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INSTRUMENTATION

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A Light Intensity Measurement System for the Analytical Ultracentrifuge

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Abstract Light intensity measurements are required by absorbance, fluorescence, turbidity and low-angle light scattering detectors in order to determine the concentration distributions encountered in analytical ultracentrifugation. By using four fast analog-to-digital converters operating in parallel, a data acquisition system has been developed for the analytical ultracentrifuge that can acquire light intensity readings from three detectors (e.g. photomultipliers, avalanche photodiodes, etc.) simultaneously. For each detector, up to forty thousand intensity readings are acquired during each rotor revolution, for up to ten revolutions. Software synchronizes data acquisition with the spinning rotor. The use of continuous light sources allows simultaneous acquisition of dozens of intensity readings from all of the samples. Data acquisition is fast, allowing rapid radial scanning of samples. This data acquisition scheme is used in the Aviv Biomedical AU-FDS fluorescence detection retrofit system for the Beckman XLI analytical ultracentrifuge. It also will be used for the updated XLI absorbance system, as well as the next generation of analytical ultracentrifuge.

Keywords Absorbance detector · Analytical ultracentrifugation · Detector systems · Fluorescence detector · Instrumentation software

Introduction

Measurement of the radial concentration distribution in a sample as a function of time is central to analytical ultracentrifugation. Amongst the optical systems used to measure concentration distributions, absorbance, fluorescence and light scattering detectors require measurements of the light intensity either passing through or emanating from a sample.

Data acquisition from the ultracentrifuge poses two particular problems. First, signal acquisition must be synchronized with the spinning rotor and, second, some means must be provided to isolate the signals from each sample. The standard Beckman Coulter XLI analytical ultracentrifuge solves the first problem by using a pair of small magnets on the rotor and a Hall effect sensor mounted on the chamber bottom to produce two closely spaced pulses, one positive going and the other negative going (Fig. 1), with each turn of the rotor. A single TTL pulse is generated at the zero-crossing point between the positive and negative Hall effect pulses, and the leading edge of this TTL pulse is used to synchronize the XLI data acquisition systems. The second problem is solved by using a pulsed light source that is triggered when a sample is aligned with the detector. Timing of the trigger signal is accomplished in hardware using counters and a fast clock as described previously [1]. In this scheme, the rotor timing signal must be jitter-free to better than one part in 4000, or else data quality suffers from poor timing of the light pulses [2]. Propagation delays in the electronics that generate the rotor timing pulse, in the clocks that produce the strobe pulse, and in the light sources themselves, require that the synchronizing systems provide rotor speed-dependent timing

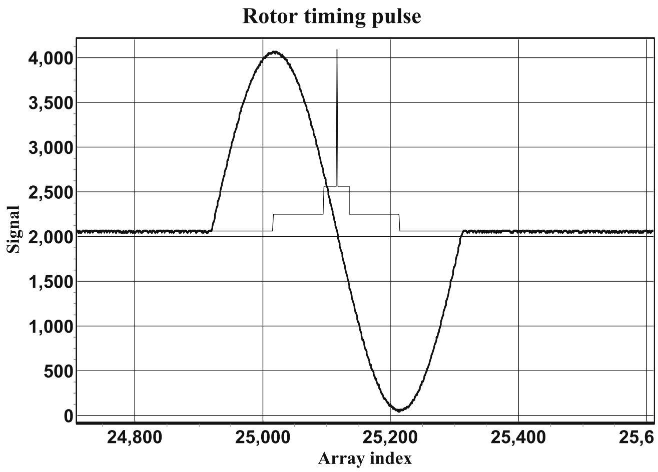

Fig. 1 Rotor timing pulse. Data are shown as the A/D values (0–4095, corresponding to ±1.0 volts) as a function of the array index (*solid line*), for a span of ∼ 20° of rotation. The *dotted line* shows the AverageSignal (∼ 2047), with *two steps* above the AverageSignal. The *first*, wider *step* shows the value of the PulseDiscriminator (= AverageSignal + 200). For clarity, this step covers the range of data between the positive and negative peaks in the rotor timing signal. The *second*, higher *step* shows the value used for the PeakDiscriminator (= AverageSignal + 300). The *spike* in the data shows the position calculated for the midpoint between the two peaks. Data were simulated for a signal to noise ratio of 40 dB

adjustments [1, 2]. Additional complications arise from the frequency dependence of the signals and propagation delays, for which the synchronizing system must compensate [1]. Given this complexity, the hardware based strobe light system of the XLI is remarkably reliable.

Despite its reliability, the current system limits the XLI absorbance data acquisition systems to one light pulse per rotor revolution. Furthermore, the maximum repetition rate for the XLI's high intensity Xenon light source is 100 Hz, so that 10 ms must elapse between light bursts. At rotor speeds above 6000 rpm, the 10 ms hiatus limits the rate at which data may be acquired, and at 60 000 rpm the light is pulsed only once for every 10 rotor revolutions. While similar lamps are available with slightly higher repetition rates (300 Hz), it is not feasible to construct lamps of this intensity that will operate at suitably higher repetition rates (> 1000 Hz). Even if such lamps were available, data acquisition is slowed by the fact that only one sample is illuminated per light pulse. If the XLI's absorbance system is being operated in the usual double-beam mode, then a second light pulse is required to illuminate the reference channel. At 60 000 rpm, a minimum of 20 rotor revolutions is required to gather the data for one absorbance reading for each sample. If the user chooses to average multiple intensity readings in order to improve data precision, the time required per reading increases proportionally.

As currently configured, the XLI performs a complete radial scan of one sample before resetting and starting to scan a second sample. At a radial spacing of 50 microns, and using one sample and one reference intensity per radial position, a radial scan of 1.3 cm length may be completed in 20–25 seconds, with the interval between scans being about 30 seconds when the time needed to reset between scans is included. For an experiment with seven samples, each sample will be scanned approximately every 3 1/2 to 4 minutes. This is a minimum time interval per scan. If the user chooses to acquire data at multiple wavelengths, or increases the number of intensity readings by either decreasing the radial spacing or increasing the number of intensity readings per radial position, the time interval between scans of a sample increases. The compromise between data quantity and data quality fundamentally limits the usefulness of the absorbance system for sedimentation velocity analysis.

One means to speed up data acquisition is to use a continuous light source, and to separate the signals from the different samples by synchronizing the detector with the spinning rotor. In this sort of scheme, those portions of the detector signal corresponding to the moments when a particular sample is in the light beam are separated, e.g. by a de-multiplexing circuit. The analog absorbance system of the Beckman Model E used this scheme to acquire data from one sample at a time. More recently, the turbidity and schlieren systems have used an analogous scheme to acquire intensity data from all of the samples with each rotor revolution [3, 4].

The system presented here uses four parallel high-speed analog-to-digital (A/D) converters to acquire data simultaneously from four separate detectors. The signal from the XLI Hall effect detector is one of the signals, and light intensity data from the absorbance, fluorescence or turbidity optical systems are the other signals that may be digitized. The software both synchronizes data acquisition with the spinning rotor and isolates the signals from each sample. With this system, it is possible to average the data for all of the detectors over several rotor revolutions for each of the samples simultaneously. Furthermore, the software can be configured to acquire data from new rotor and cell designs easily.

Description

Signals: There are four signal sources that require high-speed data acquisition: the rotor timing pulse and three light intensity detector voltages. A less expensive interface handles the lower speed analog and digital signals used to monitor device status (e.g. the presence of various optical systems, the fluorescence laser power and temperature, etc.) or control devices (e.g. fluorescence laser on/off, PMT voltages). In addition to the two interface cards, two RS-232 serial I/O ports are required. The first serial port provides communication with the XLI and is used both to control its operation and to monitor its status. The second serial port controls the stepping motors used in the optical systems. Up to six stepping motors may be present: two for the fluorescence hardware (radial position and objective lens focus), two for the absorbance system (radial position and wavelength selector) and two for the refractive detectors (camera position and schlieren bar angle). Hardware and software are included to determine which optical systems are present so that the stepping motor functions are configured properly. The software controlling the instrument uses a low level hardware abstraction layer. That is, the software is written so that function calls from common programming languages (e.g. C++, Visual Basic) remain unchanged even if the underlying hardware changes.

Hardware: Voltages from the four high speed sources are digitized using four 12-bit, 20 MHz A/D converters (PCI-DAS4020/12, Measurement Computing, Inc., Middleboro, MA, USA). This device also provides two 12-bit digital-to-analog (D/A) outputs and 24 digital input-output (DIO) lines. An internal first-in-first-out buffer memory accommodates computer latencies (e.g. hardware memory refresh, software interrupt handling) thus allowing sustained data acquisition at the full data rate. The ± 1 volt input range was used for all four A/D channels. The digitized data streams are stored as interleaved values in the computer memory as: $I_{1,1}$, $I_{2,1}$, $I_{3,1}$, $I_{4,1}$, $I_{1,2}$, $I_{2,2}$ ... $I_{1,n}$, $I_{2,n}$, $I_{3,n}$, $I_{4,n}$, where the first index refers to which A/D produced the signal and the second index indicates which reading this is in the sequence.

When fresh data are desired, the software triggers data acquisition from all four high-speed A/Ds. Once triggered, a sufficient number of data points (TotalCount) are gathered to cover several turns of the rotor, RevolutionsToAverage. The operating software adjusts the A/D clock frequency (ADClockRate) and acquisition period (TotalPeriod) so that each detector will acquire at least 10 000 values per rotor revolution (CountsPerRevolution). After each data acquisition period, fresh values ADClockRate, TotalPeriod and TotalCount are calculated so that rotor acceleration/deceleration may be accommodated. Following data acquisition, four separate data arrays are created from the interleaved data, one for each A/D channel.

A separate device (PCI-DAS6025, Measurement Computing, Inc., Middleborough, MA, USA) is used as the low-speed interface and contains 16 channels of 12-bit A/D input (at 200 KHz), two 12-bit D/A outputs, 8 DIO lines and two 16-bit counters.

All signals to and from the computer are connected to a custom-designed "system box" that contains the amplifiers, power supplies, digital signal buffers, a programmable clock and custom analog signal conditioning circuits to accommodate five optical systems (absorbance, fluorescence, turbidity, interference and schlieren). Signal conditioning includes programmable gain amplifiers that provide 1, 2, 4 or 8-fold gain for each of the light detector signals, as well as a precision voltage source used to offset the signals so that zero light intensity corresponds to -1 volt and saturating light corresponds to $+1$ volt. All of the components in the signal pathways have a frequency response > 30 MHz.

A single wiring harness connects the system box to a 100-pin connector on the XLI. A custom-designed "distribution box" within the XLI contains power supplies, the laser controller for the fluorescence optics, circuits to buffer the rotor timing pulse and vacuum signals, circuits to detect which optical systems are present, and connectors to direct the various signals to the different optical subsystems. Throughout, careful circuit layout is needed to minimize stray noise, crosstalk and ground loops.

The rotor timing pulse: All data acquisition must be synchronized to an accurate rotor timing pulse. Previous data acquisition systems use the rotor timing pulse to trigger data acquisition hardware, with subsequent transfer of the data to the computer [1–3]. The system described here acquires data asynchronously, with post-acquisition processing used to extract the light intensity signals.

One of the four high speed A/Ds monitors the rotor timing signal from the Hall effect sensor (Fig. 1). The signal is amplified to provide ± 0.95 volts when a magnet passes over the sensor, and otherwise is nominally 0 volts. One magnet produces a positive going pulse and the other a negative going pulse, with each pulse occupying ~ 18 degrees of the rotor revolution, and the two pulses being separated by ~ 20 degrees.

Because data acquisition is triggered asynchronously, rotor timing pulses may fall anywhere in the data acquisition time period. Furthermore, until ADClockRate, TotalPeriod and TotalCount are set correctly, an unknown number of rotor timing pulses may be present in the data. Thus, two situations arise: one where these parameters have not yet been determined and, more commonly, one where good estimates of these parameters exist.

When the software is first started, the rotor speed is unknown (i.e. the XLI may or may not be operating when the software is started), and default values for ADClockRate, TotalPeriod, TotalCount and RevolutionsToAverage are used. The default values for these parameters are set to acquire 20 000 data points (e.g. TotalCount = 20 000) for a TotalPeriod covering seven rotor revolutions (RevolutionsToAverage +2) at 1000 rpm. These defaults allow the system to detect rotor spinning at low rpm (e.g. during initial rotor acceleration), while still gathering data at sufficient resolution to determine an approximate rotor speed should the rotor already be spinning at 60 000 rpm. Two other default values are set: the number of clock pulses per rotor revolution (CountsPerRevolution, as TotalCount divided by the number of revolutions) and the number of clock pulses (MagnetPulseWidth) corresponding to the width (approximately 18 degrees) of one of the two peaks from the Hall effect sensor.

The software uses two values to discriminate a rotor timing pulse from background noise. The first (PulseDiscriminator) is the minimum signal amplitude required to consider the signal at any point (Signal[i]) in the data array to be part of a rotor timing pulse. The second (PeakDiscriminator) is the minimum amplitude of Signal[i] required to consider it the peak value. Comparisons are made as the absolute value of the difference between the Signal[i] and the average signal (AverageSignal, default = 2048, corresponding to 0 volts) calculated for a portion of the data that does not contain a rotor timing pulse (calculated as described below). The default values of PulseDiscriminator and PeakDiscriminator are 200 and 300, respectively. These values are stored in a database and may be changed if necessary.

The rotor timing peak finding algorithm begins by determining whether or not there is rotor timing pulse at the start of the Signal array (i.e. data acquisition was triggered when a rotor timing pulse was occurring). Data are tested for Signal[i], for i = 0 to i = twice the MagnetPulseWidth to determine if the magnitude any data point exceeds the AverageSignal. If so, the starting index for peak finding (StartIndex) is set to one-half the CountsPerRevolution to avoid dealing with partial rotor timing pulses. Because StartIndex is guaranteed to be in a region of Signal[i] devoid of a rotor timing pulse, AverageSignal is calculated as the mean value of Signal[i] from i = StartIndex to i = StartIndex plus twice the MagnetPulseWidth.

Beginning with StartIndex, Signal[i] is scanned to find the first data point having a magnitude exceeding PulseDiscriminator. At this point, StartIndex is set to i, and the portion of the Signal array from StartIndex to four times the MagnetPulseWidth (EndIndex) is searched to find the indices of the maximum and minimum values. To avoid false maxima or minima due to noise, data generated using the "reverse smoothing" algorithm of Roark [5] are searched. The values of Signal[i] corresponding to the indices for the maximum and minimum are tested to see that they exceed PeakDiscriminator, and the two indices are tested to make sure that they are not equal to either StartIndex or EndIndex (indicating that a partial rotor timing pulse is being tested). If the indices pass this test, then the index corresponding to the midpoint between them (calculated as the rounded integer of the minimum and maximum indices cast to be double precision numbers) is considered to be the position of a rotor timing pulse, and this index is added to an array (RotorPulseIndices). Once a pulse has been found, the StartIndex is set equal to the EndIndex and the peak-finding process is repeated so long as the EndIndex is less than TotalCount.

Once the rotor timing pulses have been determined, the CountsPerRevolution is calculated as the rounded integer of the average difference between entries in the RotorPulseIndices array. From CountsPerRevolution, the rotor speed (as rpm) is determined as 60*CountsPerRevolution/ADClockRate. The rotor speed calculated in this manner is accurate to better than ±5 rpm, with a precision of ±2 rpm.

Synchronizing data acquisition to the spinning rotor: The magnets used to produce the rotor timing signal are incorporated in the "over-speed disk" that is glued to the base of each rotor. The over-speed disk is positioned so that the two magnets are approximately midway between the first and last rotor hole (e.g. between rotor holes 1 and 4 for a 4-hole rotor). The magnet position is not exact, and changes every time the over-speed disc is replaced. Consequently, the rotor timing pulse provides only an approximate position, and separate means must be provided to determine the exact relationship between the rotor timing pulse and a reference position on the rotor as it passes a detector (below). Once this angle, MagnetAngle, is established at a particular rotor speed, all other angles needed to acquire data from a particular channel are fixed.

The fixed angles include: the offset angle, called the CellAngle, from the center of the first rotor hole to the center of each of the other rotor holes; the offset angle from the center of a rotor hole to the center of each channel in a centerpiece, called the ChannelAngle; and the angles separating the optical detectors, called the DetectorAngle. Values of the CellAngle are constant for a given rotor type, and are just 360*CN/CT, where CN is the cell number (starting at zero) and CT is the total number of cell holes. ChannelAngle values are constant for any given type of cell, and the angular positions of the XLI optical tracks, measured clockwise from the fluorescence detector, determine the DetectorAngle values. A database is used to store the ChannelAngle values for each type of cell. Similarly, each type of detector has a DetectorAngle value stored in the database. It is possible to accommodate new cell designs or add new detectors without changing the software by defining and storing these fixed angles.

Light intensity data typically are acquired over a small angle (e.g. 0.2 degrees, called the DataOffsetAngle) on either side of the channel center. The starting angle for data acquisition, StartingAngle, is simply the sum all of the fixed angles, plus the MagnetAngle less the DataOffsetAngle.

Determining MagnetAngle: In order to produce high-quality data, it is essential that the angle over which intensity data is gathered is held constant to within a few thousandths of a degree. Because of propagation delays in the electronics, signals arrive at the computer some time after they are produced. During the delay, the rotor continues to turn, so that the fixed geometric angles described above appear to vary with rotor speed [2]. The variation in angle is complex, particularly at rotor speeds $<\sim$ 6000 rpm, due to the frequency dependence of the components in the various signal processing circuits. Accordingly, the MagnetAngle is determined empirically. Experience suggests that the MagnetAngle should be checked whenever the rotor speed changes by 5000 rpm. The operating software monitors the rotor speed and, once the rotor speed is stable, determines the magnet angle for each optical system.

For the fluorescence system [6, and in preparation], a special calibration cell has been developed (www.camis.unh.edu) that incorporates a two-degree long by 1 mm high by 1 mm deep groove. The groove is centered at a radial position of 5.85 cm with a ChannelOffset of zero degrees. This groove is filled with a dye so that a bright fluorescent signal is produced whenever the sample is in the excitation beam. The MagnetAngle is determined as the apparent angle needed to move the "image" of the groove so that it coincides exactly with the angle predicted from the fixed angles (above). A similar scheme is used for the absorbance system, except that the image used is that of the pair of intensity pulses that occur whenever the inner calibration holes (at 5.75 cm) of the counterweight pass over the absorbance detector.

Intensity measurements: Intensity data are acquired for each channel starting from its StartingAngle and covering an angle equal to twice the DataOffsetAngle. To accomplish this, the StartingAngle and DataOffsetAngle must be converted to the corresponding number of A/D clock pulses (i.e. range of indices in the data arrays). The StartingAngle is converted to an index offset, Offset, which corresponds to the fraction of counts in one revolution: Offset = CountsPerRevolution* (StartingAngle/360). The starting indices for the data arrays are simply the value of Offset added to each of the values saved in the RotorPulseIndices array. Similarly, the range of indices, IndexRange, over which intensity data will be gathered is: IndexRange = CountsPerRevolution* (2*DataOffsetAngle /360).

Typically, the IndexRange is between 10 and 100, meaning that that many intensity readings will be obtained for each A/D channel for each rotor revolution. The software is configured to acquire data from three to ten (default five) rotor revolutions. Thus, each average intensity reading comprises 30 to 1000 individual intensity readings. The upper limit of averaging over ten rotor revolutions maintains relatively rapid radial scanning while providing good reduction of the stochastic noise. There is no reason, however, that the upper limit could not be set much higher (the software limit is 180 scans, and it could be set higher). Our experience is that only a small reduction in stochastic noise can be expected with increased averaging. However, future versions of the software will allow for more averaging.

From the moment the A/Ds complete their data acquisition until the average intensities are stored for all of the samples requires < 20 milliseconds on a 1 GHz computer. The computations are done while the radial positions of the optical systems are changed, which requires 50 milliseconds or more, depending upon the distance moved. Thus, the calculations are not the rate limiting step in data acquisition.

Intensity scans: For mechanical reasons, both the fluorescence and absorbance data acquisition systems scan from the bottom to the top of the cell. The radial increment for the scans is adjustable from 2–50 microns (default = 20 microns). After each step, the fast A/Ds are triggered and intensity data are acquired for the period determined by the RevolutionsToAverage. Using the scheme described above, raw intensity data are available for all of the detectors and all of the cells. However, not all of the data are useful, and some tests must be applied to determine which data to include in the output file for a sample.

The operating software uses five layers of logic to determine whether to include the intensity data from an optical system in the output file for a particular sample. These logic layers may be posed as questions: 1) is the optical system in use, 2) are data being acquired for this sample using the particular optical system, 3) is the radial position in the correct range (e.g. for multi-channel equilibrium cells) for the sample, 4) is the wavelength setting one that is being used for the sample, and 5) are the gain settings for the detector the correct ones for this wavelength? If all of these conditions are true, then the average intensity, along with the standard deviation, is determined and saved in a temporary array. When the scan is completed, the data files are written to disc, and the data displayed in the chart for that sample. All disc and charting operations are done during the period required to reset the detector positions to their starting positions (~ 5 seconds).

Methods

Data simulation: The operating software for the data acquisition system includes a signal simulator which allows the gains, voltage offsets, amplifier slew rates and noise level to be set for each A/D channel. The rotor timing pulse signal is simulated as a single sine wave whose angular extent and amplitude may be set. If the detector is situated at a radial position that is between the top and bottom of a channel, intensity data are simulated initially as a square wave pulse centered at the ChannelAngle for that channel. The amplitude of the pulse is adjusted according to the optical properties (scattering factor, extinction coefficient or quantum yield) and concentration of the solution components in the channel, as well as the gain setting for the A/D channel. For the A/D channels, gain settings are simulated as: $\mathrm{PGA} \cdot e^{((10^{*}V/4095)-10)}$, where PGA is the gain setting of the programmable gain amplifier (1, 2, 4 or 8) for the A/D channel, V is the D/A setting (0–4095) corresponding to the PMT high-voltage setting. The rise and fall times of the square pulse are adjusted for the slew rate for the A/D channel, and white-noise with user-adjustable amplitude (in bits) is added to the signal. In simulation mode, then, the signal to noise ratio may be controlled by the user.

Jitter determination: The effect of noise on synchronizing data acquisition was assessed using the root-mean-square (rms) variation in the difference between the indices determined for each rotor timing pulse (Fig. 1). Any variation in these positions from the correct value directly affects the portion of the signals used to acquire intensity data. Rotor timing pulses were simulated with a fixed noise level, and the average distance (in number of data points) calculated between rotor timing pulses for ten rotor pulses. The jitter was calculated as the variance (in number of data points) for a set of ten average distances, and is reported as parts per thousand of the ratio of the variance to the average distance.

Results and Discussion

Synchronizing data acquisition: Figure 1 shows the signal for a single rotor timing pulse, along with the discriminator values and the array index determined to be the "rotor timing pulse." The index for all of the intensity data during the rotor revolution will be an integer offset from this index. The number of indices, Ni, between this rotor timing pulse, Rtp[i], and the next pulse, Rtp[i+1] corresponds to one turn of the rotor (i.e. Ni = Rtp[i+1] − Rtp[i]). Hence, the angular position to the center of any sample (ChannelAngle) may be converted to an index, ChannelIndex, as:

$$\text{ChannelIndex} = \text{Rtp[i]} + \text{Ni}^{*}\text{ChannelAngle}/360\,.$$

It was found that double precision math, followed by rounding to the nearest integer, was required to minimize jitter. Likewise, any index i between Rtp[i] and Rtp[i + 1], may be converted to an angle using:

$$\text{Angle} = \text{i} - \text{Rtp[i]}/\text{CountsPerRevolution} \cdot 360.$$

Intensity data acquired over ten revolutions of the rotor, then superimposed by converting their indices to angles, are shown in Fig. 2. The variation in the position of the signal pulses is a consequence of the rotor timing signal having a signal to noise ratio of 40 dB. At 40 dB, the uncertainty in the rotor timing pulse position is 0.1 part per thousand (Fig. 3), corresponding to an angular uncertainty of ±37 millidegrees.

Radial scans: Because data may be acquired for all samples simultaneously at each radial position, significantly more data may be acquired for each sample over the course of an experiment. The time required to acquire a complete scan of the samples depends on several factors: the number of revolutions over which data are to be averaged, the rotor speed, the time needed to move the optics to the next radial position, the number of radial data positions sampled, and the time needed to reset the radial scan mechanism to the starting position. At rotor speeds above 40 000 rpm, the system requires approximately 60 seconds to acquire data at 600 radial positions (e.g. at 20 micron intervals over a 1.2 cm radial distance),

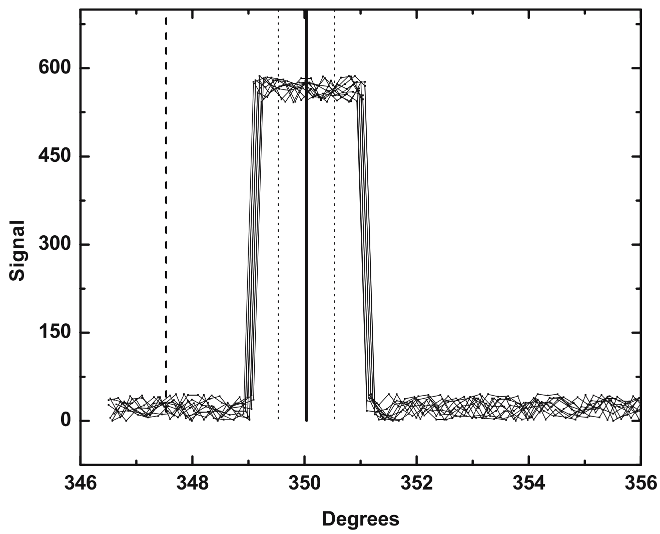

Fig. 2 Simulated raw intensity data acquired from ten revolutions of the rotor. Data were superimposed using the conversion from intensity array index to angle, as described in the text. For data shown here, the rotor timing signal had a signal to noise ratio of 40 dB, and the intensity data shown here have ±40 bits of noise. The *dashed vertical* line shows the angle calculated to be the center of the cell. The solid *vertical line* shows the angle calculated to be the center of the sample channel, and the *two dotted vertical lines* enclose the range of angles that will be used to calculate the average intensity

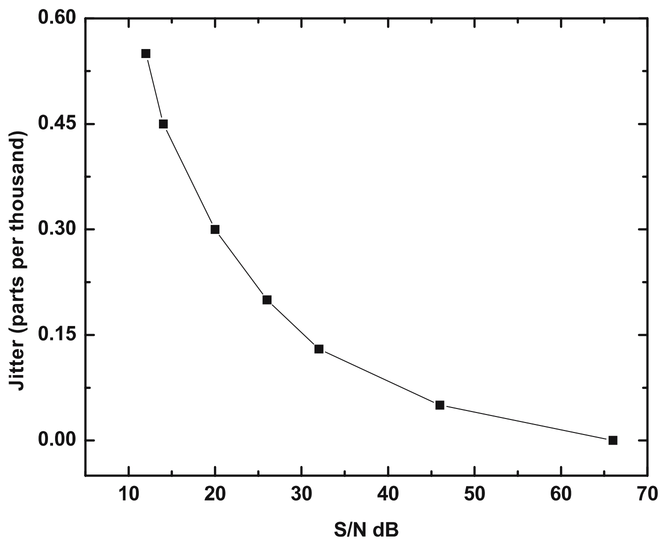

Fig. 3 Jitter as function of the signal to noise ratio of the rotor timing pulse. The jitter is presented in parts per thousand, determined as the root mean square variation in the number of data points between rotor timing pulses (Fig. 1) divided by the average number of data points between rotor timing pulses. Previous work [1] determined that a jitter less than 0.25 parts per thousand provides sufficient precision to acquire data. For the present system, jitter is acceptable so long as the signal to noise ratio is greater than 20 dB

averaging the intensities from 5 rotor revolutions at each radial position (resulting in 50 or so intensity readings per sample), then resetting the optical position to start the next scan. Using the current XLA absorbance system, approximately 720 seconds is required to gather data from three samples at the same radial spacing and averaging 5 flashes of the lamp (i.e. resulting in 5 intensities per sample). Thus, the new system provides significant speed and signal processing advantages over the current XLA absorbance optics. The shorter scan times and the use of a continuous light source in the new data acquisition system results in significantly improved data acquisition from samples containing rapidly sedimenting material.

If samples provide dramatically different signals (e.g. there is $>$ 100-fold concentration difference in fluorescent dye), it will be necessary to use different gain settings in order to optimize the signal to noise. Due to the time needed for the photomultiplier tube to settle down after changing its gain, it is not practical to change the gain at each radial position. Consequently, a separate scan must be acquired for each gain setting needed. This means that the time interval between scans of any given sample will increase in direct proportion to the number of gain settings used in a protocol.

Fluorescence intensity scans of green fluorescent protein are shown in Fig. 4a. The total signal from this sample is about 300 (on a scale from 0 to 4095) and the rms noise is about 5, yielding a signal to noise of 35 dB. For comparison, this is approximately the same signal to noise ratio that would be obtained for absorbance data with an optical density of 0.5 ± 0.01. The apparent molecular weight distribution for this sample is presented in Fig. 4b. The peak at a molecular weight of 29 570 (using an assumed partial specific volume of 0.73 ml/g) is in reasonable agreement with the protein's sequence molecular weight of 30 800.

Conclusion

The light intensity data acquisition system described here offers four advantages over current methods: 1) greater signal averaging, thus reducing stochastic noise; 2) intensity data may be acquired from all samples simultaneously,

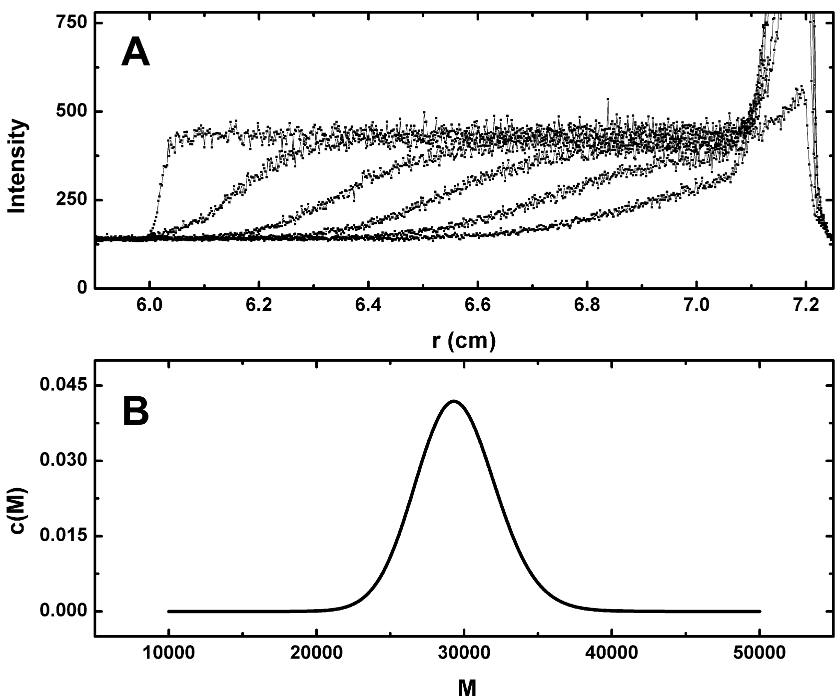

Fig. 4 Fluorescence intensity data from a sedimentation velocity (50 000 rpm, 20.0 °C) experiment. The sample is 40 nM green fluorescent protein ($M = 30\,800$) in 100 mM KCl, 20 mM Tris, pH 7.5. **a** Scans were acquired at approximately 50 second intervals, with scans taken $\sim$ 30 minutes apart shown in this panel. For each scan, intensity data were acquired at 20 micron radial intervals over the range from 5.9–7.3 cm. At each radial position, data from 5 rotations were acquired, yielding 264 individual intensity readings for each sample. The average and standard deviation of these readings is saved in the output file and presented here. All data were acquired using 73% of the maximum photomultiplier tube voltage and an 8× gain setting for the programmable gain amplifier. The photomultiplier tube gain is highly nonlinear, so that a 90% voltage setting is about 2 orders of magnitude more sensitive than the 73% setting. Hence, these data were acquired at a moderate, not high, gain setting. **b** Molecular weight distribution calculated by Sedfit [7] for the data in panel A. The peak in the distribution falls within 4% of sequence molecular weight

increasing the number of scans that may be acquired per experiment; 3) intensity data may be acquired from multiple detectors (e.g. fluorescence and absorbance) simultaneously, thus increasing the amount of information that may be obtained from a sample; and 4) where data are to be acquired (e.g. over which radii and at what angles) may be defined by the user, thus making it possible to use new centerpieces and rotors without changing the data acquisition software.

This system is available commercially in the Aviv Biomedical AU-FDS (Lakeview, NJ) fluorescence detector retrofit for the XLI analytical ultracentrifuge. It will be used for the upcoming rapid-scan absorbance system XLI retrofit, and it is anticipated that it will be used on the next generation of analytical ultracentrifuge. The full characterization of the AU-FDS optical system (e.g. sensitivity, radial resolution, scan times, etc.) will be presented in a separate publication.

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