Chapter 1
Developments in Classical Optical Spectroscopy

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Summary

An overview is given of the development of optical techniques as applied to photosynthesis research
during the last 50 years and their importance for present day research is discussed. The review concerns
the “classical” techniques, i.e. measurements of absorbance and of light-induced changes of absorbance
and fluorescence emission and excitation spectroscopy.

Abbreviations: BChl – bacteriochlorophyll; Chl – chlorophyll; FMO protein – Fenna–Matthews–Olson
protein of green sulfur bacteria

I. Introduction

In view of the key role of pigments in photosynthesis, it is not surprising that optical methods have
played, and continue to play, an important role in photosynthesis research. Engelmann (1882)
was the first to show, by means of action spectra of oxygen production, that chlorophyll and the
so-called accessory pigments are involved in photosynthesis. Progress in optical research on pho-
tosynthesis, however, was for a long time arrested, mainly because simple and sensitive
methods for measuring and recording light intensities were lacking. About 50 years ago, how-
ever, a rapid development of optical techniques set on. World War II saw the development of the
photomultiplier, and in the years that followed faster and more reliable and sensitive electronic
deVICES and new light sources became available, including pulsed lasers for flash spectroscopy as
well as computers for data processing and registration. Hand in hand with these developments
optical studies of photosynthesis have acquired growing importance for gaining insight in the mol-
ecular mechanisms of photosynthesis.

In the chapters that follow, accounts will be
given of the present state of the art, and examples
will be given of the information obtained by mod-
ern optical methods. This chapter will survey
some of the developments during the last 50 years
and will discuss some of the “traditional” me-
thods that still play an important role in photo-
synthesis research.

Pioneers of the early days were H. Kautsky
and E.C. Wassink and, at a somewhat later stage,
L.N.M. Duysens, C.S. French, B. Chance, H.T.
Witt and B. Kok. The first two studied the fluo-
rescence properties of photosynthetic material
(Kautsky and Hirsch, 1931; Kautsky and Franck,
while spectroscopy of light-induced absorbance changes was pioneered by Duysens (1952). In particular, the latter technique has proved invaluable to study the components of photosynthetic electron transport. One of the early results of such studies was, around 1960, the discovery of the two photosystems in plant photosynthesis (Duysens et al., 1961). The use of pulsed lasers was initiated in the sixties (DeVault and Chance, 1966; Wolff et al., 1969; Netzel et al., 1973) and has now progressed into the femtosecond region, enabling the study of early processes of energy transformations in excited pigments (see Chapter 4 by Jimenez and Fleming). French (French and Young, 1952) and in particular Duysens (1952) were the first to apply fluorescence spectroscopy to study energy transfer in photosynthetic organisms. French also devised various ingenious apparatus for the deconvolution of absorption spectra, the automatic recording of action spectra and for the measurement of so-called derivative spectra (French et al., 1954; French, 1955; French and Harper, 1957; Allen et al., 1960). Today, such measurements are routinely, and much more conveniently, performed with the aid of computer analysis.

In the next two sections, we shall survey these developments in some more detail, and briefly discuss the importance of these “classical” optical techniques in modern photosynthesis research.

II. Absorption and Absorption Difference Spectroscopy

Measurement of the absorption spectrum is one of the basic methods to obtain information about the characteristics of photosynthetic material. As this is normally done with commercial apparatus an extensive discussion of the method should not be necessary here. Nevertheless, absorption spectra of rather poor quality are being published occasionally even today, and this is mainly due to the fact that these commercial apparatus are not designed for scattering material. Light scattering, if not properly corrected for, not only causes an upward shift and distortion of the absorption spectrum, but it may also decrease the amplitude of absorption bands (Amesz et al., 1961; Latimer and Eubanks, 1962). Moreover, additional distortion may occur due to selective scattering near the absorption bands (Latimer, 1959). The effects can be minimized by collecting the transmitted light over a relatively large angle. Other methods that may be applied are adjusting the refractive index of the medium and the so-called opal glass method (Shibata, 1958), the latter, however, at the expense of sensitivity. In some cases reliable data can be obtained by fluorescence detected absorbance (Kramer et al., 1985). Of course, the same principles apply to more specialized absorption measurements, such as linear and circular dichroism and absorption difference spectroscopy. It should be noted, however, that even a properly measured absorption spectrum is not identical to that of the same pigments in solution, if the pigments are contained in particles that have a non-negligible absorption. This is the so-called “flattening effect” (Duysens, 1956).

Due to the presence of different “pools” of chemically identical pigments or to excitonic interactions the in vivo absorption spectra of photosynthetic pigments nearly always consist of strongly overlapping absorption bands which are, moreover, inhomogeneously broadened. French and coworkers (Allen et al., 1960) determined the first derivatives of the absorption spectra to distinguish the various in vivo absorption bands of chlorophyll. A more convenient and nowadays extensively used method to enhance the resolution of absorption (or other) spectra is by measuring the second or even fourth derivatives (Martin, 1959; Butler and Hopkins, 1970a, 1970b; see Fig. 1). Caution, however, is needed in the interpretation because the resulting bands are not only sharpened, but side bands are also generated by the differentiation.

Duysens (1952, 1957) was the first to apply the measurement of changes of absorbance, induced by illumination, to the study of photosynthesis. Since then, this method has continuously gained importance and it is still one of the most effective methods to study molecular processes in photosynthesis. Although pump-probe measurements with high time resolution are now in the forefront of research (see Chapter 4 by Jimenez and Fleming), the classical methods, using a continuous or semi-continuous measuring beam, are still being extensively used in photosynthesis research, and