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Biofilm detection and control in the food industry

The potential of Optical Coherence Tomography to analyze biofilm architecture

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INTRODUCTION Most imaging methods used to analyze biofilms are *ex situ* and destructive¹. Optical Coherence Tomography (OCT) is a **non-destructive**, **fast**, and **real-time** approach, allowing biofilm *in situ* imaging and the 400-

measurement of different parameters². Despite the advantages of this optical technique, only a limited set of image processing scripts have been developed explicitly for processing OCT biofilm images. Since the knowledge of biofilm architecture is important to understand all phenomena related to this complex lifestyle, developing novel analysis parameters obtained from OCT imaging to evaluate the biofilm structure is of paramount importance.

MAIN GOAL

Development of novel analysis parameters obtained from 2D and 3D OCT imaging to evaluate the biofilm architecture.





Surface / Biofilm bottom

Biofilm thickness (µm)

• Function of the number of pixels between the bottom of the biofilm and the upper contour line for each vertical line in the image

Contour Coefficient

- Fraction of the biofilm that is exposed to the surrounding medium
- Values close to 1 reflect a homogeneous and flat biofilm, while in biofilms with heterogeneous structures, the values are higher than 1

Total Biofilm Volume (µm³/mm²)

• Estimates the total volume of the biofilm

Biovolume (µm³/mm²)

Provides an estimate of the biomass in the biofilm

be hampered

3D Biofilm structure

0 µm

Spatial distribution of non-connected pores



Porosity (%)

 Ratio between the data obtained from the volume of non-connected pores and the total biofilm volume

Empty spaces (%)

Since porosity is a 3D concept, this represents the free spaces in 2D imaging

REFERENCES

- ¹ Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A.R., et al. (2017) Critical review on biofilm methods. Crit Rev Microbiol 43: 313–351.
- ² Wagner, M. and Horn, H. (2017) Optical coherence tomography in biofilm research: A comprehensive review Biotechnol Bioeng 114: 1386–1402.
- ³ Romeu, M.J., Alves, P., Morais, J., Miranda, J.M., de Jong, E.D., Sjollema, J., et al. (2019) Biofilm formation behaviour of marine filamentous cyanobacterial strains in controlled hydrodynamic conditions. Environ Microbiol 21: 4411–4424.
- ⁴ Faria, S., Teixeira-Santos, R., Romeu, M.J., Morais, J., Jong, E. de, Sjollema, J., et al. (2021) Unveiling the antifouling performance of different marine surfaces and their effect on the development and structure of cyanobacterial biofilms. Microorganisms 9: 1102.
- ⁵ Romeu, M.J., Lima, M., Gomes, L.C., Jong, E.D. De, Morais, J., Vasconcelos, V., et al. (2022) The Use of 3D Optical Coherence Tomography to Analyze the Architecture of Cyanobacterial Biofilms Formed on a Carbon Nanotube Composite. Polymers (Basel) 14: 4410.

500 µm

CONCLUSIONS

- A deeper knowledge of the biofilm requires a **multidisciplinary approach** since distinct techniques provide valuable and complementary information about different aspects of the biofilm's complex structure.
- The novel biofilm parameters obtained from OCT imaging are extremely important when evaluating the biofilm architecture and behavior under different scenarios, including environmental, medical, and industrial.
- This imaging tool can be applied to evaluate the effectiveness of novel antifouling surfaces and physical and chemical mitigation approaches.

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