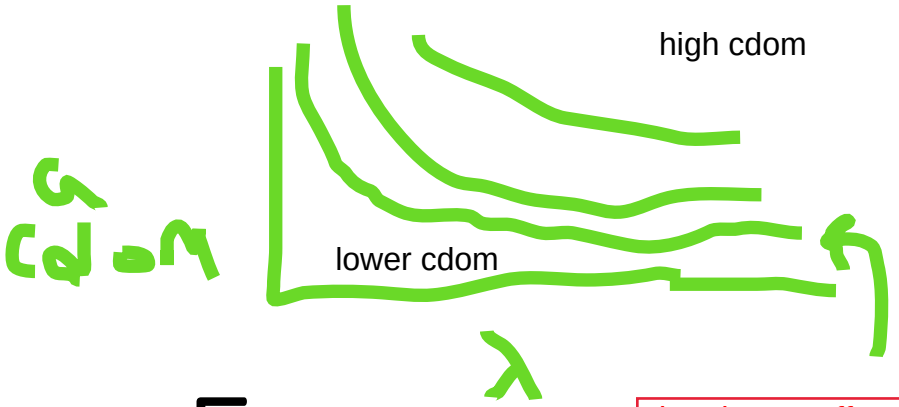
a = 2.303 (A/L(m)

same as:
log 10 (It/Io)
It = radiance incidence
Io = transmitted radiance

L = pathlength
A = Absorbance (really is measuring optical density)



absorbance offset

Lecture 5
Measuring Absorption

Collin Roesler and Emmanuel Boss

11 July 2017

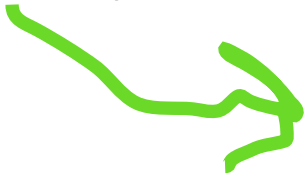
If the curve flattens at NIR, conventionally you make it to zero.

CDOM is not supposed to scatter in the NIR, so people often remove it. Emm says that sometimes that is the right thing to do and sometimes it isn't.

Ppl say that physics of absorption don't predict that flattening.

How do we measure absorption in the ocean?

- Discrete samples in the lab
  - Cuvettes
  - Quantitative filter technique
- In situ meters
  - ac meters
  - integrating cavity absorption meters

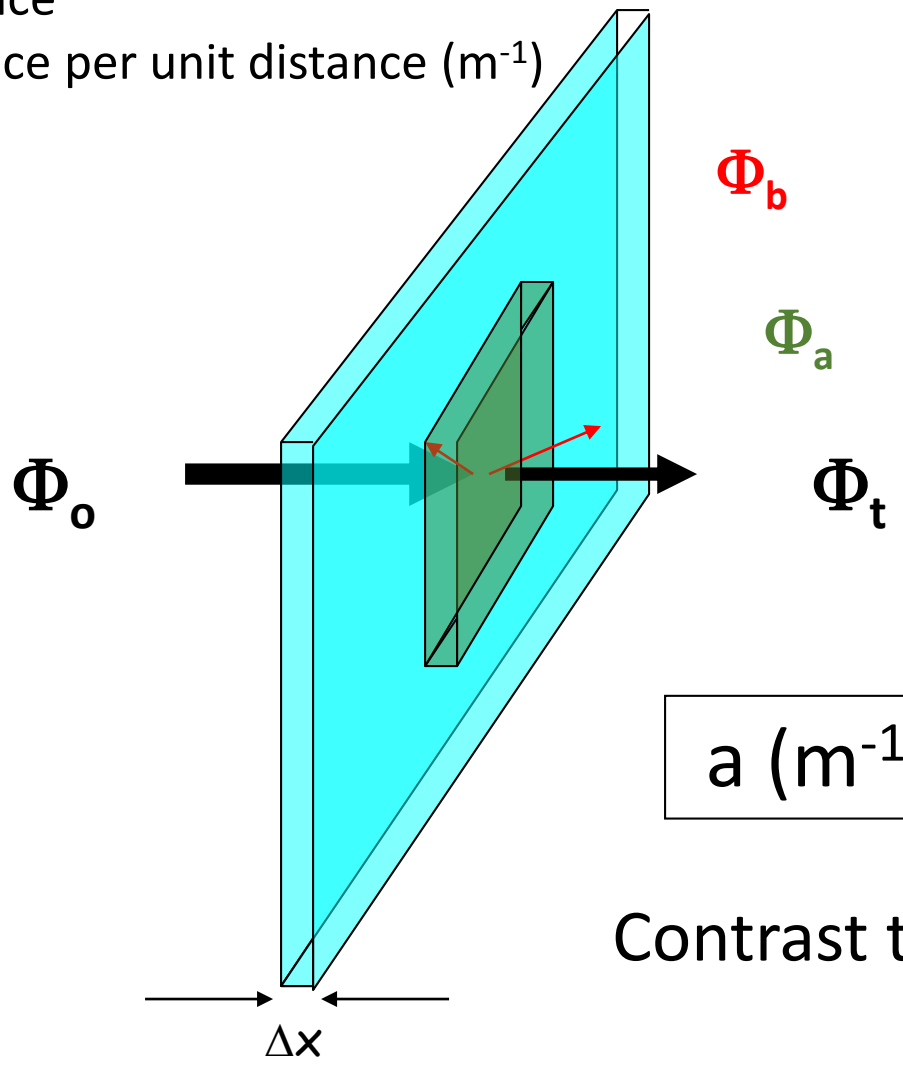


Collin says they don't work

Remember Absorption Theory

A = absorbance

a = absorbance per unit distance (m^{-1})



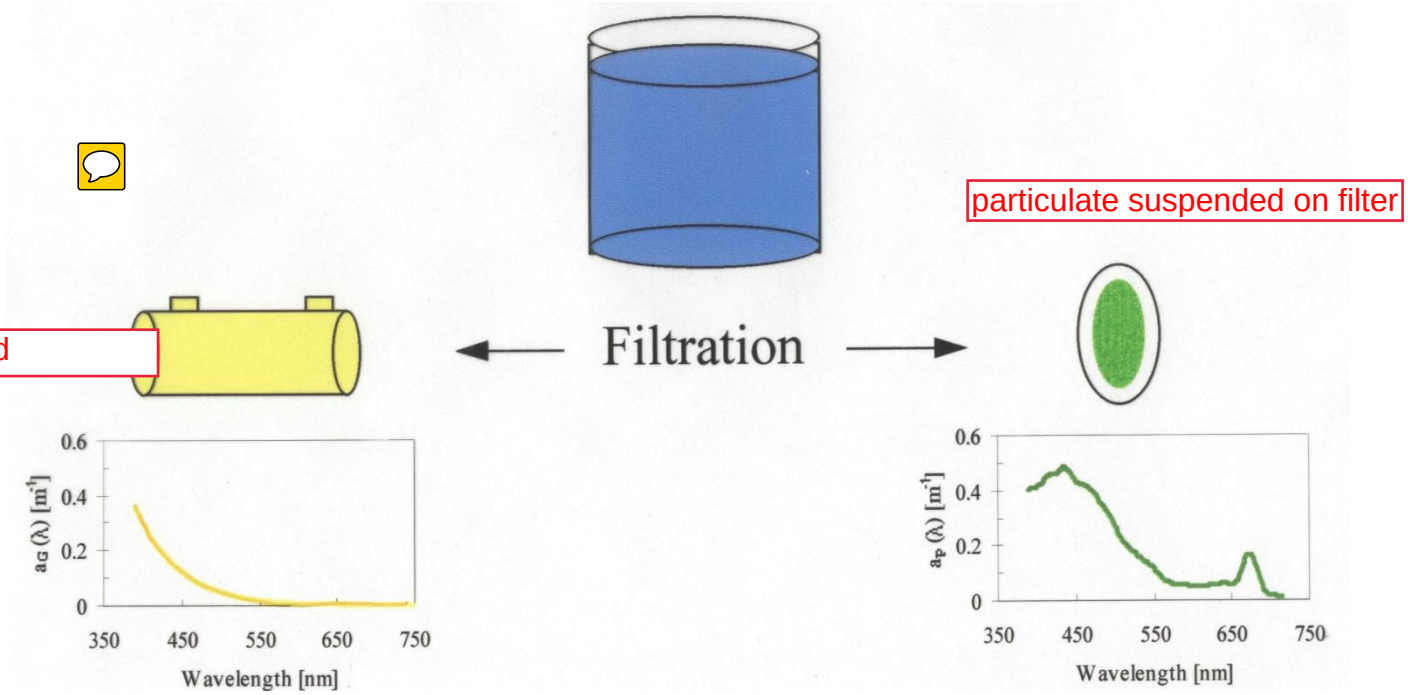
$$A = \Phi_a/\Phi_o$$

$$a = A/\Delta x$$

$$a\ (m^{-1}) = (-1/x)\ \ln(\Phi_t/\Phi_o)$$

Contrast to measuring beam c

Absorption:
Discrete spectrophotometry



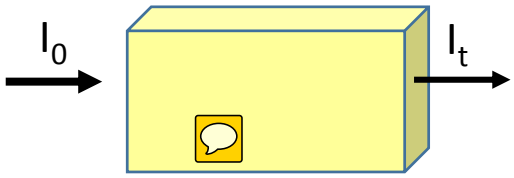
- Separates particles from *dissolved*
- Concentrates particles from dilute medium

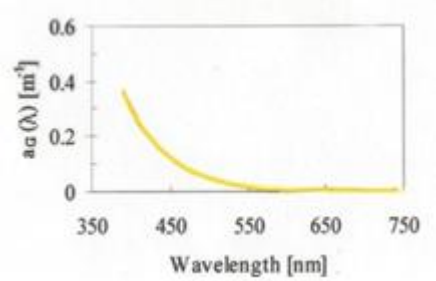
Absorption:
ved" absorption

cavity spectrophotometer

We are measuring transmittance/reflectance (no units) which is Absorbance (A) and then we will convert to absorbance (a) using the pathlength.

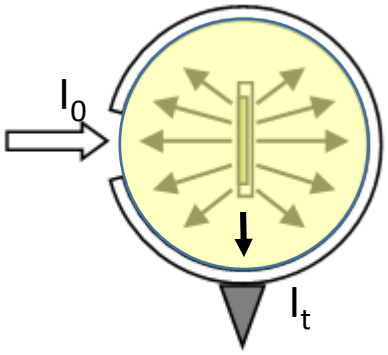
Measure fresh if you can



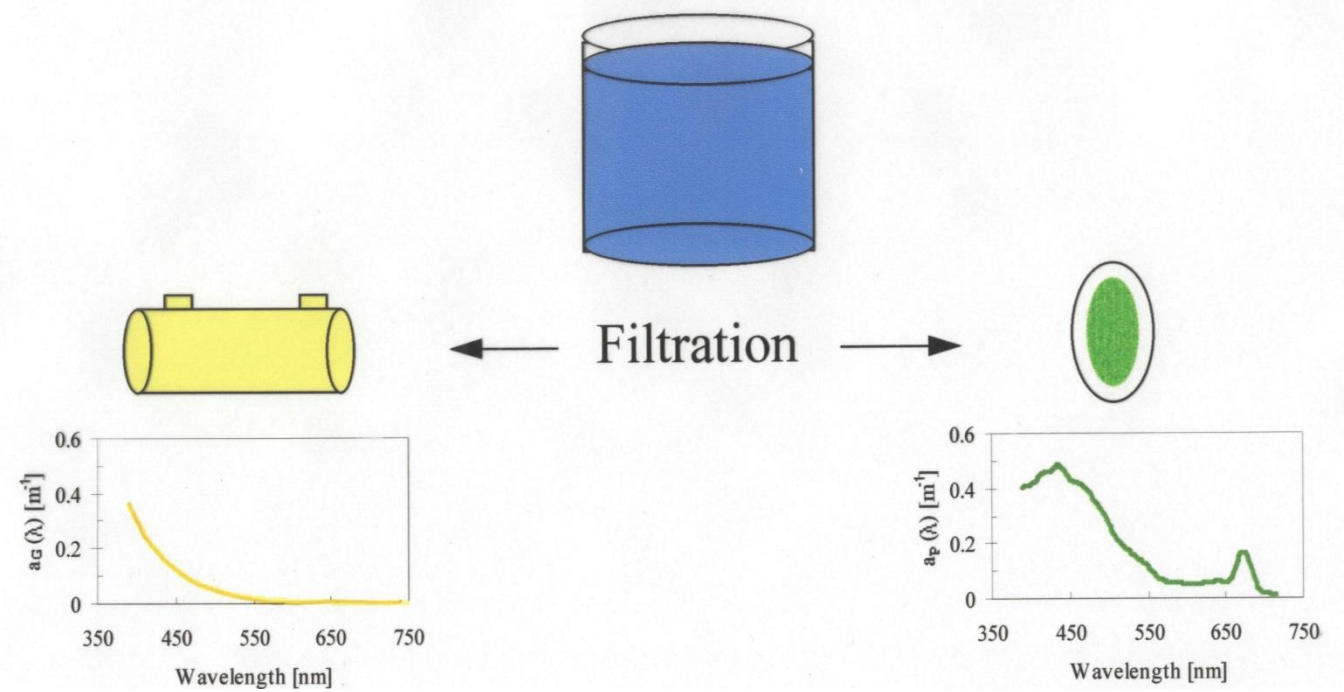


- How does spectrophotometer represent the theory?
- What are the assumptions of this method
- When might this assumption fail?

Too much CDOM, too long of a pathlength, particles that scatter light, or if scattering is not collected

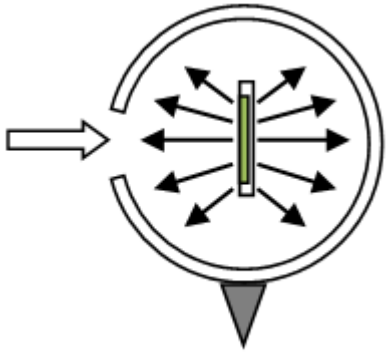


Absorption: Quantitative Filter Technique

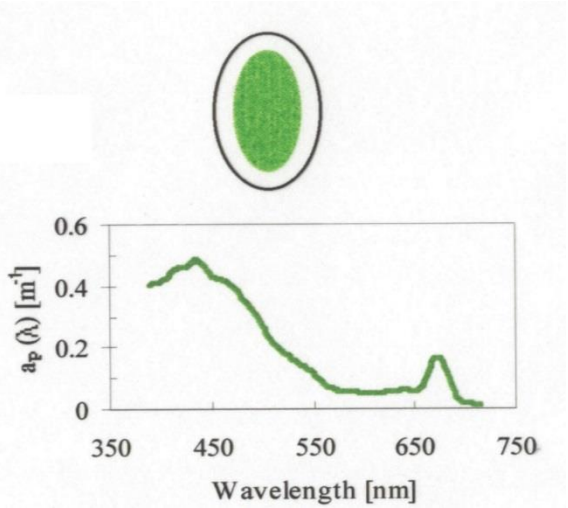


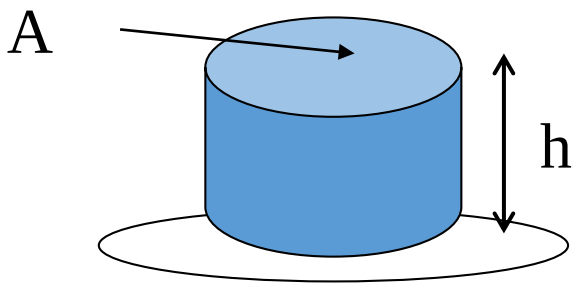
- Separates particles from *dissolved*
- Concentrates particles from dilute medium

Measure in Spectrophotometer Integrating Sphere Mode



- Baseline: mean blank filter pad scans
- Sample scans: mean of filters, rotations
- Compute absorption from absorbance
  - $a\ (m^{-1}) = 2.303 \dfrac{OD}{L\ (m)}$. What is L?



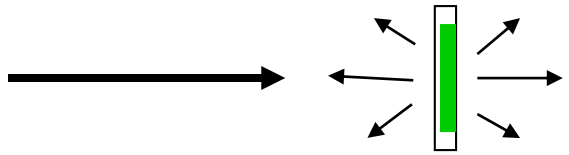


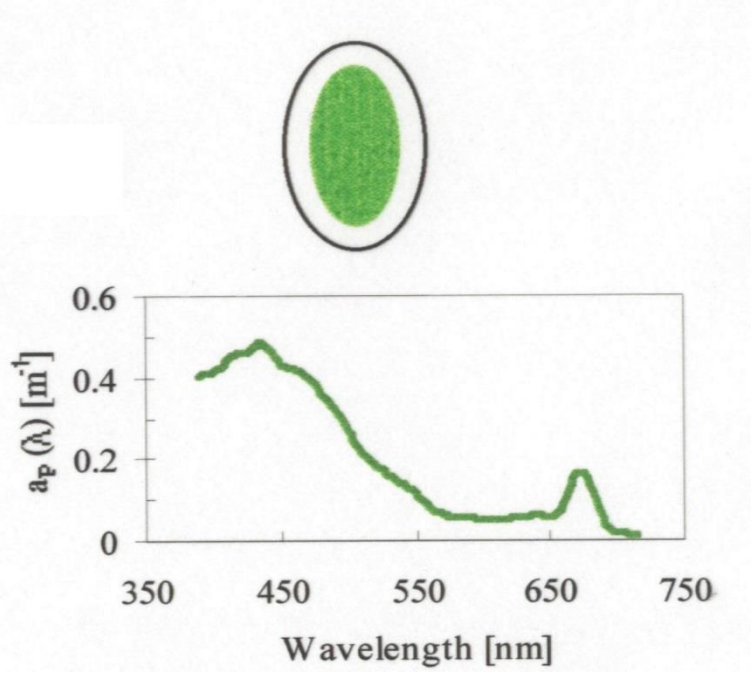
$$V_{filtered} = A_{eff}\ h$$

$$L = h = \frac{V\ (m^3)}{A\ (m^2)}$$

What about the scattering by the filter? Path length amplification

$$a\,(m^{-1}) = 2.303\,\frac{OD}{\frac{V(m^3)}{A(m^2)}}\,.$$

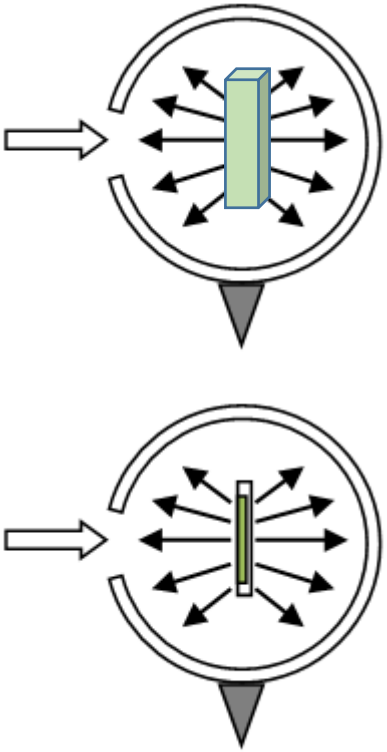


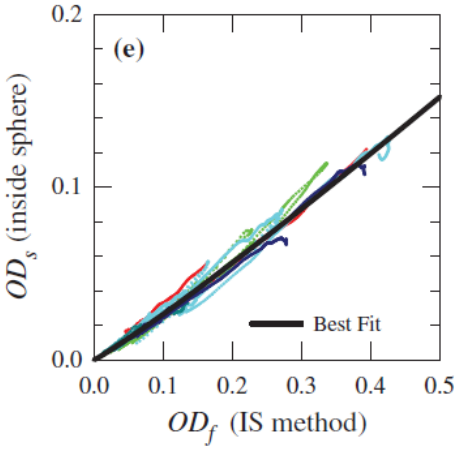


- Filter pad
  - Creates nearly isotropic light field
  - Increases optical path length
  - Increases absorption signal
  - How to correct for it?

β correction: path length amplification

- Approach
  - Cultures or samples
  - Measure absorbance in cuvette (IS-mode)
  - Measure absorbance on filter pad (IS-mode)
  - Determine ratio, $\beta = \frac{OD_f}{OD_s} = \frac{\text{optical}}{\text{geometric}}$
  - Correct OD_f, then compute a





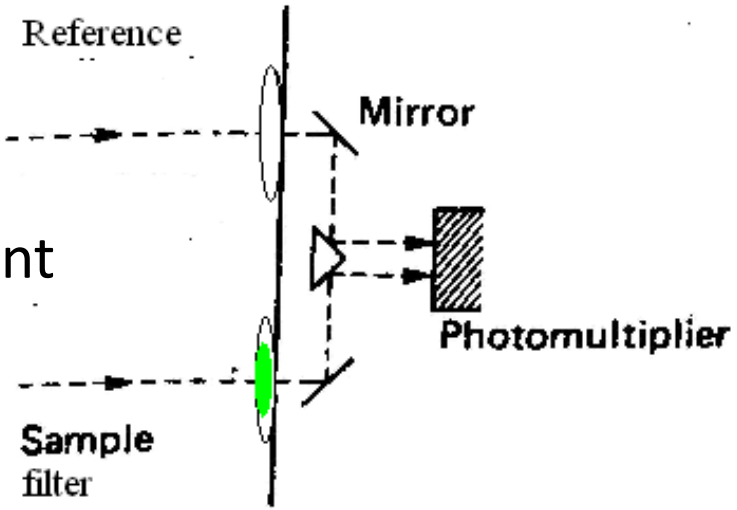
$OD_s = 0.323\ OD_f^{1.0867}$

Beta effect - used to correct for filter pad.

Stramski et al. 2015

Measure in Spectrophotometer Transmission Mode (if you don't have an integrating sphere)

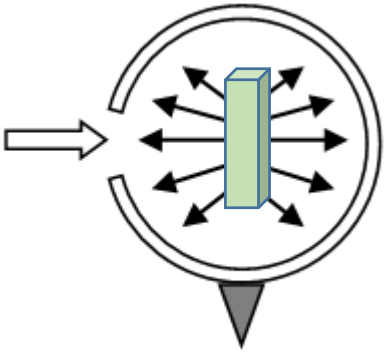
- Reference (neutral density filter)
  - Match optical density of filter pad
  - No variability
- Baseline
  - Blank filter pad in sample compartment
- Samples

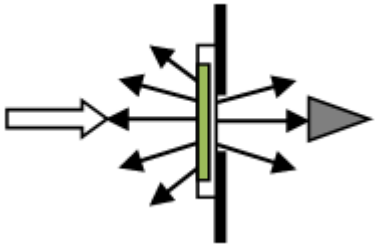


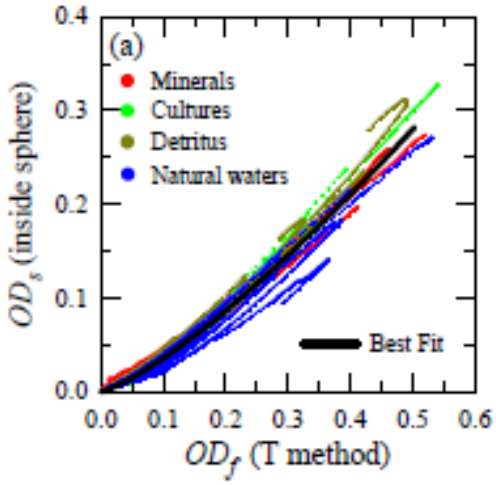
In this mode you are losing ALL the scattered light. She uses a quartz filter as a reference

β correction: path length amplification

- Approach
  - Cultures or samples
  - Measure absorption in cuvette (IS-mode)
  - Measure absorption on filter pad (T-mode)
  - Determine ratio, $\beta = \frac{OD_f}{OD_s} = \frac{\text{optical}}{\text{geometric}}$
  - Correct A_f, then compute a







$OD_s = 0.679\ OD_f^{\ 1.2804}$

Stramski et al. 2015

Uncertainty calculation

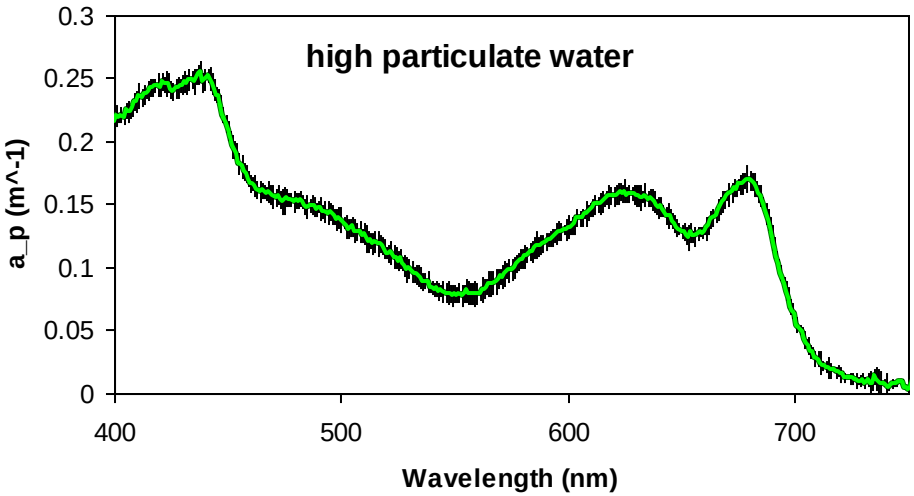
$$a\ (m^{-1}) = 2.303 \frac{OD}{\frac{V(m^3)}{A(m^2)}}.$$

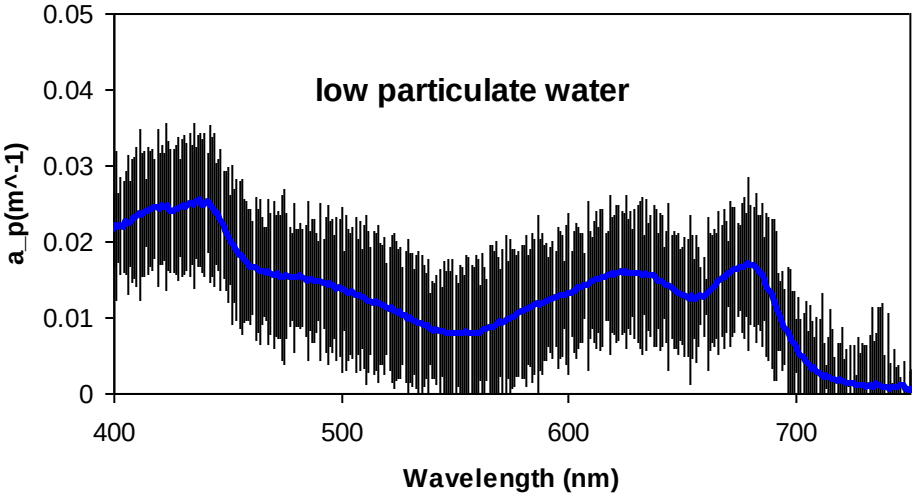
- Run three blank pads relative to your baseline
- Compute the standard deviation of the blank scans, $\sigma_{ODbl}(\lambda)$
- substitute $\sigma_{ODbl}(\lambda)$ for OD in the above equation to compute $\sigma_a(\lambda)$
- note that the uncertainty will be different for each sample:
  - V is different for every sample
  - OD is different, sample is different, so the signal:noise will be different

$$\mathbf{\sigma_a\ (m^{-1}) = 2.303 \frac{\sigma_{ODbl}}{\frac{Vsample(m^3)}{A(m^2)}}.}$$

Roesler 1998

Uncertainty example 1: impact of sample optical density

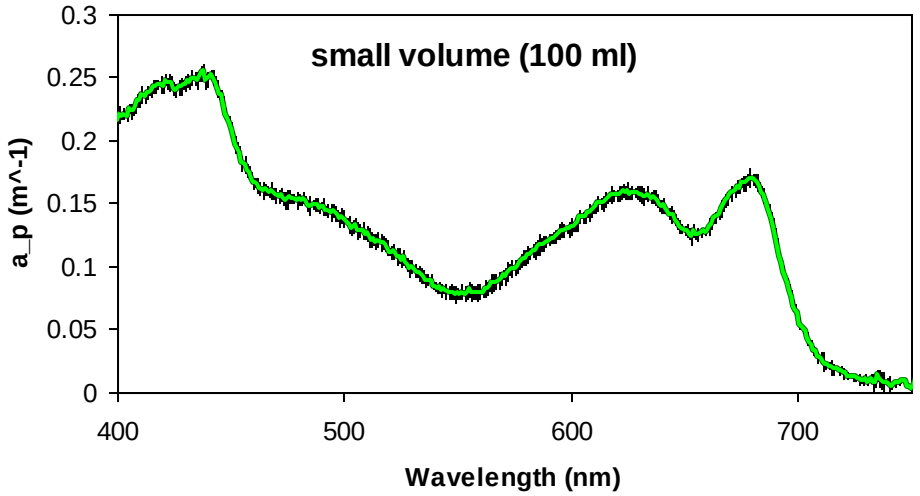


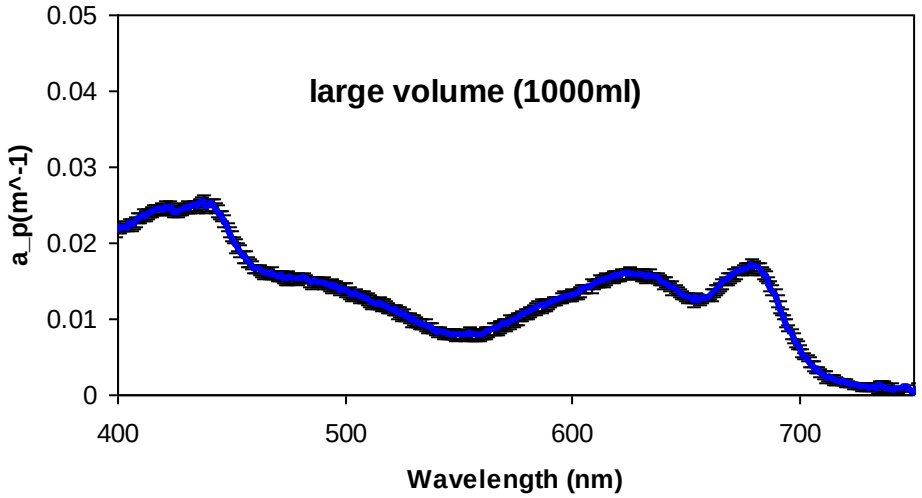


- Same volume filtered for each sample (100ml)
- $OD_{sample1}$~10*$OD_{sample2}$ (approx 0.1 vs 0.01)
- $OD_{filter\ blanks}$ ~ $OD_{sample2}$ for low particulate waters

Higher signal to noise ratio in low particulate water. It is the same magnitude of uncertainty but the relative uncertainty is larger in the low part. waters . It says you should have filtered more.

Uncertainty example 2: impact of volume filtered

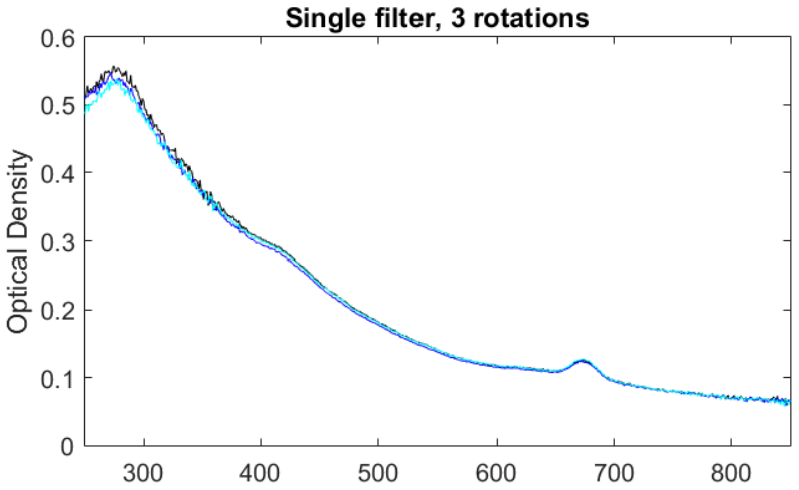




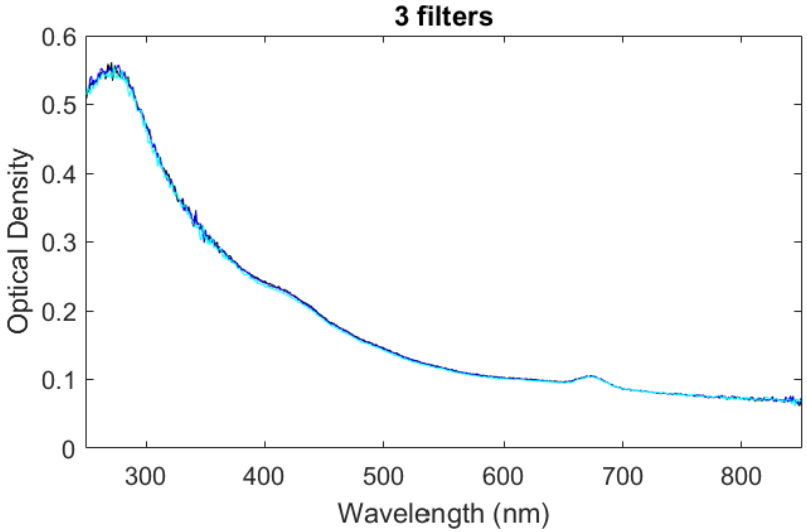
- Different V filtered for each sample (100ml vs 1000ml)
- $OD_{sample1}=OD_{sample2}$ (~0.1)
- $\sigma_{ODfilter\ blank}$~10%OD_{sample}

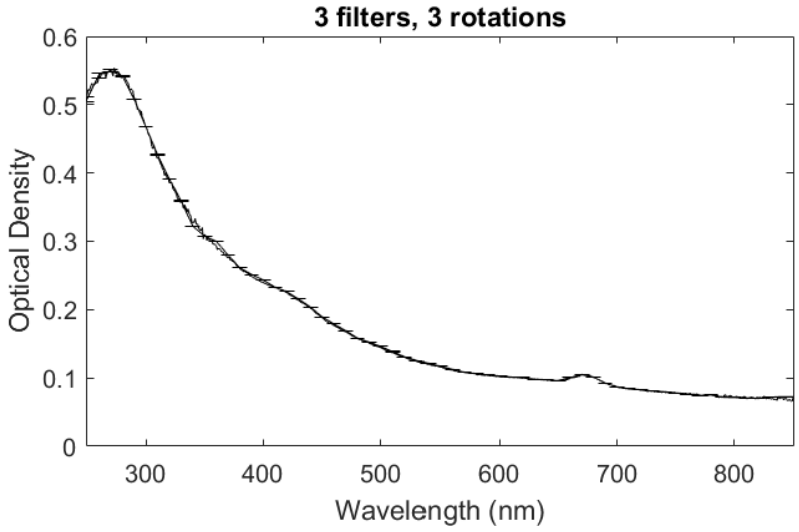
Better to **filter more volume** and obtain **higher OD_{sample}** relative to blanks

Uncertainty Budget



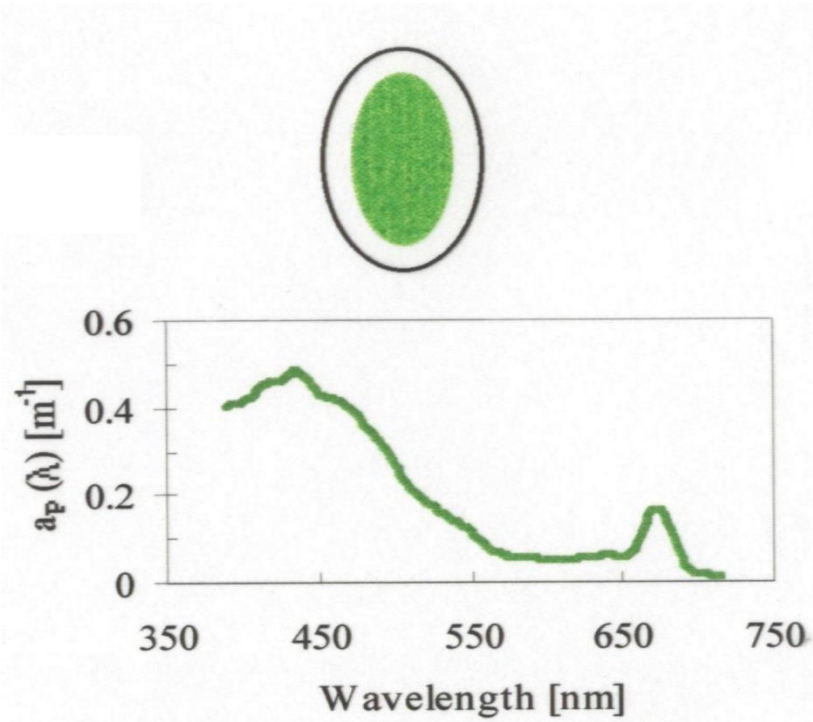
This is the blank filter pad.

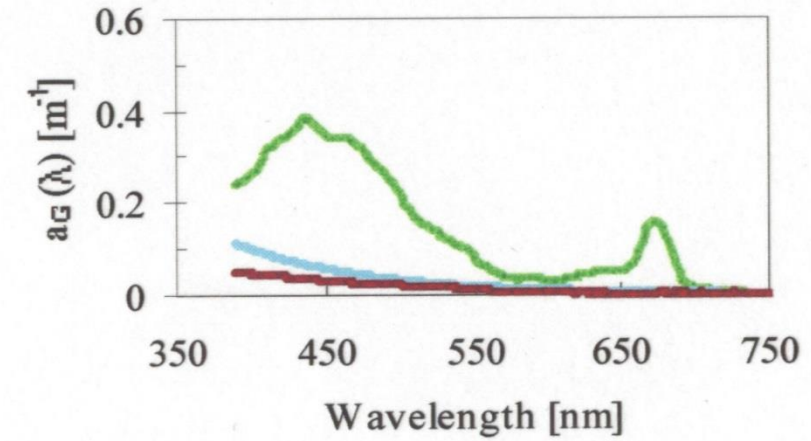




Partitioning particulate absorption

- First scan is total particles, a_p
- Extract with methanol and scan again, a_{nap} (red line)
- $a_{phyt} = a_p - a_{nap}$
- Other issues
  - Phytoplankton “parts”
  - Detrital pigments
  - Phycobilipigments
  - Inorganics





Summary Filter pad technique

- Filter sample, want high loading to overcome the variability in the blank filter pad absorption itself, but not *muddy (0.1 to 0.4 absorbance (OD))*
- What is the reference?
- Extraction to separate particulates, nap
- Computation
  - Offset correction or not? (Stramski and Babin 2002)
  - Absorption calculation, a_p and a_{nap}
  - Phytoplankton calculation, $a_{phyt} = a_p - a_{nap}$

You don't calculate it fromt he OD, you have to first translate OD to absorption

WETLabs ac9/acs sensors

Water goes through tubes then through pump. One tube measures absorption and one measures attentuation (transmission) you subtact to get b

- **Quantitative** measurements of absorption and attenuation
- Calibrated with **pure water**
- Corrections
  - Temperature and salinity of samples relative to pure water calibration
  - Non-ideal configurations for absorption and attenuation
- Strategies for robust measurements

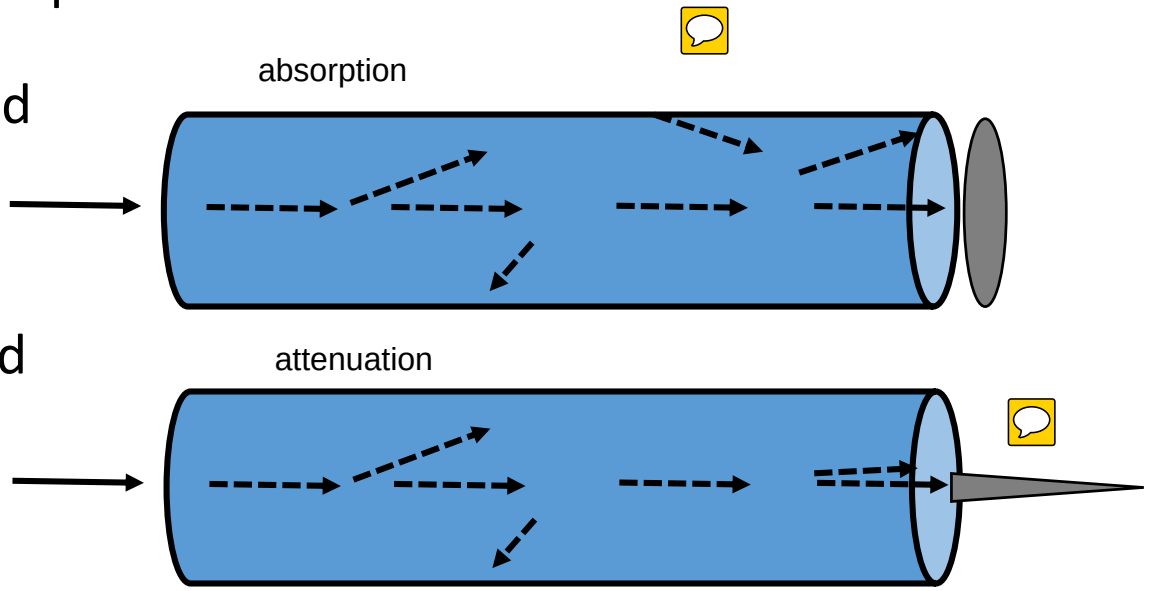
Bio-optical Sensors - Absorption

- Measurement Reality – Sensors
  - Reflecting tube absorption meters

a - Maximize scattered light collection
absorption

c – minimize scattered light collection
beam attenuation

b = c – a **scattering**



Some scattered light not collected by absorption tube, leads to overestimation of absorption →correction

Some scattered light collected by attenuation tube, leads to underestimation of attenuation → report detection angle

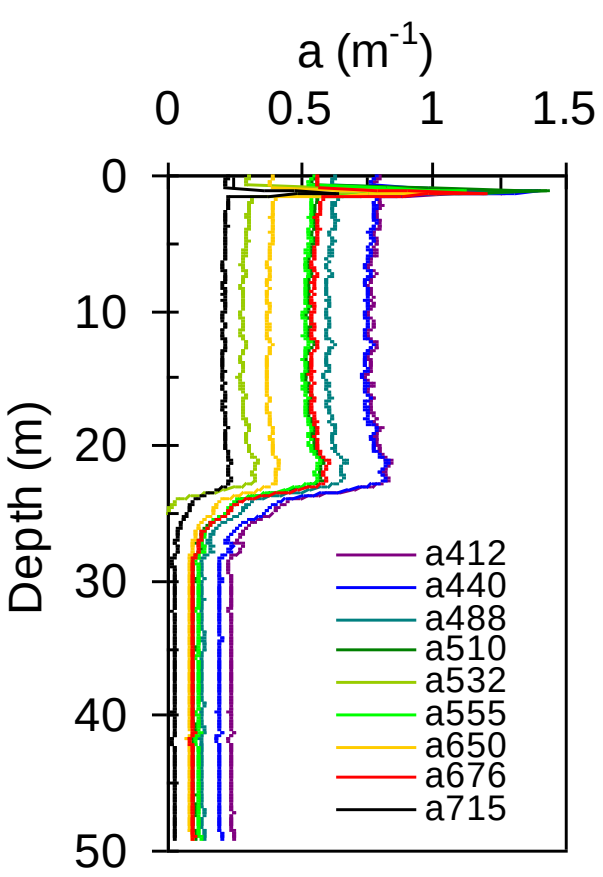
Absorption from ac9/acs

NEVER USE A FACTORY CALIBRATION

*water calibration for quantitation
air calibration to track instrument drift

- Obtain from factory
- Calibrate* in the lab
- Place in deployment configuration
  - Black tubing
  - Copper tubing
  - Air valve
  - Seat bottom
  - Bracket top
- Calibrate* on the frame
- Deploy
  - Take to depth to purge
  - Remove upcast observations (pump inversion)
- Calibrate* upon recovery

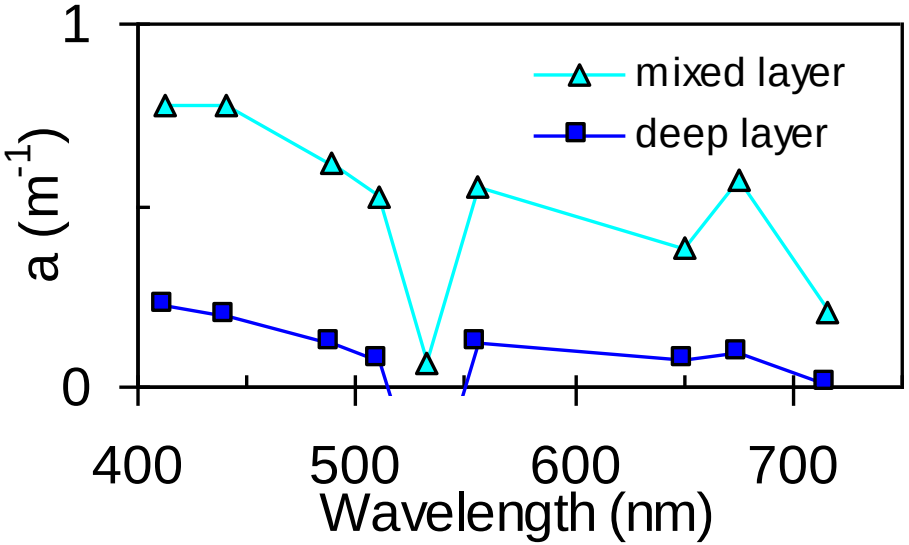
Absorption from ac9 (acs same)



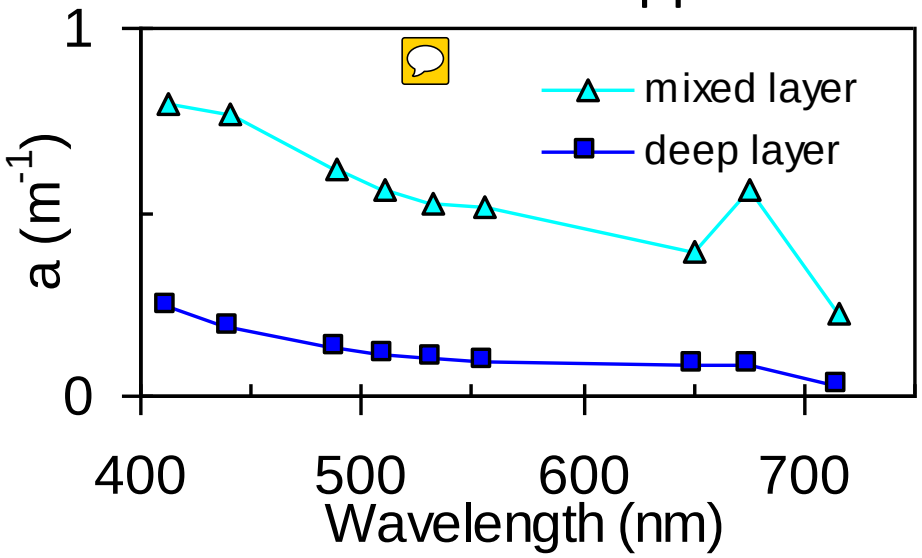
1. Pure water calibration

   $a = a_{meas} - a_{H20}$

But spectra are problematic



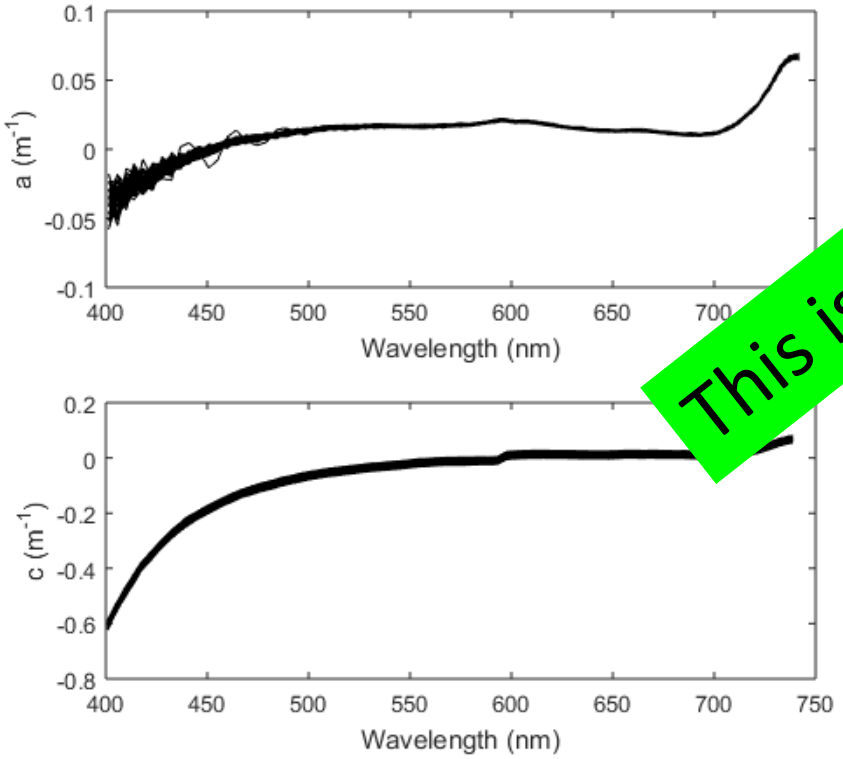
water calibration applied



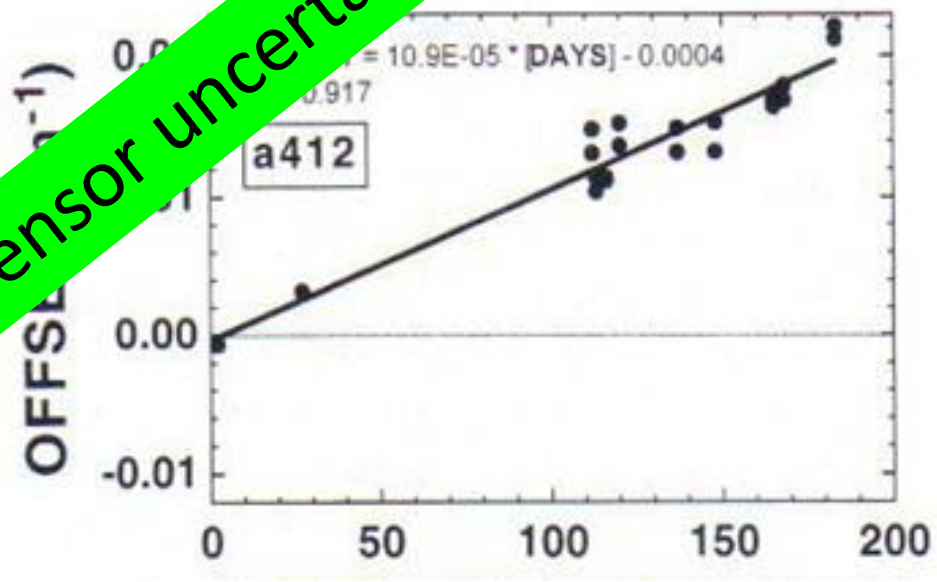
Roesler and Boss 2008

Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  1. Measure pure water scans



This is sensor uncertainty



Twardowski et al, 1997
(true for a and c)

The absorption/attenuation by water varies with temperature and salinity

If you calibrate at 25C with fresh water but measure in the ocean at 10C, you have not used a proper **calibration standard**

Temperature

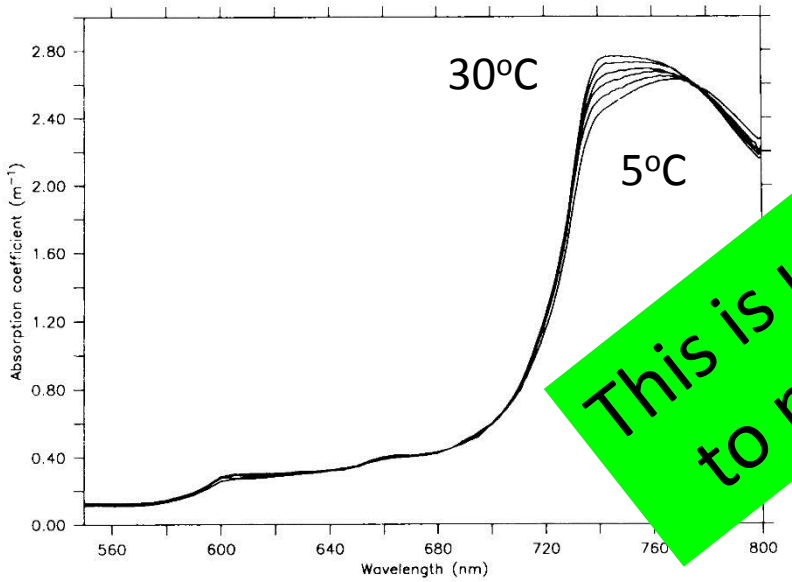
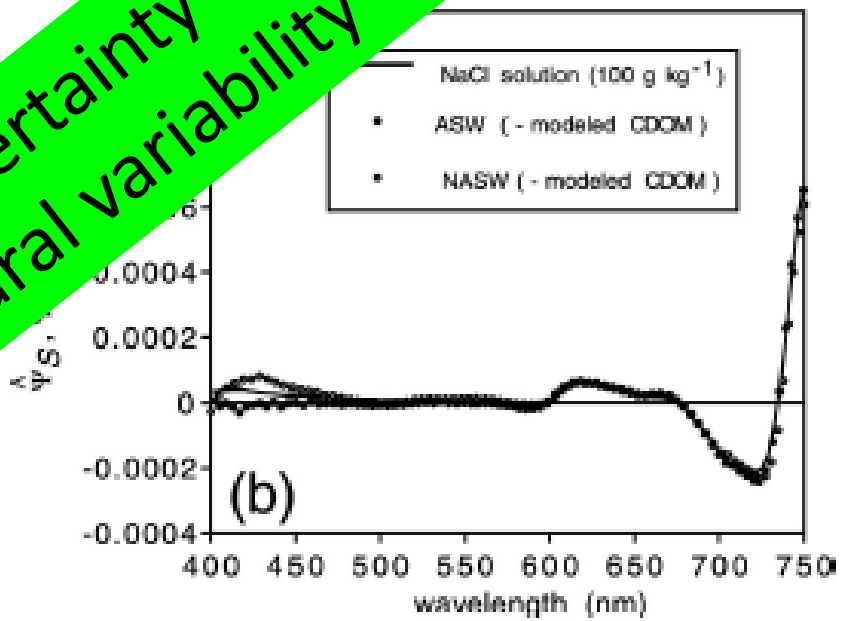


Fig. 3. Absorption coefficient from 550 to 800 nm adjusted at 685 nm to the value of Tam and Patel (1979). The curves represent absorption at temperatures of 5, 10, 15, 21, 25, and 30°C as read from bottom to top at 750 nm.

Pegau and Zaneveld 1993 Limnol Oceanogr.
Pegau et al. 1997 Applied Optics

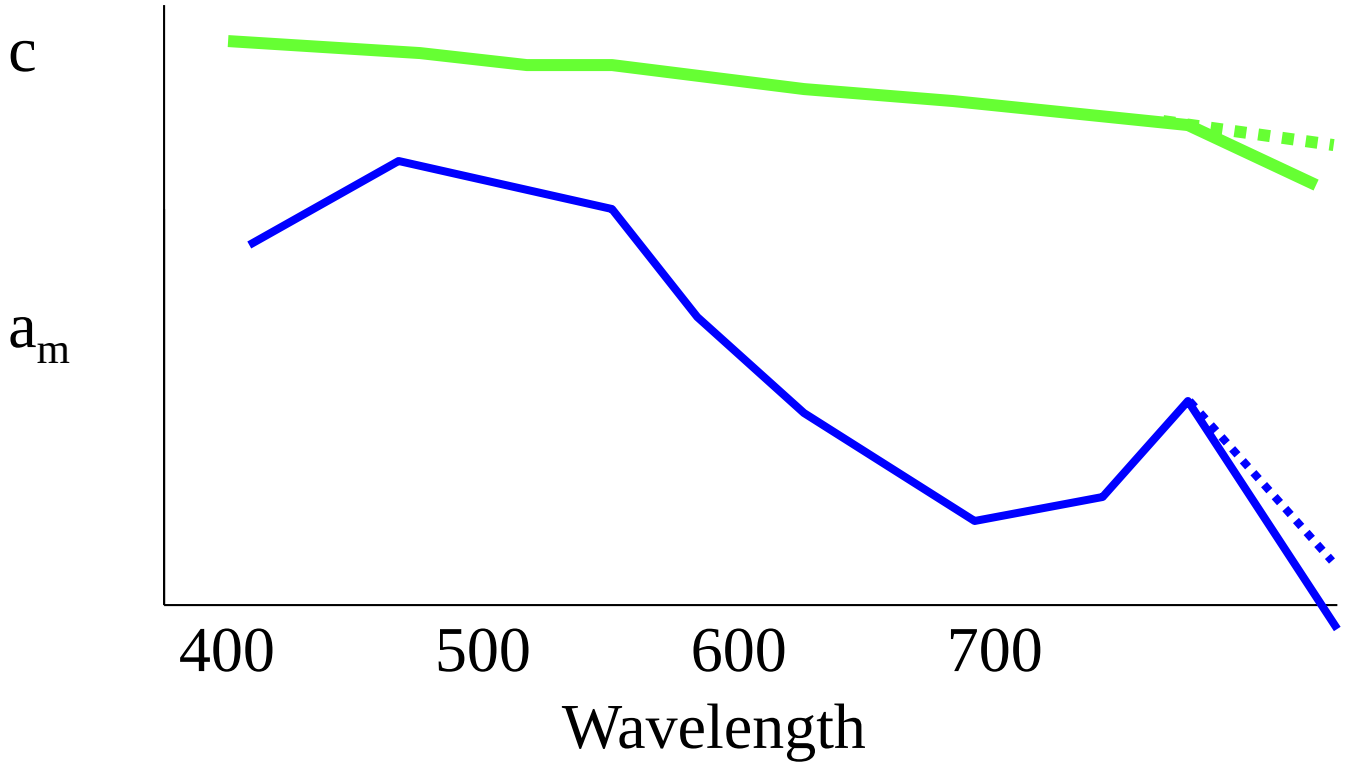
Sa

This is uncertainty due to natural variability



Sullivan et al. 2006 Applied Optics

Absorption from ac9



Peaks don't line up with the wavelengths you would expect

NUmbers are not in the right place on the X axis

Peak at 676

Why is it negative?
T and S correction - bumps it up (dotted line) but then you have an offest

2. Temperature and salinity correction

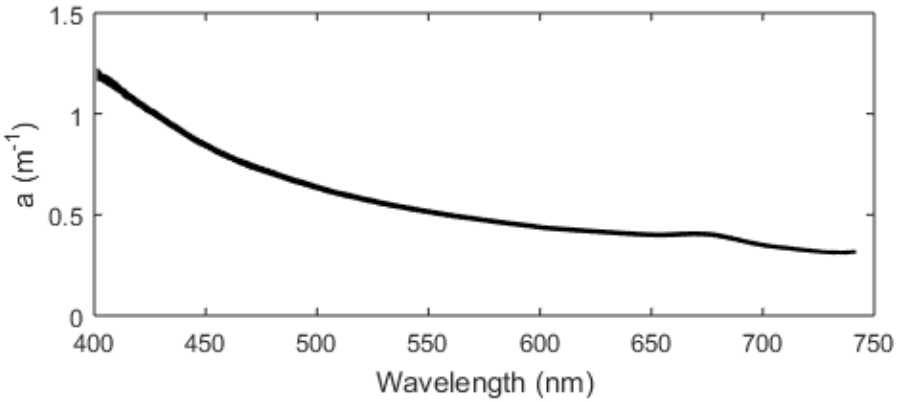
This is due to the fact that the in situ T and S are different than that of the calibration water

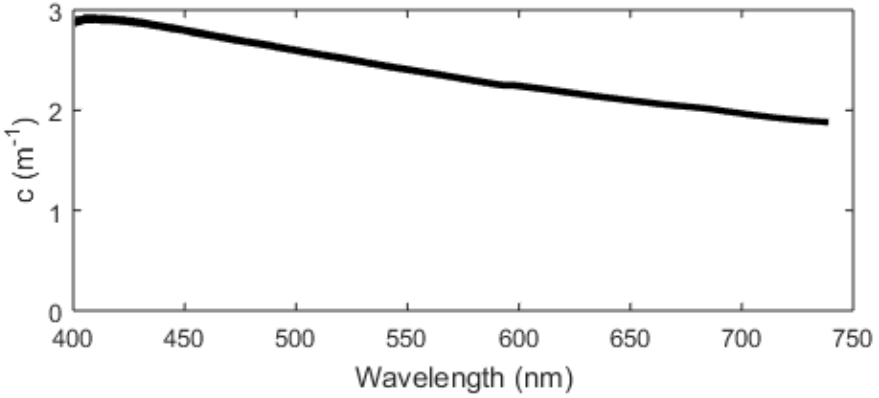
→ Requires measurement of T, S in situ

Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  - Collect sample scans

  1. correct for T, S

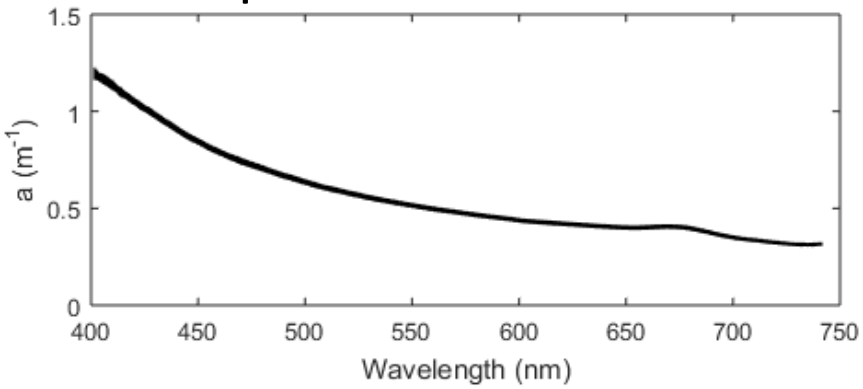


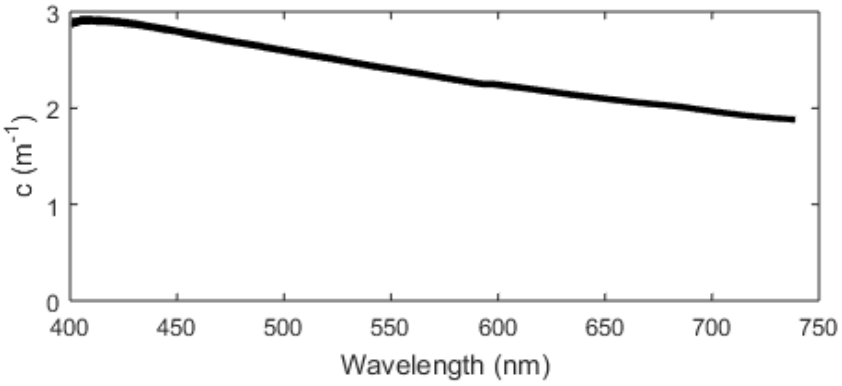


Bio-optical Sensors - Absorption

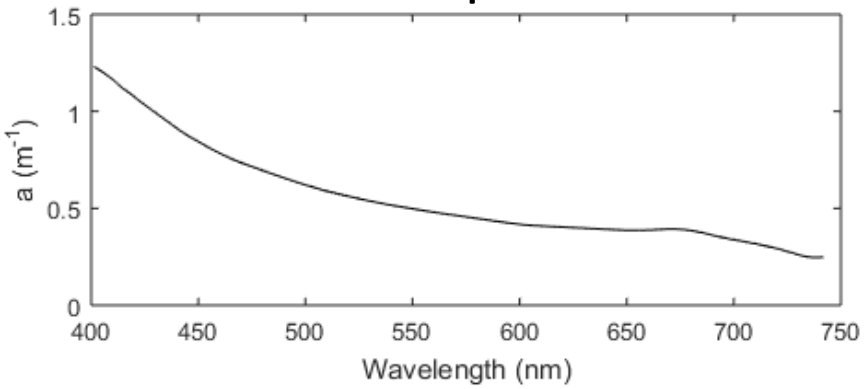
- Data Analysis and Interpretation – acs example
  2. Correct sample scans for pure water values (T, S corr)

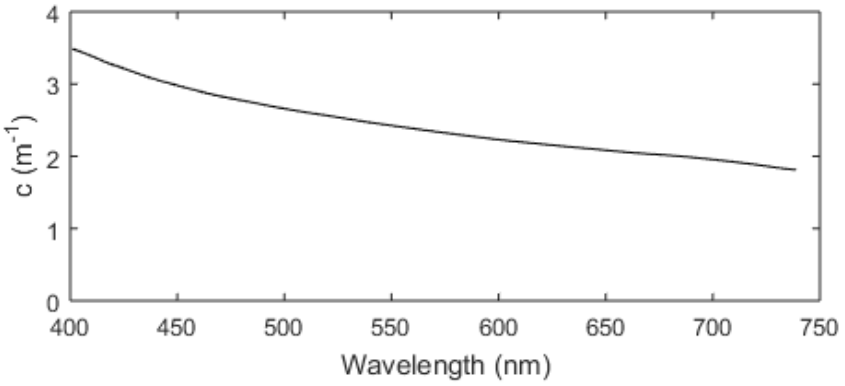
sample scan





corrected for pure water

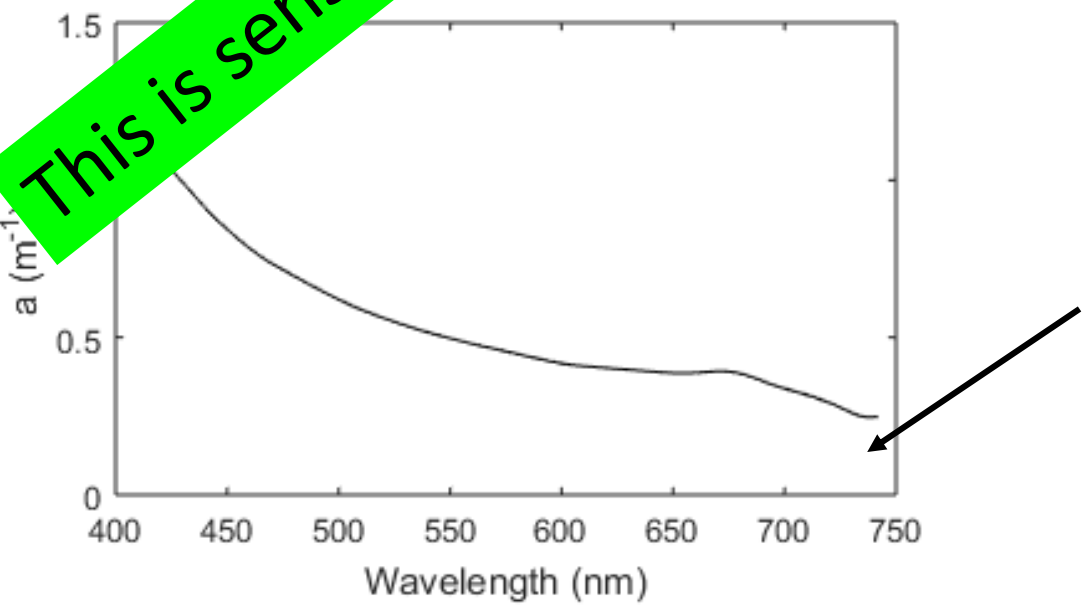




Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  3. Scattering correct the absorption spec
     find wavelength where absorption is ne
     → measured *a* is actually **scatterin**
     if T and S have been accurately d for

This is sensor uncertainty



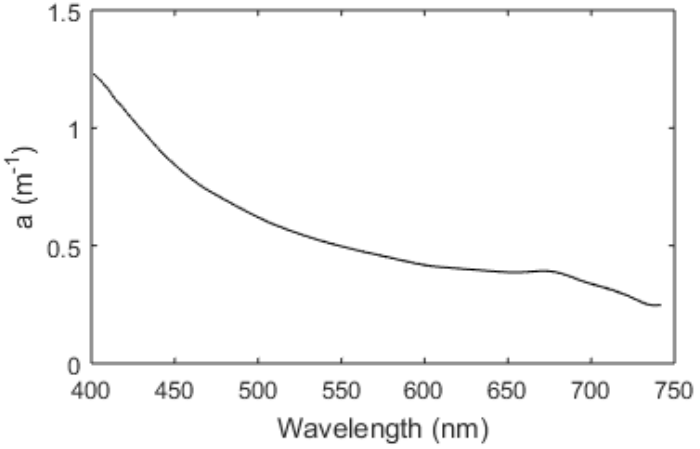
Bio-optical Sensors - Absorption

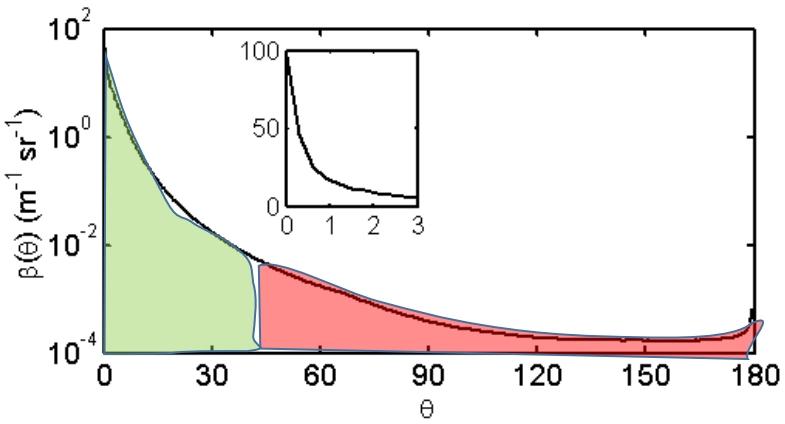
- Data Analysis and Interpretation – acs example
  3. Scattering correct the absorption spectra

     we know the ac meters collect scattered light 0 to 40^{o}

     so miss >40^{o} or back and side scattering

     how do we best correct the a for scattering loss?

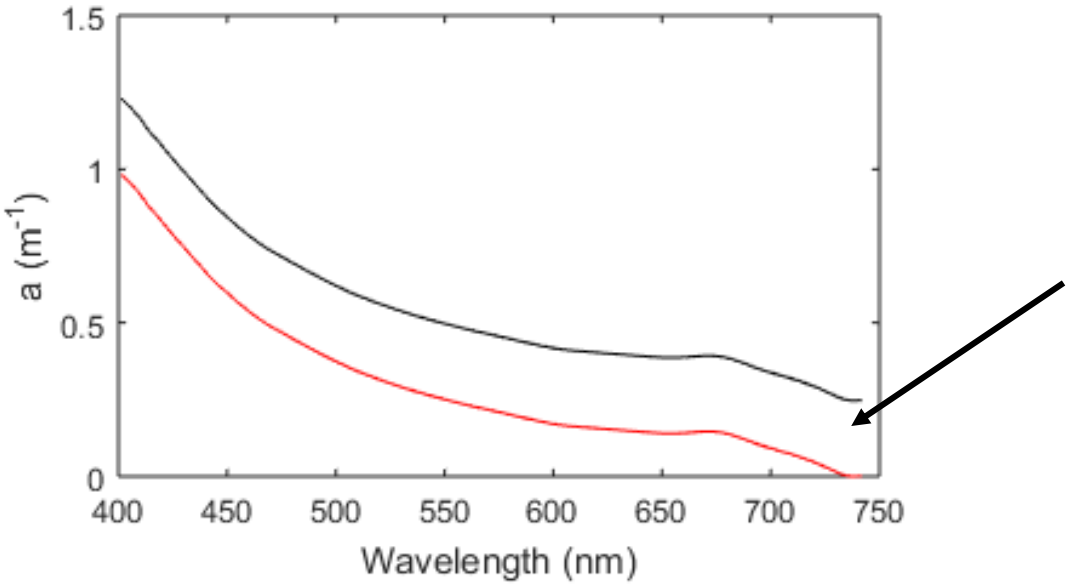




Why does scattering generate NIR absorption? IT is because the scattering is backscattering (which shows up as absorbence because it doesn't get returned to the sensor)

Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  3. Scattering correct the absorption spectra
     a. Subtract a_m(NIR) → "there is no NIR absorption"
     "b not a function of λ"
     spectrophotometric approach

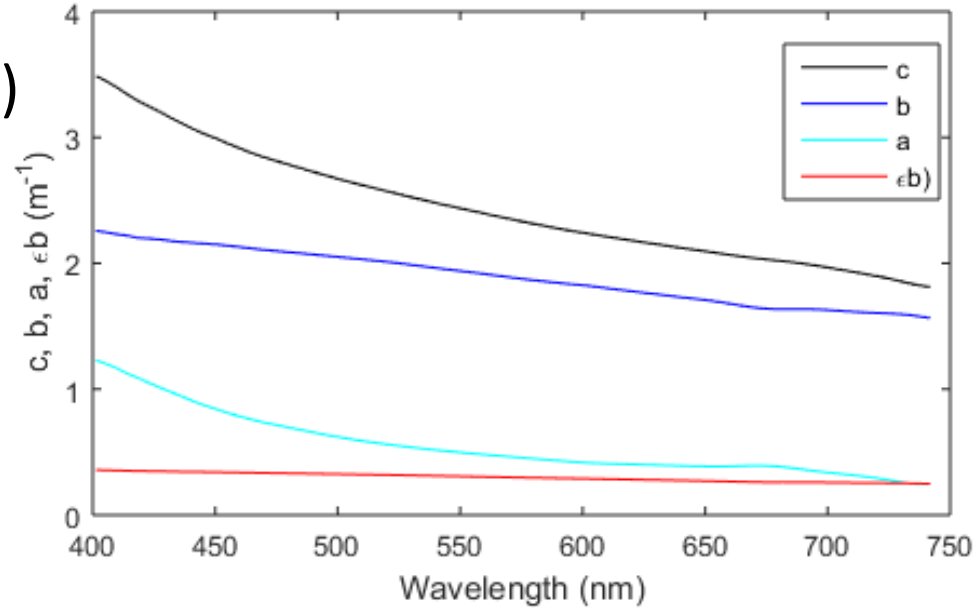


Stramski and Babin 2002

Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  3. Scattering correct the absorption spectra
     b. Subtract spectral scattering contribution, fraction of b(λ)
        "there is no NIR absorption"
        b(λ) = c(λ) – a(λ)
        if a(NIR) = 0 signal is due to scattering
        *f*b(λ) = a(NIR)/b(NIR)

$a_{corr}(\lambda) = a(\lambda) - (f b(\lambda) * b(\lambda))$



Roesler and Zaneveld 1993

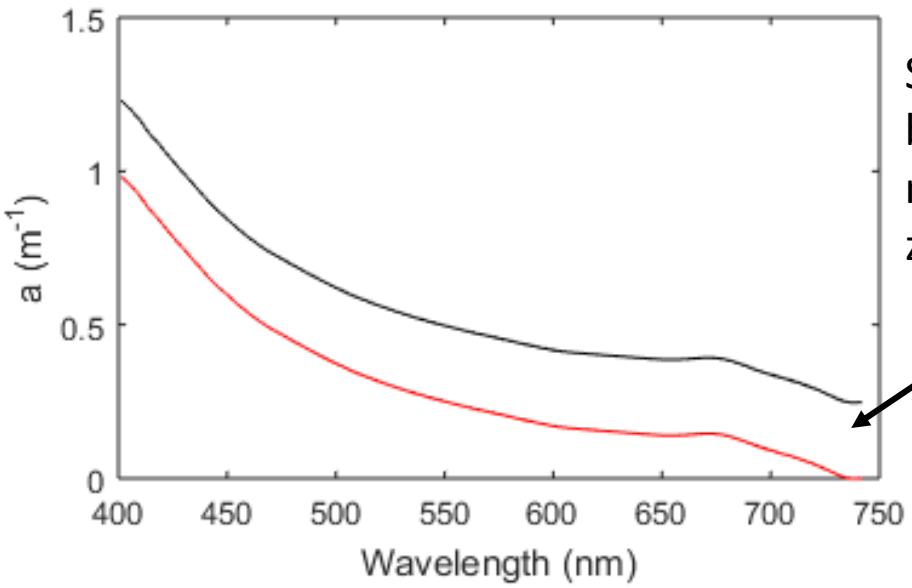
Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example

  3. Scattering correct the absorption spectra

     a. Subtract some fraction of the NIR signal → "there is some NIR absorption"

This is an active area of research!!!

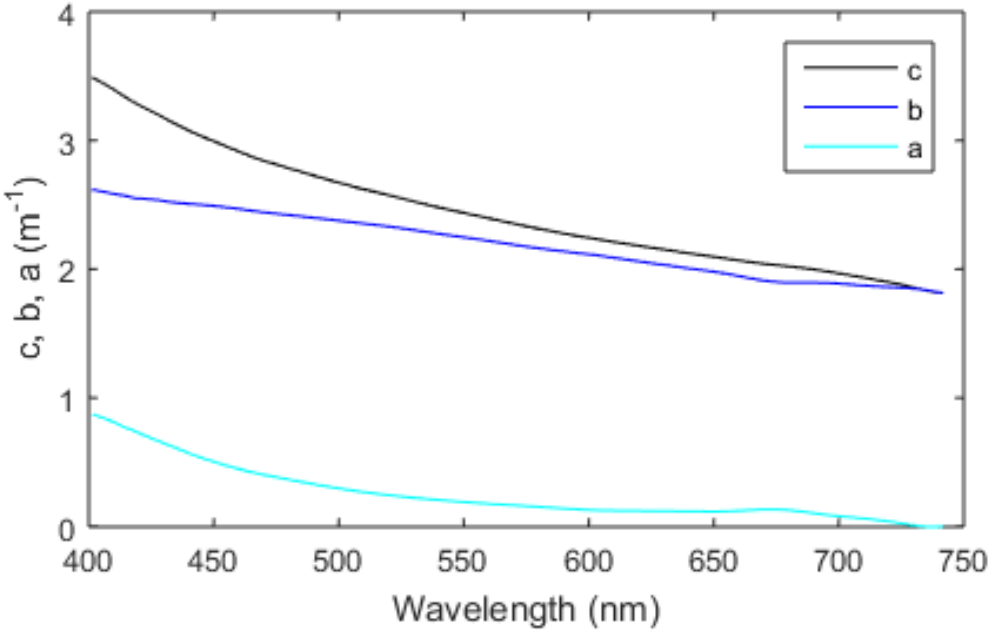


Somewhere between measured and zero

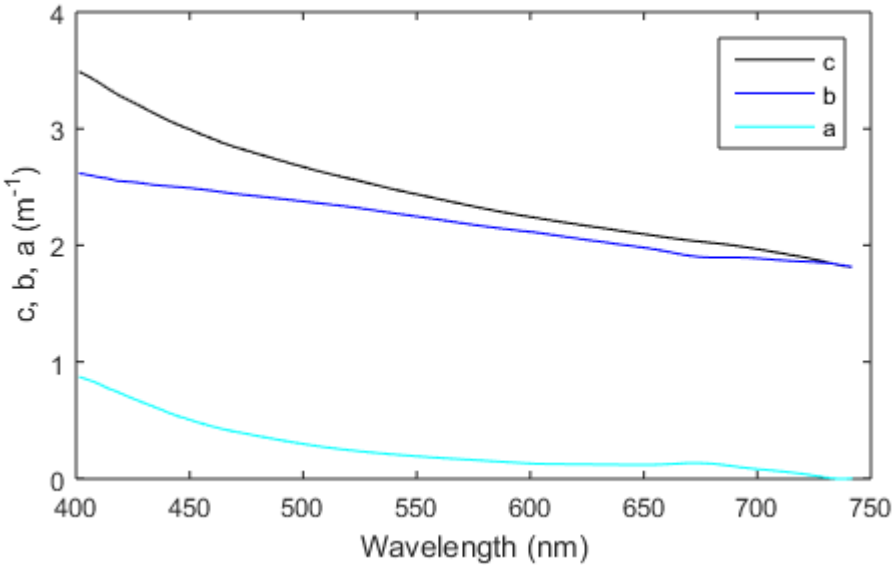
Röttgers et al 2007
Röttgers et al 2013

Bio-optical Sensors - Absorption

- Data Analysis and Interpretation – acs example
  4. Compute Scattering spectra
     $b(\lambda) = c(\lambda) - a(\lambda)$



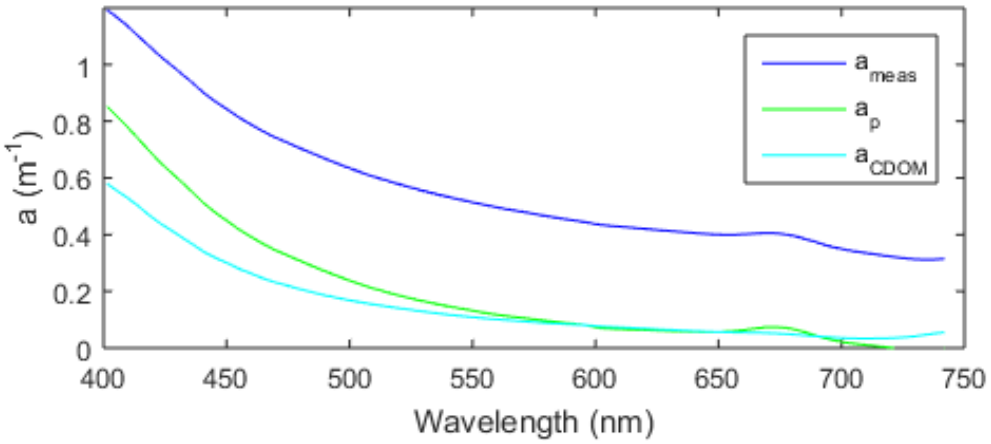
Best practices for obtaining Absorption/Attenuation from acs



- Review Data processing
  - Temperature/Salinity correct a and c of sample and calibration data
  - Subtract T,S-corrected pure water calibration from sample scans
  - Apply scattering correction to absorption
  - Compute scattering spectrum (b = c – a)

Bio-optical Sensors - Absorption

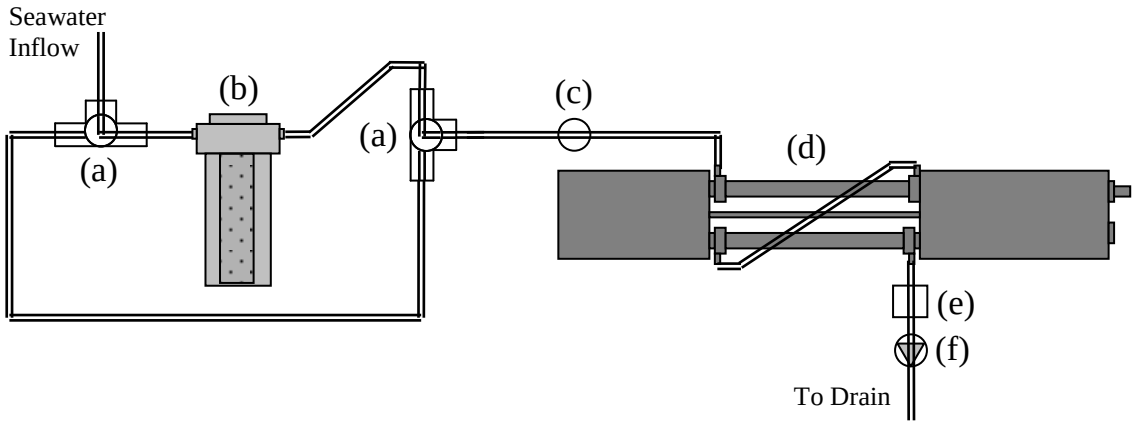
- Data Analysis and Interpretation – acs example
  - **Calibration independent** method for partitioning
  - (Slade et al. 2010)
    - Measure whole water and filtered water, a_{tot}, a_{filt}
    - Apply Temperature, Salinity correction
    - Apply Scattering correction
    - Subtract filtered water scan from whole water scan, $a_{part}=a_{tot} - a_{filt}$
    - Yields a_{CDOM} and a_{part} ***independent of calibration drift***

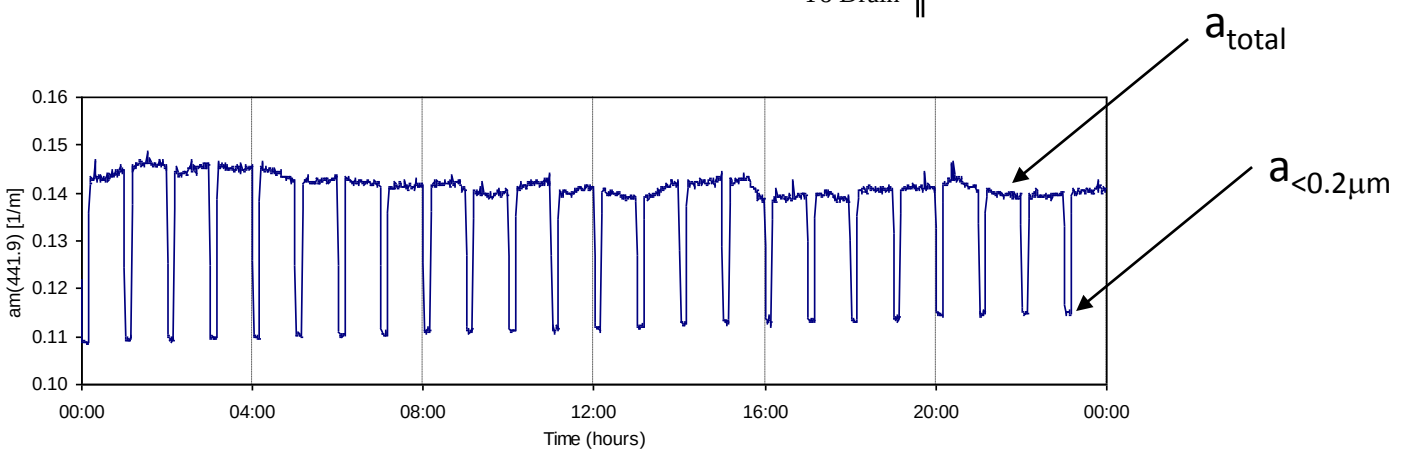


Automated shipboard flow-through method, calibration-independent

a switch that goes between
total water
and filtered water
add another line
for DI water if you want to get CDOM

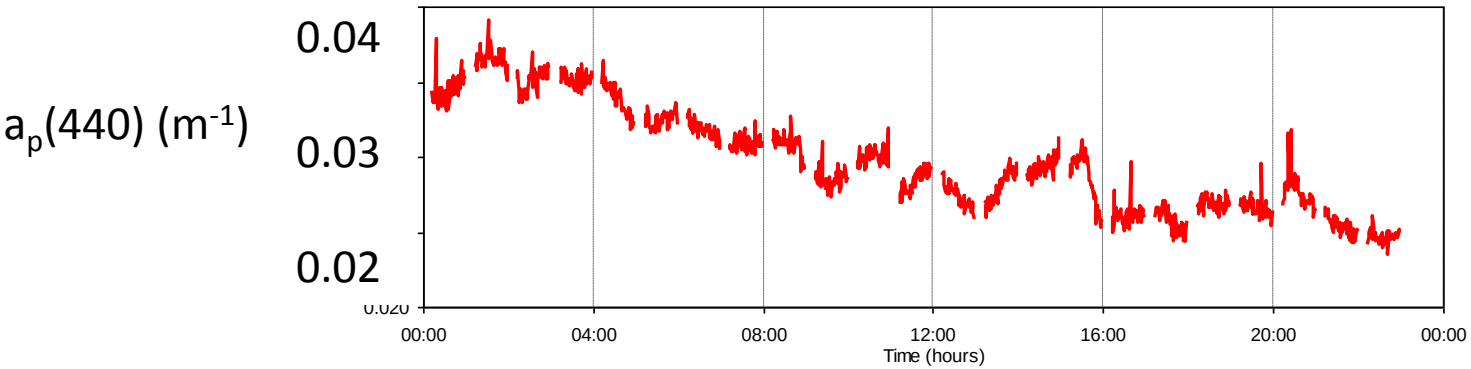
a tot = (particles + cdom)
a diss = just CDOM

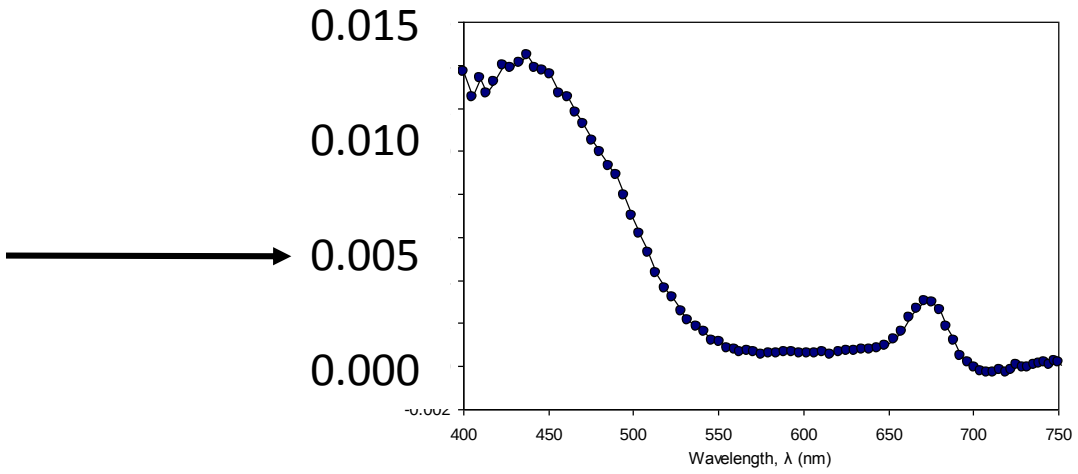




Slade et al., 2010

An example of calibration independent approach on an automated shipboard flow-through configuration





people add another switch to DI water
and that will give you CDOM and particulate

.2 microns
2 microns
20 microns

size-fractionated absorption fraction
underway

Slade et al., 2010

Today in the lab

- CDOM absorption
- Divide into two groups of 10
  - Station 1 in Lecture Hall – lab spectrophotometry
  - Station 2 in Mitchell Lab – in situ spectrophotometry
- Will take about 2 hours for each station, then we will switch