Mcat: a multispectral cluster analysis toolkit for deep learning-based segmentation and clinical biomarker quantification of MSOT data

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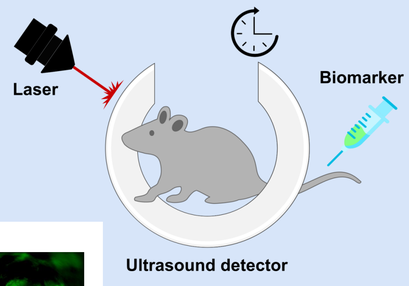
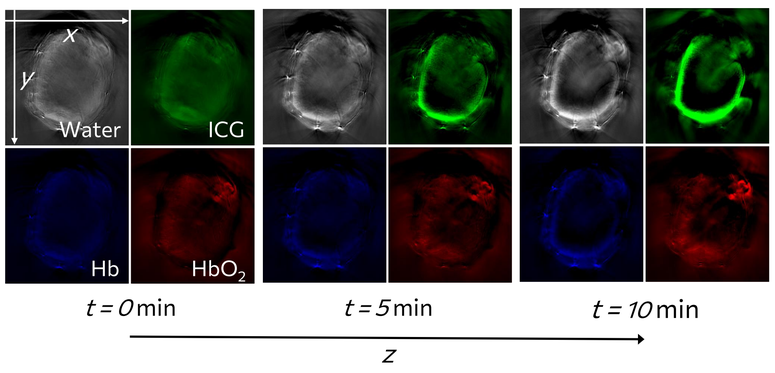
Multispectral Optoacoustic Tomography and Limitations of Current Analysis Approaches

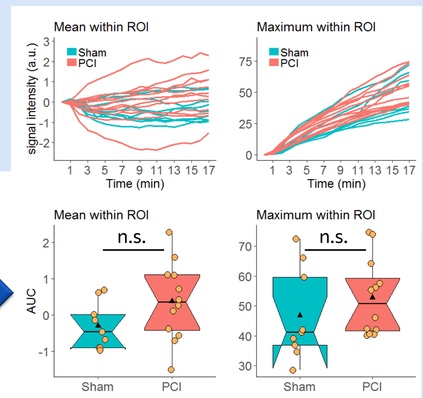
- based on light pulse illumination and thermo-elastic expansion of tissue
- gathers functional tissue information
- resolves multiple photoabsorbers in one scan
- high spatio-temporal resolution
- enables longitudinal measurements
- non-invasiveness allows *in vivo* studies
- ➢ 4-dimensional images: *x*, *y*, *z* + photoabsorber channels



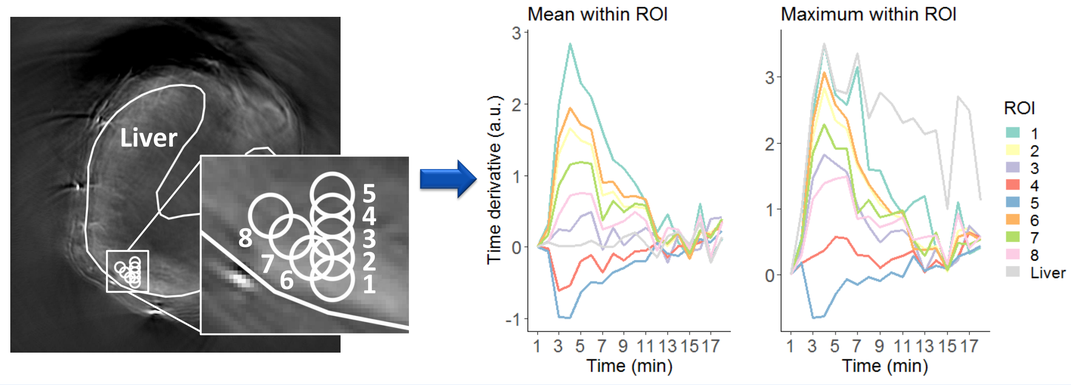


ICG – indocyanine green
Hb – deoxyhemoglobin
HbO_2 – oxyhemoglobin

- analysis of specific regions of tissue
- manually defined regions of interest (ROIs)
- extraction of basic intensity features
- area under the curve (AUC) statistics
- ➢ tissue-oriented analysis



- ➢ fails to identify differences between healthy (Sham) and septic (PCI) animals

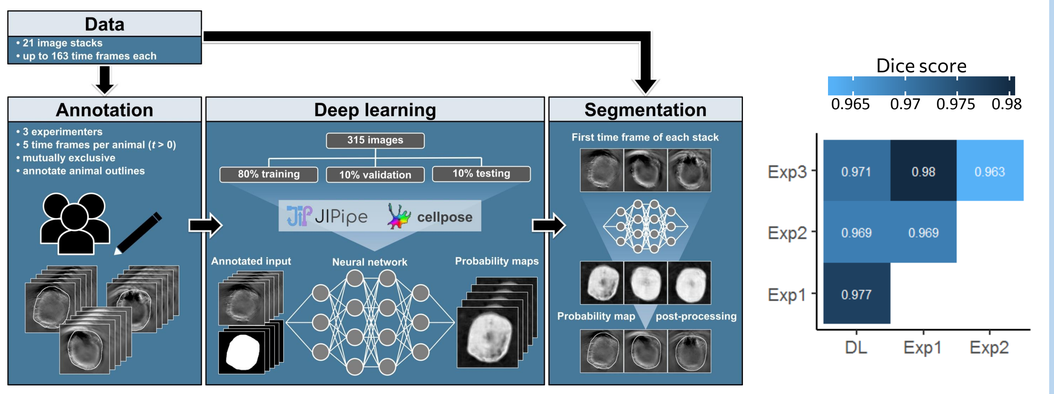


- ○ even small positional changes lead to highly different intensity values
- ➢ tissue-oriented analysis prone to user bias and often not reproducible

MSOT Cluster Analysis Toolkit – *Mcat* [1]

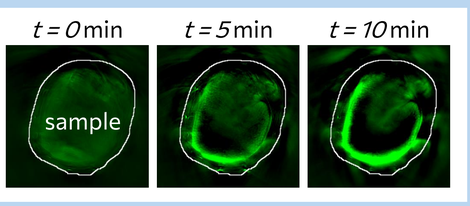
1) Deep learning-based segmentation of MSOT images

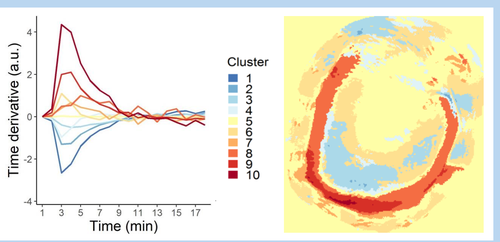
- ○ identify sample outlines from MSOT images
- ○ deep learning (DL) approach based on *Cellpose* [2] neural network architecture
- ○ retrained from scratch with manual MSOT annotations by three experts using *JIPipe* [3]
- ➢ pairwise Dice score shows high concordance of DL-based segmentation with experts



2) Signal-oriented analysis of MSOT images

- ○ pre-processing eliminates motion artifacts and inter-scan intensity differences
- ○ ROIs retrieved with DL-based segmentation
- ○ analyze signal intensities from whole sample
- ➢ signal-oriented approach



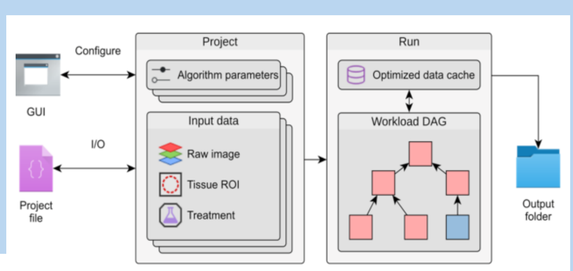


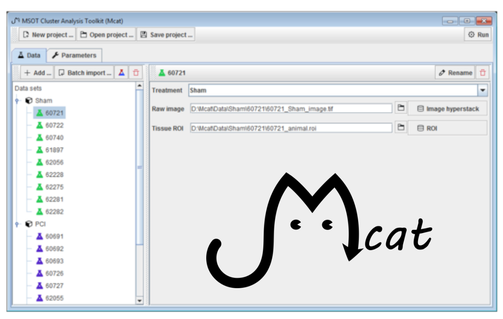
- ○ pixel-wise clustering with k-means
- ○ identifies regions of similar signal kinetics
- ○ *k* defines number of extracted kinetics

Provides:
- ➢ information about pharmacokinetics
- ➢ spatial biomarker distribution

3) ImageJ plugin *Mcat*

- ○ graph-based algorithm structure
- ○ graphical user interface (GUI)
- ○ optimized data cache structure
- ➢ easily applicable and expandable





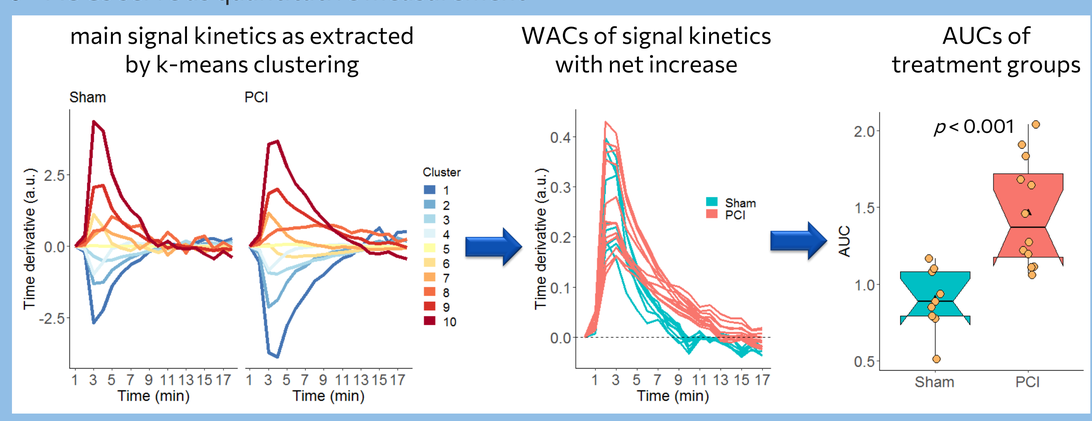
Two main sections:
1. data import and handling
2. parameter definition

- ○ multiple options to obtain ROIs
- ➢ easily adaptable for various MSOT data

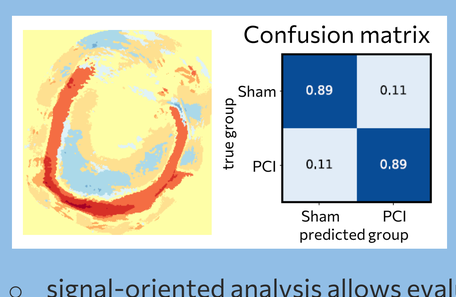
- ○ multiple parameter sets in one run
- ➢ facilitates parameter estimation

Application in Quantification of Liver Function

- ○ evaluation of liver function in preclinical sepsis model with biomarker ICG
- ○ weighted average curves (WACs) of signal kinetics with net increase for each animal
- ○ weights are pixel frequencies of the respective cluster center
- ○ AUCs serve as quantitative measurement

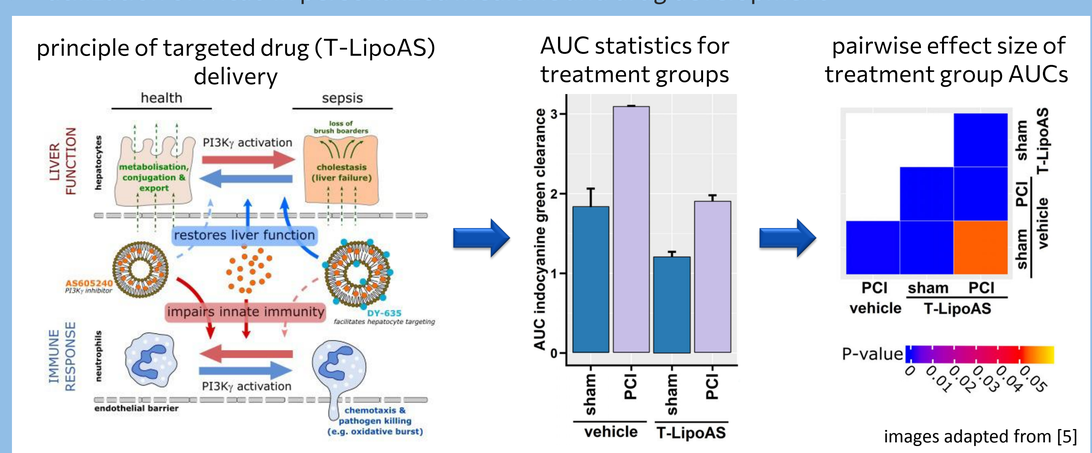


- ➢ in contrast to tissue-oriented analysis we find significant difference between groups



- ○ analysis of morphological shape descriptors
- ○ *e.g.* particle number, solidity for each signal kinetic
- ○ classification with support vector machine
- ○ high accuracy for animals with ambiguous kinetics
- ➢ spatial biomarker distribution allows discrimination of healthy and diseased animals [4]

- ○ signal-oriented analysis allows evaluation of new drugs *in vivo*
- ○ targeted delivery of PI3Kγ-inhibitor restores liver function in preclinical sepsis as found by MSOT imaging [5]
- ➢ utilization of *Mcat* in personalized medicine and drug development



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GitHub https://github.com/applied-systems-biology/Mcat

www.leibniz-hki.de

References

[1] Hoffmann *et al.*, *Photoacoustics* 26 (2022), 100361
[2] Stringer *et al.*, *Nat Methods* 18 (2021), 100-106
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[5] Press *et al.*, *EMBO Mol Med* 13 (2021), e14436