## In vivo high resolution imaging of the mouse neurovasculature

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**Introduction:** Accurate and precise imaging of neurovasculature is critical to many pre-clinical research areas. Inter alia, 1) specific vascular characteristics relate to pathologies ranging from Alzheimer's disease to tumors and brain metastases; 2) fMRI signal is strongly coupled to the vascular network and 3) development of novel therapies in the brain can benefit from the characterisation of the neurovasculature. Pathak et al. [1] already proposed a method for "multi-scale" imaging of the mouse neurovasculature: *in vivo* MRI at  $0.1 \times 0.1 \times 1$  mm<sup>3</sup>, ex *vivo* MRI at resolution of  $62 \times 62 \times 63$   $\mu$ m<sup>3</sup> and  $\mu$ CT at  $8 \times 8 \times 8$   $\mu$ m<sup>3</sup>. Here, we present a similar method for *in vivo* 3D MR imaging of the mouse neurovasculature, at the resolution of  $78 \times 78 \times 104$   $\mu$ m<sup>3</sup> for both *in vivo* and *ex vivo* scans, which enables a direct comparison of vessel sizes between data sets.

Methods: Imaging: All MR experiments were conducted on a 210-mm small animal 7T scanner (Varian Inc., Palo Alto, CA) with a dedicated mouse head-coil (RAPID MR International, OH). The *in vivo* MR imaging of healthy BALB/c mice was performed under isoflurane anesthesia with a T<sub>2</sub>\* weighted sequence (TR=50 ms, TE=25 ms, flip angle=15°, data matrix=256x256x96, field of view = 20x20x10 mm³, 2 averages) subsequent to an injection of Resovist (Bayer; injection through tail vein of 200 μl @ 200 μl/min with Fe concentration adjusted to 0.6 mM). Image acquisition started one minute after the end of the injection to let the compound equilibrate in the blood. For *ex vivo* imaging, mice were sacrificed and perfused with Microfil (Flowtech Inc, MA) using the same protocol as Pathak et al. [1]. Microfil is a silicone rubber compound that does not provide any signal in conventional MRI and which is radio-opaque. Brains were afterwards extracted, fixed in paraformaldehyde and finally placed into 1% (w/v) agarose gel for imaging. For the purpose of the comparison between *ex vivo* and *in vivo* vasculature images, a set of *ex vivo* MR images was acquired with the same parameters as for the *in vivo* images, except for the TE which was shortened to 12.51 ms to reduce T<sub>2</sub>\* artifacts, and for the number of averages which was set to 8. We also imaged the *ex vivo* brains with MR at a higher resolution and with μCT at an isotropic 5 μm resolution. Image analysis: For MR images, brains were segmented using the Brain Extraction Tool from FSL [2]. A multi-scale vesselness filter based on the Hessian matrix was applied and thresholded to 0.15 to extract the vascular network [3]. Vessels radius was determined locally using Fiji [4] local thickness algorithm. A mouse atlas [5] and the *in vivo* data set were rigidly registered to the *ex vivo* data set. Vessels size distributions and statistics were calculated for three regions which appeared to contain a significant number of vessels: the cortex, the thalamus and the caudoputamen.

**Results**: Figure 1 shows a 3D rendering of the vascular network imaged *in vivo* with the proposed method against the same rendering for the network imaged *ex vivo*. Figure 2 shows the vessel size histogram found for the *in vivo* and *ex vivo* vasculature in various areas of the brain for the mouse of Figure 1. Table 1 shows the average vessel density (the fraction of the volume occupied by blood vessels).

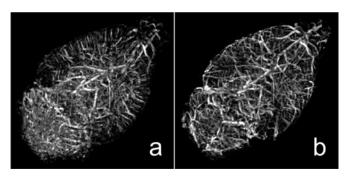


Figure 1- 3D view of the neurovasculature after thresholding the vesselness filter for the same mouse a) in vivo and b) ex vivo.

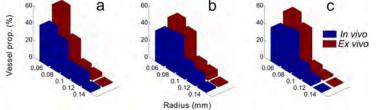


Figure 2 - Vessels radius histogram for the mouse brain of Fig. 1 for a) the cortex, b) the thalamus and c) the caudoputamen in and ex vivo.

	Vessel density (%)		Average vessel size (mm)	
	In vivo	Ex vivo	In vivo	Ex vivo
Cortex	21	12	0.16 ± 0.05	0.14 ± 0.04
Thalamus	25	21	0.16 ± 0.04	$0.15 \pm 0.04$
Caudoputamen	23	18	0.15 ± 0.03	0.14 ± 0.03

Table 1 - Vessel density and average size (mean ± std) in all three studied brain areas, for *in vivo* and *ex vivo* scans.

**Discussion:** From Figure 1, it is apparent that the proposed method allows detecting a large number of small vessels *in vivo* in the mouse brain. A noticeable point is that vessels at the brain surface are much

more apparent on the *ex vivo* scan. This is due to the fact that vessels appeared black on the original images, yielding no contrast with the skull *in vivo*, but high contrast with the *ex vivo* agarose gel. Figure 2 shows differences in the vessel distribution between *in vivo* and *ex vivo* images. Smaller vessels are more easily detected in the *ex vivo* image. Similarly, Table 1 shows that the radii and the vessel density are overestimated *in vivo* as compared to *ex vivo*. This could be explained because 1) the Resovist contrast agent effect extends beyond its physical location, thus probably yielding an overestimation of the vessels radii *in vivo* and 2) the absence of motion as well as the higher number of averages for *ex vivo* scans may allow the detection of smaller vessels..

**Conclusion:** The proposed method allows the detection of multiple small vessels (radius < 100  $\mu$ m) *in vivo*. This can be useful for many applications such as detecting vessels anomalies. As shown with comparison against *ex vivo* data, the method should be used with care to quantify vessels characteristics as it overestimates their radius. Further validation of the method with  $\mu$ CT and higher resolution MR are underway.

## References:

- 1. Pathak AP, Jones M, Zhang J (2008), Proc. Int. Soc. Magn. Reson. Med. 16. Toronto, p 103175
- 2. Smith SM (2002). Hum Brain Mapp 17:143–55.
- 3. Frangi A, Niessen W (1998) Lect Notes Comput Sci Lect Notes Med Image Comput Comput Interv MICCAl'98 1496:130–137.
- 4. Schindelin J, Arganda-Carreras I, Frise E, et al. (2012), Nat Methods 9:676–82.
- 5. MacKenzie-Graham A, Lee E-F, Dinov ID, et al. (2004). J Anat 204:93–102.