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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

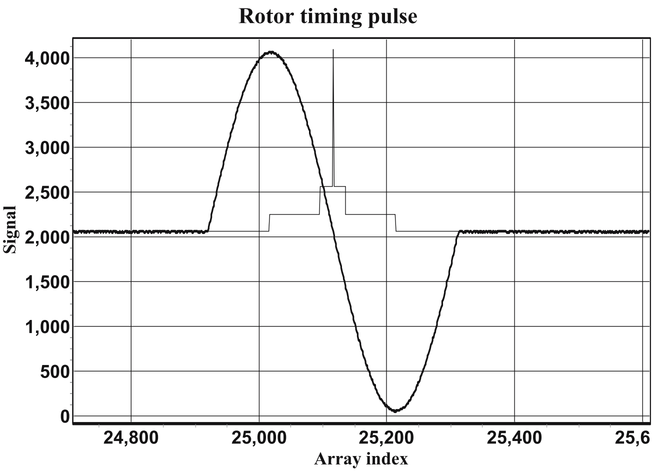

Fig. 1 Rotor timing pulse. Data are shown as the A/D values (0–4095, corresponding to ±1.0 volts) as a function of the array index (*solid line*), for a span of ∼ 20° of rotation. The *dotted line* shows the AverageSignal (∼ 2047), with *two steps* above the AverageSignal. The *first*, wider *step* shows 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