Simultaneous amplitude-contrast and phase-contrast surface plasmon resonance imaging by use of digital holography

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Abstract: The surface plasmon resonance imaging technique provides a tool that allows high-throughput analysis and real-time kinetic measurement. A simultaneous amplitude-contrast and phase-contrast surface plasmon resonance imaging method is presented. The amplitude-contrast and phase-contrast images are simultaneously obtained by use of digital holography. The detection sensitivity of amplitude-contrast imaging and phase-contrast imaging can compensate for each other. Thus, the detectable sample components may cover a wider range of refractive index values for the simultaneous amplitude-contrast and phase-contrast imaging method than for the phase-contrast imaging method or amplitude-contrast imaging method. A detailed description of the theory and an experiment of monitoring the evaporation process of a drop of NaCl injection in real time are presented. In addition, the amplitude-contrast image has less coherent noise by digital holography.

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1. Introduction

Surface plasmon resonance (SPR) is an excellent method to detect the change in refractive index of the sample near the metal surface. Biomolecular interactions can be real-time monitored by detecting changes of the refractive index. SPR-based biosensor technology has become a leading technology for label-free detection of biomolecular interactions. Surface plasmon resonance imaging (SPRI) provides the possibility of high-throughput analysis to detect hundreds of samples simultaneously [1–3]. However, the development of SPR imaging instruments for high-throughput with low detection limits is still a challenging task. Many kinds of SPR imaging system have been developed. The current imaging methods can be divided into two groups. One is the amplitude-contrast imaging method by detecting the intensity distribution of the reflected light beam directly [4,5], and the other is the phase-contrast imaging method by detecting the interference pattern between the reflected beam and a reference beam [6–8].

Phase and amplitude sensitivities in surface plasmon resonance bio and chemical sensing have been discussed in detail by Andrei V. Kabashin, Sergiy Patskovsky, and Alexander N. Grigorenko [9]. A drawback of the SPR imaging instruments is that they measure reflectivity or phase of light with a fixed incident angle. They measure either the reflectivity change or phase change instead of the change of resonance angle to obtain the refractive index change. Therefore, the differential intensity or phase scheme is used for enhanced contrast and detection sensitivity in real-time kinetic measurement of biomolecular interactions [10–12]. However, the optimal incident angle corresponding to the maximum detection sensitivity can only be found for one sample but not for all samples in high-throughput analysis. For the amplitude-contrast imaging method, the sensitivity near the minimum reflectivity is relatively small. While for the phase-contrast imaging method, the sensitivity near the minimum reflectivity is relatively large. If the amplitude-contrast and phase-contrast images can be simultaneously obtained, the detection sensitivity can compensate for each other and the detectable range of refractive index values can be expanded. The expansion will result in increasing the number of analytical samples. In other words, it will increase analytical throughput capacity. It is very important to perform high-throughput analysis.

Digital holography (DH) offers an excellent approach for simultaneous obtaining quantitative amplitude and phase distributions of an optical wave [13]. In order to obtain higher spatial resolution than total internal reflection digital holographic microscopy for
optical imaging of cell membrane, the surface plasmon resonance imaging by use of digital holography was proposed by Jingang Zhong’s group [14]. In this work, in order to realize that the phase and amplitude information can compensate for each other, a simultaneous amplitude-contrast and phase-contrast surface plasmon resonance imaging method by use of digital holography is presented. The detailed theoretical analysis and an experiment of monitoring the evaporation process of a drop of NaCl injection in real time by use of this method are shown. Evaporation of droplets on a flat substrate, a commonly observed phenomenon in our everyday life, has been a topic of continued interest for several decades because of its important role in the nature and in various engineering fields [15–17]. For the analysis of droplet evaporation, it would be beneficial in models for complex devices or systems that incorporate various sub-processes for which simplified descriptions are needed [18]. The change range of refractive index values is large in the evaporation process of NaCl injection, but neither the amplitude-contrast nor phase-contrast imaging method can monitor the full process of evaporation.

2. Theory

2.1. Amplitude and phase of reflected light in SPR imaging

The SPR system of Kretschmann configuration (Fig. 1 shows) is composed of a prism, a gold film and a layer of sample. Gold film was coating on the underside of the prism. Samples were deposited on the surface of the gold film. A beam of p-polarized light directed through the glass prism with incident angle \( \theta \) and reflected from the gold covered prism facet. The sequence of the optical media from prism to sample is denoted by medium 1, 2 and 3. According to the Fresnel’s equations, the relation of amplitude reflection coefficient \( r \) via incident angle \( \theta \) is described as follows [19,20]:

\[
\frac{r_{1,3}(\theta)}{1 + r_{1,2}(\theta) r_{2,3}(\theta) \exp(2j\theta k_{2,3}(\theta))} (j = \sqrt{-1}),
\]

\[
r_{1,3}(\theta) = \frac{\zeta_{1,3}(\theta) - \zeta_{2,3}(\theta)}{\zeta_{1,3}(\theta) + \zeta_{1,2}(\theta)} (i = 1, 2),
\]

\[
\zeta_i(\theta) = \frac{\varepsilon_i}{\kappa_i} (i = 1, 2, 3),
\]

\[
\kappa_i(\theta) = 2\pi \sqrt{\varepsilon_i - \varepsilon_i \sin^2(\theta)} \lambda (i = 1, 2, 3),
\]

where \( i \) denotes the \( i \)th optical medium, \( \kappa_i \) represents the wave number of the transmission light in the \( i \)th optical medium along \( z \) direction, \( r_{1,3} \) is the amplitude reflection coefficient of
p-polarized light at the interface between two adjacent media, \( \varepsilon_i \) is the dielectric constant of the \( i \)th medium, and \( n_i \) is refractive index of the \( i \)th optical medium, \( d_2 \) is the thickness of gold film. The reflectivity \( R \) and phase \( \varphi \) can be obtained:

\[
R(\theta) = |r_{1,3}(\theta)|, \quad (5)
\]

\[
\varphi = \arctan \left( \frac{\text{Im}(r_{1,3})}{\text{Re}(r_{1,3})} \right), \quad (6)
\]

The above equations show that the reflectivity and phase are the functions of the incident angle and the sample’s refractive index. We can calculate the behavior of the reflectivity \( R \) and phase \( \varphi \) of reflected light, which is shown in Fig. 2(a), assuming that the refractive index of the prism is 1.516 (K9), the dielectric constant of the gold film is \(-13.4 + 1.4j\), the refractive index of the sample (water) is 1.333, the thickness of gold film is 50 nm, and the wavelength of light is 632.8 nm. It can be seen from Fig. 2(a) that the resonance angle corresponding to the minimum value of reflectivity is 70.58 degree. At the resonance angle, the phase shows a very steep slope. If a light beam incidents to the underside of the prism with a 70.58 degree angle in an SPR imaging instrument, we will get the relations among reflectivity, phase and refractive index, as shown in Fig. 2(b). The refractive index corresponding to the minimum reflectivity is the resonance refractive index. As seen in Fig. 2(b), the phase changes rapidly while the corresponding reflectivity changes slowly near the resonance refractive index, and vice versa.

The sensitivities are characterized by the slope of the reflectivity curve and phase curve, respectively, and are often used for optimization in SPR measurements [21]. The sensitivity of reflectivity and the sensitivity of phase to refractive index change are defined, respectively, as:

\[
S_r = \frac{dR}{dn}, \quad (7)
\]

\[
S_\varphi = \frac{d\varphi}{dn}, \quad (8)
\]

where \( S_r \) is the sensitivity of reflectivity, \( S_\varphi \) is the sensitivity of phase, and \( n \) is the refractive index of sample. Figure 2(c) shows the sensitivities from Fig. 2(b). As can be seen in Fig. 2(c), the sensitivity of phase near the resonance refractive index is large, which is beneficial in studying the biomolecular interactions but the detectable range of refractive index values is relatively small. For the phase-contrast SPR imaging, low detectable range of refractive index values will limit analytical throughput capacity. From Fig. 2(c), the sensitivity of reflectivity

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**Fig. 2.** The relationship between reflectivity \( R \), phase \( \varphi \) of light (\( \lambda = 632.8 \) nm) reflected from a prism of K9 glass with gold film of thickness 50 nm and refractive index or incident angle \( \theta \): (a) Theoretical reflectivity \( R \) and phase \( \varphi \) curves plotted as a function of incident angle \( \theta \). The dotted vertical line represents the SPR angle. (b) Theoretical reflectivity \( R \) and phase \( \varphi \) curves plotted as a function of refractive index. (c) Calculated sensitivity of reflectivity and sensitivity of phase.
near the resonance refractive index is minimal. The amplitude-contrast SPR imaging, with a wider detectable range of refractive index values, and a low sensitivity near the resonance refractive index, leads to a discontinuous detectable refractive index, thus it is disadvantageous for the high-throughput analysis. The detected sensitivity of the two methods can compensate for each other by use of simultaneous amplitude-contrast and phase-contrast imaging. It benefits from analyzing the array samples with wider refractive index distribution range and increasing the number of analysis throughputs.

2.2. Amplitude-contrast and phase-contrast images are simultaneously obtained by digital holography

Digital holography is a new emergent imaging technology. Conventional holography uses a photochemical plate to record a hologram. Digital holography is based on the classic holographic principle with a difference that the hologram is recorded by a photoelectric sensor (such as CCD or CMOS camera), and transferred to a computer as an array of values. The reconstruction of a hologram is numerically carried out by the computer. By replacing the photochemical procedures with electronic imaging and having a direct numerical access to the complex optical wave, a wide range of new imaging capabilities becomes available. Many of them are difficult or infeasible in conventional holography. It is one of the advantages of the digital holography to obtain the phase and amplitude of complex optical wave simultaneously in real time.

The experimental setup of SPR system for measuring amplitude and phase simultaneously is presented as Fig. 3. A glass slide coated with a gold film (50 nm thick) is used as the SPR sensor chip. The SPR sensor chip is adhered to a glass prism with refractive-index matching liquid. A beam of p-polarized light becomes collimated after passing through a spatial filter and a lens. Then the collimated light is split into two beams by a beam splitter prism (BS1), one of which, serving as the object beam, is reflected by a plane mirror (M1), and illuminates the sensor chip with sample, while the other is reflected by another plane mirror (M2) serving as the reference beam. The two beams propagate through the same beam splitter (BS2) and a hologram is formed. The hologram containing the information of sample is recorded by a camera and transferred to a computer. The amplitude-contrast and phase-contrast SPR images are simultaneously obtained via numerical reconstruction of hologram.

![Fig. 3. SPR system. (SF) spatial filter consists of a 40× microscope objective and a 15 μm pinhole, (L) lens, (BS1, BS2) beam splitter, (M1, M2) plane mirror.](image)
The reconstruction of the hologram is based on the diffraction theory of optical wave. U. Schnars and W. P. Jüptner described the method used for numerical reconstruction of hologram in detail [22]. The hologram is created by the interference between two coherent waves as Fig. 4 shows: the object wave \( O(x_{H}, y_{H}) \) coming from the object, and a reference plane wave \( R_{p}(x_{H}, y_{H}) \):

\[
O(x_{H}, y_{H}) = O_{0}(x_{H}, y_{H}) \cdot \exp[j\varphi(x_{H}, y_{H})],
\]

\[
R_{p}(x_{H}, y_{H}) = R_{ph0} \cdot \exp[j2\pi(x_{H} \cos \alpha + y_{H} \cos \beta)/\lambda],
\]

where \( x_{H}, y_{H} \) are the coordinates of the hologram plane; \( O_{0}(x_{H}, y_{H}) \) and \( R_{ph0} \) are the amplitude of the object wave and the reference wave, respectively; \( \varphi(x_{H}, y_{H}) \) is the phase of the object wave; \( \lambda \) is the wavelength; \( \alpha \) and \( \beta \) are the angles between the propagation direction of the object wave and the reference wave on the \( x_{H} \) and \( y_{H} \) direction respectively. The intensity of the hologram \( I(x_{H}, y_{H}) \) can be written as

\[
I(x_{H}, y_{H}) = R_{p} \cdot R_{p}^* + O \cdot O^* + O \cdot R_{p}^* + O^* \cdot R_{p}.
\]

The hologram is irradiated by the reference wave of computer simulation, as shown in Fig. 5. The complex amplitude distribution of reconstructed wave at the hologram plane is

\[
E(x_{H}, y_{H}) = R_{p}(x_{H}, y_{H}) \cdot I(x_{H}, y_{H})
\]

\[
= R_{p}^2 \cdot R_{p}^* + R_{p} \cdot O^* + O \cdot R_{p}^* + O^* \cdot R_{p}^2.
\]

The first and the second terms form the zero order term of diffraction, namely the background intensity, and the third and the fourth terms are the virtual and the real image terms, respectively.

Employing the Fourier transform, the spectrum of the hologram at the hologram plane with \( z = 0 \) is obtained as

\[
A(\xi, \eta; 0) = F\{E(x_{H}, y_{H})\}
\]

\[
= A_{r}(\xi, \eta; 0) + A_{4}(\xi, \eta; 0) + A_{4}(\xi, \eta; 0) + A_{4}(\xi, \eta; 0).
\]

Here \( F\{\} \) denotes the Fourier transform, and \( \xi, \eta \) are the spatial frequencies. \( A_{r}(\xi, \eta; 0) \) and \( A_{4}(\xi, \eta; 0) \) represent the zero order term of the spectrum, \( A_{4}(\xi, \eta; 0) \) represents the +1 term and \( A_{4}(\xi, \eta; 0) \) is the –1 term. When employing a proper filter, the spectrum of the
virtual image $A_1(\xi, \eta; 0)$ can be obtained. Then shift the spectrum $A_1(\xi, \eta; 0)$ to the center of the spectrum. The spectrum of the reconstructed wave at the plane of distance $z$ from the hologram plane can be calculated from $A_1(\xi, \eta; 0)$ as the following:

$$
A_1(\xi, \eta; z) = A_1(\xi, \eta; 0) \cdot \exp\left[ j \frac{2 \pi z}{\lambda} \sqrt{1 - (\lambda \xi)^2 - (\lambda \eta)^2} \right].
$$

(14)

Subsequently the complex amplitude distribution of the reconstructed wave at the object plane with $z = -d$ can be obtained by taking the inverse Fourier transform as

$$
E(x_0, y_0; -d) = F^{-1}\{ A_1(\xi, \eta; -d) \},
$$

(15)

where $F^{-1}\{\}$ denotes the inverse Fourier transform and $x_0, y_0$ are the coordinates of the object plane.

So the amplitude and the phase of object wave reconstruction in the object plane are

$$
O_0(x_0, y_0) = |E(x_0, y_0; -d)|,
$$

(16)

$$
\varphi(x_0, y_0) = \arctan\left( \frac{\text{Im}[E(x_0, y_0; -d)]}{\text{Re}[E(x_0, y_0; -d)]} \right),
$$

(17)

where $\text{Re}[\ ]$ denotes the real part and $\text{Im}[\ ]$ the imaginary part.

Since the phase obtained from Eq. (17) is wrapped into the range of $[-\pi, \pi]$, the phase unwrapping algorithm must be performed. To minimize diffraction effects of aperture, an aperture apodization is often used for the hologram before numerical reconstruction.

In the SPRI system as Fig. 3 shows, the object wave is reflected wave from the gold covered prism facet. The SPR angle is greater than the total internal reflection angle. Therefore, we can assume that the reflectivity is 1 when the amplitude of object wave is a maximum, the reflectivity of amplitude $R$ can be defined as the normalization of $O_0$:

$$
R(x_0, y_0) = \frac{O_0(x_0, y_0) - \min[O_0(x_0, y_0)]}{\max[O_0(x_0, y_0)] - \min[O_0(x_0, y_0)]},
$$

(18)

where $\max[\ ]$ and $\min[\ ]$ denote the maximum and the minimum, respectively.

Coherent noise due to long coherence of the laser is inevitable. But the coherent noise in amplitude-contrast and phase-contrast images obtained by digital holography can be significantly reduced. Because numerical filtering method is employed in order to obtain the Fourier spectrum of the virtual image in the numerical reconstruction process. The +1 spectrum of the hologram is the spectrum of virtual image. Employing a proper digital filter and extracting the +1 spectrum of the hologram, the spectrum of virtual image can be obtained and the coherent noise is also filtered out at the same time. Thus, the signal-to-noise ratio (SNR) in amplitude-contrast image obtained by numerical reconstruction of hologram can be increased relative to that obtained directly by the camera.

In addition, the phase obtained by the digital holography is different from that obtained by the interferometric imaging method [6]. The reconstruction of the hologram is based on the diffraction theory of optical wave. According to diffraction theory, the complex amplitude distribution at any plane perpendicular to the optical axis can be obtained by numerical reconstruction. By use of Eqs. (16) and (17), the amplitude and the phase of optical wave obtained are at the virtual image plane near the sample surface as shown in Fig. 5. Of course, the amplitude and the phase of optical wave at the real image plane can also be obtained. This is also known as lensless imaging. But the phase obtained by the interferometric imaging method is at the camera plane. When the optical wave reflects off the sample surface and propagates to the camera plane, the sample image will blur due to the divergence of the light.
beam. Therefore, it is often to set up an imaging lens in front of the CCD or CMOS in the interferometric imaging system.

3. Experiment and discussion

3.1. Coherent noise in amplitude-contrast image

In order to demonstrate that the SNR in amplitude-contrast image obtained by numerical reconstruction of hologram can be increased relative to that obtained directly by the camera, an experiment of water droplet is carried out. A water droplet was deposited on the surface of the sensor chip with a 50 nm-thick gold film as shown in Fig. 3. Holograms are recorded by a CMOS camera (1312 × 1082 pixel, 108 fps, PhotonFocus, Switzerland) with 8-bit gray scale output. Figure 6(a) shows the amplitude-contrast images of a water drop captured by conventional amplitude SPR system (turn off the reference beam of Fig. 3 and set up an imaging lens in front of the camera). The boundary of the water drop in Fig. 6(a) is not clear. This is related to the fact that, coherent laser source is used, the coherent noises, such as speckle and parasitic interferences, are inevitable.

Figure 6(b) shows the digital hologram of water droplet obtained by the SPRI system. Figure 6(c) shows the reconstructed amplitude-contrast image of Fig. 6(b). In the experiment, the incident angle is near the SPR angle of water (about 70.58 deg. with 1.516 refractive index of the prism), so that the image captured by camera is compressed 0.33 times in one direction and the ellipse is regarded as a distortion of the circle by unequal scale factors in two directions as shown in Fig. 6(c). The boundary of the water droplet as shown in Fig. 6(c) is clear. Figure 7 shows gray scale values in the 125th column in Fig. 6(a) and 6(c) (see dashed line). From Fig. 7, we can find that the coherent noise in amplitude-contrast image obtained
Fig. 7. The gray scale values in the 125th column in Figs. 6(a) and 6(c) (see dashed line). (a) The gray scale values of the amplitude captured by camera directly. (b) The gray scale values of the amplitude reconstructed by the computer.

by numerical reconstruction is significantly reduced. Therefore, the SNR in amplitude-contrast image obtained by numerical reconstruction of hologram can be increased.

3.2. Simultaneous amplitude and phase images determine the arrays samples

From the Fig. 2(b), we can find that the reflectivity values of samples with different refractive index values may be the same, but their phase values are different. So, the sample cannot be uniquely determined from the amplitude-contrast imaging method with a fixed incidence angle. But the simultaneous measurement of reflectivity and phase can better help us determine the sample. In order to demonstrate the amplitude and phase information can compensate for each other in determination of arrays samples, an experiment of arrays samples of glucose solution is carried out.

Preparation of glucose solution: different amounts of anhydrous glucose powder (ShangHai Bio Science & Technology Co., Ltd., Shanghai China) were weighed with AL104 electronic balance (Mettler-Toledo GmbH, Shanghai, China), and the anhydrous glucose powders were dissolved in deionized water. So 3 ml glucose solutions with different concentrations were prepared. Then the refractive indexes of the glucose solutions were measured by an Abbe Refractometer (Edmund Optics). The glucose solutions with different concentrations were deposited on the sensor chip. So, 3 × 2-array droplets of solution were formed. Table 1 shows the refractive index of the solutions.

Table 1. Refractive Index of Glucose Solution

| Glucose Solution | D1     | D2     | D3     | D4     | D5     | D6     |
|------------------|--------|--------|--------|--------|--------|--------|
| Refractive Index | 1.3351 | 1.3380 | 1.3485 | 1.3362 | 1.3425 | 1.3365 |

Figure 8(a) shows the hologram of 3 × 2-array droplets of glucose solution with 472 × 351 pixels. Figure 8(b) shows the amplitude-contrast image reconstructed by computer. To observe the minimum value of the reconstructed amplitude clearly, we plot its 3D image of the negative amplitude (-R) as shown in Fig. 8(c). Figure 8(d) shows the reconstructed phase-contrast image.

Figure 8(e) shows amplitude reflectivity and phase at 100th column (see dotted lines) of Fig. 8(b) and Fig. 8(d). Figure 8(f) shows amplitude reflectivity and phase at 380th column (see dotted lines) of Fig. 8(b) and Fig. 8(d). As can be seen from Fig. 8(e), the reflectivity and phase of droplet D1, D2, D3 are different. In Fig. 8(f), the reflectivity values of droplet D4, D5, D6 are almost the same, about 0.1, but their phase values are different. It can be seen from Table 1, the refractive indexes of the three droplets D4, D5, D6 are not equal. So, the glucose solution samples cannot be uniquely determined from the amplitude-contrast imaging method.
Fig. 8. Digital hologram, the amplitude image, and phase image. (a) Hologram of the glucose solution array droplets. (b) Amplitude image reconstructed; (c) 3D representation of negative reflectivity (-R) of (b). (d) Phase image reconstructed. (e) Curve plots of the 100th column data of (b), (d). (f) Curve plots of the 380th column data of (b), (d).

with the fixed incidence angle. The simultaneous measurement of reflectivity and phase can help us determine the samples with different refractive index better.

3.3. The sensitivity of simultaneous amplitude and phase images

In order to demonstrate the detection sensitivity of the amplitude-contrast and phase-contrast images can compensate for each other in kinetic measurement, an experiment of monitoring the evaporation process of a drop of NaCl injection in real time is carried out. The experiment setup is shown in Fig. 3. The droplet was deposited on the surface of the SPR sensor chip with a 50 nm-thick gold film. The light beam from the plane mirror M1 illuminates the SPR sensor chip at an incident angle of about 74 degrees. Holograms are recorded by the CMOS camera with 8-bit gray scale output. A series of holograms of the evaporating droplet were taken every 0.48 s. The collecting time lasted for 350 seconds, and 730 holograms were captured.

The amplitude-contrast images and phase-contrast images can be simultaneously obtained by numerical reconstruction of the holograms. Figure 9(a) represents the holograms recorded at \( t = 0 \) s, \( t = 206 \) s, and \( t = 350 \) s. Figures 9(b) and 9(c) represent the corresponding amplitude-contrast images and phase-contrast images, respectively (see Media 1 and Media 2). Here the amplitude images show the negative amplitude value (-R) for easy observation. The reconstructed unwrapped phase is obtained at the base of the minimum theoretical value of phase.

To observe the relations of amplitude and phase in the evaporation process of the droplet, we plot the reflectivity and phase curves vs. time over the whole measurement period as shown in Fig. 10. The obtained reflectivity and phase are average results over 4 × 4 pixels near the center of the sample in the images. As can be seen in Fig. 10, the SPR occurs at the 206th second. Firstly the reflectivity decreases slowly and reaches the minimum at the 206th second, then increases. The reflectivity decreases very slowly with time before the 206th second, which indicates that the evaporation rate is very slow at the early stage of evaporation. At the late stage, the evaporation rate becomes faster, and the reflectivity increases sharply.

The behavior is related to the fact that, the \( V^{2/3} \) (\( V \) being the volume of the droplet) of the droplet decreases linearly with time in evaporation process [23]. That is,
Fig. 9. Refractive index changes in the evaporation process of sodium chloride solution detected by SPRI. The SPR occurred at the 206th second. (a) Digital holograms of NaCl solution recorded at $t = 0$, $t = 206$, and $t = 350$ second. (b) The corresponding amplitude images of the angular spectrum reconstruction. Here the amplitude images show the negative amplitude value ($-R$). (c) The corresponding phase images of the angular spectrum reconstruction. (See Media 1 and Media 2).

Fig. 10. Curve plots of reconstructed reflectivity and phase vs. time (second).
Fig. 11. Concentration vs. time for a droplet with the initial volume of $V_0 = 3.8 \times 10^{-10} \text{ m}^3$ and constant of $\alpha = 9.7272 \times 10^{-10}$.

where $c$ is the concentration of the solution, $m$ is the mass of the solute. Figure 11 shows the concentration vs. time for a droplet with the initial volume of $V_0 = 3.8 \times 10^{-10} \text{ m}^3$ and the constant of $\gamma = 9.7272 \times 10^{-10}$. It is known that the refractive index of solution shows an approximate linear relationship with concentration [24–26]. It means the relationship between the refractive index of evaporating NaCl solution and time approximates to Fig. 11. So the refractive index will increase more and more quickly with time in the evaporation process. As can be seen from Fig. 10, the experimental results show satisfactory agreement with Fig. 11.

Figure 12(a) shows the reflectivity sensitivity and phase sensitivity obtained from Fig. 10 vs. refractive index and Fig. 12(b) shows the sensitivity vs. time. Here the refractive indexes $n$ are calculated by an experienced formula [26] as

$$n = 1.3331 + 0.185c.$$  

(21)

Figure 12 shows that the sensitivity of reflectivity reaches the minimum while the sensitivity of phase is relatively high near the resonance time (at $t = 206s$), and vice versa near the 250th second. It means that phase differential method can be used if the refractive index changes cannot be detected by use of reflectivity differential method for the low detection sensitivity, and vice versa. Accordingly, the detection sensitivity can compensate for each other and the detectable range of refractive index values can be expanded by use of simultaneous amplitude-contrast and phase-contrast images in real-time kinetic measurement.

Fig. 12. The reflectivity sensitivity and phase sensitivity. (a) The sensitivity vs. refractive index. (b) The sensitivity vs. time (s).
5. Conclusion

A simultaneous amplitude-contrast and phase-contrast SPRI method by use of digital holography is presented. The principle is theoretically analyzed. To demonstrate the theoretical analysis, an experimental system based on SPRI and digital holography is built. The experimental results show satisfactory agreement with the theory of droplet evaporation. The proposed method has a greater advantage relative to the method of single amplitude-contrast imaging or phase-contrast imaging. When the refractive index changes can’t be detected by use of the amplitude-contrast images, the phase-contrast images may be used, and vice versa. It also means the detection sensitivity of amplitude-contrast imaging and phase-contrast imaging can compensate for each other and the detection range of refractive index can be expanded by use of the simultaneous amplitude-contrast and phase-contrast imaging method. In addition, the amplitude-contrast image has less coherent noise by digital holography. So it is conducive to observe the amplitude and phase changes in real-time kinetic measurement better. The expansion of detectable range of refractive index values will lead to an increase in the number of analytical samples or increase analytical throughput capacity in high-throughput analysis.

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