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FCMPASS - Light scatter calibration

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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.

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KEYWORDS

fcmpass, flow cytometry, EVs, calibration

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38553

PARENT PROTOCOLS

In steps of

[FCMPASS Protocol Collection](#)

MATERIALS TEXT

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

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

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Definitions: The term "SOFTWARE" throughout this agreement means the machine readable, binary, object code form, and the related documentation for FCMPASS, a software package that is designed to allow flow cytometer calibration for small particles. The term "RECIPIENT" means the party that downloads the software. The term "PROVIDER" means the National Cancer Institute (NCI), a participating institute of the National Institutes of Health (NIH), and an agency of the United States Government. By downloading or otherwise receiving the SOFTWARE, RECIPIENT may use the SOFTWARE subject to RECIPIENT's agreement to the following terms:

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- 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.



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 an 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'.



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.