

# Z2 Coulter Counter operation for Quantifying corn pollen production

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## 1. Overview

The Coulter counter provides the ability to count particles (in this case, pollen grains) relative to user-defined thresholds. This protocol briefly describes the start up procedure, calibration of the machine, operation and sample analysis, and shutdown procedures. For more details, the Beckman Coulter Z series manual is located in a drawer underneath the machine (page references are provided in this guide).

## 2. Start up procedures

- 2.1. Before turning on the Coulter counter be sure that the waste jar is empty and that the diluent jar is full with Isoton II diluent (*page 2-13*). Waste Isoton II diluent can be disposed of in the sink with abundant water.
- 2.2. Pollen counting requires a 200  $\mu\text{m}$  aperture tube. If a different aperture is in place, replace it with the 200  $\mu\text{m}$  (*pages 2-15 and 4-19*). Currently this is the only aperture we have, so this step is not required.
- 2.3. Turn on the Coulter counter.

## 3. Calibration (*page 3-1*)

Beckman recommends that the user check the size calibration for each aperture tube at least once a month to monitor for any system variations. However, when a lot of samples are being analyzed, we recommend doing so more frequently.

- 3.1. Prepare a calibration suspension by adding 5 drops of the 10  $\mu\text{m}$  standard into a beaker containing 50 ml of Isoton II diluent.
- 3.2. Place the beaker of the calibration suspension on the platform and raise it until the aperture tube, electrode and stirrer are immersed.
- 3.3. Turn on stirrer (knob on top front of Coulter counter); adjust speed to a gentle but thorough stir (stirrer will automatically stop when platform is lowered).
- 3.4. Press **Set-up** and screen **S1** will display. At **Selected units** select  $\mu\text{m}$  by using the cursor keys.
- 3.5. Press **Set-up** and screen **S2** displays. At **Select Aperture** use the cursor key to select 200  $\mu\text{m}$ . At **Metered Volume** select 1.0 ml.
- 3.6. At screen **S1**, press **CAL** and screen **C1** will display. At **Selected units** select  $\mu\text{m}$  and at **Calibrator size** use the numeric keypad to enter 10  $\mu\text{m}$ . The **Measure Aperture** field automatically changes to **Yes**.
- 3.7. Press **Start** and screen **C4** will display followed by screen **C3**.

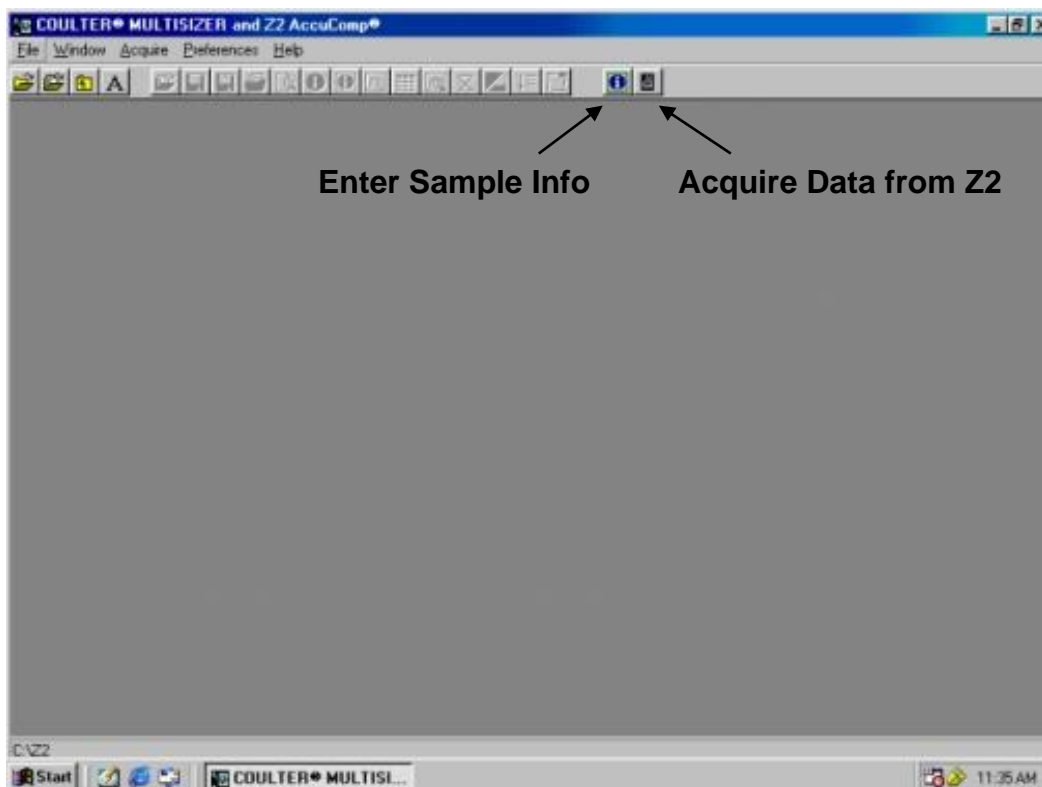
- 3.8. Press **Start** again and screen **C6** will display. Wait until the calibrating process is over.
- 3.9. Once calibration is complete, the **C7** screen will display. Press **Start** to store the new  $K_d$ .

#### 4. Operation and sample analysis

- 4.1. Raw pollen samples are normally too concentrated to be quantified directly, so dilution is required. Too low concentrations are not desirable either, as they result in lower accuracy. Choosing the correct dilution factor will depend on the operator criteria and experience. We recommend targeting for a concentration around 1000 pollen grains per ml.
- 4.2. Prepare pollen dilutions (in Isoton II diluent) in a 150 ml glass beaker. Make sure to shake the pollen sample solution until the dispersal of pollen grains is homogeneous prior to pipetting out the pollen sample. **IMPORTANT:** *Initial sample volume needs to be known for calculating total number of pollen grains.*
- 4.3. Press **Set-up** until screen **S1** displays. Check that the Aperture tube is set to 200  $\mu\text{m}$ . Set up the **Upper Size Tu** to 90  $\mu\text{m}$  and the **Lower Size TI** to 50  $\mu\text{m}$ . Set up the **Count Mode** to 'between' (page 4-2).
- 4.4. Press **Set-up** and screen **S2** will display. Be sure that **Metered Volume** is 1.0 ml.
- 4.5. Press **Output** and screen **A1** will display. In **Result type** field select between **Count** and **Concentration**. Keep in mind that If Count is selected the output will show the number of pollen grains measured in one ml of the diluted concentration. If Concentration is selected the dilution factor is required (page 4-7).
- 4.6. Place the beaker with the pollen dilution on the platform and raise it until the aperture tube, electrode and stirrer are immersed. Stirrer should be on and running at a gentle but effective speed.
- 4.7. Press **Start** to initiate an analysis. Screen **A2** will display while counting, until 1 ml of sample has been analyzed, and output screen **A3** (Concentration) or **A4** (Count) displays.
- 4.8. Output can be saved to the adjacent computer (see Saving data) or typed by hand. In any case, be aware that pressing **Start** will initiate another counting and replace the previous information.
- 4.9. In some cases, the aperture can be obstructed by pollen clumps or other impurities. This will be evident when looking through the aperture-viewing screen. In most cases, pressing **Stop** will be sufficient to unblock the aperture and the sample needs to be recounted by pressing **Start**. If the aperture remains blocked there are two options. Cleaning the orifice by using a small brush or flushing the aperture (page 7-4).

#### 5. Saving data

- 5.1. Turn on the computer and open the **Z2 AccuComp** software (there's a shortcut on the desktop).
- 5.2. Before acquiring data from the Coulter counter select the 'Enter Sample Info' icon from the toolbar to enter the sample info.



- 5.3. Select the 'Acquire Data from Z2' icon from the toolbar. The software will acquire the information displayed on the coulter counter screen. A Saving window will automatically open. Select the directory in which you want to save your file and re-check the file name.
- 5.4. Press **Save**, wait until computer have finished acquiring the data and close the output window. You are now ready to run the next sample.
- 5.5. Reports can be printed.
- 5.6. Files are only accessible by using the Z2 AccuComp software.

## 6. Shutdown procedures (page 4-16)

- 6.1. It is recommended to prime the aperture at the end of the day. Replace the last sample with a new beaker with 50 ml of Isoton II diluent. Press **Functions** and screen **F1** will display. At the line **Prime/Fill/Drain/Flush/Vent**, use the cursor key to select **Prime Aperture**. Press **Start** two times to initiate the process. At completion, screen **F1** is again displayed with the status **Aperture Primed** (page 4-21).
- 6.2. Turn of the coulter counter.
- 6.3. Place a beaker with cleaning solution or Isoton II diluent on the platform and raise it until the aperture tube, electrode and stirrer are immersed. **The aperture tube NEEDS to remain submerged.**
- 6.4. Clean the coulter counter as appropriate as well as beakers and other materials used. Return used materials to their original places.
- 6.5. Remember to sign in the Coulter Counter user log sheet.

**If you need assistance with the Coulter counter or supplies, or have any further questions please contact Maria Eckerman.**