# Of Microscopes and Microbes

## Characterising a novel nutrient uptake system in *Escherichia coli* 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 *Escherichia coli* 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 (6*x*6 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 $\mu$m [2]. The Mesolens allows for imaging relatively large samples with sub-cellular resolution throughout the dataset with no change in objective magnification.

## Methods

**Strains & Culturing;**
*Escherichia coli* (JM105) expressing GFP/HcRed1 were inoculated at a density of 1x10$^4$ cfu/ml on LB medium ([Gentamicin] = 20 $\mu$g/ml) and incubated for 18-24 hours at 37°C in custom 3D-printed imaging chambers. Specimens were mounted in sterile LB broth prior to imaging. Fluorescent stains were supplemented into solid growth medium as detailed in Results.

**Widefield Epi-fluorescence and Confocal Laser Scanning Mesoscopy;**
For widefield mesoscopy, excitation light was sourced from a pE-4000 LED lightsource (CoolLED, U.K.) at the appropriate wavelength and emission was detected via a CCD camera detector (Stemmer Imaging, U.K.). For confocal laser scanning microscopy (CLSM), 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 [3].

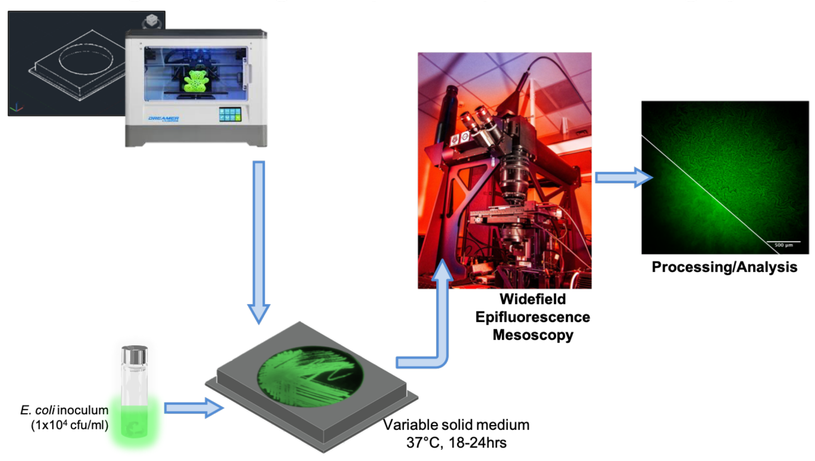


**Figure 1. General methods pipeline for this work.** AutoCAD (Autodesk, USA) was used to design a 3D-printed chamber slide which could be used to grow colony biofilms. Biofilms were grown on various solid medium supplemented with fluorescent stains/particles for each experiment. Widefield mesoscopy or CLSM was then used to assess the architecture of the colony biofilm.

## Identification of intra-colony channels

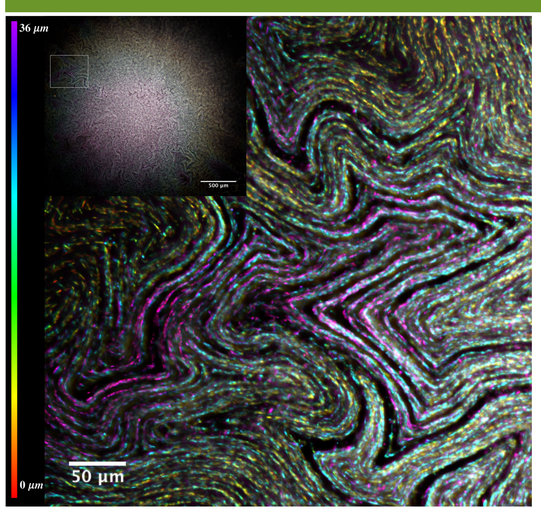


**Figure 2. Intra-colony channel systems are revealed by widefield epi-fluorescence.** Widefield mesoscopy revealed previously undocumented internal architecture of colony biofilms. A deconvolved 36 $\mu$m sub-stack with an axial look-up table is presented with an magnified region showing intra-colony channels. Intra-colony channels permeate the biofilm structure and have an 3D topology within the confines of the biofilm.

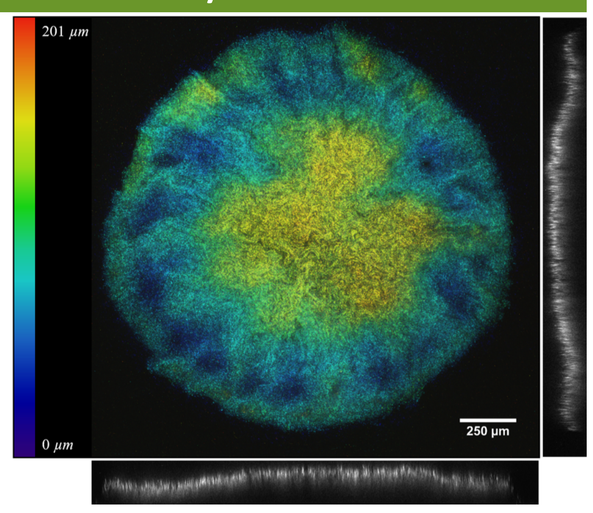


**Figure 3. Confocal laser scanning mesoscopy concurs with deconvolved widefield data.** A colour-coded maximum intensity projection of a 200 $\mu$m thick *E. coli* colony biofilm also shows the intra-colony channels we observe in deconvolved widefield datasets. Orthogonal views are also presented showing the depth profile of the biofilm. This finding shows that channels are not artefacts introduced by image processing algorithms.

## Structural assessment of intra-colony channels

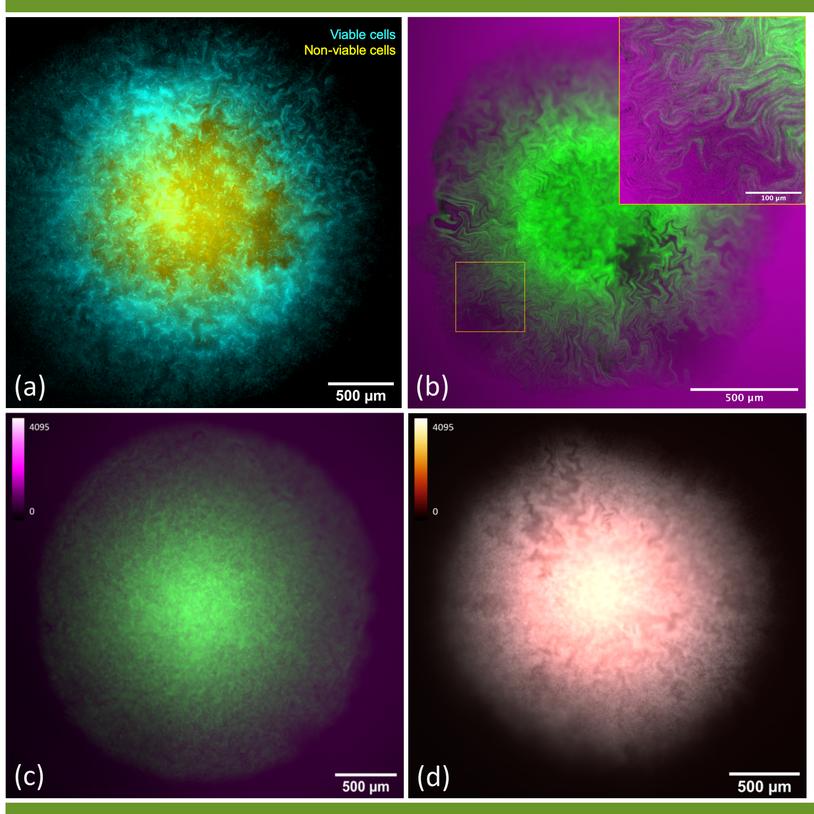


**Figure 4. Investigating the structural components of intra-colony channels. (a)** Non-viable cell distribution throughout the biofilm. We find that the channels are not occupied by non-viable/non-fluorescent cells. **(b)** Distribution of exopolysaccharides (EPS) in the biofilm. We find that channels are not composed of EPS. **(c)** Cellulose distribution throughout the biofilm. Channels are not composed of extracellular cellulose. **(d)** Lipid distribution through the biofilm. Channels are not composed of secreted lipids.

## Channels arise as a property of biofilm formation

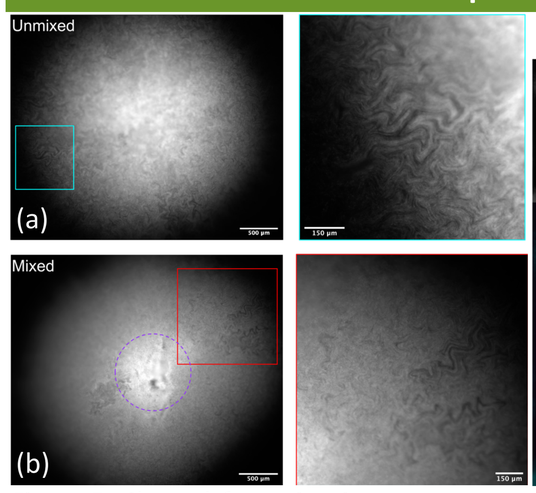


**Figure 5. Channel formation is an emergent property of biofilm development. (a)** An undisrupted biofilm imaged 20 hours post-inoculation with intra-colony channels present throughout. **(b)** After 10 hours of growth, a biofilm was disrupted to determine if channels emerge as property of biofilm development. Following a 10 hour recovery period at 37°C, channels reformed in the outgrowth. This confirms that channel formation is linked to the mechanics of biofilm development.

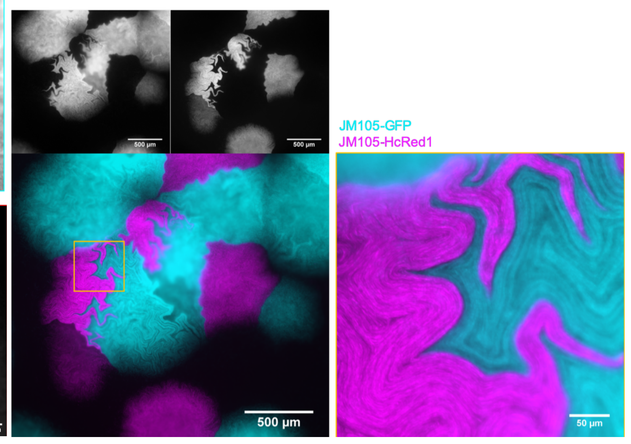


**Figure 6. Channels are not able to cross lineage boundaries.** Two isogenic JM105 strains were mixed at equal ratios, where the only genotypic difference was the expressed photoprotein. The two lineages did not mix and resulted in a fractal sectoring phenotype. Channels were unable to cross the kin-boundary between RFP and GFP-expressing strains, but were able to pass between strains which expressed the same photoprotein. This suggests that channels can be shared between homogeneous populations but not between genotypically-distinct populations.

## Functional assessment of intra-colony channels

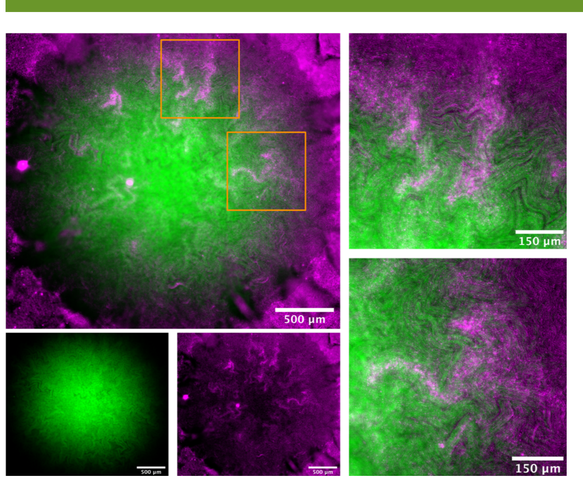


**Figure 7. Intra-colony channels facilitate uptake of fluorescent microparticles.** Prior to biofilm development a lawn of 200 nm fluorescent microspheres was prepared. Following growth of the biofilm, channels were able to facilitate uptake of fluorescent microspheres from the external environment towards the centre of the biofilm. Microsphere uptake suggests that other smaller particles (e.g. nutrients) may be transported into the biofilm via intra-colony channels.

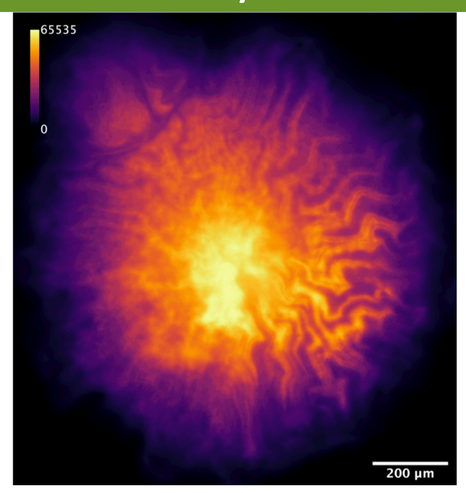


**Figure 8. The role of intra-colony channels in nutrient acquisition.** Using a fluorescent arabinose biosensor strain of *E. coli* we show that intra-colony channels present a novel nutrient acquisition system in colonial biofilms. Growth on minimal medium containing arabinose as the sole carbon source, we observe increased GFP expression surrounding the channels. This indicates a higher concentration of arabinose in the channels compared to other regions of the biofilm, indicating the role which channels play in nutrient acquisition.

## 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 and characterised an intra-colony channel system in *E. coli* biofilms which forms as an emergent property of biofilm development and plays a role in nutrient acquisition

## References

[1] Percival. *et al*, 2015. *J. Med. Microbiol.*, **64**; [2] McConnell *et al*., 2016. *eLife*, **5**; [3] Schindelin *et al*., 2012. *Nat. Methods*, **9** (7)

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