**Acoustic Focusing** 

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# The Limits of Primary Radiation Forces in Bulk Acoustic Standing Waves for Concentrating Nanoparticles

Christopher Reyes, Lin Fu, Pearlson P. A. Suthanthiraraj, Crystal E. Owens, C. Wyatt Shields IV, Gabriel P. López,\* Patrick Charbonneau,\* and Benjamin J. Wiley\*

Acoustic waves are increasingly used to concentrate, separate, and pattern nanoparticles in liquids, but the extent to which nanoparticles of different size and composition can be focused is not well-defined. This article describes a simple analytical model for predicting the distribution of nanoparticles around the node of a 1D bulk acoustic standing wave over time as a function of pressure amplitude, acoustic contrast factor (i.e., nanoparticle and fluid composition), and size of the nanoparticles. Predictions from this model are systematically compared to results from experiments on gold nanoparticles of different sizes to determine the model's accuracy in estimating both the rate and the degree of nanoparticle focusing across a range of pressure amplitudes. The model is further used to predict the minimum particle size that can be focused for different nanoparticle and fluid compositions, and those predictions are tested with gold, silica, and polystyrene nanoparticles in water. A procedure combining UV-light and photoacid is used to induce the aggregation of nanoparticles to illustrate the effect of nanoparticle aggregation on the observed degree of acoustic focusing. Overall, these findings clarify the extent to which acoustic resonating devices can be used to manipulate, pattern, and enrich nanoparticles suspended in liquids.

## 1. Introduction

Acoustic radiation forces have been extensively used to concentrate,<sup>[1-4]</sup> separate,<sup>[5–7]</sup> and assemble<sup>[8–10]</sup> microparticles and cells. Many devices that rely on the acoustic manipulation of particles employ bulk acoustic standing waves, which are obtained in a

C. Reyes, Dr. L. Fu, Dr. P. P. A. Suthanthiraraj, Dr. C. W. Shields IV, Prof. G. P. López, Prof. P. Charbonneau, Prof. B. J. Wiley NSF Research Triangle Materials Research Science and Engineering Center Durham, NC 27708, USA
E-mail: gplopez@unm.edu; patrick.charbonneau@duke.edu; benjamin.wiley@duke.edu
C. Reyes, Dr. L. Fu, Prof. P. Charbonneau, Prof. B. J. Wiley Department of Chemistry Durham, NC 27708, USA
C. E. Owens, Dr. C. W. Shields IV, Prof. G. P. López Department of Mechanical Engineering and Materials Science Duke University Durham, NC 27708, USA
The OPCID identification number(s) for the author(s) of this author.

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fixed nodes and antinodes throughout the fluid medium.<sup>[3,11,12]</sup> Such devices have been used to manipulate particles to generate complex designs in multiple dimensions,<sup>[13]</sup> to trap cells and particles in well-defined spatial regions,<sup>[14]</sup> and to separate particles,<sup>[15]</sup> e.g., cancer cells from blood<sup>[16]</sup> or solid particles from smoke.<sup>[17]</sup> Although the acoustic wavelengths used

resonating device that supports spatially

for particle manipulation are typically on the order of hundreds of micrometers, researchers have recently demonstrated such acoustic waves can be used to manipulate nanoparticles.<sup>[2,18–21]</sup> The first demonstration was by Raeymaekers et al., in which 5 nm diamond particles were concentrated.<sup>[21]</sup> Mitri et al. subsequently used an acoustic standing wave to create a periodic array of diamond nanoparticles within an epoxy matrix.<sup>[22]</sup> More recently, Mao et al. applied surface acoustic waves to induce a vortex flow via acoustic streaming to concentrate 80 nm silica and 110 nm

polystyrene particles in a narrow channel.<sup>[4]</sup> The application of acoustic radiation forces to concentrate nanoparticles has further been applied to the development of polymer composite metamaterials,<sup>[23]</sup> the alignment of metallic nanowires and carbon nanotubes,<sup>[24–26]</sup> and the contact-free separation of catalytic nanomotors.<sup>[7]</sup>

## C. E. Owens

Department of Mechanical Engineering Massachusetts Institute of Technology Cambridge, MA 02139, USA Dr. C. W. Shields IV, Prof. G. P. López Department of Biomedical Engineering **Duke University** Durham, NC 27708, USA Prof. G. P. López Center for Biomedical Engineering Department of Chemical and Biological Engineering University of New Mexico Albuquerque, NM 87131, USA Prof. P. Charbonneau Department of Physics **Duke University** Durham, NC 27708, USA



Despite the growing use of acoustics in nanotechnology, the extent to which a nanoparticle of a given composition and size can be manipulated with an acoustic standing wave has not been thoroughly addressed in the current acoustic focusing literature. As particle size and acoustic impedance diminish, the ability of acoustic radiation forces to propel particles is reduced due to competing physical effects, such as Brownian motion (thermal fluctuations), acoustic streaming, and wavelength constraints (e.g., the pressure wavelength greatly exceeds the size of the particle).<sup>[27,28]</sup> Although previous studies investigating nanoparticle acoustophoresis provide models that describe particle movement, they do not accurately describe the limits of acoustophoresis for concentration or patterning.<sup>[20,21,23,25,26,29]</sup>

This article examines the theoretical and experimental limitations of primary acoustic radiation forces for concentrating nanoparticles of a given size and composition. We first describe a simple analytical model that uses equations for Brownian motion and the primary acoustic radiation force to predict the time-dependent distribution of particles about a pressure node. Although similar models are common for describing the diffusion of particles in periodic potentials, to the best of our knowledge, this type of model has not been used to describe nanoparticle acoustophoresis.<sup>[30-33]</sup> Next, we examine the accuracy of this model by comparing the predicted fullwidth-at-half-maximum (FWHM) of the particle distribution to that of an experimentally observed system for the acoustic concentration of gold nanoparticles (Figure 1). We used gold nanoparticles because of their high density (19.3 g/cc) and low compressibility  $(5.5 \times 10^{-12} \text{ Pa}^{-1})$ , giving them a high acoustic contrast factor ( $\Phi$ ) in water, and thus a strong potential to migrate to pressure nodes.<sup>[5]</sup> In addition, the gold nanoparticles have a narrow size dispersity and a high degree of colloidal stability in aqueous suspensions (i.e., resistance to aggregation). To extend the results for gold nanoparticles to other materials, we use our model to estimate the minimum pressure amplitude necessary to concentrate spherical nanoparticles of any composition. These predictions were tested by performing a series of acoustic focusing experiments on gold, silica, and polystyrene nanoparticles, spanning three orders of magnitude in  $\Phi$ . Finally, we demonstrate that the predicted limits of concentrating nanoparticles using forces from acoustic standing waves can be overcome by inducing the aggregation of nanoparticles through a simple photochemical reaction.

## 2. Results and Discussion

Figure 1 shows a schematic of the experimental setup used for concentrating (i.e., focusing) nanoparticles in bulk acoustic standing waves. Two superimposed acoustic standing waves were produced by two lead zirconate titanate (PZT) piezoelectric transducers affixed on opposing walls of a laser-cut, rectangular acrylic chamber. In an acoustic standing wave, particles experience a primary radiation force from the acoustic potential *U*, which leads to the concentration of particles around the potential minima, called nodes. To provide insights into the extent to which nanoparticles can be focused with acoustic standing waves, we developed a simple analytical model to investigate the dynamics and equilibrium state of the system. In this



**Figure 1.** Schematic of the device used to acoustically concentrate nanoparticles. A) Gold nanoparticles suspended in water are (B) focused to nodes of the acoustic standing wave generated by two parallel piezoelectric transducers. Note that this schematic is not to scale. In experiments, the nanoparticles are much smaller than the size of the device, and the number of nodes was 125.

model, standing waves form planes with 1D periodicity. Setting the oscillatory direction along the x-axis, we write the acoustic potential as

$$U = -\frac{1}{2} V E_{\rm ac} \left[ f_1 - \frac{3}{2} f_2 + \Phi \cos(2kx) \right]$$
(1)

hence the acoustic force felt by a particle is

$$F = k V E_{ac} \Phi \sin(2kx) \tag{2}$$

with parameters defined as follows

$$E_{\rm ac} = \frac{\rho^2}{4\rho_{\rm f}c_{\rm f}^2} \tag{2a}$$



$$f_{1} = 1 - \frac{\rho_{\rm f} c_{\rm f}^{2}}{\rho_{\rm p} c_{\rm p}^{2}}$$
(2b)

$$f_{2} = \frac{2(\rho_{\rm p} - \rho_{\rm f})}{(2\rho_{\rm p} + \rho_{\rm f})}$$
(2c)

$$\Phi = f_1 + \frac{3}{2}f_2 \tag{2d}$$

$$k = \frac{2\pi f_{\rm PZT}}{c_{\rm f}} \tag{2e}$$

The system parameters and their experimental values used are listed in **Table 1**. We note that, depending on the sign of  $\Phi$ , particles focus either on the nodes ( $\Phi > 0$ , constant pressure) or the antinodes ( $\Phi < 0$ , highest pressure fluctuation) of the standing wave.<sup>[34]</sup>

The probability  $\hat{p}(\hat{x}, \hat{t})$  for a particle to be at position  $\hat{x}$  at time  $\hat{t}$  conditional on an initial distribution  $\hat{p}(\hat{x}, 0)$  can be described by the dimensionless Smoluchowski equation

$$\frac{\partial}{\partial \hat{t}} \hat{p}\left(\hat{x}, \hat{t}\right) = \hat{D}\left(\frac{\partial^2}{\partial \hat{x}^2} - \hat{\beta} \frac{\partial}{\partial \hat{x}} \hat{F}\right) \hat{p}\left(\hat{x}, \hat{t}\right)$$
(3)

where

$$\hat{x} = 2kx \tag{4a}$$

$$\hat{t} = 2k\nu_0 t \tag{4b}$$

$$\hat{D} = 2k \frac{D}{\nu_0} \tag{4c}$$

$$\hat{F} = \frac{1}{2k} \frac{1}{\nu_0^2} F$$
(4d)

Table 1. List of variables.

Variable	Definition	Value
V	Particle volume	$4/3\pi r^3$
E <sub>ac</sub>	Acoustic energy density	Equation (2a)
Φ	Acoustic contrast factor	Equation (2d) <sup>[35]</sup>
$ ho_{ m p}$	Particle density	$1.93 imes10^4~kg~m^{-3}$
$ ho_{ m f}$	Fluid density	$1.00\times10^3~kg~m^{-3}$
c <sub>p</sub>	Speed of sound in the particle	3240 m s <sup>-1[36]</sup>
c <sub>f</sub>	Speed of sound in the fluid	1484 m s <sup>-3</sup>
k	Wavenumber	2063 m <sup>-1</sup> , Equation (2e)
Р	Acoustic pressure amplitude	87.5–750 kPa
f <sub>РZT</sub>	Drive frequency of the PZT transducer	3.062 MHz
D	Particle diffusion constant	$(1.08-3.59) \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$
$\beta = 1/k_{\rm B} T$	Inverse temperature	$2.43\times 10^{20}~s^2~kg^{-1}~m^{-2}$
т	Particle mass	

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$$\hat{\beta} = \beta m v_0^2 \tag{4e}$$

The variable  $v_0$  is a scaling constant chosen such that  $\hat{\beta} = 1$  so as to simplify the calculation. Because  $\hat{p}$  is real and U is even, the Fourier series expansion gives

$$\hat{p}(\hat{x},\hat{t}) = \sum_{n=1}^{\infty} -\infty c_n(\hat{t})e^{in\hat{x}} \text{ with real } c_n = c_{-n}^*$$
(5)

which can be inserted into Equation (3) to obtain the tridiagonal recurrence relation

$$\frac{\partial}{\partial t}c_n = \frac{1}{2}\hat{D}\hat{\beta}ndc_{n-1} - \hat{D}n^2c_n - \frac{1}{2}\hat{D}\hat{\beta}ndc_{n+1, \forall n}$$
(6a)

with

$$d = \frac{1}{2\nu_0^2} V E_{\rm ac} \Phi \tag{6b}$$

In experiments, particles are initially uniformly distributed, i.e.,

$$\hat{p}\left(\hat{x},0\right) = \frac{1}{2\pi} \tag{7}$$

This uniform starting distribution is achieved experimentally by sonicating the nanoparticle solutions for 30 min prior to starting each experiment. Without loss of generality, we consider a single period ( $\lambda/2$ ) of the system in *x*, which sets the initial conditions for Equations (3) and (5) to be

$$c_0(0) = \frac{1}{2\pi}$$
 (8a)

$$c_{n\neq 0}\left(0\right) = 0 \tag{8b}$$

We then numerically solve for  $\hat{p}$  by the method of continued fractions.<sup>[37]</sup> We measure the FWHM of the particle distribution, p(x, t), to characterize the system dynamics with a single parameter to readily compare the theoretical predictions with experimental results. We note that the stationary solution to Equation (3), i.e., for  $t = \infty$ , is the Boltzmann distribution, i.e.,  $p(x) = A^* \exp(-\beta U(x))$ , where  $A = \int \frac{\pi}{k} \exp(-\beta U(x)) dx$  is a normalization factor that does not affect the FWHM. The truncation cutoff,  $n_c$ , of the Fourier series in Equation (5) is chosen such that numerical error on the FWHM for the equilibrium distribution and  $p(x, t = 10\ 000\ s)$  is below 0.2%. For P = 87.5 kPa

$$(\beta d \approx 1.1)$$
, 175 kPa  $(\beta \approx 4.2)$ , 350 kPa  $(\beta \approx 16.9)$ , and 700 kPa

 $(\ddot{B} \approx 67.6)$ ,  $n_c = 4$ , 8, 16, and 28, respectively, suffice.

By considering solely Brownian motion and the primary acoustic radiation force, we approximate the motion of the particles in a manner that is valid for ideal acoustic standing waves in the dilute nanoparticle concentration limit. To retain its simplicity and avoid the need to perform a simulation, the model explicitly does not include attractive (e.g., Van der Waals) or repulsive (e.g., electrostatic) interactions between



nanoparticles. The starting volume fraction of the particles in our experiments is generally low, between 2 and  $8 \times 10^{-6}$ , which corresponds to an interparticle separation of 4-8 µm. At these distances interparticle attractive forces are negligible, but electrostatic repulsion may be present depending on the surface charge of the particle and the ionic strength of the solution. Since electrostatic repulsion or convection would act to disperse the nanoparticles, the simple model described here can be thought of as an upper bound for the degree of focusing that can be achieved in the ideal gas limit, i.e., when interparticle forces are absent. The goal of the model is not necessarily accuracy, for such a purpose simulation is more suitable. Rather, the goal of the model is (1) to provide insights through simplicity, (2) to establish the extent to which diffusing particles in an acoustic field can be focused in the absence of interparticle forces, and (3) to set an upper bound for the degree of focusing that can be achieved for the type of bulk acoustic standing wave device that is described in this paper.

To facilitate a comparison of the results from the model with experimental results, we extracted a characteristic time,  $t_c$ , for focusing of gold nanoparticles to the pressure node

$$FWHM(t = t_c) = (FWHM(t = 0.1s) + FWHM(t = \infty))/e$$
(9)

We define the equilibrium time to focus as  $2t_c$ . This characteristic time was chosen to coincide with the times at which no significant decrease in the FWHM was observed across many experiments (**Figure 2**). The characteristic time and corresponding FWHM differ for each particle size and pressure amplitude, as shown in Tables S1 and S2 (Supporting Information).

We start our experimental comparison with the theoretical model by examining the degree to which the FWHM at pressure amplitudes of 175, 350, and 700 kPa agrees with the model predictions for FWHM at equilibrium. In a given experiment,  $\approx$ 125 parallel focusing nodes are formed within the acoustic chamber, and we imaged one node at a time (the width of the experimental panels was 242 µm; Figure 3B). Images were



**Figure 2.** Equilibrium time to focus 200 nm gold nanoparticles. FWHM of particles focused along a pressure node at various times (in multiples of  $t_c$ ). A suspension of 200 nm gold particles was focused in a bulk acoustic standing wave with applied acoustic pressure amplitudes of 175, 350, and 700 kPa, and the FWHM measurements were taken at times of  $1t_c$ ,  $2t_c$  and  $3t_c$ . Each data point represents the average of three measurements.

analyzed in ImageJ and MATLAB (MathWorks, Inc.) to obtain the FWHM of the distribution of nanoparticles about the node (see Figure S1 in the Supporting Information). Figure 3A,B indicates that the experimental observations qualitatively agree with the model predictions. Note that the scale bar in Figure 3A is set to  $\lambda_{aw}/4$ , but larger values of FWHM could be measured experimentally. Particles with diameters of 60 nm (Figure 3B, left) focused to a small extent (i.e., FWHM = 140  $\mu$ m) under the highest pressure that could be accessed with the experimental apparatus (700 kPa). The degree of focusing for particles with diameters of 100 and 200 nm (Figure 3B, middle



**Figure 3.** Acoustic focusing of gold nanoparticles. A) Theoretical plot of the equilibrium FWHM as a function of pressure amplitude and diameter of the gold nanoparticles. B) Darkfield images of 60, 100, and 200 nm gold particles (left to right) in an acoustic standing wave with pressure amplitudes of 700, 350, and 175 kPa (top to bottom) after equilibrium was reached. FWHM for 60 nm particles (left column): 140 µm FWHM at 700 kPa and no observable focusing at 175 or 350 kPa. FWHM for 100 nm particles (middle column): 43.2 µm at 700 kPa, 90.5 µm at 350 kPa, and 132.2 µm at 175 kPa. FWHM for 200 nm particles (right column): 25 µm at 700 kPa, 34.2 µm at 350 kPa, and 78.7 µm at 175 kPa. The contrast of the images in (B) was enhanced to better visualize the particle concentrations. All FWHM measurements were performed on unmodified, raw darkfield images (see the Supporting Information for details). All scale bars are 40 µm.







**Figure 4.** Equilibrium focusing of gold nanoparticles at 3.06 MHz ( $\lambda_{aw}/2=242 \mu m$ ). Theoretical equilibrium distributions for (A–D) 100 nm and (I–L) 200 nm gold particles with increasing pressure amplitude. White shading denotes regions of particle concentration. Corresponding equilibrium darkfield images from representative experiments for: E–H) 100 nm and (M–P) 200 nm gold particles. The contrast of images (E–H) and (M–P) was enhanced to better visualize the particle concentrations. All FWHM measurements were performed on unmodified, raw darkfield images. Scale bars are 40  $\mu$ m. Graphs of experimental equilibrium FWHM (black symbols) and model predictions (red curves) are shown for (Q) 100 nm and (R) 200 nm gold particles.

and right) decreased with decreasing pressure amplitude, as expected. At 175 kPa, which represents the bottom edge of the focused region in Figure 3A for particles with diameters of 100 and 200 nm, the extent of focusing was also small (i.e., FWHM = 132.2 and 78.7  $\mu$ m, respectively; Figure 3B, bottom).

**Figure 4** shows a quantitative comparison of the experimental and theoretical equilibrium FWHM of 100 and 200 nm gold particles. Theoretical distributions for 100 nm (Figure 4A–D) and 200 nm (Figure 4I–L) gold particles exhibited qualitative agreement with experimental distributions

(Figure 4E–H,M–P) for the pressure amplitudes investigated (i.e., 87.5–700 kPa). The percent difference between the model predictions and the experimental results in Figure 4 is between 20% and 50%, and is summarized in Figure S2 (Supporting Information). The experimental values for the FWHM of 100 and 200 nm particles were consistently larger than those predicted by our model (see Figure 4Q,R). We attribute this discrepancy to the neglect of forces beyond the primary acoustic radiation forces and Brownian motion considered by the model.<sup>[6,28,38,21,39–41]</sup> Given that we did not observe convection



during the experiments, we hypothesize that the larger FWHM observed in the experiments is due to electrostatic repulsion between the nanoparticles. Indeed, we will show later that when this electrostatic repulsion is removed and the nanoparticles aggregate, the nanoparticles focus to an extent greater than that predicted by the model.

Next, we investigated the kinetics of acoustic focusing by measuring the FWHM centered on a pressure node over time using 200 nm gold particles subject to a pressure amplitude of 350 kPa. Assuming a uniform distribution of particles at t = 0, we also solved the distribution p(x, t) in Equation (3) for the relevant system parameters (Figure 5A-C) and computed the FWHM as a function of time. The results are compared with experimental FWHM values obtained from triplicate measurements of darkfield images acquired at regular time intervals from a fixed region of the chamber (Figure 5D-F). As expected, the concentration of gold nanoparticles at the nearest pressure node increased with time until  $\approx 2t_c$  (34 s) was reached (Figure 5G), and the experimental results generally agreed with theoretical predictions. The particles in the experiment appear to concentrate slightly faster than what the model predicts (Figure 5G). The percent difference between the theoretical predictions is as high as ≈23%, and is summarized in Figure S2 (Supporting Information). Again, this discrepancy may be due to the fact that the simple analytical model does not include electrostatic repulsion. Given that no interparticle forces are included in the model, the agreement between the model and experimental results is remarkably good, suggesting that Brownian motion and the acoustic radiation force are the dominant factors that determine the degree and rate of nanoparticle focusing.

To overcome the limits of acoustic focusing presented by Brownian motion as well as by the design constraints (e.g., the maximum pressure amplitude that could be achieved within our device was 700 kPa), we developed a UV-light-activated aggregation technique to enable the concentration of nanoparticles beyond the theoretical limits described above. Adding a photoacid, pyranine, to the colloidal suspension increases the acidity of the solution upon exposure to UV light,  $^{\left[ 42\right] }$  which brings the surface charge of the nanoparticles past their isoelectric point.<sup>[10]</sup> The elimination of electrostatic repulsion caused the nanoparticles to aggregate, which in turn reduced their Brownian motion and allowed them to focus to a greater extent than is possible for the individually dispersed nanoparticles. In addition, since the magnitude of the primary acoustic radiation force is proportional to the volume of the particle,<sup>[5]</sup> larger aggregates will experience a larger acoustic radiation force, which results in a smaller FWHM. Analysis of scanning electron microscopy images of aggregates produced during UV-induced aggregation for 200 nm nanoparticles showed that the aggregates range in diameter from 212 nm to 1.95 µm, with a mean diameter of 596 nm (Figure S3, Supporting Information). At a pressure amplitude of 700 kPa, UV irradiation reduced the FWHM from  $32.2 \,\mu m \pm 2.1 \,\mu m$ to 12.3  $\mu$ m  $\pm$  1.3  $\mu$ m for 200 nm particles, from 121.9  $\mu$ m  $\pm$ 7.3  $\mu m$  to 60.5  $\mu m$   $\pm$  7.2  $\mu m$  for 80 nm particles, and from 141  $\mu m$   $\pm$  9.2  $\mu m$  to 105.5  $\mu m$   $\pm$  5.5  $\mu m$  for 60 nm particles, respectively, once equilibrium was reached (Figure 6A-F). Note that the 200 nm particles in Figure 6A appear brighter than the 80 or 60 nm particles because their larger size results in greater light scattering from the particles. These results indicate that the



**Figure 5.** Kinetics of acoustophoresis of 200 nm gold particles. Theoretical distribution of gold nanoparticles after: A) 0 s, (B) 30 s, and (C) 60 s of exposure to a bulk acoustic standing wave with a pressure amplitude of 350 kPa. White shading denotes regions of particle concentration. Experimental images of gold particles after: D) 0 s, (E) 30 s, and (F) 60 s of exposure to an acoustic standing wave. The contrast of images (D–F) was enhanced to better visualize the particle concentrations. All FWHM measurements were performed on unmodified, raw darkfield images. All scale bars are 40  $\mu$ m. G) Dynamical evolution of FWHM after exposure to an acoustic standing wave.



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**Figure 6.** UV-induced aggregation for enhanced acoustic focusing. Darkfield images of 200, 80, and 60 nm gold nanoparticles focused by an acoustic standing wave with a frequency of 3.06 MHz and a pressure amplitude of 700 kPa. A–C) Images of gold nanoparticles focused to an equilibrium FWHM and (D–F) after UV irradiation of the same nanoparticles. Each FWHM is the average of three measurements per experiment. All scale bars are 50 μm.

UV-induced aggregation of nanoparticles increases the concentration of nanoparticles within the acoustic node beyond what can be achieved by acoustophoresis alone. The corollary is that if the degree of nanoparticle focusing achieved in an experiment is beyond what our model predicts, then nanoparticle aggregation has likely occurred, as we discuss below.

To generalize our findings to the acoustophoresis of nanoparticles made of materials other than gold, we calculated the pressure amplitude necessary to focus nanoparticles of various sizes and acoustic contrast factors (**Figure 7**). The diagonal lines in Figure 7 show the acoustic pressure required to focus nanoparticles to an FWHM value equal to or smaller than 1/8 of the acoustic wavelength,  $\lambda_{aw}$  ( $\lambda_{aw}/8 = 60.5 \ \mu m$  in this work). There is no clear definition of focused versus unfocused particles as the degree of focusing is a continuum, so the choice of 1/8 of the acoustic wavelength is a somewhat arbitrary reference point. The vertical lines show the values of the acoustic contrast factor for polystyrene, silica, and gold, illustrating that smaller nanoparticles can be focused as the acoustic contrast factor increases.

To test these generalized predictions, we applied an acoustic standing wave with an amplitude of 700 kPa to polystyrene, silica, and gold nanoparticles, spanning three orders of magnitude of  $\Phi$  in water. The theoretically predicted diameters at which particles can no longer be concentrated to a FWHM of 60.5 µm were found to be 205, 95, and 70 nm for polystyrene, silica, and gold, respectively. We examined three different diameters for each type of particle, 200, 300, and 500 nm for polystyrene; 100, 200, and 300 nm for silica; and 80, 100, and 200 nm for gold. These sizes were chosen to observe the change in FWHM as the particle size approached the theoretical limit of focusing. As predicted by the model, limited focusing was observed for particles near the theoretical limit. Polystyrene particles with diameters of 200 nm, silica particles with diameters of 100 nm, and gold particles with diameters of 80 nm focused to FWHM values of 172.4, 160.7, and 121.0 µm, respectively. Thus, the model roughly predicts the cutoff sizes at which particles of these different compositions can no longer be focused.

Raeymaekers et al. previously reported the acoustic focusing of 5 nm diamond particles with bulk acoustic waves using a similar device, which produced a maximum pressure amplitude of 502 kPa,<sup>[21]</sup> yet our model indicates the minimum size of diamond particles that can be focused (i.e., to an FWHM of 60.5  $\mu$ m, corresponding to  $\lambda_{aw}/8$ ) with such a device is 70 nm. This finding, in conjunction with our UV-induced particle aggregation work, suggests that Raeymaekers et al. likely focused "aggregates" of diamond particles rather than individually dispersed particles. Indeed, they mentioned that the diamond nanoparticles they used may not have been uniformly dispersed prior to application of an acoustic field. After applying our image analysis procedure to the image of diamond particles focused after equilibrium, we estimated an FWHM of 100 µm was obtained in Figure 2a of the report by Raeymaekers et al.<sup>[21]</sup> Our model indicates this FWHM would be consistent with the focusing of 100-120 nm diamond particles at a pressure amplitude of 500 kPa. The report states that the diamond nanoparticles were suspended in water without a surfactant, and other work has shown that diamond nanoparticles form stable aggregates with diameters of 100-200 nm under such conditions, even after extensive sonication.<sup>[43]</sup> Although the images in the paper by Raeymaekers et al. are not of sufficient magnification to distinguish between individual particles and that of aggregates, only focusing of the latter would be possible given the insights of our theoretical model and experimental results.

## 3. Conclusions

This work aimed to clarify the extent to which nanoparticles of a given size and composition can be concentrated by bulk acoustic standing waves in a liquid by developing a simple theoretical model for predicting their acoustophoresis and validating it through experimentation. The model gives a prediction for the







**Figure 7.** Minimum pressure amplitude to focus nanoparticles of various size and composition. A) Plot showing the minimum pressure amplitude required to focus nanoparticles to an FWHM of 60.5  $\mu$ m ( $\lambda_{aw}/8$ ) as a function of their acoustic contrast factor. The operational window of our device, i.e., up to a pressure amplitude of 700 kPa, is represented by the white region. B) Experimental FWHM for particles focused with a pressure amplitude of 700 kPa. The theoretical limit refers to the smallest diameter particle that can be focused to an FWHM of 60.5  $\mu$ m ( $\lambda_{aw}/8$ ) with a pressure of 700 kPa.

degree of nanoparticle focusing (as measured by the FWHM of a focused line of nanoparticles) as a function of acoustic contrast factor, applied pressure, nanoparticle size, and time. As expected, the FWHM decreased with increasing size, pressure, and acoustic contrast factor. The predictions of the model matched with the observed time-dependent and equilibrium focusing of colloidally stable gold nanoparticles and established that the smallest individually dispersed gold nanoparticles that can be focused to an FWHM of  $\lambda_{aw}/8$  at 700 kPa are 70 nm in diameter. As gold has one of the highest acoustic contrast factors in water, this is essentially a lower limit for the diameter of dispersed nanoparticles that can be focused by primary radiation forces from a bulk acoustic standing acoustic wave with the pressure amplitudes described herein. Importantly, our theoretical model allowed us to extend these results to determine the minimum nanoparticle size that can be concentrated for a given acoustic contrast factor,  $\Phi$  (i.e., for a given nanoparticle and fluid composition). Experimental tests of these predictions with silica and polystyrene nanoparticles in water showed fair

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agreement with the theory, albeit with some discrepancy due to the fact that the model does not include interparticle forces such as electrostatic repulsion. Further, this model allows for the prediction of the sizes and compositions of particles that can be focused at pressure amplitudes higher than those used in this study. Finally, we demonstrated that UV-induced aggregation of nanoparticles could be used to focus nanoparticles to a smaller FWHM than what the model estimates.

Given the lack of predictive models for acoustic manipulation of nanoparticles, the results reported here are an important step toward the rational design of acoustic systems for the separation, concentration, and assembly of nanoparticles. This model explicitly excluded acoustic streaming, interparticle forces, and secondary radiation forces to retain the simplicity of the model and avoid the need for simulation. In addition, we worked within a regime in which streaming did not occur and secondary radiation forces are negligible. The inclusion of these forces in a future model would be increasingly important at the high acoustic pressures necessary to focus smaller nanoparticles. Such theoretical development would facilitate the development of improved systems for the acoustic manipulation of nanoparticles.

#### 4. Experimental Section

Device Fabrication and Experimental Setup: The acoustic focusing chamber was constructed from transparent acrylic (Plexiglas, McMaster-Carr, Co.) that was laser-cut into a square frame with an inner dimension of 31 mm  $\times$  31 mm, a wall thickness of 5 mm, and a height of 6 mm. Two PZT transducers (30 mm  $\times$  10 mm  $\times$  1 mm, 841-WFB, with a resonant frequency of 2.91 MHz, APC International) were fixed onto two opposing inner chamber walls using cyanoacrylate adhesive (Elmer's Products, Inc.). Electrical leads were soldered onto the transducers in a manner that prevented contact with the liquid inside of the chamber. Finally, the acrylic chamber was glued onto a 1.0 mm thick glass substrate (Nexterion Glass B, Schott, AG) using a silicone adhesive. The transducers were actuated with an AC signal from a waveform generator (AFG 3022C, Tektronix), amplified by a power amplifier (25A250AM6, Amplifier Research) to generate 125 linear pressure nodes within the chamber. Although the resonant frequency of the PZT transducer is 2.910 MHz, several preliminary experiments were performed to find the optimal resonance of the chamber. It was found that 3.062 MHz produced the narrowest FWHM at any given applied acoustic pressure. Gold nanoparticles were analyzed with a darkfield microscope (BX51, Olympus) fitted with a 20x objective (UPlanFL N, 0.50 numerical aperture, Olympus) and a camera (SC30, Olympus). Images were acquired using a constant exposure time of 4.5 ms. For each experiment, 1 mL of the stock nanoparticle suspension (undiluted) was pipetted into the chamber, the particles were allowed to settle under gravity for 15-30 min, and the microscope was focused on the settled layer of nanoparticles. This time was not sufficient to allow all of the particles to settle, especially for gold nanoparticles 100 nm or smaller. This partial settling process was done to facilitate the consistent focusing of the microscope along the bottom of the chamber, so that the plane of view was consistent across all experiments. This settling procedure helped to improve the image quality and thus the experimental consistency. Further, it was not found to influence the resulting FWHM measurements.

Determination of Amplified Drive Voltage: The amplified value of drive voltage supplied to the two PZT transducers determines the pressure amplitude generated within the chamber. To determine this drive voltage, the transducers were actuated at 3.062 MHz (corresponding to 125 half-wavelengths for our chamber dimensions 30.4 mm x 31 mm),

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the peak-to-peak drive voltage ( $V_{pp}$ ) was tuned in the waveform generator from 0.25 to 1.5  $V_{pp}$ , and the signal was read after amplification by a power amplifier using an oscilloscope.

Conversion of Amplified Drive Voltage to Applied Acoustic Pressure: In our previous work,<sup>[8]</sup> calibration experiments were performed using a hydrophone to determine the pressure amplitude generated within the same acrylic chamber, with PZT transducers actuated at 3.062 MHz. Assuming a linear relationship between the applied drive voltage and acoustic pressure, the previous results were extrapolated to convert the values of applied voltage to pressure amplitude generated within the chamber, which is given by

$$P = 7 \cdot V_{\rm pp} G \tag{10a}$$

Here, *P* is the acoustic pressure amplitude (kPa),  $V_{pp}$  is the applied drive voltage (V), and *G* is the amplifier gain (unitless). By inserting the value of voltage gain (i.e., 50) into Equation (10a), the following was obtained

$$P = 350 \cdot V_{\rm pp} \tag{10b}$$

Model to Predict Gold Nanoparticle Focusing: The dimensionless Smoluchowski equation was numerically solved as a Fourier series<sup>[37]</sup> using MATLAB (MathWorks, Inc.), and the FWHM as a function of time was then obtained over a duration of 1000 s with a resolution of 1 s. The assumptions of our model include: (1) a uniform distribution of particles inside the chamber at time t = 0 and (2) acoustic radiation forces and Brownian motion are the dominating forces governing the dynamics of nanoparticle movement (i.e., other factors were ignored). The equilibrium state was characterized by the Boltzmann distribution.

Equilibrium Time for Focusing Gold Nanoparticles: The equilibrium focusing of gold nanoparticles was characterized by measuring the FWHM of the distribution from the gray scale intensity for experimental snapshots. ImageJ was used to segment each image into rectangular regions of interest, each 40  $\mu$ m x 242  $\mu$ m. For each segment, the gray scale intensity was averaged over rows of pixels and then the distribution of gray scale intensities was fitted to a Gaussian distribution to locate the peak of the distribution. To eliminate the error caused by slight meandering of the particles line, segmentations were aligned using the image rotation tool so that the lines were 90° to the horizontal before obtaining the average of the gray scale intensity distribution of the raw image. This distribution was then subtracted by its lowest value to set the baseline to zero. Then the peak intensity value, the half-maximum, and its corresponding x-coordinates, from which the equilibrium FWHM was calculated, were determined. This procedure was repeated for each image, the mean and standard deviation from images acquired for each particle size and each acoustic pressure were computed, and those values along with the values predicted by our model were plotted.

Acoustophoresis of 200 nm Gold Particles: The kinetics of particle focusing was studied using 200 nm particles at pressure amplitudes of 87.5, 175, and 350 kPa. The model was used to predict the variation of the FWHM of the pressure node of gold nanoparticles as a function of time over the entire duration of acoustic manipulation. The camera was programmed to acquire frames at regular intervals, which varied depending upon the pressure amplitude (typically 1 s for 350 kPa and 5 s for 87.5 kPa), until equilibrium conditions were reached, with a delay of 5 s prior to acoustic actuation. The kinetics of particle concentration was characterized by measuring the FWHM of sequentially focused gold nanoparticles from ten frames for each pressure amplitude, and from a 381  $\mu$ m × 242  $\mu$ m region of interest in each frame. The experiments were repeated three times for each pressure amplitude, and the variation of FWHM with time for each experiment was plotted. The data analysis from the images was the same as for equilibrium experiments.

UV-Activated Particle Aggregation: To increase the concentration of particles in the acoustic focusing chamber, the particles were suspended in an aqueous solution of  $1 \times 10^{-3}$  M pyranine (Tokyo Chemical Industry, Japan). After the nanoparticles were focused to equilibrium, the sample was exposed to 365 nm UV light with a power density of 36 mW cm<sup>-2</sup> for 10 s. Darkfield images were taken before and after UV activation.

*Materials*—*Gold Nanoparticles*: The gold nanoparticles used in this study were spherical with diameters of 50, 60, 80, 100, and 200 nm (corresponding to G-50-100, CG-60-20, CG-80-20, G-100-100, and G-200-100, Cytodiagnostics) with coefficients of variance <10%. The nanoparticles were used in the concentrations supplied by the vendor,  $3.51 \times 10^{10}$ ,  $1.96 \times 10^{10}$ ,  $3.51 \times 10^{10}$ ,  $7.8 \times 10^9$ , and  $1.91 \times 10^9$  particles per milliliter for the 50, 60, 80, 100, and 200 nm particles, respectively, in suspensions of  $0.1 \times 10^{-3}$  M phosphate buffered solution stabilized with citric acid. These numbers correspond to volume fractions of  $2.30 \times 10^{-6}$ ,  $2.23 \times 10^{-6}$ ,  $2.10 \times 10^{-6}$ ,  $2.01 \times 10^{-5}$ , and  $8.02 \times 10^{-6}$  and weight fractions of  $4.45 \times 10^{-5}$ ,  $4.03 \times 10^{-5}$ ,  $4.06 \times 10^{-5}$ ,  $3.89 \times 10^{-5}$ , and  $1.55 \times 10^{-4}$ . Prior to each experiment, particles were agitated in an ultrasonic bath (Branson 2510) for 30 min to disperse aggregates that may have formed during storage.

*Materials—Polystyrene Nanoparticles*: The polystyrene nanoparticles used in this study were spherical with diameters of 200, 300, and 500 nm (corresponding to Polyspherex Polystyrene 200, 300, and 500, respectively) with coefficients of variance <10%. The concentration of nanoparticles was diluted to a volume fraction of  $5.3 \times 10^{-6}$ . The particles were stored in a solution provided by the manufacturer containing deionized water, a proprietary surfactant, and  $2 \times 10^{-3}$  M of sodium azide. Prior to each experiment, particles were agitated in an ultrasonic bath (Branson 2510) for 30 min to disperse aggregates that may have formed during storage.

*Materials—Silica Nanoparticles*: The silica nanoparticles used in this study were spherical with diameters of 120, 200, and 300 nm (corresponding to NanoXact 120, 200, and 300 nm, respectively) with coefficients of variance <10%. The concentration of nanoparticles was diluted to a volume fraction of  $5.3 \times 10^{-6}$ . Prior to each experiment, particles were agitated in an ultrasonic bath (Branson 2510) for 30 min to disperse aggregates that may have formed during storage.

#### **Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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#### **Conflict of Interest**

The authors declare no conflict of interest.

#### **Keywords**

acoustic focusing, acoustic radiation forces, acoustophoresis, bulk acoustic standing waves, gold nanoparticles, particle concentration

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