New generation hybrid FMT/MRI system used to assess β-amyloid plaque load on APP23 mice in vivo

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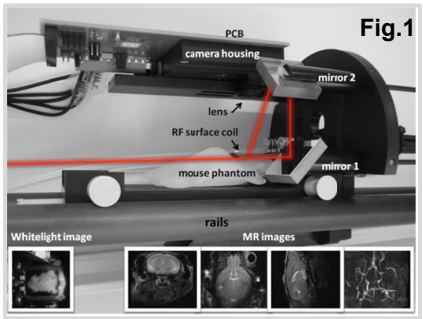
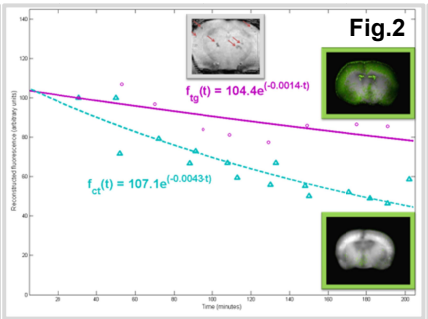
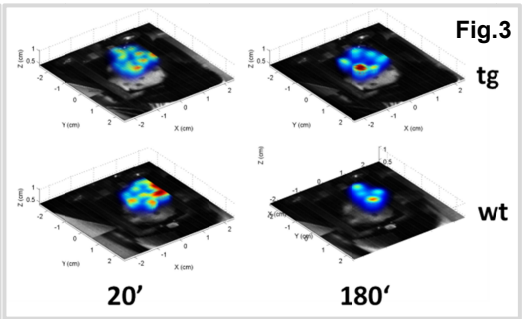
Introduction: Fluorescence molecular tomography (FMT) allows mapping of the spatial distribution of fluorescently labeled compounds. The principal limitation of FMT is photon scattering and attenuation by tissue, which limits light penetration to a few centimeters. FMT may be considered the optical analog of PET, providing quantitative molecular information with stable imaging agents at a relatively low cost. The combination of FMT with MRI has the potential to provide structural, physiological and metabolic information on a single platform for pre-clinical applications. Moreover, the structural information provided by MR can be used for improvement of FMT reconstructions. A first generation free-beam FMT/MRI using a avalanche diode array for photon detection has been described by our group and proof-of-principle has been demonstrated in a subcutaneous tumor model [1]. This system was limited by the small array size, FOV and low SNR of the camera. These issues have been addressed in a redesigned system, which was used for the first time in a neuroimaging study with the goal to estimate the β-amyloid plaque load in APP23 mice, a transgenic model of Alzheimer's disease. FMT data were collected using the dye AOI987, known to bind to aggregated β-amyloid *in vivo* [2]. The technical details of the system and the results of the *in vivo* FMT/MRI study are presented here.

Materials and Methods: **Description of FMT/MRI system:** The experimental setup consisted of an FMT excitation module located at the entrance of a Bruker 94/30 Biospec (Bruker BioSpin MRI, Ettlingen, Germany) small animal MR system operating at 400 MHz, and of an optical/MR sample detection module located in the magnet's isocenter. Fluorescence excitation consisted of a 670nm continuous wave laser (Coherent Inc., California, USA) generating a laser beam of 0.5mm diameter, which was focused on the sample with a numerical aperture-matched collimation lens (Thorlabs, Munich, Germany), an anti-reflectance coated spherical singlet lens (f=1000mm, Melles Griot, Bensheim, Germany) and two coated highly reflective front surface mirrors (Thorlabs, Munich, Germany). For scanning of the beam across the object's surface, a scan head (Scanlab, Puchheim, Germany) was used. The detection module (**Fig.1**) was designed to slide on two carbon-rod rails rigidly fixed at both ends of the magnet to ensure mechanical stability and reproducible positioning. It comprised a heated animal platform with anesthesia gas supply, a custom-made transceiver surface coil (20x24mm) for MR excitation/detection and a 256x256 CMOS detection array (CSEM, Switzerland). The detector was enclosed in a light-tight custom-made PPSU housing including a filter wheel, a mechanical filter switching mechanism and a miniature anti-reflection coated glass lens (V-4301, FL=2.1mm BFL=5.6mm, Marshall Electronics, CA, USA), yielding a 55x55mm^2 FOV at a focal distance of 400mm. High quality bandpass filters (Semrock, Rochester, USA) with peak wavelengths 660nm and 720nm were used for measurements at the AOI987 excitation and emission wavelength, respectively. For reference white light images of the sample, an electroluminescent membrane (Distrelec, Switzerland) was fixed on the housing bottom. To accommodate optical measurements in both reflection and transmission mode, three coated front surface mirrors (Edmund Optics, Karlsruhe, Germany), were used to deflect the incoming laser beam. ***In vivo* experiments:** All *in vivo* experiments were carried out in strict adherence with the Swiss law for animal protection. Two 24-month old APP23 mice (tg) and two age-matched control littermates (wt) were anesthetized using 2% isoflurane in an oxygen/air (1:4) mixture, shaved on the head and placed on the heated animal platform. Their temperature was held stable at 32C. At time t= 0, AOI987 (0.1mg/kg, 0.01mg/ml) was administered via the tail vein. FMT measurements were performed at every 20 minutes for three hours. Each FMT measurement lasted 12 minutes. MR measurements with different sequences were performed in-between the FMT measurements (**Fig.1** inlets). A reference 2D RARE sequence (TE/TR = 5ms/383ms, FOV = 2.2x2.1cm2, matrix size = 200x200, 8 averages) with 29 axial slices of 0.7mm thickness was acquired for each animal. The optical signal at 680nm and 720 nm was collected from a 4.7x4.7cm^2 ROI on the head using a 8x14 source excitation grid. After 3 hours, the animals were sacrificed and brains extracted for ex vivo validation with epi-fluorescence imaging and histology. **Data processing:** The 2D RARE data were smoothed and segmented. Isosurfaces were determined to compute the top surface height map, which was interpolated to match the optical image pixel dimensions. The height map and the optical white light image were used for co-registration. Signals from anatomical landmarks on the skull were interactively selected to compute an affine transformation between the two images. The co-registered surface was used in FMT reconstruction. Programming and visualization was done in Matlab (The Mathworks, Inc.).

Results: The quality of both MR and optical images was good (SNR=22 and 568, respectively). Cerebral microbleeds, a characteristic feature observed indicative of cerebral amyloid angiopathy [3], were detected on one tg animal with MRI (**Fig.2** gray inlet). Reconstruction of FMT data (**Fig.3**, showing one pair of tg/wt animals 20 and 180 minutes post) allowed estimation of the local fluorophore concentration *in vivo* which displayed an exponential decay upon tracer injection both in wt and tg mice **(Fig.2).** The decay constant was slower for tg ((b = -0.0014) than for wt animals (b = -0.0043), indicating a slower dye washout in tg due to dye binding to plaques. Three hours following tracer administration, significant residual FMT intensity was observed *ex vivo* in tg mice, but not in wt animals (**Fig.2** green inlets).







Discussion: A new generation of a hybrid FMT/MR was presented, featuring technical improvements. Proof-of-concept for hybrid imaging was established using a transgenic model for cerebral amyloidosis. In 22 months old APP23 mice, significant AOI987 binding indicative of cerebral β-amyloid deposition could be visualized using FMT, while MRI revealed microbleeds indicative of vascular pathology. Wildtype mice displayed no pathology. The *in vivo* findings have been confirmed using *ex vivo* analyses. In a next step, structural information derived from MRI data will be used to generate maps of optical properties (scattering and absorption), which will be used as prior information for the FMT reconstructions.

Acknowledgments: This work was financially supported by the EU FP7 FMT/XCT project. We acknowledge the contribution of M. Kuepfer in the mechanical construction of the FMT/MRI system. **References: [1]** Stuker *et al.*, IEEE Trans Med Imag, 2011 **[2]** Hintersteiner *et al.*, Nat Biotech, 2005 **[3]** Jellinger *et al.*, Eur J Neurol, 2007