Takara Bio USA, Inc.

# ICELL8® Imaging System User Manual

Cat. No. 640000
(071919)

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# I. Preface

## About this Manual

This manual provides instructions for safe and successful operation of the imaging system included with the **ICELL8 Single-Cell System** (Cat. No. 640000). Please be sure to review the information in this manual thoroughly before using the equipment.

For detailed information about performing image analysis following image acquisition, please refer to the CellSelect® Software User Manual.

**Symbols and conventions**

The following symbols and conventions are used throughout this manual.

| Symbol | Description |
|---|---|
|  | **WARNING:** Indicates a potentially hazardous situation that could result in injury to the user or damage to or destruction of the system. |
|  | **CAUTION:** Indicates a hazard that could result in loss of data or damage to the system. |
|  | Indicates the presence of an electrical shock hazard and to proceed with caution. This symbol may appear next to either a **WARNING** or a **CAUTION**. |
|  | Indicates the presence of a biological hazard and to proceed with caution. This symbol may appear next to either a **WARNING** or a **CAUTION**. |
| **NOTE:** | **NOTE:** Provides helpful ancillary information to support the use of the system. |

## Safety Information

Consult user manuals for corresponding ICELL8 Single-Cell System components for specific safety information.

**Operating conditions:**

- Operate the imaging system inside an appropriate building. Do not operate the imaging system outside or in wet environments.
- Block any direct, high-intensity light such as direct sunlight, which may affect the uniformity of the collected images.

**Instrument use:**

**WARNING:** Use of equipment and reagents for cell preparation and isolation with the ICELL8 Single-Cell System may cause exposure to toxic or biohazardous chemicals, thereby presenting a hazard. Always wear appropriate personal protective equipment (PPE), which should at minimum include gloves, eye protection, and a lab coat, when handling equipment and reagents and operating the imaging system.

**CAUTION:** Do not position the equipment so that it is difficult to operate the power switches or remove the power cords.

**WARNING:** Use only the power cords provided by the manufacturers. Do not replace the power cords with inadequately rated cords.

**Moving and lifting the system:**

**WARNING:** If you need to move system components after the system has been installed, ensure that all components are off. Use proper lifting techniques and appropriate moving equipment.

**Warning labels on the instrument:**

Note and heed all warning labels on the system components.

# II. Introduction

## Welcome to the ICELL8 Imaging System

The ICELL8 Imaging System consists of a precision microscope, camera, high-intensity light source, automated stage, and color filter control for scanning and acquiring images of live single-cell specimens dispensed into an ICELL8 chip. The Micro-Manager software takes 288 images through DAPI and Texas Red filters from 144 fields of view (6 x 6 wells in each field of view) in approximately 7 minutes. The cell specimens can be quickly returned to cold storage while the images are analyzed.

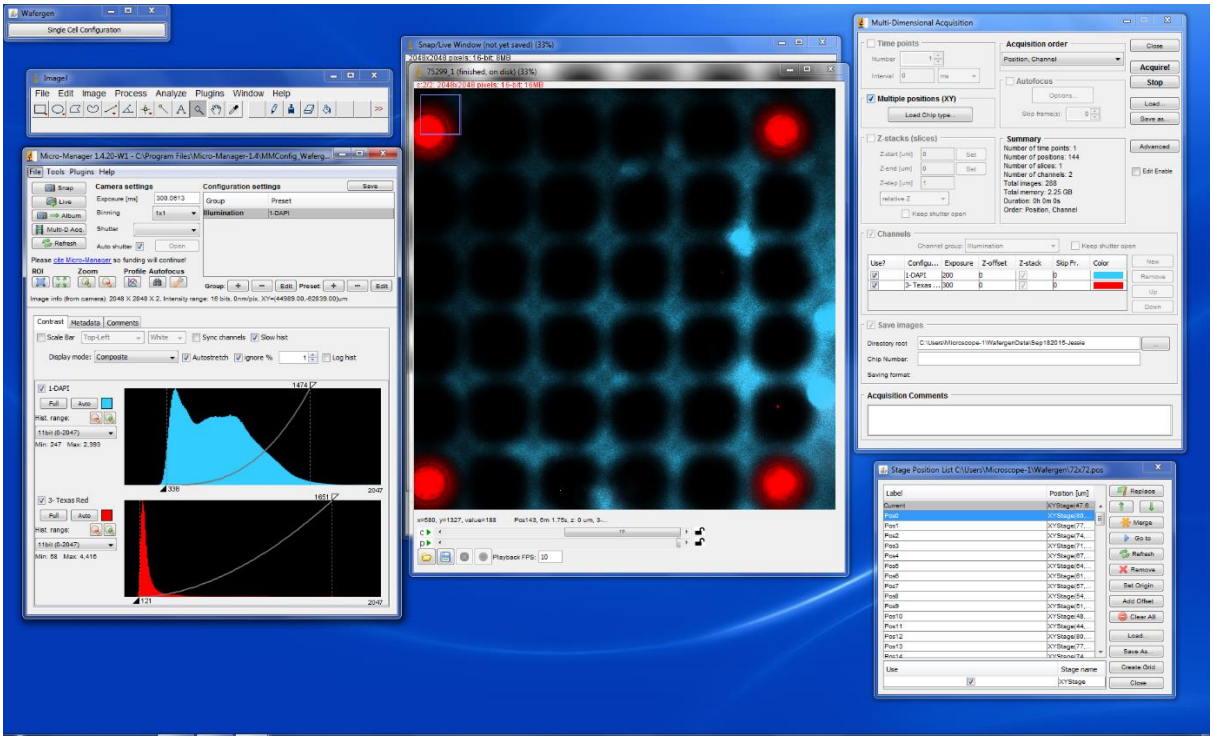


**Figure 1. Software interface for cell imaging.**

## Workflow Diagram

The imaging system described in this user manual is provided as a component of the ICELL8 Single-Cell System. For detailed information about other ICELL8 components, as well as compatible kits and reagents, please refer to corresponding documents. The schematic below depicts the role of the imaging system within a typical workflow for single-cell analysis.

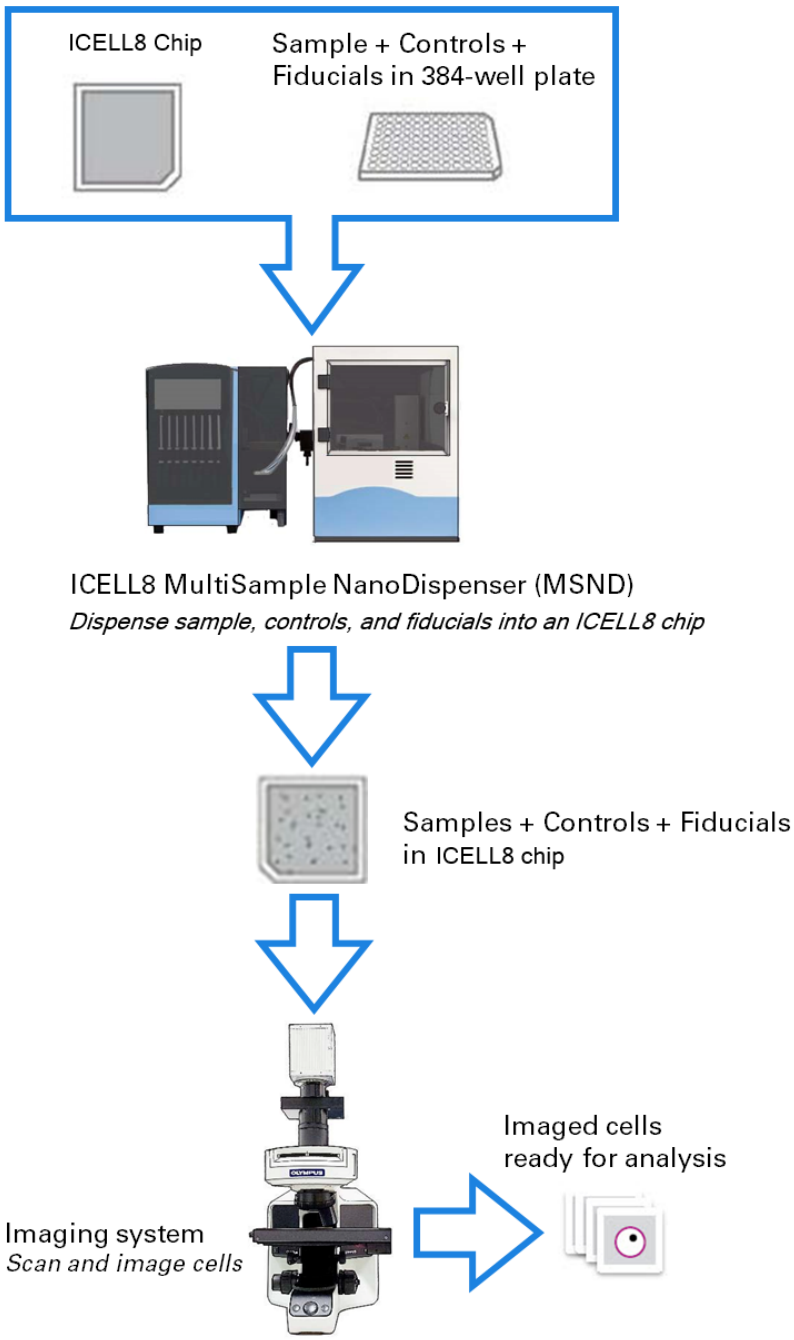



**Figure 2. Workflow diagram depicting single-cell isolation and imaging steps.**

## System Description

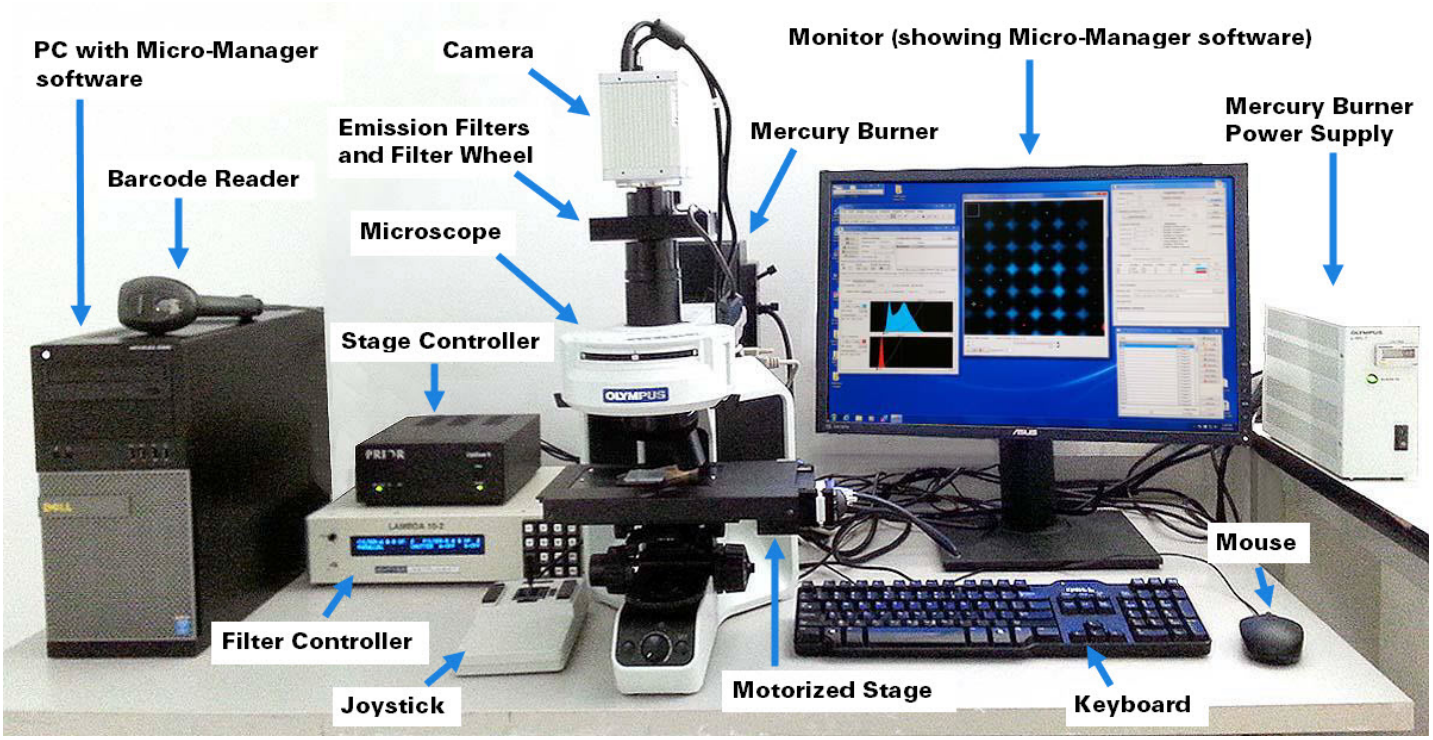



Figure 3. ICELL8 Imaging System components.

**Micro-Manager software:** Micro-Manager is open source software, created by the Vale Laboratory at UCSF and adapted for use with the ICELL8 Imaging System. Micro-Manager software controls the motorized stage, optical filters, and camera. It also acquires and manages microscope images.

**Camera:** The CMOS digital camera is mounted over the microscope and creates 4-megapixel images at 30 frames/second.

**Barcode Reader:** The hand-held Barcode Reader is connected to the computer and is used to input chip ID information by reading the barcode on the back of the ICELL8 chip.

**Motorized Stage, Stage Controller, and Joystick:** These components function together to facilitate both manual and automated stage movement. Automated stage movement occurs in synchronization with the Micro-Manager software.

**Microscope:** As the core component of the imaging system, the microscope provides 4X magnification and fine-tune focusing, and is specifically optimized for the ICELL8 system.

**Filter Controller, Emission Filters, and Filter Wheel:** The Lambda Filter Controller automates filter changing during scanning and image acquisition. The Emission Filters are housed in the Filter Wheel, which is mounted below the Camera.

**Mercury Burner and Power Supply:** The Mercury Burner provides high-intensity light to view and acquire images of sample cells.

# III. Startup

## A. Starting the Hardware

1. Turn on the Mercury Burner Power Supply (Figure 4, below) and allow it to warm up for at least 5 minutes. It will emit faint clicks before turning green. When the light is solid green, it is ready.

**NOTE:** If the light does not turn on and the faint clicks stop, turn the switch off and **wait at least 5 minutes** before turning it on again.

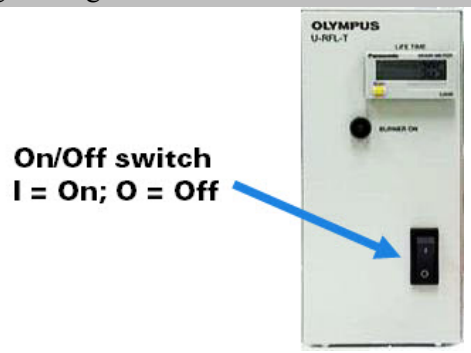



Figure 4. Starting the Mercury Burner.

**CAUTION:** The Mercury Burner is a high-intensity light source; handle carefully.

- After the burner is turned on, allow it to run for 30 minutes before turning it off.
- Wait for at least 30 minutes before turning on a previously turned-off burner.
- Do not turn the burner on and off more than 4 times in a day; it should be left on if you plan to use it frequently throughout the day.

2. Turn on the Camera and allow it to start up until the light is solid green. If you start the Micro-Manager software before the camera has completed the startup you will receive an error. If the error occurs, close Micro-Manager and restart the camera.

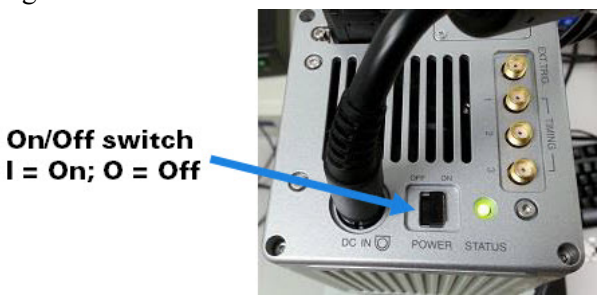
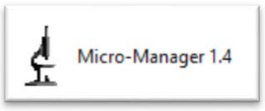



Figure 5. Starting the Camera.

3. Turn on both the Filter Controller and the Stage Controller.


Figure 6. Turning on the Filter Controller and Stage Controller.

## B. Starting the Software

1. Turn on the computer and launch the Micro-Manager software: 


**NOTE:** Wait until all hardware is on and ready before starting the software, otherwise the software will generate an error message.

2. Once the Micro-Manager *Copyright* window opens, click **OK** to continue to the *Main* window.

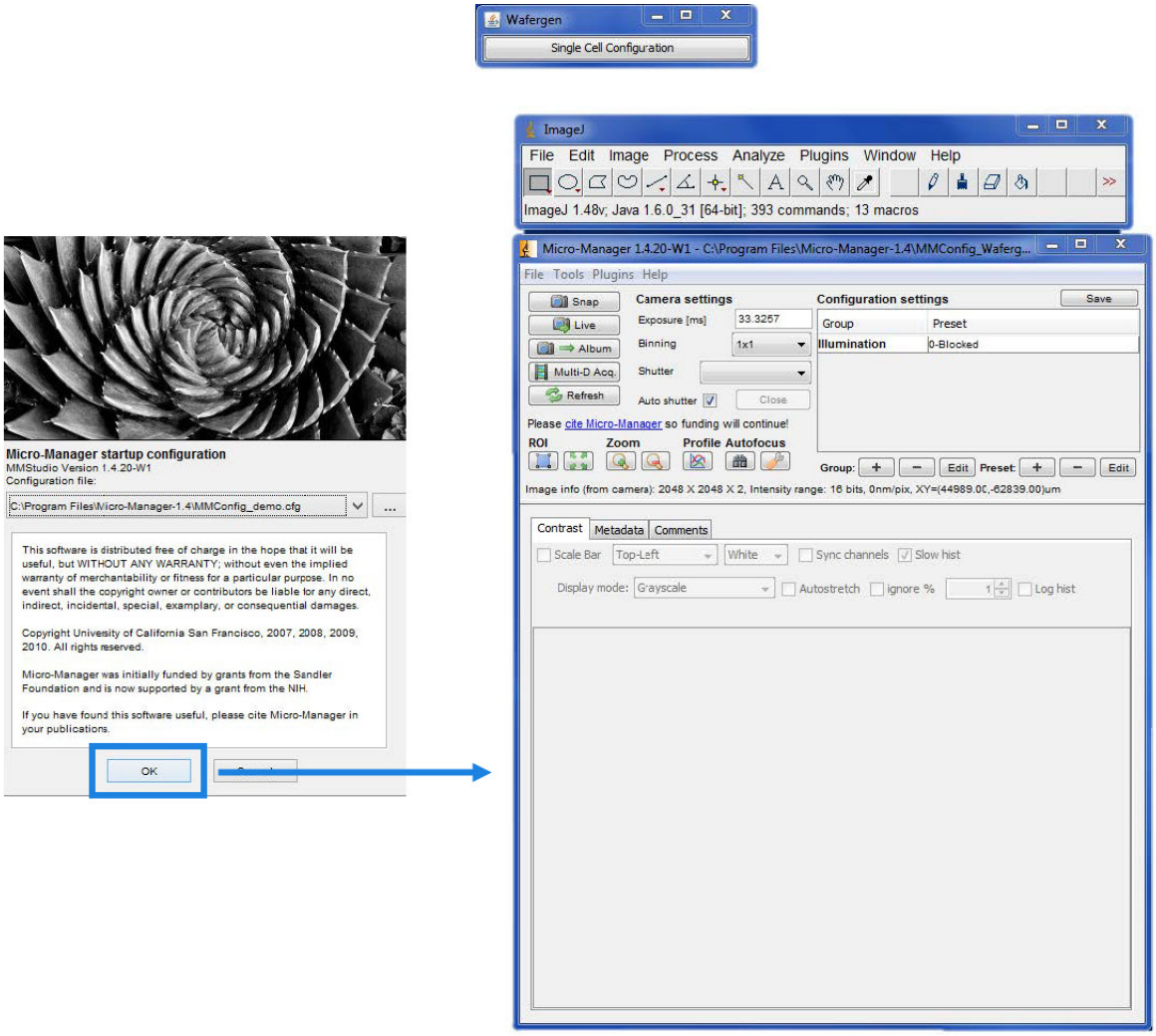



**Figure 7. Starting the Micro-Manager software: *Copyright* window and *Main* window.**

3. Ensure that the manual shutter is in the "Open" position.

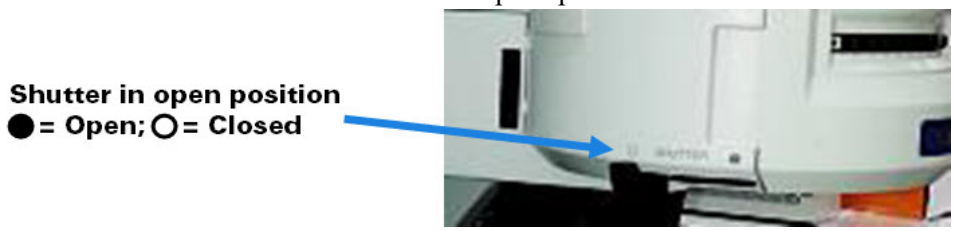



**Figure 8. Opening the Microscope shutter.**

# IV. Operation

## A. Setting Up the ICELL8 Chip

1. Click **Live** in the *Main* window. The *Live* window (right) opens and displays a continuous feed from the single ocular tube of the microscope.

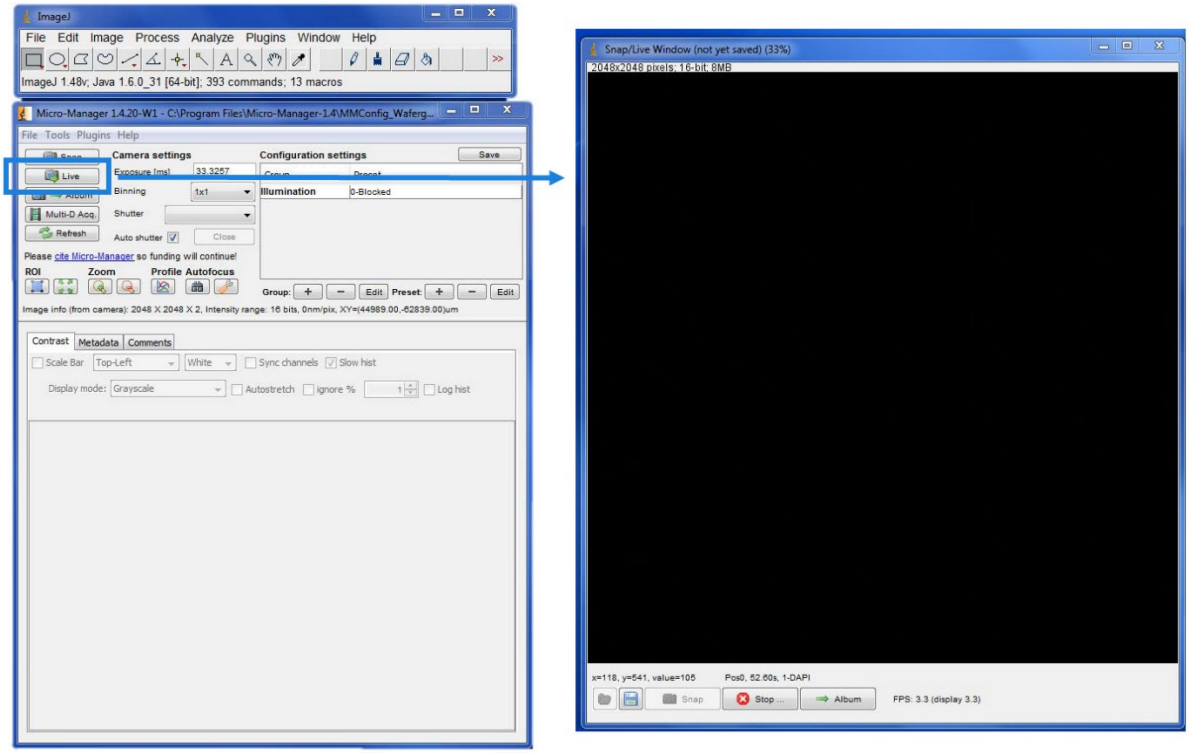



**Figure 9. *Main* window and *Live* window.**

2. Click **Multi-D Acq.** to open the *Multi-Dimensional Acquisition* window (right).

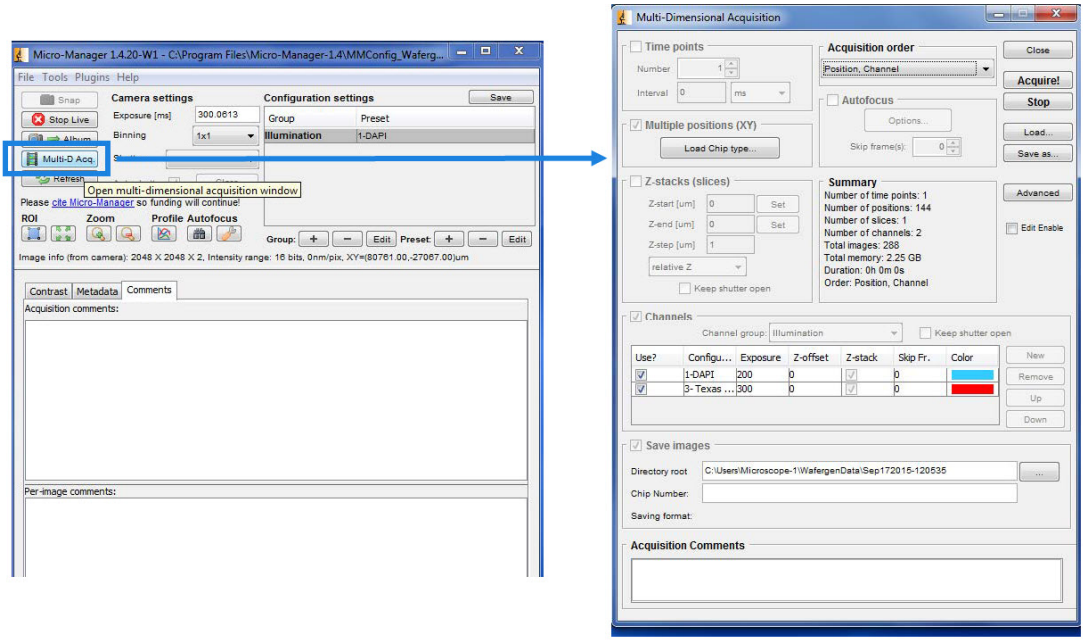



**Figure 10. *Main* window and *Multi-Dimensional Acquisition* window.** The *Multi-Dimensional Acquisition* window acquires stacks of multiple images using various parameters. For single-cell images of ICELL8 chip nanowells, the software uses the "Multiple positions (XY)" setting to acquire images of 6 x 6 well regions.

**NOTE:** The wells will appear blurry as you focus below the top of the well to find the cells near the bottom of the well.

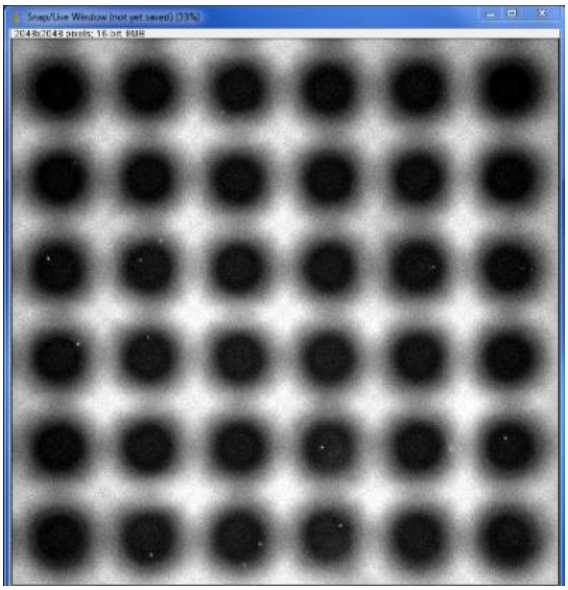


**Figure 11. *Live* window with image of 6 x 6 well region.**

3. Make sure that the **Multiple positions (XY)** box is checked (see Figure 12, below).
4. Click **Load Chip type** and load the configuration file `72.72.pos` (if it is not preloaded).
5. Position the cursor so that it resides in the **Chip Number** field (see arrow in Figure 12, below).
6. Input the chip number, or use the Barcode Reader to scan the two-dimensional barcode on the back of the chip.

**CAUTION:** Be sure to avert your eyes from the scanner light emitted by the Barcode Reader.

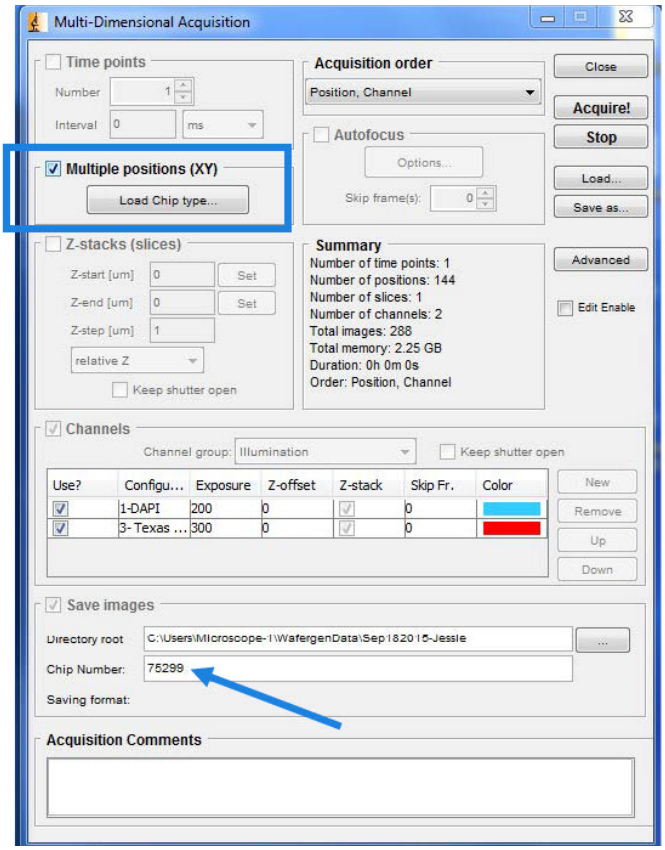



Figure 12. *Multi-Dimensional Acquisition* window.

7. Place the chip on the microscope Holding Platform with the chamfered (notched) corner facing the upper right corner (see Figure 13, below). **Ensure that the chip is sitting flat on the chuck from all sides and is in the correct orientation.**

**NOTE:** The ship should be centrifuged prior to imaging.

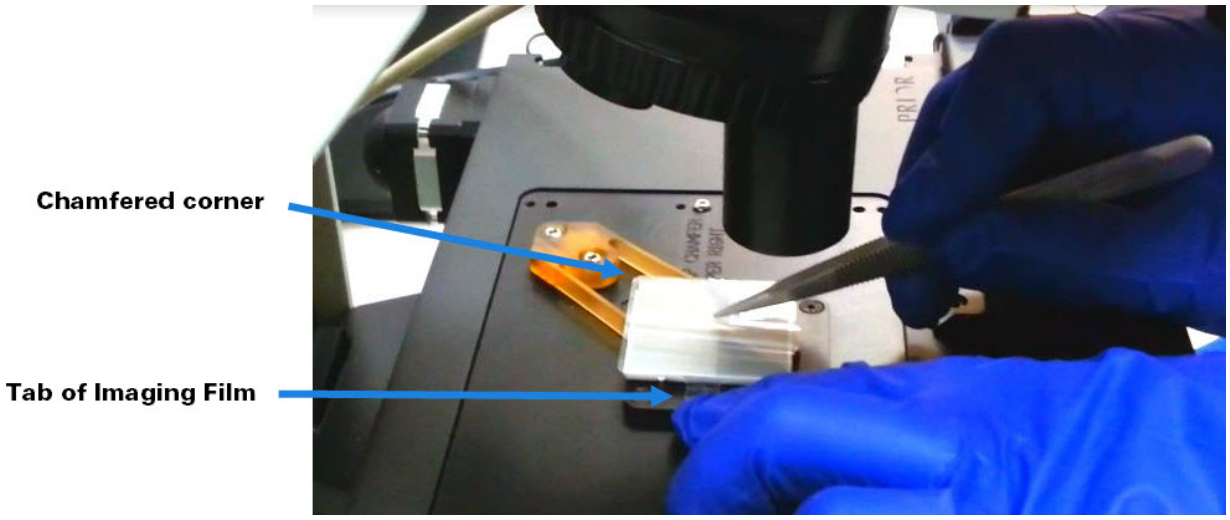



Figure 13. Seating a chip on the microscope holding platform.

8. Remove the second release liner. Hold down the tab of the Imaging Film while peeling off the top layer release liner of the Imaging Film using tweezers. Save the peeled release liner. The chip will remain sealed by the double-sided intermediate film layer.
9. Move the ocular tube over the chip (Figure 14, below). Ensure that the ocular tube is vertical and centered (you should hear/feel a slight click).


Figure 14. Positioning the ocular tube over the chip.

## B. Focusing on Cells

1. Select the row **Pos0** in the *Stage Position List* window (Figure 15, below), and then click **Go to**.

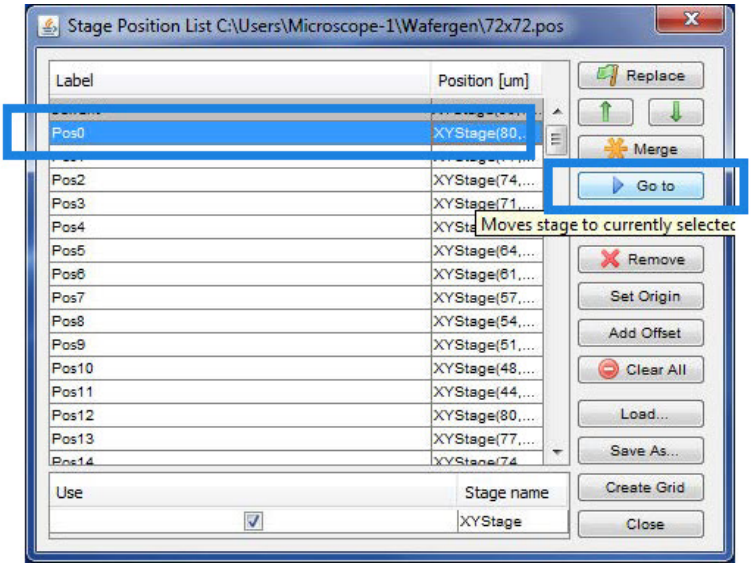



Figure 15. Moving the Motorized Stage to Position 0.

2. In the *Main* window, under "Configuration settings", select **DAPI** as the illumination channel for examination of Hoechst-stained cells.

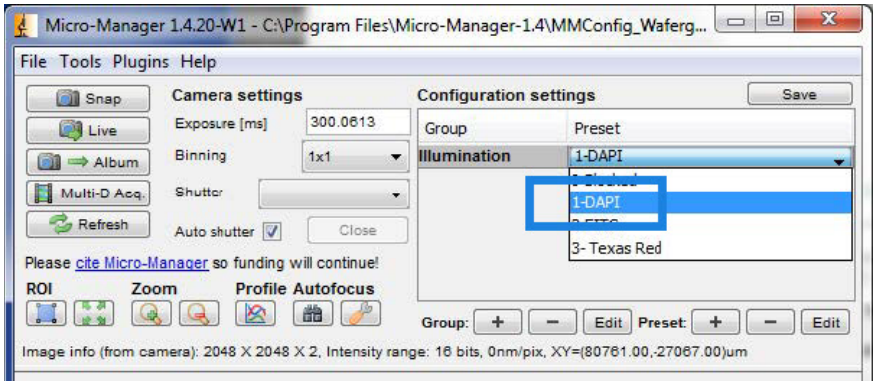



Figure 16. Setting the illumination channel to DAPI.

3. In the lower portion of the *Main* window, select the **Contrast** tab and select a value in the range of **12–14 bits** in the *Camera Depth* menu.

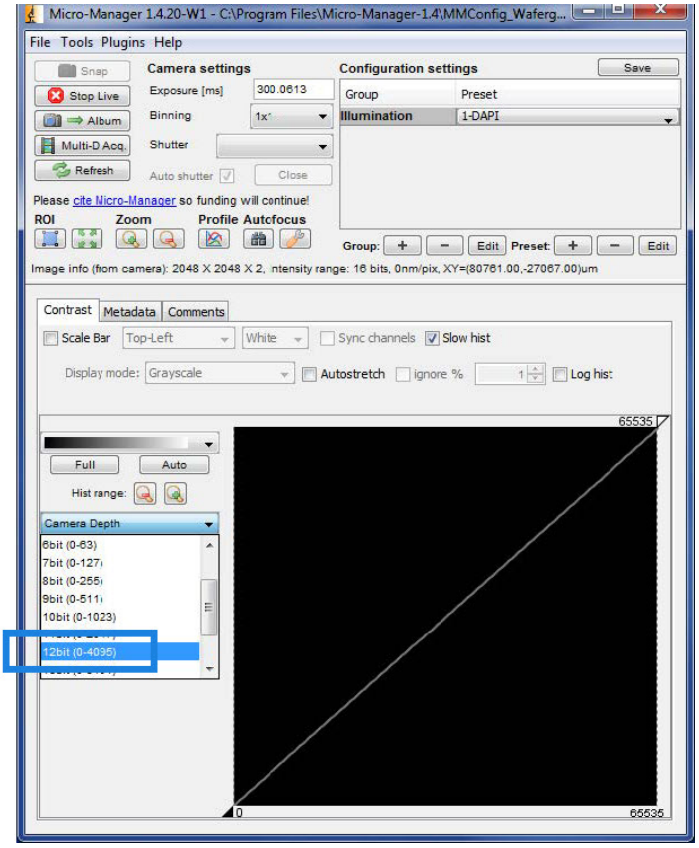



Figure 17. Adjusting camera depth.

4. Adjust the contrast of the chip image by selecting **Autostretch** and **Ignore** and a value in the range of **1–3%** for **Ignore** (see Figure 18, below). If this does not provide optimal contrast, drag the ends of the diagonal line (see arrows in Figure 18, below) closer to the histogram peaks and zoom out when satisfied with the focus in the Z-plane.

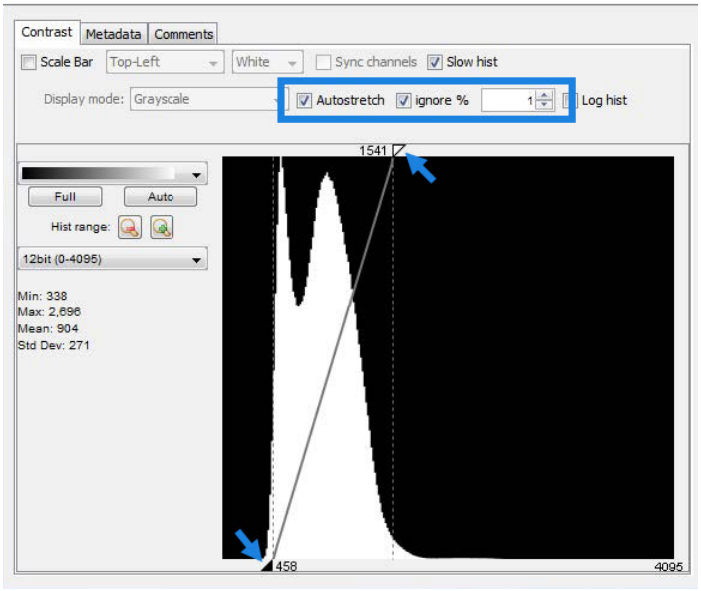



Figure 18. Adjusting contrast.

5. Use the microscope manual focus (under the stage on both sides; see Figure 19, below) to achieve the optimal Z-plane focal depth to visualize a field of single cells.

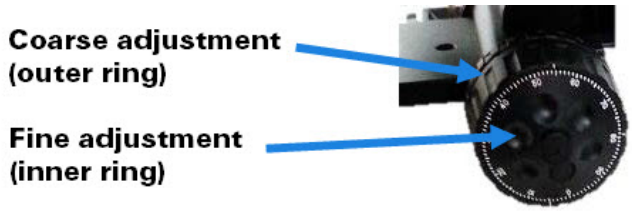
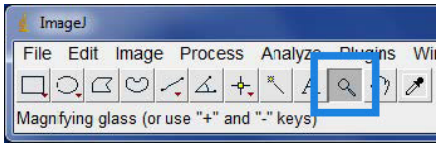



Figure 19. Adjusting microscope focus.

6. Use the zoom function in the tool bar (indicated by blue box in screenshot below) for close-ups. Once selected, use +/- keys or left/right mouse buttons to zoom in and out.




- Select a cell or couple of cells that are off center. Adjust the fine adjustment knob back and forth to find the focal point of a cell (smallest, sharpest view), or focal point between two cell depths (see Figure 20, below).

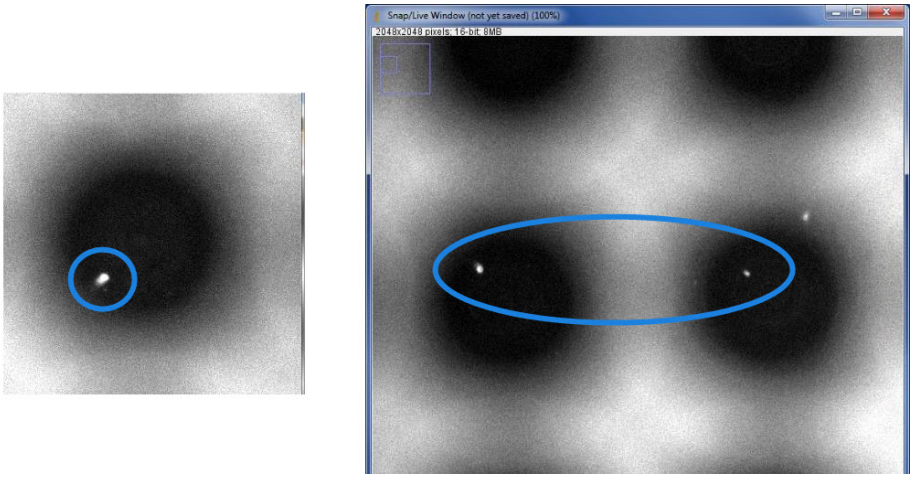


Figure 20. Finding a focal point for single cells.

- Select **Pos143** and click **Go to** (see Figure 21, below). Check the cells at Position 143 to ensure that most of the cells have good resolution.

**NOTE:** If the cells are truly out of focus, ensure that the chip is perfectly flat on the Dispense Platform.

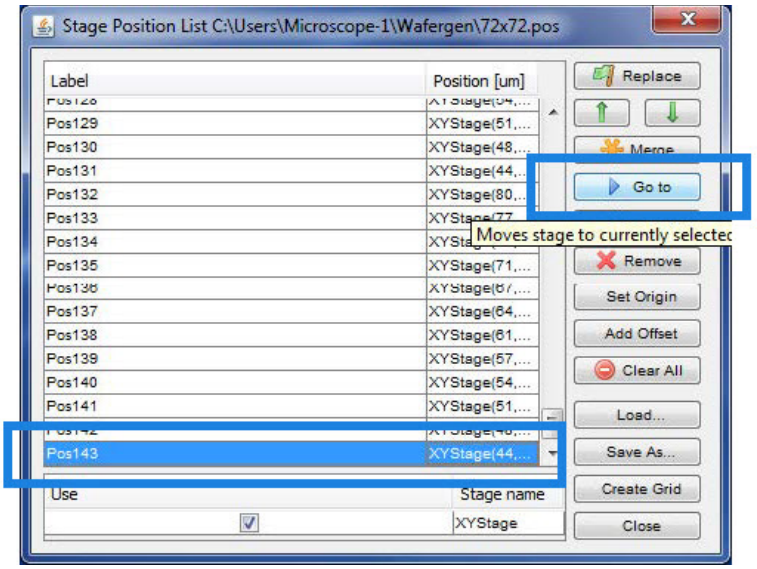



Figure 21. Moving the Motorized Stage to Position 143.

7. In the *Main* window, under "Configuration settings", select **Texas Red** as the illumination channel for examination of fiducial-containing wells.

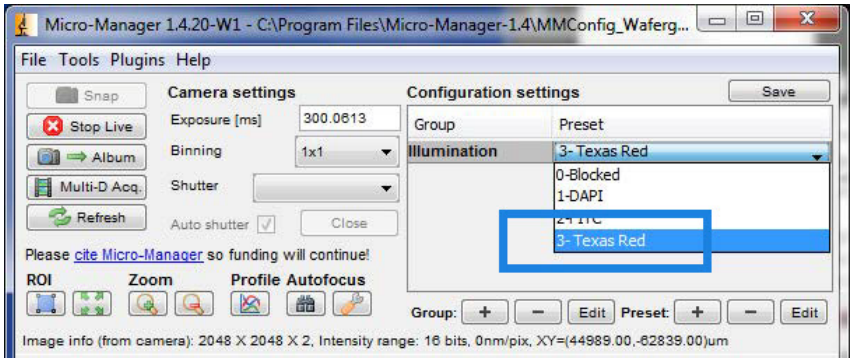



Figure 22. Setting the illumination channel to Texas Red.

8. Confirm that five fiducial-containing wells are present, forming an "X" shape at Pos0 (Figure 23, below). There are four fields of view (Pos0, Pos11, Pos132, Pos143) across an entire chip that include fiducial-containing nanowells. Each field of view includes 36 nanowells (a 6 x 6 grid).

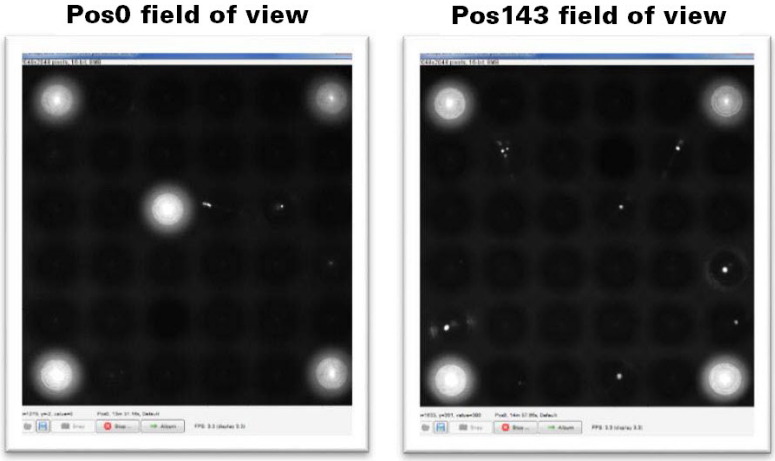



Figure 23. Brightly lit nanowells containing fiducials.

9. Select **Pos143** in the *Stage Position List* window and confirm that all 36 nanowells are properly captured in the image. There will be four fiducial-containing nanowells in the shape of a square, as shown in Figure 23 (above). Position 143 is located at the lower right corner of the stage fixture.
10. Select **DAPI** as the illumination channel for a second time.
11. Examine the field of view for Pos143 to confirm that you are satisfied with the focus. If not, check that the chip is sitting perfectly flat on the microscope's Chip Holder.
12. Close the *Stage Position List* window.
13. Under the Channels table of the *Multi-Dimensional Acquisition* window, ensure that the following stains are selected (preconfigured): **DAPI** (exposure = 200), **Texas Red** (exposure = 300). Texas Red has excitation and emission wavelengths similar to propidium iodide.

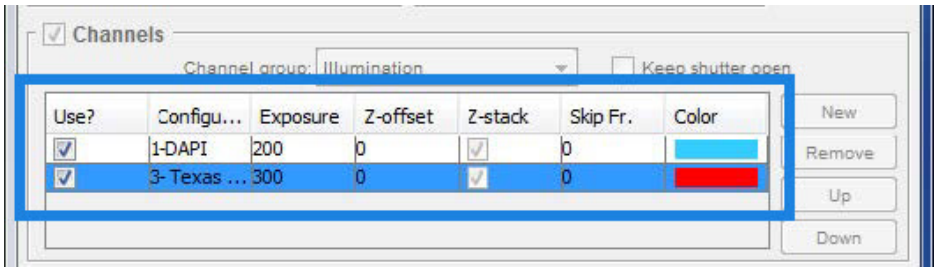



**Figure 24. Checking cell-stain channels.**

Filter colors are assigned to each channel for easy identification of the cells:

- **DAPI** (teal) indicates the presence of the cell.
- **Texas Red** (red) indicates cells with membrane leakage.

14. Enter a short experiment name after the file name prefix (`..\WafergenData\<experiment name>`) and the **Chip Number** (if you did not scan the chip earlier).

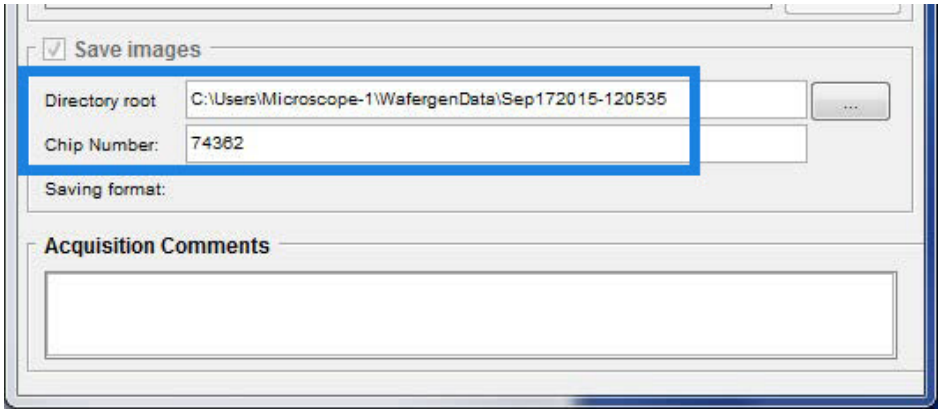



**Figure 25. Entering chip number and directory location for images.**

## C. Acquiring Images

1. Click **Aquire!** and accept the prompt to create a new folder based on the current date. The camera will take images of the fields of view with both the DAPI and Texas Red filters. The entire image folder should contain 288 TIFF files from 144 fields of view (Pos0–Pos143); each field of view has two sets of exposures, DAPI and Texas Red.

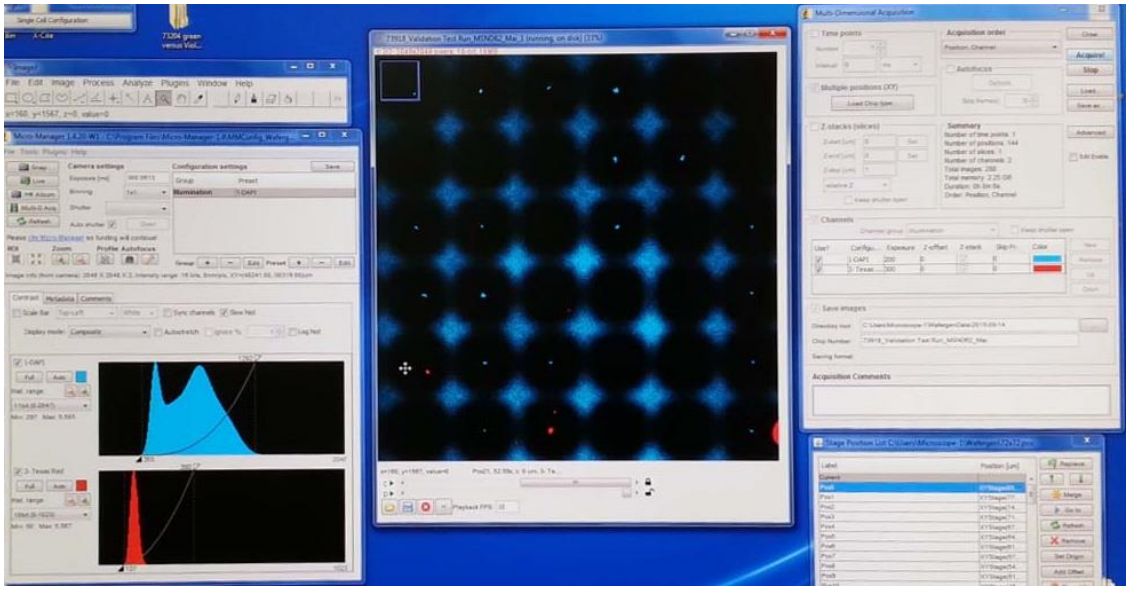


Figure 26. Acquisition of field of view images with DAPI and Texas Red filters.

2. After imaging is complete, the software will provide a prompt for converting the file. Click **Yes**. This will launch the CellSelect software. See the CellSelect Software User Manual for more information.

## D. System Shutdown and Next Steps

1. Turn off the Mercury Burner Power Supply, the Filter Controller, the Stage Controller, and the Camera (refer to Figures 4–6 in Section III.A, above, for the location of each unit's on/off switch).
2. Reapply the peeled release liner onto the top side of the double-sided intermediate film on the chip.
3. Place the imaged chip into an empty Chip Holder that has been prechilled at –80°C (Figure 27, below). Make sure that the imaging film is well sealed over the chip. The Chip Holder should click closed, indicating a proper magnetic seal.

Figure 27. Chip Holder.

4. Freeze the cells at –80°C for a minimum of 10 minutes before proceeding to the next step. Refer to the protocol or user manual specific to your application for more information.

# Appendix A: Maintenance and Troubleshooting

## Maintenance

**CAUTION:** Use of equipment and reagents for cell preparation and isolation with the ICELL8 Single-Cell System may cause exposure to toxic or biohazardous chemicals, thereby presenting a hazard. Always wear appropriate personal protective equipment (PPE), which should at minimum include gloves, eye protection, and a lab coat, before cleaning the ICELL8 Imaging System.

1. After removing the chip from the stage, inspect the stage for any debris.
2. Wipe the stage and sides of the microscope ocular tube with 70% isopropanol alcohol. Avoid touching the lens in the ocular tube.
3. Disinfect the bench or tabletop in accordance with your laboratory's cleaning procedures. Make sure that the overspray does not contact the lens in the microscope tube.

   **NOTE:** If the lens in the ocular tube requires cleaning, use recommended laboratory procedures for cleaning microscope lenses.
4. Refer to individual component user manuals for detailed information about maintenance and service procedures.

## Troubleshooting

**NOTE:** Refer to individual component user manuals for detailed information about maintenance and service procedures.

| Problem | Product |
|---|---|
| Software generates a hardware error at startup. | Shut down computer, camera, and controllers. Restart following the standard procedure. Ensure that the component is turned on and warmed up. Check cable connections to each hardware component. |
| Live window does not show an image. | Check mechanical shutter, it should be in the Open position (O). |

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