Whole Body Fluorescence Imaging in Humans

J. Mehnert[1,2*], S. Piper[1*], C. Habermehl[1], C. H. Schmitz[1,3], H. Obrig [1,2,4], J. Steinbrink[1,5]
[1]*Charité, Department of Neurology, Charitéplatz 1, 10117 Berlin, Germany*
[2]*Department for Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Stephanstr. 1a, 04303 Leipzig, Germany*
[3]*NIRx Medizintechnik GmbH, Baumbachstr. 17, 13189 Berlin, Germany*
[4]*Clinic of Cognitive Neurology, University of Leipzig, Liebigstr. 16, 04103 Leipzig, Germany*
[5]*Center for Stroke Research, Charité University Medicine Berlin, Charitéplatz 1, 10117 Berlin, Germany*
**both authors contributed equally to this work*
Jan.Mehnert@charite.de

Abstract: Whole body fluorescence imaging was performed in two adult subjects following injection of ICG. Results show that bolus tracking is very well feasible in humans and might be used for studying disturbed peripheral blood flow.
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1. Introduction

Dynamic Near-Infrared Fluorescence (DNIF) of fluorescent dyes has been extensively used to study the dynamics of diseases in small rodents [1] and has also been applied to the study of arthritis [2,3] and lesions of the brest in humans [4,5,6]. So far, fluorescence measurements in humans were restricted to localized areas of the of the periphery, such as limbs and the female breast. Recently, a study in mice has demonstrated that whole-body fluorescence imaging can differentiate the major interior organs in small animals, thus opening a wide range of conceivable application areas in preclinical research [7]. Here, we present the first experimental proof of feasibility of extending whole-body fluorescence imaging to the adult human, which we expect to have numerous applications including the study of peripheral blood supply and peripheral vascular diseases.

2. Methods

Two male healthy subjects (mean age 44) participated in the study. For imaging, each subject was positioned in the field of view of our imaging apparatus in front of a black, non-fluorescent background. The subject's clothing was removed, apart from a black, non-fluorescent undergarment (Fig. 1 b). To enhance image contrast and to reduce any background counts due to scattering, ambient light, or reflections, the entire room was completely darkened. A schematic of the setup is shown in Fig.1a. An assistant injected a bolus of the fluorescent dye of 25 mg Indocyanine Green, (ICG, by Pulsion AG, Munich, Germany) dissolved in 15 ml *aqua ad injectionem* within approximately 5 s. The subjects were asked to move as little as possible during the procedure.

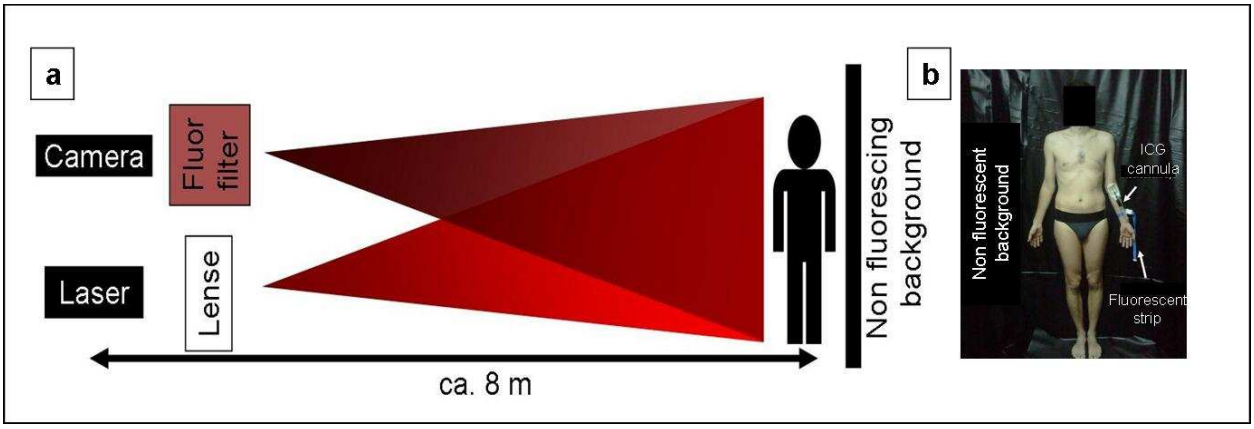

Fig. 1 a Schematic of the experimental setup. b Photo of a subject standing in a darkened room at a non-fluorescent wall with ICG injection prepared.

This scene was recorded with a near-infrared fluorescence imaging setup. The illumination source was a high-power 760 nm laser diode with integrated thermo-electric cooler (Intense Ltd., New Brunswick, NJ, USA) and a 100 µm fiber pigtail terminatied with a grin rod lens. The total excitation power was approximately 200 mW. A cylindrical lens was positioned approximately 5 cm from the grin rod lens to widen the laser beam. The subject was located about 8 m from the laser diode, where we obtained an elliptical illumination area of approx. 400 mm by 800

mm (FWHM). Detection of the fluorescent light was accomplished using a CCD camera (evolve 512, air cooled to -80°C, online gain multiplier: 1000, 512 x 512 px, Photometrics., Tuscon, AZ, USA) fitted with a camera lens (Nikkor macro lens, f = 28 mm, f/1.2, Nikon, Duesseldorf, Germany). In front of the lens, a stack of three 820-nm interference filters were blaced to prevent direct measurement of the excitation photons undergoing backscattering or reflection. For each subject a sequence of 400 images was recorded with a frequency of 2 Hz corresponding to 200 seconds total experimental duration. After an initial 30-s resting phase, a single bolus was injected (duration = 5 sec), followed by 165 seconds of recording of fluorescence dynamics. The reference time point t=0 sec was set to the end of bolus administration.

3. Results and Conclusion

Fig. 2 and 3 show a shortened image sequence of the raw fluorescence images for both subjects during 50 s following bolus injection. The apparent increased brightness of the body center compared to the head is owed to the inhomogeneous illumination profile which was roughly Gaussian. No further image processing to account for the illumination profile was used in the raw images presented below.

We restricted the images to 50 seconds after ICG injection because afterwards only minor changes in fluorescent were detectable until the end of our imaging procedure. The bolus fluorescence in the head appears at approximately 10 seconds after injection and after ca. 18 s starts spreading throughout the trunk, abdomen, and the periphery. A particularly noteworthy feature is observed around 20 s post injection. As the fluorescence enters the trunk at first, a shape outlining an internal organ – most likely tracing the intestine – is visible. This feature, which becomes especially apparent when observing the image sequence in an animated movie,, is depicted in the insert showing an enlarged view of the image frames at 20 s and 22 s.

For subject 2, the excitation illumination unfortunately did not sufficiently illuminate the head, and therefore the bolus arrival in the head cannot be seen. The fluorescence on the subject's left hand throughout all images is due to contamination with ICG solution..

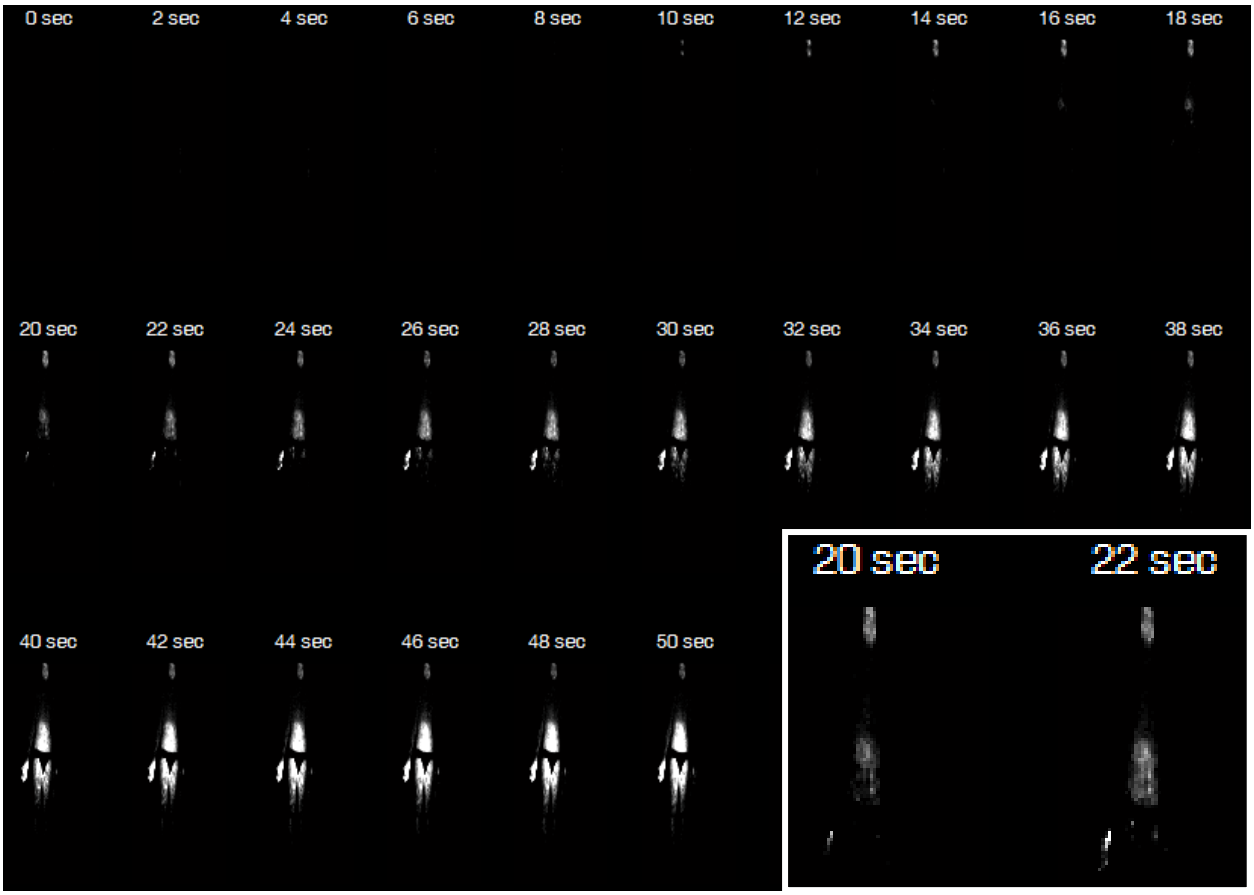

Fig. 2 Raw images for subject 1 for the following 50 seconds after ICG injection, taken every two seconds.

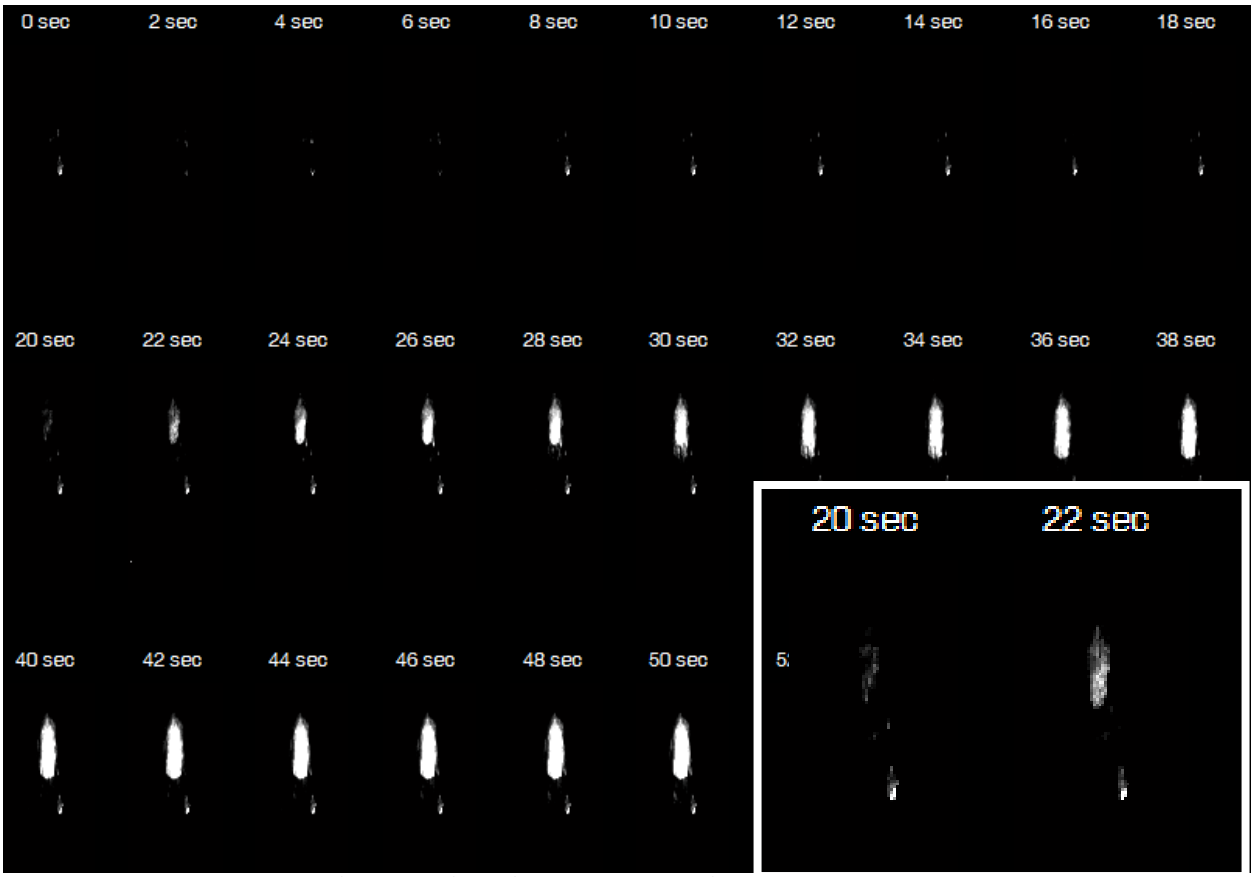

Fig. 3 Raw images for subject 2 for the following 58 seconds after ICG injection, taken every two seconds.

We take this first experiment to be a convincing demonstration of the ability to track fluorescence dyes in the whole human body non-invasively with high spatial and temporal resolution. We plan to improve on the presented approach in several ways to increase the qualitative and quantitative analysis: First, a more homogeneous illumination in conjunction with the recording of the excitation profile will aid in comparing the fluorescence intensity across the field of view and to improve the dynamic range. Secondly, post processing involving adaptive filtering and the use of a sensitivity profile will further help to reduce image noise, and to reduce the influence of spatial inhomogeneities introduced by the setup. Furthermore, multispectral imaging with different excitation wavelengths may allow a certain level of depth profiling [8]. Lastly, other fluorescent dyes [7] would be of interest for this setup. We envision future research application in the study of systemic pathologies, including peripheral vascular disease and arthritis.

4. References

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