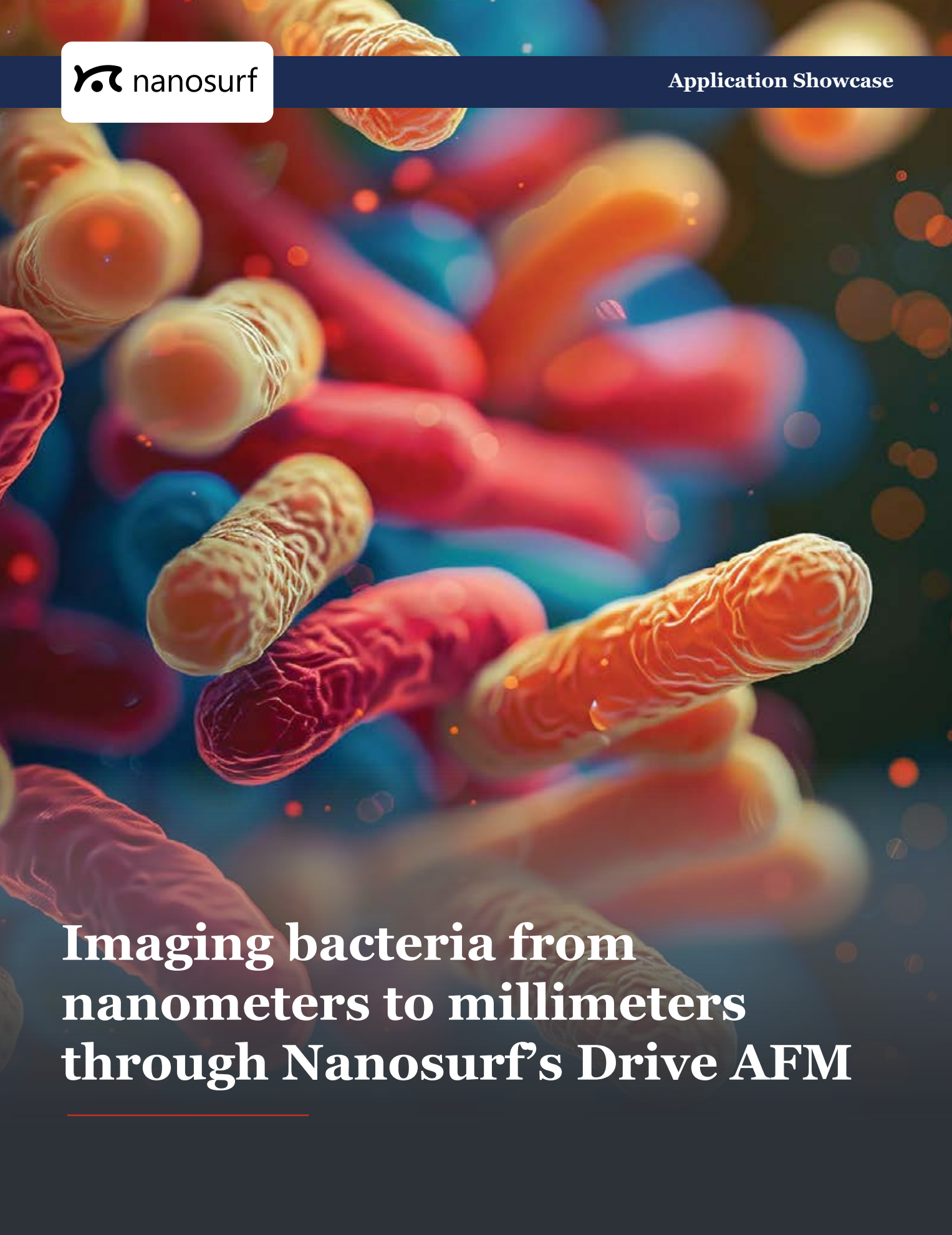




Imaging bacteria from nanometers to millimeters through Nanosurf's Drive AFM

Atomic force microscopy enables multi-scale visualization of bacterial systems, bridging molecular and community-level insights within a single workflow. High-resolution imaging reveals nanoscale surface features such as outer membrane protein organization on individual cells, while automated large-area scanning captures the structural complexity of biofilms across millimeter scales. By integrating precise force control, stable imaging, and correlative optical capabilities, this approach supports detailed investigation of bacterial architecture and its variation across length scales relevant to real-world environments.

Keywords or phrases:

Atomic Force Microscopy (AFM), bacterial imaging, biofilms, outer membrane proteins, multi-scale imaging, surface topography, Nanosurf

INTRODUCTION

With the growing health threat of antimicrobial resistance, there is a strong incentive to increase our understanding of bacterial surfaces. In this context, Nanosurf's DriveAFM and Studio offer distinct advantages for atomic force microscopy (AFM) imaging of bacteria from millimeter to nanometer length scales.



Figure 1: DriveAFM on an inverted optical microscope, enabling AFM imaging while monitoring cell viability by live-dead staining. A more compact alternative is also available, with a combination of AFM and a digital inverted optical microscope.

Compared with other available techniques, AFM stands out by offering nanometer-resolution imaging and mechanical characterization of living bacteria, as can be assessed, e.g., by fluorescent live-dead staining in a combined AFM and optical microscope setup (Figure 1).

RESOLVING OUTER MEMBRANE PROTEINS ON SINGLE CELLS

In Gram-negative bacteria such as *Escherichia coli* (*E. coli*), the bacterial cell envelope contains an outer membrane that protects the cell against harsh environments and against many antibiotics. There remain many important open questions on how this membrane is organized, how it contributes to the overall integrity and strength of the cell envelope, and how it can be disrupted by various antimicrobials.

To address these questions at molecular length scale, bacterial cells can be approximately localized on a surface by optical microscopy and next investigated at greater magnification by AFM. This is facilitated by ViewPort integration of AFM and optical microscopy in Nanosurf Studio.

Benefiting from the reliability of CleanDrive photothermal actuation of cantilevers, high resolution can readily be achieved both in dynamic mode and in WaveMode, as exemplified by the observations of networks of local, nanometer-scale depressions in the bacterial surface (Figure 2). As shown previously [1], these depressions represent individual trimers of outer membrane proteins, hence demonstrate molecular-resolution imaging on living cells.

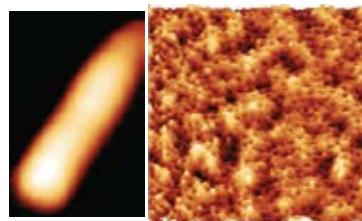


Figure 2: Images of *E. coli* acquired using Nanosurf's DriveAFM, showing the topography of an entire cell (left, scale bar: 500 nm) and of a smaller area on top of the cell (right, scale bar: 100 nm), with outer membrane protein trimers clearly resolved as local (dark/black) depressions of <10 nm wide. Courtesy: Dr William Trewby, University College London.

IMAGING BIOFILMS OVER MANY MILLIMETERS

At larger length scales, bacteria can grow and organize into biofilms covering surfaces such as catheters used in clinical settings.

Assisted by automation features such as ViewPort in Nanosurf Studio, and/or by the Python scriptability of Nanosurf products in general, it becomes possible to combine high-resolution AFM capabilities with large-area imaging such as needed for studying bacteria in real-life biofilm contexts.

Briefly, this involves the acquisition of many nanometer-resolution images of bacteria in biofilms, and next tiling and stitching of these images to generate high-resolution AFM representations that can cover many square millimeters (Figure 3).

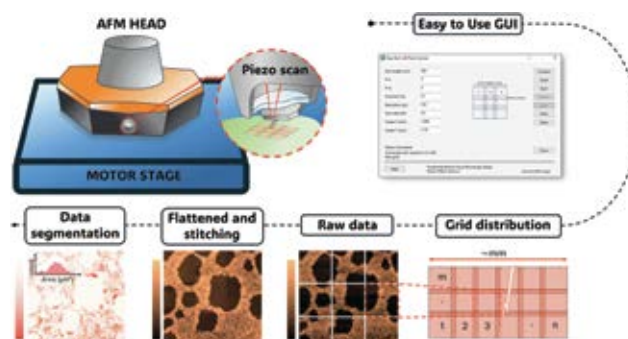


Figure 3: The large-area DriveAFM setup as implemented at Oak Ridge National Laboratory, USA. It includes an automated AFM imaging platform with a graphical interface for configuring optimal grid design and spacing. The system also features an automated processing and analysis workflow, which involves image flattening, stitching, and segmentation to generate property maps and perform statistical analyses. Reproduced from [2].

SUMMARY

Nanosurf's DriveAFM and Studio can be used for structural and mechanical characterization of single bacteria and bacterial communities in biofilms, benefiting from instrument stability, resolution and automation.

REFERENCES

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Drive AFM

