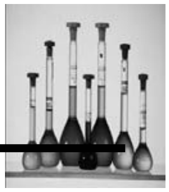
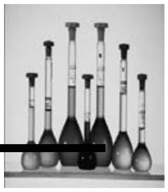
NICOMP 380 DLS



User Manual

Particle Sizing Systems, Inc.

DOCUMENT CHANGE HISTORY



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GENERAL INFORMATION

REGISTRATION

Please register your software by taking a moment to fill out the registration page provided. In keeping with our promise, we can easily provide two years of free software upgrades.

Just call us if you need information about our other products, or information about upgrading your existing system.

TECHNICAL SUPPORT

If technical support is needed please contact one of the following offices:

Particle Sizing Systems
8203 Kristel Circle
Port Richey, FL 34668

Tel: 727-846-0866
Fax: 727-846-0865

Or

Particle Sizing Systems
201 Woolston Drive, Ste. 1-C
Morrisville, PA 19067

Tel: 215-428-3424
Fax: 215-428-3429

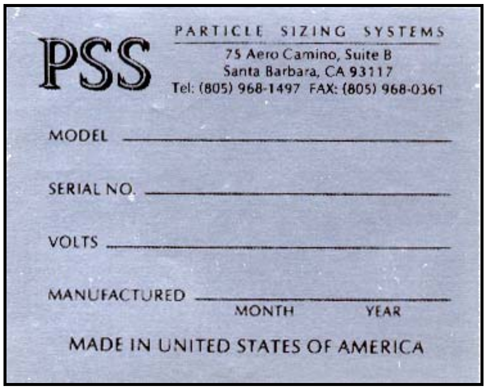
GENERAL INFORMATION

SAFETY CONSIDERATIONS

The NICOMP^{TM} (and Autodiluter) Submicron Particle Sizer, is certified to conform to the applicable requirements of 21 CFR Subchapter J, 1040.10 and 1040.11 (Radiation Control for Health and Safety Act of 1968, 42 U.S.C 263f).

As presently constructed, this instrument is designated by the Bureau of Radiological Health Class I product. Exposure to negligible levels of *Laser Radiation* during normal operation results. The two labels below are affixed to the back panel of the Nicomp 380/Autodilute. They attest to the above *Safety Certification* and also establish the place and date of manufacture of the unit.

THIS EQUIPMENT CONFORMS
TO PROVISIONS OF
US 21 CFR 1040.10
AND 1040.11



PSS
PARTICLE SIZING SYSTEMS
75 Aero Camino, Suite B
Santa Barbara, CA 93117
Tel: (805) 968-1497 FAX: (805) 968-0361

MODEL ____________________

SERIAL NO. ____________________

VOLTS ____________________

MANUFACTURED ____________________
MONTH YEAR

MADE IN UNITED STATES OF AMERICA

Important: Read carefully before attempting to operate the Nicomp

If the Nicomp is to be used with the Autodilution option, then all liquid samples will be introduced into the system by means of a syringe or tube connected to the manual sampling valve that is located on the front panel of the instrument. In this case, NO entry into the sample holder space will be required.

Alternatively, if the Nicomp is to be used without the autodilution option, then all liquid samples will be introduced into the light scattering cell using 6 mm disposable glass culture tubes or standard 1-cm cuvettes. In this case, entry into the sample cell holder space will be required.

Access to the sample cell holder, necessary for inserting or removing a sample cell, is provided by a square opening at the front left corner of the top cover of the instrument. A rectangular dust cover with handle and three thumb screws are provided to keep the scattering cell and internal optical components free of excessive amounts of dust when the unit is not in use for extended periods of time and to prevent the laser light from scattering outside the unit during operation. During normal operation this cover can be secured with one screw and swung to one side to provide easy access to the cell holder. It can be swung shut during operation to keep out stray room light and keep in beam light being scattered by the particles.

During operation of the NICOMP^{TM} Autodilute Submicron Particle Sizer, the *Top Cover* of the unit *Must Remain Closed* -- i.e. attached to the cabinet by means of the 3 screws provided. The *Warning* label on the cover warns of the possible exposure to the laser beam (a minimum of 5 milliwatts, 632.8 nm wavelength) if the top cover is removed for any reason while power is applied to the unit.

> **Important:** Any attempt to remove the front panel while the instrument is in operation may result in possible **Direct Exposure to Dangerous Laser Radiation**. Also, power must be off to the unit if the Autodilution cell is being replaced by the drop-in cell.

CE MARK

The **CE mark** (officially CE marking) is a mandatory marking on certain products, which is required if they are placed on the market in the European Economic Area (EEA). By affixing the CE marking, the manufacturer, or his representative, or the importer assures that that the item meets all the essential requirements of all applicable EU directives.

The CE mark is a mandatory European marking for certain product groups to indicate conformity with the essential health and safety requirements set out in European Directives. To permit the use of a CE mark on a product, proof that the item meets the relevant requirements must be documented. This has been achieved using an external test house which evaluates our particle size analyzers and its documentation. CE originally stood for *Communauté Européenne* or *Conformité Européenne*, French for European Conformity.

The following label is affixed to the back panel of the AccuSizer SIS to indicate that the instrument has passed CE mark testing and conforms to the European Union Directives for Electromagnetic Compatibility (EU EMC).

CE

DLS THEORY

DYNAMIC LIGHT SCATTERING THEORY

In recent years, the technique of dynamic light scattering (DLS) -- also called quasi-elastic light scattering (QELS) or photon correlation spectroscopy (PCS) -- has proven to be an invaluable -analytical tool for characterizing the size distribution of particles suspended in a solvent (usually water). The useful size range for the DLS technique is quite large -from below 5 am (0.005 micron) to several microns. The power of the technology is most apparent when applied to the difficult Particularly for diameters below 300 nm submicron size range, where most competing measurement techniques lose their effectiveness or fail altogether. Consequently, DLS-based sizing instruments have been used extensively to characterize a wide range of particulate systems, including synthetic polymers (e.g. latexes, PVCs, etc.), oil-in-water and water-in-oil emulsions, vesicles, micelles, biological macromolecules, pigments, dyes, silicas, metallic sols, ceramics and numerous other colloidal suspensions and dispersions.

PRINCIPLES OF DLS – A QUALITATIVE REVIEW

Classical light scattering: intensity vs. volume

A simplified schematic diagram of the DLS module is shown below. Light from a laser is focused into a glass tube containing a dilute suspension of particles. The temperature of this scattering cell is held constant, for reasons which will soon become apparent. Each of the particles illuminated by the incident laser beam scatters light in all directions.

The intensity of light scattered by a single, isolated particle depends on its molecular weight and overall size and shape, and also on the difference in refractive indices of the particle and the surrounding solvent. The incident light wave can be thought of as consisting of a very rapidly oscillating electric field, of amplitude E_o (frequency approx. 10^{15} Hz).

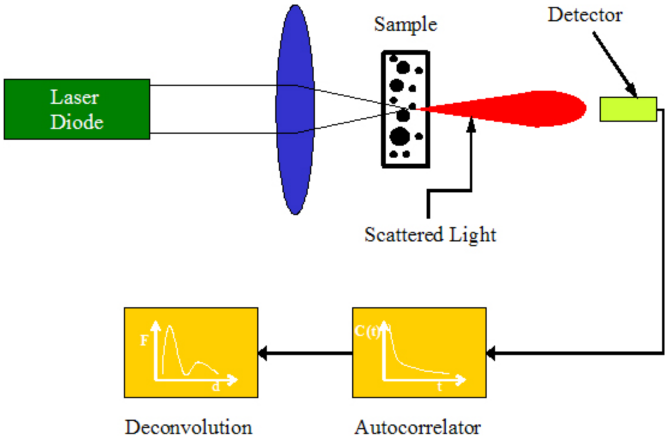


Figure 1: Simplified block diagram -- NICOMP DLS Instrument

The arrival of this alternating field in the vicinity of a particle causes all of the electrons which are free to be influenced-the so-called "polarizable" electrons -- to oscillate at the same frequency. These oscillating electrons, in turn, give rise to a new oscillating electric field which radiates in all directions- the scattered light wave. The quantity of interest in a scattering measurement is the intensity of the scattered wave, I_s, rather than its amplitude, E_s. The intensity is given simply by the square of the amplitude: $I_s = (E_s)^2$. The dependence of the scattered light intensity I_S on the molecular weight (MV) or volume (V) of the particle is particularly simple when the particle diameter is much smaller than the laser wavelength λ -- the so-called Rayleigh region. In this case, all of the polarizable electrons within a particle oscillate together *in phase*, because at any given time they all experience the *same* incident electric field. Hence, the scattered wave amplitude E_s is simply proportional to the *number* of polarizable electrons, times the incident wave amplitude, E_o. The former quantity is essentially proportional to the overall molecular weight of the particle, MW, or its volume, V (for a given particle density). The constants of proportionality that connect these various physical quantities depend on the indices of refraction of the particle (n_p) and solvent (n_n). That is, how well a given particle scatters light depends not only on MW, or V, but also on the polarizability of the particle (related to n_p) relative to that of the solvent (related to n_s). For the very small particles in the Rayleigh region, we arrive at simple expressions for the scattered intensity I_s:

$$I_s = f(n_p,n_s)\,(MW)^2\,I_o \qquad (1a)$$

or

$$r,\ I_s = g(n_p,n_s)\,V^2\,I_o \qquad (1b)$$

where I_o is the incident laser intensity, and $f(n_p,n_s)$ and $g(n_p,n_s)$ are functions of the indices of refraction of the particle and solvent, which are fixed for a given system composition (e.g. latex particles in water). For these small particles in the Rayleigh region (i.e. diameters < approx. 0.1 micron, or 100 nm), there is negligible angular dependence in the scattered intensity.

The simple expressions above must be modified when the characteristic particle dimension (i.e. the diameter, in the case of spheres) is no longer negligible compared to the wavelength of the incident light beam. In this so-called Mie Scattering region, Equations la and 1b must be altered to take account of intra-particle interference. With a larger particle, the oscillating electrons no longer oscillate together in phase; the individual scattered waves originating from different regions of the particle interfere at the distant point of detection. The resulting total scattered intensity I_s is therefore diminished relative to the values given by Equations la and b, which assume that all of the effective scattering mass is packed into a very small particle size. The expressions in Equations la and 1b can be repaired to include the effects of interference by multiplying them by a so-called Mie "form" factor; this quantity has a limiting value of 1.0 (i.e. no effect) in the Rayleigh region, but falls below unity in a non-monotonic way as the particle size grows.

Using Equation Ia or Ib, one can, in principle, determine either the molecular weight or the volume of the particles from a measurement of the scattered intensity I_s, using known calibration standards, together with empirical determinations of $f(n_p,n_s)$ and $g(n_p,n_s)$. This forms the basis for the technique of "classical" light scattering. The newer DLS method, however, departs radically from this traditional approach to light scattering. The quantity of interest is no longer the magnitude, per se, of the scattered light intensity. Rather, DLS concerns itself with the time behavior of the *fluctuations* in the scattered intensity.

Dynamic scattering: the effects of diffusion

To understand why the scattered intensity fluctuates in time, we must appreciate that it is the result of the coherent addition, or "superposition", of many individual scattered waves, each of which originates from a different particle located in the illuminated/detected volume. This is the physical phenomenon known as "interference". Each individual scattered wave arriving at the detector bears a phase relationship with respect to the incident laser wave which depends on the precise position of the suspended particle from which it originates. All of these waves mix together, or interfere, at a distant slit on the face of a photomultiplier detector ("PMT" in Figure 1), which measures the resulting net scattering intensity at a particular scattering angle (90 degrees in the DLS Module).

The suspended particles are not stationary; rather, they move about, or diffuse, in random-walk fashion by the process known as Brownian motion (caused by collisions of neighboring solvent molecules). As a consequence, the phases of each of the scattered waves arriving at the PMT detector fluctuate randomly in time, due to the random fluctuations in the *positions* of the particles that scatter the waves. Because these waves interfere together at the detector, the net intensity fluctuates randomly in time. It is important to appreciate that only relatively small movements in particle position are needed to effect significant changes in phase and, therefore, to create meaningful fluctuations in the final net intensity. This is because the laser wavelength is relatively small -- only about 0.6 micron.

The connection between the diffusion of particles and the resulting fluctuations in scattered intensity is perhaps more easily understood by considering a simplified situation in which there are only *two* particles in suspension, shown in Figure 2.

The net intensity at the detector (located far from the scattering cell, with a pinhole aperture) is a result of the superposition of only two scattered waves. In Figure 2 we have defined the two optical path lengths, $L_1 = l_{1a} + l_{1b}$ and $L_2 = l_{2a} + l_{2b}$. (More precisely, the optical path length is the distance corrected by the index of refraction, but for simplicity we assume an index of 1.0 and show L_1 and L_2 to be simple distances in Figure 2.) When the positions of the two particles are such that the difference in optical path lengths, $\Delta L = L_1 - L_2$ becomes equal to an integral multiple of the laser wavelength λ, then the two scattered waves will arrive in phase at the detector. This is called total "constructive" interference and produces the largest possible intensity at the detector.

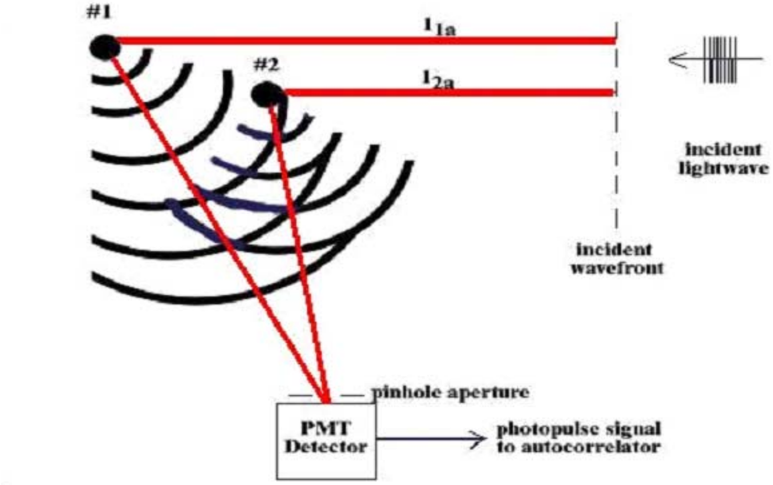


Figure 2: Simplified scattering model: two diffusing particles

At the other extreme, it is possible for the two particles to find themselves at positions such that ∆L equals an *odd* number of half wavelengths, λ/2. In this case the two scattered waves arrive at the detector totally out of phase with each other. This is total "destructive" interference, resulting in zero net intensity. Over time, diffusion of the particles will cause the net intensity at the detector to fluctuate in random fashion -- like a typical "noise" signal -- between these two extreme values. A representative total intensity signal is shown in Figure 3. The intensity varies between the maximum value and the minimum value (zero) when the optical path length difference changes (i.e. increases or decreases) by λ/2.

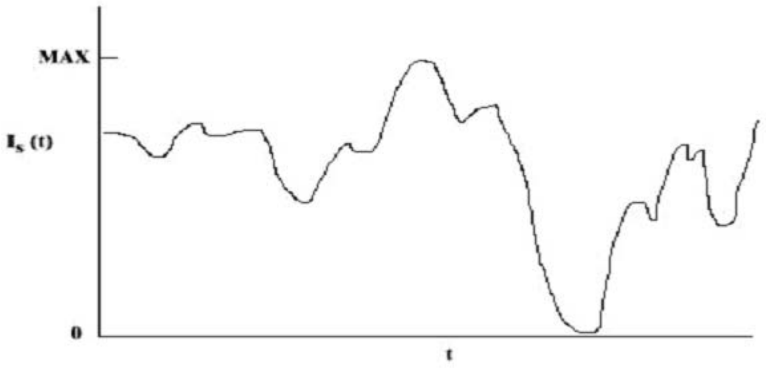


Figure 3: Typical intensity vs time for two diffusing particles

The key physical concept that underlies the DLS particle sizing measurement is the fact that the time scale of the fluctuations shown in Figure 3 depends on the *size* of the particles. For simplicity at this point in the discussion, we assume the particles to be uniform in size, with a single, well-defined diffusion coefficient. Small particles will "jitter" about in solution relatively rapidly, resulting in a rapidly fluctuating intensity signal; by contrast, larger ones will diffuse more slowly, resulting in a more slowly varying intensity.

At this point we make the simplifying assumption that the temperature of the particle suspension is held constant. We shall see that the temperature plays as important a role as the particle size in determining the diffusivity and, hence, the time scale of the resulting intensity fluctuations. In any real situation of interest, of course, there are many more than two particles in suspension which contribute to the scattered intensity signal. However, the principle of interference remains the same. The resulting signal will be observed to fluctuate average level, which is proportional to the number of particles illuminated/detected volume and their individual scattering power -- Equations 1a and 1b. The time scale of the fluctuations depends on the particle diffusivity, and hence on the particle size. This is illustrated in Figures 4a,b and c for "small", "medium" and "large" size particles (using the same time scale on all three horizontal axes). Again, it must be stressed that the fluctuations in the net scattered intensity are *not* caused by the addition or subtraction of particles in the illuminated/detected volume. Rather, they are the result of the variations in position of an essentially fixed number of particles within the scattering volume.

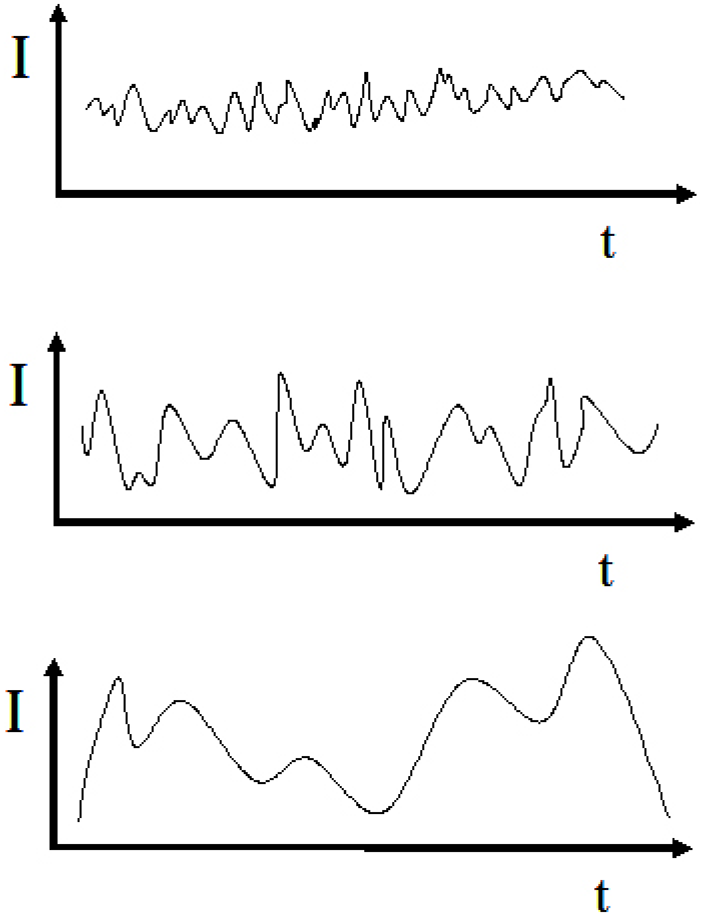


Figure 4 a,b,c: Representative intensity vs time for "small"(a), "medium"(b) and "large"(c) size particles

Obtaining particle size from the diffusion coefficient

The goal of the DLS technique is to determine the diffusion coefficient D of the particles (assumed uniform here) from the "raw" data -- i.e. the fluctuating light scattering signal, as represented in Figure 4a,b,c. From D we can easily calculate the particle radius R. using the well-known Stokes-Einstein relation,

$$D = kT/6\pi\eta R \qquad (2)$$

where k is Boltzmann's constant (1.38 X 10^{-16} erg K^{-1}), T the temperature (^{o}K, = ^{o}C + 273) and η the shear viscosity of the solvent (e.g. η = 1.002 X 10^{-2} poise for water at 20^{o}C). Thus, we see that the rate at which the particles jitter about in the suspension, as measured by D, is inversely related to the particle radius R.

From Equation 2 we see that, in general, the diffusion coefficient D of particles of a given size increases with increasing temperature T. This is due primarily to the T-dependence of the solvent viscosity η. (The fact that T is the numerator in Equation 2 is less small, in percentage, when expressed in deg. Kelvin.) For example, η for pure water falls to 0.890 X 10^{-2} poise at 25^{o}C -- i.e. more than a 10% change from the value at 20^{o}C. Clearly, the less viscous the solvent, the more rapid will be the random-walk diffusion of the particles and the faster the resulting intensity fluctuations. Hence, changes in T are *completely indistinguishable* from changes in particle radius R. as they affect D. For this reason, the sample temperature MUST be constant (and accurately known) in order to obtain a meaningful measurement of D and, hence, of R using Equation 2.

A cursory examination of the three fluctuating scattering signals in Figure 4a,b,c suggests that extraction of the diffusion coefficient from the "noise" is not a straightforward matter. Signal (b) clearly fluctuates faster than does (c), but is slower than (a); hence, its particle size must lie between the values associated with (a) and (c). However, obtaining quantitative information from these kinds of scattering signals is another matter altogether. What comes to our rescue is the mathematical operation known as *autocorrelation*.

Autocorrelation function: definition and motivation

Let us consider the autocorrelation function of the net scattered light intensity $I_s(t)$, which fluctuates in time as shown in Figures 4a, b, and c. The autocorrelation function, which we denote by C(t′) is used to study the correlation, or similarity, between the value of I_s at a given time, t, and the value of I_s at a given time, t and the value of I_s at an earlier time, t-t'. This comparison is then made for many different values of t in order to obtain a good statistical average for C(t') -- i.e. averaged over many "wiggles" of the fluctuating intensity I_s. C(t') is evaluated according to,

$$C(t') = < I_s(t) * I_s(t-t')> \qquad (3)$$

The bracket symbols < > are shorthand for a summation over many values of t. That is, one calculates a running sum of many products $I_s(t) * I_s(t-t')$, all having the <u>same</u> separation in time, t', for many different values of t.

The ability of C(t') to extract useful information from the fluctuating scattering intensity $I_s(t)$ can best be understood by considering a portion of a typical signal $I_s(t)$, shown in Figure 5. We arbitrarily choose a particular time t and record the value of I_s at that time -- $I_s(t)$. We next consider a very small value of t', equal to t_1', and evaluate I_s at this slightly earlier time, $t-t_1'$ -- $I_s(t-t_1')$. Because t_1' is presumed to be small, $I_s(t-t_1')$ must be very similar to $I_s(t)$. The reason for this, of course, is that the particles have not been able to change their positions significantly (i.e. compared to λ) under diffusion in the (presumed) short time interval t_1'. In Figure 5 $I_s(t-t_1')$ is shown to be slightly larger than $I_s(t)$.

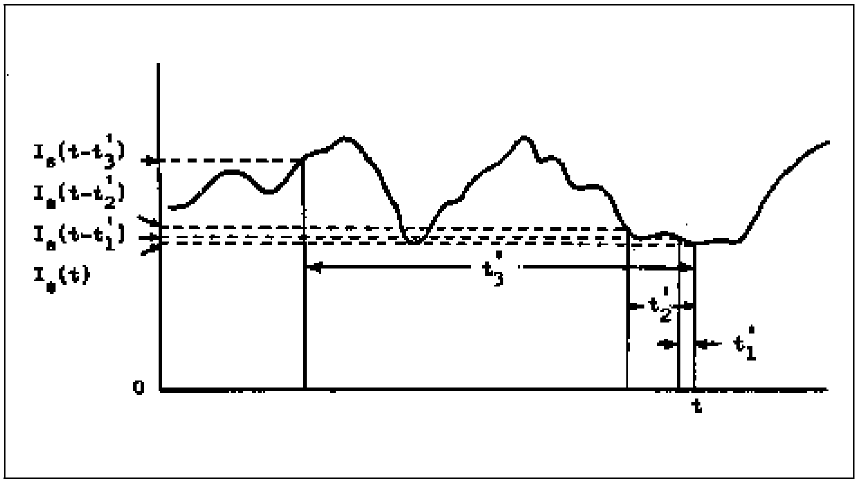


Figure 5: Computation of autocorrelation function C(t')

However, if t had been chosen differently in Figure 5, the order of the two values might have been reversed. In any case, what matters is that the two intensity values that become multiplied in Equation 3 are nearly the same. They are said to be highly correlated. Clearly, the choice of t is irrelevant -- for any value of t, $I_s(t)$ and $I_s(t-t')$ must be highly correlated (i.e. nearly the same) for a sufficiently small choice of t'.

Next, let us consider a larger value for t', equal to t_2', as shown in Figure 5. In this case, t_2' has been chosen to be large enough relative to the *time scale* of the fluctuating signal that the two sampled values of I_s -- $I_s(t)$ and $I_s(t-t_2')$ -- are now somewhat different. In this case, the two sampled intensities are less well correlated. However, there still remains some relationship between these two intensities. If t has been chosen so that $I_s(t)$ is near a minimum in the intensity, then $I_s(t-t_2')$ will still be a relatively low value. Similarly, if $I_s(t)$ lies near a maximum, then it is apparent from Figure 5 that $Is(t-t_2')$ must also be at a relatively high value (or certainly not near a minimum), given the fact that t_2' is not a very large time interval relative to the characteristic time scale of the intensity signal shown in Figure 5.

Finally, we consider a very large time interval, t_3', as seen in Figure 5. Here, we see that t_3' is so large that I_s has undergone two large fluctuations between the two sampling times, t and $t-t_3'$. It is clear, here, that the two sampled intensities will *in general* be almost completely uncorrelated for such a large choice of t_3'. The two values could easily be both high, both low, one high and the other low, or any other intermediate possibility.

We have carried out these examples assuming a single choice for time t and three different values of t'. In order to obtain a meaningful value for the autocorrelation function for a particular choice of t' -- C(t') -- one must obtain *many* products $I_s(t) * I_s(t-t')$ using many different values of t, for each value of t'. Only in this way will one average the value of C(t') over sufficiently many "bumps" and "wiggles" in the fluctuating signal I_s to obtain a statistically meaningful value of the autocorrelation function. Then, one must repeat this process for sufficiently many values of t' so as to obtain a well-defined, smooth representation of C(t') as a function of t'.

It is useful to have an idea at this point of the kinds of numbers that are involved when we use the word "many". For a typical particle size measurement of duration 5 minutes on 0.2 micron (200 nm) particles, the DLS Module performs approximately 15 *million* multiplications in order to obtain C(t') for *one* value of t' (e.g. t' = 20 microseconds for "channel" #1). The instrument makes 64 such sets of calculations simultaneously in order to obtain C(t') for 64 different values of t'.

The essential point about the autocorrelation function is that it serves as a useful probe of the characteristic lifetime, or duration, of the fluctuations in $I_s(t)$. That is, once the interval t' between two sampled intensities exceeds the average width of a major "bump", or fluctuation, in $I_s(t)$, the two sampled intensity values will cease, on average, to be correlated. At this point, the value of C(t') will have fallen substantially.

What can we say about the shape of C(t') as a function of the sampling separation t'? Without knowing anything about the physics of diffusion and its effect on $I_s(t)$, we can nevertheless say something useful about C(t') in two limiting (extreme) cases: t' $\rightarrow$0 and t'$\rightarrow$:. In the limit in which t' approaches zero, the two sampled intensities are essentially identical, because there is no time for the particles to rearrange their positions. Hence,

$$C(O) = \langle I_s^2(t) \rangle \qquad (4)$$

That is, the value of C(t') for t'$\rightarrow$0 is simply the sum over many values of t of the square of the scattering intensity.

In the opposite limit, in which the sampling interval t' becomes very large (approaching infinity), we have already seen (Figure 5) that there should be no correlation between the pair of sampled intensities. Hence, Equation 3 reduces to the square of the average scattering intensity, $I_s(t)$ -- i.e. the normalized sum of $I_s(t)$ values, taken over many values of t:

$$C(\infty) = \langle I_s(t) \rangle^2 \qquad (5)$$

It is known, and easily demonstrated, that for *any* fluctuating quantity, the average of the squares of that quantity is always larger than the square of the average:

$$\langle I_s^2(t) \rangle > \langle I_s(t) \rangle^2 \qquad (6)$$

The quantity on the right hand side of Equation 6 is the lowest value possible for the correlation function; all other values of C(t') for finite values of t' must, in principle, be larger than the square of the average of the I_s values, because of the existence of correlations. This is referred to as the *baseline* of the autocorrelation function. In practice, it can be effectively determined by evaluating Equation 3 using a sufficiently large value for t'.

Hence, we can say with certainty that the function C(t') for our situation of diffusing particles must fall from the value $\langle I_s^2 (t) \rangle$ at t'=0, to the baseline value, $\langle I_s(t) \rangle^2$ at very large t'. The problem remains -- what is the shape of C(t') between these two extreme values?

Ideal case: uniform particle size

It turns out that for random diffusion of non-interacting particles, the autocorrelation function C(t') of the fluctuating scattered light intensity $I_s(t)$ is an exponentially decaying function of time t', as shown symbolically in Figure 6. This is described by the expression,

$$C(t') = A \exp(-t'/\tau) + B \qquad (7)$$

where $A = \langle I_s^2(t)\rangle - \langle I_s(t)\rangle^2$
and $B = \langle I_s(t)\rangle^2$

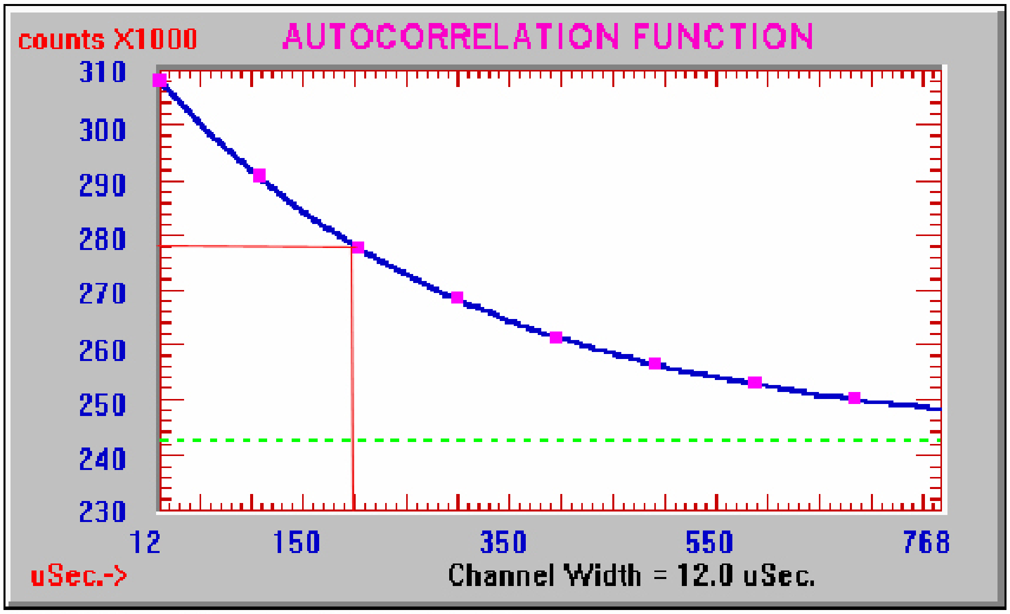


Figure 6:Autocorrelation function C(t') for diffusion of uniform particles: exponential decay

Variable τ is the characteristic decay time constant of the exponential function; τ characterizes quantitatively the speed with which the autocorrelation function C(t') decays toward the long-t' limiting value (baseline B). In effect, the value of τ describes the characteristic lifetime, or duration, of a major "bump", or fluctuation, in the scattered intensity I_s. Hence, the larger the particles, the slower the diffusivity and resulting fluctuations in I_s' and the longer the decay time constant τ.

As you might have predicted by now, we are able to obtain the diffusion coefficient D of the particles from the decay constant τ; the precise relation is,

$1/\tau = 2DK^2$ (8a)

or

$D = (1/2K_2)(1/\tau)$ (8b)

Here, the quantity K is called the "scattering wave vector". It is a constant that depends on the laser wavelength λ in the solvent and the angle θ at which scattered light is intercepted by the PMT detector. ($\theta = 90^o$ for the DLS MODULE) In effect, K acts as an absolute calibration constant, which relates the time scale of the diffusion process to the distance scale set by the laser wavelength (making interference possible). Constant K is given by

$K = (4\pi n/\lambda) \sin \theta/2$ (9)

where n is the index of refraction of the solvent (e.g. 1.33 for water). In the case of the DLS Module, with $\theta = 90^o$ and $\lambda = 632.8$ nm, K equals 1.868×10^5 cm^{-l}.

The rationale for particle sizing using the method of DLS should now be clear. We detect scattered light (at a fixed angle) produced by an ensemble of particles suspended in a solvent. The intensity fluctuates in time due to diffusion of the particles; there is a well defined characteristic lifetime of the fluctuations, which is inversely proportional to the particle diffusivity. We compute the autocorrelation function of the fluctuating intensity, obtaining a decaying exponential curve in time. From the decay time constant τ, we obtain the particle diffusivity D. Using the Stokes-Einstein relation (Equation 2), we finally compute the particle radius R (assuming a sphere).

Photon counting and digital autocorrelation functions

We now consider the practical application of the theory discussed above in an actual DLS particle sizing instrument. The first step is computation of the autocorrelation function C(t') from the scattered light intensity $I_s(t)'$ as prescribed in Equation 3. It should be apparent that the fundamental operation of multiplication is most easily accomplished if both $I_s(t)$ and $I_s(t-t')$ are expressed as digital quantities. Fortunately, it turns out that this is already the case! In our discussion thus far, we have represented I_s as an analog signal which varies continuously in magnitude as a function of time -- e.g. Figure 3 and 4a, b and c. However, in reality this is not correct. The scattering signal I_s actually consists of a series of individual "photopulses" produced by the PMT detector (Figure 1). That is, the particle suspension is sufficiently dilute that the average scattering intensity at the PMT photocathode is extremely low, resulting in a "photocurrent" which consists of discrete pulses (separated by zero baseline current), corresponding to individual photons which comprise the weak scattering signal. Hence, the DLS instrument is said to operate in the "photon counting" regime.

If I_s consists of a train of discrete pulses, rather than an analog signal, what is the quantitative meaning of the "intensity" I_s at time t? Clearly, the intensity must be represented by the number of photopulses per unit of time; the larger the number of pulses occurring in that time unit, the larger the intensity. For example, in typical operation the DLS Module might show a photopulse rate of, say, 300 kHz. This value is updated every one second and represents the number of photopulses detected in the proceeding one-second interval. The sequence of values might resemble the series 302, 297, 299, 304, 296, etc. We would therefore say that the average "intensity" is approximately 300,000 -- meaning, pulses per one-second interval. However, it would be equally valid to express the average intensity as 150,000 -- meaning per 0.5-second interval; or as 30,000 -meaning per 0.1-second interval. That is, any unit of time is as valid as any other, for the purpose of defining the average value of the scattered intensity, depending on the length of time which one wishes to use to define that average value.

Earlier we saw that it is typically necessary to sample the value of $I_s(t)$ very frequently (i.e. to choose *small* values of t' between sampled pairs) in order to obtain an accurate autocorrelation function, which is sensitive to rapid changes in I_s, caused by rapid diffusion of the particles. For this reason, it is therefore necessary to define I_s, in terms of the photopulse rate using a very small unit of time. In this way, the measurement of I_s can be made as frequently as necessary and approaches being an instantaneous value. For example, when 100 nm (0.1 micron) particles are measured by the DLS Module, the sampling of $I_s(t)$ is performed approximately every 10 microseconds. In this case, therefore, $I_s(t)$ is arbitrarily defined to be the number of photopulses which occur during a given 10 microsecond interval. This short a time interval, or smallest increment in t', is needed to compute the relatively rapid decay of C(t') versus t' which occurs for these rapidly diffusing small particles. Of course, for smaller particles an even smaller unit time interval would be needed to define $I_s(t)$.

Two observations should immediately be evident. First, given such small time intervals used to define $I_s(t)$, the resulting number of pulses must be very small. Consider our example of a typical average photopulse rate of 300,000 per second; this corresponds to an average instantaneous intensity of 3 pulses per 10 microseconds. Second, we should expect this number to change greatly from one time interval to the next, given such a small average value. If the instantaneous photon rate were to follow Poisson statistics, we would expect the rms standard deviation of the number of pulses per time interval to equal $N^{1/2}$, where N is the average number. For our example above, this gives a standard deviation of 1.7. Hence, from purely a statistical point of view we expect the "intensity" I_s per 10 microsecond time interval to vary from 0 to 5 photopulses with occasionally a 6, 7 or larger), independent of the effects of diffusion. This is simply a consequence of our having chosen a very short time interval relative to the average photopulse rate. When diffusion is added to the process, the resulting fluctuations in $I_s(t)$ become even more pronounced.

The resulting integer numbers of photopulses per small time interval are, of course, the values of $I_s(t)$ and $I_s(t-t')$ in Equation 3 which become multiplied together digitally to compute the values of C(t'). A representative sequence of photopulses is shown in Figure 7. We have subdivided the time base, t, into intervals of equal width $\Delta t'$, equal to the "channel width" of the autocorrelator. Here, the instantaneous intensity $I_s(t)$ is defined as the number of pulses in the interval $\Delta t'$ which lies closest to time t. Over each interval we have recorded the instantaneous "intensity" for that interval -- simply the number of photopulses produced by the PMT detector. (Technically, the pulses which comprise the PMT photocurrent vary substantially in height, as well as rate of Occurrence, owing to the statistical nature of the secondary-electron multiplication mechanism in the PMT.

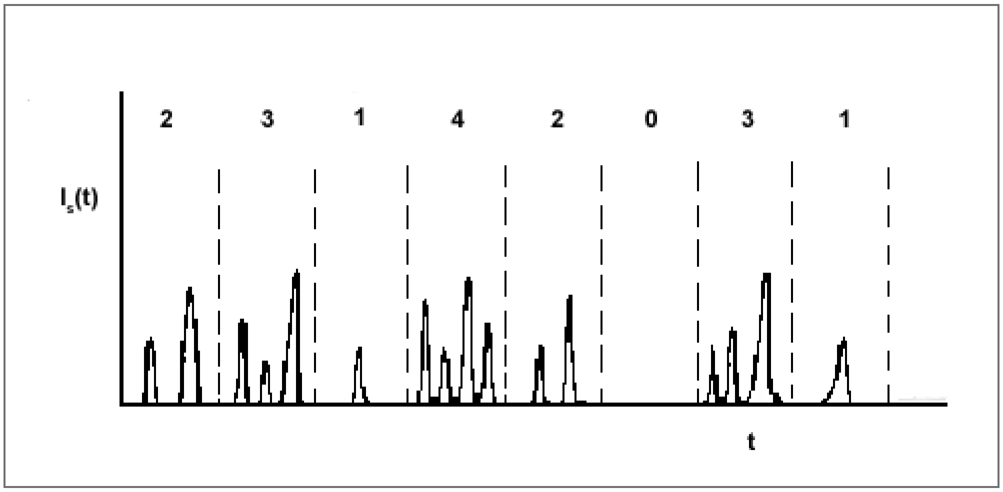


Figure 7: A typical photopulse sequence representing $I_s(t)$ divided into intervals of equal time width, t'

However, a discriminator with a low reference level is used to convert this signal to a train of pulses of uniform height, suitable for manipulation by standard integrated logic circuits in the autocorrelator.)

The procedure for computing the digital representation of C(t') should now be conceptually clear. The train of photopulses from the PMT detector is divided into intervals of equal time, or channel width, $\Delta t'$. Running sums of the products $I_s(t) * I_s(t-t')$ are then produced for many values of t' -- 64 in the case of the DLS Module. The separation times t' are "quantized" in multiples of $\Delta t'$: $\Delta t'$, $2\Delta t'$, $3\Delta t'$,...$64\ \Delta t'$. In addition, a long-delay baseline value is obtained: $t' = (64 + 1024)\ \Delta t'$.

Using the photopulse sequence shown in Figure 7, we now show how to compute the values of C(t') for the first few "channels" in t':

t' = Δt': C(t') = 2*3 + 3*1 + 1*4 + 4*2 + 2*0 + 0*3 + 3*1 + ...

t' = 2Δt': C(t') = 2*1 + 3*4 + 1*2 + 4*0 + 2*3 + 0*1 + ...

t' = 3Δt': C(t') = 2*4 + 3*2 + I*0 + 4*3 + 2*1 + ...

t' = 4Δt': C(t') = 2*2 + 3*0 + 1*3 + 4*1 + ...

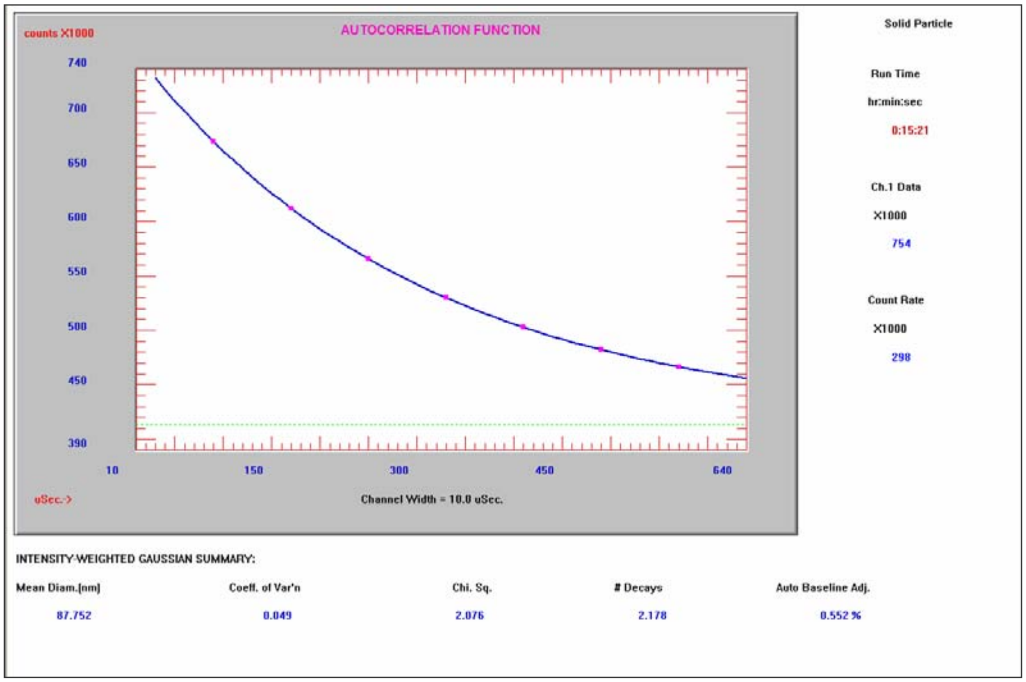
etc.

In the following section we shall generalize our discussion to include "polydisperse" systems -- those that contain a mixture of particle sizes. We shall discuss in some detail the methods by which the DLS Module is able to deal effectively with these more complex systems. These are the so-called "algorithms" for analysis of the autocorrelation function, which yield estimates of the true particle size distribution. The next section will be heavily weighted toward results -- i.e. actual pictures of screen displays and printouts -- and less concerned with theoretical details. Hence, if you have survived the proceeding discussion and equations, you have our congratulations (!) and assurance that you should experience clear sailing in the section ahead. In any case, you are STRONGLY URGED to read Section C carefully. It should help you appreciate the power of the DLS technique when applied to "difficult" particle size distributions, like those that are frequently encountered.

THE SIMPLEST APPROACH TO SIZE DISTRIBUTIONS: GAUSSIAN ANALYSIS

Uniform particle size- trivial analysis

In Figure 8a we see a video display of the 64-channel autocorrelation function obtained using DLS for a 90 nm (0.090 micron) polystyrene latex particle size standard.



Solid Particle

Run Time
hr:min:sec
0:15:21

Ch.1 Data
X1000
754

Count Rate
X1000
298

INTENSITY-WEIGHTED GAUSSIAN SUMMARY:

Mean Diam.[nm]	Coeff. of Var'n	Chi. Sq.	# Decays	Auto Baseline Adj.
87.752	0.049	2.076	2.178	0.552 %

Figure 8a: Autocorrelation function for 91-nm latex standard.

This particular sample has been chosen for this example because of its high uniformity of particle size -- i.e. it is nearly “monodisperse”.

Figure 8b shows a printout of the block of raw autocorrelation channel data corresponding to Figure 8a.

Figure 8b: Block of raw data corresponding to Figure 8a

The channel width, $\Delta t'$, for this particular run was 10 µsec (microseconds). It is instructive to verify that this autocorrelation function, $C(t')$, closely approximates the ideal result of a single decaying exponential function. To do this we plot $C(t')-B$ (B=baseline) versus t' (i.e. channel number) on semi-logarithmic graph paper. A perfectly straight line of negative slope should result, according to our previous discussion (Equation 7). This is shown in Figure 8c. The solid straight line has been drawn by eye to best approximate the slope established by the data points, $\log_e (C(t')-B)$. We have deliberately displaced the line below the points to permit the latter to be clearly seen.

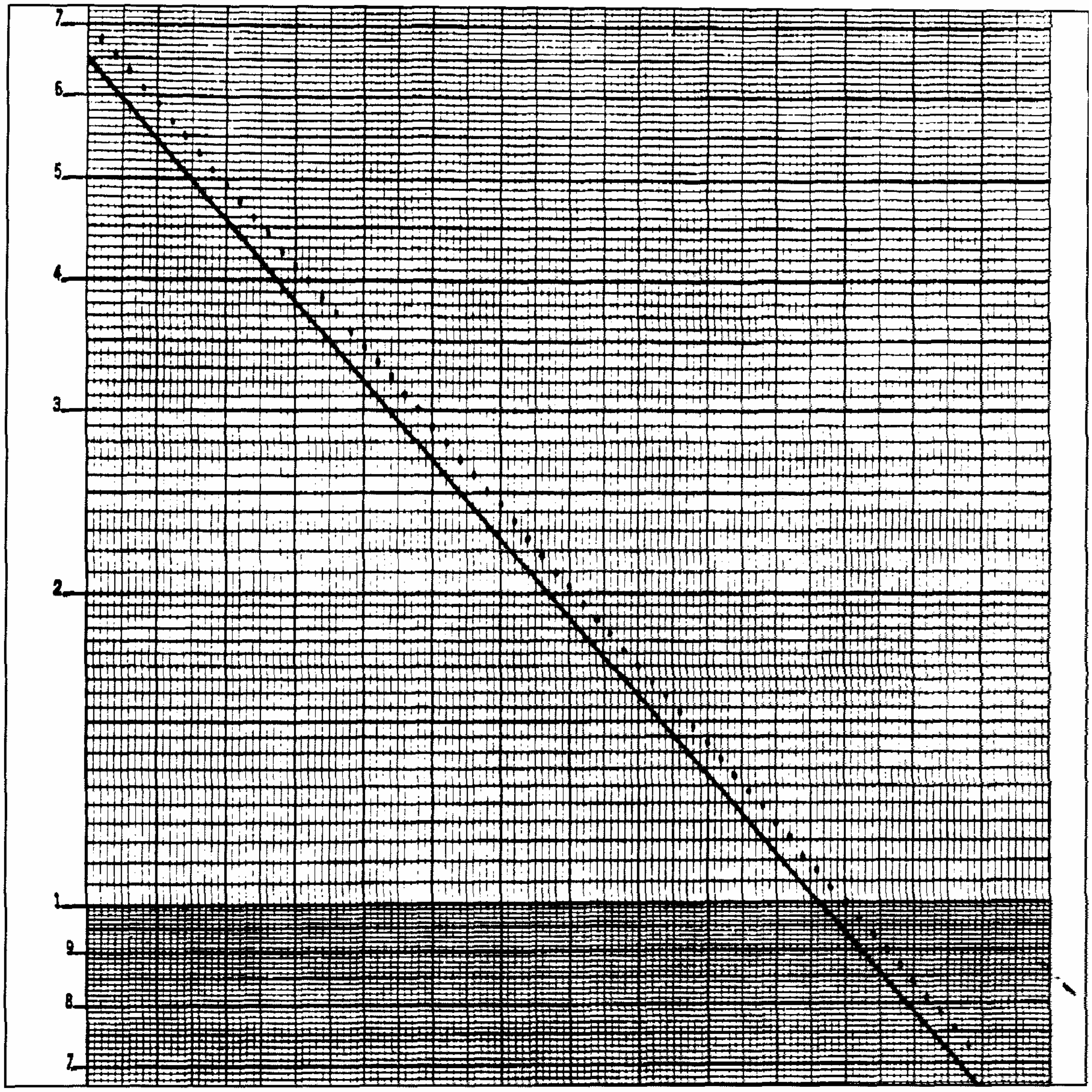


Figure 8c: Log_e(C(t')-B **)** vs t' for data of Figures 8a and 8b

Using Equations 7-9 we can now calculate the particle diameter predicted by the semilog behavior shown in Figure 8c. From Equation 7, we have

$$\log_e(\, C(t')-B\,) = 1og_e A - t'/\tau \qquad \text{(I0a)}$$
$$= \log_e A - 2DK^2 t' \qquad \text{(I0b)}$$

The slope in Figure 8c is $-2DK^2$, where $K^2 = 3.489 \times 10^{10}$cm- 2, we obtain the particle diffusivity, $D = 5.08 \times 10^{-8}$ cm^2/s. From Equation 9, with $T = 23^0$C and $\eta = 0.932 \times 10^{-2}$ poise, we obtain $R = 4.57 \times 10^{-6}$ cm, or 45.7 nm (457 Angstroms). This diameter of 91.4 nm agrees very well with the nominal size of this particular Dow polystyrene latex standard. (Its mean *volume-averaged* diameter is generally agreed to lie in the range 89-90 nary.)

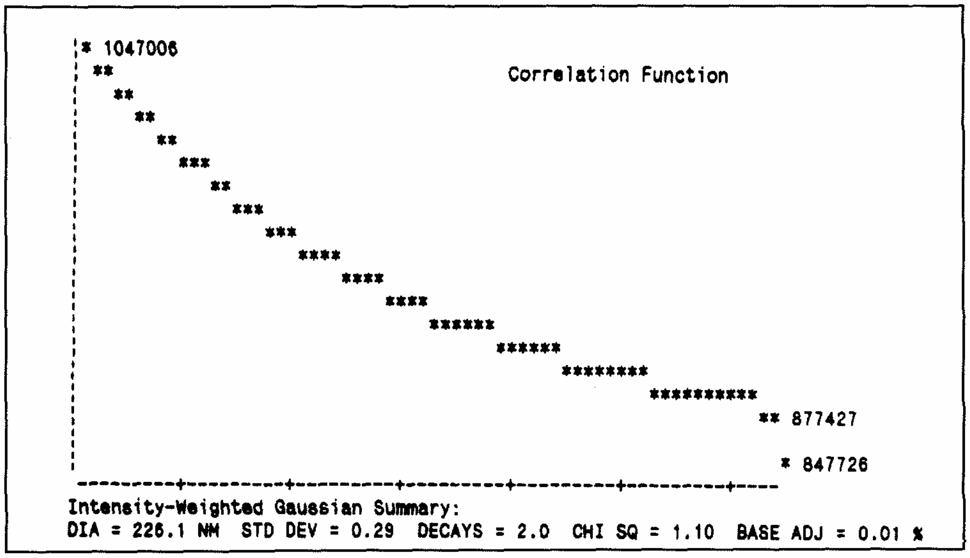
Unfortunately, the simple, straightforward analysis just discussed has only limited usefulness. As must be obvious to all but the most casual observers, most samples of practical interest differ appreciably from the uniform, “monodisperse” case discussed in the previous section. “Real” samples usually contain a range of sizes, often of substantial width, and are said to be “polydisperse”. Such a particle size distribution might be conceptually simple, consisting of a smooth, single-peak (“unimodal”) population of well defined mean diameter and width. Or, the distribution might be qualitatively more complex, resembling two discrete peaks (a “bimodal” distribution), or an even more complicated shape.

We shall see that two very different mathematical procedures, or “algorithms”, have been developed to analyze the autocorrelation “raw data”, C(t’), depending on the nature of the underlying particle size distribution. The software automatically selects the more appropriate of the two analysis procedures and provides the user with a running measure of the accuracy, or “goodness of fit”, of the computed distribution resulting from the particular analysis chosen. Nevertheless, we feel it essential to gain an appreciation of the rationale behind each of the analysis methods and to become comfortable with some typical results obtained for actual particle systems. The latter can be studied in a controlled, accurate way using polystyrene latexes, oil-in-water emulsions and other well-characterized materials.

Broad unimodal distribution Gaussian Analysis

Following the discussion in the previous section, it is now obvious that a mixture of particle sizes must give rise to an autocorrelation function C(t') which decaying exponential function is *no longer* a simple i.e. having a single, well-defined decay time constant τ, as shown in Figure 8c. The existence of more than one rate of diffusion must necessarily give rise to a mixture of decaying exponential functions, each of which has a different time decay constant τ_i corresponding to a particular diffusivity D and, hence, of particle radius R_i. The challenge which we face is to develop fast and efficient mathematical methods of analysis, whereby we can "deconvolve" C(t') and thereby extract the distribution of D values (and hence of particle diameters) from the detailed shape of C(t'). The "magic" behind the DLS Module has to do with its ability to obtain, accurately and consistently, the most useful information relating to the distribution of particle sizes in solution. To do this, the 380 must analyze precisely the deviations of autocorrelation function C(t') from single-exponential behavior. As we shall discover below, these deviations are often surprisingly slight and subtle, given the large range of complicated distributions which are encountered.

The simplest kind of complexity in the particle size distribution that we can introduce is a smooth, gaussian-like population of sizes, having a well-defined mean diameter and half width. Such an idealized distribution shape is often obtained for *emulsions,* prepared by a variety of processes, sonication, homogenization and Microfluidization^{TM}. Typically, some type of oil and water are caused to be mixed together with the aid of a dispersing agent (e.g. a non-ionic surfactant) to form a single, microscopically homogeneous phase. The result: tiny droplets of one component (e.g. the oil) suspended in the other component, or "phase" (e.g. water). The mean size and width of the resulting droplet distribution are usually sensitive functions of the stoichiometry of the starting compounds and the duration and detailed nature of the preparation technique employed. In Figure 9a we show the autocorrelation function for a fat emulsion, used for intravenous ("IV") feeding. The channel width used here was 21 usec.



```
Intensity-Weighted Gaussian Summary:
DIA = 226.1 NM  STD DEV = 0.29  DECAYS = 2.0  CHI SQ = 1.10  BASE ADJ = 0.01 %
```

Figure 9a: Autocorrelation function for an IV fat emulsion.

Let us make a visual comparison between Figure 9a and 8a, obtained for the narrow 91 nm latex standard. The shapes of the two decaying curves appear to be quite similar, which is somewhat surprising given the differences between the two samples. Qualitatively, we conclude that the average, or characteristic, particle diameter associated with Figure 9a must be roughly twice that associated with Figure 8a. The reason: both curves possess about the same number of “decays” in falling to the 64th channel, and the channel width for the latter sample is *twice* that of the former.

We can acquire a better appreciation of the subtlety of the analysis task which faces us by looking at the reduced data, C(t’)-B, on a semilog plot in Figure 9b and comparing this with the previous reduced data for the narrow 91-nm latex standard, Figure 8c. Indeed, we must look closely to find any qualitative difference between the two plots! However, on closer examination we see that the reduced data points in Figure 9b possess a slight curvature; a displaced straight line has been drawn to enable this curvature to be seen. As we shall see in a moment, this fat emulsion sample does indeed possess a substantial width, or range of sizes, in its particle size distribution. Therefore, what may seem surprising (and, perhaps, intimidating) is how relatively little deviation there is from the single-exponential behavior of C(t’) in Figure 9b, given the apparently large qualitative difference in distribution shapes between the “sharp” latex standard and the “broad” emulsion sample.

The example above illustrates the inherent difficulty which all DLS-based particle sizing instruments face: what distinguishes the shape of one computed particle size distribution from another is often a relatively subtle deviation of C(t’) from single exponential behavior. Hence, we MUST learn to appreciate the importance of acquiring data of high statistical accuracy.

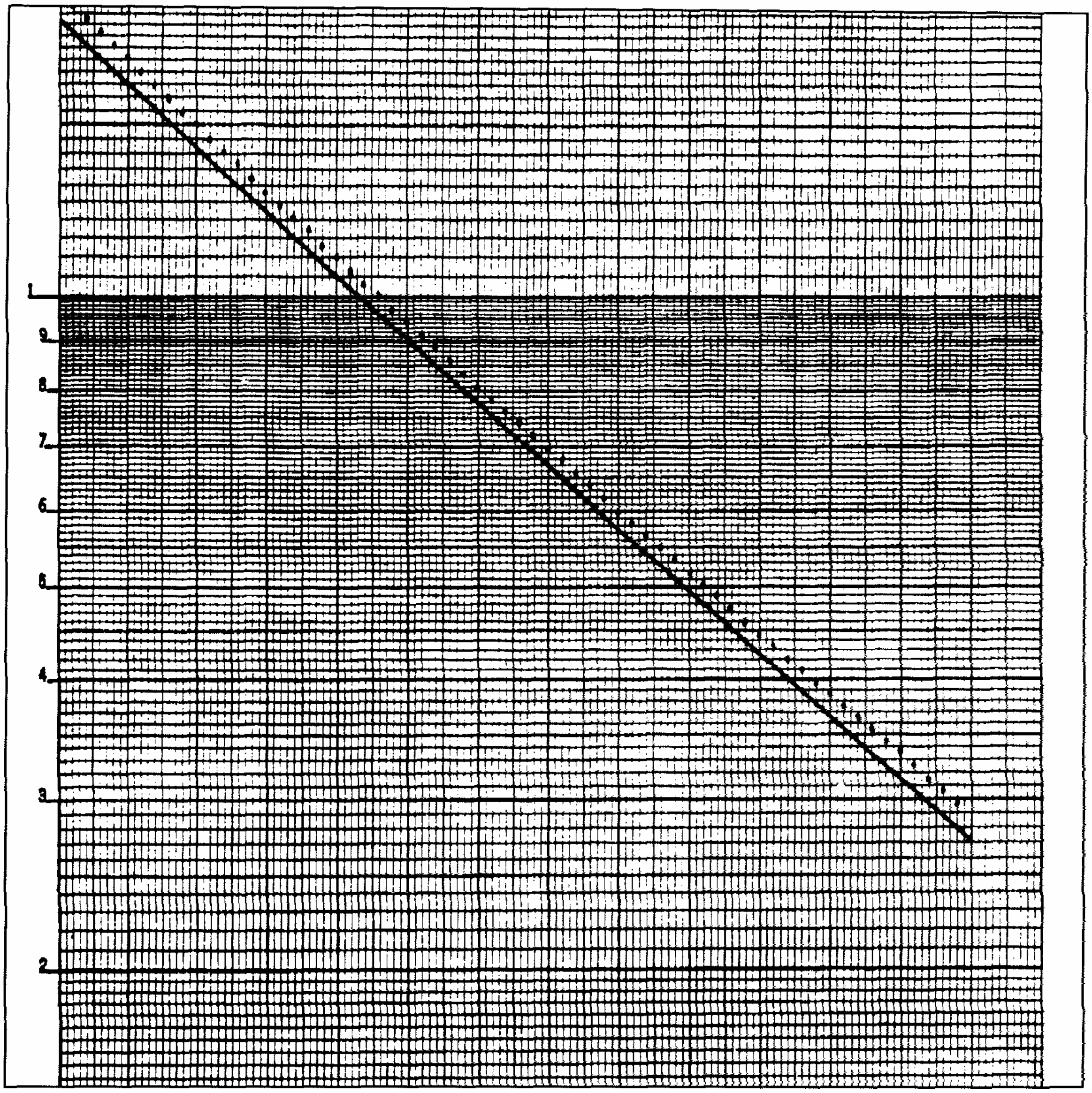


Figure 9b: Log$_e$(C(t')-B) vs t' for data of Figure 9a.

This requirement is intimately related to the run time and the efficacy of sample preparation. Not surprisingly, it turns out that there are NO 'short cuts" to obtaining good raw data and, hence, good analyses. Suffice to say, there is more than meets the eye in the successful extraction of particle size distributions using the DLS technique! More evidence will unfold below, supporting the claim that this can be a difficult business. Nonetheless, let us take courage and press on.

What is needed, clearly, is a method for dealing with the “simple” kind of polydispersity in the particle size distribution illustrated by the IV emulsion example above. The word “simple” is used to emphasize the fact that we have gone from a sharp population, consisting of essentially one size, to one which represents a smooth, not-too-wide range of sizes centered about some average. In the case of a sharp distribution, it is a simple matter to obtain the “best” straight-line fit to the logarithm of the reduced data, loge C(t’)-B) vs t’, using the well-known method of least squares. One simply adjusts the slope and intercept of the straight line to minimize the sum of the squares of the deviations, or errors, between the reduced data points and the values implied by the “theory” -- i.e. by the straight line.

The needed generalization which can deal effectively with non-exponential behavior of C(t’)-B, brought about by smooth, Gaussian-like distributions of particle diameters, is provided by the methods of cumulants. This procedure, first introduced by Koppel, has been used extensively in the past 15 years to obtain estimates of the particle size distribution from DLS. In fact, until only 5 or 6 years ago it was essentially the *only* practical method for obtaining such information. The conceptual underpinning of the cumulants procedure is simplicity itself, as will be seen below.

Suppose we consider situations for which the plot of $\log_e$ **(** C(t’)-B **)** has a relatively small curvature, representing a modest deviation from straight-line behavior. The simplest generalization of the straight-line fitting procedure is to find the *quadratic* function of C which lies closest to the reduced data points (i.e. on a least-squares basis). The prescription for carrying out a cumulants fit is, therefore, very simple, as summarized below:

$$1/2 \log_e (C(t')-B) \leftrightarrow a_0 + a_1(t') + a_2(t')^2 \qquad (11)$$

A quadratic function of C, which we’ve indicated by $a_0 + a_1(t') + a_2(t')2$, now replaces the trivial straight-line function, $b_0 + b_1(t')$. All that remains is to relate the coefficients of the quadratic function -- in particular, a_1 and a_2 -- to parameters that describe the corresponding particle size distribution.

In the simple monodisperse case discussed earlier, we recall (Equations l0a and b) that coefficient b_1 equals $-DK^2$. Hence, the value of the slope (negative), divided by the constant K^2, yields the diffusion coefficient D of the (uniform) particles. It turns out in the more general case of a quadratic fit that the distribution of diffusion coefficients D_i is approximately equal to a Gaussian, or normal, shape. This is a bell-shaped distribution that requires only two parameters for its full description (not including the peak magnitude, which is arbitrarily set equal to 100 for

all of our *relative* particle size distributions). These are the mean diffusivity, D, and the half width ΔD of diffusivity values. (Strictly speaking, the cumulants fit results in an approximately Gaussian distribution of *intensity-weighted* diffusivities. This point will be discussed later.)

The connection between coefficients a_1 and a_2, obtained from the best quadratic fit to the logarithm of the reduced data, and the parameters $\bar{D}$ and ΔD of the Gaussian distribution of diffusivities, is given by

$$a_1 = -\bar{D}K^2 \qquad (12a)$$

or, $$\bar{D} = -a_1/K^2 \qquad (12b)$$

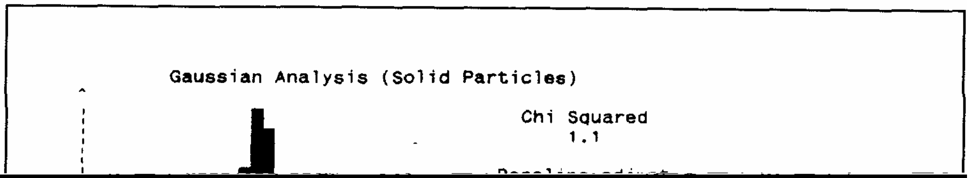
and $$\overline{\Delta}D/D = 2a_2/(-a_1) \qquad (13)$$

Equation 13 gives the normalized standard deviation (or coefficient of variation) of the diffusivity distribution -- i.e. standard deviation ΔD divided by the mean diffusivity D. Naturally, the width parameter ΔD is related to coefficient a_2, which describes the extent of curvature in the reduced data. For distributions that are very narrow -- nearly monodisperse -- we expect a_2 to be very small, so that the quadratic function effectively reduces to being a straight line.

Ultimately one wishes to have the result expressed in terms of a distribution of particle radii R (or diameters), rather than of diffusivities D. This is not a problem, of course, because the Stokes-Einstein relation (Equation 2) shows that D is simply given by 1/R times a conversion constant. Furthermore, for relatively small ranges of D a Gaussian distribution in 1/R translates into a Gaussian shape in ln R. Hence, we arrive at approximately a log-normal shape for the distribution of particle radii (or diameters). Using the Stokes-Einstein relation (Equation 2) and Equations 12b and 13 we can therefore obtain the mean particle diameter, $d = 2\bar{R}$, and the standard deviation of the diameter distribution, $2 * \Delta R$. This latter parameter is also known as the “coefficient of variation” and is equal to the square root of the “variance”; it is closely related to the half width of the particle size distribution, which is approximately a log normal in shape.

In the DLS Module we refer to this cumulants method for “inverting” the autocorrelation function as the *Gaussian Analysis.* It must be stressed that it is 2-parameter fit; that is, except for the possibility of a change in the baseline, there are only *two* variables which affect the goodness of fit of the quadratic function of C with respect to the reduced data, $\log_e (C(t')-B)$ (Equation 11). These are coefficients a_1 and a_2. (Coefficient a_0 has relatively little value in the analysis -- it is related to the contents of channel #1 of C(t’), which increases with the total run time, all other variables being equal.)

The result of the Gaussian Analysis when applied to the autocorrelation data of Figs. IV-9a,b is shown in Figure I0a.



[redacted]

Figure I0a: Intensity-weighted Gaussian Analysis corresponding to the data of Figure 9a and b.

This is the summary under the decaying curve C(t'), which is updated approximately every 30 seconds on the video display. It carries the label "Intensity Weighting" because it represents the immediate result of the cumulants calculation, before any specific type of particle weighting is taken into consideration. That is, the underlying autocorrelation function C(t') is constructed from the original scattered *intensity* values as a function of time. Hence, the quadratic fit and the corresponding Gaussian-like representation of the distribution of particle diffusivities (and, ultimately, diameters) reflect the fact that the D contributions, or R contributions, are weighted by their corresponding scattering intensities. Again, the peak shown in Figure I0a is approximately a Gaussian shape with respect to the log diameter scale -- i.e., it is approximately a log normal shape (provided the standard deviation is not excessive -- < 25%, or so, of the mean value).

There are 6 pieces of quantitative information contained in the summary display of the Gaussian Analysis (Figure I0a):

Mean Diameter	226.1 nm
Standard Deviation	30%
Chi Squared	1.1
Baseline Adjust	0.01 %
Data	1047.0 K
Mean Diffusion	2.05 X 10^{-8} cm^2/sec

The value 30% for the standard deviation means that $\Delta R/R=0.30$.

A critical piece of information contained in Figure I0a is the value of Chi Squared. Any value close to (or below) 1.0 indicates an exceptionally good fit of the quadratic function to the reduced data. Assuming that sufficient statistics have been collected in the autocorrelation function to make the value of Chi Squared meaningful (which is NOT true early into a run), a low value means that the Gaussian representation of the particle size distribution is a good assumption -- i.e. that no other distribution shape can offer a “better” fit to the data, given the limitations implied by Poisson statistics. Indeed, the test of a good Gaussian Analysis result is whether Chi Squared remains small (i.e. below 2 or 3) over the course of time, as additional intensity values are collected and incorporated into autocorrelation function C(t’). The DLS Module makes this judgment automatically and will provide an appropriate warning message if the value of Chi Squared exceeds 3, suggesting that the Gaussian Analysis result is inappropriate.

The value for Baseline Adjust shown above, 0.01 *%,* is very close to the ideal value of zero, indicating that almost no adjustment in the value of B was needed to obtain the lowest value of Chi Squared. Hence, this particular sample contained a negligible concentration of large-particle aggregates or other contaminants.

The value for Data, 1047 K, represents the contents of channel #1 of the autocorrelation function, C(t’). Such a relatively large value, exceeding one million (=1000 K), usually indicates a high degree of statistical accuracy in C(t’). At this point, the results of the Gaussian Analysis should have become stable, with relatively little change with additional run time. In the example summarized above, 1047 K was achieved in channel #1 after a run time of 14 mm 48 sec. The relationship between the value of Data and the run time will in general depend on the average scattered light intensity and the channel width $\Delta t'$.

Effects of weighting in the Gaussian Analysis

The problem with an intensity-weighted particle size distribution is that it is generally not very useful in the “real” world. Typically, one wishes to obtain a size distribution that is either weighted by particle number (a trivial “weighting”) or by particle volume, or mass (i.e. weighted by diameter cubed). The DLS Module uses the “rules” of light scattering (including corrections for Mie scattering, when intraparticle interference effects become important, for diameters greater than about 150 nm) in order to obtain number-weighted and volume-weighted diameter distributions from the starting result, which is an intensity-weighted plot. **Note:** “Volume” weighting and “mass” weighting are equivalent terms.

The volume-weighted and number-weighted video displays corresponding to the example discussed above are shown in Figure I0b and I0c, respectively.

The three display plots, Figure I0a, b and c, reveal an important property that is generally observed when using the Gaussian Analysis. That is, the value of the mean diameter can vary significantly with the choice of weighting, depending on the width of the Gaussian-like distribution. In general, the wider the distribution (i.e. the larger the percentage standard deviation), the greater the differences in mean diameter for the various weightings. In the present example we see that the mean diameter of the volume-weighted peak (209. 1 nm) is shifted almost 10% below that of the intensity-weighted result (226.1 nm). The number-weighted mean diameter is reduced even more dramatically, to 144.7 nm.

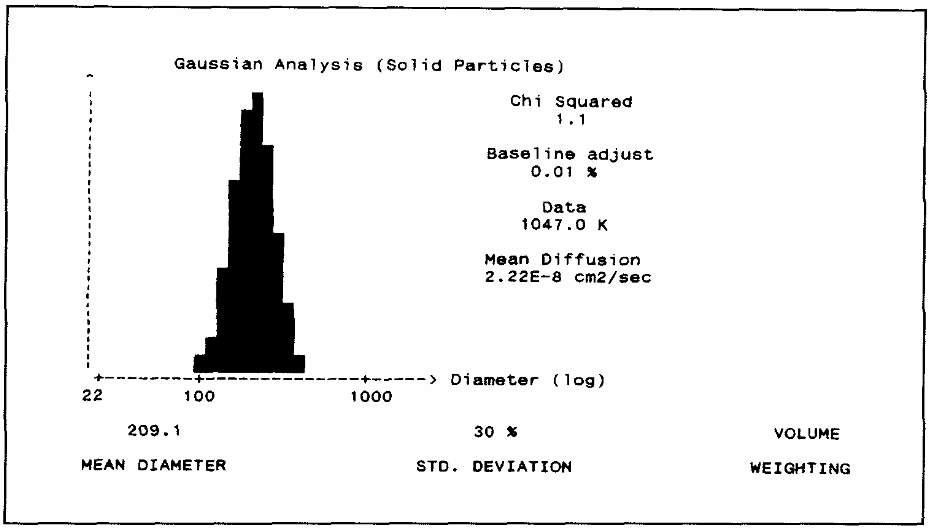
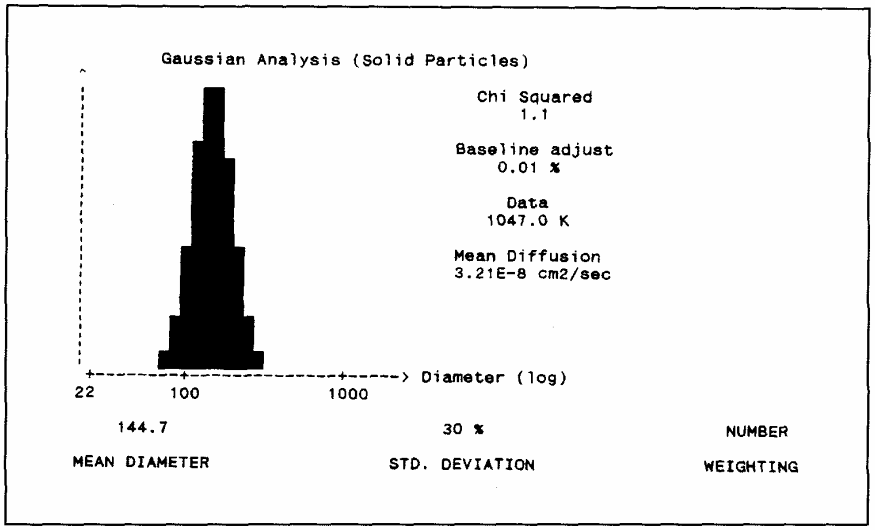


Figure I0b: Volume-weighted Gaussian Analysis corresponding to Figure I0a and data of Figures 9a and b.

This behavior can be easily understood qualitatively by reviewing the relationship between particle size and scattering intensity, discussed in the previous section. For particle sizes sufficiently small to permit neglect of the Mie "form factor" (arising from intraparticle interference of individual scattered waves), we see from Equation Ib that the contribution to C(t') of a particular decaying exponential, corresponding to a given diameter d_i, should be weighted by the factor $N_i(V_i)^2$, where N_i represents the number of particles having diameter d_i, and V_j is their individual volume, which is proportional to $(d_i)^3$. Hence, each diameter "slice" of the starting intensity-weighted distribution has associated with it this factor of $N_i(V_i)^2$



Gaussian Analysis (Solid Particles)

Chi Squared
1.1

Baseline adjust
0.01 %

Data
1047.0 K

Mean Diffusion
3.21E-8 cm2/sec

144.7
MEAN DIAMETER

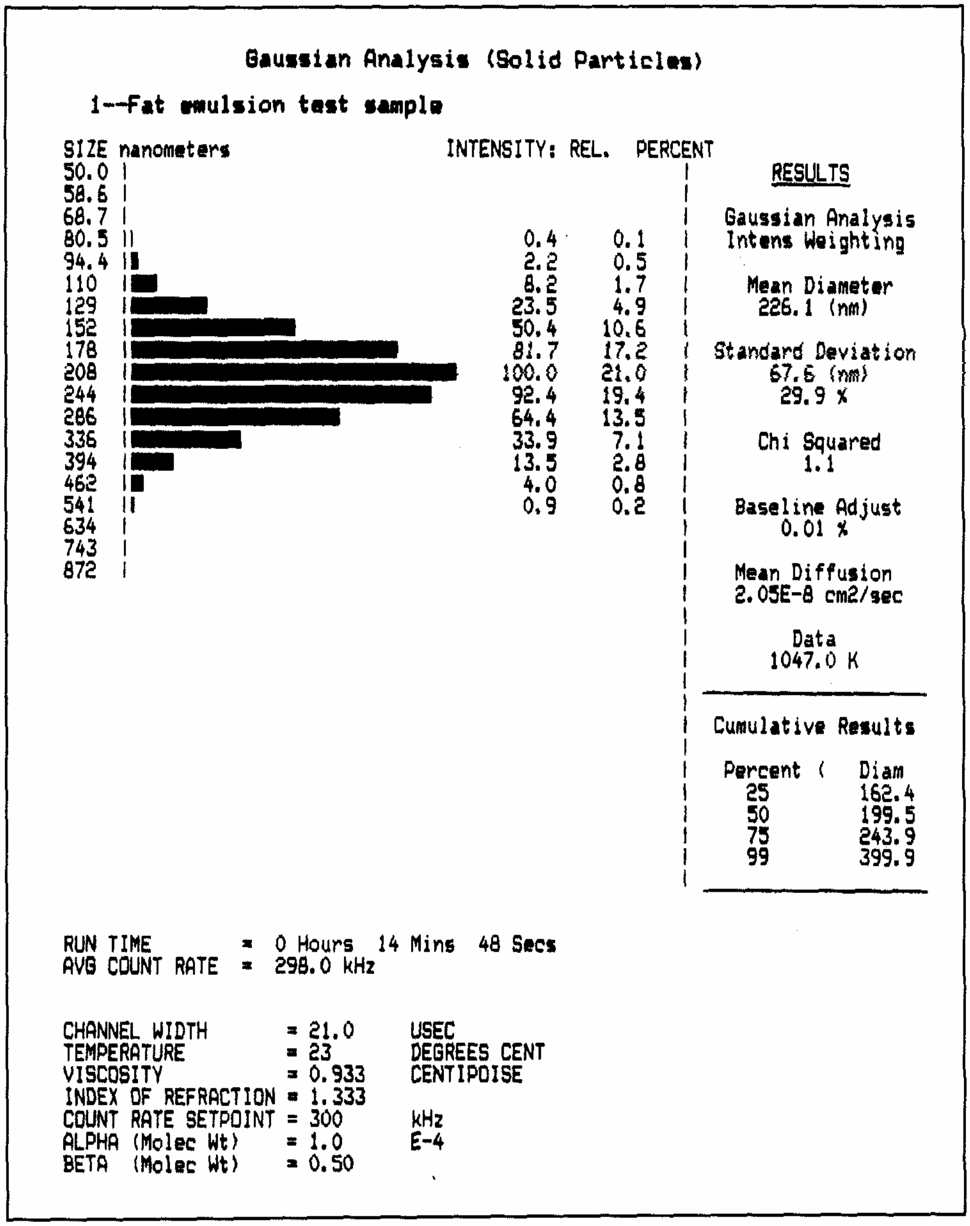
30 %
STD. DEVIATION

NUMBER
WEIGHTING

Figure I0c: Number-weighted Gaussian Analysis corresponding to Figure I0a and data of Figure 9a and b.

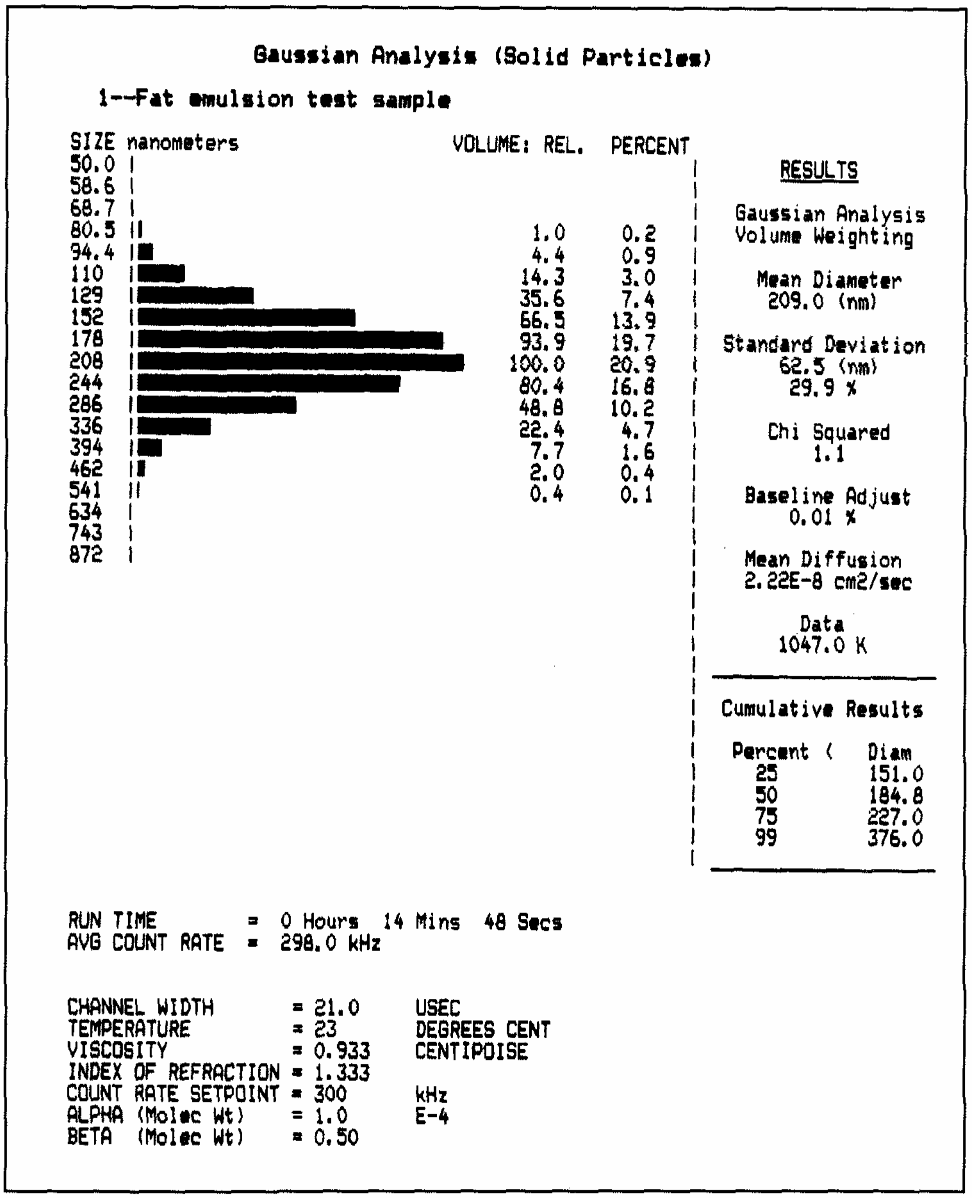
The constituent diameters of the volume-weighted distribution should each have the weighting factor N_iV_i -- i.e. the number of particles having a particular diameter multiplied by the particle volume, or mass, for that diameter. These new factors are obtained from the previous intensity-weighted factors by dividing each by V_i, or $(d_i)^3$. The resulting (approximate) distribution is shown above in Figure I0b. To obtain the number-weighted result, we perform one additional division of the weighting factors by V_i or $(d_i)^3$, to obtain a final weighting factor of just N_i, shown in Figure I0c above. In the case in which the intraparticle form factor cannot be ignored, it must first be divided out of the original intensity-weighted factors.

The printouts that correspond to the three weightings discussed above are shown in Figure 11a, b and c. The various distribution plots are labeled with two sets of numbers. The “REL” set is normalized such that the peak value is always 100. The “PERCENT” set is normalized such that the sum of all of the numbers equals 100%.



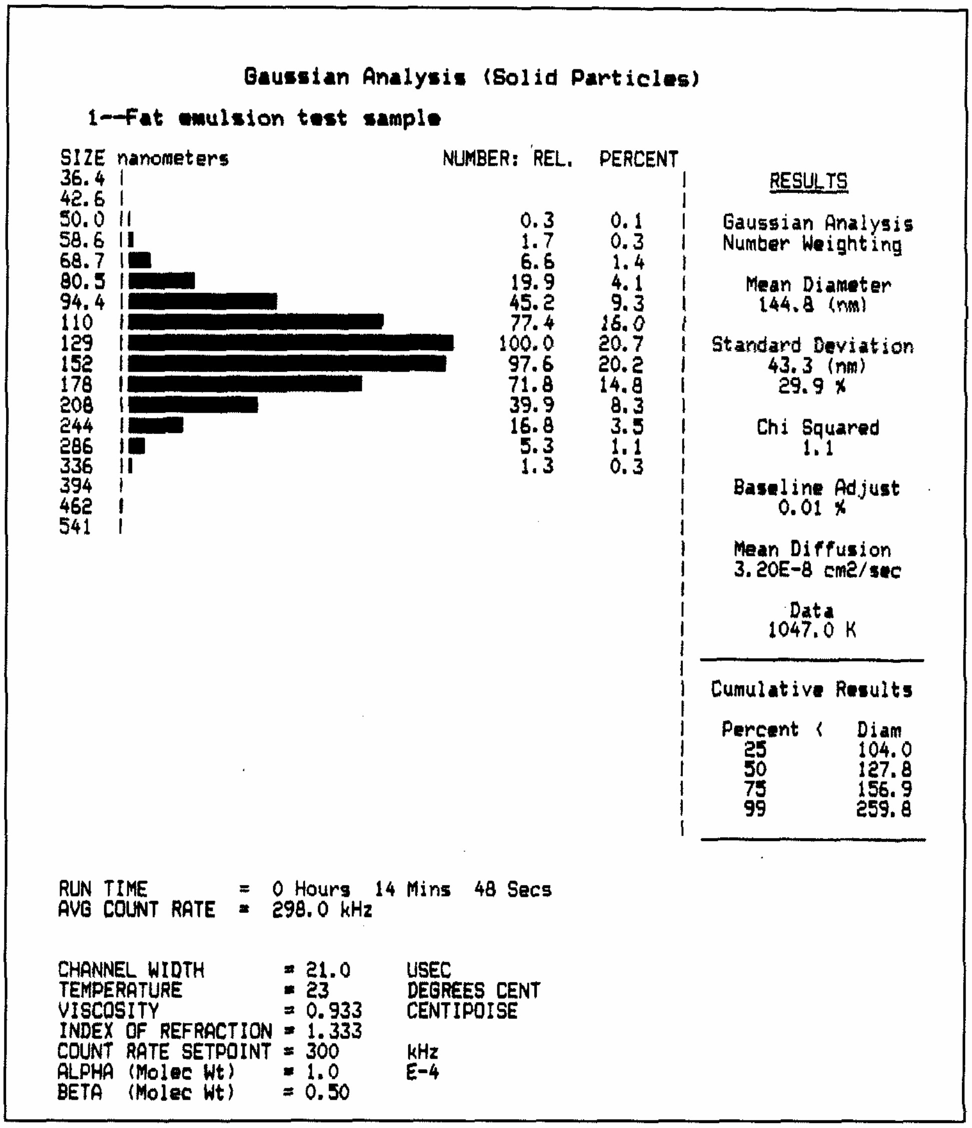
```
                Gaussian Analysis (Solid Particles)

  1--Fat emulsion test sample

SIZE nanometers            INTENSITY: REL.  PERCENT
50.0 |                                          |        RESULTS
58.6 |                                          |
68.7 |                                          |   Gaussian Analysis
80.5 ||                        0.4      0.1     |   Intens Weighting
94.4 |                         2.2      0.5     |
110  |                         8.2      1.7     |     Mean Diameter
129  |                        23.5      4.9     |     226.1 (nm)
152  |                        50.4     10.6     |
178  |                        81.7     17.2     |  Standard Deviation
208  |                       100.0     21.0     |     67.6 (nm)
244  |                        92.4     19.4     |     29.9 %
286  |                        64.4     13.5     |
336  |                        33.9      7.1     |     Chi Squared
394  |                        13.5      2.8     |        1.1
462  |                         4.0      0.8     |
541  ||                        0.9      0.2     |   Baseline Adjust
634  |                                          |      0.01 %
743  |                                          |
872  |                                          |   Mean Diffusion
                                                |   2.05E-8 cm2/sec
                                                |
                                                |        Data
                                                |      1047.0 K
                                                |
                                                | ___________________
                                                |
                                                |  Cumulative Results
                                                |
                                                |   Percent (    Diam
                                                |   25           162.4
                                                |   50           199.5
                                                |   75           243.9
                                                |   99           399.9
                                                | ___________________


RUN TIME             =  0 Hours  14 Mins  48 Secs
AVG COUNT RATE       =  298.0 kHz


CHANNEL WIDTH        = 21.0      USEC
TEMPERATURE          = 23        DEGREES CENT
VISCOSITY            = 0.933     CENTIPOISE
INDEX OF REFRACTION  = 1.333
COUNT RATE SETPOINT  = 300       kHz
ALPHA (Molec Wt)     = 1.0       E-4
BETA  (Molec Wt)     = 0.50
```

Figure 11a: Printout of volume-weighted Gaussian Analysis result for fat emulsion (See Figure I0a.)



```
                 Gaussian Analysis (Solid Particles)

   1--Fat emulsion test sample

  SIZE nanometers                VOLUME: REL.  PERCENT
  50.0                                                      RESULTS
  58.6
  68.7                                                  Gaussian Analysis
  80.5                                1.0      0.2      Volume Weighting
  94.4                                4.4      0.9
  110                                14.3      3.0        Mean Diameter
  129                                35.6      7.4         209.0 (nm)
  152                                66.5     13.9
  178                                93.9     19.7     Standard Deviation
  208                               100.0     20.9         62.5 (nm)
  244                                80.4     16.8         29.9 %
  286                                48.8     10.2
  336                                22.4      4.7        Chi Squared
  394                                 7.7      1.6            1.1
  462                                 2.0      0.4
  541                                 0.4      0.1       Baseline Adjust
  634                                                        0.01 %
  743
  872                                                     Mean Diffusion
                                                         2.22E-8 cm2/sec

                                                             Data
                                                           1047.0 K

                                                      Cumulative Results

                                                      Percent (    Diam
                                                        25        151.0
                                                        50        184.8
                                                        75        227.0
                                                        99        376.0

  RUN TIME         =  0 Hours  14 Mins  48 Secs
  AVG COUNT RATE   =  298.0 kHz

  CHANNEL WIDTH       = 21.0     USEC
  TEMPERATURE         = 23       DEGREES CENT
  VISCOSITY           = 0.933    CENTIPOISE
  INDEX OF REFRACTION = 1.333
  COUNT RATE SETPOINT = 300      kHz
  ALPHA (Molec Wt)    = 1.0      E-4
  BETA  (Molec Wt)    = 0.50
```

Figure 11b: Printout of volume-weighted Gaussian Analysis result for fat emulsion. (See Figure I0b.)



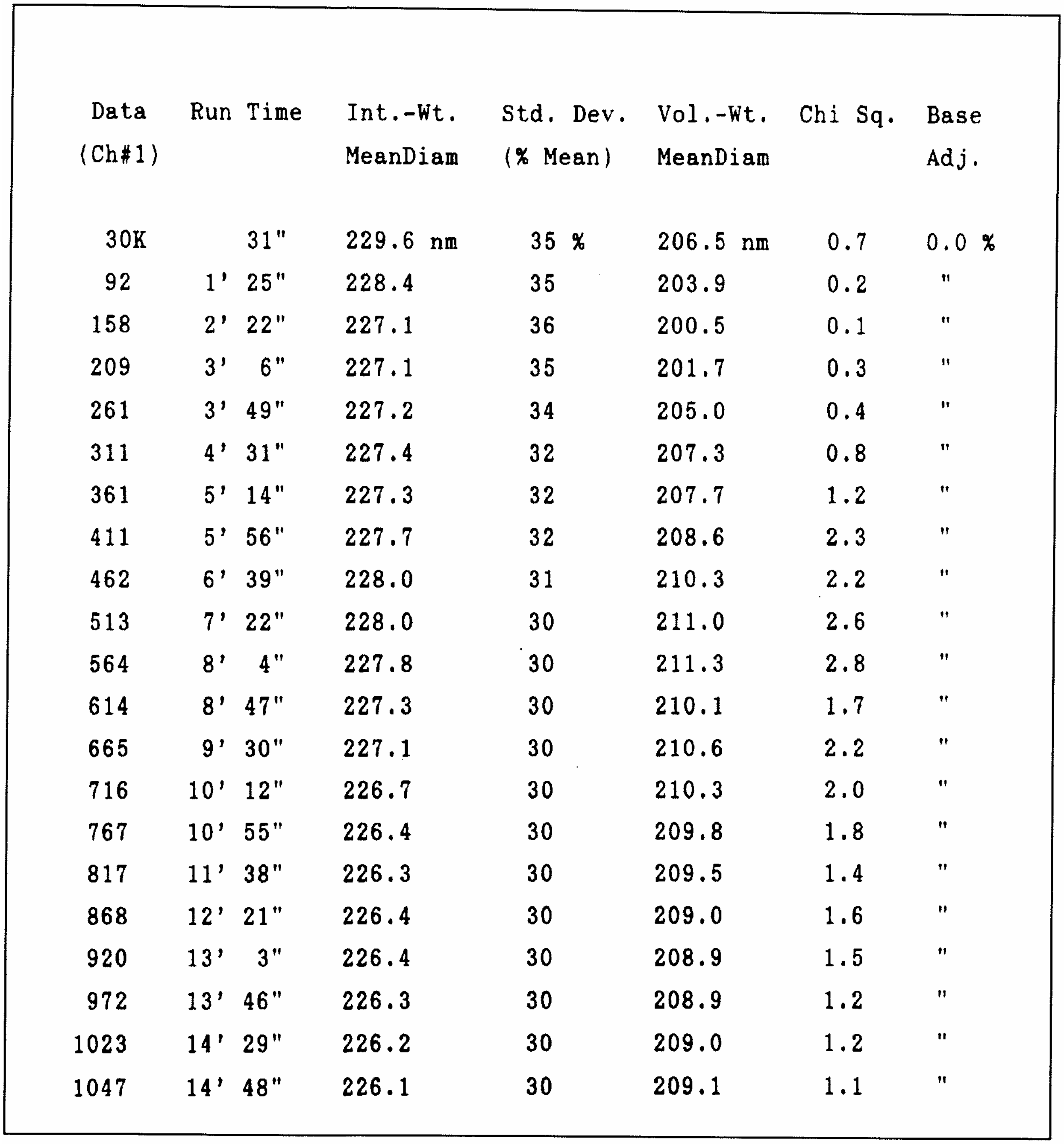
```
                 Gaussian Analysis (Solid Particles)

   1--Fat emulsion test sample

  SIZE nanometers                    NUMBER: REL.  PERCENT
  36.4 |                                                   |     RESULTS
  42.6 |                                                   |
  50.0 |                                0.3      0.1       |  Gaussian Analysis
  58.6 |                                1.7      0.3       |  Number Weighting
  68.7 |                                6.6      1.4       |
  80.5 |                               19.9      4.1       |   Mean Diameter
  94.4 |                               45.2      9.3       |   144.8 (nm)
  110  |                               77.4     16.0       |
  129  |                              100.0     20.7       |  Standard Deviation
  152  |                               97.6     20.2       |     43.3 (nm)
  178  |                               71.8     14.8       |     29.9 %
  208  |                               39.9      8.3       |
  244  |                               16.8      3.5       |     Chi Squared
  286  |                                5.3      1.1       |        1.1
  336  |                                1.3      0.3       |
  394  |                                                   |   Baseline Adjust
  462  |                                                   |      0.01 %
  541  |                                                   |
                                                           |   Mean Diffusion
                                                           |   3.20E-8 cm2/sec
                                                           |
                                                           |       Data
                                                           |     1047.0 K
                                                           |
                                                           |  Cumulative Results
                                                           |
                                                           |   Percent <   Diam
                                                           |     25        104.0
                                                           |     50        127.8
                                                           |     75        156.9
                                                           |     99        259.8
                                                           |

  RUN TIME        =  0 Hours  14 Mins  48 Secs
  AVG COUNT RATE  =  298.0 kHz

  CHANNEL WIDTH       = 21.0      USEC
  TEMPERATURE         = 23        DEGREES CENT
  VISCOSITY           = 0.933     CENTIPOISE
  INDEX OF REFRACTION = 1.333
  COUNT RATE SETPOINT = 300       kHz
  ALPHA (Molec Wt)    = 1.0       E-4
  BETA  (Molec Wt)    = 0.50
```

Figure 11c: Printout of number-weighted Gaussian Analysis result for fat emulsion. (See Figure I0c.)

It MUST be re-emphasized that the distribution shapes shown in Figure I0a,b and c and Figure 11a,b,c are obtained from the tabulations for the Gaussian, or normal, curve found in standard mathematical references. Their shape is an idealization and should NEVER be construed to offer more detailed information than can legitimately be expected from this fitting procedure. The peak shown in Figure 11b, for example, contains 13 discrete diameter slices, each evidently possessing a well-defined height, or relative volume. In a way, this is misleading; this shape has emerged from what is fundamentally just a 2-parameter fit (plus, possibly, a baseline adjust). Hence, we should NEVER be lulled into believing the actual heights of each of the slices that comprise the distribution curve. These values have originated from an idealized mathematical table, *not* from a measurement procedure which counts particles one at a time. Because the DLS technology is unable to measure particles individually, it yields only smoothed, idealized *estimates* of the actual particle size distribution curves. Nevertheless, the DLS Module will usually be found to be very sensitive to small changes in the shape of the underlying particle size distribution.

Importance of acquiring data of sufficient accuracy

A central, repeated theme of this section will be the importance of acquiring autocorrelation data of high statistical accuracy. If this is achieved, the resulting computed particle size distributions will be found to be stable in time. As we shall see, the level of statistical accuracy required for a reliable analysis depends greatly on the analysis method chosen. Because the Gaussian Analysis is only a 2-parameter fit (with the possible addition of a baseline adjustment), it will settle to a reliable result relatively quickly -- certainly faster than the NJCOMP Distribution Analysis (discussed in the next section). This simply reflects the fact that the best quadratic fit to the reduced autocorrelation data is relatively insensitive to small variations in individual data points, given a total of 64 points for each fit. A good illustration of the typical settling time of the Gaussian Analysis is provided by a tabulation of the results obtained for the IV fat emulsion discussed above. A new computed distribution was obtained approximately every *45* seconds, whenever a fresh set of analysis results was displayed on the video terminal; a summary of these results is shown below in Table 1.



Data (Ch#1)	Run Time	Int.-Wt. MeanDiam	Std. Dev. (% Mean)	Vol.-Wt. MeanDiam	Chi Sq.	Base Adj.
30K	31"	229.6 nm	35 %	206.5 nm	0.7	0.0 %
92	1' 25"	228.4	35	203.9	0.2	"
158	2' 22"	227.1	36	200.5	0.1	"
209	3' 6"	227.1	35	201.7	0.3	"
261	3' 49"	227.2	34	205.0	0.4	"
311	4' 31"	227.4	32	207.3	0.8	"
361	5' 14"	227.3	32	207.7	1.2	"
411	5' 56"	227.7	32	208.6	2.3	"
462	6' 39"	228.0	31	210.3	2.2	"
513	7' 22"	228.0	30	211.0	2.6	"
564	8' 4"	227.8	30	211.3	2.8	"
614	8' 47"	227.3	30	210.1	1.7	"
665	9' 30"	227.1	30	210.6	2.2	"
716	10' 12"	226.7	30	210.3	2.0	"
767	10' 55"	226.4	30	209.8	1.8	"
817	11' 38"	226.3	30	209.5	1.4	"
868	12' 21"	226.4	30	209.0	1.6	"
920	13' 3"	226.4	30	208.9	1.5	"
972	13' 46"	226.3	30	208.9	1.2	"
1023	14' 29"	226.2	30	209.0	1.2	"
1047	14' 48"	226.1	30	209.1	1.1	"

Table 1: Time dependence of the Gaussian analysis results for a typical unimodal sample (IV fat emulsion)

The results shown in Table 1 for a well-behaved fat emulsion nicely illustrate some of the characteristics of the Gaussian Analysis, discussed earlier. First, we notice that the parameter Chi Squared *cannot* be used to judge the stability and/or quality of the fit results if too little data has been acquired. Here, just 31 seconds into the run, the very low value of Chi Squared is potentially misleading in suggesting that the Gaussian Analysis has already produced final" results, of high quality, with settled values of the Mean Diameter and Standard Deviation. In fact, Chi Squared later *increases,* to a high of 2.8 at the later time of 8 min 4 see, when substantial amounts of additional Data have been incorporated into the autocorrelation function *(564K* in Channel #1). However, what is meaningful is the fact that this rise is obviously spurious, since it is followed by consistently lower values; Chi Squared falls back essentially to unity (1.1) after 13-14 minutes into the run. Clearly, what matters in establishing the validity of the Gaussian Analysis result for this sample is the fact that Chi Squared *remains low* with increasing data acquisition, showing no tendency to grow with time.

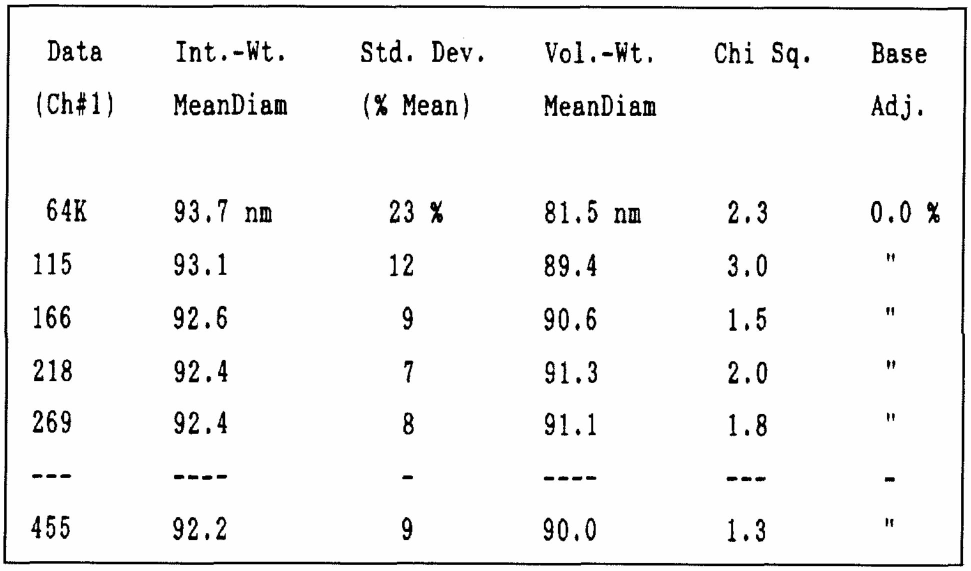
Second, we verify from Table 1 that the intensity-weighted Mean Diameter (colt 3) settles very quickly to a reliable value. After just a couple of minutes, all succeeding values are within 1 % of the "settled" value of approximately 226nm. On the other hand, the exhibits considerably initial few minutes of difference is the fact the "settled" value of approx. 226 nm. volume-weighted Mean Diameter (col. *5)* more variation (up to 4%) during the data acquisition. The reason for this that the Standard Deviation has not yet settled to a constant value. Clearly, a higher degree of statistical accuracy (i.e. signal/noise ratio) is required in the autocorrelation function to establish the value of the "curvature" coefficient, a_2, in the least-squares quadratic fit (Equation 11), than is needed to fix the value of the linear coefficient, a_1. Consequently, early into the run, we observe a 20% variation in the Standard Deviation (coming from a_2), as opposed to less than a 2% fluctuation in the intensity-weighted Mean Diameter (from a_1).

This affects the results in two ways. First, the relatively large values of the Standard Deviation (30 to 35% of the Mean Diameter) serve to "push" the volume-weighted Mean Diameter fully 10% below the intensity-weighted value, to approximately 209 nm. Second, because of the substantial fluctuation in the computed Standard Deviation in the early stages of data acquisition, the volume-weighted Mean Diameter fluctuates considerably more than does the intensity-weighted value. However, regarding these fluctuations, a simple rule applies: allow more time! Obviously, the sample represented in Table 1 is rather well behaved, requiring no Baseline Adjust and yielding good results very early into the run, close to the final, settled

values. Nevertheless, the comments above apply equally well to more “difficult” samples, for which the results of the Gaussian Analysis show larger fluctuations and require a longer time to settle.

Next, we examine another sequence of results obtained from the Gaussian Analysis as a function of accumulating data -- this time returning to the narrow, nearly monodisperse 91nm latex standard. It is useful to compare these results, summarized in Table 2, with those just discussed for the broad fat emulsion.

Again, as was found for the broad fat emulsion, the value of Chi Squared is a poor indication of the extent of settling of the Gaussian Analysis results at the beginning of a run (Data = 64K), when insufficient data has been collected. The abnormally large value of the Standard Deviation (23%) the result, again, of insufficient data -- causes the volume-weighted Mean Diameter to be pushed well below the intensity-weighted value. However, after additional time has elapsed, the Standard Deviation settles down to 8 or 9%. At this point, there is much less discrepancy between the two Mean Diameter values, based on the different weightings.



Data (Ch#1)	Int.-Wt. MeanDiam	Std. Dev. (% Mean)	Vol.-Wt. MeanDiam	Chi Sq.	Base Adj.
64K	93.7 nm	23 %	81.5 nm	2.3	0.0 %
115	93.1	12	89.4	3.0	"
166	92.6	9	90.6	1.5	"
218	92.4	7	91.3	2.0	"
269	92.4	8	91.1	1.8	"
---	----	-	----	---	-
455	92.2	9	90.0	1.3	"

Table 2: Sequence of Gaussian Analysis results obtained for the narrow 91-nm latex standard

Figures 12a and b show the displays for the intensity and volume-weighted particle size distributions obtained from the Gaussian Analysis after only 64K of accumulated Data, equivalent to between one and two minutes of running. By contrast, Figures 12c and d show the corresponding results obtained with 455K in Channel #1, after approximately 10 minutes. The latter clearly represent results having a much higher confidence level.

Finally, there is a little-recognized, but significant, attribute of the Gaussian Analysis that deserves comment here. It often turns out that the computed particle size distribution for a *broad,* unimodal population settles more quickly than for a *narrow* distribution! In the latter case, one frequently observes results for 91-nm latex after only 64K of Data.

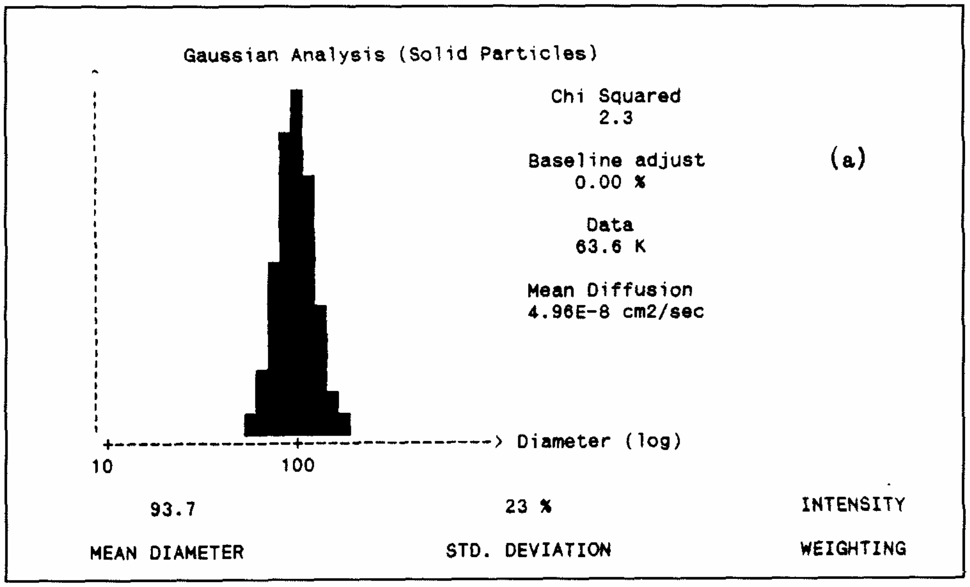
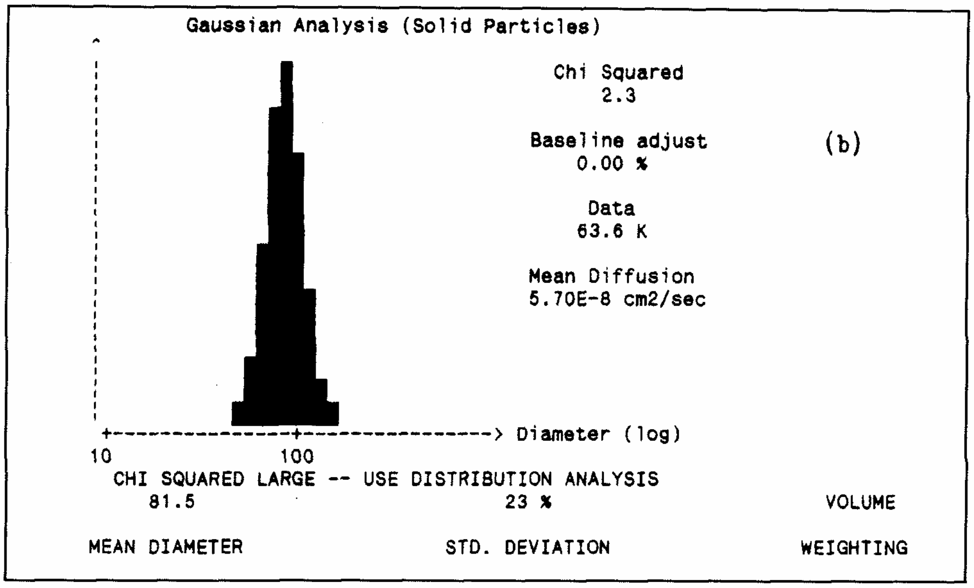
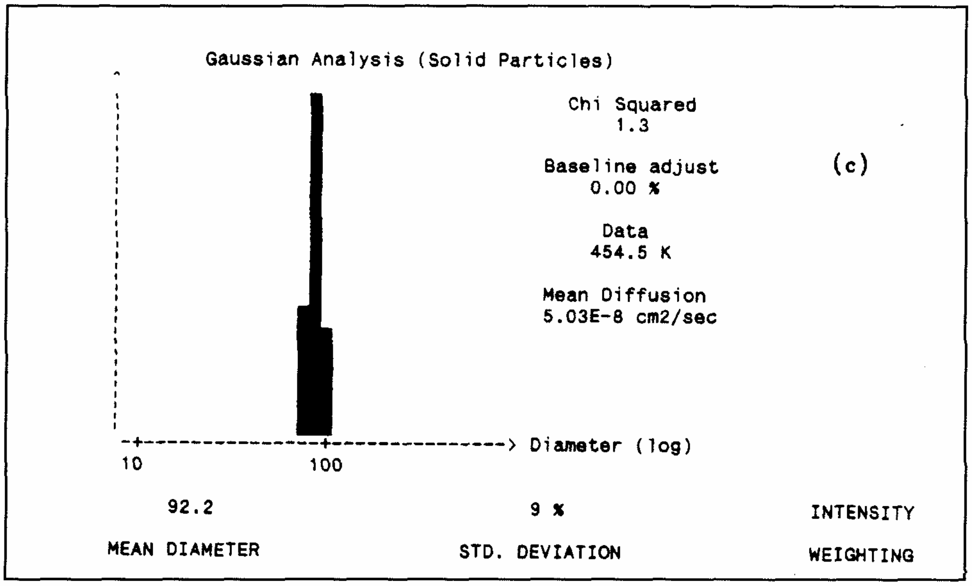


Figure 12a: Intensity-weighted Gaussian Analysis



```
Chi Squared
2.3

Baseline adjust
0.00 %

Data
63.6 K

Mean Diffusion
5.70E-8 cm2/sec

CHI SQUARED LARGE -- USE DISTRIBUTION ANALYSIS
81.5                        23 %                    VOLUME

MEAN DIAMETER           STD. DEVIATION          WEIGHTING
```

Figure 12b: Volume-weighted Gaussian Analysis



```
Chi Squared
1.3

Baseline adjust
0.00 %

Data
454.5 K

Mean Diffusion
5.03E-8 cm2/sec

92.2                        9 %                     INTENSITY

MEAN DIAMETER           STD. DEVIATION          WEIGHTING
```

Figure 12c: Intensity-weighted Gaussian Analysis

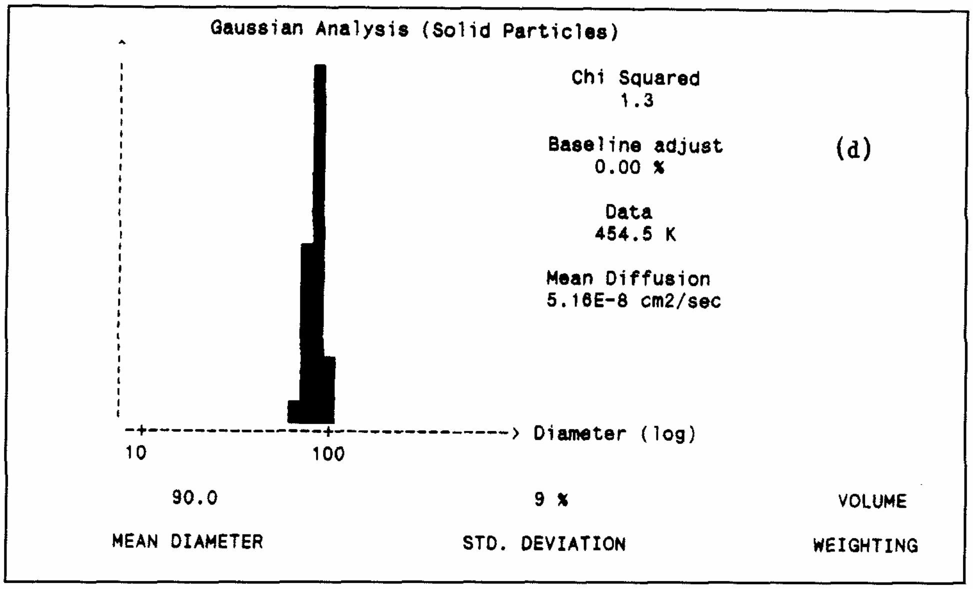


Figure 12d: Volume-weighted Gaussian Analysis results for 91-nm latex after more Data (455K).

What appear to be large fluctuations in the Standard Deviation-e.g. from 2 % to 12% -- while the Mean Diameter (with whatever weighting) remains more or less constant. While this factor-of six variation in the Standard Deviation appears, at first glance, to be very large, it really is not. In fact, all of the values within that range are effectively nearly equal. Why? Because they all describe autocorrelation functions which are very close to ideal, single decaying exponentials.

Using Equation 11, we are saying that the ‘curvature represented by coefficient a: is nearly zero for the above mentioned range of Standard Deviations. There are small changes in the least-squares quadratic fit (due to tiny fluctuations in the correlation data) which are responsible for these seemingly large changes in the Standard Deviation. Indeed, these small fluctuations may be caused by normal Poisson statistics, particle aggregates, “dirt” particles, stray scattered light, etc. By contrast, broad distributions result in substantial curvature in the quadratic fit (i.e. relatively large values of a:) and much less sensitivity to small fluctuations in the correlation data. Hence, in this case, the Gaussian Analysis results are observed to settle relatively more quickly to a final, reliable “answer”. Typically, it is necessary to start with very “clean”, well

dispersed samples and acquire data for a relatively long time (e.g. 30 to 60 minutes) to obtain reliable Standard Deviations smaller than 8 to 10% for a sample which is known to be nearly monodisperse. Under such controlled, ideal conditions, the DLS Module is certainly capable of yielding single-peak distributions of exceptionally small Standard Deviation.

NICOMP DISTRIBUTION ANALYSIS

Rationale for use of the inverse Laplace transform

In the proceeding section we saw that the Gaussian Analysis has the useful attribute that the resulting distribution settles quickly to a stable, reproducible result with increasing acquisition of light scattering data. As was pointed out, this is due to the fact that the least-squares quadratic (cumulants) fit to the reduced data is essentially just a two-parameter fit (apart from a possible baseline adjustment). Obviously, this “smooth” fit to the data is relatively insensitive to subtle changes in the autocorrelation constitutes the “good news” Analysis. function. This insensitivity associated with the Gaussian Analysis.

The “bad news”, of course, is that this method is inherently limited to describing only simple particle size distributions -- symmetric, single-peak (unimodal) populations. More complex distributions, such as highly-skewed unimodals or bimodals, are completely misinterpreted by the Gaussian Analysis; the degree of error will be either quantitative or qualitative, depending on the precise shape of the true distribution. The only indication of the existence of a more complicated distribution is the occurrence of a large (and rising) value of Chi Squared, as described previously. This “warning flag” informs the operator in unambiguous terms that the Gaussian Analysis result is inappropriate. In this case, another approach for analyzing the data is clearly needed.

The NICOMP Distribution Analysis provides the needed alternative method for analyzing the autocorrelation function. In contrast to the Gaussian Analysis, it makes NO *a priori* assumption of the shape of the final distribution. Rather than relying on only two parameters (plus, possibly, a baseline adjustment), the Distribution Analysis typically yields as many as three, or even four, independent parameters, as well as a baseline adjustment. Because the amount of information (i.e. the number of parameters) potentially provided by the Distribution Analysis exceeds that yielded by the Gaussian Analysis, considerably more data is needed in general in the autocorrelation function to obtain a reliable result. This is simply a requirement of better statistical accuracy, or signal to-noise ratio, in the autocorrelation function. In this game, which is inherently mathematical, a simple rule applies: you cannot obtain something for nothing!

Obtaining *more* information relating to a *more* complex distribution requires that more time be expended acquiring *more* scattered intensity data. While this same rule was implicit in our discussion of the settling of the Gaussian Analysis results, we shall see that it is much more critical in the case of the Distribution Analysis.

The general mathematical procedure that is utilized in the proprietary NICOMP Distribution Analysis is referred to as inversion of the Laplace transform (“ILT”). This rather sophisticated technique has also been used to analyze a variety of problems in other scientific areas, unrelated to light scattering. The specific mathematical procedure is a more sophisticated version of the least-squares calculation used in the Gaussian (cumulants) Analysis; it is referred to as nonlinear least-squares (“NLLS”) analysis.

We can appreciate the problem which must be solved by the ILT technique by considering the most general expression for the autocorrelation function, C(t’), corresponding to an arbitrary distribution of particle sizes. We recall for the trivial example of a *single* particle size that C(t’) is simply an exponentially decaying function, given by Equations 7 and 8, or 10a and b. This is now replaced by a more general expression, containing a *weighted sum* of individual exponentially decaying functions, each of which corresponds to a different particle diameter:

$$C(t') = A\left\{\sum_{i=1}^{M} f_i \exp(-D_i K^2 t')\right\}^2 \qquad (14)$$

Here, again, the baseline of the autocorrelation function ($t'\to\infty$) is given by B. and constant A simply relates to the total amount of data acquired in “channel #0” (t’ = 0) of C(t’), such that C(O)=A+B.

In Equation 14 above we have assumed a discrete distribution of particle sizes, containing M different diameters, each labeled by index i (running from 1 to M). The diffusion coefficient for each particle diameter is given by D_i, which is obtained from the radius R_i using the Stokes-Einstein relation, Equation 2. The constants f_i are the weighting coefficients which mix together the individual exponentially decaying functions, $\exp(-D_iK^2t')$, according to the relative amounts of the different particle diameters present in the sample solution (of this, more later). In the trivial case of a single, uniform particle size, Equation 14 reduces to $C(t') = A(f_1)^2\exp(-2D_1K^2t') + B$, which is exactly the same as Equation 7 or l0a and b, provided that the constant $A(f_1)^2$ replaces the previous prefactor, A.

The goal of the ILT technique is to solve Equation 14. The quantity which is known is the left-hand side, the measured autocorrelation function C(t') (where t' is given by the discrete channels $t' = \Delta t'$, $2\Delta t'$, …, $64\ \Delta t'$). The unknowns are the M individual weighting coefficients f_i, buried in the summation on the right hand side of Equation 14. This multi-variable equation must therefore be "inverted" in order to yield the "answer", which is the set of weighting coefficients fit Equation 14 is, in fact, the discrete representation of a more general *integral* equation that defines the Laplace transform. To appreciate this, we can define a new "reduced" autocorrelation function H(t'), obtained from the original C(t'): $H(t') = \{(C(t')-B)/A\}^{1/2}$.

We also define a new variable, s, which is simply proportional to the diffusion coefficient variable D : $s = DK^2$ (with K given by Equation 9). Using these new definitions we can recast Equation 14 in its integral form, in which there are an infinite number of different particle diameters. Equation 14 therefore becomes,

$$H(t') = \int_0^\infty f(s) \exp(-st') ds \qquad (15)$$

where s (or D) ranges from zero (corresponding to an arbitrarily large particle) to infinity (corresponding to a particle of diminishing small diameter). The above expression defines the integral Laplace transform of H(t'), which is the desired weighting function f(s).

Returning to Equation 14. we must use an ILT technique to obtain the best possible estimates for the weighting coefficients f_i. The details of the mathematical procedure used to perform the discrete Laplace transform inversion of the autocorrelation data in the NICOMP Distribution Analysis are beyond the scope of this manual **--** and, in any case, are proprietary. Suffice to say that the procedure represents a variation on other ILT methods discussed in the light scattering literature -- most especially, that developed by S. Provencher. The ways in which the NICOMP procedure differs from this and other methods relate mainly to the manner and extent of *smoothing* carried out within the mathematical "algorithm".

It is most important to appreciate the practical limitations inherent in *any* solution of Equation 14 using an ILT algorithm. In principle, one would like to be able to obtain, with reasonable accuracy, all values f_i (there are M of them), thereby obtaining a faithful representation of the true particle size distribution. In practice, however, one falls far short of this goal. Typically, it is possible to obtain a stable distribution containing one, two or even three peaks. In the case of two peaks, we obtain two values of peak diffusion coefficients, D_1 and D_2, , and *one* relative strength parameter, f_2/f_1, for a total of *three* independent parameters (in addition to the baseline adjust). Note, in all of the distribution results of the DLS Module, the maximum height of any distribution is always normalized to 100; hence, in the case of two peaks, only the *ratio* of their heights is an independent parameter.) In the case of three peaks, one will obtain *three* peak D values-D_1, D_2 and D_3 -- and *two* relative strength parameters, f_2,/f_1 and f_3/f_1.

The Nicomp allows one to establish the set of diameter values, *I* =1 to M, over which Equation 14 is defined. The total number, M, is given by the PLOT SIZE; the values vary from MIN.DIAM.(i=l) to RANGE * MIN.DIAM.(i=M). In most cases it is desirable to use the logarithmic diameter scale, in which the individual diameter "slices" are spaced logarithmically. Each of the diameter values is converted to a diffusion coefficient, Di; the resulting individual decaying exponential functions in Equation 14, exp(-DiK2t') , constitute the starting "basis vectors for the NICOMP ILT algorithm. The latter systematically varies the weighting coefficients fi in Equation 14 in order to determine the sensitivity of the resulting computed fit (to C(t')) to each coefficient. In this way, many, if not most, of the coefficients are set equal to zero. The non-zero values of fi which survive constitute the "solution" to Equation 14. As additional data are acquired in C(t'), this solution will change **--** either a little or a lot, depending on the current statistical accuracy of C(t').

There is an additional observation concerning the ILT fitting procedure and Equation 14 that is VERY IMPORTANT. The weighting coefficients f_i represent the *relative* scattering *intensities* contributed by particles of size type i. That is, the individual decaying exponential functions corresponding to each particle diffusivity D_i, are added together in Equation 14 with weighting factors f_i, each of which is proportional to the total scattered intensity produced by all of the particles of *that size.* From the discussions in the previous sections, we have:

$$f_i \propto N_i (V_i)^2 (G_i)^2 \qquad (16)$$

where N_i is the total number of particles of (size) type i, and V_i is the particle volume *giving rise to scattering.* In the case of a solid particle, $V_i \propto (d_i)^3$; for a thin-walled vesicle, $V_i \propto (d_i)^2$, (See the following section.) The extra factor for the additional effects of intra-particle $(G_i)^2$ accounts interference, important when the particle diameter is no longer negligible compared to the laser wavelength (Mie region). In the small diameter Rayleigh region (diameters smaller than about 50 nm), G_i is essentially unity for all particle shapes and sizes and can be ignored in Equation 16.

We have explicitly written the expression for the weighting coefficients f_i in Equation 16 to help motivate Equation 14, which is the basic starting point for Distribution Analysis. We must

remember, of course, that the coefficients f_i are the *unknowns;* they are computed by inverting Equation 14. The “answer” produced by Distribution Analysis is the intensity-weighted particle size distribution: a plot of f_i vs d_i (diameter). The d_i scale is determined by the choices of MIN.DIAM., PLOT SIZE and RANGE, as discussed earlier.

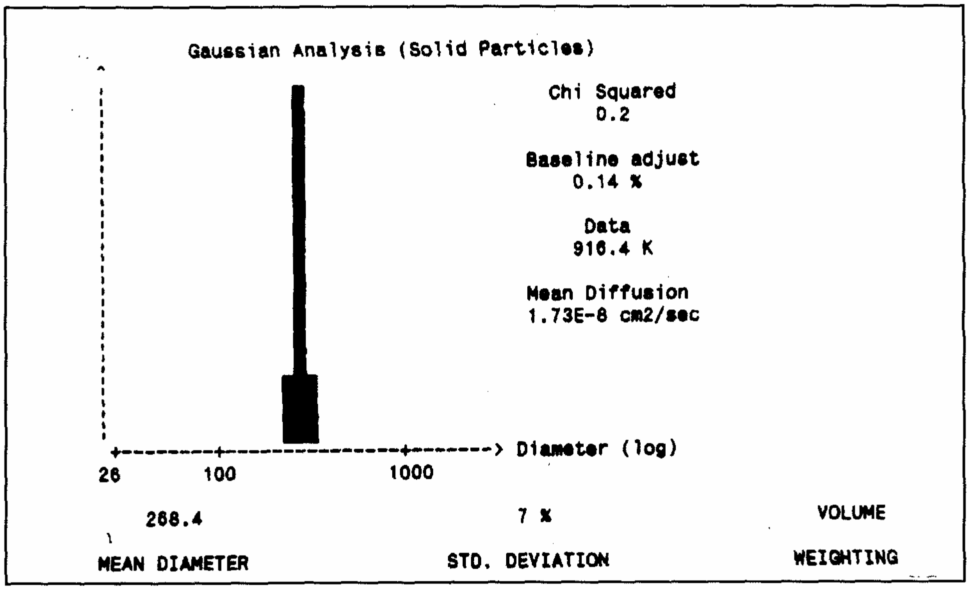
The volume- and number-weighted distributions are obtained from the intensity-weighted plot by dividing each value of f_i by $V_i(G_i)^2$ and $(V_i)^2(G_1)^2$, respectively, for each diameter “slice”. It is for this reason that Equation 16 is needed, to permit the various weightings to be obtained from the “raw” intensity weighted distribution of f_i values which emerges from the ILT algorithm.

To conclude this Section, remember that the actual plots obtained from the Distribution Analysis represent very simplified versions of whatever the true particle size distribution may be. The statistical accuracy that can be realistically achieved in the underlying autocorrelation data simply *cannot* support an analysis which *reliably* yields any more “detail” in the distribution. However, it usually turns out that the resolution and accuracy of the NICOMP algorithm are sufficient to yield a meaningful *representation* of the true particle size distribution for many systems of practical significance.

A simple test of Distribution Analysis: uniform particle size (latex)

In order to investigate the capability of the NICOMP Distribution Analysis, we must apply it to a polydisperse sample which differs *qualitatively* from those investigated earlier-i.e. those consisting of simple unimodal populations which could be well characterized using the Gaussian Analysis. First, however, we should check the performance of the new algorithm using a narrow, nearly-monodisperse polystyrene latex standard. For such a sample, it must be able to determine the mean particle diameter with high accuracy.

In Figure 13 we see the video display of the volume-weighted distribution obtained for the 91nm latex standard. The corresponding result obtained using the Gaussian Analysis was shown earlier in Figure 12d. We find a mean diameter of 88.8 nm for Figure 13, compared



Gaussian Analysis (Solid Particles)

Chi Squared
0.2

Baseline adjust
0.14 %

Data
916.4 K

Mean Diffusion
1.73E-8 cm2/sec

268.4
MEAN DIAMETER

7 %
STD. DEVIATION

VOLUME
WEIGHTING

Figure 13: Volume-weighted Distribution Analysis result for 91-nm latex standard

to 90.0 nm for the Gaussian Analysis result. This discrepancy (only 1 %*)* is excellent, considering the fact that two radically different mathematical procedures were used to obtain the two results. (It was pointed out earlier that the true diameter of this standard lies closer to 88-89 nm, rather than the nominal 91-nm value.)

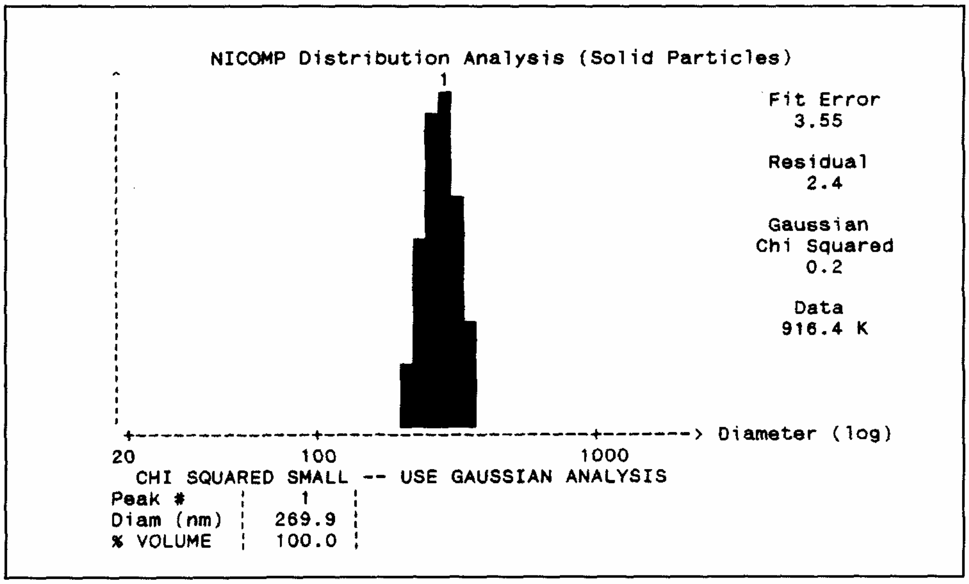
In Figure 13 we see that the Fit Error for this analysis fell to 4.60 with 480K of Data (Ch. #1). This value should continue to decrease with increasing data acquisition. It is IMPORTANT to appreciate that the Fit Error only provides an indication of how relatively stable, or settled, the Distribution Analysis results should be, but NOT on whether it is the *preferred* result, compared to the Gaussian Analysis. This judgment, of course, is provided by the value of Chi Squared. As the warning message clearly states, in this case the Gaussian Analysis must be used.

One will typically find that narrow, ideal distributions like this one will settle relatively quickly--i.e. with values of Fit Error of 3 or 4 or higher. However, in general one should run long enough to achieve a value below 2.0 in the case of a “clean’, single-peak distribution. For more complex distributions, it is usually advisable to achieve a Fit Error below 1.5*,* or even approaching 1.0, to

obtain the most accurate, reproducible results. (As a point of reference, one can typically achieve a Fit Error of 0.1 or 0.2 after 8 to 12 hours of data collection.) Again, this parameter only characterizes the stability of the resulting answer, and NOT its appropriateness vis-a-vis the simpler Gaussian Analysis result.

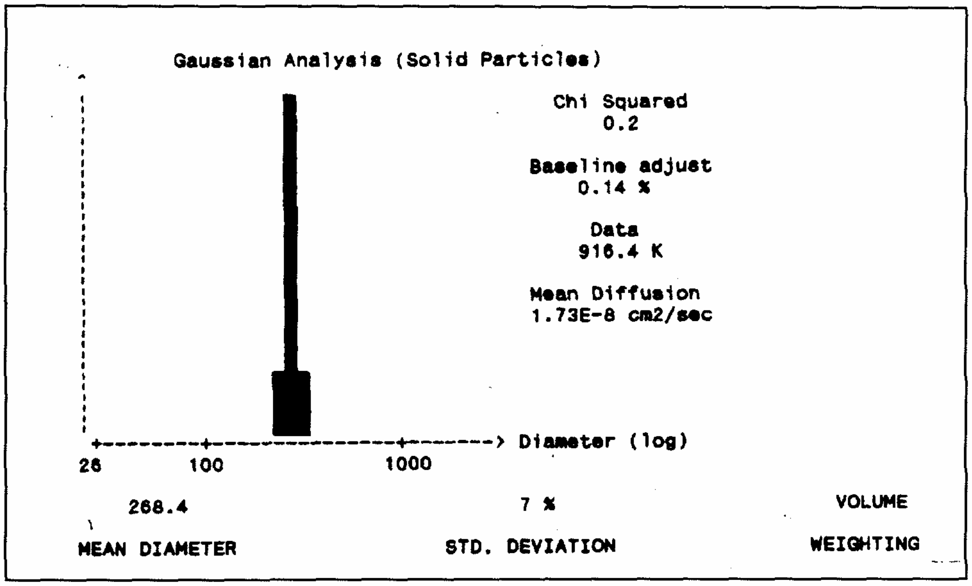
In Figure 13 we find an ideal value for the Residual: 0.0. We therefore conclude that there is a negligible concentration of aggregates or other large-particle contaminants in the sample suspension. In the event that the value for the Residual is significant, e.g. 10 or larger, one of two possibilities will occur with increased data acquisition. 1) The Residual will simply remain high, with little change in the composition of the size distribution plot; 2) The Residual will fall to zero, resulting either in a shift in the position(s) of the peak(s) (usually to *higher* diameter(s)), or in the appearance of a new peak at the high end of the diameter scale. The latter explicitly reveals the large-size component previously signaled (in #1) by the large value of the Residual. In the early stages of data acquisition these two possibilities frequently alternate in seemingly random fashion, because of inadequate statistical accuracy in the autocorrelation function. Obviously, it is desirable to achieve result #2 above -- a zero Residual. One can often hasten the transition from #1 to #2 by increasing the maximum value of the diameter scale, equal to MIN.DIAM. * RANGE. This is accomplished by increasing the value of RANGE (e.g. to 500 or 1000, if necessary).

In Figure 14a we show a typical result obtained using Distribution Analysis for a larger size polystyrene latex standard 261 nm. Here, we obtain a volume-weighted mean diameter of 269.9 nm, which agrees very well with the value found using Gaussian Analysis, 268.4 nm, shown in Figure 14b (with the same Data).



```
NICOMP Distribution Analysis (Solid Particles)

Fit Error
  3.55

Residual
  2.4

Gaussian
Chi Squared
  0.2

Data
916.4 K

CHI SQUARED SMALL -- USE GAUSSIAN ANALYSIS
Peak #     |   1   |
Diam (nm)  | 269.9 |
% VOLUME   | 100.0 |
```

Figure 14a: Volume-weighted Distribution Analysis result for 261-nm latex standard



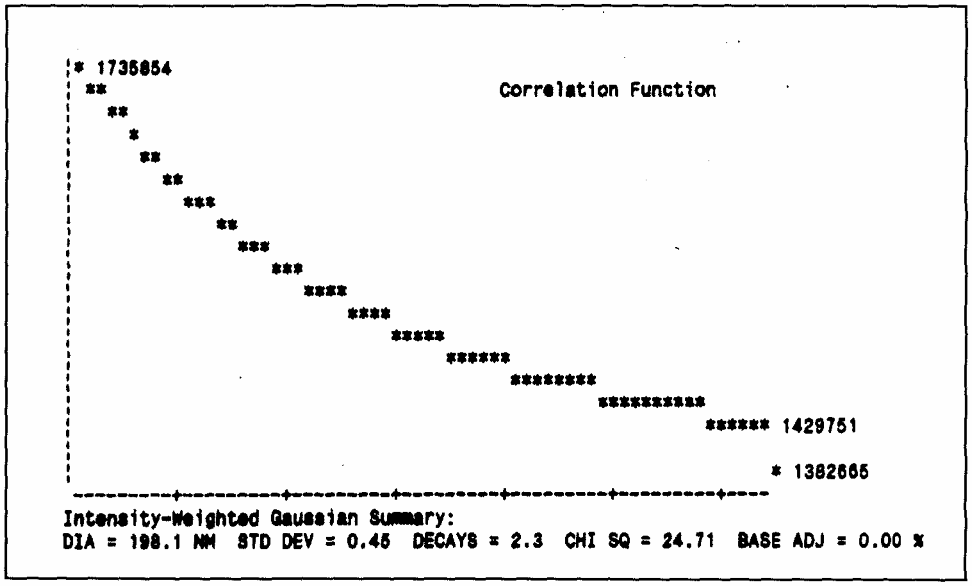
```
Gaussian Analysis (Solid Particles)

Chi Squared
    0.2

Baseline adjust
    0.14 %

Data
  916.4 K

Mean Diffusion
1.73E-8 cm2/sec

   268.4                   7 %              VOLUME
MEAN DIAMETER          STD. DEVIATION      WEIGHTING
```

Figure 14b: Volume-weighted Gaussian Analysis result for 261-nm latex standard (See Figure 14a.)

It is most important for one to appreciate the fact that reliable estimates of the *widths of* peaks in the particle size distribution CANNOT be obtained using the Distribution Analysis. The peak widths obtained from this procedure depend on details of smoothing and basis-vector coupling in the ILT algorithm, as well as the operator's choice for the SMOOTHING parameter. On the other hand, it should now be clear that the Gaussian Analysis does, indeed, yield a reliable estimate of the peak width -- as in Figure 14b -- provided Chi Squared is low, indicating a good fit.

A harder test for Distribution Analysis: a bimodal distribution (latex)

We now consider the first difficult test of the NICOMP ILT procedure -- analysis of a bimodal distribution. Figure 15 shows the autocorrelation function after 23 minutes for a bimodal sample prepared using the 91 and 261 nm latex standards investigated earlier. This test bimodal was made using a 3:1 ratio by mass (or volume) in favor of the 91 nm latex, so as to enhance the relative scattering contribution of the smaller-size component t more later). Figure 16 shows a plot of the logarithm of the reduced autocorrelation data, C(t')-B vs t' (channel #), with a straight line drawn for reference purposes.



```
Intensity-Weighted Gaussian Summary:
DIA = 198.1 NM  STD DEV = 0.45  DECAYS = 2.3  CHI SQ = 24.71  BASE ADJ = 0.00 %
```

Figure 15: Autocorrelation function for a test bimodal: 3:1 (vol.) ratio, 91 and 261 nm

latex particles

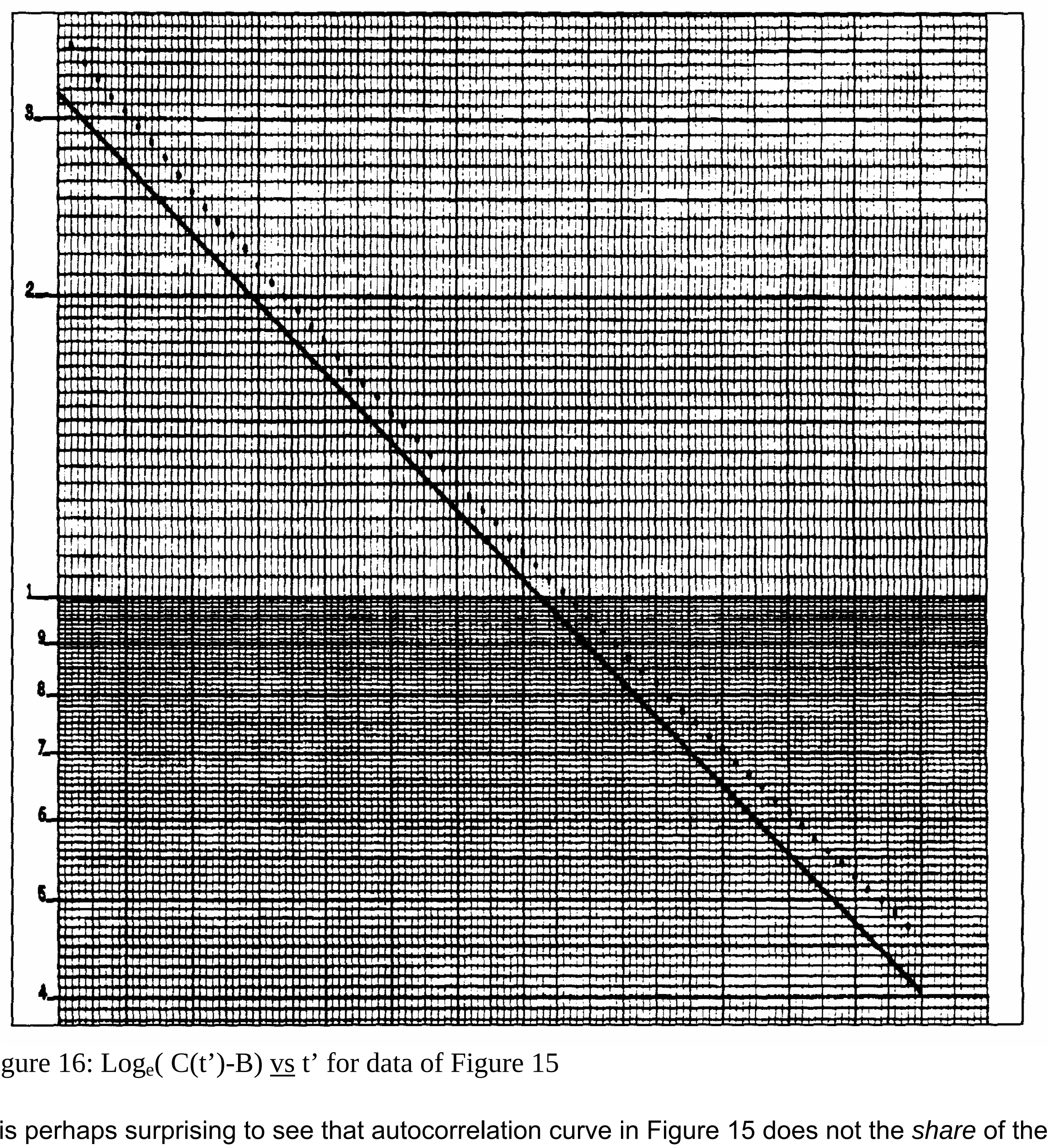
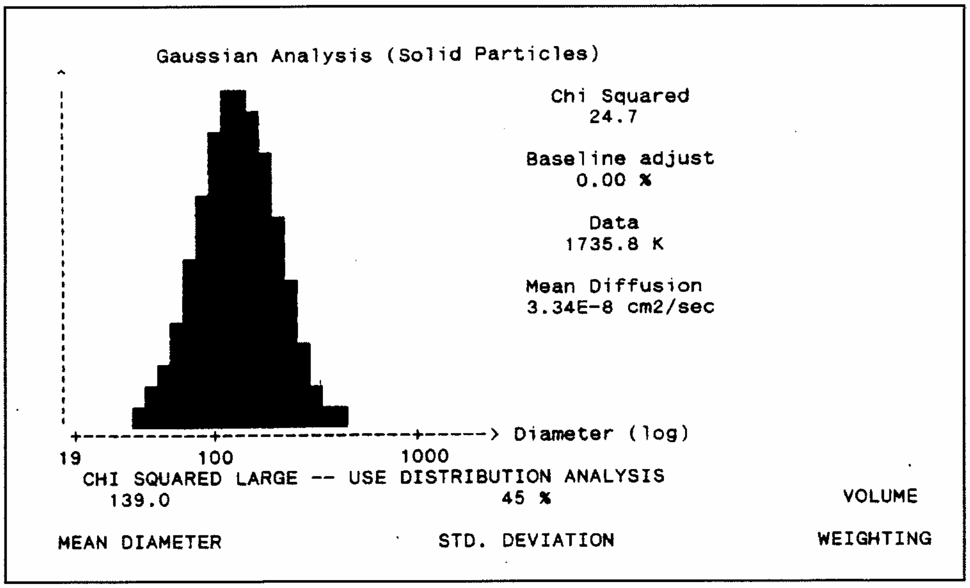


Figure 16: Log_e(C(t')-B) vs t' for data of Figure 15

It is perhaps surprising to see that autocorrelation curve in Figure 15 does not the *share* of the appear to differ appreciably from that observed previously for the broad, unimodal fat emulsion of Figure 9a. Here we have particle sizes which differ by almost a diameter. We therefore would expect to see evidence of a “breakpoint”, or “knee”, in the logarithmic plot of the reduced autocorrelation data (Figure 16), given the admixture (Equation 14) of two exponential functions whose decay time constants differ by a factor of three. However, NO such breakpoint is evident in Figure 16. Indeed, the curvature in the reduced data in Figure 16 qualitatively resembles that seen for the broad unimodal emulsion in Figure 9b.

Suppose we were to analyze this new system as we did the emulsion, using the Gaussian Analysis. From the substantial curvature evident in Figure 16, we would expect the unimodal generated by the Gaussian Analysis to possess a large standard deviation -- i.e. to be very broad. This is indeed the case, as seen in Figure 17.

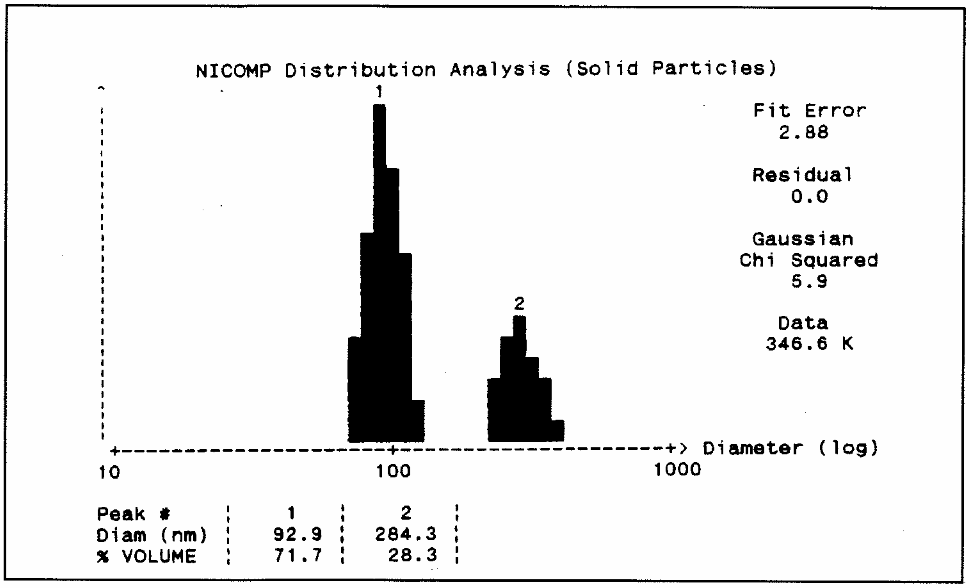


```
        Gaussian Analysis (Solid Particles)
                                        Chi Squared
                                           24.7

                                      Baseline adjust
                                          0.00 %

                                           Data
                                         1735.8 K

                                      Mean Diffusion
                                      3.34E-8 cm2/sec

 +----------+---------------+-----> Diameter (log)
19         100            1000
  CHI SQUARED LARGE -- USE DISTRIBUTION ANALYSIS
    139.0                         45 %                      VOLUME

MEAN DIAMETER               STD. DEVIATION               WEIGHTING
```

Figure 17: The volume-weighted Gaussian Analysis result corresponding to Figure 15 and Figure 16

The volume-weighted mean diameter is 139 nm, with a standard deviation of 45%. (We also obtain the expected result that the volume-weighted mean diameter is pulled down substantially below the intensity-weighted value -- 198 nm from Figure 15 -- owing to the large standard deviation.) Figure 17 would have represented the state of the art of DLS-based particle sizing technology only 10 years ago, prior to the commercial introduction of ILT algorithms for analysis of highly polydisperse distributions. Of course, we recognize that the Gaussian Analysis result is probably seriously flawed, given the large value of Chi Squared -- 24.7 -- and the fact that it was observed to increase continually (and dramatically) with increasing data acquisition. The appropriate warning message is prominently displayed in Figure 17.

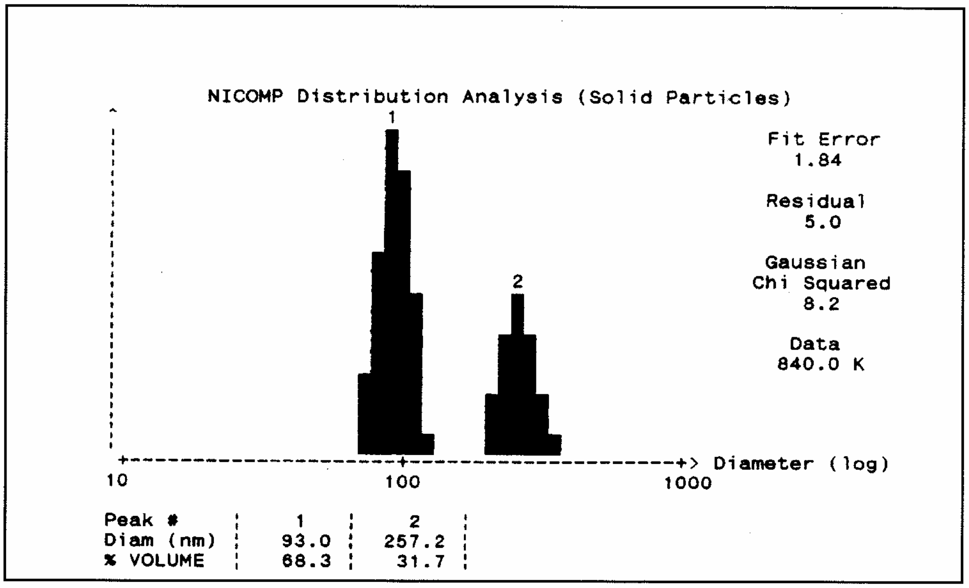
Notwithstanding the apparent qualitative resemblance of the autocorrelation data for the broad unimodal emulsion and the bimodal latex suspension, the NICOMP Distribution Analysis has little difficulty revealing the true nature of the latter sample. Figure 18a clearly shows a bimodal distribution, which is surprisingly close to the correct result, given the relatively small amount of data acquired (Data = 374K). It yields volume-weighted peak mean diameters of 92.9 and 284.3 nm, which are close to the nominal values of 91 and 261 nm. Of course, more data need to be acquired before one can be confident that the Distribution Analysis result has reached a stable solution; the Fit Error value of 2.88 is still too high, providing a cautionary signal. Nevertheless, with this small amount of data the value of Chi Squared for the alternative Gaussian Analysis has already climbed to 5.9. The values of % volume found for the two peaks, 71.7 and 28.3, are very close to the actual values of 75 and 25 % (for a 3:1 volume ratio).



Peak #	1	2
Diam (nm)	92.9	284.3
% VOLUME	71.7	28.3

Figure 18a: The volume-weighted Distribution Analysis result for the 3:1 91/261 nm test bimodal after Data = 347K

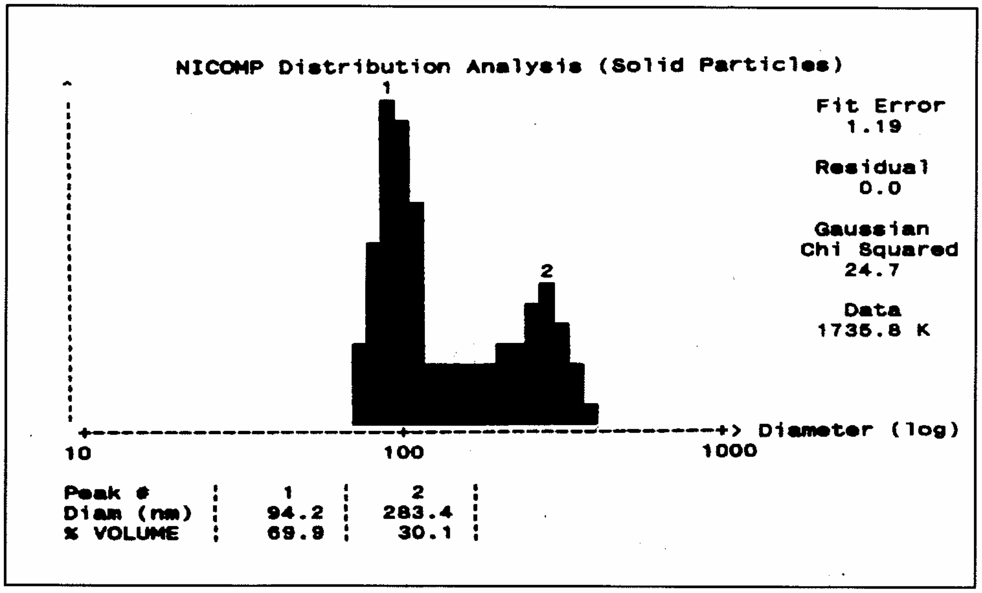
The distribution obtained after more than 10 minutes of running (Data = 840K) is shown in Figure 18b. The peak locations and relative volume percentages have changed somewhat relative to the results of Figure 18a. By this time the Gaussian Chi Squared has risen further, to 8.2, and the Fit Error has fallen to 1.84. The non-zero value of the Residual suggests that additional data acquisition would be desirable.



```
NICOMP Distribution Analysis (Solid Particles)

Fit Error
  1.84

Residual
  5.0

Gaussian
Chi Squared
  8.2

Data
840.0 K

Peak #     |   1   |   2   |
Diam (nm)  | 93.0  | 257.2 |
% VOLUME   | 68.3  |  31.7 |
```

Figure 18b: The volume-weighted Distribution Analysis result for the test bimodal after Data = 840K (10 mm.)

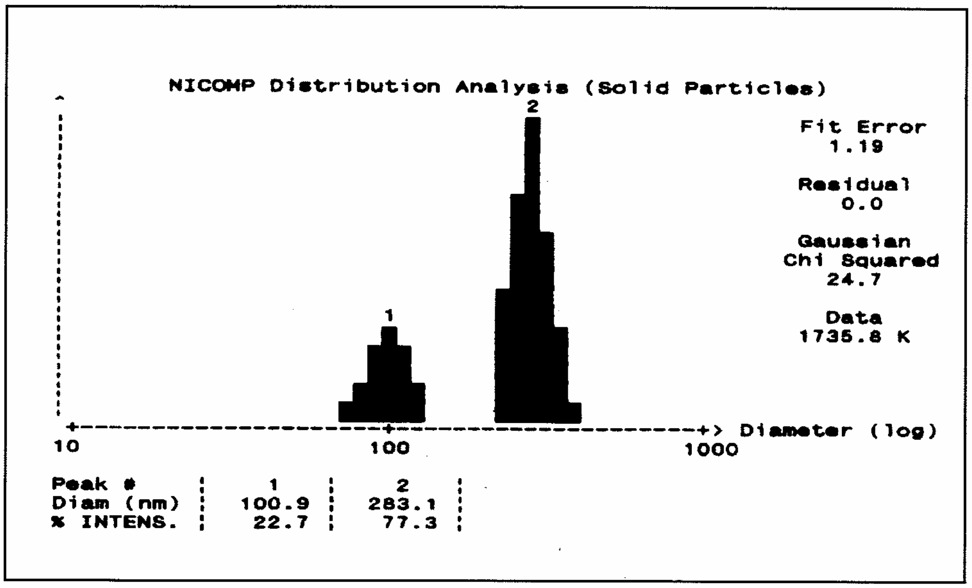
Figure 18c shows the volume-weighted Distribution Analysis result for the same test bimodal after 23 minutes elapsed time. Now, the Fit Error has fallen close to 1.0, and the Residual has returned to its ideal value of 0.0. Chi Squared has grown to 24.7, which unambiguously rules out acceptance of the Gaussian Analysis result. The mean diameters of the peaks are now at 94.2 and 283.4 nm. While these values are too high by a few percent, a closer examination of the printouts (shown below) reveals that the “error” represents diameter displacements of less than one diameter “slice” for each peak. This is an exceptionally good performance, given the difficulty of this test sample. Indeed, the alternative ILT algorithms being used today generally perform much less well when faced with a control sample like this one. We have deliberately showed *typical* results obtained by the DLS Module for this 3:1 91/261 nm test bimodal after 20-30 minutes running time; on occasion, even better accuracy has been attained.



Peak #	1	2
Diam (nm)	94.2	283.4
% VOLUME	69.9	30.1

Figure 18c: The volume-weighted Distribution Analysis result for the test bimodal after Data = 1736K (23 mm.)

Now it is instructive to show a plot of the intensity-weighted distribution -- i.e. the set of weighting coefficients f, in Equation 14 -- which corresponds to the result just discussed. This is shown in Figure 18d. As taught earlier, this is the fundamental result which emerges from the Distribution Analysis; all of the more useful weightings are *derived* from Figure 18d using the rules of light scattering (Equation 16).

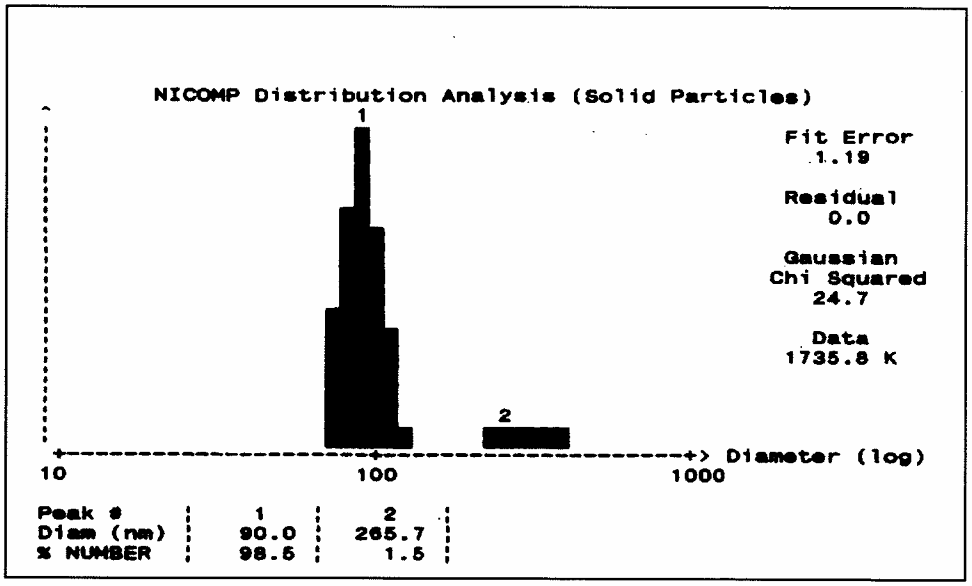


```
Fit Error
  1.19

Residual
  0.0

Gaussian
Chi Squared
  24.7

Data
1735.8 K

Peak #      !   1   !   2   !
Diam (nm)   ! 100.9 ! 283.1 !
% INTENS.   !  22.7 !  77.3 !
```

Figure 18d: The intensity-weighted Distribution Analysis result for the test bimodal, corresponding to Figure 18c

We now can appreciate why the test sample was deliberately skewed at the start toward the smaller-diameter particles. Even though there is three times as much latex mass, or volume, contained in the 91 nm component, compared to the 261 nm size, fully *three-fourths* of the total scattered light (computed by inverting Equation 14) is produced by the 261-nm fraction. Had we attempted to analyze a simple 1:1 mixture of the two sizes, the algorithm would still have found a bimodal distribution, but would have had a harder time correctly establishing the mean diameters of the two peaks, especially the weaker one (91 nm). Although we appear to have obtained the excellent results of Figures 18a-d with relative ease, the user should be warned that this analysis is a difficult one. Failure to disperse adequately the latex particles or to achieve a dirt-free suspension will in general cause the quality of the Distribution Analysis results to deteriorate markedly. Most of the commercially available DLS particle sizing instruments find it more difficult to achieve results on a routine basis like those seen in Figures 18a-d.

The number-weighted distribution corresponding to the above plots is shown in Figure 18e. It is obtained from the volume-weighted result as described earlier, using Equation 16.

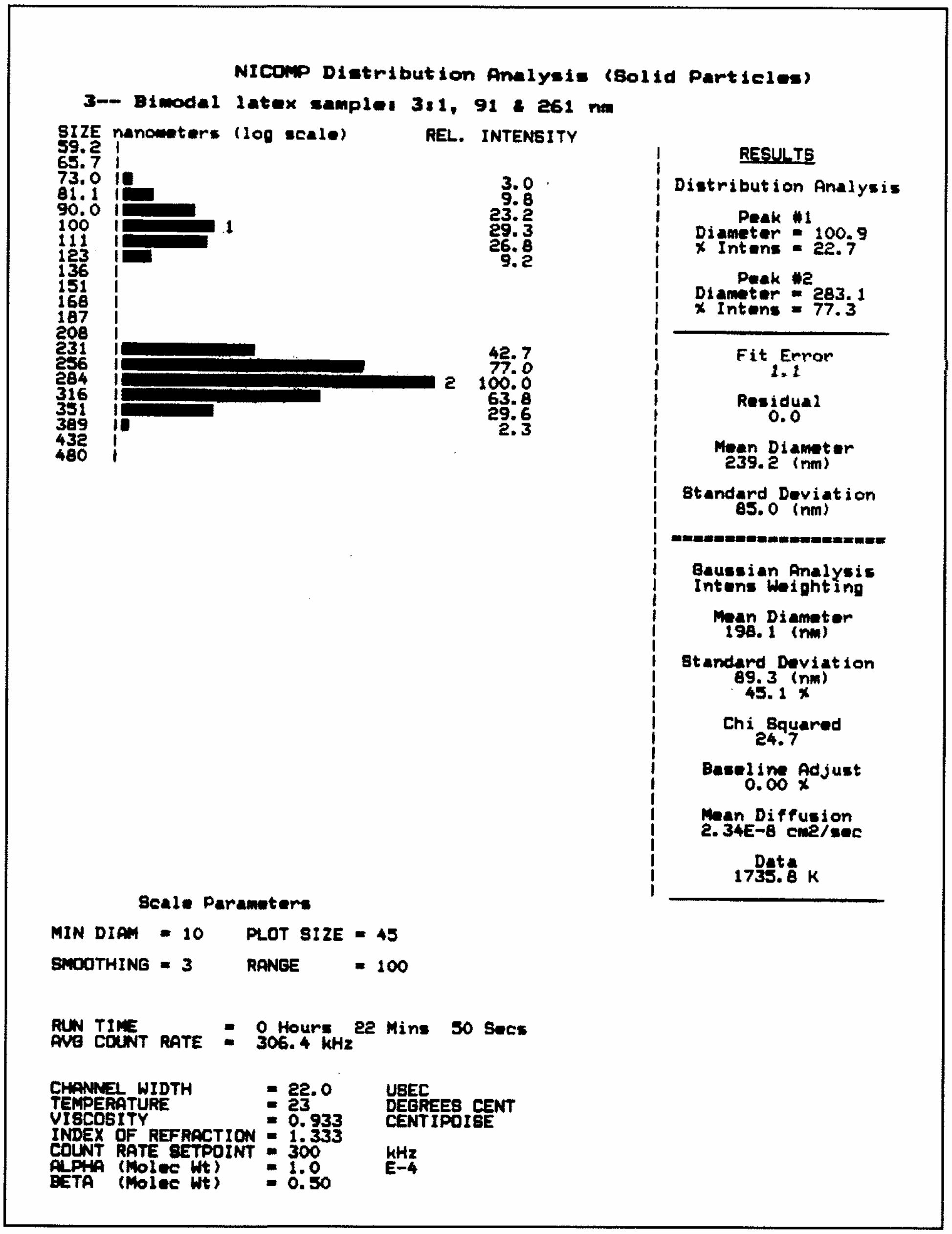


```
Fit Error
  1.19
Residual
  0.0
Gaussian
Chi Squared
  24.7
Data
1735.8 K

Peak #     :   1   :   2   :
Diam (nm)  :  90.0 : 265.7 :
% NUMBER   :  98.5 :   1.5 :
```

Figure 18e: The number-weighted Distribution Analysis result for the test bimodal, corresponding to Figures 18c,d

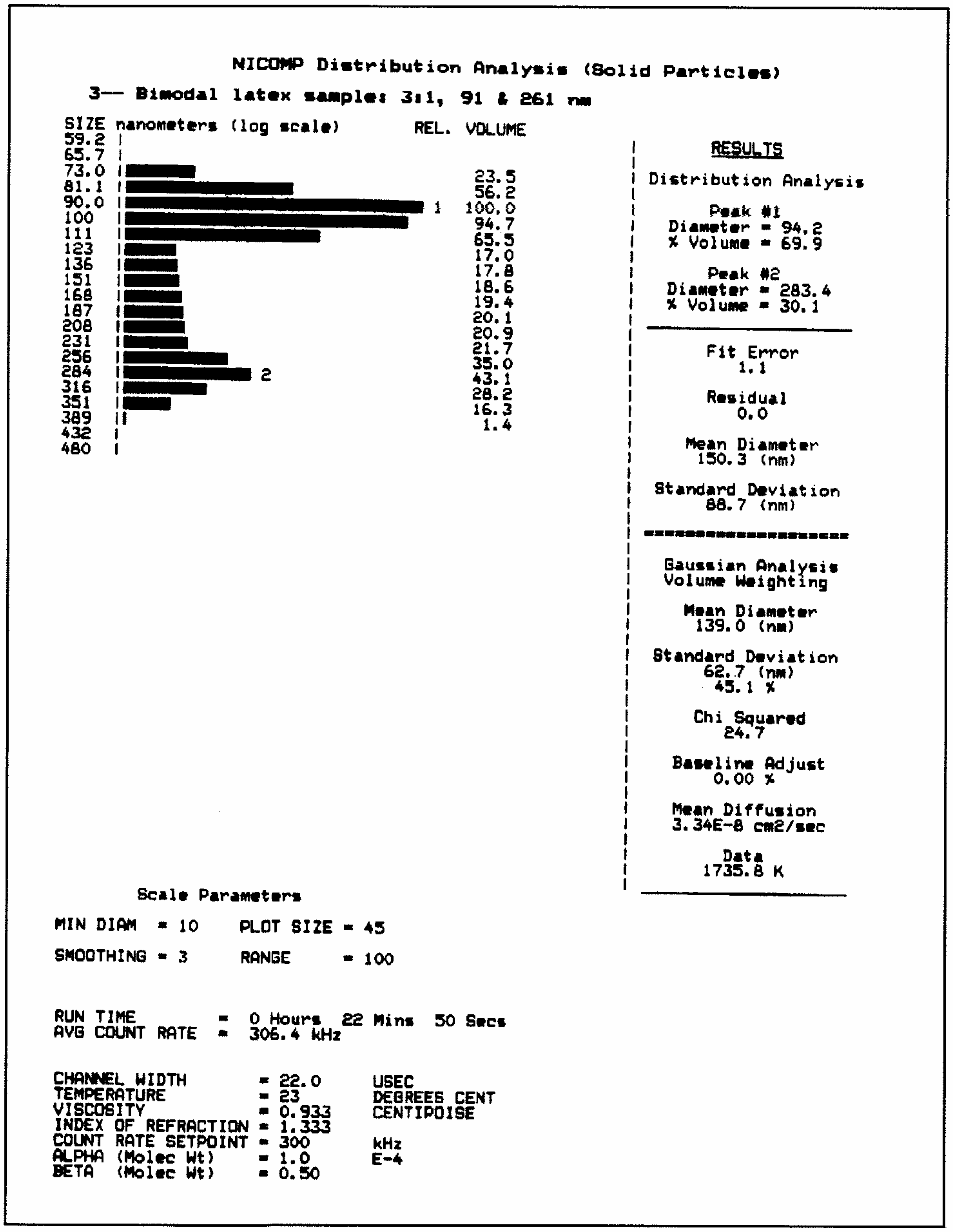
see in Figure 18e that the mean peak diameters are very close to the nominally correct values for this simplest of weightings. However, we can also appreciate why this choice of weighting is often avoided, and volume weighting chosen in its place. For any highly polydisperse distribution, such as the present one, there is usually too large a disparity between the numbers of smallest and largest particles. We find here that the 91 nm particles represent over 98% of the total number, with the 261 nm species accounting for only *1.5%.* The resulting plots on a linear scale lose accuracy because of this very strong skewing toward the smallest-particle peak in the distribution. Figures 19a,b and c show the detailed printout summaries for the three weightings corresponding, respectively, to the intensity-, volume- and number-weighted distributions.

It should be evident from our earlier discussion of the motivation behind the ILT algorithm that the closer the spacing of the two peaks in a bimodal, the more unlikely it is that the Distribution Analysis will produce the right answer. As seen in Figure 16, even when there is a 3-fold separation of the two sizes, the autocorrelation function bears a close qualitative resemblance to that obtained from a broad unimodal distribution. In practice, the NICOMP ILT algorithm is able to resolve a bimodal having a size separation of 2:1, or even somewhat closer, provided the sample is “clean” -- i.e. largely free of aggregates and other large-particle contaminants. (Of course, this capability presupposes that there is a reasonably well-balanced intensity contribution from each species.) In general, the narrower the separation, the more data are needed to permit unambiguous resolution of the bimodal, including a large value of the Gaussian Chi Squared. Even for a widely-spaced bimodal, however, one must always keep in mind the practical requirement that the weakest peak contribute adequate intensity. The “rules” of light scattering, as summarized by Equation 16, will alert one to the possibility that a bimodal cannot be measured -- NOT because of insufficient volume fraction for a given size component (typically the *smaller* size), but because that component contributes only 1 % or less to the total scattered intensity. As a general rule of thumb, we can say that a second (or third) peak will be reliably measured only if its relative intensity is at least 1-2%. Hence, whenever one is using the Distribution Analysis, it is usually a good idea to obtain a printout of the intensity-weighted result, as well as the desired volume-weighted plot.



```
              NICOMP Distribution Analysis (Solid Particles)
  3-- Bimodal latex sample: 3:1, 91 & 261 nm

SIZE nanometers (log scale)      REL. INTENSITY
59.2  |                                            |      RESULTS
65.7  |                                            |
73.0  |                             3.0            | Distribution Analysis
81.1  |                             9.8            |
90.0  |                            23.2            |       Peak #1
100   |       1                    29.3            |  Diameter = 100.9
111   |                            26.8            |  % Intens = 22.7
123   |                             9.2            |
136   |                                            |       Peak #2
151   |                                            |  Diameter = 283.1
168   |                                            |  % Intens = 77.3
187   |                                            |
208   |                                            | ____________________
231   |                            42.7            |
256   |                            77.0            |      Fit Error
284   |                    2      100.0            |         1.1
316   |                            63.8            |
351   |                            29.6            |      Residual
389   |                             2.3            |         0.0
432   |                                            |
480   |                                            |    Mean Diameter
                                                   |     239.2 (nm)
                                                   |
                                                   | Standard Deviation
                                                   |     85.0 (nm)
                                                   |
                                                   | ====================
                                                   |
                                                   |  Gaussian Analysis
                                                   |  Intens Weighting
                                                   |
                                                   |    Mean Diameter
                                                   |     198.1 (nm)
                                                   |
                                                   | Standard Deviation
                                                   |     89.3 (nm)
                                                   |      45.1 %
                                                   |
                                                   |    Chi Squared
                                                   |       24.7
                                                   |
                                                   |   Baseline Adjust
                                                   |      0.00 %
                                                   |
                                                   |   Mean Diffusion
                                                   |  2.34E-8 cm2/sec
                                                   |
                                                   |        Data
                                                   |     1735.8 K
                                                   | ____________________
          Scale Parameters

MIN DIAM  = 10     PLOT SIZE = 45

SMOOTHING = 3      RANGE     = 100


RUN TIME          =  0 Hours  22 Mins  50 Secs
AVG COUNT RATE    =  306.4 kHz


CHANNEL WIDTH          = 22.0      USEC
TEMPERATURE            = 23        DEGREES CENT
VISCOSITY              = 0.933     CENTIPOISE
INDEX OF REFRACTION    = 1.333
COUNT RATE SETPOINT    = 300       kHz
ALPHA (Molec Wt)       = 1.0       E-4
BETA  (Molec Wt)       = 0.50
```

Figure 19a Printout of the intensity-weighted Distribution Analysis result for the 3:1 91/261nm test bimodal

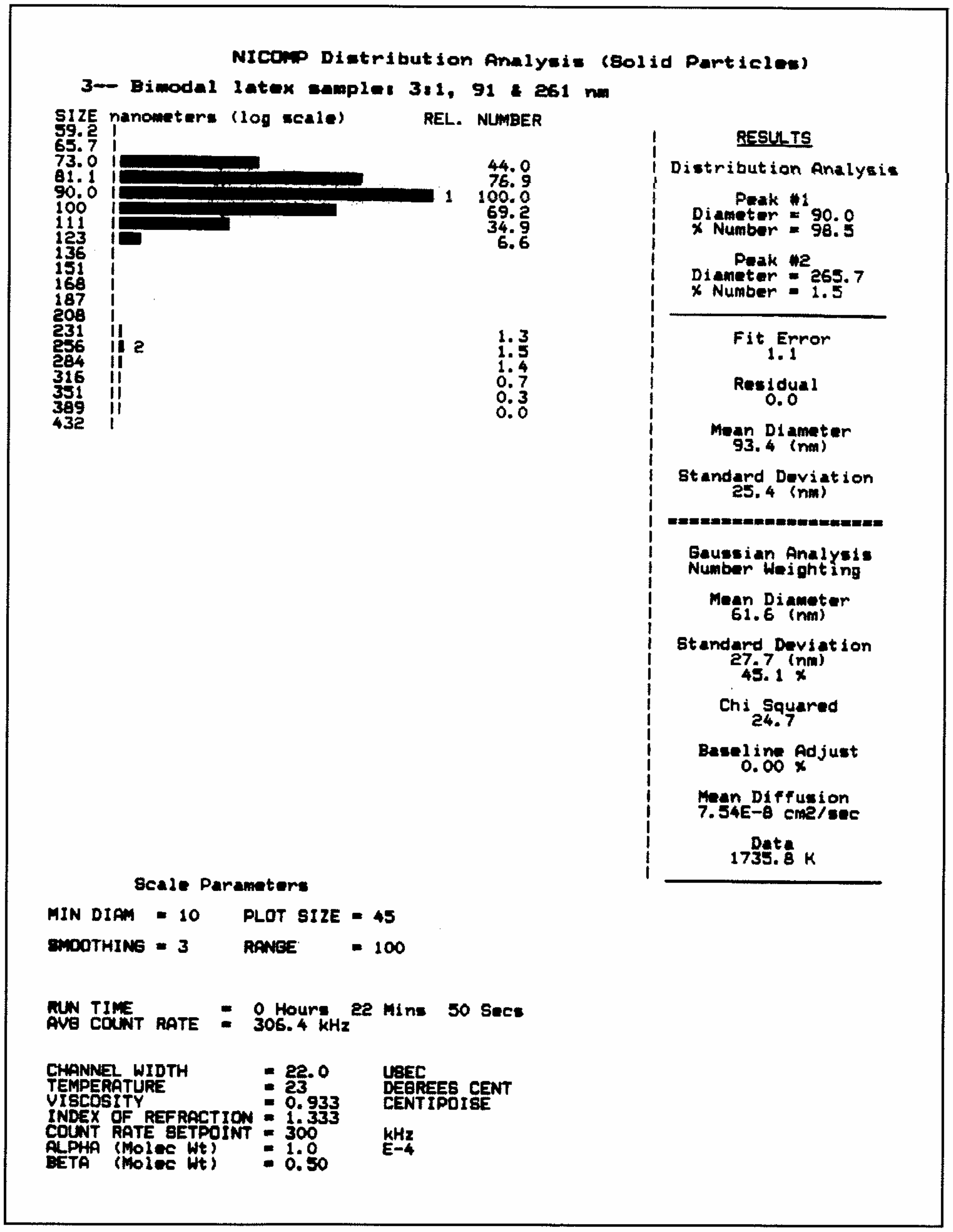


```
                NICOMP Distribution Analysis (Solid Particles)
   3-- Bimodal latex sample: 3:1, 91 & 261 nm

SIZE nanometers (log scale)      REL. VOLUME
59.2 |
65.7 |
73.0 |                                23.5
81.1 |                                56.2
90.0 |                          1    100.0
100  |                                94.7
111  |                                65.5
123  |                                17.0
136  |                                17.8
151  |                                18.6
168  |                                19.4
187  |                                20.1
208  |                                20.9
231  |                                21.7
256  |                                35.0
284  |            2                   43.1
316  |                                28.2
351  |                                16.3
389  ||                                1.4
432  |
480  |
```

```
        RESULTS

 Distribution Analysis

        Peak #1
   Diameter = 94.2
   % Volume = 69.9

        Peak #2
   Diameter = 283.4
   % Volume = 30.1
 ______________________

       Fit Error
          1.1

       Residual
          0.0

     Mean Diameter
      150.3 (nm)

  Standard Deviation
       88.7 (nm)

 ====================

   Gaussian Analysis
   Volume Weighting

     Mean Diameter
      139.0 (nm)

  Standard Deviation
       62.7 (nm)
       45.1 %

      Chi Squared
         24.7

    Baseline Adjust
        0.00 %

    Mean Diffusion
   3.34E-8 cm2/sec

         Data
       1735.8 K
 ______________________
```

```
          Scale Parameters

MIN DIAM  = 10      PLOT SIZE = 45

SMOOTHING = 3       RANGE     = 100


RUN TIME        =  0 Hours  22 Mins  50 Secs
AVG COUNT RATE  =  306.4 kHz


CHANNEL WIDTH        = 22.0     USEC
TEMPERATURE          = 23       DEGREES CENT
VISCOSITY            = 0.933    CENTIPOISE
INDEX OF REFRACTION  = 1.333
COUNT RATE SETPOINT  = 300      kHz
ALPHA (Molec Wt)     = 1.0      E-4
BETA  (Molec Wt)     = 0.50
```

Figure for the 19b: Printout of the volume-weighted Distribution Analysis result 91/261nm test bimodal



```
          NICOMP Distribution Analysis (Solid Particles)
   3-- Bimodal latex sample: 3:1, 91 & 261 nm

SIZE nanometers (log scale)      REL. NUMBER
59.2
65.7
73.0                                  44.0
81.1                                  76.9
90.0                             1   100.0
100                                   69.2
111                                   34.9
123                                    6.6
136
151
168
187
208
231                                    1.3
256     2                              1.5
284                                    1.4
316                                    0.7
351                                    0.3
389                                    0.0
432

            RESULTS

    Distribution Analysis

           Peak #1
      Diameter = 90.0
      % Number = 98.5

           Peak #2
      Diameter = 265.7
      % Number = 1.5
    ------------------------
          Fit Error
             1.1

          Residual
             0.0

        Mean Diameter
          93.4 (nm)

     Standard Deviation
          25.4 (nm)
    ====================

      Gaussian Analysis
      Number Weighting

        Mean Diameter
          61.6 (nm)

     Standard Deviation
          27.7 (nm)
           45.1 %

         Chi Squared
            24.7

       Baseline Adjust
           0.00 %

       Mean Diffusion
      7.54E-8 cm2/sec

            Data
          1735.8 K
    ------------------------

        Scale Parameters

MIN DIAM  = 10     PLOT SIZE = 45

SMOOTHING = 3      RANGE     = 100


RUN TIME          =  0 Hours  22 Mins  50 Secs
AVG COUNT RATE    =  306.4 kHz

CHANNEL WIDTH         = 22.0     USEC
TEMPERATURE           = 23       DEGREES CENT
VISCOSITY             = 0.933    CENTIPOISE
INDEX OF REFRACTION   = 1.333
COUNT RATE SETPOINT   = 300      kHz
ALPHA (Molec Wt)      = 1.0      E-4
BETA  (Molec Wt)      = 0.50
```

Figure 19c: Printout of the number-weighted Distribution Analysis result for the 91/261 nm test bimodal

As mentioned above, we can generally conclude that the wider the separation of a bimodal, the easier it is for the Distribution Analysis to perform an accurate measurement-again, provided there is adequate scattering from the weakest of the two peaks. Figure 20 shows a logarithmic plot of the reduced autocorrelation data for a 3:1 mixture (by mass, or volume) of 91 and 1091 nm latex standards. Here, for the first time, we see a very substantial curvature in the reduced data, with the initial slope (at t’ =0) significantly greater than the final slope (at t’ = 64Δt’**).** This difference notwithstanding, it is still surprising that there is no obvious “breakpoint” in the plot, despite the factor-of-12 difference in the two constituent particle sizes. However, this sample is handled with relative ease by the NICOMP algorithm. The Distribution Analysis result (video display) is shown in Figure 21. After only 7 minutes of data acquisition, the analysis finds two peaks, at 84 and 1041 nm, quite close to the correct values. Even the relative volumes are accurate: 77/23%, compared to the ideal values of 75/25%.

In conclusion, it is worth returning to the issue of statistical accuracy of the autocorrelation function and the stability of the results produced by the Distribution Analysis. Here, it turns out to be even more important than for the Gaussian Analysis that adequate data be acquired so that the ILT algorithm can settle to a reliable result. In general, the more complex the distribution, the longer the time needed to obtain a stable result. This principle is illustrated nicely by a sequence of results obtained from the 91/261 nm test bimodal discussed earlier. This sample was run again, and a time sequence of volume-weighted printouts is shown in Figure 22a-d, corresponding to run times of (approximately) 7 min., 10 min., 42 min. and 8 hours 10 min.

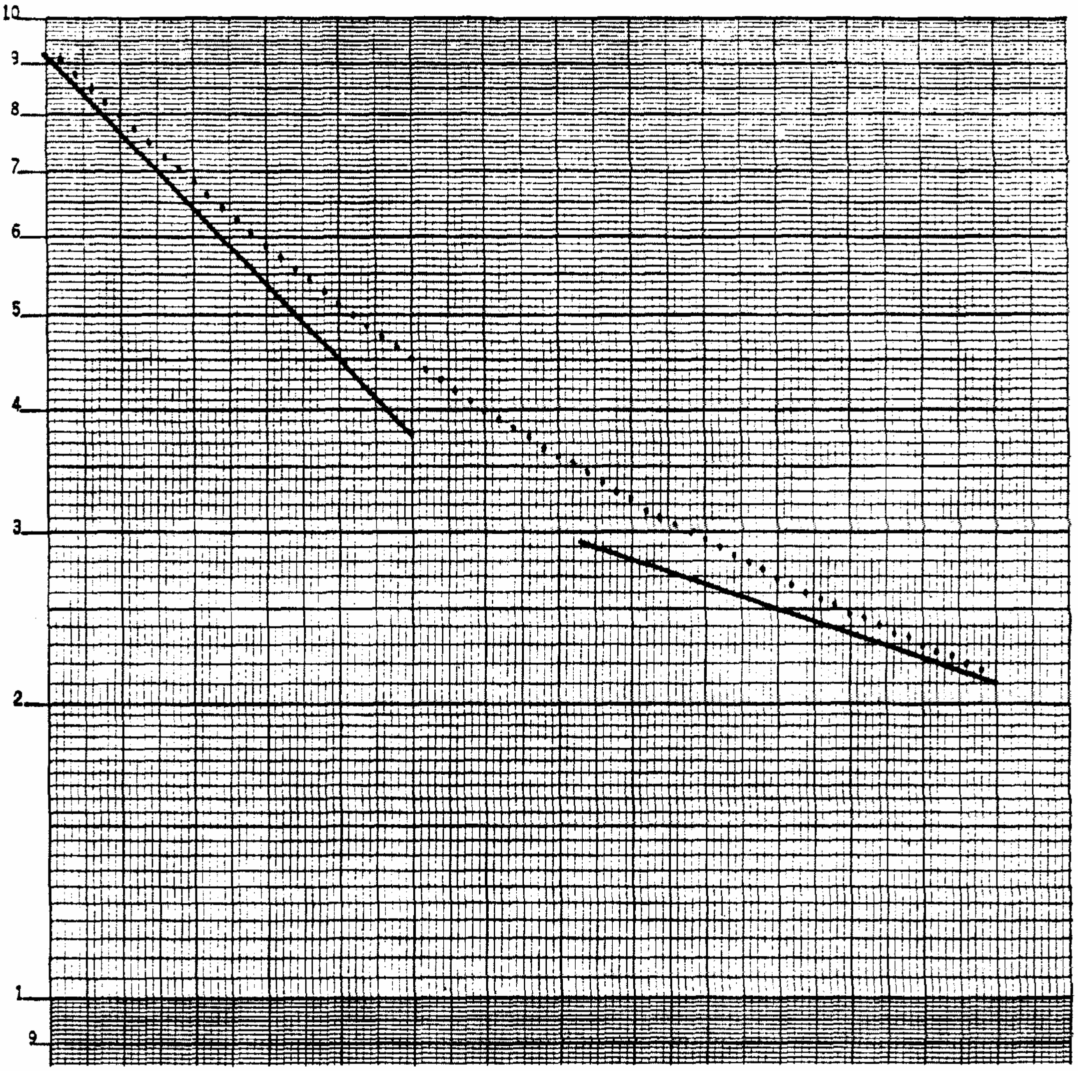
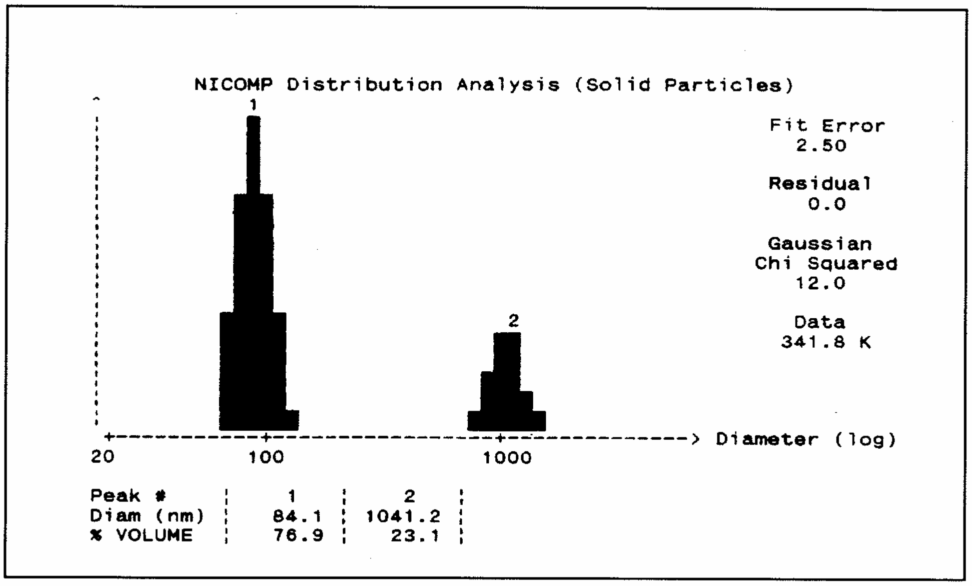


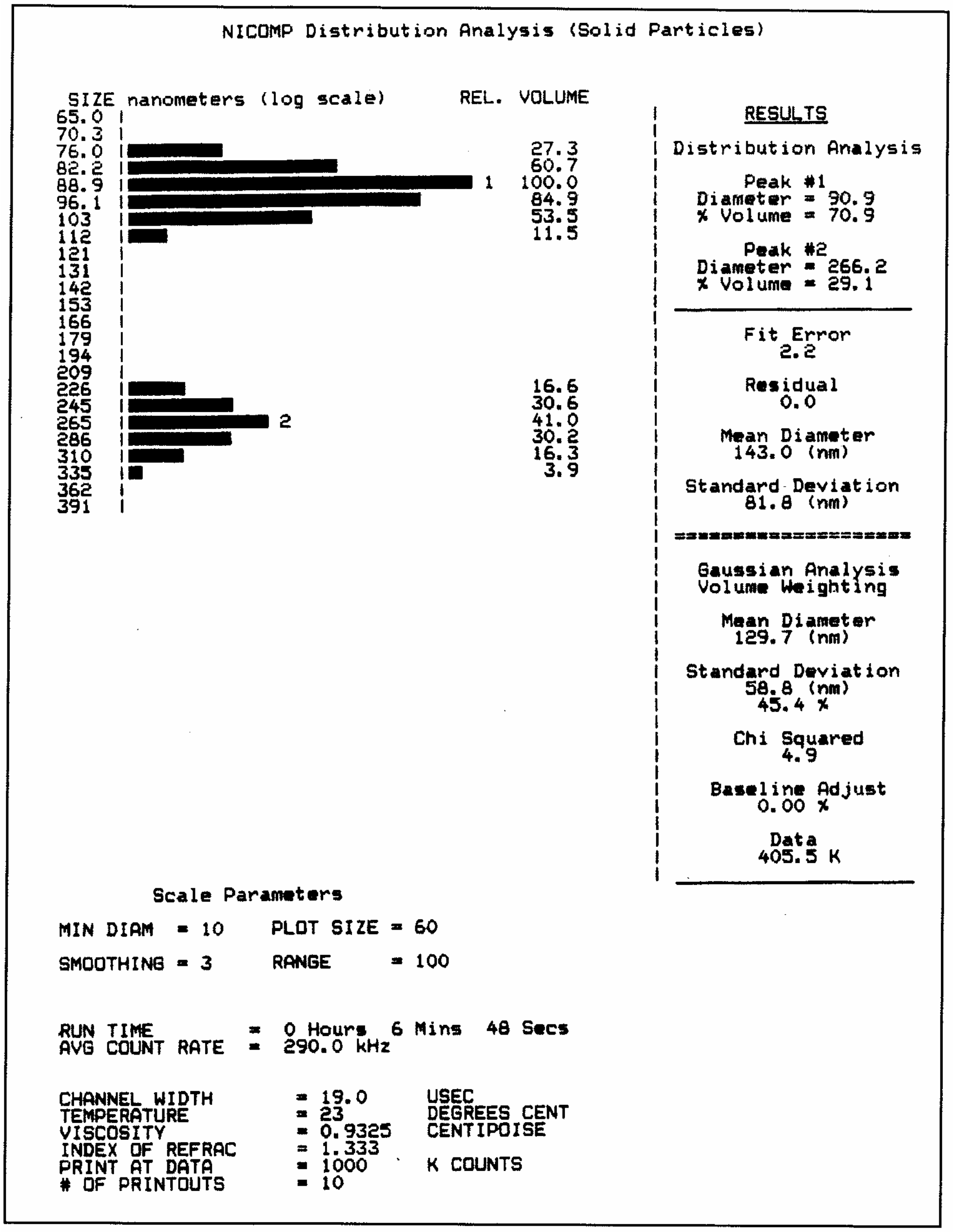
Figure 20: Log_e(C(t')-B) vs. t' for a widely-separated bimodal latex sample: 3:1 (vol.) ratio, 91 and 1091 nm



```
Fit Error
  2.50

Residual
  0.0

Gaussian
Chi Squared
  12.0

Data
341.8 K

Peak #    |   1    |   2    |
Diam (nm) |  84.1  | 1041.2 |
% VOLUME  |  76.9  |  23.1  |
```

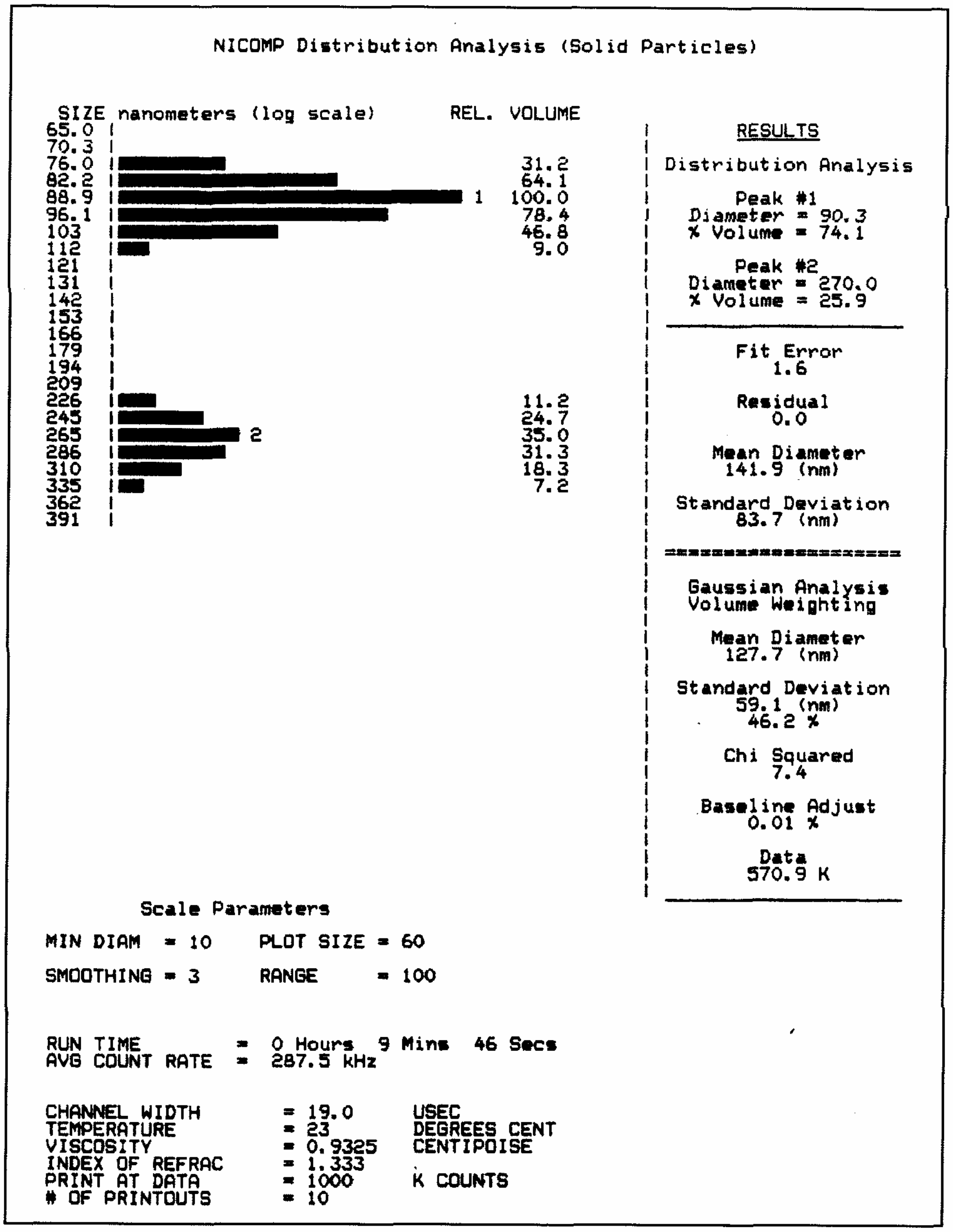
Figure 21: The volume-weighted Distribution Analysis result for the 91/1091 sample (Figure 20) nm bimodal

For this particular sample preparation, we see that the first two results agree very closely with the known situation -- indeed, more closely than could normally be expected for these short data acquisition times. After an elapsed time of 42 minutes (Figure 22c) the results have actually degraded somewhat, although the peak mean diameter values are still within a few percent of the correct answers. At this point the Fit Error has fallen to 1.0, with an ideal Residual of 0.0, which easily fits our criteria of a finished run. When we return 8 hours later (Figure 22d), we find a nearly perfect result, as regards both particle diameter and % volume for the two peaks.



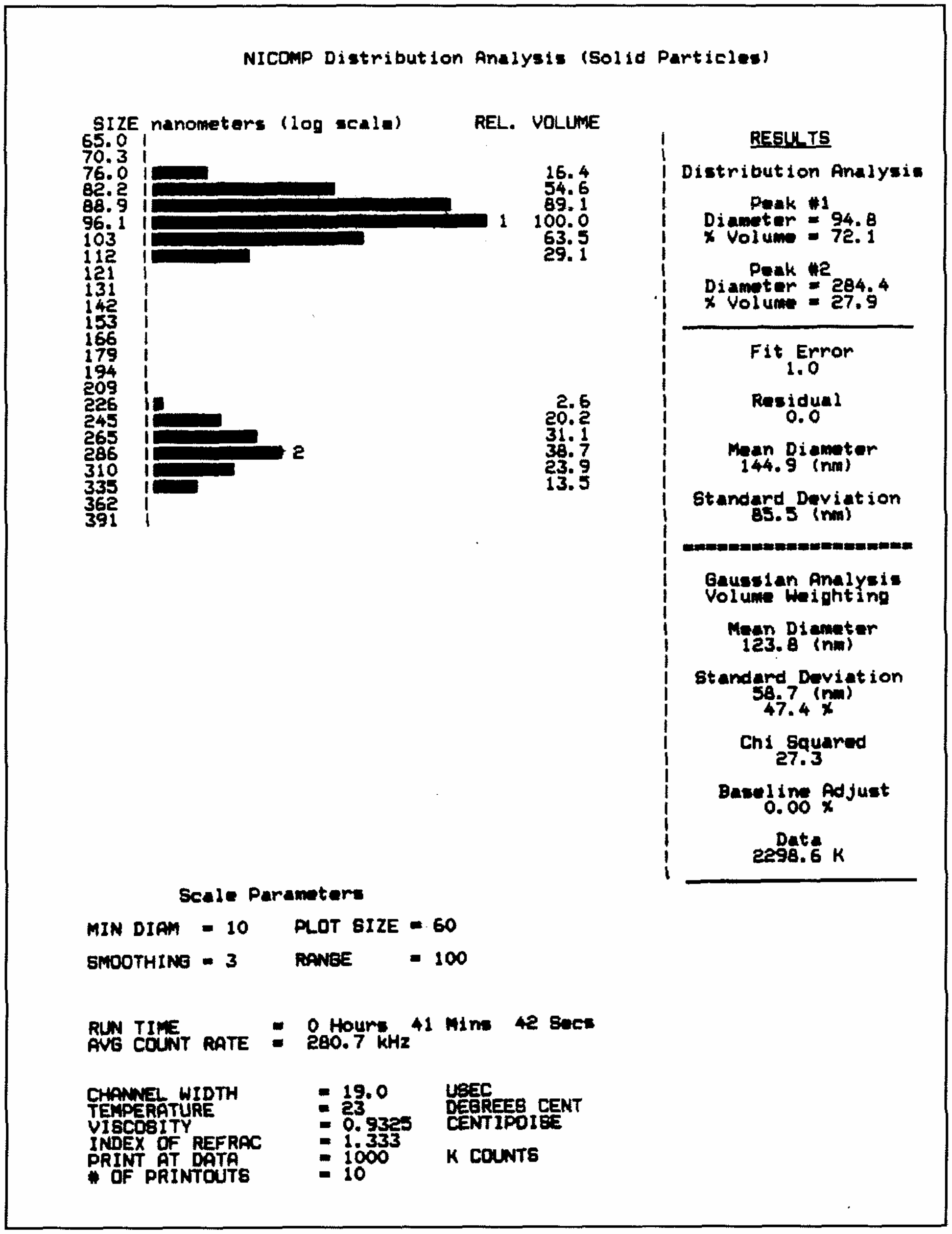
```
                NICOMP Distribution Analysis (Solid Particles)


 SIZE nanometers (log scale)       REL. VOLUME        |        RESULTS
65.0 |                                                |
70.3 |                                                |
76.0 |                                 27.3           | Distribution Analysis
82.2 |                                 60.7           |
88.9 |                           1    100.0           |        Peak #1
96.1 |                                 84.9           |   Diameter = 90.9
103  |                                 53.5           |   % Volume = 70.9
112  |                                 11.5           |
121  |                                                |        Peak #2
131  |                                                |   Diameter = 266.2
142  |                                                |   % Volume = 29.1
153  |                                                |
166  |                                                |
179  |                                                |       Fit Error
194  |                                                |          2.2
209  |                                                |
226  |                                 16.6           |       Residual
245  |                                 30.6           |          0.0
265  |          2                      41.0           |
286  |                                 30.2           |     Mean Diameter
310  |                                 16.3           |      143.0 (nm)
335  |                                  3.9           |
362  |                                                |  Standard Deviation
391  |                                                |       81.8 (nm)
                                                      |
                                                      | ===================
                                                      |
                                                      |   Gaussian Analysis
                                                      |   Volume Weighting
                                                      |
                                                      |     Mean Diameter
                                                      |      129.7 (nm)
                                                      |
                                                      |  Standard Deviation
                                                      |       58.8 (nm)
                                                      |        45.4 %
                                                      |
                                                      |      Chi Squared
                                                      |          4.9
                                                      |
                                                      |    Baseline Adjust
                                                      |        0.00 %
                                                      |
                                                      |          Data
                                                      |        405.5 K
                                                      |
          Scale Parameters

 MIN DIAM  = 10     PLOT SIZE = 60

 SMOOTHING = 3      RANGE     = 100


 RUN TIME        =  0 Hours  6 Mins  48 Secs
 AVG COUNT RATE  =  290.0 kHz

 CHANNEL WIDTH       = 19.0      USEC
 TEMPERATURE         = 23        DEGREES CENT
 VISCOSITY           = 0.9325    CENTIPOISE
 INDEX OF REFRAC     = 1.333
 PRINT AT DATA       = 1000      K COUNTS
 # OF PRINTOUTS      = 10
```

Figure 22a: Printout of volume-weighted Distribution Analysis result for the 3:1 91/261 bimodal sample after 7 min



```
                NICOMP Distribution Analysis (Solid Particles)

 SIZE nanometers (log scale)      REL. VOLUME
65.0 |                                          |        RESULTS
70.3 |                                          |
76.0 |                                31.2      | Distribution Analysis
82.2 |                                64.1      |
88.9 |                           1   100.0      |       Peak #1
96.1 |                                78.4      |   Diameter = 90.3
103  |                                46.8      |   % Volume = 74.1
112  |                                 9.0      |
121  |                                          |       Peak #2
131  |                                          |   Diameter = 270.0
142  |                                          |   % Volume = 25.9
153  |                                          |
166  |                                          |
179  |                                          |      Fit Error
194  |                                          |         1.6
209  |                                          |
226  |                                11.2      |      Residual
245  |                                24.7      |         0.0
265  |           2                    35.0      |
286  |                                31.3      |    Mean Diameter
310  |                                18.3      |     141.9 (nm)
335  |                                 7.2      |
362  |                                          |  Standard Deviation
391  |                                          |      83.7 (nm)
                                                |
                                                | ====================
                                                |
                                                |   Gaussian Analysis
                                                |   Volume Weighting
                                                |
                                                |    Mean Diameter
                                                |     127.7 (nm)
                                                |
                                                |  Standard Deviation
                                                |      59.1 (nm)
                                                |      46.2 %
                                                |
                                                |     Chi Squared
                                                |         7.4
                                                |
                                                |   Baseline Adjust
                                                |      0.01 %
                                                |
                                                |        Data
                                                |      570.9 K
                                                |
        Scale Parameters

MIN DIAM  = 10     PLOT SIZE = 60

SMOOTHING = 3      RANGE     = 100


RUN TIME        =  0 Hours  9 Mins  46 Secs
AVG COUNT RATE  =  287.5 kHz

CHANNEL WIDTH       = 19.0      USEC
TEMPERATURE         = 23        DEGREES CENT
VISCOSITY           = 0.9325    CENTIPOISE
INDEX OF REFRAC     = 1.333
PRINT AT DATA       = 1000      K COUNTS
# OF PRINTOUTS      = 10
```

Figure 22b: Printout of volume-weighted Distribution Analysis result for the 3:1 91/261 bimodal sample -- after 10 min

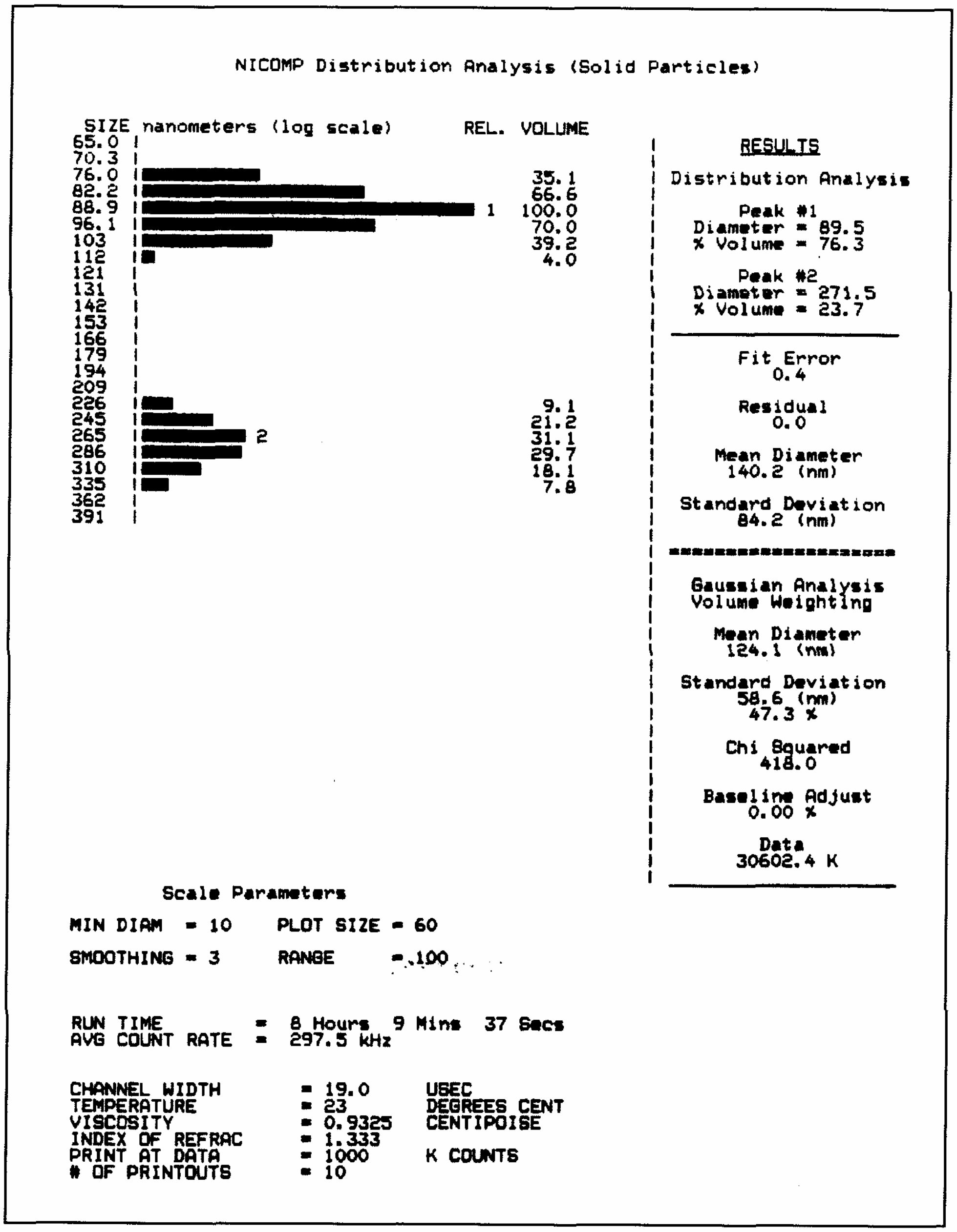


```
NICOMP Distribution Analysis (Solid Particles)

SIZE nanometers (log scale)       REL. VOLUME
65.0  |
70.3  |
76.0  |                                 16.4
82.2  |                                 54.6
88.9  |                                 89.1
96.1  |                          1     100.0
103   |                                 63.5
112   |                                 29.1
121   |
131   |
142   |
153   |
166   |
179   |
194   |
209   |
226   |                                  2.6
245   |                                 20.2
265   |                                 31.1
286   |          2                      38.7
310   |                                 23.9
335   |                                 13.5
362   |
391   |
```

```
        RESULTS

Distribution Analysis

       Peak #1
  Diameter = 94.8
  % Volume = 72.1

       Peak #2
  Diameter = 284.4
  % Volume = 27.9
 ____________________

      Fit Error
         1.0

      Residual
         0.0

    Mean Diameter
     144.9 (nm)

 Standard Deviation
      85.5 (nm)
 ====================

  Gaussian Analysis
  Volume Weighting

    Mean Diameter
     123.8 (nm)

 Standard Deviation
      58.7 (nm)
      47.4 %

     Chi Squared
        27.3

   Baseline Adjust
       0.00 %

        Data
     2298.6 K
 ____________________
```

```
        Scale Parameters

MIN DIAM  = 10     PLOT SIZE = 60

SMOOTHING = 3      RANGE     = 100


RUN TIME        =  0 Hours  41 Mins  42 Secs
AVG COUNT RATE  =  280.7 kHz

CHANNEL WIDTH        = 19.0      USEC
TEMPERATURE          = 23        DEGREES CENT
VISCOSITY            = 0.9325    CENTIPOISE
INDEX OF REFRAC      = 1.333
PRINT AT DATA        = 1000      K COUNTS
# OF PRINTOUTS       = 10
```

Figure 22c: Printout of volume-weighted Distribution Analysis result for the 3:1 91/261 bimodal sample -- after 42 min



```
NICOMP Distribution Analysis (Solid Particles)

 SIZE nanometers (log scale)      REL. VOLUME
65.0 |
70.3 |
76.0 |                                 35.1
82.2 |                                 66.6
88.9 |                          1     100.0
96.1 |                                 70.0
103  |                                 39.2
112  |                                  4.0
121  |
131  |
142  |
153  |
166  |
179  |
194  |
209  |
226  |                                  9.1
245  |                                 21.2
265  |          2                      31.1
286  |                                 29.7
310  |                                 18.1
335  |                                  7.8
362  |
391  |
```

```
        RESULTS

Distribution Analysis

        Peak #1
   Diameter = 89.5
   % Volume = 76.3

        Peak #2
   Diameter = 271.5
   % Volume = 23.7
 ______________________

       Fit Error
          0.4

       Residual
          0.0

     Mean Diameter
      140.2 (nm)

  Standard Deviation
      84.2 (nm)
======================

   Gaussian Analysis
   Volume Weighting

     Mean Diameter
      124.1 (nm)

  Standard Deviation
      58.6 (nm)
       47.3 %

      Chi Squared
         418.0

    Baseline Adjust
        0.00 %

         Data
      30602.4 K
 ______________________
```

```
           Scale Parameters

MIN DIAM  = 10      PLOT SIZE = 60

SMOOTHING = 3       RANGE     = 100


RUN TIME          =  8 Hours  9 Mins  37 Secs
AVG COUNT RATE    =  297.5 kHz

CHANNEL WIDTH         = 19.0       USEC
TEMPERATURE           = 23         DEGREES CENT
VISCOSITY             = 0.9325     CENTIPOISE
INDEX OF REFRAC       = 1.333
PRINT AT DATA         = 1000       K COUNTS
# OF PRINTOUTS        = 10
```

Figure 22d: Printout of volume-weighted Distribution Analysis result for the 3:1 91/261 bimodal sample -- after 8 hrs, 10 min

What is the lesson behind these printouts -- should samples be run for 8 hours? Certainly not! Rather, the point is that if one wishes to optimize the analytical capabilities of the instrument, they should make it a habit to obtain several intermediate printouts within the first 20 or 30 minutes of a run to verify the settling and stability of the computed distribution. Only in this way will confidence be instilled in the user that the Distribution Analysis results are close to the best that can be obtained by the DLS Module, given practical constraints on the total amount of the allotted time.

INITIAL HARDWARE SETUP

1. Connect the 25-pin male connector of the cable provided to the port identified on the back of the Nicomp instrument.

2. Connect the 9-pin female end of the connector to a serial port on the PC controller.

INITIAL HARDWARE SETUP

3. Plug the power cable provided into the back of the unit.
4. If the unit has the Autodilution feature, place the drain line into a waste bucket.
5. Apply power to the PC controller.
6. Install the Nicomp software package. Review the Software section of this manual.
7. Apply power to the Nicomp.

Software Installation

SOFTWARE INSTALLATION

The software program that is responsible for controlling the NICOMP Submicron Particle Sizer, and also the NICOMP Computing Autocorrelator, is named CW388. The version # can be identified when the program CW380 is run; it is displayed in the upper right-hand corner of the "default" display screen, which is the **Main Menu**. The software diskette that is included with the NICOMP 380 will contain four software files:

CW388.EXE: the largest of the four files. It is the executable program which controls the Instrument and initiates the running of the software.

CW388.CFG: contains the "configuration" of the 380 system. The configuration includes the identification of the serial port for communication with the 380 instrument and the parallel port for printing, as well as specification of available options, such as the flow pump for Autodilution, use of an external laser and the multi-angle accessory. In some cases the CW380 CD Rom will be missing the CW380.CFG file. This is not a mistake. Rather, after running and exiting the CW380 program, a CW380.CFG file will automatically be generated and stored in the disk drive and sub directory which are specified in the Auto Print/Save Menu (Particle Sizing Window).

CW388.TBL: includes all of the information contained in the Auto Print/Save Menu and the Control Menu (also accessed from the Particle Sizing Window). The former menu includes such parameters as Data Storage Directory, Data File Name, Printout ID, Run Time, etc. The [F3] Menu includes the following parameters, taken collectively, to define the conditions of data collection,printout and storage:

- Autoset Channel Width
- Sample Temperature
- Liquid Viscosity
- Liquid Index of Refraction

In some cases a new CW388 software diskette that is sent will be missing the CW380.TBL file. This is not a mistake. In the absence of this file, a default set of parameters will be used to define The parameters in the two menus that are accessed by pressing the [F2] and [F3] Menu keys. After the CW388 program is run and exited, a new CW388.TBL file will automatically be generated and stored in the disk drive and sub-directory where the main CW388.EXE program is located.

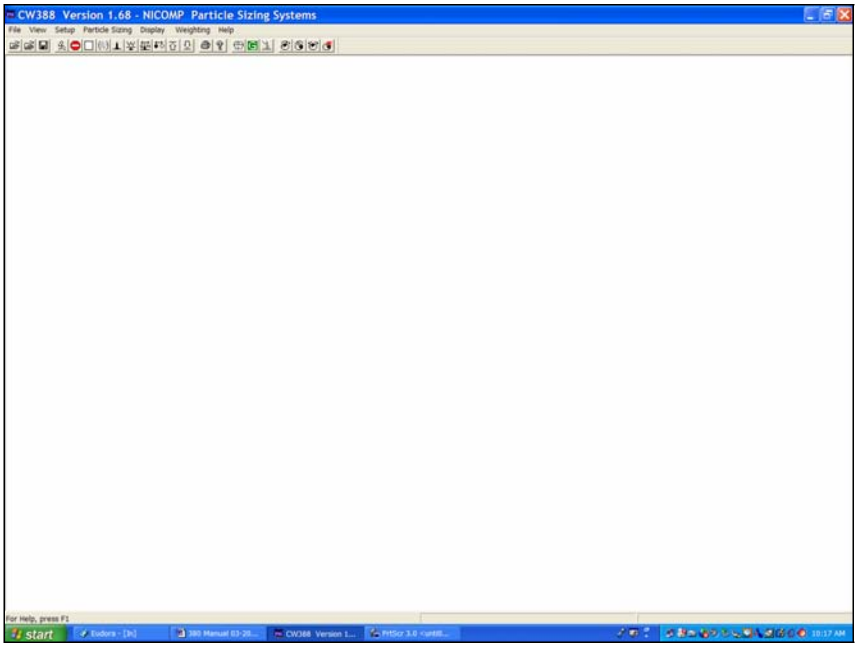
Generally it is recommended that the CW388 software files be copied to, and run from, the hard disk drive. The software on the diskette can then be saved as a backup copy, to be reloaded in the event of accidental erasure of a file or crash of the hard drive.

FILE

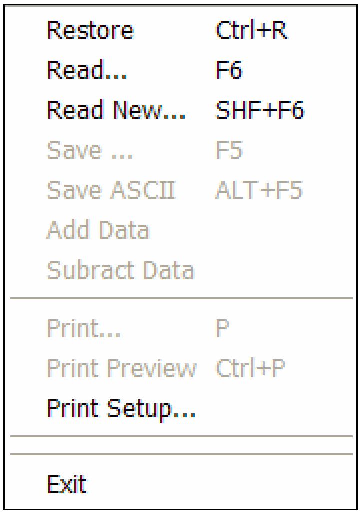
The following provides a brief summary of the File options that are used during the use of the Windows CW388 Software

Access of the File options:

1. Position the highlight over the **FILE** option and click once using the mouse. The following window of File options displays:



2. Position the highlight bar over the desired selection and click once. Following is a description of each of the options offered.



Restore

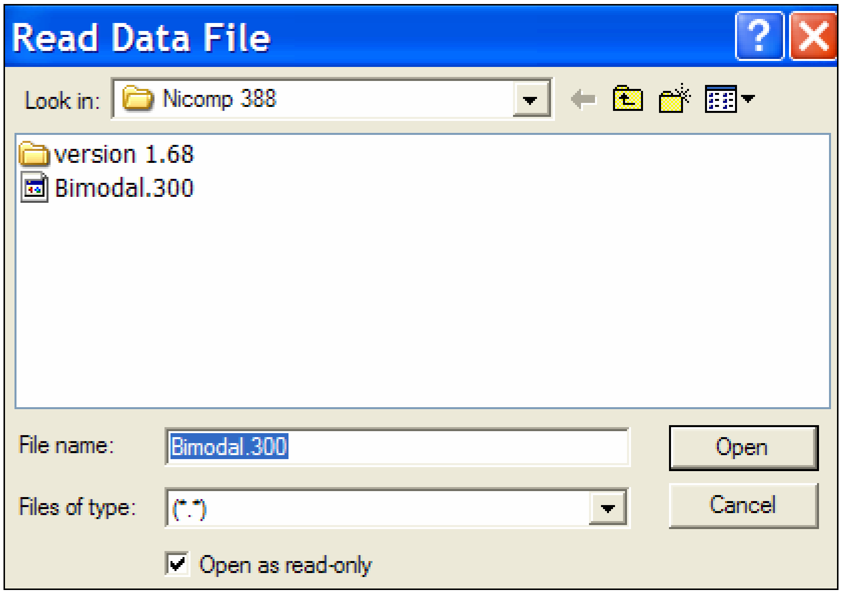
Used to restore collecting data once the autocorrelator has been halted.

Click on:

Yes: the screen will clear and data will be collected.
No: the warning screen will disappear and the autocorrelator will not start taking data.

Read

A data file that has been stored following a measurement can be retrieved to display the resulting particle size distribution (PSD), with the desired weighting. When this option is selected, a list of data files will display in the Read Data File window such as in the example below:



File name: Bimodal.300

Files of type: (*.*)

☑ Open as read-only

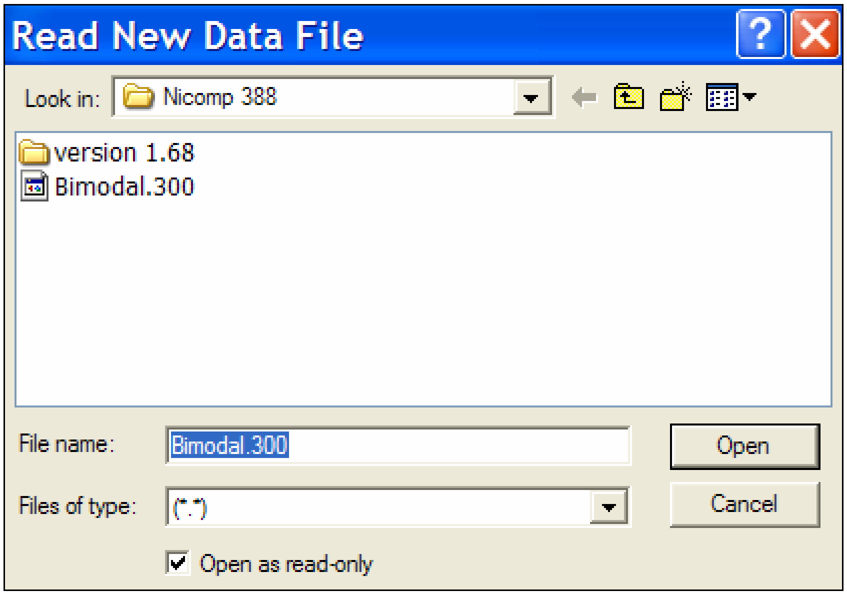
1. Position the highlight bar over the data file of interest and click the mouse once. Prior to the selected data file being retrieved from memory, the following window will display:

2. Click on:

Yes:	the data will be re-calculated.
No:	the window will disappear and the volume-weighted distribution of the file selected will display.

Read New

A data file that has been stored following a measurement can be retrieved to display the resulting particle size distribution (PSD), with the desired weighting. When this option is selected, a list of data files will display in the Read Data File window such as in the example below:



Look in: Nicomp 388

version 1.68
Bimodal.300

File name: Bimodal.300

Files of type: (*.*)

☑ Open as read-only

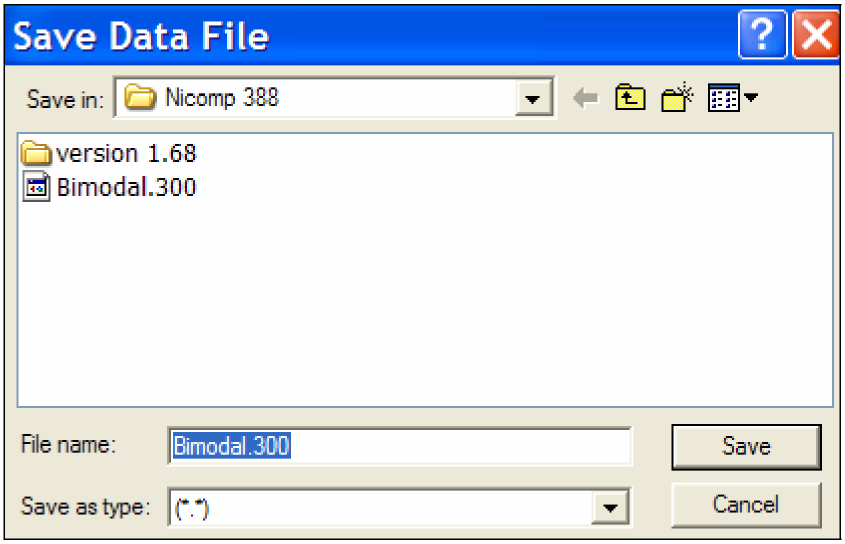
1. Position the highlight bar over the data file of interest and click the mouse.

2. Click on the **OK** button. The selected data file will be retrieved from memory. Once again the CW388 Window for re-calculating the data will re-display. Please refer to the **Read** section of this manual for additional information.

Save

This option is used throughout the CW388 software whenever a data file is to be saved. To access this option:

1. Position the highlight bar over the **File** option and click the mouse once.
2. Position the highlight bar over the **Save As** option and click the mouse once. The following screen will display:



File name: Bimodal.300

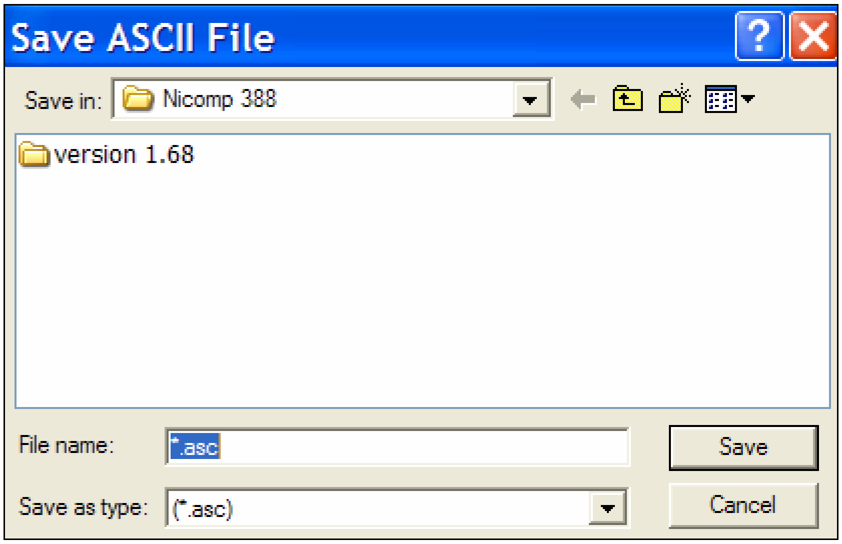
Save as type: (*.*)

Save ASCII

Use this option to save the data collected for a particular sample to an ASCII file format. The data can then be imported to a spreadsheet program for presentation.

To create new files in standard **ASCII** format to export data files into other software packages (e.g. spreadsheets), for manipulation of the original data follow these steps:

1. Select a data file.
2. Position the highlight bar over the **File** option and click once.
3. Position the highlight bar over the **Save ASCII File** option using the mouse and click once the following window displays:



Save in: Nicomp 388

version 1.68

File name: *.asc

Save as type: (*.asc)

1. Type in the desired file name. A new file in **ASCII** format will be created and stored in the **Data Directory** with the file extension **.asc**. If the same file name already exists, the following message will appear:

2. Click on:

Yes: the existing **ASCII** file having the same file name will be erased, and the new one stored in its place.
No: no new file will be stored.

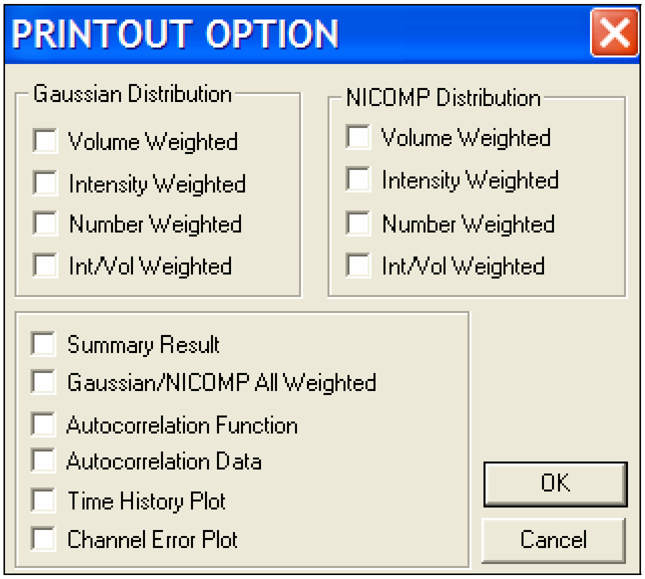
Add Data

Subtract Data

Print

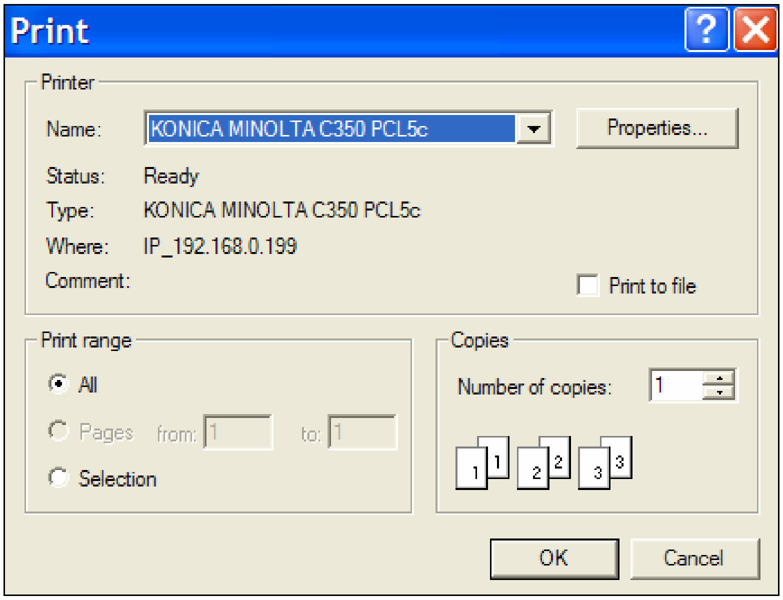
Printouts of the sample distributions that display on the computer monitor can be achieved using this option. Please refer to Appendix A for printout samples.

1. Click on the **File** Window option and position the highlight bar over the **Print** option and click. The following **Printout** Window will display:



2. Click on the square located to the left of the print selection. A black check mark will display in the box selected.

3. Click on **OK** to start printing the distributions and/or plots. The following Print window will display:



Name: KONICA MINOLTA C350 PCL5c
Status: Ready
Type: KONICA MINOLTA C350 PCL5c
Where: IP_192.168.0.199
Comment:
☐ Print to file

● All
○ Pages from: 1 to: 1
○ Selection

Number of copies: 1

1. Verify the printer type selected. If changes need to be made follow these steps:

2. Click on the Down arrow. A window of all of the printer brands and types will display. Selection of the correct printer driver software depends on the setup of this option.

3. Position the highlight bar over the **Printer** type and model that is currently hooked up to the computer being used.

4. Click the mouse once.

5. Click on the **OK** button. The Print windows will re-display with the printer and type and model selected.

6. Position the cursor over the print range desired.

   All - will print all data pertinent to the distribution being reviewed.
   Pages - will print the range of pages desired.
   Selection - will only print those pages desired.

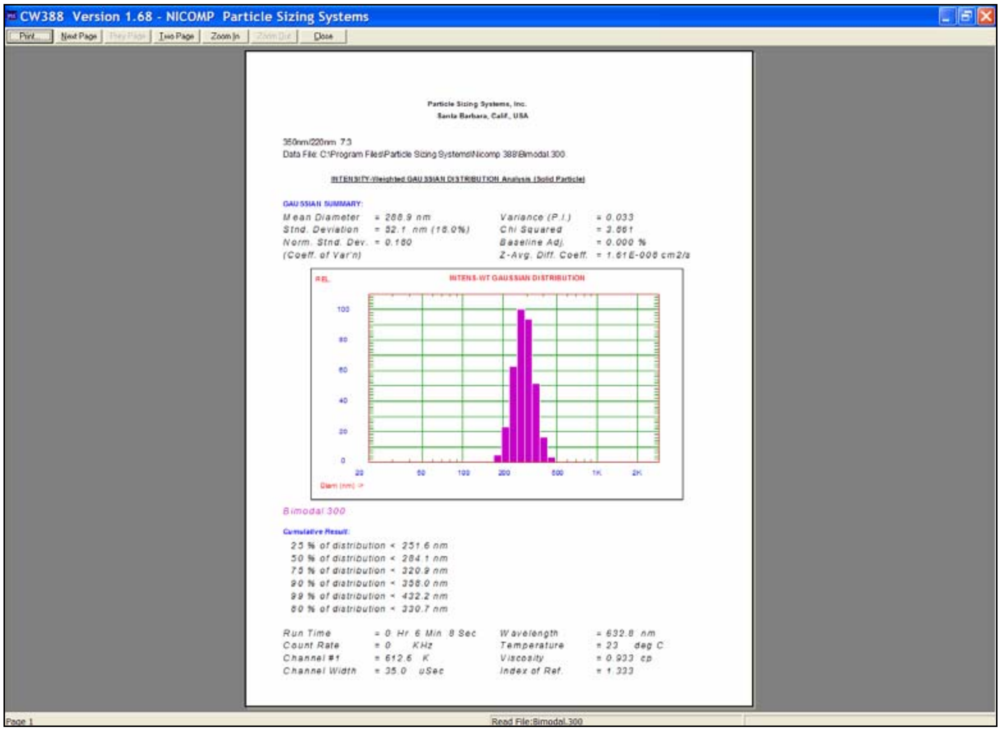
7. Position the cursor in the **Copies** option and type a number for the number of copied desired for the printout. The default is set to one.

8. Click on the **OK** button to start printing.

Print Preview

Allows the printout to be viewed prior to being sent to the printer. Following is an example of the window that will display. A data file must be accessed prior to using the Print Preview option.

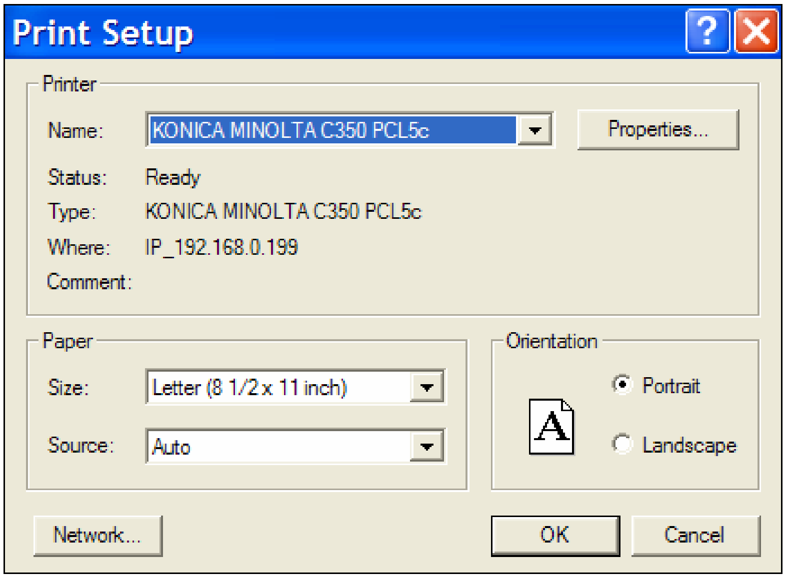
Print - will print the distribution that is being previewed
Next Page - will advance to the next page of the preview
Two Pages - will preview two pages of the same file side by side
Zoom In - provides the ability to zoom into the distribution to examine the fine details of the distribution
Close - will close this option and return to the CW388 Software Window



```
Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\Bimodal.300

INTENSITY-Weighted GAUSSIAN DISTRIBUTION Analysis (Solid Particle)

GAUSSIAN SUMMARY:
Mean Diameter    = 288.9 nm               Variance (P.I.)     = 0.033
Stnd. Deviation  = 52.1  nm (18.0%)       Chi Squared         = 3.861
Norm. Stnd. Dev. = 0.180                  Baseline Adj.       = 0.000 %
(Coeff. of Var'n)                         Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s

Bimodal.300

Cumulative Result:
  25 % of distribution <  251.6 nm
  50 % of distribution <  284.1 nm
  75 % of distribution <  320.9 nm
  90 % of distribution <  358.0 nm
  99 % of distribution <  432.2 nm
  80 % of distribution <  330.7 nm

Run Time         = 0  Hr  6 Min  8 Sec    Wavelength         = 632.8  nm
Count Rate       = 0      KHz             Temperature        = 23     deg C
Channel #1       = 612.6   K              Viscosity          = 0.933  cp
Channel Width    = 35.0    uSec           Index of Ref.      = 1.333
```

Print Setup

This option allows for the setup of the type of printer to be used, the orientation of the printout and the size paper to be used.



Print Setup

Printer

Name: KONICA MINOLTA C350 PCL5c

Status: Ready

Type: KONICA MINOLTA C350 PCL5c

Where: IP_192.168.0.199

Comment:

Paper

Size: Letter (8 1/2 x 11 inch)

Source: Auto

Orientation

● Portrait

○ Landscape

Printer

Default Printer
The printer that is used for the majority of the printing when using the computer controller. In some cases, this may be a black and white printer.

Specific Printer
Allows for the selection of another printer type such as a color printer for printing out color distributions.

Paper

Size
The paper sizes available depend on the model printer that is being used. By clicking on the down arrow located to the right of the Size window a listing of the available paper sizes for the computer being used displays. The default is set to Letter 8 1/2 x 11 since most printers accommodate for this size.

Source
The default for this option is set to Portable Sheet Feeder however, some printers have two paper trays for printing. The source of the paper feed depends on the model of printer being used.

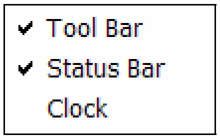
Orientation

Portrait (8 1/2 x 11 in)
The data distributions will display vertically on the paper selected.

Landscape (11 x 8 1/2 in)
The data distributions will display horizontally on the paper selected.

VIEW MENU

Click on View in the Main Window in order to pull down the View Menu. In the default condition, both the Tool bar, containing the icons near the top of the Main Window, and the Status Bar, located at the bottom of the Window, are activated (checked). They should remain activated.



Tool Bar

The Tool Bar is the bar that displays all of the CW388 icons that may be used during the use of the CW388 Windows software. Following is a summary of each of the icons displayed on the Tool Bar. A template has also been provided in this manual for ease of use.

Read Data File

A data file that has been stored following a measurement can be retrieved to display the resulting particle size distribution (PSD), with the desired weighting. When this option is selected, a list of data files will display in a the Read Data File window.

The right-most icon will overlay the PSD associated with the new, retrieved file onto existing PSDs that are already displayed, belonging to files that have already been retrieved and displayed.
Additional distributions corresponding to other data files can be accessed using this option. Each distribution curve on the screen can be identified by matching its color with that of the data file name displayed at the bottom of the screen. A maximum of eight data files can be overlaid

Save Data File

Use this option to manually save the data collected for a particular sample run.

Run Autocorrelator

The user may choose this operating mode to conduct the measurement but not save any data. The measurement will be made according to the choices made in the Control [F3] and Auto Print/Save [F2] menus of the CW388 software.

Stop Autocorrelator

Used to stop the collection of data in the autocorrelator.

Clear Correlator/Data

Clears data and closes any files that are open and displayed in the software. Prior to clearing the data a confirmation window will display prompting for action.

Start Autodilution

Starts collecting data using the proprietary Autodilution technique (U.S. Patent # 4,794,806; foreign patents pending) using this option.

Click on this tool to start Autodilution.

The automatic dilution of the sample then begins. The PUMP switches to ON (LOW SPEED) pulling diluent into the unit. When the appropriate particle concentration that results in a scattering intensity of 300 kHz.

Start Measurement

The instrument starts taking measurements once this option is initiated.

Change Weighting

Three weightings may be viewed for a particle size distribution using this option:

Volume-weighted
Displays the relative particle volume vs. diameter. The value of the volume-weighted particle size distribution is calculated assuming that the particle are spheres of uniform density which scatter light according to classical Mie Theory.

Number-weighted
Displays the relative number of particles in a sample run vs. diameter. The value of the number weighted particle size distribution is also calculated assuming that the particles are spheres of uniform density which scatter light according to classical Mie Theory.

Intensity-weighted
The result first displays from either of the autocorrelation functions. Displays the relative Intensity of scattered light vs. diameter for a sample run.

Toggle Gaussian/Nicomp Distribution

Toggles between the Gaussian and Nicomp Distribution for a sample file.

Toggle Solid/Vesicle Particle

The instrument defaults to solid particle weighting when power is first applied. The weighting may be changed using this option. The weighting will change when the results of a new analysis is displayed, at which time the title will also change, to Gaussian Analysis (vesicles). The original weighting can be re-displayed by using this option again.

Increase Intensity

Can be used to increase the sensitivity to achieve the optimum Photopulse Rate.

Decrease Intensity

Can be used to lower the sensitivity to achieve the optimum Photopulse Rate.

Print the Active Document

This option is used to print the appropriately weighted distribution displayed.
Prior to selecting the print option, pressing W will toggle among the different distribution weightings: population, number-wt., area-wt., and volume-wt.

1. Click on the Print tool. The Printout Option window will display with print options. Please refer to the FILE section of this manual for available PRINT options.

2. Position the highlight bar over the desired option.

3. Click on the box located to the left of the selection. A check mark will display to the left of the option.

4. Continue steps b and c until all print desired print options are selected. If a print option is not desired and a check mark is displayed in the box to the left of the option position the cursor on the check mark and click once.

5. Click on OK when the desired combination of printout options has been selected in the above manner. This will initiate communication with the printer, displaying the printer setup window.

6. Click on OK to start printing. Please refer to the Distribution section of this manual to view the different distribution printouts that are available.

Display Help for Current Task or Command

Provides quick on-line help for a particular operation.

Flow Pump On

This icon is used to start the flow pump and is used in flushing the system if autodilution is present.

Start Measurement

This icon is used to START the sequence of particle size measurements.

Resume Taking Data

The autocorrelator resumes collecting data for the sample that has been introduced into the instrument.

Neutral Density Adjustments

Used to increase the intensity of the incident laser light by rotating the Neutral Density Filter.

Used to decrease the intensity of the incident laser light by rotating the Neutral Density Filter.

Will show a small dialogue box that will prompt the user for a Neutral Density Filter setting.

Allows the Neutral Density Filter to automatically search out the optimal scattering intensity for the sample.

Status Bar

For Help, press F1

Collecting Data

The status bar provides pertinent information while running the Nicomp 380.

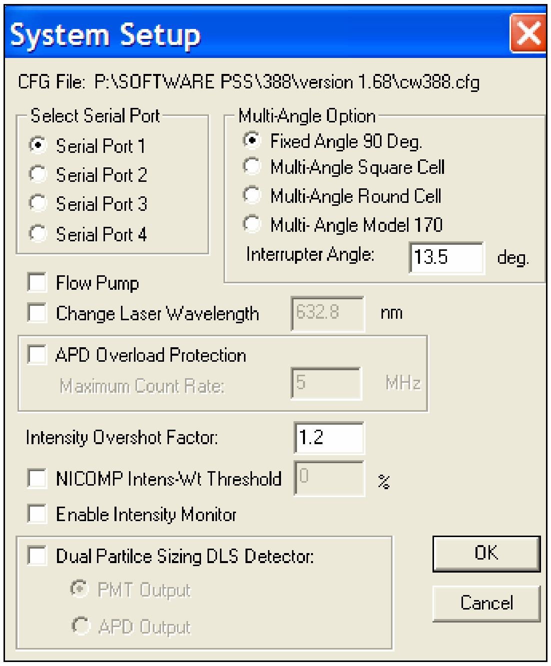
Clock

10:59:52 AM

Displays the real time clock that is set up in the windows operating system of the computer. This is the clock that is used to date and time stamp the data files that are saved.

SETUP

The Setup menu allows for communication to be established between the Nicomp and the computer controller.



CFG File: P:\SOFTWARE PSS\388\version 1.68\cw388.cfg

Select Serial Port
- ● Serial Port 1
- ○ Serial Port 2
- ○ Serial Port 3
- ○ Serial Port 4

Multi-Angle Option
- ● Fixed Angle 90 Deg.
- ○ Multi-Angle Square Cell
- ○ Multi-Angle Round Cell
- ○ Multi- Angle Model 170

Interrupter Angle: 13.5 deg.

- ☐ Flow Pump
- ☐ Change Laser Wavelength 632.8 nm
- ☐ APD Overload Protection
  - Maximum Count Rate: 5 MHz

Intensity Overshot Factor: 1.2

- ☐ NICOMP Intens-Wt Threshold 0 %
- ☐ Enable Intensity Monitor
- ☐ Dual Partilce Sizing DLS Detector:
  - ● PMT Output
  - ○ APD Output

Select Serial Port

Four serial ports are provided for setting up communications between the Nicomp and the computer.

Position the cursor over the desired selection and click on the corresponding circle. A black circle will display next to the selection and the parameter will appear in the System Setup menu.

Multi-Angle Option

This parameter is used to establish the configuration for the detection of scattered light. There are four possible configurations to choose from:

Fixed Angle 90 Deg.

Selected when using the basic Nicomp in which the scattering angle is set to 90 degrees.

Multi-Angle Square Cell

Selected if the Nicomp possesses the multi-angle option (computer controlled stepper motor (0.9 deg./step) optical fiber) and a square cuvet (either normal 1-cm or miniature) is used for the sample cell.

Multi-Angle Round Cell

Selected when the Nicomp possesses the multi-angle option and a cylindrical sample cell.

Cylindrical Cells

The true scattering angle is equal to the external angle of the stepper motor arm, provided the cell is highly cylindrical and well aligned – i.e. centered on the shaft of the stepper motor.

Multi-Angle Model 170

Designed to be used with the Nicomp 170 Computing Autocorrelator. Any value for the actual scattering angle, independent of the type of scattering cell used may be entered.

Interrupter Angle

The interrupter angle parameter is only used for the Nicomp units which include the multi-angle option.

The interrupter angle is the reference angle for the moveable arm on the stepper motor, which carries the pinhole/optical fiber receiver. When power is first applied to the Nicomp, the internal computer causes the stepper motor arm to rotate until it intersects an optical interrupter. The latter defines the reference point, or interrupter angle, which is approximately 122.4 degrees with respect to the forward direction of the laser beam (which defines the zero angle).

The stepper motor than advances in the opposite direction, at 0.9 degrees/step, until the arm reaches 90 degrees in angle. The number of steps depends on the value of the interrupter angle. Any subsequent changes in angle are made from th 90-degree “resting” angle. If the moveable arm becomes misaligned, the resting angle may differ from 90 degrees. The resulting error can easily be eliminated by resetting the value of the interrupter angle. If the resting angle is too small (e.g. 88 degrees), the interrupter angle must be decreased by the appropriate amount.

Flow Pump

The flow pump parameter indicates whether the Nicomp contains a flow pump, which is required for the Autodilution option. The flow pump may be operated manually or by automatic computer control, in Autodilution mode. Deactivate the flow pump by selecting this option.

Drop-in Cells

Use of the flow pump must be suspended when using a drop-in cell to take a measurement. If it is not, flooding in the unit will occur causing major damage to the instrument.

Change Laser Wavelength

The appropriate laser wavelength for the type of external laser being used Is entered using this option. The default wavelength is 632.8 nm which is required for the basic Nicomp with internal, 5-mW HeNe laser.

LASER	WAVELENGTH
RLD 5 MW HENE	632.5 nm
RLD 12 MW HENE	635 nm
RLD 35 MW HENE	639 nm
RLD 50 MW HENE	664 nm
RLD 100 MW HENE	664 nm
GLD 20 MW HENE	532 nm
GLD 50 MW HENE	532 nm
GLD 100 MW HENE	532 nm

APD Overload Protection

The overload protection option is selected to when trying to control the amount of scattering when too much light enters the detector.

Maximum Count Rate

Defines the maximum amount of light that can enter the detector.

Intensity Overshoot Factor

When the intensity falls to approximately the Intensity Setpoint which is set in the Conrol Menu, the pump in the Nicomp will halt, thereby stopping the flow of fresh diluent. The Intensity Overshoot Factor compensates for the proper stopping of the pump.

NICOMP Intens-Wt Threshold

Sizing is not performed until this specified level of intensity is achieved.

Enable Intensity Monitor

Provides intensity as a function of time.

Dual Particle Sizing DLS Detector

PMT Output (standard)

APD Output

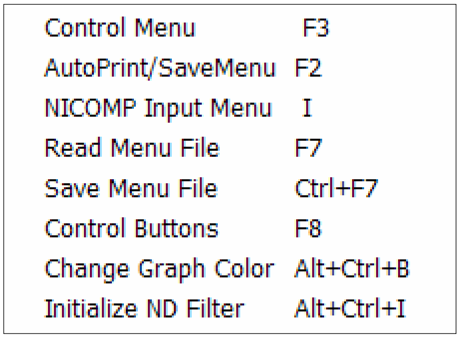
High gain detector offers 7 time the gain of a standard PMT for
sizing small nanoparticles 0.1-10 nm or low concentration colloidal solutions.

PARTICLE SIZING

The following provides a brief summary of the Particle Sizing options that are used during the use of the Nicomp Software.

Access of the Particle Sizing options:

1. Position the highlight over the Particle Sizing option and click once using the mouse. The following window displays:



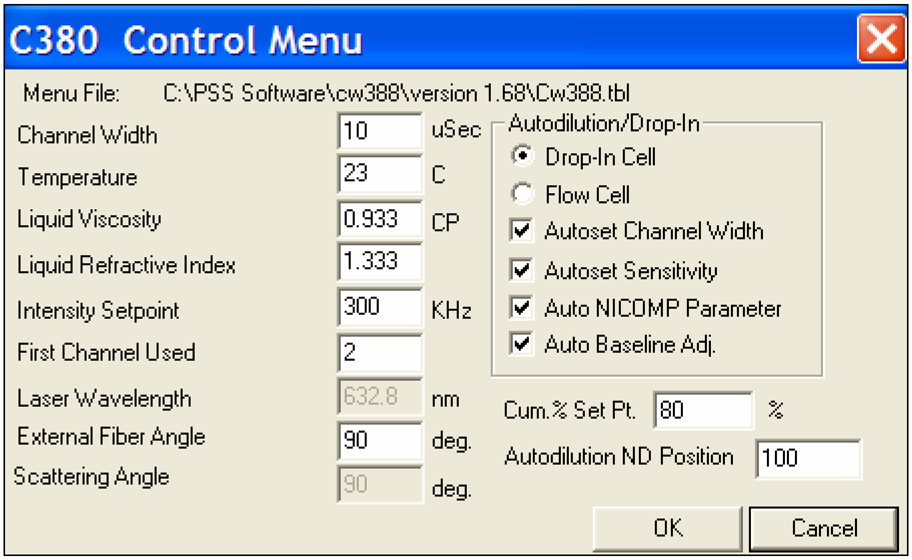
Control Menu	F3
AutoPrint/SaveMenu	F2
NICOMP Input Menu	I
Read Menu File	F7
Save Menu File	Ctrl+F7
Control Buttons	F8
Change Graph Color	Alt+Ctrl+B
Initialize ND Filter	Alt+Ctrl+I

2. Position the highlight bar over the desired selection and click once. Following is a description of each of the options offered.

Control Menu

The Control Menu determines the conditions under which raw data will be collected. Access to the Control Menu is gained using this option. This is one of two menus used to define the basic operation of the Nicomp. (The other is the AutoPrint/SaveMenu).

1. Position the highlight bar over the Control Menu option and click once. The Control Menu will display:



C380 Control Menu

Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Channel Width: 10 uSec
Temperature: 23 C
Liquid Viscosity: 0.933 CP
Liquid Refractive Index: 1.333
Intensity Setpoint: 300 KHz
First Channel Used: 2
Laser Wavelength: 632.8 nm
External Fiber Angle: 90 deg.
Scattering Angle: 90 deg.

Autodilution/Drop-In
● Drop-In Cell
○ Flow Cell
☑ Autoset Channel Width
☑ Autoset Sensitivity
☑ Auto NICOMP Parameter
☑ Auto Baseline Adj.

Cum.% Set Pt. 80 %
Autodilution ND Position 100

2. Press TAB to advance to each of the parameters on the screen.

Menu File

Specifies the file name under which this table of parameters will be saved.

Channel Width

The channel width of the digital autocorrelator in the Nicomp is reported in terms of microseconds, or µsec. In most cases the system should be allowed to set the channel width automatically. Ideally the channel width will be adjusted so that the number of decays in the autocorrelation function lies between 1.7 and 2.7.

Bypass of the automatic adjustment of the channel width can be made allowing a value to be entered manually. This option is preferred when similar samples are going to be measured on a repeat basis. Use of the same channel width for each sample measurement may improve the reproducibility of the results.

The default value for the channel width in the table-file CW388.TBL is 10 µsec, which is appropriate for a mean particle diameter of approximately 100 nanometers (nm), or 0.1 micron. If much smaller particles, such as small proteins or surfactant micelles, with mean diameters between 5 and 10 nm were to be measured, a value of about 1 µsec would be more appropriate for the channel width.

Temperature

The temperature of the sample cell, which is regulated to within ± 0.2° C by a Peltier thermoelectric element and feedback circuit can be moderated using this option. The value entered will be transmitted to the temperature regulator when the Control Menu is closed. The software includes a lower limit of 4° C and an upper limit of 60° C.

> **Important**: Sufficient time must be allowed for the temperature of the sample to reach that of the cell holder. It is the cell holder that is regulated in the Nicomp, a minimum of 5 minutes should be allowed.

Liquid Viscosity

The viscosity of the sample suspension is expressed in units of centipoise (cP). The particle suspension must be very dilute for measurements based on dynamic light scattering (DLS), in order to avoid errors due to interparticle interactions and/or multiple scattering. Therefore, the viscosity which is needed in the Control Menu is only that of the pure diluent in which the sample particles are suspended. The value of 0.933 cP, shown in the Control Menu example above, is the viscosity of water at 23°C.

The viscosity of a simple solvent can easily be determined from reference books. However, it must be remembered that the viscosity of many solvents (including water) is highly dependent on temperature. Viscosity values for various common organic solvents can be found in Appendix C of this manual.

Liquid Index of Refraction

This parameter establishes the index of refraction of the solvent, in which the particles are suspended, assuming a dilute suspension. The value of 1.333, shown in the example above, is the index of refraction of water. Unlike the viscosity, the index of refraction has very little dependence on temperature. Values for various common organic solvents can be found in Appendix D of this manual.

Intensity Setpoint

The average scattered intensity or photopulse rate, expressed in kHz, which is desired for a measurement, can be established by setting this parameter. The default value is set to 300 kHz. This value is typically recommended for most samples which scatter adequately. It is designed to optimize the efficiency of the autocorrelation process and thereby minimize the time needed to obtain reliable, accurate results for most samples.
The intensity setpoint is relevant for both modes of sample measurement -- i.e. using either Autodilution or Drop-in Cell.

Autodilution mode

The scattering intensity will increase to a relatively high value as the sample concentration initially increases, due to the flow of fresh sample and diluent through the mixing chamber and into the flow through sample cell in the Nicomp. It will then reach a maximum and then decrease as the sample concentration falls. When the intensity falls to approximately the Intensity Setpoint, the pump in the Nicomp will halt, thereby stopping the flow of fresh diluent into the flow through sample cell. The scattering intensity will then stabilize, because the particle concentration will no longer change.

Drop-in Cell mode

The sensitivity will be changed automatically in Automode operation, so that the final intensity will approximate the value stored for the Intensity Setpoint. This will be the case provided the sample concentration is not too large or small, thereby putting the initial scattering intensity outside the accessible range of the automatic system.

The sensitivity can be adjusted manually at any time one of two ways:

1. Alter the concentration of the sample.

2. Increase or decrease the incident laser intensity by adjusting the neutral density filter.

First Channel Used

This parameter is used to establish the channel number in the autocorrelation function which is chosen to be the starting channel for all calculations of the particle size distribution.

The default value is 2, which is the preferred value for most measurements. The value of 2 means that the first channel is effectively discarded; this will eliminate artifacts in the results caused by high-frequency noise, principally after-pulsing in the PMT detector.

In principle, it will be useful to increase the value of this parameter to 4 (the maximum value) in cases where the channel width is very small: i.e., < 0.5 microsec. This will reduce artifacts in the analysis results caused by PMT after-pulsing, which influences the initial 1 to 1.5 microsec. of the autocorrelation function.

Laser Wavelength

The laser wavelength, parameter is normally not accessible. The default value is 632.8 nm, which is the wavelength for the small HeNe laser used in the basic Nicomp system. Please refer to the Setup menu section of this manual for all wavelength settings. If the system includes a laser that is not HeNe, it is necessary to change this parameter in the Setup Window.

External Fiber Angle

Scattering Angle

The external angle of the stepper-motor arm and the resulting (actual) scattering angle, are only accessible when the multi-angle option is used with the system. The default value for both parameters is 90.0 degrees, which is the normal scattering angle for the basic Nicomp system.

The external angle, can be changed allowing the system to compute the resulting (actual) scattering angle, which depends on the geometry of the scattering cell. The external angle is made accessible by making the appropriate change in the System Setup Menu.

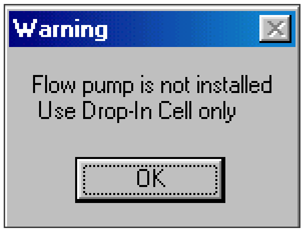
The basic operation of the NICOMP is defined using the following set of parameters.

Autodilution/Drop-in

Drop-In Cell

Used to make a measurement on a sample that is already at a good concentration using a drop-in cell.

If an attempt to use Autodilution while the Flow Pump Option has NOT been selected in the System Setup window, the option will remain selected, and the following warning will display:



Flow pump is not installed
Use Drop-In Cell only

Flow Cell

The process by which a concentrated sample is diluted to achieve the proper concentration required to make a measurement. Interactions between particles in concentrated samples can lead to significant error. Manual dilution can help eliminate this problem but it is often time consuming and can have a definite effect on sample reproducibility. The Autodilution system can automatically dilute the sample in the flow cell to optimum concentration, thus eliminating time consuming trial by error manual dilution.

> **Important**: If a change is made from Autodilution mode to Drop-in cell and the flow cell remains selected, the pump will initiate and spill water into the system. Likewise, if the drop-in cell remains selected and the pump is required to start running, Ctrl-F will not cause the pump to start running.

Autoset Channel Width

This parameter is used to establish whether the channel width of the autocorrelator will be set automatically during a measurement, which is initiated by clicking on the Autodilution icon or the Green G. The channel width can be set manually. The ideal channel width will produce a 1.7 - 2.7 decay in the correlation function.

Autoset Sensitivity

This parameter is used to establish whether the sensitivity of the PMT detector will be adjusted automatically during a measurement.

If this option is selected the sensitivity is set automatically to reach 300 kHz or whatever parameter was set in the Intensity Setpoint field of the Control Menu.

Auto NICOMP Parameters

This parameter enables the system to automatically set the range of parameters for the NICOMP^{TM} Distribution Analysis, or to permit these parameters to be preset for all subsequent measurements.

If the Automatic NICOMP Parameters option is selected the internal system computer will attempt to optimize the parameter choices. However, if this option is not selected, the parameters set in the Nicomp Input Menu will be used for analysis.

Please refer to the Nicomp Input Menu section of this manual for details about manually setting the parameters for sample analysis.

Auto Baseline Adj. (Adjustment)

Adjustment of the autocorrelation baseline function can be made automatically, in order to obtain the "best" theoretical fit, by setting this parameter.

If this option is selected automatic adjustment of the baseline will be made during each calculation cycle (30 sec.), for both the Gaussian Analysis and the NICOMP Distribution Analysis. The baseline will be permitted to rise in very small increments, in order to find the level at which the goodness of fit is optimized. In general, baseline adjustment is required for samples which contain a long "tail" of aggregates or other large, "off-scale" particles.

If this option is not selected the baseline adjustment is expressed as a percentage of the original, measured baseline, before adjustment. The value can be considered small, and therefore unimportant, if it consistently remains below 0.03%. Values larger than 0.10% provide a useful indication of the presence of significant amounts of large particles in the sample.

In the NICOMP Distribution Analysis the automatic baseline adjustment is called the Residual and is expressed as a pure number. A value of 200, which would be considered extremely large, indicates a doubling of the actual measured baseline, before adjustment. Residual values smaller than 2 or 3 can be considered negligible, while values in excess of 10 indicate the presence of significant amounts of off-scale particles. These larger values usually have the effect of pushing to smaller diameters any peaks in the size distribution, particularly in the case of bimodals.

After all of the choices have been made and reviewed in this window, exit to the Main Menu, by clicking on OK. Cancellation of all changes made may be accomplished by clicking on CANCEL.

Cum % Set Pt

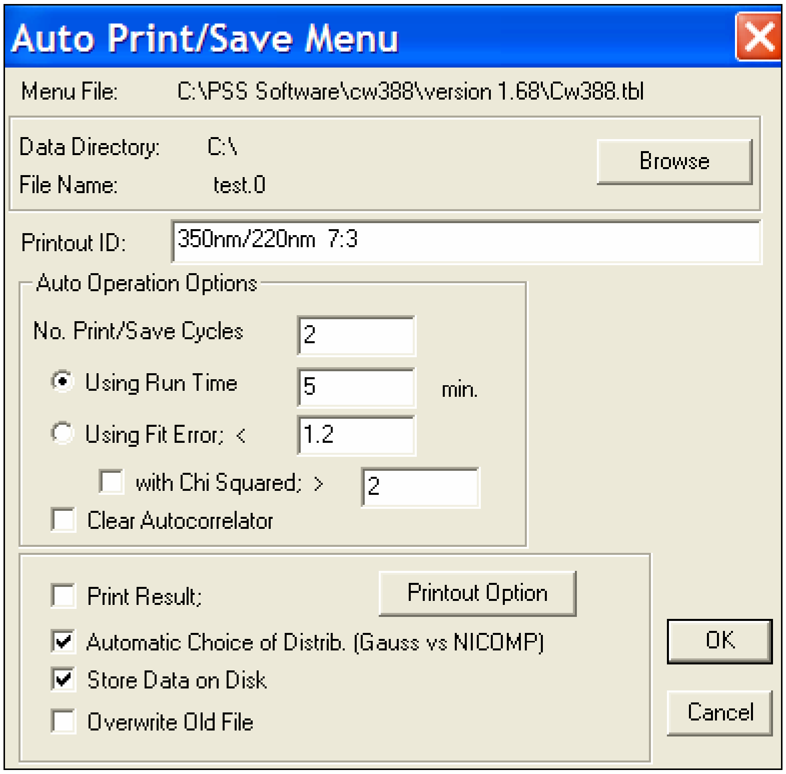
Autodilution ND Position

Sets the neutral density so it mimics a standard 5mW HeNe laser with a PMT.

Auto Print/save Menu

Access to the Edit Auto Save/Print Menu is gained using this option. This is the second of the two menus used to define the basic operation of the Nicomp.

Position the highlight bar over the Auto Print/Save Menu and click once. The Auto Print/Save Menu will display:



Auto Print/Save Menu

Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Data Directory: C:\
File Name: test.0

Printout ID: 350nm/220nm 7:3

Auto Operation Options

No. Print/Save Cycles 2
● Using Run Time 5 min.
○ Using Fit Error: < 1.2
☐ with Chi Squared: > 2
☐ Clear Autocorrelator

☐ Print Result:
☑ Automatic Choice of Distrib. (Gauss vs NICOMP)
☑ Store Data on Disk
☐ Overwrite Old File

Press TAB to advance to each of the parameters on the screen.

Menu File

Specifies the file name under which this table of parameters will be saved.

Data Directory/File Name

The data directory is used to specify the path (drive, directory) where the raw data obtained from a measurement, using either the automatic or manual save-data mode will be stored in memory. The path includes the disk drive allocation as well as any subdirectory path that is desired.

Example

To store data blocks on disk drive C, using the directory CW388 and the sub-directory, "data":

1. Double click on Browse to locate the directory in which to save the data files. This directory must be created before entering the Nicomp software.

2. Position the cursor in the File Name window and type the desired file name followed by a numerical extension. Extension numbers are automatically incremented after each analysis unless the user intervenes and renames the run.

   Note: File names are a maximum of 8 alphanumeric positions.

Printout ID

The printout caption will display on all data screens and on all printouts for a particular data file (sample run). This caption will display on all printouts until it is manually changed.

1. Edit Printout ID.

2. Position the cursor in the Printout ID window.

3. Type in or edit the existing caption in the window that displays. Use the Backspace key to delete any unwanted characters or press Ctrl and Y to delete the entire 80 character string. The caption is composed of an 80 position alphanumeric string. The caption will display exactly the way that it was typed.

4. Press ENTER when the editing is completed and the caption reads as desired. The new caption will be entered into memory.

Auto Operation Options

No. Print/Save Cycles

Use this parameter to establish the number of "Operation Cycles" which will occur after the start of the measurement process. In the example of the Auto Print/Save Menu, 2 Cycles were chosen. The cycles can be defined in two different ways.

Using Run Time

The first, and most convenient, way in which an Operation Cycle can be defined is through the Run Time.

1. Click on the circle position to the left of the Using Run Time selection. A black circle will display in the circle.

2. Enter the parameter in terms of minutes. This will define a single automatic Operation Cycle.

   A Run Time set to 5 minutes means that after approximately 5 minutes of data acquisition, the firstcycle will be completed and all of the Operating Functions which have been specified in the Auto Print/Save Menu will be implemented. If the No. Auto. Operation Cycles listed in the AutoPrint/Save Menu is larger than one, another cycle based on the Run Time will begin. This process will continue for the number of cycles specified in the AutoPrint/Save Menu. A separate file is saved for each cycle and the numerical extension is incremented by 1 each time.

Using Fit Error with Chi Squared

The other way in which an Operation Cycle can be defined is through the Fit Error Parameter alone, or a combination of the Fit Error and the Chi Squared parameters.

Fit Error

1. Click on the circle located to the left of the option and click once. The upper limit for the Fit Error will appear on the same line and to the right.

2. Enter the desired upper limit for the Fit Error. The end of the first Operation Cycle will occur when the Fit Error falls below this value, causing the pre-selected Operating Functions to be implemented.

Fit Error and Chi Squared

Distributions which are relatively complex -- e.g. bimodals and trimodals, or asymmetric unimodals having a "tail" due to large aggregates -- the NICOMP Distribution Analysis *must* be used to obtain a reasonable result. By contrast, the simple 2-parameter Gaussian Analysis will be completely inadequate to characterize such complex distributions, as generally indicated by a relatively large value for the Chi Squared goodness-of-fit parameter. In general, Chi Squared will remain close to one only when the "true" distribution is close to a simple unimodal. Consequently, when it is expected to obtain non Gaussian results, it is useful to combine the requirements of a low value for the Fit Error with a high value for Chi Squared.

1. Click on the square box located to left of the selection to include the Chi Squared parameter. The lower limit for Chi Squared will appear on the same line and to the right.

2. Enter the desired Chi Squared value.

Clear Autocorrelator

The decision to Clear the autocorrelator at the end of each Auto. Operation Cycle is made by setting this parameter. Clearing of the Autocorrelator can performed in preparation to run another sample.

Clear Autocorrelator

Click on the square box located to the left of the selection and a check will display. Each cycle then will consist of a new, independent measurement.

No Clearing of the Autocorrelator

Do not click on the box so light scattering data will continue to be acquired at the End of each cycle, thereby allowing the statistical accuracy of the autocorrelation function to improve over time. This is the preferred mode of operation.

Print/Save Options

As indicated in the previous section, after completion of an automatic Operation Cycle, one or more Print/Save options will be implemented. Each parameter may be set using this option. A brief description of each follows.

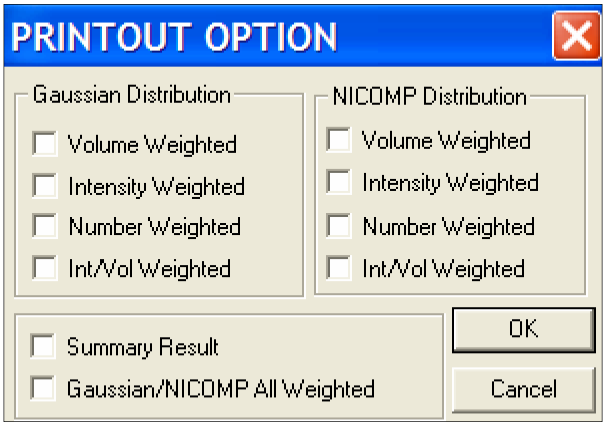
Print Result

Click on the square located to the left of the selection and a check mark will display in the box. A printout will result at the end of each Auto. Operation Cycle. If a relatively large number of Auto. Operation Cycles is required, it would be beneficial to request automatic data storage, but not printouts, for each cycle. It may be more convenient to produce the printouts later after they have been reviewed.

If this option is not selected no printout will result. However the data for the sample run will be saved if the parameter to do so has been set. The data, saved under the directory and file name specified in the Auto Print/Save Menu, can then be reviewed and printed at a later date.

Printout Option

A pre-selection of the type(s) of printout(s) desired after the completion of each Auto. Operation Cycle must be made using this option. Each analysis type and weighting which is desired can be chosen by following these steps:



1. Click on the square box located to the left of the desired print selection. A black check mark will display in the box.

2. Click on OK after all print selections has been made. Please refer to the DISPLAY section of this manual for examples of all possible types of printouts. Please refer to Appendix A for sample printouts.

Automatic Choice of Distrib.
Gaussian vs. NICOMP

Automatic selection of the Gaussian Analysis vs. the NICOMP Distribution Analysis for the printout at the end of one of the Auto. Operation Cycles can be obtained using this option.

Click on the square box next to the selection and a check mark will display in the box. The Nicomp will make this selection automatically, based on the value of Chi Squared. If Chi Squared is less than 3.0, the Volume-weighted Gaussian Analysis PSD will be printed at the end of each Auto. Operation Cycle. If Chi Squared exceeds 3.0, the Volume-weighted NICOMP Distribution Analysis PSD will be printed.

Store Data on Disk

Raw data can automatically be stored at the end of each Auto. Operation Cycle.
Click on the square located next to the selection and a black check mark will display. Data will then be stored in Data Directory and with the file name which has been specified in the Auto Print/Save Menu.

Decline saving data collected for a sample run by not making this selection.

Important: It is strongly advised to choose this Print/Save option since it is impossible to reanalyze the results of a previous measurement, or obtain a different printout, if the raw data was never saved in memory.

Overwrite Old File

Overwriting old files enables the saving of new data files over old, no longer needed, data files and conserves memory. It is recommended NOT overwrite old data files, rather copy them to disk and then manually delete them from the hard drive.

The default choice is set to N (No). If this choice is not changed then any data saving operation which attempts to create a data file having the same NAME.EXT as an existing one will cause the new NAME to be changed, by adding the character "!" (exclamation point) automatically to NAME. As a result, a new data file will be saved, having the new file name, NAME!.EXT, instead of NAME.EXT.

If a second attempt is taken to create a new file having the same file name as the original, NAME.EXT, another file will be created, this time using the added characters "!!", resulting in the new file name, NAME!!.EXT. This procedure will be invoked yet a third time, resulting in the new file name, NAME!!!.EXT. After three such substitutions, a new data file will no longer be saved. Also, no error message displays when this occurs.

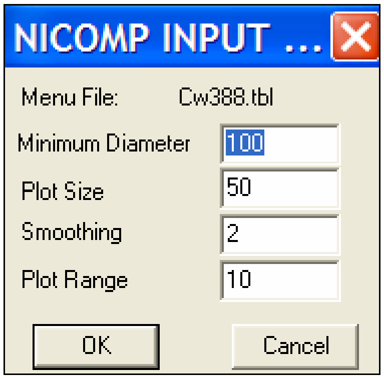
Click on the square located next to the selection and a black check mark will display. An existing file in the data directory that has the same NAME and EXT values will be overwritten.

Return to Main Menu

After all of the choices have been made and reviewed in this Menu, exit to the Main Menu, by clicking on OK.

Nicomp Input Menu

Manual setting of the NICOMP parameters may be set by specifying the following information.



Menu File: Cw388.tbl

Minimum Diameter 100

Plot Size 50

Smoothing 2

Plot Range 10

Menu File

Contains the filename of the table that contains the information from the Auto/Print Save menu and the Control menu.

Minimum Diameter

This is the smallest size diameter that is displayed. This may be any integer from 1 to 1000 nm.

Plot Size

The number of bins, or slices, into which the diameter axis is divided is provided by this parameter. A default value of 45 is initially set. The highest resolution of the diameter scale is 60. However, a more appropriate choice for most particle size distributions, especially those which are more complex than a single, narrow peak, would be 45.

Higher values of Plot Size can produce anomalous results such as "false" bimodals where a single, broad peak is more realistic, and multiple "split" peaks. These occurrences are a consequence of the complex mathematical procedure used to generate the Distribution Analysis and are important to the "overall resolution" of the size distribution (i.e. too large a number of diameter slices, Plot Size, within a given range of diameters.)

Smoothing

Establishes a bias for the Distribution analysis. At one extreme, Smoothing = 1 or 2, distribution results are favored which are either narrow single peaks or bimodals. At the other extreme, Smoothing = 5 or 6, computed fits are favored which produce broad, unimodal distributions. The default value at the start is 3, which represents a reasonable compromise until the STD.DEV and CHI SQUARED have revealed more about the probable nature of the particle size distribution. This may then suggest a better choice for the Smoothing parameter. For

example, in the case of a single narrow distribution of polystyrene latex particles, a smoothing value of 1, 2 or 3 may be used. Also, for a mixture of two different latex particles, optimal separation of the size peaks (especially for a very close spacing -- a factor of two or closer) is best achieved using Smoothing 1, 2, or 3. For a broad distribution, such as an oil-in-water emulsion made by homogenization, or milled powder, a Smoothing value of 4 to 6 should be used. Otherwise, lower values of Smoothing will tend to produce "peak splitting", leading to anomalous results like "false" bimodals.

If no prior information concerning a sample is known, i.e. whether it consists of a broad distribution of particle sizes or is, a bimodal, the results of the Gaussian Analysis can be used as a guide in selecting the most appropriate value for the Smoothing. The fit autocorrelation function, by definition, is a unimodal distribution (i.e. approximate log normal). The values of the STD.DEV. (%) and CHI SQUARED serve as guides to help decipher whether to use a low Smoothing value, or a high Smoothing value, suitable for a broad, unimodal distribution. The following table provides some suggested criteria for choosing a value for Smoothing:

STD. DEV. (Standard Deviation)	**CHI. SQR (Chi Squared).**	**Choice of Smoothing**
< 15%	< 2	Narrow, single peak **Use low Smoothing (1-3)**
> 20%	< 2	Broad, unimodal distribution **Use high Smoothing(5,6)**
> 20%	> 3	Probably a bimodal distribution **Use low Smoothing (1-3)**

Table 3: Smoothing Table

It is important to realize that a relatively high degree of smoothing in the Distribution analysis calculation, is especially useful when investigating a broad, single peak distribution, may be achieved by properly manipulating the parameters Plot Size and Range. If a low value for the Plot Size is 30 and a relatively large value for Range (i.e. for a given value of IN.DIAM.). These choices will usually improve the performance of the instrument when analyzing broad particle size distributions, such as are frequently encountered with dry milled powders (dyes, pigments, abrasives, etc.) and emulsions produced by homogenization or other procedures.

Plot Range

Determines the maximum diameter of the size scale as a multiple of the MIN diameter. The plot range can be any number from 10 to 1000. The default value is set at 50 which sets the maximum diameter to be 50 times the value of the minimum diameter.

Click on the OK button to save the parameters to memory.

There are two simple criteria which govern the selection of optimal values of MIN.DIAM., Range and Plot Size. First, it is always necessary to choose values of MIN.DIAM. and Range so that no substantial amount of the particle size population, in Intensity-weighting, appears at either edge of the plot. If the MIN.DIAM. is chosen to be too large, the small-diameter end of the distribution will be pressed against the left-hand edge of the diameter axis. In this case, the Fit Error will be seen to increase dramatically, the more MIN.DIAM. exceeds the smallest size in the distribution. Alternatively, if the Range is chosen to be too small, the large-diameter end of the distribution will be pushed into the right-hand edge of the diameter axis. Here, again, the Fit Error may increase substantially. In general, it is desirable to choose some value of MIN.DIAM. such that there remains at least (i.e., 5 or more) empty bins at the left-hand edge of the diameter axis. In this way, the resolution of the computed distribution would have to be maximized for a given value of Plot Size.

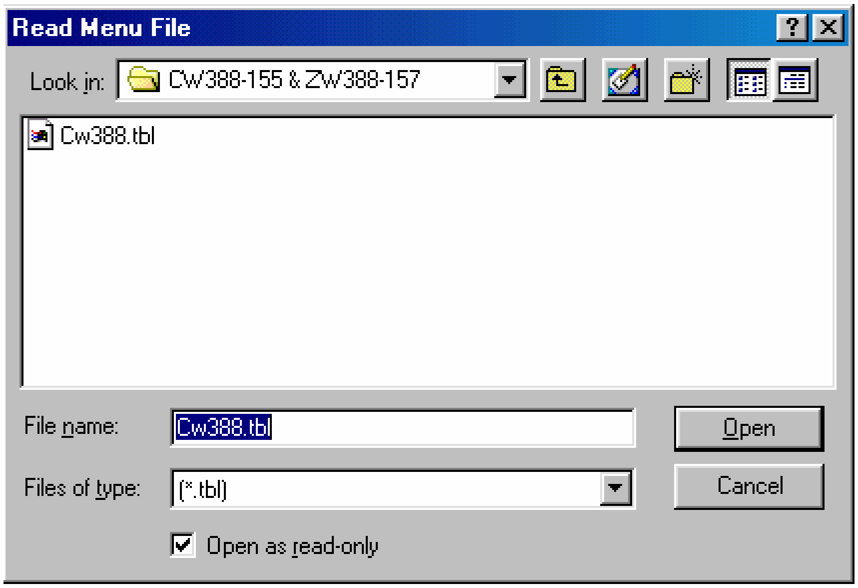
The correctness of choices of MIN.DIAM. and Range can be ascertained by examining the Intensity-weighted plot in the Distribution Analysis. What is of consequence is whether there is an appreciable intensity contribution (i.e. more than 1 or 2 units, compared to 100 units at the peak of the distribution) at either edge of the size scale. If this is the case, either the MIN.DIAM. must be decreased or the Range increased (or some combination of the two). Otherwise, two things will happen; the Fit Error will rise and, most important, the remaining portions of the size distribution histogram will become increasingly distorted and unreliable. It is vital to understand that the choices of MIN.DIAM., Range, Plot Size and Smoothing interact in a complicated way in the calculation. If the "rules" governing intensity contributions at the edges of the size scale are ignored, the choices for these parameter values may significantly, and adversely, affect the computed particle size distribution results.

The second criterion relates to the optimal selection of the Plot Size. One would like, of course, to maintain the highest possible resolution of the size histogram plot at all times by setting the Plot Size equal to 60. However, it turns out in practice that in the case of broad, highly polydisperse distributions, (whether unimodal or bimodal), this choice may result in "over-resolution" leading to an unstable fit. This condition could be recognized by the fact that the distribution "breaks up", leaving a number of "holes", where there is no apparent volume, surrounded on both sides by large relative amounts of particle volume. For example, broad, unimodal populations can be transformed into false bimodals, given too large a Plot Size and/or too low a smoothing value, for a given MIN.DIAM. and Range. In general, this over-resolution of the distribution can be rectified by decreasing the Plot Size - which does not affect the MIN.DIAM or Range.

Read Menu File

Access to complete sets of parameters that were previously saved for the Control Menu and the Auto Print/Save Menu options can be gained using this option.

1. Position the highlight bar over the Read Menu File option and click once, the following window will display:



Look in: CW388-155 & ZW388-157

Cw388.tbl

File name: Cw388.tbl

Files of type: (*.tbl)

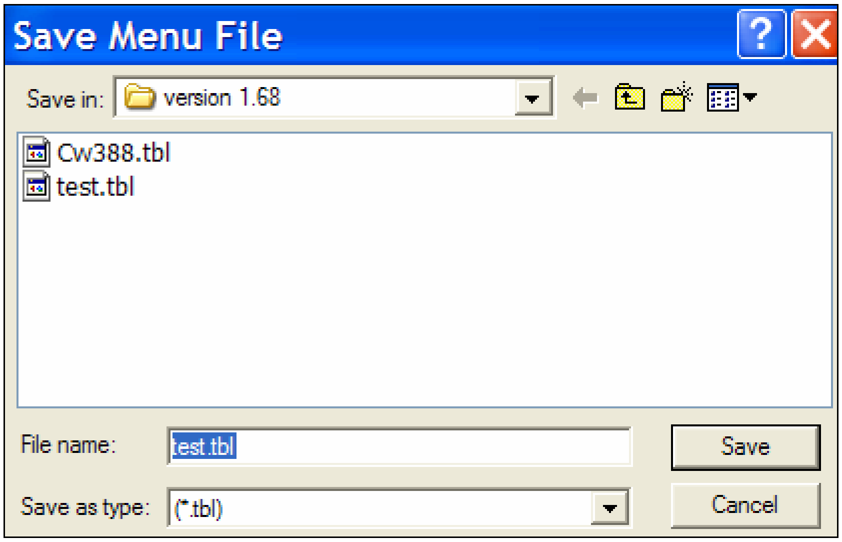
☑ Open as read-only

2. Position the highlight bar over the table file of interest and click. The selected table file with preset parameters will be retrieved and loaded from memory.

Save Menu File

This option is used to save or update an existing table of "default" values in a TBL file. The name for the table file displays in the File Name window.

1. Position the highlight bar over the table file name and click the mouse once. The following window will display:

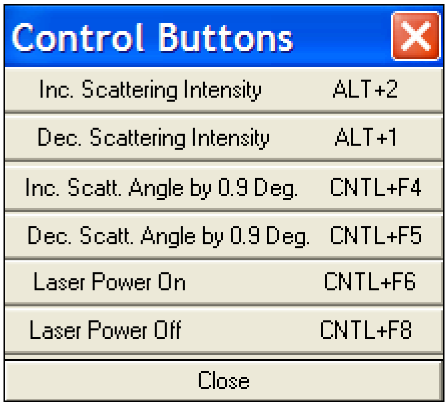


File name: test.tbl

Save as type: (*.tbl)

2. Either type the new file name that the table file parameters should be saved under or position the highlight bar over the existing table file name to be updated and click once. The parameters set in the Control Menu and Auto Print/Save menu will be saved for future use.

Control Buttons



Inc. Scattering Intensity

Increases the scattering intensity of the detector.

Dec. Scattering Intensity

Decreases the scattering intensity of the detector.

Inc. Scatt. Angle by 0.9 Deg.

Increases the scattering angle of the detector by increments of 0.9 degrees.

Dec. Scatt. Angle by 0.9 Deg.

Increases the scattering angle of the detector by increments of 0.9 degrees.

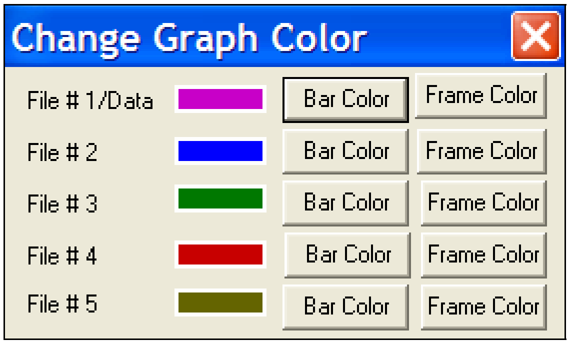
Laser Power On

Applies power to the laser.

Laser Power OFF

Powers down the laser.

Change Graph Color



A window will display all of the colors available that can be used for the bar color of the distributions of the sample runs. The new color once selected displays to the right of the file #. Selecting colors for 1 – 5 distributions is especially useful when overlaying more than one sample distribution.

a. Click on the Bar Color box to access the Color window:

b. Select the desired color for the bars of the distribution by clicking on the actual color box.

c. Click OK.

d. Click OK again once the Change Graph Color window re-displays.

e. Select a distribution to view the new color selected for the bar color.

f. Click on the Frame Color box to access the Color window:

g. Select the desired color for the frame of the bars on the distributions by clicking on the actual color box.

h. Click OK.

i. Click OK again once the Change Graph Color window re-displays.

j. Select a distribution to view the new color selected for the bar frame.

Initialize ND Filter

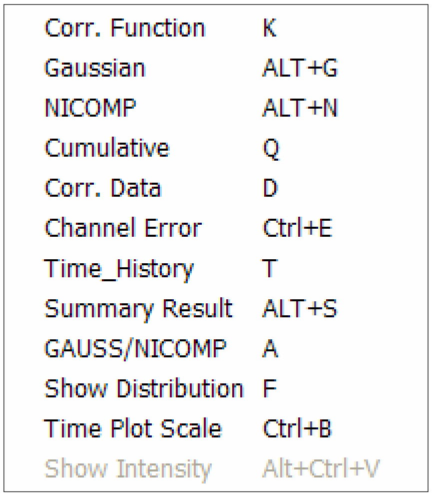
This option initializes the neutral density filter. First, it locates the zero point of the neutral density filter and then it resets the neutral density filter to the position defined in the Autodilution ND position defined in the Control menu.

DISPLAY

The following provides a brief summary of the Display options that are used during the use of the Windows CW388 Software.

Access of the Display options:

1. Position the highlight over the DISPLAY option and click once using the mouse. The following window of File options displays:

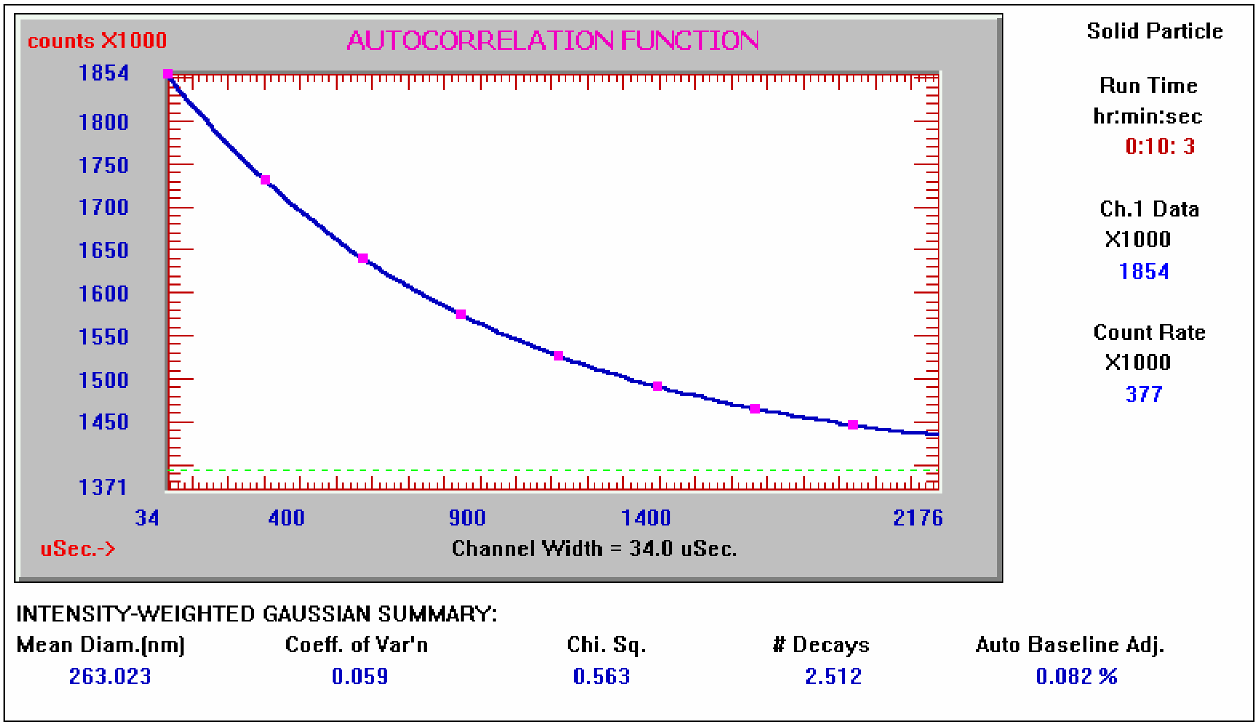


Corr. Function	K
Gaussian	ALT+G
NICOMP	ALT+N
Cumulative	Q
Corr. Data	D
Channel Error	Ctrl+E
Time_History	T
Summary Result	ALT+S
GAUSS/NICOMP	A
Show Distribution	F
Time Plot Scale	Ctrl+B
Show Intensity	Alt+Ctrl+V

2. Position the highlight bar over the desired selection and click once. Following is a description of each of the options offered.

Corr. Function

This option is used to observe the autocorrelation function produced by the scattered intensity data. The value of Decays should lie sin the appropriate range 1.7 to 2.7. This is the number of exponential decays spanned by the 64 channels of the autocorrelation function.



Solid Particle

Run Time hr:min:sec 0:10: 3

Ch.1 Data X1000 1854

Count Rate X1000 377

INTENSITY-WEIGHTED GAUSSIAN SUMMARY:

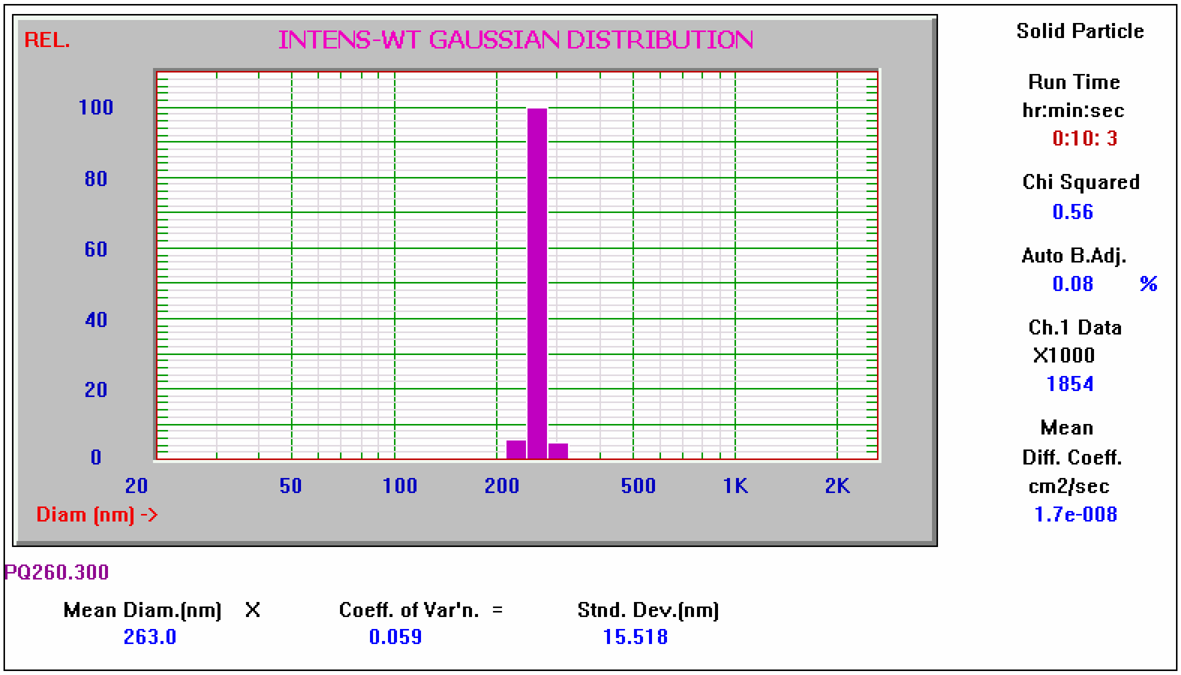
Mean Diam.(nm)	Coeff. of Var'n	Chi. Sq.	# Decays	Auto Baseline Adj.
263.023	0.059	0.563	2.512	0.082 %

Viewing the correlation function is one of the ways to ensure a measurement is progressing in the proper manner.

Gaussian

Provides the ability to view the analysis in Gaussian format.

Intensity-Weighted Gaussian



Solid Particle

Run Time
hr:min:sec
0:10: 3

Chi Squared
0.56

Auto B.Adj.
0.08 %

Ch.1 Data
X1000
1854

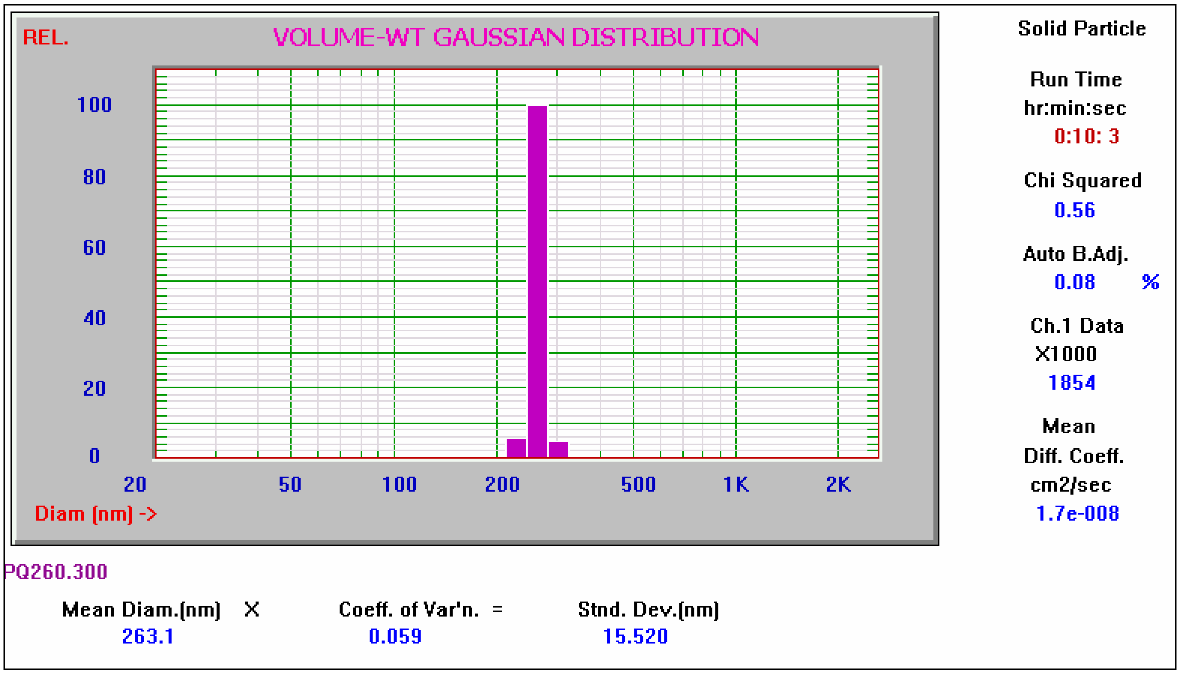
Mean
Diff. Coeff.
cm2/sec
1.7e-008

PQ260.300

Mean Diam.(nm) X	Coeff. of Var'n. =	Stnd. Dev.(nm)
263.0	0.059	15.518

When displaying the data scroll through the different weightings of the data set by clicking on the Weighting pull down menu or by pressing “W” on the key board.

Volume-Weighted Gaussian



Solid Particle

Run Time
hr:min:sec
0:10: 3

Chi Squared
0.56

Auto B.Adj.
0.08 %

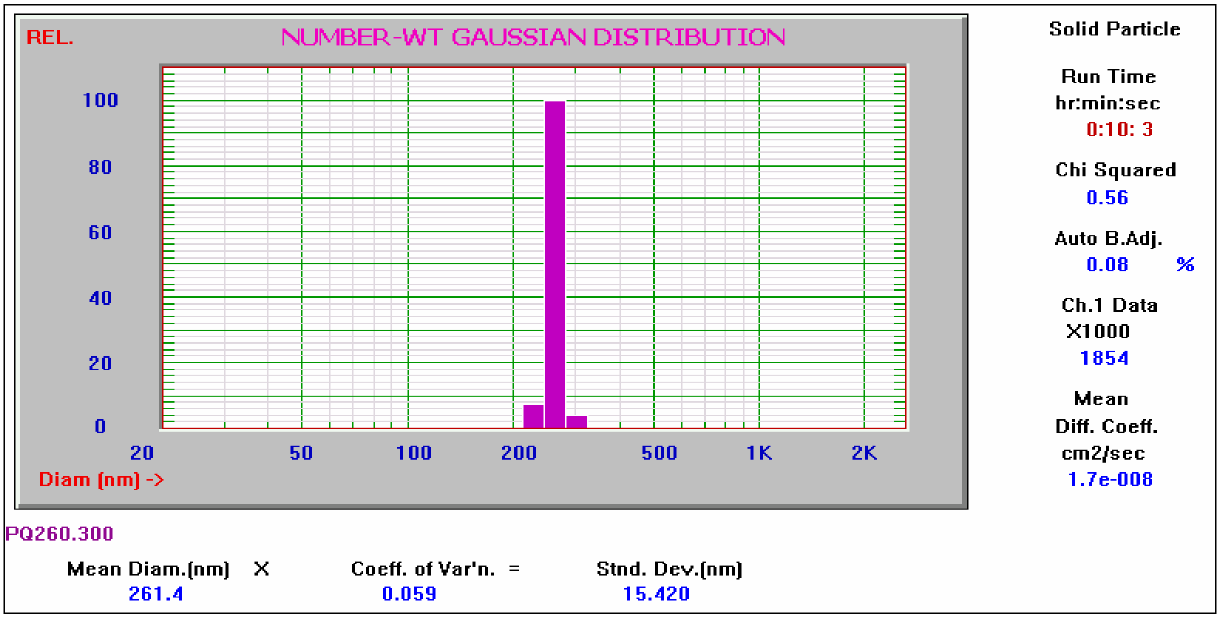
Ch.1 Data
X1000
1854

Mean
Diff. Coeff.
cm2/sec
1.7e-008

PQ260.300

Mean Diam.(nm) X	Coeff. of Var'n. =	Stnd. Dev.(nm)
263.1	0.059	15.520

Number-Weighted Gaussian



Solid Particle

Run Time
hr:min:sec
0:10: 3

Chi Squared
0.56

Auto B.Adj.
0.08 %

Ch.1 Data
X1000
1854

Mean
Diff. Coeff.
cm2/sec
1.7e-008

PQ260.300

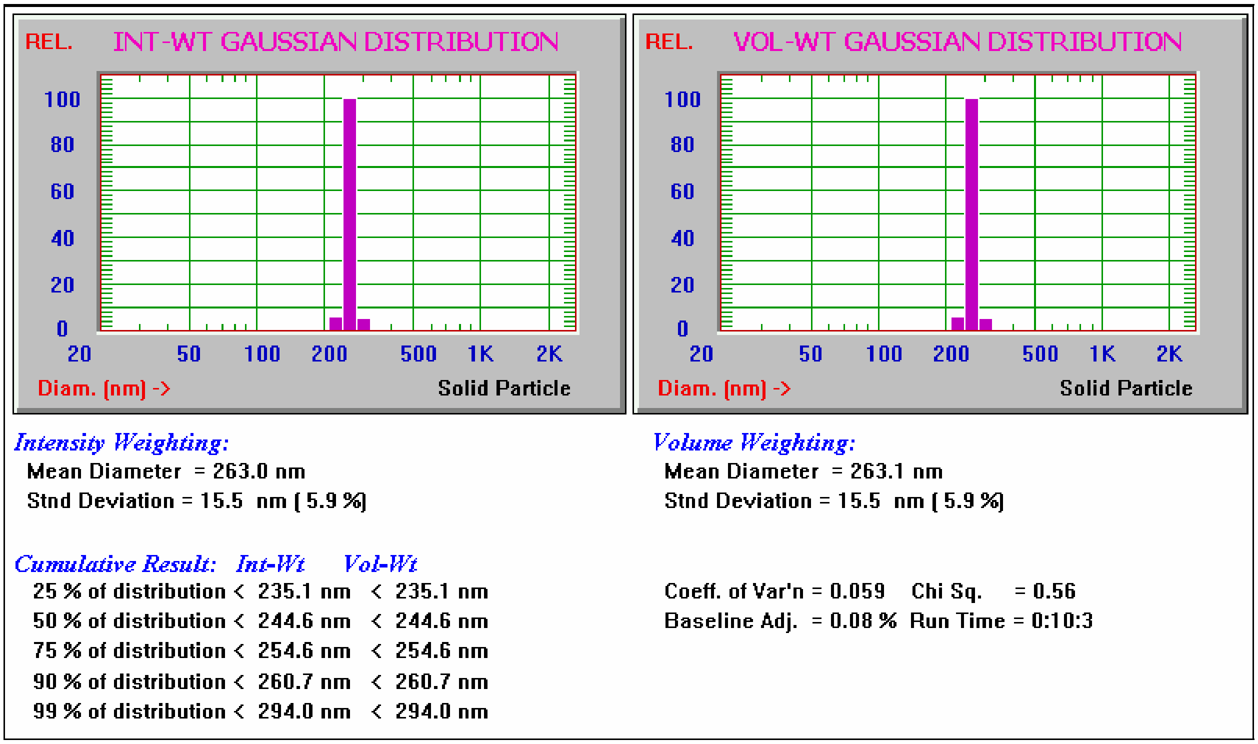
Mean Diam.(nm) X
261.4

Coeff. of Var'n. =
0.059

Stnd. Dev.(nm)
15.420

It will often be the case that the three weightings do not match in magnitude of mean diameter. This is the representative of applying the different weightings to the original data set.

Intensity and Volume-Weighted Gaussian

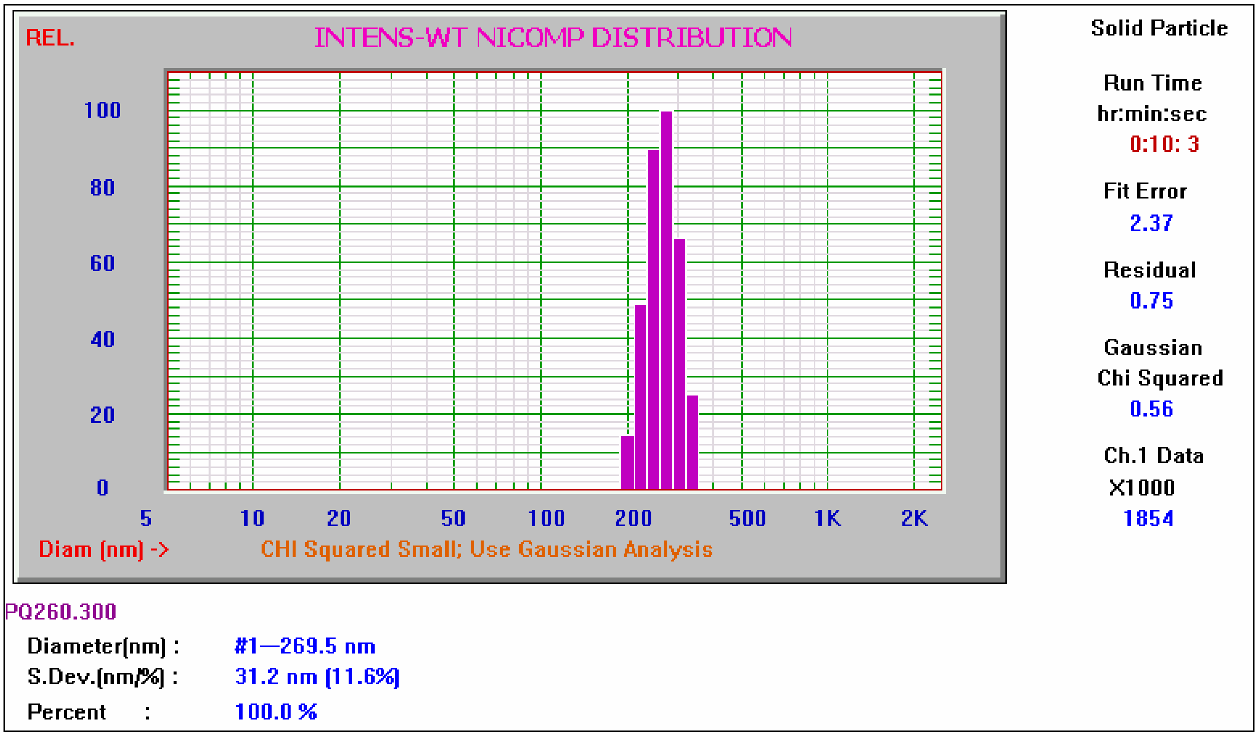


```
Intensity Weighting:                              Volume Weighting:
  Mean Diameter  = 263.0 nm                         Mean Diameter  = 263.1 nm
  Stnd Deviation = 15.5  nm ( 5.9 %)                Stnd Deviation = 15.5  nm ( 5.9 %)

Cumulative Result:  Int-Wt      Vol-Wt
  25 % of distribution <  235.1 nm   <  235.1 nm    Coeff. of Var'n = 0.059   Chi Sq.    = 0.56
  50 % of distribution <  244.6 nm   <  244.6 nm    Baseline Adj.  = 0.08 %  Run Time = 0:10:3
  75 % of distribution <  254.6 nm   <  254.6 nm
  90 % of distribution <  260.7 nm   <  260.7 nm
  99 % of distribution <  294.0 nm   <  294.0 nm
```

It is often most useful to observe both the intensity and volume weightings on the screen simultaneously.

Nicomp

Used to review the Nicomp distributions of an analyzed sample that had a chi squared value of 3 or higher. The warning on the screen will indicate which of the two data fits is most appropriate based on assessment of the Chi Squared value.



Solid Particle

Run Time
hr:min:sec
0:10: 3

Fit Error
2.37

Residual
0.75

Gaussian
Chi Squared
0.56

Ch.1 Data
X1000
1854

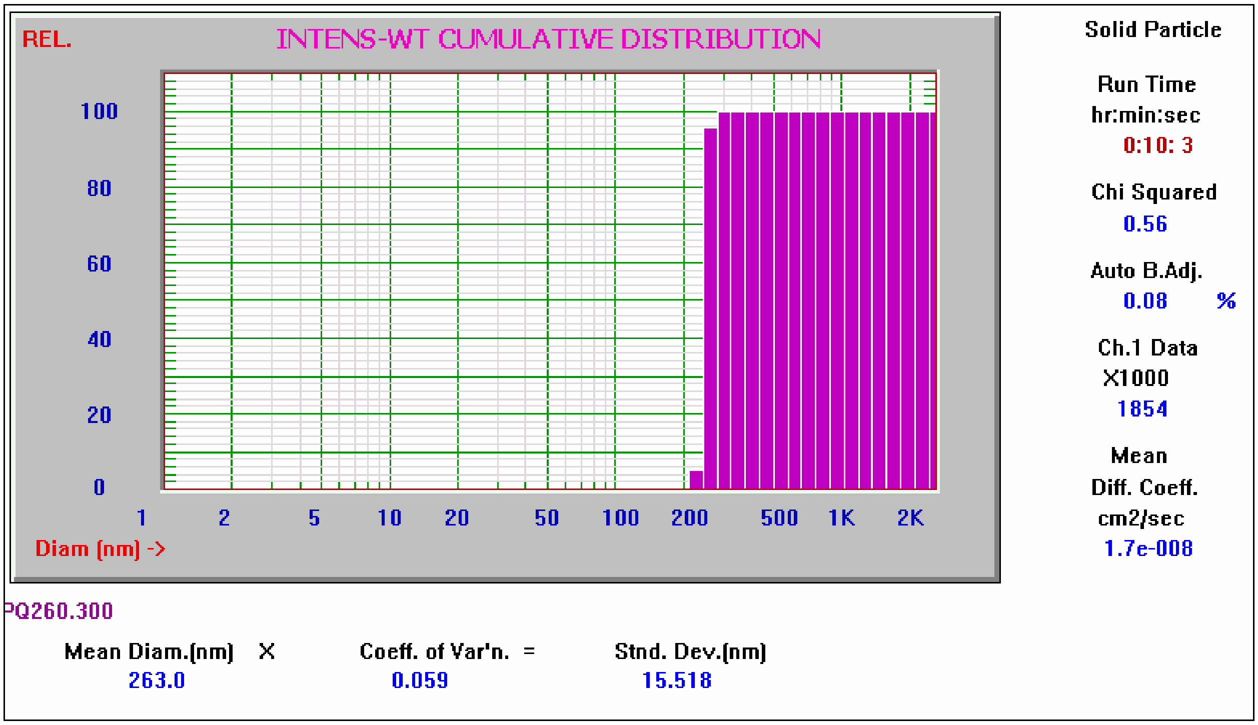
PQ260.300

Diameter(nm) : #1—269.5 nm
S.Dev.(nm/%) : 31.2 nm (11.6%)
Percent : 100.0 %

Cumulative

The coefficients of the Gaussian analysis will display as a cumulative sum starting at the lowest\ diameter and will increase towards the larger diameter.

Intensity-Weighted Cumulative Result (Gaussian)



Solid Particle

Run Time
hr:min:sec
0:10: 3

Chi Squared
0.56

Auto B.Adj.
0.08 %

Ch.1 Data
X1000
1854

Mean
Diff. Coeff.
cm2/sec
1.7e-008

PQ260.300

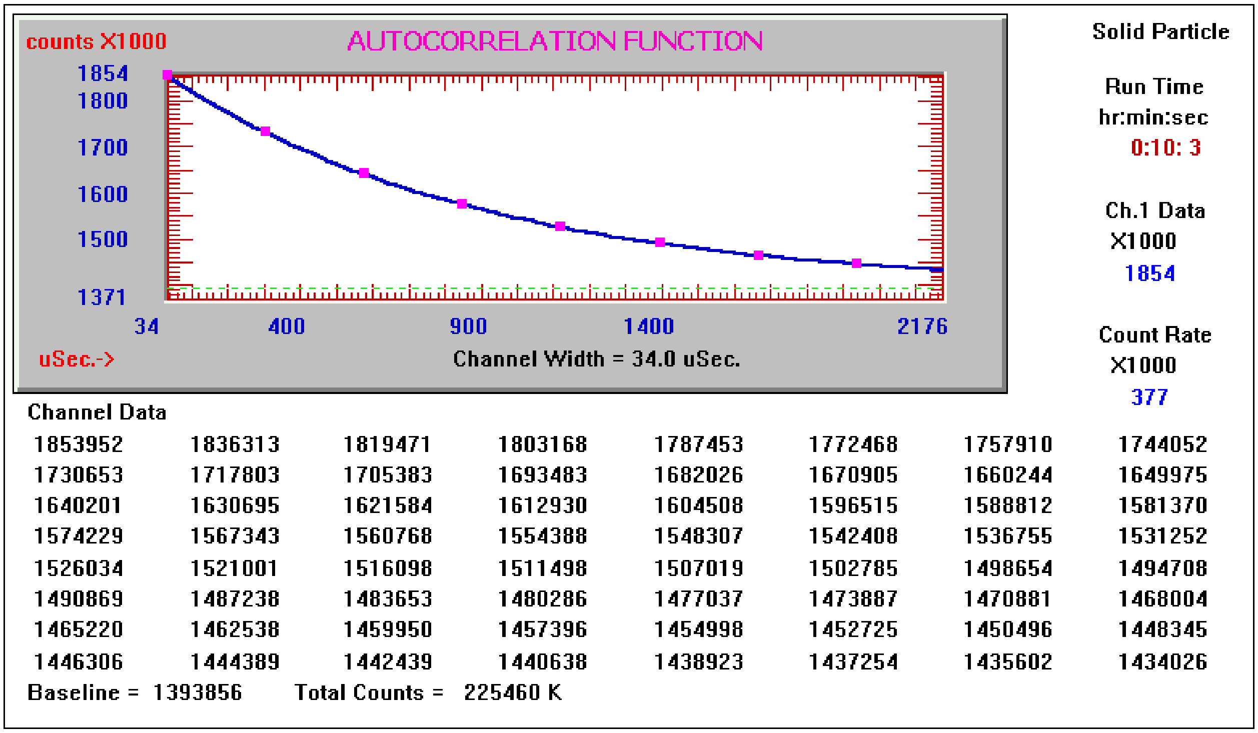
Mean Diam.(nm) X
263.0

Coeff. of Var'n. =
0.059

Stnd. Dev.(nm)
15.518

Corr. Data

Used to view the block of raw data collected from a sample run when in display mode. The data will display on the screen in the following format:



Solid Particle

Run Time
hr:min:sec
0:10: 3

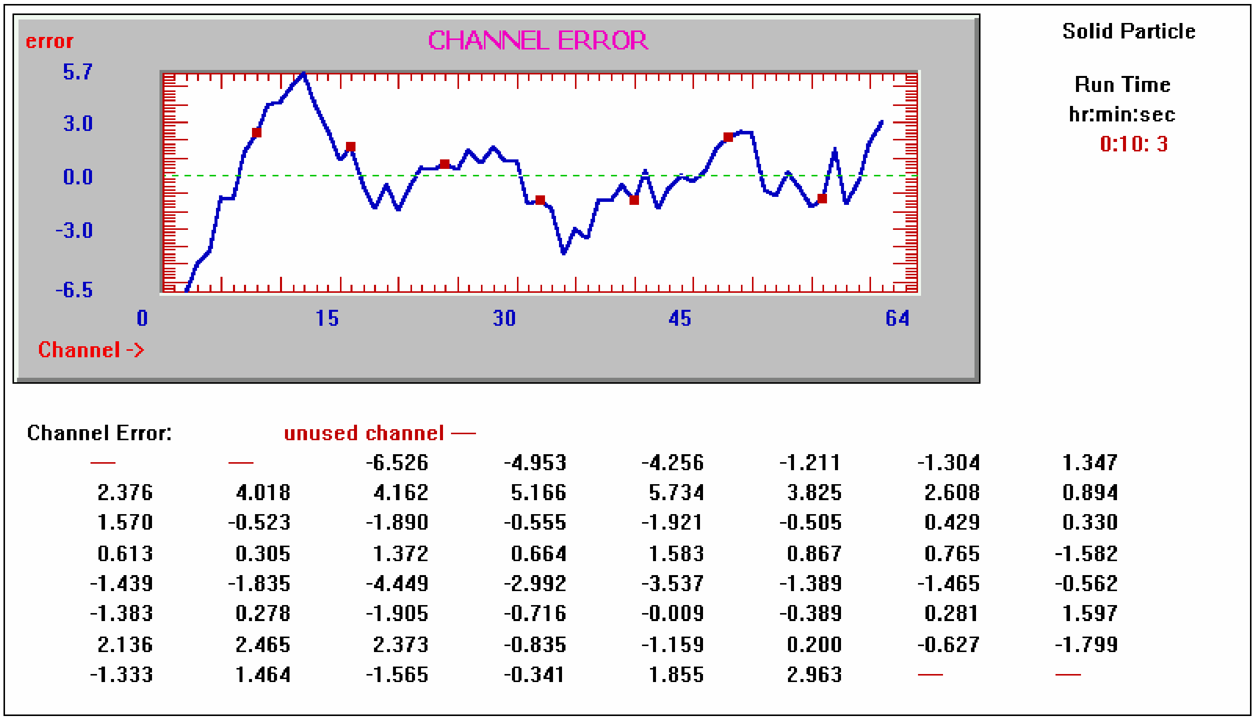
Ch.1 Data
X1000
1854

Count Rate
X1000
377

```
Channel Data
 1853952    1836313    1819471    1803168    1787453    1772468    1757910    1744052
 1730653    1717803    1705383    1693483    1682026    1670905    1660244    1649975
 1640201    1630695    1621584    1612930    1604508    1596515    1588812    1581370
 1574229    1567343    1560768    1554388    1548307    1542408    1536755    1531252
 1526034    1521001    1516098    1511498    1507019    1502785    1498654    1494708
 1490869    1487238    1483653    1480286    1477037    1473887    1470881    1468004
 1465220    1462538    1459950    1457396    1454998    1452725    1450496    1448345
 1446306    1444389    1442439    1440638    1438923    1437254    1435602    1434026
Baseline =  1393856      Total Counts =   225460 K
```

Channel Error

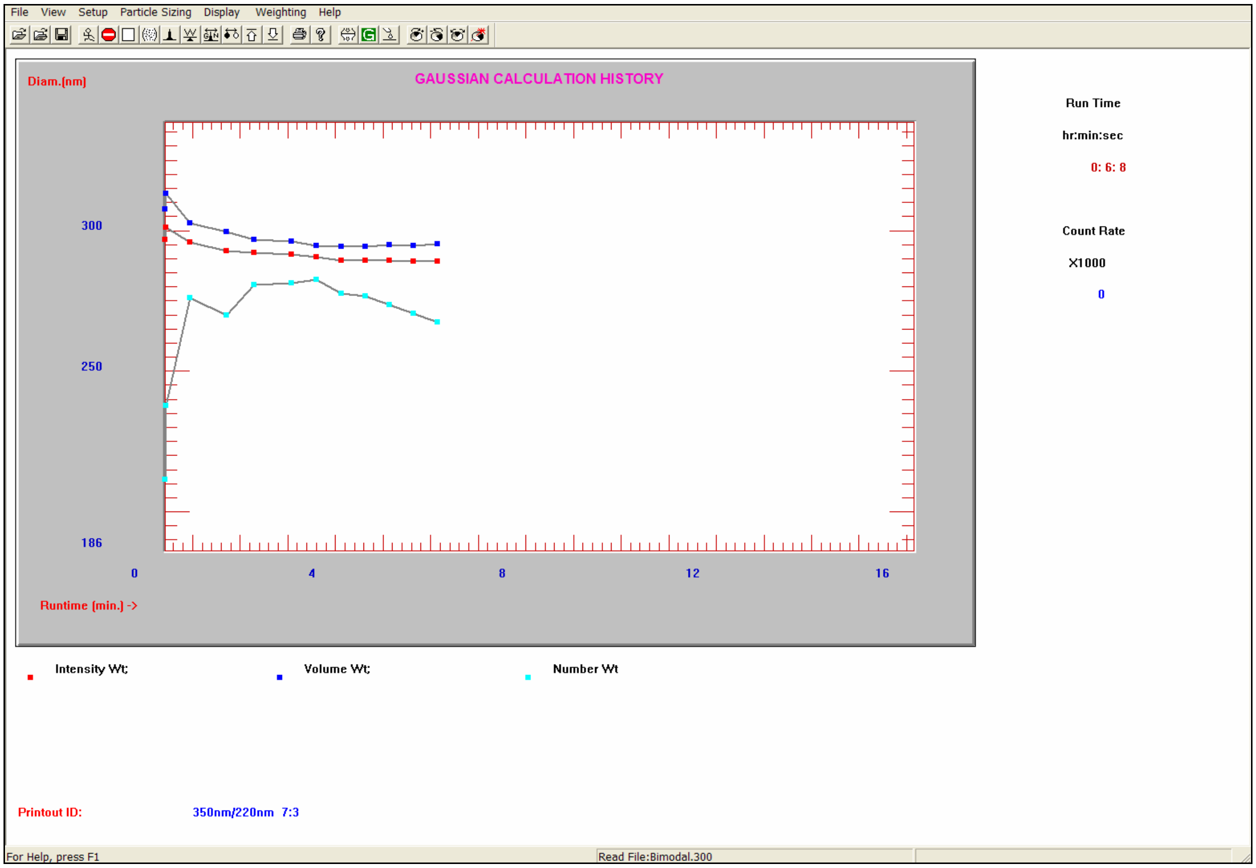
Used to compare the statistical discrepancy between the actual data and the ability to curve fit the data.



```
Channel Error:         unused channel —
   —         —       -6.526    -4.953    -4.256    -1.211    -1.304     1.347
 2.376     4.018      4.162     5.166     5.734     3.825     2.608     0.894
 1.570    -0.523     -1.890    -0.555    -1.921    -0.505     0.429     0.330
 0.613     0.305      1.372     0.664     1.583     0.867     0.765    -1.582
-1.439    -1.835     -4.449    -2.992    -3.537    -1.389    -1.465    -0.562
-1.383     0.278     -1.905    -0.716    -0.009    -0.389     0.281     1.597
 2.136     2.465      2.373    -0.835    -1.159     0.200    -0.627    -1.799
-1.333     1.464     -1.565    -0.341     1.855     2.963      —         —
```

Time History

Displays the time history plot where the stability of the data, in terms of mean diameter, is shown to be changing as a function of time.



Run Time
hr:min:sec
0: 6: 8

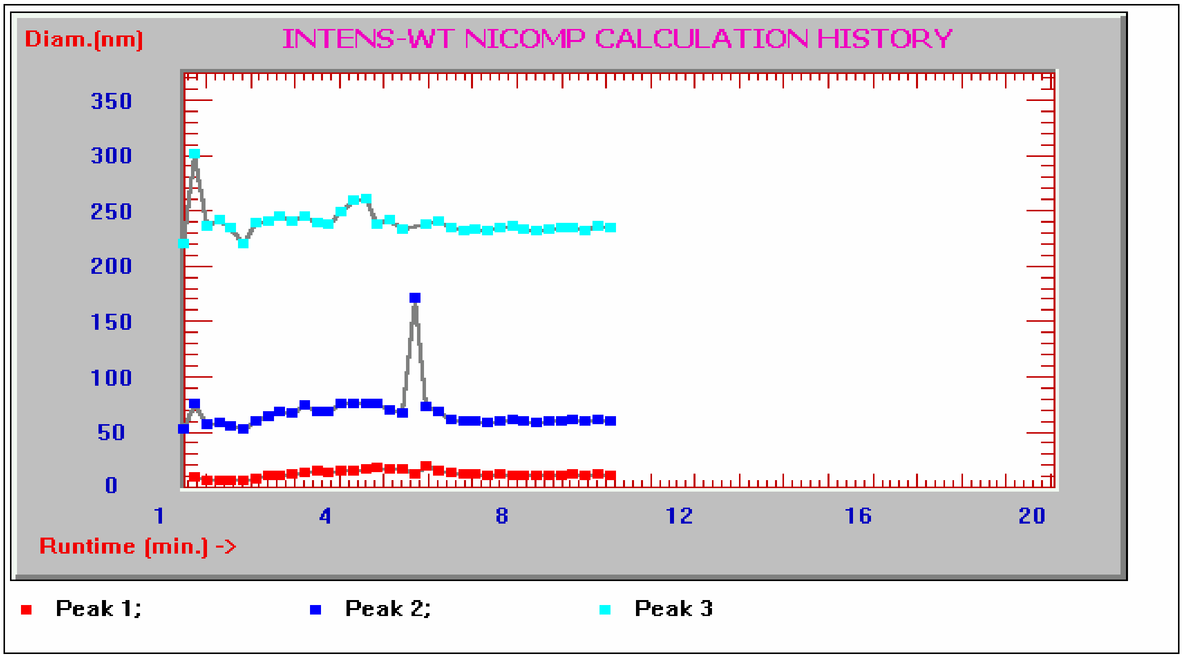
Count Rate
X1000
0

Printout ID: 350nm/220nm 7:3

For Help, press F1

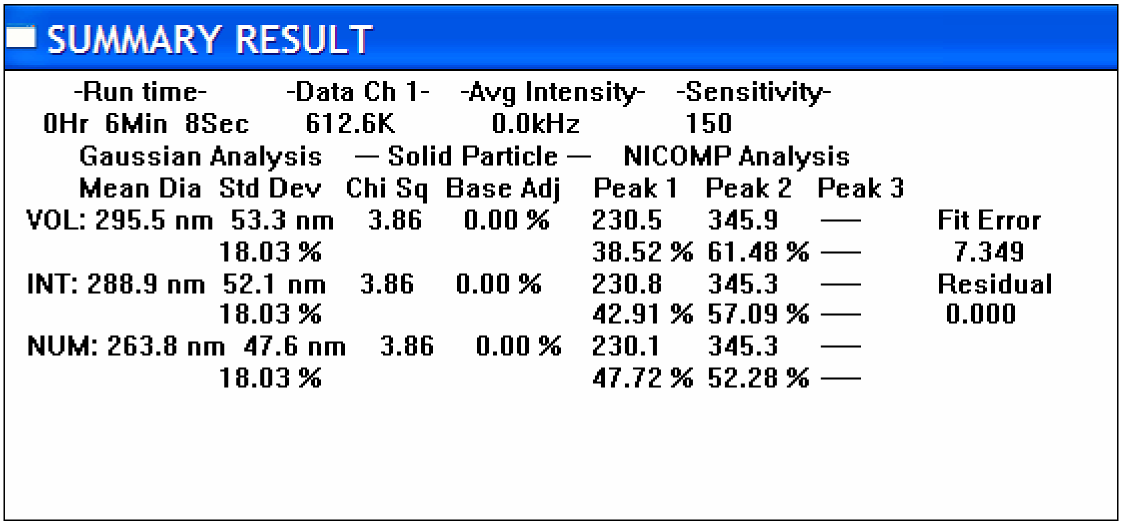
Read File:Bimodal.300

The Gaussian time history shows the intensity, volume and number weighted means on a single screen while the Nicomp time history shows different peaks. In order to view the Nicomp time history plot, select Nicomp from the Display pull doen menu.



Summary Result

Displays the Volume, Intensity and Number-weighted data for the Gaussian and Nicomp calculations simultaneously.

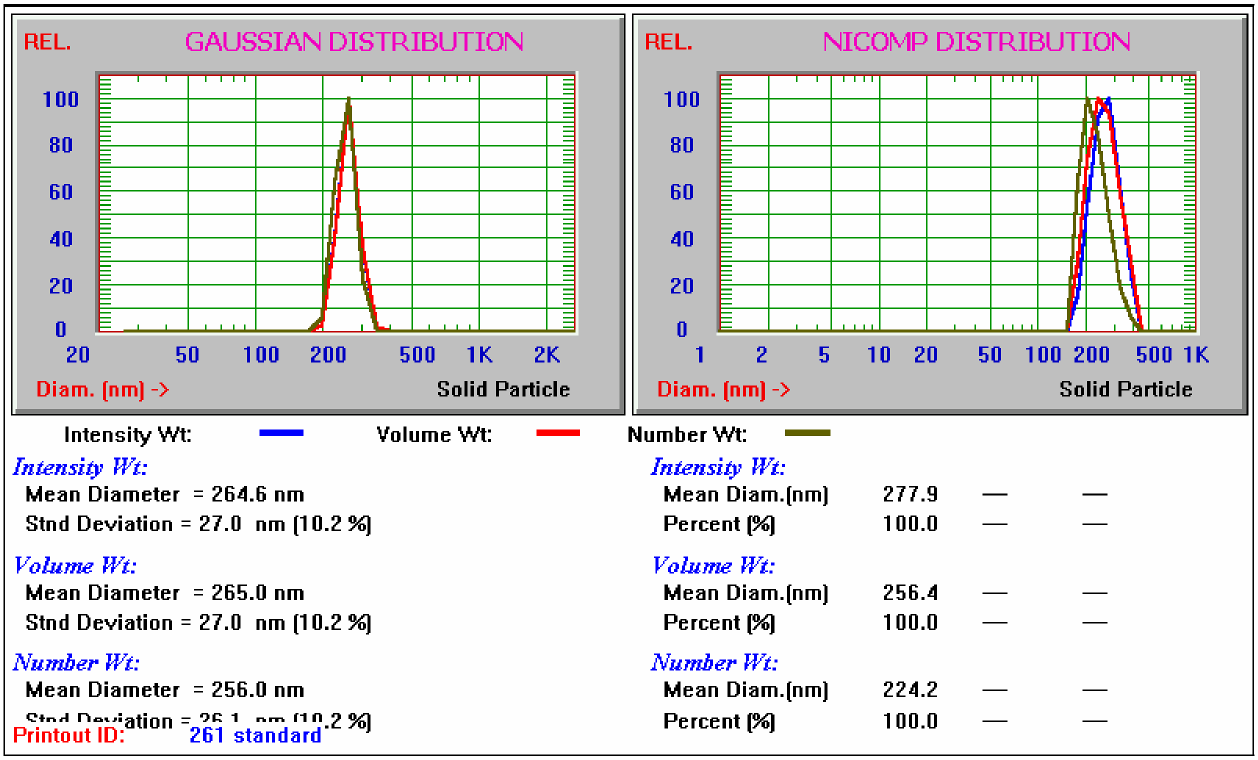


SUMMARY RESULT

```
    -Run time-        -Data Ch 1-   -Avg Intensity-   -Sensitivity-
 0Hr  6Min  8Sec       612.6K          0.0kHz            150
     Gaussian Analysis     — Solid Particle —    NICOMP Analysis
     Mean Dia  Std Dev   Chi Sq  Base Adj    Peak 1   Peak 2   Peak 3
 VOL: 295.5 nm  53.3 nm    3.86    0.00 %    230.5    345.9     —        Fit Error
                18.03 %                      38.52 %  61.48 %   —          7.349
 INT: 288.9 nm  52.1 nm   3.86    0.00 %     230.8    345.3     —        Residual
                18.03 %                      42.91 %  57.09 %   —          0.000
 NUM: 263.8 nm  47.6 nm    3.86    0.00 %    230.1    345.3     —
                18.03 %                      47.72 %  52.28 %   —
```

Gauss/Nicomp

Displays the Gaussian and Nicomp overlays of the Intensity, Volume and Number-Weighted data collected for the sample.



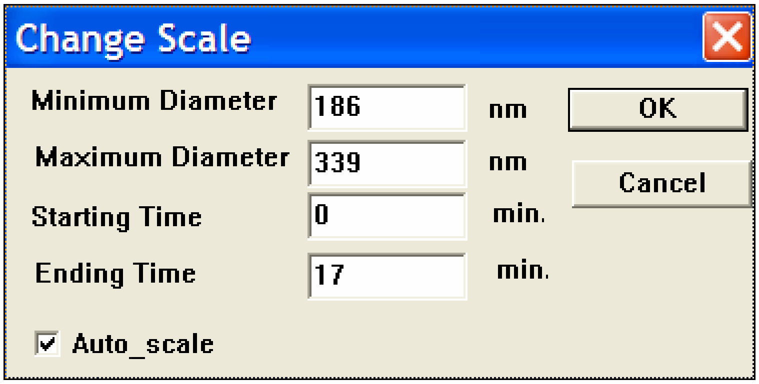
```
Intensity Wt:                                  Intensity Wt:
  Mean Diameter  = 264.6 nm                      Mean Diam.(nm)     277.9    —     —
  Stnd Deviation = 27.0  nm (10.2 %)             Percent (%)        100.0    —     —

Volume Wt:                                     Volume Wt:
  Mean Diameter  = 265.0 nm                      Mean Diam.(nm)     256.4    —     —
  Stnd Deviation = 27.0  nm (10.2 %)             Percent (%)        100.0    —     —

Number Wt:                                     Number Wt:
  Mean Diameter  = 256.0 nm                      Mean Diam.(nm)     224.2    —     —
  Stnd Deviation = 26.1  nm (10.2 %)             Percent (%)        100.0    —     —
Printout ID:  261 standard
```

Show Distributions

Returns the user to the standard distribution display.

Time Plot Scale

Is used to manipulate the Time Plot to zoom in/out on collected data. The Time History Plot must be displayed in order to use this option.



Change Scale

Minimum Diameter 186 nm

Maximum Diameter 339 nm

Starting Time 0 min.

Ending Time 17 min.

☑ Auto_scale

Minimum Diameter

Maximum Diameter

Enter the minimum and maximum diameter to isolate the range for which the time history plot is to display.

Starting Time

Ending Time

Enter the start and end time for the range that is being isolated.

The time history plot’s scale is then updated to reflect the information entered.

Auto_scale

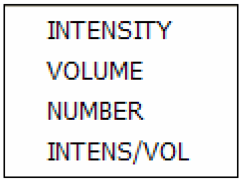
Automatically sets the min/max of the time plot scale based on the value of the graph.

Show Intensity

Shows the intensity-weighted values of the time plot.

WEIGHTING

Please refer to the Display section of this manual for examples of these distributions.



INTENSITY
VOLUME
NUMBER
INTENS/VOL

Intensity

Displays the relative intensity of scattered light vs/ diameter for a sample run.

Volume

Displays the relative particle volume vs. diameter.

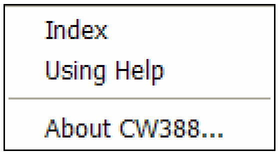
Number

Displays the relative number of particles in a sample run vs. diameter.

Intens/Vol

Displays the overlays of the intensity and volume-weighted sample runs.

HELP MENU



Index

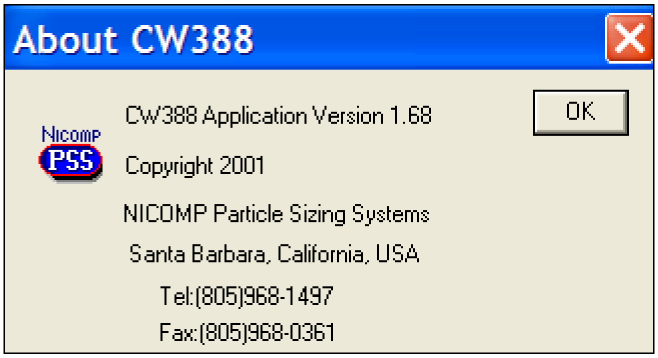
The index is a listing of the older keystroke commands that have been replaced by icons, buttons and menu choices.

Using Help

At this time the default Window help is available.

About CW388

This window will provide the user with the version number of the software in use.



CW388 Application Version 1.68

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NICOMP Particle Sizing Systems

Santa Barbara, California, USA

Tel:(805)968-1497

Fax:(805)968-0361

COMMAND KEYS

The following set of commands will display any time that **ALT_H** is accessed while operating the C380 software.

[A]: Display Gaussian/NICOMP Intensity/Volume/Number Weighted Distribution

Both the Gaussian and NICOMP distributions along with the Intensity-, Volume-, and Number-weighted information will display when this key is used.

[ALT-A]: Toggle Number Weighted/Area Weighted Distribution

Both the Number- and Area-weighted distributions for a sample analysis may be reviewed using this option.

[B]: Change Distribution to Bar Graph

The format in which data is displays can be changed from line mode to bar mode (default). It is helpful to change to line mode when reviewing overlays of the distributions.

[C]: Clear correlator

Clears the contents of the autocorrelator channels. This is part of the initialization process. For all subsequent runs it is necessary to clear the data whenever the Channel Width is changed in the input menu, or the sample is changed.

This option may also be used to restart the autocorrelator after the sample temperature has settled to its stable set point or whenever a new sample has been introduced into the scattering cell. This option may only be used in display mode.

[ALT-C]: Clock

The internal clock of the computer controller can be viewed when this option is accessed. The clock window can be removed by pressing the same keys used to access the time.

[D]: Display autocorrelator data

Used to view the block of raw data collected from a sample run when in display mode.

[E]: Edit Caption

Used to enter or edit a caption for a printout. Follow these steps to enter the caption.

a. Press **E**.

b. Type in the desired caption. A maximum string of eighty alpha-numeric characters is allowed.

c. Press **ENTER**. The string of characters entered will be saved.

[Ctrl_E]: Channel Error

Used to compare the statistical discrepancy between the actual data and the ability to curve fit the data.

[F]: Display Gaussian or Nicomp Distributions

Returns to the Gaussian Analysis Display. Will also display a list of all of the data files which have previously been created in the same data directory.

[Ctrl_F]: Turn On Flow Pump (Press ESC to Stop)

Used to flush the sample out of the system

[G]: Toggle Gaussian/Nicomp Distribution

Used to toggle between the Gaussian and Nicomp interpretations of the data.

[CTRL-G]: Reset Fiber Angle to 90 deg. (for Multi-angle system)

Aligns the fiber at the 90° mark for data acquisition. This option is used in conjunction with the Multi-angle option.

[I]: **Edit NICOMP Input Menu**

This function will change the fit of the Nicomp distribution to the new parameters specified. The input parameters, *min. diam., plot size, smoothing and range*, along with their identifying labels may be changed using this option.

[K]: **Display Autocorrelation Function Curve and Intensity-weighted Gaussian Summary**

Used to observe the autocorrelation function produced by the scattered intensity data. The value of Decays lies in the approximate range 1.7 to 2.7. This is the number of exponential decays spanned by the 64 channels of the autocorrelation function.

[L]: **Change Distribution for Line Graph**

The format in which data displays can be changed from bar mode (default) to line mode.

[M]: **Edit Correlator Control Menu**

Allows for the edit of te Control Menu. Please refer to the Particle Sizing section of this manual for additional information concerning this option.

[P]: **Print output**

The printer will begin printing the Volume-weighted Gaussian analysis plot together with a summary of the relevant parameters and a listing of the input menu. The instrument will automatically print the results when preset values of elapsed time or Fit Error are reached.

[CNTL-P]: Preview Printout

A preview of the distribution printout will display. The following options are available to click on after the distribution is reviewed:

Print: A printout of the distribution being previewed can be obtained

Zoom In: Clicking on this button magnifies the preview so that it is readable.

Close: Closes the preview of the distribution and the original distribution which displayed prior to accessing this options redisplays.

[Q]: Display Cumulative Distribution

The coefficients of the Gaussian analysis will display as a cumulative sum starting at the lowest diameter and will increase towards the larger diameter.

[R]: Run Autocorrelator

Used to exit from the input menu and begin data acquisition in the autocorrelator. It is presumed that the Channel Width listed in the input menu is appropriate for the particular sample being analyzed.

[Ctrl_R]: Resume Running Autocorrelator (Taking Data from Sample)

Restores taking data once the system is halted from collected data from a sample run.

[S]: Stop Correlator

Used to stop the collection of data in the autocorrelator. Once this option is exercised the following message will display:

[ALT-S]: Summary Result

The window of the distribution divides in two and a summary of the data displays showing the Volume-, Number- and Intensity-weighted information.

[T]: Display Time Plot

Displays the time series plot (calculation history) where the stability of the data, in terms of mean diameter, is shown not to be changing for at least three minutes as a function of time (a flat straight line).

[V]: Toggle Vesicle/Solid Particle Calculation

The instrument defaults to solid particle weighting when power is first applied. The weighting may be changed using this option. The weighting will change when the results of a new analysis is displayed, at which time the title will also change, to Gaussian Analysis (vesicles). The original weighting can be re-displayed by using this option again.

[W]: Weighting select

Three weightings may be viewed for a particle size distribution using this option:

Volume-weighted

Displays the relative particle volume vs. diameter. The value of the volume-weighted particle size distribution is calculated assuming that the particle are spheres of uniform density which scatter light according to classical Mie Theory.

Number-weighted

Displays the relative number of particles in a sample run vs. diameter. The value of the number-weighted particle size distribution is also calculated assuming that the particles are spheres of uniform density which scatter light according to classical Mie Theory.

Intensity-weighted

The result first displays from either of the autocorrelation functions. Displays the relative intensity of scattered light vs. diameter for a sample run.

[X]: Start Automatic Channel Width Adjustment

Control of setting the optimum *Channel Width* for the sample being measured is left to the instrument.

[Z]: Zero NICOMP Distribution Channels

Used to zero the three smallest diameter bins on both the computer display and printout so that they do not dominate the remaining portion of the size distribution plot. These "zeroed" bins are still included in the Distribution Analysis fit but will not be included in the calculation of the Mean Diameter which appears on the printout. These bins will appear as zeroes (000) on the printout, but their actual contributions will be printed along side the other values next to the particle size histogram; the relative numbers for the "zero" bins may exceed 100. This option does not change the computed fit to the data, only the presentation of the results.

[-]: Decrease sensitivity

Used to lower the sensitivity to achieve the optimum Photopulse Rate.

[+]: Increase sensitivity

Used to raise the sensitivity to achieve the optimum Photopulse Rate.

[Alt_T]: Stop Auto Save/Print

Used to stop the automatic saving and printing of particle size results and allows for the clearing of the autocorrelator so that a new sample may be introduced into the instrument.

[F1]: Help Menu

This option can be accessed anywhere in the software to access help.

[F2]: Auto Print/Save Menu

Please refer to the Particle Sizing section of this manual for additional information concerning this option.

[F3]: Control Menu

Please refer to the Particle Sizing section of this manual for additional information concerning this option.

[F5]: Save Data File

This option is used throughout the CW380 software whenever a data file is to be saved. Please refer to the File section of this manual for step by step instruction for saving a file.

[ALT-F5]: Save ASCII File

Use this option to save the data collected for a particular sample to an ASCII file format. This data can then be imported to a spreadsheet program for presentation.

[F6]: Read Data File

A data file that has been stored following a measurement can be retrieved to display the resulting particle size distribution (PSD), with the desired weighting. When this option is selected, a list of data files will display in the Read Data File window.

[F7]: Read Menu File

Used to access complete sets of parameters that were previously saved for the Control Menu [F3].

[CNTL-F7]: Save Menu File

Used to save or update an existing "default" TBL file, which displays in the File Name window.

[F10]: Start measurement

The instrument starts taking measurements once this option is initiated.

[Alt_F10]: Start Auto Save/Print

Used to initiate the automatic saving and printing of particle size results. Please refer to the Tool Bar section of this manual for more detail.

SAMPLE ANALYSIS RUN

SAMPLE ANALYSIS

The following are simple, step-by-step, instructions for successful use of the Nicomp Particle Sizer for the first time.

MATERIALS

Uniform latex particle reference material with a coefficient of variation less than 15% standard deviation as stated by the manufacturer.

Distilled, filtered (0.2 micron), water for dilution of reference material and cleaning glassware.

Autodilution

- 1 sterile 10cc syringe for each latex standard used
- 1 sterile 50 ml beaker
- 1 sterile pipet

Drop-in Cell

- 1 micropipet (capable of delivering 15 ul solution)
- 1 sterile 50 ml beaker
- 1 sterile pipet
- 1, 6 x 50 mm glass culture tube

Important: Reference material stated size is not an absolute. Proper storage and handling of reference material over time is essential to reduce the possibility of aggregation and contamination resulting in a high standard deviation and baseline adjust reading.

SAMPLE ANALYSIS RUN

Hardware

1. Apply power to the system in the following order:

   - Computer
   - Nicomp: power switch is located on the back right-hand panel of the instrument

The Nicomp must be powered up for a minimum of twenty minutes prior to making a measurement.

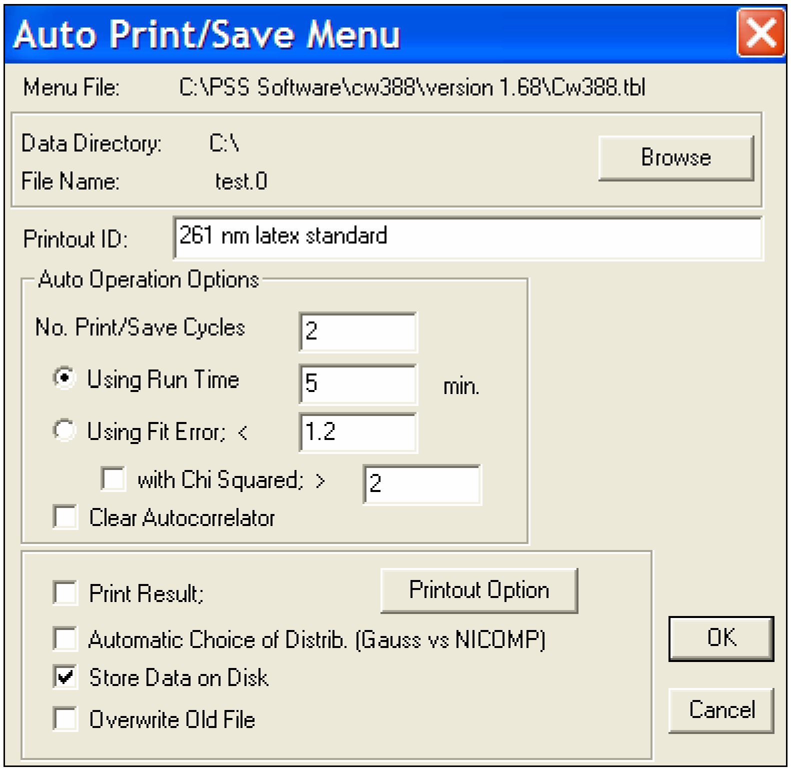
2. Access the Nicomp software.

Procedure Autodilution

1. Click on the Setup menu from the title bar and ensure that flow cell is checked on.

2. Press F2 or choose Auto Print/Save Menu from the Particle Sizing pull down menu.

3. Ensure that parameters are set according to the below:



Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Data Directory: C:\
File Name: test.0

Printout ID: 261 nm latex standard

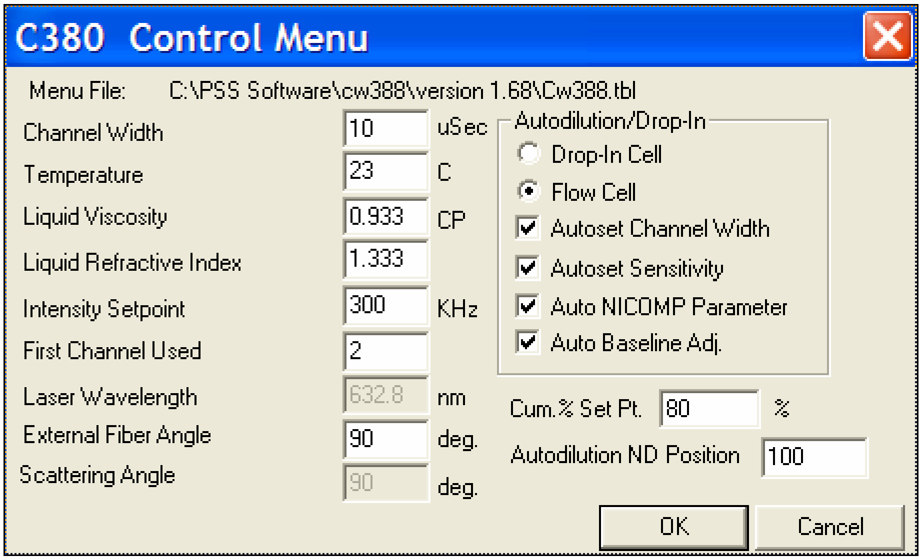
Auto Operation Options
No. Print/Save Cycles 2
● Using Run Time 5 min.
○ Using Fit Error: < 1.2
☐ with Chi Squared: > 2
☐ Clear Autocorrelator

☐ Print Result:
☐ Automatic Choice of Distrib. (Gauss vs NICOMP)
☑ Store Data on Disk
☐ Overwrite Old File

NOTE: The menu file location may be different for each computer and is unimportant for this exercise.

4. Press F3 or choose Control Menu from the Particle Sizing pull down menu

5. Ensure that parameters are set according to the below:



C380 Control Menu

Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Channel Width: 10 uSec
Temperature: 23 C
Liquid Viscosity: 0.933 CP
Liquid Refractive Index: 1.333
Intensity Setpoint: 300 KHz
First Channel Used: 2
Laser Wavelength: 632.8 nm
External Fiber Angle: 90 deg.
Scattering Angle: 90 deg.

Autodilution/Drop-In
○ Drop-In Cell
● Flow Cell
☑ Autoset Channel Width
☑ Autoset Sensitivity
☑ Auto NICOMP Parameter
☑ Auto Baseline Adj.

Cum.% Set Pt. 80 %
Autodilution ND Position 100

NOTE: The laser wavelength may differ depending on the instruments hardware setup.

6. Click on OK to return to the CW388 Software Menu.

7. Prepare the sample by gently inverting the bottle of latex material ten times.

8. Add five drops of the concentrated submicron latex standard to 25 mL of distilled, filtered water. The resulting mixture should be manually agitated to provide a uniform suspension.

9. Manually agitate the sample to provide a uniform suspension. This is confirmed by visual inspection of the sterile beaker. The suspension should be turbid (slightly white in color).

10. Draw approximately 3 mL of this turbid suspension into a sterile 3 or 10cc syringe.

11. Connect syringe to the "luer" fitting on the top of the injection valve.

12. Gently apply manual pressure to provide a finger-tight connection.

13. Point the valve handle down with the flow pattern on the handle indicating that the fluid flow path into the Nicomp is from the source of the concentrated sample in the syringe.

SAMPLE ANALYSIS RUN

NOTE: Prior to introducing the sample into the Nicomp, the flow-through system must be flushed with fresh, filtered diluent to an intensity of less than 10 kHz as displayed on the front panel screen. This can be accomplished by following the below:

a. *Click on Flow Pump icon. The system pump will begin running. An internal timer will allow the pump to run for two minutes and then stop automatically.*

   ***Important**: Press ESC to stop the pump prior to the two minute setting.*

b. *Check to see that the following two conditions are being met:*

c. *Water should be exiting from the outlet tube in a continuous flow, without bubbles.*

d. *The light scattering intensity, indicated by the digital LED display on the front panel of the Nicomp should be below 10kHz, indicating a relatively well flushed, clean scattering cell.*

If either of these conditions is not met, an additional flush cycle should be performed.

14. Click on the Autodilution icon or the Green G. The system will prompt to inject the sample.

15. Turn the valve on the front panel 90^o counter clockwise and inject approximately 1 mL of concentrated material.

16. Turn the valve on the front panel 90^o clockwise to its original position after approximately 1mL of concentrated solution has been drawn into the instrument. Sample will be drawn into the instrument; do not force all of the contents. The system will dilute the concentrated sample injected. When the proper concentration is reached the system will automatically stop the pump and begin taking data.

    The system will automatically switch to a data acquisition screen. After a few minutes of equilibration and set-up time the system will begin displaying the differential intensity-weighted distribution on the screen.

17. Press T to switch the screen to the time series plot. Allow time for the data to stabilize where the mean diameter is not changing for at least 3 minutes as a function of time. (A flat straight line)

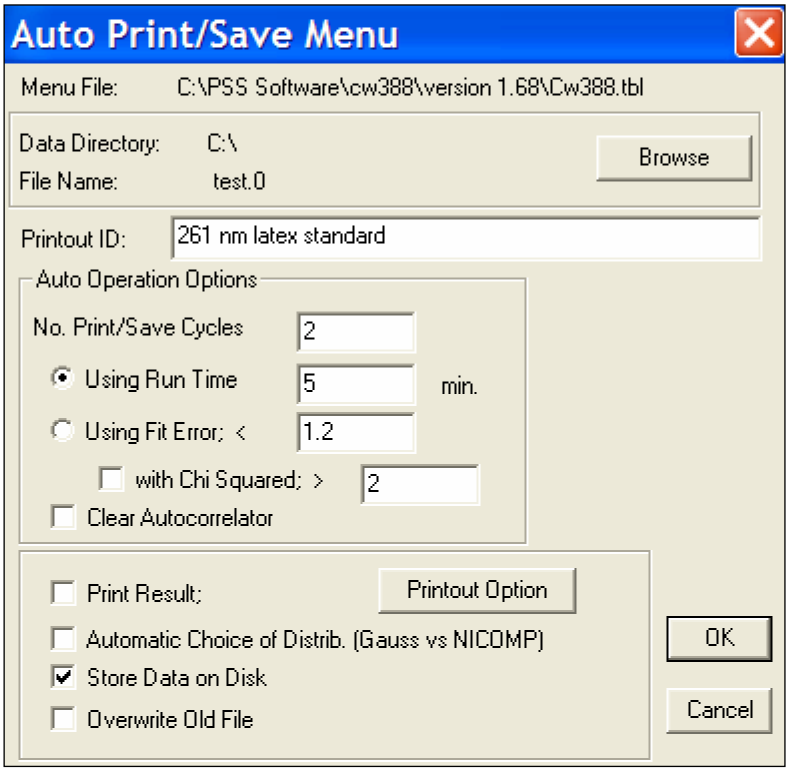
18. Press F to go back to the distribution plot in Intensity-weighted mode.

19. Verify the size standard to the reported size by following the section Interpretation of Data.

Drop-in Cell

1. Press F2 or choose Auto Print/Save Menu from the Particle Sizing pull down menu.

2. Ensure that parameters are set according to the below:



Auto Print/Save Menu

Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Data Directory: C:\

File Name: test.0

Printout ID: 261 nm latex standard

Auto Operation Options

No. Print/Save Cycles: 2

● Using Run Time: 5 min.

○ Using Fit Error; < 1.2

☐ with Chi Squared; > 2

☐ Clear Autocorrelator

☐ Print Result;

☐ Automatic Choice of Distrib. (Gauss vs NICOMP)

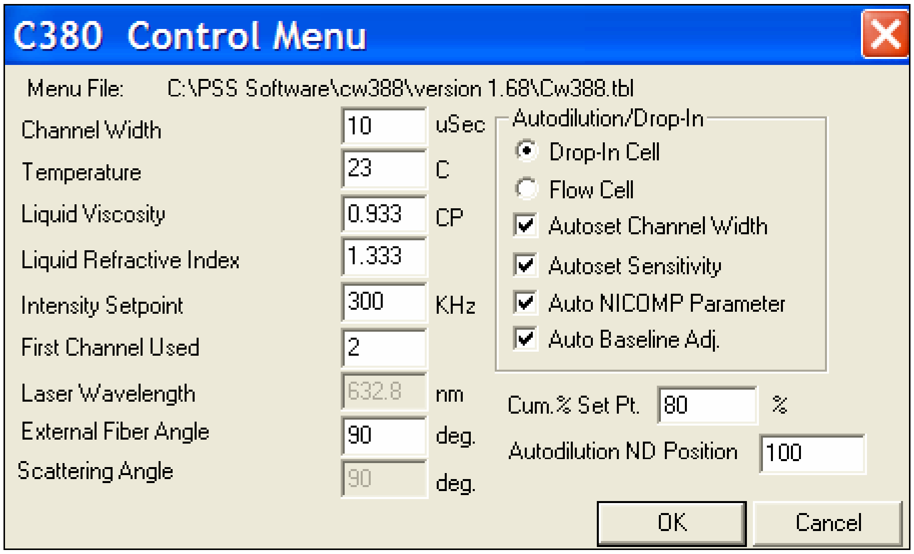
☑ Store Data on Disk

☐ Overwrite Old File

NOTE: the menu file location may be different for each computer and is unimportant for this exercise.

3. Press F3 or choose Control Menu from the Particle Sizing pull down menu

4. Ensure that parameters are set according to the below:



C380 Control Menu

Menu File: C:\PSS Software\cw388\version 1.68\Cw388.tbl

Channel Width: 10 uSec

Temperature: 23 C

Liquid Viscosity: 0.933 CP

Liquid Refractive Index: 1.333

Intensity Setpoint: 300 KHz

First Channel Used: 2

Laser Wavelength: 632.8 nm

External Fiber Angle: 90 deg.

Scattering Angle: 90 deg.

Autodilution/Drop-In

● Drop-In Cell

○ Flow Cell

☑ Autoset Channel Width

☑ Autoset Sensitivity

☑ Auto NICOMP Parameter

☑ Auto Baseline Adj.

Cum.% Set Pt.: 80 %

Autodilution ND Position: 100

NOTE: The laser wavelength may differ depending on the instruments hardware setup.

5. Click on OK to return to the CW388 Software Window.

6. Prepare the sample by gently inverting the bottle of latex material ten times.

7. Add one drop of the concentrated latex standard to 25 mL of distilled, filtered water. The resulting mixture should be manually agitated to provide a uniform suspension.

8. Manually agitate sample to provide a uniform suspension. This is confirmed by visual Inspection of the sterile beaker. The suspension should be turbid (slightly cloudy).

9. Draw approximately 1 mL of this turbid suspension into a sterile 3cc or 10cc syringe.

10. Fill the glass culture tube with latex solution and discard solution twice to clean tube.

11. Fill a glass culture tube, within 5mm of the opening.

12. Clean the tube with lint-free tissue and place the tube in the sample holder of the Nicomp.

13. A wait time of 5 minutes will allow the sample to temperature equilibrate.

14. Check the concentration of the latex solution by closing the port on top of the Nicomp and reading the intensity value on the front panel of the instrument.

15. Click the increase and decrease neutral density filter setting buttons on the tool bar until the intensity hovers at approximately 300 kHz.

16. Click on the Green G icon to start sample measurement and follow software instructions on the screen.

    Important: The instrument will adjust the channel width to the optimal value. The system will automatically switch to a data acquisition screen.

17. Press T to switch the screen to the time series plot. Allow time for the data to stabilize where the mean diameter is not changing for at least 3 minutes as a function of time. (A flat straight line)

18. Press F to go back to the distribution plot in intensity-weighted mode.

19. Verify the size standard to the reported size by following the section Interpretation of Data.

Interpretation of Data

1. Referencing data the following conditions should be met:
    a. Gaussian Int-weighted Mean Diameter within 15% of stated diameter
    b. Std Dev < 15.0%
    c. Chi Sq < 3.00
    d. Base Adj < 0.05%
2. The Nicomp is working within the manufacturer's specifications when all of these conditions are met.
3. Starting with a fresh sample repeat measurement one time if any of these conditions are not met. If on the second measurement any of these conditions are still not met, then measure a second latex standard using the same procedures.

    Important: It may not be necessary to measure both latex standards. If one works that means the instrument is operational.

Review of Completed Sample Results

1. Position the highlight bar over the File option and click once.
2. Position the highlight bar over the Read option and click once. The Read Data File Window will display.
3. Position the highlight bar over the data file to be viewed.
4. Click on OK. A second pop-up window displays prompting to re-calculate the results.
5. Press Y. The distribution will display in Number-wt mode.
6. Press W to view alternate data forms:

Volume-wt.
Number-wt.
Intensity-wt.

Print Sample Results

1. Position the highlight bar over the Print icon and click once. The Printout option window will display.
2. Click on the square box next to the desired selection. A black check mark will display.
3. Click on OK. The Print Window will display.
4. Click on OK to start printing.

Post Measurement System Flush

1. Press Ctrl-F to draw clean de-ionized water into the system via the injection port.
2. Pay close attention to the front display panel to see that the counts start to lower. Keep injecting fresh diluent until the counts fall below 10 kHz.
3. It is important to remember to clean the small injection valve on top of the system between sample runs to avoid cross contamination. This can be accomplished by following these steps:
   a. Fill a 10cc syringe with clean diluent.
   b. Inject it into the system by turning the valve mounted on the front panel of the instrument 90° counter clockwise. The system will draw in all of the diluent in the syringe.
   c. Rotate the valve 90° clockwise to its original position when approximately 5 cc's has been pulled into the system.

SYSTEM MAINTENANCE

SYSTEM MAINTENANCE

The absolute accuracy of the particle sizing technique used in the Nicomp depends on the frequency of a crystal-controlled clock and the wavelength of a laser, neither of which can "drift" over time. The only other relevant variable is the scattering angle. The optics of the Nicomp have been permanently aligned at Particle Sizing Systems' factory and should require no attention. The photopulse preamplifier/discriminator has been preset to provide optimal output pulse characteristics and requires no periodic adjustment. The thermoelectric temperature regulator has been calibrated at the factory and requires no routine attention

Preventive maintenance for the Nicomp tubing and fluidics can and should be performed on a daily basis.

MAINTENANCE

Preventive maintenance for the Nicomp Submicron Particle Sizer can and should be performed on a periodic basis.

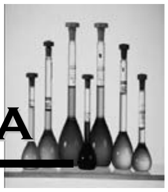
1. Flush the system adequately to achieve the same counts less than 10 mKz to display on the front panel of the instrument.

2. Visually inspect the external tubing on a weekly basis for deterioration and signs of wear. If any parts of the tubing are cracked or show signs of excessive wear they should be replaced.

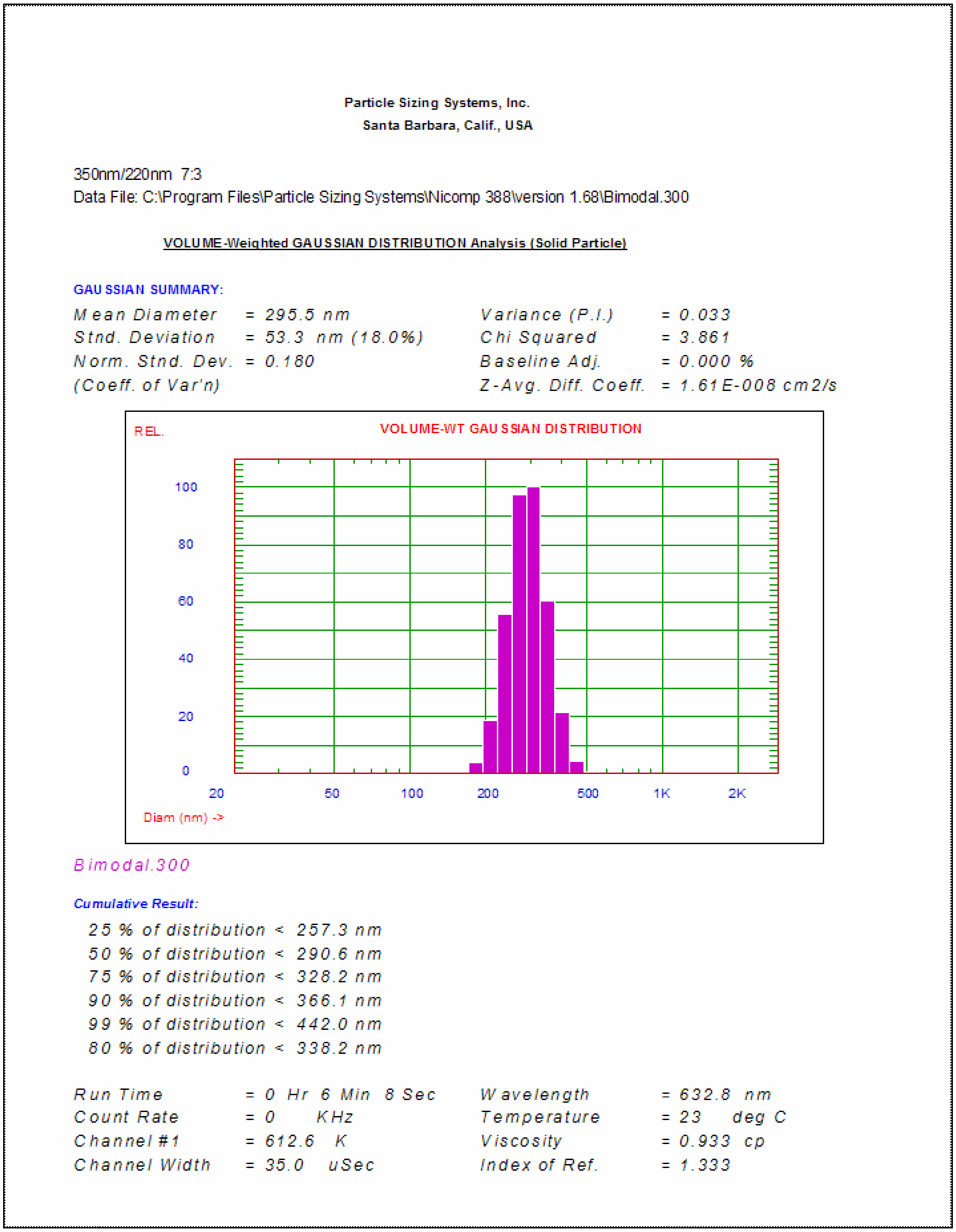
3. Visually inspect the tube section passing through the peristaltic pump for discoloration or any signs of wear.

Service related to the Laser, associated optics or the sample cell holder must be performed by the factory or a representative of Particle Sizing Systems.

APPENDIX A



VOLUME WEIGHTED GAUSSIAN



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

VOLUME-Weighted GAUSSIAN DISTRIBUTION Analysis (Solid Particle)

GAUSSIAN SUMMARY:

```
Mean Diameter    = 295.5 nm              Variance (P.I.)    = 0.033
Stnd. Deviation  = 53.3  nm (18.0%)      Chi Squared        = 3.861
Norm. Stnd. Dev. = 0.180                 Baseline Adj.      = 0.000 %
(Coeff. of Var'n)                        Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s
```

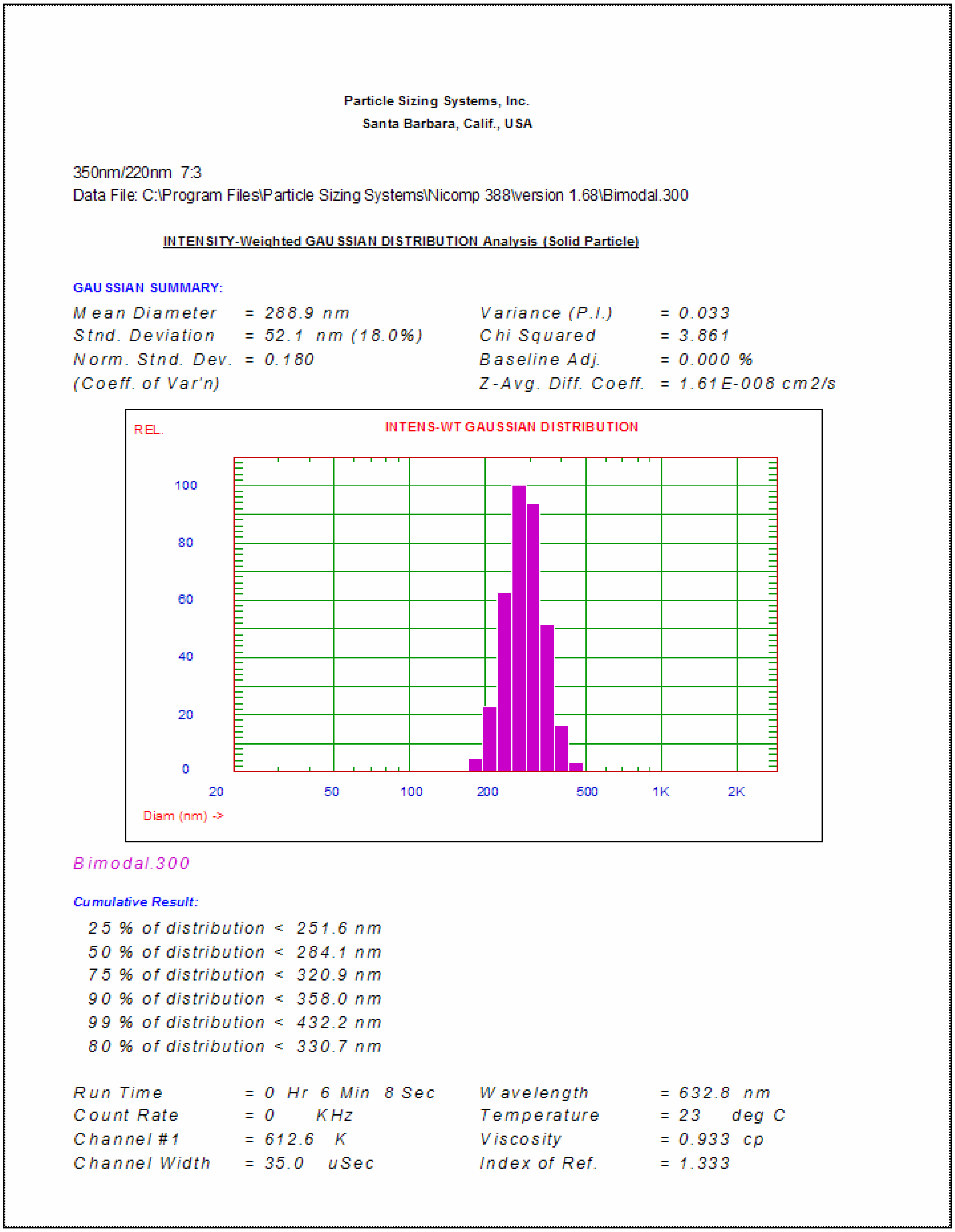
Bimodal.300

Cumulative Result:

```
  25 % of distribution < 257.3 nm
  50 % of distribution < 290.6 nm
  75 % of distribution < 328.2 nm
  90 % of distribution < 366.1 nm
  99 % of distribution < 442.0 nm
  80 % of distribution < 338.2 nm
```

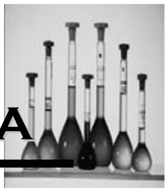
```
Run Time        = 0 Hr 6 Min 8 Sec       Wavelength       = 632.8  nm
Count Rate      = 0     KHz              Temperature      = 23    deg C
Channel #1      = 612.6   K              Viscosity        = 0.933 cp
Channel Width   = 35.0    uSec           Index of Ref.    = 1.333
```

INTENSITY WEIGHTED GAUSSIAN

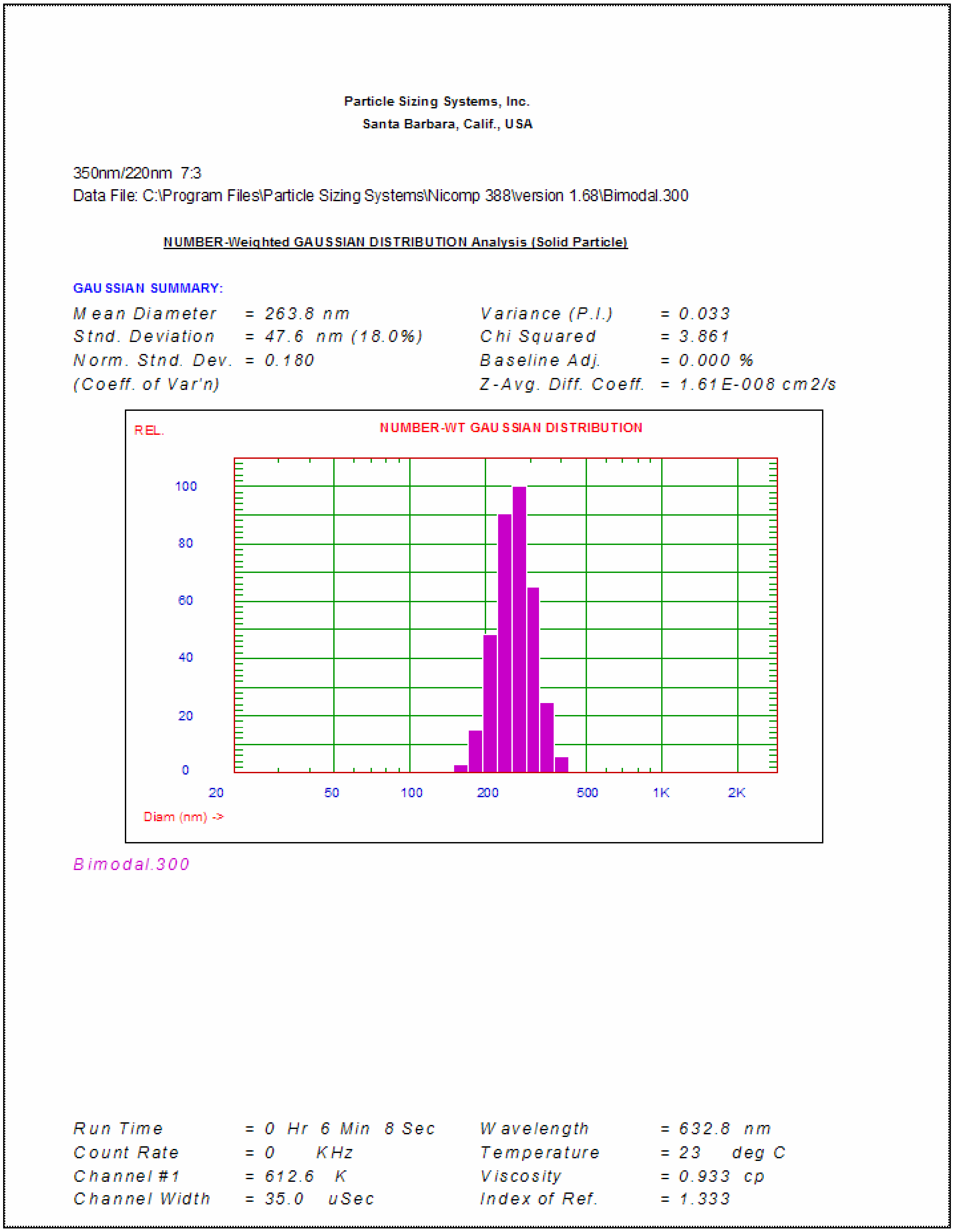


```
                    Particle Sizing Systems, Inc.
                      Santa Barbara, Calif., USA

350nm/220nm  7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

        INTENSITY-Weighted GAUSSIAN DISTRIBUTION Analysis (Solid Particle)

GAUSSIAN SUMMARY:
Mean Diameter    = 288.9 nm            Variance (P.I.)       = 0.033
Stnd. Deviation  = 52.1  nm (18.0%)    Chi Squared           = 3.861
Norm. Stnd. Dev. = 0.180               Baseline Adj.         = 0.000 %
(Coeff. of Var'n)                      Z-Avg. Diff. Coeff.   = 1.61E-008 cm2/s
```

```
Bimodal.300

Cumulative Result:
  25 % of distribution <  251.6 nm
  50 % of distribution <  284.1 nm
  75 % of distribution <  320.9 nm
  90 % of distribution <  358.0 nm
  99 % of distribution <  432.2 nm
  80 % of distribution <  330.7 nm

Run Time         = 0  Hr  6  Min  8 Sec    Wavelength    = 632.8  nm
Count Rate       = 0     KHz               Temperature   = 23    deg C
Channel #1       = 612.6   K               Viscosity     = 0.933  cp
Channel Width    = 35.0    uSec            Index of Ref. = 1.333
```



NUMBER WEIGHTED GAUSSIAN



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

NUMBER-Weighted GAUSSIAN DISTRIBUTION Analysis (Solid Particle)

GAUSSIAN SUMMARY:

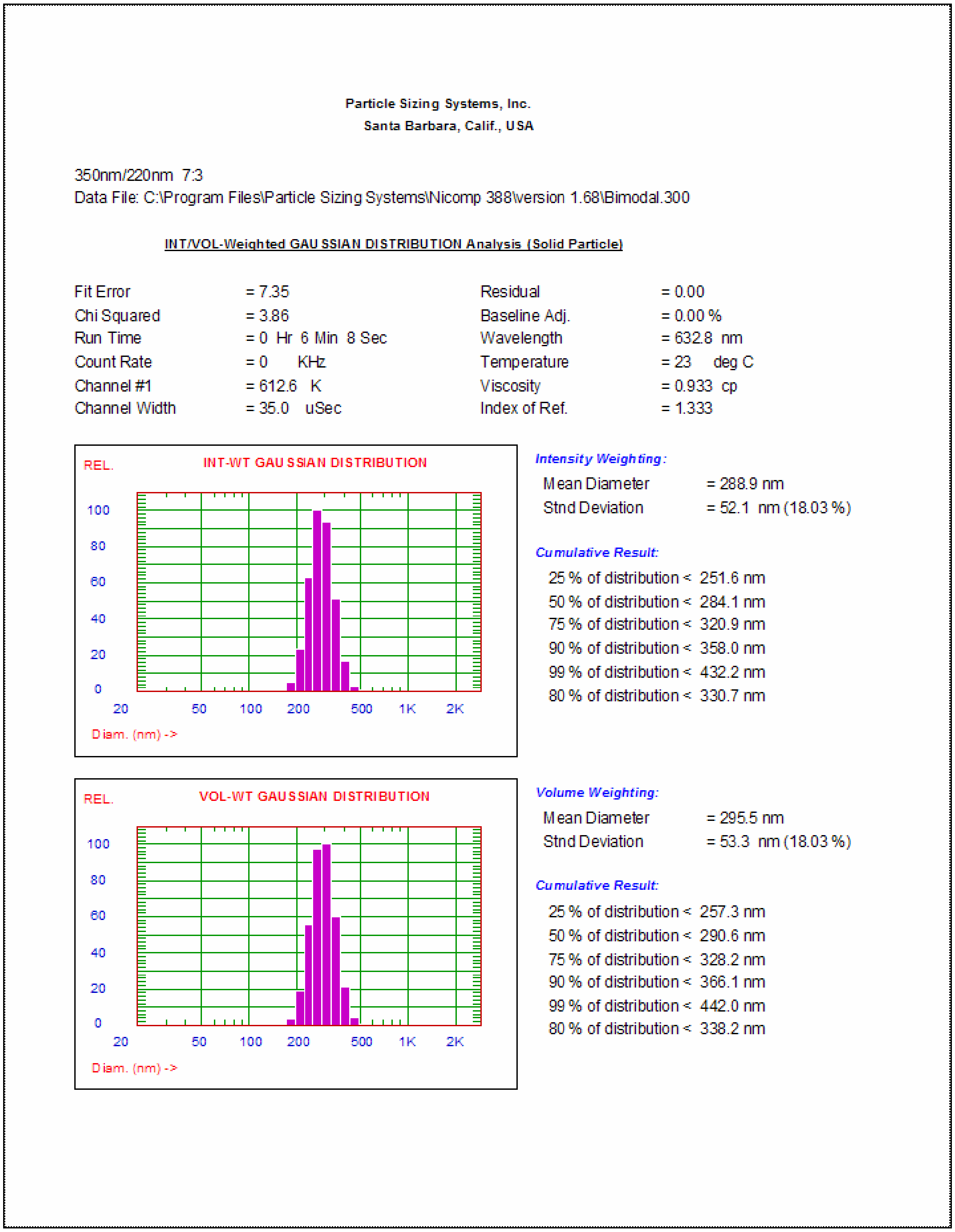
```
Mean Diameter    = 263.8 nm            Variance (P.I.)    = 0.033
Stnd. Deviation  = 47.6  nm (18.0%)    Chi Squared        = 3.861
Norm. Stnd. Dev. = 0.180               Baseline Adj.      = 0.000 %
(Coeff. of Var'n)                      Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s
```

Bimodal.300

```
Run Time         = 0 Hr 6 Min 8 Sec    Wavelength         = 632.8 nm
Count Rate       = 0     KHz           Temperature        = 23    deg C
Channel #1       = 612.6   K           Viscosity          = 0.933 cp
Channel Width    = 35.0    uSec        Index of Ref.      = 1.333
```

APPENDIX A

INT/VOLUME WEIGHTED GAUSSIAN



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

INT/VOL-Weighted GAUSSIAN DISTRIBUTION Analysis (Solid Particle)

Fit Error = 7.35
Chi Squared = 3.86
Run Time = 0 Hr 6 Min 8 Sec
Count Rate = 0 KHz
Channel #1 = 612.6 K
Channel Width = 35.0 uSec

Residual = 0.00
Baseline Adj. = 0.00 %
Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

Intensity Weighting:

Mean Diameter = 288.9 nm
Stnd Deviation = 52.1 nm (18.03 %)

Cumulative Result:

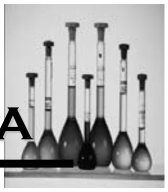
25 % of distribution < 251.6 nm
50 % of distribution < 284.1 nm
75 % of distribution < 320.9 nm
90 % of distribution < 358.0 nm
99 % of distribution < 432.2 nm
80 % of distribution < 330.7 nm

Volume Weighting:

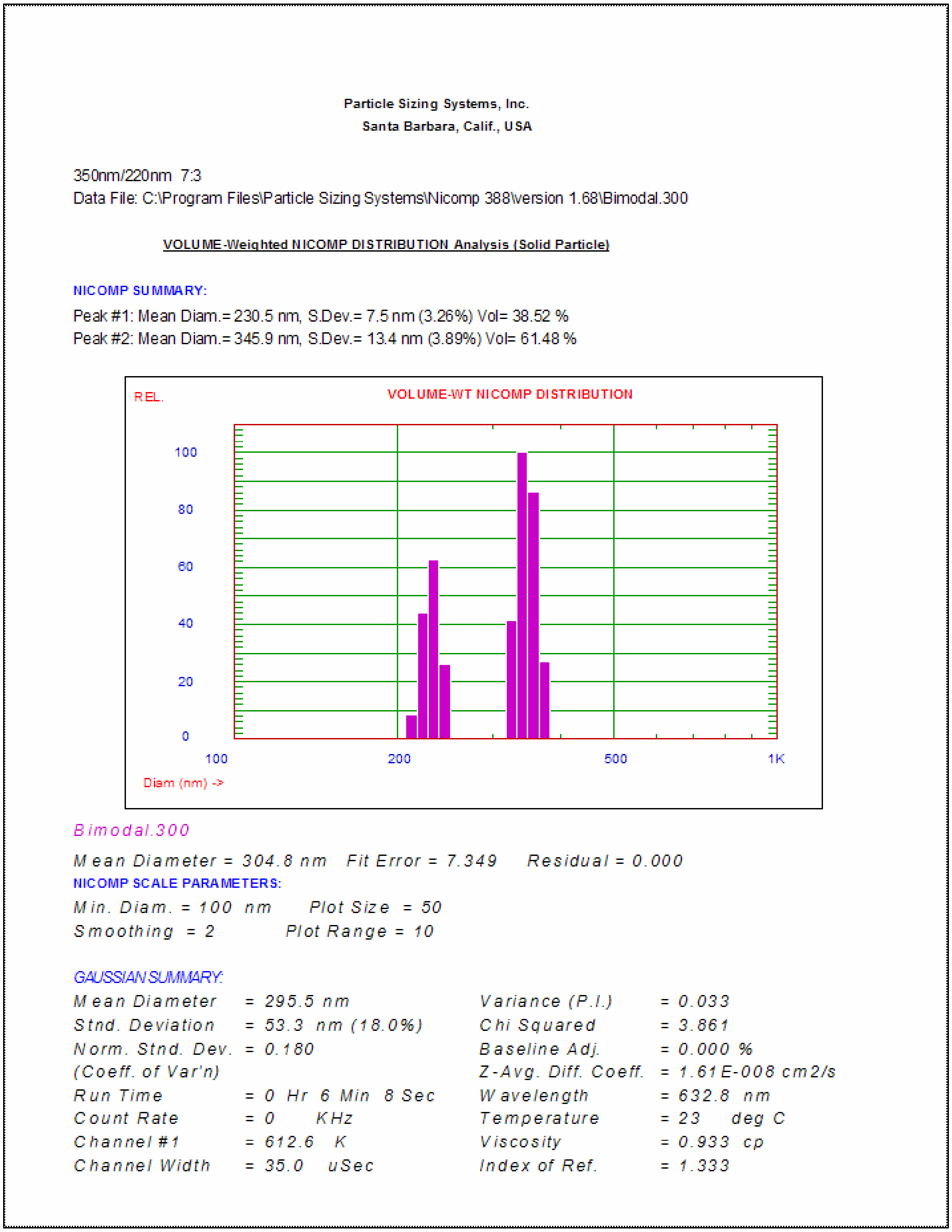
Mean Diameter = 295.5 nm
Stnd Deviation = 53.3 nm (18.03 %)

Cumulative Result:

25 % of distribution < 257.3 nm
50 % of distribution < 290.6 nm
75 % of distribution < 328.2 nm
90 % of distribution < 366.1 nm
99 % of distribution < 442.0 nm
80 % of distribution < 338.2 nm



VOLUME WEIGHTED NICOMP



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

VOLUME-Weighted NICOMP DISTRIBUTION Analysis (Solid Particle)

NICOMP SUMMARY:

Peak #1: Mean Diam.= 230.5 nm, S.Dev.= 7.5 nm (3.26%) Vol= 38.52 %
Peak #2: Mean Diam.= 345.9 nm, S.Dev.= 13.4 nm (3.89%) Vol= 61.48 %

Bimodal.300

```
Mean Diameter = 304.8 nm   Fit Error = 7.349    Residual = 0.000
```

NICOMP SCALE PARAMETERS:

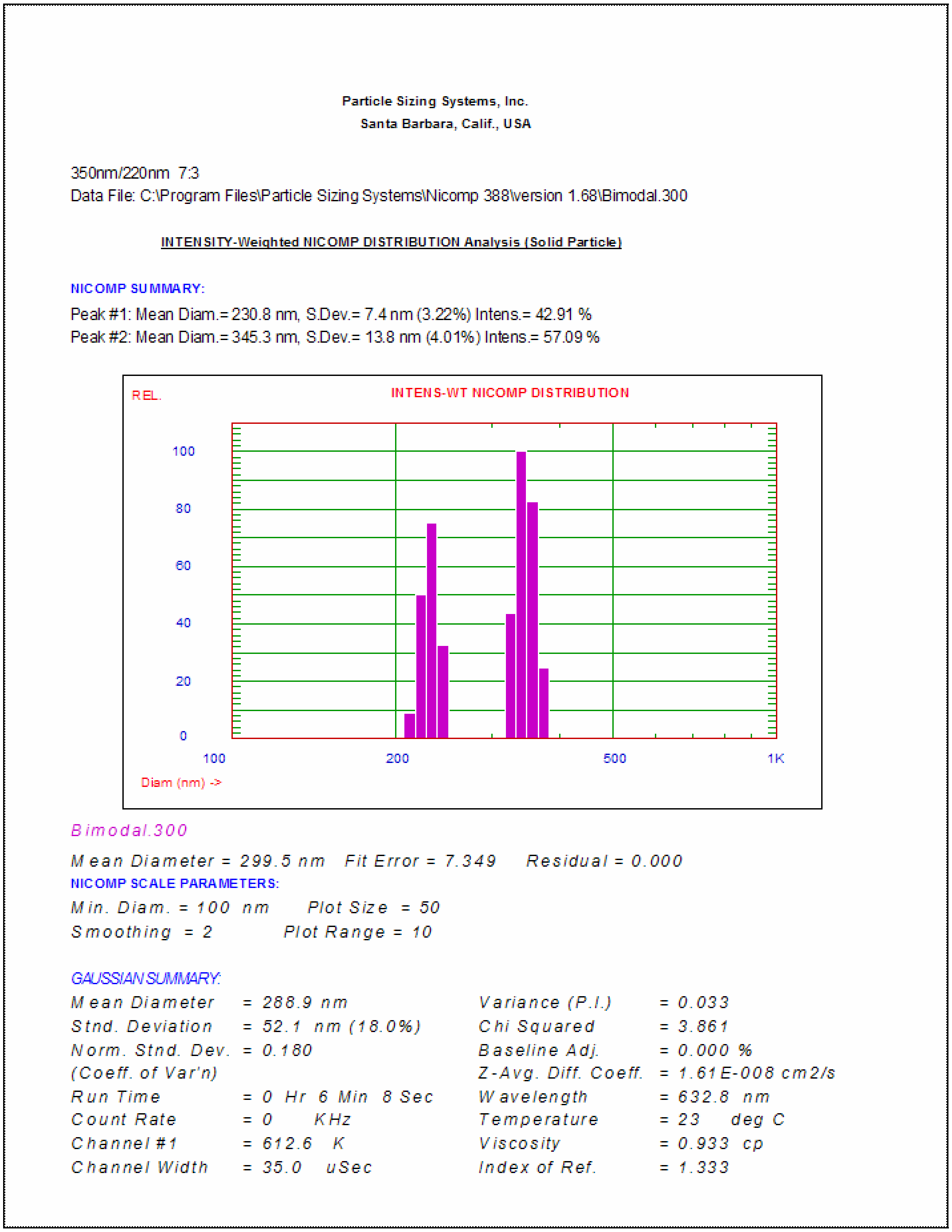
```
Min. Diam. = 100  nm      Plot Size  = 50
Smoothing  = 2           Plot Range = 10
```

GAUSSIAN SUMMARY:

```
Mean Diameter    = 295.5 nm              Variance (P.I.)     = 0.033
Stnd. Deviation  = 53.3  nm (18.0%)      Chi Squared         = 3.861
Norm. Stnd. Dev. = 0.180                 Baseline Adj.       = 0.000 %
(Coeff. of Var'n)                        Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s
Run Time         = 0 Hr  6 Min  8 Sec    Wavelength          = 632.8  nm
Count Rate       = 0     KHz             Temperature         = 23     deg C
Channel #1       = 612.6   K             Viscosity           = 0.933  cp
Channel Width    = 35.0    uSec          Index of Ref.       = 1.333
```

APPENDIX A

INTENSITY WEIGHTED NICOMP



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

INTENSITY-Weighted NICOMP DISTRIBUTION Analysis (Solid Particle)

NICOMP SUMMARY:

Peak #1: Mean Diam.= 230.8 nm, S.Dev.= 7.4 nm (3.22%) Intens.= 42.91 %
Peak #2: Mean Diam.= 345.3 nm, S.Dev.= 13.8 nm (4.01%) Intens.= 57.09 %

Bimodal.300

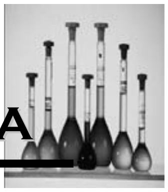
```
Mean Diameter = 299.5 nm   Fit Error = 7.349    Residual = 0.000
```

NICOMP SCALE PARAMETERS:

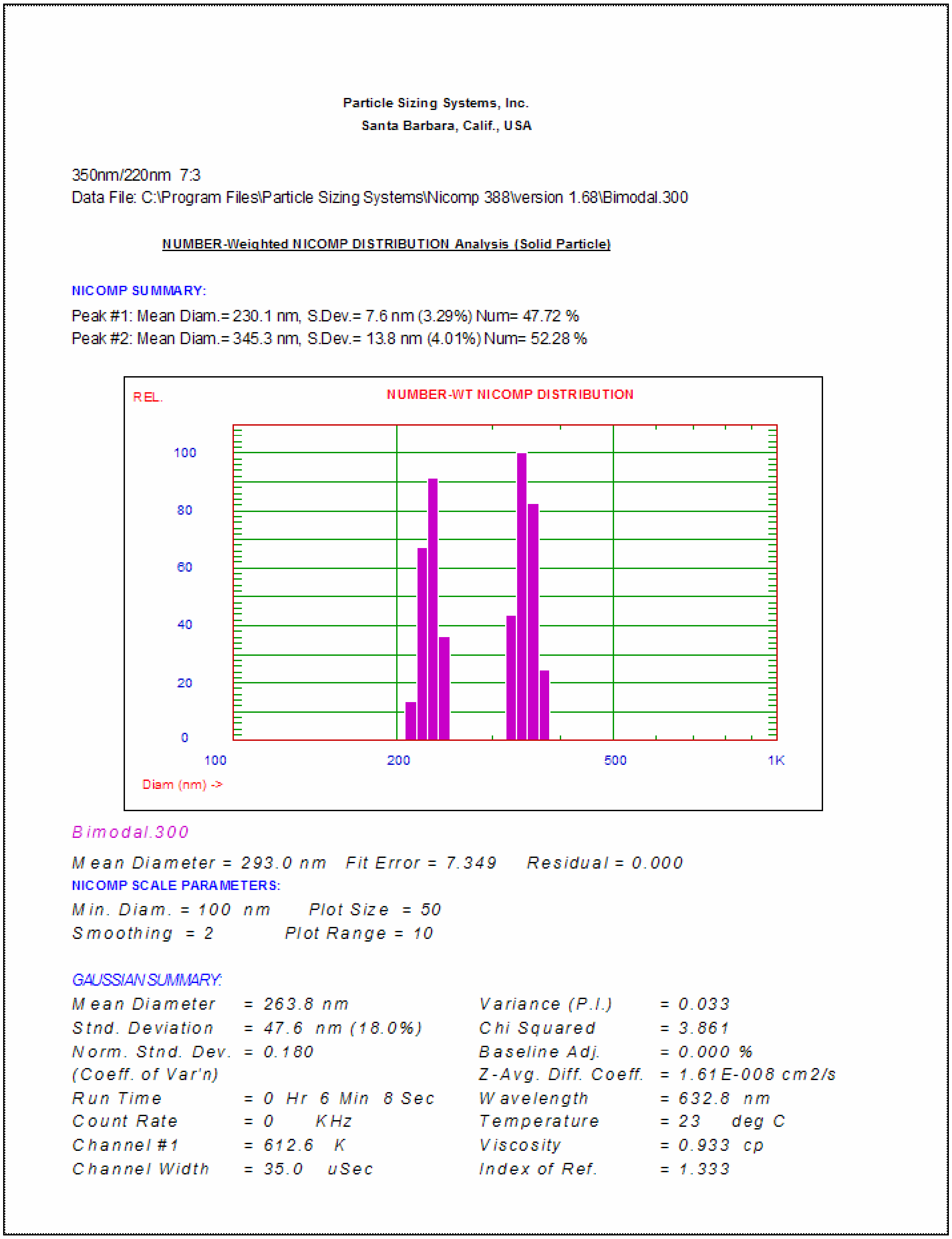
```
Min. Diam. = 100 nm      Plot Size = 50
Smoothing = 2            Plot Range = 10
```

GAUSSIAN SUMMARY:

```
Mean Diameter    = 288.9 nm              Variance (P.I.)     = 0.033
Stnd. Deviation  = 52.1 nm (18.0%)       Chi Squared         = 3.861
Norm. Stnd. Dev. = 0.180                 Baseline Adj.       = 0.000 %
(Coeff. of Var'n)                        Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s
Run Time         = 0 Hr 6 Min 8 Sec      Wavelength          = 632.8 nm
Count Rate       = 0    KHz              Temperature         = 23   deg C
Channel #1       = 612.6  K              Viscosity           = 0.933 cp
Channel Width    = 35.0   uSec           Index of Ref.       = 1.333
```



NUMBER WEIGHTED NICOMP



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

NUMBER-Weighted NICOMP DISTRIBUTION Analysis (Solid Particle)

NICOMP SUMMARY:

Peak #1: Mean Diam.= 230.1 nm, S.Dev.= 7.6 nm (3.29%) Num= 47.72 %
Peak #2: Mean Diam.= 345.3 nm, S.Dev.= 13.8 nm (4.01%) Num= 52.28 %

Bimodal.300

Mean Diameter = 293.0 nm   Fit Error = 7.349   Residual = 0.000

NICOMP SCALE PARAMETERS:

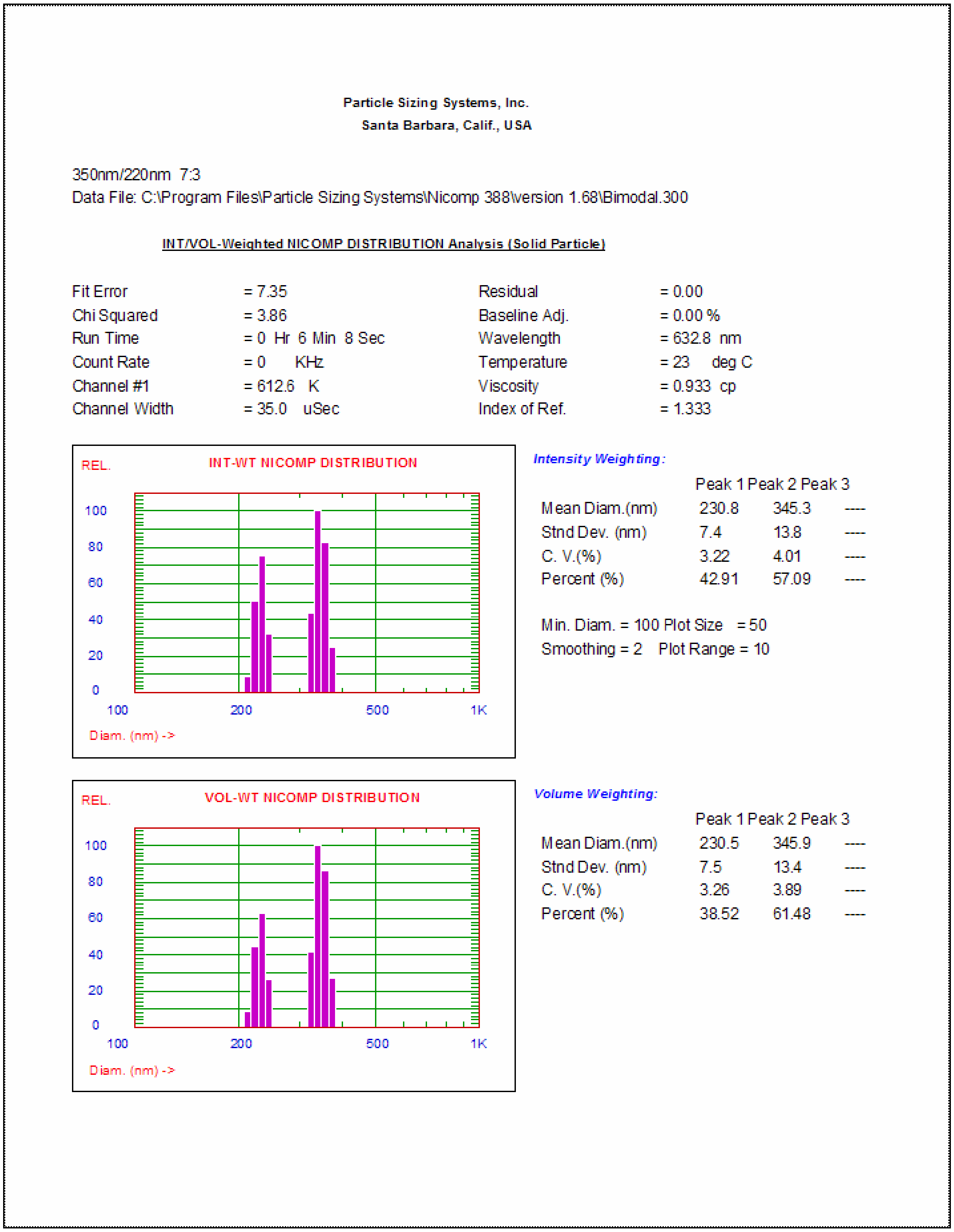
Min. Diam. = 100 nm   Plot Size = 50
Smoothing = 2   Plot Range = 10

GAUSSIAN SUMMARY:

Mean Diameter	= 263.8 nm	Variance (P.I.)	= 0.033
Stnd. Deviation	= 47.6 nm (18.0%)	Chi Squared	= 3.861
Norm. Stnd. Dev.	= 0.180	Baseline Adj.	= 0.000 %
(Coeff. of Var'n)		Z-Avg. Diff. Coeff.	= 1.61E-008 cm2/s
Run Time	= 0 Hr 6 Min 8 Sec	Wavelength	= 632.8 nm
Count Rate	= 0 KHz	Temperature	= 23 deg C
Channel #1	= 612.6 K	Viscosity	= 0.933 cp
Channel Width	= 35.0 uSec	Index of Ref.	= 1.333

APPENDIX A

INT/VOL WEIGHTED



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
Data File: C:\Program Files\Particle Sizing Systems\Nicomp 388\version 1.68\Bimodal.300

INT/VOL-Weighted NICOMP DISTRIBUTION Analysis (Solid Particle)

Fit Error = 7.35
Chi Squared = 3.86
Run Time = 0 Hr 6 Min 8 Sec
Count Rate = 0 KHz
Channel #1 = 612.6 K
Channel Width = 35.0 uSec

Residual = 0.00
Baseline Adj. = 0.00 %
Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

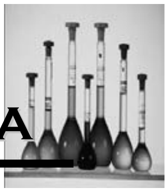
Intensity Weighting:

	Peak 1	Peak 2	Peak 3
Mean Diam.(nm)	230.8	345.3	----
Stnd Dev. (nm)	7.4	13.8	----
C. V.(%)	3.22	4.01	----
Percent (%)	42.91	57.09	----

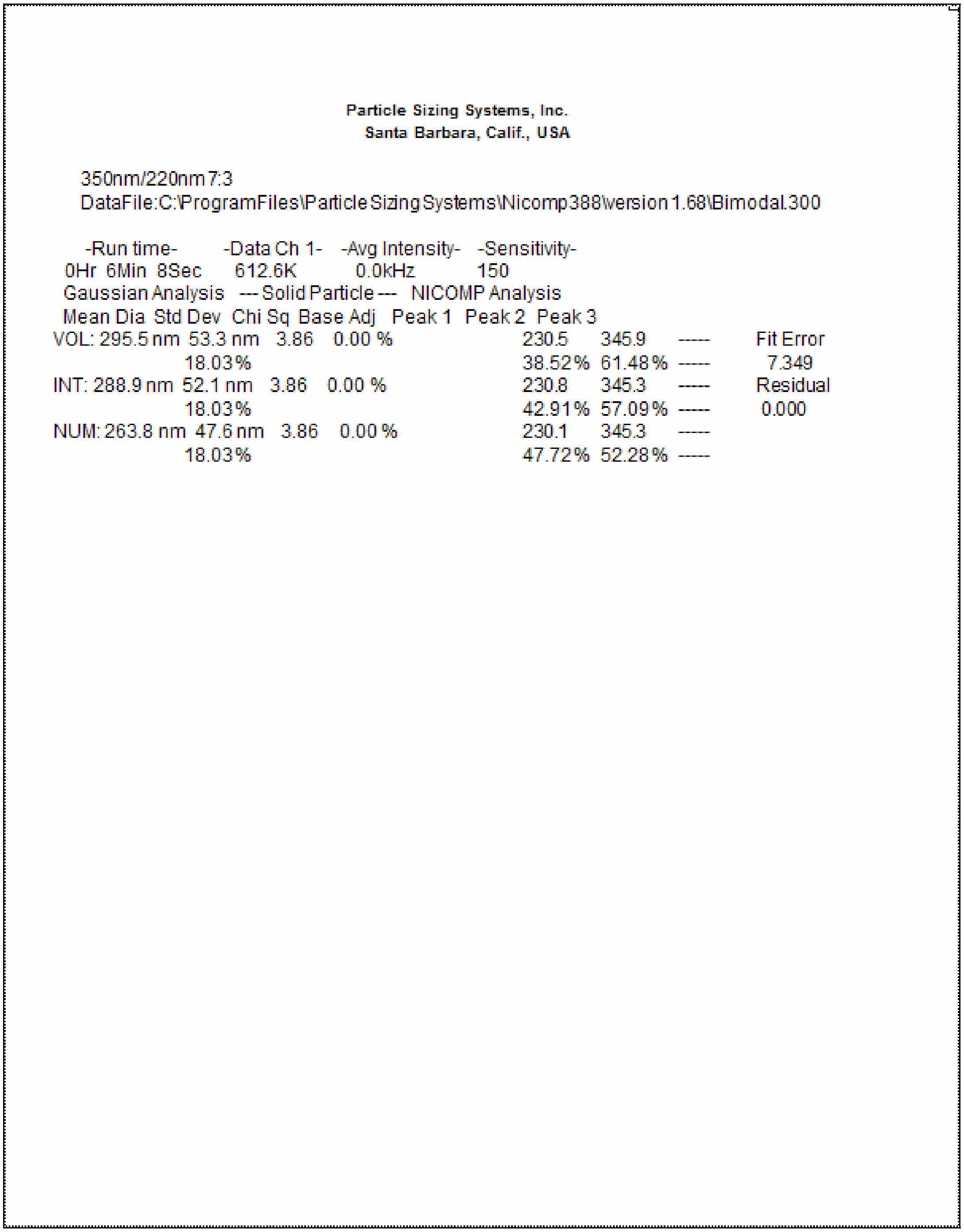
Min. Diam. = 100 Plot Size = 50
Smoothing = 2 Plot Range = 10

Volume Weighting:

	Peak 1	Peak 2	Peak 3
Mean Diam.(nm)	230.5	345.9	----
Stnd Dev. (nm)	7.5	13.4	----
C. V.(%)	3.26	3.89	----
Percent (%)	38.52	61.48	----

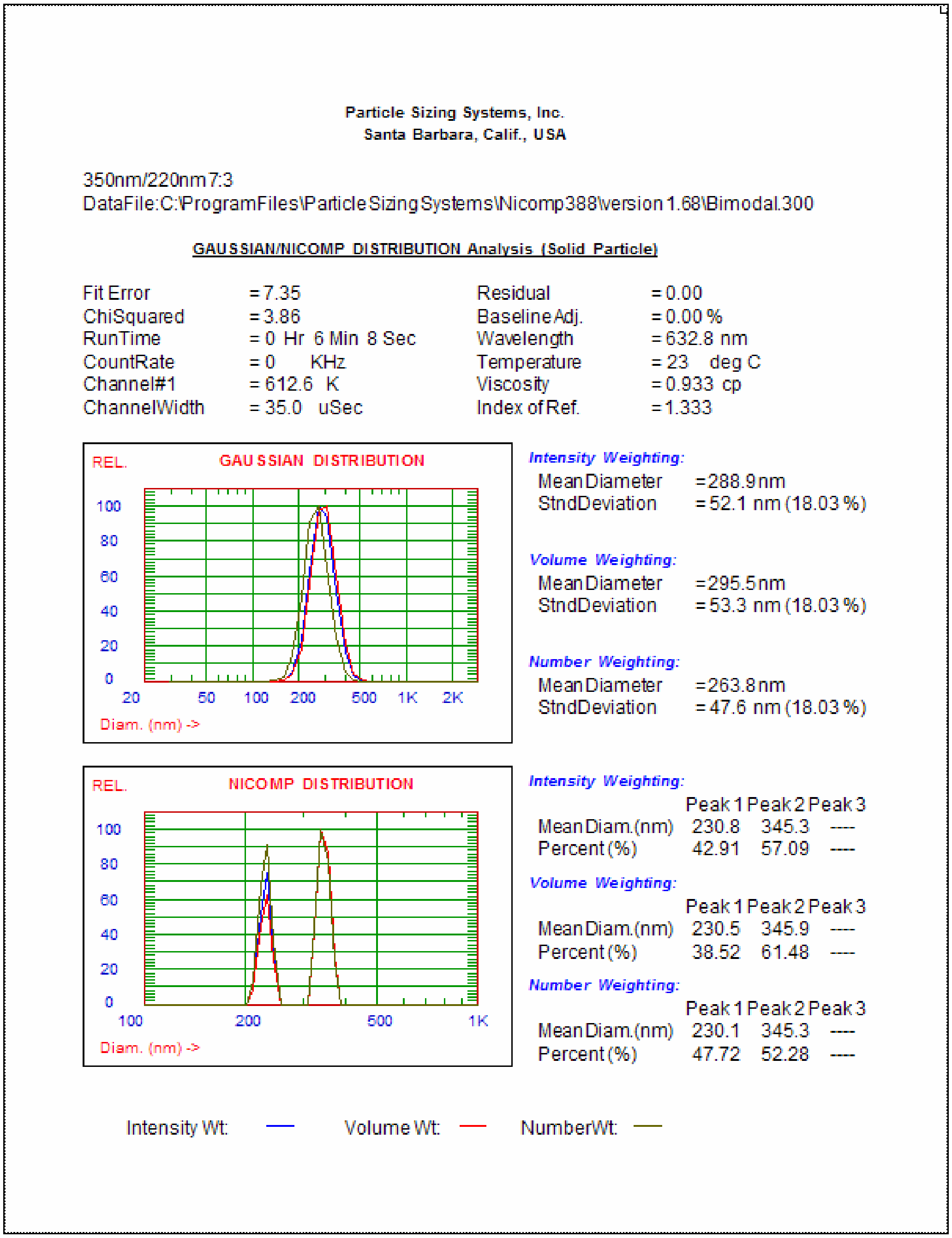


SUMMARY RESULT



```
                    Particle Sizing Systems, Inc.
                      Santa Barbara, Calif., USA

   350nm/220nm7:3
   DataFile:C:\ProgramFiles\ParticleSizingSystems\Nicomp388\version1.68\Bimodal.300

    -Run time-       -Data Ch 1-   -Avg Intensity-   -Sensitivity-
  0Hr  6Min  8Sec      612.6K          0.0kHz           150
  Gaussian Analysis   --- Solid Particle ---  NICOMP Analysis
  Mean Dia  Std Dev  Chi Sq  Base Adj   Peak 1   Peak 2  Peak 3
VOL: 295.5 nm  53.3 nm   3.86    0.00 %              230.5      345.9      -----       Fit Error
                 18.03%                              38.52%   61.48%   -----        7.349
INT: 288.9 nm  52.1 nm   3.86    0.00 %              230.8      345.3      -----       Residual
                 18.03%                              42.91%   57.09%   -----        0.000
NUM: 263.8 nm  47.6 nm   3.86    0.00 %              230.1      345.3      -----
                 18.03%                              47.72%   52.28%   -----
```

GAUSSIAN/NICOMP ALL WEIGHTED



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
DataFile:C:\ProgramFiles\Particle Sizing Systems\Nicomp388\version 1.68\Bimodal.300

GAUSSIAN/NICOMP DISTRIBUTION Analysis (Solid Particle)

Fit Error = 7.35
ChiSquared = 3.86
RunTime = 0 Hr 6 Min 8 Sec
CountRate = 0 KHz
Channel#1 = 612.6 K
ChannelWidth = 35.0 uSec

Residual = 0.00
Baseline Adj. = 0.00 %
Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

Intensity Weighting:
Mean Diameter = 288.9 nm
StndDeviation = 52.1 nm (18.03 %)

Volume Weighting:
Mean Diameter = 295.5 nm
StndDeviation = 53.3 nm (18.03 %)

Number Weighting:
Mean Diameter = 263.8 nm
StndDeviation = 47.6 nm (18.03 %)

Intensity Weighting:

	Peak 1	Peak 2	Peak 3
Mean Diam.(nm)	230.8	345.3	----
Percent (%)	42.91	57.09	----

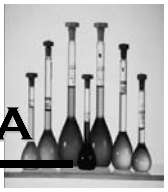
Volume Weighting:

	Peak 1	Peak 2	Peak 3
Mean Diam.(nm)	230.5	345.9	----
Percent (%)	38.52	61.48	----

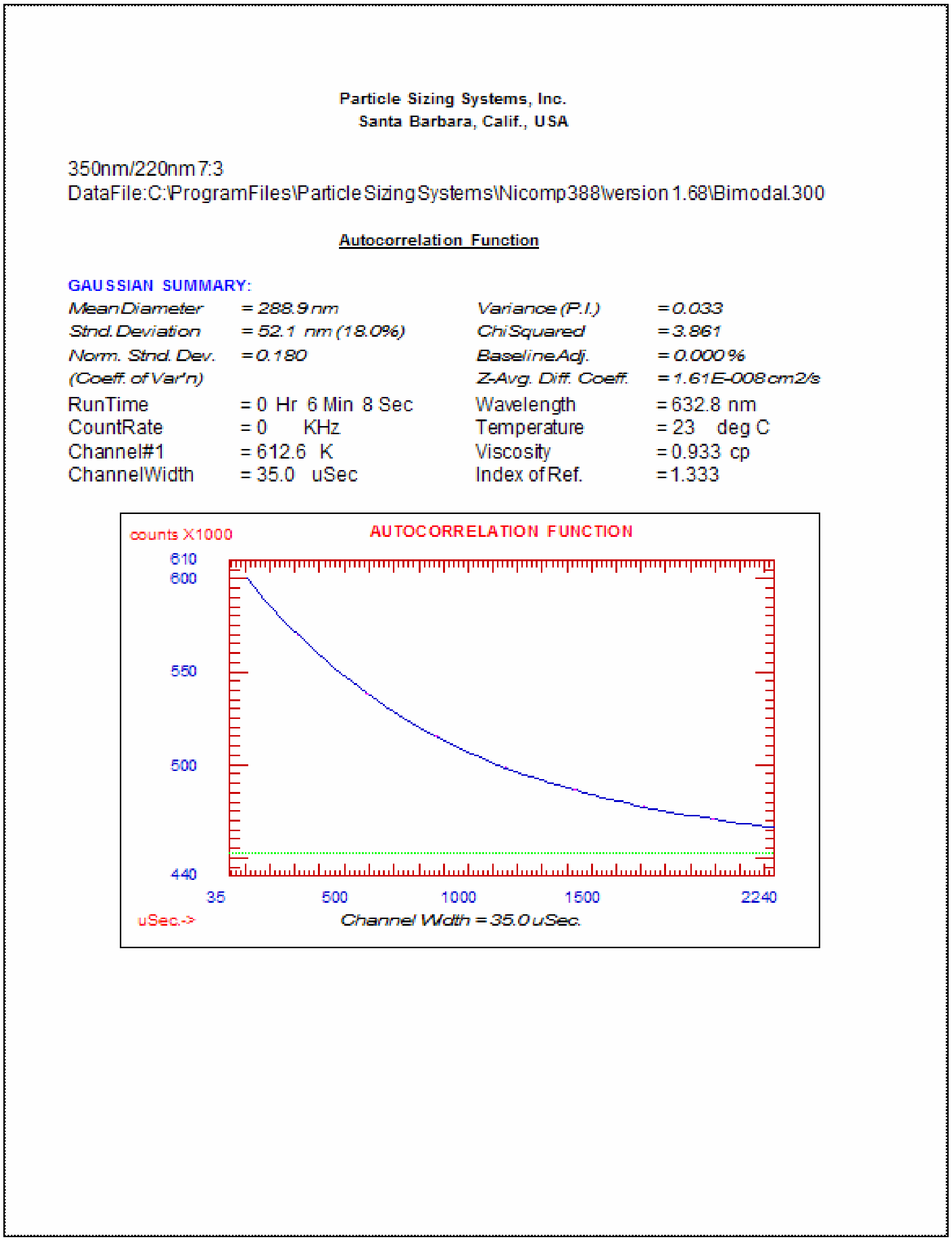
Number Weighting:

	Peak 1	Peak 2	Peak 3
Mean Diam.(nm)	230.1	345.3	----
Percent (%)	47.72	52.28	----

Intensity Wt: — Volume Wt: — Number Wt: —



AUTOCORRELATION FUNCTION



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

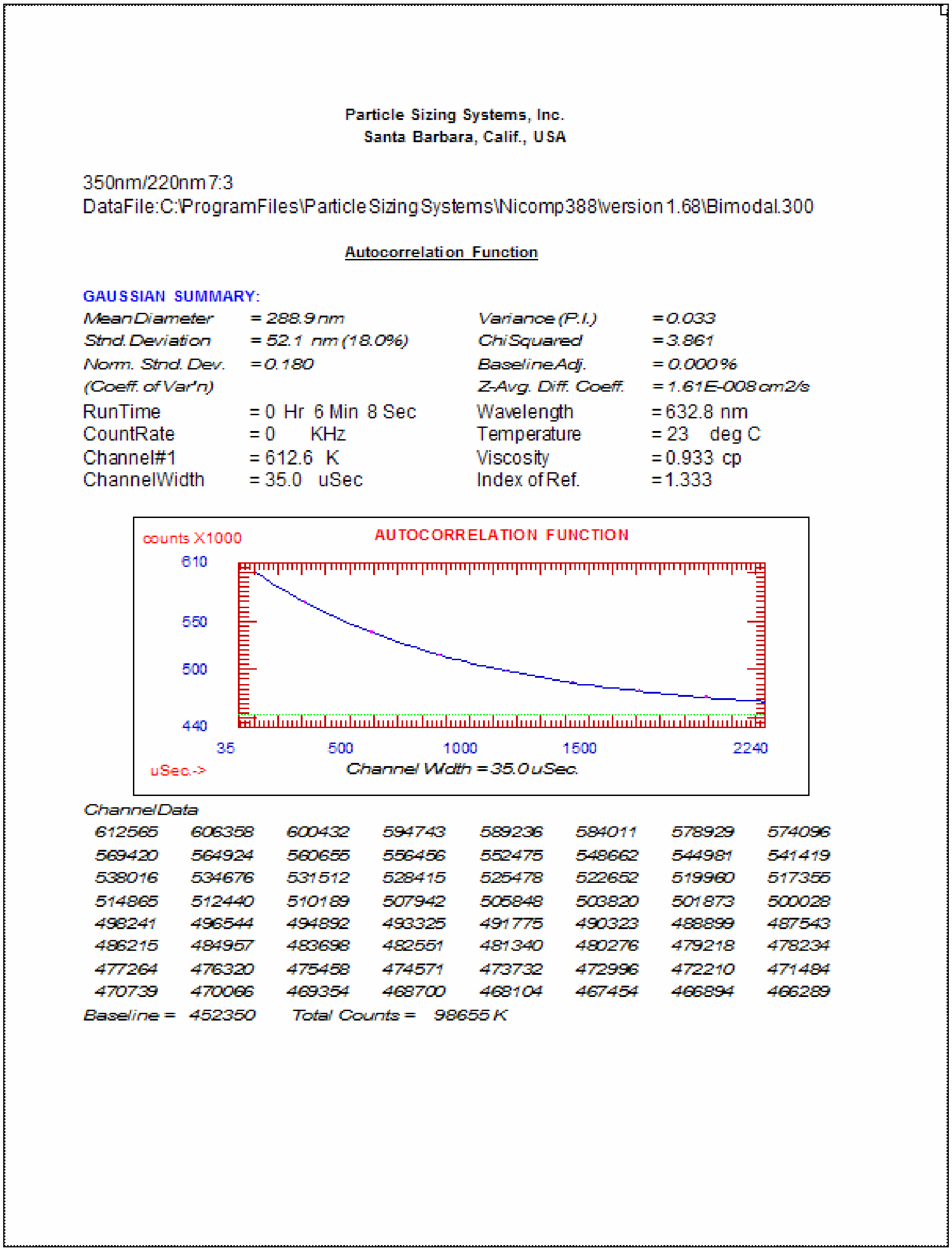
350nm/220nm 7:3
DataFile:C:\ProgramFiles\ParticleSizingSystems\Nicomp388\version 1.68\Bimodal.300

Autocorrelation Function

GAUSSIAN SUMMARY:

Mean Diameter	*= 288.9 nm*	*Variance (P.I.)*	*= 0.033*
Stnd. Deviation	*= 52.1 nm (18.0%)*	*Chi Squared*	*= 3.861*
Norm. Stnd. Dev.	*= 0.180*	*Baseline Adj.*	*= 0.000 %*
(Coeff. of Var'n)		*Z-Avg. Diff. Coeff.*	*= 1.61E-008 cm2/s*
RunTime	= 0 Hr 6 Min 8 Sec	Wavelength	= 632.8 nm
CountRate	= 0 KHz	Temperature	= 23 deg C
Channel#1	= 612.6 K	Viscosity	= 0.933 cp
ChannelWidth	= 35.0 uSec	Index of Ref.	= 1.333

AUTOCORRELATION DATA



Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

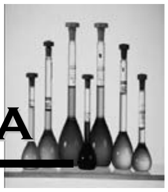
350nm/220nm 7:3
DataFile:C:\ProgramFiles\Particle Sizing Systems\Nicomp388\version 1.68\Bimodal.300

Autocorrelation Function

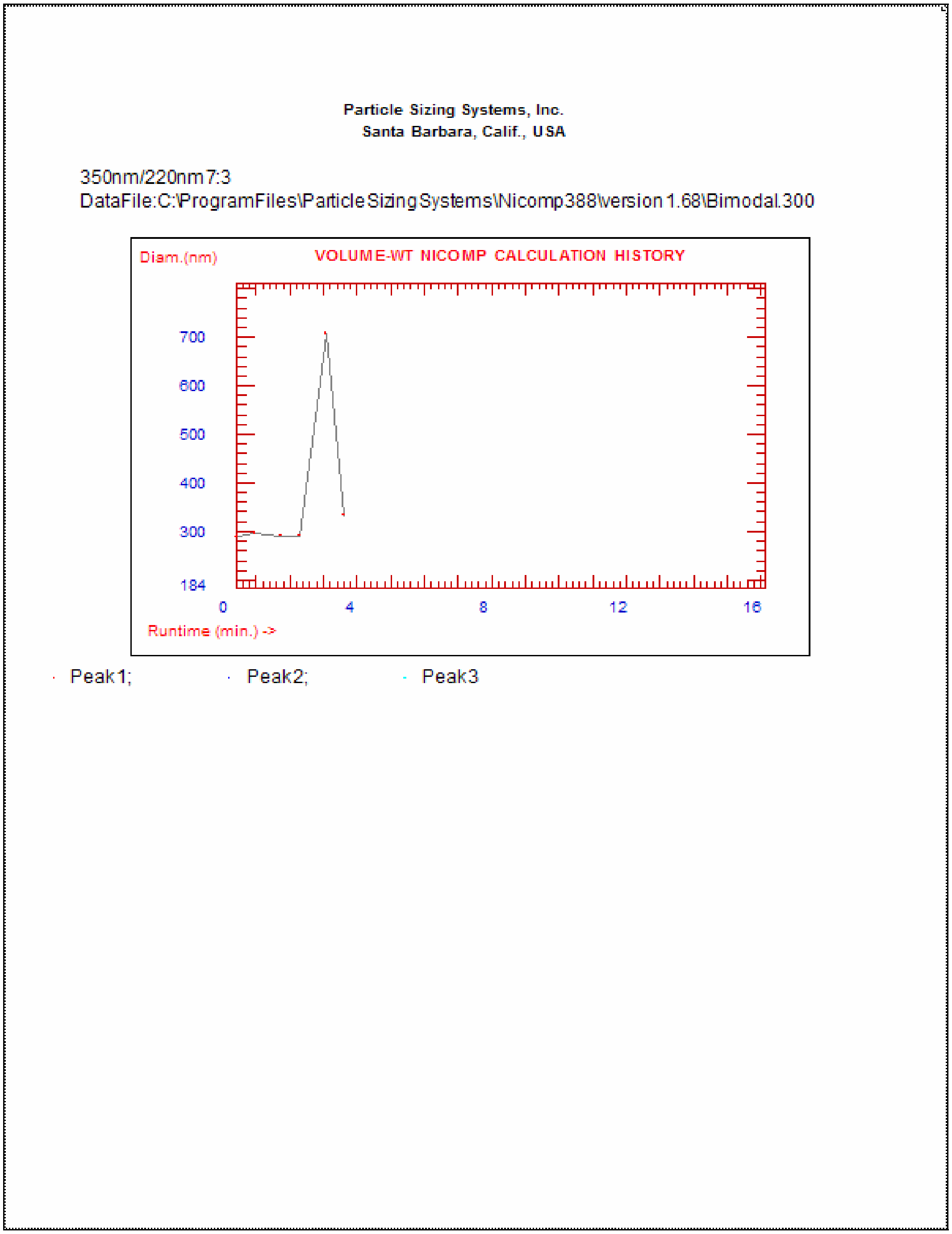
GAUSSIAN SUMMARY:

```
MeanDiameter       = 288.9 nm             Variance (P.I.)     = 0.033
Stnd.Deviation     = 52.1  nm (18.0%)     ChiSquared          = 3.861
Norm. Stnd. Dev.   = 0.180                BaselineAdj.        = 0.000 %
(Coeff. of Var'n)                         Z-Avg. Diff. Coeff. = 1.61E-008 cm2/s
RunTime            = 0 Hr  6 Min  8 Sec   Wavelength          = 632.8 nm
CountRate          = 0      KHz           Temperature         = 23    deg C
Channel#1          = 612.6  K             Viscosity           = 0.933 cp
ChannelWidth       = 35.0   uSec          Index of Ref.       = 1.333
```

```
ChannelData
  612565   606358   600432   594743   589236   584011   578929   574096
  569420   564924   560655   556456   552475   548662   544981   541419
  538016   534676   531512   528415   525478   522652   519960   517355
  514865   512440   510189   507942   505848   503820   501873   500028
  498241   496544   494892   493325   491775   490323   488899   487543
  486215   484957   483698   482551   481340   480276   479218   478234
  477264   476320   475458   474571   473732   472996   472210   471484
  470739   470066   469354   468700   468104   467454   466894   466289
Baseline =   452350     Total Counts =    98655 K
```



TIME HISTORY PLOT

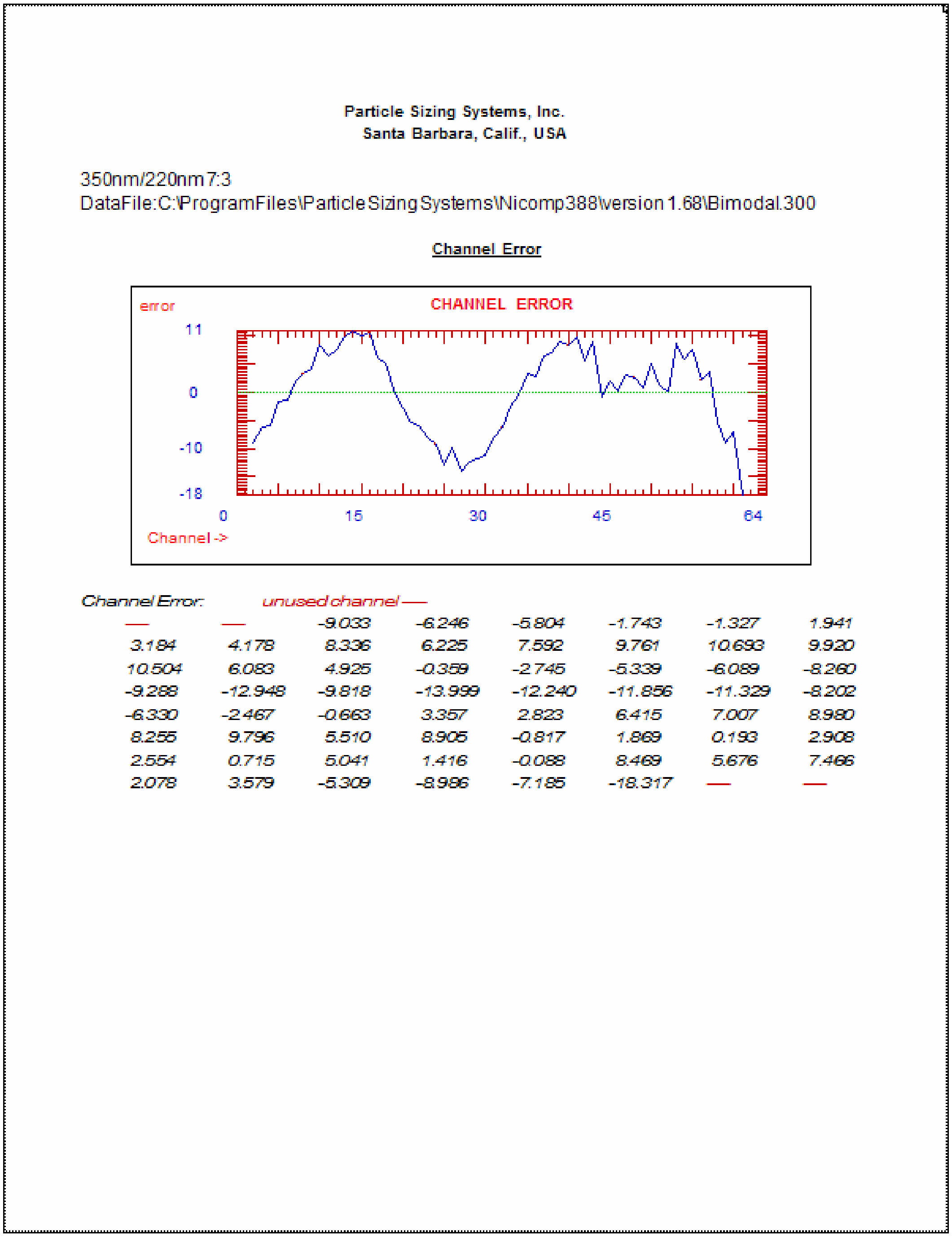


Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
DataFile:C:\ProgramFiles\ParticleSizingSystems\Nicomp388\version 1.68\Bimodal.300

· Peak1; · Peak2; · Peak3

CHANNEL ERROR PLOT



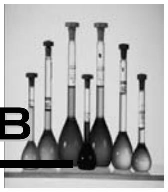
Particle Sizing Systems, Inc.
Santa Barbara, Calif., USA

350nm/220nm 7:3
DataFile:C:\ProgramFiles\Particle Sizing Systems\Nicomp388\version 1.68\Bimodal.300

Channel Error

```
Channel Error:      unused channel —
   —        —      -9.033   -6.246   -5.804   -1.743   -1.327    1.941
 3.184    4.178     8.336    6.225    7.592    9.761   10.693    9.920
10.504    6.083     4.925   -0.359   -2.745   -5.339   -6.089   -8.260
-9.288  -12.948    -9.818  -13.999  -12.240  -11.856  -11.329   -8.202
-6.330   -2.467    -0.663    3.357    2.823    6.415    7.007    8.980
 8.255    9.796     5.510    8.905   -0.817    1.869    0.193    2.908
 2.554    0.715     5.041    1.416   -0.088    8.469    5.676    7.466
 2.078    3.579    -5.309   -8.986   -7.185  -18.317     —        —
```

APPENDIX B



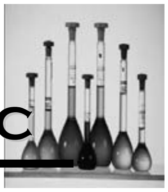
NICOMP PARTS LIST

PART	DESCRIPTION	PART NUMBER
ASSEMBLIES		
PCB, DSP Corr. Assembly		
HV Control Board		
Pre-Amp Board, 380		
Pulse Rate Board, 380		
Static Mixer Assembly		
Static Mixer Support		
Temperature Control Board		
Temperature Control Block & SS		
Terminal Block Assembly		
CABLES		
DB 25 Pin to 9 Pin	**4' Cable**	**PSS**
FANS		
Fan	**12V 4.6"**	**4710NL-o4W-B20 (NMB)**
FILTERS		
0.2 µm, Millipore	**Hydrophobic filter**	**KTGR04NP3**
Neutral Density Filter*		
*** - Only available for HPL and VHPL lasers.**		
FUSES		
Fuse fast 3A		**312003 (Littlefuse)**
Fuse Holder		**342014A (Littlefuse)**

APPENDIX B

PART	DESCRIPTION	PART NUMBER
INJECTION PORT VALVE		
Hamilton Valve	86907 HVX-2	Hamilton
Fitting, Leured	35071	Hamilton
Fitting, Barber	35072	Hamilton
Nut, Valve	35121	Hamilton
LASER		
Laser 5 mW	5mW	Uniphase
High Power Laser	35mW	Melles Griot
Very High Laser	Air cooled 75mW	UniPhase
POWER SUPPLIES		
PMT Power Supply		PMT-20CN-3 (Bertan)
Power Cord	115V	17407S (Belden)
Power Supply		MAP80-4DO2 (PowerOne)
PUMPS		
380 Pump Assembly	60 rpm, 115V Masterflex	T-7543-60
Pump Head	MasterFlex	T-7016-20
SAMPLE CELL		
Flow-thru Cell Assembly		
Sample Cell Holder, Flow-		
Sample Cell Bottom Plate		
Sample Cell Block M/A		
Sample Cell Back Plate		
Glass pieces, Autodilutor		121.114-OS (Hellma)
TUBING		
SIL,(Perox)	96400-16	Cole Parmer

APPENDIX C



NONAQUEOUS SOLVENTS FOR THE NICOMP

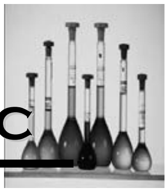
The Nicomp can be used to measure the size of particles that are suspended in solvents other than pure water. For example, certain dry powders, such as ceramic compounds, are best dispersed in organic fluids. There are two issues that must be considered when using any nonaqueous solvent for the suspending medium.

First, if the Autodilution option is to be used in the 380, be certain that the silicone tubing in the unit is compatible with the solvent in question. All of the other components in the 380 which come into contact with the diluting solvent are highly resistant to most fluids. These include the teflon injection valve, stainless steel dilution chamber, and glass flow-through scattering cell. A copy of the MasterFlex^{R} tubing compatibility chart (obtained from Cole-Parmer Instrument Company , located in Chicago, Illinois) is shown in table B-1. The relevant column which must be considered is the one labeled "S" - for silicone tubing.

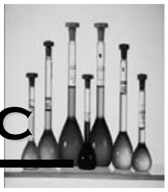
> **Important**: When considering whether to subject the fluidics system to a fluid other than water, there is a good rule to follow: When in doubt, don't! Only those fluids which are clearly marked by an "X" (satisfactory) in column "S" are safe to use in the Autodilution system of the Nicomp.

If Table B does not provide adequate information for a particular solvent, a simple experiment can be performed to test the compatibility of the tubing to the solvent. A short length of silicone tubing (e.g. the last couple of inches of the output drain line) can be immersed overnight in the solvent in question. If the physical characteristics of the tubing remain unchanged -- i.e. if there is no swelling or shrinkage, change in elasticity or color, etc. -- then it is probably safe to subject the fluidics system to the solvent. However, in any case operate conservatively. When the measurement is completed, the new solvent should be flushed out of the system (first, by running the pump -- Ctrl F -- on air, allowing most of the solvent to exit via the drain line) and then replaced with water. Run sufficient water through the system to insure complete replacement, by dilution, of any remaining solvent.

Additional replacement tubing or tubing of a different composition can be obtained directly from Cole-Parmer. The tubing is size 16.

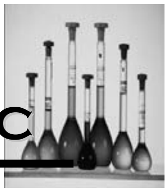


Fluid	Tubing						Head Material			
	PN	CF	S	T	TS	V	PSF	PC	PPS	SS
Acetaldehyde	A	-	B	D	D	D	D	-	A	A
Acetate LHW	A	C	-	D	D	-	D	-	A	A
Acetic Acid >5%	A	B	B	A	C	-	A	A	A	C
Acetic Acid <5%	A	C	B	B	D	-	A	C	A	B
Acetic Anhydride	A	-	D	D	D	D	D	D	A	A
Acetone	D	-	D	D	D	D	D	D	A	A
Air	A	A	A	A	A	A	A	A	A	A
Aliphatic Hydrocarbons	C	D	-	C	C	-	-	-	-	B
Aluminum Chloride	A	B	B	A	A	A	A	A	A	A
Aluminum Sulfate	A	B	A	A	-	A	A	A	A	B
Alums	A	B	-	A	A	-	-	-	-	-
Ammonia, gas, liquid	A	B	-	B	B	D	A	D	A	B
Ammonium Acetate	C	B	-	A	A	-	-	A	-	-
Ammonium Carbonate	A	B	C	A	A	-	A	-	A	B
Ammonium Chloride	A	B	C	A	A	A	A	-	A	C
Ammonium Hydroxide	A	B	A	B	D	A	A	D	A	A
Ammonium Nitrate	A	B	C	A	A	-	A	-	A	A
Ammonium Phosphate	A	B	A	A	A	-	A	A	A	B
Ammonium Sulfate	A	B	A	A	A	A	A	A	A	B
Amyl Acetate	A	D	D	D	D	D	D	D	A	A
Amyl Alcohol	B	D	D	B	B	A	A	-	A	A
Amyl Chloride	A	D	D	B	B	-	D	D	A	A
Aniline	C	-	B	D	D	-	D	D	D	A
Aniline Hydrochloride	C	-	-	D	D	D	-	D	-	D
Aqua Regina (80% HC1 ,20% H)	C	B	-	D	D	-	D	D	D	D
Aromatic Hydrocarbons	D	D	-	D	B	-	-	-	-	A
Arsenic Salts	A	B	-	A	A	-	-	-	-	-
Barium Salts	A	B	A	A	A	-	A	-	A	B
Benzaldehyde	C	D	-	D	D	D	C	C	A	B
Benzenesulfonic Acid	D	A	D	B	B	A	D	D	A	B
Bleaching Liquors	A	B	B	B	B	A	-	-	-	-
Boric Acid	A	B	A	A	A	A	A	A	A	B
Bromine	A	B	D	A	A	A	-	D	D	D
Butane	C	D	D	B	B	A	-	-	A	A
Butanol (Butyl Alcohol)	C	B	B	B	B	A	A	C	A	A
Butylacetate	D	D	D	D	D	D	D	D	A	B
Butyric Acid	A	B	D	D	B	-	-	-	A	B
Calcium Oxide	A	B	A	A	A	-	-	-	-	A
Calcium Salts	A	B	B	A	A	-	-	-	-	B
Carbon Bisulfide	D	D	-	D	D	-	-	-	-	A
Carbon Dioxide	A	B	B	A	A	A	-	A	A	A
Carbon Tetrachloride	D	-	D	D	D	A	A	D	A	B



Fluid	Tubing							Head Material		
	PN	CF	S	T	TS	V	PSF	PC	PPS	SS
Chlorine, dry	D	B	D	A	A	A	D	-	D	A
Chlorine, wet	D	B	D	B	B	B	D	-	D	C
Chloroacetic Acid	A	B	-	D	D	D	D	D	A	B
Chlorobenzene	D	D	D	D	D	A	D	D	A	A
Chlorobromomethane	B	D	D	D	D	A	D	-	-	-
Chloroform	D	D	D	D	D	A	D	D	A	A
Chlorosulfonic Acid	D	B	D	B	B	D	D	-	-	D
Chromic Acid 30%	A	B	D	A	A	A	D	D	A	B
Chromium Salts	A	B	-	A	A	-	-	-	-	-
Copper Salts	A	B	A	A	A	-	-	-	A	B
Cresol	C	D	-	D	D	A	D	D	A	A
Cyclohexane	D	D	D	D	C	A	A	B	A	A
Cyclohexaone	D	D	D	D	D	D	D	D	A	A
Diacetone Alcohol	A	B	-	D	D	-	-	D	-	B
Dimethyl Formamide	D	-	-	D	D	D	D	D	A	A
Essential Oils	A	-	C	B	B	-	-	-	-	-
Ethers	D	D	D	D	D	-	D	D	A	A
Ethyl Acetate	C	D	D	D	D	D	A	D	A	B
Ethyl Alcohol	C	B	B	B	B	A	B	B	A	A
Ethyl Bromide	D	A	D	D	D	-	-	-	-	-
Ethyl Chloride	D	B	D	D	D	A	D	D	-	A
Ethylamine	D	A	C	D	D	-	-	-	-	-
Ethylene Chlorhydrin	D	B	C	D	D	A	D	D	A	B
Ethylene Dichloride	D	B	D	D	D	B	D	D	A	B
Ethylene Glycol	A	B	A	B	B	A	A	C	A	B
Ethylene Oxide	B	B	D	B	B	D	A	D	D	B
Fatty Acids	A	B	C	A	A	A	-	C	-	B
Ferric Chloride	A	A	B	A	A	B	A	-	A	D
Ferric Sulfate	A	A	C	A	A	A	A	-	A	B
Ferrous Chloride	A	A	C	A	A	-	A	D	A	D
Ferrous Sulfate	A	A	C	A	A	-	A	A	A	B
Flouboric Acid	A	A	A	A	A	-	A	-	A	B
Flouroborate Salts	A	B	-	A	A	-	-	-	-	-
Fluosilicic Acid	A	A	-	A	A	-	A	-	A	C
Formaldehyde	A	A	B	B	B	D	A	A	A	C
Formic Acid	A	B	C	B	B	D	C	D	A	D
Freon TMS	D	D	-	D	D	-	-	D	A	-
Gasoline (high aromatic)	D	D	D	D	B	A	A	C	A	A
Gasoline (non-aromatic)	D	D	D	D	B	A	A	A	A	-
Glucose	A	A	A	A	A	A	-	A	-	A
Glue P.V.A.	A	A	A	A	A	A	-	-	-	A
Glycerin	A	B	A	A	A	A	A	A	A	A

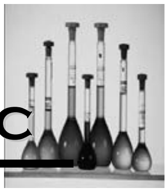
Fluid	Tubing						Head Material			
	PN	CF	S	T	TS	V	PSF	PC	PPS	SS
Hydriodic Acid	D	B	-	A	A	-	-	-	-	-
Hydrobromic Acid 30%	B	B	D	A	A	A	B	D	A	D
Hydrochloric Acid 100% conc	A	B	D	A	B	A	A	B	A	D
Hydrochloric Acid	A	B	D	A	B	A	A	B	A	D
Hydrochloric Acid med	-	A	D	B	D	-	A	D	A	D
Hydrocyanic Acid	A	A	C	A	A	A	-	-	-	B
Hydrocyanic Acid (gas 10%)	A	A	C	A	A	A	-	-	-	-
Hydrofluoric Acid 50%	-	A	D	B	B	D	-	D	A	D
Hydrofluoric Acid 75%	-	A	D	B	D	-	-	D	A	D
Hydrogen Peroxide (dil)	A	A	A	A	A	A	A	A	-	B
Hydrogen Peroxide 90%	A	D	C	D	D	-	A	A	-	B
Hypochlorous Acid	A	A	D	A	A	A	-	-	-	-
Iodine Solutions	A	D	C	A	A	-	-	D	D	D
Idoform										A
Kerosene	D	D	D	D	B	A	A	A	A	A
Ketones	D	D	-	D	D	-	D	D	A	A
Lacquers Solvents	D	D	D	D	D	D	-	D	-	A
Lactic Acids	A	A	A	A	A	A	A	A	A	B
Lead Acetate	A	B	D	B	A	-	A	-	A	B
Linseed Oil	A	D	-	A	A	A	A	A	A	A
Lithium Hydroxide	B	A	D	A	A	-	-	D	A	B
Manganese Salts	A	B	B	A	A	-	-	-	-	C
Magnesium Chloride	A	A	A	A	A	A	A	A	A	B
Magnesium Sulfate	-	A	A	A	A	A	A	A	A	B
Malic Acid	A	B	B	A	A	A	-	-	-	A
Mercury Salts	A	B	-	A	A	-	-	-	-	B
Methane	A	D	-	A	A	A	-	-	-	A
Methanol (Methyl Alcohol)	A	-	A	C	C	D	A	B	A	A
Methyl Chloride	D	D	D	D	D	A	D	-	A	A
Methyl Ethyl Ketone	C	-	D	D	D	D	D	D	A	A
Mixed Acid	B	B	-	B	D	-	D	-	-	B
Molydenium disulfide	-	B	-	-	-	-	-	-	-	-
Monoethanolamine	B	A	B	-	-	D	A	-	A	A
Naptha	D	D	D	B	B	A	B	-	A	A
Natural Gas	A	D	-	A	A	-	-	-	-	-
Nickel Salts	A	A	A	A	A	-	A	-	A	B
Nitric Acid (dil)	A	A	C	A	A	A	A	B	A	A
Nitric Acid (med conc)	C	-	D	A	B	A	C	C	-	A
Nitric Acid (concentrated)	D	-	D	B	D	-	C	D	D	A
Nitrobenzene	D	D	D	D	D	-	D	D	A	B
Nitrogen Oxides	A	B	D	A	A	-	-	-	-	-
Nitrous Acid	A	A	-	A	A	-	-	-	-	A



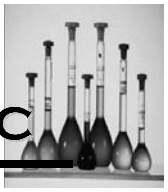
APPENDIX C

Fluid				Tubing				Head Material		
	PN	CF	S	T	TS	V	PSF	PC	PPS	SS
Oils, Animal	A	B	-	D	B	-	-	-	-	A
Oils, Minerals	D	B	D	D	B	A	B	A	A	A
Oils, Vegetable	A	-	A	B	A	-	A	-	A	A
Oleic acid	C	A	D	D	D	B	A	A	A	B
Oxalic Acid (cold)	A	A	B	A	A	A	-	B	A	B
Oxygen (gas)	A	A	B	A	A	-	A	A	-	A
Palmitic Acid										B
Perchloric Acid	D	A	D	D	D	A	D	D	A	-
Perchloroethylene	D	B	-	D	D	A	D	D	A	B
Phenol (Carbolic Acid)	-	D	D	B	B	-	-	D	-	B
Phosphoric Acid > 50%	A	A	-	A	A	A	A	B	-	A
Phthalic Acid	A	D	B	A	A	-	-	-	-	B
Plating Solutions	A	B	D	A	A	A	-	-	-	A
Polyglycol	B	B	A	A	A	-	-	-	-	-
Potassium Carbonate	B	A	-	A	A	-	A	-	A	B
Potassium Chlorate	B	A	B	A	A	-	A	-	A	B
Potassium Hydroxide (caus)	A	A	B	B	B	B	A	D	A	B
Potassium Hydroxide (med)										B
Potassium Iodide	B	A	-	A	A	-	-	-	-	A
Propyl Alcohol	C	-	A	D	D	B	B	A	A	A
Pyridine	C	A	D	D	D	D	D	D	A	A
Silicone Oils	A	A	-	B	B	A	-	A	A	A
Silicone Fluids	A	A	-	B	B	-	-	-	A	A
Silver Nitrate	A	A	A	A	A	A	A	A	A	B
Soap Solutions	A	A	B	A	A	A	A	A	A	A
Sodium Bicarbonate	A	A	A	A	A	A	A	A	A	B
Sodium Bisulfate	A	A	-	A	A	-	A	A	A	B
Sodium Bisulfite	A	A	A	A	A	A	-	A	-	B
Sodium Borate	A	A	A	A	A	A	A	A	A	B
Sodium Carbonate	A	A	A	A	A	B	A	A	A	A
Sodium Chlorate	A	A	C	B	B	-	A	A	A	B
Sodium Chloride	A	A	B	A	A	A	A	A	A	C
Sodium Ferrocyanide	A	A	-	B	B	-	-	-	-	-
Sodium Hydrosulfite	B	A	-	A	A	-	-	-	-	-
Sodium Hydroxide (dil)	A	A	A	B	D	A	A	D	A	A
Sodium Hydroxide (20%)										A
Sodium Hydroxide (conc)	-	C	-	A	D	-	-	D	A	C
Sodium Hypochlorite (<5%)	B	A	B	B	B	A	A	-	A	A
Soduim Hypochlorite (>5%)	C	A	B	B	B	B	A	-	A	C
Sodium Nitrate	B	A	D	A	A	-	-	-	A	B
Sodium Silicate	A	A	A	A	A	A	A	-	A	B
Sodium Sulfide	A	A	A	A	A	-	A	-	A	C

APPENDIX C



Fluid	Tubing						Head Material			
	PN	**CF**	**S**	**T**	**TS**	**V**	**PSF**	**PC**	**PPS**	**SS**
Sodium Sulfite	A	A	A	A	A	-	-	D	-	A
Steam	C	-	A	D	D	B	A	A	A	A
Stearic Acid	A	A	B	A	A	-	C	A	-	-
Sulfuric Acid (med)	A	A	D	A	B	A	B	C	A	D
Sulfuric Acid (conc)	A	A	D	A	B	A	B	C	A	C
Sulfuric Acid (dil)	A	A	D	A	B	A	A	A	A	D
Sulfurous Acid	A	A	D	A	A	A	A	-	A	B
Tannic Acid	A	A	B	A	A	-	A	-	A	B
Tanning Liquors	A	B	-	A	A	-	-	-	-	A
Tartaric Acid	-	A	A	A	A	A	A	B	A	C
Tin Salts	B	A	B	A	A	-	-	-	-	-
Toluene (Toluol)	D	D	D	D	D	A	D	D	A	A
Trichloroacetic Acid	D	A	D	B	D	C	-	D	A	D
Trichloroethylene	D	D	D	D	D	A	C	D	A	B
Trisoduim Phosphate	A	A	-	A	A	A	-	-	A	A
Turpentine	C	D	D	B	A	A	-	-	A	A
Urea	A	A	B	A	A	-	C	D	A	A
Uric Acid	A	A	-	A	A	-	C	D	A	A
Water, Fresh	A	A	B	A	A	A	A	A	A	A
Water, Salt	A	A	A	A	A	A	A	A	A	B
Xylene	D	D	D	D	D	B	D	D	A	A
Zinc Chloride	B	A	B	A	A	A	A	A	A	D



A safe and desirable alternative to using the Autodilution system with a nonaqueous solvent is to convert the Nicomp to disposable-cell operation. This is easily carried out by removing the flow-through cell from the cell holder (by unscrewing the four thumb screws), moving it to one side away from the scattered light detection path, reassembling the cell holder and dropping into the latter the black-anodized adapter for the disposable 6-mm cylindrical glass tubes or 1 cm glass cuvette. Following this procedure, the Nicomp is now compatible with virtually any solvent that is compatible with glass.

A second requirement when using a solvent other than water concerns the accuracy of the measurement. The same principles of light scattering, diffusion and autocorrelation hold true regardless of the solvent used. However, substitution of new values into the Input menu must be made for the physical parameters of the solvent: viscosity, η and index of refraction, n. In general, the former is highly temperature dependent, so that the value entered must correspond to the running temperature chosen. Often the reverse is true: a value for the viscosity is known for a particular temperature, (which becomes the basis for choosing the latter.) The index of refraction, on the other hand, has only a slight temperature dependence, which can be ignored.

A useful expression which shows the dependence of the computed particle diameter, d, on the solvent viscosity, η, and its index of refraction, n:

$$d = \text{constant X } (n^2/\eta)^{\tau} \qquad \text{(C-1)}$$

where τ is the characteristic decay time of the autocorrelation function (assuming a single particle size and a single decaying exponential function).

Equation C-1 may prove to be very useful when a size measurement using a solvent for which values for both η and n are unavailable at the time of the run. One may then assume the values for water, run the sample and correct the resulting diameter distribution plots later, when the correct values have been located. All size values (e.g. diameter "bin" values, peak diameter locations, mean diameters, etc.) on the plots can be scaled up or down to the correct values using the following equation obtained for Equation C-1:

$$d = (\eta_W /8\eta_S)\,(n_S/n_W)\,d_W \qquad \text{(C-2)}$$

Here, η_W and η_S are, respectively, the viscosities of water and actual solvent used (at the temperature at which the measurement is performed); n_W and n_S are, respectively, the indices of refraction of water and the solvent. The original diameter values on the printouts are denoted by d_W: the final, desired values are given by d_S above.

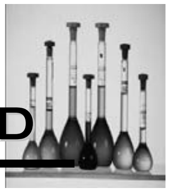
APPENDIX C

In Appendix D a list of some of the more commonly used organic solvents with their viscosities at one or more temperatures as well as their approximate indices of refraction (again, whose T-dependence can be ignored) has been provided. To operate at some different, unlisted temperature, the correct viscosity value can be estimated by linear interpolation or extrapolation. The values in Appendix D were obtained from the following references:

1. R.C. Weast, Ed., CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, FL (e.g. 1982 -- 63rd Ed.).
2. J. Timmermans, Physico-Chemical Constants of Pure Organic Compounds, Vols. I and II, Elsevier, New York (1950).

> **Important**: Exercise adequate CAUTION whenever using organic solvents. In particular, be aware of the fact that many solvents are highly FLAMMABLE and constitute a FIRE HAZARD. They are often extremely volatile; hence, all other considerations being equal, measurements should generally be carried out at REDUCED TEMPERATURES -- e.g. 15 or 20^{o}C. Also, take necessary PRECAUTIONS to ensure adequate VENTILATION exists in the area where such solvents are used; in most cases, the FUMES can be assumed to be TOXIC, dangerous to the eyes, skin and internal organs. Also, spillage of such organic fluids may remove the paint on the instrument cabinet and otherwise cause damage within the Nicomp.

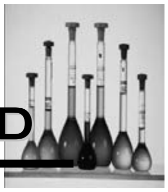
APPENDIX D



SOLVENT	TEMP (C)	VISCOSITY (cpoise)	INDEX REFRACTION
Acetaldehyde	10	0.256	1.332
	20	0.220	1.332
Acetic Acid	15	1.31	1.380
	25	1.16	1.380
	41	1.00	1.380
	59	0.70	1.380
Acetone	15	0.337	1.357
	25	0.316	1.357
	41	0.280	1.357
Acetonitrile	15	0.375	1.346
	25	0.345	1.346
	30	0.325	1.346
n-Amyl acetate	11	1.58	1.400
	45	0.805	1.400
n-Amyl alcohol	15	4.65	1.410
	30	2.99	1.410
n-Amyl ether	15	1.188	1.410
Aniline	15	5.31	1.583
	25	3.71	1.583
	35	2.71	1.583
Benzaldehyde	25	1.39	1.544
Benzene	20	0.652	1.498
	30	0.564	1.498
	40	0.503	1.498
Benzonitrile	15	1.45	1.526
	25	1.24	1.526
	30	1.11	1.526
Benzyl Alcohol	20	5.80	1.538
	30	4.65	1.538
Benzyl amine	25	1.59	1.540
Bromoform	15	2.152	1.587
	25	1.89	1.587

APPENDIX D

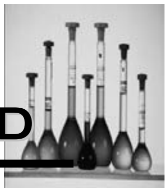
SOLVENT	TEMP (C)	VISCOSITY (cpoise)	INDEX REFRACTION
n-Butyl acetate	20	.732	1.372
	40	.563	1.372
n-Butyl alcohol	20	2.948	1.400
	30	2.3	1.400
	40	1.782	1.400
Carbon disulfide	20	0.363	1.628
	40	0.330	1.628
Carbon tetrachloride	15	1.038	1.459
	20	0.969	1.459
	30	0.843	1.459
	40	0.739	1.459
Chlorobenzene	15	0.900	1.523
	20	0.799	1.523
	40	0.631	1.523
Chloroform	20	0.58	1.444
	30	0.514	1.444
	39	0.500	1.444
Cyclohexane	15	1.06	1.426
	30	0.82	1.426
Cyclohexanol	20	68.0	1.456
	30	41.1	1.456
Cyclohexanone	15	2.45	1.450
	30	1.80	1.450
Cyclohexene	13.5	0.696	1.445
	20	0.66	1.445
Cyclopentane	13.5	0.493	1.404
n-Decane	20	0.920	1.409
	25	0.853	1.409
N,N-Dimehtylformamide	20	0.84	1.427
(="DMF")	25	0.80	1.427



SOLVENT	TEMP (C)	VISCOSITY (cpoise)	INDEX REFRACTION
Dimehtylaniline	20	1.41	1.558
	30	1.17	1.558
	40	1.04	1.558
n-Dodecane	25	1.35	1.415
Ethyl Acetate	15	0.473	1.380
	20	0.455	1.380
	25	0.441	1.380
	30	0.400	1.380
Ethyl alcohol	20	1.200	1.359
(=Ethanol)	30	1.003	1.359
	40	0.834	1.359
Ethyl benzene	15	0.697	1.495
	30	0.581	1.495
Ethyl bromide	15	0.418	1.424
	20	0.402	1.424
	30	0.348	1.424
Ethyl ether	20	0.233	1.352
	25	0.222	1.352
	40	0.197	1.325
Ethyl formate	15	0.419	1.361
	30	0.358	1.361
Ethylene bromide	17	1.950	1.538
	20	1.721	1.538
Ethylene dichloride	15	0.887	1.445
	30	0.730	1.445
Ethylene glycol	20	19.90	1.431
	30	13.35	1.431
	40	9.13	1.431
	60	4.95	1.431
Formamide	25	3.30	1.446
Formic acid	20	1.804	1.371
	30	1.465	1.371
	40	1.219	1.371

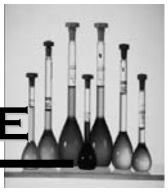
APPENDIX D

SOLVENT	TEMP (C)	VISCOSITY (cpoise)	INDEX REFRACTION
n-Heptane	20	0.409	1.388
	25	0.386	1.388
	40	0.341	1.388
n-Hexadecane	20	3.45	1.433
n-Hexane	20	0.326	1.375
	23	0.3068	1.375
	25	0.294	1.375
	40	0.271	1.375
Isobutyl alcohol	15	4.703	1.397
	30	2.876	1.397
Isopentane	20	0.223	1.355
Isopropyl alcohol	15	2.86	1.385
	30	1.77	1.385
Methyl acetate	20	0.381	1.380
	40	0.320	1.380
Methyl alcohol	20	0.597	1.326
(= Methanol)	25	0.547	1.326
	30	0.510	1.326
	40	0.456	1.326
Methyl ethyl ketone	15	0.423	1.379
(= "MEK")	30	0.365	1.379
Methyl formate	15	0.360	1.346
	25	0.328	1.346
Methylene dichloride	15	0.449	1.424
	30	0.393	1.424
Nitrobenzene	15	2.24	1.550
	20	2.03	1.550
Nitromethane	25	0.620	1.380
o-Nitrotoluene	20	2.37	1.547
	30	1.91	1.547
	40	1.63	1.547



SOLVENT	TEMP (C)	VISCOSITY (cpoise)	INDEX REFRACTION
m-Nitrotoluene	20	2.33	1.545
	30	1.77	1.545
	40	1.60	1.545
p-Nitrotoluene	60	1.204	1.533
n-Octane	20	0.542	1.395
	40	0.433	1.395
Pentane	20	0.240	1.357
Propyl acetate	20	0.59	1.382
	40	0.44	1.382
n-Propyl alcohol	20	2.256	1.385
	30	1.72	1.385
	40	1.405	1.385
1,1,2,2-Tetrachloroethan	15	1.844	1.494
Toluene	20	0.590	1.494
	30	0.526	1.494
	40	0.471	1.494
Trichlorethane	20	1.20	1.438
o-Xylene	16	0.876	1.506
	20	0.810	1.506
	40	0.627	1.506
m-Xylene	15	0.650	1.495
	20	0.620	1.495
	40	0.497	1.495
p-Xylene	16	0.696	1.493
	20	0.648	1.493
	40	0.513	1.493

APPENDIX E



ESTIMATING MOLECULAR WEIGHT

It is possible to use the Nicomp to estimate the average molecular weight, M^W, of particles or macromolecules suspended in solution. The word "estimate" is emphasized because of the fact that the measurement of molecular weight using the technique of dynamic light scattering (DLS) is not as accurate as the determination of particle size, for which all DLS-based instruments are primarily designed.

The fundamental quantity measured in a DLS-based instrument is the particle diffusivity, or diffusion coefficient, D. There is a simple empirical formula that can be used to relate the molecular weight, M^W, of a suspended particle (or macromolecule) to its diffusivity, D:

$$D = \propto M^{M\ -ß} \qquad \text{(E-1)}$$

The pre-factor constant, $\propto$, is related to the specific composition of both the diffusing particles and the surrounding solvent. Constant ß in the exponent is related to the shape/configuration of the particles and macromolecules (which may also be a function of particle and solvent composition, as in the case of polymers.

The form of Equation E-1 can be motivated by considering the simple case of solid spherical particles of uniform radius R. How is it related to M^W for such ideal particles? Using the Stokes-Einstein relation, D can be related to R:

$$D = (kT/6\Pi\eta)\ (1/R) \qquad \text{(E-2)}$$

which can be re-written:

$$D = C_1/R \qquad \text{(E-3)}$$

where C_1 is a constant: $C_1 = kT/6\Pi\eta$

For particles for a given density, molecular weight M_W is proportional to the particle volume:

$$M_W = C(4/3)\Pi R^3 \qquad \text{(E-4)}$$

where C is a constant of proportionality, related to the mass density of the particles.

It is convenient to absorb all of the components above into a new constant, C_2, given by $C(4/3)\Pi$:

$$M_W = C_2R^3 \qquad \text{(E-5)}$$

The expression for radius, R, can now be solved,

$$R = (1/C_2)^{1/3}\, M_W^{1/3} \qquad \text{(E-6)}$$

By inserting this expression in for R using equation D-3 it is possible to obtain a relationship which connects D and M_W:

$$D = C_1\, (C_2)^{1/3}\, M_W^{-1/3}$$

$$\text{or, } D = C_3 M_W^{-1/3} \qquad \text{(E-7)}$$

where single constant C_3 and C_1 and C_2: $C_3 = C_1(C_2)^{-1/3}$

Equation E-7 has exactly the form which was claimed earlier, in Equation E-1, where $\propto = C_3$ and ß = 1/3. The prefactor depends on the temperature T and solvent viscosity η (through constant C_1), as well as the mass density of the particles (through constant C_2). Exponent ß depends on the relationship between the hydrodynamic radius (R in Equation E-2) and the molecular weight; for simple spheres, ß equals 1/3.

Exponent ß has been computed theoretically for other particle "shapes"; for example, ß = 1/2 for "random-coil" molecules, and ß = 1 for "rigid-coil" molecules.

From Equation E-1, a simple, general expression for the molecular weight, M_W, as a function of the diffusivity, D:

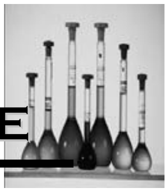
$$M_W = (\propto/D)^{1/\text{ß}} \qquad \text{(E-8)}$$

In the Nicomp, M_W is computed from Equation E-8 using the mean diffusivity D (intensity-weighted) obtained from the simple Gaussian Analysis. Contents $\propto$ and ß are obtained for the Input Menu. In order to obtain reliable estimates of the mean molecular weight of the particles, reliable estimates of constants $\propto$ and ß must be available. These quantities are related to the Mark-Houwink equation for the intrinsic viscosity, [η], of (dilute) solutions containing suspended particles or macromolecules, given by

$$[\eta] = C_4 M_W^{a} \qquad \text{(E-9)}$$

where constants C_4 and a are, respectively, a prefactor and exponent.

Equation E-9 provides one operational method for obtaining $\propto$ and ß for a given particle\solvent system. Suppose one has available several samples of the same composition but different, known



molecular weights. One can then measure and plot the experimental value of $[\eta]$ as a function of the known M_W values; the slope of a plot of log $[\eta]$ as a function of the known M_W values; the slope of a plot of log $[\eta]$ vs. log M_W yields the exponent a. The desired constant ß can then be obtained from exponent a using the equation,

$$\text{ß} = (a+1)/3 \qquad \text{(E-10)}$$

Constant ∝ can be obtained for a measurement of the diffusivity D for one of the known samples, using Equation E-1.

As an alternative to measuring the intrinsic viscosity, one could simply measure D for related ("homologous") series of particles of macromolecules of known M_W. From Equation E-1, we have,

$$\log D = \log \propto - \text{ß} \log M_W \qquad \text{(E-11)}$$

A plot of D vs. log M^W should yield a straight line; the slope is equal to (negative)ß, and the intercept yields log∝, from which ∝ is obtained.

If a series of related samples of known molecular weight is not available, consult the physical-chemical literature to locate values of ∝ and ß for the particle/solvent system under investigation. Values for a few representative polymer/solvent systems are listed below:

POLYMER	SOLVENT	T(^{o}C)	∝	ß
Poly Acrylamide	**Water**	**20**	8.46 x 10^{-4}	**0.69**
Poly acrylonitrile	**DMF**	**25**	3.2 "	**0.63**
	DMF	**35**	2.19 "	**0.58**
poly vinyl acetate	**MEK**	**20**	7.8 "	**0.63**
poly styrene	**MEK**	**25**	3.1 "	**0.53**
poly isoprene	**Chloroform**	**20**	3.5 "	**0.42**
poly methyl- methacrylate	**Ethyl Acetate**	**20**	1.61 "	**0.48**
poly acronitrile				
poly vinyl alcohol	**Water**	**20**	5.5 x 10^{-10}	**0.68**
poly 1-hexane sulfone	**Acetone**	**20**	3.0 x 10^{-3}	