FlowMie: Mie Theory Modeling in R

Wade Rogers[1,3], Jeremy Leipzig[2], Eva Silvestro[3], Richard Schretzenmair[3], Jonni Moore[3]
[1]Still Pond Cytomics, LLC
[2]Metadata Research Center, College of Computing and Informatics, Drexel University
[3]Perelman School of Medicine, Dept. of Path. and Lab. Med., Univ. of Pennsylvania

Introduction

There has been a surge of interest in the biological significance of Extracellular Vesicles (EVs). Among the various methods of detecting and characterizing EVs, flow cytometry is unique in its capability of multi-parameter EV detection and characterization. However not all instruments can perform this difficult feat. Of those that have succeeded at some level, few are capable of seeing EVs smaller than about 100 nm. Nevertheless, flow cytometry is an extremely important platform for EV characterization. Thus it is important to quantitatively understand the capabilities and limitations of instruments used for these applications.

FlowMie provides tools to calculate the light scattering distribution of small particles, including reference beads as well as EVs, along with a thorough optical description of the flow cytometer laser and detector systems, which enables a complete and accurate modeling environment to calculate and characterize the response of a flow cytometer to these very small and dim particles.

FlowMie is implemented upon the R port of the Scattnlay code by Peña and Pal. Of note is that unlike better-known codes such as that of Bohren and Huffman, Scattnlay handles the case of multi-layer spherical particles, which is important to accurately model the core/membrane structure of EVs.

FlowMie is the first non-commercial, open source package implementing the “Mie Transform”, which uses calibrated flow cytometry data to compute the diameter of each event in an FCS file and then to add that size as a calculated parameter to FCS data for purposes of gating or visualization.

Describe the Flow Cytometer

Instrument characteristics include:

- The nominal scattering angle of the detector (e.g. 90^o for side scatter)
- The detector acceptance half-angle
- The variation η across the detector acceptance aperture
- Laser polarization

This example creates a side-scatter detector with an acceptance half-angle of 60^o and linearly polarized laser perpendicular to the plane of incidence, with a uniform detector sensitivity:

```
my_detector = create_detector(theta_0 = 90, alpha = 60, psi_0 = 90,
                              pol = 1.0, eta = eta_uniform)
```

Describe the Scattering Particles

Particle characteristics include:

- Number of layers
- Radius of each layer
- Refractive index of each layer
- Refractive index of the medium in which the particle is suspended
- Incident light wavelength

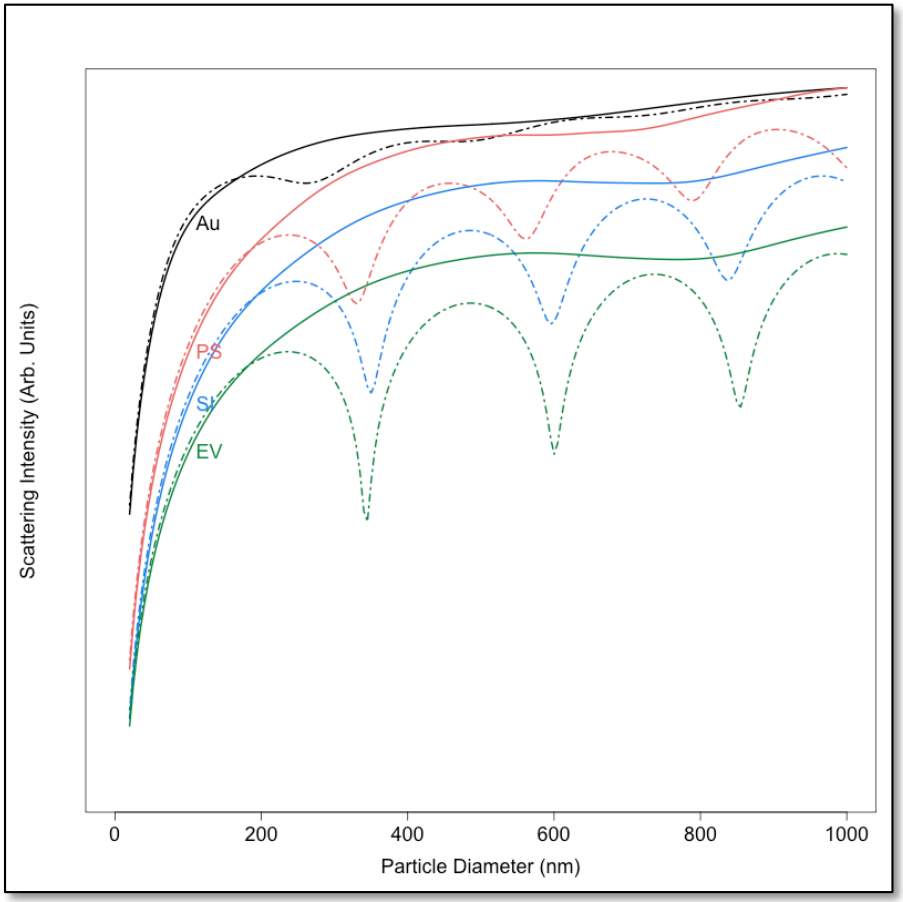
This example creates a vesicle, described as a 200 nm diameter 2-layer core/membrane structure. The first layer (core) has a radius of 95 nm and a RI of 1.38. The second layer (membrane) has a thickness of 5 nm and a RI of 1.46. The medium has an RI of 1.34 (normal saline), and the laser wavelength is 488 nm:

```
my_vesicle = create_particle(medium = 1.34, lambda = 488, n_layers = 2,
                             r = c(95, 100), n = c(1.38, 1.46))
```

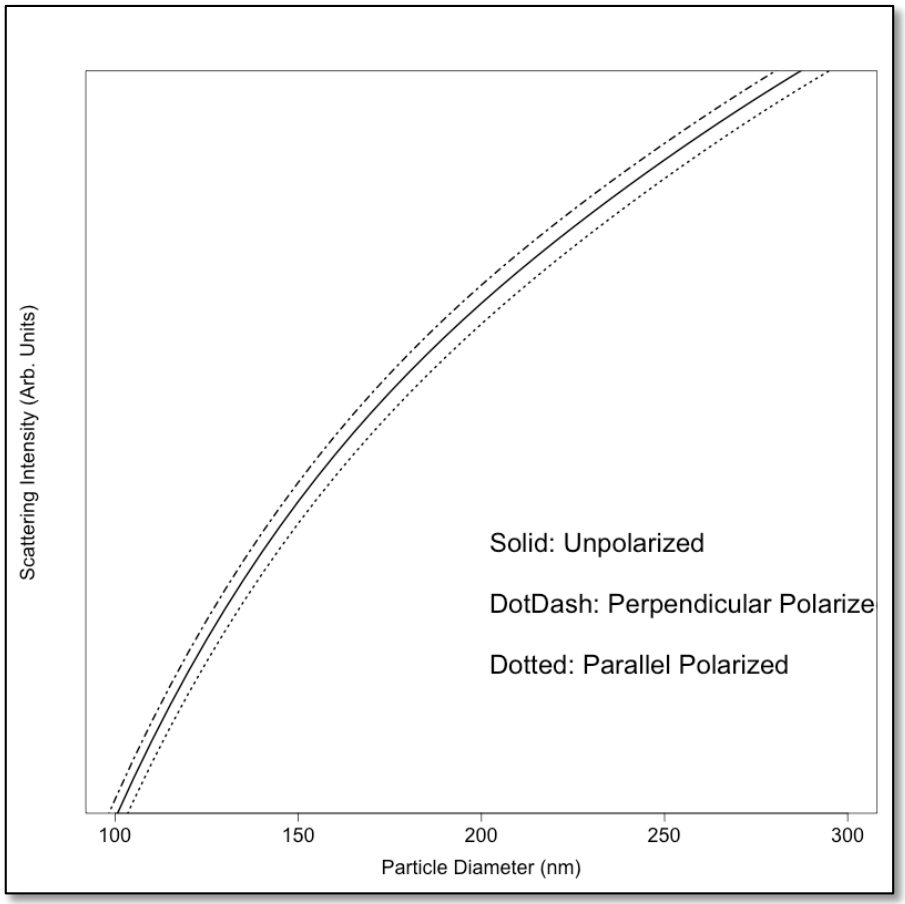
Calculate Scattering Response

Given a description of the instrument and the particle, calculate the scattering signal:

```
val = calculate_detector_response(particle = my_vesicle,
                                  detector = my_detector)
```



This figure illustrates the "smoothing" effect of a large angular acceptance (solid lines) compared with a narrow acceptance angle (dotted curves) for several structures. Note that the y-axis covers **10 decades**, emphasizing the difficulty of detecting vesicles smaller than about 100 nm. It also shows the profound sensitivity to particle composition.



This figure shows the effect of laser polarization. The differences, while relatively small, are not negligible, and can lead to systematic errors in the estimation of EV sizes. Differences between polarization in-plane versus perpendicular to the plane range from about 12% to 32%, with an average about 20% for a detector with large numerical aperture (smaller NA produces larger polarization effects).

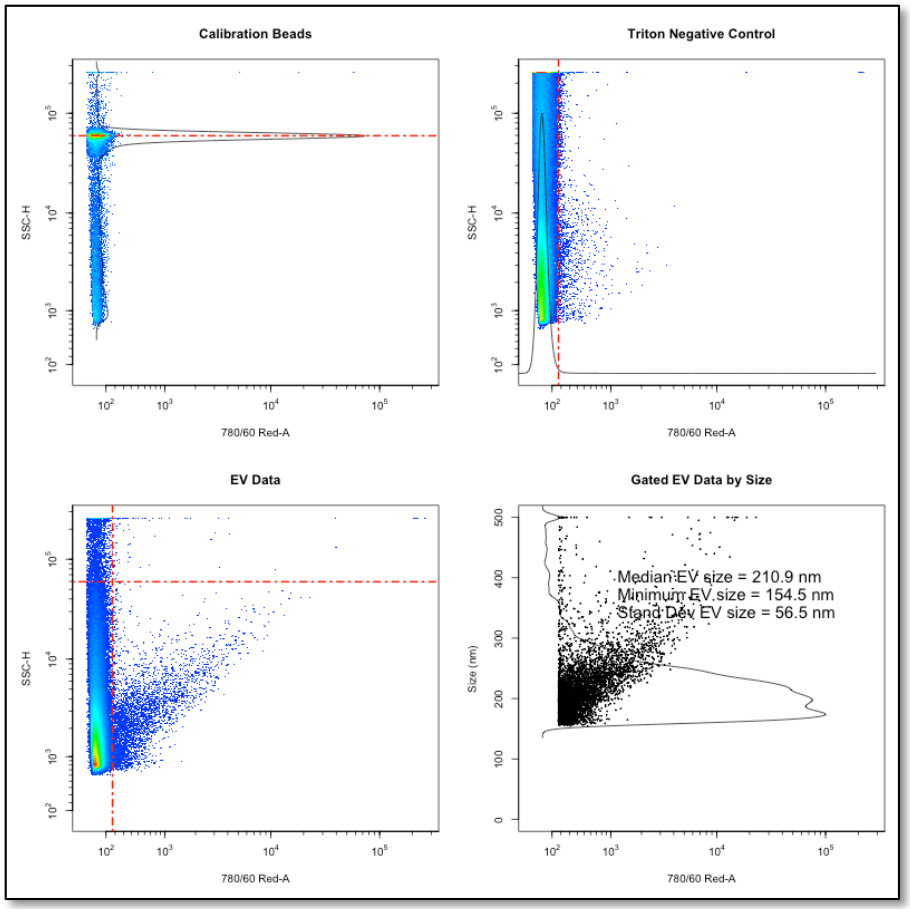
The Mie Transform

Upper Left: analysis of the 200 nm PS calibration beads.

Upper Right: Triton negative control to determine a fluorescence threshold.

Lower Left: EV sample, analyzed under identical conditions as both the bead and triton runs. The red lines indicate cut-offs used to gate the EV region.

Lower Right: gated EVs, with the Y-axis indicating particle size in nm as computed with the Mie Transform. We note that the sharp rise of the distribution at about 150 nm is an instrument effect, and not a reflection of the actual underlying EV size distribution. In this case the instrument was triggering on SSC, and the trigger threshold was high enough to reduce excess event rates, thereby limiting the lower size limit of this run. Triggering on a fluorescence signal may lower the size limit of detection. Also, use of a calibration particle with RI closer to EVs than polystyrene is advisable.



Conclusion

We introduce flowMie, an open-source, freely available R package supporting advanced modeling of light scattering from small particles, including extracellular vesicles detected by a flow cytometer. FlowMie allows for modeling the core/membrane structure of EVs, as well as a detailed description of the instrument required for accurate modeling of light scatter.

Please request that your instrument manufacturer contact us to provide instrument descriptions. We will add instrument constructors into flowMie for accurate and convenient modeling of your instrument.

FlowMie is available for download at:
https://github.com/rogerswt/flowMie

For poster discussion, contact:
wade.rogers@spcytomics.com

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