practice does not enable establishing a direct correspondence between the microscopy images and preoperative MRI images, impeding the accurate classification of doubtful regions.

The main aim of this work is to enable the reconstruction of an entire bone slice sample from a surgical specimen, stitching together congruent microscopy pictures. Structural conservation is a major concern, due to the fact that the reconstructed image will be superimposed to its corresponding slice image in a volumetric magnetic resonance of the surgical specimen. Therefore an evaluation of the introduced error is addressed as well. The direct comparison between MRI image and microscopy images might lead to a new way of interpreting MRI images, and hence determine whether there exist tumoral tissues in the region under study.

Methods

Optical acquisition

In order to acquire the images, a Carl Zeiss microscope, Primo Star model (iLED Halogen/LED) was used, supplied with a Canon Powershot A640 camera, mounted through an array of coupling lenses. The total magnification rendered on the pictures corresponds to the objective magnification (40x) added to the camera analog zoom. This gives a total measured resolution of 1270 pixels per millimeter. The sample scanning is enabled by a XY-table designed with two step motors, providing a resolution of 200 steps per millimeter of movement (6.3 pixels per step). An 8-bits word UART serial communication between the controlling system, implemented in a PC running MATLAB® 2012b (The MathWorks, Inc., Natick, Massachusetts, United States), and the XY-table controller, implemented using a Texas Instruments® MSP430G2553 microcontroller completes the closed loop system.



Figure 1– Camera coupled to microscope, focusing a bone tissue sample disposed on the XY-table.

Sample scanning process

The scanning process is divided into two main steps:

• The control system, which takes consecutive pictures in the X axis direction of movement, generates a row

of the final image, and moves in the Y axis direction, leading to a new capture of pictures.

The process of rows stitching.

Control system

Two consecutive pictures should have a transition region overlapping in a range from 40% to 60% of the capture area. Matching points between pictures are detected by searching common features with the Speeded-Up Robust Feature (SURF) algorithm [6]. Even though the movement is supposed to occur only in the X direction, there are displacements in Y due to the misalignment of the sample in the XY-table with the microscope-camera array. These displacements are compensated with consistent movements in the Y direction. During the search of features and execution of XY-table movements, a low-resolution video mode of the camera is used. Several high-resolution pictures are afterwards taken and their level of focus is measured using the Brenner gradient [7]. Once the best focused image is chosen, the process of stitching between consecutive images takes place. Afterwards, an optional blending step may be applied to match small differences in structure and luminescence in the transition zone between pictures.

Rows stitching

The rows previously obtained are then aligned one after the other, also using common features extracted with the SURF algorithm.

Optical compensations

Before starting the sampling process, luminescence compensation is performed: a sample-free RGB picture is taken (P), its mean is measured (μ), and the compensation RGB matrix (M) is calculated as follows:

$$Mij = \mu / Pij(1)$$

Geometric compensation is also carried out, which attacks the fish-eye problem caused by the microscope-camera array lenses. This deformation consists on the stretching of the image increasing with the distance to its optical axes, and may cause image discontinuities after several pictures are attached together. In order to address this issue, an inverse deformation dependent on the square of the distance was applied [8]. This inverse deformation was estimated using pictures taken to a 0.1mm depth hemocytometer.



Figure 2– Fish-eye lens distortion geometrical correction applied to a 0.1mm depth hemocytometer picture.

Visualization

The software does not generate one final slide capture, but instead hundreds of small images, and a matrix that describes how to stitch them together. This design eases future work with higher magnification, since all that changes is the amount of pictures handled by the matrix.

Stitching error measurement protocol

Eight different samples were acquired, with sizes varying from 440 mm² to 1280 mm². In order to evaluate and determine the possible misalignments generated by the stitching process, a comparison between the final stitched image and a standard image must be performed. Thus, a not-amplified-bythe-microscope picture of the sample, taken using the same camera as in the stitching and corrected with the same geometrical factors, is used as a standard for comparison. Although it lacks the grade of definition of the stitched image, it serves as a pseudo ground truth to evaluate the level of preservation of image structure.

The method used for the quantification of this error uses the Structural Similarity (SSIM) index, combined with phase shifts between the images. The SSIM index ranks locally the similarity between portions of two images. The SSIM continuous range interval is -1 for worst case to 1 for perfect match. This index can be displayed as a gray level pixel map, giving a general idea of the amount of parity between images. The index per pixel is averaged to give an overall level of similarity, whose range limits are coincident with the previously mentioned.



Figure 3– SSIM map displayed as (1+SSIM)/2 between the stitched image and the standard image, for Sample A. The map is divided in nine portions, where the corresponding index of each portion is labeled. Dark pixels mean bad correlation between images, while bright pixels correspond to good structural similarity.

The approach taken in this work to estimate the stitching error consists on registering the images, using rigid registration algorithms. Misalignment locations depend on registration process. If the correct registration and similarity congruency of a portion of the image leads to misalignments in other portions of the image, then a stitching error is identified. The stitching error in a part of an image may be compensated by adding translation offsets to the original registration. When moving one of the pictures a certain offset to the right, left, upwards, and downwards, SSIM map zones which originally appeared dark turn to white, meaning a good alignment in that part of the image, and vice versa.



Figure 4– Portion of the image from Figure 3 (Sample A) with the lowest SSIM index (0.40), shown as the stitched image (magenta) superimposed in the standard image (green). As can be seen, a better registration can be achieved by moving the standard image upwards.



Figure 5– Gray level changes in the SSIM map representation of Sample A, as a function of offset. The center image represents the better registration found between images. Right (positive) and left (negative) shifts are labeled dx, while up (negative) and down (positive) are labeled dy.

Calculating the SSIM index after every shift leads to the construction of surfaces like the one shown in Figure 6. In order to determine the error introduced by the stitching process, it is necessary to evaluate the final image misalignments produced all along the sample, compared to the standard image. This error will be quantified as the minimum radius of displacement required, in millimeters, to reach the denominated noise floor of the SSIM index in the sample. Knowing the bell-like shape of the SSIM index evolution as a function of space and being proven that it is not affected by local maximums phenomena, the SSIM map is evaluated in the initially dark areas for different shifts in X and Y directions. Strictly, the noise floor is considered reached after subsequent movements in one direction do not improve the SSIM index, but worsen it. It should be highlighted that due to its accumulative behavior, the stitching error tends to grow with the distance. Though, it is expected that the major discrepancies will appear around the edges of the image (see Figure 3 and Figure 5), assuming a rigid registration towards the center of both images.



Figure 6– SSIM index as a function of image displacements (in X and Y) with respect to the better fixing registration employed in Sample A.

dx [mm]

To evaluate an estimation of the general structural deviation of the stitched image proportions compared to the standard image ones, a Structural Deviation (SD) index is proposed for both X and Y maximum extensions (ΔX and ΔY correspondly):

$$SD_{X} = 100 (\Delta Xstd - \Delta Xstitch) / \Delta Xstd (2)$$
$$SD_{Y} = 100 (\Delta Ystd - \Delta Ystitch) / \Delta Ystd (3)$$

Results

The eight reconstructed samples (A to H) are displayed in Figure 7, and their error measurements are shown in Table 1.

Table 1-Error measurement of the samples stitched

	Size	Total	SD _X	SD_Y	SSIM	Error	
S	[mm]	images	[%]	[%]	index	[mm]	
А	40 x 32	819	0.82	2.17	0.67	0.33	
В	45 x 27	876	-3.27	-2.15	0.53	0.39	
С	28 x 21	363	0.25	-2.08	0.64	0.28	
D	20 x 22	266	-1.10	-1.44	0.73	0.10	
Е	26 x 22	364	-1.57	-0.13	0.67	0.26	
F	29 x 24	392	-0.75	-2.77	0.65	0.22	
G	23 x 23	326	-0.42	-1.45	0.75	0.16	
Н	32 x 22	378	-1.60	-0.35	0.63	0.20	



Figure 7– Images A to H showing the eight samples reconstructed using the stitching process.

Discussion

As it was shown in the results section, non-standard size (3 in $x \ 1$ in) samples could be reconstructed, with a 1270 pixels per millimeter resolution.

The results in Table 1 show an absolute maximum Structural Deviation of 3.27% in X and 2.77% in Y. In terms of stitching error, it can be seen that, in general, the higher the amount of pictures taken, the higher the error measured. Samples A and B appeared to be more deformed than the rest (0.33mm and 0.39mm), being both composed with more than three times as

many images as Sample D (0.1 mm, minimum error). This deformation was caused by the accumulated stitching error along the entire sample, mostly due to the optical distortion produced by the camera lens used for acquisition, which is not perfectly corrected. In addition, this distortion was measured higher for thicker microscope slides (more than 4.5 mm thick), which were the ones used for Samples A and B. In order to lower the error caused by this effect, it is recommended to try to use thinner slides (1 mm thick) in all cases.



Figure 8– Error variation measured in the samples, as a function of the total number of images stitched.

Although the SSIM index is a relatively good index for assessing structural differences between images, in some cases it might perform poorly for the application presented in this work. Consider for example Figure 9. In spite of the fact that both images were taken with the same camera, the stitched image (B) presents much darker regions than the standard image (A), for the same tissue. This marked difference in the contrast of the images is interpreted by the SSIM index as a major structural difference, even though it is not.



Figure 9– Although not a bone sample, this liver tissue sample clearly shows low rated areas due to contrast differences between images. (A) Standard image from sample B. (B) Stitched image from sample B. (C) SSIM map between standard image and stitched image from sample B. (D) Superposition of standard (green) and stitched (magenta) images from sample B. No significant structural differences can be seen.

Since the MRI resolution of the bone prior to becoming a surgical specimen is 1 mm, the same value will be taken as the maximum tolerable error in the complete stitched image.

It is considered essential the conservation of the original appearance of images. Although the blending between images might lead to friendlier representations for the viewer, it could alter the specialist perception of the degree of cellular disorder appreciated, as shown in Figure 10. Hence, it is believed best to simply stitch the images one after the other, obviating any kind of image processing between them, both in the rows confection as in their alignments.



Figure 10– Stitching between two wrongly matched rows. The blending applied generates an unwanted ghost effect in the final image.

Conclusion

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This work presents a new way to acquire histological images using a XY-table and a digital camera coupled to a microscope. Although being a relatively high time-demanding procedure (~30 seconds per image acquisition in average), it strongly lowers a Whole Slide Imaging Scanner budget. Measurement results presented show that the error committed is small enough for the suggested application, which aims, in the future, to help in the construction of a gold standard bone tumor samples repository.

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