**Material and methods – TEMPLATE for microscopy**

Read these very useful guidelines: [Community-developed checklists for publishing images and image analyses](https://www.nature.com/articles/s41592-023-01987-9).

Below, you can find 2 examples of Material and methods text. **It is essential that you modify them to match your own experiment.**

* The current specifications of the LCI microscopes and objectives can be found on our website, under Microscopes and under Objectives.
* All details about the acquisition settings you used can be found in the image metadata (in NIS Elements, right click in the image and select Properties). Ask us if you are unsure.
* Send us your final Material and methods text before submitting so we can review it. 😊

**Example: Crest optics spinning disk confocal**

**(can be adapted to Yokogawa CSU-X1 or single point confocal, see comments)**

The images were acquired on a Nikon spinning disk confocal running with the NIS Elements software and equipped with a Nikon Ti2 microscope, a Celesta laser light source (Lumencor), a Kinetix camera (pixel size 6.5 µm, Photometrics) and a X-Light V3 spinning disk (50 µm pinholes, 250 µm interval, Crest Optics). The images were acquired with a 20x/0.75 air objective. A 1.5x lens was inserted in the light path to ensure image acquisition at Nyquist sampling.

The excitation and emission wavelengths in nm used to image were: DAPI (ex 405, em 438/24), GFP (ex 477, em 515/30, Alexa Fluor 568 (ex 545, em 593/40). The imaging parameters were set to have no saturation in the regions of interest.

Z stacks were also acquired at Nyquist sampling using the z step recommended in the software (z step 1 µm).

For live samples, a full cage incubator (Life Imaging Services) was set to 37°C, 5% CO2 and 95% humidity. The sample was exposed to 15KJoule of illumination with an exposure time of 30 msec. Images were acquired for 3h at 10min interval.

To ensure a reproducible focus between samples, the tubulin staining was used to focus on filopodia at the bottom of the cells.

The image brightness and contrast were adjusted to allow detailed visualisation. The same adjustments were applied to images that should be compared. Pseudocolours or gamma adjustments were applied whenever specified in the figure legend.

The JOBS module in NIS Elements was used to run the image acquisition pipeline. A tiled image of the whole well was first acquired with the 4x/0.3 air objective and automatically stitched with blending in NIS elements. The regions of interest were automatically detected using a General Analysis 3 pipeline, then automatically reimaged using the 20x/0.75 air objective.

All original images and analysis pipelines are available on the BioArchive image repository with URL…

**Example: M2 Life light sheet**

The images were acquired on an Aurora Airy beam light sheet system (M2 Life) running with the M2 Cubes software and equipped with Omicron LightHUB Ultra laser light engine, two Orca Flash 4 V3 cameras (pixel size 6.45 µm, Hamamatsu) and two multi-immersion objectives (Navitar).

The samples were imaged in Cubic 2 imaging solution which has a refractive index of 1.47, leading to a final objective magnification of 16x and numerical aperture of 0.4, ensuring image acquisition at Nyquist sampling (slight undersampling for DAPI).

Z stacks were also acquired at Nyquist sampling using 0.4 µm z step.

The excitation and emission wavelengths in nm used to image were: DAPI (ex 405, em 438/24), GFP (ex 477, em 515/30, Alexa Fluor 568 (ex 545, em 593/40). The imaging parameters were set to have no saturation in the regions of interest.

Images were deconvolved (Richardson-Lucy, 100 iterations) and stitched using the M2 Life software.

The image brightness and contrast were adjusted to allow detailed visualisation. The same adjustments were applied to images that should be compared. Pseudocolours or gamma adjustments were applied whenever specified in the figure legend.

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