Sequence your cells without killing them

The Biopsy Solution on the **FluidFM®** OMNIUM platform

Product brochure

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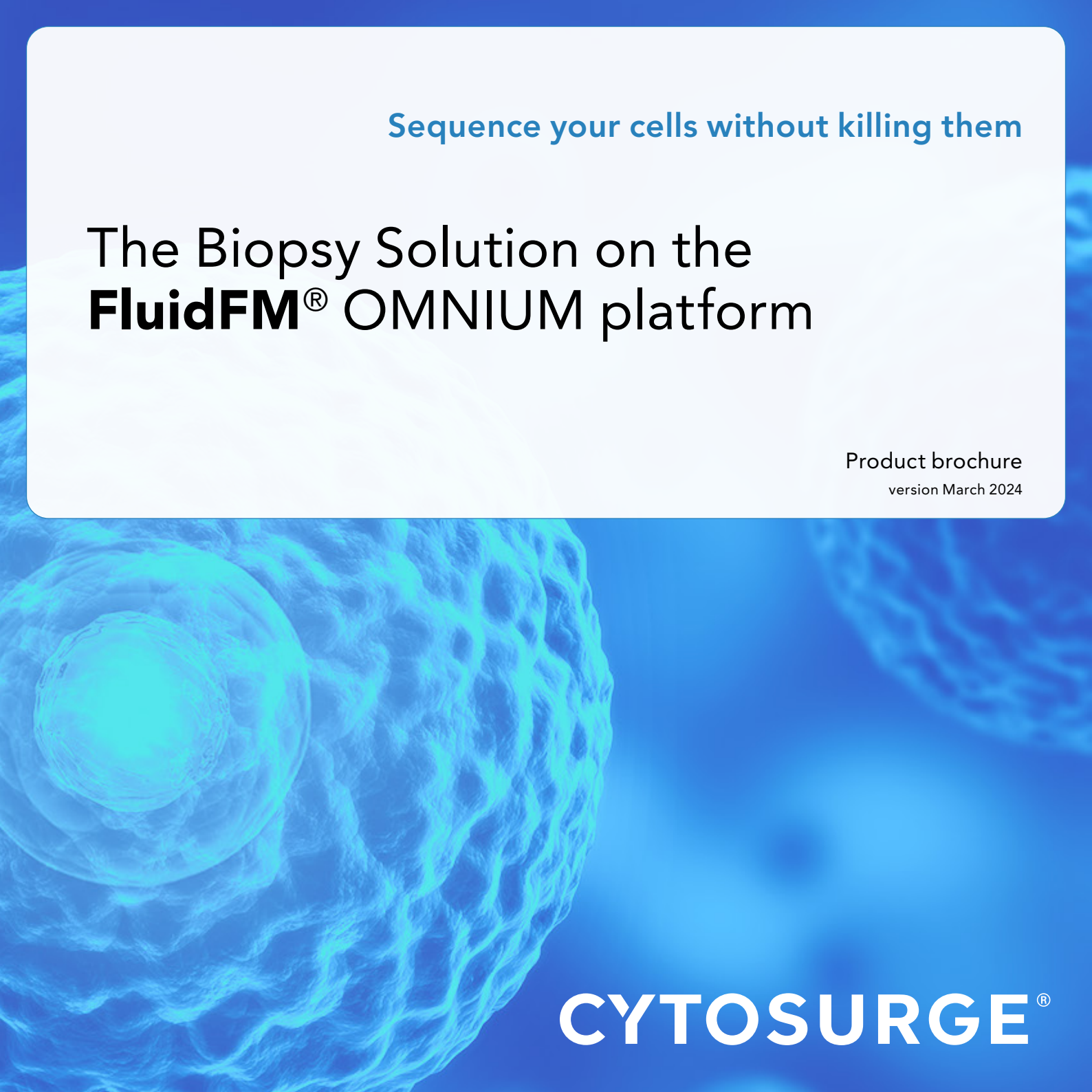


CYTOSURGE®


FluidFM® OMNIUM
Sequence your cells while keeping them alive

Content

1. The FluidFM Biopsy solution

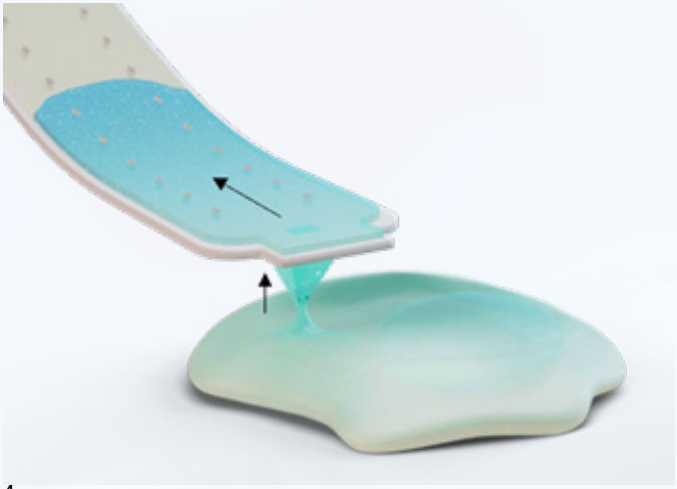
The Biopsy Solution on **the FluidFM OMNIUM platform** supports a streamlined workflow that uses a FluidFM Nanosyringe to collect cytoplasmic biopsies for live-cell sequencing or Live-seq.

The FluidFM biopsy workflow provides a delicate procedure to systematically collect a small portion of the cytoplasm of a cell of choice.

The workflow, facilitated through the FluidFM technology, enables users to precisely control the biopsy volume and pressure exerted on the cell to ensure minimal cell perturbation and high cell viability.

A biopsy is a cytoplasmic extract from a living cell, which comprises RNA that is representative of a cell's transcriptome at a given point in time.

A biopsy can be collected while the cell stays alive and therefore makes it possible to correlate the transcriptome of a specific, living cell to its future phenotype.



A FluidFM Nanosyringe, akin to a regular biopsy needle for patients, collects cytoplasmic samples from living cells.

1.1 The FluidFM OMNIUM Platform

The FluidFM OMNIUM platform is a single-cell manipulation system designed to streamline the use of FluidFM Probes in life-science applications.

The workflow is facilitated through the FluidFM technology: a combination between force microscopy and microfluidics.

Through the FluidFM OMNIUM platform, it is possible to regulate the force exerted on the cell when the Nanosyringe is gently inserted, and precisely control the biopsy volume to ensure high cell viability.

The integrated microscope and environmental conditions control enable experimentation under full visual control using epifluorescence or brightfield.

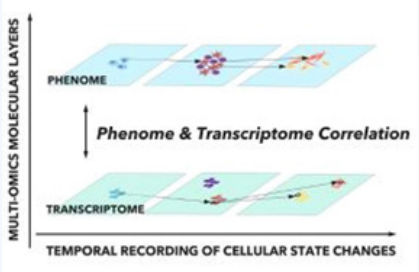
Cell selection and precise biopsy volume are controlled and monitored via the custom FluidFM ARYA software.

Sequence your cells and keep them alive

Gentle and controlled cytoplasmic extraction workflow with high cell viability

Match gene expression to a specific cell and phenotype

Visual feedback enables correlation of gene expression to cell traits within a 2D environment



Unveil real-time cell fate decisions

Record both slow and fast cel state transitions in real-time instead of pseudo-time.

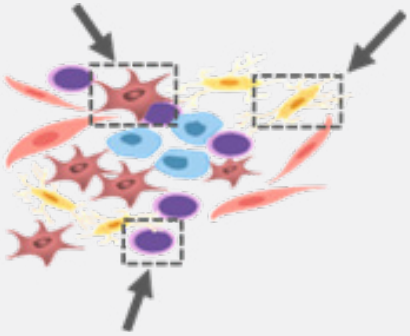
1.2 Product workflows and features

Step 1: Biopsy Collection
The cytoplasmic Biopsy is collected with a FluidFM Nanosyringe from a cell within 2D cultures of adherent mammalian cells. The cells are selected in the FluidFM ARYA software, followed by automatic insertion of the Nanosyringe into the cytoplasm of the chosen cell. Extraction is then initiated by the application of a gentle negative pressure through the microchannel of the Nanosyringe. The extraction can be monitored live and stopped once the desired biopsy volume is reached.

Step 2: Biopsy Ejection
After the extraction is completed, the system automatically inserts the Nanosyringe into a 1µL droplet of buffer and ejects the biopsy. The buffer contains RNA inhibitors to prevent RNA degradation.

Step 3: Buffer Droplet Transfer
The droplet that contains the cytoplasmic Biopsy, can be easily transferred into a tube and snap frozen for downstream analysis with a low input RNA-seq protocol such as **Luthor HD** or the **enhanced SmartSeq2 protocol as presented in Live-seq.**

2. Selected Use cases

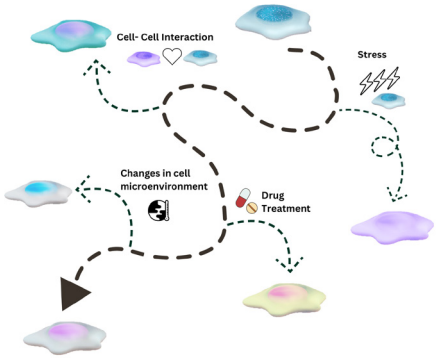


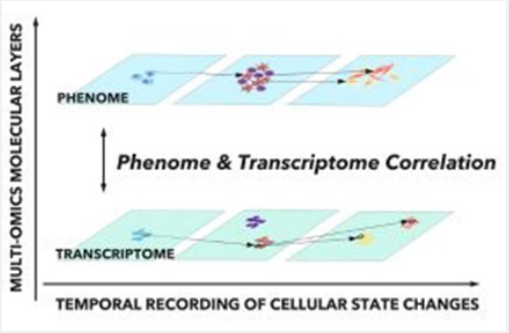
Select a specific cell for extraction

Live imaging with the integrated microscope of the FluidFM OMNIUM platform enables you to take a Biopsy from a specific cell based on its phenotype or specific cell environment.

Pinpoint the drivers of cell fate decisions

Take a Biopsy from a specific cell that has been exposed to external stimuli such as mechanical stress, changes to the microenvironment or drug treatment and assess the impact this has on the single-cell transcriptome.





Record state transitions of the same cell through consecutive biopsies

Each biopsy contains a snapshot of a cell's transcriptome at a specific time point. Because the Biopsy keeps the cell alive the transcriptome of a given cell can be directly linked to its phenotype. Subsequent biopsies can be taken to track cell state transitions in real-time.

3. The FluidFM Biopsy Solution in details

3.1. Cytoplasmic Biopsies with high cell viability

The gentle nature of the FluidFM Biopsy Solution comes from the use of force sensitive FluidFM Nanosyringes, that provide force feedback when interacting with a cell's membrane.

The FluidFM Nanosyringe has a sharp tip that facilitates gentle insertion into the cell membrane. The microchannel and nanometric opening provide fluidic access to the intracellular environment through which the cytoplasm from the cell can be extracted.

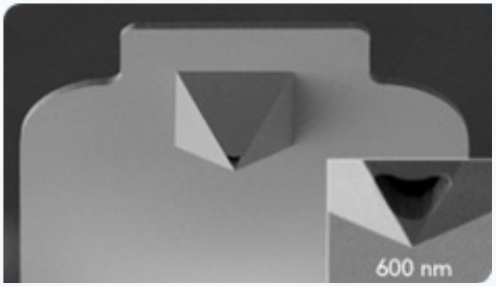
Throughout the extraction process the Nanosyringe is accurately kept in place by the OMNIUM platform, thereby minimizing physical perturbation of the cell and maximizing viability.

3.2. Precise control of the Biopsy volume

The FluidFM Nanosyringes, leveraging advanced microfabrication techniques akin to those employed in microchip production, boast unparalleled reproducibility in both the shape and size of their integrated microchannels.

This precise geometry, when paired with the ARYA software volume control and quantification module, facilitates exact control and precise quantification of biopsy volumes.

The sharp pyrimidal tip of the FluidFM Nanosyringe facilitates gentle insertion into the cell. (right)

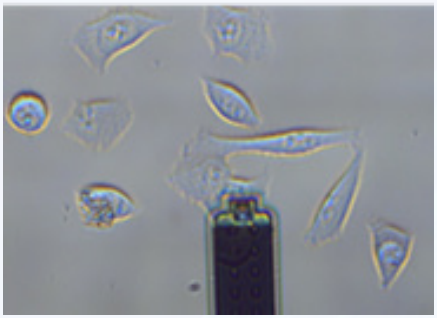


3.3. Live-cell imaging to record morphological changes of individual cells after Biopsies

The FluidFM Biopsy solution allows collecting biopsies from individual cells and tracking them over time by recording the coordinates of each cell in the ARYA software.

This allows the researcher to study the response of an individual cell to a stimulus without disturbing the cell and correlate the response to the previously collected transcriptome. Furthermore, it enables repetitive sampling of the same cell to generate time resolved transcriptional trajectories.

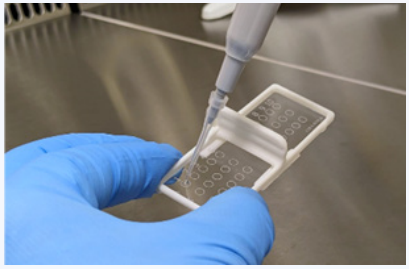
The FluidFM OMNIUM fully integrates an inverted microscope that is controlled by the FluidFM ARYA operator software. The system can be equipped with a temperature and CO2-controlled incubator for precise environment control. It is possible to collect images of the targeted cells before and after taking a biopsy to directly compare the generated transcriptome to morphological data.



Panc-1 cells are selected for extraction based on specific cell morphology.

3.4. Easy collection of single-cell biopsies for further downstream processing

The FluidFM biopsy solution comes with a custom Biopsy kit for easy collection of cytoplasmic biopsies. The picoliter extracts are ejected from the FluidFM Nanosyringe into a 1µL droplet of buffer on the droplet slide. From there, the droplet can conveniently be transferred to vials for snap freezing or direct library preparation for transcriptomic analysis.



The Biopsy kit contains:

- **35mm Ibidi® dish** with an imprinted grid to support alignment and position recovery.
- **Droplet slide and holder,** with hydrophobic surface, for precise positioning of the buffer droplet and sample collection.
- **Drying station** for automatically removing the droplet around the Nanosyringe, before and after taking the Biopsy.
- **Coating containers**

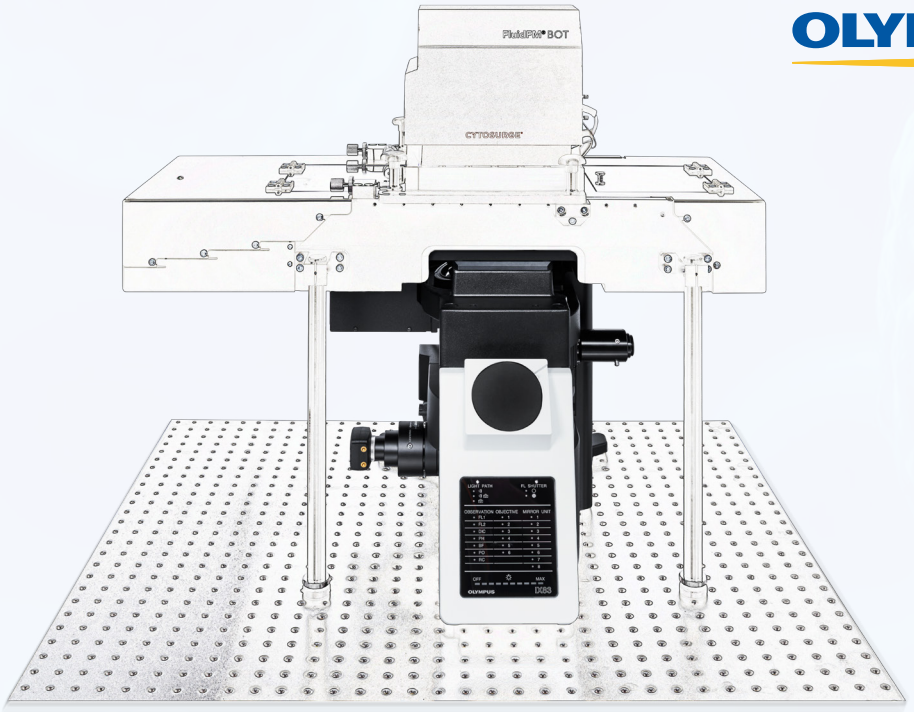
After the biopsy collection, the buffer droplet filled with the picoliter cytoplasm extract is deposited on the droplet slide coated with a hydrophobic surface.

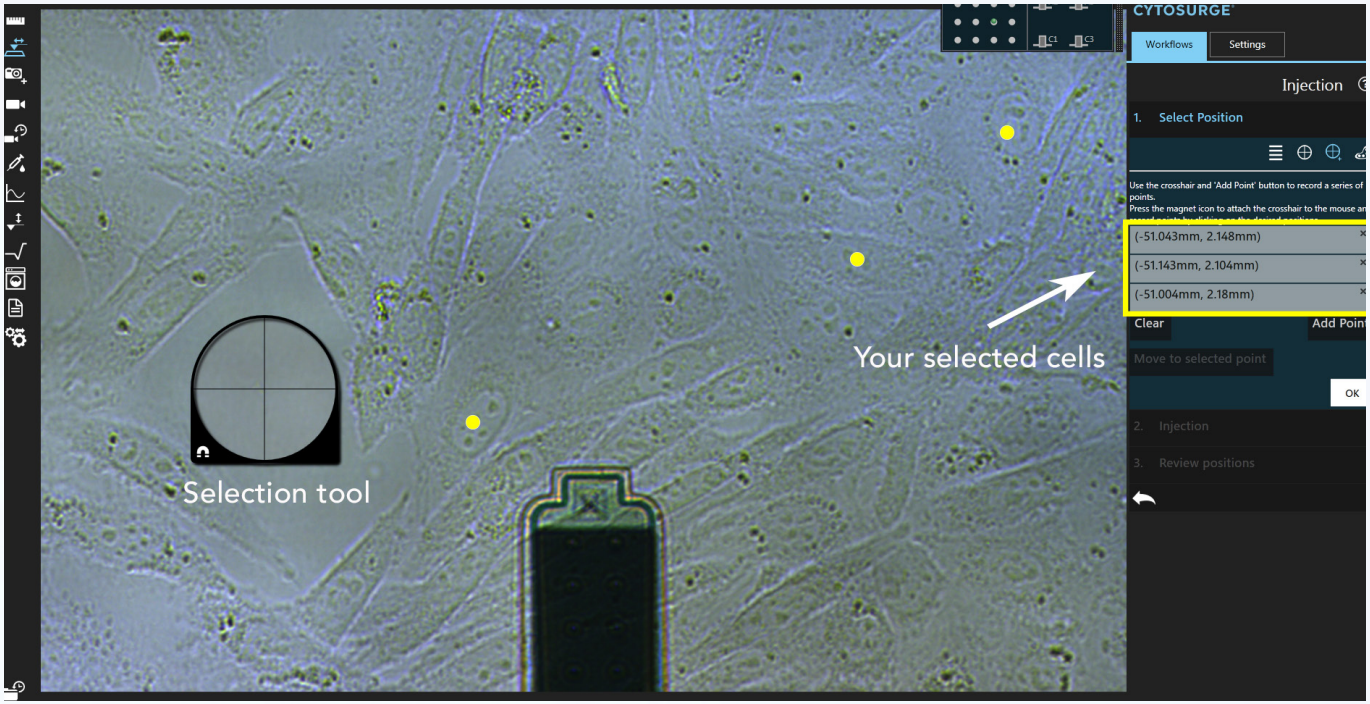
4. Product specifications

4.1 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.

The Olympus microscope has been fully integrated into the FluidFM OMNIUM 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 OMNIUM. 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.

4.2 **FluidFM®** ARYA Operator Software

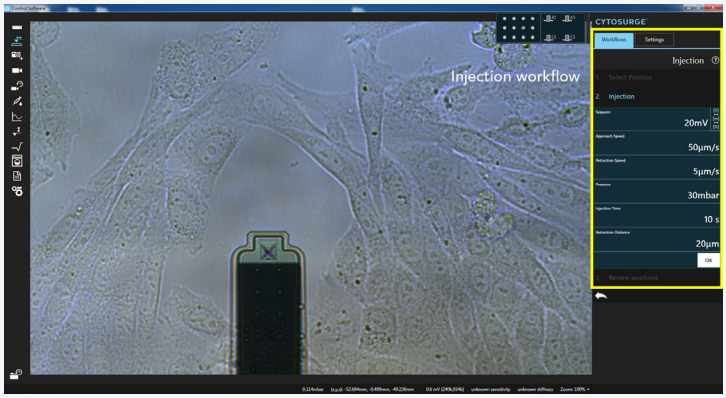
Our intuitive and user friendly software guides you step by step through the simple yet powerful application-specific workflows.

Point-and-click cell selection

Select the cells of interest according to their phenotype by simple point and click on the screen.

Full control

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



Software workflow, here parameters for the injection workflow.

Easy walkthrough procedures

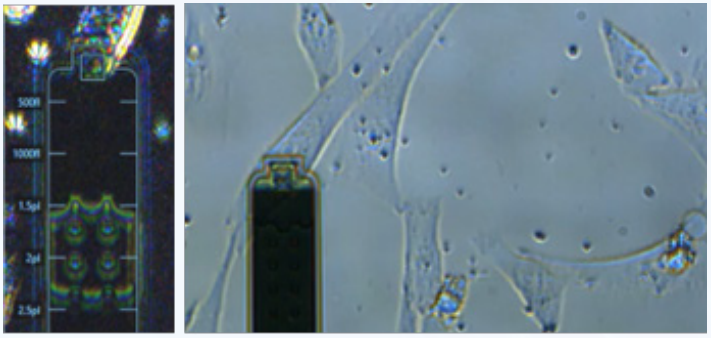
Follow the in-software suggested instructions, apply recommended settings from our protocol database and easily tailor parameters to your cells.

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 extracted volume

Our software-integrated Volume Calculator can precisely quantify with femtoliter precision the volume taken out of the cytoplasm of a cell in real-time.



A picoliter biopsy is taken from a specific HeLa cell with a FluidFM Nanopipette. The extracted volume can be onitored inside the Nanopipette. (left)

4.3 Temperature & CO_2 Controlled Incubator

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

Ideal for long-term imaging

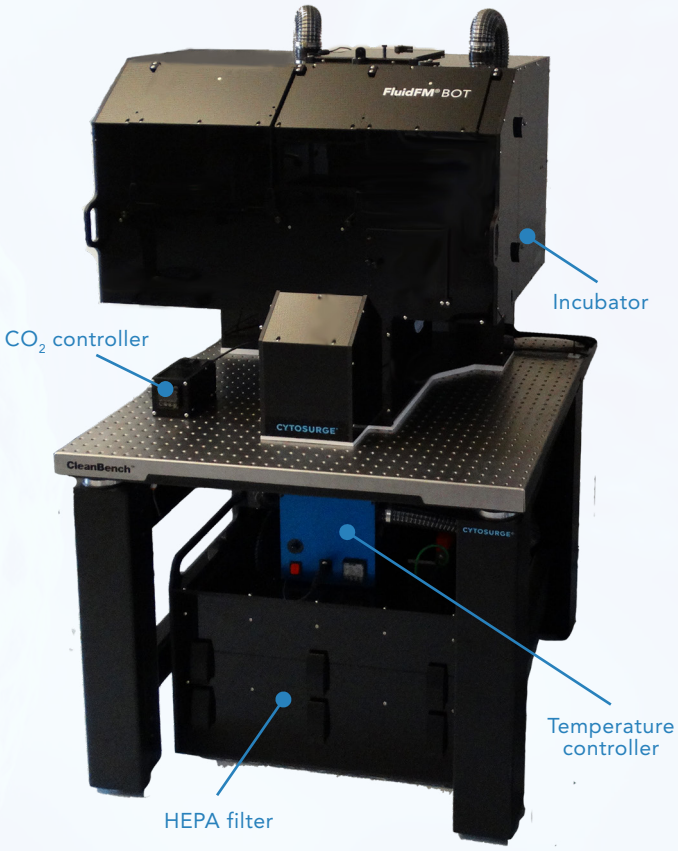
Fully enclosed system and temperature stability +/- 0.1 °C guarantees maximum thermal stabilization of all the components, thus also maximizing focus stability.

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

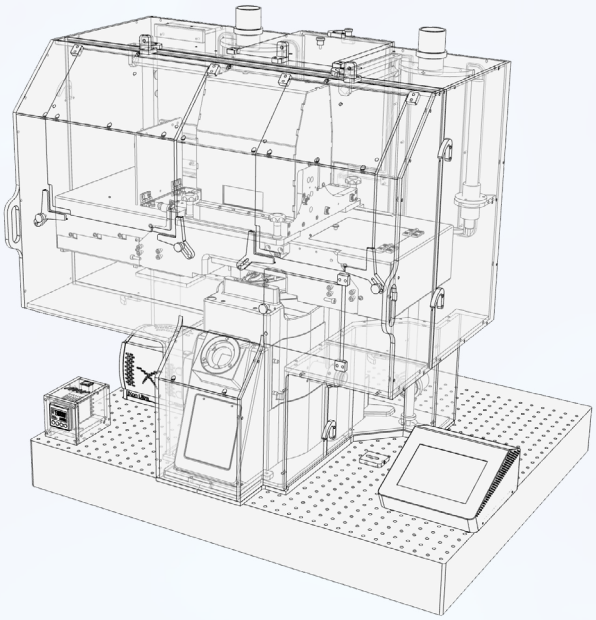
Two UV light sources with automatic life cylce monitoring and time control allow sterilization of the incubator's internal environment.

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 OMNIUM system.

5. **FluidFM®** Probes

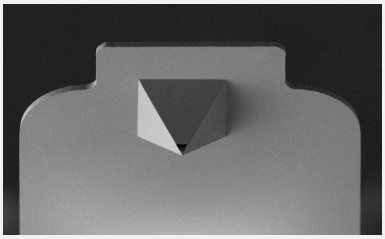
The core of the technology when performing FluidFM applications are the patented hollow FluidFM probes. The consumables are available in a broad variety of tip shapes, aperture sizes and mechanical specifications.

For single cell extraction and injection: FluidFM Nanosyringe
The very sharp apex and the around 600 nm aperture at the pyramidal tip of the FluidFM Nanosyringe enable gentle extraction and injection not compromising cell viability.

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.

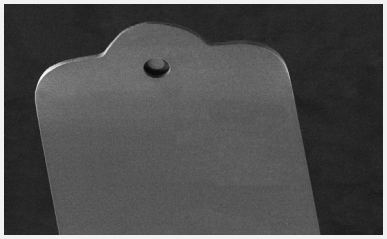
FluidFM Nanosyringe

Pyrimidal tip with 600nm aperture for cytoplasmic extraction and injection of single cells



FluidFM Micropipette

2, 4, or 8 µm aperture for cell isolation and adhesion measurements.

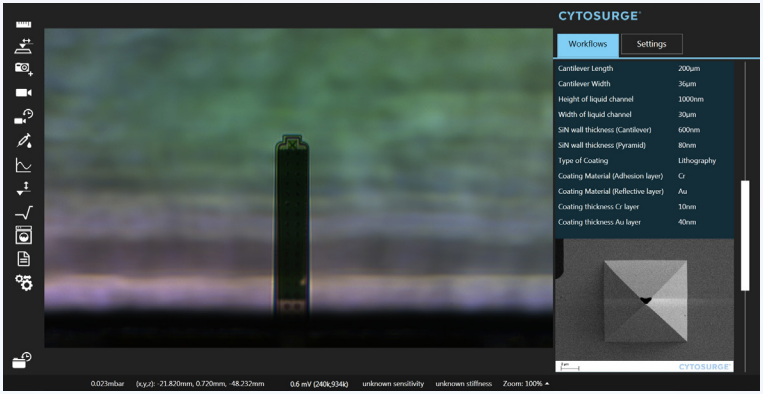


FluidFM probe.

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.



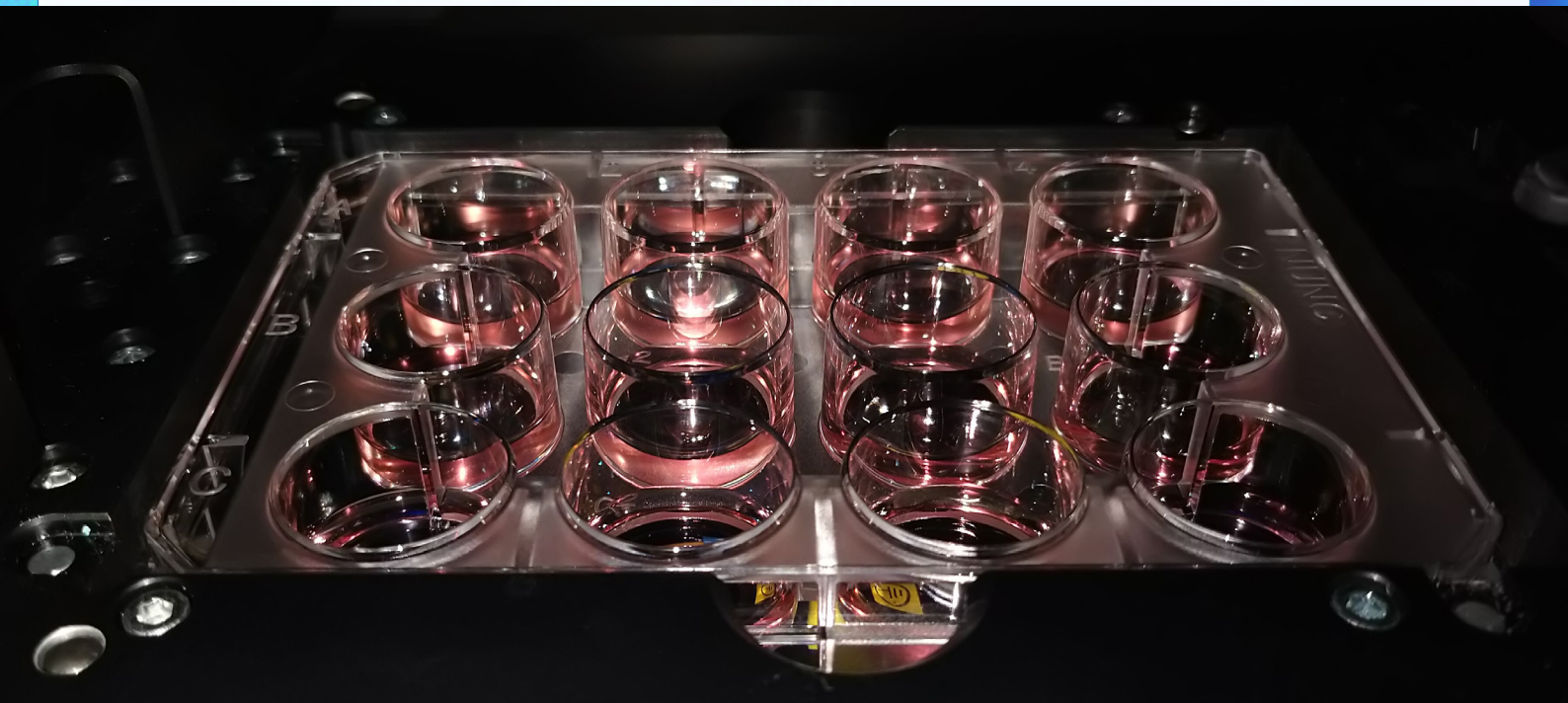
Full insight within the software. Characteristics of one specific FluidFM probe.

6. Supported Plates & Dishes

The FluidFM OMNIUM 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



7. System and 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

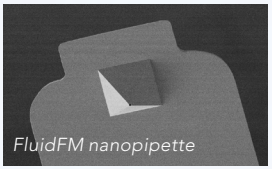
- Warranty extensions
- Maintenance contracts
- Scientific services
- Refurbishment & upgrades of system

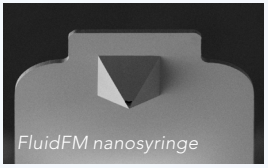
FluidFM probe discovery kit

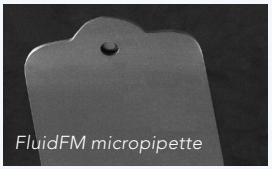
The FluidFM probe discovery kit includes a selection of 20 probes with different tips and various spring constants and apertures. The kit enables you to try diverse techniques, from cell isolation to spotting and nano-injection. The kit even includes FluidFM prototyping probes enabling you to create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

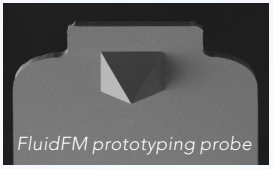
		Spring constant in N/m				
	Aperture in µm	0.3	0.6	2	2.2	4
Nanopipette	0.3		2×	2×		
Nanosyringe	0.6				4x	
Micropipettes	2	2×		2×		
	4	1×		2×		
	8	1×		2×		
Prototyping	n.a.		1x	1x		

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

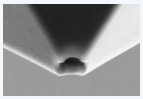
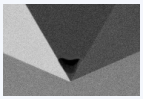
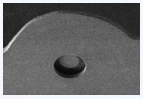
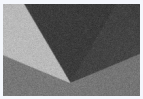

FluidFM nanopipette


FluidFM nanosyringe

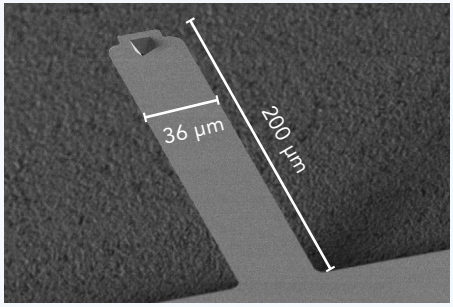

FluidFM micropipette

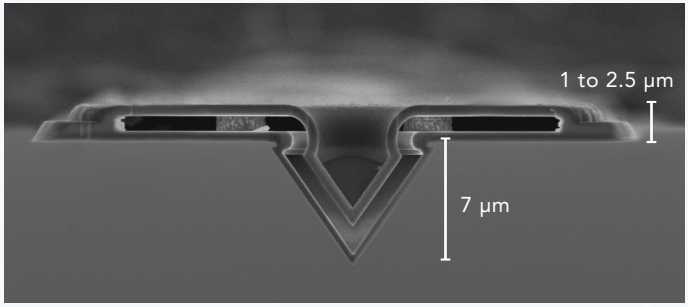

FluidFM prototyping probe

8. 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	2.2 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	HIK Robot MV-CA023-10UC and MV-CA23-10UM
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

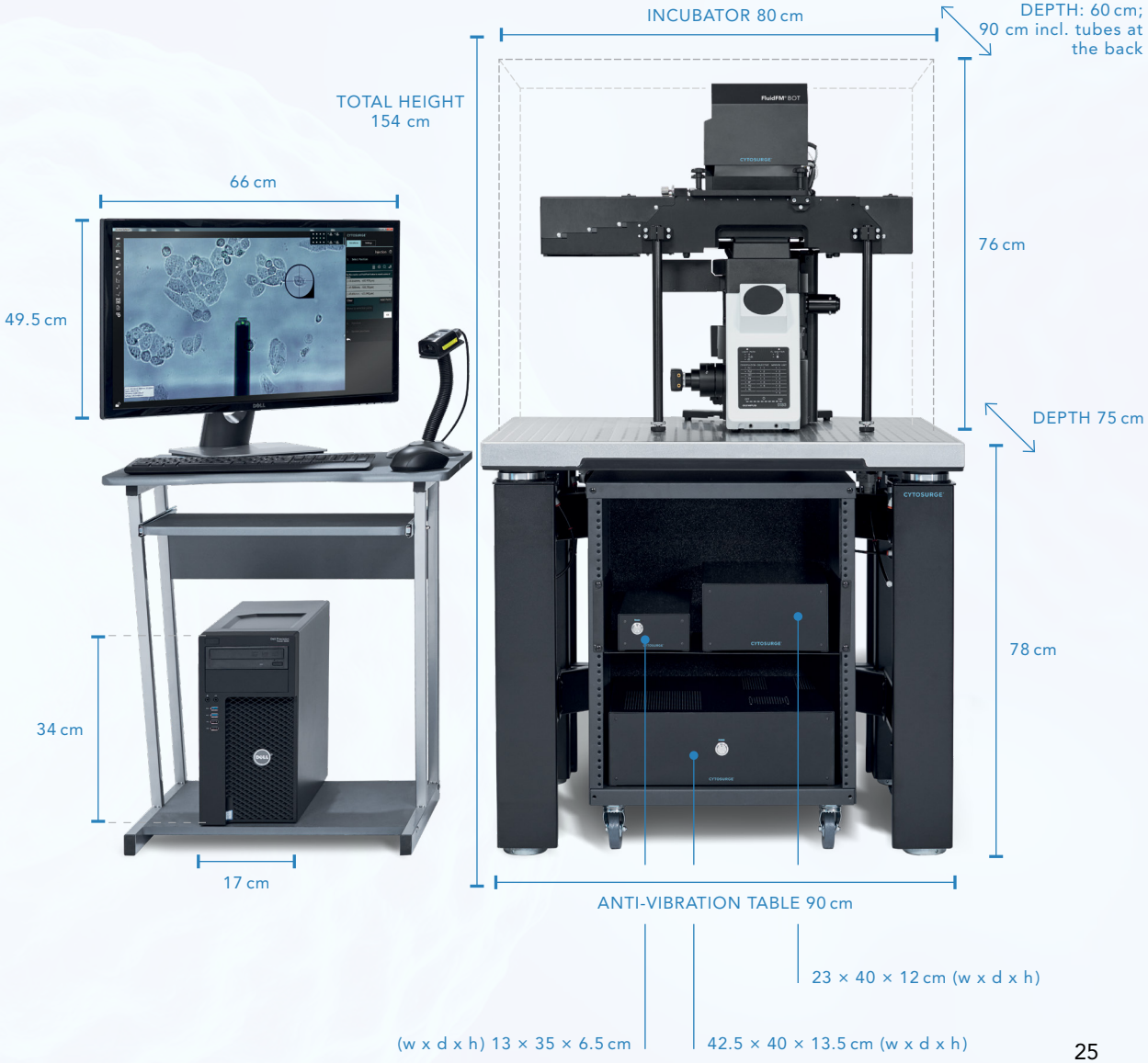
Temperature and CO_2 controlled incubator

- 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)

System Dimensions



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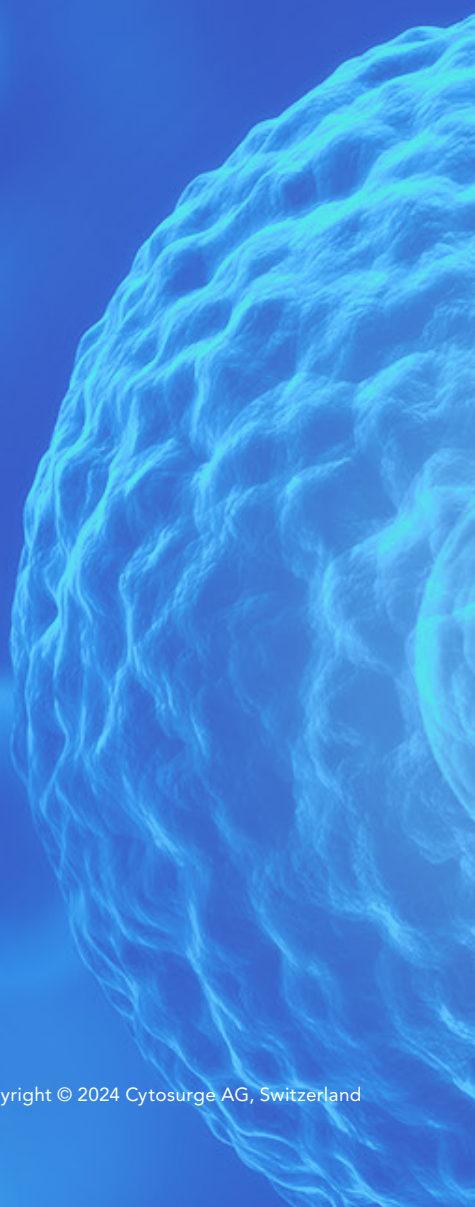

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