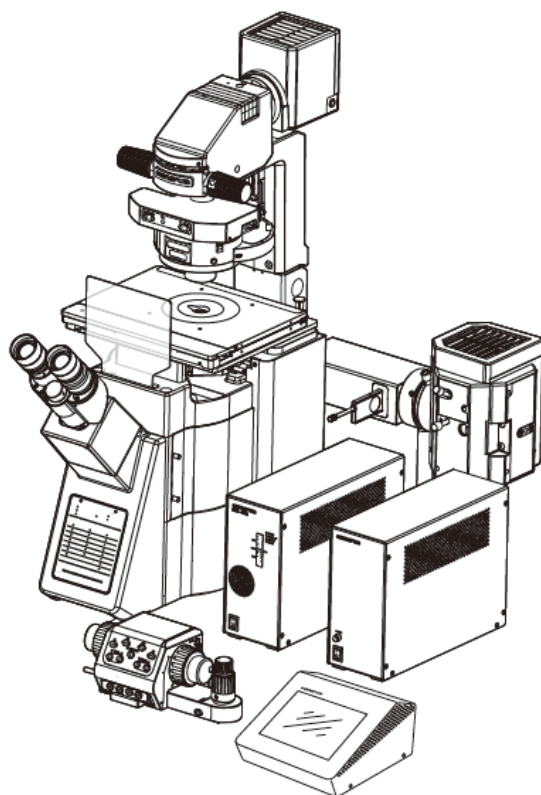


HCIimage

Olympus IX3 Microscope Guide



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Olympus IX3-ZDC

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INSTALLATION

Registered users can download the latest version of HCImage from the HCImage website using the following link - <https://hcimage.com/download/login/> (login required). For access to HCImage downloads, complete the software registration form (<https://hcimage.com/register/>), including a valid dongle number and email address, and an email will be sent with the HCImage download details.

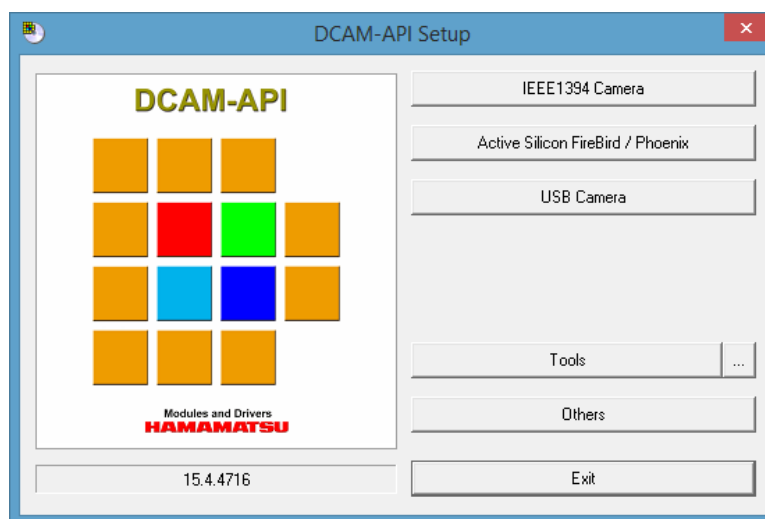
HCImage

1. Insert the HCImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click on **setup.exe**.
 2. Click **Yes**, if prompted by the User Account Controls.
 3. To begin the installation wizard, click **Next**.
 4. Follow the instructions on each installation page.
- 
5. Securely connect the dongle () to a USB port after the software installation has finished.
 6. Install the appropriate DCAM-API drivers, please see "**DCAM-API Drivers**" below.
 7. Turn the camera power on prior to launching HCImage.
 8. Click the **HCImage** icon on your Desktop to launch HCImage.
 9. Register the software to receive technical support, please go to www.hcimage.com and click **Register**.

DCAM-API Drivers

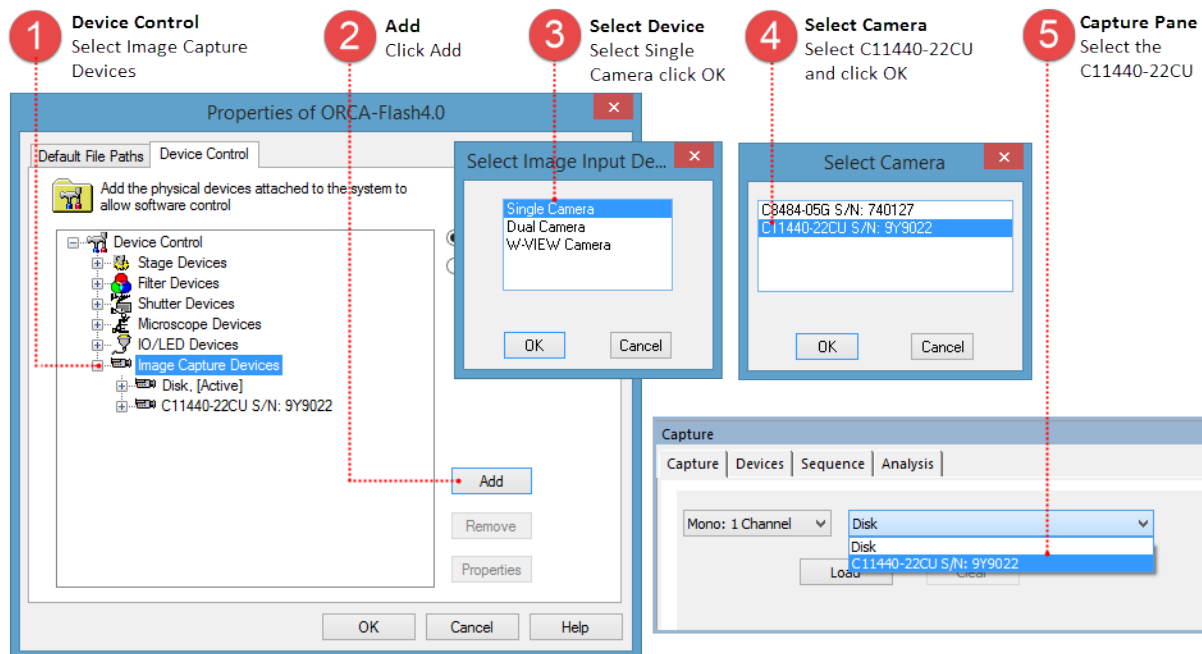
The current version of DCAM-API is available for download at <http://dcam-api.com/>. Before installing the camera driver, make sure that the camera is turned off.

1. After installing HCImage Live from the DVD, you will be prompted to install DCAM-API, click **Yes**. If you downloaded HCImage Live, please go to <http://www.dcam-api.com/> and download the DCAM-API drivers for Windows.
2. Click **Yes**, if prompted by the User Account Controls.
3. **[Camera Link]** Select the **Active Silicon FireBird / Phoenix** module. **[USB 3.0]** Select the **USB Camera** module. **[Firewire]** Select the **IEEE 1394 Camera** module.
4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.



Add the camera

Launch HCImage, go to File, select Current Profile and then follow the steps below to add a camera to the profile.



Olympus 64-bit Drivers from the HCImage DVD

Be advised that this application installs the Olympus Camera and Microscope drivers, as well as copying all of the necessary dlls into the HCImage directory.

1. Open the DVD contents in Windows Explorer and navigate to Drivers\Microscopes\Olympus\Olympus 3 Series\x64.
2. Double-click on **Olympus_x64 Install.exe** and follow the installation instructions.
3. Click **Yes**, if prompted by the User Account Controls

Note: If using a Hamamatsu 1394 camera, this driver installation may supersede the Hamamatsu driver causing communication problems. To recover from this issue, please see "**Unable to communicate with Hamamatsu 1394 camera**" on page 17.

Olympus 32-bit Drivers

Two drivers are needed, the 1394 Bus Host Controller and the Microscope I/F Device. Go to the Olympus website for the latest driver update and installation instructions (https://support.olympus.co.jp/cf_secure/en/lisg/bio/download/ga/ix3bsw/).

Install the 1394 Bus Host Controller

1. Open the **dp1394drv** folder in Windows Explorer and then open either the **i386** folder for the 32-bit version of the driver.
2. Run **DriverInstall.bat** to begin the installation.
3. A Windows Security window will open, click **Install**.

Note: If using a Hamamatsu 1394 camera, this driver installation may supersede the Hamamatsu driver causing communication problems. To recover from this issue, please see "**Unable to communicate with Hamamatsu 1394 camera**" on page 17.

Install the 1394 Microscope I/F Device

1. Open the **fsi1394** folder in Windows Explorer and then open either the **i386** folder for the 32-bit version of the driver.
2. Run **DriverInstall.bat** to begin the installation.
3. A Windows Security window will open, click **Install**.

Files necessary to run the Olympus IX3 microscopes:

Copy the files below found on the HCImage DVD in the Olympus 3 Series folder under microscope drivers (Drivers\Microscopes\Olympus\Olympus 3 Series\) and paste them into the HCImage directory (i.e., C:\Program Files\HCImage).

fsi1394.dll	gt_log.dll
msl_pm.dll	msl_pd_1394.dll
gtlib.config	

CONFIGURE THE MICROSCOPE

The microscope drivers have been installed, time to configure it using the touch panel controller (TPC) and then add it as a device in HCImage. The first step is to turn on the IX3-CBH (microscope control box) and then the touch panel controller.

Note: The "Power On" sequence for turning the equipment on before use should be: Light Source > PC > Camera > IX3-CBH > Touch Panel Controller > Launch HCImage.

Configure with the Touch Panel Controller

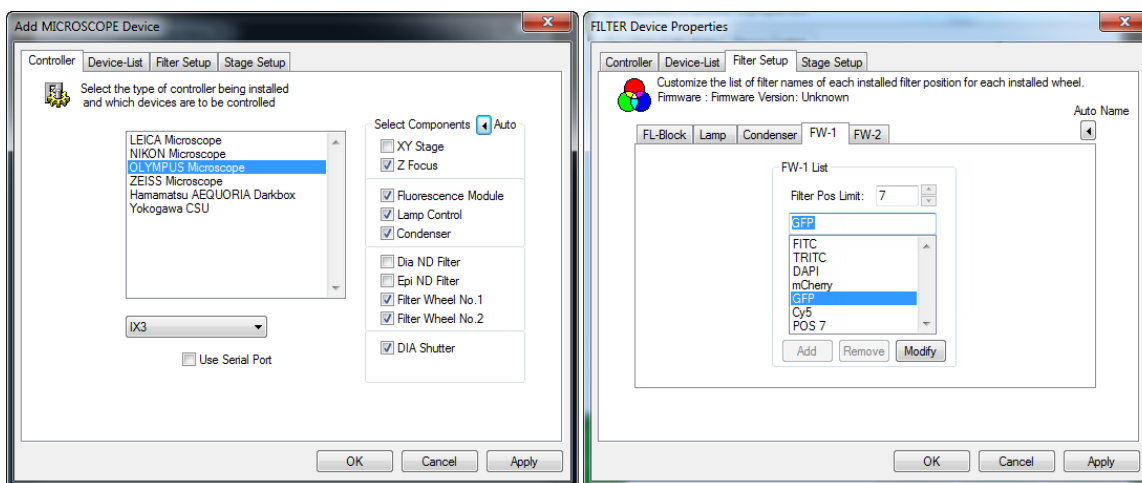
An initial system setup is required when using the microscope for the first time or after replacing one of the components. The microscope is setup and configured using the TPC.

1. Go to **System Setting** in the **Menu** screen.
2. Select **Unit**, enter the components connected to the IX83 for each module and tap **OK** to save the settings.
3. Select **Optical**, enter and configure the objectives, mirror units and condenser.
4. Select **Customized**, enter the focus limits and parfocality correction.
5. When the setup is complete, tap **X** to exit to the **Menu** screen.

Add Microscope to a Profile

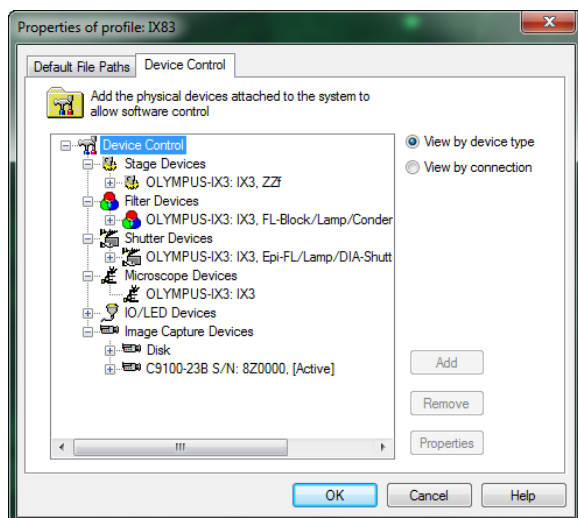
Once the microscope has been setup from the touch panel controller, the next step is to add the microscope to a profile and configure it in HCImage.

1. Launch HCImage, go to **File** and select **Current Profile**. In the **Device Control** tab, select **Microscope Devices** and click **Add**.
2. Click on **Olympus Microscope** and select **IX3** from the drop-menu. Go to the **Device-List** tab and select the **IX3** device that is listed.



3. Go to the **Controller** tab, click **Auto** and HCImage will update components list.
4. Verify that the components list is accurate, add or remove components if necessary. Go to the **Filter Setup** tab to see the settings for the individual components.
5. Click **OK** to accept the microscope settings and return to the Current Profile. The Olympus IX3 is now listed as a Microscope Device and the components are listed as Stage, Filter and

Shutter Devices. Click **OK** to save the device settings to the profile and close the window.



- Go to the **Devices** pane and expand the **Microscope Setup** panel to access the individual component controls for the fluorescence module, objectives, condenser, etc.

Microscope Setup Panel

The Microscope Setup panel is located in the Devices pane and provides access to the microscope's controls as shown below. The focus controls are available in the Z Setup panel also located in the Devices pane.

Fluorescence Shutter
Open and close the shutter

Tooltip
Hover over a filter or objective to display its properties

Objectives
Select magnification to change objective, the properties are displayed below

Condenser
Select the optical element for applicable objective and observation method

Aperture Diaphragm
Adjust the size of diaphragm opening

Transmitted Light
Turn On/Off and adjust the light intensity level (lamp voltage)

Filter Cubes
Select from available filters, details displayed below.
Right-click on filter to add color

Linked Move
Link condenser element to objective

Link to Calibration
Enable objective link to calibration

Objective Position
Escape - move the objective to the lower limit
Return - move objective to last Z position

Light Path
Displays the current light path and is controlled through the touch panel controller or the microscope stand

Shutter Controls
Polarizer - in or out of the light path
DIA - transmitted light shutter open or closed

Filter Setup Examples

Follow the steps below to configure the microscope for brightfield and fluorescence observation.

Part 1 - Filter Setup for Default Idle Positions

The screenshot shows the 'Filter Setup' dialog box with the 'Default Idle Positions' group selected. The 'Advanced Settings' section is expanded, showing 'Return to Idle On Exit' checked. The 'Filter-Shutter' section shows the 'Filters' and 'Shutters' lists. The 'Shutters' list has a dropdown menu open, showing 'Closed', 'Open', and 'Don't care' options. Red dashed lines and numbered callouts point to specific settings.

1 Advanced Settings
Enable Return to Idle On Exit, Return to Idle After Capture and Return to Idle During Delay

2 Filter Group
Select Default Idle Positions

3 Shutter Controls
Set the shutter status to Closed for the Epi-FL and the DIA shutters

Part 2 - Filter Setup for Brightfield

The screenshot shows the 'Filter Setup' dialog box with the 'BF' group selected. The 'Advanced Settings' section is expanded, showing 'Return to Idle On Exit' checked. The 'Filter-Shutter' section shows the 'Filters' and 'Shutters' lists. The 'Shutters' list has a dropdown menu open, showing 'Closed', 'Open', and 'Don't care' options. Red dashed lines and numbered callouts point to specific settings.

1 Filter Group
Select Default Idle Positions

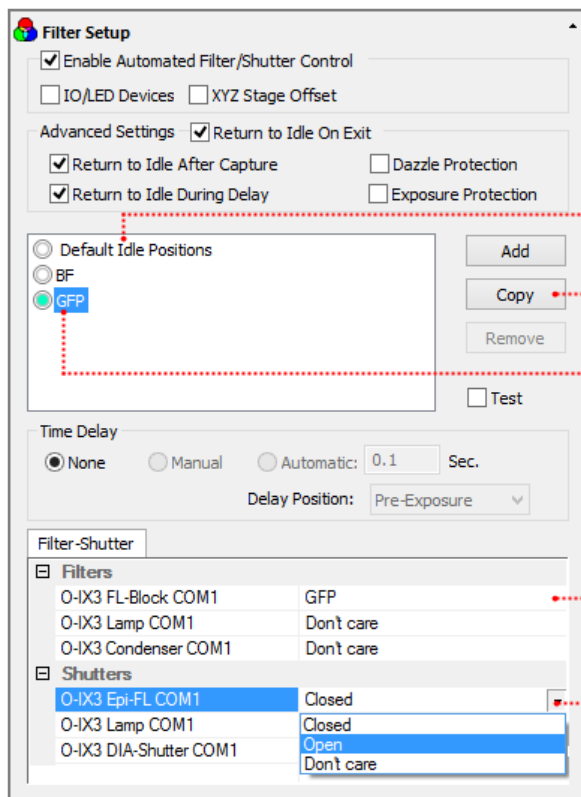
2 Copy Filter Group
Click Copy, enter BF and click OK

3 Filter Group
Select BF

4 Filter Controls
Set the FL-Block to Open and the Condenser to BF

5 Shutter Controls
Set the shutter status to Open for the Lamp and the DIA shutters

Part 3 - Filter Setup for Fluorescence



1 Filter Group
Select Default Idle Positions

2 Copy Filter Group
Click Copy, enter GFP and click OK

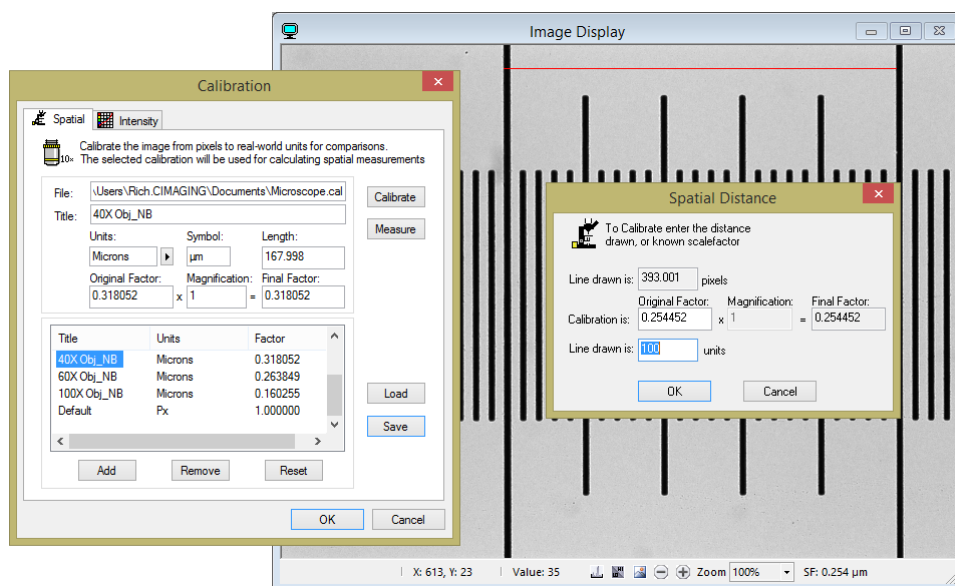
3 Filter Group
Right-click on GFP and select EGFP from the tint list

4 Filter Controls
Set the FL-Block to GFP

5 Shutter Controls
Set the shutter status to Open for the Epi-FL shutter

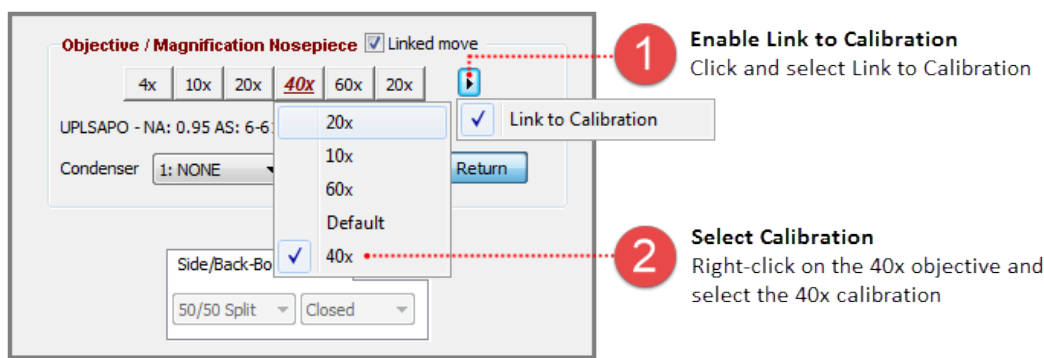
CALIBRATE OBJECTIVES

1. Starting with the lowest power objective, click **Live**, bring the micrometer into focus and center it.
2. Click **Abort** and then click on the **Calibration Properties** icon ( Calibration) in the Analysis toolbar.
3. Click **Add**, in the Title box and enter the objective magnification, contrast method and immersion type if applicable (e.g., 40x, 40x Oil, 40x DIC, etc.).
4. Select **Microns** from the **Units** drop-menu and then click **Calibrate**. Move the cursor to a starting position on the scalebar; click and drag a line to span the distance to measure.
5. Enter the known distance of the line and click **OK**. The Calibration Factor in the Spatial Calibration Menu will be updated.
6. Click **OK**, to close the **Calibration Properties** dialog and repeat the process for the remaining objectives.
7. **[Save Calibration]** Click **Save**, select file path, enter the file name and click **Save**. The Spatial Calibration is displayed in the lower right-hand corner of the image file.



Link Calibration to Objective

To link the calibration to an objective, go to the Microscope Setup panel in the Devices pane and follow the steps below.



OLYMPUS IX3-ZDC

The IX3 Z Drift Compensation module (IX3-ZDC) uses a low phototoxicity infrared laser beam to detect the correct focus position as defined by the user. The IX3-ZDC features "Continuous" and "One Shot" focus modes. The One Shot AF mode allows several focus positions to be set as desired, enabling efficient Z-stack acquisition during multiple site experiments. The Continuous AF mode keeps the desired observation plane precisely in focus, avoiding focus drift due to temperature changes or the addition of reagents.

Caution: The ZDC uses a 790 nm laser (Class 1) diode for Z drift compensation. Do not directly view the laser light. Never remove the warning and caution labels on the microscope. Please contact Olympus or see the IX3-ZDC manual for more information.

Focus Modes and Objectives

The IX3-ZDC contends with Z drift to maintain focus on the sample during extended time lapse sequences.

Note: A 35 mm glass-bottom dish with a cover glass thickness of 0.16 to 0.18 mm is required for proper focusing.

One Shot Focus Mode

In this mode, the 790 nm laser beam is first focused on the glass bottom dish's cover glass bottom surface (or on the cover glass top surface in the case of an oil- or water-immersion objective) and then moved mechanically from there to the target cell using software to reproduce the desired focus position. Although this mode requires a certain period for moving the focal point, the capability of arbitrary setting of the focus movement range makes it suitable for multiple site time lapse observation.

Continuous Focus Mode

In this mode, while focusing the 790 nm laser beam on the top surface of the cover glass of the glass bottom dish, you can focus the objective on the target cell. Continuous AF mode keeps the desired plane of observation precisely in focus, avoiding focus drift due to temperature changes or the addition of reagents.

Although this mode enables continuous observation (at the video rate - 30 fps), the focusing range is reduced. Therefore, it is suitable for observation in which the cell position does not vary greatly.

Note: The Continuous Focus mode only supports oil- or water-immersion objectives.

Note: When using the **UPLFLN60X** or **LUCPLFLN60X** objective, focus on the area just below the bottom surface of the cover glass, and click **Get Offset**.

Note: When the **UPLSAPO60XW** or **UPLSAPO60XS** objective is used, focus on the area just above the top surface of the cover glass, and click **Get Offset**.

Supported Objectives

The table below provides a list of the objectives that are supported for IX3-ZDC operation. The table also includes which observation methods and focus modes are supported for each objective. If an objective has an iris diaphragm, it should be completely open.

Supported Objectives

Objective	NA	WD	FL		DIC		PH	
			Continuous	One Shot	Continuous	One Shot	Continuous	One Shot
APON 60XOTIRF	1.49	0.10	✓	✓	X	✓	X	X
APON 100XHOTIRF	1.70	0.08	✓	✓	X	✓	X	X
UAPO 40XOI3/340	1.35-0.65	0.10	✓	✓	X	X	X	X
UAPON 100XOTIRF	1.49	0.10	✓	✓	X	✓	X	X
PLAPON 60XO	1.42	0.15	✓	✓	X	✓	X	X
PLAPON 60XOPH	1.42	0.15	✓	X	X	X	✓	X
UPLSAPO 20X	0.75	0.60	X	✓	X	✓	X	X
UPLSAPO 40X2	0.95	0.18	X	✓	X	✓	X	X
UPLSAPO 60XW	1.20	0.28	✓	✓	X	✓	X	X
UPLSAPO 60XO	1.35	0.15	✓	✓	X	✓	X	X
UPLSAPO 60XS	1.30	0.30	✓	✓	X	✓	X	X
UPLSAPO 100XO	1.40	0.13	✓	✓	X	✓	X	X
UPLFLN 20X	0.50	2.10	X	✓	X	✓	X	X
UPLFLN 40X	0.75	0.51	X	✓	X	✓	X	X
UPLFLN 40XPH	0.75	0.61	X	✓	X	X	X	✓
UPLFLN 60X *1	0.90	0.20	X	✓	X	✓	X	X
UPLFLN 100XO2	1.30	0.20	✓	✓	X	✓	X	X
UPLFLN 100XOI2	1.30-0.60	0.20	✓	✓	X	✓	X	X
UPLFLN 100XO2PH	1.30	0.20	✓	✓	X	X	✓	✓
LUCPLFLN 20X	0.45	6.60-7.80	X	✓	X	✓	X	X
LUCPLFLN 40X	0.60	2.70-4.00	X	✓	X	✓	X	X
LUCPLFLN 40XPH	0.75	0.51	X	✓	X	X	X	✓
LUCPLFLN 60X *1	0.70	1.50-2.20	X	✓	X	✓	X	X
LUCPLFLN 60XPH	0.70	1.50-2.20	X	✓	X	X	X	✓

Note: When using a large specimen such as a microplate, focusing may not be achieved due to its inclination. The focusing is guaranteed only with a glass bottom dish with a diameter of 35 mm.

Setup and Controls

Once the IX3-ZDC has been installed and connected to the microscope frame according to the procedure outlined in the IX3-ZDC Instruction Manual, the IX3-ZDC will need to be added as a microscope component through the touch panel controller.

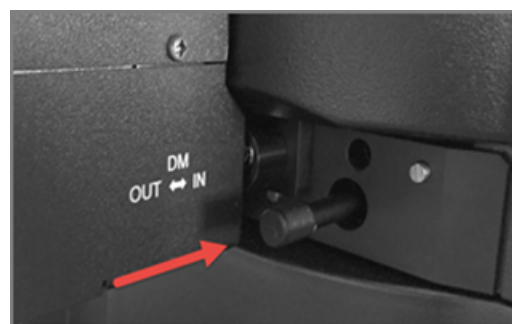
Touch Panel Controller

Follow the steps below to enable the IX3-ZDC through the touch panel controller.

1. On the **Menu** screen, go to **System Settings** and select **Unit**.
2. In the **Frame** tab, select **IX3-ZDC** from the **ZDC** list and tap **OK**.

ZDC Port Position

The ZDC port switching lever must be set to the **IN** position, as shown below, to place the dichroic mirror in the light path enabled focus operation. Pull the port lever to the **OUT** position to disengage the dichroic mirror when not using the ZDC to eliminate light loss.



Auto Focus Properties

The IX3-ZDC will automatically be included when the IX-83 is added to the Profile. The Z Setup panel, located in the Devices pane, provides access to the microscope's focus controls in the Z Focus, Auto Focus and Stage Setup tabs. The controls for the IX3-ZDC are available in the Auto Focus tab shown below. The auto focus controls are also available in the Sequence pane, as shown in the Auto Focus Examples in the following section.

Auto Focus Control
Hardware - ZDC
Software - HClmage

Active Z
Select which Z motor, coarse or fine focus, to be active

One Shot Auto Focus
Acquire focus position within a specified range at a defined interval

Get Offset
Calculates the range from the top of the cover glass to the current focus position and reports the number below

Select Focus Range
Define the search area for focusing on the sample

Focus Intensity Signal
Score is sample dependent, generally a higher value indicates the sample is more in focus

Continuous Auto Focus
Acquires and maintains focus position within a specified range

Auto Focus Button
In One Shot only mode, it activates the auto focus at the current Z position

Z Setup

Z Focus | Auto Focus | Stage Setup

Active Z: 0-IX3 IX3

☒ Hardware (ZDC) ☐ Software

Select Focus Range:

Objective Magnification: Low Med High 100 um

Focus Intensity Signal: 1515691

Green if Auto Focus successful

☒ One Shot AF

Get Offset

160

Auto Focus Examples

Continuous Auto Focus using 60x Oil UPLSAPO

Get a live image, configure the capture settings and focus on the sample, then go to the Sequence pane and follow the steps below.

1 Scan Type
Select Time Lapse

2 Auto Save
Click the ellipses icon, select CXD and enter the file location and naming convention

3 Field Delay
Select 0 Delay

4 Duration
Select Continuous

5 DISK
Select to DISK

6 Refresh Z Focus
Disable Refresh Z Focus

7 Auto Focus Options
Select Auto-Focus Properties

8 Auto Focus Type
Select Hardware (ZDC)

9 Select Focus Range
For 60x objective set to Medium (50 um)

10 One Shot AF
Disable One Shot AF

11 Offset
Click Get Offset
*A single beep will sound when focused

12 Continuous Auto Focus
Click button to enable Continuous AF

13 Save Settings
Click OK

14 Start Acquisition
Click Start

One Shot Auto Focus using 20x UPLSAPO

Get a live image, configure the capture settings and focus on the sample, then go to the Sequence pane and follow the steps below.

1 Scan Type
Select Time Lapse

2 Auto Save
Click the ellipses icon, select CXD and enter the file location and naming convention

3 Field Delay
Enter 30 s

4 End Time
Enter 3 h

5 DISK
Select to DISK

6 Refresh Z Focus
Enable Refresh Z Focus Every 1 Field

7 Auto Focus Options
Select Use Fine Focus to Autofocus

8 Auto Focus Properties
Select Auto-Focus Properties

9 Auto Focus Type
Select Hardware (ZDC)

10 Select Focus Range
For 20x objective set range to Low (200 um)

11 Offset
Click Get Offset
*A single beep will sound when focused

12 Save Settings
Click OK

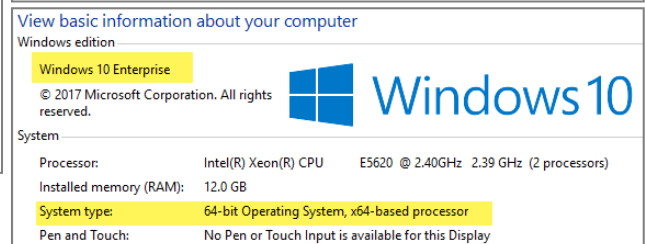
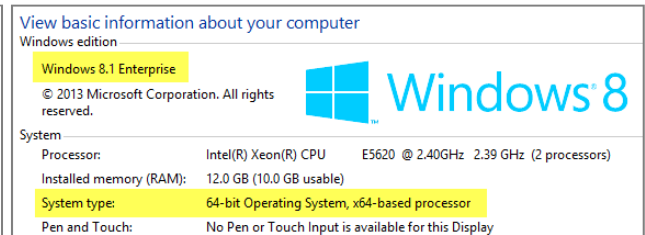
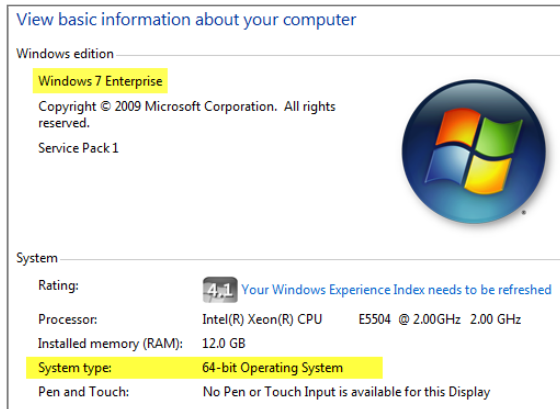
13 Start Acquisition
Click Start

TROUBLESHOOTING

System Information

What version of Windows is installed?

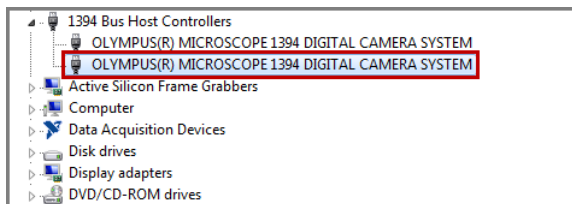
Press the **Windows Logo**  + **Pause/Break** keys to view the System Properties window. The Windows System Properties displays a basic overview of the computer including Windows edition and System type (i.e., 32-bit or 64-bit).



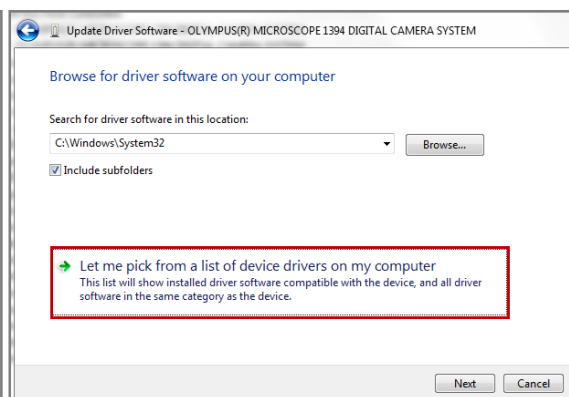
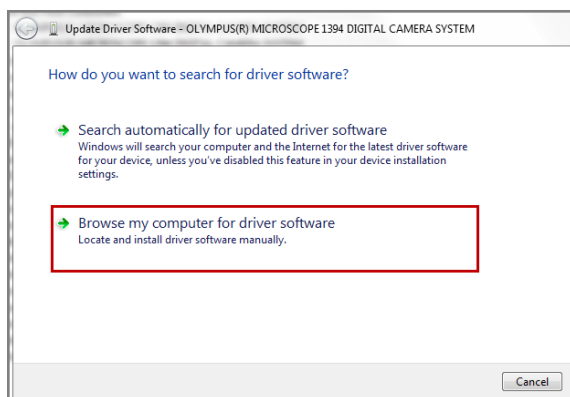
Unable to communicate with Hamamatsu 1394 camera

Was the Hamamatsu 1394 driver re-installed after installing the Olympus drivers?

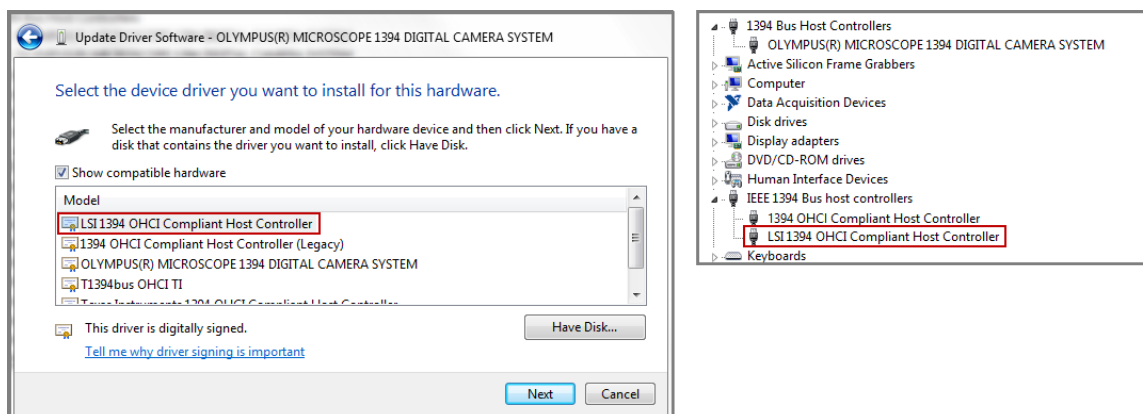
1. Right-click on **My Computer**, select **Manage** and select **Device Manager** in the System Tools list.
2. Right-click on the appropriate 1394 controller and select **Update Driver Software**.



3. Choose **Browse my computer for driver software**
4. Choose **Let me pick from a list of device drivers on my computer**



5. Choose the DCAM compatible driver - **LSI 1394 OHCI Compliant Host Controller** and click **Next** to install the driver.



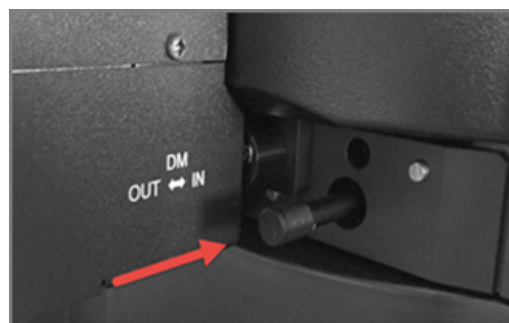
ZDC fails when Get Offset - Double Beep

What is the Focus Range setting?

For 20x and 40x objectives, the focus range should be set to an objective magnification of Low (200 μm). For 60x objectives the focus range should be set to Med (50 μm). For 100x objectives the focus range should be set to High (<10 μm).

Is the ZDC port lever in the IN position?

The ZDC port switching lever must be set to the **IN** position, as shown below, to place the dichroic mirror in the light path enabled focus operation. Pull the port lever to the **OUT** position to disengage the dichroic mirror when not using the ZDC to eliminate light loss.



Is the ZDC enabled in the Touch Panel Controller?

Go to **System Settings** in the **Menu** screen and select **Unit**. In the **Frame** tab, select **IX3-ZDC** from the **ZDC** list and tap **OK** to save the settings.

Using a glass bottom dish?

A 35 mm glass-bottom dish with a cover glass thickness of 0.16 to 0.18 mm is required for proper focusing.

Is the objective supported for ZDC operation?

Please see "**Supported Objectives**" on page 13.

Using an immersion objective?

Use the weight provided with the ZDC or attach the stage clips to keep the dish from floating. Check for bubbles in the immersion oil or water and verify that has not dried.

ZDC focuses on/at incorrect position

What is the Focus Range?

For 20x and 40x objectives, the focus range should be set to an objective magnification of Low (200 μm). For 60x objectives the focus range should be set to Med (50 μm). For 100x objectives the focus range should be set to High (<10 μm).