

# DNA Learning Center BARCODING 101

## Guide to AmScope SM Series Dissecting Scope for Sample Imaging with MU Series Camera

*The following guide is an adaptation of the [AmScope Manual](#) for use with DNA barcoding samples. Please refer to the official manual for additional detailed instructions for microscope, camera, and software use, or refer to the [product's website](#).*

### **Helpful tips before using the microscope for imaging**

1. It is best to take images of specimens immediately after collection while alive (preferable) or freshly expired. Over time, expired specimens may change in color, shape, size, etc., thereby making identification difficult or impossible.
2. Highly mobile organisms may be placed at 4°C for approximately 10 minutes to slow them down enough to take high quality photos. If pictures continue to turn out blurry due to organism mobility, take additional pictures shortly after the organism expires. A specimen may be stored in a freezer from 2 hours to overnight to euthanize.
3. Short-term storage of the specimen in the freezer in an appropriately-sized tube will help to ensure that the specimen retains most of its color and flexibility if additional pictures are required. Soft-bodied specimens, such as caterpillars, should be stored exclusively in ethanol. Freezing may cause these samples to burst.
4. Long-term storage of samples in 99%+ ethanol is preferred for preservation and DNA barcoding, though this may cause drastic morphological changes resulting in low quality specimen images. If it becomes necessary to image organisms post-ethanol preservation, please refer to recommendations below:
  - a. Place the specimen into a petri dish and add enough ethanol so that the specimen is fully submerged. This will eliminate much of the glare and light refraction caused by liquid clinging to the surface of the specimen. *Glare will interfere with the clarity of images and should be avoided.*
  - b. Specimens in ethanol will become brittle and break more easily when handled. If your specimen breaks, be sure to keep the bits together after imaging.
  - c. Use fresh ethanol for each new sample to avoid contamination.
  - d. Don't allow specimens to dry out and desiccate! *Ethanol evaporates rapidly.*

Refer to the following figures for microscope terminology in the accompanying **Guide**:

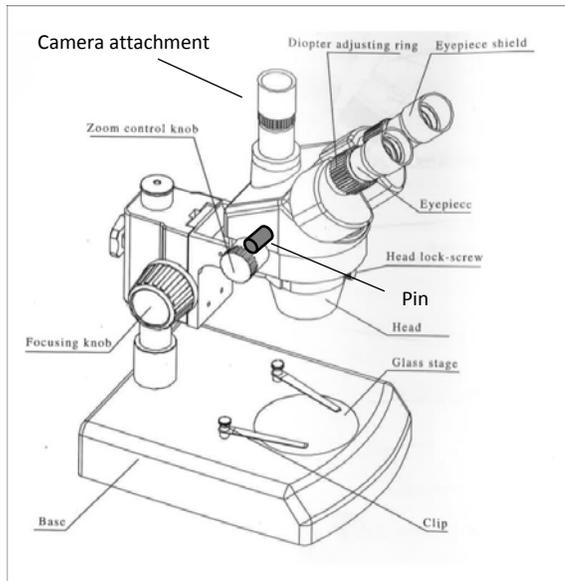


Figure 1

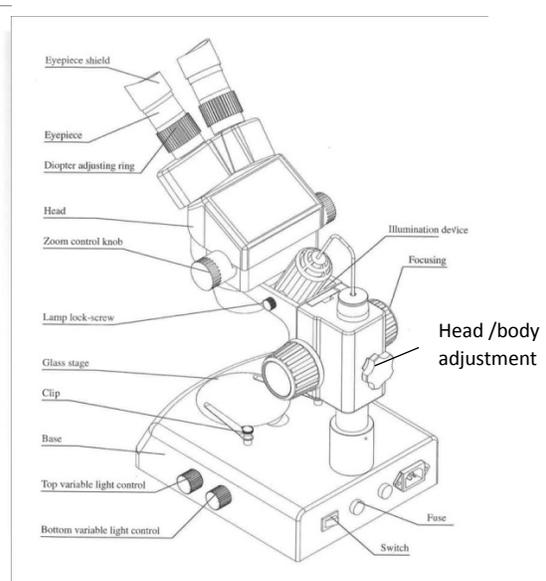


Figure 2

*\*Note: Prior to using the AmScope SM series dissecting scopes, the AmScope imaging driver and software need to be installed on your computer. Refer to the [AmScope Manual](#) for download and installation assistance.*

**Bolded red** terms throughout the guide appear in the associated figures above.

### **Dissecting Scope Set-up**

1. Plug the microscope's power cord into an outlet and connect the USB cable to the computer's USB port.
  - a. The camera should fit into the trinocular tube on the top of the microscope **head** at the **camera attachment** site (see Figure 1).
  - b. The USB cord should be connected to the camera directly.
2. Turn on the microscope using the **switch** on the back of the **base** (see Figure 2) and check if the lights are working.
  - a. Note that there is an upper **illumination device** and a lower illuminator beneath the **glass stage**/stage plate (see Figure 2).
  - b. Light intensity may be controlled for each device using the **top** and **bottom variable light controls**, respectively (see Figure 2).
  - c. The upper **illumination device** may be removed using the **lamp lock-screw** on the side of the neck of the scope (see Figure 2). This is helpful when trying to angle the light better when taking specimen images.
  - d. On the left side of the microscope **head** just in front of the **zoom control knob**, there is a silver **pin** (see Figure 1). Make sure this **pin** is pulled out from the head; if the **pin** is pushed in, the sample will not be visible through the camera.

3. The scope has an accompanying set of stage plates, which may be more or less useful depending on the type of specimen being imaged. They can be easily switched in and out as needed.
  - a. *Double-sided opaque white and black stage* – This stage allows no light from the lower illuminator to pass. This is generally the most useful when it comes to taking good surface images.
  - b. *Diffusing white plastic stage* – This stage allows some light from the lower illuminator to pass through. This is useful for seeing light or partially obscured body structures in silhouette.
  - c. *Glass stage* – This stage allows the most light from the lower illuminator to pass through. This can overpower images and obscure specimens, but may be helpful when used with an appropriate amount of upper illumination to visualize certain structures.
4. To focus a specimen, there are several adjustment tools:
  - a. **Head/body adjustment knob** – affixes the **head** and body to the microscope's pillar, which connects the **head** and body to the **base**. Loosen this knob and the **head** can be moved up and down (see Figure 2). This is useful when trying to localize the specimen in the scope's field of view. This usually does not have to be adjusted unless switching between very large and very small specimens.
  - b. **Focusing knob** – moves the head up and down at a fixed position on the pillar (see Figure 1). As the name implies, this focuses the image.
  - c. **Zoom control knob** – allows the scope user to zoom in or zoom out on a focused image (see Figure 1).
  - d. **Eyepiece** – the eyepiece can be spread apart or pushed closer together to adjust to the user's comfort (see Figure 1).
  - e. **Diopter adjusting rings** – these allow for the scope user to compensate for the differences between the user's two eyes. These can be adjusted for the user's comfort (see Figure 1).

### **Focusing with the Microscope**

1. Before attempting to image a specimen, get a feel for the specimen under the scope. Plan out what characteristics need to be imaged. Refer to the **Collection, Preservation, and Imaging Recommendations for Accurate Taxonomic Identification** guide (on [DNABarcoding101.org](http://DNABarcoding101.org)) to determine what structures should be photographed.
2. Place a specimen in a sterile Petri dish and place on the **stage** of the microscope. If the specimen is relatively immobile, keep the lid off the Petri dish to avoid glare from illumination.
3. First, find the specimen using the **head/body adjustment knob** (see Figure 2). Ensure that the specimen appears, even if it is blurry, in the field of view.

4. Next, use the **zoom control knob** to bring the specimen or specimen's feature into an appropriate field of view (see Figure 1). For an appropriate image, the specimen should occupy no less than 50% of the field of view.
5. Finally, use the **focusing knob** to focus the image appropriately (see Figure 1).
  - a. Note that for smaller specimens, it may be impossible to focus on one structure without losing focus on another. This is fine, but be prepared to take multiple *clear* images to capture all of the organism's important features.
  - b. By alternating between the **zoom control** and **focusing knob**, students should be able to image several features of a specimen without moving the specimen much (see Figure 1).
  - c. Use a *sterile* pair of forceps to maneuver the specimen around, as needed.

### Preparing the MU camera and imaging software

1. Open the AmScope program using the yellow "A" icon (likely downloaded to the desktop after software was installed) when using Windows and the yellow "T" icon when using macOS.
2. Pull out the silver **pin** on the left side of the microscope **head** just in front of the **zoom control knob** as far as it can go (see Figure 1). This will allow for camera feed.
3. On the main screen of the AmScope program, locate the "camera list" on the left side of the screen. Click on the camera name "MU1403" and a live video feed will appear in the window. A list of selections and settings adjustments is available to the left of the feed. If an image fails to appear, refer to the "Technical Image Troubleshooting" section below.

*It is recommended from this point forward that users view the specimen through the video feed in the AmScope program on the computer for focusing and zooming, rather than through the eyepiece.*

4. With the **zoom control knob**, locate the specimen (see Figure 1). If necessary, move the specimen with a pair of sterile forceps until it appears on the camera screen.
  - a. Once the organism appears in the field of view, use the **focusing knob** to further focus the image, and concentrate on specific features, as needed (see Figure 1).
5. If the video feed image has a yellow hue, it can be corrected using the "White Balance" tab on the left side of the program.
  - a. Click the double arrow pulldown menu.
    - i. A red square should be visible on the screen image. Click and drag this to an empty part of the field of view and click "White balance" in the "White Balance" pulldown menu. This should clean up the image. To return to original image, click "Defaults."

## **Imaging with attached MU Series Camera**

*For detailed information on how to use the various features in the AmScope software, refer to the [AmScope Manual](#).*

### ***Specimen Photographs***

1. When ready to image, click the double-arrow next to “Capture & Resolution” on the left side of the screen to access the pulldown menu.
2. Click the “Snap” button in the pulldown menu. A new image tab will generate along the top of the page labelled “0001\*.” Successive images will be labeled “0002\*,” “0003\*,” and so on.
3. Save images:
  - a. Select “File” on the top left of the page in the command bar.
  - b. Click “Save As.” Images should be saved as a .jpg or .jpeg files for upload to the Sample Database. To keep samples organized, save each image with the corresponding full sample ID, a brief description of the image, and the date: (organism)\_(angle the image was taken from)\_(part of specimen)\_(sample ID)\_(date in MMDDYYYY format). Ex: “Ant\_Dorsal\_Leg\_ABC-001\_09102018.jpg.”
  - c. It is recommended to save all photos for each specimen in separate folders to avoid confusion between specimens. Each folder can be placed into one main folder for the project following completion of imaging for all samples.  
*Be sure to keep track of samples in an organized manner; data analysis becomes challenging if specimen pictures do not seemingly match the sequence data.*
  - d. The images are very large files (~12 MP by default), and are too large to upload to the Sample Database. Unfortunately, image size cannot be adjusted in the AmScope app. To resize the image:
    - i. Open the file in a photo-editing program and resize the image to no larger than 4 MP. 4 MP will upload to the Sample Database without error.

### ***Specimen Videos***

4. For highly mobile organisms, it may be easier to record a video and save snapshots from the video as sample images. To record a video, click the “Record” button located next to the “Snap” button in the pulldown menu under “Capture & Resolution.” Snapshots can be taken from the videos in photo-editing software.

### ***Timing and Storage***

5. Repeat appropriate imaging steps for all organisms in your project. Ease of specimen imaging will vary depending upon the type of organism. Be aware that successful imaging can range from 5 min to upwards of 45 min per specimen to capture all important specimen features. *Inadequate images can lead to less useful DNA barcoding data. Successful imaging is extremely important for projects.*
6. Place specimens in ethanol and/or at -20° C for storage before beginning DNA isolation.

## **Troubleshooting Guide**

### ***Technical Imaging Troubleshooting***

For technical issues with the microscope or camera, refer to common problems below with associated resolutions.

#### *The specimen isn't visible in AmScope program on the computer*

- The silver **pin** (see Figure 1) may not be fully extended, therefore the specimen is still being visualized through the scope eyepiece and not through the camera feed.
- The camera may not be selected from the camera list. Ensure that the MU1403 camera has been from the camera list on the top left of the screen when first opening the AmScope application.
- The specimen may not be in the field of view. In this case, the camera is working properly, but students may not be able to see anything on the stage. Use **the head/body adjustment knob** (see Figure 2) on the back of the scope to slide the head to an appropriate distance from the specimen so that the **zoom control** and **focusing knobs** (see Figure 1) can be used to get the specimen in the field of view.

#### *The AmScope program won't open or keeps crashing*

- The software was installed before the driver. Uninstall both software and driver, and then reinstall in the appropriate order, as laid out in the AmScope software installation guide.
- Older computers may not be compatible with the AmScope software. The AmScope software is designed to work on a computer with either a Windows or Mac operating system, but older or more outdated computers may preclude or limit the functionality. The AmScope website offers several older versions of the software. Installing one of these older versions of the software may solve the problem. Otherwise, students may have to find a newer computer to carry out their imaging.

### ***Specimen Imaging Troubleshooting***

It may be difficult to obtain a clear picture that shows a specimen's color, detailed morphological features, internal anatomy, external hairs, etc., especially with mobile organisms or organisms that are difficult to position. Below are some tips to assist with common struggles:

#### *Illumination problems*

- If sample is too shadowy or is too bright, try adjusting the upper and lower illuminator intensity using the **top and bottom variable light controls** (see Figure 2). Too much or too little light can also affect focusing.

- If needed, unscrew the **lamp lock-screw** and maneuver the upper **illumination device** to eliminate shadows (see Figure 2). A flashlight or cell phone light can be also used in addition to the built-in illumination, as needed.
- Try using different stage plates to change the level of light passing through the specimen from below or the background against which the specimen sits. ***Sometimes this is one of the easiest ways to get a better image!***

#### *Glare*

- Wet specimens should be submerged in ethanol for imaging.
- To reduce ambient light, the eyepieces may need to be covered with the accompanying plastic eyepiece covers.
- Turn off lab or classroom lights and close blinds to cut down on background light.

#### *Specimen Orientation*

- Use the sides of the Petri dish to help prop-up specimens on their back or venter (underside of the organism).
- Use sterile forceps or dissecting probes to tease out a specimen's legs, wings, etc. *Work with the natural motion of organism to do this!* In other words, don't pull a leg or wing in a direction that it is not meant to extend.
- Rotate the stage plate, the image in the AmScope program, or the camera itself to get a better image.

#### *Whole specimen focusing*

- It may be difficult to get the whole organism to focus in one image. Take as many images as are needed (refer to the ***DNA barcoding 101 Taxonomic and Imaging Guide*** for recommendations).

#### *Low-quality image*

- There are many image editing features on the left hand side of the AmScope program that may be useful in producing a higher quality image - test the different features on sample images to optimize. The following are some of the particularly useful features:
  - "Color Adjustment"
  - "Exposure and Gain"
  - "Measurements" (located along the top command bar; this also may be used to insert text, such as specimen ID number, in your image)