# Nikon Ni-e & SPOT Software

## General Operating Instructions

**About this microscope:** The Nikon NI-e is a fully automated microscope with 7 different size lenses, 4x, 10x, 20x, 40x, 60x, and 100x. The condenser, aperture and f-stop settings will move automatically as you change between the different powers, they are electronically set at the best possible settings for each objective. The software used is SPOT software by Diagnostics Instruments. Special macros and setups have been installed that are specific to this microscope ease of use and to give you the best possible micrographic photos. The camera attached is a SPOT Insight camera that will take publication quality images.

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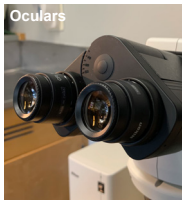
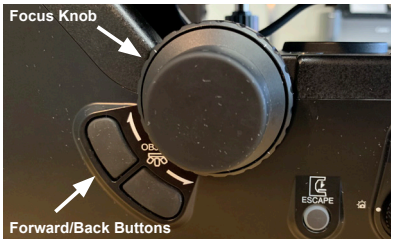




1. **Login to the computer**
   On the keyboard press control/alt and delete. In the window **Sign in with your SUNET Id** and sunet id password (*not your hospital id*, this computer is on the University network) If this is your first time logging in than it will set up a new profile for you. ***Please note: This computer is not a storage device, you should take your images and files with you or upload them to BOX as space is limited and the hard drive is cleared as it begins reaching capacity. You may also use an encrypted flash drive to back up your files as needed (this is recommended).***

2. **Turn on the microscope**
   Turn on the power (grey box to the left of scope). The green light will come on above the switch and the microscope will power up and go through automatic settings.

3. **Turn on the software**
   Click the SPOT icon on the desktop to activate the software. Click on the upper left “live view” button, this will give you a live preview of your slide.

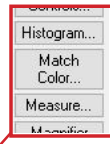
4. **Before you start**
   Adjust the oculars to match your eyesight. Each ocular can be adjusted for focus, in addition the ocular module can be widened or narrowed.

5. **Start out at a lower power**
   Start at a lower power to find your area of interest (aoi). To advance the objectives there are switches on the left and right side of the scope (see photo). NEVER advance the objectives by hand! One button will advance clockwise and the other button advances counter clockwise. ***WARNING: If on the 2x never advance backwards to the 60x power as it may break the slide.*** On the lower right bottom portion of the SPOT view window toggle to the correct objective setting, doing this will update the software to the hardware... ***IMPORTANT*** **DO THIS EVERY-TIME YOU SWITCH TO A DIFFERENT OBJECTIVE, if you do not your preview settings will not update to the new objective preview settings.** Use the fine focus knob to bring your slide into focus. Sometimes it is difficult to see that it is in focus at a lower power (2x), to help with this matter pay attention to the **“focal gauge”** to the right of the live preview as this can help you to bring the image into perfect focus. The blue and red lines should be touching as they climb up the side of the image (while you focus)...once it starts breaking up than you have gone past the “pinnacle” of the focus.

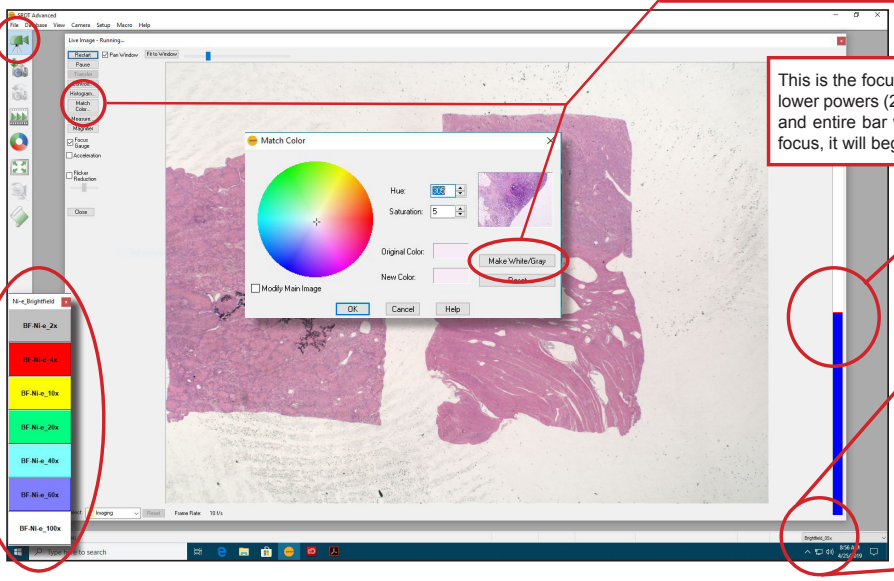
6. **Take the picture using the macro setting**
   A macro script within the SPOT software has been set up for each objective on this scope. Simply click the corresponding macro button and the script will run. The script will ask you where you would like to save your file...Best rule of thumb is to create a new folder for each session on your desktop or in the documents folder. When naming your file include the power you took your image at, for example: “non_hodgkins_pic1_40x”. Save as either a tiff or jpeg file to your folder. Take your photos with you on a flash drive or upload to BOX.

7. **Sign out when you are done with your session**
   Remember to close the application and sign out of the Windows session. To sign out of your session, go to the start menu, click on your name and “sign out”, never shut off the computer. If the computer is shut off please call me for the passphrase to boot up as the computer is an encrypted and secure Stanford University computer.

Click this icon to give you the live preview of your slide. The live preview automatically closes when you take the photo (so you can see the photo you took).



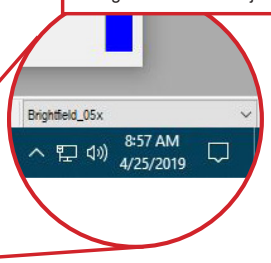

To white balance an image click on “Match Color”. A dropper tool will show up. Click the dropper tool on a white area and a box will pop up. Click “Make White/Gray” and press “OK”. The box should close and your image should now be white balanced.




Use the brightfield macro buttons to take your photos, each one corresponds to an objective on the microscope: 4x, 10x, 20x 40x, 60x, and 100x

This is the focus gauge...helpful when you are focusing at lower powers (2x and 4x). Red and blue bars should touch and entire bar will climb up the right side until you are in focus, it will begin to break up after the threshold.

Click here to update the software to the hardware as you advance through the different objectives.



# Trouble shooting

1. **The computer is off and I do not have the passphrase to logon:** This is a secure encrypted computer and you will need to call me at 415-676-1346 for the passphrase, leave a voicemail (as I will not pick up numbers I do not recognize)...I will immediately call you back with the passphrase.
2. **I am trying to login to the computer but it will not let me:** You need a SUNET id (not a hospital id) and SUNET password to login to the computer. If this is your first time logging in it will set up your own profile.
3. **There is no live preview:** You will need to click on the camera icon to give you a live preview. The live preview will turn off when you take the photo through the macro setting (it shuts off so you can have a full view of the picture you just took).

4. **I have changed the objective to a different power and now my preview does not look like what I am seeing through the lense:** You may need to update the software to the hardware. A good rule of thumb is to update the lower right hand toggle panel to the correct objective setting after you have physically changed the objective on the scope.
5. **I updated the software to the correct objective setting but the preview still does not look like what I am seeing through the scope:** You may need to click on the restart button on the live preview panel to the left, this will refresh the live view.
6. **I have done all the above but my image still looks "off color":** You may need to adjust the white balance. To adjust the white balance go to a clear portion of the slide and click on the white balance icon on the tool panel, your image should now look similar to what you see through the lense. *Do this procedure through out your session as needed, you may have to do this each time you load a new slide on the stage.*

7. **If you are are still having issues with the color:** Someone may have changed the default settings and not changed them back (Grrrrrr). Click on the "Control" button on the live preview panel...a box will pop up. Look through the settings and change back to the defaults I have provided below. You may need to reset the other control panels. If you need to reset, close the panel after you are done.