

## Recent Advances in Time-Resolved NIR Spectroscopy for Nondestructive Assessment of Fruit Quality

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Non-destructive monitoring of food internal attributes by near infrared spectroscopy (NIRS) is typically performed by the continuous wave (CW) technique, where steady state light sources (e.g. lamp or LED with constant intensity in time) and photodetectors (e.g. photodiode or charge coupled device camera) are used to measure light attenuation. Indeed light scattering can largely affect light attenuation resulting in the need of calibration for each new batch of samples. To tackle this effect time-resolved NIRS (TRS) has been proposed to improve the classical CW approach to NIRS. The main feature of TRS is its ability to retrieve information on photon path-length in a diffusive medium (generally much larger than the geometrical distance between source and detector). The use of TRS in combination with proper physical models for photon migration allows for the complete optical characterisation with the simultaneous non-destructive measurement of the optical properties (absorption and scattering) of a diffusive medium. This can be of special interest for most fruits and vegetables as well as for other foods (e.g. meat, fish, and cheese), because information derived by TRS refers to the internal properties of the medium, and is not so much affected by surface features as is the case for CW spectroscopy. In the past TRS measurements were possible only with complex laboratory instrumentation consisting of picosecond pulsed lasers, water cooled photomultiplier tubes, and electronic chain for time-correlated single photon counting. In this work we present the recent advances in TRS technology (laser, detectors and acquisition electronics) that allow the design of portable instrumentation for use in the pre-harvest (i.e. in the field) and post-harvest.

### 1. Introduction

For the last decades, optical technologies have been used in grading lines for nondestructive fruit and vegetable processing thanks to their ability to convey information on quality attributes. At first, external attributes like shape, color and presence of superficial defects were targeted by using visible (VIS) and ultraviolet (UV) radiation (Saito, 2009; Yud-Ren et al., 2002). Later on, near infrared (NIR) radiation was employed to probe the bulk tissue in a nondestructively fashion to assess the internal quality. In the NIR spectral range (700-2000 nm) light absorption, related to the presence of chromophores (e.g. chlorophyll, sugars, and water), is relatively low as compared to light scattering, related to the microscopic and/or random inhomogeneities in the refractive index properties (Nicolai et al. 2014). Therefore, NIR light can travel long paths within the medium before being eventually reemitted at the boundaries.

The description of light propagation in biological tissue is usually made in the framework of the Radiative Transport Theory in the Diffusion approximation (Martelli et al. 2010). The wave (i.e. electromagnetic) behaviour of light is disregarded in favour of a particle (i.e. photon) description. Photons migrate in a tissue described as a random medium where absorption and scattering events occur as probabilistic phenomena. The absorption coefficient  $\mu_a$  and the scattering coefficient  $\mu_s$  are defined as the inverse of the absorption and scattering mean free path in the medium, respectively. To account for anisotropic light propagation due to light scattering, the anisotropy factor  $g = \langle \cos\theta \rangle$  and the reduced scattering coefficient  $\mu_s' = \mu_s(1-g)$  are introduced.

While travelling in the medium, NIR light can be attenuated by the complex interplay between light absorption and light scattering. Light attenuation can be measured by means of standard techniques employing light sources and detectors operating in the steady state regime, also called continuous wave (CW) regime. Measurements are typically performed at multiple wavelengths; therefore, the technique is often called NIR spectroscopy (NIRS) (Contini et al. 2012).

A limitation of CW NIRS is the need of calibration against scattering changes (Zude et al., 2011). More recently, sophisticated approaches have been devised like the so-called space-resolved NIRS (SRS) and the time-resolved NIRS (also indicated as time-resolved reflectance spectroscopy, TRS). Differently from CW NIRS, SRS measure the spatial profile of the light remitted from the medium (Saeys et al. 2008), while TRS measure the distribution of photon time-of-flights (DTOF) in the medium (Cubeddu et al., 2001). Both approaches allow uncoupling light absorption and light scattering for a more comprehensive optical characterisation of biological tissue. TRS has the advantage of operating with a single pair of optodes: light is in fact injected in one point and collected in another (see Figure 1, left panel). Information on depth can be achieved by collecting photons with longer time-of-flight. Conversely, SRS requires a series of detectors at different distances from the injection point. The larger the distance the larger is the penetration depth. Typically with source detector distance  $\rho = 1-3$  cm, penetration depth is in the order of 0.5-1.5 cm.

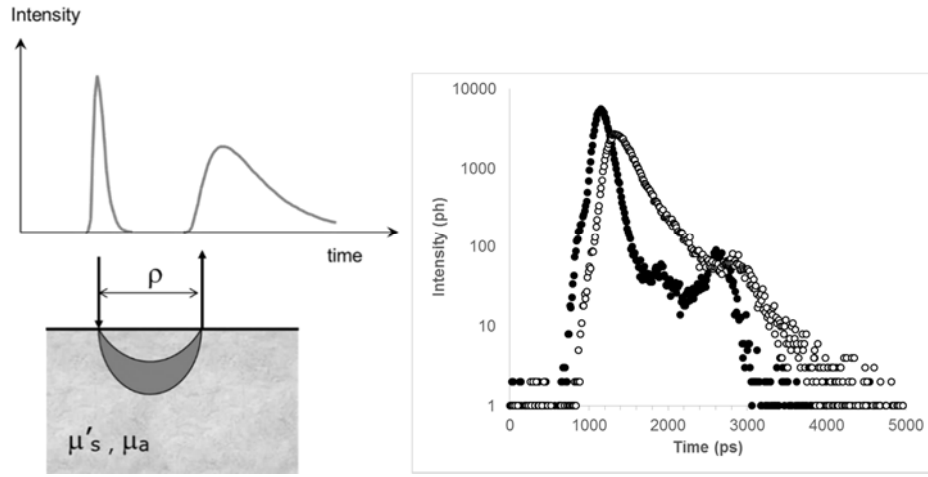


Figure 1: (Left) Scheme of time-resolved reflectance spectroscopy (TRS). (Right) Example of TRS curve (open circles) at  $\rho = 1.5$  cm and instrument response function (IRF, filled circles) at 750 nm. Data were acquired on a tomato with the system described in Figure 3.

In this work, we present the recent advances in TRS that allow the design of instrumentation for use in the pre-harvest (i.e. in the field) and post-harvest. In Section 2 we review TRS technology (laser, detectors and acquisition electronics) and present the scheme of several TRS systems. In Section 3 we present examples of results obtained with different TRS instruments.

## 2. Materials and Methods

### 2.1 Physical modelling for TRS

In TRS the DTOF can be related to the reflectance  $R(\rho, t)$ , i.e. the photon probability to be reemitted from the tissue at a time  $t$  and a distance  $\rho$  from the injection.  $R(\rho, t)$  can be expressed as:

$$R(\rho, t) = AD^{-3/2}t^{-5/2}e^{-\mu_a vt}e^{-\frac{\rho^2}{4Dvt}} \left( z_0 e^{-\frac{z_0^2}{4Dvt}} - z_p e^{-\frac{z_p^2}{4Dvt}} \right) \quad (1)$$

where  $A$  is a normalization constant,  $v = c/n$  is the speed of light in the medium,  $z_0 = 1/\mu'_s$  is the scattering mean free path, while  $z_p$  is derived from the extrapolated boundary conditions and depends on the refractive index of the tissue. The diffusion coefficient  $D = 1/(3\mu'_s)$  was taken to be independent of the absorption properties of the medium. The derivation of Eq. (1) can be found in Martelli et al. (2010). Figure 2 shows example of TRS curve from a calibrated tissue phantom ( $\mu_a = 0.1 \text{ cm}^{-1}$ ,  $\mu'_s = 10 \text{ cm}^{-1}$ ) obtained at  $\rho = 1.5$  cm.

## 2.2 Instrumentation for TRS

The range of values for the optical properties in the NIR (e.g.  $\mu_a = 0-0.5 \text{ cm}^{-1}$ ,  $\mu_s' = 5-25 \text{ cm}^{-1}$ ,  $\rho = 1-3 \text{ cm}^{-1}$ ) set the values for the attenuation in the order of  $10^6-10^8$  while the temporal dynamic spans over a 1-10 ns range. Therefore, a TRS system must be able to detect weak and faint optical signals. Since the early 1990s, time-correlated single photon counting (TCSPC) (Becker 2006) was the chosen technique to implement TRS for biomedical applications. Several research groups had already a considerable experience on TCSPC for fluorescence lifetime spectroscopy and the implementation of TRS was straightforward. Those early systems had several limitations and in the past two decades, significant advances have been implemented. Table 1 summarize the evolution of TRS components from the 1990s to nowadays. Three different generations of TRS system can be roughly considered, and a fourth can be envised.

Table 1: The evolution of TRS components

Generation	Light sources	Photo-detectors	Acquisition system
1 <sup>st</sup> (1990-2000)	gas lasers dye laser solid state laser	microchannel plate photomultiplier (PMT)	electronic chain for TCSPC with NIM module
2 <sup>nd</sup> (2000-2010)	semiconductor laser heads with external RF driver	compact metal channel dynode PMT	TCSPC electronic board
3 <sup>rd</sup> (2010-2014)	supercontinuum fiber laser	hybrid PMT	TDC module with USB controller

### 1<sup>st</sup> generation TRS

The 1<sup>st</sup> generation of TRS system employed bulky and complex laser light sources like noble gas lasers (e.g. Ar, Kr), either as standalone sources or used for the synchronous pumping of dye laser (e.g. Rhodamine). Further, solid state laser (e.g. Ti:Sapphire) were also used due to the ability to generate very narrow pulses (e.g. 1-10 ps) with high repetition rate (100 MHz) and average power >100 mW over a broad spectral range (700-1000 nm). Water-cooled microchannel plate photomultipliers (PMT) were able to provide a fast response with a very limited time-jitter, typically less than 50 ps. An electronic chain for TCSPC with NIM modules for constant fraction discriminators, delay lines, time-to-amplitude converter and multichannel analyser was used. Such a system required a few hours to warm up and reach stable conditions of operation. The system was typically hosted on an optical table and in a room with air conditioning to minimize drift due to mechanical and thermal effects. Figure 2 shows the scheme of the 1<sup>st</sup> generation TRS system developed at the Physics Dept. of Politecnico di Milano (more details can be found in Pifferi et al. 2007).

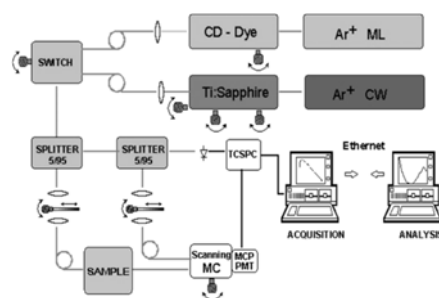


Figure 2: Example of 1<sup>st</sup> generation TRS system.

### 2<sup>nd</sup> generation TRS

In the beginning of 2000, the availability of semiconductor pulsed laser heads with compact external RF driver (e.g. LDH series, PicoQuant GmbH), of PC electronic board for TCSPC (e.g. TimeHarp, PicoQuant GmbH; SPC630, Becker&Hickel GmbH) and of compact metal channel dynode PMT with acceptable temporal resolution (time-jitter <200 ps) on one side contributed to the decrease of the overall cost of the TRS system, and on the other side paved the way to the development of transportable TRS systems. Figure 3 shows the

scheme of the 2<sup>nd</sup> generation TRS system developed at the Physics Dept. of Politecnico di Milano (more details can be found in Torricelli et al. 2008).

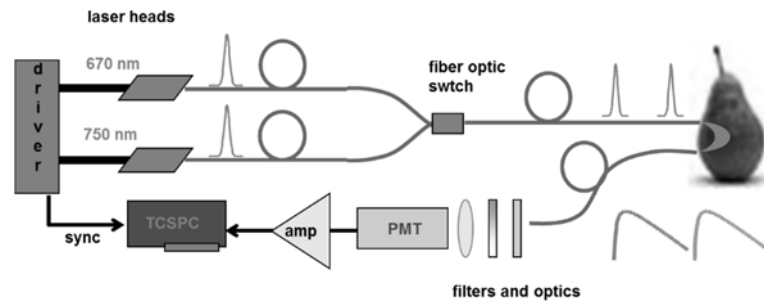


Figure 3: Example of 2<sup>nd</sup> generation TRS system.

Advantages of this configuration were an increased count rate of the TCSPC board (in the order of few MHz) as compared to the NIM module (limited to <0.1 MHz) and the robustness of the detector as compared to the fragile MCP PMT. A limitation of this approach was the limited power available with pulsed laser heads. In order to avoid broadening of laser pulses due to secondary oscillations, the average power had in fact to be limited to <1 mW to ensure a pulse duration <100 ps. Moreover, the system operated at fixed discrete wavelengths, while the extended spectral sensitivity of the detector to wavelength <900 nm, thanks to multi-alkali photocathode, was somehow hampered by the afterpulsing in the detector that could reduce the overall signal-to-noise ratio, especially at high count rate.

### 3<sup>rd</sup> generation TRS

A significant improvement was possible thanks to the availability of supercontinuum fibre laser (e.g. Fianium Ltd, NKT Photonics AG), with powerful (e.g. <6 W) emission over a broad spectral range (e.g. 500-2400 nm) of short (<10 ps) laser pulses with high repetition rate (<80 MHz). This light source in fact allowed broadband TRS measurements in a portable system. Selection of wavelengths from the supercontinuum was achieved either by acousto-optic tuneable filter (AOTF) or by classical interference filters (Farina et al. 2013).

Hybrid photomultiplier were also made available (Becker&Hickl GmbH, PicoQuant GmbH) yielding an afterpulsing free detector also with good temporal resolution (time-jitter <180 ps), large area (<3 mm diameter) and sensitivity in the NIR (quantum efficiency <15% at 800 nm). Figure 4 shows the scheme of the 3<sup>rd</sup> generation TRS system developed at the Physics Dept. of Politecnico di Milano (details can be found in Spinelli et al. 2012).

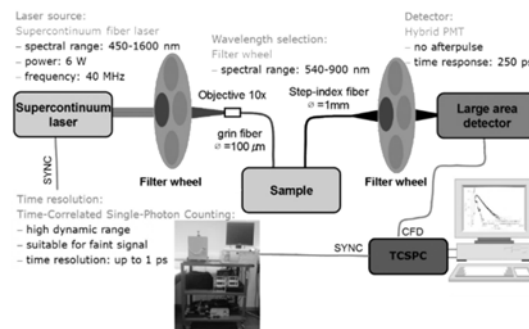


Figure 4: Example of 3<sup>rd</sup> generation TRS system.

The evolution of counting and timing electronics contributed to further advances. TCSPC instrumentation based on NIM modules or PC boards are often complex, expensive and power consuming, thus practically limiting the maximum number of channels. Vice versa, TCSPC devices based on time-to-digital converters (TDC) can nowadays be realized with high performances by using a traditional CMOS process, featuring high conversion rate (few Mcounts per second), linearity ( $\sim 1\%$  RMS of the least significant bit) and at low cost (Markovic et al. 2013). Additionally they can be easily integrated in compact modules based on field-programmable gate array (FPGA) to fast transfer the converted times to a PC through a USB connection, (Tamborini et al. 2014).

### 3. Results and Discussion

In Figures 5 and 6 examples of TRS data acquired by different TRS instruments are reported. In particular, in Figure 5 are shown the absorption and reduced scattering spectra of an apple obtained by means of the 1<sup>st</sup> generation TRS system described above. We note the extension of the spectral range that allows to appreciate the presence of the water absorption peak around 975 nm. Actually, water together with chlorophyll are the main absorbers in the visible a near-infrared spectral range. As for the reduced scattering spectra, we note that it is flat for such a kind of fruit: the small bump around the 975 nm is due to the high value of the absorption at that wavelength, which causes some artifacts in the retrieval of the reduced scattering coefficient when the diffusion approximation is exploited. Anyway, the flatness of the reduced scattering spectrum in apples means that the pulp is quite homogeneous, without microscopic structures such as fibrils.

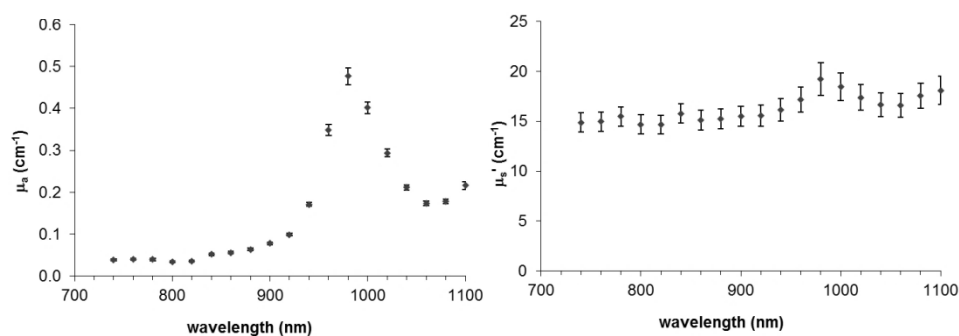


Figure 5: Absorption (left) and reduced scattering (right) spectra of an apple in the NIR obtained with the system setup described in Fig.2.

In Figure 6 examples of absorption and reduced scattering spectra for Tommy Atkins mangoes are reported. These measurements have been performed by exploiting the 3<sup>rd</sup> generation TRS system described above. The presence of the chlorophyll absorption peak at 670 nm is clearly visible in the absorption spectrum of the unripe mango. During mango maturation, the pulp chlorophyll content decreases, while carotenoids starts to accumulate: correspondently in the absorption spectrum of the ripe mango we can note a marked reduction of the absorption value at 670 nm and an increase of the absorption at 550 nm, that is in the spectral region where carotenoids are known to absorb more. Actually, TRS can follow the dynamics of these chromophores during mango ripening.

On the other hand, the reduced scattering spectrum of a mango, both ripe and unripe, is almost flat, denoting an absence of microstructures in the pulp that appear quite homogeneous like in apples.

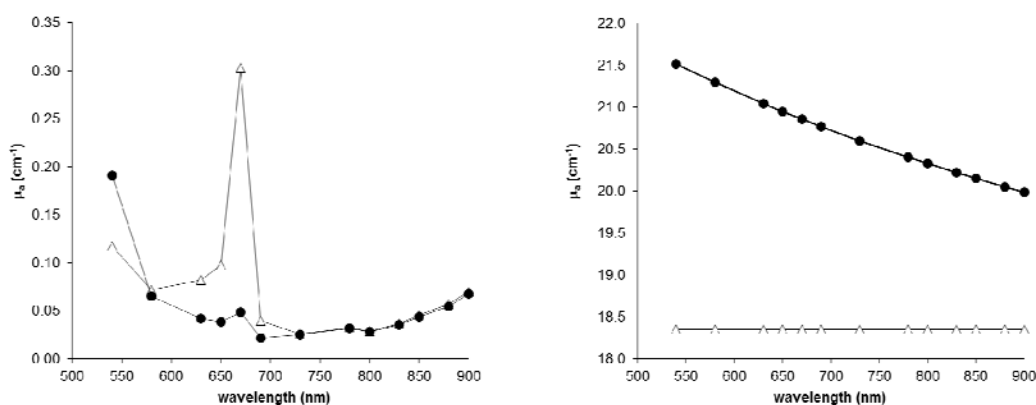


Figure 6: Absorption (left) and reduced scattering (right) spectra of ripe (closed circles) and unripe (open triangles) Tommy Atkins mango in the NIR. Data were obtained with the system setup described in Fig.4.

#### 4. Conclusions

TRS has been evolving from an expensive and sophisticated technique available in few selected laboratories worldwide towards low cost probes for a wider diffusion in a diversity of application. For example, the availability of hand-held device will allow preharvest TRS measurements for the determination of the optimal harvest date. Miniaturized TRS sensor could be mounted in the orchards for supporting precision agriculture approaches. Current research at Politecnico di Milano is focussed on the development of the next generation of TRS systems. Fostered by the plethora of applications in the biomedical field (e.g. optical mammography, functional brain imaging, monitoring muscle metabolism) we aim at increasing the parallelization of TRS acquisition with small, low cost devices. Laser-on-chip, integrated TDC module, and solid state photodetectors like single photon avalanche diode (SPAD) or silicon photomultiplier (SiPM) are the components under study.

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