Image analysis of the vessels remodelling during embryos development

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Introduction

The cardiovascular system is the first organ system to develop and reach a functional state in the mammalian embryo. This reflects the crucial need for a vascular supply for the delivery of nutrients and the disposal of catabolic products from normal tissues. The blood vessels that initially comprise this organ originate by vasculogenesis, the aggregation of de novo-forming angioblasts (endothelial precursors) into simple endothelial tubes. Angioblasts first emerge from the mesoderm and assemble a simple circulatory loop consisting of a heart, dorsal aorta, yolk sac plexus and sinus venosus. Then, the development of the embryonic vasculature involves the remodeling of a primitive capillary plexus into a more organized, highly branched vessel network (Risau and Flamme, 1995). Given the complex nature of the vascular system and the diversity of biological processes required for its assembly and refinement, it is hardly surprising that a large number of signalling pathways are employed in its development. Mutations in pathways required for vascular development frequently manifest phenotypes that result in embryonic lethality at mid gestation. The vascular activities of these pathways are not limited to this developmental time window, but extend to organogenesis, maintenance of vascular homeostasis in adulthood and states of pathological angiogenesis . Correct interpretation of how these pathways regulate vascular development between would therefore improve our understanding of how they contribute to later vascularization events. However, due to the complex nature of the vascular phenotypes, we have an incomplete understanding of the normal sequence of vascular remodelling events that occur during the development. Here, we focus on the role of the semaphorins cues in the early vasculature process (Kruger et a. 2005). In particular, we develop a suitable image analysis tool in order to detect and classify vasculature impairs due to the the inhibition of semaphorin 3A in mice embryo starting from confocal microscopy images. In Fugure 1 we show two examples of the vasculature

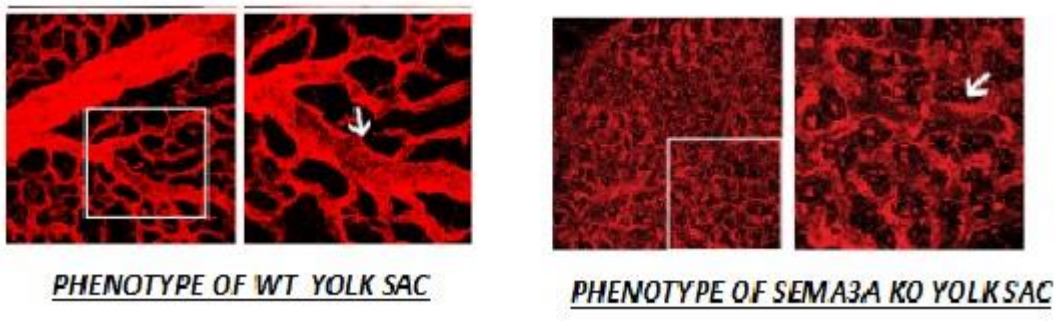

Figura 1 Different Vasculature for different Phenotypes

for different phenotype. In the wild type (WT) phenotype we can observe a normal vasculature size, dense capillary plexus, large collection vessels, and the vitelline artery is well visible. Instead in the case of mice with Semaphorine 3A knock out (KO) it appears a lack of the large collecting vessels, irregular and not remodeled vascular network, and the vitelline artery is not visible.

Materials and Methods.

The main objective of this work is to study the outcome images of the semaphorin 3A and 3F inhibition experiments in order to develop an automated analysis method. In particular, as a first step, we focus on the images of the heads of the mice at week 9.5, studying only the inhibition of semaphorin 3A, and to find a way to quantify the hierarchical structure apparent in the WT healthy samples and absent in the KO treated ones. Two criteria that have been proven to be useful for a human-made identification of this hierarchical structure are:

- long, thick and almost aligned branches near the center of the head, with long and narrow holes in between;
- more fine-grained network in the external area, with thinner vessels and well-defined holes.

In order to to analyze these features, we consider the shape and the distribution of the 'holes' formed by the vascular network.

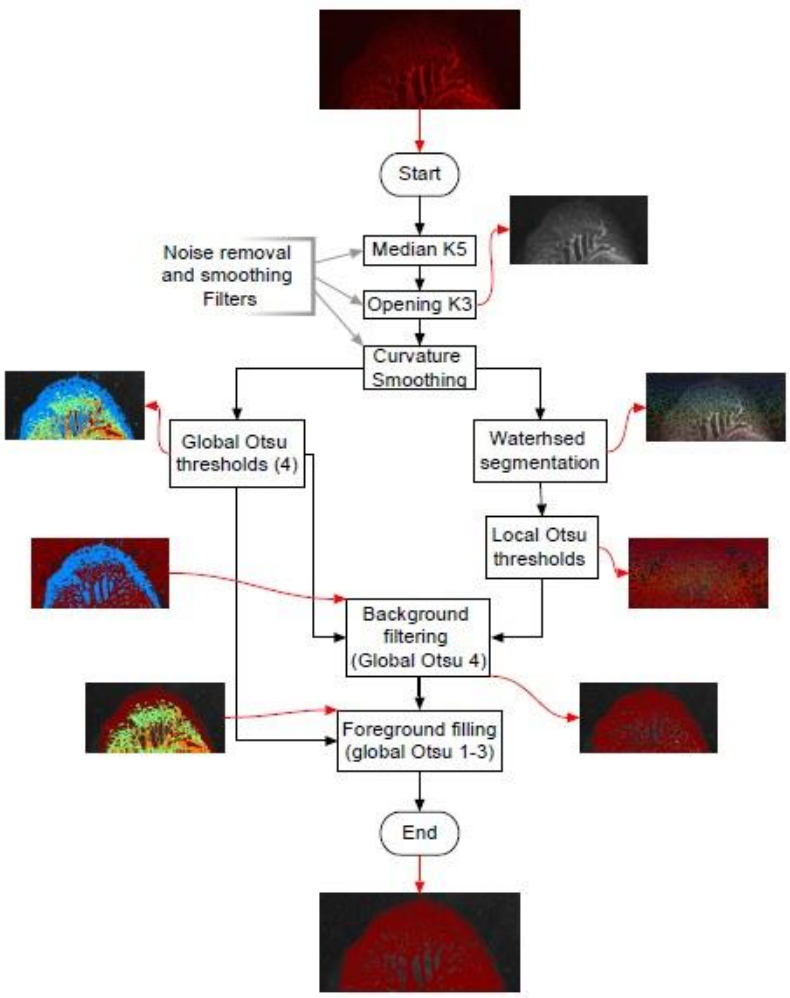

Figura 2 Flow chart of the binarization-segmentation algorithm.

The high variance in the intensity of the image makes it impossible to find a global background/foreground threshold to identify the whole topology of the vascular network. Moreover, the noise and the local characteristics of the image are such that it is difficult to find a general method to identify suitable local thresholds. In fact, both the bigger vases and the background of the image show strong irregularities, that cause many standard methods to incorrectly identify holes where only darker portion of vases are, and portions of vases in place of background noise (see Ross et al. 2001). A custom combination of a global and a local approach has then been found to obtain a binarization of the image, segmenting it in what can be considered foreground, i.e. the vascular network, and background. Then, we We have developed an algorithm which is briefly described in Figure 2.

Results and Discussion

Three features and the distributions of the holes have been studied:

- size of the hole, intended as number of pixels;
- Elongation and alignment with the vertical axes, calculated as the ratio between the vertical and the horizontal size of the enclosing rectangle with sides parallel to the Cartesian axes;
- Approximate Distance from the center of the head, calculated from the nearest corner of the same rectangle.

These measures have some limitations, as for some precision issue on the distance calculation, but they already showed good preliminary results without further refinements. One example is reported in the Figure 3 with the application of the method for a healthy and a knockouts mice at the week 9.5 E. The example involves only the distribution of the size of the holes with respect to the distance.

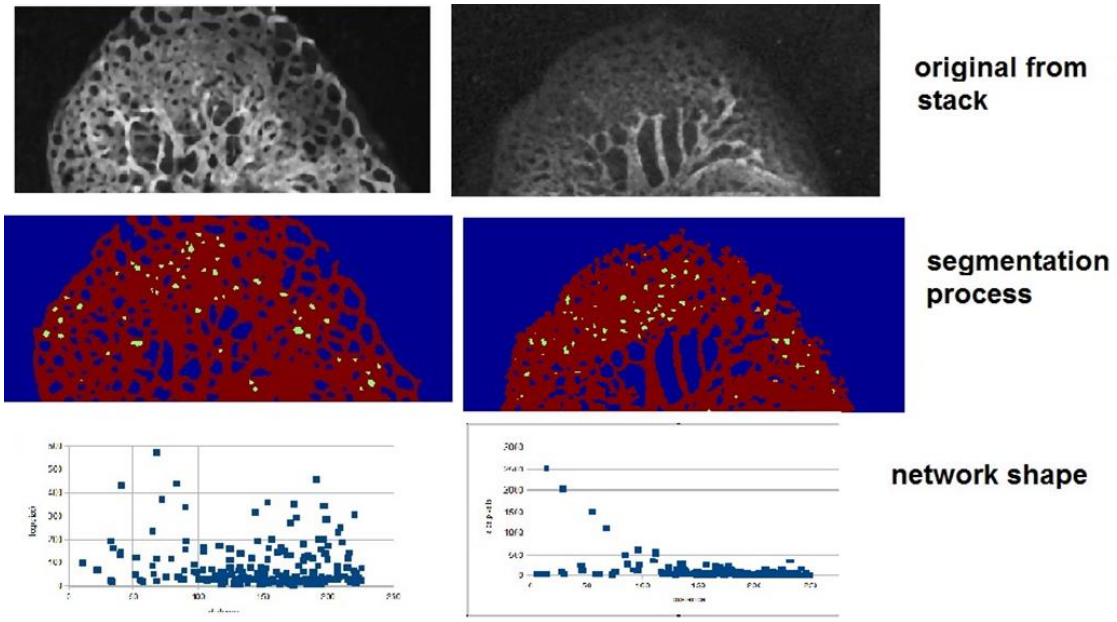

Figura 3 A comparison of the size wrt the distance distribution between two different phenotypes

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