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MEASUREMENT OF THE DYNAMICS OF ARTERIOLAR DIAMETER

J.-U. Meyer and M. Intaglietta

AMES-Bioengineering
University of California, San Diego

The diameter of the arteriolar vessels of the microcirculation undergoes a continuous variation as a consequence of vasomotion. The quantification of this process requires the implementation of spectral analysis techniques that model short data records of a finite number of superposed sinusoidal waveforms. The following techniques were tested with artificially synthetized records and actual data: the fast Fourier transform, the high-resolution autoregressive method, the maximum entropy method, and the Prony Spectral Line Estimator (PSLE). It was found that the PSLE provides the most accurate estimation of the spectral components of the dynamics of diameter changes because it does not require any assumption on the nature of the data outside the interval under analysis.

Keywords— *Vasomotion, Spectral analysis, Prony, Arterioles.*

INTRODUCTION

The diameter of small arteries and arterioles in animal experimental preparations where the microcirculation can be observed in the absence of anesthesia exhibits vasomotion, i.e., the continuous rhythmic contraction and relaxation of the blood vessels. The principal features of this activity have been described in the bat wing (13) and were recently quantified (4) in the hamster skin fold window preparation. Skeletal muscle (12), where anesthesia is necessary, and isolated arteriolar vessels of the hamster cheek pouch (5) also exhibit the phenomenon.

It is becoming increasingly apparent that this process is present in the microvasculature of most tissues, according to the data summarized by Funk and Intaglietta (7). Its principal feature is that the frequency is of the order of 1 to 3 cpm in vessels with diameter of the order of 100 μm and that it increases as the vessels become smaller. The activity is most vigorous at the level of the terminal arterioles, where it is in the range of 10 to 20 cpm. The amplitude of the vessels diameter variation is of the order of 10% of the mean vessels diameter in the 100 to 150 μm vessels and reaches 100% in the terminal arterioles, which often close completely during the contraction portion of the cycle.

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Address correspondence to Professor M. Intaglietta, AMES-Bioengineering, M-005, University of California, San Diego, La Jolla, California 92093.

OPTICAL METHODS FOR DIAMETER MEASUREMENTS

The comparatively low frequency of the time-dependent changes in diameter allow one to utilize operator-controlled methodology to obtain continuous measurement. Currently the technique of choice is based on the image-splitting concept developed by Dyson (6) and Barer (2), which was implemented as a television-measuring microscope by Baez (1). These systems optically create a second image of an object in such a fashion that it can be superposed to the original one, and moved relative to this in a lateral direction. Measurement of the object's diameter, or transverse dimension is obtained by displacing the duplicated image until the opposing edges of the object coincide.

The image-splitting method was further developed by Intaglietta and Tompkins (10) who sheared the video image along a given raster line by delaying the video vertical synchronization pulse in such a fashion that a portion of the video frame appears displaced. This technique differs from image splitting in that there is no superposition of the two images, and the measurement is made by comparing features from the opposite sides of the object along a clearly defined transverse axis, as shown in Fig. 1.

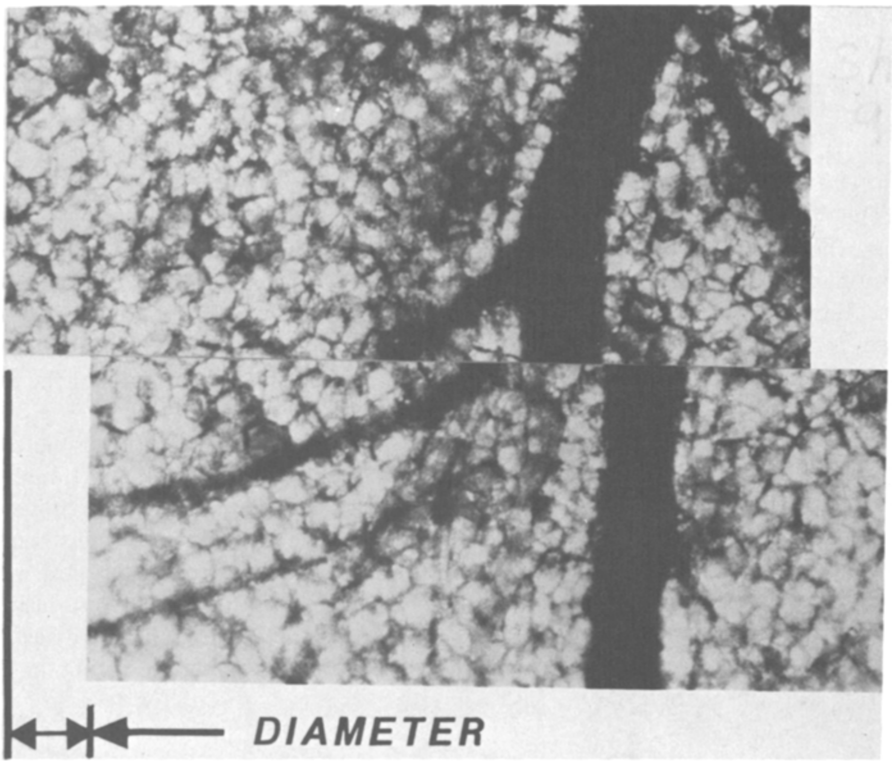

FIGURE 1. Image shearing method. The vessels are aligned in the direction perpendicular to the raster lines, and a portion of the image is moved laterally, until the two opposite edges coincide. The displacement needed to obtain the alignment is the dimension being sought.

The image-shearing technique offers the advantage that the diameter of a blood vessel, and the feature that marks its extent, can be identified by the operator using his pattern-recognition ability. This property is important in microvascular studies where the vessels tend to appear in complex backgrounds, which usually introduce spurious signals in automated measuring procedures. Furthermore, the technique is not affected by lateral motion of the object and is also independent of vertical motion if the two opposing sides are parallel. An additional advantage is that all operations are carried out in electronic format, and therefore the image can be rotated and aligned electronically without the use of additional optical components.

The accuracy and repeatability of the measurement technique is primarily a function of the operator's ability to maintain alignment of the features that were selected at the beginning of the recording procedure. Maintaining alignment is probably the principal source of errors in attempting to obtain absolute measurements, since the edges of the image are made by a gradient of shades of gray and do not constitute a step change in optical density. However, in terms of the dynamics of vasomotion, the absolute dimensions do not have the same level of significance as the relative, time-dependent variations. In this context, a keen-eyed operator appears to be quite capable of obtaining highly reproducible results for intervals of the order of 2 to 5 minutes when the frequency content of the activity does not exceed 20 to 25 cycles/min.

QUANTITATIVE CHARACTERIZATION OF TIME-DEPENDENT DIAMETER CHANGES

The phenomenon of vasomotion often presents a complex pattern of quasi-rhythmic activity that is presumed to be due to the propagation and superposition of contraction and dilation waves throughout the microvasculature, as discussed by Intaglietta (9). Furthermore, the phenomenon is continuously modulated by central and local stimuli, and it is not explicitly related to the activity of a classical mechanical oscillator, which results in harmonically related dynamics.

The time-dependent properties of vasomotion are such that the period over which it can be considered to be stationary, or at a steady state, with well-defined spectral characteristics is short and of the order of minutes. This feature poses a special challenge in attempting to obtain a quantitative description of the phenomenon, in terms of the main frequency components, their phase, and amplitude.

The fast Fourier transform (FFT) is the method of reference for power-spectrum analysis; however, its frequency resolution is limited by the reciprocal of the sampling interval. Furthermore, the FFT process implies windowing of the data, which results in a leakage of spectral energy into side lobes and therefore distorts spectral information. The effect is magnified when the data records are short. Nonuniform windowing like the Kaiser-Bessel window reduces this leakage effect but decreases the resolution of the spectral estimate.

In view of these problems we investigated alternative methods for quantifying the time-dependent features of vasomotion, utilizing as a basis the high-resolution power-spectrum programs developed by the Digital Signal Processing Committee of the Institute of Electrical and Electronic Engineers (14), and a modified version of the Prony Spectral Line Estimator (PSLE) program developed by Burkhardt (3).

The IEEE programs implemented were the FFT, the high-resolution autoregressive (AR) method, and the maximum entropy method (MEM) also referred to as

Burg's algorithm. The conventional FFT algorithm was implemented in such a fashion that 64-point data records were multiplied by a Kaiser-Bessel window. The unfiltered data were convoluted and all records were zero-padded to 128 data points.

The AR method for spectral analysis was implemented with the program AUTO of the IEEE (14). This is a feedback technique based on the identification of the principal frequencies contained in the transfer function of the data, which is derived with a special autocorrelation algorithm. This function assumes that the data are zero outside of the interval under investigation, which is implemented with the use of a Kaiser-Bessel window. This technique does not provide phase information, but is effective in modeling the frequency components with the major energy.

The MEM was implemented with the subroutines COVAR2 and CLHARM of the IEEE (14). This process minimizes the sum of the forward and backward errors in the prediction of the total energy in the model, deduced by comparing the calculated and the actual values of this parameter.

The PSLE was implemented on the basis of the program developed by Burkhardt (3). This method models data consisting of equally spaced measurements with a linear combination of complex exponentials. The PSLE is an adaptive method based on an iterative process in which an initial guess of the unknown parameters of frequency, amplitude, and phase is successively improved. This is carried out by a minimization of the square error between original and reconstructed data, as shown by Hildebrand (8).

RESULTS

The different methods were tested with both artificial data of known properties that simulate the time-dependent features of vasomotion and with actual data. One of the test records used was that given by Kay and Marple (11) in their study on comparison of the properties of different power-spectrum measuring techniques. Their test data consist of a record of 64 data points that describe three sinusoidal components with digital normalized frequencies of 0.10, 0.20, and 0.21, and with signal-to-noise ratios (SNR) of 10, 30, and 30, respectively, where the noise is due to a filtered white Gaussian process. The actual time series and the estimate given by Prony's method is shown in the top plot of Fig. 2, and the results of the implementation of each of the spectral estimation techniques is shown below.

Similar tests were conducted with artificial signals composed of 4, 5, and 7 sinusoids with varying levels of SNR that ranged from 0 to 20. The seven sinusoid signals and the results of the spectral analysis methods are shown in Fig. 3.

Application of the PSLE to actual data is shown in the examples of Figs. 4 and 5. Each of these records illustrates different types of vasomotion in terminal arterioles. It is apparent that the patterns of the activity are quite complex and that even in the reconstructed mode it is quite difficult to identify how well the two methods reproduce the original waveforms. This becomes apparent in analyzing the power spectrum and computing the correlation coefficient between original and reconstructed data. In most instances the PSLE provides the highest correlation value.

DISCUSSION

Comparison of the four spectrum analysis methods shows that the FFT method detects most of the frequencies. However, the resolution is limited by the small number

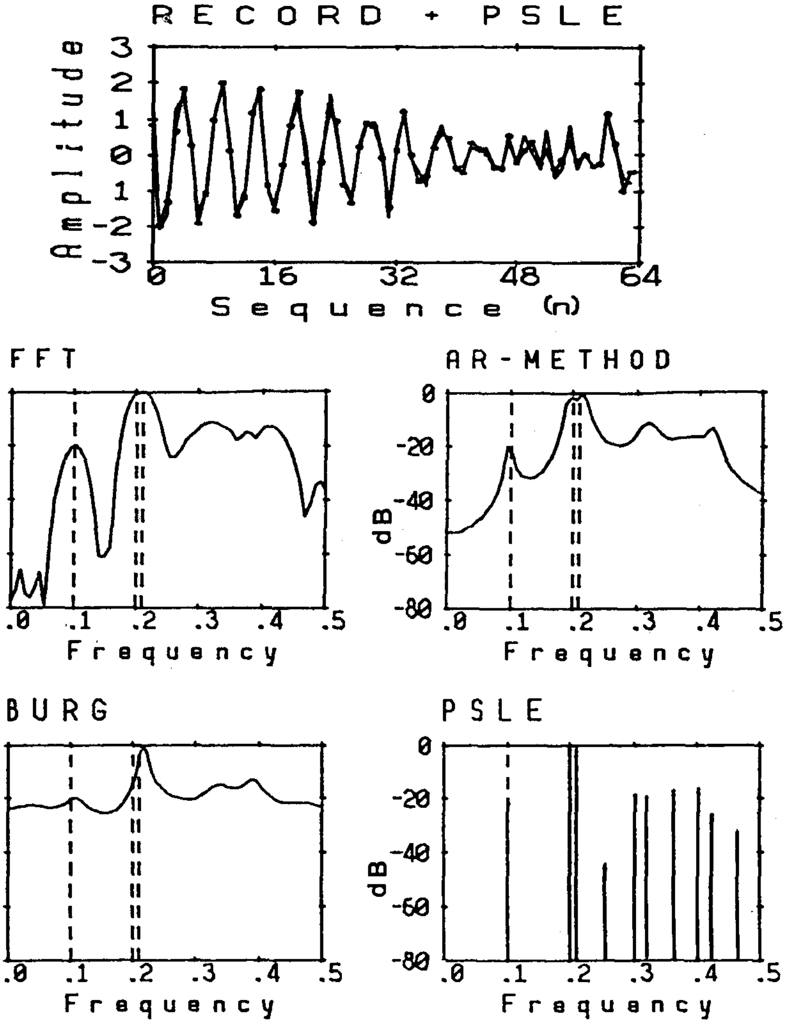

FIGURE 2. Analysis of the time series reported by Kay and Marple (11). The time series is shown as a continuous line, while the same record reconstructed with the results of the PSLE spectrum is shown as the line drawn between the discrete points. The four lower panels give the power spectrum obtained by each of the methods tested. In each case the actual frequency components are shown by dotted lines. The correlation coefficient between actual data and reconstructed data for the PSLE was 0.96.

of actual data points. Side lobe peaks that appear in the spectrum complicate the detection of a pure sinsusoid. Windowing reduces the effect of the side lobes but decreases again the resolution of the spectral estimate.

The AR is an alternative method for spectral estimation that is closely related to the linear prediction procedures used in speech processing. The AR technique gives reasonably good results for strong sinusoidal components in white noise even though it is not designed for this kind of application. Phase and amplitude information cannot be obtained using this method, and at signal-to-noise ratios less than 10 dB, the indi-

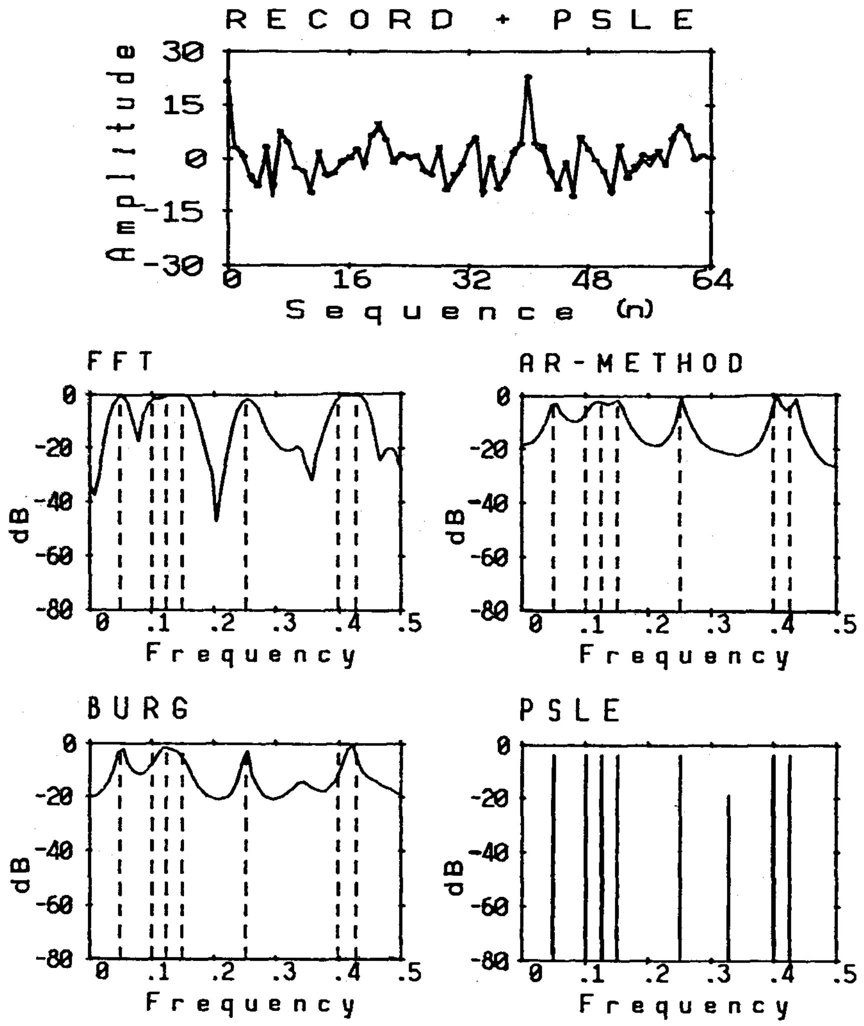

FIGURE 3. Artificial data composed of 7 sinusoids of amplitude 3.16 and frequencies 0.05, 0.1, 0.125, 0.15, 0.25, 0.40, and 0.425. The signal-to-noise ratio for each component was 10 dB. The actual data (*continuous line*) and reconstructed data obtained using the PSLE (*line joining discrete points*) are shown in the upper panel. The power spectrum obtained with each of the techniques is shown in the four lower panels. In each case the actual frequency components are shown with *dotted lines*. All the methods find the sinusoidal components as long as they are not closely spaced. The correlation coefficient between actual and PSLE reconstructed data was 0.99.

cated main frequencies are shifted toward higher values. It is stated in the literature that AR spectral estimates are inversely proportional to both the data length and the square of the SNR. Burg's algorithm shows pronounced distinct peaks for signals with an SNR ratio more than 20 dB. In contrast to reports from the literature, Burg's algorithm did not resolve very closely spaced (0.01) sinusoids.

PSLE was the most effective method for finding the sinusoids buried in white noise. This is because it is based on a least mean square optimization between the actual data

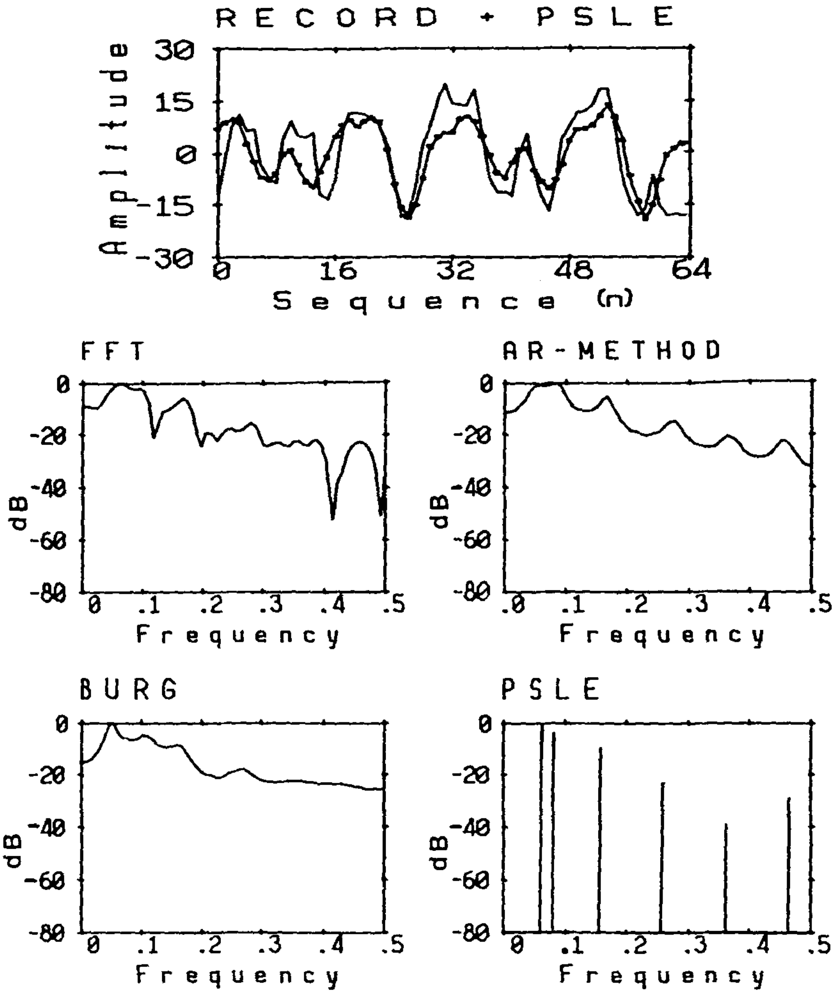

FIGURE 4. Spectral analysis of the arteriolar dynamics of an 18 μm mean diameter arterial vessel in the unanesthetized hamster skin fold. The continuous line is the record obtained with the image-shearing monitor, and the line joining the discrete points is the same record reconstructed with the results of the PSLE analysis. The results obtained with the four methods tested are shown in the lower panels. The FFT provides some indication of the spectral components present, but the results are not conclusive. The AR and Burg's method identify one major component. The correlation coefficient between actual and PSLE reconstructed data is 0.71, which is significant with $P < .01$.

and an assumed superposition of a finite number of sinusoids. It also provided adequate results when used in conjunction with the vasomotion records and was particularly efficient in finding the principal frequency components.

A key feature of the PSLE is that it requires no assumption on the nature of the data outside of the interval of analysis, and therefore no effects from this assumption are introduced in the calculation. The fact that it provides a high level of reproducibility suggests that vasomotion can be assumed to be the consequence of the activity of sinusoidal oscillators whose effects propagate and become superposed at

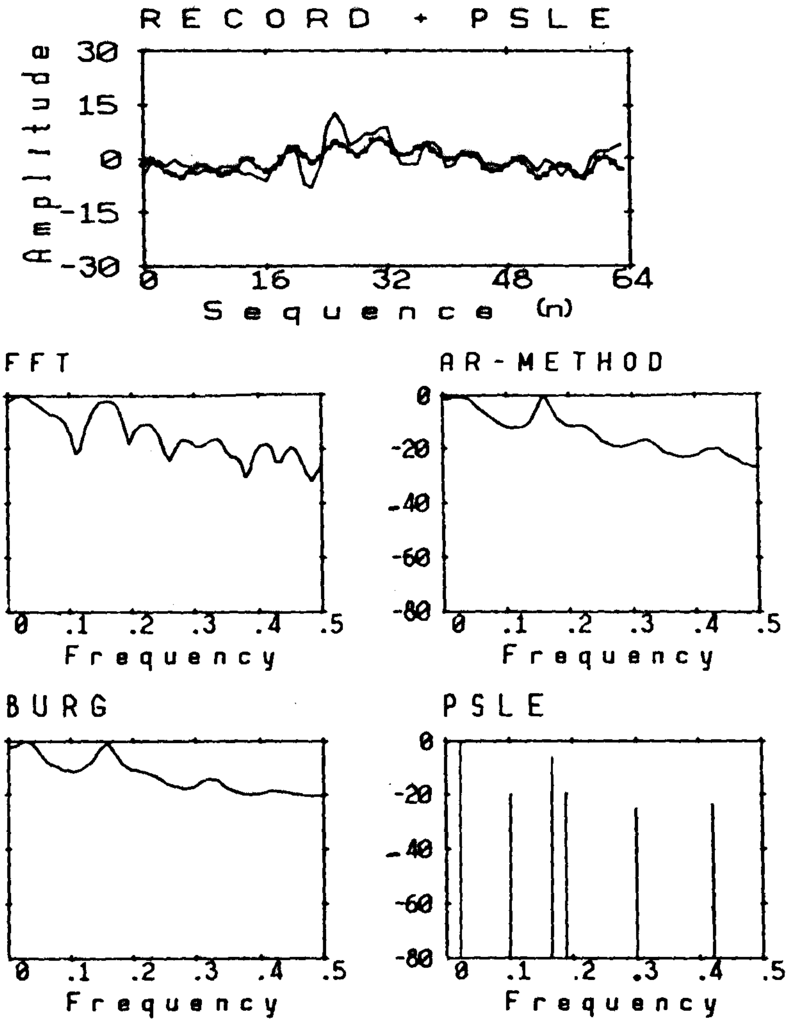

FIGURE 5. Analysis of vasomotion in a 12 μm average diameter arteriolar vessel in the hamster skin fold window preparation. Actual data are shown as a continuous line, and PSLE reconstructed record is shown in the upper panel. The presentation of results is identical to that of Fig. 4. The PSLE allows reconstructing the original data with a correlation coefficient of 0.68, which is significant with $P < .01$.

given locations in the vasculature. The verification of this model requires the identification of the location of the source of the phenomenon, which in principle could be accomplished by the high-resolution measurement of the phase of the different spectral components along the microvasculature.

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