6.2.4 *UV-excited chlorophyll fluorescence*

UV fluorimetry allows the assessment of epidermal absorptance of intact leaves in the UV spectral range. The basis of the method relies on the efficient absorption of UV by chlorophyll (Cerovic *et al.*, 1999) and on the fact that a certain fraction of leaf chlorophylls, excited by absorption of radiation, emits red fluorescence when returning to the ground state (Dau, 1994). In intact leaves, UV excitation is reduced when epidermal UV screening is high and, under such conditions, relatively weak chlorophyll fluorescence is observed (Cerovic *et al.*, 1993; Sheahan, 1996; Mazza *et al.*, 2000).

Bilger *et al.* (1997) introduced the ratio of UV-excited fluorescence to that excited by blue–green light as an accurate measurement for epidermal UV screening with the rationale that visible radiation is not absorbed by the epidermis but fluorescence excitation in the visible and in the UV is subject to the same optical peculiarities of the individual sample. Consequently, this fluorescence excitation ratio (FER) method cancels out specific optical properties of the sample except that of epidermal UV screening. Recently, two portable devices have been developed: the Dualex leaf clip (Goulas *et al.*, 2004) and the UV-A-PAM fluorimeter (Bilger *et al.*, 2001, Krause *et al.*, 2003; Kolb *et al.*, 2003, 2005). Both permit repeated measurements of epidermal UV screening of the same sample under field conditions.

The Bilger approach has been strongly supported by the parallel relationship observed between UV transmittance data from spectrophotometric measurements of isolated epidermal strips and FER data (Markstädter *et al.*, 2001). The formation of anthocyanins, however, interferes with excitation by blue light and can, therefore, restrict the use of fluorimetry in red leaves (Barnes *et al.*, 2000). This problem can be overcome by choosing another reference excitation wavelength in the red (650 nm in Dualex), which is outside the anthocyanin absorption range (Goulas *et al.*, 2004). In addition, fluorescence excited at wavelengths within the anthocyanin absorption range when divided by fluorescence excited at wavelengths of high epidermal transparency can also be used to determine the epidermal screening due to anthocyanins *in vivo* (Agati *et al.*, 2005).

6.2.5 *Fluorescence microscopy*

Fluorescence microscopy can complement data obtained with the earlier techniques by obtaining two- and even three-dimensional information on the arrangement of structures and compounds of plant surfaces. This technique can localise absorbing substances by fluorescence: such data were useful in providing an understanding of the origin of fluorescence signals of entire leaves and, also, for the interpretation of remote fluoro-sensing data.

Epi-fluorescence microscopy is widely used to analyse leaf tissues and it has been much improved in the last decade due to progress in digital imaging, confocal laser scanning and multiphoton microscopy (Blancaflor and Gilroy, 2000). Increased performance of personal computers permitted the application of image restoration