Core Equipment ID: 113572

Description: ZetaView PMX 120 Nanoparticle Tracking Analyzer (NTA)

Room: IQ Building, Rm 2500

Champion: Daniel Vocelle

1.0 Purpose

Standardize the process of control, maintenance, and ownership of the ZetaView located in IQ Building Room 2500.

1.1 ZetaView PMX 120 Nanoparticle Tracking Analyzer Capabilities

The ZetaView nanoparticle tracking analyzer (NTA) is a next generation analyzer capable of measuring particle size, concentration, and fluorescence. This instrument is capable of analyzing biological nanoparticles such as extracellular vesicles (EVs) exosomes, viruses, or virus-like particles, where particles are individually measurable in a physiological buffer. With the ZetaView fNTA system, it is also possible to conduct fluorescence measurement on a per particle basis. Automated measurements at 11 positions through the sample cell, provide a thorough interrogation of samples and increased accuracy, without need of additional accessories. The optical setup is automatically optimized by the system's software, saving the user time preparing the instrument for use and removing subjective user input bias. The ZetaView (NTA) is capable of analyzing over 2,000 particles/minute and equipped with a 520nm laser and 550LP filter. The instrument allows for the interrogation of fluorescent subpopulations within the sample. A sensitive CMOS camera, selective filters, and low bleaching performance yield high fluorescence sensitivity.

1.2 ZetaView Software Capabilities

ZetaView software controls the ZetaView PMX 120 system in order to acquire data and analyze results.

ZetaView Software provides the following features:

a. **Quality Control:** Cell quality check, daily performance check, outlier control with automatic Grubbs statistical analysis of measurement data.

b. **Live Monitoring:** Number of detected particles in scatter and fluorescence mode, scattering intensity, temperature, particle drift.

c. **Standard Operating Procedures (SOP):** Fully customizable SOPs for different samples/applications.

d. **Analysis and Reports**

i Data Analysis: particle size distribution profiles, concentration, overlays and averaging, scatter plots, zeta-potential distribution profiles, sub-population analysis (using additional 'Particle Explorer' software).

ii Data export format: AVI, TXT, CSV, FCS.

iii PDF reports containing key results.

2.0 Reason for Issue

Maintain a document that describes the Standard Operating Procedures that allow for the standard safe and optimal use of the ZetaView PMX 120 within the MSU Flow Cytometry Core Facility.

3.0 Process Description

Allow Core Facility Users within the Pharmacology and Toxicology Department to properly and effectively use the ZetaView PMX 120. The process description details the standard use of the ZetaView PMX 120. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

3.1 SOP: 113572.2500.001 for the ZetaView PMX 120, authored by Matthew Bernard, created on 02/22/2024, issued on 11/01/2024.

3.2 SOP: 113572.2500 applies to any User and / or Trainer of the ZetaView PMX 120.

3.3 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the ZetaView PMX 120. All Users are responsible for the proper use, according to defined protocol, when using the ZetaView PMX 120

a. **All Users are expected to have completed EHS training programs Bloodborne Pathogens and Biosafety Principles, as required for respective research projects.**

b. **All Users are required to complete a Biosafety Questionnaire for each cell type to be analyzed prior to use of the scheduling use of instrumentation in the facility.**

c. **All Users** must schedule equipment using the iLab Solutions portal.

d. Only covered samples may enter Room 2500. Samples must be brought to the facility in a standard **spill control box/leak-proof secondary container** that will contain any multiple tube or plate spill, per EHS standards (see Section 4.11c). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm. Spill control boxes must be labeled with Biohazard identification for BSL-2 samples.

e. Immediately after use and daily cleaning (see Section 7.3a), the ZetaView PMX 120 must be appropriately shut down (see Section 7.5).

3.4 Equipment Safety Issues

a. **Safety Issues** – The Core Facility operates at up to BSL-2 plus. Biosafety level and limitations for this facility are restricted to WHO and NIH risk groups defined as:

Risk Group 1 – Agents that are not associated with disease in healthy adult humans (no or low individual or community risk)

Risk Group 2 – Agents that are associated with disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).

Examples of risk groups 1 and 2 which may be analyzed include: 1) Plasma or serum from non-primate animals; 2) cell supernatants from cell lines of ATCC origin and those tested negative for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV); 3) primary human serum or plasma if tested for HIV, HBV, HCV, and EBV; 4) Supernatants from primary human cells if tested for HIV, HBV, and HCV; 5) Supernatants from genetically modified cell lines using third generation lentivirus systems.

Research involving BSL-3 or BSL-4 requirements are not supported, which includes WHO and NIH risk groups 3 and 4.

b. **Decontamination of ZetaView PMX 120 post-operation:**
Following the Shutdown procedures (Section 7.5) will result in appropriate daily decontamination of the flow cytometer between uses. More extensive cleaning/decontamination may be performed monthly or prior to service(see Section 7.3).

4.0 **Decontamination of work surfaces:** External surfaces in front of the ZetaView PMX 120 can be cleaned with Envirocide (or equivalent) or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent).

a. **Radioactively labeled samples are prohibited.**

b. **Under normal operating conditions, the ZetaView PMX 120 <u>does not</u> create aerosols.**

c. **All samples exposed to or infected with bacterial or viral agents must be approved by EH&S on a case-by-case basis. A related HURON Click must be submitted and approved by EH&S prior to scheduling analysis.**

d. **Spill control:**
Samples must be brought to the facility in a standard spill control box that will contain any multiple tube or plate spill (see Section 7.7c). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm.

Report spills to the Core Facility staff.

In the event of a spill for BSL-2 samples, the spill should 1st be covered with absorbent paper towel, which will then be saturated with 10% bleach and allowed to soak a minimum of 10 minutes. The wet towel should be placed in a biohazard waste receptacle after contact. The spill area will then be covered with 10% bleach, allowed to soak briefly, and then wiped up with an absorbent towel. After cleaning the spill, dispose of the absorbent material and gloves into a biohazard waste container. Squeeze bottles of 10% bleach are made fresh daily for spill control.

Report spills to the Core Facility staff.

5.0 Never place anything on top of the ZetaView PMX 120, including tube racks or kimwipes.

a. Ensure that the ZetaView PMX 120 waste container is not overfilled prior to use.

5.2 Laboratory Conditions

a. IQ 2500 is a BSL-2 research lab. The room contains a sink for hand washing, germicidal soap, emergency eye wash station, and spill control kit/equipment.

b. **Signage:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2521.

c. **Access:** Access is limited to people with permission to run samples on the ZetaView PMX 120, which has been booked through the iLabs web portal.

d. **PPE Requirements:** Standard PPE must be used at all times, which includes gloved hands, long-sleeve lab coat over full and coverage shirt and pants, and full coverage shoes with intact soles.

e. All samples will be handled with BSL-2 precautions, including proper handling, storage, transportation, disposal, and decontamination according to the MSU Biosafety Manual and BBP Exposure Control Plan.

f. **Exposure Control Plan:** Please refer to the Exposure Control Plan available on the MSU EHS website for instructions regarding what to do in the event of an exposure. The MSU Exposure Response Procedure is posted in Rm 2500.

i **Eye/Mucous Membrane Exposure:** Flush immediately at nearest eyewash station for 15 minutes.

Wounds/Needlesticks: Wash the area immediately, use warm water and sudsing soap to scrub the area for 15 minutes.

ii Notify your supervisor immediately if he/she is available.

iii Print Authorization to Invoice MSU Form to take to care facility. https://www.hr.msu.edu/benefits/workers-comp/documents/InvoiceMSU.pdf

iv Report to a Lansing Urgent Care facility for post-exposure follow-up as soon as possible. https://www.lansingurgentcare.com/

v Be prepared to provide information about the agent or cells involved in the accident. Additionally, route of exposure, dose/concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

Note: Any required follow-up visits must also take place at Lansing Urgent Care. The location in Frandor is open 24 hours.

vi Follow up by completing the Report of Claimed Occupational Injury or Illness Form with your supervisor within 24 hours.

g. Sample handling and decontamination within IQ Rm 2500 is covered in Section 3.4. All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to their lab. Waste containers are available for non-hazardous waste. A biological waste container for waste generated during a biohazard cleanup is available in the lab.

6.0 Syringes used to load samples into the ZetaView should be discarded into the sharps container next to the instrument. Ensure that sharps container does not exceed max ¾ full. Notify Core Staff if container needs to be replaced.

a. **No needles are permitted in the Core Facility.**

b. **Eating, drinking, or use of personal care products are prohibited in the facility.** Use of personal electronics will not be allowed if that use interferes with proper operation of the instrumentation in the facility. Those operating flow instrumentation in the facility must remove gloves and wash their hands before using any personal electronic device. Sani-Cloth Plus germicidal disposable wipes are available for wiping **keyboards and personal electronic devices if cross-contamination accidentally occurs.**

c. **Medical:** Users of the facility should have all current vaccinations, including those for HepB. Anyone who may be immune-compromised should visit Occupational Health before working in the facility.

6.2 Contact Information

a. **Matthew Bernard: Core Director,** Office, IQ Building, Rm 2315, (517) 355-4076

b. **Daniel Vocelle: Assistant Director,** Office, BPS Building, Rm 4198, (517)-355-1536

c. **Environmental Health & Safety:** 517-355-1053

d. **Occupational Health (University Physician's Office):** 517-353-8933

e. **MSU Police:** 571-355-2221

6.3 Quality Measures

a. **Daily:** Perform Daily QC, as described in Section 7.2.

7.0 Procedure: ZetaView PMX 120 Use

PLEASE NOTE that with the pass-through cell installed, measurement of Zeta Potential is NOT possible. Please contact Core Staff if you intend to measure Zeta Potential

7.1 Startup

a. Log into the computer. Password = 123456

b. Turn on the ZetaView instrument, then wait at least 30 seconds.

c. Double-click the ZetaView icon on the desktop and enter your name at the prompt to launch the acquisition software.

d. Make sure that the fluorescence filter is moved to the downward position and click 'OK'.

e. The ZetaView will go through an initialization including a 'Cell Quality Check', followed by a pop-up window prompt to inject water. Check the syringe containing the water to make sure air bubbles are removed prior to injecting it into the system.

Perform this step with clean milli-Q water. Clean milli-Q water is stored in a sterile 50 mL conical tube located next to the instrument. If additional water is needed, use the glass bottles placed on the shelf above the computer for the ZetaView labeled "milli-Q Water for ZetaView" and a brand-new conical tube. Please be sure to only take water from here, as this will reduce contamination.

f. Another pop-up window should confirm cell condition as "Very Good" for best results. If the condition is noted as less than "Very Good," the cell should be cleaned and the cell check repeated. Please consult with Core staff about cleaning.

g. Let the system warm up for at least 20 minutes before proceeding further. Note: if you plan on running samples cold, set appropriate temperature so that sample and cells are equilibrated.

7.2 Daily QC

a. During the warm-up period, prepare your standard reference material for AutoAlignment. The final dilution should be 1:250,000 when using the fluorescent Particle Metrix reference material (stored at 4°C), where the first dilution (1:1,000; add 5 µL of beads to 5 mL milliQ water) will be stable for ~1 week. The final dilution of 1:250,000 (add 40 µL of 1:1,000 prep to 10 mL of milliQ water) is stable for approximately 30 minutes, after which reference material will begin to aggregate.

b. For Calibration without fluorescence select 'Scatter.' For Calibration with fluorescence select 'Fluoro.'

c. After 20 minutes of warm-up, inject at least 1 mL of the standard reference material (1:250,000). Some care should be exercised to avoid bubbles. An injection rate of 0.1 mL/sec is a good rate of injection. Particle count should be ~100-200 on the display bar; dilute further if appropriate. Good settings for measuring the 100nm standard are: Sensitivity = 80, Shutter = 350, Brightness = 25, Min size = 10, Max size = 200

d. Run the 'Auto-Alignment' procedure (Step 2) under the 'Cell Check'; tab with the diluted reference standard. A pop-up window will tell you if the instrument is ready for measurements. If you do not get an acceptable alignment, then repeat or perform a cell cleaning and repeat.

e. Once auto-alignment is complete, you may proceed with sample measurements.

7.3 Maintenance

a. **Clean the instrument <u>after each use</u> (up to multiple times daily).**

i Flush with 5-10 mL milliQ WATER through the injection port.

ii Flush with 5-10 mL fresh 10% bleach through the injection port. Allow bleach to remain in the cell for 10 minutes.

iii CRITICAL - Flush with at least 10 mL of milliQ water through the injection port. And leave syringe on port when flushing has been completed.

b. **Monthly or as needed:** Record Maintenance in the electronic Instrument Maintenance Log.

i **Cleaning the flow cell:**
A detailed video walkthrough of this procedure is available here: Link

1 Place a few drops of the blue or green cleaning solution in a 50mL centrifuge tube. Add ~50mL of MilliQ water and mix well.

2 Remove the flow cell assembly from the instrument by moving the black knob to the correct position.

3 Remove the flow cell carrier from the cell assembly.

4 Wet one of the provided brushes with the cleaning solution, then insert it into the cell and pass it back and forth a few times.

5 Repeat from the other side of the flow cell.

6 Using a syringe filled with MilliQ water, rinse the inside and outside of the cell.

7 Remove the flow cell from the cell carrier.

8 Dry the cell carrier.

9 Inspect the windows on the cell. If they require cleaning use 70% ethanol or acetone and a lint free applicator.

10 Dry the flow cell with a lint-free wipe/Q-tip.

11 Reassemble in the flow cell in reverse order.

7.4 Acquisition

DO NOT RUN ORGANIC SOLVENTS THROUGH THE ZetaView

a. Click on the 'Measurement' tab.

b. For optimal counting results, inject at least 1 mL of your sample buffer (without particles) to assess background levels. Injection rate of ~0.1 mL/sec is appropriate. If background levels are acceptable. Inject at least 1 mL of your sample preparation. 'Check Drift' in lower right corner and wait for temperature to equilibrate after sample injection.

c. Check the 'No. of Detected Particles' reading (100-200 for best results). The 'Sensitivity' and 'Shutter' should be adjusted accordingly depending on the scattering behavior of the sample. The shutter value means adjusting the duration of the exposure time of the camera. The indicated value is reciprocal in seconds (e.g. 1/70 sec). High values mean short exposure time, low values represent long exposure times.

d. To ensure the best camera gain, run the 'Number of Particles vs Sensitivity' routine. This will help determine if the sample concentration is in the right range. Set the camera gain to the number indicated by the crosshairs, toggle the view to digital and

adjust the sensitivity to 3-4 numbers below the presence of noise. If there are more than 200 particles present after adjusting the sensitivity, then the sample will need to be further diluted.

e. Once settings have been optimized, click on 'Run Video Acquisition'.

f. Select or create an appropriate folder where files will be saved.

g. Select the appropriate SOP for your samples (or save current settings as a new SOP) and double check camera settings. The settings below are just a recommended starting point. Setting adjustment should be made with a positive control and your buffer/diluent particles are resuspended in to optimize sensitivity of measurement. Check the live camera view to assess noise.

h. Select appropriate file parameters for acquisition, including number of images in the Z-stack and resolution (4 filmstrip icons = highest resolution).

i. Click 'Ok' to run acquisition.

i For Exosomes (size only): Select 'Scatter.' Select the camera Sensitivity = 85, Shutter = 250 (from camera icon), start with brightness = 22, and min size = 8, max size = 800. Note: The shutter (smaller number = ability to see smaller particles), brightness (smaller number to see smaller particles) and sensitivity (higher number to see smaller particles) work together. If you adjust one, you may have to adjust others.

1 Set cycles / positions / multiple measurements according to precision you are aiming for (e.g. for more than 500 traces, use 2 cycles and 11 pos). Set the resolution to high (the third of four filmstrip icons). For camera adjustment and pre studies, use 11 positions and 1 cycle.

2 Under Admin mode, when the system starts the first analysis make sure that "Start New Traces" is unchecked.

ii For Exosomes (size and fluorescence)

1 The ZetaView is equipped with a 520 nm excitation laser and X nm filter. To perform fluorescence measurement. Calibration should be performed in 'Fluoro' mode. Select 'Fluoro' in measurement tab and when selecting sample measurement parameters.

j. Flush the cell with your buffer diluent in between samples (~3-5mL). Visualization of <10 particles on the screen typically indicates appropriate background level.

k. Please note the level of the waste bottle. If the waste bottle is near approximately 400 mL, add 50 mL of concentrated bleach. After minimum of 20-minute disinfection time discard in hazardous waste container.

l. Discard syringes in biohazard sharps container, these are not allowed in normal trash!

7.5 **Cleaning/Shutdown**

All Users should minimally perform steps 7.5a-e; 7.5g-h after running samples. If you are the last/only user of the day ensure that the ZetaView is turned off (f)

a. Flush with 5-10 mL milliQ WATER through the injection port.

b. Flush with 5-10 mL fresh 10% bleach through the injection port. Allow bleach to remain in cell for 10 minutes.

c. CRITICAL - Flush with at least 10 mL of milliQ water through the injection port. And leave syringe on port when flushing has been completed.

d. If the waste bottle is near approximately 400 mL, add 50 mL of concentrated bleach. After minimum of 20-minute disinfection time, discard in EHS-approved hazardous waste carboy in Room 2521 or 2522.

e. Close the ZetaView software after you are done cleaning.

f. TURN OFF the ZetaView instrument.

g. Disinfect appropriate surfaces with PDI wipe when you are done (computer keyboard, front of ZetaView, benchtop, waste bottle, etc.).

h. External Drives are NOT ALLOWED on Core computers. Transfer data using the OneDrive following analysis.

7.6 Records

a. **Error Messages / System Issues –** All error messages and system issues must be relayed to the Equipment Champion and the Core Facility Staff and appropriately recorded, refer to 3.3e, on the same day as equipment use.

7.7 Resource Index

a. ZetaView PMX 120 and ZetaView Software User Guide
b. ZetaView PMX 120 and ZetaView software literature and resources for the following items can be found with the ZetaView PMX 120 in the 2500 lab space.
c. ParticleMetrix Technical Support is available by calling +1 (919) 884-8376
d. ParticleMetrix Company Representatives:

Nazar Filonov
Alpha Nano Tech LLC
+1 (919) 884-8376
service@alphanano.tech

Adrian Herrera
Field Service Engineer
+1 (919) 491-1009
adrian@alphanano.tech

e. Transport of Biological Materials:
For detailed information about the transport of biological materials, see the EHS recommended procedures available at:

https://ehs.msu.edu/lab-clinic/shipping/bio-transport-local-vehic.html

8.0 Competencies, Authorization and Training

New Users must receive proper authorization from either the Equipment Champion and / or Core Facility Staff before equipment use. A new User may contact the Equipment Champion or Core Facility Staff to schedule training. Training includes SOP and flow cytometer familiarization and any additional required or specialized training. Once training is complete authorization may be issued and a system account and password may be set up. All Users are individually responsible for current SOP familiarization. All New Users must refer to 3.3a during new ZetaView PMX 120 account creation.

9.0 SOP Performance and Equipment Review

The effectiveness of the SOP: 113572.2500 will be monitored by the Core Facility Staff, Equipment Champion and All Users. Any procedural or qualitative deviations will be reflected within an updated SOP. Any Approved User should aptly report any procedural or qualitative issues and / or errors to the Core Facility Staff or Equipment Champion. The Core Facility Staff and Equipment Champion's name and contact information can be found on the Pharmacology and Toxicology Core Laboratory in iLab. Updated SOPs will be published and Approved Users will be notified. SOP: 113572.2500 review will occur every two years.

10.0 Definitions

10.1 **SOP:** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance, and ownership of the ZetaView PMX 120. The SOP number stands for (xxx . xxx . xxx) equipment serial number . room number . SOP version number.

10.2 **Originator / Author:** The individual representing the MSU Flow Cytometry Core Facility that created SOP: 113572.2500.001

10.3 **Stakeholder:** Any individual that uses or performs the task of which is the subject of the SOP, including the MSU Flow Cytometry Core Facility Department.

10.4 **New User:** An individual who has not completed the requirements of Section 3.3.

10.5 **Approved User:** An individual who uses the ZetaView PMX 120 and has fulfilled Section 3.3. This title may only be given by the Equipment Champion and / or the Core Facility Staff.

10.6 **Champion:** An individual whose direct expertise with the ZetaView PMX 120 instrument has been recognized by the MSU Flow Cytometry Core Facility Staff. This title may only be awarded by the MSU Flow Cytometry Core Facility Staff.

11.0 Approvals

The below signatures and dates are required for full SOP approval and implementation.

This SOP was written, authorized, and reviewed by:

Dr. Matthew Bernard_____ 01Nov2024

Dr. Daniel Vocelle_____ 11/13/2024

Issue Date: November 13th, 2024