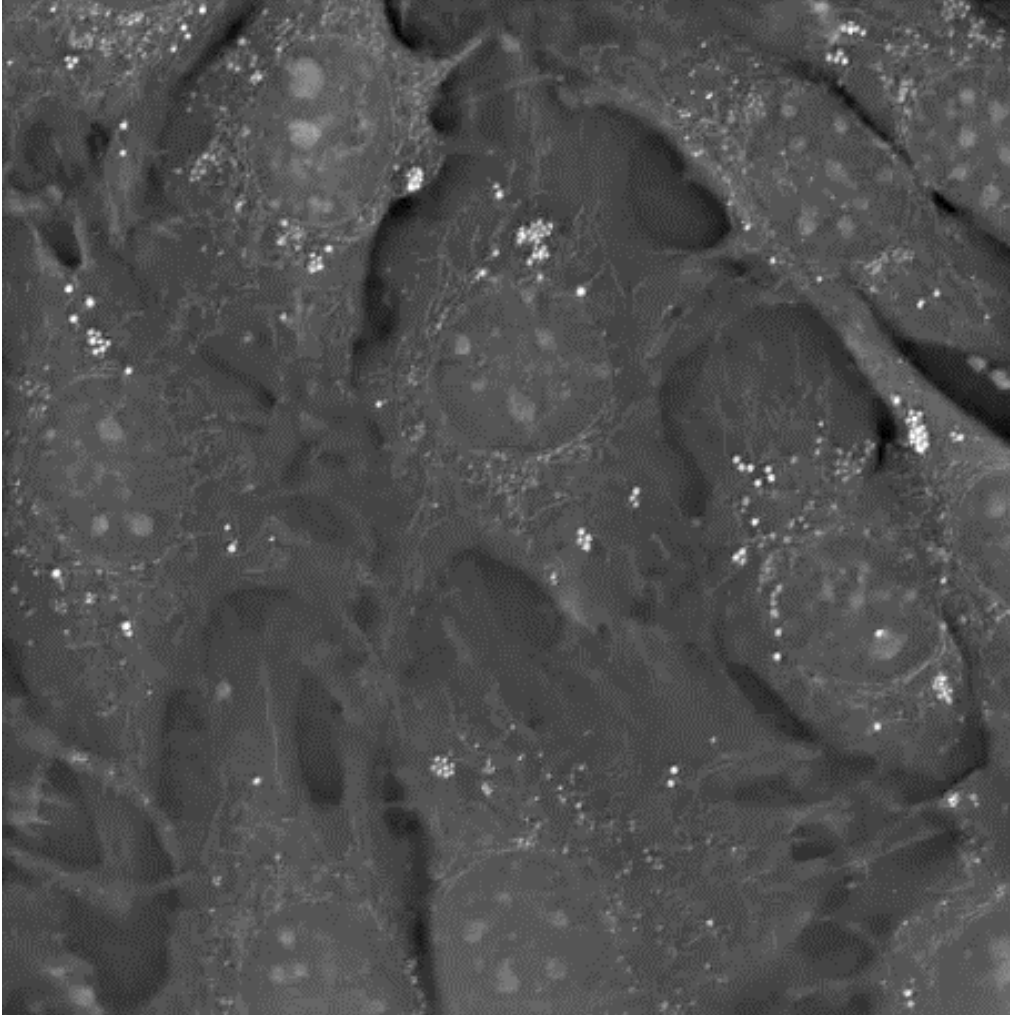




# NANOLIVE INCUBATION AND IMAGING BEST PRACTICES

- Incubator accessory applications and benefits
- Components and features
- Labware compatibility
- Live cell imaging requirements
- Troubleshooting



Mouse Preadipocytes in proliferation - **48 hours**

### On-stage incubator benefits:

- High precision temperature, CO<sub>2</sub> and O<sub>2</sub>, humidity control during experiment
- Low volume of gas required
- Minimal gas and humidity leakage
- Adaptable to different labware format (i.e. slides, 35mm dishes, multi-well plates etc.)
- Compatible with microfluidic and perfusion systems
- Easy access to cells during the experiment
- High optical accessibility and quality



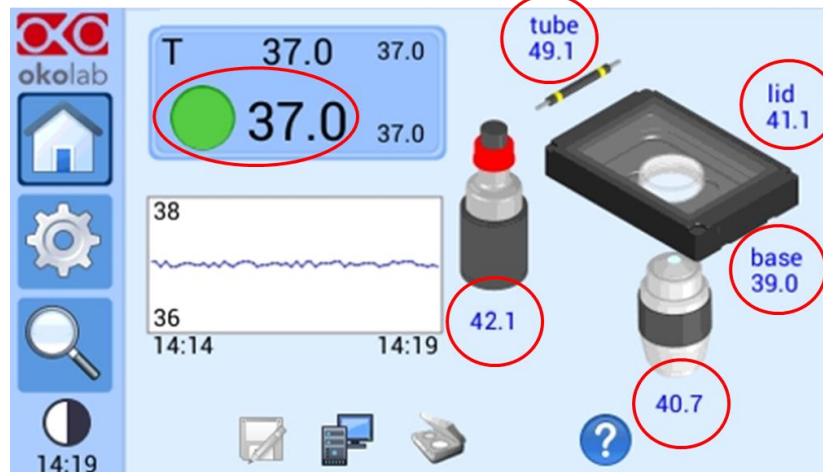
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### TOP-STAGE CONTROLLER

- Temperature Accuracy:  $\pm 0.1^{\circ}\text{C}$  in sample feedback mode and  $\pm 0.3^{\circ}\text{C}$  in chamber feedback mode
- Temperature Range: 25 to 50  $^{\circ}\text{C}$
- 4.3" touch screen interface
- Memory for data logging and mini-USB port for data download
- Includes an external T-Sensor for sample feed-back operation





### HUMIDIFIER

- Controlled via top-stage controller
- Humidification through combined action of a heated bubbling column and a heated tube
- Powerful humidifier to ensure ca. 90% of relative humidity inside the top-stage incubator



**Settings :: Humidity**

Humidity control enabled  
You can improve the performance of the Humidity Module by entering the temperature of the room

Room Temperature [C]    -    23.0    +

SP 47.8

SP 40.8

Offset    Cancel    Save

00:04



### 2GF-MIXER

- Pre-mixed gas flow rate can be regulated manually in the range 0.6-1 l/min
- CO2 range: 0-15%
- Manual Gas Mixer resolution: 1%
- Filtering device: PTFE membrane (0.2 micron pores)
- Dimensions (LxWxH, mm): 220x128x178 mm
- Weight: 3.0 kg



### GAS REGULATOR

- Pre-mixed gas flow rate can be regulated manually in the range 0.1-0.4 l/min



### 3GF-MIXER-HYPOXIA

- 3 gas mixer (Carbon Dioxide, Nitrogen, Air)
- O2 range: 2-20%





### HEATING COLLAR

- Easy installation around the objective
- Stand alone controller
- Eliminates undesired sample cooling



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**1x35-M single**  
For #1 35mm petri-dish



**1x60-M single**  
For #1 60mm Petri-dish



**1xLABTEK-M single**  
For #1 Lab-Tek 1"x2" chambered cover glass



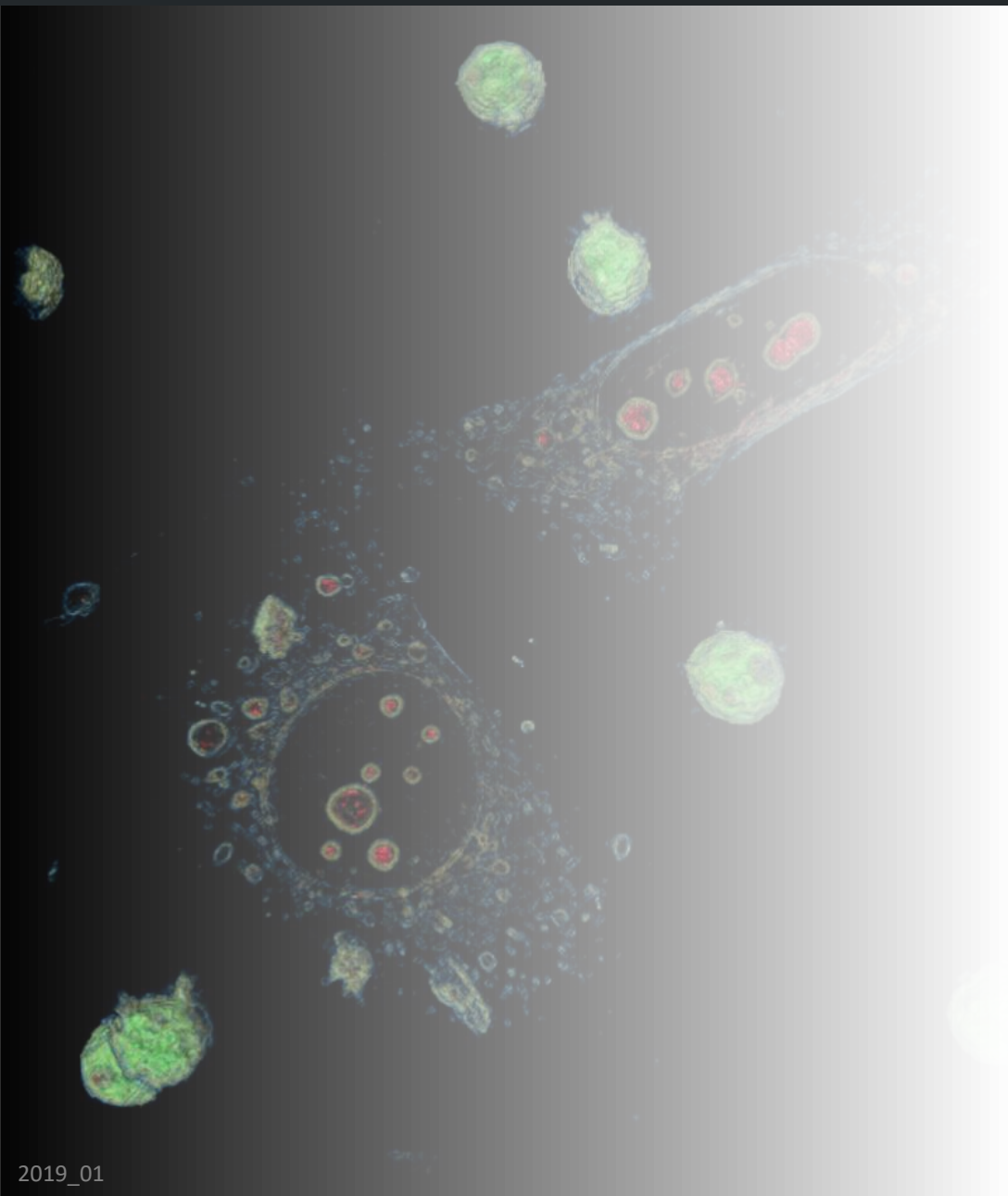
**1xGS-M single**  
For #1 1"x3" chamber slide



**1xLABTEK-II-M single**  
For #1 Lab-Tek II 1"x2" chambered cover glass

### CAMBER (H301-MINI) includes:

- Magnets for reliable holding to the stage
- Magnetic insert for **GS-M single** and **35-M single**, others available for purchase
- Glass top lid for perfect atmosphere control and sample access
- Built-in perfusion holes for insertion of tubing



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Time Lapse duration	Control	Accessory set up time	Minimum chamber equilibration time*	Minimum sample equilibration time**
Short < 3h	Temperature (T)	10'	30'	15'
Middle 3 – 12 h	T + Humidity (H) + ph (CO2) + obj heater	20'	60'	30'
Long > 12h	T + H + CO2 + obj heater Humidity extra precautions***	30'	90'	30'

\*For equilibration time we mean the required time for the controlled environment to reach and stable maintain the temperature, humidity a CO2 parameters. Please don't confuse it with calibration time, that is the required time for the microscope to center the laser into the objective before starting and acquisition.

\*\* Extra time before imaging the new living sample placed into the equilibrated chamber.

\*\* Please check extra precautions for long time lapse duration in Additional notes for Humidity control (Slide 14)

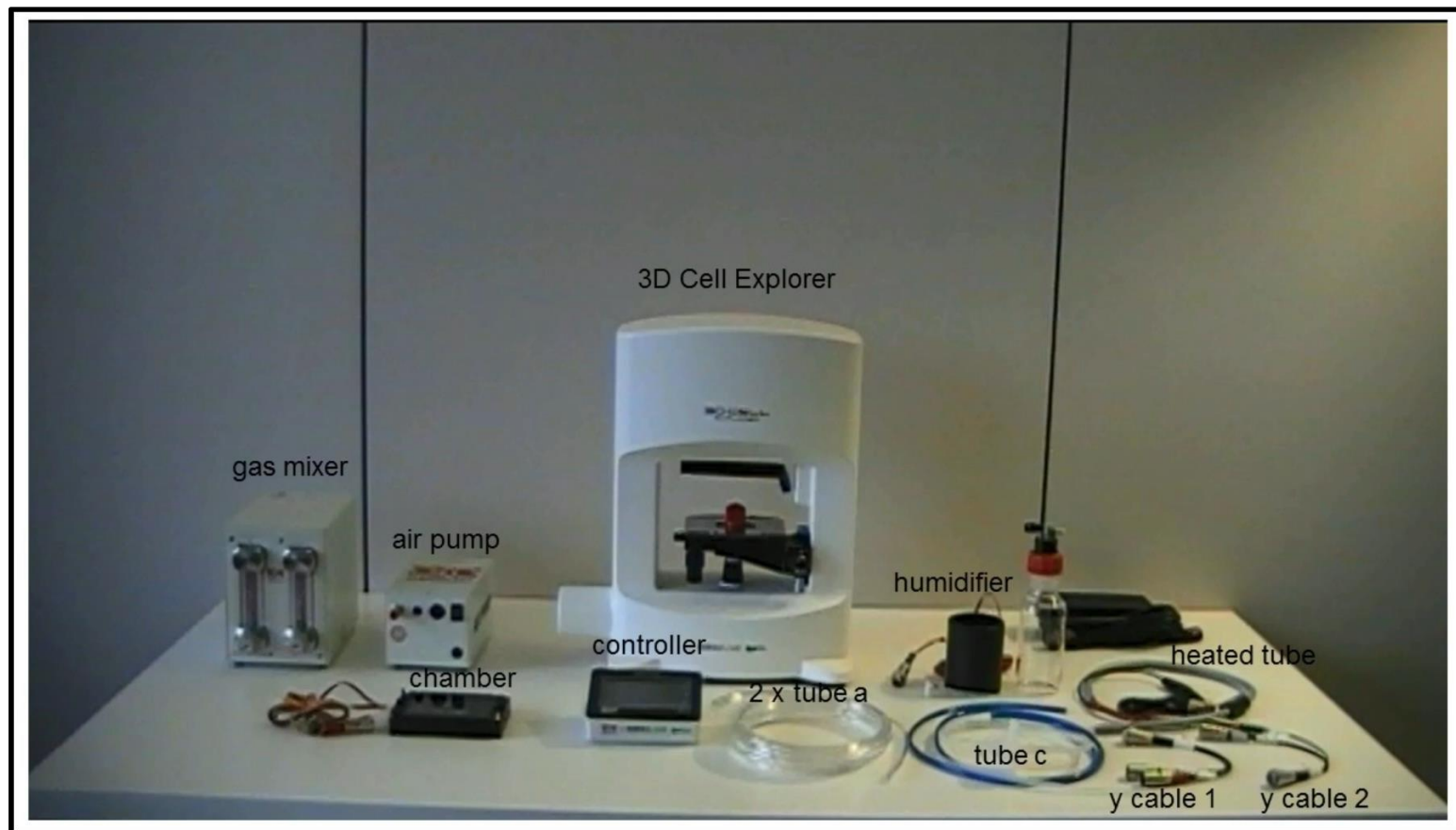
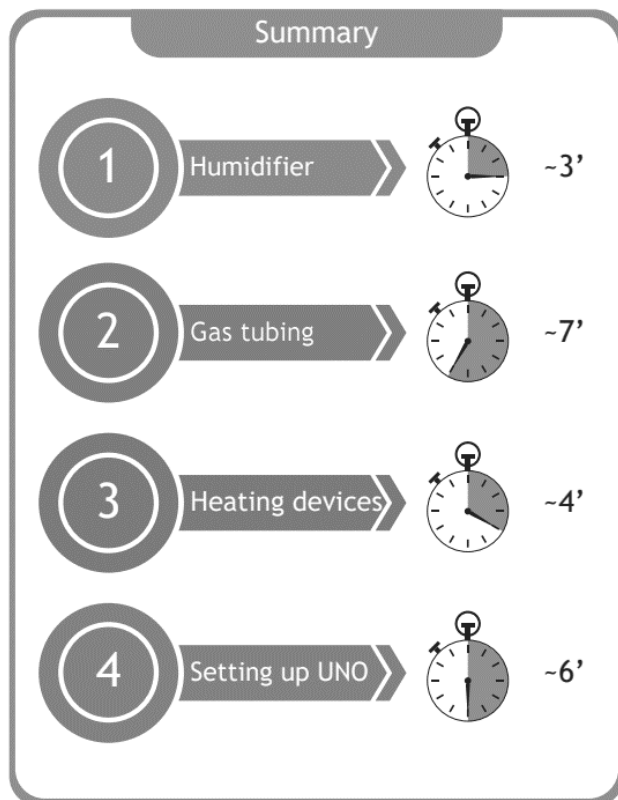
Step01 (10-30')

Set up your Nanolive stage top incubator



To install please follow the **full manual**:

<https://nanolive.ch/wp-content/uploads/UNO-STAGE-TOP-INCUBATOR.pdf>



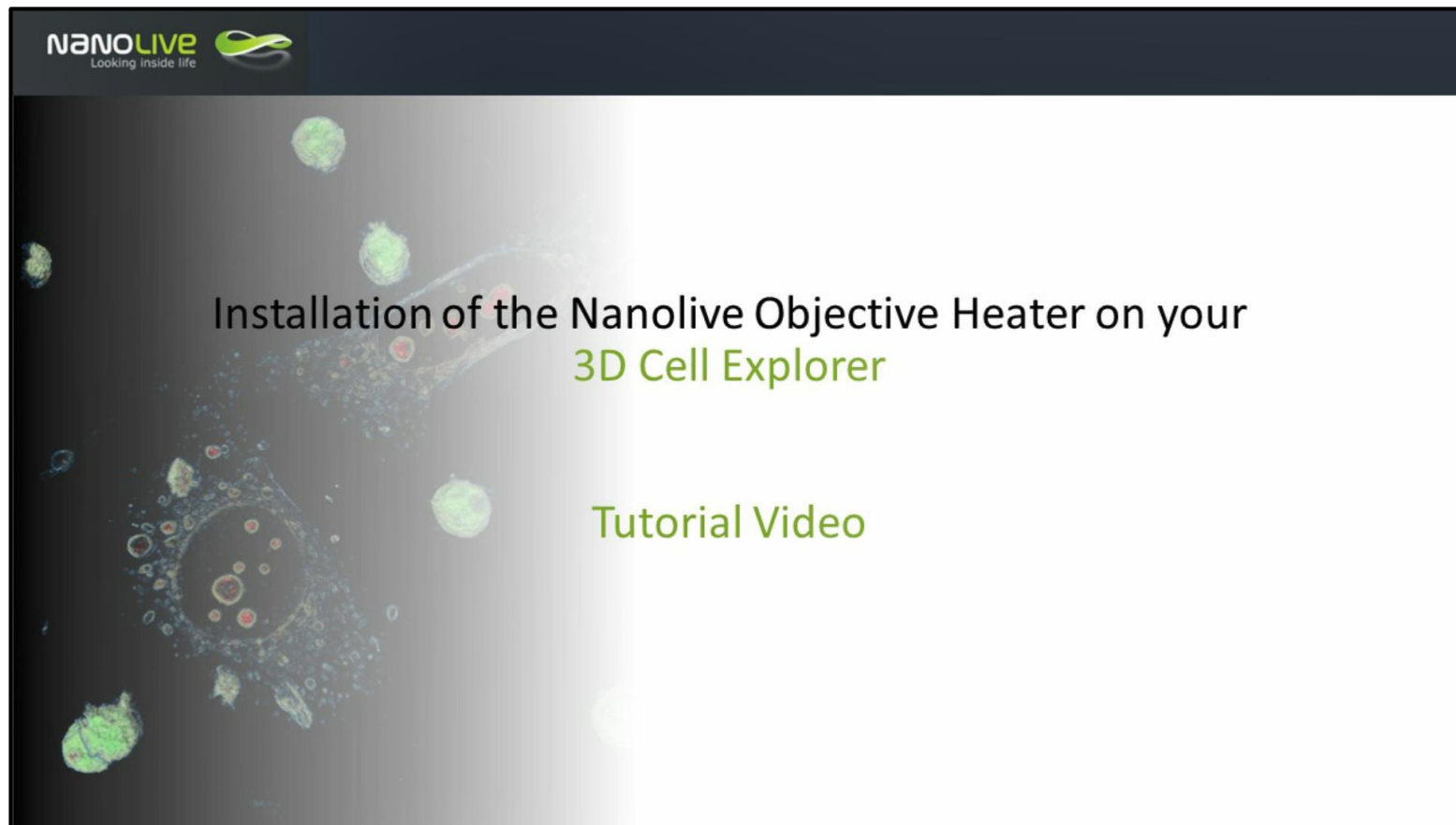
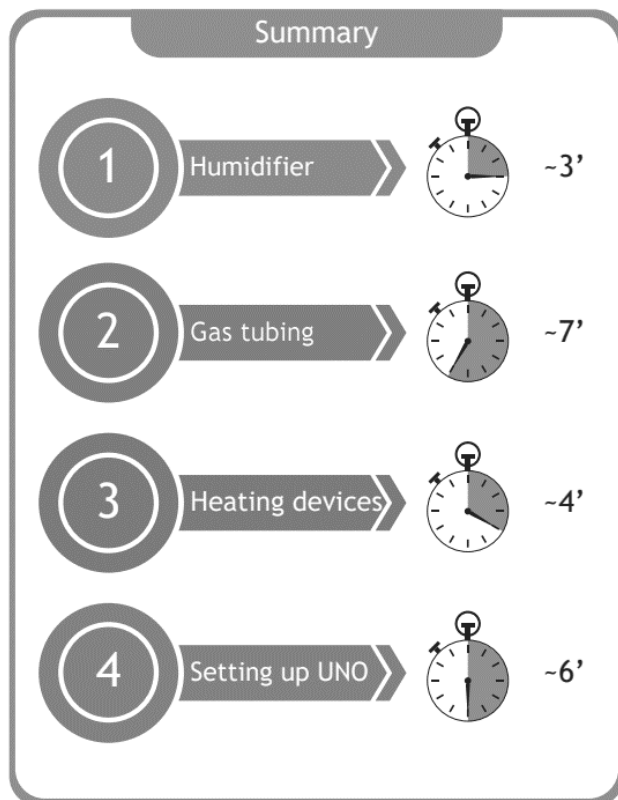
Full video: <https://www.youtube.com/watch?v=CVVSjq-hXqU&feature=youtu.be>

Step01 (10-30')

Set up your Nanolive stage top incubator



To install please follow the tutorial and guidelines, note you will need to remove your stage to install it. Once installed the objective heater can be left on the objective permanently (even when transporting unit).



- ! Be sure that the CO2 final pressure is 1bar, connected with 6mm cables.
- ! Position the air pump on the floor, far away from the instrument.
- ! Vibrations decrease image quality.
- ! We suggest to place the air pump on a rubber to reduce noise and movement.
- ! Clean carefully the chamber glass with lens paper and ethanol, maintaining the surface perfectly clean is a keep point in order to obtain high quality images.
- ! Don't bent too much the gas cables, linear cables will allow a more precise and constant humidified air flow into the chamber
- ! Hi-grade manual stage has a clip on a corner, please be sure to push it when you position chamber, **Fig. 1**. This will keep the chamber flat and stable.
- ! Press on Objective Heater icon to enter the Objective Heater menu. The Objective Heater is disabled by default, to activate it press on the Enabled icon, **Fig. 2**.

Fig. 1

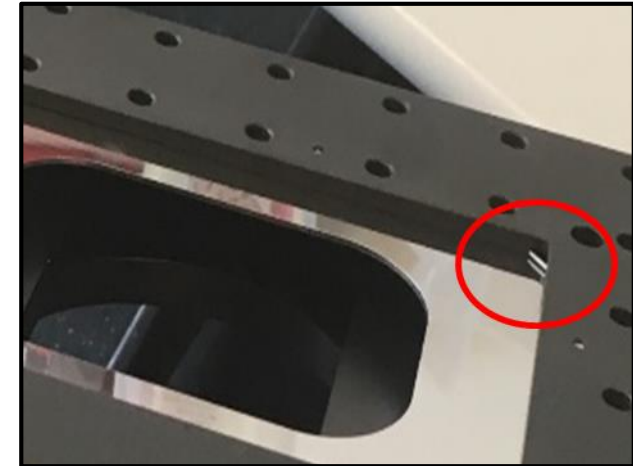
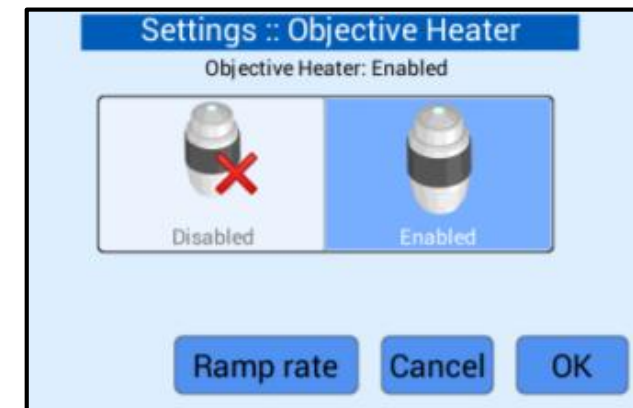


Fig. 2



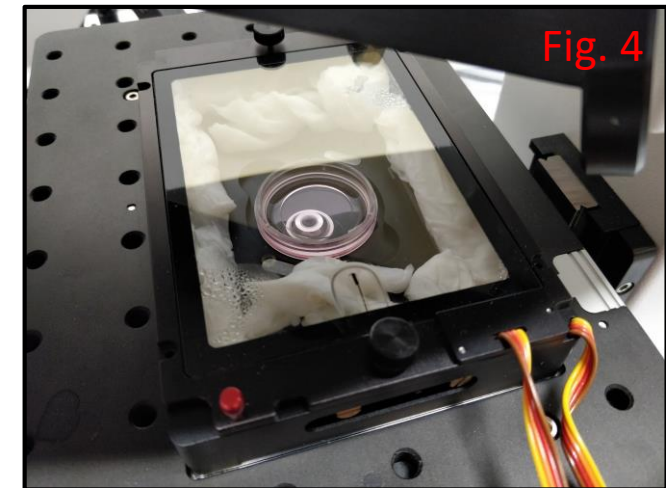
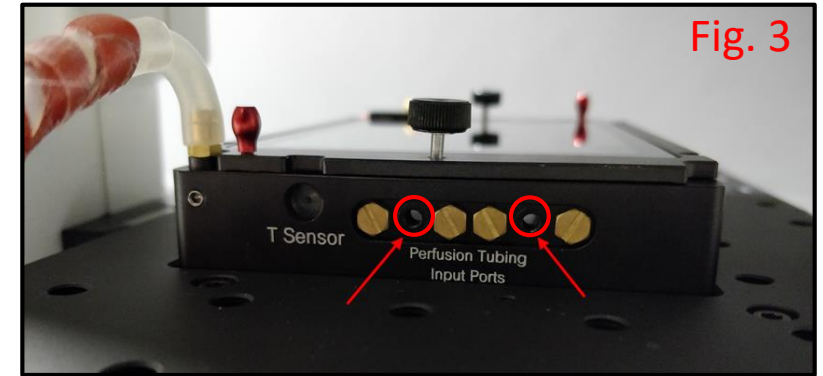


**All the required steps for the environment preparation are reported in details in the chapter 3 of [Application Note Growing and Filming Cells with the 3D Cell Explorer](#)**

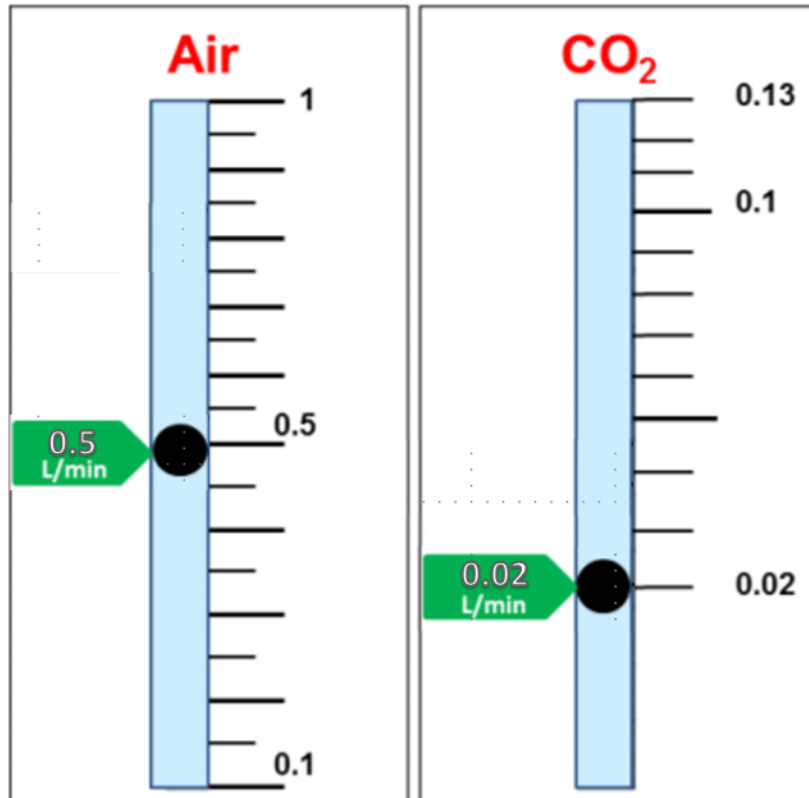
Please note that the equilibration time of the environment has to be done with:

- Chamber positioned on the microscope stage
- 35mm dish placed on the chamber adaptor, it must cover the whole hole.
- Microscope acquiring every 30s, to warm up the system and check focus drift and image quality

- ! Remove couple of screws from input or output doors, **Fig. 3**. This solution will redirect the outgoing air flow far from the objective avoiding condensation on the lens.
- ! Room dry environment (<50% air humidity) can impact on humidity retainment performance, please install the system in a room with a controlled environment.
- ! FOR LONG TIME-LAPSE DURATION (>12h), please be sure to take these **extra precautions**:
  - Start with high quantity of fresh medium, in 35mm dishes from 1,5 to 2ml.
  - Place 1 cm thick stack of Whatman or Kimtech paper all around the inner profiles of the chamber, and soak it with water, **Fig.3**.





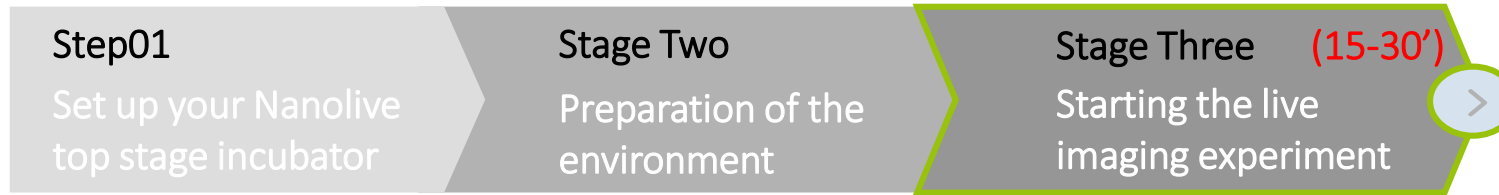


## What are the recommended values?

The suggested air and CO<sub>2</sub> values are displayed on **Fig. 4**

Note that you will see fluctuation of the metallic ball around the set values, it will take a while to stabilize. Please wait at least half of recommended equilibration time before make any further adjustment.

**These vales indicated in this doc replace the suggested values on the application note.**



**All the required steps for starting the live imaging experiment are reported in details in the chapter 4-6 of [Application Note Growing and Filming Cells with the 3D Cell Explorer](#)**

Now that all the parameters are under control, proceed with your experiment as quickly as possible to reduce the time cells are out of a controlled environment as well as the time the chamber lid is open.

Once the cells are in the chamber, we recommend waiting for at least **15-30 minutes** to let them adapt and to verify that everything is working fine. You are now ready to start a time-lapse.

In this period feel free to use bright field mode and holographic imaging and try to

- Find the best FOV,
- Calibrate the system
- Lunch short and fast time lapse to check the if there is focus drift

Don't start any long time lapse acquisition if you still observe system instability, focus drift or condensation!



**All the required steps for exporting the movie are reported in details in the chapter 7 of Application Note Growing and Filming Cells with the 3D Cell Explorer**

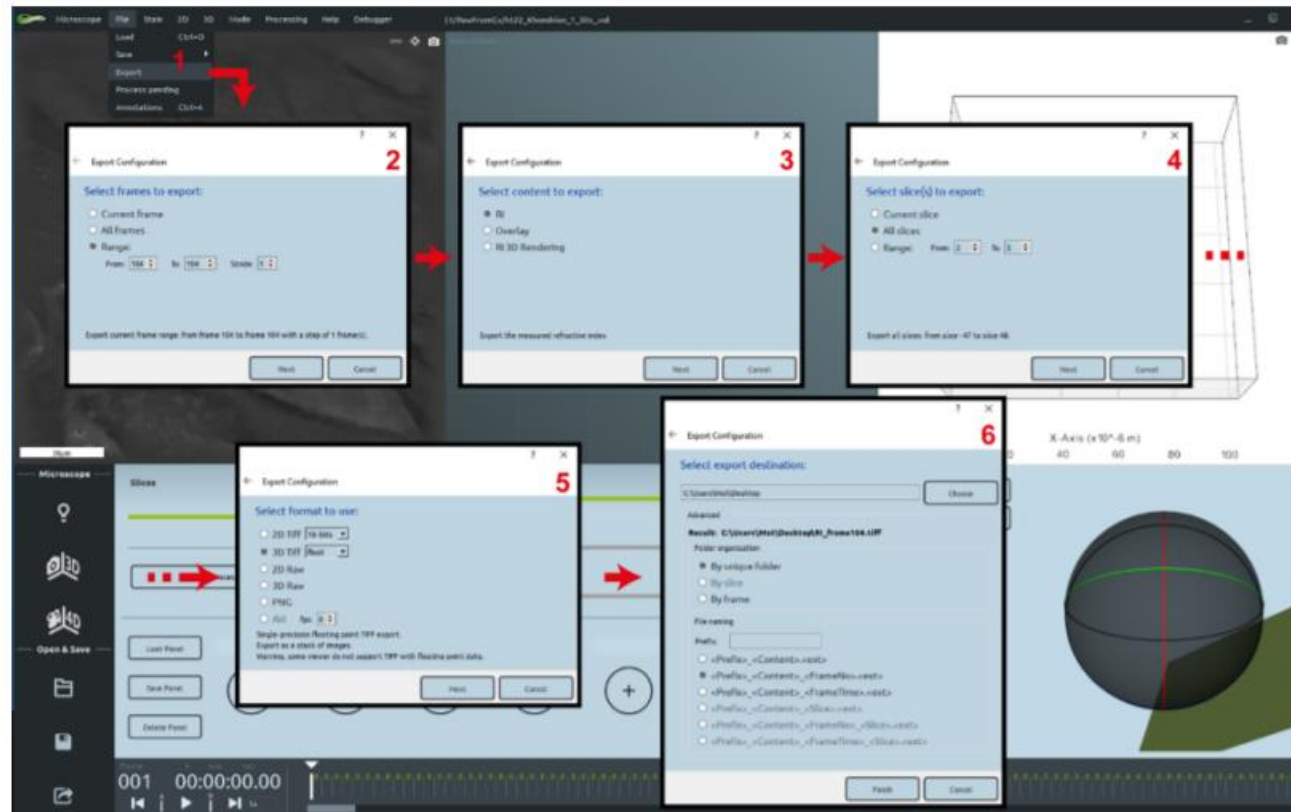
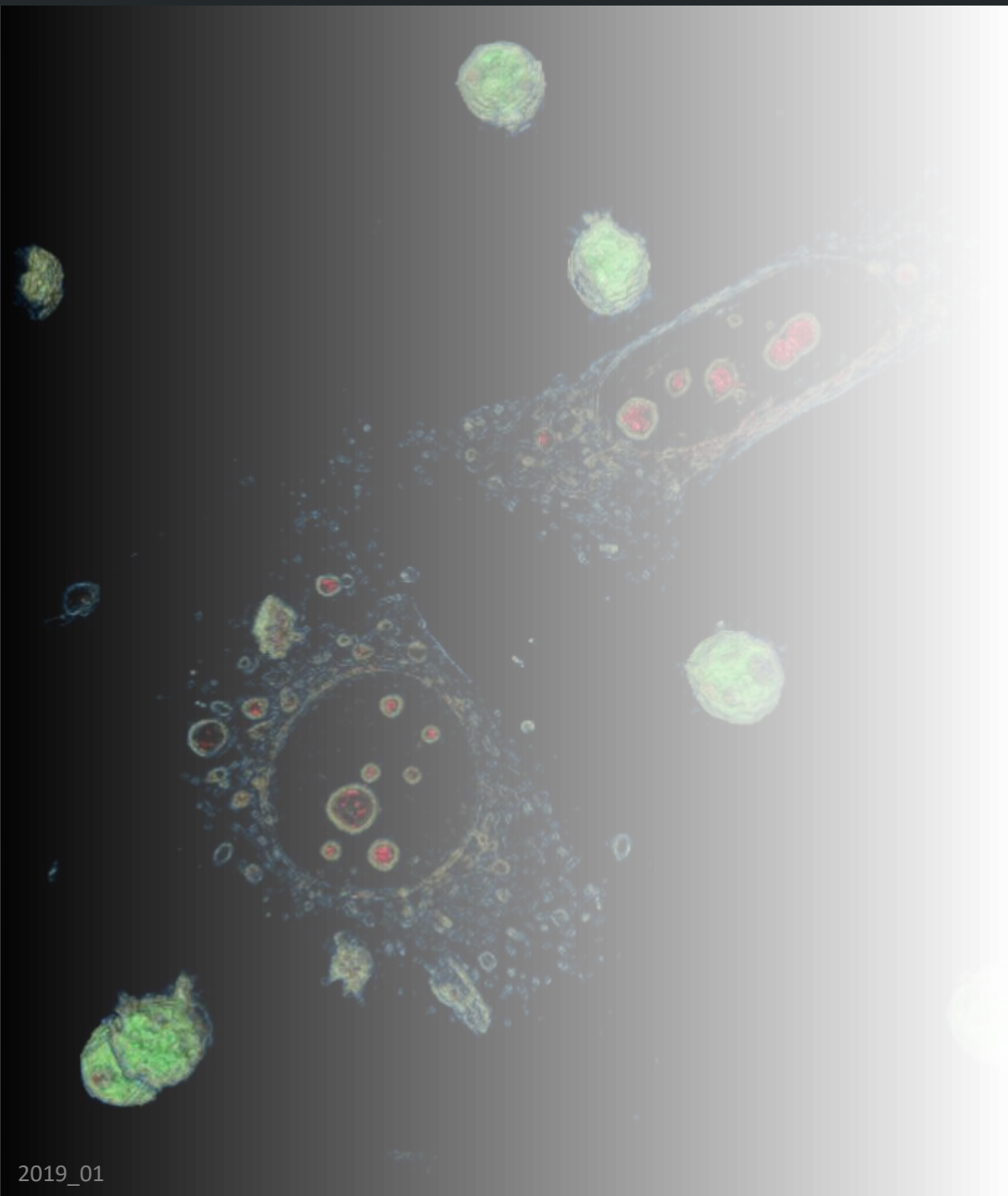


Figure 6: Exporting movies with the STEVE export tool



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Symptom	Probable cause	Remedy
Acoustic alarm sounds	The current temperature values are far temperature set-points	Check the cable connections and if the problem is still present contact Okolab
Bubbling column is not showing bubbles	Not properly inserted tubes	Strongly push the tube into the bubbling column inlet
Data download does not start	USB pendrive is not correctly formatted	Make sure that USB pendrive is FAT32 formatted
	USB pendrive is damaged	Format your USB pendrive using FAT32 file system
Calibration/alarm events are not logged	Internal memory is full (a warning icon in homepage is present)	Erase the full memory. If you need this information, download it before erasing the data
The device does not log the data on the internal memory but only on the USB pendrive	Internal memory is full (a warning icon in homepage is present)	Erase the full memory. If you need this information, download it before erasing the data
The screen does not respond properly or not at all	The display is not calibrated	Press and hold the power button for more than 10 seconds and the touch calibration will start

## Is the digital controller buzzing with a red light during the routine use?

This is probably because the temperature change sensitivity is extremely high (like 0.1C) by default option. To avoid this effect please click **settings second page > alarm > set up** the following parameters:

<b>Time [min]</b>	20
<b>Tolerance [C]</b>	2.0
<b>Transient [min]</b>	30
<b>Enabled</b>	checked
<b>Buzzer</b>	unchecked

Please check the objective heater accessory is correctly enable/disabled.