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FCMPASS - Light scatter calibration

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Joshua Welsh[1], Jennifer Jones[1]

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

Translational Nanobiolo...

Jennifer Jones
NIH

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We use this protocol and it's working

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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. By using the FCMPASS software you agree to the following terms and conditions.

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Abstract

This protocol outlines the steps required to input light scatter calibration parameters using the FCMPASS software. This is one of a number of protocols in the pipeline for performing small particle calibration using the fcmpass software package.

Materials

FCMPASS software can be accessed at https://nanopass.ccr.cancer.gov.

1 If light scatter calibration is being performed click the '+' button to add a calibration parameter to the table. If light scatter calibration is not required, click 'Next'.

1.1 If you have not yet defined the light scatter bead sets in Catalogue', click 'Catalogue' and complete as outlined in the protocol.

2 Double click the ‘Scatter Parameter’ field to change which parameter is being used for light scatter calibration.

3 Alter the ‘Scatter Wavelength (nm)’ to the relevant wavelength for the parameter being used to calibrate light scatter.

Note

You will see that the ‘Sheath RI’ field will automatically update when this is altered. In the background reference bead, core-shell model, and homogenous sphere model refractive indices will all also be updated.

4 If the selected ‘Scatter Parameter’ was used as a triggering threshold then the ‘Scatter Threshold’ field will automatically update to show the values used as thresholds in the .fcs files loaded. Select a ‘Scatter Threshold’ by double clicking the field and selecting and option from the dropdown menu. A custom entry can also be inputted.

5 Load the light scatter reference beads used by double clicking the ‘Bead Set’ field. Once loaded the beads within the set will populate the bottom table.

6 The ‘Sheath RI’ field automatically accounts for ‘Scatter Wavelength’ but can be updated manually by double clicking the field.

7 In the bottom table enter the median scatter parameter statistic for each population. The acquired CV can optionally also be completed, its use will, however, only be used for plotting purposes and not alter the model calculations.

8 Once complete click ‘Next’.

Note

Custom core-shell models, solid sphere models, plot data points, modelling parameters, and output settings can be entered or altered by clicking the 'Advanced Settings' button. By default, three EV core-shell models relating to high, medium, and low EV refractive indices are calculated. All core-shell models assume a 5 nm shell thickness.