

## Seed Morphology & Pigmentation Controlled Vocabulary Scoring Protocol

**Note:** For examples of seeds that correspond to our different controlled vocabulary terms see [this pdf](#).

### Equipment needed:

- seed stocks to be assayed
- empty seed tube box
- microscope paper stage guide/image background
- blunt-tipped forceps
- tube cap removal tool
- weigh paper (glassine) for seeds which need to be returned to the seed stock tube
- beaker & biohazard bag for discarding scored seeds
- computer with Ethernet connection and Spot Advanced software program
- portable memory stick with at least 30 MB free space
- Leica dissecting microscope (model MZ12-5) with 1x objective, polarizing lens and attached camera
- bar code scanner

### Microscope and lighting set-up

1. Remove the microscope cover and screw on the polarizing lens. **DO NOT INVERT THE LENS -- IT IS NOT ATTACHED TO THE HOUSING AND WILL FALL OUT!** The lens fits over the 1x objective at the bottom of the microscope and tightens with a thumb screw.
2. Remove the dual-beam external light source from the electric supply box and screw in the electrical jack of the polarizing lens. Adjust the light intensity on the electric supply box to exactly "70."
3. Set the microscope coarse magnification setting to 2.0
4. Turn on the camera (top of the microscope)

### Image capture set-up

1. Attach the bar code scanner to the computer.
2. Log onto the computer and make sure the wireless Ethernet connection is working properly (green and yellow "D" in Task Manager bar).
3. Create a folder for storing the seed images on the computer desktop.
4. Open the Seed Morphology Database (<http://rtsf.msu.edu/cgi-bin/seedmorph/seedmorph.cgi>), log in, choose Analyze, and then minimize the database.
5. Choose the "**SPOT Advanced**" desktop icon to open the image-capturing software.
6. In the lower right corner of the program screen, choose the "2010" protocol for photos. Adjust the live image appearance:
  - Gain: 16
  - Gamma: 1.74
  - Time: 600

### Viewing and capturing images

1. Turn off any overhead room lights. Maximize the Seed Morphology Database. To begin the database session, scan in the seed box barcode. Minimize the database and maximize the Spot Advanced program.
2. Reset the white balance after pulling out the light splitting bar:
  - a. Click camera >> Computer white balance values
  - b. Place a white background under the lens (a blank piece of white paper works well)
  - c. Click Begin >> Save in the 2010 protocol
3. Place black area of the microscope stage guide under microscope and rotate the plastic polarizing ring on the light source until the black area appears black and solid when viewed through the microscope.
4. Remove the first seed stock tube from the seed box, scan its barcode into the database, and place the tube in the working rack.
5. Open the seed tube, tap 40-50 seeds onto the sample placement guide, and remove any large pieces of chaff with a forceps. Using the forceps, distribute the seeds evenly.
6. Push in the light splitting bar for optimal viewing of the seeds via the ocular lenses. (In the "out" position, the light is split between the camera and microscope.) On the computer, click on the "Live" icon (top right).
7. Focus the image using the fine focus knob on the microscope. For photography, it is usually necessary to focus while viewing the computer monitor rather than through the microscope ocular lenses. There is a significant lag time between focusing and what is viewed on the monitor.
8. Slide the light splitting bar out before capturing the image (all light goes to the camera).
9. Select the Camera icon on the Spot Advanced window (upper right) to capture the image. Minimize the live image and view the captured image. Under Edit, choose Add Calibration Mark (a yellow horizontal bar representing ~500 microns). Move the bar with the left mouse button and position it in the top right portion of the image. Choose "Stamp" to set the bar and "lock" it in place.
10. Save the image as a .jpeg file in the desktop image folder:
  - a. File >> Save
  - b. Scan in the seed tube barcode as the file name
  - c. Be sure the file format suffix is .jpeg
  - d. Minimize the image, but do not close it until the sample is scored in the database.
11. After the image is saved, maximize the Seed Morphology Database, and score the seeds using the web form.
12. Close the image just scored.
13. Discard the scored seed sample into a small waste beaker. At the end of the assay, dispose all seed waste into a biohazard bag.
14. Place the seed stock tube into its corresponding position in the empty rack.
15. Repeat steps #3-13 for all seed stocks within the box. *Note: if a very small amount of seed is present in the tube, add the seeds to a new, unused sample placement guide, score them, and rather than discarding them afterwards, return them to the*

*seed stock tube. Make sure no other stray seeds are stuck on the forceps before this is done.*

16. When the box is complete, close the session of the database by scanning the Seed Stock box barcode. Log out of the Seed Morphology Database. The box need not be finished to close; it can be completed in multiple sessions if necessary.
17. Exit the SPOT Advanced program.
18. Copy the folder of seed images from the desktop folder onto a memory stick. Check to ensure that the images copied properly, then delete the seed image folder from the computer desktop.
19. Log off the computer, ***but do not turn off the computer! Turn off only the monitor!***
20. Turn off the camera and electric supply to the microscope. It is not necessary to remove the polarizing lens. When the camera has cooled sufficiently, replace the microscope cover.

## **Controlled Vocabulary**

The norm is defined by what is primarily normal or typical for the entire seed box, which contains seed stocks of 96 plants all grown at the same time and under the same conditions. This norm is established by viewing all the seed stocks in a box at one time by putting a small sample of each seed stock in a clear microtiter plate and quickly scanning through all the seeds in a box.

View the entire area of the distributed seed sample on the microscope stage guide. Examine the seeds using both the microscope and monitor. Score the seeds based on the characteristics of the majority of that sample's seeds to define size, color and morphology, *i.e.* ignore phenotypes represented by less than 10 seeds per ~50 seed sample. Even within a "normal" wild-type population of seeds, there are small variations in size and color due to slightly different maturation times of the seeds.

*For specific example, please refer to the file "Seed Morphology and Pigmentation phenotype examples."*

## Pigmentation

- Homogeneous population – majority of seeds are of a uniform color (default selection)
- Heterogeneous – at least 25% of the seeds are a different color variation (either lighter or darker, or a specific seed color)
- Seed coat color: normal is the default selection. Seeds can be lighter or darker than normal. When appropriate, seeds can be further characterized for color (purple, yellow or green).
- Seeds evenly colored (default selection) – if individual seeds are not uniform in surface color, select "color variations"
- Seed coat surface: this refers to characteristic of the seed coat not pertaining exactly to color or morphology (normal is the default selection). Abnormal findings include *shiny* or *dull*. For example, an occasional seed phenotype is dark brown with a waxy

appearance, but not aborted. These seeds are characterized as “darker than normal”, and “dull.”

Morphology:

- Homogeneous population – majority of seeds are of a uniform size and shape (default selection)
- Heterogeneous population – at least 25% of the seeds in a particular seed stock have some size or shape differences from the norm for that box of seeds
- Size: normal is the default selection. Seeds can be larger or smaller than normal.
- Shape: normal is the default selection. If the Abnormal box is selected, describe the abnormal shape by selecting one or more of the following: round, elongated, wrinkled, or aborted. An aborted seed appears smaller, very dark brown and shriveled.

There may be instances when there is an observable range of pigmentation or size/morphology in a seed sample which falls outside of the box norm, but the differences are not distinct enough to warrant specific vocabulary choices. In these cases, it is acceptable to score pigmentation and/or morphology as heterogeneous, without further descriptions being chosen. This will at least flag the sample as being different from the norm for that seed stock box, and allow further studies as necessary.