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Correcting speckle contrast at small speckle size to enhance signal to noise ratio for laser speckle contrast imaging

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Abstract: In laser speckle contrast imaging, it was usually suggested that speckle size should exceed two camera pixels to eliminate the spatial averaging effect. In this work, we show the benefit of enhancing signal to noise ratio by correcting the speckle contrast at small speckle size. Through simulations and experiments, we demonstrated that local speckle contrast, even at speckle size much smaller than one pixel size, can be corrected through dividing the original speckle contrast by the static speckle contrast. Moreover, we show a 50% higher signal to noise ratio of the speckle contrast image at speckle size below 0.5 pixel size than that at speckle size of two pixels. These results indicate the possibility of selecting a relatively large aperture to simultaneously ensure sufficient light intensity and high accuracy and signal to noise ratio, making the laser speckle contrast imaging more flexible.

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1. Introduction

Laser speckle contrast imaging (LSCI) is a full-field optical imaging technique for measuring changes in blood flow [1, 2]. With advancements in the last decade [3–9], LSCI has been widely applied to map blood flow in skin, cortex and brain in animals with high spatiotemporal resolution [10–15], and has been used for preclinical applications [16–19]. A speckle pattern is generated from random interference of the laser light scattered from a rough surface, and is usually spatially blurred during an exposure time due to motion of the scattering particles. Speckle contrast, defined as the ratio of the standard deviation of intensity to the mean intensity in a local region, is used to quantify such spatial blurring [20].

Practical LSCI systems always face a problem of spatial averaging due to finite speckle to pixel size used, leading to reduction of speckle contrast [20]. Such detrimental effect, if not calibrated, will cause a systematic error in the estimated correlation time $\tau$. To eliminate the spatial averaging effect, Kirkpatrick and Duncan suggested that one-dimensional speckle size should exceed two camera pixels to meet the Nyquist criterion [21]. Nevertheless, increasing speckle size requires a larger calculation region in order to ensure sufficient number of
independent speckles within the calculation region, which sacrifices spatial resolution. Alternatively, some works introduced a system factor $\beta$ to correct local speckle contrast at different degrees of spatial averaging [3–5]. Recently, Thompson et al. confirmed the linearity of $\beta$ correction for spatial averaging, making it possible to select an appropriate aperture to compromise speckle size and light intensity [22]. However, whether the $\beta$ correction works at a speckle size much smaller than one pixel size deserves further investigation, since local speckle contrast becomes very close to $\frac{1}{\sqrt{n}}$ (where $n$ means the number of speckles per pixel) in this case [20, 22]. Besides local speckle contrast, the signal to noise ratio (SNR) of the speckle contrast image is another factor which should be taken into account. In practical applications, enhancing SNR and correcting local speckle contrast could both be considered important. Since SNR is also related to speckle size [23, 24], it is therefore important to investigate the impacts of speckle size on local speckle contrast as well as on the SNR, and seek an optimum speckle size for LSCI to ensure both high accuracy and SNR.

2. Methods

2.1 Simulation of time integrated dynamic speckle images

We simulated the time-integrated dynamic speckle images based on the “copular” method [25]. We first simulated a random phasor $\varphi$ using [25, 26]

$$Z(k) = \sqrt{-2 \ln X_1 \cos \left(2\pi X_2 + \frac{\pi}{2} \frac{k-1}{n-1} \right)}$$

$$T(k) = \text{CDF}[Z(k)]$$

$$\varphi(k) = \exp[i2\pi m T(k)]$$

In Eq. (1), $Z$ is a Gaussian distributed random variable, and $T$ is the cumulated density function of $Z$, following uniform distribution; $n$ is the length of the sequence, and $k = 1, 2, \ldots, n$; $X_1$ and $X_2$ are two statistically independent random variables that are uniformly distributed on the unit interval. In this study, $X_1$ and $X_2$ were set to be of $2048 \times 2048$ pixels, and $n$ was set to 50. Through fast Fourier transform of $\varphi$ at each $k$ value, we got 50 frames of correlated fully developed speckle images. Finally, we averaged the first 20 frames of the fully developed speckle image stack to get a time-integrated speckle image.

The multiplicative factor $m$ in Eq. (1) affects the correlation coefficient of two adjacent fully developed speckle images, which further affects the correlation time $\tau$. Therefore, by changing $m$ and repeating the aforementioned procedure, we simulated a series of time-integrated dynamic speckle images which were spatially blurred in different degrees. Speckle images with various speckle sizes were simulated as below. At each $m$ value, we first simulated a time-integrated speckle image with speckle size of 4 pixels. And then, to account for the spatial averaging effect, we performed low-pass filtering of this image with various box-car convolution filters, and finally got the time-integrated speckle images with speckle sizes of 0.125, 0.25, 0.5, 1, 2, and 2 pixels.

2.2 Phantom and animal experiments

We used a He-Ne laser (Melles Griot, America; 632.8 nm and 15 mW) as the light source. A variable attenuator was used to carefully control the illumination power, so as to ensure the light level in the image plane neither too low nor saturated [27]. A beam expander working at 450 nm–680 nm (GCO-2503, Daheng, China) was used to expand the laser beam to diameter of ~12 mm for illumination. The expanded laser beam was reflected by a mirror to illuminate the sample with ~30° incident angle. A 12 bit CCD camera with dynamic range of 69.5dB
(PixelFly QE, PCO Computer, Germany; 6.45 μm × 6.45 μm per pixel, ~11 fps) was attached to a stereo macroscope (Z16 APO, Leica, Germany). A 1 × objective lens (PLANAPO, Leica, working distance = 97 mm) was used, making the system magnification range from 0.285 × to 4.6 ×. At a certain magnification, speckle size was controlled by the aperture stop inside the macroscope.

Phantom experiment was performed as below. A glass capillary tube with inner diameter of ~0.5 mm was connected to a microsyringe with a small rubber hose. 1% intralipid fluid was pushed from the microsyringe into the glass capillary tube using a syringe pump (Stereotaxic Syringe Pump, Stoelting, USA). The flow speed of the intralipid fluid was adjusted by the syringe pump, ranging from 0.5 mm/s to 3 mm/s. A piece of black flannelette was put beneath the glass tube to block the scattered light from the background. The magnification was set to 1.6. The exposure time was set to 20 ms. At a certain speed and speckle size, 50 frames of dynamic speckle images produced from the intralipid fluid were recorded. Actually, the flow speed distribution is inhomogeneous in the glass tube [28]. For simplicity, a 150 × 20 rectangular region of interest (ROI) in the middle area of the glass tube was selected for analysis, where the length 150 and the width 20 mean 150 pixels and 20 pixels along and across the glass tube respectively. Note that the width of the ROI is less than 1/6 of the inner diameter of the glass tube which occupies ~124 pixels. The light intensity was carefully controlled by the attenuator. Room temperature was maintained throughout the experiment.

In animal experiment, we used an 8-week old C57BL/6 male mouse as the sample. All the animal experiments were approved by Huazhong University of Science and Technology Animal Care Services. The mouse was anesthetized and fixed to a stereotaxic instrument. A ~10 mm × 10 mm cranial window with intact dura was formed by removing the skull with a high speed dental drill (Fine Science Tools, USA) under constant saline cooling. In vivo cortical blood flow imaging was conducted after 10 minutes of rest. The magnification was set to 0.8. The exposure time was set to 20 ms. At each speckle size, 50 frames of speckle images produced from the mouse cortex were recorded. A 700 × 700 ROI in the cortex area was selected for data analysis. The light intensity was carefully controlled by the attenuator. The body temperature of the mouse was kept constant throughout the experiment.

2.3 Data processing

As there was no marked F-numbers in our system, speckle size of an experimental speckle image was estimated as the full width at half maximum of the auto-covariance function of the speckle intensity fluctuation [29]. It should be emphasized that speckle size smaller than 1 pixel cannot be correctly estimated using the auto-covariance method. To make sure if a speckle size was smaller than or equal to one pixel, we adjusted the aperture stop continuously and check at which point the auto-covariance method becomes insensitive to the change of aperture stop.

We calculated the mean speckle contrast $\mu_K$, and the relative noise in speckle contrast $\sigma_K/\mu_K$, from the simulated and the experimental data. Note that $\sigma_K/\mu_K$ was reciprocal to the SNR of the speckle contrast image. To compromise between statistical accuracy and spatial resolution, we used a commonly used 7 × 7 region size for speckle contrast analysis of all the simulated and the experimental speckle images. For the simulated dynamic speckle images, we calculated the $\mu_K$ and the $\sigma_K/\mu_K$ values from each speckle contrast image. For the experimental speckle images, we calculated the $\mu_K$ and $\sigma_K/\mu_K$ images as below: At each speckle size, a 7 × 7 region size was used to compute the speckle contrast image from each raw speckle image, and subsequently the $\mu_K$ and $\sigma_K/\mu_K$ values in each pixel position were calculated from 50 frames of successive speckle contrast images.

For a simulated dynamic speckle image at a certain speckle size, we used the simulated fully developed speckle image as the references for local speckle contrast calibration. For the experimental data, we used the static speckle images produced from a white sheet as the
references for local speckle contrast calibration. At each speckle size, static speckle contrast image was calculated using a $7 \times 7$ region size, and then $\beta$ was obtained through averaging the static speckle contrast values over the whole static speckle contrast image. Therefore, $\beta$ not simply accounts for spatial averaging due to finite camera pixel size used, but also accounts for the effect of finite region size used in this work. Finally, the calibrated $\mu_K$ was calculated as $\mu_K/\beta$. It should be emphasized that the speckle contrast calculation is easily affected by the speckle structure, thus local speckle contrast calculated using a finite region size sometime can be larger than unity [23]. To ensure the accuracy of the calibration, speckle contrast values beyond unity should be excluded in calculation.

For the simulated dynamic speckles, we further calculated the flow index ($T/\tau$, where $T$ is the exposure time and $\tau$ the correlation time) values to verify the linear relation between $T/\tau$ and the parameter $m$ in Eq. (1). We also calculated the $T/\tau$ values for the dynamic speckles produced from the intralipid solution and verified the linear relation between $T/\tau$ and $T$. With Brownian motion assumption, $T/\tau$ can be computed using Eq. (2) [3], where $x_1$ represents $T/\tau$.

\[
K^2 = \exp\left(-2x_1\right) + 2x_1 - 1 \quad \text{(2)}
\]

The calibrated $T/\tau$ values were also calculated using Eq. (3), where $x_2$ represents the calibrated $T/\tau$.

\[
\left(\frac{K}{\beta}\right)^2 = \exp\left(-2x_2\right) + 2x_2 - 1 \quad \text{(3)}
\]

Subsequently, we calculated the $\mu_{TV}$, the calibrated $\mu_{TV}$, and the $\sigma_{TV}/\mu_{TV}$ values similar to calculating the $\mu_K$, the calibrated $\mu_K$ and the $\sigma_K/\mu_K$ values. For the dynamic speckle images produced from 1% intralipid fluid, we also checked the linear relation between $T/\tau$ and actual flow speed.

3. Results

3.1 Analysis of simulated data

As mentioned above, the parameter $m$ in Eq. (1) affects the correlation time $\tau$. A larger $m$ results in a smaller $\tau$ and a heavier spatial blurring of the speckle pattern. Subsequently, increasing $m$ results in decreased $\mu_K$ and increased $\mu_{TV}$, as demonstrated in Figs. 1(a) and 1(b).

In Fig. 1(a), it can be observed that at a certain $m$, $\mu_K$ increases with increasing speckle size ($S$) when speckle size is smaller than 2 pixels, and then decreases when speckle size is larger than 2 pixels. Such a dependence of $\mu_K$ on speckle size can be explained as below: (1) At small speckle sizes, spatial averaging is the dominant factor that affects $\mu_K$, and the spatial averaging effect is reduced with increasing speckle size; (2) When speckle size exceeds 2 pixels, the number of statistically independent speckles in the $7 \times 7$ calculation region becomes inadequate, leading to the decrease of $\mu_K$. Conversely, it can be observed in Fig. 1(b) that at a certain $m$, $\mu_{TV}$ increases with increasing speckle size at beginning, and then decreases when speckle size is larger than 2 pixels. This is reasonable since a larger $\mu_{TV}$ corresponds to a smaller $\mu_K$.

Figures 1(c) and 1(d) show the calibrated $\mu_K$ and the calibrated $\mu_{TV}$ values at a range of $m$ and speckle sizes. The calibrated $\mu_K$ curves at different speckle sizes become coincide, and the $\mu_{TV}$ curves, too. Comparing Fig. 1(c) with Fig. 1(a), and Fig. 1(d) with Fig. 1(b), we can observe the validity of $\beta$ correction in correcting $\mu_K$ and $\mu_{TV}$, even at a speckle size of 0.125 pixel. As expected, at each speckle size, the calibrated $\mu_{TV}$ shows a linear relation with $m$. The sensitivity of LSCI in detecting relative change in flow speed keeps basically constant with speckle size, which is in agreement with [30]. Figures 1(e) and 1(f) show the dependences of $\sigma_K/\mu_K$ and $\sigma_{TV}/\mu_{TV}$ on $m$ and speckle size. The $\sigma_K/\mu_K$ and $\sigma_{TV}/\mu_{TV}$ curves keep basically...
constant with $m$, suggesting that the flow speed in our simulation does not influence the SNR significantly. On the contrary, both the $\sigma_K/\mu_K$ and the $\sigma_T/\mu_T$ values increase with increasing speckle size at a certain $m$. The increased noise with increasing speckle size is attributed to decreased number of statistically independent speckles in the calculation region. The results suggest that selecting a small speckle size has the benefit of reducing noise, in other words enhancing SNR. As an example, $\sigma_K/\mu_K$ at speckle size of 0.5 pixels is only 65% of the one at typical speckle size of 2 pixels. In other words, the SNR of the speckle contrast image at speckle size of 0.5 pixels is approximately 1.5 times higher than the one at speckle size of 2 pixels.

![Fig. 1. Speckle contrast analysis of simulated time-integrated dynamic speckles. (a) Dependence of mean speckle contrast ($\mu_K$) and (b) dependence of mean flow index ($\mu_T/\tau$) on $m$ in Eq. (1) and speckle size $S$. (c) Calibrated $\mu_K$ and (d) calibrated $\mu_T/\tau$ as functions of $m$ and speckle size. (e) Dependence of relative noise in speckle contrast ($\sigma_K/\mu_K$) and (f) dependence of relative noise in flow index ($\sigma_T/\mu_T$) on $m$ and speckle size.](image)

### 3.2 Analysis of phantom experimental data

Data analysis of the phantom experimental dynamic speckle images results in two-dimensional $\mu_K$, $\mu_K/\beta$ and $\sigma_K/\mu_K$ images, and the corresponding two-dimensional $\mu_T/\tau$, calibrated $\mu_T/\tau$, and $\sigma_T/\mu_T/\tau$ images. For simplicity, each abovementioned images were spatially averaged to get the averaged $\mu_K$, $\mu_K/\beta$, $\sigma_K/\mu_K$, $\mu_T/\tau$, calibrated $\mu_T/\tau$, and $\sigma_T/\mu_T/\tau$ values.
As shown in Figs. 2(a) and 2(b), increasing flow speed results in decreased $\mu_K$ and increased $\mu_{T/\tau}$. This is attributed to heavier spatial blurring of speckles at higher flow speed. In Fig. 2(a), it can be observed that at a certain flow speed, $\mu_K$ increases with increasing speckle size when speckle size is smaller than 2 pixels, and $\mu_K$ at speckle size of 3 pixels becomes comparable with the one at speckle size of 2 pixels. The explanation of such a dependence of $\mu_K$ on speckle size is the same as in section 3.1.

Figures 2(c) and 2(d) show the calibrated $\mu_K$ and the calibrated $\mu_{T/\tau}$ values at a range of flow speeds and speckle sizes. The calibrated $\mu_K$ curves and the calibrated $\mu_{T/\tau}$ curves, too, become coincide at different speckle sizes, demonstrating again the validity of the $\beta$ correction. As expected, at each speckle size, the calibrated $\mu_{T/\tau}$ shows a linear relation with flow speed. Figures 2(e) and 2(f) show the $\sigma_K/\mu_K$ and the $\sigma_{T/\tau}/\mu_{T/\tau}$ values at a range of flow speeds and speckle sizes. Interestingly, both the $\sigma_K/\mu_K$ and the $\sigma_{T/\tau}/\mu_{T/\tau}$ curves tend to decrease with increasing flow speed. Such phenomenon may be attributed to heavier blurring of speckles at higher flow speed, making the flow speed distribution in spatial domain more homogeneous. Similar to the results in Figs. 1(e) and 1(f), both the $\sigma_K/\mu_K$ and the $\sigma_{T/\tau}/\mu_{T/\tau}$
values in Figs. 2(e) and 2(f) increase with increasing speckle size, which is attributed to decreased number of statistically independent speckles in the calculation region.

3.3 Analysis of animal experimental data

The results of data analysis of the laser speckle images produced from mouse cortex are shown in Fig. 3 and Fig. 4. Here, we only show the results analyzed from speckle contrast images. The results analyzed from flow index images, which are similar to the results in section 3.1 and 3.2, are not shown here for brevity.

The two-dimensional $\mu_K$, calibrated $\mu_K$, and $\sigma_K/\mu_K$ images at various speckle sizes are shown in Fig. 3. Images in the first line of Fig. 3 show the dependence of the $\mu_K$ image, averaged from 50 frames of successive speckle contrast images, on speckle size. Images in the second line of Fig. 3 show the dependence of the calibrated $\mu_K$ image on speckle size. The effect of the $\beta$ correction can be observed comparing the sub-images in the black rectangular ROI in the first and the second lines. Images in the bottom line of Fig. 3 show the dependence of the $\sigma_K/\mu_K$ image, calculated from 50 frames of successive speckle contrast images, on speckle size. Again, $\sigma_K/\mu_K$ shows a trend of increase with increasing speckle size. Interestingly, at a certain speckle size, vessels with higher blood flow speed tend to have smaller $\sigma_K/\mu_K$ values, as shown in the dark blue areas. This phenomenon, similar to the phantom experimental result, may be attributed to heavier spatial blurring of speckles and more homogeneous speed distribution in vessels with higher flow speeds.

Fig. 3. In vivo blood flow imaging of mouse cortex with laser speckle. Images in the first line are the mean speckle contrast ($\mu_K$) images at various speckle sizes. Images in the second line are the calibrated mean speckle contrast ($\mu_K/\beta$) images corresponding to those in the first line. Images in the bottom are the relative noise in speckle contrast ($\sigma_K/\mu_K$) images, calculated from 50 frames of successive speckle contrast images, at various speckle sizes.
Fig. 4. (a) Magnified mean speckle contrast ($\mu_K$) images (first column) and $\mu_K/\beta$ images (second column) in the ROI in Fig. 3. Granule-like noises become more and more apparent in the $\mu_K$ and the $\mu_K/\beta$ images with increasing speckle size, as shown in the circular ROI. (b) Profiles of $\mu_K$ (solid lines) and $\mu_K/\beta$ (dashed lines) along the dashed line indicated in Fig. 4(a) at different speckle sizes. $\mu_K$ and $\mu_K/\beta$ curves at speckle size <1 pixel were used as the reference curves to compare with those at other speckle sizes. The $\mu_K/\beta$ curves at different speckle sizes show good agreement. (c) Profiles of calibrated $\mu_K \pm \sigma_K$, i.e. ($\mu_K \pm \sigma_K)/\beta$, along the dashed line indicated in Fig. 4(a) at different speckle sizes. $\mu_K/\beta$ and $\sigma_K/\beta$ at a certain speckle size are calculated from 50 frames of successive speckle contrast images.

The $\mu_K$ images and the calibrated $\mu_K$ images in the rectangular ROI in Fig. 3 were further analyzed. Images in the first line of Fig. 4(a) are the magnified $\mu_K$ images in the ROI in Fig. 3, and images in the bottom line of Fig. 4(a) are the magnified $\mu_K/\beta$ images in the ROI in Fig. 3. It can be observed that, after $\beta$ correction, the $\mu_K/\beta$ images at different speckle sizes are basically in the same scale. However, the granule-like noise tends to increase with increasing speckle size, as shown in the circular ROI in the images. This result demonstrates that the $\beta$ correction only corrects the mean speckle contrast, but cannot affect the noise level at different speckle sizes. For comparison in detail, we plotted the profiles of $\mu_K$ and $\mu_K/\beta$ along the dashed line indicated in Fig. 4(a) at different speckle sizes, as shown in Fig. 4(b). $\mu_K$ and $\mu_K/\beta$ curves at speckle size <1 pixel were used as the reference curves to compare with those at other speckle sizes. As shown, the $\mu_K$ curves at different speckle size do not coincide, but the $\mu_K/\beta$ curves at different speckle sizes show good agreement with each other.

We also calculated the $\sigma_K$ and $\sigma_K/\beta$ values from 50 frames of successive speckle contrast images along the dashed line indicated in Fig. 4(a). We therefore plotted the profiles of the calibrated $\mu_K \pm \sigma_K$, i.e. ($\mu_K \pm \sigma_K)/\beta$, along the dashed line indicated in Fig. 4(a) at different
speckle sizes. It can be observed that in cortical areas where there are no apparent vessels, the \( \mu_K/\beta \) curve at speckle size <1 pixel is more flat than the curves at other speckle sizes, because of less granule-like noises. Similar to the simulated and the phantom experimental results, \( \sigma_K/\mu_K \) (\( \sigma_K/\beta \) divided by \( \mu_K/\beta \) results in \( \sigma_K/\mu_K \)) tends to increase with increasing speckle size. For comparison, at each speckle size, we averaged the \( \sigma_K/\mu_K \) values along the dashed line indicated in (a). As shown, the averaged \( \sigma_K/\mu_K \) value at speckle size <1 pixel is 0.16, whereas the averaged \( \sigma_K/\mu_K \) value at speckle size of 2 pixels is 0.20. Hence the noise level at speckle size <1 pixel is 80% of the one at speckle size of 2 pixels. In other words, the \( SNR \) of the speckle contrast image at speckle size <1 pixel is 25% higher than the one at speckle size of 2 pixels. It is worth pointing out that cerebral blood flow is probably inhomogenous in temporal domain [28]. To ensure the stability of the results, a large number of speckle images, and a relatively long time for data recording are required. In our experiment, 50 frames of images were recorded, and the total time used for recording was \( \approx 4.5 \) s.

4. Discussions

In the simulated results, Figs. 1(b) and 1(d) indicate a linear relation between \( T/\tau \) and \( m \), no matter whether the \( T/\tau \) value is calibrated or not. Although the sloop for the \( T/\tau \) curve is the highest at the smallest speckle size of 0.125, it becomes generally the same as those at other speckle sizes after \( \beta \) correction. By selecting the \( T/\tau \) value at \( m = 12 \) as the baseline value \( T/\tau_0 \), we plotted the relative changes in \( T/\tau \) as a function of the relative changes in \( m \) for different speckle sizes, and obtained an approximately 1:1 response (data not shown). Here, the 1:1 response means the relative change in \( m \) results in the same value of relative change in \( T/\tau \). Therefore, we concluded that the \( \beta \) calibration does not affect the sensitivity to speed changes. For practical applications where only relative speed change measurement is required, the results are the same with and without the \( \beta \) correction. However, since our results in Fig. 1 indicate that selecting a smaller speckle size can produce a higher \( SNR \), a small speckle size is still recommended for reflecting speed changes with relatively high accuracy. 

In [22], a very large region size was used for speckle contrast calculation, and the resulting speckle contrast values are very close to 1 at large speckle sizes. Such large speckle contrast values are practically unavailable if a small region size (for instance a 7 \( \times \) 7 region size) is used for calculation, because the number of independent speckles within the calculation region is significantly reduced at large speckle sizes. In this work, we used a widely used 7 \( \times \) 7 region size for data analyses. Hence, the \( \beta \) correction in this work not only accounts for spatial averaging due to finite speckle to pixel size, but also accounts for the impact of finite calculation region size on speckle contrast. The validity of the \( \beta \) correction has also been demonstrated when smaller region sizes such as 5 \( \times \) 5 and 3 \( \times \) 3 were used for calculation, as shown in Fig. 5. From Fig. 5, we found that all the \( \mu_{TC} \) curves can be corrected except for those at speckle size of 4 using region sizes of 3 \( \times \) 3 and 5 \( \times \) 5. This is reasonable, because in these cases, the speckle size is comparable to the region sizes used, and the number of independent speckles in the calculation region is too few. Through data analyses using various region sizes, we found that the calibration remains valid when the length of the calculation region is above 1.5 times longer than the speckle size.

Evidently, Fig. 5 also suggests the possibility of selecting a small region size to improve spatial resolution. In the condition of a small speckle size, it is also feasible to use a 3-dimensional image cube, such as a 3 \( \times \) 3 \( \times \) 5 image cube, and perform a spatiotemporal speckle contrast analysis to get speckle contrast images with higher spatial resolution [26]. Here, 3 \( \times \) 3 is the spatial region size and 5 is the number of speckle images used in temporal domain.
Fig. 5. Simulation investigation of the impacts of speckle size and region size on $\beta$ calibration. The calibration is valid when region size is above 1.5 times larger than the speckle size.

In Fig. 1, $\sigma_K/\mu_K$ keeps almost constant when speckle size is smaller than 0.5 pixel size. We explain this phenomenon from two aspects: (a) Speckle contrast image becomes highly homogeneous if speckle size is very small, hence it is reasonable that the SNR values at small speckle sizes are very close to each other. (b) Practically, $\sigma_K/\mu_K$ increases with increasing speckle size, and decreases with increasing region size. Note that Fig. 1 only shows the results calculated using a $7 \times 7$ region size. This can explain why the minimum $\sigma_K/\mu_K$ in Fig. 1 is ~0.1, but not zero. Actually, the minimum $\sigma_K/\mu_K$ can be smaller when a larger region size is used for data analyses [23].

In many clinical applications, a large field of view is required and the magnification is much smaller than 1. In such cases, speckle size for image speckle mainly depends on $F$-number of the imaging system. Considering sufficient light intensity is required, $F$-number cannot be too large, making speckle size beyond two pixels not always available. Speckle size for image speckle can be calculated using Eq. (4) [31]:

$$ r_s = 1.2 \lambda (1 + M) F. $$

Here $\lambda$ is the wavelength of the laser light, $M$ is the magnification, and $F$ is the $F$-number. Taking the instruments in our system as an example, the wavelength of the laser light is 632.8 nm, and the CCD camera contains 1392 $\times$ 1024 pixels, each with size of 6.45 $\mu$m. Suppose $M$ is set to 0.2, then the field of view is 4.5 cm $\times$ 3.3 cm. Subsequently, $F$ should exceed 14 in order to produce a speckle size beyond 2 pixels. Such large $F$-number will result in serious reduction of intensity in the image sensor. Our results indicate that it is unnecessary to set the speckle size beyond two camera pixels, and unnecessary to make a compromise between speckle size and light intensity.
As defined by Eq. (4), speckle size is dependent on both magnification and $F$-number. Since magnification is fixed in an experiment, speckle size is only determined by $F$-number. To get a small speckle size, a small $F$-number, in other words a larger aperture, should be selected. There are several advantages for selecting a relatively large aperture in LSCI. Firstly, a larger aperture results in smaller speckle size, which enhances SNR of the speckle contrast image, as has been shown in this work. Secondly, a larger aperture allows more scattered light entering the image plane, which can avoid the detrimental effect of low intensity on speckle contrast [27]. Furthermore, it makes the experiment more flexible, which no longer needs to match speckle size to one or two pixels as presented in previous works [1, 21]. Besides, since it was suggested that the number of statistically independent speckles should exceed 15 to ensure statistical accuracy [32], selecting a relatively large aperture, which results in a small speckle size, will no doubt make such requirement easier to be satisfied. However, it should be pointed out that, in practical applications, the object is usually not flat, such as the mouse cortex which has a cambered surface. In such cases, the depth of field should also be taken into account for selecting an appropriate aperture.

5. Conclusion

In conclusion, we show that correcting speckle contrast at small speckle size has the benefit of enhancing signal to noise ratio as well as maintaining statistical accuracy for laser speckle contrast imaging. The reduction in local speckle contrast, which is resulted from the spatial averaging effect and finite calculation region size used, can be corrected through dividing the original speckle contrast by the static speckle contrast. Such correction works well even at speckle sizes far smaller than one camera pixel size. Moreover, we get 50% higher signal to noise ratio of the speckle contrast image at speckle size below 0.5 pixel size than at typical speckle size of 2 pixels. The presented work suggests that we can select a relatively large aperture to simultaneously ensure sufficient light intensity and high accuracy and signal to noise ratio. The presented work also indicates the possibility of using a region size as small as $3 \times 3$ to improve spatial resolution and meanwhile maintain high accuracy in speckle contrast calculation.

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