### User Manual June 2016



### H201-LUDL 96A602

Compatible with the following Z piezo stage

Compatible with the following Okolab Controllers

Ludl 96A602

CO2-UNIT-BL

- CO2-UNIT-BL-CP2
- CO2-O2-UNIT-BL-CP2
- 02-UNIT-BL [1-20]
- CO2-O2-UNIT-BL [0-10;1-18]
- CO2-O2-UNIT-BL [0-20;1-95]
- CO2-O2-UNIT-BL [0-10;0-1]
- 2GF-MIXER
- 3GF-MIXER-HYPOXIA
- 3GF-MIXER-HYPEROXIA

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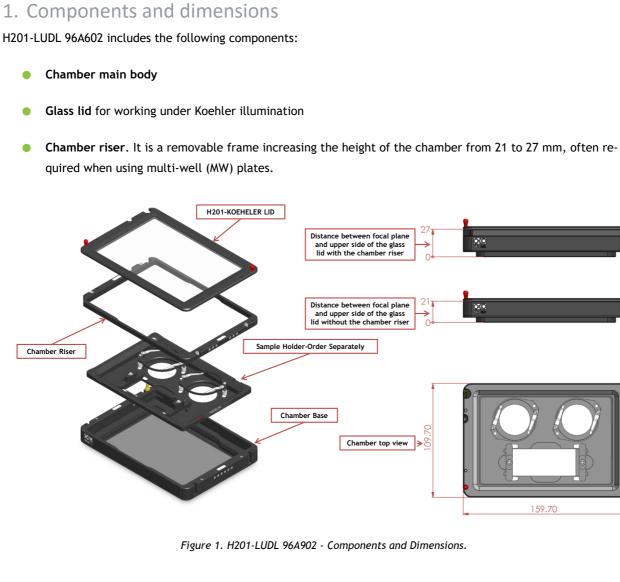
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### 2. Available Sample Holders

The following sample holders are available.

**NOTE:** Please contact <u>info@oko-lab.com</u> if you cannot find the sample holder you are looking for. We are constantly adding new inserts to the list.

1x35-M	#1 35mm Petri-dish
1xGS-M	#1 1x3in. chamber slide
1xLABTEK-M	#1 Lab-Tek 1in.x2in. chambered cover glass
1xLABTEK-II-M	#1 Lab-Tek II 1in.x2in. chambered cover glass
1x60-M	#1 60mm Petri-dish
1xT25-M	#1 Nunc and Greiner T25 flask
2x35-M	#2 35mm Petri-dish
2xGS-M	#2 1x3in. chamber slides
2xLABTEK-M	#2 Lab-Tek 1in.x2in. chambered cover glass
2xLABTEK-II-M	#2 Lab-Tek II 1in.x2in. chambered cover glass
2x60-M	#2 60mm Petri-dish



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4x35-M	#4 35mm Petri-dish
GS35-M	#1 1x3in. chamber slide and #2 35mm Petri-dish
LABTEK-35-M	#1 Lab-Tek 1in.x2in. chambered cover glass and #2 35mm Petri-dish
LABTEK-II-35-M	#1 Lab-Tek II 1in.x2in. chambered cover glass and #2 35mm Petri-dish
LABTEK-60-M	#1 Lab-Tek 1in.x2in. chambered cover glass and #1 60mm Petri-dish
LABTEK-II-60-M	#1 Lab-Tek II 1in.x2in. chambered cover glass and #1 60mm Petri-dish
GS60-M	#1 1x3in. chamber slide and #1 60mm Petri-dish
6035-M	#1 60mm Petri-dish and #1 35mm Petri-dish
H201-MW-HOLDER	Multiwell plates holder



For #1 1"x3"chamber slide and #2 35mm petri dishes



For #1 35 mm

petri dish



For #2 35 mm petri dishes and #1 60mm petri dish



For #1 60mm petri dish and #1 1"x3"chamber slide For #2 60 mm petri dishes



For #1 1"x3"chamber slide and #2 Lab-Tek 1"x2" chambered cover glass ered cover glass

For #1 60 mm petri dish



1"x2"chambered ver glass

For #1 Lab-Tek 1"x2"chambered cover glass and #1 60mm petri dish

For #1 Lab-Tek 1"x2" Chambered cover glass



For #1 Lab-Tek 1"x2" Chambered cover glass and #2 35mm petri dishes and #1 60mm petri dish



For #1 Lab-Tek 1"x2" Chambered cover glass



cover glass

For #2 1"x3"

chamber slides

I ARTEK-II-35-M For #1 Lab-Tek II and #2 35mm petri dishes

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2xLABTEK-II-M For #2 Lab-Tek 1"x2"chambered cover glass 1"x2"chambered cover glass



Figure 2. Available sample holders.

### 3. Available Lids

The following heated glass Lids are available for H201-LUDL 96A902:

- H201-KOEHLER-LID: For imaging under Koehler illumination-Included in code H201-LUDL 96A902
- H201-INJECTION-LID\*: Heated glass lid with two small openings (sealed with flexible plastic) allowing injec-tion or permanent access to the sample. Compatible only with sample holder 1x35
- H201-LASER-INTERLOCK-LID\*: Heated glass lid with safety switch. Connects to laser controller and automatically turns laser off when lid is lifted. Compatible only with sample holder 1x35

\* OPTIONAL - not included with H201-LUDL 96A602



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#### 3.1 Koehler Lid

The Koehler Lid is a heated glass lid and allows imaging under Koehler illumination. The Koehler Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 3 shows chamber dimensions with Koehler lid (with and without chamber riser).

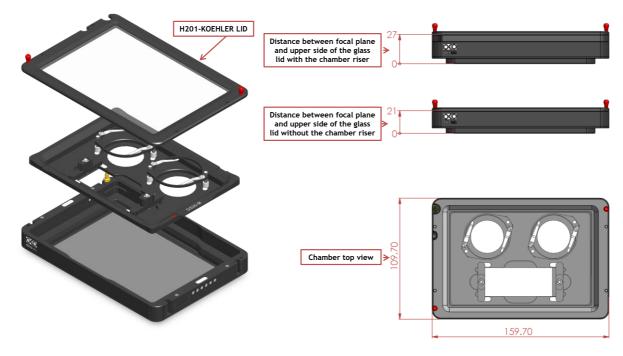


Figure 3.H201-LUDL 96A902+ H201-KOEHLER-LID

#### 3.2 Injection Lid

The Injection Lid is a heated glass lid with two small openings sealed with flexible plastic allowing injection or permanent access to the sample. NOTE: Compatible with sample holder 1x35 ONLY. The Injection Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 4 shows chamber dimensions with the Injection Lid (with and without chamber riser).

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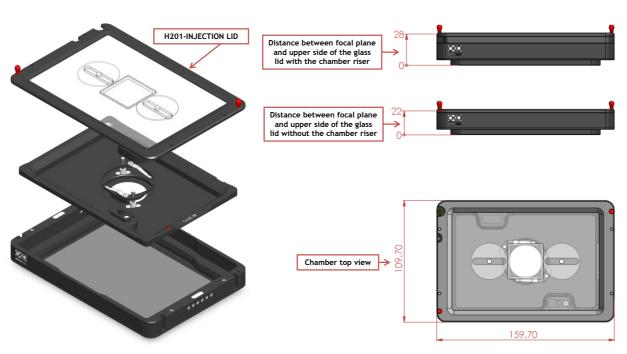
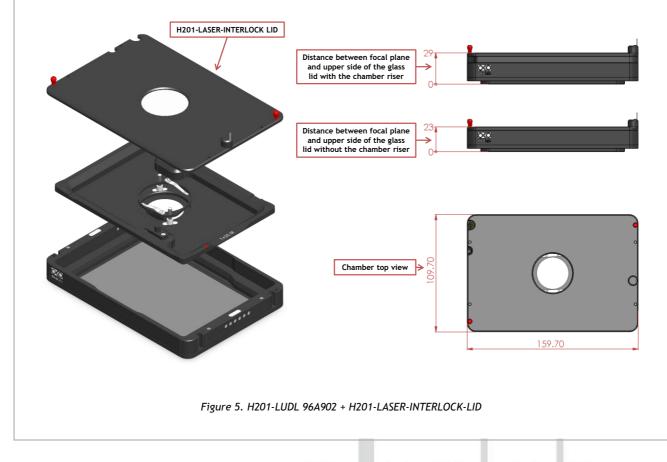


Figure 4. H201-LUDL-96A902 + H201-INJECTION-LID

#### 3.3 Laser Interlock Lid

The Laser Interlock Lid is a heated glass lid with a safety switch connecting to the laser controller. It automatically turns the laser off when the lid is lifted. NOTE: <u>Compatible with sample holder 1x35 ONLY</u>. The Laser Interlock Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 5 shows chamber dimensions with the Laser Interlock Lid (with and without chamber riser).



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

Insert the Sample Feedback Temperature Sensor through the dedicated opening located in the H201-LUDL 96A602 (see Figure 6, Frontal and 3D views).

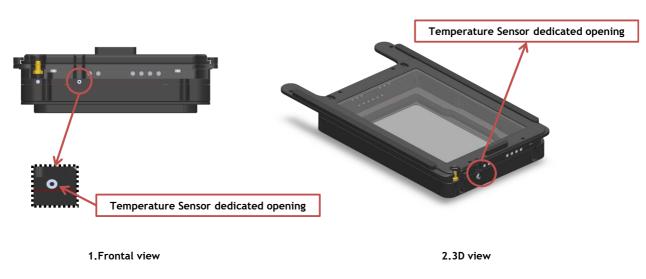


Figure 6. Insertion of the temperature sensor inside the chamber.

### 5. Insertion of Sample Holder into Chamber

Sample holders fit into the chamber base and are held in place by magnets embedded within both chamber and holder. To introduce a sample holder with the proper orientation, match the red dot on the holder to the one on the chamber base, as illustrated in Figure 7.



Figure 7. Introduction of the Specimen Holder inside the Chamber Base.

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# 6. Working with 35 or 60 mm Petri Dish – Spacing Rings and Magnetic Locks

Magnetic locks prevent movement of 35 and 60 mm dishes inside the sample holder.

Figure 8 illustrates the available magnetic locks for 35 and 60 mm dishes. Threaded magnetic posts allow adjusting holder's height.

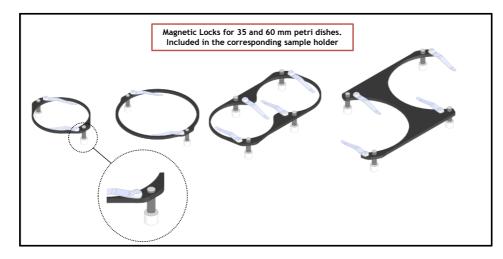


Figure 8. Magnetic locks for 35 and 60 mm dish

**NOTE:** Magnetic locks are included with sample holder.

Spacing rings accounting for variability in dish diameter from one manufacturer to another are also included. Figure 9 shows how to position the ring. Ring selection guide diagrams: rings for 35 and 60 mm dish -Figure 10 and Figure 11, respectively.

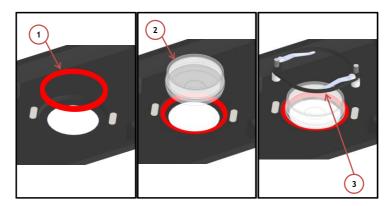
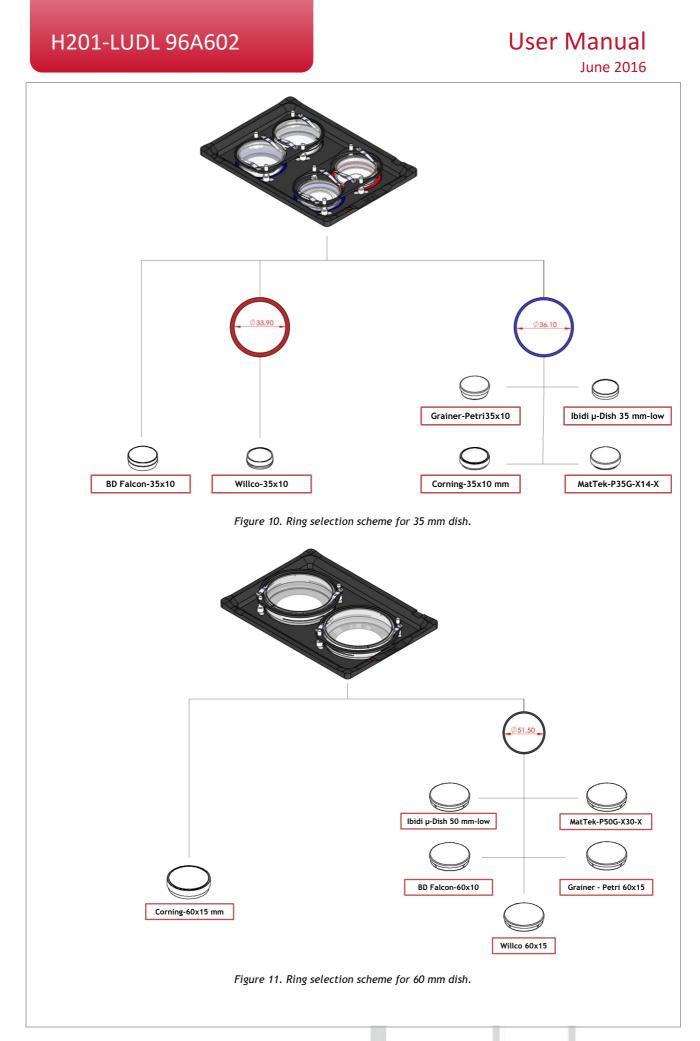


Figure 9. 1) Insertion of the ring, 2) insertion of the 35 mm dish, 3) insertion of the magnetic lock.

**NOTE:** Spacing rings are included with sample holder.

**NOTE:** Spacing rings do not block the petri inside its accommodation, but they are designed to center the petri with respect to the observation circular window.

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### 7. Working with 1x3" and 1x2" chamber slides - magnetic locks

Magnetic locks prevent movement of 1''x 3'' and 1''x 2'' chamber slides inside of the sample holder.

**NOTE:** Magnetic locks are included with sample holder.

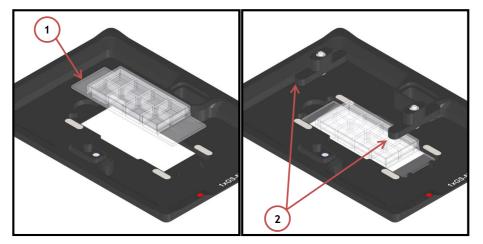


Figure 12. Magnetic lock for 1" x 3" chamber slide.

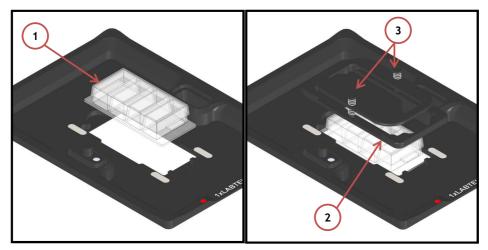


Figure 13. Magnetic lock for 1" x 2" chamber slide. To lock the slide, push simultaneously the buttons indicated with (3).



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8. Working with MW Plates - Magnetic locks and Chamber riser

Magnetic locks hold MW plates in place into sample holder illustrated in Figure 14

NOTE: MW plates holder must be ordered separately (NOT INCLUDED). Product code: H201-MW-HOLDER

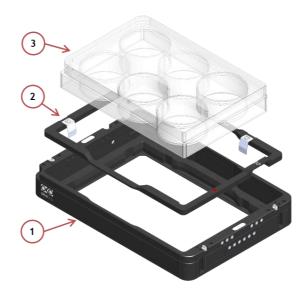


Figure 14. Magnetic locks for MW plates. To be ordered separately.

Chamber Riser increases chamber's height and is REQUIRED with 6, 12, 24, 48 MW plates, regardless of the Lid employed.

Figure 15 shows how to mount and screw the chamber riser. Screws location is indicated by letter A.

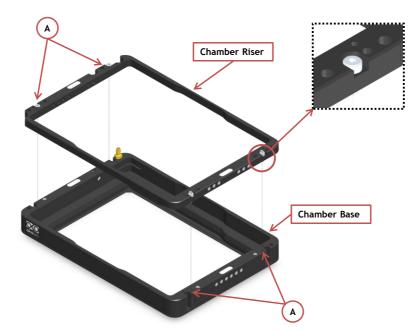


Figure 15. Riser Assembly.

### 9. Connection of the Gas Supply

A single silicon tubing carries output gas from the Okolab Gas Controller to the H201-LUDL 96A9602. Silicon tubing connects to a gas input - brass opening - located on a corner of the H201-LUDL 96A902. See Figure 16. Connect by gently pushing silicon tubing onto brass opening.

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Figure 16. Connection with gas supply.

### 10. Working with Perfusion

The Chamber Riser included with H201-LUDL 96A9602 features 12 perfusion holes for the insertion of perfusion tubing up to 2.5 mm in outer diameter. To lock the Chamber Riser into the base use a 1.5mm Allen key to secure the 4 GUTB2-2,5-6 screws as shown in Figure 17.

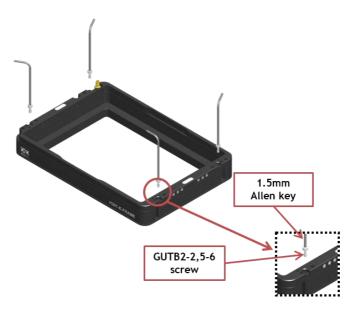
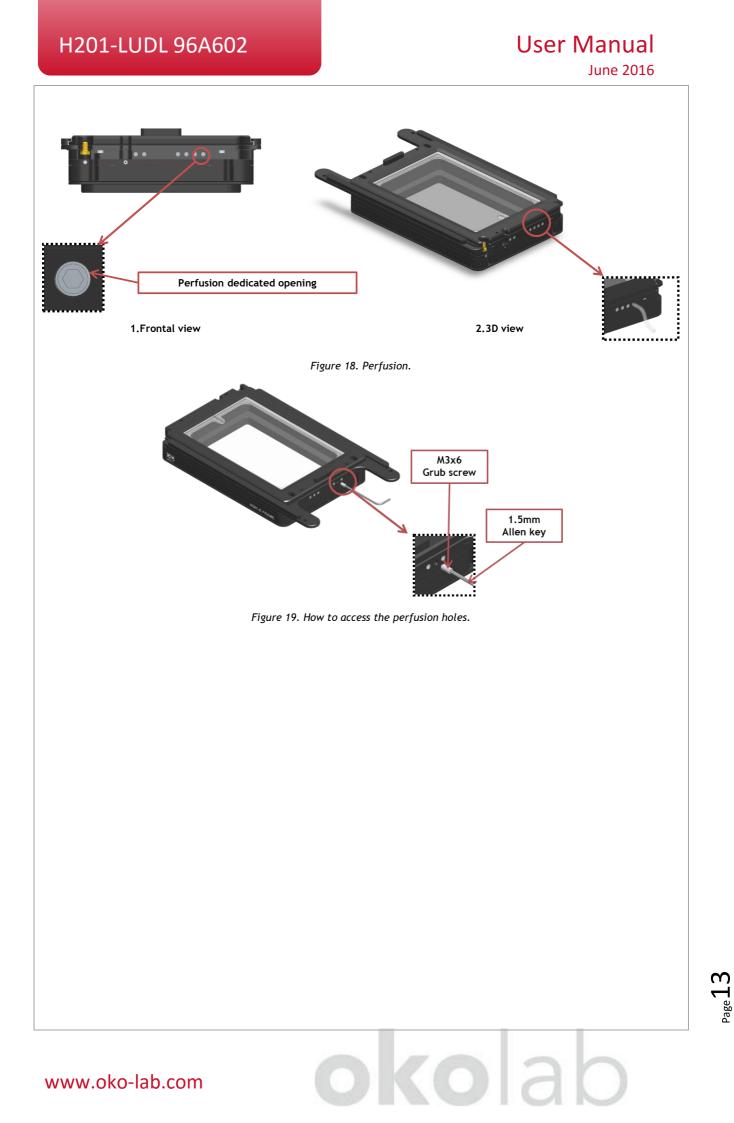


Figure 17. How to lock the Chamber Riser.

Small screws plug the perfusion holes when not in use. (Grub screws M3x6). <u>Remove small screws</u> with a 1.5 mm Allen key as necessary before introducing perfusion tubing.

Figure 18 shows location of perfusion holes.



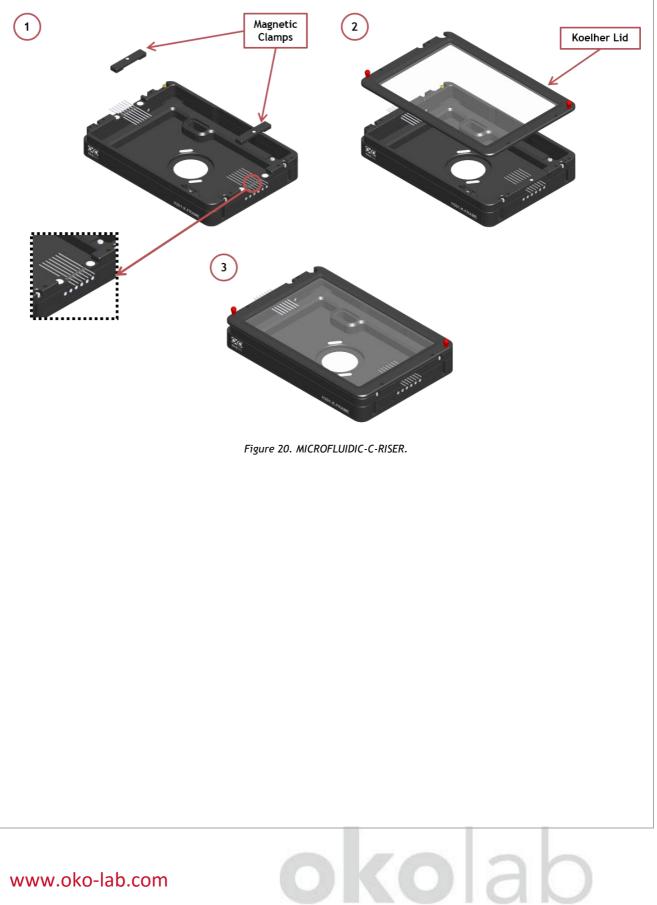


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#### MICROFLUIDIC-C-RISER (Optional)

The MICROFLUIDIC-C-RISER is a chamber riser with 19x3 mm windows (on the two short sides) to allow insertion of microfluidic tubings. Windows are magnetically closed. Compatible with most of H201 and H301 Okolab chambers. Thickness of this chamber riser is 8mm (2mm more than default chamber riser). Requires Koehler lid and single accommodation specimen holders.



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### 11. Connection of the Chamber with Z piezo stage

H201-LUDL 96A602 fits on the Z piezo Stage without any screws. To fix the chamber on the stage, follow the steps shown in Figure 21.

- 1. Place the chamber on the stage (See Image 1 of Figure 21).
- 2. Incline the chamber and slide it in direction of the springs until it snaps into the housing. (See Image 2 of Figure 21).
- 3. Image 3 of Figure 21 shows the chamber fitted on the Z piezo Stage.

