Hyperspectral wide-field time domain single-pixel diffuse optical tomography platform

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Abstract: We present the design and comprehensive instrumental characterization of a time domain diffuse optical tomography (TD-DOT) platform based on wide-field illumination and wide-field hyperspectral time-resolved single-pixel detection for functional and molecular imaging in turbid media. The proposed platform combines two digital micro-mirror devices (DMDs) to generate structured light and a spectrally resolved multi-anode photomultiplier tube (PMT) detector in time domain for hyperspectral data acquisition over 16 wavelength channels based on the time-correlated single-photon counting (TCSPC) technique. The design of the proposed platform is described in detail and its characteristics in spatial, temporal and spectral dimensions are calibrated and presented. The performance of the system is further validated through a phantom study where two absorbers in glass tubes with spectral contrast are mapped in a turbid medium of ~20 mm thickness. The method presented here offers the potential of accelerating the imaging process and improving reconstruction results in TD-DOT and thus facilitates its wide spread use in preclinical and clinical in vivo imaging scenarios.

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1. Introduction

Optical imaging is a powerful non-invasive tool to retrieve the distributions of exogenous fluorophores and/or endogenous chromophores in biological tissues. Especially, diffuse optical tomography (DOT) has found applications in numerous biomedical clinical and preclinical scenarios like breast cancer detection [1–3], functional brain imaging [4,5] and small animal imaging [6,7]. In addition, hybrid imaging systems that fuse DOT with other traditional medical imaging modalities [8–10] lead to complementary investigations of structural and functional information simultaneously or measurement cross-validations between different imaging modalities. However, the inherent ill-conditioned nature of DOT poses a great challenge to the performances of 3D reconstruction and renders it susceptible to noise and other modeling/instrumental uncertainties. Temporal modulation of light intensity, especially launching ultrashort mode-locking pulses in TD-DOT has proven to be a key factor to improve its performance. The abundant temporal information offered by time-resolved systems enables differentiating tissue absorption and scattering with minimal cross-talk [11,12] and sparse sampling of fluorescence contrast with improved accuracy [13] compared to its continuous wave (CW) counterparts. In addition, TD-DOT not only offers fluorescence lifetime as a robust and quantitative contrast for molecular unmixing but also presents improved resolution and reduced cross-talk in the 3D reconstruction of molecular information due to the combination of early-arriving photons and late-arriving highly diffused photons [14,15]. However, when there is need to acquire dense spatial, temporal and spectral hypercubes for optimal DOT performances, long data acquisition times are a limiting factor.

Recently we have proposed a novel instrumental approach to acquire such experimental hypercubes in relative short acquisition times by leveraging well-established concepts in compressive sensing and by using a double spatial light modulators (SLM) configuration.
[16,17]. The combination of structured light both in the illumination and detection channels for fast and robust DOT was first reported by Belanger et al [18]. The instrumental implementation was built around two DMD units and a single-pixel PMT detector in CW-DOT. This configuration allowed a 2 Hz frame rate acquisition in a phantom study [18]. The wide-field illumination and single-pixel detection configurations permitted reducing measurement number needed for tomography, which tremendously increased the detection SNR. This approach was further extended to TD-DOT by our group [17]. In Ref [17], the double-DMD configuration was combined with a time-resolved spectrophotometer based on a spectrally resolved multi-anode PMT detector in time domain for hyperspectral single-pixel acquisition. A Quantized Low Spatial Frequency base (QLSF) was selected as it provided best tomography results and ease of implementation experimentally. This novel instrumental paradigm that enabled the acquisition of 4D data cube ($x$, $y$, $t$, $\lambda$) was validated in fluorescence TD-DOT to resolve the relative concentrations of two dyes with lifetime and spectral contrast. This novel instrumental design was replicated recently in [19] with the addition of a stage to rotate the phantoms held in an upright position. However, if these previous studies focused on proof of concepts, none did report in details in the design of the system sub-components and their characterization.

Herein, we provide a comprehensive description of our instrumental platform and demonstrate its potential for performing hyperspectral TD-DOT. The key instrumental components of the system and the whole platform are characterized in spatial, temporal and spectral dimensions. We further validate the performance of this system with an in vitro study where the concentrations of two NIR absorbing dyes with spectral contrast suspended in a tissue-mimic liquid phantom are retrieved using the dense hyperspectral single-pixel data sets acquired in time domain with improved quantification and crosstalk. The paper is arranged as follows: in the second section, the detailed description of the system and characterization results are provided; then, the mathematical imaging model used in reconstruction is introduced in the third section; in vitro phantom study details and results are explored in the fourth section which is followed by conclusions and discussions in the last section.

2. System design and characteristics

2.1 System instrumentation overview

![Diagram of the hyperspectral TD-DOT system](image)

Fig. 1. (a) The scheme of the proposed hyperspectral wide-field time-resolved diffuse optical tomography platform (Laser and its coupling optics are not shown here). L1: lens, $f = 25.4$ mm; L2: lens, $f = 25.4$ mm; L3: lens, $f = 40$ mm; L4: lens, $f = 30$ mm; M1: plane mirror; M2, M3: concave mirrors; G: ruled reflective blazed grating, 750 nm blaze, 1200 lines/mm. (b) A photo that shows the physical implementation of the proposed system (Laser is not included).

To implement the wide-field structured illumination and hyperspectral single-pixel structured detection methodology, we proposed a system design for fluorescence molecular tomography in Refs [16,17]. Herein we report for the first time on the detailed characterization of its
components as well as its updating with a broadband supercontinuum pulsed fiber laser to enable hyperspectral functional imaging. A scheme of the proposed system is shown in Fig. 1(a) and its physical implementation (laser source not included) is depicted in Fig. 1(b). It employs two kinds of high-power pulsed lasers as light sources for delivering ultra-short NIR light pulses to the samples. Two DMD-based SLMs are implemented to enable structured light illumination and single-pixel acquisition in transmission. The spatially integrated light exiting the detection DMD is coupled into a time domain Czerny-Turner spectrometer based on a spectrograph and a multi-anode time-resolved PMT. Under each illumination and detection pair, we collect sixteen spectrally resolved temporal point spread functions (TPSFs) using TCSPC technique.

2.2 Light source

To provide a comprehensive depiction of this platform, we report its characterization of two different time-resolved light sources. For molecular imaging, we employ a Ti:sapphire mode-locked pulse laser (Mai Tai HP, Spectral Physics, CA) which is capable of generating light pulses from 690 nm to 1040 nm with ~100 fs pulse width, 80 MHz repetition rate and <15 nm spectral bandwidth (in terms of FWHM: full width at half maximum). This laser is best for fluorescence imaging as it provides high-power mono-spectral excitation. The Mai Tai output is delivered to the illumination DMD using a multi-mode fiber of 400 µm core diameter (P-400-10-VIS-NIR, Ocean Optics, FL) and a collimator (F810SMA-780, Thorlabs Inc, NJ) with ~80% transmission. For functional imaging, a supercontinuum pulsed fiber laser (SC-Pro, YSL Photonics, Wuhan, China) is employed for broadband hyperspectral excitation. It generates ultrafast supercontinuum pulses in a broad spectrum range from 400 nm to 2200 nm, with 1 MHz - 25 MHz tunable repetition rate, 150 - 200 ps pulse width and >4 W total power at 25 MHz repetition rate (>1 mW/nm spectral power density in the range of 700 nm - 820 nm). Its output is spectrally filtered using free-space optics for hyperspectral excitation before being coupled into the illumination DMD (Fig. 2(a)). The collimated output of SC-Pro is expanded by a beam expander (constructed with a microscope objective: M-10X, Newport Optics, CA and a lens: AC-254-030-B, Thorlabs Inc, NJ) and then filtered by two dichroic mirrors (FF801-Di02-25 × 36 and FF700-Di01-25 × 36, Semrock, NY) to preserve the NIR spectrum and prevent other parts of the spectrum from contaminating the measurements. Before coupling into the illumination DMD, the spectrum and total power are monitored by a spectrophotometer (USB2000, Ocean Optics, FL) and a photodetector (PDA100A, Thorlabs Inc, NJ). A typical output spectrum recorded at USB2000 is shown in Fig. 2(b).

![Diagram](image_url)

Fig. 2. SC-Pro supercontinuum fiber laser coupling. (a) The physical implementation of the coupling optics setup for the SC-Pro laser. (b) A typical output spectrum of the laser before injecting into the illumination DMD.
The setup of DMD-based illumination and detection modules. (a) The physical implementation of the illumination module. (b) The internal structure and light propagation path inside the illumination module. (c) Physical implementation of the detection module. (d) Internal structure and light path inside the detection module.

On the detection side, another DMD-based SLM (D4110 with NIR S2 + optical module, Digital Light Innovations, TX) is employed to modulate the emission light field onto wide-field detection masks with the same DMD chip as in the illumination side (see Fig. 3(c) and (d)). An 8.6-mm spacer ring is added to the detection lens to reduce its working distance to ~10 cm. Transmitted spatially modulated light signals collected by the detection DMD are coupled into an 11-mm fiber light guide (mono-fiber: core diameter = 200 μm, NA = 0.22) with a relay lens group (LB1761-B, LA1422-B and LB1757-B, Thorlabs Inc, NJ). An optical filter is usually required for fluorescence imaging and optional for hyperspectral functional investigation. The fiber light guide not only transports the emission light signals but also converts the optical aperture cross-section from a 3-mm-diameter circular aperture to a 7 × 1 mm² rectangular shape to fulfill the slit input requirement of the spectrally resolved PMT detector. The mono-fibers inside the light guide are randomly arranged from the input side to the output side to minimize the interference of spatial multiplexing on spectral multiplexing considering the relatively large slit size here. The hyperspectral detector used in the system combines a spectrograph (MS125, Newport Optics, CA) with a multi-anode time-resolved PMT (PML-16-C, Becker & Hickl GmbH, Germany) to achieve hyperspectral data acquisition in time domain. The spectrograph (focal length = 120 mm, f-number = 3.7)
employ two concave mirrors and a 1200 lines/mm ruled reflectance grating (77412, Newport Optics, CA) that blazes at 750 nm to disperse the light signals onto the active area (16 × 16 mm²) of the PMT photocathode [20]. Each of its sixteen 0.8 × 16 mm² pixels (1 mm pixel pitch) serves as a single-pixel time-resolved camera at the corresponding wavelength channel. The detection wavelength range can be adjusted by replacing gratings with different line densities and the center wavelength position can be tuned by rotating the grating.

The TCSPC technique is applied to acquire time-resolved data sets with SPC-150 module (Becker & Hickl GmbH, Germany) under the control of LabVIEW. Additionally, a NIR CMOS camera (UI-5240CP-NIR-GL, IDS GmbH, Germany) is integrated into the platform to account for the distortions, spatial intensity non-uniformity caused by the optical system by integrating the calibration results into the forward model of TD-DOT.

The system’s abilities to perform spatial light modulation of the excitation and emission light fields in time domain and signal multiplexing at the multi-anode PMT are crucial towards its goal of reconstructing functional and molecular information in preclinical samples. Thus, the platform performances are extensively reported in the spatial, temporal and spectral dimensions.

2.4 Spatial characterization

As TD-DOT is a model-based imaging technique, it is critical to capture the exact spatial distribution of the structured light illuminated on the sample and optical mask employed in the single-pixel scheme. Thus, each optical pattern is calibrated spatially by using the NIR CMOS camera. Figures 4(a)-(h) show the examples of camera captured QLSF illumination and detection masks. On the detection side, the modulated emission light field is sampled by the mono-fibers inside the fiber light guide, as is shown in Fig. 4(e)-(h). The experimental calibration of the patterns enables simulating such effect in our forward model calculations.

![Examples of illumination and detection optical masks recorded using an NIR camera.](image)

For grayscale optical masks derived from transforms like discrete cosine transform (DCT), discrete wavelet transform (DWT) and discrete Fourier transform (DFT), the linearity of light intensity coding of SLM is characterized by projecting and recording two testing optical masks in the same field of view (FOV) on the imaging plate: one full-field illumination mask and one calibration mask with linear grayscale distribution (from 0 on the left to 255 on the right, Fig. 5(a)). The calibration mask is normalized by the full-field mask to cancel out the effect of illumination non-uniformity and averaged along the vertical direction (see Fig. 5 (b)). Then, the intensity coding linearity is calculated by:

\[
\text{linearity} = \left( \frac{\delta}{255} \right) \times 100\% 
\]
where $\delta$ is the maximum deviation of grayscale values from linear distribution, and ‘255’ is the maximum level for 8-bit grayscale. This linearity mainly relies on the switch of micro-mirrors under PWM control signal thus depends on the DMD chip and corresponding electronic control circuit. For the 0.7” DMD used in this work, a <2% intensity coding linearity is observed (see Fig. 5(c)). It is worth noting that for the 0.17” DMD used for structured illumination in Ref [17], a relatively poor coding linearity of ~14.41% is observed and grayscale correction is necessary for higher projection fidelity. For example, a look-up table can be applied to generate a ~1% linearity [21].

![Fig. 5](image_url)

**Fig. 5.** Light intensity coding linearity test and results for 0.7” DMD. (a) Computer fed calibration mask for light intensity coding linearity test. (b) Recorded calibration mask normalized by recorded full-field illumination mask using the 0.7” DMD. (c) Output intensity coding level distribution (blue dashed line) from linear input (red line) for 0.7” DMD.

![Fig. 6](image_url)

**Fig. 6.** Temporal characteristics of the system. (a) IRFs captured using Mai Tai laser from 700 nm to 810 nm. (b) IRFs recorded using SC-Pro fiber laser from 740 nm to 810 nm. (c) FWHMs of the IRFs recorded using two light sources. (d) IRF FWHM stability measured using Mai Tai laser over 2-hour operation time at 750 nm. (e) IRF peak position $t_o$ variation measured using Mai Tai laser over 2-hour operation time at 750 nm.

### 2.5 Temporal characterization

The temporal performance of a time-domain imaging system is mainly characterized by its instrument response function (IRF), which is the system response to a short laser pulse. A robust IRF that is unsusceptible to time, wavelength and laser power is ideal for TD-DOT. In this platform, IRFs are measured by placing a diffusing paper on imaging plate. IRFs acquired by the system at different wavelengths using the two laser sources (Mai Tai laser and SC-Pro laser) are recorded and plotted in Fig. 6(a) and (b). Their corresponding FWHMs are
calculated after interpolating the recorded IRFs to 1 ps resolution to evaluate the temporal resolution of the system (see Fig. 6(c)). It is shown that a <170 ps FWHM is achievable throughout the spectral range considered. This indicates that the double-DMD scheme does not compromise the temporal resolution considering the ~150 ps temporal resolution of the detector itself. The temporal stability of IRF is tested by observing the variations of FWHM and peak position \( t_0 \) of the recorded IRFs over a period of >2 hours after turning on the gain voltage of PMT. Testing results using the Mai Tai laser are shown in Fig. 6(d) and (e). The FWHM drifts are within ± 5 ps around the mean value. The IRF peak position \( t_0 \) experiences larger drift during the first 20 min of operation after which \( t_0 \) drifts around the steady state mean value within a ~ ± 10 ps range.

Fig. 7. Spectral characterization results of the platform. Output powers obtained for different input powers with (a) D4110 VIS optical module, (b) D4110 NIR optical module, (c) The comparison of power transmission efficiency for the three DMD optical modules. (d) Spectral resolution test result of the PMT detector. Green line: input spectrum at 700 nm with FWHM = 1.88 nm. Blue dotted line: detected spectrum by the multi-anode PMT. Red dashed line: interpolated spectrum for analysis.

2.6 Spectral characterization

The transmission efficiency test over our primary spectrum of application mainly focuses on the SLMs. The tests are carried out by injecting different laser powers at the testing wavelengths (span from 700 nm to 820 nm) through a fiber to the DMDs and focusing their output signals onto the sensor of an optical power meter (S310C-thermal power sensor, Thorlabs, NJ) using a convex lens. The laser power at the fiber end is then measured to calculate the power transmission. For each testing wavelength, three input power values are used and the final transmission efficiency at this wavelength is obtained through linear regression. The test results for both the illumination and detection DMDs are shown in Fig. 7(a) - (c). The D4110 DMD with VIS optical module achieves >12.6% power transmission efficiency over the spectrum, while the D4110 DMD with NIR optical module offers significantly improved power transmission to allow above ~40% transmission across the testing NIR wavelength range. This is due to the NIR optical coating imposed on the projection optics and substantially reduces the energy loss for longer wavelengths. As a comparison, the Pico projector used in Ref [17] is capable of delivering >10% of the input
laser power onto the imaging plate with a full-field pattern (see Fig. 7(c)). In addition, placing the D4110 NIR module on the detection side helps to make full use of its high transmission efficiency.

The spectral resolution of the detector is independently characterized with a supercontinuum laser (WL-SC400-4, Fianium Inc, United Kingdom). An acousto-optic tunable filter (AOTF) (AOTF-V1-N2-DD, Fianium Inc, United Kingdom) is implemented to achieve an input spectrum of <2 nm FWHM. The spectrum at 700 nm is injected into the system and detected by the multi-anode detector. The output spectrum of the 16 spectral channels is interpolated and its FWHM is calculated to estimate the spectral resolution. The 1200 lines/mm grating in the spectrophotometer results in ~5.3 nm center wavelength distance between adjacent channels. The spectral resolution, in terms of FWHM, is estimated to be ~10 nm (see Fig. 7(d)). This spectral resolution is mainly determined by the blazed grating, slit width of the spectrograph and pixel size of the multi-anode PMT.

3. Optical inverse problem

In this work, a time-resolved mesh-based wide-field forward-adjoint Monte Carlo (aMC) method optimized for wide-field structured light strategies [22,23] is employed to compute the forward model and associated Jacobian matrix. We apply the Rytov approximation [24] to linearize the forward problem of TD-DOT. Suppose that the TD-DOT is performed using \( N_s \) illumination masks and \( N_d \) detection masks, and the emitted light fluence under the \( l^{th} \) \((l = 1,2,\cdots,N_s)\) illumination mask \( r_s \) and the \( m^{th} \) \((m = 1,2,\cdots,N_d)\) detection mask \( r_m \) at the \( k^{th} \) wavelength channel (center wavelength \( \lambda_k \)) and delay time point \( t \) is expressed as:

\[
U^k_l (r_s, r_m, t) = U^k_0 (r_s, r_m, t) \exp[\Phi^k_{\text{pert}} (r_s, r_m, t)]
\]

(2)

where \( U^k_0 (r_s, r_m, t) \) and \( U^k_l (r_s, r_m, t) \) are emission light fields measured without and with perturbations in optical properties. \( \Phi^k_{\text{pert}} (r_s, r_m, t) \) is the phase shift due to the perturbations and can be calculated as:

\[
\Phi^k_{\text{pert}} (r_s, r_m, t) = \ln \left( \frac{U^k_l (r_s, r_m, t)}{U^k_0 (r_s, r_m, t)} \right)
\]

(3)

At the same time, \( \Phi^k_{\text{pert}} (r_s, r_m, t) \) can be derived from the absorption perturbation \( \delta \mu^a (r) \) at space location \( r \) and \( \lambda_k \) by:

\[
\Phi^k_{\text{pert}} (r_s, r_m, t) = \int_{\Omega} w^k (r_s, r_m, r, t) \delta \mu^a (r) \, d^3 r
\]

(4)

where

\[
w^k (r_s, r_m, r, t) = -\frac{\alpha^k}{G^k (r_s, r_m, r, t)} \int_0^t dt' G^k (r_s, r_m, t-t') G^k (r_s, r_m, t')
\]

(5)

is the weight function obtained with the Green’s functions of forward and adjoint light fields \( G^k (r_s, r_m, r, t-t') \) and \( G^k (r_s, r_m, t-t') \) and the forward light field at the detection mask \( G^k_0 (r_s, r_m, t) \). \( \alpha^k \) is a constant that accounts for the system response and model-related coefficients at \( \lambda_k \) and \( \Omega \) is the imaging volume. Suppose \( \Omega \) is discretized into \( N_{\text{vol}} \) nodes and datasets are acquired with \( P \) source-detector pairs and \( T \) time gates. Then, the discretized distribution vector of \( \delta \mu^a \) within \( \Omega \) can be expressed as \( x^k \) and retrieved by solving the inverse problem of the following equation:

\[
b^k = W^{\delta \mu^a, k} x^k
\]

(6)
where $\mathbf{b}^k = [\gamma^k_{\omega}^1 \cdots \gamma^k_{\omega}^P]^T$ ($k = 1, 2, \cdots, N$), $\gamma^k_{\omega} = \ln[U_{\omega}^k (\nu_l, \nu_m, \nu_r, t)]/U_{\omega}^k (\nu_l, \nu_m, \nu_r, t)$ defines the measurement vector, and $\mathbf{W}^{\omega,k}$ is the weight matrix (for $T$ time gates) at wavelength $\lambda_k$.

Assume that the absorption perturbation $\delta \mu^k_{\omega}$ is contributed by $L$ absorbers in the medium, thus the absorption contrast $\delta \mu^k_{\omega} (r) = \sum_{n}^L \epsilon_n C_n (r) \cdot \delta \mu^k_{\omega}$ ($n = 1, 2, \cdots, L$), where $C_n (r)$ is the concentration of the $n^{th}$ absorber at $r$ and $\delta \mu^k_{\omega}$ denotes its extinction coefficient at $\lambda_k$. Then the weight function of the $n^{th}$ absorber at $\lambda_k$ in terms of concentration becomes:

$$w_n^k (\nu_l, \nu_m, \nu_r, t) = \mathbf{E}_{n,k} \cdot \mathbf{W}^k \mathbf{C}_n$$

and the inverse problem to retrieve the concentrations of $L$ absorbers using measurements from $P$ source-detector pairs, $N$ wavelengths, and $T$ time gates can be established as:

$$\begin{bmatrix} b^k \\ b^k \end{bmatrix} = \begin{bmatrix} \mathbf{W}^{\omega,k} \\ \vdots \\ \vdots \\ \mathbf{W}^{\omega,k} \\ \mathbf{W}^{\omega,k} \\ \vdots \\ \vdots \\ \mathbf{W}^{\omega,k} \end{bmatrix} \begin{bmatrix} C_1^k \\ \vdots \\ \vdots \\ C_L^k \end{bmatrix}$$

where $\mathbf{W}^{\omega,k}_n$ denotes the weight matrix for the $n^{th}$ absorber at the $k^{th}$ wavelength and vector $\mathbf{C}_k^k$ is the absorber concentration over the whole volume.

We adopted an optode calibration method [25] to suppress the artifacts induced by the fluctuations in source-detector coupling coefficients. In this method, coupling amplitude terms $s_l$ and $d_m$ ($l = 1, 2, \cdots, N_s; m = 1, 2, \cdots, N_d$) for source $\nu_l$ and detector $\nu_m$ respectively are added to each source-detector pair so Equation (4) can be updated as:

$$\gamma^k_{\omega} = \ln[U_{\omega}^k (\nu_l, \nu_m, \nu_r, t)]/U_{\omega}^k (\nu_l, \nu_m, \nu_r, t)] = \ln s_l + \ln d_m + \int_0^t w_{\omega}^k (\nu_l, \nu_m, \nu_r, \nu, t) \cdot \delta \mu^k_{\omega} d^3 r$$

Thus Equation (6) can be updated as:

$$\mathbf{b}^k = \mathbf{W}^{\omega,k} \mathbf{x}^k = \left[ \begin{array}{ccc} \mathbf{W}^{\omega,k} & \mathbf{W}_S & \mathbf{W}_D \end{array} \right] \left[ \begin{array}{c} \mathbf{x}^k/\mu_0^k \\ \mathbf{s} \\ \mathbf{d} \end{array} \right]^{T}$$

where $\mathbf{W}_S$ and $\mathbf{W}_D$ represent the involvement of source and detector masks for each measurement using “1” (source or detector used for acquiring the measurements) and “0” (source or detector not used). $\mathbf{x}^k/\mu_0^k$ is the relative absorption perturbation to the background medium, so that the reconstructed vector becomes dimensionless and the dynamic range can be reduced. Vectors $\mathbf{s}$ and $\mathbf{d}$ are coupling coefficient vectors (independent of wavelength). Similarly, the forward problems described in Equation (8) and can be updated as:

$$\begin{bmatrix} \mathbf{b}^{k} \\ \vdots \\ \mathbf{b}^{k} \end{bmatrix} = \begin{bmatrix} \mathbf{W}^{\omega,k} \\ \vdots \\ \mathbf{W}^{\omega,k} \\ \mathbf{W}^{\omega,k} \\ \vdots \\ \mathbf{W}^{\omega,k} \end{bmatrix} \begin{bmatrix} \mathbf{C}^k \\ \vdots \\ \mathbf{C}^k \end{bmatrix}$$

by stacking sensitivity matrices and updated measurements from different wavelengths. The measurements are updated based on Eq. (10) so that contributions from coupling coefficients to $\mathbf{b}^k$ are removed and $\mathbf{b}^k$ only accounts for the contributions of absorbers. The 3D distributions of two absorbers are retrieved by inverting the forward problem described in Equation (11). In this study, $L_1$-norm based regularization method [26] is implemented to solve the inverse problem. Information from both the early and late time gates are employed where reconstruction of early gate serves as the initial guess to the final reconstruction [13].
4. Phantom study and results

A liquid phantom study is conducted to validate the system performance for functional tomographic reconstruction in turbid media. Two water-soluble absorbers: India ink (Speedball Art Products, NC) and Epolight 2735 (Epolin Inc, NJ) are used to generate spectrally resolved absorption contrasts while intralipid (20% stock solution, Sigma-Aldrich, MO) is used to control the scattering property of the medium. An $80 \times 50 \times 22.5 \text{ mm}^3$ liquid phantom is prepared in a polycarbonate clear tank (see Fig. 8(a)). In the medium, the concentrations of India ink (volume concentration), Epolight 2735 (mass concentration), and Intralipid are set to 0.0024%, 0.0008%, and 0.8% respectively to generate optical properties of $\mu_a = 0.15 \text{ cm}^{-1}$ and $\mu'_s = 8.39 \text{ cm}^{-1}$ at 740 nm. Two glass tubes (10.25 mm and 10.38 mm in diameter and 16 mm center-to-center distance) are suspended at 10 mm depth in the liquid medium. Intralipid concentration is set to 0.8% in both tubes to keep the scattering properties same as the medium. In the left tube, concentrations of Epolight 2735 and India ink are set to $\sim 0.0034\%$ and $\sim 0.0024\%$ to create 3.249 times of absorption perturbation from Epolight 2735. In the right tube, the concentration of Epolight 2735 and India ink are prepared as $\sim 0.0008\%$ and $\sim 0.0092\%$ to create 2.825 times of absorption perturbation from India ink. Figure 8(b) depicts the absorption coefficient trends of the absorber mixtures in the medium and the solutions added to the two tubes over the detection wavelength range. The extinction coefficients of the two absorbing materials are calibrated using Monte Carlo-based time-resolved spectroscopy [22]. Figure 8(c) shows the absorption coefficients of 0.0008% Epolight 2735 and 0.0024% India ink obtained from the calibration tests and a clear spectral contrast could be observed.
The maximum reconstruction crosstalk and the relative concentration ratio of the two absorbers are employed to evaluate the performance of the proposed platform. The maximum crosstalk is computed as follows:

\[
Crosstalk_1 = \frac{\max[\hat{C}_2(\Omega_1)]]}{\max[\hat{C}_1(\Omega_1)]]} \times 100\%
\]

\[
Crosstalk_2 = \frac{\max[\hat{C}_3(\Omega_2)]]}{\max[\hat{C}_2(\Omega_2)]]} \times 100\%
\]

\[
Max\_Crosstalk = \max(Crosstalk_1, Crosstalk_2)
\]

Here, \(\hat{C}_1\) and \(\hat{C}_2\) are reconstructed concentrations of two absorbers normalized to their own maximum, while \(\Omega_1\) and \(\Omega_2\) are real distribution spaces of absorbers 1 and 2 at 50% isovalue. Time-resolved data of four gates: 50% rising gate, peak gate, 80% decaying gate and 60% decaying gate are applied, where reconstructions using 50% rising gate are used as initial guess of the final reconstruction. The implemented spectral information is controlled to increase from two channels (740 nm and 745 nm) to twelve channels (740 nm, \cdots, 797 nm). The retrieved absorber concentrations using two, four, and twelve channels are shown in Figs. 10 (a-c). With data from only two wavelength channels, the two absorbers are separated with relatively high maximum crosstalk \(-95.85\%\), however, by increasing the spectral information to twelve wavelength channels, the maximum crosstalk is reduced to \(35.76\%\). The relationship between maximum crosstalk and relative reconstructed concentration ratio are summarized in Fig. 11, where a decreasing trend of maximum crosstalk is easily observed.
with the increase of spectral information in reconstruction. At the same time, the quantification accuracy of the reconstructed concentration ratio increases with spectral multiplexing. The estimation error of the relative concentration of the two absorbers reduces from 81.07% (using 2 wavelength channels for reconstruction) to 0.17% (using 12 wavelength channels for reconstruction).

Fig. 10. Reconstructed absorber concentration distributions using data from four time-gates (50% rising gate, peak gate, 80% decaying gate, and 60% decaying gate) and multiple wavelength channels. (a) Reconstruction using 2 wavelength channels (740 nm – 745 nm) (maximum crosstalk = 95.85%). (b) Reconstruction using 4 wavelength channels (740 nm – 756 nm) (maximum crosstalk = 52.29%). (c) Reconstruction using 12 wavelength channels (740 nm – 797 nm) (maximum crosstalk = 35.76%). (d) Reconstruction of absorber concentrations by linear decomposition at each node using data from 12 wavelength channels. (maximum crosstalk = 51.78%). All plots are 50% isovolumic surfaces of individual absorber concentrations.

Fig. 11. The effects of spectral information on unmixing performance and quantification accuracy. (a) The relationship between spectral information and maximum reconstruction crosstalk. (b) The relationship between spectral information and retrieved concentration ratio of two absorbers.
Finally, the performance of the above method to retrieve absorber concentrations is compared with the method that performs linear decomposition at each node within the phantom volume. The results of using the reconstructed absorption maps at twelve wavelength channels with data of four time-gates to conduct linear decomposition is shown in Fig. 10(d). The result is highly compromised in terms of maximum crosstalk (51.78%) and relative quantification accuracy (reconstructed relative concentration ratio = 0.56 against ground truth = 0.38) compared to the result shown in Fig. 10(c). Linear decomposition at each node offers poor performance because it fails to exploit the correlations between nodes.

5. Discussion and conclusion

To sum up, we report on a detailed calibration of the proposed time-resolved DOT system based on wide-field structured illumination and hyperspectral single-pixel detection and validate the system performance with phantom studies. Spectral and spatial characteristics of two DMDs on illumination and detection sides show their abilities to project wide-field patterns over large area. By inputting patterns recorded during the calibration stage into our MC code, we are able to account for the non-uniformity during illumination and detection. The temporal calibration indicates that temporal resolution is not compromised by the wide-field technique.

Using a supercontinuum fiber laser to perform hyperspectral excitation of the sample offers a spectral information boost within the same data acquisition time compared to monochromatic excitation situations. Moreover, the hyperspectral wide-field single-pixel detection implementation facilitates fast collection and utilization of this abundant information. The benefits of the proposed method are also reflected by the reconstruction results where the maximum crosstalk and quantification accuracy of the relative concentration ratios retrieved through TD-DOT are improved with increasing the spectral information. Note that overall, the study reported herein is not optimized in terms of optical reconstructions strategies as well as acquisition speeds. The focus of this report is to provide a detailed characterization of the instrumental design and subcomponent of our hyperspectral dual-DMD setup which involves non-trivial elements. We opt to present the characterization of the system as usually done for time-resolved instruments with the addition of the spectral DMD characterization and validation in phantom experiments as typically done in the field. We expect overall that significant improvement in reconstructions can be achieved via more refined inverse problem methodologies, such as multimodal strategies. Similarly, we expect that significant reduction in acquisition speed can be achieved by optimizing the system or performing data postprocessing.

Indeed, the maximum photon counts allowed and data acquisition time of this study is limited by the 25 MHz repetition rate of the supercontinuum fiber laser to avoid pile-up effect during TCSPC data acquisition. Since supercontinuum fiber lasers with 80 MHz repetition rate are widely available in the market, the time needed to acquire these hyperspectral information-rich data sets could be significantly reduced to <20 min. Moreover, we have not investigated what is the required maximum photon counts in the acquired TPSF to allow for robust functional imaging. In the case of FMT, this photon counts can be reduced further, and hence, could lead to one order of magnitude in time of acquisition. Similarly, postprocessing methodologies such as gating could lead to improved photon counts without compromising the temporal information [27,28]. Moreover, the system is not fully optimized in the detection channel where we still have space for improving the transmission by replacing the inefficient components like fiber light guide and high f-number spectrograph. Furthermore, the optical masks we applied have proven to be robust and working well for in vivo imaging [29] but other sets of patterns can be considered [30,31]. For instance, we recently reported that a Hadamard base ranked by frequency provided the best results for 2D lifetime imaging when a single-pixel methodology was employed and in further studies, we will extend this work to tomographic imaging [32]. Last, one significant appeal of spatial light modulators is their...
ability to adaptively adjust illumination [21]. Such scheme is well suited to account for inhomogeneity inside biological tissue and complex geometries leading to large variation in dynamical range acquired when the animal is in a prone position as required for live preclinical studies.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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