

Performance Test of a rapid "Multiwavelength Flash Photolysis Apparatus"

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For the studying of rapid spectral changes in turbid suspensions of biological cell material we have built a "Multiwavelength flash photolysis apparatus" (MWFP) that allows to measure, store and evaluate 3000 absorbance spectra per second in the wavelength range between 340 and 1000 nm. With a time resolution of 300 μ s spectral changes of 0.0001 O.D. can be resolved. Integration of a number of consecutive data points allows to extend the amplitude resolution of the device down to about 10^{-5} O.D. Moreover, in cases where one spectrally defined intermediate is converted into another one, adding absorbance signals from the wavelength region, where a peak appears, and subtracting signals from the spectral region, where a peak disappears, not only enhances the signal to noise ratio further, but also eliminates drift problems usually inherent in a single beam design. (1)

The MWFP apparatus has been tested using two well studied biological systems which undergo a number of defined spectral changes upon flash illumination. Data will be shown on:

1.) The bacteriorhodopsin photocycle:

Rise and decay of the intermediates BR^{548} , BR^{568} , M^{412} , O^{640} , and R_1^{590} , obtained from dark- and light-adapted patches of bacteriorhodopsin containing membranes.

2.) The rhodopsin photolytic cycle:

Decay of metarhodopsin I (MI) and Rise of metarhodopsin II (MII). In this case a complete separation of spectral changes and accompanying light scattering increments has been possible.

(1) R. Uhl and B. Meyer, Biophys. Struct. Mech. Suppl. to Vol.6, 131 (1980).