Examining Phenotype Differences in Mouse Placenta with Volume Rendering and Segmentation

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***Abstract*— We present an application problem of examining phenotype differences in wildtype and retinoblastoma (Rb) knockout specimens of mouse placenta. The lack of the Rb gene causes uncontrolled tissue growth which forces infiltrations into critical sections of mouse placenta that lead to fetal death.**

We briefly describe our method for volume visualization of mouse placenta tissue level intermixing at a microscopic scale for both wildtype and Rb knockout types. Our technique combines non-trivial registration techniques, an N-point correlation classifier, and a volume rendering step. Our final volume renderings show tissue intermixing differences between both wildtype and Rb knockout specimens that are not obvious from examining the two dimensional image stack alone.

I. Introduction

In this paper we address the problem of visualizing datasets of histologically stained microscopy slides. In particular, mouse placenta with and without the retinoblastoma gene (Rb) activated. This gene is one of the first to be associated with a specific cancer (retinoblastoma) and has been studied extensively in both human and mouse cell models [1].

Mouse placenta are routinely used in these studies and are composed of three distinct layers: the *labyrinth*, *spongiotrophoblast*, and *glycogen* layers. Figure 1 shows a sample slide with the approximate labyrinth, glycogen, and spongiotrophoblast layers marked in red, yellow, and blue, respectively.

Recently, it has been shown that inactivation of the Rb gene (Rb^-) in a mouse embryo results in morphological changes in the placenta, including reduction in vascularity of the labyrinth layer. It is postulated that a decrease in vascularity contributes to fetal death at 13.5 days of gestation [3]. Cancer geneticists have immense interest in studying the three-dimensional structural changes that occur by the inactivation of the (Rb^-) gene. More specifically, the infiltrations of the spongiotrophoblast layers into the labyrinth are of great interest. These infiltrations reduce the surface area of the labyrinth resulting in fetal death. Our cancer geneticist collaborators are interested in viewing the infiltrations in their complete three-dimensional form.

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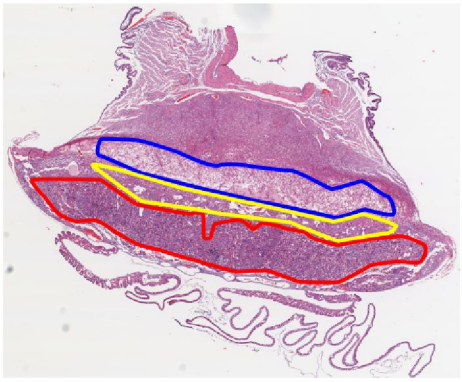

Fig. 1. An example of one of the Rb^- histological mouse placenta slides. The red, yellow, and blue markings show the approximate locations of the labyrinth, glycogen, and spongiotrophoblast layers, respectively. Tissues not marked are considered "maternal tissue".

Figure 2 shows a sample volume render of the cropped data with opacity derived off the luminance of the slides.

Our goal is to reconstruct and visualize the three-dimensional intermixing of tissue types of specimens given their sectioned sets of RGB histology slides. This problem is similar to that which radiologists experienced a decade ago in visualizing CT and MR data. However, unlike CT and MR images, our data is not co-registered, is difficult to segment, and requires the highlighting of sub-tissue level features in 3D.

II. Method

The overall technical steps for assessing the volumetric changes of various tissue types in both wildtype and knockout Rb^- mouse placenta are as follows:

1) Segment each image into regions corresponding to the three tissue types.
2) Register the serial sections of the given placenta to reconstruct a virtual placenta.
3) Build three-dimensional models of each of the tissue layers by aligning the segmented images using the information collected during the registration phase.
4) Volume render the segmented volume.

We discuss these steps in some detail below.

A. Segmentation

Our segmentation approach is based on N-point correlation functions and the lineal path which was originally introduced in the material science literature [2]. Essentially, an N-point correlation technique calculates the probability that any N points placed in the material randomly fall into two or more categories. This type of measurement gives a better measure of the distribution of heterogeneous material than the relative densities would.

B. Registration

Our registration technique follows four steps. First, defective placenta slices (such as tissues that are torn or folded) are identified and manually removed from the dataset. Due to the large number of slices in the dataset, missing slides are not noticeable in the final visualization. Secondly, we detect the tissue region in the original slide so that our registration step is not confused by background artifacts. In the third step we perform an initial alignment of the placenta slices for the final registration. We chose to use principal component analysis (PCA) based alignment since mouse placenta sections are typically elongated in shape (high width to height ratio). In the final step we transform a designated moving image slide, which is resampled onto a grid, compute a mutual information-based metric on the quality of fit and then if similar to a prior value, we stop the iterative process, else we refine the transform and start again.

C. Volume Rendering

The implication for volume rendering is that smooth three-dimensional boundaries cannot exist in the placenta data. Thus, diffuse and specular lighting across the rough three-dimensional probability surface only confuses the rendered images, rather than ascribing scope and depth. To address this issue we chose to volume render the data as an emissive volume only, thus smoothing the boundaries while still allowing the viewer to perceive depth in the three-dimensional tissue level regions. To enhance the emissive volume rendering we have also developed shape based transfer function techniques based on level sets to highlight infiltrations and extrusions from the low frequency boundary.

We take the results of the classification and map each pixel to a scalar value based on the class it belongs to. We assemble the volume by stacking the registered scalar mapped classifications onto each other, then treating the resulting data as a three-dimensional volume. Finally, we load the volume into Kitware's VolView and select transfer functions to highlight desired classes. Note that we are essentially rendering the segmentations of the volume, rather than the raw data directly.

III. Results and Discussion

In Figure 2 we show a full mouse placenta and a cropped version which shows the entire stack of images once they have been registered. Figure 3 shows a comparison between

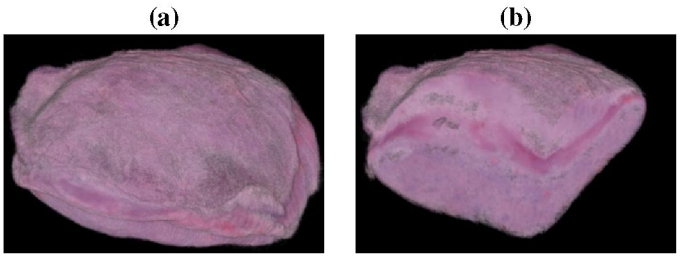

Fig. 2. **(a)** Sample volume rendering of a wildtype mouse placenta, original size is approximately 1mm^3. **(b)** The volume has been cropped so the internal structure of the placenta is exposed. The tissue specific structures (labyrinth/spongiotrophoblast mixing) are difficult to see in this view.

a knockout Rb^- and wildtype placenta. The physiological differences between the placentas are noticeable as the intermixing in the labyrinth is highlighted from blue to yellow. We observe that the wildtype labyrinth layer's intermixing layer is mostly on the outer surface of the layer, while for the Rb^- placentas intermixing occurs more freely throughout the tissue.

The changes in the phenotype are consistent with our collaborators hypothesis that the absence of the retinoblastoma gene causes uncontrolled cell duplication in the placenta thus roughening the spongiotrophoblast-labyrinth interface. Aside from identifying these phenotype changes, a full three dimensional visualization has provided our collaborators with key insights to the overall spatial organization of the spongiotrophoblast-labyrinth interface.

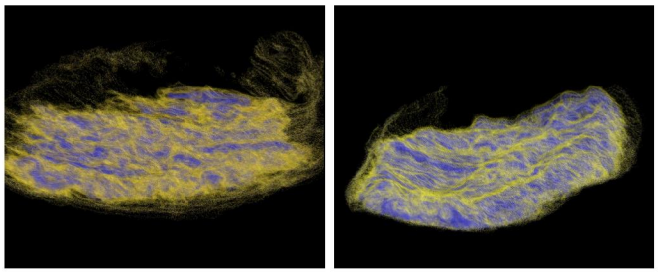

Fig. 3. This figure highlights the difference between Rb^- placenta (left), and wildtype (right). The images show the intermixing of labyrinth and spongiotrophoblast layers by increasing in opacity and coloring yellow where the labyrinth label transitions into any other tissue type. The labyrinth layer itself is colored in blue.

References

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