A HIGH PERFORMANCE MULTIWAVELENGTH FLASH PHOTOLYSIS APPARATUS

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A convenient way of studying rapidly occuring processes in biological systems is to monitor accompanying spectral changes of intrinsic or externally applied optical probes. Unfortunately these spectral changes are quite frequently obscured by underlying changes in light scattering from the membrane material studied. Thus it becomes necessary to monitor rapid changes of the complete spectrum rather than its absorption changes at a single wavelength.

For the studying of rapid spectral changes occuring in the photoreceptor cell o vertebrates, in the presence and absence of externally applie_ probes, we have devised a rapid spectrophotometer with the following design goals:
Spectral resolution: 10 nm
Spectral range: 320 nm, freely selectable between 300 and 1000 nm
Amplitude resolution: better than 0.0001 O.D.
Time resolution: 200 µsec
It should be possible to store 4000 such spectra consecutively.

The spectrophotometer consists of a quartz halogen lamp as a light source and a collimating lens system which produces parallel white light. This passes through a thermostated cuvette and is then focussed on the entrance slit of a custom made spectrograph, consisting of a concave holographic grating (f=500 mm), which creates a spectrum on its Rowland circle, where 70 photovoltaic devices produce photocurrents proportional to the light intensity at a given wavelength.Photocurrents are converted into voltages proportional to the particular absorbances by a special logarithmic amplifier device, connected to the anode of the photodiodes. To achieve an amplitude resolution of better than 0.0001 O.D., using a 12 bit Analog to Digital Converter (ADC), an automatic DC compensation circuitry has to precede another amplification step, which then amplifies only the small changes in photocurrent on top of a large DC-light level. To avoid overly long exposure to white light of the light sensitive membrane material, the DC compensation should take no more than 1 msec. Subsequently the voltages of the 32 channels are fed simultaneously into 32 sample and hold amplifiers, whose output are sequentially scanned by two analog input boards, working in parallel, of a PDP 11/23 microcomputer. The PDP 11 takes in stores and processes the data.

The performance of such a spectrophotometer design, its advantages and disadvantages will be discussed in detail.