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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 understudies. 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.

Methods

**Widefield Epi-fluorescence Mesoscopy**

![Widefield Epi-fluorescence Mesoscopy](Image)

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

![3D Reconstruction of an E. coli Colony Biofilm](Image)

**Intracolony Channel-System Topography**

![Intracolony Channel-System Topography](Image)

**Microsphere Uptake Assay**

![Microsphere Uptake Assay](Image)

**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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**Widefield Epi-fluorescence Imaging**

For widefield epi-fluorescence imaging, excitation light was sourced from a pE-4000 LED lightsource (CoolLED, U.K.) at the appropriate wavelength (λex = 490 nm) and emission detection was via a CCD camera detector (Stemmer Imaging, U.K.). For laser scanning confocal mode, excitation was sourced from 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 into a known density of 1.14×10^10 beads/ml with the JM105 inoculum and grown for 18 hours at 37°C in a shaking incubator. Samples were mounted onto a LB broth prior to imaging.

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).

**Widefield Epi-fluorescence Mesoscopy**

We hypothesise that the previously unexplored internal architecture of colony biofilms serves as an artefact. Reconstruction shows that the channel systems permeate from the basal to the apical surface of these colonies. Diameter of colony shown is ca. 3 mm.

**Three-dimensional reconstructions of colony biofilms**

Can be generated using processed 2-stack confocal laser scanning mesoscopy data. The arrangement of intra-colony channels agrees with observations in widefield mode, which shows that our deconvolution process does not introduce false spatial patterns as an artefact. Reconstruction shows that the channel systems permeate from the basal to the apical surface of the colony. This finding suggests that the channels are involved in both structural support and nutrient dissemination across the biofilm.