




QUALITY CONTROL INSTALLATIONS

**QC GUIDELINES FOR CELL SORTER, WOLF CYTOMETER G2, (#XXXXXX) AND
CELL DISPENSER N1 (#130096, #130099, #130384, #130386)**

Abstract

This document contains an illustrated step by step protocol to run a quality control test on the NanoCollect WOLF Cell Sorter G2 and N1 Single Cell Module during installations.

	<h1>Procedure</h1>
Version 0.1	Title: WOLF and N1 Quality Control at installations

1. **Purpose:** To provide step by step instructions on how to perform a quality control test of the WOLF® Cell Sorter G2 during installation at customer site.
2. **References / Quality Records:**
Each WOLF G2 unit is accompanied by its own laser alignment report. The report is delivered in paper format with each unit. The report can be downloaded from Acumatica (internal team) or the Distributor Portal.
3. **Materials Needed:**
 - WOLF Cell Sorter G2
 - Reference cartridge – *each WOLF G2 comes with a reference cartridge that serves as standard.*
 - Laser alignment report – *each WOLF G2 comes with its own report containing data generated at NanoCollect HQ using both the instrument-specific reference cartridge and a sorting cartridge.*
 - WOLF Computer (WOLFViewer software, StreamClient software).
 - Sorting cartridge
 - PBS
 - Calibration beads
 - Rainbow beads
 - Size beads
 - N1® Single Cell Dispenser (if applicable)
 - N1 alignment tool (if applicable)
 - N1 alignment plate (either 96- or 384-well standard tissue culture plate) (if applicable).
4. **Visual inspection** (Figure 1):
 - 4.1 Check for visible damages of the packaging.
 - 4.2 Check the Tip-n-Tell indicator on the side of the box (Figure 1A).
 - 4.3 Check the ShockWatch indicator on the side of the box (Figure 1B).
 - 4.4 Proceed to unpack the WOLF G2.
 - 4.5 With respect to the WOLF G2:
 - Check for dents and scratches on front and back panels, and instrument cover.
 - Check to ensure that front door remains undamaged and on its proper guide rails.
 - 4.6 With respect to the N1:
 - Check the unit and the lid for dents and scratches.
 - Remove the zip-tie locking the Z-motor using clippers prior to turning the module on. This will prevent motor damages from occurring. Be careful not to clip any other cables (Figure 1C).

Take pictures of any visible damage and/or triggered indicators for claim purpose and contact NanoCollect Technical Support at support@nanocellect.com.

Figure 1. Visual inspection during unpacking process.

Figure 1A. Tip N Tell indicators show whether the box was tipped during shipment. If tipped, blue beads would be present within upper triangle.




Figure 1B. ShockWatch indicators show whether the box was dropped during shipment. If dropped, the indicator would turn red.



Figure 1C. The orange zip tie secures the N1 single cell dispenser during shipment. It must be removed prior to turning the N1 on (arrow).



	<h1>Procedure</h1>
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5. WOLF-Computer connectivity (Figure 2)

- 5.1 Set-up the WOLF G2, N1, and computer.
- 5.2 Turn on the WOLF G2. Confirm that the indicator lights turns blue (Figure 2A).
- 5.3 Turn on computer. Log into the nanocellect account (password: nanocellect). Ensure that the computer is connected to the internet.

Note The computer should be connected to the internet to ensure that any software updates that have been released since the computer was brought up at NanoCollect HQ can be upgraded over-the-air. Software updates may occur more frequently given the novelty of this product.

- 5.4 Open StreamClient software, and go to the IP tab (Figure 2D)
- 5.5 Assess which versions of streamclient and firmware are deployed on this WOLF G2 and PC. If applicable, please upgrade firmware and streamclient according to the procedure, “How to upgrade streamclient and firmware for WOLF G2”.
- 5.6 Open the WOLFViewer folder, and assess which version of wolfviewer is present. If applicable, please upgrade software to latest version.
- 5.7 Open WOLFViewer software.
- 5.8 Confirm connectivity: the indicator lights on WOLF G2 should turn green, and connection status indicator in WOLFViewer software should also be green (Figure 2B, 2C).
- 5.9 Within WOLFViewer software, unlock the hidden “settings” tab on the menu bar by simultaneously pressing the following three buttons: ctrl + shift + m
- 5.10 Within the settings tab, select Power User settings to open a pop-up window detailing various settings associated with the software.
- 5.11 Within the power user settings, assess the status of the “homing” and “motor limits”. When using a software version >WV3.0.2016, both the motor limits and homing should be enabled (Figure 2E). If not automatically enabled, please enable the settings, and restart the WOLFViewer software.

If unable to power on the WOLF on, refer to Flowchart WOLF_01 Power On Failure

Figure 2. WOLF-computer connectivity status indicators

Figure 2A. When the series of indicator lights are blue, the WOLF G2 is now ready to connect to WOLFViewer



Figure 2B. Upon starting WOLFviewer software, the indicator lights should turn green, indicating that the WOLF G2 is on AND connected to the software.



Figure 2C. The connection status between the WOLF G2 and computer can also be assessed through connection status panel within WOLFViewer software.

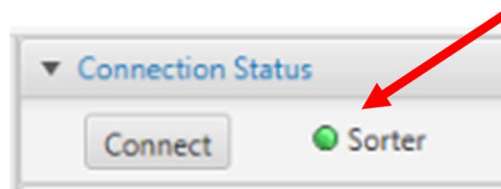


Figure 2D. Assess the streamclient version and firmware version deployed on the WOLF G2 through the use of the IP tab within streamclient.

RT Stream Client

IP Address: 169.254.89.150

Target Info:

Nanocelllect	
Status	
BoardVersion	2
Build	8
Flavor	Original
MAC	00:80:2F:28:C7:83
Product	Wolf
SerialNumber	01EA2EF7
StartupError	0
StreamReadVer	2
StreamWriteVer	2
SwVersion	2.018
TCPListening	TRUE
TCPPort	35949

WOLF Connect Time: 10:11:40 AM 12/17/2021

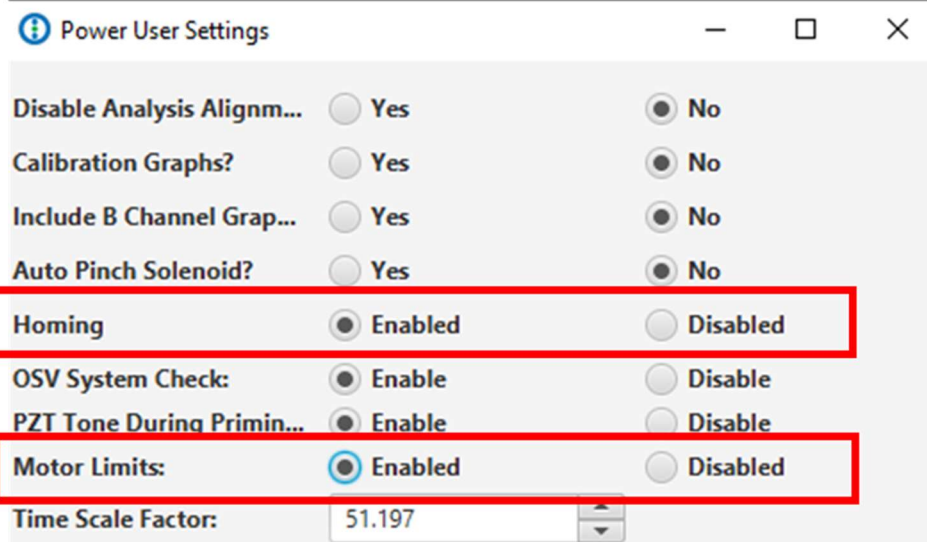
Streamclient Version: V1.055

Firmware Version = Sw Version + Build

Firmware Version: 2.18.8

Figure 2. WOLF-computer connectivity status indicators

Figure 2E. Assess the motor homing and motor limits settings within the power user settings.

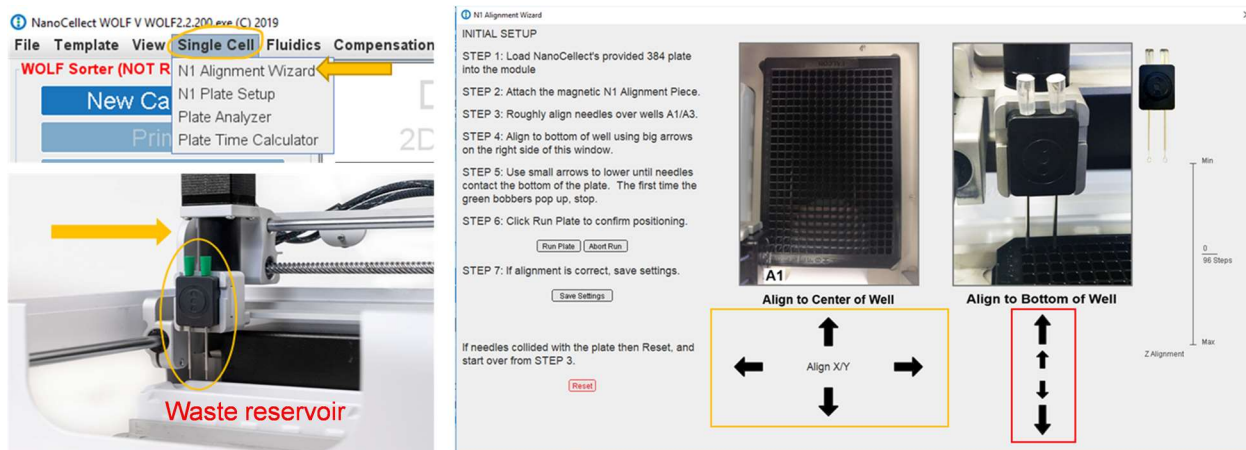


6. N1 functional test: Alignment (if applicable) (Figure 3)

- 6.1 Click on the “Single Cell” tab on the menu bar in WOLFViewer software (Figure 3).
- 6.2 Select the “N1 Alignment Wizard” from the dropdown menu.
- 6.3 Attach the N1 alignment tool on the Z motor head.
- 6.4 Insert the waste reservoir into the N1.
- 6.5 Insert the 384- or 96-well alignment plate into the N1. Plate type will be dependent on whether the customer purchased 96-well only or 96- and 384-well N1 single cell dispenser.
- 6.6 Follow software instructions to align in X, Y and Z-axis.
- 6.7 Run a full plate to confirm proper alignment. Monitor the N1 movement throughout the entire run to ensure that the alignment tool does not hit the bottom or sides of the well plate.
- 6.8 Click the “Save Settings” button.
- 6.9 If applicable, take pictures/videos of all found issues.

Figure 3. N1 Alignment Wizard

N1 alignment wizard interface and N1 tool-waste reservoir positioning.



7. WOLF G2 Reference Cartridge: Optical Alignment Assessment (Figure 4):

- 7.1 Click “New Cartridge” button, and follow the system prompts to insert the instrument-specific reference cartridge and input its ID number.
- 7.2 Click “Open Alignment” button to bring up the chip alignment pop-up window. Position the mouse on the top of the Chip Alignment GUI and type the command “ctrl+shift+m” to display the hidden “Advanced Settings” tab.
- 7.3 Go to the “Auto” tab. Turn on the Pogo-LED light (Figure 4A).
- 7.4 Move the live chip image around using the arrows or clicking in the live image to position the red cross hairs on the left XY-marker (Figure 4A).

Note: If experiencing difficulty locating the XY marker, monitor the live chip coordinates via the Advanced Settings Tab and adjust until they are similar to the values reported within the laser alignment report.

- 7.5 If necessary, adjust the focus in the Z-plane by selecting a step size and moving the arrows. (Figure 4A).

Figure 4. Reference Cartridge Set-up and Qualitative Assessments (cont.)

Figure 4A. Utilize the Auto tab to put the left XY marker into focus within the live camera field of view.

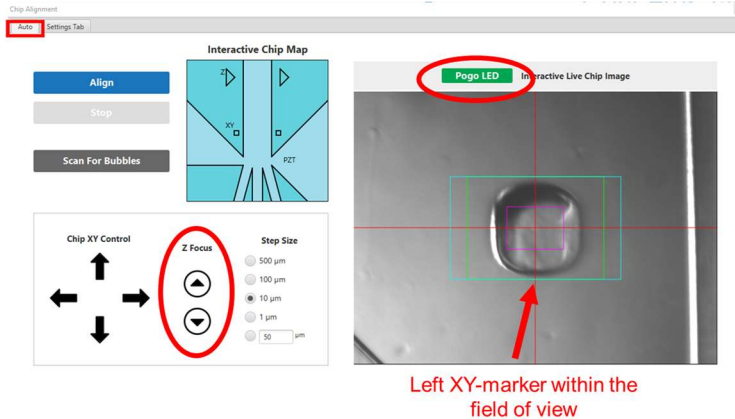


Figure 4B. Activate the focusing box by pressing the mouse center button (red arrow), when cursor is centered on the chip center.

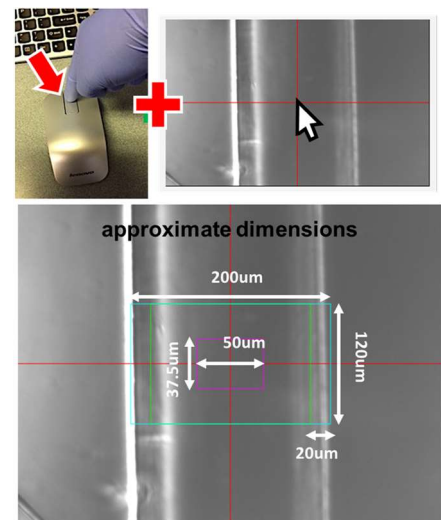
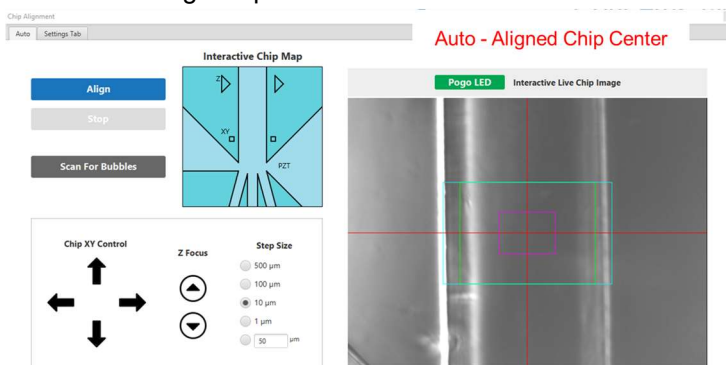


Figure 4C. Auto-align chip center.



Note: if at any point when searching for XY marker, a boundary was reached, please re-home fixture motors by pressing the button within the Advanced Settings Tab within the chip alignment window.

- 7.6 With the left XY-marker in field-of-view, press the “Align” button within the “Auto” Tab.
- 7.7 Once cartridge is aligned, position the mouse on the chip live image and click the mouse center button to enable the laser beam focusing graphic interface (crosshairs). (Figure 4B)
- 7.8 Take a screenshot of the chip center in the “Auto” tab (Figure 4C).
- 7.9 Within the interactive chip map, press the left XY marker. Take a screenshot of XY marker.
- 7.10 Proceed back to the chip center location by pressing its location within the interactive chip map.
- 7.11 Open the “Advanced Settings” tab. Take a screenshot of the chip center coordinates (Current Stage Position). The values are displayed in mm.
- 7.12 While in the “Advanced Settings” tab, de-select the auto-exposure by clicking “auto-expose” button. Change the exposure time to the value listed in the laser alignment report. Select “set exposure” button (Figure 4D).
- 7.13 Turn the 488-nm laser on by pressing the appropriate button within the “light source” panel and turn off the Pogo LED light by pressing the appropriate button within the Chip Alignment window (Figure 4E)
- 7.14 Within the “Auto” tab, change the custom step size to the value listed in the laser alignment report. Double-click the circle to select that step-size (Figure 4F).
- 7.15 Move the cartridge in the correct directions as specified on the laser alignment report by clicking the appropriate arrows (Figure 4F).

Figure 4. Reference Cartridge Set-up and Qualitative Assessments (cont.)

Figure 4D. Change the auto-exposure settings by using the advanced settings Tab.

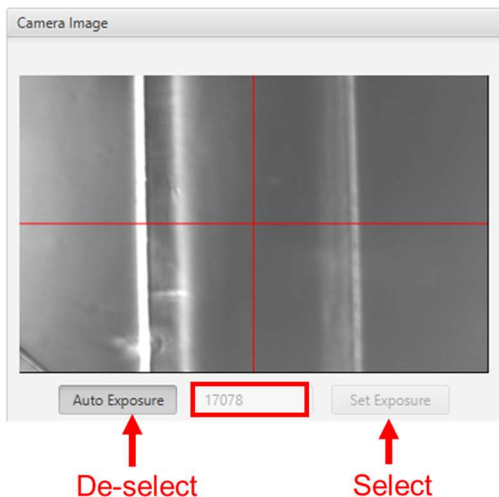
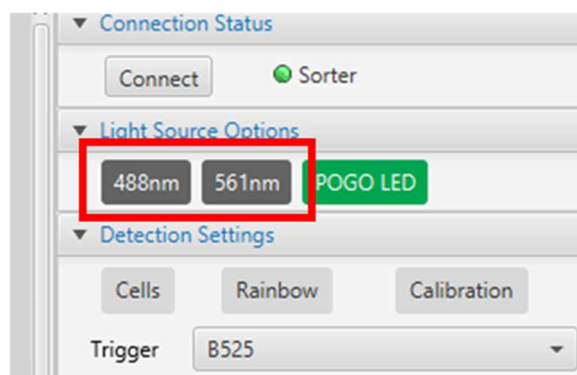


Figure 4E. Turn on the appropriate laser through toggling its button within the light source panel.



- 7.16 Take a screenshot of the laser beam with the 488-nm laser. The screenshot should have the crosshairs active to easily identify potential differences in beam position (Figure 4F).
- 7.17 Go to the Advanced Settings tab, take a screenshot of the laser beam coordinates to have a reference of the movement made when focusing the laser beam.

Figure 4. Reference Cartridge Set-up and Qualitative Assessments (cont.)

Figure 4F. Focus the 488-nm laser beam by moving the live image to the appropriate direction (red oval). The resulting image will be a picture of the focused laser beam that can be compared back to the laser alignment report.

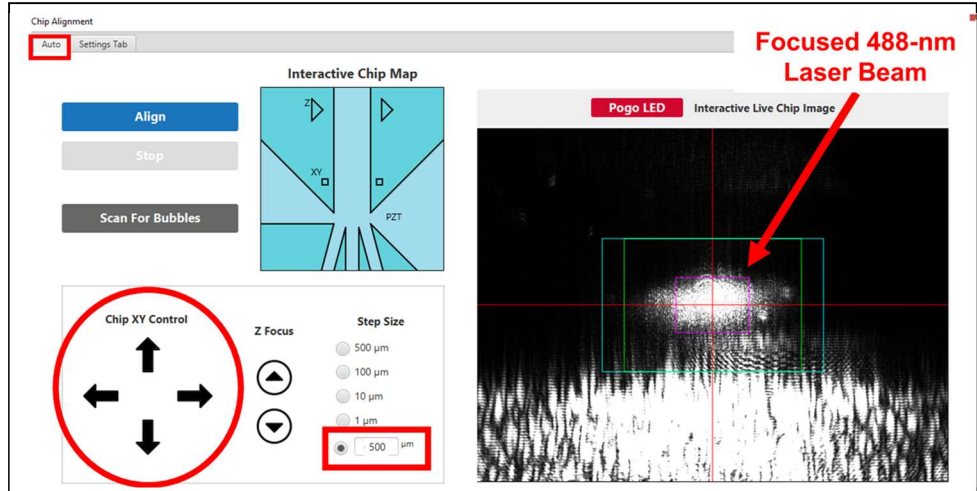
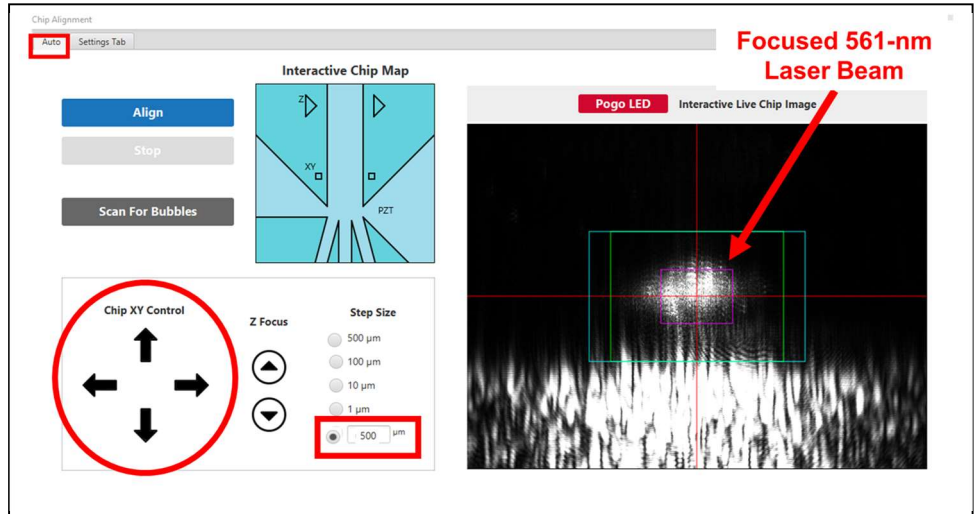


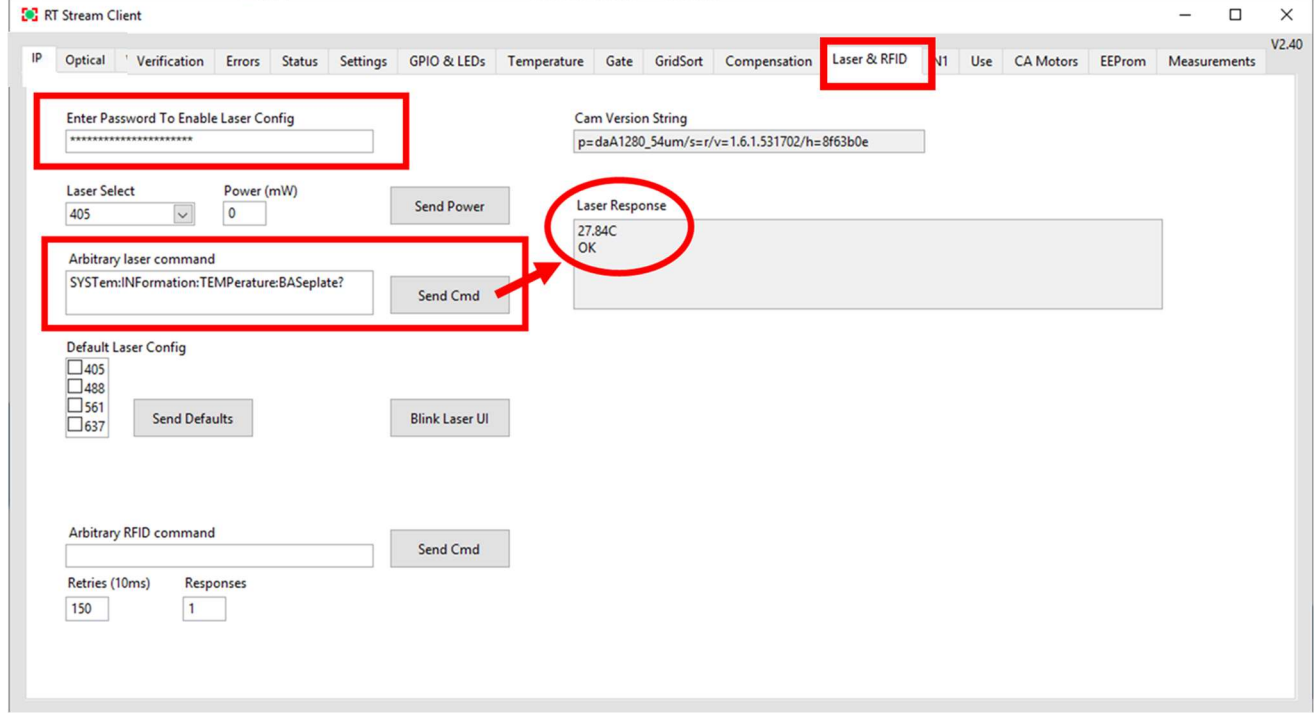
Figure 4G. Focus the 561/405/637-nm laser beam by moving the live image to the appropriate direction (red oval). The resulting image will be a picture of the focused laser beam that can be compared back to the laser alignment report.



- 7.18 Go to the light source panel, and turn off the 488-nm laser, turn on the second laser (405-nm, 561-nm, OR 637-nm) by pressing its respective button (Figure 4E).
- 7.19 Proceed back to the Auto Tab within the Chip Alignment window to capture the laser beam image of the second laser (Figure 4G).
- 7.20 Compare the laser beams visually to the image in the laser alignment report.
- 7.21 Reset exposure to “Auto-Exposure” when finished, turn the laser off.
- 7.22 Open StreamClient software by typing the word “streamclient” within the Windows search bar.
- 7.23 Within the Laser & RFID tab, enter the password to unlock the ability to send commands to the software.
 - 7.23.1 Password: I Know What I Am Doing
- 7.24 Once unlocked, enter the following prompt, and select “send cmd” button
 - 7.24.1 Prompt: SYSTem:INFormation:TEMPerature:BASeplate?
- 7.25 Record the temperature within the laser module at the time of acquiring laser beam images.

Figure 4. Reference Cartridge Set-up and Qualitative Assessments (cont)

Figure 4H. Utilizing Stream Client to measure the temperature of the laser.

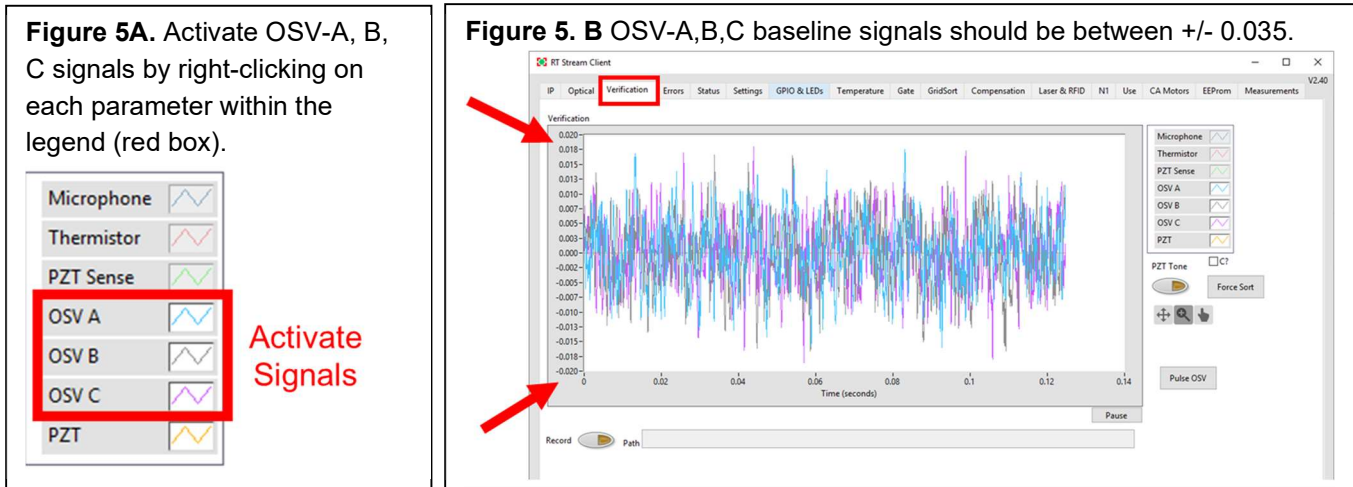


If laser beam shifted within the laser beam focusing GUI: make note of the direction and magnitude of the shift, then continue to complete the remainder of the quality control procedure.

8. WOLF Reference cartridge quality control: OSV detectors performance (Figure 5)

- 8.1 Go to the “Auto” tab of Chip Alignment window. Return to the chip center location by clicking the location within the Interactive Chip Map.
- 8.2 Open StreamClient software, and select the “Verification” tab.
- 8.3 If necessary, activate the signals in **OSV-A, OSV-B, and OSV-C channels** by right-clicking the icon in the legend. De-activate any other signals that may be activated (Figure 5A)
- 8.4 Assess the values and signal pattern of the **OSV baseline signal**. Ensure that the baseline signals **fall between -0.030 to +0.030V** (Figure 5B).

Figure 5. WOLF OSV Signals



If the baseline signal abnormal or outside of the normal range: proceed to [OSV Baseline Failure troubleshooting chart \(Chart #14\)](#).

9. WOLF Reference cartridge quality control: PZT triggering system testing (Figure 6)

- 9.1 Open StreamClient software, go to the “Verification” tab.
- 9.2 Activate the PZT plot by right-clicking on the **PZT signal** (Figure 6A).
- 9.3 Click on the “PZT Tone” button to trigger the cartridge PZT with the POGO pins.
- 9.4 Verify the **PZT triggering waveforms ranges from +30 to -30V** (Figure 6B).
- 9.5 While the PZT tone remains ON, de-activate the PZT signal, and activate the PZT Sense.
- 9.6 Monitor the signal from the **PZT sense to confirm** that current is being run through PZT. Signal from the PZT Sense should be waveforms, albeit a smaller scale than PZT signal (Figure 6C).
- 9.7 **Listen for the PZT triggering sound**. It will be a high pitch noise or use Microphone (Figure 6D).

If the PZT is not triggered, make note of the issue, and continue to complete the remainder of the quality control protocol.

Figure 6. WOLF PZT-triggering system testing

Figure 6A. Activate PZT signals by right-clicking on the parameter within the legend (red box). No PZT signal pulses are generated when 'PZT tone' is off.

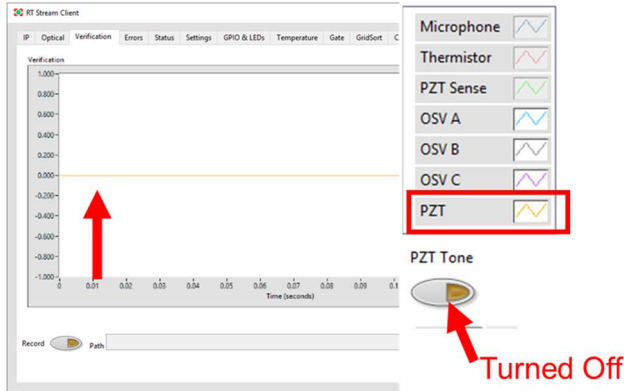


Figure 6B. PZT signal pulses are generated when 'PZT tone' is on. PZT signal pulses between +/- 30V (red arrows).

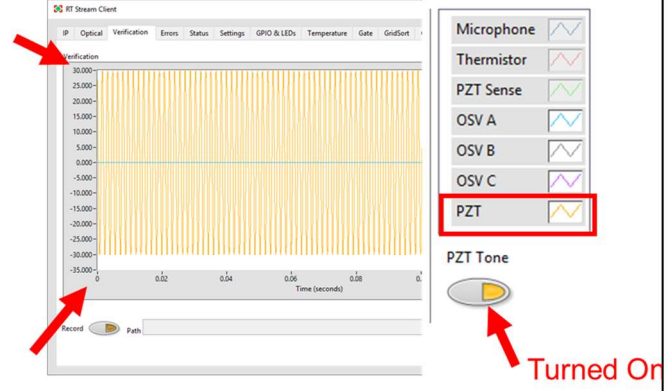


Figure 6C. Activate PZT sense signals by right-clicking on the parameter within the legend.

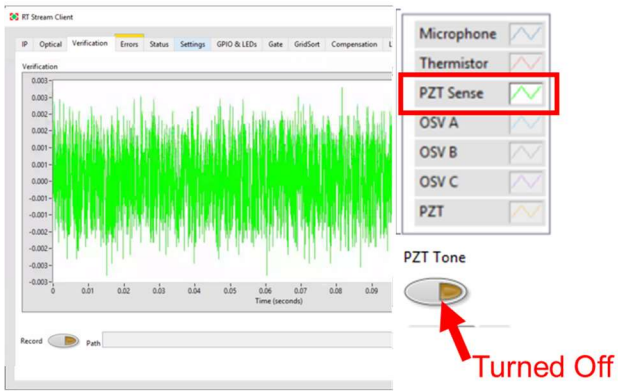


Figure 6D. PZT sense signals exhibit a waveform signal when PZT tone is on.

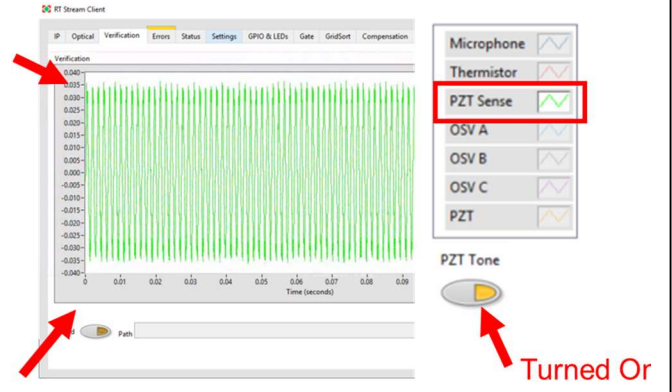


Figure 6E. Activate microphone signals by right-clicking on the parameter within the legend.

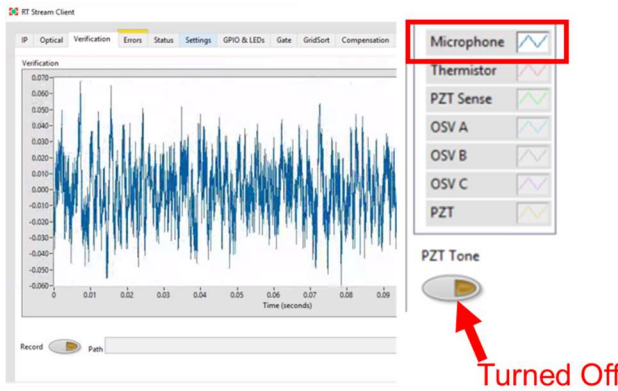
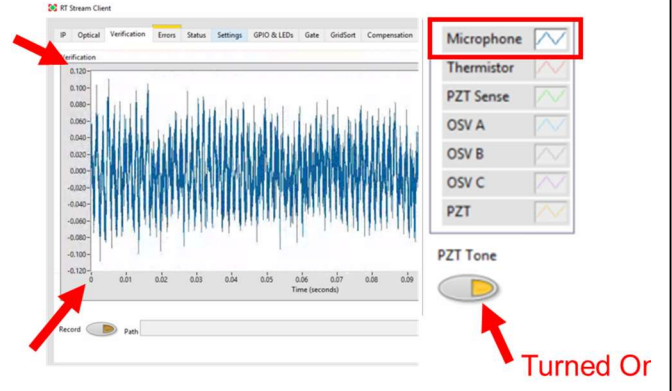


Figure 6F. Activate microphone signals by right-clicking on the parameter within the legend.



10. WOLF G2 Sorting cartridge quality control: priming, alignment and calibration (Figure 7):

- 10.1 Insert a **new bulk sorting cartridge**.
- 10.2 **Prime** the cartridge with PBS.
- 10.3 **Auto-align the cartridge**. Take a screenshot of the chip center and the chip center coordinates.
- 10.4 Within the interactive chip map, press the left XY marker. Take a screenshot of the XY marker and its coordinates.
- 10.5 **Scan for bubbles**, re-prime if needed.

If bubbles fail to dissipate after 3X re-priming, proceed to Calibration step.

- 10.6 Assess the appearance of **calibration beads** by pressing the “Analyze” button.
- 10.7 Open a 2D plot (**FSC/BSC**): **Calibration beads should appear as a tight population in the 10⁵-10⁶ range for both axes** (Figure 7A).
- 10.8 Open a 1D histogram (**#Events/FL1**): **Calibration beads should appear as a single peak in the intensity range ~10⁶**.
- 10.9 While still analyzing beads, open StreamClient, and select the “Optical” tab.
- 10.10 Within the optical tab, **Select “Volts”** from the Scaling Options dropdown menu. Right-click on the Y-axis and select “auto scale” from the pop-up menu. Right click on each parameter individually (**BSC L1, FL1 L1, FSC L1**) to activate it.
- 10.11 Monitor the signal in the “Optical” tab. As a bead passes by the laser, there should be an aligned signal pulse in all 5 detectors (Figure 7B).
- 10.12 When the average beads concentration is **≥ 20 beads/μl**, click “Calibrate” button.
- 10.13 While calibration is running, open the “**Verification**” tab in StreamClient. Right click on **OSV-A, OSV-B and OSV-C parameters** individually to activate the plot, if its not already.
- 10.14 When calibrating to channel A, peaks should appear in the OSV-A signal. Take a screenshot.
- 10.15 When calibrating to channel C, peaks should appear in the OSV-C signal. Take a screenshot.
- 10.16** When calibrating to A, minimal peaks should appear in C and vice-versa. However, regardless of which channel is being calibrated, there will always be unsorted beads flowing to channel B, thus peaks should still be appearing within OSV-B signal (gray). **The amplitude of the OSV peaks should reach at least ±110mV for all three detectors (Figure 7C).**
- 10.17** Close StreamClient. Wait for “Calibration Success” in the WOLFViewer.

If calibration fails (errors #1 to #7), follow [calibration failure troubleshooting charts \(charts #6-12\)](#).

Figure 7. Calibration optical and OSV signals

Figure 7A. Calibration beads scatter and fluorescence intensities.

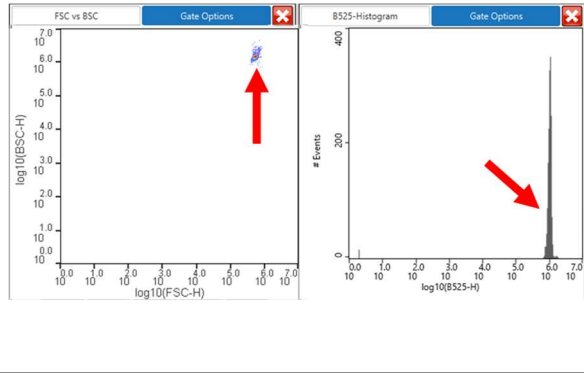


Figure 7B. Optical signal Calibration beads signals as beads pass the laser interrogation point.

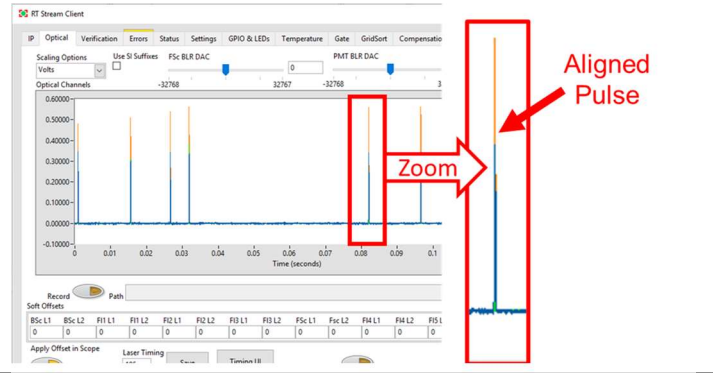
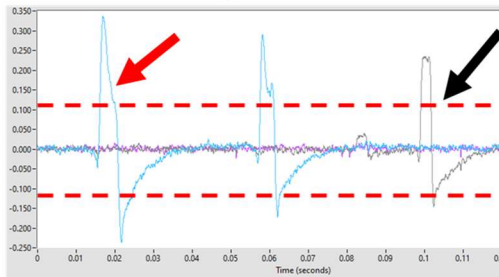


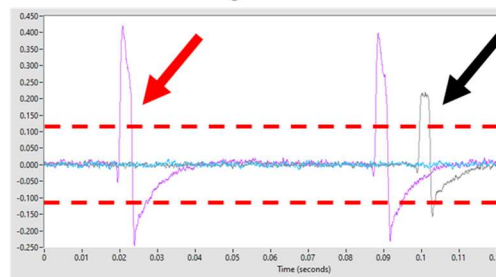
Figure 7. C


Peaks in OSV signal indicate whether a bead has been sorted to channel A, B, or C (red or black arrows). During calibration to channel A (left panel), peaks should appear in OSV-A (blue signal, red arrow). During calibration to channel C (right panel), peaks should appear in OSV-C (purple signal, red arrow). During calibration, not all beads that are detected by the laser get sorted, peaks will appear in OSV-B when calibrating to A and C (gray signal, black arrow). The amplitude of peaks should reach at least +/-110mV (red dash).

Calibrating to Channel A



Calibrating to Channel C



	<h1>Procedure</h1>
Version 0.1	Title: WOLF and N1 Quality Control at installations

11. WOLF Sorting Cartridge quality control: optical alignment test – Rainbow beads (Figure 8):

11.1 Select “Rainbow” in WOLFViewer detection settings (Figure 8A).

Note: Check the laser alignment report to ensure that the detection settings used at NanoCellect HQ are the same as the default settings.

11.2 Prepare a ultra rainbow beads solution: 1mL filtered PBS + 1 drop concentrated vial.

11.3 Analyze Ultra Rainbow beads by pressing “analyze” button. Select the “new sample” button to start sample acquisition. There is no need to flush the cartridge with PBS between running rainbow beads and calibration beads. However, be aware that tubing lines are loaded with calibration beads, so the first ~50uL of sample that passes by laser will be calibration beads.

11.4 Once Ultra Rainbow beads start appearing, press the “stop” button.

11.5 Select “Analyze” button a second time selecting “Same Sample” button instead of “New Sample” for a more stable flow.

11.6 Collect approximately 1,500 rainbow bead events, do not exceed 2,500 events (high counts numbers may lead to wider CV %s). *Note: if there is a high level of debris, the user may need to collect a much greater total number of events to acquire ~1500 rainbow events.*

Note: From this point forward, any analysis of Ultra Rainbow beads should be started by pressing “Same Sample”. Use this second analysis to assess Rainbow beads qualitatively and quantitatively.

11.7 Create the following graphs when analyzing rainbow beads:

- Open a 2D scatterplot (**FSC/BSC**): **Ultra Rainbow beads should appear as a tight population between 10^5 – 10^6 for both axes.** Draw a polygon gate around the rainbow beads excluding doublets (Figure 8A).
- Open up a series of 1D histograms, one for each fluorescent channel excited off the 488-nm laser (#Events/B525, B580, B620, B706, B760LP), and one for each fluorescent channel excited off the second laser (561-nm laser: #Events/Y580, Y620, Y706, Y760LP; 637-nm laser: #Events/R706, R760LP; 405-nm Laser: #Events/V450, V525, V580, V620, V706).
- *Note: The 488/405nm configuration will not have a B760LP channel.*
- Apply the Rainbow gate to each of the histograms.
- Qualitatively assess the appearance of beads. **Histograms should have 9 distinct peaks for B525, B580, B620, B706, Y580, Y620, and Y706, and 5 peaks in B760LP, Y760LP** (Figure 8B).
- Create a horizontal gate on the peak with the highest intensity.
- Open the statistics panel. Quantitatively assess the **CV values for the 7-9 horizontal gates.** (Figure 8C).
- **For a list of Acceptable CV values, please see table 8D**
- Save the FCS file and gating strategy.

If Rainbow bead resolution does not meet criteria, refer to Flowchart WOLF Troubleshooting Rainbow Bead Resolution issues v2.0.

Figure 8. WOLF optical alignment. Rainbow beads analysis

Figure 8A. Detection setting used to analyze rainbow beads scatter gating.

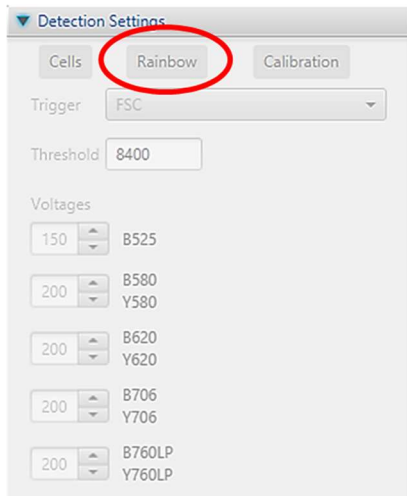


Figure 8B. Identification of a single population of rainbow beads using its scatter properties (FSC, BSC) (black polygon). Do not include doublets (2+ beads) within the scatter gate (red oval)

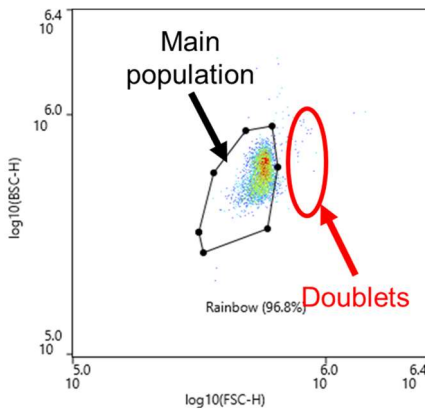


Figure 8C. Rainbow beads - fluorescent gating.

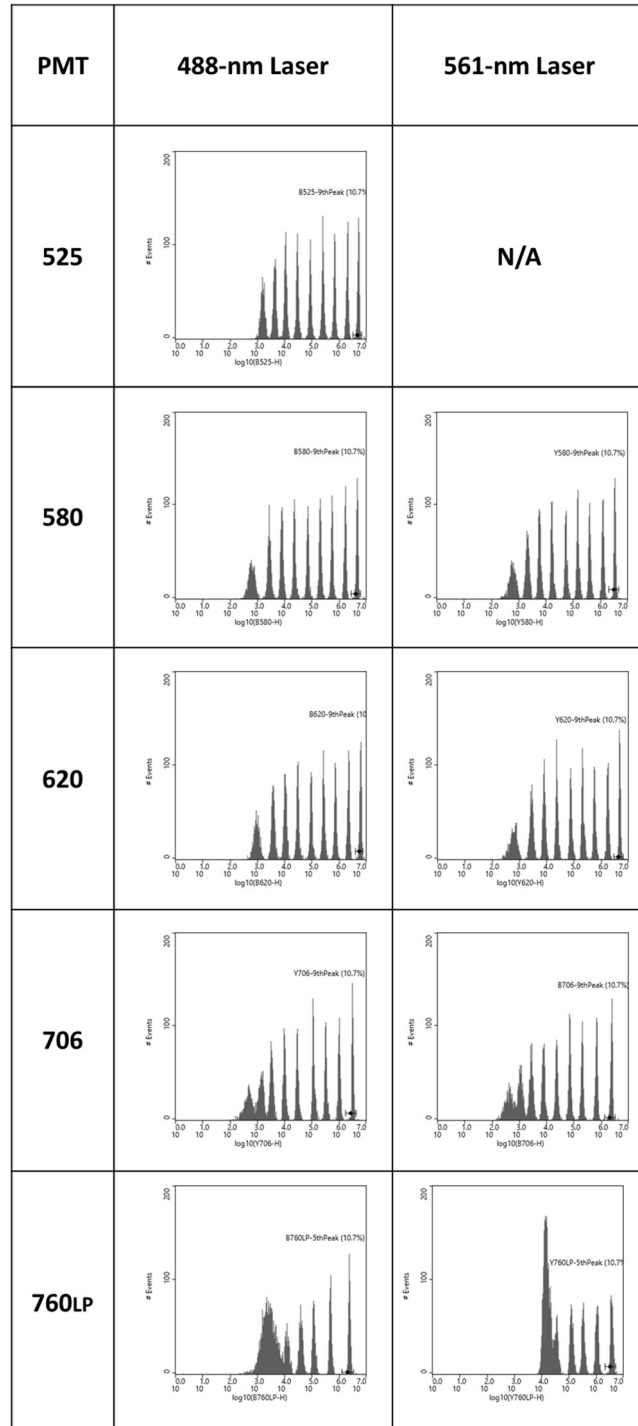


Figure 8. WOLF optical alignment. Rainbow beads analysis

Figure 8C. Statistics panel. Identify the CV of the brightest peak in each fluorescent channel.

Gate Name	Plot Name	Total Count	% of Parent	Mean FSC-H	CV FSC-H	Median FSC-H	Mean BSC-H	CV BSC-H	Median BSC-H
-	FSC_vs_BSC	4,473	100.00%	567,668	10.33%	572,372	584,055	17.62%	584,998
Rainbow	FSC_vs_BSC	4,332	96.85%	565,736	6.72%	571,319	585,060	13.62%	585,610
Gate Name	Plot Name	Total Count	% of Parent	Mean B525-H	CV B525-H	Median B525-H			
-	B525-Histogram	4,332	100.00%	938,598	182.94%	87,651			
B525-9thPeak	B525-Histogram	464	10.71%	5,461,892	7.89%	5,496,567			
Gate Name	Plot Name	Total Count	% of Parent	Mean B580-H	CV B580-H	Median B580-H			
-	B580-Histogram	4,332	100.00%	817,649	188.11%	70,622			
B580-9thPeak	B580-Histogram	464	10.71%	4,908,500	7.82%	4,933,286			
Gate Name	Plot Name	Total Count	% of Parent	Mean B620-H	CV B620-H	Median B620-H			
-	B620-Histogram	4,332	100.00%	1,085,012	187.88%	93,604			
B620-9thPeak	B620-Histogram	464	10.71%	6,533,060	7.99%	6,571,803			
Gate Name	Plot Name	Total Count	% of Parent	Mean B706-H	CV B706-H	Median B706-H			
-	B706-Histogram	4,332	100.00%	386,868	204.88%	22,961			
B706-9thPeak	B706-Histogram	463	10.69%	2,563,547	8.24%	2,580,559			
Gate Name	Plot Name	Total Count	% of Parent	Mean B760LP-H	CV B760LP-H	Median B760LP-H			
-	B760LP-Histogram	4,332	100.00%	351,215	224.40%	11,349			
B760LP-5thPeak	B760LP-Histogram	463	10.69%	2,559,013	8.52%	2,548,993			
Gate Name	Plot Name	Total Count	% of Parent	Mean Y580-H	CV Y580-H	Median Y580-H			
-	Y580-Histogram	4,332	100.00%	598,805	187.71%	53,945			
Y580-9thPeak	Y580-Histogram	464	10.71%	3,602,056	7.84%	3,581,148			
Gate Name	Plot Name	Total Count	% of Parent	Mean Y620-H	CV Y620-H	Median Y620-H			
-	Y620-Histogram	4,332	100.00%	850,119	187.67%	76,129			
Y620-9thPeak	Y620-Histogram	463	10.69%	5,107,875	7.54%	5,081,477			
Gate Name	Plot Name	Total Count	% of Parent	Mean Y706-H	CV Y706-H	Median Y706-H			
-	Y706-Histogram	4,332	100.00%	530,970	196.07%	29,274			
Y706-9thPeak	Y706-Histogram	463	10.69%	3,350,616	7.56%	3,327,045			
Gate Name	Plot Name	Total Count	% of Parent	Mean Y760LP-H	CV Y760LP-H	Median Y760LP-H			
-	Y760LP-Histogram	4,332	100.00%	619,277	200.62%	33,092			
Y760LP-5thPeak	Y760LP-Histogram	463	10.69%	4,009,733	11.02%	3,966,049			

Table 8D. CV Criteria for the brightest peak on ultra rainbow beads.

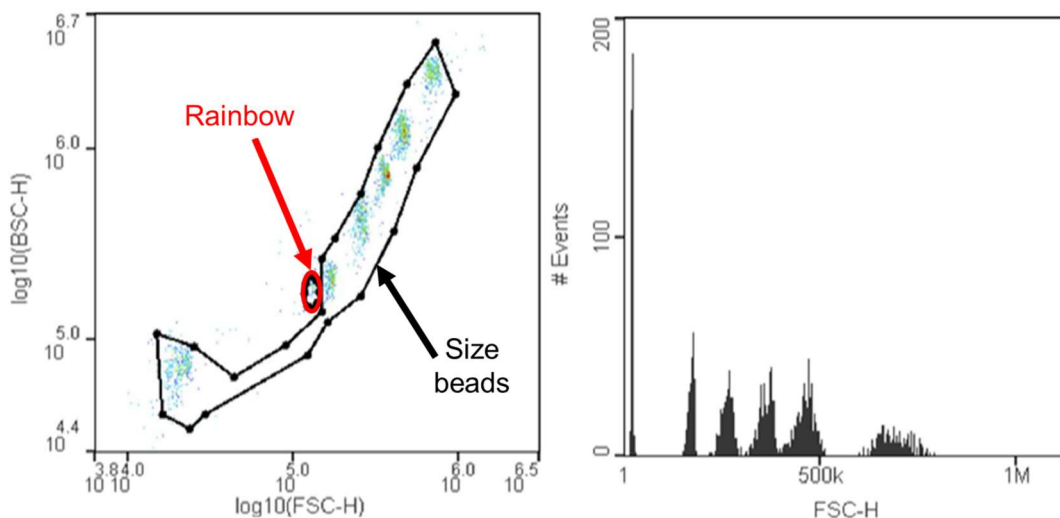
PMT	Laser Configuration					
	488	561	488	405	488	637
450	N/A	N/A	N/A	14%	N/A	N/A
525	13%	N/A	13%	14%	13%	N/A
580	13%	14%	13%	14%	13%	N/A
620	13%	14%	13%	14%	13%	N/A
706	13%	14%	17%	18%	13%	14%
760	17%	18%	N/A	N/A	17%	18%

12. WOLF wet cartridge quality control: optical alignment test – Size beads (Figure 9):

- 12.1 Utilize “Rainbow” detection settings to analyze size beads.
- 12.2 Prepare Size beads (1 drop of each size in 1mL of filtered PBS). The stock beads are available from Spherotech (CAT#PPS-6K).
- 12.3 Analyze the Size beads by pressing “Analyze” button. Select the “New Sample” button to start acquisition. There is no need to flush the cartridge with PBS. However, be aware that tubing lines are loaded with Rainbow beads.
- 12.4 Once Size beads start appearing, press the “stop” button.
- 12.5 Select “Analyze” button a second time, press “Same Sample” button. Collect approximately 1,500 events, do not exceed 2,500 events. Use this second analysis to qualitatively assess appearance of Size beads.
- 12.6 Create the following graphs when analyzing size beads:
 - Create a 2D scatterplot (**FSC/BSC**): **6 separate populations should be visible in the plot.** Draw a polygon gate around all of the size beads, making sure to exclude any remaining Rainbow beads (Figure 13A).
 - Create a 1D histogram (**#Events/FSC-H**) in linear scale. Apply the scatter gate to the histogram.
 - Qualitatively assess the appearance of size beads. The histogram **should have 6 separate and distinct peaks** (Figure 13B)
 - Save the FCS file and gating strategy.

Figure 9. WOLF optical alignment. Size beads analysis

Size Beads gating strategy.



Revision History:

	ISSUED DATE	Description of Change	Author
(A)	(02/19/2020)	Document created (V0.1)	A. Bartakova
(B)	(03/19/2020)	Edited with help from Beth Leary (V0.2)	A. Bartakova
(C)	04/24/2020	Edited in yellow highlight + comments (V0.3)	M. Bonar
(D)	05/01/2020	Edited in green highlight + answered comments (V0.4)	A. Bartakova
(E)	05/11/2020	Final edits from Beth Leary (V0.5)	E. Leary
(F)	05/11/2020	Final version (V 0.6)	A. Bartakova
(G)	05/13/2020	Final version after review by Manna Doud (V1.0)	M. Doud A. Bartakova
(H)	07/20/2020	Added instructions on how to open the Advanced Settings tab in the Alignment window (V1.1)	A. Bartakova
(I)	07/30/2020	Final version after review (V1.2)	E. Rubio de la Torre
(J)	11/09/2020	Elimination of unnecessary steps, addition of clarifying steps	E. Leary
K	11/11/2020	Review before release. Added final QC criteria.	E. Rubio de la Torre
L	5/11/2021	Modified for WOLF Cell Sorter G2	M. Doud
M	2021-08-16	Edited for WOLFG2 content	E. Leary
N	2021-12-17	Edited and addition of Fan settings	D. Califano
O	2022-01-04	Edited & removed fan content since its obsolete with new firmware version. Added in figure about how to check firmware and streamclient.	E. Leary
P	2022-01-24	Added in steps regarding taking picture of XY marker, added in CV criteria	E. Leary