BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Pod C: Keyence Microscope

Microscope in a Box

Kyle Marchuk Austin Edwards Mohammad Naser Harrison Wismer

August 2022

Contents

1 Introduction

The Keyence microscope is a self contained microscope useful for standard 4 channel fluorescence or 3 channel fluorescence and brightfield.

The objective turret houses six lenses including 2x Air, 10x Air, 20x Air, 20x Air, 40x Air, and 100x Oil.

All new users need to be trained by a BIDC staff member before independent use.

Sign up for time using the **iLab** microscope scheduler.

The available objectives are:

Mag	Immersion	Numerical Aperture	Working Distance	ID Number
2x	Air	0.10	8.5 mm	MRD70020
10x	Air	0.30	15.2	MRH20101
20x	Air	0.75	0.8 mm	MRD70270
20x	Air	0.45	8.2 - 6.9 mm	MRH48230
40x	Air	0.60	3.6 - 2.8 mm	MRH48430
100x	Oil	1.45	0.13 mm	MRD71970

2 Hardware Start-Up

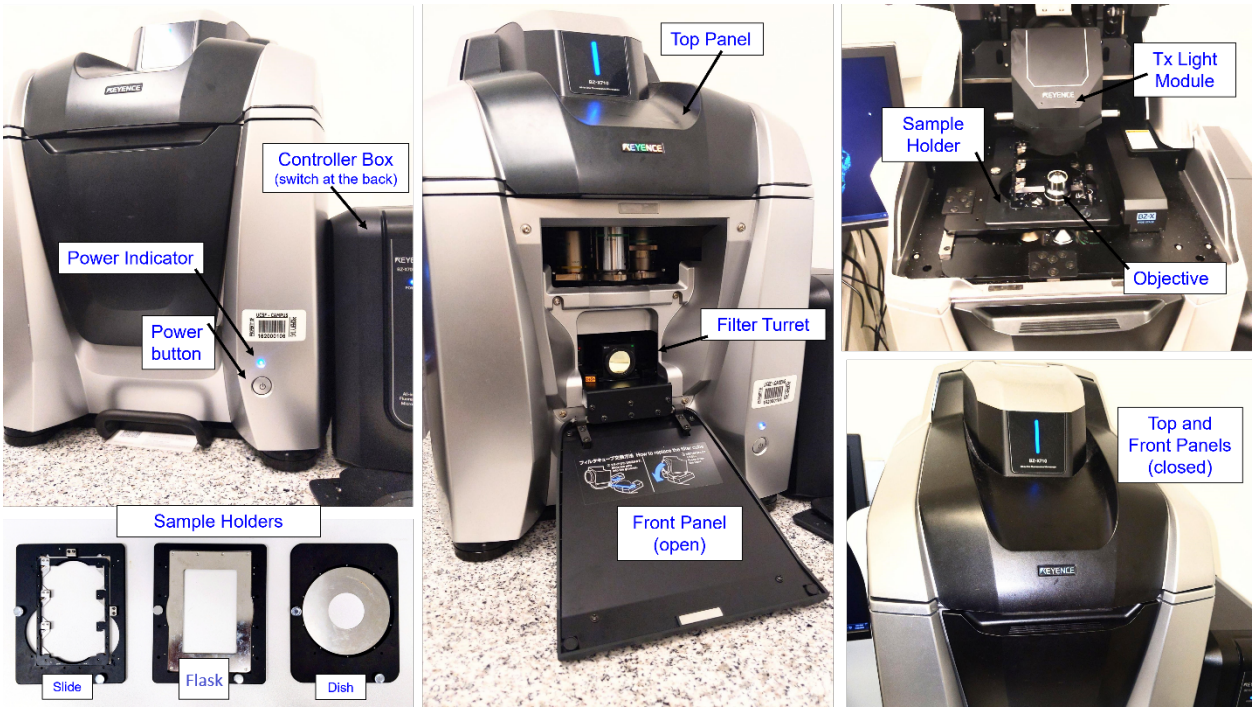

Figure 1: Overview of the Keyence System.

1. Turn on the **power switch** on the back of the controller.
2. Press the **power button** on the main unit. Power indicator should be **blue**.
3. Turn **on** the computer.

3 Software Start-Up

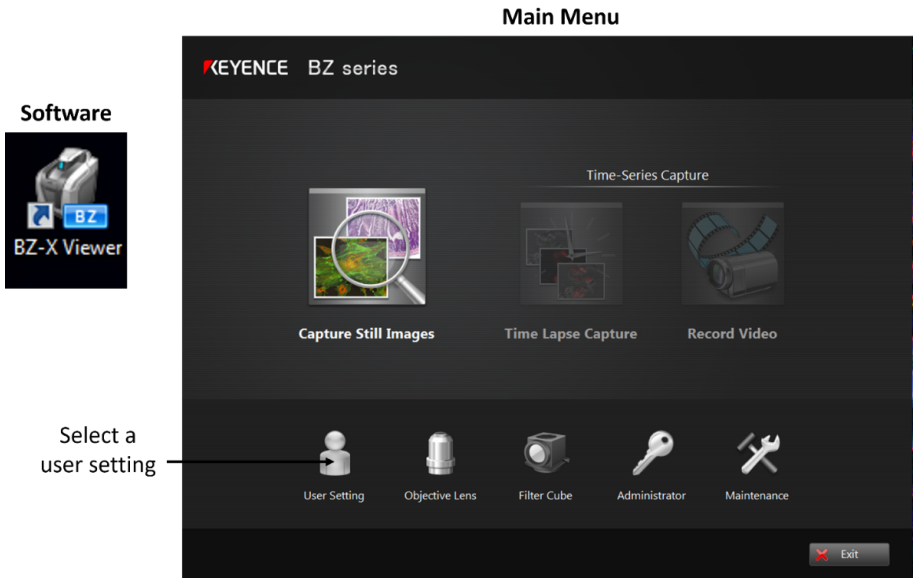

Figure 2: Options for launching the software.

1. Double-click the **BZ-X Viewer** icon on the desktop.
2. Log into the software. **No password** is required. A menu with various options should pop up.
3. Click **User Setting** and browse for **UserSettings1.bzcfg** if scanning for the first time. Otherwise, choose any previously saved settings. File location *C:\Users\USER1\Documents\BZ-X\Templates*
4. *Skip this step if using fluorescence only.*
   FOR BRIGHTFIELD ONLY: Click on the **Filter Cube** icon.
   (a) Click on **CH4** button - filter turret will rotate.
   (b) Select **Brightfield/Phase Cont.** from the drop-down menu.
   (c) Open the front panel and take the **Cy5** filter-cube out of the turret.
5. In the menu window, click **Capture Still Images**.
6. Select appropriate sample holder from the drop-down menu.
   Caution: Incorrect holder may damage the objectives.

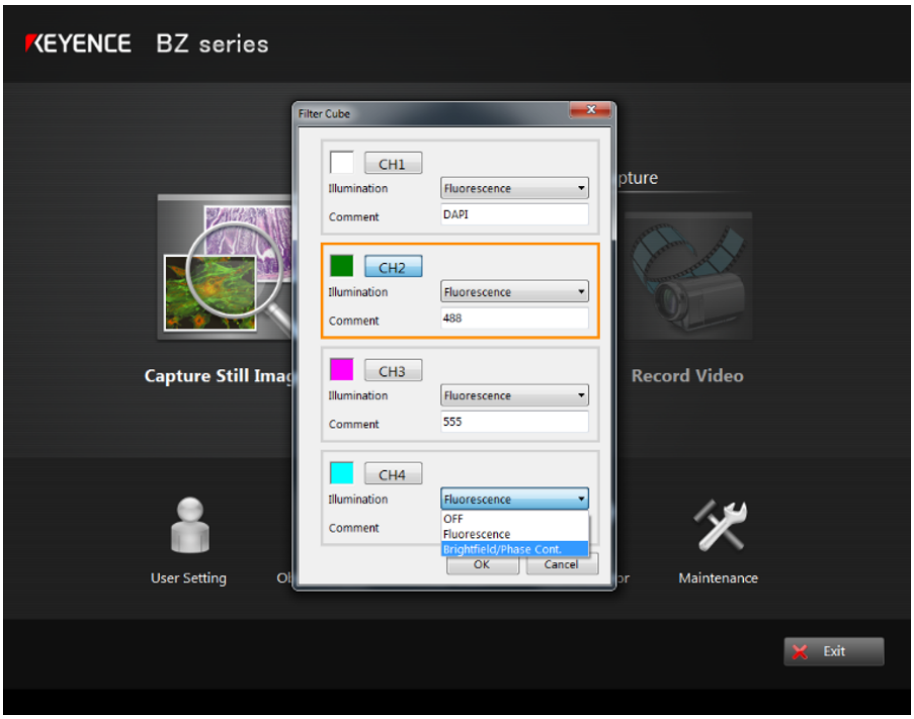

Figure 3: Brightfield Setup.

4 Loading the Sample

1. Open the **top panel** and lift the **transmitted light module**.
2. Choose the correct holder for your sample.
   (a) The slide holder is used for both regular slides as well as for 96-Well Plate imaging. If using a 96-Well Plate, remove the 3-slide insert from the slide sample holder. The well plate should slot snugly into the slot where the insert previously was housed.
3. The PlanFluor 20X and 40X objectives have correction collars that allow use with either 1.7mm plastic coverslips or .17mm glass coverslips. If using either of these objectives, be sure to adjust the correction collars accordingly.
4. Place the sample holder over the objective lens. If replacing holders, make sure it is screwed to the stage.

5 Focusing and Navigating the Sample

1. The software should start in the **Live** mode (i.e. Pause button active). Top stop **Live** feed, click on the **Pause** button.
2. At the top of the **Acquisition** panel, select a channel (for brightfield, see **Scanning Brightfield** section below).
3. In the **Microscope** tab, select a low magnification objective to create a **map** of the whole tissue or a big region of your sample. You will be able to choose a region-of-interest (ROI) to scan in higher magnification using this map as a a guide later (see next section).
4. Under the **Objective Selector** you will find a coarse schematic depicting the layout of your chosen sample holder. Click on the corresponding area that has your tissue.

5. Move the **Focusing Bar Slider** up or down to get your sample in focus.

   (a) Alternatively, you can use the **Auto Focus** button to get your sample into focus automatically. Click **Yes** to apply the **Low Photobleach** option when prompted.

   (b) You can also focus manually using the **mouse scroll wheel** as coarse adjustment and **ctr+mouse scroll wheel** for fine adjustments.

6. Click on **Navigation**; a new window will pop up.

7. In the **Navigation** window, click **Add** to create a map of the sample.

8. Select your desired higher magnification objective. Re-focus the sample.

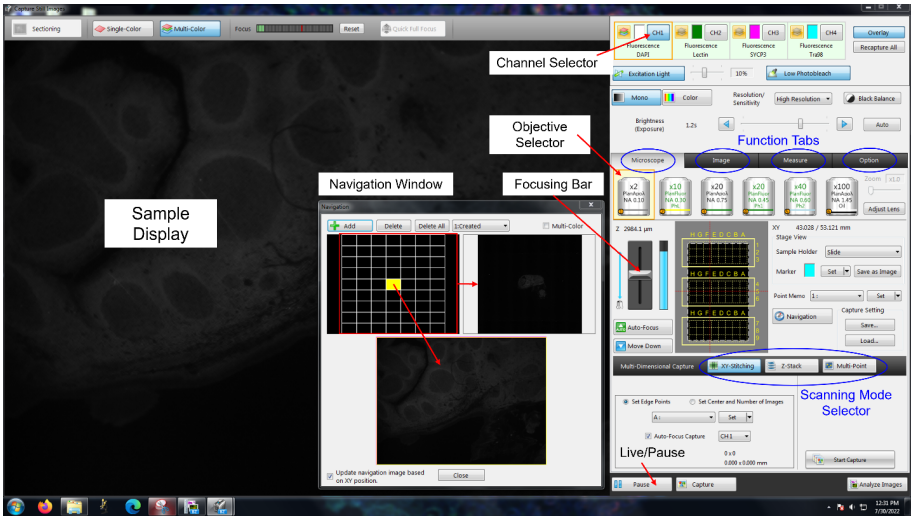

Figure 4: Overview of Focusing and Navigating the sample.

6 Scanning

6.1 Immunofluorescence

1. Select the **Mono** button for the monochromatic camera.

2. Select the individual channel and adjust **Excitation Light** and **Brightness** (exposure).

3. The **Multi-Color** option above the image display allows sequential scanning of multiple channels. To visualize all channels together, click the **Overlay** button.

6.2 Brightfield

1. Make sure brightfield mode was set up (see Step **3** in **Software Start-Up**).

2. Select the brightfield channel by click on **CH4** button on the **Navigation/Acquisition** window. You can turn off the other channels by clicking the **stack** symbol in each channel.

3. Adjust the **Transmitted Light Intensity** and **Exposure** as required.

4. Turn off **Overlay**.

5. Click on the **Color** button (instead of **Mono**).

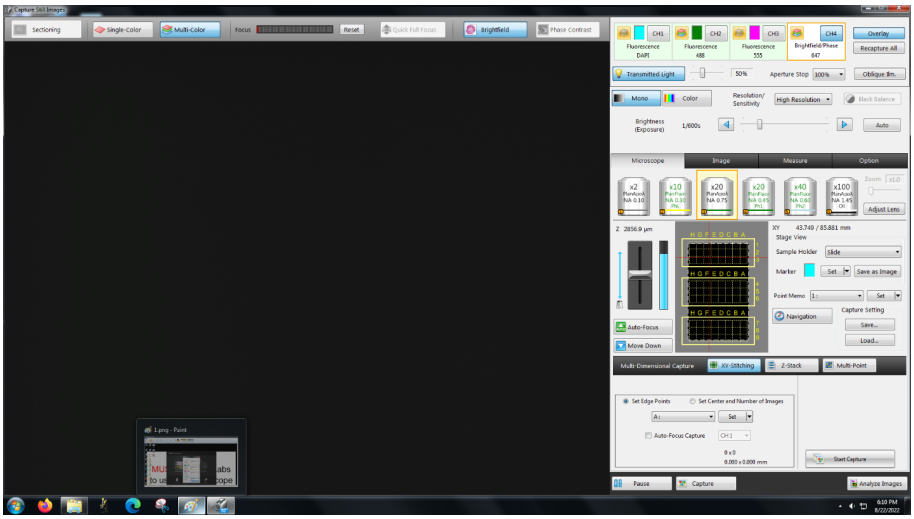

Figure 5: Brightfield scanning options.

7 Scanning Modes

Three modes available in the **Multi-Dimensional Capture** tab: **X-Y Stitching** (i.e. tile scanning), **Z-stack** and **Multi-Point** scanning. These modes can be combined or used separately.

7.1 X-Y Stitching or tile-scanning

Click on the **X-Y Stitching** button.

Option 1 - **Set Edge Points:** Image a user-defined region. Set the edge points by clicking on the grid. Click **Set** for each point to be saved. You can revisit the points by clicking **Go**.

Option 2 - **Set Center:** Scans *n*-by-*n* tiles around a user-defined point. Select the center point by clicking on the grid and then press **Set**. Default is *3*-by-*3* (9) tiles around a center point.

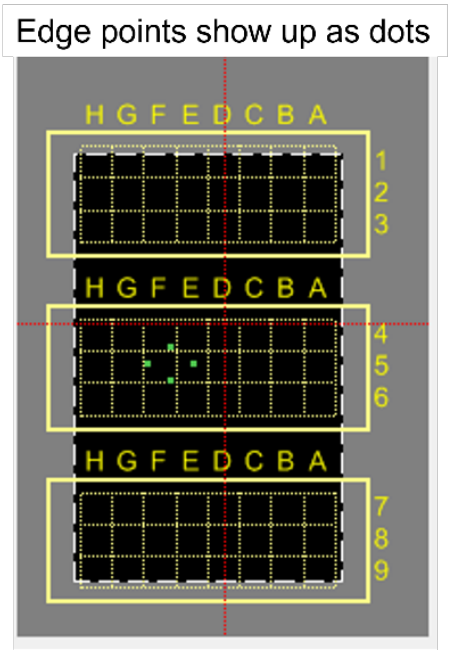

Figure 6: Stitching overlay.

7.2 Z-Stack

Click on the **Z-Stack** button.

1. With the image in focus, using the mouse wheel (or **Focusing Bar**), scroll *up* to the point where the image becomes blurry.
2. Click **Upper Limit**.
3. To set the lower limit, scroll *down* until the image becomes blurry and click **Lower Limit**.

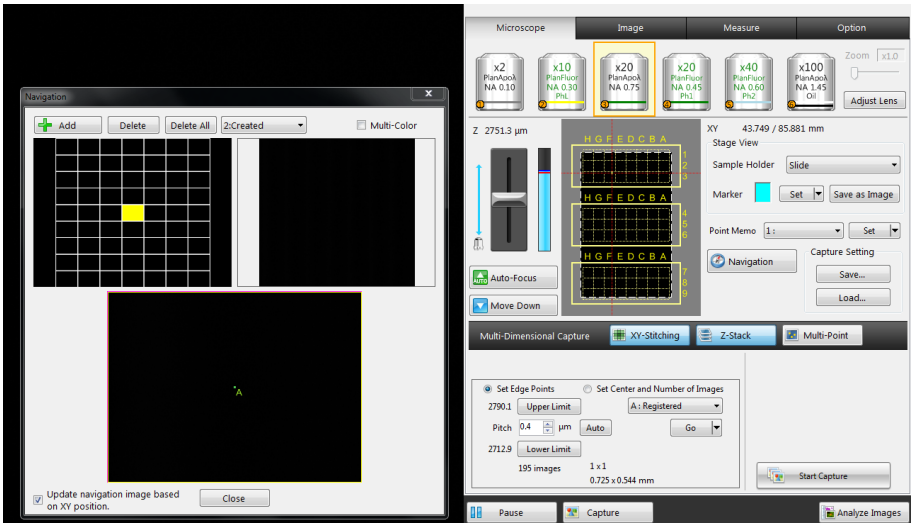

Figure 7: zStack options for acquisition.

7.3 Multi-Point

This option is useful to capture multiple tiles/regions that are not necessarily connected. For example, tissue-microarray (TMA) cores can be scanned with this mode.

1. Select a desired region on the **Navigation** grid.
2. Set **Center** and **Number of Images**.
3. Move to a different region and repeat step *a* and *b*.
   Note: All the navigated and set regions are stored in the Stitched Area menu. Both **X-Y** and **Multi-Point** modes are activated for this operation.

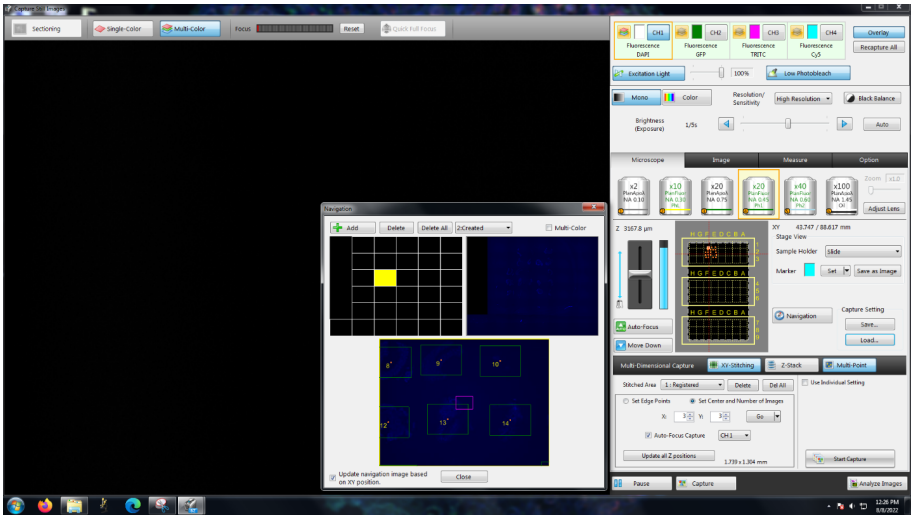

Figure 8: Overview of the Multi-Point acquisition options.

8 Capture Image

1. **Start Capture** will start scanning all saved points with the user settings.
2. **Capture** button will just capture the view on display (i.e. current point) with the selected mode settings.

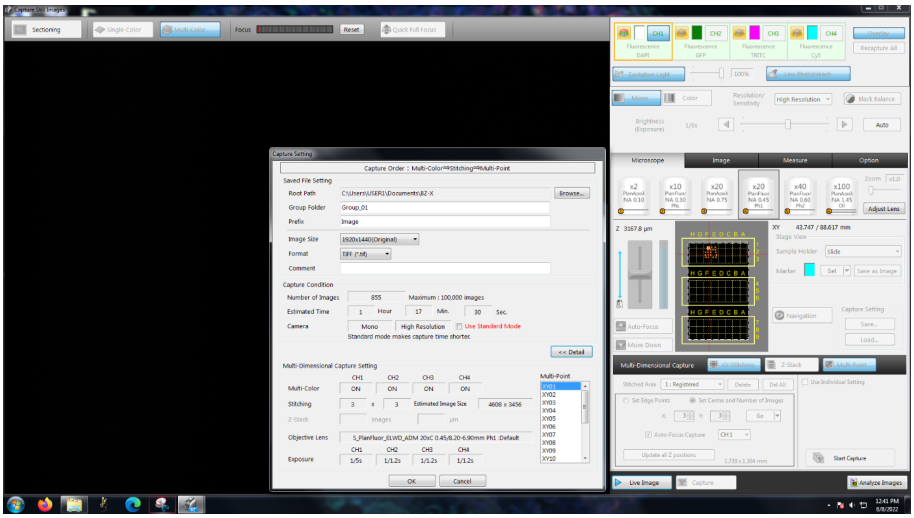

Figure 9: Capture options.

9 Saving Acquired Images

In the Capture Setting window, check if all parameters are correct before confirming.
When saving a tiled image, a folder containing the raw, unstitched tiles will be saved by default. To tile and save the full image, carry out the following steps:

1. After your X-Y stitch or multipoint is finished, a window will automatically pop up asking if you would like to open the folder containing your image tiles. Select **Open Image Folder**.
2. Within the folder containing the tiles for a particular image, open the **.bcf** file. **Note:** for multipoints, you will see multiple folders where each folder is a single XY-Stitch.
3. You should now see a window with a **Load (L)** option in the bottom right corner. Select this option.
4. A new window will appear, asking if you would like to stitch your image. Select the **Uncompressed** option before beginning stitching.
5. After the stitching procedure is completed, a final window containing the stitched image will appear. In this window, go to **File - Export in the original scale** and save your image as a TIFF or Big TIFF to your desired directory.
6. At this point, you can close out of all the preceding windows. Once only the BZ-X Analyzer windows is left open, the tiled image will pop up within the analyzer. For larger tiled images, the image in the analyzer will be automatically downscaled (by following step 5, you can save the uncompressed version of the image).

Note: Files older than **2 weeks** will be deleted without notice.

10 Shut Down

1. After saving, close the **BZ-X Viewer** software.
2. Shut down the computer using **Shut Down** option in the **Start Menu**.
3. Remove sample and **clean objectives** as needed.
4. Power **off** the main unit.
5. Turn off the **power switch** on the back of the controller.
6. Confirm the space is **clean and ready** for the next user.

11 Appendix

	CFI Plan Apochromat Lambda D 2X	CFI Plan Fluor DL 10XF	CFI Plan Apochromat Lambda D 20X	CFI S Plan Fluor ELWD ADM 20XC	CFI S Plan Fluor ELWD ADM 40XC	CFI Plan Apochromat Lambda D 100X Oil
						
Material Number	MRD70020	MRH20101 / MRH20105	MRD70270	MRH48230	MRH48430	MRD71970
Type	Plan Apochromat	Plan Fluor	Plan Apochromat	Super Plan Fluor	Super Plan Fluor	Plan Apochromat
Primary Technique	Brightfield	Phase Contrast	Brightfield	Apodized Phase Contrast	Apodized Phase Contrast	Brightfield
Immersion	Air	Air	Air	Air	Air	Oil
Magnification	2x	10x	20x	20x	40x	100x
Numerical Aperture	0.1	0.3	0.75	0.45	0.6	1.45
Working Distance	8.5	15.2	0.8	8.2-6.9	3.6-2.8	0.13
Cover Glass Thickness	0-0.17	1.2	0-0.17	0-2	0-2	0-0.17

Figure 10: Objective Specifications.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.