Real-time imaging of renal clearance using Multi-Spectral Optoacoustic Tomography

Stefan Morscher*[a,b], Neal C. Burton[a,b], Adrian Taruttis[a,b], Nikolaos C. Deliolanis[a,b], Daniel Razansky[a,b] and Vasilis Ntziachristos[a,b]

[a]Institute for Biological and Medical Imaging, Helmholtz Zentrum München

[b]Chair for Biological Imaging, Technische Universität München

ABSTRACT

Multi-Spectral Optoacoustic Tomography (MSOT) offers real time imaging that simultaneously exploits high ultrasound resolutions and strong optical contrast. It allows visualizing absorbers in tissue by using their distinct spectral absorption profiles. This work presents a non-invasive *in vivo* study of kinetics involved in the clearance of carboxylated dye in mouse kidneys. The distinction between probe uptake in different regions of the kidneys is studied, providing insights into its physiologic processes.

Keywords: Multispectral Optoacoustic Tomography, Real Time Imaging, In vivo imaging, pharmacokinetics, renal clearance

1. INTRODUCTION

In biological and pharmaceutical research, near infrared fluorescence (NIRF) is frequently used to study bio-distribution of optical contrast agents[1,2]. Key aspects that enable the performance of this approach are on the one hand the low overall tissue absorption and thus high penetration depth in the near infrared optical window, and on the other hand the sensitivity of detection. Detectable agents include a multitude of targeted agents[3], fluorescent proteins[4], and even activatable probes[5]. Performance of deep-tissue, whole-body NIRF imaging is severely limited through optical properties of tissue, most importantly intense scattering and absorption[6]. In the planar domain this leads to surface weighted imaging[6], while in tomographic geometries long acquisition times and ill-posed reconstruction problems hinder practical implementation and limit accuracy[7]. Consequently, the spatial resolution in optical imaging decreases with penetration depth, resulting in millimeter range spatial resolution for whole body imaging scenarios considered here [6].

By adding ultrasound detection to optical excitation, these physical barriers can at least partly be overcome in optoacoustic imaging[1,2,8,9]. When diffuse light excites absorbers, the subsequent heating generates transient expansion and thus generates pressure waves detectable by an ultrasound transducer positioned at the surface. In a tomographic geometries using multiple detection angles, reconstruction incorporating variety of geometric parameters yields a map of absorbed energy [10]. The acquisition process can be sped up by utilization of an ultrasound transducer array[11–13], allowing for real-time imaging capabilities, as demonstrated in this work. The inherent advantage of the modality lies in the detection of acoustic waves that are subject to far less disturbances when travelling through tissue. Most importantly, this makes it possible to retain high spatial resolutions also in whole body imaging scenarios.

Illumination at multiple wavelengths in multispectral optoacoustic tomography (MSOT) allows for separation of specific absorbers based on their spectral absorption profile[14], [15]. Specific detection has been demonstrated for imaging of fluorescent proteins in model organisms[16] and both targeted and untargeted contrast agents[11,17,18]. In addition to exogenous markers, optoacoustic imaging techniques are also able to resolve intrinsic biomarkers such as oxygenated and deoxygenated hemoglobin[18,19] and different types of nanoparticles with distinct absorption in the near infrared[12,20].

Here the MSOT is explored for *in vivo* studies of pharmacokinetics and biodistribution [21]. In particular, we injected mice with a fluorescent agent and revealed the temporal characteristics in different parts of the kidneys, attempting to explore the renal clearance pathways. Verification of results was performed using epi-fluorescence cryoslice imaging[22].

*stefan.morscher@helmholtz-muenchen.de

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2. METHODS

The imaging system utilized is described in detail in [11] and schematically depicted in Fig. 1. It consists of a Nd:YAG pumped OPO laser (Opotek Inc., Carlsbad, CA) tunable in the near infrared range (680nm – 900nm) that delivers 10ns laser pulses at a repetition rate of 10Hz as illumination. A uniform ring of illumination is delivered to the sample using a fiber bundle consisting of 10 arms. Signals are detected using a custom made 64-element ultrasound transducer array (Imasonic SAS, Voray, France) with a central frequency of 5Mhz that covers 172° around the sample at a radius of 4cm. Signals are acquired using a custom made acquisition system (Falkenstein Mikrosysteme, Taufkirchen, Germany) at a rate of 40 megasamples per second. Both transducer and illumination fiber outlets are submerged in a water tank to allow for acoustic coupling of the signals from tissue. The mouse is fixed on a holder and wrapped in a membrane to protect it from water when being submerged; anesthesia is provided using a breathing mask. The system is able to capture cross-sectional slices of mice at a rate of 10 images per second at a single wavelength.

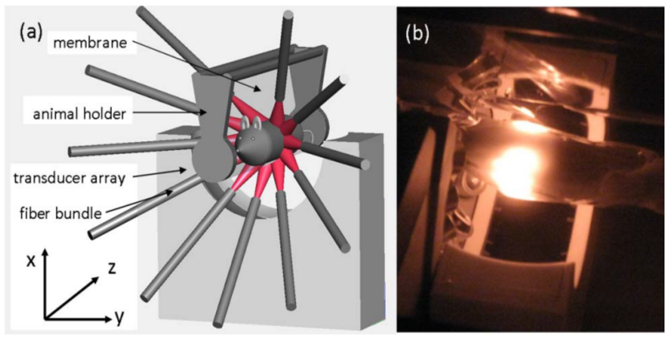

Figure 1 (a) Sketch of the imaging system. (b) Photograph of the imaging chamber with a mouse fixed in the animal holder, wrapped in a membrane and submerged in water.[11]

20nmol of IRDye800-CW (Li-Cor) were diluted in saline to a final volume of 100µl and injected intravenously into nude CD-1 mice. MSOT measurements were then performed continuously at 5 wavelengths (700nm, 730nm, 760nm, 774nm, 850nm) in a cross-sectional slice that captured both kidneys, where 50 averages were acquired for each wavelength. This protocol allowed to capture about 2 complete measurements per minute. The total number of mice imaged in MSOT was 4. In order to resolve the strong background absorbers deoxygenated and oxygenated hemoglobin from the injected fluorescent dye, we spectrally decomposed the individual sets of measurements using blind unmixing[21,23] , namely Principle Component Analysis (PCA). The spectra of the named absorbers are measured in the spectrophotometer are shown in Fig. 2.

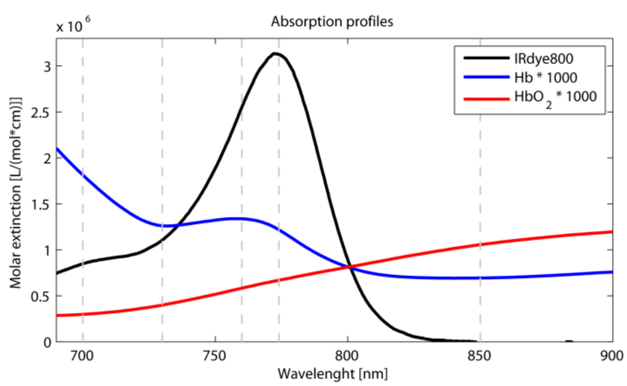

Figure 2 Absorption profiles of deoxygenated and oxygenated hemoglobin as dominant tissue absorbers (scaled by a factor of 1000) and the injected IRDye800-CW. Acquisition wavelengths marked in grey

In order to verify the achieved results, we sacrificed mice by spinal dislocation at different time points after the injection and imaged them in a dedicated epi-fluorescence cryoslicing imager[22], recording both color and fluorescence images.

3. RESULTS

The injected IRDye800-CW is rapidly filtered from the blood stream by the kidneys. The biodistribution of the dye was tracked by MSOT from the point of the injection onwards, indeed showing an accumulation in the kidneys. The results are depicted in the panel in Fig. 3. Fig. 3(a) shows measurements at 4 time points from a representative animal. The spectrally resolved signal of oxygenated hemoglobin is shown on a black and white color scheme in the background, with the specific signal of the dye overlaid in a logarithmic scale in green. Where the background clearly highlights the vascular system, the green dye signal highlights the signal of the dye in the kidneys. Fig. 3(b) shows a color cryoslice image at a comparable slice 15 minutes post injection, overlaid with the fluorescent signal in green, where the distribution matches the one resolved by MSOT.

Fig. 3(c) tracks the signals in the regions of interest marked in the rightmost image in (a), showing a fast signal pickup in the cortex (orange) and a delayed pickup in the pelvis (black). This matches the expected behavior and common understanding of the renal clearance pathways, where the filtration occurs in the renal cortex and the extracted signals towards the pelvis and thus towards urether and bladder.

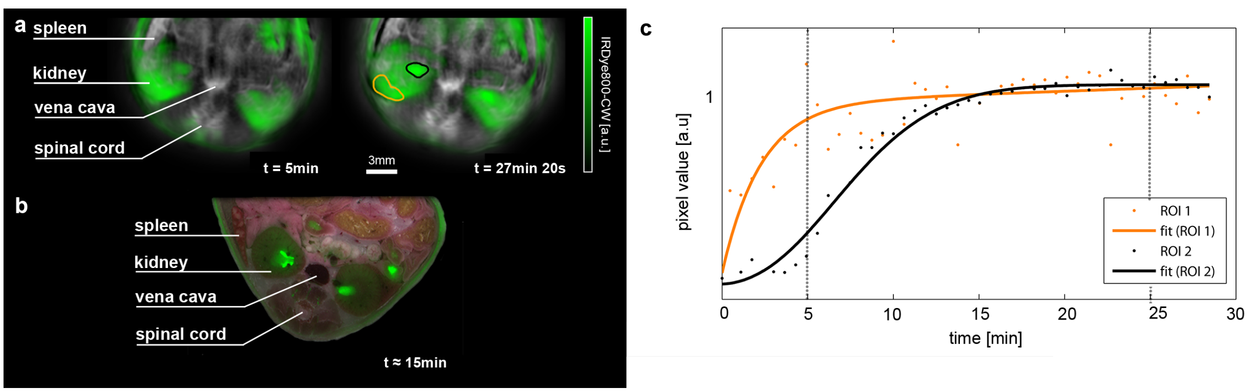

Figure 3 a) Multispectrally separated optoacoustic images of two time points after the injection. The background image shows oxygenated hemoglobin with the resolved dye signal overlaid in green. Regions of interest in renal cortex (orange) and renal pelvis (black) b) validation using cryoslicing imaging with fluorescent signal overlaid in green. c) temporal evolution of agent signal in the two regions of interest, showing an early signal pickup in the renal cortex (orange) with the delayed pickup in the pelvis (black) based on renal clearance pathways. [21]

4. DISCUSSION

The presented results showcase MSOT as a powerful tool for fast imaging of biodistribution and pharmacokinetics. Since fluorescence imaging is ubiquitously used in biomedical research, a large varietiy of different markers and biological frameworks are readily available. However, as optical resolution rapidly decreases with penetration depth, accurate visualization turns out difficult in deep tissues due to heavy scattering and absorption. In tomographic approaches such as Fluorescence Molecular Tomography (FMT), long acquisition times do not permit fast kinetic imaging of the type demonstrated here.

Fast imaging of specific absorbers has multiple potential applications in biomedical research, perhaps most importantly in tracing the bio-distribution of compounds labeled with distinct optical markers. This can provide a key tool for safety and toxicity studies of novel pharmaceuticals, allowing tracking their clearance pathways by organ accumulation, especially the excretory organs such as liver and kidney. Furthermore it can also provide biodistribution of intrinsic tissue absorbers, which paves the way for clinical applications of MSOT by allowing the assessment of tissue and blood oxygenation without additional agents that would require a lengthy approval procedure.

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