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Laboratory of Biosensors and Bioelectronics | DITET

Oil Droplets as Tunable Nanoindenters for FluidFM-based Cell Mechanics

Giacomo Paccagnan [1], Pablo M. Ruiz [2], Aiden Doyle [2], Elias Hanelt [1], Tamas Lovas [1], Tomaso Zambelli [1], Massimo Vassalli [2]

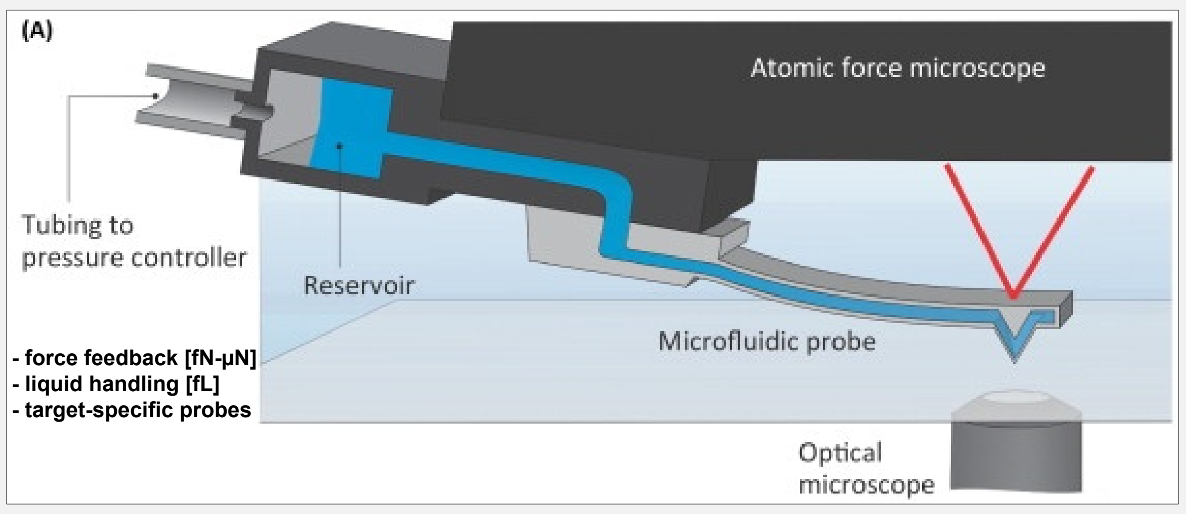
[1] Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zürich, Switzerland
[2] Cellular Mechanobiology Lab, James Watt School of Engineering, University of Glasgow, Scotland

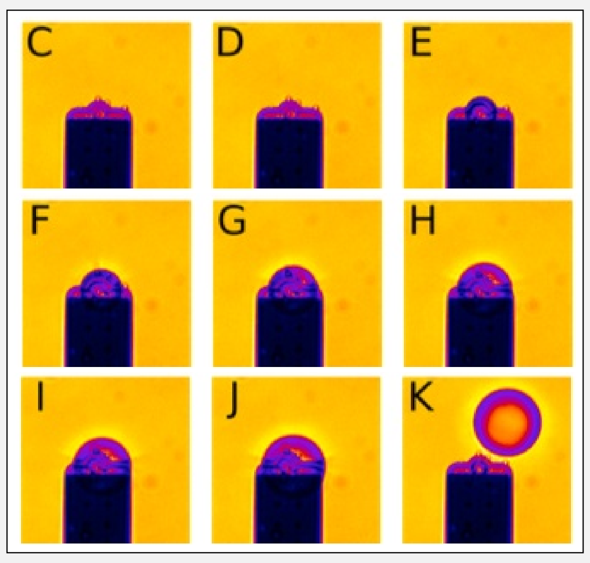
1) Introduction

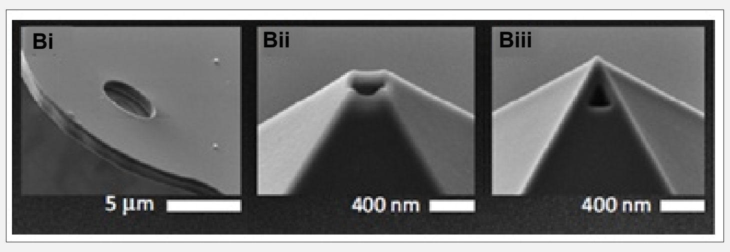
Atomic force microscopy (AFM) indentation techniques are essential for studying cell mechanics. However, traditional rigid colloidal bead indenters present limitations such as tedious attachment processes and inflexible geometry. Fluidic Force Microscopy (FluidFM), using hollow cantilevers, partially resolves these issues but remains slow due to manual bead handling. We are developing a novel approach using FluidFM-generated oil droplets as dynamic, deformable indenters. Droplets offer automated creation, quick size adjustment, and better mimic biological conditions. At the moment, we are focusing on the accurate measurement of droplet size and geometry, as it is essential for correctly determining mechanical properties such as Young's modulus.

2) FluidFM: Fluidic Force Microscope and oil droplets formation

To generate oil droplets we use the FluidFM, a force-controlled nano-/ micropipette based on an Atomic Force Microscope cantilever with an embedded microchannel, filled with mineral oil.



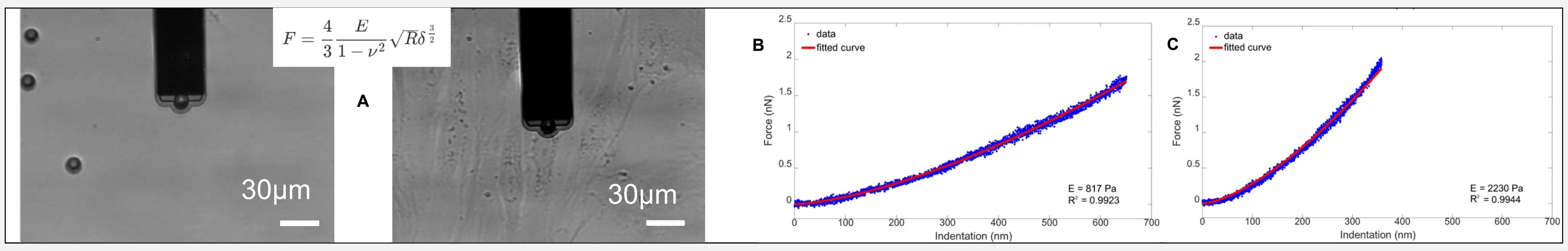




A FluidFM setup. **B** SEM micrographs of FluidFM hollow cantilever tips. From left to right: micropipette (**Bi**), nanopipette (**Bii**) and pyramidal tip (**Biii**). **A**, **B** figures adapted from [2]. **C-K** Formation of an oil droplet via an 8 µm aperture micropipette cantilever under applied positive pressure. Droplet formation initiates upon pressure application (**E**), progressively increases in size while pressure is maintained (**F–J**), and eventually detaches (**K**).

3) Classical FluidFM mediated indentation via spherical colloids for cell stiffness measurements

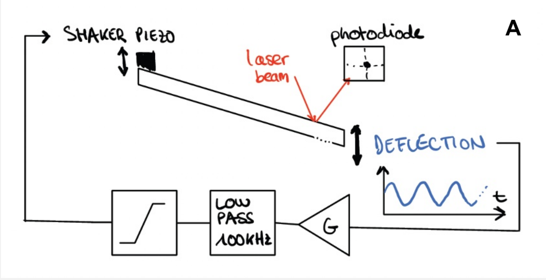
The Hertz model for a spherical indenter is used to fit Force vs Indentation depth curves and extract cells Young's modulus.



A Beads of 10µm diameter are absorbed at the cantilever aperture by applying negative pressure to the cantilever channel (left). Cells are then indented via force spectroscopy (right). **B** & **C** reproduced and adapted from [3]. Force vs indentation depth recorded during the spectroscopy discriminates between soft (**B**) vs stiff cells (**C**).

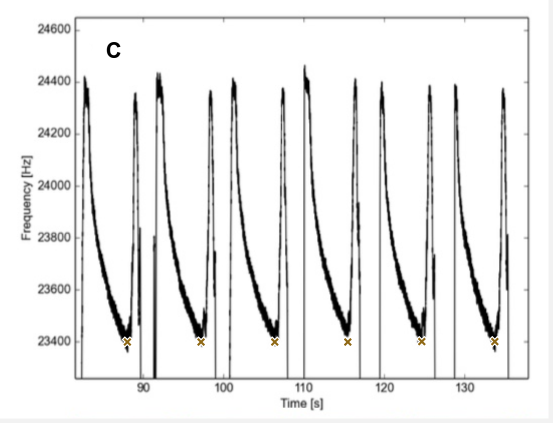
4) Self-excited cantilever strategy for dynamic droplet size monitoring.

Accurate knowledge of indenter geometry is critical for applying contact mechanics models to determine the Young's modulus. As our oil droplet indenter size varies dynamically, we implemented a self-excitation strategy to maintain cantilever resonance. The resonance frequency, tracked in real-time via an FPGA-based measurement system (RedPitaya), directly correlates to droplet size, enabling high-temporal-resolution monitoring of the indenter geometry.



B

$$f_{\mathrm{new}} = f_0 \cdot \sqrt{\frac{1}{1+\frac{m_{\mathrm{tip}}}{m_{\mathrm{eff}}}}}$$

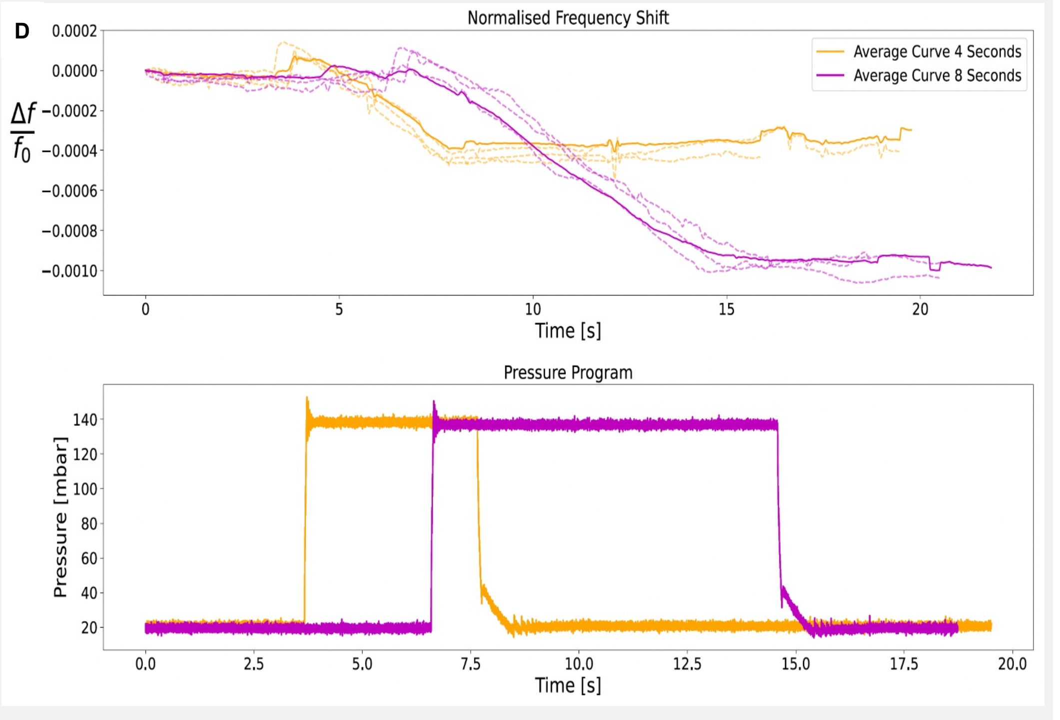


A Schematic of the engineered feedback loop maintaining the cantilever in self-excitation.

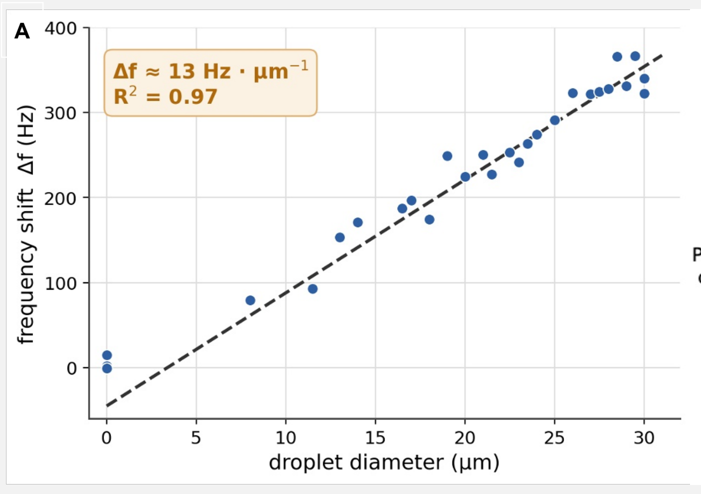
B Correlation method between cantilever resonance frequency shift and mass change at the tip. [1]

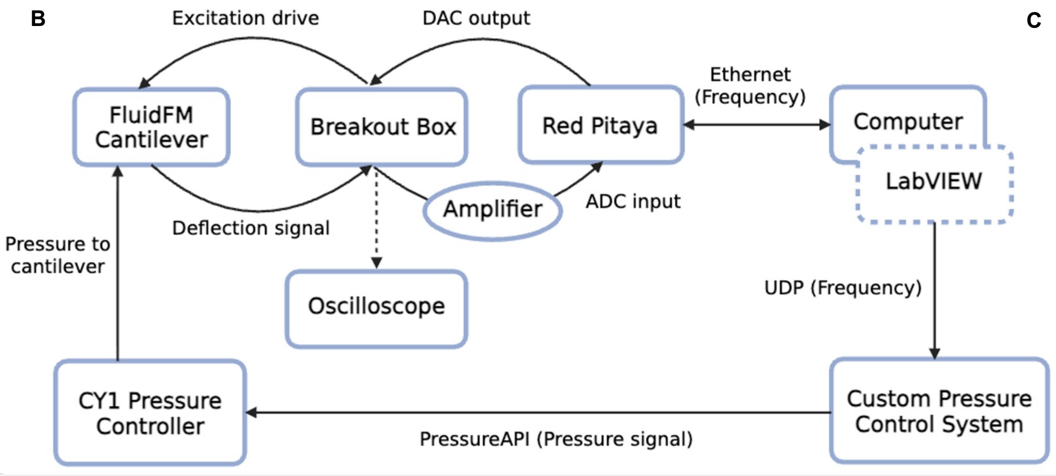
C Real-time frequency response illustrating droplet formation and detachment events.

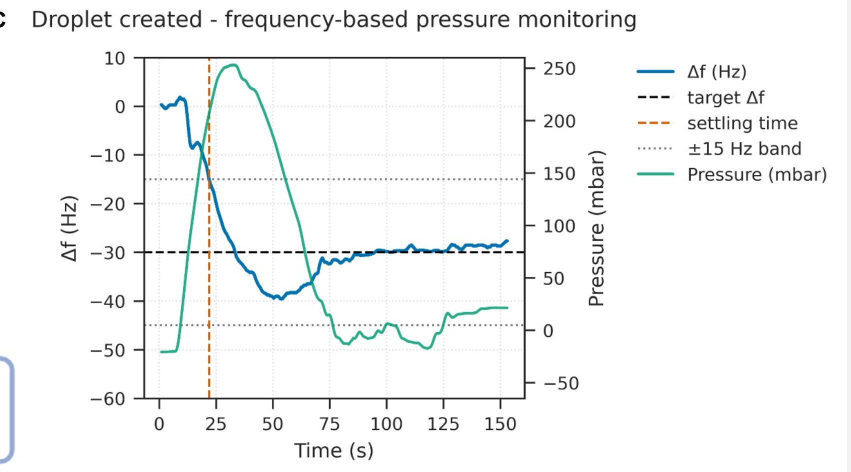
D Example demonstrating controlled generation of two droplets of different sizes and subsequent size stabilization through precise pressure adjustments.



5) Closed-loop frequency-based control of droplet size



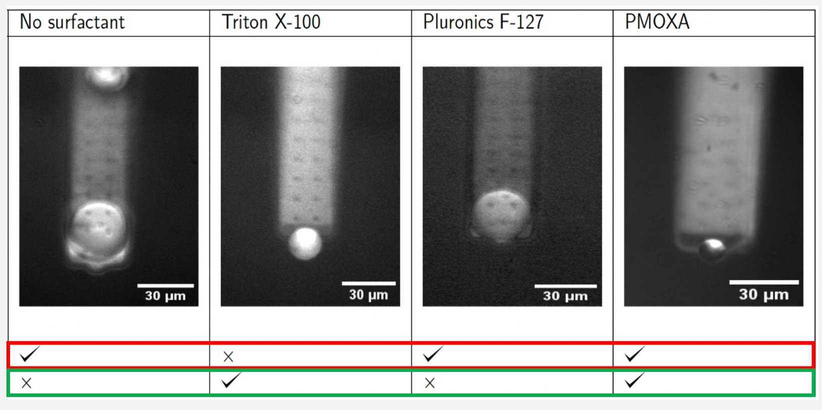




A Frequency shift Δf scales linearly with droplet diameter (≈ 13 Hz·µm⁻¹, R^2 = 0.97), giving a real-time readout of indenter size. **B** Closed-loop hardware: cantilever resonance is tracked on a Red Pitaya (FPGA) and streamed over UDP to a custom pressure controller driving the CY1. **C** Live control run — pressure is regulated until Δf reaches the target band (±15 Hz), stabilising droplet size after the settling time.

6) Droplet stabilization and shape control

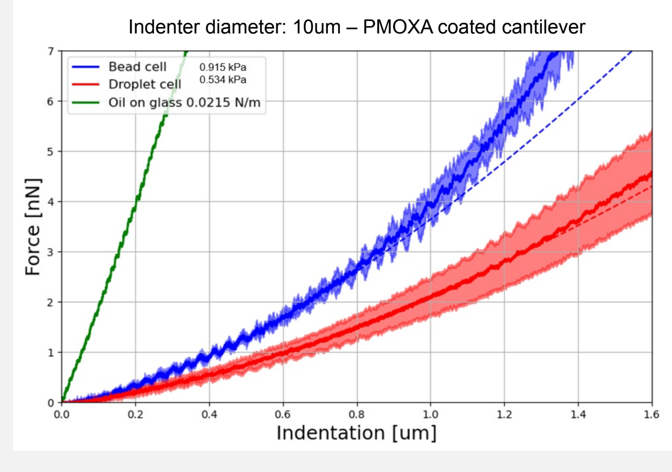
Optimization of surfactant concentration and cantilever coatings minimize oil-cantilever interactions, ensuring spherical droplet formation while maintaining biocompatibility.



Images demonstrating approaches to optimize droplet formation and biocompatibility (left to right):

(**1**) Without treatment, oil spreads on the cantilever, impeding droplet formation.
(**2**) Non-biocompatible surfactant achieves highly spherical droplets but lyses cell membranes.
(**3**) Biocompatible surfactant provides limited control over droplet shape.
(**4**) Hydrophilic antifouling cantilever coating enables formation of spherical droplets while ensuring biocompatibility.

7) Oil droplet indentation on cells



PMOXA-stabilized oil droplets of defined geometry produce clear force–indentation curves. Plot compares the same cell indented with a rigid colloidal bead (blue) and an oil droplet (red), with the corresponding Young's moduli indicated in the legend; droplet indentation on glass (green) displays a linear response.

8) Outlook

Establish a validated relationship between measurements obtained using conventional rigid indenters and those acquired using oil droplet indenters.

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References [1] Ossola, D. et al, Nanotechnology 2016, 27:415502. [2] Meister, A. et al, Nano letters 2009, 9:2501. [3] Sancho, A., et al. Sci Rep 2017, 7:46152