

WOLFVIEWER 2.4 RELEASE NOTE

Dear WOLF® Customer,

NanoCellect Biomedical is pleased to be offering you WOLFViewer 2.4, a new version of our intuitive software with several new features and improvements aimed at enhancing your experience operating the WOLF Cell Sorter.

New Features Include:

Fluidics Monitor – A simple new tool to track and warn you of your sample and sheath usage.

PZT Sorting Speed Tool – A feature that displays real-time sort speeds, allowing you to optimize sample concentration or target population.

Faster Single Cell Plating, Lower Droplet Volume and PCR Sidewall Dispensing (N1 users only) – An elective, opt-in feature which allows users to choose a faster single-cell dispensing speed for 96- and 384-well plates. These features are still considered "in-development," and is being optimized, so you will need to get into contact with your local FAS or Tech Support to access them.

We have made a number of general improvements and bug fixes as well. Keep reading for more details.

New Features

FASTER SINGLE CELL PLATING & DECREASED DROPLET VOLUME

You now can single cell plate your samples up to 3X faster! Choose from 2 speed modes which can be customized per plate. The "Standard" mode is the same settings you have been accustomed to already. To access the "Fast" mode, first go to "Single Cell" menu and choose "Single Cell Settings."





A pop-up message will appear. Choose "Enable Beta Features."



Select "Yes." To access features, <u>contact your FAS or NanoCellect Tech Support for the password.</u>

Once you enter the password, the following window will appear. Click "Apply."

Single Cell Sort Settings							
Enable Beta Features?	Yes	○ No					
PCR Sidewall Dispensin	○ Yes	No					
		_					
	Apply						

The next time you start a single cell workflow, you will see the following options under "Plate Speed" in the Single Cell grid map.

O Select 2 Wells											×												
			Clear S	elect Plate:	384Defau	itPlate		T Pro	-fill Volume	K 50	# of Ce	lls Per Wei	t 1	Dep	osit cells i	n focusing	wells Plat	te Speed:	Standard	Appl	y		
A1	A2	A3	A4	AS	A6	A7	AB	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	Standard Fast	421	A22	A23	A24
B1	B2	83	B4	85	Bő	87	BB	89	B10	811	812	813	B14	815	B16	817	818	B19	B20	821	B22	B23	B24

Choose "Fast" for a 3 min 96-well dispense time or about 9 minutes for 384-well dispense time.

Droplet volumes are about 2.5µl for 96-well plates and about 2.0µl for 384-well plates. Single cell efficiencies range from approximately 70-80% single cells per plate.

Once you have enabled "Beta features," they will appear every time you open WOLFViewer. To disable them from showing in the Single Cell Grid Map, click "No" under the Single Cell Sort Settings.



PCR PLATE SIDEWALL DISPENSING

For users who dispense into PCR plates, we have developed an optional setting that allows the needles ends to touch the side of the well wall, specifically useful for PCR plates to ensure proper droplet dispensing. Users will notice that the needle ends make a "swooping" motion in order to dispense onto the well wall and not the bottom of the well.

To choose this option, make sure to enable the feature from the "Single Cell Settings" menu as previously described. Click "Apply."



When you setup a PCR plate from the Single Cell Grid Map, (be sure that you have chosen a PCR Plate Spec file) the "Sidewall Dispensing" check should be enabled. Then you can continue in your single cell plating workflow as usual.



PZT SORTING SPEED TOOL

Click the "Tools" tab on the menu bar and click "PZT Sorting Speed."

This tool will display the sort speed for the A and C channel, as well as well as total event rate. This will allow you to adjust your sample conditions (sample concentration, target population %, if possible) to optimize for maximal sort speed. This tool is intended for bulk sorting.





FLUIDICS MONITOR

Travel to the lower right corner of WOLFViewer, under "Prepare for Transport." If you would like WOLFViewer to track your sample and sheath volume level, click "Track: On."

You will be brought to this menu:

Fluidics	Monitor			×						
-Sample-			Sheath	Sheath						
		Adjust Volume		Adjust Volume						
		+ h.0 mL Fill Amoun		+ 0.0 mL Fill Amount						
	Max : 5mL 3.0 mL 60 %	- 0.0 mL Remov	50.0 mL Max: 50mL Min : 0mL 100 %	- 0.0 mL Remove Amount						

The Fluidics Monitor is intended to track your sample and sheath volumes once you have finished calibration and are completing long sorts. This is a useful tool to avoid accidentally aspirating air from your empty sample or sheath tube.

Adjust your volumes as necessary by modifying the fields next to "Fill Amount" or "Remove Amount."

If the tool has "Track: On" enabled, then WOLFViewer will provide a pop-up warning of "Low Volume" at 200µl (0.2mL) for sample volume and 8mL for sheath. This will allow you some time to pause, and add more buffer or sample as needed for your experiments.

Make sure to adjust the volumes in the Fluidics Monitor when switching sample tubes for PBS tubes for flushing. The tool will not know when the user has switched from a cell or PBS sample without adjusting the volume.

IMPROVED WORKFLOW FEATURES



AXIS NAMING COMPATIBILITY

If you decide to change the name of your Axis (Click "View" on the menu tab then click "Axis Name"), WOLFViewer will use a nickname in the fcs file. This was completed so there is a seamless transition of the fcs files created in WOLFViewer to another FCS Software such that your analysis can continue uninterrupted in the other software.

AUTOMATIC MATRIX USAGE

When you create a compensation matrix during analysis, it will automatically be applied for sorting to avoid forgetting a compensation matrix before an important sort. Be sure that the matrix file name is the one intended for that particular sort.

GENERAL IMPROVEMENTS

- 1. PDF reports now include sample concentration.
- 2. WOLFViewer 2.4 supports all Second-Generation Cartridge priming and auto alignment algorithms.
- WOLFViewer 2.4 supports 9-digit cartridge serial codes, specific for Second-Generation Cartridges. Scan Second-Generation Cartridges as usual with the QR code reader, or type in the 9-digit code without any spaces or breaks.
- 4. At the end of a bulk sort, you will now get the notification, "Please remove collection tubes and replace with waste container" to signify the end of a sort session.



BUG FIXES

- 1. Contour plots are re-added.
- 2. Software only allows for 3 re-primes, not 4.
- 3. Width parameter name is now reflective of newly named fluorescence axis.
- 4. N1 plate times are reflective of time when stopped, not when the csv file was saved.
- 5. An extra calibration error code (07) was added to diagnose specific issues in setup process.
- 6. General background stability and robustness fixes.

QUESTIONS? support@nanocellect.com