Homogeneous Light Source for Surface Plasmon Resonance Imaging

Peter Hausler, Simon Jobst, Johannes Fischer, Carina Roth and Rudolf Bierl
Sensorik-ApplikationsZentrum SappZ, Ostbayerische Technische Hochschule Regensburg, Franz-Mayer-Str. 1, Regensburg, Germany

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Abstract: We describe how to build a homogeneous light source for Surface Plasmon Resonance Imaging (SPRi) which mainly finds its applications in pharmaceutical screening and biotechnology so far. SPR spectroscopy is a label-free, non-destructive and highly sensitive measurement principle for detecting changes in the refractive index in close vicinity of a gold surface. A transfer of this technology to a miniaturized sensor will broaden the range of possible applications. Commercial SPR assays are mainly working with a small number of sensing spots. In contrast, the SPR imaging system shown here will allow the use of an array of many sensing spots. In combination with chemical receptors designed as an artificial nose or an electronic tongue, the simultaneous detection of many analytes is envisioned. So far, lasers or other inhomogeneous light sources were used to illuminate the sensing surface, which is decreasing the systems sensitivity. We show a compact (< 60 mm), low cost, LED based light source which is providing a large area (>300mm2) homogeneous top hat profile. The combination of a high bit-resolution camera with our new light source enables a reflectivity based surface plasmon resonance imaging system with a high refractive index unit (RIU) resolution.

1 INTRODUCTION

Surface Plasmon Resonance (SPR) technology is label free, non-destructive and highly sensitive (Schasfoort, 2017). Due to these properties, SPR is an attractive measurement principle for chemical sensors. Nevertheless, there are some drawbacks limiting its applications so far: most measurement setups are designed for being used in laboratories and therefore they are very expensive. The high temperature sensitivity and the need of trained personal for its operation impede reliable in-field sensing. Miniaturized and automated systems could overcome these problems. Motivated by these prospects, many miniaturized systems were developed, (Ribeiro, 2019) includes an overview on miniaturized SPR systems. Most of these systems suffer either from missing transportability or from low sensitivity.

Beside reflectivity based SPR imaging (Fig.1) many other SPR technologies have been developed to increase sensitivity, (Wang, 2019) is a review about the state of the art and an overview of the most common technologies. However, these technologies are introducing new components which are complicating miniaturization.

In order to be able to apply reflectivity based SPR-imaging to miniaturized sensor systems we developed a compact, low cost light source, which is providing a large area, homogeneous illumination.

2 SENSING PRINCIPLE

While a thin gold film is irradiated by light, typically the entire light will be reflected (Fig. 1). However, if the light is p-polarised and the angle of incidence is altered, one can see a narrow dip in the intensity of the reflected light. This dip is indicating that at this certain angle of incidence (SPR angle) surface plasmons are excited. The SPR angle mainly depends on the refractive index in close proximity to the gold film which is deposited on a coupler, usually a glass prism. Therefore, the refractive index on one side of the gold is constant, which means that any variations in the chemical composition – and therefore in the refractive index – next to the other side of the gold film is determining the position of the SPR-angle. Selectivity to a special molecule of interest is

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generated by a chemical functionalization of the gold film by recognition elements. There are many different ways on how to utilize surface plasmons (Wang, 2019). The principle we used is reflectivity based SPR, since it has the simplest setup. Hence it is the most promising method for miniaturization. Reflectivity based SPR has no moving parts, which makes it more robust. During the design process one fixed angle at the linear region of the SPR-reflectivity slope is chosen for measurement. If the refractive index of the analyte is changing, the position of the minimum is moving and hence the reflectivity is changing (ΔR Fig.1).

3 SYSTEM SETUP

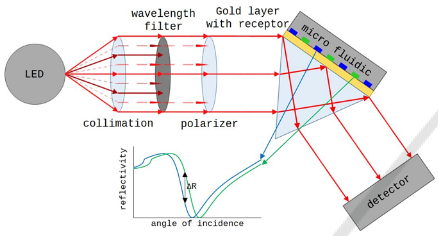

Figure 1: System-Setup, made of a LED based light source, a gold-coated prism with receptors and a detector as well as a 18 x 18 mm microfluidic chip, which is distributing the analyte.

Most single or multi-channel SPR setups are using laser light sources. For SPR-imaging, lasers are not suitable since they are known for generating speckles and diffraction patterns (Fig.2). Typical regions of interest (ROI) do have a size between 50 µm and 5 mm. While having small size ROI, the noise which is generated by the diffraction patterns is lowering the resolution of the system dramatically. Most lasers also have a Gaussian beam profile. If the sensing surface is illuminated with a Gaussian beam profile a small movement of the components will cause a movement of the steep flank thorough the ROI and hence a high change of the reflected signal. The replacement of the laser by our LED based light source overcomes this issue. LED light is very broadband compared to laser light and it is non-polarised. Therefore, a wavelength bandpass filter and a polarizer were integrated into the system. Furthermore, the beam is tailored and collimated. The modified beam illuminates a 50 nm gold film which is on top of a Schott F2 glass prism. The reflected light is collected by a 2D-camera system, which records the spatial change of the intensity of the reflected light beam (Fig.1).

Superluminescence diodes would provide similar characteristics to lasers but without spatial coherence but they are excessively expensive. The high price would impede the use of the SPR technology for infield sensors.

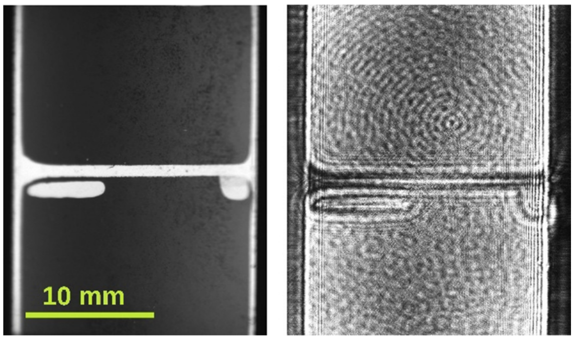

Figure 2: Comparison of a LED light source left and a laser light source right; the coherent laser light is generating diffraction patterns due to dust particle, edges and air bubbles.

The aim was to build a light source which is available at a low price and which can be miniaturized. Unstabilized lasers show mode hopping, which would introduce noise and lower the systems resolution. Speckles and other diffraction patterns would also generate some noise. Furthermore, the widening of the laser beam would require a long optical path. Therefore, lasers are not suitable for miniaturized systems.

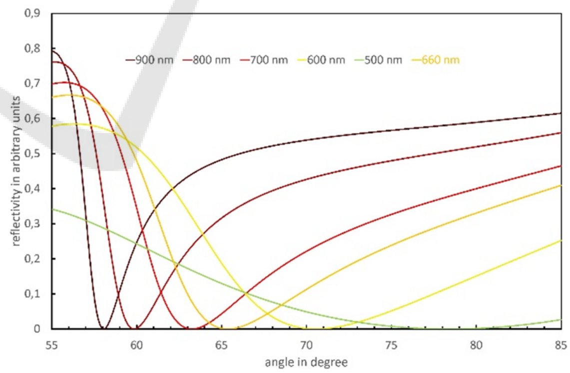

Figure 3: Simulation of an SPR signal according to changes in the excitation wavelength, the thickness of the gold layer was fitted in every simulation to achieve a minimum reflection; the SPR curve is getting steeper at higher wavelength, which raises the sensitivity; simulation was done with WinSpall software.

The SPR effect is very sensitive to wavelength shift and its sensitivity is increasing with an increasing wavelength (Figure 3). On the other hand, the sensitivity of CMOS cameras is decreasing rapidly with an increasing wavelength. Moreover, the development of a system is much easier if the wavelength is visible.

In order to have the system components available for very low price at good performance we have chosen a 660 nm LED from OSRAM (GH CSSRM2.24). It is an important wavelength for horticulture and is therefore readily available. In order to be able to alter the driving current and to have low noise at the same time, we used a Keithley Sourcemeter as a current source for the laboratory setup.

The beam shaping was done with a single 25 mm diameter plastic aspheric lenses from Edmund Optics. Depending on the desired beam characteristics, the focal length was chosen at a range between 25 mm and 75 mm (e.g. f40 #66-024). In order to avoid a wavelength shift, a custom made 2 nm FWHM filter from Chroma was used. The polarization was controlled with a 1:9000 polarizer from Edmund Optics (#85-919).

The prism is made of Schott F2 glass and has P4 polished surfaces to provide best performance.

By altering the position and the focal length of the collimation lens, the divergence of the light can be altered. If the SPR system is built with a light source with low or no divergence a camera can be used even without objective lenses.

The suitable camera should be chosen depending on the demands, concerning refractive index unit (RIU) resolution, system price and volume. The ximea MU9PM-MH with its APTINA MT9P031sensor is offering a 12 bit resolution at a low price and very low volume (15 x 15 x 8 mm) while the PCO Edge 4.2 is offering an outstanding resolution of 16 bit. The PCO Edge with its 16 bit is providing a high RIU resolution and the large chip size is enabling an acquisition of a large SPR-Image without the use of a lens. Between these two cameras, there are many different cameras, which could be used. Currently, a good compromise between costs, size and bit resolution are cameras with a Sony IMX178 sensor like the ISG Allegro.

4 RESULTS

Figure 2 shows the comparison of an LED light source (left) and a laser light source (right). Both are providing a "TopHat like" beam profile. In case of the laser, a very small area at the centre of the Gaussian beam profile was cut out. This Process is generating diffraction pattern at the edge of the lenses which were used to cut out the centre part. Furthermore, the edges of the prism and microfluidics as well as air bubbles and dust are generating diffraction patterns. The result is a very noisy illumination which could be sufficient for single or multi-channel measurement with very large regions of interest (ROI) but it is totally insufficient for high resolution SPR imaging.

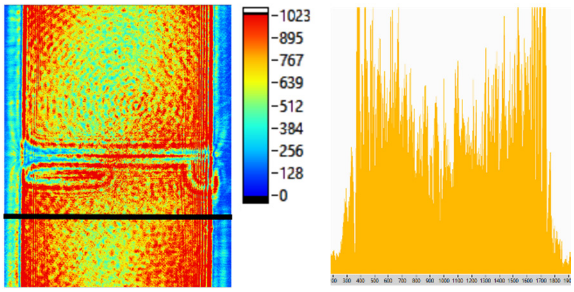

Figure 4: False colour SPR image made with a laser based light source and a Ximea CMV4000 10 bit camera; left: diffraction patterns originating from edges, dust particles and air bubbles are visible; right: cross-section along the black line from the left side, the noise which is originating from diffraction is clear to see

Figure 4 shows a SPR image which was recorded with a laser based light source. The cross-section, which was taken along the direction of the black line shows that there is tremendous noise which is lowering the RIU resolution of the SPR imaging system. To avoid this kind of noise a LED based light source was developed. LED's do not have spatial coherence, therefore they do not generate diffraction patterns.

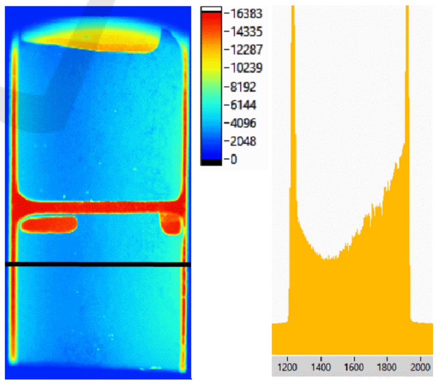

Figure 5: False colour SPR image, made with a LED light source and a ISG allegro 14 bit camera; left: the SPR active area is illuminated homogeneous, red areas are the sealing and a air bubble; right: cross section along the the direction of the black line, the cross section shows the SPR curve which is originating from the divergent illumination.

Figure 5 shows a SPR image, which was recorded with a LED based light source. The cross-section, which was taken along the direction of the black line shows the SPR curve which is originating from the light source divergence. A LED cannot be collimated like a laser, therefore a small divergence will always remain. Unlike Figure 1, which shows perfectly collimated light from a laser, a LED based light source is generating light rays, which are not perfectly parallel. Therefore, the angle of incidence is shifting slightly from one side to the other side of the sensing area. This divergence can be utilized to monitor every sensing spot at its most sensitive angle. If the SPR system does not monitor only one receptor but many different, e.g. 100 spots, it is very likely that every spot has a different refractive index. This implies that only one spot is monitored at its most sensitive SPR angle if a non-divergent light source is used, all the others are falling behind their possibilities. However, if a divergent light source is used, the angle of incidence is splayed and every receptor can be placed at its most sensitive angle of incidence.

While collimating a laser is very simple, homogenizing an LED is very complex. One could use an opal glass but this option would cause tremendous power losses on the one hand and focusing on infinity would not lead to a homogeneous beam shape on the other hand. Therefore, we developed another technique to create a homogeneous beam area. We have found an empirical equation which relates focal length of the lens, distance of the lens to the desired homogeneous surface and lens placement:

$$d_{L-FP} = (m_1 d_{L-HS} + b_1) \cdot \exp\big(f(m_2 d_{L-HS} + b_2)\big)$$

With

d_{L-FP}: Placement relative to focal plane

d_{L-HS}: Distance of lens to homogeneous surface

f: Focal length of lens

Parameter	Value
$\boldsymbol{m_1}$	$-2.5698e^{-3}$
$\boldsymbol{b_1}$	1.9311
$\boldsymbol{m_2}$	$-37.265e^{-6}$
$\boldsymbol{b_2}$	0.0383

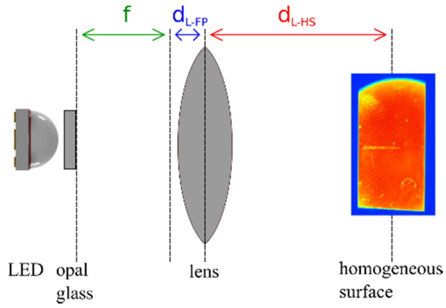

Figure 6: Placement of optical components, the 1mm aperture in front of the opal glass has been omitted in the schematic.

The SPR sensing area is inclined according to the beam direction (Figure 1) therefore the beam has to be homogeneous in a wider area along the beam direction and not only in one plane. The d_{L-HS} is the distance between the lens and the centre of the homogeneous area.

This equation is valid for focus length in the range of f 20 to f 120 and for a lens – homogeneous region distance of 40 mm up to 110 mm. The equation should be understood as an approximation, which is helping to build a homogeneous light source, which is based on a non-homogeneous LED chip. Since most LED's do have a lens on top, we placed an opal glass in front of the LED to determine the equation. However, we did the same procedure without opal glass and it lead to the same homogeneous results. The equation without opal glass has different parameters because of the added lens on top of the LED.

Placing the SPR prism, the lens and the light source according to this equation will lead to a homogeneous illumination of the prism surface. If the light is s-polarized the whole surface is iluminated homogeneously. If the light is p-polarized a gradient like it is shown in Figure 5 appears. The reduction of the reflectivity is caused by the generation of surface plasmons and the gradient of the reduction originates from the remaining divergence of the light beam. Combining this technology with a low noise current source creates a light source, which enables high resolution SPR imaging.

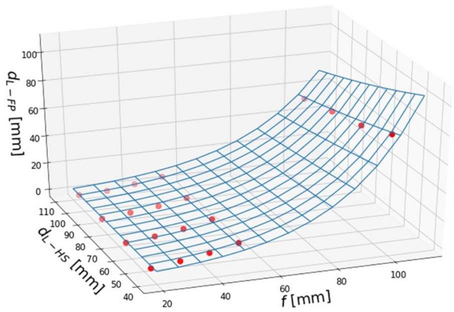

Figure 7: Plot of the equation, as a function of focal length and distance between lens and homogeneous surface; red dots are measurements conducted with the described setup.

Figure 7 shows the plot of the equation. While utilizing our method one has to deal with four parameters, which are divergence, width of the homogeneous area perpendicular to the beam direction, width of the homogeneous region along the beam direction and distance between LED and homogeneous region.

One could use a lens with low f-value to achieve a short distance between H-region and LED, while doing so, the divergence will increase and the spreading of the SPR-angle will be higher. On the other hand, one could use a high f-value to achieve a low divergence, this would increase the distance between H-region and LED. Therefore, one has to choose the best option for every task.

5 CONCLUSIONS

SPR-imaging was lacking of cheap, large area and homogeneous light sources. We described how to build a cheap, LED based light source, which is providing a homogeneous illumination over the entire sensing surface. The beam profile is homogeneous over a wide range along the beam direction and not only in one single plane. An empirical equation shows how to place lens and light source in relation to the sensitive area. We were able to reproduce the experiment many times and it is in use every day. However, the equation is not very exact and should therefore be understood as an approximation. The empirical equation is lacking a theoretical equation, thus more investigation has to be done in this field.

However, the concept can already be used and it enables high-resolution SPR-imaging on large surfaces. The light source was used for high RIU resolution experiments. Combined with a PCO Edge SCMOS camera we achieved resolutions in the 10^{-7} RIU range. The results are currently under publication.

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APPENDIX

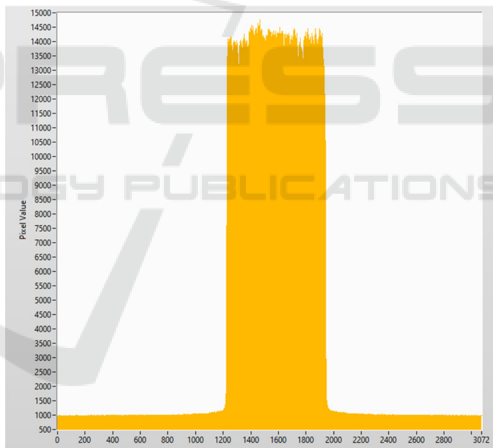

Figure 8: Cross section along the direction of the black line of figure 5 with s-polarized light.