



... Reinventing Single  
Cell Analysis

# Amphasys Introduction

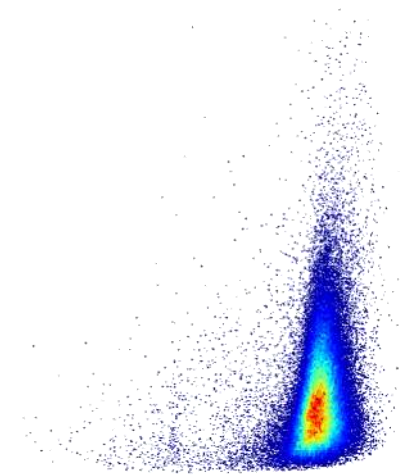


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- General Troubleshooting
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  - Buffers
  
- Known Issues/ Case Studies
  - How to identify
  - How to fix
  
- Amphasys Support



# General Troubleshooting

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- Take a step back and try to see the whole image
- 1st step: Know all facts
  - Write down what exactly happened when the error occurred
  - What is the exact problem, and what is just a symptom?
  - Send the workspace (WS) → Copy all files from the WS to a mail and send as attachment. No reports, pictures or folders, just **.meas .amph** and **.cels files**
  - Or: Zip the whole WS and send this file
- 2nd step: Know your own turf
  - Check the settings (before searching for bugs, mechanical or electrical issues) ([Recommendation list for buffers, filters, chips and settings](#))
  - Get used to/ know the Advanced Tab
  - If settings are not OK send recommendation accordingly | If not go on
- 3rd step: Identify the issue
  - See next slides

# General Troubleshooting - Fluidics

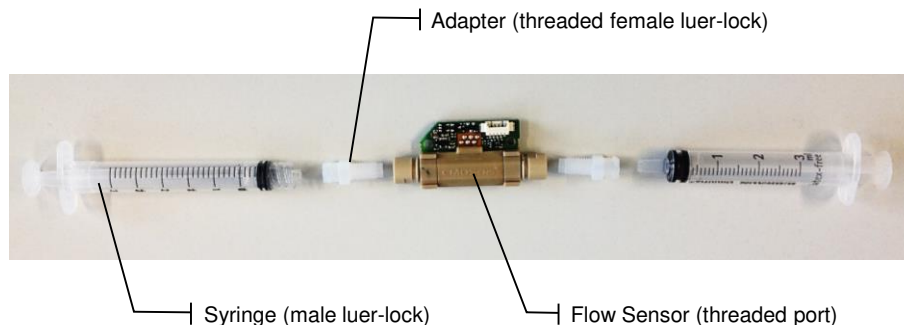


- Silicone and teflon tubes to transport the sample through the system
- Clogging, sedimentation, tube (connector) tightness
  - Clogging happens always in left side of the chip (most narrow part of fluidics)
    - Identify type of clogging (binocular or microscope)
    - Wash station, Syringe with silicone tip
    - Immersion of chip in DI-water overnight
  - Sedimentation takes place if samples are not shaken up right before measurements or pump speed is too slow
  - Speed of sedimentation is mostly dependant on pollen size
  - It can be worse for sticky pollen
    - Know if samples are difficult to handle (Maize, Cucurbits etc.)
    - Big pollen needs to be shaken seriously right before or even during the measurement
    - Sticky pollen might need additional detergent (Tween 20 or 80; final concentration of 0.05%)
  - Tube tightness
    - Constant stream of air bubbles in the sample aspiration tube
    - Check/ fix tightness of all connectors, screws and tubes

# General Troubleshooting - Fluidics



- Silicone and teflon tubes to transport the sample through the system
- Flow Sensor
  - Device which measures the aspirated volume/ time
    - Normal use of the instruments leads to unwanted coatings inside the flow sensor
    - Flow is underestimated/ displayed wrong (no effect on actual flow)
    - Since 05/2018 new ingredient in AmphaClean (Terg-A-Zyme)
    - Sophisticated cleaning procedure by Silvan Kaufmann (see additional data on your stick for details)



## Materials

- Deionized water
- Detergent (e.g. liquid soap)
- Paper tissue
- Scissors
- 2 syringes and appropriate adapters to flow sensor (e.g. threaded female luer-lock)



## Cleaning Solution Preparation

- Fill approximately 5 ml deionized water into a beaker and add some liquid soap
- Cut small (e.g. 1 mm x 1 mm) paper tissue pieces and add them to the detergent solution

# General Troubleshooting - Electronics

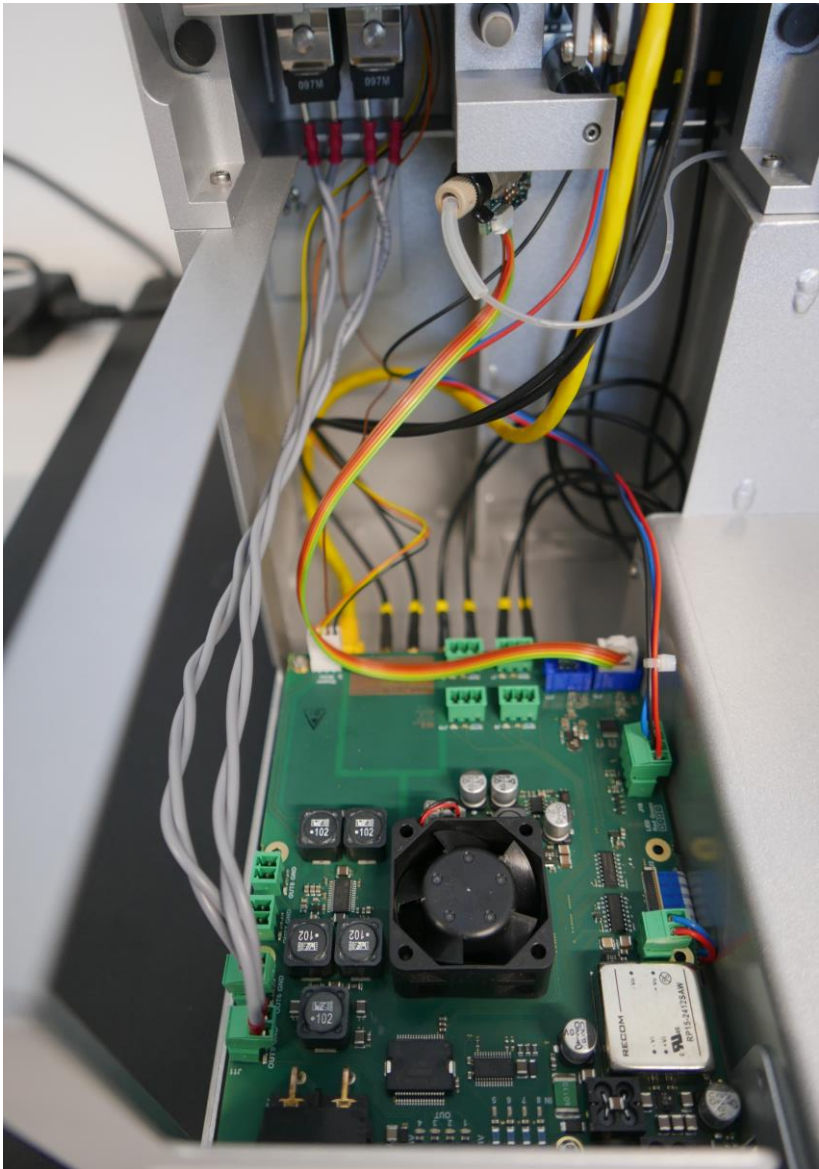
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- Most problems can be tracked back to simple causes
- Contacting
  - Neuralgic point of the system
  - Train customers to not touch pins or glass part of chips with bare hands
  - Chip recognition, signal uptake and monitoring, cleanliness of chips and contact pins
    - Clean pins with 70% Ethanol
    - Same can apply for contacts on chips - **But very careful!**
    - Rectify the pins with a tweezer
- Cables
  - Check if all cables are properly connected on periphery board after transport



# General troubleshooting





- **Ensure you have Admin rights (General and Network)**
  - In order to be able to establish a connection between instrument and computer we need to configure the USB-to-Ethernet adapter properly
  - We need to set a fixed IP address
    - Installing drivers
    - Connect USB-to-Ethernet adapter to laptop
    - Control Panel → Network and sharing center → Change adapter settings → **Realtek FE USB Family Controller** (Right Click) → Settings → Internet protocol Version 4 (TCP/IPv4) → Settings → Use following IP address
  - Afterwards we need to disable automatic shutdown by the computer (energy saving)
    - Control panel → Hardware and Sound → Devices and printers → Device manager
      - USB Controller → USB Root Hub (USB 3.0) [Right Click] → Settings → Energy options → [uncheck] Allow computer to shut down device for energy saving
      - Network adapters → Realtek USB FE Family Controller [Right Click] → Settings → Energy options → [uncheck] Allow computer to shut down device for energy saving



# General Troubleshooting - Laptop



→ > Systemsteuerung > Netzwerk und Internet > Netzwerkverbindungen

Organisieren ▾    Netzwerkgerät deaktivieren    Verbindung untersuchen    Verbindung umbenennen    Einstellungen dieser Verbindung ändern

Bluetooth-Netzwerkverbindung 2 Nicht verbunden	Drahtlosnetzwerkverbindung AVASA Intel(R) Dual Band Wireless...	LAN-Verbindung Netzwerkkabel wurde entfernt Intel(R) Ethernet Connectio...	Ethernet 5 Netzwerkkabel wurde entfernt Realtek USB FE Family Cont...
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Eigenschaften von Ethernet 5

Netzwerk    Freigabe

Verbindung herstellen über:  
Realtek USB FE Family Controller #3  
**Konfigurieren...**

Diese Verbindung verwendet folgende Elemente:

- Client für Microsoft-Netzwerke
- Datei- und Druckerfreigabe für Microsoft-Netzwerke
- QoS-Paketplaner
- Intel(R) Technology Access Filter Driver
- Internetprotokoll, Version 4 (TCP/IPv4)
- Microsoft-Multiplexorprotokoll für Netzwerkadapter
- Microsoft-LLDP-Treiber

Installieren...    Deinstallieren    Eigenschaften

Beschreibung  
TCP/IP, das Standardprotokoll für WAN-Netzwerke, das den Datenaustausch über verschiedene, miteinander verbundene Netzwerke ermöglicht.

OK    Abbrechen

Eigenschaften von Internetprotokoll, Version 4 (TCP/IPv4)

Allgemein

IP-Einstellungen können automatisch zugewiesen werden, wenn das Netzwerk diese Funktion unterstützt. Wenden Sie sich andernfalls an den Netzwerkadministrator, um die geeigneten IP-Einstellungen zu beziehen.

IP-Adresse automatisch beziehen

Folgende IP-Adresse verwenden:

IP-Adresse: 10 . 10 . 16 . 14

Subnetzmaske: 255 . 255 . 255 . 0

Standardgateway: . . .

DNS-Serveradresse automatisch beziehen

Folgende DNS-Serveradressen verwenden:

Bevorzugter DNS-Server: . . .

Alternativer DNS-Server: . . .

Einstellungen beim Beenden überprüfen

Erweitert...

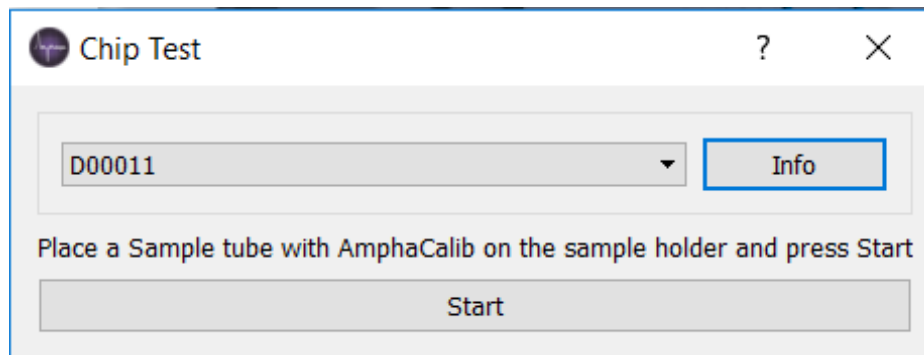
OK    Abbrechen

# General Troubleshooting - Chips



## Chip Test

- Ensure a valid measurement
- Chips need to be within specifications
- AmphaSoft User guide | Chapter 6 | Chip Tests
  - AmphaSoft Main window → Tools → Chip Test
  - Select Chip from drop-down menu
  - Place a tube with 3ml AmphaCalib on sample tube holder
  - Press start
  - [The „Info“ -button shows you how many measurements and chip tests have been performed with the chip] - with it's respective instrument
  - Result: Chip Ok – Good for further measurments
  - Result: Chip Not Ok – No further usage



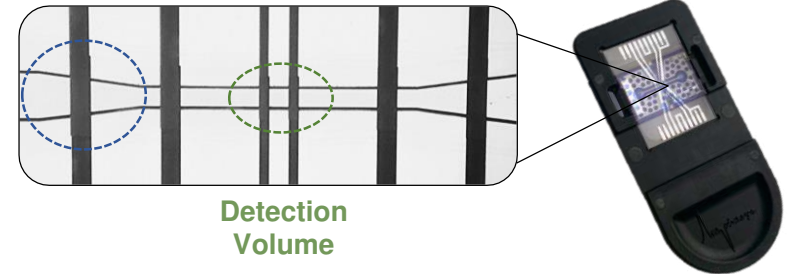
# General Troubleshooting - Chips



## Chip Clogging

- It will happen
- Stop the measurement | Click TWICE!
- Remove the chip
- Unclog the chip using the wash station
- Re-insert the chip
- Re-attach the tubing if disconnected
- Perform a rinsing

Narrowing

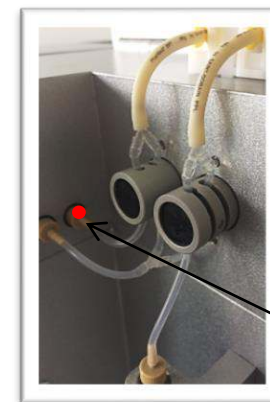


Detection Volume

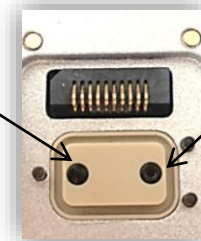


## Cleaning the chip block

- Syringe with soft tip
- Left and right port of chip block
  - Remove silicone tubing for left port
- Carefully, do not apply too much pressure



Left port



Right port

Remove this tube to clean the left port

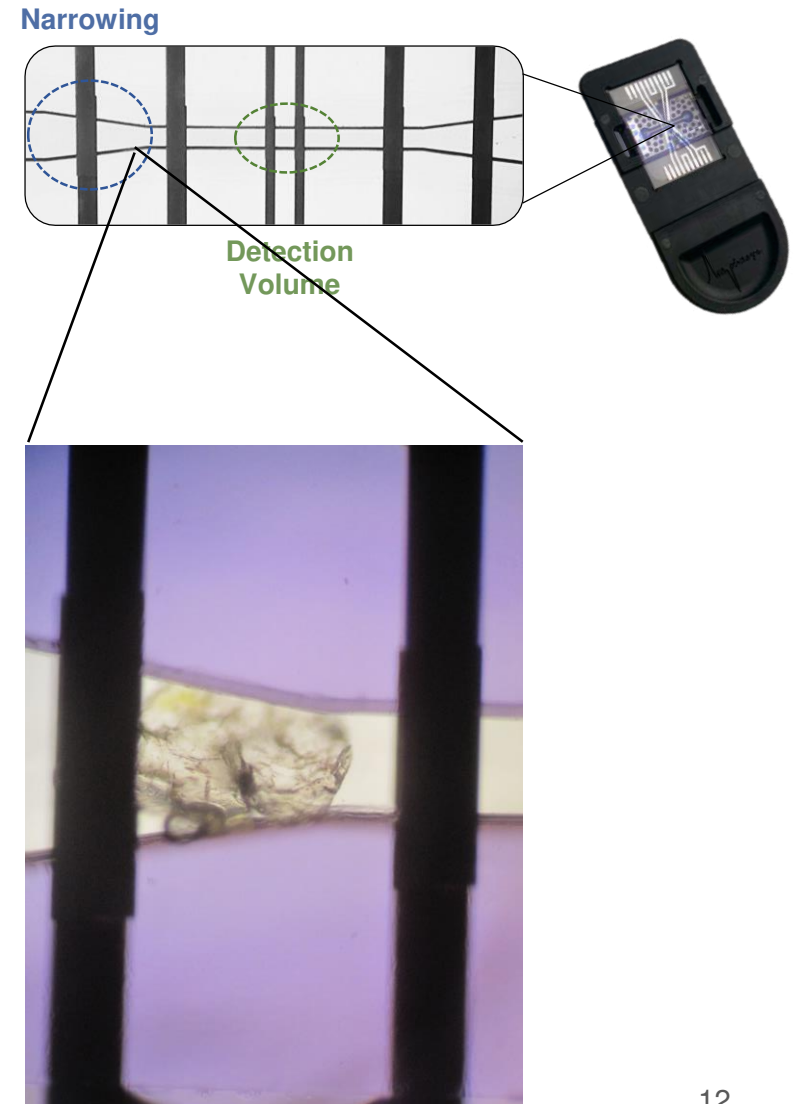
References *Ampha Z32 User Guide, Maintenance*

# General Troubleshooting - Chips



## Chip Clogging

- Identify clogging with microscope/ binocular
- Agglomerations of pollen are quite easy to remove
- Long filaments/ hairs etc. are difficult to remove and will lead to recurring cloggings
- Clean until channel is 100% free of any solid material
- Clear convex or concave cloggings is most likely dried/ crystallized buffer
  - Almost impossible to remove with chip wash station
  - Immersion of whole chip in DI water for several hours/ overnight



References *Ampha Z32 User Guide, Maintenance*

# General Troubleshooting - Chips



## Chip Detection

- Instrument does automatically recognize chip type/ channel size
- If chip is detected wrong/ not detected → Contacting issue
- Clean golden contact pins with 70% Ethanol
- If necessary rectify contact pins with tweezers - **CAREFULLY** – as shown in the video



# General Troubleshooting - Measurements



## Rejection rate / sample density

- Percentage of total cell count
- If signal does not meet quality criteria of triggering algorithm, signal is rated faulty
- < 3% Perfect | 3-5% Good | 5-9% Acceptable | > 9% too high
  - Check settings → [Recommendation list for buffers, filters, chips and settings](#)
  - Check sample density → Rule of thumb: < 1000 counts/ second

### Results

Concentration [cells/ml] 1'862  
Average Flow [ul/min] 2979.9  
Duration 00:50

Accepted	4'723
Rejected [%]	66

Start Measurement



# General Troubleshooting - Measurements



## Clogging

- Clogging probability can be minimized
  - Correct chip type/ channel size
  - Controlled sample density
  - Adding of detergent for sticky pollen
- [Recommendation list for buffers, filters, chips and settings](#)



# General Troubleshooting - Measurements



## Buffers

- Pollen is usually not very stable in our measurement buffers
  - Burst
  - Shrinkage
- Depending on the species, survivability of pollen ranges from several minutes to several hours
- For best results: Measure pollen as freshly harvested as possible
- TWEEN 20 or 80 - 0,05% final concentration
  - Commonly used polysorbates which prevent agglomeration of sticky pollen
  - Stickyness: oily sheath, spikey surface of the pollen
- Before measuring dried pollen:
  - Give the pollen time to rehydrate before adding buffer
    - Let pollen sample sit openly (a wet, non-dripping paper towel can speed up this process)



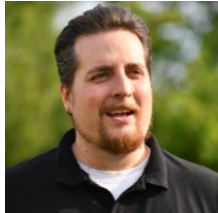
# Amphasys Support - Who you gonna call?

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- If all your efforts cannot solve the issue
- [support@amphasys.com](mailto:support@amphasys.com)
- Ensure you have all the data (WS, brief history of the error, pictures, your efforts so far) ready before approaching us [ We will ask for that! ]
- Having everything ready at your first approach saves a lot of time

# Your Contacts



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