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## **Linking biofilm spatial structure to real-time microscopic oxygen decay imaging**

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### **Abstract:**

Two non-destructive techniques, confocal laser scanning microscopy (CLSM) and planar optode (VisiSens imaging), were combined to relate the fine-scale spatial structure of biofilm components to real-time images of oxygen decay in aquatic biofilms. Both techniques were applied to biofilms grown for seven days at contrasting light and temperature (10/20 °C) conditions. The geo-statistical analyses of CLSM images indicated that biofilm structures consisted of small ( $\sim 10^0 \mu\text{m}$ ) and middle-sized ( $\sim 10^1 \mu\text{m}$ ) irregular aggregates. Cyanobacteria and EPS (extracellular polymeric substances) showed larger aggregate sizes in dark grown biofilms while, for algae, aggregates were larger in light-20 °C conditions. Light-20 °C biofilms were most dense while 10 °C biofilms showed a sparser structure and lower respiration rates. There was a positive relationship between the number of pixels occupied and the oxygen decay rate. The combination of optodes and CLMS, taking advantage of geo-statistics, is a promising way to relate biofilm architecture and metabolism at the micrometric scale.

Keywords: confocal laser scanning microscopy, real-time images of oxygen concentration, biofilm growth, planar optodes, biofilm respiration, geostatistics