# ANALYTICAL ULTRACENTRIFUGATION

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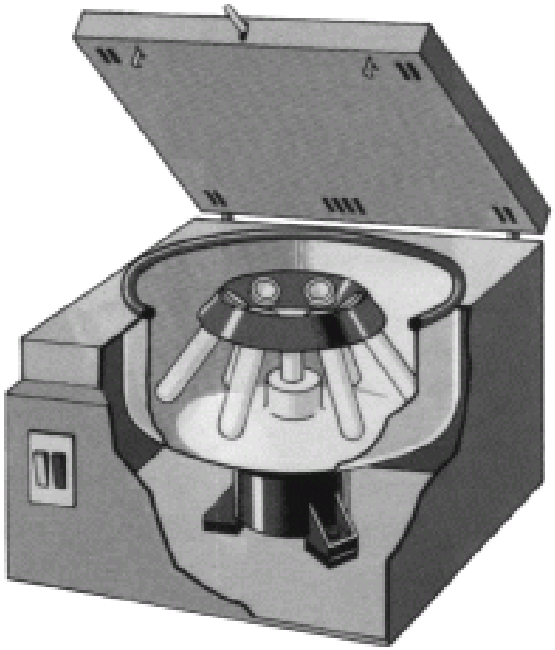
# What is an Analytical Centrifuge?



http://1.bp.blogspot.com/-qynUSyJF-_I/UOP29BV1byI/AAAAAAAAAjE/5-kyJoaWL3o/s1600/centrifuge.gif

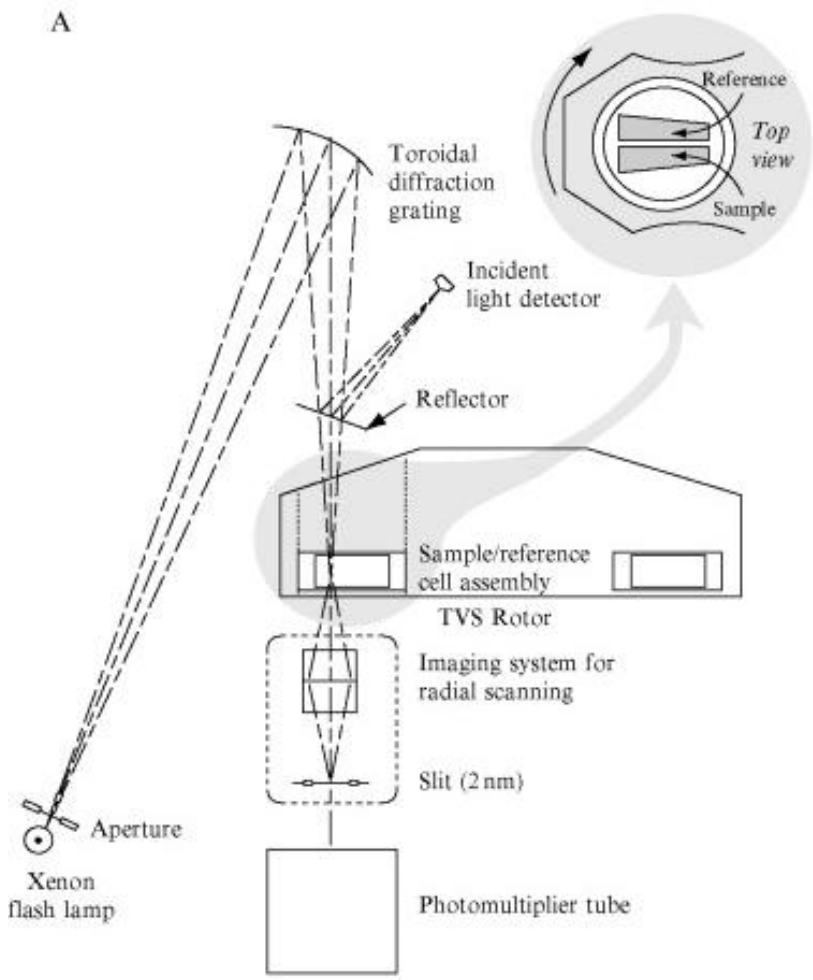
- What we typically think of as a centrifuge
- Used in biological studies to sediment and separate out components

http://bitc2-preview.sr.unh.edu/TRC/IMAGES/AUC/AUC.gif

- An analytical centrifuge
- Detectors are used to monitor the sample as the sedimentation occurs

# Rotor

- Typically spins at 60,000 revolutions per minute
- While the rotor is spinning, 1g has the apparent weight of 250 kg
- During rotation a optical receptor records data from the slide each time it passes
- There is a set counterweight to keep ensure the centrifuge is never off-balance

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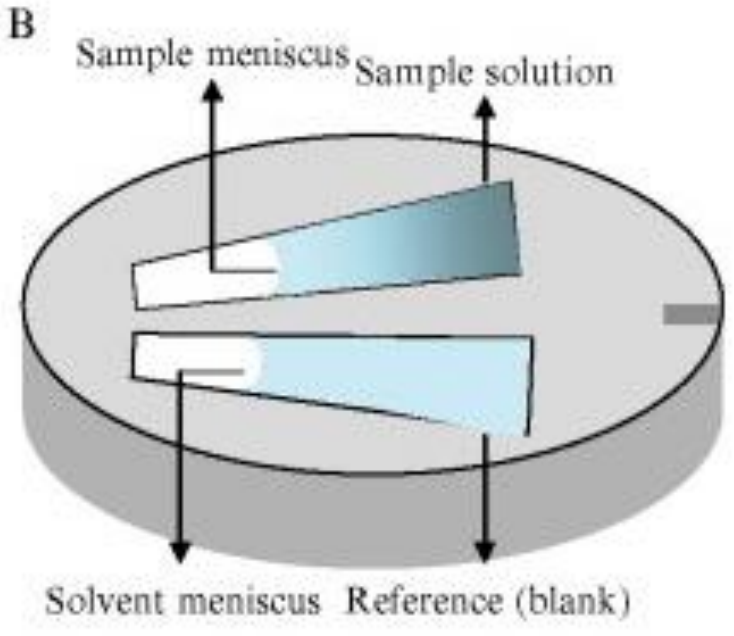
# Cells

- Cells contain a sample and a control sector
  - One contains a solution with solvent and analyte and the other just solvent
- Sectors are trapezoidal
  - The walls emanate radially from the center of the rotor
  - This is to ensure that the particles do not hit the sides of the sectors as they move radially away from the center of the rotor

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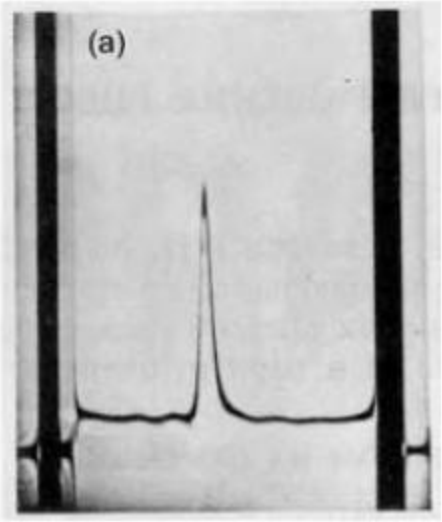
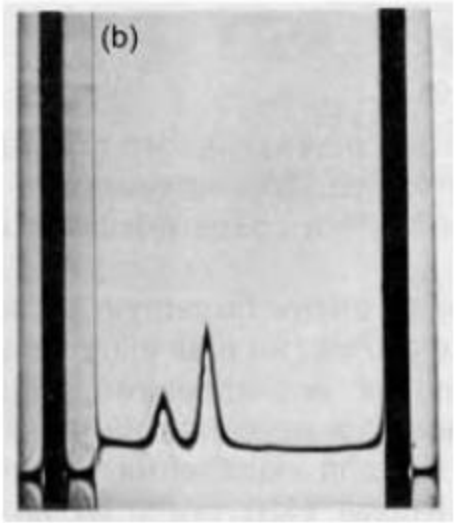
# Schlieren Optical System

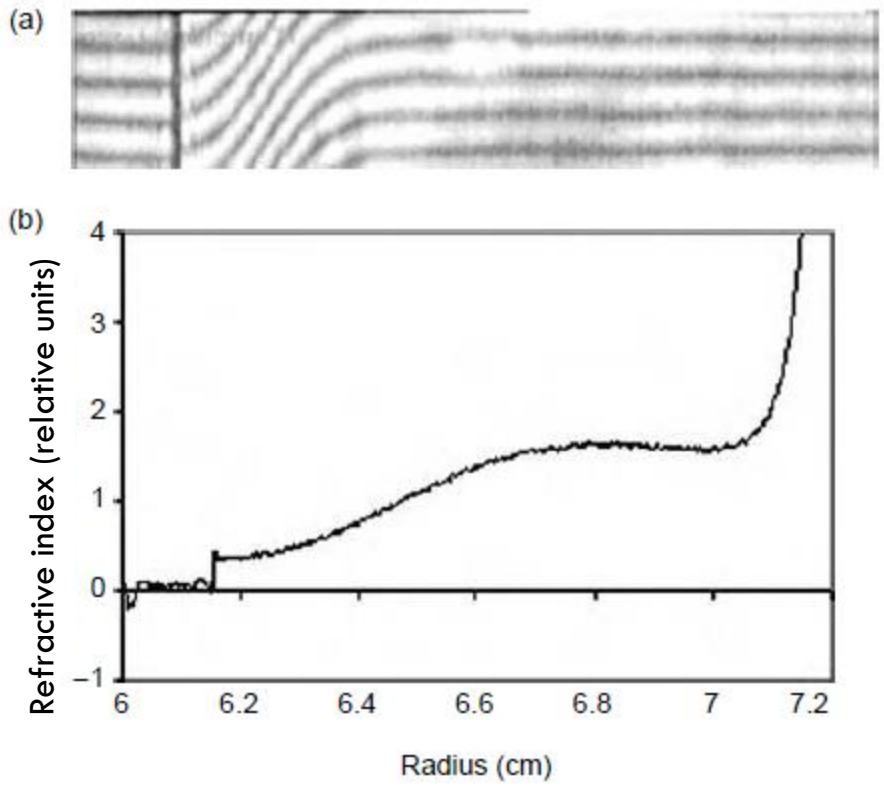
- Light is passed through the cell at a specific point
- The concentration at that point is changing due to sedimentation
- This results in a varying degree of refraction
- This change in radial distance is measured by the displacement of an image on a camera which is then plotted versus time



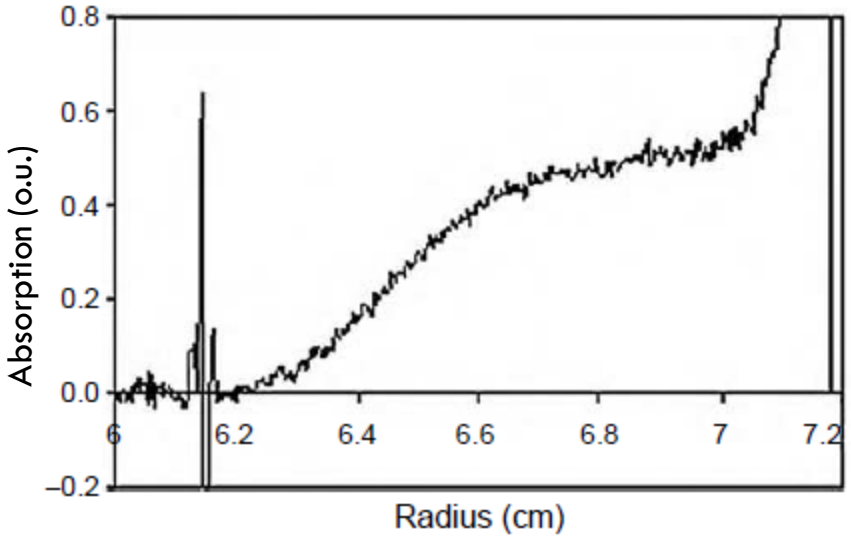
# Rayleigh Optical System

- Light get passed through the solvent and the sample and the diffraction pattern created is recorded
- In order to analyze the data, it is plotted as refraction index versus radius in the cell
- You expect to see a greater refractive index at the far end of the cell due to sedimentation
- Good for macromolecules that don't absorb in the UV range



# Absorption Optical System

- Measuring absorbance versus radius in the cell
- The higher the concentration, the stronger the absorbance
- Nucleic acids absorb very strongly at ~260 nm and proteins absorb very strongly at ~280 nm
- Good for macromolecules that absorb in the UV range



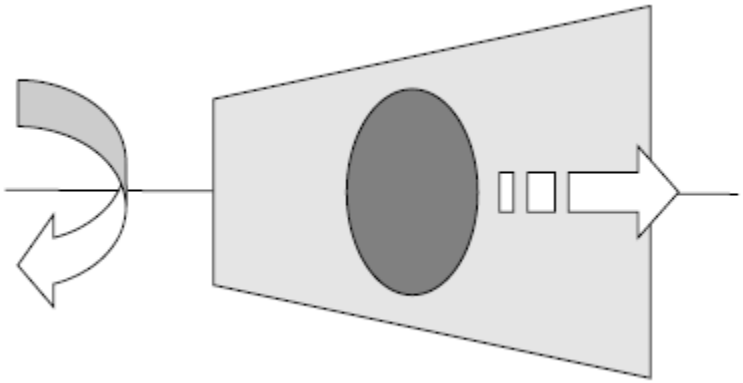
# The Lamm Equation

$$\frac{\delta C}{\delta t} = -\frac{1}{r}\left\{\frac{\delta}{\delta r}\left[\omega^2 r^2 sC - Dr\frac{\delta C}{\delta r}\right]\right\}$$

- Equation for the change in concentration over change in time
- Used to predict what the boundary shapes will look like

# Sedimentation Constant

- Two forces acting on the particle
  - Buoyant Force
    - $F_b = m_o \omega^2 r$
      - $m_o$ is the weight of the fluid displaced by the particle
  - Frictional Force
    - $F_d = fu$
- So the net force on the particle is:
  - $F_{net} = m_o \omega^2 r + fu$



$\omega$ – angular velocity
r – distance from the center of rotation
u – velocity of molecule
m – mass of the particle

# Sedimentation Constant

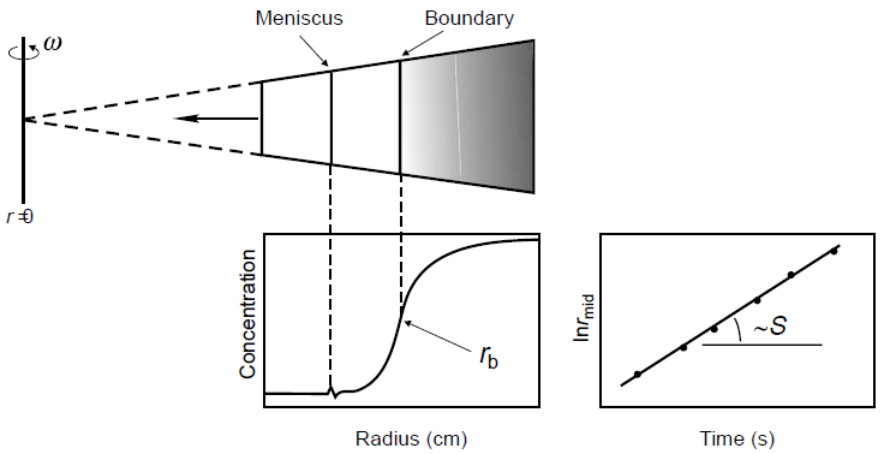
- The net force on the particle is equal to the mass times the acceleration which is this case the acceleration is the angular velocity squared times the radius
  - $F_{net} = ma = m\omega^2 r = m_o\omega^2 r + fu$
- From this we can get:
  - $(m - m_o)\omega^2 r = fu$
- The mass the particle displaces is the volume the particle takes up times the volume of the solution
  - $m_o = V_{particle}\rho_o$
- The volume of the particle is equal to the mass of the particle times the partial specific volume
  - $V_{particle} = m\bar{v}$
- We can then combine these formulas to find that:
  - $m_o = m\bar{v}\rho_o$

# Sedimentation Constant

- We can then plug this back into our net force equation:
  - $fu = m\omega^2 r - m\bar{v}\rho_o\omega^2 r = m\omega^2 r(1 - \bar{v}\rho_o)$
- We then place all of the variables that are experimentally known on the left side of the equation, and define this as the sedimentation constant
  - $s = \frac{u}{\omega^2 r} = \frac{m(1-\bar{v}\rho_o)}{f}$
- Sedimentation constant is in seconds and usually around $10^{-13}$
  - Often uses Svedberg units (S)
  - 1 S = $10^{-13}$ s

# Data Analysis

- The velocity of the concentration boundary is the change in its radial distance over the change in time and this velocity is related to the sedimentation constant from our previous definition
  - $u = \frac{dr_b}{dt} = s\omega^2 r$
- We can then rearrange this formula and take the integral of both sides
  - $\int \frac{dr}{r} = \int s\omega^2 dt \longrightarrow \ln r = s\omega^2 t$
- Using this formula we can solve for the sedimentation constant by plotting the time versus the log of the radial distance of the concentration boundary
- The slope of the plot is $s\omega^2$ and since $\omega$ is known we can find the sedimentation constant from the plot



# Analyzing Heterogeneous Solutions

- What happens when the solution has multiple macromolecules in it?
  - If the solution is spun slowly, the smallest molecules will not sediment out quickly enough (or at all) and will not produce a useful gradient
  - If the solution is spun very fast, the largest molecules sediment out too fast and will not be able to be observed
- To account for this the speed is ratcheted up after a set time step in order to be able to observe a peak for each macromolecule type in the solution

# Standardizing Sedimentation Constants

$$s_{20,w} = s_{exp} \frac{1 - \bar{\nu}\rho_{20,w}}{1 - \bar{\nu}\rho_{exp}} \frac{\eta_{exp}}{\eta_{20,w}}$$

- For biological macromolecules, the sedimentation constants are usually taken in solutions of varying density and viscosity so the sedimentation constant is adjusted to the standard of 20°C in water

- Information taken from:

Serdyuk, Igor N., Nathan R. Zaccai, and Joseph Zaccai.*Methods in Molecular Biophysics: Structure, Dynamics, Function*. New York: Cambridge University Press, 2007. Print.