

# Photoacoustic investigation of biological materials

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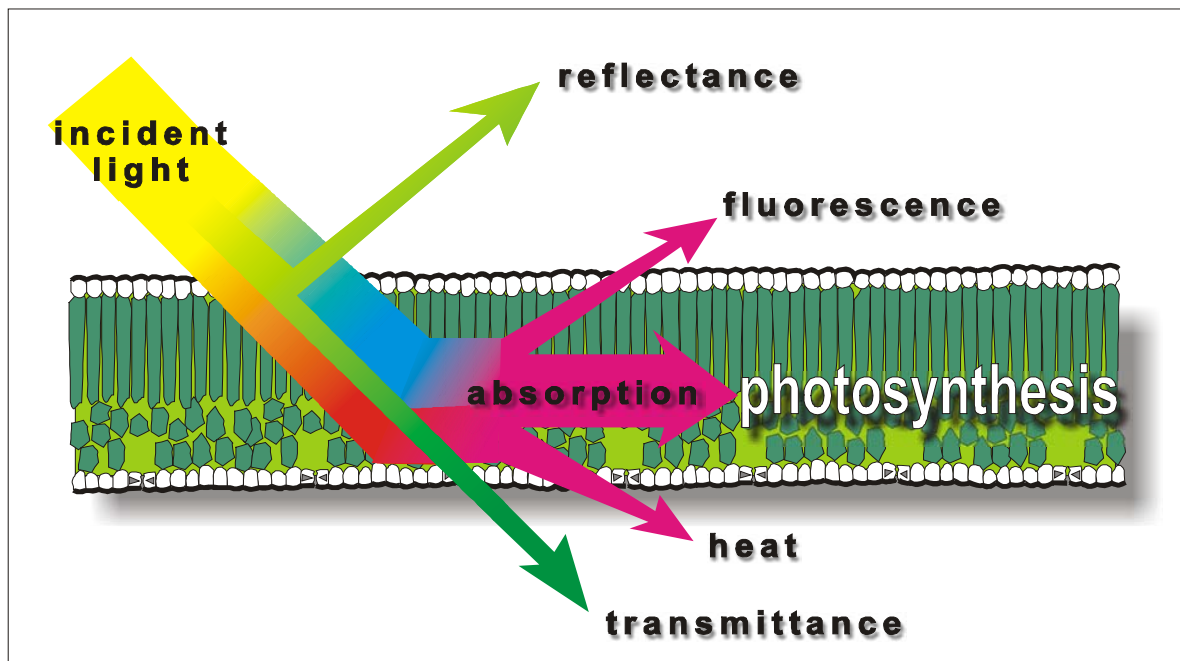
Intact, biological materials are characterized by an inhomogeneous, complex and often unstable structure. The tissue consisting of cells and aerial interspaces as well as the small organelles in the cells (nucleus, mitochondria, chloroplasts etc.) cause light scattering, internal reflectance and refraction (Fig. 1). Photoacoustic measurements are not sensitive to light scattering in comparison to other spectroscopic techniques (absorption, reflectance, fluorescence). They are, thus, suitable for measuring intact biological material without pretreatment or preparation which could destroy its normal function.

Usually photoacoustic measurements are carried out to detect thermal dissipation by non-radiative de-excitation following light absorption. In photoacoustic measurements of intact biological materials, the radiation applied for exciting thermal dissipation may be simultaneously be used also for fluorescence and

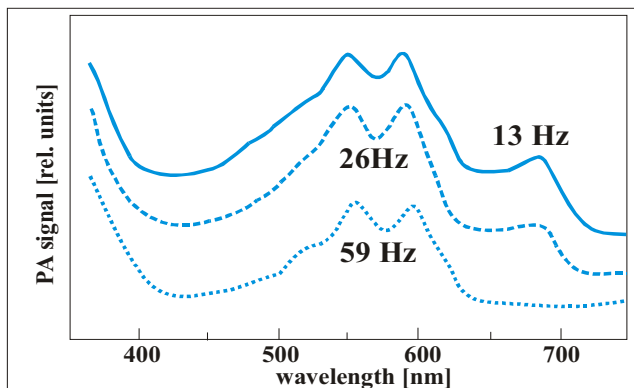
photobiochemical processes (e.g. photosynthesis in leaves, see Fig. 1) and thus the interpretation of the photoacoustic signal becomes more complicated than for non-biological samples (for reviews see [3],[5],[6],[7]).

An advantage of photoacoustic measurement is the fact that by increasing the modulation frequency of the excitation light thermally active layer contributing to the photoacoustic signal is decreased. Thus the absorption characteristics of pigments in different layers of the leaf can be detected without further preparation of the sample ("depth profiling", see example in Fig. 2 [1]).

When measuring leaves the pressure changes induced by the photosynthetic oxygen evolution may contribute to the photoacoustic signal. This can be demonstrated by the decrease of the photoacoustic signal when applying strong additional continuous light saturating photosynthesis and making oxygen evolution

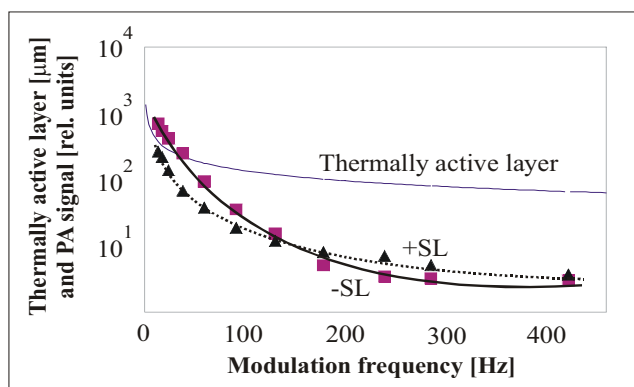


**Fig. 1.** Distribution of light energy incident on a leaf (cross section through the leaf tissue). The light is reflected at the leaf surface, transmitted through the leaf and absorbed inside the leaf. The light energy absorbed by the leaf pigments (chlorophylls and carotenoids) is transferred into photosynthesis (up to 80%), chlorophyll fluorescence (ca. 3 %) and heat (ca. 17 %).



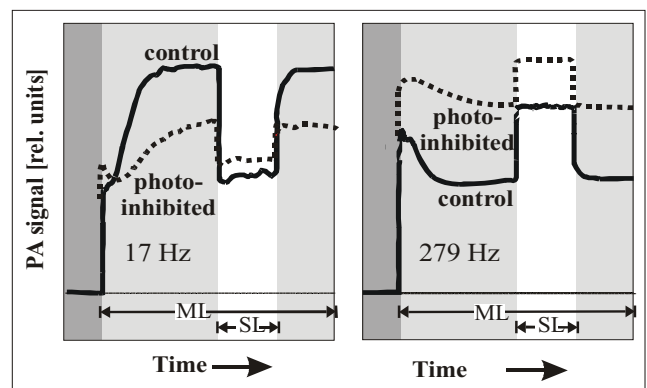
**Fig. 2:** Depth profile of a *Tradescantia* leaf [1]. At high modulation frequency only signals of the upper surface are sensed (absorption of anthocyanins in the epidermis), at lower modulation frequency the chlorophyll absorption can be seen at ca. 680 nm seen in addition.

constant (the non-modulated signal being excluded from the photoacoustic signal by the lock-in amplifier) (Fig. 3, [3]).



**Fig. 3:** Dependence of the photoacoustic signal of a radish leaf and of the thermally active layer (calculated for water and given in  $\mu\text{m}$ ) from the modulation frequency of the incident light [3]. Curves are given for the measurement without and with saturating light (SL). Below 125 Hz the signal is dominated by oxygen evolution and saturating light makes oxygen evolution constant and thus excludes it from the photoacoustic signal. Above 125 Hz oxygen evolution is no longer resolved in pulses and saturating light induces an increase of pulsed thermal component.

Photoacoustic measurements of leaves can be used to identify inhibition of photosynthetic activity as indicator of stress or decease of plants [3], [4], [5], [6], [7] (Fig. 4).



**Fig. 4.** Induction of photosynthesis of a radish leaf before and after photoinhibition [2]. With a measuring light (ML) modulated at 17 Hz (left) mainly the onset of oxygen evolution, at 279 Hz (right) the changes of thermal dissipation are sensed. For the effect of saturating light (SL) and its explanation see Fig. 3.

## References

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see also: <http://www.uni-karlsruhe.de/~botanik2/BM/H-Photoakustik.html#PASde>