Lab 2: CDOM absorption **9 July 2013**

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

INTRODUCTION

The major absorbers in seawater are water itself, chromophoric or color-absorbing dissolved organic matter (CDOM; in older literature, the term ‘g’ for Gelstoff was used; the British used Gilvin), and absorbing particles. The symbol for the absorption coefficient is [a] with units of m^{-1}, typically reported as the spectral absorption coefficient with the designation (λ). The lab also introduces the WET Labs absorption and attenuation meters (ac-9 and ac-s) and Beer’s Law.

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. For most of this class, we will use only G/FF filters.

➔Ask yourself if the difference in filter type or nominal pore size should affect the magnitude of the CDOM signal. Also ask whether the quality of the water blank matters. Ask how you could determine the water quality.

CDOM analyses should be carried out as soon as possible after water collection and filtration because colloid formation can continue after filtration; sampling and processing containers should be clean.

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

- inclusion of viruses and bacteria in the filtered fraction,
- colloidal nature of the material passing through the filter,
- changing effective pore size of the filter as a function of filter pad loading,
- role of salts and colloidal-size particles in scattering,
- quality of the ‘pure’ water blank, and
- chemical nature of the dissolved organic matter, DOM, and adsorbed minerals.

STATIONS:

1 – measure **A** (absorbance, unitless) with a spectrophotometer,
2 – measure **a** (absorption, units of m^{-1}) with ac-9 or ac-s.

WATER SAMPLES: water samples will be filtered through a nominally 0.7 μm Whatman G/FF filter. For the spectrophometric group, DRE water will also be filtered through a 0.2-μm Sartorius membrane filter. (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 (freshwater)

STUDENTS divide into six groups of 3 or 4 students per group:

	1st session	2nd session
Group 1	Station 1 – spectrophotometer in MJP Lab: 1 cm cuvette	Station 2 – Mitchell Classroom: 0.2 μm-filtered DRE water; ac-s
Group 2	Station 1 – spectrophotometer in MJP Lab: 5 cm cuvette	Station 2 – Mitchell Classroom: 0.2 μm-filtered Biscay Pond water; ac-s
Group 3	Station 1 – spectrophotometer in MJP Lab: 10 cm cuvette	Station 2 – Mitchell Classroom: 0.2 μm-filtered DRE water; ac-9
Group 4	Station 2 – Mitchell Classroom: 0.2 μm-filtered DRE water; ac-s	Station 1 – spectrophotometer in MJP Lab: 1 cm cuvette
Group 5	Station 2 – Mitchell Classroom: 0.2 μm-filtered Biscay Pond water; ac-s	Station 1 – spectrophotometer in MJP Lab: 5 cm cuvette
Group 6	Station 2 – Mitchell Classroom: 0.2 μm-filtered DRE water; ac-9	Station 1 – spectrophotometer in MJP Lab: 10 cm cuvette

STATION 1 – measure A (absorbance with unitless dimensions) with a bench-top spectrophotometer (Cary-50).

Three groups (3 or 4 students per group). Each group will perform the same measurements but will use a different pathlength cuvette (1 cm, 5 cm or 10 cm); collectively as a class, pool data to examine effect of pathlength (L) on absorbance (A).

1. run Cary-50 calibration protocol;
2. review settings – set scan limits to 300 – 800 nm at medium scan speed;
3. cuvettes have been pre-cleaned with RBS detergent; follow general guidelines:
   - don't touch optical surfaces; wipe optical surface with lens paper, NOT Kimwipes;
   - make sure all water samples are at room temperature;
   - rinse cuvette 3 times with a few mL of sample to remove previous sample;
   - look through cell to ensure that there are no visual in-homogeneities (bubbles, residual mixing/turbulence between fresh and salt water sample, particles);
   - place cell in holder in **EXACTLY** same orientation every time (dot on cuvette faces same direction, larger cuvettes are tipped in the same direction).
4. blank – use fresh but degassed Milli-Q type 1 water as baseline and store so spectrum will automatically be subtracted from all measurements;

BLANKS (& PSEUDO BLANKS) – DO ALL	**SAMPLES – DO ALL**	**PATH LENGTH**
Mill-Q water	0.2 µm-filtered DRE	1 cm (Group 1, 4)
RO (reverse osmosis) water	G/FF filtered DRE	5 cm (Group 2, 5)
tap water	0.2 µm-filtered Biscay Pond	10 cm (Group 3, 6)

STATION 2 – measure a (absorption with units of m^{-1}) with ac-meter.

Three groups (3 or 4 students per group). Each group will perform the same measurements, either on the ac-9 or ac-s. All instruments have 25-cm pathlengths.

Clean the sensor windows and tubes prior to measurements with lens paper and ethanol. Measure the temperature of every sample. Salinity of the Damariscotta River Estuary was 28 on 2 July.

➔ Focus on getting good 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.

Run the G/FF filtered water samples in both the a-tube AND c-tube of the ac-meter. Remember to save the files in your group's folder:

Group 4 – ac-s with 0.2 µm-filtered DRE water
Group 5 – ac-s with 0.2 µm-filtered Biscay Pond water
Group 6 – ac-9 with 0.2 µm-filtered DRE water

Instructions and code for processing data will be provided.

➔ ASSIGNMENTS – DIVIDE THE WORK AND CONQUER!

Come prepared to deliver a briefing tomorrow morning (feel free to reorganize as you see fit).

BLANKS:

1) Use spectrophotometer data and address whether the type of water used for the blank make a difference?

- which water gives the lowest reading: Milli-Q water or RO water or tap water? You will have zeroed the instrument with Milli-Q water, but is RO and tap water just as good? Is there a spectral variation? Why?
- how do you ensure that the cuvette is clean and properly placed?

2) For ac-s/ac-9 use only Milli-Q water as the blank:

- is the Milli-Q blank stable (e.g. when comparing between groups)? over what time interval? what might cause instability?
- how does your blank compare to the device file?
- why could your blank be different from device file (factory blank)?
- what are the symptoms of a bad Milli-Q calibration?
- how do you insure that the tubes and windows are clean?

DATA PROCESSING:

1) For the spectrophotometer samples, use A values with Milli-Q blank subtracted.

- calculate $a_{CDOM}(\lambda)$, converting A from log base 10 to natural log; ℓ is pathlength in meters:

$$a_{CDOM}(\lambda) = 2.304 \cdot A(\lambda) \cdot \ell^{-1}$$

- note that A is dimensionless, but a_{CDOM} has units of m^{-1}

2) For the spectrophotometer samples, what is the CDOM absorption coefficient at ~705-725 nm for field samples?

- are these values equal to zero (within the uncertainty of the blank)?
- is there justification for forcing these values to zero, and subtracting the average from all other wavelengths?
- use Excel or Matlab to fit an exponential function to the data. Are the slopes sensitive to the removal of the NIR value?
- Is the slope (exponent) sensitive to the method of determining them (log-linear fit vs. non-linear fit)?

3) For the ac-9/ac-s, calculate the spectral absorption coefficient, $a_{CDOM}(\lambda)$, for the field samples. First use data from only the absorption flow tube (a tube), then repeat the calculations for the attenuation flow tube (c tube). Apply:

- Milli-Q pure water calibration
- correct temperature and salinity corrections (Biscay Pond is freshwater and DRE had a salinity of 28 on 2 July)

- how would your results change if you used a temperature correction that was 2° C too high or too low?
- 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?

4) For the ac-9/ac-s, what is the CDOM absorption coefficient at ~705-725 nm (ac-s) or 715nm (ac-9) for the three field samples??
   - are these values equal to zero?
   - is there justification for forcing these values to zero, and subtracting the average from all other wavelengths?
   - use Excel or Matlab to fit an exponential function to the data. Are the slopes sensitive to the removal of the NIR value?

5) For the spectrophotometer data for the DRE (0.2 μm and G/FF filtrate), does the filter pore size affect the result (think about potential contribution by scattering)?

6) For the spectrophotometer data, is absorbance (A) a linear function of path length (Beer's Law)? Select several common wavelengths (i.e., 300, 350 and 414 nm) for the DRE and Biscay Pond (G/FF filtered water) and plot A (absorbance) from spectrophotometer vs. 1-cm, 5-cm, and 10-cm pathlengths. Is A a linear function of pathlength?

COMPARISON OF FIELD SAMPLES

1) Does the magnitude of CDOM absorption vary as a function of its source? Use 300 and 350 nm (spectrophotometers only) and ac-9 wavelengths (412, 440, 488, 510, 532, 555, 650, 676 nm) to compare a_{CDOM} for:
   - coastal waterDRE water (dock)
   - Biscay Pond (freshwater)

2) Does the spectral slope of CDOM vary as a function of its source AND/OR method to compute the slope?
   S_{CDOM} is the spectral slope of CDOM:

$$a_{CDOM}(\lambda) = a_{CDOM}(\lambda_{REF})\ e^{-S_{CDOM}(\lambda - \lambda_{REF})},$$

   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).

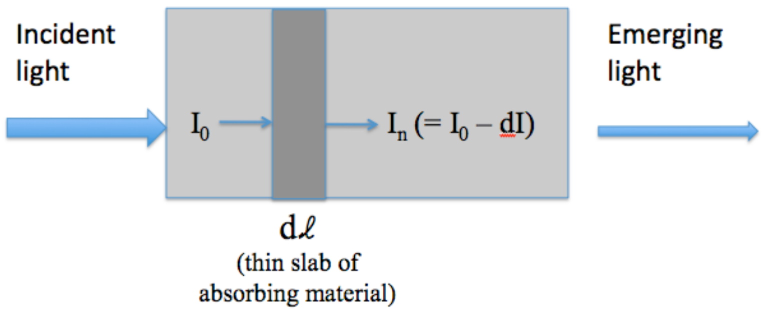
You may write your own code to determine the slope by non-linear exponential regression (we will also supply code: http://misclab.umeoce.maine.edu/software.php); OR,

less rigorously, you may determine the spectral slope for $a_{CDOM}(\lambda)$ by plotting the ln-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?*

- what is the spectral slope, S_{CDOM}, for the range 412 nm – 676 nm?
- is the slope constant over all wavelength regions?
  - what is the slope for 300 – 350 nm (spectrophotometer)?
  - what is the slope for for 350 – 450 nm (all instruments)?
- do spectral slopes vary between the water types?

APPENDICES REVIEW OF BEER'S LAW

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



$-dI$	$= I_0 \cdot (\varepsilon \cdot C) \cdot d\ell$	(-dI is fraction of light absorbed in thin slab)
$-dI / I_0$	$= \varepsilon \cdot C \cdot d\ell$	
I_n	$= I_0 \exp\text{-}(\varepsilon \cdot C \cdot \ell)$	(integrating over entire pathlength)
I_n / I_0	$= \exp\text{-}(\varepsilon \cdot C \cdot \ell)$	(also termed transmittance)
I_0 / I_n	$= \exp(\varepsilon \cdot C \cdot \ell)$	
$\ln I_0 / I_n$	$= \varepsilon \cdot C \cdot \ell = a \cdot \ell$	

where:

I_0 is intensity of light before it passes through the sample,
I_n is the intensity measured at the detector after light passes through the sample,
I_n / 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 $mole^{-1}$),
C is the concentration of the dye (mole m^{-3}), and
ℓ is the path length that the light must travel through the solution (m).

NB: Here we combine terms "ε· 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 logarithms, 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.

$$A = \log_{10} (I_0/I_n)$$
$$= \log_{10}e \cdot \ln(I_0/I_n)$$
$$= 0.434 \cdot \ln(I_0/I_n) = 0.434 \cdot (a \cdot \ell)$$
$$\mathbf{a = A \cdot (0.434 \cdot \ell)^{-1} = 2.304 \cdot A \cdot \ell^{-1}}$$

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, multiple by $\log_{10} e$ (=0.434).
To covert a base 10 logarithm to a natural logarithm, multiple by $\log_e 10$ (=2.304).

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.

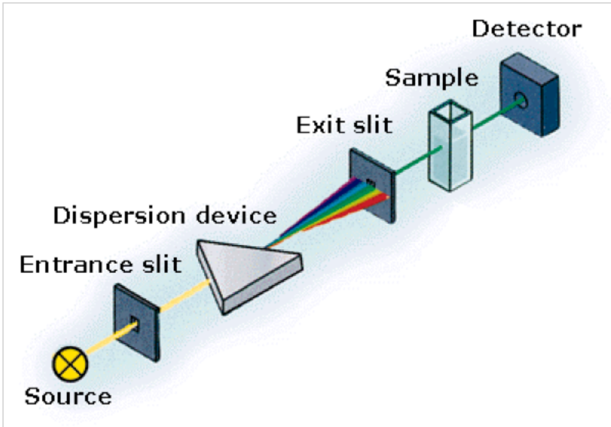
Modern spectrophotometry was pioneered by Dr. Arnold Beckman in the 1940's.

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, such as the Cary 50, have a xenon flash lamp.

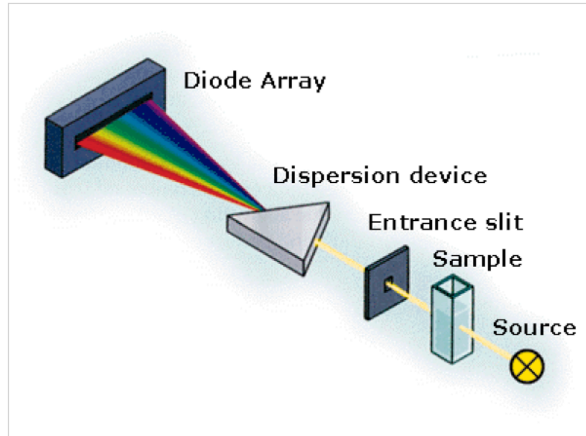
2. Dispersion of light into different wavelengths can occur before or after the light passes through the sample. The monochromator disperses light into different angles by prisms or holographic gratings. NB: with a prism, the angle of dispersion can be nonlinear and sensitive to changes in temperature. 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.

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, 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.

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.

BACKGROUND MATERIAL ON IN SITU SPECTROPHOTOMETERS – AC-9

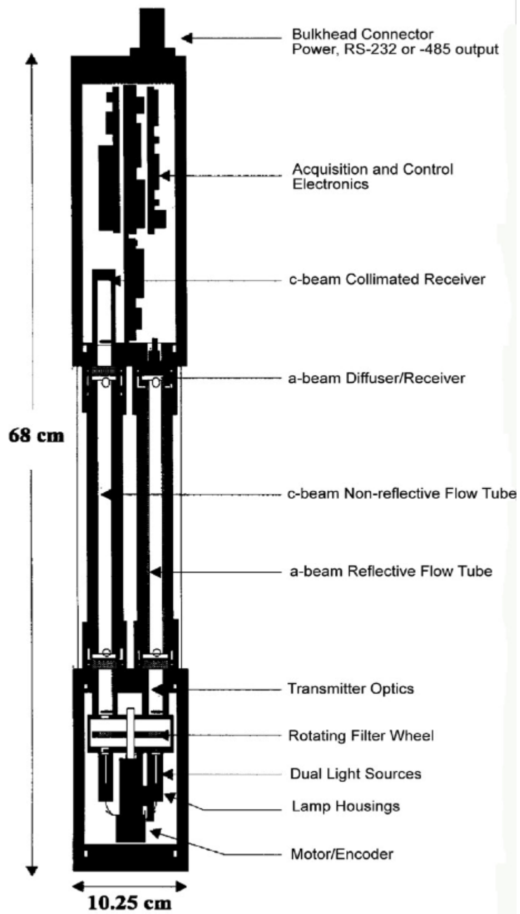


Figure 1. General schematics of WET Labs ac9.

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.

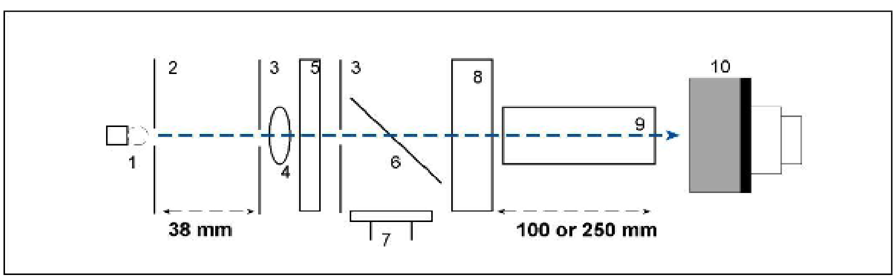


Figure 2. Schematic Representation of absorption beam optics

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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