Sales Booklet

How to sell the fluidlab R-300

Table of contents

1. Intro

1.1. Preamble

The fluidlab R-300 is one of the most convenient devices for cell counting and spectrometry on the market. Nevertheless, the portable laboratory instrument is a product that requires explanations for the customer.

In this Sales Booklet you will find everything you need to show them to better understand the advantages of working with the fluidlab R-300. The information and tips come from several years of active sales work in and around Europe and we hope that our experience can support you.

1.2. anvajo

anvajo is a German medtech company with the mission to fight modern diseases. We produce innovative solutions that revolutionize the value chain of human medicine with our disruptive technology. Whether in the laboratory for research, the development of pharmacies or the production of innovative gene therapies, we have a platform solution fitting the use case.

2. Device

2.1. fluidlab R-300

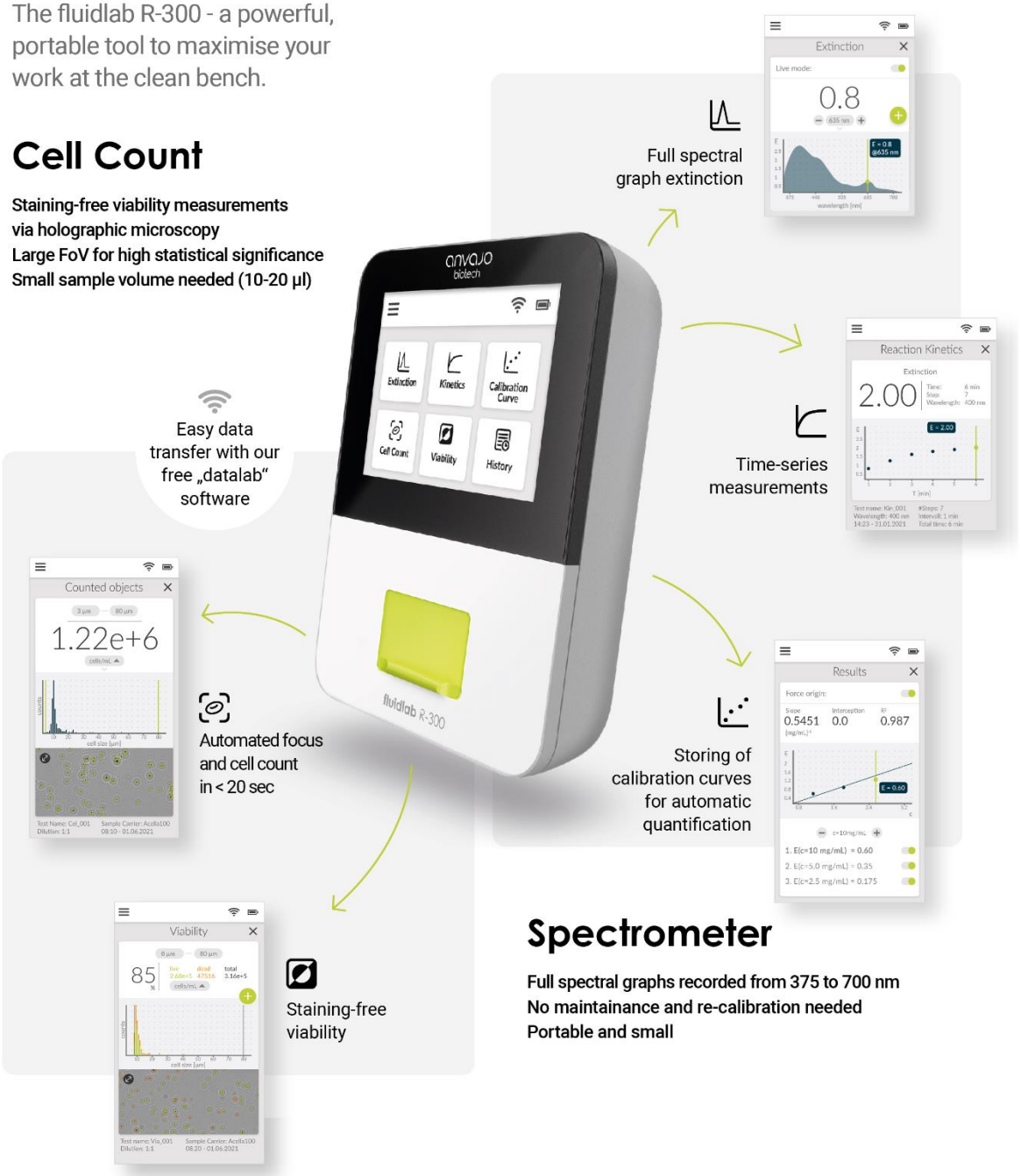
The fluidlab R-300 is our biotech product for labs. It combines a full-fledged spectrometer with an automated cell counter and enables a variety of analyses such as absorbance, scatterlight and time-series measurements, automated cell counting and staining-free viability measurements. The intuitive handling and precise measurements make the fluidlab R-300 the laboratory instrument of the future.

2.2. The device

The fluidlab R-300 - a powerful, portable tool to maximise your work at the clean bench.

Cell Count

Staining-free viability measurements via holographic microscopy
Large FoV for high statistical significance
Small sample volume needed (10-20 µl)



Spectrometer

Full spectral graphs recorded from 375 to 700 nm
No maintainance and re-calibration needed
Portable and small

2.3. Benefits and USPs

- 2 in 1: Cell counter and spectrometer in one device
- Easy to use: Small & portable
- Accurate: Reliable & reproducible
- No maintenance costs: Automated & efficient
- Rapid: < 20 sec
- Connected: Wi-Fi & hotspot
- Up to date: Free updates via Wi-Fi
- Smart algorithms: Deep Neuronal Networks and Machine Learning

2.4. The datalab

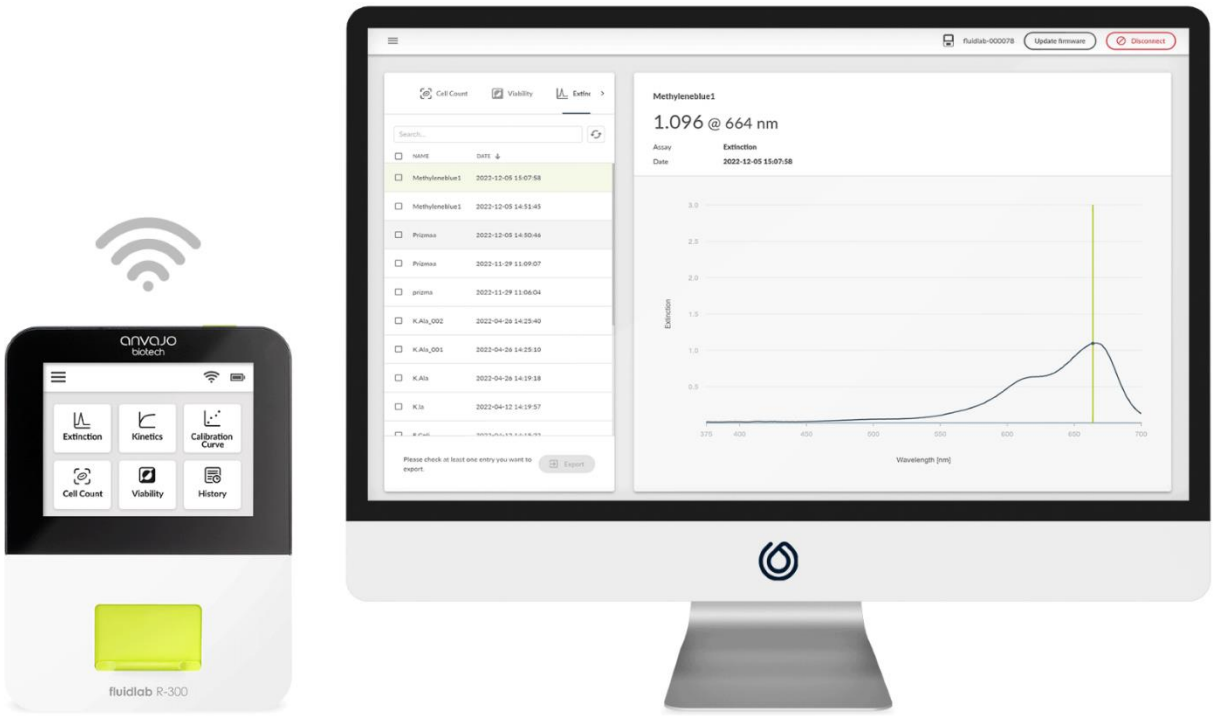


Figure 1: fluidlab R-300 with datalab

The anvajo datalab is the free desktop application developed by anvajo to assist scientists and researchers who use the fluidlab R-300. It is a modern desktop client which allows direct access to the raw data collected with the device.

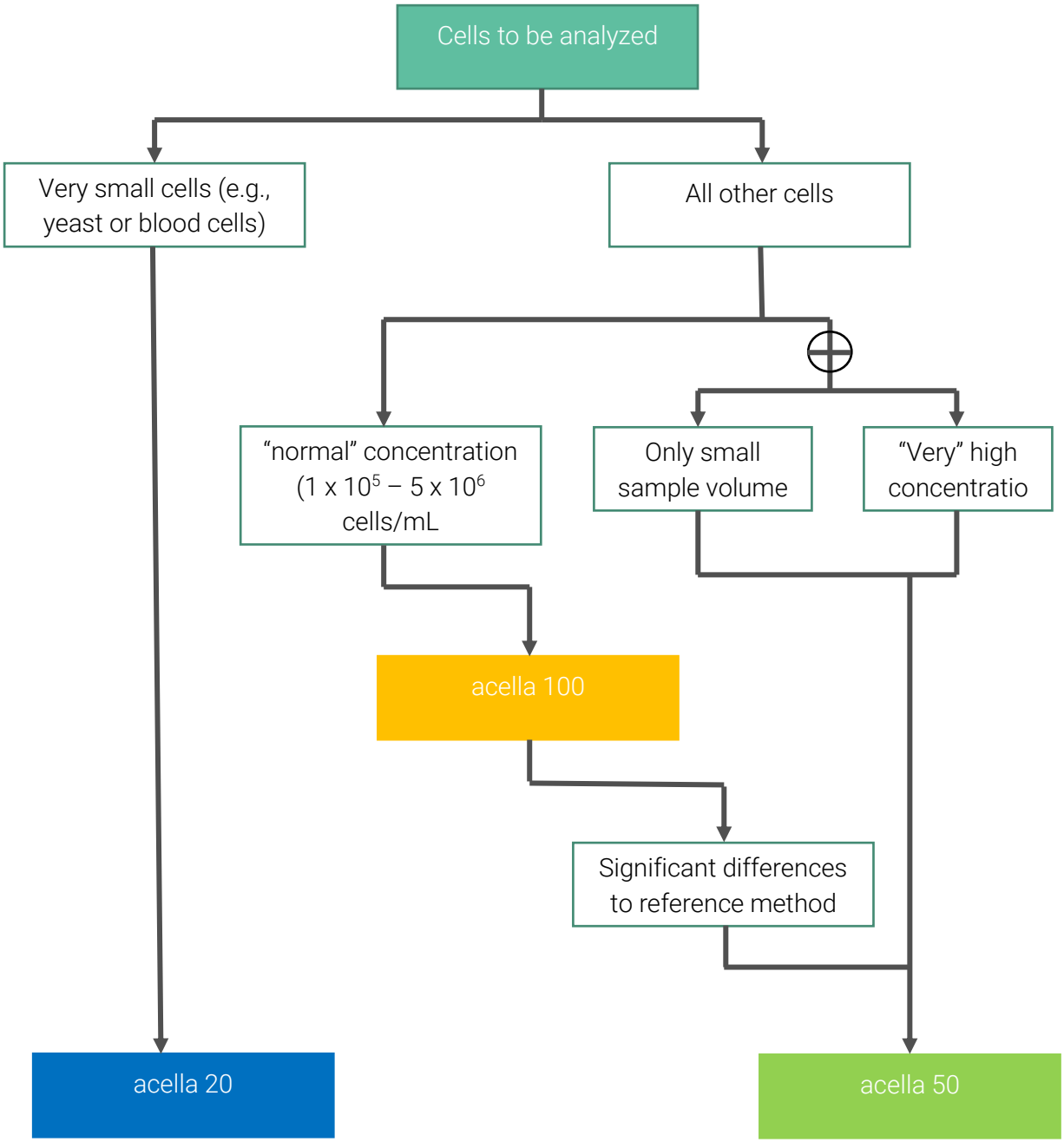
The datalab provides a comprehensive overview of all the measurements stored on the fluidlab R-300, as well as a simple preview of selected measurements to aid in data evaluation. Moreover, it grants easy export of spectrograms, microscopy images, histograms and data tables for orderly and secure laboratory record keeping. Finally, the datalab hastens customer support via direct export of digital holographic images, ensuring immediate assistance with troubleshooting.

2.5. The acella

The acella are our proprietary sample carriers for cell counting and viability assessment with the fluidlab R-300. They constitute a microfluidics device spanning between 20µm, 50µm and 100 µm in height, thus ensuring uniform filling under capillary action. Our sample carriers require only between 4-20 µl of sample to provide unparalleled accuracy and precision, making it the perfect fit to the microscopic applications of fluidlab R-300. Additionally, our acella can be used to test control samples under a regular brightfield or fluorescent microscope.

	acella 100	acella 50	acella 20
Chamber height	100µm	50µm	20µm
Sample volume	20µl	10µl	4µl
Size	75 mm x 25 mm x 1.2 mm	75 mm x 25 mm x 1.2 mm	75 mm x 25 mm x 1.2 mm
Material	Glass	Glass	Glass
Best suited for	Works with all "common" mammalian cell lines Works best with "normal" concentrations (1 x 10^5 -5 x 10^6 cells/mL)	Works will all "common" mammalian cell lines <40 -45µm Works especially well with higher cell concentrations.	Works specifically with small cells (e.g. yeast or blood cells)
When to use?	Default slide for mammalian cell lines When doing customer demos, we usually always start with the acella 100 as long as the customer does not work with very small cells (e.g. yeast or blood cells)	If the customer experienced significant differences with the acella 100 between the fluidlab and their reference method (e.g.due to very high concentrations, or very unusual cells/cell conditions), we propose to try the acella 50. Also, if the customer can only spare a smaller sample volume, the acella 50 is a very good alternative to the acella 100. This can be really communicated as a competitive advantage to other cell counters, since the combination of device and acella slide can be really individualized to the customers' needs/cell cultures.	Usually only used for smaller cells such as yeast or blood cells. Alternatively, when working with cells <15-18µm, the acella 20 is a very good alternative in case the customer can only spare a very small sample volume. acella 20 will be dis-continued

2.6. When to use which acella



2.7. Technical specifications

Table 1: Technical specifications of the fluidlab R-300

Cell Counter	
Imaging Method	Digital Holography
Analysis Method	Machine Learning Classifier
Field of View	2.3 mm x 2.3 mm (5.3 mm²)
Cell Size Limits	3 - 80 µm (cell count), 8 - 80 µm (viability)
Cell Concentration Limits	1 x 10^4 - 1 x 10^7 cells/mL
Compatible Sample Carriers	anvajo acella slides (various types available)
Spectrometer	
Light Source	Multiwavelength LED Module
Wavelength Range	375 nm - 700 nm
Spectral Bandwidth	< 2 nm
Photometric Measuring Range	0 - 2.5
Compatible Sample Carriers	Standard Cuvettes
Device	
Dimension	128 mm x 94 mm x 33 mm
Weight	240 g
Battery Runtime	5 hours*
Display	3.5“ Color Touch Screen
Connectivity	802.11 b/g/n Wireless LAN
Input Voltage	5 V DC via USB-C Power Adapter
Power Adapter	100 V - 240 V AC 50/60 Hz
Data Storage	Internal Flash Memory

* = Battery claims depend on network configuration and many other factors; actual results will vary. Battery has limited recharge cycles and may eventually need to be replaced by anvajo. Battery life and charge cycles vary by use and settings.

2.8. Technology explained

Digital holographic microscopy is a state-of-the-art quantitative phase imaging technique that allows automated staining-free analysis of cell counts and viability. In DHM, the sample is illuminated and as light passes through, some of it gets diffracted according to the sample’s refractive index while some travels through without “seeing the sample ". Then, the diffracted light interacts with the non-diffracted light, thus creating a hologram as it hits the camera. The hologram is then reconstructed digitally to retrieve an image, which contains valuable information about the cell culture sample such as membrane integrity and protein content. Finally, supervised machine learning algorithms are used to detect and count all cells within the image based on their morphology. Every cell is then classified as live or dead.

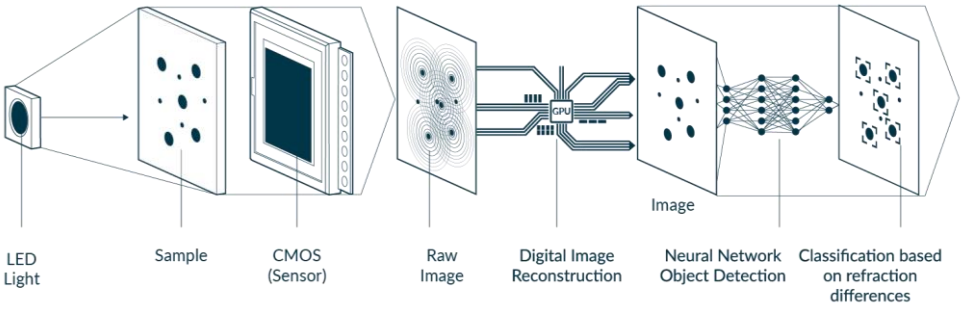


Figure 2: Sketch of Digital Holographic Microscopy (DHM)

2.9. Performed measurements

Meeting your customer for a product demo might bring up different kind of questions like reference data and performed measurements with the device. To ensure that you have a list to to those questions, we collected a broad range of cell lines.

You can find the full list in the attachments (attachment 1).

2.10. Validated Cell list

Meeting your customer for a product demo might bring up different kind of questions like reference data and how the algorithm was trained. To ensure that you have a list to to those trained cell lines, we have a list with all validated cells.

You can find the full list in the attachments (attachment 2).

3. Sales Process

3.1. Competitor Comparison

This section is a summary of the technical specifications of common competitors you are going to see in cell culture laboratories. The table summarizes applications, specification, and technology overview. You can find it in the attachments (attachment 3). Please keep in mind that those are information for internal use. Please do not share this attachment with customers, competitors or publish it online.

In our opinion, one of the biggest differentiators compared to other automated systems is the efficiency of the workflow. By using the acella as a sample carrier you do not only gain a lot of accuracy, the combination with staining-free viability assessment also reduces handling time and points of engagement to a bare minimum. This drives the risk of errors down and increases operating times. Hence, cost can be driven down although the acella is considered a pricy item.

We are convinced that this is the real reason why one should consider buying a fluidlab R-300. Performance and specifications are what you expect from an automated system and most systems deliver in that regard. But when it comes to usability, we are unmatched.

3.2. Objections

Onwards you will find some more frequent objections and how to answer them. It is of utmost important to ask as many questions as possible. Understand the customers pain to manage expectations accordingly. The competitors also do not solve all problems.

- Time per measurement
  - Our whole procedure is faster
  - No device and sample preparation (before and after)
  - Possible to prepare further samples within the measurement directly under the bench

- Less trash
  - General problem in labs, many things are single use
  - Acella made of glass not plastic
  - Carrier specially developed to achieve high-quality, reproducible results and at the same time keeping handling simple and quick (save time)

- Fluorescence
  - Do you really need that and for what?
  - We have a whole vis spectrometer for colorimetric and turbidimetric analysis

- Data transfer
  - No annoying search for a compatible usb-stick
  - Easy & fast from everywhere

- Mobility
  - Every device in the lab needs a fixed place
  - Place it next to a socket -> whole day in stand-by
  - table in front of the device where the colleagues can register when they want to use the device

- Pricing
  - High quality
  - Device is durable
  - No additional costs for maintenance, cleaning, and calibration
  - Time saving
  - Lifetime free updates
  - Device and measurement –> your results
  - Easy to share device with another working group

3.3. Overview customer groups

Summary of distinct research laboratories the fluidlab R-300 is being used. Within the given research fields you usually find cell culture facilities.

- Biochemistry
  - Medical Biochemistry and Molecular Biology
  - Biochemistry and Molecular Cell Biology
  - Biochemistry and Cell Biology
  - Biochemistry and Molecular Biology
  - Chemical biology & chemical genetics
  - Physical Chemistry and Electrochemistry
  - Institute for biophysical chemistry
  - Center for Experimental Medicine
- Pharmacology
  - Molecular Pharmacology
  - Pharmaceutical Research
  - Clinical Pharmacology
  - Clinical Pharmacology and Toxicology
- Genetics
  - Epigenetics
  - Human Genetics
  - Genetics and Microbiology
- Immunology
  - Cell Therapy and Immunology
  - Immunology in Neurodegeneration
- Colloidal & Polymer research
  - Biofunctionalised materials and (glyco)biotechnology
- Biomolekulare Systeme

3.4. Questionnaire

In preparation to a customer visit, we have a prepared a questionnaire, which helps you to evaluate the potential of a sale and makes sure everything is ready when you visit the customer in the lab to showcase the full potential of the fluidlab R-300.

You can find the questionnaire in the attachments (attachment 6)

3.5. Testimonials and references

Find some testimonials below. Additionally, we provide you with a comprehensive list of customers we won through our direct sales activities. Hence, most of them is located in Germany where our sales team was mainly active. The complete list can be found among the appendices (attachment 4).

UNIVERSITY OF CAMBRIDGE

"Combining two technologies in one portable device makes our workflow much easier. We do not have to rely on several benchtop devices and carry around or cultures. We now simply bring the instrument in need directly to our samples."

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

"The fluidlab's staining-free viability *method has enriched our work in this area. Viability measurements are very time-consuming; you have to count all cells individually beforehand and then stain them."*

"We repeated our tests with the fluidlab several times and the results were entirely accurate. There were little to no differences in the results, the repeatability was very reliable." - Taiwan University

Universitätsklinikum Hamburg-Eppendorf

"What we really like is that it can be operated intuitively. *It is kept simple so that anyone new to the lab can use it."*

Université de Paris

"We use the fluidlab because we want to seed cells under defined conditions. To be able to compare, the same number of cells must be initially placed in each well for each condition. At this point, we are very happy that we can use the handy fluidlab in pocket format."

3.6. Use cases

- Routine cell culture labs
  - Assessment of cell number and viability during routine passages
  - Dose response curves in drug testing (viability assay)
  - Protein production (eucaryotic cells)
  - Are there enough cells to transfect with a virus?
  - Are the cells viable after transfection?
- Microbiology
  - Determination of bacterial growth through Optical density (OD 600)
  - Protein production (bacteria)
    - Are there enough bacteria to produce the desired protein?
    - When do I have to induce the cells?
- Beverage industry
  - Cell count for yeasts (but not viability)
  - Determination of bacterial growth through Optical density (OD 600)
  - Colour discrimination via spectrometer

3.7. Publications

In this section you will find a curated list of publications in which the fluidlab R-300 is featured

Ronzheimer, A., Schreiner, T., & Morlock, G. E. (2022). Multiplex planar bioassay detecting estrogens, antiestrogens, false-positives and synergists as sharp zones on normal phase. Phytomedicine, 103, 154230.

Marzi, A., Eder, K. M., Barroso, Á., Wågbø, A. M., Mørch, Ý., Hatletveit, A. R., ... & Schnekenburger, J. (2022). Interlaboratory evaluation of a digital holographic microscopy–based assay for label-free in vitro cytotoxicity testing of polymeric nanocarriers. Drug Delivery and Translational Research, 12(9), 2207-2224.

Zeußel, L., Aziz, C., Schober, A., & Singh, S. (2021). pH-Dependent Selective Colorimetric Detection of Proline and Hydroxyproline with Meldrum's Acid-Furfural Conjugate. Chemosensors, 9(12), 343.

Tschorn, N., van Heuvel, Y., & Stitz, J. (2022). Transgene Expression and Transposition Efficiency of Two-Component Sleeping Beauty Transposon Vector Systems Utilizing Plasmid or mRNA Encoding the Transposase. Molecular Biotechnology, 1-9.

4. Support

4.1. Quality assessment

The quality assessment supports you in making sure that your customer is getting consistent results. If not, make sure to follow through the following steps.

Table 2: Overview

Section	Assessment	Explanation
1	Sensor check	clean sensor (status OK)
2	Sample carrier recognition	Is the sample carrier recognized?
3	Spectrometric applications	Measured values within the tolerance (of the expected value)?
4	Cell counting applications	Is the cell count according to expected value?

1) Check microscopic sensor

This test checks for any potential contamination of the microscopic sensor.
Necessary equipment: none

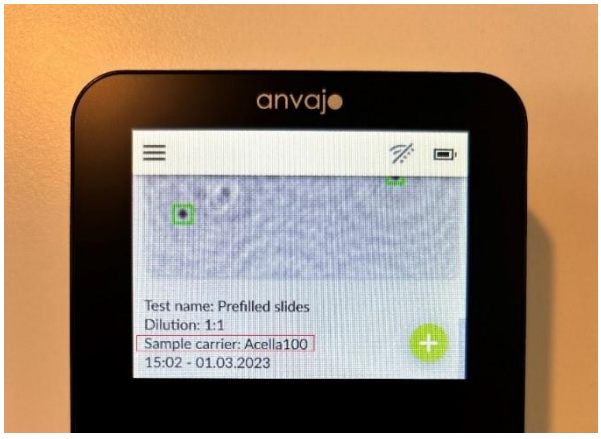


Figure 3: fluidlab R-300 screen acella check

1) Switch on the device
2) Open the settings menu of the fluidlab R- 300 by tapping the menu icon ( ).
3) Tap on the menu item “Maintenance”.
4) Tap on the menu item “Sensor Status”.
5) Tap on “Start Sensor Check”.
6) After a few seconds, the result of the sensor check will appear.

2) Sample carrier recognition

This test checks the correct recognition of the sample carrier.
Necessary equipment: acella 100

1) Use an acella 100 (red colour patch on the upper left corner)
2) Switch on the device.
3) Start cell count application.
4) Insert the slide into the device and start the cell count measurement.
5) In the result screen, scroll down to the bottom of the screen and check whether the indicated sample carrier type is equal to “Acella100”.

3) Spectrometric applications

This test checks the correct functionality of the extinction application.
Necessary equipment: neutral glass filter (F201, F203); please take care that your equipment works properly and is calibrated before performing the test.

Figure 4: fluidlab R-300 with a neutral glass filter.

1) Switch on the device
2) Select extinction application.
3) Insert adapter 03 into measuring chamber and perform blank measurement.
4) Insert F201 reference filter into adapter, making sure that the orientation is correct.
5) Start extinction measurement.
6) Compare the readout with the respected extinction value that should be obtained with the used reference filter.
7) Note the expected values are dependent on the specific filter used as each filter is calibrated individually.
8) Perform steps 4 to 6 with the filter F203.

The extinction value from the measurement shall not deviate by more than +/- 0,03 from the expected value.

4) Cell counting applications

This test checks the stable repeatability of the cell count application.
Necessary equipment: pre-filled acella slide

Figure 5: fluidlab R-300 with prefilled acella sample carrier

1) Switch on the device
2) Select „cell count“ application and ensure that the gates are set to 3 µm – 80 µm.
3) Insert Adapter 01 in the measurement chamber.
4) Use a pre-filled acella slide.
5) Insert the slide into the device and start the measurement.
6) Write down the total number of beads.
7) Measure the pre-filled slide 5 times and write down the results.
8) Compare the total number of beads of all 5 measurements given by the device.
9) The number of total beads from 5 measurements shall not deviate by more than +/- 5%.

4.2. Trouble shooting

This troubleshooting guide supports you in case your device is not properly working. Search for your problem in one of the following abstracts and find different reasons and solutions beneath.

4.2.1. General

- The device does not turn on.

A) The battery might be empty.

Charge the device for at least 5 minutes and try again by holding down the “Power on/off” button down for 2 seconds. This can happen if the device is left unused for prolonged periods of time.

B) The “Power on/off” button was not pressed long enough.

Make sure to hold down the button down for 2 seconds, release and wait for a response from the screen.

C) The software might have crashed.

Hard reset the device (**Appendix 1**).

D) The device might be overheated.

Unplug the device or remove it from direct sunlight exposure, move it to a cool and shady place, wait 15 minutes and try again by holding down the “Power on/off” button down for 2 seconds.

- The screen does not respond to the touch.

A) The user’s gloves are dirty (fatty deposits, liquids).

Change gloves and try again.

B) The screen is dirty (fatty deposits, liquids).

Clean the screen with isopropanol and try again.

C) The software might have crashed.

Hard reset the device (Appendix 1).

- The device is responding slowly.

A) The device carries an older version of the software.

Update the software to the current version (**Appendix 2**).

B) Device memory is running low.

Export the stored data with datalab and erase the entries from the device.

C) The software might have crashed.

Hard reset the device (Appendix 1).

- The device cannot connect to the Wi-Fi network.

A) Some organizations grant limited access to external devices in their networks.

Use the “Hotspot” function instead (**Appendix 3**).

B) The device carries an older version of the software.

Update the software to the current version (**Appendix 2**).

- The acella sample is not recognized.

A) The sample carrier was inserted wrongly.

Change the orientation of the sample carrier and try again.

B) The sensor status is not “OK”.

Clean the sensor with the swabs provided or isopropanol or Whatman filter paper and try again.

C) The software might have glitched.

Measure an empty acella slide and if there is no response, refresh the sensor. If this does not solve the problem, perform a hard reset (Appendix 1).

- The device cannot perform an update.

A) The device is not connected to the Wi-Fi network.

Connect to the Wi-Fi and try again.

B) The battery is running low.

Charge the device to over 50% capacity and try again.

C) The date and time are not correctly set in the device.

Set the correct time and date and try again.

4.2.2. Cell count & Viability results do not meet the expected / reference values.

This troubleshooting guide supports you in case your device is not properly working. Search for your problem in one of the following abstracts and find different reasons and solutions beneath.

- There are unrecognized objects on the image.

A) The device carries an older version of the software.

Update the software to the current version (Appendix 2).

B) The objects you are trying to measure are not in the "Verified Cells" list.

Make sure that the cells you are measuring are included in the "Verified Cells" list.

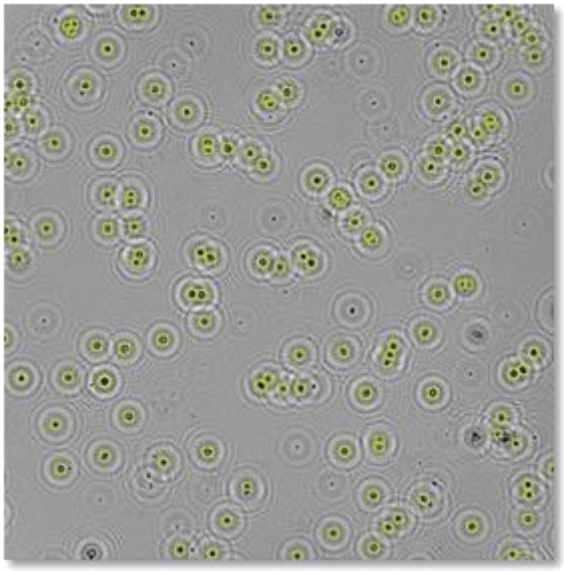


Figure 6: Detected objects are marked with a green box in the microscopic image.

- The sample is too dense or too dilute.

A) fluidlab R-300 is accurate in concentrations from 10^4 to 10^7 cells/ml.
Enrich or dilute your sample accordingly.

Figure 7: The cell density is too high , the device will show a warning with an explanation mark.

- There is an air bubble or dirt on the sample carrier.

A) Air bubbles in the counting chamber, change the distribution of the cells making the sample non-homogenous. Dirt on the sample carrier confuses the data analysis algorithm.
Use a new sample carrier.

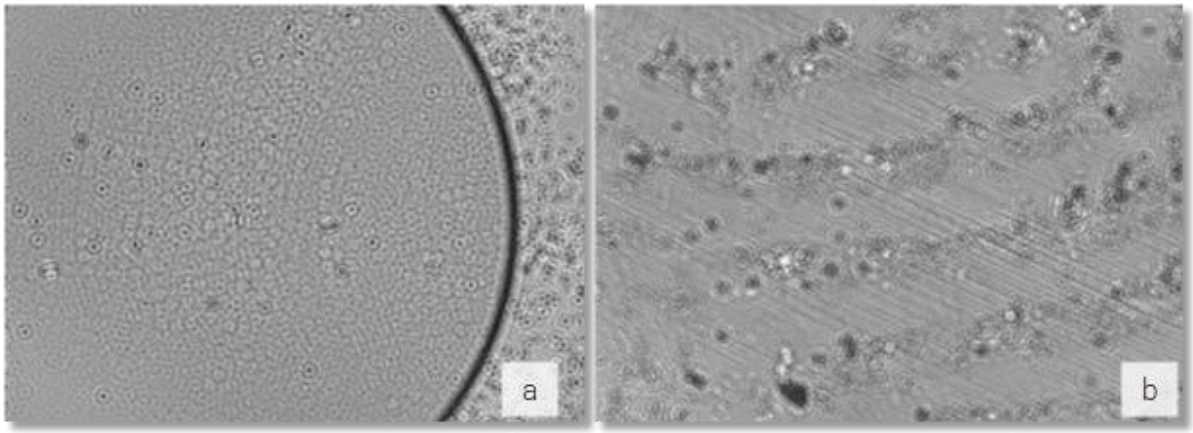


Figure 8: Air bubbles (a) and dirt (b) on the sample carrier as seen in the microscopic image.

Please watch our video on the correct handling of the acella sample carrier

- The sensor is contaminated.

A) Dirt appears on each image at the same spot affecting the autofocus function.

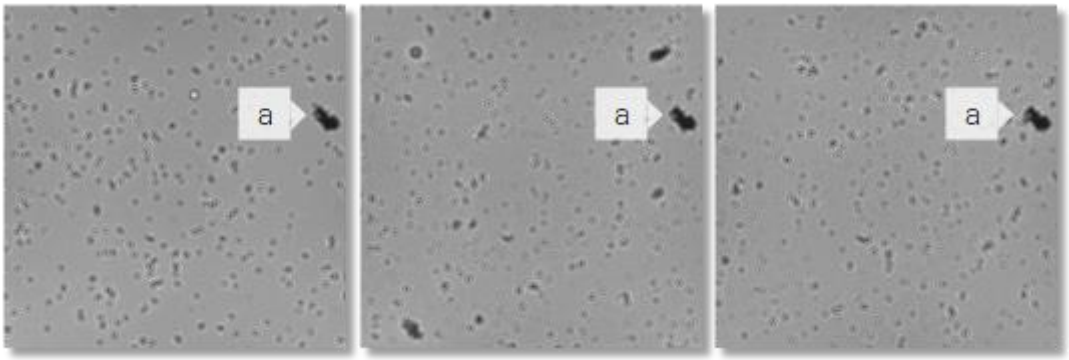


Figure 9: Dirt on the sensor (a) appears on the same spot in microscopic images.

Please watch our video on cleaning the sensor.

- The cell size range was not set correctly.

A) Setting custom cell size limits, affects the displayed result.

Make sure that you have selected the correct cell size range for your experiment.

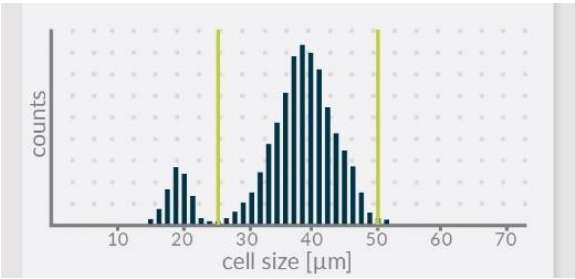


Figure 10: Limiting the cell size to a certain range will change the calculated result.

- There was an error with sample handling.
  - Was the cell suspension homogenized prior to receiving a sample?
  - Was the sample in the same medium as the reference?
  - Was the sample taken at the same time as the reference?
  - Was the reference sample over-exposed to cytotoxic agents (e.g., Trypan blue)?
  - Did the cells sink to the bottom of the sample carrier?
  - Do you use the same units for both measurements?

- Appendix 1 - Hard reset

1. Hold down the “Power on/off” button for 10 seconds. The “Power on/off” button should now be rapidly blinking (approx. 2 times per second).

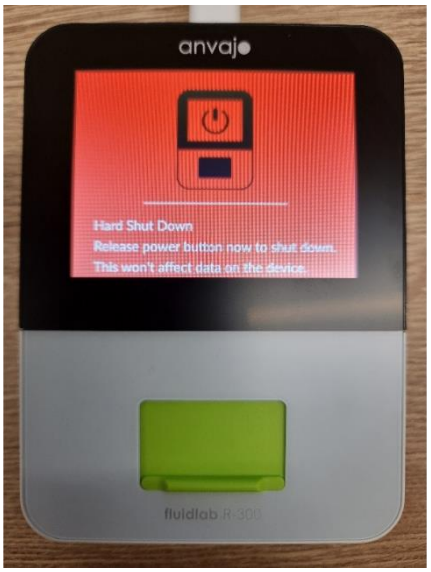


Figure 11: Hard Shut Down Screen of the fluidlab R-300

2. Release the “Power on/off” button when the red screen with the “Hard Shut Down” message appears (Figure 11). If the “Power on/off” button is not released after approximately 5 seconds of the message appearing, the red screen will not go away, and the device will be unresponsive. Resolve this by repeating “Step 1” and releasing the “Power on/off” button in time.
3. The screen will turn black, and the “Power on/off” button will be slowly blinking (approx. 1 time every 4 seconds). The device is now in Standby mode and ready to be turned on.
4. Press the “Power on/off” button once. In approx. 25 seconds the “anvajo” logo will be shown on the screen. Wait approx. 35 seconds more (for a total time of approx. 1 minute).
5. You are now on the main screen of your fluidlab R-300.

- Appendix 2 - Software update

Updates are only possible if the device is connected to the Wi-Fi.

1. Open the settings menu by tapping the icon on the top left corner once.
2. Tap on the menu item “Updates”.
3. Tap on the “Check for update” icon if it is present. If not, skip this step.
4. Tap on “Install update”.
5. The updates will be transmitted to the device. This might take several minutes.
6. The updates will be installed on the device. This might take several minutes.
7. The installation is complete once the system has automatically restarted.

- Appendix 3 - Hotspot

1. Open the settings menu by tapping the icon on the top left corner once.
2. Tap the switch to the right of “Hotspot”. When the switch turns green, hotspot mode is enabled. This will disable the Wi-Fi.
3. Tap on the word “Hotspot”.
4. The display will show the data with which your other WLAN-enabled devices (e.g., PC or laptop) can connect to the WLAN hotspot generated by Fluidlab R-300:
   Network name: *fluidlab_########*
   Default password: *fluidlab*
   IP address: 192.168.#.#
5. On your PC or laptop, when prompted to enter PIN, select “connect using a security key instead” and type in *fluidlab*.
6. Your devices are connected to the Fluidlab R-300 hotspot.

4.3. FAQ

Meeting your customer for a product demo might bring up different kind of questions about the fluidlab R-300, the acella sample carriers or the export of the results with the anvajo datalab. To ensure that you have answers to those questions, we collected the twenty most important ones customers asked us.

You can find the full list in the attachments (attachment 5).

5. Attachments

- Attachment 1: Performed measurements list

Cell lines and cell types only recognized for cell counting & viability.

Cell line	Cell type	Tissue	Organism
ympho	epithelial cells	ovary	fall armyworm
yac-1	lymphoblast	lymphoma	mouse
WBC	blood cells	peripheral blood cells	human
U373	glioblastoma cells	neuronal	human
U2OS	Osteosarcoma	bones	
T-REx™-293	epithelial cells		human
TK6	lymphoblast	spleen	human
THP1	monocytes	peripheral blood cells	human
THLE	epithelial cell	liver	human
SW620	epithelial cells (cancer)	bowel	human
sw480 sw-480	epithelial cells	colon	human
stem cells			human

skbr3	breast cancer	breast	human
SIM-A9	microglia	brain	mouse
sf21		ovary	fall armyworm
SAOS-2	epithelial cells	bone	human
RS4	Leukemia	blood	human
RO	b-lymphocytes		human
RMS	Rhabdomyosarcoma		human
RBC	blood cells	peripheral blood cells	human
RBC	blood cells	peripheral blood cells	trout
RAW 264.7	macrophages	ascites	mouse
primary asterocytes	glia cells	brain and spinal cord	human
primary	t-lymphocytes (t-cells)	peripheral blood cells	human
PDAC	pancreatic ductal adenocarcinoma	pancreas	
PC3	epithelial cells (cancer)	prostate	human
PC17	Lung carcinoma	lung	human
panc-1	epithelial cells, pancrea carcinoma	pancreas	human
NKT cells	T cells and natural killer cells		human
NIH 3T3	fibroblasts		mouse
NEK 53	epithelial cells	bone marrow	
NCM640	epitelial	Colon	human
MZPC1	pancreatic cancer	pancrea	human
MUTZ 3		skin	human
MTH53A	epithelial cells	breast epithelium	rabbit
MSC	mesenchymal stemcells	bone marrow	human
MCF7	cancer cells	breast	human
MC3T3	osteoblast	bone marrow	mouse
MB49	cancer cell line	bladder	mouse
MB49	Urothelial Carcinoma	bladder	mouse
LAPC4	cancer cell line	prostate	human
lacp4	prostate cancer cells	prostate	human
L929	fibroblasts		mouse
Keratinozyten	epidermal cells	Gingiva	human
K-562	myeloic leukemia		human
Jurkat	t-lymphocytes (t-cells)	peripheral blood cells	human
HUVEC	endothelial cells	umbilical vein/vascular endothelium	human
HUH7	hepatoma cells	liver	human
hTERT-MSC	mesenchymal stemcells	bone marrow	human

HTC-116	Epithelial-like	colon	human
HT29	colon cancer	colon	human
HT1080	epithelial cells	fibrosarcoma	human
Ht1080	Fibrosarkom		human
HS60	cancer cells	lung	human
HoxP8			
HOB	osteoblast	bone marrow	human
HN33 murine cell	hippocampal neuron × neuroblastoma	brain	murine
HL-60	acute myeloid leukemia	peripheral blood cells	human
HL1	Cardiomyocyte	cardiac muscle	mouse
HK2	epithelial cells	kidney	human
hepG2	liver cancer	liver	human
HeLa	epithelial cells	cervical cancer	human
HEK transfected	epithelial cells	embryonic kidney	human
HEK 293T	epithelial cells	embryonic kidney	human
HCT	primary colorectal carcinoma	colon	human
HCC	Hepatocellular carcinoma cells	liver	human
hbmec	epithelial cells	brain	human
glioblastoma cells	cancer cells	brain	human
FM3	Melanoma		human
FL1	bladder carcinoma	bladder	human
FKD-R	epithelial cells	small intestine	human
FB	fibroblasts	skin	rabbit
fadu	epithelial, Squamous Cell Carcinoma	Pharynx	human
DU145	epithelial cells (cancer)	prostate	human
du145	prostate cancer cells	prostate	
Dictyostelium discoideum			amoeba
D17	Osteosarcoma	bone	
CMT-13	cells of the mammary gland	breast cancer	rabbit
CHO (chinese hamster ovary)	epithelial cells	ovary	hamster
CAR-NK	killercells		
Capana-2	cancer cell	pancreas	human
Calu-3	epithelial cells	lung cells	human
Caco-2	epithelial adenocarcinoma cells from a human	lg	human
Caco-2	epithelial cells	colon	human

C2C12	myoblast	muscle	murine
C2	mast cells	cytoma stream	rabbit
BCi	Basal cells Immortalized	airway	human
Baf3	pro b cells	lymphocyte	murine
Astrocytes	astroglia	brain	murine
AML12	hepatocytes	liver	human
A549	epithelial cells	lung cells	human
A-375	melanoma cells	skin	human
A357	melanoma cells	skin	human
184B5	breast cancer	breast	human
16hbe	epithelial cells	bronchial	pri
	cancer cells	neuroblastom	human
	Dendritic cells		
	embryonic stem cells		human
	embryonic stem cells		mouse
	hepatocyte tumor cells	liver	human
	iPS cells		human
	marmacarcinoma cells	breast cancer	

Cell lines and cell types only recognized for the cell counting

Cells	Cell type	Tissue	Organism
Aspergillus fumigatus	spore		fungus
Candida albicans			yeast
Candida auris			yeast
isochrysis galbana	microalgae		microalgae
Isocrisys galbana	microalgae		microalgae
Phaeodactylum tricornuta	microalgae		microalgae
Saccharomyces cerevisiae	yeast		Yeast
Schneider S2 cells	insect		drosophila melanogaster
Tetratelmis schuii	microalgae		microalgae
Yarrowia lipolytica			yeast
	micro spore		Beta vulgaris (Zuckerrübe)
	microalgae		calciodinellum operosum
	microalgae		cyclotella cryptica
	microalgae		ditylum brightwellii
	microalgae		leonella graniferan

	microalgae		scrippsiella trochoidea
	microalgae		stephanopyxis turris
	microalgae		symbiodinium voratum
	microalgae		thalassiosira pseudonana
	microalgae		
	microalgae		desmo desmo
	microalgae		phytoplankton
	microalgae		haematococcus pluvialis
	microalgae		phaedaktylum
	microalgae		chlorela vulgaris
	microalgae		raphidocelis subcapitata
	microalgae		cosarium formasulum
	microalgae		micrasterias
	microalgae		nanochloropsis
	microalgae		cryptophyta
	microspores		hordeum vulgare (Gerste)
	microspores		zea mays (Mais)
			synura petersenii
			oily yeast
			paramecium
	saccharomyces bayanus		yeast
CTL	cytotoxic t lymphocyte	bone marrow	human
Granulosa cells	folicular cels	ovary	goat
halichondria panicea			sponge
hybridoma cells	fusion of b- and cancer cells		mouse
PBMCs	blood cells	peripheral blood cells	human
PBMCs thawed	blood cells	peripheral blood cells	human
Splenocytes	WBC	Spleen	mouse
	lung cells	lung	mouse
	spore		aspergillus oryzae
	spore		aspergillus niger
	spore		aspergillus brasiliensis
	spore		aspergillus versicolor

Attachment 2: Validated cell list

Cell name	Animal	Morphology	Tissue	Adherent/ Suspension	Permanent/ Primary	Size (µm)
HT1080	Human	Epithelial cells (fibroblasts)	Fibrosarcoma	Adherent	Permanent	12
HEK293T	Human	Epithelial cells	Embryonic kidney	Adherent	Permanent	13
HeLa	Human	Epithelial cells	Cervical cancer	Adherent	Permanent	40
MC3T3	Mouse	Osteoblasts	Calvarial bone marrow	Adherent	Permanent	20-30
hTERT-MSC	Human	Mesenchymal stem cells	Bone marrow	Adherent	Permanent	10-32
C2	Canine	Mast cells	Cystomastoma	Adherent	Permanent	20-30
MTH53A	Canine	Epithelial cells	Breast epithelial tissue	Adherent	Permanent	n/s
FKD-R	Human	Epithelial cells	Small intestine	Adherent	Permanent	n/s
CMT-13	Canine	Mammay cells	Mammary cancer	Adherent	Permanent	n/s
MSC	Human	Mesenchymal stem cells	Bone marrow	Adherent	Primary	10-32
FB	Canine	Fibroblasts	Skin	Adherent	Primary	10-15
HOB	Human	Osteoblasts	Femoral bone marrow	Adherent	Primary	20-30
Sf9	Insect	Epithelial cells	Ovarian	Suspension	Permanent	17-30
Jurkat	Human	T-lymphocytes	Peripheral blood	Suspension	Permanent	8
THp1	Human	Monocytes	Peripheral blood	Suspension	Permanent	10
*RBC**	*Human*	Blood cells	Peripheral blood	Suspension	Primary	7-9
WBC	Human	Blood cells	Peripheral blood	Suspension	Primary	8-12

Attachment 3: Competitor Comparison

	fluidlab R-300	Countess (I, II, FL, 3)	LUNA	NucleoCounter NC-3000
Application	- Cell count - Viability (staining-free) - Cell size distribution - Cell cluster recognition - Spectrometer: extinction & calibration curve - Kinetics	- Cell count - Cell viability - Cell size distribution & average cell size - Evaluate apoptosis (II FL) - Monitor fluorescent protein expression (II FL)	- Cell number - Detects live/dead cells - Cell size distribution - Discriminates cell debris	- Cell number - Cell viability (based on DAPI staining & cell lysis) - Cell size distribution - Cell cycle analysis - GFP transfection efficiency
Method	Digital holographic microscope (+spectrometer)	Brightfield microscopy	Brightfield microscopy	Fluorescence image cytometry
Used dyes	No dye necessary	Trypan blue & fluorescent dyes (II FL)	Trypan blue, Erythrosin B	Fluorophore, Acridin-Orange, DAPI
Size (mm)	128x94x33	228,6x139,7x228,6	220x210x90	290x290x310
Weight (kg)	0.24	3.63	1.2	14
Focus type	Autofocus	Autofocus (with manual focus option)	Manual focus	Autofocus
Sample Carrier	Single use glass slide	Single use /disposable slide	Single use & reusable slide - single use appr. 1,10€	Single use cassette or chamber slide - single use appr. 2€
Fluorescence yes/no	No	No (II & III), Yes (II FL)	No	Yes
Cell size	3-80 µm (cell count), 8-80 µm (viability)	4-60 µm (detection), 7-60 µm (viability)	3-60 µm	4-80 µm
Optimal concentration /cells/mL	$1x10^4$ - $1x10^7$	$1x10^4$ - $1x10^7$	$1x10^4$ - $1x10^7$	$5x10^4$-$5x10^6$
Field of view /mm²	5.3	3.48(II, II FL), 3.82 (3)		
Sample volume	4 µl, 10 µl or 20 µl	10 µl	10 µl	10 - 60 µl
Time of measurement (s)	~20	10 (II, IIFL), 30 (3)	7	90
Display	3.5“color touch screen	7“touchscreen (II, II FL), 1280 x 800 LCD screen (3)	7“ LCD display	
User protocols	No	Yes (up to 10 user profilesfor II & II FL)	No	Yes
Connectivity	Wifi, Hotspot	USB drive (II, II FL), USB, Wifi (3)	USB drive	USB drive
Data export	PNG, Excel	JPEG, TIFF, PNG, CSV files, PDF report	TIFF, PDF report	PDF report
GLP/GMP compliance	No	No	No	Yes

Attachment 4: Reference Customers

Reference Customers for the fluidlab R-300
Adian Oy
AO Research Institute Davos (ARI)
Aphea.Bio
aprentas Switzerland
Autonomous University of Barcelona - Institute of Neuroscience
BASF SE
Biohellenika
Bipolis - ADM nutrition
Boehringer Ingelheim RCV
Boehringer Ingelheim Therapeutics GmbH
Boehringer Ingelheim Veterinary Research Center
Cambridge Epigenetics
Clinical Immunology Laboratory Stöcker
College of Emden Leer
College of Esslingen - Department of Biotechnology
Cologne Audience and Biotechnology
Currenta GmbH & Co. OHG
Eurofins Ingenasa
EUROIMMUN AG
Exact Sciences Innovation
FluoretiQ Limited
Fraunhofer Institute - Life Science and Bioprocesses
Fraunhofer Institute for micro technology
Helmholtz Association - Institute for Biomaterial Research
Helmholtz Association - Institute for Biomaterial Research
Helmholtz Association - Institute for Pharmaceutical Research
Helmholtz Association - Molecular Genome Analysis
Helmholtz Association - Personalized Medical Oncology
Hull York Medical School
Humboldt University Berlin - Chemistry
KIT - Institute for functional interfaces
Leibniz Association - Hans-Knöll-Institute (HKI) - Department of Infectious Biology
Leibniz Association - Heinrich-Pette-Institute - Institute for Experimental Virology
Leibniz Association - Institute for Aging Research
Leibniz Association - Institute for Behavioral Physiology
Leibniz Association - Institute for interactive materials

Leibniz Institute for Paleobiotechnology
Leibniz Society - Research Institute Borstel - Department of Asthma and Allergy
LenioBio GmbH
L'Oréal
Max Rubner Institute - Institute for Microbiology and Biotechnology
Max-Planck-Society - Biomolecular Systems
Max-Planck-Society - Chemical Biology
Max-Planck-Society - Institute for Biology of aging
Medical School at Carl-Thiem clinics Cottbus
Private brewery Zwettl Karl Schwarz GmbH
PROGEN Biotechnik GmbH
purefluidics
Robert Bosch GmbH
Saxon Education Society for Environmental Protection and Chemical Professions Dresden mbH
Saxony University of Cooperative Education Riesa
Saxony-Anhalt State Office for Environmental Protection
Sphere Fluidics Ltd
Stanford University- Department of Pulmonary Allergy and Critical Care
State Academy of Studies Riesa
State Office for Agriculture and Rural Areas Thuringia
Technical College Cologne - Metabolon Institute
Technical College Cologne - Research Institute STEPs
Technical University Dresden - Center for Molecular and Cellular Bioengineering
Technical University Dresden - Clinic for Neurosurgery
Technical University Munich - Chair of Robotics and Systems Intelligence
Technical University of Ilmenau - Institute of Chemistry
Technical University of Ilmenau - Institute of Physical Chemistry
University Barcelona
University Barcelona - Biomedical Research Institute ; Immunogenetics of the autoinflammatory response Structural and biological mass Spectrometry
University Barcelona - Biomedical Research Institute; Systemic Vasculitis
University College London - Center for Nephrology
University Hospital Aachen - Institute of Pharmacology and Toxicology
University Hospital Dresden - Institute for Clinical Chemistry
University Hospital Essen - Institute for Humane Genetics
University Hospital Frankfurt - Biobank
University Hospital Frankfurt - Clinic for Dermatology, Venerology and Allergy
University Hospital Hannover - Institute for clinical Biochemistry
University Hospital Hannover - Institute for clinical Biochemistry
University Hospital Jena - Translational Microbiology

University Hospital Leipzig - Department of Internal Medicine, Neurology and Dermatology
University Hospital Munich - Department of Endocrinology, Diabetes and Metabolism
University Hospital of Essen - Institute for Pathology
University Hospital of Heidelberg - Institute for Pathology
University Hospital of Schleswig-Holstein - Clinic for Rheumatology and clinical immunology
University Hospital Rostock
University Hospital Rostock - Clinic for Polyclinic for Dermatology and Venerology
University of Applied Sciences Erfurt - Department of Crops
University of Applied Sciences Weihenstephan-Triesdorf
University of Cambridge - Infectious Diseases
University of Dresden - Institute of Clinical Genetics
University of Duisbur-Essen - Central collection of algae cultures
University of East Anglia - Chemistry
University of Erlangen-Nuremberg - Pharmaceutical Biology
University of Essen - Institute for Medical Microbiology
University of Frankfurt - Department of Conservation Biology
University of Freiburg - Institute of Biochemistry and Molecular Biology
University of Giessen - Institute for Human Genetics
University of Giessen - Institute of Food Science
University of Giessen - Pneumology, Infectiology and Intensive Care Medicine
University of Greifswald - Medical School
University of Halle-Wittenberg - Biocenter
University of Halle-Wittenberg - Institute for Physics
University of Halle-Wittenberg - Institute for Physiological Chemistry
University of Hamburg - Center for Structural Systems Biology
University of Hamburg - Institute of Zoology
University of Hamburg-Eppendorf (UKE) - Center for Oncology
University of Hamburg-Eppendorf (UKE) - Experimental Radiooncology
University of Hamburg-Eppendorf (UKE) - Research institute of Neurooncology
University of Hamburg-Eppendorf (UKE) - Institute of Pathology
University of Hamburg-Eppendorf (UKE) - Institute of Pharmacology and Toxicology
University of Heidelberg - Chemistry
University of Jena
University of Jena - Institute for Microbiology
University of Liège - Genetics and Physiology of Microalgae
University of Magdeburg - Clinic for Neurosurgery
University of Magdeburg - Institute for Biometry and Medical Informatics
University of Mainz - Biobank
University of Marburg - Center for Infections

University of Marburg - Core Facility of Cellular Metabolism
University of Münster - Institute of Zoophysiology
University of Münster - Biomedical Technology Center
University of Newcastle - Center for Cancer
University of Newcastle - School of Dental Sciences
University of Osnabrück - Department of Structural Biology
University of Pais Vasco - Faculty of Medicine
University of Paris Diderot - Epigenetics and Cell Fate Unit
University of Potsdam - Department of Soft Matter Physics
University of Stuttgart - Institute for Biochemical Engineering
University of Stuttgart - Institute for Microbiology
University of Würzburg - Tissue Engineering and regenerative medicine

Attachment 5: FAQ List

Category	Question	Answer
Calibration Curve	Can I export the calibration curve?	With the current version of the anvajo datalab it is not possible to export the calibration curve.
CC & VIA	What is the maximum number of cells that can be counted with the cell counter or viability tool?	The recommended concentration range is from 1 x 104 to 1 x 107 cells/ml. However, this depends on the size of the cells and the arrangement of cells. If cells are large or tend to cluster, high concentrations may be not reliably counted. If the cell concentration is too high for reliable detection, the fluidlab will show an overfull warning on the results screen.
CC & VIA	What is the upper/lower size limit of cell detection?	3µm - 80µm for cell counter, 8 - 80µm for viability
CC & VIA	Can bacteria be counted with the device?	No, because bacteria are too small, and the CC requires a cell size of at least 3 µm (in diameter).
CC & VIA	Where do the rings around the cells come from?	The images acquired with the fluidlab R-300 look different than conventional bright field (BF) images, because we employ a different microscopy approach called digital holographic microscopy (DHM). Contrary to BF microscopy, the image is not created by lenses. Instead, diffraction of light at the sample creates a hologram which is captured on the camera sensor. The hologram contains all the information about the sample, but cannot be interpreted by the human eye. Instead, the image of the sample is reconstructed numerically by a computer. The rings around the cells in the image are remaining/artefacts from the hologram but do not influence the cell recognition. As we keep improving the image reconstruction algorithms, we may be able to get rid of these rings in the future. (Analogy: Think about these rings as the water waves that form when you throw a stone into the water. The shape of these waves on the water surface will tell you something about the stone that caused them, even though you cannot see the stone anymore.)

CC & VIA	Why is the cell count in the viability measurement lower than in the cell count measurement?	The difference between the total count in the viability application and the count in the cell count application can be manifold: 1. The lower cut off of the viability application is 8µm (compared to 3µm for the cell count application). When cells are smaller than 8µm, they will not be counted by the viability application, but maybe counted in the cell counter (depending on the gating). 2. The quantification of cell viability is a two-step process: 1) All objects within the image are detected (similar to the cell counter). 2) Then, every detected cell is analysed individually and categorized one of the two categories: Living cells and dead cells. Maybe if the sample consist of a lot of cell clusters, it could be for the algorithm too difficult to distinguish these cells between live or dead.
CC & VIA	How does the fluidlab R-300 identify viable vs. dead cells?	Using holographic microscopy, the fluidlab R-300 is able to perform a staining-free/label-free differentiation between viable and dead cells. This differentiation is based on the refractive index and highlights the morphological and structural changes induced during cell death. Those visible differences are recognized by the fluidlab R-300's neural networks which then classifies cells to the respective category. For an extended explanation please see the document "Staining-free cell viability analysis with the fluidlab R-300".
datalab	When connecting the device via hotspot with the datalab, no connection can be established when typing in the name of the fluidlab. Is there another way to recognize the device?	When the fluidlab cannot be found by typing in the name, the user should try the workaround via the hotspot IP-address. The hotspot IP-address can be found when entering the menu on the device and clicking on "hotspot". For an extended explanation please see the document "Troubleshooting_Hotspotconnection".
Device software	How can saved results be deleted?	Go to the history tab and click on the box before the test name. A red trash icon appears, allowing the user to delete single or multiple results. If you click on the box in the top line, all measurements will be marked. This allows you to delete the entire history.
Device software	How many measurements can be saved on the device?	The device can save up to 1.000 measurements. The exact number can vary, depending on the type of measurements that are saved. Cell count and viability measurements use up the most memory. We recommend deleting regularly the history on the device after 250 measurements.

Device software	Why sensor Refresh?	For reliable cell counting and viability assessment, it is crucial that the correct acella sample carrier is detected. The corresponding sensor should be regularly calibrated for proper acella recognition. If acella recognition fails, it is essential to recalibrate the sensor by performing a sensor refresh.
Extinction	Why scatter light measurement for measurement of bacterial cultures?	Scattered light measurements are used to monitor the growth of bacterial cultures. The more bacteria are in the sample, the more light gets scattered and the extinction increases. Thus, the extinction over time can be used to create a bacterial growth curve.
Extinction	Can half-micro or micro cuvettes also be used for extinction measurements and the calibration curve?	We recommend using macro or half micro cuvettes for extinction measurements and the calibration curve application. The centre height of the cuvette (=height of light beam) shall be 8.5mm. When using half-micro cuvettes, we recommend using the scatter light adapter, so that light is limited to the sample and does not pass through the cuvette material.
Extinction	What is the difference between absorption and scatter light measurement?	When light travels through a sample, it can interact in different ways with the molecules/particles in the sample. Some of the incident light will get scattered, i.e. it changes its direction relative to the incoming beam (think about billard: when the white ball (=incoming light) hits the other balls (=sample particles), it deviates from its original path). The more particles are in the sample, the more the light will get scattered and less of the incoming light will reach the detector. Additionally, depending on the molecules, some of the particles will just absorb the incoming light (e.g. coloured dyes), which depends a lot on the wavelength of the light. Both scattering and absorption together determine the extinction of light as travels through the sample, i.e., the loss in light intensity between the incoming and the detected light.
General	How long is the warranty of the device?	1 Year.
Hardware	What does the "Sensor Status" option check?	The "Sensor Status" checks whether the microscope sensor is clean or dirty. Checking the sensor status is a quality control procedure for maintaining the fluidlab R-300's measuring functionality and accuracy. When the sensor status displays "dirty", the device should be cleaned according to the instructions in the user manual or the respective video in the anvajo YouTube channel. The device shall only be used for further measurements if the sensor status 'OK' is displayed.
Hardware	Why does the R-300 not require calibration (neither spectrometer nor CC)?	The fluidlab R-300 is calibrated during the production process but does not require any further calibration or maintenance at the user. This is because the fluidlab R-300 does not contain any moving parts that may get misaligned or that wear out (e.g., lenses). Moreover, sophisticated computer algorithms ensure accurate and reproducible results both for the spectrometer and the holographic microscope (CC) by controlling light exposure and focus, respectively, for each measurement.

Hardware	Can I spray the device with disinfect for cleaning?	Yes, the surface of the device can be sprayed with disinfectants. We recommend using isopropanol (99%). Check out the respective video on the anvajo you tube channel for cleaning the device.
Sample Carrier	Can the acella be used multiple times?	No. The acella is a single-use slide. It is not possible to load the acella with different samples in order to perform multiple measurements. It cannot be guaranteed that all particles will be removed from the acella by "cleaning" the chamber.
Sample Carrier	I aspired 20µl sample liquid into my pipette but the acella 100 is not completely filled up to the min mark. What should I do?	Softly knock the sample carrier on the table so the liquid sinks a little bit down. Otherwise just add a few more microliters into the carrier. When loading the acella, make sure the pipette tip is set directly at the entrance to the measuring chamber. Otherwise, a small part of the sample might remain in the half circle.

Attachment 6: Questionnaire

Topic	Researcher answer	Example
Research Area		Oncology, Cell & Gene Therapy,
Application		CC, Via, Kinetics, Spectrometer,
Cells		HEK, HeLa, CHO, Sf9, etc.
Cell size		3-80 (CC) 8-80 (Via)
Cell Concentration		$1x10^4$ – $1x10^7$
Assay for spectrometer		Bradford, OD_{600} colorimetric, turbidimetric,
Wavelength		375-700 nm
Testing period needed?		1-2 weeks
Questions?		

Sample preparation

Cell Counter + Viability measurement

- Freshly prepared cells
  - Concentration in the range
  - Well resuspended before measuring
- Neubauer chamber + trypan blue for comparison of results
- Sample volume each measurement: 5, 12 or 22 µl

Spectrometer

- Cuvettes ready
  - Standard 10x10mm (Quartz, plastic, half cuvettes)
- Solution prepared
  - Bacteria, colorimetric or turbidimetric assays
- Calibration Curve
  - Preparation of different concentrations/dilutions
- Kinetics
  - Environment prepared for different conditions or settings (e.g., CO2/O2 - chamber)

Checklist customer appointments

- (charged) fluidlab R-300 with cleaned sensor
- Power supply unit
- Laptop
- fluidlab connected to the datalab on the laptop
- Box 1: acella 100
- Box 2: acella 50 & 20 (for special cases)
- Sensor swaps
- Adapter Key
- Adapter 01,02 & 03
- “When to use which acella” – document
- “Performed measurement” – document