Physical cytometry

FOR 3D BIOLOGY

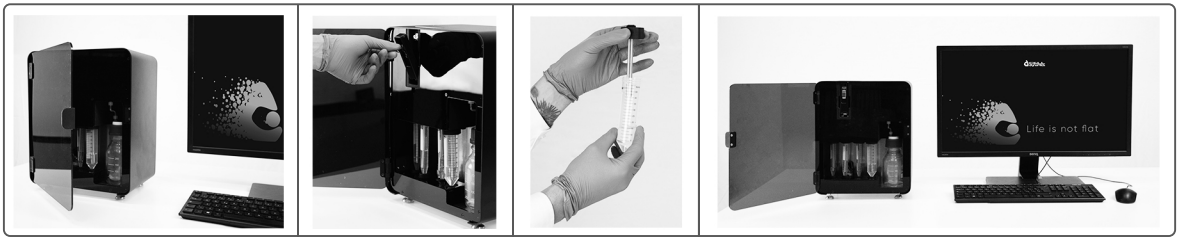
The Biophysics factor
to standardize 3D cell cultures

With the worldwide claim for uniformity in 3D cell culture methods, the focus is no longer if physical characterization will factor into your test rationale, but how can you precisely assess the homogeneity of the 3D sample populations. The scientific landscape, besides, strongly requires researchers to correlate physical parameter with the readouts coming from the commonly used in vitro tests. And most importantly, when it comes to culturing 3D cell models as spheroids or organoids, the biophysics holds a biological relevance, thereby representing a source of data variability to be considered.

Imagine if you can physically characterize your 3D cell models at once.

Lab weighing of **3D biological samples**

The W8 physical cytometer is the only full automated instrument for the physical characterization of 3D sphere-like biological samples. It covers the range from 50 µm sized small clusters up to larger spheroids or organoids with 500 µm in diameter. The combination of our groundbreaking microfluidic technology with bright-field image-based analysis provides the user with an entire data set of sample's mass density, weight and size, in just few minutes. And even more important, physical-based sorting of a target sub-population can be operated on the basis of pre-selected ranges of the aforementioned parameters.



Designed by engineers, intended for researchers

Its small size and easy handling allow the operator to place the instrument under the Biological Safety cabinet, when working with live 3D samples. After the custom selection of test settings, the instrument digitally analyses the target samples with full automation degree.

Flexible to sample size and type

Whether you are culturing cancer spheroids rather than organoids, potentially every type of sphere-like cellular aggregate in between the range from 50 µm to 500 µm of diameter fits in the sample requirements for the instrument analysis.

Physical-based gentle sorting

The patented method of automated sorting allows pure, individual target spheroids to be recovered for further downstream analysis. Flow rate and microchannel geometries are specifically designed to avoid any shear stress on the 3D samples.

Unparalleled, superior performances.

The W8 physical cytometer is equipped with the W8 chip, a specifically conceived flow-channel for the analysis of 3D samples, with sharp precision (< 0.1%) and accuracy (< 1.0%) with regards to the mass density measure. The instrument can be set up to automatically repeat the measurements up to 10 times for each sample, thereby increasing data robustness and the statistics reliability. The operator is enabled to perform a size-based pre-selection of the target 3D samples and downstream can refine them from outliers related to poorly circular samples.

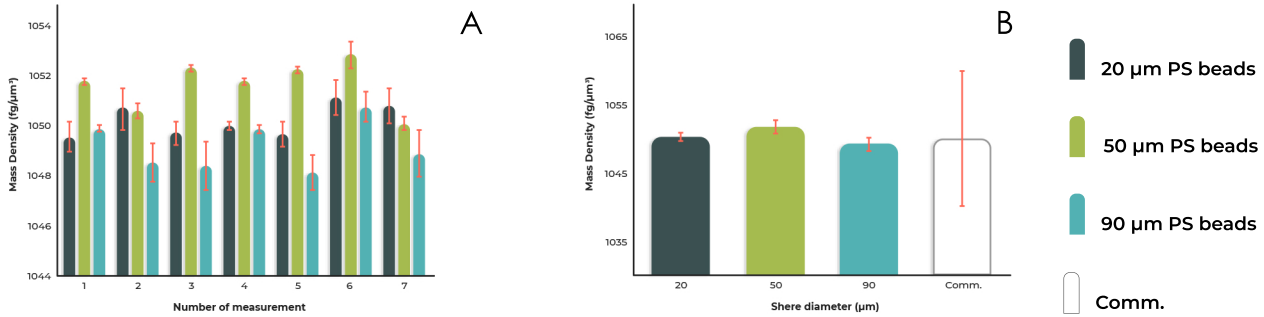
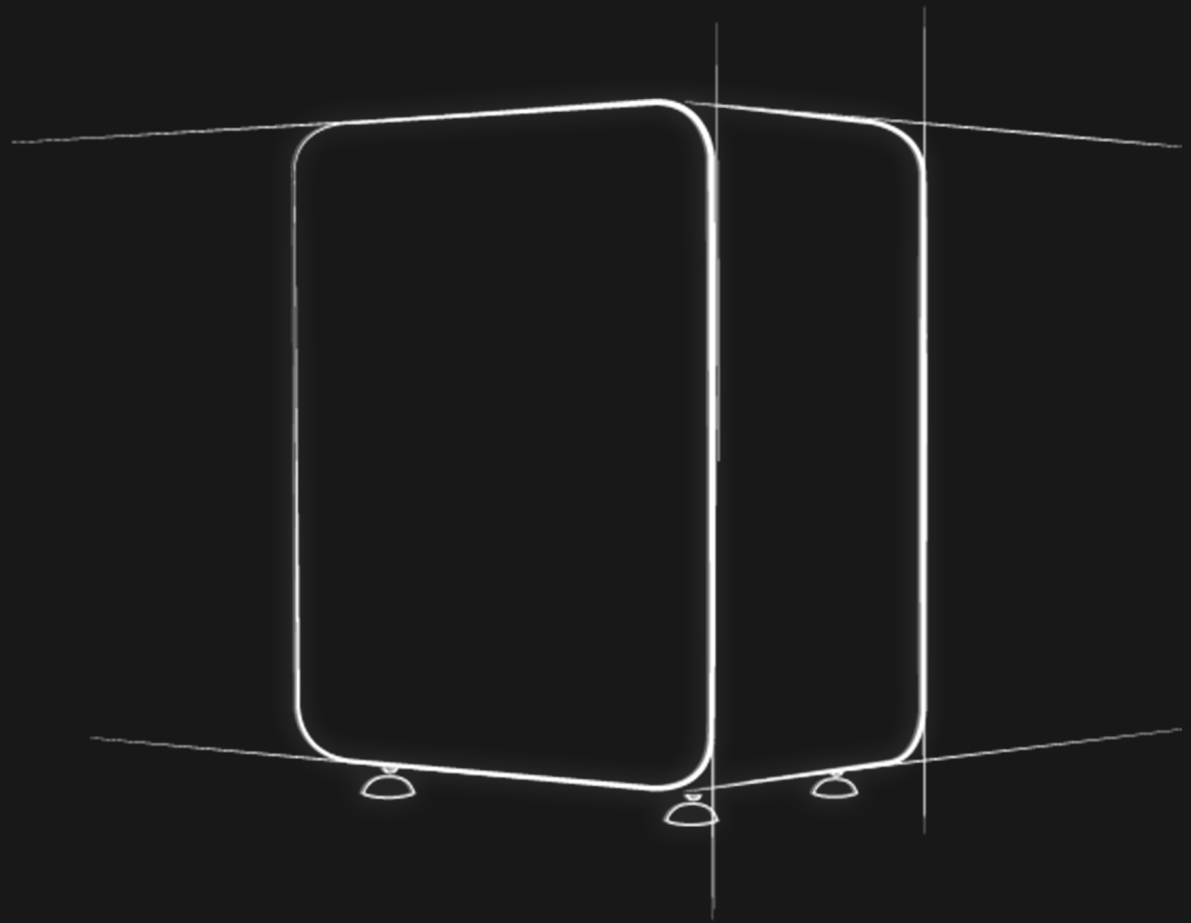


Figure 1. (A) Average mass density value and SD, over seven repetitions, for each of the seven measurements performed for the 20, 50 and 90 µm purchased Polystyene beads (blue, orange and green respectively); (B) Overall average value and SD for each bead size compared to the PS assigned commercial value and SD.

A Reliable Flow-Based Method for the Accurate Measure of Mass Density, Size and Weight of Live 3D Tumor Spheroids. Cristaldi et al., (2020) Micromachines 2020, 11(5), 465.



An innovative Quality Control Assay for 3D cell models

The W8 Physical cytometer provides a label-free, non-invasive method for monitoring and quantifying the physical properties of 3D cell cultures over time. Gathering precise information on their size, weight and mass density values is crucial to support decisions for protocols optimization and setting of the best culture conditions. For process scaling-up in cell factories, sample replicates can be analyzed at precise, regularly scheduled intervals over their growth kinetics.

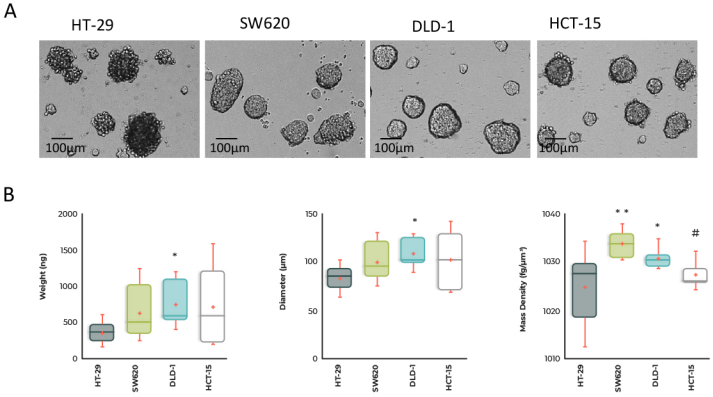


Fig. 2. Measurement of mass density, weight and diameter of colorectal cancer (CRC) spheroids. (A) CRC spheroids were generated with HT-29, SW620, DLD-1, and HCT-15 CRC cell lines cultured in ultra-low attachment flat-bottomed microplates and analyzed on day 6 by inverted microscope. Scale bar: 100 µm. (B) CRC spheroids were fixed with 4% PFA and analyzed with the W8 physical cytometer *p< 0.05 and **p< 0.001 vs HT-29. #p<0.05 vs DLD-1.

Physical Characterization of Colorectal Cancer Spheroids and Evaluation of NK Cell Infiltration Through a Flow-Based Analysis. Sargenti et al., (2020) Front. Immunol. 11:564887.

Determination of in vitro drug efficacy by means of biophysical markers

The W8 Physical cytometer constitutes an ultimate technology in drug discovery, as it provides a label-free, non-invasive analysis that helps overcome the technical limits when imaging 3D cell cultures. The precise measurement of 3D tumor spheroids' physical properties represents a key step forward to the accurate testing of new treatments' potential. While weight loss and diameter shrinkage are coherently related to decreased cell viability, mass density value proves to be a valuable marker of 3D spheroids' impaired compactness due to mechanisms of drug action.

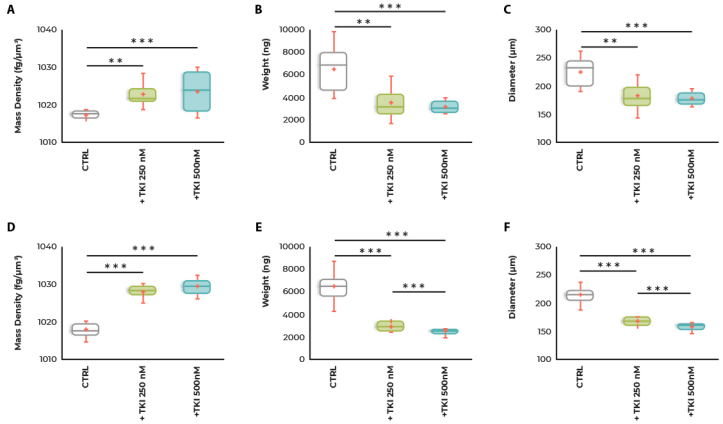


Fig. 4. Measurement of mass density, weight, and diameter of LoVo spheroids treated with tyrosine kinases inhibitor (TKI). Measurements of mass density (A and D), weight (B and E), and diameter (C and F) of live (top panels) and fixed (bottom panels) LoVo spheroid s after 10 days of treatment with TKI 250 nM (shown in red) and 500 nM (shown in blue) and relative controls (shown in green). Data are graphically depicted in box-and-whisker plots and the lines, extending from the boxes, indicate variability outside the upper and lower quartiles. **p< 0.01 and **p< 0.001.

The scientific manuscript is under review.

Physical evaluation of the in vitro killing activity of tumor-infiltrating immune cells

Immunotherapy constitutes an ever-growing trend in cancer treatment. Having a quantitative, easy assay for evaluating the in vitro efficacy would tremendously help bring together relevant insights in preclinical research. The W8 physical cytometer introduces a new method to quantify the in vitro response of 3D cancer spheroids to tumor-infiltrating immune cells, in terms of variations of their physical properties. This label-free assay consistently matches with results coming from commonly used in vitro assays, thus expanding the data sets that researchers can collect on 3D cell models.

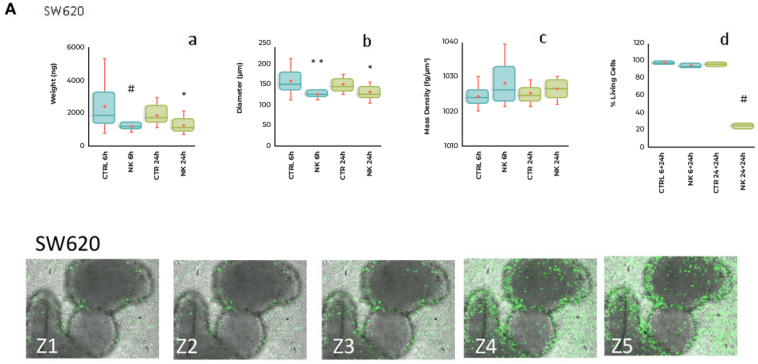


Fig. 3. Evaluation of infiltrating NK cell killing of CRC spheroids. CRC spheroids generated with SW620 (A) cell line were incubated at 37°C with NK cells at the effector:target (E:T) ratio of 1:1 for 6h or 24h. Then, samples were fixed with 4% PFA and analyzed with the W8 physical cytometer. Cytolytic activity is evaluated in parallel samples at 6h or 24h (+additional 24h to allow living cell attachment) with the Crystal Violet Cell Cytotoxicity Assay Kit (Biovision). *p<0.05; **p<0.001, #p<0.0005. SW620 spheroids was seeded into a Matrigel dome and incubated with CFSE-labeled NK cells for 24h. Samples were run under confocal microscope. Images were taken at different Z planes every 10µm with a 20x objective NA 0.40 and shown as green CFSE+ NK cells merged with bright field spheroids.

Physical evaluation of the in vitro killing activity of tumor-infiltrating immune cells

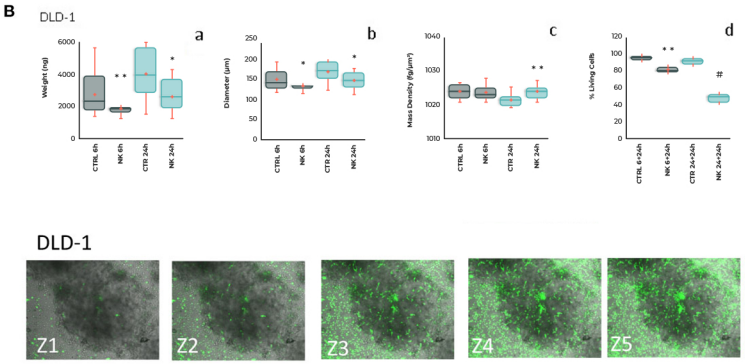


Fig. 3. Evaluation of infiltrating NK cell killing of CRC spheroids. CRC spheroids generated with DLD-1 (B) cell line were incubated at 37°C with NK cells at the effector:target (E:T) ratio of 1:1 for 6h or 24h. Then, samples were fixed with 4% PFA and analyzed with the W8 physical cytometer. Cytolytic activity is evaluated in parallel samples at 6h or 24h (+additional 24h to allow living cell attachment) with the Crystal Violet Cell Cytotoxicity Assay Kit (Biovision). *p<0.05; **p<0.001, #p<0.0005. DLD-1 spheroids were seeded into a Matrigel dome and incubated with CFSE-labeled NK cells for 24h. Samples were run under confocal microscope. Images were taken at different Z planes every 10µm with a 20x objective NA 0.40 and shown as green CFSE+ NK cells merged with bright field spheroids.

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Technical Specifications

FEATURES

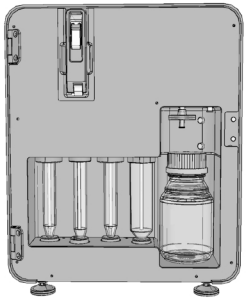
OUTPUTS	Size (µm) Weight (ng) Mass Density (fg/µm3)
SORTING	Sample recovery with a threshold of 70%
PERFORMANCE	Precision: < 0.1 % ACCURACY: < 1.0 %
SAMPLE SIZE RANGE	50 - 500 µm
SAMPLE INPUT FORMAT	15 ml tubes (16 x118,5 mm) V bottom 50 ml tubes (28,5 x 114,5 mm) V bottom

INSTALLATION

DIMENSIONS	35,7H x 28,6W x 18D cm
POWER SPECIFICATIONS	110 - 230 VAC, 50/60 Hz
WEIGHT	10 Kg
OPERATING TEMPERATURE	18 - 30 °C or 64,4 – 86 F

SOFTWARE

LIBRA	The LIBRA software is for Research Use Only. Not for use in diagnostic procedures. The License is provided to the customer as an essential element for enabling the instrument use.
LANGUAGE	English
PC REQUIREMENTS	Processor: i7-9700 Octa Core 3 GHz Ram: 16 GB Hard disk: SSD 256 GB USB ports: 3.1 Super Speed
OPERATIVE SYSTEM	Windows 10 Home or Professional or Business edition



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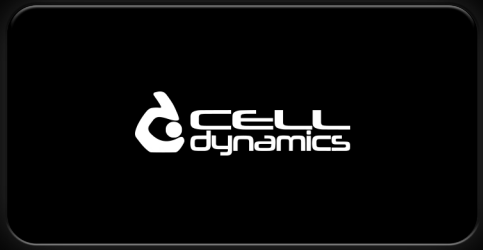
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