This protocol outlines the steps required to catalogue light scatter reference materials 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.

This protocol accompanies the following publication:


Please remember to cite the following publication along with this protocol:


This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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.

Terms & Conditions of use for FCMPASS software.

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:

1. THE SOFTWARE SHALL NOT BE USED IN THE TREATMENT OR DIAGNOSIS OF HUMAN SUBJECTS. RECIPIENT is responsible for compliance with all laws and regulations applicable to the use of the SOFTWARE.
2. RECIPIENT shall not distribute the SOFTWARE, in whole or in part without express advance written approval of PROVIDER.
3. The SOFTWARE may be used for research, academic, and educational purposes only. The SOFTWARE may not be used for commercial purposes. RECIPIENT will not license or sell or use the SOFTWARE for commercial purposes or applications.
4. The SOFTWARE that is distributed pursuant to this Agreement has been created by United States Government employees. In accordance with Title 17 of the United States Code, section 105, the SOFTWARE is not subject to copyright protection in the United States. Other than copyright, all rights, title and interest in the SOFTWARE shall remain with the PROVIDER.
5. RECIPIENT shall not modify, extend, decompile, make derivatives of, merge, publish, reverse engineer or distribute the SOFTWARE without written permission from PROVIDER.
6. RECIPIENT may publish or otherwise publicly disclose the results of using the SOFTWARE. RECIPIENT agrees to acknowledge PROVIDER's contribution of the SOFTWARE in all written publications containing any data or information regarding or resulting from use of the SOFTWARE.
7. THE SOFTWARE IS PROVIDED "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND NONINFRINGEMENT ARE DISCLAIMED. IN NO EVENT SHALL THE PROVIDER OR THE INDIVIDUAL DEVELOPERS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE. PROVIDER makes no representations that the use of SOFTWARE will not infringe any patent or proprietary rights of third parties.
8. RECIPIENT may, on an as-needed basis, send to PROVIDER reports regarding the application of the SOFTWARE and the effectiveness and problems encountered in using the SOFTWARE, without disclosing RECIPIENT's confidential information. Information from general reports may be used by the PROVIDER to enhance the capabilities of the SOFTWARE. Reports can be forwarded to the PROVIDER at one of the following addresses: joshua.welsh@nih.gov or jennifer.jones2@nih.gov
9. No indemnification for any loss, claim, damage, or liability is intended or provided by either Party under this Agreement. Each Party shall be liable for any loss, claim, damage, or liability that said Party incurs as a result of said Party's activities under this Agreement, except that Provider, as an agency of the United States, assumes liability only to the extent as provided under the Federal Tort Claims Act (28 U.S.C. Chapter 171 Sections 2671-2680).
10. RECIPIENT agrees not to claim, infer, or imply endorsement by the United States Government, or any of its organizational units, contractors or employees. RECIPIENT agrees not to use any trademarks, service marks, trade names, logos or product names of NCI or NIH to endorse or promote products derived from the SOFTWARE without specific, prior and written permission.
11. Title in the SOFTWARE shall remain with the PROVIDER. It is understood that nothing herein will be deemed to constitute, by implication or otherwise, the grant to either party by the other of any license or other rights under any patent, patent application or other intellectual property right or interest. PROVIDER reserves the right to
distribute the SOFTWARE to others and to use it for PROVIDER’s own purposes. The United States Government explicitly retains all rights to use the SOFTWARE for any purpose, to have it used on the Government’s behalf or to allow others to use it.

1. Open FCM\textsuperscript{PASS}.

2. Click ‘Catalogue’ in the top menu bar

3. Under the ’Light Scatter’ tab entry fields exist for each of the pertinent metadata for reporting with light scatter calibration.

   3.1 Diameter CV should be the percent coefficient of variation of the mean diameter provided on the certificate of analysis

   3.2 Refractive index should be the provided refractive index of the bead population on certificate of analysis

   If a refractive index is not available an approximate guide for polystyrene refractive index is 1.59 at 589 nm. Silica tends to vary more in refractive index than polystyrene but tends to be \( \sim 1.45 \) at 589 nm.

   3.3 ’RI Measurement Wavelength’ is the wavelength at which the refractive index was measured and should be provided on the certificate of analysis. This tends to be 589 nm.

   3.4 Composition can be selected as polystyrene, silica, or other. If polystyrene or silica are selected, changes in detection wavelength e.g. 488 nm to 405 nm are accounted for using the appropriate Sellmeier equations. If ’Other’ is selected then the refractive index change is made propositionally to the sheath refractive index.

   3.5 Manufacturer, Catalogue Number, and Lot Number should all be completed appropriately.

4. Once the fields have been completed for a bead population click ‘Add Bead’. The population should then appear in the table below.

5. Once the relevant beads have been added ‘Bead Sets’ can be created. A bead set are the bead populations that are
used for calibration. Any number of bead sets and combinations can be made.

5.1 In the ‘Selection’ column of the table, check all the bead populations to be included within a bead set.

5.2 Click ‘Create Set’, enter a unique Set name, and click ‘OK’.

Once your bead set has been defined you will be able to perform light scatter calibration.