Critical Point Dryer (CPD) Protocol

Materials

- Gloves
- 20µm Pipetman with tips
- Leica Automated Critical Point Dryer (CPD300)
- Leica CPD300 1/6 filler
- Leica CPD300 1/3 filler
- Leica CPD300 1/2 holder (four chambered) with metal cover
- Leica CPD300 canister and stir bar
- Leica CPD300 microfilters with lids (10 micrometer mesh)
- Leica CPD300 handle - *Items d. through i. are included in the CPD300 package*
- Forceps
- 95% ethanol - *Entire sample will need to be immersed in ethanol so the amount will vary depending on the amount of samples*
- CO_2 tank
- Samples - *Must be stored in ethanol before being loaded into the CPD*

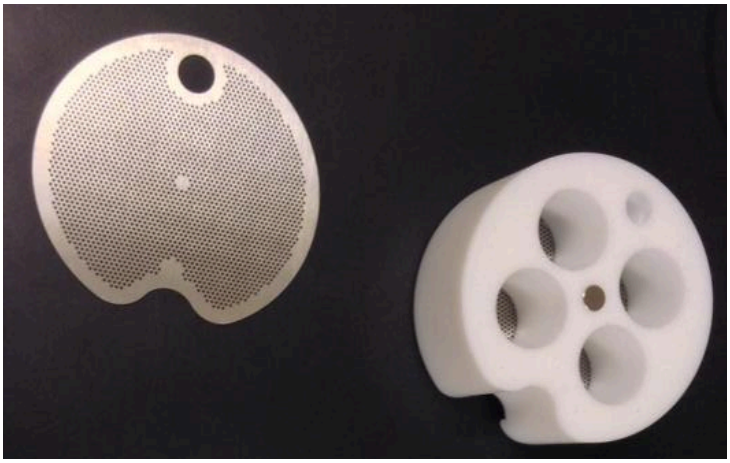
Handling Samples

1. It is extremely important in this procedure to minimize the risk of contamination of pollen samples to ensure clean mounting and imaging after the critical point drying process.
2. When transferring pollen from the vial to the filters, exercise good lab etiquette.
3. Always wear gloves.
4. Keep samples, equipment, and workplaces as clean as possible. Always wash equipment that comes into contact with pollen prior to beginning another species of pollen.
5. Additionally, good note keeping is important. Given the high volume of samples, it is important to always keep track of where the pollen is and where it is going. Knowing exactly what sample goes into each chamber of the holder is imperative.
6. NEVER at any point should the samples be allowed to air dry; it will cause the pollen to collapse making the CPD process pointless.

Loading the Microfilters

1. Before loading the samples, use tweezers to dip the microfilter (10 micrometer mesh) into the ethanol to wet the filters.
2. Use the Pipetman (set to 2µm), placed at the bottom of the Eppendorf tube sample, to remove pollen from the tube. Slowly extract the pollen from the visible portion of the sample.
3. Release the sample in the Pipetman into the microfilter slowly and carefully.

4. Using the tweezers, place the filter lid on top of the microfilter and slowly lower both parts into the bottom of one of the four chambers in the CPD300 four-chamber holder.



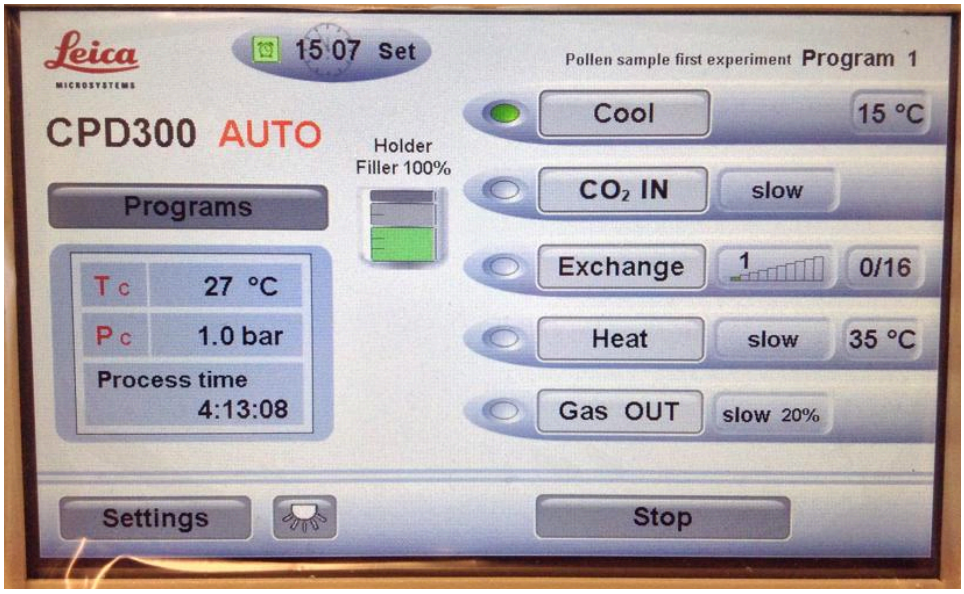
5. When the chambers are full, cover the holder with its metal grid covering and insert the handle into the hole in the holder in order to transport the holder into the bottom of the canister.
6. Load the 1/3 and 1/6 fillers into the canister.
7. Fill the canister with ethanol.
8. Place the stir bar into the bottom of the CPD opening.

9. Fill opening with enough ethanol to cover the stir bar.
10. Use the handle to transport the loaded canister finished in step f. above into the CPD opening.
11. Secure the lid of the CPD opening making sure that the lid spins on without getting caught up on anything.

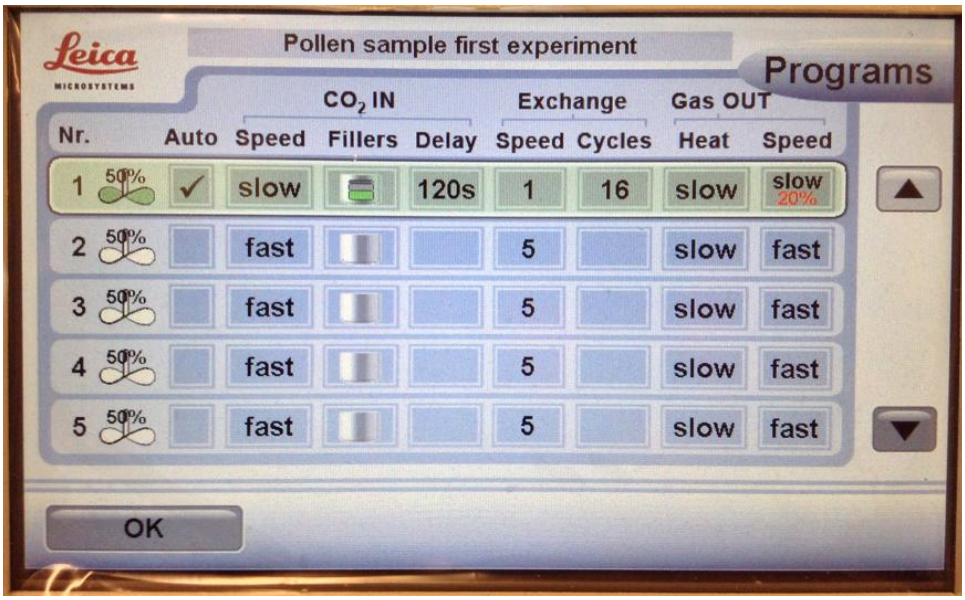
Caution: Under any circumstance, DO NOT force the lid shut if it will not close; cross threading the lid will damage the CPD.

Programming the CPD

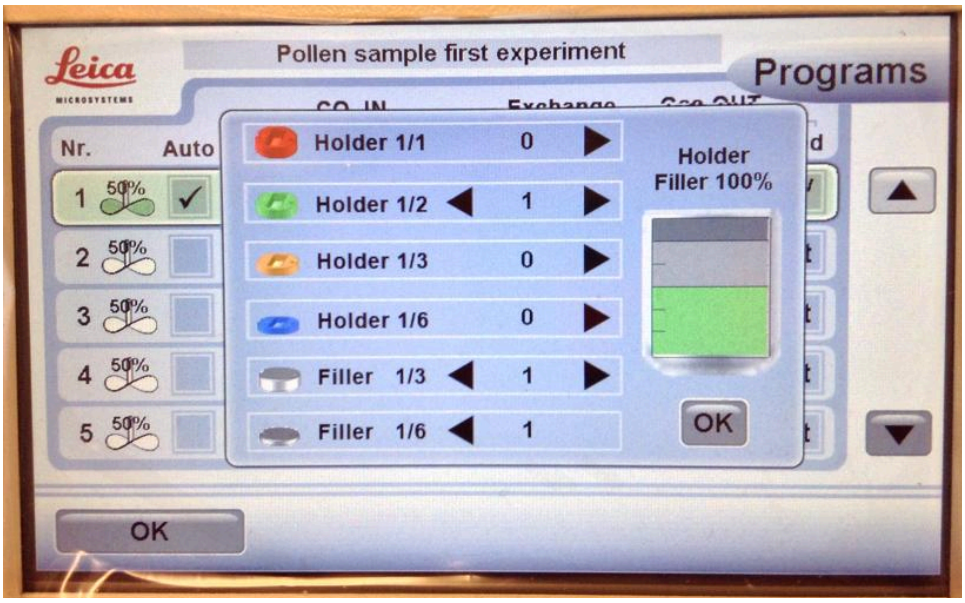
1. Press the programs button on the main screen of the CPD.



2. Set the attributes of the program by tapping on each. Set each setting as shown below.



3. In order to set the fillers, tap the fillers icon and choose the 1/2 holder, 1/6 filler, and 1/3 filler options as shown below.



4. When all settings are set, press start on the main menu of the CPD to begin the CPD process.
5. Upon completion, open the CPD and use the handle to remove the cylinder.
6. Carefully remove the microfilters using tweezers.
7. Mount the samples immediately (See mounting procedure).
8. Clean the CPD and dispose of ethanol waste to its appropriate container.