

Takara Bio USA, Inc.

# CellSelect® Software User Manual

Cat. No. 640000  
(071819)

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## I. Introduction

### Welcome to CellSelect Software

The ICELL8® Single-Cell System (Cat. No. 640000) has been engineered to dramatically increase the pace of biological discovery.

With unparalleled cell isolation, cell selection, and sample throughput, you now have control over your single-cell analyses and the ability to obtain the data you need to forward your research.

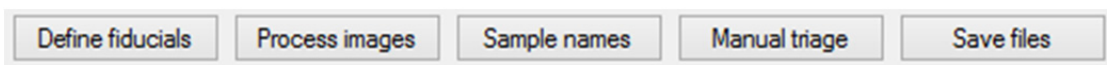
- **Power**—isolate thousands of cells of any size and process multiple chips per day.
- **Control**—choose which cells to process using image analysis software.
- **Insight**—process up to eight different samples per chip and leverage experimental flexibility for greater biological insight.

CellSelect software analyzes images generated with the ICELL8 Imaging System and provides researchers with the following capabilities:

- Automated or manual image analysis and selection of isolated cells for downstream processing
- Assessment of cell staining to determine viability (live/dead)

CellSelect software guides you through each step of the image analysis process:

- Prompts you to load images and settings into the software (directly from Micro-Manager or later from the Desktop)
- Provides functions in sequential order:



The diagram on the following page (Figure 1, below) summarizes the dispensing, imaging, and image analysis portions of the ICELL8 single-cell analysis workflow.

### Safety

Refer to safety guidelines in the user manuals for all equipment used in this protocol.



**WARNING:** Perform all experimental procedures in sterile environments with the proper personal protective equipment (PPE). Use designated UV hoods with proper ventilation for manipulating cells and setting up molecular biology reactions. Decontaminate gloves with nuclease decontamination solution, water, and ethanol. Change gloves routinely.



**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 instruments.



Note and heed all warning labels on the instruments used in this protocol.

## Workflow Diagram

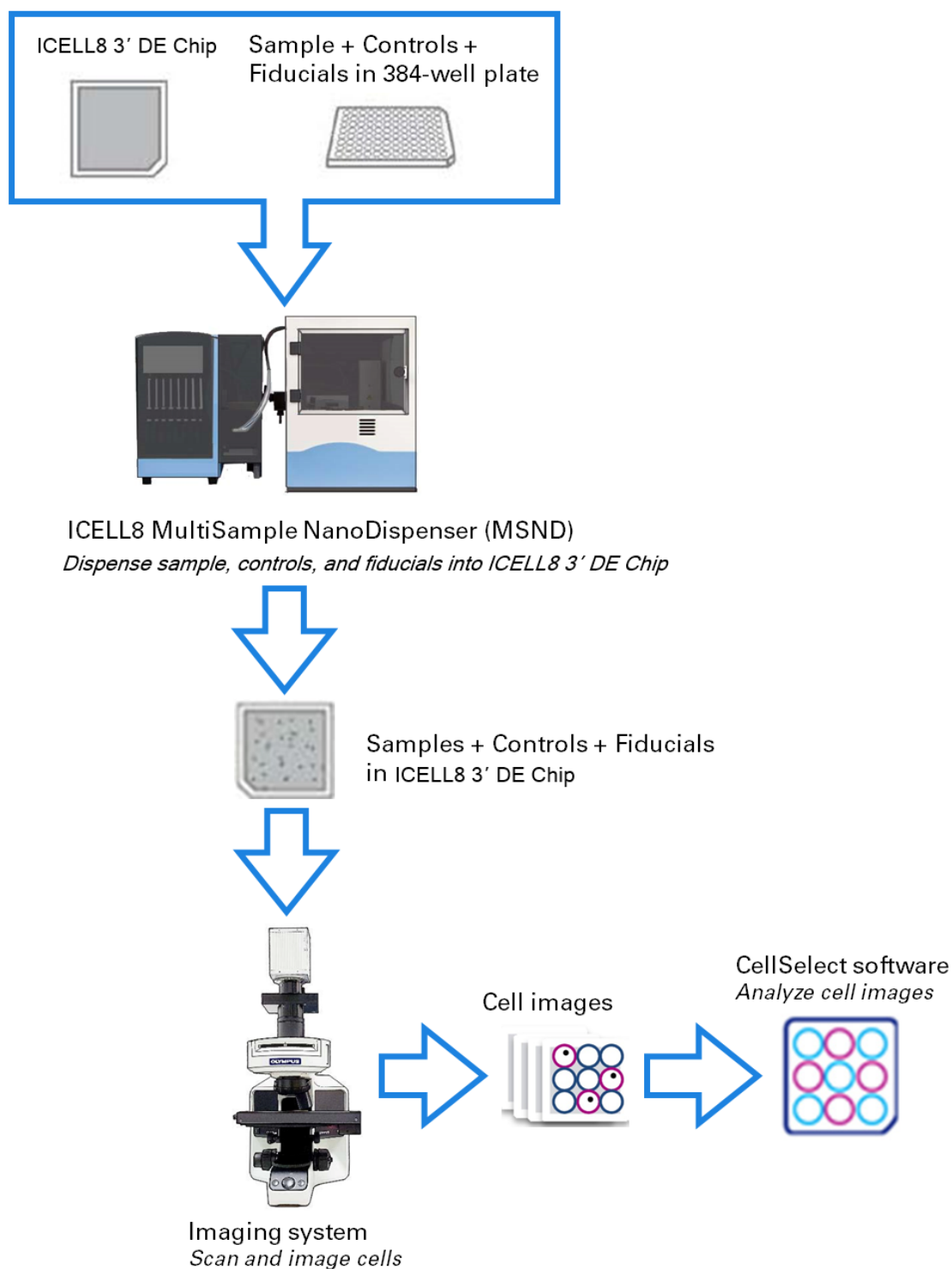


Figure 1. Workflow diagram depicting single-cell isolation, imaging, and image processing steps.

## II. Procedure

### A. Overview

In this procedure you will select nanowells using CellSelect software and then generate a filter file to be used on the MSND system for dispensing RT mix. The procedure consists of the following steps:

1. **Load Images:** Load images directly from Micro-Manager or from CellSelect software (**File** menu).
2. **Define Fiducials:** Check fiducials in the images against fiducial markers in the software.
3. **Load Barcode File:** Load the barcode file for analyzing 5,184 nanowells.
4. **Process Images:** Start image processing. The software analyzes every well image in 288 image files and determines the best candidates for future processing.
5. **Save Files:** Save details of all well analyses as well as the filter file for dispensing RT mix to selected candidate wells.

Each step, including manual nanowell analysis, is described in this procedure.

### B. Load Images

You can open the CellSelect software directly from the Micro-Manager software in the ICELL8 Imaging System, or save a file and open it later from the CellSelect software.

#### From the Micro-Manager Software

1. After imaging the cells with Micro-Manager software, click **Yes** when prompted with the question, “Run CellSelect with images from: D:\Wafergen\Wafergendata For Chip: <Chip ID>?”

**NOTE:** If you plan to analyze the images later, click **No** and close the Micro-Manager software. Proceed to the following section, From the CellSelect software, when you are ready to analyze the images.

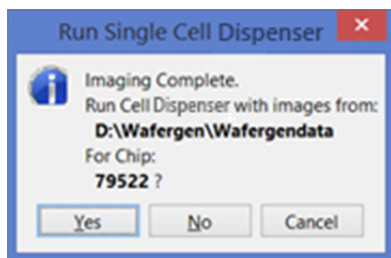


Figure 2. Converting the images.

2. Continue to Step 3 in the following section, From the CellSelect Software.

#### From the CellSelect Software

1. Launch the CellSelect software by clicking the **CellSelect** icon:



2. In the *Main* window, click the **File** tab and select **Open chip folder**.

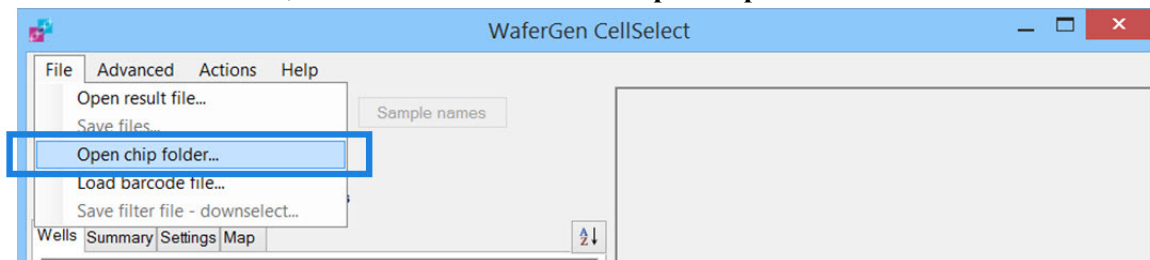


Figure 3. Opening the chip folder from the *Main* window.

3. Check the chip number when prompted for chip ID.

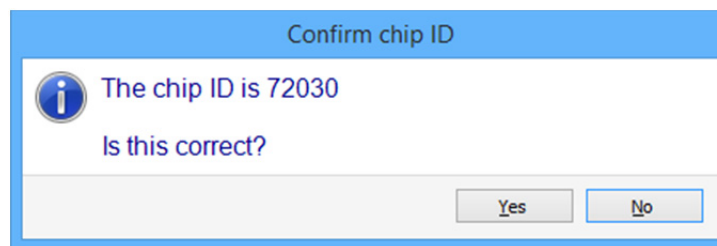


Figure 4. Entering the chip ID.

4. Add a description (if helpful) when prompted for a description.

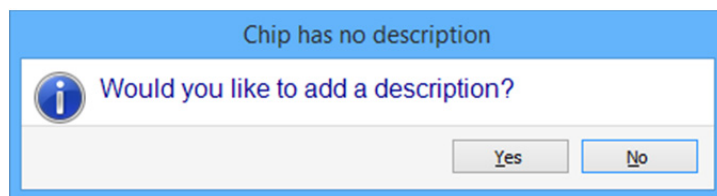


Figure 5. Adding a chip description.

5. Load the settings file, StandardCellSettings-V5.1.xml, shown below (Figure 6).

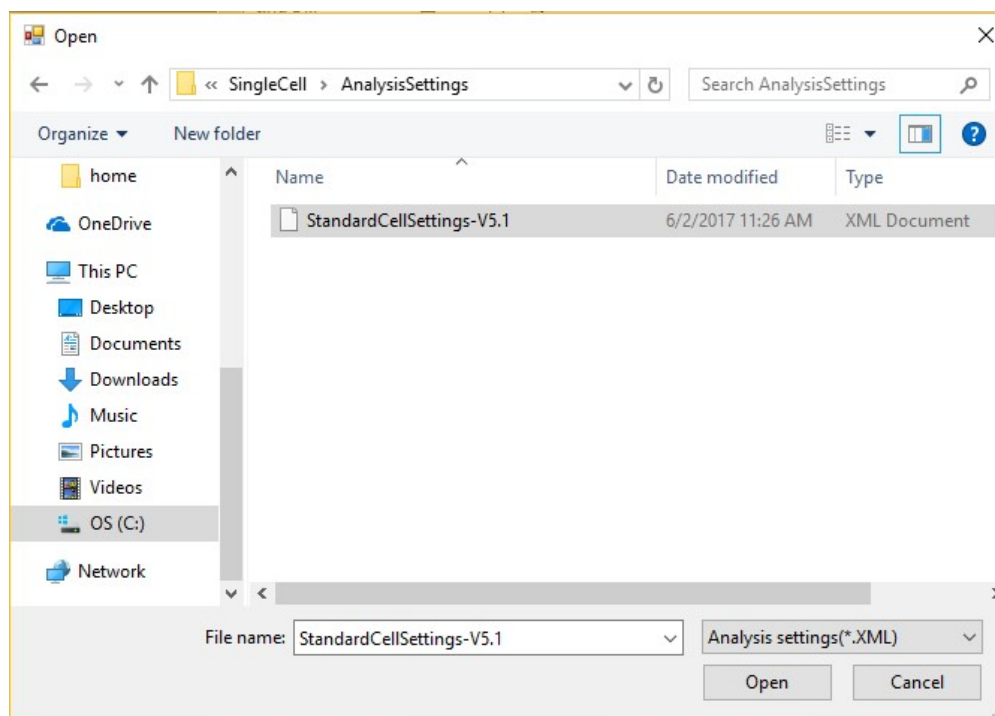


Figure 6. Loading settings.

## C. Define Fiducials

1. Click **Define fiducials**, the top-left button in the *Main* window. The software detects fiducials and prompts you with four consecutive fiducial images. The first fiducial image (Pos0) shows five green circles, while the other three fiducial images (Pos11, Pos132, Pos143) show four green circles. The green circles highlight the centers of the nanowells.

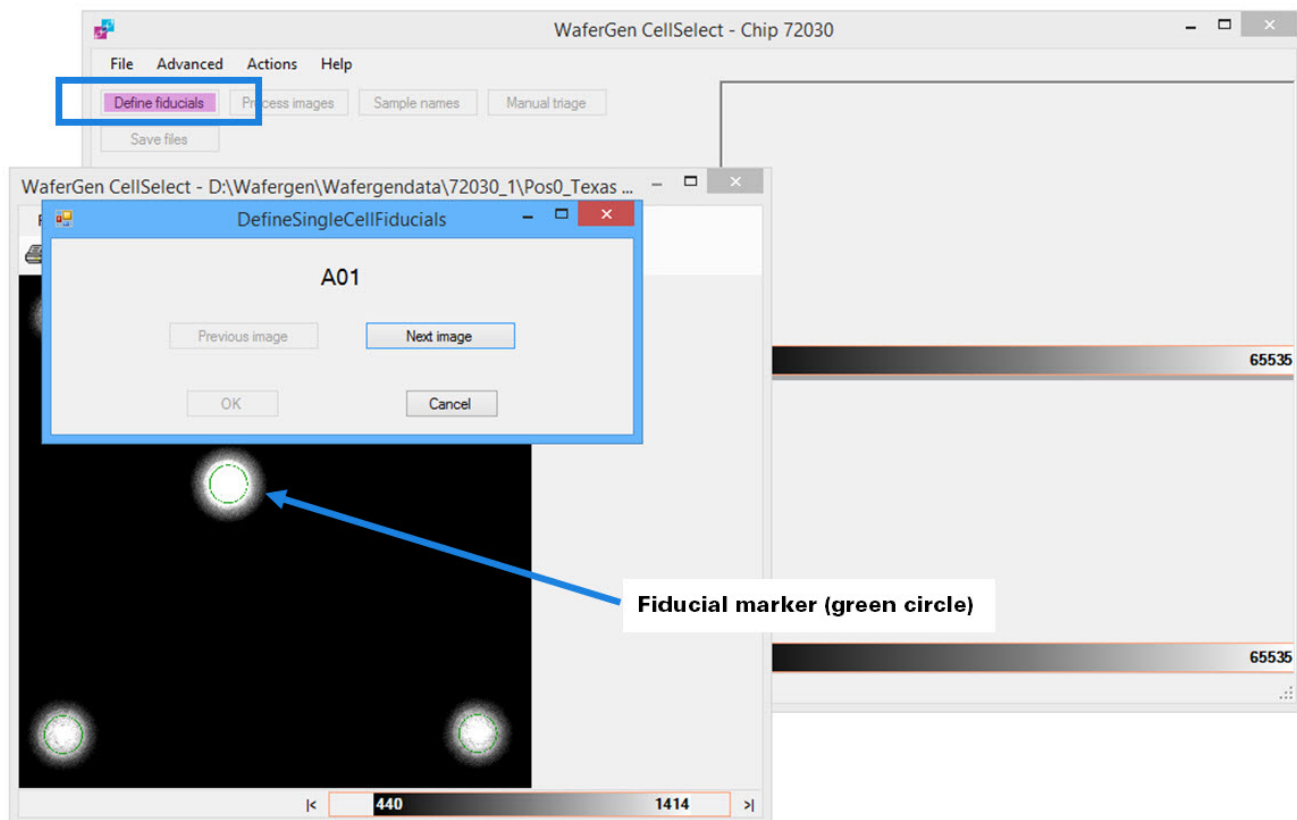


Figure 7. Defining fiducials.



2. Inspect the fiducials in each fiducial image for proper positioning.
  - a. Click and drag the *DefineSingleCellFiducials* prompt window to the side to view the fiducials image.

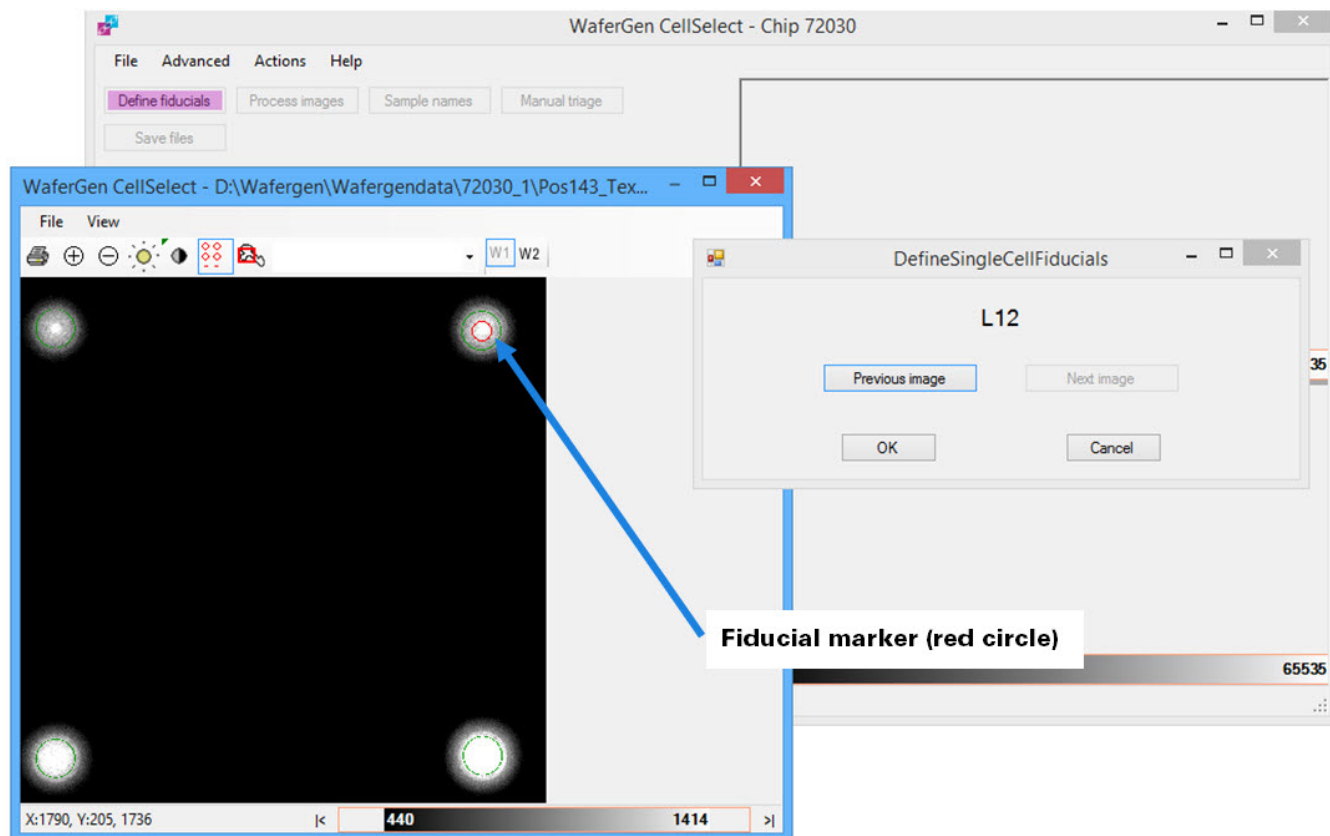


Figure 8. Inspecting and adjusting fiducials.

- b. If the green circles are not centered properly, click on the nanowell centers with the mouse pointer (changes to red circle; see Figure 8, above).
- c. If necessary, resize the fiducial image to view a 6 x 6 grid (36 nanowells). Use the following tools in the *Image Viewer* toolbar to check and correct fiducials: **Zoom in/Zoom out**, **Image contrast**, **Auto contrast**, **Well overlay**, **Wavelength 1 or 2 (W1, W2)**. Figure 9 (below) provides a legend for the *Image Viewer* toolbar icons.

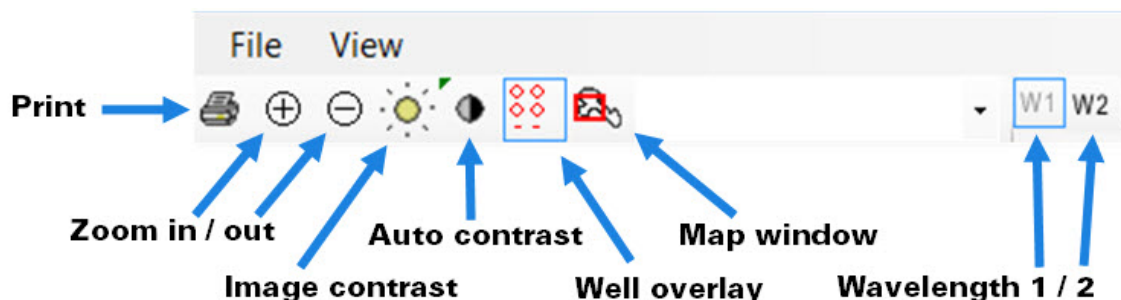


Figure 9. Image Viewer toolbar icons.

- d. Click through all four fiducial images and confirm that the chip was oriented correctly for dispensing samples. The image for the Pos0 field of view should show five fiducial-containing

nanowells, while the images for the Pos11, Pos132, and Pos143 fields of view should each show four fiducial-containing nanowells. If the layout of fiducial-containing nanowells is correct, click **OK** and close the last fiducial image. If the layout of fiducial-containing nanowells is incorrect, contact Technical Support before dispensing RT mix.

## D. Load Barcode File

1. In the *Main* window, click the **File** tab and select **Load barcode file**.

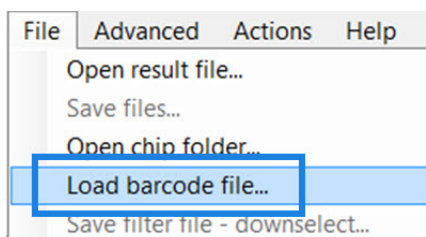


Figure 10. File menu.

2. Select the preloaded file `iCELL8_5184_v2_01232017.gal`. This GenePix Array List (GAL) file contains nanowell barcode sequences for each preprinted nanowell location.

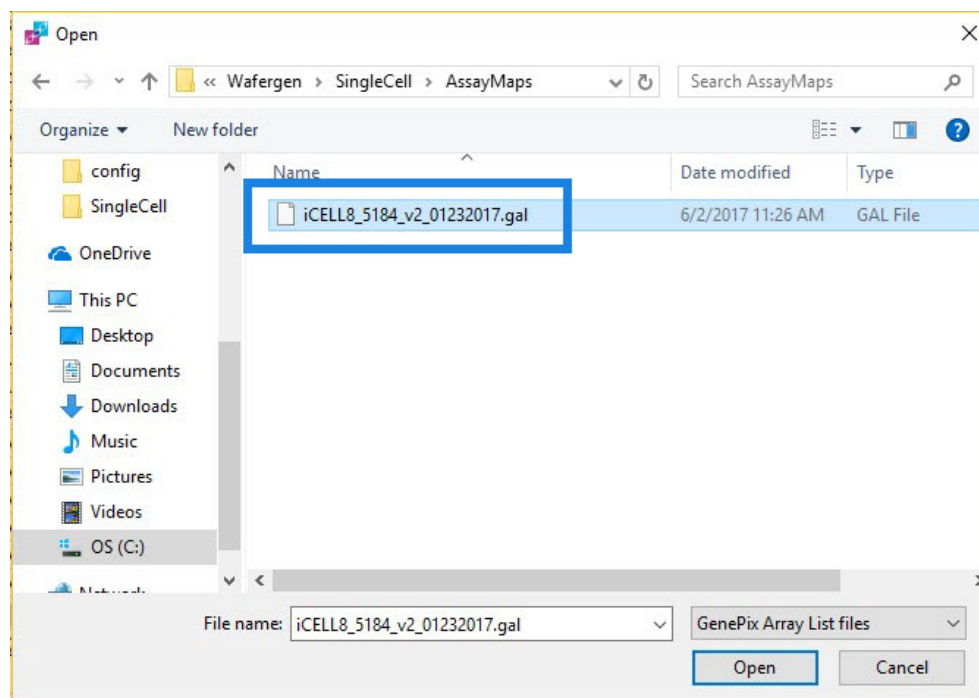


Figure 11. Loading the barcode file for the preprinted chip.

## E. Process Images

1. Click **Process images**. The software will analyze two sets of 144 multi-well images taken using DAPI and Texas Red filters, respectively, and automatically identify and select all nanowells that contain viable, single cells (i.e., “candidates”) and controls based on the parameters defined in *Settings*.

**NOTE:** For each image, the following criteria must be satisfied for the software to identify a cell as being a candidate for downstream analysis (please refer to the Appendix, below, for more details):

- The cell must appear in the DAPI channel and NOT in the Texas Red channel.
- No other cells are visible in the well.
- The cell must satisfy the size/shape parameters specified in *Settings*.

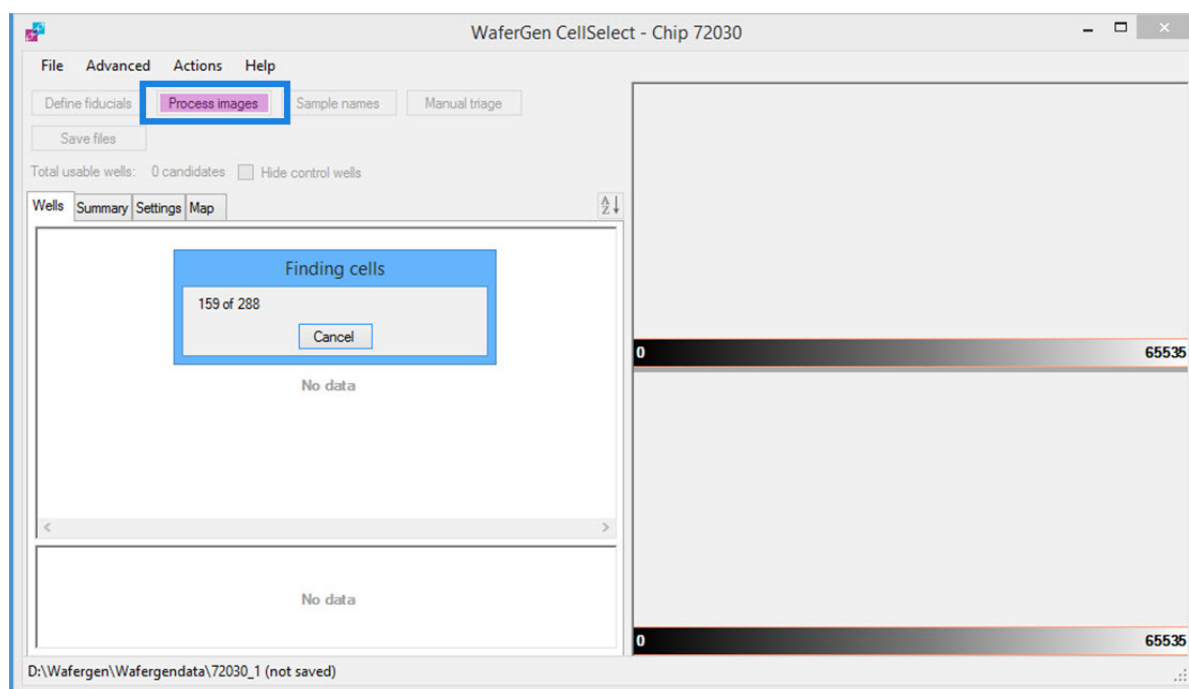


Figure 12. Having the software find cells based on parameters defined in *Settings*.

2. After processing is complete, the software will provide a prompt for saving results. Click **Yes** and input a file name prefixed with the chip number (e.g., <Chip ID>\_<Date>.wcd). The software will then do the following:
  - Select wells among all those that are checked under Candidate according to the best parameters defined in the *Settings* tab.
  - Save the result file under the specified name (e.g., 72030\_09282015.wcd). The result file will contain the selected nanowells and setting parameters.
  - Automatically generate the filter file for the dispensation of RT reagents (e.g., 72030\_FilterFile.csv). The selected wells in the filter file will be displayed in the *For Dispense* tab.

3. Each preprinted chip contains a total of 5,184 unique nanowell barcodes. You can load the results along with the settings from the saved WCD file. The software uses the following legend when examining nanowells:

- **Green** = a cell
- **Yellow** = ignored
- **Blue** = a reflection

**NOTE:** See Understanding the Software Color Code in Section II.G (below) for more information.

## F. Change Sample Names

- Click **Sample names** and enter a single sample name or use the source plate configuration layout for ICELL8 MSND dispensation.
  - The **One sample** option changes ALL names to the name you enter (the default name is “Sample”).
  - The **Multiple samples** option changes the default name (“Sample”) to grid names from the source plate—or you may edit the grid name (see Figure 13, below).

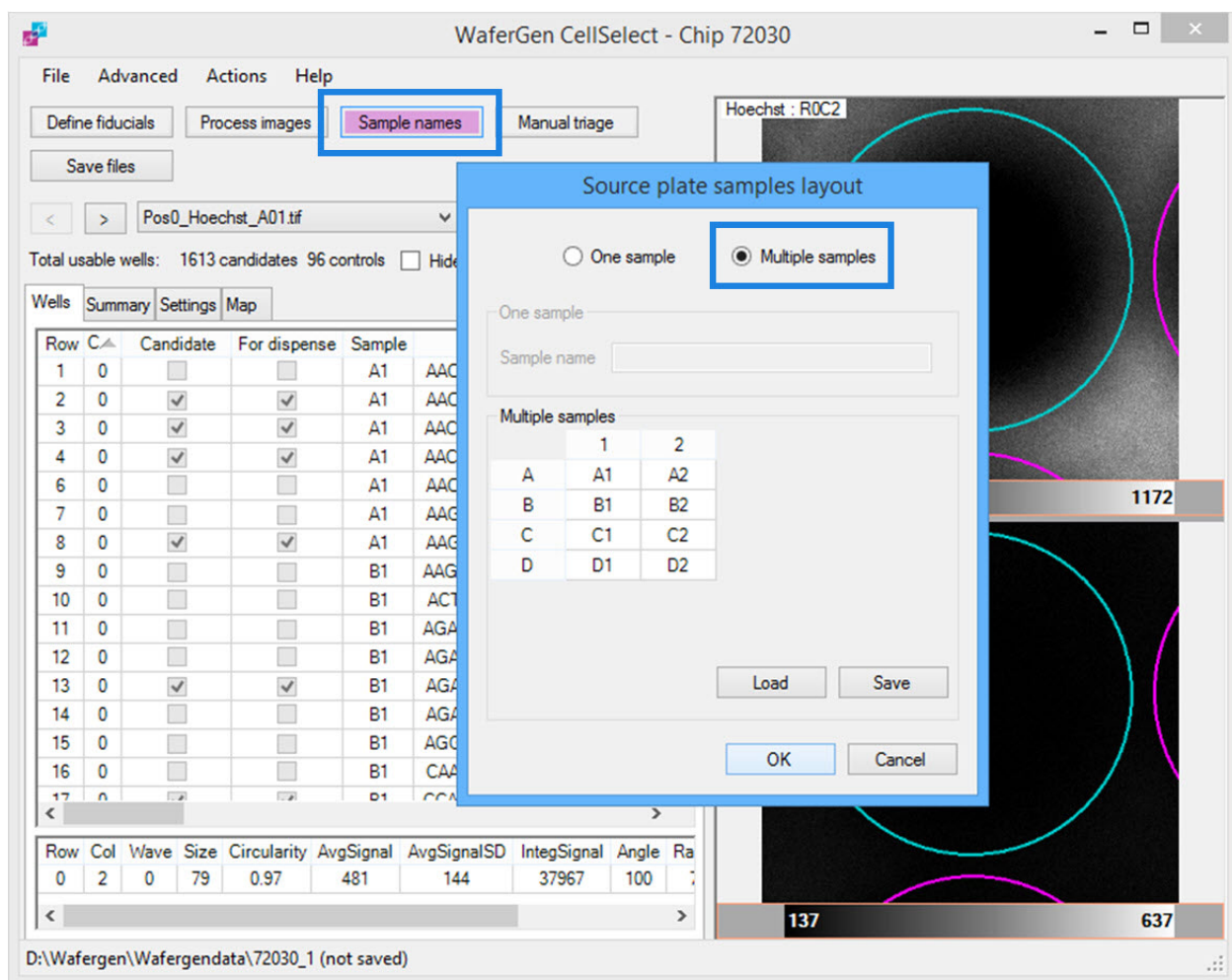


Figure 13. Inputting multiple sample names and automatically identifying control- and fiducial-containing wells.

## G. Review Images

### Consolidate All Candidate Wells to the Top

1. Click the **Wells** tab and sort the nanowell statistics in the Candidate column by clicking the **Candidate** heading (see Figure 14, below). The software will sort and consolidate all selected nanowells to the top of the table.

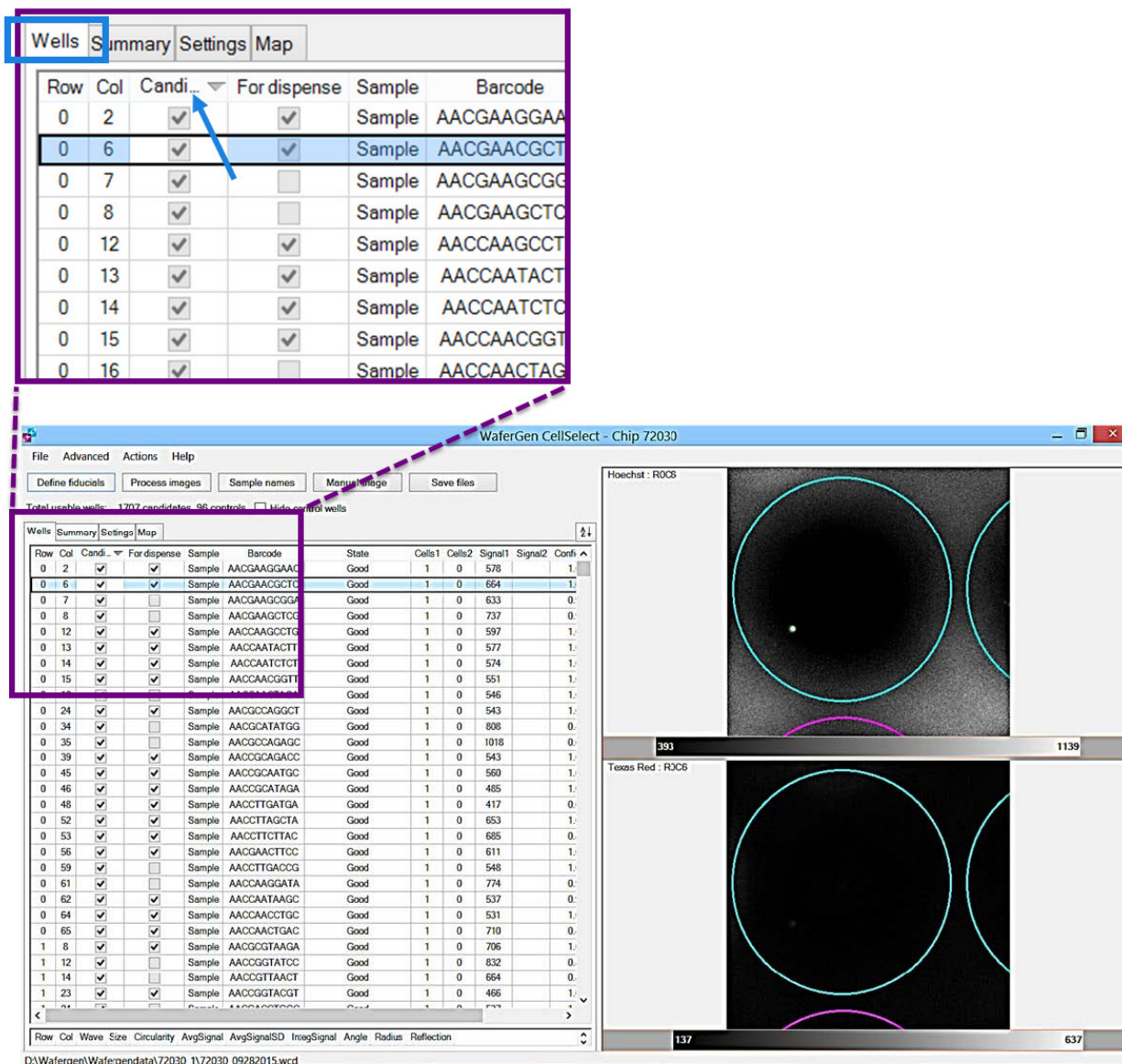


Figure 14. Sorting the Candidate column to consolidate all selected nanowells to the top of the table.

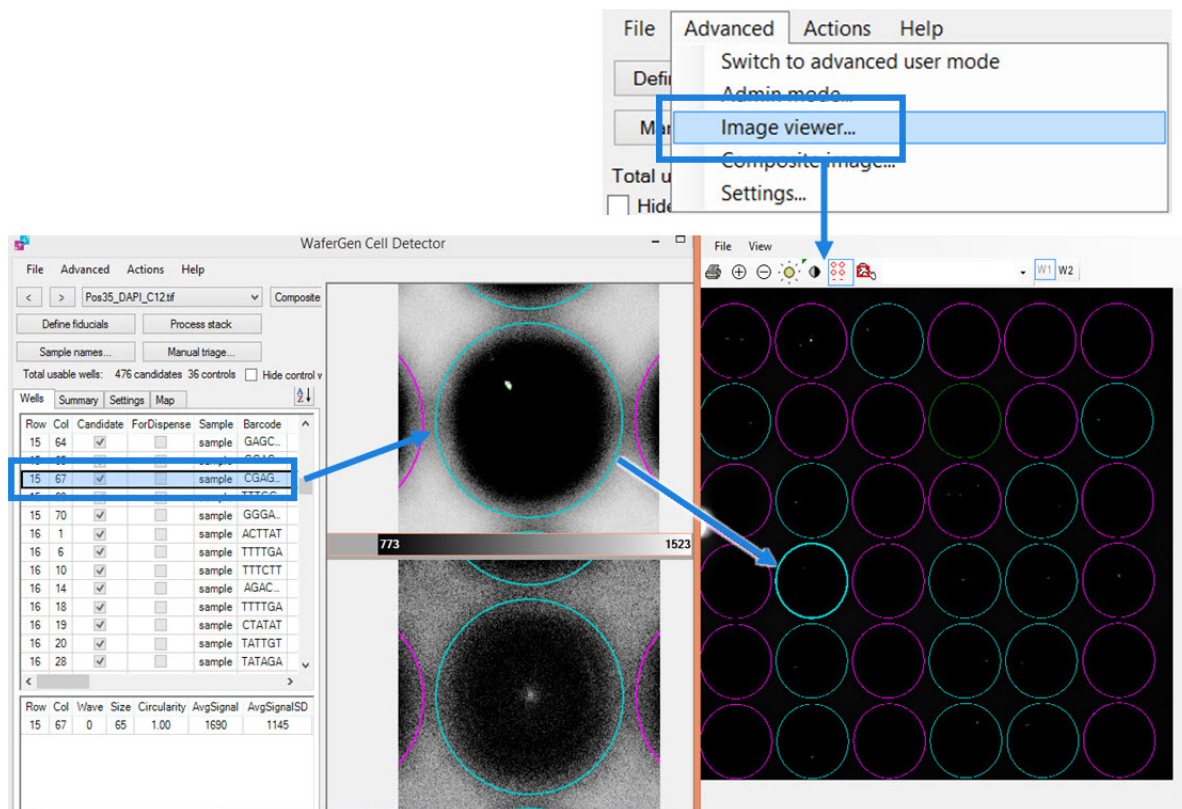
2. Click any row for closeup views of the selected nanowell using DAPI (top) and Texas Red (bottom) filters. In Figure 14 (above), the selected nanowell is located in Row 0/Column 6 of the imaged chip.



## View/Edit Results List

If desired, you can examine any selected nanowell in the Candidate column and view it as a single-well image as well as a multi-well image.

1. In the *Main* window, click the **Advanced** tab and select **Image viewer** (see Figure 15, below). A multi-well image highlighting the nanowell selected in the results table (on the *Wells* tab) will appear.
2. Arrange the windows as demonstrated in Figure 15 (below). When you click on any of the Candidate rows in the results table (on the *Wells* tab), the software will present corresponding single-well images, and display the corresponding multi-well image in the *Image Viewer* window.



**Figure 15. Opening and arranging windows for optimal image viewing.** The image on the far right is the multi-well image. The horizontally split images in the center show a single cell in a nanowell stained by Hoechst 33342 (top) and the absence of propidium iodide stain in the corresponding position (dead-cell stain; bottom). In the bottom image, the green dot in the center (if seen) marks the bottom of the nanowell and is not from a dead cell because it does not match the location of the Hoechst-stained cell.

3. Use the tools in the *Image Viewer* toolbar to critically examine the cells (see Figure 9, above, for a legend describing the toolbar icons).

4. To manually exclude one or more candidate wells, right-click the highlighted row(s) and select **Exclude selected wells** (see Figure 16, below). To exclude several candidate wells, consider using the **Manual triage** function described in the section below (Section II.H).

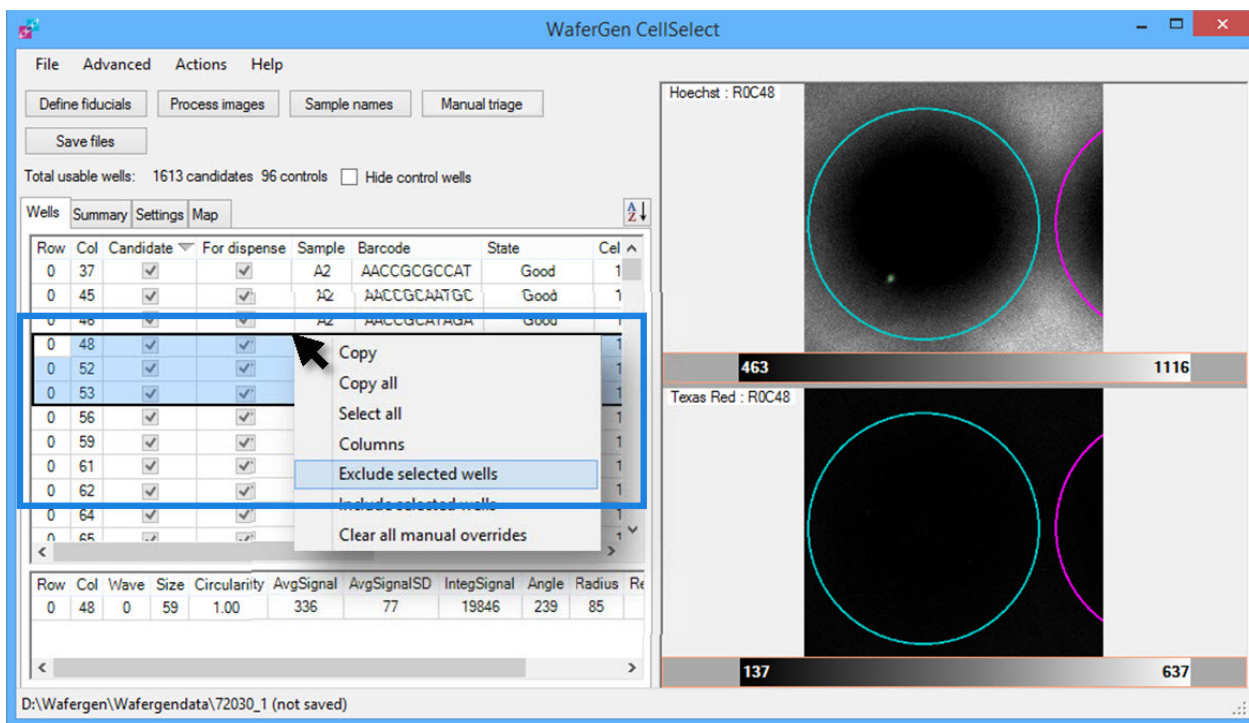


Figure 16. Excluding candidate wells.

Understanding the Software Color Code

CellSelect software analyzes images of cells taken with both DAPI and Texas Red filters. The DAPI filter is used to detect live cells (stained with Hoechst 33342), while the Texas Red filter is used to detect dead cells (stained with propidium iodide). If a single cell is visible in the DAPI filter but not in the Texas Red filter, AND meets the size and shape criteria programmed in the settings, the cell is considered a good candidate.

To make wells containing candidate cells easier to spot, the software overlays these wells with a teal-colored circle in both the single- and multi-well images, while wells containing non-candidate cells (such as dead cells, see the Status Table in the Appendix, below) are marked with pink-colored circles. A well currently displayed in the single-well image is marked with a brighter overlay than surrounding wells in the multi-well image (see example in Figure 17, below).

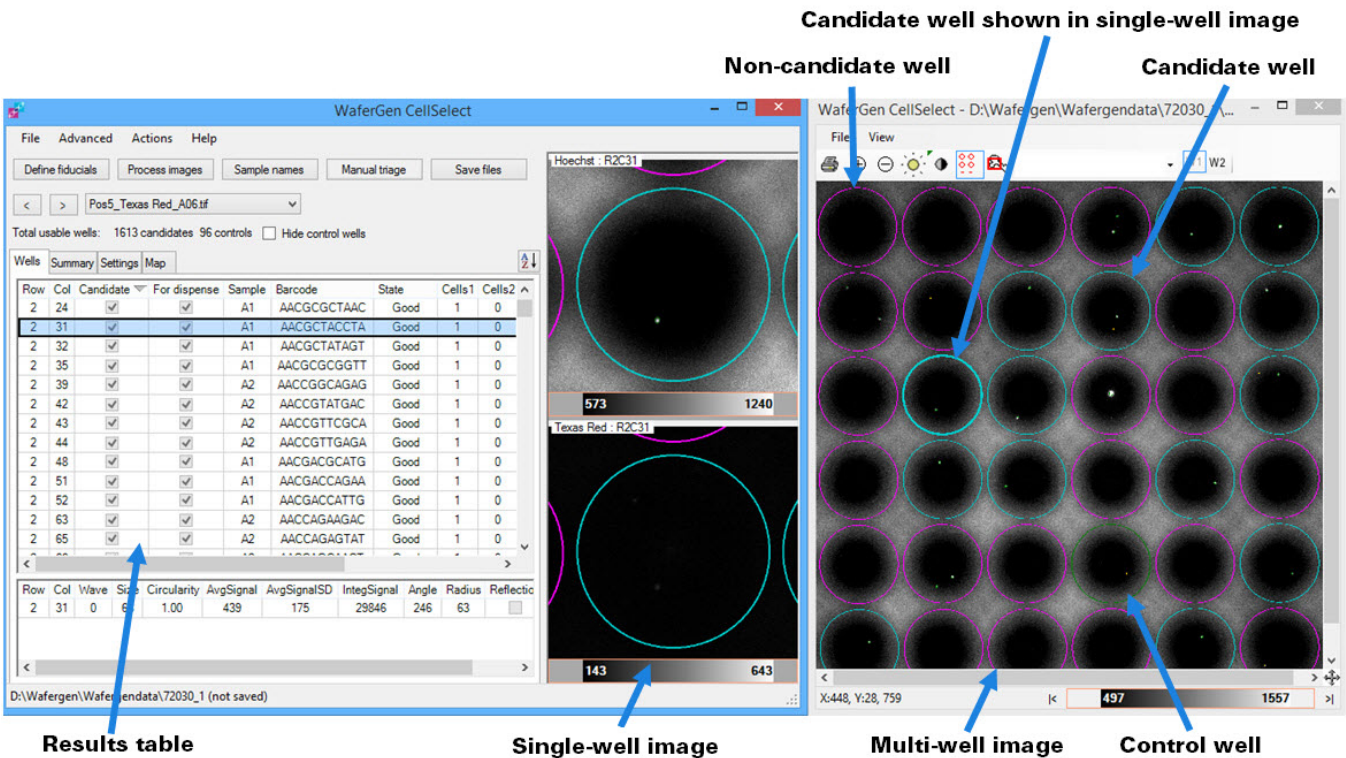


Figure 17. Understanding nanowell color codes.



If you look closely at the cells, you will also see other color indicators:

- **Green outline**—indicates that the software algorithm identifies a cell based on morphology and intensity.
- **Yellow outline**—indicates that the software algorithm identifies an artifact that is too small to be a cell and is ignored.
- **Blue outline**—indicates a rare reflection artifact.

To understand how the software determines the status of each nanowell, refer to the Status Table in the Appendix (below).

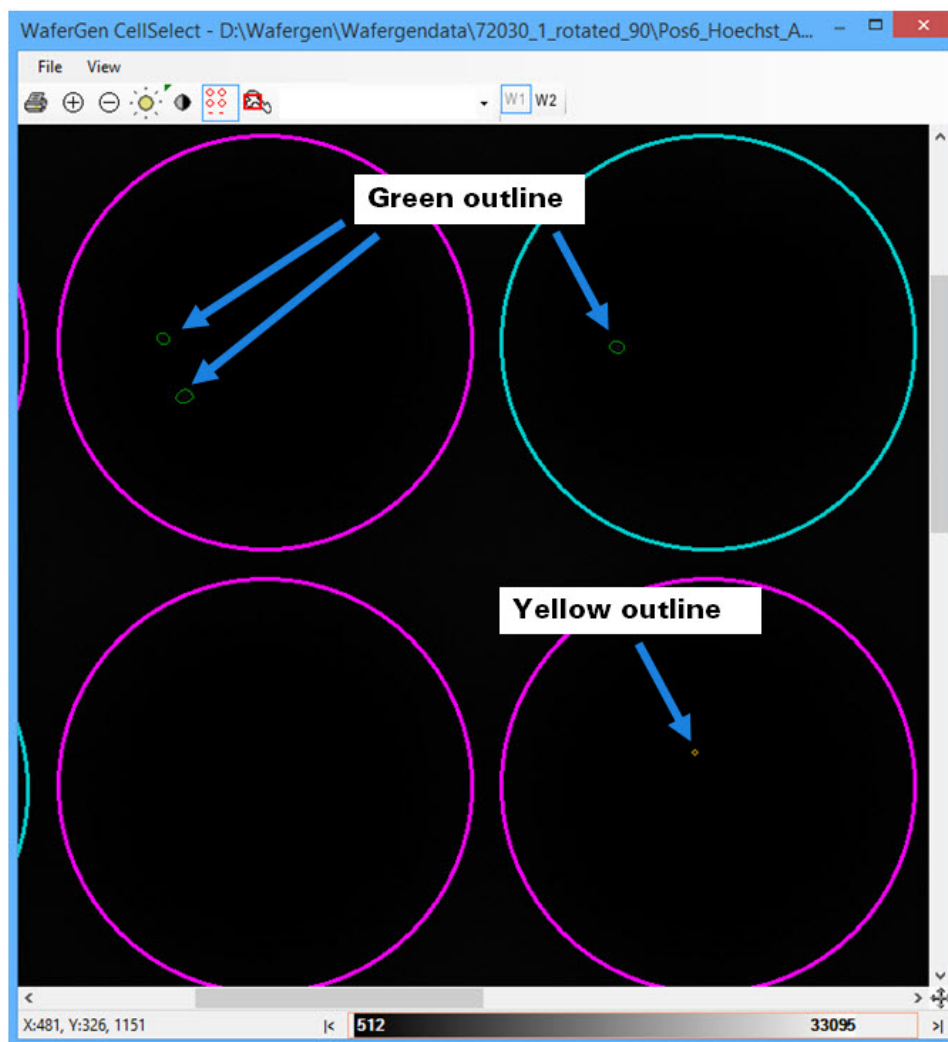


Figure 18. Closeup of wells, showing cell outlines.

## H. Use Manual Triage

The **Manual triage** function opens a dialog box to quickly review and reject (or bypass) consecutive wells down the *Wells* table (after sorting good candidates to the top of the table).

1. Click **Manual triage** to open the corresponding prompt box.
2. Examine each consecutive well image and click **Reject - Next Well** to exclude a candidate well and move on to the next candidate well, or click **Next** to retain a candidate well and move on to the next candidate well.

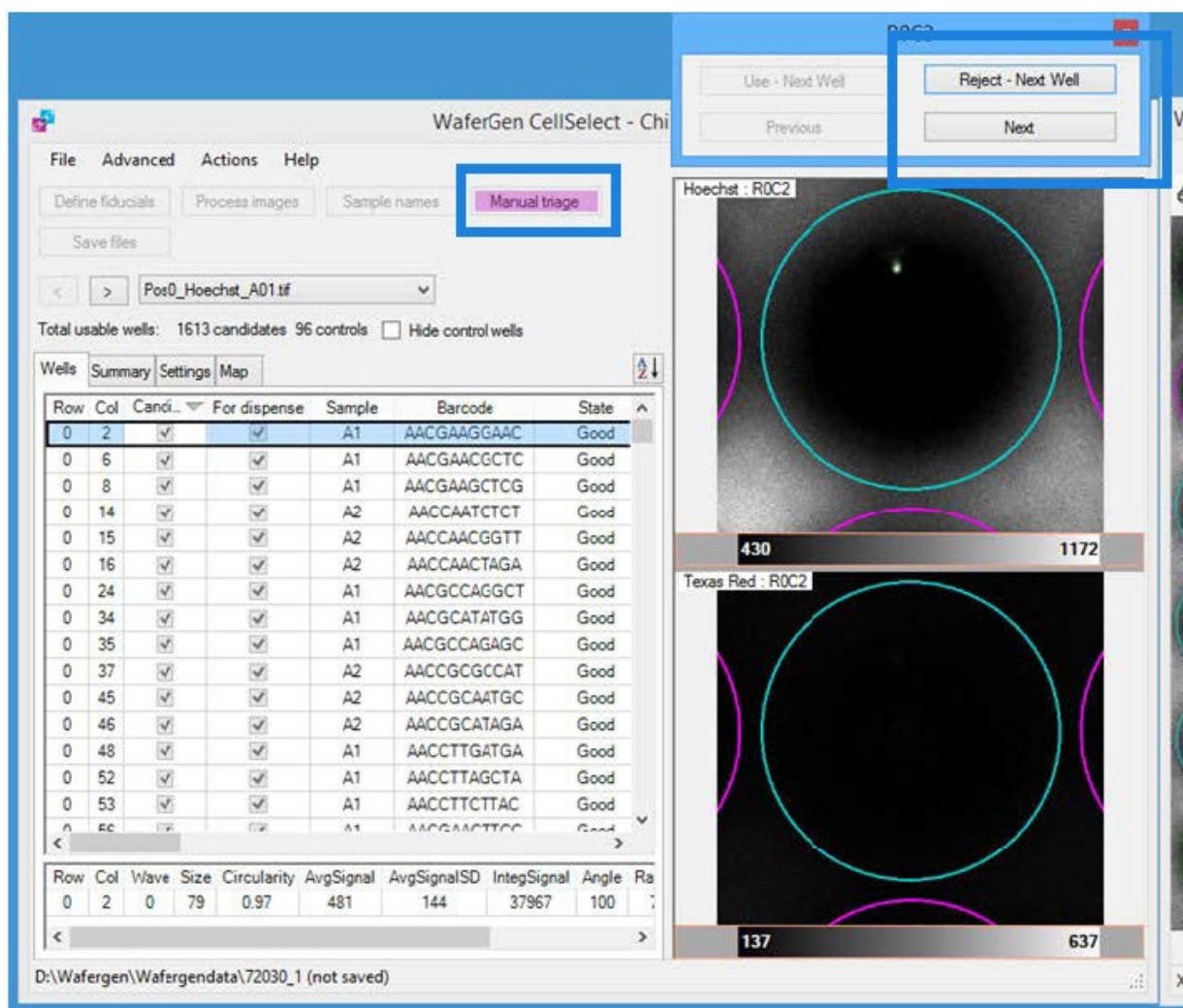


Figure 19. Manual triage.

## I. Save Files

1. Click **Save Files** and enter a new file name to save any changes you have made to the results file. We strongly recommend using a new file name to avoid overwriting the original results file. The file is saved with the .wcd file extension and stored in the Wafergendata folder.

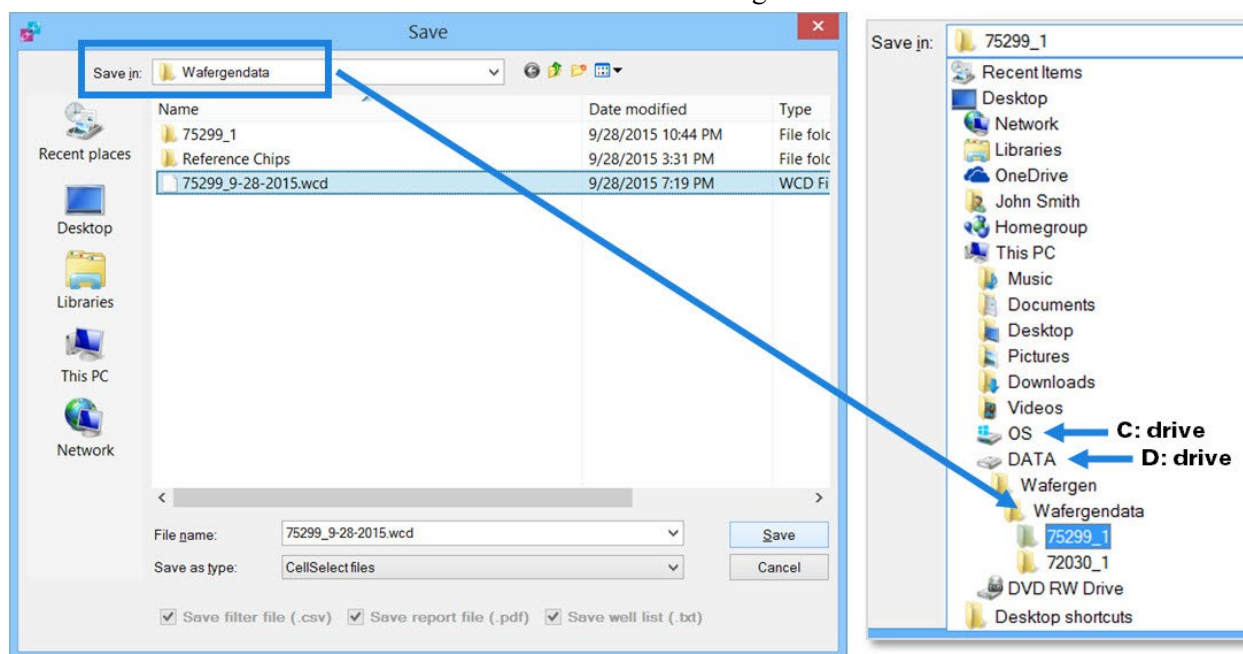


Figure 20. Saving the results file.

2. If you would like to downselect nanowells, in the *Main* window, click the **Actions** tab and select **Downselect**.

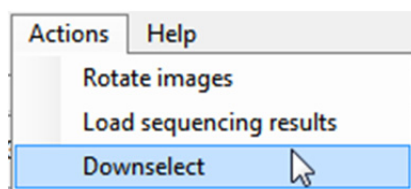


Figure 21. Saving the filter file.

3. Enter the desired number of nanowells for controls and sample wells.

**NOTE:** You can view the number of controls and samples in the *Summary* tab. However, if you enter a number much greater than the number of wells listed, the software will automatically select up to the maximum possible number (a quick and easy way to enter all the candidate wells, especially when working with multiple different samples).

**CustomFilterFileExport**

Sample	Value
Neg Ctrl	48
Pos Ctrl	48
Sample	1613

Total: 1709

OK Cancel

**CustomFilterFileExport**

Sample	Value
A1	208
A2	194
B1	197
B2	203
C1	192
C2	201
D1	208
D2	192
Neg Ctrl	48
Pos Ctrl	48

Total: 1691

OK Cancel

Figure 22. Example filter files for single and multiple sample names.

4. Save the filter file after downselecting the number of wells. A 72 x 72 grid of the entire chip will be saved as a .csv file automatically named as <Chip ID>.csv, in which nanowells to be included in subsequent dispensations are marked with a 1, and nanowells to be excluded are marked with a 0.
5. Copy the filter file (.csv) and bring it with you to the ICELL8 MSND. It will be used for dispensing the RT reaction mix.

### III. Software Reference

#### A. Main Window

The *Main* window contains all the primary functions in one place. These functions are described in detail in this section.

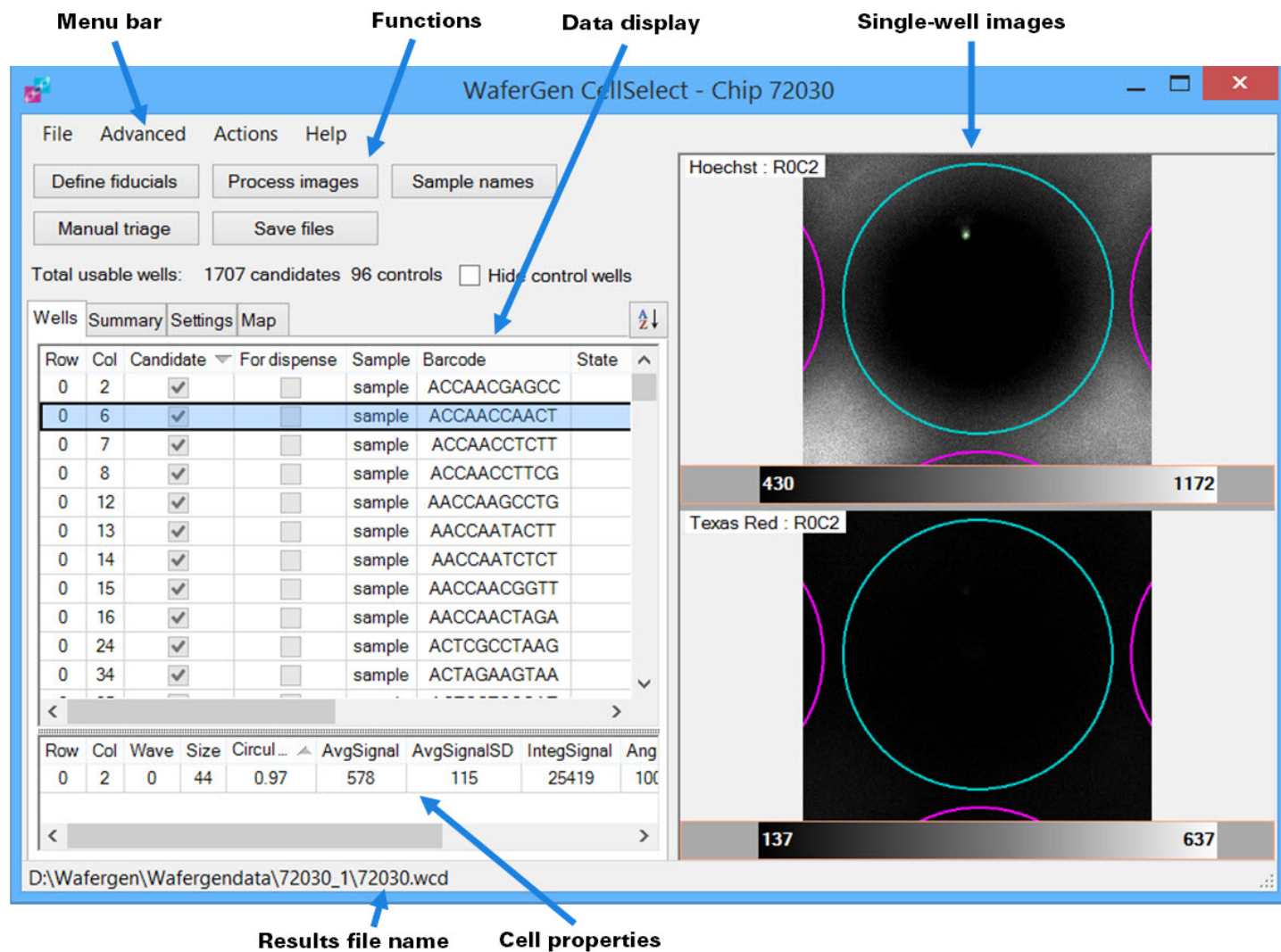


Figure 23. Main window.



## B. User Interface

CellSelect software uses tools and actions in its graphical user interface that are familiar to most users, thus enabling you to quickly learn and make the best use of the software.

**Right-click:** CellSelect software makes liberal use of the right-button mouse click to offer context-specific menu options. Users are encouraged to try right-clicking while using the software to access numerous features.

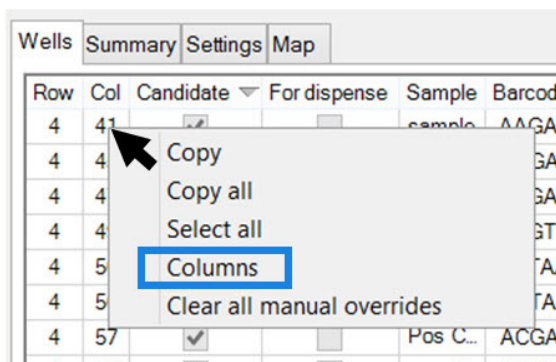


Figure 24. Example right-click menu.

**Column display:** On several data and analysis screens, you can choose the information to be displayed by right-clicking anywhere within the table (not in the column headers) and choosing **Columns** (see Figure 24, above). This action will open a *Selected fields* dialog box with checkboxes next to the information categories (see Figure 25, below). Click the checkboxes to select or deselect the columns that you want to display or hide. These selections will be remembered the next time the program starts.

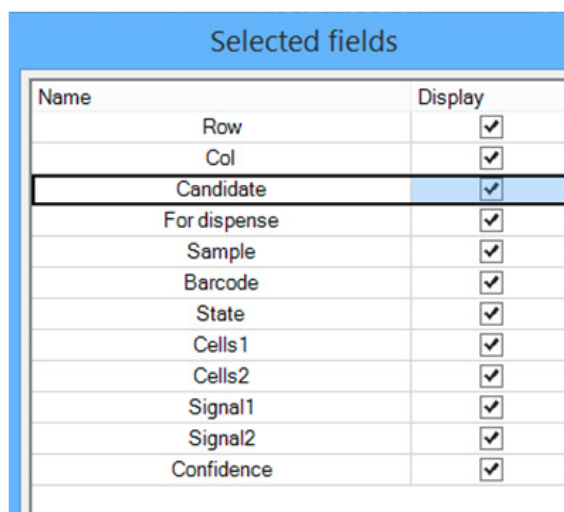


Figure 25. Column heading options.

**Column/window width:** Column widths can be adjusted by clicking the line between column headings and dragging left or right. In these instances, the appearance of the cursor changes to the  $\leftarrow| \rightarrow$  symbol. Various window/section widths can be adjusted as well. Click the left border of the *Image Viewer* window until the mouse pointer changes to the  $\leftarrow| \rightarrow$  symbol, then drag the border left or right (see Figure 26, below).

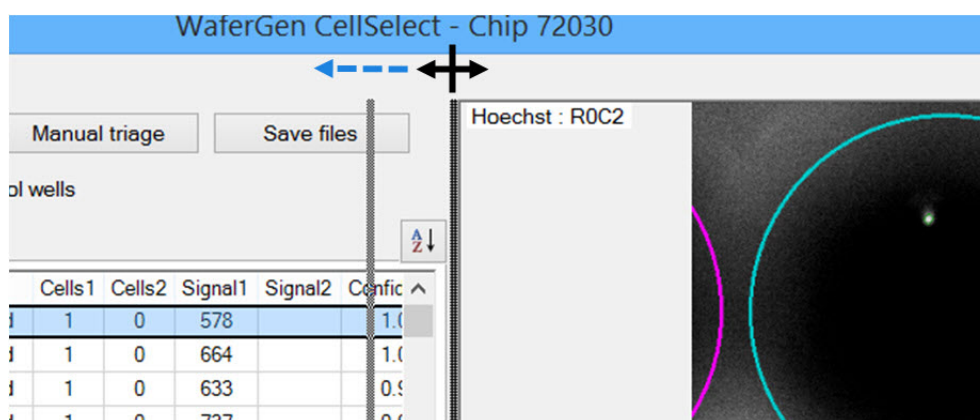


Figure 26. Adjusting the width of the *Image Viewer* window.

**Select and copy:** Right-clicking in many of the various data tables also triggers the option to select or copy the data as described in the table below:

Table I. Right-Click Commands for Data Tables in the *Main Window*.

Command	Function
<b>Copy</b>	Copies all selected rows to the clipboard.
<b>Copy all</b>	Copies the entire table, including the header row, to the clipboard.
<b>Select all</b>	Selects all rows. Click anywhere in the table to deselect all rows except the clicked row.
<b>Columns</b>	Launches the <i>Selected fields</i> window to customize the columns to be displayed.
<b>Include/Exclude* selected wells</b>	Includes rows that were not selected as candidates and/or excludes rows that were previously selected as candidates.
<b>Clear all manual overrides</b>	Removes all Include or Exclude actions.

\*Visible only when rows are selected (left click or click and drag the mouse).

**Scroll:** You can scroll in the software using the arrow keys on your computer keyboard, moving the vertical or horizontal scroll bars, or using the scroll wheel of your mouse. In some screens you may need to press the **Ctrl** key on the keyboard while moving the scroll wheel.

## C. Menu Bar

Table II. Main Window Menu Items.

Menu Item	Option	Function
<b>File</b>	<b>Open result file...</b>	Opens a previously saved result file (.wcd).
	<b>Save files...</b>	Processes and saves the results for the current chip*.
	<b>Open chip folder...</b>	Opens the chip folder containing images created by the Micro-Manager software.
	<b>Load barcode file...</b>	Loads the configuration file that identifies each well in the chip with a barcode.
<b>Advanced</b>	<b>Switch to advanced user mode</b>	Displays additional technical attributes of each well.
	<b>Admin mode...</b>	Password protected.
	<b>Image viewer...</b>	Adjusts image size, brightness, contrast, and well overlay. See Section III.H (below) for more details.
	<b>Composite image...</b>	Displays the multi-well image.
	<b>Settings...</b>	Displays the preconfigured settings for single-cell analysis.
<b>Actions</b>	<b>Downselect</b>	Selects the number of wells to be dispensed for each of the samples.
	<b>Rotate images</b>	Rotates all images 90° in the image folder and saves the rotated images to a separate folder.
<b>Help</b>	<b>About</b>	Displays the software version and End User License Agreement (EULA).
	<b>Open user manual...</b>	Opens this manual.

\*When applying changes to a results file, we strongly recommend saving the modified version under a new file name so that the original version is maintained as a backup.

## D. Functions

The following functions assist the user in adjusting and analyzing well images to identify nanowells that contain the best single-cell candidates for sequencing.

### Define Fiducials

Fiducials are dye-filled nanowells that define the corners of a 6 x 6 grid of nanowells. A TIFF image of the 6 x 6 grid (multi-well image) is generated by the imaging system and used by the CellSelect software to register the boundaries of the multi-well image. Aligning the fiducials with the CellSelect fiducial markers ensures that images are correctly analyzed.

The first function in the *Main* window of the software, **Define fiducials**, runs an automated program that finds and presents the four fiducial images for user inspection. If any software fiducial marker is slightly off center, the user can reenter the fiducial position by clicking the center of the misaligned well with the mouse pointer (red circle in Figure 27, below).



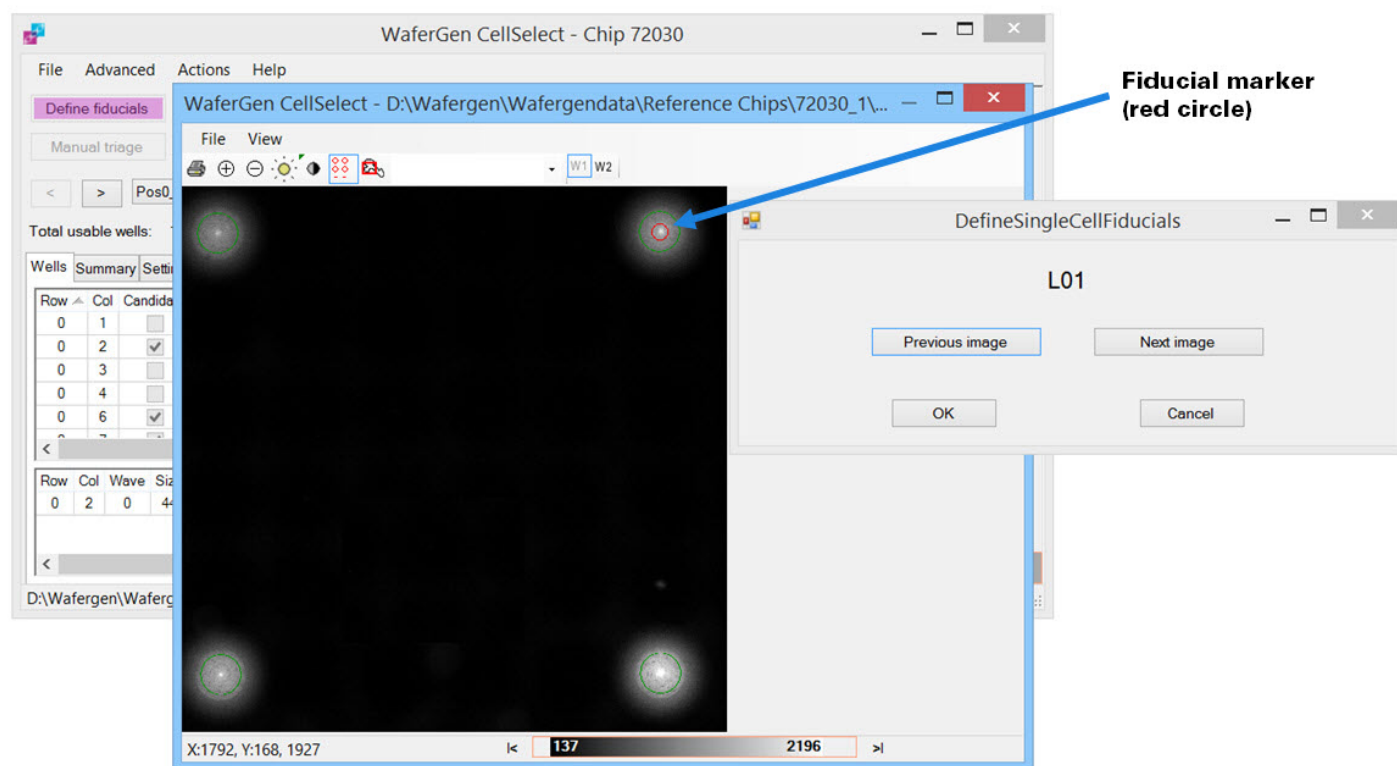


Figure 27. Inspect and adjust fiducials.

If necessary, the **Zoom in/Zoom out** functions on the *Image Viewer* toolbar (see Figure 28, below) can be used to view a closeup of the 6 x 6 grid (36 nanowells). Other functions on the toolbar enable printing of multi-well images (warning: printing the images consumes a lot of ink!), adjustment of image brightness/contrast, removal of the fiducial marker, verification of well location (if only a portion of the multiwell image is displayed), and changing of wavelengths (W1/W2). See the section entitled *Image Viewer Toolbar Icons* in Section III.H (below) for a detailed description of each tool.



Figure 28. *Image Viewer* toolbar icons.

## E. Process Images

The **Process images** function analyzes all 5,184 nanowell images in the 288 TIFF image files generated for a chip.

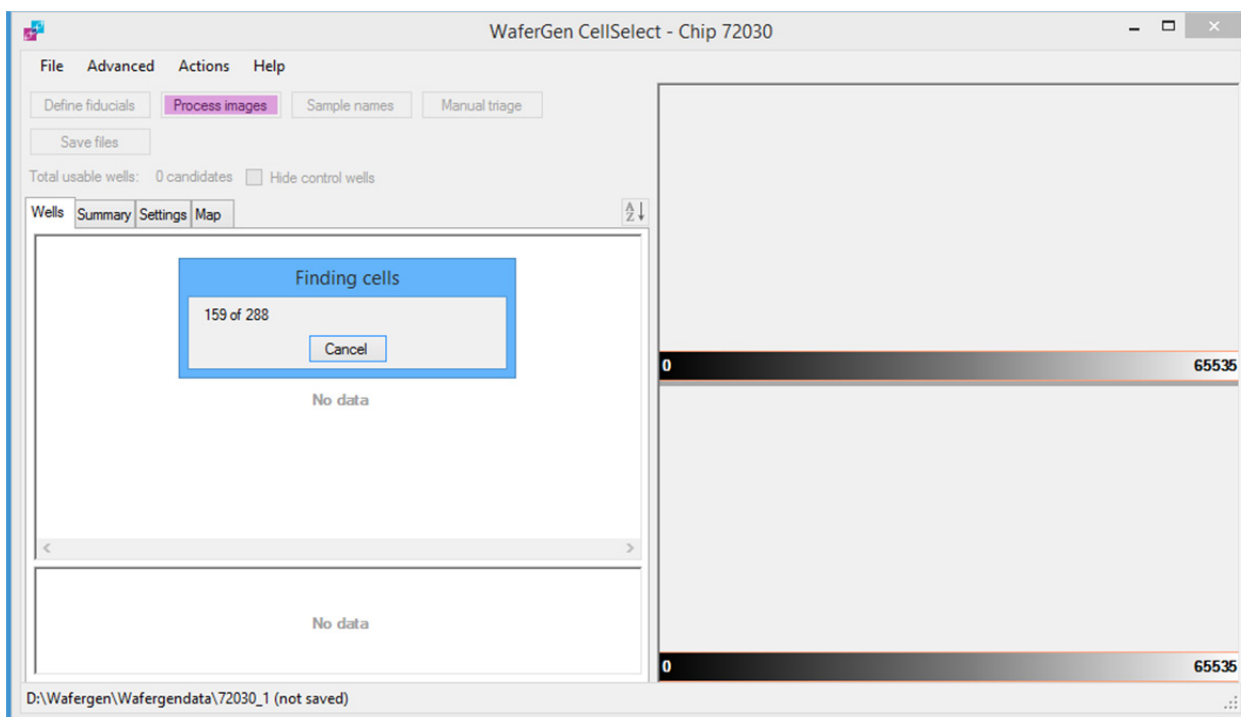


Figure 29. The software analyzes well images.

After the software finishes the analysis, it prompts you to save the results file. When you click **Yes** to the prompts and enter a file name, the software will do the following:

- Select wells among all those that are checked in the Candidate column (*Wells* tab) according to the parameters defined on the *Settings* tab. See the section entitled Settings in Section III.H (below).
- Save the result file. The result file will contain the selected nanowells and setting parameters under a new file name (e.g., 72030\_10202015.wcd), as demonstrated in Figure 30 (below). The results file contains all the data shown on the *Wells* tab.
- Automatically generate the filter file for dispensing RT reagents (e.g., 72030\_FilterFile.csv). The selected wells in the filter file will be checked in the “For dispense” column in the results on the *Wells* tab. The filter file is a simple map of all well positions in the chip. Each well position receives a “1” if it is marked as a candidate in the results file and a “0” if it is not a good candidate.

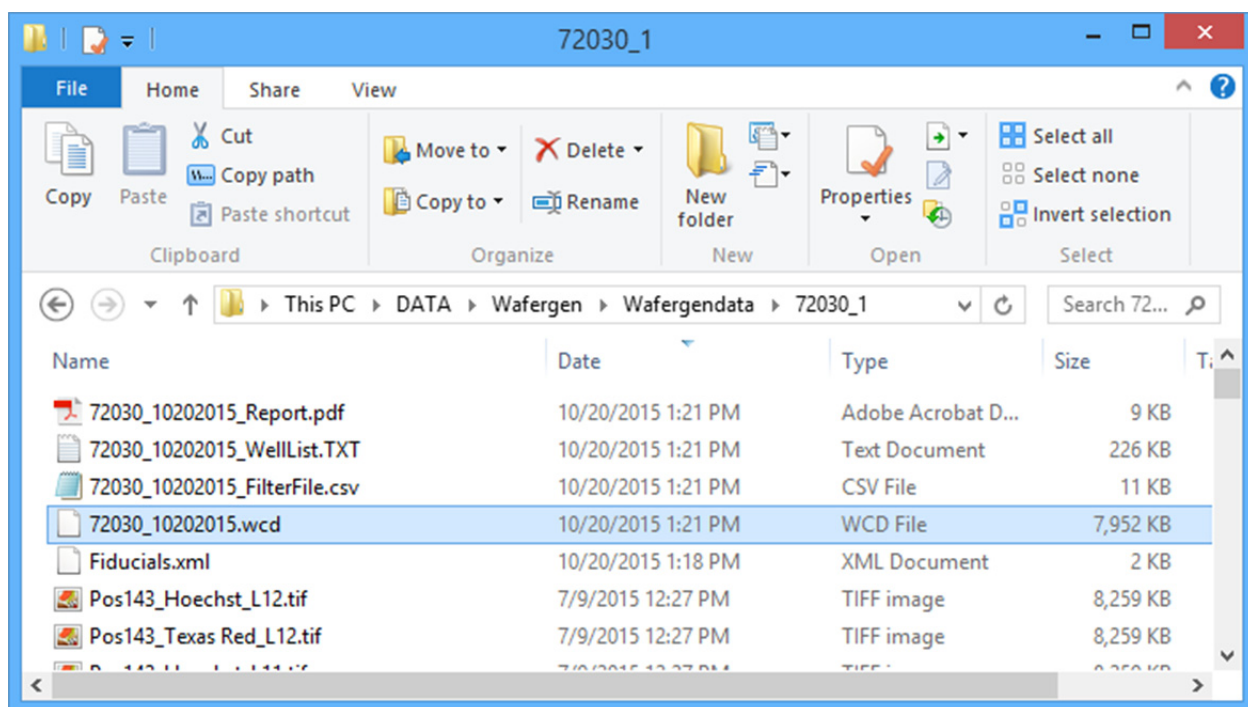


Figure 30. Files generated by CellSelect software. Each preprinted chip contains a total of 5,184 unique nanowell barcodes. You can load the results along with the settings from the saved WCD file.

## F. Data Display

The data section of the *Main* screen includes the following tabs: *Wells*, *Summary*, *Settings*, and *Map*.

### Wells

The data table on the *Wells* tab lists all the wells in the chip and statuses of any cells found.

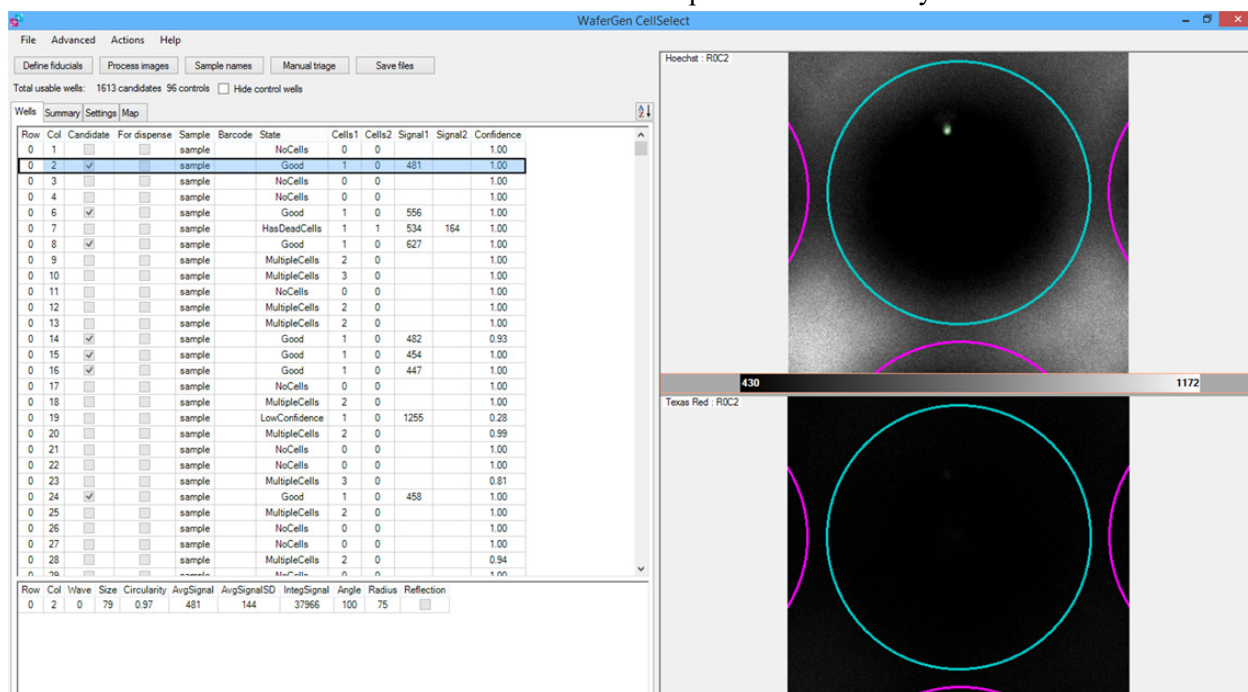
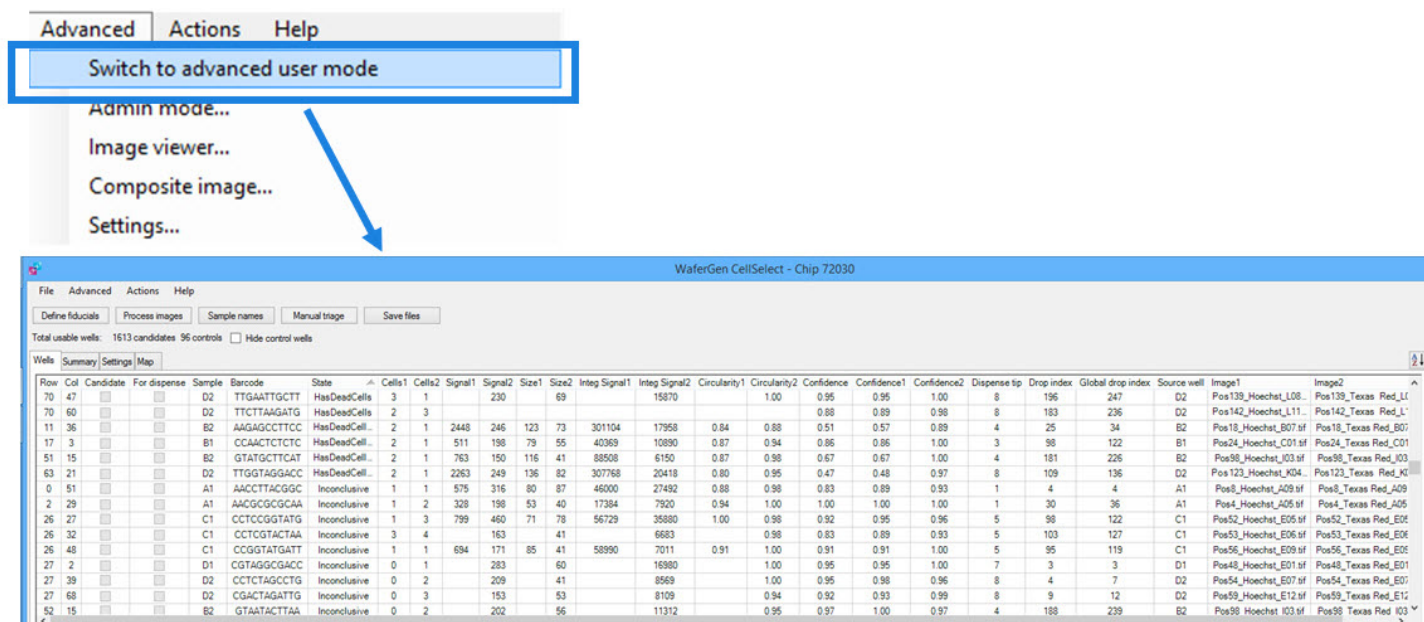


Figure 31. Wells data table.

You can view additional data by selecting **Switch to advanced user mode** from the **Advanced** menu.



**Figure 32. Additional well information in Advanced User Mode.**

## Summary

The data table on the *Summary* tab displays tallies for controls, samples, and all categories in the results table.

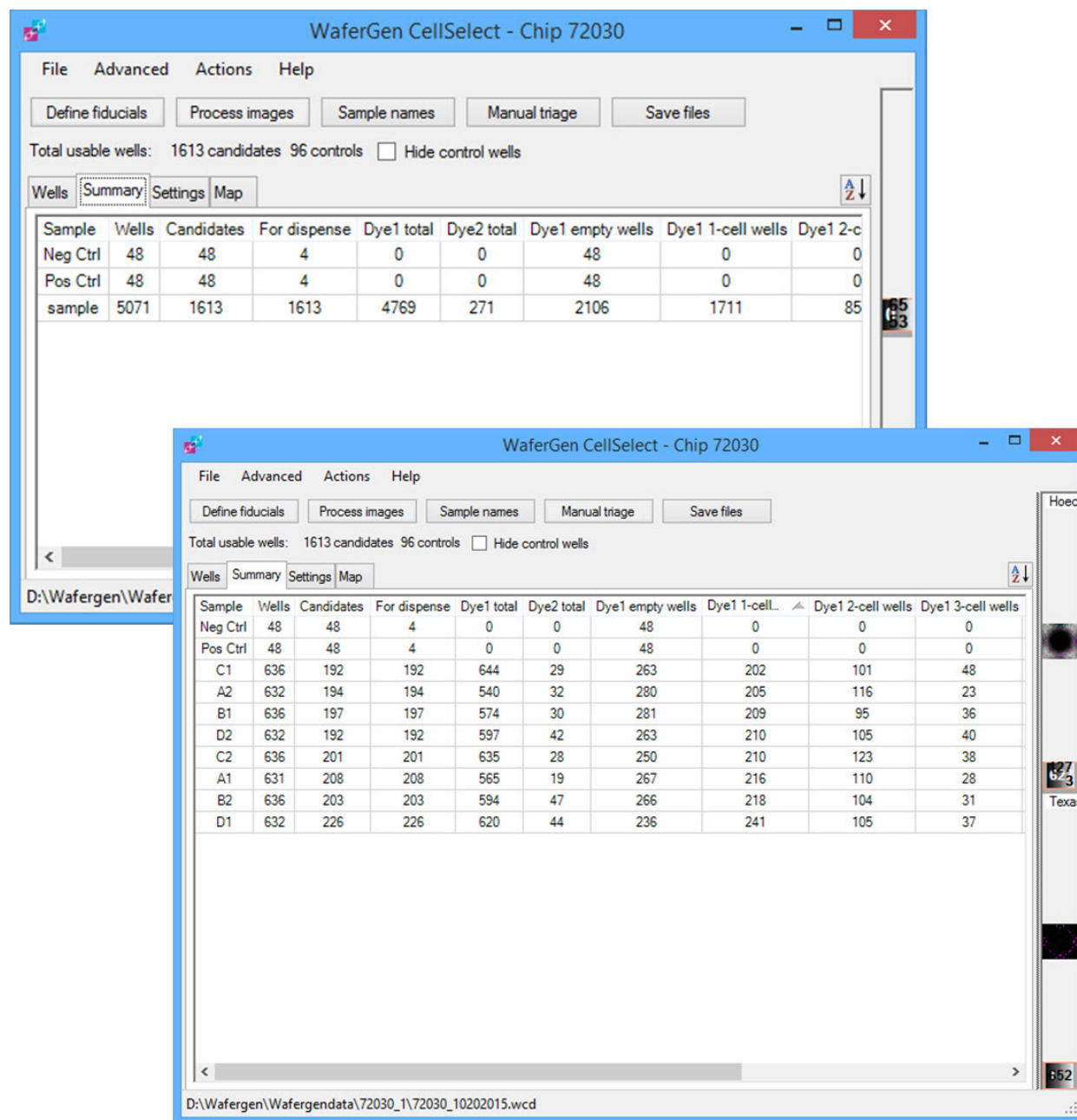


Figure 33. Summary data table.

## Settings

The following settings have been preconfigured for single-cell analysis. You DO have the option to edit the settings; however, we strongly recommend that you become very familiar with all the setting parameters before editing the file.

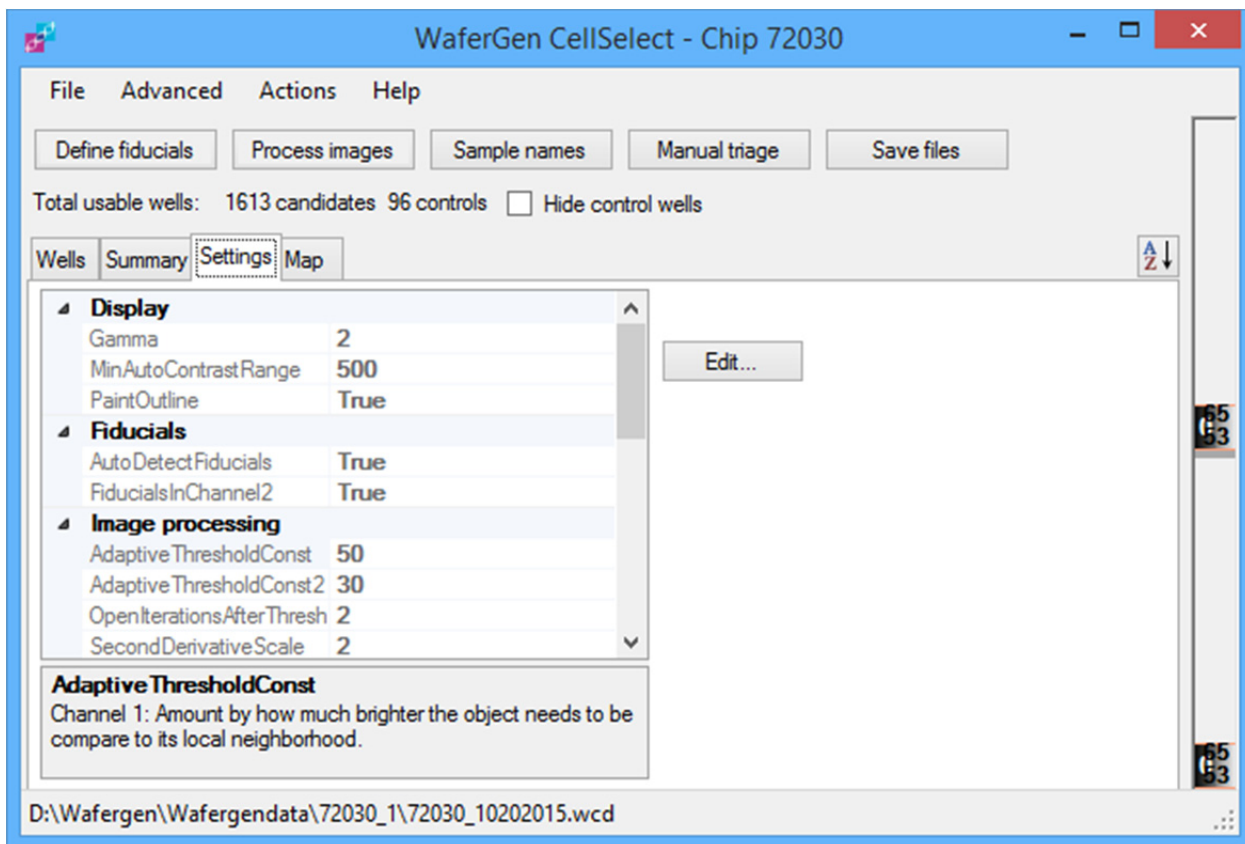


Figure 34. Settings tab.



If you edit the Settings file and wish to return to the standard settings for single-cell analysis:

1. Click **File** and select **Load** (see Figure 35, below).
2. Select StandardCellSettings-V5.1.xml or the latest version.

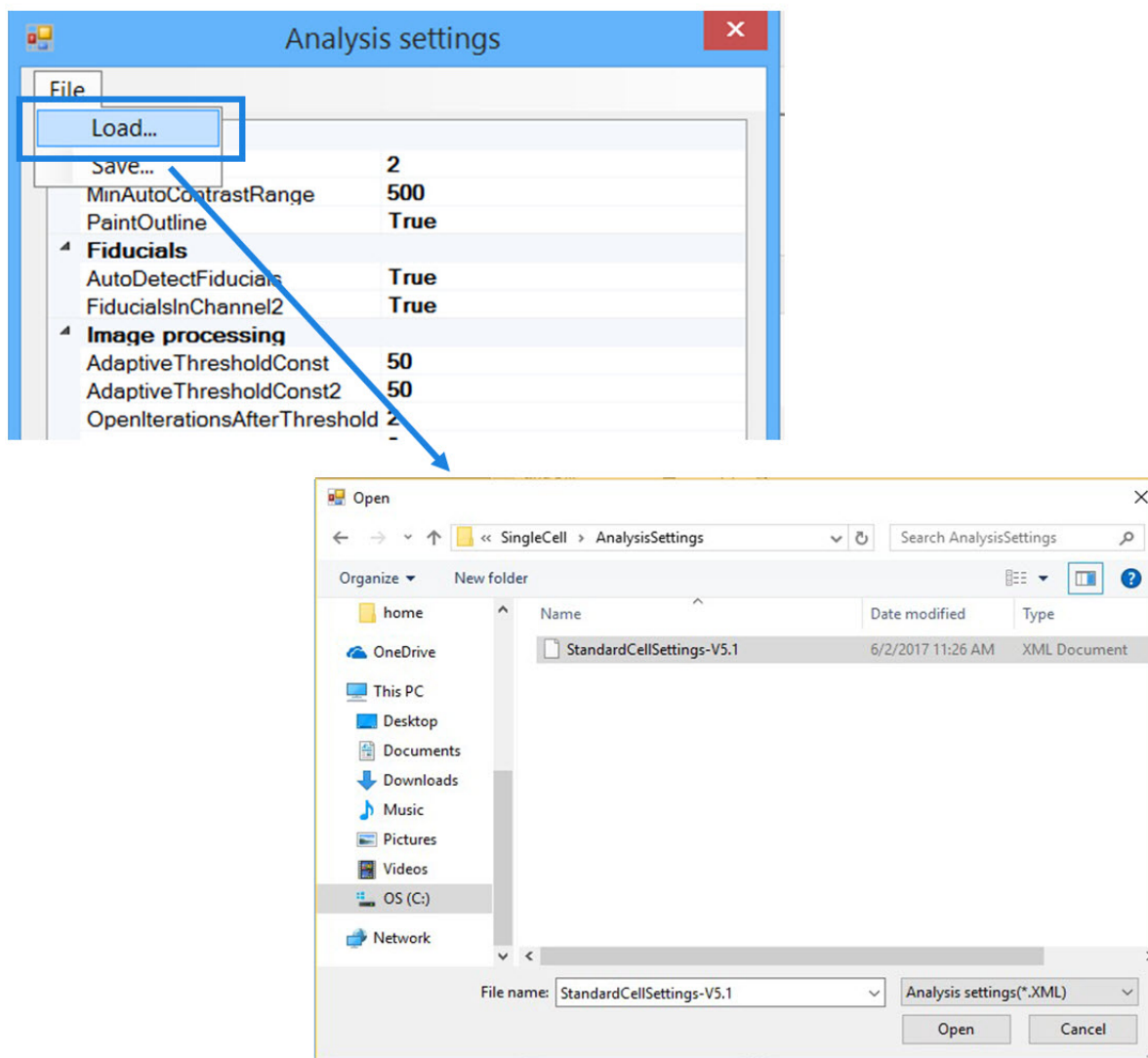


Figure 35. Loading/reloading the settings file for single-cell analysis.

## Map

The data table on the *Map* tab displays different data categories in bar graphs and graphic maps. Click the drop-down menu and graph icons to select the data category and graph type (see Figure 36, below).

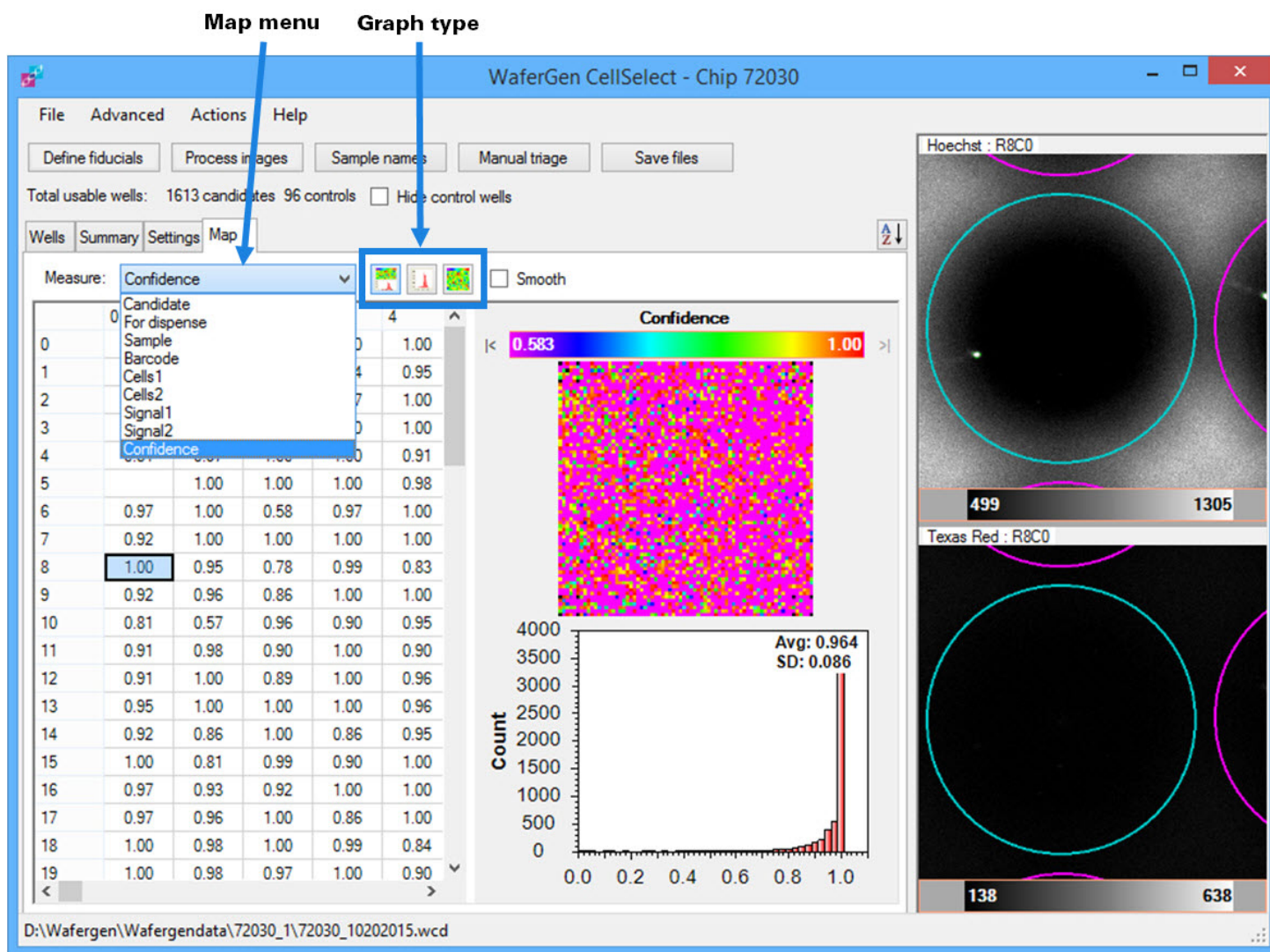


Figure 36. *Map* tab.



## G. Well Images

**Top view:** The top view shows cells that have absorbed Hoechst stain, which indicates the presence of one or more live cells—given that the cell(s) match the designated parameters. A live cell will not absorb propidium iodide and therefore will not appear in the bottom view. A good candidate is a single cell that fits the shape and size parameters of a live cell and appears in the top view only.

**Bottom view:** The bottom view shows cells that have absorbed propidium iodide, which occurs if a cell is dead. Cells that appear in the bottom view, or both the top and bottom views, are either dead or an artifact (considered inconclusive).

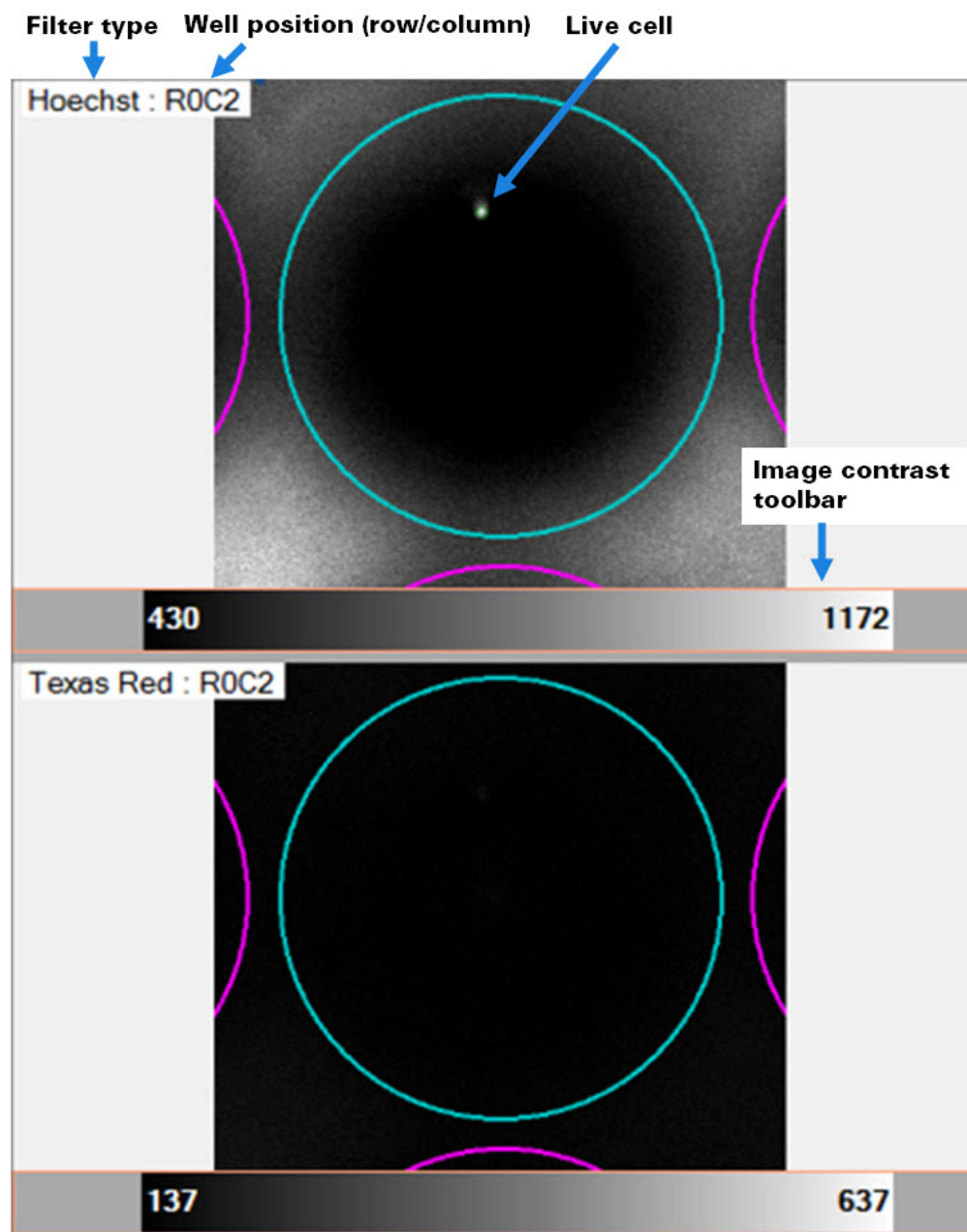


Figure 37. Single-well image.

### Image Contrast Slider

In addition to the **Contrast** icon on the *Image Viewer* toolbar, the slider at the bottom of each single- and multi-well image can be used to adjust image contrast (see Figure 38, below). Simply click and drag the left and right edges of the grayscale bar or the whole bar itself. Scrolling the mouse wheel while the mouse is over the center of the grayscale bar changes the shape of the curve and is equivalent to dragging the center control point in the image contrast tool. Clicking the buttons to the left and right of the grayscale bar quickly resets the mapping limits to 0 and 65,535, respectively. Alternatively, you can also double-click on the left or right edges of the bar. Right-clicking on the toolbar changes the underlying color map.

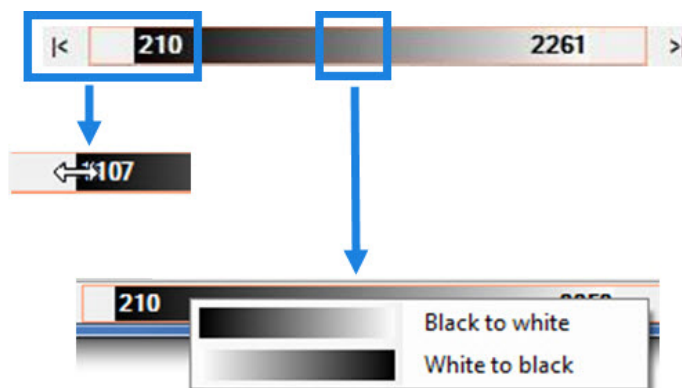


Figure 38. Adjusting image contrast using the slider.

## H. Image Viewer

The *Image Viewer* window is accessible via the **Advanced** dropdown menu in Analysis mode, and displays multi-well images (6 x 6 nanowells).

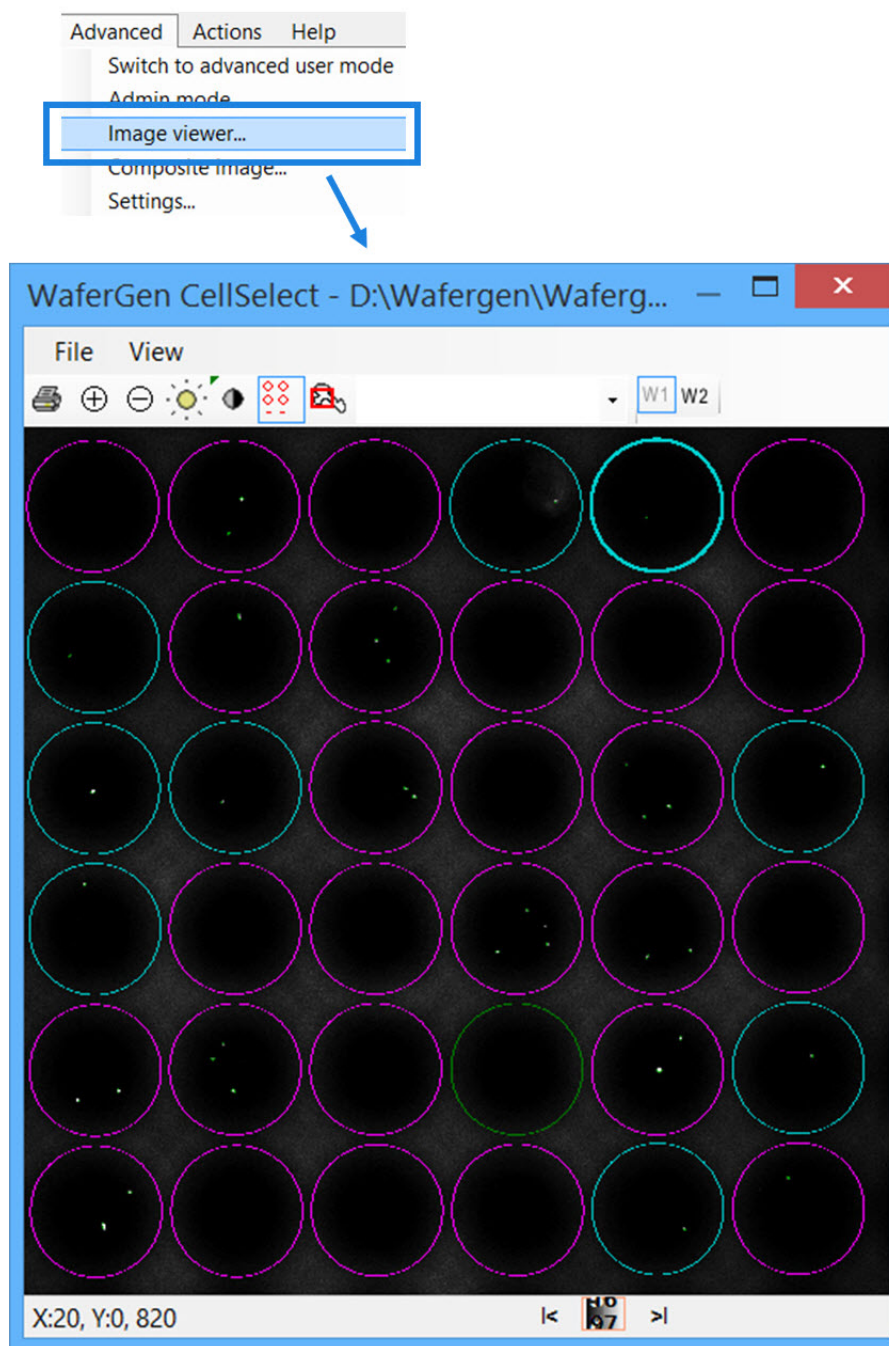


Figure 39. *Image Viewer* window.

## Image Viewer Toolbar Icons



Figure 40. Image Viewer toolbar icons.

**Zoom Icons**

The **Zoom** icons are for increasing or decreasing the size of the multi-well image. Clicking the **Zoom in** icon while holding down the **Shift** key triggers the *Image Viewer* window to resize itself to occupy the same area as the displayed image. Another way to resize the image is to press the **Ctrl** key while turning the scroll wheel on the mouse.

**Image Contrast Icon**

Fluorescence images generated by the instrument typically have a very large dynamic range. Clicking the **Image contrast** icon opens the *Image contrast settings* window, which allows you to adjust the mapping of the 65,535 image intensity levels to 256 shades of gray (see Figure 41, below). Drag the control points with the mouse to adjust the mapping.

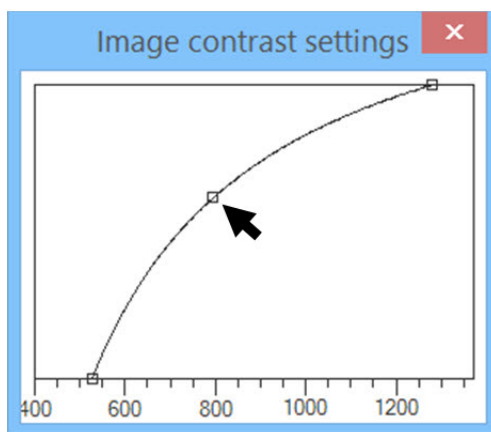


Figure 41. Image contrast settings window.

**Auto Contrast Icon**

Use the **Auto contrast** icon to adjust the upper and lower image contrast, and to specify when and how the Auto contrast operation is applied. Clicking the **Auto contrast** icon while holding down the **Ctrl** key opens the *Auto contrast settings* window, which allows you to adjust the settings.

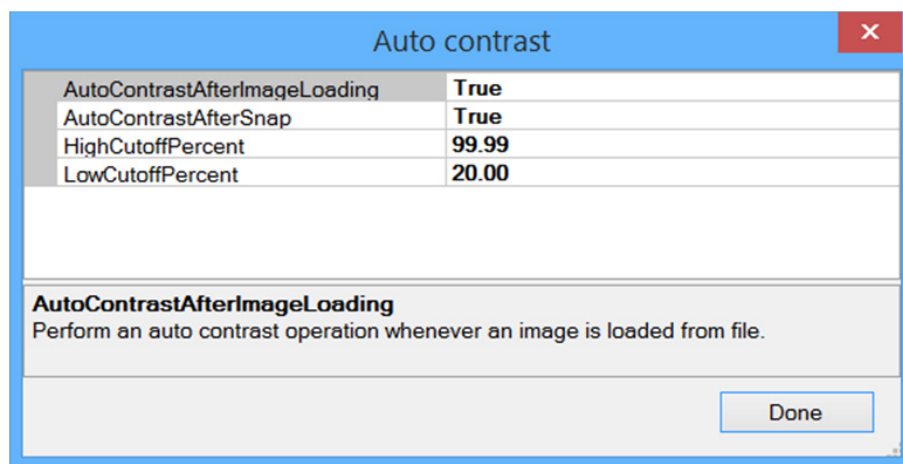


Figure 42. *Auto contrast settings* window.

Table III. Auto Contrast Settings.

Option	Function
<b>AutoContrastAfterImageLoading</b>	Perform an auto contrast operation whenever an image is loaded from a file.
<b>AutoContrastAfterSnap</b>	Perform an auto contrast operation whenever an image is taken with the camera.
<b>HighCutoffPercent</b>	The high threshold is set such that all pixels above the HighCutoff percentage are colored white.
<b>LowCutoffPercent</b>	The low threshold is set such that all pixels below the LowCutoff percentage are colored black.



### Map Window Icon

Use the **Map window** icon to display the entire image. After selecting this icon, drag or resize the red rectangle to change the viewed portion of the image.

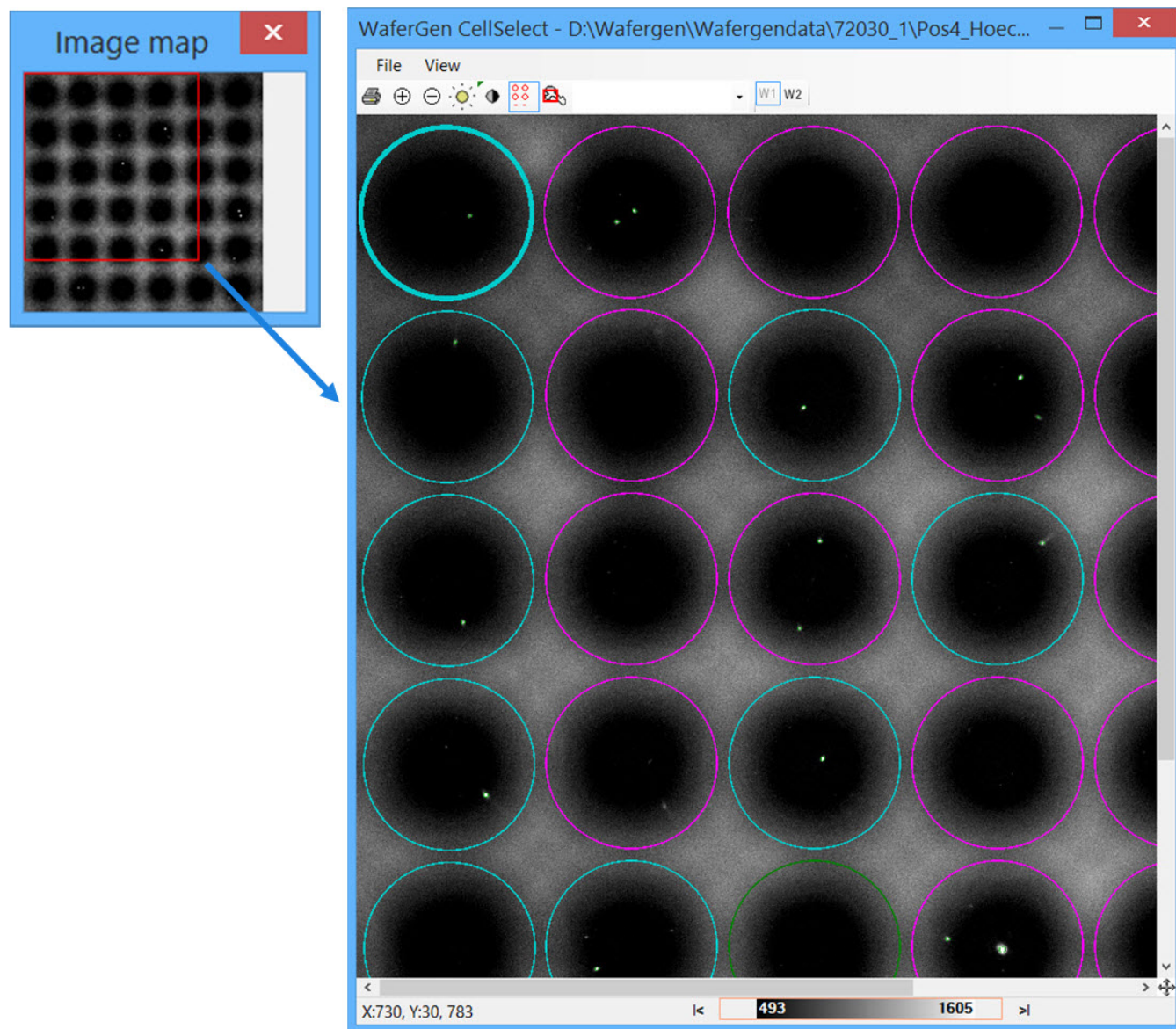


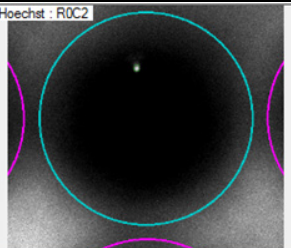



Figure 43. Viewing a subsection of an image using the Map window icon and *Image map* window.

Appendix: Status Table

**Top view:** Hoechst staining indicates the presence of a live cell, provided that the object meets established size and shape parameters and does not appear in the bottom view.

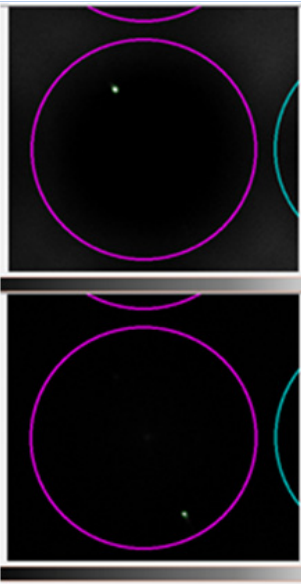
**Bottom view:** Propidium iodide staining indicates the presence of a dead cell, a well bottom, or an artifact.

Table IV. Nanowell Status Descriptions and Sample Images.

State	Conditions (all conditions must be met)	Image Example
Good	Single cell that meets the designated parameters appears in the top view (DAPI filter), but not in the bottom view (Texas Red filter).	<div> 430 1172</div> <div> 137 637</div>
Inconclusive and Has Dead Cells	Cell appears viable in top view but is also visible in bottom view.	<div> 597 2864</div> <div> 144 644</div>

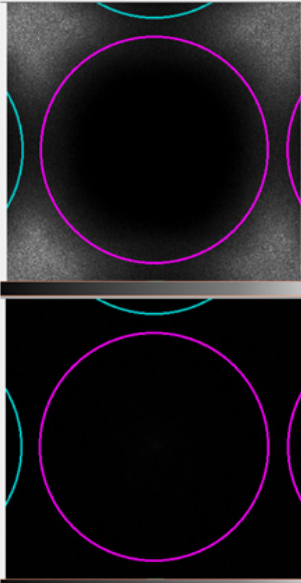
**Inconclusive**

One cell appears in the top view, one or more cells appear in the bottom view.  
No cells occupy the same location in both views.



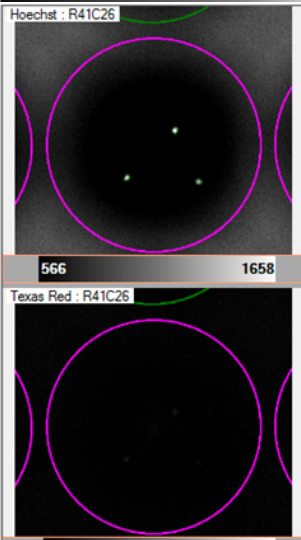
**NoCells**

No cells present in either view.



**MultipleCells**

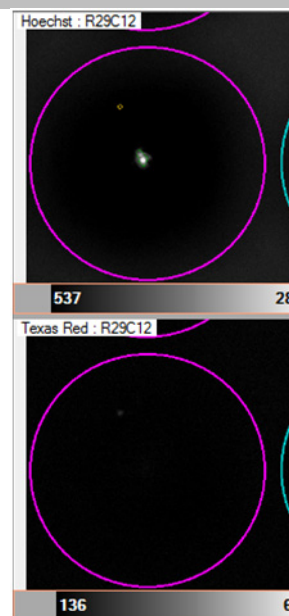
Multiple cells appear in the top view, no cells appear in the bottom view.





**Has Dead Cells** At least one cell in the top view has a colocalized signal in bottom view.

**Low Confidence** Cell is not clear.



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