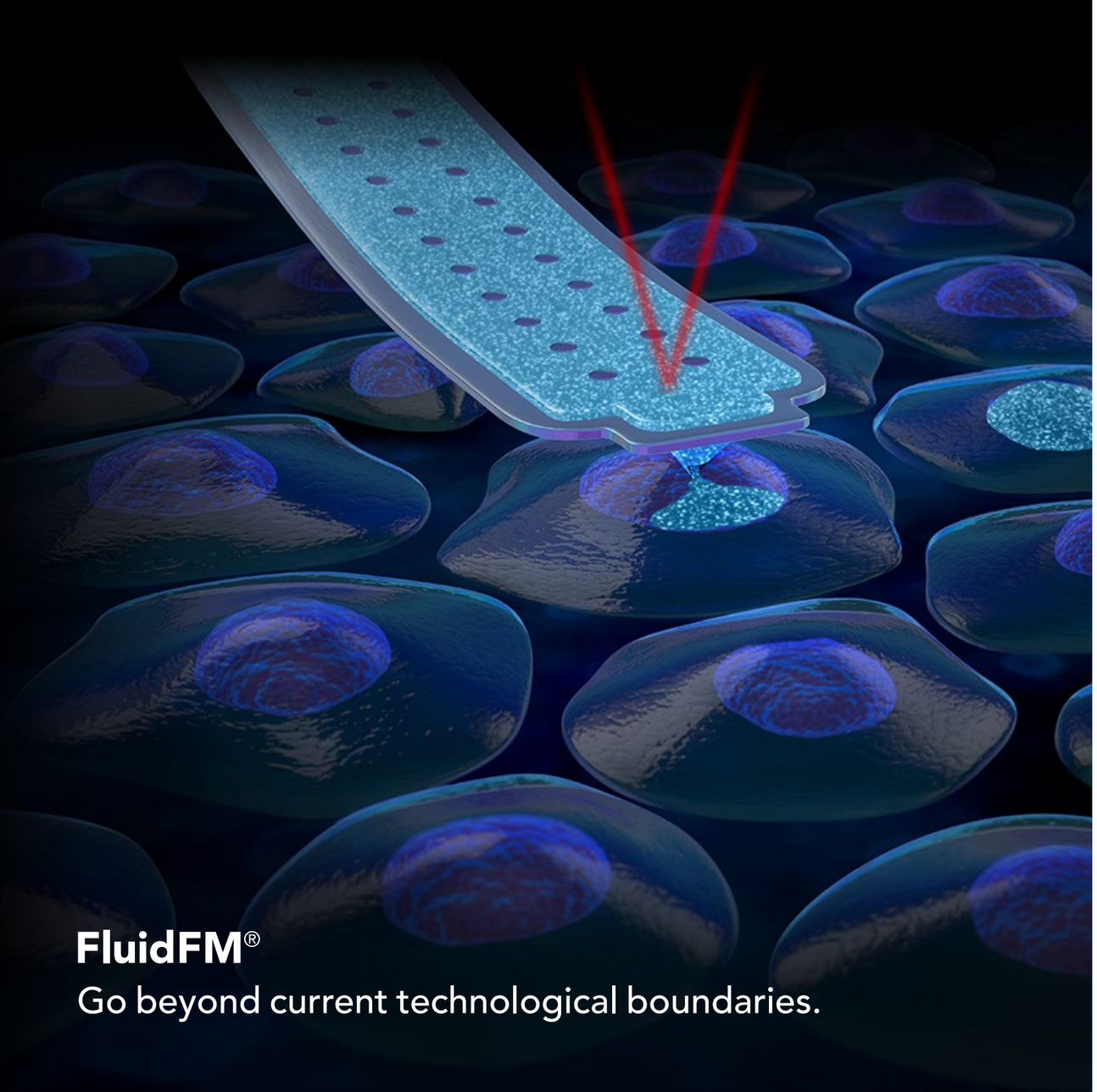
CUTTING-EDGE SYSTEM FOR SINGLE CELL RESEARCH.

FluidFM® BOT BIO SERIES

SYSTEM BROCHURE.

CYTOSURGE®



FluidFM®

Go beyond current technological boundaries.

CONTENT

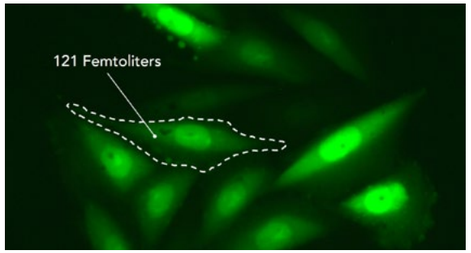
CUTTING-EDGE.

1. SYSTEM APPLICATIONS

The FluidFM BOT BIO Series offers solutions for single cell research – from single cell gene editing to drug research and toxicology.

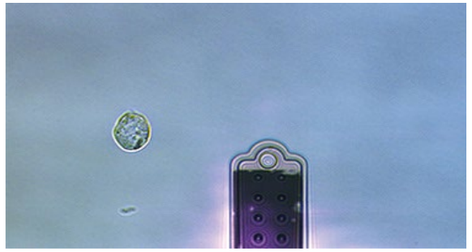
Single cell CRISPR delivery

Direct intra-nuclear CRISPR delivery. Place your complexes directly where they are required: in the nucleus.



Single cell drug assays

Deliver compounds directly into the cell. A suitable delivery method for any compound.



Cell isolation

Select which cell you want – place it where you want. For suspended and adherent cells. No unnecessary cell stress: directly from the culture plate.

More

Exploit the versatility of FluidFM technology to the fullest. Perform nano-printing, single cell adhesion measurements and more.

1.1 SELECTED USE CASES

Single cell CRISPR delivery & cell isolation:

Generation of multiple KO clones. One instrument, two weeks.

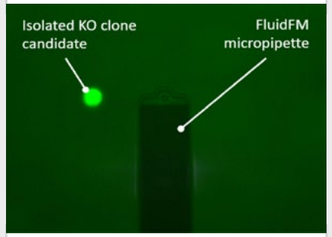
1. Injection of multiple gRNAs & Cas9

A fluorescent marker is co-injected into CHO cells to verify successful delivery.

- Multiple gRNAs are simultaneously delivered
- Viability: >95%

2. Isolation of the clone candidate

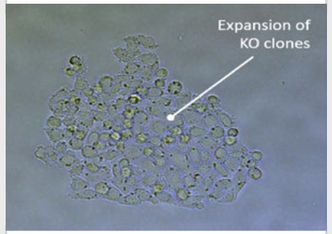
Selected cells are isolated into separate wells.



- Proof of monoclonality: visual confirmation of the deposition of a single cell
- Viability: >95%

3. Clone expansion and analysis

Monoclonal colonies are expanded and the DNA sequenced. 50% of the colonies show a mutation in all the targeted loci.



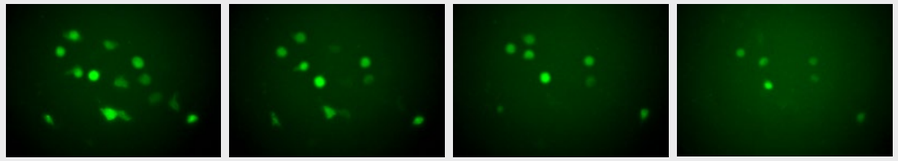
- Extreme reduction in complexity and development time
- Monoclonal cell line generated starting from few nano-injected cells

Single cell drug assays:

Protein degradation observed within 5 hours

Drug clearance experiment:

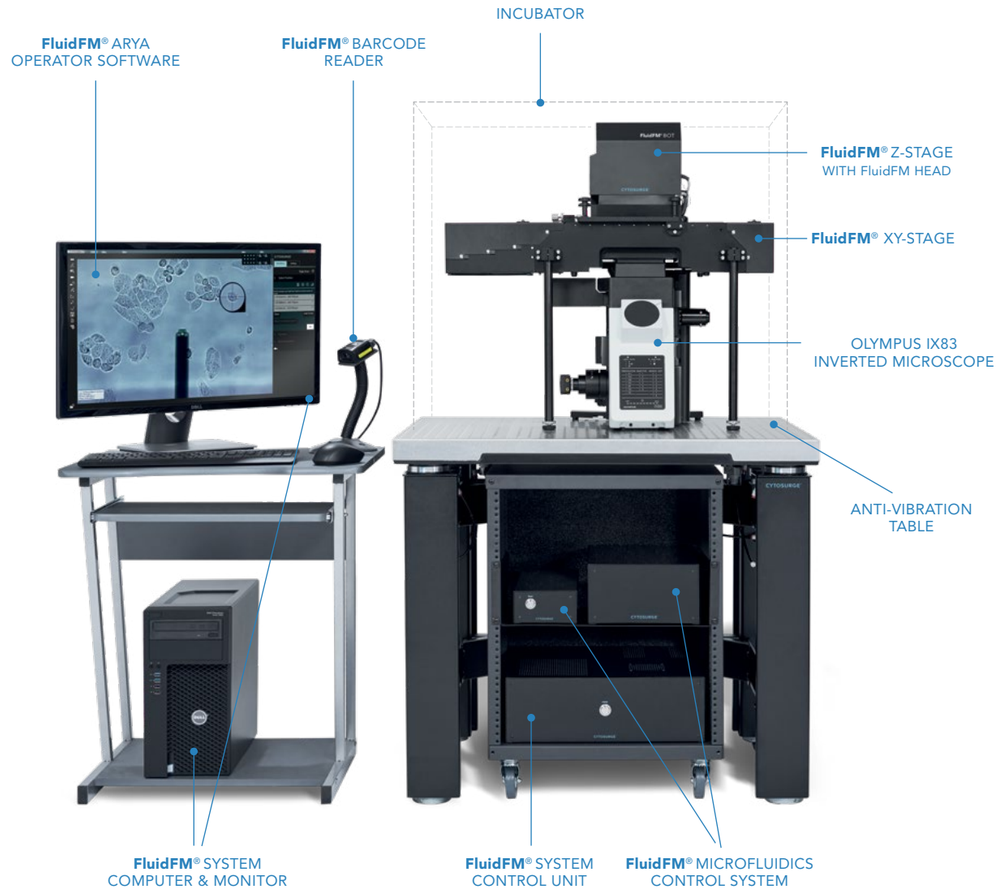
- An average of 50 fL of GFP solution injected into HEK293T cells, no impact on cell viability
- Fluorescent signal followed during 16h, drug clearance observed within 5h



Protein degradation. Time-lapse over 5 hours.

DEVELOPED FOR YOUR NEEDS.

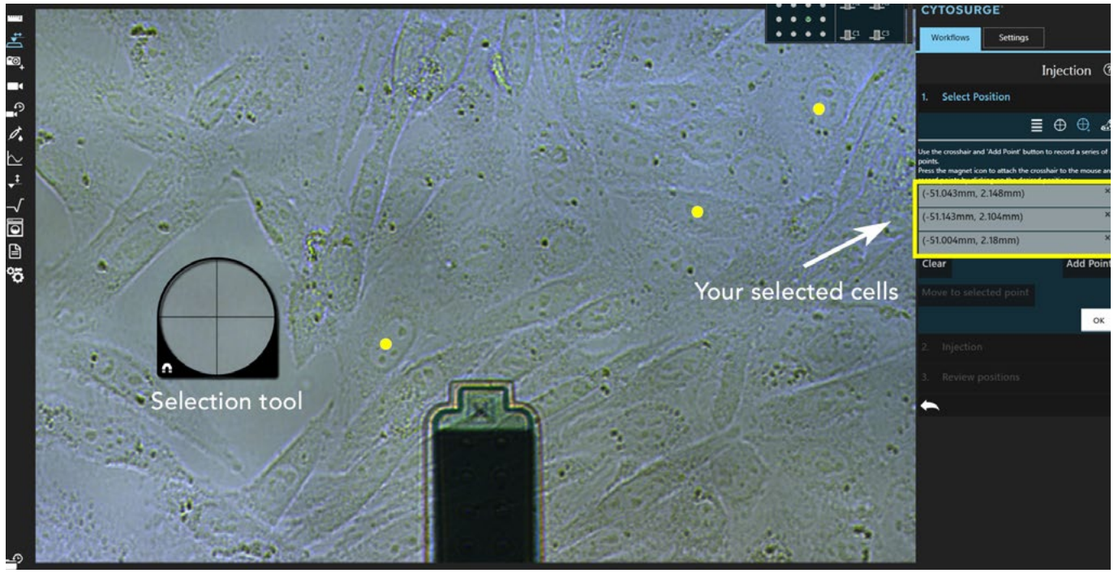
2. SYSTEM COMPONENTS



2.1 FULLY MOTORIZED MICROSCOPE FRAME BY OLYMPUS

Olympus' fully motorized inverted IX83 microscope provides long-term imaging solutions that greatly enhance the efficiency of this powerful and versatile system.

Olympus OEM components have been integrated into the FluidFM BOT BIO Series, for maximum imaging quality and performance. The IX83 inverted microscope is ideally suited for live cell imaging offering exceptional quality both in transmitted- and reflected-light imaging modes. This ensures high-quality images even with standard cell culture vessels. Equipped with a selection of three long-distance objectives (10x, 20x and 40x) and with epifluorescence capabilities, the Olympus IX83 inverted microscope maximizes the functionality and usability of the FluidFM BOT BIO Series. Each microscope component is controlled by the FluidFM ARYA operator software,ensuring a seamless operation experience and readiness to automated FluidFM workflows.



Easy point-and-click cell selection.

2.2 **FluidFM®** ARYA OPERATOR SOFTWARE

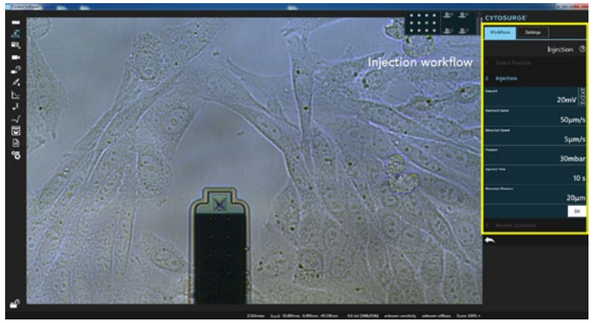
Our intuitive and user friendly software guides you step by step through the simple yet powerful application-specific workflows.

Point-and-click cell selection

Select the cells of interest according to their phenotype by simple point and click on the screen. When performing injection, selectively target for the nucleus or cytoplasm.

Full control

Appreciate full control over all components within the software, from cell selection to imaging and data management.



Software workflow, here paramters for the injection workflow.

Easy walkthrough procedures

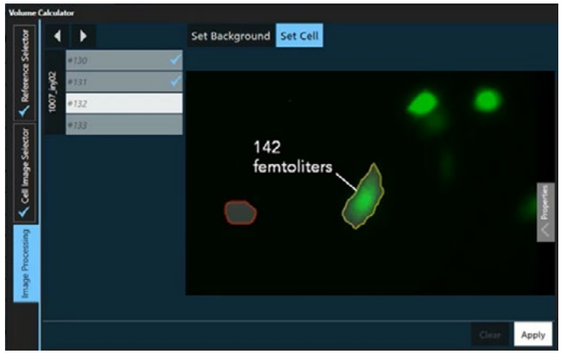
Follow the in-software suggested instructions, apply recommended settings from our protocols database and easily tailor parameters to your cells.

Observe & follow up

The complimentary FluidFM data management software EDNA enables to revisit and analyse your FluidFM experimental data at any time. See all relevant data grouped by the coordinates of each cell. Instruct the system to take the required images with predefined settings in few clicks and perform long-term observation with multi-channel time-lapses.

Calculation of injected volume

Our software-integrated Volume Calculator can precisely quantify with femtoliter precision the amount of injected compound for each single cell, when co-injected with a fluorescent marker. The Volume Calculator guides you step-by-step towards a self-generated report.



Volume Calculator interface.

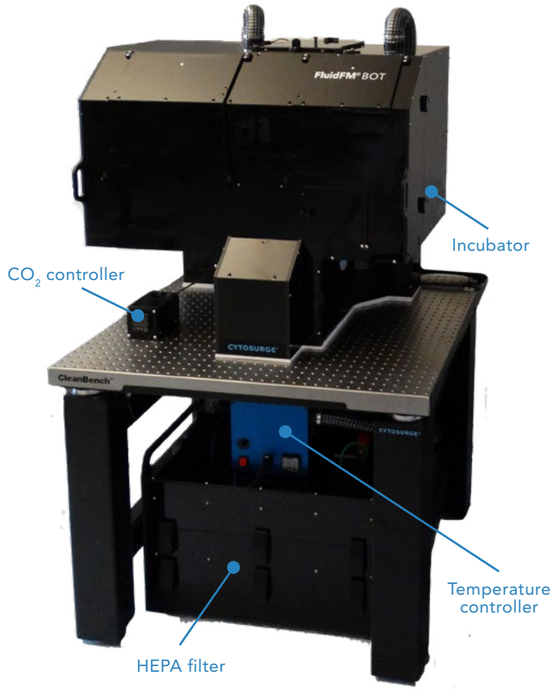
2.3 TEMPERATURE & CO_2 CONTROLLED INCUBATOR

Conduct your experiments in a cell-friendly environment to minimize disturbance on living cells. Designed around the FluidFM BOT BIO Series, our incubator offers the highest usability and performance.

Ideal for long-term imaging
Fully enclosed system and temperature stability +/- 0.1 °C guarantees maximum thermal stabilization of all the components, thus also maximizing focus stability.

CO_2 control
The incubator comes with CO_2- control capabilities, with adjustable CO_2 concentration levels between 1 and 15% (+/- 0.1% accuracy). Conduct experiments in physiological-like conditions.

Recirculating air and long lasting HEPA filters
A closed, recirculating air circuit ensures fast reaction to temperature disturbances and lowers contamination probability. With the addition of the HEPA filtering unit, your samples are protected form any airborne contamination.

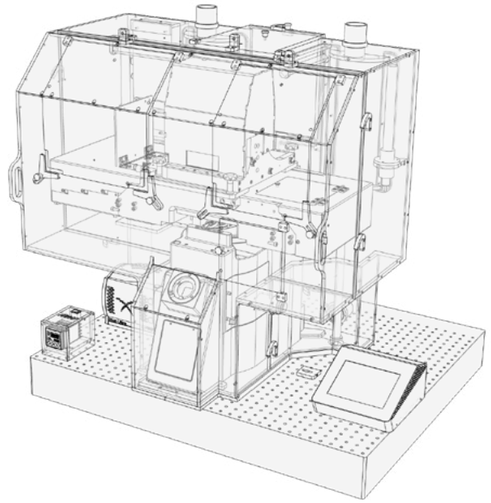


Incubator components.

Built-in UV decontamination lamps
Two UV light sources with automatic life cylce monitoring and time control allow sterilization of the incubator's internal environment.

LED inside
Large access panels and bright LED illumination ensure optimal accessibility to the system both for the experiment preparation phase and for routine preventive maintenance tasks.

Dark room
Black polycarbonate panels creates a dark environment ideal for fluorescence applications.



Incubator built around the FluidFM BOT BIO Series system.

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CONSUMABLES.

3. **FluidFM®** PROBES

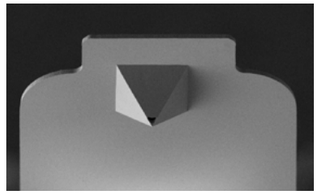
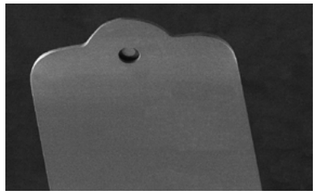
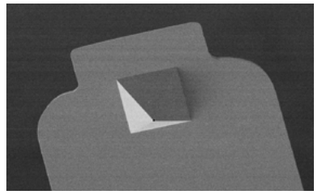
The core of the technology when performing FluidFM applications are the patented hollow FluidFM probes. The consumables are available in a broad variety of tip shapes, aperture sizes and mechanical specifications.

For single cell CRISPR delivery & drug assays: FluidFM nanosyringe

The very sharp apex and the around 600 nm aperture at the pyramidal tip of the FluidFM nanosyringe enable gentle injection not compromising cell viability and make it possible to work with compounds with differing molecular weights and densities. The FluidFM nanosyringe can be loaded with any liquid with viscosities ranging from 1 to 10000 cP (water to honey).

For cell isolation: FluidFM micropipette

With its flat circular aperture available with diameters of 2, 4 and 8 micrometers, the FluidFM micropipette is the ideal choice to pick and place suspended cells and objects of different sizes. For bacteria and sub-micron particles, the FluidFM nanopipette with its 300 nanometer aperture can be used.

FluidFM nanosyringe 600 nm aperture	FluidFM micropipette 2, 4, 8 µm aperture	FluidFM nanopipette 300 nm aperture
		
Nano-injection into single cells	Cell isolation	Spotting
Nano-extraction from single cells	Adhesion of single cells	Nano-printing

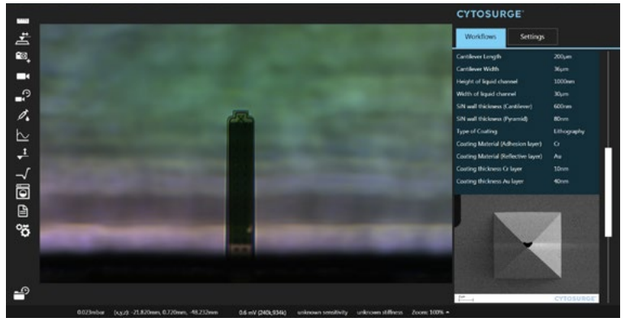
FluidFM probe types. If a customized probe designs is required, a prototyping probe is available to create an aperture of your choice.

FluidFM probe.

1 mm

High quality. Full control.

FluidFM probes are produced according to a patented, high-precision micro-fabrication process and are pre-assembled and individually wrapped in a tailor-made blister package. The QR-code printed on the package gives convenient access to all relevant probe characteristics, most importantly to a unique SEM image of each individual FluidFM probe taken during our quality control process. You can see exactly how your probe opening looks.



Full insight within the software. Characteristics of one specific FluidFM probe.

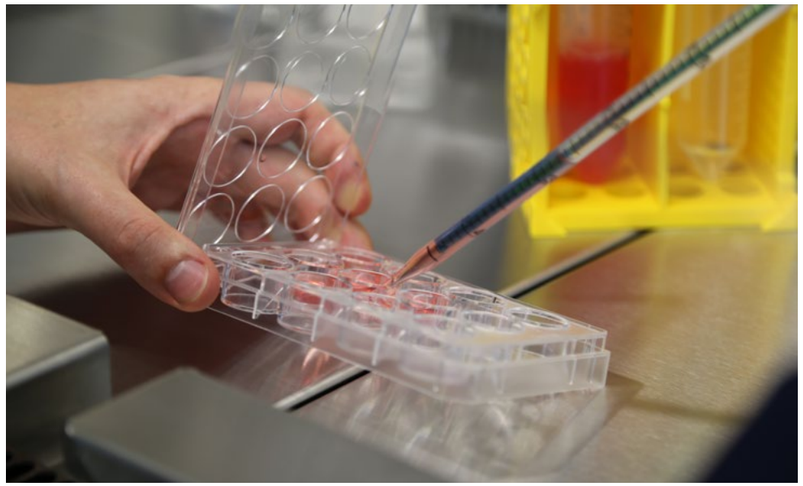
COMPATIBILITY.

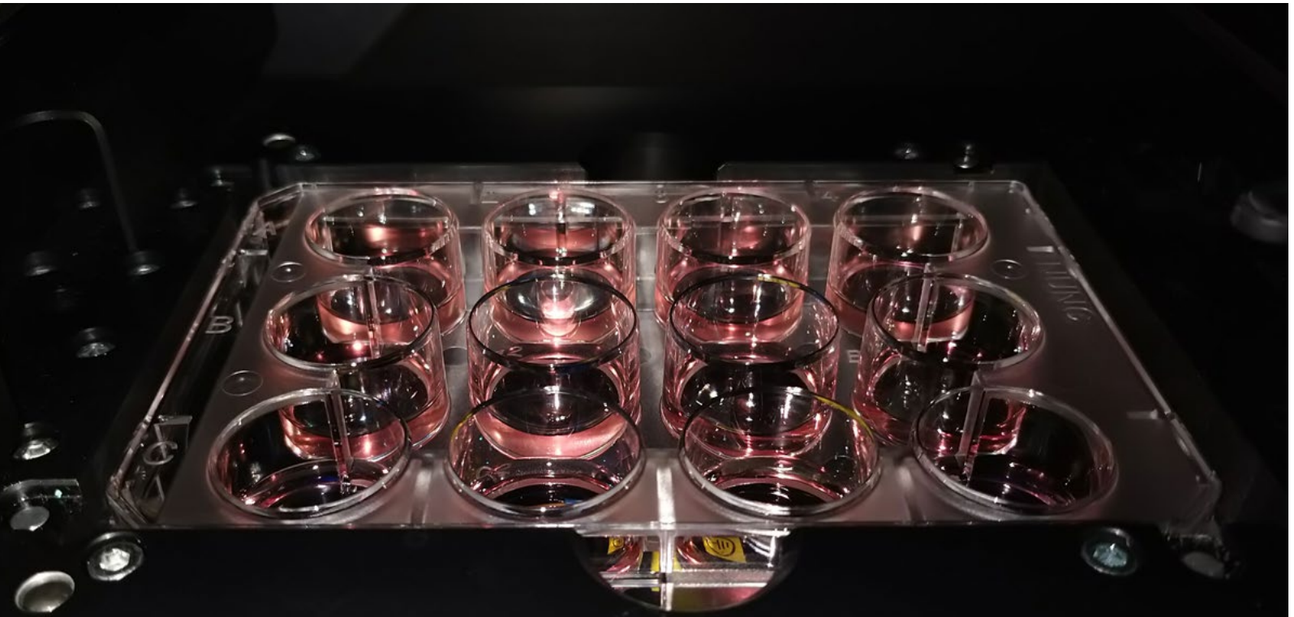
4. SUPPORTED PLATES & DISHES

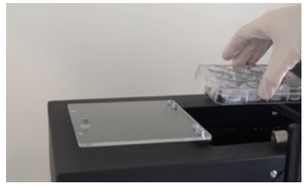
The FluidFM BOT BIO Series system already supports a wide range of plates and dishes. The smallest standard well size supported is that of 24 well plates. Consequently 12 and 6 well plates are also compatible, as are standard petri-dish sizes of various formats.

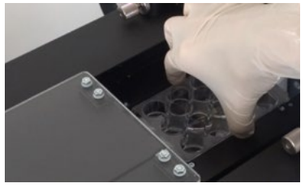
Specific examples of already supported plates:

- 24 well plate: VWR® Tissue Culture Plate
- 12 well plate: Thermo ScientificTM NunclonTM Delta surface treated
- 6 well plate: Corning Costar®
- 50 mm WillCo or Matek dishes (e.g. WillCo Type 5040)
- Microscopy slides, 0.5-1.5 mm thick









Morevover, the plate editor integrated in the FluidFM ARYA operator software, allows the user to add his own well-plate or dish. We can also create a special configuration for your preferred container. The physical holder for custom plates can either be created by the user or is available on request from Cytosurge (subject to charges).

5. SYSTEM & OPTIONS

System

- ✓ Automated FluidFM head unit
- ✓ Nanometer precise FluidFM XYZ-stages
- ✓ FluidFM microfluidics control system
- ✓ FluidFM system control unit
- ✓ FluidFM ARYA operator software
- ✓ FluidFM system computer & monitor, pre-configured
- ✓ FluidFM essential accessories (including FluidFM probe dicscovery kit (p. 17), barcode reader and more)
- ✓ Olympus IX83 inverted, motorized and fully integrated microscope
- ✓ Anti-vibration table

Options

Application specific modules

Epi-fluorescence module

- White light fluorescence LED illumination, 25'000 hours lifetime
- IX83 motorized fluorescence set, 3 HQ filters
- Fully integrated into ARYA software – 1 click setting changes and automated observation
- Option: Mercury (HG) lamp

Incubation module

- Temperature control
- CO_2 control
- Low-noise, high stability incubation
- HEPA filters
- UV lights integrated
- Option: no CO_2 control

Accessories

- Diverse FluidFM coating kits for different applications
- FluidFM coating container

Services & warranty

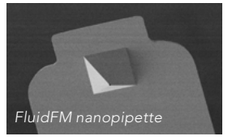
- Warranty extensions
- Maintenance contracts
- Scientific services
- Refurbishment & upgrades of system

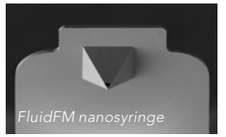
FluidFM probe discovery kit

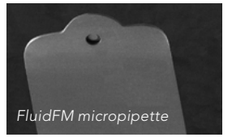
The FluidFM probe discovery kit includes a selection of 20 probes with different tips and various spring constants and apertures. The kit enables you to try diverse techniques, from cell isolation to spotting and nano-injection. The kit even includes FluidFM prototyping probes enabling you to create the aperture of your choice.

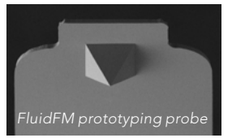
		Spring constant in N/m			
	Aperture in µm	**0.3**	**0.6**	**2**	**4**
Nanopipette	**0.3**		2×	2×	
Nanosyringe	**0.6**			4×	
Micropipettes	**2**	2×		2×	
	4	1×		2×	
	8	1×		2×	
Prototyping	**n.a.**		1x	1x	

FluidFM probe discovery kit. The table shows the number of each FluidFM probe type contained in the kit.



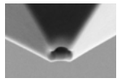
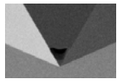
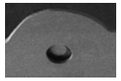
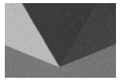




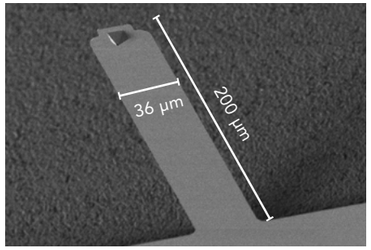


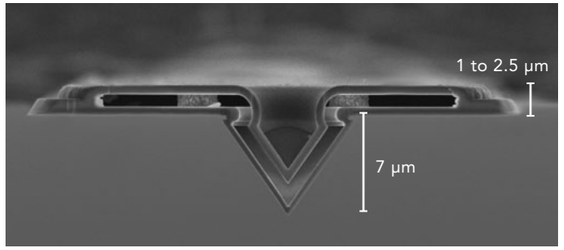
KNOW THE DETAILS.

6. TECHNICAL SPECIFICATIONS

FluidFM probe types	Aperture diameter	Main application(s)	Tip shape / aperture shape	Avl. spring constants (channel height)
 **Nanopipette**	300 nm	Spotting, nano-printing	Pyramidal/circular	0.6 N/m (500 nm), 2 N/m (1000 nm)
 **Nanosyringe**	600 nm	Nano-injection, nano-extraction	Pyramidal/triangular side aperture	2 N/m (1500 nm)
 **Micropipette**	2, 4, 8 µm	Cell isolation, cell adhesion	Flat/circular	0.3 N/m (500 nm), 1 N/m (500 nm), 2 N/m (1000 nm), 4 N/m (1500 nm)
 **Prototyping**	closed	Custom	Pyramidal/custom	0.6 N/m (500 nm), 2 N/m (1000 nm)

FluidFM probe dimensions





FluidFM® components	
Motorized FluidFM XY-sample-stage	– Bidirectional XY repeatability <500 nm over full range – Inset system allows to work with standard and custom sample containers (e.g. with two standard multi-well plates, 240 x 74 mm)
Motorized FluidFM Z-stage	– Bidirectional Z repeatability <5 nm – Z-axis jitter <5 nm
FluidFM head	– Motorized optics for beam deflection sensing – Motorized FluidFM probe exchange mechanism – Integrated pneumatics for FluidFM – Transmitted LED light source – Interchangeable for facilitated maintenance
FluidFM system control unit	– Control unit for FluidFM XY- and Z-stages and FluidFM head – Power consumption 450 W
FluidFM® microfluidics control system V2	– Pressure Range -800 to +1000 mbar – Differential pressure resoultion 0.5 mbar – Transient response time < 2.5 s, for full-range changes < 500ms for ΔP < 100 mbar – Steady state max. SD < 1 mbar – Steady state average error (Δt=5s) < 1 mbar – Integrated flow sensor for pneumatic tightness monitoring – APIs https://documentation.cytosurge.com/cora/api/ – Power consumption 50 W
System computer + monitor	– Windows 10 Enterprise version – 27" high resolution monitor – Power consumption 300 W
FluidFM ARYA Operator software	– All available FluidFM application modules – Multi-user support – Complimentary FluidFM EDNA data management software – Pre-installed – Free software updates for 12 months

Optical microscope		
Microscope frame	**Model**	Olympus IX83 P2ZF 2 ports frame
	Revolving nosepiece	Motorized sextuple revolving nosepiece (DIC slider attachable), simple waterproof structure
	Focus	Stroke: 10.5 mm, Minimum increment: 0.01 µm, Maximum nosepiece movement speed: 3 mm/s
Transmitted lightsource		LED in FluidFM head
Long-distance objectives NA: Numerical aperture WD: Working distance FN: Field number		– 10x UPLFLN 10X2: NA: 0.30, WD: 10 mm, FN: 26.5 – 20x LUCPLFLN 20X: NA: 0.45, WD: 6.6–7.8 mm, FN: 22 – 40x LUCPLFLN 40X: NA: 0.60, WD: 2.7–4 mm, FN: 22
Operating environment		– Indoor use – Ambient temperature: 5° to 40°C (41° to 104°F) – Maximum relative humidity: 80% for temperatures up to 31°C (88°F), decreasing linearly through 70% at 34°C (93°F), 60% at 37°C (99°F), to 50% relative humidity at 40°C (104°F) – Supply voltage fluctuations: Not to exceed ±10% of the normal voltage
Power consumption		– Max. 755 W

Standard fluorescence equipment	
Fluorescence LED light source	– White light fluorescence LED illumination – Wavelength Range: 360-770 nm – Peaks (nm): 365, 430, 475, 545, 650, 735 – Lifetime: 25’000 hours
Motorized fluorescence mirror turret EF: Exciter filter BS: Beam splitter BF: Barrier filter	Motorized turret with 8 positions, built-in shutter; – U-F39004 Red: EF: 527-552 nm, BS: 565 nm, BF: 577-632 nm – U-F39002 Green: EF: 465-495 nm, BS: 505 nm, BF: 515-555 nm – U-FUNA Blue: EF: 360-370 nm, BS: 410 nm, BF: 420-460 nm
Fluorescence illuminator	Straight design with field iris diaphragm
Motorized attenuator wheel	Time to shift another filter 300 ms (rotation time until next hole on the wheel)

Microscope Camera	
Model	IDS UI-3060CP-C-HQ Rev.2
Exclusively used by	FluidFM ARYA operator software
Sensor type	CMOS Color
Sensor size	1/1.2 inch
Resolution	2.35 MP
Aspect Ratio	16:10
ADC	12 bit
Cooling	Passive
Interface	USB 3.0

Temperature and CO_2 controlled incubator

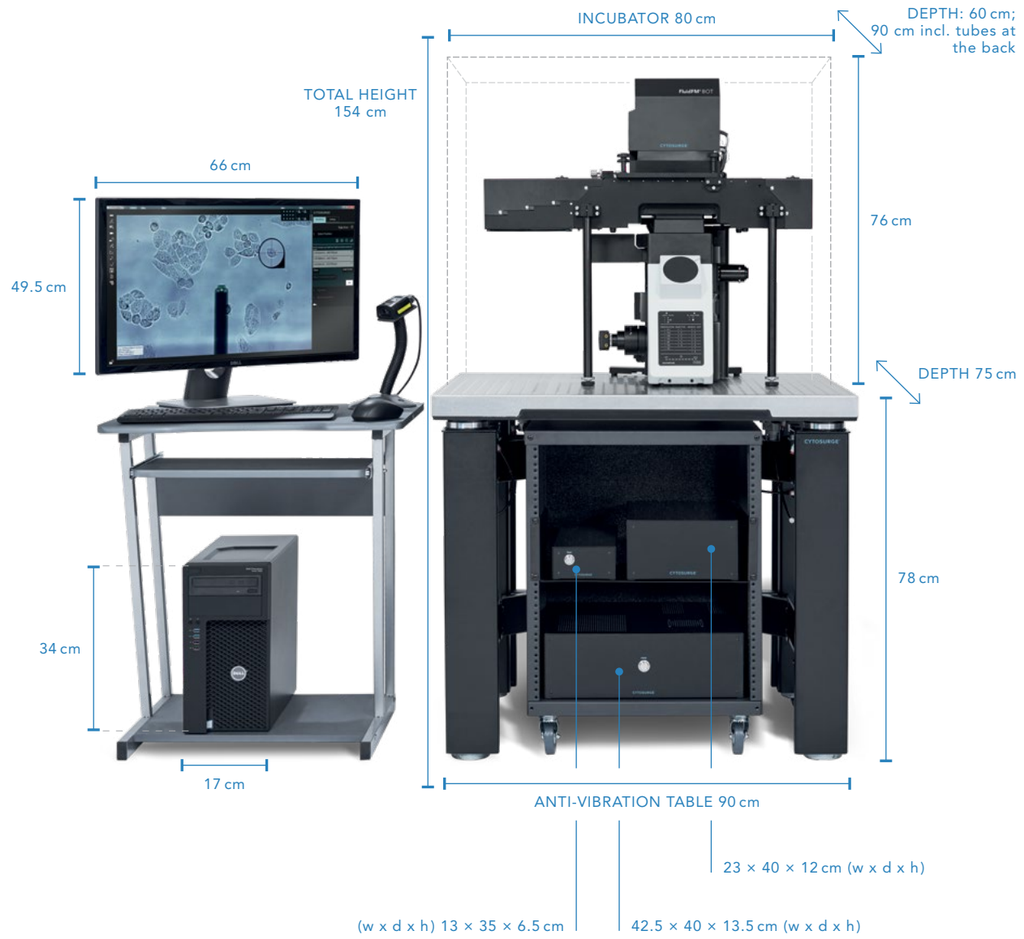
- Temperature control (+/- 0.1°C)
- Integrated CO_2 control: Adjustable CO_2 concentration levels between 1 and 15% (+/- 0.1% accuracy) (Option: no CO_2 control)
- Integrated airflow control system
- UV sterilization: 2 x 10W UV decontamination lamps with automatic life cycle monitoring & time control
- High Efficiency Particulate Air (HEPA) filter
- Polycarbonate casing for optimal fluorescence imaging
- Internal LED illumination
- Tailor-made doors for easy access to main FluidFM stage and microscope areas
- Power consumption up to 600 W

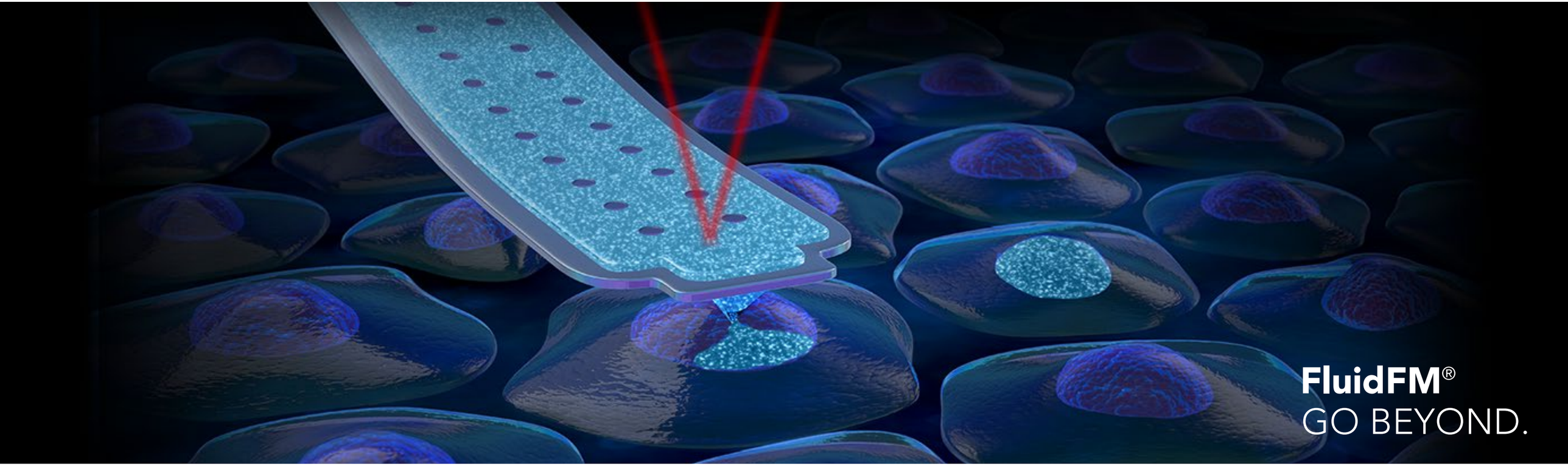
Anti-vibration table

Isolator natural frequency	High Input: vertical = 1.2 Hz, horizontal = 1.0 Hz Low Input: vertical = 1.5 - 2.0 Hz, horizontal = 1.2 - 1.7 Hz
Isolation efficiency @ 5 Hz	Vertical = 70 - 85%, horizontal = 75 - 90%
Isolation efficiency @ 10 Hz	Vertical = 90 - 97%, horizontal = 90 - 97%
Recommended load capacity	350 lb (160kg)
Facilities required	80 psi (5.4 bar) nitrogen or air
Shipping weight	Approx. 600 lb (272 kg)
Height control valves	Repeatability standard valve +/-0.050 in. (1.3 mm) Precision valve +/- 0.005in. (0.13 mm)
Power consumption	207 W (if operated with included Jun Air Compressor)

KNOW THE DETAILS.

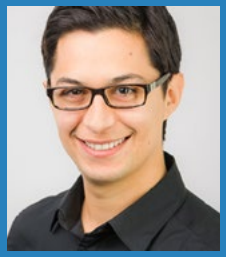
7. SYSTEM DIMENSIONS





CONTACT US.

We offer comprehensive application and technical support. Please contact us with your specific requests or application ideas. We will be delighted to support you and help you make your vision come true.



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Our BIO Lab (BSL-2) is fully equipped for a wide range of FluidFM cell experiments. Request a live on-site demo of your application and take away the results of what you have achieved. We also offer live remote demos around the globe.

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