

DC2™ Alignment Procedure

This document provides a straightforward, step-by-step outline of the alignment procedure for the Photometrics® DC2 imaging system. As some combinations of microscopes and objectives, particularly in some newer Olympus and Zeiss stand/objective configurations, have displayed vignetting, it is recommended that the DC2 be tested for compatibility with your system. If use of the procedure does not produce alignment within the specifications listed for the DC2 system, please contact the Photometrics Support team at support@photometrics.com.

NOTE: PLEASE READ THE ENTIRE PROCEDURE BEFORE YOU BEGIN ALIGNMENT OF THE DC2.

Install DC2 on Microscope

1. Attach your cameras to the DC2 by screwing the female C-mounts on the cameras onto the male C-mounts on the DC2 (**Figure 1a**).



(a)



(b)

Figure 1. (a) The DC2's two C-mounts for mounting two CCDs. (b) Microscope c-mount attached to female c-mount of DC2.

2. Remove the C-mount adapter from the video port of your microscope and screw the adapter into the female C-mount on the DC2 (**Figure 1b**). The DC2 is designed to work with a 1x C-mount adapter without additional lenses. Use of any other C-mount adapters may alter imaging performance.
3. Attach the "cameras/DC2" combination to the microscope by sliding the C-mount adapter into the video port of the microscope. Position the DC2 so that its logo faces either towards the ceiling (for side-port microscopes) or towards you (for top-port or bottom-port use).

Before starting alignment:

1. Make sure there are no sample slides in place on the microscope.
2. Turn on (only) the brightfield/transmitted light on the microscope.
Do not use the incident fluorescence source.
3. Make sure there are no microscope filter cubes in the light path of the microscope.
4. Make sure you are using an objective with a magnification of at least 40x.
5. Make sure the objective is of the PlanApo variety and that it is not a phase contrast objective.
6. Pull the DC2 filter slider halfway out of the tube (Bypass Mode).



Figure 2. Filter cube in Bypass Mode position.

The filter slider should click into the Bypass Mode position (**Figure 2**).

5-Step Alignment Procedure

The alignment of the DC2 is performed using a 5-step process:

1. Insertion and orientation of the calibration grid
2. Focusing of the bypass image
3. Focusing of the second camera's image
4. Positioning and aligning CCD mask
5. Alignment using software feedback

Step 1: Insertion and orientation of the calibration grid

1. Remove the calibration grid supplied with the system from its case. The grid is a glass substrate that has a metallic coating on one side of the substrate. Please note which side has the coating.
2. The objective you will be using will normally require a coverslip of the appropriate thickness. Please place a coverslip on top of the coating side of the grid and tape it to the grid. If the objective you are using is an immersion objective, please make sure to use the appropriate immersion liquid when doing this alignment.

3. Always place the grid on your microscope stage with the coating/coverslip facing the objective. Rotate the grid until the grid pattern is perfectly horizontal and vertical. If the grid is not placed in the proper orientation, you will not be able to produce a focused image of the grid.
4. The DC2 filter holder should be in Bypass Mode (as described above).
5. Send the light to the microscope eyepieces and adjust the microscope focus until the image of the grid is in focus when viewed through the eyepieces.

Step 2: Focusing of the bypass image

1. Using the software of your choice, display a live, full-resolution image on your computer screen.
2. The region of interest should be the “full chip”.
3. Make sure you are not binning.
4. Make sure you do not have “auto-scaling” of the display turned on.
5. The image of the grid should appear in the field of view captured by the camera. You may need to adjust the exposure settings to match the dynamic range of the camera to the illumination intensity.
6. The image of the grid may be out of focus. Using the focus adjustment dial on the left C-mount port of the DC2 (**Figure 3**), adjust the focus until the image of the grid is crisply in focus.

Step 3: Focusing of the second camera's image

1. Push in the filter cube so that the image is split by the dichroic to both cameras.
2. Rotate the second camera until the orientation of the grid is identical in both images. To do so, loosen the three $\frac{5}{64}$ " set screws with the supplied ball driver. Rotate Camera 2 until the orientation of the grid is correct and then retighten the set screw.
3. Using the focus adjustment dial on the right side of the DC2 (**Figure 3**), bring the image from Camera 2 into crisp focus. Both images should now be parfocal to the image as viewed through the eyepieces.

Bypass focus adjustment dial

Camera 2 focus adjustment dial

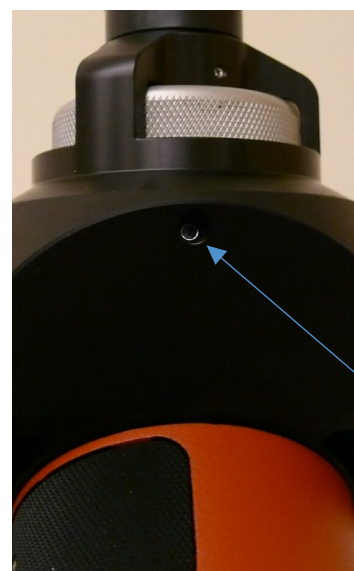


Figure 3. The DC2's bypass focus adjustment dial, Camera 2 focus adjustment dial, and Camera 2 camera lock set screw.

Step 4: Positioning and aligning CCD mask

1. The DC2 is equipped with four CCD aperture blades, one for each side of the chip.
The position of this mask is adjusted with the lower set of 0.05" hex screws on each of the four aperture adjustment panels on the DC2.

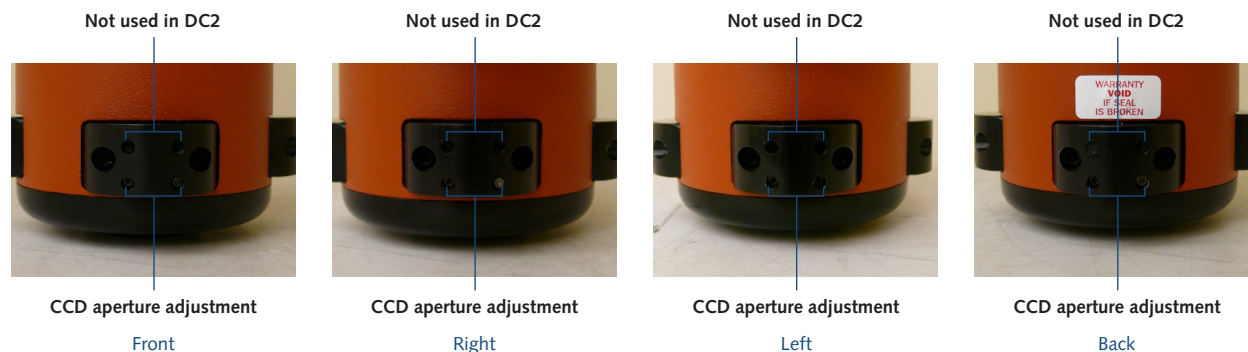


Figure 4. Aperture adjustment panels.

2. Place the DC2 filter cube into Bypass Mode (**Figure 2**) mode and have the cameras displaying a live preview image. Starting with the "front" aperture adjustment panel (**Figure 4**) use the supplied 0.05" hex key and the CCD aperture adjustment screws to adjust the position of the front CCD aperture blade so that it is just at the top of the field of view and then adjust it so it is just out of the field.
 - a. Each CCD aperture blade has two adjustment screws. One that adjusts the left half of the blade and one that adjusts the right half.
 - b. To adjust the position of the blade, adjust one half first and then the other half, thus "walking" the blade into the desired position.
 - c. Once the position of the blade is close to the desired position, the precise angle of the blade can be adjusted by turning one or the other adjustment screws slightly until the desired angle is reached
3. Repeat these adjustments for right, left and back aperture blades.

DO NOT OPEN THE CCD APERTURE BLADE BEYOND THE EDGE OF THE CAMERA BEING USED.

Step 5: Alignment using software feedback

To align the two images to each other to within a few pixels, you will need to use your software's alignment module. This module has been specially written to work with the DC2. It will allow you to subtract the two DC2 images and display the resulting subtracted image live to the computer screen while you fine-tune the R/L and U/D knobs on the DC2.

If the real-time subtracted composite image is not available, you can perform the alignment by viewing a real-time color overlay of the two images of the calibration grid and adjusting the DC2 so that the grid bars are overlapping in respect to the x and y axes of the images.

The DC2 optics produce images that can be aligned to each other at a single pixel level.

1. Using the special alignment module in the software, view a live subtracted image.
2. Adjust the images' right/left positions first. For now, ignore any up/down-related misregistration. Using only the R/L knob on the right side of the DC2 (**Figure 5**), fine-tune the right/left alignment of the second image until the vertical grid bars in the two images are as precisely aligned as possible.

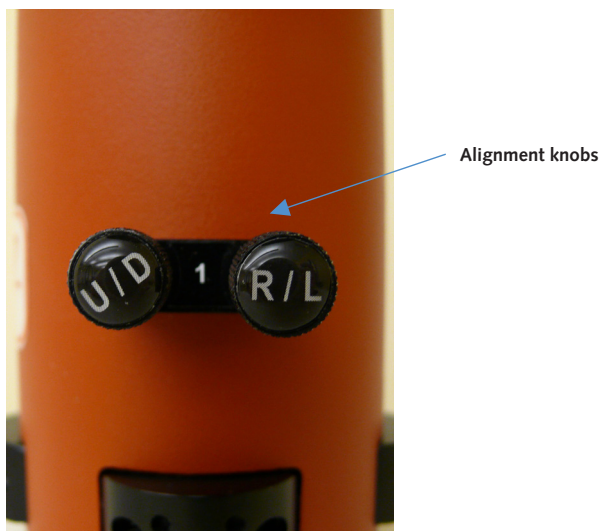


Figure 5. The up/down and right/left alignment knobs are used to bring the field of view captured by the second camera into precise registration with the field imaged by the primary (bypass) camera.

3. Now use only the U/D knob (**Figure 5**) and fine-tune the up/down alignment of the second image until the horizontal grid bars are precisely aligned with the horizontal grid bars in the first image. When the R/L and U/D adjustments are done correctly, the image of the grid should vanish from the real-time subtracted image, as the image from one camera is subtracted from the other. You may need to adjust the contrast of the images independently to achieve a completely “black” composite image.
4. As you adjust the up/down position of the second image, a slight rotation of the image will occur. This rotation can be corrected by loosening the three $\frac{5}{64}$ " set screws for Camera 2, rotating the camera until the image rotation is eliminated, and retightening the set screw.
5. Adjust the focus adjustment dials to achieve pixel alignment across the two camera images. Start by turning one camera's focus adjustment dial in single 360 degree turns followed by U/D and R/L knob readjustment to check for alignment. Continue with half-turns or adjustment of the other camera's focus adjustment dial if needed to achieve alignment.
6. On days that you require full-field imaging (i.e., when you do not want to use the DC2 for dual-field imaging), you do not have to remove the DC2 from the microscope. Instead, you simply need to click the filter assembly halfway out of the DC2 (Bypass Mode position) in order to send all of the light coming back from the sample to Camera 1.