



## Technical Specifications

Accuracy on sample T	$\pm 0.1^{\circ}\text{C}$ in sample feedback mode
Operation mode	Sample feedback and chamber feedback. Option is selected via Touch Screen
Specimen T range	From ambient T to $50^{\circ}\text{C}$
Temperature Sensors	Chamber base Chamber lid Humidity module (code H.M.1, to be ordered separately) Objective Heater Free Sensor (for Sample feedback operation or self calibration) Room Temperature Sensor
Controlled Devices	Top Stage Chamber: i) base; ii) lid Humidity Module (code H.M.1, to be ordered separately) Objective Heater (code OBJ-T-BL, to be ordered separately)
Voltage	110 (50 Hz) or 220 (60 Hz)
Power	150 W
Dimensions, mm	319 x 230 x 117
Weight, g	3700 g
Compatible with	Any OKOLAB gas controller Objective Heater (code OBJ-T-BL, to be ordered separately) Smart Box (code SM-BL, to be ordered separately)
User interface	OKO-Touch (code OKO-TOUCH, to be ordered separately)
Web Operation / Data Logging	Via Smart Box (code SM-BL, to be ordered separately)
SDK	Available for download through website
Certification	CE



Objective heater



Humidity Module (H.M.1)



Smart box



## Sample and Chamber Feedback Operation Chamber Feedback Self Calibration Objective Heater Self Calibration



**Sample feedback:** a temperature sensor placed inside a reference petri dish provides feedback to the controller. The temperature sensor must be attached to the bottom of a petri dish and covered with water. If the sample is placed in a covered petri, the dish with the temperature sensor should be covered as well. Given the symmetry of the system, sample and reference petri will be at the same temperature. **The sample feedback operation mode ensures a stability of  $\pm 0.1^\circ\text{C}$**

**Chamber feedback:** a temperature sensor embedded into the incubating chamber provides feedback to the controller. In this operation mode, a calibration is needed between the temperature of the chamber and the temperature of the sample. Such a calibration depends on: i) chamber type, ii) room temperature iii) desired set point value. Okolab Top Stage Incubators are calibrated at  $23.0^\circ\text{C}$ , with set point  $37.0^\circ\text{C}$ . Calibration data are stored in the Touch Screen device and are recalled by selecting the chamber and sample holder in use. **If room temperature is different than  $23^\circ\text{C}$  or the desired set point is different from  $37.0$ , the calibration can be updated by performing the Self Calibration Routine.**

**Self-Calibration Routine:** This routine, improves the accuracy of the chamber feedback operation mode by searching for the proper temperature of the Chamber and, if present, of the Objective Heater, to achieve the desired sample set point temperature in the sample, regardless of room temperature. To run the self calibration it is necessary to place a petri with a temperature sensor inside the incubator (as done to operate the incubator in Sample Feedback mode) and to start the routine from the touch screen.

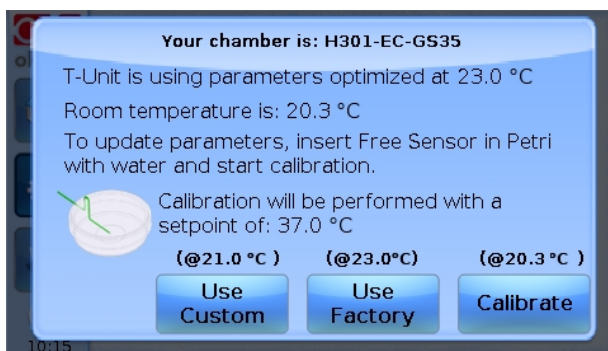
### Chamber Self Calibration

In this step the Self Calibration Routine modifies chamber temperature until the set point value is reached inside the petri with the temperature sensor (i.e. in the sample).

In this step the Objective should not be in oil contact with the Petri.

### Objective Heater Self Calibration

This step can be performed after the calibration of the chamber (if the system is operated in chamber feedback mode) or as the only calibration step (if the system is operated in sample feedback mode.) The objective should be brought into oil contact with the Petri containing the temperature sensor. The routine searches for the proper temperature of the objective heater to keep the sample at set point value.



## Temperature Stability

Temperature stability was assessed, both in sample feedback and chamber feedback mode, by following sample temperature as a function of time for 12 consecutive hours. Room Temperature was allowed to fluctuate in the range 22.60-25.50 °C.

### Equipment Tested

#2 Electric Top Stage Systems H301-EC-BL with specimen holder H301-EC-2x35 in **Sample Feedback mode** and **Chamber Feedback mode**.

### Experimental Protocol

Each Top Stage Incubator was loaded with two plastic 35 mm Petri dish with 2 ml of water. A temperature sensor was attached with adhesive tape in each Petri. One system was operated in **Sample Feedback mode**. One Petri was dedicated to provide feedback to the controller and the other one was considered as the Petri in which the Sample would be placed. The other system was operated in **Chamber Feedback mode** and was loaded as described above. In this case, feedback to the controller was provided by the chamber and both Petri were considered as Samples. To improve accuracy, the system was calibrated by running the Self Calibration Routine.

Room Temperature was controlled with an Air Conditioner set at 24°C and was monitored with a dedicated thermocouple.

### Results

**Temperature Uniformity** between the two neighbouring Petri in each chamber was always better than 0.1°C, regardless of the Feedback mode.

**Temperature accuracy and stability** is reported in Figure 1, where Sample Temperature is plotted as a function of time for the system operated in **Sample Feedback (left axis)** and **Chamber Feedback (right axis)** mode. **Room Temperature**, plotted in Orange, varied in the range 22.60°C - 25.50°C.

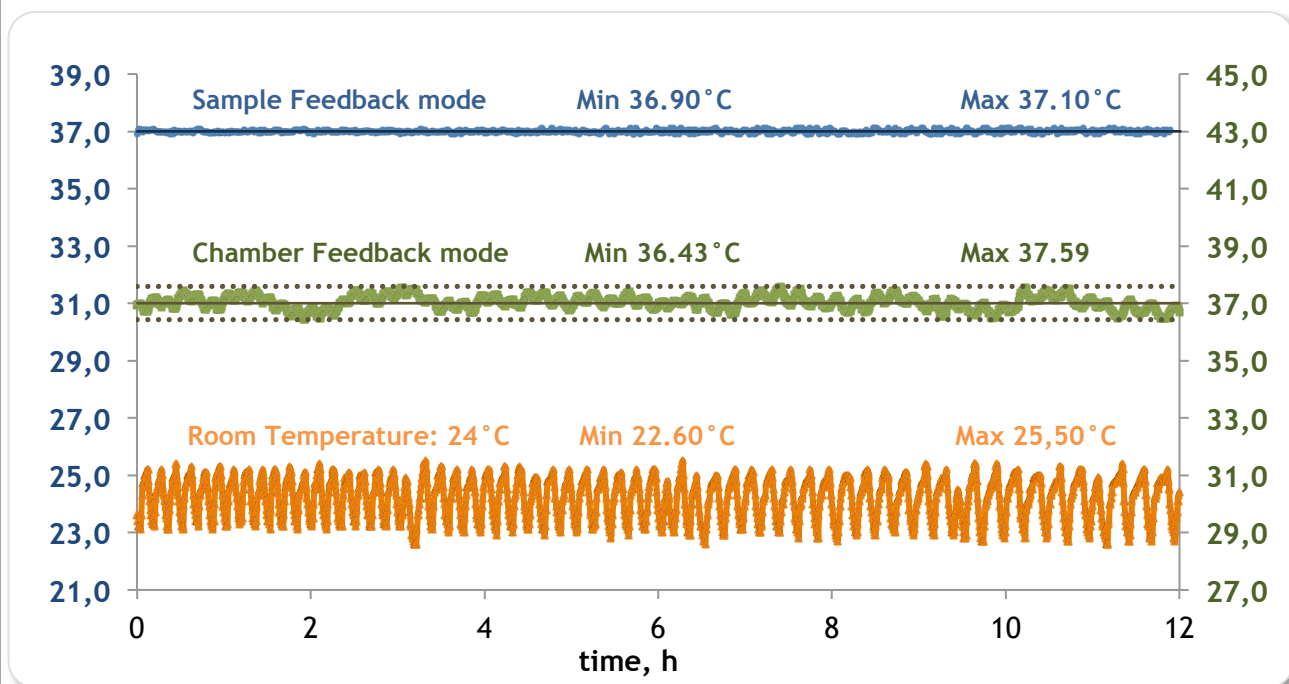


Figure 1 -Sample Temperature Stability Assessment. **Sample Feedback** and **Chamber Feedback** mode.

### Conclusions on Temperature Stability

In a room with temperature fluctuations of  $\pm 1.5$  °C the systems showed the following Sample Temperature stability:

Specimen Feedback mode:  $37.0 \pm 0.1$  °C  
 Chamber Feedback mode:  $37.0 \pm 0.5$  °C

## Objective Heater – Effect of Sample Temperature

### Equipment Tested

Top Stage: **H301-EC-BL** in specimen feedback mode  
 Specimen Holder: **H301-EC-2x35**  
 Objective Heater: **OBJ-H-BL** mounted on Objective Zeiss Neofluar 100/1.30 Oel  
 Petri 1 and 2: Willco Glass Bottom Dish, 0,17mm,  $\phi$  22mm

### Experimental Protocol

As schematized in Figure 2, the incubator was loaded with two Petri dish, each one equipped with a T sensor attached on the 0.17mm glass and loaded with 2 ml of water. Petri 1 was used to provide feedback to the T controller (operating in Sample Feedback mode) and Petri 2 was used to follow Sample Temperature profile as a consequence of the contact with the oil immersion objective

The experimental protocol was as it follows:

- Step 1:** Objective not in contact with Petri 2; Objective Heater Off.
- Step 2:** Objective in oil contact with Petri 2; Objective Heater Off.
- Step 3:** Objective in oil contact with Petri 2; Objective Heater on, Offset value 5.3 °C.

During the self calibration routine, the offset value of the objective heater is progressively increased, until T2 reaches the desired set point temperature of 37.0 °C.

Figure 3 reports the profile of T1, T2, and of Objective Heater Temperature during the experimental campaign described above.

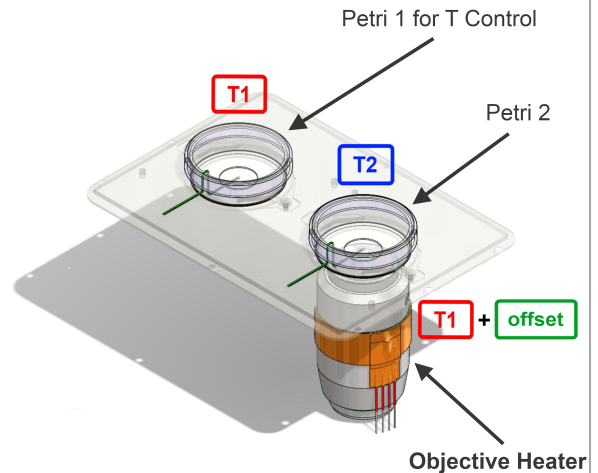


Figure 2 -Schematic view of Petri in Top Stage Incubator

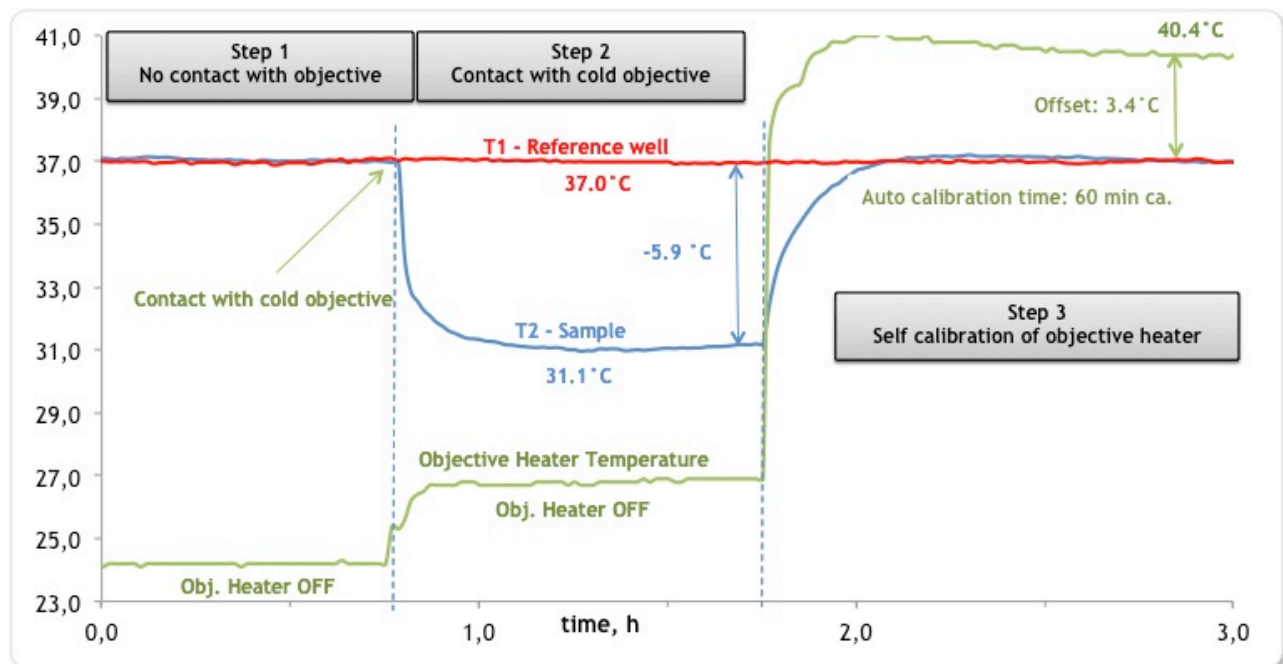


Figure 3 -Sample Temperature Profile during oil contact with a cold objective. Effect of the Objective Heater.

### Conclusions

- As a consequence of contact with the cold objective. **Sample Temperature drops to 31 °C, ca.**
- **The objective heater allows rise Sample Temperature back to 37.0 °C**