Measurement of Optical Properties of Fruits and Vegetables: A Review

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Abstract

This paper provides an overview of the principles and theory of measuring optical properties of biological materials. It then presents the instrumentation and data analysis procedures for implementing several emerging optical techniques, including spatially resolved, time-resolved, and spatial-frequency domain, along with the standard integrating sphere method. Applications of these techniques for optical property measurement, maturity and quality assessment, and defect detection of fruits and vegetables are then reviewed, followed with discussions on issues and challenges that still need to be addressed for these emerging optical techniques. While these optical techniques are overall more sophisticated in instrumentation and computation, they are based on solid radiative transfer theory or diffusion approximation theory. Hence, measurement of optical absorption and scattering properties has the potential of providing more complete, objective information for quality evaluation of horticultural products. At present, these techniques are still slow in measurement, and prone to errors due to modeling and instrumentation deficiencies. Further research is therefore needed in using a better mathematical

modeling approach, improving data acquisition accuracy and speed, and developing more robust inverse algorithms for optical property estimations.

Keywords: Spatially resolved, time-resolved, spatial-frequency domain, postharvest quality, fruit, vegetable.

1. Introduction

Optical imaging and spectroscopic techniques now are widely used for property, quality and safety assessment of horticultural and food products. Most of these techniques rely on measurement and direct analysis of the output optical signals (i.e., spectra and/or images in the form of reflection or transmittance) acquired from food samples under a specific form of lighting (i.e., diffuse or uniform lighting, point lighting, patterned lighting, etc.). This type of approach, which may be termed the direct approach, overall is simpler, faster and easier to implement for online or offline applications, compared to the optical measuring methods reviewed in this paper, which belong to the inverse or indirect approach. Conventional visible and near-infrared (Vis/NIR) spectroscopy employs the direct approach to measure the aggregate amount of light either reflected from or transmitted through a biological or food material, resulting from the combined effect of absorption and scattering of photons by the tissues. The acquired spectral data are then processed, via mathematical techniques, for establishing a quantitative or qualitative relationship with the property or quality parameter(s) of interest. Once the calibration model is established and validated, it can then be used for predicting new samples. There are, however, some inherent shortcomings with conventional Vis/NIR technique and the direct approach in general, because the measured values of reflectance or transmittance are extrinsic or phenomenological values (like force and pressure in mechanical measurements), and they are influenced by such factors as type of instrument, sensing mode (reflectance, transmittance, etc.), and light source/detecting probe setup. It thus presents great challenges in transferring the Vis/NIR calibration models between different instruments or comparing the performance of different instruments.

In recognizing the shortcomings or limitations of conventional Vis/NIR technique or the direct approach in general, researchers have long sought alternative approaches to measure optical absorption and scattering properties of food and biological materials. They are two intrinsic material properties that characterize the behavior of light interaction with biological tissues. Hence, the measured values for the two optical property parameters are, in principle, independent of instrumentation type and lighting/detecting probe setup, which would make it easier to compare the data acquired from different instruments for different studies. In this paper, the terms 'optical properties' and 'bulk optical properties' are used interchangeably, both referring to the average effect of different optical processes occurring in tissues at the microscale. The light interaction with tissues can be described withtwo optical parameters related to the two basic processes, i.e., absorption and scattering. Absorption is largely determined by the chemical composition of the tissue, while scattering is dependent on density and tissue structures (e.g., particle size, distributions, etc.). Hence, measurement of the two optical parameters could provide more complete, objective information about the structural and chemical properties of samples.

Early efforts had been made by Birth and colleagues in the late 1970s and early 1980s on measuring the optical absorption and scattering properties of food products, based on the empirical model (i.e., Kubelka-Munk model) for two-dimensional turbid slabs (Birth, 1978, 1982; Birth et al., 1978). However, due to the limitations of optical and computer technologies available at that time, the measurement procedure, which involved both reflectance and transmittance, was quite tedious and time consuming. Nevertheless, these early studies provided an alternative approach for optical property measurement, and they also demonstrated some merits of using optical absorption and scattering parameters for assessing properties and quality attributes of food products.

From the late1980s to the 2000s, rapid advances were made in Vis/NIR technique in both hardware and software (i.e., chemometrics). A large variety of Vis/NIR instruments became available, from expensive benchtop instruments to low-cost, miniaturized handheld devices. Chemometric techniques also evolved from conventional linear modeling to nonlinear modeling and to artificial neural networks. Thanks to these developments, Vis/NIR spectroscopy has spread into different areas of food and agriculture applications as well as many other industries (e.g., pharmaceutical and chemical), as shown by the exponential increase in scientific publications over that period of time (Nicolaï et al., 2007). In comparison, little progress was made on optical property measurement of food and agricultural products in the 1990s, with only a few, sporadic scientific publications. However, the situation was completely different in biomedical optics research. Since the mid-1980s, significant advances have been made in noninvasive (also called *in vivo* in the biomedical optics field) techniques for measuring optical absorption and scattering properties of biological materials, thanks to major breakthroughs in analytical solutions to the theory of light transfer as well as optical detection and laser technologies. Specifically, analytical solutions to the diffusion approximation theory, a simplification to the radiative transfer theory, became available for several special, important illumination conditions (i.e., steady-state or continuous-wave point lighting, pulsed point-lighting and frequency modulated lighting) (Farrell et al., 1992; Kienle and Patterson, 1997; Patterson et al., 1989, 1991). Based on these analytical models, several new techniques, including spatially resolved (SR), time-resolved (TR) and frequency-domain (FD), were developed for measuring optical properties of biological materials during the 1990s and 2000s (Tuchin, 2007).

The interest in these emerging optical property measurement techniques for food and agricultural products began to grow from the early 2000s, owing to the availability of more affordable optical instrumentation. TR technique was first applied to measure the optical properties of apples and other fruits (Cubeddu et al., 2001a, 2001b). Subsequently, a series of studies were reported by a group of researchers in Italy (Rizzolo and Vanoli, 2016) on using different TR instrumentation configurations (i.e., single wavelength, multi-wavelength and broad spectral region) to assess quality, maturity and internal defects of fruits and vegetables. Around that time period, several research groups in the U.S. and Europe also started to actively pursue SR techniques for measuring optical properties of fruits, vegetables and other food products. Researchers with the U.S. Department of Agriculture Agricultural Research Service (USDA/ARS) at Michigan State University in Michigan, USA developed a hyperspectral imaging-based SR technique for fast measurement of optical absorption and scattering properties of horticultural and food products over a broad range of wavelengths (Cen and Lu, 2010; Qin and Lu, 2007, 2008). Researchers at the University of Missouri, Columbia, Missouri, USA developed a SR technique, based on single-fiber spectroscopy, for measuring optical properties of meat muscles and meat analogs (Xia et al., 2007, 2008). Researchers from KU Leuven in Belgium, used the integrating sphere method (IS), coupled with an inverse adding-doubling (IAD) algorithm (hereinafter called IS-IAD), to measure the optical properties of apple, potato, and meat tissue components (López-Maestresalas et al., 2015; Saeys et al., 2008; Van Beers et al., 2017b; Zamora-Rojas et al., 2013). Later, the group also developed other SR sensing systems, based on single-fiber, multi-fiber and imaging-based configurations, for measuring fruits and food products (Aernouts et al., 2015; Nguyen Do Trong et al., 2014a, 2014b; Van Beers et al., 2015). More recently, researchers from the University of Georgia at Athens, Georgia, USA used IS-IAD to measure optical properties of onions and other horticultural products (Wang and Li, 2013, 2014; Zhang et al., 2019).

In recent years, new optical property measurement techniques have continued to emerge in the biomedical optics field. Researchers from the University of California at Irvine, USA reported on a new spatial-frequency domain (SFD) technique for wide-area imaging of the optical properties of biological tissues (Cuccia et al., 2005). The SFD technique was used to detect healthy and bruised apple tissues (Anderson et al., 2007). Recently, researchers at Zhejiang University, Hangzhou, China and the USDA/ARS laboratory in Michigan, USA, used SFD technique to measure optical properties of fruits and food products composed of one or two homogeneous layers (Hu et al., 2016, 2018).

This paper is therefore intended to give an overview of the principles, theory and modeling of light transfer in biological materials, followed by a brief introduction to the instrumentation and data analysis approaches for IS-IAD, SR, SFD, and TR techniques. Applications of these techniques for quality assessment of fruits and vegetables are then reviewed. Finally, discussions are given on critical issues, challenges and future research needs for these techniques. This review is aimed to help researchers and practitioners gain a better understanding of these emerging optical property measurement techniques and their advantages and limitations, thus stimulating further research in optical property measurement of horticultural and food products.

2. Principles and Theory of Light Transfer

2.1 Light transfer in biological materials

Biological or plant tissues are a complex system formed by cells and their extracellular matrix. These cells and extracellular matrix contain different components like membranes, cytoskeletons, organelles, etc., each of which has different structural, chemical and optical characteristics. Hence, the actual process of light interaction with the biological tissue system is rather complex at the microscale. However, in studying light transfer in biological or plant tissues, they may be treated as being primarily composed of scattering (e.g., the organelles and cellular membranes) and absorbing (e.g., chromophores) particles. Under this simplified treatment, light transfer in biological tissues mainly involves the process of photon interactions with the scattering and absorbing particles. As photons enter the plant tissues, they will move straightforward until encountering a scattering or absorbing particle. The photons will change the travelling direction (called scattering) upon hitting the scattering particle, or they would be absorbed, if the particle is an absorbing particle and when the level of energy for the photons matches that of the particle according to quantum theory. The actual change in the scattering direction of the photons depends on the optical properties of the scattering particle. After the particle is hit by a large quantity of photons, the angular scattering profile can be described by the scattering phase function [designated as $p(\theta)$], which is unique for each particle and is determined by the size, shape and orientation of the particle. Specifically, the fraction of photons that would be scattered to a specific direction is determined by the anisotropy factor, designated as g. Values of g range between -1 and 1, where g=-1 is total backward scattering; g=0 is isotropic scattering (i.e., photons scatter equally in all directions), and g=1 represents total forward scattering. For most food and plant materials, g values range between 0.7 and 0.9, indicating that forward scattering is dominant. The absorption process can be characterized by the absorption coefficient, normally designated as μ_a. On the other hand, the scattering process is defined by the scattering coefficient (μ_s) and the anisotropy factor (g). Hence, with the knowledge of μ_a, μ_s and g, one can, in principle, describe the transfer of photons in biological tissues. As shown in the following section, when scattering is dominant (i.e., $\mu_s >> \mu_a$), the anisotropy factor can be lumped into the scattering coefficient, which leads to a new optical parameter, called the reduced scattering coefficient [i.e., $\mu_s{}' \equiv (1-g)\mu_s$] . This means that for most plant materials like fruits and vegetables, it would be sufficient to use μ_a and $\mu_s{}'$ for characterizing the interaction of light with the plant material.

2.2 Diffusion approximation theory

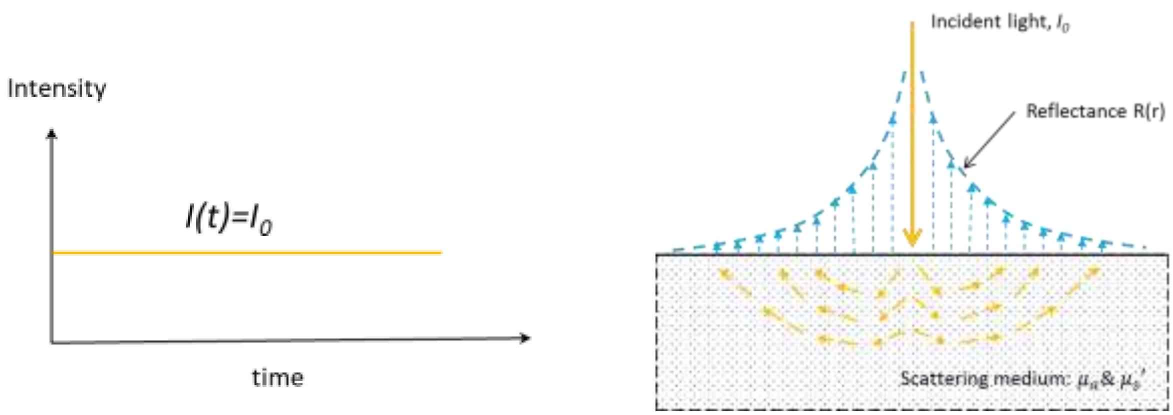
The transfer of light in biological materials is best described by radiative transfer theory. The radiative transfer equation can be derived by employing the principle of conservation of energy. A detailed description on the derivation of the radiative transfer equation can be found in Lu (2016). Since the radiative transfer equation is expressed in an integro-differential form with six variables, it cannot be solved analytically, except for a few special, restricted situations. Subsequently, the assumption of dominant scattering in turbid biological materials is used to approximate it as by a diffusion equation:

$$\frac{\partial \Phi(\vec{r},t)}{\partial t} = \nabla \cdot [D \nabla \Phi(\vec{r},t)] - \mu_a \Phi(\vec{r},t) + S(\vec{r},t) \qquad (1)$$

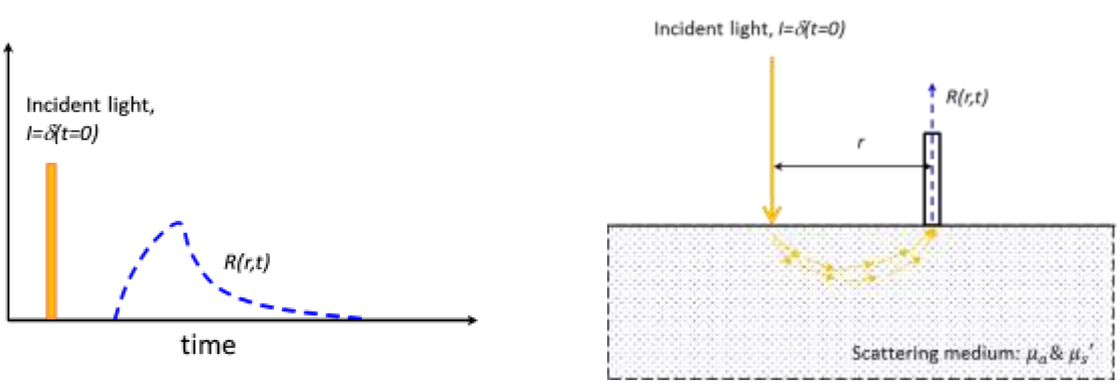
where $\Phi(\vec{r},t)$ is the fluence rate, $\vec{r}$ is the location vector, t is the time variable, $S(\vec{r},t)$ is the isotropic source in the medium, and D is called the diffusion coefficient, which is given by

$$D = \frac{1}{3[\mu_a + (1-g)\mu_s]} = \frac{1}{3(\mu_a + \mu_s{}')} \qquad (2)$$

where $\mu_s{}'$ is the reduced scattering coefficient. The diffusion equation only contains two independent optical parameters, i.e., the absorption and reduced scattering coefficients, and it has been solved analytically for several special, important illumination situations, as shown in Figure 1, which include continuous-wave or steady-state point lighting (Figure 1a), pulsed point lighting (Figure 1b), frequency-modulated point lighting (Figure 1c), and spatially-modulated area lighting (Figure 1d) (Lu, 2016). These analytical solutions form the theoretical basis for the SR, TS, FD, and SFD techniques described in Section 3.



(a)



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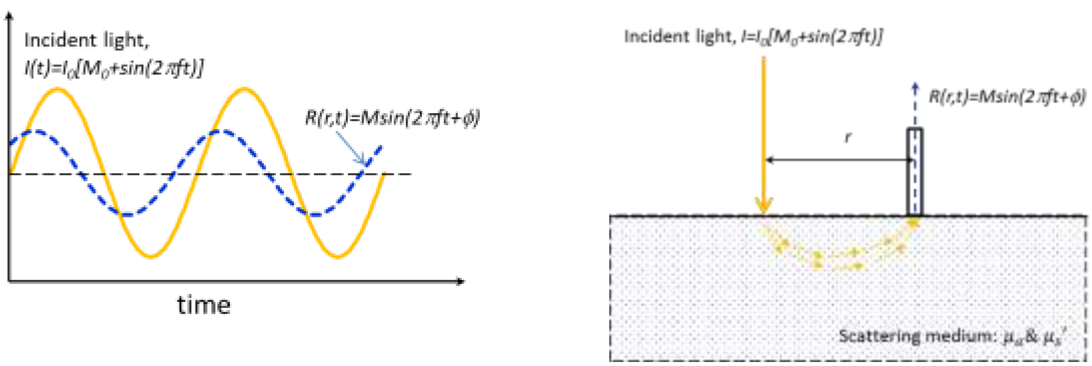

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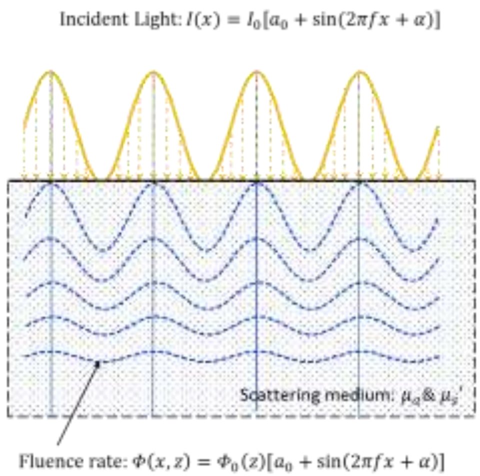

(d)

Figure 1. Four special cases of light illumination at the surface of a semi-infinite turbid medium, for which analytical solutions to the diffusion approximation equation are available: a) continuous-wave point lighting (time-independent), b) short-pulsed lighting, c) frequency-modulated point lighting, and d) spatially-modulated area lighting (time-independent), where symbol I represents the intensity of the incident light, f is the frequency either in the time (t) or spatial domain. It should be noted that in cases a) through c), the spatial dimensions are expressed in the two-dimensional polar coordinate system, while in case d), they are in the two-dimensional Cartesian coordinate system.

2.3 Numerical simulations

Although the diffusion equation is widely used with SR, SFD and TR/FD techniques for estimating optical properties of biological materials, it is only applicable when scattering is dominant over absorption ($\mu_s >> \mu_a$) and if the radiance is only considered at a sufficiently large distance from the point of illumination (>> one mean free path) (Martelli et al., 2010). In the Vis/NIR region of the spectrum, these assumptions do not always hold for biological tissues, due to the high absorption by biological chromophores, of which chlorophyll, carotenoids, anthocyanins and water are dominant in plant materials. To overcome these limitation researchers have used numerical methods, such as adding-doubling (AD) and Monte Carlo (MC) methods, to simulate light propagation in biological tissues (Prahl et al., 1993; Simpson et al., 2001; Tuchin, 2017; Wang et al., 1995).

The AD method, which was first introduced by van de Hulst (1980), is used to numerically solve the radiative transfer theory (RTT) for an infinitely thin and single-scattering homogeneous layer by calculating the angle-dependent reflection and transmission of that layer (Prahl, et al., 1993). With the assumption of single scattering, the RTT can be solved with relative ease for a series of bulk optical properties (BOP) (i.e., μ_a, μ_s, and $p(\theta)$). By adding layers with different optical properties, dissimilar slabs with internal reflections at the boundaries can be accounted for. Likewise, the differences in refractive indices *n* between two materials, causing refraction and reflection at the boundaries, can be accounted for, respectively, using Snell's and Fresnel's laws. Eventually, for a specific sample slab, the AD routine results in angular reflectance and transmittance functions per wavelength. Integrating these functions over the different conical segments provides the total reflectance and total transmittance of the sample slab. The equations and algorithm of the AD method have been described in Prahl (1995). The AD approach is fast and accurate, but it is restricted to layered geometries and cannot be used to retrieve information on the spatial distribution of the reflected and transmitted photons (Aernouts et al., 2014). Moreover, each layer should have homogeneous BOP. The routine has been extended to estimate the spectral and angular distribution of the scattered radiation of (fluorescent) materials (Leyre et al., 2012), while it can also be inverted to retrieve the BOP for thin sample slabs from the measured total transmittance and total reflectance, as described in Section 3.1 (Aernouts et al., 2013; Prahl et al., 1993).

MC simulation is widely used for modeling light propagation in turbid biological tissues, owing to its flexibility and simplicity in simulating the energy transfer process in arbitrary geometries with complex boundary conditions or spatial localization of inhomogeneities. MC methods provide a probabilistic approach to simulate the random walk of photons in absorbing and scattering media (Watté et al., 2016). Photons are traced through a turbid medium until they exit at the sample surface or until they are absorbed. Photon movement from one photon-tissue interaction to the next is described by probability functions using the tissue's optical properties. Repeating this process for a large number of photons results in an estimation of photon distributions in the tissue (Wang and Jacques, 2000). Classic MC algorithms, such as the MC code for multi-layered media, allow to simulate light propagation in multi-layered, semi-infinite slabs (Wang et al., 1995). Moreover, the MC method can also be used in a structured finite mesh approach, in which photons move in a tetrahedral mesh (Watté et al., 2015a). This approach allows to describe more complex biological structures, including targets with curved boundaries or locally refined structures, like tissue cells and intercellular spaces. Finally, meshless approaches can be studied using particle-based simulations, in which photons and scattering particles are defined as particles, while the interaction of light with spherical scattering particles can be determined using the exact Mie solution. However, because of the large number of photons (typically 10^4 to 10^6) that need to be simulated to reduce the effects of stochastic noise, MC simulations are generally computationally intensive (Tuchin, 2007).

To overcome some shortcomings of the previously described MC techniques (e.g., long computational time and oversimplifications in describing the photon-particle interactions) and to better deal with noise, which is inherently present when modelling data retrieved from optical measurements, stochastic data-based surrogate models, often referred to as 'metamodels', have been introduced as a computationally cost-effective alternative for solving the RTT for light propagation (Aernouts et al., 2015; Watté et al., 2013). These metamodels directly establish the link between the design space (input parameters) and the performance space (output parameters) (Simpson et al., 2001). These models would be trained by using the data obtained from MC simulations or optical measurements on samples with known BOP. Building a metamodel based on MC simulations would provide high flexibility. However, the estimation procedure can be simplified by linking optical measurements on known samples directly with the BOP. This has the advantage that in future predictions, using the same optical measurement technique, both light propagation characteristics and measurement geometry used are accounted for (Watté et al., 2013). To achieve this goal, so-called optical phantoms, designed with known optical properties, are measured with the same optical setup as that used for the actual measurements on the desired products. By doing this, a possible mismatch between simulation and measurement setup can be avoided. To use these phantom measurements for building a metamodel, a set of phantoms describing a wide range of absorption and scattering properties should be designed so that they are representative for the BOP of the measured products. Three different key components should be considered when designing optical phantoms: (1) the matrix material, (2) the type of absorber and (3) the scattering agent (Pogue and Patterson, 2006). The phantom ingredients should be chosen carefully to avoid interactions between the components, as these might affect the estimation of the BOP.

An example of creating a set of liquid optical phantoms by combining Naphthol Blue Black (NBB) as an absorber (peak absorption at 618 nm) and Intralipid® 20% as a scattering agent, is shown in Figure 2. The rows (from number 1 to 7) have an increasing level of absorption, while the columns (from letter B to H) have an increasing level of scattering. The reference BOP values, measured using a double integrating sphere (DIS) setup (Aernouts et al., 2013), served as an input for building a forward metamodel, linking bulk optical properties to the diffuse reflectance values at different distances from the point of illumination. The diffuse reflectance was measured using a contactless hyperspectral scatter imaging setup.

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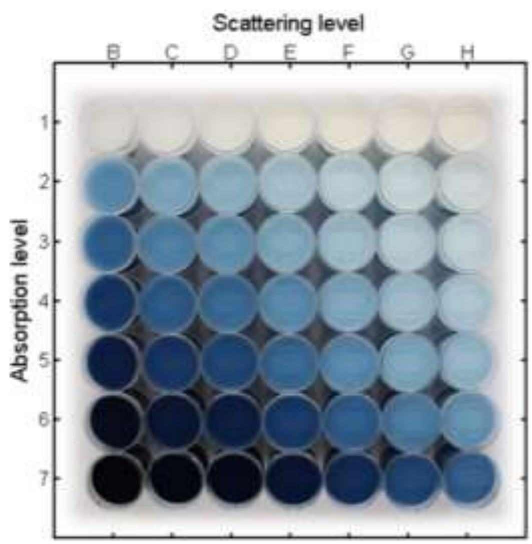


Figure 2. Set of 49 calibration phantoms made by combining Intralipid® 20% (IL) as a scattering agent, Naphthol Blue Black (NBB) as an absorber and water as a dilution agent. The concentration of IL increases from left to right (level B to H), while the concentration of NBB increases from top to bottom (level 1 to 7).

A metamodel combining 30 source-detector distances was evaluated using a set of validation phantoms. The performance of the metamodel for the BOP estimations of 8 validation phantoms at 91 different wavelengths (from 550 nm to 1000 nm in steps of 5 nm) is illustrated in Figure 3. The bulk absorption coefficient was predicted with an R^2 of 0.998 and a root mean squared error for validation (RMSE) of 0.200 cm^{-1} (Figure 3a), which are in agreement with the results reported by Watté et al. (2015b), when a translation-stage SR setup was employed. Predicted values for μ_s', especially above 40 cm^{-1}, are less accurate, with an R^2 of 0.957 and an RMSE of 3.212 cm^{-1}. This could have possibly been caused by the design of the set of calibration phantoms, as values close to the edges of this design showed larger prediction errors.

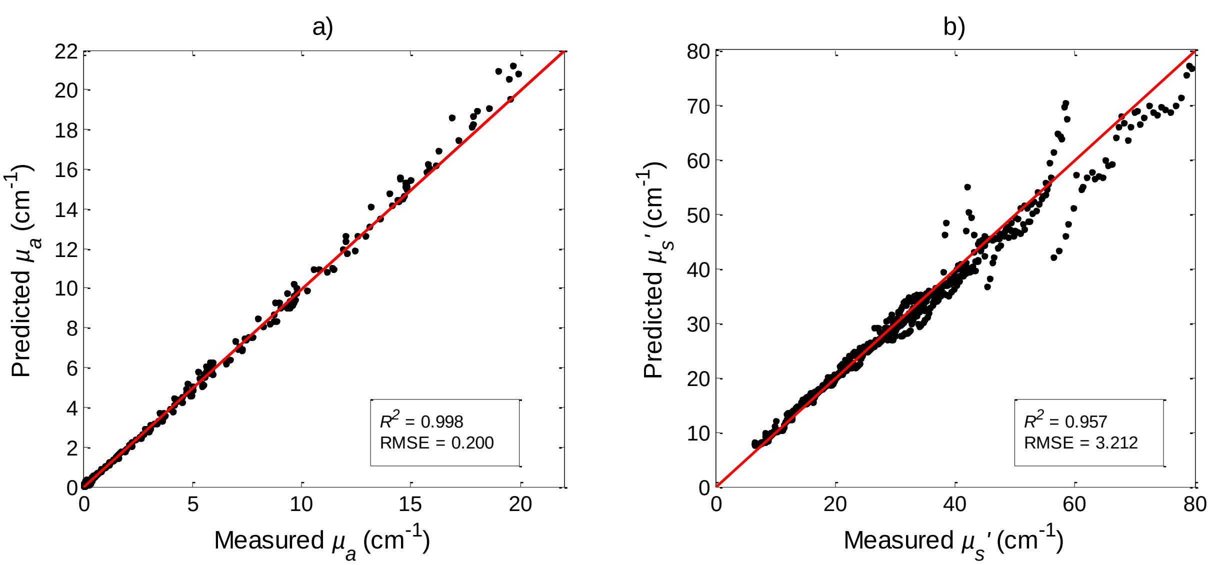


Figure 3. Scatter plots of predicted versus measured (a) bulk absorption coefficient μ_a and (b) reduced scattering coefficient μ_s' for the validation phantoms. The red lines indicate the 1:1 line.

Finally, Aernouts et al. (2014) proposed and validated a flexible tool for simulating the BOP of polydisperse spherical particles in an absorbing host. A microscale model was used as the base for a multiscale model predicting the BOP of polydisperse systems. By using the Mie solution for Maxwell's equations, the optical properties of a spherical particle in an absorbing host were simulated. Polydispersity of the particle systems was then supported by discretization of the provided particle size distributions. The number of intervals was optimized automatically in an iterative procedure. As a result, the BOP of the polydisperse system could be obtained in a flexible way. Two aqueous nanoparticle systems and an oil-in-water emulsion (Intralipid® 20%) were used for validating the developed tool. The simulated BOP values were compared to the reference BOP measured using a DIS and unscattered transmittance setup (described in Section 3.1). This study showed that this type of simulation based on the particle size distribution of the scattering particles matched closely ($R^2 \geq 0.899$) with the BOP values obtained by the reference measurements. Postelmans et al. (2018) implemented this microscale light propagation tool in an inverse estimation algorithm to develop a shape dependent method for the estimation of particle size distributions from bulk scattering coefficient spectra. They successfully validated this method on simulated data for polystyrene in water suspensions and investigated its sensitivity to measurement errors. They found that a correct estimate for the refractive index mismatch between the particle and the medium is most critical.

3. Measurement Techniques

In this section, we first describe the integrating sphere technique (i.e., IS-IAD), which is often used as a reference method for evaluating other new techniques, and then the three nondestructive measurement techniques (i.e., SR, TR, and SFD), based on the light illumination conditions presented in Figure 1. All the techniques have been used for measuring optical properties of fruits and vegetables.

3.1. Integrating sphere

3.1.1 Instrumentation

Integrating spheres have been commonly used as an optical calibration and measurement tool and in particular they have been successfully used to measure optical properties of tissues (Jacquez and Kuppenheim, 1955; Tuchin, 2007). The inner surface of an integrating sphere is uniformly coated with highly reflective diffuse materials (e.g., reflectivity $\rho = 0.98$) to achieve homogenous distributions of light radiation at the sphere's inner wall. A light beam falling on the inner surface of an integrating sphere is evenly scattered to all directions (i.e., Lambertian reflections) and the light fluxes are evenly distributed (spatially integrated) on the homogenous inner surface of the sphere after multiple Lambertian reflections. A standard integrating sphere usually has three ports: input port, exit port, and a third port for detector. Plugs with highly reflective materials are also needed to cover the port that should be closed. For real integrating spheres, the surfaces do not have perfect Lambertian reflection. To prevent measurement errors by specular reflection, baffle(s) coated with a highly reflective material is often placed inside the sphere to further diffuse the specular reflection and avoid the direct reflection from reaching the detector. In certain applications, the fourth port is also used so that the specular reflection beam can go out from the sphere in a light trap.

There are several advantages of using integrating sphere techniques to measure the spectral reflectance and transmittance of fruit and vegetable tissue samples, in comparison to directly measuring the sample by a spectrometer. First, in a regular spectrometer measurement where the incident light directly impinges on the sample surface, the detected reflectance often has a dependency on the angle and distance between the incident beam and the detector. When an integrating sphere is used, the fluxes reflected on the sample are all captured and normalized by the sphere. Thus, the angular dependency is no longer an issue. Second, the detector-object distance is often fixed in the integrating sphere measurement. Even if there is a small change between the sample-sphere distance, it will not affect the results of the measurements as long as all reflected light bounces back into the sphere. Additionally, by using integrating spheres, the spectral measurements are less dependent on the shape of the light beam and the homogeneity of the sample, since both incident light beam and the reflected/scattered light will be normalized on the inner surface of the sphere before being captured by the detector.

Figure 4a shows an example of the instrumentation setup of an integrating sphere system for collecting the spectra of vegetable tissues (Wang and Li, 2014). The system consisted of an integrating sphere, a spectrometer, a light source, optical fibers, a collimator, and a slab of tissue sample sandwiched between glass slides. The integrating sphere (model 4P-GPS-060-SF, Labsphere, Inc., North Sutton, NH, USA) had an internal diameter of 152 mm and four 25.4 mm diameter ports at 0^{o}, 90^{o}, 180^{o}, and the north pole. The sphere was coated with a highly reflective material (Spectraflect®, Labsphere, Inc., North Sutton, NH, USA), whose reflectivity was greater than 98% in the test spectral range. A Vis/NIR spectrometer (model USB4000, Ocean Optics, Dunedin, FL, USA) and a NIR spectrometer (model CD024252, Control Development, Inc., South Bend, IN, USA) were used to measure light signals for the two spectral ranges of 550 – 880 nm and 950 – 1650 nm, respectively. An optical fiber (400 μm diameter and 0.37 numerical aperture) (model M32L02, Thorlabs, Newton, NJ, USA) was used to deliver the light to the spectrometer from the integrating sphere. The light source was provided by a DC-regulated fiber optic illuminator (model DC-950, Dolan-Jenner Industries, Boxborough, MA, USA) with a goose neck light guide. The collimator mounted in front of the light guide was used to collimate the divergent light of the fiber optic illuminator to a 1.5 mm diameter light beam. Each vegetable tissue sample was sandwiched between two pieces of Borofloat glass slide (transmittance > 90%), covering the whole entrance or exit port of the integrating sphere.

The total transmittance T was measured when the sample was placed in front of the entrance port of the sphere and the opposite exit port was covered by a Spectraflect plug (Figure 4b). The total reflectance R was measured when the sample was placed behind the exit port (Figure 4c). To measure collimated transmittance T_c, a 1-mm diameter iris was placed between the collimator and the sample to constrain the position and the size of the incident beam, and the other iris in front of the detector blocked the ambient scattered light from entering the spectrometer (Figure 4d) (Prahl, 2011).

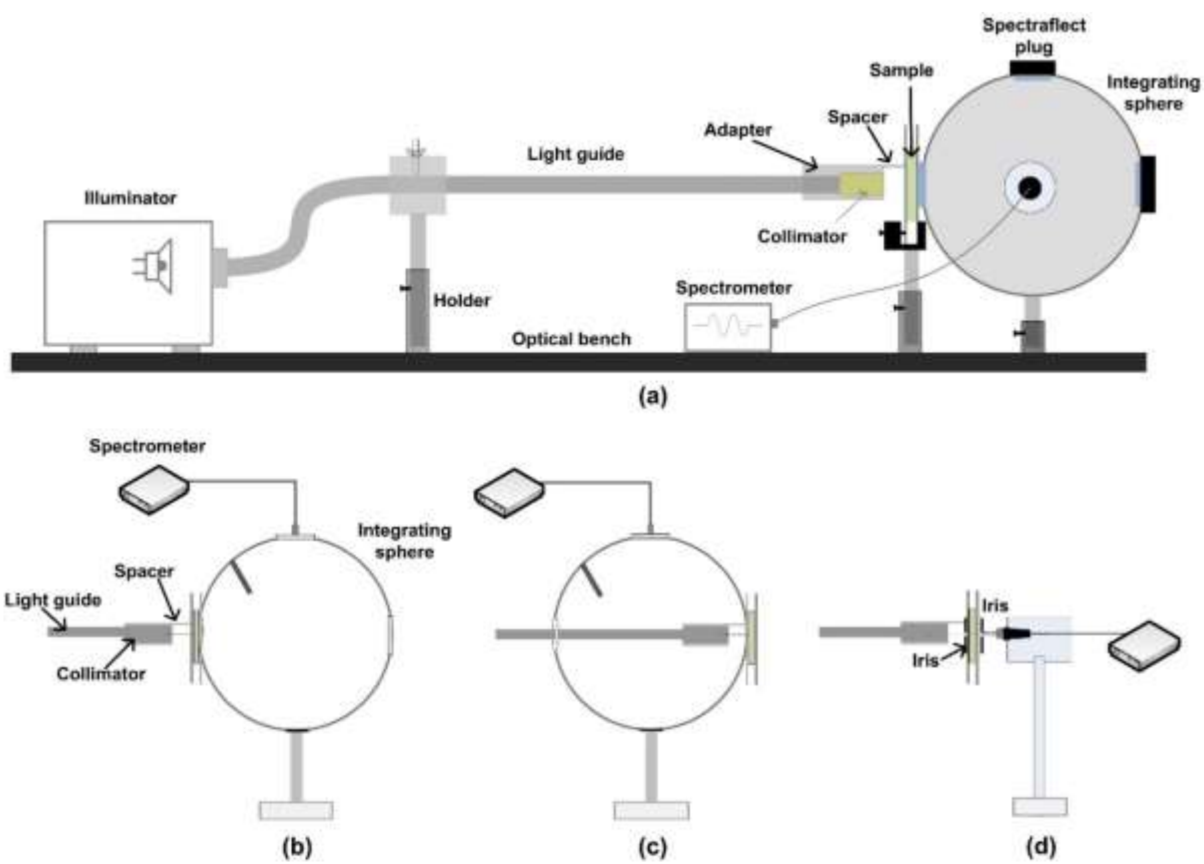


Figure 4. The hardware components of the integrating sphere-based spectroscopic system (a), and the schematics for measuring the three types of spectra: the total transmittance *T* (b), the total reflectance *R* (c), and the collimated transmittance *Tc* (d) (Wang and Li, 2014).

3.1.2 Data analysis

The optical responses (R, T, and T_c) measured using the spectrometer and the integrating sphere are processed by the inverse adding-doubling (IAD) algorithm to obtain the optical properties of fruit and vegetable tissue samples. IAD (Pickering et al. 1993; Prahl et al., 1993) is one of the most common and accurate methods to calculate the light scattering and absorption coefficients of samples based on their reflectance and transmittance (Tuchin, 2007). It is the inverse algorithm of the adding-doubling method, which has been described in Section 2.3. The general procedure of the IAD algorithm is illustrated in Figure 5.

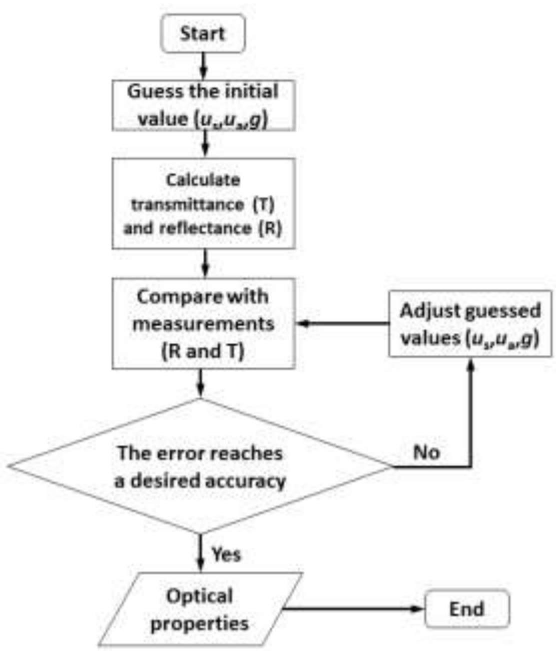


Figure 5. Flowchart of the inverse adding-doubling (IAD) algorithm.

In implementing the IAD method, it is essential to measure the reflectance and transmittance of the sample by integrating spheres. In addition to the method introduced in the previous section using a single integrating sphere to measure reflectance and transmittance, double integrating sphere setups can also be used to obtain both reflectance and transmittance measurements simultaneously (Pickering et al., 1993, Aernouts et al., 2013). The double integrating sphere setup with single beam is simple to construct and use, but it requires two integrating spheres.

In the past decade, the IS-IAD method has been used to measure the optical properties of food items. For instance, Saeys et al. (2008) and Van Beers et al. (2017b) measured the μ_a, μ_s and *g* of the apple skin and flesh in 350-2200 nm using a double integrating sphere configuration in combination with the IAD method for different apple cultivars and at different maturation stages. Lopez-Maestresalas et al. (2015) measured the optical properties of potato flesh in the 500-1900 nm range. Wang and Li (2013) measured the optical properties of the skin and flesh of four common types of onions at 633 nm and reported that the optical properties of onion tissues were significantly different between onion cultivars. The group also used the IS-IAD method to investigate the optical properties of healthy and diseased onion tissues in a broad spectrum (550-880 nm and 950-1650 nm) (Wang et al., 2014). Fang et al. (2016) used a similar method to measure absorption and scattering coefficients of kiwifruit tissues at the wavelength of 633 nm. The IS-IAD method also provides good reference measurements for the development of other techniques (e.g., SR, TR and MC) for measuring optical properties of food items (Cen et al., 2013; Lu, 2008; Qin and Lu, 2009).

3.2 Spatially resolved

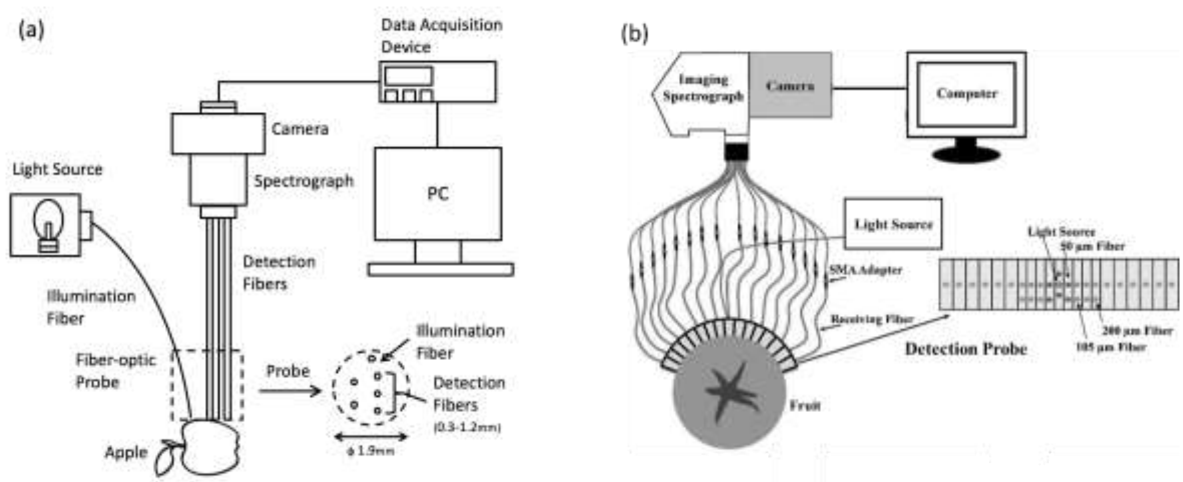
Spatially resolved (SR) technique was first developed by Reynolds et al. (1976) for understanding light propagation in turbid media. Later, Langerholc (1982) and Marquet et al. (1995) reported that SR measurements can be used to determine optical properties of biological tissues. In this method, a small continuous-wave light beam perpendicularly illuminates the sample's surface, and the reemitted light is measured at different distances from the light source (Figure 1a). The absorption coefficient (μ_a) and the reduced scattering coefficient (μ_s') can then be extracted from the measured SR reflectance profiles by using a numerical method or an appropriate analytical solution to the diffusion equation, coupled with an inverse algorithm.

3.2.1 Instrumentation

SR technique is well suited for use in postharvest applications thanks to its low instrumentation cost, easy implementation and nondestructive measurement setup. Hence, many different SR measurement configurations have been developed for horticultural and food products. Optical fiber arrays and non-contact reflectance imagery are two typical sensing configurations in SR measurement (Doornbos et al., 1999; Fabbri et al., 2003; Malsan et al., 2014; Pilz et al., 2008), which can be implemented with fiber optic probe (FOP), monochromatic imaging (MCI), and hyperspectral imaging (HSI). Figure 6 shows four instrumental setups with SR technique for measuring optical properties of fruits and vegetables. In the FOP measurement, a single spectrometer, multiple spectrometers, or a spectrograph-camera combination coupled with multiple detection fibers can be used to measure diffuse reflectance at different distances from the light incident point (Nguyen Do Trong et al., 2014a). Figure 6a shows one of the FOP configurations used for measuring optical properties of fruit and food products, which consists of five optical fibers arranged at different distances. The rigid FOP only covers the maximum spatial distance of 1.5 mm and hence it can only measure tissues of homogeneous properties or the superficial layer of the sample. Since most fruit and vegetable products are of curved or irregular shape, a rigid FOP would have problem maintaining good contact with the sample during measurement. Moreover, it is also desired or needed to measure the sample tissue at greater depth. To overcome the shortcomings of a rigid FOP, a flexible FOP with 30 optical fibers covering a spatial distance range of 0-30 mm was developed for measuring the optical properties of fruits and vegetables (Huang et al., 2017). The optical fibers are coupled to a multichannel hyperspectral imaging system, which allows simultaneous acquisition of 30 SR spectra from the sample. The use of three different sizes of fibers (50, 120 and 200 μm) for the probe also effectively expands the dynamic range of the camera, allowing to acquire spectra from the sample at greater distances.

As a non-contact method, MCI is more suitable for measuring optical properties of fruits and vegetables at one single wavelength. A laser diode or a combination of a supercontinuum laser and monochromator can be used to illuminate the sample at a specific wavelength (Baranyai and Zude, 2009; Van Beers et al., 2015). SR diffuse reflectance is then acquired with a CCD camera (Figure 6c). This SR configuration is simple and relatively easy to implement. The acquired 2-D scattering images are reduced to 1-D scattering profiles by radial averaging (assuming the scattering images are axisymmetric with respect to the laser incident point). However, this assumption does not hold for anisotropic tissues where the light is guided by the tissue fibers. For example, in the case of bovine muscle tissue, the effect of the fibers resulted in scatter spots with a rhombus shape (Van Beers et al., 2017a). Measurement at multiple wavelengths requires sequential wavelength scanning. In addition, a substantial portion of the signal of each pixel comes from the surrounding areas, which can affect measurement accuracy. Characterization of the point-spread function (PSF) could be helpful to obtain accurate intensity values for the image data interpretation (Du and Voss, 2004; Pilz et al., 2008).

In hyperspectral imaging, spectral and spatial information is acquired simultaneously. This is therefore advantageous for measuring SR diffuse reflectance profiles over a broad spectral range (e.g., 400-1000 nm). Figure 6d shows a hyperspectral imaging-based SR system in line scan mode, which mainly consists of a high-performance CCD camera, an imaging spectrograph, a zoom or prime lens, a light source, and an optical fiber coupled with a focusing lens for delivering a small broadband beam to the sample (Cen et al., 2012b). This SR configuration allows fast acquisition of SR spectra from the sample at high spatial resolution, and it typically covers a spatial distance range of about 10 mm for plant products like apples and peaches. In the HSI configuration, two key factors, i.e., light beam and source-detector distance, need be carefully considered in order to meet the requirements of the diffusion approximation theory (Cen and Lu, 2010).



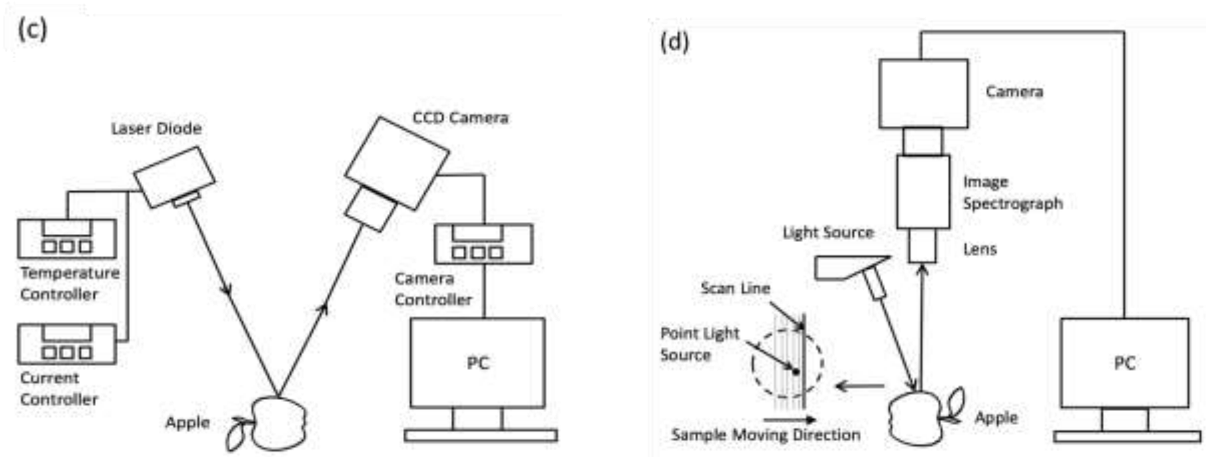


Figure 6. Schematic illustrations of (a) a fiber-optic probe with a rigid plate (Nguyen Do Trong et al., 2014a), (b) a fiber-optic probe with a flexible plate mounted with 30 optical fibers (Huang et al., 2017), (c) monochromatic imaging (Baranyai and Zude, 2009), and (d) hyperspectral imaging-based spatially resolved systems (Cen et al., 2012b).

Various studies have been reported on using different configurations of SR technique for measuring fruit and vegetables. FOP has been used for estimating optical properties of dried apples (Nguyen Do Trong et al., 2014b), fresh apples (Nguyen Do Trong et al., 2014a), and tomatoes (Huang et al., 2018). MCI technique was used for optical characterization of apples, kiwifruit, pears and oranges (Adebayo et al., 2017; Baranyai and Zude, 2009; Lorente et al., 2013; Mollazade and Arefi, 2017), while the HSI-based SR technique has been used for measuring apples, peaches, pickling cucumbers, tomatoes, and sugar beets as well as for evaluating their quality attributes (Cen et al., 2012a, 2012b; Qin et al., 2009; Van Beers et al., 2015; Zhu et al., 2015).

3.2.2 Data analysis

As an indirect method for optical property measurement, computation of the optical parameters from the SR measurements usually requires sophisticated modeling based on the radiative transfer theory, diffusion approximation, or MC simulation, coupled with appropriate inverse algorithms. Numerical methods are generally required for solving the radiative transfer equation or using inverse MC simulation. These methods need no or fewer physical approximations on photon transport in the media, but they could be subjected to statistical uncertainties during the estimation of the reflectance. One major drawback with the numerical methods is that they require substantial computational time. Therefore, it has been proposed to build a library of MC simulated SR profiles for a grid of μ_s, μ_a and g values. This library can then be used either as a look-up table (Hjalmarsson and Thennadil, 2007; Sharma et al., 2014) or for training a neural network (Hjalmarsson and Thennadil, 2008). As discussed earlier, databased models, such as metamodels, can also be used to model the relation between diffuse reflectance and BOP (Watté et al., 2015). This has been demonstrated both for FOP-based SR (Watté et al., 2015) and HSI-based SR (Aernouts et al., 2015) systems. A popular and faster approach is to use the analytical equation derived by Farrell et al. (1992) or Kienle and Patterson (1997), coupled with an appropriate inverse algorithm, to obtain the estimates of μ_a and μ_s' from the acquired SR diffuse reflectance profiles (Cen et al., 2012a, 2012b; Erkinbaev et al., 2014).

Accurate estimation of the optical parameters by inverse algorithms is not an easy task due to the complexity of the analytical solutions and potential experimental errors in measuring diffuse reflectance from the medium. In general, optical parameter estimation can be defined as a nonlinear least squares optimization problem with several important assumptions (i.e., constant variance errors, uncorrelated errors, and the Gaussian distribution of errors). The results will not be valid if these assumptions are violated. Cen et al. (2010) recommended using data transformation and weighting methods as a pre-processing approach before implementing the inverse algorithm. Since the optical parameters are sensitive to the SR reflectance profile, high noise level and improper selection of the profile region could result in large estimation errors. In addition, for estimating the optical parameters of layered media, the increased number of free parameters can dramatically increase the computational time, further exacerbating the estimation of optical parameters, and/or causing ill-posed problems. Different strategies, such as a multi-step method, sensitivity analysis and statistical evaluation, have been proposed to optimize the inverse algorithms and improve the estimation accuracies (Cen et al., 2010; Hu et al., 2019; Wang et al., 2017a; 2017b).

In contrast to the above inverse approach for estimating the optical absorption and scattering coefficients of fruits and vegetables, researchers have also proposed several direct approaches to characterize the obtained SR profiles for quality assessment of fruit and vegetables. With these direct methods, the acquired SR profiles (usually after corrections for the dark and instrument responses) are fitted with some empirical mathematical equations (e.g., Gaussian and Lorentzian functions, etc.) (Peng and Lu, 2006, 2007) or by extracting image features (including mean reflectance, image histogram, scattering size or area, etc.) from the 2-D scattering images (in the cases of monochromatic or multispectral scattering images) (Qing et al., 2008; Lu, 2004; Romano et al., 2011; Tu et al., 2000). While these direct methods are faster and simpler in extracting the features from the SR profiles and also have yielded good results in predicting quality of fruits and vegetables, they are highly dependent on type of instrumentation and light source/detector setup.

3.3 Time-resolved

In TR technique, ultrashort laser pulses are injected into a turbid sample, and temporal responses of the reemitted light at a certain distance away from the laser incident point are recorded (Figure 1b). The temporal responses are a function of time. After acquisition of the time-resolved reemitted light intensity signals, the absorption and reduced scattering coefficients are then estimated by fitting the acquired TR data using an inverse algorithm for an analytical solution for the diffusion equation.

3.3.1 Instrumentation

Figure 7 shows the schematic of a TR system for measuring optical properties of turbid biological samples. The two key components for the TR system are a laser (or lasers for a multiple wavelength system, or a tunable laser for a broad spectral region), which can generate ultrashort pulses at the repetition frequency up to 100 MHz, and a time-correlated single photon counting (TCSPC) device, which counts the number of photons arriving at the detector for different time intervals. Hence, the detection system essentially records the histogram of the detection times for the reemitted photons from the sample. To accurately measure the optical properties of tissues, the TCSPC device needs to have the capability of providing sufficient time resolution down to picoseconds (10^{-12} s) or even femtoseconds (10^{-15} s). A high temporal resolution and high sensitivity are critical to the performance of a TR system. Temporal resolution is related to both the width of laser pulses and the timing accuracy of the detection electronics. To achieve high sensitivity, it is desirable to have a high-power laser (or lasers), but a laser with excessive power can cause damage to biological tissues and pose safety concerns. Hence, there is a delicate balance between sensitivity and safety in choosing an appropriate level of laser power for a TR system. Over the years, many different TR techniques have been developed for biomedical applications (Tuchin, 2007, Wang and Wu, 2007). Three TR instrumentation configurations were developed by a group of researchers in Italy for measuring the optical properties of biological tissues and horticultural products over a broad spectral range, at a single wavelength, and at select discrete wavelengths (Rizzolo and Vanoli, 2016). TR imaging systems have also been reported for 3-D imaging of biological tissues (Hebden et al., 2004; Pifferi et al., 2003).

Compared to SR and SFD methods, the TR method is considered to be more accurate in measuring optical properties and able to interrogate deeper tissues, which is important in assessing quality and internal defects of horticultural products with a relatively thick surface layer (i.e., skin or rind). However, TR techniques are expensive and complex, even for a portable TR instrument. Moreover, it is important to have good contact of the detection probe with the sample during the measurement, which may not be easy in working with intact fruit and vegetable products of curved or irregular shape.

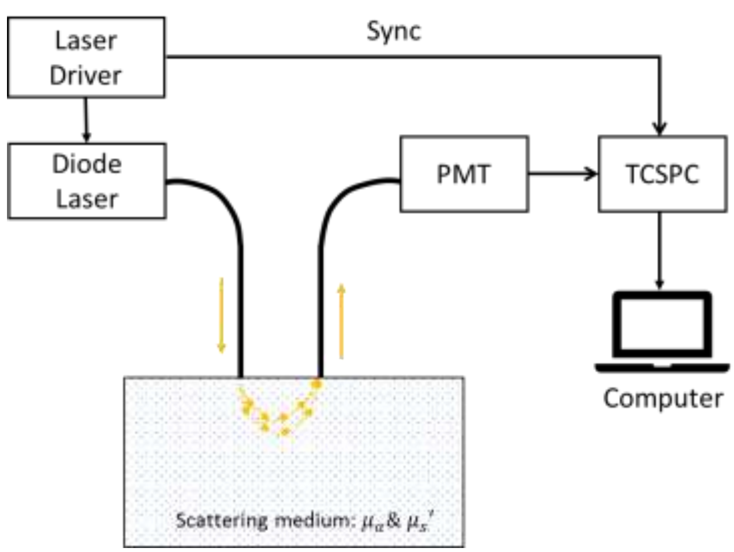


Figure 7. Schematic of a single-wavelength time-resolved system for measuring optical properties of biological tissues, where PMT is a photomultiplier tube for detecting single photons

and TCSPC represents a time-correlated single photon counting device synchronized with the laser driver.

3.3.2 *Data analysis*

In principle, once the temporal response curves have been obtained for samples, values of μ_a and μ_s' can be obtained by fitting the data using a nonlinear inverse algorithm for an analytical solution for the diffusion approximation equation (Patterson et al., 1989). However, like the case discussed earlier for SR technique, due to the complex instrument response to optical signals, a direct curve-fitting approach could result in large errors in the estimation of optical parameters.Torricelli (2009) proposed to convolute the theoretical TR reflectance with the instrument response function is first calculated, which is then used to fit the experimental TR reflectance curve. According to Torricelli (2009), this approach yielded better results compared to the approach of directly using the acquired TR data.

3.4 *Spatial-frequency domain*

Spatial-frequency domain (SFD) technique was first reported by Cuccia et al. (2005) as a means for estimating the optical absorption and scattering properties of turbid media. The technique is different from SR and TR in that it allows wide-field mapping of optical properties in turbid biological materials. Thus, it has the capability of 3-D imaging of biological tissues. Instead of using a point light source for SR and TR techniques, SFD requires using special patterns of 2-D illumination (usually sinusoidal patterns). To estimate the optical properties, reflectance images are acquired from the sample with different spatial frequencies of illumination, and the acquired images are then demodulated to obtain direct component (DC) and alternating component (AC) images. The amplitudes of reflectance from the demodulated images are then used for the inverse curve fitting by an analytical diffusion equation (Cuccia et al., 2009) to obtain the estimated values of μ_a and μ_s'.

3.4.1 Instrumentation

Figure 8 shows the schematic of an SFD system for measuring optical properties of fruits and food products. The system mainly consists of a high-performance CCD camera, a liquid crystal tunable filter (LCTF), which allows selecting specific wavelengths for imaging, a polarizer that blocks specular reflectance from the sample, and a digital light projector (DLP), which is controlled by computer and connected to a DC light source via a fiber optic cable. The DLP can generate different patterns of illumination through computer programming. For optical property measurements, sinusoidal patterns of illumination are used to illuminate samples. For each spatial frequency, three patterned images, corresponding to three phase-shifted sinusoidal illumination patterns (120 degrees apart), are usually needed. To estimate the absorption and reduced scattering coefficients, images should be acquired for a range of spatial frequencies. To ensure accurate measurement of optical properties of turbid food samples, several calibration procedures have to be carried out for an SFD system (Bodenschatz et al., 2014). First, it is important that the camera system has good linearity responses. Second, under the ideal situation, the illumination patterns should be sinusoidal. However, this may not always be realized due to optical system imperfections and a specific setup of the light source. Hence, careful calibrations should be done on standard reflectance panels (e.g., the reflectance panels by Labsphere, Inc., North Sutton, NH, USA). For the SFD setup configuration shown in Figure 8, the light illumination is not incident onto the sample from the vertical or normal direction, which would create nonuniform illumination on the sample and should thus be corrected. In addition, the light illumination along the second axis should be constant in intensity, but in real situations, this may not always be the case. Hence, careful calibration of the system is required to eliminate or minimize these effects.

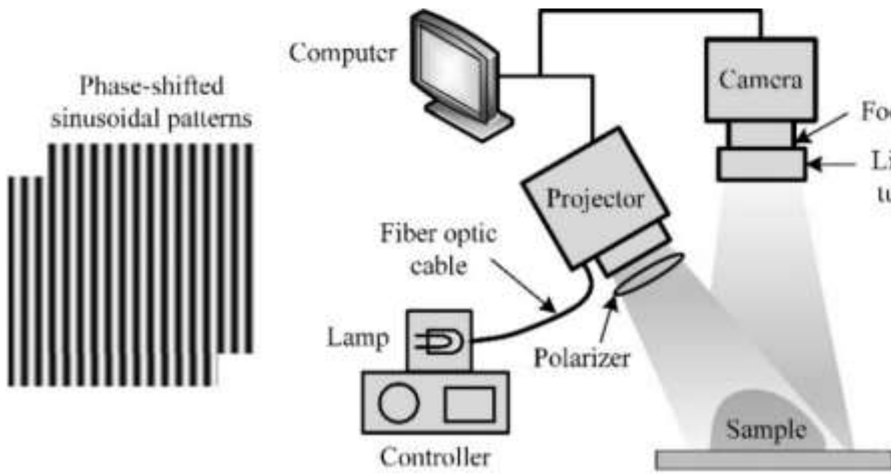


Figure 8. Schematic of a spatial-frequency domain imaging system for measuring optical properties of horticultural products (Lu et al., 2016a).

3.4.2 Data analysis

Figure 9 shows a typical procedure for implementing the SFD technique for estimating optical absorption and reduced scattering coefficients. First, two or three phase-shifted images are acquired for each spatial frequency at each wavelength. The number of spatial frequencies used varied in different studies; some used more than 12, while others only used a few (e.g., 4 to 6). The next step is to perform demodulation of the acquired pattern images, from which DC and AC images are obtained. The conventional demodulation method requires three images, while using new methods, such as spiral phase transform and Gram-Schmidt orthonormalization, two patterned images would be enough (Lu et al., 2016b, 2016c). After the demodulation, the reflectance values (the amplitude) are extracted. The extracted reflectance profile versus spatial frequency is then fitted by the diffusion model with an appropriate inverse algorithm, from which the absorption and reduced scattering coefficients are estimated for each wavelength. In performing the inverse algorithm for curve fitting, one should be aware that the analytical solution for the SFD method is derived based on two basic assumptions: 1) scattering is dominant (i.e., $\mu_s >> \mu_a$), and 2) the spatial frequency is much smaller than the transport coefficient (i.e., $\mu_{tr} = \mu_a + \mu_s'$). Studies have shown that SFD technique is prone to errors due to the complexity of the diffusion model and low signal-to-noise ratio for measured data at large spatial frequencies. It is therefore important that proper inverse algorithm implementation procedures are utilized, which include data smoothing, selection of proper spatial frequency range and start and end frequencies. An SFD system consists of many optical components (lens, tunable filter, detector, etc.), each of which has different optical response characteristics. The optical response of the optical assembly will have important ramifications on the acquired reflectance images. Hence, proper calibrations of the optical system are needed to reduce or minimize errors in estimating the optical properties. Hu et al. (2019) suggested that the most effective method for image correction is to use reference samples covering a range of known properties to calibrate the acquired or demodulated reflectance images. Two-step and stepwise optimization methods, including the reference-sample based correction procedure, were proposed to improve the optical parameter estimation by SFD technique (Hu et al., 2018, 2019).

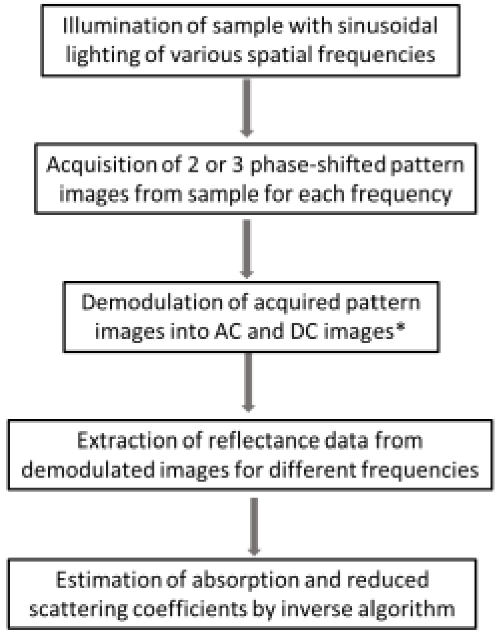


Figure 9. The procedure of implementing spatial-frequency domain technique for measuring optical properties of fruit and vegetable samples (* AC and DC denote amplitude and direct components, respectively).

So far, only limited studies have been reported on using SFD technique for optical characterization of fruits and vegetables, including apples, mangoes, and pears (Anderson et al., 2007; He al et., 2017, 2018; Hu et al., 2016).

Similar to SR technique, a direct approach for SFD technique, called structured-illumination reflectance imaging (SIRI), has been proposed as a new imaging modality for quality evaluation of horticultural products (Lu, 2018; Lu and Lu, 2017b; Lu et al., 2016a). The SIRI system shares the same optical configuration as that for the SFD system as shown in Figure 8, and the same demodulation procedure is used to obtain DC and AC images. Instead of using the inverse algorithm to estimate the optical absorption and reduced scattering coefficients, SIRI directly applies image processing procedures on the DC and AC images to extract image features for evaluating quality of fruits and vegetables. Studies (Li, et al., 2018; Lu et al., 2017b) showed that AC images, which are unique to the SIRI technique, provide higher spatial resolution and image contrast and can reveal subsurface tissue features at specific depths, compared to DC images, which are equivalent to the ones acquired under uniform, diffuse illumination. SIRI was found to be especially useful for detecting subsurface defects (e.g., bruising in apples and tomatoes) and early developments of fungal infection in peaches and other fruits (Sun et al., 2019), which are difficult to ascertain by other conventional imaging techniques.

4. Applications

Considerable research has been reported in recent years on using IS-IAD, SR, TR and SFD techniques to measure optical absorption and scattering properties of fruits and vegetables as well as on using these optical properties for maturity and quality assessment and for disease and defect detection. As discussed in Section 3, direct approaches for analyzing SR and SFD signals also have been proposed for evaluating quality of horticultural and food products. However, our discussion in this section is mainly focused on the application of optical absorption and scattering properties for quality assessment of fruits and vegetables.

4.1 Absorption and scattering spectra for fruits and vegetables

Figure 10 shows typical mean absorption coefficient (μ_a) spectra for the apple skin (Figure 10a) and cortex tissues (Figure 10b) of three apple cultivars (‘Braeburn’, ‘Greenstar’, and ‘Kanzi’) in the spectral region of 500-1850 nm, which were measured using an IS-IAD technique (Van Beers et al., 2017b). The insert in Figure 10b shows a close-up of the absorption coefficient for the apple cortex in the 500-1000 nm region. Several absorption peaks are observed for the apple skin over the visible region of 550-680 nm, which are attributed to carotenoids (around 500 nm), anthocyanins (550 nm – 600 nm) and chlorophyll (mainly chlorophyll a at 678 nm). Absorption peaks around 678 nm are also noticed for the apple cortex; however, their values are at least 10 times smaller than those for the apple skin, which indicates the presence of chlorophyll and carotenoids, albeit at much lower concentration levels, in the apple cortex. In addition, the OH bonds in water also have an important effect on the absorption coefficient for both apple skin and cortex in the NIR region around 970 nm, 1200 nm and 1450 nm (Hale and Querry, 1973; Lancaster et al., 1994; Merzlyak et al., 2003). Differences between the cultivars can also be observed. For example, absorption at 550 nm is much lower for 'Greenstar', a green cultivar with little anthocyanins in the skin.

Absorption spectra reported for other fruit and vegetables (apple, blueberry, kiwifruit, mango, onion, peach, pear, pickling cucumber, plum, tomato, zucchini squash) are also largely dominated by water in the NIR range and chromophores like chlorophyll, carotenoids and anthocyanins in the visible range (Cen et al, 2012; Cen et al., 2013; Cubbedu et al., 2001; Huang et al., 2018b; Lu et al., 2011; Nguyen Do Trong et al., 2014; Nicolai et al., 2008; Qin and Lu, 2008; Saeys et al., 2008; Spinelli et al., 2012; Vanoli et al., 2011; Wang et al., 2014; Zhang et al., 2017a). For example, in immature tomatoes (i.e., in the green and breaker stages) chlorophyll dominates the visible spectral region, while for ripe, red tomatoes, chlorophyll disappears, and carotenoids (mainly lycopene) increase significantly (Clément et al., 2008; Huang et al., 2018).

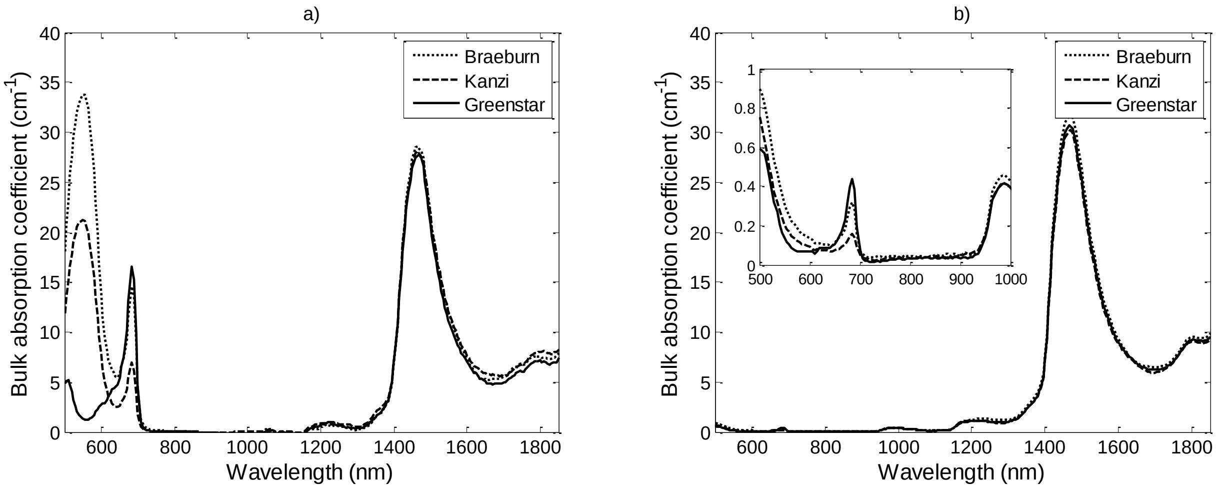


Figure 10. Mean absorption coefficients of the apple skin (a) and cortex (b) tissues of three apple cultivars (Van Beers et al., 2017b).

The mean reduced scattering coefficient (μ_s') spectra for the apple skin and cortex tissues of the same three cultivars are illustrated in Figure 11(Van Beers et al., 2017b). The reduced scattering coefficient decreases exponentially with the increasing wavelength, which is typical for biological tissues (Bashkatov et al., 2005). Again, differences are noticed between the different cultivars and between the tissue types. These differences in the scattering behavior typically relate to differences in the microstructure, like differences in the cell structure and porosity in both tissue types. Other researchers also reported μ_s' values for different fruits, ranging between 0 cm^{-1} and 20 cm^{-1} (Cen et al., 2012, 2013; Huang et al., 2018; Nguyen Do Trong et al., 2014; Qin and Lu, 2008; Rowe et al., 2014; Saeys et al., 2008; Seifert et al., 2015).

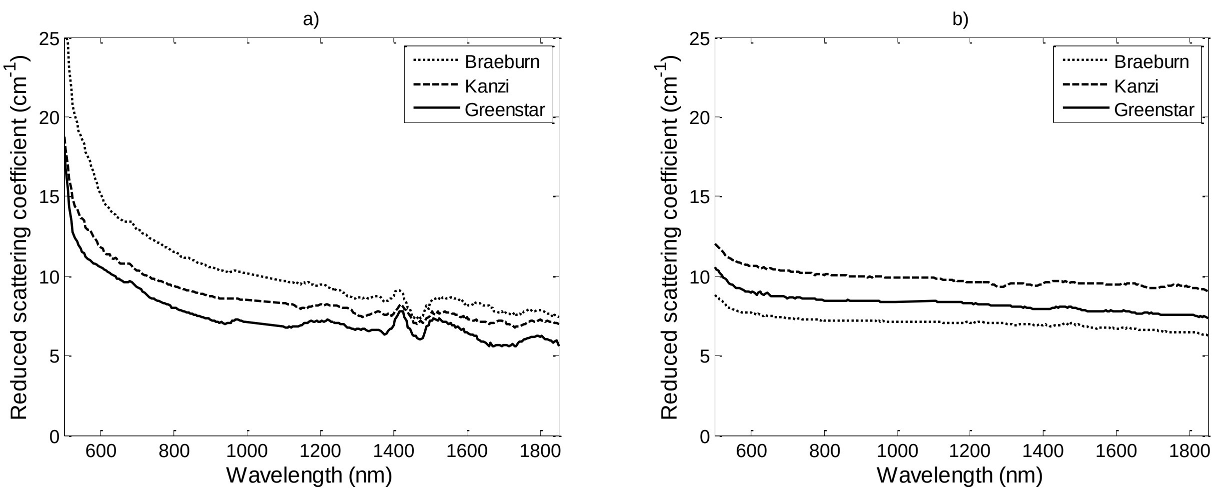


Figure 11. Mean reduced scattering coefficients of the (a) skin and (b) cortex tissue of three apple cultivars (Van Beers et al., 2017b).

In studying the effect of pre-harvest maturation on the optical properties, Van Beers *et al.* (2017b) found significant changes in the optical behavior of the apple tissues. The absorption by anthocyanins was found to increase, while the scattering coefficients (μ_s) of the apple cortex decreased. It was hypothesized that this evolution in scattering could be related to cell elongation during maturation, reducing the volume fraction of cell walls and air pores encountered by the light (Van Beers et al., 2017b).

In studying the effect of bruising on the absorption and scattering properties of apples over time, Lu et al. (2010) reported that while no consistent pattern of changes in the absorption spectra over time was observed, there was, however, a steady decreasing trend in the values of μ_s' over time. A similar trend of changes in the absorption and reduced scattering coefficients for the spectral region of 950-1400 nm was also reported for healthy and bruised blueberries when IS-IAD was employed (Zhang et al., 2017a). These studies suggested that bruise detection could be enhanced by utilizing the scattering properties of fruit. Wang et al. (2014) measured the absorption and reduced scattering coefficients for healthy and diseased onion tissues in the spectral region of 550-1650 nm. They reported that values of the reduced scattering coefficient for dry skin were 10 times higher than that of onion flesh. Likewise, large differences in the absorption coefficient values over the spectral region of 550-1300 nm were observed between dry skin and flesh onion tissues.

4.2 Maturity and quality assessment

Maturity determines when fruits or vegetables should be harvested and how they should be stored or marketed after harvesting. Maturity directly influences postharvest quality and shelf life of horticultural products. Hence, nondestructive measurement of product maturity before and at harvest is critical for the fruit and vegetable industries. Generally, maturity assessment requires measuring multiple quality attributes. Different fruits and vegetables may have different requirements or standards for assessing maturity. For instance, in assessing the maturity of apples, multiple quality parameters, including fruit surface color, soluble solids content (SSC), starch pattern index, flesh firmness and even titratable acidity, are often used. On the other hand, tomato maturity is mainly judged based on the surface or flesh color distributions, upon which tomatoes are classified into six maturity or ripeness grades. The maturation of fruits or vegetables is accompanied with changes in the chemical composition and structural characteristics. Consequently, the absorption and scattering properties also change during the maturation process, although the trend and magnitude of changes for the two optical properties are different. Hence, optical absorption and reduced scattering coefficients are useful for assessing multiple maturity parameters of fruits and vegetables. Table 1 lists some recent studies on assessing maturity and quality of fruits and vegetables, including apple, banana, kiwifruit, mango, nectarine, peach, pear, plum and tomato, by using IS-IAD, SR, TR and SFD techniques.

Not surprising, most studies were focused on using the two optical parameters measured by SR and TR techniques to predict firmness and SSC, the two most important quality attributes for a variety of fruits. These studies have demonstrated that both absorption and reduced scattering coefficients were related to firmness, SSC, flesh color, and starch index. However, the absorption coefficient overall was better for predicting these quality parameters, which could be attributed to the fact that pigments and other chemical composition change during maturation, accompanied with changes in the cellular structures. In most cases, it was also found that combination of the two optical parameters tended to improve predictions of the fruit maturity/quality parameters (Barzaghi et al., 2009; Cen et al., 2012; Huang et al., 2018a, 2018b; Qin and Lu, 2009; Vanoli et al., 2011). However, Nguyen Do Trong et al. (2014a) reported the best results for SSC prediction based on the absorption coefficient spectra. While few studies have been reported on directly comparing the performance of SR and TR techniques with conventional Vis/NIR spectroscopy, the overall results appeared to be comparable between the two approaches (Barzaghi et al., 2009; Huang et al., 2018a, 2018b). The current optical measurement techniques are still prone to errors in data acquisition as well as during the inverse algorithm extraction of optical parameters, thus preventing them from achieving full potential in assessing maturity and quality of fruits and vegetables.

The softening of apples during postharvest storage is often accompanied with the structural changes in the fruit tissues and their optical properties. In studying the relationship between the optical properties of apples and the cell structures and firmness, Cen et al. (2013) reported that during the accelerated softening of apples at room temperature, the reduced scattering coefficient for the measured apples of ‘Golden Delicious’ and ‘Granny Smith’ varieties for the spectral region of 500-800 nm showed a consistent pattern of decrease over a period of 30 days, while the change of the absorption coefficient was more complex. They further reported that the absorption and reduced scattering coefficients at 675 nm, which corresponds to chlorophyll absorption, were strongly correlated with tissue firmness as well as the area or diameter of tissue cells. This is because the decrease of firmness during the accelerated softening is also accompanied with a decrease in the chlorophyll content. Eccher Zerbini et al. (2006, 2011, 2015) developed mathematical models relating the softening of apples, mangoes, and nectarines with the absorption coefficient at 540 nm and 670 nm.

In using a SR sensing probe to measure the optical properties of dried apple slices, Nguyen Do Trong et al. (2014b) reported that the scattering coefficient was related to the processing condition, microstructure and textural quality. Rizzolo et al. (2011; 2014b) also reported that the absorption coefficient of ‘Cripps Pink’ and ‘Golden Delicious’ apples at 670 nm measured at harvest could be used to classify raw and dried apple rings for different quality grades.

Table 1. Assessment of maturity and postharvest quality of fruits and vegetables by using optical measurement techniques

Product	**Maturity/Quality Parameter***	**Measuring Technique****	**Reference**
Apple (Fresh)	Firmness, SSC, anthocyanins, chlorophyll, carotenoids, starch index, softening, sensory profile	IS, SR, TR	Barzaghi et al. (2009); Cen et al. (2013); Nguyen Do Trong et al. (2014a); Qin et al. (2009); Rizzolo et al. (2010b, 2014a); Sun et al. (2017); Van Beers et al. (2017b); Vanoli et al. (2011, 2015)
Apple (dried or processed)	Hardness, firmness, elasticity, crispness, snapping work, browning index, flesh color, porosity	SR, TR	Nguyen Do Trong et al. (2014b); Rizzolo et al. (2011, 2014b)
Banana	Firmness, SSC, ripeness	SR	Adebayo et al. (2016)
Kiwifruit	Firmness, SSC, acidity	TR	Valero et al. (2004)
Mango	Firmness, pulp color, softening	TR	Eccher Zerbini et al. (2015); Pereira et al. (2010); Spinelli et al., (2012)
Nectarine	Firmness, softening	TR	Eccher Zerbini et al. (2006, 2011); Rizzolo et al. (2010a); Tijskens et al. (2007a, 2007b)
Peach	Firmness, SSC	SR, TR	Cen et al. (2012b); Rizzolo et al. (2013)

Pear	Firmness, SSC, softening	IS, SR, TR	Adebayo et al. (2017); He et al. (2016); Nicolai et al. (2008);
Tomato	Firmness, SSC, surface color, flesh color, ripeness	SR	Huang et al. (2018); Qin and Lu (2008); Zhu et al. (2015)

* SSC =soluble solids content. ** IS=integrating sphere, SR=spatially resolved, SFD=spatial-frequency domain, TR=time-resolved.

4.3 Defect detection

Fresh fruits and vegetables are susceptible to a variety of physiological and pathological disorders and mechanical damage during growth, harvest and postharvest handling and storage. Surface defects are relatively easy to detect, while many internal defects still cannot be effectively detected by using the existing optical techniques. Products with defects, either external or internal, tend to have poor marketability and could be rejected by consumers. Hence, it is critical that defective fresh products, especially those with internal defects, be removed or separated during postharvest packing operations. Over the years, many optical techniques (e.g., Vis/NIR, fluorescence imaging, hyper- and multi-spectral imaging, X-ray imaging, and magnetic resonance imaging) have been used for detecting defects of fruits and vegetables (Lu et al., 2017; Lu and Lu, 2017). For instance, Vis/NIR spectroscopy is now being used in some fruit packinghouses for detecting and segregating fruit with internal defect. However, the commercial Vis/NIR systems still cannot fully meet industry needs because of high false positive/negative rates. Hence, new, more effective inspection technologies are especially needed for internal defect detection.

Physiological disorders often cause changes in the chemical and structural properties of products, which would subsequently induce changes in the optical absorption and scattering properties. For instance, internal browning is a common symptom for apple and many other fruits. Apples with internal browning were found to have higher values for the absorption coefficient at 750 nm and lower values for the reduced scattering coefficient at the same wavelength (Vanoli et al., 2009). Due to its ability of penetrating tissues at greater depth, TS technique has been used to detect internal browning and/or internal bleeding of apples, nectarines, and plums (Lurie et al., 2011; Vangdal et al., 2012; Vanoli et al., 2009, 2012). Mealiness alters the cellulosic structure of apple tissues and hence the optical properties. It was thus feasible to differentiate mealy apples from normal ones based on the absorption and reduced scattering coefficients (Vanoli et al., 2009). Likewise, water-cored apples were found to have higher values for the absorption coefficient at 790 nm and lower values for the reduced scattering coefficient (Vanoli et al., 2009). Studies also showed that diseased onions showed significantly different absorption and scattering characteristics compared to normal onion tissues (Wang et al., 2014). Table 2 lists some recent studies on using the various optical measuring techniques for detecting defects for a variety of fruits and vegetables. While these studies have demonstrated that absorption and reduce scattering coefficients can be used for differentiating normal and defective tissues, there still exist considerable challenges in implementing SR, TR or SFD for real-time, practical inspection of fruits and vegetables for internal defects. First, these techniques are still too slow to be suitable for real-time sorting and grading. Second, both SR and TR techniques are not suitable for detecting defects that are localized or deep inside fruit or vegetable products. TR technique allows greater penetration of light into tissues, but its measurement can only interrogate a small section of the sample tissue. While SFD technique has potential for wide-area, 3D mapping of optical properties for horticultural products, it has limited capabilities of imaging tissues at no more than 1-2 mm deep.

Table 2. Detection of defects of fruits and vegetables using optical absorption and scattering properties.

Product	**Type of Defect**	**Measuring Technique***	**Reference**

Apple	Bruising	IS, SR, SFD	Anderson et al., (2007); Lu et al. (2010); Zhang et al. (2017b)
	Internal browning	SFD, TR	Hu et al. (2016); Lurie et al. (2011); Vanoli et al. (2009, 2012);
	Mealiness	TR	Valero et al. (2005); Vanoli et al. (2009)
	Watercore	TR	Vanoli et al. (2009)
Blueberry	Bruising	IS	Zhang et al. (2017a, 2019)
Cucumber (pickling)	Bruising	SR	Lu et al. (2011)
Nectarine	Internal bleeding, internal browning	TR	Lurie et al. (2011)
Onion	Sour skin, neck rot	IS	Wang et al. (2014)
Orange	Decay	SR	Lorente et al. (2015)
Pear	Brown heart	TR	Eccher Zerbini et al. (2002)
Plum	Internal browning	TR	Vangdal et al. (2012)

* IS=integrating sphere, SR=spatially resolved, SFD=spatial-frequency domain, TR=time-resolved.

5. Issues and Challenges

Over the past 15 years, we have seen significant research efforts in the development and application of new SR, SFD, and TR techniques, along with IS-IAD, for optical characterization of horticultural and food products. While these emerging techniques offer new opportunities for quality and safety assessment of horticultural and food products, there still exist considerable issues and challenges in using these techniques due to increased sophistication and investment in instrumentation and algorithms, compared to conventional spectroscopic techniques. First, many of these techniques are based on the diffusion approximation theory (except for data-based models or MC libraries discussed in Section 2). As discussed earlier, the diffusion approximation theory is only applicable to samples, in which light scattering is dominant over absorption (i.e., $\mu_s >> \mu_a$). This condition is not always met satisfactorily as many biological materials like fruits and vegetables show strong absorption at specific wavelengths or spectral regions due to biological chromophores (anthocyanins, carotenoids, chlorophyll, water, etc.). For these spectral regions, the diffusion theory would introduce large errors or is completely inadequate. Second, accurate estimations of optical absorption and scattering properties are also hindered by our ability of acquiring reliable, high-quality spatially- or time-resolved reflectance signals from horticultural samples, which are sensitive to noise and imperfect sample conditions (e.g., irregular geometry, limited sample dimensions, presence of local defects or abnormalities, etc.). With SR techniques, a small, collimated, and normally-incident light source is critical to proper measurement of spatially-resolved reflectance. However, it is difficult to meet all the requirements in actual application situations. Likewise, with TR techniques, the equipment used to generate short laser pulses and acquire the reflected signals over the time scale of 10^{-12} s to 10^{-9} s are complex and expensive. Accurate measurements of optical properties are further complicated by the fact that many fruits and vegetables are heterogeneous in structure and are of irregular shape or uneven surface. Specifically, the surface layer (i.e., skin or rind) of horticultural products has distinct optical properties and its presence could cause problems for the measurement of sublayer optical properties, which are usually of major interest for quality assessment (Saeys et al., 2008; Van Beers et al., 2017b). Hence, it is desirable or even necessary to consider the heterogeneity properties in measuring fruit and vegetable products. Preliminary efforts have been made on using SR and SFD techniques to measure the optical properties of fruit skin and flesh by treating them as two layers of homogeneous tissues (Cen and Lu, 2009; Hu et al., 2019; Wang et al., 2017a). However, considerably more challenges would be encountered in obtaining acceptable estimations of the optical properties of individual layers, due to the increased number of unknown optical parameters and much more complicated mathematical models for two-layer turbid media. Moreover, in all SR, TR and SFD systems, the acquired reflectance signals are closely related to the instrument response. It is thus important that the reflectance signals be properly corrected or calibrated, before being used for inverse estimation of optical properties. Currently, no standard procedures have been established for calibrating these optical systems. Consequently, the optical measurement results are influenced, to a great extent, by the calibration procedure. Many a time, reference samples of known optical properties are needed during the calibration process. Proper procedures for preparation and selection of reference samples are thus critical to the calibration of an optical system. Furthermore, many SR, TR and SFD measurement configurations require contact of the light source and/or detection probe with the sample to be measured. The measurements are not carried out in real time and/or rapidly. For horticultural and food products, fast and noncontact measurements are often needed or desirable. Hence, much research is still needed in further development and/or improvement of these emerging optical property measurement techniques for better assessment of horticultural and food products. Finally, proper preprocessing procedures for the acquired reflectance data are important for the inverse estimation of optical parameters. Different preprocessing treatment methods and procedures can have significant ramifications on the estimation of optical parameters (Cen and Lu, 2010, 2011; Hu et al., 2018, 2019; Wang et al., 2017a, 2017b).

Overall, the inverse approach to optical property estimation is a complicated process, and it relies on an appropriate mathematical model, precise instrumentation setup and calibrations, and rigorous inverse algorithms. Large estimation errors for the optical parameters could be incurred, if the measuring system is not carefully calibrated, procedures for the inverse algorithm are not properly implemented, or samples are of irregular shape or contain defective or irregular tissue spots within the area of measurement. The presence of these issues and challenges also provides opportunities for further research for these emerging techniques. Further research should also be directed at the development of low-cost, portable or even miniaturized optical measuring systems that can perform optical property measurements in real time, in the field, and under online situations.

In view of the more complicated, time-consuming procedures for inverse estimation of the optical properties, researchers have also proposed simpler and faster direct approaches to analyze the reflectance data acquired from SR and SFD techniques for quality and maturity assessment and defect detection of fruits and vegetables. For instance, simple empirical mathematical models or scattering image features have been used to describe the 1-D scattering profiles or 2-D scattering images acquired by SR technique in FOP (Huang et al., 2018c), MCI (Baranyai and Zude, 2009; Lu, 2004; Mollazade et al., 2013; Peng and Lu, 2005, 2006; Qing et al., 2008), or HSI modes (Huang and Lu, 210; Peng and Lu, 2008), which were found to correlate well with quality attributes such as fruit firmness and SSC. For the SFD technique, SIRI has been used as a new imaging modality for detecting subsurface defects of apple, peach, and other fruits (Li et al., 2018; Lu et al., 2016a; Lu and Lu, 2017; Sun et al., 2019). However, these direct approaches are highly dependent on instrumentation setup and the models developed for different systems are generally not interchangeable and cannot be compared directly for different studies.

6. Conclusions

Optical absorption and scattering properties are directly related to the chemical and structural properties of fruits and vegetables, and thus are useful for evaluating maturity, quality and defects of products. Over the past 15 years, several emerging optical property measuring techniques have been made available for assessing fruits and vegetables and other food products. IS-IAD technique is widely used as a standard method for measuring optical absorption and scattering properties. The technique is, however, destructive and requires careful preparation of samples with specific dimensions for measurement. SR technique is relatively simple in instrumentation, low in cost and faster in measurement. Thus, different SR sensing configurations have been developed for measuring fruits and vegetables. However, accurate estimation of optical absorption and reduced scattering coefficients by SR technique is still challenging, due to measurement errors in SR reflectance and challenges in the inverse algorithm implementation. TR technique, on the other hand, can interrogate tissues at greater depth, which is important for detection of internal quality or defects in horticultural products. However, the technique needs sophisticated and expensive instrumentation, which could limit it for wide practical applications in quality inspection of fruits and vegetables. SFD technique offers the unparalleled capability and potential of measuring and mapping optical properties of fruit and vegetable products, but its imaging depth and resolution is still limited. Considerable research is thus needed in both hardware and software (i.e., mathematical modeling and data processing) for improving accuracy and reliability in measurement of the optical properties by these emerging optical techniques. Moreover, research should also be devoted to the development of low-cost, portable or miniaturized optical property measurement systems and for implementing these emerging techniques for real-time, online applications for quality assessment of horticultural products.

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