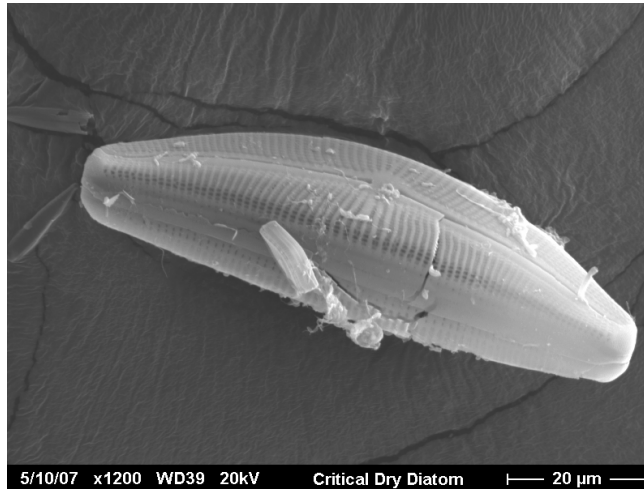


CRITICAL POINT DRYER USER GUIDE

SAMDRI-795, Tousimis Research Corporation, Rockville, MD



Imaging and Chemical Analysis Laboratory at Montana State University
Barnard Rm. 339A
(406) 994-4199, ical@sympa.montana.edu

I. BASIC INFORMATION – preservation of delicate ultrastructure

Why use critical point drying (CPD)? During normal evaporative drying, liquid (water or alcohol) goes into the gas phase across a *liquid-vapor* phase boundary with extreme changes in density. Such an abrupt phase transition introduces unwanted morphological changes in the delicate 3-D structure of a sample due to surface tension of the liquid. To avoid such structural changes, the sample must be brought from the *liquid phase* to the *gas phase* without crossing a *liquid-vapor phase boundary*. This is done by going “*around*” the critical point of the working fluid, liquid CO₂, by manipulating pressure and temperature. Liquid CO₂ is brought to the *supercritical fluid* phase and then to the *vapor phase*, avoiding abrupt changes in density, allowing the structural integrity of the tissue to be maintained.

How is it done? First the sample must be pretreated (e.g. gluteraldehyde, osmium fixation) and brought to the lab in an alcohol bath. Then the sample is re-immersed in 100% alcohol in the CPD unit. This alcohol is then replaced with liquid CO₂, which is compressed to above the *critical pressure* and then heated to above the *critical temperature* of LCO₂. This brings the CO₂ into the *supercritical fluid* region. The supercritical CO₂ is then decompressed and brought to the *ambient pressure* without ever crossing a *phase boundary*.

II. SYSTEM OPERATION

NOTE: system is semi-automatic; some steps are automatic and others require user to press the next stage LED button switch.

1. **Turn** power switch “ON”.
 - Green LED on VENT button will illuminate. Unit is now “standing by” in the VENT mode.
2. Let Samdri stand for 3–5 minutes to “warm-up”.
3. Open the main LCO₂ tank valve.
4. Pour enough 200 proof alcohol into the opened process chamber to cover your sample (10–15 mL).
 - Transfer the sample into the open process chamber *quickly* in order to avoid exposure to air and moisture.
 - Secure the chamber lid down over the chamber using the 3 knurl nuts; tighten each by hand with equal pressure, just “hand tight”.
5. Once the lid is secured, set the PURGE timer, located to the right of the push button switches. Positions on the purge timer are calibrated at 5-minute intervals: position #1 will give you a 5-minute purge time, #2 is 10 minutes of purge time, etc. Maximum purge time is position #9 for 45 minutes. “Purge time” settings are best determined by the individual operator. In general, purge times for various chamber alcohol levels within the 795 are the following:
 - ¼ chamber = 5 minute purge time
 - ½ chamber = 7 minute purge time
 - ¾ chamber = 10 minute purge time (typically sufficient for the majority of sample types)
6. After initial warm up **press** the COOL button.
 - The COOL LED light will go on and the VENT light will turn off.
 - You will hear LCO₂ circulating through the unit as the 795 cools and chamber temperature reaches 0°C (± 5 °C). At this point, cooling will **automatically** shut off. Observe temperature.
7. **Press** the FILL button and the 795 will begin to fill the process chamber with LCO₂ and continue for 2 minutes.
8. After the 2 minute FILL mode expires, the 795 will **automatically** advance into the PURGE mode, indicated by the illumination of the PURGE LED.
9. The 795 will remain in the PURGE mode for the time pre-set via the PURGE timer (step 5). The waste alcohol will exit the 795 Chamber via the PURGE/VENT exhaust line.

10. Upon completion of the PURGE mode, the PURGE LED will turn off and the HEAT LED will illuminate. The HEAT mode is the stage whereby the samples are carried through the “*Critical Point*”. BOTH the pressure and temperature will steadily rise.

11. At the end of the “*tousimis equilibrium*”[®] (see NOTE) period, **press** the BLEED switch.

- BLEED LED will illuminate. The flow rate should read 8–10 SCFH on the flow meter initially. **OBSERVE THIS!**
- The temperature will automatically remain above 31 °C in the chamber during decompression (100–150 SCFH/min).

NOTE: *tousimis equilibrium is the point during the critical point passage in which both the pressure and temperature are maintained above the critical point within the chamber for a period of 4 minutes prior to the user advancing Samdri into the BLEED mode.*

12. At approximately 360–400 psi, **press** the VENT button to advance into VENT mode.

- The chamber comes to atmospheric pressure in approximately 3 minutes

14. When chamber is at 0 psi range (see mark on gauge), **remove** the chamber lid by alternatively and evenly loosening the knurl nuts. (NEVER attempt to ‘force’ opening).

15. The sample(s) can be removed from the chamber for further processing. **Seal** the chamber with the lid to help keep it clean and moisture free.

16. **Turn** the 795 power off using the ON/OFF switch. The VENT LED will remain lit for a few seconds.

17. **Turn** LCO₂ main tank ON/OFF valve **OFF**.

18. Fill in the LOG BOOK information.

NEED HELP?

Sara Zacher, ICAL

406-570-3064

sarazacher@montana.edu