The choice of an image processing algorithm for increasing sensitivity of the surface plasmon resonance method

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Abstract. The paper describes the principles of the surface plasmon resonance imaging method in application to investigation of heat and mass transfer processes. It is shown, that one the main obstacles to increase its sensitivity is low signal-to-noise ratio due to speckles and stray interference structures in the experimental images. An algorithm of image processing is developed to overcome this obstacle. Several ways to do it is discussed in the paper.

1. Introduction
It is obvious that in order to provide the development of almost all scientific areas it is needed to conduct measurements. And the more accurate, sensitive, convenient and demonstrative the used measurement technique is, the more productive results it allows to receive. Thus, the task of development of measurement methods, techniques and devices always remains relevant. The most advanced measurement methods for many characteristics are optical ones. The optical method considered in this paper is the surface plasmon resonance imaging (SPRI) method [1], which allows determining the change in refractive index in a thin boundary layer of investigated medium with thickness of few hundred nanometers. Typically, this method is used to visualize biochemical reactions and to measure the binding constants in them [2]. In this particular work, the method is used for visualization of processes of heat and mass transfer such as evaporation, cooling, mixing liquid droplets [3]. To expand application area and provide more widespread using to this method it was decided to consider some possible ways to increase its sensitivity [4]. And one of them is corresponding algorithm of image processing, the choice of which this paper is devoted.

2. Surface plasmon resonance imaging method
As it is well known, surface plasmons are oscillations of the density of free electrons on a metal surface, excited by an external effect, usually an evanescent wave, produced by the total internal reflection of polarized light inside a glass prism. Under certain conditions (providing the required values of the metal film thickness, wavelength, polarization state and angle of incidence of the exciting light, the ratio of the refractive indices of the glass prism, metal and medium under study), a so-called surface plasmon resonance (SPR) occurs, at which, despite the condition of total internal reflection, the intensity of the reflected light equals to zero. When any of the parameters, for example, the refractive index of the medium, deviates from the resonance value, the efficiency of generation of surface plasmons decreases and reflected light appears, which is a signal of violation of resonant conditions. Thus, if a wide collimated laser beam is used as the exciting light, then on the screen in
reflected light one can observe a change in the refractive index of the medium by changing the intensity of the light incident on the screen. A change in the refractive index of a medium may be due to a change in its temperature, phase composition (formation, for example, of ice or gas bubbles in water), concentration of certain substances in a solvent, etc. Knowing the dependence of the refractive index on the changing parameter, it is possible to determine the magnitude of the change in this parameter in the corresponding place of the boundary layer.

In order to demonstrate possibilities of the SPRI method, an experimental setup was developed and created, described in detail in [3]. It consists of a semiconductor laser module with a wavelength of 655 nm and a power of 5 mW, a beam expander, a polarizer, a rectangular glass prism, on the base of which is placed a glass plate with a deposited gold film 50 nm thick, screen or lens and a digital video camera. Investigated drop is placed on the surface of the film illuminated by a wide collimated laser beam and the image of the reflected beam is recorded. As it was shown above, when the refractive index of a thin layer of a drop bordering a film changes, the intensity changes at the corresponding place on the screen. According to the results of processing the images obtained during the process being studied, we can judge about the change in the parameter of interest.

3. Stages of image processing

Each algorithm of image processing consists of several stages. Usually it is preprocessing, filtration, processing itself (calculation of required quantities from image matrix) and sometimes postprocessing (smoothing, plotting, visualization, etc.). Let's consider it sequentially.

3.1. Preprocessing

In our case it consists of selection of desired number of images from received pack of them, then alignment and cropping all selected images in the same way and then renaming in accordance with the resulting sequence. There are no essential features in this stage except for the difficulty of full automation.

3.2. Filtration

The most important part on which we will focus in more detail because that is what determines the quality of information that we get from experimental images. As it can be seen in the examples of experimental images, the main problem is speckle-noises caused by using laser as an excitation light source. This problem can be solved by using moving (e.g., rotating) screen [5], but it entails additional difficulties and is not covered in this paper. Another way of solving this problem is averaging or smoothing. There are few approaches how to do it: use of filters with finite impulse response (FIR-filters), Fourier filtering, wavelet filtering, averaging by few rows or columns of an image and some others. They are needed to be compared in application to our problem.

Speckle-noise is a high-frequency noise that is why we need to use low-frequency filters. Low-frequency spatial filters attenuate high-frequency components (small areas with strong intensity changes) and leave the low-frequency components of the image unchanged. They are used to reduce noise and remove high-frequency components, which improves the accuracy of studies of the content of low-frequency components. As a result of applying these filters, we obtain a smoothed or blurred image. Let us consider the most useful types of low-frequency filters [6].

3.2.1 Arithmetic mean filter. This filter gives the mean value of intensity for a pixel within a local region of an image with help of using mask with equal coefficients. In English literature the name box-filter is also used. The arithmetic mean is defined as

\[ G(x, y) = \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} F(i, j), \]

where \( G(x, y) \) – intensity of a resulted image pixel with coordinates \( x \) and \( y \), \( F(i, j) \) – intensity of corresponding pixel of an original image within mask, \( m, n \) – width and height of a mask correspondingly. An arithmetic mean filter operation on an image removes short tailed noise such as
uniform and Gaussian type noise from the image at the cost of blurring the image. The larger the filtering mask becomes the more predominant the blurring becomes and less high spatial frequency detail that remains in the image.

3.2.2. Geometric mean filter. Geometric mean value is calculated by the following formula:

\[ G(x, y) = \left( \prod_{i=1}^{m} \prod_{j=1}^{n} F(i, j) \right)^{\frac{1}{mn}}. \] (2)

The effect of applying this filter is similar to the previous method, however, individual objects of the original image are less distorted.

3.2.3. Harmonic mean filter. This filter is based on the next expression:

\[ G(x, y) = \frac{mn}{\sum_{i=1}^{m} \sum_{j=1}^{n} \frac{1}{F(i, j)}}. \] (3)

The filter suppresses “salt” noise well and does not work with noises like "pepper".

3.2.4. Counterharmonic mean filter. This filter is based on the expression

\[ G(x, y) = \frac{\sum_{i=1}^{m} \sum_{j=1}^{n} F(i, j)^{Q+1}}{\sum_{i=1}^{m} \sum_{j=1}^{n} F(i, j)^{Q}}, \] (4)

where \( Q \) is the filter order. The counterharmonic filter is a generalization of mean filters and at \( Q > 0 \) suppresses noise like “pepper”, and when \( Q < 0 \) — noise like “salt”, however, the simultaneous removal of white and black dots is impossible. When \( Q = 0 \), the filter becomes arithmetic, and when \( Q = -1 \), it becomes harmonic.

3.2.5. Gaussian filter. At this type of filtration, the two-dimensional Gaussian filter is used:

\[ G(x, y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}}. \] (5)

The larger the parameter \( \sigma \), the more blurred the image. Commonly, the filter size equals \( 3\sigma \). In this case the mask size \( 6\sigma + 1 \times 6\sigma + 1 \).

3.2.6. Median filter. This is a non-linear filter which is very useful at suppression of additive noise, especially if the noise is impulse. The method is very simple, does not require customization and therefore has received wide distribution. The procedure of image processing by the median filter is that for each position of the mask, the pixels that fall into it are ordered by increasing intensity. The average count in this ordered sequence (there must be an odd number of them) is called the median and is recorded as the center value of the mask window. As a result of applying this filter, sloping areas and sharp drops are preserved, and impulse noise, whose duration is less than half of the window, will be suppressed. The larger the window, the larger the details will be blurred. There are also other modifications of a median filter, such as adaptive median filter and weighted median filter, but they are usually used in some specific cases and will not be considered in this paper.

Should be added that it has recently become increasingly widespread use of neural networks and deep learning to increase signal-to-noise ratio in corrupted images (e.g., [7]). However, this question is beyond the scope of this work and may be the subject of further research by the authors.
Different studies give different ratings and recommendations for choosing filters [8-10], so they are needed to be compared in relation to the SPRI images. The table 1 shows the results of application of these filters to the example of experimental images with three different sizes of mask (3×3, 5×5 and 7×7) and three different amplitude filtering thresholds (50, 100 and 150) for Fourier filtration.

**Table 1.** The results of application of different filters to an example of experimental images.

| Filter          | 3×3 mask | 5×5 mask | 7×7 mask |
|-----------------|----------|----------|----------|
| Arithmetic      | 0.076    | 0.069    | 0.069    |
| Geometric       | 0.085    | 0.061    | 0.060    |
| Harmonic        | 0.061    | 0.066    | 0.066    |
| Median          | 0.037    | 0.047    | 0.047    |
| Gaussian        | 0.077    | 0.064    | 0.068    |
| Threshold       | 50       | 100      | 150      |
| Fourier         | 0.289    | 0.206    | 0.102    |

In order to choose the optimal filter, the criterion of the smallest standard deviation in an image area corresponding to homogeneous water droplet was chosen. The table 2 shows the results of its estimation for rectangular area 50×20 pixels in the center of matrix obtained by dividing an image with droplet by the image without it (in other words, matrix of reflectance coefficient). The standard deviation of the same pixel values for non-filtered images is 0.292.

**Table 2.** Estimation of standard deviations of pixel values in filtered images.
Thus, it can be seen that the optimal filter for available images is median filter with mask size $3 \times 3$. Apart from minimum standard deviation it allows to get better visual perception and not to lose possible small-scale details in images. For very noisy images an adaptive median filter may be used.

3.3. Processing

After filtration we get images ready for processing. Now it is time to extract quantitative data from these images. For this we take two images obtained with the same experimental parameters (angle of incidence of induced light, intensity distribution across laser beam, recording options, etc.) except absence or presence of investigated liquid on the top of the metal film: in one of them there is an image droplet and the other one is without it (reference image). To calculate reflectance coefficient we divide values of intensity in each pixel by corresponding values in reference image. So we obtain matrix of distribution of reflectance coefficient over the interface between the metal film and investigated medium which corresponds to distribution of refractive index in thin boundary layer in close proximity to this film.

Use of reference image allows us to neglect uneven distribution of intensity by cross-section of a laser beam. But the main difficulty here to provide absolutely the same conditions for registration in order to speckle structure does not change. If we could meet this condition, it would be even unnecessary to do filtration, because it would have been performed automatically. Unfortunately, it is too difficult to fulfill this condition with our technical capabilities, because the SPRI method is very sensitive not only to change of refractive index, but also to the slightest displacements or deformations of parts of the setup. That is why any vibrations or even air flows cause to change in the speckle pattern. This makes it important to choose proper filtering algorithm.

On the other hand, this circumstance can be used to get rid of speckles. Indeed, if we record several images with and without contact of the film and investigated medium with different speckle and stray interference patterns due to vibrations, for example, we can average them and it will have the same effect as physical averaging with help of rotating screen.

In order to get more sensitivity from the same images the controlled contrast enhancement method may be used. This method can allow to artificially increase the reflection coefficient difference between two images of droplets with different refractive indices. The problem is that dependence of reflectance coefficient on refractive index is not linear and for correct interpretation we need to exactly know on which part of this dependence we are working. However, it may be successfully applied for qualitative visualization of change in refractive index of an investigated medium, when it is required.

3.4. Postprocessing

Thus, as a result of processing, we have a matrix of distribution of refractive index in thin boundary layer of an investigated medium in close proximity to the metal film. Sometimes it is needed to reconstruct the distribution of some other value (e.g., temperature, concentration, phase composition, etc.). Then we have to know the dependence of the required value on refractive index of medium and knowing it we can easily recalculate distribution of refractive index into distribution of required value. The next step is plotting smooth graph using experimental points. To do this, we select appropriate values, and sometime spend an average.

4. Conclusion

Based on the above mentioned material, the next algorithm of image processing for experimental images of the surface plasmon resonance imaging method was chosen. At first, selected images should be cropped in exactly the same way so that only the region of interest remains in the image field. Then cropped images should be filtered using median or in some cases adaptive median filter. After filtering the processing begins, which consists of dividing the images by corresponding reference ones in order to get the reflectance coefficient matrices, then averaging by several rows or columns, then calculating a required parameter for each processed image. And finally an approximation curve of dependence of this parameter on time or on coordinates in image should be plotted.
The novelty of the work lies in the fact that all the main modern techniques of image processing were considered and compared in application to the surface plasmon resonance image processing.

It is planned as the nearest future work to implement this algorithm in the Python programming language. The next step may be to use neural networks for image processing in order to reduce noise.

Acknowledgments
This work was financially supported by the Russian Federation President Grant No. MK-6361.2018.8.

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