Mcat: A toolkit for deep learning-based segmentation and automated analysis of MSOT image data

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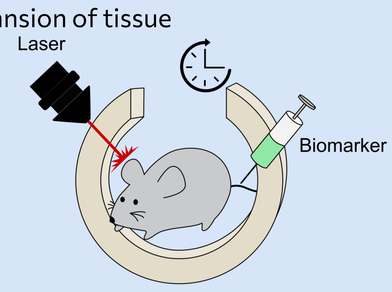
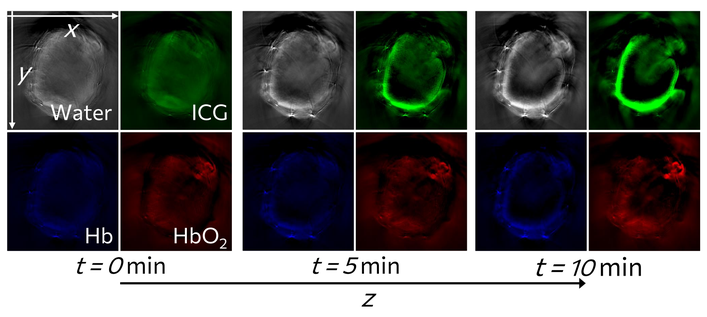
Multispectral Optoacoustic Tomography and Limitations of Current Analysis Approaches

- 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



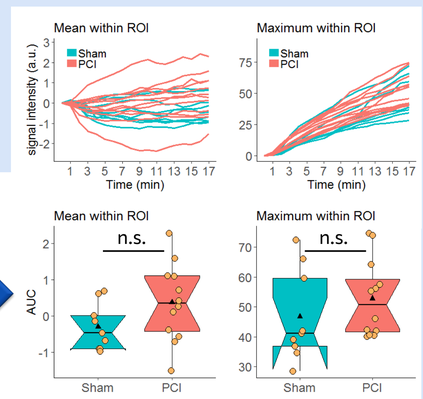
➢ **4-dimensional images: *x*, *y*, *z* + photoabsorber channels**



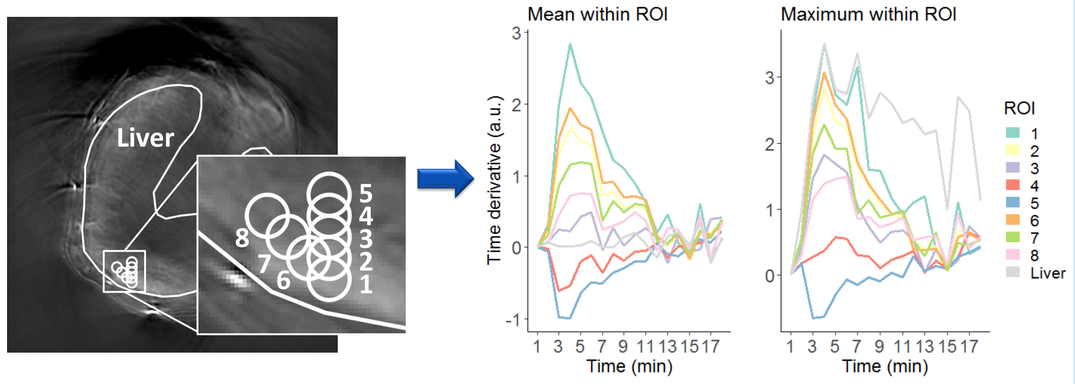
ICG – indocyanine green
Hb – oxygenated blood
HbO_2 – deoxygenated blood

- 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**



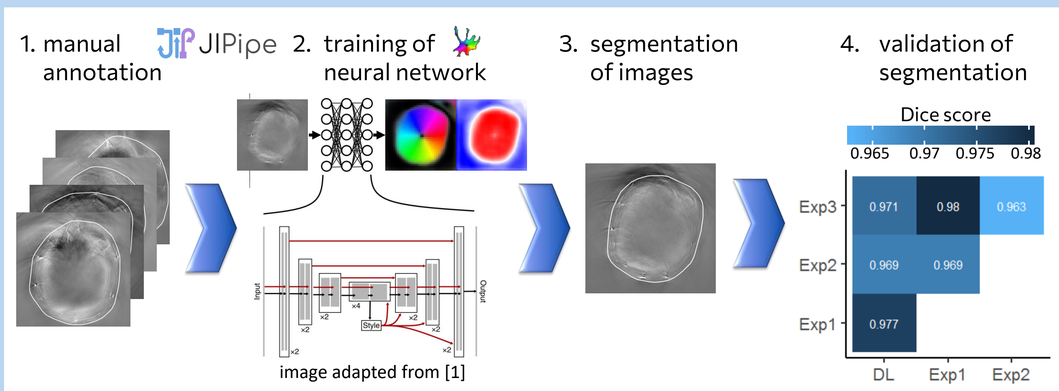
- even small positional changes lead to highly different intensity values

➢ **tissue-oriented analysis prone to user bias and often not reproducible**

Deep Learning-based Segmentation of MSOT Images

- identify sample outlines from MSOT images
- deep learning (DL) approach based on *Cellpose* [1] neural network architecture
- retrained from scratch with manual MSOT annotations of three experts using *JIPipe* [2]

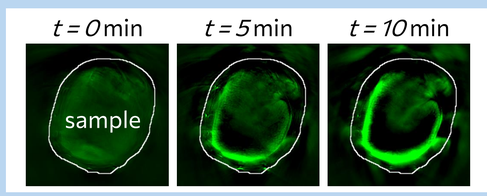
➢ **pairwise Dice score shows high concordance of DL-based segmentation with experts**

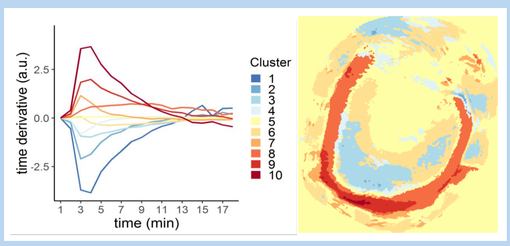


Signal-oriented analysis of MSOT images

- 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

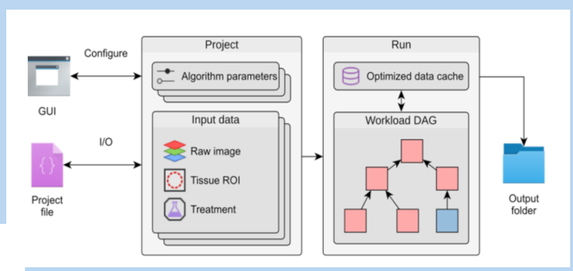
Provides:

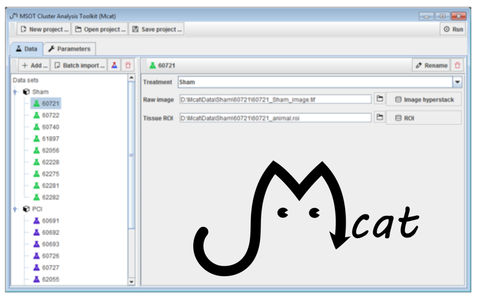
➢ **information about pharmacokinetics**
➢ **spatial biomarker distribution**

MSOT Cluster Analysis Toolkit - *Mcat*

- implementation as ImageJ plugin
- graph-based algorithm structure
- graphical user interface (GUI)
- optimized data cache structure

➢ **easily applicable**
➢ **easily expandable**





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

- multiple options to obtain ROIs

➢ **easily adaptable for various MSOT data**

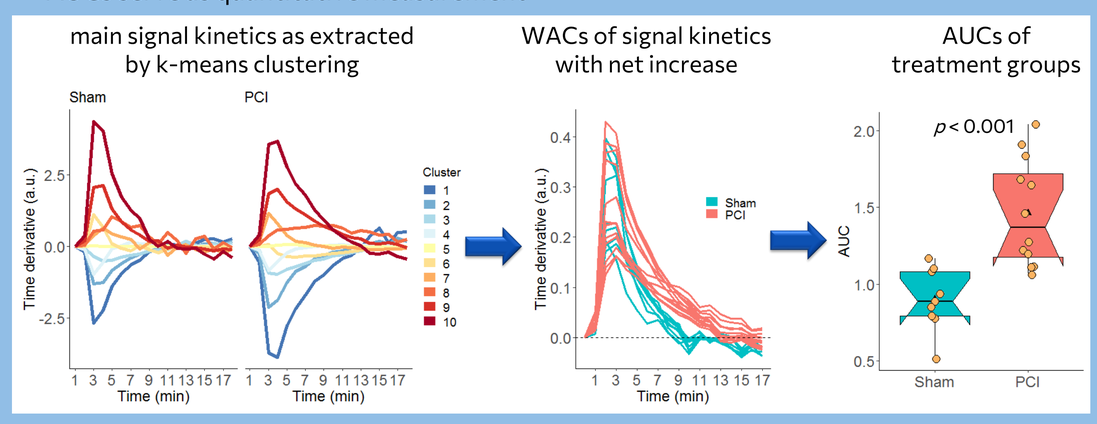
- multiple parameter sets in one run

➢ **facilitates parameter estimation**

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

Application in Quantification of Liver Function

- evaluation of liver function in preclinical sepsis model with biomarker ICG [3]
- 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 also 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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References

[1] Stringer *et al.* 2021. *Nat Methods*. 18:100-106
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