User guide for counting oyster seed using open-source software and hardware

This user guide contains instructions on how to use open-source software and hardware to assist with counting oyster seed in a nursery. Through the use of open-source technology, this project aimed to increase the accuracy and efficiency of counting oyster seed. The software component creates an image segmentation program that can accurately count oyster seed, replacing manual counting methods. The hardware component provides physical devices to assist with consistently capturing high-quality images for software analysis.

The software and hardware components are meant to be used in combination. The relevant files can be downloaded from the following GitHub repositories: software at https://github.com/LSU-Devision/GUI/releases, and hardware at https://github.com/lil-squid/LSU_Seed_Counting_Hardware. This document will take users through the process of downloading the software and hardware files from GitHub, installing the software, 3D-printing the hardware, and using the software and hardware to assist with counting oyster seed.

This project was funded by NOAA (NOAA-OAR-SG-2024-24794) and was developed by Louisiana Sea Grant (under the guidance of Dr. Sarah Bodenstein and Dr. Elizabeth Robinson) with collaboration from the Oyster Team at the LSU Department of Mathematics during the 2024 and 2025 school years.

Table of Contents

Section 1: Downloading and Installing Software

Note: this software will only run on PC or Linux computers and requires 1.5 GB of space to download.

1. Go to https://github.com/LSU-Devision/GUI/releases and click the .zip folder named "v1.5-DV-oyster-windows.zip" to download (Figure 1.1).

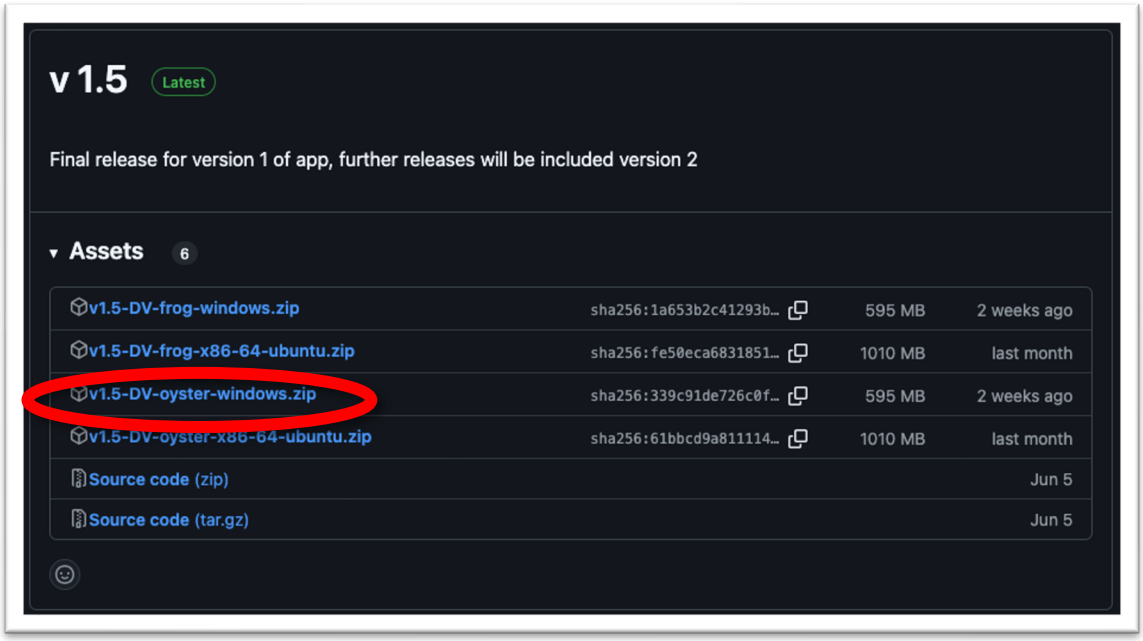


Figure 1.1. The "v1.5-DV-oyster-windows.zip" folder hosted on GitHub.

2. Once the zip folder is downloaded, it is recommended you move it out of "Downloads" and into your computer's "Desktop" or "Documents" directories.

3. Double click "v1.5-DV-oyster-windows.zip" and inside you should see a folder named "main". Open this folder and inside you should see the files shown Figure 1.2.

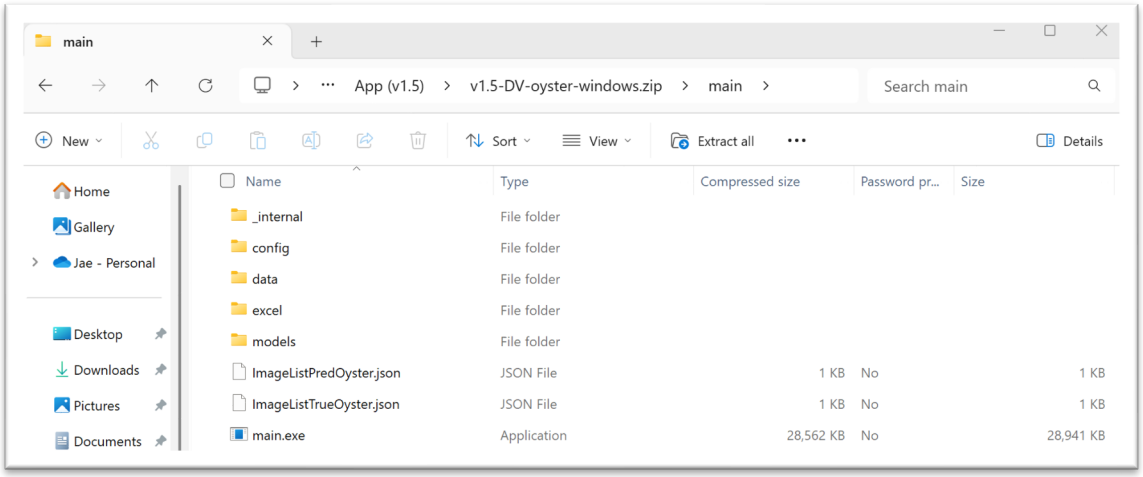


Figure 1.2. The files inside of the "main" folder.

4. Double click on “main.exe” to launch the software. A pop-up window will appear, click “Extract all” (Figure 1.3).

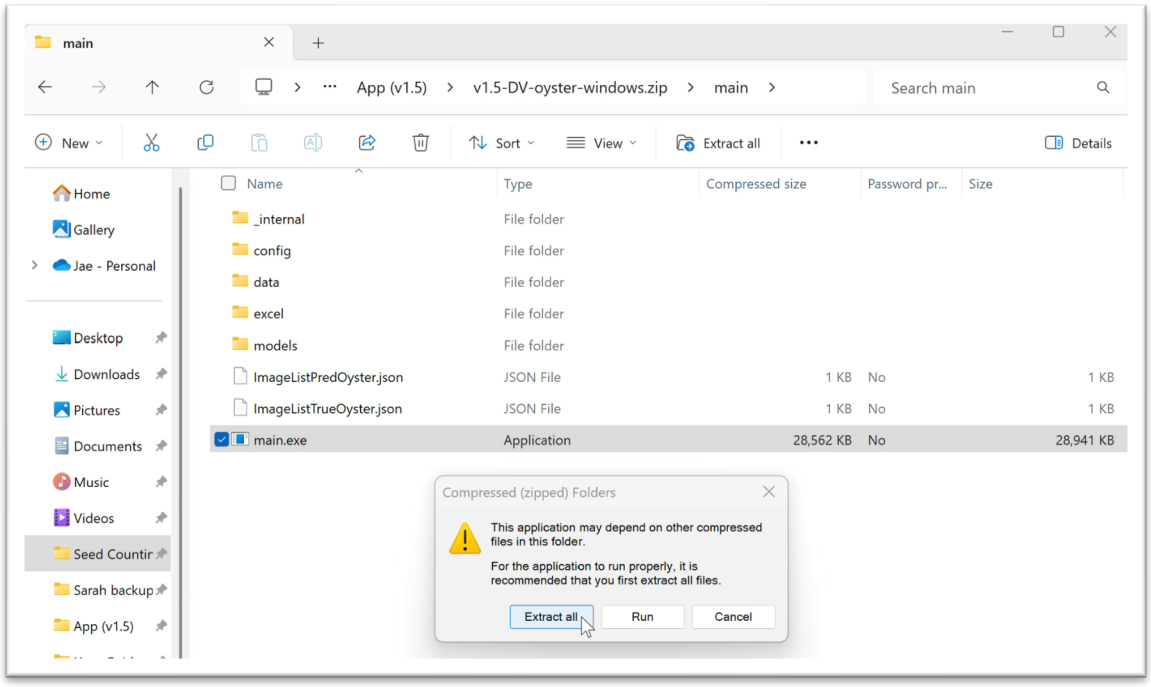


Figure 1.3. Extracting files to run main.exe.

5. Save the extracted files to the default folder your computer suggests (it should be the same folder you are currently in) by clicking “Extract” (Figure 1.4).

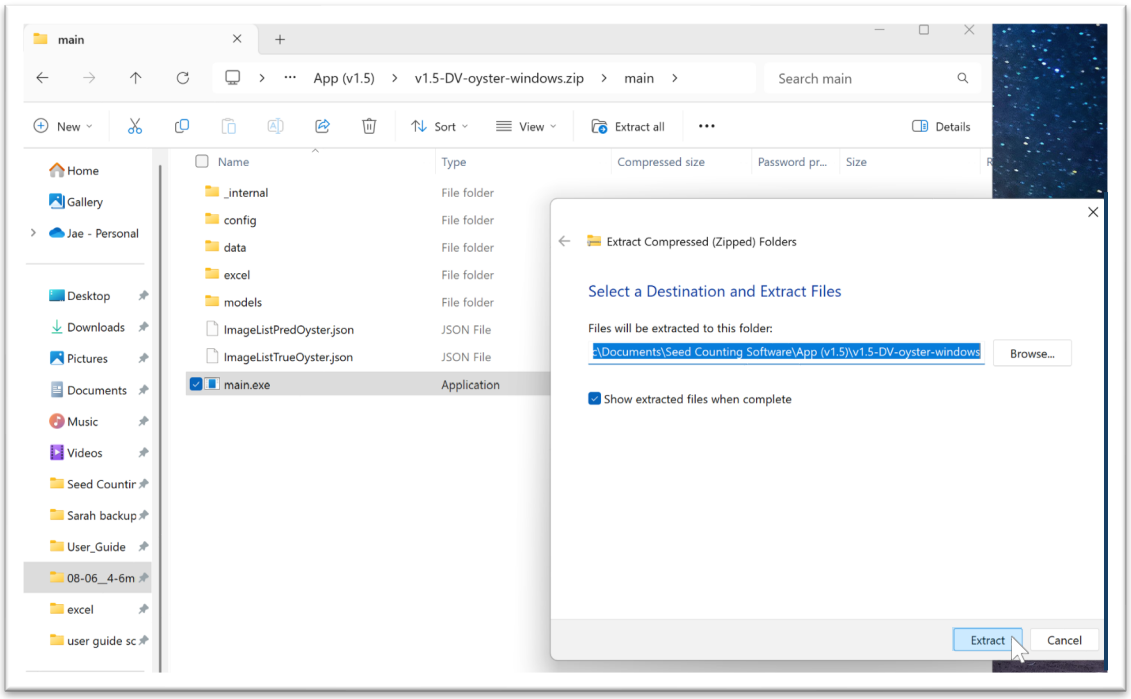


Figure 1.4. Saving extracted files.

6. The files will be extracted and saved to your computer (Figure 1.5). This may take several minutes.

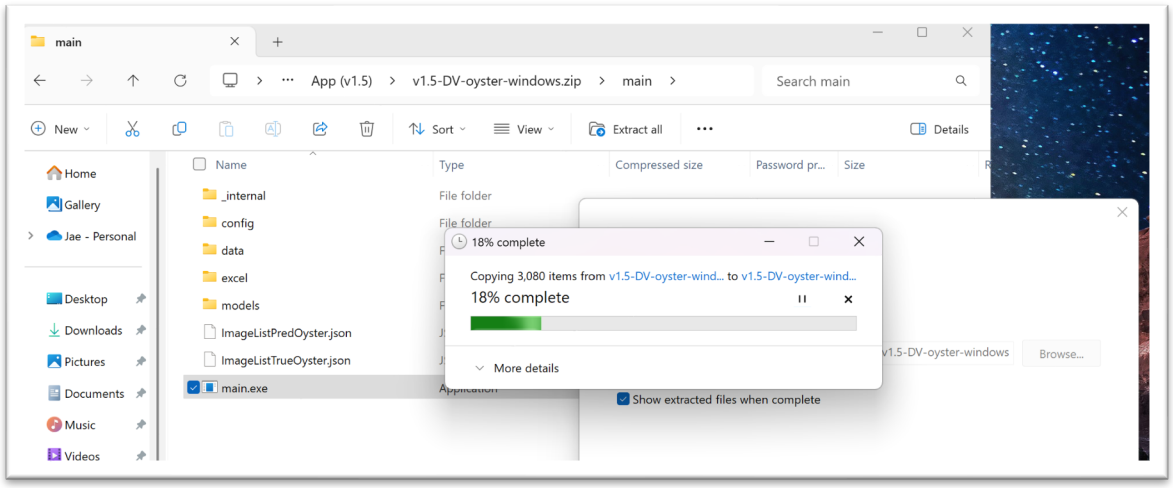


Figure 1.5. The files being extracted.

7. Go back to the folder you saved the "v1.5-DV-oyster-windows.zip" file in. There should now be a folder called "v1.5-DV-oyster-windows". Open that folder and then open the "main" folder within. Inside you should see the files shown Figure 1.6. Create a new folder called "images" in the main folder to load future subsample images. Inside the images folder, create a new folder called "annotations" to store the future images the software has analyzed.

8. Right click on "main.exe" and click "Run as administrator (Figure 1.6).

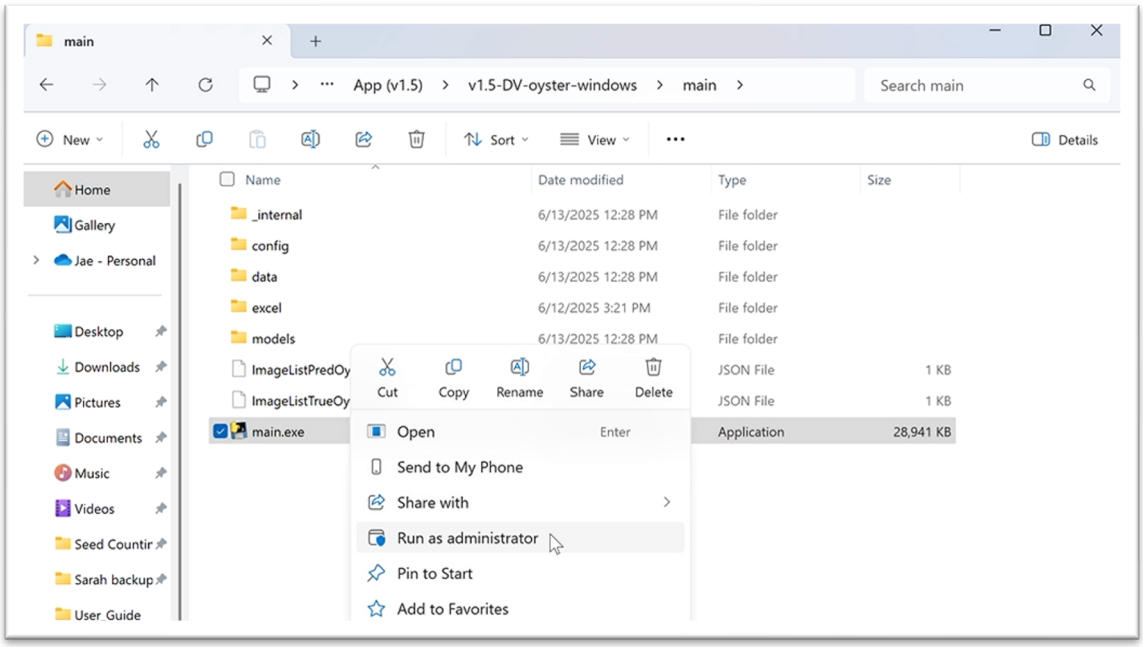


Figure 1.6. Running the software as an administrator.

9. Your computer may indicate that this software is “unrecognized”, click “Run anyway”. Your computer will ask if the software can make changes, click “Yes”.

10. A black command window will appear and begin to install the necessary programs to run the software. Then the main software window will appear (Figure 1.7).

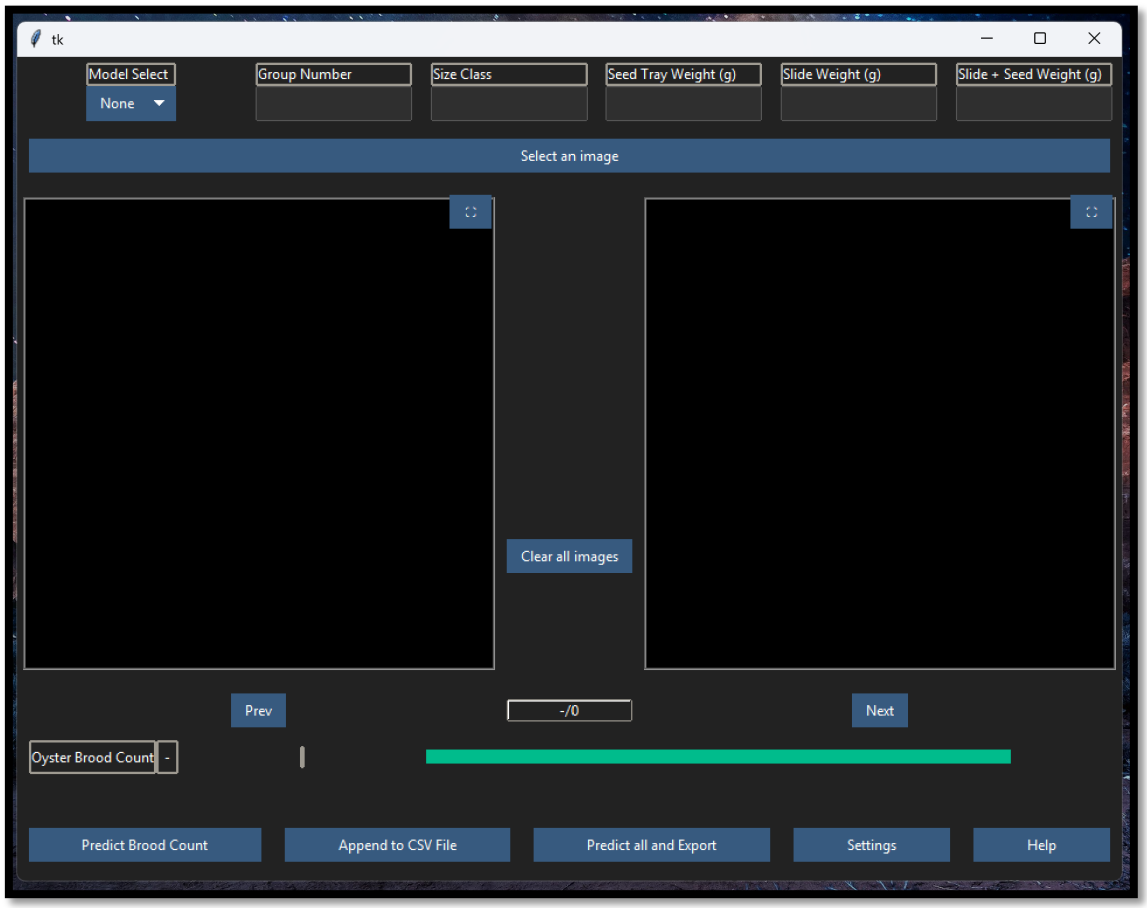


Figure 1.7. The open software window.

Section 2: Downloading and 3D-Printing Hardware

1. To download the 3D-printable hardware files, go to: https://github.com/lil-squid/LSU_Seed_Counting_Hardware/tree/main

2. Navigate to the "Print-Files" folder (Figure 2.1) and there you will find instructions on how to download and print the files (see Print Instructions), and the following 3D files:
    a. a set of scoops (2, 5, and 10 mL volumes) for collecting seed subsamples and placing them on counting slides or weigh boats
    b. a circular counting slide for counting 4 – 6mm seed,
    c. and the top, bottom, and posts to construct a phone stand to capture consistent images of seed subsamples.

3. To download files, click on the file name you want to download. Once you are on the file page click the three dots (Figure 2.1) in the upper righthand corner of the page and select "Download". The file should appear in the Downloads folder on your computer.

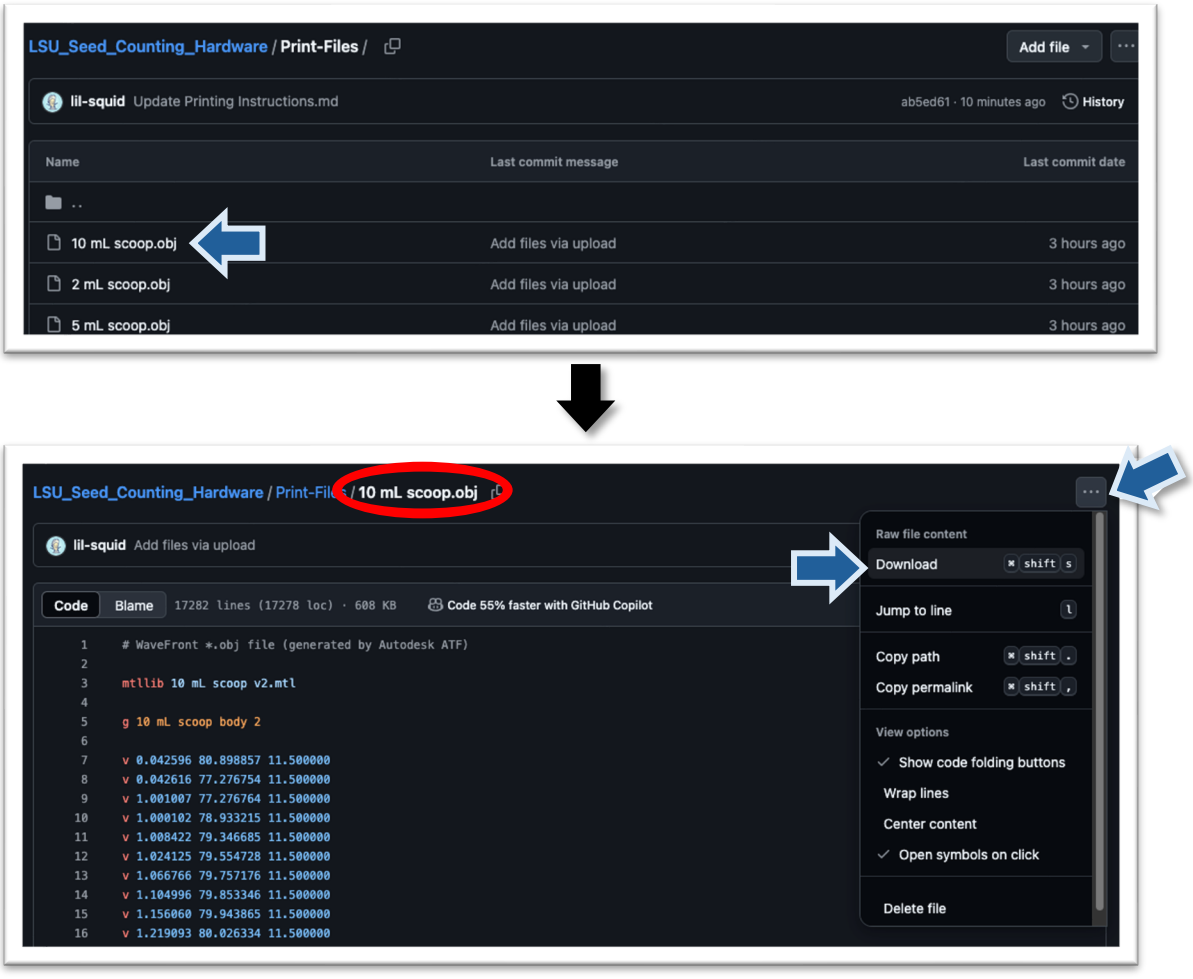


Figure 2.1. Click the name of the file you want to download (top image) and then click the three dots in the upper righthand corner and select download (bottom image).

4. Full instructions on how to prepare downloaded files for printing can be found in the Print-Files folder in the Print Instructions.md document.

5. In short, “slicer” software is needed to convert the downloaded .obj files into instructions a 3D printer can understand, called G-code files. The recommended slicer is Cura by Ultimaker (https://ultimaker.com/software/ultimaker-cura), a free and easy to use option.

6. For more information on how to use Cura, please visit: https://ultimaker.com/learn/how-to-use-a-3d-printer/

7. For more information on how to use a 3D printer, please visit: https://www.creality.com/blog/how-to-use-a-3d-printer

Section 3: Collecting Seed Data using 3D-printed Hardware

Note: the software can only count seed in the 2 – 4 mm and 4 – 6 mm size classes

1. After installing the software and 3D-printing your hardware devices, clean and size grade your seed. Make sure that seed is collected into individual sieves by size class and genetic or ploidy group (Figure 3.1). Shake out excess water and allow sieves to drain for 15 – 20 minutes.

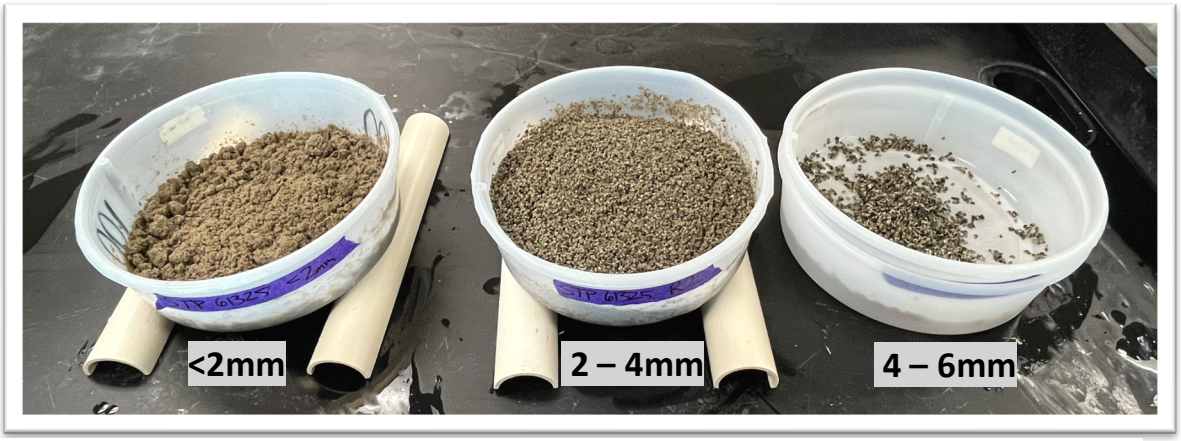


Figure 3.1. Three size classes of oyster seed sorted into individual sieves.

2. Gather the necessary supplies and 3D-printed devices to assist with seed counting (Figure 3.2). The following supplies are recommended (3D-printed devices are indicated with red text):
    a. A bench scale with a capacity of at least 3,000 g.
    b. Counting slides or weigh boats to hold seed subsamples. A **circular counting slide** is available for download on GitHub. This slide can be used to hold seed subsamples in the 2 – 4mm and 4 – 6mm size classes. The slide must be printed with dark filament to provide high contrast when capturing images.
        i. Non-3D-printed options include a plastic counting slide for 2 – 4mm and aluminum weigh boats (65 mL) for 4 – 6mm seed. Slides and weigh boats must have dark backgrounds for high-contrast images. It is recommended to cover aluminum weigh boats in dark tape.
    c. Scoops or spatulas to collect seed subsamples. **Scoops with 2, 5, and 10 mL** volumes are available for download on GitHub. Scoops assist with sampling consistent quantities of seed and ensure that seed are not overcrowded on the counting slide. The 2mL scoop is recommended for 2 – 4mm seed, and the 5 or 10 mL scoops are recommended for 4 – 6mm seed. Scoops can also assist with spreading seed out on the slide before capturing the image.
        i. Non-3D-printed options include laboratory scoop and spatula sets.
    d. Labeling tape to keep track of which seed group each subsample below to.

e. A phone stand to ensure that captured images are of consistent quality. Parts to print and build a **phone stand** are available for download on GitHub. It is recommended to avoid using zoom features on your smartphone when capturing images and to take photos from approximately 5 – 6 inches away from the slide.
    i. Images can be captured without a phone stand, but for best results captured images should be taken from a consistent distance from the counting slide.

f. A smartphone to capture samples images.

g. A computer with the seed counting software installed.

3. The Total number of seed in each group will be calculated using $Equation\ 1$ below:

$Equation\ 1$

$$Total\ Number\ of\ seed\ = \frac{Subsample\ Count}{(Slide\ \&\ Seed\ Weight) - Slide\ Weight} \times Seed\ Tray\ Weight$$

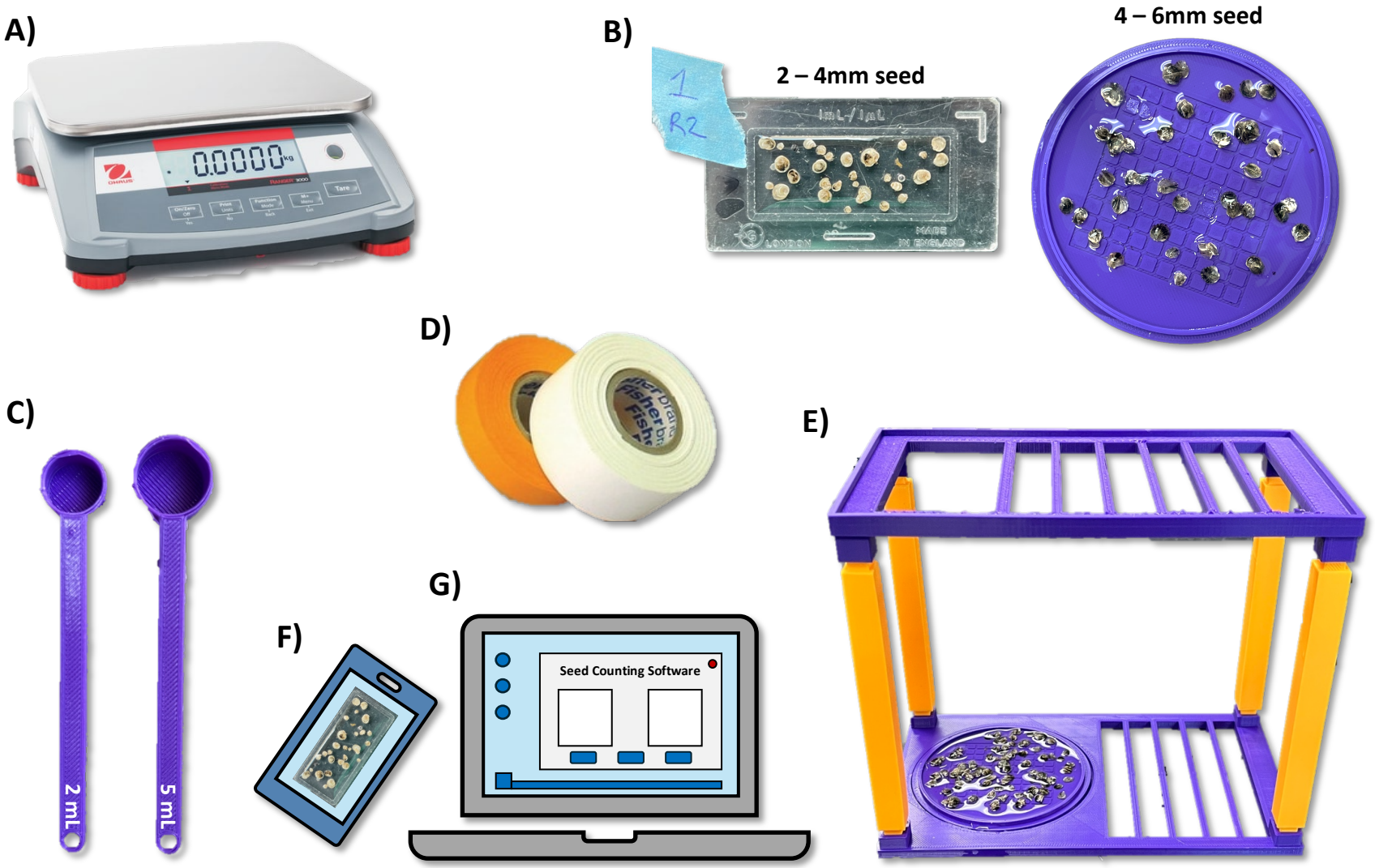


Figure 3.2. The recommended supplies, including 3D-printed hardware, for counting oyster seed: A) A benchtop scale, B) seed counting slide options, including a plastic counting slide (left) and 3D-printed counting slide (right), C) 3D-printed scoops, D) labeling tape, E) a 3D-printed phone stand, F) a smartphone to capture images, and G) a computer with the seed counting software installed.

4. After allowing water to drain from the sieves for 15–20 minutes, tare the scale with an empty, dry sieve, then weigh the sieve containing the seed from the first group and size class. This is the $Seed\ Tray\ Weight$ for $Equation\ 1$.

5. Weigh an empty counting slide or weigh boat. This is the $Slide\ Weight$ for $Equation\ 1$. You can also create a label with labeling tape to help keep track of each subsample.

6. Collect a small subsample of seed from the sieve, place on the slide or weigh boat, and weigh. This is the $Slide\ \&\ Seed\ Weight$ for $Equation\ 1$. Make sure to record all weights to input into the software.

7. Spread out the seed on the slide or weigh boat to minimize overlapping seed. If using the 3D-printed phone stand, place the circular slide on the bottom rack and the phone facing down on the top rack. Take a picture of the subsample with a smartphone. Before uploading to a computer, crop the image to remove excess background noise from the image (Figure 3.3).
    - Note: The slide or weigh boat should have a dark background so the seed stand out in the picture.

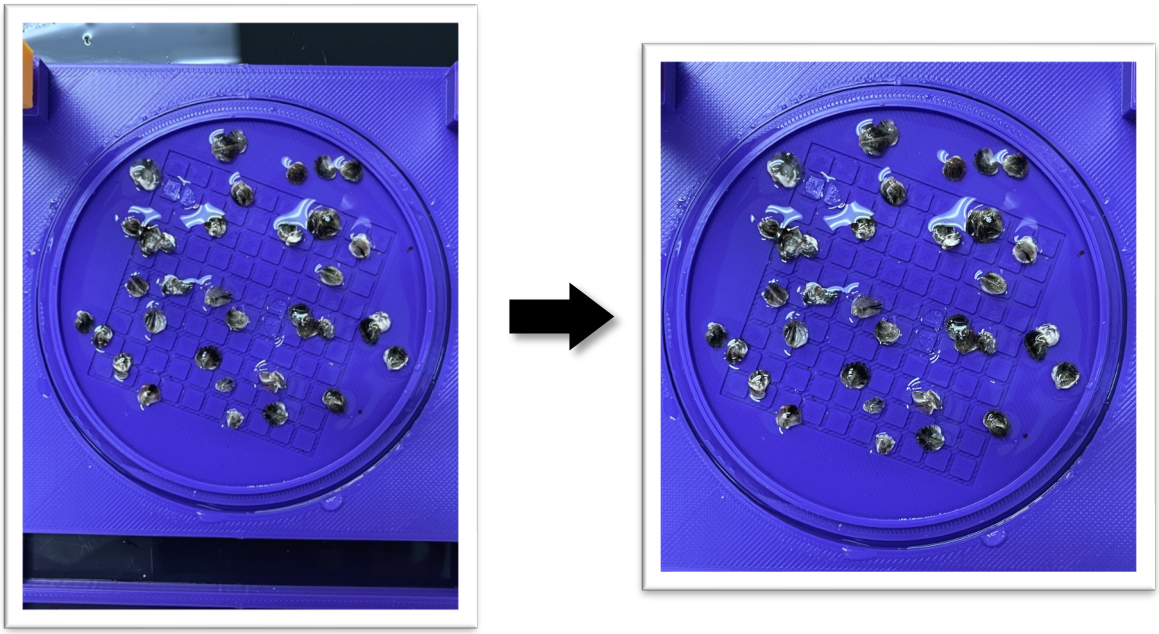


Figure 3.3. On the left is a picture taken of a seed subsample in the 4 – 6 mm size class on the 3D-printed circular counting slide. The seed have been properly spread out to minimize overlapping. On the right is a suggested crop of the image to reduce the chances of the software mistakenly counting background clutter as seed.

8. Collect 2 – 3 additional subsamples from the same sieve (i.e. from the same group of seed) and repeat Steps 5 – 7.

9. If there are other groups of seed (i.e., other sieves) for which the total seed count needs to be estimated, repeat Steps 4 – 8.

Section 4: Counting Oyster Seed using the Software

1. Open the main folder and transfer the subsample images you captured into the images folder. Make sure images are labeled for easy identification, which could include subsample number, group name, and date.

2. Launch the software by right-clicking on “main.exe” and running the program as an administrator. Click “Yes” when your computer asks if the software can make changes. The software must be open this way every time.

3. First, the command window will appear, followed by a new window displaying the seed counting software (Figure 4.1).

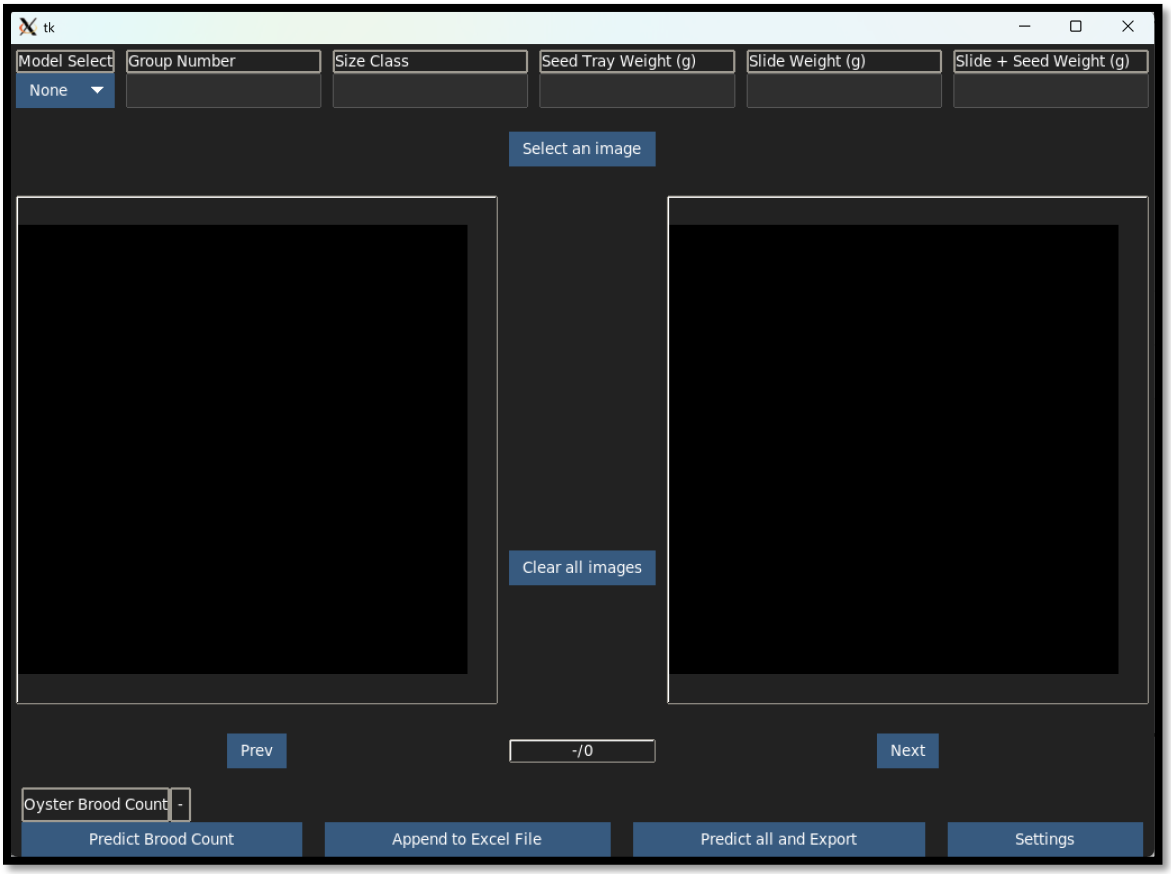


Figure 4.1. The open seed counting software window.

4. Upon first use, click the Settings button in the lower right-hand corner to open the settings menu. In the menu, go to the Defaults tab. Double-click the space next to “CSV Output Directory” and select the folder named excel within the main folder in the pop-up window (Figure 4.2). Next, double-click the space next to “Annotation Output Directory” and select the annotations folder you created inside the images folder. Finally, for the recommended settings, double-click the active button next to “Auto-export CSV file”, “New CSV when clearing”, and “Auto-save images” to change them to inactive.

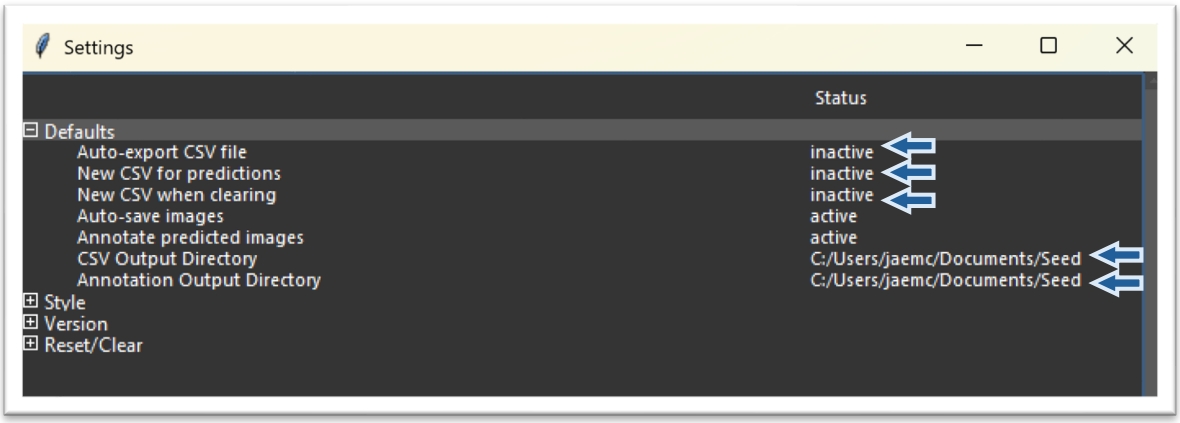


Figure 4.2. The settings window with recommended changes indicated with arrows.

5. After closing the Settings window, click on “Select an image” located near the top center of the screen (Figure 4.1). This will open a pop-up window where you can choose the images you want to analyze from your “images” folder. Multiple images can be selected at once. Once selected and loaded into the software, you should see the image on the lefthand side of the screen. You can cycle through and view all images by clicking “Prev” and “Next”.

6. For each image, in the upper lefthand corner, click the drop-down menu under “Model Select” and select which size seed you’d like the software to count. Select either the 2 – 4mm model or the 4 – 6mm model.

7. Label each image with a Group Number (what group the subsample belongs to, must be labeled with numerals only) and Size Class at the top of the window. Also for each image, enter the weights for the total seed quantity of the group ($Seed\ Tray\ Weight$), the slide ($Slide\ Weight$), and the slide with seed ($Slide\ \&\ Seed\ Weight$).

8. Once all images are loaded and labeled, go back to the first image and click “Predict Brood Count” on the left bottom side of the window. The command window will display the progress as the seed count is being predicted. It will take ~30 – 45 seconds to analyze each image.

9. When the analysis is finished, an annotated version of the image will appear on the righthand side of the screen and the predicted count will appear near the bottom lefthand corner of the screen (Figure 4.3). Annotated images show which seed were counted by outlining them in red. Annotated images should be autaomatically saved in the "annotations" folder.

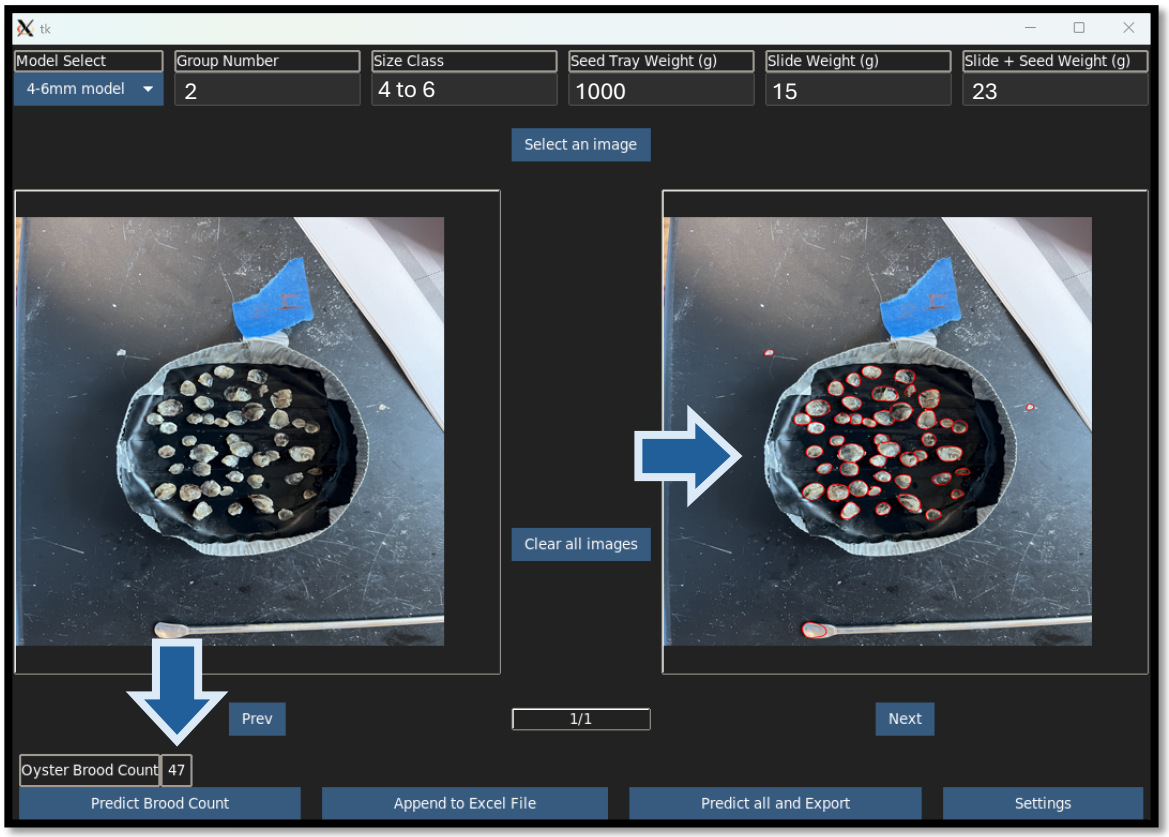


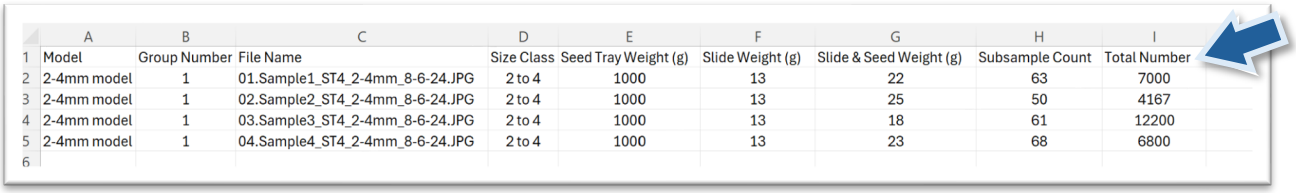
Figure 4.3. The seed counting software displays the original image on the left and the annotated image on the right (marked with an arrow) with counted seed outlined in red. The predicted seed count appears in the bottom left corner, marked with an arrow. Note: In this example, the software has incorrectly identified several pieces of background clutter as seed.

10. Repeat Steps 8 – 9 for each image loaded into the software.

11. Once all images have been analyzed, click "Predict all and Export" at the bottom of the screen. This will create a CSV file in the "excel" folder that includes: the data you assigned to each image, the predicted seed count, and the estimated total number of seed in the group based on the recorded weights.

12. To view the results, navigate to the excel folder and open the newly created CSV file. The file will be named something similar to "data01.csv".
    - Note: The CSV files with "stats" in their name do not contain data for the predicted total number of seed.

13. The CSV file will contain the Model used to analyze each image, the Group Number, and the File Name. It will also include the entered data: Size Class, Seed Tray Weight (g), Slide Weight (g), and Slide & Seed Weight (g). Finally, it will list the predicted Subsample Counts and the Total Number of seed based on those predictions (Figure 4.4). The equation used to calculate the Total Number of seed is shown below (Equation 1).



	A	B	C	D	E	F	G	H	I
1	Model	Group Number	File Name	Size Class	Seed Tray Weight (g)	Slide Weight (g)	Slide & Seed Weight (g)	Subsample Count	Total Number
2	2-4mm model	1	01.Sample1_ST4_2-4mm_8-6-24.JPG	2 to 4	1000	13	22	63	7000
3	2-4mm model	1	02.Sample2_ST4_2-4mm_8-6-24.JPG	2 to 4	1000	13	25	50	4167
4	2-4mm model	1	03.Sample3_ST4_2-4mm_8-6-24.JPG	2 to 4	1000	13	18	61	12200
5	2-4mm model	1	04.Sample4_ST4_2-4mm_8-6-24.JPG	2 to 4	1000	13	23	68	6800
6									

Figure 4.4. The Excel file output including the Total Number of seed calculated (marked with an arrow).

$$Equation\ 1$$

$$Total\ Number\ of\ seed\ = \frac{Subsample\ Count}{(Slide\ \&\ Seed\ Weight) - Slide\ Weight} \times Seed\ Tray\ Weight$$

14. Once all images have been analyzed, exit the software window and the command window by click “X” in the top left corner.