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SINGLE FIBER LASER-DOPPLER FLOWMETRY—DEPENDENCE ON WAVELENGTH AND TIP OPTICS

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ABSTRACT

Single fiber, laser-Doppler flowmetry can be used for blood flow measurement in deeply located tissue structures by the insertion of optical fibers into the tissue. The geometry of the monitored volume has been estimated at two different wavelengths and when using two types of fiber tips, one of which has been modified with a lens formed at the fiber end surface. Physical models as well as intramuscular measurements have been used in the experiments. The scattering image was studied in latex solutions of three different scatterer concentrations. The wavelengths 632.8 and 750 nm were used. At higher concentrations of scatterers, the near infrared (NIR) wavelength gave a larger scattering area. At the lower concentration, the difference between the areas was smaller or nonexistent. The NIR wavelength also showed an increased monitoring depth than that of the He–Ne laser in an experimental model study. The properties of the tip optics were evaluated in a flow-through model where the distance between the fiber tips and the flow channel was varied. The flat tip fiber has a sensitivity maximum close to its end surface, whereas the modified fiber (''pear'' tip) showed a sensitivity maximum 1.5 mm from the end surface. This property may decrease the influence caused by the insertion trauma in intramuscular measurements. [S1083-3668(98)00903-4]

Keywords single optical fiber; monitored depth; scattering image; modified fiber tip; wavelength; digital signal processor.

1 INTRODUCTION

Laser-Doppler flowmetry (LDF) for blood flow measurement was introduced by Riva et al.,[1] and Stern[2] and was first used by Öberg et al.[3] to measure the microvascular blood flow in skeletal muscle. The single fiber technique introduced by Salerud and Öberg[4] opened up the possibility to study deep tissue perfusion under various physiological conditions. This technique was further developed by us and applied for research purposes and clinical use.[5–10]

A disadvantage of the single fiber technique is that the monitored tissue volume is relatively small.[11] This, in combination with the heterogeneous perfusion of the skeletal muscle, may lead to unreliability in the obtained muscle perfusion data. In order to obtain more accurate perfusion measurements we developed an optical system for detection from larger tissue volumes. This system is based on a near infrared (NIR) laser diode with a wavelength of 750 nm which is less scattered in the tissue than the He–Ne light of 632.8 nm, a wavelength that we used earlier.[5–9] In addition, we modified the tip of the optical fiber to obtain a ''pear'' type lens[12] at the fiber tip which can detect deeper tissue volumes than the traditional flat fiber tip.

The aim of this paper is to evaluate the single fiber technique at the He–Ne and NIR wavelengths with flat tip and modified ''pear'' tip fibers by using experimental models and *in vivo* measurements.

2 MATERIALS AND METHODS

2.1 HE–NE SINGLE FIBER PROBE

A Periflux Pf1d (Perimed, Stockholm, Sweden) was modified to fit a single fiber probe (cladding/core $\varnothing = 0.72/0.42$ mm). A He–Ne laser launched light (632.8 nm) into an optical fiber. The laser light was absorbed, scattered and reflected in the tissue. Part of the light was brought back to two photodetectors via an optical coupler.

Two different signals, both related to the flux of erythrocytes, are available in the laser-Doppler Pf1d instrument.[13] First, the unprocessed (raw) Doppler signal can be used for calculation of flux via the emulated standard algorithm. Second, a flux-related signal can be obtained via rectification and low-pass filtering.

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This module is called ''the He–Ne module'' in this paper.

2.2 NEAR INFRARED SINGLE FIBER MODULE

This module has previously been presented by us.[14] A laser diode (Sharp LT030MD0, 3 mW, 750 nm) feeds a single optical fiber (glass, step-index fiber cladding/core $\varnothing = 0.72/0.42$ mm) via launching optics. The laser diode is temperature stabilized within 0.1 °C. The optical module also includes a photodetector (Silicon Detector Corporation SD 100-41-11-231). The detector current is converted into an analog voltage. The ac portion of the signal is sampled and used for flow calculations.

This module is named ''the NIR module'' in this paper.

2.3 SCATTERING IMAGE ASSESSMENT AT 632.8 AND 750 NM

The comparison of the scattering images at the two wavelengths in the same medium can give important (qualitative) information about the transmission properties of diffuse light at the chosen wavelengths. The scattering images were compared in an *in vitro* model at 632.8 and 750 nm. Ordinary milk (containing 1.5% fat), diluted with different percentages of water to obtain different scatterer concentrations, was used as scattering medium in a cylindrical glass beaker. The optical power output of each light source was adjusted to 0.08 mW. The optical fibers were dipped into the diluted milk and the fiber tips were positioned in the middle of the beaker. A high resolution black-and-white charge-coupled device (B/W CCD) camera (ICD-40E Ikegami CCD) was used to image the scattering zone. The experiment was performed in a dark room and at normal room temperature. To avoid ''cross-talk'' between the two wavelengths the video images were recorded in one sequence.

The video signal was extracted by using a QUICKIMAGE 24 video frame grabber card in an Apple Macintosh IIcx. The software QUICKIMAGE 24 v.2.0 (MASS Microsystems', Inc.) was used for image acquisition. By using NIH IMAGE software (National Institute of Health, Bethesda, MD) the image processing was performed as well as the scattering ''area'' calculations. B/W images were recorded. In the NIH IMAGE software, the image was color coded into 32 colors, each with a relation to the intensity of scattered light. Then, the area of the high intensity (central part of the image) was calculated automatically by the software used.

2.4 A PC-BASED SIGNAL PROCESSOR

A PC-based signal processor was designed to carry out the signal processing for the comparison of wavelengths and fiber setups. The signal processor consists of a PC with an analog-to-digital converter and a Doppler signal processor (DSP) board, which was used for sampling the Doppler signal and calculating its power spectral density and corresponding fluxes. These results were then transferred from the DSP32C local memory to the PC, using the internal direct memory access controller from a DSP C-program.

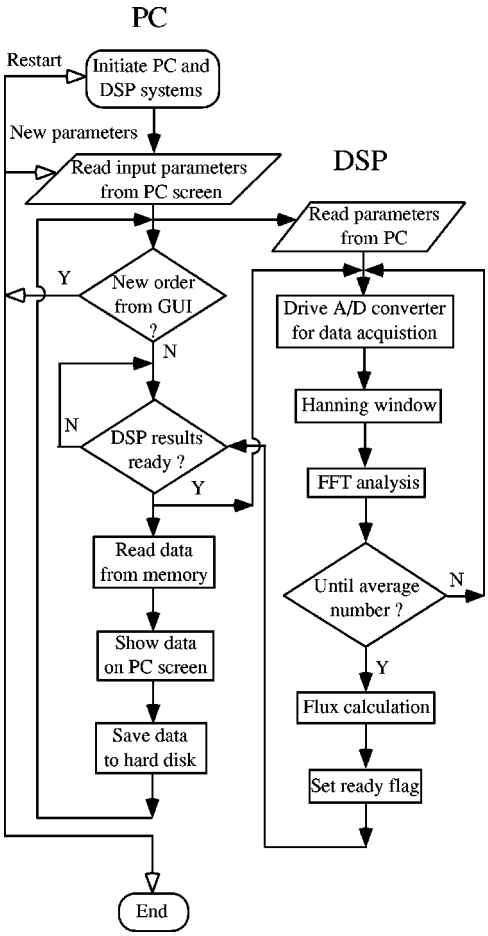

Fig. 1 Flowchart of the algorithm for DP- and PC-based digital signal processor.

A C-program was developed for the host PC in Turbo C++ software (Borland International, Inc.). It co-worked with the DSP board by calling a DSP C-program which controls the data acquisition and processing.[14] All results were presented by means of a graphic interface within LABWINDOWS (National Instruments Corporation, Austin, TX) software. The host PC and DSP board can work independently, which makes it possible to process and show fluxes in real time. The software flowchart is shown in Figure 1.

The algorithm used for calculating the flux of erythrocytes is[15]

$$\text{Flux} = K_o \cdot \int_{\omega_2}^{\omega_1} \omega \cdot p(\omega) \cdot d\omega - N(t), \qquad (1)$$

where $\omega_1 = 2 \cdot \pi \cdot 25$ Hz and $\omega_2 = 2 \cdot \pi \cdot 12.5$ kHz are the lower and upper cutoff frequencies of the filter.

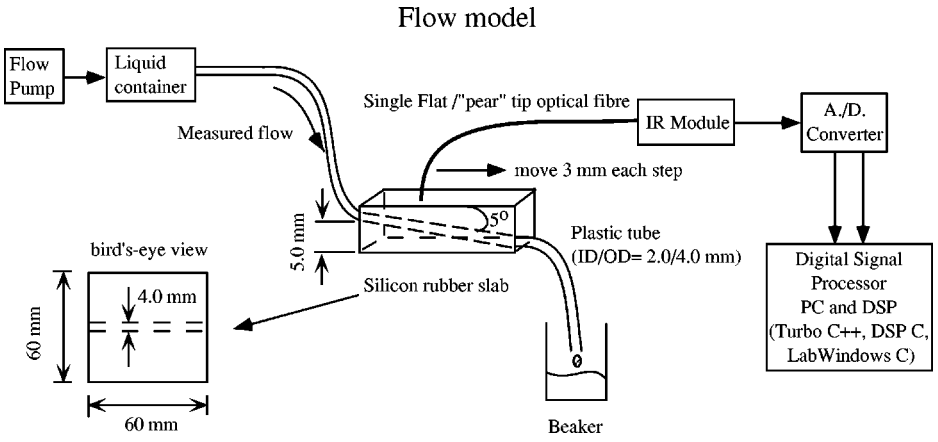

Fig. 2 Experimental setup for depth sensitivity measurement.

P is the calculated power spectral density from the Doppler photocurrent signals. K_o is a constant. $N(t)$ is a noise signal. Flux is related to the product of the average speed and concentration of scatterers in the sampled volume.

2.5 MODEL SETUP FOR FLUX STUDIES

A flow model was used for evaluation of the wavelength dependence and tip geometry properties of the two single fibers. A flow-through model which permits the study of depth sensitivity versus tip geometry was used. In these measurements, we used a PC-based digital signal processor as described in Sec. 2.4. A calculated flux value was based on 177 sampled data, obtained during 15 s. The single fiber laser-Doppler modules used for these measurements are described in Secs. 2.1 and 2.2. The fiber tip was moved horizontally along the flow model.

A polyethylene tube (i.d./o.d.=2.0/4.0 mm) (Figure 2) was molded into a silicone rubber slab (dimensions of 60×60×10 mm), the latter serving as a static scattering medium (Rhodorsil RTV 1556 Transparent, Sikema AB, Sweden). The polyethylene tube formed an angle of 5° with the horizontal surfaces of the slab. The optical fiber with a flat/''pear'' tip was placed above the plastic tube perpendicular to the upper surface of the slab at a distance of 1.0 mm. As the fiber tip was moved in steps of 3 mm along the tube, the distance between the fiber tip and the monitored flow tube was increased continuously. The flow velocity was fixed at 4.88 mm/s in the experiment. $N(t)$ in Eq. (1) was determined at zero velocity.

Hollow spheres' (Potters Industries Inc. Carlstadt), serving as scattering particles, were mixed with 98% water. The flow velocity was controlled by a pump (Mek. lab-konstruktioner, Västra Frölunda, Sweden). The ''pear'' fiber used in this study had a modified tip of width/length=1.0/1.5 mm.

2.6 INTRAMUSCULAR MEASUREMENT

A percutaneous single ''pear'' tip fiber was connected to the NIR module and the PC-based digital signal processor was used for continuous recording of brachioradial muscle microcirculation. The optical fiber was introduced into the brachioradial muscle 10 cm below the elbow joint via a plastic cannula (Venflon 2 i.v. cannula, 1.2 mm outer diameter, Viggo, Helsingborg, Sweden) after anesthetizing the skin locally. 180 samples were taken during each 1 min long flow recording.

The following three blood flow levels were studied:

1. the brachioradial blood flow at rest;
2. the blood flow in the contracted muscle when the subject pressed a bar tightly; and
3. the recovery flow level after the subject loosened his/her grip.

3 RESULTS

3.1 SCATTERING VOLUME VERSUS WAVELENGTHS AND SCATTERER CONCENTRATIONS

Video images of the scattering zone around the fiber tip processed by the NIH software are presented in Figure 3.

The areas were calculated and normalized with respect to NIR 750 nm and 0.185% fat as in Figure 4.

3.2 MONITORING DEPTH VERSUS WAVELENGTH

The flux normalized with respect to the recordings at a distance of 0.5 mm, are illustrated in Figure 5.

The two-sample t-test gives significant differences ($P<0.001$, two-tailed) between two wavelengths at all distances. The results of the distance experiment show that, at all distances, the NIR

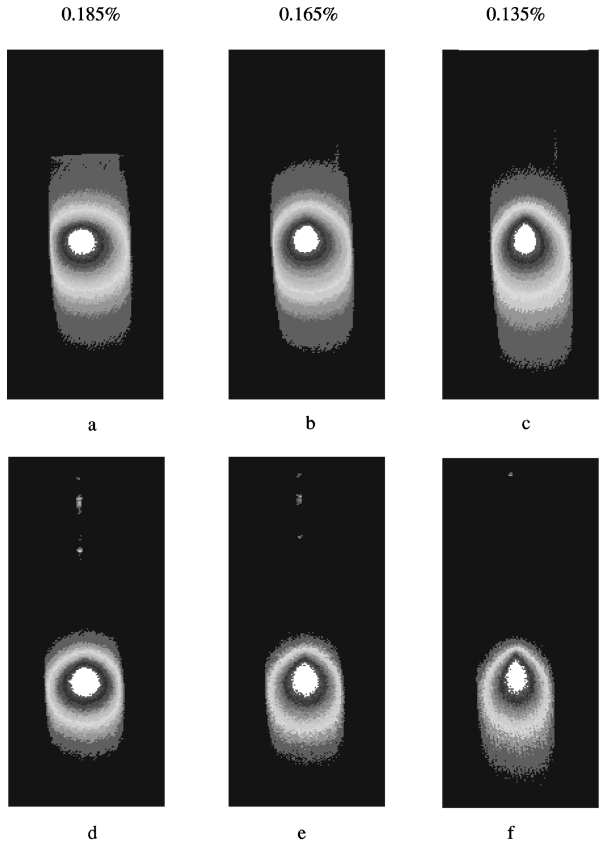

Fig. 3 Images at different scatterer concentrations at He–Ne [632.8 nm, (a), (b) and (c)] and NIR [750 nm, (d), (e), and (f)].

wavelength gives the larger signal value when methodological differences (except for the wavelength) have been compensated for.

3.3 MONITORING DEPTH VERSUS FIBER TIP OPTICS

Figure 6 shows the results normalized with respect to the 1.0 mm recordings of this experiment. The

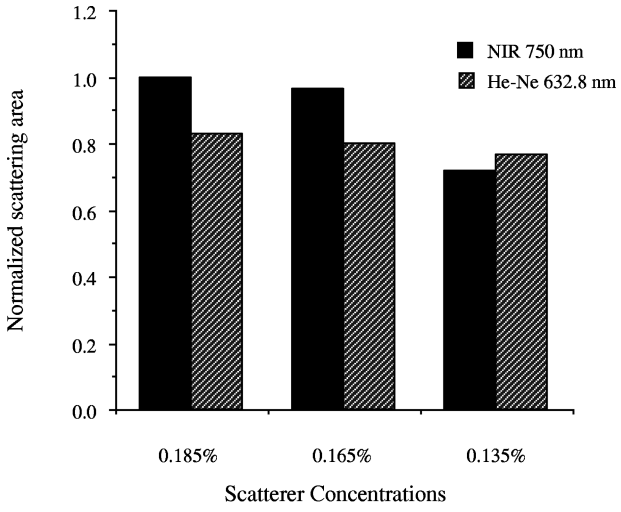

Fig. 4 Relative scatterer area/volume at two wavelengths.

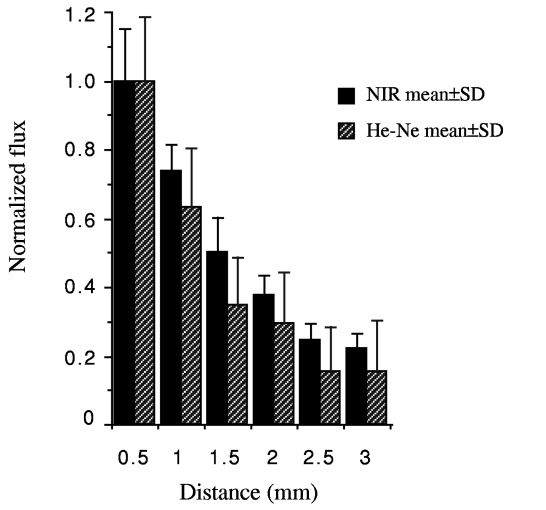

Fig. 5 Normalized flux as a function of the distance between rotating disk and flat fiber tip (He–Ne and NIR wavelengths).

flat end fiber gives a flow signal that continually decreases with increasing distance to the flow channel. The ''pear'' tip, however, gives a maximum signal when the distance has increased to approximately 1.5 mm. At longer distances a signal decrease takes place. However, the signal level is generally larger than the one associated with the flat fiber tip. Methodological differences, except for the differences in fiber tip geometry, have been compensated for.

The two-sample *t*-test shows significant differences ($P<0.001$, two-tailed) between the two tips.

3.4 INTRAMUSCULAR RECORDING USING MODIFIED TIP

Figure 7 shows the measured microcirculatory blood flow levels at rest, during muscular contraction and recovery. The vasomotor waves can be clearly identified.

It is probable that the ''pear'' type fiber used in this experiment records blood flow from a region at a distance from the bottom of the wound channel. The vasomotor waves indicate that this region is unaffected by the tissue trauma that might exist in the close vicinity of the wound channel.

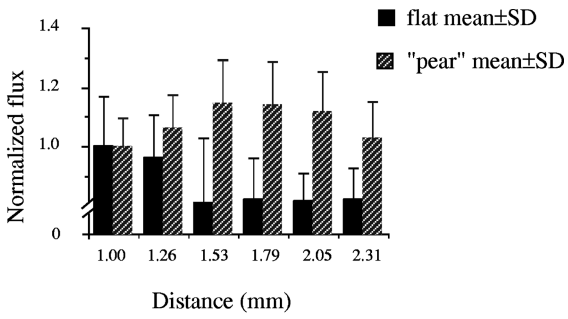

Fig. 6 The influence of fiber tip optics (flat, ''pear'' tip) for the depth sensitivity of the probe.

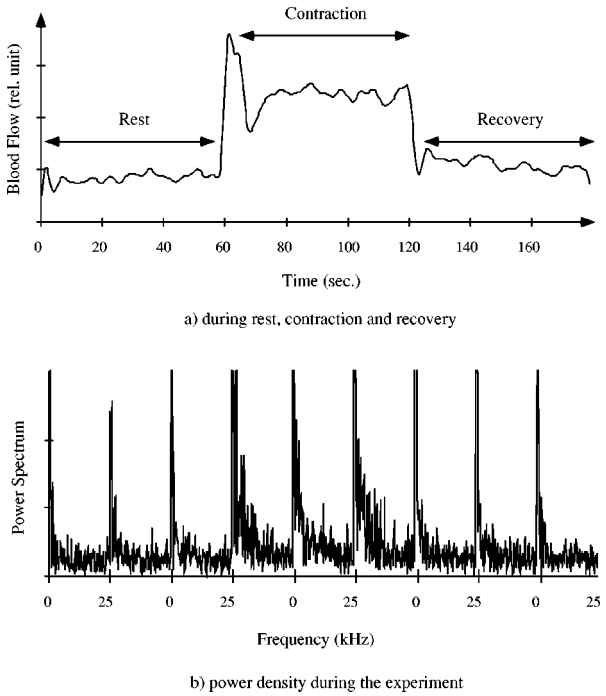

Fig. 7 Intramuscular blood flow: (a) during rest, contraction, and recovery, and (b) power density during the experiment.

4 DISCUSSION

Real time, continuous intramuscular recording of blood flow is an important application of laser-Doppler flowmetry that can be of considerable value when trying to understand muscle blood flow conditions in health and disease. The standard, multifiber optical probes have usually larger total diameters. They are less suitable for deep perfusion measurements because of the trauma created. To avoid trauma as much as possible we have been using the smallest diameter fiber available, i.e., a single fiber probe. The single fiber technique allows assessment of blood flow deep in the tissue. This is a convenient way to continuously monitor the microcirculation in the muscle. Extra physiological information may be obtained by combining the single fiber technique with other types of measurements.[16–18]

The single fiber method also has a disadvantage in that the monitored tissue volume is rather small. For human skin, the single fiber with a diameter of 0.12 mm had a mean sampling depth of <0.1 mm calculated by Jakobsson[19] who compared the size of the sampled volume in a number of probe configurations. With such a small sampling volume there is a risk that one monitors blood flow in the trauma zone in addition to flow in intact tissue away from the wound channel. The extravasated blood in the wound channel probably contributes more to the signal if the sensitivity maximum is located close to the fiber end. The small sampled volume around the fiber tip, in combination with the tissue trauma caused by the probe insertion and the normally found heterogeneity of blood flow, may give rise to a considerable variability of the blood flow data obtained.

To avoid these disadvantages of the single fiber method we have investigated two methods of increasing the sampling volume and depth:

1. a longer wavelength; and
2. a modified fiber tip.

4.1 MONITORING AREA/VOLUME VERSUS WAVELENGTH

The calculated relative areas in Figures 3 and 4 show that by increasing the wavelength we can increase the scattering area/volume, since longer wavelengths are less scattered in tissue.[20,21] In Figures 3(a), 3(b), and 3(c) the He–Ne wavelength shows a more spherical scattering image compared to the images produced when using the NIR wavelength.

In Figure 3 we can study the less scattered NIR light in the shape of the scattered area. NIR penetrates deeper into the monitored volume which results in a more longitudinal scattered area and, thus, an ellipsoidal scattering volume.

In Figure 5, we alternately used the NIR and He–Ne lasers and changed the distance between the fiber tip and the rotating disk. Calculated flux according to Eq. (1) for both the NIR and He–Ne wavelength is reduced when the distance is increased. The NIR wavelength (compared to the He–Ne) shows higher signal levels at corresponding distances. For example, the signal magnitude of the He–Ne module at a distance of 2 mm is 29% of the initial one compared to 38% with the NIR module. We conclude that a longer wavelength is to be preferred at a longer distance between the probe and the tissue under study.

4.2 MONITORING DEPTH VERSUS FIBER TIP OPTICS

Using a modified fiber tip we can increase the scattering area at the fiber end, which has been proven by a ray-trace simulation program in our previous study.[22] In this paper, a new flow model (Figure 2) was used for evaluating if it is possible, using a modified tip, to make recordings from deeper located volumes.

Figure 6 shows that it is possible to localize the most ''sensitive'' point away from the probe end by modifying the tip of the fiber. In comparison with the signal magnitude at 1.5 mm away from the fiber tip, the flat fiber decreases by 19% whereas the ''pear'' fiber gives its maximum sensitivity there. This is an important improvement of the original technique. First, recordings can be made from a tissue volume away from the wound channel, thereby obtaining a recording not affected by the trauma. Second, the unavoidable blood pooling around the fiber tip will contribute to a lesser extent to the flux signal. This blood volume is not stationary but has

a Brownian motion of the erythrocytes, contributing to the laser Doppler signal without being considered as intramuscular blood flow. The amount to which this blood volume contributes to the signal is unknown today.

Theoretically, the depth sensitivity could probably be further increased by increasing the lens diameter. However, the trauma caused by introducing the larger tip into tissue will also increase. A compromise between a larger tip diameter and the trauma caused by the tip must be found.

4.3 INTRAMUSCULAR BLOOD FLOW MEASUREMENT

The calculated flux during muscle static contraction was larger than that when the muscle was at rest. The 3 min blood flow recording in Figure 7(a) shows smooth and regular fluctuations (oscillations) which are called vasomotion,[22] i.e., rhythmic variations in blood flow with a frequency of about six cycles per minute. This phenomenon was also observed in our earlier studies.[5,14] The presence of vasomotion is often interpreted as a sign that the tissue is not affected by the trauma caused by insertion of the optical fiber.[23] The number of moving blood cells in the sampled volume should increase as soon as the normal subject contracts the muscle because of increased perfusion. This can be clearly seen in Figure 7(b) (periods 4, 5, and 6). After contraction for 1 min the muscle fatigues and responds with increased blood flow.

After finishing 1 min of contraction the muscle then recovers. The flow levels decrease to resting levels.

5 CONCLUSIONS

By increasing the laser wavelength and modifying the single fiber optical tip with a lens formed at the end surface, the fiber can sample from larger and deeper volumes, which will reduce the risk of sampling from less representative volumes.

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