3-D APPLE SKIN AUTOFLUORESCENCE STUDIED WITH TWO-PHOTON EXCITATION MICROSCOPY

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Non-destructive fluorescence imaging methods are being developed for rapid physiological screening of the complete skin surface to detect storage diseases like bitterpit and storage scald. Fluorescence from Chlorophyll, NADH and flavin-nucleotides is used to elucidate the physiological state of the fruit. Earlier work showed steady-state spectra of selected areas of apple skin (Sowinska et al., SPIE Proc., 3382, 100 1998). Now the autofluorescence of selected volumes of intact apple skin has been studied with two-photon excitation (TPE). Scans were located on the equator and centered on the red and green colored side of all the fruit. Apple varieties included Malus Domesticus Borkh. x, Granny Smith and Jonagold. The home-built microscope setup (Sytsma et al., J. Microsc. 191, 39 1998) was equipped with a 1.2 NA, 60x, water objective; a 100 fsec, 80 MHz, Ti:Sa laser running at 772 nm and a fiber coupled CCD camera for 2-D spectral scanning. Scan depths extended down to several tens of µm over an area of 64 µm2. Various structures show characteristic lifetimes between <1 and >5 nsec. Both fluorescence lifetime resolved images (FLIM) and steady-state spectra are presented. Their physiological significance is discussed. This work is supported by the project “Fonds Slimme Regio” of the Province of Limburg.