

**FINAL REPORT**

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**EVALUATION OF ADVANCED LIGHT SCATTERING  
TECHNOLOGY FOR MICROGRAVITY EXPERIMENTS**

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## 1. INTRODUCTION

The objective of this program was to evaluate the capabilities of modern light scattering equipment and the uses it might have in studying processes in microgravity. Initially an extensive series of tasks were to be undertaken, but the original program had to be reduced in scope because of budget and time curtailment. While the early stages of the research followed the schedule closely, when informed of the imminent reduction in available time and funds the work concentrated on the resolution of polydisperse systems. This choice was made since a major use of light scattering was expected to be the study of crystal growth of macromolecules in low gravity environments.

UAH was to evaluate a modern photon correlation spectrometer, while UAB was to evaluate a Mie spectrometer. The results obtained at UAH with a Brookhaven Instruments photon correlation spectrometer will be discussed first, followed by a report submitted by UAB on evaluation of a Wyatt Dawn multi-detector instrument.

## 2. BROOKHAVEN SPECTROMETER AND SUBSIDIARY INSTRUMENTATION

The Brookhaven Instrument Corporation (BIC) model BI-200SM light scattering goniometer was installed on a four by eight foot optical table. A four by five foot annex was built over one end of this table to carry the Spectra Physics model 2020/11 krypton laser, the model BI-2030AT digital correlator, two BIC high voltage photomultiplier power supplies, a reference photomultiplier and housing, a gear pump and filter for the index matching fluid, a Newport model 815 digital power meter, and a Spectra Physics Corporation temperature controller model 482 for the model 583 oven-controlled etalon. This two-level arrangement is shown in figure 1. At approximately the same height as the annex table a Neslab circulating thermostat was installed on a separate wall shelf to isolate the goniometer from the vibration of the thermostat's compressor. Insulated tygon tubing was used to convey the thermostat fluid to the heat exchange coils in the scattering chamber. A major advantage of this two-level arrangement is that all the necessary connections from the accessory equipment are kept off the optical table and those that attach to the rotatable detector swing freely, rather than drag over the table. The spectrometer and cable arrangement is shown in figure 2. While this arrangement is convenient for homodyne measurements, it probably would introduce too much vibration for heterodyne experiments even though the annex table is extremely sturdy.

The system as supplied by BIC was modified in three ways: The index matching fluid was changed from the usual toluene to tetrahydronaphthlene (decalin) for a better index match to the sample cells as discussed earlier in a semiannual progress report. The peristaltic pump supplied by BIC was exchanged for a gear pump to reduce the time required for filtering the index matching fluid. A heat exchanger was built around the photocathode end of the photomultiplier case to keep the photocathode at the temperature of the scattering chamber. This last modification was necessary because the temperature control of the laboratory in which this equipment is located is very poor. During the course of a few hours the dark count would vary by factors of three or four. Thermostating the photomultiplier reduced the dark count variation to a few counts. In any light scattering laboratory airborne particulates are a problem, in this laboratory they were disastrous because the ceiling was cellotex. This problem was reduced to a manageable degree by enclosing the spectrometer with vinyl sheets hung from the sides of the annex table and putting a small electrostatic air filter inside this tent.

The optical path of the spectrometer is shown in figure 3. The etalon limits the emergent laser beam to single mode (100) and the combination of a rotatable half-wave plate and polarizing cube provide an variable attenuator which produces a vertically polarized beam. The beam is then split by a non-polarizing beamsplitting cube for measurement of the power incident on the scattering sample. This configuration provides a well polarized beam whose intensity can be varied without changing the laser power. The power incident on the sample was stable to within 1 mW for periods of 10 hrs. The major source of power instability was large variations of room temperature.

The remainder of the system, as shown, is the standard BIC optics. Because BIC had difficulties in supplying a 647.1 nm laser line filter the actual detector system was modified by inserting a 647.1 nm filter between the adjustable aperture and the first lens of the detector and using the no-filter position of the filter wheel.

When the solvent suspending the scattering particle differs from pure water significantly it is necessary to determine its viscosity and refractive index. A J & L Instruments model FNV-200 falling needle viscometer and a Milton-Roy Abby refractometer were used to measure these properties.

### 3. EXPERIMENTAL

#### 3.1 STANDARD SUSPENSIONS

For the evaluation of the BIC spectrometer suspensions of polystyrene spheres were obtained from Duke Scientific Corporation. These particle size standards are certified for mean diameter and traceability to the National Bureau of Standards (National Institute of Standards and Technology). In this work the primary interest was in the nanometer size range and a set of suspensions containing spheres from nominal diameters of 20 to 800 nm were used. The properties of the suspensions are given in table I.

Photon correlation spectroscopy (PCS) measurements require these suspensions to be diluted to concentrations suitable for light scattering measurements. Dilutions were made using Hamilton Company micro-syringes of appropriate size to remove the standard suspension from its dropping bottle. A measured volume of sphere-containing suspension was injected into a known volume suspending solution. Suspending solutions consisted of 0.001M KCl or NaCl, purified by ion exchange, made with type-1, biological grade water to which sufficient sodium dodecylsulfate was added through a 0.22 micron filter to produce a 1 ppm solution. The highest concentration suspension was prepared first and subsequent lower concentrations were prepared from it by dilution.

Suspensions prepared as described are stable for long periods and over normal temperature ranges for particle with diameters of 800 nm or less. Larger particles coagulated after the suspensions aged. Ultrasonification has been recommended as a means of dispersing hydrophobic suspensions. On properly prepared suspensions of small particles we have seen no effect.

Table I. Standardized Particles from Duke Scientific Corporation, Polystyrene Spheres

SIZE (nm)	N/ml	STD. DEV. (nm)	%SOLIDS	LOT BATCH	CERT. DATE PACK. DATE
21+/-1.5	1.9E15	NOT SPECIF	2	8948 3020-001	7/01/87 6/24/88
40+/-1.3	5.7E14	NOT SPECIF	2	9166 3040-001	7/01/87 9/14/88
63+/-2.8	1.4E14	NOT SPECIF	2	8050 3060-001	7/01/87 6/24/88
83+/-3.2	6.6E13	NOT SPECIF	2	9256 3080-001	7/01/87 10/10/88
107+/-3.0	3.0E13	4.3 (4%)	2	9329 3100-001	7/01/87 10/26/88
220+/-6.0	3.4E12	3.5 (1.6%)	2	9257 3200-001	7/01/87 10/10/88
298+/-3.0	1.4E12	4.3 (1.5%)	2	9150 3300-001	7/01/87 9/02/88
398+/-4.0	5.8E11	5.5 (1.4%)	2	9260 3400-001	7/01/87 10/10/88
798+/-7.0	7.2E10	7.0 (1%)	2	9332 3800-001	7/01/87 10/27/88
1040+/-21	1.9E10	12 (1.2%)	0.63	9915 4010-001	9/22/86 5/11/89
2062+/-25	1.0E8	22 (1.1%)	0.48	10066 4202-002	10/16/86 6/19/89
3983+/-30	1.0E8	70 (1.8%)	0.35	9759 4204-001	10/14/86 3/17/89
9870+/-57	6.0E6	80 (0.8%)	0.32	10004 4210-003	3/03/89 6/05/89
19580+/-100	1.0E6	300 (1.5%)	0.44	9952 4220-003	11/11/87 5/19/89
29400+/-120	4.0E5	150 (0.5%)	0.49	9968 4230-005	12/05/88 5/24/89

On aged suspensions of large particles we have observed mixed results, sometimes coagulation, sometimes dispersion. In general the Duke Scientific Co. standards were found reliable with two exceptions. One 20 nm vial was contaminated with visible fibers. A 60 nm sample was obviously mislabeled because it did not yield the assigned diameter. Considering the large number of standards and replacement vials obtained from Duke Scientific two faulty vials did not seem excessive. However, some caution is required in using such standards.

### 3.2 BASIC PARTICLE MEASUREMENTS

The theory and practice of PCS have been thoroughly discussed<sup>1-8</sup>. Hence, we will give here only a brief discussion with emphasis on its implementation in the BIC instrument. A convenient, fully detailed discussion has been given by Schatzel<sup>8</sup> on whose article this discussion is based.

In light scattering studies based on Brownian motion, such as the ones performed here, the amplitude correlation function  $G_1(t)$  is given by

$$G_1(t) = \langle A(0)A^*(t) \rangle = N \langle |A|^2 \rangle \langle \exp[iq \Delta x_j(t)] \rangle, \quad (1)$$

neglecting any particle interactions and assuming a monodisperse suspension. The absolute single particle amplitudes are assumed to be slowly varying, thus the phase averages can be performed separately. In equation (1)  $G_1(t)$  depends on the time lag,  $t$ , through the average particle displacement  $x(t)$  only. Einstein found the average particle displacement to be normally distributed with variance or as usually written

$$\langle \Delta x(t)^2 \rangle = 2Dt. \quad (2)$$

This is valid when the lag time is large compared to the hydrodynamic relaxation time. Taking the Fourier transform into  $q$ -space of the Gaussian displacement density gives the familiar expression

$$G_1(t) = N \langle |A|^2 \rangle \exp(-q^2Dt), \quad (3)$$

where  $q = [4\pi n \sin(\theta/2)]/\lambda_0$ . From equation (3) the diffusion coefficient,  $D$ , can be obtained. This exponential correlation corresponds to a Lorentzian spectrum.

Knowing  $D$ , the diameter,  $d$ , of the diffusing particle can be estimated using the Stokes-Einstein relation,

$$D = kT/(6\pi\eta d), \quad (4)$$

if the temperature,  $T$ , and the viscosity,  $\eta$ , of the solvent are known. Typically diameters from several nm to a few  $\mu\text{m}$  or band widths from 1 Hz to 1 MHz can be observed.

While the amplitude or first order correlations are the simplest to discuss, all experimental measurements are based on intensity measurements which yield the second-order correlation functions,  $G_2(t)$ . This can be expressed by

$$G_2(t) = \langle A(0)A^*(0) A(t)A^*(t) \rangle = \langle I(0)I(t) \rangle. \quad (5)$$

When a large number of particles is in the scattering volume this simplifies to the Siegert relation<sup>9</sup>

$$g_2(t) = G_2(t) / \langle I \rangle^2 = 1 + |g_1|^2 \quad (6)$$

which holds for Gaussian amplitude statistics. The lower case  $g$  denotes normalized correlations. For identical, non-interacting particles equation (6) also gives a single exponential decay which may be written as

$$g_2(t) = 1 + \exp(-2q^2Dt). \quad (7)$$

Note that the intensity correlation decays faster than the amplitude correlation by a factor of 2 and contains an additional background term.

In actual experimental measurements the coherence at the detector is finite and the exponential decay term must be reduced by a factor,  $b$ . The value of  $b$  depends on the optical characteristics of the instrument.

When the solvent contains independent particles of several sizes the normalized intensity correlation function can be written as

$$g_2(t) = 1 + \sum b_i \exp(-2q^2D_i t) \quad (8)$$

Such a sum of exponentials is ill-conditioned. It can only be approximated numerically. If the scattering system contains asymmetric particles equation (8) will contain similar terms for rotational diffusion. These are not of interest in this work, fortunately, as homodyne PCS is not the best method for their study.

In practice it is more convenient to count photons than measure intensities, and all modern PCS instruments do so. For monodisperse systems  $G_2(t)$  is linearized by taking the logarithm of equation (8), which gives

$$\ln [C_m(m \ t) / B - 1] = \ln b^{1/2} - 2q^2D(m\Delta t). \quad (9)$$

The notation has been changed to better represent the quantities the instrument measures. Here  $C_m$  is the count in channel  $m$ ,  $m\Delta t$  is the product of channel number and the sample time (lag time), and  $B$  is the value of the correlation function at very long time after the starting time, that is, it corresponds to the average intensity squared.

The sample time should be chosen such that  $G_2(t)$  decays completely,  $(dC/dm) = 0$  as  $m$  approaches the maximum number of channels in the correlator,  $M$ , so that the curvature is spread over as many channels as possible. When the particle size is known the sample time can be estimated by calculating  $D$  from the Stokes-Einstein relation (4), then  $t = 1/MDq^2$ . The normalization factor  $B$ , called a baseline, is provided by the BIC correlator as a measured value equal to the average of eight

channel delayed to the equivalent of channels 1024 to 1031, and a calculated baseline equal to the correlation function extrapolated to infinite time. Comparison of the measured and calculated baselines provides a good indicator of the cleanliness of the sample. The fluctuation due to a large particle will decay slowly. The measured baseline will be larger than the calculated baseline. In a satisfactory measurement these should be within 1% or less of each other. Figure 4 illustrates two correlation functions for 40nm and 220nm particles and that of a mixture of them.

The BIC correlator/computer has five data reduction programs. The most elementary is built in with dedicated keys. It fits the logarithm of the correlation function by linear regression to a single exponential and a cumulant fit to the second order. It reports the experimental parameters and the results of these two calculations. The four other programs are software options and used for distribution analysis. These consist of a cumulant fit to the quartic order<sup>10</sup>, a double exponential fit<sup>11</sup>, an exponential sampling program<sup>12</sup>, and a non-negatively constrained least squares program<sup>13</sup> (NNLS).

The application of these analyses to monodisperse suspensions was discussed in the second semiannual report in some detail. Figure 5 illustrates a series of NNLS measurements on standard spheres. The vertical error bars represent the spread in five successive measurements. The horizontal error bars represent the stated uncertainty in the reported diameter. As observed in the earlier report the cumulant analysis gives good agreement with the standardized diameters if a sufficient number of channels are used in the analysis and the duration of the experiment sufficient for the four orders of this calculation to converge. The double exponential analysis will only converge if the correlation function is extremely noise free. Therefore it is not especially useful for short duration measurements of interest in studies of dynamic processes such as encountered in crystal growth. Consistently wider size distributions were produced by the exponential sampling program from samples known to be nearly monodisperse than any of the other analytical methods. The most reliable results were obtained from NNLS analysis. It will be the favored data reduction method in the more advanced studies discussed below.

From the above studies we conclude that PCS is a reliable technique for particle sizing of monodisperse suspensions composed of nearly spherical non-interacting particles provided the suspension is sufficiently dilute that multiple scattering can be neglected. The majority of problems that have been reported are most likely due to poor sample preparation or improper use of the method.

### 3.3 POLYDISPERSE SUSPENSIONS

When the suspension contains particles of several sizes equation (8) describes the system if the particles are spherical and non-interacting. The normalizing factor B of equation (9), in the ideal case, becomes the sum of the square of the average intensities for each size particle and the logarithmic term contains a sum of contrast terms,  $b_i$ , for each particle size  $i$ . The final term contains all the diffusion coefficients,  $D_i$ . It is the latter that must be determined by fitting the correlation function. An additional fluctuation occurs in polydisperse systems that arises from compositional variation of the particle size distribution in the scattering volume. In most measurements on polydisperse systems it is assumed not to vary. This is a reasonable assumption if the the number of particle of each size in the scattering volume is large.



In section 3.2 the data reduction programs supplied with the BIC correlator were discussed. When such programs are developed their ability to resolve a polydisperse mixture is experimentally tested using a suspension made from equal volumes of suspensions of different size particles which have nearly equal scattering intensities. Usually the particles differ by a factor of ten in diameter. In such a mixture the contribution to the baseline  $B$  is equal for each component and the contrast  $b_i$  is most favorable. Thus such mixtures produce the most favorable conditions for the resolution of polydisperse suspensions, but provide no guide to the question of the number concentration,  $\langle C \rangle$ , of scatterers of a given size that can be detected in a particular mixture. This quantity is of prime importance in knowing if dynamic light scattering can be used to control or study processes such as crystal growth.

To gain some knowledge of the numbers of scattering particles in resolvable polydisperse suspensions a series of intensity measurements on suspensions containing a known number of particles of known diameter were made. For a series of suspensions of particles with diameter  $d$  prepared in identical solvents the scattered intensity,  $I_s$ , can be expressed as

$$I_s = (w + s \langle C_d \rangle) P I_0, \quad (10)$$

where  $s$  is the intensity scattered per particle (i.e., a particle cross section)  $\langle C_d \rangle$  is the number of particles per  $\text{cm}^3$ ,  $P$  is an optical factor for the scattering system containing all quantities of both the suspension and instrument that remain constant through the series of measurements, and  $w$  represents the portion of the detected light that is independent of  $\langle C \rangle$ , but not of the incident intensity  $I_0$ , for example flair. If no higher order scattering processes occur the slope of a plot of the scattered intensity vs the number of scatterers will give  $s$ . When  $\langle C \rangle$  varies over several orders of magnitude  $I_0$  must be varied to avoid overflow of the counter while maintaining acceptable counting statistics. Such groups of measurements will be displaced from each other at each  $I_0$  by  $w P I_0$ . If the concentration ranges of each set overlap the displacement can be subtracted from the data by choosing one data set as a reference and adjusting the others to smoothly join the reference set at the overlapping concentrations. The remaining  $w P I_0$  term can be removed by utilizing the fact that  $I_s$  must be zero at  $C=0$ . After these adjustments the data can be refitted by linear regression to obtain the final value of  $s$  and the quality of the fit can be judged from the regression correlation coefficient and the deviation of the intercept from zero at  $\langle C \rangle = 0$ .

A series of suspensions with concentrations usually ranging over several orders of magnitude were prepared by dilution of the Duke Scientific Corporation polystyrene spheres with nominal diameters from 20 to 800nm. The supplier's number concentration was used to calibrate the most concentrated sample and all other samples were prepared by diluting that sample using Eppendorf pipets. Even if the initial calibration was in error the difference between successive samples should contain only the very small dilution errors. An error in the initial calibration will cause an error in the zero intercept on the final regression fitting of the adjusted data. None of the data sets exhibited significant error. Since sample time should not effect the intensity measurements, these measurements were made as though PCS data were being taken (see figure 6). This allows us to compare measured with calculated baselines and the measured size with the standardized size as a means of detecting excessive noise in the measurement. The total number of counts was adjusted for photomultiplier dead time and divided by the duration of the experiment to obtain  $C_{ms}^{-1}$  which is proportional to  $I_s$ . Figure 7 shows raw data for a typical group of measurements on 107nm particles. Each point represents the

average of five successive measurements and the error bars correspond to the 95% confidence limit for the five measurements.

The final results of the matching procedure are shown in figure 8. The slope gives the counts per second per particle. I refer to this quantity as a PCS cross section,  $s$ . It is not equivalent to a formal Rayleigh or Mie cross section, but the terminology is convenient. Such measurements were made for particles from 21nm to 798nm. This size range extends from the Rayleigh into the Mie scattering domains.

Figure 9 presents the final result of the cross section measurements. As a reference, Mie scattering factors are also shown. While these two quantities should not be identical they should resemble one another and they do. These curves clearly show the transition from Rayleigh scattering at the smallest diameters to the Mie region. These cross sections give  $I_s$  as

$$I_s = sNP I_0, \quad (12)$$

where  $P$  contains all the factors of the instrument and suspensions that are constant. The values of  $s$  are given in table 2 along with details of the measurements used to obtain them. These cross sections are for 90 degree measurements. A limited number of similar measurements were made at 30, 45, 60, 120, and 150 degrees for several particle sizes. The ratio of the cross section at a given angle to the 90 degree cross section is very nearly equal to the Mie angular dependence factor. Therefore the Mie angular correction can be applied to the PCS cross section. The number density required to produce equal scattering is given by the relation

$$N_1 s_1 = N_2 s_2. \quad (13)$$

Ratios of 500 21nm particles to one 40nm particle or  $1.14E5$  21nm particles to one 220nm particle would produce equal scattering intensities. Of course the actual number of particles of all sizes in the scattering volume must be sufficient such that compositional fluctuations do not occur.

With these cross-sections relative correlation functions for mixtures of polystyrene spheres can be constructed, then adjusted for  $I_0$  to compare them with measured correlation functions. Figure 10 illustrates the effect of the number concentration on polydisperse correlation functions. These logarithmic plots show that the range of compositions that can be expected to be resolvable into two particle sizes is limited.

An extensive series of solutions of 40nm and 220nm particles was prepared by dilution. Starting with equal scattering solutions, the number fraction of 220nm to 40nm particles was varied in each direction until we were no longer able to resolve the mixtures. Similar, but less extensive measurements were made on 220-21nm and 220-63nm mixtures. The results of these measurements are shown in figures 11 and 12 for 220-40nm mixtures at 90 and 45 degrees, in figure 13 for 220-21nm mixtures, and 220-63nm mixtures in, both at 90 degrees. For all measurements the measured and calculated baselines were within 1%. The data points represent the maximum in the distribution

Table 2. Cross Section Measurements

Diameter d(nm)	Cross Section s(cm <sup>3</sup> )	Correlation Coefficient,r	Particle Density Range (N/cm <sup>3</sup> )	Number of Measurements
21	2.2e-10	0.99755	6.8e10 - 5.7e12	8
40	1.1e-7	0.99848	9.3e10 - 5.7e12	8
63	5.7e-6	0.99942	8.9e9 - 2.1e12	9
82	6.6e-6	0.99825	4.1e9 - 1.3e12	7
107	5.04e-7	0.99735	6.0e9 - 5.9e11	10
220	2.5e-5	0.99498	2.8e8 - 6.7e11	10
298	1.4e-4	0.99837	5.8e7 - 3.1e10	8
343	1.5e-4	0.99978	7.1e6 - 9.0e8	6
398	6.2e-5	0.99996	2.17e6 - 5.7e9	9
460	2.8e-4	0.9912	3.7e8 - 7.4e8	4
502	3.7e-4	0.99744	2.0e8 - 5.8e8	5
597	7.8e-4	0.97798	1.2e8 - 2.1e8	4
705	8.8e-4	0.99307	9.2e7 - 2.0e8	5
798	1.1e-4	0.99962	8.0e6 - 1.1e10	10

produced by the NNLS analysis. The error bars show the half-width of the distribution at approximately half-height.

At 90 degrees the  $220 \pm 6$  particles are reported with the distribution maxima at 215 or 260nm. With one exception the width of the distribution associated with the larger size is greater than that found in the correlation functions that give the nearly correct size. At their extremes the distributions include both sizes. The  $40 \pm 1.3$ nm particles exhibit maxima between 35 and 80nm. These are not correlated with the 220 maxima. The distributions are less broad than those of the larger particle. At 45 degrees the small sized particles are reported with reasonable accuracy, but the 220nm particles show a much greater deviation toward larger sizes than the 90 degree measurements.

The suspensions mixed to form the polydisperse sample were prepared as described previously and the particles measured correctly in the individual suspensions. These data were obtained with relatively short term measurements. Therefore the correlation function is noisier than those obtained in very long term measurements. As expected the noise in the higher channels contributes a larger fraction of the correlation function than in the early channels. This causes the size variation in the large particles that the program reports.

Similar measurements were made on 21-220nm mixtures. These are the usual sizes used in resolution studies with long duration experiments. As can be seen from figure 13, short term experiments with this mixture behave as the 40-220nm mixtures did.

The factors which affect the ability to resolve particles in a mixture is their relative diffusion coefficients as that determines the per channel contribution of the particle to the correlation coefficient. A simple index for this is the ratio  $D_1/D_2 = d_2/d_1$ . For the above mixtures, taking the larger particle to be component 2, these are approximately 10, 5.5, 3.5. All could be resolved within a limited concentration range. Measurements were made on two mixtures in which each component had equal scattering intensity and a diffusion ratio of 2. Such a mixture of 21-40nm particles could frequently be resolved into 17nm and 45nm bands with sample times of 2 and 4 microseconds using a duration of 500 seconds. Similar mixtures of 107-220nm particles could not be resolved with sample times of 10 and 20 microseconds and 500 second durations. The latter produced only a band with a maximum at 177nm and a mean around 167nm.

#### 4. CONCLUSIONS

The Brookhaven Instruments Corporation goniometer is satisfactory for simple light scattering measurements. Its weakest feature is the detector optics. Since the instrument used in these studies was delivered they have made major improvements in the detector optical system. The accessories such as the index filtration pump and the index matching fluid recommended BIC have been changed to those used in this report. The correlator is satisfactory. It is the better component of the instrumentation. While the software contains most current analysis techniques, it seems designed more for industrial applications than research on complex systems, but has many useful features. It would be difficult to modify software to the extent that more versatility was incorporated, such as measurements on nonspherical particles.

The equipment as designed would not be suitable for any kind of mobile experiment, it was not designed for such work. A multidetector design incorporating fibers and other detectors could be made rugged enough to withstand severe vibration, then be used in a noisy environment so long as the sample could be protected from it.

Photon correlation spectroscopy is excellent for simple sizing of nearly monodisperse samples. It does not consistently give true sizes for polydisperse samples in measurement times short enough or over a wide enough concentration range for the study of dynamic processes that have process "rates" that are of the order of 10 to 15min. However it can give useful information on changing systems, through the collective diffusion coefficient. Investigators often over interpret the data. In all cases PCS is extremely sensitive to dirty suspensions or solutions.

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## 6. UAB FINAL REPORT

In light of the initial proposal, we completed the following task: First was the installation of the Wyatt Dawn-B laser light scattering instrument. The accompanying software INSTALL-B, DAWN-B, SKOR-B, CHANLOOK, and PC-LAB and AUORA were installed. The unit was tested using polystyrene spheres which were 1.09 microns and it seemed that the system was operating satisfactorily. Then we proceeded to use lysozyme in the batch mode since the unit could only handle large quantities of solution. All this was reported in the second semi-annual progress report.

It is now ready to be used as soon as we obtain polydispersed samples (through UAH) which have been characterized by using the Brookhaven laser light scattering system.

In an effort to automate the monitoring of protein crystal growth over extended periods of time in the order of days, we utilized the CHANLOOK program. This program has possibilities, but analysis of the raw data obtained by CHANLOOK has proven to be very tedious.

The chamber provided by UAH caused drastic flare into the forward and rearward angles resulting in data which was not usable. However, other methods such as placing a shim under the sample vial allowed us to reduce the sample volume from 15 ml. to 5 ml.

We have not decided on a method for attaining temperature control of the sample during measurement. There is a fear that any gross method of changing the temperature would affect the alignment of the detectors due to expansion or contraction of the read head. Such considerations have been taken in account by the Wyatt and thus insulation and installation of the read head would amount to an added cost of \$10,000.

The unit is presently being installed in my lab in the School of Optometry where we are developing a facility for practically monitoring protein crystal growth by various methodology.

Since an important part of this subcontract was assessment of the DAWN-B, we have concluded the following: (1) The conceptual design of the unit is adequate for making angular dependent intensity measurements rapidly. (2) The compactness of the unit is an advantage when considering the unit for use in a zero-g environment. (3) In principal the software should allow the development of a turnkey system where samples could be placed in the unit and an analysis performed automatically, but in reality the software has been found to contain bugs that Wyatt is working on.

In conclusion, the Wyatt is not at present amenable to monitoring lysozyme crystallization even though it did successfully detect changes in  $I(\theta)$  over time during the amorphous precipitation of lysozyme.

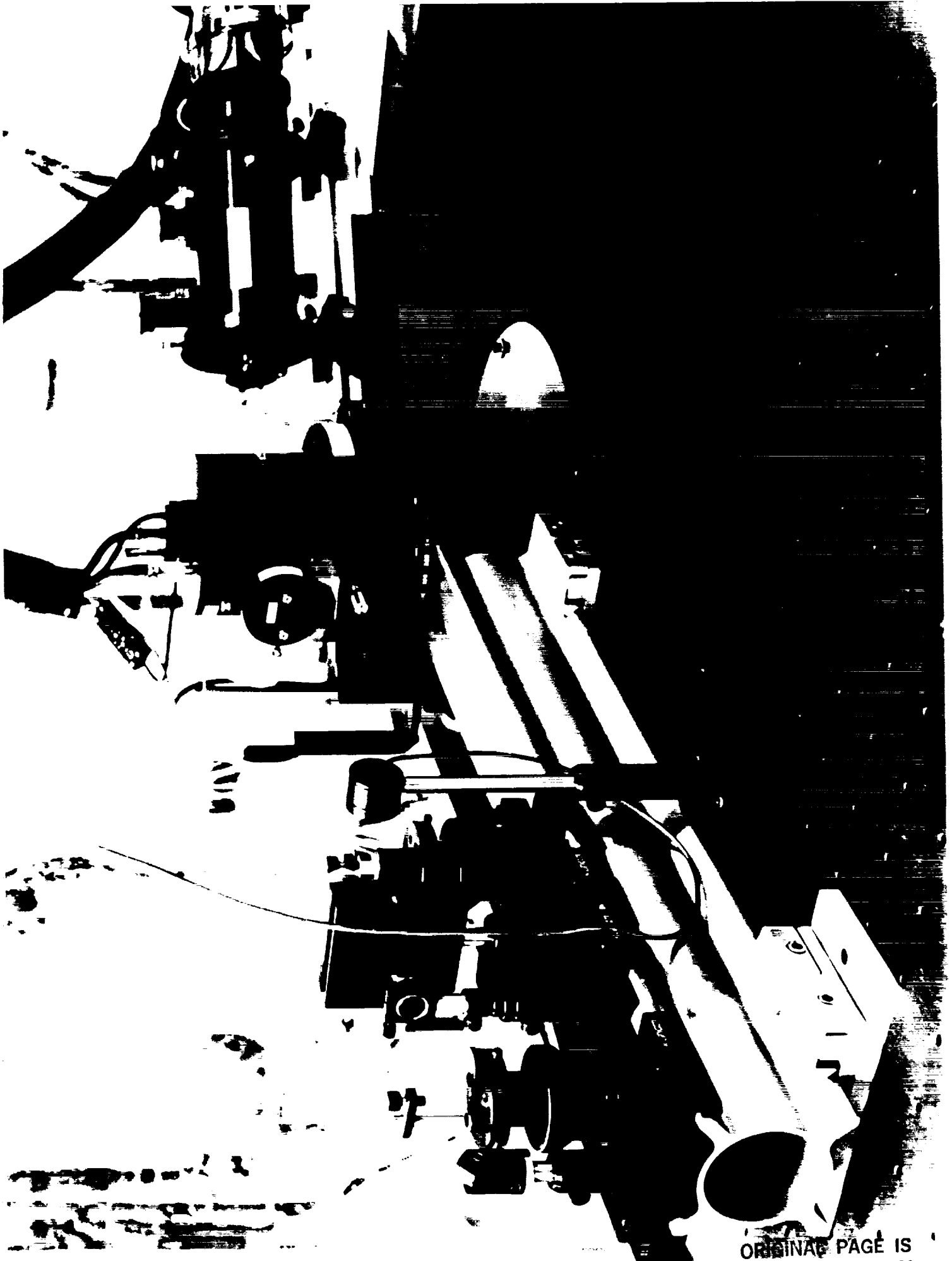
Figure 1. View of apparatus showing the annex table and a portion of the equipment mounted on it. The plastic dust shields are in place causing the apparent distortion of the goniometer and laser power supply.



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Figure 2. The goniometer and input optics. From left to right are the turret mirror, auxiliary beam splitter table, half-wave plate, polarizing cube, non-polarizing beam splitter, power meter detector, focus and steering lenses, scattering cell with stationary connections extending from its top, detector optics, photomultiplier and heat exchanger. Below the photomultiplier a corner of the electrostatic dust filter can be seen. This is not run during measurement.



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Figure 3. The optical path of the photon correlation spectrometer.

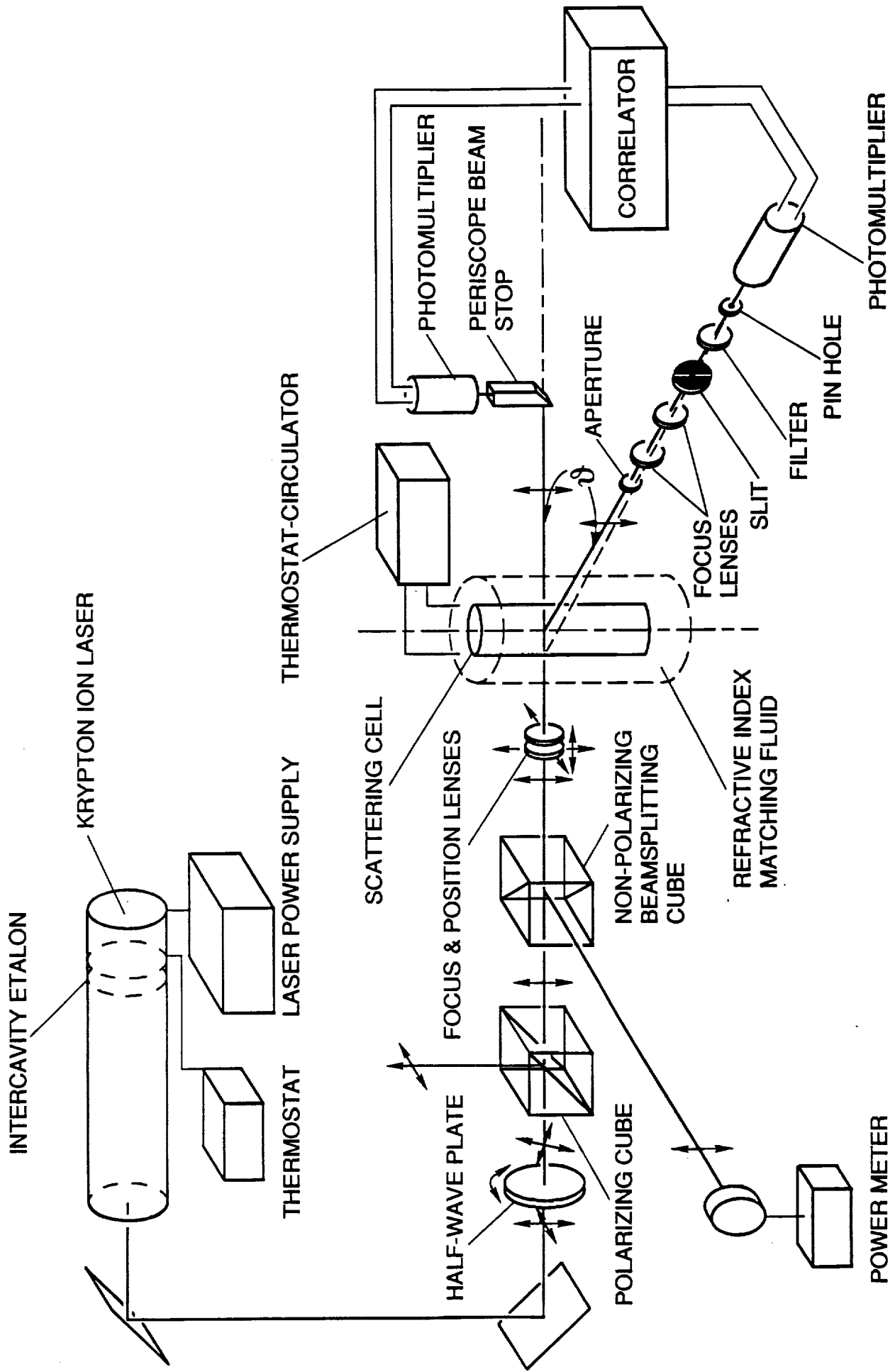


FIG. 3. Optics of photon correlation spectrometer.

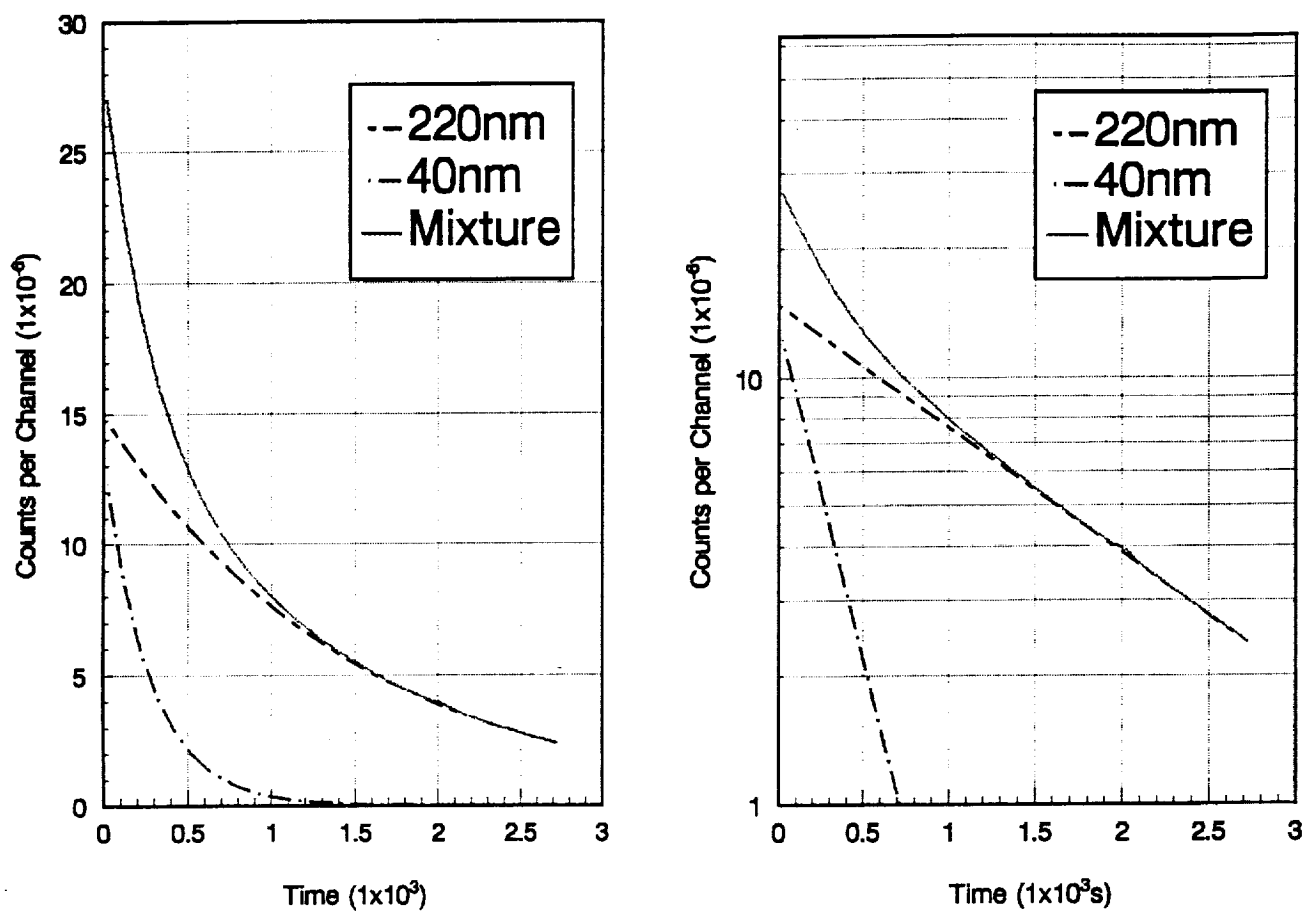


Figure 4. An illustration of correlation functions and their semi logarithmic plot for 40 and 220 nm particles. The solid line illustrates their mixture. A sample time for 40 nm particles was used in this calculation.

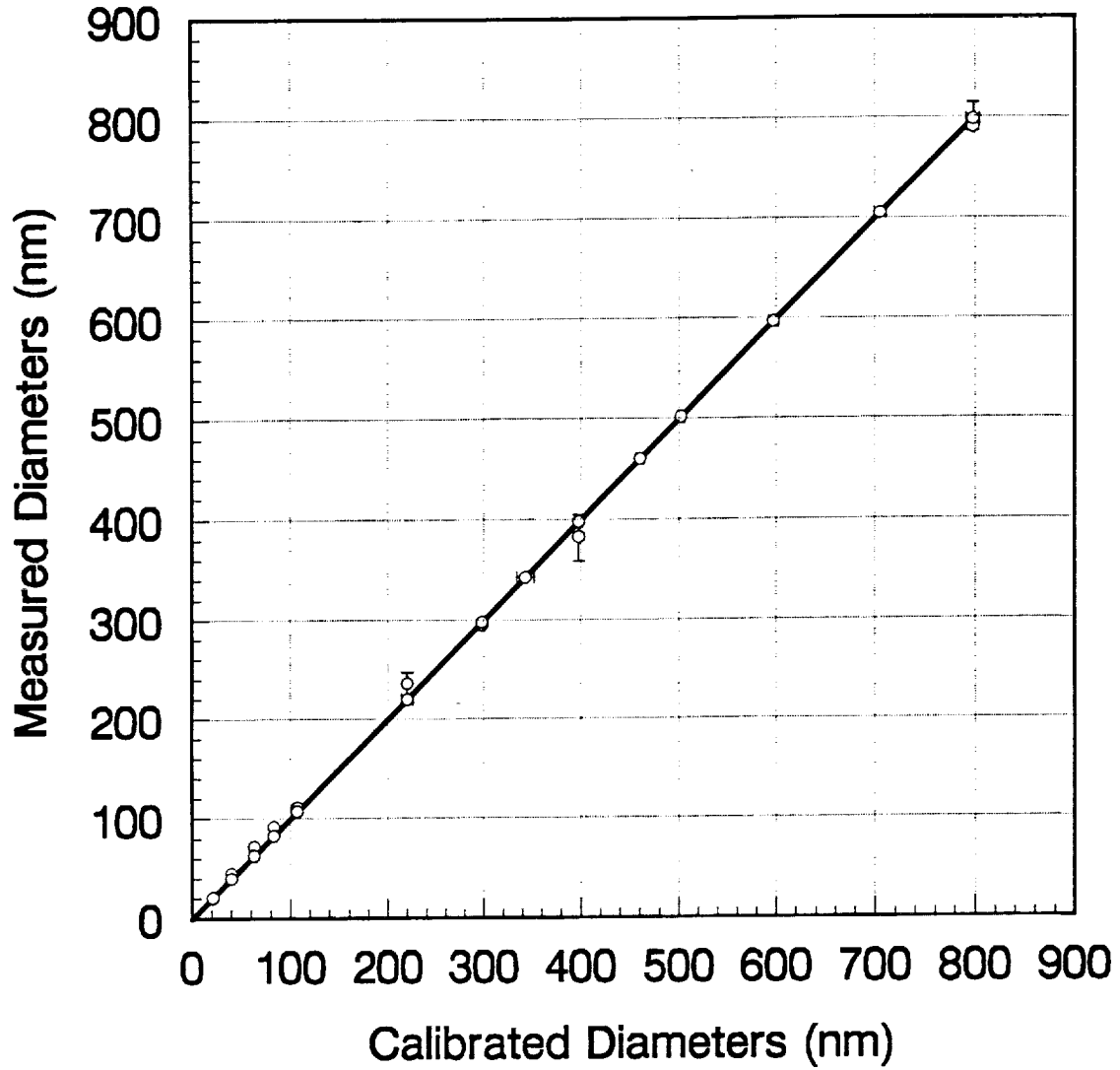


Figure 5. Comparison of calibrated and measured diameters of polystyrene particles. Vertical error bars represent the 95% confidence level. Horizontal error bars represent the size uncertainty given by Duke Scientific Corporation.

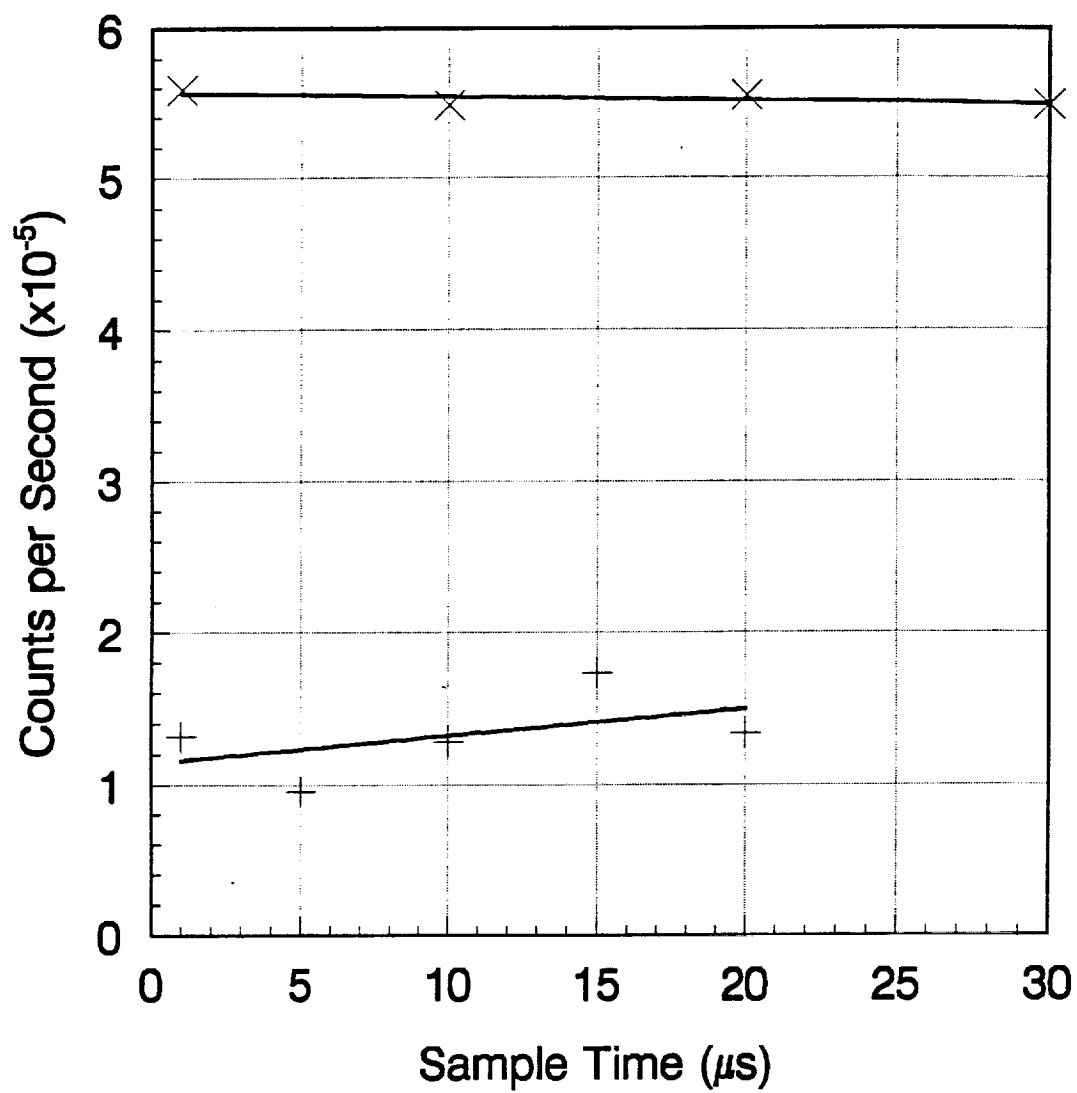


Figure 6. The intensity (counts/second) is independent of the sample time. 21nm particles shown by + and 220nm particles by x. The proper sample times for these particles are 2 and 21 microseconds, respectively.

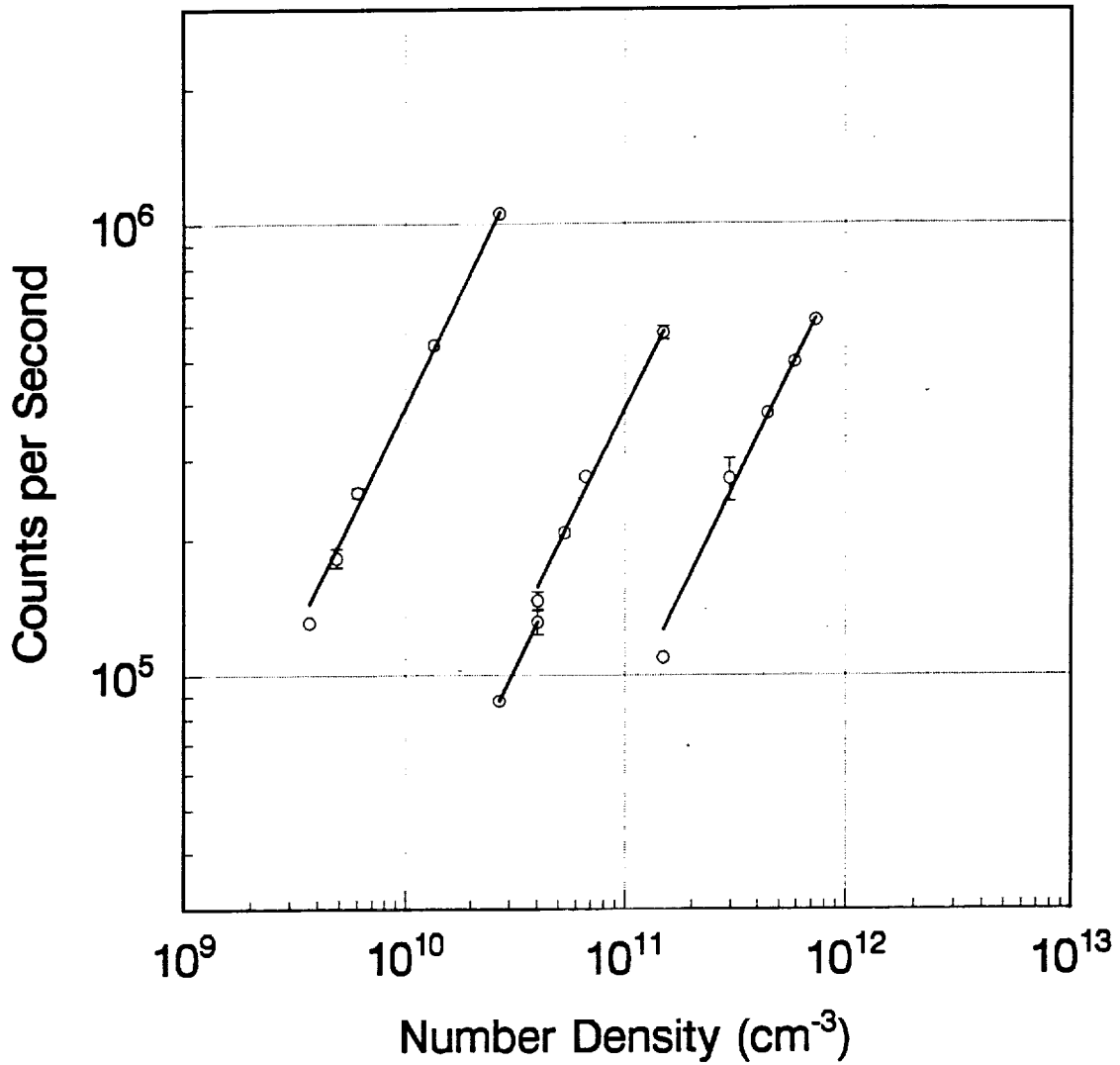


Figure 7. A typical set of cross section measurements before matching over lapping concentrations. When secondary scattering occurs the higher concentration range will have a much lower slope.



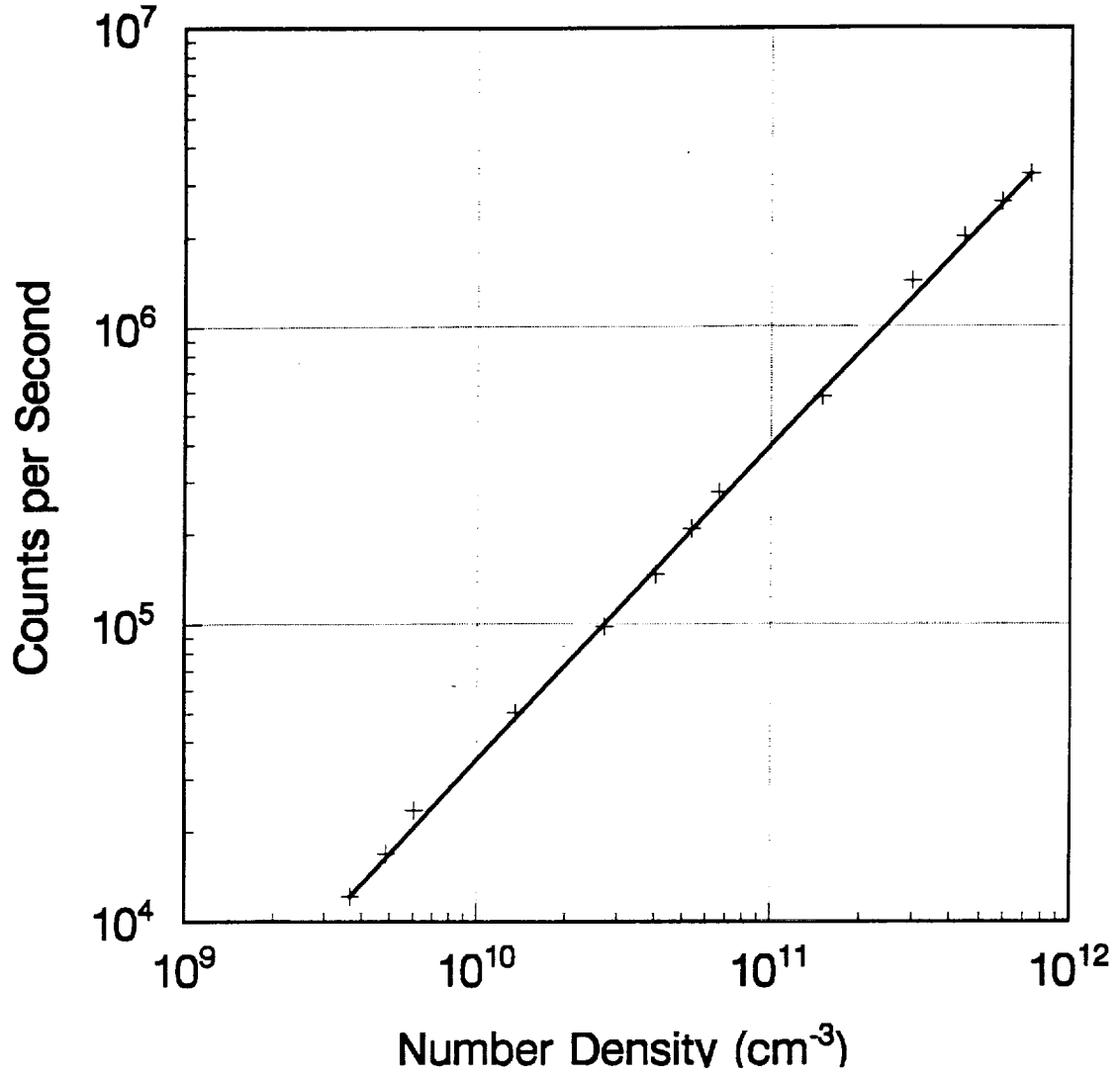


Figure 8. The data shown in figure 7 after all adjustments and matching of the raw data. The slope is the PCS cross section.

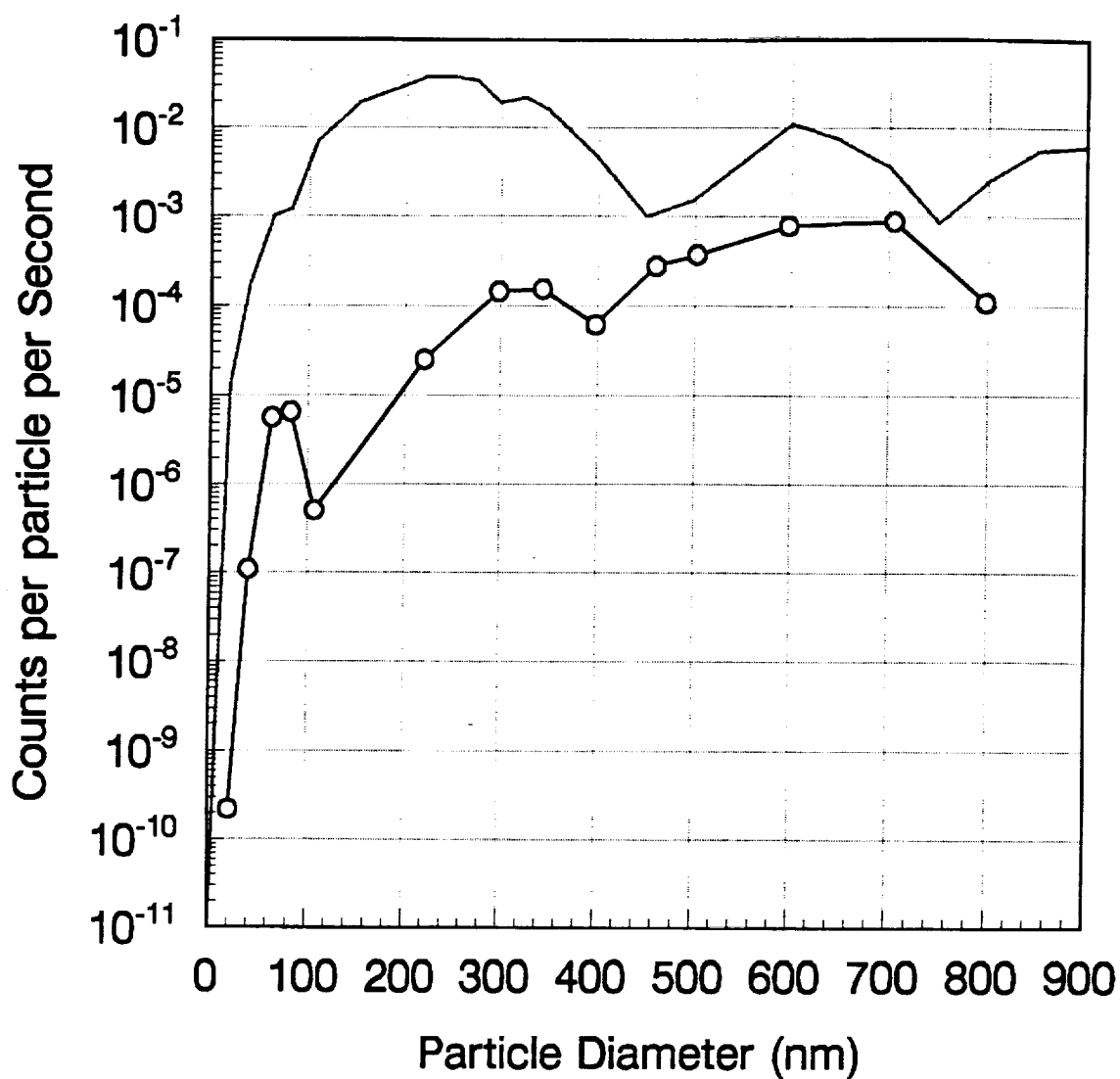


Figure 9. The PCS cross sections expressed as counts per second per particle at 90 degrees for polystyrene spheres and 647.1nm light. The top curve is the Mie scattering factor calculated for the same conditions.

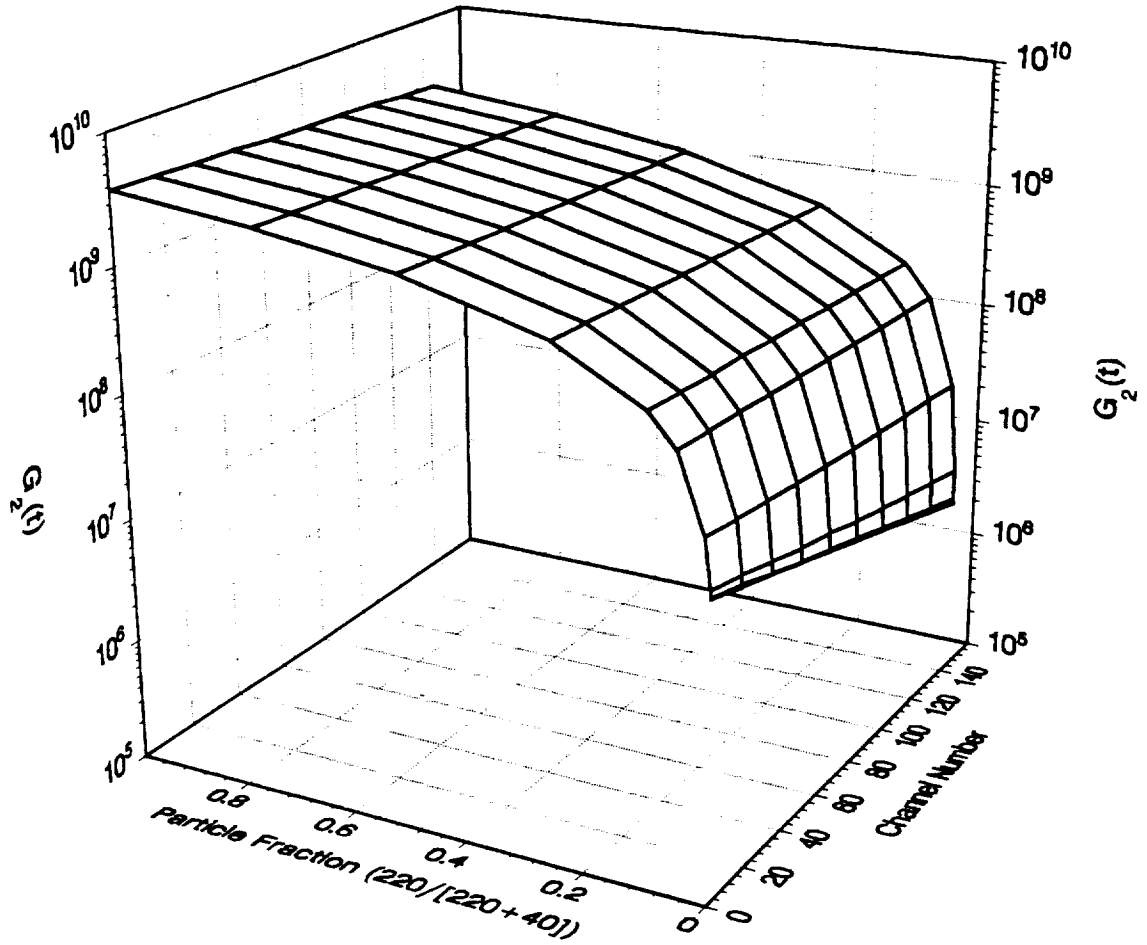


Figure 10. A representation of logarithm of  $G_2(t)$  expressed as counts per second per channel for an arbitrary  $I_0$  for mixtures of 40nm and 220nm spheres for mixtures of various compositions. Note the relatively small range in which the particle mixtures produce curvature.

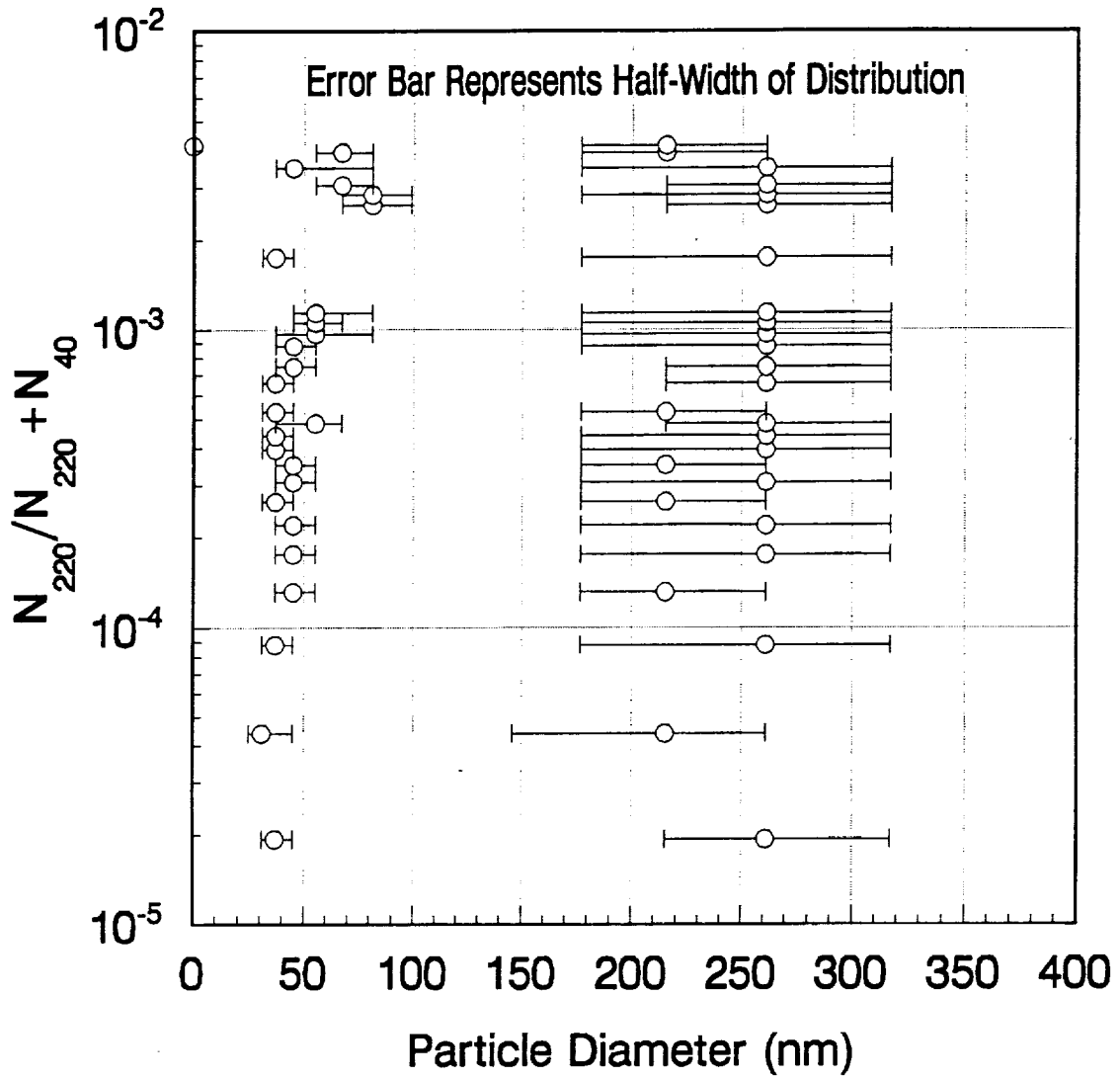


Figure 11. Resolution of 40 and 220nm polystyrene spheres at 90 degree scattering angle with 647.1 light using sample times of 5 and 20 microseconds with a duration of 500 seconds. The extremes of the number ratio represent the resolution range for these conditions.

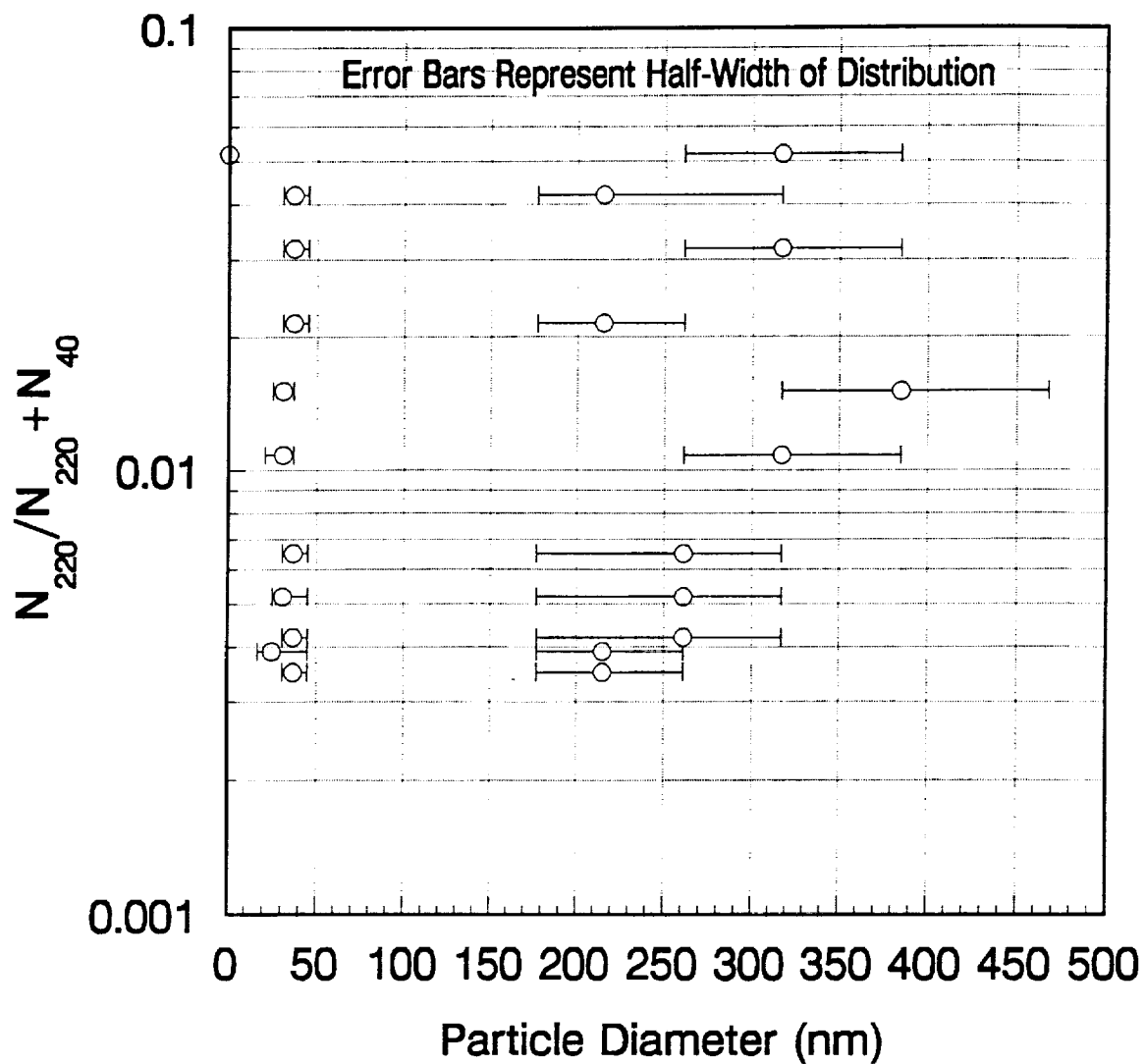


Figure 12. Resolution of 40nm and 220nm polystyrene spheres at 45 degree scattering angle with 647.1nm light. The sample times were 20 and 80 microseconds with a duration of 500 seconds.

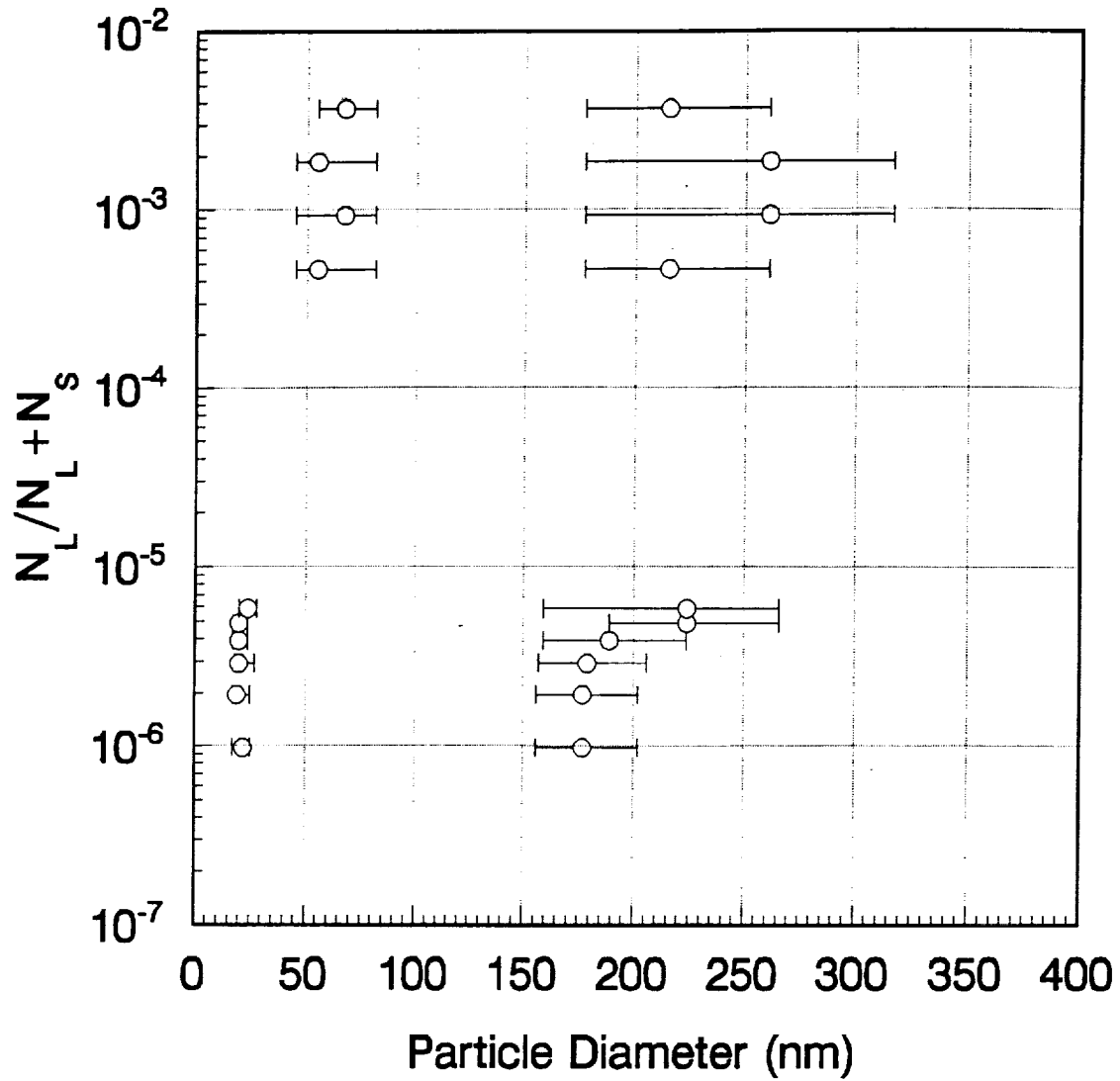


Figure 13. Comparison of the resolution of 21nm and 63nm particles mixed with 220nm particles. For the 21-220nm mixture the sample times were 2 and 16 microseconds with a duration of 100 seconds. The 63-220 measurements were made with sample times of 6 and 24 microseconds with a duration of 60 seconds. Both measurements were at 90 degrees.