Electronic Supplementary Material (ESI) for Lab on a Chip.

ESI for the paper:

IR-Live: Fabrication of a low-cost plastic microfluidic device for infrared spectromicroscopy of living cells

By

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1. Resist primary master.

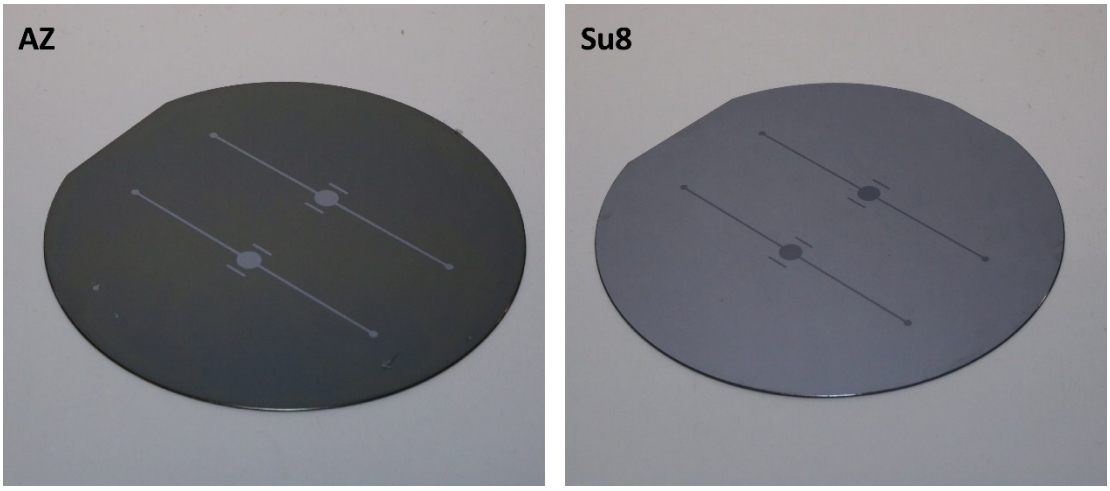

Figure S1 – This pictures shows an example of the UV-lithography prepared moulds, with positive tone resist (left) and negative tone resist (right).

2. SEM characterization of the NOA73 slab with calcium fluoride window embedded

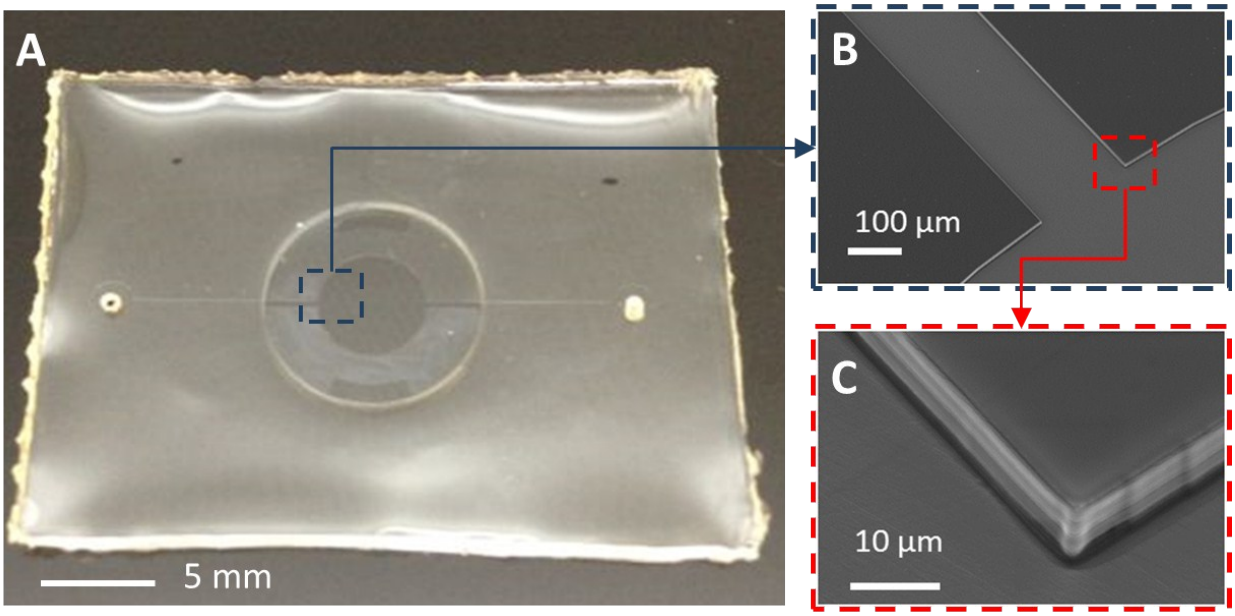

Figure S2. A) A photo of the top half of the device with the structured NOA 73 layer. B) An SEM image of the in-let area on the device. C) A close-up SEM image of the in-let area shown in B. The high fidelity of the moulding process is revealed by the scalloping of the walls, which arises from the dry etching of the silicon master mold. The scratches on the CaF2 surface are produced during the surface polishing by the manufacturer.

3. FTIR characterization of the sealing quality

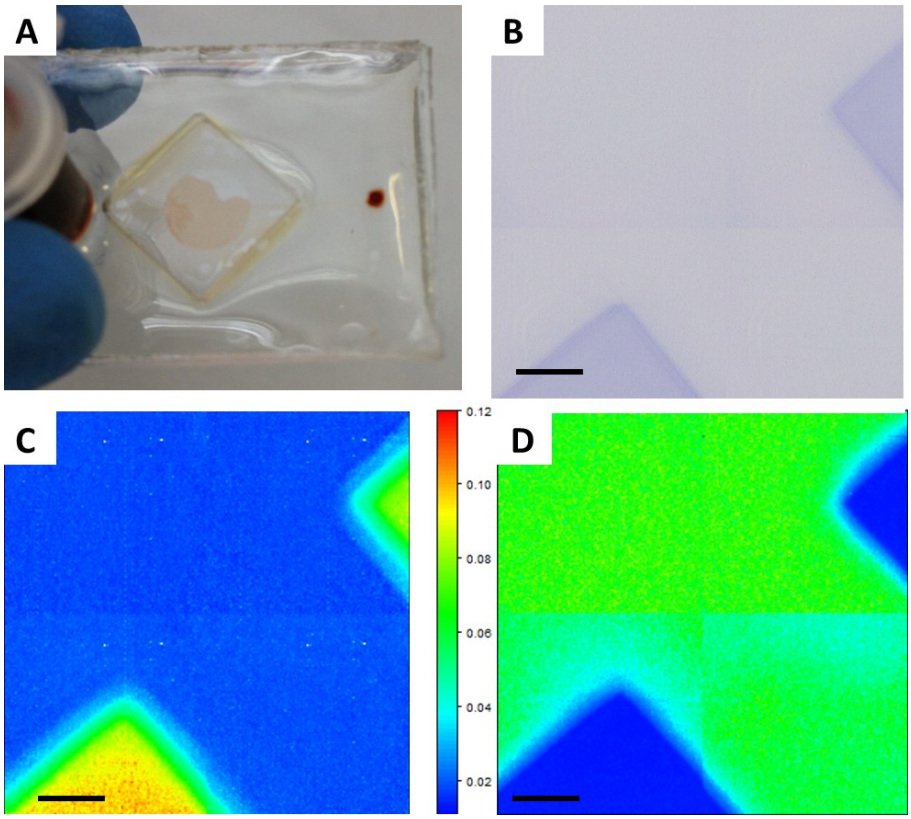

Figure S3. A) An image of a device with a central chamber being filled with a red-color dye. B) An optical microscope image of the water filled device taken with the Vis-IR microscope Hyperion 3000 Vis-IR Bruker microscope at BSISB. C) A heatmap of the water filled device acquired with the FPA at BSISB and reconstructed by integrating the polymer peak (CH2-CH3 stretching bands at 2800-3000 cm-1). D) A heatmap of the water filled device acquired with the FPA at BSISB and reconstructed by integrating the water peak (O-H bending band centred at 1642 cm-1). Scale bars = 50 µm

4. Materials viability: Optical images of REF52 cells cultured on calcium fluoride and NOA-embedded calcium fluoride windows for 2 days

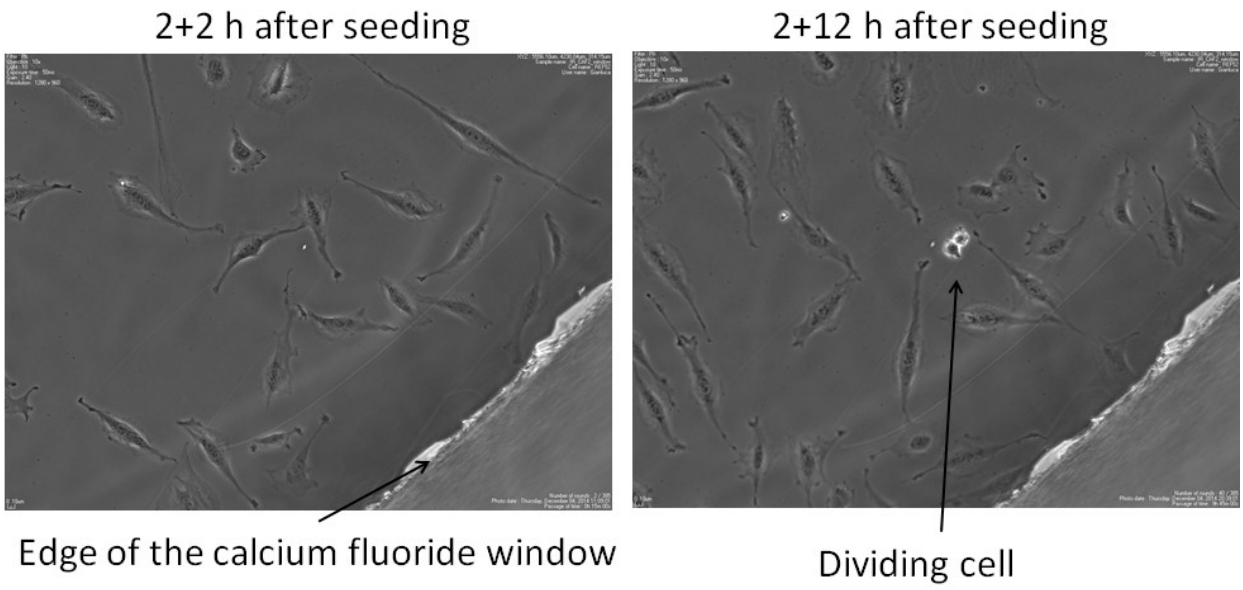

Figure S4. REF52 cells seeded on top of a calcium fluoride window, which is placed on a petri dish filled with culture medium.

In figure S4 we show REF52 cells cultured on the surface of a calcium fluoride window, which is placed at the bottom of a standard petri dish filled with medium. After placing the window on the petri dish, 5 ml of fresh medium was added and then cells seeded. The petri dish containing the substrate was then kept 2 h inside a standard incubator to let the cell attach. Afterward, the sample was places in the IM-Q Biostation and image acquisition started. Left picture shows the cells after 2+2 h (2 h in the Biostation), the right one is taken 12 h later.

5. Device viability: cells culturing test monitored by Nikon IM-Q Biostation

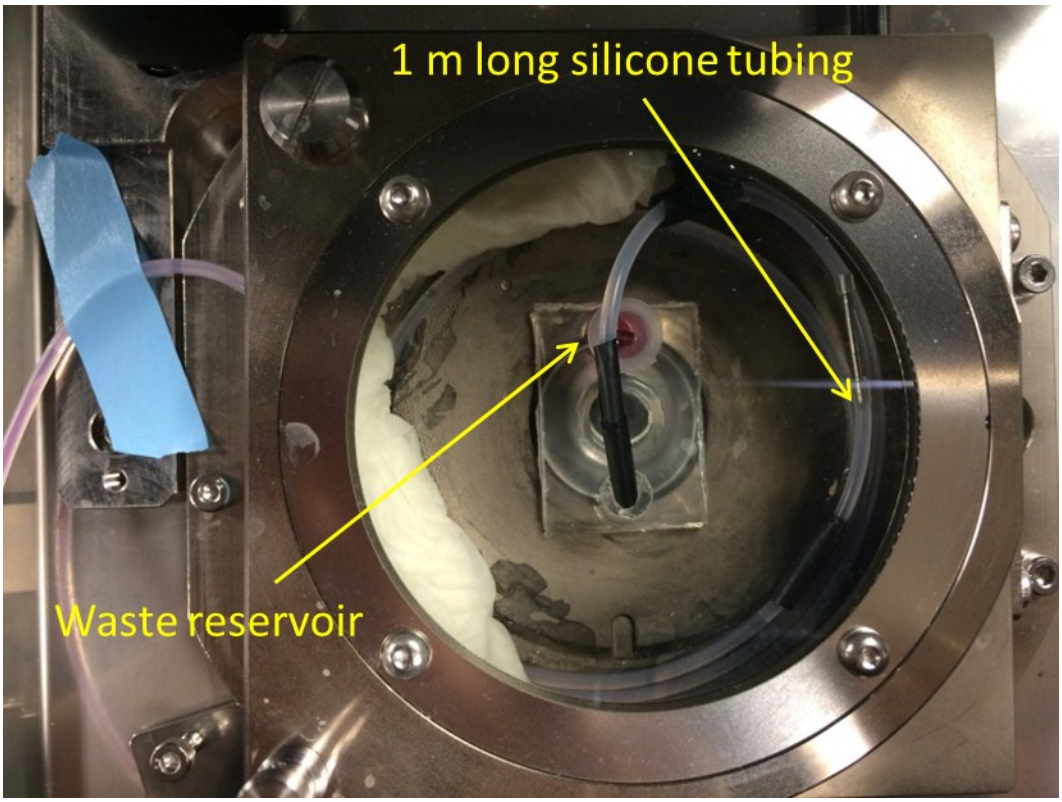

Figure S5. . An IR-live device placed in the environmental chamber of the Nikon IM-Q Biostation. It was used to observe the behaviour of REF52 cells injected and maintained inside the device. The 1-m long silicone tubing was coiled inside the chamber to allow equilibrate with the CO2 in the medium because of its permeability to gases.

6. REF52 visible microscope image

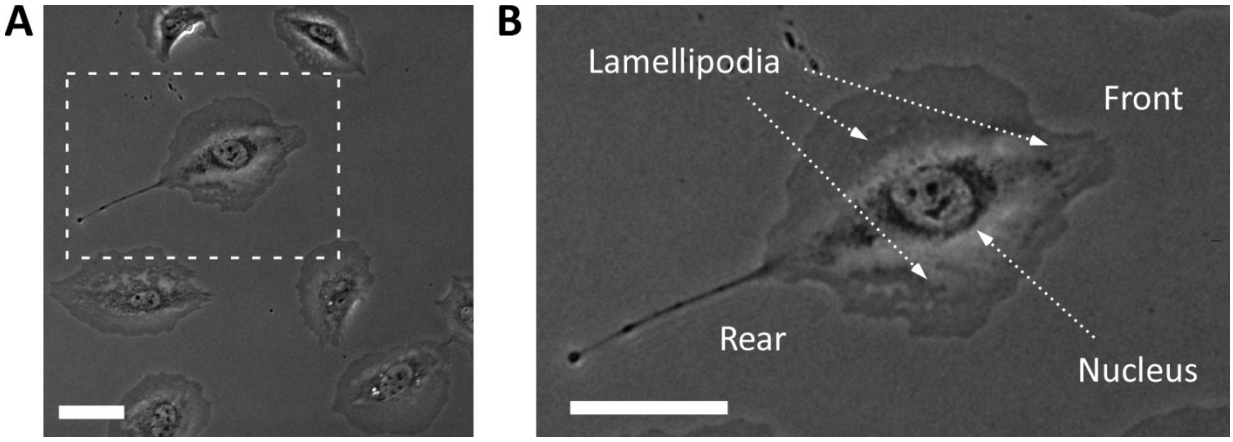

Figure S6. A) An image of REF52 cells taken with an Olympus IX71 microscope with a 20x air objective. B) A close-up image of the same cell indicated in (A). Scale bars = 10 μm

7. PCA analysis

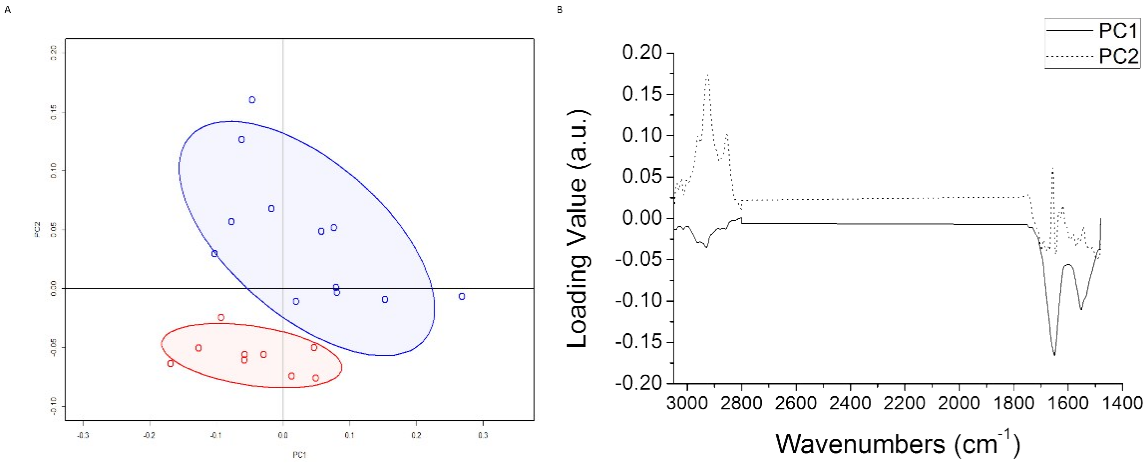

Figure S7 A.. A) A PCA plot of IR spectra of REF-52 cells. Red are spectra from cells at t0=0, blue represent the spectra of cells acquired after 24h inside the device, t1=24h. B). Loadings of the PC analysis; solid line = PC1 (proteins), and dotted line = PC2 (lipids).