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Blood flow measurements at different depths using photoplethysmography and laser Doppler techniques

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Abstract

Background/purpose: This study has evaluated a multi parametric system uniting laser Doppler flowmetry and photoplethysmography in a single probe for the simultaneous measurement of blood flow at different depths in the tissue. This system will be used to facilitate the understanding of pressure ulcer formation and in the evaluation of pressure ulcer mattresses.

Methods: The blood flow in the tissue over the sacrum was measured before, during and after loading with 37.5 mmHg respectively 50.0 mmHg. The evaluation of the system consisted of one clinical part, and the other part focusing on the technicalities of the probe prototype.

Results: An increase in blood flow while loading was the most common response, but when the blood flow decreased during loading it was most affected at skin surface and the blood flow responses may be different due to depths of measurement. Reactive hyperaemia may occur more frequently in the superficial layers of the tissue.

Conclusion: The study showed that the new system is satisfactory for measuring tissue blood flow at different depths. The laser Doppler flowmetry complements the photoplethysmography, and further development of the system into a thin flexible probe with the ability to measure a larger area is required.

Key words
Pressure ulcers, blood flow, Photoplethysmography, laser Doppler Flowmetry, non-invasive.

Running head
Blood flow measurements using PPG and LDF techniques
**Background**

A pressure ulcer is a complication related to the need for the care and treatment of primarily disabled and elderly people. Living with a pressure ulcer affects a person’s life physically, socially (1, 2) and mentally (2, 3), and is often associated with pain (1-4). It is important in nursing care to be able to identify patients who are at risk of developing pressure ulcers. The extrinsic factors known to be related to pressure ulcer development are pressure, time (5), temperature (6), moisture, friction and shear (7, 8).

Two of the more important aspects of prevention of pressure ulcers are the lowering of interface pressure using mattresses or overlays that optimize weight distribution (9) and pressure-relieving measures like turning the patient or using alternating-pressure mattresses (10). The problem with using this equipment is the poor level of evaluation in this area (11, 12), which makes it difficult to provide the most effective and cost-effective prevention to a patient. External pressure affects individuals very differently. Young, healthy people are less affected by loading than are patients with hemiplegia (6), and geriatric and paraplegic patients are more likely to have decreased skin blood flow than are young and healthy people (8, 13). Therefore, it is preferable to measure tissue blood flow instead of interface pressure (12).

Blood perfusion is crucial in fulfilling the tissue’s requirement for nutrition and oxygen, and in the transportation of waste products from the tissue. Blood perfusion also has an impact on an individual’s blood pressure, blood volume and temperature regulation. Regulation of blood flow is strongly linked to vasomotion, the constriction and dilatation of blood vessels (14). Vasodilatation leads to an increase in blood flow and is thought to protect the tissue from ischemia during mechanical loading (15-17). A rapid reduction in or elimination of external pressure that has caused ischemia in the tissue results in a considerable increase in skin blood
flow to above baseline levels (16), called reactive hyperaemia. Findings indicate that there are many mechanisms involved in pressure-induced vasodilatation and, despite the development of pressure-induced vasodilatation, the pressure application can impair blood flow (15).

The laser Doppler flowmetry (LDF) and photoplethysmography (PPG) techniques are appropriate methods, as they reflect the blood flow directly at different tissue levels (12). If these two techniques were to be combined into a single probe for measuring blood flow at different depths simultaneously, there would be new, improved possibilities to explore the area of pressure ulcer formation. LDF is an optical, non-invasive method for monitoring microvascular blood flow. It is a method used in many areas such as leg ischemia, diabetes, connective tissue diseases, drug effects in patients (18), thermal injury and plastic surgery, and in many organs such as the skin, brain, liver and kidney (19). The advantages of LDF are its relatively low price, its ease of use (which leads to low operator bias) and its being well validated (18).

Like LDF, PPG is an optical, non-invasive technique for measuring blood flow in tissue (20). The clinical applications of PPG are widespread in different areas, for example monitoring physiological responses such as heart rate, respiration and blood oxygen saturation (21). PPG has also been used for vascular assessment of arterial disease, endothelial function, microvascular blood flow and tissue viability. Another area of use is for measurement of autonomic functions like vasomotor function, thermoregulation and neurological assessments. PPG is also used in commercially available medical devices due to its low-cost technology and non-bulky components (21).
**Objective**

The aim of this study was to evaluate a multi parametric system combining laser Doppler flowmetry and photoplethysmography into a single probe, for the simultaneous measurement of blood flow at different depths in the tissue over the sacrum when the tissue is exposed to external loading. This new system will be used to facilitate the understanding of pressure ulcer formation.

**Methods**

**LDF**

LDF is based on the principle that monochromatic light incident on the tissue is scattered and, if reflected by a moving scatterer, is Doppler broadened. This frequency shift is detected and presented in arbitrary units (Volts) as an estimate of the perfusion. The perfusion is linearly related to the velocity ($v_{RBC}$) and the concentration of moving red blood cells ($c_{RBC}$), provided there is a low blood cell concentration (22-24):

$$\text{perfusion} = <v_{RBC}> \times c_{RBC}$$

A laser Doppler flowmeter with a HeNe laser, wavelength 632.8 nm, was used (PeriFlux Pf2b, Perimed, Järfälla, Sweden). The depth of measurement was approximately a few hundred micrometres.

**Photoplethysmography**

A light source emits light of a certain wavelength towards the tissue of examination. The light is absorbed, scattered and reflected in the tissue and the blood, and a part of the reflected light is detected by a photo detector. The depth the light penetrates depends on the wavelength and
the distance between the light source and photo detector (25). Green light is suitable for measurements of superficial blood flow and the near infrared (IR) (880 nm) for measurements of muscle blood flow deeper in the tissue (26). The PPG signal can be divided into two separate parts, an AC and a DC signal. The AC signal correlates directly to blood flow and is synchronous with the heart rate. It reflects the arterial blood flow in the vascular bed (27) in terms of both pulsatile blood volume variations and orientation of RBCs (28, 29). The DC signal is a baseline reflecting the total blood volume (27) and varies slowly based on vasomotor activity, respiration and thermoregulation (21). Factors that may affect the amount of light received by the detector include blood volume, movement of the vessel wall and the orientation of the RBC (28, 29).

A three-channel PPG instrument (Department of Biomedical Engineering, Linköping University, Linköping, Sweden) was used and green light (560 nm) and near infra red light (810 nm) were used to penetrate the tissue at different depths.

**The optical probe**

The probe consisted of three pairs of light emitting diodes (LED) placed symmetrically around a photo detector and one laser Doppler (LD) fibre optic probe inserted between the IR LEDs (Figure 1). The distance from the photo detector was 5mm for the green LEDs, and 10 mm and 25 mm for the IR LEDs. With this combination of wavelengths and distances, the depths of the measurements were assumed to be approximately 2 mm, 8 mm and 20 mm. The probe was a prototype and the components were integrated in a silicone plate that was fixed in a stiff plate of 10*10 cm. The probe was integrated in the test bench and did not exert a pressure itself on the subject’s tissue.
Figure 1: The design of the optical probe with light emitting diodes, LEDs, placed symmetrically around a photodetector and a laser Doppler fibre fixed in a black silicone plate.

Subjects

Seventeen individuals of both sexes aged over 60 years were recruited to participate in this study. The participants considered themselves as healthy.

Approval for this study was granted by the Research ethical committee at Linköping, Dnr M166-06.

Procedures

All measurements were performed in the same room during the day. The participants’ height and weight were noted. Subjects were supine in a quiet room and the temperature of the room
was measured. They rested for 15 minutes and their blood pressure, pulse, body temperature and skin temperature were noted. The blood flow in the tissue over the sacrum was measured while the subjects were lying on their stomach during two periods of loading. Five kilograms (approximately 37.5 mmHg) and 7 kg (approximately 50 mmHg) were chosen on the basis of an earlier study. In geriatric patients, loading of the tissue in the sacrum and the gluteus maximus with 11-50 mmHg has been shown to impair blood flow measured using laser Doppler (6). Pressure is the force per unit area (N/m²), where 1 Newton (N) is equivalent to 1 Pascal (Pa) and 1 millimetre of Mercury (mmHg) is approximately 0.13 kPa.

First, a baseline measurement of 5 minutes without loading was performed, followed 5 minutes of loading with 37.5 mmHg, then 5 minutes without loading, further loading with 50.0 mmHg in 5 minutes, and finally 5 minutes without loading. After the measurements were taken, skin temperature was registered. During the session the subjects were asked to lie as still as possible.

**Data collection and analysis**

Blood flow was recorded continuously on a computer for a session of 25 minutes using a Labview program (Labview 6.1, National Instruments, Kista, Sweden) at a sampling frequency of 75 Hz. On one occasion, the session was shortened to 20 minutes because the participant had difficulty lying on his stomach. Therefore the periods of unloaded tissue for this participant were reduced while the period of loading remained the same (5 minutes for each loading) as for the other participants.

All the adjustments on the instruments were noted on a protocol together with background data and skin temperature. Blood flow was analysed further by a computer program (IMT, Linköping University, Linköping, Sweden) that computed mean amplitudes for the AC
signals and mean value for the DC signals and LD signal. Mean amplitudes and mean values were computed from seven occasions during each session: during the first unloaded period, during load at 37.5 mmHg, directly after removing the weight, just before loading with 50.0 mmHg, during load at 50.0 mmHg, directly after removing the weight and, finally, just before ending the session. The time periods that were computed were between 15-20 seconds and were chosen based on the quality of the signal. When the signal showed no pulsations, blood flow was assessed as closed.

The measurements were validated through palpation of the radial artery pulse while recording blood flow; the pulsations were always controlled against the computer view to control the agreement between manually checked pulsations and the visual pulsation curve on the computer screen.

Body temperature was measured using the Braun ThermoScan 6022 (Kronberg, Germany), skin temperature using a Raytek Raynger ST IR thermometer (Santa Cruz, California, USA), and room temperature with a Schwille Elektronik type 565 digital thermometer (Kircheim, Germany). Blood pressure was measured with a manual device from Speidel & Keller (Jungingen, Germany) and the pulse was counted manually.

All the physiological responses described in the result were analysed individually for each participant as relative change in per sent in blood flow on the basis of blood flow at baseline.

**Statistics**

Background variable values are presented in terms of mean ± standard deviation. Differences in age and body mass index (BMI) between genders are compared using an independent
sample T-test, and differences in skin temperature prior to and post measurement are compared using a paired sample T-test. P < 0.05 was considered to be significant.

The relative changes in per cent for the different depths are presented as a single box plot for each variable on the category axis. Box plots show the median, quartiles and extreme values for the variable. In these, the relative changes in per cent are shown on the y-axis and the points in time on the x-axis for each depth.

**Results**

There were 17 participants, 11 women and 6 men. Mean age was 68.5 ± 7.1 years and mean BMI was 24.3 ± 2.4; there were no significant differences between the genders. All participants had a normal pulse of 65.7 ± 7.4, systolic blood pressure of 129.1 ± 13.6 and diastolic blood pressure of 74.4 ± 8.8. Skin temperature varied from 32.3 ± 1.8 °C prior to measurement to 32.7 ± 0.8 °C after measurement, and the difference was not significant (Table 1). One participant had an irregular pulse, and one was a smoker. None of the participants experienced discomfort or pain during the periods of loading.

**Table 1: Overview of subject measurements, presented as mean ± SD**

<table>
<thead>
<tr>
<th>Background variables</th>
<th>N=17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient temperature (°C)</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.4 ± 0.4</td>
</tr>
<tr>
<td>Skin temperature prior to measurements (°C)</td>
<td>32.3 ± 1.8</td>
</tr>
<tr>
<td>Skin temperature after measurements (°C)</td>
<td>32.7 ± 0.8</td>
</tr>
<tr>
<td>Differences in skin temperature (°C)</td>
<td>0.5 ± 1.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129.1 ± 13.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.4 ± 8.8</td>
</tr>
<tr>
<td>Pulse</td>
<td>65.7 ± 7.4</td>
</tr>
</tbody>
</table>
The most common pattern of blood flow response during the measurement in all individuals is described as “the standard appearance”. The standard appearance for the PPG channels for the participants was seen in 10 individuals. The blood flow increased during load at 37.5 mmHg and 50.0 mmHg, and the increase was higher at 50.0 mmHg than at 37.5 mmHg for seven individuals. During unloading, the blood flow decreased to a level near baseline or slightly over or under this level. During these periods of unloading, the blood flow either increased or decreased moderately (Figure 2).

Figure 2: Example of the standard appearance in a subject exposed as the relative change in per cent at the three PPG signals. t1 is baseline, t2 is the point in time during loading with 37.5 mmHg, t3 is directly after removing the 37.5 mmHg weight, t4 is just before loading with 50.0 mmHg, t5 is during loading with 50.0 mmHg, t6 is directly after removing the 50.0 mmHg weight and t7 is just before ending the session.
The overall relative change using green light PPG showed increases in blood flow during loading. The median for the relative change at 37.5 mmHg load was 31.1% with q₁ = 9.5% and q₃ = 37.1%. The median for the relative change at 50.0 mmHg load was 29.2% with q₁ = 14.1% and q₃ = 46.8% (Figure 3).

**Figure 3**: Relative change in per cent at green light at PPG with median, quartiles and extreme values at the 7 points in time. t₁ is base line, t₂ is the point in time during loading with 37.5 mmHg, t₃ is directly after removing the 37.5 mmHg weight, t₄ is just before loading with 50.0 mmHg, t₅ is during loading with 50.0 mmHg, t₆ is directly after removing the 50.0 mmHg weight and t₇ is just before ending the session.
The overall relative change using superficial IR light PPG was larger than with the green light. The increase at 50.0 mmHg load was larger than at 37.5 mmHg load. The median for the relative change at 37.5 mmHg load was 43.6% with $q_1 = 8.8\%$ and $q_3 = 54.9\%$. The median for the relative change at 50.0 mmHg load was 59.9% with $q_1 = 21.2\%$ and $q_3 = 99.6\%$ (Figure 4).

Figure 4: Relative change in percent at superficial IR light at PPG with median, quartiles and extreme values at the 7 points in time. $t_1$ is base line, $t_2$ is the point in time during loading with 37.5 mmHg, $t_3$ is directly after removing the 37.5 mmHg weight, $t_4$ is just before loading with 50.0 mmHg, $t_5$ is during loading with 50.0 mmHg, $t_6$ is directly after removing the 50.0 mmHg weight and $t_7$ is just before ending the session.
The overall relative change using deep IR light PPG was similar at the two sessions of loading and the variances were the greatest of the three PPG measurements. The median for the relative change at 37.5 mmHg load was 52.3% with $q_1 = 20.5\%$ and $q_3 = 72.8\%$. The median for the relative change at 50.0 mmHg load was 53.9% with $q_1 = -1.5\%$ and $q_3 = 110.6\%$ (Figure 5).

![Deep IR light](image)

Figure 5: Relative change in per cent at deep IR light at PPG with median, quartiles and extreme values at the 7 points in time. t1 is base line, t2 is the point in time during loading with 37.5 mmHg, t3 is directly after removing the 37.5 mmHg weight, t4 is just before loading with 50.0 mmHg, t5 is during loading with 50.0 mmHg, t6 is directly after removing the 50.0 mmHg weight and t7 is just before ending the session.
The blood flow measured using the LDF showed more variance than the PPG signals and no standard appearance was found. The median for the relative change at 37.5 mmHg load was 14.0% with $q_1 = -28.2\%$ and $q_3 = 106.9\%$. The median for the relative change at 50.0 mmHg load was -6.9% with $q_1 = -54.2\%$ and $q_3 = 26.4\%$ (Figure 6).

**Figure 6:** Relative change in per cent at laser Doppler with median, quartiles and extreme values at the 7 points in time. $t_1$ is base line, $t_2$ is the point in time during loading with 37.5 mmHg, $t_3$ is directly after removing the 37.5 mmHg weight, $t_4$ is just before loading with 50.0 mmHg, $t_5$ is during loading with 50.0 mmHg, $t_6$ is directly after removing the 50.0 mmHg weight and $t_7$ is just before ending the session.
**Decreases in blood flow**

Three individuals showed decreases in blood flow using PPG while loading. One of these individuals showed decrease only at load at 50.0 mmHg, but at all three channels. The other two showed decreases in blood flow at 37.5 mmHg and 50 mmHg using superficial IR light and at 50 mmHg using deep IR light. But due to interference in the measurements, data from loading using green light in these two individuals were not available, as was the case with deep IR light on one occasion, but the measurements during unloading do not contradict the possibility that there could be decreases in blood flow even at these depths and loads.

Thirteen individuals had a decrease in blood flow using LD while loading, and this considerable variance is reflected as relative change over time. Five of the individuals only had a decrease loading with 37.5 mmHg, while four of them had decreases at both 37.5 mmHg and 50.0 mmHg loads. Three of the individuals had total occluded blood flow using LD, two of whom showed occlusion at both 37.5 mmHg and 50.0 mmHg load.

**Reactive hyperaemia**

Reactive hyperaemia was seen in four individuals on the PPG channels. Two of these individuals showed reactive hyperaemia at both 37.5 mmHg and 50.0 mmHg loads using green light. The third individual showed reactive hyperaemia at both 37.5 mmHg and 50.0 mmHg load using green light and superficial IR light, and at 50.0 mmHg using deep IR light. The last individual showed reactive hyperaemia on one occasion, at 50.0 mmHg load using superficial IR light.

Reactive hyperaemia was seen in 14 individuals using laser Doppler flowmeter. In four individuals a different pattern appeared: there was araid increase and a high level was
reached but instead of a gradual decrease, the blood flow increased during the entire period of unloading, i.e. 5 minutes (Figure 7).

Figure 7: Reactive hyperaemic response with laser Doppler in one individual. After removal of the 37.5 mmHg weight, the response is normal. After removal of the 50.0 mmHg weight, a gradual increase of the blood flow during the whole period of unloading is shown.

One individual showed no response in blood flow using PPG while loading with 37.5 mmHg, but showed a considerable decrease in blood flow to a level far below baseline at all three depths. At 50.0 mmHg load, the blood flow increased to a level above the baseline but decreased again to an even lower level than before while unloading.
Discussion

The aim of this study was to evaluate a multi-parametric system combining LDF and PPG in a single probe for the simultaneous measurement of blood flow at different depths. The tissue over the sacrum was chosen because it is an area prone to pressure ulcer development and is exposed to external loading to a large extent in immobile individuals. Further development of the system may lead to a clinical application for measuring tissue blood flow in the sacrum area while a patient is lying in bed, for example.

The evaluation of the system consisted of two parts: a clinical part, focusing on the ability of the system to detect relevant physiological responses in blood flow at different depths and validation of the responses, and another part focusing on technical aspects and limitations of the probe prototype.

Physiological responses

The main physiological findings in this study are that the blood flow increases using PPG when the subject’s tissue is exposed to mechanical load at 37.5 mmHg and 50.0 mmHg. This result was expected, mainly due to three reasons. The subjects in this study are healthy and active in their daily life. Other studies point out that the state of illness, rather than age, is important (30, 31). Healthy subjects may respond to the applied pressure with a compensatory increase in blood flow to protect the tissue (16, 32). The fact that the tissue is compressed while loading affects the signal. When measuring with PPG on a compressed tissue, compared to an unloaded tissue, the light penetrates more deeply into the tissue to a certain depth, and therefore reaches deeper and larger vessels. The result is an increase in the PPG signal.
Blood flow seems to be most effected at skin surface, shown by LDF. At this depth, a severe reduction in blood flow while loading with 37.5 mmHg and 50 mmHg was found in 13 individuals, and blood flow was totally occluded in three of these individuals. This is unexpected, considering that this study encompassed healthy individuals and the tissue was exposed to relatively low pressure. Previous studies have shown that sitting, healthy individuals (mean age 34 years) required pressure values exceeding 120 mmHg for blood flow occlusion, but the geriatric, hospitalized group experienced occlusion below 40 mmHg (8), and pressure over 60 mmHg most likely changes skin blood flow in both healthy individuals (mean age 39 years) and patients, but lower values can decrease blood flow in patients (6). The lower pressure values that led to occlusion in this study may be due to the higher age of the participants, despite their healthy condition.

Reactive hyperaemia seems to occur more frequently in the superficial layers of the tissue because LDF following by green light most often detected the phenomenon. This study has detected two types of reactive hyperaemia. The most common response was classical reactive hyperaemia, described in the literature as the rapid increase in blood flow to a high level immediately after unloading, and a gradual decrease to normal level. But another type of reactive hyperaemia was seen in 4 individuals using LDF: after the rapid increase in blood flow, the flow continued to increase during the entire period of unloading, i.e. 5 minutes. Three of these subjects were men, and the woman was a smoker. Previous studies have shown that smokers have a depressed hyperaemic response (33).

One interesting finding was related to the fact that one subject did not show a large increase in blood flow using PPG while loading, but when unloading the tissue the blood flow decreased to a level far below baseline. Is it a negative pattern that makes a person predisposed to
pressure ulcer development, or does this person not require the compensatory mechanism that the hyperaemic response provides?

Validation of the measurements by palpation of the radial artery pulse while recording the blood flow was unproblematic; agreement between manually checked pulsations and the visual pulsation curve on the computer was total, and it was not a difficult procedure. The subject who had a normofrequency atrial fibrillation showed irregular pulsations using PPG and LDF, as well as when the pulse was manually checked, and they were totally synchronous with each other. This validation proves that the arterial pulsative blood flow was measured using LDF and PPG.

The prototype of the probe

Reactive hyperaemia is a strong indicator of previous ischemia in the tissue, and there are no other phenomena that can explain this condition. Therefore, there are grounds to conclude that if reactive hyperaemia is detected, the tissue has been exposed to ischemia even if there is no decrease in previous blood flow during loading. Likewise, if there is an occlusion or a decrease in blood flow detected while loading but no reactive hyperaemia is seen, ischemia may still be present in the tissue. It is unlikely that the subjects are unable to respond with reactive hyperaemia, as LDF detected this response in the skin surface. Reactive hyperaemia was always seen in the superficial layers if it was detected in the deeper layer, except for in one subject, in whom there was a single occasion of reactive hyperaemia in superficial IR, and in this case it is likely that the probe had been dislocated. The reason for these problems is likely the design of the probe. The probe is a solid stiff plate with only one detector each for PPG and the LDF, resulting in measurements at only one point. Depending on the subject’s body constitution, the probe had a tendency to move the skin surface in relation to underlying tissue. The detector may have been dislocated during loading and therefore did not measure
the exact same spot while loading and unloading the tissue, respectively. Because of these problems with the movement of the probe, it is important to further develop the probe prototype for future measurement. The probe needs to be flexible and thin so it does not influence the tissue, and it is desirable to have a matrix of detectors in the probe so a larger area can be measured instead of a single point as in the present prototype, which would make it easier to detect changes in blood flow.

The green light of PPG measures blood flow at a depth of approximately 2 mm, and LDF measures at an approximate depth of 0.5-1 mm. These two techniques do not fully agree; it was only in 4 individuals that the same pattern was seen with both techniques. Therefore, it is necessary to continue the development of the probe involving both the LDF and the PPG technique, as they complement each other.

In this study, loss of data has occurred periodically due to disturbances in the PPG, which prevented analysis in four cases of loading in three individuals. This may have been due to inaccuracy of the PPG instrumentation in handling large variations in signal strength; further development is needed in this area. In three individuals it was difficult to obtain a clear baseline signal at the beginning of the measurements using deep IR light, which may be because the probe was not in good contact with the skin surface. Further development of a flexible probe will hopefully solve this problem.

**Conclusions**

The study concludes that the new system with integrated LDF and PPG is satisfying for measuring tissue blood flow at different depths. All the main blood flow responses were expected and well documented in previous literature and therefore support the fact that the tissue blood flow had been measured. An increase in blood flow while loading at 37.5 mmHg
and 50.0 mmHg was the most common response in the study, but when the blood flow decreased during loading it was most affected at skin surface.

The possibility to measure blood flow at different depths provided new interesting findings and indicates that the blood flow responses may be different due to depths of measurement. Reactive hyperaemia may occur more frequently in the superficial layers of the tissue. Two types of reactive hyperaemia were shown using LDF: the “classic” rapid increase after unloading the tissue, and a slow gradual increase during the entire period of unloading.

This study has shown that the LDF complements the PPG, and further development of the system into a thin flexible probe with the ability to measure a larger area and handle larger variations in signal strength is needed.
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