ABSTRACT
Accurate knowledge of the thermal conductivities of biological tissues is important for thermal bioengineering, including applications in cryopreservation, cryosurgery, and other thermal therapies. The thermal conductivity of biomaterials is traditionally measured with macroscale techniques such as the steady longitudinal heat flow method or embedded thermistor method. These techniques typically require relatively large, centimeter-scale samples, limiting their applicability to finer biological structures. They are also vulnerable to errors caused by thermal contact resistances and parasitic heat losses. In contrast, the thermal conductivity of inorganic solids such as semiconductor wafers and thin films is commonly measured using the “3 omega method” [1-3]. This frequency domain technique is robust against thermal contact resistances and parasitic heat losses. It routinely has sub-millimeter spatial resolution, with theoretical limits down to tens of microns. Here we adapt the 3 omega method for measurements of biological tissues. Thermal conductivity measurements are made on both frozen and un-frozen samples including agar gel, water, and mouse liver, including samples with sub-millimeter thicknesses. The measurement results compare favorably with literature values and span the range from around 0.5 to 2.5 W/m-K. This study demonstrates the promise that this technique holds for thermal measurements of bulk tissues as well as fine sub-millimeter samples.

INTRODUCTION
Thermal therapies are used in several areas of medical treatments. For example, thermal ablation has been used to treat hepatic cancer and has been shown to be superior to other treatment methods [4,5], while cryoablation has been used to treat prostate, breast, and renal cancer [6-8]. In cardiology, cryopreservation of heart valves and blood vessels is used to maintain tissue function for grafts and transplants [9-12], and controlled heating and cooling of blood vessels is used to treat atrial fibrillation, peripheral artery disease, and renal hypertension [13-16]. All of these techniques rely on repeatable and predictable cooling and heating of biological tissues. In the case of ablation, the thermal necrosis volume depends critically on the thermal properties of the tissue. Under-estimating the thermal conductivity of local tissues risks creating a larger thermal necrosis volume than intended and damaging nearby healthy and potentially vital tissue, while over-estimation risks failing to kill all cancerous cells leading to a future relapse after treatment. In the case of cryopreservation, controlling the rate of cooling also depends on the thermal properties of the tissue. Cooling too slowly risks dehydrating cells, while cooling too rapidly risks the formation of intracellular ice, both of which can cause undesirable damage or even death to the tissue being preserved [17,18].
Several techniques currently exist for measuring the thermal conductivity of biological tissues, such as the guarded hot plate method, the cut bar method, and the embedded thermistor method [18]. However, there are inherent limitations to these techniques. They are susceptible to parasitic heat losses to the environment, thermal contact resistances, and size limitations requiring that samples must be at least approximately one centimeter large in all dimensions in order to be measurable [19-21]. This is important, because there are many tissues with characteristic lengths much smaller than 1 cm, such as heart valves (1 - 2 mm), the pulmonary vein (1 - 3 mm), the phrenic nerve (< 1 mm), the esophagus (1 - 3 mm), small diameter arteries (1 mm), and fascia (0.1 mm). Any of these tissues could be at risk when thermal therapies are applied to surrounding tissue, and this risk cannot be accurately quantified or controlled until the thermal conductivity of such thin (< 5 mm) tissues can be measured.

The “3ω” (or “3 omega”) method is a frequency domain electrothermal technique traditionally used to measure the thermal conductivity, \( k \), of inorganic solids such as semiconductor wafers and thin films, and which overcomes all of the above-mentioned difficulties of parasitic heat losses, contact resistances, and small (down to tens of microns) sample sizes [1-3]. However, the traditional 3ω method has its own limitations. For one, it requires samples to undergo a harsh microfabrication process during which a heater line is deposited on the sample. Such a process involves solvents, vacuum, and temperatures high enough to destroy biological tissues or permanently change their physical properties. Another limitation is the requirement that every sample must have its own dedicated heater line independently and permanently deposited.

In order to address these limitations, here we report a “supported 3 omega method,” in which we perform all the microfabrication on a separate known substrate. We then place the sample of interest on this substrate for thermal conductivity measurements. This adaptation of the 3 omega method has been done before, such as recently for nanofluid conductivity measurements. This adaptation of the 3 omega method has been done before, such as recently for nanofluid conductivity measurements. This adaptation of the 3 omega method has been done before, such as recently for nanofluid conductivity measurements. However, there are inherent limitations to these techniques. They are susceptible to parasitic heat losses to the environment, thermal contact resistances, and size limitations requiring that samples must be at least approximately one centimeter large in all dimensions in order to be measurable [19-21]. This is important, because there are many tissues with characteristic lengths much smaller than 1 cm, such as heart valves (1 - 2 mm), the pulmonary vein (1 - 3 mm), the phrenic nerve (< 1 mm), the esophagus (1 - 3 mm), small diameter arteries (1 mm), and fascia (0.1 mm). Any of these tissues could be at risk when thermal therapies are applied to surrounding tissue, and this risk cannot be accurately quantified or controlled until the thermal conductivity of such thin (< 5 mm) tissues can be measured.

The traditionnal 3ω method is a well established technique that has been in use for over 20 years, and has been extensively described in References [1,2]. Here, we briefly present the basic idea. A small metal heater line with dimensions 65 \( \mu \)m wide by 0.2 \( \mu \)m thick by 2000 \( \mu \)m long (L) is microfabricated directly onto the substrate to be measured, with current and voltage contacts in a four-probe configuration as in Fig. 1(a). A sinusoidal current \( I = I_0 \sin(\omega t) \) is sent through the heater line, with driving angular frequency \( \omega \). The electrical resistance of the heater line causes joule heating, which dissipates the electrical energy as heat at a rate proportional to the square of the current, resulting in a constant DC power output plus an AC power output at twice the driving frequency, \( Q = Q_0 (1 - \cos(2\omega t)) \). This causes the temperature of the heater line to oscillate at frequency 2\( \omega \). The thermal properties of the substrate determine how large of a temperature gradient is required to transport this heat away from the heater line and into the substrate. Therefore, the magnitude of the heater line’s temperature oscillations contains information about the thermal properties of the substrate.

### METHODS AND MATERIALS

1. **Background: Traditional 3 Omega Method**

<table>
<thead>
<tr>
<th>Current Techniques [18]</th>
<th>Traditional 3ω</th>
<th>Supported 3ω</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can Measure Samples &lt; 1 cm</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Negligible Parasitic Heat Loss</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Measurement Independent of Thermal Contact Resistance</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Usable on Bio Tissues</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Reusable Sensor</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Average Measurement Duration &lt; 1 hour</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>Does not Require Microfabrication</td>
<td>✓</td>
<td>✗ (necessary for every sample)</td>
</tr>
</tbody>
</table>
The oscillating temperature gradient results in a thermal wave that propagates radially away from the heater line into the substrate and decays exponentially with increased distance from the heater line. The frequency-dependent penetration depth, \( \lambda = \sqrt{D/\omega} \), of the thermal wave is a measure of the characteristic length scale for the exponential attenuation of the amplitude of the thermal wave. \( D \) is the thermal diffusivity of the substrate.

The \( 2\omega \) temperature oscillations of the heater line cause its electrical resistance to oscillate at \( 2\omega \), which combines with the \( 1\omega \) driving current to produce an oscillating voltage with a \( 3\omega \) frequency component. This \( 3\omega \) voltage signal, therefore, contains information about the thermal properties of the substrate and can be measured directly using a lock-in amplifier set to monitor the voltage at the third harmonic of the driving frequency. When the portion of this \( 3\omega \) voltage signal that is in phase with the driving frequency is plotted as a function of the logarithm of the driving frequency, the slope of the resulting line is inversely proportional to the thermal conductivity, \( k \), according to [1,2],

\[
k = \frac{V_{1\omega,\text{rms}}^2 I_{1\omega,\text{rms}}}{4\pi L \frac{dR}{dT}}
\]

where the equation has been expressed in terms of measurable quantities. \( V \) is voltage, \( I \) is current, \( dR/dT \) is the rate of change of the heater line’s electrical resistance with respect to temperature (discussed further in Section II), and subscripts denote harmonics and root-mean-square values.

The \( V_{1\omega,\text{rms}} \) voltage signal across the heater line combined with the known \( I_{1\omega,\text{rms}} \) driving current was used to calculate the average resistance of the heater line, which depends on the heater line’s temperature. The heater line was therefore also used as a thermometer to measure the temperature of the sample exactly where the \( k \) measurement was happening (discussed further in Section II).

Measurement of thin samples can be achieved by using high frequency driving currents that create short thermal wavelengths, whose penetration depth into the sample is controlled to be much less than the sample’s thickness. Because the measurement is transient, operating in the frequency domain, the thermal wavelengths are localized within the sample minimizing the effect of steady-state parasitic heat losses to the environment. We chose frequencies to ensure that \( \lambda \) was always at least 5x smaller than the sample thickness in all directions.

Another important feature of Eq. (1) is that thermal contact resistances will show up as a constant offset of the in-phase \( V_{3\omega,\text{rms}} \) versus \( \ln(\omega) \) line, not affecting the slope and hence not affecting the measured value of \( k \) [23]. We chose frequencies to ensure that \( \lambda \) was always at least 90 \( \mu \text{m} \) in both the sample and the sensor. Because the 1 \( \mu \text{m} \) dielectric layer was much smaller than \( \lambda \), it appeared as a contact resistance and did not affect the slope in Eq. (1).

II. Supported 3 Omega Method: Sensor Construction and Calibration

We begin the supported \( 3\omega \) method by preparing a sample according to the traditional \( 3\omega \) method, on an amorphous SiO\(_2\) substrate (1 mm thick glass). Using standard photolithography and liftoff microfabrication techniques, we patterned a gold heater line with dimensions 65 \( \mu \text{m} \times 0.2 \mu \text{m} \times 2000 \mu \text{m} \), on top of a 5 nm thick chromium adhesion layer. 76 \( \mu \text{m} \) diameter bare copper wire was attached to the four electrodes using conducting silver epoxy.

A 1 \( \mu \text{m} \) insulating dielectric layer was then deposited to insulate the heater line and electrical leads from samples that may be electrically conducting. This was accomplished by dissolving polystyrene pellets in toluene at a concentration of 15 mg / mL. This solution was dropped directly on top of the area to be insulated, and the surface was held vertical to
allow the excess solution to run off, while the remaining solution evaporated leaving behind a thin layer of polystyrene. This technique was tested on glass microscope slides and the repeatable thickness of the coating layer of polystyrene verified using a stylus profilometer. The glass substrate, heater line, and dielectric layer assembly is referred to as a “sensor.” After the sensor was prepared, samples were placed directly on top of the heater line to be measured as in Fig. 1(b).

Once constructed, each sensor was characterized by calibrating its thermal conductivity and electrical resistance, both as functions of temperature. First, the resistance was measured. A 30mA current at frequency $\omega/2\pi = 15$Hz was sent through the sensor while the entire structure was held at a constant temperature (temperature control is described in section III), and the $V_{10,\text{rms}}$ voltage signal was recorded. This was repeated at 5 to 10 different temperatures ranging from approximately -20°C to 25°C. From this, a plot of resistance ($R = V_{10,\text{rms}}/I_{10,\text{rms}}$) versus temperature was obtained, and a slope $dR/dT$ was extracted from a least-squares linear regression fit.

30mA allowed for strong signal-to-noise ratios, but we later realized that it caused non-negligible self heating of the heater line, of around 3 °C. Due to this offset uncertainty in the R(T) curve, another, higher accuracy measurement of the electrical resistance was performed at room temperature, $T_0 = 22.9$ °C. This was done by sweeping the current magnitude and plotting the resistance ($V_{10,\text{rms}}/I_{10,\text{rms}}$) versus the square of the current. This plot is linear, with the intercept ($R_0$) corresponding to the heater line electrical resistance in the limit of zero joule heating. The sensor temperature was measured before and after the sweep, and was found to be the same. Temperature was measured using a Keithley 2700 multimeter with a K-type thermocouple. Consistent with the known physics of R(T) for gold over our applicable temperature range, we assumed that our previously measured slope, $dR/dT$, was constant, and combined this slope with the known physics of $R(T)$ for gold over our applicable set of measurements.

After calibrating $dR/dT$, the thermal conductivity of the sensor was measured at five temperatures from approximately -15°C to 30°C, using Eq. (1). A quadratic fit was applied to the resulting plot to obtain a $k_{\text{sensor}}(T)$ function, as illustrated in Fig. 3.

For the traditional 3ω method, Eq. (1) calculates the thermal conductivity based on the rate of heat conduction from the heater line into the substrate below. In the case of the supported 3ω method, heat is conducted out of the heater line into both the substrate below and the sample above. If the sample were made of the same material as the substrate, it would double the heat loss and Eq. (1) would calculate twice the thermal conductivity. In the case when the sample and the sensor both have similar thermal diffusivities (within a factor of 10 of each other), this can be generalized to [24]
dielectric-sample interface, the greater the fraction of total heat directed down into the substrate. The total upper thermal impedance is a series sum of the dielectric layer thermal impedance [27], contact resistance [27], and the sample thermal impedance [28]. This combines in parallel with the lower substrate thermal impedance [28].

The majority of the error of using Eq. (2) is due to the mismatch between the sample and substrate’s thermal diffusivities. For our heater line geometry, for samples having a $D$ within an order of magnitude of $5 \times 10^{-8}$ m$^2$/sec, and for frequency ranges $\leq 20$ Hz, there can be a maximum total error of 3% in Eq. (2) as compared to the exact solution [25,26]. For our particular measurements we calculated a maximum error of no more than 2% from Eq. (2), and thus the corrections were left out of our calculations below.

The thermal flux directly from the sample to the substrate, laterally outside the footprint area of the heater line, is neglected in favor of the “boundary mismatch” model [24], which is a reasonable assumption when the thermal diffusivities are similar [25].

All samples were unfrozen and very hydrated when originally placed on the sensor and therefore can be assumed to be in intimate thermal contact with the dielectric layer. Accordingly, order of magnitude values [29,30] for the thermal conductance at this interface directly above the heater line were used to quantify its effect. The combination of this contact thermal resistance with the thermal impedance of the dielectric layer itself was found to contribute cumulatively negligible error toward the determination of $k_{\text{sample}}$ (at most 0.2% error but usually less than 0.02%) and was also left out of our calculations below.

### III. Sample Preparation and Mounting

The sensor was mounted on top of a Peltier module for temperature control with a thin layer of Omegatherm 201 thermal paste. The sensor-Peltier sandwich was housed inside an aluminium cavity with lid (wall thicknesses 1 cm) to shield against outside electrical noise and to reduce moisture evaporation from the sample. Metal spring clips clamped the sensor to the Peltier module and in turn to the cavity floor. A small hole in the cavity housing allowed access to electrical leads while padding helped mechanically anchor these wires. For subzero measurements, the entire system was placed inside a commercial freezer. The freezer’s rate of temperature drift due to cycling was measured to be no more than 0.25 °C/minute, whereas the temperature controller could correct temperature on the order of 1 °C/second, ensuring the sample’s temperature would be held constant. This setup is favorable over a Peltier-cooled sample in room temperature setup, because putting the entire housing in a freezer minimizes thermal gradients within the aluminium housing cavity, decreasing uncertainty in the temperature of the finite thickness sample. For suprazero measurements, the housing was placed in ambient atmosphere at room temperature. For all measurements, the temperature of the sample, the internal temperature of the freezer, and the ambient room temperature were recorded continuously throughout the experiment.

Samples were carefully placed directly on the heater line of the sensor, ensuring the sample extended laterally at least 0.5 mm around the perimeter of the heater line in all directions as in Fig. 1(b). Samples were held in place by their own weight and were not clamped. Because all samples were soft, clamping them in place would risk creating asymmetric pressures and altering their physical properties. No samples shifted position during measurement. All frozen samples (including ice) were initially placed on the heater line in their thawed form, which conformed to the surface of the sensor and heater line, and were then frozen in place. All samples were also carefully peeled off and inspected after each measurement to look for signs of trapped pockets of air, incomplete or non-uniform contact, or significant geometry changes during the experiment. None of these were ever observed.

We will now briefly discuss the preparation of each of the three different types of samples measured: ice, agar gel, and mouse liver. Deionized water was used for ice measurements. Water was boiled for 5 minutes prior to freezing. We found that placing a 30 μL bead of water on the heater line and then flash-freezing it from below using the Peltier module was the best way to minimize internally trapped air bubbles, especially near the bottom close to the heater line.

Agar gel was prepared by dissolving agarose powder in tap water preheated to approximately 65°C. The solution contained 0.5% agar gel by weight. The heated solution was stirred for 20 minutes until clear and colorless, and then allowed to set in a refrigerator over night.

Fresh mouse liver, stored in phosphate buffered saline, was used as the biological tissue to be measured. Thin samples were prepared by placing the bulk tissue in a recessed surface constructed from 1 mm thick microscope slides, while a microtome blade was used to slice across the top of the recession. All liver measurements were done within 3 days of receiving the fresh tissue, which was stored in the refrigerator in phosphate buffered saline when not being handled.

All thawed samples were covered with a layer of plastic wrap to minimize evaporative losses from the sample during the measurement. First, the samples were placed on the heater line, then a small square of plastic wrap was placed directly on top, large enough to cover both the sample and the sensor, and gently pressed down to fit the shape of the sample. Small quantities of water (for agar gel) or phosphate buffered saline (for liver) were injected between the plastic wrap and the sample to expel trapped air bubbles if necessary.

## RESULTS & DISCUSSION

A representative set of raw $\delta \omega$ data for 1 mm liver tissue at -15.7 °C is plotted in Fig. 4. The low frequency portion of the curve was used to calculate $k$. All thermal conductivities were calculated from within the frequency range $1 \text{ Hz} < \omega/2\pi < 20 \text{ Hz}$, due to physical limitations. In the
low frequency limit, \( \lambda \) approaches the thickness of the sample or sensor’s substrate. In the high frequency limit, \( \lambda \) approaches the width of the heater line. Both of these limits violate the analytical model used in deriving Eq. (1). These upper and lower bounds on \( \omega \) agreed with the raw data plots and with which frequency intervals were linear.

The experimental results are summarized in Fig. 5. All of the 3 mm liver measurements were done using the same sensor (sensor 1), while all the ice, agar gel, and 1 mm liver measurements were done using a second sensor (sensor 2). This demonstrates the reusability of the sensors. When measurements were done at suprazero temperatures, it took approximately 10 minutes to stop recording data for one measurement, remove the old sample, clean the sensor, put in a new sample, and begin recording data for the second measurement. In contrast, for the traditional 3\( \omega \) method each new sample would have to independently undergo the microfabrication process of having its own devoted heater line deposited before it could be measured.

All measurements in Fig. 5 reflect sample sizes 3 mm thick or thinner. All samples were at least 2 mm wide x 3 mm long, to ensure sufficient coverage of the heater line. The subzero 1 mm liver \( \lambda \) measurement in Fig. 5(b) lies within the range of values defined by the set of 3 mm subzero measurements, indicating that the measured thermal conductivity is insensitive both to the thickness of the sample and to the sensor used.

Water and agar gel measurements showed close agreement with literature, with an average error of 2% and 7% (compared to water) respectively. Liver tissue measurements also showed good agreement, but were consistently lower than reference values. Compared to the literature, measured subzero liver samples had an average error of 11%, and suprazero liver samples had an average error of 8%. We speculate that this may be because the liver reference is a convenient piecewise-fitted function to a spread of data, including samples from five different kinds of animals (pig, dog, human, cow, rabbit) none of which are the same as the animal (mouse) measured in this work.

**SUMMARY**

We discuss the application of the supported 3\( \omega \) technique for biological tissue thermal conductivity measurements. This is an adaptation of the traditional 3\( \omega \)
ACKNOWLEDGEMENTS

We thank Shannon K. Yee for introducing us to the polystyrene-toluene insulation technique.

REFERENCES


[26] Lubner, S. D., Dames, C., unpublished work.
[36] CRC Handbook of Chemistry and Physics. 90th ed. CRC Press