Discovery-1™

Compensating for Changing Focus between Wavelengths

Document ID
D50003

Product
Discovery-1, version 5.0

Created
11-Nov-2002

Last Reviewed
28-Oct-2003

Abstract

This document explains how to create a journal that automates changing the focus between wavelengths when acquiring images using Discovery-1, version 5.0. Because different fluorescent probes often focus at different Z positions, you must compensate for this change in focus when acquiring images using several wavelengths. You can do this by auto focusing each individual wavelength, or by using the journal described below to automatically change the focus based upon the wavelength. The following procedures are explained in this document and must be completed in the order shown below:

- Creating the Journal
- Enabling the Journal

Note: This procedure only applies to version 5.0 of the Discovery-1 application. The procedure is not needed for versions 6.1 and above of the application. The described functionality is built into the acquisition process for these versions of the application.

Instructions

Creating the Journal in Version 5.0

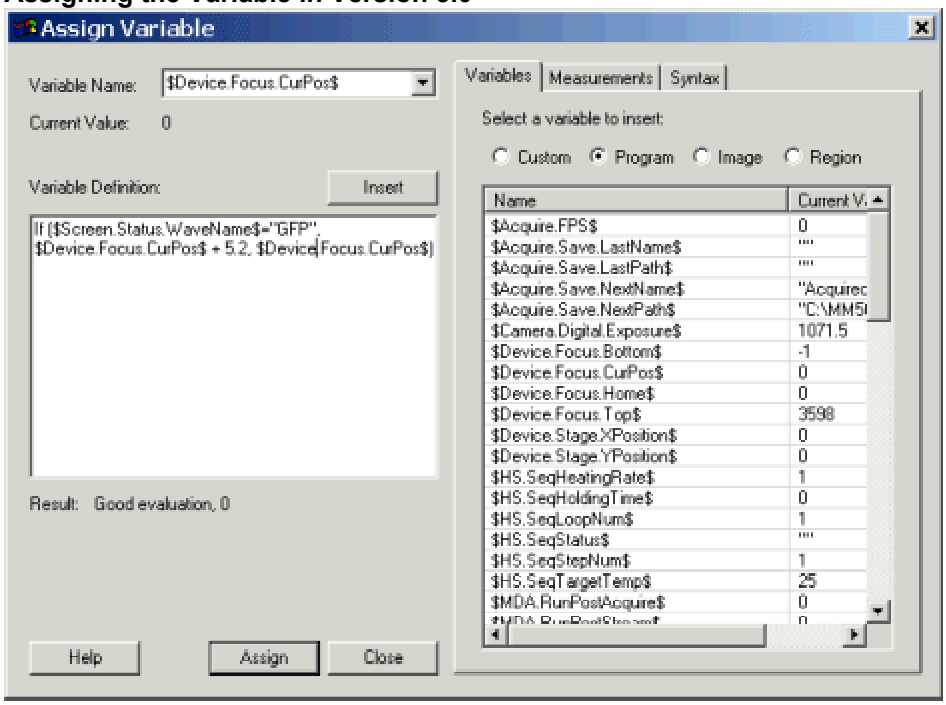
In the following example journal, the GFP wavelength is the second wavelength and it focused 5.2 microns above the first wavelength. The experiment is set up to auto focus using the first wavelength. Complete the following steps to create a journal that moves the Z motor for a specific wavelength:

Note: For your own application, replace GFP with the name of the second wavelength acquired and replace 5.2 with the Z step difference between the two wavelengths used.

1. Start Discovery-1.
2. Select Start Recording from the Journal menu. The Discovery-1 Imaging System title bar will display the message [Recording].
3. From the Journal menu, select Variables > Assign Variable. The Assign Variable dialog box opens.
4. In the Variable Name box type: **$Device.Focus.CurPos$.**
5. Clear the Variable Definition field and type: **if ($Screen.Status.WaveName$ ="GFP", $Device.Focus.CurPos$ + 5.2, $Device.Focus.CurPos$)**, as shown in Figure 1:

Keywords: screening acquisition focus v5 wavelength
Issue Type: acqn acquisition

Figure 1
Assigning the Variable in Version 5.0



6. Click Assign.
   Note: For more than two wavelengths repeat steps 3 - 6.
7. Click Close to close the Assign Variable dialog box.
8. From the Journal menu, choose Stop Recording. The Save Journal As dialog box opens.
9. Type **ScreenPreAcquire.jnl** in the *File Name* text box, navigate to the correct folder (see note below) and click Save.

   Note: The location where you save the journal depends on whether Discovery-1 is configured to start in Single User Mode or Multiple User Mode:

   - **Single User Mode** (you are not specifying a user when you start the software): Save the journal in *C:\MM\Groups\Discovery-1*.
   - **Multiple User Mode** (you specify a user when starting the software): Save the journal in a sub folder of *C:\MM\Groups\Discovery-1\Users*. For example, if you start the Discovery-1 software and choose John Doe as the user, the correct folder will be *C:\MM\Groups\Discovery-1\Users\JohnDoe*.

Enabling the Journal in Version 5.0

In order to use the journal you created, you will need to disable auto focusing for the GFP wavelength and enable the journal to run. Use the following procedure to disable auto focusing for the GFP wavelength:

Note: For your own application, replace *GFP* with the name of the second wavelength acquired.

1. From the Apps menu, select Screen Acquisition. The Screen Acquisition dialog box opens.
2. Click the *Wavelength* tab and select the GFP wavelength from the Wavelength dropdown box.
3. Ensure that neither Wide nor Narrow are checked in the *Auto Focus Acquisition* field. If either is checked, deselect one or both.
4. Click *Close*.

Use the following procedure to enable the Journal in version 5.0:

1. From the Journal menu, choose Variables > Assign Variable. The Assign Variable dialog box opens.
2. In the Variable Name box type: **$Screen.RunPreAcquire$.**
3. In the Variable Definition field type: *1.*
4. Click *Assign.*
5. Click *Close.*