Procedure for Making Camera and Microscope Eyepieces Parfocal Charles Krebs 12/10/10 v1.5

The procedure discussed below is the method I use to make a SLR camera (or other lens-less camera body) that is mounted above the trinocular tube parfocal with the microscope eyepieces. I have always used SLR cameras of this type mounted independent of the microscope with no "hard" contact between them. This is done to minimize any deterioration of the image quality due to shutter or mirror vibration. Done properly, this arrangement also allows the camera to be raised or lowered in very fine increments, which is extremely important in order to make the camera accurately parfocal with the eyepieces. With some more recent camera bodies (such as Canon bodies with <u>EFSC</u>) the vibration issue has been reduced to the point where I am comfortable mounting the camera directly to the microscope. When this is done, it is still extremely valuable to have some method of permitting the camera body alone to be adjusted in fine increments up/down relative to the microscope. The custom adapters I have made for mounting directly to the microscope incorporate a focusing helical to permit this adjustment. The trinocular tube will hold an eyepiece that will project the image onto the camera sensor. The procedures given here should be effective with any camera where the microscope image can be projected directly onto the sensor.

Preliminary Preparation:

Be sure microscope viewing eyepieces are properly set up, and adjusted for your eyesight. If, on your microscope, the focus will change with a change in interpupillary distance setting, or there are individually focusing eyepieces, be sure they are properly set and these settings remain constant. If they are prone to move accidentally you might consider taping them in place so that the settings do not change. If they are inadvertently changed after following this "parfocal" procedure, you may need to run through it again.

It is best to use a 4x or 10X objective for this procedure. Higher power objectives will give less accurate results. It's somewhat counterintuitive, but the higher the power of the objective, the greater the depth-of-**focus** (at the film/sensor plane) even though the depth-of-**field** (at the subject plane) decreases dramatically. Since the purpose of this procedure is to position the camera body for most accurate focus, it is desirable to perform it with minimal depth-of-focus, which is obtained with low power objectives.

If you will be using an eyepiece designed as a "projection" or "photo-eyepiece" in the trinocular head (such as an Olympus FK or NFK) start at Step 1-A

If you will be using a normal viewing eyepiece in the trinocular tube, start at Step 1-B

Step 1-A

First set up a static subject slide that has a very fine detail and high contrast. Focus carefully on a specific detail through the eyepieces. *DO NOT CHANGE THE FOCUS DURING THE REMAINDER OF THIS STEP.*

If the trinocular tube is adjustable in height (some are, most are not), remove one of the viewing eyepieces and place it into the trinocular tube. While viewing through this eyepiece, adjust the trinocular tube height up/down until the subject appears in sharp focus. Leave the trinocular tube at that height. Put the "viewing" eyepiece back into the eyepiece tube, and place the photo-eyepiece into the trinocular tube.

If you have a camera body that has a "live-view" capability, go to STEP 2-B, if not, continue directly below.

Now, place the camera body above the trinocular tube, and while looking through the camera viewfinder

raise/lower the camera body until it appears you have achieved best focus in the camera viewfinder. (Not as easy as it sounds... but at this point, being as close as possible is good enough). We're done using the camera viewfinder for focus from now on.

Go to STEP 2-A: Three shot procedure.

Step 1-B

This procedure uses a regular viewing eyepiece in a way that causes it to project an image into a camera body. It does work, and the results can be of good quality so it may be worth a try if you want to use a DSLR without the lens. You are actually using the eyepiece in a manner quite different from its intended use. If possible, it would be better to locate appropriate "projection" photo-eyepieces that were made specifically for projecting the intermediate image onto the camera sensor/film. See Note 1 at end of this page.

First set up a static subject that has a very fine detail and high contrast. Focus carefully through the eyepieces. **DO NOT CHANGE THE FOCUS DURING THE REMAINDER OF THIS STEP.**

Place the eyepiece you will use as the photo relay into the trinocular tube and while viewing through it, adjust the trinocular tube up/down until the subject is also in focus through that eyepiece.

This is your starting position for the trinocular eyepiece. It will never be positioned lower than this point. At this "base" position, it will provide a "virtual" image that would require a lens to convert it to a "real" image capable of being recorded on a sensor or film. The way we can accomplish this without adding additional optics is to "pull" this eyepiece "out" slightly so that the distance between this eyepiece and the objective is increased. (This places the intermediate image formed by the objective slightly in front of the eyepiece diaphragm, and it will now project a real image.) If the trinocular tube is adjustable, you can do this by increasing its length to some degree. If it can not be adjusted you will need to place shims or spacers under the eyepiece to elevate it. After this eyepiece has been elevated you should no longer try to assess focus or magnification by looking through the trinocular eyepiece with your eye. (Reminder... the microscopes actual focus should not be changed). You now need to place a lens-less camera that can be raised and lowered above this eyepiece and proceed to "rough-in" the desired magnification based on the following rules:

As the eyepiece in the trinocular tube is moved up (increasing the distance between this eyepiece and the objective) the relay magnification will decrease. The camera must be moved so that image as seen through the camera viewfinder looks as if you have achieved best focus on the camera focusing screen. (Not as easy as it sounds... but at this point, being close is good enough).

If the magnification still appears to be too large through the camera viewfinder (that is, you are recording much less of the subject than desired compared to what can be seen through the viewing eyepieces) you must increase the distance that the eyepiece in the trinocular tube is raised... which will then necessitate lowering the camera until it looks as sharp as possible through the camera viewfinder. *(see Note 1 at bottom)*

(If you have ever used a slide projector the following analogy might be helpful. Think of the camera sensor as the screen, the eyepiece in the trinocular tube as the projector lens, and the microscope slide as the slide. If you focus the lens on a slide projector so that it moves farther way from the slide, the screen (sensor) must be moved closer to be in focus, and the projected image becomes much smaller).

Once you have "roughed in" the magnification and positioned the camera so that it appears that you have achieved best focus on the camera focusing screen you are ready to move on to the next step. We're done looking through the camera viewfinder for focus from now on.

If you have a camera body that has a "live-view" capability, go to STEP 2-B

If your camera does not have "live-view" capability, Go to Step 2-A: Three shot procedure

Step 2-A: Three shot procedure:

Take a series of three pictures.

1) The first should be taken at what appears to be the best focus through the viewing eyepieces.

2) For the second shot, move the fine focus and raise the subject up towards the objective just the slightest amount so that it now appears very slightly "out-of-focus" through the viewing eyepieces. (Your real point of visual "best" focus is now placed slightly "below" the subject).

3) For the third shot, go back and set visual best focus. Then using the fine focus, very slightly "lower" the subject stage so the subject now appears very slightly "out-of-focus" through the viewing eyepieces. (Your point of visual "best" focus is now placed slightly "above" the subject).

Now examine the three images very carefully at 100% or 200%. Find the one in which the subject appears best. (Chances are none will be that great, but you will be able to tell which is "closest".

-- If the "best" picture result came from the image where the subject is defocused slightly toward the objective (point of visual "best" focus falls slightly "below" the subject), the camera is too high... you need to lower the camera very slightly.

-- If the "best" result came from the image where the subject is defocused slightly away from the objective (point of visual "best" focus falls slightly "above" the subject), the camera is too low... you need to raise the camera very slightly.

Run through this 3-shot procedure until you consistently get the best results with the first shot... the one that looks "dead-on" focused through the eyepieces. It may take a few iterations. Try it with several subjects to confirm. If done with care, this procedure should allow you to have your camera over the trinocular tube be accurately parfocal with the view through the eyepieces.

Step 2-B: For cameras with "Live-View" capability:

Mount the camera onto or above the trinocular tube. With "live-view" turned on, be sure the subject from Step 1 is centered on the viewing screen (if not, center it using the camera buttons, not the x-y microscope stage controls). Then adjust the height of the camera up/down until best focus is achieved in the camera body. That's it! Live-view has greatly simplified this procedure!

It's always best to try a few different subjects and check to be sure that you consistently get proper focus in the viewing eyepieces and camera simultaneously.

Note 1: If available, it might be preferable to use a "viewing" eyepiece of lower than 10X power. This would require raising the eyepiece a shorter distance in order to get acceptable relay magnifications. My personal experience with this method is limited. I have always used Olympus NFK photo-eyepieces with my Olympus (and a few Zeiss) objectives. However I was curious how using a regular eyepiece would compare. Some testing that I have done has shown that the results can indeed be excellent... the NFK photo-eyepieces yielding what I felt were just slightly better results. For these tests I used an Olympus 10X eyepiece that provided the proper chromatic corrections for my LB series Olympus objectives. The 10X had to be "lifted" a fair amount in order to get a sufficiently low relay magnification that would record a good portion of the field of view. I don't own a lower power "normal" Olympus eyepiece.