

Fluidic force microscopy

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Abstract

Fluidic force microscopy is a versatile bionanotechnology platform that integrates atomic force microscopy with microfluidic probes. This hybrid approach enables precise measurement and application of forces across sub-nanonewton to micronewton ranges while simultaneously dispensing or sampling sub-femtolitre to picolitre volumes, all with real-time optical visualization at sub-micrometre resolution. In recent years, fluidic force microscopy has emerged as an enabling tool for minimally invasive single-cell manipulation, subcellular analysis and high-resolution nanoprinting applications. This Primer describes the fundamental principles underlying the combination of atomic force microscopy with microchannelled cantilevers, providing a comprehensive framework for understanding the unique capabilities of fluidic force microscopy and its rapidly expanding range of applications in biological research and nanotechnology.

Sections

[Introduction](#)[Experimentation](#)[Results](#)[Applications](#)[Reproducibility and data deposition](#)[Limitations and optimizations](#)[Outlook](#)

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Introduction

The manipulation and analysis of biological systems at single-cell and subcellular levels represent a fundamental challenge in biology and bioengineering. Such investigations require minimally invasive tools that integrate mechanical precision with well-regulated microfluidics and nanofluidics. Fluidic force microscopy (FluidFM) addresses this need by combining the mechanical sensing and force application capabilities of atomic force microscopy (AFM) with aperture-gated, pressure-driven microfluidics. FluidFM connects an AFM cantilever with an embedded microchannel ending with a tip aperture to a microfluidics controller¹.

The conceptual foundation of FluidFM builds upon several decades of progress in single-cell manipulation tools. Glass micropipettes, pioneered by Marshall Barber at the beginning of the twentieth century for bacterial isolation and inoculation^{2,3}, established the principles of cellular manipulation. These capillaries enable single-cell operations such as microinjection for targeted delivery of biomolecules⁴, aspiration for gene-expression analysis^{5,6} and patch clamping for electrophysiology⁷. Concurrently, glass micropipettes became essential in electrochemical additive manufacturing, providing localized electrochemical control for precise metal deposition at the microscale^{8,9}. Recent advances have further broadened these applications^{10–21}. Advances in AFM revolutionized surface analysis by enabling controlled nanoscale mechanical interactions. Beyond imaging, AFM became a quantitative tool for force measurements, from colloidal surface interactions to single-molecule spectroscopy^{22–24}. The technique expanded to nanolithography, enabling mechanical surface patterning²⁵ and molecular deposition^{26,27}. Transforming AFM cantilevers into pipette-like devices represented a milestone. Early implementations, such as the nanodispensing tool, featured hollow

pyramidal tips with an apex aperture^{28,29} but suffered from rapid liquid evaporation. Later designs introduced AFM cantilevers with embedded microchannels linking tip apertures to external open reservoirs, fabricated either by the sacrificial layer^{30,31} or the thermal bonding^{32,33} processes, both producing hollow silicon-based cantilevers.

FluidFM builds on the aforementioned technological developments, achieving a force-controlled pipette through integration of microchannelled AFM cantilevers with a closed reservoir coupled to an external pressure controller¹ (Fig. 1). The modification of standard AFM functionality by drilling a precise hole in the probe holder and generating a watertight seal between the microchannelled probe and a pressure controller was the key innovation, establishing a continuous fluidic conduit from the pressure source to the tip aperture, thus enabling both overpressure and underpressure for dispensing and aspiration¹. Critically, AFM feedback responds rapidly to pressure pulses, compensating for the induced cantilever deformation while maintaining stable contact with the underlying surface³⁴. Because of the sealed fluidic path, FluidFM can be operated in both air and liquid. The AFM head can be mounted on the *x–y* stage of an inverted optical microscope and optionally placed in an incubator with temperature and CO₂ control. An electrode can be inserted in the fluidic channel with a corresponding electrode in the solution to measure the ionic current flowing through the embedded microchannel³⁵, and the system can be made compatible with three-electrode electrochemical cells³⁶. FluidFM's intrinsic versatility can be used to address various fundamental questions in cell biology, materials science and bioengineering.

Robotic FluidFM – known as the OMNIUM or BOT system – represents an evolution of the standard FluidFM platform towards full automation and scalability. Although conventional FluidFM requires substantial user intervention, many manual steps and a strong dependence on operator skill, robotic FluidFM offers a user-friendly and automated solution that minimizes variability and enables high-throughput workflows. Incubator-equipped robotic systems can automatically target cells over millimetre to centimetre scales and assess multiple Petri dishes simultaneously, featuring automatic probe-washing and exchange capabilities. These advanced set-ups have been used in large-area printing³⁷, high-throughput single-cell adhesion measurements for studying cellular force distributions^{38–40}, single-cell injection⁴¹ and force calibration of indirect high-throughput devices^{38,42,43}. Although standard FluidFM remains ideal for custom, small-scale and exploratory experiments requiring high flexibility and direct user control, as well as real-time live-cell imaging, robotic FluidFM platforms are better suited for reproducible, large-scale studies and automated biological workflows.

In this Primer, we present FluidFM as a force-controlled nanofluidic platform integrating the precision of AFM with microfluidic control, enabling minimally invasive single-cell manipulation and high-precision nanoprinting. We describe its instrumentation and experimental approaches, survey applications spanning cell biology and materials science, and discuss reproducibility, limitations and future directions to guide researchers in adopting and extending this versatile technology across both the life sciences and the physical sciences.

Experimentation

This section provides practical guidance for performing FluidFM experiments, covering equipment, probe choice and preparation, general considerations, protocols, and software. A key initial step is selecting

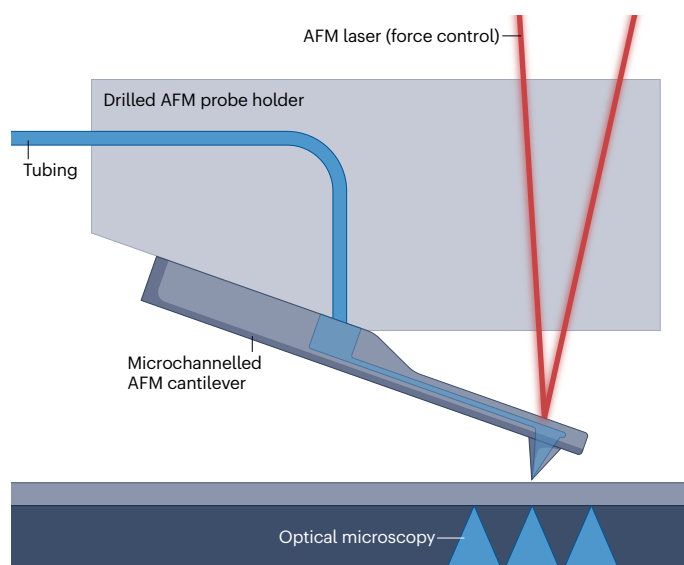


Fig. 1 | Basic principles of FluidFM. Schematic showing a microchannelled cantilever chip fixed to a drilled atomic force microscopy (AFM) probe holder at the chip reservoir. That watertight junction (up to 7 bars) establishes a continuous pipeline from the pressure controller to the aperture at the cantilever extremity, creating either an overpressure or an underpressure. Both the probe holder and the chip can be immersed in a bath containing either the same or a different liquid as that in the microchannel. Cantilever deflection is detected with a standard laser beam, and the AFM is mounted on the *x–y* stage of an optical microscope in inverted configuration. FluidFM, fluidic force microscopy.

a compatible AFM platform and probes, as the diversity of set-ups and probe types reflects the broad range of FluidFM applications.

Equipment

AFM. Fundamentally, FluidFM is an AFM-based probe technique. Probes can be *a priori* mounted on any AFM head after adaptation of the probe holder to integrate the micro/nano fluidics. For biological applications, FluidFM can be implemented on AFM systems conceived specifically for studying biological samples and soft matter under near-physiological conditions, known as a bioAFM. These systems are compatible with Petri dishes, coverslips and biochips, while integrating environmental controls such as temperature and gas. BioAFM is designed to integrate with advanced optical microscopy techniques⁴⁴, enabling correlated imaging and multidimensional analysis of biological samples. They offer specialized measurement modes for biological applications, such as high-speed imaging for molecular dynamics and force spectroscopy mapping for cell mechanics, while the software includes data analysis features relevant to soft-matter research, such as quantifying cell stiffness, elasticity and adhesion. Mounting the AFM head on the *x*-*y* stage of an inverted optical (fluorescence) microscope is paramount for many applications, because it facilitates the process of selecting a particular region of the substrate or cell in a Petri dish to be manipulated, and of positioning the FluidFM probe on top of it with micrometre precision.

FluidFM probes. Probes containing an embedded channel and an aperture at the apex are at the core of FluidFM. Probe geometry enhances the versatility of the tool in controlling force, pressure and ionic current. Presently, there are three main types of probes: tipless, pyramidal and cylindrical (Fig. 2a–f). FluidFM tipless and pyramidal probes are commercially available whereas the cylindrical ones are at the prototype stage (Table 1). Probes are fabricated using silicon-rich nitride deposition with a lithographical wafer-scale process including a (poly) silicon sacrificial layer. Etching forms the microchannel (with height in the 0.5–4.0 μm range), which together with the wall thickness (typically 100–600 nm) determines the stiffness of the probe. Probes may be further shaped by a focused ion beam to adjust the aperture size, sharpen the apex or compensate for the $\sim 10^\circ$ angle of the probe mounted on the probe holder. Probe backsides are coated with a 30 nm-thick reflective gold layer for standard optical beam detection⁴⁵. Commercial FluidFM probes are glued to a plastic clip to facilitate fixation to probe holders of some AFMs. A purpose-built connector enables fastening to the microfluidic system and external pressure control. To fill the microchannel, several tens of microlitres of the solution of interest are pipetted into the designated volume of the clip, which is then connected to the pressure controller. By applying an overpressure, microchannel filling is achieved within seconds (minutes in case of apertures smaller than 300 nm). The microchannel can also be filled by plunging it into a droplet of the desired solution and applying an underpressure⁴⁶.

Tipless probes, featuring a circular aperture of 2–8 μm at the cantilever extremity, are suitable for experiments involving cell adhesion and indentation upon picking up a colloidal microsphere, as well as for dispensing with a spatial resolution of approximately 5 μm limited by transport phenomena related to the finite aperture-to-surface distance. Pyramidal probes with basal sides of 7 μm and with a triangular aperture on a facet close to the apex are used for injection–extraction experiments requiring cell-membrane puncturing, whereas pyramidal probes with a 300 nm aperture at the apex are used for dispensing experiments with sub-micrometre resolution. Cylindrical probes with

0.5–2 μm diameters and 7–15 μm heights are used in patch-clamp experiments mimicking the geometry of the apex glass capillaries, as well as for cell-puncturing experiments⁴⁷.

Preparation of FluidFM probes

Chemical anti-fouling coating of probes. Biofouling – non-specific adhesion of cells, proteins and biomolecules – can impair FluidFM manipulations by damaging cells, obstructing fluid flow, reducing measurement accuracy or hindering cell release (Fig. 2g and Supplementary Fig. 2). Anti-fouling coatings are commonly applied to the probe exterior, or to both the exterior and the microchannel, helping to prevent non-specific adhesion and ensuring consistent performance. The two main classes of coatings are electrostatically adsorbed polymer brushes and vapour-phase silanization.

For polymer-brush coating of FluidFM probes, two effective hydrophilic polymers are commonly used, poly(L-lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG)^{48,49} and poly(acrylamide)-*g*-(poly(2-methyl-2-oxazoline), 1,6-hexanediamine, 3-aminopropyltrimethylsilanol) (PAAm-*g*-(PMOXA, NH₂, Si))⁵⁰. To form anti-fouling monolayers, the probe surface is first activated by a brief (1 min) air plasma treatment, followed by filling the microchannel with a 0.1–0.5 mg ml^{−1} polymer solution and immersion of the probe in the same solution for 30–60 min. If only the probe exterior requires coating, the microchannel can instead be filled with experimental buffer. After adsorption, the probe is dipped in ultrapure water to remove unbound polymer from the exterior surface. For single-cell force spectroscopy or isolation experiments, in which no defined solution is needed in the microchannel, the polymer solution can remain inside the microchannel. For injection experiments, the polymer solution in the microchannel is replaced by the solution to be delivered; the probe-holder reservoir is emptied, the new solution is loaded, and overpressure is applied until the polymer solution is completely displaced. In this case, the internal coating is gradually washed away by the injection solution itself; adding a separate water rinse would be time-consuming, and it would substantially increase the risk of introducing air bubbles or particles that could clog the probe. PLL-*g*-PEG has been used in mammalian and yeast cell injections^{51,52}; for adhesion-force assays with yeast, bacteria and mammalian cells^{53–56}; and for mammalian cell isolation⁵⁴. Its performance and resistance to degradation improves when coating is performed at a temperature of 80 °C (ref. 54). Although PLL-*g*-PEG relies solely on electrostatic interactions, PAAm-*g*-(PMOXA, NH₂, Si) combines electrostatic binding with covalent anchoring, resulting in more durable coatings that have been successfully used for mammalian adhesion-force assays^{57,58}.

Vapour-phase deposition of Sigmacote (Sigma-Aldrich) provides an alternative to polymer-brush coating that does not require liquid to flow through the microchannel. Sigmacote forms a covalently bound hydrophobic siloxane layer by vapour deposition. After plasma activation, probes are placed in a sealed chamber together with an open dish containing Sigmacote solution (approximately 500 μl in a 20 cm-diameter desiccator). Vacuum is applied through the chamber, while Sigmacote adsorbs for at least 60 min. To coat the microchannel, the inlet is connected to a 20 ml syringe holding a drop of Sigmacote (50–100 μl), and overpressure is applied to deliver Sigmacote-saturated air through the channel. The probes are then cured at 80–100 °C for 60 min in a dry oven to enhance cross-linking and coating stability. This silane polymerization produces a thicker, more defect-tolerant film than polymer brushes and can compensate for low-efficiency surface activation within the microchannel. Applied under dry conditions,

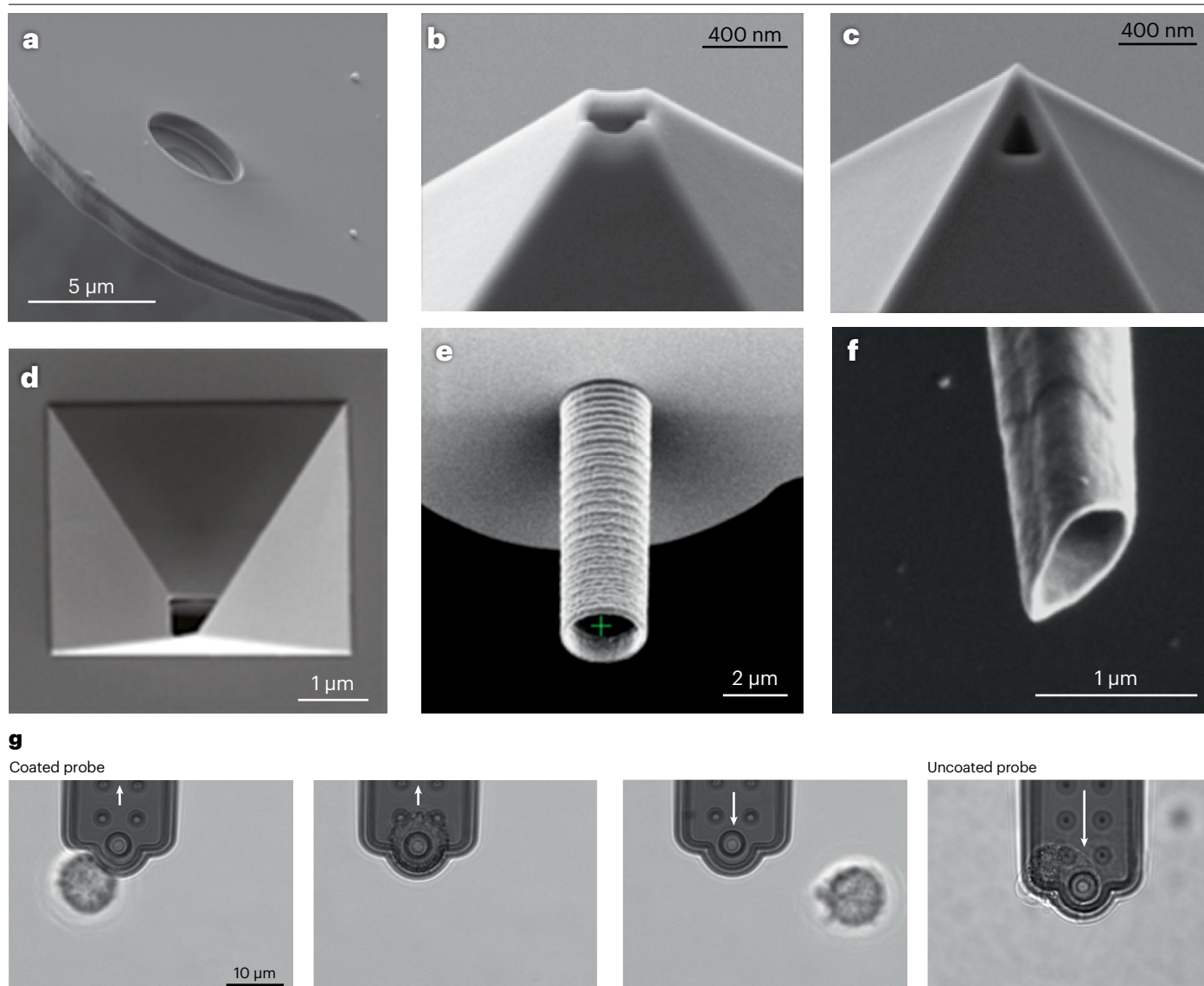


Fig. 2 | Scanning electron micrographs of FluidFM probe tips. **a**, Tipless probe with a circular aperture (2, 4 or 8 μm), known as micropipette. **b**, Pyramidal tip with a 300 nm aperture at the apex, known as nanopipette. **c**, Pyramidal tip with a 300 nm triangular aperture close to the apex, milled via focused ion beam or obtained with a wafer-scale process, known as nanosyringe. **d**, Pyramidal tip with a 900 nm double triangular aperture close to the apex, milled by a focused ion beam. **e**, Cylindrical tip with a 1.5 μm aperture. **f**, Cylindrical tip with a 500 nm aperture sharpened to a 45° angle with a focused ion beam. **g**, An example of antifouling probe coatings using sequential images to show a coated fluidic force

microscopy (FluidFM) probe during single-cell manipulation: the HeLa cell is gently captured at the aperture by aspiration and subsequently released with a pressure pulse, leaving both the cell and probe intact. The uncoated probe causes adhesion of the HeLa cell to the bare probe surface, preventing release with a pressure pulse, resulting in cell damage and irreversible probe fouling. White arrows indicate the direction of applied pressure through the microchannel: upward for underpressure and downward for overpressure. Parts **a–c** reprinted with permission from ref. 201, Elsevier. Part **d** adapted from ref. 82, CC BY 4.0. Part **e** reprinted from ref. 87, Springer Nature Limited.

the coating also supports extended storage of prepared cantilevers for up to several weeks. It is used in injection and extraction experiments for both mammalian and fungal cells^{39–61}, as well as in adhesion-force assays with mammalian, yeast and bacterial cells^{62–64}.

Calibration. Accurate force measurements with FluidFM require precise calibration of two key parameters of the cantilever: the inverse optical lever sensitivity (InvOLS) and the spring constant (k).

Both parameters directly influence the conversion of the measured photodetector voltage into quantitative force values. FluidFM utilizes the laser beam reflection method to measure cantilever deflection⁴⁵: a laser beam is pointed onto the back side of the cantilever, while the reflected beam hits a two-segmented position-sensitive photodetector (Fig. 3a). At each moment t , the force F (in newtons) on the cantilever is characterized by the measured voltage function $U(t)$ (in volts) on the position-sensitive photodetector and can be calculated by using

Hooke's law (equation 1), in which k (in newtons per metre) is the spring constant of the cantilever, $\delta(t)$ (in metres) is its deflection, which is determined by scaling the measured voltage ($U(t)$ (in volts)) with the InvOLS (in metres per volt)^{65,66}.

$$F(t) = k \times \delta(t) = k \times U(t) \times \text{InvOLS} \quad (1)$$

The InvOLS of a cantilever is usually quantified by acquiring force versus distance curves^{23,24,67} on an ideally hard and flat surface, for example that of cell-culture dishes⁶⁶. In this case, the mechanical deformation of the surface can be neglected. Hence, pressing the probe to that hard surface results in only the cantilever bending: a linear signal in the position-sensitive photodetector versus the piezo position is obtained, known as the sensitivity approach curve (Fig. 3b). InvOLS is usually calculated as the slope of this linear approach curve as in equation 2, in which Δz is the piezo movement in the surface normal direction.

$$\text{InvOLS} = \frac{\Delta z}{\Delta U} \quad (2)$$

The cantilever's spring constant k is measured through the Sader method^{68,69} in numerous FluidFM configurations. Importantly, the Sader method relies on the measured position and quality factor (Q) of the first fundamental resonance frequency (ω_R) of the cantilever⁷⁰:

$$k = 0.1906\rho\omega^2LQ\Gamma_i(\omega_R)\omega_R^2 = 0.1906\rho\omega^2L\Gamma_i(\omega_R)\omega_R^3\text{FWHM}^{-1} \quad (3)$$

in which ω and L are the width and length of the cantilever, respectively, and ρ and Γ_i are the density and imaginary part of the dimensionless hydrodynamic function of the fluid (or air), respectively, evaluated at the resonant frequency⁶⁹. By definition, $Q = \omega_R\text{FWHM}^{-1}$. ω_R and FWHM are experimentally inferred by recording the thermal noise spectrum of the unloaded cantilever in air and finding the position and full width at half maximum (FWHM) of the resonance peak from a Lorentzian fit (Fig. 3e). Any optical noise affecting the resonance spectrum influences the determination of the spring constant. Importantly, the measured force scales linearly with the spring constant and InvOLS; therefore, their calibration is essential to gain reliable, comparable and consistent results. The hollow nature of the FluidFM cantilevers causes interference effects, resulting in increased optical noise compared with conventional AFM cantilevers^{66,71}. When properly calibrated, the hollow nature of the FluidFM cantilevers does not affect the force spectrum⁷².

Both the obtained InvOLS and spring constant values are dependent on the position of the laser spot on the FluidFM cantilever⁶⁶ (Fig. 3c,d). Well-defined and reproducible fluctuations of $\pm 30\%$ were observed in InvOLS with a consequent error from -13 to $+20\%$ of k , strongly depending on the laser spot position, found to be in

correspondence of the first pair of pillars of the hollow cantilevers⁶⁶. An alternative method delivering precise k values (with variations below 0.2%) independent of the laser spot position on the cantilever backside uses the first two resonances measured in air and water to determine the hydrodynamic cantilever function⁷¹, upon insertion of geometrical parameters like the length and width of the cantilever^{70,71}. Here, the k value is determined using the air and water resonant frequencies, the regression coefficients of the generalized hydrodynamic function, and geometrical parameters of the cantilever. This simplification reduces errors in k below 3%. For extended force measurements, tracking instrumental drifts – particularly changes in InvOLS – is crucial^{45,73}. Using the measured k , a fully contactless InvOLS calibration for FluidFM cantilevers was introduced based on hydrodynamic principles⁷³ taking advantage of the cantilever's internal microfluidic channel and external pressure control, achieving calibration in under 1 min. The cantilever deflection under pressure is quantitatively described using the thrust equation and finite element simulations. This hydrodynamic approach matches the accuracy of classical calibration methods, and it is rapid and easily adaptable for high-throughput robotic FluidFM^{38–40}, helping to detect clogging during measurements caused by cell debris, and hindering cantilever contamination due to its noncontact nature. From such calibration, stiffness values were inferred down to 0.2 N m^{-1} , allowing for force measurements with sub-newton resolution^{56,74–76}.

Protocols

The fully integrated FluidFM OMNIUM contains built-in routine applications and a user-friendly interface that require minimal expertise. This streamlined system has been developed based on foundational work from several research laboratories. OMNIUM can be useful for specific, well-established applications, although conceptual understanding of the principles presented here remains necessary. Here, we focus on interested readers and users who aim to fully capitalize on the versatility and opportunities offered by this technology, whether they are applying established methods or developing new ones. Advanced applications and novel implementation routines demand a deeper investment in understanding the operation principles of the technique and mastering its multidisciplinary aspects.

FluidFM provides three measurable parameters: cantilever deflection (quantifying force interactions between tip apex and target surface), pressure inside the embedded channel (controlling fluid flow) and, optionally, ionic current (enabling electrochemical measurements). Simultaneous control of these parameters enables diverse experimental approaches (Fig. 4). Force regulation allows gentle surface approach and adhesion-force measurements. Pressure control through the sub-micrometre aperture enables liquid delivery (positive pressure) or uptake (negative pressure) with precision of

Table 1 | Overview of the FluidFM probe system

Probes	Commercial	Aperture (μm)	Stiffness (N m^{-1})	Applications
Tipless (micropipette)	Yes	2, 4 or 8	0.3, 1, 2 or 4	Picking, dispensing
Pyramidal — apex (nanopipette)	Yes	0.3	0.6 or 2	Picking, dispensing
Pyramidal — syringe (nanosyringe)	Yes	0.6 or 0.8	2.2	Piercing
Pyramidal (closed)	On demand	–	2.2	Custom aperture and tip designs
Cylindrical	Not at time of publication	0.5, 1, 1.5 or 2	1.5–2	Dispensing, piercing, ionic current

FluidFM, fluidic force microscopy.

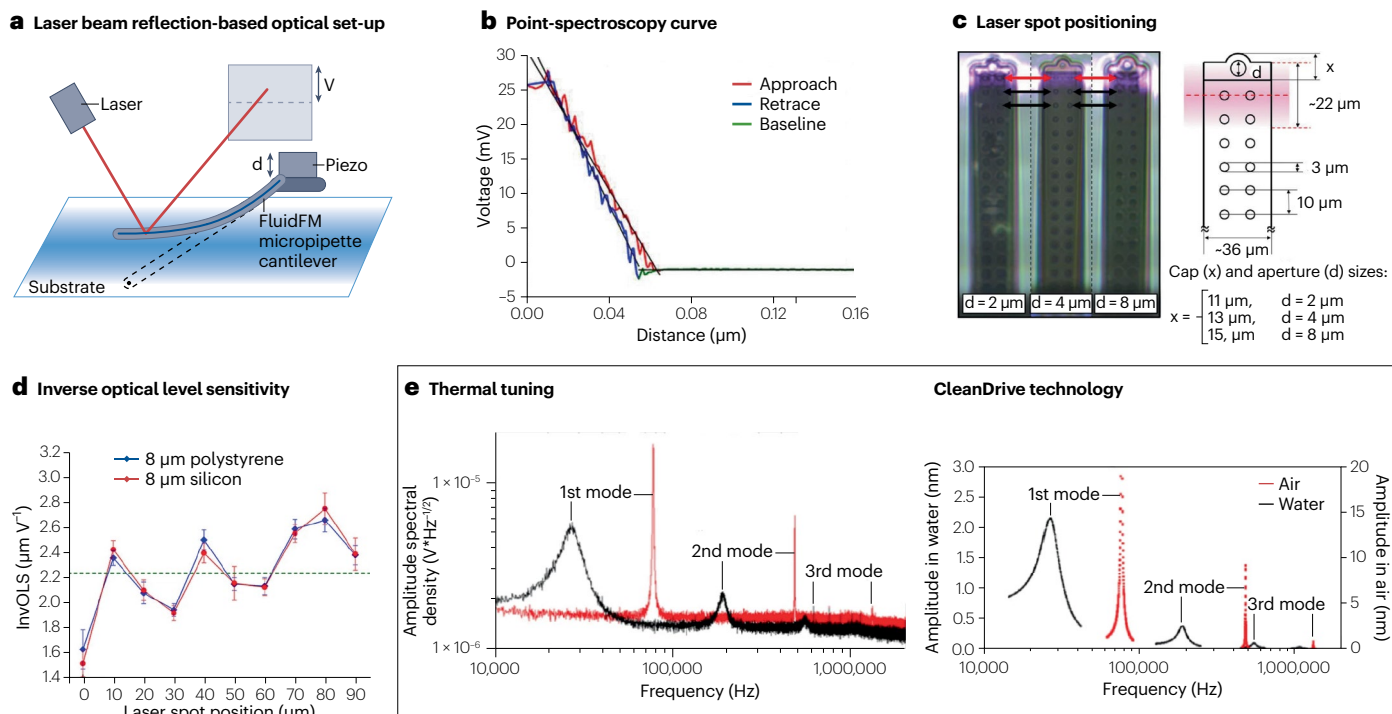


Fig. 3 | Calibration of the FluidFM system. **a**, Schematic of the laser beam reflection-based optical set-up. **b**, A sample point-spectroscopy curve, performed as calibration using a polystyrene substrate. **c**, Micropipette cantilevers with the laser spot positioned at the reference position, directly over the first pair of pillars (indicated by red arrows, second and third pair of pillars are indicated by black arrows). The illustration includes the geometrical parameters of the cantilevers, in which d is the aperture size and x is the cap size. The label indicates the various cap size requirements of different aperture sizes. **d**, Inverse optical lever sensitivity (InvOLS) values measured in 10 μm steps on

polystyrene (blue) and silicon (red) substrates as functions of the laser position for cantilevers with 8 μm aperture. The solid lines represent the average and standard deviation of ten measurements at every position, and the dashed line indicates the average of all data. **e**, Tuning spectra of a fluidic force microscopy (FluidFM) micropipette cantilever in air and with a channel filled with water, measured with a Nanosurf Drive atomic force microscope; thermal tuning and the company's CleanDrive technology, in which the amplitude of the spectrum measured in water has been multiplied by 5. Parts **a–d** reprinted from ref. 66, CC BY 4.0. Part **e** reprinted from ref. 71, CC BY 4.0.

approximately 100 aI, supporting applications from single-cell injection and extraction to 2D patterning and 3D layer-by-layer printing. Negative pressure enables grasping objects at the tip aperture for detachment from surfaces in adhesion experiments or for use as a colloidal sphere in subsequent indentation experiments whereas positive pressure allows for the object to be released. Control of the ionic current enables force-controlled electrophysiology experiments.

In this section, we detail the main experimental considerations, whereas the systems assessed with these protocols are described later. General advices, regardless of the protocol envisaged, can be found in Box 1.

Local dispensing. Tipless probes with apertures of 2 μm or 4 μm are suited to delivering molecules or nanoparticles onto cells, whereas cylindrical probes and pyramidal probes with 300 nm apertures at the apex are better-suited for 2D patterning and 3D printing experiments (Fig. 2). For all kinds of probes, the microchannel is filled with a solution containing the molecule or ion to be dispensed. The tip is positioned above the desired location under optical guidance, followed by the launch of the AFM approach. Upon contact, when the force reaches the setpoint, an overpressure pulse is applied to deliver the solution; the magnitude and duration of the overpressure pulse (typically of

the order of tens of millibars and hundreds of milliseconds) must be optimized for every system.

In 2D patterning and 3D printing experiments, the AFM scanner or the x – y stage is moved according to predefined lithography routines in the AFM software or custom LabVIEW codes. These codes synchronize the AFM controller to regulate tip–surface contact; movement along the x , y and z directions; and the pressure controller for material dispensing. Printing experiments carried out in air are much more delicate than those conducted in liquid because of the strong influence of capillary forces and surface tension, requiring probe coating to compensate for wetting⁷⁷.

Underpressure-mediated indentation and adhesion. The application of underpressure in FluidFM facilitates the reversible capture of micrometre-sized objects – like cells, bacteria or colloidal particles – at the cantilever aperture. Colloidal particles and cells must first be adsorbed onto a surface to allow their aspiration. Objects must be larger than the aperture to form a tight seal; therefore, the diameters of particles and cells determine the maximum aperture diameter of the tipless probe. Microchannel height must also be considered, as rigid microbeads must not touch the upper wall of the microchannel⁷⁸. The probe is positioned using the optical inspection above the object

on the surface. For cells, the AFM approach is initiated with a setpoint of about 10 nN. Approximately 10 s after contact is established, an underpressure of 700 mbar is applied. The object is tightly aspirated against the aperture and subsequently detached with backward force spectroscopy $F(z)^{23,24,67}$ for adhesion experiments⁵³. The retraction speed must be controlled and optimized for each mammalian cell type.

Captured colloidal spheres may be used as indenters for cell-indentation experiments using forward force spectroscopy $F(z)^{23,24,67}$. In this set-up, the spheres are added to the same dish as adsorbed cells. A pulse of overpressure releases the captured sphere and prepares the probe for acquiring the next one. This experimental approach can also be used to evaluate the adhesion of colloidal particles to the substrate⁷⁹.

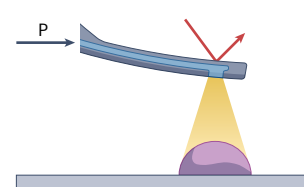
Membrane puncturing. Forward force spectroscopy is used with pyramidal or slanted cylindrical probes to pierce cell membranes and the nuclear envelope of living cells, allowing access to the cytoplasm or nucleus for pressure-driven delivery of selected cargos. The aperture is fully inserted by driving the tip apex down to the substrate beneath the cell, using setpoint forces typically ranging from tens to hundreds of nanonewtons, depending on cell size and stiffness.

Using low-stiffness probes (approximately 0.2 N m^{-1}) enables detection of perforation events such as penetration of the plasma membrane or nuclear envelope via the $F(z)$ curves; these are represented as subtle relaxation peaks^{47,51,52}. A constant applied force holds the tip in place, whereas a brief pressure pulse (tens of millibars for a few seconds) releases tens to hundreds of femtolitres directly into the cytoplasm or nucleus^{1,51}. Injected volumes can be quantified using fluorescence, either of the molecule itself or a co-injected fluorescent marker. HeLa cells, for example, tolerate injections of up to 800 fl, representing 20% of their volume, without noticeable morphological changes, whereas larger volumes cause vesiculation and cell death⁵¹. The combination of real-time force feedback, pyramidal tip geometry, anti-fouling coatings and precise pressure control ensures maximal injection efficiency while preserving cell viability.

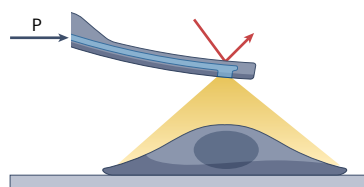
FluidFM also enables the extraction of intracellular fluid by applying underpressure after tip insertion⁸⁰, with applications ranging from sampling intracellular molecules for downstream analyses⁸¹ to organelle transplantation between cells⁸². Pre-filling the probe with oil or perfluorooctane prevents dilution of the extracted fluid by confining it at the front of the microchannel for later dispensing. The probe aperture acts as a molecular sieve: standard 550 nm openings

a Local delivery ($P > 0$)

2D and 3D lithography

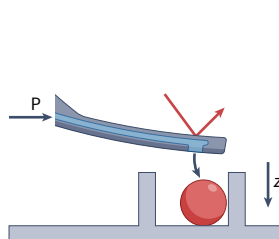
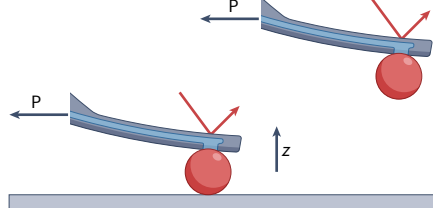


Delivery of active molecules

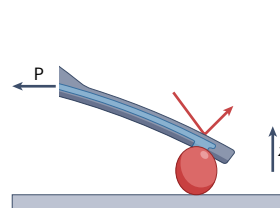


b Grasping an object ($P < 0$)

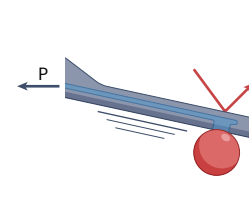
Pick and place



Adhesion colloidal probe

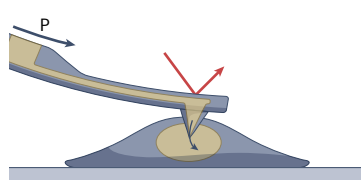


Microbalance

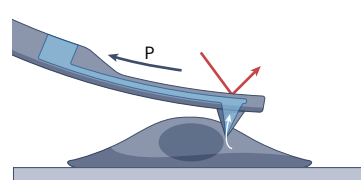


c Cell puncturing

Injection ($P > 0$)



Extraction ($P < 0$)



d Ionic current

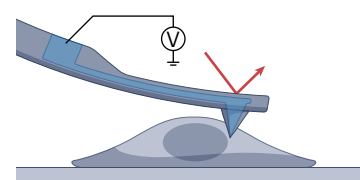


Fig. 4 | FluidFM operating modes. Force, pressure and ionic current, combined with varying tip geometries, facilitate a broad range of experiments. In all panels, p indicates the applied pressure and the red arrows represent the laser beam for force control. **a**, Local delivery of payloads with an overpressure ($P > 0$) from micropipettes or nanopipettes. These types of experiments include lithography and delivery of active molecules. **b**, Grasping an object, such as a cell or a colloidal sphere, using micropipettes or nanopipettes applying underpressure ($P < 0$).

These protocols are used for pick-and-place, adhesion-and-indentation and weighing experiments. **c**, Cell membranes can be punctured using nanosyringes, which can be used either for injection with overpressure ($P > 0$) or extraction with underpressure ($P < 0$). **d**, Measurement of ionic currents is achieved by inserting an electrode into the fluidic force microscopy (FluidFM) pipeline for patch-clamp, scanning ion-conductance microscopy and nanopore experiments.

Box 1 | General recommendations before performing a FluidFM experiment

Basic principles

Fluidic force microscopy (FluidFM) is an atomic force microscopy (AFM) technique; new users must learn the principles of AFM, at least in contact mode (used for imaging and force spectroscopy)^{23,24}. FluidFM experiments involve a combination of AFM, electrochemistry, microfluidics and cell biology, requiring some prior experience among users.

Technical considerations

Users should verify the integrity of the fluidic connection between the clip and the tubing leading to the pressure controller, to ensure proper flow control and the absence of leaks. This connection must be monitored when applying underpressure to prevent aspirated liquid coming into contact with the pressure controller. Installing a liquid trap between the system and the controller eliminates this risk.

All solutions to be loaded into the probe should be filtered using 0.22 µm pore filters to prevent clogging of the microchannel.

At the end of an experiment, the used probe can be stored for future reuse by immersing the clip in double-distilled water. Applying a slight overpressure can help to maintain flow, reducing the likelihood of clogging.

Experimental troubleshooting

Probe clogging can render the probe non-functional and halt the procedure. The addition of a fluorescent dye to the filling solution helps to monitor flow during the experiment. The dye can be visualized as a fluorescent cloud following an overpressure pulse, revealing partial or full flow obstruction.

If the interaction forces are of quantitative importance, the inverse optical lever sensitivity (InvOLS) and spring constant k must be determined with the highest possible accuracy.

Relocating the same cells after incubation between FluidFM manipulations can be challenging, particularly over long intervals.

Gridded or patterned substrates provide fixed spatial references that facilitate cell tracking. Both manual stages with micrometre screws and automated stages with stored coordinates enable precise repositioning, even though accuracy can decline if the culture dish shifts in the stage holder between sessions. Cell movement and morphological changes over time add another layer of complexity. Culturing cells in confined areas, such as microstructures or inserts, and fluorescently labelling selected target cells during the initial manipulation can greatly improve tracking⁸¹.

Injection experiments

Successful injection requires that the nanosyringe aperture be fully inserted into the cell; otherwise the injected solution will leak into the surrounding medium. To minimize this risk, the triangular aperture is positioned as close as possible to the apex of the probe. For cytoplasmic injection in well-spread cells, the puncture should be made near the nucleus, where the cell is thicker. Adding a fluorescent dye to the channel helps to monitor the injection process.

Ionic current experiments

For $I(V, z)$ curves, an electrode (usually an Ag/AgCl wire) is custom-mounted into the connector from the tubing side. Proper electrical insulation of the connection between the electrode and the internal reservoir is crucial: a water-tight seal alone is insufficient, as small leakage currents can still occur. Additional insulation with wax or glue is therefore required. A Faraday cage is mandatory to shield the electronics, and the Petri dish should be fixed to the x-y stage with a metal ring to ensure stability.

Lithography experiments

Experiments performed in liquid are generally reliable. By contrast, experiments in air are challenging because of wetting, surface tension and capillarity effects, which are difficult to control and tune.

selectively extract soluble molecules such as metabolites, proteins and transcripts⁸⁰, whereas larger, custom-designed apertures can retrieve entire organelles like mitochondria or endoplasmic reticulum⁸². Substantial cytoplasmic volumes have been extracted without causing permanent damage to the cell. For example, HeLa cells maintained 82% viability after removal of up to 4 pL of cytoplasm⁸⁰, and smaller macrophages tolerated extraction of up to 70% of their volume with 86% viability⁸¹, as assessed by live–dead staining and time-lapse microscopy post-extraction. No significant effects on cell growth, division, transcriptomic profiles or cellular functions were detected after extraction, underscoring the method's minimal invasiveness⁸¹.

Ionic current. FluidFM-based single-cell electrophysiology experiments require two Ag/AgCl electrodes, prepared by electroplating in 3 M KCl (ref. 35) or using automatic chlorination devices⁸³. One electrode is placed in the bath solution connected to the ground, and the other is inserted into the reservoir of a probe holder connected to a patch-clamp amplifier⁸⁴. As in standard AFM experiments, FluidFM-based single-cell experiments require cells to be firmly attached to the substrate. Adherent cells naturally spread onto the substrate; non-adherent cells, such

as suspended animal cells, require immobilization on substrates containing microfabricated structures^{85,86}. The probe is positioned over a selected cell by optical microscopy visual guidance and is approached to the cell surface in contact mode (1–50 nN). While the contact force between the probe and the cell is maintained constant by the AFM force-feedback controller, force and ionic currents are recorded simultaneously; during such monitoring, an underpressure is eventually applied to form a stable seal, which is mandatory for patch-clamp measurements⁸⁷.

Software

FluidFM combines an AFM system, operated with an inverted optical microscope, with a pressure controller and potentiostat or patch-clamp amplifier. Each of these instruments exploits proprietary software both for experiments and for data analysis. In many cases, external signals, such as those from patch-clamp amplifiers, can be acquired simultaneously in additional data channels by means of break-out boxes available from the instrument manufacturers. As FluidFM is commonly implemented on commercial instruments, third-party open-source software (for example, Gwyddion) can be used for data analysis.

Custom LabVIEW and Python routines synchronize the instruments and ensure a common temporal origin for the data series recorded by different devices. Available software like Cytosurge and Arya for the robotic FluidFM integrate force spectroscopy, the pressure controller settings and optical microscopy image acquisition. As such, a streamlined workflow for single-cell force spectroscopy can be readily achieved for new users.

Results

As an AFM-based instrument, FluidFM can both measure observables associated with an object and physically manipulate that object. This dual capability is reflected in the types of data produced and in the software tools used to analyse them. This section outlines the main readouts associated with each operating mode and explains how to interpret them, following progression from raw signals to basic quantitative descriptors and, in manipulation experiments, to the molecular or phenotypic outcomes generated.

FluidFM as measuring tool

Topography. Surfaces can be scanned using a pyramidal probe in contact or oscillating modes to image topography, like a standard AFM^{23,24}. The approach location can be determined with higher resolution when using the syringe-like version (Fig. 2c,d) than when using the optical microscope (Fig. 5a). A sufficient spatial resolution can be assured even with a 300 nm aperture at the apex when using one of the small tips (Fig. 2b). AFM topography images are acquired using the software that controls the AFM and can be processed either within that same software or with programs specialized for AFM data analysis²³.

Force spectroscopy F(z). FluidFM is suited for both indentation and adhesion experiments. The raw forward and backward F(z) curves each contain two columns – a z-position and cantilever deflection – which can be analysed directly with the AFM's software or with AFM-specific programs²³, or it can be imported in MatLab or Python. From the approach curve, Young's modulus of the indented object can be derived^{88–93}, although applying the Hertz model to complex, viscoelastic soft matter remains debated⁹⁴. The extracted Young's modulus is highly sensitive to the choice of contact point, making its accurate selection crucial^{95–98}.

Conversely, retraction curves quantify the adhesion of an object (such as a colloidal sphere or a cell) to the surface^{99–101}. In these curves, the minimum (most negative) force corresponds to the maximum adhesion force, and the area enclosed between the retraction curve and the z-axis represents the energy required to detach the cell from the substrate.

Although many research groups still rely on custom-written software to analyse large sets of force curves, an increasing number of suitable open-source tools is now available.

Ionic current. In patch-clamp, scanning ion-conductance microscopy (SICM) and nanopore experiments, the resulting ionic current is measured with a patch-clamp amplifier, as for electrophysiology experiments. The corresponding raw data – voltage and deflection versus time (Fig. 5b) – are recorded with LabView and analysed with Python routines.

FluidFM as manipulating tool

When using FluidFM for printing experiments and single-cell perturbation experiments, the output data involves both the deflection signal during manipulation and the read-out used to characterize the effect of the manipulation.

Printing. Immediately after 2D patterning by dispensing molecules or colloids – optionally under electrochemical control – the resulting structures can be inspected in situ using AFM topography^{36,102}. The 2D patterns and 3D structures can also be examined at any time with scanning electron microscopy. Because fluorescence imaging is convenient and versatile, it is often beneficial to dispense biomolecules or nano-objects labelled with a fluorescent tag, which allows the 2D patterning to be visualized in situ by optical microscopy. The corresponding images are processed using the software associated with the scanning electron or fluorescence microscope.

Single-cell perturbation. Following FluidFM-based single-cell manipulations, downstream readouts are tailored to the specific perturbation and therefore vary widely across studies. These readouts track the fate of the manipulated cell, the delivered cargo or both, and fall into three broad categories: live phenotypic imaging, bulk analysis after clonal expansion and picolitre-scale biopsy omics.

For cells left in situ, fluorescence microscopy is the most commonly used readout, as it provides a straightforward means of dynamic phenotyping. Examples include monitoring the translation of injected mRNA or plasmid reporters^{41,51}, Ca²⁺ transients through mechano-activated channels⁸⁷, quantum-dot traction maps on confocal traction force microscopy substrates^{58,103} (Fig. 5c), membrane-tension fluorescence lifetime imaging microscopy with Flipper-TR^{47,87} (Cytoskeleton Inc.; Fig. 5d) and inflammasome activation after cytosolic *Salmonella* delivery^{59,104}. Fluorescence imaging can also track the cargo itself – for instance, the integration of transplanted mitochondria into the host network⁸², the subcellular localization of injected extracellular vesicles⁴¹ or the growth of injected bacteria^{61,105}.

When a single manipulated cell can be isolated and clonally expanded, bulk assays become possible. Examples include Sanger genotyping of CRISPR-edited clones⁶⁰ and mitochondrial genome sequencing after organelle transfer⁸². Such clonal readouts relax the sensitivity constraints inherent to low-input single-cell assays and allow for broader molecular analyses.

Finally, FluidFM can extract picolitre-scale cytoplasmic biopsies for low-input molecular profiling, enabling full-length single-cell RNA sequencing (RNA-seq)⁸¹ or matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) metabolite profiling¹⁰⁶ without destroying the cell. Because the sampled material represents less than the content of a single cell, these analyses require highly sensitive, dedicated protocols adapted from the single-cell field to ensure sufficient analytical sensitivity.

Applications

In this section, we discuss established applications of FluidFM across four core modalities: local dispensing and printing, picking for indentation and adhesion, membrane puncturing for injection and extraction, and force-controlled ionic-current measurements. Representative case studies illustrate the applications of FluidFM and highlight typical outcomes and use contexts.

Local dispensing

2D patterning. AFM enabled fabrication and characterization of 2D patterns of nanostructures, for example through nanolithography techniques such as nanografting^{107–109}, dip-pen nanolithography^{26,27,110} and the nanodispensing tool NADIS^{111–113}. FluidFM, which combines

the technical advantages of AFM-based nanolithography with microfluidic material delivery, enables true 2D nanolithography by design¹⁰².

Its operation is analogous to a chart recorder but at far smaller scale: the probe follows a predefined trajectory while ink is steadily delivered to the tip apex and deposited onto the substrate. During

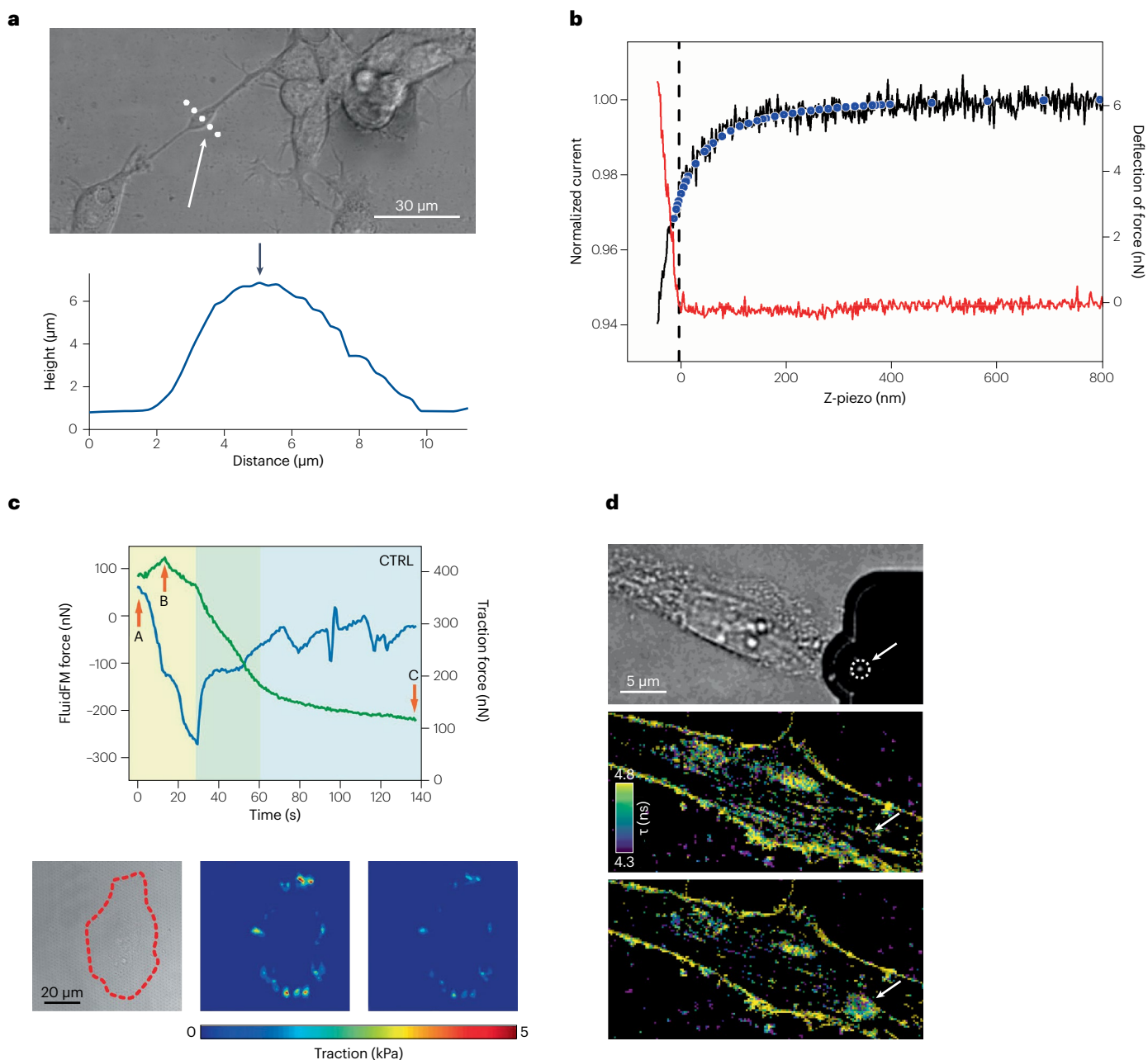


Fig. 5 | Example of experimental results. **a**, An optical image of two neuroblastoma cells connected by a neurite forming a varicosity-like structure (white arrow) obtained using the differential interference contrast method. The corresponding atomic force microscopy (AFM) profile is shown along the white dotted line (oscillating mode with a constant amplitude of 50 nm, scan rate of 0.1 s). The black arrow indicates the point on the profile where the tip was precisely approached using AFM control. **b**, Simultaneous force (red) and ionic current spectroscopy (black) using a nanopipette probe in a 150 mM KCl solution. Overlaid on the ionic current curve is an approach curve simulated with the finite element method (blue dots). **c**, Representative fluidic force microscopy

(FluidFM) force curve (blue) and generated traction (green) over time during detachment of HeLa cells using a micropipette probe. The corresponding bright field image of the HeLa cell is provided (cell is highlighted in red), together with the two traction force maps at time points A (left) and B (right). **d**, Representative bright-field and fluorescence lifetime images of a cell loaded with Flipper-TR before (middle) and after (bottom) indentation with a 25 nN force using a cylindrical probe (white circle and arrow). C, complete detachment; CTRL, control. Part **a** reprinted with permission from ref. 1, ACS. Part **b** reprinted with permission from ref. 158, APS. Part **c** adapted from ref. 58, CC BY 4.0. Part **d** reprinted from ref. 87, Springer Nature Limited.

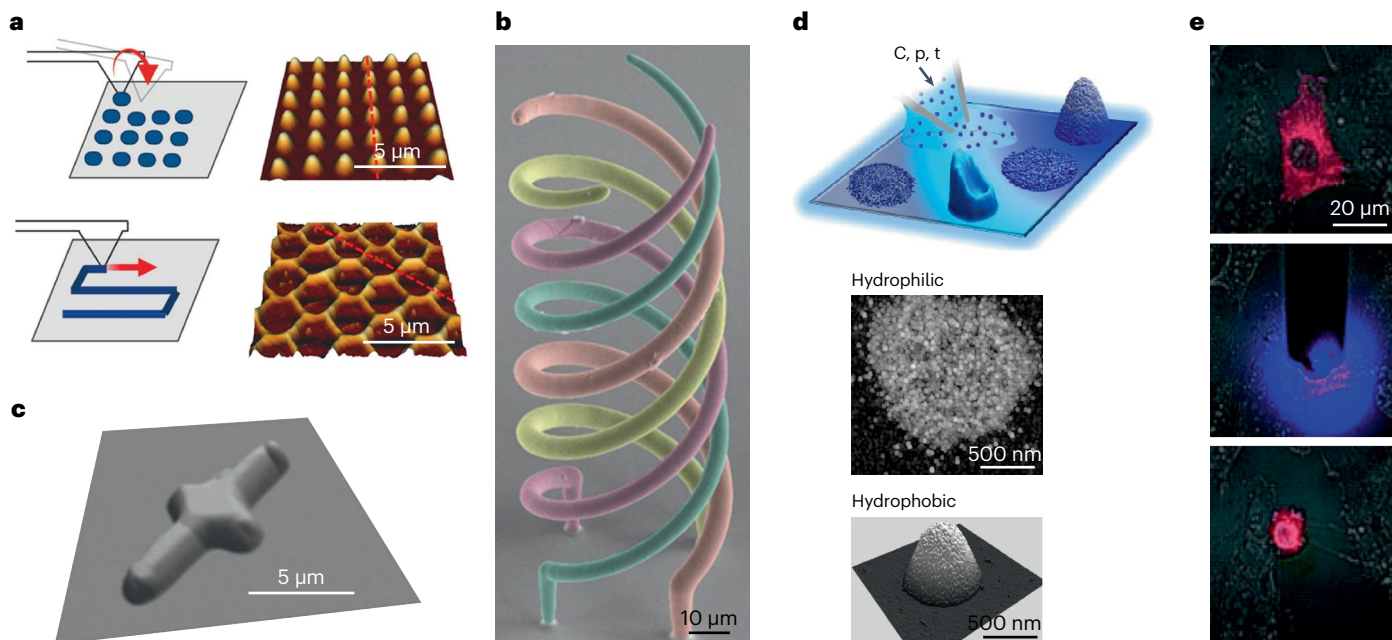


Fig. 6 | Local dispensing experiments. **a**, Surfaces are patterned with a commercial ink (hybrid acrylate-based ultraviolet (UV)-curable Loctite AA3491 (Henkel)) utilizing a fluidic force microscopy (FluidFM) nanopipette followed by UV curing for polymerization. Dispensing is performed either in force mapping mode for the hemispherical grid structures or in lithography mode for the honeycomb pattern. **b**, Scanning electron microscopy images (coloured) of four intertwined coils printed with a 500 nm FluidFM nanopipette as nozzle, each coil printed at a different pressure to highlight the flexibility and the ability to change and control the voxel size within the same structure during the printing process; 3,056 voxels were printed in 25 min to obtain the 180 μm -tall structures. **c**, Atomic force microscopy topographic image of a cross structure of asymmetric 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine crosses. **d**, Top, schematic diagram of liquid delivery controlled by pressure (p), concentration of solute (C) and contact time (t). Printing star [(polystyrene)₃₄poly(N,N-dimethylaminoethylmethacrylate)₄₀]₃₉, a molecule carrying net positive charges in aqueous solution due to the primary amine termini: star polymers form disk structures on a hydrophilic *N*-(6-aminohexyl)-aminopropyltrimethoxysilane/glass surface, whereas they form mounds on a hydrophobic octadecyltrichlorosilane/glass surface. Scale bars, 500 nm. **e**, Single-cell detachment by controlled trypsin release. Glass-cultured HeLa cells with a fraction of them transfected with p(monomeric red fluorescent protein (mRFP)-actin) (shown in red). The microfluidic probe is positioned 5 μm above the mRFP-actin-transfected cell. Trypsin and the fluorescent marker Cascade Blue were released using a pressure pulse. The targeted cell was detached from surrounding cells. Panel **a** reprinted with permission from ref. 119, ACS. Panel **b** reprinted with permission from ref. 123, Wiley. Panel **c** reprinted from ref. 128, CC BY 4.0. Panel **d** reprinted with permission from ref. 116, ACS. Panel **e** reprinted with permission from ref. 54, RSC.

patterning, the contact force (or AFM load) is set to a specific value – for example, 15 nN for gentle contact – and used as the feedback signal. As in contact-mode AFM imaging, the force remains constant throughout printing, ensuring steady contact between tip apex and surface. Together with the aperture size, the interplay among force setpoint, the applied overpressure (typically only a few millibars) and the writing speed (from $<1 \mu\text{m s}^{-1}$ to hundreds of micrometres per second) determines the final pattern¹⁰².

In general, the writing resolution is limited by the aperture size and cannot be smaller than the aperture; however, writing resolution can be improved upon exsiccation. Compared with conventional AFM nanolithography, FluidFM offers several advantages, including a broader range of deliverable materials and fine control over deposition via adjustments to pressure, contact time, contact force and the local environment. A unique feature of FluidFM is its ability to perform nanolithography across scales – producing millimetre-scale features with nanometre precision. Material delivery is routinely combined with local chemistry in ambient or liquid environments, including electrochemical conditions.

Using FluidFM, 2D patterns have been printed from various materials, ranging from small molecules and nanoparticles to hard materials

and soft polymers^{36,77,102,114–119} (Fig. 6). In addition, patterns have been formed within thin films via selective dissolution by delivering an etching solution¹²⁰.

3D printing. A challenge for performing 3D nanolithography with AFM is the difficulty of depositing a second material layer on top of the first with high positional accuracy. AFM drift can shift the tip relative to previously printed features, leading to poor inter-layer alignment and reduced fidelity. FluidFM overcomes this limitation by enabling 3D printing via 2D nanolithography combined with precise inter-layer registry achieved with force-based feedback.

In a typical set-up, a FluidFM cantilever is immersed in a three-electrode electrochemical cell and filled with a metal-ion solution whose flow is pressure-controlled. The probe tip acts as a localized ion source within a supporting electrolyte: when the tip contacts the working electrode at a sufficiently cathodic potential, the dispensed ions are reduced, and metal is electroplated in a confined volume beneath the tip. As the voxel grows, it eventually touches the tip apex, generating a cantilever deflection (tens of nanonewtons) that is detected and used as a feedback signal to retract the probe and move to the next position¹²¹. Therefore, force feedback has a dual role: establishing initial

probe–surface contact and sensing voxel growth. This approach enables layer-by-layer 3D metal printing of complex structures made from copper, gold, silver, nickel, and Ni–Mn and Ni–Co alloys^{121–126} (Fig. 6b).

FluidFM can also be operated analogously to commercial 3D nano-printers but in a highly miniaturized format with superior spatial resolution, again leveraging force feedback. During printing, the contact force (AFM load) is set to a fixed value (for example, 15 nN), which remains constant throughout the process, similar to contact-mode AFM imaging. High inter-layer registry was also demonstrated by 3D printing a photopolymer in a design consisting of three stacked grid layers, achieving 4.8 nm precision over a 1 mm feature range¹²⁷.

Another mode of operation involves dynamic movement of the probe during dispensing and drying along predefined trajectories with nanometre precision: materials are deposited along the path traced by the tip¹²⁸ (Fig. 6c). In practice, either printing mode – or a combination of both – can be used to realize complex 3D structures.

New chemistry via spatial confinement. FluidFM enables the delivery of ultrasmall (sub-attolitre) droplets. The influence of experimental parameters – such as aperture size, delivery pressure, contact time and local surface tensions – on droplet volume has been systematically characterized to allow precise tuning of the dispensed volume^{116,117}. With sub-attolitre droplets, molecular assembly is primarily governed by the initial droplet geometry, because evaporation occurs rapidly. The liquid–air interface geometry during drying directly shapes the resulting molecular assembly. Under these kinetically dominated conditions, molecular packing deviates from equilibrium structures and instead depends on transient local concentration and curvature. By varying the solution concentration and manipulating the tip near the phase boundary, molecular packing within the droplet can, in principle, be regulated. This concept of controlled assembly is demonstrated in Fig. 6d, in which both the overall feature geometry and intra-feature molecular packing of star polymers – for example, star [(polystyrene)₃₄-(poly(N,N-dimethylaminoethylmethacrylate)₄₀)]₃₉ – were precisely controlled^{116,117}.

Cell-selective delivery. FluidFM has also been applied for single-cell-level delivery of substances on adherent cell layers to assess how cellular responses depend on the delivered dose and environmental factors like buffer composition and cell-layer confluence. For example, viruses deposited onto individual cells were used to quantify infection probability as a function of the number of virus particles, revealing a cooperative effect¹²⁹. The microchannel was loaded with trypsin to isolate an individual cell upon its detachment from the adherent culture⁵⁴ (Fig. 6e). Spiking activity in primary rat hippocampal neurons was studied by releasing the excitatory neurotransmitter glutamate while simultaneously measuring electrical and optical signals¹³⁰. Immune responses were probed by delivering specific agonists to macrophages plated on surfaces¹³¹.

Indentation and adhesion

FluidFM has been widely applied to force spectroscopy to map the interaction potential between the probe and the sample surface^{132,133}. From these measurements, interfacial and material properties such as adhesion and Young's modulus can be extracted. In liquid environments, the $F(z)$ curves reveal several characteristic regimes (Fig. 7A). Upon approach, long-range interactions such as van der Waals forces and diffuse layer overlap act before physical contact, corresponding to the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) forces in colloid

science¹³². In the case of fuzzy interfaces, additional steric (repulsive) or bridging (attractive) forces may appear, for example from extracellular matrix components¹³⁴. Once the probe contacts the sample, further approach leads to indentation, which enables determination of elastic properties⁸⁹. During withdrawal, adhesive properties are measured¹³⁵.

Accurate quantitative analysis of forward $F(z)$ requires well-defined probe and sample geometries, motivating the development of the colloidal probe technique^{136,137}. In colloidal probe-AFM, a micrometre-sized colloidal particle is glued to the end of a conventional AFM cantilever (Fig. 7B). This technique has proven powerful for measuring particle–particle interaction forces, elastic moduli of cells, and other soft materials and adhesion forces¹³³.

FluidFM addresses the main limitation of the classic colloidal probe technique – the permanent attachment of colloids – by enabling reversible immobilization of colloidal particles through aspiration at the cantilever aperture⁷⁸ (Fig. 7C). This eliminates the glueing step and allows rapid exchange of probes⁴². As a result, statistically robust datasets can be obtained using multiple colloids, avoiding systematic errors from probe roughness variations⁷⁹. Aspirating a series of microgel particles further accelerates the determination of their Young's modulus compared with indenting pre-adsorbed particles with a classic colloidal probe¹³⁸. Colloids can also be aspirated from suspensions undergoing adsorption¹³⁹. Exchanging probes additionally prevents contamination, such as protein adsorption, often transferred in conventional contact measurements¹⁴⁰.

Because immobilization does not require glue or optical manipulation, much smaller colloids can be used – down to 300 nm (refs. 72,74) (Fig. 7D). A conductivity measurement through the internal channel can verify successful probe attachment⁸³. Importantly, colloidal probe-FluidFM enables the study of particles that cannot ordinarily be immobilized because they lack rigid anchoring points, such as core–shell particles⁷⁴, as well as oil droplets¹⁴¹, gas bubbles¹⁴² and hydrogel microspheres¹⁴³.

FluidFM indentation has expanded mechanical analysis of soft materials, including disentangling the mechanical contributions of the actin cortex⁹⁸. In contrast to classical AFM, FluidFM also enables inverse indentation, in which a sample (like protozoa) is aspirated at the aperture and pressed against a substrate¹⁴⁴. The mass of the aspirated object can be quantified by comparing cantilever resonance frequencies with and without the object¹⁴⁵.

The most transformative application, however, has been measuring cell adhesion on solid substrates. FluidFM enables aspiration of individual cells followed by controlled detachment^{38–40,53,56,63,146–151} (Fig. 7E), overcoming limitations of single-cell force spectroscopy approaches that rely on growing or chemically attaching cells onto cantilevers^{67,99}.

The analytical power of FluidFM-based force spectroscopy increases even further when combined with techniques such as confocal traction force microscopy or fluorescence lifetime imaging (Figs. 5c,d and 7f). These combinations allow mechanical stimuli – indentation or aspiration – to be linked to biochemical responses at the cellular level^{87,103}. Membrane-tension-dependent activation of mechanosensitive ion channels such as Piezo1 can be probed directly⁸⁷. Traction force microscopy enables correlation of the FluidFM-applied stimulus in the z direction with lateral (x – y plane) substrate coupling^{58,103}.

Puncturing the cell membrane

Mammalian cell injection. FluidFM nanoinjection (Fig. 8A) has been successfully applied to many adherent cell types, including those that are difficult to transfect^{41,59,152}. Cargoes that can pass through the

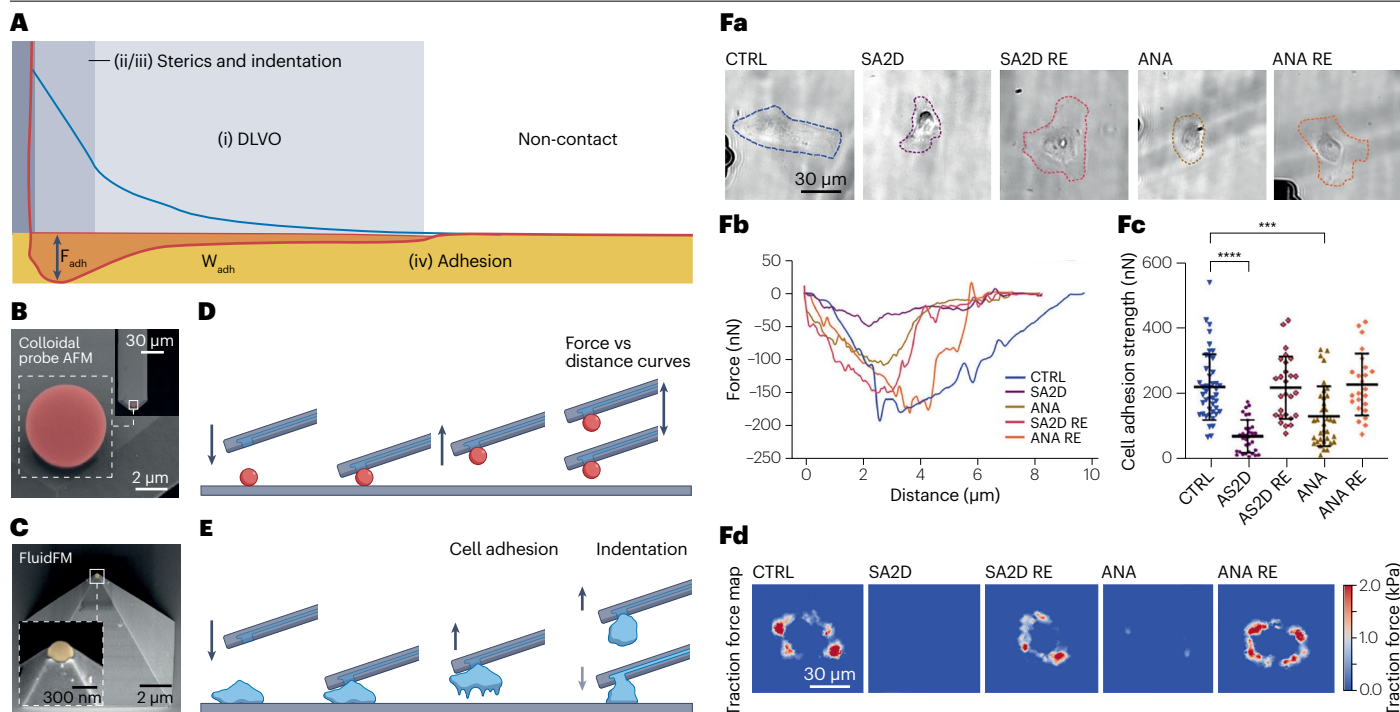


Fig. 7 | Indentation and adhesion experiments. **A**, A schematic representation of a force F versus distance z as typically acquired by atomic force microscopy (AFM). The blue line represents the force during probe approach, whereas the red line represents the force during probe retraction. The force $F(z)$ acting on the probe is determined as a function of the piezo displacement. In fluidic force microscopy (FluidFM), $F(z)$ curves are analogous to those obtained using conventional AFM measurements, so the algorithms used to convert piezo displacement to probe distance or indentation apply. **B**, **C**, Scanning electron microscopy images of colloidal probe-based FluidFM with a micropipette (part **B**) and with a nanopipette (part **C**, 300-nm aperture), which are used to determine interaction profiles between colloidal particles or for indentation and adhesion experiments. **D**, Schematic of colloidal particle aspiration,

force spectroscopy and ejection. **E**, Schematic of cell adhesion behaviour measurement. **F**, Chromatin remodelling regulates cell adhesion strength and traction force. **Fa**, Representative brightfield images of PtK2 cells before detachment with FluidFM. The outline of the cell area is labelled in blue for CTRL, purple for SA2D, pink for SA2D RE, yellow for ANA and orange for ANA RE. **Fb**, Representative force–distance curves of cell detachment from cells in panel **Fa**. **Fc**, Quantification of cell adhesion strength in nanonewtons. **Fd**, Corresponding traction force maps (kilopascals) of cells in panel **Fa**. Red indicates a large contractility area, and blue a small contractility area. CTRL, control; DLVO, Derjaguin–Landau–Verwey–Overbeek forces. For ANA, ANA RE, SA2D, and SA2D RE, see ref. 151. Parts **B** and **C** reprinted with permission from ref. 74, Wiley. Part **F** adapted with permission from ref. 151, ACS.

600–800 nm nanosyringe aperture can be delivered, including proteins (such as RNase¹⁵²), nucleic acids (for example, plasmid DNA^{41,51} and mRNA^{41,60}) and CRISPR–Cas9 ribonucleoproteins⁶⁰, and mixtures thereof. Larger biological entities that exceed electroporation limits can also be introduced: intact extracellular vesicles (300–400 nm) using standard probes⁴¹, and entire organelles⁸² or live bacteria^{59,105} using cylindrical probes. Direct cytoplasmic injection enables studies of intracellular fate and interactions while bypassing endosomal uptake routes or allowing implantation of bacterial strains incapable of natural internalization.

Mammalian cell extraction. Picolitre-scale biopsies allow molecular analyses of living cells. Nuclear biopsies have been examined by electron microscopy and quantitative PCR; conversely, cytoplasmic extracts support enzyme assays, metabolite profiling and transcriptomics^{80,81,106}. Live-seq generates transcriptomes from 1–2 pl cytoplasmic samples that faithfully reflect whole-cell profiles. By preserving cell viability, it enables sequential transcriptome measurements from the same cell, capturing dynamic state transitions (Fig. 8C) and linking molecular signatures to eventual phenotypes.

Although FluidFM uniquely enables subcellular molecular readouts from living cells, analytical sensitivity remains a key limitation due to the extremely small sample volumes. Advances in single-cell omics, together with techniques developed for nanoscale biological materials such as extracellular vesicles and secretomes, are expected to broaden targeted and omics-scale analyses of transcripts, proteins and metabolites from FluidFM biopsies.

Extraction can be coupled with injection to transplant organelles or other cellular components between cells⁸². For example, mitochondria transferred via FluidFM exhibit higher integrity and more efficient integration into host networks than those delivered using conventional purification-based methods (Fig. 8D).

Injection and extraction in fungi. FluidFM protocols have been adapted to fungi, including yeasts and filamentous species, which are approximately ten times smaller than mammalian cells and encased in a rigid cell wall⁵². Adaptations include smaller apertures, increased insertion forces up to 2 μ N (10–100 $\times$ those used for mammalian cells) and higher injection pressure (>1 bar) to overcome turgor. Fluorophores and labelled histones have been injected from a few to several hundred

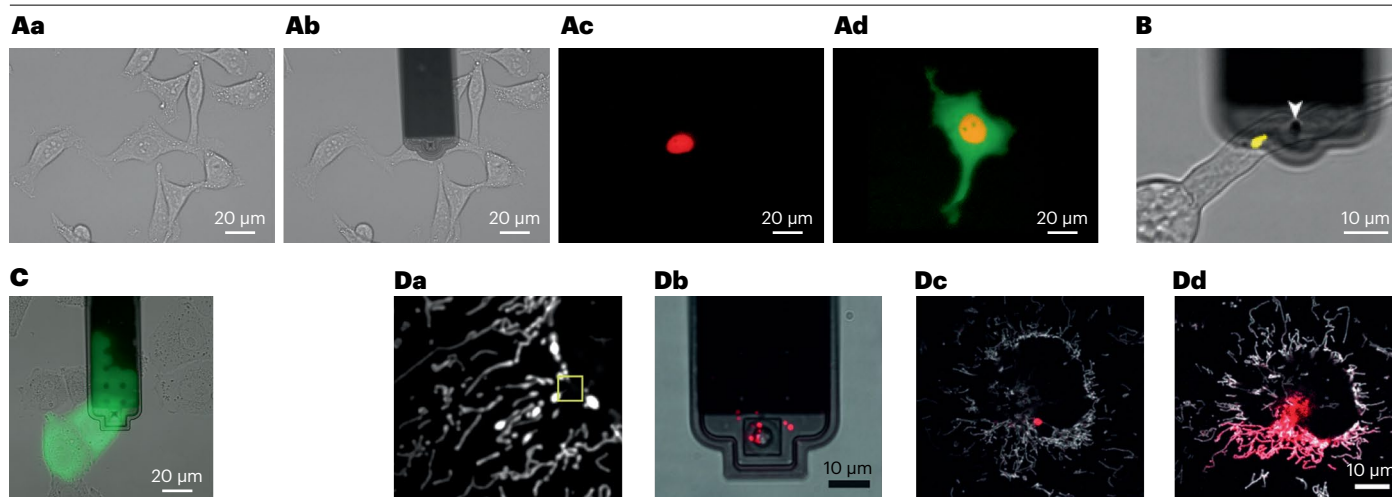


Fig. 8 | Injection, extraction and transplantation experiments. **A**, Nuclear injection into HeLa cells before (part **Aa**), during (part **Ab**) and immediately after (part **Ac**) nuclear injection of pmaxGFP, followed by the same cell 48 h later (part **Ad**) expressing the encoded GFP (green). A dextran–TRITC tracer (red) was co-injected and remained confined to the nucleus. **B**, Bacterial implantation. Delivery of *Escherichia coli* (yellow) into the filamentous fungus *Rhizopus microsporus*. The white arrowhead marks the apex of the probe inserted in the germling. **C**, Cytoplasmic extraction of cytoplasmic fluid from a GFP-expressing HeLa cell, with extracted material visible in the probe channel. **D**, Organelle transplantation using extraction and injection. **Da,Db**, Extraction of multiple

mitochondria from a viable U2OS donor cell. Part of the mitochondrial network (su9-BFP) of the donor cell during extraction (part **Da**); the cantilever apex is indicated by the yellow box. Extracted mitochondria collected inside the FluidFM probe (part **Db**). The scale bar applies to parts **Da** and **Db**. **Dc, Dd**, A single transplanted mitochondrion (su9-mCherry, red) delivered into a U2OS recipient (mitochondrial network su9-BFP, white). Immediately after delivery (part **Dc**). Integration of the transplanted mitochondrion into the host network after 87 min (part **Dd**). The scale bar applies to parts **Dc** and **Dd**. Part **A** reprinted with permission from ref. 51, Wiley. Part **B** adapted from ref. 61, Springer Nature Limited. Part **D** adapted from ref. 82, CC BY 4.0.

femtolitres across diverse species without compromising cell viability. Cylindrical-tip probes have enabled direct cytosolic injection of living bacteria into filamentous fungi (Fig. 8B), facilitating the artificial induction and tracking of early endosymbiosis in *Rhizopus microsporus*⁶¹. *Escherichia coli* triggered septation and blocked symbiosis, whereas *Mycetohabitans rhizoxinica* propagated and was partially vertically transmitted. Long-term selection primarily drove fungal genomic adaptations that improved symbiotic stability. More recently, FluidFM was used to implant the free-living bacterium *Ralstonia pickettii* into *R. microsporus*, revealing a transcriptional shift from antagonism towards commensalism as the induced endosymbiosis stabilized across fungal generations¹⁵³. Nanoextraction has also been demonstrated in fungi⁵² and has been applied to study fungal defence mechanisms by mimicking nematode predation⁵².

Force-controlled ionic current

Patch-clamp measurements. Ion channel-mediated ionic fluxes are fundamental to cellular physiology¹⁵⁴, and patch clamp remains the principal method to measure these currents with molecular resolution^{155,156}. However, conventional micropipettes lack force feedback, making controlled approach to cells difficult. FluidFM provides a force-sensitive pipette, enabling reliable whole-cell recordings on pulsatile cardiomyocytes³⁵. Automated force control allows the probe to maintain a stable seal during contractions, enabling continuous recording of periodic electrical activity (Fig. 9A). A current limitation is the difficulty in routinely achieving gigaseals.

SICM. SICM is a powerful technique for high-resolution, non-contact topography and electrochemical imaging^{11,12,157}. The same FluidFM configuration used for patch clamp can perform SICM,

enabling simultaneous measurement of force and ionic current during approach¹⁵⁸ (Fig. 5b). FluidFM-based SICM provides high-quality topographical imaging of delicate structures such as neuronal filaments, which are difficult to image by AFM due to their weak substrate adhesion. The measured force can also serve as an auxiliary feedback signal: non-contact SICM is used on smooth surfaces, and force feedback is activated if probe deflection exceeds a threshold, enabling safe navigation of steep height gradients and preventing probe collisions¹⁵⁹.

Scanning nanopore microscopy. An approximately 5 nm pore sculpted at the apex of a pyramidal tip yields a scanning nanopore microscope. The force-controlled approach creates tunable nanoconfinement, enabling controlled biomolecular translocation⁸⁴ (Fig. 9B). This FluidFM-based nanopore can record extracellular protein secretions and ion-channel activity at arbitrary membrane sites, and – via force-controlled penetration – probe intercellular translocation events (such as at the nucleus). Moreover, unlike fixed-size nanopores, FluidFM can create pores of tunable diameter (2–20 nm) by force-driven deformation of the tip against a soft substrate¹⁶⁰ (Fig. 9C). Functionalization with DNA aptamers enables recognition of specific amino acids upon translocation, facilitating identification of peptides containing phenylalanine from structurally similar controls¹⁶¹. Integrating such nanopores with mass spectrometry in the future could facilitate analysis of secreted biomolecules at the single-cell level, aiding in diagnostics and therapeutic-target discovery¹⁶².

Reproducibility and data deposition

In this section, we outline the main factors affecting reproducibility in FluidFM experiments, practical strategies to enhance consistency,

as well as current data sharing practices and the development of standards for transparent reporting and common formats.

Reproducibility

FluidFM is a highly versatile tool that supports a wide range of applications across diverse experimental systems. Now used in more than 100 laboratories worldwide, its various applications have been repeatedly reproduced, adapted and optimized in independent settings.

As a relatively recent AFM adaptation, FluidFM can be custom-assembled or purchased from several manufacturers and combined with numerous complementary tools. Successful operation requires

continuous monitoring and adjustment of AFM and microfluidic parameters, along with readiness to troubleshoot fluidic components. Skill, dedication and day-by-day experience are essential – particularly given the intrinsic variability of biological samples.

Scanning probe microscopy performance depends on atomic-scale tip properties (for example, geometry and cleanliness), which are inherently outside of user control²³. FluidFM probes fabricated with a wafer-scale lithography also exhibit unavoidable variability in features such as microchannel dimensions, apex radius and aperture size; for this reason, each probe is supplied with a scanning electron microscope image of its aperture.

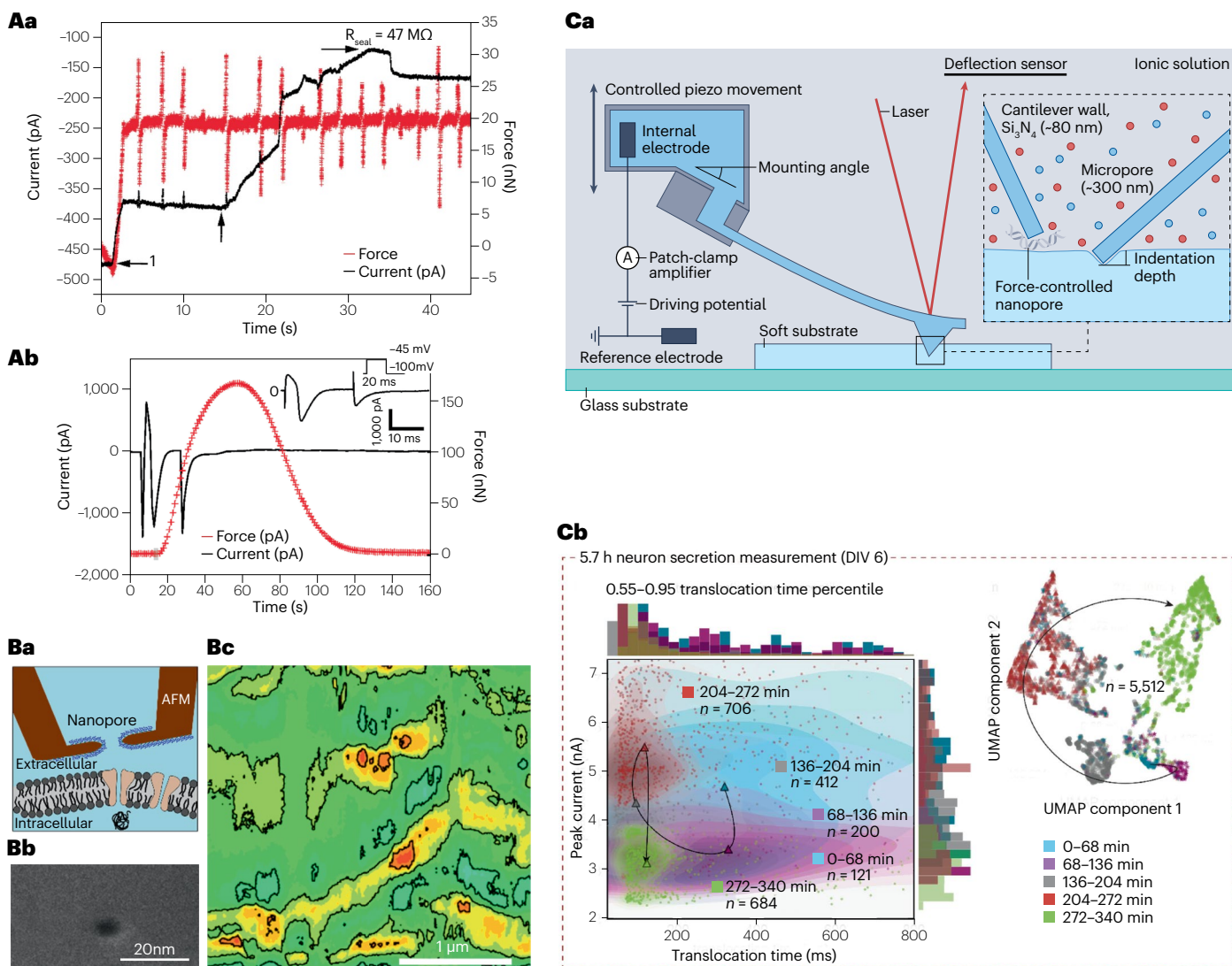


Fig. 9 | Ionic current experiments. **A**, Simultaneous whole-cell current recording and contraction-force measurement on a beating cardiomyocyte using fluidic force microscopy (FluidFM)-based patch clamp. **Aa**, Force and ionic current recording during approach on a contracting cardiomyocyte with enabled force control. **Ab**, With the force control disengaged, the effective contraction amplitude is measured simultaneously to evoked transmembrane currents. **B**, FluidFM-based scanning nanopore microscopy for localized detection of ions and biomolecules from single cells. **Ba**, **Bb**, Schematic illustration of extracellular recordings of protein secretions by a cell (part **Ba**) and a scanning electron

microscopy image of the nanopore of a FluidFM tip (part **Bb**). **Bc**, An ionic current map of HEK293 cells with excess of Piezo1 ion channels. **C**, Force-controlled nanopore with controllable sizes. **Ca**, Schematic diagram showing the tunable nanoconfinement at the interface between the FluidFM tip and the soft polymer substrate. **Cb**, Long-term (5.7 h) measurement of the secretome of a neuron. AFM, atomic force microscopy; DIV 6, six days in vitro culture; R_{seal} , resistance of the seal; UMAP, uniform manifold approximation and projection. Part **A** adapted with permission from ref. 35, ACS. Part **B** adapted from ref. 84, Springer Nature Limited. Part **C** adapted with permission from ref. 160, ACS.

When working with biological or other soft materials, aperture-edge contamination is common and can affect both dispensing pressure and membrane-puncture force setpoints. Anti-adhesive probe coatings are therefore crucial for reproducibility in sequential protocols, even though coating quality can vary.

When measuring forces, FluidFM inherits the same limitations as classical AFM. Two parameters are critical: the InvOLS and k , which together determine force-measurement accuracy. On soft cell-culture substrates, InvOLS cannot be calibrated in the standard way, leaving issues such as drift uncorrected; new calibration protocols offer promising improvements. Spring-constant calibration also remains a constant error source: a cross-laboratory test showed that accuracies better than 10% are rare¹⁶³. Good practice is to use two independent calibration methods (for example, Sader, Hutter–Bechhöffer, Cleveland).

Finally, protocols require adaptation to the specific system under study. In cell-adhesion measurements, for example, parameters such as contact setpoint, dwell time and retraction speed strongly depend on cell type. Therefore, publications should include as many protocol details as possible when reporting methodology to ensure reproducibility.

Data deposition

There are currently no standards for data repositories and formats. Common data formats are also missing in the AFM community as a whole²³. The increasing use of datasets based on the hierarchical data format (for example, HDF5) may represent an avenue for standardization in the future.

Limitations and optimizations

In this section, we discuss technical and practical constraints in FluidFM experiments, as well as mitigation strategies. Limitations are found at the probe, protocol and data analysis levels, affecting sensitivity, throughput and interpretability. Continued optimization of probe design, workflows and analysis is steadily increasing feasibility and experimental throughput.

Probes and tools

Because of their embedded channel – and because cantilever stiffness scales with the cube of thickness^{164–166} – FluidFM probes are relatively stiff. Although ultra-soft cantilevers (0.01 N m^{-1}) can reach piconewton sensitivity when noise is controlled, the softest FluidFM probes (0.2 N m^{-1}) achieve approximately 100 pN sensitivity. This is sufficient for cell-adhesion experiments but not for single-molecule rupture or unfolding experiments possible with bioAFM. The pyramidal probe apex typically has a radius of approximately 100 nm, which prevents atomic-resolution imaging but remains sufficiently sharp for membrane puncture. Focused ion beam sharpening of the aperture and apex provides flexibility but requires specialized equipment, and it is time-consuming.

Probe coating is essential, yet no universal and fully reliable protocol exists. Clogging may occur – often during mammalian cell adhesion or electrodeposition experiments – and is irreversible. If a mammalian cell remains stuck to the aperture edge, it may be mechanically removed by gently scratching a clean Petri dish region with the tipless part of the cantilever, but residual fragments degrade the seal in subsequent manipulations. The microchannel itself extends 1 mm from the cantilever to the chip's macroaperture, producing a high access resistance of approximately 18 MΩ in 150 mM KCl.

Current robotic set-ups typically use electric motors rather than piezos for vertical probe motion, prioritizing speed and range at the expense of resolution. Future systems could recover higher resolution by adopting advanced piezo actuators.

Protocols

FluidFM enables sequential single-cell adhesion and injection–extraction experiments with a single probe, but throughput remains limited to tens to hundreds of cells per day, depending on the workflow. As with AFM-based single-cell manipulation more broadly, this relatively low throughput largely reflects the heavy reliance on manual operation and operator expertise. The user must precisely position the FluidFM probe at specific subcellular locations (such as the nucleus or cytoplasm) before dispensing, injection or extraction. Prolonged experiments can lead to operator fatigue, affecting accuracy, and once the probe is positioned above the cell, it often obscures the view, making it difficult to verify correct placement.

A second fundamental limitation is the inherently serial nature of single-probe operation: each cell must be processed individually. Throughput therefore varies by application, from a few seconds per cell for adhesion or injection workflows to several minutes for subcellular extraction, plus additional time for biopsy transfer when molecular analyses are required. Semi-automated systems (like OMNIUM) that incorporate artificial intelligence (AI)-based cell recognition already accelerate routine workflows, and further developments in automation are expected to continue increasing throughput. Synchronization of system components is typically achieved through custom LabVIEW or Python scripts.

Although physiological conditions (such as temperatures of 37 °C) can be maintained during FluidFM manipulation of single living cells – and CO₂-independent media eliminate the need for 5% CO₂ – maintaining sterility throughout the experiment remains a significant challenge.

Data analysis

Results from using theoretical models (like the Hertz model) to fit indentation curves depend on the choice of contact point, which may be ambiguous. This limitation applies to FluidFM and AFM in general. The validity of the Hertz model for complex materials like mammalian cells has been questioned^{167,168}. The resulting Young's modulus should be considered a relative apparent value for comparison with values obtained from the same material under different conditions¹⁵¹.

Outlook

This section explores the future of FluidFM, from innovations in probe fabrication and self-sensing designs to advances in automation, multimodal integration and real-time imaging. These developments are expected to extend the precision, throughput and robustness of FluidFM experiments, lowering barriers for use and enabling more complex manipulations. Looking ahead, applications are likely to expand beyond single-cell studies to include organelles, tissues and therapeutic contexts, enhancing the role of FluidFM in quantitative cell biology and precision medicine.

Probes

Evolution of current optical beam detection probes. As with traditional AFM, the sensitivity of a FluidFM probe is dependent on its spring constant; the spring constant can be reduced – and sensitivity correspondingly increased – by acting on the length and width of the

cantilever, the height of the microfluidic channel, and/or the thickness of the channel walls. The polysilicon sacrificial technique for fabrication can achieve cantilever stiffness of $0.02\text{--}190\text{ N m}^{-1}$. Using polymer or other softer materials in the channel walls rather than silicon nitride is another option for reducing the probe spring constant.

Current FluidFM probes contain a single microfluidic channel. Integrating multiple channels into a single probe would enable new capabilities, such as combined injection and extraction through one tip insertion, delivery of multiple solutions in a single step, or even simultaneous 3D printing of different materials using a multi-channel design in which each channel terminates in its own nano-aperture at the pyramidal tip apex (Supplementary Fig. 3).

Modifying tip geometry expands the design space for FluidFM. Hybrid mosquito-style tips that combine pyramidal and cylindrical elements offer alternative geometries with potentially improved fluidic behaviour and subcellular access (Supplementary Fig. 4). Similarly, funnel-shaped tips with highly polished interiors provide a different interface for patch-clamp configurations and may facilitate gigaseal formation (Supplementary Fig. 5).

The current fabrication method also supports integrating active elements into the probes. For instance, metal electrodes can be incorporated on or within the cantilever and used for electrical, thermal or electrochemical sensing (Supplementary Fig. 6), with a small exposed region at the tip apex serving as the active electrode surface.

Self-sensing probes towards multiprobe FluidFM. Commercial bio-AFMs currently rely on optical beam deflection to measure cantilever bending, which restricts operation to a single probe per controller. Enabling multiple FluidFM probes – each dedicated to a specific stimulation or task on the same cell – will require self-sensing detection^{169–176}, an exciting direction for future development.

A fabrication process for polymeric AFM cantilevers with embedded microchannels and integrated silver nanowires as piezoresistive sensors has been demonstrated using SU-8; however, the limited spatial resolution of SU-8 microfabrication prevented integration of a sharp tip¹⁷⁷. In a notable advance, a multilayer approach combined the advantages of soft (SU-8) and hard (silicon, ceramic) materials by incorporating a piezoresistive semiconductor strain sensor into an AFM cantilever along with a sharp tip¹⁷⁸. Extending this strategy could enable the development of self-sensing FluidFM probes.

Coating and cleaning. Coating strategies must be improved to extend probe lifetime. One possibility is wafer-scale deposition of materials such as Teflon at the end of fabrication; another is the development of new polymer formulations that do not interfere with probe attachment to the plastic holder or degrade the gold reflective layer on the cantilever. Concurrently, cleaning protocols need to be optimized so that probes can be immersed in solutions harsh enough to remove biological residues while still preserving the adhesive bonds, securing the cantilever and the integrity of its reflective coating.

Protocols

Patch clamp. FluidFM was originally conceived as a force-controlled pipette to facilitate patch-clamp experiments. Ironically, this is the area in which progress has been slowest. The formation of gigaseals with glass capillaries is itself not fully understood^{179,180}; both glass composition and tip morphology have empirically been shown to be critical^{181,182}. This may explain the difficulty of forming gigaseals with FluidFM probes, which are made of silicon-rich nitride and are likely covered by a 10 nm SiO₂

layer in air and aqueous solutions. Current efforts focus on cylindrical probes designed to better mimic the geometry of glass capillary tips.

SICM. Because of viscous drag, the nearly horizontal orientation of the FluidFM probe is not well-suited for fast z-axis motion, unlike the vertical geometry of glass capillaries used in SICM^{183–186}. Nevertheless, for non-contact imaging, a dual-feedback controller that concurrently processes ionic-current and cantilever-deflection signals would be valuable. In such a scheme, the SICM controller would operate as the primary controller, whereas the AFM controller would act as a high-gain P controller with a minimal setpoint, taking over when collisions are detected. The main challenge is to characterize bandwidth and phase effects to avoid instability.

Glass capillaries routinely achieve $<10\text{ nm}$ diameters⁹ – difficult to match with FluidFM probes⁸⁴ – so standard SICM will generally remain superior for high-resolution imaging. However, combining FluidFM with SICM could be advantageous for correlated measurements on the same cell, such as FluidFM indentation for stiffness experiments⁷⁸ and localized hydrostatic pressure application in SICM mode^{187–190}.

Automation of cell protocols. Increasing automation and throughput of FluidFM remains a priority. User-friendly, automated robotic FluidFM platforms already support large-area printing³⁷, high-throughput single-cell adhesion measurements^{38–40}, single-cell injection⁴¹ and force calibration for indirect high-throughput devices^{38,42,43}. A promising direction is the integration of FluidFM with image-recognition workflows. Recent work has shown that deep-learning-based, label-free recognition of cellular structures in bright-field images enables operator-independent high-throughput AFM single-cell mechanical analysis¹⁹¹. Another study demonstrated fully automated AFM measurements and data analysis using AI to distinguish healthy from diseased cells or tissues¹⁹².

Future deep-learning approaches could identify subcellular structures directly from optical images and use this information to guide automated probe positioning, stage movement and pressure control. Such developments will significantly improve efficiency and usability, expanding FluidFM applications in life sciences. Cell attachment could also be monitored electrically by measuring channel currents⁸³. This points to new ways of increasing throughput by automating cell selection from optical images, probe positioning and the subsequent FluidFM protocol (indentation, adhesion, injection or extraction).

Moreover, the emerging field of single-cell surgery requires high-precision tools for manipulating organelles to study cellular dynamics, subcellular functions and disease mechanisms¹⁹³. Combining FluidFM with microrobotics and nanorobotics will open new opportunities for single-cell and subcellular therapies, advancing precision medicine at the cellular level.

Lateral view. Real-time visualization of FluidFM manipulations from a lateral cross-sectional perspective would be highly beneficial. Although confocal imaging can provide side-view images^{194–196}, it is neither real-time nor free of photobleaching concerns. Constructing a side-view optical microscopy module^{197,198} would enable real-time lateral observation during FluidFM experiments (for example, of the inside of the tip) and provide additional information to improve experimental control and interpretation.

Applications

Cell cultures. Standard 2D cell cultures have been a major focus of FluidFM experiments, and several opportunities exist for expanding

applications. FluidFM is particularly well suited for longitudinal single-cell studies, in which the same cell is repeatedly probed and observed over time – for example, to track responses to perturbations⁸¹ or to correlate molecular signatures with eventual phenotypes such as differentiation or drug resistance. In addition, as automation and throughput improve, FluidFM could become a routine tool for functional genomics screens requiring precise, low-volume delivery of CRISPR reagents or other cargoes into defined cell populations. Co-culture systems offer another frontier: FluidFM could selectively manipulate one cell type in the presence of another to dissect signalling, immune interactions or competitive dynamics. Similarly, combining FluidFM manipulation with complementary techniques – such as live-cell fluorescence imaging, traction force microscopy or electrophysiology – on the same cell will yield multimodal datasets linking mechanical, molecular and functional readouts.

Tissue slices and organoids. Although FluidFM studies have so far focused mainly on standard 2D cell cultures, extending the technology to 3D tissue slices^{188,189} and organoids^{199,200} would be highly valuable, as these systems better preserve native architecture, cell–cell interactions and physiological functions. FluidFM has already been applied to flattened organoids, in which intestinal enteroids are first grown in 3D to establish epithelial organization and then opened into a 2D plane to allow probe access. In this format, the intestinal enteroids retain key tissue-specific programs and innate immune pathways that are typically lost in conventional 2D cell lines, enabling FluidFM perturbations to reveal epithelial immune responses not observable in monolayer cultures^{59,104}.

Tissue slices similarly maintain 3D cellular architecture, microenvironmental cues and functional heterogeneity, making them useful for studying drug responses, development and disease mechanisms.

Applying FluidFM to intact tissues poses two major challenges: optical targeting in highly scattering samples and mechanical access to subsurface cells, which requires traversing overlying layers. Current cylindrical tips are 10–20 µm-long; longer tip geometries, along with improved 3D-aware imaging and targeting approaches, may eventually enable true 3D FluidFM manipulation within intact tissues and organoids.

Conclusion

FluidFM has established itself as a unique platform that bridges AFM and microfluidics, enabling simultaneous force control and fluid manipulation at the single-cell and subcellular level. Over the past decade, the technology has matured into a robust method supporting diverse applications, ranging from quantitative cell adhesion measurements and minimally invasive injection and extraction, to high-precision nanoprinting and force-controlled electrophysiology. The ability to probe, perturb and sample individual living cells while preserving their viability is a unique strength of FluidFM. Ongoing advances in automation, AI-driven workflows and probe engineering continue to enhance throughput and reliability. These developments will expand the impact of FluidFM across the life sciences and materials science.

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Competing interests

E.S. oversees the production of FluidFM probes and is employed by Bruker Nederland BV (NL). The other authors declare no competing interests.

Additional information

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