DIGITAL MICROARRAY INSTRUMENT

STANDARD OPERATING PRODCEDURE

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# Before you start

* Always wear gloves when using the instrument, even when using just the keyboard and mouse.
* Immediately report instrument malfunction, unexpected operation or other abnormalities by email to [derin@bu.edu](mailto:Derin@bu.edu).
* Help to keep the area clean by not storing samples on the instrument bench or shelves, putting away tools and disposing of waste before you leave.

# 1 – Startup

1. Fill out sign-in sheet: name, date, and objective(s).
2. Remove the dust cover and place it to the left of the instrument
3. Switch on the ASI stage with the switch on the back panel.
4. (Optional) Change the microscope objective:
   1. Fully retract stage: On the ASI stage controller, press HOME and wait for movement to complete.
   2. Remove the transparent plastic lid from the desired objective.
   3. Unscrew the objective from black plastic cap, but do not remove it. Place it on the table with the objective pointing upwards.
   4. Gently unscrew the objective in the microscope (to remove, screw the objective clockwise when looking from above). Always use two hands, alternating hands so it does not drop.
   5. Rotate the old objective upwards and hold in one hand. Pick up the new objective in your other hand, placing the old objective in its place.
   6. Gently screw the new objective into the microscope. Always use two hands. To avoid cross-threading, gently push the objective upwards into the threads and turn clockwise (when looking from above) until it is properly seated (i.e., oriented perfectly vertically). Then, turn the objective counter-clockwise. Do not tighten: the fit is overtight if it cannot be easily loosened with just two fingers.
5. Turn on the LED: turn the main knob completely clockwise. Ensure the switch is
6. Start Micro-manager on the computer:
   1. Log in to Windows as:

|  |  |
| --- | --- |
| **User** | ece-ultra-pc01\irisuser |
| **Password** | irisuser2 |

* 1. Close all programs and windows.
  2. Open *Micro-manager 2.0beta* using the desktop icon.
  3. In the startup menu, select the ‘defaultMM2’ configuration. It is saved at “D:\User\_Scratch\\_MM2 files\configs\defaultMM2.cfg”. Wait for the Micro-manager windows to appear.

# 2 – Loading a chip

Loading a dry chip

1. Fully retract stage: On the ASI stage controller, press HOME and wait for movement to complete.
2. Place your chip with the correct orientation on the stage.
3. Turn on the vacuum line to secure the chip.

# 3 – Data acquisition

1. Adjust the polarization optics to the ‘off’ position:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **L1** | **Q1** | **Q2** | **L2** | **Exposure time** |
| 0o | 60o | 351o | 0o | 10 ms |

1. Move the chip into focus:
   1. Open the Micro-Manager *Stage Control* window.
   2. Move the XY stage using the left-hand controls until the objective is above the region of interest.
   3. Observe the current stage position on the ASI stage controller. The correct focus position is at about -10 mm.
   4. Open the Live Preview window.
   5. Coarse adjustment: adjust the objective position using the right-hand controls on the Stage Control window, in increments of 1,000 microns or less.
   6. You may adjust the displayed brightness in the live preview window by adjusting the look up table maximum and minimum settings.
   7. Fine adjustment: adjust the objective position using the knob on the ASI stage controller. Do not move the objective too close to the sample.
2. Select the appropriate acquisition script from the following list:

|  |  |  |
| --- | --- | --- |
| **Script Name** | **Description** | **Use it for** |
| EndpointArea | Automatically acquire images and detect particles across a large rectangular region of interest using preconfigured settings | * Automated scanning of wet or dry chips |
| AcquireZStack | Acquire a z-stack of images at the current position with default or custom settings | * Save raw data from a single FOV * Performing measurements of particle properties |
| RealtimeGNRs | Acquire z-stacks and perform particle detection within a single region of interest, over time | * Dynamic detection of gold nanorods |
| RealtimeBioNPs | Acquire images of a single region of interest, over time. | * Dynamic detection of biological nanoparticles or spherical gold particles |

# 4 – Shutdown

1. Unload the chip and clean tubing.
2. Quit the Micro-manager application by clicking the Windows red X.
3. Fully retract stage: On the ASI stage controller, press HOME and wait for movement to complete.
4. Switch off the ASI stage controller with the switch on the back panel.
5. Turn off the LED: turn the main knob completely counter-clockwise, until it clicks.
6. Cover the instrument with the dust cover.
7. Turn off the camera:
   1. Open ***Point Grey Flycap 2*** using the desktop icon.
   2. Click ***Configure Selected*** and uncheck the ***Power*** checkbox.
8. Immediately copy acquisition results to your USB drive or another computer.

# 5 – Data analysis

Count particles in spots

1. Open ***FIJI***
2. Run the ***SPANDEX Particle Counter***plugin.
3. Follow the on-screen instructions.

Analyzing images from AcquireZStack

You can use the *Spandex Stack* ImageJ plugin to measure the properties of nanoparticles in a image stack.

1. Open ImageJ using the link on the desktop
2. Open the image stack in ImageJ.
3. Run the *Spandex Stack* plugin