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Wideband fluorescence-based thermometry by neural network recognition: Photothermal application with 10 ns time resolution

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Neural network recognition of features of the fluorescence spectrum of a thermosensitive probe is exploited in order to achieve fluorescence-based thermometry with an accuracy of 200 mK with 100 MHz bandwidth, and with high robustness against fluctuations of the probe laser intensity used. The concept is implemented on a rhodamine B dyed mixture of copper chloride and glycerol, and the temperature dependent fluorescence is investigated in the temperature range between 234 K and 311 K. The spatial dependence of the calibrated amplitude and phase of photothermally induced temperature oscillations along the axis of the excitation laser are determined at different modulation frequencies. The spatial and frequency dependence of the extracted temperature signals is well fitted by a 1D multi-layer thermal diffusion model. In a time domain implementation of the approach, the gradual temperature rise due to the accumulation of the DC component of the heat flux supplied by repetitive laser pulses as well the immediate transient temperature evolution after each single pulse is extracted from acquired temporal sequences of fluorescence spectra induced by a CW green laser. A stroboscopic implementation of fluorescence thermometry, using a pulsed fluorescence evoking probe laser, is shown to achieve remote detection of temperature changes with a time resolution of 10 ns. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4935277>]

I. INTRODUCTION

Temperature, one of the fundamental thermodynamic quantities of many-particle systems, plays a key role in the behavior of matter. The temperature-sensitive nature of fluorescence provides a non-contact, all-optical, and emissivity-independent approach to determine the temperature of a system of interest.¹ Fluorescence-based thermometry circumvents many of the limitations encountered in other traditional thermometry techniques,² based on thermoelectric devices, electrical resistance based probes, emitted infrared (IR) radiation, etc. Additionally, by employing fluorescent nanoparticles, fluorescence-based thermometry allows for local temperature measurements down to submicron/nano-scale.^{3–6} As a consequence, fluorescence-based thermometry is receiving increasing attention in the framework of various kinds of scientific research and industrial processes.^{7–14}

Fluorescence-based thermometry methods can be classified into several different categories¹⁵ on the basis of the characteristic parameter of fluorescence that is utilized for temperature readout, e.g., intensity, band-shape, spectral

position, polarization, lifetime, and bandwidth of fluorescence. Intensity-based methods, involving the measurement of the fluorescence integrated intensity and/or peak intensity, require relatively simple instrumentation and data analysis, and therefore, tend to be the most cost-effective approach to implement. The accuracy of the acquired temperature, however, is hampered, due to the fact that the fluorescence intensity is also affected by mechanical, electrical, and optical instabilities of the measurement arrangement, such as drifts of optical element positions and fluctuations of the power of the probe laser used to evoke the fluorescence.¹⁶ In order to partially bypass such issues, ratiometric fluorescence thermometry,^{17–23} based on the intensity ratio of fluorescence at two selected emission bands, or based on the fluorescence anisotropy, extracted from the fluorescence intensities of two orthogonally polarized components, has been proposed. Recently, neural network (NN) recognition, a powerful tool for solving inverse problems and retrieving information of interest from experimental data in many applications,^{24–26} has been reported to provide an alternative approach to improve the measurement accuracy of fluorescence-based thermography.²⁷ This was accomplished by exploiting multiple features of fluorescence spectra as parameters for

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temperature determination, resulting in a robust information extraction algorithm.

In this work, a detailed investigation of the application of NN recognition to fluorescence-based thermometry is performed on a rhodamine B (RhB) dyed mixture of copper chloride (CuCl_2) and glycerol sample. Different types of NNs, based on the same set of fluorescence spectra recorded at steady state temperatures ranging from 234 K to 311 K, are trained by using different compact sets of spectral features for NN inputs. An approach for performing spatially resolved extraction of photothermally induced temperature oscillations in a semitransparent sample is proposed, both in a frequency and time domain implementation.

The remainder of this article describes the procedure followed to acquire and pre-process temperature dependent fluorescence calibration data (Section II), to feed them into the NN, train the NN for temperature determination (Section III), and implement the NN recognition approach in spatially resolved photothermal experiments in the frequency (Section IV) and time domains (Section V). Particular attention is given to stroboscopic fluorescence thermometry (Section VI) with respect to applications for ultrafast temperature detection. Conclusions and perspectives for future applications of the approach are given in Section VII.

II. TEMPERATURE DEPENDENT FLUORESCENCE

RhB has been employed as a marker for thermal sensing in many works^{11,22,28} due to its high and strongly temperature dependent fluorescence quantum yield.²⁹ Particularly, with respect to the photothermal application, RhB has been employed to determine the laser induced temperature increment and its spatial distribution around a pump laser beam.³⁰ Here, RhB was chosen as a dopant in glycerol, to which, in view of the particular photothermal implementation of the setup (Section IV), CuCl_2 was added in order to increase the infrared absorption coefficient of the sample for the wavelength of a pump laser (1064 nm). The absorption coefficient of the sample at 1064 nm was measured by the optical transmission method and found to be $\beta_{\text{IR}} = 5.0 \text{ Neper cm}^{-1}$ at room temperature, compared to $0.1 \text{ Neper cm}^{-1}$ for the sample without CuCl_2 .

The temperature dependence of the fluorescence spectrum of the sample was investigated by using the setup shown in Fig. 1. To excite the fluorescence of RhB, a CW 532-nm laser (Samba 100, Cobolt[®]) was used and slightly focused to the sample by a lens with focal length of 300 mm. The entrance beam diameter to this lens was 2.5 mm, while the spot diameter at the focal point was approximately $128 \mu\text{m}$. The emitted fluorescence was collected by a lens system with numerical aperture $\text{NA} = 0.22$ and sent via fiber to a USB 4000 spectrometer (Ocean Optics[®]), where the spectrum was recorded. The sample cell was placed inside an optical cryostat (Optistat-DN-V, Oxford Instruments[®]), where the temperature could be regulated by an accompanying temperature controller (ITC 503, Oxford Instruments). In order to determine possible temperature differences between the cold finger and the sample holder (inset, Fig. 1), a resistive PT1000 thermometer (read by an HP34401A[®]

FIG. 1. Calibration setup. The sample cell was placed inside a vacuum optical cryostat. A CW 532-nm laser was used to evoke fluorescence in the sample. The fluorescence emission spectra were recorded by a spectrometer. The sample temperature was measured by a combination of a platinum resistor (PT1000) and a type T thermocouple.

multimeter) was glued to the sample holder, monitoring its temperature, while a type T thermocouple, attached near the region of interest at the cuvette wall, was used to measure the temperature difference between the temperature in the optical detection region and the sample holder. In this way, information on the temperature in the region of interest in the sample, which was needed for the calibration of fluorescence thermometry, was continuously available. The concentrations of RhB and CuCl_2 were $5.4 \times 10^{-4} \text{ M}$ and $1.4 \times 10^{-1} \text{ M}$, respectively. The optical power of the probe laser evoking the fluorescence was 3 mW.

It is noteworthy that photobleaching, the rapid loss of fluorescence due to the photochemical destruction of fluorophores upon exposure to an excitation light source, of the RhB fluorophore has been reported in many works.^{4,22,31} It was thus important to check for possible photobleaching of the system prior to performing fluorescence-based thermometry experiments. This was carried out by continuously illuminating the sample by probe laser light and monitoring the fluorescence intensity for a long time (60 min) at room temperature. A typical time evolution is presented in Fig. 2. The integrated fluorescence intensity over time was normalized to an initial value 1 min after switching on the probe laser. Only very negligible intensity decay was observed in our

FIG. 2. Photobleaching rate of the RhB fluorophores at room temperature under continuous illumination of the fluorescence evoking probe laser. The probe laser intensity was 23 W/cm^2 and the sample was sealed in a cuvette in a vacuum cryostat.

system, 0.5% in 60 min. This is because the laser intensity used was moderate (23 W/cm^2) and the sample was sealed in a cuvette which was in a vacuum cryostat (oxygen-free). The fluorescence signal was found to start recovery around 45 min, which is probably related to translational diffusion of the RhB molecules, which leads to refreshing of fluorophores under illumination of the green laser spot. For three-dimensional isotropic diffusion, the translation time (τ_T) of a molecular can be estimated by Eq. (1a), with a the size of the spot ($64 \mu\text{m}$ in this work) through which the molecules diffuse, and D the translational coefficient defined by the Stoke-Einstein equation as shown in Eq. (1b), where k_B is Boltzmann's constant ($1.38 \times 10^{-23} \text{ J/K}$), T is the absolute temperature (room temperature in this work, 293 K), η is the viscosity ($1.3 \text{ Pa}\cdot\text{S}$ (Ref. 32)), and R is the hydrodynamic molecular radius (around 0.57 nm for RhB (Ref. 33)). By substituting those values into Eq. (1), the estimated translation time is 39 min, close to the 45 min duration observed in Fig. 2

$$\tau_T = \frac{a^2}{6D}, \quad (1a)$$

$$D = \frac{k_B T}{6\pi\eta R}. \quad (1b)$$

Fluorescence spectra of the sample were collected at 11 different steady state temperatures in the range from 234 K to 311 K. The temperature dependence of the fluorescence spectrum of the sample is summarized in Fig. 3. Both the integrated and peak (left axis, inset) intensities evolve

FIG. 3. Temperature dependent fluorescence spectra (upper) of RhB in a mixture of CuCl_2 and glycerol for a selection of temperatures. The lower figure shows the temperature dependence of the integrated intensity (solid squares), the peak intensity (hollow squares), and the ratio of the integrated intensity to the peak intensity (stars, right axis).

substantially in a non-monotonic way, with a maximum around 288 K. The observed decrease in fluorescence intensity above 288 K with rising temperature can be attributed to a decrease of the quantum yield of RhB with temperature. This decrease is due to an increase of the rate constants for internal conversion or intersystem crossing, which compete with the fluorescence when the temperature is increased.¹ The opposite evolution below 288 K might be due to thermally activated recovery from the long-lived dark state, the radical anion of RhB,^{34,35} or the electron transfer from glycerol to the excited rhodamine. However, the ratio (solid line) of the integrated intensity to the peak intensity increases as the temperature increases (right axis, inset) due to the emission bands becoming narrower towards low temperatures. Also the shape, width, and maximum position of the spectrum turn out to be strongly temperature dependent.

In order to highlight the temperature dependence of the spectral shape in Fig. 4 (right axis), the spectra in Fig. 3 were normalized to their peak value. One normalized absorbance spectrum representative of the dilute solution of this sample was measured at room temperature (293 K) by a UV-visible spectrometer. The results are shown in the left axis of Fig. 4, with the absorption maximum around 563 nm. The emission maximum around 595 nm monotonically shifts to shorter wavelengths with decreasing temperature, as shown in the inset (left axis). The full width at half maximum (FWHM) broadens (inset, right axis) as the temperature increases, which is consistent with the behavior of the ratio of the integrated intensity to the peak intensity depicted in

FIG. 4. Temperature dependent normalized fluorescence (upper) of RhB in a solution of CuCl_2 in glycerol, for a selection of temperatures (right axis) and normalized absorbance spectrum measured at 293 K (left axis). The lower figure shows the redshift of the emission maximum (solid circles, left axis) of the fluorescence spectra, and broadening (hollow circles, right axis) of the FWHM as the temperature increases.

the inset of Fig. 4. The blue-shift covers 9 nm over a temperature range of nearly 80 K, around 0.1 nm/K, comparable to the observation in some quantum dots (QDs) which have also been used for accurate thermal sensing in the past.^{36,37} This shift occurs because the rate of relaxation of the fluorophore to a solvent³⁵ is strongly dependent on temperature because the solvent viscosity increases dramatically with decreasing temperature.³⁸ At high temperatures, the relaxation time is much shorter than the fluorescence decay time. However, at low temperatures the relaxation time can no longer be neglected compared to the fluorescence decay time (due to increased solvent viscosity), and fluorescence from non-relaxed solvent configurations occurs, leading to a blue shift of the emission spectrum. Briefly speaking, the increase of viscosity with cooling leads to increasing fluorescence contributions of non-relaxed states and thus blue-shift.^{39,40}

In addition to the main peak, the spectra seem to contain an underlying lower and broader peak with a maximum around 640 nm, which becomes more pronounced with decreasing temperature. The emission band around 640 nm corresponds to the absorption band around 530 nm, which is shown in the left axis of Fig. 4. This can indicate a vibrational progression of an involved transition from the zeroth vibrational level of the excited state to the first vibrational level of the ground state (a difference between 595 and 640 nm corresponds to 1182 cm^{-1} , which is close to the distance between the 0–0 and 0–1 transitions in the absorption spectra, 1106 cm^{-1}). In this case, the more pronounced 640 nm band at low temperatures is merely due to the fact that bands become narrower at low temperatures. However, effects of dimerization cannot be excluded,^{41–44} as the concentration of the RhB in the strongly hydrogen bonding solvent is quite high. In this case, the emission band around 640 nm can be due to dimers. Decreasing the temperatures will, on the one hand, increase the dimerization and, on the other hand, increase the fluorescence quantum yield of the dimers, increasing the importance of the 640 nm band at lower temperatures. It is at present difficult to determine to which extent each of both scenarios play a role.

In any case, spectral features like FWHM, emission maximum, and shape are independent of the laser power fluctuation and drifts of the optical elements. They can therefore be used as a reliable source of information for temperature extraction.

III. TEMPERATURE RETRIEVAL BY NN RECOGNITION

Fluorescence-based thermometry requires one calibration procedure to link the employed spectral information and the corresponding temperature. In the literature, it is mostly done by polynomial fitting the curve of temperature dependent fluorescence intensity, decay time, or line shift. Recently, an alternative approach on the basis of the NN recognition algorithm has been reported.²⁷ This approach allows for the combined exploitation of different features of fluorescence spectra simultaneously, rather than being limited to only one correlation, thus improving the temperature retrieval performance. In NN recognition, parameters of an *ad hoc*-chosen NN function are optimized in order for the function to

adequately convert numerical input information to a numerical output, namely, the parameter to extract. In the case of fluorescence-based thermometry, the numerical fluorescence information serves as NN input, while the NN output yields the sample temperature to be recognized. The search for the optimum set of NN parameters can be done iteratively by minimizing the sum of square differences at each iteration, as defined in Eq. (2), between the predicted temperature values, T^{NN} (on the basis of the corresponding input fluorescence data, FL) and the respective true temperature values, T^{true} (known from the calibration). In Eq. (2), N_{tr} represents the number of training examples.

Fig. 5 shows the one-hidden-layer NN architecture used in this work. It consists of N_{ne} hyperbolic tangent input neurons and one linear output neuron, as defined in Eq. (3). For a given number of NN inputs (N_{in}) and a set number of neurons, the NN is fully determined by the weights of the hidden neurons ($v_{j,k}$) and output neurons (w_k). $v_{0,k}$ and w_0 represent the bias nodes in the hidden layer and output layer, respectively. In this work, the number of hidden neurons, N_{ne} , was fixed at 2.

$$e^2 = \sum_{i=1}^{N_{tr}} \{T_i^{NN}[FL] - T_i^{true}\}^2, \quad (2)$$

$$T_i^{NN}[FL] = w_0 + \sum_{k=1}^{N_{ne}} w_k \times \tanh \left[v_{0,k} + \sum_{j=1}^{N_{in}} v_{j,k} \times FL(j) \right]. \quad (3)$$

In our implementation, a large set (8250) of experimentally obtained pairs of spectra and temperatures were used as examples to train and test the NN. The spectra were recorded while stabilizing the temperature at 11 values between 234 K and 311 K, in a stepwise temperature scan, by means of the setup shown in Fig. 1. 750 pairs of spectra and temperatures were recorded at each target temperature. 80% of the examples ($N_{tr} = 6600$) were randomly taken for training the NN and the remaining 20% constituted the test set used for validating the NN. This procedure, which involved randomly shuffling the training and test examples, was repeated several times for cross-validation thus to avoid the possibility of over-training.

Three types of NNs, discriminated by utilizing different compact sets of fluorescence spectral information from the emission band between 575 nm and 675 nm, were investigated.

FIG. 5. Data flow of a NN architecture with one hidden layer used in the current fluorescence-based thermometry implementation.

The first type of NN (from here on referred to IP NN or Integrated and Peak NN) was based on the integrated intensity and peak intensity of the emission band only (input neurons $N_{in}=2$), which is representative of the conventional calibration procedure of polynomial fitting of the fluorescence integrated/peak intensity. The second type (from here on referred to as SP NN or shape NN) made use of spectral shape associated information, which contains the emission maximum, FWHM, and values of the normalized spectra at 60 wavelengths between 575 nm and 675 nm (input neurons $N_{in}=62$). In the third type of NN (multi-band NN), the fluorescence emission spectrum from 575 nm to 675 nm was split into five bands with a bandwidth of 20 nm, and the integrated intensities of the each of the five bands was employed as the NN input (input neurons $N_{in}=5$).

The reconstruction performance of each NN is depicted by box-whisker-plots of the reconstruction errors ($T_{NN}-T_{true}$) at each calibration temperature, as shown in Figs. 5(a)–5(c), respectively, for IP NN, SP NN, and multi-band NN. Training examples are presented in the upper figure of each plot and test examples in the lower. In our box-whisker-plots, the top/bottom dots represent the maximum/minimum or range of reconstruction error, the diamond represents the mean value of reconstruction errors for the examples located at the same target temperature, and the standard deviation (1σ) determines the height of the box. The horizontal line inside the box represents the median reconstruction error, verifying that the reconstructed data is not skewed.

Fig. 6 shows that the spectral shape-based NN (SP NN) and multi-band NN provide similar reconstruction performances, and that both of them are better than the intensity based NN (IP NN). This is due to the spectral shape being substantially temperature dependent while essentially not affected by mechanical instabilities of the measurement arrangement. It should be noted that the bandwidth of the spectral shape based thermometry is limited by the spectrometer since a longer acquisition time is required, in

comparison with the short timescales associated with photo-detectors used in an intensity-based thermometry. However, the similar reconstruction performance yielded by the multi-band NN suggests a compromise between high bandwidth and low accuracy with the potential to implement a tunable filter to switch the emission band being detected to enable multi-band NN based thermometry. Provided the constitution of the sample is not changed and data are acquired and pre-processed in the same way, the test error is representative for the performance of the trained NN for new spectra. Thus, the mean value of σ at different temperatures for the test examples (0.54 K, 0.27 K, and 0.25 K, respectively, for IP NN, SP NN, and multi-band NN) can be used to describe the overall reconstruction accuracy in the calibration range. Taking into account the integration time (4 ms) of the spectrometer and the number (5) of spectra that were averaged before being fed to the NN, the averaging σ values of test samples correspond to $76 \text{ mK}\cdot\text{Hz}^{-1/2}$, $38 \text{ mK}\cdot\text{Hz}^{-1/2}$, and $35 \text{ mK}\cdot\text{Hz}^{-1/2}$, respectively. The estimated accuracy for both IP NN and SP NN is consistent with the one reported in Ref. 27, $65 \text{ mK}\cdot\text{Hz}^{-1/2}$ and $21 \text{ mK}\cdot\text{Hz}^{-1/2}$, despite using a different RhB concentration (making the fluorescence spectrum quite different) and experimental configuration (in this work the fluorescence light was collected along the path of the transmitted probe laser beam, while in Ref. 27 it was collected with the collecting fiber positioned sideways and perpendicular with respect to the probe beam. This indicates that the reported technique can serve as a very reliable calibration tool in fluorescence-based thermometry.

In order to further evaluate the reconstruction performance of the proposed method, we have also implemented the setup in Fig. 1 to monitor dynamic temperature changes. An infrared pump laser (Vector, Coherent®) was used to photo-thermally induce a temperature change, by taking advantage of the enhanced absorption of the sample to the infrared light by CuCl_2 . The power of the laser light, containing collinear infrared (wavelength 1064 nm) and green (532 nm) beams,

FIG. 6. Performance comparison of three types of NNs based on different compact sets of spectral features. The box-whisker-plots in (a), (b), and (c) represent the temperature reconstruction error on the basis of IP NN, SP NN, and multi-band NN, respectively, for training examples (upper) and test examples (lower) at different calibration temperatures. Plot (d) shows the reconstruction of photothermally induced temperature evolutions (left axis), under sinusoidal modulation (pump intensity right axis), by IP NN (light gray), SP NN (dark gray), and multi-band NN (black).

was modulated by using an IntraAction[®] acousto-optic modulator (AOM). The first-order diffracted beam exiting the AOM was split into a 1064 nm pump beam and a 532 nm reference beam. The infrared component illuminated and heated the sample and the transmitted light through the sample was blocked by a shortpass filter. The 532 nm reference beam was sent to the fiber detector entrance as well, so that the corresponding spectral peak could be used to monitor the pump laser modulation via the spectrometer. The power of the pump laser was sinusoidally modulated at 0.1 Hz between 42 mW and 123 mW. The laser power was measured by a PM100D, Thorlabs[®] power meter placed just in front of the optical window of the cryostat. The pump laser beam, with diameter 3 mm, was aligned to overlap the green probe laser in the bulk sample cuvette (optical path 1.0 mm, with a total surface $45(L) \times 12.5(W)$ mm²). While photothermally exciting the sample, the spectrometer was continuously collecting spectra, which contained both a fluorescence and reference contribution, at a sampling frequency of 50 Hz. The left axis in Fig. 6(d) shows the temperature evolutions reconstructed by using IP NN (light gray line), SP NN (dark gray line), and the multi-band NN (black line). The black line in the right axis is, for reference, the pump intensity in arbitrary units. The reconstructed temperature evolutions by different types of NNs are overlapping. However, the fluorescence multi-band NN and SP NN yield a lower noise level compared to the IP NN, confirming the interpretation of the temperature reconstruction error in Figs. 6(a)–6(c). Note that the found temperature evolutions could not be validated by thermocouple measurements. The response time of thermocouples is too slow to be synchronous with the solvent temperature, and the temperature registered by a thermocouple in the pump laser beam would be affected by additional heating due to direct illumination.

IV. PHOTOTHERMAL APPLICATION OF FLUORESCENCE BASED THERMOMETRY IN FREQUENCY DOMAIN

By probing and analyzing optically excited temperature changes in materials, photothermal techniques⁴⁵ are used to determine thermal and/or optical properties of a sample in a wide range of in material characterization applications,⁴⁶ chemical analyses,^{47,48} and environmental research.^{49–51}

In the following, we demonstrate the concept of spatially resolved detection of photothermally induced periodical temperature changes in a semitransparent sample by fluorescence-based thermometry, by taking advantage of its feasibility for remote, local, and fast temperature monitoring. The experimental configuration in Ref. 27, shown in Fig. 7, was employed. An infrared pump laser (Vector, Coherent), whose intensity was sinusoidally modulated by using an acousto-optic modulator, was used to excite the photothermal response of the sample, while a green laser (532 nm, Samba 100, Cobolt) was used to evoke fluorescence in the sample, a RhB (2×10^{-6} M) and CuCl₂ (0.1 M) dyed glycerol solution. The sample was sealed in a cuvette (optical path length 10.0 mm, total surface $45(L) \times 12.5(W)$ mm²), which was embedded in a copper sample holder, situated inside an optical cryostat. The temperature

FIG. 7. Experimental setup for spatially resolved detection of photothermally induced temperature oscillations in a semitransparent sample. The sample cuvette (10 mm optical path) was situated inside a vacuum cryostat. The path of the green excitation laser (circled in the inset) was imaged by a lens (focal length = F) onto the entrance of the spectrometer fiber, which was mounted on a translation stage scanning the Z axis. M, mirror; IF, interference filter; L, lens; DM, dichroic mirror; BT, beam trap; and BP, band-pass filter.

control of the sample cell was accomplished using the same approach as in Fig. 1, except for the PT1000 thermometer read by an HP34401A multimeter, which here was directly attached to the wall of the cuvette with no thermocouple present. The path of the green excitation laser (inset, Fig. 7) was imaged from the right side (perpendicular to the incident light) by a lens ($F = 100$ mm, $D = 50.8$ mm) with $NA = 0.25$, onto the entrance of the spectrometer fiber, and sent through a fiber to a spectrometer (USB 4000 Ocean Optics), where the fluorescence spectrum was recorded. Both the object distance and image distance were 200 mm ($2F$ configuration) in order to have an equal-sized image. The spectrometer was mounted on a micrometer-driven translation stage, allowing for scans of the detection location of temperature oscillations at different distances from the cuvette-sample interface, where the pump and excitation laser were entering the sample liquid. The power of the probe laser was about 3 mW and the pump laser power was modulated sinusoidally between 0 mW and 300 mW. The probe and pump laser beams, with diameters 0.8 mm and 5 mm, respectively, were aligned to overlap in the bulk of the cuvette. While photothermally exciting the sample, the spectrometer continuously collected the spectra at a sampling frequency of 50 Hz. The recorded spectra contained both a fluorescence and reference contribution, similarly as in Section II.

The frequency dependent photothermal response at three locations at different distances (Z_1 , Z_2 , and Z_3) from the sample from the front cuvette-sample interface where the probe and pump beam entered the sample compartment was determined by the proposed fluorescence-based thermometry approach. The distance between each position was fixed at 0.5 mm by the micrometer of the translation stage. Given that the thermal diffusion length at 0.05 Hz, calculated by Eq. (4)

$$\mu = \sqrt{2\alpha/\omega} \quad (4)$$

(with $\alpha = 9.5 \times 10^{-8} \text{ m}^2/\text{s}$ the thermal diffusivity of glycerol and $\omega = 2\pi f$ the modulation angular frequency) is 0.78 mm, much smaller than the radius of pump beam (5 mm), the thermal diffusion model of a 1D multilayered flat⁵² could be employed to fit the experimental data. Fig. 8(a) shows the frequency dependent amplitude and phase signals at distance Z2 (circles) and Z3 (triangles), normalized to the signal at Z1, and corresponding best fitting curves (solid lines). The data were normalized by amplitude division and phase subtraction. Two fitting parameters were used for the fit: the distance Z1 from the cuvette-sample interface, and the optical absorption (β) coefficient, which was measured to be 3.8 Neper cm^{-1} by the optical transmission method at room temperature. The thermal properties of glycerol (thermal conductivity $0.28 \text{ W m}^{-1} \text{ K}^{-1}$ and thermal diffusivity $9.5 \times 10^{-8} \text{ m}^2/\text{s}$) and the cuvette material (thermal conductivity $1.38 \text{ W m}^{-1} \text{ K}^{-1}$ and thermal diffusivity $8.2 \times 10^{-7} \text{ m}^2/\text{s}$), along with the cuvette wall thickness (1.25 mm) were fixed as known parameters. The contour plot in Fig. 8(b), representing the dependence of the normalized sum of squared fitting errors on the two fitting parameters, reveals a satisfactory fitting quality. This confirms the feasibility of fluorescence-based thermography to determine the photothermally induced temperature variations at different locations, with the spatial resolution determined by the entrance aperture of the spectrometer used (in this work the fiber aperture was $100 \mu\text{m}$), and to extract the underlying thermal and optical material parameters.

V. PHOTOTHERMAL APPLICATION OF FLUORESCENCE BASED THERMOMETRY IN TIME DOMAIN

The thermal impulse response of a sample to heat input can be obtained by applying an impulse perturbation and directly monitoring the evolution of its temperature response. Methods involving pulsed light sources have also been widely used in photoacoustic and photothermal spectroscopy, in particular for spectroscopy of weak absorption samples, due to the fact that the high peak power of the laser pulse is capable of producing a good signal-to-noise ratio for samples with low optical absorption density. By simply replacing the CW pump laser in Fig. 7 with a pulsed ND: YAG laser (Lab-130-10, Quanta-Ray[®]), as shown in Fig. 9,

FIG. 9. Experimental setup for time-resolved photothermal and photoluminescent spectroscopy. M, mirror; L, lens; DM, dichroic mirror; and BP, bandpass filter.

we have tried to demonstrate the concept of using fluorescence thermometry to perform pulsed laser induced photothermal detection in the time domain.

In Fig. 9, the probe and pump laser beams, with diameters of 0.8 mm and 3 mm, respectively, were aligned to overlap in the bulk of the cuvette, the probe laser power was 3 mW, and the pulse energy of the pump laser, measured by a laser pulse energy meter (NOVA II, OPHIR[®]), was 65 mJ. Fig. 10 depicts the gradual temperature rise resulting from repeated pulsed laser heating, on a long time scale, up to 100 s. The pump laser pulse repetition frequency was 10 Hz. In principle, the sampling frequency of the spectrometer used can be up to 115 Hz in free-running mode. However, since we were monitoring the temperature evolution on a long time scale in order to keep the amount of stored data within reasonable limits, a function generator was employed to externally trigger the spectrometer to record spectra at a relatively low frequency around 8 Hz. From the acquired sequence of fluorescence spectra, the temperature evolution of interest was reconstructed off-line by using the trained SP NN depicted in Fig. 6. The reconstructed temperature evolutions at different initial temperatures are all characterized by a rapid initial increase due to accumulation in the probed

FIG. 8. Experimental data and the best fit of the photothermal signal at two distances from the cuvette wall through which the probe and pump beam entered the sample compartment. (a) Frequency dependent amplitude (upper) signals and phase (lower) signals at distance Z2 (circles) and Z3 (triangles), normalized to Z1 by amplitude division and phase subtraction, and the corresponding best fit (solid line) by a 1D layered thermal wave model. (b) Contour plot of two fitting parameters Z1 ($0.77 \pm 0.03 \text{ mm}$) and absorption coefficient β ($334 \pm 14 \text{ m}^{-1}$).

FIG. 10. Reconstruction of temperature evolution induced by repetitive laser pulses at several initial temperatures. The fluorescence spectra from which the sample temperature evolution was reconstructed were collected at 8 Hz sampling rate. Due to the high repetition rate of the laser pulses (10 Hz), the used time scale of several tens of seconds does not allow to resolve the individual temperature increase steps.

sample region of the repeated heat flux inputs supplied by the optically absorbed laser pulses. Gradually the temperature evolutions tend to stationary value, which is determined by the balance between the laser heat supply on the one hand, and heat losses by conduction and convection out of the illuminated region towards cooler parts of the sample and the sample holder on the other. The demonstrated remote temperature monitoring approach can, e.g., be useful for monitoring the temperature evolution during a chemical reaction in a semitransparent system.⁵³ Spatially resolved (3D) temperature monitoring can also be useful in the emerging research field of photothermal therapy,^{54,55} in which laser light, ultrasound, or microwave absorption are induced heat to kill targeted malignant cells. Real time monitoring of the temperature distribution of the target region would be highly beneficial for tuning the intensity and duration of the excitation light source such that the heating is localized in the target tissue, with minimum impact on the surrounding healthy tissue.

We have also verified the feasibility of detecting the temperature decay in glycerol shortly after individual pump laser pulses, as shown in Fig. 11, where the measurement was initialized at 247 K, the spectrometer was free-running at its maximum sampling rate of about 115 Hz, while the pump laser was controlled by a homemade Labview program to send a pulse every 7 s, thus allowing sufficient time for the laser induced temperature change to totally vanish. Fig. 11 reveals a temperature jump of about 1 K induced by a single pulse, which is consistent with the estimation, 1.2 K, as calculated by Eq. (5)

$$\Delta T = \frac{Q\beta}{\rho C \pi a^2} \quad (5)$$

with $Q = 65$ mJ the pulse energy, $\beta = 3.8 \text{ cm}^{-1}$ the optical absorption coefficient, $a = 1.5$ mm the beam radius, $\rho = 1260 \text{ kg/m}^3$ the density of glycerol, and $C = 2400 \text{ J/kg/K}$ the specific heat capacity of glycerol. The decay of the

FIG. 11. Reconstruction of time-resolved temperature evolution after a single laser pulse and best fit (red) by a single exponential decay.

temperature after the initial sudden rise is due to the supplied heat flowing away from the pump laser light illuminated area towards the surrounding fluid, and from there to the sample holder. The decay time τ_c of approximately 1.4 seconds obtained by exponential fitting (cf. Fig. 11) can be expected to be of the order of the thermal diffusion time needed for heat to diffuse from the middle of the pump beam to the surrounding liquid. Using thermal diffusivity of glycerol $\alpha = 9.5 \times 10^{-8} \text{ m}^2/\text{s}$, a characteristic diffusion distance of $\sqrt{4\tau_c \alpha} = 0.7$ mm is obtained, which is reasonable, given the actual pump radius of $a = 1.5$ mm.

VI. ULTRAFAST OPTICAL THERMOMETRY BY STROBOSCOPIC FLUORESCENCE SPECTRUM ACQUISITION

With respect to applications requiring the monitoring of faster temperature evolutions, the main limitations of the proposed spectral shape-based thermometry approach are (i) the finite fluorescence time, (ii) the 115 Hz sampling rate of the spectrometer in free-running mode, and, for frequency domain applications, (iii) the roughly $1/\omega$ -decrease with frequency of the temperature oscillation magnitude and signal to noise ratio. The first limitation is not very stringent, since the 2.5 ns fluorescence time, measured at 280 K by the time-correlated single photon counting (TCSPC) technique,⁵⁶ of RhB in our sample allows, in principle, for a very large temperature data acquisition bandwidth of 500 MHz. We have circumvented the second and third limitation by employing a stroboscopic approach, which makes the effective time resolution independent of the sampling rate of the spectrometer, and which exploits the possibility of using a pulsed laser approach in order to supply all the optical energy to heat the region of interest within the short time span of interest.

Fig. 12 shows the experimental configuration implementation of the stroboscopic ultrafast fluorescence thermometry approach that we have used for detecting photothermally generated temperature jumps in a RhB ($2 \times 10^{-6} \text{ M}$) and CuCl₂ (0.1 M) dyed glycerol solution. During the measurement the ND: YAG pump laser was working in single shot mode. The flash lamp was continuously firing at 10 Hz, but the trigger for the Q-switch that initiated a laser pulse was only issued about every 4 s (0.25 Hz). The TTL signal that was synchronous with the 10 Hz lamp firing, was sent to a digital delay generator (DG535, Stanford Research®), in

FIG. 12. Experimental setup (a) and timing scheme (b) for stroboscopic fluorescence thermometry.

order to produce, with controllable delay, a TTL signal that was used to trigger the 1 ns probe laser (532 nm PNG-002025-140, JDS Uniphase[®]) pulse that temporarily evoked the fluorescence of the sample. Since the Q-switch buildup time (after trigger) of the probe laser pulse (140 μ s) was shorter than that of the pump laser pulse (170 μ s), this timing scheme allowed the probe pulse to be timed from 30 μ s before until any delay after the pump pulse. The pump beam and probe beam were aligned coaxially inside the sample and the diameter of the pump beam was around 3 mm, substantially larger than that of the probe beam (0.8 mm), in order to approximate a situation of uniform laser heating and temperature field on the time scale of interest. The region trespassed by the probe laser was imaged by a lens ($F = 100$ mm, $D = 50.8$ mm) with $NA = 0.25$ onto the entrance of the fiber of the spectrometer, which was also triggered by the 10 Hz TTL reference signal. The intensity of the probe laser light transmitted through the sample was monitored, together with the fluorescence spectrum, by the spectrometer, with the goal of indicating, synchronous with the acquisition of the fluorescence spectra, whether a pump laser pulse had been fired or not. This approach exploited the thermal lens effect evoked by the pump laser heating on the probe laser beam divergence, which was reflected in the collected probe laser light as an intensity drop.

With the used pump and probe laser types, we had to deal with jitter on the Q-switch buildup time of the respective laser pulses after the electronic trigger. The jitter on the probe and pump pulse timing was, respectively, about 300 ns and 7 ns. This resulted in an initial uncertainty of about 307 ns on the pump-probe delay time. In order to bypass this issue and reduce the effective uncertainty on the value of the

time delay of the probe laser pulse after (or, if negative, before) the pump laser pulse, a fast oscilloscope (LC564A, Lecroy[®]) and two photodetectors (DET364A, Thorlabs) were used to monitor extracted parts of the respective laser beams and accurately record the actual time delay (Δt) between the pump and probe pulses. By virtue of a synchronized acquisition of spectra and oscilloscope signals, each spectrum could be associated with the accurate effective delay time extracted from the oscilloscope signal traces.

At each measurement cycle, as shown in Fig. 12(b), a fixed amount of pump laser pulse energy was deposited (during the 10 ns pulse duration) to the sample, produced an upward temperature step (ΔT). As mentioned in Section V, the probed temperature gradually decayed back to its value before the pump pulse before the next pump pulse was fired. The spectrometer was continuously recording spectra at 10 Hz during the entire measurement. Hence, given the pump laser Q-switch repetition rate of 0.25 Hz (one pump pulse every $t_0 = 4$ s, or equivalently, every $M = 40$ reference periods), M spectra were recorded for each cycle n at time delay values $t_i = \Delta t_n + 100 \text{ ms} \times i$ ($i = 0, 1, 2, \dots, M-1$). For measuring N cycles, a set of $N \times M$ spectra was collected at the following times after the beginning of the cycle

$$\left\langle \begin{array}{c} \Delta t_1 + 100 \text{ ms} \times i \\ \dots \\ \Delta t_n + 100 \text{ ms} \times i \\ \dots \\ \Delta t_N + 100 \text{ ms} \times i \end{array} \right\rangle_{i=0,1,2,\dots,M-1} \quad (6)$$

with Δt_n chosen so as to achieve probe-pump time delays varying between -30μ s and $+10$ ms (see later in this section).

The temperatures for the collection times were reconstructed from the respective spectra by using the spectral shape neural network, as described in Section III. The first column of the reconstructed temperature matrix (for $i = 0$), $T(\Delta t_1, \Delta t_2, \dots, \Delta t_n, \dots, \Delta t_N)$, represents a fast temperature evolution between Δt_1 and Δt_N . Temperatures from the 2nd column to the last column at each row of the matrix represent the slow temperature decay, approximately from 100 ms to 4 s, of each cycle, in steps of 100 ms. Due to the fact that the temperature rise evoked by each pump laser pulse disappears completely before illumination by the next pump pulse, the data acquisition scheme allows one to average the time evolution of the temperature after pump laser excitation by combining data from multiple cycles $T(i \times 100 \text{ ms})$. By merging and sorting temperatures reconstructed from all matrix columns, the full temperature evolution, $T(\Delta t_1, \Delta t_2, \dots, \Delta t_n, \dots, \Delta t_N, 100 \text{ ms}, 200 \text{ ms}, \dots, 100 \text{ ms} \times i, \dots, t_0)$, induced by a nano-second laser pulse could be obtained.

Fig. 13 shows the result of a measurement sequence performed according to the above algorithm, in RhB and CuCl₂ dyed glycerol at 240 K. The effective delay time was chosen between -350 ns (Δt_1) and $+10$ ms (Δt_N), in logarithmic steps with 250 values in total. The integration time of the spectrometer was set at 40 ms, larger than $\Delta t_N = 10$ ms, so that the spectrometer integration period included the periods during which the (delayed) probe pulse was evoking the

sample to fluoresce. The red curve in Fig. 13(a) shows the full reconstructed temperature evolution, which consists of a series of values at the mentioned 250 short probe-pump time delays between Δt_I (−350 ns) and Δt_N (40 ms), and 50 averages of temperatures reconstructed from spectra acquired at long probe-pump delay times between 100 ms and 4 s in steps of 100 ms. The total acquisition time of the data was 250×4 s or about 17 min. Adequate averaging of the 250 stroboscopically obtained values for long delay times requires the repeatability of the temperature evolution after the pump pulse during the 17 min acquisition period.

Fig. 13(a) also shows the time evolution of a reference temperature (blue), which was taken every 4 s for a probe-pump-delay of −100 ms, thus long after the previous pump laser pulse, after the disappearance of the laser induced temperature rise, and shortly before and not affected by the pump laser pulse of interest. The purpose of collecting the reference temperature evolution is to monitor the long term evolution of the sample temperature during the 17 min that were needed to collect the data needed to reconstruct the time dependence of the pump laser pulse induced temperature transient. By subtracting the reference temperature trace from the transient temperature trace of interest, fluctuations of the sample temperatures could be removed from the data, as illustrated in Fig. 13(b). The temperature response to a pump laser pulse is characterized by a sudden jump that happens within about 10 ns, and only after about 100 ms, the temperature starts to decay as a consequence of heat diffusing out of the excitation area to the cooler surroundings. By averaging multiple measurements of 20 times, a noise reduced signal (black) with error bars is presented in Fig. 14. The noise on the (not averaged) temperatures acquired for the 250 short time delays is about 200 mK rms. This can be compared with the 38 mK rms noise in the frequency domain data for 1 Hz bandwidth, by taking into account the number of probe laser photons used to evoke fluorescence in both cases. In the pulsed case, the number of photons per pulse was $N_{ph} = 25 \mu\text{J}/(h\nu) = 6.7 \times 10^{13}$, with h Planck's constant and ν the optical frequency of the pulsed probe laser. Translating to an equivalent frequency domain experiment with $P_{probe} = 3$ mW as probe laser power, this

FIG. 13. (a) Reconstructed temperature evolution for probe-pump delay times between −350 ns and +4 s (red). 200 early times between 0 ns and +10 ms (Δt_N) are logarithmically spaced. 50 times average of longer delay times were linearly spaced between 100 ms and 4 s. The corresponding reference temperature (see text) is shown in blue. (b) The subtracted temperature evolution.

FIG. 14. Noise reduced signal by averaging multiple measurement of 20 times.

would correspond to an equivalent acquisition time $\tau_{acq,FD} = N_{ph}h\nu/P_{probe}$, and an acquisition bandwidth $f_{FD} = 1/\tau_{acq,FD} = 120$ Hz. The noise level of 200 mK rms for this 120 Hz bandwidth thus corresponds with a noise level of $200 \text{ mK}/120 \text{ Hz}^{1/2} = 18 \text{ mK}/\text{Hz}^{1/2}$.

The proposed method for fast stroboscopic thermometry offers an adequate approach to investigate the heat capacity relaxation behavior of glass forming liquids⁵⁷ that results from the finite relaxation time needed for cooperative molecular motions to respond to a stimulus (e.g., mechanical force, aligning electric field, heat input,...) in a glassy network.⁵⁸ With the current setup, the temporal resolution of 10 ns results in a bandwidth of 100 MHz, which is 100 times higher than state of the art techniques to determine the frequency dependence of the heat capacity of relaxing materials.⁵⁷ As mentioned above, by employing a probe and pump laser type that allows for more adequate timing control and synchronization with a minimum of jitter, the bandwidth offered by the stroboscopic approach is only limited by the laser pulse duration and the fluorescence time of the thermochromic dye, the latter being of the order of a few nanoseconds.

VII. CONCLUSION

The application of NN recognition in fluorescence-based thermometry was demonstrated on a RhB dyed mixture of CuCl_2 and glycerol. The approach exploits the advantages of NN recognition, taking into account temperature dependent spectral features in an optimum way. Three types of NN were presented: an intensity based NN representing the conventional fluorescence intensity based thermometry, a spectral shape based NN utilizing the combination of spectral shape associated fluorescence features (emission maximum, FWHM, and a selection values of the normalized spectrum), and a multi-band based NN where the emission spectra is split into 5 bands and the integrated intensities of each band are used. The spectral shape based NN shows higher reconstruction accuracy ($38 \text{ mK} \cdot \text{Hz}^{-1/2}$) than the intensity based NN ($76 \text{ mK} \cdot \text{Hz}^{-1/2}$). The similar reconstruction accuracies of the multi-band NN to the spectral shape based NN, $35 \text{ mK} \cdot \text{Hz}^{-1/2}$, provides a compromise between the high accuracy and low bandwidth of temperature measurement, since the spectral shape based thermometry requires a longer data acquisition time. A proof of concept of spatially resolved detection of photothermally

induced temperature oscillations, with spatial resolution determined by the entrance aperture of the fluorescence collecting optical fiber (100 μm in this work), was demonstrated by determining the frequency dependent photothermal response of the sample at different depths inside the sample, based on fluorescence-based thermometry. The satisfactory fitting of the experimental data suggests that the technique has the perspective to be applied to profile the thermal properties and optical properties of semitransparent systems and can further be extended to perform 3D tomography. We have also demonstrated the concept of using fluorescence thermometry to perform time-resolved photothermal detection, which is, on one hand, the determination of a gradual temperature rise due to the thermal accumulation of heat flux portions supplied by repetitive laser pulses and, on the other hand, the determination of the transient temperature evolution after a single pump laser pulse. With respect to applications for faster temperature evolutions, the proposed spectral shape-based thermometry approach in a CW-probe configuration is limited by the 115 Hz sampling rate of the spectrometer and by the signal to noise decay with frequency due to the $1/\omega$ dependence of photothermally induced temperature modulation signal in the case of uniform heating. However, by making use of pulsed laser excitation or by implementing multi-band thermometry with a tunable filter, larger temperature variations combined with a large bandwidth can be achieved, offering the possibility for fast thermometry in a stroboscopic implementation. The presented stroboscopic approach allows remote temperature detection in a very large temporal bandwidth, from 10 ns to 4 s, where the lower limit reflects the specifications on the timing control of the used pump and probe laser pulse triggering, but which is intrinsically only limited by the pump and probe laser pulse duration and the fluorescence time of the used thermochromic agent (here 2.5 ns for RhB). Fast stroboscopic thermometry can be used to study the temperature response of glassforming liquids to impulsive photothermally induced heating, and to derive therefrom the broadband relaxation behavior of the heat capacity. It should be noted that the proposed approach for remote fluorescence-based thermometry is generic. Research on an implementation that makes use of thermosensitive luminescence is ongoing. The proposed technique offers the possibility of detecting and monitoring relaxation behavior of the temperature response in glycerol and other glass forming materials, where it can be expected that the pulsed optical heating first induces a larger temperature rise followed by a decay of the temperature due to the slow degrees of freedom taking up thermal energy. The onset time of the decay corresponds to the relaxation time of the heat capacity of the system.⁵⁹ The investigation of this relaxation phenomenon and its dependence on the DC temperature is the topic of ongoing research.

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