# Semiconductor light-emitting devices with in-built bioreaction chambers

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**Abstract**

We demonstrate complete integration of a fluorescence-based assay in that the analyte well is also an optical emitter. Laser machining is used to create 'active micro-wells' within semiconductor light emitting diode and laser structures. These are then used to optically excite fluorescently-labelled beads in solution within the well. The results show efficient illumination on a par with traditional lamp-based excitation. This technology therefore provides active micro-well plates with completely localized excitation, confined to the analysis well, that can be engineered via the micro-well geometry. The micro-wells have also been machined within the cavity of lasing semiconductor structures and coherent emission maintained. Thus lasing multi-well plates are also realizable.

Key words – fluorescence, optoelectronics, semiconductor lasers

## 1. Introduction

The application of optoelectronic components within biophotonic systems is increasingly popular. In addition to their compact size semiconductor based light emitters and detectors provide an unrivalled ability for customization of photonic performance via controlled epitaxial growth of crystal composition and dimension [1]. Therefore optical biochips which provide a complete and integrated platform of photonics and biological analytes are now very much a reality and increasing in complexity [2]. Here we report on the development of optical biochips in which the integration is taken to its limit in that the analysis well is within the semiconductor material i.e. the bio-analysis takes place *inside* the light emitter. This arrangement provides maximal coupling of excitation light to the sample and allows novel control mechanisms for a given assay. As an example of this we demonstrate geometrical control of fluorescence excitation intensity through design of the spatial extent of micro-wells that are laser machined into a semiconductor LED wafer.

Sensing of chemical or biochemical analytes using fluorescence has been demonstrated to be extremely useful for a wide range of applications. In some form fluorescence sensing is utilised in environmental monitoring, clinical chemistry, DNA sequencing and genetic analysis, cell identification in flow cytometry and in numerous imaging techniques. At the most basic level, a number of assays can be performed by simply monitoring the reaction of the fluorescence intensity of a probe to an analyte. Advances in micro-engineering have seen increasing interest in developing highly portable and even disposable lab-on-a-chip (or bio-chip) devices [3] for fluorescence sensing in point-of-care applications. Recent innovations have seen cell handling via electrophoresis [4], optical elements [5], micro-fluidic elements [6] and even excitation sources and detectors being incorporated into such micro-scale systems [7,8]. In the majority of biochips the optical excitation source, detector and biological sample remain as distinct and separate entities i.e. micro-scaling and integration brings these components closer together but their operation within a fluorescence assay platform remains essentially the same as in macro systems. The use of semiconductor

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optoelectronics provides a technology in which micron-scale structuring is commonplace and highly evolved and so the potential is there for a true symbiosis in which the biological material is analysed *within* the photonic structures. A highly successful example of this is the pioneering work by Paul Gourley and co-workers on intra-cavity laser analysis of blood cells [9]. A more recent example has demonstrated integrated micro-fluidics within the semiconductor crystal [10].

In this paper we present work on the development of micro-wells within semiconductor light emitting diode (LED) structures to form intra-cavity fluorescence assays. Laser machining is used to drill micron scale wells within a GaInP based LED structure which provides optical excitation at ~ 630 nm. These fluorescence micro-wells are lined with an optically clear epoxy layer to mechanically and electrically isolate the semiconductor crystal from any analyte. A simple fluorescence experiment is demonstrated using dye-labelled, Flow Cytometry beads within micro-wells of varying diameter. The paper concludes with an initial demonstration of the technology in laser structures and consideration of the benefits to be gained from the use of resonant photonic cavities.

## 2. Laser machining and fabrication

Laser micromachining as a manufacturing technique, has emerged from the development of micro and nanotechnologies over the past two decades. While laser micromachining is still considered a new process in many areas of micro engineering, it has become an established manufacturing method in niche application areas such as inkjet printer nozzle drilling [11] and flat panel display patterning [12]. Accurate laser micromachining tends to use pulsed lasers at wavelengths where heating and melting-based surface disruption is minimal. By controlling the number of laser pulses, and hence the total incident radiation, precise machining depths can be achieved while minimal thermal distortion occurs at the edge of the exposed region. In this work laser micromachining using femtoecond pulsed infrared (800nm) Ti:Sapphire lasers has been used to create analysis wells within a light-emitting semiconductor diode wafer.

Direct write laser micromachining makes use of a laser beam tightly focused to a small spot which is moved over the surface of the workpiece during machining. Typically direct write machining uses a laser with a Gaussian beam profile. Good beam quality and a low $M^2$ factor allow the beam to be focused to a small spot using simple optical components and to give a beam fluence at focus that is above the ablation threshold of the workpiece material. Control of the beam movement over the surface of the workpiece allows arbitrary 2D patterns to be machined. By overlaying machining runs it is also possible to create 3D structures. Machining depth control during workpiece movement is achieved by synchronizing laser pulse or power output with workpiece stage position. The direct write machining system used in this work was an Exitech M2000F Laser Micromachining Workstation (Exitech Ltd., UK) incorporating a Spectra Physics Hurricane Ti:Sapphire laser with a pulse duration of 120fs and a beam power density of up to $3.5 Wcm^{-2}$. In this system beam delivery components allowed control of beam power density and focusing. Typically the beam was focused to a 20µm spot delivering power densities of up to $0.3 MWcm^{-2}$. The workpiece was held on a micropositioning stage with 1µm resolution (Aerotech Inc., USA). Movement of the workpiece and beam on/off control was through a PC based motion controller (Unidex 500, Aerotech Inc. USA).

The accurate quantification of fluorescence can only be achieved if the exciting radiation is quantified and illumination is optimized. Using femtosecond laser micromachining it is possible to machine semiconductor diode and laser wafers in a manner that allows arbitrary shaped 2D light sources to be created. The delivery of high energy laser pulses in time periods close to 100fs leads to an ionizing ablation process capable of cleanly removing only the region of the workpiece illuminated by the tightly focused laser beam. In the case of light emitting semiconductors where light emission occurs in a defined junction region typically located within 1.5µm of the surface of the wafer, the ionizing ablation process allows the junction to be cleanly machined without disturbing the electrical diode junction. The lack of large physical debris from the machining process reduces the likelihood of the semiconductor junction being short circuited and the tightly focused beam restricts machining to a highly defined area or path.

## 3. Application to fluorescence assays

Using the laser machining processes described above we have fabricated micro-wells within red-emitting semiconductor crystals. With a peak emission wavelength of 630nm these wafers are suitable for excitation of red fluorophores such as Cy5, Nile Blue and also the new far-red / near I.R. inorganic, quantum dot probes. Our ultimate goal is application of this technology within live cell environments and so the focus on far-red wavelengths is well suited, minimising tissue absorption and cell auto-fluorescence. The wafer used here is a commercially available semiconductor diode material (IQE plc, UK). The light emitting structure was grown by metallic organic vapor phase epitaxy (MOVPE) on a silicon doped <100> n-type substrate misorientated at $10^0$ to the <111A> plane. The light generating region consists of three undoped GaInP quantum wells surrounded with 0.2 µm $(Al_{0.7}Ga_{0.3})_{0.51}In_{0.49}P$ barrier layers.

Figure 1a shows a series of cross-shaped light sources formed by machining the upper 10µm of a wafer. Figure 1b shows a laser machined circular source used to provide ring illumination to latex microbeads held in a laser micromachined well within the surface of the wafer. Using a solid state Ti:Sapphire femtosecond laser operating at 800nm and 5kHz, the wells were machined using a direct write machining process with a pulse fluence of 3.2 J $cm^{-2}$ at the wafer surface. Wells were formed by trepanning the final well diameter starting at the centre of the upper surface entrance circle. Control of the work piece velocity was used to produce wells of different depths. A typical well 100µm deep and 100µm diameter took approximately 6 seconds to machine.

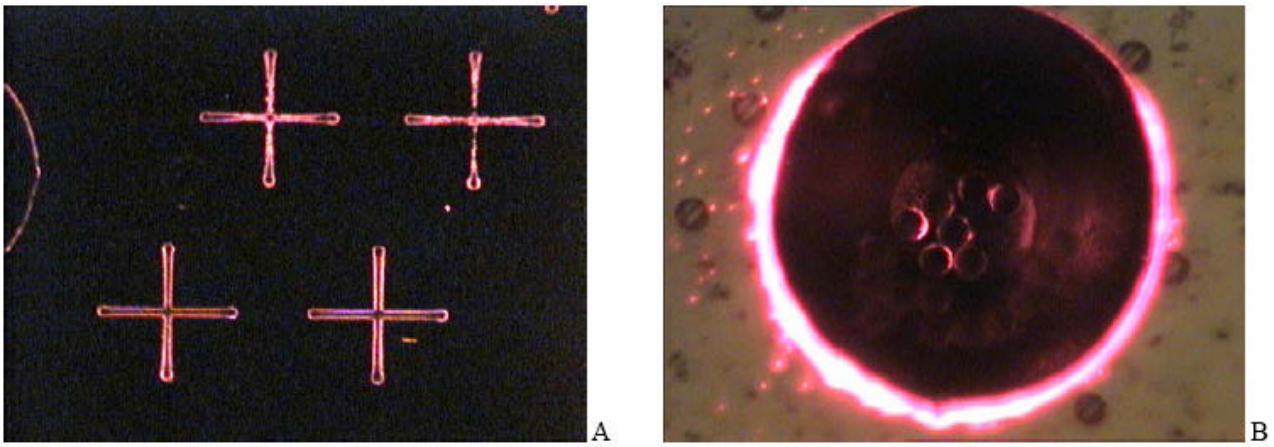


*Fig. 1: Light wells created within the surface of a semiconductor wafer. (A) Cross-shaped illumination sources machined directly into the wafer. (B) Electrically isolated microfluidic, in-chip wells with integrated ring illumination used to quantify latex microbeads.*

To use the light well structures within an optical biochip, the exposed wafer junction must be electrically isolated from the biochip sample fluid to prevent short circuiting of the junction. Light well wafers are first encapsulated by spin coating an SU-8 epoxy resist layer over the surface of the machined wafer to a thickness of typically 20µm. The SU-8 is

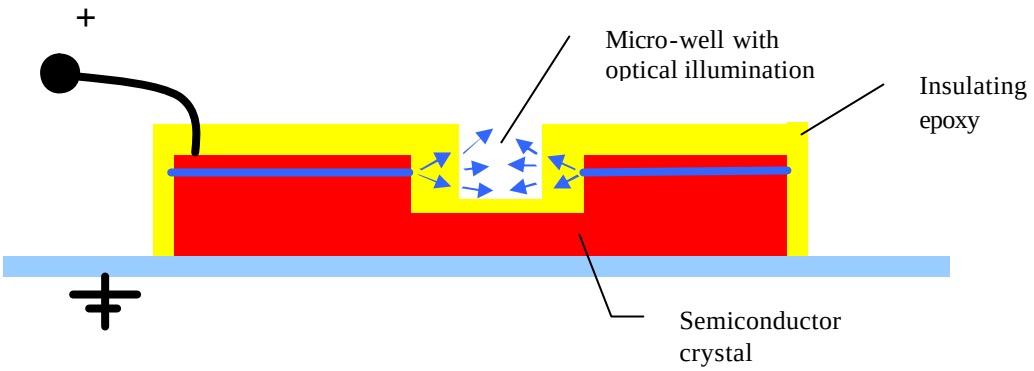


*Figure 2: Schematic of the laser machined and encapsulated micro-well within the semiconductor crystal.*

blanket exposed using a mask aligner and baked to produce a hard, electrically insulating, film over the surface of the wafer. During the spin coating process the laser machined well structures become planarized. Electrically isolated light wells are reformed to a diameter less that than the original well using an excimer laser at a wavelength of 248nm and a pulse fluence of 150 mJ $cm^{-2}$. Using a mask projection machining approach to aperture the workpiece beam to the diameter of the desired well, multiple low energy pulses can be used to precisely control the depth of the final well. A schematic cross-section of the finished device is shown in figure 2. The encapsulation of the semiconductor within the epoxy provides a sealed environment and allows integration of the 'wet' environment into the optical chip, *i.e. microfluidics within a chip rather than on a chip.*

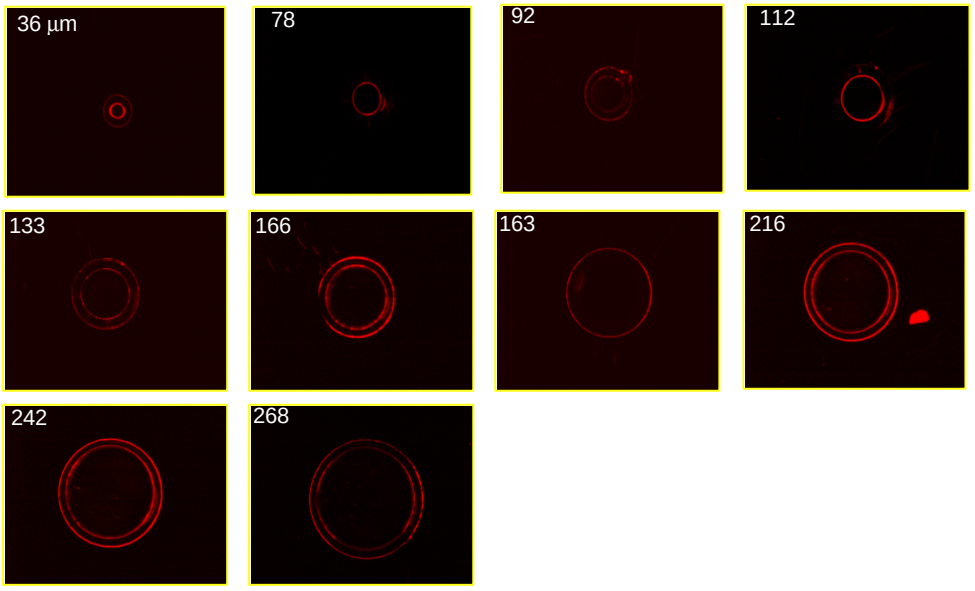


*Figure 3: Top-view of micro-wells, visible via scattered light from the semiconductor wafer. The dimensions in the top left of each image give the micro-well diameter in microns.*

To demonstrate the use of these micro-wells within a fluorescence experiment we fabricated a set of 10 wells with diameters varying from 36 to 268 µm. These were machined in the same way as the devices shown in figure 1 but in this case the depth of the wells was 4 µm. Figure 3 shows images of the light emission from the wells. These are viewed from above with the laterally-emitted light visible due to scatter at the machined interfaces.

The 10 wells are machined into a single piece of wafer and so all are illuminated simultaneously as current flows through the semiconductor. The current density and hence the light generation is uniform across the set and so the variation in diameter leads to a variation in optical power density as the micro-well area to circumference ratio alters. This provides the potential for excitation control within a fluorescence assay by geometry. To test the suitability of these wells for fluorescence experiments we dropped 2.5 µm diameter flow beads in solution onto the top surface. These had a 633 nm excitation maxima and 660 nm emission peak. The micro-wells were then imaged, i. in bright field illumination to get a physical image, ii. in fluorescence mode with the excitation light filtered out and epi-illumination from a fluorescence lamp (i.e. excitation from above) and iii. in fluorescence mode with excitation from the well itself.

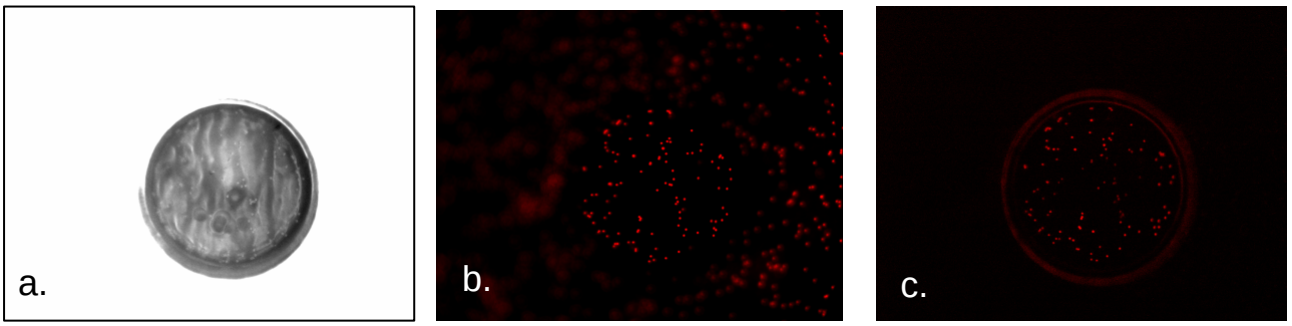


*Figure 4: Top view of micro-well, i. bright field image, ii. fluorescence under epi-illumination, iii. fluorescence under self-illumination.*

The images from the 242 µm diameter device are shown above in figure 4. The bright field image is focused on the bottom of the well. The insulating epoxy is visible as an annulus around the outer edge and within the structure produced by the laser machining the micro-beads can be seen as bright white points. The fluorescence from the beads is clearly visible in figure 4b. and those within the well clearly distinguished from those in the surrounding medium due to the different focal plane and the circular outline of the well produced by the shadow of the optical epoxy. The beads are also clearly distinguishable when illuminated directly by the micro-well, the signal in this case is reduced by approximately a half in comparison to the lamp illumination. The use of the semiconductor to provide fluorescence excitation is thus efficient enough to provide signal levels of the same order of magnitude as traditional fluorescence techniques. The geometrical aspect of the well illumination can be seen in the relative decrease of signal at the centre in contrast to the even fluorescence seen under epi-illumination. The light will diffract strongly as it emerges from the semiconductor ($\Delta\theta \sim 30^0$) and so we attribute the dimness of the central beads to a reduced optical intensity as the illuminating beam expands. Obviously the smaller diameter devices should provide higher and more even excitation intensity and this is indeed the case. Figure 5 shows results from the 92 µm diameter device. The bead signal is even across the whole of the well in this case and extremely similar to that achieved under lamp illumination. Comparison of figures 5a. and b. highlights another advantage of direct illumination within the well. This provides spatial delineation of the excitation in the z-axis (normal to the surface) thus only the beads within the well are illuminated and background signal from beads within the surrounding media and stray light are removed. Within a micro-well based assay this would provide high noise rejection and unambiguous identification of the measured signal with the sample.

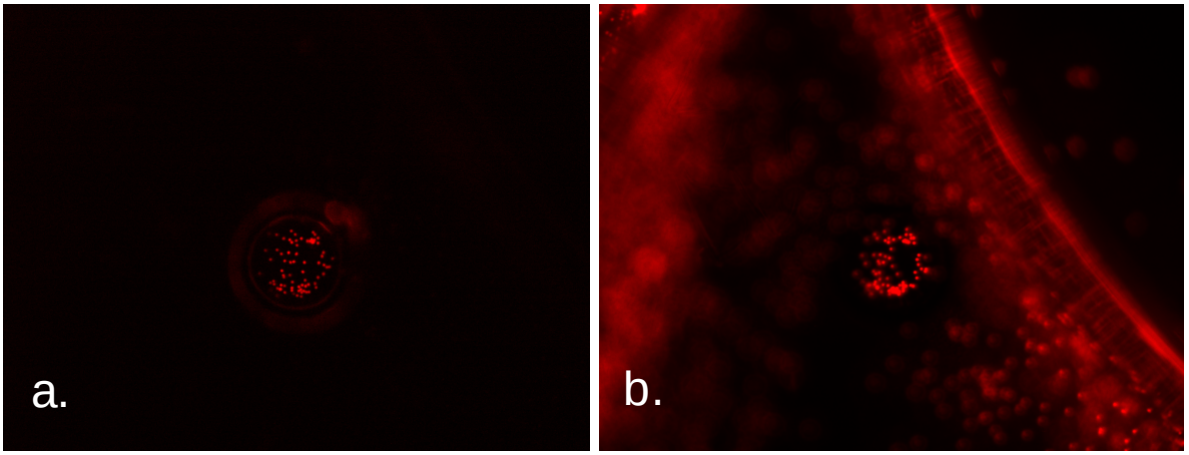


*Figure 5: a. Self-illuminated and b. epi-illuminated fluorescence from the 92 mm diameter well.*

These experiments show that total integration in which a micro-well provides optical excitation as well as sample containment is feasible. The use of such micro-scale engineering also provides unique properties in the spatial confinement of the excitation within the plane of the wells and the ability to customize the light signal through the geometry of the micro-well.

## 4. Integration within semiconductor laser cavities

Whilst the experiments shown above are exciting enough they do not exploit the full potential of the semiconductor optoelectronics. In particular the use of LED structures provides relatively broad-band excitation at low power and does not use the photonic structuring potential of semiconductor structures. The fabrication technology is independent of the semiconductor wafer design and hence the micro-wells can just as easily be fabricated within a laser structure. Fabrication of the wells within a laser would provide high power, monochromatic excitation signals and, perhaps more importantly, enable signal enhancement via interaction with the resonant optical cavity. A typical semiconductor laser linewidth of 10 MHz equates to a Q-factor of $46 \times 10^6$ for the cavity resonance at an emission wavelength of 650 nm. This equates to a coherence length of 1.36 m *i.e. the optical path length and hence the interaction length with the analyte is over a metre due to the multiple (> 1000) round trips of photons within the laser cavity*. Intra-cavity sensors of this type are well-established in solid-state lasers where the large scale, discrete optical cavity allows easy insertion of an analyte within the beam path. Integration within the micron-scale, monolithic cavity of a semiconductor laser is not so easy.

As a first stage in the development of lasing micro-wells we have investigated the influence of laser machined holes on the performance of oxide-isolated stripe, lasers emitting at 630 nm wavelength. These are similar in design to the LED structures used for the micro-wells but with an integrated waveguide. Images of the laser chips, viewed from above, are shown in figure 6. The semiconductor chip is 300 µm in width and 1 mm in length and lasing takes place within a 50 µm wide central area through which the current flows. The left hand side images, 6a and c show devices under bright-field illumination with holes and slots machined down into the semiconductor. The depth of these is 3-5 µm and so they go through the laser active region and all of the optical waveguide of the structure. The right hand side images, b and d show the devices under lasing with image illumination from the scattered laser light. Light scattered from the waveguide and out of the machined apertures can be clearly seen. In figure 6d the edge of the trenches which cuts into the lasing stripe is illuminated whilst the rest is dim as this area of the semiconductor has no current injection and hence no light generation. The influence of the machined structures, which have dimensions of 10-50 µm, on the laser threshold is surprisingly small. This increases by 10-20% due to the reduced amplification length and increased optical loss introduced by the holes. The laser machining is of real benefit in this fabrication in that it allows real time monitoring of the laser whilst the holes are ablated. Thus the structures can be machined to produce a given increase in threshold

current. Alternative techniques such as lithographic masking and etching could not provide this immediate feedback and flexibility. The modest increases seen in the laser threshold make the use of lasing structures for fluorescence micro-wells highly viable and we are currently pursuing this research.

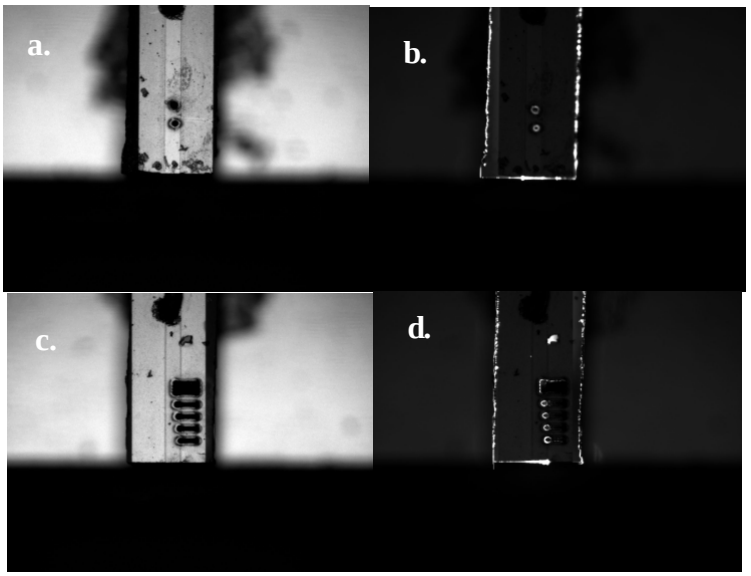


*Figure 6: Top-view of machined semiconductor lasers. a. and c. are under bright-field illumination, b. and d. are images under laser illumination from the devices themselves.*

Insertion of a fluorescently labeled sample into the cavity of a laser provides novelty in terms of the read-out signal in that it allows sample analysis via the optical characteristics of the laser light rather than the fluorescent signal. Interaction of the internal lasing field with fluorophores via absorption will change the intensity and spectrum of the coherent light. These changes can be used as a read-out with the advantage that the detection required is then of an intense laser signal rather than a weak and diffuse fluorescence. Insertion of a saturable absorber within a laser can introduce dynamic effects such as optical pulsing [13] and so intra-cavity sensing of fluorophores could also provide temporal read-outs linked to the fluorescence lifetime.

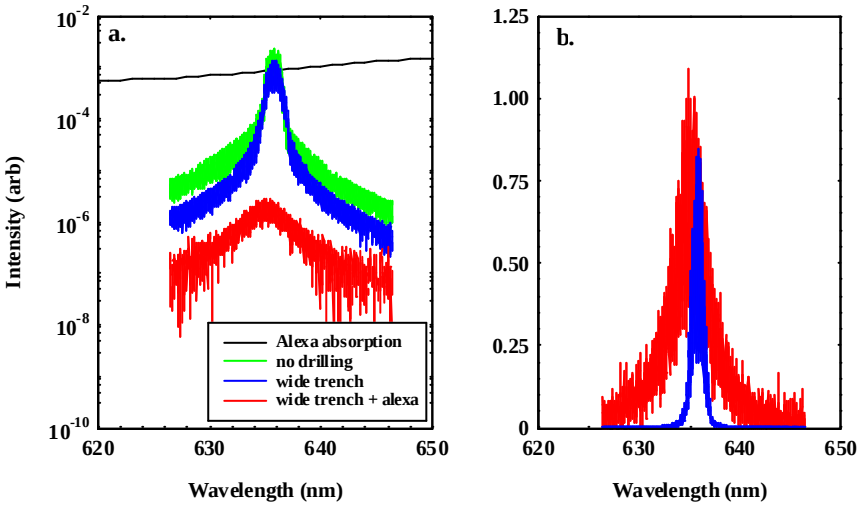


*Figure 7: Lasing spectra from the device shown in figure 6c. illustrating the effect of absorption from an Alexa dye.*

To demonstrate the potential of these laser-fluorophore interactions we have used the structure shown in figure 6c and Alexa in CyGelof ??? dye over the surface. The presence of the dye within the machined apertures increases the laser losses and thus raises laser threshold by 10-20 %. It also changes the laser spectrum at a fixed injection current as shown in figure 7. In 7a spectra are shown from the undrilled device, the drilled device alone and the drilled device with dye. In 7b the spectra with and without the Alexa dye are shown on a normalized scale to highlight differences between the two. There is a blue-shift and a broadening of the spectrum of the spectrum with the dye present. Spectral signatures of this type could be used to infer the presence of an active fluorophore within a lasing micro-well.

## 5. Summary

We have used femtosecond laser machining to produce micro-wells within optically active semiconductor crystals. These wells are electrically isolated from any analyte by potting them with an optical epoxy and re-drilling the holes. Formation of the wells by laser drilling provides a rapid and flexible fabrication which can easily be re-configured and which allows continuous monitoring of the semiconductor wafer viability via monitoring of its light output. Fluorescence excitation of 630nm flow beads has been achieved thus demonstrating that total integration of sample and light source can be achieved and fluorescence assays carried out inside an LED. This direct excitation by a ring around the circumference of the micro-well provides extremely high excitation efficiency and a spatial localization of signal in three dimensions. The areal density of the excitation can also be controlled via the well geometry rather than externally via the LED drive current. This potentially provides a means to excitation dosing of multiple wells simultaneously. Micro-wells have also been created within semiconductor laser structures and in this case lasing is maintained but with an increased threshold requirement. All of the technological steps required to produce lasing micro-well plates have therefore been demonstrated and the first steps taken towards the use of laser beam measurement to inform on fluorophore presence and characteristics.

**Acknowledgements**

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