# Spatially-resolved spectroscopy: a model-based approach for separating the information on microstructure and composition of Braeburn apples

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## Introduction

The change of Vis/NIR radiation when propagating through biological material is the result of a complex process of molecule-specific absorptions and multiple light scattering caused by the interaction of photons with the sample microstructure.[1] This microstructure is equally important for food product quality as chemical composition.[2] In this study, diffuse reflectance measurements in the range 400–1100 nm at different distances from the incident light beam (spatially-resolved) were combined with light propagation models to estimate both the compositional and microstructural properties of Braeburn apples represented by absorption and reduced scattering coefficients, respectively.

## Materials and Methods

### Spatially-resolved spectroscopy (SRS) setup

A set-up for SRS measurements in the 400–1100 nm range has been built (Figure 1); it consists of a contact probe with accurately placed fibres which is linked to a spectrograph for simultaneous measurement of reflectance at different distances by a CCD camera. The fibres used are Thorlabs multimode silica fibres with a numerical aperture of 0.22 and a core diameter of 200 μm. The detection fibres (n = 7) are placed at various distances from the illumination fibre, ranging from approximately 0.3 to 1.2 mm with a step of about 0.15 mm. The illumination fibre of the probe is connected to an AvaLight-DHc halogen lamp (Avantes, Eerbeek, The Netherlands) through an optical switch. The detection fibres from the SRS probe and a fibre from the optical switch of the light source are aligned in the entrance slit of a CP200 133 $g.mm^{-1}$ spectrograph (Horiba Jobin-Yvon, New Jersey, USA) which splits the light from each of these fibres into its spectral components and projects these onto a C7041 CCD camera with a S7041-1008 detector (Hamamatsu, Louvain-La-Neuve, Belgium). The signal from this camera is transferred to a computer by means of a PCI MIO-16E-4 data acquisition card (National instruments, TX, USA). Control of the light source, optical switch and camera is performed in LabView software (National Instruments, TX, USA).

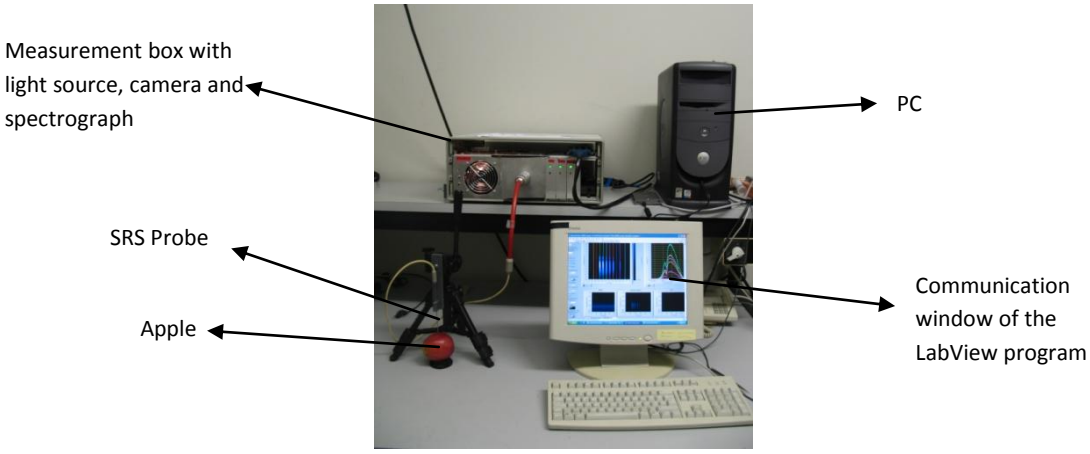


**Figure 1.** The setup for spatially-resolved spectroscopy.

### Samples

Thirty Braeburn apples were harvested at the same orchard following the normal cultivation method in Flanders, Belgium and were transported to the lab. In the lab, these apples were measured with the SRS setup (day 1) and then stored under shelf-life conditions (18°C in a controlled temperature storage cell) for 2 weeks. SRS measurements were carried out at different positions on the equator at both sides of each apple

Reference paper as:
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(most reddish and most greenish side). In this way, both the variations in optical properties within an apple and between different apples were assessed. After a shelf-life of 2 weeks, SRS measurements were repeated at the same sides for each apple. The experiment aimed at investigating the changes in microstructure and composition of Braeburn apples, as represented by absorption and reduced scattering coefficients of the apple tissues following 2 weeks of storage.

### Estimation of optical properties

For each sample and each wavelength, the optical properties (absorption and reduced scattering coefficient) were estimated by fitting a solution of the radiative transport equation for light propagation in turbid media based on the diffusion approximation[3] to the spatially-resolved reflectance profile acquired by the SRS setup by means of a trust-region non-linear least squares fitting algorithm in Matlab (version 7.5, The MathWorks Inc., Natick, USA). Before applying this procedure to the apple data, the accuracy of the fitting procedure was validated by implementing the same procedure on 16 solid phantoms with known optical properties.[4] These solid phantoms contained both $TiO_2$ powder (serving as scatterer) and black toner ink (acting as absorber ) in an epoxy resin medium.

## Results and Discussion

### Validation of the procedure for optical properties estimation on solid phantoms

Figure 2 shows the spatially-resolved reflectance profiles acquired for a solid phantom ($\mu_s'$ = 20 cm$^{-1}$, $\mu_a$ = 0.14 cm$^{-1}$) in the range 450–1050 nm (high signal-to-noise regions). The unit on the vertical z-axis is relative reflectance calculated as the ratio of the dark-corrected intensity acquired for the sample by the dark-corrected intensity collected in an integrating sphere. In this way, the measured signals are compensated for dark noise, variations of light source intensity due to sensor sensitivity, differences in efficiencies of different pixels of the camera and differences in efficiencies of the detection fibres. Fibre 1 is the closest fibre and fibre 7 is the furthest one from the illuminating fibre. A clear decrease of the relative reflectance with increasing fibre number can be observed. This can be explained by the fact that light exiting the sample at a larger distance from the incident light beam has travelled a longer path through the sample and thus had more chance to be absorbed or scattered. The quite flat spectrum observed at each fibre position is a good indication that the black ink absorbs all wavelengths in the range 450–1050 nm.

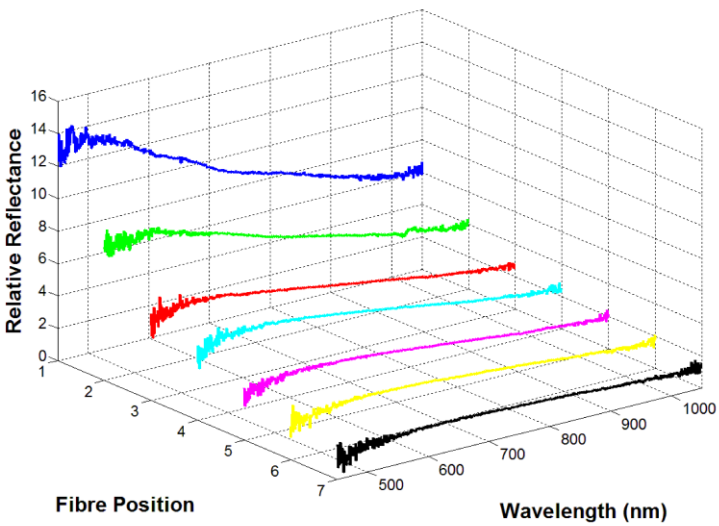


**Figure 2.** Spatially-resolved reflectance profiles of a solid phantom ($\mu_s'$ = 20 cm$^{-1}$, $\mu_a$ = 0.14 cm$^{-1}$).

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In Figure 3 the optical properties for these 16 solid phantoms estimated by the aforementioned fitting procedure are presented together with and against the optical properties calculated from the recipe.[4]

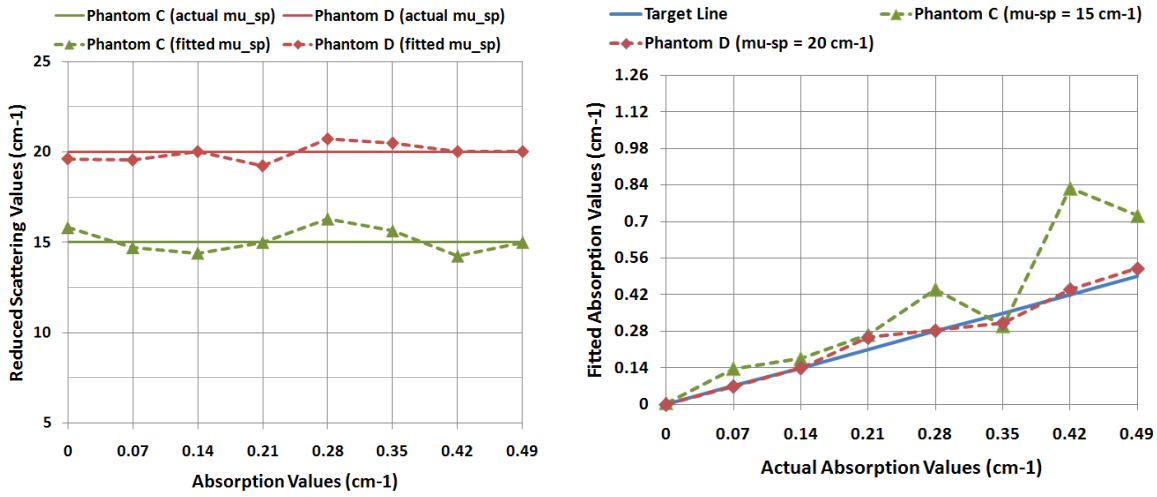


**Figure 3.** (Left) Fitted reduced scattering coefficients of 16 solid phantoms. Two levels of actual reduced scattering values are denoted by C ($\mu_s'$ = 15 cm$^{-1}$) and D ($\mu_s'$ = 20 cm$^{-1}$). At each scattering level there are 8 phantoms (denoted by numbers from 1 to 8) representing 8 actual absorption values. (Right) Fitted absorption coefficients of 16 solid phantoms. The blue diagonal line represents the target line.

The results obtained indicate quite good accuracy of the estimated optical properties for these solid phantoms which are considerably more scattering than absorbing ($\mu_s' >> \mu_a$). In Figure 3, relatively bigger differences between the fitted and actual values can be observed for the phantoms with higher absorption values. This can be explained by the fact that the diffusion approximation only holds for samples with considerably higher scattering than absorption ($\mu_s' >> \mu_a$). When the absorption coefficient increases, but the scattering coefficient remains the same, larger errors can be expected. Another contributing factor could be the loss of a fraction of the diffusely reflected light which has not been captured by the detection fibres due to imperfect contact to the phantom surfaces which subsequently results in an over-estimation of the absorption values.

## Optical properties of Braeburn apples

Figure 4 shows typical spatially-resolved reflectance spectra acquired at one position of a Braeburn apple at harvest (day 1) in the range 500–1050 nm. Regions of lower reflectance values can be observed around 670 nm and 970 nm, corresponding to the absorption peaks of chlorophyll and water in the apple tissue. The relative reflectance values at the different wavelengths clearly decrease from fibre position 1 to fibre position 7 because the number of photons captured at a larger distance will be smaller due to scattering and absorption phenomena in the apple tissue.

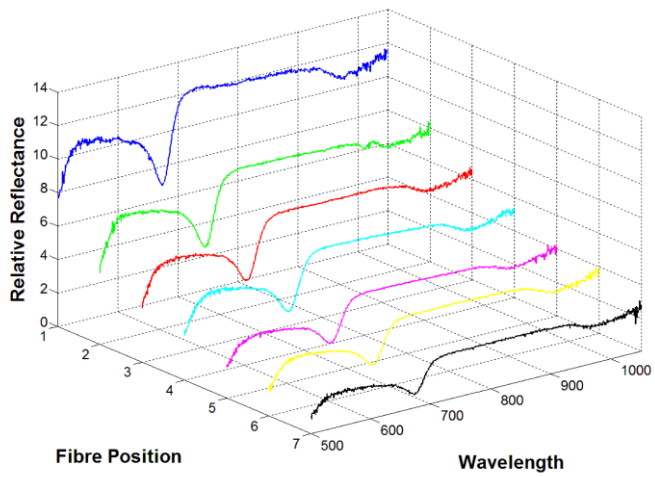


**Figure 4.** Spatially-resolved reflectance spectra of a Braeburn apple (at harvest; day 1).

Reference paper as:
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Figure 5 shows typical results for the average absorption and reduced scattering coefficient spectra estimated for the Braeburn apples at harvest (day 1) and after 14 days of shelf-life storage.

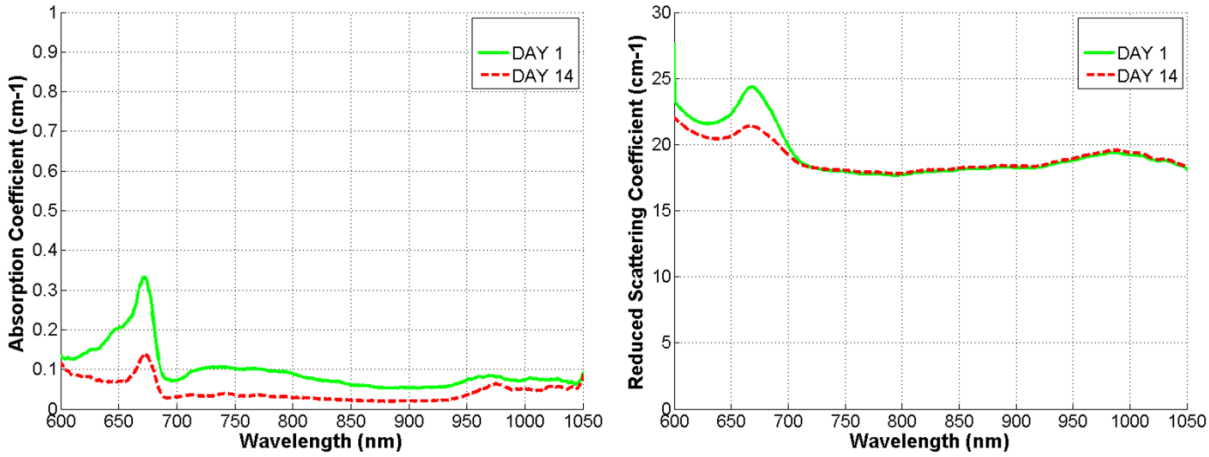


**Figure 5.** Average spectra of absorption (left) and reduced scattering coefficients (right) of Braeburn apples at harvest (day 1) and after 2 weeks of shelf-life storage (day 14).

A significant drop in the absorption at 670 nm by chlorophyll can be observed in Figure 5 (left), which can be attributed to the rapid degradation of chlorophyll during shelf-life storage at 18°C. A decrease in the water absorption at 970 nm can also be observed, indicating water loss during shelf-life storage. For scattering, the change in the reduced scattering coefficient spectrum after 14 days of shelf-life storage is rather limited (Figure 5, right). 'Peaks' of estimated reduced scattering coefficients appear unexpectedly around 670 nm probably due to violation of the assumption of much larger scattering than absorption in the regions with high chlorophyll absorption.

## Conclusion

A spatially-resolved spectroscopy set-up based on a fibre-optic probe was elaborated in the lab. A model based on the diffusion equation was successfully employed for describing the light propagation in apple tissue. The accuracy of the algorithm for estimating the optical properties from the acquired SRS profiles was successfully validated on solid phantoms with known optical properties. This method was then used to investigate differences in the absorption and reduced scattering spectra of Braeburn apples before and after 2 weeks of shelf-life. Decay of the chlorophyll content and water loss were observed from the absorption coefficient spectra while no clear changes in the reduced scattering spectra could be observed.

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