

Using the Motic Camera

Important Points:

- Do not wipe/touch any of the lenses with anything except lens paper. Kimwipes are NOT lens paper!
- Turn the eyepiece head so the word “Nikon” is right side up, then remove the right eyepiece from the microscope and set it down on the table on the end that goes into the microscope, NOT the end you look into. This could scratch the lens.
- When removing the eyepiece, grip the polished black ring that is beneath the plastic cap.
- Put the camera in the eyepiece socket so that you can read the label “Moticam”. Fasten the camera with the set screw.
- Put all camera/eyepiece equipment BETWEEN the computer and the microscope, not near the edge of the table! You run the risk of knocking the eyepiece off the table if it is set near the edge!
- Turn the focusing knob (front side) up to move the microscope stage closer to the objective.
- Turn the focusing knob (front side) down to move microscope stage farther from the objective.

Focusing between your eyes and the camera display:

4x Objective (RED):

When this is focused for your eyes, you need to turn the knob (front part) DOWN to focus the camera display

10x Objective (YELLOW):

Same as for 4x.

40x Objective (BLUE):

When focused for your eyes, it should be close to the correct focus for the camera display, make small adjustments.

Step-by-Step Guide to Taking a Photo:

1. Attach the camera to the RIGHT eyepiece and plug it into a USB port on the back of the Mac.
2. Click the Motic Images Plus icon on the desktop to open the program.
3. Go to File > New
4. Choose “Live Video” and click “Ok.” The window will open to show what the camera sees in the microscope.

5. Go to File > Video Settings to open up the camera adjustment controls.
 - a. To adjust the exposure (lighting) for the camera, click “Auto Exposure” and wait about 5 seconds. If you want the computer to do this automatically from now on, check off “Full Auto.” “White balance” will correct for the yellowness of the light. If you want to adjust the settings manually, turn off “Full Auto” and move the sliders to tweak the various lighting/color functions.
 - b. Set the size of the image to its maximum (probably 1280 x 1024). After you click OK, maximize the live video window with the green button in the upper left corner.
6. The image on the computer screen will probably not be in focus, because the camera and your eyes are at different “distances” from the slide.
7. To adjust the focus for the computer image, **SLOWLY** turn the focus knob on the microscope in the correct direction for the objective you are using (4×, 10×, or 40×). It is **IMPORTANT** to turn the knob **VERY SLOWLY**, because it takes time for the camera image on the computer to refresh. By focusing too quickly, you may go right past the point where the image is focused.
8. To make a photo, look at the top of the window at the three icons. Click the middle icon (it looks like a small digital camera with a clock next to it).
9. Change the ‘save’ location to the desktop.
10. To the right of where “File Name” appears in the dialog box, click the drop down box to save the image in the jpg format and NOT in the “.mix” format.
11. Leave the capture interval settings alone for now, but change the number of Total Images to “1” instead of “5.” You don’t need five copies of the same picture, right?
12. Click “OK” and the new file will appear on the desktop.

Putting a Scale Bar on your Image

To begin this task, take some nice images of cells. Now you will calibrate your microscope so you can determine the sizes of cells and structures within them. To do this, you will use a calibrated stage micrometer, a slide that has a very fine ruler etched into it (Figure 1).

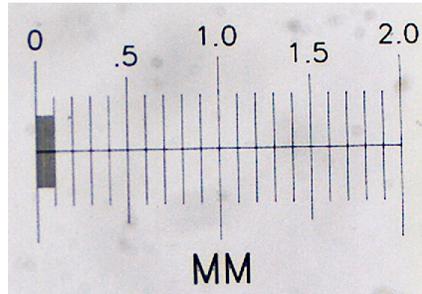


Figure 1. Stage micrometer from Ward's Science (part # 949910)

1. Return the 40× objective as the active lens. Remove the cell slide and replace it with the calibrated stage micrometer slide. Notice which side the stage micrometer is on. That side faces the objective lens.
2. Locate the stage micrometer by eye, center it, and get it into focus. If you are having trouble locating the micrometer, look at it from above the microscope stage. You should be able to make it out with your eyes. Move it to near the center of the objective lens and then try to find it in the microscope again.
3. Get the camera image of the stage micrometer, optimize it and be sure it is in good focus.
4. Take a single image of the stage micrometer and save it.
5. Open your micrometer image in ImageJ. Click on the line symbol and draw a straight line from one stage micrometer hash mark to another. It will be most accurate if you draw the line from hash marks as far apart as possible. (Why?)
6. From the “Analyze” pull down menu select “Set scale...”. The window that pops up will show you the distance in pixels of your line. You need to type in the actual distance between the two hash marks that you used. If you can't figure this out, ask for help. Be sure to use the correct units of measurement in the “Set Scale” box. (1 cm = 10⁴ μm.) Leave the aspect ratio set at 1. Your camera has square pixels, so the aspect ratio (height:width) is 1.
7. Put a scale bar on one of your yeast images. Use “Analyze > Tools > Scale Bar”. Save this in a separate file, appropriately named, using the “Save as” button. Make another Word or Open Office file with this image and an appropriate caption. When a scale bar appears in an image you should always give the dimensions of the scale bar, in the caption instead of adding that number to the image. The scale bar is usually positioned in one corner of the image where it will not obscure any important image detail.

8. Determine the size that a camera pixel represents when the 40× lens is used. You can do this by dividing the known distance between hash marks in part 6 by the distance in pixel units from part 6. This value is the number of μm per pixel (or cm per pixel if you prefer). This tells you how much of the specimen area falls on each pixel in the camera. This value will remain the same for any future use of this objective lens and camera in this microscope. The value will be different for different lenses (and for different cameras, if you happened to use a different one).