TOWARDS MEASURING OXYGEN CONCENTRATIONS IN BACTERIAL BIOFILMS USING TRANSIENT STATE MONITORING

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Biofilms are structured bacterial communities in which cells are held together within a matrix formed by self–secreted polymeric compounds [1]. They are notoriously hard to remove once formed, and are responsible for up to 80% of all bacterial infections [2]. Oxygen is required for respiration in aerobic bacteria and hence plays an essential role in the generation of energy, necessary for cell maintenance and growth. The distribution of oxygen within a biofilm can be heterogeneous and is expected to change during their life cycle. Most commonly, measurements of oxygen are carried out using electrodes. Despite being fast and robust, they are invasive in nature and may alter the micromechanical properties of the sample, giving rise to artefacts.

Here, we apply Transient State (TRAST) monitoring [3], a non-invasive method that can measure the relative populations of molecules in the triplet states by averaging the fluorescence intensity under time-modulated illumination. We use a Single Plane Illumination Microscope (SPIM), which allows optical slicing, suitable for imaging thick samples such as biofilms (Figure 1a). We present, for the first time, a map of triplet relaxation times measured inside a *Pseudomonas* aeruginosa biofilm, non-destructively, at the *μm* range (Figure 1b). We further investigate the effect of microenvironmental factors (viscosity, oxygen uptake) on the behavior of fluorescence, towards providing a protocol for quantitative oxygen concentration measurements. Our first results indicate that completely anoxic zones lie within the colonies [4], and oxygen concentration gradients are formed near the boundaries of the biofilm that extend outside the areas of high cell density (Figure 1c).

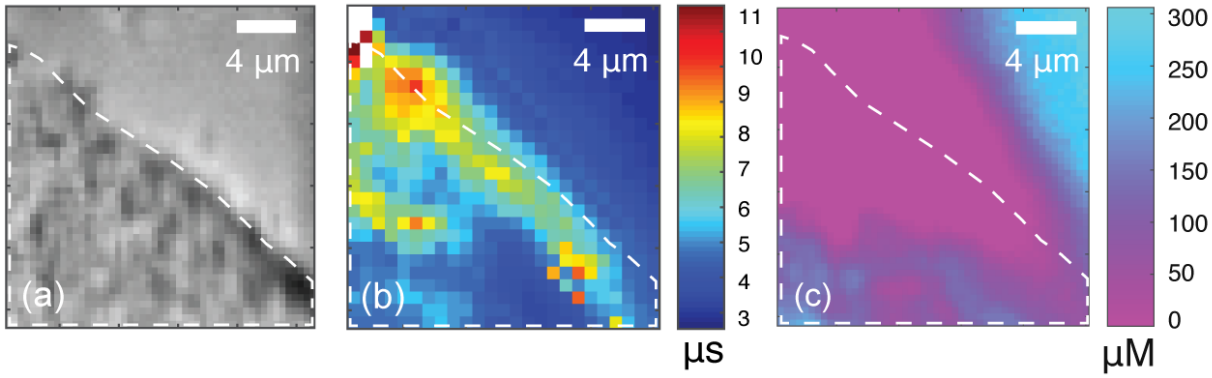

Fig 1. Imaging a P. aeruginosa biofilm microcolony (~80 h post–inoculation). (a) SPIM image. (b) Triplet relaxation time map. (c) Oxygen concentration map.

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