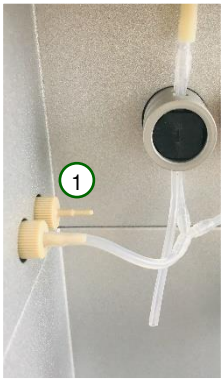
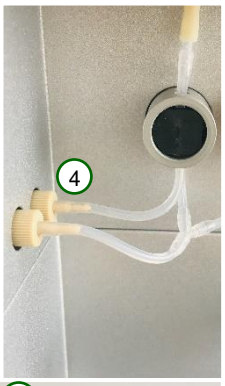
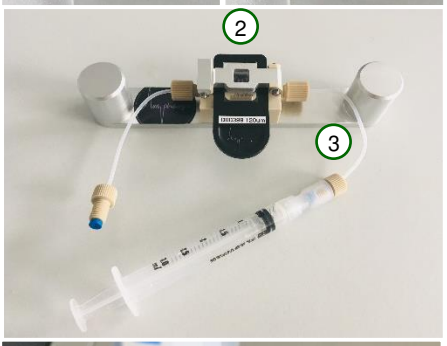
Chip and chip head deblocking

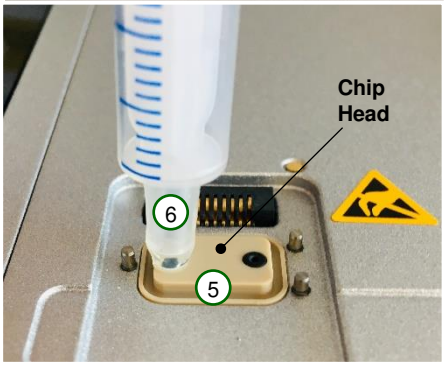
Chips can be blocked by cells or other large particles in the sample. In case of a chip blocking, please follow the following procedure:

1. When a chip is blocked, the pressure in the fluidic system increases until a silicone tubing pops off the adapter (Fig. 1).
2. Stop the current process, e.g. click on **Stop Measurement** in the AmphaSoft measurement tab. In case the flushing process starts automatically thereafter, click **Stop Measurement** again.
3. Remove the measurement chip and place it in the Chip Wash Station (Fig. 2).
4. Unblock the chip by flushing water from the outlet port (right) towards the inlet port (left) (Fig. 3).
5. After deblocking, carefully clean and dry the chip with a lint-free tissue.
6. In case the silicone tubing popped off the adapter, reattach the tubing (Fig. 4).
7. Carefully clean the chip head of the instrument (Fig. 5) with a lint-free tissue and then place the chip back in the instrument.
8. Perform an instrument rinsing by clicking on **Instrument Control** in the **Navigation** panel, selecting the **Basic** tab and clicking **Start Rinsing**.
9. If the tubing pops off during the rinsing, the chip head may be clogged too.
   a. Switch off the instrument.
   b. Manually detach the silicone sample tubing from the adapter (Fig. 1).
   c. Take the syringe with rubber tip, fill it with deionized water and carefully flush the left fluidic port of the chip head (Fig. 6). Do not apply too much pressure to prevent water leakage between syringe tip and chip head. Cover the golden electrodes with a lint-free tissue. When flushing, water will come out of the tubing adapter (Fig. 1).
   d. After flushing thoroughly, reattach the silicone sample tubing on the adapter (Fig. 4) and dry the chip head with a lint-free tissue (Fig. 5).
   e. Put the chip back in the instrument, switch the instrument on and perform an instrument rinsing in the **Basic** tab.









Tip: Preventing Chip Clogging

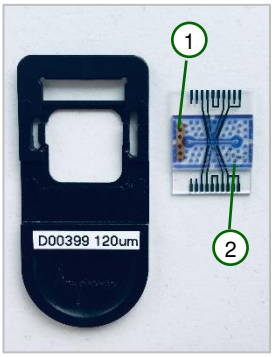
To reduce the risk of chip clogging, please consider the following tips:

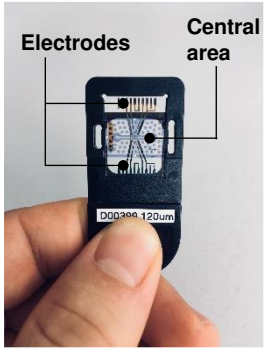
- Reduce the cell concentration of the sample. The detection rate should not be higher than 1000 cells / second.
- Make sure you are using the appropriate filter and chip. Recommendations can be found in the ***Pollen Analysis Instructions*** at www.amphasys.com/download
- In case of hydrophobic and clump-forming cells or cells adhering to tubes, add 0.05 % Tween 20 to the measurement buffer aliquot.

Putting the glass chip back into the plastic frame

The glass chips can be separated from the plastic frame. Clip them back using the following procedure:

1. Pick up the glass chip. Make sure you only touch the central glass area and not the exposed electrodes.
2. Position the chip in a way that the serial number is located on the left (Fig. 1) and the Amphasys logo is in the bottom right position of the glass chip (Fig. 2).
3. Carefully insert the left edge of the glass chip into the left clip of the plastic frame.
4. Press the right edge into the right clip.
5. Carefully clean the glass chip surface with a lint-free tissue.



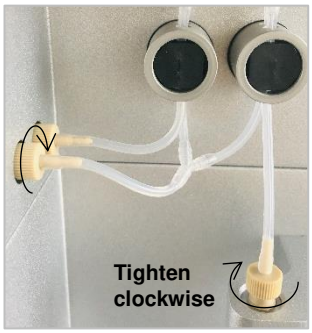


Air bubbles in the tubings

Air bubbles in the fluidic system interfere with the measurement. If you observe many air bubbles while rinsing or measuring, please try the following steps:

- If a tubing adapter is loose, tighten it by hand (clockwise).
- If an external silicone tubing has a defect, replace it or contact Amphasys support for a replacement.
- If a vacuum is created at the bottom of the FACS tube while aspirating the sample, lower the position of the FACS tube.

Contact Amphasys support if none of these steps works.



High rejection rate

The rejection rate is a key quality criterion for a measurement. The rejection rate is the percentage of particle signals that did not meet the predefined criteria. The rejection rate of a measurement should be below 10 %. Possible reasons for higher rejection rates are:

- Lots of small particles (e.g. debris) in the sample. Try a nondestructive sample preparation method or increase the triggering level to exclude those particles from the analysis. Contact Amphasys support for help with sample preparation or the configuration.
- Non-default instrument settings. Wrong configuration of the triggering algorithm or wrong configuration of the pump speed. Send the zipped workspace to Amphasys support.
- Fluidic leakage leading to lower flow rate. Try to localize the leakage (without opening the instrument) and contact Amphasys support. In case of a leakage, unplug and do not use the instrument anymore.
- Air bubbles. See chapter *Air bubbles in the tubings* in this *Quick Guide*.

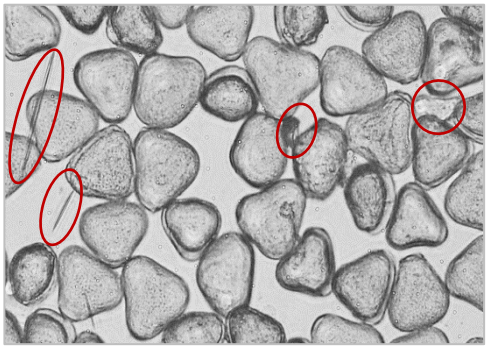
A lot of debris in the sample

Debris are small particles, such as cell fragments and dust. Due to their small size, they have a low impedance amplitude (y-axis of the scatter plot). Depending on the configuration of the measurement (triggering algorithm), those particles are counted or ignored.

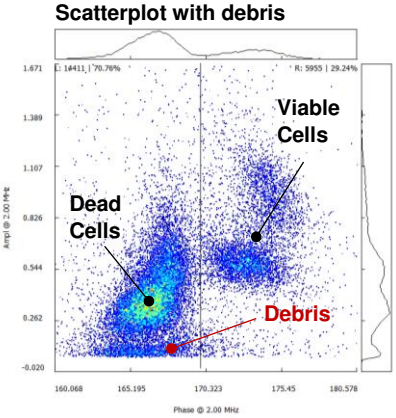
There are several ways to deal with debris:

- Hide the debris cloud using the AmphaSoft *Hide Cells* feature.
  - Create a polygon gate around the debris cloud.
  - Left click into polygon to highlight it > right click > **Gating** > **Hide** > **Cells**.
- Try a nondestructive method for sample preparation. Contact Amphasys support for ideas.
- Increase the triggering level to ignore debris signals. Contact Amphasys support for help with this configuration.

Pollen sample with debris



Scatterplot with debris



Scatterplot with hidden debris