HelmholtzZentrum münchen
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Listening to Molecules

Assessing PK Parameters using dynamic contrast enhanced multispectral optoacoustic tomography (DCE-MSOT)

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Abstract

Multispectral Optoacoustic Tomography (MSOT) is an emerging hybrid imaging modality that allows for imaging of optical contrast in the near-infrared at high spatial and temporal resolutions based on ultrasound detection. Using multi-spectral imaging capabilites it can resolve distinct spectral absorbers such as intrinsic (hemoglobin, melanin) and extrinsic (fluorescent dyes, fluorescent proteins, nanoparticles) tissue biomarkers, allowing a wide range of biomedical applications.

Image acquisition at a rate of up to 10 images per second permits the collection of a multispectral data set of a single, cross-sectional slice within seconds. The resulting imaging speed can be used for pharmacokinetic imaging of marker uptake in specific regions of tissue, allowing very similar performances as e.g. fMRI. A time series of images is modeled on a per-pixel basis using an one-compartmental model, resulting in a set of parametric maps. Important applications are drug toxicity studies and cancer targeting as demonstrated in this work.

Methods

Nude mice (some of which bearing an 4T1 orthotopic tumor) were injected with targeted and non-targeted fluorescent agents. For MSOT measurements, images were acquired at 6 wavelengths between 700 nm and 900 nm to enable separation of absorbers with distinct spectral absorption profiles. Spectral unmixing was performed using spectral fitting using a Moore-Penrose inverse matrix.

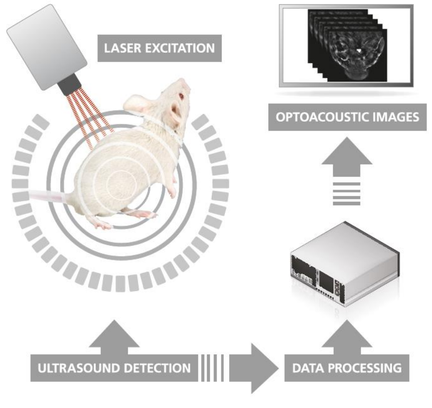
For Figure 1, a nude mouse was injected i.v. with a hydrophilic dye conjugate that gets cleared through the kidneys.

For Figure 3, ICG (Pulsion Systems) was injected i.v. while monitoring a slice showing the orthotopic tumor (cropped).

In order to cope with motion, the resolution of all images was reduced to 300µm. Each pixel was then individually modeled using pharmacokinetic models, with some of the resulting parameters visualized as parametric maps.

Ex-vivo validation was obtained using cryoslice analysis.

PRINCIPLE OF MSOT OPERATION



Pulsed light of multiple wavelengths illuminates the tissue of interest and establishes transient photon fields in tissue that are being absorbed by photoabsorbers. In response, acoustic responses are generated via the photoacoustic phenomenon, which are then detected with acoustic detectors. Tomographic images can then be generated and spectrally unmixed to yield the biodistribution of reporter molecules and tissue biomarkers.

Results

In vivo MSOT imaging was naïve nude mice as well as such bearing an orthotopic 4T1 tumor. Based on imaging at multiple excitation wavelengths and the presented calibrated spectral unmixing, the agent specific signature could be extracted and localized (see Fig. 1). In case of Fig. 1 the injected agent was hydrophilic, resulting in a clearance scheme through the kidneys. Subsequent kinetic modelling of per-pixel behavior over time with a simplistic one-compartmental model (see Fig. 2) yields modeled pixel-behavior and parametric maps (see Fig. 1 and 3) that allow assessment of organ function. Similar procedure was applied in Fig. 3, where a mouse bearing an orthotopic 4T1 tumor was injected with untargeted ICG as a perfusion marker to characterize vascularization of the tumor.

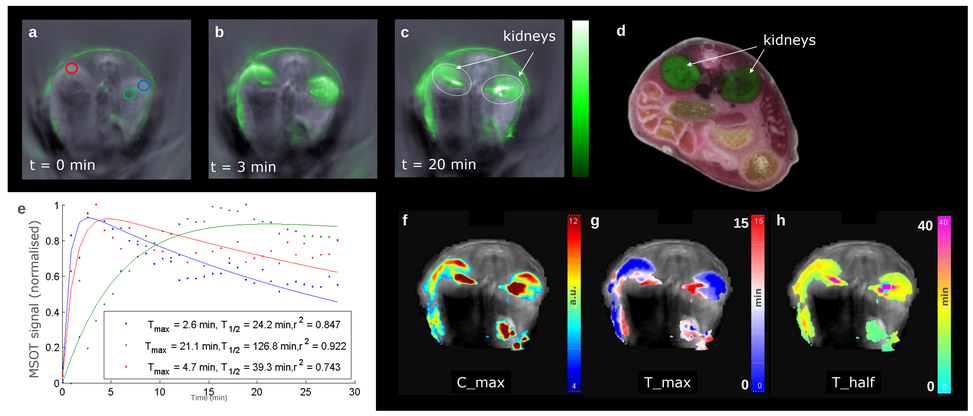
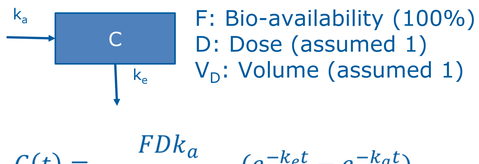


Fig. 1: Detection of hydrophilic fluorescent agent in the kidneys

A naive nude mouse was injected with a fluorescent dye that gets cleared through the kidneys. (a) through (c) show some snaphots, with the localization of the dye overlaid in green. (d) shows a color photograph from a cryostat overlaid in green with a fluorescence image in the same setup validating the biodistribution of the dye. (e) shows the temporal profile in three areas marked in (a) and the corresponding modeled line. Parametric maps are created from the fit of the model in Fig. 2, where (f) shows the peak intensity for each pixel, (g) shows the time-to-peak and (h) shows the half-life of the probe. This clearly visualizes an early filtration in the cortex, followed by a delayed uptake in the pelvis.



$$C(t) = \frac{FDk_a}{V_D(k_a - k_e)}(e^{-k_e t} - e^{-k_a t})$$

$$T_{max} = \frac{\ln(k_a/k_e)}{k_a - k_e}$$

Fig.2: Schematic of simple one-compartmental model.

Every pixel is assumed to be dominated by either vascular or interstitial space. More complex models are to be evaluated in the future.

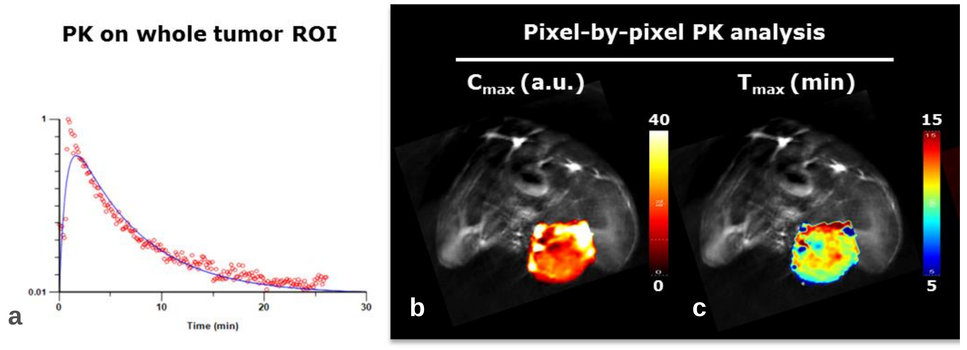


Fig.3: Perfusion of ICG in an orthotopic 4T1 tumor

Cropped images of a 4T1 tumor in the area of the hind limbs overlaid with parametric maps generated after ICG injection. (a) shows the temporal profile in the whole tumor area. (b) shows the peak concentration over time and clearly highlights the vascularized area. (c) displays time-to-peak and shows early perfusion in the vascularized regions, while less perfused areas show delayed uptake through the EPR effect.

Conclusions

This work demonstrates the ability of MSOT to capture fast processes in vivo with molecular specificity and high spatial and temporal resolution. Subsequent per-pixel analysis allows fitting of a pharmacokinetic model and calculation of parametric maps that enable various applications with similar if not superior performance as compared to fMRI. Examples include perfusion studies of tumors and brain tissue as well as studying of agent clearance profiles in various organs or specificity studies of targeted markers in tumor models. The simple one-compartmental model utilized here serves the purpose of illustrating the potential of MSOT as the underlying imaging modality, whereas more advanced applications with more complex models are under investigation.