Placental Fetal Stem Segmentation in a Sequence of Histology Images

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ABSTRACT

Recent research in perinatal pathology argues that analyzing properties of the placenta may reveal important information on how certain diseases progress. One important property is the structure of the placental fetal stems. Analysis of the fetal stems in a placenta could be useful in the study and diagnosis of some diseases like autism. To study the fetal stem structure effectively, we need to automatically and accurately track fetal stems through a sequence of digitized hematoxylin and eosin (H&E) stained histology slides. There are many problems in successfully achieving this goal. A few of the problems are: large size of images, misalignment of the consecutive H&E slides, unpredictable inaccuracies of manual tracing, very complicated texture patterns of various tissue types without clear characteristics, just to name a few. In this paper we propose a novel algorithm to achieve automatic tracing of the fetal stem in a sequence of H&E images, based on an inaccurate manual segmentation of a fetal stem in one of the images. This algorithm combines global affine registration, local non-affine registration and a novel 'dynamic' version of the active contours model without edges. We first use global affine image registration of all the images based on displacement, scaling and rotation. This gives us approximate location of the corresponding fetal stem in the image that needs to be traced. We then use the affine registration algorithm "locally" near this location. At this point, we use a fast non-affine registration based on L^2-similarity measure and diffusion regularization to get a better location of the fetal stem. Finally, we have to take into account inaccuracies in the initial tracing. This is achieved through a novel dynamic version of the active contours model without edges where the coefficients of the fitting terms are computed iteratively to ensure that we obtain a unique stem in the segmentation. The segmentation thus obtained can then be used as an initial guess to obtain segmentation in the rest of the images in the sequence. This constitutes an important step in the extraction and understanding of the fetal stem vasculature.

Keywords: placenta images, histology images, segmentation, registration, fetal stem vasculature.

1. INTRODUCTION

Understanding the three dimensional structure of placental fetal stems can help doctors identify where prenatal development diverged from normality. This could potentially help lead to earlier diagnoses of significant life-long diseases.[5,6] However, the process of identifying fetal stems in the placenta is currently impossible. Manual extraction of the fetal stems would be costly and extremely time-consuming; an expert must hand-trace the fetal stems in each histology slide of the three dimensional volume. By automating the detection of fetal stems, we hope to increase efficiency and reduce costs for emerging placental research.

Hematoxylin and eosin (H&E) stained images are commonly used for understanding the tissue structures. The H&E images are obtained by slicing the placental tissue vertically near the area of interest. The digitized images thus obtained are high resolution images, where the cell nuclei are stained blue and the other structures in various shades of red, pink and orange.

H&E staining serves a fundamental purpose in the study of tissue structure in a single slide. An approach based on a study of a single slide does not capture understanding of the movement of the blood vessels inside

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a human organ. Any study aiming to analyze the vasculature of a placenta needs to incorporate knowledge incorporated in the consecutive slices.

Nevertheless, to extract the vasculature structure, it is essential to successfully follow the blood vessels from one slide to the other. This problem is difficult because no two consecutive slices are registered. The slices may not have the same orientation, scaling and there may be a linear translation between two consecutive slices. To overcome this, we propose to use affine registration of the slices with four parameters: angle of rotation (θ), scaling (α), translation in the x and y directions (t_{x_1} and t_{x_2}). Such rigid registration produces a sequence of globally registered H&E images, which could be viewed as a movie, depicting a fluid motion of the placental tissues.

At this point we should note that the affine registration should be done only at a 'global' level, not at a local level. This is because of the fact that the blood vessels move inside the placenta. So the location of a cross section of a blood vessel does not match the same blood vessel's cross section in the next slide. Affine registration at a local level will actually align these cross sections, where in reality they should not be aligned.

Once the entire sequence is registered with affine registration, we would like to follow the blood vessels at a local level. Our approach here is to start with some initial knowledge of the location of a blood vessel, and then follow this blood vessel through the sequence of H&E slices. This is the segmentation step in our algorithm.

The additional difficulty here is that the blood vessels (and any other tissue, in general) change shape nonlinearly in space. For example a blood vessel may change its thickness, or may be deformed. Thus, only parametric affine registration is not sufficient in following the blood vessels along the H&E sequence. A global affine registration then should be followed by a non-affine registration.

2. DETAILS OF THE METHODS

In this paper we try to solve the problem of tracking the placental fetal vessels, through a sequence of H&E images. This is a very difficult problem. Existing algorithms based on edge detection or even on texture segmentation would not work, due to the complexity of the data. We propose a method made of several steps, as explained next.

2.1 Global multilevel parametric registration

As described before, we need to perform affine registration of the sequence of H&E images. We postulate that the images are scaled, rotated and translated. To obtain a sequence of registered images we register the second image (target image, T) with the first image (source image, S) to obtain a new registered second image. Then the third image is taken as the target image and it is registered to the newly registered second image and so on, giving us a sequence of globally registered images.

To this effect we use a parametric registration, with four parameters: the angle of rotation (θ), scaling factor (α), translation in the x_1-direction (t_{x_1}), and the translation in the x_2-direction (t_{x_2}). The target image, T, is transformed into $T(R(\mathbf{x}))$ where R is the following transformation

$$R(\mathbf{x}) \equiv R(x_1, x_2) = \alpha \begin{pmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} + \begin{pmatrix} t_{x_1} \\ t_{x_2} \end{pmatrix}. \tag{1a}$$

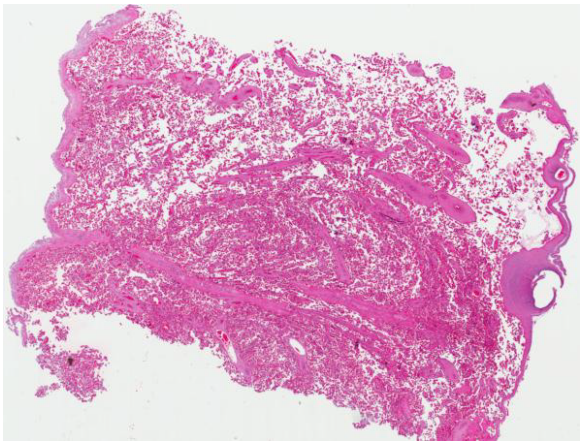
To find an optimal vector, $\mathbf{z}$ of parameters $\mathbf{z} = (\alpha, \theta, t_{x_1}, t_{x_2})^T$ in order to register a target image T to the source image S, we minimize the following energy functional,[4]

$$\mathcal{E}(\mathbf{x}, \alpha, \theta, t_{x_1}, t_{x_2}) \equiv \mathcal{E}(\mathbf{x}, \mathbf{z}) := \int_\Omega |S(\mathbf{x}) - T(R(\mathbf{x}))|^2 \, d\mathbf{x} \tag{1b}$$

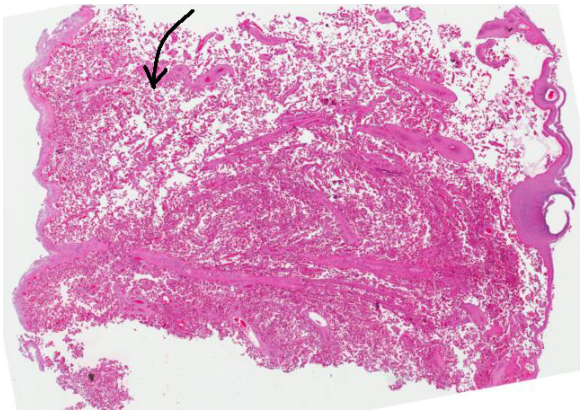
over all affine transformations R. This can be done using Newton's method which takes the form of the following iteration

$$\mathbf{z}_{k+1} = \mathbf{z}_k + (\nabla^2_{\mathbf{z}} \mathcal{E}_k)^{-1} \nabla_{\mathbf{z}} \mathcal{E}_k. \tag{1c}$$

Here we face another problem, the fact that the images are extremely large. Their size affects the optimization speed. Thus we opt for a multilevel approach in the registration, in the sense that we first downsample the given image by a factor of 2^n. We then perform the minimization (1) to obtain the parameter vector $\mathbf{z}^{(n)} = (\alpha^{(n)}, \theta^{(n)}, t_{x_1}^{(n)}, t_{x_2}^{(n)})^T = \text{arginf}_{\mathbf{z}}\, \mathcal{E}(\mathbf{x}, \mathbf{z})$. Then we perform the minimization with the downsampling factor 2^{n-1}, using $\mathbf{z}_0^{(n-1)} = (\alpha^{(n)}, \theta^{(n)}, 2\, t_{x_1}^{(n)}, 2\, t_{x_2}^{(n)})^T$ as the initial guess. We can then repeat the process till we reach the full resolution image. This process produces a sequence of globally registered H&E images. The result of this registration is depicted in Figure 1.



(a)



(b)

Figure 1. (a) The second image in the sequence, (b) The second image after the affine registration.

2.2 Manual tracing of fetal stem and local parametric registration

The next step is the segmentation of the fetal stem in the sequence obtained from the previous step. We opt for a semiautomated process to do this, where we use one H&E slide where a blood vessel is traced manually. This tracing is done after the global parametric registration. This tracing need not be very accurate, see Figure 2(b). We use this tracing for two purposes. Firstly, it gives us an approximate location and size of the blood vessel in the next image. This is possible due to the global registration. Secondly, the manual tracing will later serve as an initial guess for the active contours model without edges.[1,2]

Now that we have an approximate location and size of the blood vessel that we intend to follow, we extract two smaller regions of interest from the two consecutive slides to be registered. At this point due to the movement of the blood vessels, the locations of the blood vessels do not match. This can be corrected by performing an

affine registration. As the sizes of these regions are much smaller, we need not use a multilevel approach to achieve this. We note that in this step the size of the blood vessels is distorted, but this is later reversed as we know the parameters of this registration.

2.3 Local diffusion registration

Even after the affine rigid registration, the two blood vessels are not likely to match completely. This is due to the non-linear change of shapes of the tissues from one slide to the other. In order to follow the blood vessels, we need the blood vessels from two consecutive slides to match as precisely as possible. To this effect we should allow for nonlinear change in shapes. In non-parametric registration we are looking for a transformation of the target $T_{\mathbf{u}}$, where $T_{\mathbf{u}}(\mathbf{x}) := T(\mathbf{x} - \mathbf{u}(\mathbf{x}))$. The problem here is to look for an optimal flow $\mathbf{u}(\mathbf{x}) = (u_1(\mathbf{x}), u_2(\mathbf{x}))$ such that the warped target image $T_{\mathbf{u}}$ is close to the source image S with respect to some measure, i.e. we need to minimize $\mathcal{D}(S, T_{\mathbf{u}})$ over all $\mathbf{u}$. A direct minimization of the distance has some drawbacks: the problem is ill-posed since small changes of the input data may lead to large changes of the output data. The solution is not unique since the problem is not convex and the deformation may not be continuous. This leads us to use a regularizing term $\mathcal{R}(\mathbf{u})$. We then look for an optimal flow $\mathbf{u}$ that solves the following minimization problem

$$\inf_{\mathbf{u}} \alpha\mathcal{R}(\mathbf{u}) + \mathcal{D}(S, T_{\mathbf{u}}). \tag{2}$$

In the current application we use L^2 distance measure, i.e. $\mathcal{D}(S, T_{\mathbf{u}}) = \|S - T_{\mathbf{u}}\|_{L^2(\Omega)}$. There are several ways[4]to formulate the regularizer $\mathcal{R}$. Some of them are linearized elastic potential, static elastic potential, diffusion potential, diffusion potential, biharmonic potential, etc. For our application we chose diffusion regularization $\mathcal{R}(u_i) := \|\nabla u_i\|_{L^2(\Omega)}$ introduced by Fischer et al.[3]

$$\inf_{u_i} \alpha\|\nabla u_i\|^2_{L^2(\Omega)} + \|S - T_{u_i}\|^2_{L^2(\Omega)} \tag{3}$$

The idea behind this regularizer is to privilege smooth deformations while minimizing oscillations of the components of the displacement. The Euler-Lagrange differential equations associated with this problem are

$$\alpha\triangle u_i + (T_{u_i} - S)\nabla T_{u_i} = 0. \tag{4}$$

These can be discretized and solved using the steady state of the corresponding time dependent differential equations

$$\frac{\partial u_i}{\partial t} = \alpha\triangle u_i + (T_{u_i} - S)\nabla T_{u_i}. \tag{5}$$

2.4 Dynamic active contours without edges

After the registration we transfer the manually traced curve from the source image onto the transformed regions of interest in target image. This curve $\mathcal{C}_0$ should be close to the tracing around the corresponding blood vessel in the transformed target image. We use this curve as an initial curve for the active contours without edges model,[1,2] where we solve the following minimization problem for the transformed target image T_0:

$$\inf_{c_1,c_2,\mathcal{C}} \mu\,\mathrm{Length}(\mathcal{C}) + \lambda_1 \int_{inside(\mathcal{C})} |T_0(\mathbf{x}) - c_1|^2\, d\mathbf{x} + \lambda_2 \int_{outside(\mathcal{C})} |T_0(\mathbf{x}) - c_2|^2\, d\mathbf{x}.$$

Here, $\mathcal{C}$ is the unknown curve, c_1 and c_2 are unknown average constants of the image inside and outside the curve respectively.

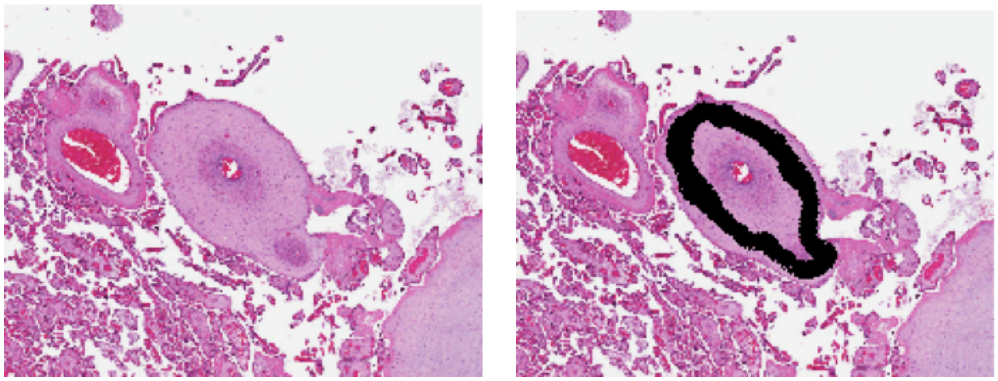
We know that the segmentation should give a unique stem. Thus, coefficients of the fitting terms, i.e. μ, λ_1 and λ_2 are computed iteratively to ensure that we obtain a unique 'stem' in the segmentation. Thus we get a contour $\mathcal{C}$ that gives a reasonable segmentation of the fetal stem in the next consecutive image.

2.5 Transferring of the tracing

Now that we have the contour traced out in the target image, we then invert the non-affine diffusion map $\mathbf{u}$ to obtain a tracing of the blood vessel which is locally registered using non-rigid registration. Then we use the local affine deformation map to transfer this contour onto the local region of interest of original target image and onto the entire target image consequently. This process now can be repeated where we can use the target image and the resultant contour $\mathcal{C}$ to obtain segmentation in the next slide in the histology sequence.

3. NUMERICAL RESULTS

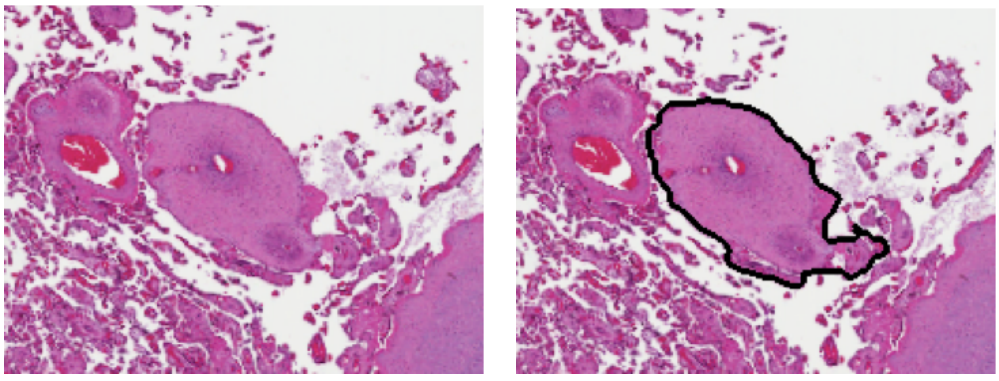
In our paper we approach the problem of tracing of the blood vessels in multiple stages. We use parametric registration, non-local registration and Chan-Vese segmentation in combination to segment the blood vessels. Due to the dynamic computation of coefficients in the Chan-Vese segmentation[1,2] our method succeeds even if the initial curve is inaccurate. In Figure 2 we show the details of the initial tracing in the source image.



(a) (b)

Figure 2. (a) Details of the blood vessel in the source image, (b) Initial manual tracing of the blood vessel in the source image.

In Figure 3 we see that the blood vessel is accurately segmented, even though the tracing in the initial image is not accurate. Furthermore, the target image used here is not even the consecutive image, it is in fact the fifth image in the sequence.



(a) (b)

Figure 3. (a) Details of the blood vessel in the target image, (b) Result of the segmentation in the target image.

3.1 Conclusion

The proposed algorithm was devised as a first step in the tracing of fetal stems in the sequence of placental H&E slides. It uses manual tracing in one of the images as a starting point and then tries to follow the shape in the consecutive images. The significance of this method lies in the fact that it can be used to trace any other tissue type in the human body as the identification of the fetal stem itself is not done automatically, making this method very adaptable for many other types of H&E slides. In the future we would like to incorporate the branching of the blood vessels. In the current version, manual intervention is needed to take the branching into account. In the H&E sequence used here, most of the maternal blood was drained out. The algorithm makes use of this fact in the segmentation step. This can not be guaranteed in general and the H&E protocol needs to take this into consideration.

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