Kelvin Force Microscopy Used to Study Electronic Properties of Materials

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A combination of new scanning probe techniques has been developed over the last few years to meet the need for understanding the relationship between microstructure and extrinsic properties. These combinations are used in a two-pass mode of operation to obtain the structural detail with the corresponding map of the property of interest. An example of one such successful technique is used in the magnetic force microscope, which takes advantage of the fact that magnetic interactions are long-range. A line-by-line measurement is made in tapping mode first on the surface, then at a fixed height above the surface, outside the range of Van der Waals interactions, to obtain magnetic information from the stray field emanating from the surface. The same is true for Kelvin force microscopy, which also employs a combination of alternating contact and noncontact scans to simultaneously obtain topography and contact potential variation maps. Kelvin force has been used to reveal a variety of new electronic information such as variations of work function on surfaces and discontinuities at grain boundaries.

Multi-photon spectroscopy of plant tissues

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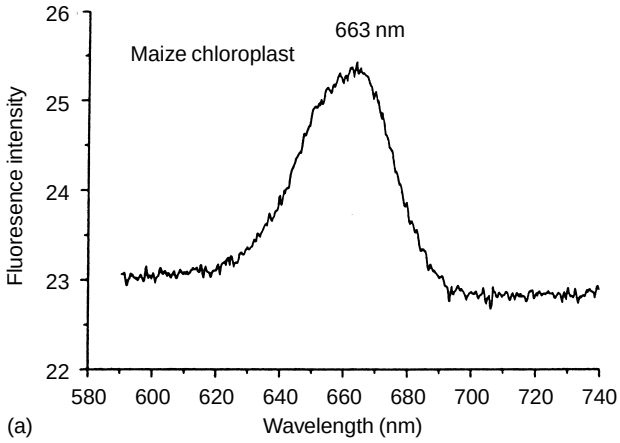
Considering its non-linear nature, two-photon excitation may generate very different spectral response in samples when compared with single-photon excitation. It is thus necessary to measure the two-photon spectra of samples, so that the fluorescence images can be properly interpreted[1]. However, fluorescence spectra obtained from bulk samples may not provide adequate information for microscopy. For instance, due to the relatively small contribution to the total fluorescence intensity, a small number of fluorescent particles in a generally fluorescing specimen may escape detection when the spectrum of the specimen as a whole is obtained. In addition, signals resulted from second harmonic generation (SHG) may be mixed with low-level broadband background auto-fluorescence commonly



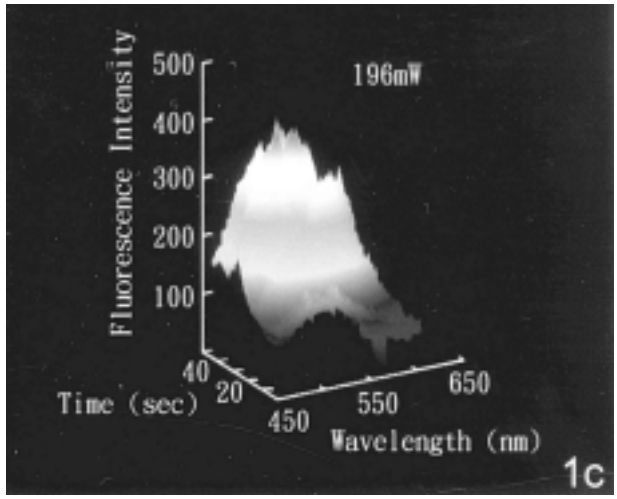


FIG.1 (a and b) Two-photon excited fluorescence of a single chloroplast in maize. (c) Three-dimensional plot of fluorescence emission spectra vs. time showing the changes in spectrum and intensity of the green fluorescence from a chloroplast under intense NIR(Ex=780nm) illumination.

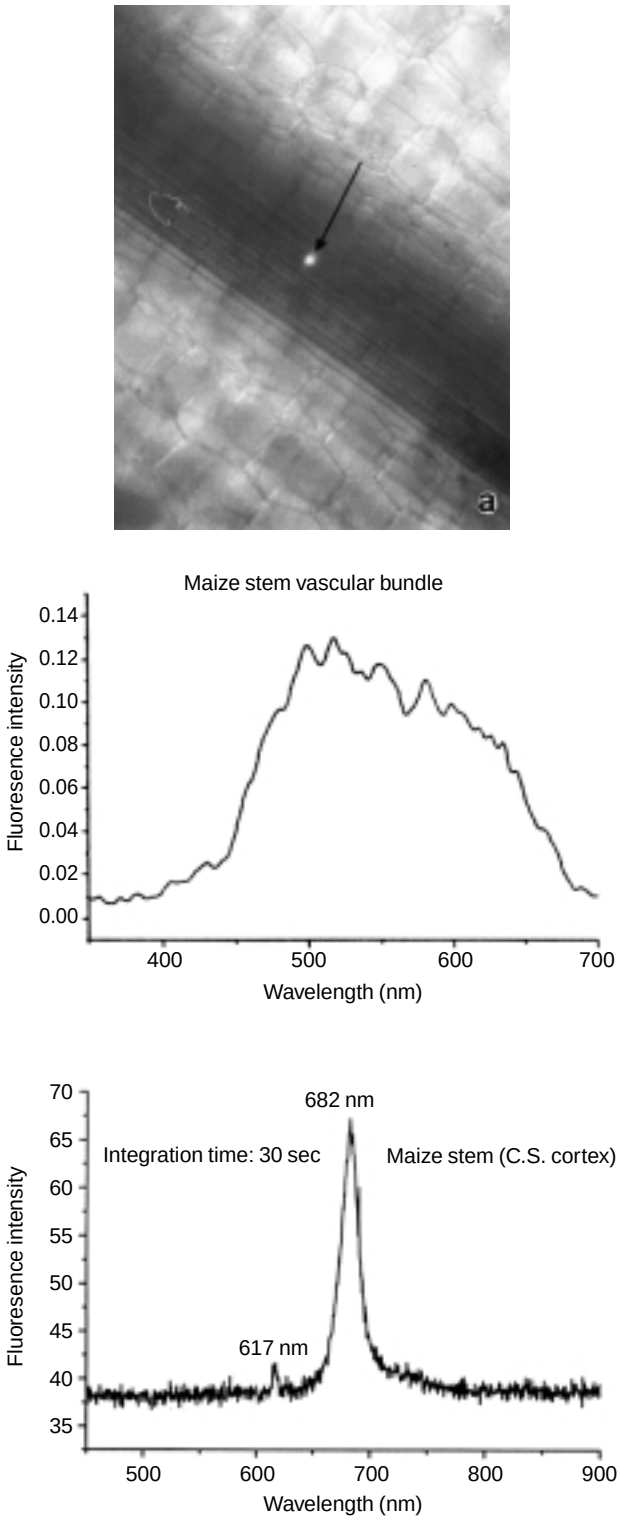


FIG.2 (a) Transmission micrograph of a longitudinal section of maize stem. Arrow in the center of the micrograph indicates two-photon excited fluorescence emission generated by a tightly focused IR beam. (b) The fluorescence spectrum obtained from the spot shown in (a). (c) Spectrum obtained from the cortex in maize stem (cross-section) excited by 1234nm IR. Note the red fluorescence at 682nm and SHG at 617nm.

found in biological specimen. Therefore, measuring fluorescence spectrum from a micro-focused volume is essential to properly interpret multi-photon fluorescence images. In this study, leaf protoplasts and stem slices of maize were used as samples to address this issue in a micro-spectroscopic set-up.

Leaf protoplasts of *Zea mays* L. (Ohio43) were isolated and suspended in culture medium according to the methods of Huang and Chen[2]. Unstained stem slices of maize were also used. Two-photon induced fluorescence spectra were measured with a SpectraPro-500 spectrometer equipped with a TE-cooled PMT. The excitation laser beam (780nm, NIR) is derived from a mode-locked Ti-sapphire laser operating at a repetition rate of 82MHz with a pulse width approximately 100fs. A dichroic beam-splitter (ChromaTech-650DCSP) was used to achieve epi-illumination and on-axis fluorescence detection in a modified Olympus BX microscope. Two IR cut-off filters (Edmond Scientific, K53-710) were installed in front of the entrance slit of the monochromator to reject scattered IR from the sample. Excitation intensity of 10^{12}W/cm^2 was reached at the focal point. A second set-up using a Chromium-doped Forsterite laser, operated at 120MHz and 130fs pulse, was used for 1234nm infrared excitation.

Figure 1 shows two-photon excited fluorescence spectra obtained from a chloroplast. A prominent red fluorescence peak with emission maximum at 663nm was observed (Fig. 1a). A broadband green fluorescence emission, peaked at 532nm/550nm, was measured (Fig. 1b). The 663nm emission is the result of chlorophyll fluorescence while the origin of the 532/550nm emission is yet to be determined. Fig. 1c is a three-dimensional plot of spectra against time showing the spectral and intensity changes of the green auto-fluorescence of a chloroplast under intense NIR illumination. The technique allows detailed study of cellular damages in multi-photon microscopy. Fig. 2a is a transmission micrograph of a maize stem. Two-photon excited yellow-green fluorescence generated by a focused IR beam is clearly visible in the center of the micrograph (arrow). The fluorescence spectrum obtained from the spot is illustrated in Fig. 2b. When excited with 1234nm IR, a spectrum with red fluorescence peaked at 682nm was obtained from the cortex of maize stem. A small peak at 617nm was also observed which is likely a result of SHG in the plant cell wall (Fig. 2c).

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Effects of Milk Proteins on Frozen Dough Microstructure Visualised with CSLM

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The gluten network is the main structural component in yeasted dough and is responsible for gas retention. A major problem encountered with frozen dough is dough weakening which involves disruption of the gluten network by ice recrystallisation and reducing compounds from dead yeast cells. This results in excessively long proof times, low bread volume, and poor texture. Additives, especially dough strengtheners, which interact with the gluten network, are used in frozen dough to counteract weakening. Dairy proteins are added to bread for their nutritional and functional properties. Incorporation of dairy ingredients into frozen dough which improve baking quality would be beneficial compared to use of synthetic additives because of their high nutritional value and natural origin. Sodium caseinate (SC), untreated whey protein concentrate (WPC), and heat-treated whey protein concentrate (WPCHT) were incorporated into frozen dough. Baking properties were measured over a 15-week frozen storage period. Confocal scanning laser microscopy was used to view frozen dough microstructure after 10 weeks of frozen storage. Nile blue was used to visualise protein and starch. SC had a positive influence on frozen dough baking properties. It decreased proof time, increased bread volume and reduced crumb firmness. CSLM images showed that SC appears to interact with the gluten network to produce an enhanced, more continuous gluten network compared to the control. WPC increased proof time, decreased volume and increased crumb firmness. CSLM images showed that the gluten network of frozen dough with WPC appeared to be fragmented and less continuous than the control, suggesting that WPC interferes with the gluten network. Heat treatment of WPC improved its baking properties, and frozen dough with WPCHT had shorter proof time and produced bread with higher volume and lower crumb firmness compared to frozen dough with WPC. CSLM images showed that heat treatment eliminated the negative effects of WPC on the gluten network.

The Effect of pH on Heat Denaturation and Gel Forming Properties of Glycinin

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Soy glycinin is the major protein in soybean, representing ca. 30% of total protein. Glycinin consists of an acidic and a basic polypeptide, linked by a single disulphide bridge, with molecular weights of ca. 38 kDa and 20 kDa, respectively. In this research we studied heat denaturation, gelation behaviour and the resulting gel structure of soy glycinin at pH 7.6 and pH 3.8 at an ionic strength of 0.5.

Glycinin was isolated from Williams'82 soybeans according to Thanh and Shibasaki (1976) by isoelectric precipitation at pH 6.4. Heat denaturation was determined by differential scanning calorimetry (DSC). Gel formation of 10% glycinin was followed by dynamic measurements in a Bohlin reometer (strain 0.01, freq. 0.1 Hz). After heating from 20-95°C at 1°C/min the samples were held at 95°C for 30 min (glycinin), and subsequently cooled down to 20 °C at 1°C/min.

"Non-network protein was defined as the protein in the supernatant obtained by centrifugation of the gel. In order to obtain the "network" protein slices of 40 (m were cut from a gel. Next, the soluble fraction was allowed to diffuse out of the slices during 24 hours by adding excess buffer. From the protein concentration of the non-network protein fraction the total proportion of non-network protein was calculated. The composition of the protein (ratio acidic versus basic polypeptides) was obtained by SDS PAGE followed by densitometric analysis. CSLM pictures were obtained with a LEICA TCS SP microscope with a 63(objective. A data restoration procedure was performed afterwards to remove blur and noise in order to recover relevant detailed information that is present but concealed in the unprocessed data.

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