DCAMCAP
Introduction Manual

⚠ **CAUTION**	• The manual describes the correct handling method of the system and provides cautions in order to avoid accidents. Read this manual carefully before and use the system correctly. • After reading the manual, store in a location where you can refer to it at any time.

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HAMAMATSU PHOTONICS K.K.

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Contents

1. INTRODUCTION

The DCAMCAP software is a tool to adjust and save the back focus position, the X,Y shift and rotation correction parameters between CCD1 and CCD2 of C11254-10B (ORCA-D2).

1-1 SYSTEM EQUIPMENTS

The system requirements for this software are as follows.

Type of computer	PC-AT compatibles
OS	Windows 7 and Vista (32 bit and 64 bit) Windows XP SP3 (32 bit) Windows XP SP2 (64 bit)
Interface	IEEE1394b interface
Drive	CD-ROM drive
Additional software	DCAM-API June 2010 or later

1-2 TRADEMARK

Window XP, Windows Vista, and Windows 7 are the registered trademarks or trademarks of Microsoft Corporation in the United States and other countries. Other brand names are the trademarks or registered trademarks of each company.

1-3 WORD DEFINITIONS IN THIS MANUAL

CCD1	CCD1 means the fixed CCD chip in C11254-10B.
CCD2	CCD2 means the CCD chip whose Z position can be adjusted with the linear stage in C11254-10B.
X,Y shift	X,Y shift means the horizontal and vertical differences between CCD1 and CCD2 images.
Rotation	Rotation means the rotation difference between CCD1 and CCD2 images.
Back focus position	Back focus position means the linear stage position where CCD2 can acquire an image with similar focus to CCD1 based on best contrast in the image. This position is defined for each combination of optical block and objective lens.
Calibration slide for bright field	The calibration slide for bright field is included in the standard components of the C11254-10B. This slide has optical pattern for calibration. This slide is used for both transmission and fluorescence illumination on the microscope.
Calibration slide for fluorescence:	The calibration slides for fluorescence are included in the standard components of the C11254-10B. There are three slides (Red, Green, Blue). Select the color slide that most closely matches the excitation / emission properties of the optical block used in the camera during calibration.
Memory	Memory means the storage area on the camera control unit which saves back focus position and X,Y shift and rotation correction parameters in camera control unit.
Application software	Software other than DCAMCAP for operating the camera, capturing images and image analysis. Examples are HCImage, MetaMorph, Nis-Elements, Slidebook, µManager, Labview and other commercially available packages that are DCAM compliant.

2. OVERVIEW

The C11254-10B (ORCA-D2) consists of with two CCDs and a selection of interchangeable optical blocks that split an incoming image into two components based on differences in wavelength (Dichroic optical blocks) or two equal components of the same spectral range (50/50 beam splitter). Since many optical components, especially lenses or microscope objectives have some residual chromatic aberrations, the images on the two CCDs may not have the same focal planes or alignment characteristics when combined in a new image. To compensate for these differences, CCD1 is permanently fixed in place and CCD2 is attached to a moveable stage in the camera head. By moving CCD2 in the Z axis it is possible to adjust the focus to match that of CCD1 or in the case of the 50/50 beam splitter to create a second, separate focal point for CCD2. The lateral displacement of the two images is corrected by shifting the image electronically on the CCDs.

To correct accurately, the calibration software depends on the user to create a high contrast image of the calibration slide on CCD1 using an application software of your choice and proper illumination conditions. The application software is then closed and DCAMCAP is opened. Following the instructions in this manual will allow the DCAMCAP software to adjust and store the necessary parameters for CCD2 to create the best possible image match to CCD1.

The purpose of the following calibration procedure is to adjust and store these parameters for each optical block and objective combination on a microscope or other optical system. The alignment needs to be done for each objective if high quality results are desired.

3. PARAMETER ACQUISITION PROCEDURE

The DCAMCAP software will acquire and store the following parameters by using the microscope and calibration slide together.

- Automatically acquire Back focus position (parameter) of CCD2 in C11254-10B.
- Automatically acquire correction parameters of the X,Y shift and rotation difference between CCD1 and CCD2 in C11254-10B.

3-1 PREPARATIONS FOR CALIBRATING OPTICAL BLOCKS

3-1-1 CAMERA PREPARATIONS

- Make sure the Camera's power is OFF first.

(1) Connect the C1254-10B to the microscope and adjust the support foot under the rear of the camera head by rotating the knurled ring until the camera head is supported. Confirm this is the same position that you will use to acquire images in an experiment.
(2) Be sure to insert and tighten the screws on the optical block before the main power of the camera (C11254-10B) is turned on.
(3) Make sure that the C11254-10B is the only camera connected to the computer and the Firewire cable is fully inserted and tightened at both ends.
(4) Turn ON the camera main power.
(5) Check for normal operation of the camera CCD1 with application software (HCImage or others).
(6) Close the application software. Keep the camera main power ON.

3-1-2 MICROSCOPE PREPARATIONS

Calibration setup is different for transmitted light and epi-fluorescent applications but the calibration results from either may be used for both.

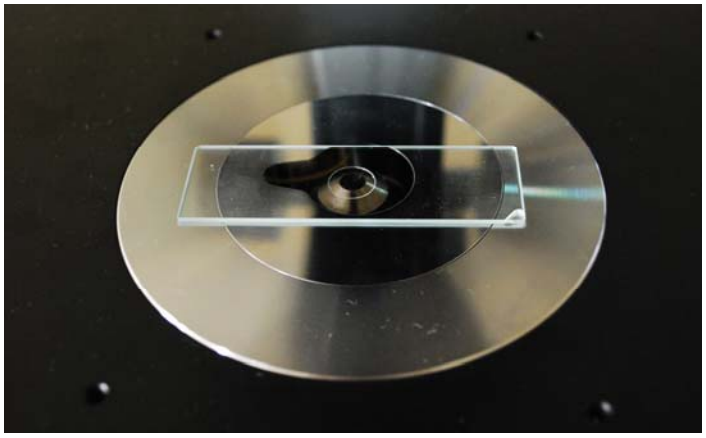
3-1-2-1 Using transmission illumination on the microscope

(1) It is very important to follow Kohler illumination principles when using transmitted illumination for calibration.

Step 1 - Be sure to set the eyepieces of the microscope to the Zero diopter position. Failure to do this will result in difficulty calibrating the optical block for more than one objective

Step 2 - Focus the calibration slide sharply and confirm the field diaphragm of the microscope is focused and centered in the same field of view.
Step 3 - Open the condenser diaphragm (aperture diaphragm) fully to ensure the full resolution of the microscope is used and the depth of focus is as shallow as possible.
Step 4 - Confirm that any color fringing at the edges of the image of the calibration slide markings are uniform in the field of view. If not, check the alignment of the transmitted light lamp and center it.

(2) With the calibration slide properly focused in the microscope follow the procedures below to calibrate the optical block. The images shown are based on the use of an inverted microscope but the procedure is the same for an upright microscope.



(3) Start the application software (not DCAMCAP) and get a live image of the calibration slide. Center the pattern in the field.

(4) Adjust Z-focus on the microscope and find the sharpest visual focus position. Compare this with the image from CCD1 in the camera. A good way to confirm the sharpest focus on CCD1 is to use the image histogram and look for the maximum standard deviation value for the whole field. If the best visual image and the maximum standard deviation value do not match, go back and check the eyepiece setting and the aperture diaphragm setting.

(5) Close the application software.

- If the microscope is a manual microscope, it is easy to keep the focus plane but if the microscope is motorized or under computer control, the application software may change the Z-focus. Please make sure the Z-focus is correct before proceeding

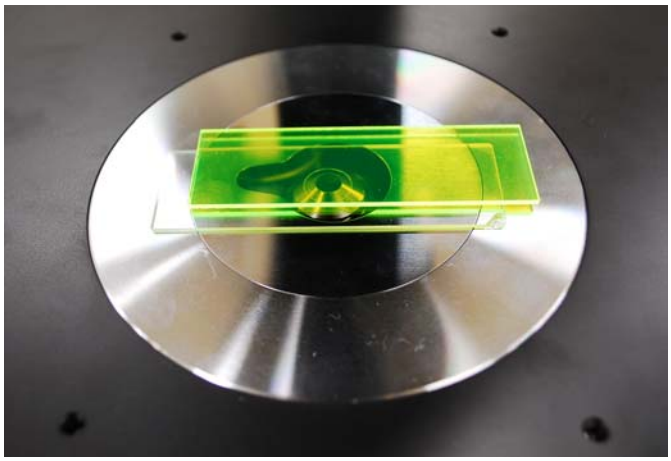
- When step (4) is completed and checked proceed with the back focus and X,Y shift and rotation correction using DCAMCAP. If step (4) is not appropriate, the resulting correction parameters in DCAMCAP will not be correct.
- If the application software used does not display standard deviation values for the images and you cannot confirm the maximum contrast in step (4) you can still proceed. You can confirm the contrast by using DCAMCAP later in the procedure.

3-1-2-2 Using fluorescence illumination on the microscope

(1) Be sure to set the eyepieces of the microscope to the Zero diopter position. Failure to do this will result in difficulty calibrating the optical block for more than one objective.

(2) Adjust and align the fluorescence light source on the microscope and confirm the illumination is even across the field of view.

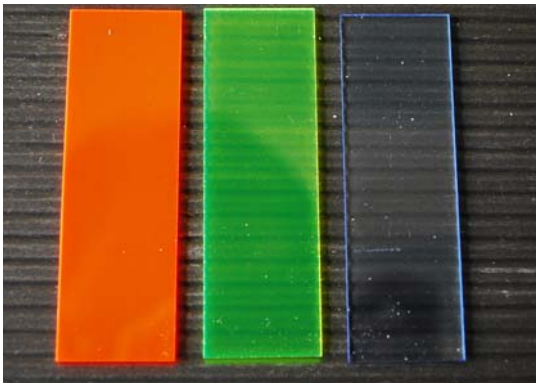
(3) Stack the bright field calibration slide and the appropriate color fluorescence slide together so that the cover slip on the calibration slide is on the outside of the stack. Then place the stack on the microscope with the cover glass facing the microscope objective as seen the image below. (Example using an inverted microscope.)



Note

- If using an inverted microscope it may help to place a dark cover over the slides to ensure an evenly dark background for the calibration procedure.

The appropriate fluorescence calibration slide depends on the optical block that is used. Refer to the below table.



Optical Block	Calibration slide for fluorescence
A11400-02	Green Plate
A11400-03	Green Plate
A11400-04	Green Plate
A11400-05	Red Plate
A11400-08	Green Plate

Proper calibration in fluorescence illumination requires an appropriate match between the optical block in the camera and the fluorescence filter set in the microscope. It may not be possible to calibrate without this. HPK recommendations are shown in the table below.

Camera	Method	Filter set at microscope		
Optical Block		Excitation	Dichroic mirror	Emission
A11400-02	-	-	-	-
A11400-03	1 Ex, 2 Em	FF01-438/24-25	FF458-Di01-25	-
	2 Ex, 2 Em	FF01-416/501-25	FF440/520-Di01-25	FF01-464/547-25
A11400-04	2 Ex, 2 Em	FF01-468/553-25	FF493/574-Di01-25	FF01-512/630-25
A11400-05	1 Ex, 2 Em	FF01-531/40-25	FF562-Di01-25	-
	2 Ex, 2 Em	FF01-534/635-25	FF560/659-Di01-25	FF01-577/690-25
A11400-08	2 Ex, 2 Em	FF01-468/553-25	FF493/574-Di01-25	FF01-512/630-25

Note

- The above filter sets are Semrock products and part numbers.

(4) Start the application software (not DCAMCAP) and get a live image of the calibration slide. Center the pattern in the field.

(5) Adjust Z-focus on the microscope and find the sharpest visual focus position. Compare this with the image from CCD1 in the camera. A good way to confirm the sharpest focus on CCD1 is to use the image histogram and look for the maximum standard deviation value for the whole field. If the best visual image and the maximum standard deviation value do not match, go back and check the eyepiece setting.

(6) Close application software.

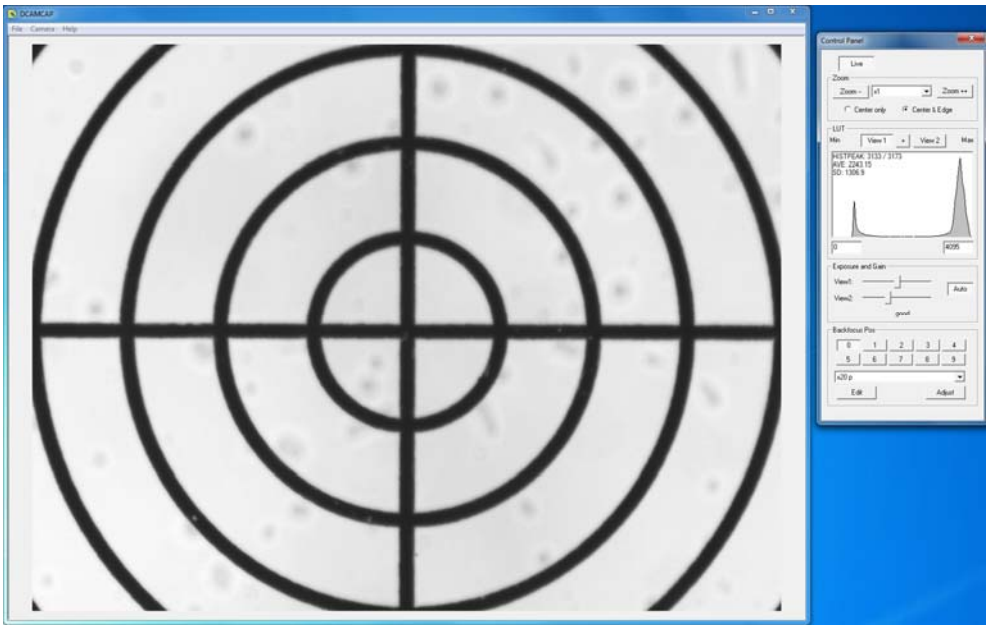
3-2 BACK FOCUS POSITION (PARAMETER)

After completing and checking the microscope setup for either brightfield (section 3-1-2-1) or fluorescence (section 3-1-2-2) the calibration procedure for CCD2 can begin.

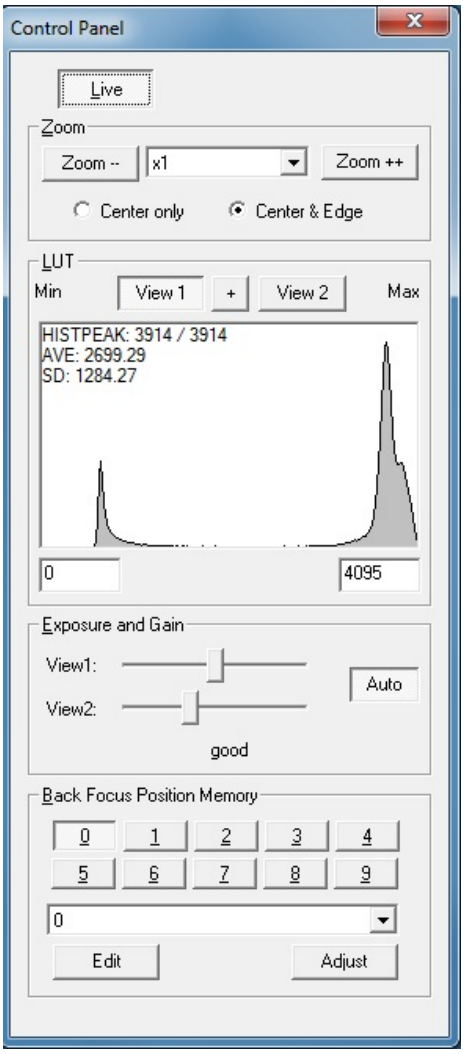
(1) Startup DCAMCAP

Double click “DCAMCAP” to execute it.

You will see the following Control Panel and display the CCD1 live image.



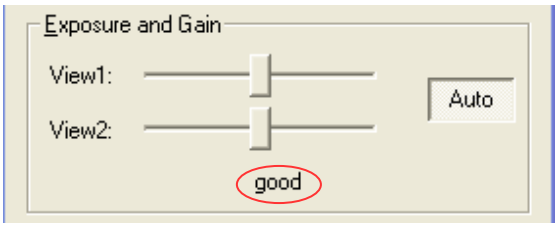
[Control Panel]



Live	Start live image display from CCD1 and CCD2 and stop.
Zoom	
Pull down menu	The menu will enlarge or contract the display image by x1, x2, x4, x8 factors
Zoom++	This will enlarge the display image in steps of x1, x2, x4, x8 factors
Zoom --	This will contract the display image in steps of x1, x2, x4, x8 factors
Center only	These change the image display view. Refer to page 11 for details.
Center & Edge	
LUT	
View 1	This displays the CCD1 image.
View 2	This displays the CCD2 image.
+	This displays the merged images of CCD1 and CCD2. Refer to Page 8 and 9 for details.
Histogram	This shows the histogram of the display image.
HISTPEAK XXXX,YYYY	XXXX is the maximum frequency value of either the latest 100 images or images within 3 s.
AVE	Shows mean value of the display image.
SD	Shows the standard deviation of display image.
Min	Min is minimum value of LUT setting. (enable 0 to 4095)
Max	Max is maximum value of LUT setting. (enable 0 to 4095)
Exposure and Gain	
View 1	This sets exposure time and gain setting of CCD1.
View 2	This sets exposure time and gain setting of CCD2. If bar position is right, exposure time is long and gain is high.
Auto	If Auto button is pushed, DCAMCAP automatically controls exposure time and gain of CCD1 and CCD2 and tries to acquire appropriate images for correction. Refer to Page 8 for details.
Result	This show result message.
Back focus Position Memory	
0-9	These buttons are Memory numbers.
Pull down menu	This displays description text of selected Memory. Refer to Page 10 in details.
Edit	Used to edit description text of Memory. Refer to Page 10 for details.
Adjust	This shows detail dialog for parameter acquisition. Refer to Page 11 for details.

3-2-1 PREPARATION FOR AUTO ACQUISITION OF BACK FOCUS POSITION (PARAMETER)

DCAMCAP detects C11254-10B when it is executed. It also displays a live image on the monitor when detection of C11254-10B is completed. An Error message will appear if detection fails.
DCAMCAP can automatically control exposure time and gain for both CCD1 and CCD2 to acquire appropriate calibration images. Auto control function is enabled as the default setting. Click the Auto button (button will pop up) to disable the auto control function.
DCAMCAP displays messages about illumination conditions in the Exposure and Gain Control Panel window. According to the displayed message, adjust the intensity of the microscope until the displayed message becomes 'good'.



(1) Adjust the X,Y, stage control of the microscope to center the calibration slide pattern in the center of the image display. Confirm that all the conditions and settings of the microscope have not changed from the microscope setup in section 3-1-2-1 (brightfield) or section 3-1-2-2 (fluorescence).

(2) Adjust the Z stage control of the microscope to acquire a good contrast image of the calibration slide pattern.

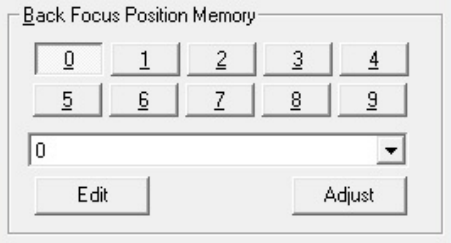
(3) Confirm that the best visual contrast and the maximum standard deviation value of live image occur at the same Z setting. The standard deviation value is displayed in LUT of Control Panel window.

- If steps section 3-1-2-1(4) and 3-1-2-2 (5) are not done properly, the auto acquisition of a correct back focus position will not be appropriate.

(4) This completes the preparation for auto acquisition of back focus position (parameter).

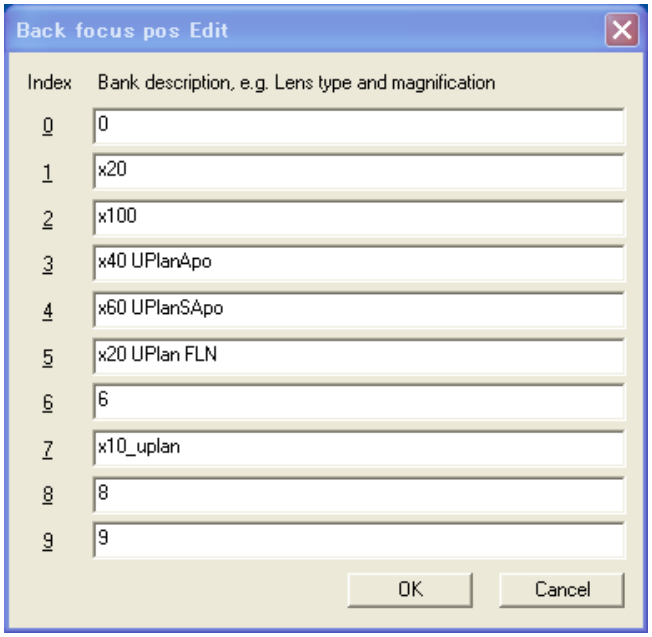
3-2-2 SELECTION OF BACK FOCUS POSITION (PARAMETER) MEMORY

The back focus position is defined by the combination of objective lens and optical block, if you use C11254-10B with a microscope. The C11254-10B can automatically recognize individual optical block information but cannot recognize objective lens information. For this reason, there are 10 memory positions in each optical block that can be assigned to different microscope objectives. After calibration the CCD2 moves to the appropriate memorized position by selecting this Memory number.



C11254-10B has 10 back focus positions for each optical block. DCAMCAP has 10 buttons in the Back Focus Position Memory. The currently selected button appears depressed and there is descriptive text for each selected button in a pull down menu.

Push the Edit button in Back focus Position Memory Control Panel to open the Back focus Position Memory Edit window to edit Memory description text. This descriptive text information is displayed in the pull down menu and in application software.

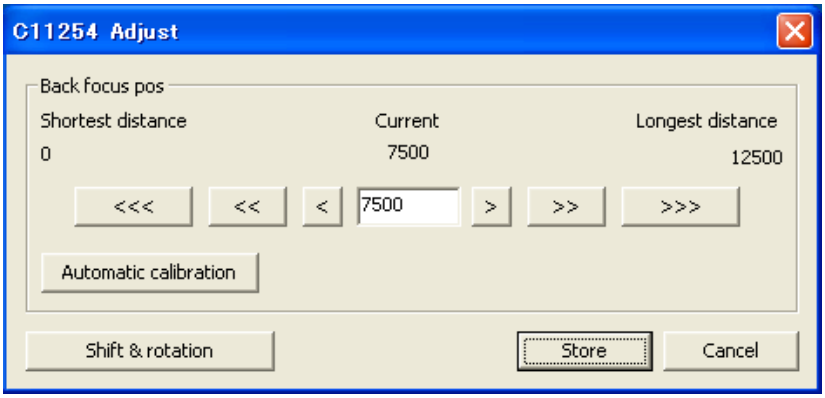


Note

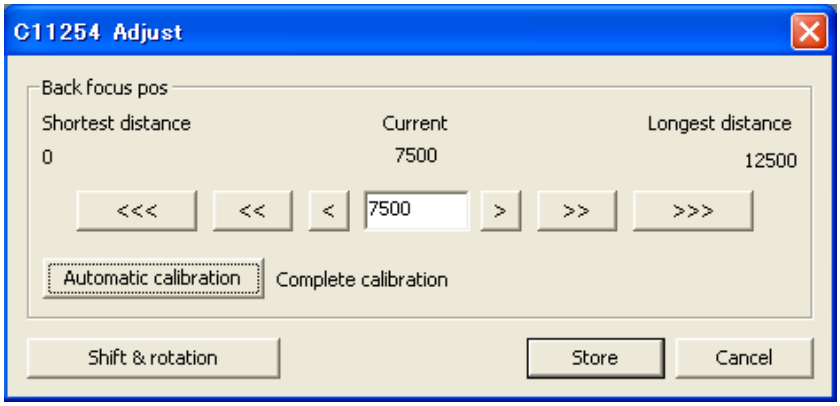
- It is suggested to include details of each objective in the memory since it is part of the memory of each optical block and the description is accessible to the application software when the optical block is inserted in the C11254-10B.

3-2-3 AUTOMATIC BACK FOCUS ACQUISITION

Push the Adjust button in Back focus Position Memory Control Panel to get the dialog window below. Push the Automatic calibration button to start the back focus position acquisition automatically. This process will take about a minute.



The message “Complete calibration” is shown when the calibration is finished.

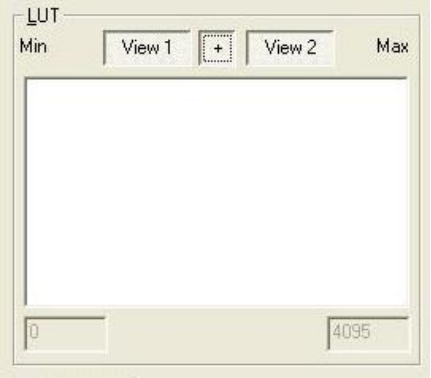


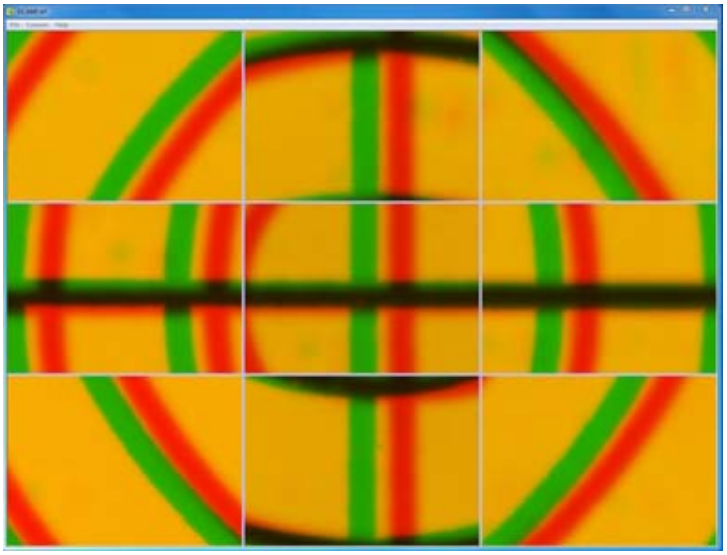
Push the Store button in order to memorize this back focus position (parameter) in the selected Memory.
Push the Cancel button to forget this back focus position and return to the Control Panel window.

3-3 X,Y SHIFT AND ROTATION CORRECTION

3-3-1 IMAGE DISPLAY METHOD

Push + button in LUT of the Control Panel window and then display the merged image of CCD1 and CCD2. This display method is useful for checking alignment.(In this setting, histogram does not display.)

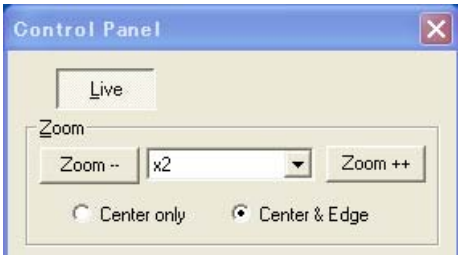


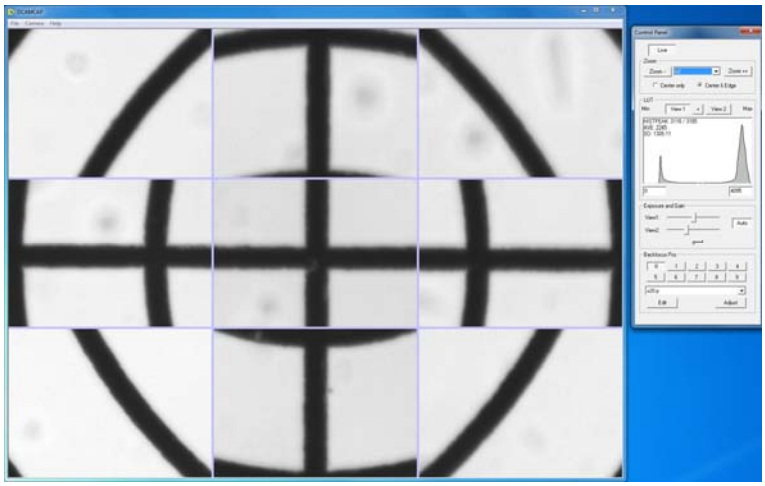


(Green: CCD1, Red: CCD2)

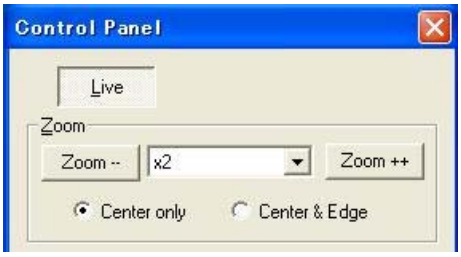
Select Center & Edge to display a single image with 9 portions of the entire image in one view. The image center, left, right, top, bottom, and the four corners make it easy to confirm alignment between CCD1 and CCD2 with the Zoom function.

The combination of Zoom x2 and Center & Edge are as shown in the image below.



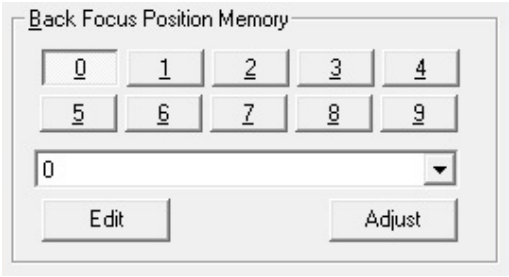


The combination of Zoom x2 and Center only are as shown in the image below.



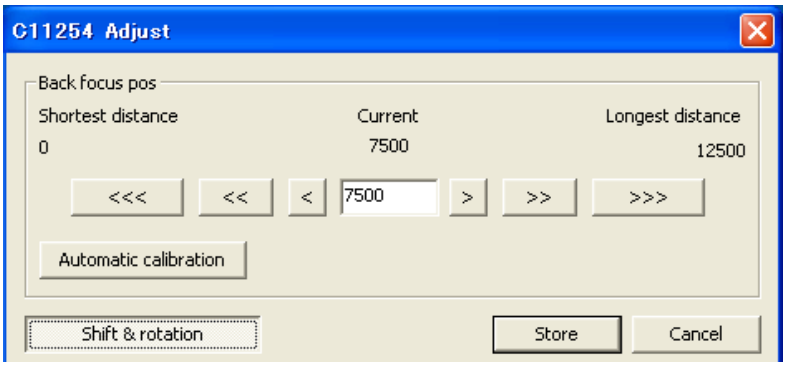
3-3-2 SELECTION OF X,Y SHIFT AND ROTATION CORRECTION PARAMETER MEMORY

For each memory position, the memory includes not only the Z-axis position but the X,Y shift and rotation parameters as well. Select the appropriate memory for the objective and proceed with the X,Y shift and rotation correction.

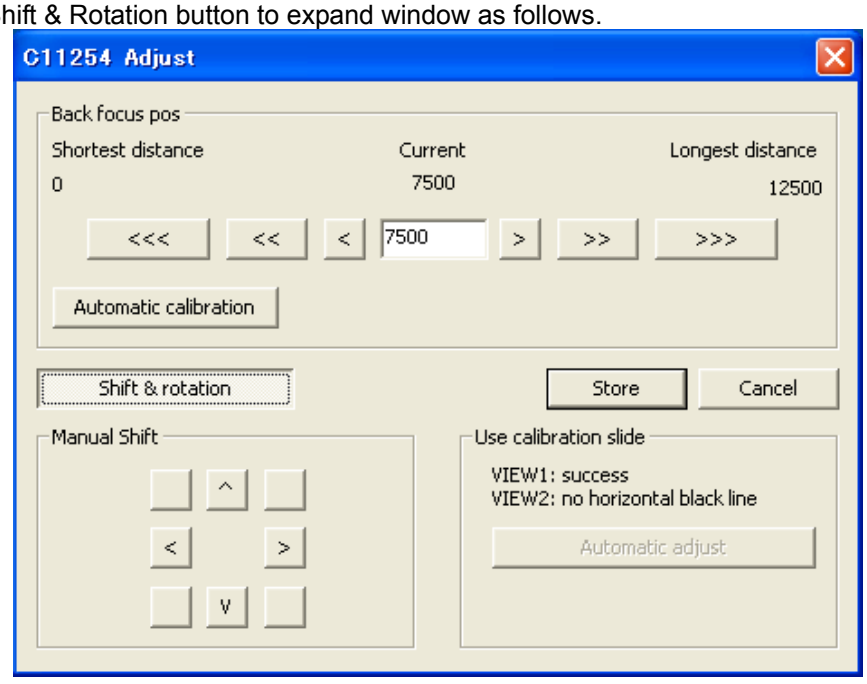


3-3-3 X,Y SHIFT AND ROTATION CORRECTION

Push the Adjust button and open the dialog window shown below.

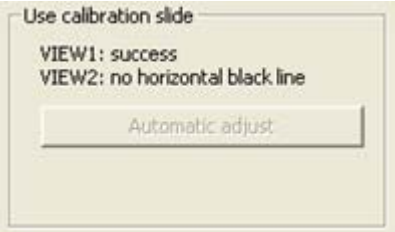


Push the Shift & Rotation button to expand window as follows.



3-3-3-1 Calibration slide setting

In the Use calibration slide window, there are comments that indicate if the calibration slide setting is correct or not. “Success” means that the slide setting is satisfactory for calibration condition. “No horizontal or vertical black line” means the slide setting is not satisfactory for calibration. If so, adjust the X,Y stage of the microscope until the “success” comment appears in both VIEW1 and VIEW2.

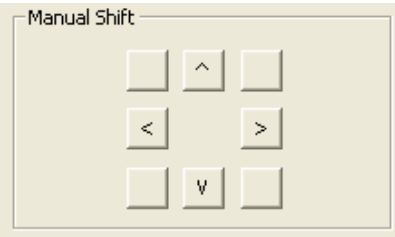


3-3-3-2 Automatic X,Y Shift and rotation correction

Push the Automatic adjust button in the Use calibration slide window. Automatically acquisition of the correction parameters of X,Y Shift and rotation begins.
Check the display image and push the Store button if you are satisfied with this result. The correction parameters will be stored to select Memory.
If you are not satisfied with this result, push the Cancel button in order not to save these parameters.

3-3-3-3 Fine adjustment of X,Y Shift

If you are not satisfied with the result by automatic correction, you can make fine adjustments of X,Y Shift in Manual Shift. Push the button in Manual Shift and adjust alignment of CCD1 image and CCD2 image.
If you are satisfied with the result of this fine adjustment, push the Store button. If not, push the Cancel button.



- After pushing the Manual shift button wait until the displayed image is stable before pushing the next button

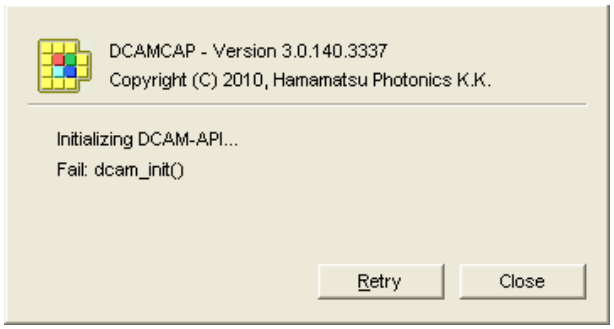
Note

- Pushing the Cancel button returns to the previous status before this dialog was opened.

4. OTHER DIALOG BOX INDICATION

(1) No camera is found

The following dialog indicates there is no camera connected or DCAM-API is not installed.

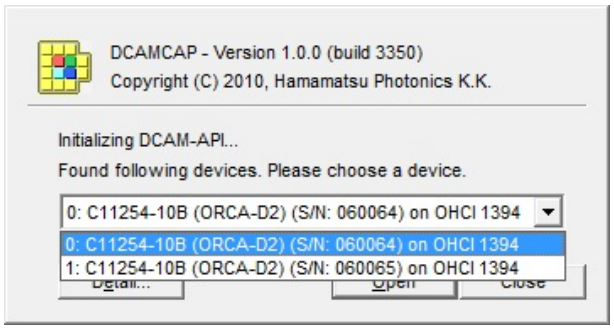


Note

- Please see “3-1” PREPARATIONS FOR CALIBRATING OPTICAL BLOCKS.

(2) Two or more cameras are connected

You will see following dialog if two or more cameras are connected.



You will see the camera model name and serial number. Please press [Close] button to close this software. Turn off the power to the other cameras and the computer and disconnect the other cameras. Restart the computer with only the C11254-10B camera connected, and then run DCAMCAP again.

(3) Other dialogs

If you see other dialog or message boxes, it means DCAMCAP has encountered other problems. Please refer to Section 3 (Setup) of this manual again and check all the settings.

If you see same dialog many times, please contact your Hamamatsu local subsidiary.

Note

- Please refer “5 CONTACT INFORMATION”.

5. CONTACT INFORMATION

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