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Fluorescent Molecules as Transceiver Nanoantennas: The First Practical and High-Rate Information Transfer over a Nanoscale Communication Channel based on FRET

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Nanocommunications via Förster Resonance Energy Transfer (FRET) is a promising means of realising collaboration between photoactive nanomachines to implement advanced nanotechnology applications. The method is based on exchange of energy levels between fluorescent molecules by the FRET phenomenon which intrinsically provides a virtual nanocommunication link. In this work, further to the extensive theoretical studies, we demonstrate the first information transfer through a FRET-based nanocommunication channel. We implement a digital communication system combining macroscale transceiver instruments and a bulk solution of fluorophore nanoantennas. The performance of the FRET-based Multiple-Input and Multiple-Output (MIMO) nanocommunication channel between closely located mobile nanoantennas in the sample solution is evaluated in terms of Signal-to-Noise Ratio (SNR) and Bit Error Rate (BER) obtained for the transmission rates of 50 kbps, 150 kbps and 250 kbps. The results of the performance evaluation are very promising for the development of high-rate and reliable molecular communication networks at nanoscale.

Nanotechnology has opened up the opportunity to manipulate individual molecules to design molecular-size machines, i.e., nanomachines, that can perform very basic tasks such as sensing, actuating, computing and data storing. However, the limited operational capabilities of individual nanomachines are not sufficient to realise advanced applications that nanotechnology promises such as real-time health monitoring and precise drug-delivery. To extend the application range, it is crucial to provide coordination through communication among nanomachines with different capabilities as well as between nanonetworks and macroscale remote controller units[1]. But the rather unique characteristics of nanoscale environments hinder the implementation of conventional communication techniques, and thus, lead researchers to investigate the feasibility of bio-inspired nanocommunication methods such as molecular communications.

Molecular communications is based on the idea of using molecules as information carriers[2]. Following a bio-inspired approach, it is envisioned that information can be encoded into concentration or type of molecules, and molecular messages can be delivered through diffusion in a fluid medium. However, as the case for most of the biological processes, transmission, propagation and reception of molecules are very slow, which results in very-low rate communications and limits the range of possible applications. Moreover, implementing molecular communications requires cell-like complex nanomachines to be devised. Therefore, it is not surprising that studies on molecular nanonetworks so far have been limited to theoretical investigations.

The progress in nanotechnology has also enabled the design of several photoactive nanomachines, some of which are single molecular, such as photosynthesizers[3], fluorescence biosensors[4], all optical molecular transistors[5], photochromic memory units[6], excitonic logic gates and switches, e.g., bistable [2]rotaxane[7]. The functionalities of these devices mainly rely on the trade of optical excitation energy among nanounits, or between nanounits and macroscale controllers such as laser source[8]. These developments have led us to investigate the

feasibility of a radically different molecular communication technique which can reveal the cooperative functions of these nanomachines. The proposed communication paradigm is based on Förster Resonance Energy Transfer (FRET)[9], which is the exchange of excited state energies between fluorescent units in resonance and promising for enabling communication between simple, even single molecular, photoactive nanomachines, without the need of designing complex device architectures and communication protocols.

FRET is a pairwise non-radiative energy transfer process observed among fluorescent molecules, i.e., fluorophores, such as organic dyes, fluorescent proteins, and semiconductor nanoparticles, e.g., Quantum Dots (QDs), which have spectral similarities and are located in close proximity[10]. The phenomenon has been widely used in biotechnological research including fluorescence microscopy, molecular biology and optical imaging, since it provides a significant amount of structural and spatial information about molecules by means of optical signals with nanoscale resolution[11]. FRET also intrinsically meets the very basic requirement of any communication system, i.e., the transportation of energy between distant functional units. Basically, FRET requires three conditions to be satisfied: (i) spectral similarity between the fluorophores, i.e., the emission spectrum of the donor and the absorption spectrum of the acceptor should have sufficient overlap; (ii) proximity of the fluorophores, i.e., the energy donor and acceptor should be in close proximity such as 0–10 nm; (iii) the transition dipole moments of the donor and acceptor should not be orthogonal to each other. If the conditions are satisfied, the donor is expected to transfer the excited state energy to a nearby acceptor.

FRET-based nanocommunications, firstly proposed in[12], is based on encoding information into the energy states of fluorescent molecules, which are ground and excited state. The exchange of energy states between an excited and a ground-state fluorophore through FRET establishes a short-range nanocommunication. Our initial studies focused on the information theoretical analysis of the communication channel for different configurations such as point-to-point, broadcast and relay[12–14]. The rate of communication is ultimately limited by the energy transfer rate which is on the order of 10^7–10^9 s^{-1} depending on the system parameters like fluorescence lifetime of the molecules, intermolecular distance and Förster radius[10]. This was validated by our realistic Monte Carlo simulations which we obtained very high data transmission rates on the order of 10 Mbps for practical cases including the effect of Inter-Symbol Interference (ISI)[13,14]. The results underlined that FRET-based nanocommunications can pave the way for high-speed molecular information processors and computers. We also investigated the FRET-based mobile molecular ad hoc sensor/actor networks in which information is propagated in a stochastic manner among mobile fluorescent molecules with basic sensing and actuating functionalities[15,16]. The main design principle of these networks is to take the intelligence from the network nodes, which have limited capabilities, to the external control units that communicate with the nanonetwork through an optical interface. The communication theoretical studies showed that this type of collaboration between mobile functional units without the need of any protocol can provide significant practical advantages especially for *in vivo* applications, such as target detection and removal, in terms of extended coverage range and throughput.

In this study, we aim to experimentally validate the feasibility of FRET-based nanocommunications. We implement a communication system composed of macroscale instruments, which are connected to Internet, and FRET-based nanoscale communication channel. The channel is established in an ethanol solvent with a high concentration of Fluorescein (Fl) and Rhodamine B (RhB) dye molecules as the transmitter and receiver nanoantennas (TNs and RNs), respectively. The molecules are densely deployed and randomly moving. The TNs receive an On-Off Keying (OOK) modulated optical signal from a laser source and transmit the information into the channel in the form of excitons, i.e., excited state. RhB molecules, as the RNs, receive the transmitted information through the FRET mechanism, and send the detected information to a distant photodetector, which is a macroscale receiver hardware, in the form of fluorescence signals. The topology of the channel connections with multiple TNs and RNs distributed randomly is analogous to the collaborative Multiple-Input and Multiple-Output (MIMO) scheme which is extensively implemented in conventional wireless communications[17]. However, it is not possible to analytically model the behavior of the channel due to the high level of randomness originated from both the transfer characteristics intrinsic to FRET as well as the mobile and complex topology of the channel connections. Nevertheless, this study aims to empirically analyse the performance of the nanocommunication channel focusing on the resultant transmission errors and SNR for communication rates of 50 kbps, 150 kbps and 250 kbps. The unique contributions of this work can be summarised as follows

- To the best of our knowledge, this is the first demonstration of a controlled information transfer through a molecular nanocommunication channel.
- The study is also the first experimental investigation of FRET phenomenon from a communication theoretical perspective.
- The paper provides the experimental setup and the methodology to analyse a FRET-based nanocommunication channel, which can pave the way for the design, test and implementation of future nanonetwork applications.
- This study also introduces an optical interface between a macroscale network and a nanonetwork, which has not been implemented before.

Methods

Experimental Setup. A digital communication system is composed of basically three major components: sender, channel and receiver. The overall aim of the system is to transfer any kind of information from the source node to the destination node through the communication channel with minimum error. Figure 1 demonstrates the basic functional units of each communication block. The source encoder encodes the information into a binary data sequence which is then transmitted to the channel encoder which adds redundancy bits to combat with the possible transmission errors. Based on a predefined modulation scheme, the modulator shapes a carrier signal according to the bit-stream modified by the channel encoder. The transmitter antenna, which is the interface between the transmitter hardware and the channel, converts the modulated signal into a suitable energy form that can be transmitted through the physical channel. The transmitted waveforms propagate along the channel and presumably reach to the receiver antennas with additional noise whose extent mostly depends on the physical characteristics of the channel. The waveforms detected at the receiver antennas are transmitted to the demodulator which converts the signal into a binary bit stream according to a predefined demodulation scheme. The function of the channel decoder is the removal of the redundant bits. The source decoder converts the binary stream into the symbols, and transmits them to the destination as the recovered information.

For the aim of demonstrating the feasibility and evaluating the performance of a FRET-based nanocommunication channel, we implement a simple and yet practical communication system whose block diagram is presented in Figure 2. Note that we do not implement any channel coding mechanism to explicitly reveal the information transfer characteristics of the channel. Each functional block of the system is expressed in detail as follows.

Source and Source Encoder. In the source PC, the information to be transmitted through the channel is prepared in the form of continuous binary (digital) sequence. If the information is analog, then it requires to be converted into digital by the source encoder based on a predefined quantisation scheme.

Modulator. The bit sequence generated in the source encoder is received by the Data Acquisition Card (DAQ) through serial communications, and stored at this unit. Once triggered by the user through the instrument control program NI-LabVIEW, the DAQ generates Transistor-Transistor-Logic (TTL) signal at its digital output representing Bit-1 by 5 V-level, and Bit-0 by 0 V-level, and transmits it to the High-Voltage Amplifier (HVA) at a user-defined transmission rate. The HVA amplifies the modulating TTL signal to the voltage level required for the input of the Electro-Optic Modulator (EOM). The operation of EOMs are based on the electro-optic effect, i.e., Pockels effect, which defines the modulation of the refractive index of crystal materials by the applied electric field. The modulation of the refractive index is

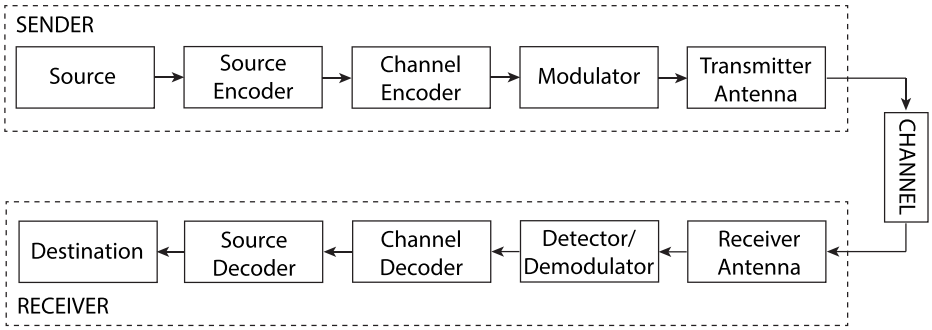

Figure 1 | Block diagram for a digital communication system.

reflected by the EOM into the polarization of the optical input. We use a visible-spectrum broadband electro-optic amplitude modulator (New Focus, model 4102) which operates with 0–195 V digital modulating input and supports operation frequencies ranging from DC to 200 MHz. The optical input of the EOM is supplied by an Argon-Ion laser whose emission is at 488 nm (with ~30 mW optical power), which is close to the maximum absorption wavelength of the Fluorescein molecules employed as the FRET donors, i.e., TNs. The input laser beam is vertically polarized, and thus, the EOM changes the polarization from vertical to horizontal when high-voltage signal representing Bit-1, i.e., 195 V, is applied to its modulation input. The Polarizing Beam Splitter (PBS) located at the output of the EOM is aligned to allow the passage of only horizontally polarized beams, thus, it reflects the laser beams whose polarization is not altered by the EOM. This setup provides an ON/OFF Keying modulation of the laser output based on the binary information supplied by the DAQ. The modulated optical output of the PBS is transferred at the same rate to the optical microscope through the band-pass filter which filters out the undesired optical contributions. The function of the microscope is to condense the optical input passed through the filter and send it directly to the molecular sample, i.e., nanocommunication channel, consisting of the donor and acceptor molecules, i.e., TNs and RNs, respectively.

Nanoantennas and Communication Channel. The overall communication link from the sender to the receiver is completed through the FRET channel between the TNs and the RNs, which constitutes the main focus of this study. The function of the TNs is to represent the modulated optical signal by the molecular energy states (ground state for Bit-0 and excited state for Bit-1), which are then transferred via FRET. The RNs do the inverse, i.e., they convert the excited state of the acceptors, which are sensitised through FRET if Bit-1 is transmitted, into fluorescence which is then collected by an external photodetector. In the case of Bit-0 transmission, the TNs stay in the ground-state, and thus, the RNs are not sensitised and do not release any photon.

For the TNs and RNs, we use a commonly used FRET pair, i.e., Fluorescein (Fl) and Rhodamine B (RhB), whose emission and absorption spectra with the resonance overlap between them are shown in Figure 3. We prepare a bulk ethanol solution of these organic dyes with the concentrations of 3 mM for each type of molecule. The single molecular nanoantennas are not bound to any structure, thus, they freely translocate and rotate in the solution. The high spectral overlap between the emission of Fl and the absorption of RhB results in a Förster radius of 5.7 nm[18]. Note that the configuration of the channel in this work is substantially different from the ones in the previous theoretical studies which consider the communication between a single donor (TN) and a single acceptor (RN) molecule[12–14]. We prefer using bulk solution of fluorophores instead of a single pair of donor-acceptor molecules due to the very low time resolution of single-molecule FRET (smFRET) measurements with the state-of-the-art instruments which limit the sampling rate of fluorescence signals to the range of 1–10 kHz[19]. Employing multiple donor and acceptors increases the resultant fluorescence intensity, and allows sampling at higher frequencies up to MHz, which we require for demonstrating a real-time communication with data transmission rates on the order of kbps.

The FRET mechanism, which is one of the relaxation pathways for the excited donors, constitutes a virtual communication channel between the TNs and RNs. The topology with multiple transmitter and receiver antennas which are randomly mobile is analogous to the cooperative MIMO scheme applied for electromagnetic wireless communications where multiple transmitter antennas collaborate to transmit the same information. However, the information transmission through the MIMO channel in this experiment has a high degree of randomness which results in significant amplitude and timing distortions over the transmitted signal and makes it impossible to analytically model the received signal. In addition to the stochastic nature of FRET and the inevitable noise originated from the hardware, the signal distortions are mainly resultant from the following characteristics of the channel:

- The molecules, i.e., nanoantennas, are freely moving and rotating in the solution. Therefore, the intermolecular distances, the relative orientations of the molecular transition dipole moments, and the number of possible relaxation pathways for the TNs, all of which have significant effects on the extent of the energy transfer, are rapidly fluctuating.
- The donor molecules (TNs) are not expected to be simultaneously excited by the laser. Moreover, they can be randomly excited and relaxed multiple times during a single Bit-1 transmission, especially if the symbol duration is larger than the mean donor lifetime.
- The received signal is the superposition of the fluorescence signals of multiple acceptors (RNs). The acceptor molecules cannot be expected to concurrently get excited through FRET and fluoresce. Moreover, they can get excited and fluoresce multiple times during a single symbol transmission. Furthermore, due to the diversity in the direction of the photon release, it is not possible to detect all of the fluorescence resultant from the sensitisation of RNs.
- Organic dyes are known to photobleach after approximately 10^5–10^6 excitation-relaxation cycles[10]. Therefore, especially for long data transmissions, photobleaching of molecules can contribute to the distortions.
- Since the solution includes high concentration of molecules, random collisional quenchings, which result in the loss of information carrying excitons on the excited molecules, are expected to contribute to fluctuation of the received signal.

Demodulator. The fluorescence of the system resultant from the transmission is collected by the microscope and transmitted optically to the photodetector after

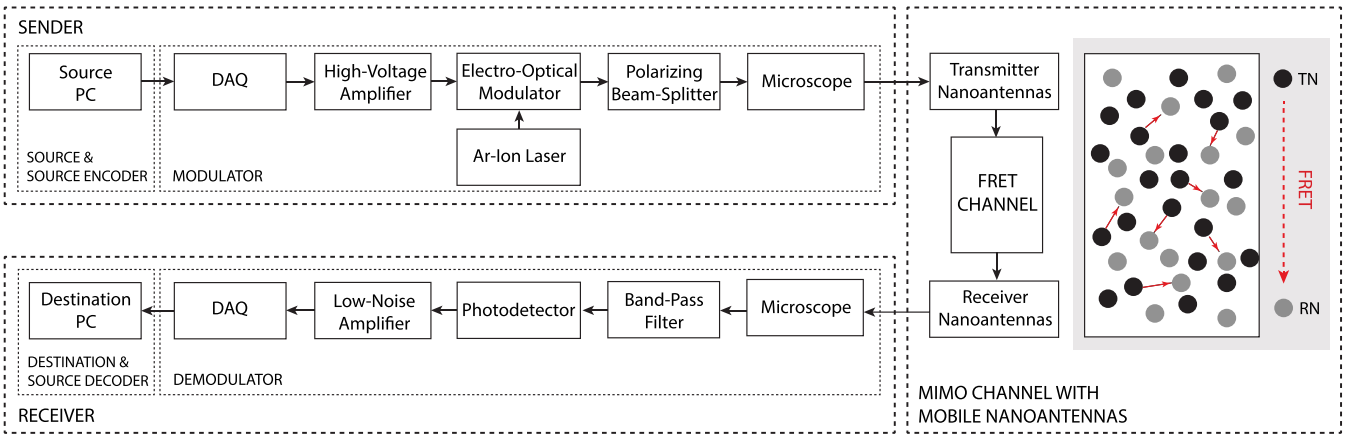

Figure 2 | Block diagram for the experimental setup.

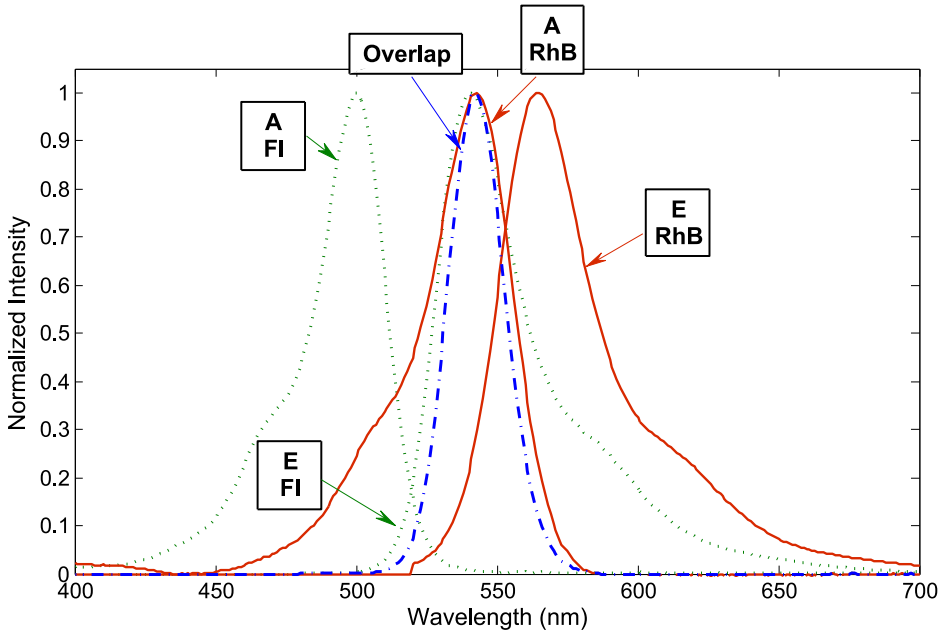

Figure 3 | Normalised absorption (A) and emission (E) spectra of Fluorescein (Fl) and Rhodamine B (RhB), and the calculated overlap function.

passing through the band-pass filter. The photodetector converts the optical signal to electrical signal which is then amplified by a low-noise amplifier. DAQ samples the continuous analog output of the amplifier at a predefined frequency and demodulates the sampled signal based on OOK modulation scheme by comparing the voltage level to a threshold. If the signal amplitude is higher than the threshold voltage, it decides Bit-1; otherwise, it decides Bit-0. The generated binary information sequence as a result of the demodulation is sent serially to the destination PC for source decoding.

Source Decoder and Destination. Source decoding is performed at the destination PC, and the binary sequence received from the DAQ is converted to an analog signal based on the uniform quantisation scheme applied in the source PC.

3-Cube Method for Signal Extraction. The first step in experimenting FRET is to excite a sample solution consisting of donor and acceptor fluorophores with an optical signal, the wavelength of which is in the absorption spectrum of the donor. The second step is to detect the resultant fluorescence signal of the acceptor after passing it through a band-pass filter whose pass-band overlaps with the emission spectrum of the acceptor. In most cases, especially when the donor and the acceptor have very similar optical characteristics, the absorption spectra of the donor and acceptor may overlap. The same situation may apply to their emission spectra as well. If there is an overlap of the absorption spectra, the acceptors in the system may also be directly excited with the optical input. In a similar way, any overlap in the emission spectra results in ambiguity between the acceptors' sensitised emission resultant from FRET, and the natural fluorescence of the donor molecules without any contribution of FRET. These contaminations are termed the Acceptor's Spectral Bleed-Through (ASBT) and the Donor's Spectral Bleed-Through (DSBT) to the FRET signal for the former and latter cases, respectively, and both complicate the detection of the contribution of FRET on the resultant fluorescence signal[20]. Fortunately, with a proper methodology including a post signal processing on the received fluorescence, the FRET signal can be revealed.

In this work, in order to remove the spectral bleed-through, we apply the 3-cube method which is regarded as the most effective procedure in the literature[21]. In the 3-cube method, three solutions, i.e., three cubes, which contain only donor (C_D), only acceptor (C_A) and both donor and acceptor (C_{DA}) should be prepared. Each solution is separately excited by the same laser source with a wavelength which is close to the maximum absorption wavelength of the donor[21]. The resultant fluorescence signals are then transmitted through one of two different optical band-pass filters selected based on the type of the excited sample. One of the band-pass filters is the Donor Filter (DF) which allows only the signals with the wavelengths near the maximum emission wavelength of the donor to pass. The other filter is named the Acceptor Filter (AF), and allows the signals with the wavelengths close to the maximum emission wavelength of acceptor to pass. In this experiment with the donor-acceptor pair of Fl-RhB, the pass-bands of the filters are determined as 525/50 nm and 607/67 nm for the DF and AF, respectively. The fluorescence signals that achieve to pass through filters are then observed by a photodetector and recorded for the post signal processing. Using the data obtained, the contribution of FRET can be extracted based on the following equation

$$S_{FRET} = S_{DA,AF} - S_{A,AF} - S_{D,AF}\frac{I_{DA,DF}}{I_{D,DF}} \quad (1)$$

where $S_{DA,AF}$, $S_{A,AF}$ and $S_{D,AF}$ are the continuous fluorescence signals received from the samples C_{DA}, C_A and C_D, respectively, through the filter AF. $I_{DA,DF}$ and $I_{D,DF}$ are the fluorescence intensities for the samples C_{DA} and C_D, respectively, detected through the optical filter DF. To apply the 3-cube method, we prepared ethanol solutions of 3 mM Fl as the sample C_D, 3 mM RhB as the sample C_A, and 3 mM Fl + 3 mM RhB as the sample C_{DA}.

Results and Discussion

FRET Efficiency. We first observe the overall energy transfer efficiency of the system to evaluate its suitability for the further communication experiments. Donor's fluorescence intensity in the absence of any acceptor decreases when acceptor molecules are introduced to the sample because of the energy transfer. The level of decrease in the donor's fluorescence reveals the efficiency of FRET which can be calculated as follows

$$E_{FRET} = 1 - \frac{I_{DA,DF}}{I_{D,DF}} \quad (2)$$

We obtained the fluorescence intensities by exciting each sample, i.e., C_{DA} and C_D, with a stationary optical pulse. We observed the resultant fluorescence signal through photodetector by recording its electrical voltage output which is proportional to the fluorescence intensity of the corresponding sample. The output voltages are measured as 0.130 V and 0.715 V for the samples C_{DA} and C_D, respectively. Using equation (2), we calculate the FRET efficiency of the system as ~81.7%.

The high efficiency is expected because in the sample C_{DA}, where the TNs and RNs communicate through FRET, an individual donor is presumably surrounded by more than one acceptor in its close proximity, which significantly increases the probability of FRET for an excited donor compared to a single-pair FRET system. Therefore, our system with the specified molecular concentrations provides a very efficient, thus suitable, environment to test the information transmission characteristics of FRET.

Random Data Transmission. The performance of a serial digital communication system is best evaluated by observing its eye

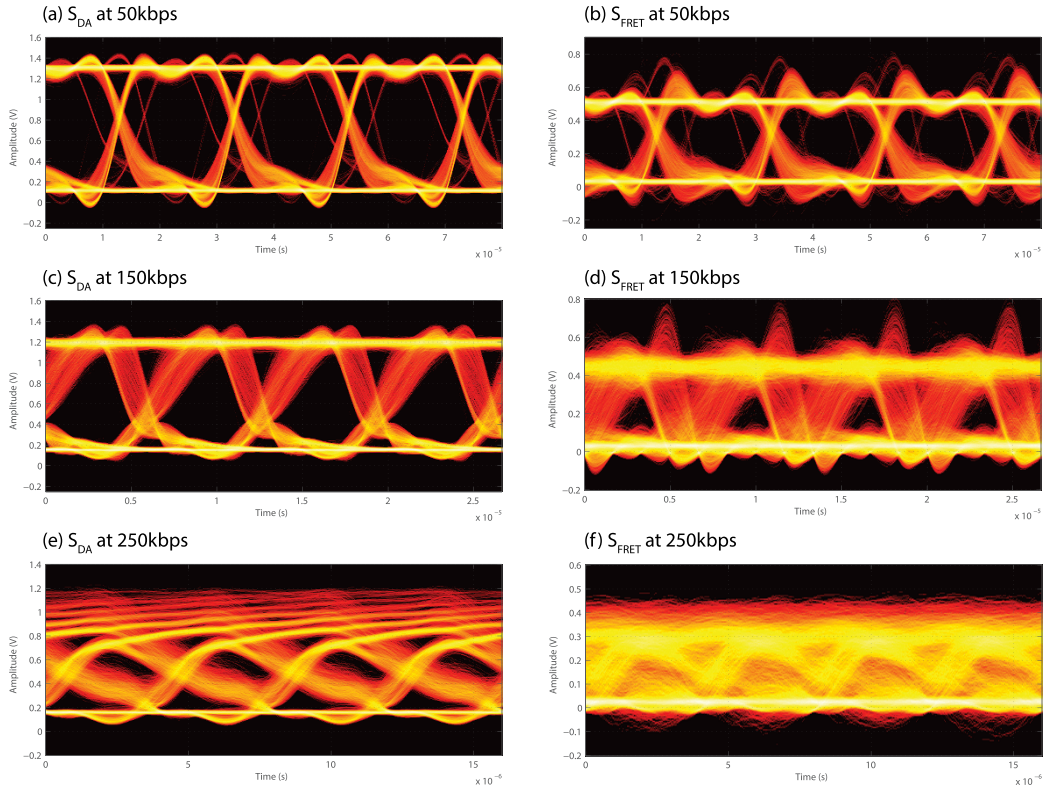

Figure 4 | Eye diagrams of S_{DA} and S_{FRET} signals for several information transmission rates.

diagram which is the superposition of all Bit-0 to Bit-1 and Bit-1 to Bit-0 transitions, as well as the constant Bit-0 and Bit-1 levels. Eye diagrams provides a rich set of information about the transmission characteristics of the channel such as the noise level and the jitter caused by the random fluctuations in the fall and rise times.

To generate the eye diagram, we prepare a pseudorandom binary sequence with 2^{19} bits at the source PC to be transmitted through the channel at various transmission rates, i.e., 50 kbps, 150 kbps and 250 kpbs. Following the 3-cube method, for each rate, the binary sequence is transmitted through each of the three samples, and the resulting fluorescence signals are detected by the photodetector after they pass through the appropriate bandpass filter (see Section II). The amplified electrical outputs of the photodetector are sampled by the DAQ at the sampling rates of 400 kHz, 1.2 MHz and 2 MHz (8 samples per bit) for 50 kbps, 150 kbps and 250 kbps transmission rates, respectively; and recorded at the destination PC. Note that no source encoding/decoding scheme is applied, because the generated pseudorandom information sequence is already binary.

The obtained eye diagrams which store 1000 traces of 4 bit-length sequences for the observed signal S_{DA} and the calculated signal S_{FRET} are demonstrated in Figure 4, for different transmission rates. Note that the pure FRET signal S_{FRET} is obtained using the 3-cube equation (1). To increase the resolution of the eye diagrams, the sampling rates of the observed signals are increased 32 times through lowpass interpolation in MATLAB; however, further analysis of BER and SNR are realised on the original waveform without interpolation.

The eye diagrams clearly demonstrate the transitions among all sequential combinations of Bit-1 and Bit-0's. If the signal had been ideally transmitted, i.e., without noise and any time distortion, the waveforms would have looked like sequential rectangular boxes. However, as in all communication systems, the stochastic nature of the communication channel together with the thermal noise of the employed hardware causes amplitude and timing distortions, i.e., jitter, in the received signal. The extent of the distortion in the amplitude can be revealed from the eye closure in the vertical axis; and the timing distortions can be observed through the eye closure in the horizontal axis. As is seen in Figure 4, all of the received waveforms at each transmission rate contain both type of the distortions in some extent. As the transmission rate increases from 50 kbps to 250 kbps, jitter resultant from the distortions in the finite fall and rise times of the fluorescence signal completely closes the eyes, and thus, complicates the detection. The jitter is mainly originated from the high level of randomness in the timing of the excitation of the donors and the emission of the acceptors. We also observe that the amplitude of the waveforms decreases as the transmission rate increases. This is because the decreasing bit interval does not allow the fluorescence intensity reach to higher levels.

Comparing to the transmitted binary sequence, we also obtain the optimal decision threshold voltage and optimal sampling point, i.e., decision point out of 8 sampling times per bit, which minimise the errors at the end of the demodulation process for each transmission rate. Table 1 presents the results of this analysis with the corresponding BER and SNR values. At the rate of 50 kbps, the communication

Table 1 | Optimal Sampling Settings and Resultant BER and SNR

Transmission Rate (*kbps*)	Optimal Threshold (*V*)	Optimal Sample Point	SNR (dB)	BER (bit^{-1})
50	0.248	3/8	12.16	1.9074×10^{-6}
150	0.202	5/8	7.10	5.7221×10^{-5}
250	0.167	7/8	3.34	3.0449×10^{-2}

is realised with an average of 1.9 bit errors per 1 Mbits transmission, and with an SNR of approximately 12 dB, which makes the channel very suitable for the envisioned nanonetwork applications. At the rate of 150 kbps, the channel is in a fairly good condition with an acceptable SNR of 7 dB. However, as the transmission rate increases to 250 kbps, SNR decreases to 3 dB, which makes the communication very problematic. This can be validated by the low BER value such that on average, 3% of the transmitted bits are erroneously decoded.

Conclusion

In this work, we realised the first data transfer exploiting FRET as a communication means at nanoscale. We implemented a practical communication system in which macroscale devices are connected over a nanoscale MIMO communication channel between fluorophore-based nanoantennas. The performance of the channel was evaluated by transmitting pseudorandom binary bit sequences and observing the resultant BER and SNR for different transmission rates. The experiments revealed that the FRET-based communication channel provides acceptable reliability for nanonetworks up to 150 kbps transmission rates, even when the location of nanoantennas are not fully controllable. The easy-to-implement test setup and the simple methodology presented in this paper will surely benefit the nanocommunications literature for designing, testing and improving the nanonetworks based on FRET, and developing high-rate and reliable nanonetwork applications.

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Author contributions

O.B.A. led the project. A.K. and O.B.A. supervised the experiments. M.K. conducted experiments and measurements and fulfilled data analysis and physical interpretations. M.K., A.K. and O.B.A. discussed the results. M.K. wrote the manuscript.

Additional information

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