MSOT cluster analysis toolkit to objectively assess pharmacokinetics and biodistribution of clinical biomarkers Bianca Hoffmann¹, Ruman Gerst^{1,2}, Zoltán Cseresnyés¹, WanLing Foo^{3,4}, Oliver Sommerfeld^{3,4}, Adrian T. Press^{3,4}, Michael Bauer^{3,4} and Marc Thilo Figge^{1,2}

¹ Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute (HKI), Jena, Germany
 ² Faculty of Biological Sciences, Friedrich Schiller University Jena, Jena, Germany
 ³ Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany
 ⁴ Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany

Multispectral Optoacoustic Tomography (MSOT)

- based on light pulse illumination and thermo-elastic expansion of tissue
- gathers functional tissue information
- resolves multiple photoabsorbers in one scan
- high spatio-temporal resolution



New signal-oriented analysis approach

- signal intensities are analyzed from whole sample (animal-ROI)
- signal-oriented approach



Cluster - 1 = 2 = 3 = 4 = 5

- enables longitudinal measurements
- non-invasiveness allows in vivo studies



Ultrasound detector

4-dimensional images
x, y, z (width, height, time)

photoabsorber channels:

- water
- oxygenated hemoglobin (Hb)
- deoxygenated Hb (HbO₂)
- indocyanine green (ICG)





identifies regions of homogeneous signal kinetics (k cluster centers)

> pharamcokinetics differ between groups



- Study aim:
- quantify liver function based on signal of biomarker ICG in MSOT images
- peritoneal contamination and infection (PCI) model of sepsis
- healthy (Sham) and diseased (PCI) animals

MSOT image pre-processing

- needed to resolve issues specific for MSOT image data
- rigid registration based on water channel to reduce motion artifacts
- z-transformation of intensity values for inter-scan comparability
 smoothing by averaging and downsampling (smoothing factor *s*) in time domain to reduce breathing artifacts
- biodistribution of signal kinetics can be visualized for each animal
- weighted average curves (WAC) of cluster centers with net increase
- for each animal: weights are frequencies of pixels in cluster centers
- AUCs of WACs serve as quantitative measurement



• conversion to time derivatives to deal with negative intensity values

Limitations of current MSOT image analysis

current analyses concentrate on specific regions
 tissue-oriented approach

manual definition of regions of interest (ROIs) in specific tissue regions



area under the curve (AUC) statistics
 no significant differences between groups



- > Sham and PCI show significant differenences in pharmacokinetics
- high accordance of results for animal-ROIs drawn by three different experimenters
- no expert knowledge necessary



Implementation as ImageJ plugin Mcat

> MSOT cluster analysis toolkit

- graph-based algorithm structure
- graphical user interface (GUI)



findcat

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MSOT Cluster Analysis Toolkit (Mcat)				
🗘 New project 🖹 Open project 🖺 Save project				🗇 Run
Data & Parameters				
+ Add 🖟 Batch import 👗 📋 👗 60721 🔗 Rename 📋				
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Sham	Raw image	D:WcatData\Sham\60721\60721_Sham_image.tif	1	Image hyperstack
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even small positional changes lead to highly different intensity values
 tissue-oriented analysis prone to user bias and often not reproducible



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Vroject file Treatment

- supports parameter estimation
- multiple parameter sets in one run
- effect size between Sham and PCI stable over wide range of smoothing factor s and k-means k



Contact: bianca.hoffmann@leibniz-hki.de