Olympus BX53 Digital Microscope User Guide

A. System Start-up.

1. **Sign into the log book on the desk (start/end time).**
2. Turn on (or wake up) the computer and select "USER" and enter the password on the log-in window.
3. Turn on the microscope ①, the main control unit②, the Prior remote z-stage controller③, DP26 color camera④, and Xenon lamp⑤ (only if you need it for fluorescence imaging).
4. Start **cellSens Dimension** program.

B. Hardware Control and Setting.

1. **Light path bars**⑥ directs light to eyepiece, camera, or both.
2. Select an objective lens by turning the **nosepiece turret**⑦ by hand.
3. **Halogen lamp dial knob** ⑧controls the brightness of transmission light for bright field viewing. The transmission

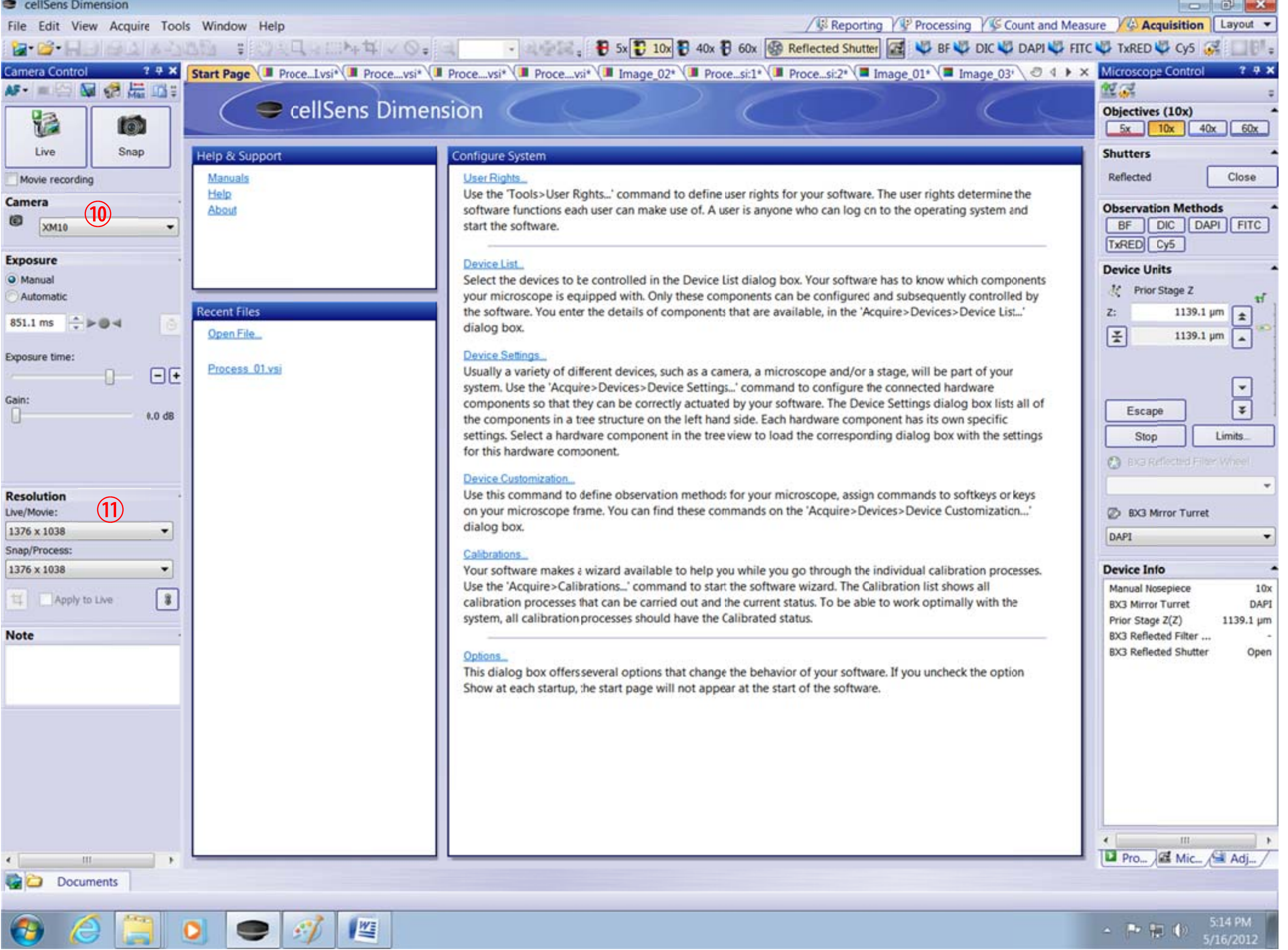
light will be visible from the condenser lens.

4. **Prior Z-stage Control Box**③ moves the stage up or down to focus on specimen. The **UP** button brings the stage up close to the objective and **DOWN** button brings it down quickly. Finer movement of stage can be controlled with the knob; the extent of the fine movement can be selected High (H), Medium (M), or low (L) by pressing "SPEED" button. The selected speed appears on the LED screen (**Note: DON'T USE the focus knob on the microscope and the ZERO button on the controller**.)

5. **Remote controller box** ⑨ controls the selection of imaging mode (i.e. BF or Fluorescence (FL)). Use the **Mirror** buttons to select an appropriate filter mirror cube for different colors of fluorescence. Press the **FL Shutter** button to open or close the mercury arc lamp light shutter.

C. Overview of cellSense program and basic image acquisition.

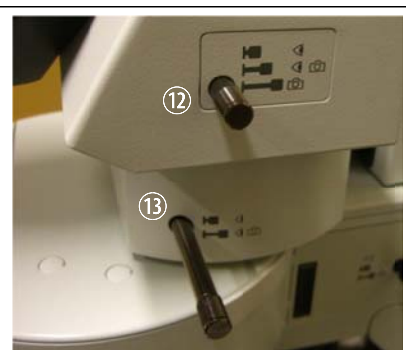
1. When the program opens, first set up the program for your imaging needs (i.e. bright-field imaging for histochemically stained samples or fluorescence imaging for fluorescently labeled samples). Click the **Acquisition** window tab Acquisition.



2. Select an objective (start with 5x or 10x objective) by pressing an objective 5x 10x 40x 60x button. The stage will lower and a message window will pop up. Turn the objective **nosepiece turret** ⑦ by hand to put the matching objective in place. Click **OK** on the message window (*It is critical to match the magnification with the objective to obtain correct scale bar*).
3. Click one of BF DIC DAPI FITC TxRED Cy5 buttons on the **Observation Methods** panel for brightfield or fluorescence viewing.
4. Focus onto the sample by moving stage with **Prior z-stage controller** or by clicking arrowheads in the **Device Units** panel.

5. Select a camera from the **Camera** list⑩: **XM10** monochrome camera for fluorescence or **DP26** color camera for bright field. Select **Manual** or **Automatic** exposure to obtain better images. With DP26 camera selected, select **Automatic** from the white balance mode list.
6. Select **Resolution**⑪ as desired. (*Note: You can choose a lower resolution for **Live/Movie** for fast update of live (active) scene.*)

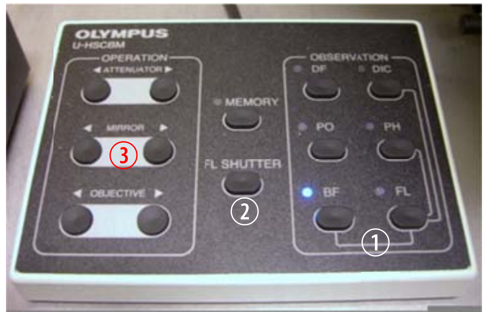
D. Bright field (or DIC) Image Acquisition



1. Make sure that DP26 camera is **ON** and selected from the **camera list** and that the **upper light path bar**⑫ is pushed **in** and the **lower bar1**⑬ is pulled **out**.
2. Click **Live** button and obtain proper exposure and white balance (**Automatic exposure**⑭ with **Spot 30%** region and **automatic white balance**⑮ **work** fine for most cases). Click the button again to stop live viewing.
3. When the setting is satisfactory, click **Snap** button and save the image as .vsi or .tif format by clicking the right mouse button over the image tab.

E. Fluorescence Image Acquisition

1. Click the **FL button**① on the remote controller box to use mercury arc lamp light for fluorescence. The light can be on or off by pressing **FL Shutter** button② on the remote box.



2. Select an appropriate fluorescence mirror cube by pressing **Mirror** buttons③ in the controller box or clicking any one of the **Observation Methods** buttons [BF] [DIC] [DAPI] [FITC] [TxRED] [Cy5] (except BF and DIC). The selected mirror will be in position④. Make sure that the **light path bars**⑤ are in correct position (the upper bar at the mid or extended position and the lower bar pushed in) and **XM10** camera is selected.

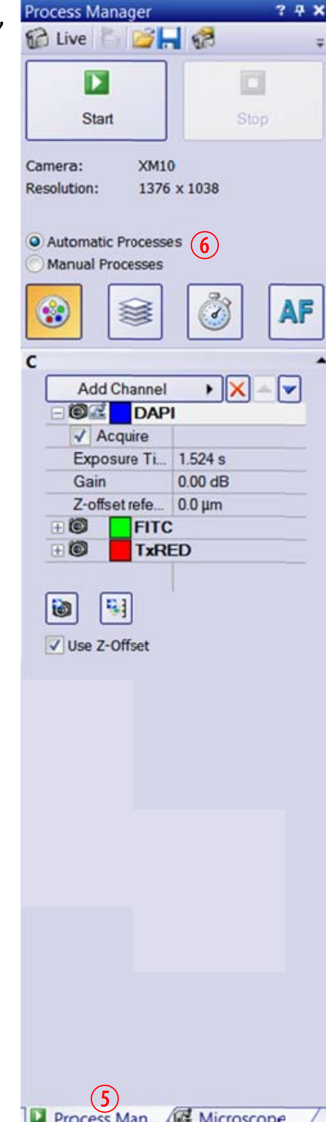
3. Let's take multicolor fluorescence image acquisition with an example stained with DAPI, FITC, and TxRed fluorophores.

4. Click the **Process Manager** tab⑤ and check the **Automatic Processes** ⑥. Click the multicolor wheel button. Click the Add Channel button and select DAPI (blue) in the list. Repeat twice to select FITC (green) and TxRed (red) channels. Click the "**plus**" sign next to the camera icon; it will expand to show current exposure setting. Click the **Live** button. If Automatic exposure (with Spot 30%) is used, let the program find the appropriate exposure time. Once the exposure conditions are set, click icon to set these exposure conditions for DAPI.

5. Click the FITC section on the list and use the Automatic exposure. Wait until it finds an optimal exposure time and click icon to read the setting. Repeat once more for TxRED channel. Once all the settings are obtained, click the Start button. It will take three shots for each channel automatically. The resulting multichannel image, by default, will be saved as .vsi file in the **Libraries>Pictures** folder as soon as the acquisition has been completed. Save the original image files as .vsi format without any manipulation or processing.

6. Images can also be saved as .tiff files. Select ***Image>Mode>8 bit/Channel*** menu command to change the image into 8-bit image. Use ***File>Save as…*** menu command to save the image as one image containing multiple channels. Or use ***File>Export to>tiff series…*** command to save the image as multiple images for each channel.

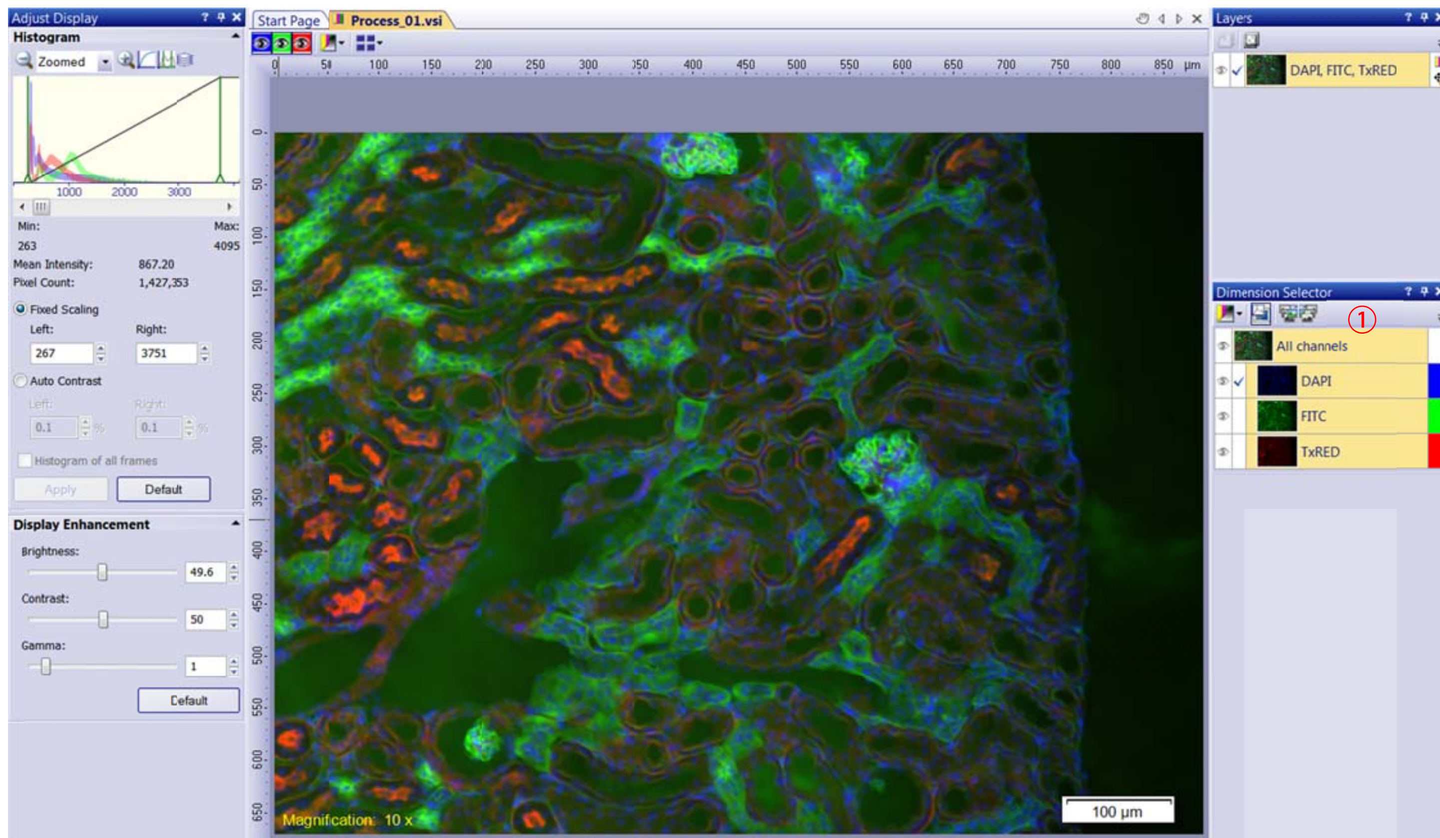
7. Scale bar can be viewed with ***View/Scale bar*** command and the magnification info can be displayed with ***View/Info Stamp*** command. These annotations can't be saved with your tiff image. Save the annotated image as JPEG(JFIF)(*.jpg).



F. Image Processing

1. Once you save the original file, make a duplicate if it is necessary to make any changes in the image for presentation or publication. Click the **Processing** window tab Processing from the main window tabs, in which the image histogram and the brightness and contrast of the display can be adjusted.

2. In default, the adjustment of the **Histogram** or the **Display Enhancement** applies to all the visible channels. On the **Dimension Selector** panel①, you can choose the channels to be visible (eye symbol indicates "visible" and grey-out eye symbol indicates "invisible"). Click the color channels you want to show and click on the eye symbols to hide unwanted channels. Then, you can adjust the histogram and the brightness/contrast of the selected channel only.

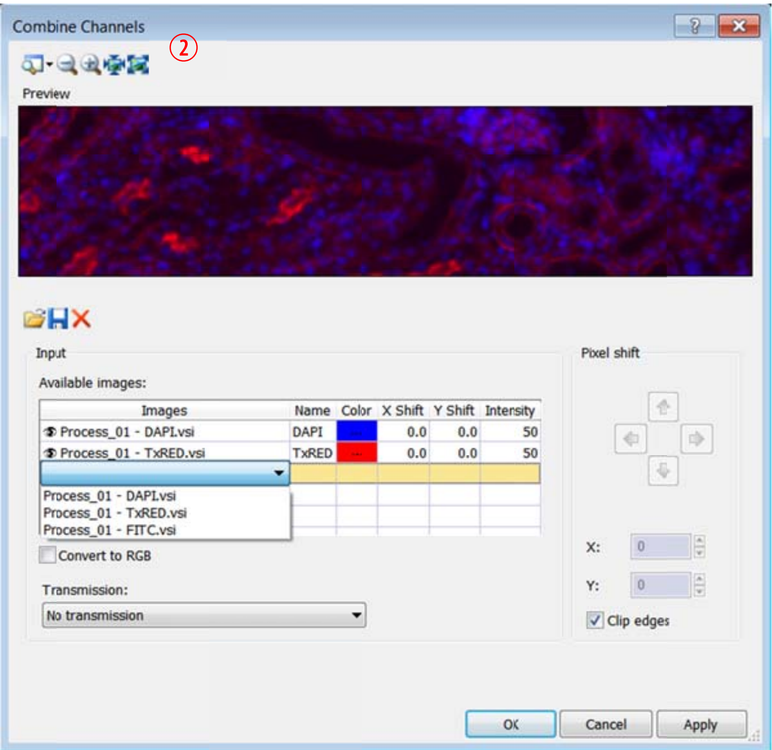


3. To save some overlaid images from a multichannel image you just made as above, there are three ways (*note: these operations will automatically change the image type from 16 bit grayscale to 8 bit color*):

   (1) Make a composite image as above. From the **Edit** menu, use ***Edit/Select All***, then ***Edit/Copy***, and then ***Edit/Paste As/New Image***. This operation will generate a composite image with the same scale bar.

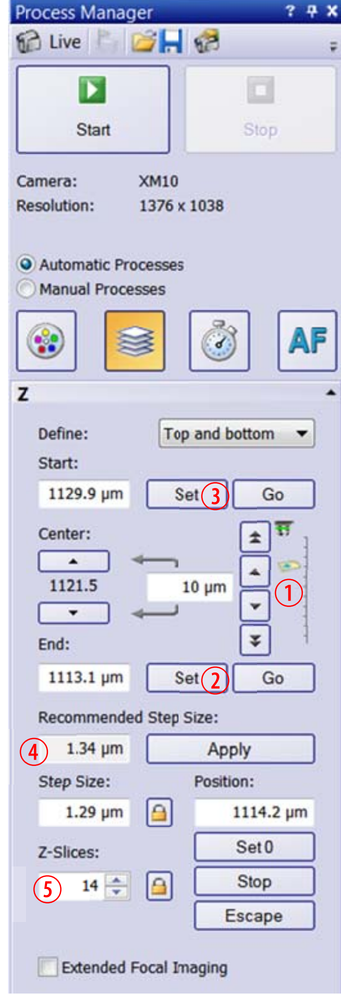
   (2) Make a composite image as above. Use ***Edit/Copy Display to Clipboard*** and then ***Edit/Paste As/New Image***. This will create a new image with scale bar in pixel unit (losing some dimensional information).

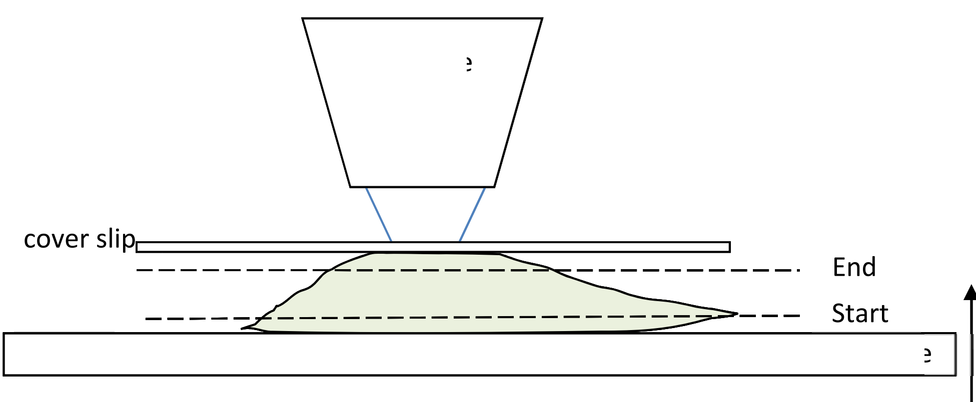
   (3) Select all channels from the **Dimension Selector**. Use ***Image/Separate/Channels*** command to separate the multichannel image into individual channel images. Then use ***Image/Combine Channels***②. In the window, select channels to be combined and click **OK**.



F. Acquisition of Z-stack (z-series)

1. A z-stack image contains multiple frames acquired at different focus positions. Click z-stack button to display **Z** stack panel. To set the **Start** and **End** limits of a z-stack, move the stage using the Prior stage controller or by clicking the arrowheads ① in the **Process Manager** window. First, lower the stage to focus onto a region of interest close to the cover slip and then click **Set** button②, which will be the **End** position. Next, bring up the stage to focus onto a deeper region close to the slide and click **Set** button③, which will the **Start** position. (*Image acquisition will begin at the Start position.*)

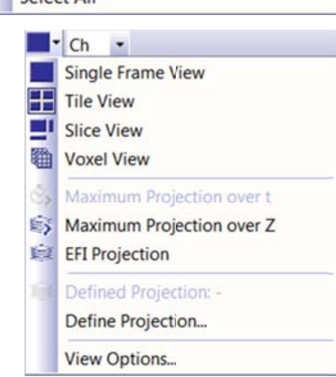




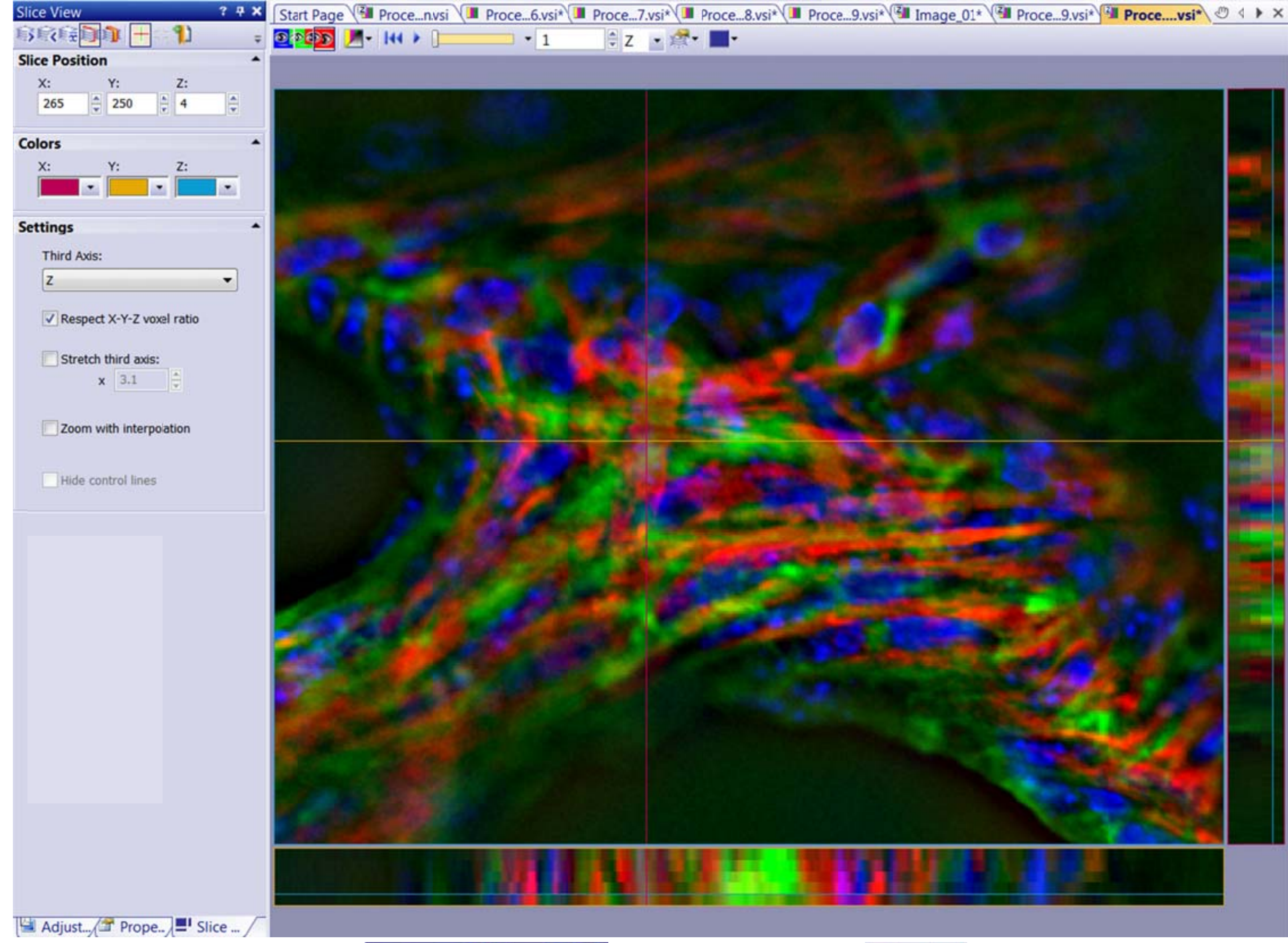
2. The program will automatically recommend a step size ④for z-series according to the objective. Click **Apply** button to accept this step size or change as you want and apply. It will calculate the number of z-slices⑤ accordingly.
3. Click the **Start** button to capture a z-series. You can combine this function with multicolor imaging. Save the images.

G. Displaying and Converting z-stacks

1. In the **Processing** tab, the z-stack image can be manipulated in various ways to generate different views.
2. With the navigator bar, you can navigate different focal frames of a z-stack.
3. You can select a single frame from a z-stack by clicking the right-mouse button in a single frame view and selecting ***Extract Current Frame*** from the context menu.
4. You can also select a short range of frames within the stack by using **Set Selection** buttons⑥; determine a new starting position within the stack and click ***Set Selection Starting Position*** and choose a new end position and click ***Set Selection End Position***. Then choose ***Tile View***. Make sure all the channels are visible (selected). Over an image, click the right-mouse button and select ***Extract*** from the context menu. A new z-stack containing only selected frame will be generated as a .vsi file. Save the image.



5. You can project all the z-stack frames into a single 2D image by selecting ***Process/Projections/Maximum Z*** command from the main menu or ***Maximum Projection Over Z*** on the navigator bar menu.
6. With the ***Slice View*** menu, you can display a cross-sectional z-dimension view of an x and y plane. You can view anywhere by moving the xy cross-hairline to the desired position.



7. With the slice view menu options , such as **Extended Slice** **,** you can visualize z dimension view of extended x-y slice position.