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Signal processing for a laser-Doppler blood perfusion meter

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Abstract

Two signal processing methods for laser-Doppler perfusion velocimetry are presented. The methods are based on the calculation of the moments of the frequency power spectrum. The first uses $\sqrt{\omega}$ -filtering (ω is the frequency) with analogous electronics, the second uses signal autocorrelation with digital electronics. Comparison is made with a third instrument: a spectrum analyzer coupled to a computer, using Fourier transform techniques. The performance of these setups (sensitivity, limit sensitivity and accuracy) are investigated. We propose a calibration standard for signal processors to be used for blood perfusion measurements. The analogous instrument proved to be the cheapest but the digital instrument had the best performance.

Zusammenfassung

Zwei Methoden für die Behandlung der Signale von laser-Doppler Geschwindigkeitsmessung der Durchblutung von Gewebe sind vorgelegen. Die Methoden sind basiert auf der Berechnung der Momente des Frequenzvermögensspektrum. Die erste wendet $\sqrt{\omega}$ -Filterung an (ω ist die Frequenz) mit analogische Elektronik, die zweite benutzt Autocorrelation der Signale mit digitalische Elektronik. Eine Vergleichung mit ein drittes Instrument ist angestellt: eine Spektralanalysateur gekuppelt mit einem Rechenautomat, mit Anwendung von Fourier Transformationstechnike. Die Leistung dieser Apparate (Empfindlichkeit, Grenzemfindlichkeit, Genauigkeit) sind untersucht. Wir stellen eine allgemeine Methode für die Eichung von Signalprozessoren, für Anwendung in Messungen der Durchblutung, vor. Das analogische Instrument zeigt sich als preiswert, aber das digitalische hat eine bessere Leistung.

Résumé

Deux réalisations différentes de la chaîne de traitement du signal d'un appareil à laser pour mesurer la perfusion du sang par effet Doppler sont présentées. Ces méthodes sont basées sur le calcul des moments de la densité spectrale de puissance. Le premier utilise un filtre en $\sqrt{\omega}$ (ω étant la fréquence) et de l'électronique analogique; le deuxième utilise la fonction d'autocorrelation avec de l'électronique digitale. Ces deux appareils sont comparés à un analyseur de spectre par transformée de Fourier couplé à un ordinateur. Les performances de ces trois appareils sont examinées (sensibilité, seuil

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de sensibilité et précision). Nous proposons une méthode générale de calibration pour des appareils semblables, mesurant la perfusion du sang. L'appareil analogique se montra être le meilleur marché, mais l'appareil digital le plus performant.

Key words: Diode-laser; Laser-Doppler; Velocimetry; Anemometry; LDA; Blood perfusion; Microcirculation; Blood flow; Blood velocity; Signal processing

1. Introduction

In this paper we present two different signal-processing methods to derive the blood perfusion measured with a laser-Doppler perfusion meter. The instruments are compared with a third method: direct Fourier transform using a frequency spectrum analyzer, coupled to a computer. The performance of the different instruments such as accuracy, sensitivity and limit sensitivity will be discussed, and we will show results from measurements on skin tissue and from model experiments using a signal generator and a laser-Doppler signal simulator. The accuracy is defined here as the maximum relative deviation from linearity, the sensitivity is defined as the output per unit of input signal and the limit sensitivity is defined as the smallest measurable signal with given accuracy.

A performance test procedure for a sensitivity calibration for a laser-Doppler signal processing unit is introduced. This sensitivity calibration enables a more objective comparison of measurements performed with different blood flow meters.

Laser-Doppler velocimetry provides a method for non-invasive measurement of the perfusion of tissue. The tissue is illuminated with a monochromatic light source and back-scattered light from the tissue is collected at a detector at a nearby site (see Fig. 1). Due to interaction with moving red blood cells, some photons may be frequency-shifted which causes the intensity of the detector to fluctuate because of interference. These fluctuations provide the information from which a measure for the perfusion can be derived [1, 10]. In the spectral region of interest the intensity fluctuations approximately show an exponential decay.

For the analysis of perfusion data the moments M_n and the weighted or normalized moments $M_{w,n}$ of the spectral power density $S(\omega)$ of the frequency distribution of intensity fluctuations due

to the Doppler effect are important. In the following we will use the notation:

$$M_n(a, b) = \int_a^b \omega^n S(\omega) d\omega; \quad (1)$$

$$M_{n,w}(a, b) = M_n(a, b) / M_0(a, b),$$

with a and b expressed in Hz.

Bonner and Nossal [1] showed that the first moment F is linearly proportional to the root-mean-square velocity of moving red blood cells:

$$F = M_1(0, \infty) \sim \sqrt{\langle v^2 \rangle}. \quad (2)$$

They also showed that the first moment F is linearly proportional to the concentration of red blood cells provided this concentration is small (negligible multiple scattering), which is the case for tissue perfusion (red blood cells ≈ 0.025 vol%) [1]. In practice this first moment F is assumed to be linearly proportional to the perfusion defined as the average blood cell concentration time the average cell velocity.

Since the total signal power is also linearly proportional to the concentration of red blood cells, the weighted moment F_w , defined by

$$F_w = M_{1,w}(0, \infty), \quad (3)$$

can be shown to be linearly proportional to the average cell velocity $\langle v \rangle$. By calculating both the weighted and the unweighted first moment from measurements, three relative parameters can be derived: the average perfusion, the average red blood cell concentration and the average cell velocity [2], respectively denoted by $F = M_1$, $D = M_0$ and $R = M_{1,w} = F/D$.

The results of laser-Doppler flow measurements presented in literature are almost always expressed in Volts. These measurements normally refer to the unweighted first moment (clinically often referred to as: 'flow') [1] or the unweighted first moment

Fig. 1. Setup of the optical probe, placed on typical skin tissue. In the jacket a laser diode and two photodiodes are included. Light scattered at moving blood cells and at scatterers at rest is mixed at the detectors. For temperature stabilization of the laser a Peltier element can be included. Note the different dimensions used for the probe and skin tissue.

corrected for multiple scattering and homodyne mixing introduced by Nilsson et al. [5]. In addition, arbitrary units (the so-called perfusion units) are used [9, 11]. Gush et al. [3] presented a signal processing method calibrated in Hz using a time-correlation technique. With other measurements on human skin with a TSI laser-Doppler flow meter model BPM403 [8] the function F (blood flow) was calibrated in $\text{ml min}^{-1}/100 \text{ g}$, D (blood volume) is erythrocytes/ $\text{mm}^3 \times 1.2 \times 10^4$ and R (blood velocity) in Hz. Although the absolute calibration of the numerator F and the denominator D is perhaps somewhat arbitrary, the accurate calibration of the fraction R gives the possibility of comparison with other blood flow meters.

The signal processing instruments under investigation calculate one or more of the frequency moments mentioned above. The first instrument, an analogous device, provides a real-time measurement of the first weighted moment integrated over the frequency region of practical relevance. For

tissue perfusion measurements this region ranges from 30 Hz to 30 kHz.

In the second instrument, a digital device, signal processing is performed by a correlation technique which provides signals proportional to the square of the average perfusion, the average red blood cell concentration and the average cell velocity. The duty cycle of the measurements ($\approx 20 \text{ ms}$) is short enough to provide approximately real-time perfusion measurements. This duty cycle is dependent upon the type of microprocessor used.

A third instrument, used for calibration, is a HP 3561A spectrum analyzer connected to a Personal Computer with an IEEE-488 interface. The spectral amplitude $A(\omega)$ is measured with the spectrum analyzer and sent to the computer by which the moments are calculated. This instrument is able to calculate only one measurement every two or three seconds in a limited frequency range. This is mainly caused by the communication time (handshaking) needed.

In Section 2 the instruments will be discussed. Section 3 contains calibration measurements and blood perfusion measurements of tissue. In Sections 4 and 5, discussion and conclusions are given.

2. Description of the instruments

2.1. The optical probe

During blood flow measurements and preceding calibration tests the same optical measuring probe was used for the three analyzing instruments. The probe we used here consists of a 780 nm, 5 mW diode laser which is temperature-stabilized with a Peltier element. Two photo diodes are present with current-to-voltage converters. The photo diodes collect the light from two similar areas of the sample. This light contains contributions from Doppler-shifted scattering at moving blood cells and non-Doppler-shifted scattering at structures at rest, mixed at the detectors. The signals from the diodes are fed into the analogous and digital signal processors, and also into the computer setup through a differential amplifier. A sketch of the optical probe is given in Fig. 1.

2.2. The analogous instrument

The analogous signal processor calculates the moments F_a and F_{wa} ('a' stands for 'analogous'):

$$\begin{aligned} F_a &= M_1(30, 30 \text{ k}) \text{ and} \\ F_{wa} &= M_1(30, 30 \text{ k})/M_0(30, 30 \text{ k}). \end{aligned} \quad (4)$$

In order to prevent an influence of the photoplethysmographic effect on the signal (which normally will not exceed 30 Hz [11]), the lower frequency boundary was chosen to be 30 Hz. According to the Nyquist theorem the cut-off frequency of 30 Hz allows the analysis of signals with a period of approximately 0.1 s, so well below the heart-beat frequency. The upper frequency boundary was chosen to be 30 kHz since tissue perfusion normally does not contribute to signals above this frequency.

It can be shown from measurements at the physical and biological zero-signal level that the noise is

white for the frequency region of interest and beyond, up to 100 kHz. Therefore, an averaged noise level is obtained from measurements at an adjacent frequency region (e.g. from 30 to 60 kHz), where no signal is present, and subsequently subtracted from the Doppler signal. The noise-corrected weighted first moment F_{wac} is given by

$$F_{wac} = \frac{M_1(30, 30 \text{ k}) - M_1^n(30, 30 \text{ k})}{M_0(30, 30 \text{ k}) - M_0^n(30, 30 \text{ k})}, \quad (5)$$

where the superscript n indicates that in Eq. (1) $S(\omega)$ has to be replaced by $S^n(\omega)$ obtained from the noise measurement, as indicated above.

The block diagram of the analogous instrument is given in Fig. 2. From the input signal $x(t)$:

$$x(t) = \int_0^\infty A(\omega) e^{i2\pi\omega t} d\omega, \quad (6)$$

where $A(\omega)$ stands for the spectral amplitude, related to $S(\omega)$ by $S(\omega) = |A(\omega)|^2$, the following output signals are derived:

$$\begin{aligned} F_a &= \bar{M}_1(30, 30 \text{ k}); & D_a &= \bar{M}_0(30, 30 \text{ k}); \\ N_a &= \bar{M}_0(30 \text{ k}, 40 \text{ k}); & R_a &= F_a/D_a, \end{aligned} \quad (7)$$

or (optionally) the noise-corrected functions, according to Eq. (5). Here the bar on top of symbol M denotes the replacement of $S(\omega)$ in Eq. (1) with $\langle S(\omega) \rangle_\tau$. The symbol τ stands for the time constant for averaging. In the present instrument τ can be varied from 0.1 to 4 s. So on one hand details of the heart beat can be followed and on the other an averaging over several heart beats can be obtained. The integrations are performed electronically using a $\sqrt{\omega}$ -multiplication filter (for F_a), RMS-to-DC converters and voltage squarers. The signal D_a equals the denominator in Eq. (4). The signal N_a is used to correct F_a and D_a from noise contributions. This is allowed in case white noise is present, which can be verified using a 'black' sample or with a tissue sample under physical-zero condition. The ratio of the noise-corrected signals F_a and D_a (see Fig. 2) is equal to the first weighted moment F_{wac} (Eq. (5)).

The proper noise-correction factors β and γ (representing the amplification factors of the operational amplifiers in the noise correction part, see

Fig. 2. Block diagram of the analogous signal processor. OP, optical probe; DA, differential amplifier; SA, summing amplifier; AMP, amplifier; TB, total backscattered light; BPF, band pass filter (upper 30 Hz–30 kHz, lower 30–60 kHz (analogous instrument), lower 30–40 kHz (digital instrument)); SQ, square-root filter; RD, RMS-to-DC converter; SCA, scaling amplifier; VS, voltage squarer; FA, output signal F_a ; RA, output signal R_a .

Fig. 2) have to be checked experimentally by placing the probe head on a stationary object and adjusting the output to zero. In case of white noise these values are 1 and $\frac{1}{3}$ for the 0th and the 1st moment, respectively, assuming the frequency limits mentioned in Eq. (7).

The signals collected by the two photodiodes in the optical probe are fed into a differential amplifier. After subtraction the resulting signal is almost free from disturbances due to external light sources. The two signals from the probe are also fed into a summing amplifier. With this summing amplifier we can measure the total amount of diffuse backscattered light that reaches the two photodiodes.

The output signal of the differential amplifier feeds two fourth-order bandpass filters. The first filter selects the Doppler band from 30 Hz to 30 kHz. The second filter filters the so-called noise band from 30 to (actually) 40 kHz. After filtering the Doppler band, the resulting spectrum is frequency-weighted by a $\sqrt{\omega}$ -filter. This means that the transfer function of this filter has a constant slope of -3 dB/octave within the Doppler band.

The RMS value of the resulting spectrum is calculated using a RMS-to-DC converter. The averaging time constant of the converter is switch-selectable. The signal derived in this way is squared by an analogous multiplier and the result after

additional amplification is the unweighted first moment F_a as defined in Eq. (4).

To obtain the weighted first moment, a second RMS-to-DC converter is connected to the output of the Doppler filter and the resulting signal is squared by an analogous multiplier. After scaling by an additional amplifier the signal is used as the denominator of Eq. (4). The unweighted first moment is the numerator of Eq. (4). The two signals are then divided with an analogous divider and the result is the weighted first moment F_{wa} as defined in Eq. (4). The noise spectrum in the band of 30 to 40 kHz is treated in the same way as above. Eventually the analogous instrument yields three noise-corrected output signals. The first output signal is the corrected signal F_a and is assumed to be proportional to the average blood flow. The second is the corrected signal D_a , assumed to be proportional to the average blood cell concentration. The third output signal is the corrected signal F_a divided by the corrected signal D_a , proportional to the average cell velocity. We will refer to this ratio as R_a .

2.3. The digital instrument

The digital signal processing is based on a correlation algorithm in the time domain, enabling the calculation of the second moment $F^{(2)}$ of the

spectral power density $S(\omega)$, defined as

$$F^{(2)} = M_2(0, \infty). \quad (8)$$

If the input signal is exponential (which approximately is the case for blood perfusion measurements) the following relation between the first moment F and the second moment $F^{(2)}$ holds exactly:

$$F = \sqrt{(F^{(2)}/2)}. \quad (9)$$

Then the average root-mean-square velocity of moving red blood cells is linearly proportional to the square root of the weighted second moment $F^{(2)}$. The noise-corrected weighted second moment $F_{\text{wdc}}^{(2)}$ ('d' stands for digital) for the digital instrument is defined as

$$F_{\text{wdc}}^{(2)} = \frac{M_2(30, 30 \text{ k}) - M_2^n(30, 30 \text{ k})}{M_0(30, 30 \text{ k}) - M_0^n(30, 30 \text{ k})}. \quad (10)$$

In Appendix A it is shown that this function can be calculated from the measurements using

$$F_{\text{wdc}}^{(2)}(i) = \frac{R_{yy}(0, i) - \varepsilon R_{nn}(0, i)}{R_{xx}(0, i) - \delta R_{nn}(0, i)}, \quad (11)$$

where R_{xx} , R_{yy} and $R_{nn}(0, i)$ are the digitized zero-time autocorrelation functions of the signal $x(t)$, its time derivative $y(t)$ and the noise contribution $n(t)$ at time interval i . The constants ε and δ are related to the way of calculation of the noise correction, and turn out to be equal to 3 and 0.74, respectively for the present setup.

The block diagram of the digital instrument is given in Fig. 3. The two signals from the optical probe are fed into an AC-coupled instrumentation amplifier with a -3 dB cutoff at 3 Hz. The voltage gain is 100x. With these settings the output voltage of the amplifier has an amplitude of about 200 mV. Because of the use of two photodiodes in subtraction mode, in practice the output of the instrumentation amplifier contains Doppler information only. Laser noise and signals caused by external light sources such as room lamps (50 or 60 Hz and their higher harmonics) are mainly eliminated.

This signal is fed into the same type of Doppler and noise filters are used in the analogous instrument. The only difference is that the noise-bandpass filter has its -3 dB cutoff's at, respectively, 30 and 40 kHz. This noise filter is followed

by an additional amplifier, because the noise amplitude is much smaller than the Doppler signal amplitude. In this way the dynamic range of the analog-to-digital converter for the noise signal will be used optimally. The input of each filter can be selected by a processor-selectable analogous switch.

After passing the analogous switch the signal is coupled into the input of two cascaded processor-controlled programmable amplifiers (PRAMs), enabling the selection of gain factors of 2^n with $0 \leq n \leq 7$. The PRAMs are included to prevent the bipolar input signal to exceed its limiting amplitude of 2.5 V, and are set such that the dynamic range of the ADCs is optimally used. The gain is automatically adjusted every 20 ms. The effect of amplification is corrected for afterwards, at the end of the processing. The signal resulting after amplification is fed into a 12-bit DAC for further processing.

The heart of the digital processing is the TI 32025 digital signal processor (DSP). In the present instrument this 16-bit processor contains 8 kbytes of ROM, 2 kbytes of fast Data-RAM and 2 kbytes of program-RAM. The processor communicates via the system bus with the processor/device interface board which controls the PRAMs with monitoring electronics and the analogous switch which selects between Doppler and noise band. Additionally the system bus is the communication path to the processor control panel board (the PCP). Using this PCP, instructions about the mode of operation are entered to the processor.

Four output signals are available:

- The numerator of the second weighted moment, considered to be linearly proportional to the square average blood flow (F_d);
- The denominator of the second weighted moment, linearly proportional to the average blood cell concentration (D_d);
- The square root of the second weighted moment itself (numerator divided by the denominator), considered to be linearly proportional to the average blood cell velocity (R_d);
- The total intensity of back-scattered light (Doppler shifted and non-Doppler shifted light). This 'total reflection' signal is of no further interest for this paper.

The calculated signals are available at the outputs of four digital-to-analog converters. These DACs are

Fig. 3. Block diagram of the digital signal processor (see also Fig. 2) AS, analog switch; PR, programmable amplifier; ADC, analog–digital converter; MC, monitor circuit for PR; DAC, digital–analog converter; PDI, processor device interface; PCP, processor control panel; DSP, digital signal processor; PRAM, program RAM; DRAM, data RAM; PROM, program ROM.

connected to the system bus. With the present instrument the signals from the DACs are connected to a four-channel pen recorder for parallel on-line recording.

The measurements in this work refer to the digital instrument as a stand-alone unit. As a further development, this device was implemented on a computer card, to be inserted into a free slot in a personal computer.

2.4. The computer setup

The computer setup, used for calibration, consists of a Hewlett–Packard 3561A spectrum analyzer coupled to a personal computer with a National Instruments IEEE-488 interface. The computer is equipped with a numerical coprocessor. The spectrum analyzer samples the input signal x during a period T , taking P samples. A discrete spectral amplitude density $X(n\Delta\omega)$ is calculated using

$$X(n\Delta\omega) = \frac{T}{P} \sum_{k=0}^{P-1} x(k\Delta t) e^{-jkn2\pi/P}, \quad (12)$$

where $0 \leq n \leq P$, $\Delta\omega = 2\pi/T$, $\Delta t = T/P$ and $j^2 = -1$.

This discrete spectral amplitude density $X(n\Delta\omega)$ is transferred into the computer where the discrete spectral power density $S(n\Delta\omega)$ is calculated according to

$$S(n\Delta\omega) = X^2(n\Delta\omega). \quad (13)$$

The spectrum analyzer has two settings for the span and the resolution, of interest for the present: 10 kHz span with 25 Hz resolution, and 12.5 kHz span with 31.25 Hz resolution, respectively. These spans are comparable to the spans used by the analogous and digital instrument. It was not necessary to select a larger frequency range, more equal to those of the analogous and digital instrument, since the signals used in the calibration did not exceed 12.5 kHz. The quantization errors in the weighted and unweighted first moments of the spectral power density are smaller than 0.2% for a 10 kHz span and smaller than 0.3% for a 12.5 kHz span.

The spectrum analyzer renders also the possibility of averaging the spectral amplitude density $X(n\Delta\omega)$ several times before transferring into the computer.

Noise can be subtracted by the spectrum analyzer if the noise can be measured separately from the signal. The noise is stored in a buffer which is subtracted from the spectral amplitude density $X(n\Delta\omega)$ after transfer into the computer.

The time necessary for one calculation of the weighted and unweighted first moment of the spectral power density depends on the number of averages. Without averaging one calculation takes about 3 s (primarily due to the handshaking between computer and analyzer). Although no real-time tissue measurements can be done with this setup, the high accuracy gives the possibility of gauging the analogous and digital instrument under static conditions. Short-term variations (as can be found in the exponential noise source) can be eliminated by averaging, in order to maintain static condition.

3. Performance tests

3.1. Introduction

Both the analogous and digital signal processors were calibrated (Fig. 4) with the analyzer + computer setup using two kinds of input signals:

- a sine function with a constant amplitude and a variable frequency (signal generator Advance Instruments);
- a noise source with a fixed exponential decay in the spectral domain (a fixed -3 dB frequency), but with a variable amplitude.

The noise source (a 'laser-Doppler flow-meter signal simulator') consists of a white-noise source (a constant signal in the frequency domain) coupled to

a series of first-order band filters, leading to the signal

$$N(t) = A_n \exp(-f/f_0), \quad (14)$$

with A_n the amplitude at $f = 0$ and f_0 the -3 dB frequency. The exponential behavior is reproducible after averaging: less than 1% amplitude variation after 100 times root-mean-square averaging. This is done since the characteristics of noise can only be determined after averaging. Two such noise sources were used, with -3 dB points at $f_0 = 3.0$ and 6.0 kHz, respectively. In Table 1 the dependence of the relevant moments upon the amplitude or the frequency of the two kinds of sources is summarized.

The unit of all output signals of both the analogous and digital instrument is Volt. The amplitude $A(\omega)$ measured with the spectrum analyzer is also given in Volt. This determines the dimensions of the relevant frequency moments and of the sensitivity (defined as the output per unit of input signal) of the moments. The dimensions are mentioned in Tables 2 and 3, together with the measured values (to be dealt with in the following sections).

To calculate the limit sensitivity (or the minimal detectable signal), the smallest acceptable amplitude was determined with the sinusoidal input signal for both the analogous and digital instrument and verified with the computer setup for several frequencies.

The characteristic time constant τ during measurements was 4 s for the analogous instrument and 5 s for the digital instrument.

Fig. 4. Block diagram of the performance test setup during measurements.

Table 1

Dependence of the output signals with respect to the performance test input signals

Source	Sinusoidal	Exponential
Variable	Frequency	Amplitude
Fixed	Amplitude	Frequency
F_a	$\sim f_0$	$\sim A_n^2$
D_a	constant	$\sim A_n^2$
R_a	$\sim f_0$	constant
F_d	$\sim f_0^2$	$\sim A_n^2$
D_d	constant	$\sim A_n^2$
R_d	$\sim f_0$	constant

Table 2
Sensitivity and accuracy of F_a , D_a and R_a of the analogous instrument

	Range	Sensitivity	Accuracy
F_a	0–120 V ² Hz ²	$(4.09 \pm 0.05) \times 10^{-3} \text{ V}^{-1} \text{ Hz}^{-2}$	$\pm 1.2\%$
D_a	0–0.03 V ² Hz	$(2.66 \pm 0.04) \text{ V}^{-1} \text{ Hz}^{-1}$	$\pm 0.8\%$
R_a	0–3 kHz	$(1.42 \pm 0.02) \times 10^{-3} \text{ Hz}^{-1}$	$\pm 1.4\%$

Table 3
Sensitivity and accuracy of F_d , D_d and R_d of the digital instrument. With R_d two different measurements are shown

	Range	Sensitivity	Accuracy
F_d	0–350 $\times 10^6 \text{ V}^2 \text{ Hz}^3$	$(2.02 \pm 0.02) \times 10^{-8} \text{ V}^{-1} \text{ Hz}^{-3}$	$\pm 1\%$
D_d	0–0.15 V ² Hz	$(52.7 \pm 0.5) \text{ V}^{-1} \text{ Hz}^{-1}$	$\pm 0.9\%$
R_d	2–8 kHz	$(8.56 \pm 0.03) \times 10^{-4} \text{ Hz}^{-1}$	$\pm 0.4\%$

Since we use well-defined input signals for calibration, free of noise such as shot noise and dark-current noise from an optical probe, in the present experiments noise subtraction was not necessary and was not used.

3.2. The analogous instrument

Measurements with the analogous instrument are shown in Figs. 5–7. The measurements of F_a and R_a were performed with the sinusoidal input signal (variable frequency) and the measurement of D_a is done with the exponential noise input signal (variable amplitude). Of course absolute values of measurements depend on chosen settings of the amplifiers of the output signals. Therefore the sensitivity depends on an amplification factor f (here $f = 1$) and the accuracy is given in percentages.

The results of the measurements with the analogous instrument are listed in Table 2. The limit sensitivity of F_a turns out to be linearly dependent upon the frequency: $1.0 \times 10^{-3} \text{ V}^2 \text{ Hz}^2 / \text{Hz}$. The limit sensitivity of R_a is frequency independent: $1.0 \times 10^{-3} \text{ V}^2 \text{ Hz}$.

In Fig. 8 a typical measurement of the blood perfusion of skin tissue [4], performed with the analogous instrument, is shown. The optical probe was attached to the third finger of a healthy person. The characteristic time constant τ is equal to 0.1 s. The

Calibration numerator analog setup.

Fig. 5. The F_a output signal of the analogous instrument in V as a function of the numerator F output signal of the computer setup in $\text{V}^2 \text{ Hz}^2$ (measured with the signal generator).

pulsation with a period of approximately 1 s in the non-occluded part of the recording is caused by the heart beat. The fluctuations in F_a with a time scale of about 10 s are due to the inhalation of air (indicated in the figure) followed by holding the breath for a moment, to an occlusion and to vasomotion (unvoluntary motions of the veins), respectively.

In general the output signals F_a , D_a and R_a can be multiplied with an electronic amplification factor f . The amplification factor f is not necessarily the same for F_a , D_a and R_a (similar for the digital instrument: F_d , D_d and R_d) and is variable (although

Fig. 6. The D_a output signal of the analogous instrument in V as a function of the denominator D output signal of the computer setup in $V^2\text{Hz}$ (measured with the exponential noisesource).

Fig. 7. The R_a output signal of the analogous instrument in V as a function of the fraction R output signal of the computer setup in Hz (measured with the signal generator).

$f = 1$ holds for the settings regarding the results presented in this paper).

3.3. The digital instrument

Measurements with the digital instrument are shown in Figs. 9–11. The results of the measurements are listed in Table 3. Although the sensitivity of F_d and D_d also depends on chosen settings of the output amplifiers (amplification factor f), R_d is independent of those settings. This gives the

Fig. 8. Recordings of F_a (nominator, proportional to the flow; upper panel), D_a (denominator, proportional to the concentration; middle panel) and R_a (fraction, proportional to the averaged velocity; lower panel) as a function of time, processed with the analogous instrument. In F_a and R_a the heart beat can be seen. The fast fluctuations in D_a are analyzed to be noise. Slower fluctuations (time scale 10 s) are due to breath inhalation (indicated by 'In'), arterial occlusion of the upper arm (indicated by 'Oc'), or vasomotion (unvoluntary motions of the veins). With an occlusion D_a drops to almost zero, and therefore R_a becomes out of range. Further explanation can be found in the text.

Fig. 9. The F_d output signal of the digital instrument in V as a function of the square of the numerator F output signal of the computer setup in $V^4\text{Hz}^6$ (measured with the signal generator).

opportunity of gauging R_d (the second weighted moment) absolutely in V per Hz.

For the ranges mentioned in Table 3, the limit sensitivities for F_d (given per Hz; i.e. linearly

Calibration denominator digital setup.

Fig. 10. The D_d output signal of the digital instrument in V as a function of the denominator D output signal of the computer setup in $V^2 \text{ Hz}$ (measured with the exponential noise source).

Calibration fraction digital setup.

Fig. 11. The R_d output signal of the digital instrument in V as a function of the fraction R output signal of the computer setup in Hz (measured with the signal generator).

dependent upon the frequency) and D_d of the digital instrument are $1.6 \times 10^{-3} V^2 \text{ Hz}^3 / \text{Hz}$ and $1.6 \times 10^{-3} V^2 \text{ Hz}$, respectively.

In Fig. 12 a measurement of blood flow in skin tissue is shown [4]. The measurement was performed similar to the measurement of the analogous instrument. The characteristics time constant τ is equal to 0.5 s. The variations in F_d are similar to those of the variations in F_a of the analogous instrument. Note that D_d which is a measure for the number of red blood cells does not give an accurate

Fig. 12. Recordings of F_d (nominator; upper panel), D_d (denominator; middle panel) and R_d (fraction, lower panel) as a function of time, processed with the digital instrument. A similar response as with the analogous instrument (Fig. 8) can be seen. For further explanation see the text.

number during an occlusion. This is due to the fact that during an occlusion no moving particles are present in the probe volume and therefore D_d and R_d are of no use in this range. F_d probably still gives a usable signal in this range for clinical use (i.e. the absence of flow).

4. Discussion

When we compare the performance of the analogous and digital instrument, the digital instrument is to be preferred. The digital instrument is more accurate and has a larger dynamic range. The larger dynamic range is caused by the automatic optimization of the amplification of the input signal. The limit sensitivity of the analogous instrument is comparable to that of the digital instrument but due to the automatic amplification of the input signal the digital instrument is very user-friendly. In addition to this the amplification factor f of the output signals in the digital instrument is limited to a known number of settings of the DACs, whereas the amplification factor f in the analogous instrument is set by potentiometers and its value is difficult to verify.

The only advantage of the analogous instrument is a lower production price due to cheaper components. Remarkable, however, is the offset in R_d of the digital instrument below 2 kHz (see Fig. 11)

which limits the range of maximum accuracy. Although it is caused by an offset in F_d , it is more easily noticed in R_d due to the way of calculation. The error probably occurs in the calculations of the time derivative $y = dx/dt$ (see Eq. (A.12)) and requires further research.

When the results have to meet very high quality demands, a similar setup as our computer setup with a spectrum analyzer is necessary. Although no data are given on sensitivity, accuracy and limit sensitivity of this setup, these are probably only limited by the errors due to quantization of the input signal. These errors are estimated to be always smaller than 0.3%. A disadvantage, however, is the long time needed for one measurement and the high costs for such a setup.

Finally when we look upon the units of the numerator F , the denominator D and the fraction R of both the first and second weighted moment of the computer it is obvious that these units do not refer to the physical units of the first and second moment. We introduced these units only to describe correct signal transfer functions from the Doppler input signal to the signal processor output signals.

5. Conclusions

In this paper we presented different signal processing setups for laser-Doppler perfusion meters: an instrument based on analogous electronics, an instrument based on digital electronics and a setup consisting of a HP3561A spectrum analyzer coupled with a National Instruments IEEE-488 interface to a personal computer. The sensitivity, limit sensitivity and accuracy of these setups are determined and measurements with all three setups are presented. The results are presented in this paper. For each setup the advantages and disadvantages are discussed. In short the analogous instrument is the cheapest but has a very small dynamic input signal range, the digital instrument has a good performance and is user-friendly and the computer setup is the most accurate but very expensive and does not measure in real time. As a further development, the algorithm of the digital instrument was implemented on a processor card to be inserted into a free slot of a personal computer.

We proposed a performance test procedure for laser-Doppler flow-meter signal processors in order to make several processors and measurements comparable.

According to this procedure the output of signal processors should be calibrated in $V/V^2 \text{ Hz}^2$ for the numerator F , $V/V^2 \text{ Hz}$ for the denominator D and V/Hz for the fraction R of the weighted first moment of the spectral power density $S(\omega)$. Calibration for the biological relevant blood flow, blood volume and velocity is likely to be very dependent on the specific construction of the optical probe, the actual size and optical parameters of the tissue measuring volume and parameters such as temperature and degree of humidity and should therefore for every new measurement to related to the standard calibration we presented above. We have to note that comparison of signals measured with instrumentation based on processing the first moment of the spectral power density of the Doppler signal to signals measured with instrumentation based on processing the second moment of the spectral power density of the Doppler signal, is only possible according to Eq. (9), if the signal shows an exponential decay in the spectral region. Since in literature always exponential signals are reported both in theory and in measurements [1, 6] the assumption of exponential signals seems justifiable.

Appendix A

For the derivation of Eq. (11) we define the signal functions $x(t)$ and $\phi(\omega)$ as a Fourier pair,

$$x(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \phi(\omega) e^{i\omega t} d\omega. \quad (\text{A.1})$$

According to the Wiener-Khinchine theorem the autocorrelation function $R_{xx}(\tau)$ of $x(t)$, defined as

$$\begin{aligned} R_{xx}(\tau) &= \langle x(t)x(t+\tau) \rangle \\ &= \lim_{T \rightarrow \infty} \frac{1}{T} \int_{-T/2}^{T/2} x(t)x(t+\tau) dt, \end{aligned} \quad (\text{A.2})$$

and the power spectrum $S(\omega) = |\phi(\omega)|^2 = \phi^*(\omega)\phi(\omega)$ are a Fourier pair also. Then one can write

$$R_{xx}(0) = \frac{1}{2\pi} \int_{-\infty}^{\infty} S(\omega) d\omega. \quad (\text{A.3})$$

Using a discrete input signal $x(t)$ the autocorrelation function at zero time is given by

$$R_{xx}(0) = \frac{1}{N} \sum_{i=0}^N x^2(t_i), \quad (\text{A.4})$$

where $x(t_i)$ is the signal strength of sample i of N samples in a single measurement.

Since a multiplication of a harmonic frequency function with ω corresponds with a differentiation in the time domain, one can write

$$R_{yy}(0) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \omega^2 S(\omega) d\omega, \quad (\text{A.5})$$

in which the signal $y(t)$ is obtained by differentiating $x(t)$: $y = dx/dt$. Using a similar expression for the noise contribution $S_n(\omega)$ to $S(\omega)$, it follows:

$$F_{\text{wdc}}^{(2)} = \frac{R_{yy}(0) - kR_{nn}(0)}{R_{xx}(0) - kR_{nn}(0)}, \quad (\text{A.6})$$

where k stands for the constant ratio between the noise terms in the numerator and in the denominator. The numerical value of k can be derived by evaluating the noise terms in Eq. (10). A uniform white-noise distribution in the frequency domain of interest ($S_n(\omega) = C = \text{constant}$), leading to

$$\int_{30 \text{ Hz}}^{30 \text{ kHz}} \omega^2 C d\omega = k \int_{30 \text{ Hz}}^{30 \text{ kHz}} C d\omega, \quad (\text{A.7})$$

yields $k = 3 \times 10^8$. Eq. (A.6) can be evaluated using autocorrelation techniques in the time domain. For real-time measurements, Eq. (A.4) is not applicable. Then the autocorrelation function can be approximated using moving-average techniques [7]:

$$R_{xx}(0, i) = (1 - \alpha)^N R_{xx}(0, i - N) + \sum_{k=0}^{N-1} \alpha(1 - \alpha)^k x(t_{i-k}). \quad (\text{A.8})$$

Taking only the first-order term in Eq. (A.8) yields

$$R_{xx}(0, i) = (1 - \alpha)R_{xx}(0, i - 1) + \alpha x^2(t_i), \quad (\text{A.9})$$

where α denotes the moving time-average factor over a characteristic time length τ and is given by

$$\tau = N/(\alpha f_s), \quad (\text{A.10})$$

where f_s stands for the repetition rate of the AD converter used for the input signal x . The relative error ε_r in Eq. (A.9) is given by

$$\varepsilon_r < \frac{N\alpha^2}{\alpha(1 - N\alpha)} \approx N\alpha. \quad (\text{A.11})$$

In the present instrument τ -values in the range 0.1–5 s were used ($N = 64$). The choice of α and N is a compromise. A rather large value of τ is desirable and can be obtained by introducing a large N and a small α . However, if N is chosen too large it would result in a large sample time and if α is chosen too small it would result in large numerical errors in the calculations. The choice of α is discussed at the end of this section.

The algorithm to obtain the value of $y(t_i)$ from $x(t_i)$ is given by

$$y(t_i) = -a_0 y(t_{i-1}) + a_1 [x(t_i) - x(t_{i-1})], \quad (\text{A.12})$$

where a_0 and a_1 are constants. The values for a_0 and a_1 can be calculated using the theory for discrete differentiation in z -domain representation. In the z domain the transfer function $H(z)$ for a differentiation can be written as

$$H(z) = \frac{a_1(1 - 1/z)}{1 + a_0/z}, \quad (\text{A.13})$$

where

$$a_0 = \frac{\pi f_{3 \text{ dB}} - f_s}{\pi f_{3 \text{ dB}} + f_s} \quad \text{and} \quad a_1 = \frac{2\pi f_{3 \text{ dB}}}{\pi f_{3 \text{ dB}} + f_s}. \quad (\text{A.14})$$

For a value of the cut-off frequency $f_{3 \text{ dB}}$ slightly above the upper limit of the frequency range (40.5 kHz) and with $f_s = 100$ kHz the constants a_0 and a_1 amount to 0.1199 and 1.1199, respectively. Then a magnitude of $y(t_i)$ which is a good approximation of $dx(t)/dt$ in the frequency range 30 Hz–30 kHz can be obtained (see [7]).

Using Eq. (A.8) we can now write

$$\begin{aligned} R_{xx}(0, i) &= (1 - \alpha)R_{xx}(0, i - 1) + \alpha x^2(t_i), \\ R_{yy}(0, i) &= (1 - \alpha)R_{yy}(0, i - 1) + \alpha y^2(t_i), \\ R_{nn}(0, i) &= (1 - \alpha)R_{nn}(0, i - 1) + \alpha n^2(t_i). \end{aligned} \quad (\text{A.15})$$

When the input signal $x(t)$ is measured in the 30 Hz–30 kHz interval and the noise signal $n(t_i)$ in the 30–40 kHz interval we can write for Eq. (A.6):

$$F_{\text{wdc}}^{(2)}(i) = \frac{R_{yy}(0, i) - \varepsilon R_{nn}(0, i)}{R_{xx}(0, i) - \delta R_{nn}(0, i)}, \quad (\text{A.16}) = (11)$$

where δ and ε correct for the different frequency intervals for the signal and the noise. Using a similar assumption for the noise as for the calculation of k in Eq. (A.6) (uniform noise distribution), we can write for δ and ε :

$$\begin{aligned} \delta &= \int_{30}^{30 \text{ k}} C d\omega \bigg/ \int_{30 \text{ k}}^{40 \text{ k}} C d\omega; \\ \varepsilon &= \int_{30}^{30 \text{ k}} \omega^2 d\omega \bigg/ \int_{30 \text{ k}}^{40 \text{ k}} \omega^2 d\omega, \end{aligned} \quad (\text{A.17})$$

where C is a constant, yielding $\delta = 3$ and $\varepsilon = 0.74$.

In the present instrument the value of $F^{(2)}(i)$ is obtained every 20 ms by evaluating the signal during 15 ms:

$$\langle x^2(t_i) \rangle_\tau = (1 - \alpha) \langle x^2(t_{i-1}) \rangle_\tau + \alpha x^2(0), \quad (\text{A.18})$$

and the noise data during 3 ms. During the remaining 2 ms the necessary calculations are done. With the values of τ and α chosen (α in the range from 1.28×10^{-4} to 6.4×10^{-3}) the errors are smaller than 0.2% during operation compared to the autocorrelation function mentioned in Eq. (A.4) (see Eq. (A.11)). The error due to digitalization of the input signal x is smaller than 0.7% (calculated for an exponential spectrum $E(\omega) = A \exp(-0.0002 \cdot \omega)$ where A is the amplitude and ω is in Hz, which is a good approximation for the input signal x).

References

- [1] R.F. Bonner and R. Nossal, "Model for laser-Doppler measurements of blood flow in tissue", *Appl. Opt.*, Vol. 20, 1981, pp. 2097–2107.
- [2] F.F.M. De Mul, J. Van Spijker, D. Van der Plas, J. Greve, J.G. Aarnoudse and T.M. Smits, "Mini laser-Doppler (blood) flow monitor with diode laser source and detection integrated in the probe", *Appl. Opt.*, Vol. 23, 1984, pp. 2970–2973.
- [3] R.J. Gush, T.A. King and M.I.V. Jayson, "Aspects of laser light scattering from skin tissue with application to laser-Doppler blood flow measurements", *Phys. Med. Biol.*, Vol. 29, 1984, pp. 1463–1476.
- [4] H.W. Jentink, Laser Doppler velocimetry using diode lasers, Ph.D Thesis, Twente University of Technology, Enschede, 1989.
- [5] G.E. Nilsson, "Signal processor for laser-Doppler tissue flowmeters", *Med. Biol. Eng. Comput.*, Vol. 22, 1984, pp. 343–348.
- [6] G.E. Nilsson, T. Tenland and P.A. Öberg, "A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy", *IEEE Trans. Biom. Engrg.*, Vol. 27, 1980, pp. 12–19.
- [7] L.R. Rabiner and B. Gold, *Theory and Application of Digital Signal Processing*, Prentice-Hall, Englewood Cliffs, NJ, 1975.
- [8] M. Rendell, T. Bergman, G. O'Donnel, E. Drobny, J. Borgos and R.F. Bonner, "Microvascular blood flow, volume, and velocity measured by laser-Doppler techniques in IDDM", *Diabetes*, Vol. 38, 1989, pp. 819–824.
- [9] R. Ruth, D. Haina and W. Waidelich, "The determination of a blood-flow parameter by the Doppler method", *Optica Acta*, Vol. 31, 1984, pp. 759–766.
- [10] M.D. Stern, "In vivo evaluation of micro circulation by coherent light scattering", *Nature*, Vol. 254, 1975, pp. 56–58.
- [11] R.W. Wunderlich, R.L. Folger, D.B. Giddon and B.R. Ware, "Laser Doppler blood flow meter and optical plethysmograph", *Rev. Sci. Instrum.*, Vol. 51, 1980, pp. 1258–1262.