Stitching Algorithms for Automatic Assembly of Hyperspectral Histological Images

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***Abstract*—Whole Slide Imaging refers to the digitalization of entire histology slides and it is becoming an improvement for pathology workflows. Moreover, hyperspectral imaging is an emerging technology in the biomedical field because it can improve histological disease detection. However, its spatial information is usually lower. This paper describes different methodologies for microimage stitching, offering an approach for expansion of the field of view (FOV) of hyperspectral images. The goal is to find the optimal combination of parameters for the input frames which provide an accurate mosaic of the whole slide.**

Keywords- Hyperspectral imaging (HSI), Computational Pathology and Stitching.

I. INTRODUCTION

Nowadays, the study of histology slides is regarded as the gold standard for the clinical diagnosis of cancer, and the trend is to digitalize histology slides for further histology image analysis. Histopathologists visually examine cell shapes and tissue distributions, determine whether tissue regions are cancerous, and determine the malignancy level [1]. However, interpretation of these images is often subjective due to limitations in human vision to distinguish subtle color differences, particularly because of spatially overlapping emissions.

Spectral technology improves on color camera performance by expanding the number of channels beyond the RGB (Red, Green, Blue) palette. The higher content of information provided by a hyperspectral (HS) image can be analyzed to detect objects and patterns as well as the chemical composition of the materials which are present at the scene [2]. However, the spatial information provided by a hyperspectral imaging (HSI) frame is usually lower compared to a traditional RGB frame. Microimage stitching offers an approach for expansion of the field of view (FOV), aiding visualization of microscale features across macroscopic areas of tissue.

After an exhaustive study of the state of art, we concluded that the number of studies applying stitching to HS images in the literature is very limited. Lang *et al.* [3] developed a multichannel mosaicking algorithm which could process square cubes up to 10 channels and 94% overlap between them. It was hypothesized that input parameter could be optimized and still obtain accurate results (e.g. overlap between frames or number of channels per frame).

II. METHODOLOGY

The database used in this research work belongs to the ITHaCA project [4]. The specimens investigated in this research work consist of human biopsies extracted during brain tumor resection procedures and captured with a customized HS microscope system.

A quality assessment protocol needs to be designed for the stitched images. The problem arises when trying to measure the quality of the alignment of several small FOV images, as such large FOV image does not exist. We addressed this problem by designing an approach based on the one proposed by Wald in 1997 [5]. The steps shown in Figure 1 are further explained.

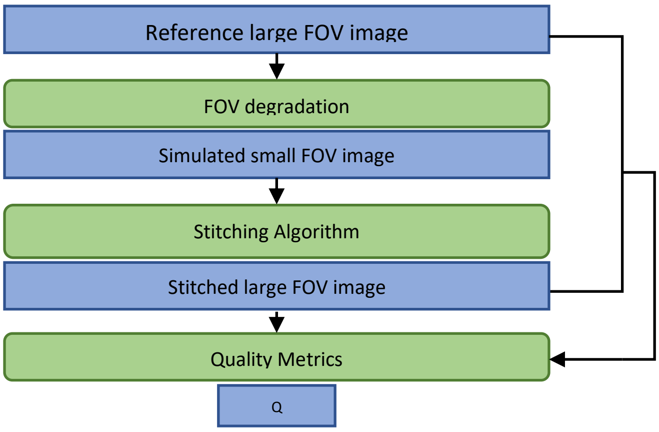


Figure 1. Block diagram of the Wald Protocol.

A. *Field of View Degradation (FOVD)*

When working with microscopes, the FOV is mainly determined by the used magnification. Pathologists, usually work in 20× to detect an event on a slide [6], and so, to simulate this magnification, we should apply a FOV degradation (FOVD) of 20. However, for simplicity of the tests, it was decided to work with a FOVD of 2. However, such a perfect degradation cannot be achieved in real life. When working with the platform of our microscope, we must deal with 3 µm translational errors. Thus, when degrading the FOV, errors were simulated by translating the small FOV images a random number of pixels within the range -8 to 8.

Another variable to consider was the overlap between the small FOV images (*Nsi*). It is defined as the percentage that one image *i+1* shares with its predecessor, *I* (1). It is possible to have different overlaps for *x* and *y* axis. However, for simplicity of the test, we are going to set the same overlap for both axes.

$$Overlap = \frac{Nsi - FOVD}{Nsi - 1} \qquad (1)$$

The last variable is the number of frames (*Nf*) taken. To reconstruct the whole original image, *Nsi* number of frames are needed. However, if a smaller number of frames are taken, less area is reconstructed. Thus, the number of frames taken *Nf*, goes from the FOVD (minimum number frames possible), to *Nsi* (number of frames that reconstruct the original image) (2). Overlap function (1) has an asymptote in 1 thus, tests stopped at the graph's plateau, *overlap=0.92* (*Nsi=13* for *FOVD=2*).

$$FOVD \leq Nf \leq \frac{overlap - FOVD}{overlap - 1} \qquad (2)$$

B. Stitching Algorithm

In this study we decided to employ two different stitching methods, namely manual and automatic stitching. The manual stitching consisted of just mosaicking the images one next to each other, without any kind of further processing. However, for the automatic stitching a more complex algorithm, developed by Lang *et al*. [1], was used.

C. Quality Metrics

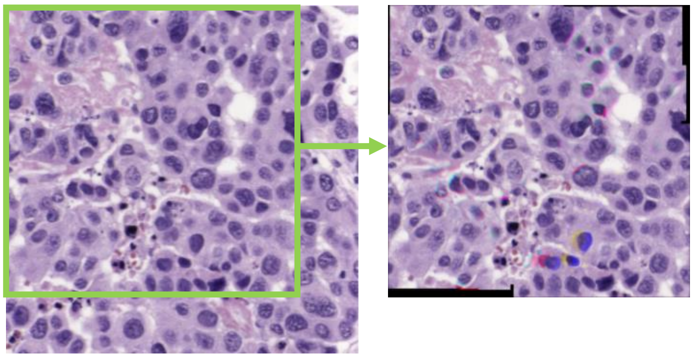
Image quality evaluation methods can be subdivided into objective and subjective [7]. Subjective methods are based on human judgment and operate without reference to explicit criteria. In any case, it is also necessary to establish quantitative measures to quantify the effects of image stitching algorithms on image quality. Root Mean Square Error (RMSE) [8], Peak Signal-to-Noise Ratio (PSNR) [9] and the Structural Similarity Index (SSIM) [10] were employed.

III. RESULTS

On the one hand, we obtained the results for the manual stitched images consisting of mosaicking the images one next to each other, without further processing. Quantitatively, metrics for this kind of stitching algorithms were not bad. However, qualitatively, the image quality was really poor in the joint areas between the original frames.

On the other hand, we obtained the results for the automatic stitched images. Firstly, the selected overlap and number of frames (*Nf*) were tested. This meaning that for every image we had, it was tested all the overlaps between 50% and 92% and all the possible frames for a determined overlap. We can see an example of a graph obtained for SSIM in Figure 3.a.

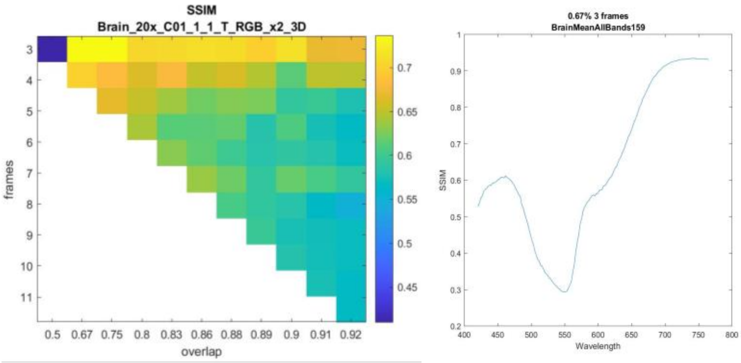
Results clearly show the most repeated number of frames to be 3, and thus the selected one. And although there is no clear number for the overlap values it was chosen the one which better reconstructed the original image from the selected frames, 67% (reconstructs 83%). In Figure 2, we can appreciate that qualitatively the mosaics show good results.



(a) (b)

Figure 2. Brain histology images. a) Original image. (b) Reconstructed 83% of the original image from 3 frames at 67% overlap.

Secondly, we proceeded to test each band individually. From pre-processed images containing 159 bands, single band stitching was realized. Then, for each metric it was perform the mean for each band (of the 5 brain histology images), as shown in Figure 3. The most accurate stitching results would be given from bands in the range of wavelength 650 to 750 nm. Finally, some band configurations were evaluated. Cubes of 10 and 3 bands were conformed using wavelengths between 650 and 750 nm. The mosaics resulting from these cubes offered SSIM values between 0.88 and 0.94. Computational resources of this algorithm required ~0.5 MB of memory and 100 seconds of execution time per each band stitched.



(a) (b)

Figure 3. (a) SSIM of a Brain Histological Image for all overlaps and its corresponding frames. (b) Plot of mean SSIM vs wavelength for 5 Brain Histology images (159 bands) at 67% overlap and 3 frames.

IV. CONCLUSIONS

Spatial information provided by an HSI frame is usually poor, but microimage mosaicking improves microscopic resolution while macroscopic FOV. In this project the state-of-art referring the stitching algorithms of HS histology images was studied. Experimental tests were performed, employing manual and automatic algorithms. Although, manual tests showed good results metrics, the joint area between images were not as good. Lang *et al.* [3] algorithm offered good results when applying a 67% of overlap between frames and 3 frames to reconstruct the original image. Single band stitching was also performed, finding a range of wavelength between 650 and 750 nm that provided accurate mosaics. Several combinations of bands within this range were tested and proved to be quantitative and qualitative good. In conclusion, a final stitching algorithm have not been developed, however, several important discoveries have been made to create our own algorithm in a close future.

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