Fiber-Based Laser Speckle Imaging for the Detection of Pulsatile Flow

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Background and Objective: In endodontics, a major diagnostic challenge is the accurate assessment of pulp status. In this study, we designed and characterized a fiber-based laser speckle imaging system to study pulsatile blood flow in the tooth.

Study Design/Materials and Methods: To take transilluminated laser speckle images of the teeth, we built a custom fiber-based probe. To assess our ability to detect changes in pulsatile flow, we performed *in vitro* and preliminary *in vivo* tests on tissue-simulating phantoms and human teeth. We imaged flow of intralipid in a glass microchannel at simulated heart rates ranging from 40 beats/minute (bpm) to 120 bpm (0.67–2.00 Hz). We also collected *in vivo* data from the upper front incisors of healthy subjects. From the measured raw speckle data, we calculated temporal speckle contrast versus time. With frequency-domain analysis, we identified the frequency components of the contrast waveforms.

Results: With our approach, we observed *in vitro* the presence of pulsatile flow at different simulated heart rates. We characterized simulated heart rate with an accuracy of and >98%. In the *in vivo* proof-of-principle experiment, we measured heart rates of 69, 90, and 57 bpm, which agreed with measurements of subject heart rate taken with a wearable, commercial pulse oximeter.

Conclusions: We designed, built, and tested the performance of a dental imaging probe. Data from *in vitro* and *in vivo* tests strongly suggest that this probe can detect the presence of pulsatile flow. LSI may enable endodontists to noninvasively assess pulpal vitality via direct measurement of blood flow. Lasers Surg. Med. 47:520–525, 2015.

Key words: cold test; dental photoplethysmography; electric pulp test; endodontics; leached fiber bundle; pulpal vitality; pulsatile blood flow; root canal

INTRODUCTION

Tooth viability critically depends on blood flow within the dental pulp. Maxillofacial injuries or dental caries may induce a loss in pulpal blood flow, resulting in loss of viability, and the need for root canal surgery [1,2]. To assess tooth vitality, clinicians currently use liquid CO_2 or electrical pulp testing to test the innervation of the tooth. The accuracy of these sensibility tests depends on the cooperation of the patient, and the tests themselves can be painful [2,3]. These tests have low sensitivity values, because they rely on the clinician's interpretation of a patient response. In particular, cold testing, heat testing, and electric pulp testing have sensitivities of 0.83, 0.86, and 0.72, respectively [3]. Therefore, these tests are associated with a high rate of false negatives, which leads to unnecessary root-canal treatment procedures [3].

A critical need exists for a pulpal vitality test with improved sensitivity and specificity. Assessment of blood flow is expected to better reflect the health status of a tooth [4]. To monitor blood flow, research groups previously studied use of optical methods, such as laser Doppler flowmetry [5] and photoplethysmography [6]. Unfortunately, these methods suffer from key limitations that preclude them from routine clinical use. To maximize signal integrity, custom molds are required for each

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interrogated tooth [7]. Each mold requires considerable time and patient cooperation. The process of mold construction may prove to be difficult with patients who have a sensitive pharyngeal reflex [8].

We previously reported on the use of laser speckle imaging (LSI) to measure flow in excised human teeth [9]. Specifically, we demonstrated that transillumination LSI enables determination of flow. We based this transillumination approach on the previous observation that dentin tubules effectively act as light-guiding conduits that direct light primarily in the original propagation direction [10,11].

Our overall objective is the development of a fiber-based LSI probe that enables measurements of pulsatile blood flow. Nadkarni *et al*. [12] described the use of leached fiber bundles for LSI of atherosclerotic plaques. They reported minimal leakage of light among the individual fibers, a characteristic that decreases the severity of artifacts induced by motion of the bundle itself. Based on this report, we designed and built a leached fiber-based imaging probe and compared its performance to an established open-air LSI system [13]. We developed an image analysis method based on temporal speckle contrast [14] and frequency-domain processing, and assessed the *in vitro* accuracy of our probe to simulated changes in heart rate. We finally report on an *in vivo* proof-of-principle validation of the performance of the probe.

MATERIALS AND METHODS

Imaging System

We developed a LSI probe (Fig. 1a) based on a 1mm diameter leached fiber bundle (Schott, Elmsford, NY) coupled to a CCD camera (Flea3, Point Grey, Richmond, BC) [15]. We used a custom-machined adjustable lens holder consisting of a hollowed out 5/16 hex bolt and a threaded plastic cap (Fig. 1b). The cap contained a 2 mm diameter drum lens ~5 mm past the threads. The leached fiber bundle was fixed in the bolt, which was threaded through the plastic cap. The cap was rotated to adjust the distance between the tip of the fiber and the drum lens to enable control of the fine focus of the imaging probe on the tooth. We used a 4× objective (Olympus, Center Valley, PA) and a mirror to image the fiber bundle on the camera sensor [15]. We used FlyCap software to collect all image sequences at 15 frames/second, using an exposure time of 10 ms, and 24 dB gain.

Fiber-Bundle Versus Open-Air LSI

To characterize the ability of LSI through an imaging fiber bundle to measure flow, we first collected data from an *in vitro* flow phantom consisting of a glass microcapillary tube (inner diameter ~0.65 mm). We embedded the tube at the surface of a silicone block containing TiO2 to mimic the optical scattering ($\mu s' = 1\,mm^{-1}$) of soft tissue [16]. We used a syringe pump (Harvard Apparatus, Holliston, MA)

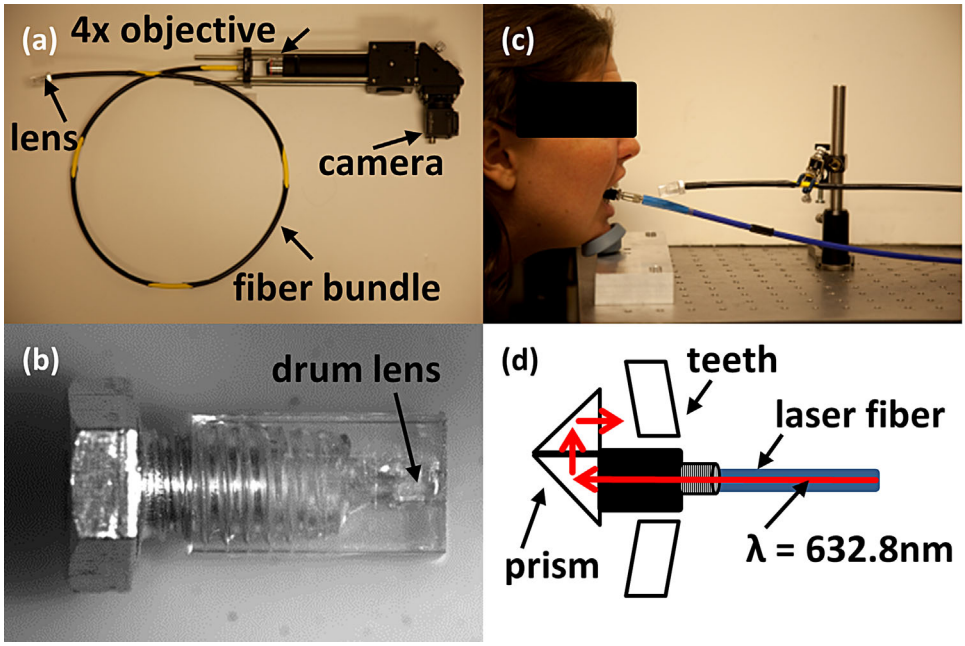


Fig. 1. **a**: The fiber-based LSI probe is composed of a leached fiber bundle with a lens at one end. The fiber bundle directs light through a 4× objective to the camera sensor. **b**: A custom-built adjustable lens holder contains a 2 mm drum lens. The imaging fiber fits through a hole down the center of the metal and plastic pieces. The plastic end rotates to allow for fine focusing by adjusting the distance between the end of the fiber bundle and the drum lens. **c**: The imaging fiber bundle (black) is placed ~50 mm from the subject. The laser fiber (blue) transmits laser light into the mouth, to the lingual side of the tooth. **d**: The laser fiber (blue) is coupled with an SMA adapter to the retroreflector (black). This component contains two right-angle prisms (white) to redirect the light towards the lingual side of the tooth hence towards the imaging fiber bundle.

to infuse a solution of 5% Intralipid (Baxter, Deerfield, IL) through the tube at speeds ranging from 0 to 2 mm/second, which spans the range from no-flow to an estimated average speed of blood in the tooth [17]. For these measurements, we used an epi-illumination configuration, in which light from a 632.8 nm HeNe laser irradiated the phantom and the imaging fiber bundle was placed on the same side of the phantom. We compared these measurements with data collected using a standard open-air LSI system employing a scientific-grade, thermoelectrically cooled CCD camera (Retiga EXi, QImaging, Surrey, BC) [13].

In vitro Evaluation of LSI Probe

To assess the performance of LSI with an imaging fiber bundle to characterize pulsatile flow, we collected additional *in vitro* image sequences of the flow phantom described above. We used a custom-built pulsatile pump to infuse 5% Intralipid with a pulse rate varying from 0.67 to 2.00 Hz, which corresponds to the range for physiological human heart rates [18].

In vivo Evaluation of LSI Probe

For *in vivo* transillumination LSI of a tooth, we first developed an optical delivery device suitable for use in the mouth (Fig. 1c). We coupled coherent light from a 632.8 nm HeNe laser into a 1mm diameter optical fiber (Ocean Optics, Dunedin, FL). The fiber transmitted light into a custom 3D-printed plastic retroreflector containing two right-angle prisms (Fig. 1d). We coupled this device to the laser fiber via a SMA adapter. We designed the retroreflector to re-direct the light by 180° to irradiate the lingual side of the tooth (Fig. 1d). As a first demonstration, with the fiber-bundle LSI probe, we collected *in vivo* image sequences from the buccal side of the upper front incisors of three healthy subjects. The subject bit down on the plastic piece to hold it steady behind the tooth of interest (Fig. 1c and d). We collected the data as part of a study approved by the Institutional Review Board at the University of California, Irvine.

Data Analysis

We used both spatial and temporal speckle analysis approaches, all written in MATLAB (The Mathworks, Inc., Natick, MA), to analyze the image sequences. We computed spatial speckle contrast maps with the use of a standard 7×7 sliding window algorithm [19]; we then calculated an average spatial speckle contrast map as the average of 30 individual speckle contrast maps. We also used a standard temporal contrast algorithm [14] to compute temporal speckle contrast values across the same 30-frame sequence. To compute pulsatile speckle contrast waveforms, we used a rolling temporal algorithm. We computed the temporal contrast from the first five frames of the sequence ($n = 1$–5), then for frames $n = 2$–6, etc., for the entire sequence. We linearly detrended the resultant contrast waveform to remove DC noise and applied a fast fourier transform (FFT) to the signal. We analyzed the frequency-domain representation of the pulsatile waveform.

RESULTS

Fiber-Bundle Versus Open-Air LSI

Figure 2a shows a brightfield image taken with the fiber-based LSI probe. The honeycomb pattern apparent in the image (and shown magnified in the inset) represents the individual 8.5 μm diameter fibers that comprise the leached fiber bundle. With the lens placed ~50 mm away from the subject, the standard usable field-of-view of the probe is ~14 mm in diameter (Fig. 2a).

We compared the contrast at different flow speeds using the fiber-based LSI probe and the open-air LSI system describe above. We calculated contrast using both spatial and temporal processing algorithms to assess the effects of the honeycomb artifact on contrast values. Spatial and temporal contrast values with the standard open-air system are identical, and decreased as expected when

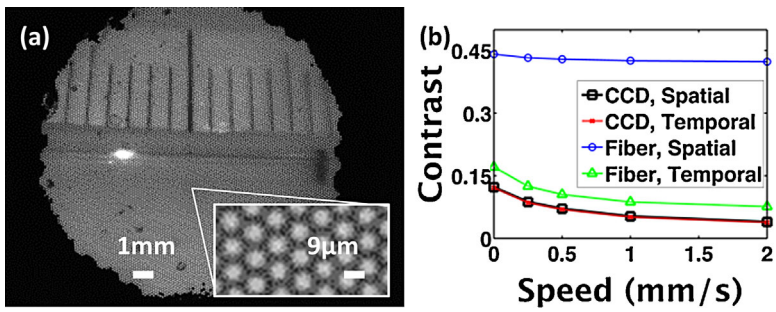


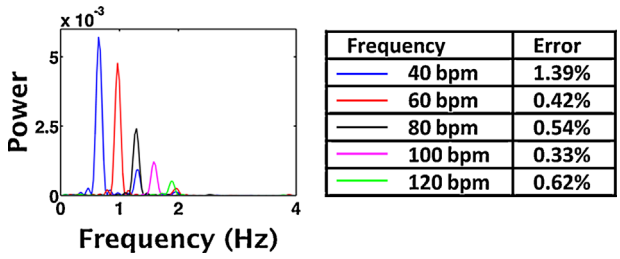
Fig. 2. **a**: Representative broadband image of a millimeter ruler through the fiber bundle. The inset shows the honeycomb pattern formed by the individual fibers (8.5 μm diameter) comprising the bundle. **b**: Comparison of contrast versus flow speed in the fiber-based system and the conventional open-air CCD-based system using spatial and temporal processing methods (with the CCD system, the spatial [black] and temporal [red] curves directly overlap). With the spatial algorithm, the fiber-based system is less sensitive to changes in flow speed. However, the temporal contrast curve for the fiber-based probe has the same contrast response to flow speed as the CCD-based system.

flow speed increased (Fig. 2b). The temporal contrast values were slightly higher using the fiber-based system, but they follow the same trend and have a similar slope as those measured with the open-air system. However, the spatial contrast values using the fiber-based LSI probe are significantly higher and do not demonstrate the same dynamic range as the temporal algorithm or the scientific CCD camera-based system. Using the CCD system as the gold-standard for LSI measurements, if we compare the slopes between each subsequent measurement for the fiber-based spatial contrast curve relative to the scientific system, the fiber-based system slopes are associated with an error greater than 75%.

Evaluation of Fiber-Based LSI to Characterize In vitro and In vivo Pulsatile Flow

The combination of our fiber-based LSI probe and rolling temporal processing algorithm enables *in vitro* identification of the presence of pulsatile flow at different frequencies (Fig. 3). Figure 3 shows results from our *in vitro* pulsatile pump tests at frequencies ranging from 40 to 120 bpm (0.67–2.00 Hz), to span the physiological range of human heart rates [18]. Based on qualitative analysis of the *in vitro* contrast waveform, we observed a pulsatile component. The corresponding frequency domain analysis (Fig. 3) confirmed the presence of the expected frequencies. With stationary Intralipid, the signal strength is predominantly at DC (0 Hz). The power of the FFT peak decreased as the frequency of the signal increased; however, at all frequencies it is clearly distinguishable from the no-flow signal at 0 Hz, and the peak is present at the expected frequency with an accuracy of $\geq$98% (Fig. 3).

We also collected measurements from the upper incisors of three subjects with healthy teeth (Fig. 4). In the first subject, the main frequency signal detected was slightly above 1 Hz, at a frequency of 69 bpm (Fig. 4a), which matched the recorded heart rate measured with a wearable commercial pulse oximeter. The second subject had a higher recorded heart rate, and we detected the main frequency signal at 90 bpm (Fig. 4b). We detected the main frequency peak in the physiological range to be 57 bpm for subject three (Fig. 4c), which also matched their measured heart rate.



Frequency	Error
40 bpm	1.39%
60 bpm	0.42%
80 bpm	0.54%
100 bpm	0.33%
120 bpm	0.62%

Fig. 3. Frequency analysis of *in vitro* contrast waveforms resulting from use of the rolling temporal algorithm. We can clearly identify each of the different pulsatile frequencies (0.66 Hz [40 bpm] – 2.00 Hz [120 bpm]) of the pump. The error between the detected peak frequency and the actual frequency is less that 1.4% for all the measured frequencies.

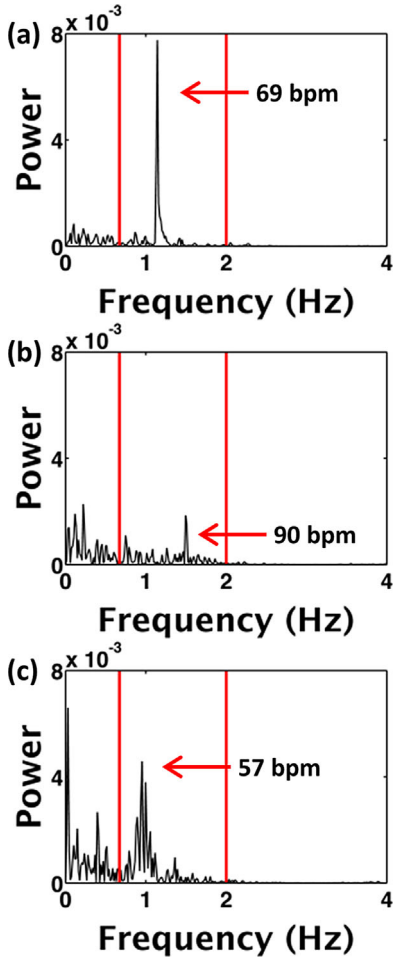


Fig. 4. Frequency analysis of signal from the healthy upper incisor of three subjects. The range of physiological heart rate frequencies (0.67–2.00 Hz) is bounded by the red lines. **a**: There is a peak just above 1 Hz (at 69 bpm) that corresponds to the measured heart rate of the subject. **b**: This subject had a higher resting heart rate, and the peak in the physiological range is present at 1.5 Hz (90 bpm). **c**: This subject has a heart rate peak present near 1 Hz (at 57 bpm). There is some low frequency noise present in the signal, below the physiological range, which is probably due to motion artifacts.

DISCUSSION

We built and validated a fiber-based LSI probe, to detect pulsatile flow (Fig. 1). We compared two different processing algorithms to compute speckle contrast from the raw image data (Fig. 2). We demonstrated the ability of the probe to characterize pulsatile flow in both an *in vitro* system (Fig. 3) and *in vivo* in human subjects (Fig. 4). Collectively, our findings demonstrate the ability of the LSI methodology to assess the presence of pulsatile blood flow.

Current methods for diagnosing pulpal vitality are called sensibility tests. These tests involve the application of a stimulus, usually either heat, cold, or an electric pulse,

to the tooth and rely on an assessment of the patient's response by the clinician [1–3]. These tests can be painful, and are a subjective measure of innervation rather than blood flow. Additionally, primary and immature teeth lack the neurons that respond to thermal and electrical tests, making these tests inaccurate in children and teenagers [20]. These tests have low reported sensitivity values: 0.86, 0.83, and 0.72 for heat, cold, and electric pulp testing, respectively [3]. Therefore, there is a high rate of false negatives, meaning root canal treatments are performed on teeth that still had blood flow and were viable. Our results suggest that LSI can detect pulsatile flow in teeth, which we postulate will lead to a painless, quantitative, objective assessment of pulpal vitality.

Temporal speckle contrast was more sensitive to changes in flow than the spatial contrast (Fig. 2b). We believe this effect is due to the honeycomb pattern associated with the individual fibers of the leached fiber bundle (Fig. 2a). This pattern artificially introduces zero intensity values into the image, which increases the standard deviation of intensity values in the sliding window, while simultaneously decreasing the mean intensity in the same region. This has the effect of increasing contrast values, and decreasing the influence of the desired blood-flow component of the signal. However, with the temporal algorithm, the honeycomb pattern is fixed in space, mitigating its effect on subsequent analysis of the signal of interest (Fig. 2a).

We processed the raw speckle data with both the spatial and temporal algorithms to assess if we could use a single deterministic value to threshold between "flow" and "no-flow" conditions. This approach previously was proposed as a potential method for measuring pulpal vitality [9]. Here, we found a slightly higher sensitivity to changes between "no-flow" (0.0 mm/second) and "flow" (0.25–2.0 mm/second) conditions when we used the temporal algorithm as compared to the spatial algorithm (27% relative change vs. 2% relative change, respectively), further confirming that temporal processing was more suitable for LSI with a fiber bundle. This maximum difference in "flow" and "no-flow" contrast values is small, even under ideal, controlled conditions. We observed that the change in average *in vivo* contrast values from the upper incisors of a single subject collected during separate imaging sessions, consistently differ (>50% of the time) by more than the contrast value change measured *in vitro* between simulated no-flow and flow conditions (Fig. 2b).

Hence, our data suggests that the use of a single contrast value to diagnose pulpal vitality is flawed. As an interferometric technique, LSI is extremely sensitive to motion. Since LSI typically measures relative changes in motion rather than absolute values, we determined that using a single threshold speckle contrast value, as proposed previously, [9] is not feasible *in vivo*. Any external movement or vibrations of the fiber probe lead to changes in the absolute contrast values. Changes in the orientation of the device relative to the tooth, or the uniformity of the laser illumination may also cause a change in the measured contrast. We also previously demonstrated that speckle contrast values may even vary intratooth due to local variations in tooth thickness [21].

Due to this result, we instead investigated the ability of frequency-domain analysis of speckle contrast data to assess pulpal blood flow. With our probe and analysis method, we accurately characterized the pulsatile flow both with an *in vitro* flow system (Fig. 3) and *in vivo* in three human subjects (Fig. 4). We distinguished between the presence and absence of a pulsatile signal in the *in vitro* system by analyzing the contrast waveform in the frequency domain. By taking data from healthy human incisors, we demonstrated that our system is also capable of detecting pulsatile flow *in vivo* (Fig. 4). Figure 4a shows a clear-cut result from a subject with a high signal to noise waveform, and the peak at 69 bpm is clearly visible. The subject measured in Figure 4b had a higher resting heart rate of 90 bpm, which demonstrates that the technique is sensitive to different frequencies. There is more low frequency noise present in Figures 4b and c, which we believe is due to motion artifacts such as motion of the subject's head relative to the imaging fiber bundle, or incidental movement of the laser fiber. However, these additional frequency spikes lay outside of the physiological heart rate range, indicated by the red lines [18], and could potentially be removed with more advanced signal processing methods. Another potential source of noise is demonstrated in Figure 4c. There is a clear peak at 57 bpm, however, other frequencies surrounding this peak in the FFT spectrum exist and may be due to the fact that heart rate *in vivo* is not perfectly constant, and fluctuates during the measurement period by a few beats/minute. We postulate that endodontists can use such frequency-based information to assess the presence of blood flow in a tooth, making this a critical step in achieving a clinic-ready solution to this diagnostic challenge.

Although the current fiber-based probe enables flow characterization in teeth, we propose that design improvements will make the probe more robust. Since LSI is inherently sensitive to motion artifacts, further efforts should involve improvement on the fiber design, removal of the honeycomb artifact, or perhaps avoid use of fibers altogether for this specific application. With continued progress made in consumer-grade optical and electronic components, miniaturization of the probe is a desired outcome, and is the subject of future work. For example, a miniaturized probe that clips onto a tooth or fits into a dental mouthguard may help reduce motion artifacts as well as improve our access to other teeth such as the molars.

In conclusion, here we describe a first-generation LSI probe that enables characterization of pulsatile flow. We proposed the use of frequency-domain analysis of temporal speckle contrast waveforms to assess the pulsatile frequency of blood flow, both *in vitro* and *in vivo*. Our data demonstrate the potential of LSI as a simple pulpal vitality test that avoids noxious stimuli and directly assesses blood flow as opposed to pulpal innervation.

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