Rev. June 11, 2010

Automontage imaging guidelines

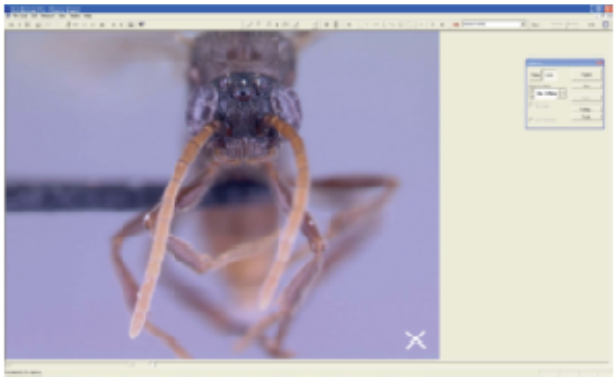
System Set-Up:

Z6 lens + 0.5X adaptor	Z6 lens + 2X adaptor	Z16 lens + 2X adaptor

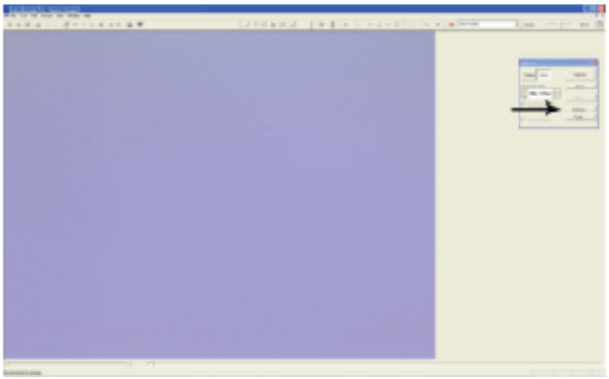
There are three lens and C-mount adaptor combinations that produce the sharpest images of ants. The Z6 lens and 0.5X adaptor are used for imaging the largest ants (5.6-21.5mm), the Z6 lens and 2X adaptor are used for imaging average-sized ants (4.0-5.6mm), and the Z16 lens is used with the 2X adaptor for the tiniest ants (0.34-4.0mm).

White Balance:

Before you begin imaging, first check the white balance. It is important that all of the images on AntWeb have a uniform light gray background.



Yuck! The white balance is way off here. The background should be light gray, not purple.



To correct the white balance, first move the specimen out of the frame for an unobstructed view of the background. Next, click the **Settings** button (see arrow) in the **Capture** window to bring up the **Capture Chain Properties** window.

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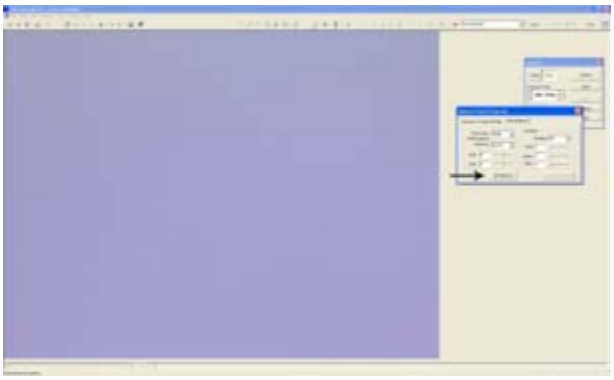
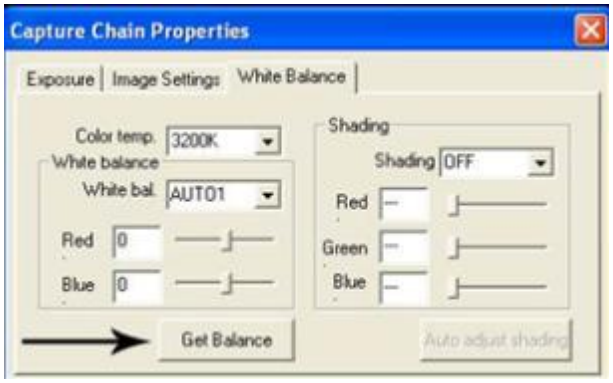
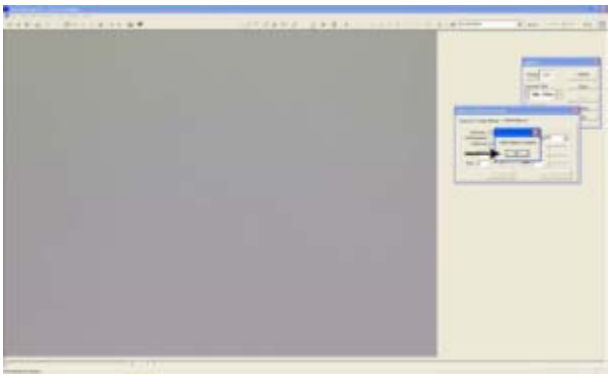


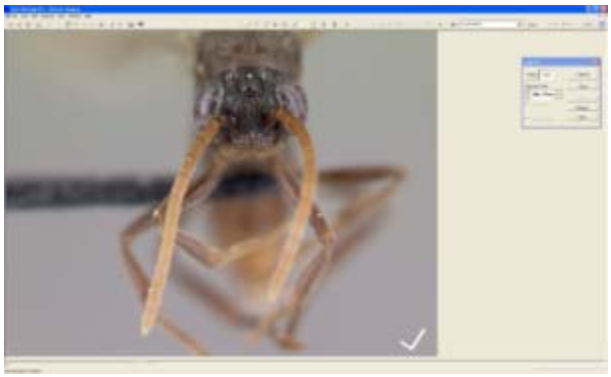
Click on the **White Balance** tab.



Make sure the settings match this window. Click the **Get Balance** button (see arrow).



Click **OK** and move your specimen back into the frame.



Fixed! This is what your background should look like.

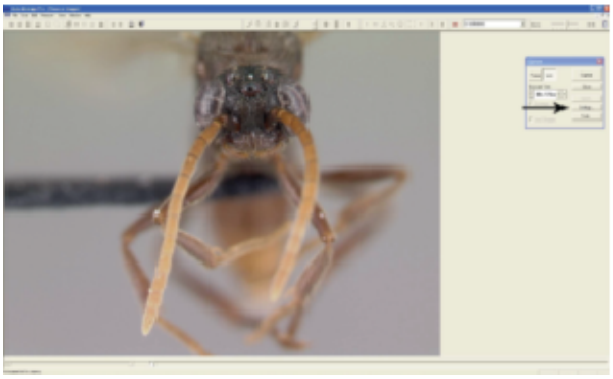
Important settings:

- COLOR TEMP = 3200K
- WHITE BALANCE = AUTO 1
- BE SURE TO CHECK THAT THESE SETTINGS DO NOT GET RESET!

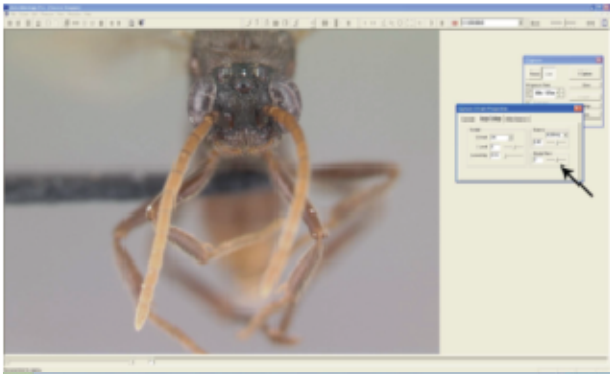
This can happen if new software is installed on the computer that you are using, so try to do a white balance at least once a week and check that the White Balance Settings are still correct.

Master Black:

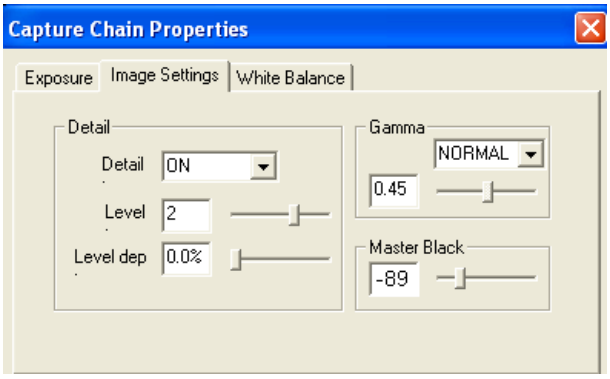
Master Black changes the saturation of your image. If your specimen looks washed out or slightly fuzzy, check the master black settings.



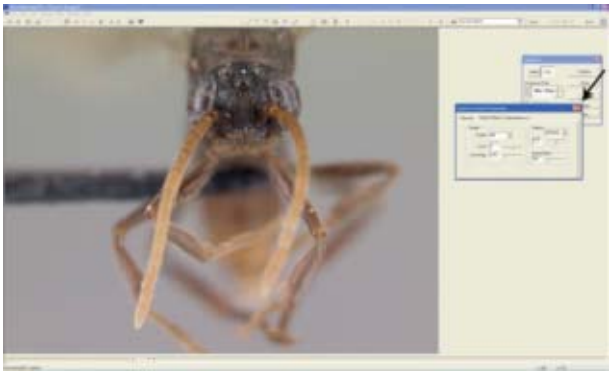
Click **Settings** (see arrow) to open the **Capture Chain Properties** window.



In the **Capture Chain Properties** window, click on the **Image Settings** tab.



Make sure the settings match this window. Moving the scale lets you view your specimen at different **Master Black** values. I usually leave the Master Black at -89, but switch to -99 for smaller yellow specimens.



Closing the window automatically saves your settings. You can now begin positioning.

Important settings:

- DETAIL = ON
- LEVEL = 2
- LEVEL DEP. = 0.0%
- GAMMA = Normal; 0.45
- MASTER BLACK= between **-99 & -89.** For yellow small ants = -99.
  For black shiny ants = -89. Play with this to see what suits your preference.
- MAKE SURE TO CHECK THAT THESE SETTINGS DO NOT GET RESET!

Positioning:

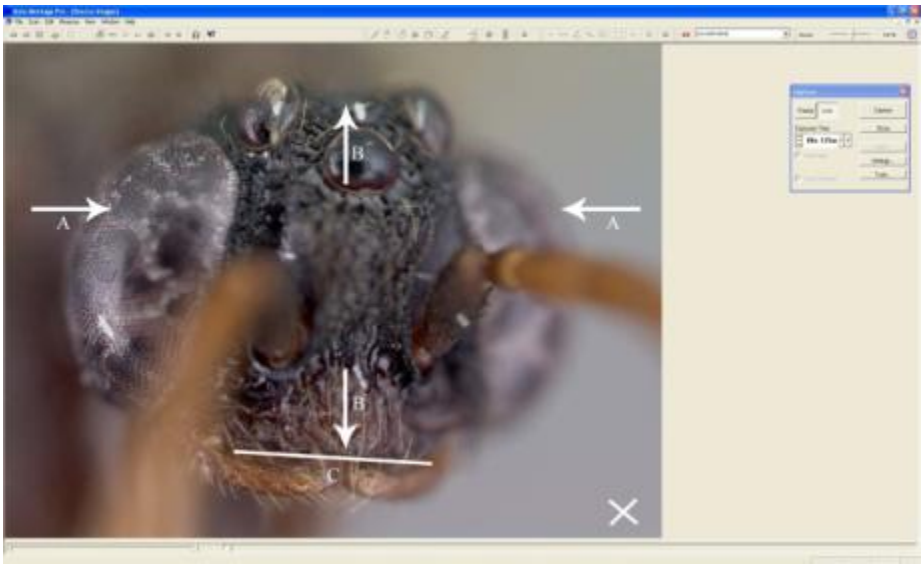
AntWeb is a great resource if you have questions about positioning a particular specimen. Look up the genus on AntWeb and you will find an example of an imaged specimen that you can use as a guide.

Examples of bad positioning from the archives:

The head is tilted so far back that you can't see the top. Also, more of the left side of the head is visible than the right.

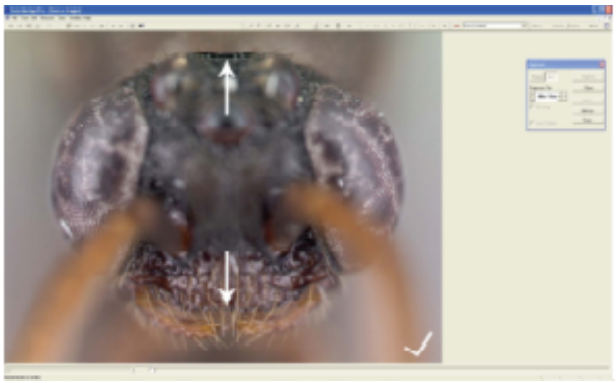
This specimen is tilted too far back so that too much of the area under the thorax is visible.

Head (_H) : This is the most difficult step. Think of the head as a box. Make sure the top of the head and the clypeus are in the same plane of focus. Next, make sure the sides of the face are even and that you can see the same amount of each eye on each side.

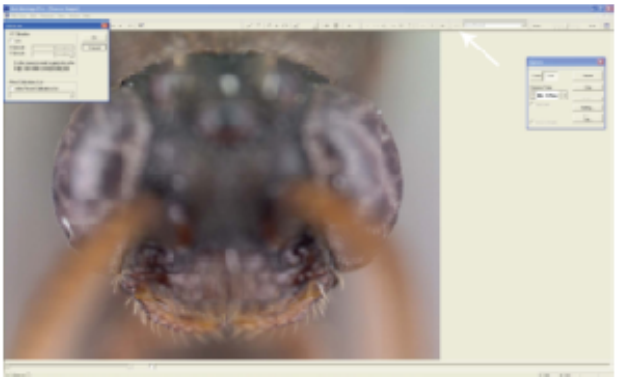


This ant head is NOT positioned correctly under the microscope before montaging. One eye is in focus, the other is not. The clypeus is in focus but the top of the head is not.

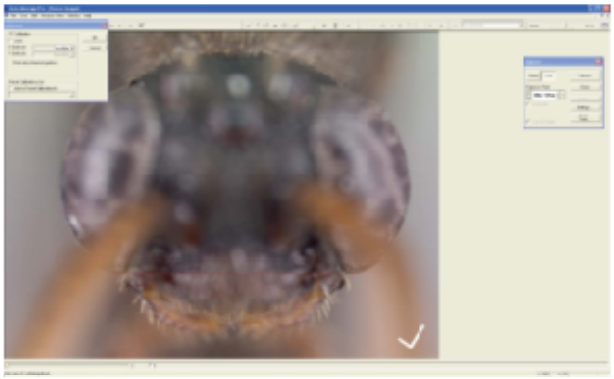
The eyes should be in the same plane of focus as demonstrated here.



The top of the head and the bottom of the clypeus should be in the same plane of focus as demonstrated here.

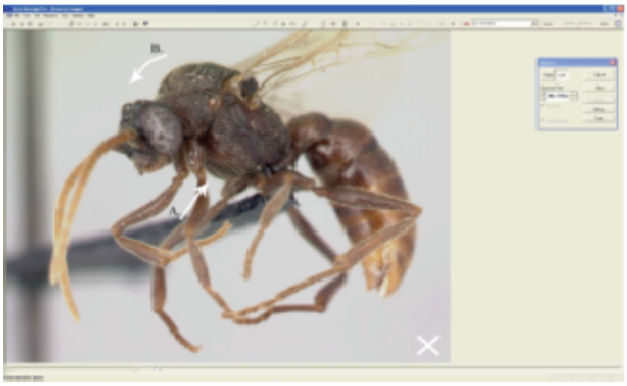


To make sure the head isn't tilted, use the **red ruler** (see arrow) and draw a line connecting the bottom of each eye.

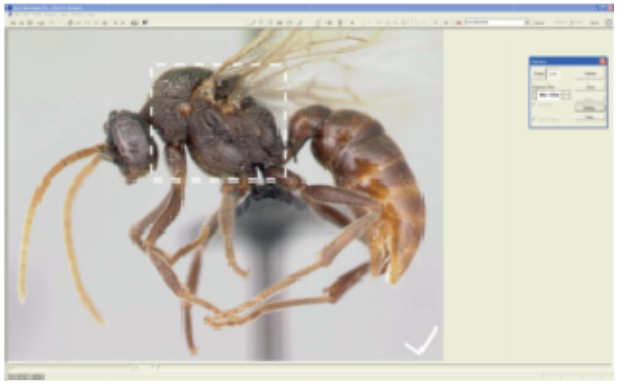


This is the CORRECT position for the head because the red line is horizontal.

Profile (P_1): Think of the thorax as a box. First look at the two hind coxae and make sure that they are in a straight line. Next, look at the top and bottom of the thorax and make sure you are getting a dead on lateral view. Make sure that features such as spines line up. Finally, look at the front and rear of the thorax to make sure they are in the same plane and the thorax is not spun towards or away from you.



The specimen is NOT positioned correctly. Too much of the underside of the thorax (A) and the dorsal (B) are visible. The specimen needs to be moved to the left and down.

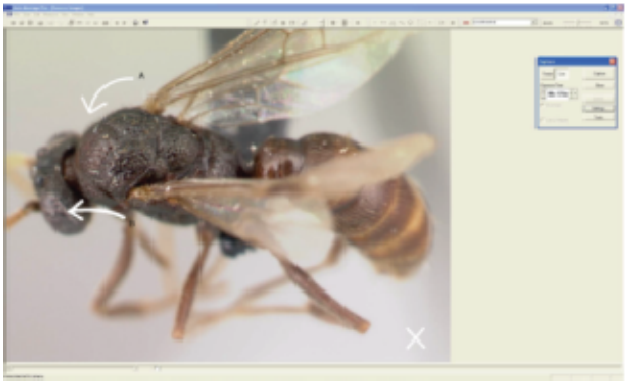


This is the CORRECT position for the profile shot.

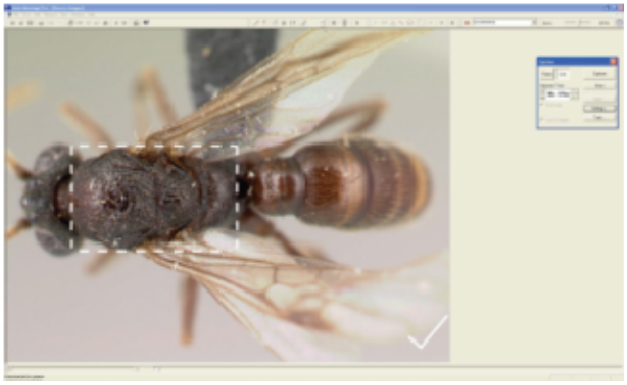
Wings (P_2):

This is the CORRECT position for the wing shot. Get as close as possible while keeping a safe margin around the wing. Position the wing at a 45 degree angle, to get a closer picture and avoid too much empty space.

Dorsal (_D): Think of the thorax as a box. Make sure it is not tilted too far forward or backward, and that the top plane of focus is in the middle. Check that you can see the same number of coxae on each side of the ant.

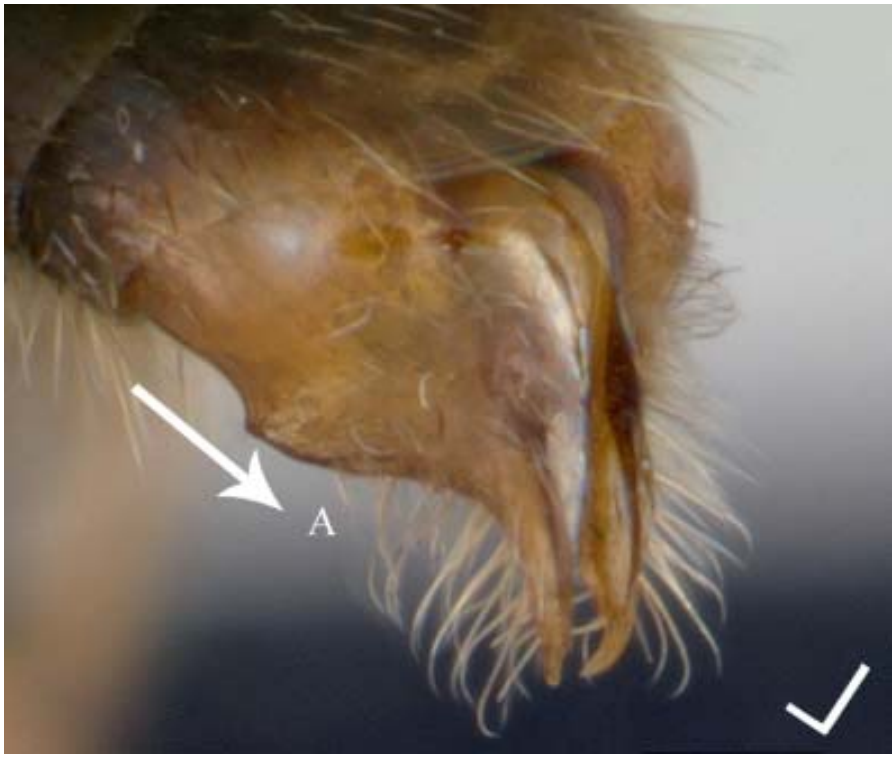


This ant is NOT positioned correctly for the dorsal shot. The base of the left and right forewing should be in the same plane of focus. The pronotum and the propodeum should be in the same plane of focus. To correct this, move the specimen up (A) and left (B).



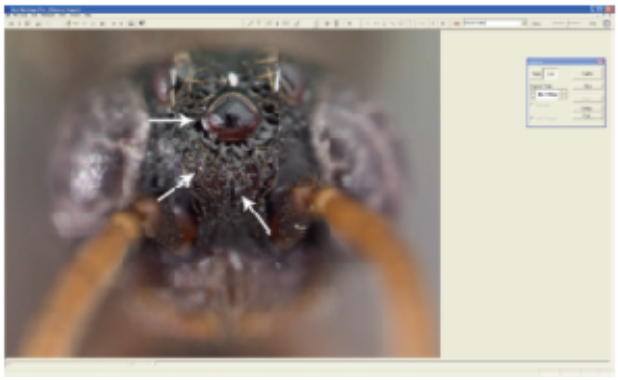
This is the CORRECT position of the dorsal shot.

Genitalia (P_3):

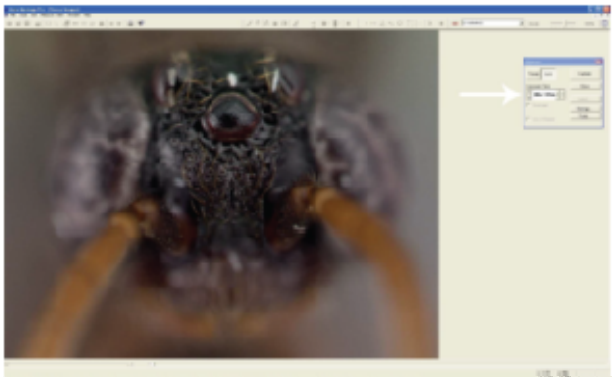


The genitalia shot is difficult to position. I like to keep the specimen at a 135 degree angle (see arrow A).

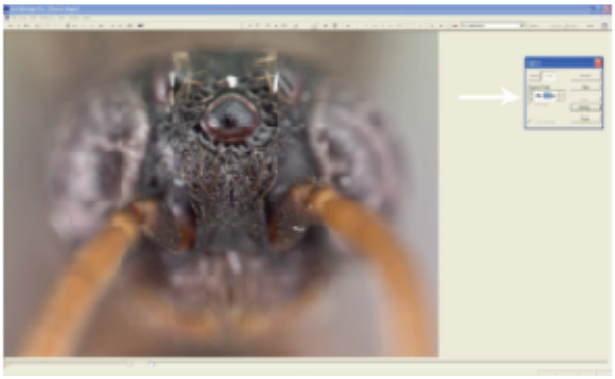
Lighting:



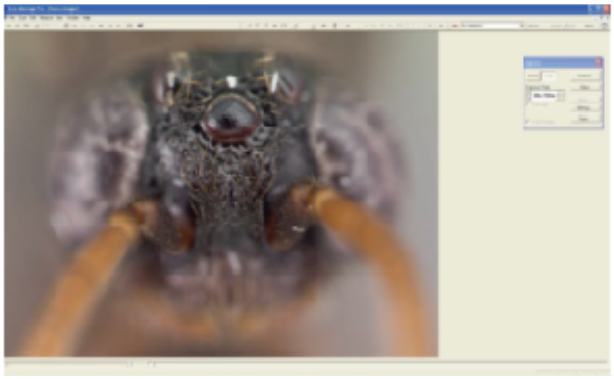
Avoid glare spots when lighting the specimen (see arrows).



If your specimen is too dark, change the exposure time to 250 ms (see arrow).



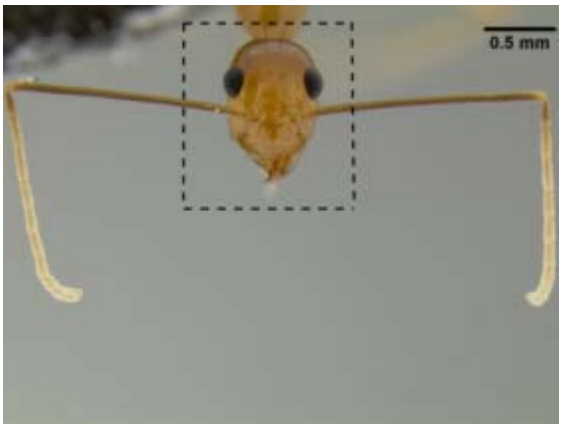
If changing the exposure washes out your specimen, adjust the lighting.



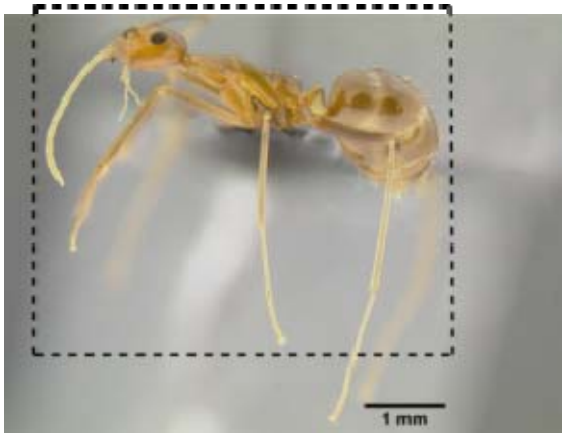
This is the CORRECT lighting for imaging a specimen.

Framing:

Examples of bad framing from the archives:

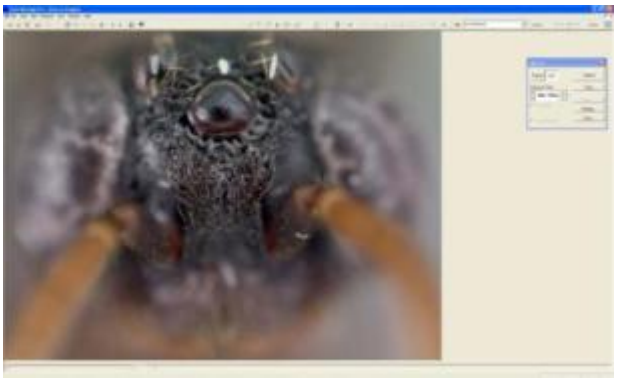


This photo was taken from much too far away. I only include antennae in the head shot if they are short and close to the head; they are often better covered in the profile shot. The dotted line indicates the correct framing for this specimen.

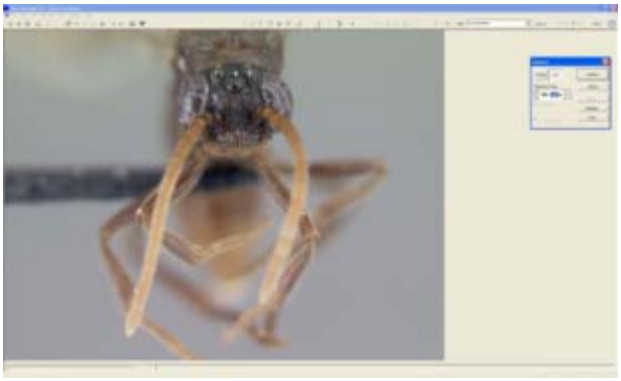


This photo was also taken from much too far away. Be sure to get at least one full leg and both antennae, if visible, in the profile shot. The dotted line indicates the correct framing for this specimen.

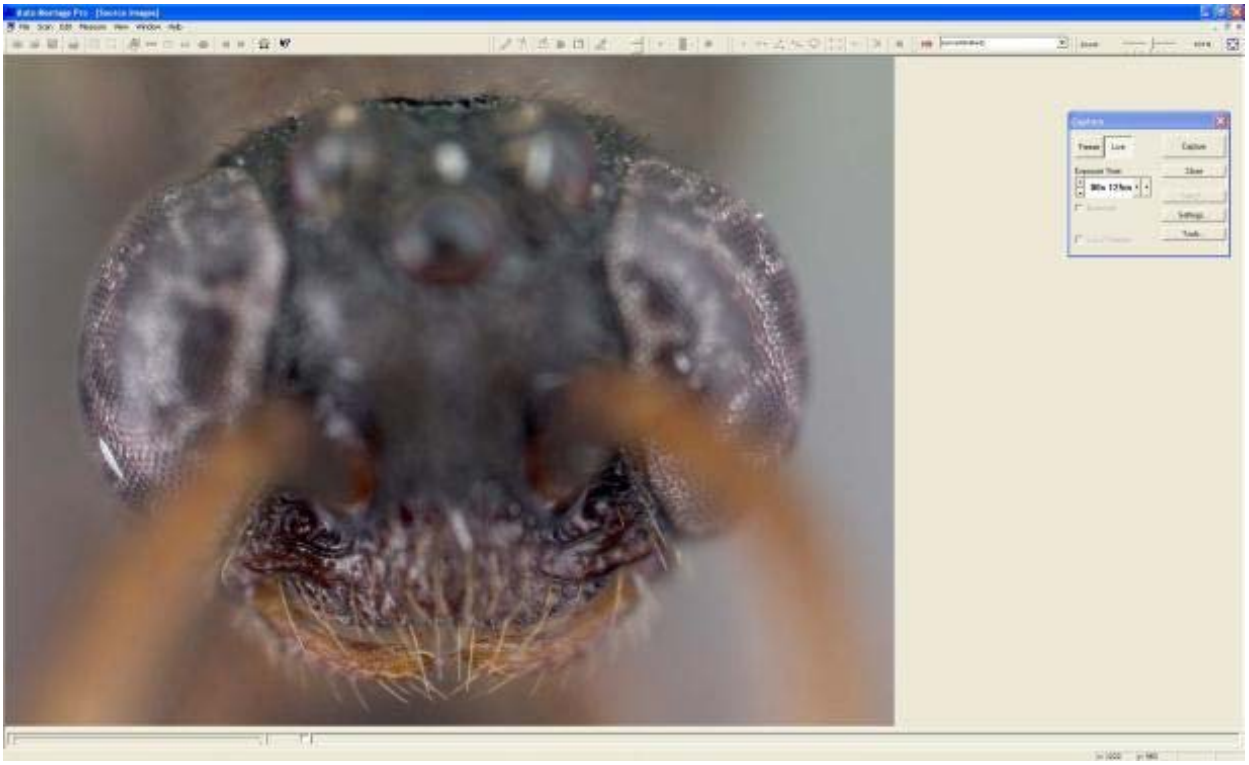
Head:



This head shot was taken too close up. I like to keep a comfortable margin around the head.

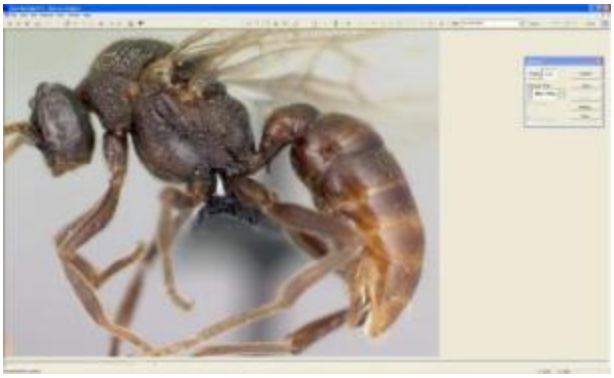


This head shot was taken from too far away. Don't worry about including long antennae in head shots.

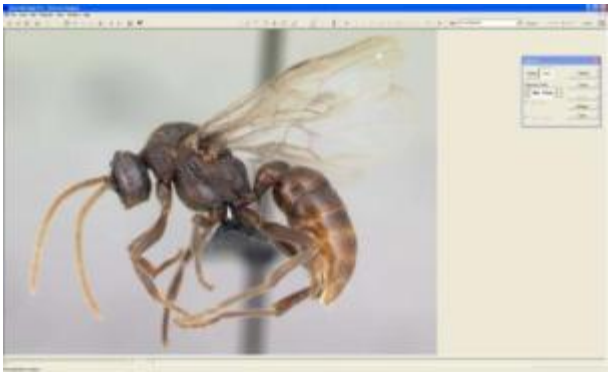


This is the correct framing for the head shot. You can see the hair on the top of the head and the hair on the mandibles.

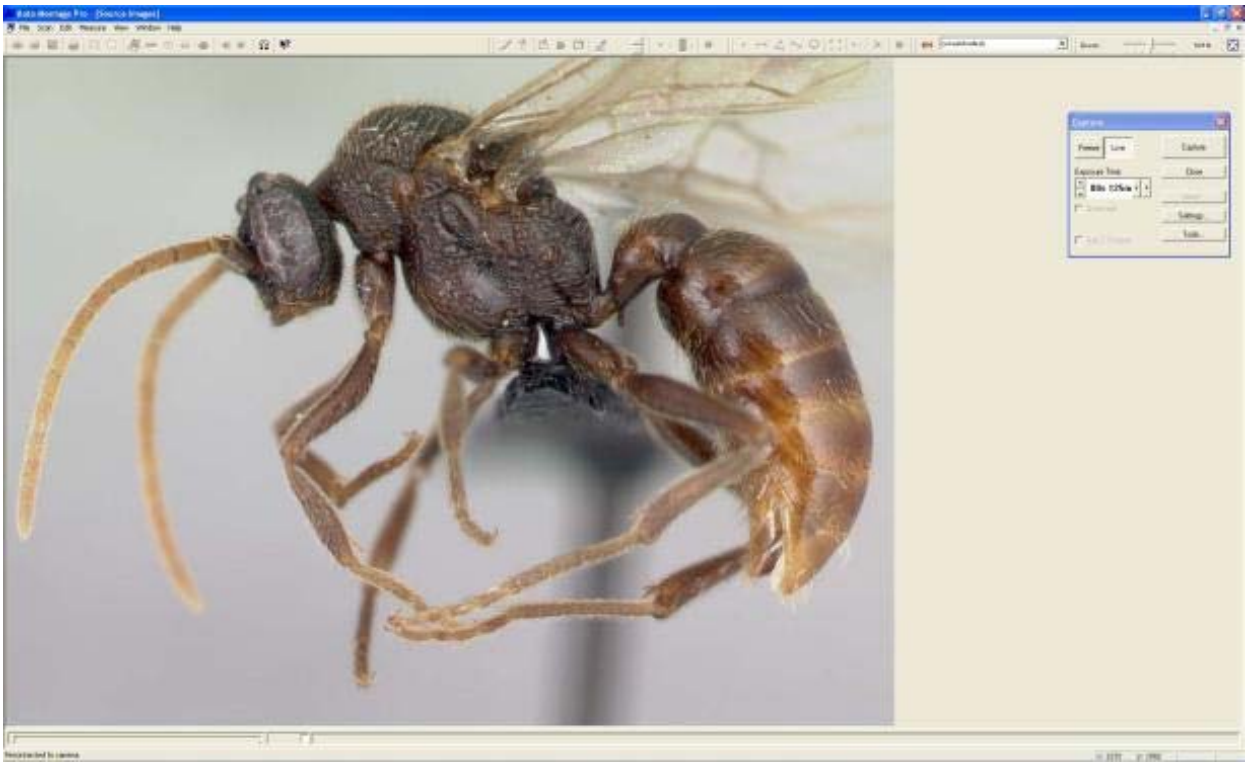
Profile:



This profile shot is too close up. I make sure to get at least one full leg and all visible antennae in the profile shot.

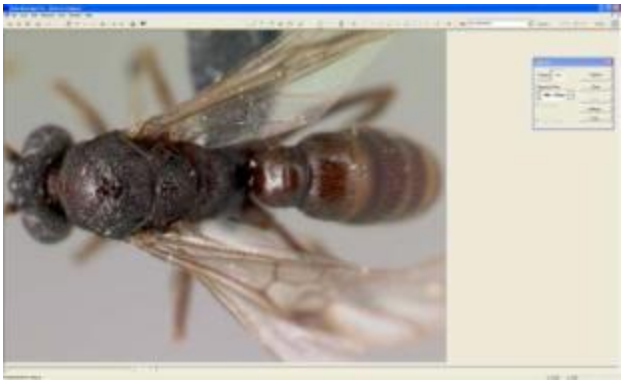


This profile shot is was taken from too far away. I should already have a separate wing shot, so we can get closer than this.



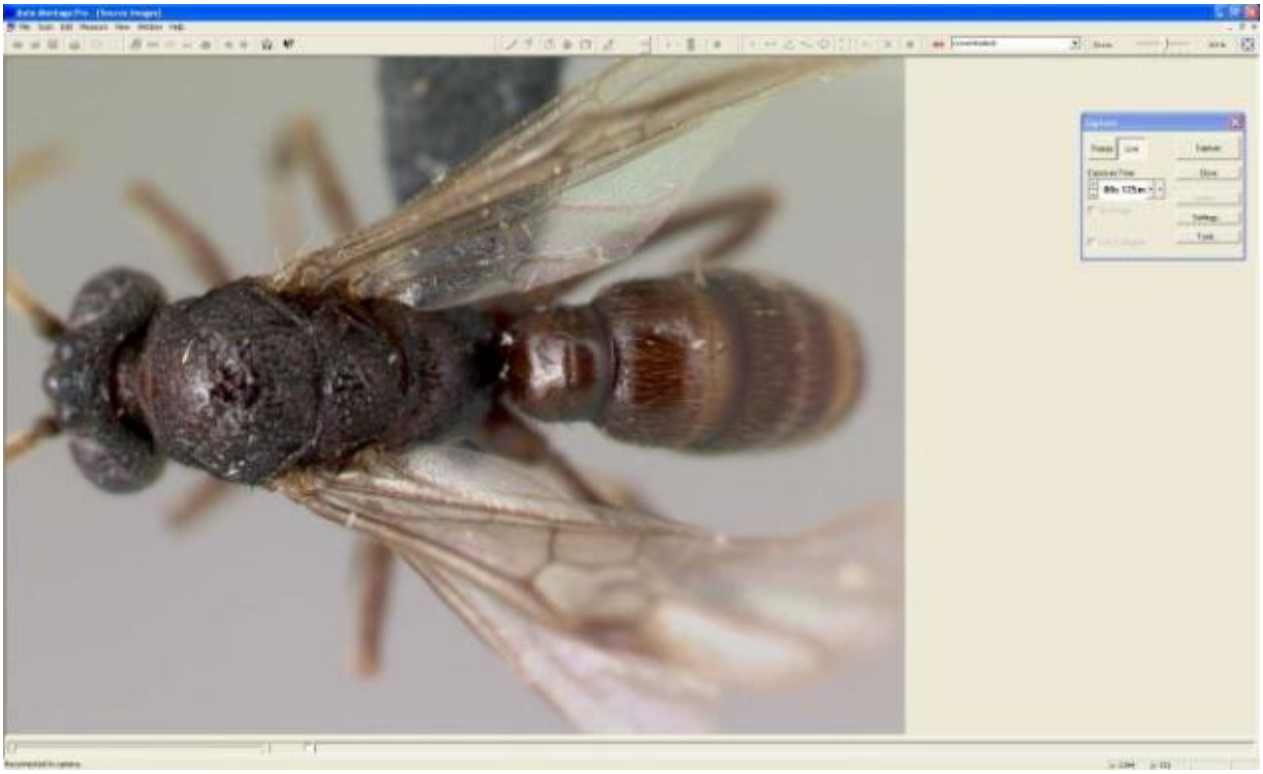
This is the correct framing for the profile shot. I can see both antennae, at least one full leg, and there's a comfortable margin around the body.

Dorsal:



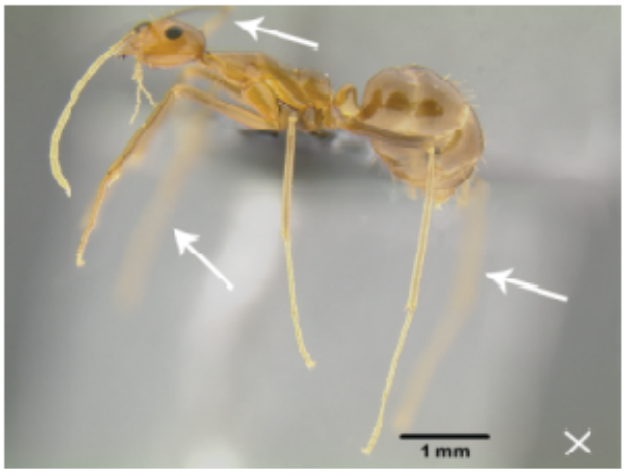
This dorsal shot is too close up. I like to keep a safe margin between the gaster and the edge of the frame and the top of the head and edge of the frame.

This dorsal shot was taken from too far away. I should already have a wing shot and depicted the antenna in the profile shot, so zoom in around the gaster and head.

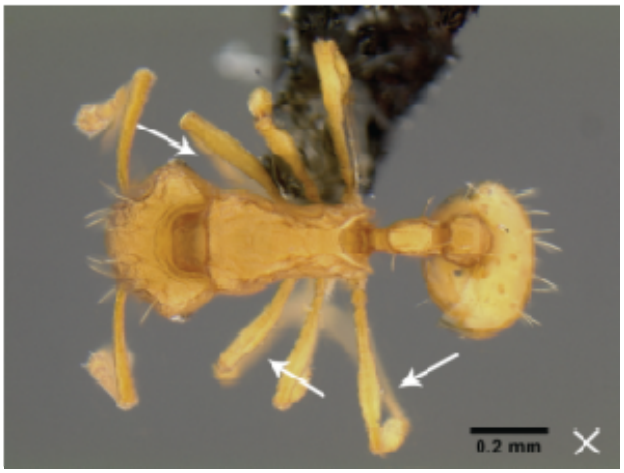


This is the correct framing for the dorsal shot. There is a comfortable margin around the gaster and head.

Blurring:

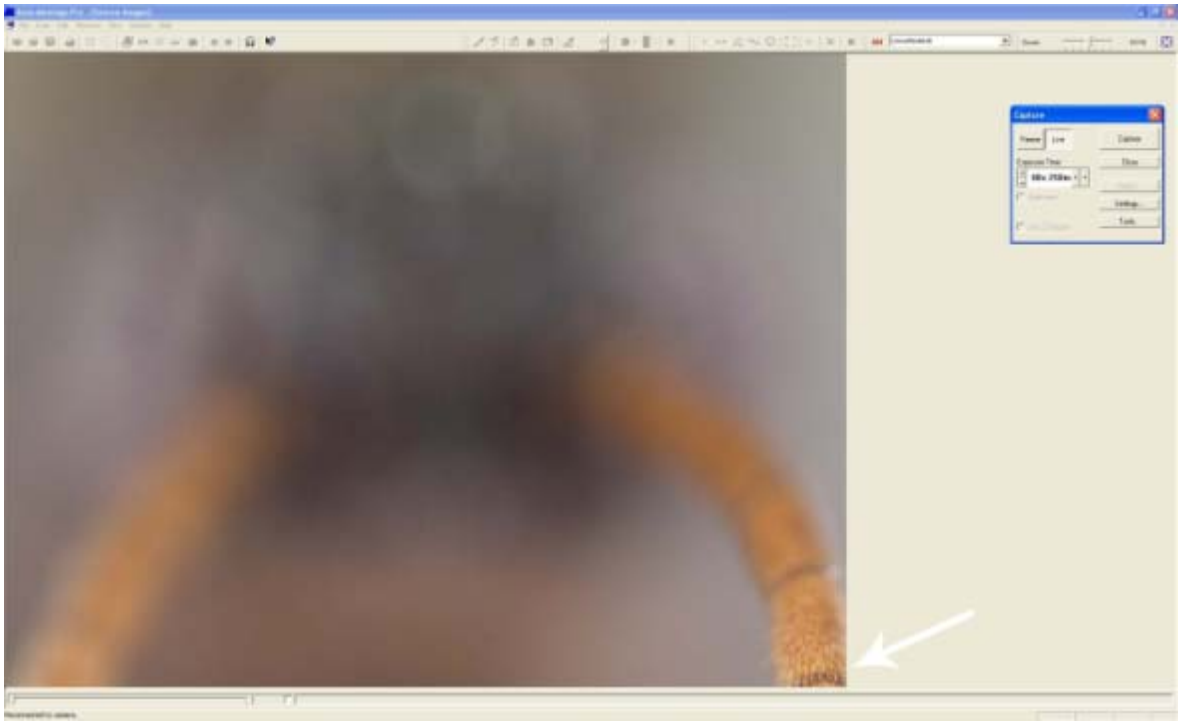


Blurring is unnecessary! Get as much of the body in focus as you can. To take a few extra shots, even if you think they're not needed. It takes little additional time and it's easier to edit clear legs than blurred legs.

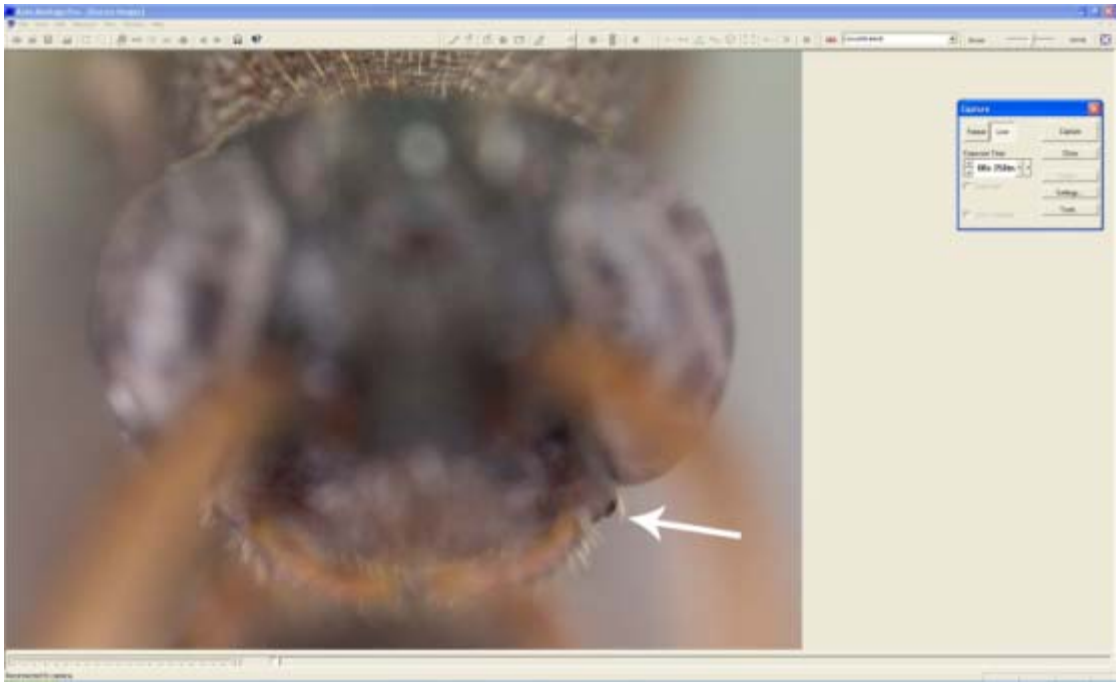


A poorly focused and blurry shot. You can't see the tips of the antennae or the bottom of the legs. You lose information and it takes time to edit out the blur.

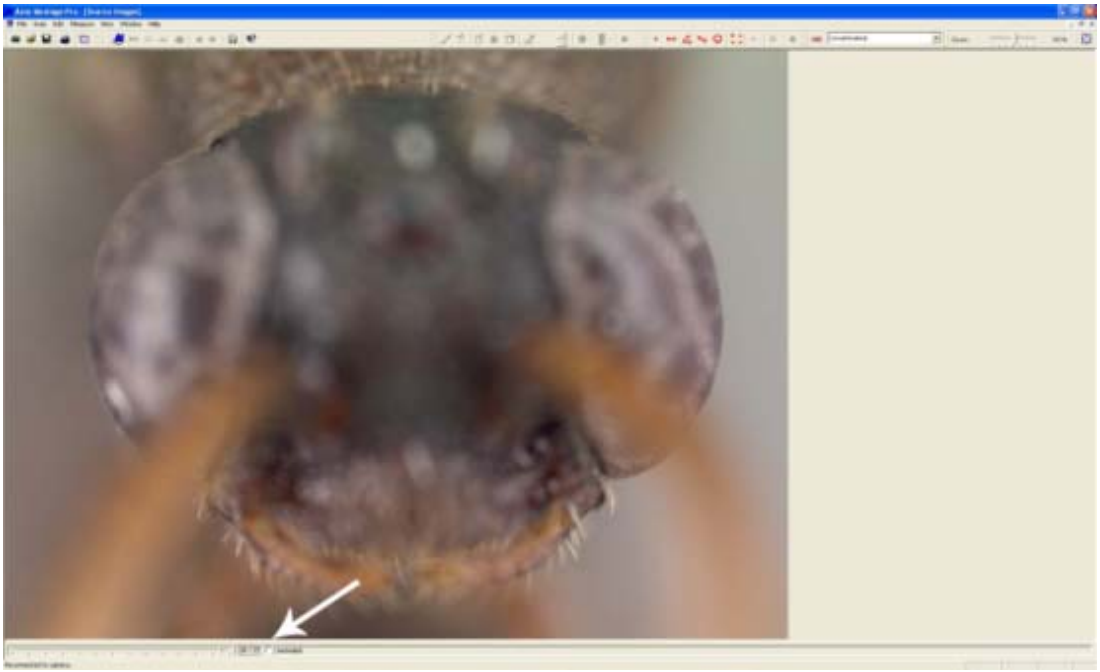
Imaging and Editing



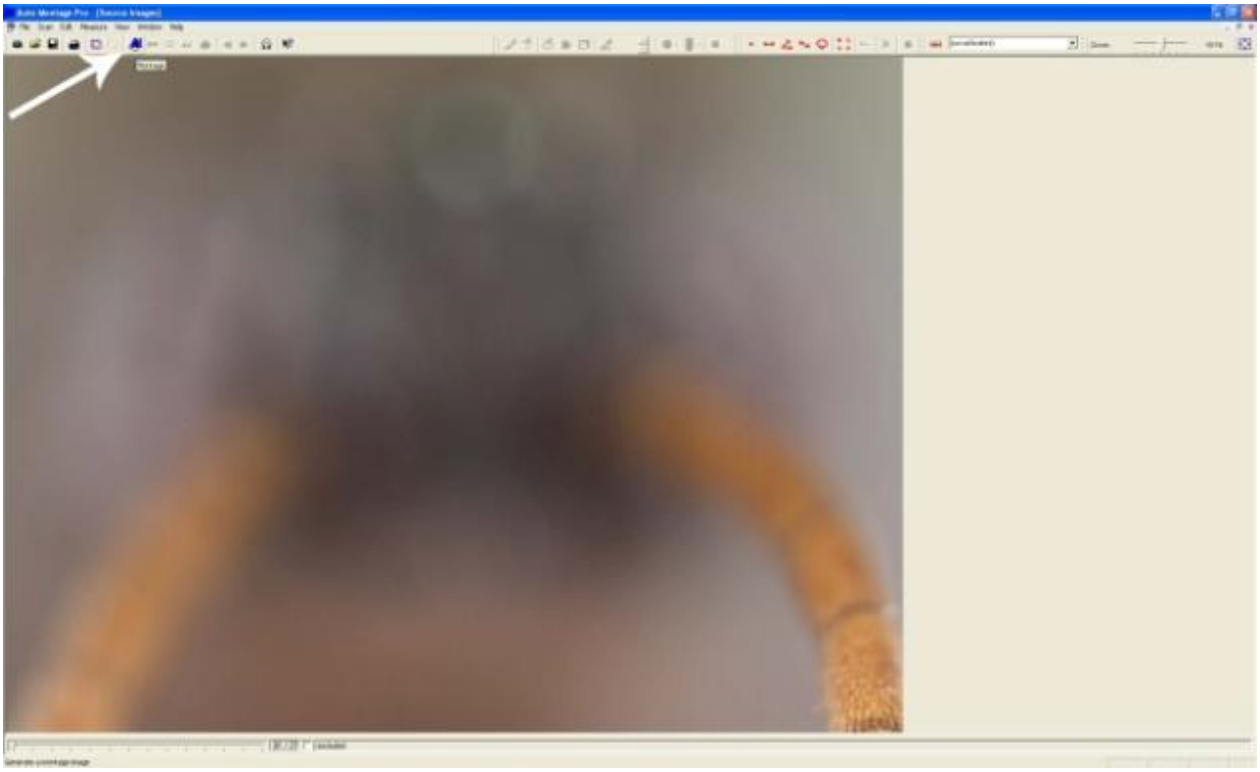
Click on the **Camera** icon, and select **Live** view. Start by focusing at the top of the specimen. For the headshot, this is usually the top of the antenna (see arrow).



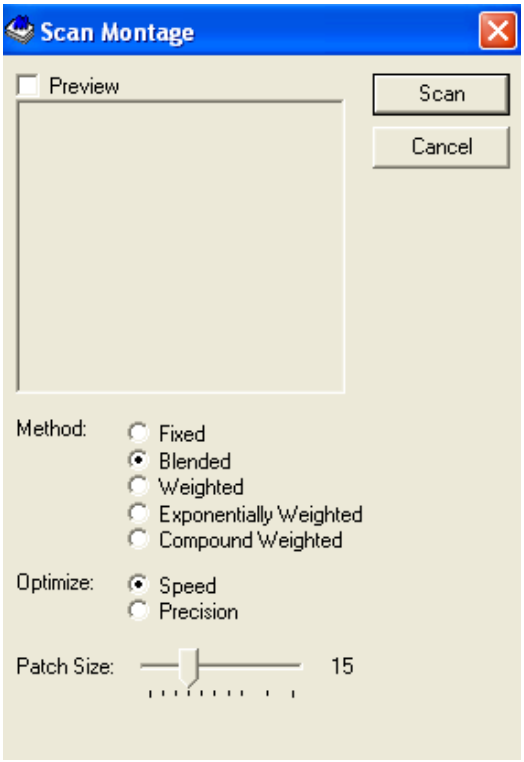
Click **Capture** at a steady pace, trying to focus on equal portions of the ant as you move down the specimen. Stop when you get to the bottom of the specimen. For the head shot, the last image is usually the back of the clypeus. Close the capture box.



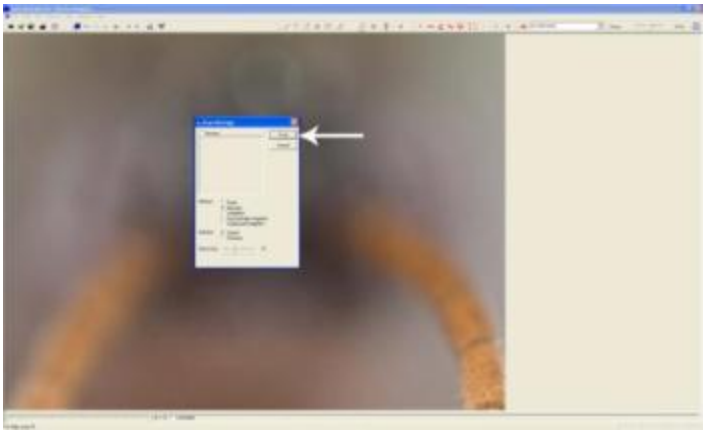
Scroll through your images to make sure you have everything in focus. You can exclude unnecessary images where you don't gain any extra detail by clicking the box at the bottom of the window (see arrow).



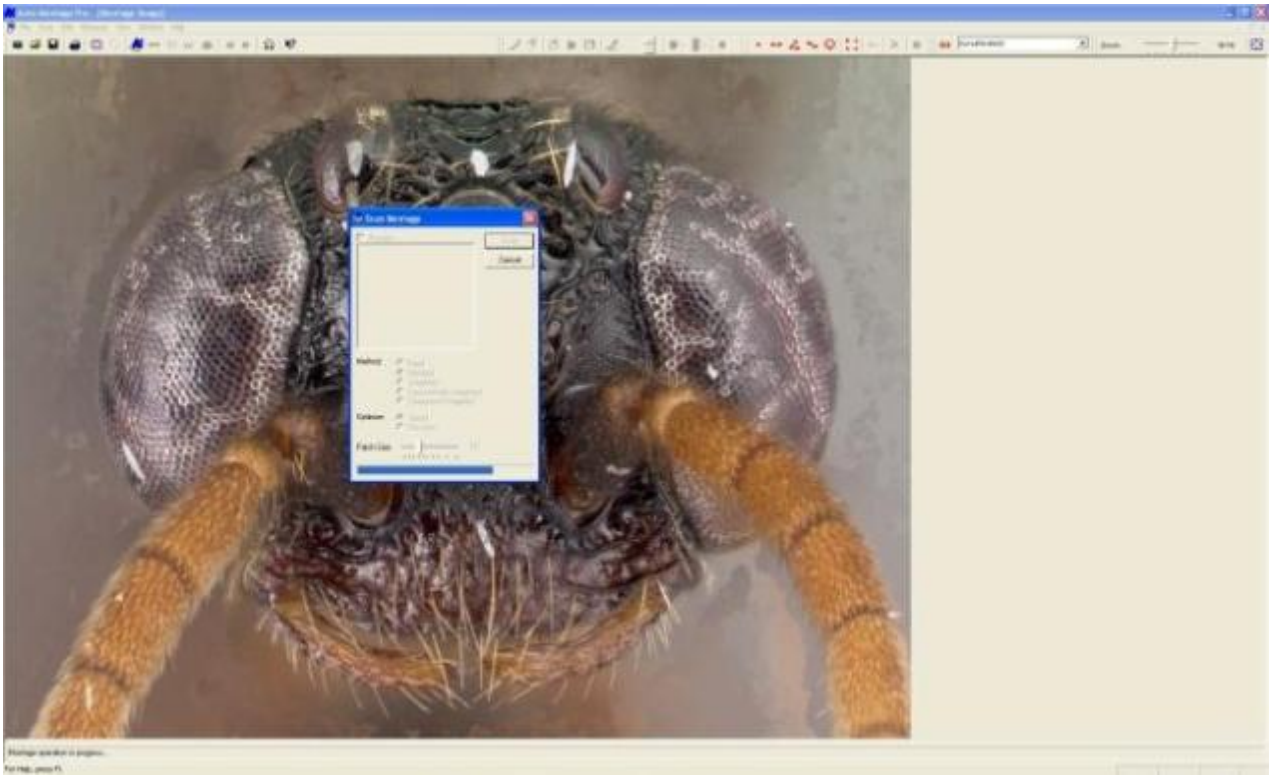
After scrolling through the source images and excluding unnecessary shots, you're ready to montage. Click the big blue M in your toolbar (see arrow).



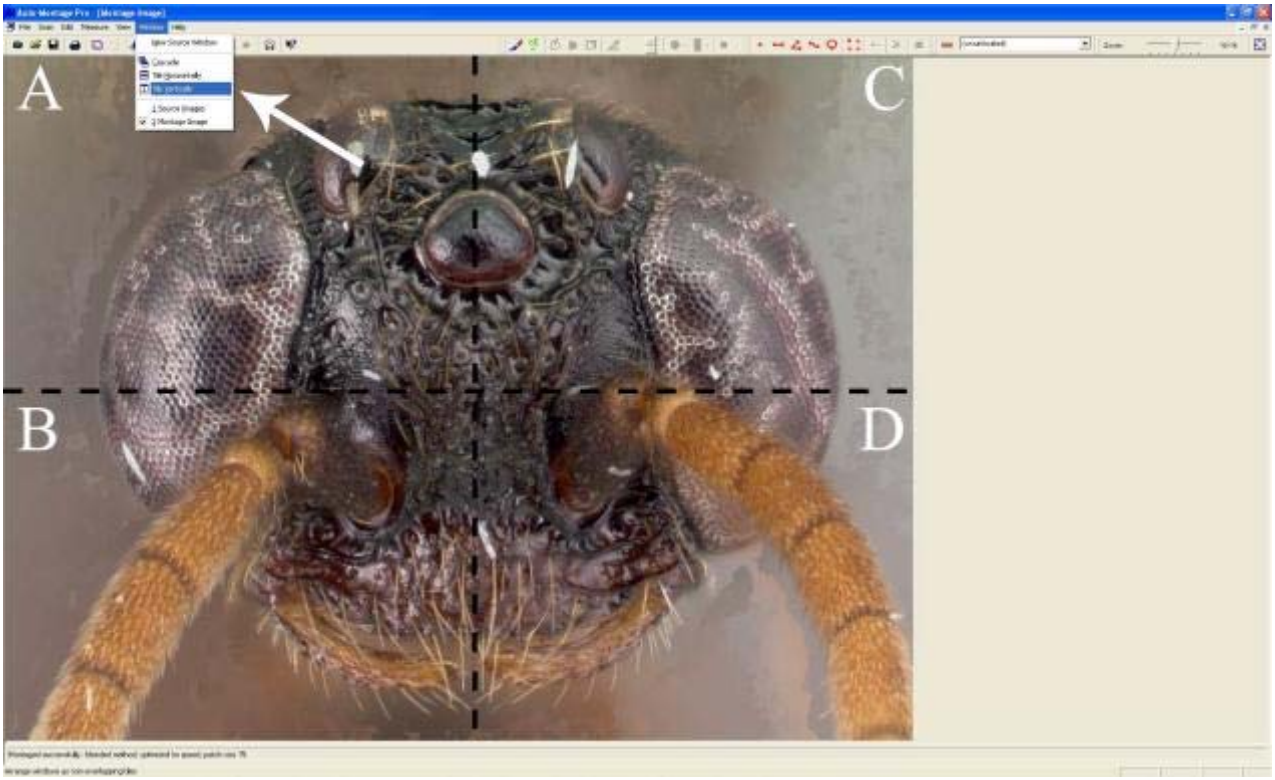
These are the settings we've used for years. Play with them to see what suits your preferences.



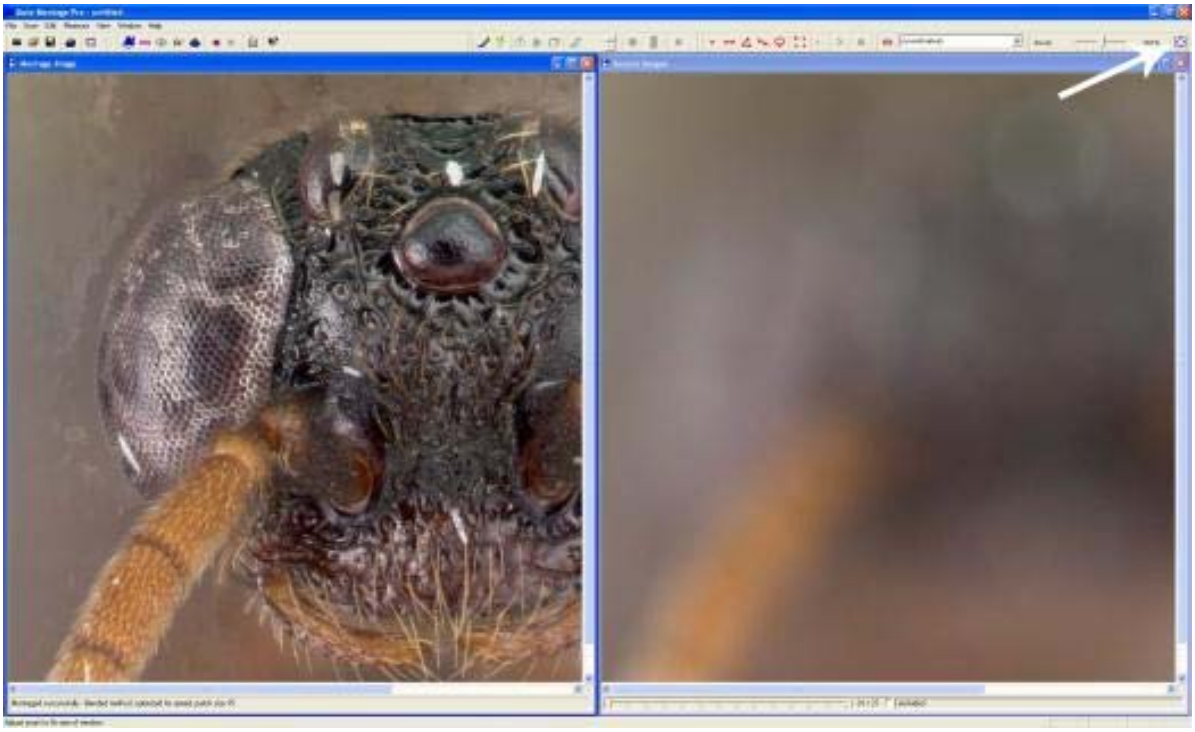
Click scan to begin montage.



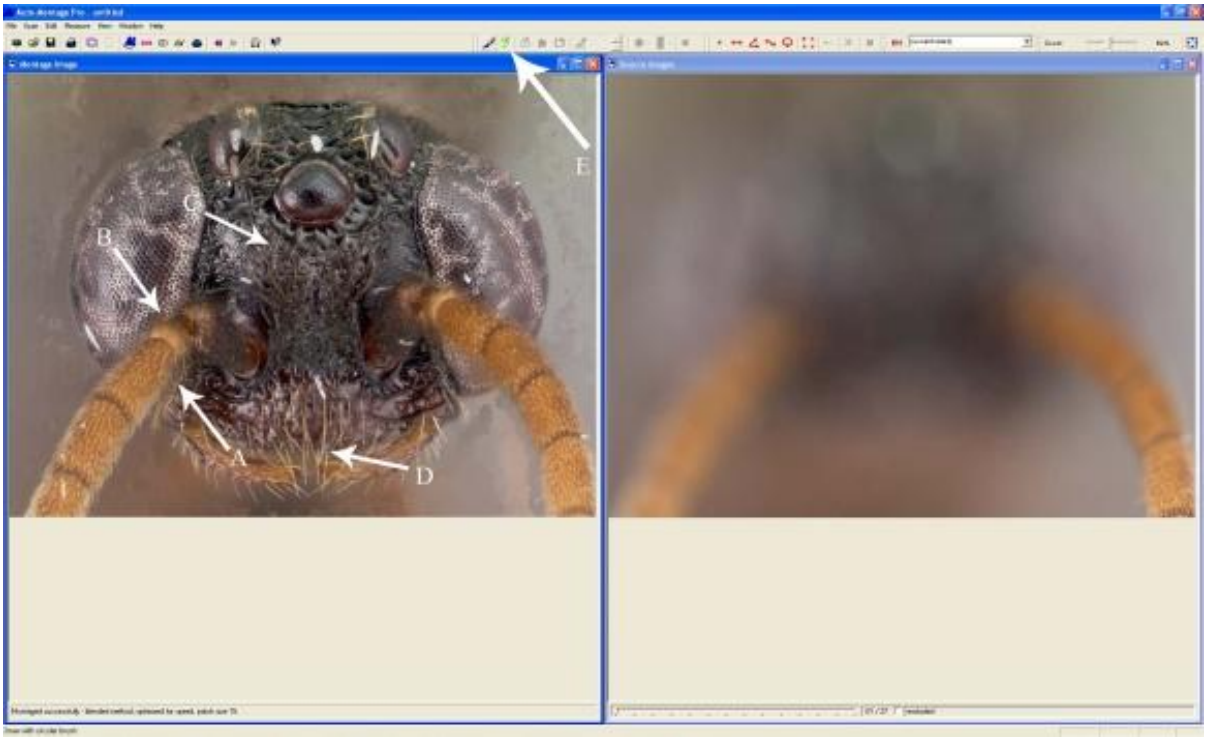
Montage in progress



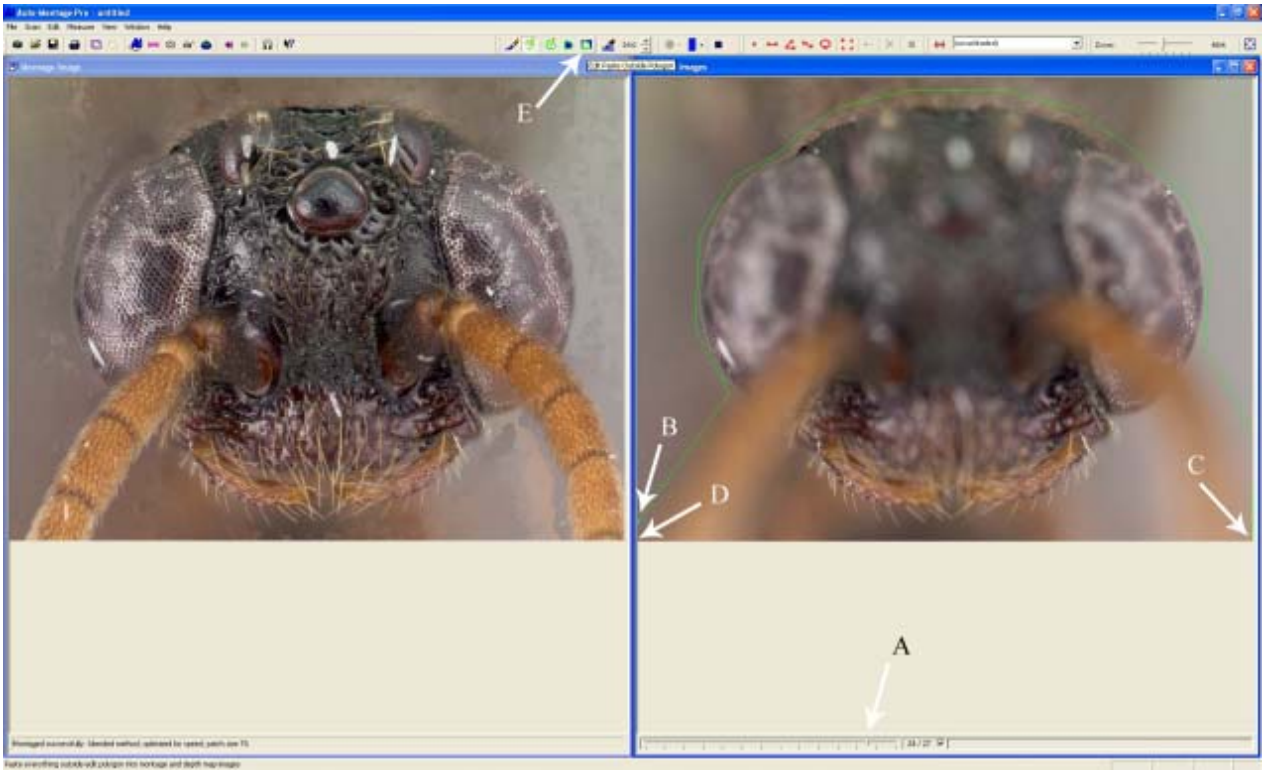
This is the resulting montaged image. Not pretty, but you can fix that. Start by visualizing four quadrants, A->D. To begin editing, click **Window** in the toolbar. Select **Tile Vertically**.



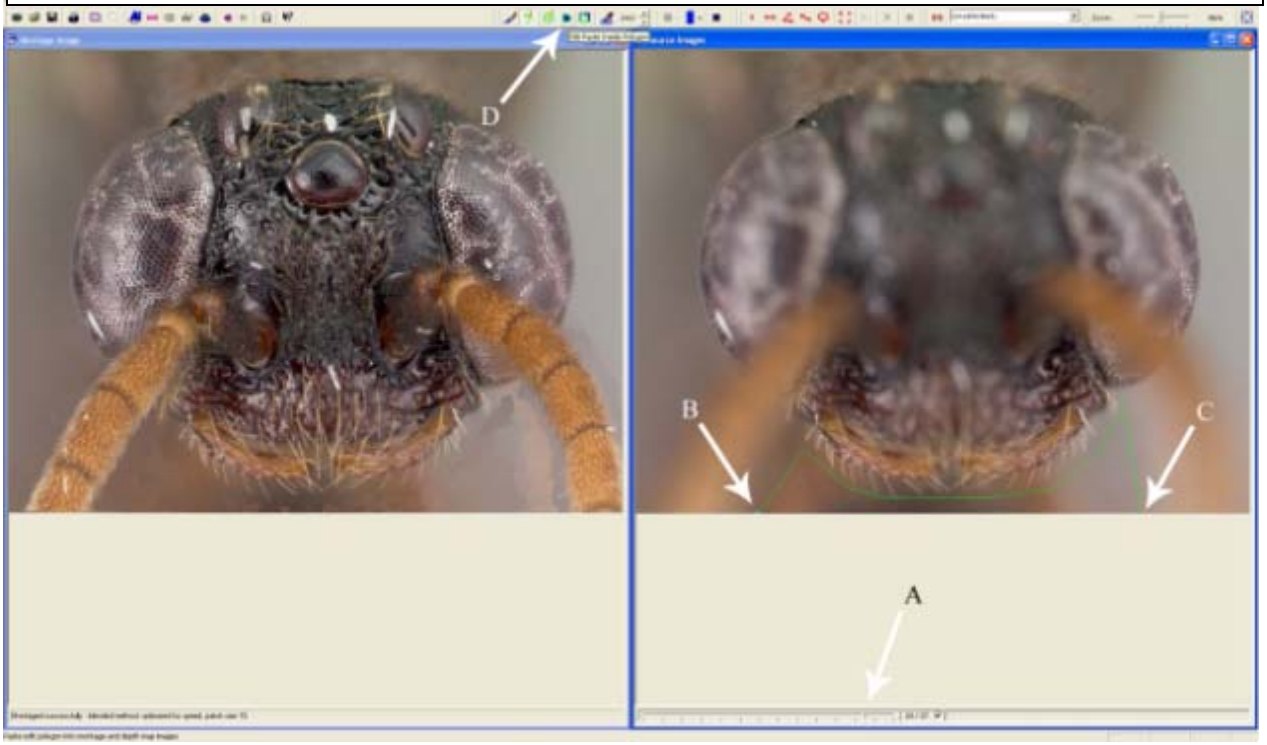
Start the editing process by cleaning up artifacts in the background first. Click the square in the top right corner of the window (see arrow) to see the full montaged image next to the source images.



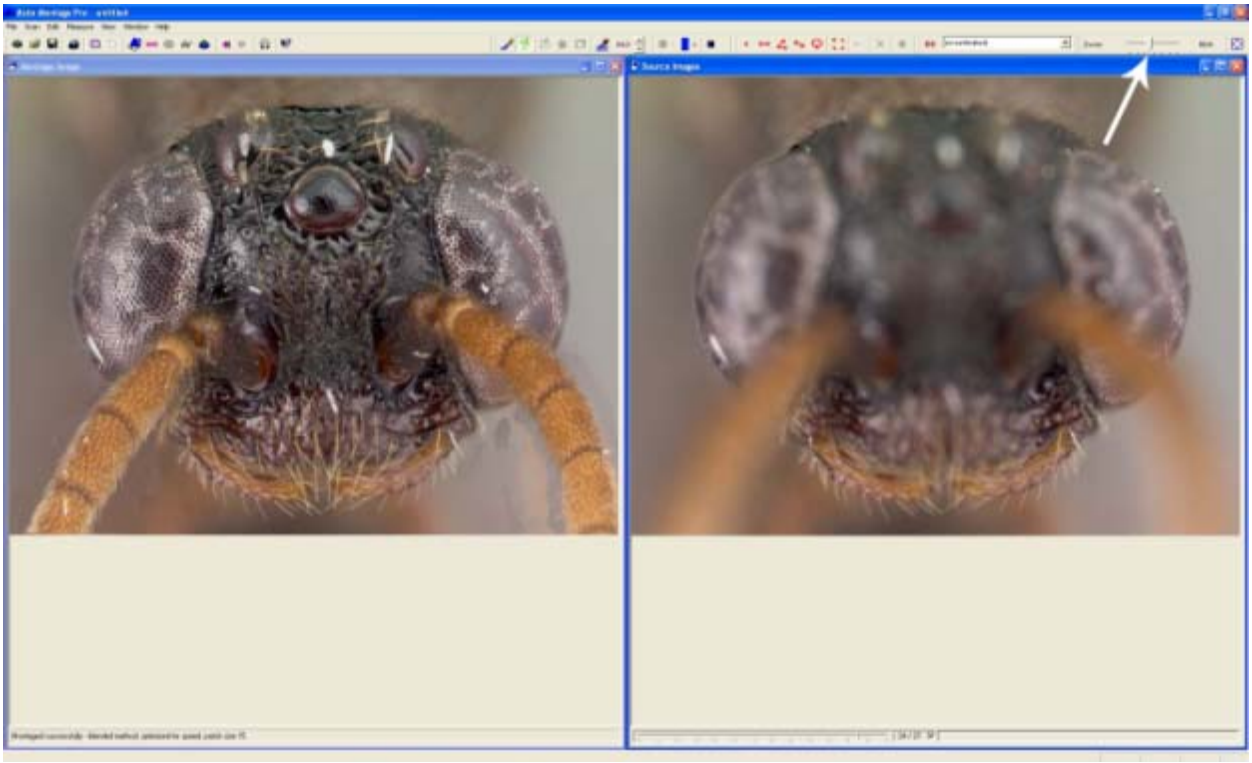
The arrows indicate problem areas. Dead spots surrounding overlapping areas (A), parts of the antenna that got cut out (B), hairs on the front of the face that didn't show up and need to be drawn in (C), and dead spots on the clypeus that need to be drawn in (D). Start fixing these by clicking the **Polygon** tool (E).



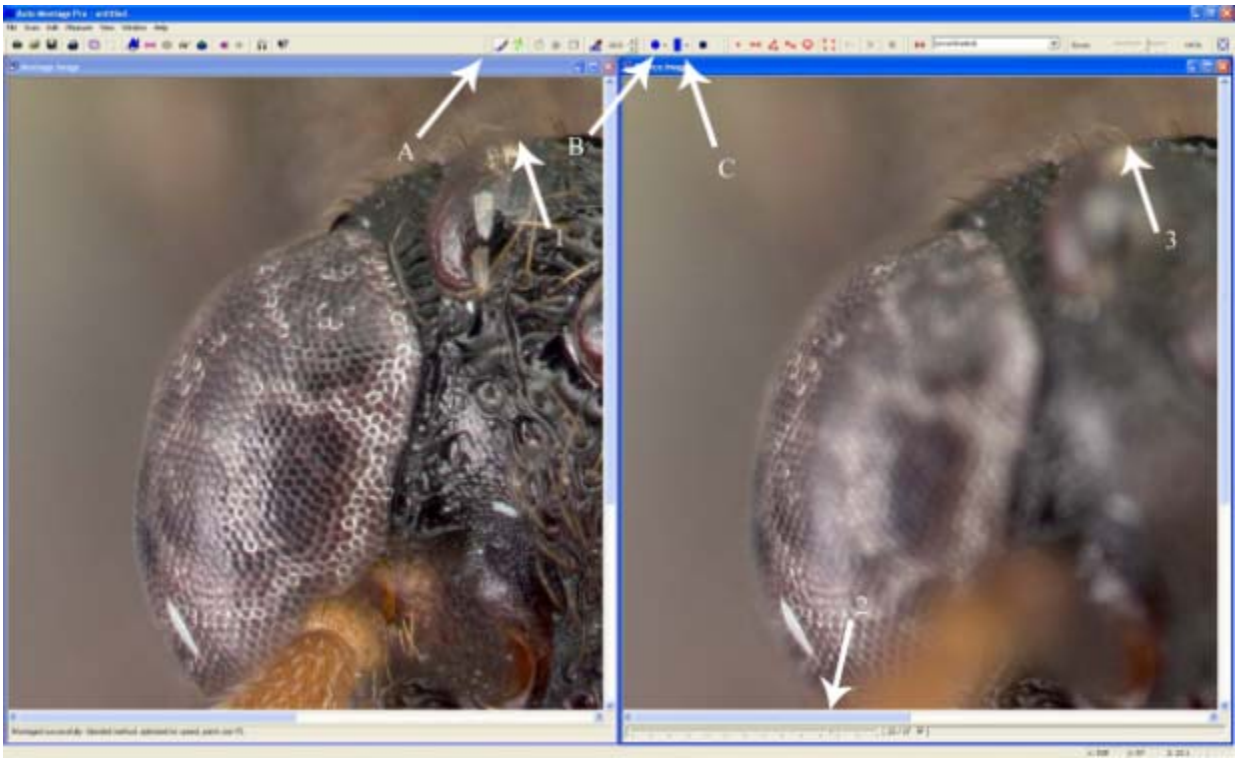
Review the source images to find one with the edges of the head in focus (A) (each tick of the sliding bar represent a different potential source image). Start the **Polygon** tool on the source image at the left side of the left antenna (B) and draw closely around the head to the right side of the right antenna (C), and along the edge of the window to the bottom left corner of the window (D). Select



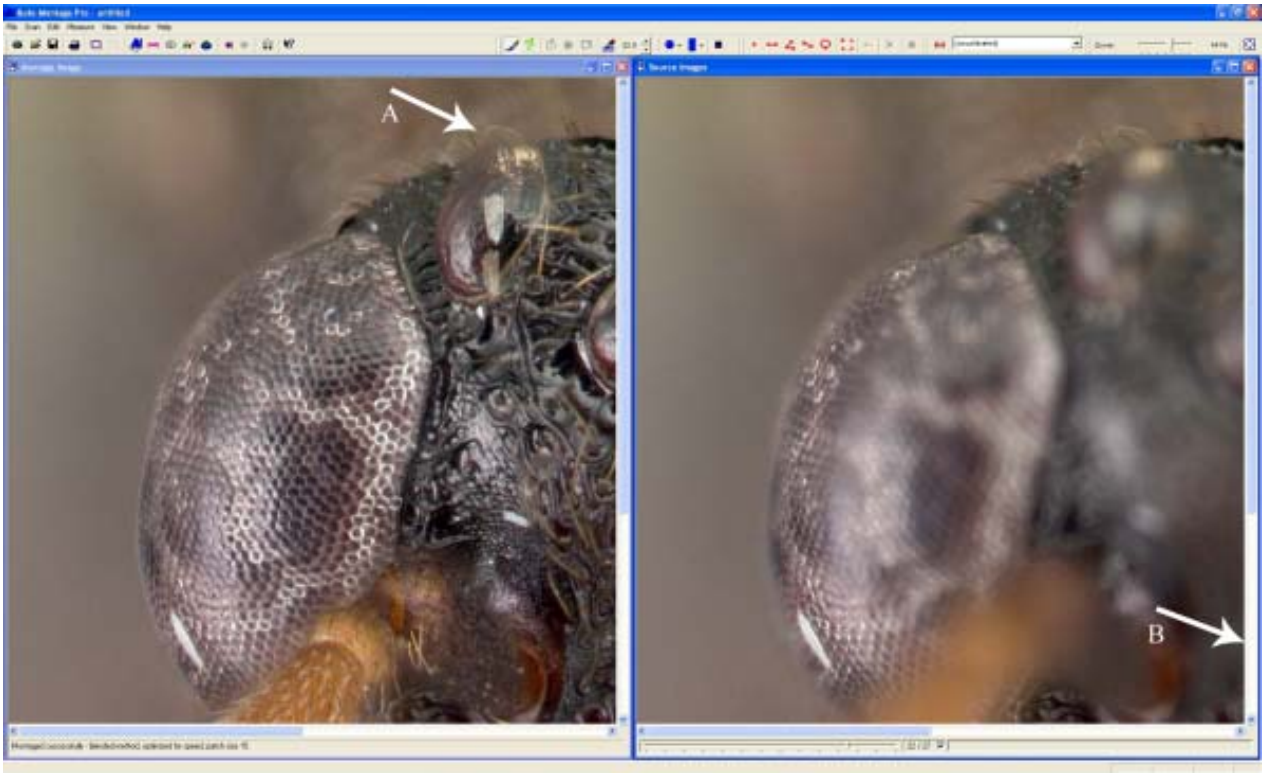
To clean up the area inside the antennae, make sure you're on the correct source image (A). Then click the polygon tool on the source image to the right of the left antenna (B), draw closely around the bottom of the clypeus, and end to the left of the right antenna (C). Select **Edit Paste Inside Polygon** (D).



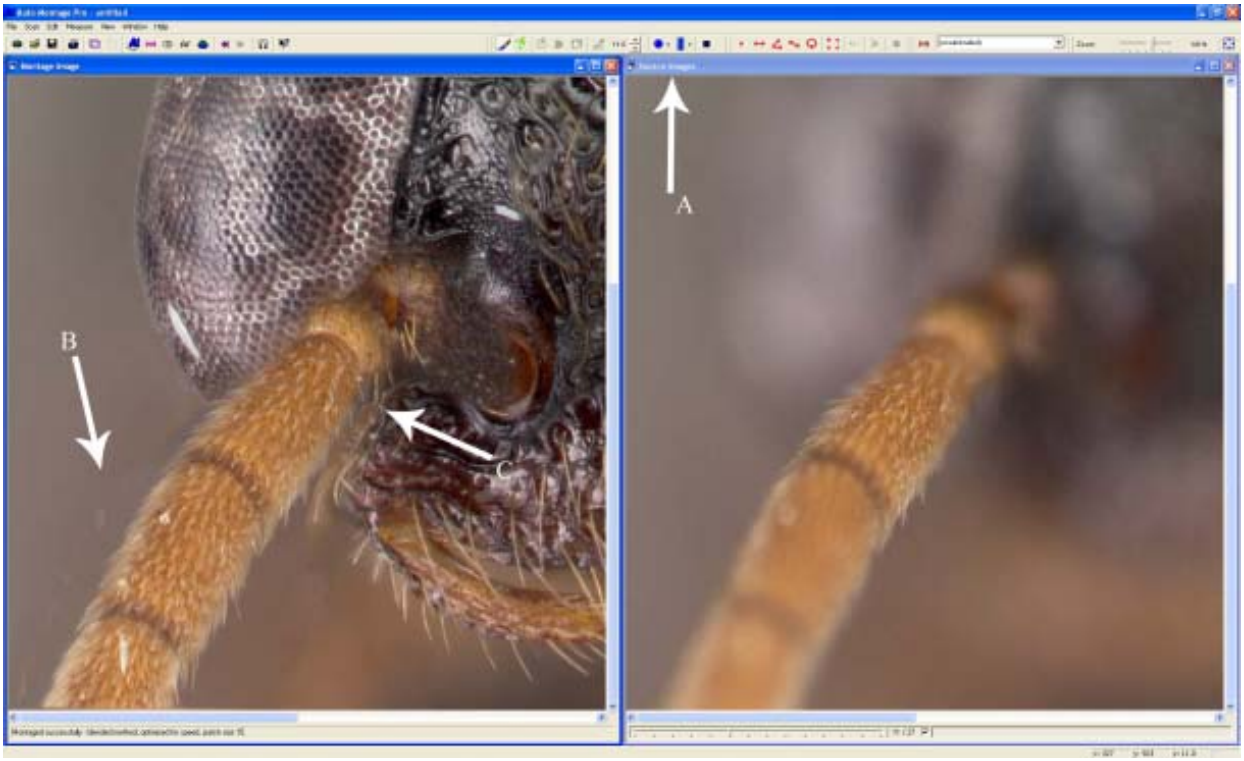
Once you've taken care of the artifacts in the background, go on to tackle the nitty gritty details. Begin by selecting the **zoom scrollbar** and zoom in to 100% (see arrow).



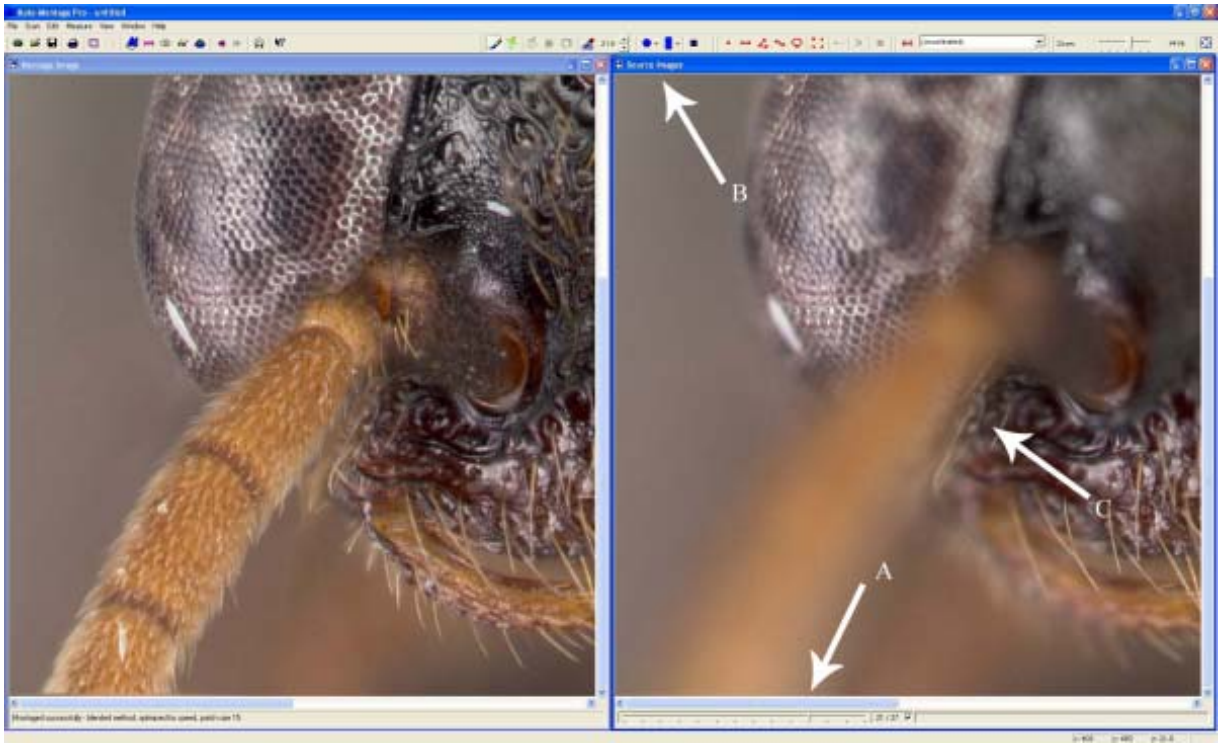
Start in the top left corner of the image (quadrant A). Detailed editing calls for the **Brush** tool (A). Start with a thick brush (B) with soft edges (C). The hairs on the head with bits missing jump out at me first (1). Scroll through the source images to find one where the missing bits are in focus (2). Draw on the source image what you would like pasted in the montage image (3).



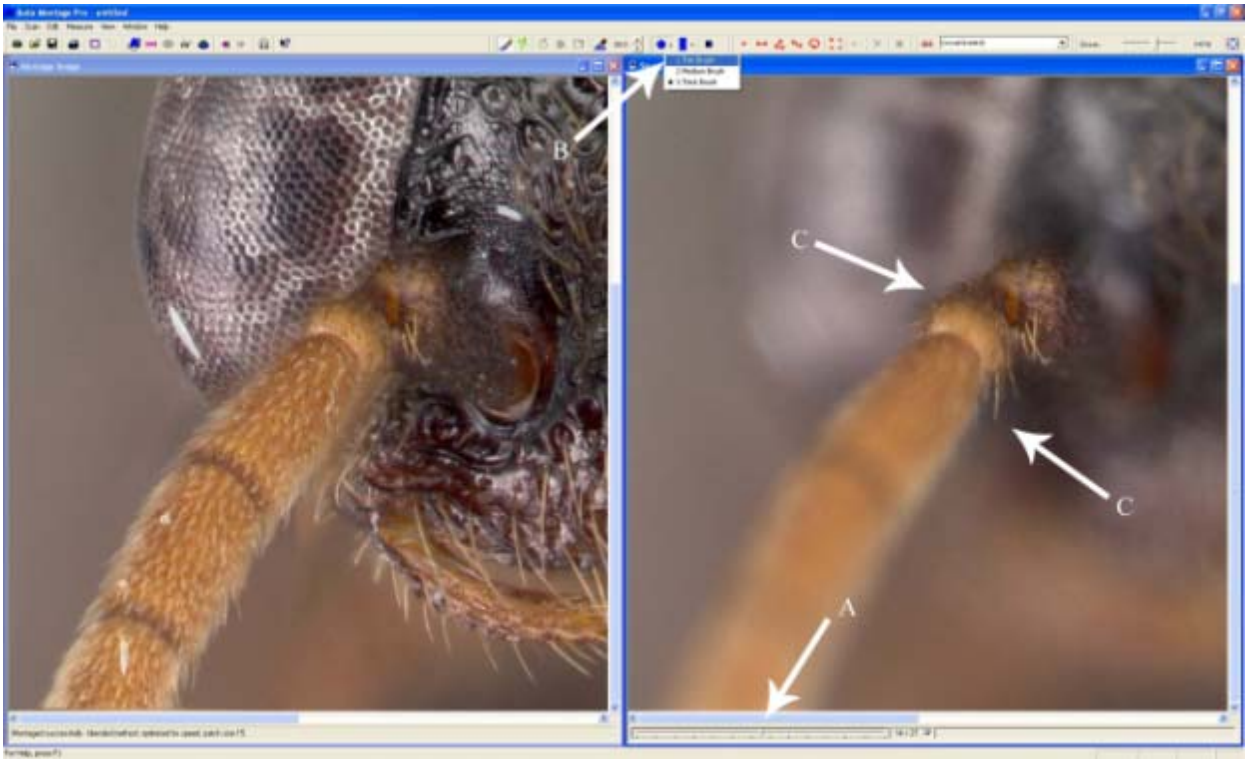
Ta da, the hair is complete in the montage image (A)! Move to quadrant B by scrolling down to the bottom left of the image (B).



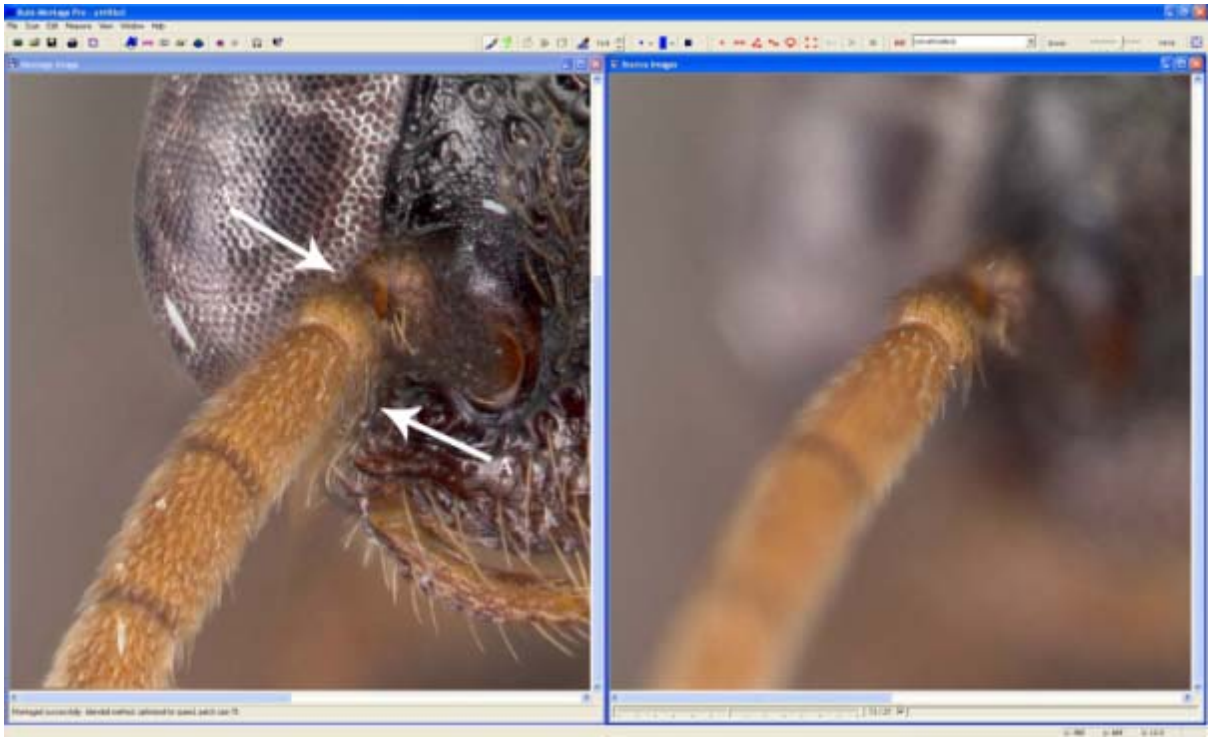
A few background artifacts have persisted (B) along with dead spots under the antenna (C). Get rid of these using a large brush with soft edges (A).



The background artifacts are fixed. Now scroll through the source images to find one where the base of the antenna is in focus (A). Select a medium brush with soft edges (B) and draw under the antenna (C).



Now that the base of the antenna is in focus, scroll through the source images to find one with the hairs on the antenna in focus (A). Select a thin brush with soft edges (B) and draw in hairs (C).



The hairs are in focus. Now move on to quadrants C and D, repeating these steps.

When all of the dead spots have been erased and hairs have been drawn in, you are done editing. Double-click on the Montage image. Select the **Stop Edit** icon in your toolbar. Insert the correct scalebar, making sure it is in the bottom right corner. Right-click on the screen and select **Export Image to File**. This will save only the montage image, not the source images.

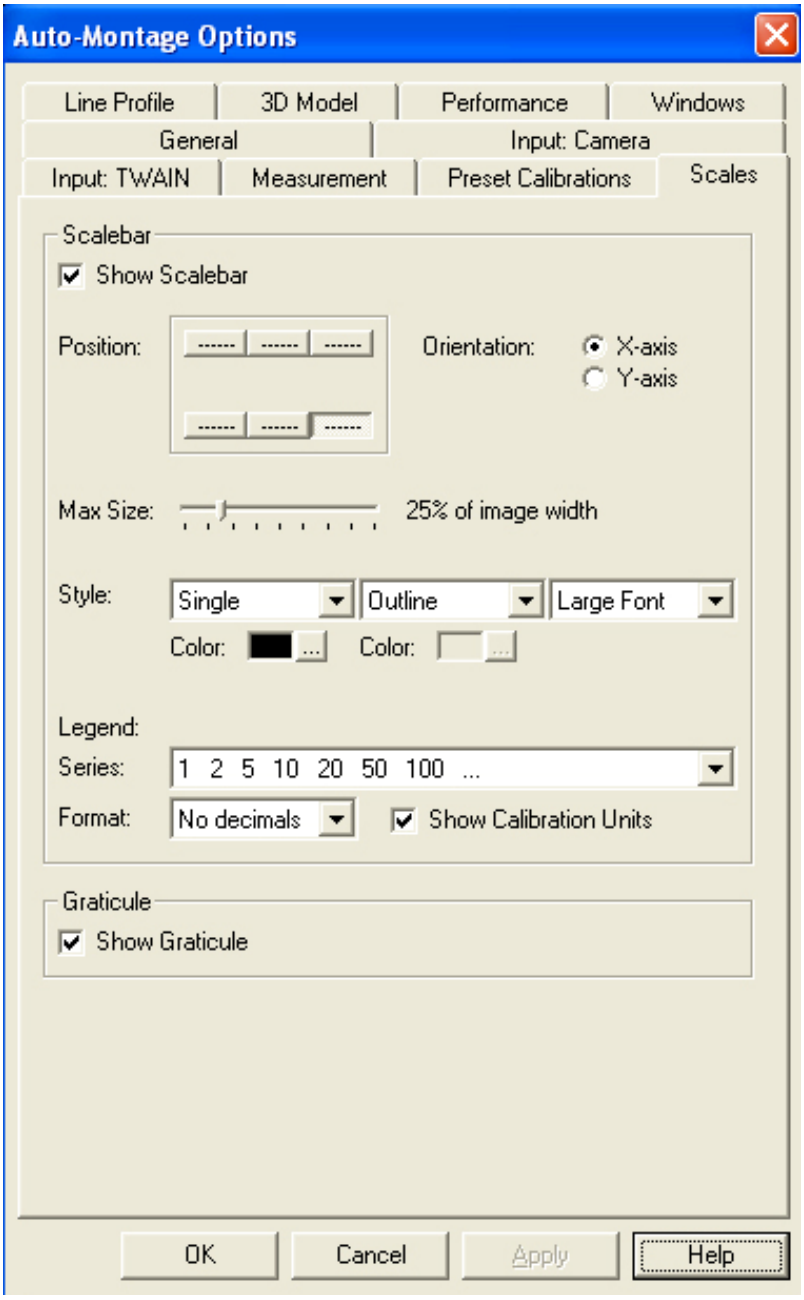
Important Information:

- Image ants using the **Live** function so that you can see how much distance you are covering with each turn of the knob.
- Take approximately **8-11** images for wing and genitalia shots, **15-20** images for head shots, and **19-23** images for profile and dorsal shots.
- There are three brush sizes (1-3) and four brush textures (0-3) to choose from.
- The polygon tool is used for editing large portions of an image (generally the background), while the brush tool is used for more detailed editing.

Scalebar:

Before you can start imaging specimens, you will need to calibrate measurements for each magnification of the microscope, repeating this task for each lens and each C-mount adaptor. Save these settings in the drop-down menu so that you can import a scale-bar onto each Montage image.

1. Open camera icon and obtain a live image.
2. Focus on your ruler.
3. Select the red scale bar icon (**{------}**).
4. Trace your ruler with the red scale bar.
5. Select the correct units (mm) in the dialog box.
6. Select the box **Add to Preset Calibration List** in the dialog box.
7. Name the measurement (lens-adaptor-magnification), for example: (Z16 2X 9.2).
8. The next time you image, your scale bar will be in the drop-down menu in the upper right hand corner of your window.
9. NOTE: a glitch in the software displays all measurements in **mu** instead of **mm** when first used. To correct this, go to the **Options** menu (it looks like as set of headphones) after saving your measurements, and select **Preset Calibrations.** Change the **Display Units** from **mu** to **mm.**



Make sure your scalebar settings match this window.

Important Information:

- Position = Lower Right Corner
- 25% of image max in length
- Style = Single, outline, large font
- Color = Black. (Change this to white if black is too hard to see.)
- Series = 1 2 5 10 20, etc.
- Format = No decimals