QV2™ Alignment Procedure

This document provides a straightforward, step-by-step outline of the alignment procedure for the Photometrics® QV2 imaging system. If use of the procedure does not produce alignment within the specifications listed for the QV2 system, please contact us at support@photometrics.com.

NOTE: PLEASE READ THE ENTIRE PROCEDURE BEFORE YOU BEGIN ALIGNMENT OF THE QV2.

Install QV2 on Microscope

1. Attach your camera to the QV2 by screwing the female C-mount on the camera to the male C-mount on the QV2. Be careful not to overtighten.

2. Remove the C-mount adapter from the video port of your microscope (**Figure 1a**) and screw the adapter into the female C-mount on the QV2 (**Figure 1b**). The QV2 is designed to work with a 1x C-mount adapter without additional lenses. Use of any other C-mount adapters may alter imaging performance.

(a)   (b)

Figure 1. (a) Remove the C-mount adapter from the camera port of the upright microscope. (b) Attach the C-mount adapter to the QV2.

3. Attach the "camera/QV2/adapter" combination to the microscope by sliding the C-mount adapter into the video port of the microscope (**Figure 2**). Position the QV2 so that its logo faces either towards the ceiling (for side-port microscopes) or towards you (for top-port or bottom-port use).

Figure 2. The QV2/camera combination mounted on the top port of the microscope.

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 fluorescence.

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 there is no phase
   ring in the objective.

6. Place bypass filter cube in the system.

7. Slide the bypass lever to QV2 Mode (**Figure 3**).

8. The microscope should be set to direct the image to the camera, not to the eyepieces.

9. Using the software of your choice, display a live, full-resolution image on your
   computer screen.

   a. The region of interest should be the "full chip".

   b. Make sure you are not binning.

   c. Make sure you do not have "auto-scaling" of the display turned on.

Filter cube in
QV2 Mode

Bypass lever in
QV2 Mode

Figure 3. The QV2 with the bypass lever in the QV2 Mode position.

5-Step Alignment Procedure

The alignment of the QV2 is performed using a 4-step process:

1. Positioning and aligning CCD mask

2. Orientation and centration of the 4-blade aperture

3. Insertion and orientation of the calibration grid

4. Alignment of the QV2 images

5. Fine alignment using software feedback

Step 1: Positioning and aligning CCD mask

1. When the camera is acquiring, you should see an image of the 4-blade aperture. Black areas indicate where light is blocked from reaching the camera. The white or grey area indicates where light is reaching the camera. This white or grey area is an image of the internal QV2 aperture. If the area is white, you may be saturating the camera. Please lower the intensity of the brightfield light until the white area becomes a light grey color.

2. The light area of the image may appear rotated if the camera and the QV2 are not rotated correctly in respect to each other (**Figures 4a** and **4b**). When both systems are properly rotated, the 4-blade aperture in the QV2 will appear vertical for the left/right aperture blades and horizontal for the top/bottom aperture blades (**Figure 4c**).
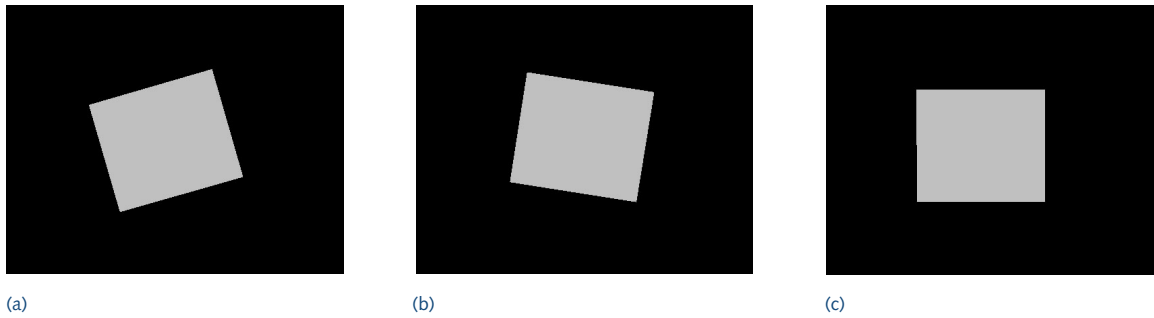
(a)   (b)   (c)

Figure 4. (a, b) Images of the QV2 aperture show that the aperture and camera are not aligned with each other. (c) Aperture edges are vertical/horizontal when the camera is properly aligned to the QV2.

3. The QV2 is supplied with a C-mount adapter that can rotate independently from the rest of the QV2 body. This option allows you to properly orient the internal 4-blade aperture in the QV2 in respect to the camera. The screw that allows you to lock and unlock the QV2 C-mount adapter is located in a recessed hole labeled with the words *camera lock* found at the top of the QV2 (**Figure 5**).

Camera lock

Figure 5. The *camera lock* on the QV2 can be unlocked to rotate the C-mount adapter.

4. Using the 0.05-inch ball driver that is supplied with the system, loosen the *camera lock* screw to unlock the QV2 C-mount adapter.

5. Rotate the QV2 C-mount adapter, along with the camera, clockwise or counterclockwise in respect to the QV2 body until the top and bottom aperture edges are horizontal and the left and right aperture edges are vertical (**Figure 4c**).

6. Once the aperture edges are exactly vertical and horizontal, tighten the *camera lock* screw to lock the C-mount adapter on the QV2.

7. Slide QV2 slider to Bypass Mode

8. The QV2 is equipped with four CCD aperture blades, one for each side of the chip. The position of this mask adjusted with the lower set of 0.05 hex screws on each of the four aperture adjustment panels on the QV2.
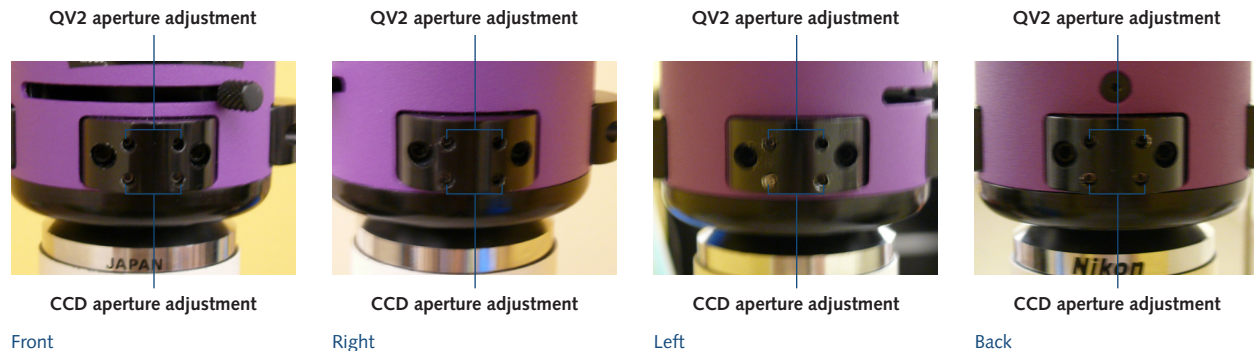
QV2 aperture adjustment　　QV2 aperture adjustment　　QV2 aperture adjustment　　QV2 aperture adjustment

CCD aperture adjustment　　CCD aperture adjustment　　CCD aperture adjustment　　CCD aperture adjustment

Front　　　　　　　　Right　　　　　　　　Left　　　　　　　　Back

Figure 6. Aperture adjustment panels.

9. Place the system in Bypass Mode and have the camera displaying a live preview image. Starting with the "front" aperture adjustment panel (**Figure 6**) 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 adjustment 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

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

Step 2: Orientation and centration of the 4-blade aperture

1. Move QV2 slider to QV2 Mode position.

2. Now use the aperture screws on the left and right aperture adjustment panels (**Figure 6**) to adjust the location of the aperture edges. The resultant image should look like **Figure 7**. It is very important that you execute this step in a highly precise manner.

   a. Adjust the left edge of the aperture until it is located ¼ across the field of view (from left).

   b. Adjust the right edge of the aperture until it is located ¾ across the field of view (from left).

   c. Adjust the top edge of the aperture until it is located ¼ across the field of view (from top).

   d. Adjust the bottom edge of the aperture until it is located ¾ across the field of view (from top).
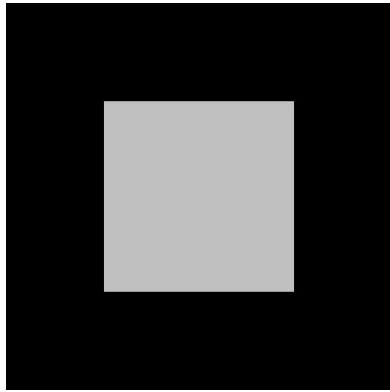
Figure 7. The correct positioning of the QV2 aperture blades.

For example, for a 1024 x 1024 array, the left/right/top/bottom aperture blades should be positioned as follows:

    a. Left aperture blade: pixel 256 from the left edge of the CCD

    b. Right aperture blade: pixel 768 from the left edge of the CCD

    c. Top aperture blade: pixel 256 from the top edge of the CCD

    d. Bottom aperture blade: pixel 768 from the top edge of the CCD

    When positioned properly, the aperture blades will define a region of the CCD that is exactly ¼ of the CCD area (512 pixels by 512 pixels), located directly in the center of the CCD.

Step 2: Insertion and orientation of the calibration grid
With the completion of Step 1, the internal aperture of the QV2 has been centered in respect to the camera attached on the back. Step 2 will properly orient the calibration grid provided with the system. You will use the image of the grid for the fine image alignment in Step 4.

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. If the grid is not placed in the proper orientation, you will not be able to produce a focused image of the grid.

4. Make sure the slider lever is in the QV2 Mode position (**Figure 3**).

5. Ensure that QV2 bypass cube is in place.

6. Adjust the microscope focus until the image of the grid is in focus on the computer screen. You should see one image of the grid in the exact center of the image field. An example is shown in **Figure 8**.
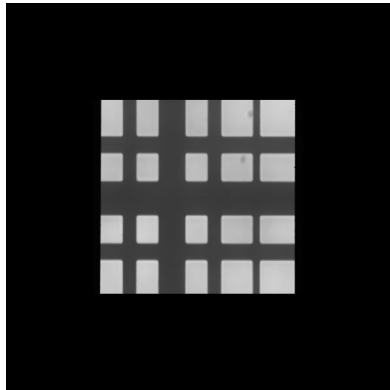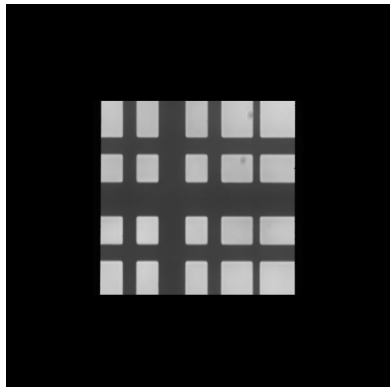
Figure 8. Image of the grid in the center of the image field. You can see that the grid is not properly oriented because the lines are not horizontal and vertical.

7. If the lines of the grid are not horizontal and vertical, then proceed as follows:

   a. If your microscope stage has rotation adjustments, rotate the grid until the lines are horizontal and vertical in the image (**Figure 9**).

   b. If your microscope stage does not have rotation adjustments, loosen the screws of the microscope C-mount adapter and rotate the microscope C-mount adapter with the QV2 attached until the lines are horizontal and vertical (**Figure 9**).

Figure 9. Image of the grid in the center of the image field. You can see that the grid is properly oriented because the lines are horizontal and vertical.

Step 3: Alignment of the QV2 images
Step 3 ensures that the right/left and top/bottom positions of the images on the camera are correct.

1. Keep the slider lever in QV2 Mode (**Figure 3**).

2. Insert the QV2 filter cube into the QV2 tube.

3. You will now see four images in the image field. If you do not see anything, or if the images are very dim, increase the intensity of the brightfield light until the images are visible on the computer screen. These four images correspond to the same 512 x 512 field of view that you created in Step 1. The four images are separated into four color channels and each is positioned near its own quadrant of the CCD (**Figure 10**).
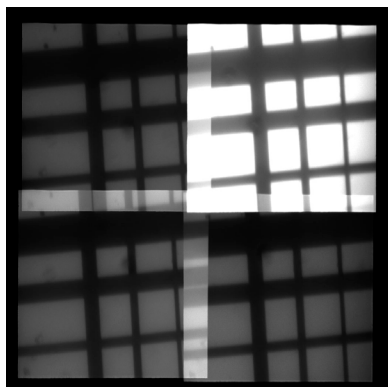
Figure 10. Example of how the four images will look when the QV2 filter cube is inserted.

4. Using the U/D and R/L knobs associated with the *lower left image*, move the image so that its bottom edge is aligned with the bottom of the CCD and its left edge is aligned with the left edge of the CCD (**Figure 11**).
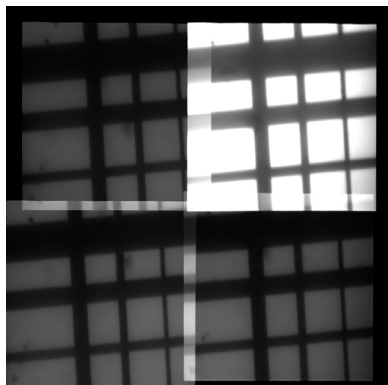

Figure 11. Correct positioning of lower left image.

5. Using the U/D and R/L knobs associated with the *lower right image*, move the image so that its bottom edge is aligned with the bottom edge of the CCD and its right edge is aligned with the right edge of the CCD (**Figure 12**).
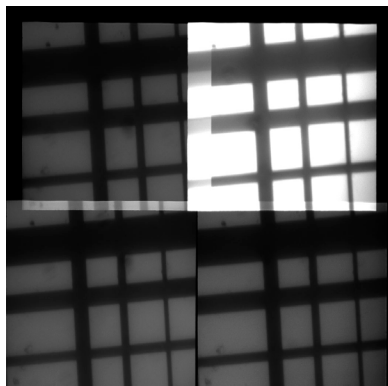

Figure 12. Correct positioning of lower right image.

6. Using the U/D and R/L knobs associated with the *upper left image*, move the image so that its top edge is aligned with the top edge of the CCD and its left edge is aligned with the left edge of the CCD (**Figure 13**).
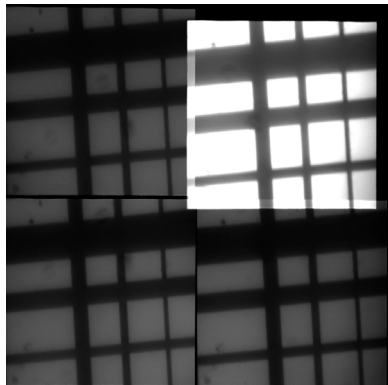

Figure 13. Correct positioning of upper left image.

7. Using the U/D and R/L knobs associated with the *upper right image*, move the image so that its top edge is aligned with the top edge of the CCD and its right edge is aligned with the right edge of the CCD (**Figure 14**).
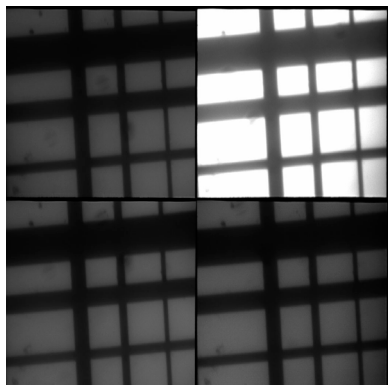

Figure 14. Correct positioning of upper right image.

Step 4: Fine alignment using software feedback

To align the four 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 QV2. It will allow you to subtract the four QV2 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 QV2.

The QV2 optics produce images that can be aligned to each other at a subpixel level. The most misalignment you will see will be on the order of 1/4 to 1/2 of a pixel in the extreme corners of the image.

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 knobs, fine-tune the right/left alignment of the images until they are as precisely aligned as possible.

3. Now use only the U/D knobs and fine-tune the up/down positions of the images until they are precisely aligned.

4. On days that you require full-field imaging (i.e., when you do not want to use the QV2 for split-field imaging), you will use the QV2 in its Bypass Mode. To do so, you simply need to have:

   a. The slider lever in the Bypass Mode position (**Figure 14**)
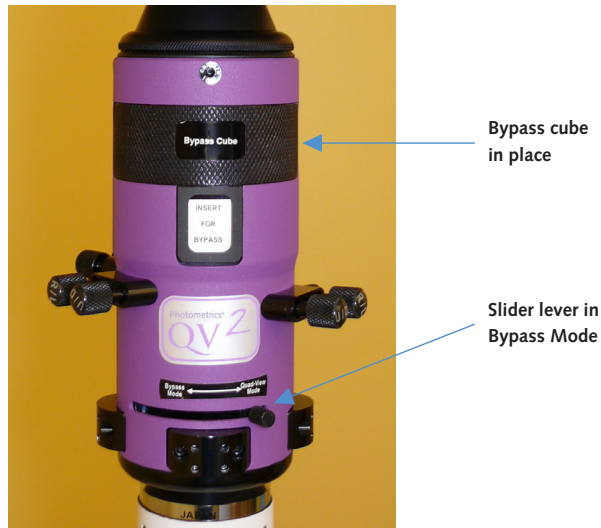
   b. The QV2 filter cube removed from the system

Figure 14. The QV2 with the slider lever in the Bypass Mode position.