

Nikon TE300 (Inverted) Microscope

The TE300 inverted microscope can be used to collect either phase contrast or fluorescent images. In addition, it is capable of ratiometric imaging of FURA2 fluorescence. The following provides a standard set of instructions for using the microscope for both visual microscopy and digital imaging. Individuals wanting to use the microscope should obtain some instruction from the facility manager in the layout of the microscope and the software. Individuals interested in more advanced configurations should consult with the facility manager.

System startup

1. If using fluorescence, turn the mercury lamp power supply on (power supply labeled Mercury-100W). This MUST be done first to avoid electronic damage to the camera and computer.
2. Turn on halogen lamp power supply (power supply labeled Nikon)
3. Turn on Ludl MAC5000 filter wheel and shutter control box (red box on left of microscope). Do not adjust the smaller metal toggle switches on the face of the box.
4. Turn on the Hamamatsu camera power supply (located underneath the Ludl control box).
5. Turn on the computer.
6. Open the Micro Manager program. During program initialization, a startup configuration Window will open. Check to make sure that the configuration file labeled MMConfig_ham_ludl_focus2010 is selected; click "OK" (circled in red).
7. Mount sample on the specimen stage with the coverslip facing the objective lenses. The 40x, 60x, and 100x lenses require immersion oil.



Brightfield/Phase Contrast Microscopy and Image Acquisition

1. To view samples through the oculars, set the knob on the right side of the microscope base to position A (100% oculars).
2. Set the phase settings on the condenser to the appropriate position (Ph1 for the 10x and 20x objectives; ??? for the 40x objective; and Ph3 for the 60x and 100x objectives;). Turn on the halogen lamp by pushing the green square button on the left side of the microscope. Lamp intensity is controlled by the intensity knob next to the green switch. Focus the sample using the focusing knobs.

3. To capture an image on the camera, set the knob on the right side of the microscope base to position C (100% camera).

4. Adjust the halogen light intensity knob on the left side of the microscope base to the position marked with the camera icon (6V 30 W)

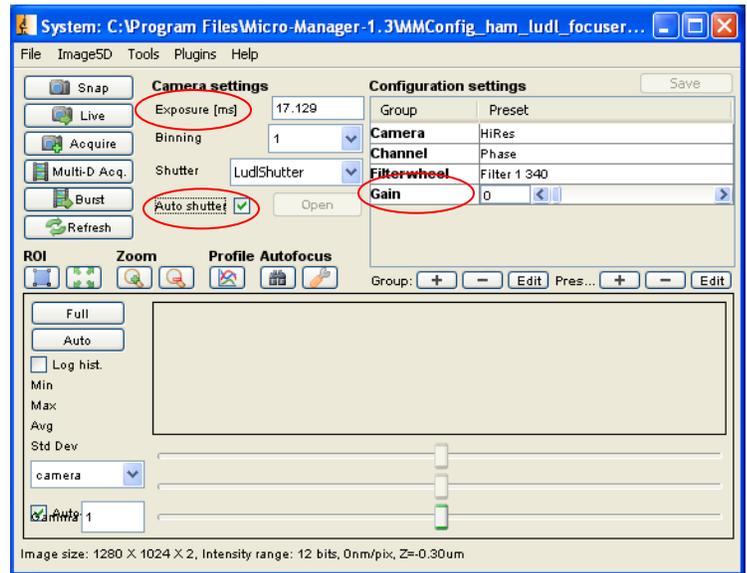
5. Unselect "Auto shutter" in the Micro-manager window on the computer screen.

6. Adjust camera exposure to 50 ms.

7. Select  to get an image on the screen. Image intensity can then be adjusted using either the intensity knob, exposure time or camera gain (see figure). For brightfield/phase microscopy it is unlikely that the camera gain will have to be increased. Focus and then select Live again to stop the image capture.

8. Select  to get a snap shot of the image.

9. The acquired image can be saved by selecting  on the lower left side of the image window.

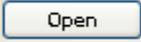
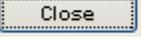


Fluorescence microscopy and image acquisition

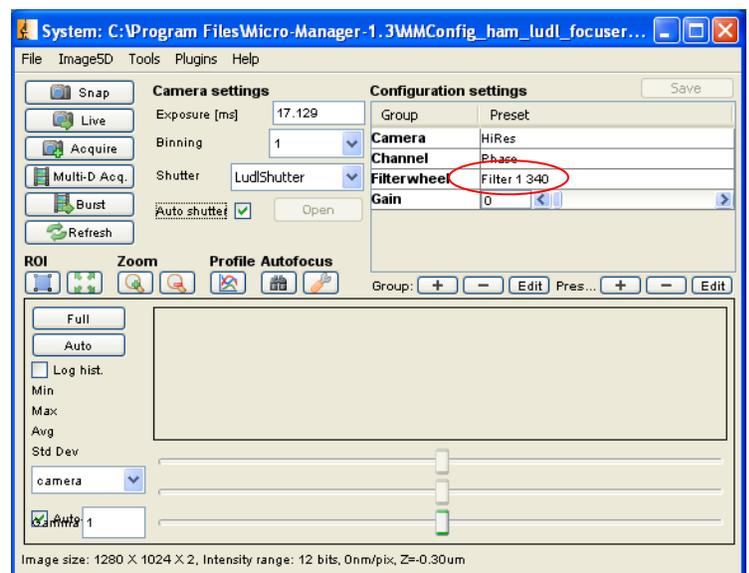
10. To view samples through the oculars, set the knob on the right side of the microscope base to position A (100% oculars).

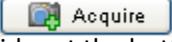
11. Select the appropriate excitation/emission filter for the fluorochrome being viewed using the filter slider on the right side of the microscope adjacent to the objective lens turret.

12. Set the filter wheel position in the Micro-manager Configuration settings to Filter 3 by selecting the drop down box (outlined in red).

13. Unselect "Automatic shutter" and click  to illuminate the sample. View samples through the oculars. Click  to turn off the Illuminating light.

14. To capture an image on the camera, set the knob on the right side of the microscope to C (100% camera).



15. Reselect “Automatic shutter” in the Micro-Manager program window. The Open button will become inactive allowing the software to control the mercury bulb shutter automatically.
16. Click  to focus the image on the computer screen and to adjust the camera exposure and gain settings. Select stop once you’re satisfied with the focus and camera settings. Seek assistance from the facility manager if you are uncertain about the camera settings.
17. Click  to capture your single image. If taking images of multiple probes; move the filter cube slider to the appropriate position for the next image, select  again to fine tune the camera settings and then select  to capture the second image. Both images will be in the same image window and the slider at the bottom of the image window will allow you to scroll between the images.
18. Save the image by selecting  at the bottom left of the image window. Once you name your file and select OK, a folder with that name is created and all of the individual images are saved as tif files in that folder.
19. For users wishing to do more advanced operations such as time series and more automated multiple color acquisitions you will use the Multi-Dimensional Acquisition feature by pressing . For further instruction on how to operate these functions please contact the facility manager.

System Shutdown

Close Micro-manager and shut down the equipment in the following order:

1. Computer
2. Camera
3. Ludl filter wheel and shutter box
4. Halogen lamp power supply
5. Mercury lamp power supply

IMPORTANT: TURN OFF MERCURY LAMP LAST