Research article

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Nanosecond-order long–short fluorescence lifetime switchable encryption with enlarged coding capacity

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Abstract: The turn-off fluorescent photoswitches for information encryption are constantly being developed. However, there are no reports about time-switchable (fluorescence lifetime-switchable) encryption to overcome the limitations of tunable encoding numbers in spectrally and temporally encoded libraries. Based on the double-exponential fitting of fluorescence lifetime, we propose, a fatigue-free and highly flexible switch between the amplitude-weighted average fluorescence lifetime (τ_m) and the intensity-weighted average fluorescence lifetime (τ_i), which will realize the supermultiplexed fluorescence lifetime switchable encryption. The potentially enormous library of different fluorescent lifetime combinations would facilitate the development of information security.

Keywords: anti-counterfeiting; fluorescence lifetime; optical multiplexing; photo-switch; time-switch.

1 Introduction

Optical encryption strategies generally utilize fluorophores and play an important role in encryption technology [1, 2]. To expand the number of encoding levels and improve

the security of fluorescence encryption, a method was developed that can generate a turn-off photoswitchable fluorescence property, unlocking additional multiplexing levels unattainable by conventional decoding methods. At present, photoswitching fluorescence encryption is popular based on the principles of Forster resonance energy transfer (FRET) [3], luminescence resonance energy transfer, the excited-state intramolecular proton transfer (ESIPT) process [4, 5], photochromic compounds (photocyclization reaction), such as spiropyrans, diarylethenes, and spirooxazines and photobleaching (by varying combinations of different photostable dyes) [6–8]. The above-mentioned photoswitches are mostly carried out under alternative illumination of UV and visible light; therefore, the irradiation time, thermo- and photo-stability, cyclability (photo-reversibility), fatigue resistance, switching speed and efficiency due to the energy transfer crosstalk [9] need to be considered. In addition, a single-fluorophore supramolecular system also can generate multicolor switchable fluorescent emissions at solvent control (amphiphilic self-assembly) and chemical signal control (γ -cyclodextrin mediated host-guest recognition) [10].

Similar to color, fluorescence lifetime is an intrinsic property of fluorescence, which is independent of light irradiance and intensity. A precise tailoring of lifetime at an emission band (or color) can entail a virtually unlimited number of unique temporal codes [11, 12]. To date, a tunable fluorescent lifetime switch has not been proposed for information encryption. A selection of organic dyes of varying types and the use of lanthanide-doped upconversion nanoparticles (UCNPs) and perovskite nanoparticles (NPs) with size- and dopant concentration-engineered lifetimes have been reported for lifetime-encoded or temporal multiplexed imaging, allowing time-domain discrimination of these codes in cells and in patterned structures [13–17]. However, the weak absorption and long radiative lifetimes of UCNPs may significantly limit their use in fast imaging applications, and a robust approach is required to rapidly and accurately measure the luminescence lifetimes from the relatively slow-decaying signals.

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Therefore, Liu et al. [18] achieved fast upconversion emission (sub-2 μ s lifetime) by coupling the emission of UCNPs to gap-mode plasmonic nanoresonators. Jin et al. [19] used an algorithm to rapidly and accurately measure the luminescence lifetimes for the microsecond region.

Similar to changing the doping ratio of the lanthanide of UCNPs or adjustment of the halide ion of perovskite NPs, changing the solvent of the dye can also adjust its fluorescence lifetime to realize lifetime-based encryption. Additive mixing, that is, changes in the overall emission color of a sample achieved by mixing two or more individual fluorophores in different ratios has been successfully applied [20]. Huang et al. [21] tuned the emission color of a single-component molecular crystal by varying the excitation wavelength, allowing to visually detection of specific wavelengths in the UV region. In addition, UCNPs and perovskite NPs with multiple emissions can achieve photoswitchable encryption by adjusting different excitation wavelengths, for example, UCNPs emission spectra of the $\text{NaYF}_4\text{: xYb, 0.2\%Tm}$ nanoparticles ($x = 20, 50, 80, 99.8\%$) under 980 nm excitation resulted in four emission peaks being detected in the range of 300–500 nm. Moreover, by polar solvents (methanol) impregnation and halide salt conversion (MABr spraying), as the encryption and decryption agents, respectively, the luminescence of the perovskite NPs can be quenched and recovered, leading to reversible on–off switching of the luminescence signal for multiple information encryption and decryption processes [22]. However, the multicolor emission of UCNPs renders the optical crosstalk between barcodes and label dyes difficult to avoid [23]. At the same time, UCNPs and perovskite NPs can also carry out the corresponding fluorescence lifetime acquisitions for lifetime switchable encryption. However, to obtain the fluorescence attenuation at different energy levels for the single nanomaterial, it is necessary to change the excitation several times or to adjust the corresponding receiving wavelength. Time-tunable and multicolor emissions under a single excitation (976 nm pulsed laser) have also demonstrated that the longer decay times of the emissions from Tb^{3+} ions in comparison to the emissions from Tm^{3+} guarantee a large delay between the blue and green emissions from the single core–shell UCNPs system [24]. However, the proposed devices require special cameras for the detection of the emissions or pumping sources with tunable powers or pulse widths, which can be expensive [25, 26].

Under the premise of a double exponential fitting, the amplitude-weighted average fluorescence lifetime (τ_m) and the intensity-weighted average fluorescence lifetime (τ_i) can be obtained at the same time. Previous studies found that the commonly used τ_m is more biased to short

fluorescent lifetime components and τ_i is dominated by the longer fluorescence lifetimes component [27], but usually only one of them is used to indicate the fluorescence lifetime of the studied subject.

Here, we report a reversible fluorescence lifetime switch of a single dye (eosin) with different solvents. We acquire quickly and freely between τ_m and τ_i to add the fluorescence lifetime switching property. Such fluorescence lifetime-switchable systems have great potential for application in various optical switches and optical antifake labels.

2 Results and discussion

2.1 Long (τ_i)–short (τ_m) switchable lifetime

Xanthene dyes are commonly used as dyestuffs in the food, cosmetics, and textile industries. Eosin is a xanthene derivative and finds many applications, but its high fluorescence emission is often overlooked. As shown in Figure 1, following the addition of different volume ratios of propylene glycol (10, 30, 100% v/v) or N,N-Dimethylformamide (DMF) (10% v/v) to change the polarity of the eosin aqueous solution (100 $\mu\text{g/mL}$), the fluorescence attenuation of eosin can be adjusted (Figure 1A). We use τ_m (Figure 1B) and τ_i (Figure 1C) to obtain the fluorescence lifetime histograms of the binary mixed solutions consisting of propylene glycol or DMF and eosin aqueous solution, respectively. Interestingly, each curve of the τ_m and τ_i histograms was pairwise nonoverlapping (Figure 1D), and can switch independently from each other. The τ_m and τ_i histograms with the change in polarity of the binary mixed solutions can be represented visually for the sparse coding. Previous studies incorporated single color fluorophores into beads and then generated barcodes by conjugating or mixing beads of different colors together [28]. In this work, the full width at half maximum (FWHM) of the normalized τ_m and τ_i histogram was defined as the fluorescence lifetime barcode. In Figure 1B and C, we found that τ_m was shorter than τ_i for the same eosin solutions, and τ_i of eosin solutions with different solvents had a larger time shift than that of τ_m . It is well known that τ_m is directly proportional to the fluorescence quantum yield (and inversely proportional to the extent of quenching). Nevertheless, τ_i is not directly proportional to the fluorescence quantum yield [27]. The calculated τ_m lifetimes were shorter than those of τ_i , especially when short lifetime components were present in intensity decays, such as DY547-SA and Alexa Fluor 532-SA, where the short components contributed significantly to the total emission. Previous studies have shown that the τ_m

Figure 1: Schematic representation of the long (τ_l)-short (τ_s) lifetime switchable encryption.

(A) Different volume ratios of propylene glycol (10, 30, 100% v/v) or DMF (20% v/v) were added into the eosin aqueous solution to control the fluorescence decay of eosin. (B) Double-exponential fitting to obtain the normalized amplitude-weighted average fluorescence lifetime (τ_m) histograms versus different solvents of eosin solutions. (C) Double-exponential fitting to obtain the normalized intensity-weighted average fluorescence lifetime (τ_l) histograms of eosin in different solvents. Short (Long) I is the τ_m (τ_l) histogram of eosin mixed with water and 10% v/v propylene glycol, short (long) II is the τ_m (τ_l) of eosin mixed with water and 20% v/v propylene glycol, and so on. (D) The combination of τ_m and τ_l of eosin in different solvents can be used for switchable encoding. (E) The combination of τ_m and τ_l can be switched for lifetime multiplexing, and the long/short lifetimes can be used to encrypt based on the sparse coding.

decreased approximately tenfold for most fluorophores. The τ_l did not decrease as much as it was dominated by the long lifetime components [29]. Therefore, the use of eosin τ_m in combination with τ_l for supermultiplexed lifetime imaging seems to be a promising strategy to realize long–short fluorescence lifetime switchable encryption (Figure 1E). In addition, different volume ratios of propylene glycol (0, 10, 30, 70, 100% v/v) or DMF (20% v/v) were mixed with eosin aqueous solution (100 $\mu\text{g/mL}$). As shown in Fig. S1, long–short lifetime switching (reversible) provides a method for confidential information encryption and decryption by fluorescence lifetime imaging. To the best of our knowledge, no reports have demonstrated lifetime turnable optical cryptography by switching the τ_m and τ_l lifetimes.

2.2 Long (τ_l) and short (τ_s) lifetimes multiplexing

As shown in Figure 1, a combination of τ_m and τ_l histograms of the binary mixtures of propylene glycol (10, 30,

100% v/v) and eosin aqueous solution (100 $\mu\text{g/mL}$) showed that there was still free available time-domain multiplexing in the range of 1–2.5 ns. In Figure 2, different volume ratios of propylene glycol (0–100% v/v) or DMF (10, 20, 30% v/v) were carefully mixed with eosin aqueous solution (100 $\mu\text{g/mL}$). The fluorescence emission spectrum (Figure 2A1) and decay curve (Figure 2A2) of eosin were measured with the change of polarity of the above binary mixed solutions. A red shift in the emission spectra of eosin was observed with decreasing solvent polarity. Relatively small changes in the fluorescence spectrum were observed for the different solvents, the shortest peak wavelength was found in water and the longest in propylene glycol (100% v/v). Changing the solution solvent provided tunability between ~ 554 nm in water and ~ 563 nm in propylene glycol. For the conventional dye multiplexing system, spectral overlapping is unavoidable because the possibility of overlapping is highly related to the degree of multiplexing in the spectral domain. A higher spectrum resolution system can be adopted to decode and separate highly overlapping spectra [30].

Figure 2: Long (τ_l) and short (τ_m) lifetimes multiplexing.

Different volume ratios of propylene glycol (0–100% v/v) or DMF (10, 20, 30% v/v) were added into eosin aqueous solution (100 $\mu\text{g}/\text{mL}$) for lifetime multiplexing. (A) Changes in fluorescence emission spectrum (A1) and fluorescence decay curve (A2) of eosin solution with the variation of solvent. (B) The binary mixed solutions were injected into the “BiouphotncsGpr” microfluidic alphabet chips to obtain the normalized τ_m (B1) and τ_l (B2) histograms of the corresponding alphabet. (C) The eosin solution parameters injected into the alphabet chips corresponded to the τ_m and τ_l histograms of each alphabet. (D) Fluorescence gray-scale intensity (D1), τ_m (D2) and τ_l (D3) color-coded images of eosin injected into the alphabet chip. The accumulated photon number of the brightest dot in the gray-scale intensity images was 1200. The lifetime pseudocolor image was color-coded from 1 (blue) to 2.5 ns (red). The scale bar is 400 μm .

Moreover, the binary mixtures of eosin aqueous solution and different volume ratios of propylene glycol or DMF were injected into each alphabet of the “BiouphotncsGpr” microfluidic alphabet chips. Figure S2 shows the schematic diagram of the chip size and fluorescence lifetime imaging. The chips were then excited by single photon excitation at 495 nm, and the fluorescence was detected from 520 to 580 nm. Eosin with different solvents which exhibited multidistinguishable emission signals under a single wavelength excitation can minimize the complexity of fluorescent measurement, thereby remarkably simplifying the instrumentation requirements, and allowing a simultaneous excitation of all fluorophores involved in the chips.

Moreover, eosin solution with a variation in solvent had a pronounced double-exponential decay. The normalized τ_m (Figure 2B1) and τ_l (Figure 2B2) histograms of different eosin solutions injected into each alphabet chip were obtained. It can be seen that, both eosin τ_m and τ_l of the binary mixed solutions became longer with increased volume ratio of propylene glycol or DMF. The fluorescence lifetime is the inverse of the sum of the radiative rate constant and the nonradiative rate constants for the excited state depopulation processes. The increase of the solvent polarity decreases the fluorescence lifetime value of eosin owing to an increase in the rate of nonradiative deactivation [31]. Figure 2C shows the injected solvent parameters and these

parameters corresponded to the curves of lifetime histograms. The resulting decay data were then analyzed by fitting a double-exponential decay to the decay for each pixel in the field of view to generate a false-color lifetime image. The eosin injected into each alphabet chip is thus endowed with a range of identifiable color codes individually assigned to a specific solvent. From top to bottom, Figure 2D shows the fluorescence gray-scale intensity (Figure 2D1), τ_m (Figure 2D2), and τ_i (Figure 2D3) color-coded images of different eosin solutions in the “BiophotncsGpr” microfluidic alphabet chips, respectively. It can be seen from the comparison of these false-color images, differences are clearly visible, and each alphabet had a shorter τ_m and a corresponding longer τ_i .

2.3 Switching the long–short lifetimes for supermultiplexed lifetime

To demonstrate the applicability of controlling the fluorescence decay of a single dye by adding organic solvent and using τ_m and τ_i to achieve lifetime switching, the robustness of the combination of τ_m and τ_i for the lifetime switch is illustrated by the compatibility of differently substituted organic solvents, as well as DMSO. As shown in Fig. S3, the fluorescence emission spectra (Fig. S3A1) and decay curve (Fig. S3A2) of the binary mixed solutions of eosin aqueous solution (100 $\mu\text{g/mL}$) and different volume ratios of DMSO (10–100% v/v) or glycerol (0, 10% v/v) were measured. In the same way, the above-mentioned binary mixtures were injected separately into the “urGscnthpoiB” microfluidic alphabet chips, and the normalized τ_m (Fig. S3B1) and τ_i (Fig. S3B2) histograms of the different eosin solutions injected in each alphabet were obtained. Figure S3C illustrates the injected eosin solution parameters. Figure S3D shows the corresponding fluorescence gray-scale intensity (Fig. S3D1), τ_m (Fig. S3D2) and τ_i (Fig. S3D3) color-coded images of the “urGscnthpoiB” chips, respectively. For the subsequent optimization of fluorescence lifetime multiplexing for different eosin solvents, we changed the lifetime color-coded range of the alphabets “Biophotonics Group” in Figure 2D2 (τ_m) and Figure 2D3 (τ_i) from 1–2.5 to 1–3.3 ns (Fig. S4), the color represents the fluorescence lifetime. The flexibility in organic solvents emphasizes the generality and modularity of this approach and may potentially also allow combination with other classes of photoswitches or photolabile protecting groups [32]. However, color crosstalk exists in a short lifetime range that diminishes the color gamut, resulting in reduced resolution. It is noteworthy that the captured fluorescence intensity images from the lifetime

measurement belong to grayscale, the false color diagram of fluorescence lifetime can be considered the intensity image encryption.

As shown in Figure 3, the different volume ratios of propylene glycol or DMF were mixed with eosin aqueous solution and then injected separately into “BiophotncsGpr” alphabet chips (Figure 3A). Using τ_m (Figure 3A1), τ_i (Figure 3A2) alone and a combination of τ_m and τ_i (Figure 3A3), the pairwise distinguishable normalized lifetime histograms and the corresponding lifetime color-coded images from the “BiophotncsGpr” alphabet chips were obtained. It should be pointed out that τ_m combined with τ_i to achieve fluorescence lifetime multiplexing has the following multiple typical alphabet combinations: “BBiiooupohntncpr” (Figure 3A3). The τ_m and τ_i multiplexing of the binary mixed solutions of different volume ratios of propylene glycol or DMF and eosin aqueous solution was mainly in the lifetime range of 1–2.5 ns. In order to make the most of fluorescence lifetime space of 1–3.3 ns, the different volume ratios of glycerol or DMSO were mixed with eosin aqueous solution and then injected separately into “urGscnthpoiB” alphabet chips. To make full use of the nanosecond-order lifetime range, we combined the “BiophotncsGpr” with the “urGscnthpoiB” alphabet chips, proper utilization of τ_m (Figure 3B1) and τ_i (Figure 3B2) alone, or both (Figure 3B3), and the numbers of the pairwise distinguishable normalized lifetime histograms and their corresponding lifetime color-coded images were increased in the lifetime range of 1–3.3 ns. Current optical multiplexed technology is limited by a multiplexing ceiling due to existing optical materials. Min et al. achieved 20 distinct Raman frequencies, as a “carbon rainbow”, for optical supermultiplexing by rational engineering of polyyenes, the FWHM of the Raman peaks in the silent spectral window still overlapped [33]. However, as shown in Figure 3B3, we do not need the complicated preparation process and imaging method to obtain the lifetime supermultiplexing (24 distinct fluorescence lifetimes) without the overlapping and ambiguous FWHM.

2.4 Cryptography strategy based on switching the lifetimes

As shown in Figure 4, switching the long (τ_i)-short (τ_m) lifetime realized fluorescence lifetime encryption of the alphabets “Biophotonics Group”. The binary mixed solutions of different volume ratios of propylene glycol or DMF and eosin aqueous solution were successively injected into the “BiophotncsGpr” alphabet chips. The τ_m (Figure 4A1, top) and τ_i (Figure 4A1, bottom) color-coded image of each

Figure 3: Switching the long (τ_l)-short (τ_m) lifetimes for supermultiplexed lifetime.

Different volume ratios of propylene glycol or DMF (light blue, the lifetime range from 1 to 2.5 ns) were mixed with eosin aqueous solution (100 μg/mL) and then injected separately into the "BiophotncsGpr" alphabet chips. (A) Using τ_m (A1) and τ_l (A2) alone or both (A3) to obtain the normalized lifetime histogram of pairwise distinguishable alphabets and their corresponding lifetime color-coded images. (B) τ_m was combined with τ_l to achieve lifetime multiplexing. The eosin solutions dissolved in different volume ratios of glycerol (DMSO)/water mixtures (black, the lifetime range from 1 to 3.3 ns) were injected separately into the "urGscnthpoiB" alphabet chips, and their corresponding τ_m and τ_l histograms were filled with the corresponding colors. For supermultiplexed lifetime imaging of eosin in different volume ratios of propylene glycol, DMF, glycerol or DMSO, the τ_m of the "BiophotncsGpr" alphabet chips was combined with the "urGscnthpoiB" alphabet chips (B1), or the τ_l of the "BiophotncsGpr" alphabet chips was combined with the "urGscnthpoiB" alphabet chips to obtain pairwise distinguishable normalized histograms and their corresponding lifetime color-coded images (B2). The τ_m and τ_l of the "BiophotncsGpr" alphabet chips combined with the "urGscnthpoiB" alphabet chips (B3) were obtained. The black line represents the FWHM.

alphabet chip was used to compose the alphabets "Biophotonics Group", respectively. The FWHM of the normalized τ_m and τ_l histograms of each alphabet constituted the barcodes of the alphabets "Biophotonics

Group", and then the overlapping parts of the FWHM were integrated to obtain the final encrypted barcode (Figure 4A2). As shown in Fig. S5A, the τ_m color-coded images of the alphabets "Biophotoi" and the τ_l color-coded

images of the alphabets “csGroup” can also be used to form the alphabets “Biophotonics Group” (Fig. S5A1) and the corresponding encrypted barcodes (Fig. S5A2). In addition, Fig. S5B1 (B2) shows the use of the “Boponsp (iuhtcGr)” alphabets τ_m color-coded images and “iuhtcGr (Boponsp)” alphabets τ_i color-coded images to form the alphabets “Biophotonics Group” and accordingly the final encrypted barcodes.

In Figure 4A and Fig. S5, the fluorescence color bar was 1–2.5 ns, and the alphabets “o” and “i” were repeatedly

used to form the alphabets “Biophotonics Group”. In order to obtain the 17 fluorescence lifetimes corresponded to the 17 alphabets of the “Biophotonics Group”, the τ_m and τ_i color-coded images of the “BiophotonicsGpr” and “urGscnthpoiB” alphabet chips were used together in the lifetime range of 1–3.3 ns. In Figure 4B1 (top), the alphabets “Biophotonics Group” were composed of the τ_m color-coded images of the “ioo” and the τ_i color-coded images of the alphabets “iuoncpr&GsthpoB”. The alphabets “ioo” and “iuoncpr” were from the “BiophotonicsGpr” chips,

Figure 4: Cipher cryptography strategy based on switching the long (τ_i)-short (τ_m) lifetimes.

(A) Different volume ratios of propylene glycol or DMF and eosin aqueous solution were mixed and then injected into the “BiophotncsGpr” alphabet chips. The alphabets “Biophotonics Group” are formed by using the τ_m (A1, top) and τ_i (A1, bottom) color-coded images, respectively, and the corresponding barcodes are composed of the FWHM of the normalized τ_m and τ_i histograms, respectively. The overlapping part of the lifetimes is integrated to obtain the final encrypted barcode (A2). The τ_m FWHM values of the alphabets “tncdGpr” overlapped with each other (A1, top), so we merged the overlapping values as a lifetime barcode (A2, bottom). The τ_i FWHM values of the alphabets “tncdGpr” showed the same result. (B) Different volume ratios of glycerol or DMSO were mixed with eosin aqueous solution and then injected separately into the “urGscnthpoiB” alphabet chips. The τ_m color-coded images of the alphabets “ioo”, the τ_i color-coded images of the alphabets “iuoncpr” from the “BiophotncsGpr” alphabet chips and the τ_i color-coded images of the alphabets “GsthpoB” from the “urGscnthpoiB” alphabet chips were used to form the alphabets “Biophotonics Group” with the corresponding lifetime barcodes (B1, top) and final encrypted barcodes (B2, top). Similarly, the τ_m color-coded images of the alphabets “ioo”, the τ_i color-coded images of the alphabets “iponcr” from the “BiophotncsGpr” alphabet chips, the τ_m color-coded images of the alphabets “uh”, and the τ_i color-coded images of the alphabets “GsthpoB” from the “urGscnthpoiB” alphabet chips were also used to form the alphabets “Biophotonics Group” with distinguishable fluorescence lifetime of each alphabet, the corresponding 17 lifetime barcodes (B1, bottom) and final encrypted barcodes (B2, bottom) are also shown. The τ_m FWHM value of the alphabet “o” from the “BiophotncsGpr” alphabet chips overlapped with the τ_i FWHM value of the alphabet “s” from the “urGscnthpoiB” alphabet chips (B1, top), so the overlapping FWHM values were regarded as a lifetime barcode (B2, bottom). The τ_i FWHM values of the alphabets “oB” from the “urGscnthpoiB” alphabet chips overlapped each other, so the overlapping FWHM values are integrated to be a lifetime barcode. The alphabets from the “BiophotncsGpr” alphabet chips were represented by light blue, the alphabets from the “urGscnthpoiB” alphabet chips were represented by black.

which were injected into the binary mixed solutions of different volume ratios of propylene glycol or DMF and eosin aqueous solution. The alphabets “GsthpoB” are from the “urGscnthpoiB” chips, which were injected into the binary mixed solutions of different volume ratios of glycerol or DMSO and eosin aqueous solution. Figure 4B2 (top) show the corresponding encrypted barcodes of the alphabets “Biophotonics Group”. Similarly, as shown in Figure 4B1 (bottom), the alphabets “Biophotonics Group” were also composed of the τm color-coded images of the alphabets “ioo&uh” and the τi color-coded images of alphabets “iponcr&GstpoB”. The alphabets “ioo&iponcr” and “uh&GstpoB” were from the “BiouphotncsGpr” and “BiouphotncsGpr” chips, respectively. Figure 4B2 (bottom) shows the corresponding encrypted barcodes of Figure 4B1 (bottom). As shown in Fig. S5C, we can realize the fluorescence lifetime switchable encryption of the alphabets “Biophotonics Group” by measuring changes in the τm and τi of eosin in different solvents. In short, eosin solutions with different solvents were injected into the microfluidic alphabet chips. Using τm and τi alone or both can obtain the different lifetime color-coded images of the alphabets “Biophotonics Group” and the corresponding encrypted barcodes. Upon alternation of τm and τi , the alphabet chip system achieves 100% switching long–short fluorescent lifetime from the eosin solutions with different solvents.

2.5 Switching the long–short lifetimes for cryptography puzzles

The confidentiality of eosin with different solvents can be further enhanced by combining it with graphical encoding and pattern encryption enabled by the microfluidic alphabet chips. As shown in Fig. S6, two distinguishable barcodes were obtained using the FWHM of the normalized τm and τi histograms. For eosin in the “BiouphotncsGpr” alphabet chips (left), using τm and τi alone obtained 7 and 10 pairwise distinguishable barcodes, respectively. However, the combination of τm and τi obtained 14 pairwise distinguishable barcodes. Similarly, for eosin in the “urGscnthpoiB” alphabet chips (right), it was conservatively estimated that using τm and τi alone obtained 10 and 9 pairwise distinguishable barcodes, respectively, while the combination of τm and τi obtained at least 15 pairwise distinguishable barcodes. As shown in Fig. S7, the “BiouphotncsGpr” and “urGscnthpoiB” alphabet chips were combined to obtain the alphabets “uuriioou-psohntncprhthpoB”, and the fluorescence lifetimes between these alphabets were distinguishable from each other in the lifetime range from 1 to 3.3 ns. Moreover, the

alphabets whose normalized lifetime histograms overlap each other and their corresponding lifetime color-coded images are also shown. Optical techniques for image security have triggered considerable interest due to their unique advantages, such as parallel processing and multiple dimensional capabilities. The τi can be calculated from the τm without additional equipment, sample preparation and lifetime acquisition. Consequently, the combination of τm and τi significantly reduced the calculation load in image encoding and decoding (reduced by almost 1/2) and increased the encoding capacity (by approximately twofold) compared with previous studies (τm or τi alone).

To further illustrate the switching of long (τi)-short (τm) lifetime can realize the encryption and hiding fluorescence lifetime information, eosin solutions (100 $\mu\text{g/mL}$) were dissolved in propylene glycol (DMF)/water mixtures and injected into the “BiouphotncsGpr” alphabet chips. As the normalized τi histogram of the alphabet “t” overlaps with the normalized τm histograms of the alphabets “csG”, the encoded alphabets “Group” was visible only when the fluorescence lifetime was calculated using the τm , but if the τi was used to decode these messages, this just produced a subsequent steganography of the encoded message. As shown in Figure 5A, the true information obtained from the τm color-coded lifetime image of the alphabets “Group” can be hidden as cryptography puzzles “troup”, “croup” and “sroup”, respectively. In addition, the normalized τm histograms of the alphabets “pr” overlapped with the τi of the alphabet “n”. The real information obtained from the τm color-coded image of the alphabets “roup” in Figure 5B can be hidden as “noup”, “roun”, and “noun” puzzles, respectively. Likewise, the normalized τm histogram of the alphabet “u” was very similar to the τi of the alphabet “o”, which could also be used for encryption by hiding the message; thus, the real information obtained from the τm color-coded image of the alphabets “out” can be hidden as “oot”. By combining the τm color-coded image of the alphabets “pr” in Figure 5B with the τi color-coded image of the alphabet “n”, the real information “oops” in Figure 5C can be hidden as fake alphabets “ours” and “oont”, respectively. This lifetime-switched method can be combined with Julius Caesar’s method, who employed one of the first known ciphers, a system that involved a shift three letters to the right: for example, a plain text Z would become a C, an A a D, and so on. Long (τi)-short (τm) switches significantly improve the anti-counterfeiting performance, offering a method for multi-level authentication. We developed a long–short time-switchable steganography scheme to hide secret messages, opening an era of fluorescence lifetime cryptography that

Figure 5: Switching the long (τ_l)-short (τ_s) lifetimes for cryptography puzzles.

One alphabet has long (τ_l)-short (τ_s) two fluorescence lifetimes. Different alphabets can obtain the same fluorescence lifetime by adjusting the short-long lifetimes. The eosin solutions dissolved in propylene glycol (DMF)/water mixtures were injected into the “BiouphotncsGpr” alphabet chips. The alphabets with the overlapping normalized τ_m or τ_i histograms were used to realize hidden information and encryption. (A) The normalized τ_i histogram of the alphabet “t” overlaps with the τ_m of the alphabets “csG”, and the real information obtained from the τ_m color-coded images of the alphabets “Group” can be hidden as alphabets “troup”, “croup” and “sroup”, respectively. (B) The normalized τ_m histograms of the alphabets “pr” overlap with the τ_i of the alphabet “n”, and the real information obtained from the τ_m color-coded images of the alphabets “roup” can be hidden as the “noup”, “roun” and “noun”, respectively. (C) The τ_m of the alphabet “u” overlaps with the τ_i of the alphabet “o”, the real information obtained from the τ_m color-coded image of the alphabets “out” can be hidden as “oot”. Moreover, using the alphabets “prn” in Figure 5B, “oops” can be hidden as “ours” and “oont”, respectively. The fluorescence lifetime pseudo-color image was color-coded from 1 (blue) to 2.5 ns (red).

involved τ_m and τ_i switching to create data encryption keys to ensure message confidentiality.

To verify the repeatability of long–short fluorescence lifetime switchable cipher cryptography by changing the solvent properties of a single dye and switching the τ_i and τ_m , the fluorescence lifetimes of eosin in the “BiouphotncsGpr” and “urGscnthpoiB” alphabet chips was continuously and repeatedly measured 5 times. The stability of the normalized τ_m and τ_i histograms of the corresponding eosin solutions injected into each alphabet chip was studied at room temperature (20 °C). As shown in Fig. S8, the fluctuation range of the FWHM of the long (τ_l)-short (τ_s) lifetime histograms was much smaller than the FWHM itself, the lifetime CVs (coefficient of variation, STDEV/average FWHM) was around 0.03–0.3%; thus, it was feasible to switch the long (τ_l)-short (τ_s) lifetime to realize the fluorescence lifetime cryptography of a single dye.

Earlier studies found that the τ_i is very well determined with an accuracy $\sim 1\%$, with little dependency on the parameters of data acquisition or quality of the fitting procedure [34]. Hence, it can be considered as the main intrinsic characteristics of the fluorescence decay data. In contrast, the value of τ_m was much less stable. Particularly unpleasant is the dependency on the fitting model. For data with a lower number of detected photons, the number of exponentials in the fitting model may not be correctly recognized without some *a priori* information, and can result in a largely erroneous τ_m determination. Therefore, τ_m values should be considered very carefully, with respect to the data quality and fitting model used. Earlier

studies revealed that the main intrinsic characteristic of the fluorescence decay is the τ_i . This value can also be recovered with high accuracy by a very simple, model-free, calculation directly from the histogram, which does not require a time-demanding search for the optimal fitting model using deconvolution procedures, and can be easily automated. In brief, τ_i is very robust characteristic, in contrast to the amplitude-weighted characteristic, and its value is dependent on the data quality and particularly on the fitting model used.

Conventional organic dye encoding is limited by photobleaching and spectral overlap, thus restricting the number of distinguishable codes that can be used in practice. To this end, we report a green sustainable method to maximize the time-resolved encoding capacity of a single dye. By simply switching the τ_i and τ_m of eosin with different solvents, at least 24 distinct populations of fluorescence lifetimes were obtained within a nanosecond-order lifetime range of 1–3.5 ns, which in turn increased the difficulty of duplication and provide extra high-level security protection. While eosin fluorescence is used to demonstrating the approach, this method can be applied to other materials. In general, in order to obtain the 24 distinct fluorescence lifetimes, the use of τ_i or τ_m alone requires the preparation of 24 distinguishable fluorescent dyes and the corresponding number of tests. However, the combination of τ_i and τ_m requires only a small amount of fluorescent dyes, and does not need to be tested 24 times. Herein, an encoding strategy for a single dye with a high-capacity multiplexed fluorescence lifetime was established. This is a fluorescence lifetime switching strategy has been

introduced into optical encoding, which not only greatly simplifies the preparation process of encrypted fluorescent dye, but also provides more alternatives for the decryption process at one time. Our investigation not only provides a fundamental method to adjust the fluorescence lifetime of a single dye by simply changing the dye solvent, but also realize the lifetime tunable encryption of a single dye by switching between long (τ_l)-short lifetime (τ_m). We believe that this method is also suitable for UCNPs and other luminescent nanomaterials that require double exponential fitting. Compared to existing approaches, the demonstrated long (τ_l)-short (τ_m) lifetimes switches represent a promising method for production of on-demand tags with the potential to supersede conventional lifetime-encoding in many ways. We believe that long (τ_l)-short (τ_m) lifetime switches will play an important role in fluorescence lifetime-based encryption technology with the development of wide-field [35, 36] and kilohertz frame-rates [37] fluorescence lifetime technology.

3 Conclusions

In conclusion, we demonstrated the possibility of increasing time-resolved encoding capacity, specially designed for a single dye without the need for tedious synthesis. The lifetime supermultiplexing barcodes were achieved by the switching long (τ_l)-short (τ_m) lifetime of a single dye. Remarkably, the proposed long (τ_l)-short (τ_m) lifetime switchable encryption was simple, fast, flexible, and low-cost, which is superior to the previous photo-switchable and temporal information encryption. Our proof-of-concept work imparts greater accessibility to the construction of a high-capacity multiplexed platform, which can reduce substantial technical and economic barriers such as limited barcode numbers, and the need for considerable experimental iterations. We believe the potentially enormous library of different fluorescent lifetime combinations would facilitate the development of information security.

Author contributions: T. L. and T. Z. conceived the project. T. L. designed the microchip device and Y. Z. assisted to fabrication. T. L. and Y. Z. performed the microfluidic experiments. T. L. analyzed the data. T. L. and T. Z. prepared the manuscript. J. Q. supervised the project.

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