Absorbance Reference

Absorbance mode.

OOIBase32 uses an equation to determine the concentration of a species in solution (illustrated below). The software uses this equation to evaluate each pixel on the detector and produce the absorbance spectrum:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

Where:

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

The concentration of a species in a solution directly affects the absorbance of the solution. This relationship, known as Beer's Law, is expressed as: $A_\lambda = \varepsilon_\lambda c \ell$

Where:

A = Absorbance at wavelength λ,

ε_λ = Extinction coefficient of the absorbing species at wavelength λ

c = Concentration of the absorbing species and l is the optical path length of the absorption.

Click the Absorbance mode () icon or select **Spectrum | Absorbance Mode** to enter Absorbance mode.

As a reference, here some calculated numbers at 266nm

Protein	Space group	conc(M)	eps ($M^{-1}cm^{-1}$)	Absorbance 100µm crystal
elastase	P212121	0.0309	45410	14
insulin	I213	0.0843	4820	4
hewl	P43212	0.0610	32717	20
pyp	P65	0.0592	10518	6
rnaseA	P3221	0.0433	6998	3
thaumatin	P41212	0.0592	24823	15
trypsin	P212121	0.0315	30478	10

Abs=conc*eps*l , l=path length crystal