Received: 6 December 2022 | Revised: 18 January 2023 | Accepted: 18 January 2023

DOI: 10.1111/jmi.13172

Journal of Microscopy RMS

THEMED ISSUE ARTICLE

Addressing multiscale microbial challenges using the Mesolens

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Abstract

We provide a brief review of the development and application of the Mesolens and its impact on microbiology. Microbial specimens such as infected tissue samples, colonies surfaces, and biofilms are routinely collected at the mesoscale. This means that they are relatively large multimillimetre-sized samples which contain microscopic detail that must be observed to answer important questions across various sectors. The Mesolens presents the ideal imaging method to study these specimens as no other optical microscope can thanks to its unique combination of low magnification and high numerical aperture providing large field-of-view, high-resolution imaging. We demonstrate the current applications of the Mesolens to microbial imaging and go on to outline the huge potential of the Mesolens to impact other key areas of microbiology.

KEYWORDS

biofilm, high-content imaging, mesoscopic imaging, microbiology

From Louis Pasteur's disproof of spontaneous generation to Robert Koch's germ theory, our understanding of microorganisms has crucially depended on optical microscopy. Microscopes, essentially like those used by Pasteur and Koch, are still indispensable today as a complement to biochemical and molecular methods in the diagnosis of infection.

However, despite its widespread application in microbiology, the optical microscope has not changed in basic design for over 100 years. The ratio of magnification to numerical aperture (NA) has been set to a fixed value of approximately 40:1 for all microscope objectives. The reason for this is wholly historical: this proportion gives an image in which the detail is suited to the capacity of the human eye.[1] In microbiology, a 4× NA 0.1 objective is often used for searching for an appropriate area on the slide for study before switching to an objective of 60×, NA 1.3 for resolving individual bacteria. The magnification-to-NA ratio is the same for both objectives, but forces researchers to choose between imaging a few microbes in detail or thousands so poorly resolved that their shape and connectivity cannot be seen.

We are exploring the utility of mesoscale imaging in microbiology by applying the Mesolens* to imaging of large and complex specimens. The Mesolens, in which the ratio mentioned previously is 8:1 rather than 40:1, can resolve more spatial information than the human eye can accommodate.[2] Its design was originally specified for imaging 12-day-old rodent embryos, but it has also been used successfully to image a diverse range of specimens,

from blood films and immunolabelled cell lines to intact insects such as adult *Drosophila*[3] and *Tribolium* adults and larvae (mealworms).[4] The lens was designed to provide a field of view of up to 6 mm in diameter with a 3 mm working distance while giving sub-cellular resolution throughout this unusually large imaging volume. It is also chromatically corrected throughout the entire visible spectrum, and it has been used as an objective lens in brightfield, darkfield, widefield epifluorescence, confocal point-scanning,[2] HiLo,[5] lightsheet[6] and total internal reflection fluorescence mesoscopy.[7]

Here we report our progress on the application of the Mesolens and image analysis methods compatible with the large datasets it generates to advance our understanding of single-species and dual-species bacterial colonies growing on agar.[8,9] These microbial communities are notoriously difficult to study by optical microscopy because of their inherent structure; all such biofilms absorb and reflect light because they are densely packed with cell bodies and complex extracellular polymers. Moreover, biofilms exhibit phenotypes which often manifest in morphological changes to overexposure to light or the introduction of aqueous mountants. Routine microscopy fails to reveal their internal structure, but the Mesolens has proved highly useful. The ability to acquire a single high-resolution image of an entire intact biofilm overcomes the traditional drawbacks of lengthy acquisitions by stitching and tiling. These methods often introduce unwanted inhomogeneous intensity artefacts which can complicate analysis. In addition, because biofilms are prone to shedding, it is imperative to image them as quickly as possible. The Mesolens can capture approximately one full-resolution image of the entire field every 15 seconds We conclude by suggesting other applications where the Mesolens are likely to immediately impact microbiology.

1 | REVEALING THE ROLE OF CHANNELS IN MATURE COLONY BIOFILMS

The internal architecture of whole live *Escherichia coli* mature colony biofilms was investigated using widefield and confocal fluorescence mesoscopy with the Mesolens.[8] An example optical cross-section is shown in Figure 1A, where contrast is provided by the constitutive expression of a chromosomally encoded photoprotein. A network of channel-like structures is clearly visible: this permeates throughout the biofilm and links the centre of the colony to the leading edge. To discover whether these channels were functional, we prepared an agar substrate with a lawn of 200 nm fluorescent microspheres and we used the Mesolens to visualise the uptake of these microspheres into the colony biofilm. Figure 1A also shows a single optical section image taken close to the midsection of the colony, with *E. coli* cells in cyan and microspheres in magenta. From these data, it was evident that the microspheres were transported into the biomass, suggesting that the channels were active. Moreover, the physiological role of the transport channels was investigated using a fluorescent nutrient biosensor – which led us to conclude that these newly discovered channels may well assist biofilms to transport nutrients centripetally.

2 | QUANTIFYING THE ROLE OF NUTRIENT AVAILABILITY ON CHANNEL ARCHITECTURE/MORPHOLOGY

Armed with the new discovery of these functional channels being involved in nutrient transport, we hypothesised that their morphology may be dependent on their local environmental conditions. *Escherichia coli* colonies were grown in different nutrient conditions by controlling the carbon and nitrogen concentrations in the growth media and were imaged using fluorescence mesoscopy with the Mesolens (Figure 1B).[9] Using a simple custom image analysis pipeline based on the open-source software FIJI and the Python programming language (Figure 1B), it was possible to measure intra-colony channel width at various positions inside the biofilm. These measurements were used to quantify the effect of nutrient availability on intra-colony channel architecture. Our results show that channels are on average 25% wider inside carbon-limited biofilms compared to nitrogen-limited biofilms, and that channel width increases non-linearly with radial distance from the centre of the biofilm.

3 | UNDERSTANDING THE INTERACTION BETWEEN PATHOGENS IN DUAL-SPECIES MATURE COLONY BIOFILMS

Microorganisms are not commonly found in the planktonic state but predominantly form biofilms composed of two or more pathogens. Bacteria in multispecies biofilms can cooperate, compete, or have neutral interactions, depending on the species involved. Two common skin microorganisms, the bacterium *Staphylococcus aureus* and the fungus *Candida albicans*, are often found together in biofilm infections in those with underlying medical conditions. Coinfections with *C. albicans* and *S. aureus* show increased virulence and greater resistance to antimicrobial drugs than infections with either species alone, making

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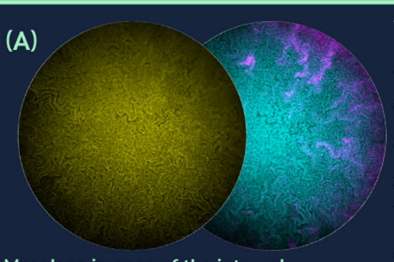
Bacterial biofilms are grown from diluted liquid cultures in custom designed imaging chambers. Specimens are then mounted in fresh liquid growth medium and imaged by widefield or scanning confocal mesoscopy using the Mesolens.

Workflow for multi-scale high-resolution imaging of mature colony biofilms using the Mesolens

(A)



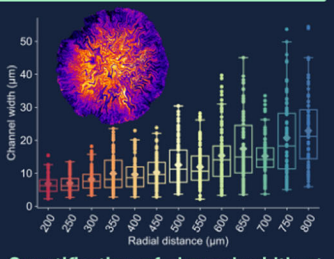
Mesolens images of the internal architecture of *E. coli* colony biofilms

The unique combination of large imaging volume and sub-cellular spatial resolution in three dimensions allows for visualisation of the internal architecture of *E. coli* biofilms at unprecedented scales.

We observed a self-organised channel system capable of transporting small particles and nutrients inside the biofilm.

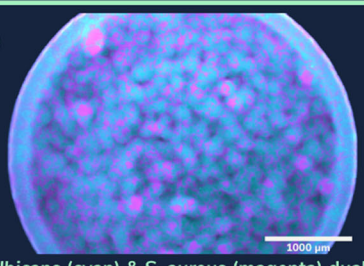
(B) We used the Mesolens to image and quantify changes in biofilm internal architecture under various environmental conditions.

Nutrient availability and substrate stiffness altered internal biofilm morphology at both the mesoscale and microscale. Transport channels were proportionally larger at the leading edge of the biofilms.



Quantification of channel width at different radial positions

(C)



***C. albicans* (cyan) & *S. aureus* (magenta) dual-species biofilm on agar, 6 hours post-inoculation**

We study interkingdom biofilms of the fungus *Candida albicans* and the bacterium *Staphylococcus aureus*, two commonly co-isolated pathogens. We use the Mesolens to investigate the link between global biofilm architecture and the increased virulence and antimicrobial resistance associated with *C. albicans* and *S. aureus* co-infection.

Looking forward: How can the Mesolens help the Microbiologist?

Microbial Ecology

Infection & Healthcare

Astrobiology

them hard to treat, and often resulting in poor patient outcomes.

The Mesolens has been used to reveal the microscopic and macroscopic structures formed by *C. albicans*/*S. aureus* dual-species biofilms (Figure 1C). The Mesolens allowed visualisation of a large area of biofilm with subcellular resolution, providing a global view of biofilm structure, and allowing identification of biological features that would normally be missed by routine microscopy. Community biofilms of *C. albicans*/*S. aureus* spanning 3.5 mm in diameter, which expressed different fluorescent photoproteins, were imaged as they developed over time. Imaging revealed the spatial distribution and compartmentalisation of both *C. albicans* and *S. aureus* subpopulations and the emergence and maturation of biofilm architecture. These types of imaging experiments have great potential in, for example, understanding the dynamics of polymicrobial infection or monitoring the response to new antimicrobial therapies in dual-species systems.

4 | BEYOND MATURE COLONY BIOFILMS

In this short review we have focused on the use of the Mesolens in the study of mature colony biofilms, but we believe that it has many potential uses in microbiology. These include:

1. The study of microorganisms in large-scale three-dimensional (3D) arrangements. These include spacings and zonation in more complex biofilms, and physiological confinement to diffusion zones, e.g., that of *Thiovulum* spp. and other organisms in a sulphide gradient.[10] Dynamic pattern formation also occurs, as in *Tetrahymena*,[11] and large-scale structures can be formed by association of amoeba, as in the grex of cellular slime moulds or the parallel association of ciliates.[12] Spatial patterning in microbial communities has been exploited in biotechnology and biomedical research[13] – understanding their 3D organisation could inform new potential applications.
2. The study of quorum sensing may benefit from the ability to image a volume of growth medium of hundreds of cubic millimetres, in which the response of individual bacterial cells can be seen as well as patterns of enhanced or suppressed growth.[14]
3. Rarely observed events, such as cell division or stages in sporulation, could be measured with good statistics because of the huge number of cells in the photometric volume. Pathogens could be located even if present in sputum or body fluids at very low concentrations.
4. Astrobiology is at the frontier of environmental microbiology and visualising the spatial organisation of microbes in harsh environments is crucial to modelling and understanding extra-terrestrial life. The survival of extremophiles in harsh environments can be studied in great detail, and billion-year-old fossilised biofilms can be visualised to understand aspects of primordial life on Earth.[15] Insight into potential life in other areas of the Solar System could be probed by mesoscopic imaging of samples returned from exploration of Mars, Europa, and Enceladus, which will help further our knowledge of the origins of life.
5. Understanding the community architecture of microorganisms involved in biogeological and bioremedial processes could contribute to improved sustainability and remediation practices in ecosystems compromised by human activity.[16] For example, visualising the stratification of marine microbial mats can provide means of predicting the impact of climate change and natural disasters.[17] Furthermore, biofilms established deep in bedrock can be displaced because of hydraulic fracking.[18] Understanding the spatial organisation, physical properties, and potential for displacement of these biofilms informs their potential for biofouling.
6. The large capture volume and high spatial resolution of the Mesolens make it ideal for studying host-pathogen interactions and inflammation in optically cleared bulk tissue or small model organisms. For example, understanding the colonisation pattern of trypanosomes and neural remodelling in intact mouse brains using light-sheet mesoscopy,[19] or understanding the host inflammatory response in excised chronically infected human tissue.

FUNDING INFORMATION

LMR was funded by the University of Strathclyde and the Leverhulme Trust. BB was funded by the University of Strathclyde. KB was funded by a Medical Research Council-sponsored Daphne Jackson Trust Fellowship and a Tenovus Small Pilot Grant, S21-14. WBA was funded by a Leverhulme Trust Emeritus Fellowship. PAH was funded

FIGURE 1 Addressing multiscale microbial challenges using the Mesolens. An overview of current applications of the Mesolens to biofilm imaging and potential future applications. A typical workflow for biofilm imaging is presented (top) followed by three use cases. (A) Using the Mesolens to observe intra-colony transport channels in *E. coli* biofilms. (B) Using the Mesolens to understand and quantify the response of biofilm transport channels to their surrounding environment. (C) Observing interkingdom interactions with the Mesolens in polymicrobial biofilms

by the Royal Academy of Engineering, RCSRF2021\11\15. GM was funded by The Medical Research Council, MR/K015583/1, and the Biotechnology and Biological Sciences Research Council, BB/P02565X/1 and BB/T011602/1.

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ENDNOTE

* 'Mesolens' is a registered trademark of the manufacturer, Mesolens Ltd.

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How to cite this article: Rooney, L. M., Bottura, B., Baxter, K., Amos, W. B., Hoskisson, P. A., & McConnell, G. (2023). Addressing multiscale microbial challenges using the Mesolens. *Journal of Microscopy*, 1–5. https://doi.org/10.1111/jmi.13172