Algorithm for confrontation assay quantification (ACAQ): Label-free image analysis supported by Hessian filtering
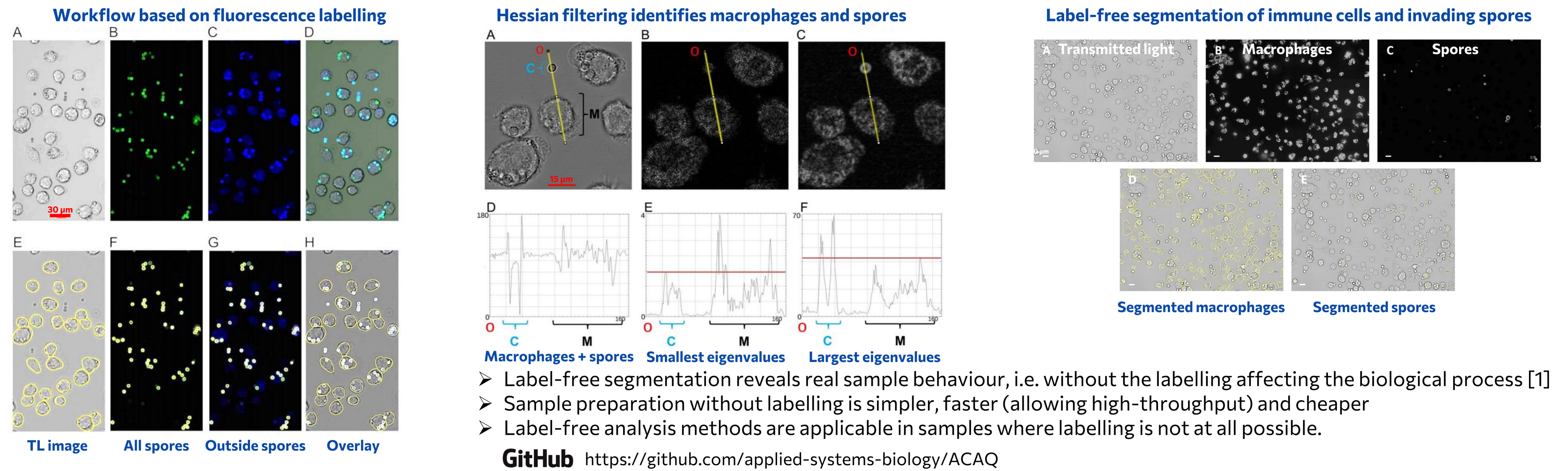
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[1] Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute, Jena, Germany
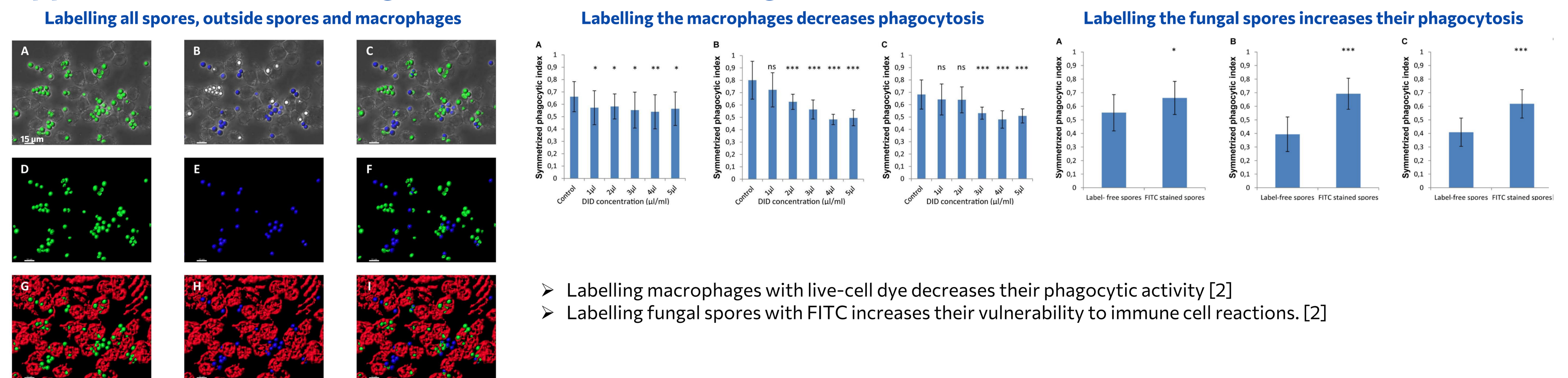[2] Faculty of Biological Sciences, Friedrich-Schiller-University Jena, Germany
[3] Institute of Microbiology, Faculty of Biological Sciences, Friedrich-Schiller-University Jena, Germany
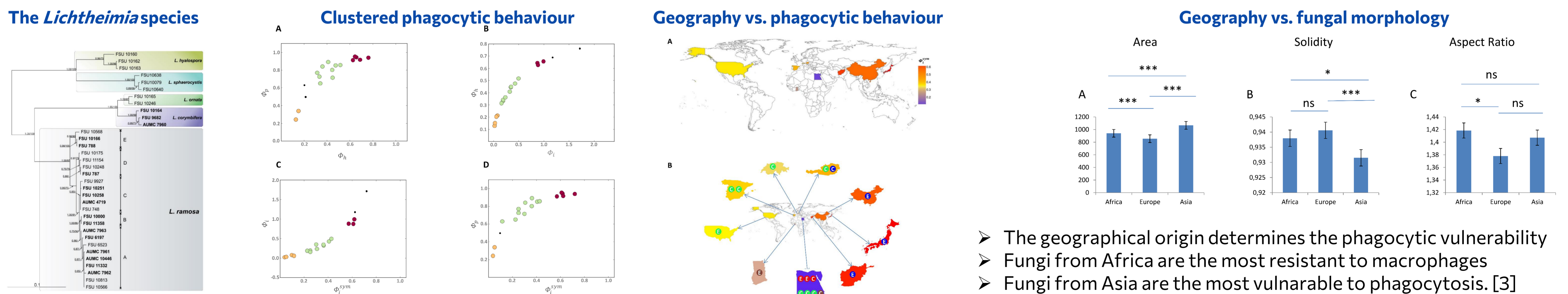
Label-free image segmentation

Workflow based on fluorescence labelling

TL image   All spores   Outside spores   Overlay

Hessian filtering identifies macrophages and spores

Macrophages + spores   Smallest eigenvalues   Largest eigenvalues

Label-free segmentation of immune cells and invading spores

Segmented macrophages   Segmented spores

- Label-free segmentation reveals real sample behaviour, i.e. without the labelling affecting the biological process [1]
- Sample preparation without labelling is simpler, faster (allowing high-throughput) and cheaper
- Label-free analysis methods are applicable in samples where labelling is not at all possible.

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

Application 1: direct biological effects of cell labelling

Labelling all spores, outside spores and macrophages

Labelling the macrophages decreases phagocytosis

Labelling the fungal spores increases their phagocytosis

- Labelling macrophages with live-cell dye decreases their phagocytic activity [2]
- Labelling fungal spores with FITC increases their vulnerability to immune cell reactions. [2]

Application 2: the geographical origin determines the vulnerability of fungi

The *Lichtheimia* species

Clustered phagocytic behaviour

Geography vs. phagocytic behaviour

Geography vs. fungal morphology

- The geographical origin determines the phagocytic vulnerability
- Fungi from Africa are the most resistant to macrophages
- Fungi from Asia are the most vulnarable to phagocytosis. [3]

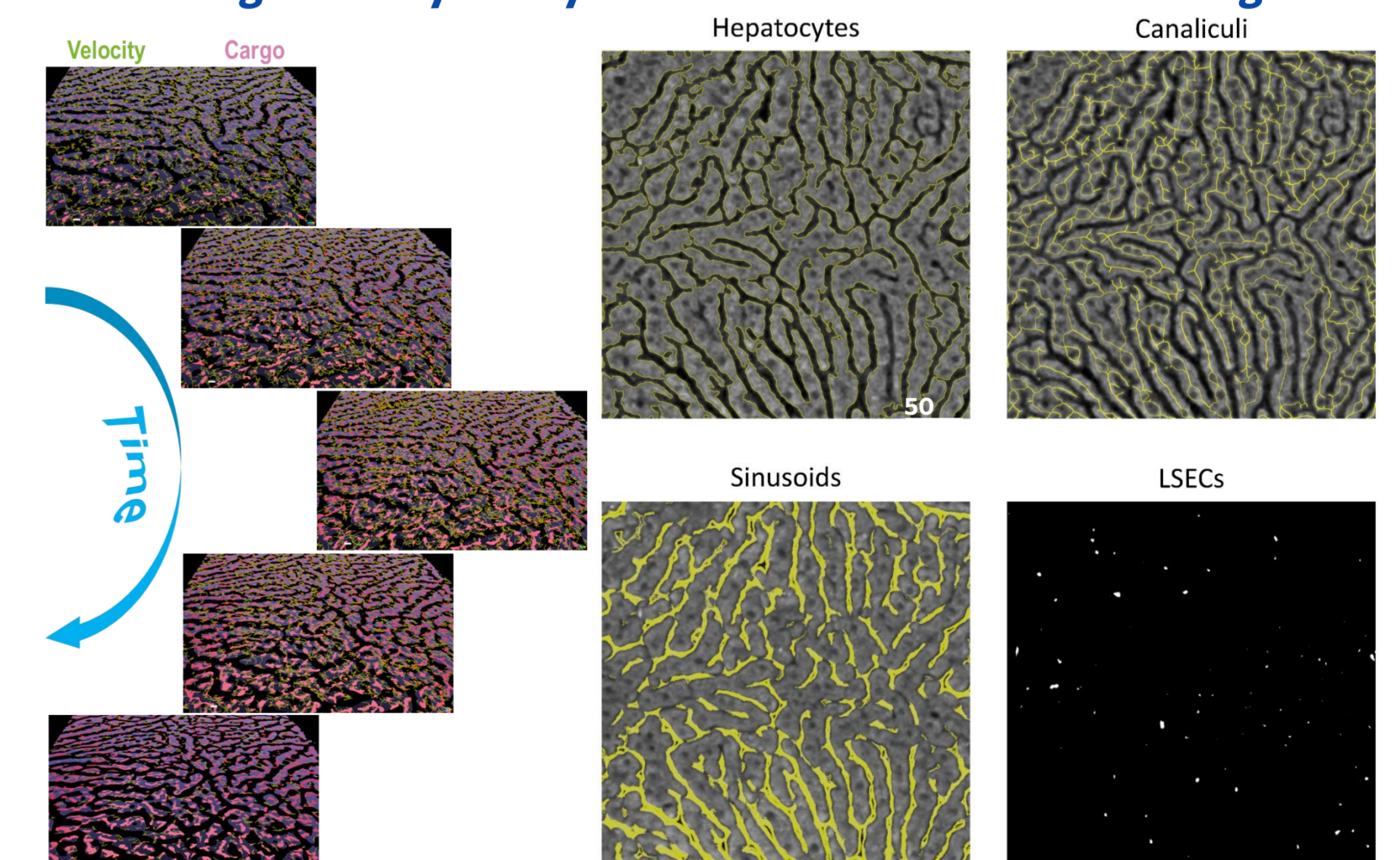## Application 3: tracking of unlabelled nematodes

Label-free analysis of *C. elegans* activity quantifies unique worm-fungus-bacteria endosymbiosis

Raw image   Hessian

Li threshold w/o outliers   Filtered outlines

Frame 1   Frame 26

Worm 10   VR = 2.04 ± 0.38

Worm 24   VR = 1.24 ± 0.12
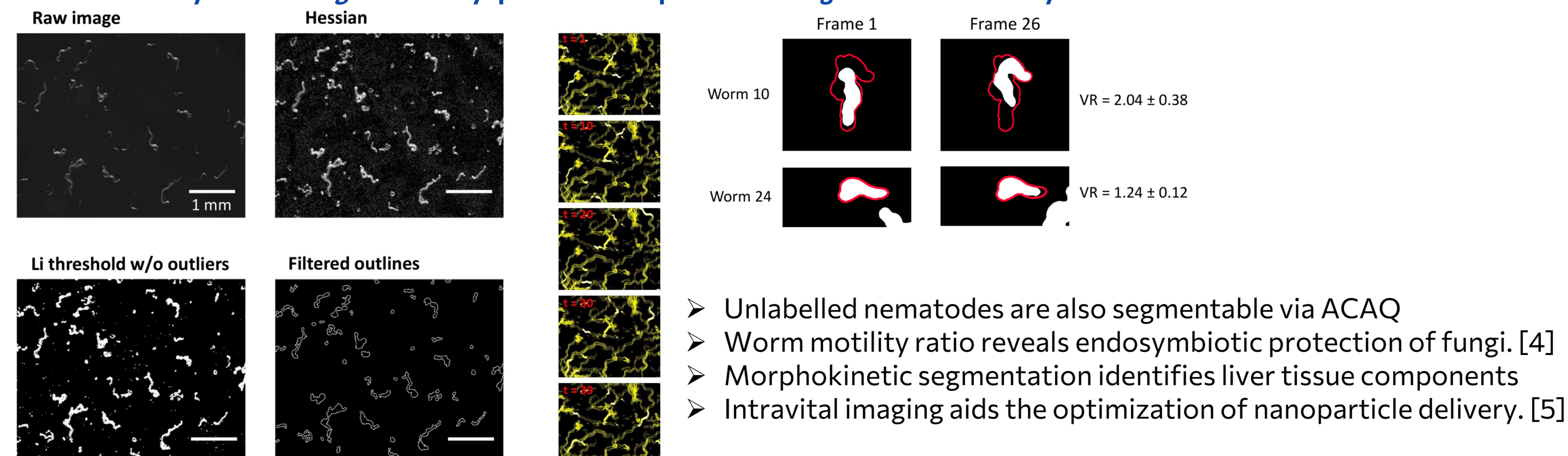
Application 4: intravital imaging of the liver

Drug delivery study without direct tissue labelling

Velocity   Cargo   Hepatocytes   Canaliculi

Time

Sinusoids   LSECs

- Unlabelled nematodes are also segmentable via ACAQ
- Worm motility ratio reveals endosymbiotic protection of fungi. [4]
- Morphokinetic segmentation identifies liver tissue components
- Intravital imaging aids the optimization of nanoparticle delivery. [5]

References
[1] Cseresnyes *et al.*, 2018. *Cytometry A.* 93(3): 346-356
[2] Cseresnyes *et al.*. 2020. *Front Microbiol.* 11: 1193
[3] Hassan *et al.* 2019. *Environ Microbiol.* 21(12): 4563-4581
[4] Büttner *et al.* 2021. *PNAS.* 118(37):
[5] Muljajew *et al.* 2021. *ACS Nano.* 15(7): 12298-12313