Supplementary material

A Methodology for Characterising Nanoparticle Size and Shape Analysis using Nanopores

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The sizes of the particles used in the first experiment were studied using electron microscopy. The pulse magnitude in RPS experiments is known to be proportional to the particle volume, and here we wished to scrutinise the ability of the RPS method to distinguish between rod and sphere for particles of comparable size. A 158 nm polystyrene nanosphere and a hematite nanorod were chosen as they had comparable sizes. To confirm the particle size distributions for the two sets of particles, measurements carried out via electron microscopy for the rod length, width and spherical particle diameter, Figure 1b, were converted into a predicted particle volume. The volumes of the sphere and rod particles were determined and overlaid with the predicted volumes from S/TEM, Figure A.



Figure S1 Distribution of particle volume versus frequency for the nanorods and sphere based upon measured and predicted volume of the spheres and rods using RPS and T/SEM respectively.



Figure S2 Plot of Pulse Frequency versus concentration of nanorods

We studied experimental conditions under which the best predictive models could be created. PU pores are known to stretch and change their dimensions over time. We also noticed that a calibration run at t=0, i.e. a new pore, would not work on the same pore that had been in use for several hours. This is likely due to the relaxing of the pore shape and size. The pore can be recalibrated after a period on stretch, but we noticed that the overall ability to discriminate between rods and spheres diminished over the pore stretch period as well as over the pore lifetime. An illustration of the change in pore shape over time is given in Figure B; the changes in the pulse shape for the same spherical particles is evident.



Figure S3: The average pulse shape recorded for spherical particles over several runs on the same pore. Numbers on x-axis represent the time points within each pulse. Red = run 1 on first day, yellow = sequential runs 2-14 on second day, green = run 15 on third day.

We investigated the misclassified rods and spheres for models A (depth only) and E (fixed normalised splines) for the final replicate for pore 3. First, we can see from the tables below that the size only (A) and fixed normalised spline models (E) are not classifying particles in the same way—that is, it is not merely residual size data in the normalised spline coefficients (eg, through signal width) that we are detecting in model E.

For spheres:	E pred rod	E pred sphere	total
A pred rod	24	54	78
A pred sphere	84	389	473
total	108	443	551
For rods:	E pred rod	E pred sphere	total
A pred rod	240	153	393
A pred sphere	77	54	131
total	317	207	524

Table C: confusion matrixes for shape prediction of spherical and rod particles by models A and E

Of the first 50 rod signals that were misclassified by model E, 9 were cases in which two peaks were captured in the same 301 time selection. The rest of these signals were reasonable quality, so it is not clear why immediately obvious why they were misclassified. Of the first 50 sphere signals that were misclassified by model E, 12 were poor quality signals (Figure C) and four were double peaks.



Figure S4: Examples of poor quality sphere signals

Supplementary material – not included here but will be available to down load submitted with manuscript

- 1. Extracted pulse datasets from runs described in this paper
- 2. R code for extracting pulses and shape data using raw instrument data and blockade file
- 3. R code for constructing models A-E