Class context: Week 1 roadmap

Absorption part 1: Theory, context, overview

Absorption part 2: Measurements and practical considerations

Wednesday-Thursday: Scattering, beam attenuation, and particle size

Friday: Phytoplankton and fluorescence

Tuesday lab: absorption measurements without particles

Wednesday lab: absorption measurements with particles

Thursday lab: scattering and beam attenuation measurements

Friday lab: fluorescence measurements
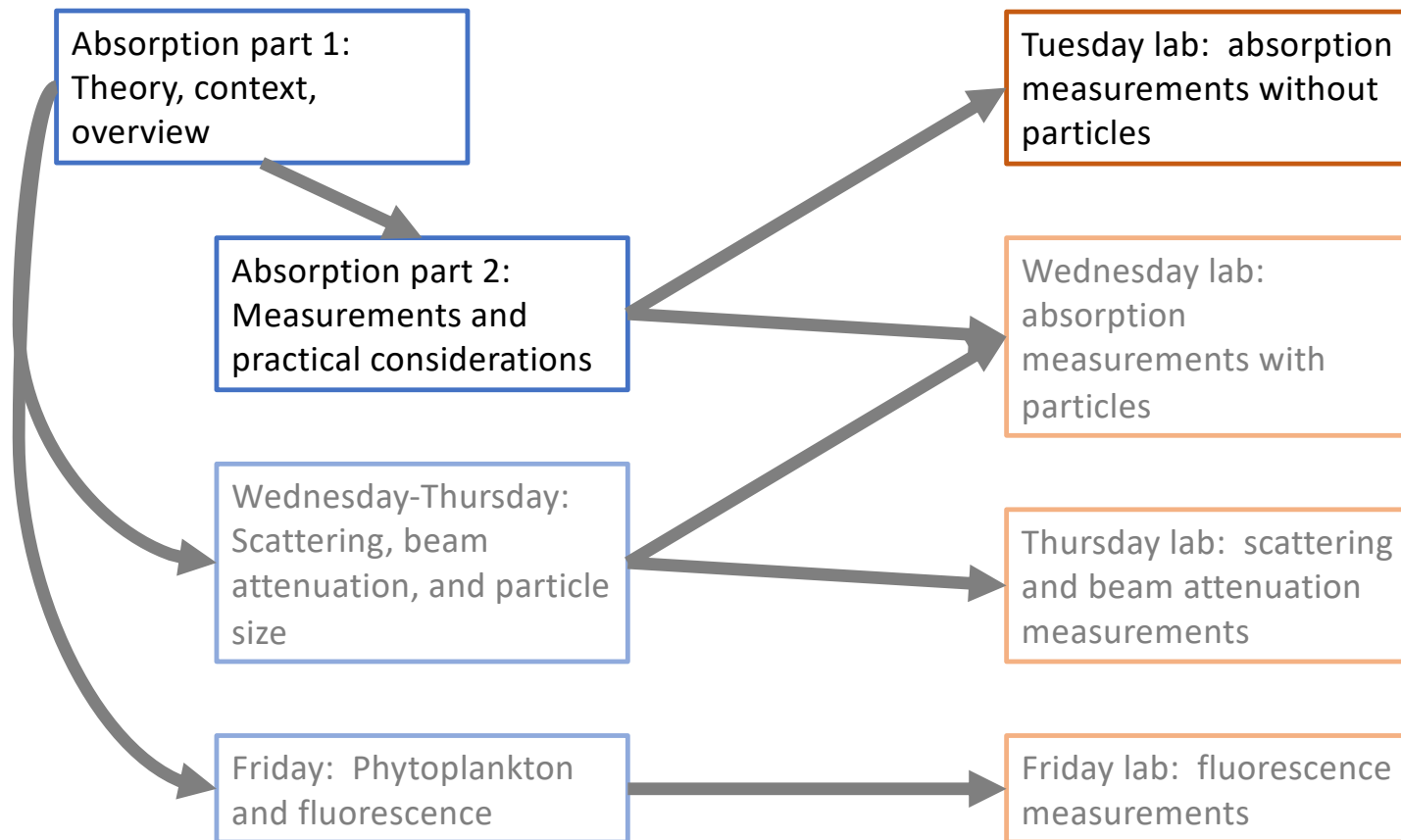
Absorption, part 2

- Beer-Lambert-Bouguer Law
    - Conceptual
    - Realistic
- Survey of measurement techniques
- Lab overview

Beer's Law

… or Beer-Lambert-Bouguer law – see Mobley et al. 2022, p. 357 footnote for historical dates

- Bouguer and Lambert: Light decays exponentially through a medium

$$L(r) = L(0)e^{-a(r)}$$

where L is the radiance, r the pathlength, and a is the absorption coefficient.

- Beer: The decay coefficient of the exponential decrease is proportional to the concentration of the absorbing substance
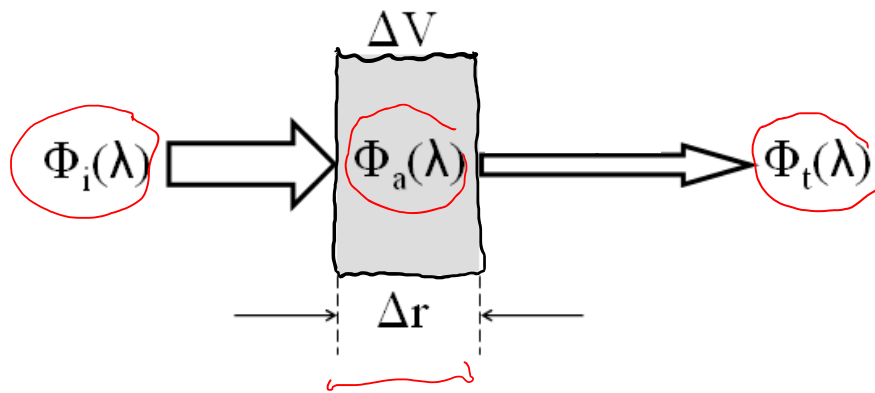
$$a = a^* \times C$$

where a^* is the concentration-specific absorption coefficient and C is the concentration of the absorbing substance.

- If there are multiple absorbing substances, we can simply sum them up:

$$a = \sum_i a_i^* \times C_i$$

Deriving Beer's Law

Consider a volume of water with thickness Δr and incident radiance Φ_i (everything is a function of wavelength, not written for brevity)

Φ_a is the radiance absorbed in the volume
$\Phi_s(\Psi)$ is the radiance scattered into angle Ψ
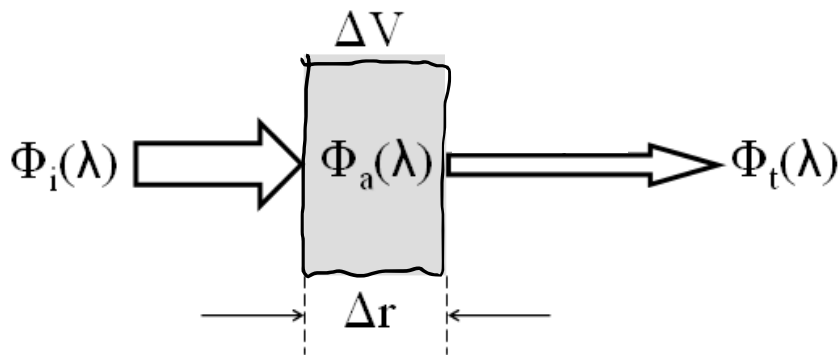Φ_t is the radiance transmitted through the volume

Conservation of energy:
$\Phi_a + \Phi_s + \Phi_t = \Phi_i$

Now assume (for now) that there is no scattering so $\Phi_a = \Phi_i - \Phi_t$

ΔV

$\Phi_i(\lambda)$ $\Phi_a(\lambda)$ $\Phi_t(\lambda)$

Δr

Figure: Mobley et al. 2022, *The Oceanic Optics Book,* Fig. 3.1

Deriving Beer's Law

We define the absorption coefficient as the fractional radiance loss *per unit distance* through the volume.

$$a = \lim_{\Delta r \to 0} \frac{-\Delta\left(\Phi_i - \Phi_t/\Phi_i\right)}{\Delta r} = \frac{-\frac{d\Phi}{\Phi}}{dr}$$
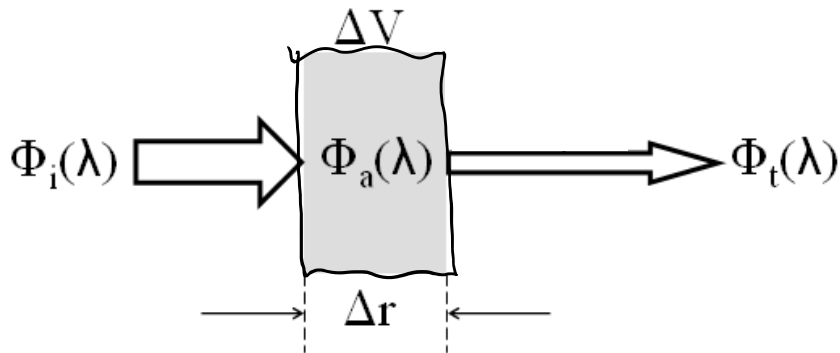
Now we can rearrange the above expression

$$a\, dr = -\frac{d\Phi}{\Phi}$$

and integrate both sides

$$\int_0^r a\, dr = \int_0^r \frac{1}{\Phi} d\Phi$$

Deriving Beer's Law

Integrating...

$$\int_0^r a\,dr = -\int_0^r \frac{1}{\Phi}d\Phi$$

$$a(r - 0) = -\big(\ln\big(\Phi(r)\big) - \ln\big(\Phi(0)\big)\big)$$

Simplify left-hand side, substitute actual Φ values in right-hand side:

$$ar = -(\ln(\Phi_t) - \ln(\Phi_i))$$

and solve for a

$$a = -\frac{\ln\frac{\Phi_t}{\Phi_i}}{r}$$

Units?

equivalently: $\Phi_t = \Phi_i \exp(-ar)$

Other names and symbols...

- We just derived the *absorption coefficient*

$$a = -\frac{\ln \frac{\Phi_t}{\Phi_i}}{r} \quad \text{[m}^{-1}\text{]}$$

- Spectrophotometers (such as you will use this afternoon) often report Absorbance (A), where

$$A = -\underline{\log_{10}}\left(\frac{\Phi_t}{\Phi_i}\right) \qquad \text{Units?}$$

$$a = \ln(10) * A / r \qquad \text{unitless}$$

$$= 2.303 * A/r$$

Note that sometimes A is also called "optical density" or OD.

OK, but what about scattering? We want to measure real samples, in real instruments!

- Option 1: Remove the scatterers from the sample

- Option 2: Collect (nearly) all of the scattered light and measure a known fraction of it

- Option 3: Make well-justified assumptions about the scattered light and apply a correction
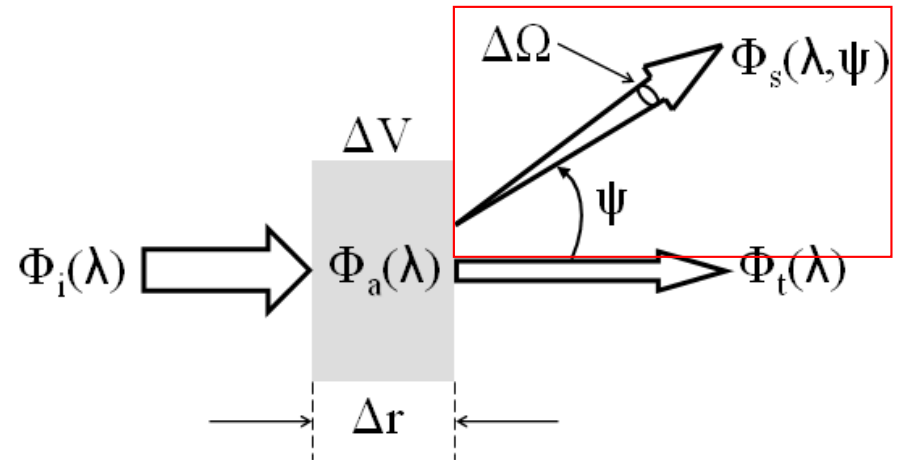
Absorption measurements when scattering is negligible

- Benchtop spectrophotometry
- Double beam and single beam configurations

1. Measure transmittance by both the sample and a reference (what should it be?)

2. Compute the sample absorbance by difference

   $A_{corr} = A_{samp} - A_{ref}$
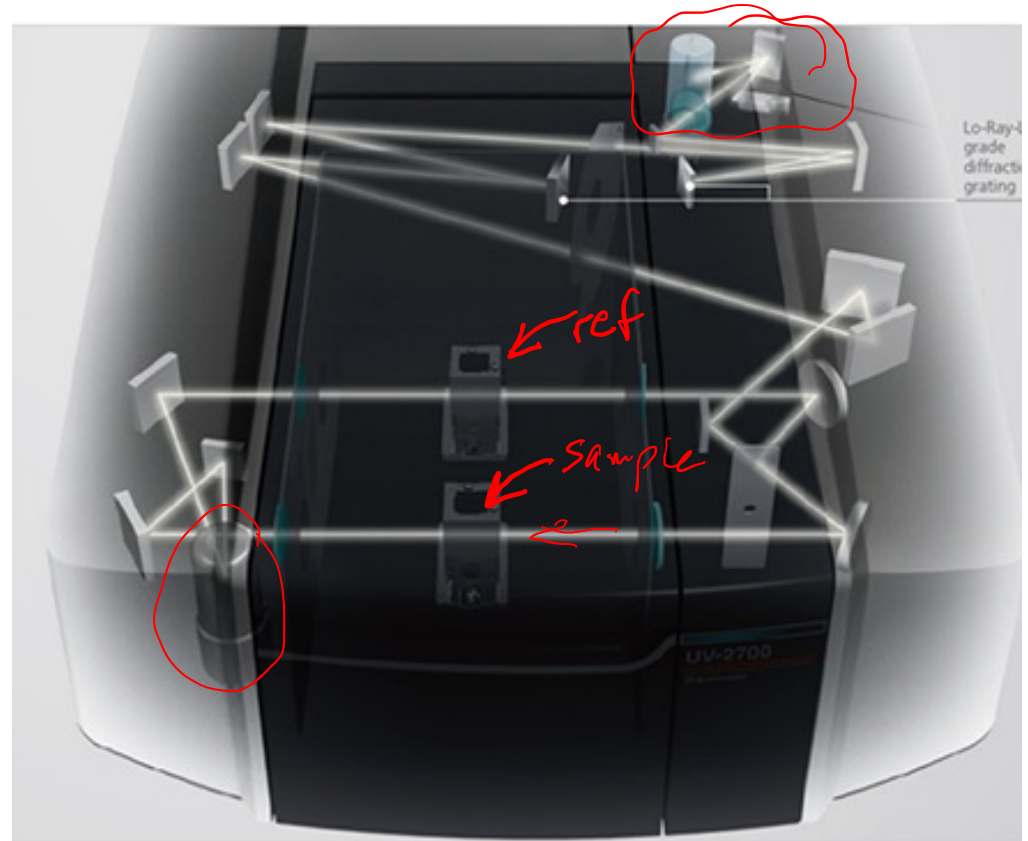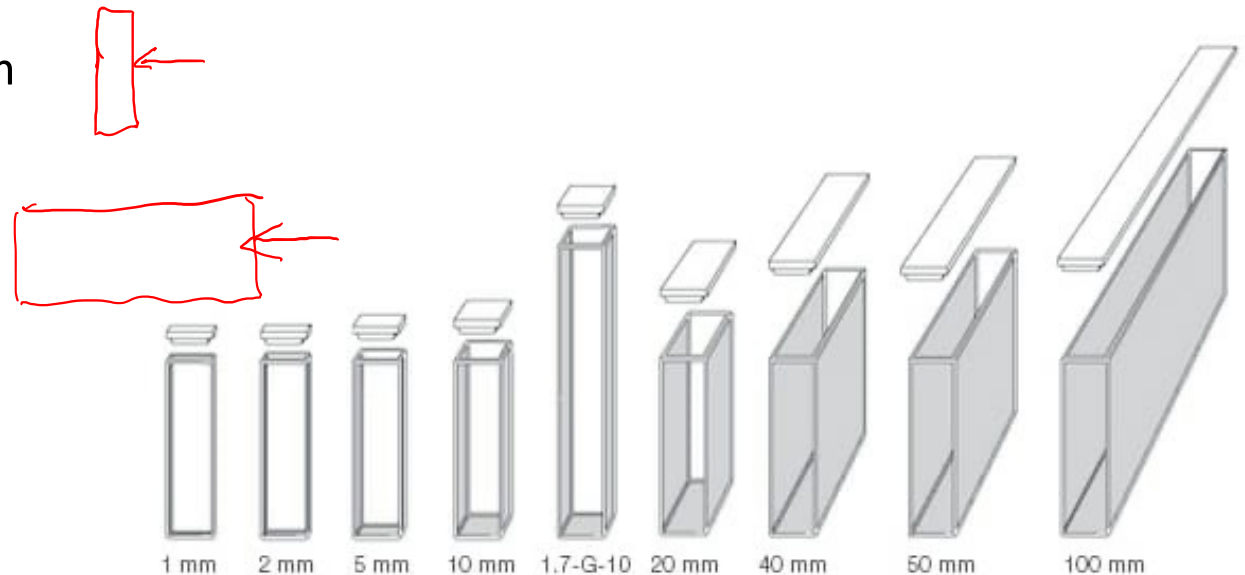 $= -\log_{10}(\Phi_{t,samp}/\Phi_{t,ref})$

Figure: https://www.ssi.shimadzu.com/service-support/faq/uv-vis/instrument-design/25/index.html

Absorption measurements when scattering is negligible

- Benchtop spectrophotometry
- Double beam and single beam configuration
- Pathlength through sample

Recall that A = $-\log_{10}(\Phi_t/\Phi_0)$

How will the spectrophotometer's measurement of A change if the pathlength is large?
If it is small?

1 mm 2 mm 5 mm 10 mm 1.7-G-10 20 mm 40 mm 50 mm 100 mm

https://www.ssi.shimadzu.com/service-support/faq/uv-vis/cuvettes/1/index.html

Rule of thumb: A should be between ~0.1-0.4. Outside this range, the sample must be diluted or the pathlength changed.

Absorption measurements when scattering is negligible

- Benchtop spectrophotometry
- Double beam and single beam configuration
- Pathlength through sample

Liquid core waveguide (aka Liquid Waveguide capillary cell)

Measure small sample volumes (mL) in pathlengths of 0.5 m or longer

Very sensitive to bubbles, particles, careful cleaning required (eg., Floge et al. 2009, 10.4319/lom.2009.7.260)

(a) Optical fibre (illuminating) — Optical fibre (to detector) — Reflective surface — Glass capillary — Liquid capillary

(b) Liquid core waveguide — T-junction — Optical fibre B — OUT — IN — Liquid capillaries — Optical Fibre A
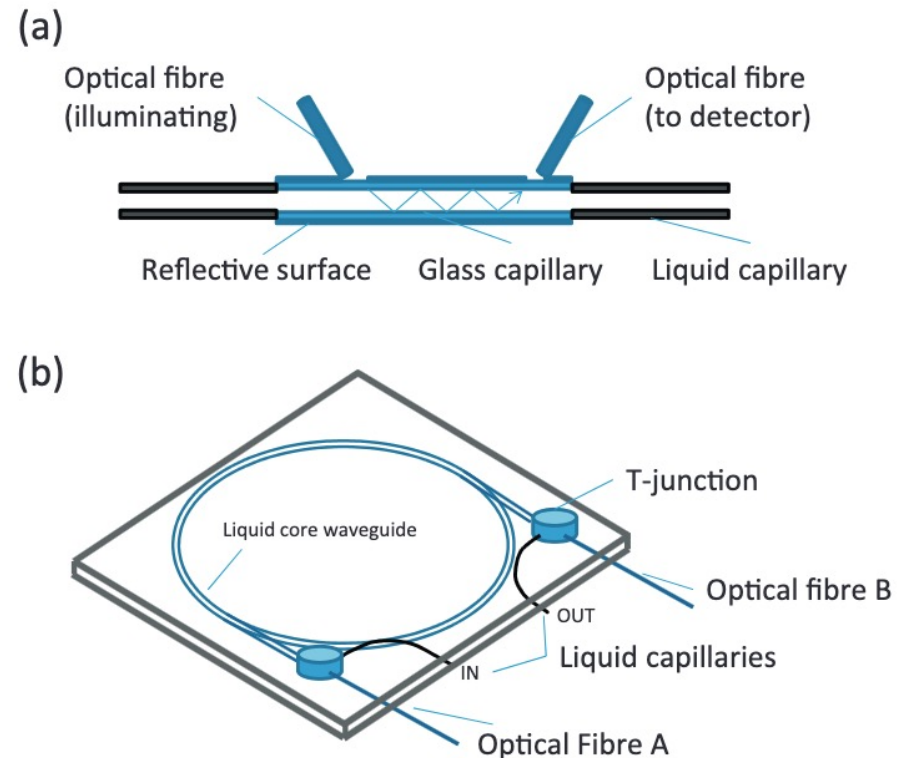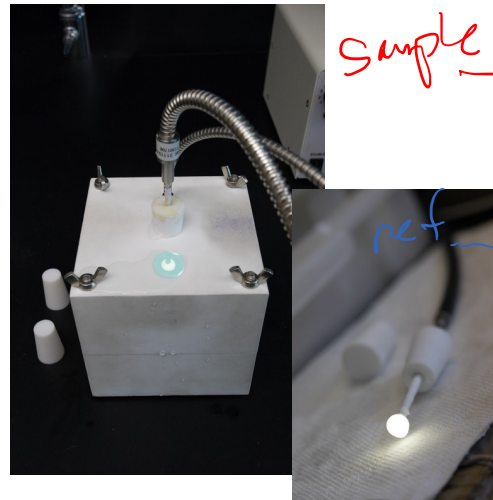
Figure: Pàscoa et al, 2012. 10.1016/j.aca.2012.05.058

Absorption measurements when scattering is not negligible (and/or a_p is of interest)
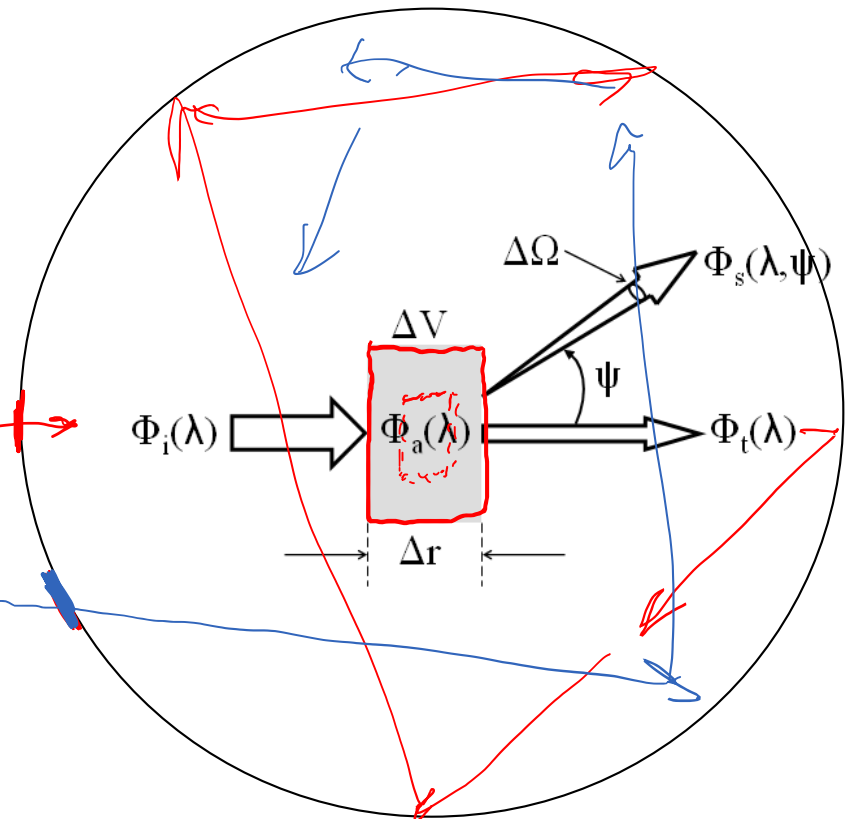
- Collect all (or nearly all) of the scattered light inside an *integrating sphere* with reflective inner surface. Measure a known fraction.

- What is the pathlength traveled by the light?

- Measure $\Phi_a = \Phi_i - (\Phi_s + \Phi_t)$

Point source integrating cavity absorption meter (PSICAM)

https://www.trios.de/en/oscar.html

https://sunstonesci.com/product/psicam

$\Phi_i(\lambda)$ $\Phi_a(\lambda)$ $\Phi_t(\lambda)$

ΔV $\Delta \Omega$ $\Phi_s(\lambda, \psi)$ ψ Δr
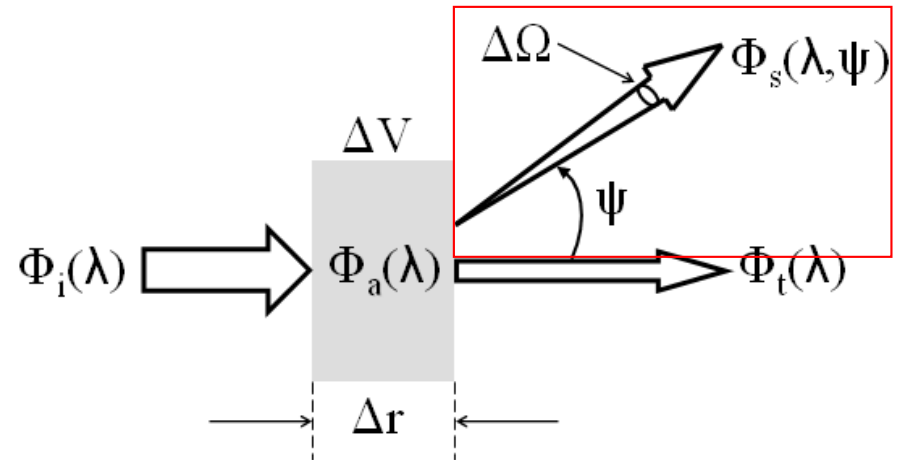
sample

ref

OK, but what about scattering? We want to measure real samples, in real instruments!

- Option 1: Remove the scatterers from the sample

- Option 2: Collect (nearly) all of the scattered light and measure a known fraction of it

- Option 3: Make well-justified assumptions about the scattered light and apply a correction

Filter-pad absorption measurements

- Determine the geometric pathlength from the volume filtered and the area of the filter that the sample passed through

- Put the filter over the detector port of the spectrophotometer, at the entrance/exit of an integrating sphere, or in the center of an integrating sphere, and measure the absorbance.

- Also measure and subtract the absorbance of a similarly-moistened blank filter. Collect blank scans in triplicate to estimate uncertainty.

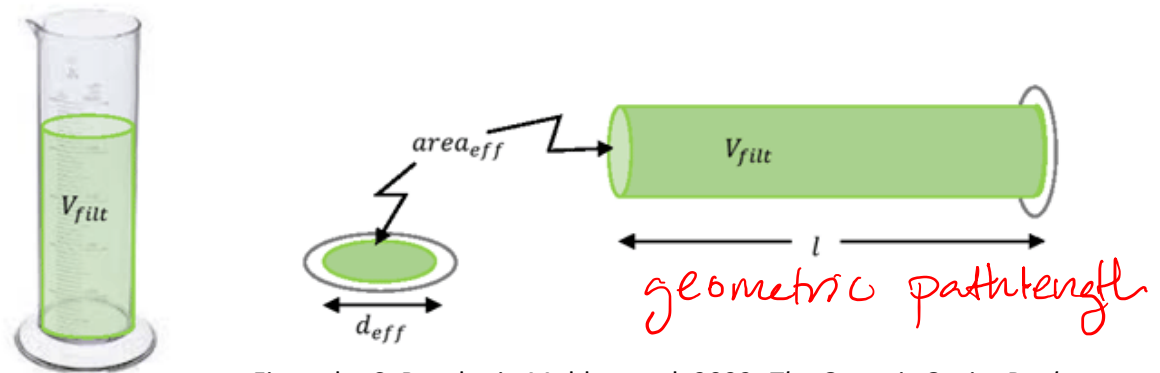- **Is simply subtracting the absorbance (which is mostly scattering...) of a blank filter sufficient?**

Figure by C. Roesler in Mobley et al. 2022, *The Oceanic Optics Book*
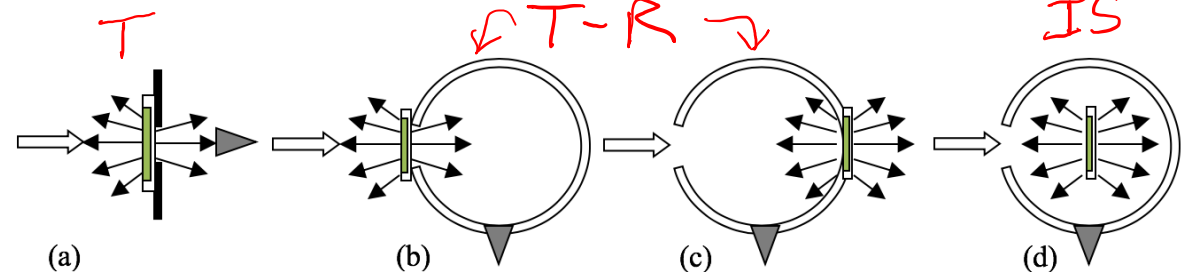
(a)   (b)   (c)   (d)

Figure 5.1. Spectrophotometric configurations for determining filter pad optical density: (a) transmittance mode (T-mode), (b and c) transmittance and reflectance mode measured with an integrating sphere with externally mounted samples (T-R-mode); (d) internally mounted sample in integrating sphere (IS-mode). Open arrow indicates incident beam, black arrows indicate beams scattered from filter, grey cone indicates detector for the generalized model.

Figure: Roesler, Collin S., Dariusz Stramski, Eurico J. D'Sa, Rüdiger Röttgers and Rick A. Reynolds. "Chapter 5: Spectrophotometric Measurements of Particulate Absorption Using Filter Pads." (2017). in Neeley et al., 2018. doi:10.25607/OBP-119.

Filter-pad absorption measurements

Is simply subtracting the absorbance (which is mostly scattering...) of a blank filter sufficient?

- There is additional scattering off the filter fibers which causes pathlength amplification.

- Even after subtracting the absorbance of the blank, pathlength amplification still increases the probability of absorption relative to the same sample in suspension

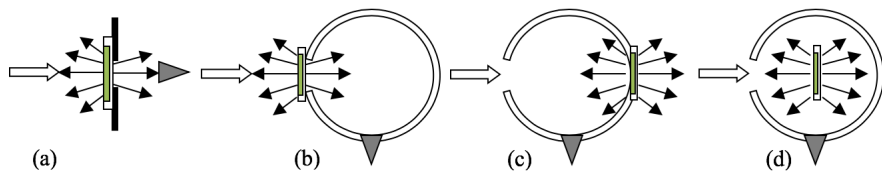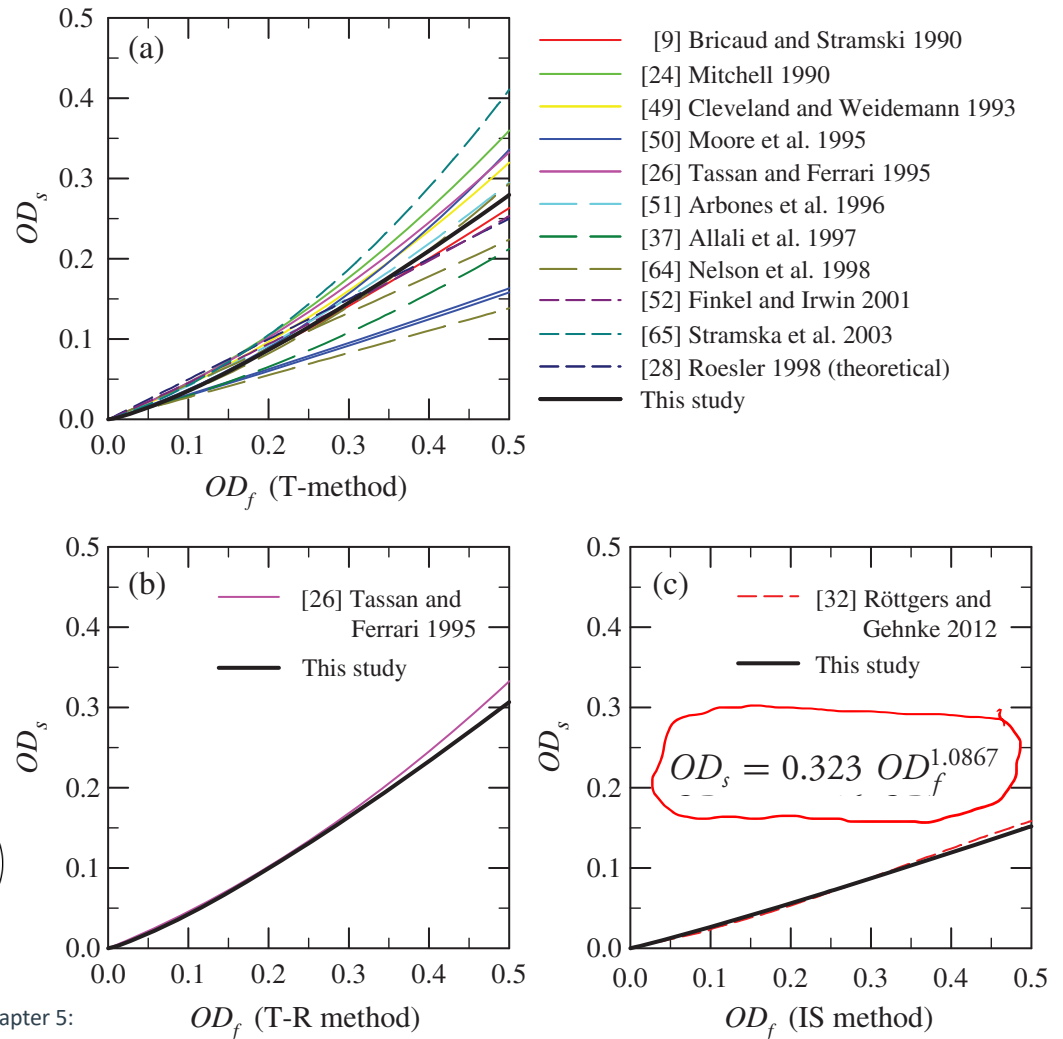Solution: Apply an empirical correction relating A on the filter pad to A in suspension.

Figure: Neeley et al., 2018. doi:10.25607/OBP-119.
Roesler, Collin S., Dariusz Stramski, Eurico J. D'Sa, Rüdiger Röttgers and Rick A. Reynolds. "Chapter 5: Spectrophotometric Measurements of Particulate Absorption Using Filter Pads." (2017).

Figure: Stramski et al. 2015, 10.1364/AO.54.006763

(a)

- [9] Bricaud and Stramski 1990
- [24] Mitchell 1990
- [49] Cleveland and Weidemann 1993
- [50] Moore et al. 1995
- [26] Tassan and Ferrari 1995
- [51] Arbones et al. 1996
- [37] Allali et al. 1997
- [64] Nelson et al. 1998
- [52] Finkel and Irwin 2001
- [65] Stramska et al. 2003
- [28] Roesler 1998 (theoretical)
- This study

OD_f (T-method)

(b)

- [26] Tassan and Ferrari 1995
- This study

OD_f (T-R method)

(c)

- [32] Röttgers and Gehnke 2012
- This study

$$OD_s = 0.323\ OD_f^{1.0867}$$

OD_f (IS method)

Reflecting-tube absorption & attenuation meters

- Measure absorption and beam attenuation simultaneously. Use the beam attenuation measurement to correct for undetected scattered light in the absorption measurement. Compute scattering by difference.

- Absorption measured in a cylinder with reflective interior and a diffuser in front of the detector. Only light scattered through angles > ~42° is lost.

- Attenuation measured in a cylinder with black, non-reflective walls and a detector with a narrow field of view

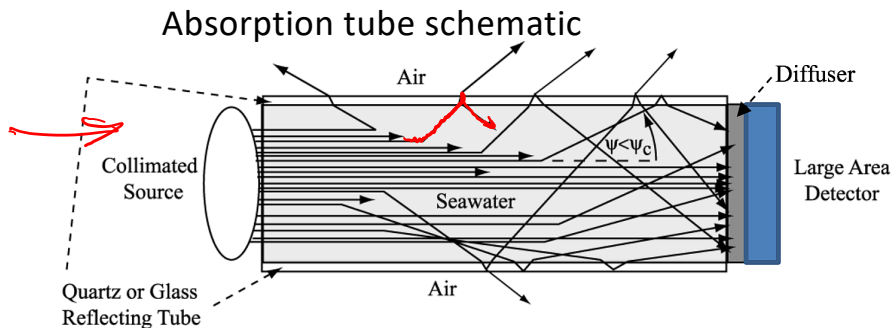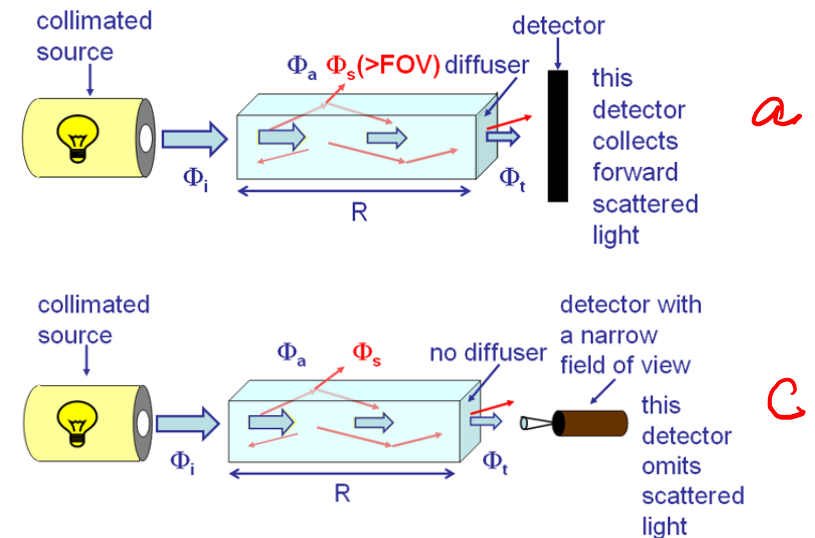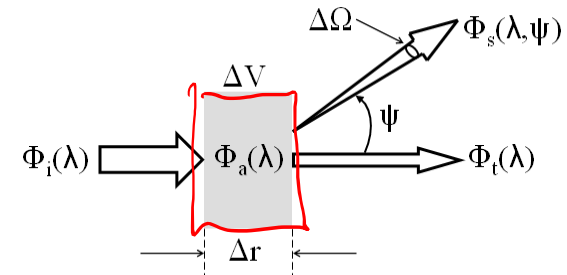$\psi_c \cong 42°$

$\psi_c < \psi \leq \frac{\pi}{2}$

$[1 - \rho_g(\psi)]$

$\psi \geq \frac{\pi}{2}$

Absorption tube schematic

Figure: Neeley et al., 2018. doi:10.25607/OBP-119
Twardowski et al, "Ch. 2: Reflective Tube Absorption Meters"

$\psi \leq \psi_c$

$\psi_c < \psi < \frac{\pi}{2}$

Figures 3.8-3.9, Mobley et al. 2022, *The Oceanic Optics Book*

Reflecting-tube absorption & attenuation meters

b = scattering
c = beam attenuation
$b = c - a$

But!

Absorption is overestimated because not all the scattered light is detected in a-tube

Attenuation is underestimated because a little bit of scattered light *is* detected in c-tube

$a_m(\lambda)$, $b_m(\lambda)$, $c_m(\lambda)$ = measured spectra

Photo: ac-s datasheet, https://www.seabird.com/asset-get.download.jsa?id=54627862140
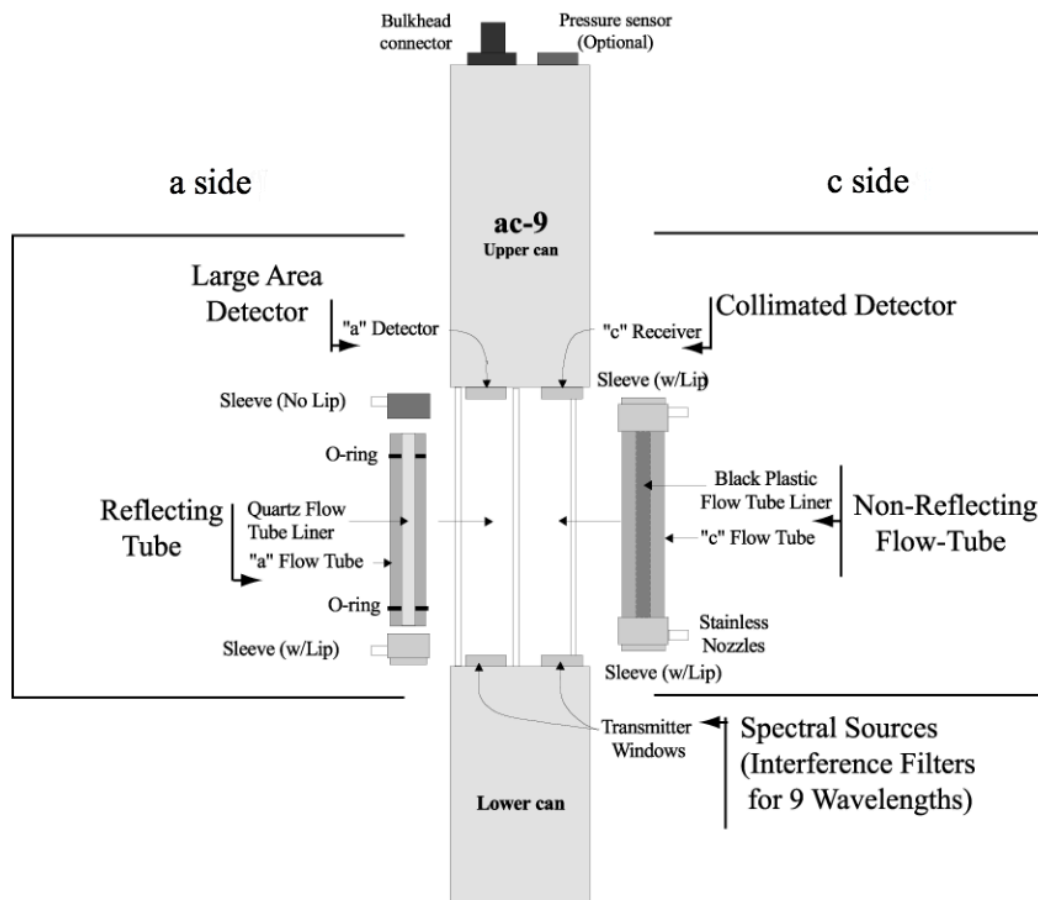
ac-s

Figure 2.3. Schematic illustration of the ac-9 beam attenuation and absorption meter (courtesy of Sea-Bird Scientific).
Figure: Neeley et al., 2018. doi:10.25607/OBP-119

Reflecting-tube absorption & attenuation meters

- $a(\lambda)$ = true absorption spectrum (measured in suspension in an integrating sphere)
- For measured $a_m(\lambda)$, $c_m(\lambda)$:
    - temperature-salinity corrections performed
    - pure water absorption already removed

Use the calculated scattering $b_m(\lambda)$ to estimate the scattering *correction* ε (Zaneveld, 1994). Assume $a_m(\lambda_r)$ in the near infrared (NIR) is zero...
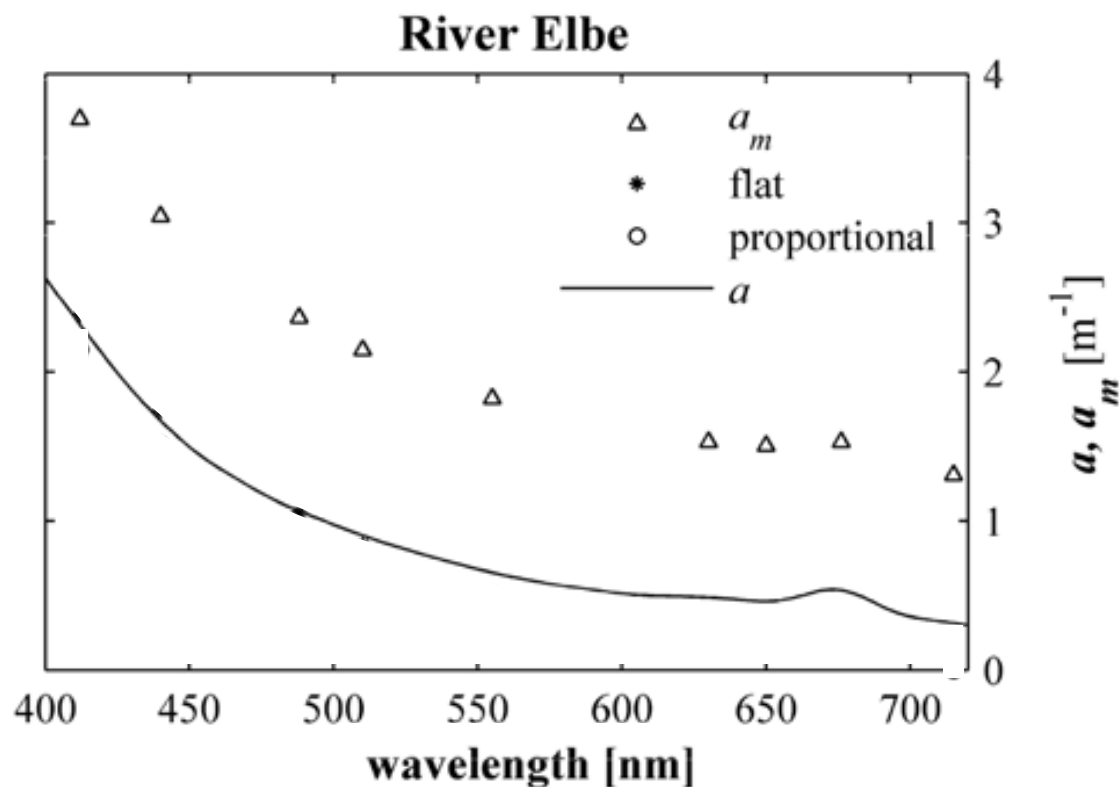

Figure: Röttgers et al., 2013. 10.1016/j.mio.2013.11.001

Reflecting-tube absorption & attenuation meters

- $a(\lambda)$ = true absorption spectrum (measured in suspension in an integrating sphere)
- For measured $a_m(\lambda)$, $c_m(\lambda)$:
  - temperature-salinity corrections performed
  - pure water absorption already removed

Use the calculated scattering $b_m(\lambda)$ to estimate the scattering *correction* ε (Zaneveld, 1994). Assume $a_m(\lambda_r)$ in the near infrared (NIR) is zero...

Option 1 "Flat": ...and that ε is the same at all wavelengths.

Option 2 "Proportional": ... or that ε is equal to a constant *proportion* of the measured scattering
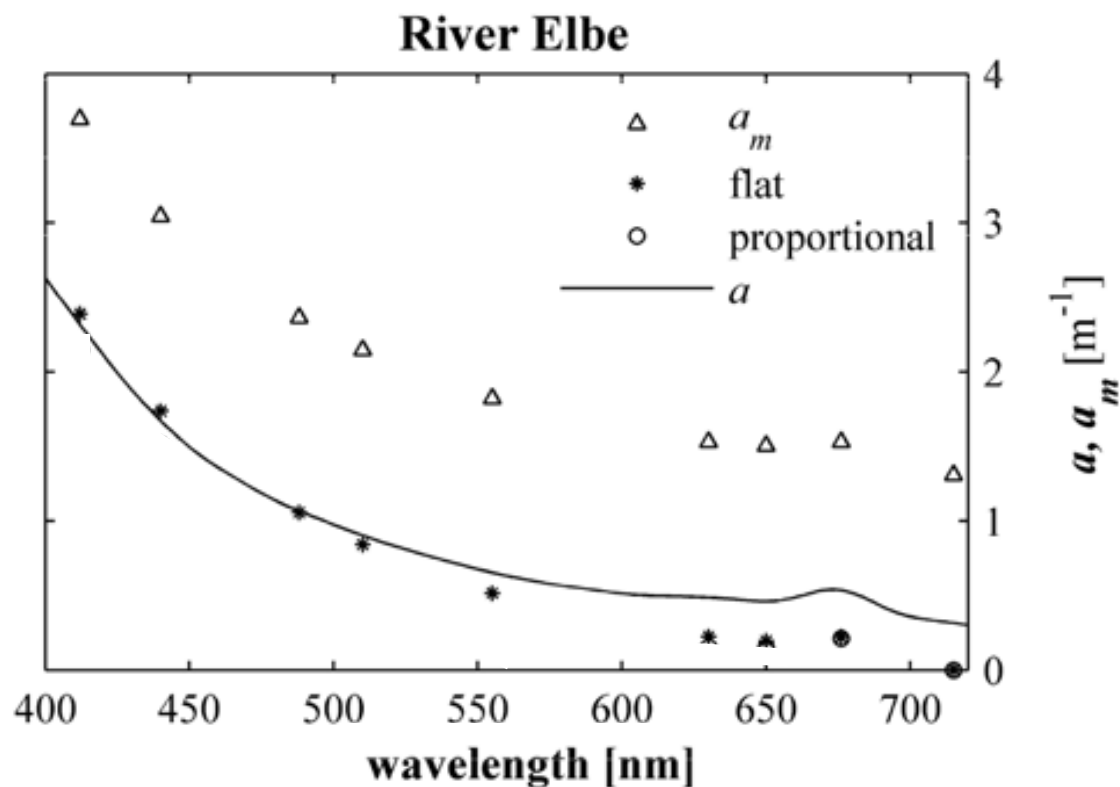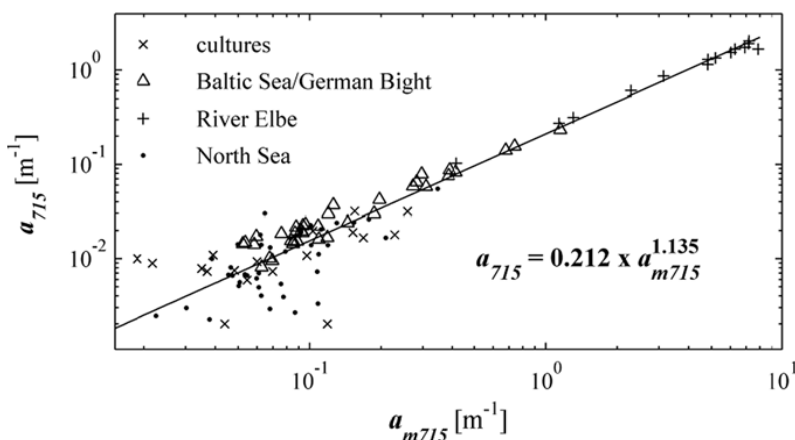

Figure: Röttgers et al., 2013. 10.1016/j.mio.2013.11.001

Reflecting-tube absorption & attenuation meters

What if you don't want to assume that $a_m(715)$ is zero?

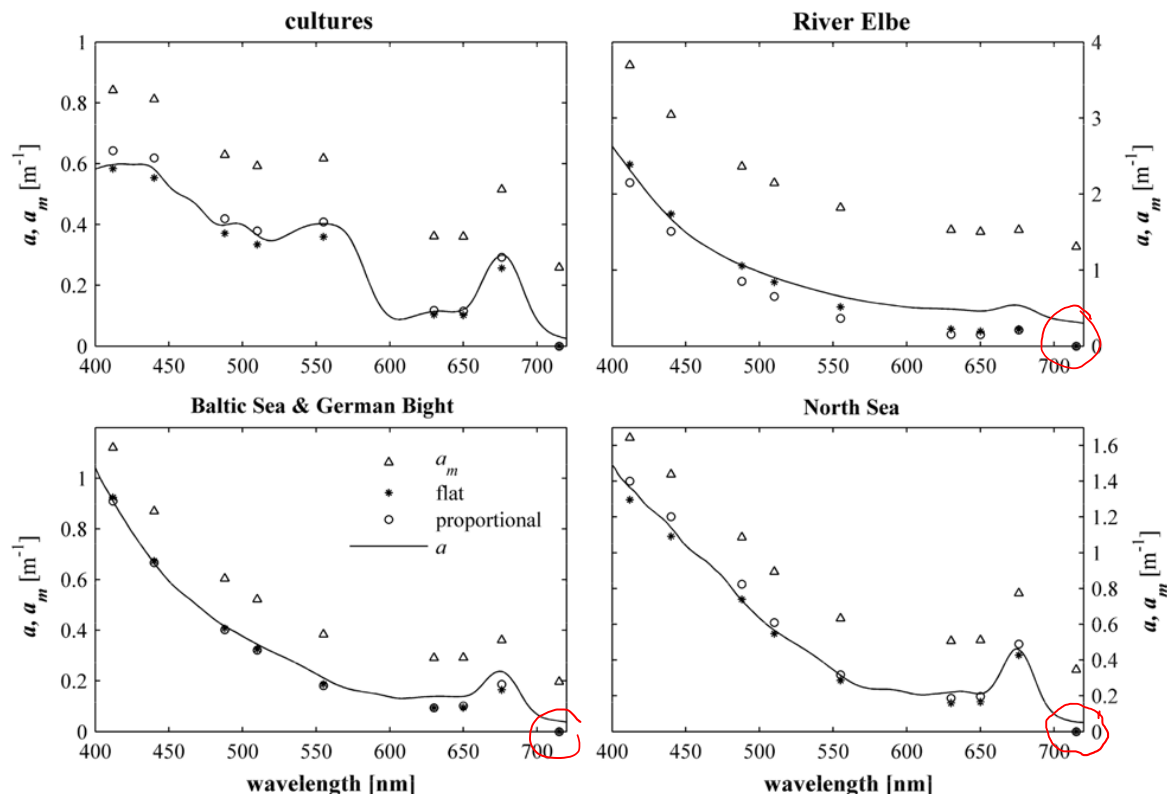Empirical correction of Röttgers et al. 2013:

1. Compute $a(715)$ from empirical fit to measured and "true" absorption (in suspension, in an integrating sphere):

$$a_{715} = 0.212 \times a_{m715}^{1.135}$$

2. Correct $c(\lambda)$ for underestimation due to finite detector acceptance angle (more tomorrow!)

3. *Then* do "proportional scattering correction"

? If you know the VSF

Figures: Röttgers et al., 2013. 10.1016/j.mio.2013.11.001

After $a(\lambda)$ and $c(\lambda)$ have been scatter-corrected (by any method), you need to recompute $b(\lambda)$.

Closure

Kostakis et al. (2021): multi-investigator intercomparison of absorption measurements in relatively high-absorption coastal water in Florida.

Think-pair-share: Look at the terms/parameters/methods in the table and figures. Do you feel like you have the vocabulary to read Kostakis et al?

| Instrument	Method	Number of sensor systems	Original spectral range (resolution)	Absorption parameter(s) directly measured	Absorption parameter derived
PSICAM	Bench top integrating sphere	1	360–728 nm (2 nm)	a_{nw}, a_{CDOM}	a_p
Turner Designs ICAM	In situ integrating cavity	2	365, 440, 488, 510, 532, 555, 590, 630, 676	a_{nw}, a_{CDOM}	a_p
WET Labs AC-s	In situ reflective tube	1	AC-s 1: 399–741 nm (mean: 4 nm) AC-s 2: 401–726 (mean: 3.7 nm)	a_{nw}	—
WET Labs AC-9	In situ reflective tube	2	412, 440, 488, 510, 532, 555, 650, 676, 715	a_{nw}	—
TriOS irradiance quartet	Gershun	1	320–900 nm (2 nm)	a_t	a_{nw}
ISFP	Bench top spectrophotometer with filter pad inside integrating sphere	2	ISFP 1: 400–750 nm ISFP 2: 300–850 nm (both 1 nm)	a_p, a_{nap}	a_{ph}
QFT-ICAM Filter pad	Bench top integrating sphere with filter pad inside cavity	1	340–844 nm (2 nm)	a_p, a_{nap}	a_{ph}
LWCC	Bench top capillary waveguide	1	240–750 nm (2 nm)	a_{CDOM}	—
Cary E3 UV–VIS spectrophotometer	Bench top dual-beam spectrophotometer with 10-cm cuvettes	1	400–750 nm (1 nm)	a_{CDOM}	—
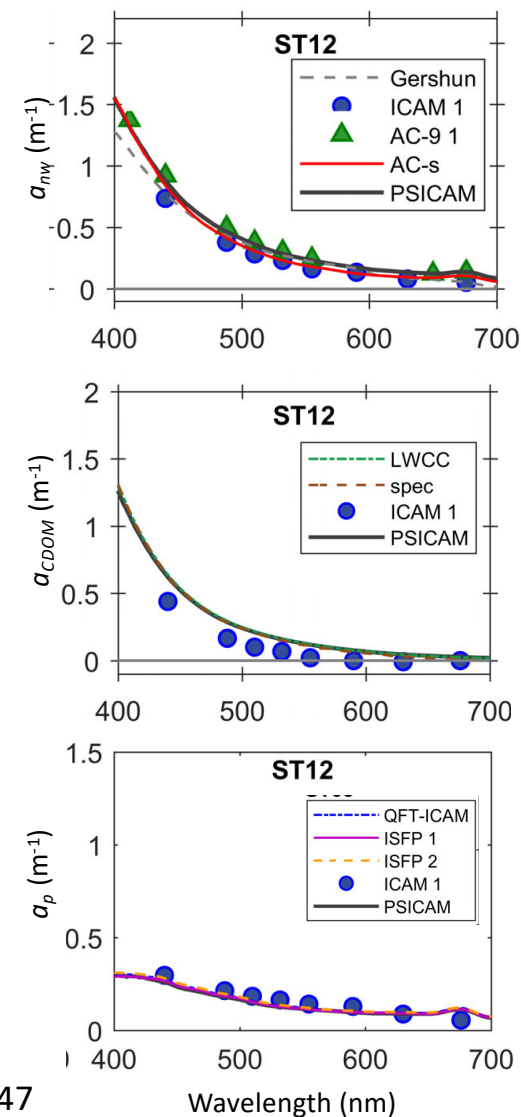
Figure and table: Kostakis et al (2021). 10.1002/lom3.10447

Absorption, part 2

- Beer-Lambert-Bouguer Law
  - Conceptual
  - Realistic
- Survey of measurement techniques
- Lab overview
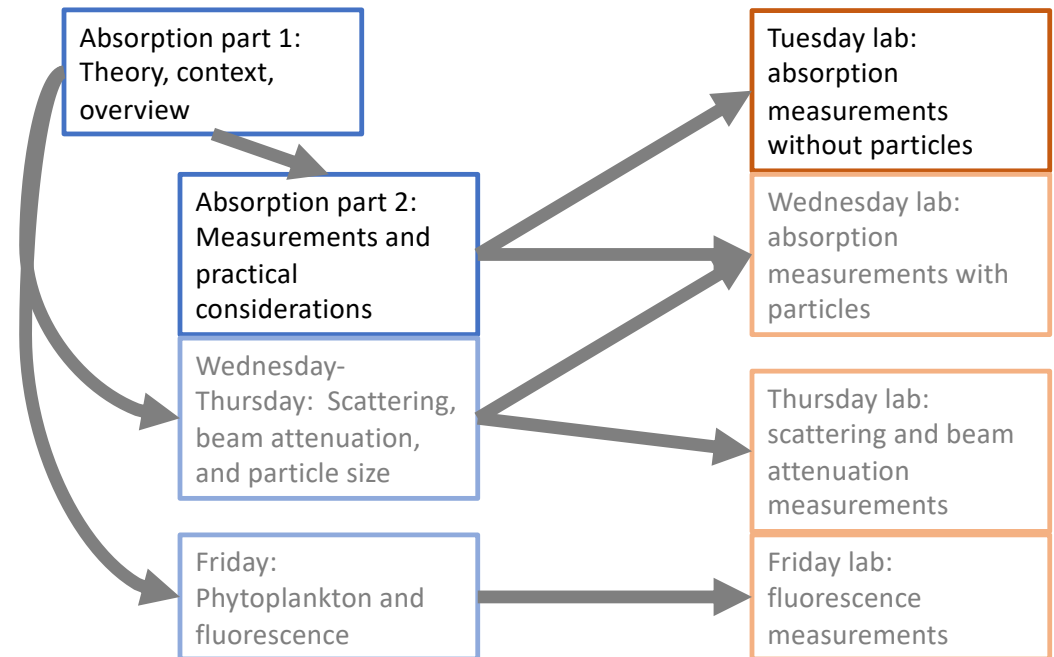
Lab today: absorption by CDOM

Group 1

- Beer's Law demonstration (simple, hands-on)
- Benchtop spectrophotometer
  - Effect of salinity and temperature
  - Effect of geometric pathlength
  - How to
    - Clean and care for cuvettes and the instrument
    - Collect absorbance measurements relative to appropriate reference solutions

Group 2

- ac-meters
  - Effect of salinity and temperature
  - Effect of pathlength
  - How to
    - Clean and care for the instrument
    - Acquire pure-water readings
    - Correct for temperature and salinity effects

45 min

90 min

Lab today: absorption by CDOM

Broad questions to answer as a group, presented at the start of the day tomorrow:

1. *How does the absorption spectrum of purified water vary with temperature?*
2. *How does the absorption spectrum of purified water vary with added salt (salinity)?*
3. *How does the dye concentration (in the Beer's Law demonstration) affect the absorbance of a solution?*
4. *How does the geometric pathlength impact the measured absorbance and the derived absorption coefficient?*

You will need to combine measurements from all of the small group work this afternoon to answer the questions!

Lab tomorrow: absorption by particles

- Filter-pad absorption measurements inside an integrating sphere
- Partitioning particulate absorption into phytoplankton pigment and non-algal components
- Acquiring and correcting ac-meter measurements of unfiltered seawater and cultures