

Keyence Box Microscope:

Turn-on Sequence

- 1) If computer is off, turn it on and log in to Windows.
- 2) Log in to BRC Login.
- 3) If blue light is off (arrow), push button to turn on microscope. Wait until the blue light stops flashing.
- 4) Start the BZ-X800 viewer.
- 5) At this initial screen, make sure you have your objective correction collar set correctly and you have the correct filter packs in the system (see back). If not, you can always come back to this screen by exiting the acquisition.



- 6) Choose acquisition app and click Change Sample (top menu of viewer) to lower objective and bring stage to the front.

Turn-off Sequence

- 1) Click Change Sample to lower objective and bring stage to the front.
- 2) Remove your sample
- 3) Exit all the way out of the software
- 4) If no one else is waiting to use the system, click button at lower right-hand corner of box (arrow). Blue light will turn off. Orange light on the controller indicates standby mode.
- 5) Log out of BRC Login.

Objectives

<i>Magnification</i>	<i>NA</i>	<i>Type</i>	<i>Phase</i>	<i>Coverslip thickness (mm)</i>	<i>Working Distance (mm)</i>	<i>Scaling (S_{11}) at Zoom#=1 with no camera binning^{&} ($\mu\text{m}/\text{pixel}$)^{**}</i>
4X	0.13	Plan Fluorite	PhL	---	16.5	1.88721
10X	0.3	Plan Fluorite	Ph1	0-2	14.5	0.75488
20X	0.45	Plan Fluorite	Ph1	0-2 Adj*	8.8-7.5	0.37744
40X	0.6	Plan Fluorite	Ph2	0-2 Adj*	3.3-2.2	0.18872
20X	0.75	Plan Apochromat	---	0.17	0.6	0.37744
40X	0.95	Plan Apochromat	---	0.11-0.23 Adj*	0.25-0.17	0.18872

*Adj indicates an adjustable correction collar. When exiting the system, please leave these in default mode (0.17).

#Zoom slider is next to objective on the acquisition screen (can only be adjusted in single channel mode).

&Camera binning is adjusted under camera settings->Resolution/Sensitivity->Custom.

**Actual scaling is given by $S=S_{11}*(\text{binning}/\text{zoom})$

Filter packs

<i>Cube Name</i>	<i>Excitation Bandwidth (nm)</i>	<i>Emission Bandwidth (nm)</i>	<i>Dichroic 50% Transmission Wavelength (nm)</i>
BZX DAPI	360/40	460/50	400
BZX GFP	470/40	525/50	495
BZX TRITC	545/25	605/70	565
BZX Texas Red	560/40	630/75	585
BZX Cy5	620/60	700/75	660

Other notes

For scaling info: When you are in the analysis software, go to Insert->Scale. You can put a scale bar on your image, but you don't need to. Just go to the bottom section under calibration and choose the "setting..." button. This will give you the scale in $\mu\text{m}/\text{pixel}$.

Zoom: The zoom slider to the right of the objective can only be adjusted which you are in single channel mode. Once you do this, it will remain fixed after changing back to multichannel mode.

Live imaging recommendations:

- 1) For the incubation chamber, you will need 30 ml of H₂O/lysol (stored in the incubator). If you are doing a multi-day series, you probably need to add ~15 ml/day. Be CAREFUL! Add this slowly so as not to get water in the microscope mechanics and electronics below.
- 2) Keep fluorescence illumination power as low as possible. You can turn up the camera gain if necessary (camera settings->Resolution/Sensitivity->Custom).
- 3) Use focus tracking on the brightfield/phase channel to stay in focus.