Optical devices and methods for distributed lab-on-a-chip analyses

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“The mind is everything.
What you think you become.”

(Buddha)
Abstract

Lab-on-a-chip (LOC) technologies entail the miniaturization of analytical systems, and the reduction of required sample and reagent volumes. LOC devices offer compact alternatives to classical instrumentation while delivering comparable performance and disposable formats. These aspects make disposable LOC a clear candidate to support distributed chemical sensing applications; however, the need of accessory services and readout obstructs the materialization of pervasively distributed LOC solutions.

In this thesis methods and devices to solve this problem are investigated. A distinctive aspect of this work is the pursuit of solutions based on disposable LOC elements specifically conceived to exploit ubiquitous infrastructure for readout and evaluation.

Consumer electronic devices, such as cell phones are ubiquitous platforms with residual capabilities that can be used for chemical sensing, if properly interfaced. This work investigates elements and tools needed to empower cell phones as readers of disposable LOC devices and commercial disposable tests.

Access to flexible fabrication of LOC devices at low cost is an important requisite for testing ideas and implementing customized solutions. A first contribution in this thesis is the development of a platform for mask less fabrication of 3D microstructures, which coexists on a routine fluorescence microscope. This microscope projection lithography system (MPLS) is capable of controlled 3D micro structuring, including cavities and cantilever geometries, and the sealing of monolithic micro cavities to glass substrates as well as the connection to large scale service areas. This fabrication platform and other fabrication methods were used along this thesis to provide disposable optical and fluidic components.

Besides custom-made LOC solutions there are well-established commercial disposable devices, which are essentially compatible with decentralized diagnosis, except for the use of specialized readers that confine them to medical centers. The implementation of high dynamic range (HDR) imaging with standard cell phones, using the phone screen to control exposure, shows that sensitivity and resolution can be boosted to permit robust evaluation of this type of disposable tests, in decentralized scenarios.

Solutions employing commercial tests, which have not been designed for cell phone evaluation, are typically suboptimal and the investigation of customized LOC components occupies a central role in this thesis. Accordingly, one important aspect to evaluate LOC
devices in compact configurations is to be able to image the LOC at a close distance from the phone camera, a condition for which phones cameras are not able to focus.

In addition, different phone brands and models have different optical specifications, and a practical refocusing solution should adapt to all of them. In this work an adaptive lens concept, complemented by phone time-lapse acquisition, which can be integrated in disposable LOCs, is demonstrated.

The implementation of sensitive detection methods, such as surface plasmon resonance (SPR), which is compatible with label free protocols that simplify sample conditioning, is central to the materialization of ubiquitous LOCs readable with cell phones. In this thesis a disposable optical coupler, conditioning illumination taken from the phone screen, is used to create an angle resolved SPR signal from a LOC, which is read with the phone front camera. Tested performance is comparable with commercial compact SPR modules and detection of \( \beta_2 \) microglobulin, which is an established marker for cancer, inflammatory disorders, and kidney disease, is within the diagnostics range for blood and urine.

Finally, fluorescence detection within classical LOC devices is tailored to be detectable with consumer cameras. In this case a disposable optical coupler and fluidics is designed to condition laser illumination into total internal reflection excitation, while DSLR and phone cameras capture optically separated fluorescence. The system configuration supports a broad dynamic range and HDR imaging enables localized resolution boost at selected detection ranges. Detection of free fucose, a diagnostic marker for liver cirrhosis and several cancer forms, is shown feasible with a HDR implementation, as one last example of practical LOC detection schemes for decentralized scenarios.
Populärvetenskaplig sammanfattning

Optiska komponenter och metoder för distribuerad analys med "lab-on-a-chip"

Lab Denna avhandling visar hur speciellt anpassade "laboratorier-på-en-bricka" (lab-on-a-chip, LOC) kan användas för en analys av en rad parametrar, inte minst av intresse för hälsokontroll. LOC är i sig föremål för en intensiv forskning och utveckling. Avhandlingen beskriver design, tillverkning och tillämpningar av LOC för engångsbruk dedicerade för att användas tillsammans med en mobiltelefon, där telefonens kamera och skärm ingår som komponenter i ett optiskt detektionssystem. Avancerade optiska analyser demonstreras i avhandlingen med LOCs tillverkade med en metod som inte behöver komplicerade masker för fotolitografi utan där tredimensionella mikrostrukturer, inkluderande flödeskanaler, byggs upp genom en programmerad belysningssekvens i ett mikroskop.

I avhandlingen beskrivs också hur en mobiltelefon har använts för kommersiellt tillgängliga tester baserade på kontrastförändringar. Ett sådant exempel är detektion av en markör för hjärtsvikt (NT-pro-BNP), där för första gången en mjukvara som tillåter avbildning inom ett stort dynamiskt exponeringsområde (high dynamic range photography, HDR) används för sensorändamål för att ge ökad känslighet och upplösning.


HDR har använts tillsammans med en annan LOC för att detektera fluorescens med hjälp av mobiltelefonens kamera. LOC ser till att excitation med hjälp av en laser sker genom total intern reflektion i en mikroflödeskanal och att endast fluorescensen träffar kameran. Detektion av fukos i urin (markör för bl.a. skrumplever) vid kliniskt relevanta
koncentrationer ges som ett sista praktiskt exempel på resultaten av den forskning som avhandlingen avser.
Sammanfattningsvis så visas på möjligheten att använda etablerade analytiska metoder för medicinsk diagnostik med hjälp av engångskomponenter, en mobiltelefon och lämplig programvara.
Preface

The work presented in this doctoral thesis is a result of my PhD studies between March 2008 and November 2012 at the Optical Devices Laboratory at Department of Physics, Chemistry and Biology (IFM), Linköping University (LiU). This thesis mainly concerns the methods and devices for disposable lab-on-a-chip (LOC) devices and optical elements, which are used to support distributed chemical sensing applications conceived to operate on cell phones. My main supervisor has been Assoc. Prof. Daniel Filippini and my co-supervisor has been Prof. Ingemar Lundström. This work has been supported by a PhD scholarship from Thammasat University (TU) of Thailand and three grants from Linköping Center for Life Science Technology (LIST). The results are presented in five papers that collect the scientific output of my work.

This thesis is divided in two parts. The first part provides a general introduction to LOC technologies and describes the background, including microscope photolithography systems (MPLS), sessile drops lenses, high dynamic range (HDR) imaging, surface plasmon resonance (SPR) and total internal reflection fluorescence, with their associated applications. The second part is a collection of the five papers included in this thesis.

Pakorn Preechaburana
Linköping, November 2012
List of papers and contributions

Papers included in this thesis

I. Fabrication of monolithic 3D micro-systems

P. Preechaburana, D. Filippini


(My contribution: optical design, assembly, optimization, microfabrication, characterization and writing)

II. HDR imaging evaluation of a NT-proBNP test with a mobile phone

P. Preechaburana, S. Macken, A. Suska, D. Filippini

*Biosensors and Bioelectronics, 2011, 26, 2107-2111.*

(My contribution: part of the experimental work, data analysis and writing)

III. Embedded adaptive optics for ubiquitous lab-on-a-chip readout on intact cell phones

P. Preechaburana, A. Suska, D. Filippini

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(My contribution: microfabrication, characterization, sessile drop imaging and writing)

IV. Surface Plasmon resonance chemical sensing on cell phones

P. Preechaburana, M. Collado Gonzalez, A. Suska, D. Filippini


(My contribution: optical design, fabrication, assembly and integration, characterization, and writing)

V. Disposable total internal reflection fluorescence lab-on-a-chip for medical diagnosis

P. Preechaburana, P. Erlandsson, E. Åström, P. Pålsson, D. Filippini, N. D. Robinson

*In manuscript, 2012.*

(My contribution: optical design, devices fabrication, optical measurements, HDR data processing and writing)
Other papers not included in this thesis

VI. Snapshot mask-less fabrication of embedded monolithic SU-8 microstructures with arbitrary topologies
P. Preechaburana, D. Filippini
*Procedia Chemistry*, 2009, 1, 778-781.

VII. Mobile phone analysis of NT-proBNP using high dynamic range (HDR) imaging
P. Preechaburana, S. Macken, A. Suska, D. Filippini

VIII. Fast prototyping of monolithic micro-system on epi-fluorescence microscopes
P. Preechaburana, D. Filippini
Conference contributions

Snapshot mask-less fabrication of embedded monolithic SU-8 microstructures with arbitrary topologies
P. Preechaburana, D. Filippini
*Eurosensors XXIII*, 6-9 September 2009, Lausanne, Switzerland, Poster.

Mobile phone analysis of NT-proBNP using high dynamic range (HDR) imaging
P. Preechaburana, S. Macken, A. Suska, D. Filippini
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Fast prototyping of monolithic micro-system on epi-fluorescence microscopes
P. Preechaburana, D. Filippini
*Eurosensors XXIV*, 5-8 September 2010, Linz, Austria, Poster.

3D mask-less photolithography on epi-fluorescence microscopes
P. Preechaburana, D. Filippini

Generic optical coupler for angle-resolved SPR imaging on lap-on-a-chip devices
P. Preechaburana, D. Filippini
*Lap-on-a-Chip European Congress*, 30 June - 1 July 2011, Hamburg, Germany, Poster.

Adaptive disposable lens for cell phone lab-on-a-chip readout
P. Preechaburana, D. Filippini
*Lap-on-a-Chip European Congress*, 30 June - 1 July 2011, Hamburg, Germany, Poster.

Cell phone-based surface plasmon resonance imaging for lab-on-a-chip devices
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Part I

An introduction to the field
Chapter 1

Introduction

Lab-on-a-chip (LOC) devices are a particular category of micro electro mechanical systems (MEMs). LOC, also referred to as micro total analysis system (µTAS), miniaturize and integrate single or multiple laboratory operations in a compact format [1]. LOC technologies comprising microfluidic systems deal with the handling of fluids using microchannels, which permit analytical detections with small sample and reagent volumes, and high performance. Continuous development of the LOC technologies offers other essential benefits including faster analysis times, better process control and lower manufacturing cost [2-4].

Early demonstration of MEMs can be traced back to the 1960s. Miniaturized pressure sensors of micrometer and sub-micrometer sized silicon structures were demonstrated by several groups in the early 1960s, while one of the first µTAS devices was a gas chromatographic air analyzer developed by S. C. Terry et al. at Stanford university and published in 1979 [5]. In the late 1980s and early 1990s, numerous microfluidic handling devices, such as micropumps and flow sensors were developed, and sample conditioning processes such as mixing and separation steps were integrated in µTAS devices.

In the mid 1990s, µTAS technologies were employed for molecular biology applications and for military purposes aiming at deployed warfare agent detection. The term lab-on-a-chip was introduced from this breed of applications, which is not only restricted to analyses carried out in laboratory facilities.
In contrast to more costly silicon MEMs devices, LOC employs alternative materials such as SU-8 photoresists and polydimethylsiloxane (PDMS), which are cheaper and easier to use for microstructures fabrication [2, 6]. Microfluidic components such as channels [7-9], valves [10], pumps [11] and optics [12-14] integrated in LOC devices have been demonstrated in both SU-8 and PDMS. Especially in the case of optical devices these materials are convenient since their optical characteristic are similar to glass.

Dedicated microfabrication methods have been developed for LOC devices; however, they are typically limited to experts and specialized facilities [15]. Conventional photolithography using chrome binary masks is widely used to fabricate microstructures and PDMS templates, although this method involves relatively high cost of masks, which additionally need to be entirely replaced if modifications, addressing improvements in the design, need to be introduced [16, 17].

Alternatively mask-less photolithography is capable to incorporate changes in the fabrication process in a more effective way. Several mask-less photolithography methods such as gray-scale microfluidic photomasks [18], printed color mask [19], binary gray-scale physical masks [20] and multi photon absorption polymerization [21] have been developed to fabricate microstructures. One successful technique to produce complex 3D microstructures is micro-stereo lithography (µSL) [22-24], which utilizes precision 2D scanning of a focusing light beam over a photo-curable resist, thus building 3D microstructures layer by layer.

A more versatile mask-less instrumentation, the microscope projection lithography system (MPLS) utilizes liquid crystal displays (LCD) [25, 26] or digital micromirror devices (DMD) [27, 28] working as spatial light modulators (SLM) for pattern generation, thus enabling direct conversion of 2D gray-scale layouts to 3D polymer microstructures in just one exposure step.

In Paper I, a fabrication process implemented as a DMD-based MPLS that overcome some obstacles including cavity formation, alignment and sealing procedures, which affect existing methods [29-31], is demonstrated. This system co-exists as a modulated light source on a Zeiss Axiovert 40 CFL inverted routine microscope, and is able to configure complex 3D microstructures of SU-8 including monolithic cavities, suspended cantilever structures and sealed microchannels to glass substrates, as well as the connection to service areas. This fabrication technique and also regular soft-lithography methods for PDMS are used in this thesis to construct the optical and fluidic components.

Accurate diagnosis is a prerequisite for effective treatments and early detection of diseases. Diagnosis and health monitoring typically involve time-consuming and costly
procedures; with most services centralized in healthcare centers. Alternatively, distributed diagnosis entailing LOC devices and other technologies have been proposed as a cost-effective and easy to use alternative [32-36]. In this scenario patients can use these decentralized tools in absence of sanitary infrastructure and specialists to seek early diagnosis or routine monitoring by themselves.

One example of this paradigm is based on non-standard LOCs, such as 3D microfluidic paper analytical devices (µPADs) [37, 38], which are used for point-of-care analysis and configured for visual readout.

In this thesis conventional LOC devices, fabricated from SU-8 and PDMS, compatible with high analytical performance, are configured as disposable devices to support distributed chemical sensing applications that can be accurately evaluated with cell phones. A distinctive aspect of this thesis is the pursuit of solutions based on disposable LOC elements specifically conceived to exploit ubiquitous infrastructure for readout and evaluation.

Consumer electronic devices (CEDs), such as scanners [39, 40], web cameras [41, 42] and cell phones [43, 44] have been demonstrated for readout of chemical sensors. Among the CEDs, cell phones are ubiquitous platforms with residual capabilities that can be used for chemical sensing, if properly interfaced. This work investigates elements and tools needed to empower cell phones as readers of disposable LOC devices and commercial disposable tests.

Heart failure is a serious medical condition that allowedly reduces patient mobility, while simultaneously requires routine monitoring of health indicators, which typically demands to attend to health centers for evaluation. Decentralized monitoring of this marker would make the procedure more comfortable for the patients, contribute to the compliance and adherence to treatment and help to release medical centers from routine tasks.

The N-terminal proBNP molecule (NT-proBNP) is a cleavage product of the precursor protein B-type natriuretic peptide (BNP), the concentration of which in blood is a key predictor of cardiovascular mortality in patients with diagnosed heart failure [45].

In Paper II the evaluation of a disposable commercial sensing test, well-established for NT-proBNP (Roche Cardiac proBNP), which is essentially compatible with decentralized use, except for the use of specialized readers, is demonstrated using a regular cell phone.

Since in the present case, the detection consists of quantifying the contrast level of a region of interest (ROI) on the NT-proBNP test, a high dynamic range (HDR) [46] image acquisitions procedure was implemented. By using the phone screen for controlled
illumination and employing only the most basic resources from Java ME to control image acquisition, a solution compatible with most mobile phones was achieved.

HDR techniques record an extended dynamic range by composing and tone mapping a number of images acquired under different exposures [47], and in this case enables quantitative determinations with a substantial increase in the sensitivity and resolution, which foster the usability of cell phones for detection.

Disposable LOC devices have been demonstrated for numerous sensing and clinical applications [48]; however, their dissemination is restricted by the instrumentation required for readout. LOC solutions for point of care (POC) or other distributed detections [37] are typically associated with dedicated and specific off-chip readers [2].

Thus, although LOC devices can be disposable and deployable to a large scale, the availability of readers and their specific characteristics restrict the dissemination of analyses based on this technology. Therefore, if disposable LOC devices could be evaluated using generic and common platforms, such as cell phones, the benefits of this technology could be made ubiquitous.

Solutions employing commercial tests, which have not been designed for cell phone evaluation, are typically suboptimal and the investigation of customized LOC components occupies a central role in this thesis. Among the multiple challenges posed by this goal, one important aspect to evaluate LOC devices in compact configurations is to be able to image the LOC at a close distance from the phone camera, a distance at which phones cameras cannot focus.

In Paper III off-chip readout of disposable LOC devices on cell phones is investigated in a configuration without additional accessories and using adaptive optics that can be integrated within the LOCs. The device sits on the camera surface, which provides a reliable mechanical support, and the necessary optical coupling. This PDMS device temporarily sticks on the camera during evaluation and is disposed afterwards.

Since cell phone cameras cannot focus at such short distances, the LOC entails a refocusing element that permits to image the micrometric detection area typical in LOC devices. Simple fixed lenses can be implemented for a particular camera type [50]; however, since different brands and models have slightly different optical designs, a generic solution to this problem demands to adapt to all of these conditions with a unified concept. Adaptive optics is central for autofocusing and can be implemented in different ways [51–54] as...
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dedicated components, but in this work we pursued a solution that could be embedded in disposable LOC devices, such as a sessile drop [55–57] complemented by data analysis.

The implementation of sensitive detection methods, such as surface plasmon resonance (SPR), which is compatible with label free protocols that simplify sample conditioning, is central to the materialization of ubiquitous LOCs readable with cell phones.

In Paper IV angle-resolved surface plasmon resonance (SPR), which is an established label-free detection method [58], is migrated to cell phones as a disposable interface.

The SPR coupler central to this implementation is compatible with regular lab-on-a-chip (LOC) technology and gently adheres to the phone screen surface during the measurement; it couples and conditions the illumination from the screen and directs the SPR image to the phone camera. After the measurement the device can be detached and disposed of, thereby leaving the phone intact.

SPR detection is illustrated with a commercial assay for \( \beta_2 \) microglobulin (\( \beta_2 \)M) [59], an established marker [60, 61] for cancer, inflammatory disorders, and kidney disease, which are deemed candidates for complementary monitoring in decentralized conditions; moreover SPR detection is also illustrated with a custom-made chip including embedded calibration, a key concept in ubiquitous sensing.

Several human diseases, including liver disease and cancer, are characterized by an increased synthesis and degradation of carbohydrate structures containing the monosaccharide fucose. As a result, free fucose can be found in the urine of these patients [62].

Regular tests for free fucose in urine are typically laborious and costly, whereas a simple test for urinary fucose that can be used bed-side to give clinicians a fast and cheap way to determine whether further investigation of liver disease or cancer is necessary, would constitute a valuable resource.

Conditioning the fluorescence detection to the resolution compatible with consumer and cell phone cameras is a necessary step to make the decentralized instrumentation simple, affordable and versatile.

In Paper V, fluorescence detection within classical LOC devices is tailored to be detectable with DSLR and cell phone cameras. In this case a disposable optical coupler and fluidics is designed to condition laser illumination into total internal reflection excitation,
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while the phone camera captures optically separated fluorescence. The system configuration supports a broad dynamic range and HDR imaging enables localized resolution boost at selected detection ranges. Detection of free fucose, a diagnostic marker for liver cirrhosis and several cancer forms, is shown feasible with this system.

This and the other results in this thesis constitute evidence that minimally modified or even intact cell phones can offer an appropriate platform for accurate chemical analyses, running on conventional disposable LOC technologies or even adapting to existing commercial tests.

For optimal results, the design of disposable LOC and optical elements, conceived to operate on cell phones, introduce a key advantage to materialize convincing ubiquitous LOC diagnosis.
Chapter 2

Microscope projection lithography system

Fabrication of complex 3D microstructures is a central aspect to microelectromechanical systems (MEMS), micro total analysis systems (μTAS), micro-fluidic devices, miniaturized sensors, and optical devices [63-66]. Conventional fabrication methods entail binary photolithographic techniques, which require costly chromium masks and involve time-consuming multiple step procedures to fabricate complex microstructures [6, 7]. Fig. 2.1 illustrates increasing levels of complexity in 3D microstructures fabrication. Fig. 2.1(a) corresponds to 3D microstructures without shape control along the height, which can be fabricated with a 2D binary mask, whereas a structure fully configured in 3D (Fig. 2.1(b)) requires a more sophisticated technique. Finally, the fabrication of sealed 3D controlled cavities (Fig. 2.1(c)) entails a higher level of sophistication.
Chapter 2. Microscope projection lithography system

Figure 2.1 Scheme of 3D microstructures involving increasing fabrication difficulties (a) 3D structures created with binary masks (b) 3D positive geometries (c) 3D negative geometries sealed to substrate.

To reduce the running costs and accelerate the process iteration, mask-less photolithography techniques have been recently developed for exploratory research, including gray-scale microfluidic photomasks [18], printed color masks [19] and multiphoton absorption polymerization [21]. One successful mask-less technique to produce complex 3D microstructures is micro-stereo lithography (μSL), which utilizes 2D scanning of a beam over a photocurable resist and operates the procedure layer by layer to configure 3D structures [22-24]. An alternative mask-less instrumentation, the microscope projection lithography systems (MPLS) utilizes a liquid crystal display (LCD) [25, 26] or a digital micromirror device (DMD) [27, 28] working as a spatial light modulator (SLM) for a pattern generation presenting a direct conversion of 2D gray-scale layouts to 3D polymer microstructures.

In this thesis, a DMD-based MPLS is implemented as co-existing element on a routine fluorescence microscope that enables the fabrication of simple channels (2D), positive 3D structures and sealed monolithic 3D cavities of arbitrary geometry as well as suspended 3D structures of any cross section. Concurrently, the MPLS platform is capable of integration at two-dimensional scales involving few fabrication steps.
Chapter 2. Microscope projection lithography system

2.1 MPLS design

The developed MPLS uses a spatial light modulator as virtual gray-scales mask generator projected at the focal plane of the microscope. In this way a routine epi-fluorescence microscope is transformed into a micro-fabrication platform with several benefits, such as complete 3D control of positive and negative geometries as well as the versatility to coexist with regular microscope uses [16, 28].

![Figure 2.2](image)

**Figure 2.2** (a) Schematic of the fabrication setup. Epi-fluorescence routine inverted microscope used as a mask-less 3D micro-fabrication platform using a DLP projector providing patterned illumination. (b) Spectral radiance of white illumination from the projector. Adapted from *Paper I* by permission of The Royal Society of Chemistry.

The MPLS setup implemented in this thesis (Fig. 2.2(a)) consists of an inverted routine microscope (Zeiss Axiovert 40 CFL), a digital light processing (DLP) projector (Optoma EP1690) with 2500 ANSI lumens and contrast ratios of 2500:1, and a camera prime lens (Nikkor 50mm f/1.8). Every component can be reassembled in their original configurations since there are no permanent modifications to the setup. This system exploits the commercial projector, which comes with an DMD array (1024×768) serving as the virtual photo mask generator. The high image contrast ratios of DMD-based projectors are well suited with the lithographic system to generate effective and well allocated exposure [28].

The UV filter glass placed in front of the ultrahigh pressure lamp, which is the light source of the projector, was removed to increase intensity in the near UV region. The projector was aligned with the fluorescence illumination path, through the prime lens fixed at its maximum aperture.
Fig. 2.2 (b) illustrates the spectral radiance of the light source measured at the microscope stage through the glass slide. Regular negative tone photoresist (SU-8) is sensitive to the broadband near UV radiation in the range of 350 - 400 nm, with a recommended 365 nm (i-line) [67]. In this setup, a substantial part of the UV light is useful at absorbed by the projector which is designed for the visible range. The intensity of i-line is very low (0.4 mW/cm²), but still enough to be useful at acceptable long exposures.

Photoresist to become microstructures is spin coated on one side of a glass slide (Menzel-Glaser) and placed on the microscope XY traverser during the fabrication process. Filter cubes (Zeiss FT510 in a SET15 filter cube) in the microscope filter block slider are customized for observation and exposure. During the focusing and alignment process, a green excitation filter (Zeiss BP 546/12) is mounted in one filter cube to avoid the exposure of the photoresist. The different focal planes, which can be chosen at the photoresist/air interface and at photoresist/glass interface, are manually selected using the microscope focusing screw.

If the pattern observed through the eyepiece is perfectly focused in green light, it may be blurred in near UV because of an axial (longitudinal) chromatic aberration [28, 68]. This aberration can be corrected by finely adjusting the position between the camera lens and the projector [28].

Once perfectly focused without the aberration, the sample is exposed by switching to the next filter cube, which has the excitation filter removed.

Different resolutions and working areas can be achieved by switching objectives. For a 5× objective the resolution is 30 µm with a working area of ~50mm², whereas a 10× objective handles a working area of ~4mm² with the resolution of 5 µm.

### 2.2 Gray-scale photolithography

In order to contextualize the contribution of MPLS, alternative procedures are described in this section. Conventional photolithography (Fig. 2.3(a)) uses binary chrome masks selectively blocking UV light to generate 2D layouts of a given height [69]. In order to create 3D microstructures, multi-layer photoresist with several masks and multiple exposure steps are required [70].

Alternatively, gray-scale lithography creates precisely controlled 3D surfaces in a one step exposure procedure. This technique uses the same protocol for resist preparation i.e. film coating, soft baking and development process as in the conventional lithography, but the resist is exposed by a modulated single exposure dose. A low exposure dose cross link, a
resist at a shorter depth than a region receiving a high exposure dose. After development, the resist is dissolved in the proportion of the exposure, thus generating a 3D topology [69, 71] as illustrated in Fig. 2.3 (b).

![Figure 2.3 Comparison of photolithographic techniques (a) conventional binary mask photolithography and (b) gray-scale photolithography.](image)

An important feature of gray-scale lithography is the number of gray levels which the system can generate. The spatial light modulator created by the projector used in our MPLS provides 8 bits resolution. However, the photoresist absorption is nonlinear, and the full dynamic range of 256 gray-levels can neither be achieved. Accordingly, illuminating patterns must be calibrated to compensate nonlinearity and range [72].

### 2.3 Fabrication of 3D microstructures

SU-8 has very low optical absorption (high transparency) above 360 nm, which contributes to its conventional use to fabricate nearly vertical sidewall structures in thick resist films [73]. However, this makes the precise control of exposure depth in the SU-8 difficult to archive and embedded channels or suspended structures cannot be fabricated with the light source used in this thesis.

To address this issue, the properties of the SU-8 are modified by adding a light absorber in the available spectral range [74, 75].
Figure 2.4 Spectral radiance of the light source measured on the microscope stage through a glass slid, through 30 μm of SU-8 on glass and through 30 μm of SU-8/S1818 on glass. Adapted from Paper I by permission of The Royal Society of Chemistry.

In this thesis, the SU-8 is mixed with 10% volume of the positive photoresist S1818. The fabrication process for the mixture (SU-8/S1818) is the same as pure SU-8 and the behavior of the mixture is like that of SU-8. Unexposed structures are removed upon development and the increased absorbance in the region of 400-450 nm (Fig. 2.4) provides the required control of adsorption depth for the available source. Thus complex 3D monolithic suspended microstructures such as the cantilevers and sealed negative controlled geometries, i.e. vaulted channels, can be fabricated as illustrated in Fig. 2.5.
Figure 2.5 3D images of monolithic microstructures (a) vaulted channels sealed to a glass substrate and aligned with a positive plano-convex lens. (b) Cantilevers of arbitrary cross sections. Adapted from Paper I by permission of The Royal Society of Chemistry.
Chapter 2. Microscope projection lithography system
Real world scenery can exhibit extreme dynamic ranges, which refers to the differences between the brightest and the darkest level of light, than that can be captured by existing recording media. Loss of information in dark and saturated areas is a consequence of this limited dynamic range recording. On the other hand, the details in bright areas of the scene can be captured using short exposure times, whereas the details in dark areas can be recorded for long exposure times [76].

High dynamic range (HDR) imaging is a photographic technique that enables capturing the complete contrast range of the real scenes. Concurrently, displaying media such as computer screens and printouts support even shorter dynamic ranges than recording media. The nonlinear projection of HDR images into these low dynamic range (LDR) media, known as tone mapping, permits realistic rendering despite the contrast limitations.
3.1 HDR image acquisition

To acquire HDR images, a multi-exposure method is used in this work. A sequence of images taken with different exposure times is reconstructed in a post processing stage to generate a HDR image [77] (Fig. 3.1).

![Diagram](image)

**Figure 3.1** A scheme exposure bracketing to acquire a HDR image. A sequence of regular images is taken with different exposure times and is combined to generate a HDR data set.

The sequence of images of the same scene is captured using a common configuration, in which the camera aperture \(N\) is fixed in order to maintain the depth of field constant, and the exposure time \(\tau\) is varied (Fig. 3.2). These parameters are related to the exposure value \(EV\) by:

\[
EV = \log_2 \left( \frac{N^2}{\tau} \right) \tag{3.1}
\]

The dynamic range is described in terms of \(EV\) difference \((\Delta EV)\) between the brightest and darkest areas of the images. This \(EV\) difference is related to the contrast ratio, \(C\), by:

\[
C = 2^{\Delta EV} \tag{3.2}
\]

Thus a regular reflex camera capable of 11 stops dynamic range \((\Delta EV = 11)\) can acquire a contrast of 1:2048, while real scenes can be in excess of 1:100000.
Chapter 3. High dynamic range imaging

Figure 3.2 A series of LDR images of the same scene taken with different exposure values (EV = -2, 0, +2).

Figure 3.3 HDR image reconstructed from a series of LDR images in Fig. 3.2 using PhotomatixPro 3.2.7. The image was tone mapped in tone enhancer.
Chapter 3. High dynamic range imaging

3.2 Tone mapping
The dynamic range in HDR images exceeds the capabilities of displaying devices, which have lower dynamic range. Hence, these displaying media devices cannot render the complete range of captured light. An image reproduction technique i.e. tone mapping is required for the purpose to project the information into the LDR range [78], while the details of the HDR image including the localized contrast between neighboring pixels are maintained. Thus, fine details in the resulting image are enhanced in dark and bright areas as shown in Fig. 3.3.

Specific implementations of tone mapping depend on the applications. In some cases, the target of tone mapping is to maintain a brightness match between HDR scene and the tone mapped result, while other situations can emphasize as much of fine details in image as possible. The nature of HDR/tone mapping technique is the flexibility to decide in the post processing which aspect to highlight.

3.3 HDR photography
The idea of using several exposures was firstly developed by Gustave Le Gray in 1850 [79]. Le Gray attempted to render seascapes showing the strong contrast between the sky and the sea, which was at that time using standard photography. He made it possible using two negatives: one exposed for the sky and another one longer exposed for the sea, and then combined them to create a single photographic print.

In the 1940s, Charles Wyckoff developed wide dynamic range film (XR film) which was composed of three layers with different sensitivity to light. This film was used to capture historic images of the first nuclear explosions.

In 1993, Steve Mann introduced a method to generate HDR images from digital multi-exposure bracketing.

The concept of tone mapping is even older than HDR, and can be observed in attempts of extending contrast by combining indigo with gold leaf contrast in paints from Cimabve (1240-1302). Remarkably, 19th century impressionists highlighted the focus of theses artists on capturing the light on the canvas by dismissing the accurate rendering of the figures.

Today established software packages, such as Photomatix [80] and Photoshop [81], combine the capabilities of multi exposure merging and tone mapping in a single environment.
Scientific uses of HDR imaging include ultra-high contrast amplification in bright field microscopy of low contrast phase-object, such as unstained cells and micro-organisms [82]. In this thesis, HDR processing is used to improve sensitivity and resolution in the evaluation of commercial NT-proBNP tests using standard cell phones.
Chapter 3. High dynamic range imaging
A sessile drop is a drop of liquid deposited on a horizontal solid substrate where the wetted area is confined by a three-phase contact line. The drop geometry is defined by drop height, contact radius and contact angle. It is found that the contact angle plays an important role in characterizing surface properties of solid substrate [83, 84] such as the surface energy in contact angle measurements.

**Figure 4.1** The equilibrium of a liquid drop on solid substrate, and acting surface tensions.
4.1 Contact angle and wetting phenomenon

Fig. 4.1 shows a liquid drop deposited on a solid surface. In equilibrium, the drop is balanced by three forces acting along the three interfaces between the solid, liquid and vapor phase. These three forces are the surface tensions between solid and liquid ($\gamma_{SL}$), between solid and vapor ($\gamma_{SV}$), and between liquid and vapor ($\gamma_{LV}$). The contact angle ($\theta$) is defined as the angle formed between the solid/liquid interface and the liquid/vapor interface or alternately described as the angle between the solid surface and the tangent plane to the surface of liquid.

The contact angle is related to the interfacial tensions by the Young equation [85, 86]:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta$$  \hspace{1cm} (4.1)

Wetting of solid substrates by liquid is a surface phenomenon, which results from the difference in the surface tensions between the solid and liquid. The degree of wetting (wettability) can be characterized by measuring the contact angle [87].

If the contact angle is greater than 90º, the solid surface is considered hydrophobic, which indicates a low surface energy and corresponds to a low degree of wetting. Hydrophilic surfaces on the other hand correspond to high surface energy and wettability, whereas contact angles are lower than 90º [87-89]. Fig. 4.2 illustrates the comparison between the hydrophobic and hydrophilic surfaces. Completely wetting and a completely non-wetting conditions occur at the extremes of $\theta$=0º and $\theta$=180º, respectively.

**Figure 4.2** Comparison of the hydrophobic and hydrophilic surface specified by the contact angle.
4.2 Evaporation of sessile drops

The evaporation of a sessile drop involves simultaneous heat and mass transfer processes, including conductive heat transfer to the substrate, convective heat transfer, which is affected by the surface tension, and temperature gradients inside the drop [55]. The sessile drop can be described as a spherical cap shape when the drop is smaller than the capillary length (~2.7 mm for water), for which the surface tension has more effect than the gravity [90-92]. The contact angle ($\theta$), the contact radius ($r$), the drop height ($h$) and the radius of curvature ($R$) are used to characterize the spherical cap geometry as shown in Fig. 4.3.

![Figure 4.3 Cross section of the spherical cap shape of a small sessile drop. The contact angle ($\theta$), the contact radius ($r$), the drop height ($h$) and the radius of curvature ($R$) are used to characterize the spherical cap shape.](image-url)
Each of these parameters can be written in the function of other two parameters by considering the geometry as [55]:

\[
R = \frac{r}{\sin \theta} \tag{4.2}
\]

\[
h = \frac{R}{1 - \cos \theta} \tag{4.3}
\]

\[
\theta = 2 \tan \left( \frac{h}{r} \right) \tag{4.4}
\]

The volume \((V)\), the drop cap surface area \((A)\), and the radius of curvature \((R)\) can be expressed in terms of \(r\) and \(h\):

\[
V = \frac{\pi h (3r^2 + h^2)}{6} \tag{4.5}
\]

\[
A = \pi (r^2 + h^2) \tag{4.6}
\]

\[
R = \frac{r^2 + h^2}{2h} \tag{4.7}
\]

There are three possible modes of evaporation: the constant contact angle mode, the constant contact radius mode and the mixed mode [92] as shown in Fig. 4.4. During evaporation in the constant angle mode (Fig. 4.4(a)), the contact angle remains constant and the drop shape is also unchanged, whereas the contact radius is continuously reduced until the end of the evaporation. The constant contact radius mode (Fig. 4.4 (b)) is characterized by a constant contact radius, whereas the contact angle decreases while the evaporation takes place. For the mixed mode of evaporation (Fig. 4.4 (c)), the contact angle and the contact radius are altered through the whole evaporation process.
Figure 4.4 Comparison of liquid evaporation modes: (a) constant contact angle mode (b) constant contact radius mode and (c) mixed mode.

4.3 Application for optical detection

In this work, sessile drop evaporation is used as a disposable morphing lens for off-chip readout of LOC devices on any cell phone. Fig. 4.5 illustrates an integrated adaptive focusing element using a water lens for imaging microscopic detection regions of lab-on-a-chip (LOC) devices in contact with cell phone cameras. The proposed concept demonstrates a simple and generic device for optical detection, which can operate across brands and models of intact cell phones.
Chapter 4. Sessile drops

Figure 4.5 (a) Design of the morphing lens. The forward distance ($d_F$) and the back focal length (BFL) are the parameters setting the lens magnification and describing the adaptive focusing capabilities. (b) PDMS used for the substrate of the lens, which is attached to the rear cell phone camera of a Nokia 6720. (C) 3D scheme of the measuring device. Reproduced from Paper III under the open access license.

The lens is a drop of distilled water delivered from a 0.5 mm needle on a 150 µm thick PDMS substrate, which mildly adheres to camera surface providing the reliable optical coupling between these elements.

The PDMS substrate is separated from the LOC devices by a forward distance ($d_F$) less than 2 mm providing a compact configuration. During the measurement the device temporarily sticks on the camera and is easily disposed after the measurement leaving the camera intact.
Figure 4.6 Collection of sessile drop images captured with a stereomicroscope at a 15 s interval. The evaporation regimes (indicated as 1, 2 and 3) are highlighted. Reproduced from Paper III under the open access license.

The evaporation of a water drop from a PDMS substrate measured at 15 s interval at room temperature shows the three regimes of evaporation (Fig. 4.6). For about half of the recorded time, the constant contact angle is observed in the first regime (1 in Fig. 4.6) with the changes of the drop curvature by reducing the contact radius and the height of drop. Mixed mode occurs in the second regime (2 in Fig. 4.6), the contact radius and the height keep changing, whereas the contact angle changes accelerate. In the final regime (3 in Fig. 4.6), the constant contact radius mode is observed, with shape changes accelerating even further, with the drop pinned at the substrate. In this conditions, a large range of increasing R (and correlated BFLs) result in the conditions exploited to adapt LOC focusing on diverse phone cameras.
Chapter 4. Sessile drops
Chapter 5. Surface plasmon resonance

Surface plasmon resonance (SPR) is an optical phenomenon that can be used as a sensing tool to accurately examine refractive index changes in the very near vicinity of a metal surface. Common applications of SPR include biomolecular interactions analysis, for which SPR is a sensitive and label-free method [93-95].

5.1 SPR principle

Surface plasmons (SP) are transverse magnetic (TM) waves which travel along the interface between a dielectric material and a highly conductive metal, and exponentially decay in the perpendicular direction to the interface [96, 97]. Surface plasmons arise from a collective oscillation of free electrons at a metal surface [98, 99] (Fig. 5.1) which in the case of gold films can be excited at visible frequencies. SPR is the resonant excitation of a surface plasmon wave along the dielectric-metal interface [100, 101]. This resonance occurs from the interaction between excitation light and free electrons in the metal only under appropriate conditions.
Chapter 5. Surface plasmon resonance

Figure 5.1 Schematic illustration of the charge distribution and electric field $E$ of a surface plasmon at a metal/dielectric interface.

**Excitation of SP by light**

The wave vector, $k_{sp}$, of the surface plasmon traveling along the metal/dielectric interface can be expressed as [100]:

$$k_{sp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$  \hspace{1cm} (5.1)

where $\omega/c$ is the wavevector of light in vacuum, $\varepsilon_m$ and $\varepsilon_d$ are respectively the dielectric permittivity of metal and dielectric medium.
Fig. 5.2 shows the dispersion relation of the surface plasmon curve in relation with those of light traveling in the dielectric medium and in a prism. For the dielectric medium [102, 103]:

\[ k_d = \frac{\omega}{c} \sqrt{\epsilon_d} \]  \hspace{1cm} (5.2)

Accordingly, the surface plasmons cannot be excited in the dielectric medium by directly shining light on the metallic surface [103], hence a coupling mechanism is necessary to match the wavevector; a prism with high dielectric constant (Fig. 5.2) is utilized to couple the excitation to the surface plasmons [102].
Fig. 5.3 illustrates the Kretschmann prism coupling geometry which is the most widely used technique to attain the SPR phenomenon. The base of the prism is coated with a thin metal film of approximately 50 nm thick [104], which is kept in direct contact to the dielectric sample of lower refractive index. Gold is the most common metal used in SPR sensors because of its stability and relative chemical inertness [105]. P-polarized light is directed through one side of the prism at an incidence angle $\theta$ greater than the critical angle at the prism/dielectric interface. Total internal reflection creates an evanescent wave, which in-plane component of wavevector ($k_p$) is given by [100, 102]:

$$k_p = \frac{\omega}{c} \sqrt{\varepsilon_p} \sin \theta$$  \hspace{1cm} (5.3)

where $\varepsilon_p$ is the dielectric constant of the prism. When the propagating constant of the evanescent wave matches that of the surface plasmons at a certain incident angle, which is called SPR angle ($\theta_{SPR}$), the excitation of surface plasmon occurs [102] with the resonance condition given by:

$$\frac{\omega}{c} \sqrt{\varepsilon_p} \sin \theta_{SPR} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$  \hspace{1cm} (5.4)
If the reflected light is measured as a function of incidence angle $\theta$, then a sharp dip at $\theta_{SPR}$ is observed as the conversion of energy to the surface plasmons occurs (Fig. 5.4).

**Figure 5.4** A typical SPR spectrum showing the dip at the resonant angle $\theta_{SPR}$.

**Penetration depth**

The penetration depth of surface plasmons into the sensing medium is defined as the distance in the perpendicular direction to the interface at which the intensity of the electric field decays to $1/e$, which is given by [98]:

$$\delta_{SPR} = \frac{\lambda}{2\pi} \sqrt{\frac{\epsilon'_m + \epsilon_d}{\epsilon_d^2}}$$

(5.5)

where $\epsilon'_m$ represents the real part of the dielectric constant of metal. For example, if incident light with $\lambda = 633$ nm is excited on gold film with $\epsilon_m = -11.6 + 1.2i$, then the penetration depth $\delta_{SPR} = 328$ nm into air medium and 177 nm into water medium [103].
Chapter 5. Surface plasmon resonance

5.2 Design of coupling optics for SPR sensing on cell phone

Since cell phone displays in current models provide wide-angle illumination with intensities typically between 300 and 500 nits (equivalent to 43.9 and 73.2 $\mu$W/cm$^2$sr at 555 nm), they suffice to illuminate angle-resolved SPR experiments. Additionally, modern front cell phone cameras are typically of VGA resolution which is sufficient to capture angle-resolved SPR images, with good accuracy.

The coupler used in this work is a disposable optical element made of an optical grade epoxy (EPO-TEK 301-1, $n=1.5$) and polydimethylsiloxane (PDMS, $n=1.4$). In order to capture the SPR dip for a gold/water interface under red illumination, the epoxy with matching refractive index to the glass slide ($n=1.5$) is needed to achieve the necessary illuminating angles. The glass slide coated by 45 nm of thermally evaporated gold is attached to the epoxy surface using immersion oil. Simple fluidics or more advanced lab-on-a-chip (LOC) devices on the surface can be utilized for sample conditioning.

The light source is provided by an image displayed (a red rectangle in this case) in the cell phone screen, and a white frame is used for the optical coupler placement as shown in Fig. 5.5(a). The screen illumination is collected by PDMS plano-cylindric element and conveyed to an 8 mm long region on the gold surface. The angle-resolved SPR signal reflected from the gold interface is collected by a PDMS cylindrical element and deflected towards the camera by total internal reflection in a PDMS prism as shown in the ray-tracing (Fig. 5.5(b)).
Chapter 5. Surface plasmon resonance

Figure 5.5 (a) 3D scheme of the setup for angle-resolved SPR optically coupled by a disposable device with screen illumination and front camera detection (b) 2D ray-trace of the coupler element showing the light path from screen to camera. Adapted from Paper IV with permission from Wiley-VCH.
Chapter 5. Surface plasmon resonance
Chapter 6

Total internal reflection fluorescence

Total internal reflection fluorescence (TIRF) exploits an evanescent wave to excite fluorophores in the limited region adjacent to the interface of two optical media, such as a glass substrate and a liquid sample [106-110]. Fluorophores in the bulk of the sample are thus not excited because the evanescent field is extinguished away from the interface. Thus TIRF provides a powerful technique to selectively excite a functionalized surface and to optically separate fluorescence from excitation in compact geometries, which can be convenient for optical chemical sensing [111-114].

6.1 Fluorescence principle

The fluorescence process is illustrated in the Jablonski diagram shown in Fig. 6.1. A fluorescent substance, i.e. a fluorophore absorbs light and an electron is raised from a ground state \(S_0\) to an exited state \(S_1\) by absorbing the photon energy.

The excited electron loses part of its energy by dissipation of non-radiative energy to the surrounding environment and then relaxes to the lowest excited state \(S_1\). This phenomenon is called internal conversion. From this excited state \(S_1\), the electron will return to the ground state \(S_0\) and simultaneously emit the fluorescent light with a longer wavelength than that of the exciting photon.
Chapter 6. Total internal reflection fluorescence

Figure 6.1 Simplified Jablonski diagram. The diagram illustrates the electronic transition used to describe the phenomenon of fluorescence.

The Stokes shift is the difference in wavelength of the peak between excitation and fluorescent emission spectra. The excitation energy $E_{ex}$ is given by:

$$E_{ex} = \frac{hc}{\lambda_{ex}}$$  \hspace{1cm} (6.1)

and emission energy $E_{em}$ is given by:

$$E_{em} = \frac{hc}{\lambda_{em}}$$  \hspace{1cm} (6.2)

where $\lambda_{ex}, \lambda_{em}$ is the wavelength of the excitation and emission spectra peaks respectively.
Chapter 6. Total internal reflection fluorescence

Figure 6.2 Stokes shift between the absorption and emission spectra of maxima.

Due to energy lost in the internal conversion, the emission energy is smaller than the absorption energy i.e. $E_{em} < E_{ex}$. The emission spectrum maximum is shifted to longer wavelength ($\lambda_{em} > \lambda_{ex}$) compared to the excitation spectrum which is illustrated in Fig. 6.2.

6.2 Total internal reflection principle

Total internal reflection (TIR) can occur when light propagates from an optical medium of larger refractive index to a medium of smaller refractive index. Fig. 6.3(a) illustrates how light behaves at an interface of two medium with different refractive indexes. A beam of light traveling in the higher refractive index medium i.e. glass ($n_2 = 1.51$) reaches a medium of lower refractive index i.e. aqueous solution ($n_1 = 1.33$–1.38 [116]). For illuminating angles smaller than the critical angle given by the Snell’s law:

$$\theta_c = \sin^{-1}(n_1/n_2), \quad (6.3)$$

the light beam is refracted in the second medium. If the angle of incidence is greater than $\theta_c$, total internal reflection occurs, and the light beam is reflected back within the glass. Nevertheless, some of the energy of the light beam penetrates through the interface generating an evanescent electromagnetic wave, which can excite the fluorophores adjacent to the interface.
Chapter 6. Total internal reflection fluorescence

The intensity of evanescent wave exponentially decays away from the interface restricting the excitation to the glass surface region and providing an efficient mechanism for optical separation of fluorescence from excitation (see Fig. 6.3(b)). The intensity $I$ at the distance $z$ from the interface is given by:

$$I_z = I_0 e^{-z/d}$$  \hspace{1cm} (6.4)

where $I_0$ is the intensity of the evanescent wave at the interface ($z = 0$). The penetration depth $d$ at which the evanescent wave decays to 37% of $I_0$ is given by [116]:

$$d = \frac{\lambda_0}{4\pi} \sqrt{\frac{1}{n_s^2 \sin^2(\theta) - n_i^2}}$$  \hspace{1cm} (6.5)

where $\lambda_0$ is the wavelength of the excitation light in vacuum.

Typical values of the depth are in the range of about 60-200nm [106, 108, 109]. This depth depends on various parameters including refractive index of sample and coupling medium, wavelength of excitation and angle of incidence. The penetration depth decreases as angle of incidence increases or the wavelength of excitation decreases.

In practical cases, such as those considered in this thesis, the wavelength of the excitation and refractive index of coupling medium are fixed and the incident angle, is adjusted to exceed the critical angle, which is adjusted for the refractive index of the sample.
Figure 6.3 Comparison of TIRF and transmitted fluorescence setups. (a) TIRF technique, when angle of incidence $\theta_i$ is greater than the critical angle, the excitation is reflected while the fluorophores near the interface are excited by the evanescent wave. (b) In transmitted fluorescence arrangements, the fluorescent signal is embedded in the excitation.
6.3 Optical design

There are diverse configurations to convey the excitation to the sample, in some cases using an objective lens [117-119] and in others optical coupler such as a prism [120-122]. The latter has several benefits such as inexpensive setup, greater range of incident angles and a compact geometry that can be adapted to disposable devices such as the planar waveguide associated to this thesis. Fig. 6.4 illustrates the cross section of the TIRF excitation. The illuminating laser beam is refracted by the prism and propagates further along the planar waveguide. In order to configure a disposable version of this geometry, the prism was fabricated in PDMS rubber and out of the shelf glass slides constituted the planar waveguide. Concurrently the PDMS element inherently provides the necessary adhesion to the glass surface required for a proper mechanical and optical matching, without additional processes. If $\theta_2$ is larger than $\theta_s$, TIR occurs and the illuminating beam propagates in a zigzag path confined to glass slide, whereas the evanescent wave, which decays exponentially from the interface, selectively excites the fluorophores in this region.

![Figure 6.4 2D cross section of the prism-based TIRF coupler.](image)

The relation among $\theta_1, \alpha, \gamma$ is obtained from the geometry of the assembly as:

$$\theta_1 = \alpha + \gamma \quad (6.6)$$

The other two relations are deduced from the considering the Snell’s law at the air/prism interface

$$n_0 \sin \theta_0 = n_p \sin \gamma \quad (6.7)$$

where $n_0 = 1$, and
Chapter 6. Total internal reflection fluorescence

\[ \gamma = \sin^{-1} \left( \frac{\sin \theta_0}{n_p} \right) \]  
(6.8)

However, \( \theta_1 \) can be expressed in term of \( \theta_0 \) by substituting Eqn. 6.8 in Eqn. 6.7. So that:

\[ \theta_1 = \alpha + \sin \left( \frac{\sin \theta_0}{n_p} \right) \]  
(6.9)

Considering the Snell’s law again at prism/coupling interface:

\[ \sin \theta_2 = \frac{n_p}{n_m} \sin \theta_1 \]  
(6.10)

Hence,

\[ \theta_2 = \sin^{-1} \left( \frac{n_p}{n_m} \sin \theta_1 \right) \]  
(6.11)

6.4 Integrated optical setup

Fig. 6.5 shows the implementation of the TIRF setup used in this thesis. The microscope glass slide (Menzel-Glaser) with \( n_m = 1.51 \) and 45° PDMS (Dow Corning Sylgard 184) prism with \( n_p = 1.4 \) constitute the excitation coupler. Since the refractive index of the aqueous samples (\( n_r \)) considered in this thesis are about 1.33, the angle of incidence at glass slide/sample interface \( \theta_i \) needs to be larger than 61.7°, the minimum critical angle. Accordingly, the incidence angle \( \theta_i \) in Fig. 6.5(a) needs to be above 40°.

A blue laser at 473 nm illuminates the PDMS prism as indicated in Fig. 6.5(a), confining a 3 mm wide beam within the glass slide and exciting fluorophores at the glass surface. Emitted fluorescence light is captured across the glass slide by a DSLR camera (Canon EOS 500D) and a cell phone camera (iPod touch 4th generation). An emission filter (Zeiss BP 515-565) is inserted in front of the camera to remove the scattered excitation in the PDMS fluidic. In the present case optimum HDR collection is not required and matching of fluorescence in the iPod camera dynamic range is accomplished by direct imaging of the glass surface. Fig. 6.5(d) shows fluorescence proportional to fucose concentration for a sample in a 100 \( \mu \)m wide channel as captured by the iPod camera.
Figure 6.5 The experimental arrangement used for TIRF measurements. (a) Cross section of the setup. (b) 3D scheme of the experimental assembly. (c) The picture of the actual setup. (d) The picture of fucose modulated fluorescence light measured with a phone camera.
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