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Applying the Mesolens to Microbiology
Visualising Biofilm Architecture and Substructure

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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\(^2\). 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 (4x) 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**

- Intra-colony channel systems are revealed by widefield epi-fluorescence mesoscopy.

**Widefield Epi-fluorescence Mesoscopy**

![Widefield Epi-fluorescence Mesoscopy](image1)

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

- Confocal laser scanning mesoscopy concurs with the results of deconvolution of widefield data.

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

![3D Reconstruction of an E. coli Colony Biofilm](image2)

**Intracolony Channel-System Topography**

- Topological mapping reveals 3D organisation of the intra-colony channel system.

**Intracolony Channel-System Topography**

![Intracolony Channel-System Topography](image3)

**Microsphere Uptake Assay**

- Translocation of fluorescent microspheres correlates with spatial organisation of intra-colony channels.

**Microsphere Uptake Assay**

![Microsphere Uptake Assay](image4)

**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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