Handling multi-dimensional images with Sync_Windows and Image5D

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ABSTRACT

ImageJ by itself handles image data with up to three dimensions. That means that 3D image stacks or time series of 2D images can be viewed and processed. A number of plugins are available to handle image data with multiple color-channels, slices and/or time-points. Two of these tools are presented here in detail: SyncWindows and Image5D.

Sync_Windows synchronizes cursor movements, mouse clicks and movement of the slice selector between selected images and image stacks. When images from multiple color-channels are synchronized, the same region of interest can be selected and measured in all channels. An extension of SyncWindows, SyncMeasure3D, allows simultaneous measuring of volumes and centers of hand-selected 3D-objects. It is also useful for measuring the shift between images of an object in different color channels ("chromatic shift").

Image5D allows browsing of time series of multi-channel image-stacks. The color channels can be overlaid in selectable colors and quickly switched on and off. Existing plugins see and process the currently selected z-stack of an Image5D as a normal image-stack. Additionally, there are plugins to make projections and montages from all or selected channels.

Keywords: multi-channel, stack, sequence, time-series, display, analysis, chromatic shift

1. INTRODUCTION

Modern microscopic imaging systems enable their users to acquire three-dimensional image data of their specimen. Data from those instruments can comprise multiple color channels (up to 32 in confocal microscopes from Zeiss and Nikon). Furthermore, time-series of such 3D multi-channel data can be obtained. Visualization and, to a variable degree, processing of such data can be done with the control software supplied with the microscope systems. For offline work with the data, often additional licenses of this software can be obtained. Software for offline-analysis can also be purchased from independent software companies. Moreover, most microscope manufacturers offer free versions of their acquisition software with a reduced functionality.

ImageJ[1] has established itself as a free alternative to commercial scientific image processing software, and in many respects surpasses the functionality of available commercial software when the appropriate plugins are installed. ImageJ's capability to handle multi-dimensional data is limited. Two-dimensional images and stacks of two-dimensional images are supported, where the stack can either be a z-stack, a time series or a color (RGB) stack. Up to three channels of 8-bit data can also be addressed by the RGB image type. Thus, ImageJ can at most address z-stacks or time-series of up to three 8-bit color channels.

To overcome this limitation, several plugins have been developed. The UCSD confocal microscopy plugins[2] open and view multi-channel 12-bit images acquired with Olympus Fluoview confocal microscopes and quickly select and deselect certain channels for display. They include the "SyncWindows_" plugin, which synchronizes cursor movements and mouse clicks in selected windows. The HyperVolumeBrowser plugin[3] interprets the additional dimension of an image stack as composed from two extra dimensions instead of one, and provides two scrollbars to browse these dimensions. The WiscScan Data Browser[4] is adapted from the HyperVolumeBrowser for opening and browsing of data

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acquired with WiscScan laser-scanning microscopes. The Image Layering Toolbox[5] overlays multiple 2D-images with selectable colors for each image.

Here, we present two plugin-collections maintained by one of us (J.W.) for displaying and analyzing multi-dimensional image data. Sync_Windows/Sync_Measure_3D can be used to perform measurements synchronously in several grayscale z-stacks interpreted as color channels of a multi-channel data-set. Image5D is a new image type for ImageJ that holds time series of z-stacks with an arbitrary number of channels of 8-bit, 16-bit or floating point image data. A collection of plugins (the "Image5D-plugins") open, save and do processing of Image5Ds.

# 2. METHOS AND RESULTS

2.1. Sync_Windows / Sync_Measure_3D

Sync_Windows is an enhanced version of the SyncWindows_ plugin by Partrick Kelly, which is part of the UCSD confocal microscopy plugins package. With Sync_Windows one can synchronize cursor movements and mouse clicks in multiple images. This can be used to conveniently select the same region of interest (ROI) in different color channels of the same data set. Sync_Windows also synchronizes the displayed z-slice in image-stacks. Sync_Measure_3D extends SyncWindows and adds the option to measure 3D-gravity centers, volumes and intensities of signals in synchronized stacks.

2.1.1. Installing Sync_Windows and Sync_Measure_3D

To install Sync_Windows/Sync_Measure_3D, download the file "Sync_Win.jar" from the ImageJ plugins page (http://rsb.info.nih.gov/ij/plugins/sync-windows.html), place it into the plugins directory of your ImageJ installation and restart ImageJ. The plugins are accessible in the *Analyze/Tools* submenu.

2.1.2. Browsing multi-channel data with Sync_Windows

- Open the images to be synchronized.
- Run Sync_Windows from the *Analyze/Tools* submenu of the ImageJ menubar.
- The Sync_Windows control window opens (Fig. 1). It contains the following elements
  - o A list with names of all open image windows, in which windows can be selected by a single click on the respective names.
  - o A checkbox "Synchronize Cursor" to select, whether cursor movements and mouse-clicks are synchronized in the selected windows.
  - o A checkbox "Synchronize z-Slices" to select, whether the current slice should be the same in all stacks.
  - o A checkbox "Image Coordinates" to select the type of synchronization of the cursor. If the checkbox is selected, cursors in synchronized windows are at the same position in the image relative to the coordinate system of the image, even if the synchronized windows have different zoom factors or different regions of the images are zoomed out. If the checkbox is deselected, cursors in synchronized images are at the same position relative to the coordinate system of the screen.
  - o Two buttons "Synchronize All" and "Unsynchronize All" for selecting/deselecting all windows at once for synchronization.
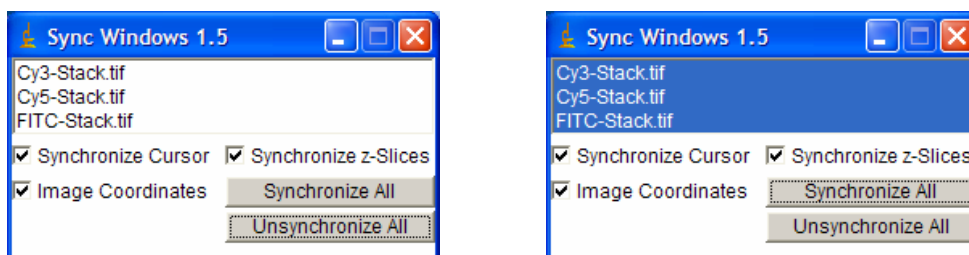

Figure 1. The Sync_Windows control window. Left: no window synchronized, right: all windows synchronized.

2.1.3 Doing Measurements with Sync_Measure_3D

The use of Sync_Measure_3D will be explained using an example. Measuring of the chromatic shift of a microscope requires position measurements (intensity gravity centers) of fluorescent beads imaged in the different color channels of the instrument. In this example, the same beads appear in three color channels. Their positions in the different channels are slightly different due to chromatic aberrations of the microscope objective.

- Open the images of all three color channels and specify their pixel height/width and voxel depth in the *Image/Properties* dialog.

- Run Sync_Measure_3D from the *Analyze/Tools* submenu.

- Select all windows for synchronization.

- Press the "Start Measurements" button. A panel with a slider for every synchronized window appears. (Fig. 2)

- Select a suitable threshold for each of the synchronized windows using the sliders. (Fig. 2)

- To measure the positions of a bead image in all image stacks simultaneously, draw a ROI around the bead of interest and double-click into the ROI. A text window that contains the results of the measurement in one line appears.

- Measure the positions of the other beads. A line for every measurement is added to the results window. For every image stack the line contains the intensity weighted center of the measured bead, the volume and total intensity of the bead, the selected threshold, and the distance of the bead to all preceding beads.

- Press "Stop Measurements". The threshold sliders disappear, and two more lines appear in the results window (Fig. 3). For every stack the first line, labeled "Shift", contains the average shift relative to the first stack (in this case the "Cy3" stack) in x-, y- and z-direction. Positive values mean that the image in the respective stack is moved to the right, down and to higher slice numbers relative to the reference stack (i.e. the first stack).
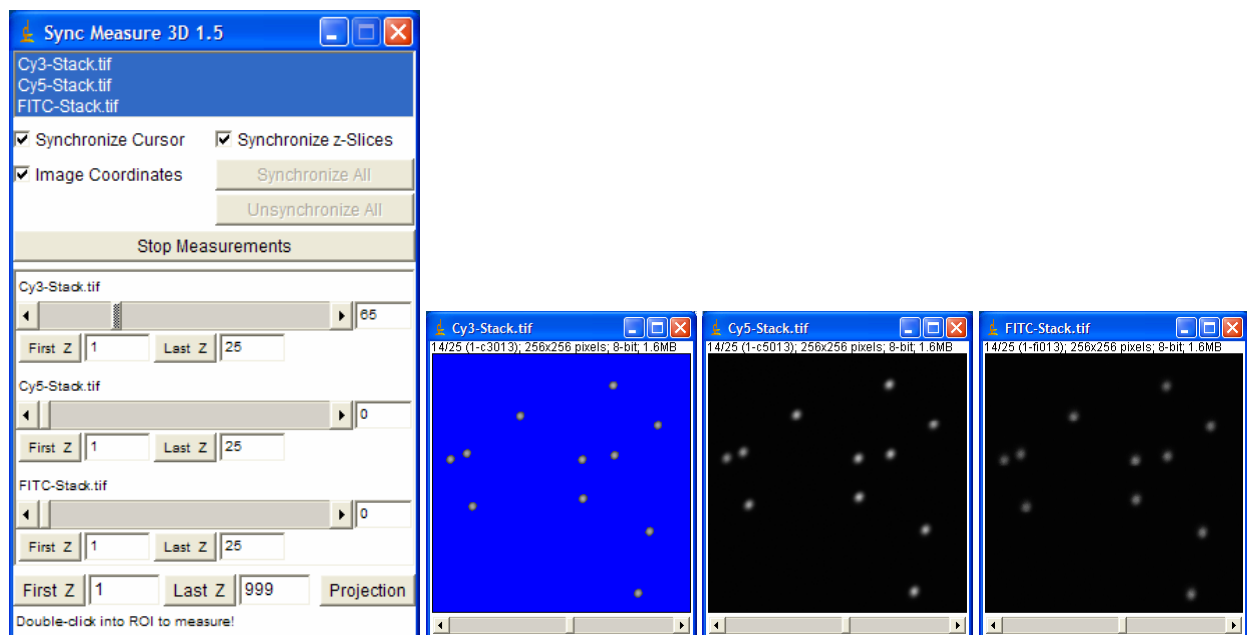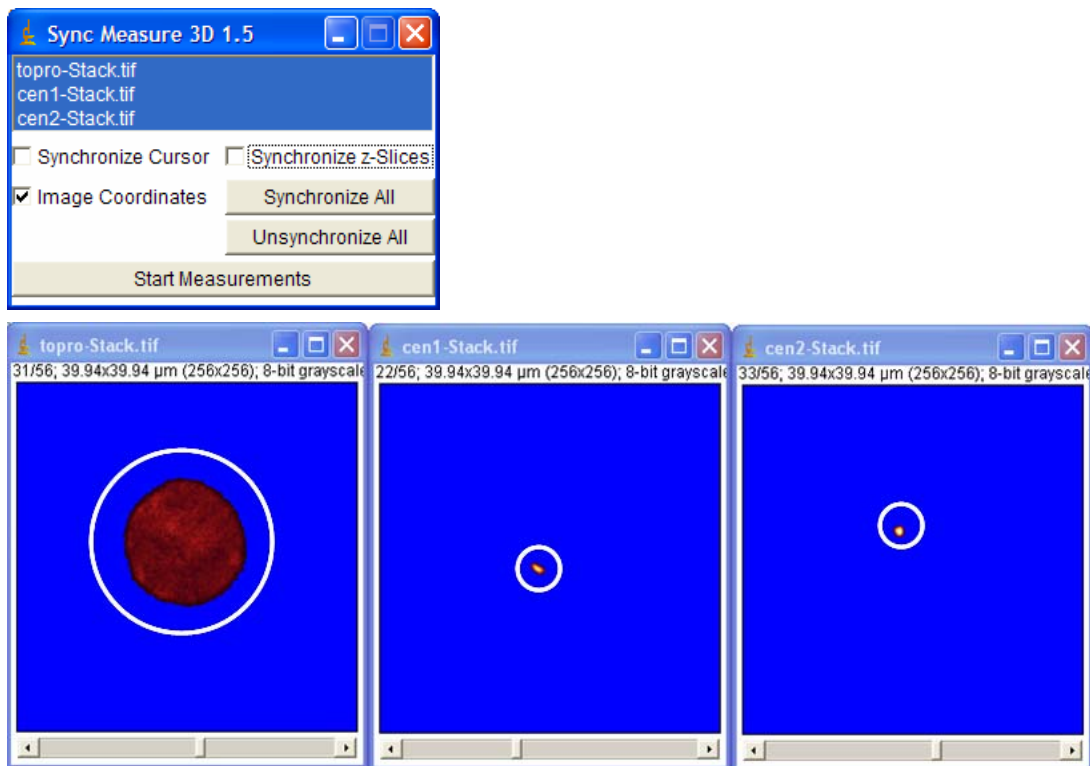

Figure 2. The Sync_Measure_3D control window and the three image stacks corresponding to three color channels. All windows are synchronized, measurement is started and the threshold for the first image (Cy3-Stack) is selected.

Figure 3. The Sync_Measure_3D results window for the measured three image stacks.

Sync_Measure_3D can also be used to measure 3D-distances between non-identical objects in different color-channels. Fig. 4 shows, how the positions of two centromeres in nuclei of human lymphocytes are measured. The "Synchronize Cursor" and the "Synchronize z-Slides" checkboxes in the control window are deselected. Therefore, ROIs and the slices for drawing them can be independently selected in the three stacks. Measurement are performed as in the synchronized case by double-clicking into one of the ROIs. For further analysis, the data can be copied to the system clipboard by selecting *Edit/Copy All* in the menubar of the results window. From the clipboard the data can be inserted into a spreadsheet window.

Figure 4. top: The Sync_Measure_3D control window with the Synchronize Cursor checkbox deselected. bottom: Image stacks of the nuclear counterstain ("topro") and the two centromere signals ("cen1", "cen2"). For explanations see text.

2.2. Image5D

### 2.2.1. Viewing Data with Image5D

Image5Ds are displayed in a window that displays x/y sections of the Image5D at the currently selected slice, frame and channels (see Fig. 5). Below the image, there are sliders for selecting the displayed z-slice, and time-frame. To the right of the image there is a control for selecting the displayed channel or channels ("channel-control").

The channel-control contains a dropdown box for changing between three modes to display channels: grayscale, color, and overlay mode. In grayscale mode, a single channel is displayed at a time in gray. In color mode, each channel is displayed with its colormap applied. It is possible to assign a different colormap to each channel. A vertical scrollbar is used to select the currently displayed channel in both modes.

In overlay mode, all channels are displayed in the window, each channel shown with its colormap applied. There is a radiobutton (round) and a checkbox (square) for each channel. The background of these controls is filled with the color corresponding to the currently selected colormap, and the label of the channel is displayed to the right of the checkbox. The checkboxes are used to toggle the display of the respective channel. Deselecting a checkbox switches its associated channel off. Pressing the button "All" above the controls for the first channel switches all channels on, and the button "None" switches all channels off.

To plugins, which are not designed for using Image5D, an Image5D appears as a grayscale image-stack, which contains all the z-slices of the currently selected color-channel at the current time-frame. E.g. the *Image/Stacks/Z_Project* command of ImageJ does a z-projection of the current color-channel. In grayscale and color modes, the current channel is selected by the channel-scrollbar. In overlay mode, the radiobuttons are used to select the currently active channel, although a "current" channel is not necessary for managing the display in overlay mode.

In color and overlay mode, clicking the "Color" button on the very top of the channel-control opens a panel, which allows a convenient selection of the colormap for the current channel ("color-chooser"). The biggest part of the color-chooser is occupied by a field, which contains little squares in different colors. Clicking on one of the squares assigns the color of this square to the current channel. To the left of this field, the current colormap of the channel is displayed. Above the field, there is a checkbox "Grayscale". Selecting this checkbox temporarily switches the display of the current color-channel to grayscale. When the checkbox is deselected, the previous colormap is restored. The buttons "Edit Color" and "Edit LUT" open dialogs for selecting the color with RGB-sliders or editing the colormap in detail to obtain e.g. a rainbow-colormap.
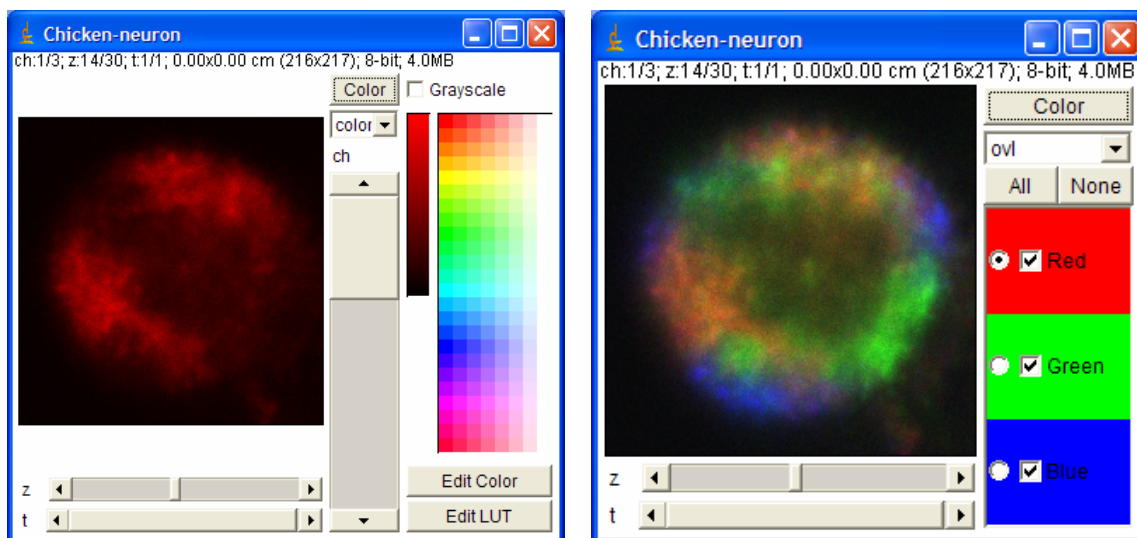
Figure 5. Image5D of a FISH-stained chicken lymphocyte cell. Left: displayed in color mode with the color-chooser panel opened; right: in overlay mode ("ovl"), color-chooser panel closed.

2.2.2. Installing Image5D

Download the file "Image5D.jar" from the ImageJ plugins page (http://rsb.info.nih.gov/ij/plugins/image5d.html), place it into the plugins directory of your ImageJ installation, and restart ImageJ. The Image5D plugins are accessible in the *Plugins/Image5D* submenu.

2.2.3. Creating, Opening, and Saving Image5Ds

To create an Image5D, use the plugins "Open Series as Image5D", "Stack to Image5D" or "RGB to Image5D". Open Series as Image5D" opens a series of images or stacks from hard-disk and converts it to an Image5D. "Stack to Image5D" converts an already opened stack to an Image5D, and "RGB to Image5D" converts an RGB-image or stack to an Image5D.

Image5Ds can be saved to hard-disk with the plugin "Save Image5D" and reloaded with "Open Image5D". Image5Ds are stored as ImageJ TIFF-stacks with some extra metadata. Colormaps for each channel are retained during saving and loading.

Ordered series of (2D-) images can be opened as "Virtual Image5D". In this mode, only the currently displayed images are loaded into memory. All the other images are only present on the hard-disk. In this way, large stacks or time series can be opened and browsed, that would exceed the size of available RAM.

2.2.4. Processing Image5Ds

As explained above, plugins which are not designed to use Image5Ds treat them as image-stacks. For example, the *Image/Stacks/Z_Project* function and the *Image/Stacks/Make_Montage* function of ImageJ make z-projections and montages of only one channel. In principle, one can make projections and montages of all channels separately, and then combine them to a single RGB-image. But as this is tedious, plugins are provided in the Image5D collection, which make projections and montages of Image5Ds.

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