Principles of Operation

System Operation

The following simplified functional description outlines the operation of the DW-2000. For details of a particular mode of operation, refer to MODES OF OPERATION later in this section.

The DW-2000 contains two lamps: a tungsten (VIS) lamp and a deuterium (UV) lamp. When both lamps are operating and the selected scan dictates the use of both lamps, the DW-2000 automatically changes from one lamp to another at 330 nm (unless otherwise specified in the Olis software).

Mirror M2 collimates the impinging beam(s) from the UV or VIS lamp onto either grating 1 (for one wavelength) or onto gratings 1 and 2 (when two wavelengths are used). Each grating is blazed at 300 nm to enhance UV sensitivity. The diffracted (monochromatic) beam(s) produced at the grating is focused by mirror M3 on the exit slit.

After passing through the exit slit, the beam(s) passes through the zoom lens and beam-balance dual attenuator. The zoom lens keeps the aperture image focused in the center of the sample compartment, regardless of the wavelength. The attenuator is used to equalize beam intensities. The beam(s) is then reflected by the rotating chopper mirror and mirrors M5 or M6, depending on the operational mode. In the double-beam mode, the incoming single beam is split in two. One beam passes through the sample cuvette and the other beam passes through the reference cuvette. In the dual-wavelength modes, both beams pass through the sample cuvette and shutter 2 is closed to block the beam produced by mirror M5. The beam-balance split attenuator is used in the double-beam mode to attenuate either the sample or reference beam.

After the beam passes through the sample or reference cuvette, the beam enters the beam scrambler. The beam scrambler consists of an elongated light-confining tunnel with a diffuser at the entrance. Light entering the beam scrambler is diffused to form a uniform field of illumination at the exit end of the tunnel. The optical mixing of light beams eliminates any special differences between the beams when they strike the PMT cathode at the output of the beam scrambler.

When using highly scattering samples, you may remove the diffuser from the beam scrambler to increase light throughput. The beam scrambler is most effective during the double-beam mode of operation and is not essential in the dual-wavelength mode. When using the optional magnetic stirrer, the beam scrambler should be in place to eliminate the influence of the magnetic field on the PMT. The beam scrambler cannot be used with the Stopped-flow accessory or the Total Fluorescence accessory.

The output of the PMT is converted from a current to a voltage signal and applied to the wide band acquisition amplifier. Since the PMT signal contains both sample and reference signals at different times, these signals are separated by timing synchronization (sync) pulses referenced to the rotating chopper mirror. When the sample beam is present, a sample sync pulse is applied which enables this circuit to accept and amplify the input signal voltage. Likewise, when the reference beam is present, the reference sync pulse is applied to the circuit.

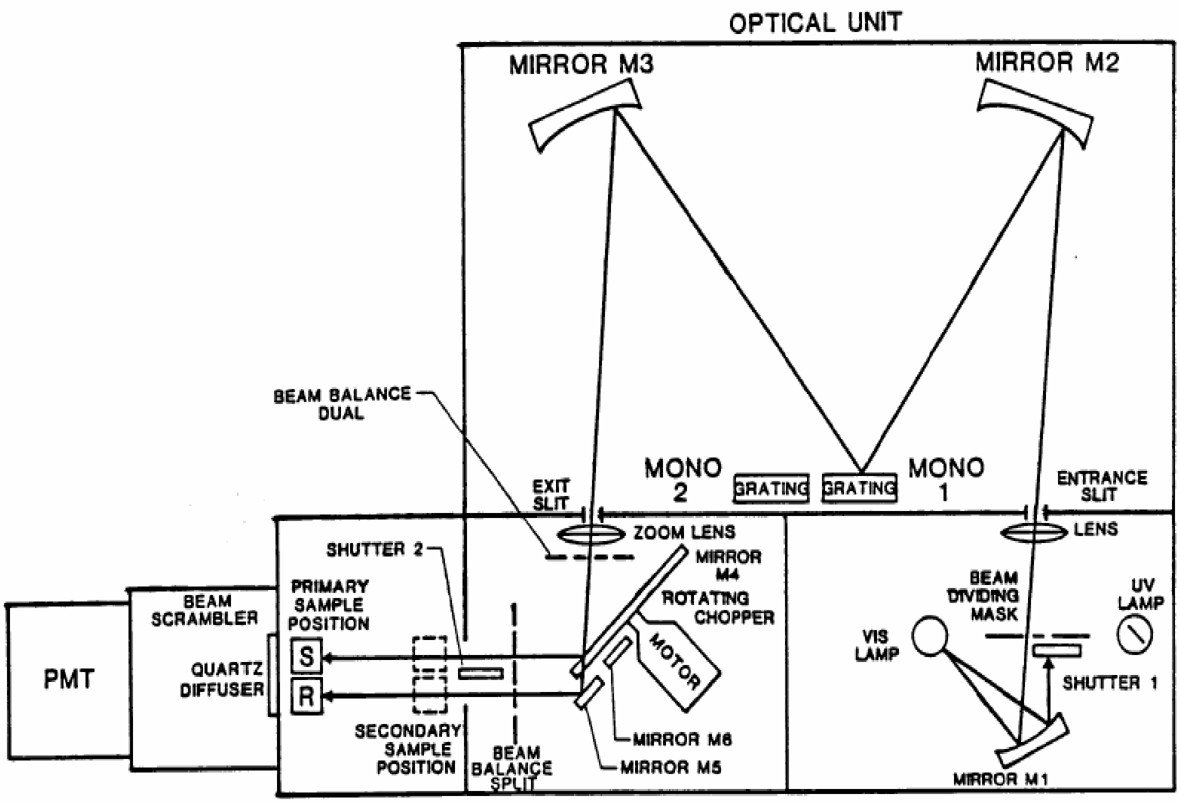
Modes of Operation

Double-Beam Scanning and Non-Scanning Mode

In the double-beam mode, differences or absolute spectra are recorded in absorbance, % transmittance, external input and absorbance, and external input and %T, with a single monochromator scanning across the wavelength range of interest or at a single wavelength of interest. To record a baseline, fill reference and sample cuvettes with identical solutions and place them in the sample compartment cuvette holders. A single monochromatic light beam is chopped by the rotating chopper mirror and reflected alternately through the reference and sample cuvettes. This chopped radiation from one beam minimizes any inequalities between the sample and reference beams and produces a relatively flat baseline. After storing the baseline, the sample is altered chemically or physically and the spectra taken.

Optical and Electrical Signal Paths in Double-Beam Mode

Mirror M1 focuses radiation from either the VIS or UV lampoon the entrance slit to monochromator 1. In double-beam mode, shutter 1 is positioned so that only one beam passes through the beam dividing mask. Mirror M2 collimates the beam and MONO 1 grating diffracts it. Mirror M3 focuses the resulting monochromatic radiation on the exit slit. The zoom lens corrects for chromatic aberration and automatically maintains the beam focused at a fixed point in the sample compartment, regardless of wavelength. The rotating chopper mirror M4 and the stationary mirror M5 alternately reflect the beam to the sample and reference cuvettes, respectively. The beam-balance split attenuator is adjusted to attenuate the reference or sample beam to equalize the signals of both beams.



Aminco DW-2000 double-beam mode functional diagram

Dual-Wavelength Non-Scanning Mode

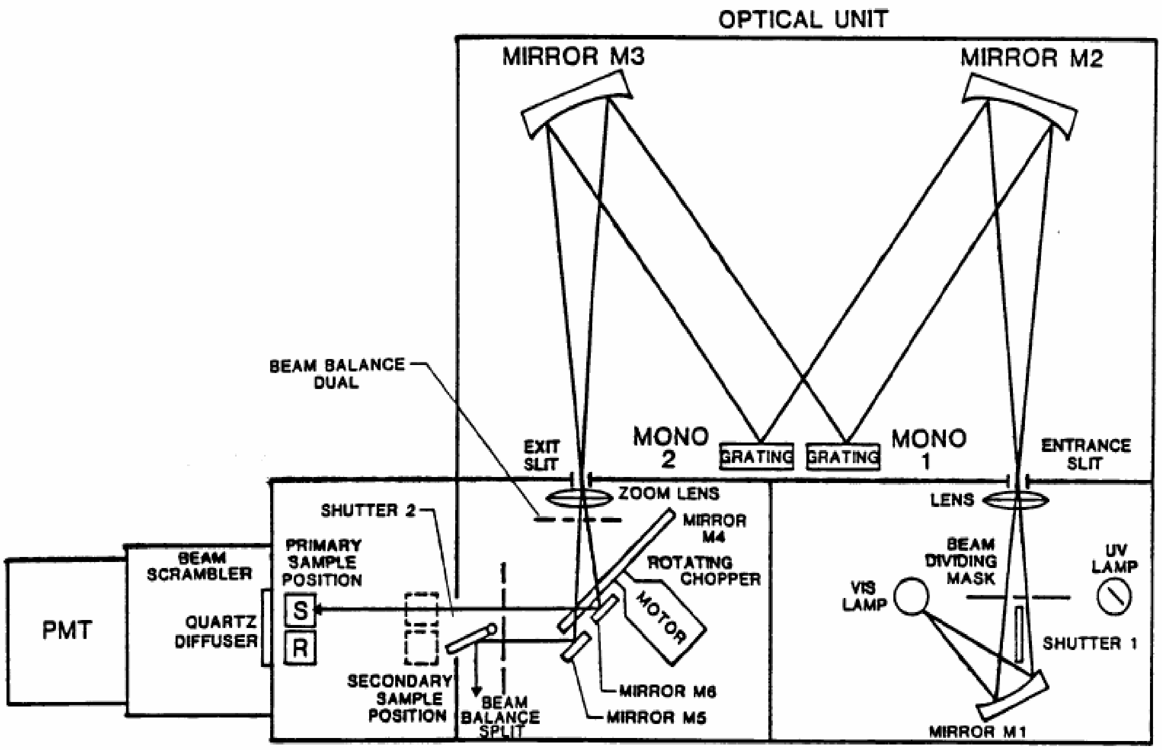
In the dual-wavelength mode, differences or absolute spectra are recorded in absorbance, % transmittance, external input and absorbance, and external input and %T by passing two monochromatic light beams of different wavelengths alternately through a single cuvette. This method allows monitoring the kinetics of small absorbance changes which would normally be hidden in a highly absorbing and/or highly scattering background. Since both beams pass through the same portion of a single cuvette, the effects of sample settling and scattering are cancelled. Generally, an isobestic point (wavelength at which the absorbance of the sample is the same for the two chemical states) is selected as the reference wavelength and a nearby absorption peak or valley is chosen as the sample wavelength. The intensities of the two beams are equalized by attenuating the higher intensity beam. When the sample is either physically or chemically changed, the absorbance value at the reference (isobestic) wavelength remains constant. Starting with zero difference, it is possible to measure very small absorbance changes that occur in the sample.

Optical and Electrical Signal Paths in Dual-Wavelength Mode

Mirror M1 focuses radiation from either the VIS or UV lamp on the entrance slit to monochromators 1 and 2. In the dual-wavelength modes, the position of shutter 1 allows two beams to pass through the beam dividing mask. Mirror M2 collimates these beams. The MONO 1 grating diffracts one of the beams and the MONO 2 grating diffracts the other beam. Mirror M3 focuses the resulting monochromatic beams on the exit slit. The two beams are equalized in intensity by the beam-balance dual attenuator. This equalization negates the absolute absorbance of the sample at the selected wavelengths and minimizes the effects of scattering. After being equalized, the two beams are alternately reflected through the sample cuvette as follows:

The rotating chopper mirror has four separate blades, evenly spaced. Each revolution of the motor corresponds to four cycles. One half of each cycle consists of the time one of the blades is intercepting the beam, and the other half cycle when the beam is passing between two blades. During a portion of the chopper mirror M4 cycle, the chopper mirror reflects the MONO 1 beam to the sample cuvette. At this time, the chopper mirror blocks the MONO 2 beam. When the chopper mirror passes out of the path of the two beams, mirror M6 reflects the MONO 2 beam into shutter 2 which blocks the MONO 1 beam. Thus, during one half of the chopper mirror cycle, the MONO 1 beam is reflected to the sample cuvette and during the other half of the cycle, the MONO 2 beam is reflected to the sample cuvette.

After passing through the sample cuvette, the beam scrambler shapes the MONO 1 and MONO 2 into uniform fields of illumination which strike the cathode of the PMT. The PMT generates two time-separated current signals which are converted to sample and reference voltage signals in the wide band preamplifier stage. The rotating chopper mirror produces synchronization pulses which are used to gate the reference and sample signals.



Aminco DW-2000 dual-wavelength mode functional diagram