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The Chlorophyll Fluorescence Imaging and its Application in Plant Science and Technology

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Seeing is believing

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We have come a long way since Hans Kautsky observed with his own eyes, in the 1930s, the red chlorophyll (Chl) *a* fluorescence changes with time and related it to Otto Warburg's measurements on photosynthesis. This was, perhaps, the first recorded remotely sensed Chl *a* fluorescence transient in a control and a poisoned leaf (see a digital video of the Kautsky effect at http://www.life.uiuc.edu/govindjee/movkautsky.html and a 3-D presentation at http://www.greentech.cz/science/lapi/3DKautsky). Chl *a* fluorescence is a non-destructive, non-invasive, and highly sensitive probe of photosynthesis. Extensive researches in the laboratories of Eugene Rabinowitch, Lou Duysens, Warren Butler, Jean Lavorel, among others, brought before us most of the basics needed to understand its application to photosynthesis (Govindjee 1995). The plant Chl fluorometry was upgraded to an imaging technique by Omasa *et al.* in 1987. The imaging is indispensable whenever the experimental object exhibits a substantial heterogeneity of fluorescence emission over its area. Application of fluorescence to "physically" sort photosynthetic mutants, to study heterogeneity of photosynthesis in leaves, to monitor localized infection by viruses and other pathogens (before visible symptoms appear), to study the local effects of irradiance, temperature, and heavy metal stress, and to investigate the physiology, biochemistry and biophysics of single algal and bacterial cells, and even single chloroplasts, requires the construction of original fluorescence imaging instruments. The specificity of the plant fluorescence imaging instruments compared to instruments used in other biomedical fields and in astronomy or material science is dictated by the dual role of photons in photosynthesis serving to excite fluorescence and, at the same time, changing the photochemical state of the observed object. The first imaging instrument of Omasa *et al.* (1987) was followed soon thereafter by Daley *et al.* (1989), who recorded data on magnetic videotapes, and by Fenton and Crofts (1990) who provided a relatively inexpensive instrument. In 1994, Genty and Meyer (1995) quantitatively mapped photosynthesis yields in a leaf. In the same year, Balachandran *et al.* (1994) were able to diagnose virus infection in tobacco leaves. This was followed by the application of fluorescence imaging on effects of various stresses on leaves using not only emission of Chl but also of other fluorescing substances (Lichtenthaler *et al.* 1996). Ning *et al.* (1995) described an instrument to monitor pathological and physiological change in plants, and Scholes and Rolfe (1996) monitored infection of crown rust on leaves. Siebke and Weis (1995) imaged photosynthetic oscillations in leaves that reflect a negative feedback control of photosynthesis that is occurring with varying frequency in neighboring leaf compartments and that can be observed only using an imaging instrument. Oxborough and Baker (1997) resolved photochemical and non-photochemical processes affecting the Chl *a* fluorescence and mapped them by leaf imaging.

In this special issue, Editors have brought before the photosynthesis community ten papers on plant imaging from eleven laboratories. One paper dealing with non-imaging applications of Chl fluorescence (Kurasová *et al.*) of plants is also included. The papers demonstrate the capabilities of the method in very diverse fields: early detection of fruit infection, mutant screening, phytoplankton research, lichen physiology, chloroplast structure research, and light-shade acclimation as well as in discrimination between the life-time and absorption factors of emission at microscopic level. Two papers describe the first imaging applications of pulse-amplitude modulated (PAM) method to capture images of F_0, F_M, and F_V emission in a way that has so far been used only by non-imaging instruments (Nedbal *et al.* and Küpper *et al.*). Since changes in fluorescence intensities can be due to either changes in the absorption cross section of photosystem (PS) 2, or to changes in quantum yield of fluorescence, it becomes necessary to image lifetimes of fluores-cence that can distinguish between the two. Indeed, an instrument for fluorescence lifetime imaging microscopy (FLIM) has now been constructed and applied to measurements on leaves and single algal cells (see Holub *et al.*). Vácha *et al.* report on discrimination of PS2 and PS1 fluorescence in isolated chloroplasts frozen to 77 K using a laser scanning microscope. Varotto *et al.* screened for *Arabidopsis* mutants using mapping of plant arrays by a robotized PAM fluorometer. The review on multicolor fluorescence imaging (Buschmann *et al.*), an application of the multicolor fluorescence imaging (Langsdorf *et al.*), and the case study relating Chl fluorescence and reflectance measurements in a canopy (Méthy) are important in relating various other techniques with Chl fluorescence imaging. Barták *et al.* and Lichtenthaler *et al.* demonstrate use of the Chl imaging fluorometry in an investigation of sun and shade acclimation of beech and of hydration in lichens, respectively. Nedbal *et al.* used imaging fluorometry for an early detection of lemon fruit infection.

We hope that this special issue will stimulate progress in introducing the powerful method of Chl fluorescence imaging (both intensity and lifetime) in agriculture, horticulture, floriculture, viticulture, forestry, and biotechnology. Wé also anticipate that, in the present post-genome era, the plant fluorescence imaging will rapidly become a powerful tool with a profound impact in mutant screening. The fluorescence screening is expected to go far beyond PS2 mutants, to source-sink coupling, to sensitivity to phycotoxins, and other biotic and abiotic stressors. Obviously, the high-throughput requirement of the screening will stimulate development of intelligent software tools (Tyystjärvi *et al.* 1999) to increase the reliability of the screening process among large number of wild type and mutant plants. The same tools are anticipated to contribute to an application of the plant fluorescence imaging in precision farming where, for example, discrimination between weed and crop plants may be a decisive factor to reduce financial and environmental cost of the pesticide treatment.

We invite you to discuss these and related topics of the plant fluorescence imaging during the next Plant Fluorescence Imaging Workshop to be held at Nové Hrady, Czech Republic, July 15-21, 2002 (see http://www.greentech.cz/international/icbte/index.html, applications at nedbal@greentech.cz

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