# Blood volume fraction measurements using MRI: a correlation between two-photon and in silico MR estimates

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## Introduction

Several MR methods allow quantification of microvascular characteristics such as blood volume, vessel size index **[1]** or blood oxygenation level **[2]**. Although these methods have shown good correlation with other techniques, the absolute quantification requires further investigation **[3].** Recently, it has been shown that MR signal from complex microvascular networks could be obtained in silico **[4]**. In the present study, brain cortical microvascular networks are digitized using a two-photon microscope. Blood volume fraction (BVf) is then obtained in two ways. First, BVf is quantified from the microscopic data using morphologic tools. Second, a steady-state MR protocol designed for measuring BVf **[1]** is performed in silico, using a MR simulation approach recently published **[4-5]**. BVf estimates obtained with the two techniques are eventually compared.

## Material and methods

*Animal preparation*: Mice (n=11) were anesthetized with a mixture of xylazine/ketamine and placed on a stereotactic frame. A craniotomy of 3mm in diameter was carried out above the left parietal cortex. The bone was removed and the exposed cerebral cortex was protected by an agarose gel. A 70kD fluorescein-dextran solution (Sigma-Aldrich) was then injected in the tail vein of the mouse in order to highlight all cortical vessels.

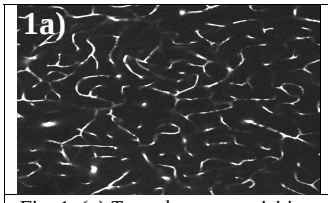
*In vivo two photon microscopy*: two-photon laser scanning microscopy was performed with a confocal microscope consisting of a Biorad (MRC 1024) scanhead and an Olympus BX50WI microscope. The beam scanned the x-y plane to acquire a 512*512 image (0.9s/image). The z-scan (variation of the observation depth) was realized by vertical motion of the motorized objective. The z-step between scans was 5µm. The final field of view was 600*600*300 µm, in the range of standard MR voxel sizes.

*Preprocessing of two-photon microscopy data:* images were analyzed in the Matlab environment and using home-made software. A Gaussian filter was first applied to remove isolated pixels and morphological treatments (erosion / dilatation) were used to fill holes in the vessels. A semi automatic 3D threshold was used for image segmentation (blood/tissue).

*Estimation of BVf (*BVf,2hµ*) using morphological tools:* BVf,2hµ was computed as the ratio between the number of pixels in a vessel and the total number of pixels.

*Estimation of BVf (*BVf,MR*) using a simulation of a steady-state MR approach*: A diffusion coefficient ($D=10^{-9}m^2s^{-1}$) and a magnetic susceptibility – that of blood or of tissue – was associated to each point of the preprocessed two-photon microscopy data. The magnetic field distribution was computed at 4.7T using a Fourier based approach **[4]**. Relaxation and diffusion of water were accounted for using a deterministic approach **[5]**. The MR signal (i.e. the gradient echo transverse magnetization as a function of echo time) is then computed. In absence of contrast agent, blood and tissue susceptibility were considered as equal. In presence of contrast agent, the blood susceptibility was 0.231 ppm. BVf,MR was computed using: $BV_{f,MR}=3/(4\pi)\ \Delta R_2^*/(\gamma\Delta\chi B_0)$, were $\Delta\chi$ represent the change in blood susceptibility induced by the contrast agent. The proposed simulation approach was initially validated using straight cylinders (blood volume fraction: 3%, vessel radius: 3µm, no preferential vessel orientation).

## Results



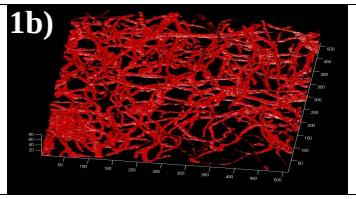




*Fig. 1. **(a)** Two-photon acquisition of a mouse brain cortex (1 slice) **(b)** 3D representation of one two-photon dataset (600*600*300µm).*

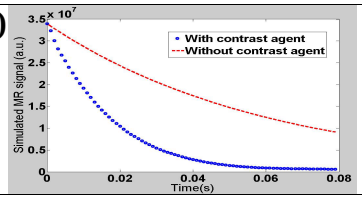




*Fig. 2. (a) Magnetic field estimation in presence of contrast agent (1 slice) (b). Simulated gradient echo signal from one sample (with & without contrast agent).*

| Samples | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BVf,2hµ (%) | **6.5** | **5.9** | **6.6** | **6.7** | **3.1** | **3.6** | **3.0** | **5.1** | **4.1** | **2.1** | **2.9** | **11.4** | **12.9** | **8.9** |
| BVf,MR(%) | **8.5** | **5.1** | **5.6** | **5.8** | **4.2** | **4.9** | **3.2** | **7.7** | **6.6** | **2.6** | **2.6** | **13.7** | **12.4** | **9.9** |
| Error (%) | **31.3** | **12.4** | **15.9** | **12.7** | **36.9** | **38.2** | **5.9** | **52.5** | **60.6** | **26.4** | **9.6** | **16.5** | **4.5** | **10.6** |

*Table 1. BVf estimates obtained using morphological tools and using the in-silico MR approach as well as the corresponding error= (BVf,2hµ-BVf,MR)/BVf,MR.*

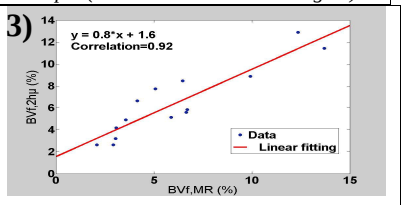



*Fig. 3. Correlation between BVf,MR and BVf,2hµ. $R^2=0.92$. $y=0.8x+1.6$.*

Using straight cylinders as a reference, the proposed simulation approach provides a BVf estimate with less than 2% error. Fig. 1 shows an example of two-photon data. Fig. 2. shows an example of the magnetic field distribution in a plane computed from the two-photon data (Fig. 2a) and corresponding gradient echo MR signals (Fig. 2b). BVf estimates using morphological tools and using the in-silico MR approach are given in Table 1. They show constant error (mean=23%) in the determination of BVf,MR. Fig. 3 shows the correlation ($R^2=0.92$) between the two modalities.

## Conclusion

This work suggests that MR quantification of BVf correlates with the corresponding physiological BVf. However our results shows a systematic error (20% on average) on absolute quantification, possibly induced by the approximations made in the model used in MR to represent the microvascular network. Further studies on restrictions and assumptions of microscovasculature model such as vessel density, vessel orientation or vessel shape are required to determine the origin of this systematic error.

## References

[1] I Troprès et al, *Magn Reson Med*, 2001. [2] X. He and D. A. Yablonskiy, *Magn Reson Med*, 2007 [3] S Valable et al, *NMR Biomed*, 2008. [4] J. P. Marques and R. W. Bowtell,.*NMR Biomed*, 2007. [5] L. M. Klassen and R. S. Menon, *Biophys J*, 2007.