Analytical Ultracentrifuge User Guide

Volume 1: Hardware

Center for Macromolecular Interactions

Center for Analytical Ultracentrifugation of Macromolecular Assemblies

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1 History

Centrifugation (think swinging a bucket by a rope) has been around for centuries, but high speed ultracentrifugation became a reality in the 1920's. The ultracentrifuge was initially developed by Theodor Svedberg [1–3] (Fleräng 1884 – Örebro 1971, Sweden) and co-workers in the nineteen twenties (Figure 1.1 panel A) to study gold particle size distributions [4]. For his work on the ultracentrifuge, Svedberg was awarded the Nobel Prize for chemistry in 1926. The ratio of the sedimentation velocity and centrifugal or gravitational acceleration is expressed in units of Svedberg ($1\,\mathrm{S} = 1 \cdot 10^{-13}\,\mathrm{s}$).

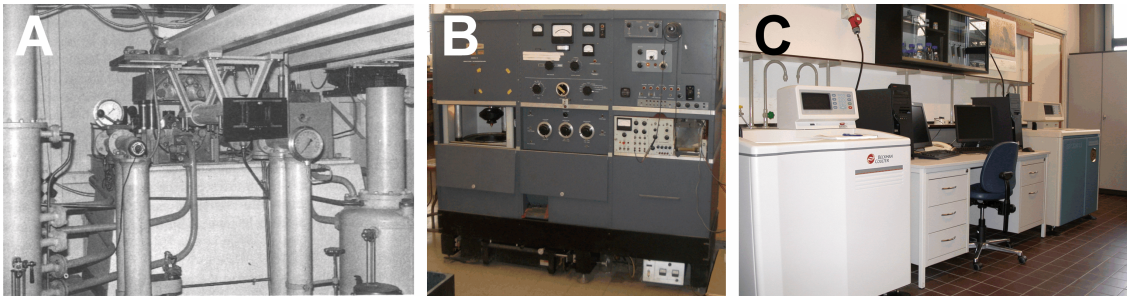
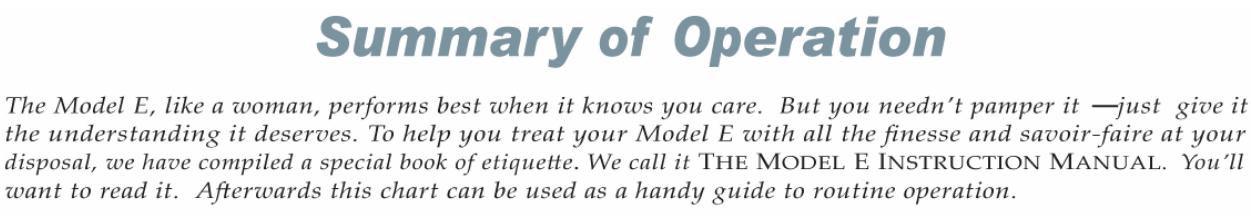


Figure 1.1: Images of various analytical ultracentrifuges. An early (oil turbine) analytical ultracentrifuge (reproduced from ref. [5] with kind permission of the Royal Society of Chemistry) as employed by T. Svedberg (image A). Image B shows the Specialized Instruments Corporation (Spinco/Beckman) Model E analytical ultracentrifuge. To date, the Beckman Coulter^{TM} Optima^{TM} XL-A (on the right of image C) and XL-I (on the left of image C) analytical ultracentrifuges are employed for sedimentation experiments. The AUC facility of our Van 't Hoff Laboratory in 2008 is shown in image C.

Edward Pickel, in the 1940's, developed a method of performing ultracentrifugation under vacuum, thus greatly reducing heat from frictional interactions. He started a company, Spinco (Specialized Instruments Corporation), which later was sold to Beckman (now Beckman-Coulter). The Spinco division of Beckman produced centrifuges and ultracentrifuges including the commercially available Model E analytical ultracentrifuge (Figure 1.1 panel B). The Model E was a large, cumbersome beast and difficult to maintain, see Figure 1.2 for the summary of operation of the Model E analytical ultracentrifuge. Analog data was collected on film and manually interpreted. The structure of DNA and isolation of the polio virus are linked to work on the Model E.



Summary of Operation

The Model E, like a woman, performs best when it knows you care. But you needn't pamper it —just give it the understanding it deserves. To help you treat your Model E with all the finesse and savoir-faire at your disposal, we have compiled a special book of etiquette. We call it THE MODEL E INSTRUCTION MANUAL. *You'll want to read it. Afterwards this chart can be used as a handy guide to routine operation.*

Figure 1.2: Summary of operation of the Spinco Model E analytical ultracentrifuge instruction manual.

The use of analytical ultracentrifugation fell into decline until Beckman introduced the Optima XL-A (Figure 1.1 panel C) in 1992 based on the outward design of their preparative ultracentrifuges. These instruments produce digital absorbance data, are smaller and more easily maintained and serviced. The need to study proteins and other macromolecules in solution led to the rapid growth of analytical ultracentrifugation as a research tool. Since the instrument's introduction, Rayleigh interference and fluorescence optical detection systems have been added and the software to analyze the data has grown and developed tremendously.

2 The AUC Instrument

2.1 General

In the following sections various parts of the Beckman Optima XL-A/I AUC are discussed. A protocol to check the performance of the Beckman Optima XL-A AUC (absorbance optics only) can be found in Appendix A. You may find it useful to read Chapter 2 of the PhD thesis *Analytical Ultracentrifugation of Inorganic Colloids* [6] for a detailed discussion on the Beckman Optima XL-A/I instrument and the basic experiments that can be performed with this AUC. Also practical tips on how to set-up and to perform your experiments can be found in that chapter. The thesis is, free of charge, accessible via the internet (click *here* to navigate to the latter document, Adobe® Reader® 5.0 or higher required).

2.2 Chamber

A high strength structural steel door covers the chamber and can be opened with the power on and the vacuum off. The inside chamber is constructed of epoxy-coated aluminum with a drive spindle and safety plate visible on the bottom. The slit-assembly, required for the absorbance optical system, which is driven by a motor pot is integrated in the safety plate. Also on the bottom the condenser lens used in Rayleigh interference optical systems, the index pulse detector (Hall-effect sensor) which determines the rotor position, a photoelectric device which determines the maximum speed of the rotor based on the alternating reflective and non-reflective surfaces of the over speed ring, and the radiometer which is activated at a vacuum less than 100 microns (100 μm Hg) and measures the temperature of the rotor are visible. A thermistor (underneath the safety plate and therefore not visible) measures the temperature of the chamber above 100 microns. The monochromator mounting receptacle is positioned on the bottom of the chamber outside the safety plate.

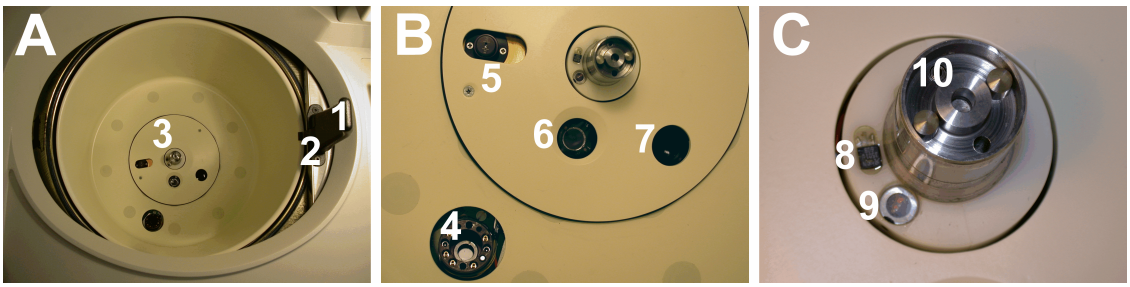


Figure 2.1: Top views of a XL-I rotor chamber. Panel A is an overview with the handle (1) and the lid (2) to close the chamber and on the bottom the safety plate (3). Panel B shows the monochromator mounting receptacle (4) with either 3 (XL-A) or 5 (XL-I) pins, the absorbance slit assembly (5) which can mechanically change its radial position, the radiometer (6) and the condenser lens (7) for the interference optics. Panel C is a zoom on the magnetic position sensor or index pulse detector (8, also called Hall effect sensor), the photoelectric device (9) of the overspeed system and the drive spindle (10).

2.3 Vacuum System

The Optima XL-A/I uses an oil pump running in series with a diffusion pump to lower the chamber pressure to less than 5 microns. The system is activated once the "ENTER" and "START" buttons are pressed or by the "VACUUM" key on the console. It must be noted that the AUC should be operated from the controller (PC), except for the vacuum pump activation, to prevent possible communication errors. The system is also activated if the "START SINGLE" or "START METHOD" scan is selected in the software.

When a run is finished, the oil in the diffusion pump must be allowed to cool sufficiently to prevent blow back of vapor into the chamber. A notation on the lower left side of the console will tell the operator to *Press Vacuum to Open Door* once the diffusion pump oil has cooled or *Holding for DP Cooling* if it has not. Even under normal circumstances, oil vapor will collect on the bottom and the side of the rotor chamber. The oil should be transparent for the diffusion pump and relatively clear (yellowish) for the vacuum pump oil. Brownish color oil in the chamber may indicate a problem with the drive and the instrument must be serviced before use. A turbo molecular pump may be purchased and installed to prevent most of the oil vapor problems associated with the standard combination of an oil and diffusion pump.

Any leaks in the vacuum system prevent the system from pulling an almost complete vacuum. The discharge of oil vapor into the room in which the instrument is housed is a giveaway. If the operator determines that a vacuum is not being formed inside the chamber, there are several areas to check after turning off the system and disconnecting the power plug. The first area to check would be the Xenon flash-lamp assembly. There is an O-ring on the top of the flash lamp which must be intact and in place. Check the gasket on the underside of the sliding steel door (the lid (2) in Figure 2.1). It should be intact, clean, and lightly covered with vacuum grease. A third area to check would be the photomultiplier tube assembly. Only a service engineer or an experienced operator should check this area. If the problem persists, take the instrument out of service until an engineer can check the instrument.

Once a run is started and a large change in chamber pressure occurs accompanied by a change in temperature, a leak in one or more of the cells is the culprit. Stop the run and check all the cells before resuming.

2.4 Temperature System

The Optima XLA/I is an air-cooled system without refrigerant. A series of fans are used to cool motors and electrical systems within the instrument. A heat sink lies beneath the chamber on which a series of Peltier cells heat and cool the chamber and, consequently, the rotor. This system allows for the running of samples between 0 and 40 °C although extremes of temperature affect oil vapor and may cause clouding of some of the optical devices inside the chamber. Here is an example of anticipating the rotor cooling due to adiabatic stretching to speed up the set-up of a sedimentation velocity run: If a sedimentation velocity run at for example 20.0 °C and 60,000 rpm is to be performed, the cells are loaded into the rotor. After activating the oil pump (press the "VACUUM" key) the rotor is accelerated to 3,000 rpm. At

this speed the diffusion pump imediately starts and a vacuum is reached in approximately 15 minutes. Now suppose that the temperature is 20.5 °C at $\leqslant$ 5 microns, the machine starts cooling. If the temperature reaches 20.3 °C the "Start Method Scan" (sedimentation velocity) at 60,000 rpm key should be activated. Due to stretching (adiabatic) of the rotor, the rotor temperature drops to the desired 20.0 °C.

2.5 Console

The console (Figure 2.2) consists of the activation hard-keys on the right, a keypad, parameter hard-keys, the video display, and soft-keys on the bottom with labels on top of them. A red power off key is located at the bottom right and revolutions counter is located on the bottom left. The speed, time, and temperature of the run can be controlled from the parameter hard-keys. The right side of the video display shows set parameters while the left side shows real-time values. Rotor selection and printer options can be selected through use of the soft-keys.

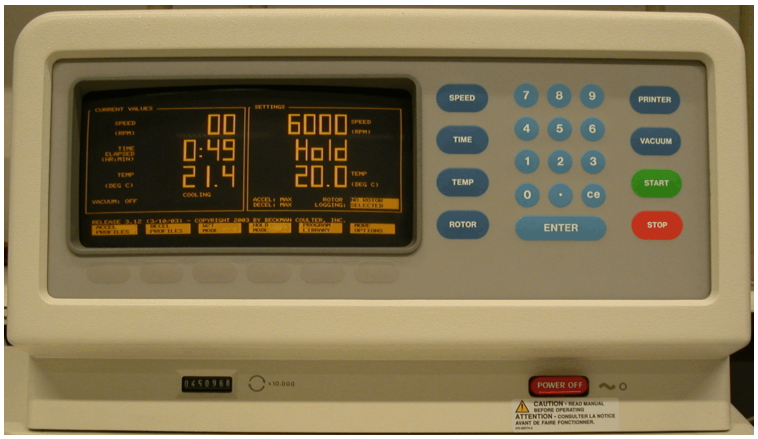


Figure 2.2: Console of the Beckman Coulter XL-I AUC.

2.6 Drive

A vacuum encased, direct drive induction motor powers the drive shaft. The motor is brushless and frequency controlled. It is cooled by oil internally and forced air externally. The drive can handle speeds ranging from 1000 rpm to maximum rated rpm of the instrument, since the rotor speed after stabilization at or above 100 rpm is within 20 rpm of the selected speed.

2.7 Overspeed System

Overspeed disks are located on the bottom of all rotors and they must be maintained. The alternating reflecting and non-reflecting sectors pass over the photoelectric device generating

a series of electrical pulses that are picked up by the circuitry and software. After the rotor reaches 1000 rpm, the set speed is checked against the overspeed disk. If the set speed is greater than the maximum speed permitted by the disk, the speed setting is automatically lowered to the disk's maximum speed, but the run continues without interruption. An error message is displayed to alert the operator to the change.

3 Detection Systems

3.1 General Remarks

Generally, the absorbance optics is used if (1) selectivity is required, (2) the concentrations of solutes absorbing light with a wavelength in between 200-600 nm is relatively low and (3) if the sample cannot be dialyzed. Contrary, the interference optics should be employed if (1) the reference solvent or buffer absorbs light of a wavelength below 675 nm (light source interference optics), (2) the solutes or particles do not absorb significantly and (3) a relatively high precision is required. The choice of the appropriate optical system for a given experiment is discussed in detail in ref. [7].

3.2 Absorbance Optics

The absorbance optical system, Figure 3.1, is composed of a (1) Xenon flash lamp in a housing located under the chamber which emits light from 190 nm to 800 nm, (2) the monochromator, installed in the chamber on a mount (mounting receptacle), which selects the proper wavelength and reflects the light from the flash lamp down through the (3) sample cell, the (4) slit assembly which mechanically moves in and out to track changes in absorbance radially, and finally the (5) photomultiplier tube (PMT) which collects the light and sends the generated electrical current to an analog to digital (A/D) converter. As will be discussed later, data can be collected as absorbance or as intensity data and mathematically converted into pseudo-absorbance data.

Absorbance or attenuance data is based on intrinsic properties of molecules such as peptide bonds at 230 nm, aromatic side chains of amino acids found in peptides at 280 nm, nitrogenous bases found in DNA or RNA at 260 nm, chromophores such as heme which absorb around 420 nm or light scattering as is the case for non-absorbing colloids. The Xenon flash lamp is especially useful in producing high intensity light in the UV range. In order to fall within the linear limits of Beer's Law, the absorbance of any sample should usually not exceed 1.0 OD at any chosen wavelength. Remember the light path of the analytical ultracentrifuge is 12 mm compared to 10 mm for standar cuvets used in spectrophotometers, thus the optical density will be higher by a factor of 1.2 compared to the absorbance recorded with a spectrophotometer.

3.2.1 Care of the Xenon Lamp

Diagnostic wavelength scans recorded in intensity mode (Figure 3.2) are used to show deterioration of the lamp output caused by oil and dirt build-up on the surface. The intensity of the 229 nm peak should be the highest in the emission spectrum. Depending on the instrument this 229 nm emission peak should generally be 15,000 counts or higher.

After turning off the power, the Xenon flash lamp assembly can be removed noting the orientation of the lamp assembly by marking it with tape or ink. The cover is removed and the lamp cleaned with toothpaste and a cotton tip swab or a pencil eraser (Figure 3.3). The

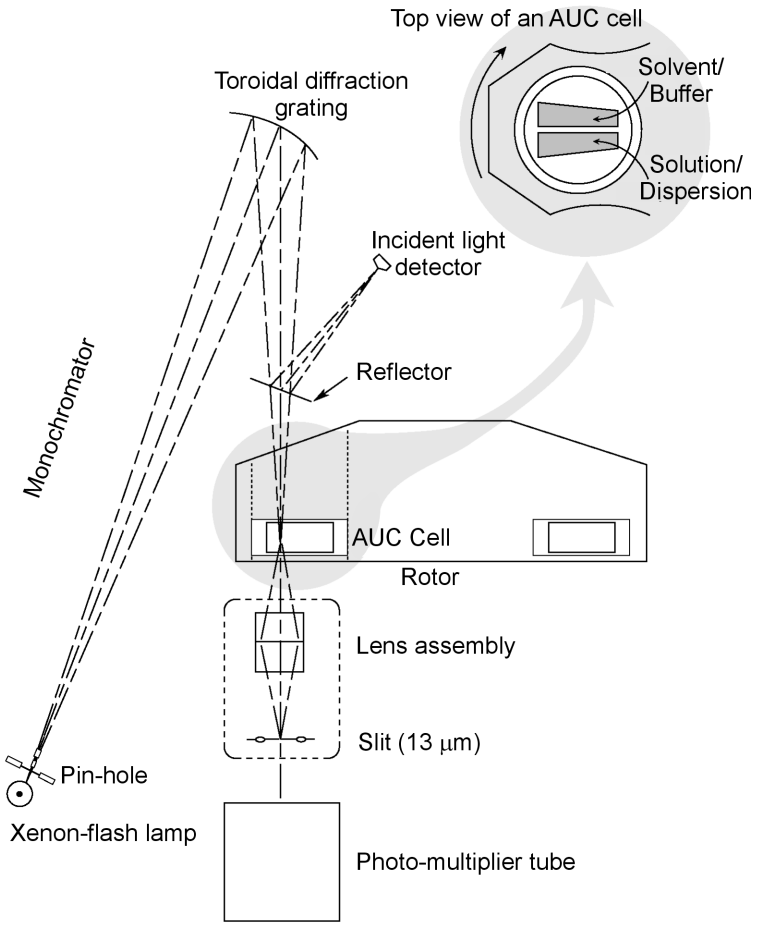


Figure 3.1: The light path in an absorbance optical system (Beckman Coulter^{TM} Optima^{TM} XL-A and XL-I AUC, reproduced from ref. [8] with kind permission of Beckman Coulter^{TM}). The inset shows a top view of an AUC cell, contained in the rotor, with the reference (solvent/buffer) and sample (solvent/buffer with solutes or colloids) sectors.

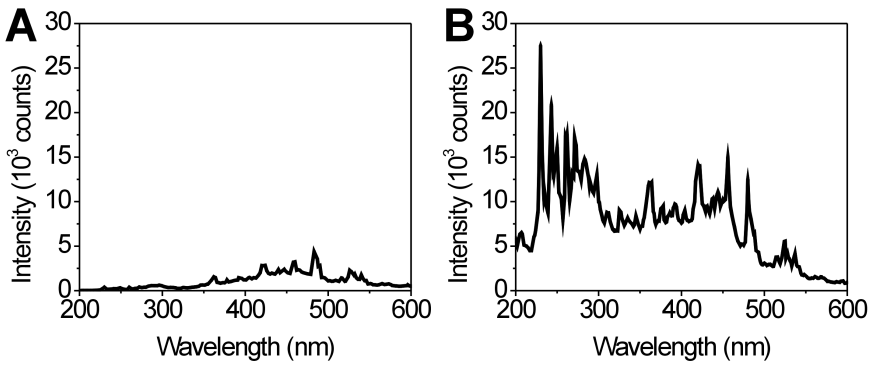


Figure 3.2: Emission spectra for a Xenon-flash lamp (electric discharge) with oil deposited onto the light bulb (graph A) and after lamp cleaning with a cotton tip swab, toothpaste, water and ethanol (graph B).

lamp is cleaned further by ethanol and milli-Q water, and reassembled. The lamp is replaced in the same orientation as it was removed. Another diagnostic wavelength intensity scan can be performed for confirmation. Lamp cleaning is essential for a high signal-to-noise ratio to obtain quality data and can, in principle, be performed by the user. A trained service engineer or lab technician can guide a new user through the procedure.

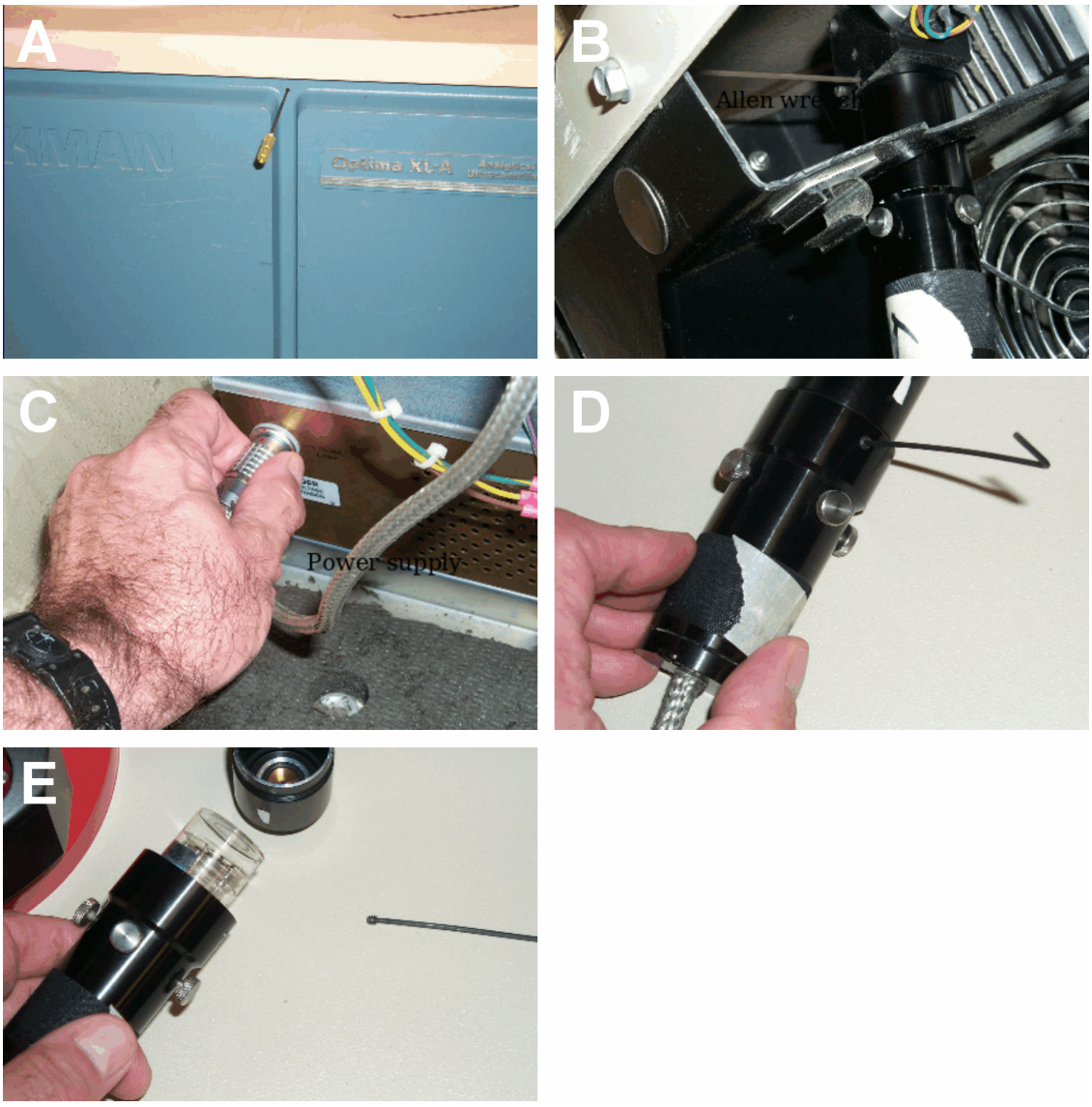


Figure 3.3: Turn off power, unplug the instrument and remove the front panel (A), locate the lamp assembly and find the recessed hex screw (B). Unplug the lamp from the power supply (C), loosen the hex screw and pull down the lamp (D). Remove the cover (E), clean the lamp, replace the cover, and re-insert.

3.3 Rayleigh Interference Optics

Interference optics provide a way to examine concentrated samples in which the optical density is too high using absorbance optics, such as studies looking at conditions for crystallization or NMR. It is also useful in studying systems in which the buffer or some other component, such as nucleotides, absorb. Figure 3.4 schematically represents the various components and the light path of the Rayleigh interference optical system.

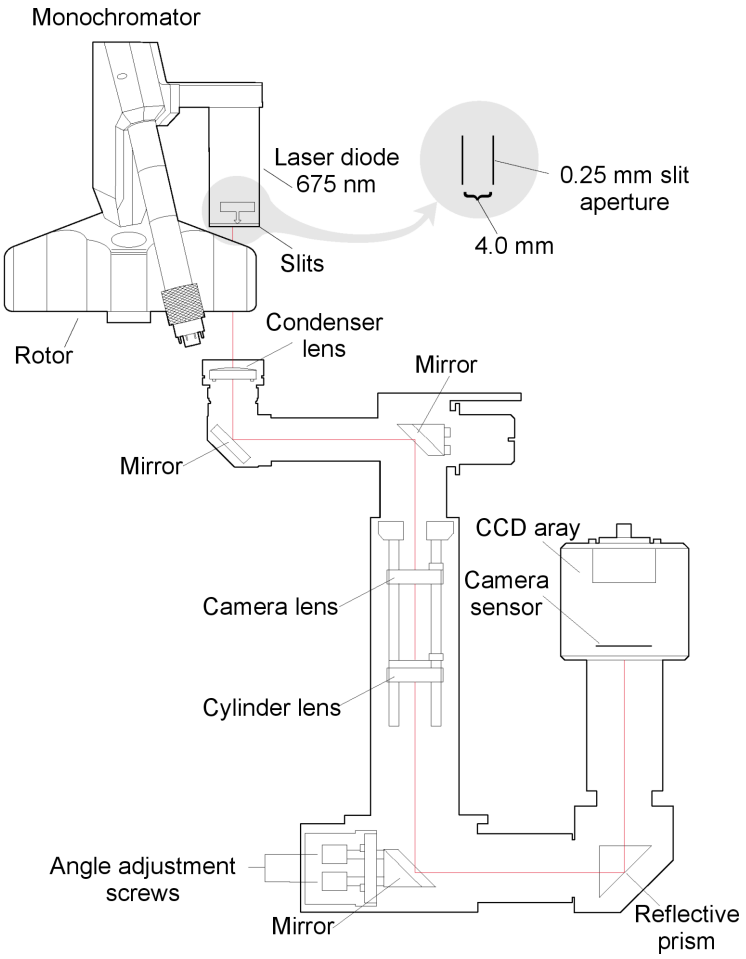


Figure 3.4: The light path of the Rayleigh interference optical system (Beckman Coulter^{TM} Optima^{TM} XL-I AUC, reproduced from ref. [8] with kind permission of Beckman Coulter^{TM}). Note that the laser diode is mounted onto the monochromator that is part of the absorbance optics. Shown is the light path of the Rayleigh interference optical system. The interference pattern is captured on a CCD which is displayed on the top part of the screen. The data undergoes FFT and is displayed as a scan.

3.4 Absorbance and Interference Optics Light Sources

The monochromator housing for absorbance optics and laser housing for interference optics (if available) are joined (Figure 3.5). The base of the monochromator will have either

3 (absorbance only) or 5 (absorbance and interference) gold pins, two thick, aluminum guide pins, and a hole in which the wavelength selector pin on the mount inserts to select the appropriate wavelength by tilting the difracting grating unit in the monochromator. Because it is mechanically driven, the system is not always able to change and reset the wavelength accurately. A sedimentation velocity run is typically performed on a single sample when employing the absorbance optics, to prevent the monochromator's incorrect wavelength resetting and to enhance the scan rate.

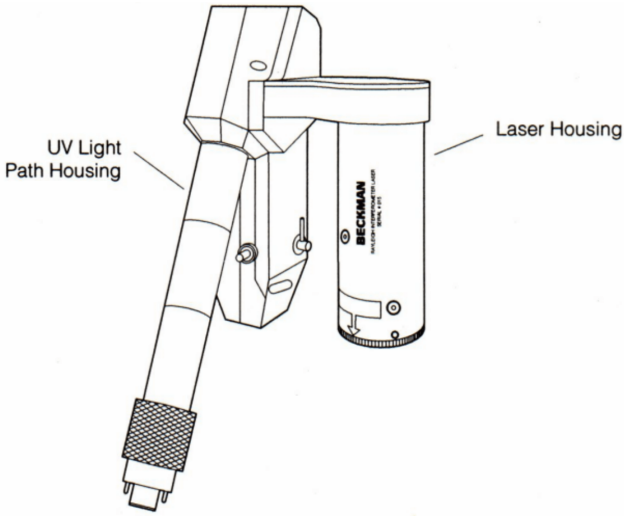


Figure 3.5: Monochromator and laser housings (reproduced from ref. [8] with kind permission of Beckman Coulter^{TM}).

The monochromator is inserted onto a mount on the bottom of the chamber using the index pins (Figure 3.6). The red ring on the bottom of the monochromator is secured by hand tightening. The threads of the mount can be treated with SpinKote® periodically. Be careful not to strip the threads on the mount. If using a wavelength above 400 nm, turn the adjustment perpendicular to the stem of the monochromator and parallel for work below 400 nm (Figure 3.7).

3.5 Fluorescence Detection System

In fluorescence scanning, a degassed sample containing a natural fluorophore or tag (i.e. SybrGreen, fluorescein) are centrifuged. The excitation laser (488 nm) and emission detector ($< 505\,\text{nm}$) are located above the rotor (Figure 3.8). Because there is no need for blanks, both sectors of a 2-sector centerpiece can be used for samples. It is important to match the relative concentrations of two samples in the same cell so that the gain settings will be comparable. Fluorescence scanning allows picomolar concentrations to be tested and allow for examination of complex solutions were only one macromolecule is labeled. The fluorescence scanning technology is licensed to Aviv Biomedical NJ.

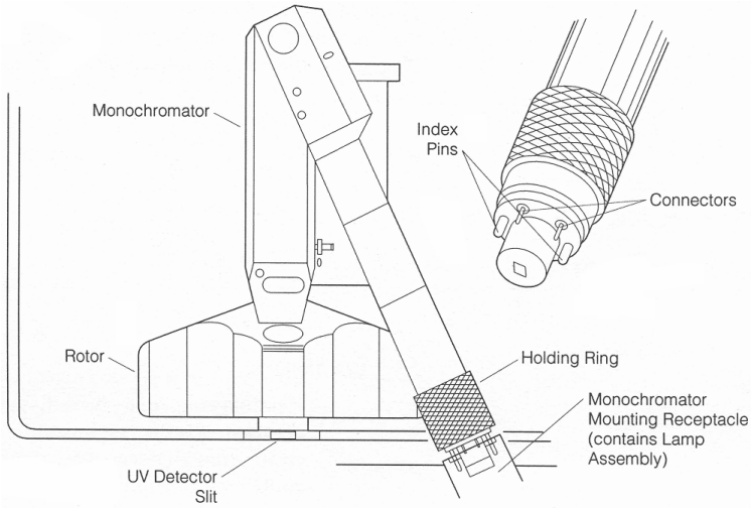


Figure 3.6: Installing or mounting the monochromator (reproduced from ref. [8] with kind permission of Beckman Coulter^{TM}).

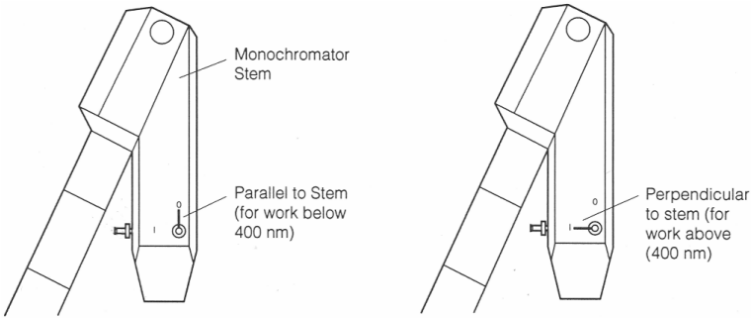


Figure 3.7: Monochromator filter lever positions (reproduced from ref. [8] with kind permission of Beckman Coulter^{TM}).

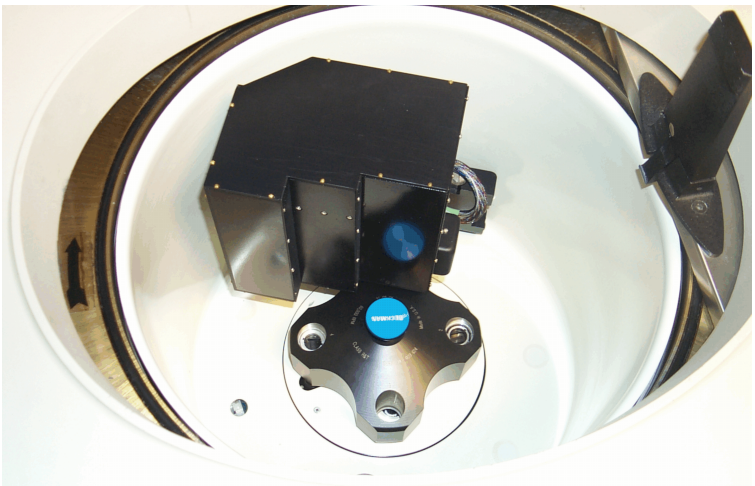


Figure 3.8: Fluorescence detection system, installed in the rotor chamber.

4 Rotors

There are two anodized titanium rotors (Figure 4.1) used for most experiments. The An-60 Ti (Beckman 361964, overspeed disk 360256) rotor has four holes (three sample cells and one counterbalance) and is r(ot)ated to 60,000 rpm. The An-50 Ti (Beckman 363782, overspeed disk 363784) rotor has eight holes (seven sample cells and one counterbalance) and is r(ot)ated to 50,000 rpm. There is also an An-55 Ti rotor, no longer sold, r(ot)ated to 55,000 rpm. The rotors can be cleaned periodically with mild soap and water. Check the overspeed disk at the bottom for scratches or broken magnets.

Rotors should be check at least yearly, especially older rotors, by Beckman service for safety. Depending on use, rotors generally have a life span of 10-years.

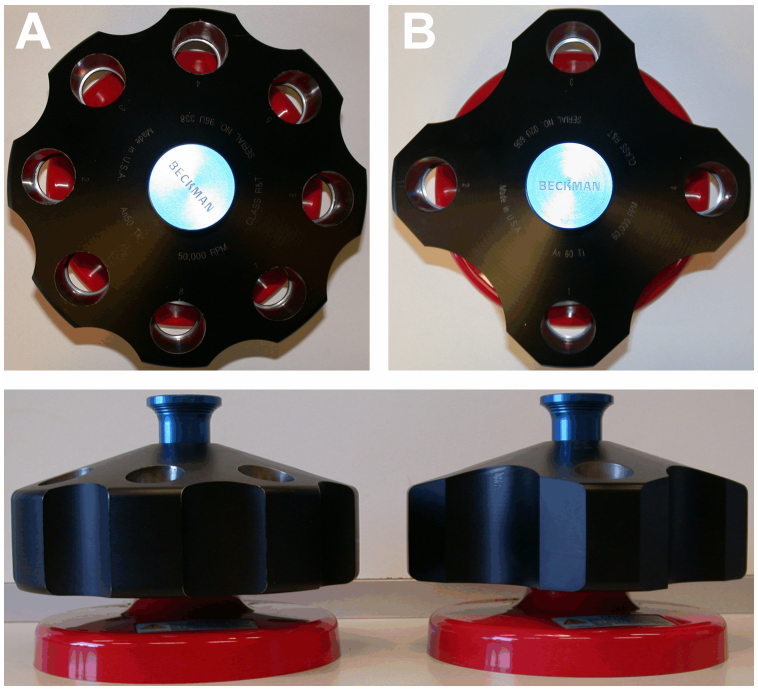


Figure 4.1: The An-50 Ti (panel A) and An-60 Ti (panel B) rotors.

5 Counterbalance

The counterbalance (Figure 5.1) is a red anodized "cell". It is inserted in rotor position 4 in the An-60 Ti or in position 8 in the An-50 ti rotor. The top of the counterbalance has a set screw for securing the counterbalance in the rotor, inner and outer reference holes (one set is wider than the other – this set would face the outside of the rotor). An arrow is pointed to the outside of the rotor as well. In the center, a screw hole is available to add appropriate weight for balance.

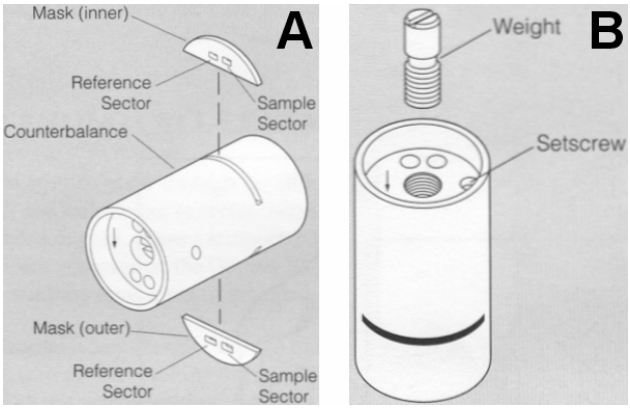


Figure 5.1: Inserting masks (panel A) and weights (panel B) into the counterbalance.

On the bottom of the counterbalance, the set screws to hold the masks are visible as well as the center hole. Only a 0.5 g weight can be added to this side. All other weights must be added to the top. For the proper insertion of the counterbalance into the rotor see Figure 5.2.

The instrument calibrates radially, in absorbance mode, by looking at the light and dark areas of the counterbalance. The edges are at 5.85 cm on the inside and 7.15 cm on the outside. When the user selects Radial calibration before first scan in the "OPTION" box, the instrument looks for these edges and sets the inside and outside radial distances based on what it "sees" (for the sequence of calibrations see Appendix B) . A radial scan should be done when changing the counterbalance, rotor, or weekly. If performing a velocity run, it is better to perform a radial calibration before the run with a "dummy" cell and the counterbalance so that no time is lost at the beginning of the velocity run. For equilibrium runs, the latter procedure is not required since it does affect the experiment (always perform a radial calibration at 3,000 rpm to avoid any off-set due to rotor stretching).

Radial calibration of the interference optical system is done manually. Viewing the counterbalance using the interference optics while in the "INTERFERENCE" menu under Radial calibration, the user clicks on a point on the raptor screen representing the inside edge of the counterbalance and clicks on "Inside radius" and does the same for the outer edge. Match menisci from both absorbance and interference scans of water to make sure both systems are calibrated the same way (Figure 5.3).

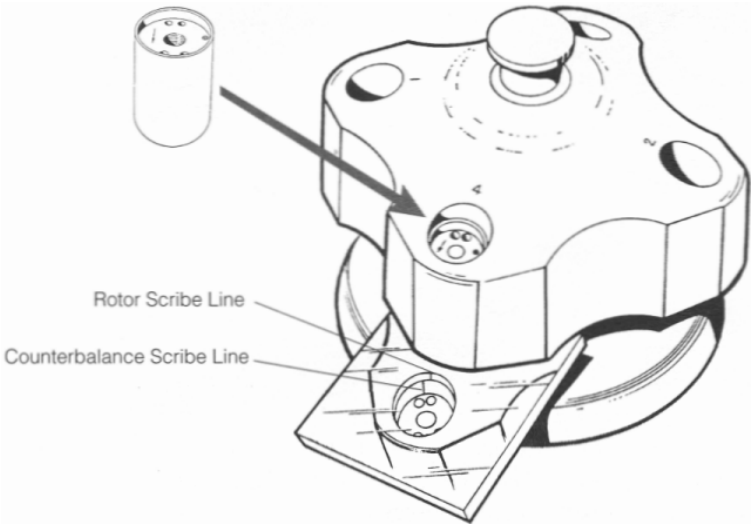


Figure 5.2: Loading the counterbalance into a An-60 Ti rotor. Scribe lines are reflected in the mirror.

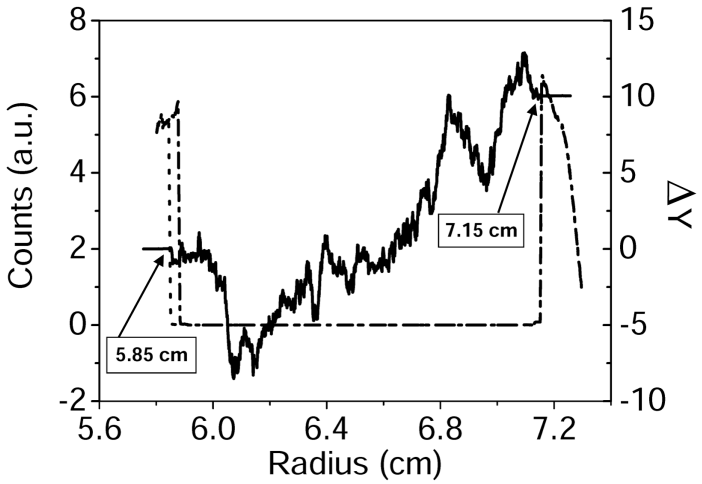


Figure 5.3: Overlay of the reference (·····) and sample (– · –) intensity (×1000) at 400 nm and total fringe displacements ΔY (——), all recorded through the counterbalance to check that the radial calibration values were set properly for the interference optics.

6 AUC Cells

6.1 General Remarks

In this chapter the various AUC cell components, see Figure 6.1, are discussed after which, in the next section 6.5, the cell assembly is described.

6.2 Windows

There are two types of windows quartz for absorbance and fluorescence scanning and sapphire for interference scanning. Sapphire windows may also be used for absorbance scanning above 240 nm. Sapphire windows are more durable and weigh approximately 2 g more than quartz windows. Do not mix sapphire and quartz windows. These windows can be distinguished by their weight. Sapphire windows weigh $\approx$ 5.64 g (range VIS and IR, density is 3.98 g cm^{-3}), and quartz windows weigh $\approx$ 3.76 g (range UV and VIS, density is 2.65 g cm^{-3}). Several sources provide windows, Table 6.1. Meller Optics sells to Beckman and want to keep their sales to others under the radar.

6.3 Centerpieces

Centerpieces come in many flavors. Commonly, 2-(double)-sector, 12 mm centerpieces are used for sedimentation velocity experiments while 6-sector, 12 mm centerpieces are used for sedimentation equilibrium experiments. The centerpieces may be made of Epon charcoal-filled, anodized aluminum, titanium, or older centerpieces may be made of Kel-F or other materials. Metal centerpieces require gaskets on both sides to seal and protect the windows. In contrast to equilibrium centerpieces, standard two channel (double-sector) centerpieces are sector shaped with the wider end facing the outside of the rotor.

Generally, the speed rating for epon-charcoal centerpieces is 42,000 rpm while metal centerpieces are rated to the maximum of 60,000 rpm. Chemical compatibility/resitance tables are available to check solvents against various types of centerpieces.

Other centerpieces are available. Go to spinanalytical.com for a wide variety of commonly used and specialty centerpieces as well as novel tools. For titanium centerpieces see Table 6.2.

Table 6.1: Window distributors

	Beckman	Meller Optics	Linos	Kyburz AG
Quartz	301730	SCD0267-03C		NA
Sapphire	307077	SCD0267-01C	NA	

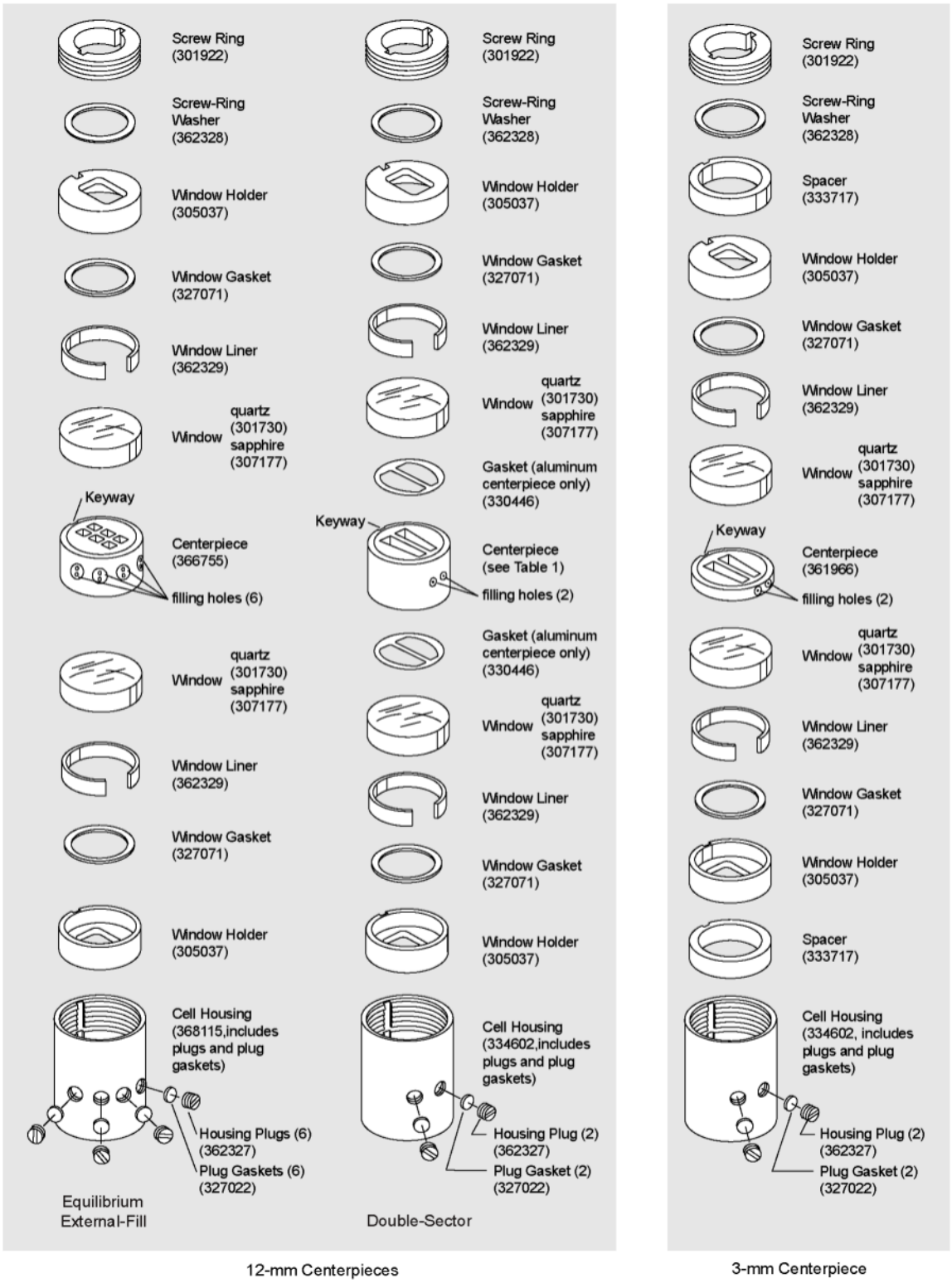
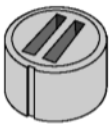
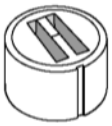


Figure 6.1: Exploded view of cells with different centerpieces.

Type	Material	Part Number	Fill Volume (mL)	Recommended Maximum Speed (rpm)
Standard Double Sector 	aluminum	334623	0.45 per sector	60 000
	12-mm Epon charcoal-filled	306493	0.45 per sector	42 000
	3-mm Epon charcoal-filled	361966*	0.12 per sector	42 000
	Epon aluminum-filled	305685	0.45 per sector	42 000
Synthetic Boundary Capillary-type 	Epon charcoal-filled	331431	0.4 solvent 0.15 sample	42 000
	Epon aluminum-filled	306076		
Band Forming 	Epon charcoal-filled	331359	0.01 per well 0.35 per sector	42 000
Equilibrium Six-channel 	Epon charcoal-filled	331376†	0.12 per channel	48 000
Equilibrium Eight-channel 	Epon charcoal-filled	361851	0.025 per channel	50 000
Equilibrium Six-channel with External-Fill 	Epon charcoal-filled	368116‡	0.12 per channel	48 000

* Two spacers (333717) required.

† Housing kit (335402) required.

‡ Housing kit (368115) required.

Figure 6.2: Centerpieces for the An-50 Ti and An-60 Ti cells.

Table 6.2: Titanium centerpieces

	Nanolytics GMBH
Titanium, 2-channel, 12 mm[a]	810-12
Titanium, 6-channel, 12 mm[a]	810
Titanium, 2-channel, 3 mm[b]	810-03

[a] Due to the weight, cells with titanium centerpieces require a titanium counterbalance.

[b] Requires 2 spacers. Nanolytics also sells shorter housings which do not require spacers but can only be used for 3 mm centerpieces.

6.4 Cleaning

The cells are disassembled after use (see section 6.6). The windows and centerpieces are rinsed with warm water and placed in a tray for ultrasonic cleaning (Figure 6.3) with a mild detergent (LiquiNox) for 60 minutes. The windows are rinsed with water, ethanol, and milli Q water and then allowed to dry in a standing position on lens paper. The centerpieces are rinsed thoroughly to remove any detergent and rinsed with ethanol and left to dry standing on lens paper. The windows are gently polished with lens paper and reassembled into the window housing as shown below (Figure 6.6). The whole cell is then assembled and placed in a covered tray. Cell housings can be wiped with a Kim Wipe dampened with WD-40.

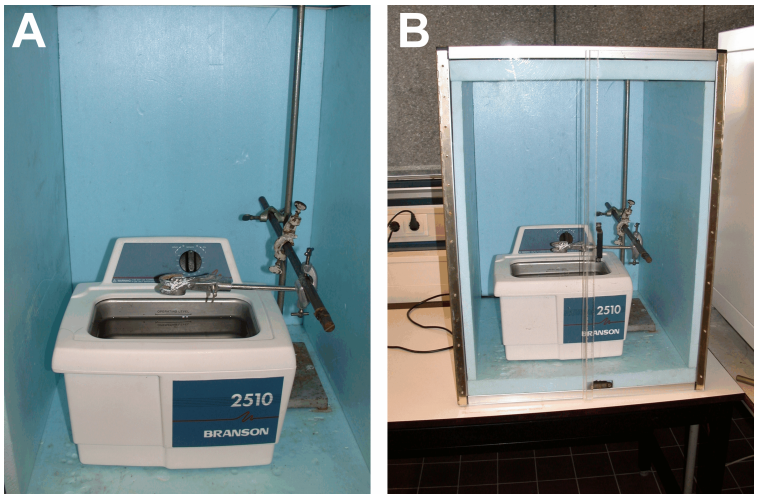


Figure 6.3: Ultrasonic bath (panel A and B). Note that ultrasonic sound may lead to impaired hearing or even loss of hearing, therefore the ultrasonic bath should be placed in a well isolated closet (panel B).

6.5 Cell Assembly

AUC cells for the An-50 Ti and An-60 Ti are most easily assembled when using the assembly tools shown in Figure 6.4.

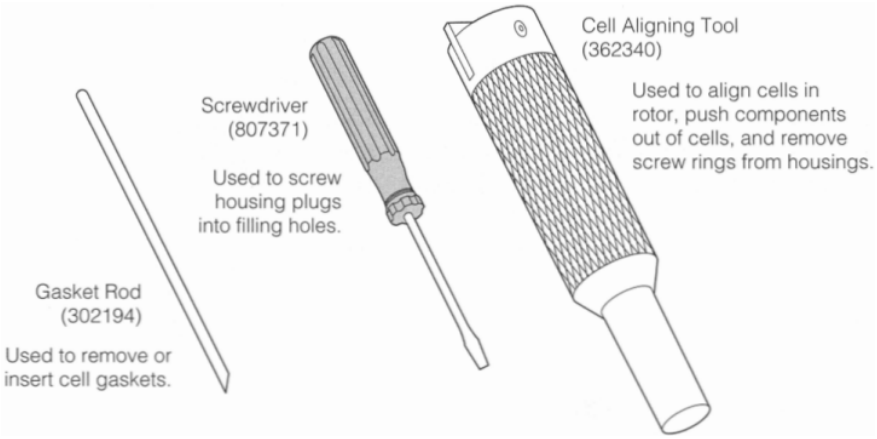


Figure 6.4: Required tools for cell assembly.

Also very convenient is a nitrogen pistol and a table for assembling and filling cells. For an example see Figure 6.5.

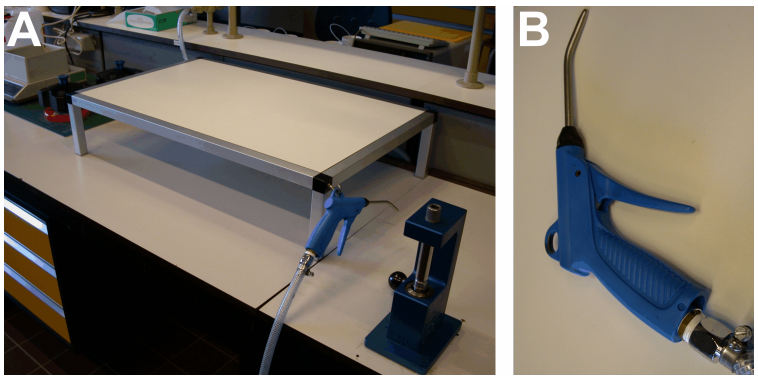


Figure 6.5: Convenient cell assembly facility. Panel A shows an overview (from left to right) of the table with a balance, rotors on their stands, cell assembly table with a hang-up for a nitrogen pistol (panel B) and the collet for torquing cells (also see Figure 6.10).

The windows are assembled as shown in Figure 6.6. The window gasket should be checked and changed when it becomes transparent indicating compression (Figure 6.6). The window liner is placed so that the open end is opposite of the alignment mark and the window inserted as shown with the arrow lined up with the keyway. The window should fit snuggly and the liner changed if it does not.

The bottom window assembly is pushed down to the bottom of the housing, window side up aligning the keyway with the key, the centerpiece inserted part number right side up, and the top window assembly inserted (Figure 6.7).

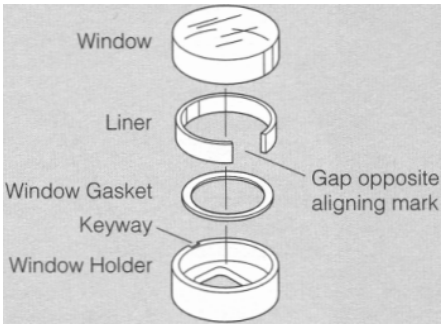


Figure 6.6: The assembly of the window in the holder. Window gaskets need to be changed periodically. Check to see if it has become transparent from compression.

f

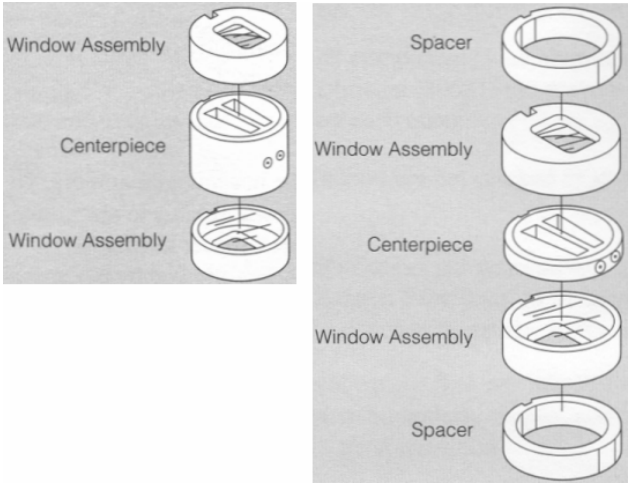


Figure 6.7: Stacking a standard double-sector centerpiece (left) or a 3-mm centerpiece, window assemblies with or without spacers.

Place the screw ring gasket and screw ring into the top and torque to 120 in-lbs (inch pounds). Over torquing may result in the warping of the cell or crushing of windows.

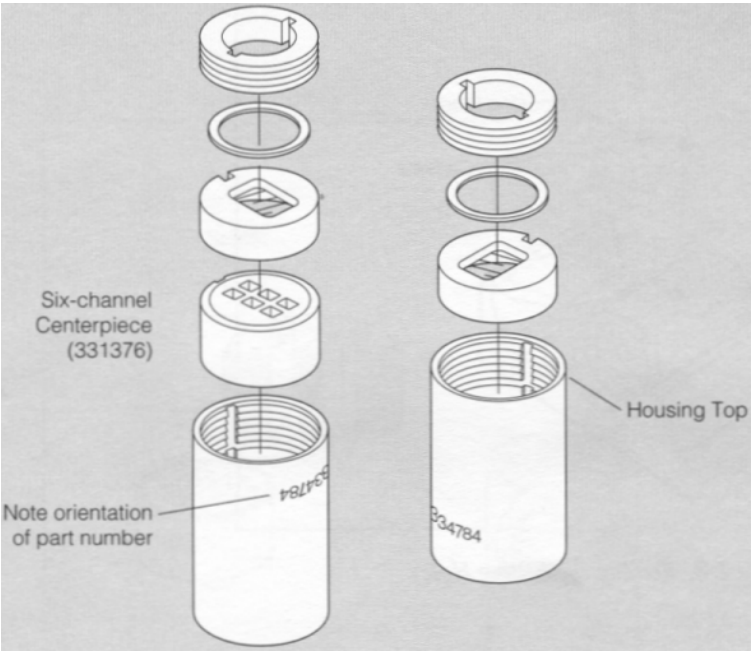


Figure 6.8: The assembly of six-channel cells. The bottom centerpiece is inserted from the bottom as shown and a window assembly placed in position. A screw ring gasket and screw ring are tightened to 60 in-lbs (inch pounds). The cell is inverted right side up and the part number placed to the left. The front row holds the reference buffer while the back row holds the three samples. The top window assembly is inserted and the top screw ring gasket and screw ring positioned and torqued to 120 in-lbs (inch pounds). The center of the keyway is used to align the cell in the rotor as there are no scribe marks.

Cell housing for 6-channel centerpieces have screw rings on both sides (Figure 6.8) unlike housing for 2-channel centerpieces. The centerpiece is inserted as shown in Figure 6.8 and the bottom window assembly is added. The screw ring gasket and screw ring and put in place and tightened to 60 in-lbs (using a torque wrench with a collet). The reference and samples are added as described and the top window inserted and then the screw ring gasket (screw ring washer) and screw ring (Figure 6.9). The entire assembly is torqued to 120 in-lbs (inch pounds) using a collet to hold the cell and a torque wrench for tightening (Figure 6.10). Use of SpinKote® aids in the proper torquing of the cells, but should be used sparingly.

6.6 Cell Disassembly

After centrifugation the cells need to be disassembled as soon as possible to prevent, in the case of Epon charcoal-filled centerpieces, the swelling or degradation of centerpieces. Essentially there are two three different types of cells namely, the external fill double-sector and six-channel centerpieces and the six channel centerpieces without external filling holes.

The cells with external filling holes can be emptied after removal of the plug screws and gaskets and before disassembling the cells. Generally, stable samples can be recovered this

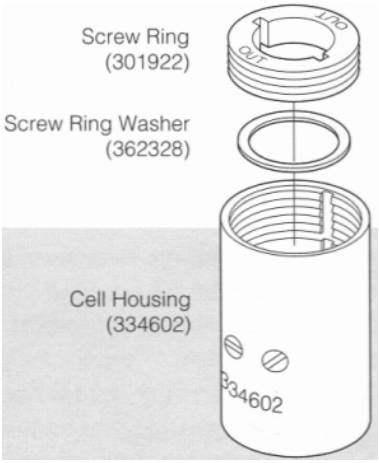


Figure 6.9: The screw ring washer and screw ring. Apply light coating of SpinKote® to gasket and screw ring. Torque to 120 inch-pounds.

Figure 6.10: Torque wrench (1) to tighten the assembled cell and a collet (2) to hold the cell in place.

way and this then makes AUC a non-destructive analysis method.

The six-channel equilibrium cells without external filling holes are a little more difficult to disassemble when the sample should be recovered, or when the cells contains harmful chemicals to which one should not be exposed. The disassembly is as follows. Untighten the bottom screw ring using the torque wrench and collet. Holding the cell horizontally, untighten the upper screw ring and take it out together with the screw ring washer (gasket). Now take out the bottom screw ring and washer. Push with your (index) finger the upper window down into the cell while holding it still horizontally. You can now take out the upper window holder together with the window gasket and window liner. Use the alignment tool (Figure 6.4) to push the upper window and everything beneath out of the bottom of the cell supporting the bottom window assembly with the left hand. Holding the cell assembly vertically, take of the upper window and recover your sample.

7 Cell and Rotor loading

Figure 7.1 panel A shows two different, accepted methods of loading cells. For pipetting, the user can piggy-back (stacking) at round, gel-loading tip onto a 1000 μL tip to load 400500 μL of reference buffer or sample. Air must be allowed to escape from the chambers as fluid is pushed into the centerpiece; otherwise, the fluid will be pushed out. Concentrated samples and those containing detergents are more difficult to load. A continual, slow injection will help to prevent problems. Once the reference buffer and/or samples are loaded, a small gasket covers the hole and a small brass screw is placed in the hole and tightened (Figure 7.1 panel B). Over-tightening of the brass screw can cause warping of the cell housing and make it difficult to push into the rotor hole.

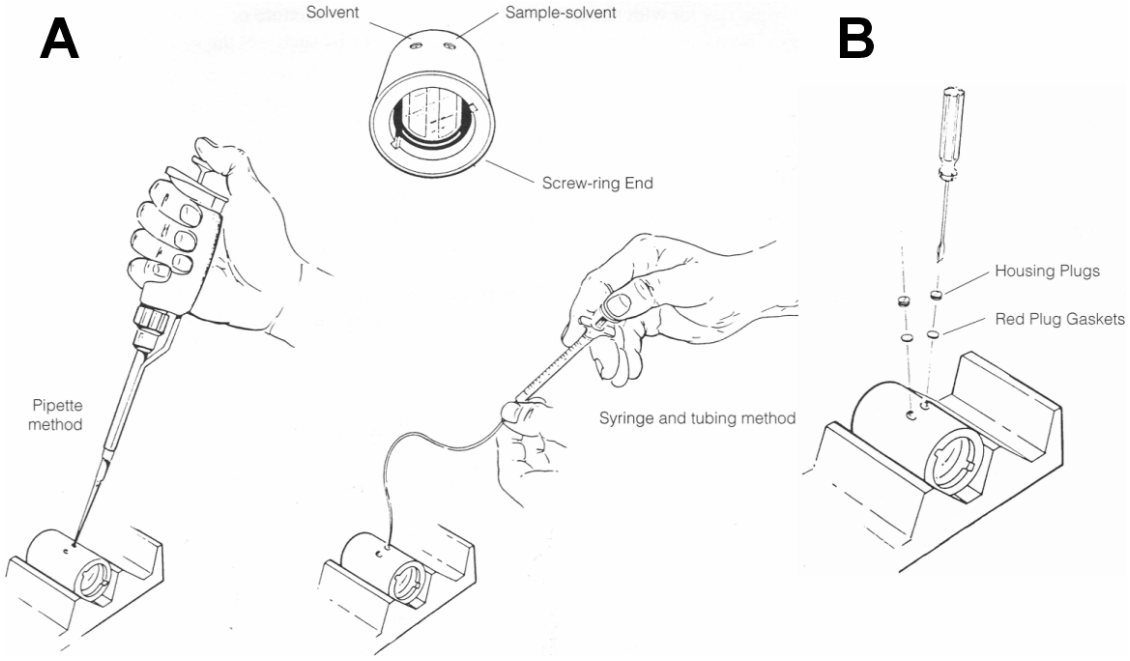


Figure 7.1: Cell loading techniques (panel A) and the sealing of the filling holes of a cell (panel B).

The cell is inserted into the proper hole of the rotor (the holes are labeled on the rotor). The fill holes are to the inside and the wider end of the sector shaped centerpiece is to the outside. A cell alignment tool (Figure 6.4) assists in inserting the cell. The cell is then aligned with the scribe marks of the cell aligning with those on the rotor. Spin Analytical sells a tool for cell alignment. Nitrogen gas (Figure 6.5 panel B) is used to blow off dust and the rotor is placed on the spindle and the monochromator inserted and secured. The door is closed and the vacuum turned on.

To load a band forming centerpiece, the bottom window of a standard housing is inserted and the centerpiece inserted with the small well on the top. Approximately 15 μL of sample is injected into the well using a gel loading pipet tip. The top window is inserted and then the screw ring gasket (washer) and the screw ring. After torquing, 390 μL of reference buffer

is pipetted into the reference chamber and 375 μL of buffer containing D_2O or other suitably dense buffer is pipetted into the sample chamber. Cover the holes with gaskets and screws as before (Figure 7.1). Do not spin the cell until the run is ready to start. Once started, the sample is forced out of the well and onto the dense buffer.

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A Performance Check of the AUC

1. Intensity scan (Xenon-flash lamp emission spectrum) at 6.5 cm: the peak at 229 nm must have an intensity of 15,000 counts or higher, otherwise clean the lamp.
2. Check the wavelength calibration: peaks should be at 229 and 527 nm (see scan recorded in step 1). If not OK then calibrate the wavelength emission spectrum.
3. The intensity scans (through windowless cell) at 5.9, 6.5, and 7.1 cm must overlay.
4. The intensity scans at 5.9, 6.5, and 7.1 cm must overlay.
5. Intensity scan at 280 and 400 nm must not deviate by more than 20% throughout the cell and intensity values for sample and reference must be the same.
6. Perform a noise check (absorbance mode) at 190, 400, and 800 nm through a windowless cell. The standard deviation of the OD, (B(Y) column if using Origin®: spreadsheet program shipped with the Beckman AUC), should be no more than listed in Table A.1.
7. Perform a Radial calibration check (velocity intensity scan through the counterbalance). Top should be at 5.85 cm (Reference only) and bottom at 7.15 cm. Lines should be vertical, otherwise the slit may be polluted.
8. Check for stray light using a $9\,\mathrm{g\,L^{-1}}$ NaBr "cut-off" solution against HPLC-grade water reference. This solution does not pass any wavelength less than 200 nm. At a wavelength of $195 \pm 4\,\mathrm{nm}$, the AU (absorbance unit) of the trace should rise sharply to a value of $\geqslant 1.7\,\mathrm{AU}$. Stray light causes the AU to maximize at about $\geqslant 1.7\,\mathrm{AU}$. Since $\%\mathrm{T}10^{-A} \cdot 100$, or $\mathrm{A} = \log(100\%\mathrm{T})$, this represents less than 2%T. If no stray light were present, the OD below 200 nm would rise to infinite AU. Below 200 nm values should be more or equal to 1.7 AU. Excessive stray light is most probably caused by a dirty grating. You must then replace the monochromator assembly with an exchange unit.
9. Perform an OD check using the photometer Check Solutions Kit.
10. If everything is fine than you are ready to perform your measurement!

Table A.1: Standard deviations ODs through windowless cell

λ[a](nm) (nm)	σ[b] (OD)
190	0.010
400	0.009
800	0.019

[a] Wavelength.
[b] Standard deviation.

B Sequence of Calibrations

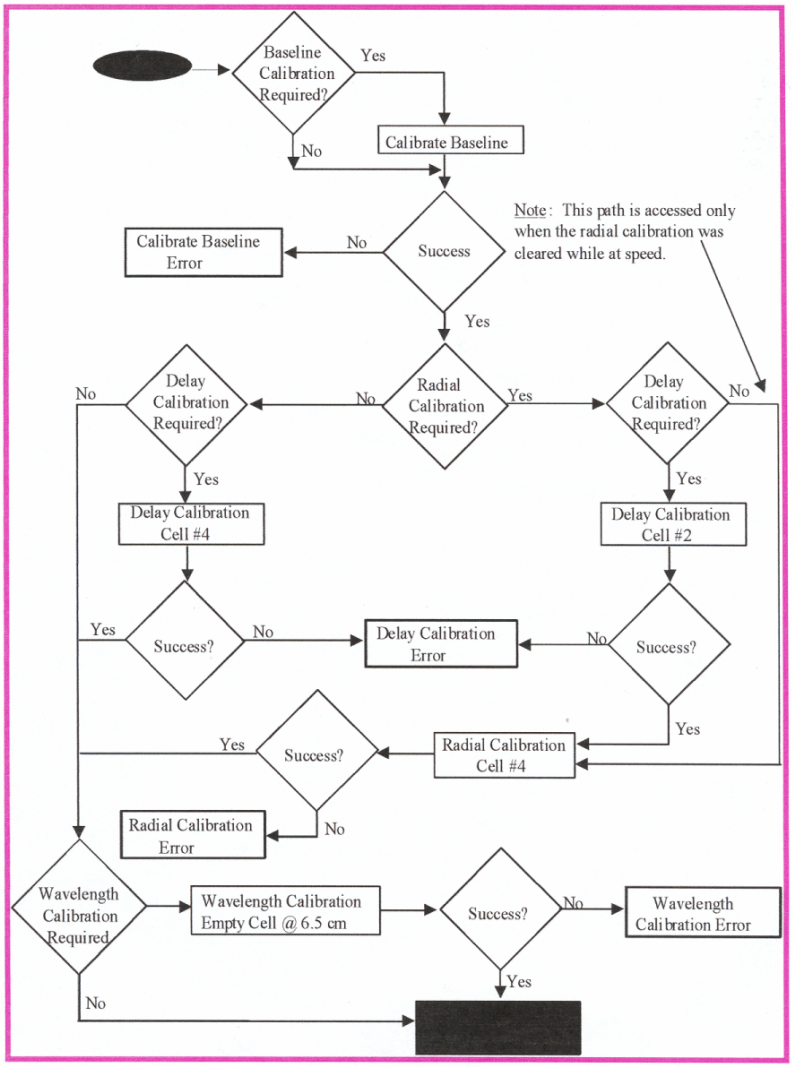

Figure B.1: Sequence of calibrations that are performed prior to a sedimentation run. Note that the rotor hole through which a calibration is performed depends on the rotor (An-50 Ti or An-60 Ti) and on the type of calibration. Calibrations are performed at 400 nm. This scheme represents the calibrations performed if using an An-60 Ti (4 hole rotor).