NanoSight NS300

Operation instructions

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Injection/flushing brief overview:

1. Do not exceed flow of 1 ml per 20 seconds or one drop per second to the waste bottle
2. Inject two 1 ml syringes with nano-pure or DI water.
3. If the water does not come out in a waste line after 1 ml of injection, please, stop and report the observations.
4. If water elutes to waste bottle, inject two 1 ml syringes with your solvent/buffer.
5. Inject your sample. (Delay 10, Syringe pump 35, Delay 20).
6. Place less than half filled syringe with your sample in a pump.
7. Run you sample.
8. Flush with 2 syringes (2 ml) of your buffer.
9. Flush with 5 syringes (5 ml) of nano pure or DI water.
10. Inject nanopore water with little azide and leave syringe connected.

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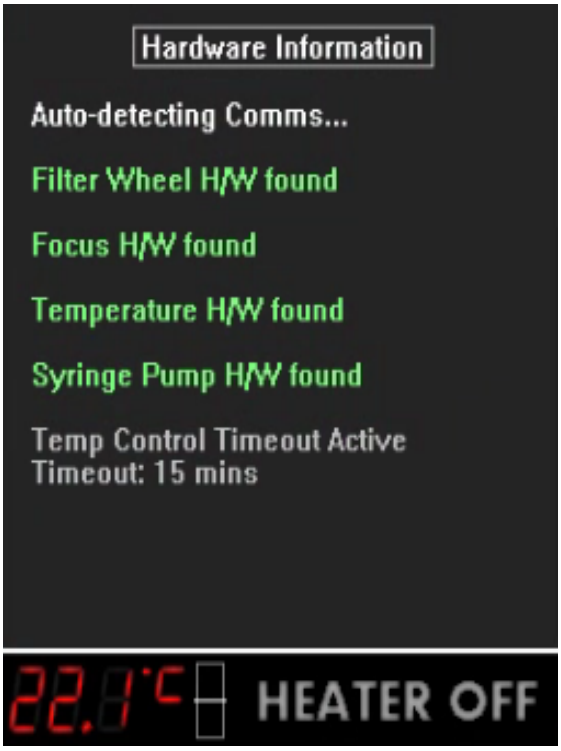
1. The instrument is always in Stand By mode. Both camera and laser become active once the software is on.

2. Connection status is detailed in the Hardware Information window. Any available instrument hardware, such as the syringe pump, will be automatically detected by the software. If there any error reported by the system, please, do not troubleshoot, unless directed. Report any error.



3. Make sure that outlet line is connected or inserted into the waste bottle. Make sure the outlet line is not dipped into the water.

4. The line usually kept dry between instrument uses. Insert a 1 ml syringe of liquid (water or buffer) into the Luer port and push all the liquid through the inlet tubing. Please, maintain the speed of 1 ml per 20 second. Do not continue if you feel much back pressure and report the observations.

5. **Do not perform unmounting of the cell or laser unit unless instructed to or received permission. Do not perform manual cleaning of the cuvette surfaces.**

6. When flushing the system with pure water (preferably nano pure), make sure no air bubbles are observed on camera.

7. When changing syringes, ensure that liquid-to-liquid contact is maintained at the syringe port. Before changing syringes, have the next syringe prepared, ensuring there are no air pockets present at the tip and that there is a small positive meniscus (bead of liquid)

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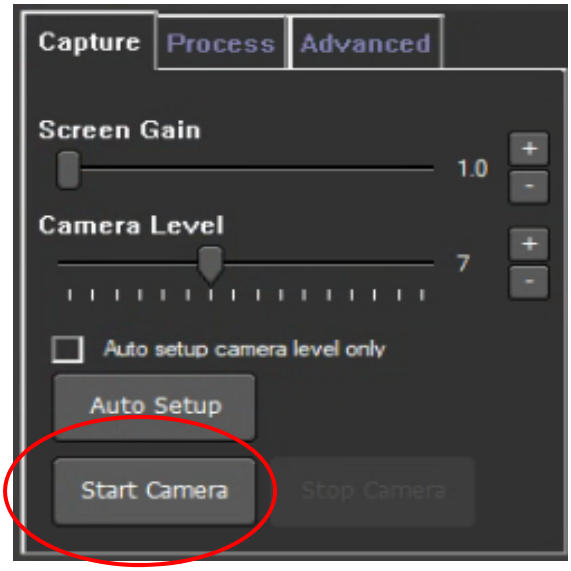
protruding from the syringe. Keep the Luer port as low as possible (at bench level) when changing syringes to prevent liquid draining from the Luer port. Remove the old syringe from the Luer port and insert the new syringe into the Luer port (keeping syringes and Luer port horizontal) such that the two meniscuses combine without trapping an air bubble. You may need to drop liquid into Luer port to fill it before attaching syringe.

8. **Liquid should be introduced into the NS300 system for sample loading (and cleaning) using a 1ml syringe, at speeds no faster than 1ml in 20 seconds (0.05ml per second).**

9. Introduce the sample into the system using the same solvent (water, buffer) used for initial system flushing.

10. Make sure the camera is on. Press Start Camera button to engage the camera.



11. This is an iterative process between Camera Level, Sample Concentration, Beam Position, and Focus. Different sample types will require different final settings.

12. If you do not see particles in the field of view, set camera level to 16. Look for the bright area above or below the field of view. Adjust laser beam position by clicking left mouse button and dragging the image up or down.

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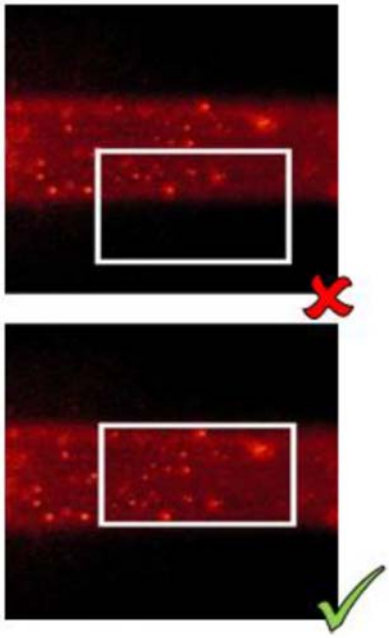
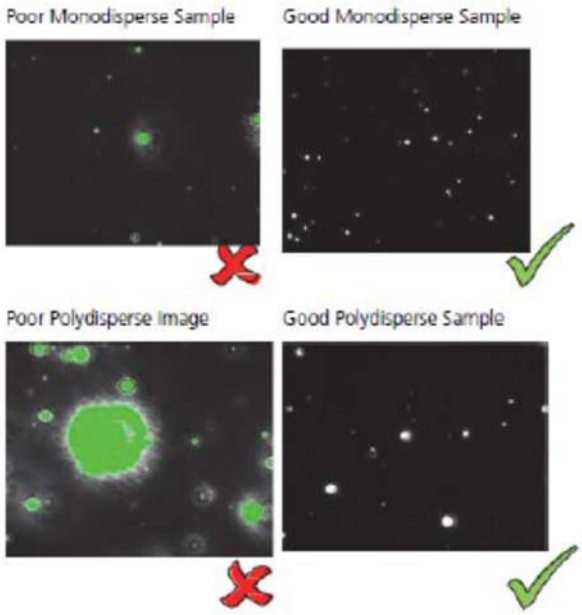


13. Correctly setting up the camera and sample image prior to capturing the video are essential to achieving valid results. Increase the Camera Level until all of the particles in the sample can be seen clearly but no more than 20% are saturated (colored pixels).



14. Initial focus is set with the manual control on the side of the NanoSight NS300, adjust for fine focus within the software Indistinct particles, as in a) will give inaccurate results. Ideally, particles should appear as in b) but those in c) will be acceptable if that is the best image that can be achieved.

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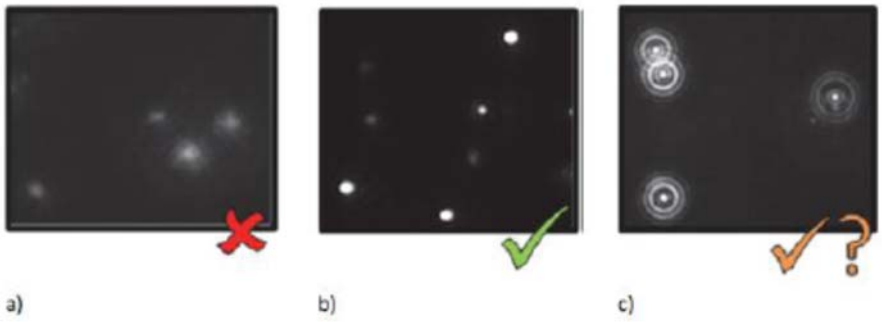
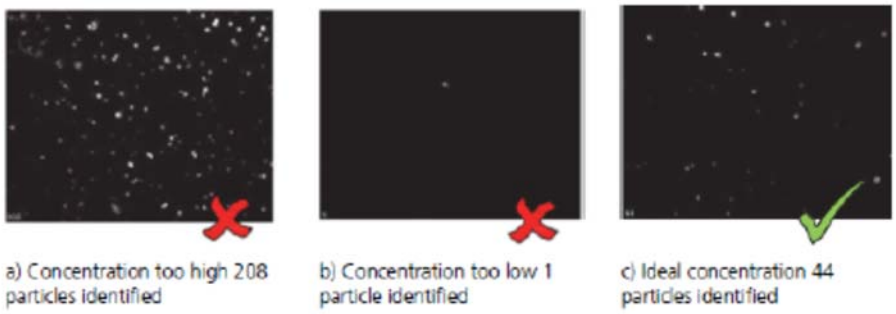
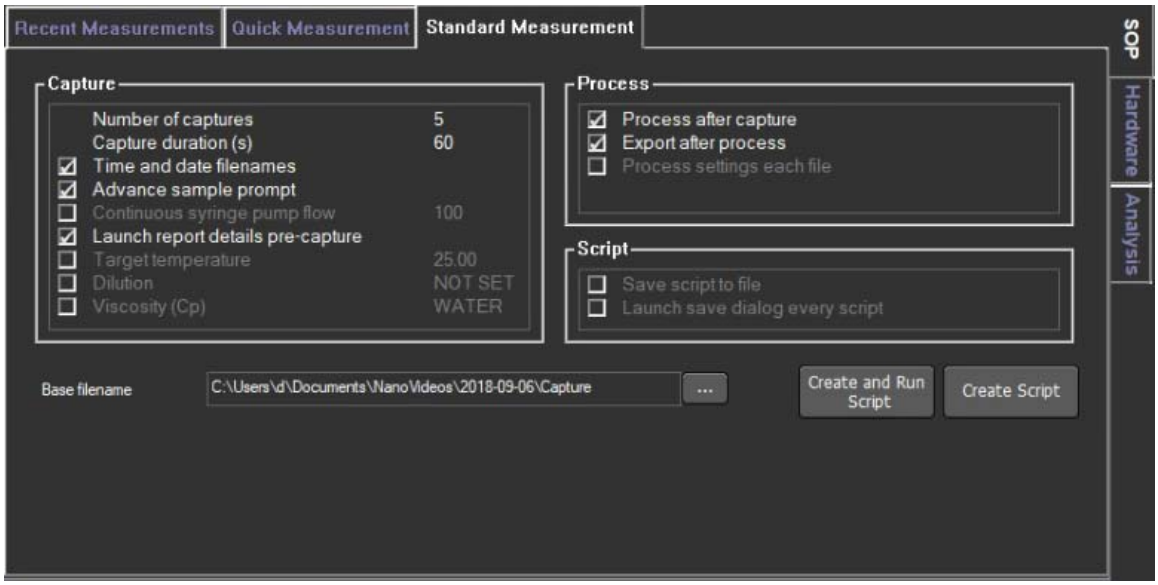


15. Concentration. The instrument can work with particle concentrations in the range of ~10^6-10^9 particles/ml, which is approximately 20-100 particles in the field of view. Too high a sample concentration may prevent accurate particle tracking. Lower concentrations require longer capture and analysis time to produce statistically significant results. Using the NanoSight syringe pump will improve results by sampling more particles.



Taking a Measurement

16. On the SOP tab, Select Standard measurement.



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a. Number of Captures: when using the syringe pump, 3 repeats of sufficient length to ensure acceptable reproducibility is the default recommendation. When using static measurements, select at least 5 repeats.

b. Capture duration: 60 s (standard)

c. Advanced sample prompt. Used for static measurement without pump.

d. Continuos syringe pump flow. Recommended to collect better statistics. Used with pump.

17. Base filename. Please, create or select your own directory. Due to high size of the files, make sure you pick up your data (especially video files) after your experiments. The hard drive will be purged periodically.

18. Press Create Script. The script will appear on left of the window:

19. Drag the script window to the center to enable editing:

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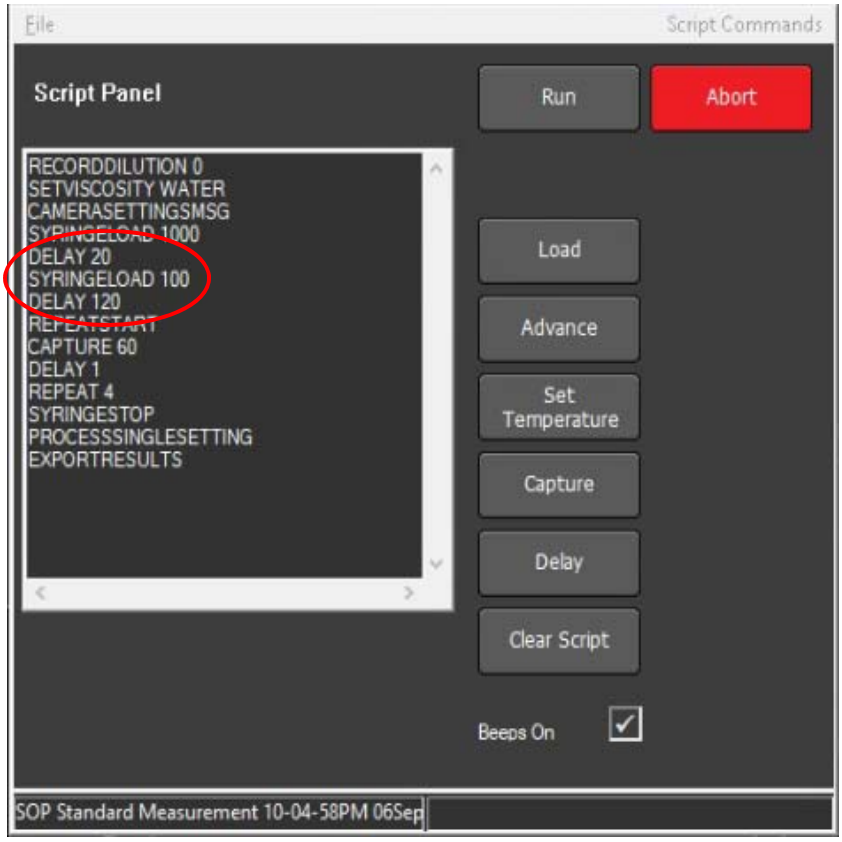
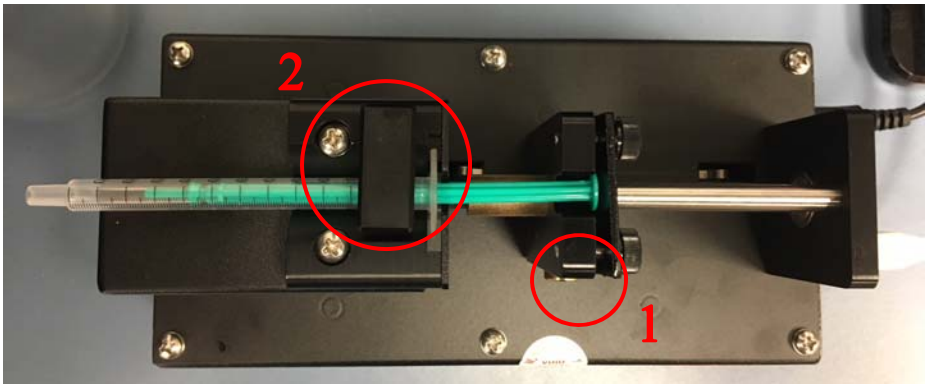


20. Change fist delay from 20 s to 10 s, second syringe load from 100 to 35 and second delay from 120 s to 20 s.

21. Follow on-screen prompts as needed.

Syringe pump

22. Please, follow in person training instructions how to load syringe in the pump:



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23. Pressing button 1 will allow smooth movement of the syringe bottom pusher.

24. Syringe is fixed on pump by locking clock-wise lever 2 and unlocking with slight elevation counter-clock-wise.

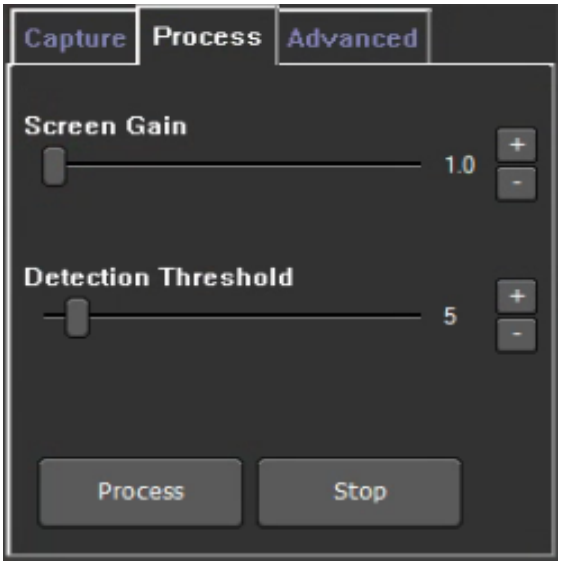
25. Syringe pump pulsing or inconsistent flow can be caused by bubbles in the syringe or tubing.

26. Certain syringe types are more likely to have stiction and move in an inconsistent fashion. Please, use only 1 ml syringe.

27. **Do not start with the syringe loaded more than half full as the shaft can flex.**

Data Processing and Export

28. Adjust detection threshold. The lower the setting the more centers will be found; however if it is too low, 'noise' can be tracked. If the setting is too high, dimmer particles will be excluded.



29. For the best analysis, identify the center of each particle by reducing the Detection Threshold to a level to include as many particles as possible and within the following restrictions:

    a. In the bottom right of the image is a count of the number of red crosses. This should be between 10 and 100 for good conditions; ideally between 20 and 50.
    b. When considering the image by eye, some of the red crosses may not appear to be distinct particles. Ideally there should be <10 such crosses.
    c. There may also be blue crosses on the screen. Ideally there should be <5.
    d. View the setting at multiple time points of the video file to ensure a reliable setting for the full video length.

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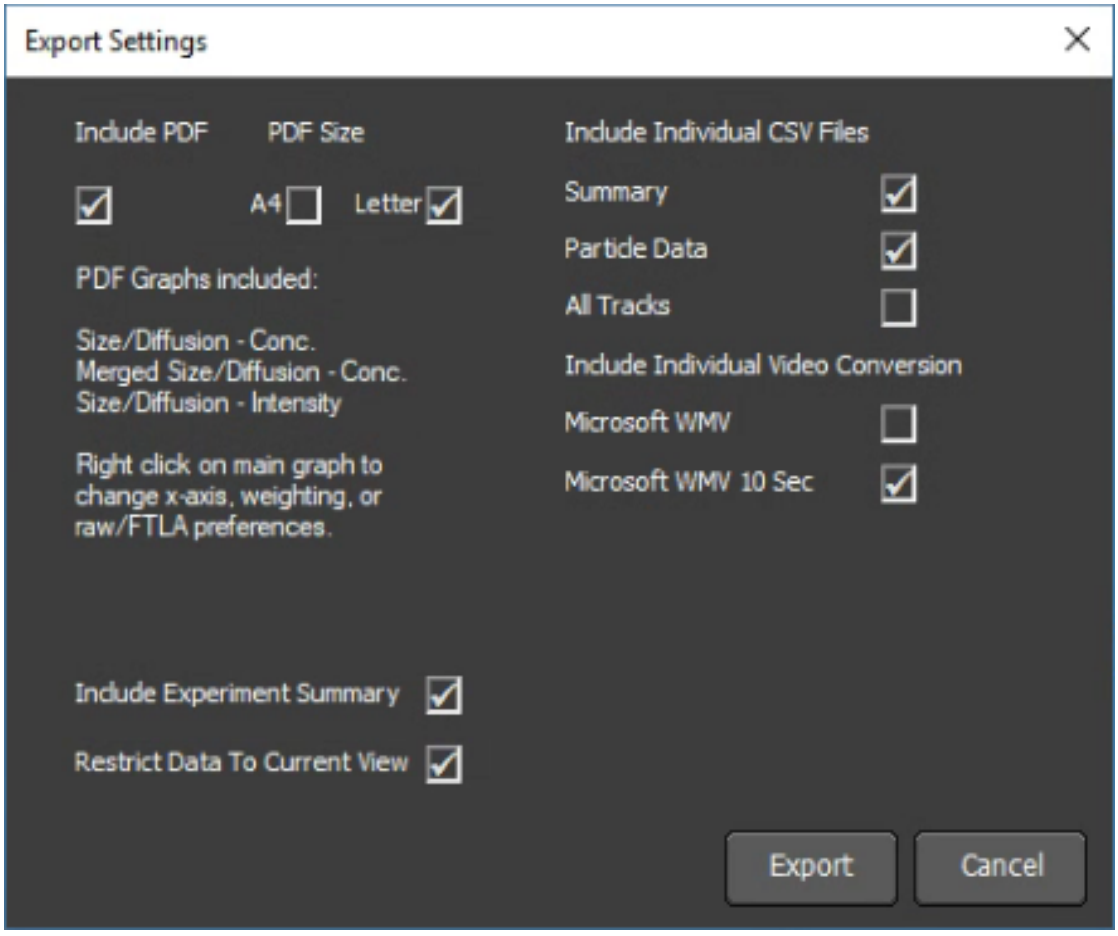
30. At the completion of processing the software automatically opens the Export Settings options window.

    a. Experiment Summary is the single spreadsheet file (.csv) for all repeat measurements of the sample, the average, and standard error. Separate files for each repeat can be exported if desired.

    b. Select "PDF report" to export a short summary report with graphs.

    c. Select individual video conversion to export in (smaller file size) .wmv format or a 10 second .wmv clip (~1MB file size).



31. The experiment can be re-analyzed and re-calculated.

32. Open experiment, select the run or multiple runs to process:

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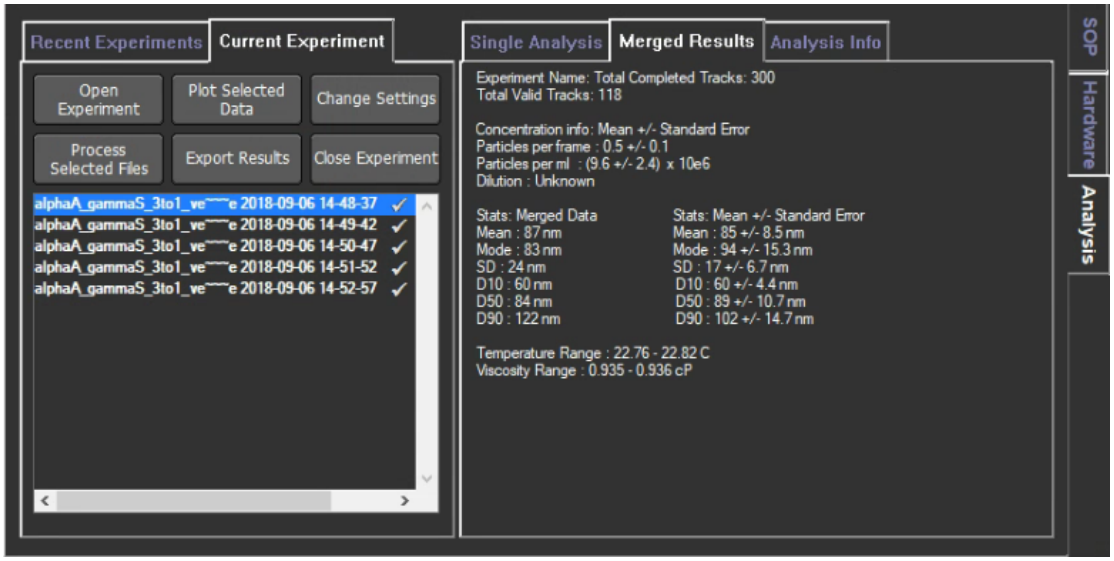

Cleaning

33. Between samples, push at least 1 ml of water or diluent through the sample chamber to remove any particles present. This ensures carry over of less than 1%.

34. **Do not perform unmounting of the cell or laser unit unless instructed to or received permission. Do not perform manual cleaning of the cuvette surfaces.** A manual clean is only necessary if there is visible cloudiness or sample residue stuck to the optical flat or the glass window in the gasket component. Report if this occur.

35. A dirty optical surface will make bubbles difficult to dislodge. To avoid this, a mild detergent solution can be used to clean the system. A diluted (10%) window cleaning solution (i.e. Windex) can be an effective alternative.

36. For disinfection or for cleaning, a brief flush with 10% ethanol followed by thorough water rinse of at least 10 ml can clean and wet the cell surfaces well.

37. For disinfecting following biological samples, Virkon, 2% Decon 90, or other mild biocide is recommended. Diluted bleach (10% or <0.5% sodium hypochlorite) can be used for a brief flush. In all cases, immediately flush thoroughly with water. **Please, receive permission prior doing so.**

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Shutdown

38. Flush system with clean diluent first.

39. After use, the system should be flushed to with clean water to clean all tubing and optical surfaces. Please, use at least 2 ml of water.

40. Once all tubing assemblies are flushed clean, empty the fluidics by pushing a 1ml syringe full of air through the system (**do not exceed maximum rated flow speed of 1ml in 20 seconds**).

41. The NTA software should be shut down while NS300 power switch turned on.

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