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Optical fluorescence of biological samples using STJs

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Abstract

We have recently reported on the first use of tantalum/aluminium single pixel Superconducting Tunnel Junctions (STJs) in biological fluorescence measurements [G.W. Fraser, et al., Rev. Sci. Instrum. 74 (2003) 4140]. In this paper, we present optical spectra from multiple biological fluorophores bound to DNA. The data confirm, in a real biological context, the advantages of STJs over conventional photomultiplier or avalanche photodiode based detectors. The STJ's, resolving power $E/\Delta E > 10$, together with appropriate band-pass filtering, allows the preferential detection of individual fluorophores and permits the removal of auto-fluorescent emission from the microscope lens.

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Keywords: Optical fluorescence; DNA; Superconducting tunnel junction

1. Introduction

A common biological problem is the measurement of the optical emission from spatially coincident fluorophores (dyes). Imaging the functional components of a living cell demands the registration of multiple fluorescent markers. Quantifying the hybridisation of labelled nucleic acids (probes) to immobilised target molecules in a microarray ("gene chip") also requires the simultaneous detection of multiple-component fluorescent spectra [1].

Current detector technology based on charge coupled devices (CCDs) or photomultiplier tubes (PMTs) only provides colour information when combined with narrow band filters or dispersive gratings. Recent progress in the field of fluorescent microscopy has been incremental: better optics, better filters and brighter fluorophores. Fundamental problems still remain. Increased illumination causes irreversible photobleaching of samples. Increasing sample concentrations makes the hybridisation response non-linear. Optical photon counting with Superconducting Tunnel Junctions (STJs) has the potential to alleviate all these problems by measuring the entire spectrum, on a photon-by-photon basis, with a sensitivity ~100 times greater than that of conventional systems [2].

We have previously demonstrated proof-of-concept by measuring the spectral responses of pure fluorophores [2]. In this paper, we extend the analysis to labelled DNA samples, thus demonstrating an approach to real biology.

2. Measurements

The detector was a $30 \times 30\,\mu m^2$ Ta/Al STJ [2], operated at ESTEC in a Heliox sorption cooler at 300 mK. The STJ had a measured resolving power of 14.1 at 600 nm. Samples were stimulated with a Leica microscope with mercury excitation light. Preferential selection of colour from fluorophore samples was made using Omega triple filter set (combining both input and output filters), which gave

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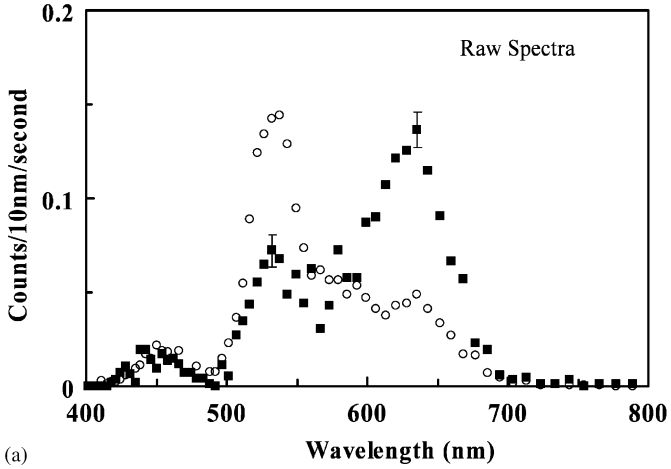
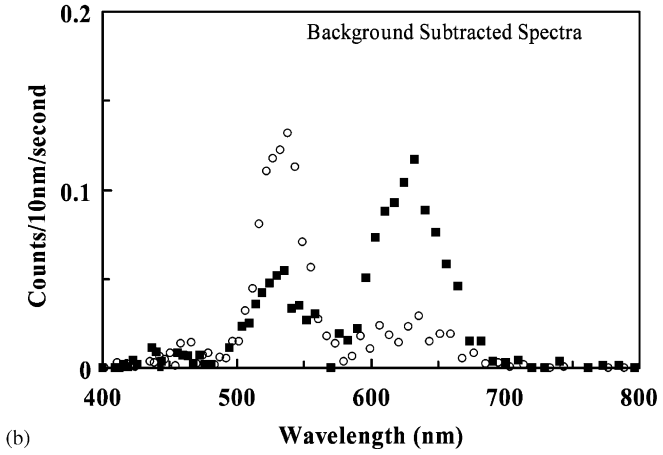
transmission in narrow bands centred on 450 nm (blue), 520 nm (green) and 620 nm (red). The integration times were all 30 s.

Figs. 1(a,b) show the overlaid spectra for the fluorophores Alexa 488 and Cy3, separately attached to DNA. In Fig. 1(a), there is in both spectra significant background counts outside the narrow pass bands of the Omega Optical filter; these events are due to the autofluorescence of the microscope objective lens. In Fig. 1(b), we use the photon counting capability of the STJ to remove this background, by subtracting in each spectral bin the count rate measured with the DNA samples absent.

Fig. 2 shows the results of a *dilution experiment*, where the DNA was simultaneously labelled with Alexa 488 and Cy3 in different ratios (4:1, 1:1, 1:4). The peak count rates in the two longer wavelength channels of the Omega filter give a measure of the relative concentrations of the two fluorophores—the relative *degrees of expression* in a microarray context. Fig. 3 shows that the ratio of counts in the two channels is indeed a linear measure of the relative fluorophore concentration—but only after proper background subtraction.



(a)



(b)

Fig. 1. (a) (top) Overlaid raw spectra of Alexa 488 + DNA (circles) and Cy3 + DNA (squares). (b) (above) Overlaid spectra after subtraction of auotofluorescent emission from the microscope objective.

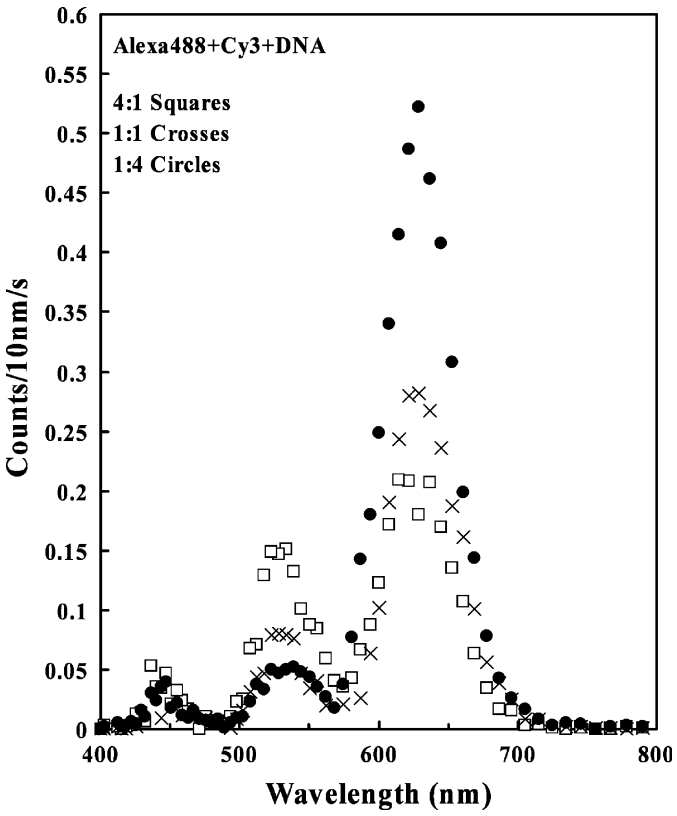


Fig. 2. STJ spectra measured from DNA labelled with Alexa 488 and Cy3 in the ratios shown in the inset.

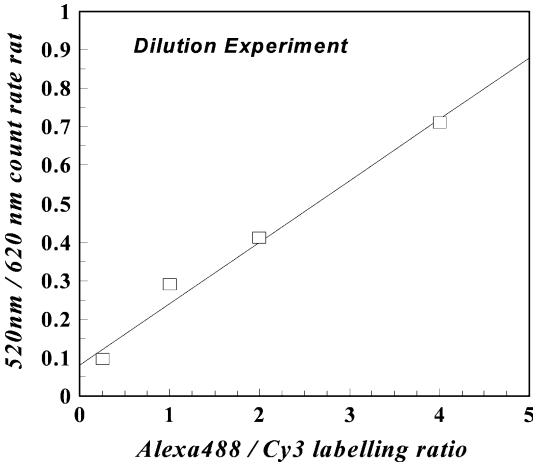


Fig. 3. Calibration curve for the expression ratio. The curve does not pass through zero because both fluorophores produce signals in both channels.

Other measurements, to be reported in full in a later contribution, showed strong evidence for the environmental influence on the fluorophore emission spectrum. The emission from Alexa 488 + DNA in solution gave a measured spectrum red-shifted from the expected manufacturer's peak for the dye in solution but also shifted from that of Alexa 488 samples dried on a microscope slide. The dried samples also show a broadening of the spectrum, not consistent with that expected after convolution with the STJ energy response.

3. Conclusions

We have demonstrated the spectroscopic capabilities of STJs coupled with the appropriate band-pass filtering to make simultaneous measurements of multiple fluorescent labels attached to DNA. The present Ta/Al devices (resolving power ~10–20) are capable of simultaneously measuring at least four well-separated fluorophores. In the longer term, smaller band gap, lower operating temperature, STJ devices with better resolving power—Hf with R~80 or Mo with R~40—will become important. The modest throughput of single pixel STJs will be improved by the continued development of large format arrays.

Acknowledgements

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