

ZetaView® NTA system Training Quick Guide

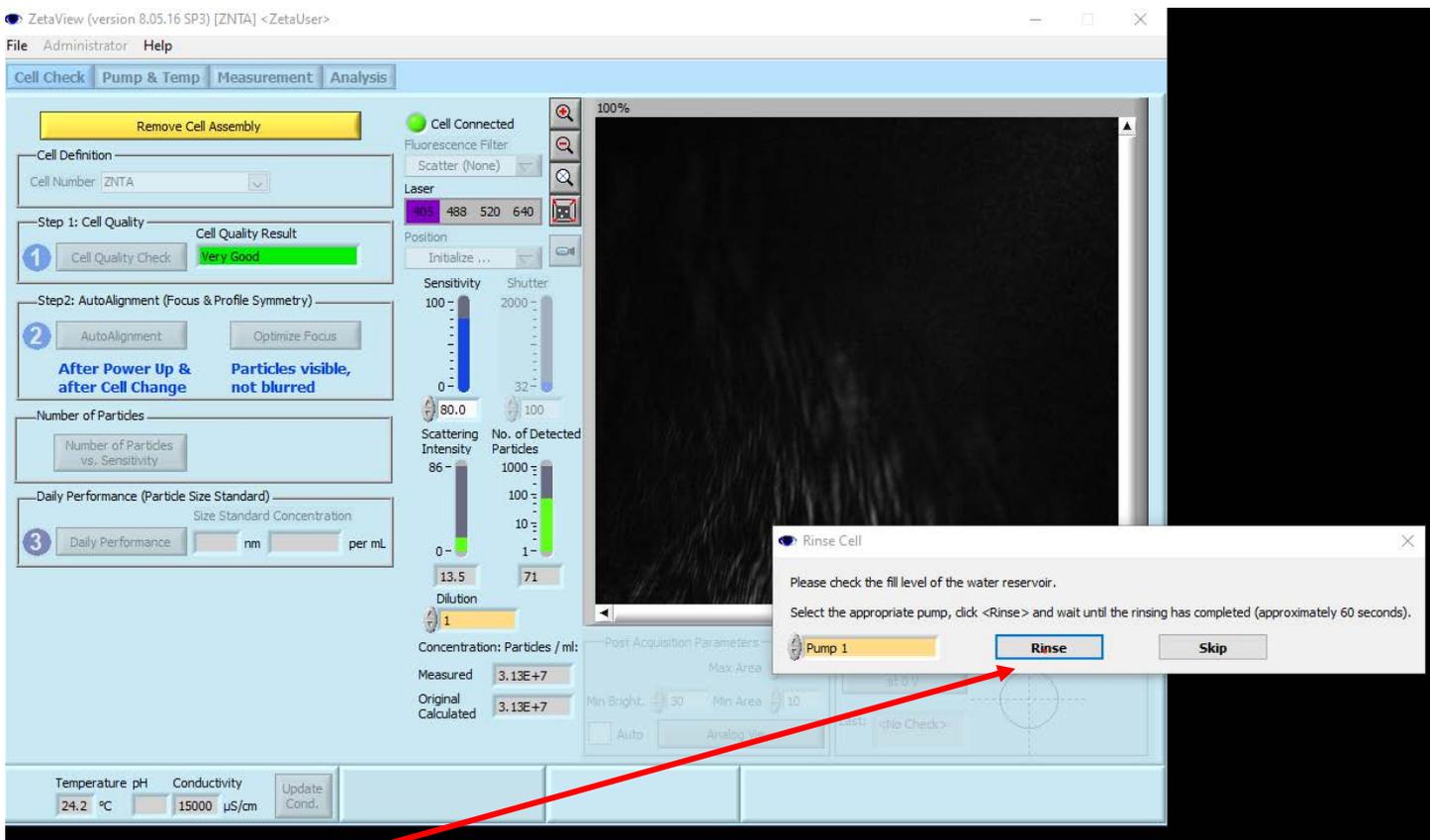
1) Turn system ON:

- (a) turn ON the switch at the back side of the blue box;
- (b) then turn on the computer – Windows Password is 123456.
- (c) Turn ON the ZetaView software

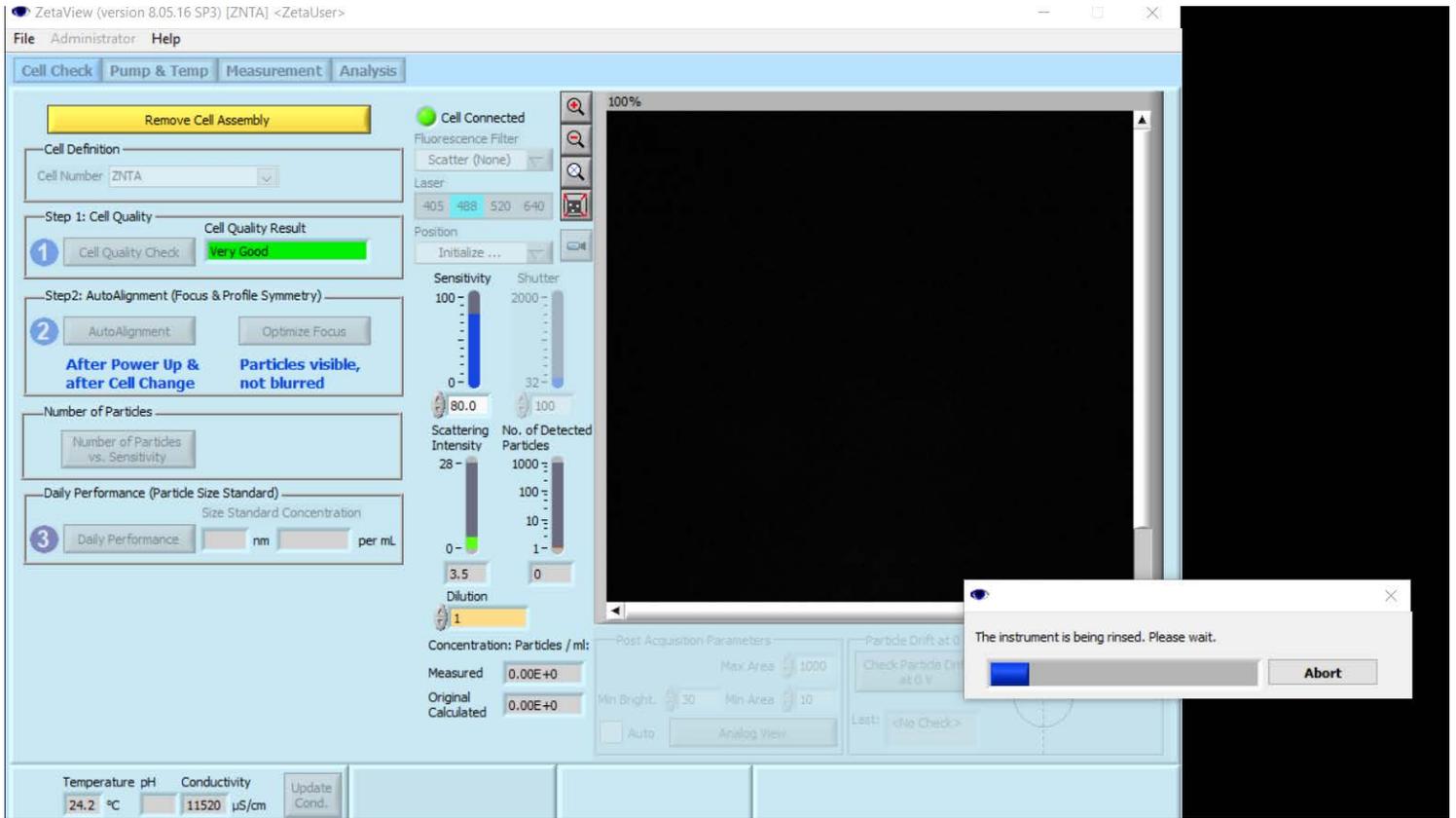


- (d)
- (e) The system will initialize for about a minute, so wait for a prompt from the software

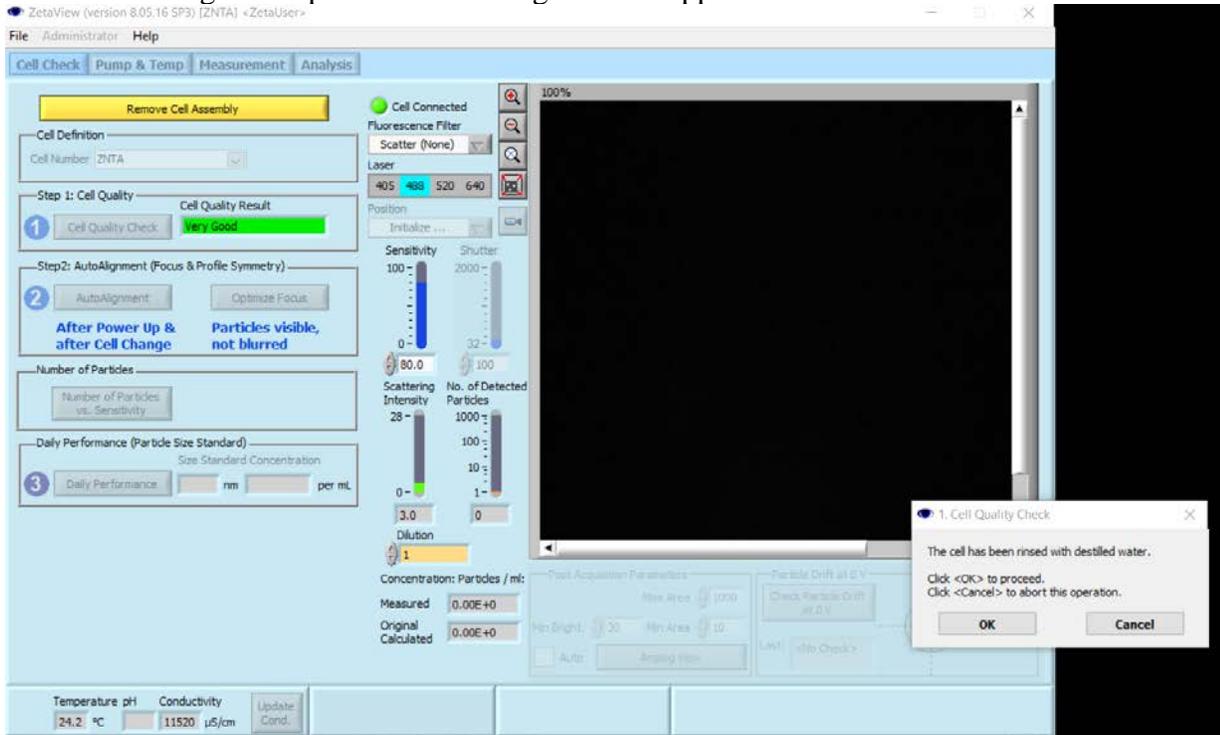
2) Allow software to prime the sample cell with water from Pump 1 (the following window will appear)



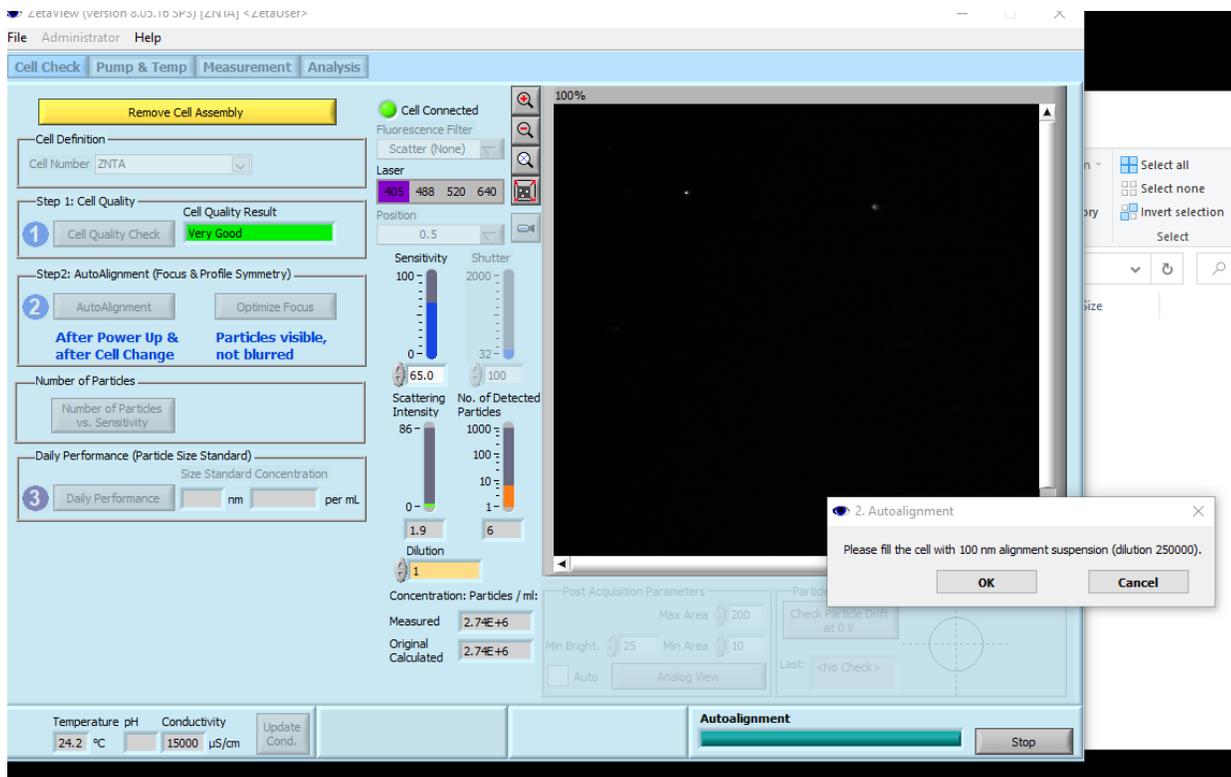
Choose “Rinse” the bottle attached to pump #1 should contain water; the deionize water from the white faucet (house DI water) is sufficiently clean to use on the system.



3) After the rinsing is complete the following window appear:



4) After the Cell quality is assessed (should read Very Good) the following window appears:



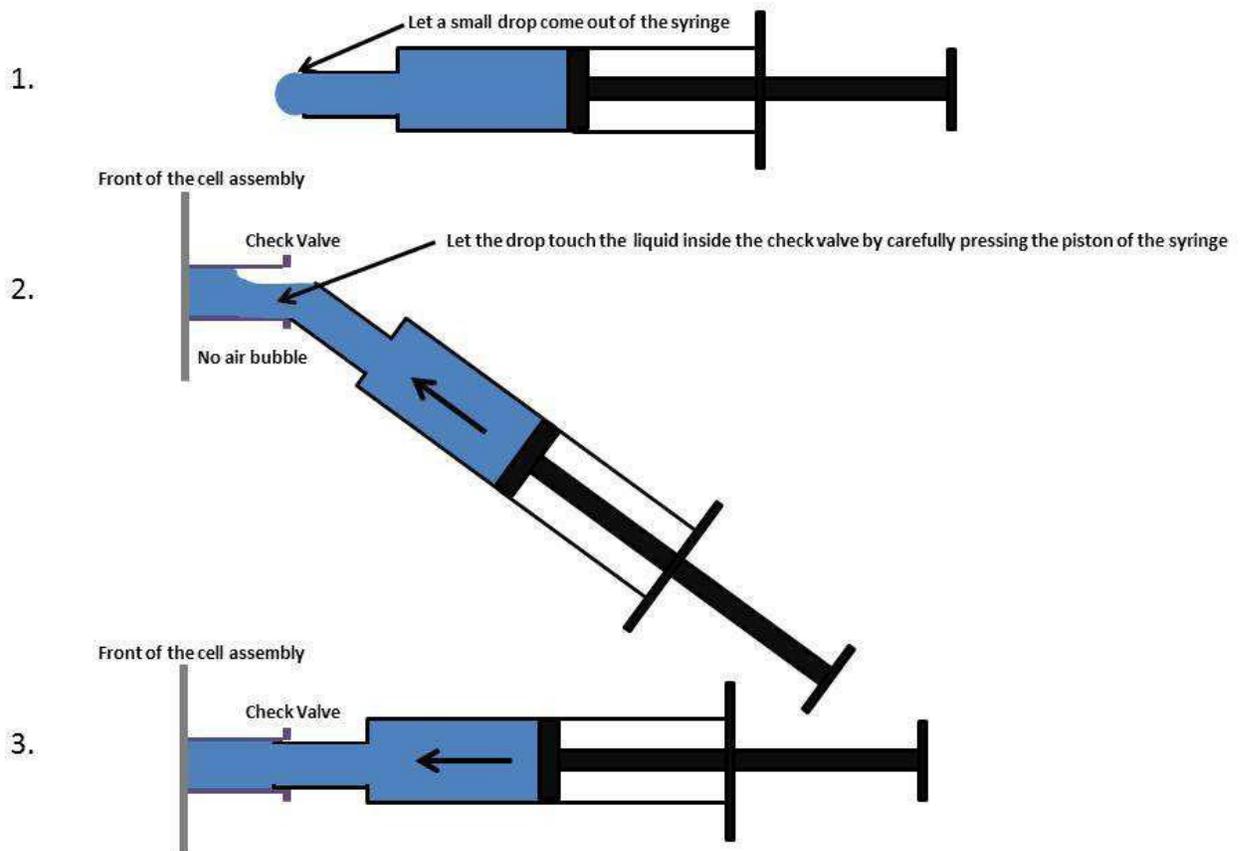
5) You may rinse the cell with water from the sample loading port.

6) **ABSOLUTELY NO LEUER LOCK SYRINGES**



a) Before proceeding further, practice the “art of” injecting a sample into the cell by making a droplet at the end of the sample loading syringe (per figures below).

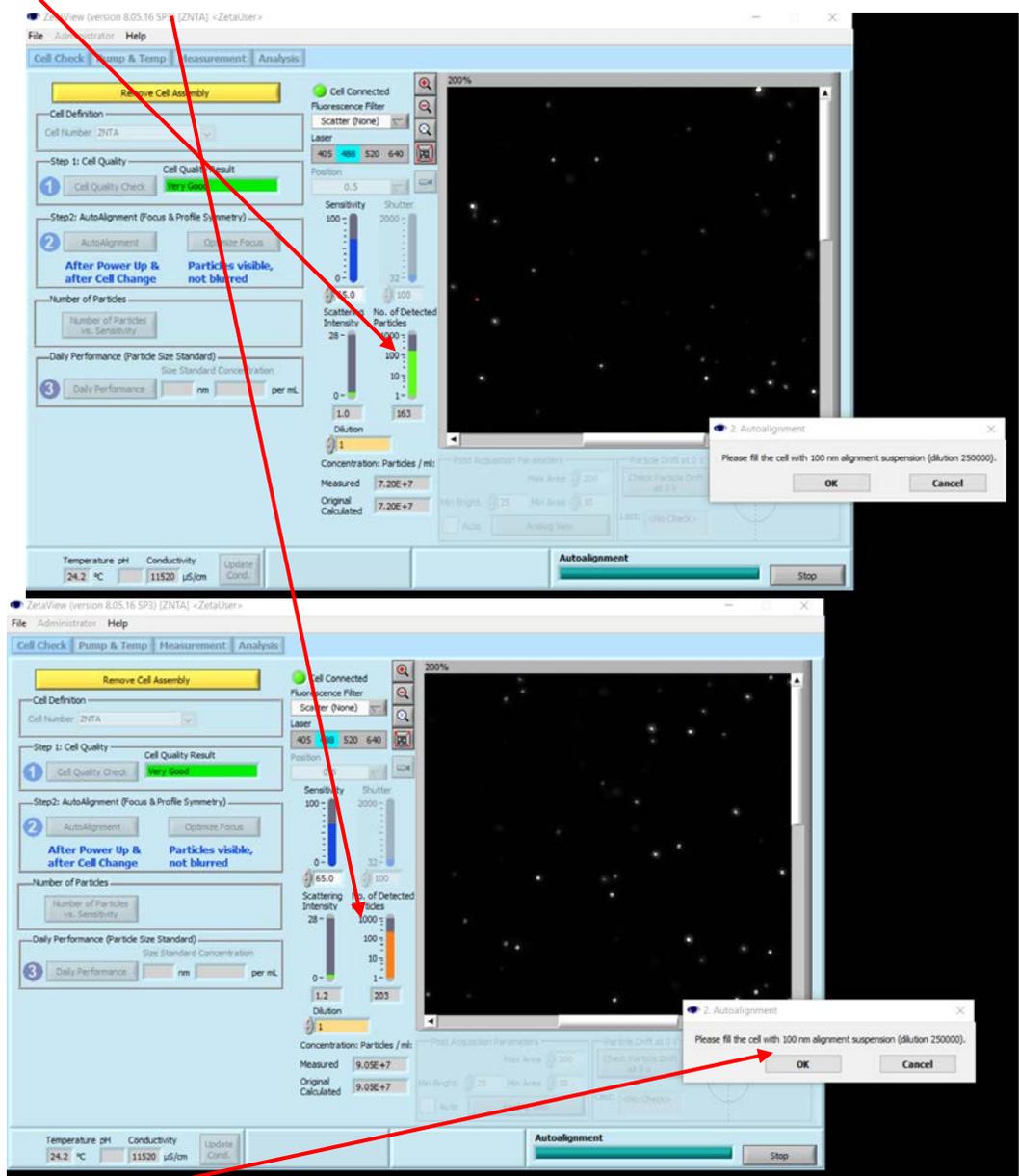
Try to inject your sample in this way, here you are not creating air bubbles!



- 7) **Run cell Quality Check** as prompted. Results should be reported as ‘Very Good’ under the cell check tab - if not Very Good, the flush syringe and/or water source are likely unclean, so replace them both and repeat
- 1) **Prepare 100 nm standard** suspension (250,000x dilution) for AutoAlignment / Optimize Focus, as prompted by Software, and then inject the standard sample into the cell. **Use a 1 mL syringe to inject 250k times diluted 100nm polystyrene (PS) standard** (stock and 1000x dilution are kept in the fridge- bottom shelf, left corner)

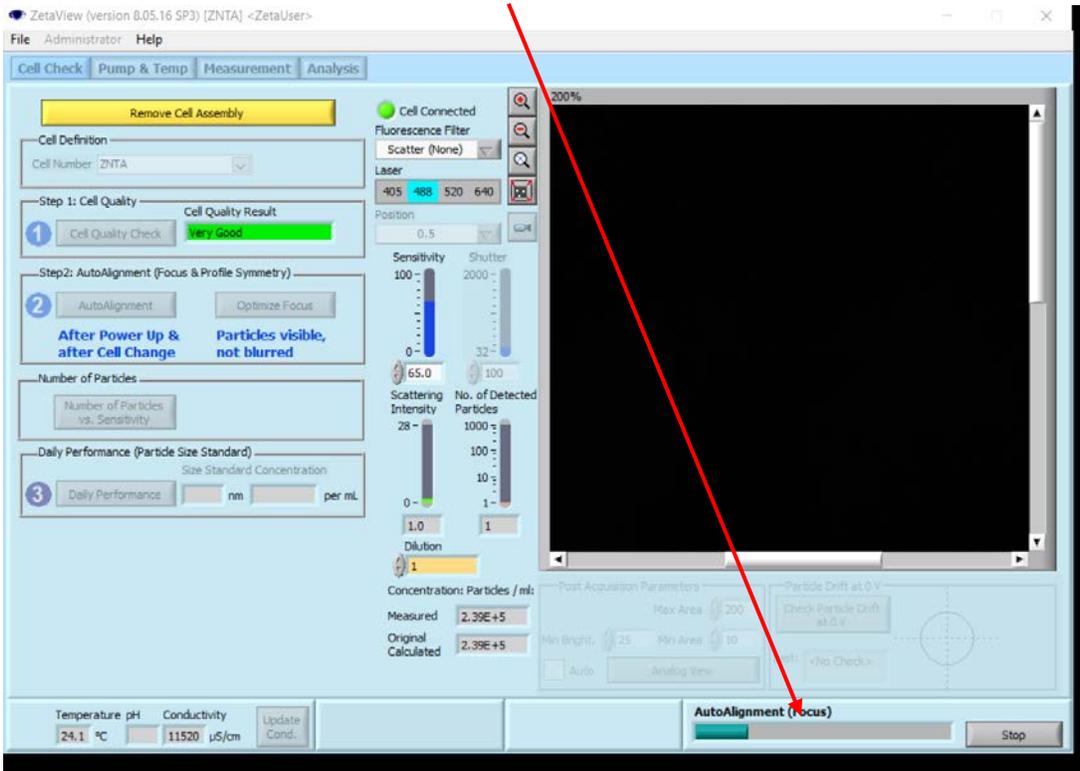
Side Note: A primary 1,000x stock of 100 nm PSL standard is typically usable for 2 months (i.e., for making a final dilution for daily instrument Start-Up checks); however, the fully diluted standard is only good for a couple hours, so always have some 1,000x stock available to prep a full 250kx dilution for Start-Up checks.

- 2) Once the cell is filled with the PS 100nm standard, you should monitor the particle number to make sure that they are either in green or orange range; if too concentrated, make an additional 2x dilution:

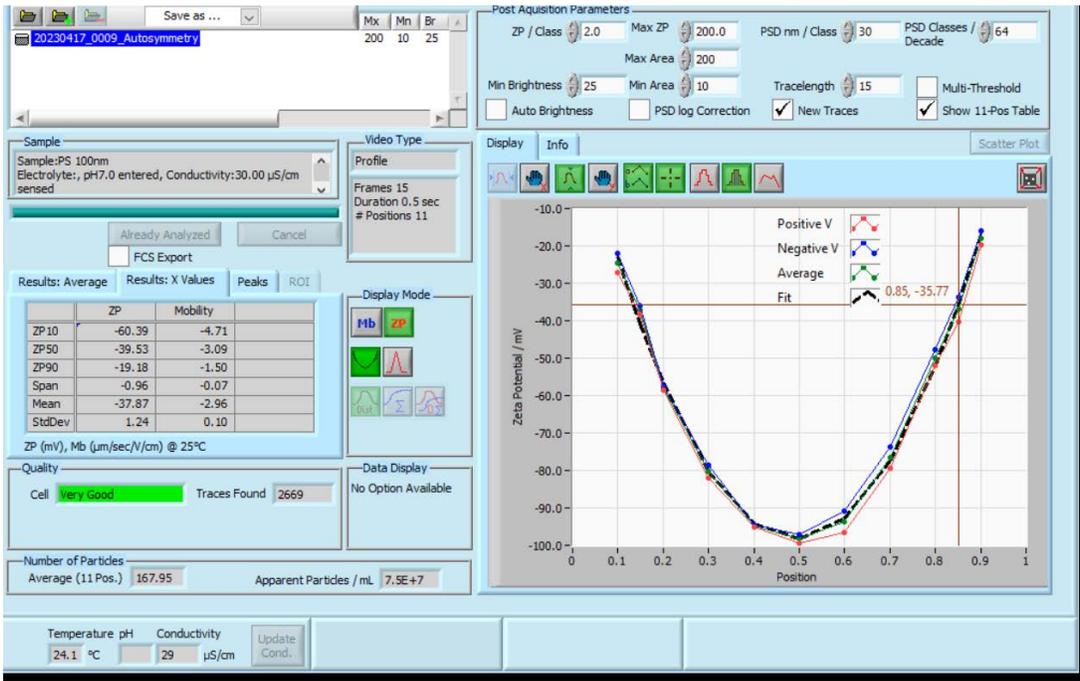


Please ensure that the system has been ON for ≥ 20 minutes to get it up to temperature ($> 21^{\circ}\text{C}$). Choose **OK**

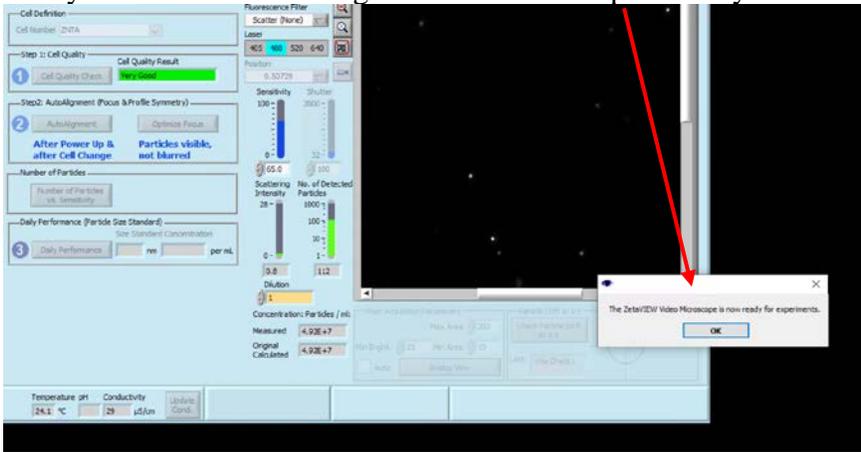
3) The system will start autoalignment and focusing:



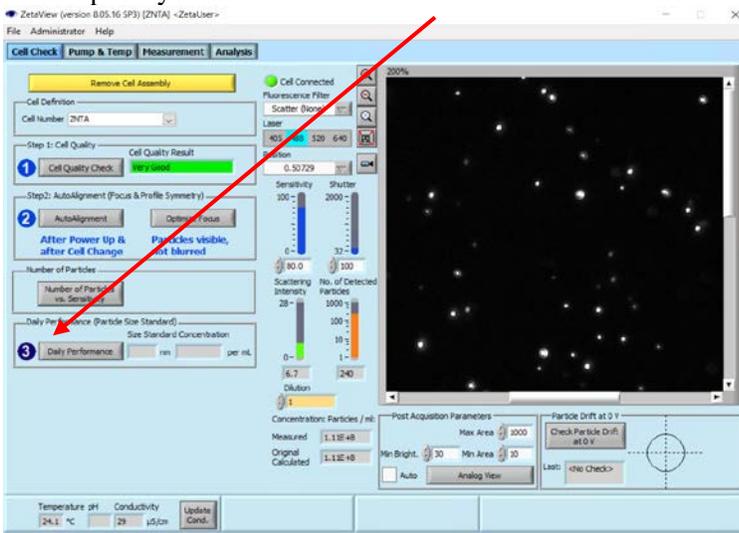
4) You should see the parabola appeared after this step is completed:



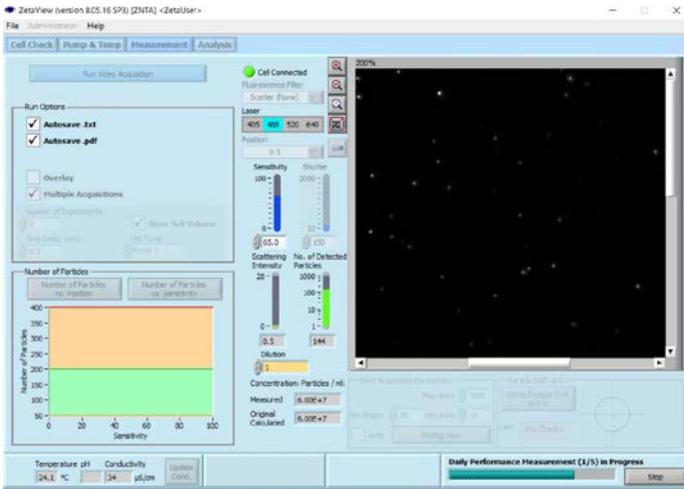
5) And you will see the message that the microscope is ready



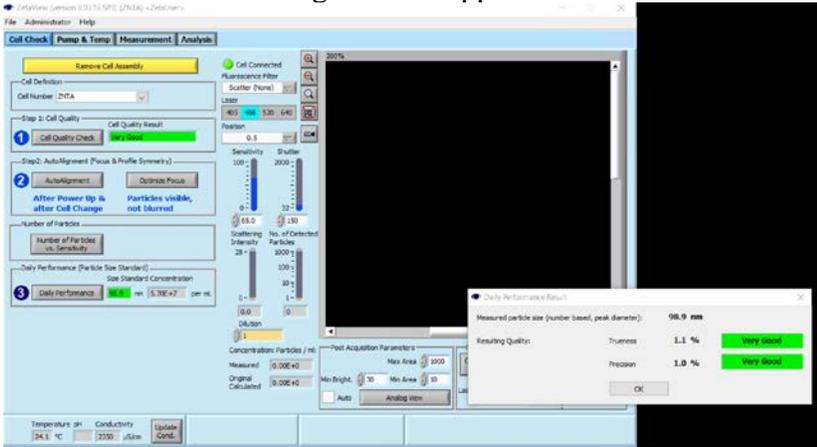
6) At this point you should run **DAILY Performance** –



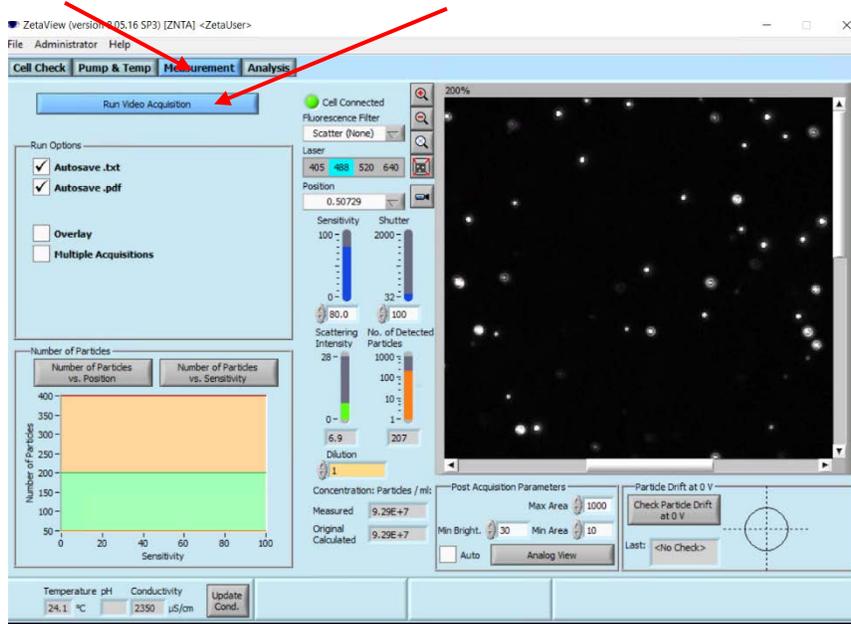
name the results file with the date for reference



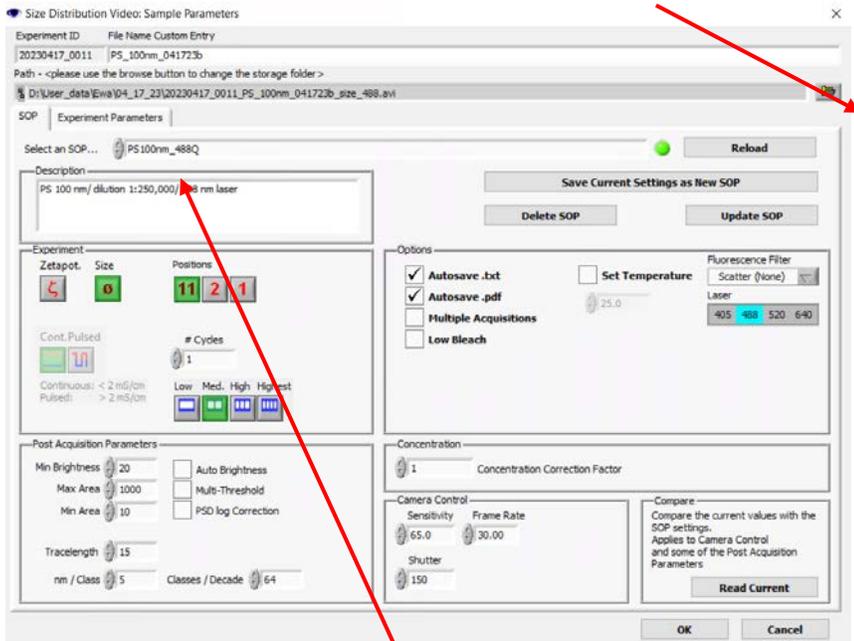
7) When done the following window appear



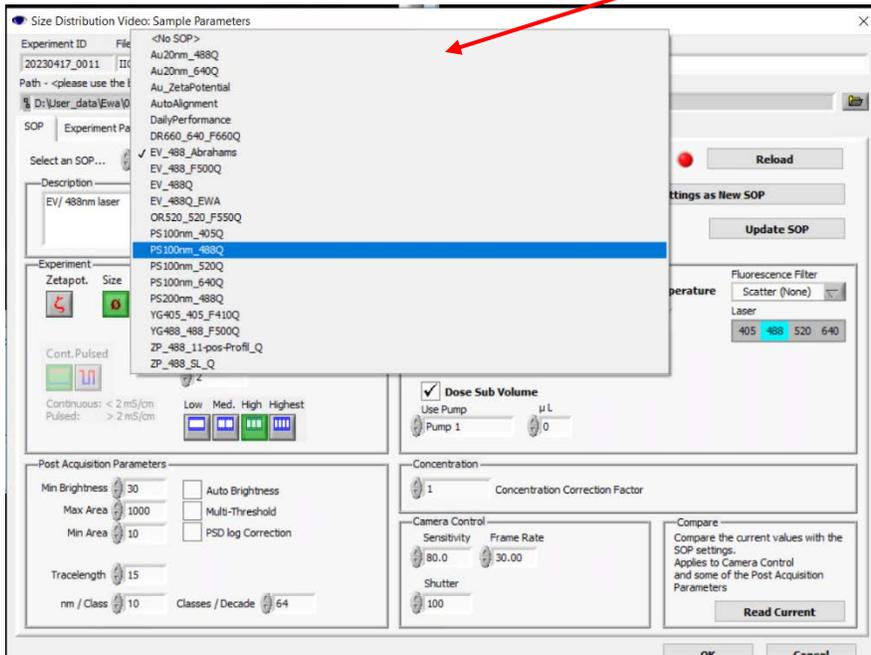
8) Even though the daily performance was done, the results are not accessible for reference, so it is a good practice to run the measurement as a regular experiment, so the result can be saved with your other data; choose Measurement and Run Video Acquisition



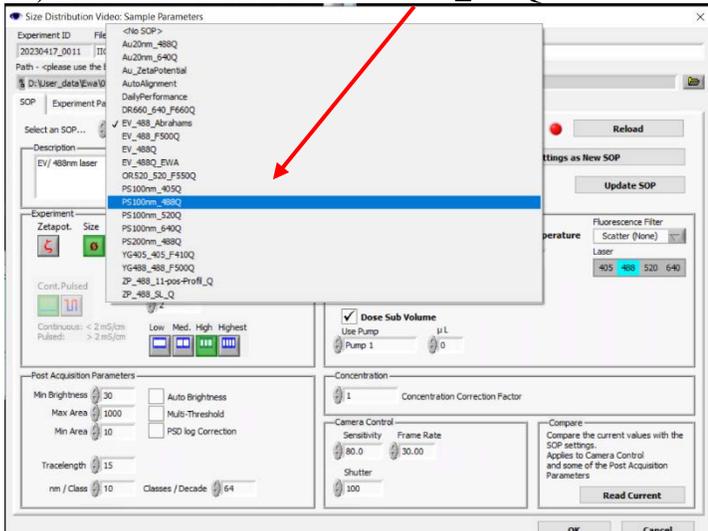
9) The following window pops up; choose data storage folder: and name the results' file



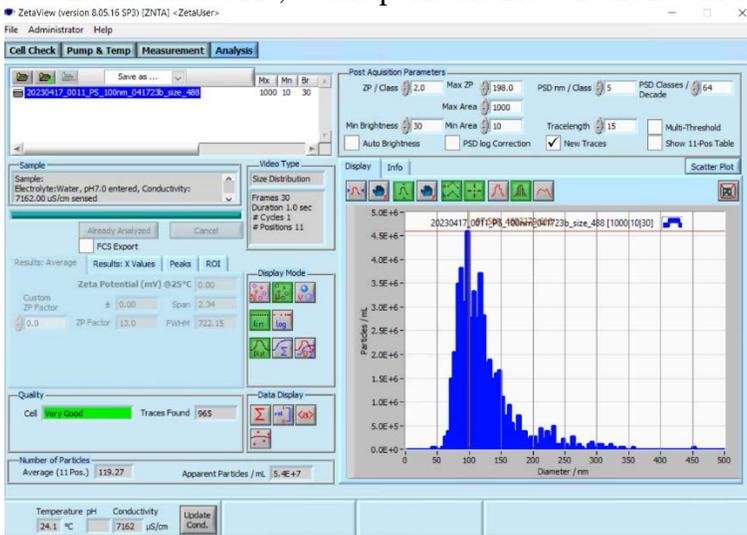
10) You will need to choose SOP (standard operating procedure) for this measurement from the pull down menu



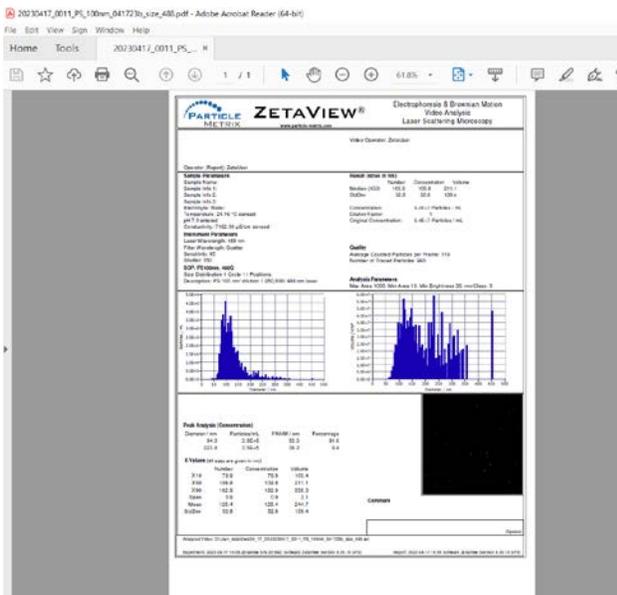
11) For PS standard use: PS 100nm_488Q



and choose “OK”; at this point the measurement will start and when done the result’s window appears:

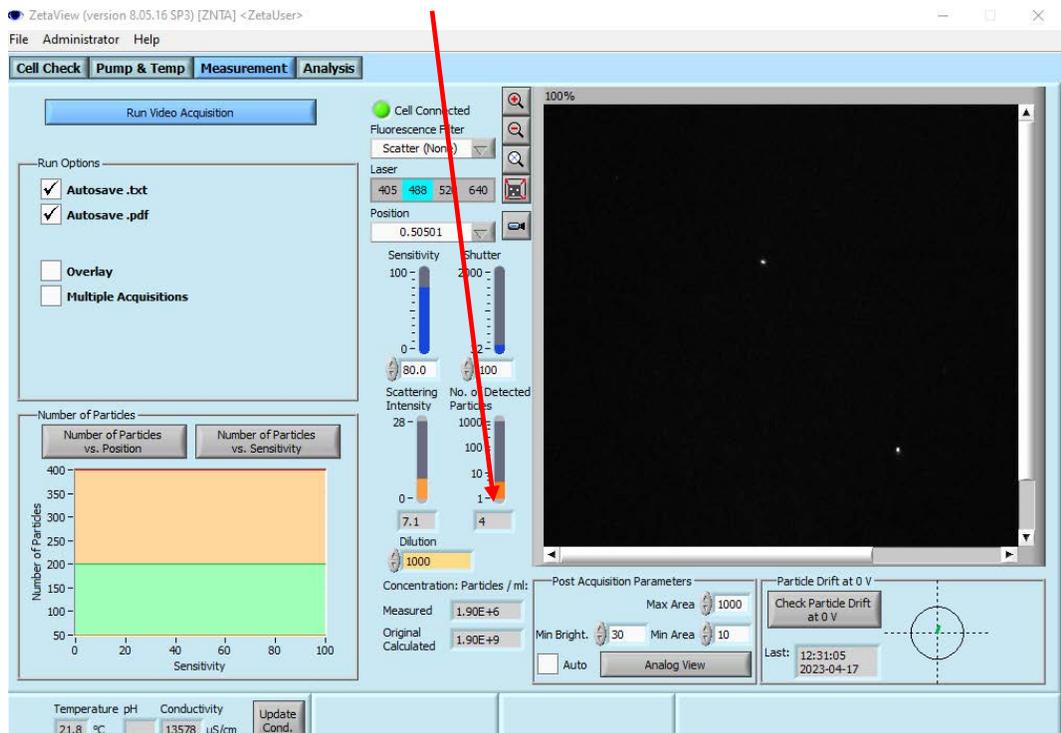


12) The *.pdf with results report will appear as well:

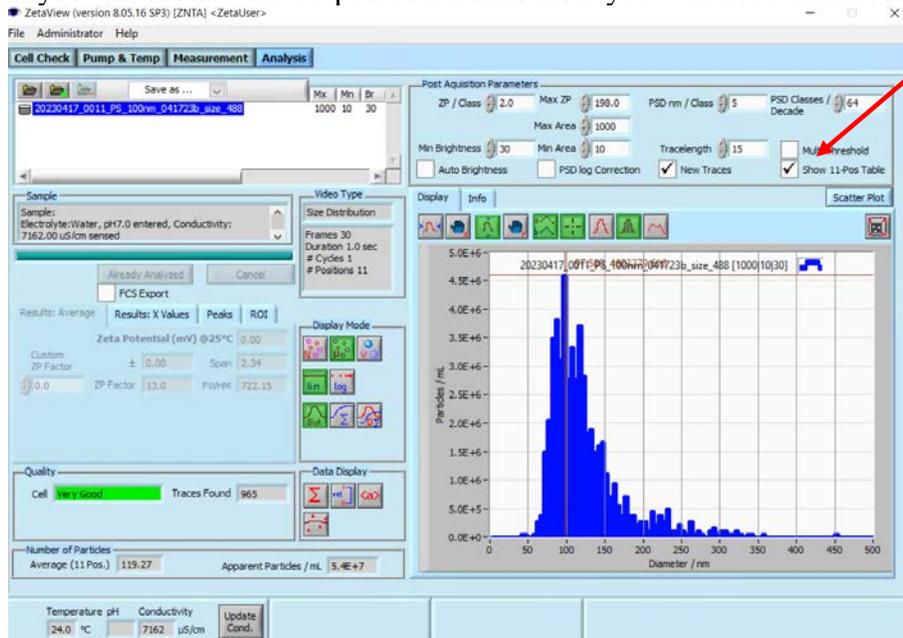


13) At this point the instrument is ready for analysis of samples of interest. Rinse the cell with water and

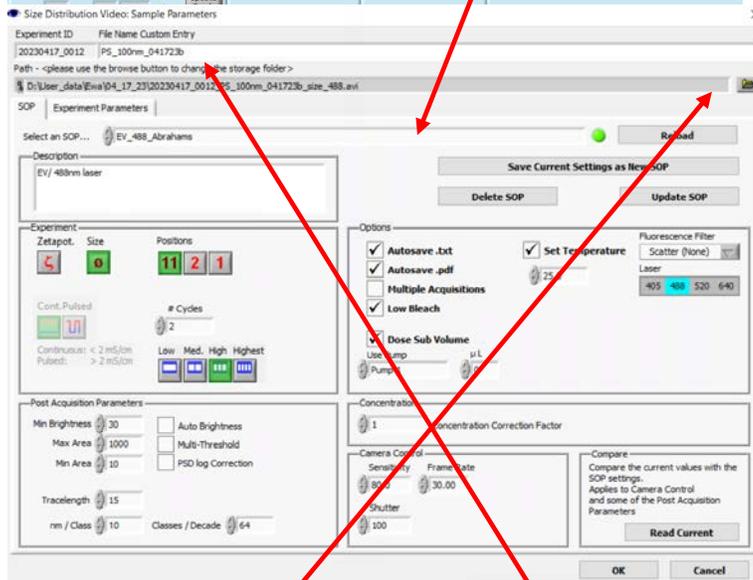
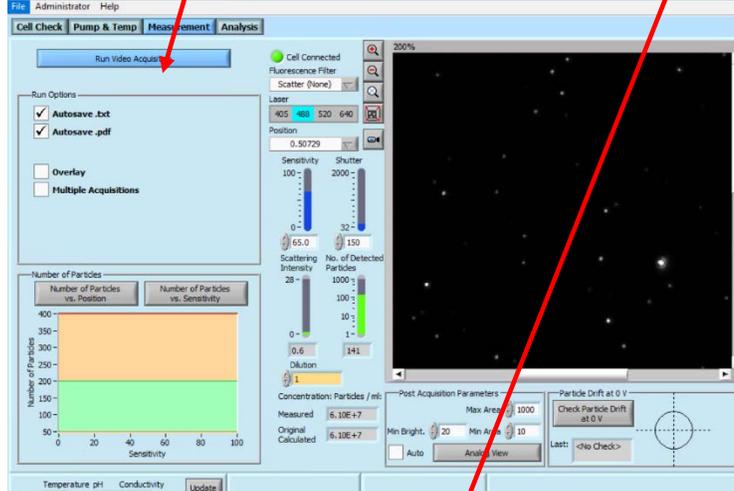
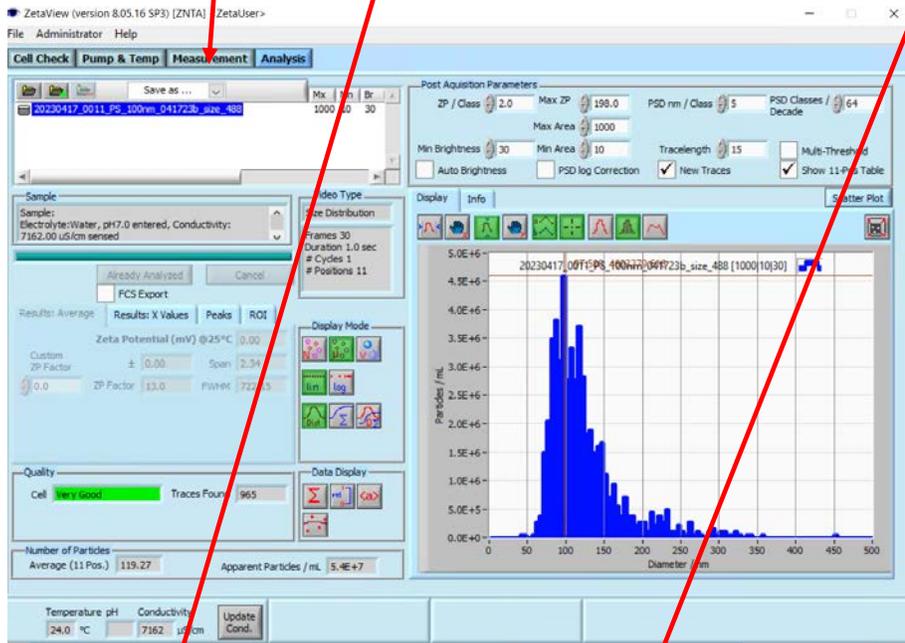
your buffer until there are just a few particles detected by the camera:



14) If you want to see the 11-position results table you will need to choose this option under Analysis tab:

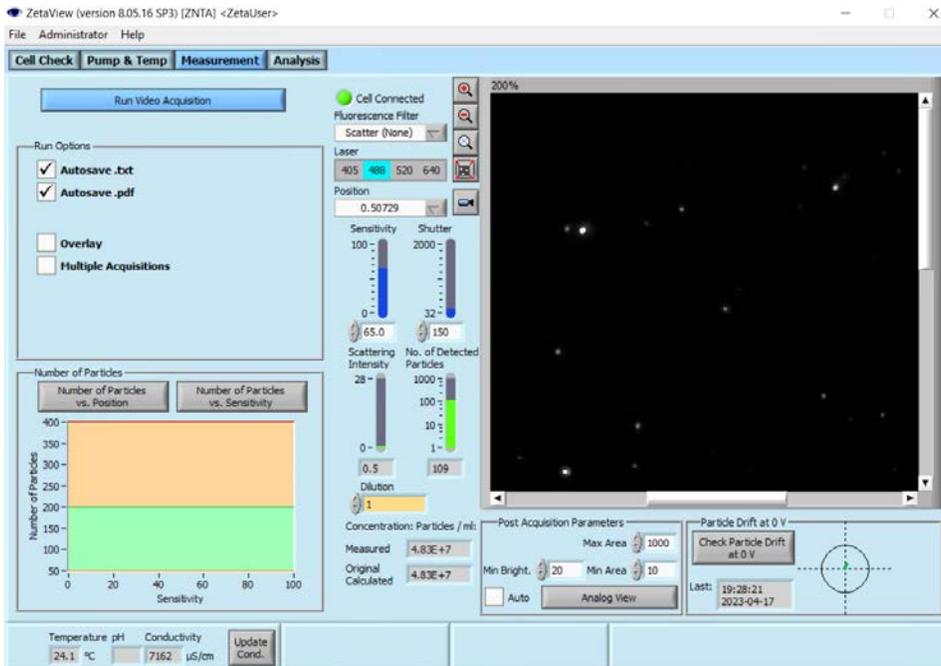


15) Select: **Measurement**, **Run video acquisition** and choose appropriate **SOP** from the acquisition window

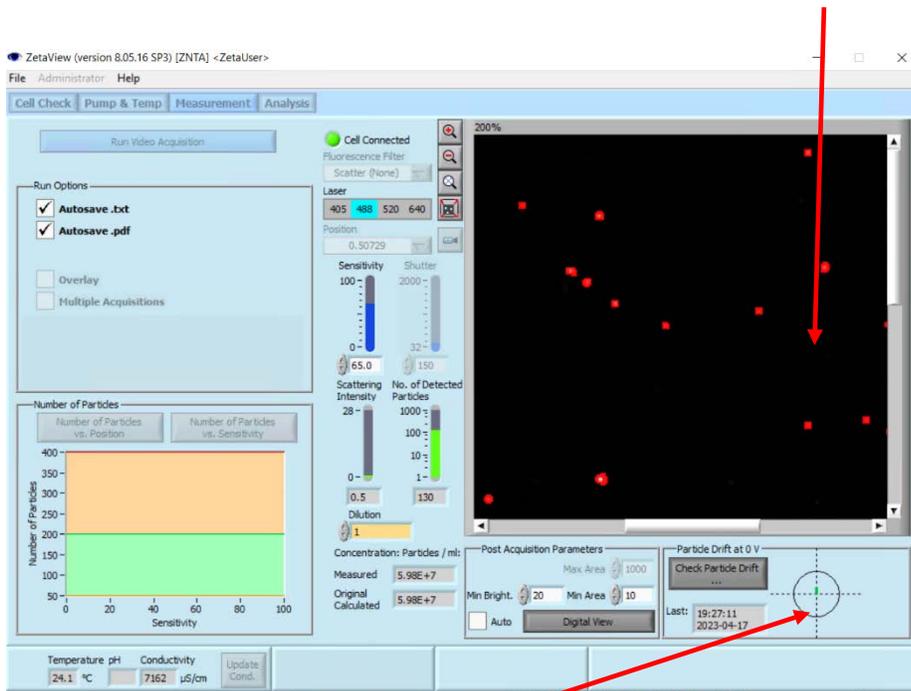


16) Update the data storage folder and the name of the results file and choose OK.

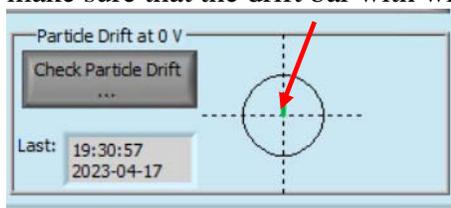
17) Once the sample is loaded; check the drift before the data are collected:



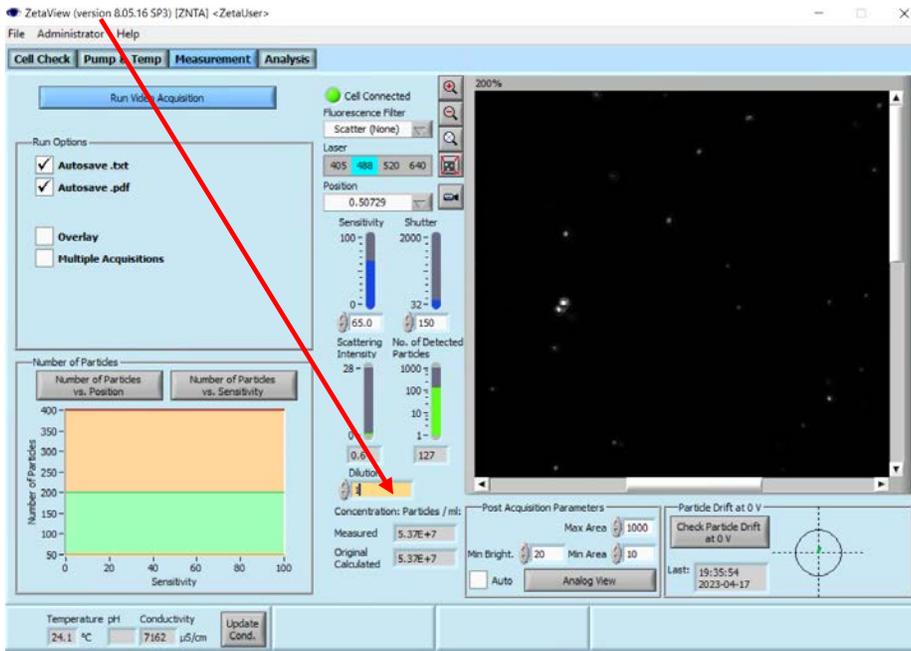
18) Once you choose this option, the instrument will start tracking the drift in the following display;



make sure that the drift bar with within the circle and shown in green.



19) Deselect “Check Particle Drift” and start measurements. Before measurement, you may update the dilution factor for your reference:



20) Once the measurement is finished; the 11-position table will appear for your review:

File Administrator Help

Cell Check Pump & Temp Measurement Analysis

Save as ...

Post Acquisition Parameters

ZP / Class 2.0 Max ZP 198.0 PSD nm / Class 5 PSD Classes / 64

Positions Overview

Use	Position	Mean Int.	Av. No of Particles	Conc. (p./ml)	Orig. Conc. (p./cm ³)	No. of Traces	X50 (nm)	Peak ø (nm)	Span	Drift (µm/s)	Removal
X	0.10	0.6	91.0	4.1E+7	4.1E+7	72	109.2	104.1	1.2	6.3	
X	0.15	0.6	118.0	5.3E+7	5.3E+7	94	110.4	109.3	1.1	9.2	
X	0.20	0.3	101.0	4.5E+7	4.5E+7	85	103.8	93.9	1.1	10.2	GRUBBS_MI
X	0.30	0.8	129.0	5.8E+7	5.8E+7	93	112.8	119.1	1.0	10.8	
X	0.40	0.7	135.0	6.1E+7	6.1E+7	97	115.4	120.5	0.9	11.0	
X	0.50	0.6	130.0	5.8E+7	5.8E+7	87	99.0	93.8	0.9	8.7	
X	0.60	0.5	134.0	6.0E+7	6.0E+7	86	111.5	93.4	1.0	5.3	
X	0.70	0.6	158.0	7.1E+7	7.1E+7	109	106.0	96.4	1.0	4.0	
X	0.80	0.7	129.0	5.8E+7	5.8E+7	86	103.2	100.1	0.9	0.6	
X	0.85	0.6	121.0	5.4E+7	5.4E+7	79	111.9	93.4	1.1	0.3	
X	0.90	0.7	127.0	5.7E+7	5.7E+7	90	109.2	88.0	0.9	0.8	
	Mean	0.7	127.2	5.7E+7	5.7E+7	89.3	108.8	101.8	1.0	5.7	
	St.Dev.	0.1	16.7	7.5E+6	7.5E+6	10.1	4.9	11.2	0.1	4.2	
	Rel.St.Dev.	11.6	13.1	1.3E+1	1.3E+1	11.3	4.5	11.1	10.0	73.5	

Continue

Quality: Cell Very Good Traces Found 978

Data Display

Number of Particles: Average (11 Pos.) 124.82 Apparent Particles / ml 5.6E+7

Temperature pH Conductivity

24.1 °C 9383 µS/cm

Update Cond.

1.5E+6

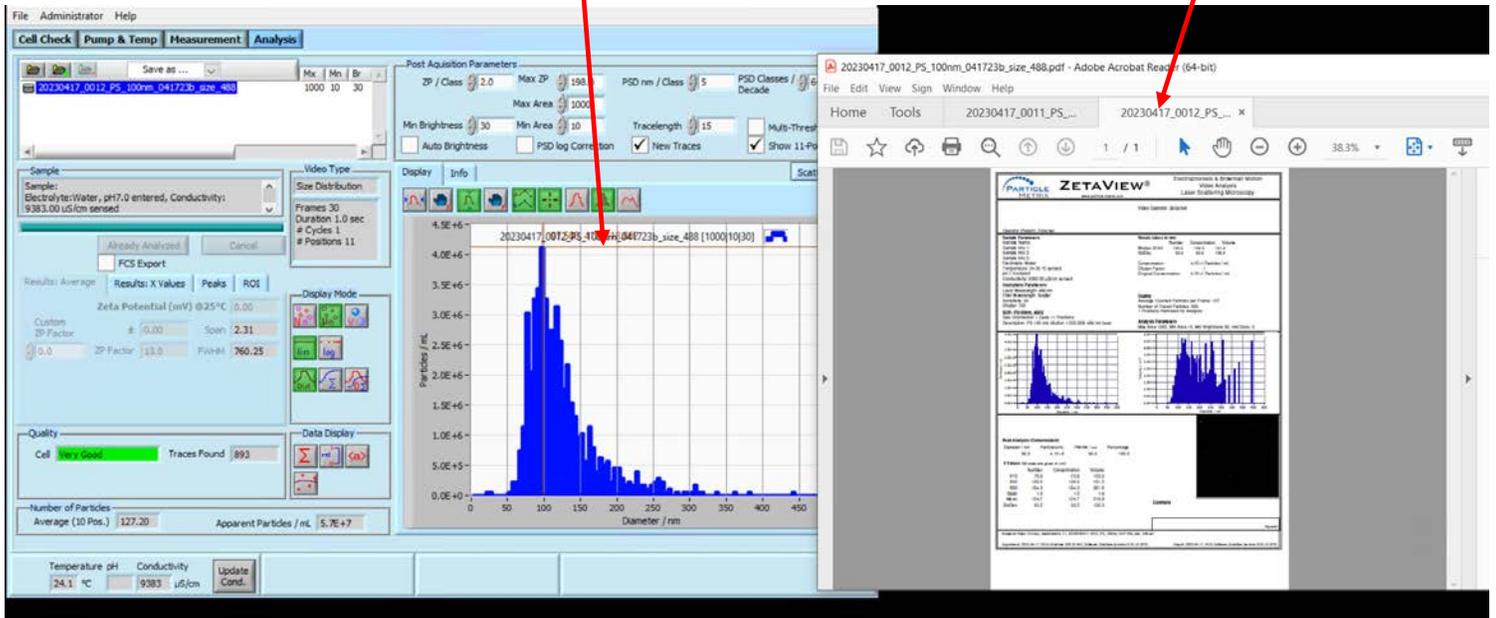
1.0E+6

5.0E+5

0.0E+0

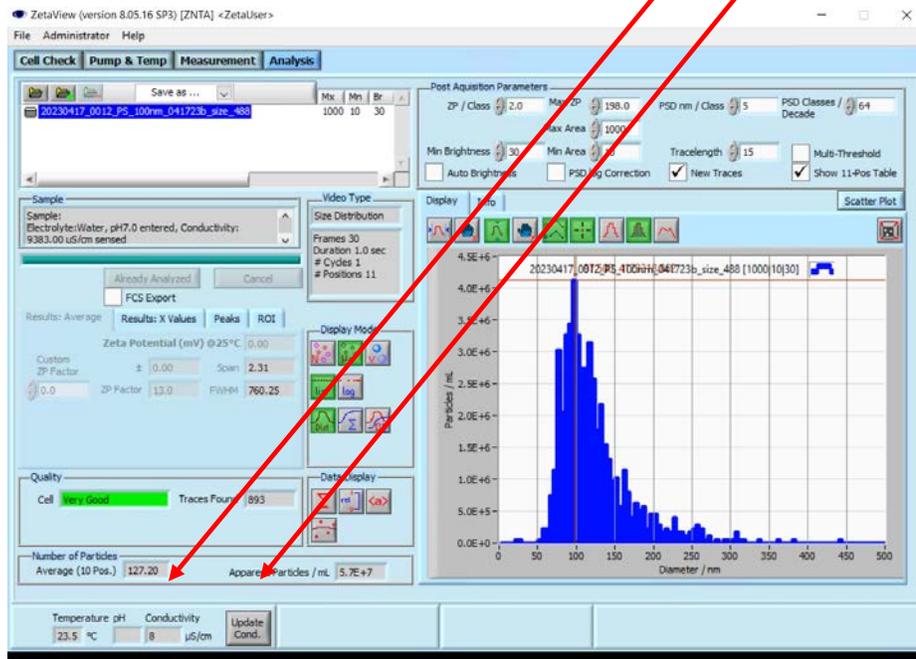
Diameter / nm

And once you choose “Continue” the results will pop up and the report will show up as a *.pdf file.



SHUT DOWN

- 21) Rinse cell with buffer in which the samples were prepared and finally with water.
- 22) Check conductivity recorded in the sample cell by clicking “Update Conductivity” button. Observe the number; it should change from a few thousand, to below 20 for clean water.
- 23) Keep on rinsing with water, but if this is not sufficient, you may soak the cell for 1-2 minutes with diluted HELMMA detergent (in 100ml bottle on the shelf above the instrument) and rinse with water again.



- 24) Once the cell is clean and the conductivity is low; push 10ml of air to empty the cell.
- 25) Close the software.
- 26) Shut down the instrument (power it down- the blue light at the right corner should shut down **IMPORTANT**).
- 27) **EMPTY WASTE** bottle and **bottle with water used for rinsing** (attached to pump #1).

Additional information:

- 28) **Interpretation of NTA Data Analysis** – *explain the following parameters, details in manual pg. 161-179:*
 - a) 11 position analysis – *briefly describe the fields in the 11-pos table, with removal criteria (pg 117-122)*
 - b) X50, mean, peak analysis – *make sure to point out the 3 main types of particle size values reported*
 - c) Vol vs. frequency plot – *explain obsolescence of Volume Plot, except for those correlating to DLS values*
 - d) Peak summary – *manual pg. 173 ... explain the spotty peak-picking algorithm, often missing smallest*
 - e) ROI - *manual pg. 175 ... very useful to complement data exported to graphing tools ... quick & easy!*
 - f) Scatter plots – *pg. 188-190, show where to set FCS file conversions in SOPs & convert via post-analysis*
 - g) Run peak overlay – *the manual describes all the peak comparison tools but without explicitly showing how to do a simple peak overlay with the buttons ... this needs to be demonstrated.*
 - h) Ave, relative view, smoothing – *pg 166-172 ... quickly demonstrate.*

- 29) **Modifying, or building own SOP's** – *details found in the manual from pg. 144-160*
 - a) per section 8, initiate a “trial run” using a starting SOP - hit OK, then hit Cancel
 - b) Go to Measurement tab, enter digital mode, then optimize Camera Sensitivity & Shutter for application:
 - (i) for particles > 200 nm, set Sens. = 70, Shutter = 175 then reduce Sens. to optimal, minimizing noise
 - (ii) biological particles < 80 nm, Sens. = 84, Shutter = 110, reduce Sens. to optimal, minimizing noise
 - c) What do all the different options do?
 - i) Movie length: *silly film strip icons: for 30 fps, they are ½, 1, 2, 3 secs. then double that for 15 fps*
Note: the manual's description (pg. 98) is false and therefore is basically useless.
 - ii) # of positions – *explain using SL1 & 2 for ZP, or for quick NTA reconnaissance analyses.*
 - iii) # of cycles - *explain when to increase to 2 or more (i.e., for on-screen particle counts < 100).*
 - iv) Size vs. Zeta Pot - *explain when to increase to 2 or more (i.e., for on-screen particle counts < 100).*
 - v) Laser selection and mode selection –*show the buttons ... important for fluorescence SOPs*

- 30) **Running f-NTA measurements** - *Manual pages 200-218*
 - a) Switching between scatter and fluorescence manually.
 - b) Switching between the two by loading SOP's
 - c) Teach necessity of setting **focus manually** in **Admin Mode** (and setting Min Bright < 20 for SOPs)

d) Explain & demonstrate the low bleach option using labelled beads

Offer the “f-NTA low-bleach cheat sheet” as a separate, detailed Quick Guide for using f-NTA.

e) Spend a few minutes pointing out our sample prep labeling SOPs (manual pages 219-224)

31) Running zeta measurements

a) Check conductivity of your sample by clicking “update conductivity” in the lower left corner of the “Cell Check” window.

- For sample conductivity < 2000uS, use Continuous Current mode (select appropriate SOP icon)

- For sample conductivity > 2000uS, use Pulsed Current mode (select appropriate SOP icon)

- If your sample is in 1x PBS the conductivity will be too high, so dilute buffer at least 3x

- to get salinity so that conductivity < 2000uS, you will need to dilute PBS by 20x with water.

b) **Always run zeta measurements last** – ZP passes a charge through your sample and, which can change the apparent particle size; i.e., this electro-chemical experiment slightly *cooks* biological particles

c) **Always make sure the sample cell is clean** - the cell ZP calibration (AutoAlignment) depends on it.

32) **Data output** – show the file types created (incl. FCS) and show file structure of histogram *.txt files.

33) **Removing and servicing the Sample Cell** – acquaint users with our Instructional YouTube videos.

a) Review the video for removing cell, *then demonstrate it.*

https://www.youtube.com/watch?v=Fl1ycEq6Fkg&list=PL_sY_BH4vS4BddT2eSAW3b2cKGKTzjsU8&index=6

b) Review the video for cleaning the sample cell, *then demonstrate it.*

https://www.youtube.com/watch?v=LmzrKtsiXmY&list=PL_sY_BH4vS4BddT2eSAW3b2cKGKTzjsU8&index=4

34) Trouble Shooting

a) Identifying and **removing air bubbles**: *review the video then demonstrate.*

https://www.youtube.com/watch?v=2lat7fGSBHU&list=PL_sY_BH4vS4BddT2eSAW3b2cKGKTzjsU8&index=14

- *If bubbles stubbornly remain, then clean the sample chamber.*

b) Manual focusing

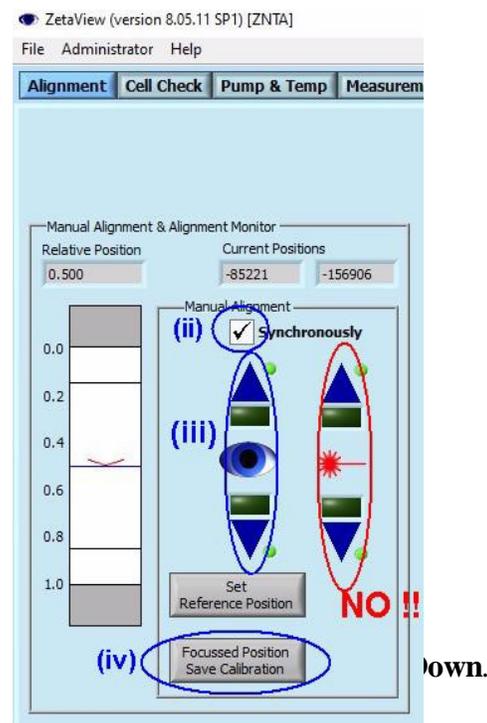
i) Go into Admin Mode (06charly13) then click the Alignment tab
(located far left of the screen) ... *per figure at right.*

ii) Un-check button “Synchronously”,
this decouples the Lens movement from Laser positioning

iii) Adjust only the lens positions (buttons located @ left with the
eyeball icon). Do NOT adjust the laser buttons!

iv) Click the button “Focussed Position Save Calibration”
(*ignore the gem quality typo*), this sets your new focus position.

v) Re-check the button Synchronously – *you’re now ready*



a) If a user just ran 10 EV samples, clean sample cell per section 14

b) After cleaning the cell, run water through the sample cell w. Pump 1, for > 15 seconds

c) Then flush the cell twice with water from a 3-5 ml syringe

d) If the system will be used within the next 1-5 days, then the system can be left as is (wet)

e) If the system won't be used for a while, you can blow the cell clean with air, but you **MUST** ensure that the sample cell is clean, otherwise any organics pasted to the cell walls will dry into a hard crust!

f) Do NOT leave the sample cell with any sample material in it, nor with PBS in it ... this is bad practice

g) Turn OFF the ZetaView software, then turn OFF the blue instrument box (switch @ backside)

** Clean the connections to the sample cell once a week, or sooner if needed, to remove buffer from pins