

# MANTA S.O.P

## **Instrument:**

Viewsizer 3000 from Horiba

## Software:

ViewSizer3000

## **Purpose:**

For direct measurements of Brownian motion through image processing, providing size and particle concentration information. Advanced techniques are also available for Kinetic, Fluorescence and Sedimentation assays.

## To Begin:

## **Know the specifications of the instrument.**

Specifications	Viewsizer 3000		
NTA Size Range	10nm-1um		
Sedimentation Size Range	1um-1um		
Lasers	445nm, 520nm, 635nm		
Spectral Resolution	Better than 0.09cm1		
	10 nm to 15 μm		
Range of Particle Sizes			
Measured*			
Typical Sample Volume	350 µL to 2.5 mL		
Typical Sample	105 – 109 particles/mL		
Concentration*			

- For more tips on focusing and stirring, consult the "Tips for Using Viewsizer 3000" document next to the instrument.
- A note about solvents and NTA: NTA is an extremely sensitive technique that can see particles at low concentrations. As such, some unfiltered solvents that are suitable for DLS are not suitable for NTA measurements. Excess background noise may occur if your solvent is not free of stray particulates.
  - Make sure your solvent has been filtered to be free of particles larger than 100 nm before sample dilutions. Running a "blank" will not work if the number of excess stray particles is above the saturation detection limit and are close in size to your particles of interest.
  - o Unfiltered PBS is a common culprit for noisy backgrounds.



Filtered PBS

Unfiltered PBS



#### Steps to startup the instrument

**NOTE:** The instrument is powered off after use. If powering on, immediate software connection may not occur. Allow the system and lasers to warm up for 1 minute before opening software.

#### > Power On Instrument

- 1. The instrument power switch is located on back left corner.
- 2. Log on to the computer
- > Open Viewsizer Software (VU-3000 Measure)
  - Upon initialization of software, confirm connection by enabling video view. The software may need a minute upon bootup to fully connect to the camera

#### Prepare your sample

#### Clean the Cuvette

- The quartz cuvette for the NTA should be cleaned before and after each sample is tested.
- Use the cuvette cleaner to rinse the cuvette
  - Flip the cuvette upside down onto the spout of the cuvette cleaner.
  - Turn on the vacuum line to start pulling air through the cuvette
  - Add Water and IPA to the solvent introduction funnel
    - DO NOT DUMP WASTE IN THIS FUNNEL, YOU WILL CONTAMINATE THE CLEANER FOR EVERYONE
  - Use the vacuum of the cleaner to dry out the cuvette
  - Turn off the vacuum line and remove your cuvette.



Cuvette cleaner loaded with cuvette and washed with cleaning solvents. Make sure the vacuum line is turned on during use. Do not dump waste into the cuvette cleaner.

#### Clean the stir bar and the cuvette insert

• Rinse the stir bar and the cuvette insert with IPA and water into a designated rinse waste centrifuge tube. Avoid dropping/damaging the insert and stir bar.



## Load the Cuvette

• Add the stir bar to the cuvette and then use the magnetic cuvette holder to hold the stir bar in the center of the cuvette.



• Gently slide the insert into the cuvette, keeping the stir bar in the center.



- $\circ$  Add 400 µL of your sample to the cuvette using a micropipette.
- If there are air bubbles trapped in the insert, use the vortex spinner to gently shake the air bubbles out.

#### > Insert the Cuvette into the Viewsizer 3000

- Open the lid of the Viewsizer and remove the laser safety cap from the cuvette chamber.
- Place your cuvette inside the sample chamber, making sure to orient the cuvette so that the L-shaped channel is facing the camera and that only a small square hole is facing towards the lasers.
- Replace the laser safety cap (make sure to align the pins so that the cap sits flush against the cuvette chamber) and close the lid of the Viewsizer.



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This side should face the camera



### > Acquiring data with VU-3000

For more detailed tips on focusing, laser power, camera settings, processing settings, and stirring: consult the "Tips for Using Viewsizer 3000" document located next to the instrument.

		8~		Green	Red	# of
Particles	Diameter	Dilution	Blue Laser	Laser	Laser	videos
-	nm	Х	mW	mW	mW	
	200 nm down to 70					
PSL	nm	40000	70	12	8	25
PSLK	50 nm	100000	210	12	8	25
Silica	200 nm and above	40000	70	12	8	25
Silica	100 nm and below	40000	210	12	8	25
nano-diamonds	60 nm	-	100	0	0	25
nano-diamonds	30 nm and below	-	250	0	0	25
Gold	60 nm	-	70	12	8	25
Gold	30 nm and below	-	0	0	25	25
Exosomes	100 nm and below	-	210	12	8	50

#### **Typical Settings for Different Kinds of Particles**



## > Set up your experiment and sample

- To make new experiment setting presets, select Method Settings.
  - Recording Settings:
    - Here you can pre-specify laser powers, identify your dispersant, set up stir times, preset your video settings, etc.
    - Select the mode for your experiment using the dropdown menu in the upper left corner.
      - If using fluorescence mode, you will need to have the fluorescence filters installed. Contact facility staff to help you do this.



- Processing Settings
  - Here you can set your video processing parameters and decide if the videos will automatically be queued for processing
    - It is recommended to turn off "Queue for Processing" if running more than one sample in a session.
  - Display and Plotting can be ignored until you get your results.
- You can name your sample in the sample name box and then open "Sample Information"
  - Here you can label this sample as a sample or as a blank.
    - If running a blank, your sample needs to be labeled as a blank to apply the correction to other samples.
    - Excessively noisy blanks or blanks with particles that are similar in size to your sample can skew your statistics.
  - You can also specify more information about your sample and set where the data for this sample will be saved.
    - NTA results are sometimes GBs in size, so choose a hard drive that is at least 30% empty
    - **DILUTION NOTE:** If you specify the sample dilution, the final concentration measurement the NTA tells you is impacted. Make sure this information is accurate when analyzing your results.
  - You can also choose to queue the sample for processing in this tab as well.

## Visualizing your Particles

- Make sure that you have selected appropriate laser powers for your sample. Consult the tables on the previous page for a guide on laser powers with common sample types
- Select the "Start Streaming" button to pull up a live camera feed.
- To focus the camera on your particles, you can use the arrow buttons in the "Camera" panel to move the camera closer and further away from the cuvette.
  - If your sample is polydisperse, try to focus on particles that are around 100-200nm in diameter. In very polydisperse samples, try to focus on the smallest particles.
  - Most samples will have a focus distance around 13.64, though this will change with your average particle size.
  - You've found a good focus when most of the particles on screen no longer have a halo effect.
- Modify the gain, brightness, contrast, and gamma so that you can get a good image of your particles.
  - Avoid excess gain as artificially brightened individual pixels will be mistaken as particles by the software. This is usually the case if detection preview thinks there are thousands of particles on screen when you can see only a hundred.



- Once you have a good image of your particles, select "Detection Preview". This will count how many particles are in a single frame. You want to aim for around 100-150 particles on screen if possible.
  - Lower than ideal particle counts will need more and longer videos to be taken for good statistics.
  - Higher than ideal particle counts will lead the software to not accurately track the motion of all particles on screen.



Fig. 5 Standard of 300 nm PSL diluted 1000x (too much concentrated), properly diluted at 20,000x and over diluted 400,000x

- Once you have a good image of your particles, ensure that a video is streaming and then select "Start Acquisition"
  - Your total sample acquisition time is dependent on the number of videos taken, their total frames, the frames per second, and any stir wait times that have been set.
- Once all the videos are taken, the videos will be sent to your data folder.
- If it was selected to queue for processing, the Video processing tab will open.
  - Video Processing times can be slow depending on the size and number of your videos, so try to process results at the end of your session if running multiple samples in one session.
  - To add results to the processing queue, select the data folder for your sample from the directory panel and select the "Add Sample" button. When all your samples are queued for processing, select "Start Processing"

## Data Analysis

- The size data analysis tab can be used to visualize your size and concentration data.
- To add more data sets to this window, add them from the directory panel on the left-hand side of the screen.
- Here you can select the display binning style, choose to save the results to a PDF, and export your statistics.
- If you ran a blank that needs to be factored into your samples, you can select the

"Set Blank" button in the upper left corner. It will have you select the appropriate blank file for your data set

• You cannot set other samples as blanks for your data sets. It must be a "Blank" sample type.



• To omit outliers in your statistics, or just focus in on a specific size range, you can select the "Range" button the will let you set the bounds on your histogram that the software will process information from.

## <u>To Finish:</u>

- Make sure the sample prep area and instrument are clean for the next user.
  - Clean the quartz cuvette, insert, and stir bar using the same procedure as earlier.
  - Properly dispose of your waste. Samples left behind may be disposed of by staff.
  - Turn off the instrument and close all sample chambers to limit dust contamination.
- The software can now be closed.
- Collect your data using a thumb drive. Be sure to properly eject your thumb drive to avoid any data corruption.