

# Research on LASCA and Denoising of Blood Vessel images of Small Animals

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*Abstract:* -A denoising approach is proposed that based on the combination of wiener filtering, order-statistic filtering and wavelet, which denoise the LASCA(LASer Speckle Contrast Analysis)image of blood vessels in small animal. The approach first performs laser spectral contrast analysis on cerebral blood flow and brain blood flow in rat, get their spatial and temporal contrast images. Then, a denoising filtering method is proposed to deal with noise in LASCA. The image restoration is achieved by applying the proposed admixture filtering , and the subjective estimation and objective estimation are given to the denoising images.

*Key-Words:* - Cerebral blood flow, Brain blood flow, Wiener filtering, Order-statistic filtering, Wavelet, Laser speckle contrast imaging, Hybrid filtering.

## 1 Introduction

Laser speckle contrast imaging (LSCI) technique is a new modality to monitoring blood flow dynamics with high spatio-temporal resolution. It records the full-field spatio-temporal characteristics of microcirculation without the need of scanning in real time [1]. Laser speckle contrast analysis (LASCA) is a non-scanning, non-invasive technique that produces 2-D map of blood flow by analyzing speckle images captured by CCD camera [2]. Our research is to develop the image quality of low frame image and make it reach the quality of high frame image by using image processing techniques. Laser speckle contrast imaging applies the 1st order spatial statistics analysis , the method of spatial statistics of Time-varying Speckle was proposed by Briers in 1980s [3]. It calculates contrast value on the local image (subimage or statistics window), and convert into pseudo color image [4]. In fact, it can be considered as the 1st order spatial statistics analysis on speckle image , and the temporal statistics analysis is first proposed in 1976 by Ohtsubo and Asakura [5]. Serov applied this method

in Laser doppler perfusion imaging and use CMOS fast imager, the sampling rate can be 9000 pps [6].Cheng applied this in LATAC to gain the two dimension imaging of blood vessel distribution, which performs temporal statistics on each point around m frames images [7].

However, when applying LASI, the details of time-varying speckle is difficult to discriminate. It needs for spatial or temporal statistics to denoising while preserving the details. Because the time cost in spatial LASCA and the space cost in temporal LASCA are huge, it becomes an important research objective for us that use less frames of time-varying speckle to gain higher quality imaging.

Wiener filtering is a kind of adaptive filtering , which can effectively noise restraining and protect the edge, and is widely used in image processing. However, badly in detail discriminate , it would easily cause thin line, curve, etc lost and damaged [8].The nonlinear filters are proved to be effective in suppressing or eliminating fix-value impulse noise [9]. Moreover, nonlinear filters preserve the details and edges of an image during the process of

denoising [10]. Wavelet is an effective tool for signal restoration, which has good performance in denoising and preserving details, but cause edge fuzzy for its soft threshold [11].

Therefore, we propose a novel hybrid filtering method which combine wiener filtering, nonlinear filtering and wavelet, for denoising of LASCA images. This approach first performs wiener filtering, add nonlinear filtering with the result, change the luminance; Meanwhile, makes wavelet transform on the noisy image, and via inverse wavelet transform. Finally, the result is the fusion of wavelet and those two. The experimental results show that the hybrid filtering has better performance than each single filtering, which denoising while preserve the edge and other details of images.

## 2 Laser Speckle Contrast Analysis on small animals' blood vessel

### 2.1 LSCI system

The schematic setup for the experiment is shown in Fig 2.1. A rat was anesthetized and fixed in a stereotaxic instrument. An approximately  $5.0 \times 5.0$ -mm cranial window with intact dura was formed by removing the skull overlying one side of the parietal cortex with a high speed dental drill (Fine Science Tools, USA) under constant saline cooling. A beam of He-Ne laser (Melles Griot, America; 632.8 nm and 15 mW) was expanded and collimated to illuminate the cranial window at about 30-degree incidence. 30 frames of statistically independent laser speckle images were acquired by a 12-bit charge-coupled device (CCD) camera (PixelFly QE, PCO Computer, Germany; pixel size =  $6.45 \times 6.45 \mu\text{m}$ ) attached to a microscope (Z16 APO, Leica, Germany; working distance 97 mm) for data processing. The CCD exposure duration was 20 ms and the frame interval time is approximately 87 ms. The system magnification is adjusted to  $3.15\times$ , and the aperture diaphragm is well controlled to ensure the average speckle size of the images to be approximately two pixels. A variable attenuator was used in the light path to ensure the light intensity within the dynamic range of the CCD camera. The whole setup was placed on a vibration-isolator table (VH3036W, Newport)

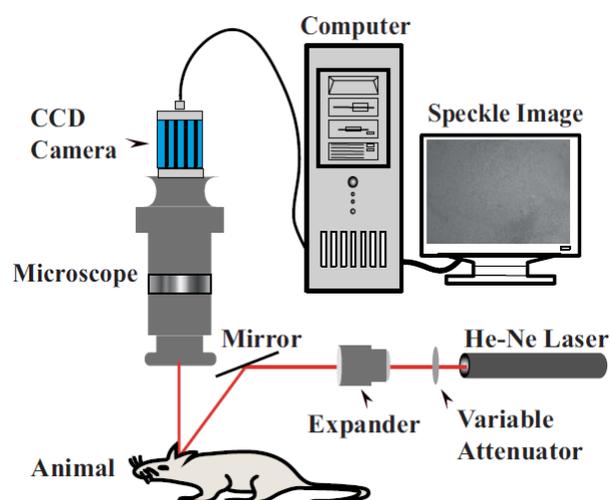


Fig 2.1 Scheme of laser speckle contrast imaging system.

System constituted by laser light source, microscope, CCD camera and computer.

The experimental setup for laser speckle contrast imaging is very simple. Diverging laser light illuminates the object under investigation, which is imaged by a CCD camera (or equivalent). The image is captured by a frame grabber (or equivalent) and the data passed to a personal computer for processing by custom software.

### 2.2 Spatial LASCA and Temporal LASCA

As illustrated in Fig. 2.2, the laser speckle contrast analysis can be performed based on spatial statistics and temporal statistics. The spatial LASCA performs speckle contrast calculation in the spatial domain using a spatial window. It achieves high temporal resolution with the loss of spatial resolution, impeding its application on monitoring blood flow changes in small vessels. The temporal LASCA method, which is based on temporal statistics, computes speckle contrast images using a sequence of speckle images acquired along a few time points instead of using a spatial window. It preserves the original spatial resolution by sacrificing the temporal resolution, making it inappropriate in applications where video frame rate visualization of blood flow is required.

### 2.3 Application of LASCA on blood vessel images of small animal

From Fig 2.3 to 2.5, it shows the spatial, temporal(10 Frames) and temporal (100 Frames) LASCA images, respectively. TABLE 2.1 shows objective estimation between spatial and temporal LASCA with Base Image.

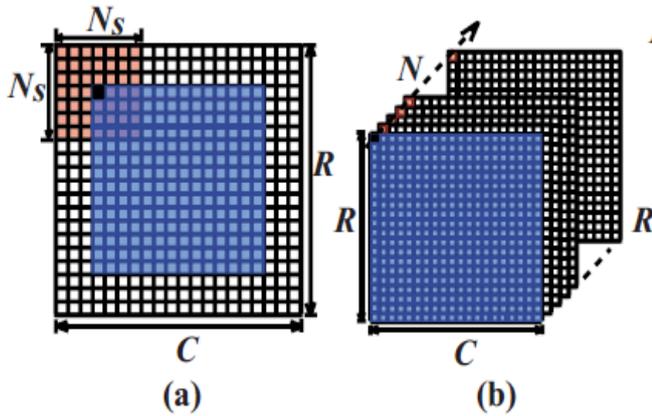


Fig 2.2 (a) Spatial LASCA and (b) Temporal LASCA

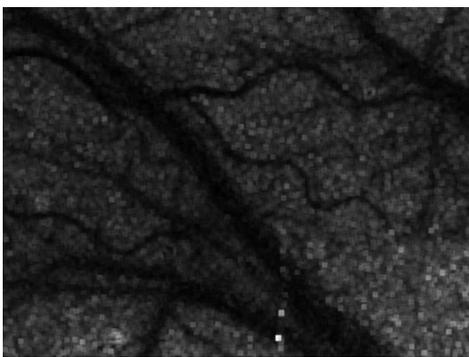


Fig 2.3 spatial LASCA

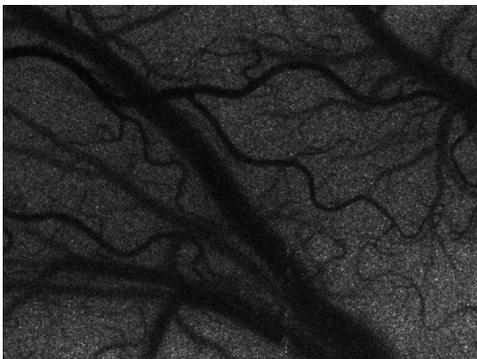


Fig 2.4 temporal LASCA 10Frames

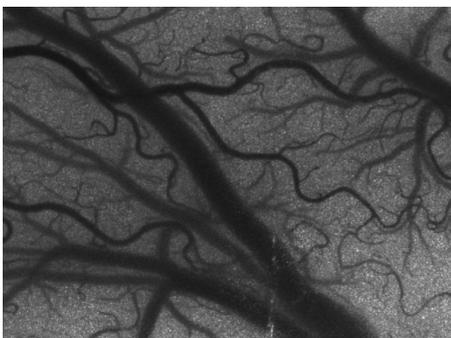


Fig 2.5 temporal LASCA 100Frames (Base Image)

TABLE 2.1 Objective Estimation between spatial and temporal LASCA with Base Image

	Spatial LASCA	Temporal LASCA (10Frames)
MSE	0.14349	0.12686
SNR	1.5838	1.4046
PSNR	15.964	16.304

### 3 Denoising of LASCA images by hybrid method combined with wiener, nonlinear and wavelet filtering

#### 3.1 wiener, nonlinear and wavelet filtering

##### 3.1.1 wiener filtering

Suppose the signal model is:

$$X=S+N \quad (1)$$

In this equation S is the true signal , x is the noisy signal recorded , N is Gaussian white noise , mean value is zero , variance is constant  $\sigma_N^2$  , and be independent with signal S. The objective is searching filter  $\hat{\lambda}$  , which make mean square error is least [12].

$$MSE = E[(S - \hat{\lambda}X)^2] \quad (2)$$

From Eq (2)

$$MSE = E[S^2 - 2\hat{\lambda}SX + \hat{\lambda}^2X^2] = E[S^2] - 2\hat{\lambda}E[SX] + \hat{\lambda}^2E[X^2] \quad (3)$$

make derivation of (3),

$$\hat{\lambda} = \frac{E[SX]}{E[X^2]} = \frac{E[S(S+N)]}{E[X^2]} \quad (4)$$

because s and N are independent ,and  $E[N]=0$

$$\hat{\lambda} = \frac{E[SX]}{E[X^2]} = \frac{E[S^2]}{E[X^2]} \quad (5)$$

And

$$E[X^2] = E[(S+N)^2] = E[S^2] + E[N^2] = E[S^2] + \sigma_N^2 \quad (6)$$

So

$$\hat{x} = \frac{E[X^2] - \sigma^2}{E[X^2]} \quad (7)$$

The estimate signal is

$$\hat{x}_{ij} = u_{ij} + \hat{\lambda}_{ij} (x_{ij} - \mu_{ij}) \quad (8)$$

$\mu$  is the mean value of signal. When filter is least mean value, the  $\hat{x}_{ij}$  be the optimal solution.

### 3.1.2 Nonlinear Filtering

A nonlinear filter is a signal-processing device whose output is not a linear function of its input. Terminology concerning the filtering problem may refer to the time domain (state space) showing of the signal or to the frequency domain representation of the signal. When referring to filters with adjectives such as "bandpass, highpass, and lowpass" one has in mind the frequency domain. When resorting to terms like "additive noise", one has in mind the time domain, since the noise that is to be added to the signal is added in the state space representation of the signal. The state space representation is more general and is used for the advanced formulation of the filtering problem as a mathematical problem in probability and statistics of stochastic processes.

According to a negative definition, a nonlinear filter is any filter that does not meet the criteria of linearity. For a linear system  $\xi$ , given the two signals  $\{x\}$  and  $\{y\}$  and a constant  $a$ , two familiar conditions must hold:

$$\xi(ax) = a\xi(x) \quad (9)$$

$$\xi(x) + \xi(y) = \xi(x + y) \quad (10)$$

In contrast, the nonlinear filter can be written in the general form

$$y_n = f(x_n, \dots, x_{n-N}, y_{n-1}, \dots, y_{n-M}) \quad (11)$$

Where  $f$  is a nonlinear function.

### 3.1.3 Wavelet Filtering

The wavelet filter is good at removing gaussian-type noise, while it can leave some kind of photon noise (very hot pixels for example). Thus an option is provided in the form of an optional adaptive median filter. This filter will detect pixels that differ from their context by more than a given multiple of the neighborhood's standard deviation. If marked as outlying, the pixel value is replaced by the median value of the neighborhood. A suggested default value is  $1.6 * sd$ . The idea behind this filter is that if

an adequate sampling was chosen upon acquisition, no such outlying (extreme value) pixels should be found.

In practical image processing in computer, we use binary discrete wavelet(DWT) to make continuous wavelet and its transform meaningful. In other words, the next discussion would be the discretization of continuous wavelet  $\psi_{a,b}(t)$  and continuous wavelet transform  $W_f(a,b)$ . Consider function:

$$\psi_{a,b}(t) = |a|^{-1/2} \psi\left(\frac{t-b}{a}\right) \quad (12)$$

Here,  $b \in R, a \in R^+, a \neq 0$ , then the compatibility condition is:

$$C_\psi = \int_0^\infty \frac{|\hat{\psi}(\bar{\omega})|}{|\bar{\omega}|} d\bar{\omega} < \infty \quad (13)$$

Therefore, the corresponding discrete wavelet function

$$\psi_{j,k}(t) = a_0^{-j/2} \psi\left(\frac{t - ka_0^j b_0}{a_0^j}\right) = a_0^{-j/2} \psi(a_0^{-j} t - kb_0) \quad (14)$$

The discrete wavelet transform coefficient is

$$C_{j,k} = \int_{-\infty}^\infty f(t) \psi_{j,k}^*(t) dt = \langle f, \psi_{j,k} \rangle \quad (15)$$

The reconstruction equation is

$$f(t) = C \sum_{-\infty}^\infty \sum_{-\infty}^\infty C_{j,k} \psi_{j,k}(t) \quad (16)$$

## 4 Proposed Hybrid Filtering

Based on the comparison of several filtering methods discussed above, considering their advantages, we put forward a hybrid denoising method oriented for LASCA images, which combined with wiener, nonlinear and wavelet filtering.

The process is shown below:

(1) Apply spatial statistic analysis or temporal statistic analysis on LASI (time-varying speckle) images, to gain  $f_{01}(i,j)$  from spatial LASCA and  $f_{02}(i,j)$  from temporal LASCA for small number of frames (no larger than 10 frames). They are all noisy images.

(2) Perform wiener filtering on  $f_{01}(i,j)$  (or  $f_{02}(i,j)$ ) to gain processing image  $f_w(i,j)$ .

(3) Perform order-statistic filtering and grayscale processing on  $f_w(i,j)$  to gain  $f_{NL}(i,j)$ .

(4) Divide  $f_{NL}(i, j)$  into 2 layers and perform wavelet transform to gain  $W_{T1}(i,j)$  and  $W_{T2}(i,j)$ , then inverse transform for each. Finally, reconstruct wavelet denoising image  $f_i(i,j)$  from those two.

(5) The result of admixture filtering is the denoising image we gain.

The framework is shown as below.

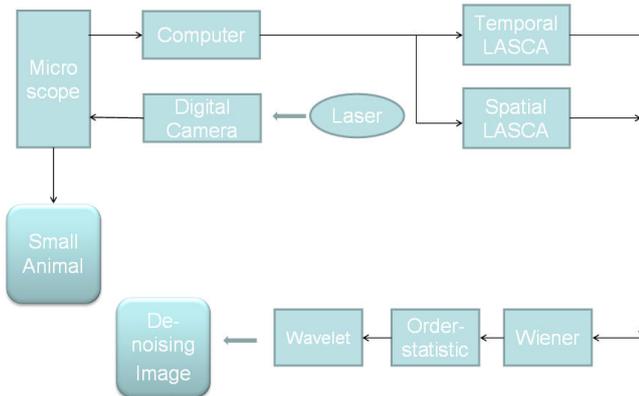


Fig 4.1 Framework of Blood Vessel Imaging and Denoising

## 5 Experimental Results and Analysis of LASCA image Denoising

### 5.1 Evaluate Criterion

Because the Temporal Contrast Analysis (100Frames) has a fine performance, in our research, it is considered as the base image, which is used in objective estimations --- MSE, SNR and PSNR.

The above three objective measurements are selected and used for our research study. Definition:  $x(m,n)$  denotes the base image,  $\hat{x}(m,n)$  denotes the de-noising image. M and N are number of pixels in row and column directions, respectively.

#### (1) Mean Square Error (MSE)

The simplest of image quality measurement is Mean Square Error (MSE). In statistics, the MSE of an estimator is one of many ways to quantify the difference between values implied by an estimator and the true values of the quantity being estimated. MSE is a risk function,

corresponding to the expected value of the squared error loss or quadratic loss. MSE measures the average of the squares of the "errors." The error is the amount by which the value implied by the estimator differs from the quantity to be estimated. The large value of MSE means that image is poor quality. MSE is defined as follow:

$$MSE = \frac{1}{MN \sum_{m=1}^M \sum_{n=1}^N (x(m,n) - \hat{x}^{m,n})^2}$$

#### (2) Signal to Noise Ratio (SNR)

Signal-to-noise ratio (often abbreviated SNR or S/N) is a measure used in science and engineering to quantify how much a signal has been corrupted by noise. It is defined as the ratio of signal power to the noise power corrupting the signal. A ratio higher than 1:1 indicates more signal than noise.

SNR is defined as follow:

$$SNR = \frac{P_{signal}}{P_{noise}}$$

where P is average power.

#### (3) Peak Signal to Noise Ratio (PSNR)

The phrase peak signal-to-noise ratio, often abbreviated PSNR, is an engineering term for the ratio between the maximum possible power of a signal and the power of corrupting noise that affects the fidelity of its representation.

The small value of Peak to Noise Ratio (PSNR) means that image is poor quality. PSNR is defined as follow:

$$PSNR = 10 \log \frac{255^2}{MSE}$$

As a part of estimation, we do in this way: mainly compare the subjective performance

between the base image and our hybrid de-noising image. Also, we provide the performance of 3 single de-noising methods we apply, in order to ensure the effectiveness of our hybrid method. 2 former researchers' works (Med and Linear Filters) are added in our comparison at last.

## 5.2 Experimental results for each filtering method on rat's cerebral blood flow

From Fig 5.1 to 5.6, it shows denoising result applied on Fig 2.3 and Fig 2.4 by using single filters --- Wiener, nonlinear and wavelet. From Fig 5.7 to 5.8, it shows the result by using our proposed filter.

For objective estimations, we choose MSE, SNR and PSNR to be criterions. In computing, the base image (temporal LASCA 100 Frames) is used to be compared with. TABLE 5.1 and 5.2 show the specific data of those three.

### ( 1 ) Results of Single Filtering

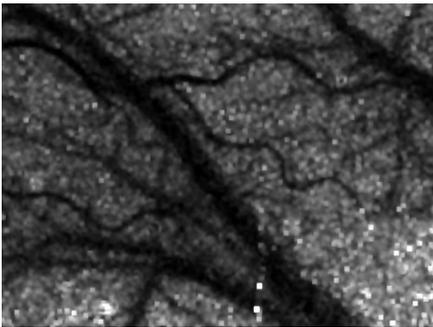


Fig 5.1 Result for Fig 2.3(Wiener)

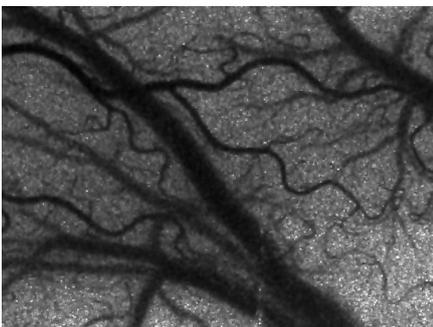


Fig 5.2 Result for Fig 2.4(Wiener)

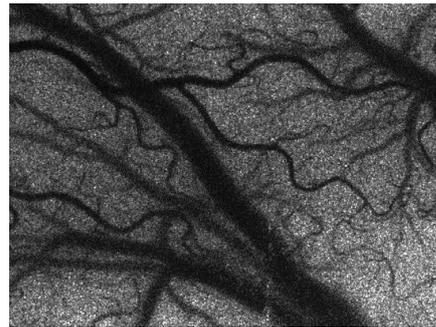
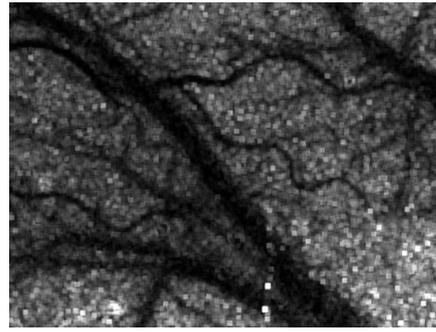


Fig 5.4 Result for Fig 2.4(Nonlinear)

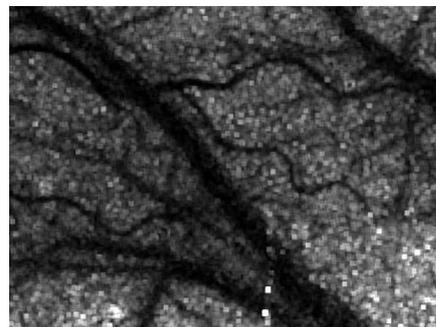


Fig 5.5 Result for Fig 2.3(Wavelet)

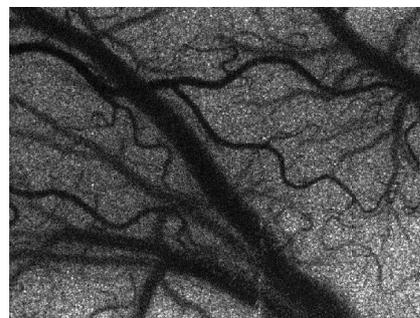


Fig 5.6 Result for Fig 2.4(Wavelet)

### ( 2 ) Result of Hybrid Filtering

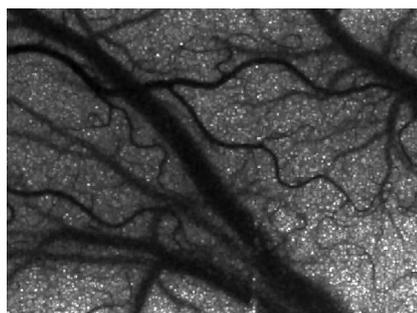
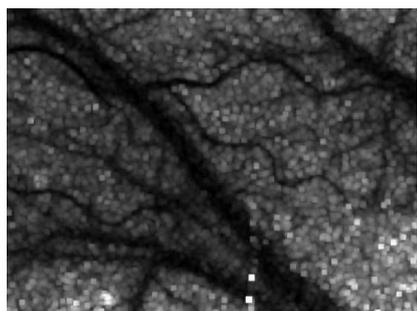


Fig 5.8 Result for Fig 2.4

( 3 ) Objective Estimation for each filtering method

TABLE 5.1 Objective estimation of Fig 2.3

	Original	Hybrid	Single		
			Wiener	Nonlinear	Wavelet
MSE	0.14349	0.016318	0.032712	0.03317	0.033109
SNR	1.5838	8.1698	9.4593	6.4932	6.5077
PSNR	15.964	20.243	18.822	20.024	20.029

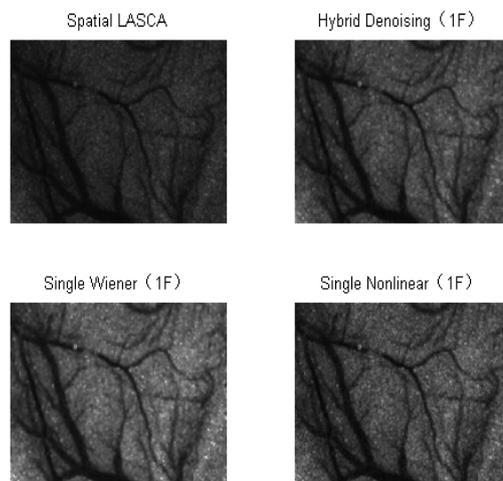
TABLE 5.2 Objective estimation of Fig 2.4

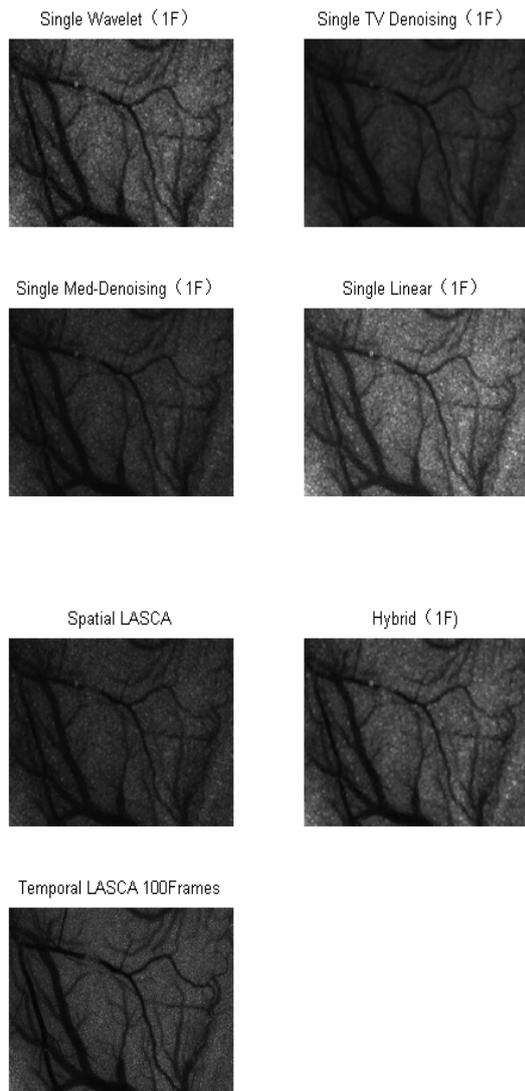
	Original	Hybrid	Single		
			Wiener	Nonlinear	Wavelet
MSE	0.12686	0.001201	0.059582	0.0031516	0.002892
SNR	1.4046	13.3	12.655	9.0205	9.0134
PSNR	16.304	22.538	20.125	19.925	19.918

5.3 Experimental results for each filtering method on rat's brain blood flow

5.3.1 Experimental results and analysis of Spatial LASCA image denoising

Similarly as last section, here, we show the results of each denoising filter --- from original (noisy image), our proposed filter to each single filter, also, we add other researcher's method such as TV denoising and so on. From the widely compare, it is firmly shown that, our hybrid denoising have the best objective performance. Moreover, from TABLE 5.3 to 5.5 show the specific data of objective estimations.





### 5.3.2 Experimental results and analysis of temporal LASCA

( 1 ) Experimental results and analysis of Temporal LASCA (5F) image denoising

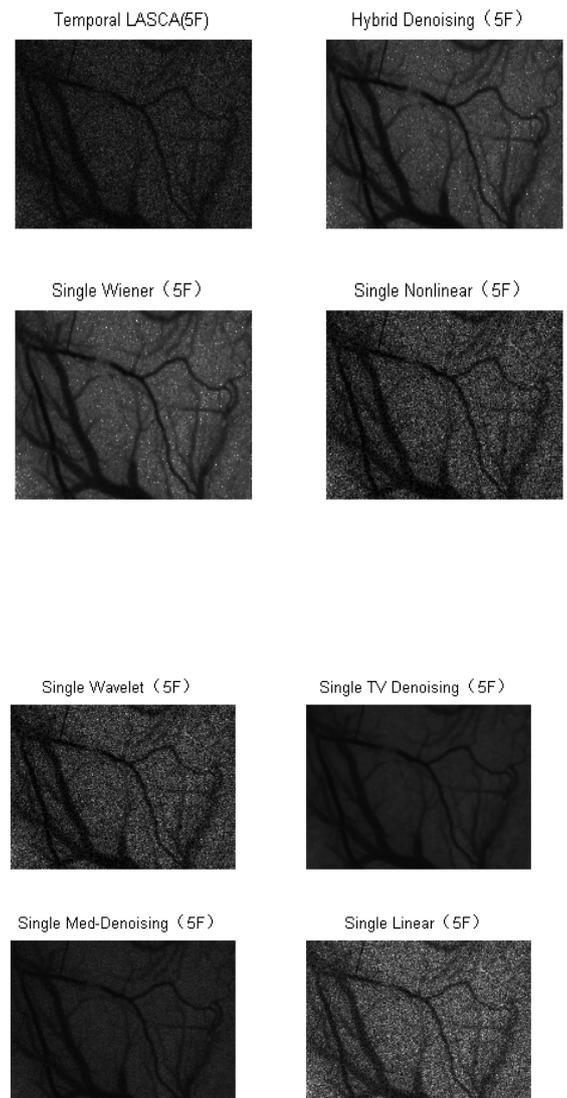


Fig 5.9 Results of Spatial LASCA image denoising

TABLE 5.3 Estimation of denoising (Spatial)

	Original	Hybrid	Single			Others		
			Wiener	Nonlinear	Wavelet	TV	Med	Linear
MSE	0.0477	0.0383	0.1138	0.0525	0.0525	0.0485	0.0479	0.1487
SNR	8.3454	11.9030	7.7906	10.3830	10.3926	50.3915	8.3791	5.7848
PSNR	15.0007	20.1797	16.3509	19.3908	19.4030	43.8904	14.5771	14.9426

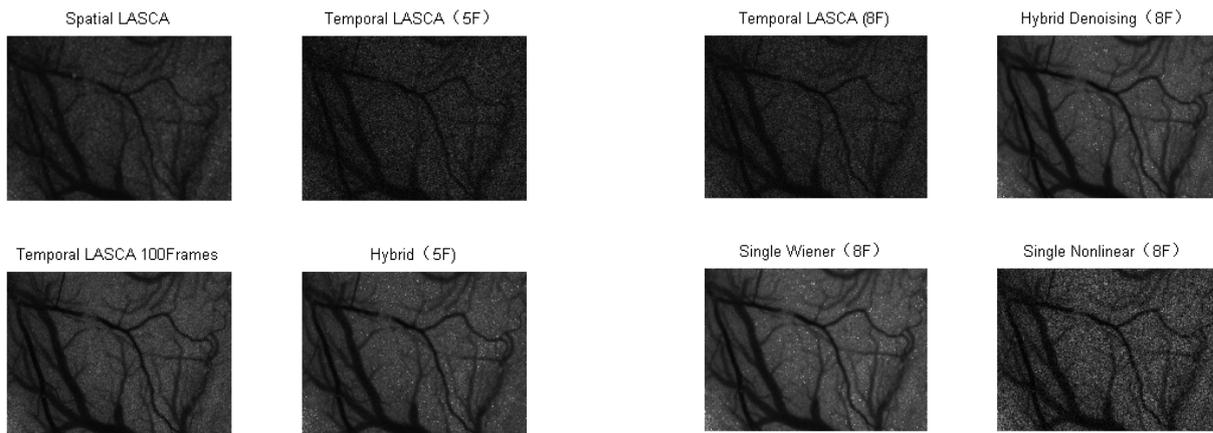


Fig 5.10 Results of Temporal LASCA (5F) image denoising

TABLE 5.4 Estimation of denoising (5 Frames)

	Original	Hybrid	Single			Others		
			Wiener	Nonlinear	Wavelet	TV	Med	Linear
MSE	0.07667	0.0119	0.0373	0.0077	0.0076	0.0802	0.08056	0.076
SNR	3.9292	14.0343	11.9009	6.8384	6.8502	52.2700	3.4655	5.9091
PSNR	14.0777	26.6278	24.2052	21.0845	21.0770	48.6558	7.9792	17.8743

( 2 ) Experimental results and analysis of Temporal LASCA (8F) image denoising

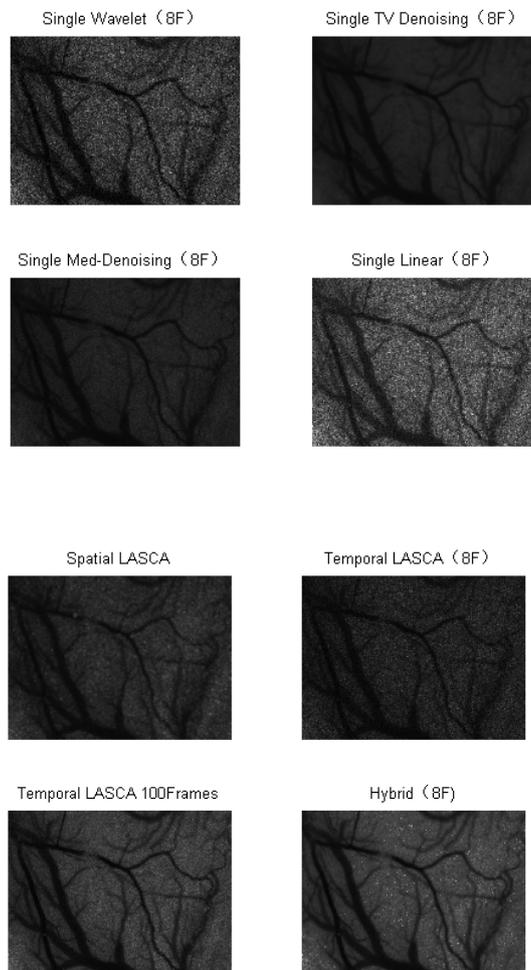


Fig 5.11 Results of Temporal LASCA (8F) image denoising

TABLE 5.5 Estimation of denoising (8 Frames)

	Original	Hybrid	Single			Others		
			Wiener	Nonlinear	Wavelet	TV	Med	Linear
MSE	0.0719	0.0230	0.0500	0.0022	0.0022	0.0747	0.0750	0.0884
SNR	4.9965	13.9797	11.3387	8.9911	9.0024	51.8801	4.5431	6.4365
PSNR	13.7477	25.9501	23.5541	21.8760	21.8966	46.2378	9.5101	18.3410

Comprehensive compared with 3 objective estimation parameters(MSE SNR PSNR) and subjective estimation on image quality, our proposed hybrid filtering method has improved performance than single filtering and other filtering.

## 6 Conclusion

This paper proposes a LASCA method for blood vessel imaging of small animals. We also propose a novel hybrid filtering for LASCA in denoising of medical images. Regrouping Wiener filtering, Nonlinear filtering and Wavelet denoising technology, the laser speckle imagings based on spatial contrast or temporal contrast has been, from the experiment, we can conclude that

1. The proposed hybrid filtering for spatial and less frame contrast image (5F 8F) has high value of PSNR and good subjective performance.
2. The hybrid filtering absorbs all the benefits of each filtering.
3. From the results, both subjective and objective performance, the hybrid filtering is the best or one of the best. Therefore, it is considered that it has better performance than other single filtering.
4. The results show that the performance of our proposed "LASCA in small number of frame and the denoising" is very similar with the performance of LASCA in high number of frames (100 F). Thus our proposed method can provide an effective and prompt blood vessel image processing.

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