Takara Bio USA, Inc.

ICELL8® CellSelect® Software User Manual

Cat. Nos. 640198, 640000, 640188, 640189 software v1.5 (110519)

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I. Introduction

A. Welcome to the ICELL8 CellSelect Software

The ICELL8 cx Single-Cell System (Cat. No. 640188, 640189) 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.

ICELL8 CellSelect Software analyzes images generated with the ICELL8 cx instrument 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).
- Modify parameters and rerun analyses with the new settings.

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

- Prompts you to load images and settings into the software.
- Provides functions in sequential order:

Define fiducials	Process images	Sample names	Manual triage	Save files	
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The diagram on the following page (Figure 1) summarizes the dispensing, imaging, and image analysis portions of the ICELL8 cx single-cell analysis workflow.

B. 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.

C. 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 ICELL8 CellSelect Software and then generate a filter file to be used for dispensing RT mix. The procedure consists of the following steps:

- 1. Load Images: Load images directly from the ICELL8 cx Software when scanning, or later from the ICELL8 CellSelect Software (File menu).
- 2. Load a Different Barcode File (optional): Barcodes are already selected when you scan images from the ICELL8 cx system. However, this menu item allows you to associate a different barcode file to the run for analyzing 5,184 nanowells.
- 3. **Process Images:** Start image processing. The software analyzes every well image in 288 image files and determines the best candidates for future processing.
- 4. **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.

Icon for ICELL8 cx Software (for dispensing)



Icon for ICELL8 CellSelect Software (for image analysis)

Figure 2. Icons for the ICELL8 cx System software and ICELL8 CellSelect Software.

B. Load Images

You can open the ICELL8 CellSelect Software directly when scanning from the ICELL8 cx Single-Cell System or save a file and open it later from the ICELL8 CellSelect Software.

From the ICELL8 CellSelect Software

1. Launch the ICELL8 CellSelect software by clicking the CellSelect icon:



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

Open result file Save files	Sample names	
Open chip folder		
Load barcode file Save filter file - downselect	•	

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

Alternatively, you can drag and drop a folder from Windows File Manager into the application

- 3. If the images in that folder were not previously analyzed, you will be prompted to supply the necessary information.
 - a. If there is one .wcd file in the folder, that file will be loaded.
 - b. If there are multiple .wcd files in the folder, you will be prompted to select the file you want to load.

New stack info			
Chip ID	117735		
Analysis settings	AnalysisSetting_250nL_chip xml - Predefined \sim		
		🔜 Load file	
Barcodes	3'DE.xml \checkmark	Choose the file that you want to load.	
Chip comment		→ 92532.wcd	
		➔ 92532_Test.wcd	
	OK Cancel		(

Figure 4. Loading the settings file.

C. Load a Different Barcode File (optional)

Barcodes are already selected when you scan images from the ICELL8 cx system. However, this menu item allows you to associate a different barcode file to the run.

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

🔡 Ta	karaBio CellSe	lect - Chip	1097
File	Advanced	Actions	He
	Open result fil	e	
	Save files		
	Close		
	Open chip fol	der	
	Load barcode	file	
-			

Figure 5. File menu.

2. Select one of the preloaded XML files: 3' DE.XML, Smart-seq_SetA.XML, or Smartseq_SetB.XML. These XML files contain nanowell barcode sequences for each preprinted nanowell location. The default folder is:

C:\ProgramData\Wafergen\SingleCell\AssayMaps\

Pen Open						×
\leftarrow \rightarrow \checkmark \Uparrow Wafergen \Rightarrow SingleCell \Rightarrow As	ssay	'Maps 🗸	Ō		Search AssayMaps	Q
Organize 🔻 New folder						•
🔒 SingleCell	^	Name	^		Date m	odified
AnalysisSettings		📓 3'DE.xml			10/18/2	018 5:30 PM
🔥 AssayMaps		📔 Smart-seq_Set	tA.xr	ml	7/18/20	18 3:28 PM
		📓 Smart-seq_Set	tB.xr	ml	11/1/20	18 1:14 PM
Log	Ŷ	<				>
File name:				~	Assay map files (*.XML)	~
					Open Ca	ancel

Figure 6. Loading the barcode file for the preprinted chip.

D. 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.
- It must be the only one visible in the well.
- It must satisfy the size/shape parameters specified in *Settings*.

File Advanced Actions Help Define fiducials Process images Sample names Manual triage Save files Total usable wells: 0 candidates Hide control wells Wells Summary Settings Map Eincline cells Eincline Cells	
Isg of 288 Cancel No data	0 65536
No data	0 65535

Figure 7. 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 or 72030 09282015.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 dispense of RT reagents (e.g., 72030_09282015_FilterFile.csv). The selected wells in the filter file will be displayed in the *For Dispense* column.
 - The software will also generate a PDF file called 72030_09282015_Report.pdf which contains a short summary of the results.
 - Also, a file 72030_09282015_WellList.txt is generated which contains the content of the *Wells* table in a form that can be read easily by downstream analysis software.

- 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

See "Understanding the Software Color Code" in <u>Section II.F</u> (below) for more information.

NOTE: The user can re-process images after changing the analysis settings.

E. Change Sample Names

- 1. Click **Sample names** and enter a single sample name or use the source plate configuration layout for ICELL8 cx 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 8, below).

🔡 Tal	karaB	io CellS	elect	- Chip 92	532							_	
File	Adv	vanced	Ac	tions	Help								
Defin	e fidu	ciale	Pro	cess imag		Sample name	e M	anual triane		Hoechst : R12C6	9		
TakaraBio CellSelect - Chip 92332 - × File Advanced Actions Help Define fiducials Process images Sample names Manual triage Hoechst : R12C69 Save files Source plate samples layout One sample Multiple samples Image: Control of the sample sample sample sample name Image: Control of the sample sample sample sample sample sample sample name Image: Control of the sample samp													
Sa	ve file	es								- /			
<	>	Pos3	5_1 S	ource pla	ite samp	les layout							
Total us	able \	wells:	828			samole	Multir	ole earrolee					
Wells	Sumo	any Sat	tine		0 0110	oumpio	() Initiating	ole samples					
TakaraBio CellSelect - Chip 92532 File Advanced Define fiducials Process images Save files Source plate samples layout > Total usable wells: 82 Wells Sumple name Nutliple samples One sample One sample Multiple samples 12 63 13 4 13 10 13 14 13 15 13 15 13 14 13 15 13 14 13 14 14 26 13 34 13 37 13 37 13 37 14 26 13 37 14 26 14 26 14 26 15 11 16 0.85 14 26 15 161													
TakaraBio CellSelect - Chip 92532 File Advanced Define fiducials Process images Save files Source plate samples layout <		/											
12	56	yes	_	Sample	name								/
12	64	yes	_	Makinta								/	
12	69	yes		Multiple	samples	1			2				
13	4	yes	_	Δ		А1			A2	157			1220
13	10	yes	_	В		B1			B2	497	_		1338
13	15	yes	_	С		C1			C2	Red : R12	C69	-	
13	24	yes	_	D		D1			D2				
13	34	yes	_										
13	35	yes	-										
13	37	yes	-										1
13	4/ cc	yes	-					Load	Save				
13	20	yes	-					2000	0010				
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12	69	1	118	0.85	0.43	0.97	760	220	8963			/	/
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l:\Expe	erime	nts\SC\	92532	2 (not s	aved)								

Figure 8. Inputting multiple sample names and automatically identifying control- and fiducial-containing wells.

F. 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 9, below). The software will sort and consolidate all selected nanowells to the top of the table.

🔡 Ta	karal	Bio Cell	Selec	t - Chip 9253	2										-		
File	Ad	lvanced	I A	ctions He	lp												
Defir	ne fidu	ucials	Pr	ocess images	S	ample name:	s	/anual t	riace	Sav	ve files		Hoechst : R2C0				
<	>	Post)_1-H	oechst_A01.tif		~									~		
Total u	sable	wells:	828 c	candidates 🔽	Hide	control wells							/				
Wells	Sum	mary Se	ttinas	Map								Ą↓				1	
	0011	C			0			0.11.1	0 11 0	C 14	0.01	2.					
Row	Col	Candi.		For dispense	State	C 1		Cells1	Cells2	Signal1	Confidence	^			۲		
	29	ye	s	yes		Good		-	0	840	0.82						
	50	ye	5	yes		Good		-	0	1233	0.84						
	59	yes	s	yes		Good		1	0	861	1.00						
	61	ye	s	yes		Good		1	0	581	0.92					1	
	62	ye	S	yes		Good		1	0	938	0.82						
1	64	ye	S	yes		Good		1	0	900	0.87					1	
1	65	ye	S	yes		Good		1	0	868	0.84				/		
2	0	yes	S	yes		Good		1	0	1144	0.87						
2	1	yes	5	yes		Good		1	0	955	0.82		a faith a search				
2	9	yes	s	yes		Good		1	0	464	0.99		593			109	3
2	16	ye	s	yes		Good		1	0	1141	0.75		Texas Red : R2C0				
2	27	ye	S	yes		Good		1	0	373	0.81						
2	29	ye	s	yes		Good		1	0	942	0.80			-	 _		
2	35	yes	5	yes		Good		1	0	376	0.89		Section 2	/	-		
2	60	ye	5	yes		Good		1	0	1414	0.87		/				
3	5	yes	5	yes		Good		1	0	856	0.87		/				
3	6	yes	5	yes		Good		1	0	718	0.76		/			1	
3	17	yes	5	yes		Good		1	0	659	0.79						
3	30	ye	5	yes		Good		1	0	1091	0.78						
3	33	yes	5	yes		Good		1	0	820	0.87	~					
Row	Col	Maya	Size	Circularity	Inertia	Convertity	AvaSian	al Ave	SignalS	D Inters	ianal Radiu	P					
2	0	1	27	0.84	0.45	1.00	Avg3igi		/59	201	1010 110000 1011 110000	5 11				1	
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															/		
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<												>	170			67	0
H:\Exp	erime	ents\SC	\9253	32_2\92532_Te	est.wcd	ł											

Figure 9. 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 9 (above), the selected nanowell is located in Row 2/Column 0 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 10, 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 10 (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. Similarly, by double-clicking on a well in the *Imager Viewer* the corresponding row in the *Wells* table will be highlighted.



Figure 10. 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 10, 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 11, below). To exclude several candidate wells, consider using the **Manual triage** function described in the section below (Section II.G).

👪 Ta	karaE	Bio Cell	Select	- Chip 9253	32							_		
File	Ad	vanced	A	ctions He	elp									
Defin	e fidu	iciale	Pro	ocess images	S	ample name		Manualti	riane		Hoechst : R12C69			
Denn	e naa	icidia	110	indgea		ampie name.		- and a d	lidge					
Sa	ive file	es												
<	>	Pos3	5_1-H	loechst_C12	tif	~								
Totalus	able	welle:	828 c	andidates 🛛	Z Hida	control wells								
rotar as		Wella.	020 0			CONTROL WEIS								
Wells	Sumr	mary Se	ttings	Мар						Ż↓	/ \ -			
Row	Col	Candi.	. 🔻	For dispense	e State			Cells1	Cells2	Signal 🔺			/	
12	56	yes		yes		Good		1	0	803			/	
12	64	yes		yes		Good		1	0	1023		/		
12	69		C	opy		<u> </u>	-	1	0	760				
13	4		0	opy all				1	0	990				
13	10			u u u				1	0	1183	457		133	3
13	15		Se	elect all				1	0	406	Texas Red : R12C69			l
13	24		Co	olumns				1	0	961				
13	34	~	Υ Aι	utosize colu	mns			1	0	718				
13	35		Ex	clude select	ed well	s		1	0	1055				
13	37		In	clude select	ed well	s		1	0	1173				
13	47		CI	lear all man	ual ove	rrides		1	0	392				
13	66	yes	-	yes	-	0000		1	0	1079				
14	26	ves		ves		Good		1	0	815 *				
Row	Col	Mave	Size	Circularity	Inertia	Convertity	AvaSia	nal Ave	SignalSi) IntenSi			/	
12	69	1	118	0.85	0.43	0.97	760	an Avg	220	8963				
				0.00	55	0.07								
<										>	151		65	5
		ntel CC	0252	2.2 (not	ad)									-
in (Expe	inne	ants (SC	(9205)	2_2 (not sav	euj									

Figure 11. 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 tealcolored circle in both the single- and multi-well images, while wells containing non-candidate cells (such as dead cells, see the Table VI in the <u>Appendix</u>, 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 12, below).



Candidate well shown in single-well image

Figure 12. 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 Table VI in the <u>Appendix</u> (below).



Figure 13. Closeup of wells, showing cell outlines.

G. 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.
- 3. Add a comment to the selected well by typing it into the "Comment" field and pressing [Enter]. You can also double-click on one of the already defined comments in the list to edit the contents.

Barcode	State	Cells1 Cells2	Signal1	Signal2	Confidence	Comment
ACCAACCG	PACE				1.00	
ACCAACGA	RUCS		×		0.79	Comment1
AAGCCAAG	Use - Next Well	Reject - Next We	ell		0.92	Comment2
AAGCCATA		-			1.00	
ACCAACCA	Previous	Next			0.49	
ACCAACCT	Undo				0.78	
ACCAACCT	Crido				0.91	
AAGCCAAC		~ .			0.96	
AAGCTTCT	Insert Comment	Clear comment			0.54	
AAGTTGGT	Comments:				0.86	
AACCAAGC	Comment2				1.00	
AACCAATA	Comment1				1.00	
AACCAATC	Comment2				0.80	
AACCAACG					1.00	

Figure 14. Adding comments to selected wells.

H. 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. You should always save the wcd file in the same folder where the associated images are located. When you copy or move files to another hard drive or a network drive you should always move or copy the entire folder with all the files it contains.

🔡 Save					×
Save in:	92532_2		G 👂 😕 1	···· •	
Quick access	Name 92532.wc 92532_Tes	d st.wcd	Date 2/26/2017 10:35 PM 5/22/2018 4:26 PM	Type WCD File WCD File	Size 5,708 KB 6,352 KB
Desktop					
Libraries					
This PC					
Network	< File <u>n</u> ame:	92532_Test.wcd		~	> <u>S</u> ave
	Save as type:	CellSelect files		~	Cancel
	Save filter f	ile (.csv) 🗹 Save	e report file (.pdf) 🗹 Sav	e well list (.txt)	.:1

Figure 15. Saving the results file.

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

Actions	Actions Help								
Rota	Rotate images								
Load	Load sequencing results								
Dow	/nselect 🔓								

Figure 16. 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 samples).

Downselect		
Select the maximum number of wells to	be dispensed for each of t	he samples.
Neg Ctrl	Þ 💠	Max: 47
Pos Ctrl	0 ≑	Max: 48
sample	0 ≑	Max: 779
Total: 0	ОК	Cancel

Figure 17. Enter the number of nanowells for controls and samples.

- 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>_FilterFile.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. Use the filter file (.csv) for dispensing the RT reaction mix on the ICELL8 cx unit.

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 18. 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 contextspecific menu options. Users are encouraged to try right-clicking while using the software to access numerous features.

۷	Vells	Sumr	nary	Set	tings	Мар							
	Row	Col	Ca	ndid	ate	For dis	pense	ense Barcode					
	0	1		no		n	0	TCAGGTCGCCG					
	0	2			0								
l	0	3				ру			N				
	0	4			Co	opy all							
	0	6			Se	lect all							
l	0	7			Сс	olumns							
	0	8		~	Au	ıtosize	colum	ns					
	0	9			Ex	clude s	elected	wells					
	0	10		Include selected wells									
	0	11		Clear all manual overrides									
	0	12		по		n	0	TGGCCAGCAGT					

Figure 19. 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 19, above). This action will open a *Selected fields* dialog box with checkboxes next to the information categories (see Figure 20, 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.

lame	Display
Row	✓
Col	✓
Candidate	1
For dispense	✓
Sample	✓
Barcode	✓
State	✓
Cells1	✓
Cells2	✓
Signal1	✓
Signal2	✓
Confidence	~

Figure 20. 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 ++ 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 ++ symbol, then drag the border left or right (see Figure 21, below).

				-		-		-	→	
	Manua	triage		Save fil	es]		Hoechst : R0C2	
Ы	wells					[₹↓			
-	Cells1	Cells2	Signal1	Signal2	Cor	nfic	^			
i	1	0	578			1.(
i	1	0	664			1.(
i	1	0	633			0.5				
1	1	0	737	1		0 4				

Figure 21. 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:

Function
Copies all selected rows to the clipboard.
Copies the entire table, including the header row, to the clipboard.
Selects all rows. Click anywhere in the table to deselect all rows except the clicked row.
Launches the <i>Selected fields</i> window to customize the columns to be displayed.
When turned on, the software will automatically adjust the column widths to accommodate the length of the data being displayed.
Includes rows that were not selected as candidates and/or excludes rows that were previously selected as candidates.
Removes all Include and/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 2. Main Window Menu Items.

Menu Item	Option	Function		
File	Open result file	Opens a previously saved result file (.wcd).		
	Save files	Processes and saves the results for the current chip*.		
	Open chip folder…	Opens the chip folder containing images created by the Micro- Manager software.		
	Load barcode file	Loads the configuration file that identifies each well in the chip with a barcode.		
Advanced	Switch to advanced user mode	Displays additional technical attributes of each well.		
	Admin mode	Password protected.		
	Image viewer…	Adjusts image size, brightness, contrast, and well overlay. See Section III.G (below) for more details.		
	Composite image	Displays the multi-well image.		
	Settings	Displays the preconfigured settings for single-cell analysis.		
Actions	Downselect	Selects the number of wells to be dispensed for each of the samples.		
	Downselect – clear	Removes existing Downselect settings.		
	Rotate images	Rotates all images 90° in the image folder and saves the rotated images to a separate folder. (This action is only needed if a mistake was made during dispensing. Contact technical support at <u>technical support@takarabio.com</u> before taking this action.)		
	3D – stack	Opens a 3-D viewer to inspect images from individual z-planes.		
Help	About	Displays the software version and End User License Agreement (EULA).		
	Open user manual	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. Process Images

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



Figure 22. The software analyzes well images.

After the software finishes the analysis, it prompts you to save the results file. When you click [Yes] the software will prompt you to enter a file name and will then 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 "Settings" in <u>Section III.G</u>.
- 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 23 (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_10202015_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.
- The software will also generate a PDF file called 72030_09282015_Report.pdf that contains a short summary of the results.
- Also, a file 72030_09282015_WellList.txt is generated that contains the content of the Wells table in a form that can be read easily by downstream analysis software.

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Copy Paste Copy path Clipboard	Move to • X Delete • Copy to • Organize New	Properties Open	Select all Select none Invert selection Select							
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Name	Date	Туре	Size Ti ^							
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📋 72030_10202015_WellList.TXT	10/20/2015 1:21 PM	Text Document	226 KB							
72030_10202015_FilterFile.csv	10/20/2015 1:21 PM	CSV File	11 KB							
72030_10202015.wcd	10/20/2015 1:21 PM	WCD File	7,952 KB							
Fiducials.xml	10/20/2015 1:18 PM	XML Document	2 KB							
Pos143_Hoechst_L12.tif	7/9/2015 12:27 PM	TIFF image	8,259 KB							
🛃 Pos143_Texas Red_L12.tif	7/9/2015 12:27 PM	TIFF image	8,259 KB							
C 1011 1 1 141.7	7/0/2015 12 27 014	TICE -	>							

Figure 23. Files generated by ICELL8 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.

Ε. **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.



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

Adva	nce	ed Ad	tior	ns Hel	р																		
	Swi	itch to a	dva	inced use	er mode																		
	Adi Ima Coi Set	min mo age viev mposite tings	de ver : im	 age																			
S												Wa	ferGen Ce	IISelect -	Chip 72030)							
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me Adva Define fiduci sal usable w ells Summa Row Coll C 70 47 70 60 11 36 17 3 51 15 63 21 26 27 26 32 26 32 26 32 27 2	ials [ells: 11 ary Setti	Process images 613 candidates 96 image Map For dispense	Sample D2 D2 B1 B2 B1 B2 D2 A1 A1 C1 C1 C1 D1	Ne names Mar Hide control well TIGAAITIGCTT TITGTAAGATG CCAACTECTCC GTATGCTCAT TIGGTAGGACC ACCTCACGGCAA CCTCCGGTATGAT CCGGGAGCGCACA	Is State A HasDeadCells HasDeadCells HasDeadCells HasDeadCell HasDeadCell Inconclusive Inconclusive Inconclusive Inconclusive Inconclusive	Save fil 3 2 2 2 2 2 1 1 1 3 1 0	Cells2 1 3 1 1 1 1 2 3 4 1 1	Signal1 Sign 23 2448 24 511 19 763 15 2263 24 575 31 328 19 759 46 16 694 17 28	al2 Sia 5 11: 3 7 0 1 1 3 1 5 8 3 5 7 3 1 8 3	ze1 Siz 65 23 73 79 55 16 41 36 82 80 83 33 40 71 78 41 85 41 85 41	a2 integ Signal1 3 301104 5 40069 8 8508 2 307768 4 6000 9 17284 5 56729 1 5 8990	Integ Signal2 15870 17958 10890 6150 20418 27492 7420 35880 6683 7011 16980	Circularity1 0.84 0.87 0.87 0.87 0.88 0.94 1.00 0.91	Circularity2 1.00 0.88 0.94 0.98 0.95 0.98 1.00 0.98 0.98 1.00 0.98 1.00	Confidence 0.95 0.88 0.51 0.86 0.67 0.47 0.83 1.00 0.92 0.83 0.91 0.95	Confidence1 0.95 0.89 0.57 0.86 0.67 0.48 0.67 0.48 0.69 1.00 0.95 0.89 0.91 0.95	Confidence2 1.00 0.98 1.00 1.00 0.97 0.93 1.00 0.96 0.93 1.00	Dispense tig 8 4 3 4 1 1 5 5 5 5 7	Drop index 196 183 25 58 181 109 4 30 58 103 95 3	Global drop index 247 236 34 122 226 136 4 36 126 126 126 122 127 127 13 3	Source well 02 02 82 81 82 02 41 41 41 61 61 61 01	Image1 Pos133_Hoechst_L08. Pos142_Hoechst_L08. Pos18_Hoechst_B0731 Pos18_Hoechst_B0731 Pos18_Hoechst_B0731 Pos19_Hoechst_B0731 Pos19_Hoechst_B0731 Pos19_Hoechst_B0731 Pos19_Hoechst_E0531 Pos53_Hoechst_E0531	Image2 Post 33_Texas Red_LL Post 42_Texas Red_D2 Post 3_Texas Red_D2 Post 3_Texas Red_d20 Post 3_Texas Red_d20 Post_Texas Red_d26 Post_Texas Red_d26 Post 3_Texas Red_D26 Post 3_Texas Red_D26 Post 3_Texas Red_D26 Post 3_Texas Red_D26
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Figure 25. 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.

fiduc	cials	Process in	nages S	ample names	Manual	triage S	ave files					
>	Pos	33 1-Hoechst	C10.tf	~								
Ja u	valle.	828 candidat	ee 67 contro	• 🗆 Hida a	antrol unalla							
	none.		Co or contro	• [] hide c	UNU WEIS					AL		
JULIU	nary Se	ettings Map								Z.		
ple Wells		Candidates	didates For dispense Dye1 total		Dye2 total	Dye1 empty wells	Dye1 1-cell we	lis Dye1 2-cell well	Is Dye1 3-cell wells	Dye1 4-cell wells		
ri ri	48	30	30	12	0	30	10	4	0	0		
	5071	840	828	2225	15	3329	1319	367	48	7		
	73											
	[Takar	aBio CellSe	lect - Chip	92532							
			duanced	Actions	Help							
		File A	advanced –									
		File A	uvanceu	Actions			1					
		Define fi	ducials	Process in	lages S	ample names	Manual tri	iage Sav	e files			
		Define fi	ducials	Process in 1-Hoechst	ages S	iample names	Manual tr	iage Sav	e files			
		File A	ducials Pos33	Process in	ages S	ample names	Manual tr	iage Sav	e files			
		File A Define fi < > Total usabl	ducials Pos33 e wells: 8	Process in _1-Hoechst	ages S _C10.tif es 67 control	iample names	Manual tri	iage Sav	e files			
		Total usabl	ducials Pos33 e wells: 8 mmary Sett	Process in _1-Hoechst, 28 candidat	ages S _C10.tif es 67 control	iample names	Manual tr	iage Sav	e files			
		Total usabl	ducials Pos33 e wells: 8 mmary Sett	Process in _1-Hoechst 28 candidat ngs Map andidates	ages S _C10.tif es 67 control	ample names	Manual tri ntrol wells	iage Sav	Dye1 1-cell wells	Dye1 2-cell wells	Dye1 3-cell wells	Dye1 4-cell
		File A Define fi < > Total usabl Wells Su Sam	ducials Pos33 e wells: 8 mmary Sett Wells C 631	Process in _1-Hoechst, 28 candidat ngs Map andidates 108	ages S _C10.tif es 67 control For dispense 107	iample names	Manual tr ntrol wells Dye2 total Dy 1	ve1 empty wells	Dye1 1-cell wells 164	Dye1 2-cell wells 54	Dye1 3-cell wells 4	Dye1 4-cell
		File A Define fi Control usable Wells Su SamA A1 A2	ducials Pos33 e wells: 8 mmary Sett Wells C 631 632	Process in _1-Hoechst, 28 candidat ngs Map andidates 108 106	For dispense 107 105	e Dye1 total 288 266	Manual tr ntrol wells Dye2 total Dy 1 2	ve1 empty wells 408 419	Dye1 1-cell wells 164 163	Dye1 2-cell wells 54 45	Dye1 3-cell wells 4 3	Dye1 4-cell 1 1
		File A Define fi <	ducials Pos33 e wells: 8 mmary Sett Wells C 631 632 636	Process in 1-Hoechst 28 candidat 28 candidat 28 candidates 108 106 116	ages S _C10.tif es 67 control For dispense 107 105 114	e Dye1 total 288 266 297	Manual tr ntrol wells Dye2 total Dy 1 2 2	ve1 empty wells 408 419 402	Dye1 1-cell wells 164 163 179	Dye1 2-cell wells 54 45 48	Dye1 3-cell wells 4 3 6	Dye1 4-cell 1 1 1
		File A Define fil C 2 Total usabl Wells Su Sam_A A1 A2 B1 B2	ducials Pos33 e wells: 8 mmary Sett Vells C 631 632 636 636	Process in 1-Hoechst, 28 candidat ngs Map andidates 108 106 116 94	ages S _C10.tf es 67 control For dispens- 107 105 114 92	e Dye1 total 288 266 297 258	Manual tr ntrol wells Dye2 total Dy 1 2 2 4	ve1 empty wells 408 419 402 434	Dye1 1-cell wells 164 163 179 152	Dye1 2-cell wells 54 45 48 45	Dye1 3-cell wells 4 3 6 4	Dye1 4-cell 1 1 1 1
		File A Define fi Cotal usable Wells Su Sam/ A1 A2 B1 B2 C1	ducials Pos33 e wells: 8 mmary Sett Wells C 631 632 636 636 636	Process in 1-Hoechst, 28 candidat ngs Map andidates 108 106 116 94 103	ages S _C10.tf es 67 control For dispense 107 105 114 92 102	e Dye1 total 288 266 297 258 263	Manual tri ntrol wells	ve1 empty wells 408 419 402 434 422	Dye1 1-cell wells 164 163 179 152 171	Dye1 2-cell wells 54 45 48 45 37	Dye1 3-cell wells 4 3 6 4 6 6	Dye1 4-cell 1 1 1 1 0
		File A Define fi Contal usable Wells Su Sam_A A1 A2 B1 B2 C1 C2	ducials Pos33 e wells: 8 mmary Sett Wells: 631 632 636 636 636 636 636	Process in 1-Hoechst, 28 candidat andidates 108 106 116 94 103 105	ages S _C10.tf es 67 control For dispense 107 105 114 92 102 102	ample names → Hide con e Dye1 total 288 266 297 258 263 269	Manual tri ntrol wells Dye2 total Dy 1 2 2 4 0 0 0	ve1 empty wells 408 419 402 434 422 428	Dye1 1-cell wells 164 163 179 152 171 158	Dye1 2-cell wells 54 45 48 45 37 41	Dye1 3-cell wells 4 3 6 4 6 7	Dye14-cell 1 1 1 1 0 2
		File A Define fi Control usable Wells Su A1 A2 B1 B2 C1 C2 D1	ducials Pos33 e wells: 8 mmary Sett Wells C 631 632 636 636 636 636 636 636 636	Process in 1-Hoechst, 28 candidat andidates 108 106 116 94 103 105 108	res 67 control For dispense 107 105 114 92 102 102 107	ample names → Hide con Pe Dye1 total 288 266 297 258 263 269 302	Manual tri ntrol wells Dye2 total Dy 1 2 2 4 0 0 0 1	xe1 empty wells 408 419 402 434 422 428 403	Dye1 1-cell wells 164 163 179 152 171 158 170	Dye1 2-cell wells 54 45 45 48 45 37 41 46	Dye1 3-cell wells 4 3 6 4 6 7 7 12	Dye1 4-cell 1 1 1 1 0 2 1
		File A Define fi Control usable Wells Su A1 A2 B1 B2 C1 C2 D1 D2	ducials Pos33 e wells: 8 Wells C 631 632 636 636 636 636 636 632 632 632	Process in 1-Hoechst 28 candidat 28 candidat 28 candidat 28 candidates 108 106 116 94 103 105 108 100 100	hages S C10.tif	ample names → Hide con P P P P P P P P P	Manual tri ntrol wells Dye2 total Dy 1 2 4 4 0 0 1 5	ve1 empty wells 408 419 402 434 422 428 403 413	Dye1 1-cell wells 164 163 179 152 171 158 170 162	Dye1 2-cell wells 54 45 45 45 37 41 46 51	Dye1 3-cell wells 4 3 6 4 6 7 7 12 6	Dye1 4-cell 1 1 1 1 0 2 1 0
		File A Define fi C 2 Total usable Wells Su Wells Su A1 A2 B1 B2 C1 C2 D1 D2 Neg Ctr	ducials Pos33 e wells: 8 Wells C 631 632 636 636 636 636 636 636 632 632 1 48	Process in 1-Hoechst 28 candidat 28 candidat 28 candidat 28 candidat 28 candidat 108 106 116 94 103 105 108 100 30	res 67 control For dispense 107 105 114 92 102 102 102 107 99 30	ample names	Manual tri ntrol wells Dye2 total Dy 1 2 2 2 4 0 0 0 1 1 5 0 0	xe1 empty wells 408 419 402 434 422 428 403 413 30	Dye1 1-cell wells 164 163 179 152 171 158 170 162 13	Dye1 2-cell wells 54 45 48 45 37 41 46 51 4	Dye1 3-cell wells 4 3 6 4 6 7 12 6 0	Dye1 4-cell 1 1 1 1 1 1 2 1 0 2 1 1 0

Figure 26. *Summary* data table.

The Summary data table includes the barcode filenames and downselect information.

ells Sum	mary S	ettings Map					
Sample	Wells	Candidates	For dispense	Dye1 total	Dye2 total	Dye1 empty wells	[
Neg Ctrl	48	47	47	0	1	48	
Pos Ctrl	48	48	48	0	0	48	
sample	5071	600	400	4907	141	2292	
<							
< Barcode	file: 3 Requ	'DE.gal ested items	For Dispense				
< Barcode Sample	file: 3 Requ	'DE.gal ested items 400	For Dispense 400	_	_		
< Barcode Sample Sample Neg Ctrl	file: 3 Requ	DE.gal ested items 400 47	For Dispense 400 47				

Figure 27. Summary data table with barcode filenames and downselect information.

If you have to replicate barcodes, the system may not always be able to satisfy the full number of requested wells without selecting duplicate barcodes. In that case, the number of wells for dispense may be less than what was requested.

NOTE: The Downselect table is only visible if downselect is in effect.

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.

	DADI	NOT To Bad		
Dye	es: DAPI co-located with	: NUT TexasRed		
	Gamma	2	^	Esta
	Min AutoContrast Range	500		Edit
	PaintOutline	True		
>	PaintOutlines	False		
~	Fiducials			
	Auto Detect Fiducials	True		
	FiducialsInChannel2	True		
~	Image processing			
	ExtraSegmentations	6		
	OpenIterationsAfterThresh	2		
	Scale Steps	6	~	
Op	en Iterations After Thres	hold		
Nur	mber of 'open' operations.			

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

- 1. Select **File** > **Load** (see Figure 29, below).
- 2. Select the appropriate XML file for the type of ICELL8 chip being used.

Table 3. Chip XML file selections.

Chip type	Cat. No.	XML file name
ICELL8 250v Chip	640183	AnalysisSetting_250nL_chip.xml
ICELL8 350v Chip	640019	AnalysisSetting_350nL_chip.xml

	Analysis settings	×				
	File Load Styve NOT TexasRed					
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	Comment Comment Display Fiducials Image processing					
	Extra Segnentations 6					×
	← → · ↑ 🔤 « SingleCell → AnalysisSettings	: >	predefined 🗸 Ö	Search predefine	d	م
	Organize 🔻 New folder			-	== - [
	SingleCell	^	Name		Date mo	dified
	AnalysisSettings		AnalysisSetting 250nl	chip.xml	12/3/201	8 4:16 PM
	predefined		AnalysisSetting_350nL_	chip.xml	2/4/2019	7:19 PM
	AssayMaps					
	Hardware					
	Log					
	PostRun					
	Protocols					
	SampleLayouts	J	<			>
	File name: AnalysisSetting_250n	L_cł	hip.XML ~	Analysis settings	;(*.XML) Car	

Figure 29. Loading/reloading the settings file for single-cell analysis.

The distinction between live and dead cells is defined in the Dye Assignment dialogue box. Here you select what constitutes a "good" cell.

- 1. Select the **Master** dye used to identify cells.
- 2. If necessary, check the **Invert Dye2** check box to indicate that candidate cells should not be visible in the second dye.
- 3. The bottom of the box will display your final rule. The screenshot below (Figure 30) indicates that we want objects that are visible in FITC, but invisible in Texas Red.
- 4. Click [OK].

Dyes FITC co-located with: NOT Texas Red					
Dye Assignment	Dye Assignment				
Dyes					
FITC	Master	O Dye2			
Texas Red	⊖ Master	Dye2			
Co-location					
✓ Invert Dye2					
NOT Dye2					
FITC co-located	with: NOT Texa	as Red			
			ОК	Cancel	

Figure 30. Dye Assignment dialogue box.

Back in the *Settings* window, you can set PaintOutlines, Thresholds, and MinDistanceFromWellCenter for the two dyes.

1. Expand the item to edit the individual values.

~	Thresholds	250 100
	All	
	Master	250
	Dye2	100

Figure 31. Expanded "Thresholds" option in the Settings window.

2. If you want all channels to have the same value, type that value into the All field.

\mathbf{v}	Thresholds	50
	Al	50
	Master	50
	Dye2	50

Figure 32. Selecting the Settings, "Thresholds", "All" option to customize it.

3. When the setting is collapsed, you will see two values separated by a space. If all items have the same value, you will only see one value listed.



Figure 33. The *Settings* "Threshold" section, displaying multiple sub-level values in the collapsed view.

You can also access settings for ScaleSteps and SecondDerivativeScale.

- 1. Cells of significantly different sizes can be detected using the scale space approach (<u>https://en.wikipedia.org/wiki/Scale_space</u>).
- 2. The **SecondDerivativeScale** value indicates the filter kernel size for the first scale level.
- 3. For each additional **ScaleStep**, the image is blurred with a Gaussian kernel of radius sqrt(2).

ScaleSteps	3
SecondDerivativeScale	1.80

Figure 34. The "ScaleSteps" and "SecondDerivativeScale" options in Settings.

Мар

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 35, below).



Figure 35. Map tab.

F. 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 36. Single-well image.

G. 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 37. *Image Viewer* window.

File View Print Image contrast Map window Zoom in / out Auto contrast Map window Image contrast Well overlay Wavelength 1 / 2

Figure 38. Image Viewer toolbar icons.

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 39, below). Drag the control points with the mouse to adjust the mapping.



Figure 39. Image contrast settings window.

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 40, 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 40. Adjusting image contrast using the slider.

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.

Auto contrast					
AutoContrastAfterImageLoading	True				
AutoContrastAfterSnap	True				
HighCutoffPercent	99.99				
LowCutoffPercent	20.00				
AutoContrastAfterImageLoading Perform an auto contrast operation whe	enever an image is loaded from file.				
	Done				

Figure 41. Auto contrast settings window.

Table 4. Auto Contrast Settings.

Option	Function
AutoContrastAfterImageLoading	Perform an auto contrast operation whenever an image is loaded from a file.
AutoContrastAfterSnap	Perform an auto contrast operation whenever an image is taken with the camera.
HighCutoffPercent	The high threshold is set such that all pixels above the HighCutoff percentage are colored white.
LowCutoffPercent	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 42. Viewing a subsection of an image using the Map window icon and *Image map* window.

H. 3-D Stack Control

When scanning a chip, the ICELL8 cx system acquires images at different z-planes to capture cells that may not be at the bottom of the well. The cx System software combines (flattens) the images from the various z-planes to create a single image that will be further analyzed.

1. Enter the 3-D Stack Control from the Action > 3D - stack menu to review the images from the individual z-planes.

10	Karabio Cense	sect - 020	13
ile	Advanced	Actions	Help



2. You will be prompted to specify the **parent folder** that contains the z-plane images sub-folders. In most cases, this should be the chip folder.

🔜 Select Folder			×
\leftarrow \rightarrow \checkmark \uparrow \Box << Experiments \rightarrow SC \rightarrow Biodot \rightarrow 82879	~ Ō	Search 82879	م
Organize 🔻 New folder			== • ?
2016.06.22.12.43-75674-blank ^ Name ^		Date	Туре
75674-2016.06.22.14.16-no-centrifuge		7/8/2016 10:15 PM	File fold
77777-SmallSteps z2		7/8/2016 10:19 PM	File fold
82879 z3		7/8/2016 10:19 PM	File fold
82879_extras		7/8/2016 10:20 PM	File fold
85049_Olympus1		7/8/2016 10:21 PM	File fold
85049 Olympus2		7/8/2016 10:22 PM	File fold
96404 pre		7/8/2016 10:23 PM	File fold
20160620 85049 1p03			>
Eolder 82879			
		Select Folder	Cancel

Figure 44. Example of the location of the z-plane images sub-folders, shown in Windows Explorer. 82879 is the ID of the chip and therefore the chip folder; folders z1–z7 are the image sub-folders.

3. Next, you will be asked to select the z-planes that you want to examine. It is recommended that you select all z-planes, but you can check or uncheck the folders to include and/or exclude, if needed. Click [Ok] to proceed; the *3D Stack Control* dialogue window will pop up.

Select items
 H:\Experiments\SC\Biodot\82879\z1 H:\Experiments\SC\Biodot\82879\z2 H:\Experiments\SC\Biodot\82879\z3 H:\Experiments\SC\Biodot\82879\z4 H:\Experiments\SC\Biodot\82879\z5 H:\Experiments\SC\Biodot\82879\z6 H:\Experiments\SC\Biodot\82879\z7
Select all Deselect all
OK Cancel

Figure 45. Selection window for inclusion/exclusion of the z-plane images sub-folders.

3D Stack Control dialogue window

🖷 3D Stack Control	×
Z: 2 - Pos: 7 - Wave DAP	I ~
Flatten All Flatten	0 🜲
Set root Show flat Calc shift	Align

Figure 46. Close-up of the 3D Stack Control dialogue window.

Table 5. 3D Stack Control dialogue window options.

Option	Function	
Z	Value indicates which z-plane image file to display.	
Pos	Specifies the position (i.e., well) image to display.	
Wave	View the DAPI or Texas Red channels.	
Flatten All	Flatten all images and save the resulting images in the parent (root) folder. IMPORTANT: Existing images in the root folder will be overwritten.	
Flatten	Flatten the z-plane images for the current position ("Pos") and display the resulting image.	
Set root	Re-loads the root (parent) folder and returns to the z-folder selection screen (step 3, above).	
Show flat	Shows the flattened image that exists in the root folder.	
Calc shift	See the "Image shift" section, below.	
Align	See the "Image shift" section, below.	

eriments\SC\Biodot\82879\z1\Pos7_1-DAPI_A8.tif	A	В
© D Stack Control Z: 1 → Pos Flatten All Set root S	× 7 ♥ Wave DAPI ♥ Ratten 0 ♥ how flat Calc shift Align	Image: Set root Show flat Calc shift Align
K 1031 € 3D Stack Control	5680 C	D 3D Stack Control
⊘ Z: 3 → Pos: Ratten All Set root Sh	7 Wave DAPI Flatten 0 ow flat Calc shift Align	Z: 4 Pos: 7 Wave DAPI Ratten All Ratten 0 Set root Show flat Calc shift Align

Figure 47. Example of viewing the same well through its z-planes. From A–D, the image cycles through layers 1–4, showing a different view at each layer.

Image shift

A problem that sometimes occurs is that, due to vibrations, individual images in a z-stack are not perfectly aligned, but instead are shifted by one or more pixels. On rare occasions, when a single cell is equally in focus in two z-planes but it is shifted by a large amount on the x- and y-axes, the resulting flat image would appear to contain two separate cells.

The [Calc shift...] button in the *3D Stack Control* dialogue window initiates an alignment procedure between the image in the current z-plane and the one above it; the estimated shift between the two images is reported.

To fix the shift problem, the "Align" option corrects for the shift during flattening.

NOTE: During a chip scan, the images are flattened by the cx instrument software, not CellSelect. Therefore, the "AlignImagesBeforeFlattening" option is also present in the cx software.

If you've upgraded from a previous version of the CellSelect cx software, the option can be turned off (set 'False') to maintain consistency with previous scan results. For the 3' DE / TCR application, it is off by default.

o App	p p	roperties		
Ap	op	SMART-Seq		~
• •	-	Filters		_
		FilterConfiguration	DAPI-TexasRed	
	/	Focus		
		AlignImagesBeforeFlattening	True	\sim
1		DepthIntoWell	2.2	
2		ExtraFocusDelta	0.06	

Figure 48. The "AlignImagesBeforeFlattening" Focus option in the ICELL8 cx System software.

IMPORTANT: If the "Align" button toggle is changed, you will need to click [Flatten All] to regenerate the flattened images.

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 6. Nanowell Status Descriptions and Sample Images.







Cell appears viable in top view but is also visible in bottom view.

Inconclusive and

Has Dead Cells







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.

NoCells



Low Confidence Cell is not clear.

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