H201-K-FRAME

Compatible with the following XY stages

- ASI: all models, including z-piezo
- Leica: all models with 160x110 opening
- Ludl: all models with 160x110 opening
- Marzhauser: all models
- Olympus: all models with 160x110 opening
- Prior H107
- Zeiss: all models with 160x110 opening

Compatible with the following Okolab gas modules

- CO2-UNIT-BL
- CO2-UNIT-BL-CP2
- CO2-O2-UNIT-BL-CP2
- O2-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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1. Components and dimensions

H201-K-FRAME includes the following components:

- **Chamber main body**

- **Sliding glass lid** for easy pipetting

- **Chamber riser**. It is a removable frame increasing the height of the chamber from 24 to 30 mm, often required when using multi-well (MW) plates
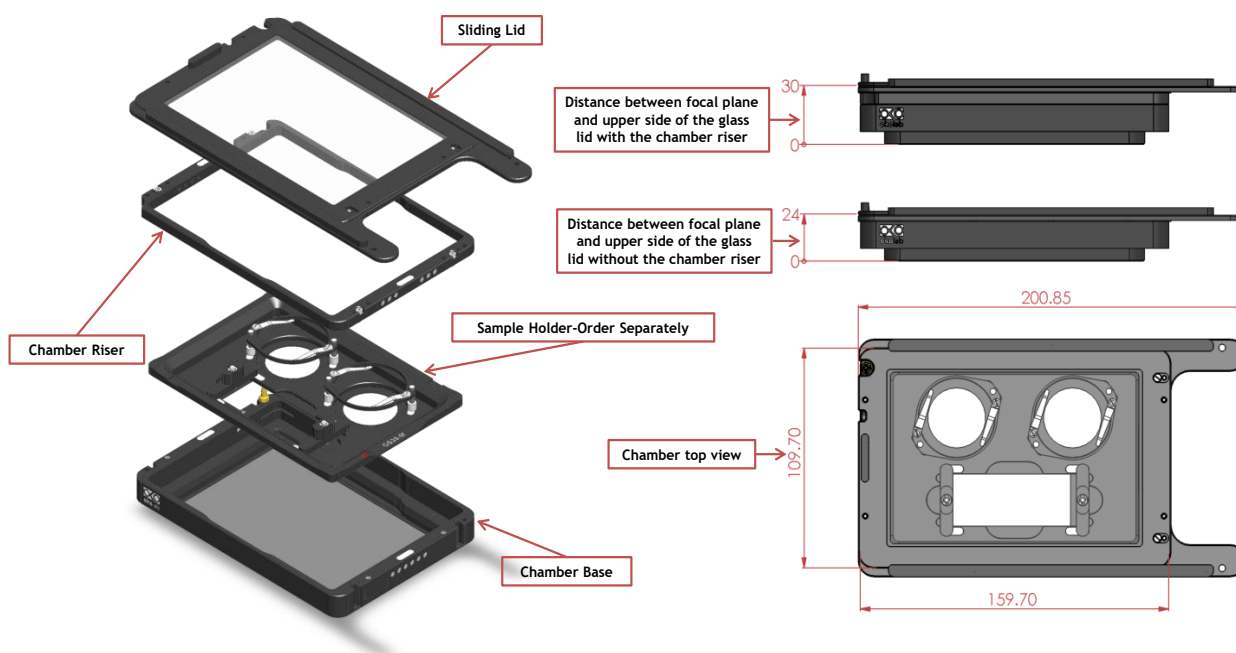

Figure 1. H201-K-FRAME - Components and Dimensions.

2. Available Sample Holders

The following sample holders are available.

NOTE: Please contact info@oko-lab.com 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
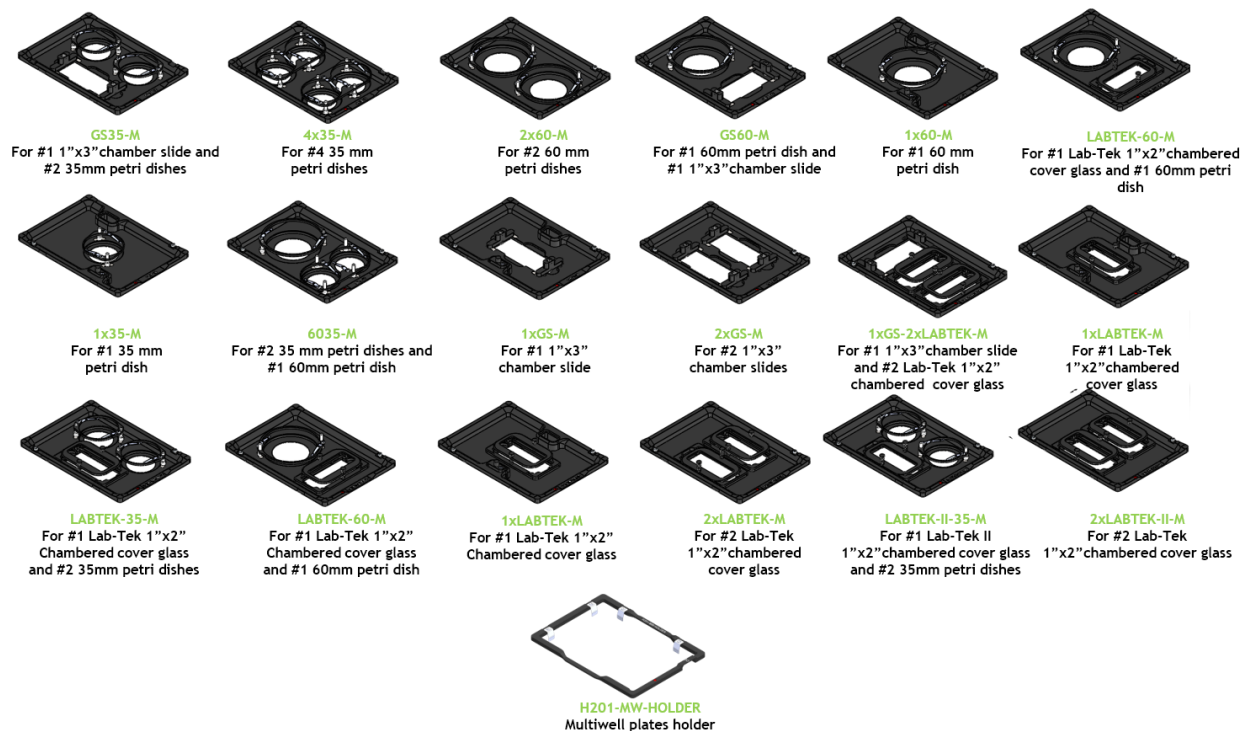
Figure 2.Available sample holders.

3. Available Lids

The following heated glass Lids are available for H201-K-FRAME:

- **H201-SLIDING-LID**: For easy sample loading and pipetting – **Included in code H201-K-FRAME**

- **H201-KOEHLER-LID***: Reduces chamber height to 21 mm and allows imaging under Koehler illumination

- **H201-INJECTION-LID***: Glass lid with two small openings (sealed with flexible plastic) allowing injection or permanent access to the sample. Compatible only with sample holder 1x35

- **H201-LASER-INTERLOCK-LID***: 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-K-FRAME

3.1 Sliding lid

The Sliding Lid is a glass lid allowing for easy sample loading and pipetting. The Sliding Lid is screwed onto the chamber.

Figure 3 shows chamber dimensions with the Sliding Lid (with and without chamber riser). Figure 4 illustrates how to remove Sliding Lid, when a different Lid is necessary.

NOTE: Sliding Lid MUST BE REMOVED when using any other Lid. The Sliding Lid is fixed onto the chamber with 4 screws. Screws location is indicated by letter A in Figure 4 (image 2 and 3). Keep Sliding Lid fully closed to access screws labeled A in image 2. Open Sliding Lid to access screws labeled A in image 3.
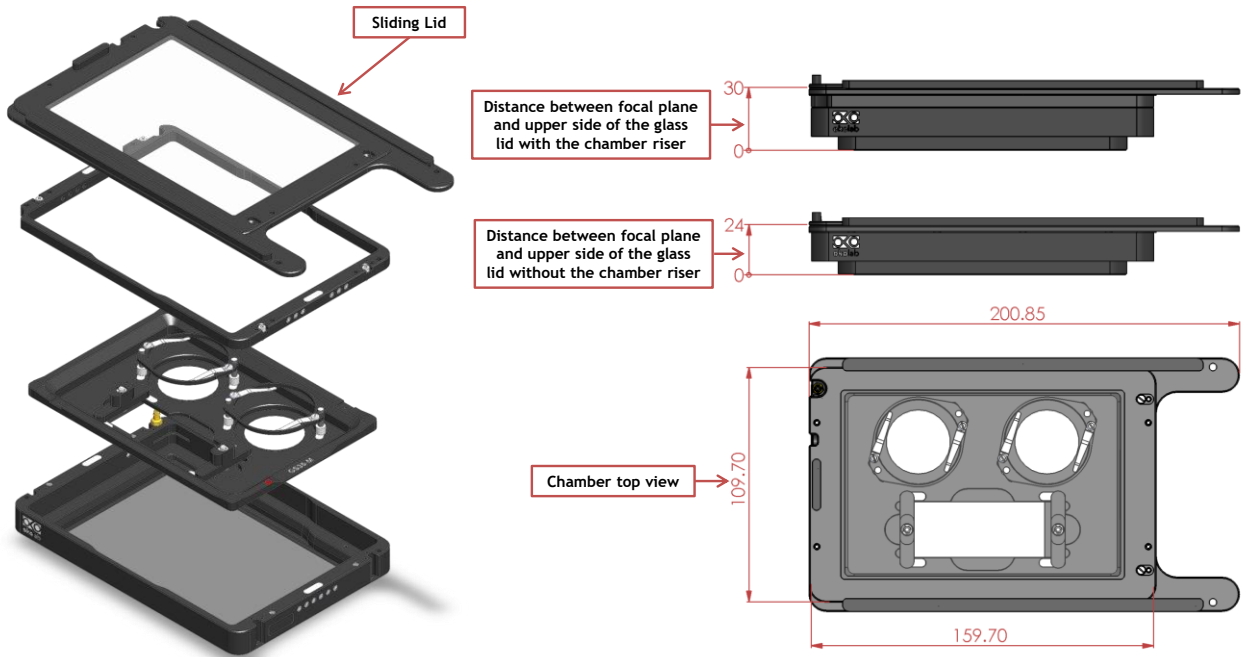

Figure 3. H201-K-FRAME (Sliding Lid comes as standard)

Figure 4. Assembly of the sliding lid

3.2 Koehler Lid

The Koehler Lid is a glass lid reducing the chamber's height to 21 mm and allowing imaging under Koehler illumination.

The Koehler Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

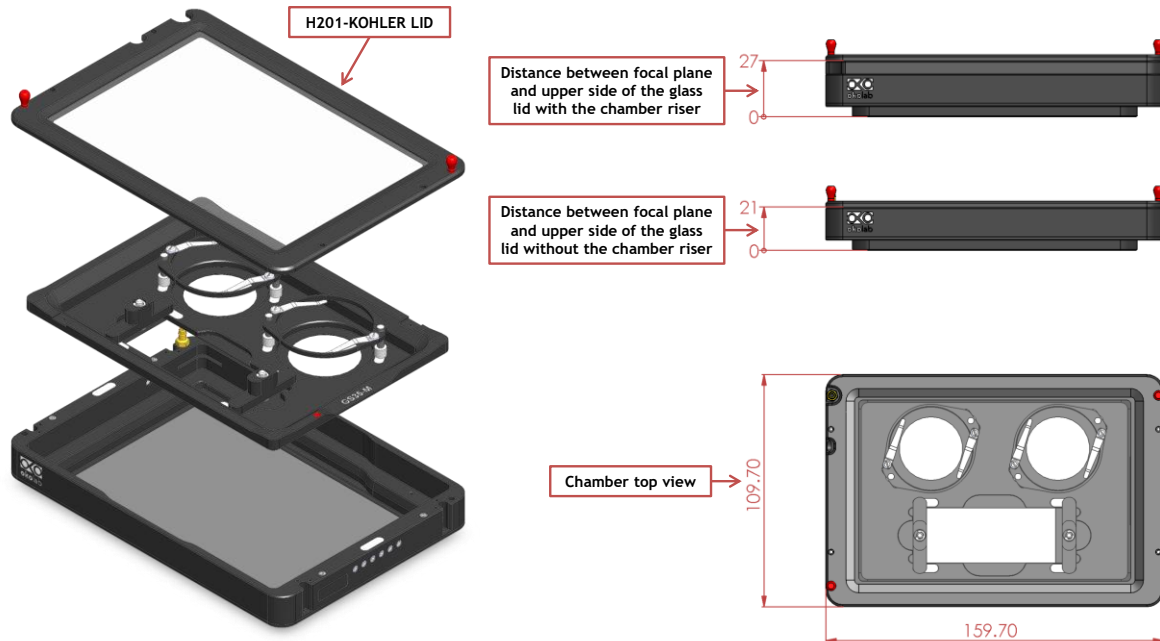Figure 5 shows chamber dimensions with Koehler lid (with and without chamber riser).

Figure 5.H201-K-FRAME + H201-KOEHLER-LID

3.3 Injection Lid

The Injection Lid is a 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).

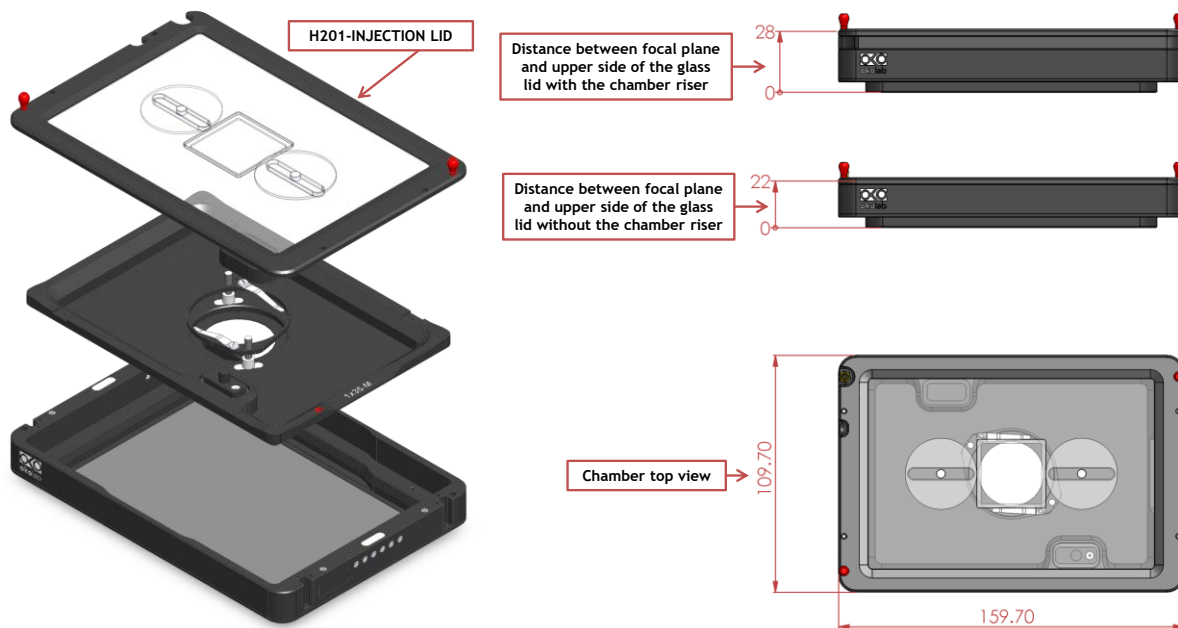Figure 6 shows chamber dimensions with the Injection Lid (with and without chamber riser).

H201-INJECTION LID

Distance between focal plane and upper side of the glass lid with the chamber riser — 28 / 0

Distance between focal plane and upper side of the glass lid without the chamber riser — 22 / 0

Chamber top view

109.70

159.70

Figure 6. H201-K-FRAME + H201-INJECTION-LID

3.4 Laser Interlock Lid

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

Figure 7 shows chamber dimensions with the Laser Interlock Lid (with and without chamber riser).
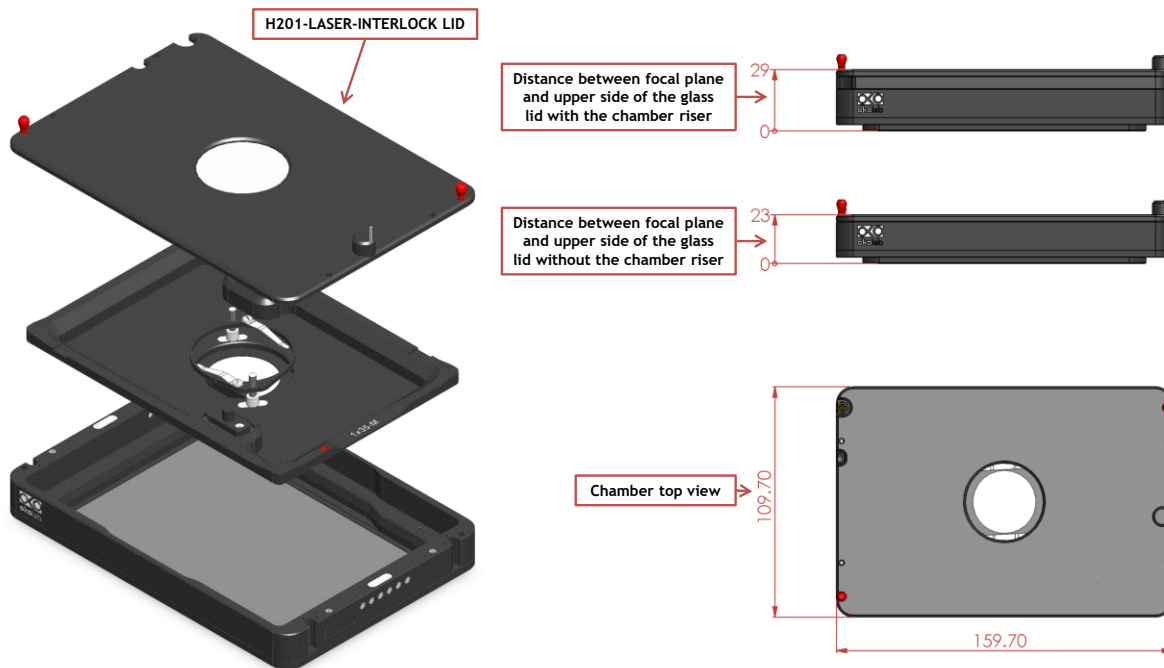

H201-LASER-INTERLOCK LID

Distance between focal plane and upper side of the glass lid with the chamber riser — 29 / 0

Distance between focal plane and upper side of the glass lid without the chamber riser — 23 / 0

Chamber top view

109.70

159.70

Figure 7. H201-K-FRAME + H201-LASER-INTERLOCK-LID

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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-K-FRAME (see Figure 8, Frontal and 3D views).
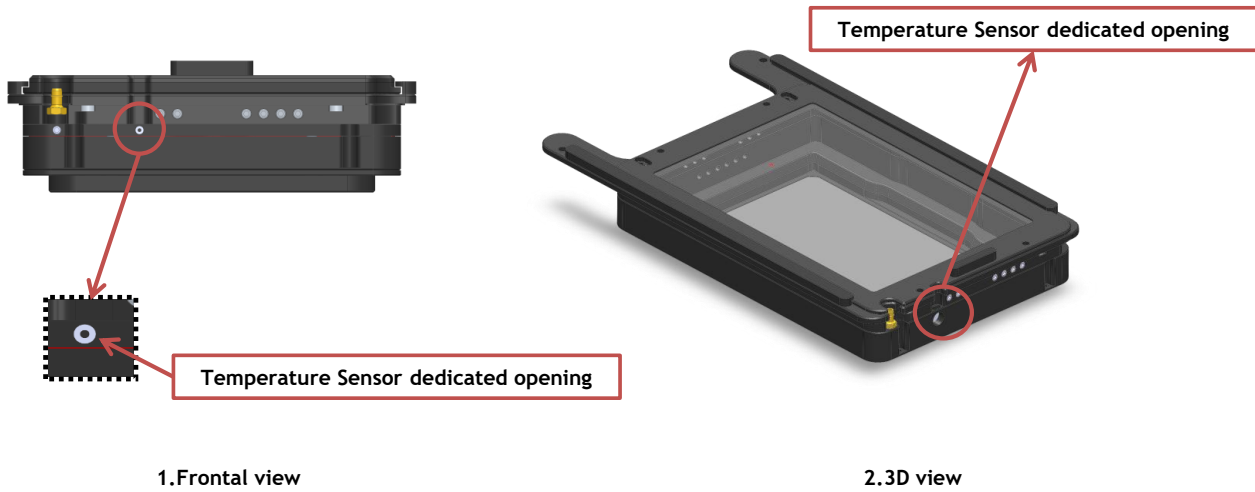

Temperature Sensor dedicated opening

Temperature Sensor dedicated opening

1.Frontal view   **2.3D view**

Figure 8. 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 9.

Insert the adapter by superimposing the red points
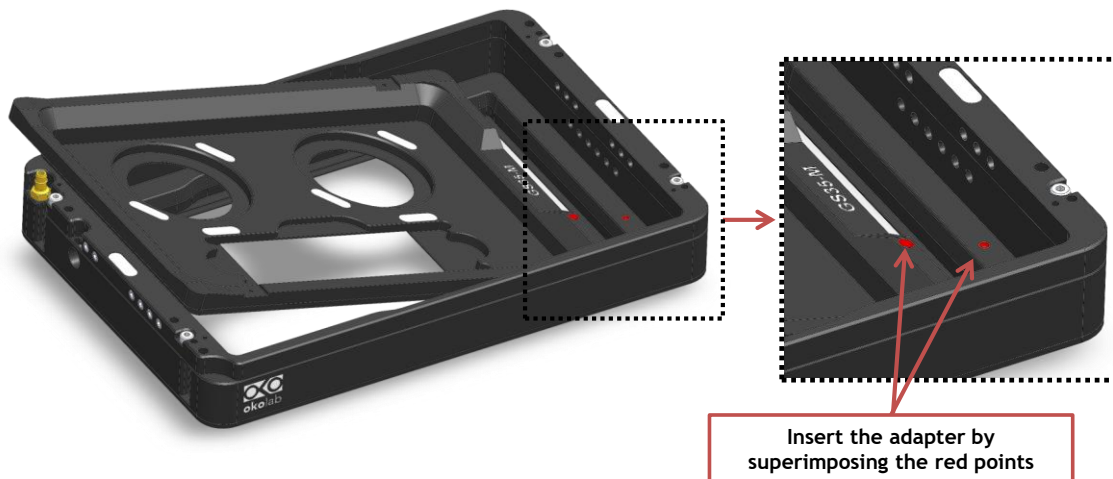
Figure 9. Introduction of the Specimen Holder inside the Chamber Base.

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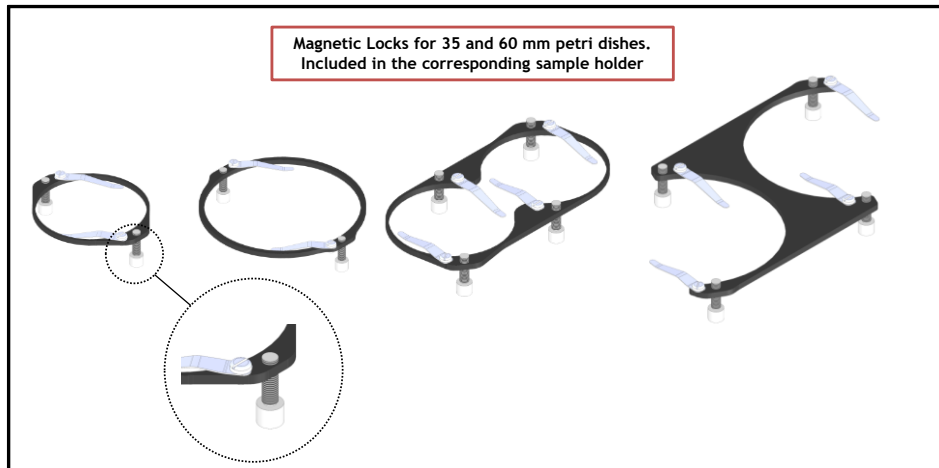

Figure 10.Magnetic locks for 35 and 60 mm dish

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 11 shows how to position the ring. Ring selection guide diagrams: rings for 35 and 60 mm dish -Figure 12 and Figure 13, respectively.
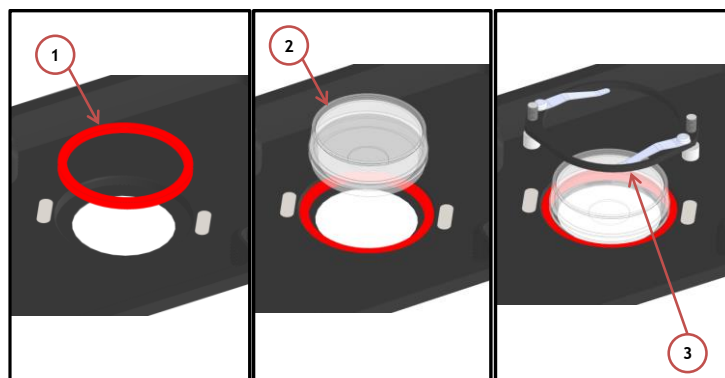
Figure 11. 1) Insertion of the ring, 2) insertion of the 35 mm dish, 3) insertion of the magnetic lock.

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.
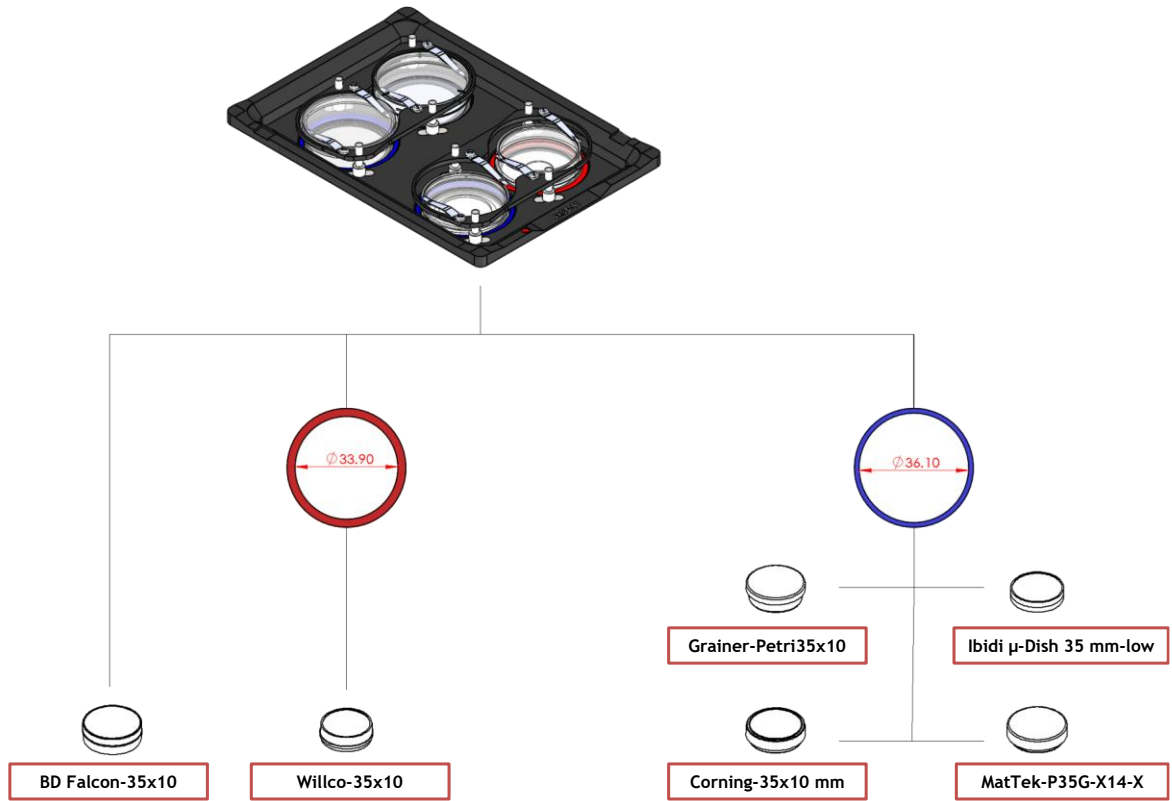
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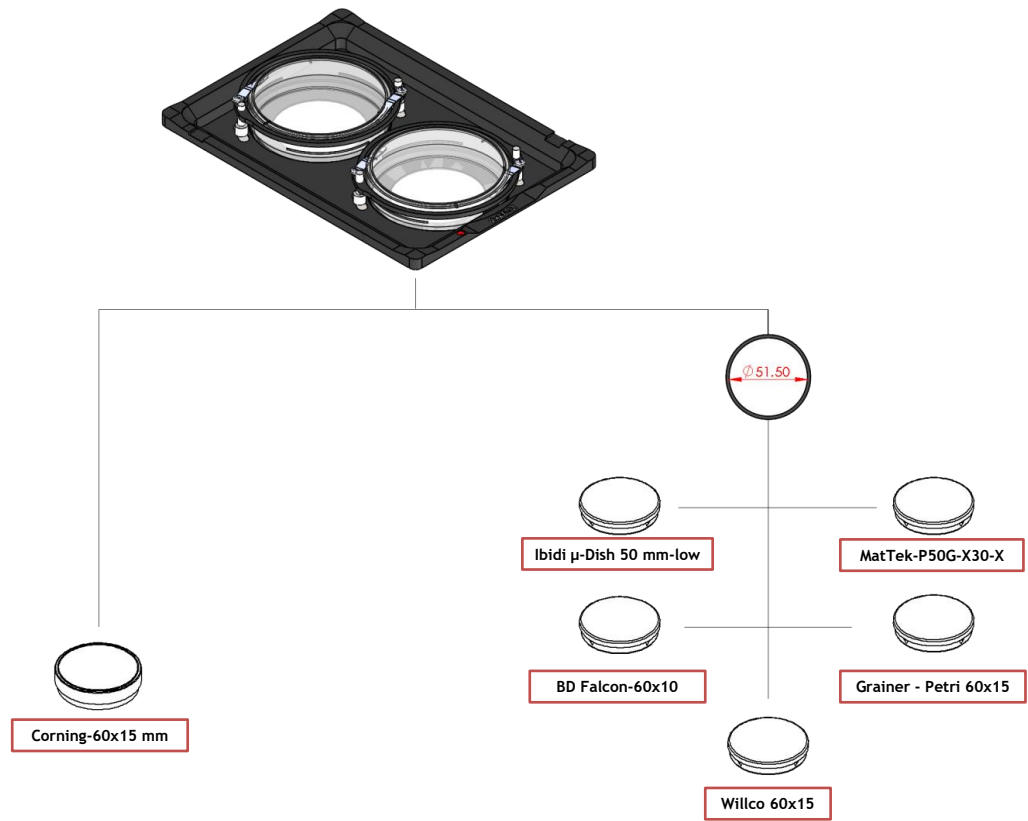*Figure 12. Ring selection scheme for 35 mm dish.*

Figure 13. Ring selection scheme for 60 mm dish.

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.

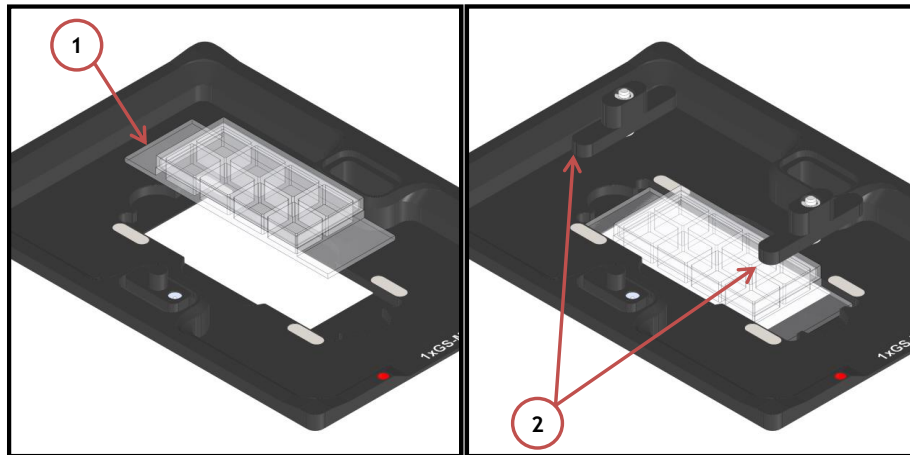**NOTE:** Magnetic locks are included with sample holder.

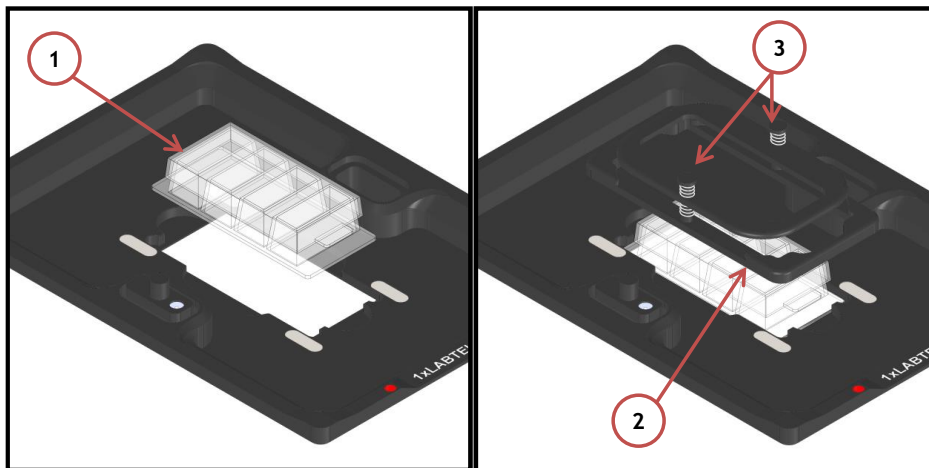*Figure 14. Magnetic lock for 1" x 3" chamber slide.*

Figure 15. Magnetic lock for 1" x 2" chamber slide. To lock the slide, push simultaneously the buttons indicated with (3).

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

Magnetic locks hold MW plates in place into chamber illustrated in Figure 16

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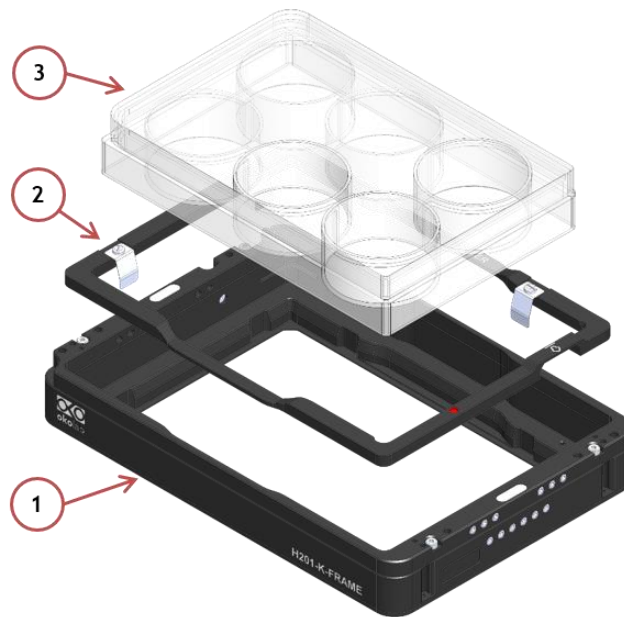

Figure 16. Magnetic locks for MW plates. To be ordered separately.

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

Figure 17 shows how to mount and screw the chamber riser. Screws location is indicated by letter A.
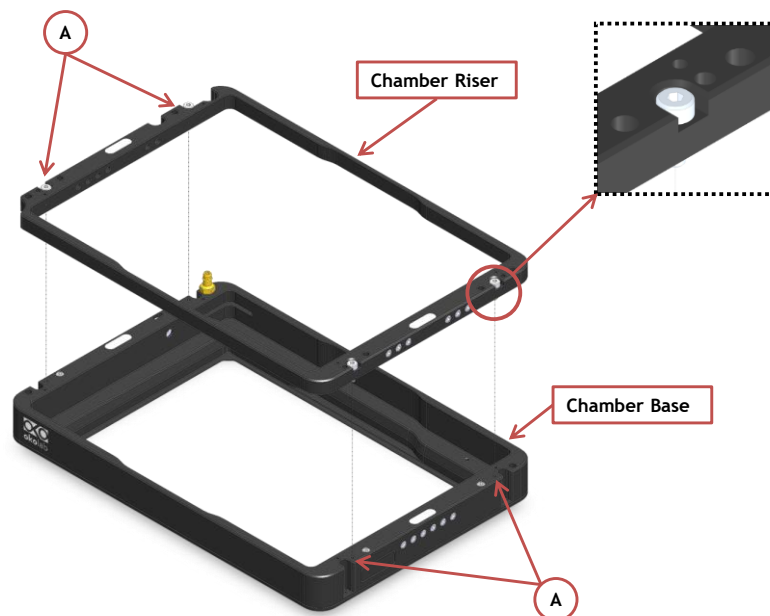

Figure 17. Riser Assembly.

9. Connection of the Gas Supply

A single silicon tubing carries output gas from the Okolab Gas Controller to the H201-K-FRAME. Silicon tubing connects to a gas input - brass opening - located on a corner of the H201-K-FRAME. See Figure 18. Connect by gently pushing silicon tubing onto brass opening.

Figure 18.Connection with gas supply.

10. Working with Perfusion

The Chamber Riser included with H201-K-FRAME 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 19.
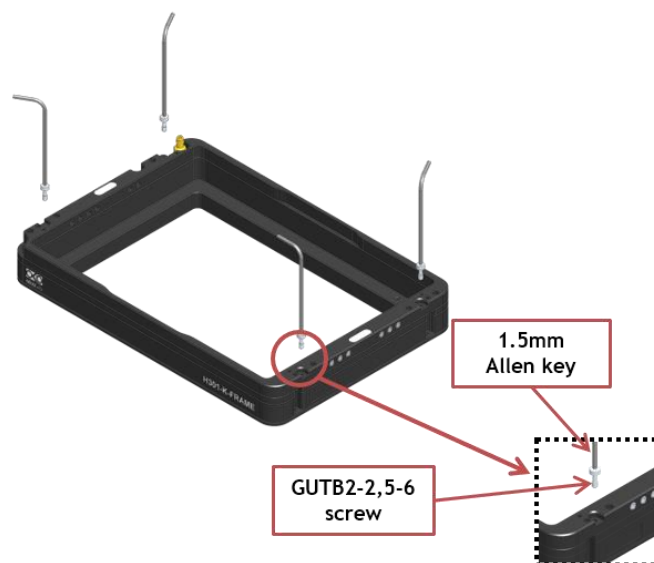

Figure 19. How to lock the Chamber Riser.

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

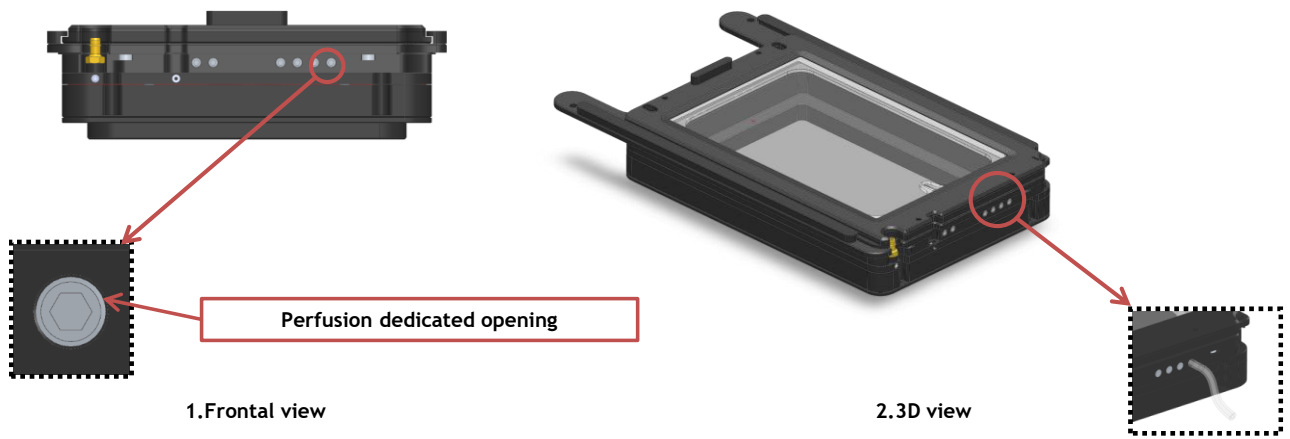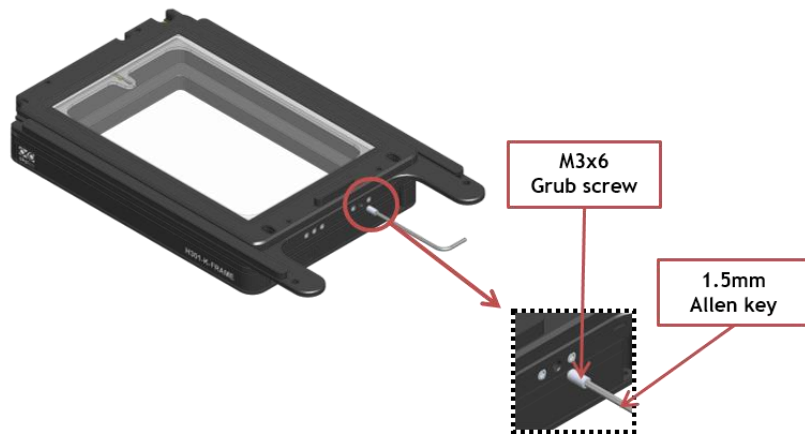Figure 20 shows location of perfusion holes.

Perfusion dedicated opening

1.Frontal view

2.3D view

Figure 20. Perfusion.

M3x6
Grub screw

1.5mm
Allen key

Figure 21. How to access the 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.
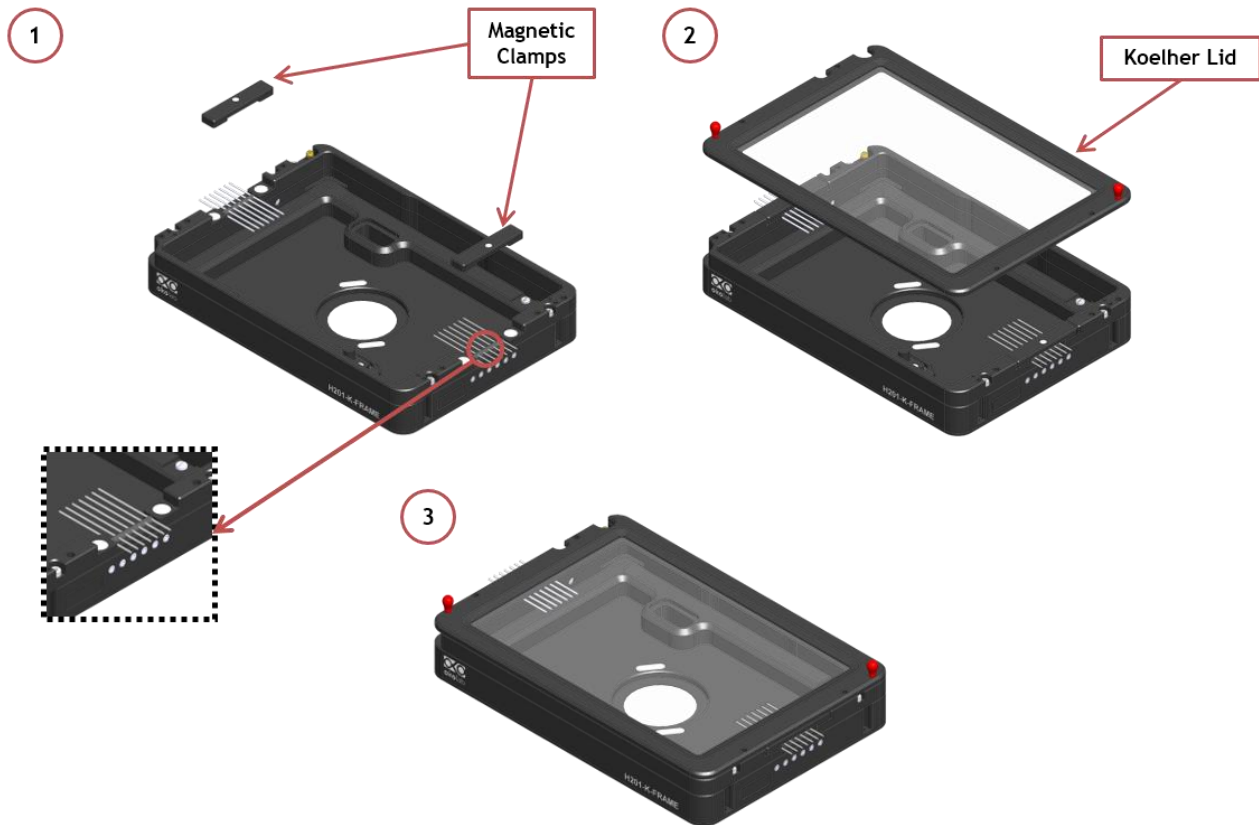

Figure 22. MICROFLUIDIC-C-RISER.

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11.   Connection of the Chamber with XY stage

H201-K-FRAME fits on the XY Stage without any screws. To fix the chamber on the stage, follow the steps shown in Figure 23.

1. Place the chamber on the stage (See Image 1 of Figure 23).
2. Incline the chamber and slide it in direction of the springs until it snaps into the housing. (See Image 2 of Figure 23).
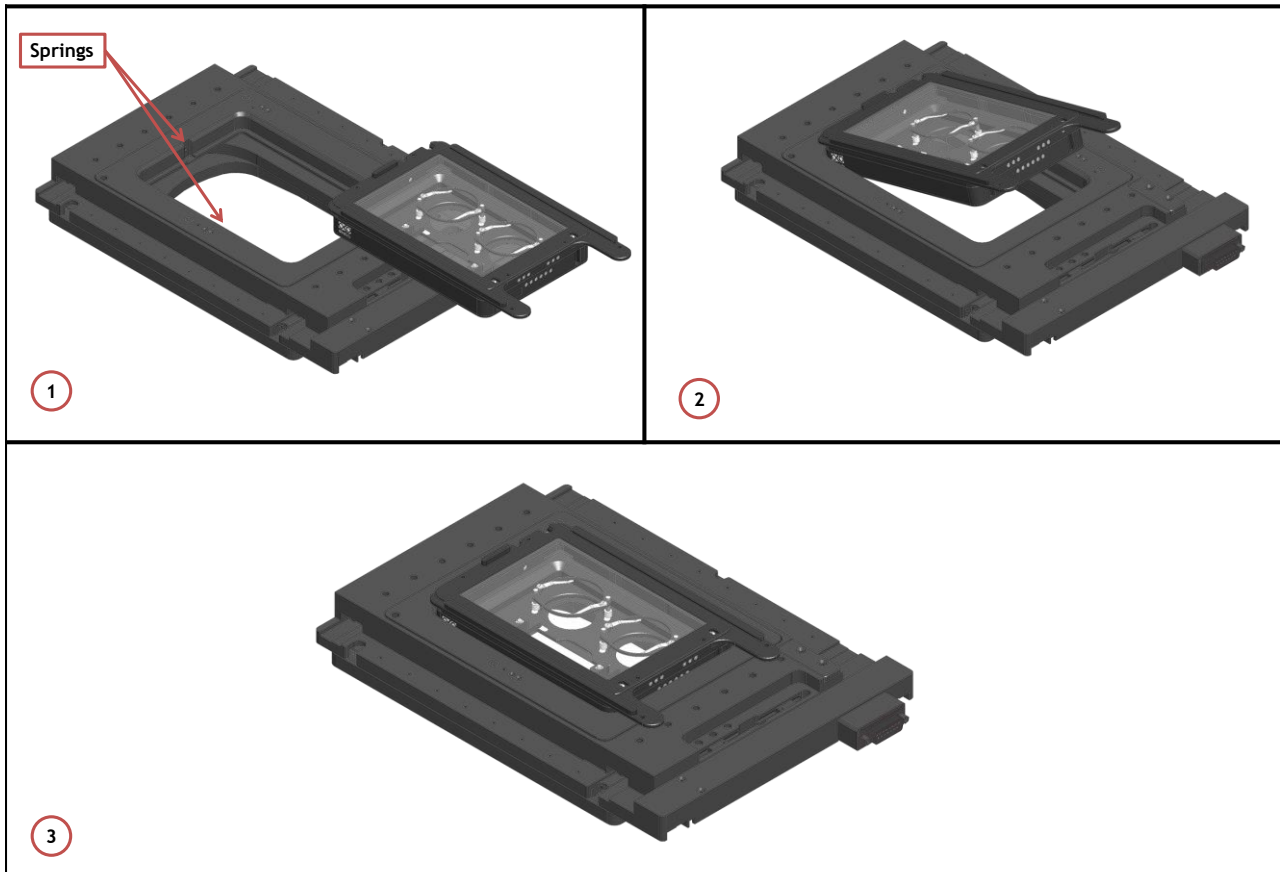3. Image 3 of Figure 23 shows the chamber fitted on the XY Stage.

Figure 23. Connection of the Chamber with XY Stage

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