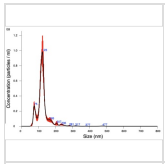

Version 2

Oct 20, 2020

Nanosight LM10 V.2

Joshua A Welsh[1], Bryce Killingsworth[1], Jason Savage[1], Tim Traynor[1], Jennifer Jones[1]

[1]Translational Nanobiology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health

1 *Works for me* This protocol is published without a DOI.

Translational Nanobiology Section

Jennifer Jones

ABSTRACT

This is a protocol for performing nanoparticle tracking analysis measurements on the Translational Nanobiology Sections Nanosight LM10 (405 nm) module.

PROTOCOL CITATION

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extracellular vesicles, EVs, exosomes, nanoparticle tracking analysis, NTA, nanosight, malvern, LM10

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PROTOCOL INTEGER ID

43422

GUIDELINES

The optical flat of the instrument is very sensitive, make sure to only wipe it with lens paper and dry using compressed air

MATERIALS

NAME	CATALOG #	VENDOR
DPBS	14190	Invitrogen - Thermo Fisher
Lens Paper, 4L x 6 in. W (10.1 x 15.2cm); Sheets per book: 50	11996	Thermo Fisher
Covidien Monoject™ Rigid Pack 12mL Syringes	22-652-090	Fisher Scientific
Air-Tite™ All-Plastic Norm-Ject™ 1 mL Syringes	14-817-25	Fisher Scientific
Micro-90® Concentrated Alkaline Cleaning Solution	M-9050-12	International Products Corporation (IPCOL)

MATERIALS TEXT

Nanosight LM10
Nanoparticle Tracking Analysis
Malvern **NA**

SAFETY WARNINGS

Do not turn the laser on when the metal plate covering the optical flat is removed

DISCLAIMER:

This protocol summarizes key steps for a specific type of assay, which is one of a collection of assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

ABSTRACT

This is a protocol for performing nanoparticle tracking analysis measurements on the Translational Nanobiology Sections Nanosight LM10 (405 nm) module.

BEFORE STARTING

Gather PBS, an empty waste container, 12 mL syringes, and 2 mL low protein-binding Eppendorf tubes

Equipment Start Up 15m

1

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Ensure that the black power strip is turned on.

2

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Turn on the computer on the shelf, login using the password written above screen

3 Turn on the Nanosight microscope, you should hear the camera fan turn on

4 Remove the storage lens paper which is positioned between the stainless steel cover and the optical flat of the LM10 module

5 Inspect the optical flat of the module and window in the steel cover to see if there are any obvious smudges which require immediate cleaning

6 If immediate cleaning is necessary:

6.1 Examine translucent window component of steel cover to see if there are any particles or smudges. The outside part of the window shouldn't need to be lceaned of smudges but may have dust and link which can be blown off using a can of compressed air

6.2 The inside of the window can be cleaned by blowing off the dust and pouring a small amount of 1% MICRO 90 (diluted in sterile HPLC water) into the cap of a 50 mL conical and dipping the end of a folded piece of lens paper into it. Gently clean the surface, and repeat this same procedure using a new piece of lens paper dipped into DPBS. Dry the window with a piece of lens paper and set the steel cover aside.

6.3 The optical flat can be cleaned with 1% MICRO 90. Pour it into the cap of a 50 mL conical and dip a piece of folded lens paper into it. Wipe the optical flat, focusing on the area of the optical flat that contains a translucent rectangle. PRess firmly perpendicularly to the edge of the optical flat and parallely, not in circles.

6.4 Quickly place the steel cover back onto the module and screw it in, tightening the screws in a diagonal pattern and ensuring not to over-tighten

7 Quickly load 10 mL of DPBS into a 12 mL syringe

8 Flush the LM10 module with DPBS by inserting the syringe into the white plastic port on the side of the module. One side of the stainless steel has the entry port, while the other side has the exit port. The exit port is directly adjacent to another dark metal port while the entry port is the only port on its side. Hold the module as vertically as possible and slowly inject DPBS, ensuring that no bubbles form on the viewing window

9 Once liquid can be seen filling up the fluid exit port, flip the Nanosight module so that the syringe is standing straight up and slowly press on the plunger until all of the DPBS has passed through the module and into a waste container

10 If bubbles are formed, DO NOT REVERSE THE SYRINGE to remove the DPBS, but rather push it all the way through

11 Once the syringe is empty, return the Nanosight module to its original vertical position

12 Draw out the remaining DPBS that remains int he viewing window of the Nanosight slowly, ensuring that minimum fluid is left behind

13 Discard the DPBS in the syringe in a waster container

14 Draw up another 6 mL of DPBS into the syringe. If there were bubbles present in the initial flush, push the entire volume through and add another 6 mL of DPBS to the syringe

15 Flush the NanoSight with ~5 mL of DPBS, leaving less than 1 mL remaining in the syringe

16 Slide the LM10 module into the grooved slot on the microscope table

17 Make sure the 20X lens is in use on the microscope

18 Open up the most recent version of the NanoSight software (3.4 as of January 2020)

19 Turn the camera on inside the NTA software by clicking "Start camera"

20 Adjust the settings to those optimal for the instrument. These settings are subjective depending upon instrument parameters such as the wavelength and module alignment, along with the sample.

20.1

These settings are specific to the Translational Nanobiology Section's instrument

A guide for the 405 nm LM10 module
Capture -
Screen Gain: 1
Camera Level: 14
Process -
Screen Gain: 1
Detection Threshold: 4
Advanced -
Uncheck all "AUTO" boxes
Min Track Length: 8

21 Plug the power source into the side of the LM10 module

22 Turn on the laser by flipping the silver switch on the right side of the LM10 module

23 Use the microscope focusing knobs/stage mover to ensure that you are in the correct viewing window.

23.1 Make sure that the "thumb-print" is centered in the microscope window. The thumb will look like a large, purple oval that takes up the majority of the screen

23.2 Once centered using the thumb-print, move the screen to the right until you see a large, vertical line. The viewing frame for sample analysis should be as close to this line as possible without having the line

being in the actual viewing window (position the viewing frame immediately to the right of the line)

23.3 If the line is not vertical, the camera module on top of the microscope may need to be slightly rotated

24 With DPBS loaded, ensure that there are no particles remaining in the NanoSight from the previous samples

24.1 If there are particles, turn off the laser and disconnect the power source. Get a new aliquot of PBS and a new syringe (at the user's discretion) and repeat the flushing process

24.2 If there are large, highly fluorescent smudges, or significant amounts of white backgroup noise, turn off the laser and disconnect the power source. Clean the optical flat and viewing window and flush the module as described in steps 6-15.

24.3 If the NanoSight is clear, turn off the laser and pull the remaining PBS out with the syringe, ensuring to go slow and leave behind as little fluid as possible

Sample Analysis 10m

25 Dilute samples as needed in a 2 mL low protein-binding Eppendorf tube

26 Vortex samples thoroughly

27 Draw up sample in 1 mL syringe, pulling down the syringe plunger to remove any bubbles

28 Load sample into the LM10 module through the fluid entry port

29 Turn on the laser

30 Ensure the NanoSight is in the correct viewing window, adjust as necessary

31 Ensure that the sample is in the correct viewing window and properly focused

32 Ensure the sample is not too dilute or concentrated

32.1 It is too dilute when there are too few (<20) particles on screen at any given time

32.2 It is too concentrated when there is significant fluorescent background or particles cannot be distinguished from one another

33 To adjust settings, press:
SOP -
1. Standard Measurement
2. Number of Captures: 3
3. Capture Duration(s): 30
4. Current temperature: Measure using thermometer on table

34 To begin recording, press "Create and run script." Enter the sample name and the dilution amount, then hit "OK" on the windows that pop up

35 When the fan turns off, the instrument is recording. Ensure that nothing bumps, vibrates, or moves the table while the recording is taking place and refrain from leaning over the table.

36 After each 30-second capture, you will be prompted to advance the sample and press "OK." Do this by carefully pressing the plunger in until you see the particles on the screen have moved.

37 At the end of the 3 captures, allow the software to automatically analyze the data

38 As soon as the data analysis begins, turn the laser off

39 When data analysis is complete, hit "Export" on the box that pops up

40 If you need to measure another sample:

40.1 Flush the device with at least 2x12 mL syringes of DPBS (they need not be entirely full, technique is more important than quantity of DPBS flushed through)

40.2 Check the viewing device to ensure that the viewing frame is particle free and does not have smudges or excessive background noise

40.3 IMPORTANT NOTE: if there are particles remaining, the module was flushed insufficiently and will need to be opened and cleaned as described in steps 6-15

Device Shutdown 15m

41 After you've finished with all of your samples, the device must be opened and cleaned as described in step 6 with the following changes:

41.1 After wiping with MICRO 90, obtain another piece of lens paper and gently wet it with pure water, then wipe down the optical flat. Use another piece of dry lens paper to wipe off the water.

41.2 ALL COMPONENTS of the module which may have come into contact with DPBS must be wiped gently with a Kim wipe or a lens paper wetted with pure water, then dried gently with compressed air. This includes the sides of the module, especially the corners and edges where DPBS can pool and crystalize

41.3 Water should NOT be poured or pipetted onto the LM10 module as it could enter the electronic components and cause damage. Only use a wetted piece of lens paper if you need to apply fluid to the module

42 Place a piece of folded, dry lens paper between the optical flat and steel cover plate

43 Ensure that all power sources are turned off

File Export 5m

44

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To transfer data to a flash drive, click:

1. This PC
2. J'D (H:)
3. Nanosight files
4. Nanovideos
5. Date of recording