PROTOCOL:

Critical Point Drying (CPD) with the K850

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

Critical point drying is an established method of dehydrating biological tissue prior to examination in the Scanning Electron Microscope (SEM).

What to Do Before Starting

- Make sure all valves are closed, including the CO_2 tank.
- Place processed specimens in designated CPD holders and in final dehydrant.

NOTE #1: Dehydrants, especially acetone/propylene oxide, are volatile and specimens cannot be allowed to dry **at Step #3.1**.

Procedure *(see photo of K850 on page 2)*

Open the Liquid CO_2 tank valve.

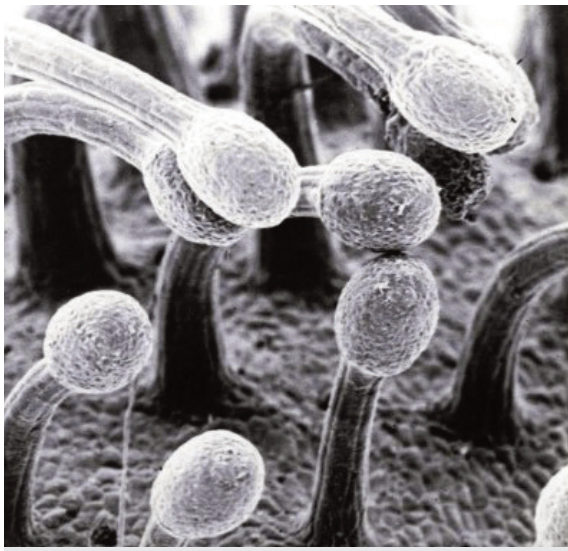
1. Pre-cool the chamber to +5°C using the **Blue "COOL" valve**, opening it in 30-second bursts followed by a 15-second pause to allow the temperature gauge to respond. Close when complete.
2. Unscrew the chamber lid retaining knobs and remove the lid.
3. Transfer the specimen holders from the final dehydrant into the chamber. See NOTE #1 above.
4. Replace the lid and securely tighten the retaining knobs.
5. Open the **Green "INLET" valve** and fill the chamber to the top of sight glass. Leave inlet open!
6. Allow to soak for 2 minutes.

NOTE # 2: The rotating stirrer should be switched off 1 minute prior to doing Step #7, as both acetone and ethanol are more dense and will sink to the bottom of the chamber, which is designed to be bottom draining and top filling.

NOTE #3: During Step #7 below, the fluid level may drop. Do not let the level fall below the middle of the sight glass by restricting the volume of exhaust.

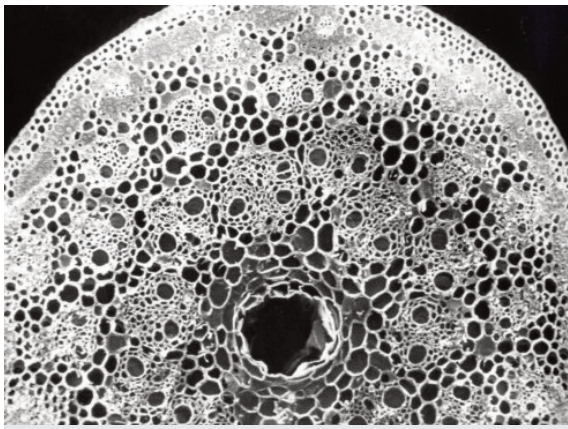
7. (See Notes #2 and #3 above before continuing.) Allow fresh CO_2 into the chamber by opening the **Black "EXHAUST" valve**. Do this for 2 minutes or until the odor of the final dehydrant is no longer detectable. This is done by using a small disc of filter paper placed into the exhaust stream of gas and smelling it.
8. Close exhaust valve and let soak for 5 minutes. (**NOTE:** For very large specimens, the soak period should be double). During this period, the stirrer may be used to enhance solvent exchange.
9. Repeat steps 7 and 8 – twice – allowing the chamber to be fully purged of dehydrant and samples fully saturated with CO_2.
10. Ensure that the meniscus is at the center of the viewing window. Close all valves. For added safety, also close the carbon dioxide cylinder valve.
11. Switch on heater and allow stable conditions to be reached of approximately 37–40°C (±2°C) and ≥1200 psi. This will take about 15 minutes. **DO NOT** let pressure exceed 1300 psi by opening the fine needle valve to the left of the **RED "Bleed" valve**.

continued on page 2...



Glandular Trichomes on Modified Leaf Surface of Butterwort

An insectivorous plant, the butterwort (Pinguicula vulgaris) has modified leaves which bear tiny granular trichomes which trap insects. The capitate head of the trichome then secretes protease enzymes to digest the insect parts.



Bamboo Stem: Transverse fracture of the stem of young bamboo (Bambusa sp), demonstrating xylem and phloem bundles and heavily thickened (lignified) epidermal and hypodermal cells.

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Procedure (continued)

12. After the above critical points are reached, let sit for 5 minutes while monitoring pressure.

13. After 5 minutes, the chamber can be depressurized by opening the using the **RED "Bleed" valve** and needle valve at an approximate rate of 100 psi/minute. For delicate specimens, a slower rate may be desirable.

14. When pressure has been equalized (about 15 minutes), the specimen can be removed and subsequently treated. In all cases, this should be maintained in dry conditions.

15. The K850 can now be re-used or shut down.

Appearance of the leech body wall at sensillum SI. The central area with numerous white projections is surrounded by a 20-Mm-wide ring, which like the central area is devoid of pores.

The Biological Bulletin, Authors: Marine Biological Laboratory (Woods Hole, Mass.); Marine Biological Laboratory (Woods Hole, Mass.). Annual report 1907/08-1952; Lillie, Frank Rattray, 1870-1947; Moore, Carl Richard, 1892-; Redfield, Alfred Clarence, 1890-1983

K850 Operation and Functions

Stirrer Switch: Switches on motor, which is located on the base of chamber, this has a magnetic coupling to a stirrer bar located inside the chamber.

12V DC LED: Indicates 12V DC control voltages are present.

Heater Control LED: Indicates when heater is on or off under control mode.

Heater Switch: Switches power on to heater control system.

Pressure Gauge: Monitors direct pressure in chamber.

Flow Gauge: (Optional) Allows monitoring and fine control of depressurization.

Temperature Gauge: Monitors temperature of chamber.

Inlet Valve (Green): Allows gas direct to chamber.

Exhaust Valve (Black): Allows direct exhausting of gas from chamber.

Bleed Valve (Red): Allows bleeding of gas from chamber.

Cool Valve (Blue): Allows controlled flow of gas to adiabatic cooling system — the change in pressure giving a cooling effect to the chamber — the adiabatic cooling system does not let gas into the chamber.

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