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Noise reduction in Surface Plasmon Resonance images

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Some of the most important problems reported when performing Surface Plasmon Resonance Imaging (SPRI) measurements with modular systems consist in image imperfections. Finding a solution to this challenging aspect is essential since the reproducibility and the sensitivity of the method are based on an accurate image analysis. The aim of this paper is to compare some simple algorithms to improve the quality and the amount of information in an SPR image. Two sections are highlighted: noise reduction and combination of information. The first one deals with the improvement of the contrast in the images (this means, difference of intensity between spots and background) and the second one with the possibility of combining the information obtained through two different images in a single one by using a false-colour system.

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1. Introduction

Surface Plasmon Resonance (SPR) phenomenon is based on the absorption of light by the surface plasmons present in a thin metallic layer. This absorption takes place when a beam of monochromatic polarised light is incident with a specific angle at the surface. Summarising briefly the process, it can be said that the amount of absorbed light is related to its wavelength, to the incidence angle, to the optical properties of the surface and to the refractive index (and therefore to the mass) of what is bound/deposited on the metallic surface, this means, the analyte immobilised on the surface. This technique has been widely used as sensor transducer for biological or chemical interesting products [1-3].

If the laser beam is expanded with an optical lenses system and the reflected light is detected with a CCD camera, it is possible to use the same SPR phenomenon to create an image of the whole metallic surface – SPR imaging (SPRI). This allows the obtainment of information of different samples located at different spots of the sensor surface in the same time.

Fig. 1 summarises the set-up of a common SPRI system. Briefly, the laser beam is expanded through a collimating system and then the light is p-polarised. A motorized tilting mirror is responsible for changing the beam direction (by controlling the angle of the mirror – θ_{Mirror}) in order to ensure the incidence angle of the light on the sensor surface corresponding to the occurrence of the SPR phenomenon. The relationship between the angle of the mirror (θ_{Mirror}) and the SPR angle (θ_{SPR}) is presented in Figure 1. β represents the angle of the prism. The reflected light is directed to a CCD camera recording the SPR image characteristic to the whole surface of the sensor.

Multiple analysis protocols could be exploited. Among these, the most used consists in the imaging at the gold SPR angle, that will result in an image with a dark background characteristic to the gold surface (minimum reflectivity for gold) and lighter spots corresponding to the presence of immobilised molecules on the gold. In a similar way, the analysis at the SPR angle of the biological samples immobilised on the metallic surface will lead to dark spots (minimum reflectivity of the biological molecules layer) on a brighter background characteristic to the metallic surface of the sensor.

Further biological interaction between the immobilised species and other molecules could be also monitored, at the chosen incidence angle (usually at the gold SPR angle) by analysing the difference in the reflectivity compared to the control image taken before the interaction occurrence. Of course, as mentioned elsewhere [4], the variations of reflectivity will correspond to different intensities of the biological or chemical interactions and the reflectivity of the signal will increase with increasing the occurrence of these specific affinity events. This will provide SPR images with variable ratios between the background and the spots that could be quantitatively explored, leading to important conclusions without using any molecular labels.

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To resume, the obtainment of an SPR image is based on the differences in the light reflectivity depending on the samples on the metallic layer. The dependence of the reflectivity both with the incidence angle and with the refractive index of the sample are shown on the Fig. 2. For creating the image an angle is selected, usually that in which the reflectivity for the neat gold is minimum. At gold SPR angle, for instance, as already mentioned above, the image of the sensor will be as schematically presented in Fig. 2 panel a. at protein SPR angle, respectively, the SPR image of the sensor will look similar to the scheme shown in Fig. 2 panel b, with the minimum reflectivity corresponding to the protein spots.

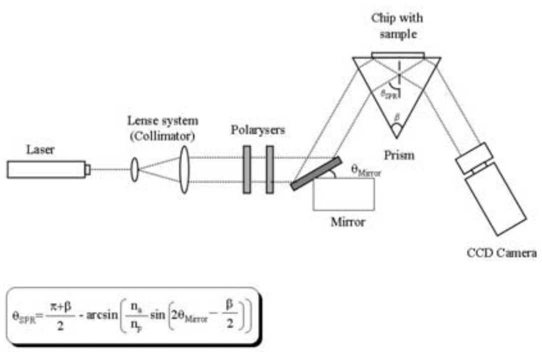

Fig.1. Schematic presentation of a SPRI device.

Sometimes, the quality of the image is not as good as desired, especially when using modular SPRI devices. In addition to the inherent noise to every scientific measure, there are extra noise sources such as inhomogeneities in the expanded beam due to the optics or to the laser itself, as well as due to not well defined edges of the spots, or to the hollow appearance of the latter.

The aim of this paper is to compare some simple algorithms to improve the quality and the amount of information in an SPR image. The work is subdivided in two sections: noise reduction and combination of information. The first one deals with the improvement of the contrast in the images (this means, difference of intensity between spots and background) and the second one with the possibility of combining the information obtained through two different images in a single one by using a false-colour system.

2. Experimental

The SPR image is taken as a grey-level photograph with 256 different levels (0 is black, 255 is white). In this report, four different algorithms were tried in order to improve the contrast between the spots containing the sample. This problem was focused from two different points of view: reducing the noise of the system, understood as the standard deviation of the grey intensity within either one spot or the background and homogenising the background. Both possibilities are presented more in detail in the section 3. Results and discussion.

The images used in this study were recorded with a modular SPRI system built up by our own team. The functioning principle respects the scheme presented in Fig. 1 and it was detailed elsewhere [4]. The only difference used in the set-up compared with Fig. 1 consists in the positioning of a second tilting mirror after the prism, to improve the recording of the images on the CCD camera. Monochromatic light (632.8 nm) was supplied by a HE-Ne laser LGK (LASOS GmbH, Ebersberg, Germany) and the images were acquired on a CCD camera (Retiga 1300 Qimaging Burnaby, British Columbia, Canada).

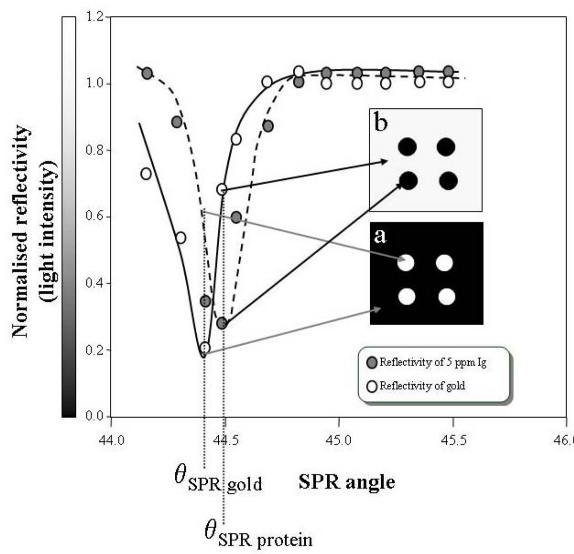

Fig. 2. Reflectivity versus SPR angle for gold (○) and protein Ig (concentration 5 ppm) immobilized on gold (●).

The substrate used for the study was represented by classical gold SPR chips (sSens, Netherlands), 10×10 mm, 0.3 mm thickness, based on borosilicate glass (n = 1.51). The gold adherence on glass was improved by the manufacturer by using a thin titanium layer. The slides were fixed on the prism using a drop of oil with the same refractive index as the glass (n = 1.51). The prism was made in BK7 glass (n = 1.51) with an angle of 90 °. The gold surface of the slide was rinsed with Piranha solution for 1 minute prior to use, to remove any contaminants without changing the surface roughness.

3. Results and discussion

3.1 Homogenising the background

In this algorithm, a grey value is selected as threshold. All the levels equal or lower to this one are considered as background and converted to 0 (black), while all the other grey levels are kept as they were. Visually, the contrast is improved since all the non-spot part of the photograph is transformed into black colour.

There are two different ways of selecting this threshold value, originating in two different algorithms.

1) The first one is based on the ability of estimating the percentage of the image, which is background. Then a threshold value T is selected in order to accomplish the following equation:

$$\int_0^T H(g)\cdot dg = A\int_0^{255} H(g)\cdot dg \qquad (1)$$

where H(g) is the histogram, g the grey level from 0 to 255, T the threshold value and A the ratio of the area belonging to the background (ranging from 0 to 1).

2) Alternatively, a second algorithm can be designed by estimating the noise in a piece of the image where there are no spots. In this case, the standard deviation of the grey level is then calculated, and the threshold selected as fifteen times the standard deviation of the greys within the selected region.

$$T=15\cdot\sigma \qquad (2)$$

Although this method is statistically better, as it does not depend in the ability of the user to guess the ratio of image being background, it presents a big disadvantage if the image is too noisy (big □ in Fig.3). In this case, the threshold value can be too high, and part of the real spots can be discarded as background.

3) As a last algorithm, the contrast tried to be improved by drastically reducing the amount of information (grey levels) in the image. To do so, the histogram was divided in N sections and a single colour was assigned to every section. In this way, 256 grey-levels are converted to N different colour. These sections could be either length-equivalent (256/N grey-levels pro section) or area-equivalent. In the latest case, every section *i* from grey-level $g_{i,0}$ to $g_{i,1}$ with $g_{i,1}=g_{i+1,0}-1$ or $g_{i,1}=255$ was selected accomplishing with the following equation:

$$\int_{g_{i,0}}^{g_{i,1}} H(g)\cdot dg = \frac{1}{N}\int_0^{255} H(g)\cdot dg$$

This algorithm would provide good results when the standard deviation of grey-levels in a small area is low. Otherwise, consecutive pixels can belong to very different sections and, therefore, has different colours. If this happens, the contrast is not properly improved.

Fig. 3 shows how an image changes when using the three different algorithms.

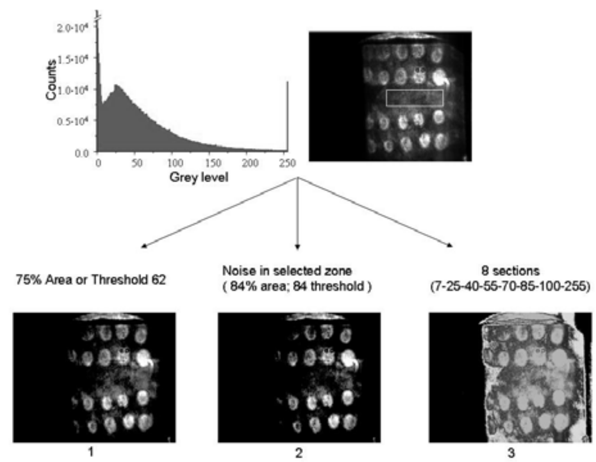

Fig. 3. Noise reduction by selection of area (1), background (2) or dividing the histogram in 8 sections (3).

3.2 Reducing the noise of the image

The last alternative explored in this work was to reduce the noise by using chemometric tools.

It is well known the property of the Principal Components (PC) to compress the information. In fact, few PCs can explain most of the variance of the system so the other PCs are usually related to the noise in the system [5].

The fundamentals of Principal Component Analysis (PCA) relays on the algebra of matrixes. According to PCA theory, every matrix **X** with dimension m x n can be expressed as a product of other two matrixes: **U** (scores) and **V** (loadings) with dimensions m × i and i × n respectively:

$$\mathbf{X} = \mathbf{U} \times \mathbf{V}$$

$$(mxn) = (mxi) \times (ixn)$$

where i is the total number of PCs.

However, the matrix X can be approximately recomposed using the same matrix product but only with a few number of PCs, a (a<i). If we do so, the previous equation would be transformed into:

$$\mathbf{X'} = \mathbf{U'} \times \mathbf{V'}$$

$$(mxn) = (mxa) \times (axn)$$

The matrix **X'** is not exactly equal to **X** but, depending on how many PCs were taken (how big was a) the most of the amount of information contained in X is retained in **X'**, loosing mainly the information related to the noise. How many PCs must be used can be determined using statistical criteria [5, 6].

If we consider the image as a matrix with m and n as the width and height in number of pixels, this algorithm could be used as an alternative to reduce the noise. Figure 4 shows an original SPR image and how it does change when de- and re-composed with different number of PCs.

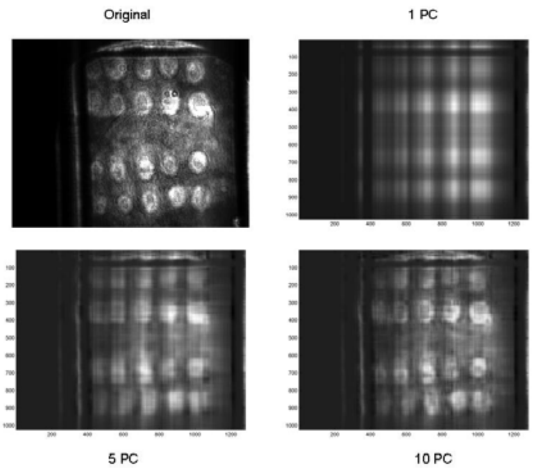

Fig. 4. Noise reduction by recomposing the image with 1, 5 or 10 principal components (PCs).

3.3 False colours - Combining information

The most common ways of extracting information of a sample through the surface plasmon resonance phenomenon is either by checking the angle at which the reflectivity of the sample is minimal, as done in the "classical" SPR, or by analysing the different reflectivity of the samples with a constant angle, usually selected for the minimum reflectivity of the background. The last option is what is usually done in the SPRI.

Since SPR imagers are able to change and control the incidence angle, this technique has the possibility of combining both ways of extracting information: the angle, and the reflectivity. In principle, this would be done by taking different images at different angles.

However, this would provide more than one image that should be compared one with each other in order to provide the maximum amount of information.

Here we propose an alternative to this comparison, by compressing the information of up to three different images in a single one.

From the point of view of a computer, colour can be expressed as a three-dimension vector c = (r, g, b) where r, g and b are the amount of red, green and blue of the colour c, everyone ranging from 0 to 255. Vectors (0,0,0) and (255,255,255) would be respectively black and white, and every vector such as r = g = b = |c|, would correspond to a grey colour. So, every image is a width x height matrix of three dimensional vectors, although when using gray level images we can convert this complex matrix in a simple width x height matrix of escalars by substituting every colour vector for its module |c|, as done when working with PCs.

In this way, if there are three different gray level images, all of them could be easily combined without loosing information by converting one to green, one to red and one to blue and simply adding them.

This algorithm can be mathematically expressed as:

$$F_{i,j} = {}^{1}X_{i,j}\begin{pmatrix}1\\0\\0\end{pmatrix} + {}^{2}X_{i,j}\begin{pmatrix}0\\1\\0\end{pmatrix} + {}^{3}X_{i,j}\begin{pmatrix}0\\0\\1\end{pmatrix}$$

where ${}^{1}X$, ${}^{2}X$ and ${}^{3}X$ are the three images that are being combined, F is the final image and i and j are ranging from 1 to width and length, respectively.

The main advantage of this procedure is the better ability of the eye to appreciate small differences in the image. Only the parts of the pictures which remain the same in the three original ones would keep a grey colour. Differences in the image will be easily remarked by appearing in blue, red, green or linear combinations of these colours.

4. Conclusions

As important as the biological or the chemical work for obtaining SPR images is the treatment of these images.

When developing a modular SPRI system, the operators should ensure that the device is capable of performing highly reproducible experiments, with a high sensitivity and determinations' accuracy. These are the main attributes that should be provided for a successful molecular label-free analytical tool.

Besides other problems, noise is usually the biggest issue to deal with, although statistics and principal components help to reduce it and to improve the quality of the images. Of course, part of the noise is due to the optical set-up used in the measurement, and there are very strict demands to be respected for obtaining reliable reproducible results. Partially, the noise could be the effect of a lack of spot-to-spot reproducibility due to the widely used pipeting in stead of specialized spotting robots. This usually leads to difficulties in identifying the edges between background and spots and also to a nonhomogeneous aspect of the spots, appearing as grey level irregularities that diminishes the accuracy of the experiment.

Although these aspects are very important and should be seriously considered and resolved when designing a modular SPRI set-up, the remaining challenge that made the object of this study was dedicated to the improvement that could be made by means of the image analysis. This is useful mainly in getting maximum of visual information that could be quantified in terms of biomolecular interactions, helping to identify species or affinity events of interest by using only few microliters of very diluted sample. This aspect is of maximum importance and one of the simplest but very powerful example that could be used for a better understanding: the early cancer diagnosis. Most of the biological species - tumour markers - useful for cancer diagnosis are detectable through classical detection methods only when their concentration in the organism is quite high, as a result of tumour formation. The use of SPRI technique allows the detection of the same tumour markers immediately after their presence or concentration is higher than the parameters characteristic in healthy people. Nevertheless, the technique is very simple and very efficient due to the possibility of simultaneous exploring of an important number of samples spotted on the same sensor surface at the time.

Furthermore, the combination of the information obtained with the SPR phenomenon, both reflectivity and incidence angle, can be easily combined with a simple mathematical algorithm to provide a false-colour image with a more visual way of showing complex information.

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