Let's make this DNA visible!

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Background

We present a novel approach to **increase the detection sensitivity** of trace amounts of **DNA** in a sample by employing Förster Resonance Energy Transfer (FRET) between intercalating dyes. **Two intercalators** that present efficient FRET were used to enhance sensitivity and improve specificity in detecting minute amounts of DNA. Comparison of steady-state acceptor emission spectra with and without the donor allows for simple and specific detection of DNA (acceptor bound to DNA) down to **100 pg/µl**.

If we use as an acceptor a dye with a significantly longer lifetime (Ethidium Bromide bound to DNA),we can use **multi-pulse pumping** and **time-gated detection.** These techniques enable imaging/visualization of picograms of DNA present in a microliter of an **unprocessed DNA sample**.

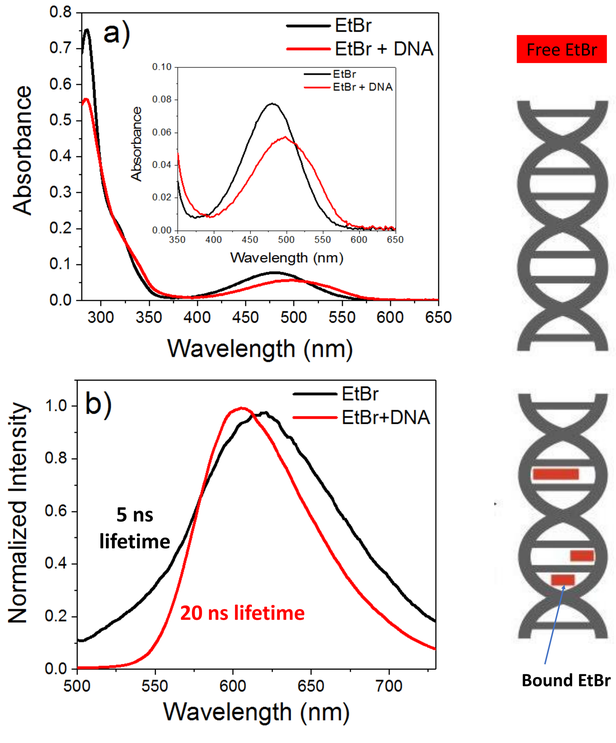
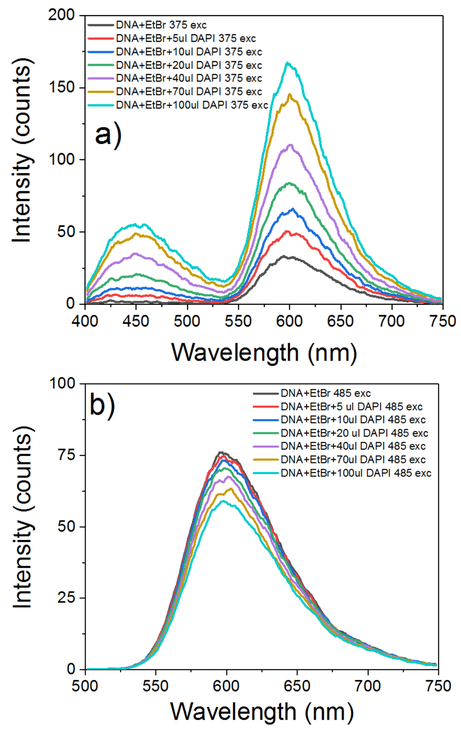


Figure 1. Absorption (a) and normalized emission (b) spectra of free and bound to DNA EtBr

FRET experimental approach



375 nm wavelength excites DAPI

↓

DAPI transfers energy to bound EtBr (FRET)

↓

Bound EtBr glows brighter

485 nm wavelength does not excite DAPI

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Adding DAPI doesn't induce any change

Figure 2. Measured emission spectra for increasing concentrations of DAPI in a DNA-EtBr solution with 375 nm excitation (a) and 485 nm excitation (b).

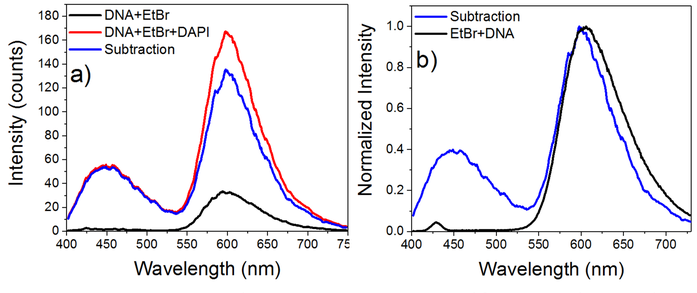

Figure 3. Emission spectra with 375 nm excitation measured for a DNA solution with EtBr only (black), EtBr and DAPI (red), and spectrum obtained by subtracting the signal of EtBr only from the signal of EtBr and DAPI (Blue) (a), and spectrum of DNA solution with EtBr normalized to the differential spectrum (b).

Subtracted spectrum = fully bound EtBr

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Adding DAPI increases only the signal of bound EtBr

Results

Is FRET really happening?

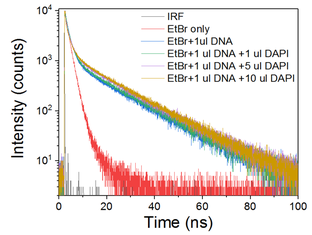


Figure 4. Intensity decays for a solution containing **EtBr only (no DNA)**, a solution of **EtBr with DNA**, and a solution to which an increasing amount of **DAPI** was added.

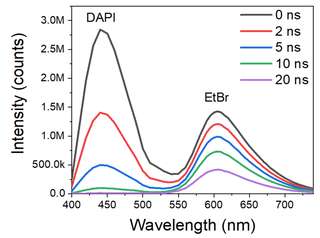
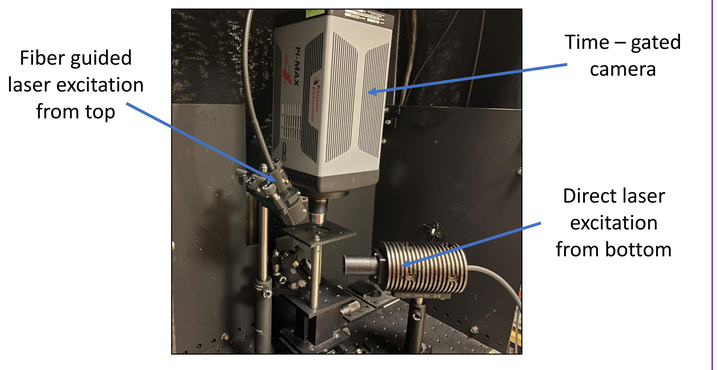


Figure 5. Time resolved emission spectra (TRES) for a 100 pg/ul DNA solution stained with EtBr and DAPI. The spectra have been obtained with increasing observation delays of 0, 2, 5, 10 and 20 ns.

As easy as taking a picture!



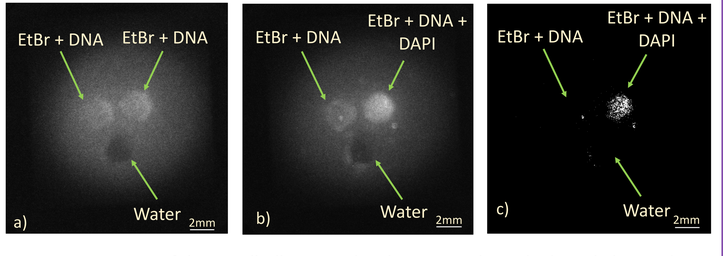


Figure 6. Images of three wells illuminated with a 375 nm laser diode and observed through a 580 nm long pass filter.

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