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Clip-Chips: 3D printed microfluidic and characterization modules for cell culture applications

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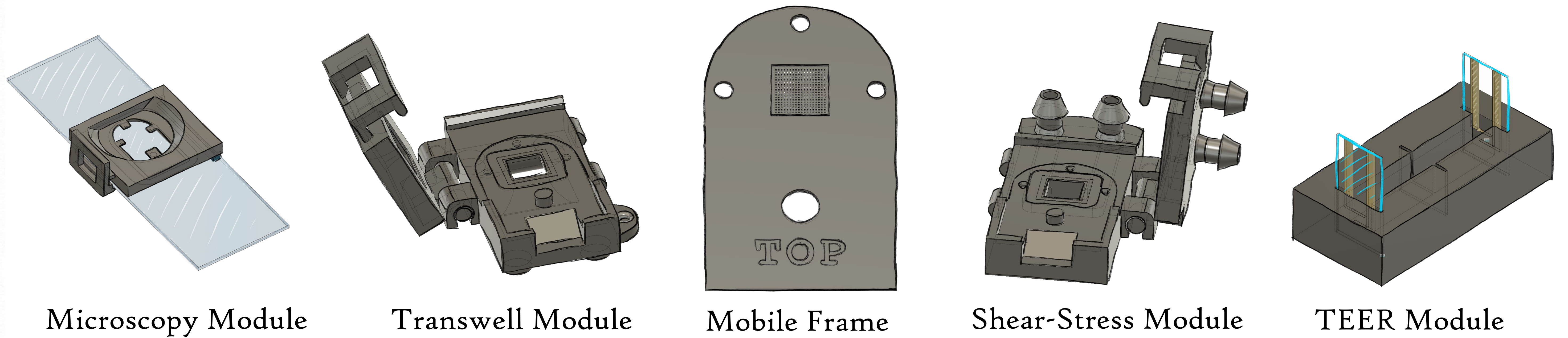
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The Mobile Frame and its Modules

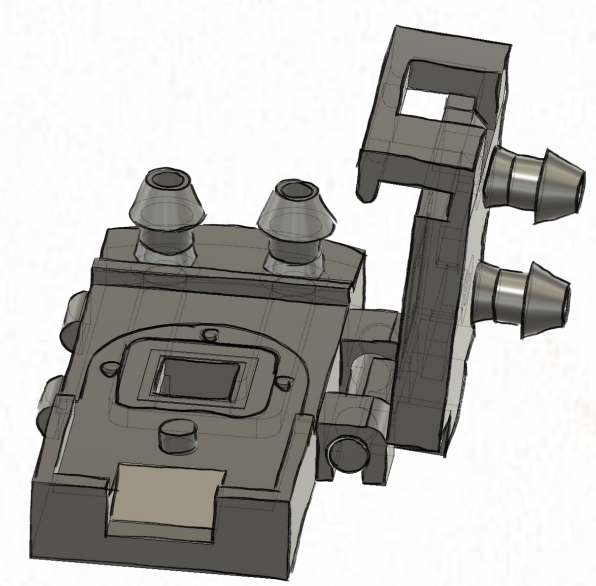
Mobile frames with a perforated membrane as a support for 2D and 3D cell culture inserted in modules with dedicated functions



Clip-Chips

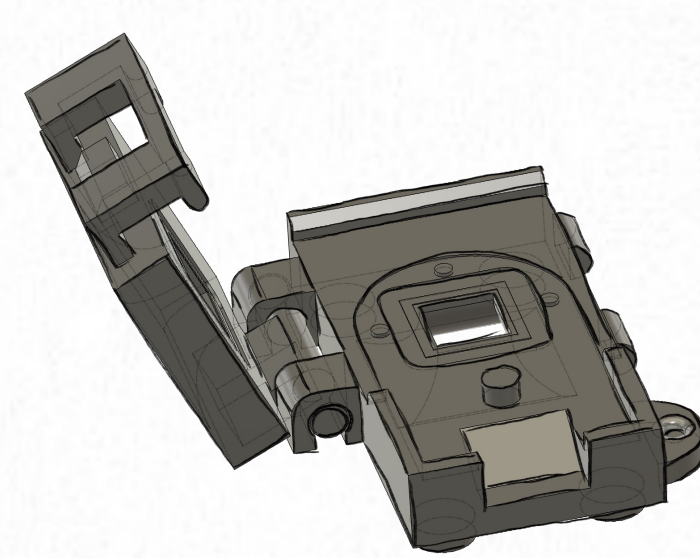
3D printed microfluidic chips with hinges and a clip-based locking mechanism for a reversible closing of the system.

Shear Stress Module



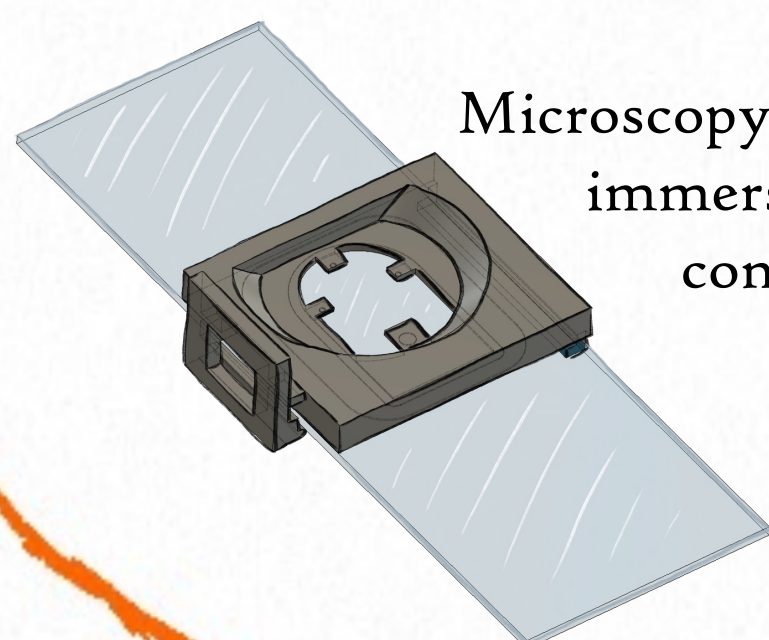
Microfluidic chip for an apical shear stress stimulation and an automated basal medium change on cultured epitheliums

Transwell Module



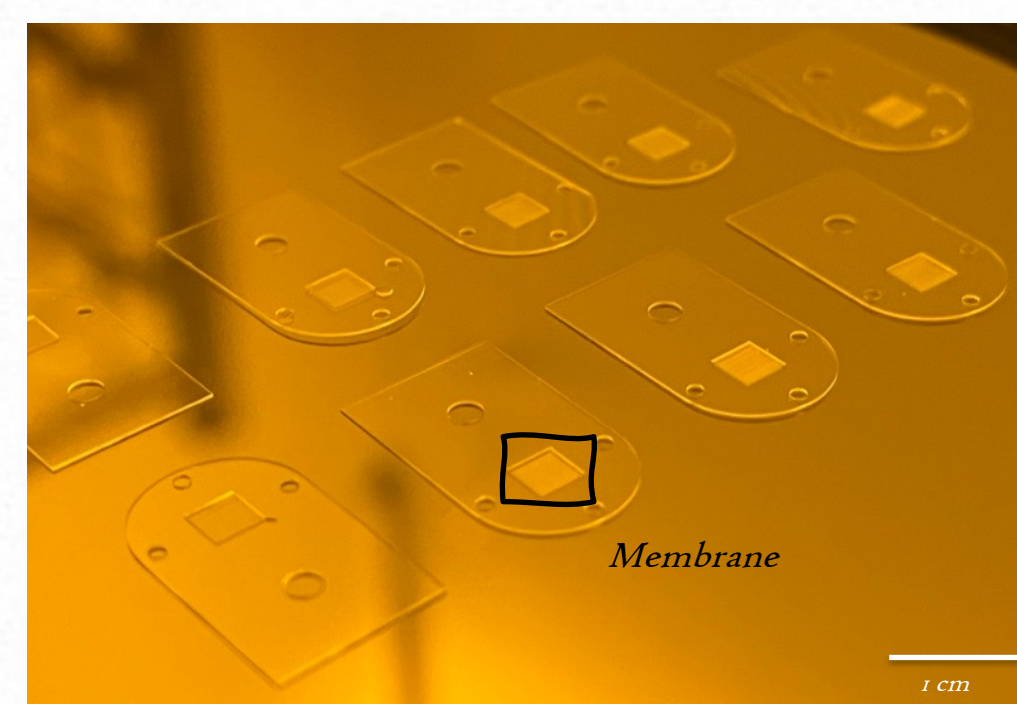
Transwell-like cell culture for well-plate use and translocation assays

Microscopy module for immersed upright confocal microscopy



Mobile Frames

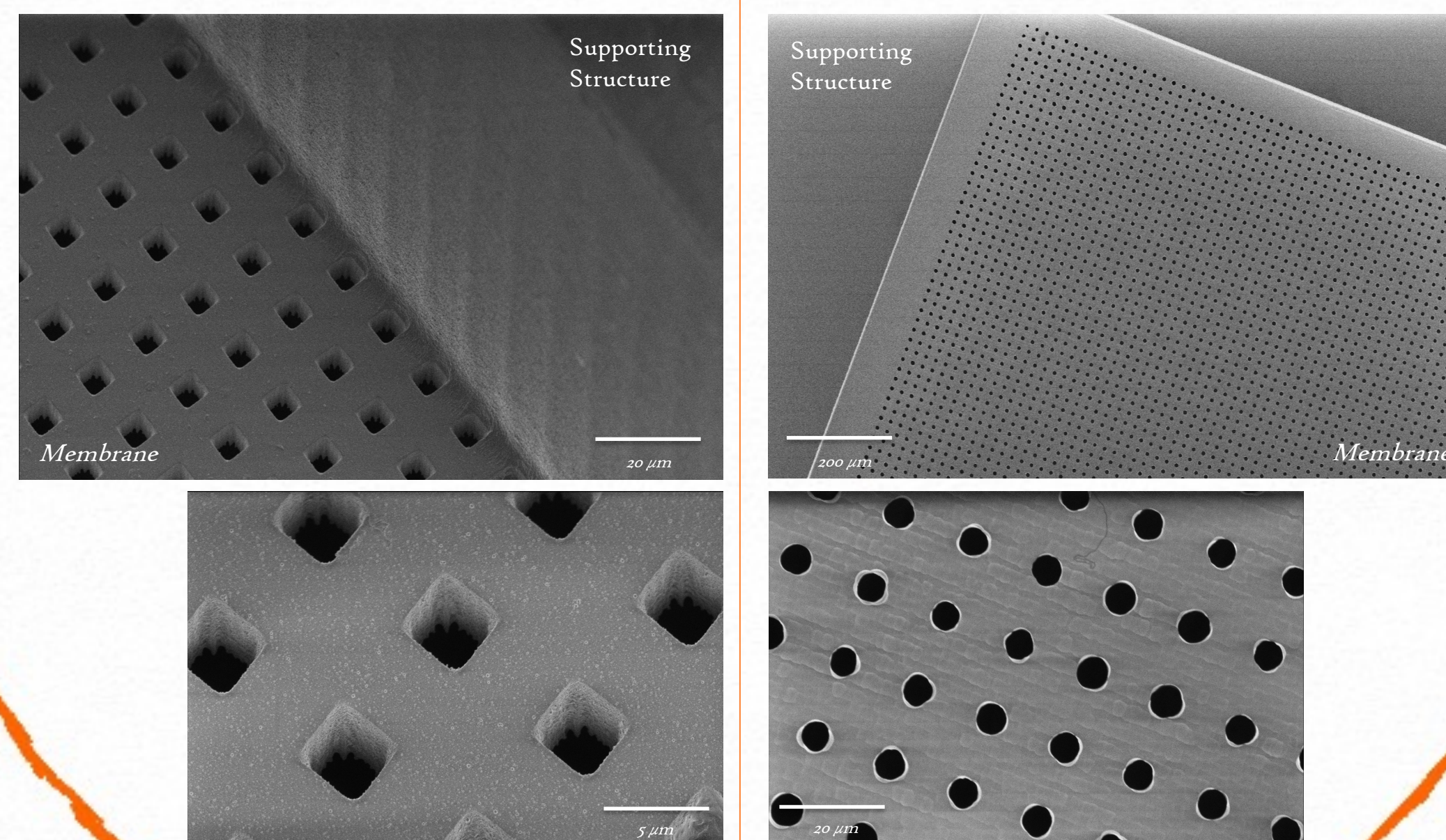
SU8



Glass

Two-Step Photolithography Process

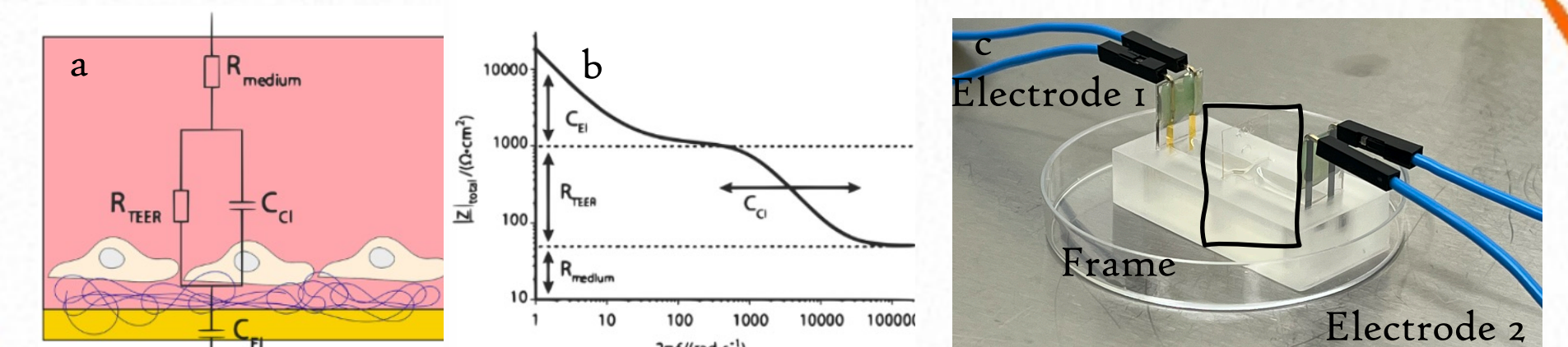
Femtosecond Laser Irradiation and Chemical Etching



SEM micrographs of the 9 mm² SU8 (left) and Glass (right) membranes with respectively 7 μm and 13 μm thickness, 5 μm pore size and 90 000 pores.

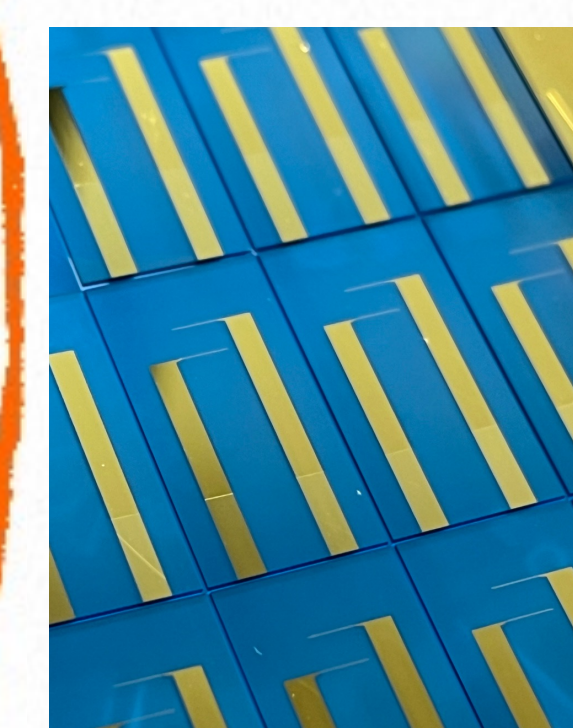
TEER Reader

Slide-in system for TEER measurement on mobile frames (Trans epithelial Electrical Resistance Measurement)



Equivalent circuit (a) and typical impedance spectrum (b) of cells in 2D composed of :
 1. R_{TEER} Transepithelial Electrical Resistance (Para-cellular pathway, cell tight junctions)
 2. C_{CL} Cell Layer Capacitance (Trans-cellular pathway, membrane topology)
 3. R_{MEDIUM} and C_{EL} representing respectively the medium resistance and the interfacial (electrode electrolyte) capacitance.
 The arrows on the impedance spectra (b) represent the impact of each electrical component on said spectra

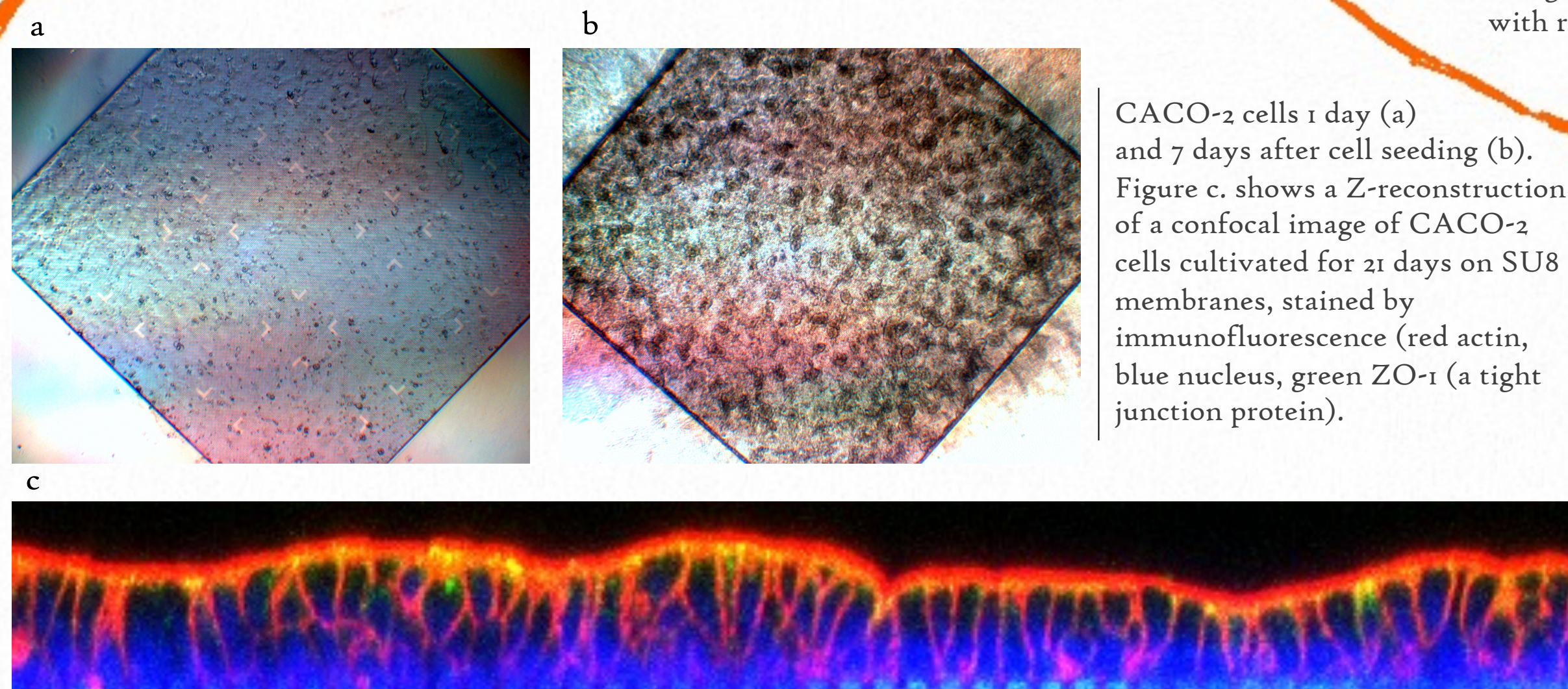
This system allows for fast measurement (15 seconds per frame) of all the above-mentioned parameters of an epithelium cultivated on the mobile frames. These measurements are self-calibrating as information about the medium and the sensor is embedded in the spectrum, thus being suitable for all kinds of cell culture substrate



Microfabricated Electrodes
 1. PVD Deposition Cr/Au (200nm)
 2. Electrode Patterning by laser etching
 3. Electrical insulation (Perminex)
 4. Dicing

Caco-2 Culture

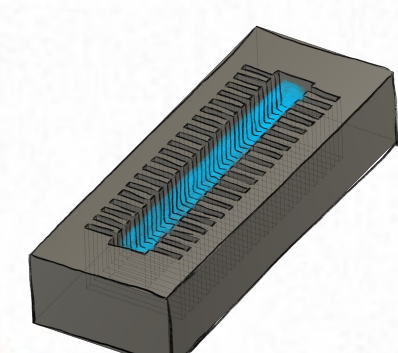
Immortalized colorectal adenocarcinoma cells cultivated on SU8 membranes for 21 days.



CACO-2 cells 1 day (a) and 7 days after cell seeding (b). Figure c. shows a Z-reconstruction of a confocal image of CACO-2 cells cultivated for 21 days on SU8 membranes, stained by immunofluorescence (red actin, blue nucleus, green ZO-1 (a tight junction protein)).

(Very) Simple Cell Seeding Protocol

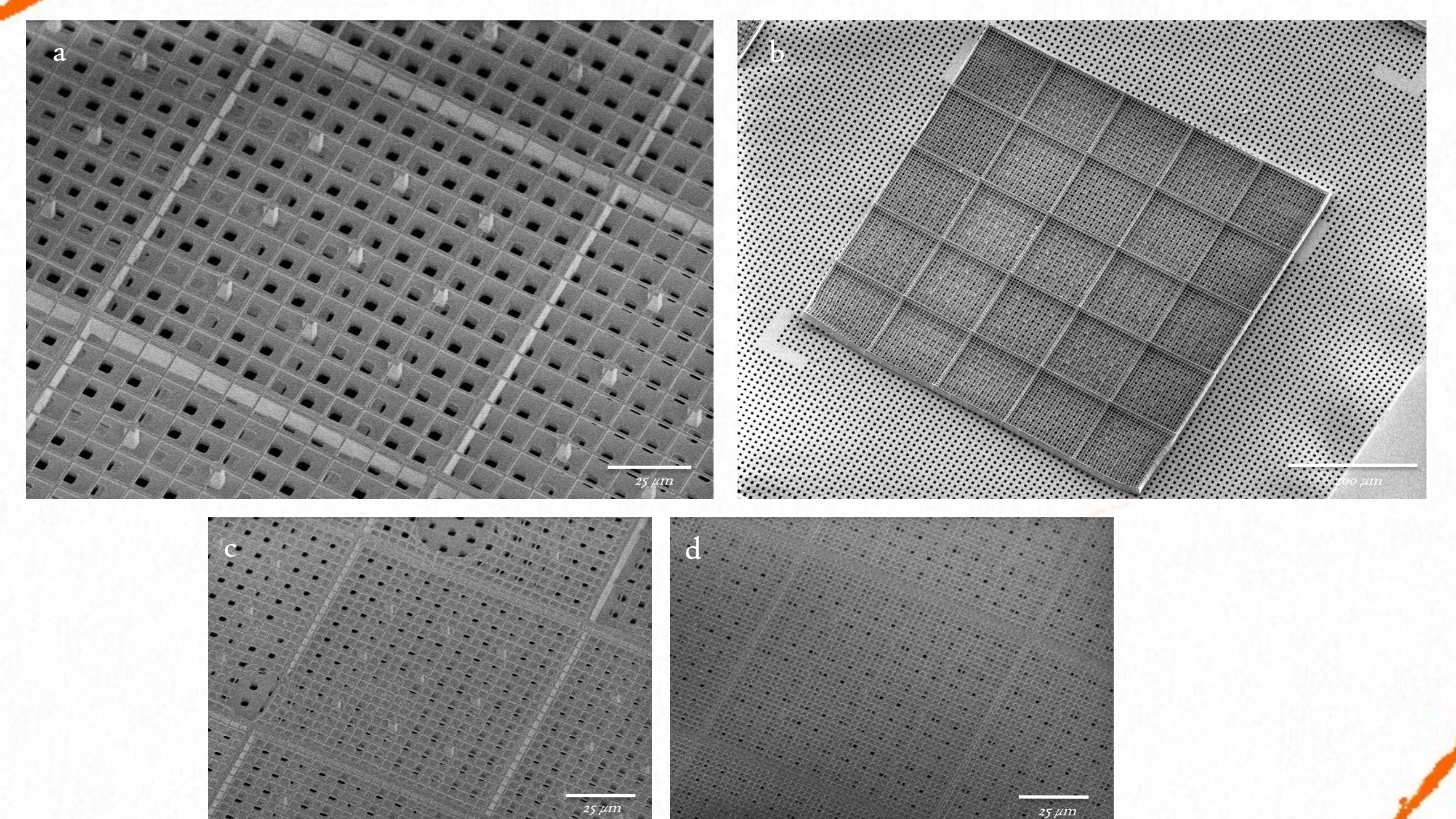
1. Hard bake (150°C - 2h) and O₂ Plasma (5 min, 15 sccm, 0,6 bar)
2. Laminin Coating (6μg/mL) for 4 hours
3. Deposit a 15 μL drop of medium with cells on the membrane
4. After 1 hour of incubation, top up with medium



Frame holder for sterilization and coatings steps.

3D Net Printing

2-Photon Printing with Nanoscribe GT2 Printer IP-Dip acrylate Resist



SEM Micrographs of the 3D printed nets with 100 μm sides and 1 μm fibers on top of the SU8 membrane with varying pore size: 10 μm (a and b), 5 μm (c) and 3 μm (d).

Advantages :

- Frame handling through modules is ergonomic and allows for a high experimental throughput
- High versatility of the frames which can accommodate membranes of different materials: PET, GelMa, SU8, Glass, ...
- Implementation of a new functionality = Development of a dedicated module = No increase in overall system complexity