Serial Section Alignment of Histopathological Images stained with different Biomarkers

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Abstract

Registration of whole slide images (WSI) allows simultaneous analysis of multiple biomarkers and colocalization of regions of interest from consecutive tissue segments. This is a key process in digital pathology. In this paper, we propose a novel registration system for digitized stained segments that faces the drawback of working with high resolution (HR) WSI. To this end, a global to grid-based registration framework on a Low Resolution (LR) version of the original sample is applied. We first compute a downsampling of the HR virtual slides under analysis and apply a global rigid registration based on Mutual Information. The accuracy of this rigid registration is enhanced by using the demons non-rigid registration algorithm, over square blocks of the rigid registration result image. Finally, we transfer the transformation obtained from both LR rigid and deformable registrations to the original HR images. The results obtained show that this technique yields satisfactory registration results over HR slides in a valid operational time and ensuring color constancy. Thus, improving the quality and performance of related works. Besides, this proposal allows an accurate WSI registration, which is difficult to obtain when working directly at the original slide resolution.

1. Introduction

Pathologists generate a huge amount of data samples from tissue sections. These tissue sections are stained with different biomarkers according to the clinicopathological features to analyse. The stains allow to examine multiple proteins to extract relevant information from the complex biological processes involved in serious pathologies. For instance, in our research, to detect cancer subtypes the set of biomarkers considered are: immunohistochemistry (IHC) with Ki-67, expression of estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (Her2) and the tumor histology conformed by Hematoxilin-Eosin (HE) stain. **Figure 1** shows an example of these biomarkers in a serial slide analysis.

Advances in digital pathology has partially solved the performance of examining each consecutive stained sample to get a diagnosis for each set of biomarkers. The digitalization of the tissue slides into High Resolution (HR) Whole Slide Images (WSI) allows pathologists to visualize the serial slides on a screen by just opening each individual WSI. Ideally, the set of stained WSIs for each tissue should be correctly registered in terms of position, rotation and shape to allow pathologists to compare the stained sections in a single view. However, the process of serial section alignment in WSI is far from being automatic and still presents some drawbacks:

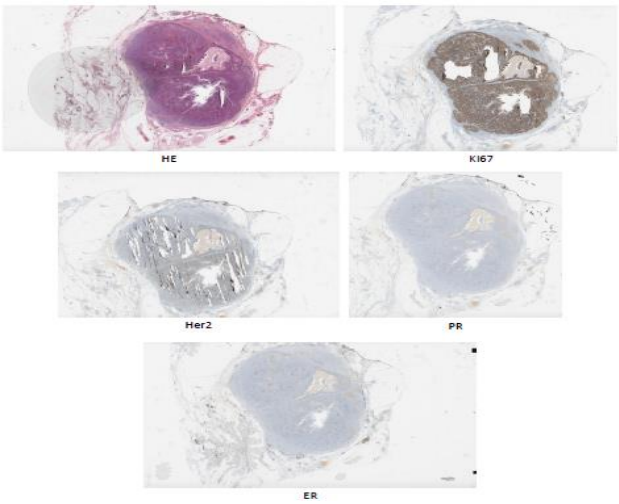

Figure 1 *Example of serial WSIs stained with different biomarker: Ki67, Her2, PR and ER stains. The example shows the variability in terms of spatial, color and tissue structures present in the different sections.*

-Spatial differences in terms of shape and position of the structures, which can include: distortions, deformations or even folds and tissue breaks.

-Colour differences due to the stain used in each section.

-Large size of WSIs (GigaByte images), which makes manipulation and processing difficult.

In **Figure 1**, we can observe an example of the high variability present in one WSI set, and the difficult scenario we are facing.

1.1. Image registration survey

Registration has been used in several image processing applications, including medicine (mainly in radiology). In [1] the authors give a comprehensive survey of different registration methods. Another survey maybe found in Goshtasby *et al.* [2], and more recently in Sotiras *et al.* [3].

The application of registration techniques to microscopic images has started relatively late, about 7 years ago. Most of the registration methods consist of the following four steps: feature detection, feature matching, transformation estimation and re-sampling.

In [4], the authors propose a system to register prostate MR images to pathological serial sections. In [5], Mueller *et al.* use two-step registration on low-resolution images applied to the tissue region under analysis. Lopez *et al.* in [6], show a WSIs registration method based on the Elastix registration framework ([7]) to register multimodal WSIs.

In [8], Deniz *et al.* presented a Multi-Stained WSIs alignment based on rigid registration transformation and Mutual Information. Recently, Trahearn Schwier *et al.* propose in [9], a framework for robust registration of serial sections stained with different markers. Finally, in [10], the authors propose a patch-based registration for gigabyte WSIs, which work with a low-resolution version of the WSI. The main drawback of these systems is that they need huge computational time to compute the registration of the High Resolution (HR) WSI. Besides, some of them does not deal with the color distortion that appears after the deformation applied to each patch.

In this paper, we propose an image registration method applied to digital pathology area that achieves a fast computation and solve color distortion problems associated to accurate deformable registrations. We propose a WSI registration that follows a coarse to fine work-flow, starting to a Low Resolution (LR) version of the original HR WSI. We use a global Intensity-based rigid transformation based on Mutual Information, and a deformable registration applied to blocks of the image, which allow the parallelization of this costly process. The deformable registration that we use is the diffeomorphic demons algorithm proposed by Vercauteren *et al.* in [11] and based on the demons algorithm [12].

The remainder of the paper is organized as follows. Section 2 describes the materials and database. Section 3 presents the proposed method, detailing the functionality of each step. Quantitative and qualitative results are shown in Section 3. Finally, conclusions are drawn in Section 4.

2. Materials

Experiments have been performed with a dataset created by expert pathologists. The dataset is composed of 36 breast tissue samples extracted from incisional biopsies to detect cancer regions. The tissue sample preparation previous to the WSI digitalization is the regular systematic process used in any pathology laboratory. The process is characterized by:

- Formalin-fixed paraffin-embedded (FFPE) tissue samples.
- Section thickness of 4 μm.
- The serial sections from each case were stained with five biomarkers: Ki-67, ER, PR, Her2 and HE.
- Slides digitized at 20x magnification with an Aperio ScanScope XT scanner. Thus, a dataset composed of 36 WSI ranged between 1GB to 10 GB was used.

3. Method

We propose a registration method for WSIs that exploits the advantages of both rigid and non-rigid registration to achieve a correct alignment between serial sections. Since HR WSIs have Gigabyte sizes, the computational cost of any process or transformation is too high to consider working directly with these slides. Therefore, we propose to use a LR version of the original sections to compute the registration process in an acceptable time. In a posterior step, we apply all the image transformations to the HR WSIs, thus keeping the HR quality in the final registered slides.

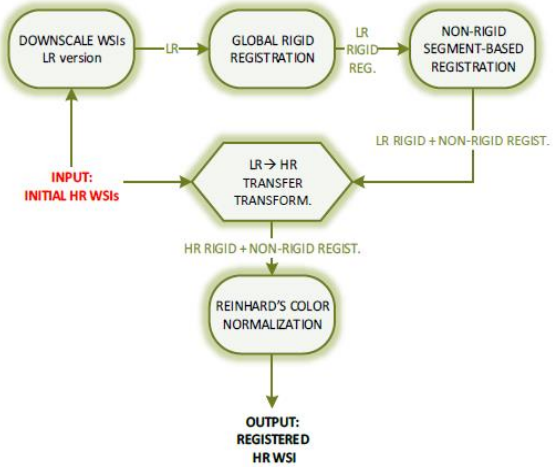

Figure 2 *Work-flow of the proposed registration method.*

Given the high cost that non-rigid registration presents, even for LR WSIs, we decide to apply it in a block-based framework to register image blocks in parallel, thus speeding up this costly process. Besides, since this registration adds a color distortion according to the deformation applied, this block-based approach results in a color modification for each of the blocks. Hence, we propose to apply a color normalization method, in a final step, to keep the color constancy.

The improvements of the proposed method in WSI registration are twofold: First, we achieve an accurate registration for HR WSIs that combines rigid and deformable registration methods and solves color distortion. Second, the registration is fast since we work with LR versions of the original WSIs, and we apply the non-rigid registration to square blocks.

The work-flow of the proposed method is shown in **Figure 2**, with the following steps:

1. Use a Low Resolution (LR) version of the WSIs to register: The HR WSIs to process have a huge size (20x magnification, with sizes starting from 20k x 10k pixels to 70k x 50k pixels). We work with low resolution (LR) images with a magnification of 2x.

2. Apply rigid registration to LR image based on mutual information.

The iterative steps followed by the rigid registration are:

- Rigid image transformation (rotation, translation).
- Bilinear Interpolation. The image is interpolated with a bilinear filter used to construct the final resampled moving image from the geometric transformation estimate.

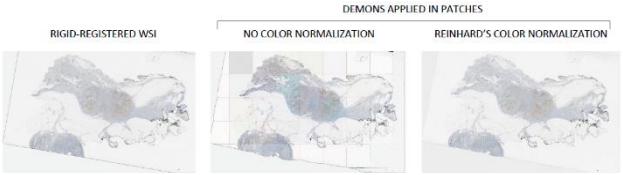

Figure 3 *Color normalization using Reinhard's technique.*

- Mutual Information. Mutual Information (MI) is used here to evaluate the similarity that each reference and moving image pairs present along the optimization iterations. High MI value denotes that images are well aligned, i.e. the values of one set of pixels match to similar values in the other image.
- Optimizer. The one plus one evolutionary algorithm [13] is used to change the parameter set to produce the geometric transformations along the iterations through the multi-resolution levels (from coarse to fine). The stop criterion is set according to a maximum MI value and a maximum number of iterations. In our case, the maximum number of iterations is 100.

3. Refine the coarse rigid registration by applying diffeomorphic demons non-rigid registration in square regions or blocks to allow parallelization.

These techniques are defined as non-parametric spatial transformations. They specify a different displacement *U(X)* for each pixel of the image *X* to get the transformed image *T(X)*, correctly aligned with the reference one. Hence:

$$T(X) = X + U(X), \qquad (2)$$

The goal of the registration is to find the transformation *T* that maps each point of *X*.

With the aim of reducing the computational cost of the diffeomorphic demons registration, we propose the following strategies:

-Divide the LR WSIs in squared blocks and apply the deformable algorithm in parallel for each block. According to our experiments, an area of 1/30 of the reference LR WSI area yields correct results. Thus, on average of the dataset considered the size of the tissue blocks is 512x512 pixels. Since each block is registered separately, merging artifacts can be observed at the borders of the blocks. Although we do not address these situations in our implementation, to avoid its computational cost, slightly overlapping blocks and stitching techniques among them can be used to avoid these situations.

-Avoid the processing of squared blocks that do not contain any tissue. In order to detect these regions, we compute a tissue segmentation for each LR WSIs by means of background segmentation.

4. Transfer resultant rigid+deformable LR registration to the entire HR WSI. We transfer the Rigid + Deformable registration to the HR moving slide. We apply it in the same order as it was computed.

5. Finally, a color normalization based on Reinhard's method [14] is applied to correct the color variations that all spatial deformations add to the registered HR WSI. **Figure 3** illustrates the effect of registration without color normalization and how color normalization corrects it.

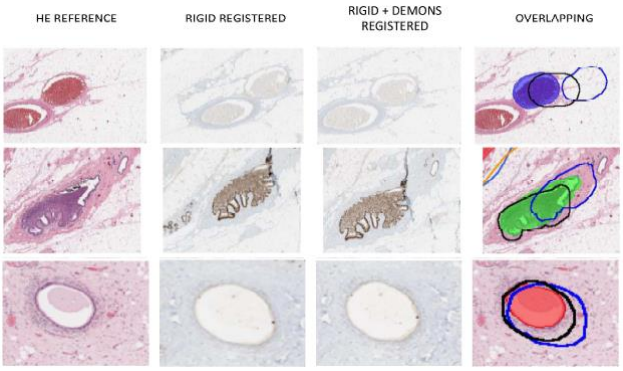

Figure 4 *Qualitative region evaluation. 1st column: reference HE region; 2nd column: biomarker region or moving image after rigid registration; 3rd column: registered region with the proposed method. And 4th column displays the overlapping of all three regions: coloured region is the HE region, blue line is the rigid registered region, black line is the final registered region.*

The resultant HR WSI preserves the quality and fidelity of the physical slide and makes the method suitable for digital pathology analysis.

4. Results

30 different registrations from 9 different sets of WSIs were analyzed, each slide stained with a biomarker belonging to HE, Ki-67, ER, PR or Her2. The WSI stained with HE is used as reference image, and we registered the other four WSIs, stained with Ki-67, ER, PR and Her2, to this reference. To evaluate the registration quality, we have segmented three regions per slide where zones of pathological interest and structures for registration analysis have been masked. In **Figure 4**, we can observe three of these results extracted from the final registered slides. These samples show how our registration approximates better to the HE reference region thanks to the demons non-rigid algorithm, which allows the deformation of the region towards the reference position/size. When rigid registration achieves a correct alignment of the slides, our approach improves even more the alignment if it is possible.

To compare the improvement of the proposed system with a rigid registration, we have done a comparison between the overall proposal and the rigid registration used in the second step of the proposal. **Figure 5** shows this comparison. We have computed the intersection percentage between the reference image regions *A,B,C* and their counterpart in the moving image *A'*, B', C'. Therefore, $A \cap A' = 100\%$ means that both regions are completely aligned. On the other hand, $A \cap A' = 0\%$ means that the registration has failed and no alignment has been done. As we can observe, the proposed rigid registration achieves the overlapping of regions in all the evaluations. Some difficult sections perform worse than the rest of the slides, as in the case of wsi6.1, wsi6.2 and wsi9.4, due to the bad quality of the extracted sections

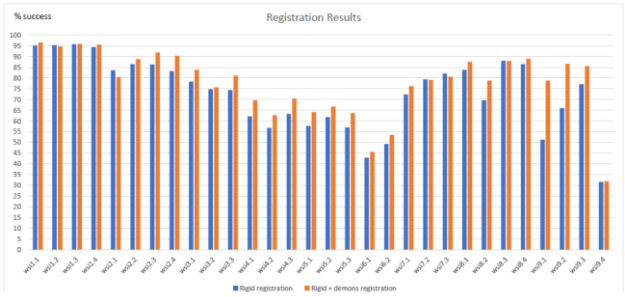

Figure 5 *Rigid registration (blue bars) and Rigid + Demons registration (orange bars) results.*

and the high differences present in serial sections. Rigid + deformable registration achieves an improvement of the registration results almost in all the slides. The magnitude of the improvement depends on several factors like how well rigid registration achieve to register both slides, and the quality of the samples analyzed.

When the slides are correctly extracted, but rigid registration cannot achieve a good percentage of alignment, demons algorithm helps to improve the results, as we can see in slides like wsi9.1, where the improvement reaches a 27\%, or wsi9.2, wsi4.1, wsi4.3, wsi2.3, wsi2.4, wsi4.1 with significant improvements.

In situations where rigid registration achieves a correct alignment of the slides, non-deformable algorithm does not show a significant improvement, as we can see in wsi1.1, wsi1.2, wsi1.3 or wsi8.4. In wsi2.1, we can observe the only case where demons algorithm performs worse than rigid registration. In this slide, the poor stain and the lack of image contrast results in a 3\% decrease of the rigid registration accuracy.

Averaging all the dataset results, the complete registration that we propose achieves an overall improvement of 4.88% over the rigid registration results. Regarding the computational cost, we have processed HR slides of 20k x 10k pixels using an Intel Xeon CPU ES-2690 with 2.90 GHz, 64 GB of RAM and 16 CPUs. Using our approach, with 10 CPU parallel processes to compute block-based demons registration, we compute the registration in 4.5 minutes. In order to compare the improvements in terms of computational performance, we have processed the same image at 20x magnification utilizing rigid and demons registration over the whole slide without any parallelization. The computational time obtained with this setup is 28 minutes. Therefore, our approach gives us a registration 6.22 times faster than working directly with HR WSI.

5. Conclusions

In this paper, we have presented a novel system for the registration of WSIs. Our proposal takes the most of both rigid and deformable registration to achieve an enhanced alignment of pathological slides. Since WSIs present difficulties to be processed, due to their size (order of GigaBytes), we perform first the registration at LR level (2x magnification), and then apply the transformations to the HR level (20x magnification). The strategy to deal with square blocks to speed up the non-rigid registration step while keeping the quality of the demons alignment is also presented. The results show that the proposed method allows a correct registration between the WSIs, preserving the color and resolution fidelity with the original slides, while solving computational cost problems present in reference techniques.

Acknowledgements

This project has received funding from the European Union's FP7 programme under grant agreement no: 612471. (http://aidpath.eu/).

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