

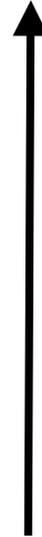
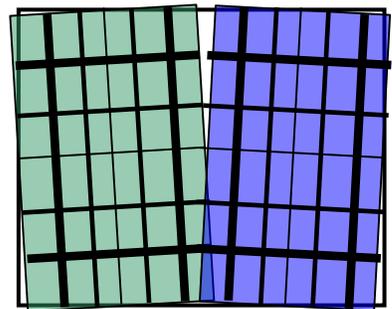
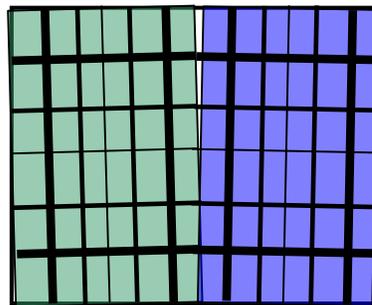
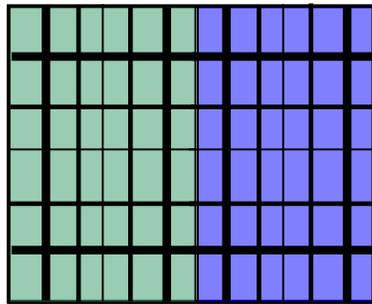
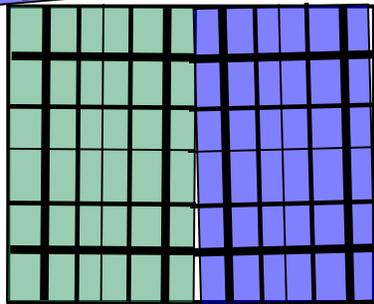
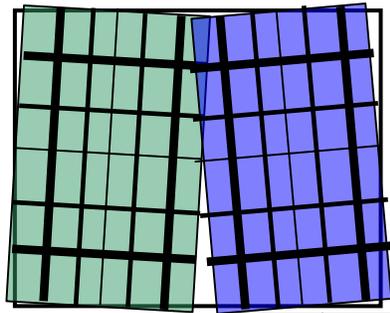
DUAL-VIEW™ ALIGNMENT INSTRUCTION SHEET

- (1) Attach your camera to the Dual-View™ Micro-Imager by screwing the female C-mount on the camera to the male C-mount on the Dual-View™. Make sure the camera and the Dual-View™ are screwed tightly together, but be careful not to over tighten.
- (2) Attach the microscope C-mount to the Dual-View™. Screw the male side of microscope C-mount into the female C-mount on the Dual-View™.
- (3) Attach the “camera/Dual-View™/microscope C-mount adaptor” combination to the microscope by sliding the microscope C-mount adaptor into a video port of your microscope. Position the Dual-View so its logo is either facing up towards the ceiling or towards you. Tighten any screws that hold the microscope C-mount adaptor into your microscope.
- (4) Turn on the brightfield/transmitted light only (do not use fluorescence) on your microscope.
- (5) Make sure you do not have ANY microscope filter cube in the light path of the microscope.
- (6) Pull the Dual-View™ filter slide half-way out of the tube (called the “Bypass Mode”).
- (7) Using the software of your choice, display a live, full field image to your computer screen. Make sure you are not “binning” and you do not have “auto scaling” on.
- (8) You should see 2 black stripes and one white or grey stripe on your computer screen. The black stripes indicate where light is being blocked from reaching your camera and the white strip indicates where light is being allowed to reach your camera. The white stripe is an image of the internal Dual-View™ aperture.
- (9) These 3 stripes may appear skewed if your camera and the Dual-View™ are not corrected rotated with respect to each. When both system are properly rotated, the internal aperture in the Dual-View™ will appear straight up and down because it will be parallel to the edges of your camera chip.
- (10) The Dual-View™ is supplied with a C-mount adaptor that can rotate independently from the rest of the Dual-View™ body. This option allows you to properly orient the internal aperture in the Dual-View™ with respect to your camera. (The setscrew that allows you to lock and unlock Dual-View™ C-mount adaptor is located in a recessed hole labeled with the words: “camera Dual-View™ lock” found at the top of the Dual-View™ tube.
- (11) Using the 0.05-inch yellow-handled ball driver that is supplied with your system, loosen the “camera lock” setscrew to unlock the Dual-View™ C-mount adaptor. Once this is unlocked, you will be able to twist or rotate the Dual-View™ C-mount adaptor with the camera attached.
- (12) Rotate your camera and Dual-View™ C-mount adaptor clockwise or counterclockwise with respect to the Dual-View™ body until the black and white stripes appear to be very straight up and down.
- (13) Lock the “camera lock” set screw. Note that now the logo on the Dual-View™ and the logo on the camera will not necessarily be facing the same direction. However, you will want to position the Dual-View™ so its logo is either facing up towards the ceiling or towards you as you sit at the microscope.
- (14) Now you will use the A1 and A2 knobs to adjust the aperture until it is centered and its opening matches ½ the width of your camera. You will need to have:

- a. Slider lever in "Dual-View Mode" position
 - b. Dual-View™ filter holder in "Bypass Mode" position (half-way out of tube).
- (15) *(PLEASE NOTE: it is very important that you take the time to be very precise about this step.)* Adjust the A1 and A2 knobs until the **left** aperture (the black stripe located on the left) ends EXACTLY at $\frac{1}{4}$ of the way across the field and the **right** aperture (the black stripe located on the right) ends EXACTLY at $\frac{3}{4}$ of the way across the field. To do this, you will need to know EXACTLY how many pixels your camera has in the x-direction. Divide the number of camera pixels by 4 to get the pixel number that corresponds to the $\frac{1}{4}$ position and then multiply that pixel number by 3 to get the pixel number that corresponds to the $\frac{3}{4}$ position.
- (16) Now you will adjust the R/L knobs to put the Dual-View™ images in their proper left and right positions on your camera. You will need to have:
- a. Slider lever in "Dual-View Mode" position
 - b. Dual-View™ filter holder inserted all the way into the Dual-View™ tube.
- (17) Now, you should see 2 stripes that dominate the image field. (You may need to increase the level of your brightfield/transmitted light to be able to see these 2 stripes.) Each stripe will usually be a different grey level. You may also see a bright stripe in the middle of the image field where these 2 stripes overlap. You may also see 2 or 3 narrow black stripes located on the far left of the image, on the far right of the image field, and/or in the center of the image field.
- (18) Using the R/L knobs **ONLY** position the 2 grey stripes left and/or right until you do not see any black stripes in the image and there is no overlap in the center.
- (19) Now you will adjust the U/D knobs to put the Dual-View™ images in their proper up and down positions on your camera. You will need to have:
- a. Slider lever in "Dual-View Mode" position
 - b. Dual-View™ filter holder in "Bypass Mode" position (half-way out of tube).
- (20) Place the alignment grid that is included with the Dual-View™ system on your microscope stage. Adjust the microscope focus until the grid is in focus on your computer screen. You should see one image of the grid in the exact center of your image field.
- (21) If the horizontal lines of the grid are not horizontal, then:
- a. If you have rotation adjustments on your stage, rotate the grid until the horizontal lines are horizontal.
 - b. If you do not have rotation on your stage, loosen the screws of your microscope's C-mount adaptor and rotate the microscope C-mount adaptor with the Dual-View™ attached until the horizontal lines are horizontal.
- (22) Move your microscope stage until you can see an edge of the grid either at the **top** or the **bottom** of your image field. You will know you are at the edge of the grid, when you see all black instead of any grid lines.
- (23) Place your mouse pointer on one of the horizontal lines in your image, as far to the left on the image as possible. Be very careful to not touch or move your mouse now because you will be using its position as a reference point.
- (24) Insert the Dual-View™ filter holder all the way into the Dual-View™ tube. You should now see 2 images of the grid, one on the left and one on the right. One image will usually be brighter than the other image due to the different bandpasses of the emission filters. (You may need to increase the level of your bright field to be able to see these 2 images of the grid.)

- (25) Look **ONLY** at the image on the **left**. Using the mouse pointer as your reference, check to see if this image is too far up or too far down, as compared to the previous image you saw in step (23). Use the U/D knob that controls the **left** image to move this image either up or down until it is in the proper location (according to the mouse pointer).
- (26) Pull the Dual-View™ filter holder half-way out of the Dual-View™ tube so you once again only see one image of the grid in the center of the image field. (You may need to decrease the level of your bright field to be able to see this single image of the grid.)
- (27) Keeping your mouse pointer on the same horizontal line in your image, move as far to the right on the image as possible. Be very careful to not touch or move your mouse now because you will again be using its position as a reference point.
- (28) Insert the Dual-View™ filter holder again all the way into the Dual-View™ tube. Now look **ONLY** at the image on the **right**. Using the mouse pointer as your reference, check to see if this image is too far up or too far down, as compared to the previous image you saw in step (23). Use the U/D knob that controls the **right** image to move this image either up or down until it is in the proper location (according to the mouse pointer).
- (29) Now you are within a few pixels of having the 2 Dual-View™ images aligned and registered to each other.
- (30) To get your 2 images aligned to each other to better than a few pixels, you will need to use your software's alignment module. This module has been specially written to work with your Dual-View™. This module will allow you to subtract the 2 Dual-View™ images and display the resulting subtracted image live to your computer screen while you fine tune the R/L and U/D knobs on the Dual-View™.
- (31) The Dual-View™ optics produce images that can be aligned to each other at a subpixel level. The most misalignment you should experience will be in the extreme corners of your image and will be on the order of $\frac{1}{4}$ to $\frac{1}{2}$ of a pixel.
- (32) Using the special alignment module in your software, view a live subtracted image.
- (33) You will first adjust the images right and left. So, for right now, ignore any up/down related misregistration you may have. Using only the R/L knobs, fine-tune the right/left alignment of the images until they as precisely aligned as possible.
- (34) It is important to understand that when you move the images up and down using the U/D knobs, the images move up and down on an arc (see diagram on next page).
 - As the right image is moved up, you will see that its top starts to rotate in to the left.
 - As the left image is moved up, you will see that its top starts to rotate in to the right.
 - As the right image is moved down, you will see that its bottom starts to rotate in to the left.
 - As the left image is moved down, you will see that its bottom starts to rotate in to the right.

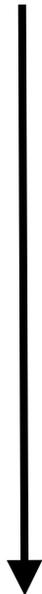
Therefore if you do not position the 2 images in their proper up/down location, they will be rotated relative to each other. There is an "ideal location" up/down where there will be no rotation between the images.



As the images are moved up, their tops rotate in toward each other

Images in ideal up/down location

As the images are moved down, their bottoms rotate in toward each other



- (35) Now use only the U/D knobs and fine-tune the up/down alignment of the images until they are precisely aligned. If there appears to be some rotation between the 2 images, you will need to move both images up together at the same time (by a slight amount) and see if the rotation improves. If it does not improve, then move both images down together (by a slight amount) and the image rotation will improve.
- (36) On days that you require full-field imaging (you do not want to use the Dual-View for split field imaging), you do not have to remove the Dual-View™ from your set-up. You will use the Dual-View in its “Bypass Mode”. You will need to have:
- a. Slider lever in “Bypass Mode” position
 - b. Dual-View™ filter holder in “Bypass Mode” position (half-way out of tube).