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ARTICLE

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The XL-I analytical ultracentrifuge with Rayleigh interference optics

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Abstract The Optima XL-I analytical ultracentrifuge with integrated Rayleigh interference and ultra-violet/visible optics, and how it provides a modern, convenient platform for acquiring hydrodynamic data, is described. With a choice of optical systems that can be used either alone or in combination, together with an easy to use software control interface, its usefulness in simplifying the analysis of interacting molecular systems and description of particle size distributions is indicated.

Key words Analytical ultracentrifuge · Interferometry

Introduction

Analytical ultracentrifugation in the 1990s has been augmented by the great advances in information technology of the past two decades, and now offers the possibility of new applications in addition to the traditional analyses of molecular weights, self-associating systems, and hydrodynamic shape. These include analyses of particle size distribution (Müller and Herrmann, 1995), heterogeneous associations (Lewis et al., 1995; Kim et al., 1995), and the selection of appropriate associative models for systems analyzed by other methodologies, such as surface plasmon resonance spectroscopy (Hensley, 1996).

To better address these and other applications, Beckman has recently released a Rayleigh Interference optical system for the XL-A analytical ultracentrifuge. This system can be used independently or in conjunction with the existing UV-visible absorption optical system. Dubbed the Optima XL-I, for Integrated optics, this system provides rapid, high-precision data acquisition for samples at a broad range of concentrations. This article will describe the optical system and the PC-based user interface software.

In addition to higher precision and to the ability to measure samples at a broader range of concentrations, Rayleigh optics offer several advantages for work in the analytical ultracentrifuge. The Rayleigh system can be applied to molecular species which do not provide a significant absorbance in the UV-visible range. Unlike absorbance, the refractive index change observed with increasing sample concentrations is fairly independent of the sample identity, providing a better measure of concentration in mixed systems. And unlike previous Beckman refractometric systems, such as the one offered in the Model E, the XL-I system allows both absorption and refractive measurements to be gathered from the same experiment.

Rayleigh optics: creation of interference pattern

The XL-I interference optical system is based upon the interferometer described by Lord Rayleigh in 1896, and originally adapted to analytical ultracentrifugation by Philpot and Cook (1948). An interference pattern is produced by splitting a beam of coherent light and passing it through paired sectors of a cell in a spinning rotor. The speed of the light will be altered as it passes through these sectors, to an extent dependent upon the refractive index. When the beams are merged after passage through the sectors, the waves combine to form an interference pattern. If both sectors are identical in length and contain identical solutions, a pattern of straight interference fringes with be produced when the beams are recombined. However, if one sector contains a reference solvent and the other contains the same solvent as well as the sample of interest, the greater concentration in the sample sector will alter the light vector as it passes through that solution more than will be the case in the reference sector. When these beams are recombined, the fringe pattern will be shifted to an extent that corresponds to the concentration difference between the sectors. The fringe shift is related to the concentration by the clas-

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sical relationship

$$J = a(\Delta n)/\lambda$$

where J is the fringe shift, equivalent to the differential number of wavelengths between the solvent and the solution, a is the length of the light path through the sample or solvent compartments (12 mm for the rotors available for the XL-I), λ is the wavelength of the light, and Δn is the refractive index difference between the solvent and solution. Since the refractive index is generally proportional to concentration, the fringe shift can be used to follow the redistribution of sample as a function of radius in the sample sector. Thus, Rayleigh optics track concentration by means of refractivity, where the sample's refractive index provides the proportionality constant in a manner analogous to absorbance optics, which track concentration by means of optical density, where the proportionality constant is the molar extinction coefficient. An advantage of the refractometric approach is that, for reasonably shallow gradients (Lloyd, 1974), the refractive index shift is linear over a very broad concentration range and does not vary nearly as much does the extinction coefficient among different proteins.

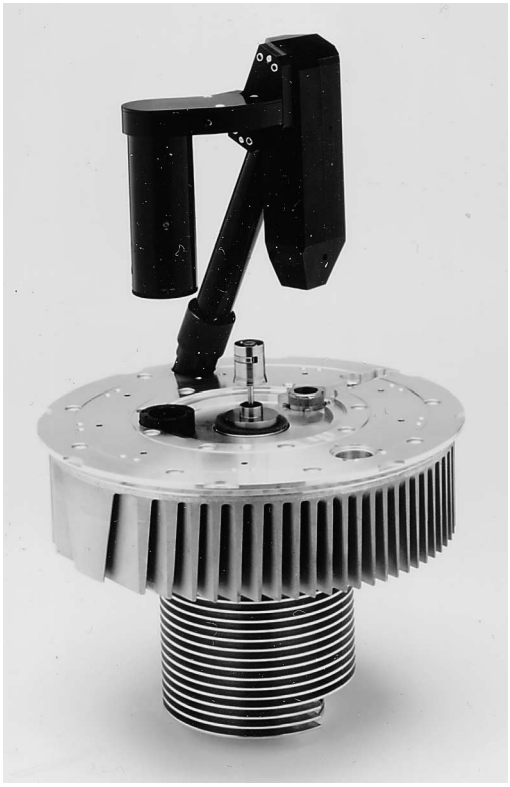

Fig. 1 The XL-I Optical System. The photograph shows the laser light source for the Rayleigh optical affixed to the "periscope", or toroidal diffraction grating which provides monochromatic light for the UV-visible absorption optics. Both systems are shown here mounted on the heat sink which normally resides beneath the floor of the centrifuge chamber

Optima XL-I optical system

Laser

The refractive optics system is designed to be used either independently or in conjunction with the absorbance optical system. The absorbance optics employ a Xenon flash lamp and a toroidal diffraction grating monochromator, which is mounted on the floor of the centrifuge chamber and extends above the rotor in the form of a gantry, commonly referred to as a periscope (shown in Fig. 1). The light source for the Rayleigh system is a 30-mW, 675-nm laser that is mounted on the gantry by means of captive screws. The laser is easily removable for servicing, or to limit exposure to oil vapors when the centrifuge is to be used extensively for absorbance runs.

The laser output is passed through a pair of 0.25-mm slits spaced 4 mm apart. These separate the light into a pair of beams which are then passed through the sample and reference sectors of each cell in the rotor. The slits are machined into a disk that screws into the laser mounting. These disks are removable for cleaning or exchange. For the precise positioning required in order to align the laser and slit assembly, the original XL-A gantry design (Giebeler 1992) has been modified with the addition of a second guide pin where it is mounted into the chamber floor.

Firing of the laser is controlled electronically by the system software. The position of each pair of sample and reference sectors is determined by monitoring rotation of the rotor with a magnet mounted in the rotor body. Unlike the case for absorbance optics in the XL-A, once a radial calibration has been made it is not necessary to dedicate one sample cavity to a reference sector when only the Rayleigh optics are to be used. The system will provide a flash duration corresponding to approximately 1° of rotation at 60,000 rpm. The timing can be adjusted manually or automatically to center the flash within the sample compartment. The software then adjusts automatically for any changes in rotor speed that might be programmed to take place during the course of a run.

Light path

The remaining elements of the optical system are located beneath the floor of the centrifuge can, and are not visible to the user without removal of the front panel. The assembly is designed to permit service access without the need to remove the centrifuge drive. The details of this portion of the optical system are shown schematically in Fig. 2, to which the reader should refer for the following discussion.

The system consists of a tubular light path in which the interacting laser beams are modified by a series of mirrors and lenses. The first lens shown in Fig. 2, the condenser lens, acts to prevent any loss of signal from the system. Light passing through the sample sector may be diverted

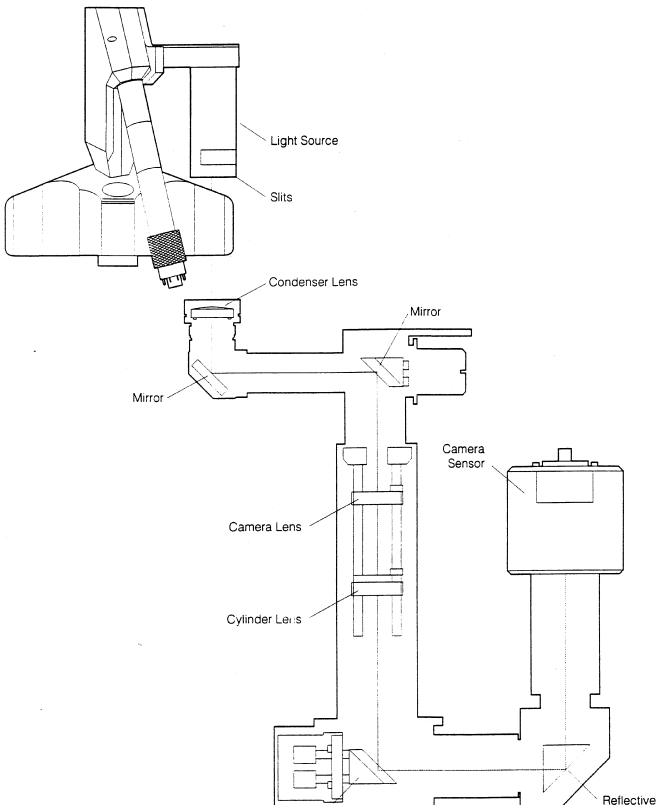

Fig. 2 The XL-I Light Path. The rotor and light source are inside the centrifuge chamber. The heat sink and floor of the centrifuge chamber are situated just above the condenser lens. For a complete description, please see the text

by the refractive index gradient produced by the sample concentration gradient. If this gradient is sufficiently steep, the light may hit the walls of the light path channel and be lost to further analysis. The condenser lens returns divergent beams to a path that will be contained within the tubular light path, so that none of the radial data is lost.

The light is then diverted by a series of mirrors until it impinges upon the camera and cylinder lenses. The camera and condenser lenses focus the image of the sample sector on the CCD camera. A plane ⅔ down the sample compartment is imaged. The cylinder lens combines the beams from the sample and reference cells at the CCD camera to produce the fringe pattern for subsequent analysis. These lenses are provided with focusing knobs, but do not normally require adjustment by the user. None of the other mirrors or lenses require focusing or adjustment by the user.

The entire light path from the condenser lens to the CCD camera is airtight and may be evacuated by means of a port located behind the mirror immediately below the condenser lens. Although this evacuation is possible, it does not appear to be necessary. Comparisons made of data obtained with air or with vacuum in the system have shown no difference in quality. The system is shipped with the light path at atmospheric pressure.

CCD camera

The data is acquired digitally when the interference pattern produced by the cylinder lens impinges upon the elements of a CCD camera. The camera consists of an array of 96×2048 pixels, providing a length sufficient to acquire the signal from the entire radial distance subtended by the sample cell. This permits extremely rapid scan times, limimited only by the speed of the computer. Typical scanning rates are of the order of 10 s with a 75-MHz Pentium microprocessor. To provide sufficient exposure for signal accumulation at the CCD camera, multiple flashes are accumulated from every second turn of the rotor. Each scan of the sample thus corresponds to a typical exposure of 100–200 μs. The system is specified to provide a minimum of 20 pixels per fringe, offering a minimum data precision of f/20.

Light intensity data is sent from the CCD array to the data acquisition board, where it is organized by comparison with timing signals corresponding to each pixel and to each row. During operation of the instrument, the controlling software shows an image of the fringe pattern reported from the cell, as well as an immediate single frequency finite Fourier transform, based on the algorithm of DeRosier et al. (1972), providing a graphical representation of fringe displacement as a function of radius. An example of these data representations is shown in Fig. 3.

Software interface

An advanced, Windows-based data acquisition software system provides control of the centrifuge and of both the absorbance and Rayleigh Interference optical systems. In addition, rapid links are provided to the Beckman-Origin data analysis software. Rather than a complete description of the software, which would be beyond the intended scope of this article, an overview of the major functions will be given here.

A centrifuge control window allows one to start and to stop the centrifuge and to set the speed and temperature from the computer console. One need access the centrifuge directly only to insert or to remove the rotor and the gantry-laser unit. This window can be kept visible to report the status of the run. A separate window is also available to report the status of scans in progress.

Each experiment is controlled by a “Scan File”, which specifies the scanning method (see below) for the run, and which can be saved to create a new directory for the experiment. Subordinate windows within the scan file allow for adjustment of the laser, radial calibration, and specification of the parameters for interference or absorbance scans.

Scans can be set up individually, or as a series of scans to take place throughout the run. A series of scans is re-

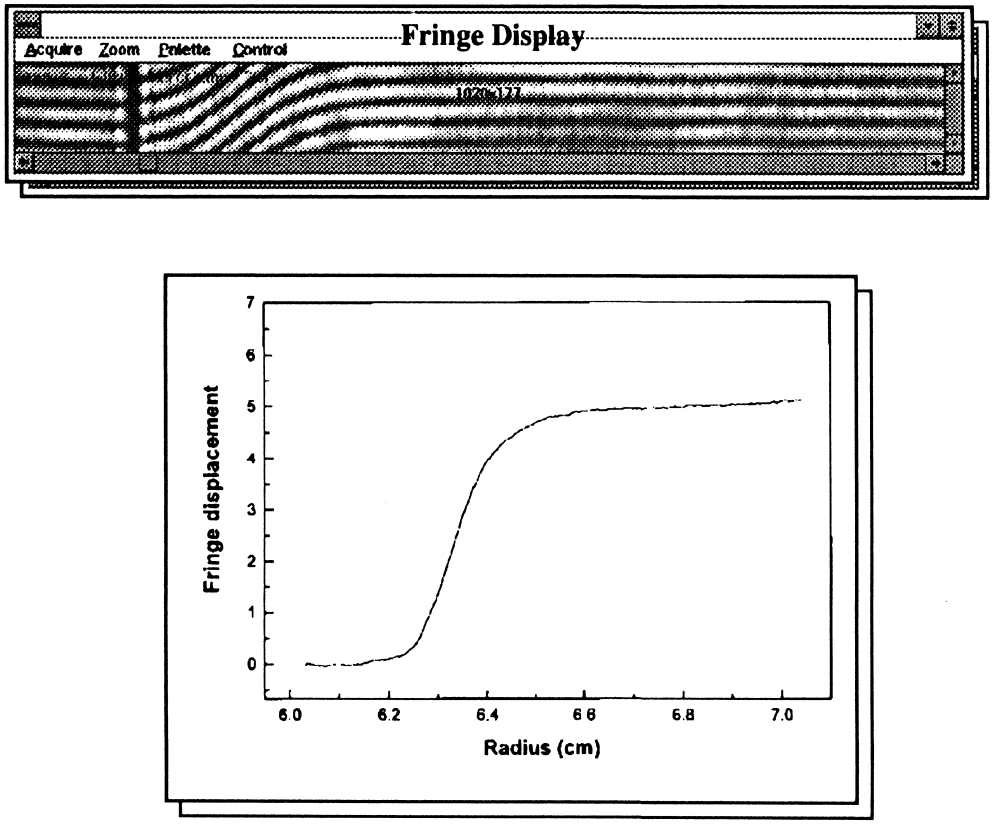

Fig. 3 Representative Data from the XL-I Rayleigh Interference Optical System. Bovine serum albumin at 2 mg/mL was run at 50 000 rpm in the AN-8 rotor in a sedimentation velocity experiment. In this experiment, data from an air-air blank position was subtracted from the sample data, although this step is not normally required. Both the fringe pattern and the corresponding Fourier transform are shown on the computer screen during normal operation

ferred to as a "method", and the software allows the programming of two types of methods. For the "Velocity Method", a series of up to 99 scans can be scheduled to take place in close succession beginning at any time during the run. This facilitates data acquisition for methods such as Stafford's "Time Derivative" analysis (Stafford, 1992) which requires data from large numbers of closely-spaced scans. The "Equilibrium Method" provides an alternative programming procedure in which the run can be broken up into individual stages of varying speed or temperature, and scans scheduled to take place at specified intervals. Both methods allow the collection of large numbers of scans and the acquisition of data from both the Rayleigh and the absorbance optical systems. When a scan protocol calls for the use of both optical systems, Rayleigh scans – which are very rapid – are acquired first at each time point, and the absorbance scans follow.

Scan data is saved automatically to a directory structure that has been designed to prevent loss through accidental overwrites. All data is saved to a directory with a name corresponding to the current date, for example "960831". Within this directory, a new subdirectory is started each time a method or individual scan is initiated. These subdirectories will have names corresponding to the time the method is started, for example "114500". A log file is saved to each subdirectory with a record of the scans it contains. Separate log files are created for absorbance and Rayleigh scans.

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