

Chlorophyll fluorescence: the signal hidden behind leaf reflectance. A new technique for the study of plant status by remote sensing

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Abstract. Incident solar light in terrestrial vegetation can be either reflected, absorbed or transmitted by a leaf. The absorbed light photons can be used for photosynthesis or can be dissipated into heat or emitted as fluorescence photons. In the latter case, photons are brought in an excited state of chlorophyll and emitted with a longer wavelength than light before excitation. The chlorophyll fluorescence is emitted, at leaf and canopy level, in the region 650-850 nm, with a peak located at 685-690 nm and another one at 735- 745 nm. The emitted fluoresced radiance is always super-imposed on the background reflected signal, but with a much lower intensity than the reflected radiance from the leaf, therefore it has been necessary to develop techniques that allow separating both signals from remotely sensed radiance. This fluorescence signal is instantly related to the functional status of the photosynthetic apparatus. Monitoring of this excess energy dissipation pathway provides a tool for detecting early stress symptoms of vegetation - that is, before damage is irreversible. This responsive feature of fluorescence also supports practical applications such as agronomic crop improvement and forest vegetation assessment in the face of climatic instability.

The growing interest on the potential of measuring chlorophyll fluorescence from remote sensing on a global scale recently led to the initiation of the Fluorescence Explorer (FLEX) mission. FLEX, currently under development phase A/B1 of the 8th European Space Agency (ESA) Earth Explorer Program, aims to be the first space mission primary dedicated to the estimation of chlorophyll fluorescence at a global scale. Our group is involved in various FLEX-related research activities and a brief overview of their latest status will be presented.

Keywords: FLEX, Fluorescence, Photosynthesis, Chlorophyll.

1. Fluorescence in photosynthesis

Photosynthesis is the plant process that synthesizes organic compounds from inorganic substances in the presence of sunlight. The most important step, chemically, in photosynthesis is the conversion of carbon dioxide and water into carbohydrates and oxygen. The first photosynthesis part is an energy conservation phase that includes the formation of an electron transfer redox chain and the consequent generation of a protons concentration gradient. Thylakoids (sheet structures inside chloroplasts) contain protein complexes that perform the management of electrons and protons necessary for energy conversion. The protein complexes in thylakoid included are four; two of them are photosystems, namely, systems capable of absorbing and transforming energy photon in redox electrochemical energy. They are called photosystem I (PSI) and photosystem II (PSII). Each one has a different spectral absorption shape of chlorophyll-*a* in the reaction centre. The chlorophyll of photosystem I, called P700, absorbs light at infrared wavelengths (735-745 nm) and the PSII chlorophyll called P680, absorbs at red wavelengths (650-850 nm) (Figure 1a). The third complex is a connector between the two systems, called b₆f cytochrome complex. Finally there is a fourth protein complex not involved in the transfer of electrons, but using the proton gradient generated the other complex through the thylakoid to synthesize. When the chlorophyll-*a* molecule present in PSI and PSII is irradiated with electromagnetic radiation, is able to absorb a certain quantity of the same energy corresponding to a photon with the same energy quantity that is necessary to excite an electron from the molecule of chlorophyll-*a* and cause the jump of the electron to a higher energy orbital, leaving the

chlorophyll-*a* molecule in an excited state (Figure 1b). The chlorophyll-*a* molecule is not stable in that excited state and must return to its fundamental energy state, and therefore, must transfer the excitation energy. There are three ways in which the electron recovers its fundamental state: 1. Photochemical processes, photosynthetic activity, included CO₂ assimilation, consuming the ATP and NADPH generated in the initial phase of photosynthesis. 2. No photochemical processes, mainly the thermal dissipation (no radiant) of the excitation energy. 3. Light re-emission (fluorescence (F)). As seen, the F signal is related to photosynthesis, allowing a direct estimate of the actual photosynthetic activity of the vegetation. In fact, F emission occurs in competence with photosynthesis and heat dissipation (Figure 1c). Under stress conditions, plants show a decrease of photosynthetic activity due to a change in the distribution of the energy absorbed in the three scope processes. However, F measurement is difficult because it represents a small amount of the total radiance measured by a sensor to be masked by the light reflected by the plant itself (Figure 2) (Gomez-Chova et al., 2006).

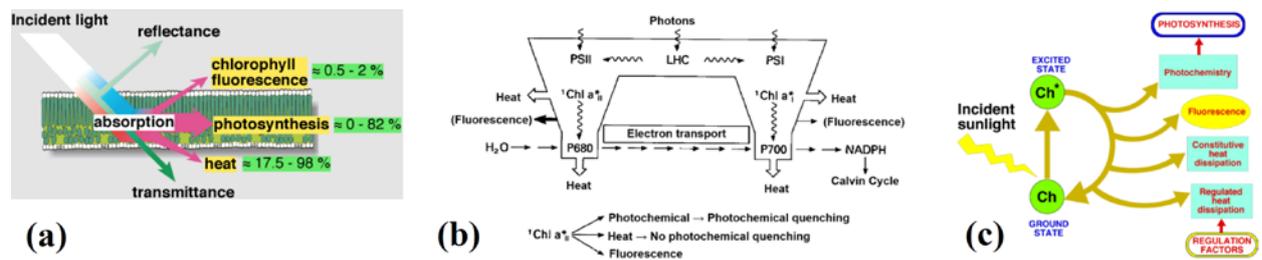


Figure 1. (a) The fate of light within leaves (ESA, 2008). (b) The different processes of light energy dissipation in photosynthesis (Cendrero, 2010). (c) Scheme of light-energy capture by chlorophyll molecules and the different dissipation mechanisms used to return to the ground-state energy-level (ESA, 2008).

The PSI and PSII are responsible for the F emission and as each of them has one shape of chlorophyll-*a* in the reaction centre spectrally different, therefore, the light re-emission is spectrally different. P700 absorbs light at infrared wavelengths and therefore is responsible for the F emission peak in the infrared (F740) and P680 absorbs at red wavelengths and therefore is responsible for the fluorescence emission in the red peak (F690) (Figure 2a).

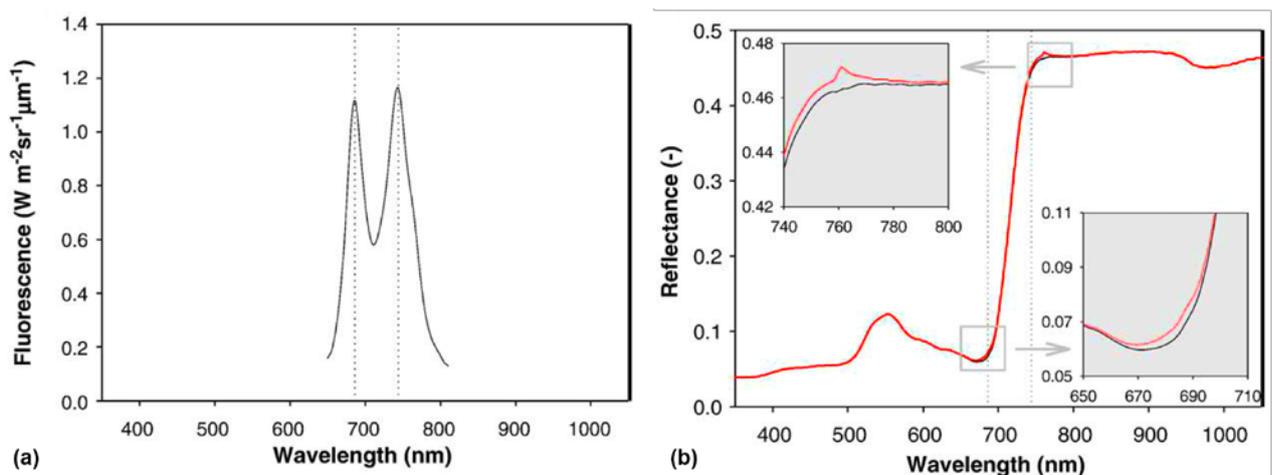


Figure 2. Solar-induced Chl fluorescence and reflectance spectra of a sugar beet leaf. (a): F spectrum in the region between 650 and 800 nm. (b): The actual reflectance (black curve) was computed as the ratio of the reflected and incident fluxes; the apparent reflectance (red curve) was computed as the ratio of the total upwelling flux (reflection plus F) and the incident flux. The difference between the two quantities is highlighted in the grey boxes, showing the responses surrounding the F emission maxima (vertical dotted lines) (Meroni et al., 2009).

Although fluorescence, photosynthesis and non-photochemical protection are closely connected, the translation of fluorescence data to photosynthesis is not trivial. In fact, while under low light unstressed conditions (no non-photochemical protection mechanisms activated) a negative correlation exists between fluorescence and photochemistry, most studies have observed that in the presence of plant stress and high light conditions, fluorescence declines with photosynthesis as a result of protective mechanisms which take place in the leaf to prevent damage caused by harmful radicals formed in such stress conditions (Van der Tol et al., 2009).

2. Fluorescence and Remote Sensing

The study of solar induced fluorescence (SIF) (also known as ‘passive’ fluorescence) is one important research objective for space-based platforms. In some ways it is a more mysterious phenomenon than actively-induced fluorescence because a given signal level cannot be unambiguously interpreted without consideration of aspects such as atmospheric and environmental information, or the pattern of SIF behavior over time (necessitating repeat measurements). SIF typically has been studied with respect to the changes in the ratio of the Red:NIR peaks that occur with physiological fluctuations. The ratio has been shown to be responsive to chlorophyll content, decreasing with increasing chlorophyll as a result of greater re-absorption of the red F in the leaf. SIF also responds to photosynthetic fluctuations independently of chlorophyll content (Lichtenthaler & Rinderle, 1988). Comparisons between active and passive F measurements, photosynthesis yield, and photosynthesis rate have also been made (Carter et al., 1990; Moya et al., 2004; Meroni and Colombo, 2006). SIF has been successfully correlated to photosynthesis at the leaf scale, and in a preliminary way at the canopy scale. Simple ratios of the two peaks or other features such as the numerical value of the integral between peaks normalized over a parameter such as absorbed or incident PAR are examples of how SIF information may be considered for use in characterizing photosynthetic performance and resilience.

The capacity to observe SIF presents a complete novel option for space-based remote sensing, by extracting information on the real-time workings of the photosynthetic machinery of plant canopies. Because the production of CF is related to the function of the two photosystems active during photosynthetic initiation in green plants, it provides an actual glimpse into the dynamics of the photosynthetic process. As a photosynthesis indicator, F may be envisaged for use in:

- tracking vegetation responses to stress, including resilience and recovery from natural or human-induced perturbations
- quantifying photosynthetic efficiency, which can serve as a proxy for light use efficiency in gross primary productivity (GPP) and global carbon models
- investigating implications for photosynthetic rate, and possibly even more directly helping to quantify GPP

Plant F under solar illumination adds a weak signal to reflected solar radiation. If both fluorescence emission and surface reflectance are assumed to follow Lambert's law, the radiance upwelling from vegetation (L) at ground level is therefore composed of two coupled contributions, one reflected ($r E/\pi$) and the other emitted (F) (Meroni et al., 2009):

$$L(\lambda) = \frac{r(\lambda)E(\lambda)}{\pi} + F(\lambda) \quad \left[\text{W m}^{-2} \text{ sr}^{-1} \mu\text{m}^{-1} \right] \quad (1)$$

where λ is wavelength, r is reflectance (free of the emission component), and E is total irradiance incident on the target. Even though F contributes to the signal detected by a remote sensor, its magnitude is small (1–5% of the reflected radiation in the near infrared) thus making the decoupling of the two contributions difficult. Zarco-Tejada et al. (2000) first recognized the effect of F on reflectance and demonstrated that it is possible to detect the F signal using reflectance measurements. In fact, the reflectance factor usually computed by the RS community as the ratio between upwelling and incident fluxes is indeed polluted by the F contribution (Meroni et al., 2009):

$$r^*(\lambda) = \frac{\pi L(\lambda)}{E(\lambda)} = r(\lambda) + \frac{\pi F(\lambda)}{E(\lambda)} \quad [-]. \quad (2)$$

This quantity, referred here as apparent reflectance, r^* , differs from the pure reflectance (r) as indicated by the right hand side of Eq. (2) and as shown in Figure 2b.

The F signal is comparably stronger and can be detected passively in narrow dark lines of the solar and atmospheric spectrum in which irradiance is strongly reduced (the so-called Fraunhofer lines). In the visible and near-infrared, the solar spectrum at ground level exhibits three main “Fraunhofer” features which have been exploited for F estimation: $H\alpha$ due to hydrogen (H) absorption in the solar atmosphere (centered at 656.4 nm) and two telluric oxygen (O_2) absorption bands in the Earth atmosphere, O_2 -B (687.0 nm) and O_2 -A (760.4 nm). The incident at-surface irradiance at different spectral resolutions (0.005, 0.1 and 1.0 nm Full Width at Half Maximum, FWHM) and at the spectral regions around these three absorption features is shown in Figure 3. Irradiance data were simulated by means of the Matrix Operator Model (MOMO) (Fell & Fischer, 2001). Among other inputs, the sun zenith angle was set to 30° , with a mid-latitude summer atmosphere, the surface at sea level, and the aerosol optical thickness at 550 nm equal to 0.2. Resampling to the different spectral resolutions was performed assuming Gaussian-like spectral response functions with the given FWHM (Meroni et al., 2009).

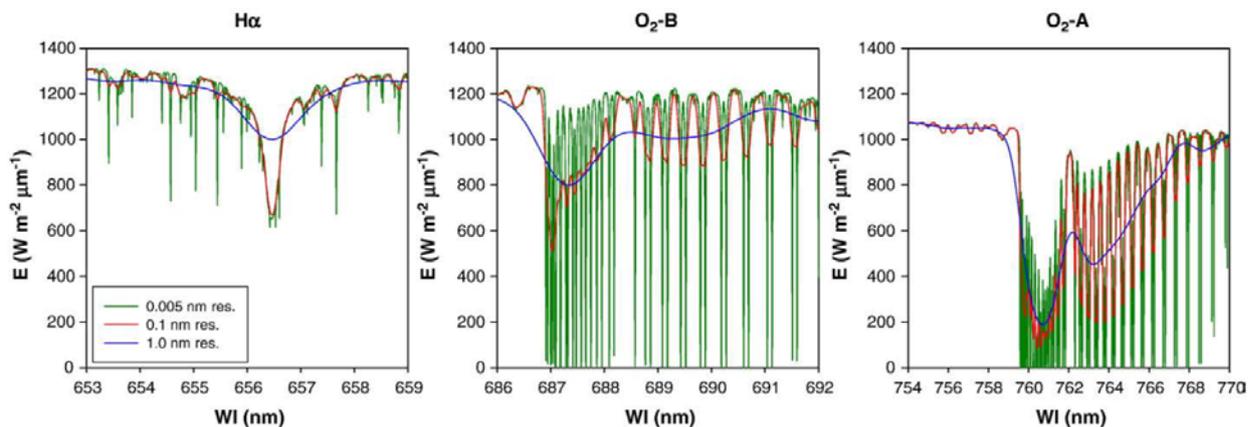


Figure 3. Incident irradiance (E) at ground level at the three absorption lines normally used for F retrieval and at three different spectral resolutions (Meroni et al., 2009).

Recently, alternative retrieval algorithms based on the spectral fitting of hyperspectral radiance observations, Spectral Fitting Methods (SFMs), have been proposed. FLD and SFMs were used to estimate F starting from Top Of Canopy (TOC) fluxes at very high spectral resolution (0.12 nm) and sampling interval (0.1 nm), exploiting the O_2 -B (687.0 nm) and O_2 -A (760.6 nm) atmospheric oxygen absorption bands overlapping the fluorescence emissions at the red and far-red spectral window, resulting that violation of FLD assumptions results in a positive bias in F estimation at both oxygen absorption bands that cannot be avoided even at the high spectral resolution considered (Meroni et al., 2010).

3. Past projects

Projects carried out over the past decade have served as valuable proof-of-concept studies for remote measurement of SIF:

- SIFLEX (Solar Induced Fluorescence Experiment, executed over Finland in 2003) demonstrated that SIF was discernible even in boreal conditions using tower-based or airborne instrumentation.
- SEN2FLEX (Sentinel-2 and FLEX Experiment in 2005) used a new airborne instrument AirFLEX and the CASI (Compact Airborne Spectrographic Imager) to reveal that the fluorescence signal contains information not apparent in classical remote-sensing observation.
- CEFLES 2 (CarboEurope, FLEX and Sentinel-2, carried out in Southern France in 2007) showed that canopy fluorescence is indeed closely correlated with ecosystem carbon uptake and that fluorescence data improve diurnal model predictions of GPP. On the regional scale, the fluorescence signal could be correlated to regional airborne measurements of carbon fluxes.
- FluorMOD project (Miller et al. 2005), was a seminal work funded by ESA, which undertook to model leaf and canopy fluorescence. An integrated model was developed, comprising the sub-models FluorMODleaf and FluorSAIL. The focus was to create a model for unstressed vegetation, though it did accommodate diurnal downregulation. The decision to emphasize fluorescence under benign conditions permitted understanding what ‘normal’ fluorescence behavior looks like, so that one application of the FluorMOD results could be to help identify the existence of stress based on the deviation of measured results acquired from space against an unstressed ‘baseline’ or ‘control’. FluorMOD predicted that SIF might, indeed, be measurable from a space-based platform. Developments linked to the FluorMOD work include an improved leaf emission spectrum taking into account emission of the two photosystems involved in the initial states of photosynthesis (Pedrós et al., 2008), and a further elucidation of FluorMODleaf that incorporated re-absorption of emitted fluorescence (Pedrós et al., 2010). This model simulates apparent spectral fluorescence yield of a fresh leaf between 640 and 840 nm for any excitation light of the visible spectrum.
- In other recent work (separate from FluorMOD), a computational procedure was developed to resolve the respective contributions of PSI and PSII to the leaf chlorophyll fluorescence emission spectra at room temperature (Palombi et al., 2011). The approach used Principal Component Analysis of fluorescence emission in the O-I kinetic induction phase. A single-band spectrum for fluorescence emission peaked at about 735 nm for PSI, and a 2-band spectrum with maxima at 685 and 740 nm for PSII was identified. A linear combination of only these two spectral shapes yielded a good fit for any measured emission spectrum of the leaf under investigation (*Ficus benjamina*) and may be useful for discerning the fluorescence emission contributions of the photosystems under different conditions. Thus, the dynamics of energy distribution between the two photosystems may be monitored at physiological (~room) temperatures rather than having to use -196°C to study PSI emission as has been done in the past. Separation of the PSI and PSII emission components can improve our understanding of the changes in fluorescence signal induced by environmental factors or stress conditions, and may be helpful for interpreting remotely captured fluorescence.

4. FLEX mission

FLEX will be the first space mission designed to optimize remote observation of the chlorophyll fluorescence in terrestrial vegetation. The ESA mission will undertake to provide global maps of vegetation-fluorescence emissions. These might then be converted into photosynthetic efficiency for use in global monitoring of health and stress resilience. The Photosynthesis Study will provide useful recommendations for generation of spatial-temporal maps of vegetation health and stress status in near real-time.

FLEX will take advantage of additional measurements provided by the Ocean and Land Colour Instrument (OLCI) and the Sea and Land Surface Temperature radiometer (SLST) onboard Sentinel-3. The combined information is critical to resolve the disagreements between different

land models that attempt to simulate future atmospheric CO₂ concentrations through interactive terrestrial carbon balances.

The objective is a global coverage mission, monitoring vegetation photosynthesis along the seasonal cycles and the activation/deactivation of the photosynthetic mechanisms where photochemistry is adjusted to a lesser or greater level of activity. Global coverage should be achieved for all land surfaces between -56° and +75° latitude including major islands.

FLEX will be a satellite flying in tandem with Sentinel-3. Observations of FLEX and S-3 will be acquired within 6 to 15 s from each other. Payload is the FLuOREscence Imaging Spectrometer (FLORIS) measuring fluorescence in O2-A and O2-B bands plus reflectance over red-edge and Photochemical Reflectance Index (PRI) spectral ranges. FLORIS operates in pushbroom mode with a swath of 100 to 150 km (contained within one of the S-3 OLCI camera swath) and a pixel size of 0.3 x 0.3 km². Data in four spectral bands: O2-A (750-777 nm); O2-B (660-714 nm); red edge (714-750 nm); PRI (520-660 nm) with sampling of 0,1 nm in oxygen bands and 0,5 to 2 nm in red edge and PRI bands calibration relies on both cross-calibration with the equivalent bands of OLCI and dedicated devices. FLEX and S-3 images co-registered by on-ground processing through correlation algorithms. Vega launcher as baseline and mission duration of nominally 3,5 years (to cover at least 3 full vegetation growth cycles) with goal of 5 years.

The optimal spatial resolution for observing land targets and monitor surface processes is difficult to define, since almost all spatial scales are important. Land-atmosphere interactions can hardly be modeled in high detail for large areas and in most cases the surface—atmosphere coupling is represented at reduced spatial resolution. In the particular case of carbon exchanges between surface and atmosphere, the combination of requirements provides an optimum spatial resolution in the range of 100-300 m, which is detailed enough to identify vegetated patches while resolving interactions at scales comparable to typical heights of the atmospheric boundary layer. Here a resolution of 100—300 m would be reasonable as well and in line with other data sources and modeling efforts.

5. BIOHYPE project

Biomonitoring of natural vegetation allows the acquisition of well-defined samples at an affordable cost and allows determining air pollution at different time-scales. It reflects longer-term changes of environmental quality, because plant leaves accumulate pollution over months, or even years for evergreen species. In the BIOHYPE project, biomonitoring is organized at three tree structural levels: (i) the sub-leaf level, (ii) the leaf level and (iii) the canopy level. An airborne hyperspectral based biomonitoring is applied to cover the goals at canopy scale. The overall research goal is to develop, test and validate a passive biomonitoring methodology based on hyperspectral observations and based on a field experimental approach which lapses over several spatial scales as well as over several tree structural and temporal levels, depending on the parameter considered. One of main goals of the project is the investigation at sub-leaf, leaf and canopy level of the spatial distribution of chlorophyll fluorescence. These objectives will be fulfilled by two broad field and flight campaigns in two cities of two different climatic zones: Ghent (Belgium) and Valencia (Spain). The scientific outcome of the project will foster thorough knowledge of the correlation between tree characteristics at the sub-leaf, leaf and canopy levels and air pollution. Moreover, a more profound knowledge can be acquired concerning the correlation between the different tree level structural characteristics, and quite specifically the relevance and biomonitoring potential of airborne hyperspectral, as well as chlorophyll fluorescence data. Up and downward fluorescence yield are, to some extent, linearly related to each other, but depending on the leaf anatomy and chlorophyll content, the ratio or fitting can differ. It is known that chlorophyll is one of the drivers of the first fluorescence peak, hereby reflecting the activity of photosystem II (Figure 4) (SR/00/131, 2012). Results also show that SIF acts as a more sensitive indicator of (traffic) pollution stress compared to more static biochemical variables such as chlorophyll content (Van Wittenberghe et al., submitted).

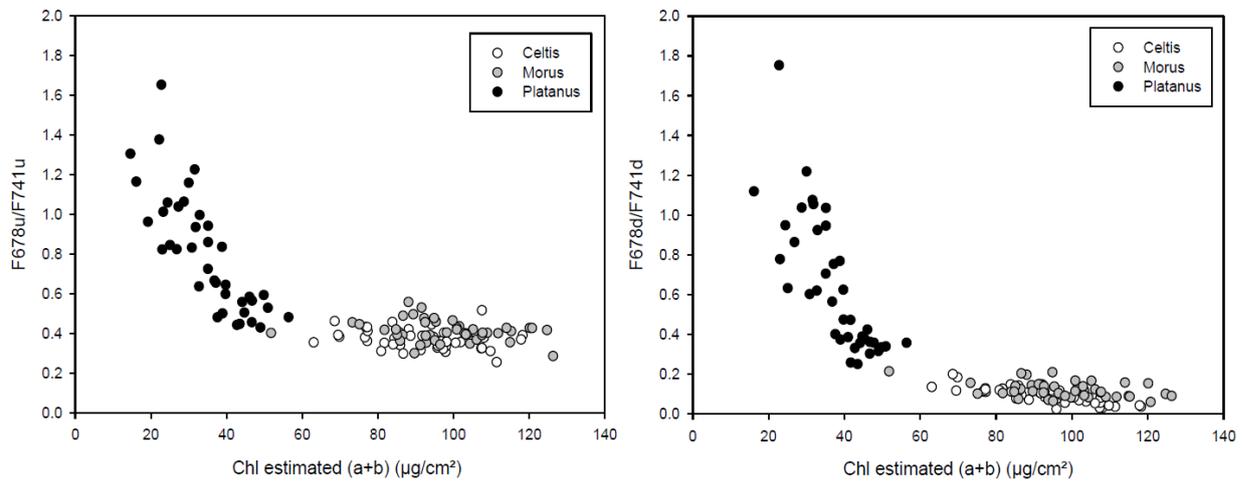


Figure 4. The fluorescence ratio F678/F741 upward and downward versus the estimated chlorophyll content.

6. Example of estimation of solar-induced vegetation fluorescence from space measurements

In this example, a methodology for the estimation of solar-induced chlorophyll fluorescence from spaceborne and airborne sensors is presented. The fluorescence signal is included in an atmospheric radiative transfer scheme so that chlorophyll fluorescence and surface reflectance are retrieved consistently from the measured at-sensor radiance. This methodology is tested on images acquired by the Medium Resolution Imaging Spectrometer (MERIS) on board the ENVIRONMENTAL SATellite (ENVISAT) taking advantage of its good characterization of the O₂-A absorption band. Validation of MERIS-derived fluorescence is carried out by applying the method to data acquired by the Compact Airborne Spectrographic Imager (CASI-1500) sensor concurrently to MERIS acquisitions (Figure 5). CASI-derived fluorescence is in turn compared with ground-based fluorescence measurements, a correlation coefficient R^2 of 0.85 being obtained (Guanter et al., 2007).

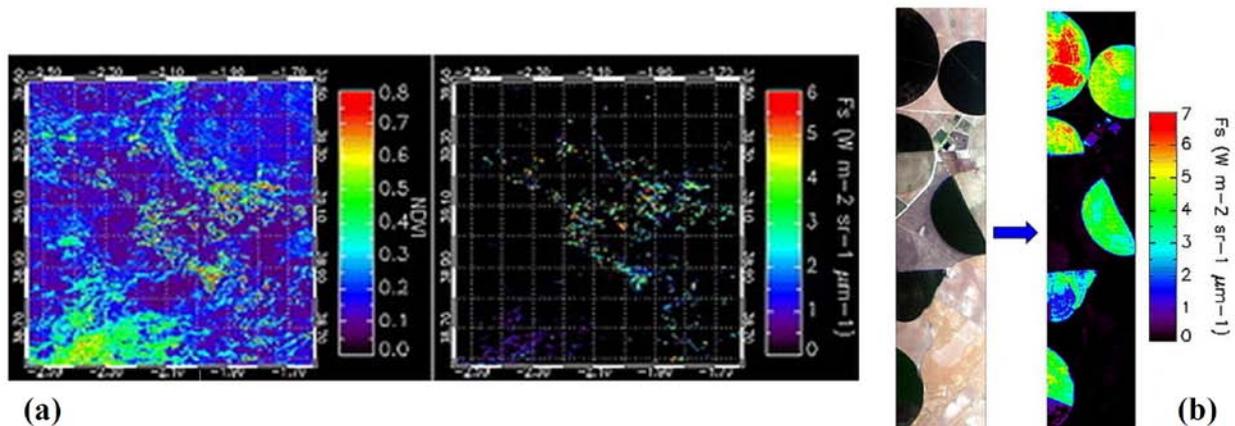


Figure 5. Fluorescence maps obtained from a MERIS (a) and CASI (b) images (Guanter et al., 2007)

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