Using FRET Networks to Identify Molecular Signatures of Tumors

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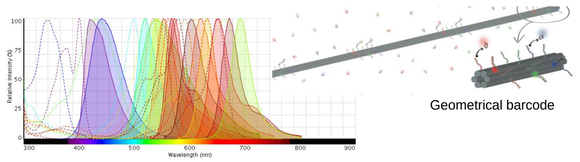
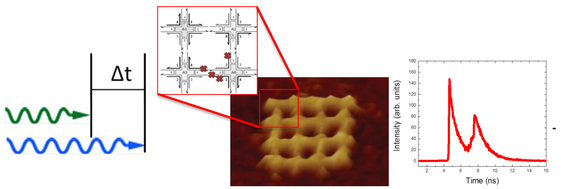
Summary

Fluorescence microscopy is one of the most widely used assays in biological systems. However, the technique suffers from limited multiplexing capability with previous attempts at detecting more than 11 fluorophores simultaneously resulting in barcodes that are too big for *in vivo* analysis, expensive and involve time-consuming detection schemes. Here, we introduce DNA self-assembled FRET networks that provide a unique, optical output when probed by a series of light pulses. Markov and entropy modeling of the nanoscale FRET sensors show that 125 fluorophores can be observed simultaneously. Furthermore, experimental analyses of over 1200 time-resolved fluorescence signatures on 300 prototypical networks show that the optical responses are repeatable 99.48% of the time and that minor variations between FRET networks can be discriminated resulting in a total of 10^{375} unique responses. This enormous increase in spatial information density enabled by FRET networks allowed us to identify molecular signatures in lung and breast cancer tumors.



Geometrical barcode

FRET Network



Input Laser Pulses → FRET Network → Fluorescence Output

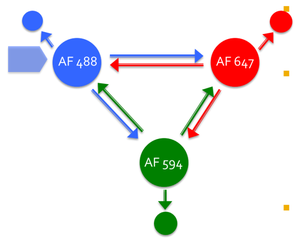
The FRET sensor we designed, self-assembles DNA probes labeled with acceptor fluorophores to the target DNA/RNA secondary structure forming an optical network. A DNA strand labeled with a donor fluorophore triplex binds to a unique sequence adjacent to the secondary structure. When the donor fluorophore is excited, the interaction between the input light and network of fluorophores on the DNA grid results in resonance energy transfer between the fluorophores and the rate of transfer is given by:

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{Ro}{r}\right)^6$$

This interaction results in time-resolved fluorescence output unique to the fluorophore network and hence the underlying secondary or tertiary DNA/RNA structure. Advantages of the sensor at structure detection over other *in situ* sensors include:

- The sensor makes use of DNA/RNA probe strands, which makes it very specific to the target secondary/tertiary structure.
- The small size of the fluorophores enables high spatial resolution.
- The optical nature of detection enables picosecond time resolution and is inexpensive.
- The use of oligos allows easy access to highly condensed regions in the nucleosome.
- The FRET sensor is essentially a nanoscale optical computing system. Therefore, when the fluorescence output indicates that a cell is malignant, the output of the sensor can be used to drive therapeutic agents, such as photosensitizers, to kill the cell.

Markov Model



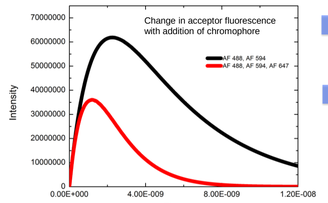
- Fluorophores modeled as random variables with predefined probabilities of transfer
- Transfer rate given by Förster's equation:

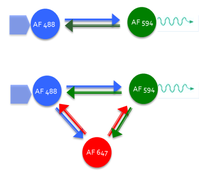
$k_T(r) = 1/\tau_D \cdot (Ro/r)^6$

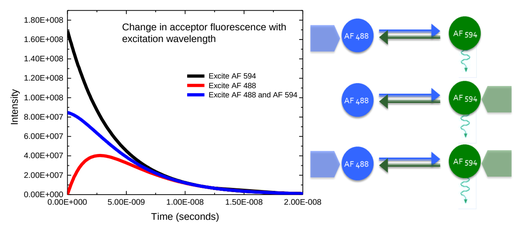
where, Ro is the Förster radius, r is the distance between the donor the acceptor, τ_D is native lifetime of the donor.

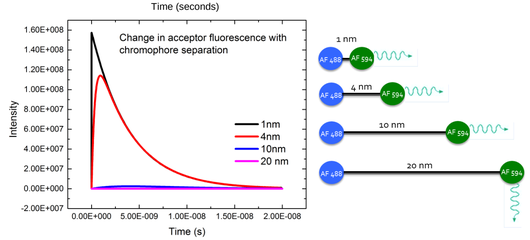
- Fluorescence is the absorbing state

The results of the Markov model, below, show that adding a single fluorophore to a network, changing the position of a fluorophore by a few nm or changing the excitation wavelength gives rise to a unique output.

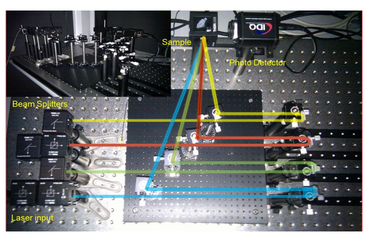








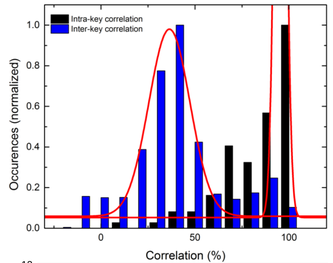
Experimental Set-Up



In order to probe the sensor with multiple light pulses, incoming white light is split into 4 parts with varying wavelengths and delays. The beams excite the sample and the fluorescence decay is measured using a single photon optical detector.

Experimental Results

In order to validate our Markov model, we undertook the most extensive and systematic survey of FRET networks to date.

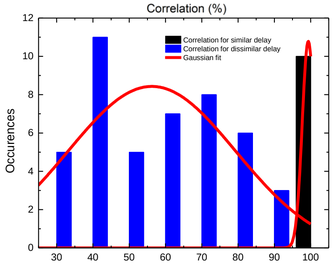


Change in signature with sensor

We deliberately introduced minute variations to a single network (e.g., moving a fluorophore by 1nm or by interchanging locations) and observed the change in output:

-High reproducibility: Responses from identical networks were 99.48% reproducible, from 1200 experiments.

-Unique outputs: From over 65,000 comparisons, the number of collisions between outputs of dissimilar networks was only 0.39%.

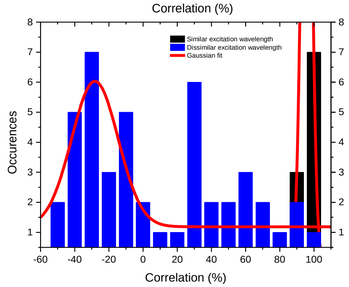


Change in output with delay

-Excitation delay is varied but excitation wavelengths and network are unchanged.

-As little as 100 ps delay gave rise to a new output.

- 125 excitation decays can be resolved using our instrument between 0-12.5 ns.



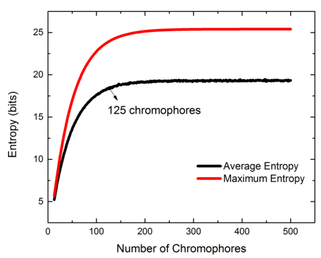
Change in output with wavelength

-Excitation wavelength is varied but excitation delays and network are unchanged.

-As little as 2.1nm wavelength difference gave rise to a new output.

- 190 excitation wavelengths can be resolved using our instrument between 400-800 nm

Large Number of Unique Outputs



The entropy of a network is frequently used to determine the information content of a network and is calculated using $H = -\sum_{i=1}^{n} p_i log_2 p_i$, where p_i is the weight of each edge and n is the total number of edges in the network. Here we see that the entropy of the system scales up to 125 fluorophores.

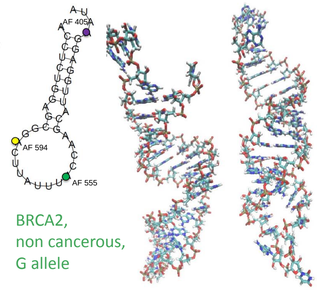
From experimental data, we can reliably detect a 8% change in amplitude from 100-200,000 peak counts and a 14% change in lifetime between 0.1-6ns. Therefore,

Number of unique single exponential decays: 100 amplitudes x 20 lifetimes = $2x10^3$

Total number of outputs: Number of unique single exponential decays$^{\text{Number of resolvable delays}} = (2x10^3)^{125} = 10^{375}$

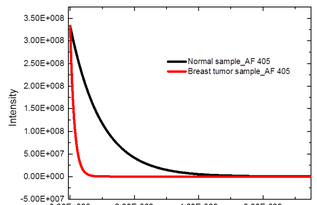
Breast Cancer Detection

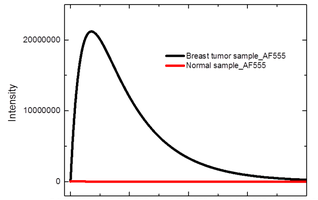
We know cancer cells contain aberrant DNA/RNA secondary structure in the regulatory regions of some genes. The FRET network self-assembled to the target structure results in a different optical signal based on the presence of the wild type or the aberrant secondary structure. Here, we identify breast and lung cancer cells with high specificity and repeatability.



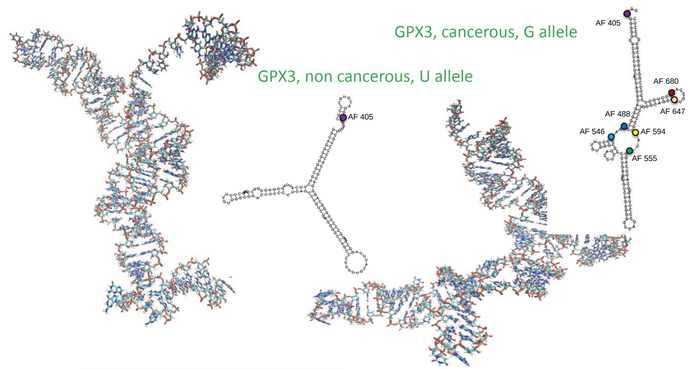
BRCA2, non cancerous, G allele

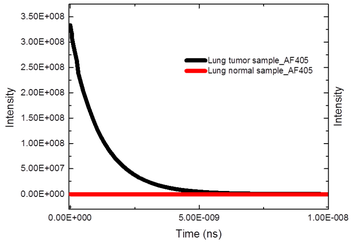
BRCA2, cancerous, A allele

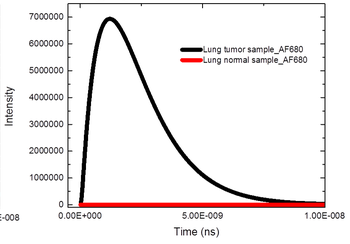




Lung Cancer Detection







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