Lab 2: CDOM absorption **11 July 2017**

LABORATORY SAFETY ISSUES – isopropyl alcohol for cleaning ac-meters; general laboratory safety

INTRODUCTION

The major absorbers in seawater are **water** itself, chromophoric or colored dissolved organic matter (**CDOM**; in older literature, the subscript 'g' was used for gelbstoff – yellow substance– or gilvin), and **absorbing particles** (including phytoplankton and non-algal particles, NAP). This lab introduces Beer's Law and its application in a standard benchtop spectrophotometer and the WET Labs absorption and attenuation meters (ac-9 and ac-s).

The benchtop spectrophotometer (Station 1) provides estimates of absorbance, **A**, which is dimensionless and based on log 10. The in situ absorption meters (Station 2) provide estimates of the absorption coefficient, a, with units of m^{-1} and based on the natural log. Typically a is reported as the spectral absorption coefficient with the designation (λ). Please see Appendix 1 for review of Beer's Law. Note the conversion between A (log 10) and a (natural log) is:

$$a = A \cdot (0.434 \cdot L)^{-1} = 2.304 \cdot A \cdot L^{-1}$$

where 'L' is the pathlength of the instrument measured in m. The spectral absorption coefficient of CDOM ($a_{CDOM}(\lambda)$) is operationally defined as the absorption of seawater or freshwater that has been passed through a filter MINUS the absorption of a high quality water blank (such as Milli-Q type 1 water with a UV oxidizing cartridge <http://www.millipore.com/lab_water/clw4/type1>). Typically a 0.2 μm plastic filter such as a Sartoris filter or a glass fiber filter such as a Whatman G/FF filter with a nominal pore size of 0.7 μm is used. The choice of filter pore size is important and dependent upon the question you are asking.

CDOM analyses should be carried out as soon as possible after water collection and filtration because colloid formation can continue after filtration. Typically samples are collected in amber glass bottle. Sampling and processing containers should be clean.

Several things to consider in the filtration and analysis of the CDOM sample are:

- inclusion of viruses and small bacteria in the filtered fraction,
- colloidal nature of the material passing through the filter (how does a colloid form, and could colloids form while you are processing?),
- effective pore size of the filter (0.2 μm vs. 0.7 μm),
- does the effective pore size of the filter change as a function of filter loading?
- role of salts and colloidal-size particles in scattering,
- quality of the 'pure' water blank (how pure is pure? how do you know it is pure?),
- chemical and absorptive nature of the dissolved organic matter (DOM) and adsorbed minerals.

WATER SAMPLES: water samples will be filtered through a nominally 0.2 μm water.

Water samples should be at room temperature. Record the temperature.
(Save water in white buckets for fluorescence lab on Friday; store in dark).

1. DRE - Damariscotta River Estuary water (collected at dock)
2. Biscay Pond (a freshwater 'tea' lake)

STUDENTS divide into six groups of 3 or 4 students per group. Three groups will start in the lecture classroom (Station 1), three groups will start in the Mitchell classroom (Station 2). They will switch after ~2 hours.

Station 1: Investigating the role of pathlength and concentration on Beer's Law
Part A. Each group will investigate the role of *pathlength* on Beer's Law
Each group is responsible for measuring the absorbance of the water samples in the spectrophotometer using a single cuvette (either 0.5 cm, 1 cm, 2 cm, 5 cm, or 10 cm). Collectively the class will pool their data in order to examine the effect of geometric pathlength on absorbance, **A**, and absorption, **a**. Check off the cuvette size used on the sheet by the spectrophotometer to ensure that data from all are used.

- Spectrophotometer Setup: Menu: **wavelength scan**; Mode: **Abs**; scan from **850 nm** to **390 nm** at **1 nm intervals** with **slow** scan speed.

- Cuvettes are pre-cleaned and ready for use. Clean them when you have completed your scans. Wear gloves and never touch the optical faces of the cuvettes. Use only lens paper, not Kim Wipes. Rinse cuvette with sample 3 times before filling 75% full for scanning. Always start with your cleanest, lowest CDOM water samples, transitioning to your highest CDOM samples. Visually inspect the cuvette to ensure there are not particles or bubbles in the sample and that the windows are clean and dry. Ensure the cuvette is always placed in the cuvette holder in the same orientation.

- Fill cuvette with fresh Milli-Q water, place in holder. Run 100% Abs scan as a baseline.

- When completed, press Start to run the Milli-Q water as a baseline corrected scan (this is the minimum resolution achievable).

- Measure the absorbance spectra of the following water samples: reverse osmosis (RO), tap, DRE and BP. For each sample save the scan for later analysis using the naming convention of water type, pathlength and date, e.g. DR-0p5-20170711 for DRE measured in a 0.5-cm cuvette today, or RO-10p0-20170711 for reverse osmosis measured in a 10-cm cuvette.

- Derive the absorption coefficients at the specified wavelengths. For each of the natural waters, plot the absorbance, A, versus the geometric pathlength for the cuvettes. On a separate graph, plot the absorption coefficient, a, versus geometric pathlength. Plot the results for the wavelengths 300 nm, 412 nm, 500 nm, 600 nm and 712 nm on each graph using distinct symbols.

Part B. Each group will investigate the role of *concentration* on Beer's Law

Position a microscope light on one side of the tank and a light detector on the other.
Measure the incident (I_0) and transmitted light (I_t) before you begin adding dye.
Add 0.5 mL drop of green dye to the tank, mix, measure and record transmitted light (I_t).
Continue adding dye until $I_t = 0.5\ I_0$. Plot I_t/I_0 vs. mL dye.

Incident, I_0, light reading _____

$V_{dye\ added}$ (ml)	**Cumulative $V_{dye\ added}$**	**Transmitted Light reading, I_t**	**I_t/I_0**
0	0		
0.5 mL	0.5 mL		
0.5 mL	1.0 mL		

Station 2: Measuring CDOM absorption spectra with an in situ absorption meter
Each group will be assigned to either a WETLabs ac9 or acs instrument. They will prepare the instruments by cleaning them, running a sequence of pure water calibrations (with Milli-Q water), then measuring the absorption spectra for the assigned natural water samples (0.2 μm-filtered Damariscotta River Estuarine water –DRE– or 0.2 μm-filtered Biscay Pond –BP– water). Note that all instruments have 25-cm geometric pathlengths.

Clean the sensor windows and tubes prior to measurements with lens paper and ethanol or isopropyl alcohol. Measure the temperature of every sample. If salinity of the Damariscotta River Estuary is not available, assume a salinity of 28.

➔ Focus on getting good pure water calibrations: each student should run her/his own Milli-Q water cal (either a-tube or c-tube of the ac-meter, or both). Save files in your group's folder. Note the magnitude and shape of the pure water spectra; they should be repeatable to within the instrumented resolution between calibrations (i.e. 0.005 m-1 for absorption; 0.01 m-1 for attenuation).

Run the filtered water samples in both the a-tube AND c-tube of the ac-meter. Collectively you will be able to compare the CDOM absorption of DRE water between an ac9 and an acs, the CDOM absorption of DRE and BP waters with an acs, and the CDOM absorption of both waters with in situ versus benchtop spectrophotometers. Remember to save the files in your group's folder:

Group A – acs with 0.2 µm-filtered DRE water
Group B – ac9 with 0.2 µm-filtered DRE water
Group C – acs with 0.2 µm-filtered Biscay Pond water

Instructions and code for processing data will be provided at the lab.

ASSIGNMENTS – DIVIDE THE WORK AND CONQUER! Come prepared to deliver a briefing tomorrow morning (feel free to reorganize within/among groups as you see fit).

BEER'S LAW:

CONCENTRATION The tank is an analogue spectrophotometer with light source, sample (water with green dye) and detector. Unlike the Varian benchtop spectrophotometer, the light is not strictly monochromatic.

(1) Plot I_t/I_0 vs. volume of dye.

(2)How does absorbance depend on concentration?

PATHLENGTH

(1) Plot absorbance, **A**, from the benchtop spectrophotometer vs. the width of the cuvette (the geometric pathlength) at 300 nm, 412 nm, 532 nm.

(2) Convert the ac-meter absorption coefficient at 412 nm and 532 nm to absorbance (natural log to log base 10). Recall that a (m^{-1}) = 2.303 * A/ L(m) and that the pathlength of the ac meters is 25 cm, so:

$A(\lambda)= [a_{CDOM}(\lambda) * 0.25\ m)]/ 2.303$.

Add these data to the plot of **A** vs. pathlength for 412 nm and 532 nm.

(3) How does absorbance, **A**, depend on geometric pathlength? How does it compare amongst the three wavelengths?

SPECTROMETER BLANKS:

All spectrometers (Varian, ac-meters, etc.) depend on a pure water blank.

1) For the Varian benchtop spectrophotometer, compare the RO and tap water spectra measured against the MilliQ water baseline. Would RO and tap water be sufficient as a blank? Is that true for all wavelengths?

2) For the ac-meters, you used Milli-Q water as the blank.

- how does your blank compare to the device file?
- why could your blank be different from the device file (factory blank)?
- what are the symptoms of a bad 'pure' water calibration?
- what, in addition to bad water, could contribute to a poor calibration?

TEMPERATURE EFFECT

1) For the ac-meters, calculate the spectral absorption coefficient, $a_{CDOM}(\lambda)$, of DRE and Biscay Pond waters for both the absorption flow tube (a tube) and the attenuation flow tube (c tube). Perform or consider the following:

- Apply the Milli-Q pure water calibration
- Perform corrections for temperature (at the time of measurement) and salinity (Biscay Pond is freshwater and assume DRE has a salinity of 28)
- How would your results change if you used a temperature correction that was 2º C too high or too low? Is there a diagnostic pattern that suggests an incorrect temperature correction?
- How would your results change if you used a salinity correction that was 1 unit too high or too low? 5 units too high or too low?
- Is there any difference between the $a_{CDOM}(\lambda)$ for the **a** tube vs. the **c** tube? Are these consistently different between or among samples?

2) Convert 10 cm Varian absorbance values to absorption coefficients for 705 nm to 725 nm: $a_{CDOM}(\lambda) = 2.303 \cdot A(\lambda) / L$. Compare corrected ac-meter and Varian data for the field samples in this waveband.

- Are these values equal to zero?
- Is there justification for forcing these values to zero, and subtracting the average from all other wavelengths? See below on spectral slopes (are they sensitive to NIR values?).

SPECTRAL SLOPE:

Calculate the spectral slope of the CDOM absorption coefficient, S_{CDOM}, for DRE and Biscay Pond water. For the ac-meters, the slope will be for the visible range only. Convert the Varian spectrophotometer values of A to a. The rule of thumb is that absorbance values should be maintained between 0.1 and 0.4. Are all of your CDOM absorbance spectra within this range?

- using data from the Varian benchtop spectrophotometer, compute the spectral slope over the ranges 390nm – 700 nm; 412nm – 676 nm, 412 nm – 712 nm
- using the ac meter data, compute the spectral slope over 412 nm – 676 nm
- how does the computed spectral slope vary:
    - with wavelength range?
    - with absorbance magnitude?
    - Between the ac-meters and spectrophotometer for the range 412 nm – 676 nm?
    - depending upon the computation method (non-linear vs linearized estimates)
    - if wavelengths longer than 700 nm are omitted vs included?
    - between water sources (natural variations)?

Equation for and calculation of spectral slope:

$$- S_{CDOM} (\lambda - \lambda_{REF})$$

$$a_{CDOM}(\lambda) = a_{CDOM}(\lambda_{REF})\ e$$

where $a_{CDOM}(\lambda)$ is the amplitude of the absorption coefficient at any wavelength λ (Jerlov, 1976) or at the reference wavelength, λ_{REF} (usually 412 or 440 nm). See Carder et al. (1989) and Blough and Del Vecchio (2002) for a discussion of the interpretation of the spectral slope.

The best method to calculate the slope is to minimize the square difference between the exponential model and the data (possibly weighed by a different error in each wavelength if the uncertainty varies as function of wavelength, e.g. due to variability in source intensity as function of wavelength). The relative (percent) error is not constant spectrally; in the red the absorption is low and the signal-to-noise high. Slope measurements often exclude red wavelengths due to its sensitivity to temperature (e.g. the 715 nm channel in the ac-9).

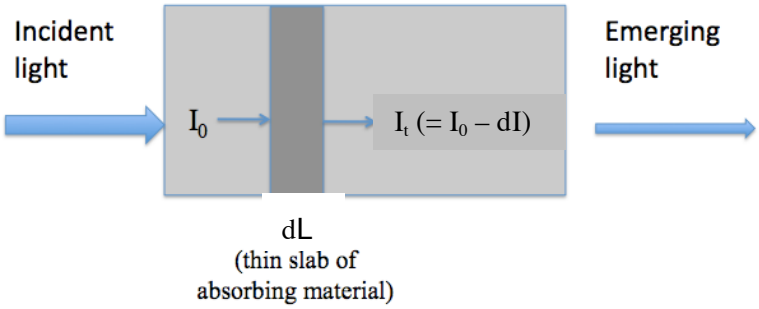
You may write your own code to determine the slope by non-linear exponential regression (we will also supply code: http://misclab.umeoce.maine.edu/software.php);

OR,

less rigorously, you may determine the spectral slope for $a_{CDOM}(\lambda)$ by plotting the natural-log-transformed values of a_{CDOM} vs. wavelength using Excel and adding a trend line (this is the same as if you fit an exponential curve in Excel, try it). If you use the latter method, *is the slope linear?*

APPENDIX 1 REVIEW OF BEER'S LAW

http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm



-dI	$= I_0 \cdot (\varepsilon \cdot C) \cdot dL$	(-dI is fraction of light absorbed in thin slab)
$-dI / I_0$	$= \varepsilon \cdot C \cdot dL$	
I_t	$= I_0 \exp-(\varepsilon \cdot C \cdot L)$	(integrating over entire pathlength)
I_t / I_0	$= \exp-(\varepsilon \cdot C \cdot L)$	(also termed transmittance)
I_0 / I_t	$= \exp(\varepsilon \cdot C \cdot L)$	
$\ln I_0 / I_t$	$= \varepsilon \cdot C \cdot L = a \cdot L$	

where:

I_0 is intensity of light before it passes through the sample,

I_t is the intensity measured at the detector after light passes through the sample,

I_t / I_0 is unitless,

ε is the molar absorption coefficient – a measure of how much light a 1 M solution of dye will absorb (m^2 mol^{-1}),

C is the concentration of the dye (mol m^{-3}), and

L is the path length that the light must travel through the solution (m).

NB: Here we combine terms "$\varepsilon \cdot C$" into a single term "a", the absorption coefficient (m^{-1}).

Notice that the Beer's Law equation is written in log base e (natural logarithm, ln). However, spectroscopists historically used log base 10, rather than log base e. The principle is the same but A, the absorbance output from the spectrophotometer, is log base 10. Also, chemists include pathlength in A, because all measurements are typically made using same pathlength; hence, A is reported with as dimensionless and the value of A will change with pathlength.

A	$= \log_{10}(I_0/I_t)$
	$= \log_{10}e \cdot \ln(I_0/I_t)$
	$= 0.434 \cdot \ln(I_0/I_t) = 0.434 \cdot (a \cdot L)$
a	$\mathbf{= A \cdot (0.434 \cdot L)^{-1} = 2.303 \cdot A \cdot L^{-1}}$

Remember from calculus, that when changing log bases: $\log_a X = \log_b X \cdot \log_a b$.
To covert a natural logarithm to a base 10 logarithm, multiply by $\log_{10} e$ (=0.434).
To covert a base 10 logarithm to a natural logarithm, multiply by $\log_e 10$ (=2.303).

Limitations of the Beer-Lambert law
The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- deviations in absorptivity coefficients at high concentrations due to electrostatic interactions between molecules in close proximity
- pathlength amplification due to scattering of light by particulates in the sample
- fluorescence or phosphorescence of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

Source: http://www.chemistry.adelaide.edu.au/external/soc-rel/content/beerslaw.htm

BACKGROUND MATERIAL ON SPECTROPHOTOMETRY
General principles of operation of spectrophotometer:
Web sites that present good reference material on the fundamentals of UV-visible spectrometry:
http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/UV-Vis/uvspec.htm#uv1
http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/InfraRed/infrared.htm

Across the Spectrum: Instrumentation for UV/Vis Spectrophotometry
Slightly modified and shortened from Shane Beck, 1998, *The Scientist*, 12(3): 20.

Modern spectrophotometry was pioneered by Dr. Arnold Beckman in the 1940's.

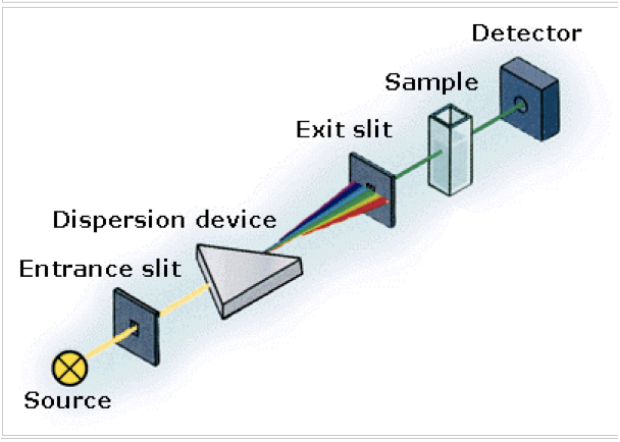
1. Light source: typical UV/Vis spectrophotometers utilize two light sources: a deuterium arc lamp for consistent intensity in the UV range (190 to 380 nm) and a tungsten-halogen lamp for consistent intensity in the visible spectrum (380 to about 800 nm). Some spectrophotometers have a xenon flash lamp.

2. Dispersion of light into different wavelengths can occur before and/or after the light passes through the sample. The monochromator disperses light into different angles by prisms or holographic gratings. NB: with a prism, the angle of dispersion can be nonlinear and sensitive to changes in temperature. In contrast, holographic gratings eliminate nonlinear dispersion and are not temperature sensitive; they are glass blanks with narrow ruled grooves. The grating itself is usually coated with aluminum to create a reflecting source. Gratings do require filters since light is reflected in different orders with overlapping wavelengths.

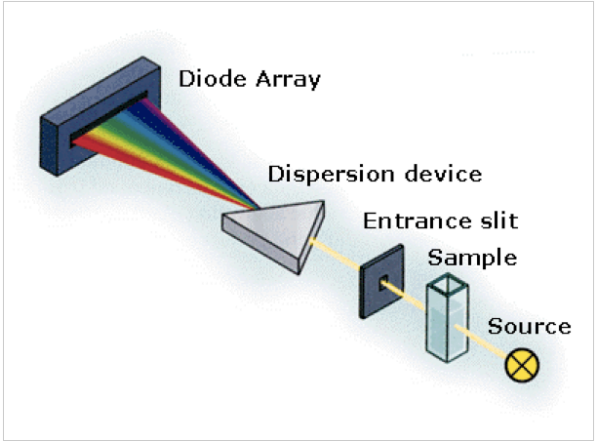
3. Light passing through the monochromator exits as a band. The width of this band of light at half the maximum intensity is the spectral bandwidth. Bandwidth comes in to play with regard to accuracy, since the accuracy of any absorbance measurement is dependent on the ratio of the spectral bandwidth to the natural bandwidth of the substance

being measured. The natural bandwidth is the width of the absorption band of the sample at half the absorption maximum. As a rule, a ratio between spectral bandwidth and natural bandwidth of 0.1 or less will generate absorbance measurements 99.5 percent accurate or better. Above this, accuracy deteriorates.

Adapted from Hewlett-Packard's "Fundamentals of Modern UV-Visible Spectroscopy" publication number 12-5965-512E, 1996.



Adapted from Hewlett-Packard's "Fundamentals of Modern UV-Visible Spectroscopy" publication number 12-5965-512E, 1996.



4. Sample absorbance is determined by comparing the intensity of the light passing through the sample and hitting the detector vs. intensity of light passing through a blank. Detectors include: a) photomultiplier tube, with good sensitivity throughout the UV/Visible spectral range and highly sensitive at low light levels; or b) photodiode with a wider dynamic range, and consisting of a semiconductor and a capacitor to charge the semiconductor. As light hits the semiconductor, electrons flow through it, thereby lowering the charge on the capacitor. The intensity of light of the sample is proportional to the amount of charge needed to recharge the capacitor at predetermined intervals. Often the detector is composed of a photodiode array, with photodiode detectors arranged on a silicon crystal so a spectral scan is instantaneous.

In single-beam spectrophotometers, like the Variance, the blank and sample are not measured simultaneously. Interspersing measurements of samples and blanks are needed to correct for lamp drift. Dual-beam spectrophotometers utilize a "chopper" or beam splitter that alternates the light path between the reference optical path and sample optical path to the detector at a speed that minimizes medium- or long-term effects of lamp drift. Some dual beam instruments scan continuously so that the sample, blank and dark reference are actually performed at different wavelengths (leading to a skewing effect as a function of wavelength, dependent upon scan speed); in others there is a phase locked wavelength drive so that the sample, blank and dark reference readings occur at the same wavelength. Blanks should be refreshed to prevent sample warming, or kept in a cooled holder.

5. If the sample is not a pure solution, scattering can occur. An integrating sphere can be used to collect scattered light, and correct the instrument reading to provide true absorption. The coatings on integrating spheres are highly scattering so as to ensure that the light field within the sphere is isotropic and therefore measuring a small portion of that light is equivalent to measuring it all. However, the coatings are also particularly absorptive of UV and blue radiation, which limits their utility in the UV range.

APPENDIX 1 - BACKGROUND MATERIAL ON IN SITU SPECTROPHOTOMETERS

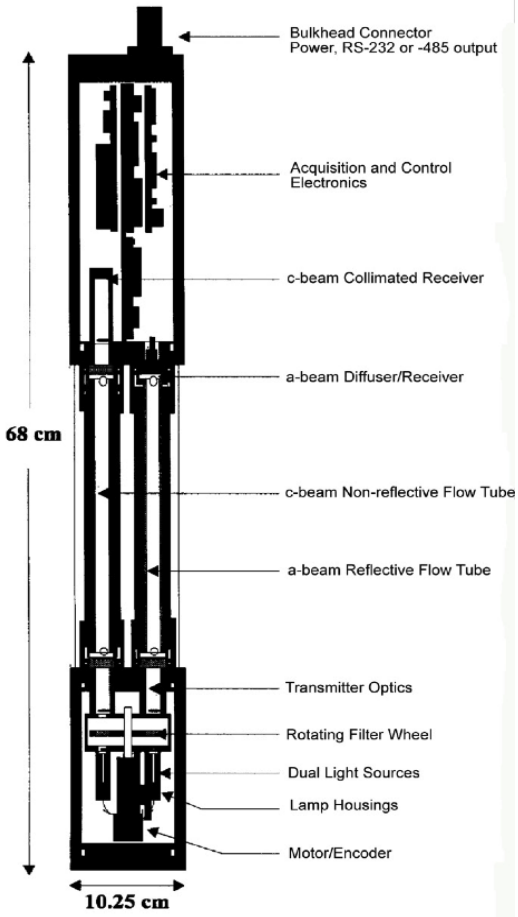


Figure 1. General schematics of WET Labs ac9.

General principles of operation of the ac-9:
The only commercially available mature *in situ* absorption meter is manufactured by WET Labs (Figure 1) http://www.wetlabs.com.
Some important issues related to using the ac-9 can be found in Pegau et al. 1995; Pegau et al. 1997; Bricaud et al. 1995; Zaneveld et al (1994); Twardowski et al., 1999; Roesler and Boss (2007); Leymarie et al., (2010).

Schematics (Figure 2)
1. Light source: Incandescant bulb
2. Dispersion of light into different wavelengths is done by a filter wheel with 9 filters. The filter wheel spins at 6 Hz yielding 6 spectra per second. The filter band width is 10 nm.
3. A collimated beam of light passes through the sample and onto a diffuser and a single diode detector (in the case of a; to maximize the capture of forward scattered light) or into a narrow angle detector (in the case of c, to minimize the capture of forward scattered light).
4. Sample absorption is determined relative to a pure water calibration provided by the factory (contained in the device file, ac-90nnn.dev, where nnn is the instrument serial number). Given the tendency for drift and alignment issues, it is standard practice to run your own pure water calibration prior and subsequent to each experiment.

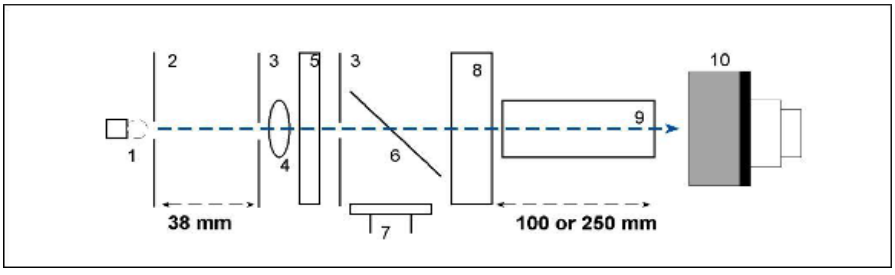


Figure 2. Schematic Representation of absorption beam optics

1 Lamp
2 1 mm aperture
3 6 mm aperture
4 38 mm singlet lens
5 Interference filter
6 Beam splitter
7 Reference detector
8 6 mm quartz pressure window
9 Reflective flow tube
10 Diffuser/Signal detector

Figure 3. Filter wheel of acs.

The hyperspectral version of the ac-9 is called the ac-s. Although similar in design the filter wheel holds two sections of a Linear Variable Filter (LVF), centered 180 degrees from each other on the filter wheel (Figure 3). The two filter sections are cut from a single LVF such that a portion of the spectrum around 550 nm is covered by both filters. This overlap is to allow for merging of the data from both filter sections (data generally display a slight error at this overlap that needs to be corrected for). Each filter covers approximately a 72 degree section of the beam path across the filter wheel. The filter wheel rotates at a tightly controlled 8.0 rps, such that the shorter wavelength of each filter section is traversed before the longer wavelength.

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