Integrated Optical Solutions for Ubiquitous Sensing

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During the course of the research underlying this thesis, Stephen Macken was enrolled in Forum Scientium, a multidisciplinary doctoral programme at Linköping University, Sweden.

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To all those who mean something to me
Abstract

Ubiquitous chemical sensing aims at exploiting consumer electronic devices as temporary chemical sensing platforms. These devices are a highly disseminated infrastructure, not presently being exploited for chemical sensing purposes. The goal of the ubiquitous chemical sensing field is to create the methodologies and adapted devices to profit from this unexploited resource.

DVD drives, flat bed scanners and computer screens used in combination with web cameras, and mobile phones have been demonstrated as measuring platforms, during the past 10 years.

In particular the combination of computer screens with web cameras, a technique called the computer screen photo-assisted technique (CSPT), is the most versatile approach to support multiple types of optical detection phenomena, and together with mobile phones are the most ubiquitous type of platforms.

The versatility of CSPT comes from the ease with which one can configure a spectrally controlled, 2D micro-positioned, linearly polarized, wide angle and internally modulated illumination with a programmable screen, just by controlling the displayed contents. Concurrently, the imaging devices in CSPT enable one to capture arbitrary assay layouts, the evaluation of which can then be numerically customized.

The full exploitation of the CSPT platform requires optically adapted sensing interfaces and specialized methods, in order to increase sensitivity and make use of all available information, for a thorough and complete chemical sensing analysis. In this thesis I have explored the foundations of CSPT sensing on integrated devices, developing methods and sensing’s interfaces that provide enhanced optical detection customized to the CSPT platform.
Populärvetskaplig Sammanfattning

Allmänt utbredd kemisk detektion (ubiquitous chemical sensing) syftar till att utnyttja hemelektronik som tillfälliga kemiska sensorplattformar. Hemelektronik är vitt spridd men utnyttjas för närvarande inte för detta ändamål. Målet är att skapa metoder och anpassad utrustning som kan dra nytta av denna outnyttjade resurs för kemisk analys.

DVD-enheter, ”flat bed scanners” och datorsärmar som används i kombination med webbkameror samt mobiltelefoner har vid forskningslaboratorier fungerat som mätplattformar för allmänt tillgänglig kemisk analys under de senaste 10 åren.

Kombinationen av datorsärmar med webbkameror, en teknik kallad the computer screen photo-assisted technique (CSPT), är den mest mångsidiga strategin som möjliggör flera typer av optisk detektion. Tillsammans med mobiltelefoner är det den mest utbredda typen av hemelektronik plattformar.

Mångsidigheten hos CSPT kommer från den lätthet med vilken man, med en programbar skärm, kan konfigurera en spektalt kontrollerad, 2D styrbar, linjärt polariserad, vidvinkel och internt modulerad belysning genom ett enkelt styreprogram. CSPT tillåter en mängd olika utseenden på de prover som ska analyseras och erbjuder en utvärdering anpassad till proventa.

Ett fullständigt utnyttjande av CSPT plattformen kräver optiskt anpassade avkänningsytor och specialiserade metoder för att öka känsligheten och för att kunna använda all tillgänglig information, för en noggrann och fullständig kemisk-sensorisk analys. I denna avhandling har jag undersökt grunderna för CSPT avkänning på integrerade enheter samt utvecklat metoder och avkänningsytor som ger förbättrad optisk detektion anpassad till CSPT plattformen.
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List of Publications

The following list covers all of my published papers. Papers 1-5 make up the work of this thesis while papers 6 and 7 are related to, but not included in any discussions herein.

Paper 1

Optical properties of microstructures for computer screen photoassisted experiment.


Authors Contribution

I was involved in all of the planning of this work. I performed all of the experiments and result evaluation. I also wrote the manuscript.

Paper 2

Towards integrated devices for computer screen photo-assisted multi-parameter sensing


Authors Contribution

I was involved in all of the planning of this work. I performed all of the experiments and result evaluation. I also wrote the manuscript.

Paper 3

Imaging fingerprinting of excitation emission matrices


Authors Contribution

I was involved in the planning of this work. I also contributed with the computer modelling as well as helping with performing the experiments.
Paper 4

Monolithic SU-8 microcavities for efficient fluorescence collection

Authors Contribution
I was involved in all of the planning of this work. I performed all of the experiments and result evaluation. I also wrote the manuscript.

Paper 5

Colour Moiré Interferometry for Physical Sensing Using A Standard Web-Camera

Authors Contribution
I was involved in all of the planning of this work. I performed all of the experiments and result evaluation. I also wrote the manuscript.

Paper 6

Optically addressable field effect nano-environments for chemical imaging

Paper 7

HDR imaging evaluation of a NT-proBNP test with a mobile phone
P. Preechaburana, S. Macken, A. Suska and D. Filippini, Biosensors and Bioelectronics (In Press)

Conferences

Eurosensors XIX in Göteborg, 2006, Poster contribution.
Eurosensors and Transducers XX in Lyon, 2007, Poster contribution.
Eurosensors XXII Laussane, 2009, Poster contribution.
Eurosensors XXIII Linz, 2010, Oral contribution
Chapter 1: Preamble and a Prior Art

This first chapter will introduce the area of ubiquitous sensing and recap the status of the concept prior to my work.
Ubiquitous Sensing: An Introduction

Ubiquity is a term used to describe the widespread presence of something. An object can achieve this status when it has pervaded society to such an extent that it is considered commonplace to have. A good example of this, in the Western world, is the computer and its peripherals e.g. CD drives. Since its inception in the 70’s [1] computer systems have evolved at a staggering rate. They are at a point today where computer sets are sophisticated and versatile platforms capable of an ever expanding number of interactions with the surrounding environment e.g. DVD [2, 3, 4], flat bed scanner [5, 6, 7, 8], mouse [9] and visual aid control platform for assisted environments [10].

Conventional chemical sensing approaches are based on dedicated instruments that provide specific solutions for restricted targets [11, 12, 13, 14]. Ubiquitous optical sensing, in contrast, aims at expanding the capabilities of consumer electronic devices to support chemical sensing on a widespread infrastructure. These familiar devices though, must be adapted to fully exploit their potential as ubiquitous sensing platforms. Fortunately, consumer electronics are continuously evolving tools always incorporating an increasing number of powerful functions, thus providing versatility to these potential sensing platforms, from being able to surf the Internet, take pictures, watch films, make purchases, play games etc, all with a intuitive user interface [15].

There is, of course, a trade off that must be made for convenience, such as in resolution and speed. However that gap between specialised high-spec and versatile low-spec products is an ever shrinking one with each new product iteration. This is a trend present in almost all industries, as the consumer demands continuously stimulate the evolution of advanced products that are more compact and versatile.

These three aspects of ubiquity, chemical sensing and versatility come together into the field of ubiquitous chemical sensing. By taking the ubiquitous technology, mentioned in the beginning of this chapter, and expanding their already developed physical sensing abilities into being able to process chemical input, promises affordable, versatile, robust and user-friendly chemical sensing platforms. This melding of fields is highly advantageous as focus can now be put onto the development of various sensing methods. While the instrumentation can remain largely unchanged lending to a greater integration of sensing formats within a single device.
For the past ten years several groups have been investigating these ubiquitous sensing strategies. Suslick’s is one such group who make use of flatbed scanners to analyse chemical sensing colorimetric arrays [5, 6, 7, 8]. The fundamental idea being to use an array of deposited metalloporphyrins to detect volatile hazardous chemicals. When these porphyrins are exposed to certain gases they will change colour. By using a flatbed scanner to scan an image of the array pre-exposure to a gas, and then once again post-exposure, a subtraction of the image data (post-exposure minus pre-exposure) will provide a detection of the gas. As each of the porphyrin drops has different cross sensitivities to different aspects of the gases, a unique pattern signature is then generated for each gas.

Potyrailo’s group has also explored sensing technology using products in a way they were not specifically designed for [2, 3, 4]. This group have developed an analysis technique whereby it is possible to use a DVD optical drive to sense different chemicals. The main advantage of this technique is the sophistication of the laser reader in the DVD drive, allowing for very precise measurements.

This “Lab-on-a-DVD” platform makes use of a LabVIEW program to control the optical reading head of the DVD drive, with the data being collected in an installed data acquisition card connected to the DVD drive. By coating optical discs with different sensing materials and exposing their sensitive layers to a sample they have shown that it is possible to evaluate these discs to determine concentration of Ca⁺ ions [2], chlorine [4] and currently up to 54 different types of ions in water, as well as the thickness of the deposited films [3] by exploiting the different wavelength lasers present in an optical drive [4].

There are, however, disadvantages to these techniques. Potyrliao’s platform requires hardware access to the DVD drive making this approach an essentially dedicated instrument. Suslick’s concept is also restricted to a rather large apparatus, namely a flat bed scanner, which are neither very portable nor so ubiquitous. In any case both systems also require a computer to control and operate the peripheral as well as process the measured data. There are, though, another two peripherals that can be considered standard equipment for a computer set - the computer screen and the web-camera.
The combination of a screen and web-camera is not only the most ubiquitous but also the most versatile arrangement for this type of chemical sensing [16]. Even though all of these peripherals require specific drivers to function, because of the intrinsic usage of a computer screen; delivering arbitrary graphical information, it is easy to deliver spectrally controlled illuminations that are in a wide angle, with 2D micro-position resolution, that is linearly polarized, intensity modulated and of a variable area size. Thus providing a very flexible and compact light source without moving parts. Concurrently, imaging devices are able to capture arbitrary information from 2D or 3D objects in its field of view [17]. This information can then be numerically represented allowing for the mathematical evaluation of recorded images. Also due to the cameras overlapping sensing channels objects can be discriminated due to their spectral composition.

This chemical sensing platform using computer screens and web-cameras has been given the name “The Computer Screen Photoassisted Technique” (CSPT). The development of this concept and the accessory elements required to progress this technology has been the focus of my work the past four years and will be the subject of this thesis.
**CSPT: Prior Art**

The computer screen photo-assisted technique (CSPT) is a technique first put forth in an issue of Chemical Communications in 2003 [16]. In this paper the authors demonstrated the ability to discriminate between different substances, figure 2, and showed a possible bio-chemical analysis by performing a MTT cell viability test.

![Setup for CSPT in 2003 for the use of a MOS detector to detect transmitted light through a cuvette in contact with a computer screen.](image)

This paper introduced the concept of using web-cameras in conjunction with computer screens to perform simultaneous analysis of multiple targets, in this case a cell viability test in a multi-well microtiter plate. Before my own work began in 2006 this technique received a thorough mathematical grounding and performance evaluation [18, 19, 20, 21, 22, 23], where the aim was to develop the analysis of the spectral information captured by the web-camera, optimizing it and evaluating the best way to examine the measured results. Continuing on from the initial paper above the analysis of colour and colour-strips [24, 25, 26, 27] and the system as a microplate biosensor [28] was also explored.

The platform was also tested in a variety of other fields of analysis such as chemical/fluorescence/produce detection [29, 30, 31, 17], ellipsometry [32, 33], evaluation of ELISA tests [34] and DNA detection [35]. These helped to really expand the toolbox of measurement capabilities of the CSPT platform into different areas of optical chemical sensing.
Comparable results to the otherwise dedicated instrumentation were also obtained throughout. The red line going through all the CSPT work that provided these successful results was, amongst other things, the analysis technique developed for the system called “spectral response fingerprinting” as well as the capturing of the “Polychromatic Excitation Emission Matrix” (PEEM) (explained in Ch. 2).

**CSPT: The next phase**

By 2006 the CPST platform was an emerging technology in the field of ubiquitous sensing. However, there were a number of issues that needed to be addressed to make the platform user-friendly. Specifically the sensing elements needed miniaturization and an incorporation of more sophisticated functions, in order to give a more customized solution adapted to the platform. The platform needed to be pushed into different areas of measurement, expanding its toolbox of capabilities. There was also an incomplete understanding of how the platform was capable of capturing spectral fingerprints of fluorescent substances.

To this end I was involved in the development of the polychromatic excitation emission matrix (PEEM) fingerprinting [36, paper III] analysis method. Developed in tandem with my first papers, this work shows that using a polychromatic light source, as in a computer screen, and a tri-chromatic image detector, as in a web-camera, can provide comparable levels of fluorescence identification as using fluorescence spectrometry. Theory section 1 will go through this method of fluorescence identification as well as explaining the other methods of spectral response fingerprinting that have been mentioned previously.

In regard to the issue of miniaturization and customization of the sensing elements my first two papers explored the use of microstructures as possible substrates for indicator analysis. The investigation showed how microstructures, made from a photo-curable polymer called SU-8, could provide optical separation to a degree that benefits fluorescence identification. By enabling fluorescent light to break substrate confinement while at the same time selecting the amount of excitation light reaching the detector, the balance needed for PEEM was achieved allowing the detection of small amounts of fluorescent indicator. Paper II would take this technique to the next level by using a micro-dropper to deposit drops of indicators on to individual microstructures, thereby
permitting the analysis of multiple indicators, confined to 100 μm diameter regions, simultaneously.

Optical simulations were created to explain the effect the microstructures had on fluorescent light confinement. The theory of which, along with their 2D/3D models, will form the second part in the theory chapter. The analysis of paper II showed an extensive spatial interference effect. In this paper we took advantage of this effect aiding the discernment between different fluorescent indicators. Section 3 of the next chapter will go through the origin of this effect and how it applies to web-camera systems. The last of my papers, paper V, took advantage of this interference effect allowing the further expansion of the CSPT toolbox to the evaluation of weight displacement, which is also covered here.

The concluding section of theory will focus more on the design and manufacturing aspects of microstructures that provided an efficient collection of fluorescent light in solution. These closed vaulted microstructures containing fluorescent indicators in solution, when measured in a fluorescent microscope, provided an enhancement of 15 times the level of fluorescence compared to indicators on freestanding microstructures.

Chapter 3 will then discuss the results and conclusions of my papers, demonstrating the logic behind them and providing a more complete picture of my work. In conclusion I will discuss the possible future applications of my work and round of the picture of my doctoral research as the facilitating mechanism for the progression of the Computer Screen Photo-assisted Technique towards becoming a truly versatile ubiquitous sensing method.
Chapter 2: Expounding Theory

This chapter will cover the various theoretical areas that I developed and used during my doctoral research.
**PEEM: Fingerprints**

The CSPT concept, which I first came into contact with in 2006, was an emerging ubiquitous sensing strategy. The main analytical process in use at the time, and even now, is spectral fingerprinting \[30, 31\]. A CSPT spectral fingerprint is a unique signature from a substance. It is formed by illuminating the substance to be evaluated with a sequence of different polychromatic spectral radiances while using a web-camera to detect the resulting signal.

A computer screen is a polychromatic light source made up of the overlapping spectral radiances of red, green and blue pixels \[R(\lambda), G(\lambda), B(\lambda)\]. This means that any colour \(i\) generated by the computer screen is a composition of the three polychromatic spectral radiances, whose amplitudes are represented by the triplet weights \(r_i, g_i, b_i\), which will result in a total spectral radiance:

\[
c_i(\lambda) = (r_i R(\lambda) + g_i G(\lambda) + b_i B(\lambda))
\]

where \(\gamma\) is the correction for the non-linearity of the intensity with the displayed colour value \[36\]. A fluorescent substance illuminated by this source will modulate the transmitted light with absorption and fluorescence contributions \(\Psi_i(\lambda)\), which are a function of the wavelength and the particular illuminating radiance.

A colour web camera is a detector array with an overlaying checkerboard pattern of red, green and blue (RGB) filters. The intensity \(I\) of a given pixel \(j\), in the camera is for a given screen illumination \(c_i(\lambda)\),

\[
I_{r_i}^{(j)} = \int \lambda \psi_i^{(j)}(\lambda) F_r(\lambda) D(\lambda) d\lambda
\]

\[
I_{g_i}^{(j)} = \int \lambda \psi_i^{(j)}(\lambda) F_g(\lambda) D(\lambda) d\lambda
\]

\[
I_{b_i}^{(j)} = \int \lambda \psi_i^{(j)}(\lambda) F_b(\lambda) D(\lambda) d\lambda
\]

where \(D(\lambda)\) is the spectral response of the detector, and \(F_r(\lambda), F_g(\lambda)\), and \(F_b(\lambda)\) are spectral windows of the RGB camera filters.
A spectral fingerprint is then built up by illuminating the sample area with $c/(\lambda)$, using a sequence of colours on the computer screen. The web-camera is positioned to receive the light coming from the sample area, and a video of the sample under these conditions is composed. The spectral information of the substance ($\Psi$) is thus captured in this video.

Post-processing extracts the information from the video sequence and constructs the spectral fingerprints used for analysis. The composition of the fingerprints varies with the application, but they share in common the selection of regions of interests (ROI) on the images. It is from these that the intensity profiles for each illuminating colour, unfolded in the three camera channels can be obtained. Figure 3 shows an example of the concatenation of those intensity profiles. A CSPT fingerprint of a given ROI is then constructed as the difference between an intensity profile for a target, such as fig. 3, minus a reference intensity profile, an example of which is seen in figure 4.

A reference profile can take on many forms depending on the application. For the work presented in this thesis the reference profile is obtained by measuring the spectral fingerprint of an area where no other substances are present on the substrate. Other methods include performing a pre-exposure fingerprint of a substance. Where here one records the response of the substance pre-exposure to a reacting agent that, upon exposure, will change the fingerprint response of the substance post-exposure [29, 37, 38].
Figure 3: CSPT intensity profile.

Figure 4: Spectral response fingerprint, both with their web-camera channels highlighted.
**PEEM: The polychromatic excitation emission matrix**

Spectral response fingerprinting can identify fluorescent indicators using the CSPT platform. Numerous demonstrations and applications of this concept have been described [16, 29, 30, 31]. However, at the beginning of my PhD the fingerprinting of fluorescence substances was not well described and a model of the CSPT capable of predicting such fingerprints was clearly missing. It was not fully understood how a polychromatic light source and a tri-chromatic image detector could provide such comparable spectral identification with spectroscopy.

Figure 5: (a) Spectral radiances of the 50 colours displayed on the computer screen. (b) Excitation emission matrix of fluorescein. (c) Measured spectra under polychromatic illumination provided by the computer screen. Ten spectra are highlighted using the corresponding colours used for illumination. Black solid lines are the modelled spectra, which describe them as fingerprints of the EEM. The spectral windows of the camera channels are also displayed with black lines. (d) CSPT fingerprint composed as the concatenated intensity differences in the red, green and blue camera channels for all illuminating colours. Open circles are the experimental result, with colours corresponding to illuminating colours and the solid black lines are the modelled response.

Reprinted from [41]. Copyright (2009), with permission from Analytica Chimica Acta.
The solution came with the realisation that the spectral response fingerprint was a fingerprint of a polychromatically stimulated Excitation Emission Matrix (EEM). In total spectroscopy the emission spectra of every excitation band is composed in a single 3D space, which produces a complete characterisation of a fluorescent sample [42, 43]. An example of one such contour plot for fluorescein is seen in figure 5 (b).

Usually a fluorescence spectrometer is used for EEM analysis. This instrument has a broadband white light source and measures the emission of a fluorescent indicator for each excitation wavelength. In our case, as I described in the previous section, a computer screen illuminates with a polychromatic radiance $c_i(\lambda)$ that can be varied for each $i$ index, figure 5 (a). When measuring fluorescence in a CSPT platform the emission and transmission are entangled in the acquired fingerprints.

In contrast with standard spectroscopy, where the sample absorption is unique, in CSPT, depending on the spectral radiance used for illumination, gaps in the excitation can occur, figure 5 (a). In order to describe the transmittance measured with such a light source we modulate the sample absorption ($A(\lambda)$) with the particular radiance used for illumination.

$$a_i(\lambda) = \overline{c_i}(\lambda)A(\lambda) \quad (3)$$

where $\overline{c_i}(\lambda)$ is the normalized screen spectral radiance and $A(\lambda)$ is the spectroscopic absorption, with a transmittance:

$$\tau_i = 10^{-a_i(\lambda)} \quad (4)$$

Each illuminating colour is polychromatic and accordingly each measured emission signal will be the result of multiple excitation lines. The result of this is that each illumination probes a portion of the total EEM information of the fluorophore. If $\rho(\lambda, \lambda_{em})$ is the normalized EEM of the substance, the CSPT emission $e_i(\lambda_{em})$ can be calculated as:

$$e_i(\lambda_{em}) = \int \overline{c_i}(\lambda)\rho(\lambda, \lambda_{em})d\lambda \quad (5)$$
If we now calculate the total transmittance of a fluorescent sample under polychromatic illumination we will get that the measurable signal $\Psi$ at a particular position $x$ is

$$\Psi_i(\lambda, x) = n_1(x) \tau_i(\lambda) + n_2(x) \varepsilon_i(\lambda_{em})$$  \hspace{1cm} (6)

where the weights $n_1$ and $n_2$ are variables that depend on the setup used for measurement. This measurable signal can then be directly inserted into equation (2), determining the intensity of pixels $j$ in the red, green and blue channels of the webcam for fluorescence measurement eq. (2). It is these channels that are concatenated to form the spectral fingerprints ($\xi_j(\psi_i)$) as in figure 3. Quite often a reference subtraction is performed, as in figure 4, in order to highlight the fluorescent signal of the sample and this results in a fingerprint:

$$\phi_j(\psi) = \frac{\xi_j(\psi_{sample})}{\xi_j(\psi_{ref})} - 1$$  \hspace{1cm} (7)

**Paper III** formulates this theory and proves its plausibility by fitting the whole set of PEEM’s for various substances using only two free parameters $n_1$ and $n_2$, figure 5 (d). This result is further strengthened by the comparison of the PCA [44] evaluation pattern using either traditional fluorescence spectroscopy or the CSPT platform and the above model, where discrimination between different fluorescent substances is obtained to a comparable level, figure 6 (a). Figure 6 (b) demonstrates the needed balance between emission and excitation. The CSPT fingerprints being, this time, created from a purely fluorescent signal. This results in a poor discrimination between the porphyrins, which can be seen as the grouping together of their PCA scores. The results therefore show the necessity to measure not just the excitation or emission profiles but a balanced combination of the two.
Figure 6: (a) First two principal components score plot of the spectra (blue asterisk), CSPT fingerprints (black squares) and modeled CSPT fingerprints (red diamonds) measured from a balance excitation/emission region. (b) Idem to (a) but for a pure fluorescence region. Blue and black lines are a help to the eye, highlighting the classification pattern.
Fluorescence and Prior Art

Fluorescence as a phenomenon is the result of an electron, being excited from the ground state by a photon. The excited electron then decays down again to the ground state by emitting its’ energy in the form of light [45]. Fluorescent indicators themselves are used as labels in numerous types of assays [46] and as markers in fluorescent microscopy [47]. In sensing applications these indicators can also be the functional material by changing its optical characteristics after exposure to a target environment [37]. The focus of my work has been on developing a technique to enhance the detection of fluorescence signals in the context of CSPT detection.

In particular, fluorescence emission on dielectric surfaces implies that the greater portion of the emitted signal goes into the media with the higher refractive index [48, 49, 50, 51, 52]. It also does so at angles above the critical angle of that media [θ_c as, figure 7]; the critical angle being the angle above which no light can escape from a media and is such totally internally reflected (TIR). This essentially means that light emitted by a fluorophore on a substrate, that has a higher refractive index than the ambient, will do so into the substrate it is sitting on at primarily at angles such that very little light will actually come out the other side.

![Figure 7: Model of fluorescence emission, where “as” is the air/substrate interface and “Is” is the indicator/substrate interface.](Reproduced from [53].)

Integrated solutions dealing with this issue have been proposed previously [53]. By using microstructures with sloped walls, made in PMMA, TIR fluorescent light emitted from a chemical marker, on the tip of the structures, was redirected through the base. In order to block the excitation light, they made use of a filter allowing the collection of high levels of fluorescence.
This is a drawback for our applications, since it requires the filter to be adapted for different fluorophores, thereby limiting the versatility to sense diverse indicators. Also, as was described in the previous section, the CSPT platform is dependant on a balance of excitation and emission for its fingerprint evaluation and so we need to introduce optical separation for this purpose.

We work with SU-8, which is a UV curable polymer having similar properties to glass. This makes it a suitable element for our problem. By depositing it as a thin film on a substrate and exposing it to UV light, microstructures of any shape can be generated provided a suitable mask can be created [54, 55, 56, 57, 58, 59, 60]. These structures thus provide multiple micro-testing areas and even the possibility to perform microfluidics experiments [61].
SU-8 Microstructures

There are different procedures to configure SU-8 microstructures on substrates [62] but we wanted to make use of glass due to its availability, low cost and optical properties. We therefore became interested in well-defined structures at a glass/SU-8 interface such as those demonstrated in [63], figure 8. In this procedure the exposure mask is transferred to a transparent substrate (i) and then depositing the SU-8 on top of this transferred mask (ii), and exposing the SU-8 through the substrate (iii) creates well-defined microstructures (iv).

This provides better adhesion and control of the base of the microstructures, as this is the area that receives the strongest intensity of UV light, while also allowing the creation of stable high aspect ratio structures. The structures would also taper at their tips due to the Fresnel diffraction, which results in a profile of transmitted intensity with increasing distance from the surface. This aids in the use of the SU-8 microstructures as a moulding template (SU-8 being mostly used as a template for replica moulding in other materials, such as PDMS [64]).

Adapting the process to our needs, I introduced a fifth step in the above process that removed the mask layer. This I did in order not to block or disrupt the optical properties of the sample. The exact parameter detail of each step was also optimized for our

Figure 8: i) A thick photoabsorptive mask layer is first deposited on the substrate, in this case amorphous silicon on a glass wafer. ii) Pattern and etch the mask. iii) Spin on a layer of SU-8 photoresist exposed to UV light. iv) Develop SU-8.
patterns. This is because the quality of the final result is highly dependent on the exact process conditions for particular layouts e.g. larger patterns with larger spacing develop much quicker than patterns that are small and tightly packed. The specific recipes I used for the generation of microstructures in papers I, II and IV can be found detailed in the supplementary material section. Also, as can be read there, I did not use Amorphous Silicon as a mask or glass wafers as a substrate, as in [63], instead choosing Chromium as a mask and normal microscope slides as a substrate. Further modifications of this fabrication process lead us to the monolithic roofed structures described at the end of this chapter.

**Paper 1: 2D Modelling**

An optical model, using 2D and 3D raytracing software [65, 66], was developed to examine the illumination of a SU-8 pillar arrayed microstructured substrate (figure 9).

![Raytrace of light passing through a SU-8 microstructure.](image)

Figure 9: Left: Raytrace of light passing through a SU-8 microstructure. Right: Image of SU-8 hexagonal microstructures on glass with a spot of fluorescein under cyan illumination.

The 2D raytracing showed that light incident, at an angle, on our substrate will be refracted and conducted in two distinct directions. The majority of the light is transmitted straight through but a small fraction will totally internally reflect (TIR) from the microstructure wall and be directed towards the camera. It is this small fraction that makes the structures visible even when the camera is not looking directly at the screen through the structures, figure 9. This optical separation thereby allowing the selection of a fraction of the transmitted signal.

To examine this behaviour with fluorescent substances a similar model was developed but this time the focus was on fluorescent emission, figure 10. The emitted fluorescent
light is approximated here as point sources of light emitting above the critical angle of the substrate it was resting on, which is the media with the higher refractive index [48] (in this case the SU-8 microstructure or glass).

Due to the vertical walls light that is emitted at the critical angles of the SU-8, will refract when passing into the glass substrate and change their dispersion angle in the glass, escaping TIR. Meanwhile fluorescence, which is emitted from the tip of a microstructure, will experience refraction through air before being refracted through the substrate leading it to be collected by a web-camera perpendicular to the surface.

![Reprinted with permission from [40]. Copyright (2006), American Institute of Physics.](image)

Figure 10: Fluorescence emission model of fluorescent material on SU-8 and a glass substrate.

**Paper 1: 3D Modelling**

The above models were adequate to get a basic understanding of how SU-8 microstructures on a glass substrate can enhance fluorescent detection and provide optical separation. However, they can’t visualise what occurs when microstructures of different shapes and spacings are illuminated and how the 3D optical light paths will develop through a glass substrate. Therefore a 3D raytracing model was developed. Here light rays could be visualised and the effect of objects on their light paths modelled. Two models were thus generated that contained to-scale representations of the groups of SU-8 pillars used in paper 1, i.e. hexagonal and circular.

The microstructured areas were essentially a bound repetition of a smaller scale pattern. Therefore the model contains just a small group of structures at the four corners of the microstructured areas. This provides the boundary limits of how light would be affected by passing through a glass substrate with microstructured elements, with all other light effects contained within these limits.
Figure 11 shows the 3D models illuminated with a monochromatic light source. The inset shows a close up of one of the microstructure clusters at a corner limit. Making use of a detector in the model environment enables us to see how the light is dispersed after travelling through the sample, fig. 11 (a) and (b). The results, seen in fig. 12, show quite different dispersion patterns for the different structures, with the hexagonal structures directing the light to three distinct areas while the circular structures scatter the light in a circular pattern.

If we consider a cross-section of each structure this result is a natural consequence of the shape of each pillar. If the light coming in is at an angle to the pillars only half of the pillar will reflect this light. This we see in the 2D model, figure 9, as the light that is directed towards the camera. Therefore light hitting the hexagonal pillar will reflect from its three flat sides, thereby reflecting the light to three distinct areas (2, 3 and 4 of fig. 12 (a)), whereas the circular pillars having curved side’s results in the light being reflected in an arc (fig. 12 (b)).
Figure 12: (a) detector representation showing dispersion of excitation illumination and fluorescence emission from hexagonal pillars. (b) Idem. to (a) except for circular pillars.

When the fluorescent light is emitted from the surface of the microstructures, as in figure 10, it will disperse in all directions and be transmitted through the glass substrate. However, the shape of the microstructure also affects this, as the curved walls of the circular pillar will act as a lens causing the fluorescent light to diverge from a focal point. Due to limitations in the modelling program this effect is not seen here but is estimated with spot points. In reality the fluorescent light will be spread out in a similar way to the transmitted excitation light for the circular pillars.

Therefore the hexagonal structures are a better candidate for PEEM evaluations as one has a greater flexibility in controlling the ratio of excitation to emitted light to areas of local maxima or minima. This was later confirmed in the results of paper I.
**Paper 2: Moiré Patterns**

*Paper II* studied the evaluation of multiple fluorescent indicators on microstructures. In pushing the web-camera to such a limit of its focussing ability a collateral effect was encountered in that the pictures taken by the web camera contained coloured bands. These bands were a result of moiré interference [67, 68, 69, 70, 71, 72].

A moiré pattern is created when two periodic structures spatially interfere with one another. Figure 13 shows two examples of this; one where two patterns of concentric circles are shifted with respect to one another and another where two rectangular mesh grids are rotated with respect to one another.

![Figure 13: Examples of moiré interference.](image)

In a web camera, as I mentioned above, a similar effect can be seen when imaging microstructures with a discrete imaging array. The basis for this specific imaging effect of digital moiré interference is to be found in the very principles of digital imaging, which I will now introduce.
On the market today are two main types of digital image recorders: Charged Coupled Devices (CCD) and Complementary Metal Oxide Semiconductor (CMOS) devices. They both detect photons and transform this analogue signal into a digital one, but in slightly different ways [73]. However they function under the same principle; light hits a photosensitive cell or pixel which, in the case of a 24-bit colour detector, outputs a signal with a resolution of 0 – 255 intensity values per channel. As I described previously in “PEEM: Fingerprints” the photosensitive cells are covered in three types of filters. The pattern in which they cover the cells is known as a Bayer pattern [74] and separates the detector into three channels representing each of these colours, with twice as many green pixels as red and blue (mimicking the sensitivity of human vision). This is a way of minimizing cost and size of the detector by only needing one multi-filtered detector instead of three individually filtered ones. The output signal from the various pixels is then associated with its respective colour filter.

It is this intensity value and coloured grid position that results in what is called a Bayer image. If we now consider a simple object like that of figure 14 (a) and point a colour digital image detector at this object, figure 14 (b). We will produce a Bayer image as in figure 14 (c), which will then be subsequently sent to the detector processor. Due to the cost-saving channel separation of the Bayer filter, the red and blue channels contain the intensity information for one quarter of the image each while the green channel contains half of the intensity information.

Further processing is now performed called demosaicing [75]. This process can be done using a number of different mathematical methods [76, 77]. The simplest and quickest of which is bilinear interpolation.
Bilinear interpolation is a process that takes the average of the pixel intensity surrounding unknown values in a specific channel and assigns this average value to them.

The same approach is then applied to the unknown pixel intensities of the blue and green channels. After this process is completed the final image is then composed of these three layers of the interpolated red, green and blue channels, which when combined together reproduces a digital image of the initial object, figure 14 (d).


Moiré And The CSPT Platform

The previous method works very well in most cases and with regard to a web-camera, whose primary area of use is in imaging faces and rudimentary shapes, this method is very effective and cheap. However, when we now try to use these devices outside of their normal realm of use, as in imaging microstructures, it gives rise to a moiré pattern.

In paper II 100 μm hexagonal SU-8 microstructures on glass were imaged through a web-camera under computer screen illumination. The web-camera was placed at a distance of 1 cm from the structured substrate. However, even at this short distance the resolution of the camera was insufficient to prevent a moiré pattern appearing on the digitised image of the microstructures, figure 16.

By changing the distance between the camera and the microstructures the projection of the image on the detector changes, thus generating different moiré patterns that highlight different regions of the Bayer filter. Thereby enabling the Bayer information to be decoded in the demosciced image. This relationship of distance and moiré bands is one widely used in industry for positioning [78, 79, 80] and is even used for touch screen technology [81]. Paper V takes advantage of this effect by using the difference between two imaged moiré bands to infer a change in weight.

Figure 16: Image of microstructures under white illumination with the deposition areas indicated 1. Old Rhodamine, 2. Old Fluorescein, 3. Ge-Tcp, 4.Zn-Tpp, 5. Rhodamine, 6-8 Fluoresceins and an illustration of the pixels selected for analysis in the areas 4 and 8 is shown to the side.
Paper 2: Bayer Image Fingerprinting

There is though an advantage to this effect in that we now have access to a Bayer like image. Ideally, one would always want to use the un-demosaiced Bayer image for spectral analysis. This is because due to the interpolation done to obtain the demosaiced image, a lot of averaging is performed, which degrades the acquired spectral information. This is disadvantageous for analysis purposes, as you would want to retain as much of the pure original signal as possible. The Bayer image can be accessed through a dedicated driver or directly produced by digital imaging. So as the CSPT platform is considered to be a ubiquitous user-friendly platform, being able to access Bayer like information without modifying the setup will greatly increase the functionality of the platform.

Returning now to the issue of fluorescent fingerprinting on microstructures, we find ourselves in a situation whereby information of the Bayer pattern is overlaid on the final image. Recalling from previously, a spectral fingerprint is composed of the concatenated intensity values from the three camera channels in a specified ROI. If we were to use this method to try to evaluate a fluorescent indicator on a single microstructure in figure 16, discrimination would be very poor as the sample will sit on either a red, green or blue Bayer region. The chosen configuration in paper II resulted in mostly red or blue regions only.

A new spectral fingerprint evaluation procedure was subsequently developed to take advantage of this new information and in paper II discrimination between two different fluorescent indicators and two porphyrins as well as discrimination between newer and older depositions of the same indicators was achieved. All of which had been deposited on groups of individual 100 μm hexagonal microstructures, figure 16.

The new method involved extracting similar information from individual pixels within each ROI, which consisted of the groups of similarly coated micropillars as indicated in figure 16. As before the area was illuminated with a sequence of 50 colours from a computer screen. To extract and group the pixels that had similar spectral responses in each ROI a PCA of all the pixels in a ROI was plotted, figure 17.
This process of selection was performed on image 16 as under white illumination it is easier to discriminate between red and blue pillars as well as ensuring that the pixel selection is performed correctly. As can be seen in figure 17 a large proportion of the image is black (representing the black background) and then as you move to the right of the plot a clear division between blue and red coloured pillars is seen. By selecting two sets in this plot that exhibit similar spectral responses it becomes possible to choose the pixels that provide the best fluorescent signals discriminated in the two decoded Bayer regions.

By combining the spectral fingerprints of red and blue Bayer regions into a single spectral fingerprint for each substance, one can naturally exploit the Bayer decoding, thus improving the discrimination between indicators. Figure 18 is an example of one such combination.
Figure 18: Spectral fingerprint of one red and one blue pixel from ROI 1.

By collecting together 50 such red/blue pixels pairs for each ROI and normalizing to reduce noise it is possible to discriminate between all regions. In figure 19 it is even possible to see a difference in fluorescent signal between older and more recently deposited indicators, with older indicators having more quenched emissions and thereby represented here as a tendency to drift left in the PCA. If one attempts to discriminate the same amount of information but by using just one of the above sets the top PCA of figure 19 shows that discrimination then becomes more difficult, further supporting the benefits of Bayer decoding.
Figure 19: PCA of the selected pixels from ROI 1 to 8 of figure 19 where NRh = new rhodamine, ORh = old rhodamine, OFL = old fluorescein, FL 1–3 = fluorescein 1–3 with the top PCA showing how by using only one channel discrimination between multiple fluorescent signals becomes difficult.
Paper 5: Moiré Balance

As previously mentioned a periodic object will create different moiré patterns at different distances from a web-camera. This means that through knowledge of a few basic parameters i.e. camera field of view (FOV), number of pixels in sensor etc. it becomes possible to have an indirect measurement of distance. **Paper V** takes this information and uses it to make a physical sensor out of a web-camera.

First a calibration measurement was performed to obtain a response curve of the change in moiré pattern with distance. Then a spring-loaded platform was mounted on the web-camera and, through loading the spring with water in a container, it was possible to ascertain a displacement in weight down to 240 \( \mu \text{g} \).

For simplicity the same hexagonal pattern was used again but this time in the form of just the Cr mask on a glass substrate. As the setup was intended to be used in a vertical position with light being transmitted straight through, the use of a Cr mask provided a simple resilient way to replicate the effect seen with the SU-8 microstructures. The sample was then mounted on a motorised manipulator allowing for the displacement of the sample with a resolution of approx. 90 \( \mu \text{m} \). The sample was moved over a distance of 1 cm from 2.5 – 3.5 cm from the camera lens ring and pictures were taken at each 90 \( \mu \text{m} \) step.

Figure 20 shows examples of the moiré patterns recorded at three positions. Using the theory discussed previously a model based on the movement of a hexagonal patterned grid was created and the subsequent moiré patterns, at key positions, across the calibration range were generated. These were compared to the calibration data and, as the profile data in figure 20: column 3 shows, matched with this data quite well.

In order to extract a calibration curve from this information the \( \frac{1}{2} \) period (\( \phi \)) of just one of the colour bands was evaluated for each moiré pattern over the 1 cm displacement range. When plotted against the known distance that the moiré pattern was recorded at, a curve can be produced as in figure 21, and a system resolution evaluated. Now placing the patterned Cr mask in the setup of figure 22 and adding 1 ml of water in 100 \( \mu \text{l} \) steps it is possible with the help of the calibration curve of figure 21 to evaluate a displacement due to weight, which in this case gave a resolution of 240 \( \mu \text{g} \).
Figure 20: Column 1 and 2: Illustration of modelled moiré image compared to measured moiré image for the Z-distances, with alternating blue and red stripes. Column 3: Examples of the single line sinusoidal pixel intensity modulations of the measured moiré images (red/solid line) and model (black/dashed line) in the marked regions A – A’. These have been compensated for the phase difference between the model and measured pictures to illustrate their matching quality in $\phi$.

Figure 21: Graph of $\phi$ for the calibration (black dots and best-fit black solid line) and model (best-fit green star marked line) with 3 $\sigma$ error lines (red circle marked lines). Weight measurements (x) calibrated to the best-fit line. The blue line with square markers is the resolution of the system over the investigated range of distances.
Figure 22: Setup for measurement of weight displacement due to moiré pattern shifting in digital images of a Cr patterned glass substrate.
Roofed Microstructures

This final section of this chapter will discuss a method to create monolithic 3D microstructures in SU-8.

As light passes through an aperture it will be diffracted, with a greater diffraction effect being seen as the wavelength of the light approaches the size of the aperture. In our SU-8 microstructure manufacturing process holes as small as 30 μm are used to create structures of such a diameter. As we are using an ultra violet light source with wavelengths around 375 nm a noticeable diffraction effect is present.

This is seen and commented on in [63] as a tapering of the tips of the microstructures and a firm attachment in the base of the structure. In our work we use the diffraction pattern along the 30 μm thick SU-8 film, which is being illuminated from the aperture (mask) at the glass/SU-8 interface to the SU-8 surface. By playing with the shape and the coordination of the aperture (mask pattern) we can control the 3D profile of the illuminating pattern and even have a higher intensity in the distal region of the polymer. This strategy allows us to cross-link the polymer at the distal surface while providing empty inner spaces of controlled geometry (paper IV).

Following the manufacturing procedure in the second section of the supplementary material, by exposing SU-8 through a mask that it is in intimate contact with over a range of intensities from 180 – 252 mJ cm⁻², one can produce cavities of various forms. Figure 23 shows a simplified model of this process for a single aperture, with figure 23 (b) illustrating the intensity profile distribution from an aperture of the same size as in (a).
This intensity distribution $I_z$ after passing through the aperture is calculated from [84],

$$I_z = \frac{I_0}{4} \left\{ \left[ C(u_z) - C(u_1) \right]^2 + \left[ S(u_z) - S(u_1) \right]^2 \right\}$$  \hspace{1cm} (8)

where $C(u)$ and $S(u)$ are the Fresnel integrals;

$$C(u) = \int_0^u \cos \left( \frac{\pi u'^2}{2} \right) du'$$ and $$S(u) = \int_0^u \sin \left( \frac{\pi u'^2}{2} \right) du',$$  \hspace{1cm} (9)

with
\[ u = \frac{b}{2} \sqrt{\frac{2}{\lambda \cdot z}} \]  

(10)

at a distance \( z \) (0 - 30 \( \mu \)m) from the aperture and a aperture width \( b \), using a wavelength \( \lambda \), in this case of 365 nm.

Figure 23 (c) shows the cross-linking profile achievable between two adjacent apertures when the photon energy is increased above 170 mJ cm\(^{-2}\) for 30 \( \mu \)m thick SU-8, thus creating cavities in the non-crosslinked areas. Three such cavity structures created using square, hexagonal and circular apertures at different photon energies are shown in figure 24: column 1. To better understand the 2D distribution of the diffracted UV light that created these cavity structures, a Matlab model for 2D Fresnel diffraction [85] figure 24: column 3 was made.

This time the output 2D diffraction profile is based on the convolution of an input wave and an aperture structure, which according to Fourier optics in [85]

\[ \Psi_p(x,y,z) = \Psi_{p_0}(x,y) * h(x,y,z) \]  

(11)

where \( z \) is 0 - 30 \( \mu \)m as before and,

\[ h(x,y,z) = e^{i \frac{\pi x^2 y^2}{2z}} \]  

(12)

with \( k_0 = 365 \) nm.

Clearly seen in the simulation is the degree of overlapping that emanates from the edges of all the apertures. It is this overlap that provides the required photon energy to cross-link the SU-8 in the tips of the structures while leaving the area around the bases free.

In order to test the cavity structures a roofed microstructure based on circular pillars, figure 25, was filled with a solution of rhodamine B. These structures were then able to collect a fluorescent signal 15 times larger than the corresponding open (not roofed) structures.
Figure 24: Column 1: SEM images of partially closed cavities formed using (a) square apertures, (b) hexagonal apertures and completely closed cavities formed using (c) circular apertures. Column 2: selected symmetry of partially closed cavities formed using (d) square apertures, (e) hexagonal apertures and completely closed cavities formed using (f) circular apertures. Column 3: 2D model of diffraction pattern for the considered symmetries in column 2. Column 4: intensity profile in the selected segments indicated in column 2.

Figure 25: A peel back showing circular pillars attached only in their tips forming a roof structure.

This can be seen in figure 26 where the intensity profile across an “open” pillar structure coated in rhodamine is compared to a “closed” cavity structure.
Figure 26: (a) 0.5 mM rhodamine on 25 μm wide and 30 μm tall: (a1) open circular pillars, (a2) open circular pillars with a mirror in the back and (a3) closed circular structures with embedded Cr mirror. (b) Profiles taken from indicated white line, across (a1) and (a3), comparing fluorescent signal in the green channel for open (dashed black line) and closed (solid black line), circular patterned SU-8.

If we look over the distance B – B’ in each picture a1 – a3, two distinct features can be compared between the open structures and the roofed ones. The first of these is the signal in the distance between adjacent pillars. Comparing the opened structures to the roofed one, G2, sees a 15 fold increase in the detected fluorescence. This is the signal from the bulk rhodamine that is contained within the cavities of the roofed structured. As explained previously fluorescence near a dielectric is emitted above the critical angle of the dielectric leading to poor transmission. This is the result we see in a1 and a2.
This also occurs in a3 except now there is a great deal of contained bulk rhodamine in liquid form as in region B of figure 27 (b) providing a greater fluorescent signal. The other feature areas are the walls of the pillars, seen as the rings in a1 and a2. G1 is the comparison of this signal, which is 3 times larger in the roofed structures of a3 compared to the open ones. The enhancement here is explained purely through the geometry of the microenvironment. To further illustrate these effects 2D and 3D models were then created to investigate this effect, figure 27.

Figure 27: (a) Section of a closed microcavity circular structure taken from fig. 26 (a) a3. (b) Raytrace model of a closed microcavity. Light sources A represent fluorescent light emitted into the SU-8 along the SU-8/acetone critical angle $\theta_c = 31.4^\circ$ and light source B represents the fluorescent light emitted from the bulk rhodamine/acetone solution within the microcavity and radiating in all directions. (c) Profile in the black solid line is from the CCD response of (b), as modelled in CyberRay 2.4, and the profile in the black dashed line is an extract from the profile plot of fig. 26 (b).

In figure 27 (b), the model demonstrates that as the fluorescent light is emitted into the media with the highest refractive index, which in this case is the SU-8, high levels of fluorescence can escape. As demonstrated in paper I open pillar structures only transmit fluorescence from the pillars, with any fluorescent light on the glass substrate being TIR. Therefore in this case with the roof on the cavity, it is possible to have the sample upside down so that light transmitted through the SU-8 roof can be detected from the bulk solution B and the internal surface of the cavity A.

3D raytracing was then used to model and interpret the transmission profile that is seen in figure 26 (b), i.e. large intensities at the edges and low or zero intensity within the pillar structure. Figure 28 illustrates this model showing how multiple light sources emitting above the critical angle of the SU-8/ethanol interface into the SU-8 produces areas of high and zero intensity. In figure 27 (c) this CCD profile is compared to the one from figure 26 (b) showing an exact match.
This concludes the theoretical portion of this thesis. The next chapter will discuss the various scientific contributions provided by the presented research as well as some thoughts on the future development of the CSPT principle.
Chapter 3: Drawing to a close

Concluding remarks and discussions around the contributions of this work.
The CSPT platform is a collection point for optical techniques. Instead of having diverse instruments that have a few specific functions the CSPT platform has an adaptability that allows it to adopt optical techniques for analysis in the visual spectrum.

By taking microstructures that are normally used for microfluidics in lab-on-a-chip applications I developed a method that allowed for a significant increase in fluorescence detection in the CSPT platform. Looking to the final results, shown here in figure 30, the introduction of a microstructured substrate increased fluorescent signals by a factor of 5 and 3.16 for hexagonal and circular pillars respectively.

![Figure 30](image-url)

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Figure 30: (a) CSPT spectral fingerprints of fluorescein deposited on hexagonal pillars for illuminating angles from 6° to 40°. The inset shows a 4 mm area of pillars illuminated with a cyan colour. The white circles indicate the regions of interest selected to compose the fingerprints. The dark frame of the inset is the image of the glass surface without pillars. (b) Idem as (a) but for circular pillars.

The angle of the sample to the screen was varied during this experiment in order to find the best balance in the PEEM. This occurs at 40° as this is the point beyond which light will start to enter directly into the camera and cause too much background illumination thereby disrupting the PEEM balance and leading to poorer signals. For comparison a spot of fluorescent material was deposited on bare glass and measured in the same way at the various angles. The black dashed line is the result of this and remains low and constant throughout, demonstrating the clear advantage of the SU-8 microstructures.

This paper was the first use of micro pillar arrays to provide simple, flexible and compact optical separation compatible with PEEM. This development furthermore
introduced a more sophisticated alternative to CSPT experiments and was an essential step towards more advanced detections.

**Paper 2**

Continuing on from the work of paper 1 this paper took the detection of fluorescent material to the next logical phase i.e. the detection and discrimination between multiple indicators simultaneously. As discussed in Ch. 2 this process provided us with a number of challenges, with the eventual development of an adaption of the fingerprinting process to exploit the presence of moiré fringes. Interestingly these fringes were present in the first paper however they were removed simply through defocusing the lens slightly. At this 1 cm distance from the web-camera the fringes can be easily removed using this technique without any great loss in resolution. It is only when, as in this paper, we wished to have individual pillar resolution that a sharp focus was needed and thus required the development of the new evaluation process.

**Paper 3**

This paper, which was developed alongside the previous two, forms the backbone of the whole fluorescent detection theory on the CSPT platform. By showing that a spectral fingerprint captures the excitation emission matrix associated to a fluorescent indicator, a consistent explanation to the identification capabilities seen in the first two papers was developed. This also showed for the first time that the CSPT platform is supported by a robust detection concept on par with those used in more advanced instrumentation.

**Paper 4**

Normally when working with microstructures their main purpose is to be used in combination with microchannels in a micro-fluidic system. It is therefore crucial that the system be completely sealed before any liquid is sent through. This is usually achieved through creating the channels and microstructures on one substrate and then attaching a separate roof piece using some form of sealant. This is a very sensitive procedure as the channels could become filled with sealant or an incomplete seal could be made such that the system leaks when filled with liquid.
If instead one creates a micro-fluidic system that has a roof already, much time, cost and resources can be saved. By exposing SU-8 beyond its recommended dosage, and using controlled diffraction, it becomes possible to create vaulted cavities with a roof in just one exposure step. What was often considered something to be avoided now becomes a very useful method for the fast production of lab-on-a-chip devices.

**Paper 5**

The last of my papers to be published showed the versatility of the CSPT platform by demonstrating the use of a web-camera as a weight displacement sensor. This work constitutes a first explanation of the CSPT concept outside the chemical sensing field and extends its applicability to physical parameters (such as weight or distance), which can have important complementary uses aside from its main use as a chemical sensor. It was also while working on this paper that the full model of moiré fringe generation was developed, providing a testing platform for the development of other patterns for fringe analysis at shorter or farther distances.
Outlook

The future of any research is always hard to predict as there are so many variables that can affect its development. From grant money, to manpower, to availability of resources all these things guide and shape all scientific research. However a good guess can be made and for the CSPT platform to evolve further a jump to mobile phone technology is the next obvious step. With their availability and increasing complexity mobile phones are more and more like mini computers with built in cameras, thus making them ideal for the CSPT concept. The first demonstration of this is [86], where we have shown how a standard medical test can be evaluated with the aid of a just a mobile phone.

Other possibilities are open now due to my last paper allowing the CSPT platform to explore physical sensing. Moiré interference can be induced in a web-camera using any type of high contrast periodic structure so monitoring the movement of flexible micro-pillars can provide e.g. flow measurements. Rhythmic patterns of movement like breathing could even be monitored using this technique. Where the movement to be monitored is transduced into movement in a camera with respect to a fixed pattern.

The true beauty with the CSPT technology is its accessibility and connectivity. By using everyday electronics and simple sample interfaces the measurement of chemical substances can be performed by any layperson in similar ways to pregnancy tests or urine tests today. This will most likely lead to its usage in hereto-untried fields like botany for examining and recording new species to criminal forensics for on the spot analysis of crime scene evidence.

It is easy to see the progress towards more mobile versions of the CSPT platform, as I mentioned above, but as a stationary and easily managed measurement tool it also has the possibility of being used for environmental monitoring. By placing CSPT platforms at key points of interest and with the possibility to connect to the Internet, CSPT measurements could be geo-tagged, providing fast and accurate analysis of e.g. the water quality throughout an entire river system or the spread of an air-born virus throughout an entire building.
SU-8: Microstructure generation paper 1 and 2

SU-8 is an epoxy resin that contains a photosensitive cationic salt. When this salt is exposed to UV radiation, between 350 - 400 nm, it becomes a powerful acid. This in turn will cause the SU-8 resin to cross-link its’ molecules when heated \cite{87}, thereby leaving hardened SU-8 resin. This hardened resin is resistant to developing agents thus allowing the removal un-exposed regions \cite{88} and making SU-8 a negative-tone photoresist.

SU-8 can also be purchased in various viscosities. This is for the convenience of the end user as the diluter of SU-8 is fairly toxic. For all of the work presented here SU-8 (10) was used, which can provide structures approximately 30 μm high. This we chose merely out of convenience as SU-8 with higher viscosity gives very thick layers that require a much longer process time \cite{60} and lower viscosity SU-8 will only create very small structures \cite{88}, which was not helpful for us as we needed something large enough for optical testing. What follows is the procedure I developed for creation of 30 μm high microstructures with various shapes and spacing.

Standard microscope glass slides were cleaned in H$_2$O : H$_2$O$_2$ : HCl (6:1:1) for 10 mins to remove any residue and provide a good surface for SU-8 attachment. After drying with N$_2$ gas, 150 nm of Cr were deposited through thermal evaporation, from tungsten boats, onto one side of the slides.

Cr films were then coated with S1818 by spin on a disk at 4000 rpm for 30 sec. Directly after spinning the samples were baked at 100°C for 1 min before being exposed for 10 sec at 3.6 mJ cm$^{-2}$ by a UV mask aligner system, through a patterned quartz mask. After development the exposed Cr, after the removal of S1818, was etched using a wet-etching chemical process resulting in the dissolution of the exposed Cr film. Washing with acetone and water to remove the un-exposed S1818 gave a Cr patterned glass slide, which could then be used as a mask for SU-8 micro-fabrication.

When the samples were thoroughly dried a layer of SU-8 (10) was spun onto the slide by first spinning at 500 rpm for 15 sec and then at 1000 rpm for 30 sec. This two-step
spinning procedure is required to evenly coat the sample and to provide a film thickness between 25-30 μm [89]. The samples are then pre-baked at 65°C for 4 min and 95°C for 8 min, to remove the solvent from the SU-8, before finally being exposed through the back.

Once exposed to a minimum energy of 170 mJ cm⁻² [89] the samples are post-baked for 2 min at 65°C and 4 min at 95°C to reinforce the cross-linking initiated by the exposure process. The final step was then to develop the samples in MR. DEV 600 for 5 mins before washing in isopropanol and drying on a baking plate at 100°C.

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Figure 31: Blow-up of SU-8 microstructures on a glass slide from paper I.
As stated before, for a depth of 30 μm a minimum exposure of 170 mJ cm\(^{-2}\) is required to cross-link the SU-8. However, this dosage is used to cross-link the directly exposed SU-8 and not the area in between the mask features where the intensity is much lower. Also the manufacture of SU-8 microstructures is a many stepped process and each step has an influence on the final result i.e. the length of time spent developing the structures, the spacing between mask features, as well as the pre/post exposure baking conditions. Therefore, in order to produce microcavities a specific set of process parameters must be found.

Pre/post exposure baking conditions provide stability to the structure and ensure that the SU-8 roofs cross-link, which has been shown for the bulk SU-8 elsewhere [90]. A two-step baking procedure, where the pre-baking conditions were to eliminate the solvent in the SU-8 and the post-baking conditions were to selectively cross-link the exposed SU-8 [89], was used: 4 min at 65 °C and 8 min at 95 °C pre-baking / 2 min at 65 °C and 4 min at 95 °C post-baking. Also due to the weak exposure of the roofs, it was found that excessively long development could almost completely remove the roof structure. Therefore a 5 min development time with an isopropanol wash at 2.5 min was considered optimal.

As expected, similar mask features with different spacing's could form a cap at different exposure times. For example, a mask feature with a spacing of 30 μm formed roofs with 252 mJ cm\(^{-2}\), with a spacing of 20 μm roof formation occurred with 216 mJ cm\(^{-2}\) and with a spacing of 10 μm roof formation occurred with 180 mJ cm\(^{-2}\).
Figure 33: Column 1: SEM images of partially closed cavities formed using (a) square apertures, (b) hexagonal apertures and completely closed cavities formed using (c) circular apertures. Column 2: selected symmetry of partially closed cavities formed using (d) square apertures (e) hexagonal apertures and completely closed cavities formed using (f) circular apertures.

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