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3D-HyTracer: Quantitative hyphal growth analysis through hyphae-to-graph transformation

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Biological background





Organ-on-chip models allow fungal growth analysis under realistic conditions

A more physiological approach to study the interactions between fungi and host [1]

- Hyphal growth
- Hyphal branching
- Niches and microcolonies formation







Automated tracing is hindered by

- image quality due to varying signal-to-noise ratio
- crowded structures due to touching/crossing hyphae

Objective from image-based systems biology: Automated tracing of fragmentary hyphae and reconstruction into a graph structure to provide quantitative analysis



How does the infection process take place? Can we alter hyphal invasion by manipulating chemical environment?



Tracing hyphae manually is extremely time-consuming

Image analysis for hyphal growth characterization Simulation of synthetic 2D / 3D data

Analysis of 2D images to obtain characteristics of hyphal growth of *C. albicans* such as:

- Branching angles
- Distances between the individual branches



Transmitted light microscopy experiment



Segmented Hyphae with drawn characteristic



Using hyphal growth characteristics to generate ground truth data with the following properties:

- ✓ Arbitrary high temporal resolution
- ✓ High variability for endpoint experiments
- ✓ High-resolution in all dimensions (x, y, z)
- ✓ Various densities of yeast cells / hyphae
- ✓ Generation of rare experimental occurrences
- ✓ Embedding other elements (membrane)
- ✓ Individual labeling for each hypha

Reconstruction of fungal hyphae

Further development of Rivulet2 algorithm [2] for neuron tracing to **3D-HyTracer**: Adaptation of Rivulet2 for tracing fungal hyphae enables

Reliable correction of incorrect hyphal traces



Tracing output



✓ Robust compensation of interrupted hyphae
 ✓ Reliable correction of incorrect hyphal traces
 ✓ Reconstructed hyphae in a graph structure



- 2) Transition to a inverted cost map using nDT (optional: weights W) $C(x) = (nDT(x)^4 + 10^{-10}) \times W(x)$
- 3) Get 'travelling time map' (a) to approximately solving Eikonal-equation

$$C(x) = \frac{dx}{dT}, \qquad |\nabla T(x)| = \frac{1}{C(x)}, \qquad T(x_0) = 0$$

4) Gradient-based tracing

Performance on simulated data

Outlook

Application and validation on real images

> Dynamic implementation for application to 2D / 3D data





Tracing requires occasionally correcting branching and end points and the dissection of simulated hyphal structures from multiple spores

Post processing after reconstruction



Dice Score and F1-score of branching points (B) and tips (T) of 3D-HyTracer output from disrupted and undisturbed simulated 2D input. A point is considered correct if a corresponding point is within 2r in the simulation. The average hyphal radius is described by r.



Quantifying the effect of the physiological environment:

- Macrophages
- > Bacteria
- Changes in chemical environment
- Candida albicans growth in absence/presence of Lactobacillus rhamnosus
- Improving pellet segmentation
- Application and comparison with deep learning based techniques

C. albicans pellet in an organ-on-chip model

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References

[1] Maurer *et al.* 2019. *Biomater Sci.* 220: 119396
[2] Liu *et al.* 2018. *IEEE Trans Med Imaging.* 37(11): 2441-2452

