Topological Analysis of the Vasculature of Angiopoeitin-Expressing Tumours through Scale-Space Tracing

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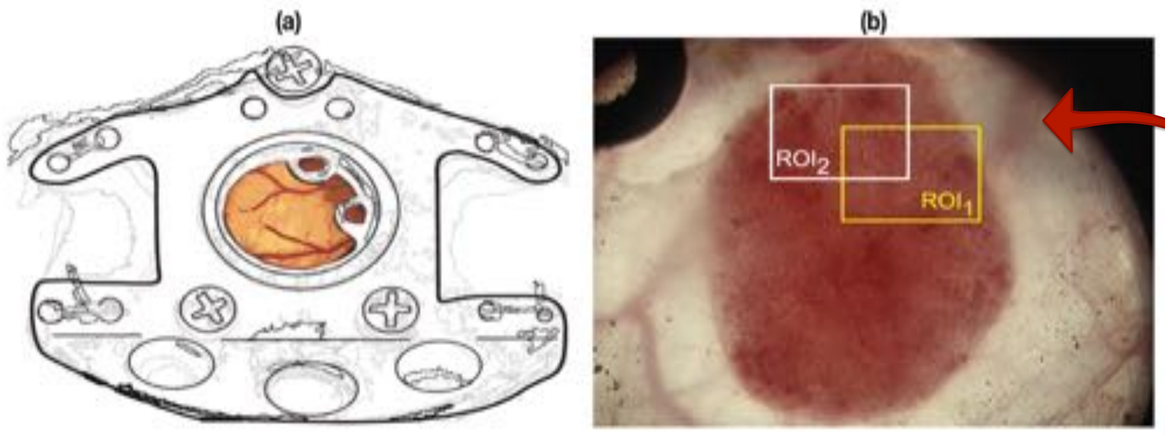


(a) Illustration of a dorsal skin-fold window chamber, which is implanted in the dorsal skin of a mouse and through which the vasculature of a tumour (centre) can be observed over time. (b) One representative image of a tumour acquired with a ×2.5 microscope objective. Red colour corresponds to the vessels that supply blood to the tumour. Lighter regions are tissue into which the tumour has not spread. The size of the regions of interest to be captured with a ×10 microscope objective is illustrated with boxes.

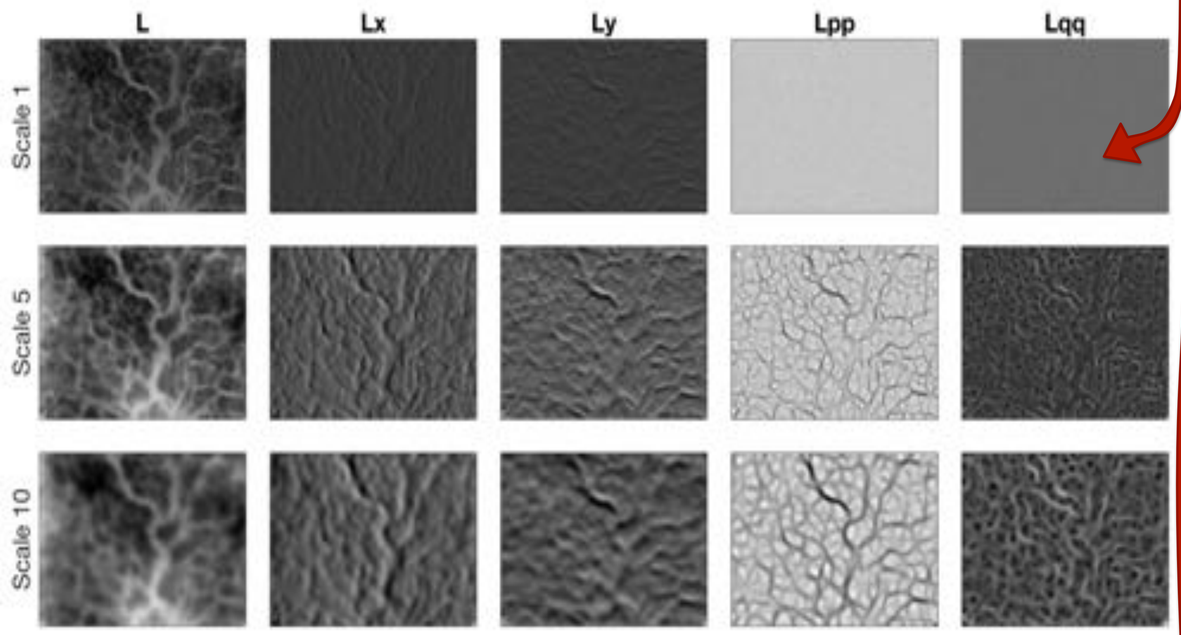
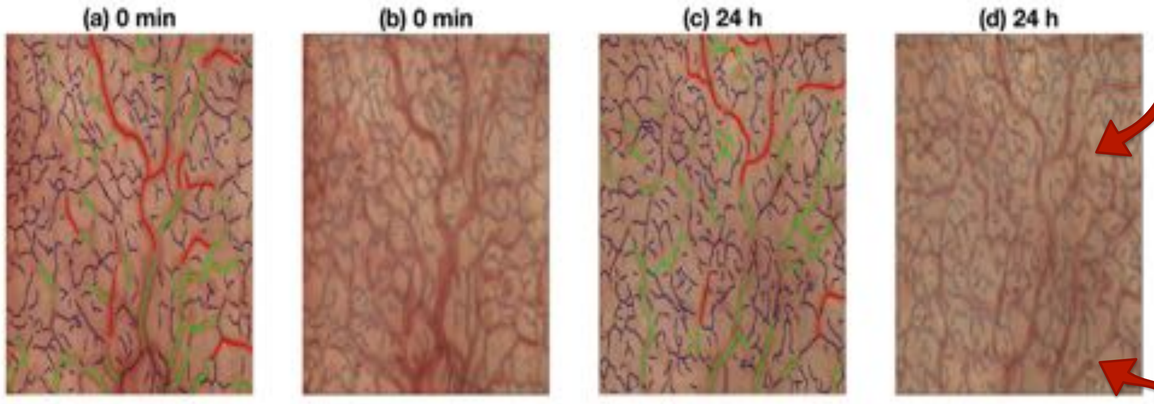


Illustration of a Scale-Space process where the intensity of an image is filtered at different scales (vertical) and derivatives in different directions highlight features at each scale.



(a,c) Traced vessels overlaid on two time points from a single tumour. The vessels have been ranked and the top 10 are traced in red, 11–50 are traced in light green and the rest in thinner black lines. Notice the changes of the vascular topology over time. (b,d) Traced vessels and the inferred thickness of each vessel denoted by dark shading. A large number of measurements can be obtained from the traced vessels, but for this work we concentrated on the following:

1) number of vessels, 2) average vessel length,
3) average vessel diameter, and 4) relative area covered by vessels.

The length and number are direct measurements. The diameter is estimated as proportional to the scale where the ridge was selected. The area is derived from the combination of diameter and the length of each traced vessel. The relative area is the ratio of the area previously calculated over the total area (shaded pixels in b,d) of the tumour tissue in the ROI. In addition, it is possible to rank the vessels by their saliency, i.e. the contrast between the vessel itself and the surrounding regions.

Abstract

This work describes:

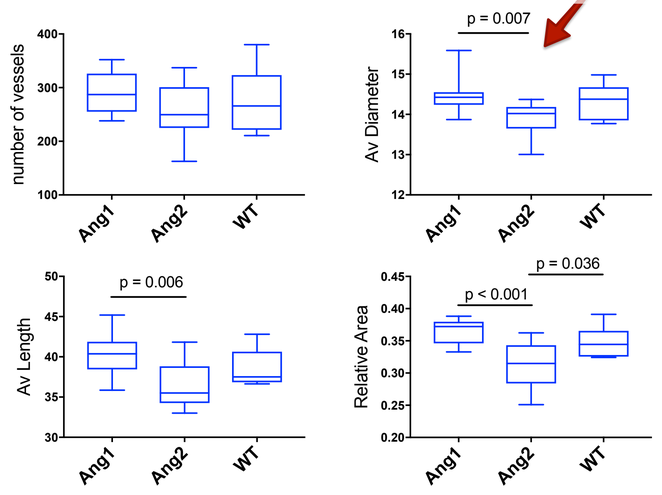
- Topological analysis of **the vasculature of tumours**.
- Analysis is performed with **a scale-space technique**,
- Scale-space **traces vessels as topological ridges** of the image intensities
- A **series of measurements** (length, width, density, etc.) are obtained, which are used to **compare the vasculatures**.

Tumours of **SW1222 human colorectal carcinoma xenografts** were observed when growing **in dorsal skin-fold window chambers in mice**.
Three variants of the tumours expressing either

- **endogenous levels** of **angiopoeitins** (WT) or
- **over-expressing** either **angiopoietin-1** (Ang-1) or
- **angiopoietin-2** (Ang-2)

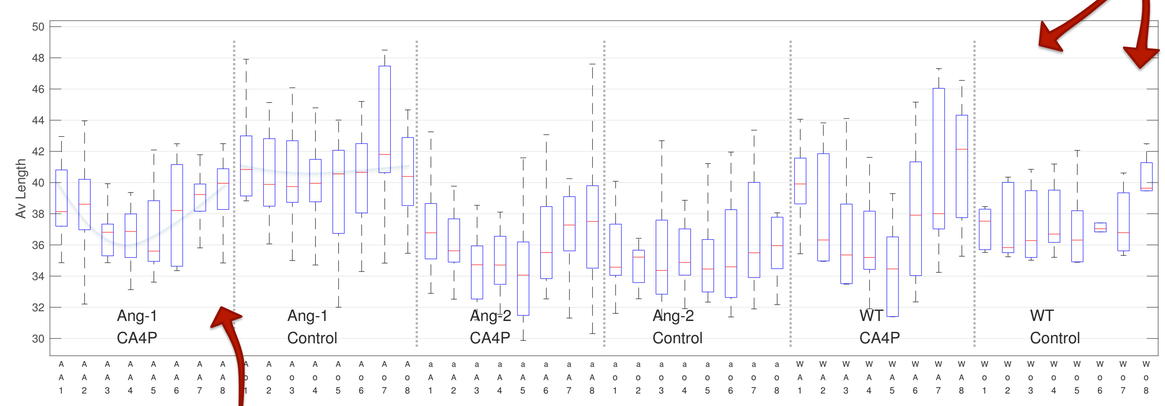
were assessed **with/without vascular targeted therapy** (combretastatin A4 phosphate (CA4P; fosbretabulin)).

The measurements reported **statistically significant differences between the three tumour types** thus confirming the **topological analysis as a suitable technique to analyse changes in vasculature**.

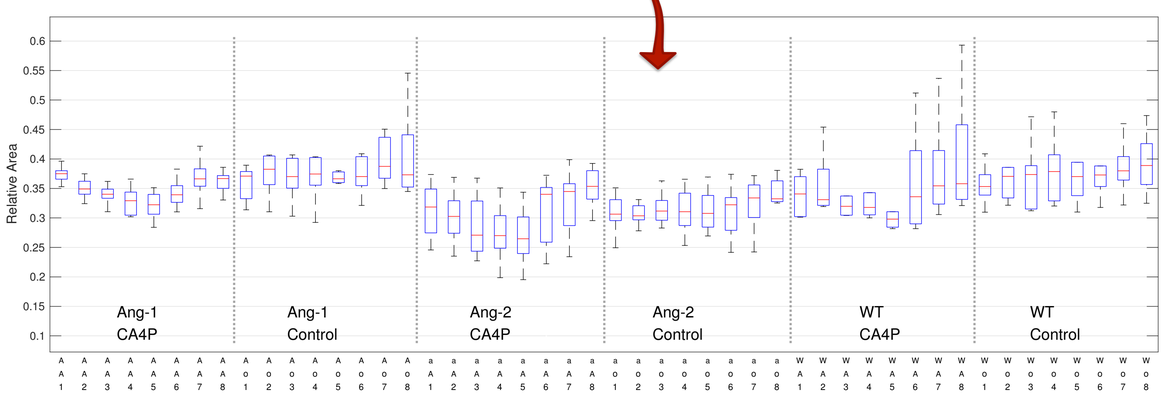


Boxplots summarising the statistical difference between the three tumour types at time t = 0, before the start of treatment. The *p* –values for 1-way ANOVA followed by a Tukey post-test are included within the figures. There was a statistical difference between Ang-1 and Ang-2 for the diameter, length and relative areas, and between Ang-2 and WT for relative area only.

Moreover, the measurements obtained confirmed the **effects of angiopoietins** as previously reported in the literature.



Boxplots of the *Length* [pixels] *and Relative Area* as determined by the tumour type Ang-1, Ang-2 and WT (A/a/W), treatment for CA4P or control (A/o) and the time of acquisition (1–8).



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