

Discovery-1 Kinetic Data: Analysis of Kinetic Data

Abstract

In a non-kinetic assay, a single image is opened for each wavelength and then the assay is run. This process is repeated for each site in the plate. For example, this process is repeated 96 times for a 96 well plate with a single site, or 384 times for a 96 well plate with four sites-per-well. In a kinetic assay, a stack¹ of images is opened for each wavelength, and then the assay is run. Since the images are opened as a stack, the following strategies should be used.

Instructions

Strategy 1. Process Entire Stacks Whenever You Can

For a Fura-2 experiment, you normally divide the 340 image stack by the 380 image stack (multiplied by some scale value). Since arithmetic allows you to select the entire stack, all time points can be processed with one operation.

Strategy 2. Loop for All Planes

Once stacks have been processed, all measurements should be carried out on a plane-by-plane basis. If you are working with multiple regions of interest, you can either loop for all regions and then loop for all planes (for data by object and then by time point), or you can loop for all planes and then loop for all regions (for data by time point object and then by object). In the first option, you can log values on one line and each object will have its values in the same row - increasing columns = increasing timepoints. In the second option, you can log each object in a separate column, and subsequent rows are subsequent time points. The option that you choose depends primarily on which data format is more usable.

Strategy 3. Use the Journal Function Set Time Point for Database Logging

As you proceed through your stacks of images (time points), you need to notify the database which results correspond to which time points. The specific journal function to do this is *Set Time Point for Database Logging* (Apps menu > Review Screen Data). Whichever method of looping you choose, you must use this journal command if you want to store your data in the database with the correct timepoint. The most common way of doing this is to create a variable (for example, Time). Assign to your variable the definition *Image.ActivePlane* (the active plane of the stack at which you are looking).

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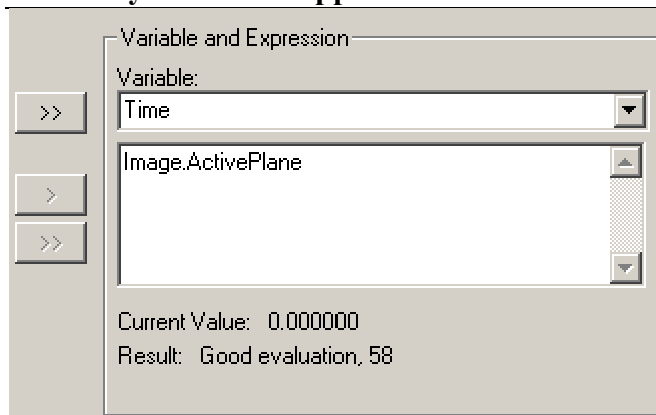
Product
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Use this variable with the *Set Time Point for Database Logging* function by choosing *Select Settings to Override* **Select Settings To Override** in the journal editor. Override the Time Point parameter with your variable. The journal lines should look like this:

```
1: Time = Image.ActivePlane
2: Set Time Point for Database Logging()
   ↳ Time Point = Time
```

Suggested Method for Handling Fura-2 Data

1. Subtract the background from the 340 and 380 images.
2. Ratio the 340 and 380 images. Use Arithmetic to divide the 340 stack by the 380 stack but multiply the numerator and denominator by a set value to keep the dynamic range in your data. For example, if you expect a ratio between 0.1 and 15 you can multiply the numerator by 4000 and still stay below the 65536 gray scale limit. You can also subtract.
3. Segment your image to find areas of interest. This can be from either wavelength and can use the stack maximum or a specific plane of the stacks.
4. Create Regions of interest around the areas of interest.
5. Loop through all of the planes -
 - a. Set the Time Point for Database Logging.
 - b. Loop through all regions of interest
 - i. Measure the intensity in the ratio image using the region of interest (you can also measure the 340 and 380 intensities).
 - c. Go to the next row in the spread sheet.

Or

6. Loop through all of the regions of interest -
 - a. Loop through all of the planes.
 - b. Set the Time Point for Database Logging.
 - c. Measure the intensity in the ratio image using the region of interest (you could can also measure the 340 and 380 intensities).
 - d. Go to the next row in the spreadsheet.

¹ A stack of images is a collection of images associated with a single image window and stored in a single file. Just like a stack of pancakes, a stack of images has a top (the first image plane) and a bottom (the last image plane). And just as you can put your fork through the whole stack of pancakes you can perform operations on the whole stack of images simultaneously or go through the stack one image plane at a time. In a kinetic experiment each plane represents a different time point.