# An on-line interferometer for the XL-A ultracentrifuge

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*Abstract*: As a result of the renewed interest in analytical ultracentrifugation, the Beckman XL-A was released recently. This instrument automates spectrophotometric measurements of the concentration distribution of molecules in a gravitational field. Presented here is the design and the performance characteristics of a Rayleigh interferometer for the XL-A ultracentrifuge. The interferometer consists of a laser diode light source, imaging optics providing a 1.4-fold magnification of the cell, and a solid-state television camera detector. The source and detector are interfaced to a personal computer which synchronizes data acquisition for up to four cells, performs data reduction, and allows data analysis. About 1700 data points, at a radial spacing of $\sim 9\ \mu m$, are acquired over a standard double sector cell image. Data are then stored on the disk and presented as a graph on the screen. The complete sequence of data acquisition, storage, and presentation requires about 15 s. The interferometer has a precision of about $\pm 0.003$ fringe, and can be used for both equilibrium and velocity sedimentation. The design can be adapted for Schlieren detection.

*Key words*: Analytical ultracentrifuge – interferometry – instrumentation – equilibrium sedimentation – velocity sedimentation – methods – hydrodynamics – thermodynamics

## Introduction

The principle measurement needed for any analytical ultracentrifuge experiment is the solute concentration as a function of radial position. As currently configured, the Beckman XL-A contains a superb spectrophotometer. However, it is desirable to complement this optical system with refractometric detection. The Rayleigh interferometer provides a cell image in which the refractive index difference between sample and reference at each radial position is given by the vertical displacement of a set of evenly spaced horizontal fringes [1, 2]. This image is well-suited for automated data acquisition [3–6].

Absorbance and interference (refractive) optical systems provide complementary ways to determine concentration distributions in the centrifuge. The merits of the absorbance system are that it can provide sensitive and selective solute detection. For many biochemical systems these virtues are needed and appreciated. However, it is less useful when solutes do not absorb significantly or when solvents do. For these cases refractive detection offers several advantages over absorbance measurements. Moreover, the interferometer provides greater accuracy [7], higher radial resolution [8, 9], a greater concentration range, and the ability to trace very steep concentration gradients [8].

Presented here is the description of a Rayleigh interferometer suitable for use with the Beckman XL-A analytical ultracentrifuge. This instrument is suitable for use in both sedimentation equilibrium [8–10] and sedimentation velocity analysis [11].

## Description of the on-line interferometer hardware

### *Overview*

A schematic of the on-line Rayleigh interferometer is presented in Fig. 1. The two periscopes

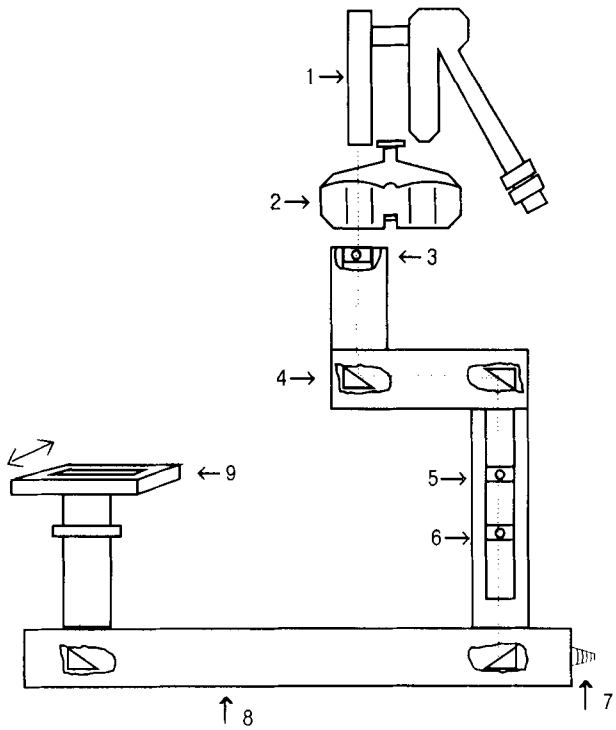


Fig. 1. Overview of the XL-A interference optical system. 1) light source, 2) rotor, 3) condensing lens, 4) top periscope, 5) cylinder lens, 6) camera lens, 7) vacuum port, 8) bottom periscope, and 9) television camera sensor. The heat sink of the XL-A was machined to accept the condensing lens holder and top periscope. For safety reasons, two separate vacuum systems are used, one for the rotor chamber and a second one to evacuate the optical track

are necessary to avoid obstacles and to fold the optical path to fit inside the machine. This design is based on prototypes constructed using the Model E ultracentrifuge [10]. Wherever possible, stock optical components from the Spindler & Hoyer Microbench (Göttingen, FRG) system were used. The optics consist of a pulsed laser diode light source, beam expansion and collimation optics, slits to provide the reference and sample beams, the centrifuge cell, a condensing lens to mix the two beams, a cylinder lens and a camera lens to focus the cell image and fringes, respectively, on the CCD television camera. The entire optical track, except for the television camera, can be kept under a vacuum. This eliminates distortion of the image due to the Schlieren effects caused by convective air currents. Both the laser and the camera are operated by computer so that their functions can be synchronized to one another and to the spinning rotor. The computer also is used to acquire images, calculate the concentration distribution, store information, and to edit and analyze the data.

### *Lightsource*

The light source consists of a Toshiba TOLD 9215 10 mwatt, 670 nm, index guided laser (Fig. 2). A pulsed, constant-current circuit was developed for driving the laser (Fig. 3). Two lenses, a 16-mm focal length achromat mounted approximately 5–8 mm from the laser diode, and an 80 mm achromat provide a collimated beam of light sufficiently large to illuminate the Rayleigh mask. The custom Rayleigh mask, two 0.5 mm slits, 2.5 cm long and separated by 0.8 cm (kindly supplied by Beckman Instruments) is mounted in a rotatable holder in front of the 80 mm achromat lens. The light source mount is attached to the XL-A monochromator arm, and both optical

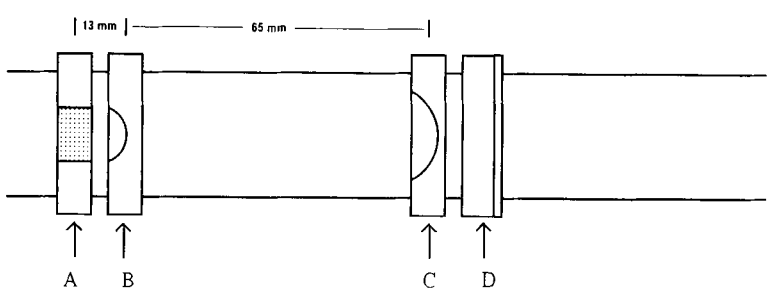


Fig. 2. Schematic of the XL-A light source. Lenses were chosen to expand and collimate the laser beam. The resulting beam is a 1.2 cm × 3 cm ellipse. Distances shown are nominal, with the final positions chosen to provide the best collimation, as monitored by a shear-plate [10]. Components include: A) the 10 m watt, 670 nm, index-guided laser diode mounted in a Delrin holder for electrical isolation, B) a 16-mm fl 6 mm $\phi$ achromat, C) an 80-mm 31.5 $\phi$ achromat and D) Rayleigh mask holder. All components, except the Rayleigh mask, are from Spindler & Hoyer, and are mounted on 150-mm-long Microbench stainless steel rods cut to the appropriate length