

Dual-Stoichiometry Copper Sulphide Nanoparticles by Laser Ablation in DMSO: Synthesis and Biomedical Applications for Enhanced Photothermal Therapy and Photoacoustic Imaging

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Copper sulphide nanoparticles are synthesized by laser ablation of a copper target in DMSO by a 527 nm nanosecond pulsed laser. These nanoparticles have double stoichiometry (CuS and Cu_{1.8}S) and crystalline structures, sizes under 30 nm, and they present substantial absorbance in the second near-infrared window and photoluminescence in the visible region. The nanoparticles are used as photothermal and photoacoustic agents at 1080 nm and 1064 nm, respectively. Utilizing as a photothermal agent, these nanoparticles rapidly exhibit local heating, photothermal stability, and a temperature change of 52.2 °C within 300 s at 1 mg mL⁻¹ concentration and

3.23 W cm⁻² laser intensity. On the other hand, while employed as a photoacoustic agent, they enhanced the contrast significantly and increased the brightness proportional to their concentrations when compared to ultrasound imaging. Additionally, the biocompatibility properties of these nanoparticles were tested with cancer cells, and they were subjected to laser ablation to assess their photothermal effects. In this article, we demonstrate that copper sulphide nanoparticles synthesized by pulsed laser ablation hold great promise for photothermal and photoacoustic applications, especially in biomedical applications.

Introduction

Nanoparticles have garnered significant attention with the emergence of nanotechnology, and remarkable demands in their usability in different areas such as electronics,^[1] solar cells,^[2] targeted drug delivery,^[3] agriculture,^[4] biosensing,^[5] and many other areas, and continue to increase. One of the reasons for this increased attention to nanoparticles is their changing optical properties compared to their bulk counterparts. The optical properties of nanoparticles may change depending on their size and shape.^[6]

On the other hand, the therapeutic window, also known as the near-infrared window, is the optical range of wavelengths from 650 nm to 1350 nm due to enhanced penetration inside the tissue.^[7] According to this, the use of near-infrared lasers in medical applications has become prominent. Nanomaterials with near-infrared light absorption are needed to make use of these lasers inside the tissue. Moreover, these materials need to be non-toxic to be used inside living organisms.

As an example, copper sulphide nanoparticles (CSNP) meet such requirements and offer to be a suitable option since they have absorption in the near-infrared area^[8] and have low toxicity compared to gold nanostructures.^[9] CSNPs have become prominent due to these attributes. Considering the literature, research is still going on using CSNP in areas such as photothermal ablation,^[10] photoacoustic imaging,^[11] drug delivery,^[12] DNA detection,^[13] positron emission tomography (PET),^[14] magnetic resonance imaging^[15] or immunosensors.^[16]

Covellite nanoparticles (CuS NPs), digenite nanoparticles (Cu_{1.8}S NPs) and nonstoichiometric phases of copper sulphides (Cu_{2-x}S, 0 < x < 1) are synthesized by various methods such as sol-gel,^[17] sonochemical,^[18] microemulsion method,^[19] reflux condensation^[20] and laser ablation in the liquid method.^[21,22] Among them, the laser ablation in liquid (LAL) method excels at producing pure nanoparticles in a simple way without any ligands or by-products. Simply put, the technique relies on the immersion of precursor materials into the liquid and focusing a pulsed laser beam onto the surface of the material to synthesize nanoparticles.

The LAL method also allows the synthesis of nanoparticles different from the bulk material used in the process by reacting the liquid and the bulk material to produce nanomaterials

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different from the target material. The LAL method can be utilized to synthesize and realize the potential use of copper sulphide nanoparticles in optical applications.

One of these optical applications is photothermal therapy, which is a type of therapy that employs heat generated by a material absorbing in the near-infrared region. This heat is utilized to damage or destroy cells through hyperthermia. Various materials, such as gold nanoparticles^[23–26] and iron oxide nanoparticles^[27–29] are employed for the light-to-heat conversion process in photothermal therapy. Molybdenum disulfide nanostructures,^[30,31] dyes,^[32,33] liquid thermal crystals,^[34] and copper sulphide nanoparticles.^[35,36] CSNPs are a convenient choice for photothermal therapy since they are easy and inexpensive to produce, have promising nontoxicity, and their localized surface plasmon resonance (LSPR) in the NIR region can be tuned according to requirements by controlling their average size and the composition of Cu_{2-x}S NPs.^[37]

In addition, photoacoustic imaging (PAI) is another promising optical application for CSNP. PAI has attracted attention recently due to its high resolution and ability to image tissue at a deeper level by uniting ultrasound and optical imaging.^[38–40] PAI works on the concept of ultrasonic wave production by the rapid heating of light-absorbing molecules. To achieve enhanced contrast in PAI, contrast agents are used that absorb light in the near-infrared region. Similar to the ones used in photothermal applications, the nanoparticles used in PAI are made up of nanoparticles such as organic nanoparticles,^[41] gold nanoparticles,^[42,43] upconversion nanoparticles,^[44] polymer nanoparticles,^[45] and copper sulphide nanoparticles.^[46,47] CuS and Cu_{2-x}S NPs are also suitable choices for photoacoustic imaging due to their adjustable absorption and favorable biodegradability. Herein, we report the synthesis and comprehensive characterization of copper sulphide nanoparticles *via* laser ablation in DMSO liquid through a 527 nm nanosecond pulsed laser, the photothermal and photoacoustic properties of these copper sulphide nanoparticles, and their applicability as photothermal conversion and photoacoustic contrast agents when excited by near-infrared lasers. We then evaluate any

photothermal effects of these nanoparticles on breast cancer cells through cytotoxicity and live/dead assays, thereby demonstrating their potential in the biomedical realm.

Results and Discussion

Properties of Copper Sulphide Nanoparticles

Nanoparticles are synthesized by a pulsed laser ablation process, and the experimental setup is depicted in Figure 1a. The crystal structure of the synthesized nanoparticles was first confirmed by an XRD study and presented in Figure 1b. The positions of the peaks are at the 2θ values of 27.79° , 29.24° , 31.69° , 32.22° , 32.79° , 46.24° , 47.89° , 52.70° , 54.80° , and 59.40° . These values can be indexed as CuS (101) and $\text{Cu}_{1.8}\text{S}$ (111) at 27.79° , CuS (102) at 29.24° , CuS (103) at 31.69° , $\text{Cu}_{1.8}\text{S}$ (200) at 32.22° , CuS (006) at 32.79° , $\text{Cu}_{1.8}\text{S}$ (220) at 46.24° , CuS (110) at 47.89° , CuS (108) at 52.70° , $\text{Cu}_{1.8}\text{S}$ (311) at 54.80° , and CuS (116) at 59.40° according to CuS (ICDD No 03-065-3556) and $\text{Cu}_{1.8}\text{S}$ (ICDD No 04-003-4437). This suggests that CuS is in a hexagonal shape with $a=b=3.7960^\circ\text{\AA}$, $c=16.3600^\circ\text{\AA}$, belonging to space group $P6_3/mmc$, and $\text{Cu}_{1.8}\text{S}$ is in a cubic shape with $a=b=c=5.5640^\circ\text{\AA}$, belonging to space group $Fm\bar{3}m$. These results confirm that the output of the laser ablation process is compounds with different crystalline structures and phases, namely, covellite (CuS) and digenite ($\text{Cu}_{1.8}\text{S}$). To the best of our knowledge, this is the first synthesis of $\text{Cu}_{1.8}\text{S}$ nanostructure with the LAL method. The reason for the synthesis of not just CuS NPs but CuS NPs together with $\text{Cu}_{1.8}\text{S}$ NPs might be attributed to the rapid sulfurization of the copper target surface due to the high energy per pulse and long pulse duration (100 ns) from our laser, resulting in very high temperatures at the focal point of the laser. Changes in the target surface during the ablation process might have caused the synthesis of different nanoparticles when sulfurated surfaces interact with the laser beam again.

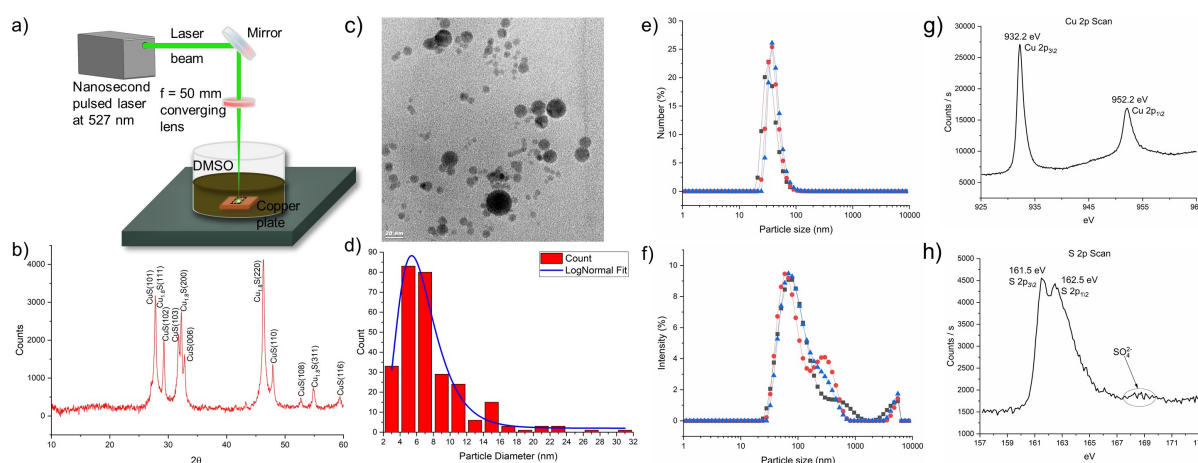


Figure 1. a) Experimental setup of CSNPs synthesis, b) XRD patterns of synthesized copper sulphide nanoparticles (scale size: 20 nm), c) TEM image synthesized copper sulphide nanoparticles (scale size: 20 nm), d) size histogram of the nanoparticles, e) intensity and f) number weighted particle size of CSNPs, g) Cu 2p XPS scan, and h) S 2p XPS scan of CSNPs.

In our study, we used TEM and DLS techniques to examine the CSNPs. An analysis in TEM was done to determine the shapes, sizes, and elemental composition of the nanoparticles. Figure 1c shows a TEM image of the NPs fabricated by pulsed laser ablation in DMSO. It is shown that the NPs can be considered to be in spherical shape. For accurate size distribution, the diameters of 282 nanoparticles are measured using ImageJ software, and the histogram of the sizes of the nanoparticles is drawn in Figure 1d. Most of the nanoparticles are in the range of 5–10 nm or smaller, but the diameters of NPs range from 1 nm to 30 nm, and the size distribution of nanoparticles can be fitted with a lognormal curve. According to this result, on average, our NPs are smaller than copper sulphide NPs synthesized by the LAL method in the literature.^[21,22] Large sizes of nanoparticles and the effects of inorganic substances are causes of toxicity in live tissues.^[48] Nanoparticle synthesis by laser ablation produces chemical-residue-free nanoparticles with small sizes. This increases their biocompatibility since there are no additional toxic chemicals or ligands, and their size allows them to be cleared through renal clearance. Figure S1 (Supporting Information) shows the crystal-line structure of the synthesized NPs, proving the crystallinity of the nanoparticles. Figure S2 shows the EDX spectra of the nanoparticles, and the inset shows the high-angle annular dark field (HAADF) image. The EDX spectra acquisition area is shown in the red square. The EDX spectra indicate that the NPs are formed of Cu and S elements. The C peak can be linked to the carbon coating of the Cu grids used for imaging in TEM. According to the DLS results in Figure 1e–f, the average size of the CSNPs, based on intensity-weighting, was 91.3 ± 3.7 nm, and the polydispersity index (PDI) ranged from 0.3 to 0.4. Intensity-weighted size refers to the distribution of sizes based on the intensity of scattered light, providing information about the relative abundance of particles of different sizes. On the other hand, the number-weighted size distribution represents the relative population of particles with different sizes, or PDI. The number-weighted particle size was approximately 30 nm, which closely matched the results obtained from the TEM images in Figure 1c.

Considering electrical charge, the outer layer of metal sulfide particles usually consists of two types of functional groups, i.e., sulfido-groups ($>SH$) and hydroxo-groups ($>OH$).^[49] Consequently, the electrical charge present on the surface of a metal sulfide particle relies on the concentration and individual electric charge of these respective sulfido- and hydroxo-groups. The surface charge strictly depends on how the atoms are arranged in the crystal lattice structure and how metal (Cu in this study) and sulfide ions interact with each other. The threshold value of zeta potential differs significantly among the other metal sulfide systems due to variations in the Hamaker constant, which is the coefficient accounting for Van der Waals interactions between two materials.^[50] As a result, the solid fraction of metal-sulfide nanoparticles varies, impacting their colloidal stability, which is associated with the zeta potential of the nanoparticle system. For instance, previous studies have suggested that aggregation tends to dominate within a zeta potential range from -15 mV to $+15$ mV.^[51]

Furthermore, it has been established that the colloidal stability of a suspension containing CSNPs can be attributed to a highly negative zeta potential of approximately -38 mV at a pH of 6.^[52] In our study, the CSNPs displayed a zeta potential of -11.6 ± 2.12 mV, and this would be further improved for the colloidal stability of our particles.

The graphs in Figure 1g–h show the elemental Cu and S scans of our sample by XPS. In Figure 1g, there are two peaks at 932.2 eV and 952.2 eV, which can be attributed to Cu $2p_{3/2}$ and Cu $2p_{1/2}$ states, respectively. In figure 1h, the scan for S 2p is shown. The doublet peaks of S 2p are found at 161.5 and 162.5 eV. These peaks are attributed to S $2p_{3/2}$ and S $2p_{1/2}$ states respectively. In addition to these peaks, between 168 and 169 eV, there is evidence of a small amount of SO_4^{2-} . As explained in the experimental section, the samples are produced with a heat treatment, and thus, the formation of sulfate groups on the surface is expected after the heat treatment.

In addition, Figure 2a shows the absorbance spectrum obtained from the colloid solution of CSNPs from 230 nm to 1600 nm, and the inset graph shows the range between 400 nm and 1600 nm. The absorbance spectrum exhibits a broad absorbance spanning from 1000 nm to 1600 nm. The absorption peak is at 1183 nm and has a wide absorption range in the NIR region. Absorbance at 1080 nm, which is the wavelength for our photothermal study, is at 92.7% of the peak at 1183 nm, and absorbance at 1064 nm is at 90.8% of the peak absorption in the NIR region, which is the wavelength of common and a majority of near-infrared lasers. Also, most of the high absorbance is in the second near-infrared region (1000–1700 nm), where biological tissues have low absorption and scattering of photons.^[7] This suggests that CSNPs can be used for the absorption of the light of NIR lasers, and CSNPs can image deeper in live tissues than commonly used gold nanostructures^[53] or carbon-based nanomaterials^[54] where their absorption peaks are in the first near-infrared region (700–1000 nm). The incorporation of $Cu_{1.8}S$ NPs seems to increase the peak wavelength of the LSPR since the LSPR of CuS NPs is around 900–1000 nm.^[46,55,56] Our CSNPs have an absorbance peak at 1183 nm, which is shifted to higher wavelengths when compared to copper sulphide nanoparticles produced by other methods.^[46,56] This can be attributed to the effect of $Cu_{1.8}S$ nanoparticles. Higher absorbance of the laser light can increase the photothermal conversion and photoacoustic imaging efficiencies of CSNPs compared to other methods. This increase in absorption increases the efficiency of application when NIR lasers at wavelengths 1064 nm and 1080 nm, where biological tissues have minimal absorption compared to 808 nm NIR lasers.^[7] To study the bandgap of the nanoparticles, the Tauc plot is drawn from the absorbance graph.

The corresponding Tauc plot for bandgap estimation is shown in Figure 2b. Tauc relation is used to estimate the bandgap value, which is given by:^[57]

$$(\alpha h\nu)^n = K(h\nu - E_g) \quad (1)$$

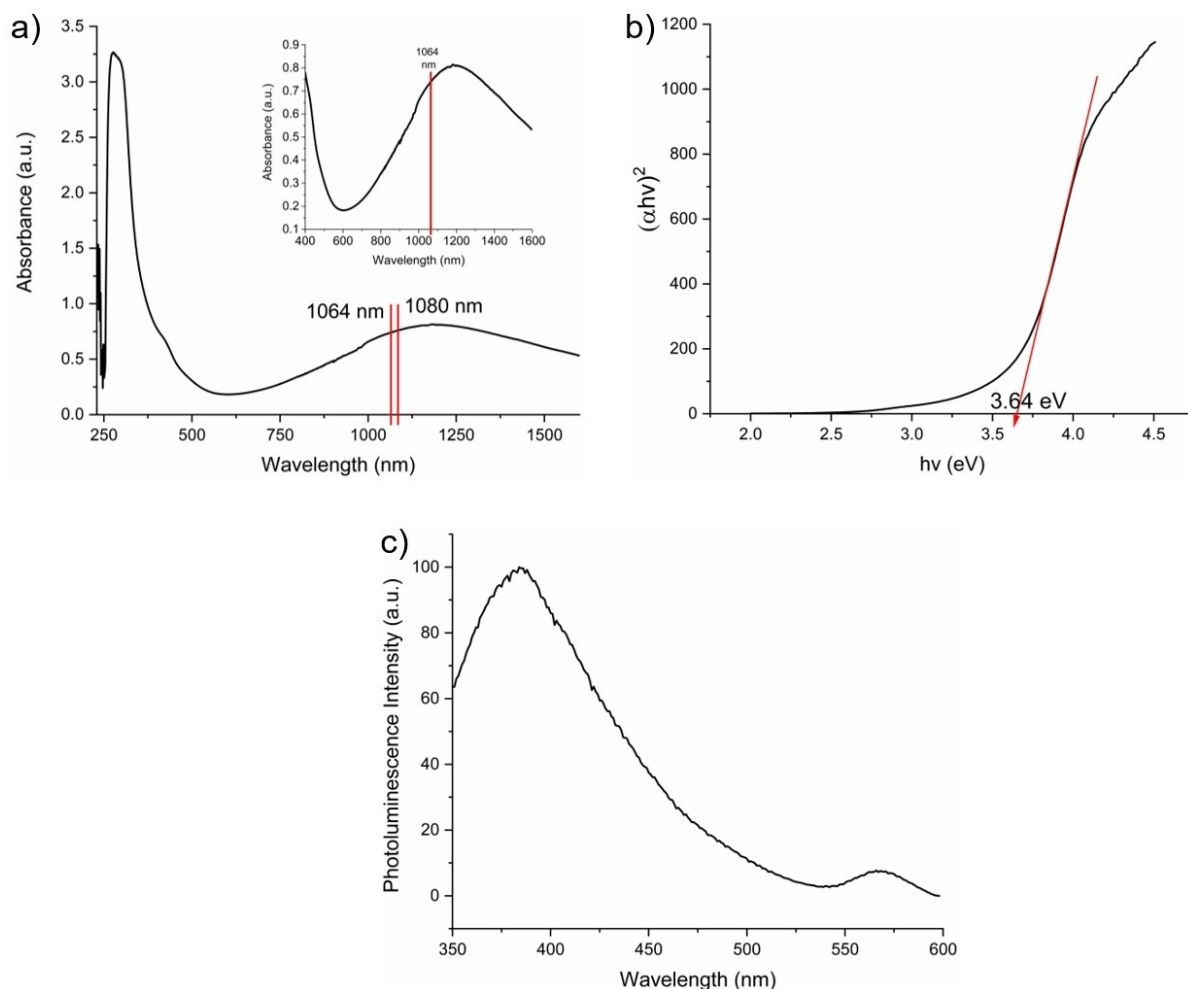


Figure 2. a) Absorbance spectra of CSNPs (inset shows the absorbance between 400–1600 nm), b) The corresponding Tauc plot of the absorbance plot, c) Graph of photoluminescence intensity of CSNPs excited at 320 nm.

where α is the absorption coefficient, h is Planck's constant, ν is the frequency of light, $n = 2, \frac{1}{2}$ for direct and indirect transitions respectively, K is a constant and E_g is the bandgap. Using this relation, the bandgap is found to be 3.64 eV for direct transitions.

Due to the substantial usability of copper sulphide NPs in photo-applications, photoluminescence behavior is studied. In addition, the laser ablation process can produce deficiencies in the crystalline structure of the nanoparticles.^[58] On account of these, the photoluminescence properties of the NPs are recorded. Figure 2c depicts the photoluminescence (PL) behavior of the synthesized CSNPs. The PL spectra are recorded between 350–600 nm and NPs are excited at 320 nm wavelength from the xenon lamp of the spectrophotometer.

The spectrum has 2 peaks, the first at 383 nm and the second at 570 nm wavelengths. The first peak at 383 nm might be coming from defects and vacancies in the crystal structure or electron-hole recombination in the surface states.^[59–61] The second peak at 570 nm may be attributed to the recombination of sulfur vacancies of the Cu^{2+} ion.^[62] Both emissions in the blue region and the green region are compatible with the

literature.^[59–63] Considering these results, these nanoparticles have potential to be used in photo imaging for their photoluminescence response, especially in the blue and green regions.

Photothermal Properties of Nanoparticles

The photothermal properties of CSNPs are studied with a 1080 nm continuous-wave laser. An illustration of the experimental setup is given in Figure 3a. To investigate the photothermal response of the sample to the laser beam, we subjected the sample's top surface to a 4 mm-spot-sized laser beam of 1080 nm wavelength with a laser power intensity of 3.23 W cm^{-2} and a laser power of 406 mW, as shown in the representative images in Figure 3b. The temperature was measured every 5 seconds for 300 seconds. The heat expansion became apparent throughout the laser exposure duration. The scope of the NIR thermal camera was adjusted to capture the region of the sample with the highest temperature. Significant temperature increases were reported where the initial temper-

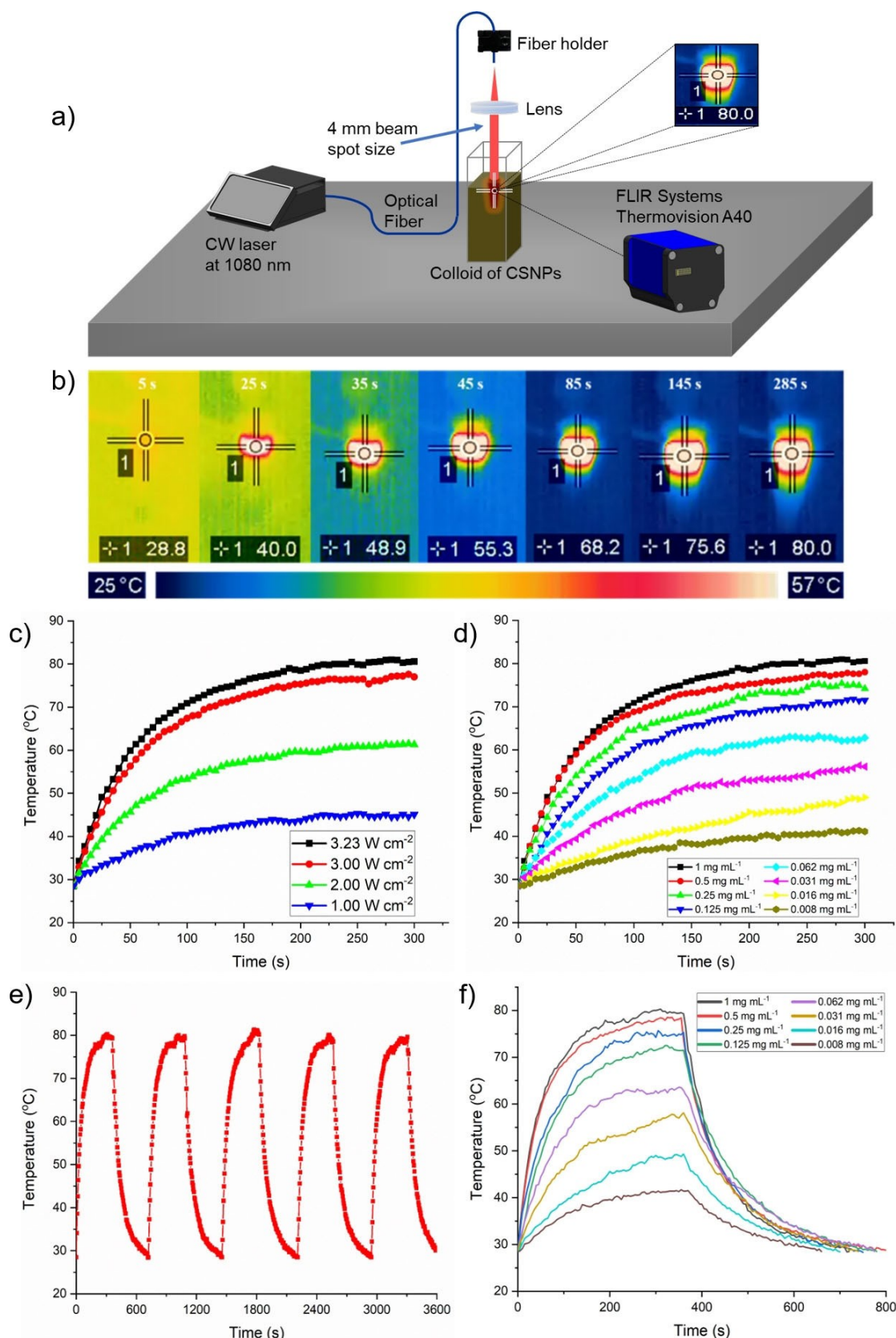


Figure 3. a) Illustration of the photothermal experiment setup, b) Time-dependent IR thermal camera captures of CSNPs placed into a microcentrifuge tube where the center region of the sample is exposed to the laser beam. The initial temperature was 28.4 °C. c) The temperature of CSNPs recorded under continuous laser beam irradiation with a 4 mm-spot sized at different laser power intensities. d) The temperature of CSNPs with different concentrations was measured every 5 s for 300 s under exposure to the 4 mm-spot sized at an intensity of 3.23 W cm⁻². e) Five heating-cooling consecutive cycles for photothermal stability of CSNPs measured for 300 s under exposure to the 4 mm-spot-sized laser beams with a concentration of CSNPs of 1.00 mg mL⁻¹ at an intensity of 3.23 W cm⁻². f) Concentration-dependent temperature change of 1.00 mg mL⁻¹ of CSNPs with cooling curves.

ature was 28.4 °C and reached 80.6 °C in 300 seconds, resulting in a 52.2 °C temperature change. At various laser intensities, 1.00 mg mL⁻¹ of CSNPs were irradiated with a 4 mm laser spot of 1080 nm wavelength and a spot size of 4 mm (varying from 1.00 to 3.23 W cm⁻²). In the process of the laser intensity-dependent temperature change of 1.00 mg mL⁻¹ of CSNPs, the initial temperature of the CSNPs was maintained at room temperature (28.4 °C) before the application of each laser intensity. Under the laser irradiation with different intensities of 1.00 W cm⁻², 2.00 W cm⁻², 3.00 W cm⁻², and 3.23 W cm⁻², the latest temperatures reached were recorded as 45.0 °C, 61.3 °C, 77.0 °C, and 80.6 °C, respectively. Therefore, it can be deduced that the maximum heat release occurred when the highest laser intensity was applied as provided in Figure 3c, where temperature increased with an increase in the laser intensity. To demonstrate the concentration-dependent temperature change of 1.00 mg mL⁻¹ of CSNPs, the laser intensity was set to 3.23 W cm⁻² and irradiated in all concentrations (from 1.00 to 0.008 mg mL⁻¹). As presented in Figure 3d, the photothermal response of nanoparticles displays a gradual increase in temperature between each concentration as the illumination is initiated. The latest temperature reached for the least dense (0.008 mg mL⁻¹) of CSNPs with the highest irradiation laser intensity was 41.1 °C, while the temperature for the highest dense solution reached 80.6 °C. It is clear that the denser sample has not only more of the CSNPs exposed to the laser beam but also a higher light absorption intensity. In this way, the CSNPs confirm a concentration-dependent temperature increase at a constant laser intensity. In addition, we also confirmed that CSNPs have photothermal stability.

As shown in Figure 3e, five consecutive heating-cooling cycles under repeated laser ON/OFF actions were performed. The sample with a 1.00 mg mL⁻¹ concentration was subjected to excitation at 1080 nm wavelength with the 4 mm-spot-sized laser beams for 300 s and kept cooling down to room temperature. Since there was no discernible change in behavior between repeated cycles and CSNPs could reach the same maximum temperature (about 80 °C) for each repeated cycle, it can be concluded that CSNPs preserve their reversible photothermal properties. Besides the heating response of all concentrations with a laser intensity of 3.23 W cm⁻², cooling behaviors reflect consistency and are presented in Figure 3f. In conclusion, the synthesized CSNPs show that with even low concentrations, large temperature changes can be achieved and the NPs can be used repeatedly. It can be seen that copper sulphide NPs show great promise for photothermal applications.

Cellular Uptake, Cytotoxicity Assay, in Vitro Photothermal Application of CSNPs and Live/Dead Analysis

Initially, the cellular uptake of CSNPs was investigated with nucleic acid staining and confocal microscope imaging. MCF-7 (breast cancer) and HEK 293 (human embryonic kidney 293) cells were cultured on the cover slide (22 mm×22 mm) in a 6-well plate. Once the cells adhered, they were treated with CSNPs and incubated overnight. After the excess number of

particles was washed and removed, cells were fixated with paraformaldehyde and dyed with Hoechst 33342 nucleic acid stain. Confocal microscope images for HEK293 and MCF-7 were presented in Figure 4. Due to the size of particles at the nanoscale (Figure 1d–f), only aggregated particles at the same location were visible. Herein, green luminescent particles were located inside the cell cytoplasm and in the nuclear membrane. Confocal microscope images prove that the process of cellular absorption of CSNPs was effective. Lateral z-axis images were also considered (Figure 4c) to evaluate the exact positions of these particles. The distance between cell nuclei and CSNPs was smaller than 300 nm, pointing out that CSNPs might not be located on the cell surface but be accommodated inside the cell. As a result, both healthy (HEK 293) and breast cancer (MCF-7) cells achieved to internalize the particles. Since the only mechanism here was size-dependent absorption and there was no active targeting element such as affibody-conjugated nanoparticles^[64] or magnetically driven particles,^[65] it was expected to achieve similar results with healthy cells as well. In this study, MCF-7 cells were utilized as a model cell type, and the major strategy was to demonstrate cell death due to the hyperthermic environment after the mutual integration of CSNPs and the laser application. By modifying the surface of these particles, they will potentially become targeted and more selective to the cell type and tumor microenvironment.

The cytotoxicity of CSNPs in DMSO was investigated *via* flow cytometry. Cultured MCF-7 cells were treated with (i) only DMSO as the control group and (ii) CSNP in DMSO as the treatment group. Cells were collected with trypsin-EDTA and stained with propidium iodide (PI). Dye emissions were taken under the phycoerythrin (PE) filter (see Supporting Information in Figure S4), and normalized cell viability values were presented in Figure 5a. Considering the results, it was observed that DMSO affects cell viability at high concentrations and should be reduced.^[66] This was recorded for further experiments. Upon normalizing the data, it was noted that the particles had a minor cytotoxicity impact (with 93.7% cell viability) and there was no significant difference ($n=3$, $p>0.05$) compared to the control group (only DMSO).

In addition, we continued to evaluate the concentration effect of CSNPs on cytotoxicity over MCF-7 cancer cells in Figure 5b. The experiment was designed with six groups, including 1) untreated cells (control), 2) cells treated with 0.250 mg mL⁻¹ of CSNPs concentration, 3) cells treated with 0.125 mg mL⁻¹ CSNPs concentration, 4) cells treated with 0.0625 mg mL⁻¹ CSNP concentration, 5) cells treated with 0.025 mg mL⁻¹ CSNP concentration, 6) cells treated with 0.0125 mg mL⁻¹ CSNPs. To assess the cytotoxicity, we measured cell viability in each group after applying the respective CSNPs treatments. Per the results, although we observed some fluctuation in the cell viability data for each experimental set, there was no statistically significant difference observed in cell viability among the groups for each CSNPs-treated and untreated MCF-7 cells ($n=4$, $p>0.05$). This result encouraged us to further evaluate the photothermal effect of CSNPs over same cell types while culturing these cells on a layer of PDMS mimicking a 3D environment.

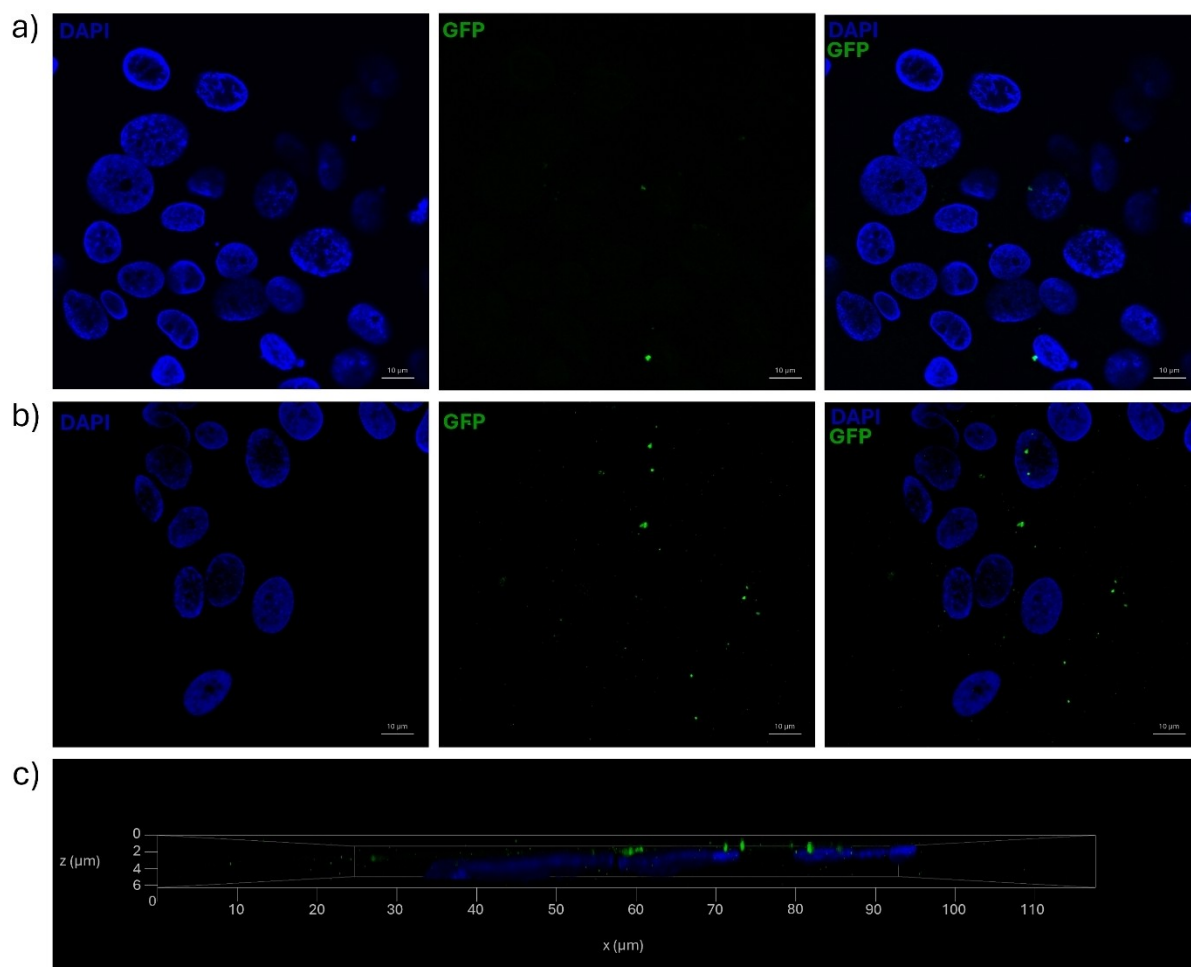


Figure 4. Confocal microscope images of CSNPs treated and Hoechst 33342 stained a) HEK 293 and b) MCF-7 cell lines. c) Lateral z-axis image of MCF-7 cell lines.

Herein, we investigated *in vitro* photothermal application of CSNPs on MCF-7 cells in Figure 5c–h. The cells were treated with different concentrations of CSNPs, followed by exposure to 1080 nm CW laser ablation. We designed four different experimental sets containing different concentrations of CSNPs and applied the same photothermal effect (3.23 W cm^{-2} , 5 min duration). The concentration of CSNPs were (i) 0.250 mg mL^{-1} , (ii) 0.125 mg mL^{-1} , (iii) $0.0625 \text{ mg mL}^{-1}$, and (iv) 0.025 mg mL^{-1} . Our results revealed a substantial effect of the CSNPs in inducing cell death when combined with laser ablation. In all treated populations, a significant number of dead cells were observed (down to 77.5% of cell death), indicating the potential of the photothermal therapy approach using CSNPs. According to the live/dead cell analysis using ImageJ, the cell viabilities were calculated as 22.5%, 34.5%, 34.9%, and 42.8% from the highest concentration to the lowest concentration. Notably, the highest concentration of CSNPs, 0.250 mg mL^{-1} , exhibited a particularly potent effect, resulting in a significantly low number of live cells (22.5% cell viability). This finding suggests that higher concentrations of CSNPs may enhance the photothermal ablation efficacy against MCF-7 cells. The remarkable cell death observed in this group indicates the promising therapeutic

potential of CSNPs in combination with laser ablation for cancer treatment. Furthermore, our results demonstrated that the 0.025 mg mL^{-1} CSNPs treated group exhibited a lower number of dead cells compared to the other three groups. Although not as pronounced as the 0.250 mg mL^{-1} CSNPs concentration, the observed decrease in cell death indicates that lower concentrations of CSNPs can still contribute to the photothermal therapy's efficacy. This finding suggests that the optimal concentration of CSNPs for photothermal treatment of MCF-7 cells may lie within the range of 0.025 mg mL^{-1} to 0.250 mg mL^{-1} .

In order to determine specific fatal effects of laser treatment alone, we also compared the results with two additional control groups: (i) laser-irradiated cells without CSNPs treatment (control-1) and (ii) cells without laser and CSNPs treatments (control-2). In Figure 5, the laser irradiated cells (control-1) had a cell viability of 97.7%, while the control-2 group had a cell viability of 98.7%. Remarkably, both of these control groups demonstrated cell viability, pointing out that the cells remained unaffected by laser treatment alone. Statistical analysis revealed no significant difference between the two control groups, indicating that the absence of laser treatment had a compara-

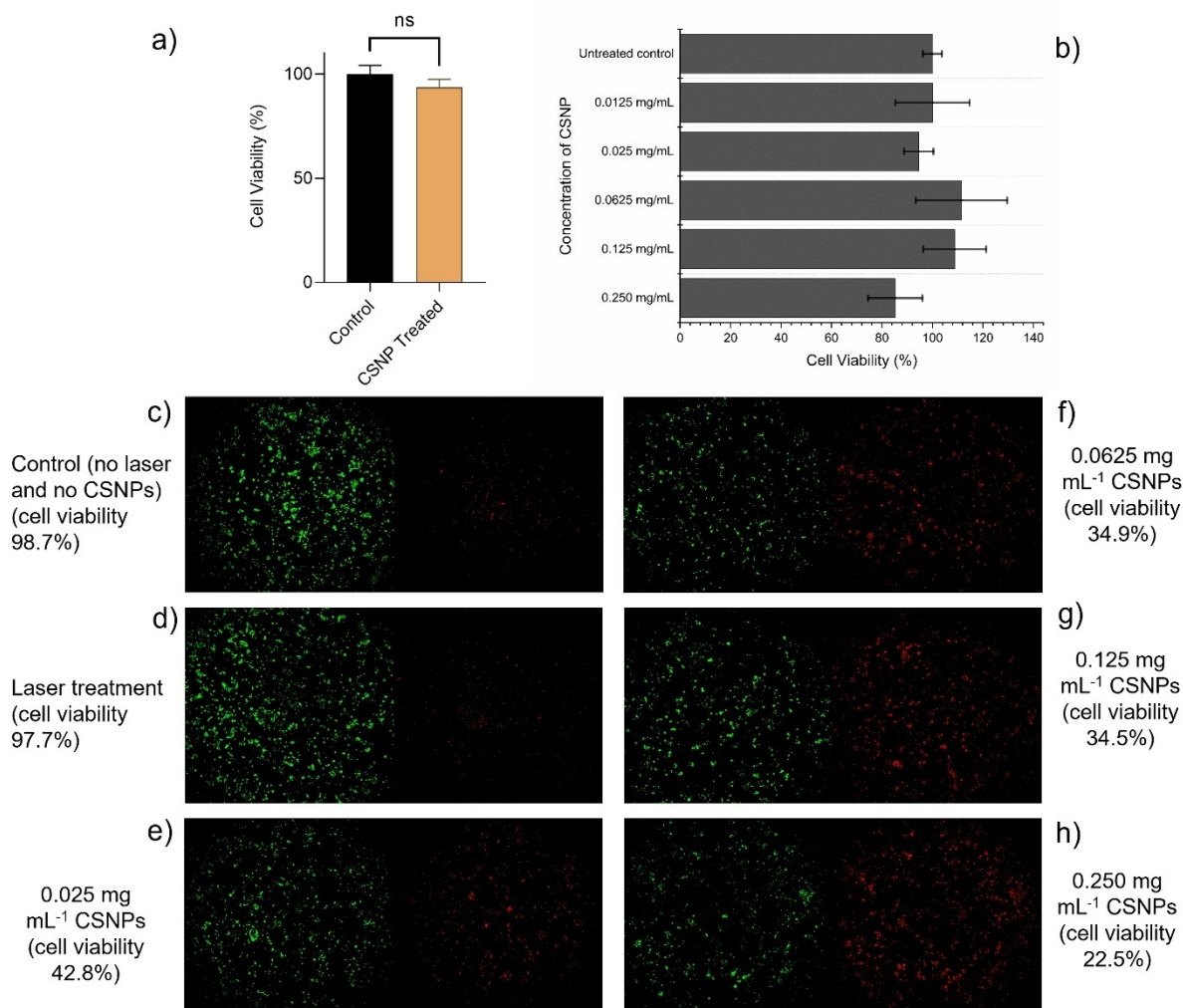


Figure 5. a) Normalized live/dead MCF-7 cell viability analysis for DMSO applied control group and CSNP treated experiment group. The data received was analyzed using t-test and analyses without significant differences were indicated as “ns” (non-significant) ($n = 3$, $p > 0.05$). Error bars represent standard deviation of the mean. b) The viability of CSNPs-treated MCF-7 cells, c) The control group (without laser irradiation and nanoparticle treatment), d) Laser irradiation control group, e) cells treated with CSNPs with the concentration of 0.025 mg mL^{-1} CSNPs, f) $0.0625 \text{ mg mL}^{-1}$ CSNPs, g) 0.125 mg mL^{-1} CSNPs, and h) 0.250 mg mL^{-1} CSNPs.

ble effect to the absence of both laser treatment and CSNPs treatment ($n = 3$, $p > 0.05$). This suggests that laser irradiation on cells did not induce substantial phototoxicity in MCF-7 cells. These findings contribute to the understanding of *in vitro* photothermal applications of CSNPs and provide insights for the development of novel approaches to cancer treatments in the near future.

Photoacoustical Properties of Copper Sulphide Nanoparticles

In photoacoustic imaging, a pulsed laser illuminates the region of interest, and the absorbed energy induces a thermo-elastic expansion, which generates acoustic waves. These acoustic waves are then received by an ultrasound phantom and post-processed to construct a photoacoustic image.

In this study, the acoustic waves generated due to the thermo-elastic expansion of the tube in response to the firing from the pulsed laser are received by a phased array transducer. An illustration of the experimental setup can be seen in Figure 6a. The received signals are then beamformed using a delay-and-sum receive beamformer, a commonly used technique in literature, to form the image scan lines. Next, we employed Hilbert transform-based envelope detection on the scan line data to obtain the envelope information, which was subsequently used for image construction.^[67]

To understand the motion of the tube caused by the thermo-elastic expansion, we first examined the beamformed signal along the axis of the wave propagation. Figure 6b presents the beamformed signal when the transducer is positioned such that its axis is perpendicular to the wave propagation axis. We obtained this beamformed signal in the case of a 0.250 mg mL^{-1} concentration of CSNPs, a 1064 nm

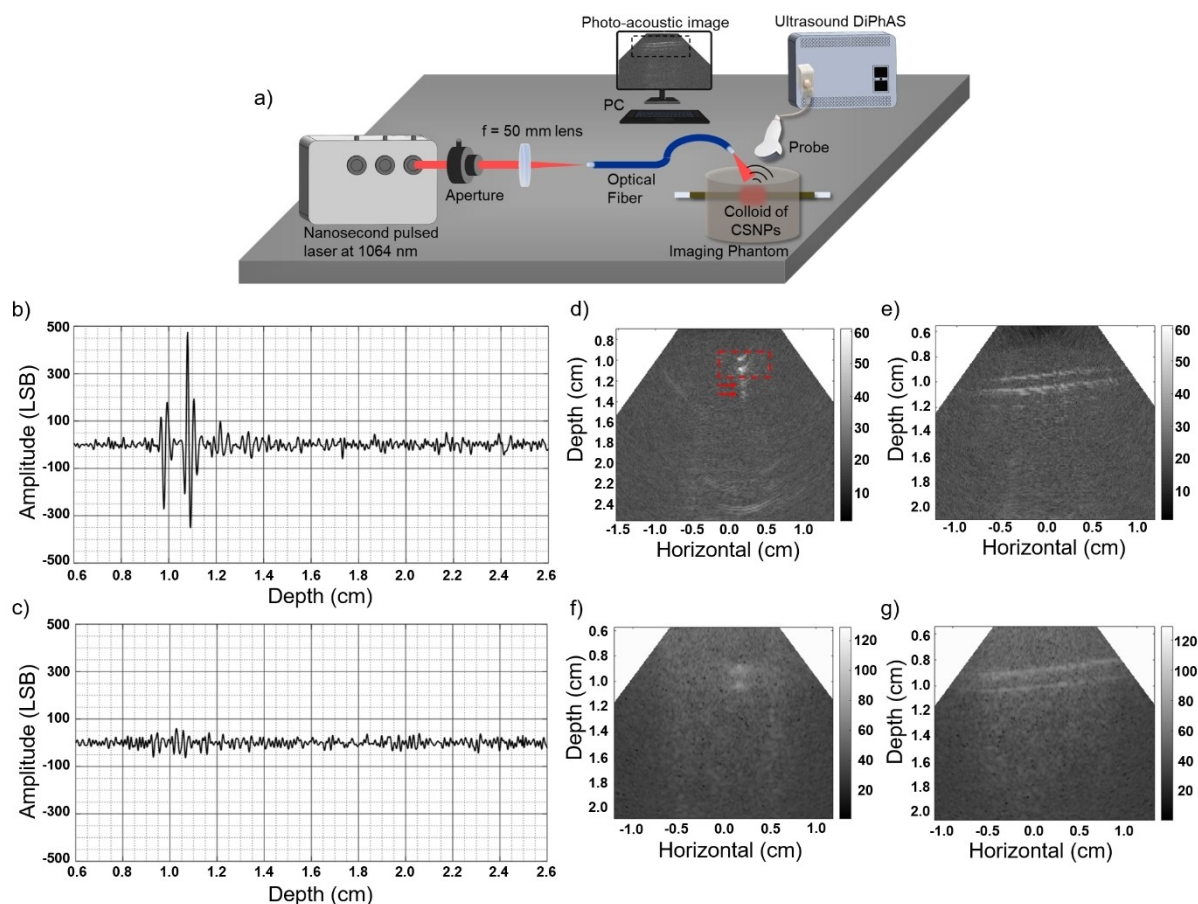


Figure 6. a) Illustration of photoacoustical experiment setup, b) Beamformed signals where the transducer is positioned such that its axis is perpendicular to the wave propagation axis. c) Beamformed signals where the transducer is positioned such that its axis is parallel to the wave propagation axis. The amplitude unit is the least significant bit (LSB) of the analog-to-digital converter in DiPhAS. Perpendicular (d) and parallel (e) photoacoustic images with perpendicular (f) and parallel (g) ultrasound images. Photoacoustic images were taken at the concentration of 0.250 mg mL^{-1} , the excitation wavelength of 1064 nm , and the average energy of 5.0 mJ .

excitation wavelength, and a 5 mJ average energy. As seen in Figure 6b, thermo-elastic expansion of the tube volume results in two signals being formed. It is observed that the former signal comes from a depth of 0.95 cm as expected since the depth of the tube is approximately 1 cm . The acoustic waves generated due to the motion of the upper wall of the tube cause this signal to appear at the beamformer output. It is also observed that the acoustic waves generated due to the motion of the bottom wall of the tube cause the latter signal with a polarity opposite to that of the former signal. This latter signal comes from a depth of 1.05 cm , which is compatible with the depth of the bottom wall of the tube.

When the ultrasound probe orientation is changed such that its axis is parallel to the wave propagation axis, the amplitude of the beamformed signal decreases significantly, and it becomes barely visible, as shown in Figure 6c. It is also obvious that the two signals coming from the upper and bottom walls of the tube cannot be easily distinguished from each other when compared to those shown in Figure 6b. The reason is that the volume of the tube subjected to the optical firing of the laser increases in this orientation. In this case, the thermal expansion results in acoustic wave generation over an

increased volume, in which the acoustic waves are destructively added up during their propagation toward the ultrasound probe. This destructive addition causes a significant decrease in the amplitude of the beamformed signal.

To gain a better understanding of the tube motion due to the thermo-elastic expansion, we also benefited from the correlation. For this purpose, we correlated the beamformed signals shown in Figure 6 with the beamformed signal generated due to the motion of the upper wall of the tube. Note that the energy of the beamformed signal generated due to the motion of the upper wall of the tube is normalized to unit energy, which means that the correlator gain is unity. Figure S3 compares the correlator outputs for the perpendicular and parallel orientations of the probe as a function of depth. The two signals generated due to the motions of the top and bottom walls are again distinguishable in Figure S3a, and the latter signal has an opposite polarity compared to the former signal. The correlator output shown in Figure S3a has a higher amplitude than that shown in Figure S3b, which confirms the adverse effect of the destructive addition of the propagating waves in the case of parallel probe orientation. The correlator output shown in Figure S3 is in good agreement with the

results obtained from beamformed signals shown in Figure 6b–c. Consequently, the beamformed signals and the correlator outputs well describe the motion of the tube under the laser excitation.

Next, we presented the photoacoustic images of the target, a polymer tube filled with 0.250 mg/mL CSNPs, positioned approximately at 1 cm depth in Figures 6d,e for different transducer orientations. These images were obtained with a 1064 nm excitation wavelength and 5.0 mJ average energy. For comparison purposes, we also constructed ultrasound images for different transducer orientations, shown in Figures 6f,g, as reference images. We used these images for both subjective evaluations and to examine whether or not the results derived from the beamformed signals and correlator outputs are also observable in the images.

The photoacoustic image when the transducer is positioned such that its axis is perpendicular to the wave propagation axis is shown in Figure 6d. It can be easily seen from Figure 6d that the target is detected and clearly visible, while it is barely visible in the ultrasound image shown in Figure 6f. The received acoustic waves generated from the expansion of the top and bottom walls of the tube manifest themselves in Figure 6d as two separate point-like targets at approximately 1 cm and 1.1 cm depths due to the transducer orientation. The distance between these two point-like targets, shown in dashed rectangular in Figure 6d, was measured as 1 mm, which is compatible with the distance between the top and bottom walls of the tube. In addition, Figure 6d shows more detail compared to Figure 6f, such that the effects of the acoustic waves generated during the damping of the expansion of the tube are also visible, which are shown with arrows in Figure 6d. These effects are observed in the deeper region, as expected, at 1.2 and 1.4 cm depths, compared to point-like targets.

These results shown in Figure 6 confirm that the quality of the photoacoustic images is better than that of the ultrasound images.

When the ultrasound probe orientation is changed such that its axis is parallel to the wave propagation axis, the quality of the photoacoustic image decreases significantly, as shown in Figure S5b, compared to Figure 6d. The reason is the destructive addition of the acoustic waves in this probe orientation during their propagation toward the ultrasound probe. The significant decrease in the beamformed signal shown in Figure 6e manifests itself as poor quality in the photoacoustic image. Moreover, the parallel orientation of the transducer leads to the detection of the target as a line target instead of a point-like target. This is because, in this parallel orientation, the laser illuminates a longer section of the tube along its length. Despite this, the photoacoustic image shown in Figure 6e has a better image quality than the ultrasound image shown in Figure 6g in the case of parallel transducer orientations as well.

Figure S5 presents the photoacoustic and ultrasound images of the same polymer tube but with a different concentration of CSNPs, which is 1 mg mL⁻¹. We observed that the images shown in Figure 6 and Figure S5 have similar image qualities. However, the increased concentration causes an increased brightness around the surface and the bottom of the

detected target in both Figures S5a and S5b compared to Figures 6d,e. As in Figure 6, the quality of the ultrasound images is significantly low compared to the photoacoustic images illustrated in Figure S5. Moreover, the transducer orientation has the same effect on both photoacoustic and ultrasound images in Figure S5 and Figure 6.

Conclusions

In conclusion, we demonstrated the synthesis of CSNP through nanosecond pulsed laser ablation in DMSO liquid in two different stoichiometries: (i) covellite (CuS) and (ii) digenite (Cu_{1.8}S), in hexagonal and cubic orientations, respectively. Herein, we aimed that rapid sulfurization of the target surface during long ablation times have caused this dual synthesis. The synthesized nanoparticles were further characterized by XRD, TEM, UV-Vis-NIR spectroscopy, and photoluminescence measurements. These nanoparticles were smaller than 30 nm in size (mostly around 5–10 nm); they had high absorbance in the second near-infrared window, as well as a wide bandgap energy corresponding to 3.64 eV and two photoluminescence peaks at 383 nm and 570 nm when excited at 320 nm. In photothermal experiments, we achieved rapid local heating in laser irradiation regions, power and concentration-dependent heating, and photothermal stability upon heating-cooling cycles.

Next, we evaluated whether various concentrations of CSNPs would have any effects on the cell viability of MCF-7 breast cancer cells, regardless of photothermal activity. MTT analysis in this respect denoted that there was no statistically significant impact of these particles on cell viability, in spite of some fluctuations in the data. In the experiments, we evaluated the concentrations of CSNPs as a small cohort for testing, spanning from 0.025 mg mL⁻¹ to 0.250 mg mL⁻¹. Yet, this concentration range would be broadened to understand any biological effects, such as any up-regulations or down-regulations of specific genes in these cells, although this was not the scope of this study. In addition, as clearly stated, CuS nanoparticles have been reported to be safe to use *in vivo* in previous studies.^[68] In our study, MCF-7 cells might have demonstrated resistance to the cytotoxic effects of CSNPs at the tested concentrations. Further investigations would be warranted to explore the underlying mechanisms behind the observed lack of cytotoxicity in MCF-7 cells treated with CSNPs. On the other hand, other than varying concentrations, exposure duration, laser intensity, and cell type would be evaluated for these particles in the near future, providing valuable insights into the potential cytotoxic effects of CSNPs on MCF-7 cells. Later on, we explored the photothermal effect of these particles while culturing MCF-7 cells in a 3D environment formed by a layer of PDMS. Per the live/dead assay results, the increments in the concentrations of CSNPs led to the killing of the cells, pointing out that there might be an enhancement in photothermal ablation efficacy against MCF-7 cells. To validate this point, we also designed two sets of control experiments: (i) laser irradiated cells without CSNPs treatment and (ii) cells without laser and CSNPs treatments. In both cases, cell viabilities were

similar and close to the cells without any particle and laser ablation, stating that laser irradiation on cells did not lead to any considerable cytotoxicity against MCF-7 cells. These findings contribute to the understanding of *in vitro* photothermal applications of CSNPs and provide insights for the development of novel approaches to cancer treatments in the near future. The lack of statistical significance suggests that the cytotoxic effect of CSNPs on MCF-7 cancer cells was not observed within the range of concentrations tested in this study. It is important to note that the absence of statistical significance does not necessarily imply the absence of any biological effect. Other factors, such as the exposure duration, nanoparticle size, and cell type may influence the observed cytotoxicity. The absence of statistical significance does not guarantee the absence of biological effects, as other factors, such as exposure duration, laser intensity, and cell type, can influence observed cytotoxicity. However, the cytotoxicity of these nanoparticles may vary depending on the specific cancer cell line, concentration, and experimental conditions employed. In our study, the MCF-7 cells might have demonstrated resistance to the cytotoxic effects of CSNPs at the tested concentrations. Further investigations are warranted to explore the underlying mechanisms behind the observed lack of cytotoxicity in MCF-7 cells treated with CSNPs. Additionally, expanding the concentration range and exploring longer exposure durations could provide valuable insights into the potential cytotoxic effects of CSNPs on MCF-7 cells.

Moreover, in photoacoustic imaging experiments, we achieved significant contrast enhancement in perpendicular transducer placement and lesser contrast enhancement in parallel transducer placement due to increased volume and the destructive addition of acoustic waves. These results are also verified with correlator outputs. With these results, we can deduce that photoacoustic imaging from deeper depths is within possibility with appropriate nanoparticle concentration and laser pulse energy.

Experimental Methods

Synthesis of Copper Sulphide Nanoparticles

NPs are synthesized by immersing a high-purity copper plate into DMSO liquid and ablated with a Q-switched nanosecond pulsed laser (Empower, Spectra-Physics) at 527 nm obtained by a frequency-doubled emission of Nd:YLF crystal and have a 1 kHz repetition rate with a 100 ns pulse width. The laser was operated at 15 W average power during the ablation process, corresponding to 15 mJ energy per pulse. Copper targets were cleaned with distilled water and isopropyl alcohol before the ablation process. The laser light was focused onto the surface of the copper plate with a 50 mm converging lens, and the ablation process lasted about 3–4 minutes to achieve a 1.00 mg mL⁻¹ concentration level. Lower concentration levels were prepared by diluting the 1.00 mg mL⁻¹ batch. An illustration of the experimental setup is shown in Figure 1a. Nanoparticles were synthesized ligand-free due to cell studies done in this work since purity is vital for cytotoxicity assays of nanoparticles. Researchers have shown that even biocompatible ligands, such as citrate, hinder toxicity experiments.^[69,70]

Characterization of Nanoparticles

The crystal structure of the nanoparticles is identified by X-ray diffraction analysis (Panalytical X'Pert PRO Multipurpose Diffractometer). The radiation source for the X-rays is CuK α , the operating voltage was 45 kV, and the used current was 40 mA. The preparation of the sample was done by centrifuging the CSNPs colloid for 2 minutes at 10000 rpm, and the precipitated nanoparticles were quickly dried on a hot plate at 200 °C on a silicon wafer to avoid oxidation during sample preparation. The images of the nanoparticles were taken using a high-resolution transmission electron microscope (HR-TEM, FEI-Tecnaï G2F30). The operation voltage was 300 kV. The samples were prepared by drop casting the CSNPs colloid prepared by laser ablation to a carbon-coated Cu grid for TEM imaging. The optical absorption spectra were taken by using the synthesized CSNPs colloid. The spectra of the CSNPs colloid were recorded in a quartz cuvette using a spectrophotometer (Varian Cary 5000 UV/Vis/NIR spectrophotometer). The photoluminescence spectra were recorded to characterize the photoluminescence properties of the nanoparticles by using a fluorescence spectrophotometer (Horiba Scientific Jobin Yvon, Fluorolog, FL-1073). The characterization of the elemental composition of the synthesized nanoparticles was performed via X-ray photoelectron spectroscopy (XPS). The measurements were carried out using the monochromatic instrument K-Alpha (Thermo Scientific), which operates at 12 kV and 2.5 mA. The sample was prepared by dripping CSNPs colloid onto a Si substrate at 200 °C heat treatment on a hot plate for approximately 5 minutes.

Photothermal Experiments

A fiber-coupled diode (LD-1080-FBG-400) continuous wave laser was used for the photothermal excitations at 1080 nm, and a thermal camera (FLIR Systems, Thermovision A40) was used to observe and record the change in temperature of nanocolloids. To investigate the photothermal response of the sample to the laser beam, we exposed a 4 mm-spot-sized laser beam of 1080 nm wavelength to the top surface of the colloidal sample. The power of the laser was adjusted by using the Thorlabs driver from 1.00 W cm⁻² to 3.23 W cm⁻². Temperature was recorded for 300 seconds for every 5 seconds. Temperature values are recorded for concentrations ranging from 1.00 mg mL⁻¹ to 0.0078125 mg mL⁻¹. Concentrations lower than 1 mg mL⁻¹ are prepared by diluting the 1.00 mg mL⁻¹ batch. Nanocolloids are used inside 2 mL Eppendorf tubes. An illustration of the experimental setup is shown in 3a.

Physicochemical Analysis of CSNPs

The average size (Z-ave), intensity, and number-weighted size distribution of the synthesized CSNP nanoparticles were determined using the dynamic light scattering technique. The measurements were conducted with a Zetasizer NanoZS instrument (Malvern) employing a backscattering configuration at a scattering angle of 173°. A He/Ne laser emission with a wavelength of 633 nm and a power source of 4.0 mW was utilized for the analysis of CSNP nanoparticles. The refractive index and absorption values chosen were 2.12 and 0.1, respectively. Before measurement, all samples were diluted in ultrapure water. The zeta potential of the nanoparticles was determined using Laser Doppler Anemometry. The measurements were performed in triplicate at a temperature of 25 °C in ultrapure water, employing the Zetasizer NanoZS instrument (Malvern).

Cellular Uptake of CSNPs

The MCF-7 breast cancer cell line was cultured in DMEM medium supplemented with 10% FBS and 1% penicillin and streptomycin under a humid atmosphere at 37°C and 5% CO₂. 22 mm×22 mm cover slides were rinsed with 100% ethanol and cleaned under UV light for 15 minutes. Slides were placed in a 6-well plate, and 50,000 cells were seeded on each slide. After cells adhered and became confluent on the glass cover slide, they were rinsed with DPBS, and 2 µg mL⁻¹ CSNPs in cell culture media (DMEM-F12 with 10% FBS and 1% Penicillin-Streptomycin) with 1% DMSO were applied. Particles were incubated overnight with the cells, and after incubation, slides were rinsed with DPBS. For imaging, cells were fixed with 4% paraformaldehyde for 5 minutes and permeabilized with 0.1% Triton X-100 for 10 minutes. The nuclei of the cells were stained with 1.5 µg mL⁻¹ Hoechst 33342 for 5 minutes and rinsed 3 times with DPBS. Cover slides were placed on the microscope slides with glycerol and imaged with DAPI and GFP filters.

Effect of CSNPs on Cell Viability

We initially assessed any effects of CSNP over cell viability, and on the course of this consideration, we did not apply photothermal activity to the nanoparticles. For the flow cytometry live/dead analysis, cultured MCF-7 cells were treated with (i) only DMSO and (ii) CSNPs in DMSO and incubated overnight. After incubation, they were rinsed with DPBS once to remove excess particles from the environment. Then they were detached from the surface with trypsin-EDTA and centrifuged for 5 minutes at 2400×g. Cell pellets were dispersed, washed with PBS, and counted with flow cytometry. 1,000,000 cells were stained with a final concentration of 50 µg mL⁻¹ in 200 µL PI and incubated for 30 minutes in the dark. Following incubation, the samples were washed once with PBS to eliminate any surplus dye from the surroundings, and samples were acquired in the PE-H (PI) channels at 488 nm excitation and 572 ± 28 nm detection of the flow cytometer. Three replicates were used for both control and treatment groups, and a t-test was applied for statistical assessments.

For the cytotoxicity assay, an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) agent from ThermoFisher was used. Briefly, cells were seeded at a concentration of 5×10⁴ cells/well in 200 µL of culture medium in 96-well microplates with a flat bottom and incubated for 24 hours at 37°C with 5% CO₂ to allow attachment. CSNP nanoparticles were then added at varying concentrations of 0.250 mg mL⁻¹, 0.125 mg mL⁻¹, 0.0625 mg mL⁻¹, 0.025 mg mL⁻¹, 0.0125 mg mL⁻¹ while maintaining a DMSO concentration of 0.5%. For the *in vitro* validation of CSNP nanoparticles on cells, we performed tests at the given concentrations as recommended in a previous study.^[56] After the incubation period, 5 mg of MTT was dissolved in 1 mL of PBS. 10 µL of the MTT labeling reagent (final concentration of 0.5 mg/mL) was added to each well, and the microplates were incubated for an additional 5 hours in a humidified atmosphere at 37°C and 5% CO₂. Finally, 100 µL of the biological grade DMSO was added to each well to facilitate the solubilization of formazan crystals and enable the measurement of assay results, and the absorbance values were measured using a plate reader at 570 nm.

In Vitro Photothermal Application of CSNPs

In order to mimic an *in vitro* tissue environment, we utilized a layer of polydimethylsiloxane (PDMS) for cell attachment, and this would enable us to generate a more complex environment compared to the 2D cultures. Compared to the earlier section, we were able to understand the photothermal activity of CSNP over cancer cells

cultured in 3D condition. In this scenario, PDMS was initially prepared by thoroughly mixing the base polymer and curing agent at a ratio of 10:1 to obtain a homogeneous mixture. The mixture was then degassed to remove any air bubbles and poured into microplates with 24 wells and a flat bottom, creating a thin layer of PDMS on each well. To enhance temporary hydrophilicity and facilitate the attachment of mammalian cells, the PDMS layers were treated with O₂ plasma using parameters of 50 sccm O₂, RF power of 100 W, chamber temperature of 20°C, and a cycle time of 2 minutes. Once the PDMS surface became hydrophilic, the cells were seeded onto the PDMS layers and allowed to reach 70% confluency. Subsequently, the cells were treated with CSNPs at concentrations of 0.250 mg mL⁻¹, 0.125 mg mL⁻¹, 0.0625 mg mL⁻¹, 0.025 mg mL⁻¹, and 0.0125 mg mL⁻¹ (in a 10% DMSO solution), followed by a 20 minute sedimentation period. After removing the medium, fresh medium was gently added to the wells to avoid disturbing the CSNPs. Subsequently, the cells were exposed to a CW laser operating at 1080 nm at a power density of 3.23 W cm⁻² at a 4 mm beam diameter for 5 minutes, and cell viability was confirmed using a live/dead viability assay.

Live/Dead Assay

The live/dead assay protocol involves staining the cells with a combination of two fluorescent dyes: calcein-AM and ethidium homodimer-1 (EthD-1). Calcein-AM is a cell-permeant dye that labels live cells with green fluorescence, indicating their viability. EthD-1, on the other hand, is a cell-impermeant dye that enters only cells with compromised membranes, such as dead or dying cells, and labels them with red fluorescence, indicating cytotoxicity. To perform the protocol, cells were first prepared by washing and suspending them in DMEM. The cell suspension was then treated with a working solution of the calcein-AM and EthD-1 dyes (1:4), followed by incubation for 30 min at room temperature. After the incubation, the cells were observed under fluorescence and confocal microscopes.

The taken images were further processed in Fiji using the live-dead quantification macro. In order to quantify the signal in the green (live cells) and red channels (dead cells), the images were split and treated separately. It was ensured that the images were in 8-bit space. To reduce background noise, the brightness and contrast were adjusted accordingly. Next, the grayscale image was converted into a binary black-and-white image. The green channel was selected, and the threshold was adjusted. To separate the individual cells that appeared as one, the image was selected, and it was processed using the "watershed" command. This created thin separation lines between the objects detected as separate by the software. Finally, the prepared image was subjected to automatic counting by selecting "Analyze Particles" from the analyze menu. The percentage of live cells was computed by dividing the number of spots detected in the green channel by the total number of green and red cells. This quotient was then multiplied by 100% to express the result as a percentage.

Photoacoustic Imaging Experiments

The imaging setup consists of a pulsed laser source, a phased array system, an ultrasound transducer, and a photoacoustic phantom. A pulsed laser (Ekspla-NT350) system was used to expose the materials, as seen in Figure 6a. In the system, the pulsed laser light was at a wavelength of 1064 nm, and its energy was 5 mJ with a frequency of 10 Hz and a pulse width of 5 ns. The laser light was collimated and coupled to the optical fiber. Digital Phased Array System (DiPhAS, Fraunhofer IBMT, Frankfurt, Germany) is used to collect data.

A phased array ultrasonic transducer operating at 7.5 MHz center frequency is used in the experiments, where the output end of the optical fiber is mounted at a 45° angle to the ultrasonic transducer. The transducer has a 70 % fractional bandwidth.^[71] The transducer is equipped with 128 elements with a 0.1 mm element pitch. DiPhAS sends an external trigger signal to the laser system. After the laser system is triggered, optical pulses are initiated, and PA waves are generated inside the phantom. Using the ultrasound transducer and DiPhAS, samples of the PA waves are acquired at an 80 MHz sampling frequency for a 95 μ s record length. In the optoacoustic phantom, a 1 mm diameter polymer tube was filled with CSNPs in DMSO, which we prepared in two different molarities of 0.25 and 1.00 mg mL⁻¹, and positioned at a depth of 1 cm. The outer diameter of the polymer tube we use here is 1.5 mm, and the inner diameter is 0.8 mm. The experiments were carried out in two configurations. For the first configuration, the transducer is positioned such that its axis is perpendicular to the axis of the acoustic wave propagation. The other configuration is parallel to the axis of acoustic wave propagation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: copper sulphide nanoparticles · laser ablation in liquid · photothermal · photoacoustic · cancer · cell viability

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