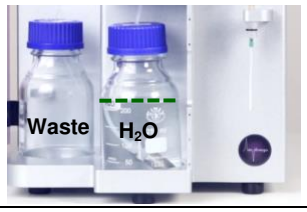
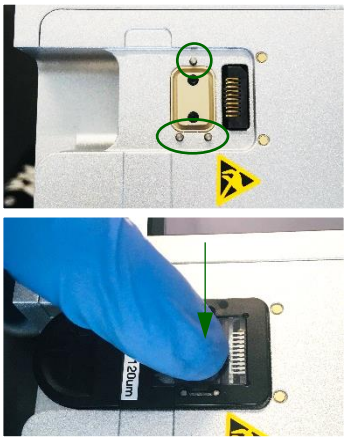
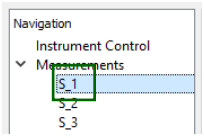
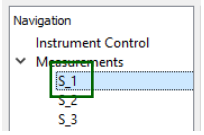
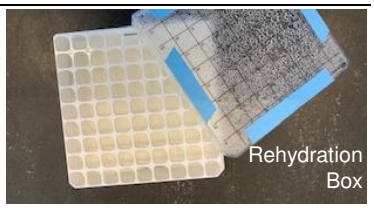
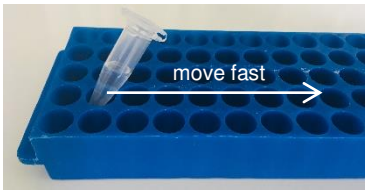
QUICK GUIDE 1: INITIALIZATION & SAMPLE PREPARATION

Initialization

1. Check bottles		• Fill the water bottle with deionized water • Check that the waste bottle is empty ***TIP:*** *Adding a drop of soap to the waste bottle helps to keep it clean*
2. Switch instrument on		• Make sure the instrument is connected to your laptop via ethernet cable and ethernet-to-USB adapter • The power switch is at the rear of the instrument
3. Copy template		• Create a copy of the measurement template and rename it. This will be your workspace where the measurements are saved.
4. Start AmphaSoft		• Double click the AmphaSoft 2.0 icon on the desktop
5. Open template		• In the AmphaSoft menu click on **Workspace** > **Load**. Navigate to the workspace, select the folder by clicking on it and click **Select Folder**.
6. Place chip		• Place the chip in the instrument using the 3 positioning pins (◯) and gently push on the middle of the chip (▼) without touching the electrodes • Carefully close the lid
7. Perform initial rinsing	 	• Click on **S_1** in the left **Navigation** panel and make sure the correct chip number is configured in the measurement tab. If not, select the correct chip from the **Chip Id** dropdown menu. • Click on **Instrument Control** in the **Navigation** panel • Select the **Basic** tab and click on **Start Rinsing** The software will first connect to the instrument (green progress bar at the bottom) and then initiate the rinsing process • After successful connection, a popup message appears. Make sure an empty container is placed below the sample holder and confirm by clicking **Continue Rinsing** twice ***TIP****: If the water filter is dry or contains air bubbles, perform a few rinsing steps until the tubing is filled with deionized water*
8. Start measuring		• Return to the measurement view by selecting the first measurement (**S_1**) in the **Navigation** panel on the left • Enter the sample name and start with the sample preparation

Sample Preparation

1	Buffer Preparation	• Take the buffer bottle out of the fridge. Check the buffer quality. It must be particle free and completely transparent. • Prepare a 50 ml buffer aliquot • Put the buffer bottle back into the fridge. • Let the aliquot equilibrate to room temperature ***TIP:*** *The buffer recommendations list on www.amphasys.com/download contains the recommended buffers by species* ***TIP:*** *For hydrophobic pollen, add Tween20 (final concentration 0.05 %) to the buffer aliquot to facilitate suspension*
2	Pollen Collection	**PURE POLLEN** • Add the quantity of pollen for one measurement into an 1.5 ml Eppendorf tube • Recommended quantity:  **ANTHERS** • Add anthers into an 1.5 ml Eppendorf tube • Number of anthers: Wheat 3, Pepper 3, Potato 1, Brassicas 6, Cucumber 3, Melon 3, Watermelon 3 **WHOLE FLOWERS** • Add flowers into an 1.5 ml Eppendorf tube • Recommended quantities: Fennel: 1 umbellet Carrot: 1 umbellet ***NOTE:*** *High cell concentration lead to a higher risk of chip clogging and inaccuracy of the data.*
3	Rehydration (if required)	• If the sample material is dehydrated, rehydrate for 30 min. Place the open Eppendorf tube with the sample in a rehydration box and close the box. ***TIP****: The Quick Guide Measurement & Tips shows how to determine the optimum rehydration time* 
4	Buffer	***TIP:*** *For anthers: If pollen cannot be released from the anther by shaking, slice them with a scalpel before adding the measurement buffer or squeeze them with tweezers after adding the buffer (step 5)* • Add 1 ml measurement buffer
5	Extraction and Suspension	• Suspend the pollen by finger flicking or moving the tube over a tube rack (see image) ***TIP:*** *For anthers: In case the pollen cannot be released from anthers by shaking, use tweezers to squeeze the anthers* 
6	Filtration	• Use the recommended filter type (see buffer recommendations list on *www.amphasys.com/download)*. Filters must be clean and dry.
7	Dilution	• F chip: Add 1 ml measurement buffer • D chip: Add 1 ml measurement buffer • E chip: Add 2 ml measurement buffer
8	Equilibration	• Let the pollen equilibrate for 2 – 3 minutes in the buffer Exceptions: Sensitive pollen, such as wheat, brassica, artichoke. These samples must be measured immediately. ***TIP:*** *The stability of the cells in the buffer can be tested using the experiments suggested in the Quick Guide Measurement & Tips, page 2.*
9	Mixing	• Slowly invert the FACS tube to distribute sedimented cells and immediately start measuring ***TIP:*** *Seal the FACS tube with Parafilm before inverting*