Filters and Shutters
Reference Manual
**Printing History**

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Filters and Shutters

Overview

This module allows you to control a number of filterwheels, shutters and diaphragms remotely from your computer. In some cases devices will only be available if you also have the Microscope module and are using a particular type of microscope.

This module is not part of the core product, but may be purchased separately. Once you install the module, you will be able to display three new palettes on the screen. These palettes allow you to select the device that you wish to control.

- The Filterwheel Palette allows you to define filter settings for your device, if appropriate, and select them. You will have a separate palette for each filterwheel that you require.

- The Shutter Palette allows you to control and name the shutter on your selected device.

- The Aperture Palette allows you to control and name the diaphragm on your selected device.

This chapter describes the Shutter, Aperture and Filterwheel Palettes, and how to use them.

Filter Wheels

Openlab supports filter wheels both as excitation and emission devices. With emission filters there are two options:

- A traditional filterwheel, such as a LUDL device, which is typically used for FRET imaging.

- A CRI RGB filter, which is typically used for brightfield high-resolution color imaging.

Both the traditional filterwheel and the CRI RGB filter connect via the serial port on the Macintosh and require the appropriate Hardware Support module. They are controlled from the Filters and Shutters module, but must first be enabled in Openlab’s Serial Preferences dialogue.
See this guide for information about using the Filters and Shutters module and the “Serial Devices Guide” for further details about connecting serial devices and setting up preferences. Refer to page 15 for a detailed description of setting up the CRI RGB filter and using it in an automation.

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**The Filterwheel Palette**

The Filterwheel Palette allows you to select the device that you wish to control, and filters for each Filterwheel position, where appropriate.

![Diagram of Filterwheel Palette](image)

Indicates the current filter

Filter wheel, as selected in the Filters menu

Name of filter

Click and hold for pop-up menu of available filters

Click and drag to extend palette

---

**Setting up the Filterwheel Palette**

In order to use the Filterwheel, you need to:

- Select the appropriate Filterwheel palette.
- If necessary, edit the characteristics of each filter.
- Set up filter sets on the Filterwheel palette (see “Using the Filterwheel Palette”).
Selecting the palette

When you run Openlab, the software checks which hardware controller you have and opens the maximum number of palettes for that controller. For example, it opens three palettes for a LUDL controller. The information at the foot of the palette identifies the device to which the settings apply.

- Select the required Filterwheel palette so that you can set up the filter settings. The entries will be blank when you run the module for the first time.

Edit Filter Settings

1. Select Edit... from the Filters menu on the Filterwheel Palette.

2. The Optical Filters Setup dialogue is displayed. You can define new optical filters, as required. The entries that you define in this dialogue are displayed in the pop-up menu on the Filterwheel Palette.

Note: You can insert a blanking plate into a position on the filter and use it as a shutter.

3. Click on the New Filter button to create the entry. This creates an untitled entry in the list of filters in the left-hand window.

4. Click on the entry to select it and type a name for the filter in the box underneath the scrollable list.
5. Select the **Neutral** or the **Color** button, and type an appropriate value into the adjacent box. The selection that you make is reflected underneath the filter name in the list of filters, and in the filter icon.

In the example, we have created a **Blanking** filter. This is so that we can set up one of the filter slots with a solid plate that transmits no light, which can be used as a shutter.

6. If you want to edit an existing entry, select it from the list in the left-hand window and edit the name and characteristics, as appropriate.

6. Repeat steps 3 to 5 until you have set up all optical filters. A typical setup may have entries similar to the example below.

7. If you want to delete an entry, select it from the list in the left-hand window and click on the **Delete** button.

8. Click on **Save** to save the settings. They will now be available from the pop-up menu on the Filterwheel palette.
**Using the Filterwheel Palette**

1. Select the Filterwheel Palette that is appropriate for your device.

2. Check that the filter information is correct and amend, as appropriate.

   For each slot in the Filterwheel, select a filter from the pop-up menu. The icon is filled and a name appears next to the icon. (The filters that appear in the menu are set up in the *Edit...* dialogue.)

3. Click once on the left-hand side of the required icon to move the Filterwheel to that position. The arrow indicates the current position of the filter wheel.

**Editing names from the Palette**

You can also edit the filter name from the Filterwheel Palette. Double click on the name to display the Set Name dialogue. Type in the new name and click on **OK**. The name is changed on the palette. You will need to use the Optical Filters Setup dialogue if you want to change the name that appears in the pop-up menu.
Shutters Palette

When you run Openlab, the software creates a single palette for all shutters. The shutters may be on different devices. Before you start to use the software, you need to identify which shutter relates to which shutter icon on the palette. You can then use the Shutters Palette to open or close the shutter.

Using the Palette

1. Click on the first Shutter icon to see which device it controls.

2. Double-click on the shutter name to display the Set Name dialogue. Type in a meaningful name for your shutter, for example LUDL brightfield, and click on OK. You can now identify the shutter on the palette and in an automation, see page 11. You can edit the name at any time.
3. Click on the shutter icon to open or close the shutter.

4. If you want to change the order in which the shutters are displayed on the palette, click on the View pop-up menu and select the appropriate option.

Apertures Palette

The Apertures Palette allows you to control the size of the diaphragm's aperture. When you run Openlab, the software creates a single palette for all diaphragms. The diaphragms may be on different devices. Before you start to use the software, you need to identify which diaphragm relates to which diaphragm icon on the Apertures Palette.

Using the Palette

1. Click on the first Aperture icon and move the handle to see which diaphragm it controls.
2. Double-click on the Aperture name to display the Set Name dialogue. Type in a meaningful name for your diaphragm and click on OK. You can now identify the diaphragm on the palette and in an automation, see page 12. You can edit the name at any time.

3. Click on the appropriate Aperture icon and move the handle until you achieve the required transmission percentage.

4. If you want to change the order in which the apertures are displayed on the palette, click on the View pop-up menu and select the appropriate option.

Filter Assembly and Automator tasks

If you have the Filterwheel Assembly module, you will be able to use Filterwheel, Aperture and Shutter Control tasks in your automation. The tasks will appear in the Task List in the Automator Window.

Note: You will only be able to use the Set Aperture task if your microscope supports this function.
This section describes the Filterwheel, Aperture and Shutter Control tasks. Refer to the Automator Reference Manual for further details about creating and running automations.

**Move Filterwheel Task**

This task allows you to select a Filterwheel and rotate it to the required filter position. You specify the Filterwheel and the filter position in the Setup dialogue.

Select the appropriate filter wheel

Enter a value for the filter position

---

**New Variable...** **Cancel** **OK**
Open Shutter Task
This task allows you to open a shutter that you specify in the Setup dialogue.

Close Shutter Task
This task allows you to close a shutter that you specify in the Setup dialogue.
Set Aperture Task

This task allows you to select a diaphragm and specify a value for the % light transmission in the Setup dialogue.

[Diagram of the Set Aperture task with labels:
- Select the appropriate aperture
- Enter a % light transmission value]
An Example Automation

The following example illustrates how you can use the Filters and Shutters tasks to capture a series of brightfield and fluorescent images.

- The **Target image window** task targets the image window for the captured image.
- The **Aperture** task sets the aperture for the brightfield image.
- The **Move filterwheel** task moves the filterwheel to the fluorescent filter.
- The **Loop** task sets up the loop for the number of images that you intend to capture.
- The first **Set Video Control** task sets the exposure for the brightfield image.
- The **Open Shutter** task opens the brightfield shutter.
- The **Capture Layer** task captures the brightfield image.
- The **Close Shutter** task closes the brightfield shutter.
The two Null tasks are cosmetic. They make the automation easier to read.

The second Set Video Control task sets the exposure for the fluorescent image.

The Open Shutter task opens the fluorescent shutter.

The Capture Layer task captures the fluorescent image.

The Close Shutter task closes the fluorescent shutter.

The Delay task allows you to specify a time delay before the next sequence of brightfield and fluorescent images are captured.
CRI RGB Filter

Overview

The CRI RGB filter enables images captured in black and white to be converted into color images. This means that you can use a monochrome camera to capture color images. This process is best run using an automation, because each time you move to a new position or to a new slide, you need to capture three pictures via the RGB filters and then merge them into a color layer. This section describes how to use the CRI RGB filter within an automation. There are three main stages:

- Set up the optical filters.
- Calculate exposure values manually.
- Set up the automation.

Setting Up Optical Filters

You need to set up the CRI RGB filter palette for Red (650 nm), Green (520 nm) and Blue (450 nm) wavelengths and make sure that they correspond to the Red, Green and Blue indicators on the front of the CRI controller.

1. Select Edit... from the Filters menu on the Filterwheel Palette.

2. The Optical Filters Setup dialogue is displayed. Set up three filters for Red (650 nm), Green (520 nm) and Blue (450 nm), as described on page 4.
3. Save the settings. They will now be available from the pop-up menu on the Filterwheel palette. "Red" corresponds to filter position 1, "Green" corresponds to 2 and "Blue" to filter position 3.

4. Either manually adjust the color temperature of your microscope, or use the Microscope Palette to set the color temperature to the correct setting.

**Calculating Exposure Values**

Each time you move to a new position or to a new slide, the intensity will change. Rather than recalculate all exposure values each time you move position for each image, you can use the following process, which is summarized below and then described step-by-step:

- Capture an image using each of the three filters manually.
- Find the Blue exposure value, and use that as the reference value.
Find the Red and Green values, and express them as a percentage of the Blue value.

Use these values in your automation, so that when you move to a new position you only have to recalculate the blue exposure—the red and green values are fixed percentages of that blue value.

**Note:** If you change the lamp or restart the experiment, you need to recalculate the exposure values manually.

1. Select the Blue filter in the CRI filter palette and move the slide to an area of background (hence white area). Set the "Colorization" to 10 levels in the Video Controls Palette and adjust the exposure of the image so that you can just about see some red where the image is saturating.

2. Use the HSI Colorspy to choose an area on the image and note the pixel intensity, for example 2800.

3. Record the pixel intensity against the exposure. (You may like to use a table similar to the one shown in step 5.)

4. Repeat for the Red and Green filters. Keeping the pixel intensity in the same area at the same value as before, adjust the exposure so that the background is relatively white (BUT not saturated). Then note the exposure values required for the R and G filters.

5. Using the Blue filter as the reference value, calculate the other filters as percentages of the blue filter. For example, if the blue exposure was 700 ms, the red exposure was 896 ms and the green exposure was 500 ms, the percentages would be as follows:

<table>
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<th>Pixel Intensity</th>
<th>Exposure</th>
<th>Percentage</th>
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<tr>
<td>Red</td>
<td>2800</td>
<td>896</td>
<td>896/700 = 128%</td>
</tr>
<tr>
<td>Green</td>
<td>2800</td>
<td>500</td>
<td>500/700 = 71%</td>
</tr>
<tr>
<td>Blue</td>
<td>2800</td>
<td>700</td>
<td>700/700 = 100%</td>
</tr>
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**Using the Values in an Automation**

You will now use the values that you have calculated to set up an automation.

- The **Target image window** task targets the image window for the captured image.

- The **Move Filterwheel** task moves the filter wheel to the CRI RGB filter position 3. This is the Blue filter.
The **Input** task asks you to provide a name for your images. This name will be used with a number variable, once you start to capture images.

![Setup task “Input”](image)

The **Loop** task defines the number of sets of images that you will capture.

The first **Tell** task asks the user to set the exposure to just below saturation. Check the **Use a non-modal alert** box so that the automation will wait until the user clicks on **OK** to close the alert dialogue.

![Setup task “Tell”](image)

**Note:** When the automation is run, you need to set the "Colorization" to 10 levels in the Video Controls Palette and adjust the exposure of the image so that you can just about see some red where the image is saturating. When you have done this, click on **Continue** and the remaining tasks will capture the red, green and blue channels and merge them.

The **Loop** task defines the number of sets of images that you will capture.
The **Get Video Control** task captures the exposure value for the Blue filter and puts it into a number variable called **Exp_Blue**.

The **Set Video Control** task sets the red exposure, expressing it as the percentage of the blue exposure that you calculated manually.

The **Move Filterwheel** task moves the filter wheel to the CRI RGB filter position 1. This is the Red filter.
The **Capture layer** task captures the layer using the red filter, and names it. It uses the loopcount variable to identify it as a unique “Red” layer within the sequence.

The **Set layer info** task sets the layer type as a red channel.

The second **Set Video Control** task sets the green exposure, expressing it as the percentage of the blue exposure that you calculated manually (exp_blue*0.54).

The second **Move Filterwheel** task moves the filter wheel to the CRI RGB filter position 2. This is the Green filter.

The second **Capture layer** task captures the layer using the green filter, and names it. It uses the loopcount variable to identify it as a unique “Green” layer within the sequence.

The second **Set layer info** task sets the layer type as a green channel.

The third **Set Video Control** task sets the blue exposure to the variable exp_blue.

The third **Move Filterwheel** task moves the filter wheel to the CRI RGB filter position 3. This is the Blue filter.
The third **Capture layer** task captures the layer using the Blue filter, and names it. It uses the loopcount variable to identify it as a unique “Blue” layer within the sequence.

The third **Set layer info** task sets the layer type as a Blue channel.

The **Merge** task merges the three layers into a single RGB layer. It will use the name that the user enters when prompted by the Input task at the beginning of the automation, and the loopcount variable as a unique identifier for the layer within the loop sequence.

The **View layer** task displays the merged layer in the Document Window.

The **Tell** task asks the user if it is OK to continue. It uses a non-modal alert dialogue so that the automation will wait until the user clicks on **OK**.