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Authors
Fantini, Sergio
Franceschini, Maria-Angela
Maier, John S
et al.

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Frequency-Domain Multi-Source Optical Spectrometer and Oximeter

Sergio Fantini*, Maria Angela Franceschini*, John S. Maier, Scott A. Walker, and Enrico Gratton

Laboratory for Fluorescence Dynamics,
Department of Physics, University of Illinois at Urbana-Champaign,
1110 West Green Street, Urbana, Illinois 61801-3080

*Also with: Isituto di Elettronica Quantistica - Consiglio Nazionale delle Ricerche
Via Panciatichi, 56/30, 50127 Firenze, Italy

ABSTRACT

We have designed and constructed a near-infrared spectrometer for the non-invasive optical study of biological tissue. This instrument works in the frequency-domain and employs multiple source-detector distances to recover the absorption coefficient (μ_a) and the reduced scattering coefficient (μ_s') of tissue. The light sources are eight light emitting diodes (LEDs) whose intensities are modulated at a frequency of 120 MHz. Four LEDs emit light at a peak wavelength of 715 nm (λ_1), while the other four LEDs emit at a peak wavelength of 850 nm (λ_2). From the frequency-domain raw data of phase, DC intensity, and AC amplitude obtained from each one of the eight light sources, which are located at different distances from the detector fiber, we calculate μ_a and μ_s' at the two wavelengths λ_1 and λ_2. The concentrations of oxy- and deoxy-hemoglobin, and hence hemoglobin saturation, are then derived from the known extinction coefficients of oxy- and deoxy-hemoglobin at λ_1 and λ_2. The statistical error in the measurement of the optical coefficients due to instrument noise is about 1-2%. The accuracy in the determination of the absolute value of the optical coefficients is within 10-20%. Preliminary results obtained *in vivo* on the forearm of a volunteer during an ischemia measurement protocol are presented.

1. INTRODUCTION AND BACKGROUND

In the study of biological tissue for medical diagnostic, monitoring, and therapeutical purposes, optical methods can achieve relevance if they provide a quantitative determination of the tissue optical properties. Specifically, the quantitative determination of the optical absorption coefficient (μ_a) at localized tissue sites enables one to detect and determine the concentration of specific endogenous (hemoglobin, cytochrome aa_3, melanin, bilirubin, etc.) or exogenous (drugs, chemical markers for tumors, photosensitizing agents for photodynamic therapy) chromophores. The relatively high penetration depth of near-infrared light in tissue renders near-infrared spectroscopy a potentially effective technique for the optical study of tissue. Near-infrared spectroscopy employs non-ionizing radiation, resulting in harmless and non-invasive measurement. In addition, this technique is cost effective, compatible with compact bedside instrumentation, and can provide real time information. On the other hand, the optical study of tissue in the near-infrared is complicated by the strong scattering experienced by photons in tissue. In these conditions, light intensity attenuation is caused by both absorption and scattering, and the average optical path length is unknown. It has been recently

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demonstrated that time resolved methods, either in the time-domain[1,2] or in the frequency-domain,[3-6] are able to separate the contribution of absorption and scattering to the intensity attenuation. Optical spectroscopy of turbid media, such as tissue, is thus feasible. A powerful application is the determination of hemoglobin saturation in tissue. By assuming that oxy- and deoxy-hemoglobin contribute more than other chromophores to the absorption of near-infrared light in tissue, one can obtain the concentrations of oxy- and deoxy-hemoglobin, and hemoglobin saturation from a measurement of μ_a at two distinct wavelengths.[7]

Working in the frequency-domain, we have employed light emitting diodes (LEDs) as light sources. We have previously shown the capability of LEDs of being intensity modulated in the 100 MHz frequency region, and we have pointed out the properties that render LEDs suitable devices for medical applications.[8] The theoretical background for light propagation in turbid media is provided by diffusion theory.[9] To model the non-invasive configuration where light sources and detector fiber are placed on the surface of the tissue, we apply semi-infinite medium boundary conditions.[2,10] Our instrument implements a multiple source-detector distances protocol, which is particularly effective in independently and simultaneously measuring the absolute values of μ_a (absorption coefficient) and μ_s' (reduced scattering coefficient) of strongly scattering media.[11,10]

2. DESCRIPTION OF THE SPECTROMETER

The block diagram of the frequency-domain tissue spectrometer is shown in Fig. 1. The general principle of operation is that of frequency-domain spectroscopy. The intensity of the light sources (located in the measuring head together with the detector optical fiber) is modulated at a frequency of 120 MHz, the time varying signal being provided by a frequency synthesizer (Marconi Instr. Model 2022A) through a radio frequency amplifier (ENI Model 403 LA). A second frequency synthesizer, synchronized with the first one, modulates the gain function of the photomultiplier tube (PMT) (Hamamatsu R928) at a frequency of (120 MHz + 400 Hz). The frequency mismatch of 400 Hz between the signal which modulates the light source's intensity and the signal which modulates the detector's gain function is the so-called cross-correlation frequency.

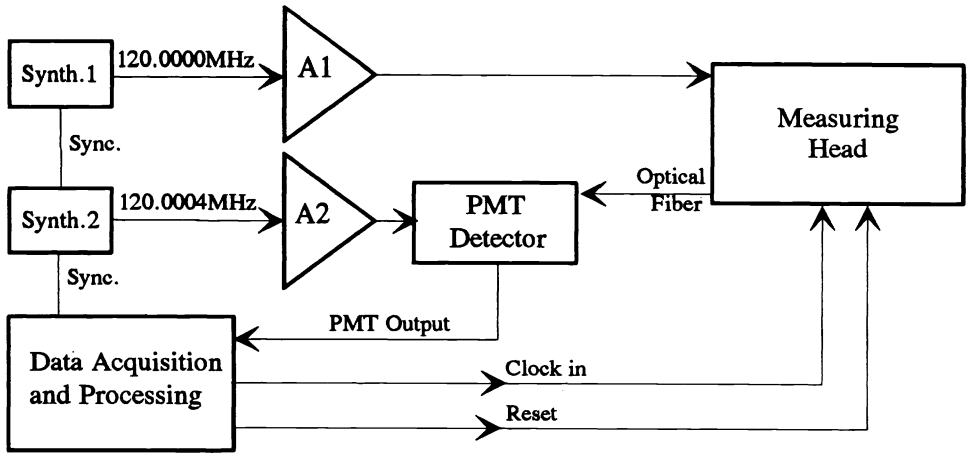

Fig. 1 Block Diagram of the frequency-domain tissue spectrometer and oximeter

The output signal of the PMT is the result of a beating between the detected signal at 120 MHz and the PMT's response function at (120 MHz + 400 Hz). Hence, the PMT output contains a strong frequency component at 400 Hz which carries all the information of average intensity (DC), amplitude of intensity oscillations (AC), and phase of the detected signal at 120 MHz. The 400 Hz component of the PMT output is digitally filtered and processed by the computer.

The measuring head of the tissue spectrometer contains the eight LEDs, the multiplexing electronics which drives them, and the detector optical fiber which collects light and delivers it to the PMT detector. Figure 2 shows the design of the measuring head.

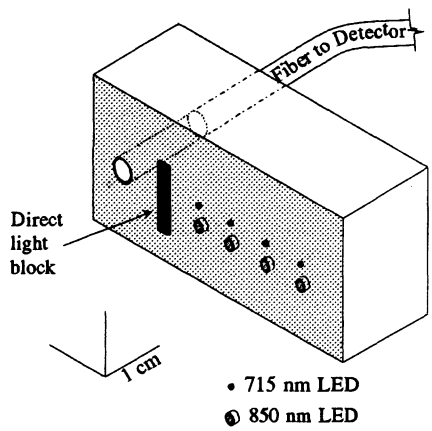

Fig. 2. Design of the measuring head of the tissue oximeter. In a measurement, the shaded surface, on which the eight LEDs and the tip of the detector optical fiber are located, is placed in contact with the sampled medium. The light shield between sources and detector is used to block the light reflected at the surface of the medium.

The 715 nm LEDs (Hewlett Packard HEMT-6000) and the 850 nm LEDs (Motorola MFOE1203) are placed in two rows containing four LEDs each. The distances between LEDs and detector optical fiber range from 1.5 to 3.5 cm, and a light shield is placed between sources and detector to prevent detection of photons which are reflected from the tissue's surface. The detector fiber bundle has an overall diameter of 3 mm and a numerical aperture of 0.56. In a measurement, the face of the measuring head containing LEDs, light shield, and optical fiber, is placed on the surface of the tissue to be examined. This approach is totally non-invasive. Figure 3 shows the schematic of the multiplexing circuit employed to drive the eight LEDs. Since we want to measure DC, AC, and phase obtained from each one of the light sources, we want only one LED to be on at a time. To achieve this, we have placed a relay (MAGNECRAFT W171 DIP-2) in series with each LED. An LED is on only when the corresponding relay is closed. Each relay is driven by an output line of a demultiplexer (74HCT238), and since only one demultiplexer line is enabled at a time, we obtain the result that only one LED is on at a time. The switching between enabled demultiplexer lines is controlled by a counter (RCA CD4024) which is driven by a clock signal sent by the computer. The computer also sends a reset pulse to start the sequence with the same LED every time. This multiplexing scheme enables us to implement the multiple source-detector distances protocol by employing a number of light sources located at different distances relative to the optical detector. This procedure is faster, more precise,

and more practical than using a multiple detector fiber scheme (which would require either a number of PMTs or a mechanical shutter to select which optical fiber is coupled to the PMT) or a mechanical movement of a single light source (which would introduce a larger uncertainty in the position of the source). To exploit the whole dynamic range of the analog-to-digital converter of the data acquisition card, we put a resistor in series with each LED to control the current in the light sources. In this fashion, we accomplish a light source equilibration that yields comparable light intensities at the detector fiber from each LED. As a consequence of this operation, we must calibrate the light intensity and the phase of the eight light sources. This calibration also accounts for the unknown source terms of each LED.

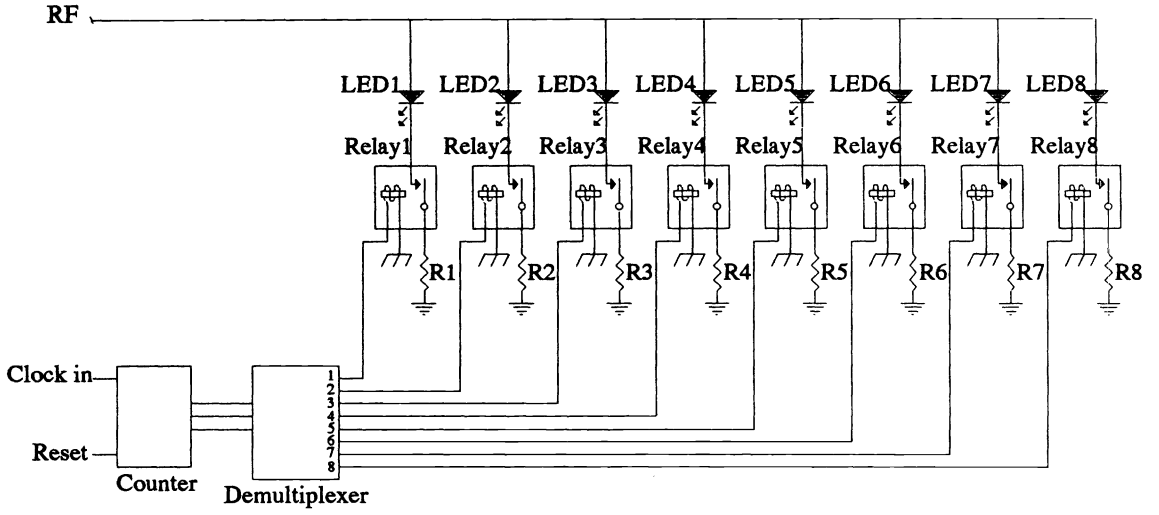

Fig. 3. Schematic of the multiplexing circuit employed to drive the 8 LEDs of the frequency-domain oximeter. The counter (RCA CD4024) counts the number of waves of the clock signal, sent by the computer, and continuously transmits this number (mod. 8) to the demultiplexer (74HCT238). The enabled line of the demultiplexer, which closes the corresponding relay (MAGNECRAFT W171 DIP-2), is then changed at the frequency of the clock. The reset pulse is sent at the beginning of the measurement to start the sequence always with the same LED.

The calibration procedure is performed by placing the measurement head on a solid block of a substance with known absorption and scattering coefficients. Multiplicative factors for the DC and AC intensities, and additive factors for the phases of the light sources are introduced in the computer in order to reproduce the known values of μ_a and μ_s'. Note that this calibration is performed with the purpose of determining the intensity and the phase of each light source. This calibration procedure should not be confused with the calibration of most commercial oximeters which need to be calibrated according to 0-100% baselines and statistical tables based on the spectroscopic characteristic of the particular tissue to be measured. We also periodically checked the light source calibration for drifts in the light source characteristics.

3. RESULTS

3.1. *In vitro*

We conducted a systematic *in vitro* study to characterize our instrument. We employed a tissue-like phantom consisting of an aqueous solution of Liposyn 20% (from Abbott Laboratories) and black India ink. The medium was held in a 3.8 *l* cylindrical container 20 cm in diameter. We employed several concentrations of Liposyn (13 to 50 m*l*/*l*) to vary μ_s' and different concentrations of a prediluted black India ink solution (0.26 to 1.06 m*l*/*l*) to vary μ_a. In this way we tested the instrument over a range of values of μ_a and μ_s'. Specifically, we conducted eight measurements. The first four relative to four different Liposyn concentrations in the absence of black India ink. The last four relative to four different ink concentrations and the same Liposyn concentration of 50 m*l*/*l*. We measured the optical coefficients of the liquid phantom by placing the measuring head on the surface of the medium. To evaluate the accuracy of the measurements, we have also measured μ_a and μ_s' in a quasi-infinite geometry by deeply immersing a single LED and the detector optical fiber inside the medium. In this case, multiple source-detector separations were accomplished by physically moving the LED to different positions relative to the detector fiber. This technique has already been described and it provides accurate values for μ_a and μ_s'.(Ref. 11) The experimental results for μ_a and μ_s' at 850 nm are shown in Fig. 4. Since the results in the infinite geometry are known to be accurate, Fig. 4 provides information on the accuracy of the instrument, on its capability to separate the absorption from the scattering properties of the medium, and on the lack of correlation when one of the two parameters is changed while the other is kept constant.

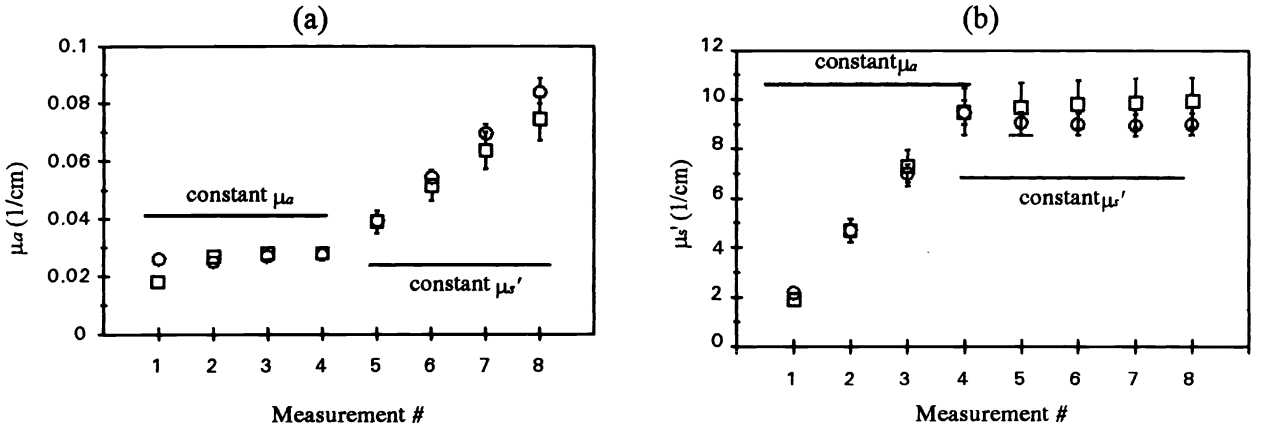

Fig. 4. Measurement of (a) μ_a and (b) μ_s' of a model scattering and absorbing medium (aqueous solution of Liposyn 10% and black India ink) using the 850 nm LEDs. In meas. #1-4 the solution contains no ink, whereas in meas. #4-8 the scatterer content is kept constant. The values measured with our instrument (❑) are compared with the values obtained with a single LED deeply immersed in the medium (○).

3.2. *In vivo*

We tested the frequency-domain tissue spectrometer by measuring the temporal behavior of the oxygenation of the forearm skeletal muscle during ischemia. For this purpose the instrument was

placed on the forearm of a volunteer and a pneumatic cuff was used to induce a vascular occlusion causing ischemia. In the measurement protocol, after one minute of baseline acquisition, the cuff was inflated to a pressure of 240-260 mmHg and kept inflated for eight minutes. Then, the cuff was released and data acquired for three more minutes. The acquisition time was set to 1 sec. The result for the temporal evolution of hemoglobin saturation during this experimental protocol *in vivo* is shown in Fig. 5.

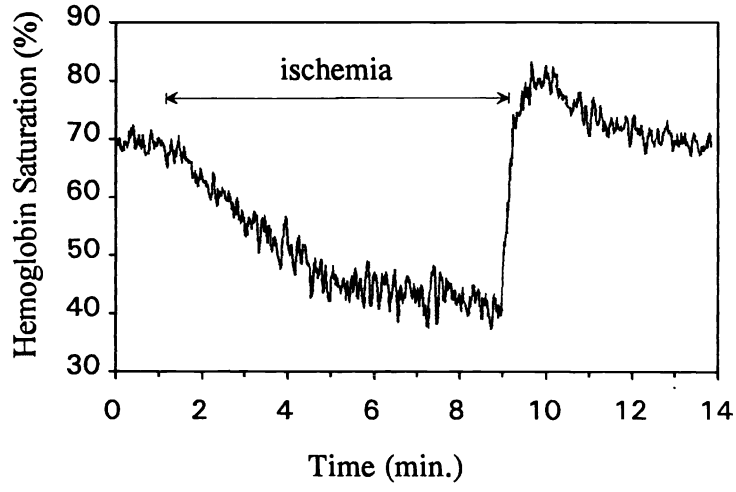

Fig. 5. Temporal dependence of hemoglobin saturation of the forearm skeletal muscle during the ischemia protocol described in the text.

4. DISCUSSION

The precision of the μ_a and μ_s' determination depends on the level of detected signal and on the acquisition time. We already mentioned that we are sending more current through the more distant LEDs to have comparable signals for all of the light sources. We define the acquisition time (t_a) to be the time required to obtain μ_a and μ_s' at both λ_1 and λ_2. We have verified that the instrument is shot noise limited, so that the precision varies as the square root of the product of detected signal and acquisition time. To quantify the precision of the instrument, we have measured μ_a and μ_s' at a signal level of about 10% of the full scale signal (i.e. the signal which saturates the analog to digital converter) and for an acquisition time of 1 sec. In these conditions the statistical error in the measurement of the optical coefficients due to instrument noise is about 1-2%.

The accuracy of the instrument is the capability of measuring values of μ_a and μ_s' that reproduce the actual values of μ_a and μ_s' of the medium. Several factors influence the accuracy:
1. Validity of the physical model;
2. Optical coupling between the light sources and the medium;
3. Wide spectral emission of the LEDs.

1. The accuracy of the employed relationships between the frequency-domain parameters (DC, AC and phase) and the optical properties of the medium (μ_a, μ_s' and the index of refraction) relies on

the validity of the assumptions made in deriving them. These assumptions are the macroscopic homogeneity of the medium and the semi-infinite geometry.[10] Neither condition is rigorously satisfied in the case of *in vivo* measurements. Nevertheless, it has been proposed that some tissues can be treated as homogeneous media.[2,12] Exceptions are given by the presence of macroscopic inhomogeneities whose optical properties are strongly different from those of the surrounding tissue, as is the case of tumors, large blood vessels or the proximity of bones. Moreover, the tissue must be at least several centimeters thick to permit one to apply the equations for the semi-infinite geometry. The actual air-tissue interface is not a geometrical plane, and source and detector are not located exactly on the boundary plane. However, we have demonstrated that the measured phase, DC and AC photon fluxes are largely insensitive to the precise geometrical configuration at the boundary.[10] The overall accuracy of the measurement in the macroscopically homogeneous, semi-infinite medium in the diffusion model has been shown to be better than 4% for μ_a (in the range 0.02-0.40 cm^{-1}) and better than 15% for μ_s' (in the range 4-16 cm^{-1}).[10]

2. The optical coupling between the light sources and the medium strongly affects the amount of detected light. Nonetheless, when the LEDs are all evenly coupled to the medium, the effect on the measured values of μ_a and μ_s' is on the order of a few percent. The reason for this relatively small effect is the insensitivity of the straight lines associated to DC, AC and phase (which are employed for determining μ_a and μ_s') to the light source intensity. In regard to *in vivo* applications, we can state that the physical contact of the LEDs with the skin is not critical. By contrast, when the optical coupling is not even for the eight LEDs, the measured values of μ_a and μ_s' can be affected by systematic error. This error, estimated to be less than about 20%, must be considered when the LED-skin contact is varied unevenly over the 8 LEDs.

3. Rigorously, the equations utilized to calculate μ_a and μ_s'(Ref. 10) are valid only if monochromatic light is employed. The wide spectral emission of the LEDs (about 40 nm FWHM) introduces contributions to the measured frequency-domain parameters which are not taken into account by our theoretical model. However, the single wavelength equations are still exact if the absorption and reduced scattering coefficients of the medium are wavelength independent. In tissues, μ_s' is actually a weak function of λ,(Ref. 7) but μ_a is wavelength dependent (even if the opposite spectral dependencies of HbO_2 and Hb in the region from 700 to 900 nm partially compensate). We have simulated a realistic condition for biological tissues in conjunction with a light source spectral distribution typical of an LED and with the spectral response of our detector. We have found that by using the single wavelength equations in these experimental conditions, μ_a at 715 nm is overestimated by about 5% while μ_a at 850 nm is underestimated by about 3%. The effect on μ_s' is less than 1%. We note that the systematic error due to the wide spectral emission of the source is only a function of the spectral shapes of $\mu_a(\lambda)$, $\mu_s'(\lambda)$, $S(\lambda)$ and $F(\lambda)$ (where S is the source intensity and F is the spectral response of the detector). Any scale factors in S and F, which may depend for instance on the LED-tissue optical coupling or on the voltage supplied to the PMT, have no effect on the calculated values of μ_a and μ_s'. Such scale factors cancel out in the derivation of μ_a and μ_s'.
The use of laser diodes as the light sources would eliminate this wavelength bandwidth dependent contribution to the instrumental accuracy.

5. CONCLUSION

The instrument we have designed provides quantitative absolute measurements of the absorption and scattering coefficients with relatively high accuracy. This permits one to determine the absolute values of the concentrations of oxy- and deoxy-hemoglobin, and of the oxygenation in tissues. The measured quantities are displayed directly on screen, providing a simultaneous real time monitoring of such tissue parameters. Time derivatives and time integrals of the measured quantities can be monitored in real time as well. Our instrument can also be made compact in size. All the required instrumentation shown in Fig. 1 can fit in a light, portable unit.

There are several characteristics of our instrument that can be changed without affecting the underlying principle ideas:
- The number of light sources can be augmented or decreased depending on the particular application. For hemoglobin saturation measurement, 8 sources are adequate. However, the same multiplexing principle can be applied to a much larger number of sources for the simultaneous determination of the concentration of more chromophores, for adding another wavelength, or for imaging applications.
- We have used light emitting diodes as the light sources. It is possible to substitute them with laser diodes with minor modifications of the driving electronics. The advantage of using laser diodes over LEDs is that laser diodes emit monochromatic light, provide higher intensity and can be modulated at higher frequencies. However, for the purpose of the proof of concept of a multiplexed tissue oximeter, LEDs are adequate both in terms of light intensity and modulation frequency.
- The value of 400 Hz for the cross-correlation frequency was selected for convenience. We have experimentally tested cross-correlation frequencies from 40 Hz to 1500 Hz with comparable results. The use of higher values of cross-correlation frequency allows for a better detection of faster processes.
- The light source multiplexer was constructed using mechanical relays (see Fig. 3). However, solid state switches can be used as well if multiplexing speeds faster than 2.5 ms are required.

6. ACKNOWLEDGMENTS

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