Platform for enhanced detection efficiency in luminescence-based sensors

R. Blue, N. Kent, L. Polerecky, H. McEvoy, D. Gray and B.D. MacCraith

Luminescence-based biochip measurement platforms are employed in a wide range of biological applications, such as biomedical diagnostics. Based on an understanding of the anisotropic emission properties of luminescence emitters close to a dielectric interface, a simple strategy for producing a better than 25-fold enhancement of the detected luminescence is presented. This strategy is demonstrated for low cost polymer platforms compatible with mass-production.

Introduction: There is an increasing requirement to detect a wide range of analytes with high sensitivity in areas such as biomedical diagnostics, environmental monitoring, food quality testing and bioprocess monitoring. Biosensor and chemical sensor technologies play a significant role in these fields [1]. Of the variety of sensing schemes developed, optical techniques are widespread and, within these, luminescence-based systems are the most widely used owing primarily to their better sensitivity than alternative approaches. Fluorescent labels (such as cyanine dyes and quantum dots) are employed routinely in bio-assays to transduce the biomolecular binding event.

Array biosensors, which typically comprise patterned arrays of biorecognition elements (such as antibodies) on a planar dielectric surface, provide multi-analyte and/or multi-replicate measurement capability. Such sensors, often referred to as microarray biochips, are of significant interest, especially in applications such as point-of-care testing, water quality monitoring and biowarfare detection [2]. Applications such as these are driving the demand for low cost, portable instruments combined with microarray biochips. The biochips are typically fabricated from glass or polymer, often incorporate microfluidic functionality, and are treated as single-use disposable components.

In this Letter we report for the first time a novel platform which provides a dramatic enhancement of the detected luminescence signal from an emitting species located on or close to a dielectric substrate. This platform is generic in nature and is compatible with microfabricated, mass produced biochips, especially in the case of polymer materials. The enhancement strategy presented here has major significance for the future design and fabrication of low-cost, high sensitivity platforms. In particular, it will lead to lower limits of detection and enable the use of lower cost detection systems, such as CMOS in preference to CCD cameras. In this work we will refer in general to luminescence although most biosensors employ emitting species with short lifetimes, in which case the more specific term fluorescence applies.

Theoretical background: Experimental evidence of the anisotropic emission properties of electric dipoles close to a dielectric interface has been reported experimentally by a number of authors [3, 4], together with a qualitative theory of the observations. More recently, a more rigorous electromagnetic theory has been proposed [5] to explain the phenomenon. We have subsequently expanded upon this work and developed a theory [6] that predicts the anisotropic emission pattern from systems employing thin luminescent films on planar dielectric substrates, which are used widely in the area of optical chemical sensors and biosensors. The theory models the spatial distribution of the luminescence radiated by randomly distributed luminescent molecules across multilayer systems comprising arbitrary linear, isotropic media.

For example, the particular case where the thin luminescent layer is sol-gel-derived silica doped with a luminescent indicator and deposited on a glass substrate is shown in Fig. 1. This Figure illustrates the spatial emission pattern as a 2-D cross-section. A comparison of Fig. 1*a* and *b* indicates the sensitivity of the mission pattern to the superstrate refractive index. In both cases, however, it is clear that a substantial portion of the luminescence is radiated into the glass substrate, which has a higher refractive index than the environment above it. Of most significance is the fact that the majority of the light that is radiated into the substrate is so-called supercritical angle luminescence, or more specifically supercritical angle fluorescence (SAF) [7], i.e. it propagates at angles above the critical angle corresponding to the environment (e) substrate (s) interface

$$\theta_c^{es} = \arcsin(n_e/n_s) \tag{1}$$

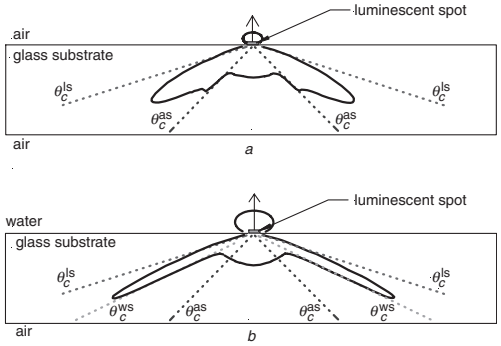

Fig. 1 *Angular properties of luminescence radiated from a thin luminescent spot deposited on a glass substrate*

The substrate is surrounded by air below and by air (*a*) or water (*b*) above. The solid lines represent the angular distributions of luminescence radiated from luminescent spots of thickness $t_l = 0.1\ \lambda$, and the dotted lines the location of the relevant critical angles, where l = layer, a = air, and w = water

Consequently, in the case of planar substrates with parallel sides as shown, this light will undergo total internal reflection at the bottom interface and become substrate-confined (SC) or trapped. In a 'conventional' detection technique, where a detector is placed above or below the substrate in the vicinity of the luminescent layer, it is clear that a significant amount (the majority) of the generated luminescent signal will be waveguided away as SC modes and will go undetected.

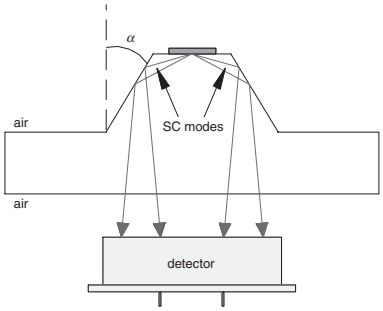

Fig. 2 *Cross-section of the configuration facilitating improved efficiency of the luminescence capture*

The SC modes radiated from the luminescent spot are redirected towards the detector placed below the substrate by means of a total internal reflection at the interface A

Enhanced luminescence capture system: The phenomenon described above is very significant when one considers the widespread employment of planar substrates coated with one or more fluorescence-based sensors. It is clear that conventional systems, which do not take account of SAF, are highly inefficient as a result. To address this issue, we have developed a range of sensor configurations [8] to produce a much greater capture efficiency. These configurations can be realised easily in a wide range of polymers using standard micro-fabrication techniques (such as micro-injection moulding). In each case, our theoretical model, combined with knowledge of the relevant refractive indices (polymer substrate, superstrate), enables us to calculate the spatial emission pattern of the fluorescent molecules. Here we describe one such configuration that has been implemented. The concept is shown in Fig. 2. Injection moulding is used to produce chips with an array of frustrated cones such as shown in Fig. 2. The cone angle α is chosen so that SC modes radiated from the luminescent spot undergo total internal reflection at the side-walls of the cone. The advantage of this configuration is two-fold. First, the total internal reflection applies to all the SC modes and, second, the redirected SC modes impinge on the bottom interface of the substrate at angles close to 0°. Consequently, a large fraction of the power is transmitted out of the substrate towards the detector.

Experimental results: As an initial proof of principle for this configuration, a thin film of sol-gel-derived silica (methyltriethoxysilane precursor) doped with a luminescent ruthenium complex (tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride) was spotted onto the full top surface of each of an array of frustrated cones and, for comparison purposes, also onto the planar substrate surface between the cones. All spots were approximately circular with a diameter of ca. 1 mm. The polymer (polymethyl-methacrylate—PMMA) chip was placed in front of a CMOS camera (Silicon Video 1310), with the camera lens focussed to collect an image of the emission area. Blue (470 nm) LEDs were used to excite the ruthenium complex which emits luminescence at around 610 nm (orange-red). A gelatin filter (Lee Filters No. 135) placed in front of the camera blocked the passage of reflected blue light.

Fig. 3 shows a typical image of an illuminated chip with four spotted cones. The three dull spots across the centre result from the luminescence captured from the doped spots located on the flat substrate surface between the cones. The images collected from the cones, however, exhibit two distinct features. In the centre of each such image one can see a dull spot of a similar level of brightness to that obtained from the conventional spots. The second feature, which provides striking visual evidence of the concept underlying this configuration, is the bright ring surrounding each spot. Each ring (or annulus) is a result of the captured luminescence that would normally be lost as SC modes.

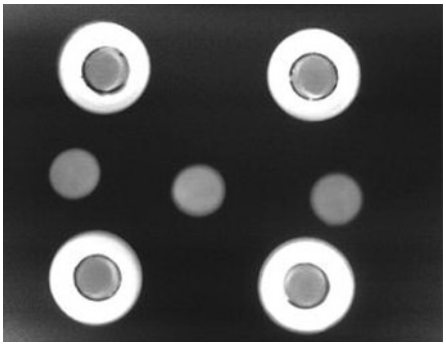

Fig. 3 *Image of enhanced luminescence capture (bright rings surrounding spots) from four spotted frustrated cones compared to image obtained from three spots on unmodified planar substrate*

Using straightforward image processing software, a mean enhancement factor of 25.8 was calculated for the four cones, implying that the luminescence intensity detected from each of the spots on the frustrated cones was more than 25 times higher than that obtained from the similar sized spots on the planar portion of the substrate. More recent work in this laboratory, which has focussed on refining this technique, has led to even greater enhancement factors. For example, careful analysis shows that the enhancement can be improved dramatically by coating a fraction of the cone top and depositing each spot at the centre of a cone using high-precision printing. When the size and location of the spots were controlled in this manner, enhancement factors of greater than 80 were obtained.

Conclusion: We have reported the design and experimental verification of a low-cost platform that can enhance the luminescence capture efficiency by over 80 times from radiating molecules upon planar substrates. Moreover, this platform is compatible with mass production using conventional polymer microfabrication techniques. The principles underlying this platform are generic in nature and have significant implications for the design of high sensitivity biochips and other luminescence-based platforms for a wide range of applications. Future publications from this laboratory will report improved platforms with even higher sensitivity together with embodiments in biochip and microfluidic systems.

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References

1 Eggins, B.R.: 'Biosensors: an introduction' (John Wiley & Sons, New York, 1998)
2 Rowe, C.A., *et al.*: 'Array biosensor for simultaneous identification of bacterial, viral and protein analytes', *Anal. Chem.*, 1999, **71**, pp. 3846–3852
3 Stuart, H., and Hall, D.: 'Enhanced dipole-dipole interaction between elementary radiators near a surface', *Phys. Rev. Lett*, 1998, **80**, pp. 5663–5666
4 Lee, E.H., *et al.*: 'Angular distribution of fluorescence from liquids and monodispersed spheres by evanescent wave excitation', *Appl. Opt.*, 1979, **18**, pp. 862–868
5 Enderlein, J., Ruckstuhl, T., and Seeger, S.: 'Highly efficient optical detection of surface-generated fluorescence', *Appl. Opt.*, 1999, **38**, pp. 724–732
6 Polerecky, L., Hamrle, J., and MacCraith, B.D.: 'Theory of the radiation of dipoles within a multilayer system', *Appl. Opt.*, 2000, **39**, (22), pp. 3968–3977
7 Ruckstuhl, T., and Verdes, D.: 'Supercritical angle fluorescence (SAF) microscopy', *Opt. Express*, 2004, **12**, pp. 4246–4254
8 Polerecky, L., and MacCraith, B.D.: 'A luminescence-based sensor', 2002, PCT Patent Application, WO 02/059583 Al