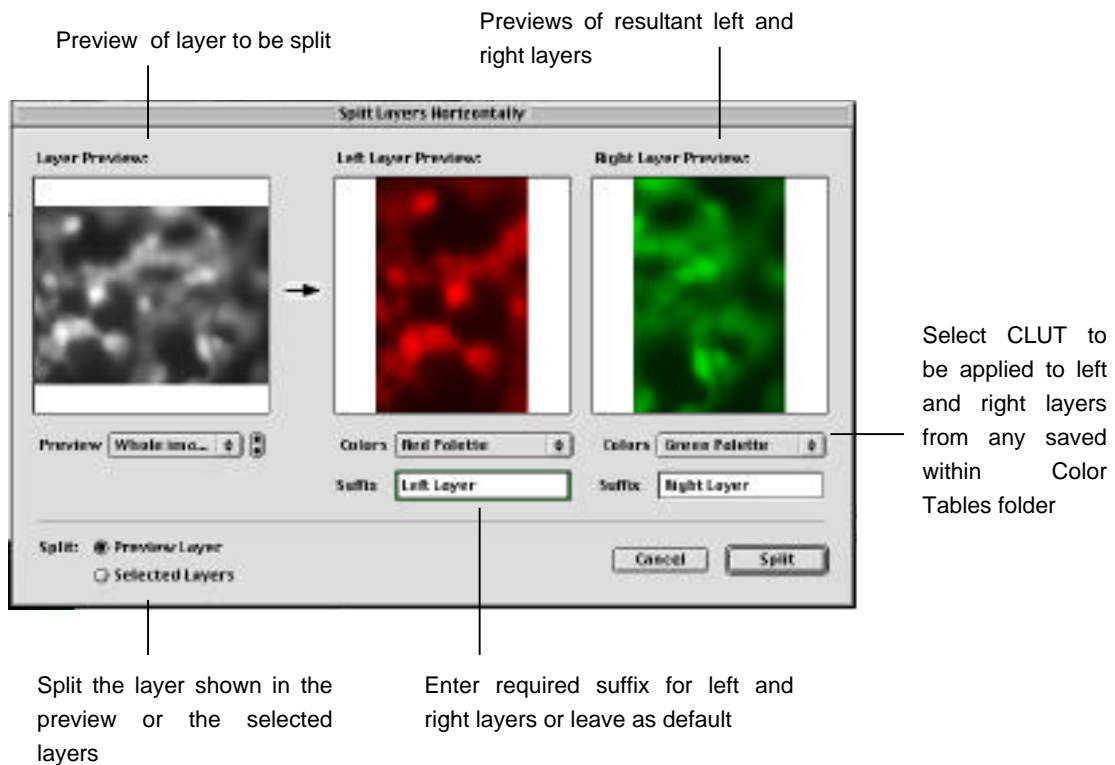## Topic

This technical note describes the use of the Openlab Image Splitter module and Automator task. The Image Splitter module takes layers within image documents and splits them along the horizontal axis to produce a new image document containing twice as many images of half the original width. This functionality makes it ideal for use with optical devices that simultaneously image two fluorescent channels to the same digital camera. This technical note also describes the alignment procedure for two such devices, the Optical Insights MicroImager and the Hamamatsu W-Viewer.

## Discussion

This module is used only to split images, it will not register or align the images. The Openlab module Registration may be a useful addition to a system using such a device.

The Image Splitter module can act on the current layer or selected layers. There is a menu item; **Split Horizontally...** in the Layers menu, and an Automator task **Split Layer Horizontally** in the **Layer Splitting** section of the Automator tasks.

Choose **Split Horizontally…** from the Layers menu:

Preview of layer to be split

Previews of resultant left and right layers

Select CLUT to be applied to left and right layers from any saved within Color Tables folder

Split the layer shown in the preview or the selected layers

Enter required suffix for left and right layers or leave as default

Alt-click to zoom in on any of the three previews.

When adding suffixes to the resultant images both halves will keep the original image name, but must be suffixed. You cannot have two images in one image document under the same name.

The resultant, split images are created in a new image document half the width of the original.  The original images remain in the original image document.  Save the new image document to save the split images.

**Split Layers Horizontally Automator task**

The **Split Layers Horizontally** Automator task is found in the Layer Splitting section of the Automator tasks.

Double click on the task to access the set up dialog:

**Split Layers Horizontally**
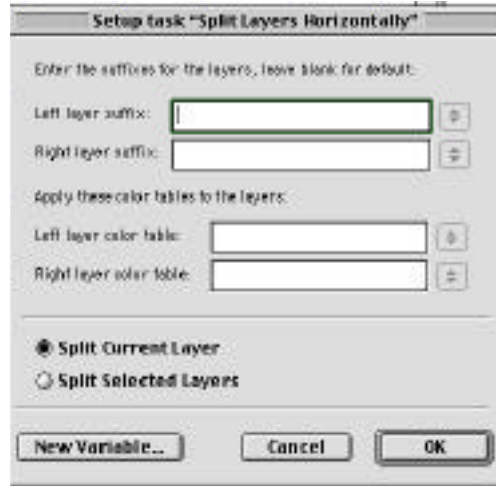
Enter the suffixes for left and right layers, if required. The software will automatically suffix with **Left Layer** or **Right Layer** if either of these fields is left blank.

Enter the name of the CLUT that is to be applied to the left and right layers. This must be one of the CLUTs found within the Color Tables folder inside the Openlab folder on the hard disc. The software will automatically use the CLUT of the original source image if either of these fields is left blank.

Choose to split the current layer or selected layers. An automation containing this task will therefore first require either, an image to be made current using a **View Layer** task, or a group of layers to be selected using a **Select Layers** task.

**Alignment of MicroImager or Hamamatsu W-Viewer devices using Openlab.**

**Optical Insights MicroImager**

Prepare a sample of fluorescence beads, which express both fluorescence emissions at the same time.

In an open image document view live video. First check that both halves are perfectly squared along the vertical axis. Use brightfield illumination for this.

The camera body will need to be turned to correct any misalignment.  There may be an Allen key to loosen the c-mount fixing to enable you to turn the camera. Once you have checked the tracking, take an image.

Using image registration, horizontally shift the image by half its total width. Select the shifted image in the Layers palette click and drag to place above the Video Preview.  Change the Opacity level for the image you have just moved, so the Live Preview is visible through the shifted image.  What you are seeing is the left side over the live right side.

Using an Allen key, (see the MicroImager manual for more information) move the right side dichroic/morror, until the images on the right hand side of the image window are in perfect alignment.

**For the Hamamatsu W-Viewer**

Prepare a sample of fluorescence beads, which express both fluorescence emissions at the same time.

View live video in an image document. First check that both halves are perfectly squared along the vertical axis. Turn on the brightfield for this. The camera body will need to be turned to correct any misalignment.  There is an Allen screw that will loosen the C-Mount to enable you to do this. Once you have checked the tracking, take an image.

Using image registration, horizontally shift the image by half its total width. Next, select, click on and drag the recently captured image in the Layers Manager palette, and place it above the Video Preview.

Change the Opacity level for the image you have just moved, so the Live Preview is visible through it.

Temporarily swap the emission filters over in the W-Viewer. What you will see is the left path though the W-Viewer over the right path.  Using the adjustments on the W-Viewer for the right path/side, move the mirror until you get the two fluorescent objects you see on the right side of the image window to overlap.