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3D Integration of a μ Sieve for Particle Filtration Combined with real-time *in-situ* Analysis within Complex Media

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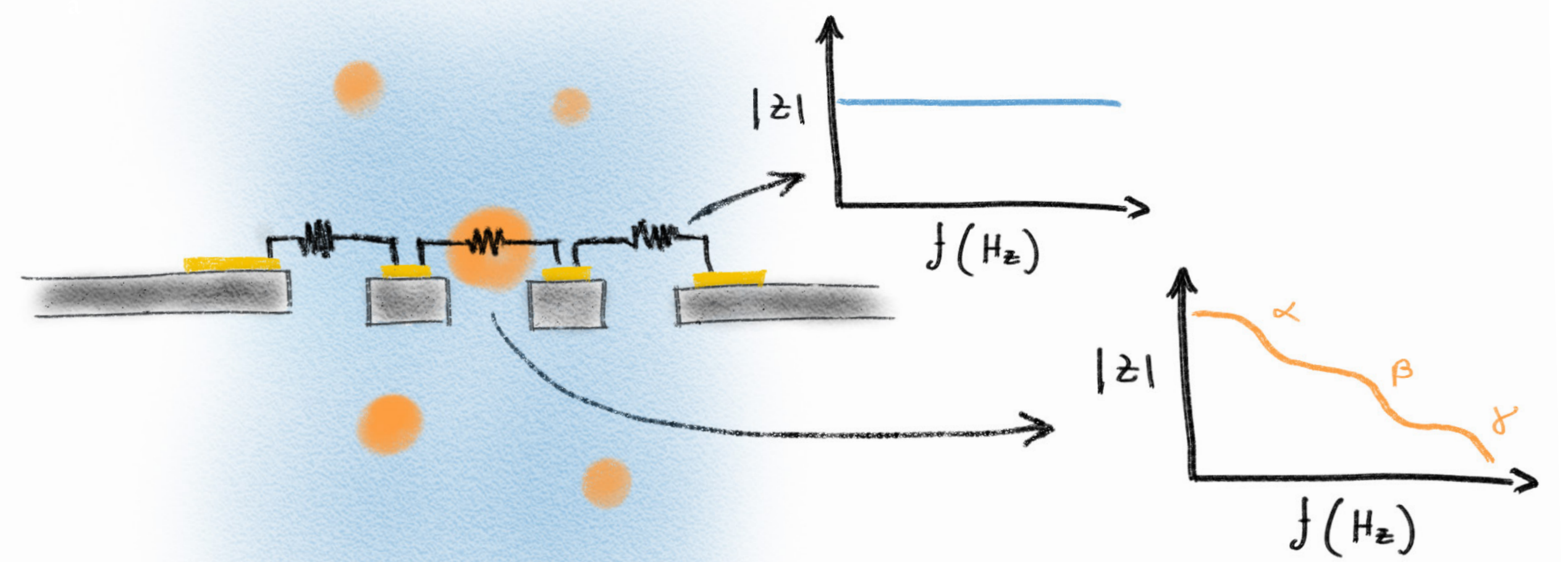
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Particle Filtration and Analysis Within Complex Fluids

Context

In microfluidic filtration systems, two of the leading obstacles to large volume sampling in complex media are flowrate and retrieval of the filtrate for characterization. We introduce a novel microfluidic sieve allowing for large flowrate thanks to surrounding fluidic slits, together with a dual (electrical and optical), real-time *in-situ* characterization of the filtrate, thus avoiding the challenging step of retrieval for analysis. The real-time characterization challenge is addressed by implementing sensing electrodes directly onto the μ Sieve together with the integration of this device within a 3D printed transparent microfluidic chip allowing for live optical control. Moreover, *in-situ* cell culture post filtration is demonstrated, which adds substantial interest and could bring new insights in liquid biopsy applications, particularly for the analysis of rare biological elements such as circulating tumor cells in complex media.

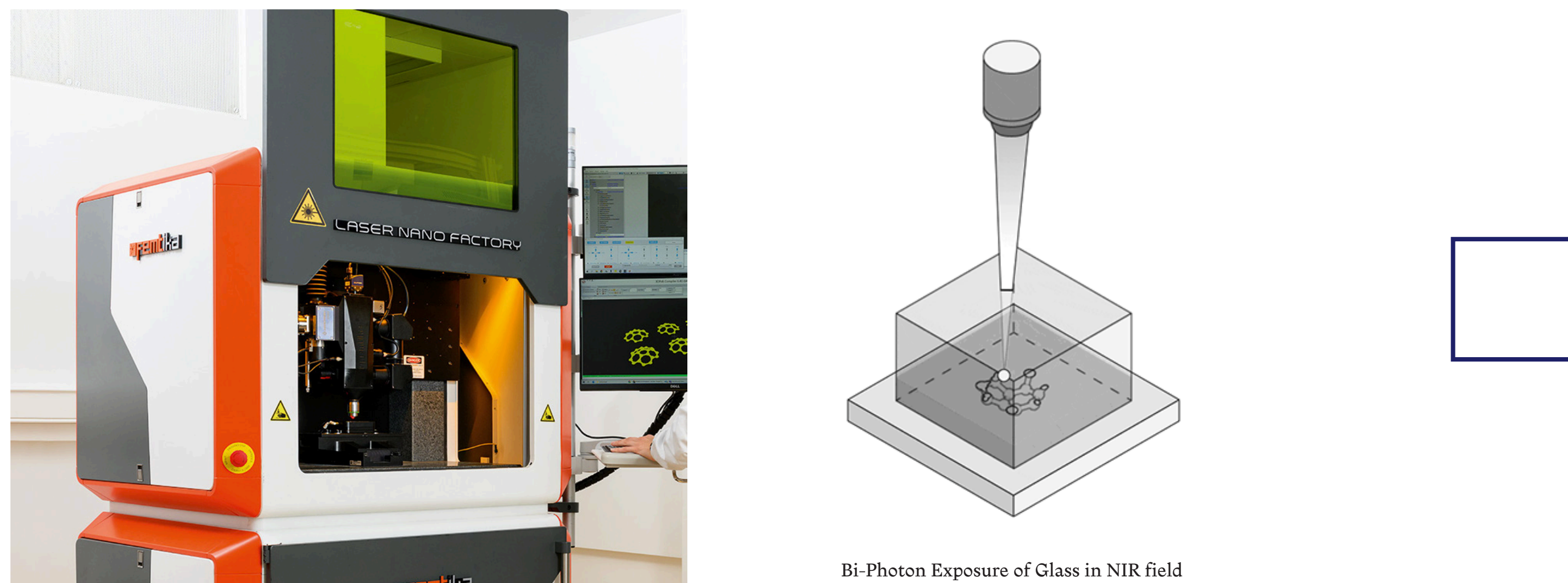
Principle of Electrical Analysis



Impedance spectroscopy is implemented on the μ Sieve through the microfabricated electrodes. Impedance Spectroscopy returns a complex impedance value for each frequency probed which allows to understand and model frequency dependant behaviours. In the case of cell sieving, biological elements exhibits frequency dependant (or electrically dispersive) electrical properties. This electrical dispersion is closely related to the physical properties of the cells, thus, being able to observe this dispersion and characterize the key transition frequencies may give access to the cell physical properties: size, roughness, cytoplasm composition...

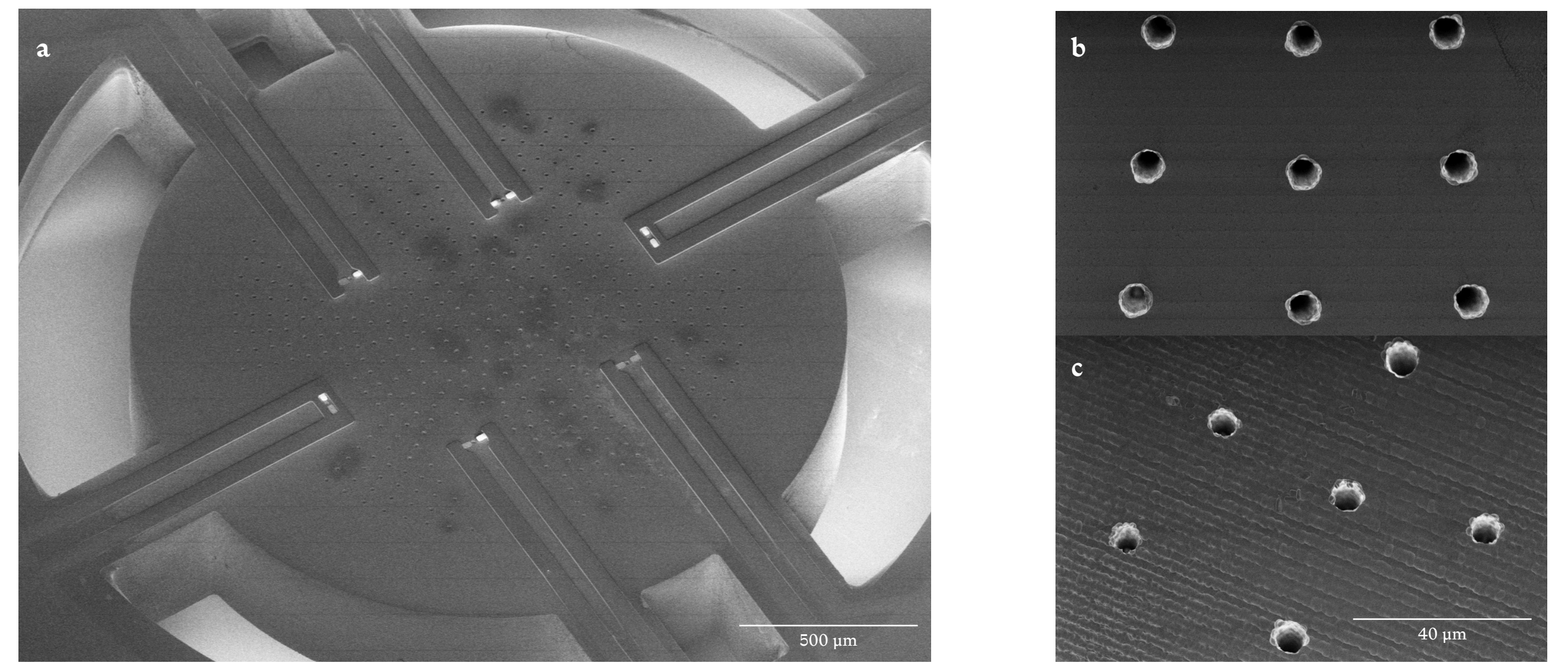
Glass Micro Sieve Fabrication and Integration Process

Selective Laser Etching

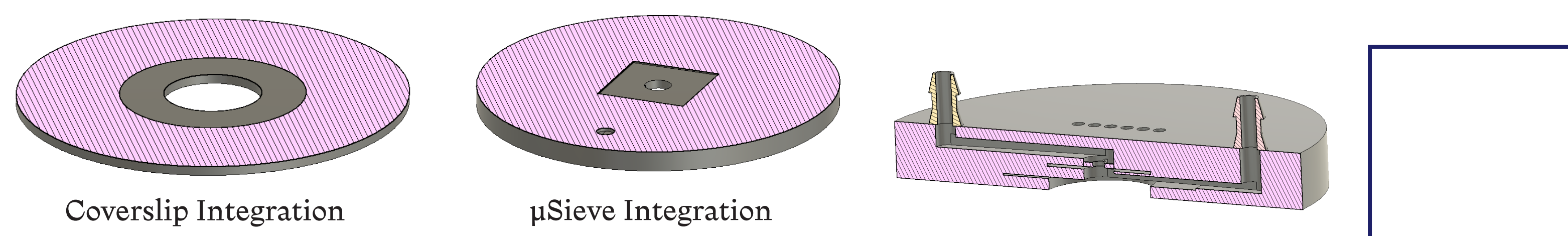


Selective Laser Etching machine commercialized by Femtika is shown in a. Its working principle, described in b is relying on two fabrications steps: femtosecond laser irradiation and subsequent chemical etching. Tightly focused femtosecond laser beam induces modifications of glass material within the focal point of laser beam. The sample is then immersed in an etchant solution (KOH), which etches out preferentially laser modified areas.

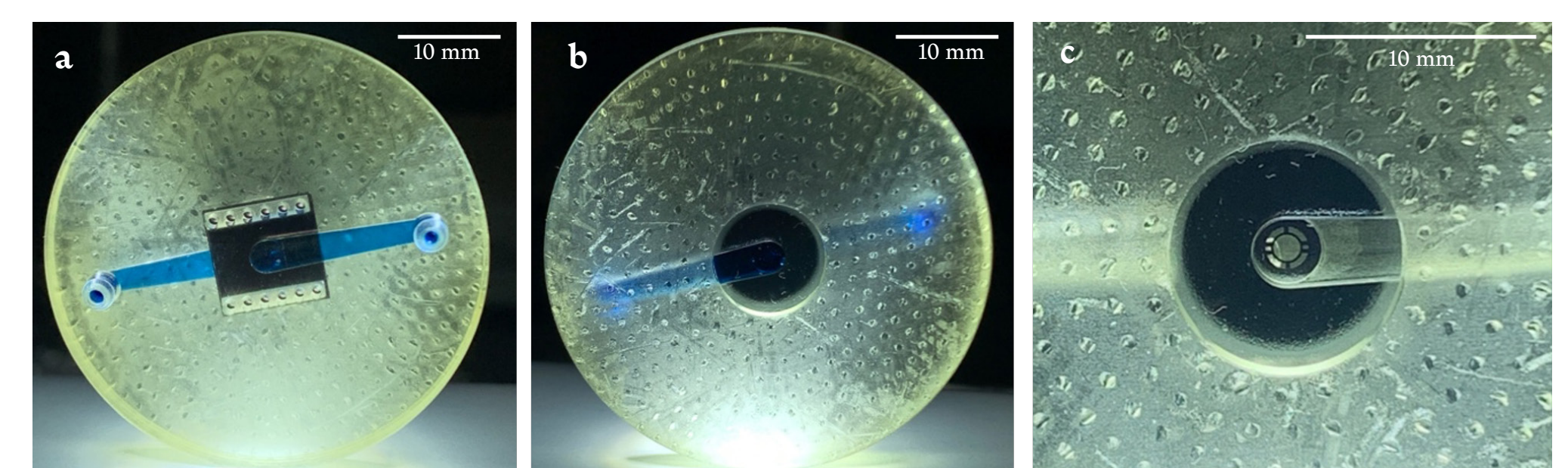
Fabricated Micro Sieve



Scanning Electron Micrographs of the fabricated μ Sieve. a Shows the perforations in the center part with sensing electrodes on top and surrounding fluidics slits. The thickness of the whole glass structure is 300µm with traversing 10µm pores. The electrodes are defined from a Cr/Au 200nm layer with a standard UV photolithography step followed by a plasma etching (Ar). b and c exhibits the 10µm pores respectively on the front side and the back side (aspect ratio 1:30).



Print Pause Print (PPP) Process allowing the integration of the microfabricated sieve. First, a donut shape is printed, corresponding to the bottom side of the chip with the inner part of the donut being the optical access. The printing is paused and a silanized glass coverslip is deposited on top of the donut, the printing then resumes, and the coverslip gets trapped in the polymerized resist. Fluidic channels are printed on top of the coverslip and, after a second pause, the μ Sieve is incorporated and gets embedded by the polymerized resists. Finally, resist development is done by flushing ethanol through the fluidic channel and is followed by a 15min UV curing. This process is done on a DWS printer with DS3000 biocompatible resist and conventional printing parameters.

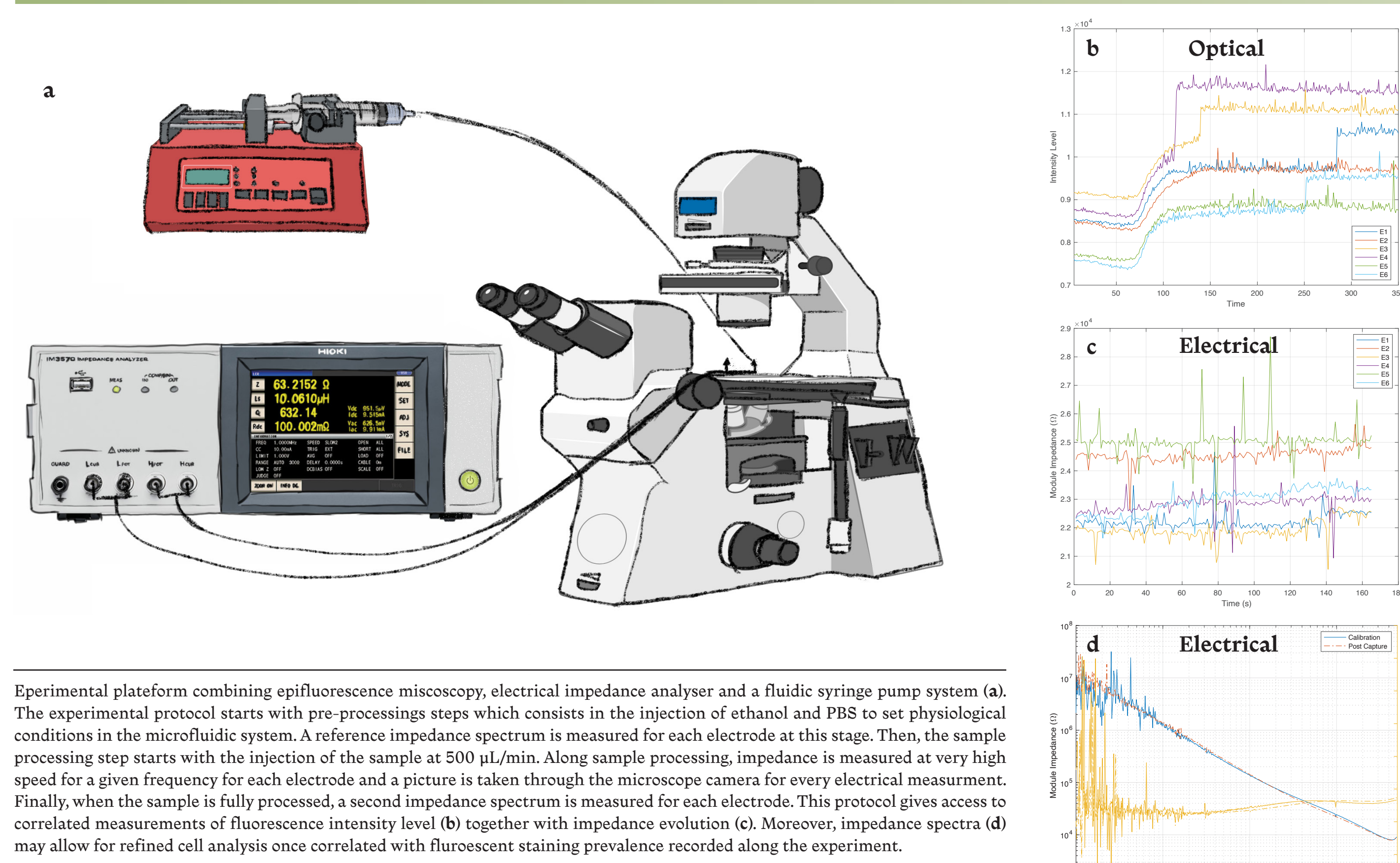


Integration of the cleanroom fabricated device with optical, electrical, and fluidic access in a 3D printed chip. The printed chip has an overall diameter of 40 mm, the fluidic channel has a cross-section ranging from 1.5 to 2.25 mm² and is filled with a blue dyed solution on photographs a and b. a Top side of the chip exhibiting fluidic connectors and the integrated device in its centre with access to the electrical contact pads through the 12 holes. b Bottom side of the device with optical access, to the fluidic canal and the integrated transparent sieve through a glass coverslip. c Close up on the centre of the chip, showing the channel, the integrated sieve and the fluidic slots surrounding it.

Print Pause Print Integration Process (SLA)

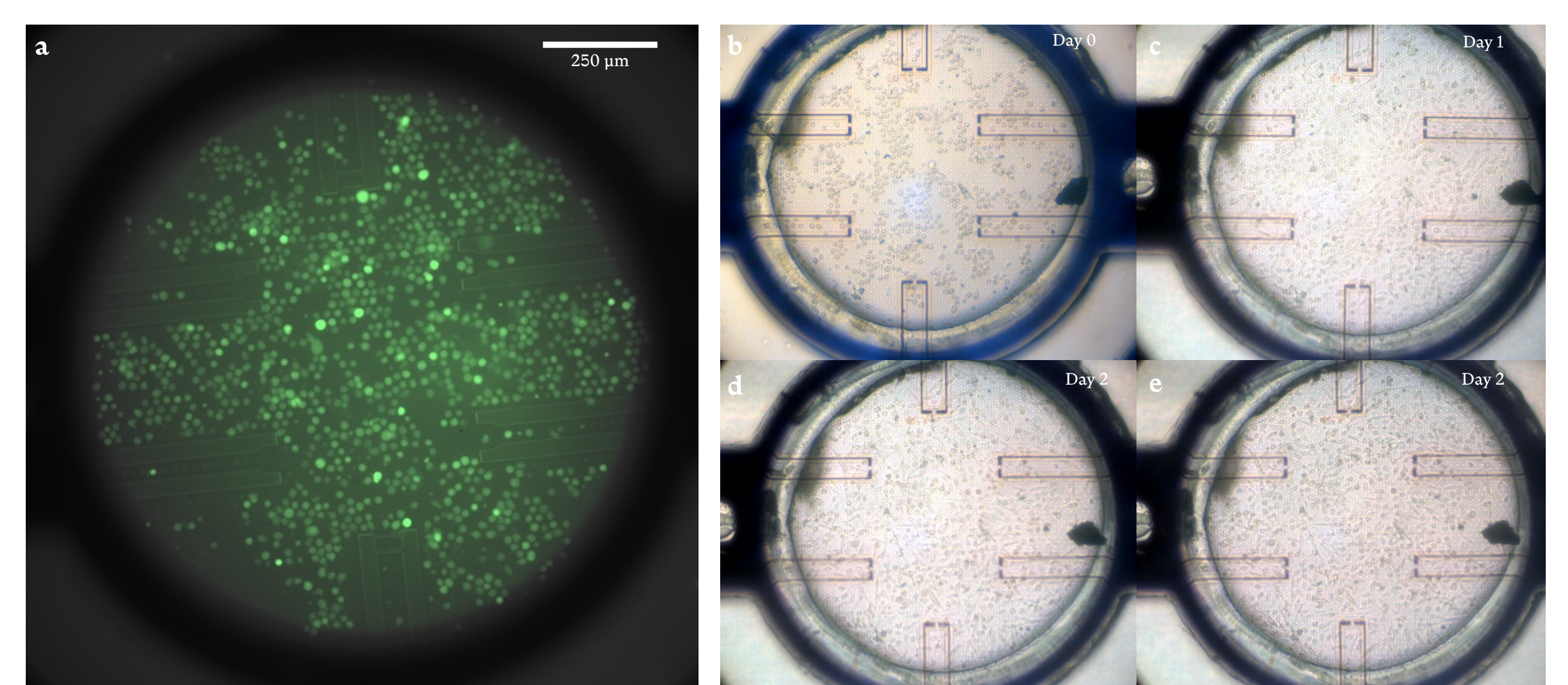
3D Printed Microfluidic Chip

Experimental Platform & Cell Sieving Analysis



Experimental platform combining epifluorescence microscopy, electrical impedance analyser and a fluidic syringe pump system (a). The experimental protocol starts with pre-processing steps which consists in the injection of ethanol and PBS to set physiological conditions in the microfluidic system. A reference impedance spectrum is measured for each electrode at this stage. Then, the sample processing step starts with the injection of the sample at 500 µL/min. Along sample processing, impedance is measured at very high speed for a given frequency for each electrode and a picture is taken through the microscope camera for every electrical measurement. Finally, when the sample is fully processed, a second impedance spectrum is measured for each electrode. This protocol gives access to correlated measurements of fluorescence intensity level (b) together with impedance evolution (c). Moreover, impedance spectra (d) may allow for refined cell analysis once correlated with fluorescent staining prevalence recorded along the experiment.

Cell Sieving & *In-situ* Culture



Cellular Capture followed by *in-situ* culture of prostatic cancer cell line (PC3 - GFP). The μ Sieve is first incubated in a fibronectine solution for 4 hours in order to promote cellular adhesion. a exhibits a single frame of a video recorded during a sieving experiment. b, c, d and e represent photographs of the sieve at different culture stage: after the capture (b), after a day of culture (c) and after two days of culture, before (d) and after (e) a medium change. This experiment demonstrates the feasibility of *in-situ* cell culture after sieving from a sample which can be followed by optical microscopy, both in the bright field and fluorescent domain. The culture medium can be interchanged without impacting cell adhesion which suggests a potential use for long-term culture.