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#### User Manual REV 02

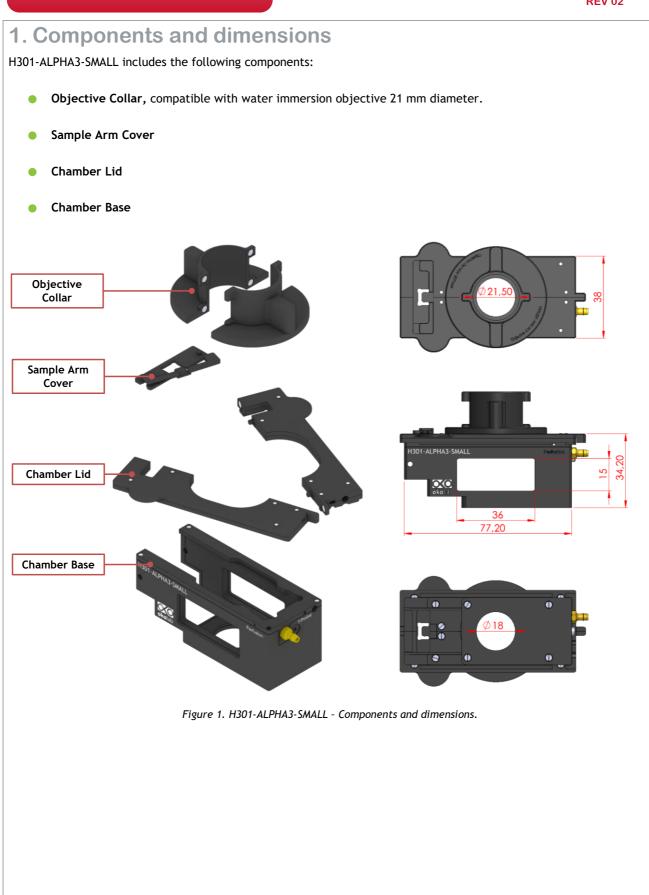
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#### 2. Insertion of the Sample Feedback Temperature Sensor

Insert the Sample Feedback Temperature Sensor through the dedicated opening located in the H301-ALPHA3-SMALL (see Figure 2).

To read the temperature of the culture medium and realize a self-calibration of the chamber, fix the Temperature Sensor tip with adhesive tape on the sample holder inside the H301-ALPHA3-SMALL. For more information about the self-calibration, please refer to the User Manual of the Okolab Controller in use.

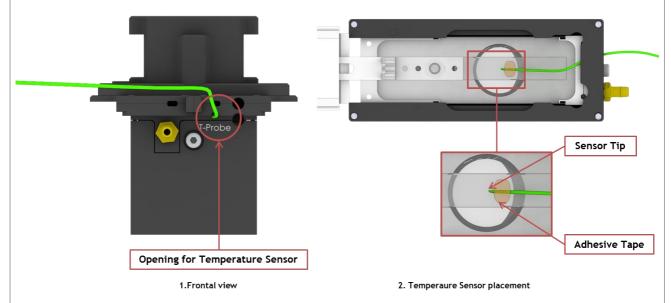


Figure 2. Insertion of the temperature sensor inside the chamber.

#### 3. Connection of the Gas Supply

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A single silicon tubing carries output gas from the Okolab Gas Controller to H301-ALPHA3-SMALL. Silicon tubing connects to a gas input - brass opening - located on a corner of the H301-ALPHA3-SMALL (see Figure 3). Connect by gently pushing silicon tubing onto brass opening.

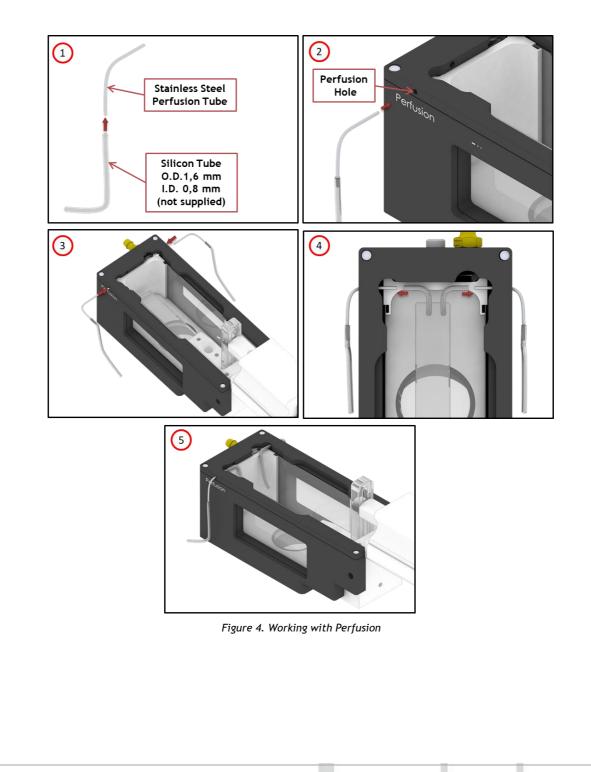


### 4. Working with perfusion

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The Chamber H301-ALPHA3-SMALL features 2 perfusion holes on the sides for the insertion of perfusion tubing up to 1 mm in outer diameter. Follow the instruction below to work with perfusion:

- 1. Use the supplied Stainless Steel Perfusion Tubes. Attach a Silicon tubes O.D. 1,6mm I.D. 0,8mm (not supplied) to the Stainless Steel Perfusion Tubes (see image 1 in Figure 4).
- Insert the Stainless Steel Perfusion Tubes in the perfusion holes (see images 2 and 3 in Figure 4).
  Insert a Silicon tubes O.D. 1,6mm I.D. 0,8mm (not supplied) to the free end of the Stainless Steel Perfusion Tubes (see image 4 and 5 in Figure 4).



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# 5. Installing the H301-ALPHA3-SMALL around the sample holder

To install the H301-ALPHA3-SMALL on the stage, please follow the steps below:

- Insert the chamber base by sliding into place as shown in image 1 and 2 in Figure 5.
  Note: Please pay attention to the sample holder glass.
- 4. Tighten the captive screw M2 to fix the chamber base around the sample holder by using the provided hexagonal key (see image 2 in Figure 5).
- 5. Place the magnetic lid on the chamber base (see image 3 in Figure 5).
- 6. Insert the sample arm cover (see image 4 in Figure 5), and close it (see image 5 in Figure 5). It allows to reduce the brightness into the sample.
- 7. Place the magnetic objective heater in place as shown in image 6 in Figure 5.

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