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Aerosol sample preparation methods for X-ray diffractive imaging: Size-selected spherical nanoparticles on silicon nitride foils

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1 **ABSTRACT** We are developing aerosol generation and processing methods for x-ray analyses of
2 nanoscale materials using conventional synchrotron radiation sources and using the newly operational
3 soft x-ray free-electron laser in Hamburg (FLASH) at the Deutsches Elektronen Synchrotron. Charge-
4 reduced electrospray, differential mobility analysis and an electrostatic precipitator were used to prepare
5 samples consisting of size-monodisperse spherical nanoparticles deposited on 20 nm thick silicon nitride
6 foils supported by silicon frames. 97 and 102 nm diameter spheres were selected from a broader
7 distribution of 98 nm spheres using differential mobility. We measured the size distribution of the
8 spheres using forward scattering from 1.65 nm light at the Advanced Light Source (ALS) in Lawrence
9 Berkeley National Laboratory and scanning electron microscopy (SEM). The full-width half maximum
10 (FWHM) of the size-distribution of the size-selected spheres was as narrow as 5.4 nm when measured
11 by SEM, as compared to 16 nm for the non-size-selected distribution. Forward scattering measurements
12 of the 97 nm diameter size-selected spheres fit a size distribution with a FWHM of 4 nm and allowed us
13 to validate the methodology for use in future diffraction imaging experiments at FLASH.

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15 Keywords: electrospray, differential mobility, nanoparticle, x-ray diffractive imaging
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17 18 **1. Introduction**

19 Scattering of ultraviolet (UV) or visible light, as described by Mie theory, is a standard approach for
20 characterizing the properties of spherical particles (van de Hulst, 1981). Angle-resolved measurements
21 of the scattered light intensity provide a way to calculate particle size and index of refraction. The
22 primary goal of this study is to validate the use of aerosol methods for preparation of size-monodisperse
23 spherical nanoparticles that will be used as targets in future experiments designed to study laser-matter
24 interaction at the world's first free electron laser functioning in the soft x-ray regime, the free electron
25 laser in Hamburg (FLASH). One of the goals of the FLASH experiments is to determine the change of
26 particle properties (size and refractive index) from the diffraction pattern recorded from single x-ray
27 pulses, as a function of pulse fluence. This will enable us to study the response of materials, on a
28 submicron length scale, as they are rapidly heated to hot (60000 K) dense plasma (Chapman *et al.*,

1 2006) by the FLASH pulses. That is, the scattering pattern from the single pulse will be used to
2 examine the effect of that pulse on the material over the pulse duration. With lower fluence non-
3 damaging pulses, these types of samples will also be useful for studying ultrafast dynamics of materials
4 (for example, near phase transitions). Spherical nanoparticles offer both modeling and experimental
5 advantages as test objects because interpretation of their diffraction patterns is well understood. A
6 spread in the initial diameters of the spheres causes line broadening in the diffraction pattern that
7 reduces the resolution of the measurement of changes in particle size and optical properties. These
8 measurements therefore require samples prepared with size-monodisperse spheres to optimize the
9 resolution. Furthermore, signal levels in FLASH experiments will depend on the magnitude of the
10 photon fluence and the number of spheres irradiated. Thus a protocol for creating samples with different
11 numbers of spheres (estimated to be 1-1000 particles per FLASH pulse, corresponding to a range of
12 3×10^{-3} to 3 particles/ μm^2) is required.

13 We addressed these requirements by preparing size-monodisperse spherical nanoparticle samples
14 using electrospray differential mobility size-selection and electrostatic deposition (Fissan *et al.*, 2003).
15 Polystyrene spheres ~ 100 nm in diameter are deposited onto silicon nitride (Si_3N_4) foils (Anastasi &
16 Burge, 1990) that are thinner than the sphere diameter and transmissive to x-rays, thus facilitating
17 diffraction imaging experiments. Diffraction imaging is elegant in its experimental simplicity: a
18 coherent X-ray beam illuminates the sample and the far-field diffraction pattern of the object is recorded
19 on an area detector (in this case a bare CCD chip). In this work, x-rays scattered from particles
20 positioned on the foil are transmitted through the 20 nm thick Si_3N_4 foil and are detected by a CCD
21 camera (Figure 1). The x-ray scattering data collected with conventional synchrotron radiation serves as
22 a proof-of-concept for this sample preparation methodology for future FLASH diffraction imaging
23 experiments.

24 Under its initial operating parameters, FLASH emits intense 25 fs, 4×10^{13} W/cm² pulses containing
25 10^{12} photons at 32 nm wavelength. Interaction of a single pulse with a nano-structured non-periodic
26 object produces a coherent diffraction pattern that can be recorded using specially designed x-ray optics
27 and a CCD. An iterative algorithm, Shrinkwrap (Marchesini *et al.*, 2003), uses the information in the

1 diffraction pattern to reconstruct the image of the object irradiated by the single pulse. The first
2 demonstration of this experiment, femtosecond diffractive imaging (Chapman *et al.*, 2006), confirmed
3 predictions (Bergh *et al.*, 2004; Hau-Riege *et al.*, 2004; Jurek *et al.*, 2004; Neutze *et al.*, 2000) that the
4 diffraction pattern created from a non-crystalline object irradiated by such a pulse would be
5 representative of the structure of the object before the manifestation of damage. The spherical
6 nanoparticle samples prepared and characterized here will be used as targets for x-ray pump-probe
7 measurements using the ultrafast laser pulses that will enable us to measure the dynamics of the
8 explosion on very short time scales.

9 10 **2. Experimental**

11 12 *2.1 Nanoparticle Aerosolization and Deposition*

13 The nanoparticles used in this work are polystyrene spheres (PostNova, Germany) with a reported
14 diameter of 98 nm and a coefficient of variance of 5.4 %. They are provided in a surfactant-free solution
15 at a concentration of 1.5×10^{14} particles/ml (p/ml). Solutions that contain surfactants to stabilize the
16 spheres were also studied but solution-viscosity modifications due to the surfactant destabilized the
17 electrospray process used to aerosolize the spheres. When solutions containing surfactants are
18 electrosprayed, particles <10 nm in diameter are detected in addition to the spheres. These particles are
19 the residues of droplets that contain no spheres. The formation of these residue particles suggests that
20 electrospraying solutions of spheres that also contain surfactants results in spheres coated by surfactants
21 (Kaufman, 2000). To eliminate the potential for coated spheres, only surfactant-free spherical
22 nanoparticle solutions are used to prepare samples for x-ray studies.

23 To characterize the sphere size distribution and to reduce their size-polydispersity we use charge-
24 reduced electrospray and differential mobility analysis technology (Lenggoro *et al.*, 2002). Figure 2A
25 describes the apparatus and procedure for collecting size-selected spheres onto Si_3N_4 foils. An
26 electrospray aerosol generator (TSI model 3480) is used to aerosolize solutions of 1.5×10^{12} to 1.5×10^{13}
27 p/ml prepared in 25 mM ammonium acetate in water. Similar to previous studies (Bacher *et al.*, 2001;
28 Chen *et al.*, 1995) 25-100 μL of sample in a 1.5 ml plastic microcentrifuge tube is placed in the

1 cylindrical pressure chamber of the electrospray generator. A capillary of 40 μm inner diameter and a
2 platinum wire at a voltage of 2.0 kV make contact with the sample. A differential pressure of 1.2 psi
3 forces the solution through the capillary at about 0.1 $\mu\text{L}/\text{min}$. The voltage applied to the sample creates
4 a Taylor cone at the tipped exit of the capillary. These electrospray conditions produce primary droplets
5 150 nm to 200 nm in diameter. (Chen *et al.*, 1995)

6 The electrospray droplets are introduced into a flow of 1.5 lpm air and 0.1 lpm CO_2 to minimize
7 corona discharge. The droplets are immediately charge-reduced by exposure to ionized air created by a
8 ^{210}Po α -source and allowed to evaporate to dryness, resulting in an aerosol of discrete aerosolized
9 spheres with a known charge distribution. The charge carried by the spheres is predominantly zero,
10 while a small fraction is singly-charged (positively or negatively), and an even smaller fraction is
11 doubly-charged (again, positively or negatively), as predicted by Fuchs' particle charge distribution
12 (Fuchs, 1963; Hoppel & Frick, 1986), also called a Boltzman charge distribution (Liu & Pui, 1974). The
13 size-polydisperse spheres pass into a differential mobility analyzer (DMA) (TSI, model 3081) via a
14 conductive silicone tube and are size-classified based on their electrical mobility. A portion of the size-
15 selected aerosol is sampled into a condensation particle counter (CPC) (TSI, model 3786) to monitor the
16 gas phase particle concentration ($\text{number}/\text{cm}^3$) and the remaining aerosol is delivered to a nanometer
17 aerosol sampler (NAS) (TSI, model 3089) for electrostatic capture onto a substrate (Dixkens & Fissan,
18 1999). Silicon wafers (1 cm^2) supporting the Si_3N_4 foils are positioned with electrically conductive tape
19 onto a 1 cm diameter electrode (-10 kV) in the NAS. The particle size distribution was measured by
20 scanning the DMA electrode voltage while counting the particles entering the CPC. The DMA data was
21 not corrected for the finite width response of the DMA.

22 During sample preparation, aerosol concentration (C) of size-selected spheres indicated by the CPC is
23 used as a real-time diagnostic of the stability of the electrospray conversion of the spheres in solution to
24 an aerosol. From C , the total number of spheres that enters the NAS for collection on the foil, (N), is
25 calculated using the relationship $N = Cft$, where f is the flow rate of the gas carrying the aerosol into the
26 NAS and t is the total exposure time. N is used to provide an estimate of the number of spheres on the

1 surface of the sample. SEM is used to measure the actual number of spheres on the foil. The total
2 number of spheres in an area of $20\ \mu\text{m}^2$ is counted in 5-10 images to provide an average number of
3 spheres/ μm^2 on the surface.

4 Figure 2B describes the method used to coat a single sample with different densities of spherical
5 nanoparticles by exposing discrete regions of the sample to a different absolute number of spheres. In
6 this example, a sample with four regions is created. The number of regions that can be made on a
7 sample surface can be increased or decreased to fit experimental requirements. In step 1, a mask is used
8 to cover a portion of the foil. The uncovered area is exposed to a known number of spheres N_1 . The
9 spheres are electrostatically deposited on the sample to a resultant coverage of D (spheres/ μm^2). In step
10 2, the mask is moved so that a larger region, that includes the first region, is exposed to another known
11 number of spheres N_2 . Thus two regions are created, the first is exposed to $N_1 + N_2$ spheres and the
12 second to N_2 spheres. Continuing in this manner for n steps produces n discrete regions on the foil with
13 decreasing exposures of spheres as n increases, i.e. $D_1 > D_2 > \dots D_n$.

14 2.2 X-ray analysis

15 The thin foils (thickness less than the radius of the spheres) are designed to cause minimal
16 interference from x-rays scattered from the foil. The foils are prepared on $300\ \mu\text{m}$ thick Si wafers
17 following the procedure of (Anastasi & Burge, 1990). The basic procedure starts by coating both sides
18 of a standard Si wafer with Si_3N_4 . One side of the wafer is then coated with photoresist, masked and
19 exposed to UV light. Si_3N_4 in areas once covered by photoresist is removed by hydrofluoric acid etching
20 or plasma etching. Uncovered Si is removed by potassium hydroxide anisotropic etch to leave free
21 standing Si_3N_4 foils. Foils can be created with a large variation in dimensions but for experiments
22 reported here they are $20\ \text{nm}$ thick and $1750\ \mu\text{m} \times 50\ \mu\text{m}$. A thickness of $20\ \text{nm}$ is chosen because it
23 represents a functional balance between high x-ray transmission, fabrication ease, and operational
24 integrity. The transmission of a $20\ \text{nm}$ thick foil at a wavelength of $1.65\ \text{nm}$ is $97\ \%$. Si-supported Si_3N_4
25 foils are also commercially available (SPI Supplies Structure and Probe Inc., PA).

1 X-ray scattering experiments were performed at beamline 9.0.1 at the ALS. The experimental vacuum
2 chamber in which the experiments were performed has been described in detail (Beetz *et al.*, 2004;
3 Howells *et al.*, 2002). An off-axis zone-plate monochromator is used to select the third harmonic of a
4 10-cm-period undulator beamline. The 1.65 nm light is passed through a 5 μm pinhole and diverges to
5 illuminate about a 10 μm diameter spot on the sample with a flux of $\sim 10^9$ photons $\text{s}^{-1} \mu\text{m}^{-2}$ (Figure 1).
6 An in-vacuum backside illuminated CCD camera (MTE-2, Roper Scientific, Inc) with a 1340x1300
7 array of 20 μm square pixels is used to capture the scattering signal at a position 11.8 cm from the
8 sample. This enables detection of scattering light over an angular range of about 7° on either side of the
9 optical axis. A translatable opaque beamstop prevents saturation and radiation damage of the center
10 CCD pixels while collecting the higher angle scatter. Individual samples are translated into the beam
11 using motion controllers, until the foil supporting the spheres is illuminated and then the scatter signal is
12 optimized. CCD integration time is 3-20 minutes. Background corrected data was compared to Mie
13 theory using a refractive index of 0.9956411 with an imaginary component of 6.1915517×10^{-5} .

14

15 **3. Results and Discussion**

16 *3.1 Size-monodisperse spherical nanoparticles on Si_3N_4 foils*

17 The size distribution of the 98 nm polystyrene spheres obtained from PostNova was measured using a
18 DMA (Figure 3A). A Gaussian fit of this data shows the most abundant diameter of the particles by
19 percent concentration is 99.9 ± 0.2 nm, and the full width at half maximum (FWHM) of the distribution
20 is 16 nm. While the SEM measurement of the particle size distribution (Figure 3B) agreed with the
21 DMA measurement, the manufacturer's reported FWHM is 12.5 nm, assuming a normal distribution.
22 Nonetheless, the width of this size distribution is too large for future FLASH experiments because this
23 width will broaden (smear) the sphere diffraction patterns. We want to demonstrate that in future
24 experiments at FLASH we will be able to measure changes in sphere diameter as small as a 5 %
25 expansion, while a particle explodes. To do so we need starting distributions to be as narrow as possible.

1 We used commercially available aerosol processing technology to size-select spherical nanoparticles,
2 reducing the polydispersity to an acceptable level.

3 To demonstrate the sample preparation method and x-ray scattering measurements, an example of two
4 samples that were prepared with size-selected spherical nanoparticles collected onto foils with up to 3.2
5 nanoparticles/ μm^2 will be used. The stock solution of 98 nm diameter spheres was electrosprayed and
6 the DMA was set to transmit a specific diameter by changing the voltage applied to the DMA (-2465.3
7 V and -2704.9 V for 97 and 102 nm diameter spheres, respectively). Comparison of SEM images of
8 spheres from the polydisperse source aerosol and spheres size-selected from the same aerosol illustrates
9 the capability of the DMA to transmit a narrow size distribution of spheres (Figure 4). For example, in a
10 randomly selected foil coated with non-size-selected spheres, four adjacent spheres had nominal
11 diameters of 73, 80, 93, and 100 nm (Figure 4A) whereas six adjacent spheres on the sample prepared
12 with size-selection all had measured diameters of 97 nm (Figure 4B). Other key features revealed in
13 these micrographs are the local uniformity of the Si_3N_4 foil and the absence of contaminant particulate
14 matter. All material irradiated by the x-ray beam contribute to the diffraction image so clean surfaces
15 are essential for generating high quality images.

16 The diameters of >200 spheres for both sizes selected were also measured by SEM. Histograms of the
17 size-selected sphere diameters (Figure 3C) show the efficacy of DMA size-selection when compared
18 with the non-size-selected population in Figure 3B. For comparison, the size-selected histogram data
19 were converted into percent concentration and Gaussian fits were overlaid onto Figure 3A. The
20 FWHM of these fits were 5.4 and 6.5 nm for the 97 and 102 nm diameter particles, respectively. This
21 data provides a clear representation of the improved monodispersity due to DMA particle size-selection
22 under these conditions from the original size distribution with a FWHM >10 nm.

23 SEM images were also used to determine the extent of coverage of nanoparticles on the Si_3N_4 foil. In
24 this example, a single image of each of the four discrete regions was taken at equal magnification. The
25 four regions contained 86, 39, 10, and 3 spheres, respectively (Figure 4 C-F), confirming that the mask
26 procedure did result in $D_1 > D_2 > D_3 > D_4$ (refer to Figure 2B). For measurements made from additional
27 samples prepared over a period of weeks, D was found to correlate linearly with N ($R^2 = 0.97$). The

1 resulting relationship, $D = (-0.03 \pm 0.04) + (6.5 \pm 0.04) \times 10^{-9}(N)$, was used to determine an estimate of D
2 in real time, eliminating unnecessary and timely measurements of D by SEM during preparation of
3 samples with a specific desired number of spheres on the surface. The time required to prepare a sample
4 varies from 5 to 600 minutes or higher depending on the desired D and the aerosol concentration.

5 In all images analyzed, greater than 98.5 % of 97 nm spheres occupied distinct positions on the Si_3N_4
6 foils. The remaining 1.5 % existed as pairs in contact. Very rare instances of 3 spheres contacting were
7 observed. If the sphere deposition followed Poisson statistics, a higher occurrence of pairs in contact is
8 expected. This deviation is likely due in part to the repulsion forces present from the like charges on the
9 spheres. Distinct positioning of the nanoparticles is a desirable characteristic of the sample preparation
10 because the x-ray penetration depth into polystyrene is about 15 μm so a pile of ~ 150 spheres would
11 distort the scatter signal observed. In the areas analyzed to prepare the histogram of non-size selected
12 spheres diameters, several clusters of spheres $\sim 1 \mu\text{m}$ in diameter and 1 cluster of $\sim 8 \mu\text{m}$ were observed.
13 These large aggregates are much larger than the droplet size produced by the stable mode of
14 electrospray thus were likely formed during an unstable pulsing mode of the electrospray process.
15 Preparation of samples using DMA size-selection has no risk of such aggregates existing on a sample
16 and only at unreasonably long deposition times could a layer of spheres thicker than the penetration
17 depth be formed. By contrast, the aggregation resulting from direct deposition from solution is much
18 more likely to result in localized nanoparticle aggregates thicker than the x-ray penetration depth.

19 *3.2 X-ray measurement of DMA size-selected spherical nanoparticles*

20 Scatter signal was integrated for 21 and 11 minutes for 97 and 102 nm diameter spheres, respectively
21 (Figure 5A and 5B). It is estimated that three hundred spheres occupied the area irradiated by the x-ray
22 beam, providing sufficient the signal-to-noise ratio of the scattering intensity while retaining a
23 diffraction pattern consistent with that of a single sphere. Furthermore, the Si_3N_4 foil preparation
24 method produces sufficiently thin and clean substrates as to not interfere with the sphere diffraction. A
25 plot of the radial average of the CCD signal as a function of angle (radial pixel position) facilitates
26 visualization of the change in the minima of the diffraction pattern due to particle diameter change

1 (Figure 5C). Minima for the 102 nm diameter spheres are at smaller angles than minima for the smaller
2 spheres (2.30 and 2.37 for the 3rd minimum, respectively – see inset Figure 5C), thus the particles were
3 prepared with sufficient monodispersity to differentiate a 5 nm change in diameter using the diffraction
4 patterns. Seven minima are observed in the radial averages, suggesting the population of spheres
5 measured is highly size monodisperse.

6 Mie theory was used to determine the size distribution of spheres that best fit our experimental results.
7 Summarized in Figure 6, this analysis showed that distributions with a FWHM of 10 nm clearly
8 resolved too few minima and thus DMA size-selection increased monodispersity to less than 10 nm
9 FWHM. The best fit for our data was a FWHM between of 4 nm (inset Figure 6). This measurement is
10 in general agreement with the SEM data, further supporting the usefulness in these experiments of the
11 size-selection capability of the DMA. Background scattering from the Si₃N₄ foil was a variable in our
12 measurement and may account for the difference with the SEM. A more accurate measurement of the
13 size distribution with more rigorous background corrections is possible. Nonetheless, the reduction in
14 size-polydispersity of the aerosol imparted by DMA size selection has been validated by SEM and x-ray
15 scattering measurements of samples that can be used as targets for future FLASH experiments.

16 **4. Discussion**

17 Both the SEM and x-ray measurements presented here confirm that samples consisting of a
18 population of ~100 nm diameter spherical nanoparticles with FWHM <5 nm can be prepared on Si₃N₄
19 foils. Note that the methodology is not limited to the sphere sizes in the example reported here.
20 Spherical nanoparticles of 88 and 140 nm in diameter have also been size-selected with similar results
21 (data not shown). Furthermore, the method is applicable to any nanoscale biomaterials, such as viruses,
22 amenable to electrospraying (Hogan, 2006). Samples created using this method will act as standards for
23 the characterization of FLASH pulse interaction with matter in pump-probe experiments and for
24 femtosecond diffractive imaging of nanoparticles. The ALS x-ray scattering measurements reported
25 here can differentiate between the two diameters of size-selected nanoparticles with a 5 nm difference
26 verified by scanning electron microscopy. Thus, in future FLASH experiments it will be possible to
27 detect similar changes in sphere diameter due to any explosion. In short, we have used the

1 measurements on two sets of size-selected spheres to mimic a diameter change due to an explosion that
2 may be measured at FLASH in the future. During such experiments the 20 nm thick Si₃N₄ foil will
3 transmit at only 42 % instead of 97 % because of the change in wavelength from 1.65 nm to 32 nm.
4 Future upgrades of FLASH to 13 and 6 nm will allow transmission of 75-85 %. The ability to control
5 the number of spheres positioned on the Si₃N₄ foils, as demonstrated here, will be critical to preparation
6 of samples to meet these changing criteria.

7 New methods for controlled delivery of free nanoparticles to x-ray free-electron-lasers must also be
8 developed to remove substrate effects from the x-ray measurements of individual nanoparticles. Thus,
9 future work will also include adaptation of the method for injection of free nanoparticles to the FLASH
10 pulses. In this approach, the size-selected nanoparticles will be delivered into a differentially pumped
11 aerodynamic focusing system that will generate a beam of nanoparticles that can be steered into the
12 FLASH pulses. The successful characterization of nanoparticles using the new intense x-ray sources
13 such as FLASH and the Linac Coherent Light Source at the Stanford Linear Accelerator Center will rely
14 heavily on adaptations of existing aerosol sample preparation methods such as those described here.

15

16 **5. Conclusions**

17 Aerosol methods for preparing size-monodisperse spherical nanoparticles as fixed target standards
18 for x-ray diffractive imaging were validated using conventional synchrotron radiation and SEM. The
19 number of spheres in a specific area of a Si₃N₄ foil can be controlled and essentially all of the spheres
20 occupy distinct positions, ensuring the integrity of the diffraction pattern recorded. SEM and x-ray
21 scattering measurements showed that DMA filtered polystyrene particles have size distributions
22 adequately narrow (FWHM ~5 nm at ~100 nm diameter) to produce high quality scattering images. This
23 aerosol method will be used to prepare samples for femtosecond diffractive x-ray imaging of
24 nanoparticles using x-ray free-electron lasers.

25

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26 **Figure Captions**

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Figure 1. X-ray diffraction experiment at ALS. The sample of spherical nanoparticles positioned on a
29 Si_3N_4 foil (A) supported in a silicon frame (B), is positioned into the pulsed x-ray beam (C). The
30 resultant diffraction pattern (D) is recorded using a CCD camera (E) and the direct beam is stopped by a
31 beamstop (F). The silicon chip is depicted as a cross-section to reveal the thickness of the foil (20 nm)
32 relative to the chip (300 μm thick). This figure is not drawn to scale.

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Figure 2. A) Instrumental setup for aerosolization, size-selection, and collection of spheres on Si_3N_4
34 foils. **B)** Strategy for varying the exposure time (t) to different areas of the foil to control sphere
35 coverage (D) on the surface.

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Figure 3. (A) Size distribution of the electrosprayed stock solution of 98 nm polystyrene spheres as
38 measured using differential mobility analysis. The dotted line is a Gaussian fit (FWHM = 16 nm) to the
39 DMA data (filled squares). The red and green traces are Gaussian fits to SEM data obtained when the
40

1 DMA was set to transmit 97 (red) and 102 (green) diameter spheres. (B) Histogram of SEM diameter
2 measurements of 299 non-size-selected spheres (before DMA filtering). (C) Histograms of SEM
3 diameter measurements of 237 DMA size-selected 97 nm spheres (open red bars) and 231 102 nm size-
4 selected spheres (solid green bars) used to create traces in (A). Sphere diameters were separated into 1
5 nm bins for all histograms.

6
7 **Figure 4.** Scanning electron micrographs of spherical nanoparticles (A) without size-selection and (B)
8 with the DMA set to transmit 97 nm diameter particles. Scale bars are 100 nm long. Wider field
9 micrographs of a single foil with four regions exposed to decreasing numbers of 97 nm diameter
10 particles: (C) $D_1=1.10$, (D) $D_2=0.45$, (E) $D_3=0.11$, and (F) $D_4=0.02$ particles/ μm^2 . The scale bar in (C)
11 represents 2 μm . Image contrast and brightness was modified to enhance visualization of the particles.

12
13 **Figure 5.** CCD images of forward scattered 1.65 nm radiation from (A) 102 nm diameter particles at
14 3.2 particles/ μm^2 and (B) 97 nm diameter spheres at 3.2 particles/ μm^2 positioned on 20 nm thick Si_3N_4
15 foils. The black area represents an area removed from radial average calculations because it either
16 contained scatter from the edges of the foil or contained no signal due to the beam stop. (C) Radial
17 averages of the angle-resolved scatter signal for 102 nm diameter (green) and 97 nm diameter (red)
18 spheres. Dotted lines show angle-resolved scatter intensity after background correction. The inset
19 highlights the change in angle of the 2nd minimum due to a 5 nm difference in sphere diameter.

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21 **Figure 6.** Comparison of experimental diffraction results for the 97 nm diameter spheres (solid blue
22 line) with Mie theory calculated for a distribution of spheres with FWHM of 3, 4, 5, 10 nm (green, red,
23 black, and grey dotted lines, respectively). The inset is a closeup of the third minimum, showing that the
24 intensity at the minimum fits best to Mie calculations based on a 4 nm FWHM particle size distribution.

25
26 Figures

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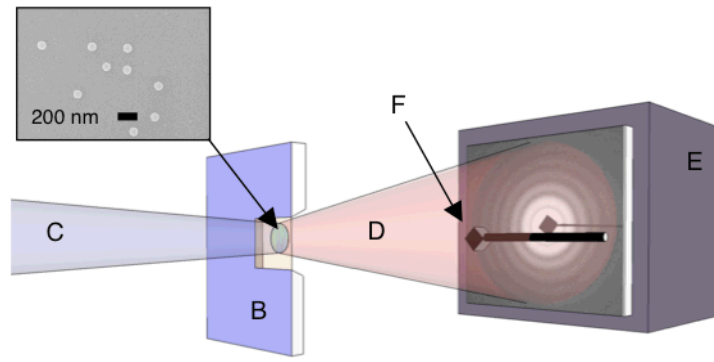
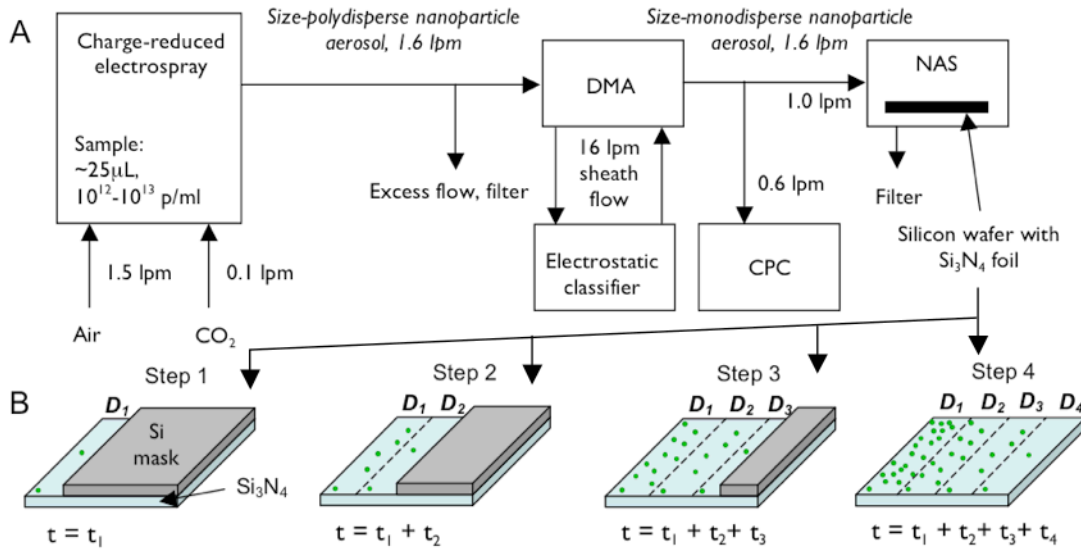
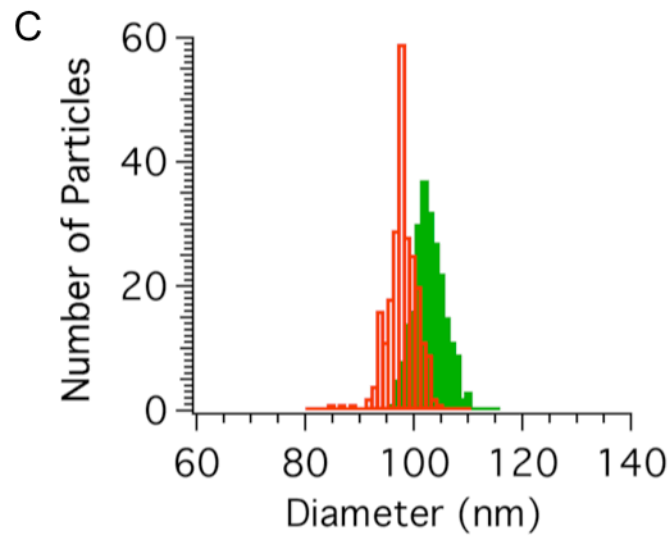
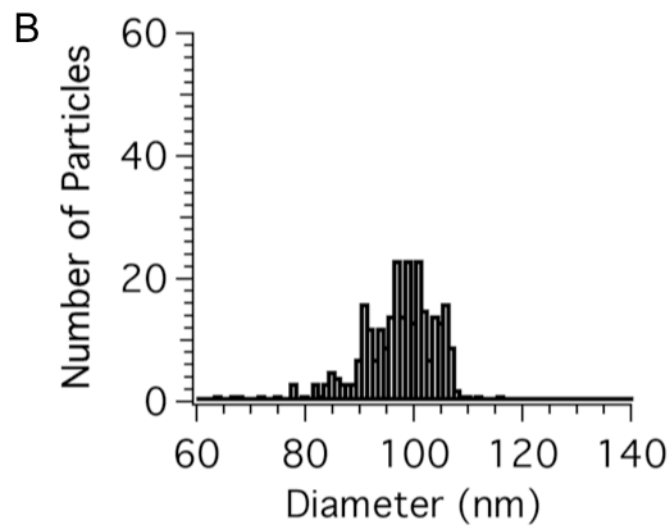
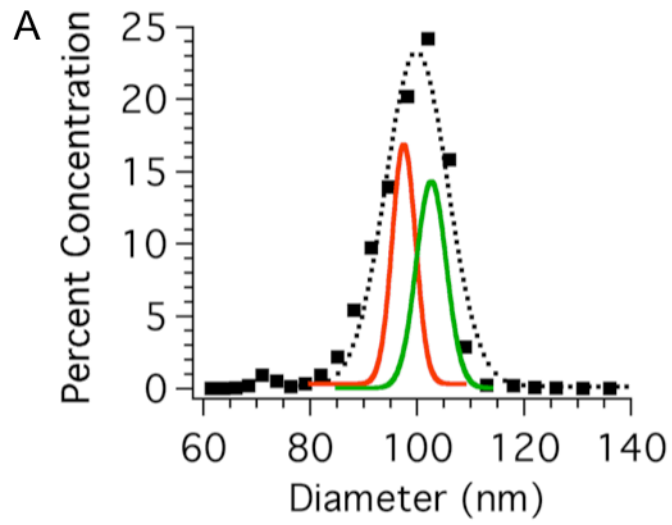


Figure 1



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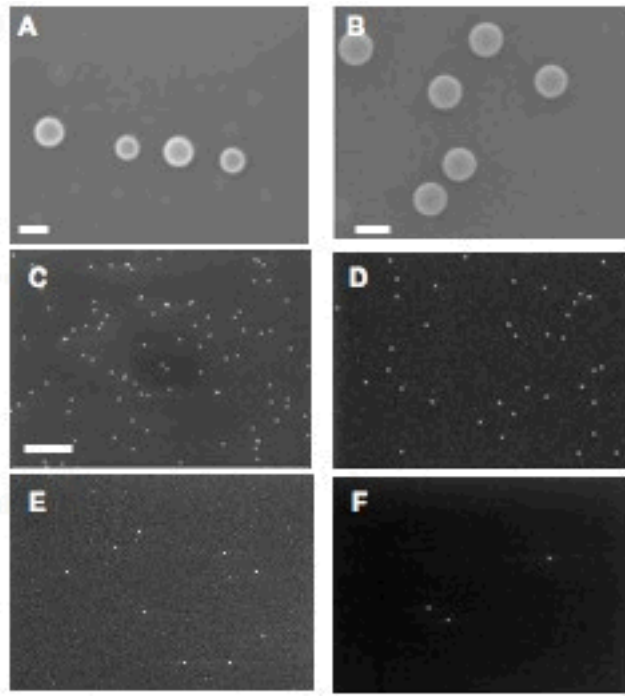


Figure 4

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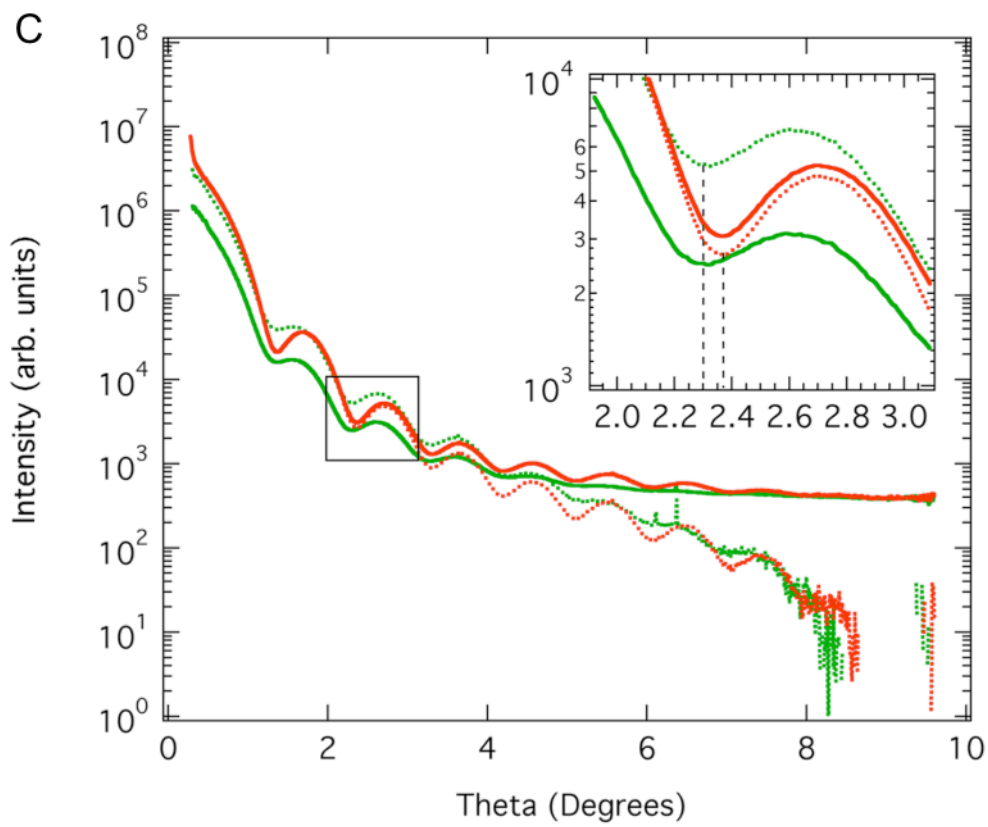
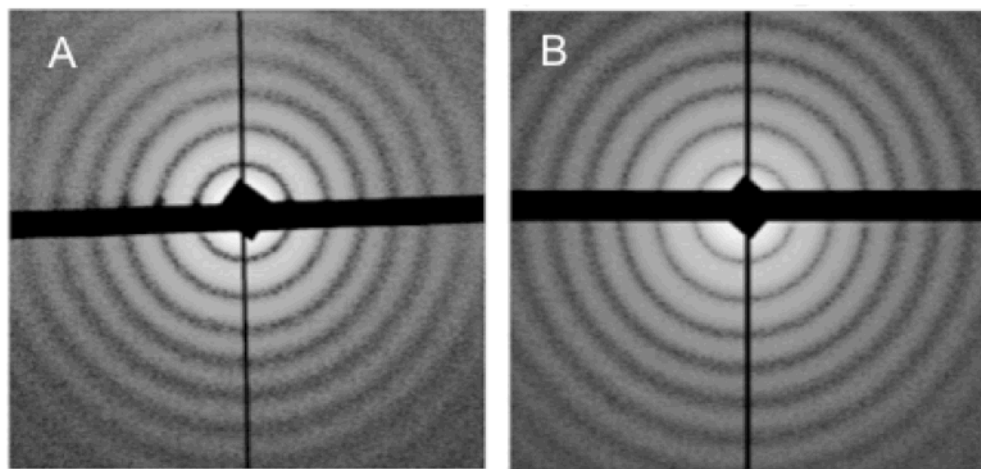


Figure 5

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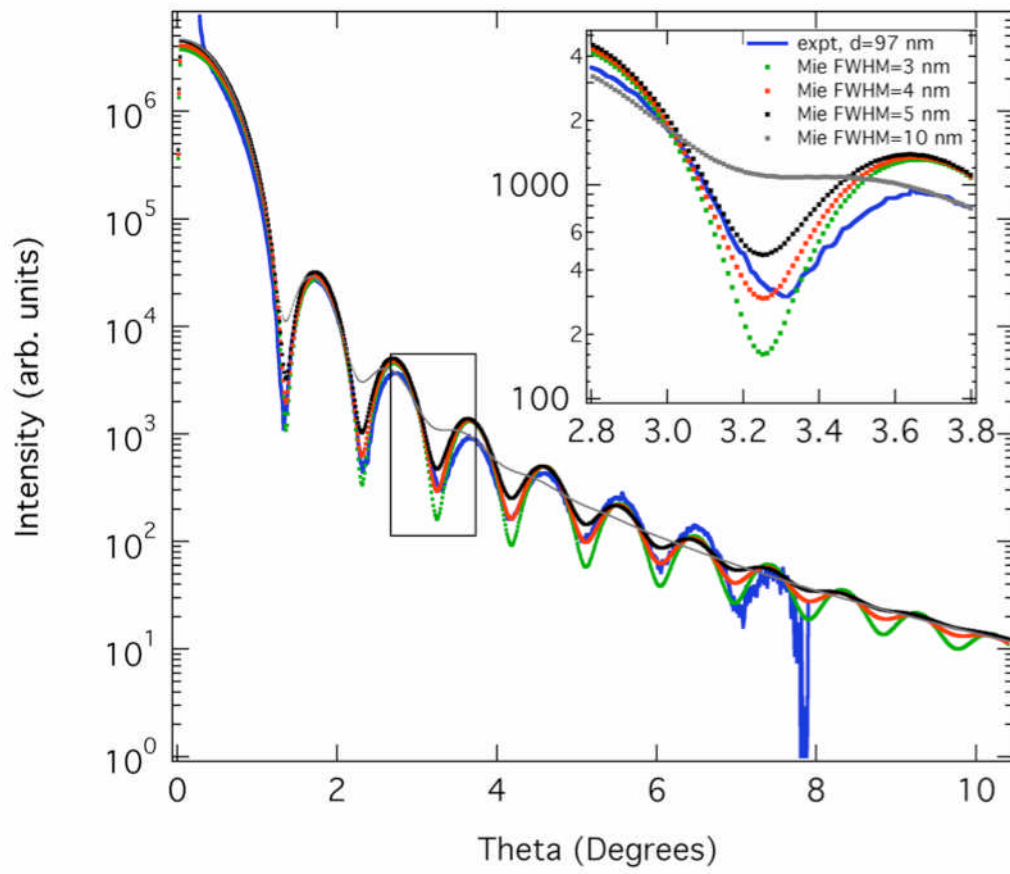


Figure 6

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