Wide-bandwidth photon time of flight spectroscopy for biomedical and pharmaceutical applications.

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Introduction

Diffuse optical spectroscopy (DOS) is an important component of the biophotonics toolbox that readily finds its applications in biomedical optics [1-3]. Furthermore DOS is also used in numerous applications within the pharmaceutical [4, 5], food [6, 7], wood [8] and agrichemical industries. It is applied in new product R&D, fabrication process monitoring and quality control. To further advance applications, development of novel DOS instrumentation capable of delivering highly accurate and complete spectroscopic data at high speeds relevant for such time-critical applications as real-time treatment response monitoring [9] or in-line process control [10] is needed.

The main challenge for DOS spectroscopy of highly-scattering samples is to determine sample light absorption, dependent on chemical composition, in the presence of varying amounts of light scattering, arising from sample morphology. The light extinction measured in conventional DOS experiments with CW light results from the interplay of absorption and scattering which cannot be easily decoupled from each other. The standard experimental procedure to allow isolation of the absorption contribution to extinction includes data pre-treatment algorithms in combination with chemometric calibration of experiments. Although this works well in various specific cases, no universal pre-treatment algorithm exists. Furthermore, this approach will only work within the calibration space spanned by a pre-defined design of experiments. For unexpected variations, there will always be some remaining uncertainty about the validity of such a model.

Recent advances in photonic technologies have enabled the development of new, highly precise and elaborate spectroscopic techniques which are free from limitations inherent to CW DOS. Therefore, it is becoming possible to independently determine absorption and scattering of spectra of virtually any heterogeneous material. This eventually leads to increased accuracy and a reduced cost for determination of chemical composition and physical properties of a sample.

In this contribution, we present state-of-the-art performance characteristics of a novel photon time-of-flight spectrometer for analysis of turbid media. We discuss the performance of the instrument in a number of prospective applications and review newly-developed methods for calibration and verification performance of the instrument.

Material and methods

The spectrometer is based on a broadband, super-continuous source (SCS) providing short (ca. 30 ps) optical pulses at a frequency of 80 MHz. Spectrally-narrow probe pulses are sliced from the broadband super-continuum using electronically tunable acousto-optic tunable filters (AOTFs). Depending on the selected probe wavelength, either of two broadband single photon counting detectors is used (Figure 1). The detectors are operated in time-correlated single photon counting mode that enables precise monitoring of the photon time-of-flight (PTOF) distribution through the turbid sample in the appropriate spectral range. In the wavelength range up to ca. 1000 nm, we employ Si SPC APD (MPD, Italy) while in the longer NIR wavelength, an extended MCP PMT (Hamamatsu, Japan) is utilised. The PTOF data accrued by TCSPC electronics is collected by PC for further analysis. Evaluation (fitting) of the PTOF distribution with either an analytical (diffusion) or Monte Carlo model for photon diffusion enables independent reconstruction of absorption and scattering coefficients of the sample.

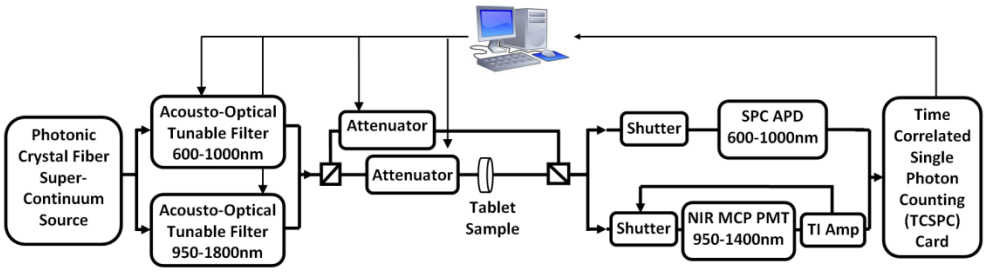

Figure 1. PTOF spectrometer system diagram. PCF SCS is used in combination with either AOTF to generate tunable probe pulses.

The pulses are sent to sample while a small fraction of the power is split off and directly routed to the detector for timing stabilisation. Signal levels are adjusted by attenuators. Either of two SPC detectors is used in combination with TCSPC electronics for precise monitoring of PTOF distribution. The setup is controlled by PC. (TI Amp – tans-impedance amplifier).

In order to suppress the effect of temporal drifts and enhance precision of determination of the optical parameters, we developed a special double path optical scheme that enables temporal stabilisation of the system. In a nutshell, a tiny fraction is stripped from the probe signal prior to the sample or IRF spacer and routed towards the detector over a dedicated optical path. This enables a timing reference (TR) signal be recorded simultaneously with the PTOF distribution or IRF. The timing reference signal is used for precise synchronisation of PTOF distribution and IRF prior to fitting of the experimental data with an appropriate model for turbid light propagation. Diverse scattering samples were used in the present study in order to verify spectroscopic performance of the setup and demonstrate its applicability in a number of prospective applications.

Phantom pharmaceutical tablets with highly stable optical properties were prepared from bi-component epoxy glue doped with titanium dioxide to obtain the desired scattering level and crushed BG36 glass powder to obtain wavelength dependent absorption (Table 1). The pilot test tablet set was prepared from a mixture of ibuprofen (pain-killer drug) and mannitol (sugar) representing an active pharmaceutical ingredient (API) and tablet filler material respectively. Depending on the purpose of the study, API concentration was varied between 15% and 35%; tablets were compressed using 4 ton compression force. In order vary the scattering level of the tablets, filler material of different partial sizes was used.

Table 1 Tablet ingredients

Role	Product name	Particle mean diameter
API	Ibuprofen art no MF1100065, ba R11-002845AZ	
Filler	PEARLITOL® 50C Mean diameter	
Filler	Parteck M200	
Filler	PEARLITOL® 400DC	360 µm

One of main difficulties that complicate development of the instrument and validation of its performance is that, to date, no “gold standard” technique exists for scattering measurements. As a result, it is neither possible to independently verify accuracy of the measurements nor to design a calibrated set of test samples for e.g. checking instrument linearity. The novel approach that is suggested in the framework of the present study is to ensure linearity of the measurements by checking self-constancy of the absorption spectra measured at highly different scattering levels.

Results and discussion

Factors that determine (or limit) the spectroscopic performance of a PTOF spectrometer are manifold [11-13]. In a direct analogy to the conventional (i.e. non-turbid) absorption spectroscopy, the spectral resolution of the instrument is limited by the spectral width of the probe pulses which is in turn determined by the AOTF[13]. Compared to conventional CW transmission spectroscopy, the spectral distortions in PTOFS can be even more severe due to an in-proportionally high contribution from the probe side-lobes falling outside comparably narrow absorption bands. Another performance limiting factor of the spectrometer is related to the temporal stability of the source. PTOFS as a time-resolved technique heavily relies upon an accurate measurement of instrumental response function (IRF). The absorption/scattering spectral data provided by PTOFS is thus highly sensitive to the temporal drifts of the source. An uncertainty (or error) in the temporal shift between the IRF and recorded photon time-of-flight distribution leads to errors in determination of the absorption and scattering coefficients of the media tested. Continuous monitoring of the IRF position shows that typical temporal drifts in the present setup are ca. 3-4 ps/hour, which are most probably associated with the source drifts. The effect of such drifts upon the relative errors in determination of the absorption (µa) and reduced scattering (µs’) coefficients are, in principle, dependent on the magnitude of the optical parameters. In a typical pharmaceutical tablets case, that is

when μa << 1 1/cm and μs' is ~ 400 1/cm, the relative errors in estimation of μa and μs' can easily exceed 2-3 %.

In order to suppress effect of temporal drifts and enhance precision of determination of the optical parameters, we developed a special double path optical scheme that enables temporal stabilisation of the system. The distinct advantage of the present technique is that it enables a drastic reduction in the errors caused by temporal drifts and eventually allow greater precision in the determination of the absorption and scattering up to 0.5%.

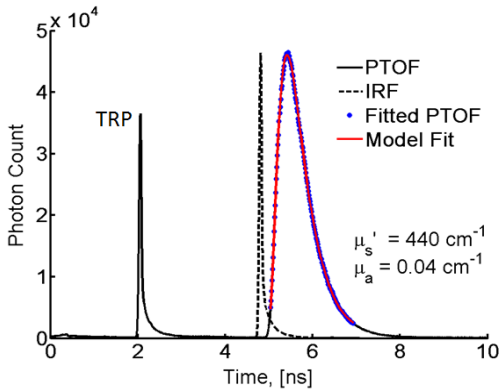

Figure 2. An example of the recoded PTOF distribution and IRF both having timing reference pulse (TRP) inscribed. Model fit is also shown as s red line.

An example of the experimental data and corresponding analysis is shown in Figure 2. There, a photon time-of-light distribution is fitted by the convolution of the instrumental response function with an appropriate model for turbid light propagation. Timing reference pulse is used for synchronisation of IRF and PTOF distributions.

Linearity is another key performance characteristic of the PTOFS system. We tested this property by performing a solution of series experiments as shown in Figure 3. In a nutshell, absorption or scattering of the solution tested was increased in a stepwise manner by adding graduated amounts of scatterers (intralipid) or absorbers (ink). The linear relationship obtained between the volume fraction of scattering or absorbing solution and evaluated sample absorption and scattering coefficients validate the linearity of our setup.

Spectroscopic analysis of pharmaceutical solids and semisolids [14, 15] can be strongly facilitated by employing advanced optical techniques that enable independent evaluation of absorption and scattering within such samples. Absorption can be used for assessment of the chemical composition whereas scattering is characteristic of structural parameters such as ingredient particle size etc.

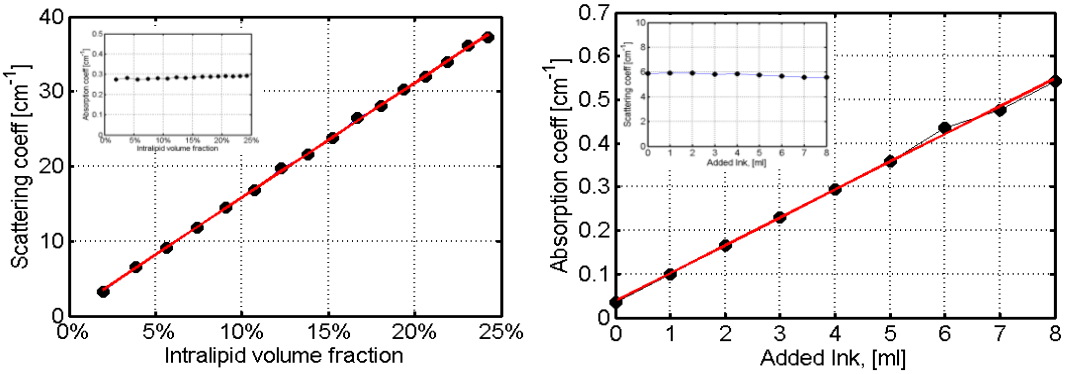

Figure 3. Added scatterer (left) and absorber (right) solution series. Corresponding inserts show stability of the complementary parameter i.e. absorption and scattering on the left and right respectively.

In order to test the suitability of the present setup for pharmaceutical analysis, we evaluated absorption and scattering spectra of a test set comprising 14 tablets mixed in different proportions using ibuprofen as API and one of three different qualities of mannitol as filler. Absorption and scattering spectra of four tablets compressed from pure ingredients used were also measured. A characteristic example of data obtained is presented in Figure 4 in which we plot absorption and scattering spectra of pure ibuprofen, one type of mannitol and two corresponding mixed tablets.

Observed absorption spectra exhibit overtones of CH_2/CH_3 absorption bands; notably, absorption spectra of mixed tablets are a superposition of absorption spectra of the pure ingredients. Moreover, our analysis shows that absorption spectra of all mixed tablets can be highly accurately fitted with a linear combination of absorption spectra of the ingredients. Fitted curves corresponding to respective mixed tablets are also plotted in Figure 4.

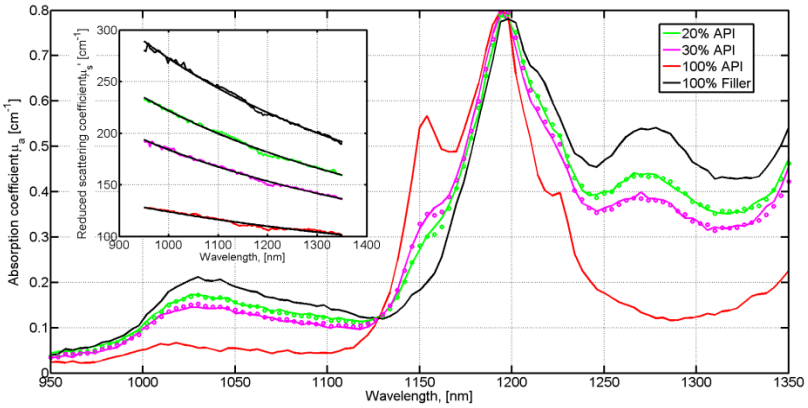

Figure 4. Absorption spectra of ibuprofen (red), mannitol (black) and two characteristic tablets with 20% and 30% ibuprofen content. Mixed tablet absorptions fit a linear combination of the ibuprofen and mannitol spectra, shown as open circles. Inset shows correspondent scattering spectra fitted with Mie type-dependence.

Scattering coefficient (μ_s') spectra for the four selected tablets are depicted in the insert of Figure 4. Each of them can be accurately fitted by $\mu_s' \sim A(\lambda/\lambda_0)^{-\beta}$ dependence ($\lambda_0 = 1nm$), typical for Mie type scattering. As may be expected, tablets compressed from the larger particles (ibuprofen) exhibit lower scattering than tablets composed of smaller particles (mannitol). The fact that mixed tablet spectra can be accurately fitted by spectra of the ingredients is also important for verification of the performance and linearity of the present setup. Indeed, as may be noted form Figure 4, ingredient absorptions are measured at highly different scattering levels; in fact, scattering in the pure mannitol sample is five times larger than in the pure ibuprofen sample. Nonetheless, as shown by the fitting, the absorption measurements are self-consistent. This to our understanding verifies linearity of the present setup.

Conclusions

In this contribution, we present state-of-the-art performance characteristics of a novel photon time-of-flight spectrometer for analysis of turbid media. The broad spectral range of the instrument facilitates advance structural sample characterisation due to the extended scattering data available. Implementation of advanced stabilisation techniques allowed us to attain superior precision in measurements of absorption and scattering coefficients, down to 0.5%. As an illustration of the instrument capabilities, we have demonstrated that the absorption spectra of a mixed tablet can be readily represented by a linear combination of the ingredient absorption spectra measured separately and at highly different scattering levels. We hold that this clearly validates the linearity of our instrument.

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Hard Quantitative Calibration Transfer Across Multiple New Handheld NIR Spectrometers.

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Introduction

Calibration transfer is important for wide scale deployment of NIR spectrometers, particularly for a large scale consumer-focused application. Hard calibration transfer, or direct transfer, takes place when a calibration created on one system, the primary system, is copied to another system(s) without any form of data adjustment or offset correction. This paper explores the development of quantitative calibration for microcrystalline cellulose (MCC), a common pharmaceutical excipient, measured with the MicroNIR™ 1700 (**Figure 1**), a new low-cost handheld NIR spectrometer developed by JDSU. The spectrometer specifications and working principle have been reported in a previous publication [1]. A calibration model was investigated and developed across the 1100-1620 nm wavelength region on one primary MicroNIR spectrometer and subsequently transferred to 6 additional secondary MicroNIR spectrometer systems.

Materials and Methods

Seven MicroNIR 1700 spectrometers were used for this study; one was randomly selected as the 'primary' spectrometer and the other six were deemed 'secondary'. The identification numbers of the seven spectrometers are provided in **Table 1**. Each spectrometer was tested for photometric linearity and photometric noise including both High Flux RMS noise and Low Flux RMS noise in accordance with United States Pharmacopeia (USP) General Chapter <1119> Near Infrared Spectroscopy guidelines. The results are summarised in **Table 2**.

Figure 1 MicroNIR 1700 Spectrometer

Table 1. Instrument DetailsFifteen samples of a pharmaceutical

Instrument Serial Numbers	Integration Time (µs)	Scans Averaged	Total Scan Time (S)
S1-2012-00115	10,000	50	0.5
S1-2012-00116	10,500	50	0.55
S1-2012-00117	10,500	50	0.55
S1-2012-00118	11,000	50	0.55
S1-2012-00119	9,750	50	0.5
S1-2012-00120	11,250	50	0.55
S1-2012-00121	10,250	50	0.5

Blend with known concentrations of microcrystalline cellulose were used for the analysis. Samples were collected and transferred into standard glass vials (15 mm x45 mm, 4 mL, 1 mm flat bottom- Borosilicate Type I Class A, w/PE Cap, ThermoFischer Scientific) for near infrared (NIR) analysis . Samples were compacted and sealed to minimizs sample variability.

Table 2 Instrument Photometric Response Details

Instrument	SN 115	SN 116	SN 117	SN 118	SN 119	SN 120	SN 121
Avg HF RMS noise (e-03)	0.054	0.046	0.051	0.037	0.039	0.050	0.035
Max HF RMS noise (e-03)	0.126	0.127	0.134	0.103	0.125	0.149	0.085
Avg LF RMS noise (e-03)	0.372	0.476	0.377	0.296	0.380	0.345	0.221
Max LF RMS noise (e-03)	1.079	1.825	1.037	0.775	0.936	0.883	0.381
Photometric Linearity (R)	0.975	0.977	0.978	0.978	0.977	0.978	0.978
Y Intercept (R)	0.023	0.020	0.020	0.019	0.022	0.019	0.019
Photometric Linearity (A)	0.936	0.945	0.953	0.958	0.948	0.951	0.955
Y Intercept (A)	-0.004	-0.004	-0.007	-0.006	-0.006	-0.004	-0.005