Applying the Mesolens to Microbiology
Investigating the Structural Organisation of Bacterial Biofilms

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Introduction

Biofilms pose an increasing public health risk due to their ability to confer chemical, mechanical and environmental protection to the constituent bacteria[1]. Previous studies have shown complex fractal patterning and chirality in multi-strain colony biofilms; however, the architecture and substructure of single-strain communities is somewhat understudied. We aim to use the Mesolens to image the previously unexplored internal architecture of an intact colony biofilm to better understand spatiotemporal organisation of a live bacterial community.

The Mesolens is a large objective lens with a low magnification (x4) lens capable of imaging a large field of view (6x6 mm) with a 3 mm working distance in either widefield epi-fluorescence or laser scanning confocal modes. A high numerical aperture (N.A. = 0.47) results in lateral resolution of 700 nm and axial resolution of 7 µm [2]. The Mesolens allows for imaging relatively large samples with sub-cellular resolution throughout the dataset with no change in objective magnification.

Using the Mesolens we have observed the internal architecture of *Escherichia coli* colony biofilms and documented previously unreported channel systems. We hypothesise that these channels are involved in structural support and nutrient dissemination throughout the biofilm.

Widefield Epi-fluorescence Mesoscopy

Strains & Culturing:
*Escherichia coli* (JM105s) expressing EGFP were inoculated at a density of 1x10^8 cfu/ml on LB medium ([Gentamicin] = 20 µg/ml) and incubated for 18 hours at 37°C in custom 3D-printed imaging moulds. Specimens were mounted in sterile LB broth prior to imaging.

Widefield Epi-fluorescence and Confocal Laser Scanning Mesoscopy:
For widefield epi-fluorescence imaging, excitation light was sourced from a pE-4000 LED lightsource (CoolsLED, U.K.) at the appropriate wavelength (λex = 490 nm) and emission was detected via a CCD camera detector (Stemmer Imaging, U.K.). For laser scanning confocal mode, excitation was sourced from a 488 nm line from a multi-line laser system (Cairn Research, U.K.) and the emission signal was detected using a photomultiplier tube (Thorlabs, USA) with a source-blocking filter. Image analysis was performed using Fiji.

Fluorescent Microsphere Uptake Assay:
Multi-excitatory fluorescent microspheres (dia. 200 nm) were seeded at an approximate density of 1.14x10^11 beads/ml with the JM105 inoculum and cultured for 18 hours at 37°C in an imaging mould. Uptake was observed by widefield epi-fluorescence mesoscopy as above, but used dual-excitation and emission of the EGFP (λex = 490 nm, λem = 512 nm) and the microspheres (λex = 560 nm, λem = 612 nm).

**Figure 1.** Flow diagram illustrating the methods used in this work. AutoCAD (Autodesk, USA) was used to design a 3D-printed chamber slide which could be used to grow colony biofilms. Widefield epi-fluorescence mesoscopy or confocal laser scanning mesoscopy was then used to assess the architecture of the colony biofilm.

3D Reconstructed of an *E. coli* Colony Biofilm

**Figure 2.** Widefield epi-fluorescence mesoscopy revealed the previously undocumented internal architecture of colony biofilms. Applying a deconvolution algorithm to the raw data (right) improved the image quality and revealed a network of channel-like structures radiating throughout the biofilm. Each channel measures approximately 15 µm wide.

3D reconstruction of *E. coli* colony biofilm

Microsphere Uptake Assay

**Figure 4.** A deconvolved 36 µm sub-stack selected from the centre of a 150 µm thick widefield z-stack of a colony biofilm. The applied colour map corresponds to the axial position of each optical section in the sub-stack. The magnified region reveals the 3D organisation of the biofilm channel system which appear as ridges protruding from the base of the colony. Dissemination systems involved in the colony formation process are also detected.

Conclusions

- The Mesolens offers a novel imaging method for studying large microbial populations with sub-cellular resolution throughout the three-dimensional dataset.
- We have observed an intra-colony channel system which we suggest plays a role in both structural support and nutrient dissemination throughout the biofilm.

References


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