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Supplementary information

High-resolution imaging of bacterial spatial organization with vertical cell imaging by nanostructured immobilization (VerCINI)

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SUPPLEMENTARY INFORMATION: High-resolution imaging of bacterial spatial organisation with Vertical Cell Imaging by Nanostructured Immobilisation (VerCINI)

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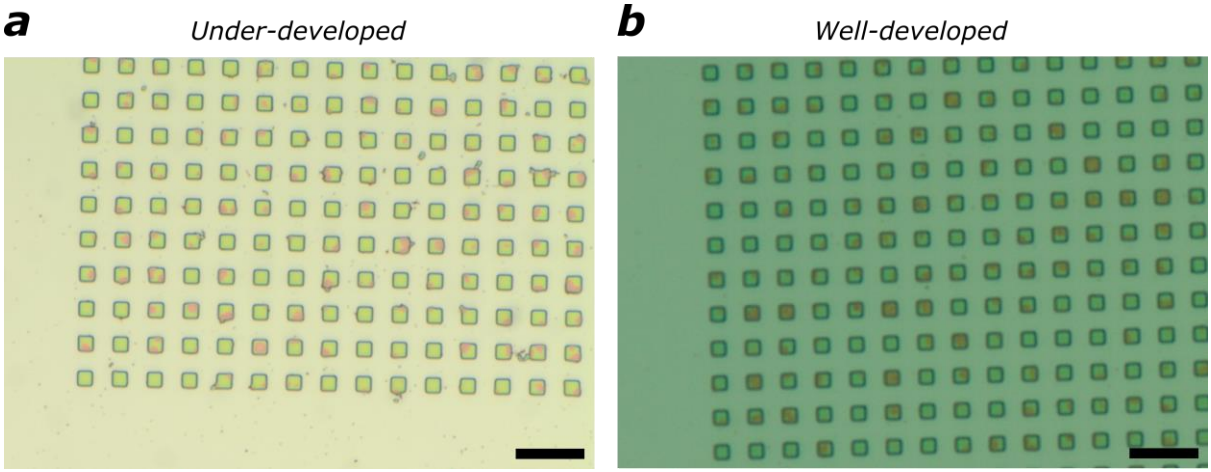
SUPPLEMENTARY FIGURES



Supplementary Figure 1: Examples of patterned resist that have and haven't developed properly. (a) A pattern that has not been developed long enough. Bits of resist are still visible between squares, and many squares have clumps hanging off of them, distorting their shapes. Image taken with a 100× microscope objective lens. (b) A pattern that has been developed properly. Little to no resist is visible between squares, and the squares have the correct shapes. Image taken with a 50× microscope objective lens. Colour differences between panels (a) and (b) are the result of slightly different illumination when images were taken. Scale bars in both panels: 10 µm.

SUPPLEMENTARY METHODS

Sample preparation for the results provided in Figure 2

Bacillus subtilis HS48 (*amyE::spc* P_{xyl}-*tlpA-gfp*)[1] cells were grown from single colonies overnight in Time Lapse Medium (TLM) at 30°C. Overnight cultures were diluted 1:5 in Chemically Defined Medium (CDM) and grown at 30°C until they reached OD_{600} ~0.25. Cells were then induced with 0.25% xylose and continued to grow for 1 hr. To image horizontal cells (Figure 2b, top), cells were then spotted onto a 2% agarose pad made of CDM with 0.25% xylose and imaged, following a previous protocol[2]. To image cells with VerCINI (Figure 2b, bottom), cells were loaded into the holes of a 6% agarose pad made of CDM with 0.25% xylose as described in the protocol text.

Imaging for the results provided in Figure 2

Imaging was done with a custom-built inverted microscope equipped with a 100× TIRF objective (Nikon CFI Apochromat TIRF 100XC Oil). Cells were illuminated with a 488 nm laser (Obis), through a

multi-band dichroic (Chroma, ZT405/488/561/640rpc-UF2) and single channel emission filter (Chroma, ET-500lp). A 200 mm tube lens (Thorlabs TTL200) and Prime BSI sCMOS camera (Teledyne Photometrics) were used, giving effective pixel size of 65 nm/pixel. Imaging was done with a custom-built ring-TIRF module[3] using a pair of galvanometer mirrors (Thorlabs) spinning at 200 Hz to provide uniform, high SNR illumination. Horizontal cells (Figure 2b, top) were imaged with this system operating in ring-HILO mode with power density of 0.32 W/cm^2. Vertical cells (Figure 2b, bottom) were imaged with this system operating in ring-TIRF mode with power density 3.2 W/cm^2. Both were imaged with a 1 s exposure with 1 frame/s interval.

Data analysis performed on the example in Figure 2

Videos were denoised using the ImageJ plugin PureDenoise[4] and registered using the ImageJ plugin StackReg[5]. TlpA-GFP clusters in the vertical cell (Figure 2b, bottom) were tracked using TrackMate[6] and the (x,y,t) coordinates exported to MATLAB for analysis. In MATLAB, Cartesian coordinates (x,y,t) were transformed to polar coordinates (ρ,θ,t) to produce Figure 2c. Mean squared displacements (MSDs) were calculated for each cluster and fitted to the generalized diffusion equation $\langle r^2(t)\rangle = K_\alpha t^\alpha$, where K_α is the generalized diffusion coefficient. The three clusters shown in the figure yielded $\langle K\_\alpha\rangle = 5 \cdot 10^{-7} \pm 6 \cdot 10^{-7}$ μm^2/s$^\alpha$ and $\langle\alpha\rangle = 1.7 \pm 0.3$ (mean ± SD).

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