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Photothermal studies of single molecules and gold nanoparticles : vapor nanobubbles and conjugated polymers

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Enhanced photothermal contrast using near-critical xenon as transducing medium

Abstract– We experimentally show that the photothermal contrast of 20 nm gold nanoparticles can be greatly enhanced up to thousand times using xenon close to the critical point as the transducing medium. The dependence of the photothermal signal on temperature and pressure of xenon as well as on the modulation frequency is examined and compared with the common liquid glycerol. The enhanced photothermal signal could be useful for single-molecule detection since molecules could survive for longer times in studies at lower excitation powers.

5.1. Introduction

Photothermal microscopy is a high-sensitivity method based on the optical absorption and thermal properties of a sample [40]. Upon optical excitation, molecules absorb energy from incident photons, and subsequent non-radiative energy dissipation (into the local environment) results in sample heating and changes in the temperature, refractive index, and density of the surrounding medium. The heat-induced temperature/index gradient or thermal lens, which can diffract a second incoming beam, is ultimately the basis behind photothermal microscopy. Photothermal microscopy has several advantages when it is used as a detection modality. First, it is only sensitive to the heat that is dissipated by the absorbing centers. Weak scattering in the sample is discriminated from the signal [42]. Second, not all the absorbing molecules are fluorescent when they are excited, but they all dissipate energy from incident photons into their environment. Thus, photothermal microscopy can be used to detect non-fluorescent absorbing nano-objects. For gold nanoparticles, the scattering cross section scales as the sixth power of particle size, while the absorption cross section only goes as the third power of size. Therefore, photothermal microscopy is particularly suitable for probing small gold nanoparticles. Over the past decade, photothermal microscopy has enabled the study of different types of nano-objects such as noble metallic nanoparticles [43, 44, 49], quantum dots [109], single organic molecules [45], and single-wall carbon nanotubes [110].

In principle, the photothermal signal is proportional to the electromagnetic field scattered by the refractive index gradient induced by the modulated heating, and can be written as follows [42]:

$$S \approx \frac{1}{\pi\omega_0} n \left. \frac{\partial n}{\partial T} \right|_p \frac{1}{C_p \lambda^2 \Omega} \frac{\sigma_{abs}}{A} P_{heat} P_{probe} \Delta t, \quad (5.1)$$

where S is the photothermal signal, ω_0 is the focal radius of the probe beam, n is the refractive index of the surrounding medium, λ is the probe wavelength, Ω is the modulation frequency, σ_{abs} is the absorption cross section of the target object, A is the focal area of the heating beam, P_{heat} and P_{probe} are the heating and probe power at the sample respectively, and Δt is the integration time of the lock-in amplifier. If the noise in the experiment is dominated by the shot noise (ideal case), then the signal to noise ratio (SNR) can be written as:

$$SNR \approx \frac{1}{\pi\omega_0} n \left. \frac{\partial n}{\partial T} \right|_p \frac{1}{C_p \lambda^2 \Omega} \frac{\sigma_{abs}}{A} P_{heat} \sqrt{\frac{P_{probe} \Delta t}{h\nu}}, \quad (5.2)$$

where h is Planck's constant, ν is the frequency of probe beam. By optimizing the above parameters such as heating and probe powers, integration time and

by choosing a good transducing medium with large $\partial n/\partial T$, the photothermal contrast can be optimized [42].

Common liquids such as water and glycerol typically have $\partial n/\partial T \approx 10^{-4} \text{ K}^{-1}$ (in the following, we denote the isobaric coefficient as $\partial n/\partial T$). However, close to the critical point, fluids exhibit large changes in compressibility, density, specific heat, and thermal conductivity in response to small changes in temperature or pressure. Thus, $\partial n/\partial T$ of fluids diverges at the critical point. Therefore, we expect a larger photothermal signal if a critical fluid is used as the transducing medium for the photothermal microscopy. Carbon dioxide and water are commonly used as supercritical fluids to dissolve and extract substances [111, 112]. Xenon is an inert gas with its critical point at 289.733 K, 5.842 MPa [65], conditions which are not too difficult to implement experimentally. So we choose (supercritical) xenon in our experiments as the transducing medium.

In this chapter, we show the design of a high-pressure cell which can be integrated onto a conventional confocal microscope with a high N.A. objective, and experimentally demonstrate that photothermal contrast can be greatly enhanced (up to thousand times) using xenon close to its critical point as the transducing medium. We use small gold nanoparticles as the absorbing objects, and characterize the photothermal signals of gold nanoparticles in xenon under variable conditions of temperature and pressure. We also compare the signal in supercritical xenon with that in the common liquid glycerol, and examine the dependence of the photothermal signal on the modulation frequency. Enhanced photothermal contrast could have many applications, such as single-molecule detection, since much less excitation powers can be exploited to get a good photothermal contrast while the molecules do not suffer severe photobleaching.

5.2. Evaluation of $-\partial n/\partial T$ and enhancement factor in xenon

We first evaluate two terms that are important in determining the photothermal signal in the supercritical xenon. Those are the derivative of the refractive index with respect to temperature: $-\partial n/\partial T$, and the ratio between $-\partial n/\partial T$ and the thermal conductivity κ of xenon. We will use the data from the standard chemical database NIST website [65]. The derivative $-\partial n/\partial T$ characterizes the thermal expansion of a transducing medium, while a smaller thermal conductivity κ will lead to a better thermal confinement, and to a larger signal. Therefore, we use the ratio $-(\frac{1}{\kappa})\frac{\partial n}{\partial T}$ to characterize the enhancement in photothermal signal. We call it enhancement factor in what follows.

In Fig.5.1, we plot a set of isobaric curves showing the density of xenon as a

function of temperature at different pressures near the critical point.

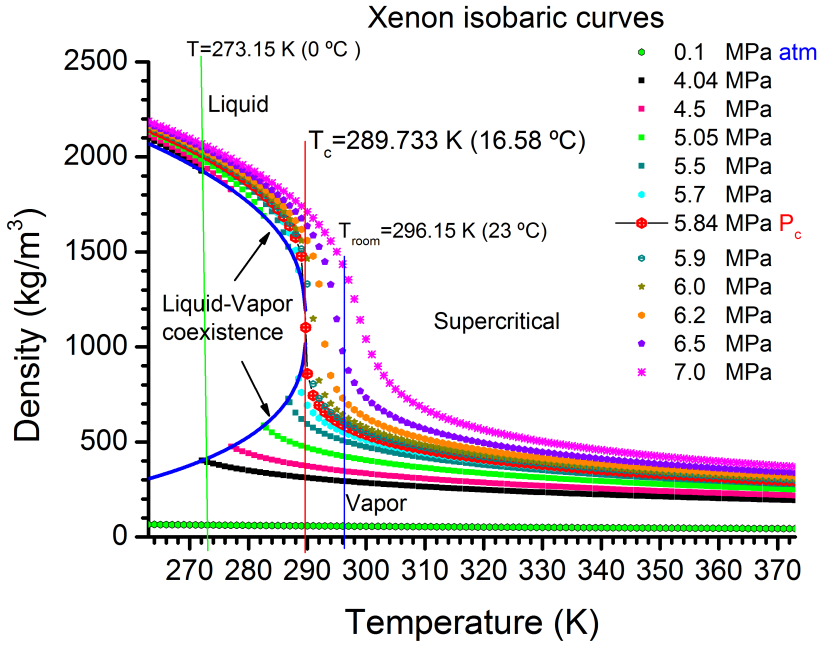


Figure 5.1: A set of isobaric curves (density vs. temperature) of Xe close to the critical point. Different colors and labels represent different pressures. The blue curve is the liquid-vapor coexistence curve. Above this curve, xenon is in the liquid phase; below the coexistence curve, xenon is in the vapor phase. To the right of coexistence curve, xenon is in the supercritical state. The three vertical lines are the three typical temperatures: 273.15 K (green), critical temperature of xenon 289.733 K (red), and the ambient temperature 296.15 K (blue). Data are from ref. [65].

The slope of each isobaric curve, representing $\partial\rho/\partial T$ at a particular temperature and pressure, can be evaluated from these curves. $\partial\rho/\partial T$ can be converted to $-\partial n/\partial T$ using the Lorentz-Lorenz function [113]. Then we will get $-\partial n/\partial T$ as a function of temperature at different pressures. The Lorentz-Lorenz function (LL) states that the refractive index n of homogeneous non-polar medium is related to its density ρ and polarizability α . It is written as follows:

$$\frac{n^2 - 1}{n^2 + 2} \frac{1}{\rho} = \frac{4\pi N_0 \alpha}{3M} = LL, \quad (5.3)$$

where n is the refractive index, ρ is the density in kg m^{-3} , M is molecular weight and N_0 is Avogadro's number. The LL function has been measured in xenon over a wide range of density and was found about $8 \times 10^{-5} \text{ kg}^{-1} \text{ m}^3$ at the critical point [113]. We will use this number in the following discussions. By differentiating the above equation on both sides with respect to the temperature T , we

can get the following expression [114]:

$$\frac{\partial n}{\partial T} = \frac{3LL}{2\sqrt{(1+2\rho LL)(1-\rho LL)^3}} \frac{\partial \rho}{\partial T}, \quad (5.4)$$

The derivative $-\partial n/\partial T$ and the enhancement factor calculated with Eq.5.4 and the data from Fig.5.1 are shown in Fig.5.2.

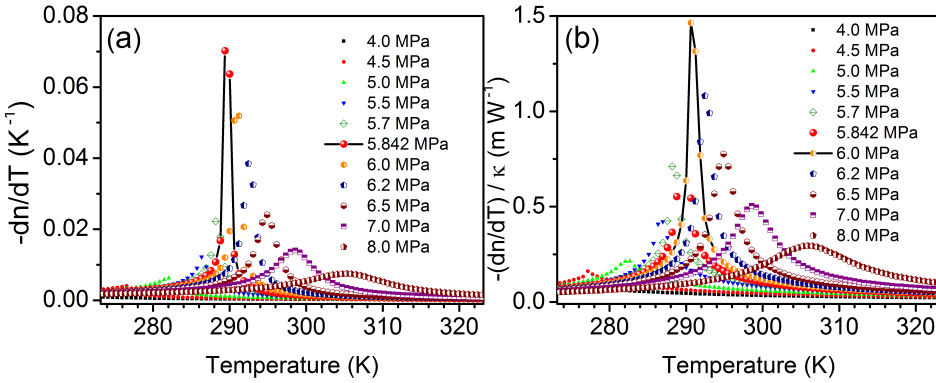


Figure 5.2: A set of isobaric curves showing $-dn/dT$ and enhancement factor in S.I. units as functions of Xe temperatures at different pressures. (a): temperature dependence of $-dn/dT$ under variable pressures (in different colors and labels). The solid curve labels are under the critical pressure, at 5.842 MPa. Because the density and thermal conductivity close to the critical point are not available from NIST, there are less data points plotted on the curves close to the critical point. (b): the enhancement factor in xenon as a function of temperature under variable pressures.

As we can see from Fig.5.2, when temperature and pressure of xenon approach the critical point, both $-\partial n/\partial T$ and the enhancement factor increase and $-\partial n/\partial T$ diverges at the critical point. This is a characteristic property of a supercritical fluid. Close to the critical point, a small change in temperature and pressure can lead to a large difference in density due to the large compressibility of the supercritical fluid. Compared with 10^{-4} K^{-1} values of conventional transducing media, supercritical xenon exhibits at least two orders of magnitude higher $-\partial n/\partial T$. Thus a larger photothermal signal is expected when supercritical xenon is used as the transducing medium. However, we also need to consider the thermal conductivity of Xenon, which defines the thermal diffusion length at a given frequency. Fig.5.2 (b) shows the enhancement factor as a function of temperature at different pressures. We can see that the maximum of the enhancement factor slightly shifts to a higher temperature and pressure compared with the critical point. This is because the thermal conductivity of xenon also increases rapidly as temperature and pressure come close

to the critical point. When the temperature and pressure move away from the optimal conditions (about 5.9 MPa, 290.6 K), the enhancement factor drops significantly but still remains much higher than in normal transducing media such as glycerol and water at ambient conditions.

Note that there are uncertainties in the evaluation of $-\partial n/\partial T$ and enhancement factor in xenon when data from NIST are used, because of the lack of data close to the critical point and the numerical errors in the derivative calculations. So Fig.5.2 should only be seen as a qualitative prediction of the thermal properties of xenon.

5.3. Experimental details

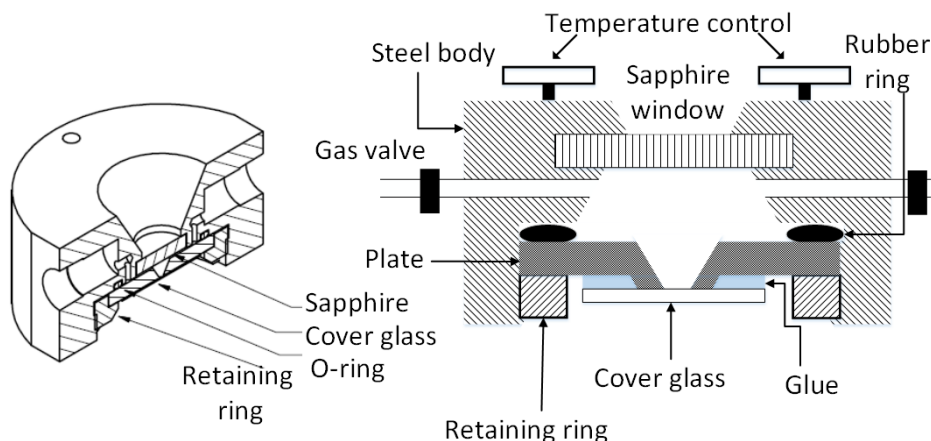


Figure 5.3: Scheme of the pressure cell used for the photothermal measurements in supercritical xenon. Left: top view of the cell; right: the cross section of the cell

The detailed description of the photothermal setup and sample preparation can be found in Chapter 3. Briefly, for sample preparation, single gold nanoparticles with diameter 20 nm are spin-coated on a clean cover glass. We adopt the design of ref.[115] and fabricate a high-pressure cell, which can be integrated into an inverted microscope with a high N.A. objective (Olympus, 60 $\times$, N.A.= 1.45). The scheme of the pressure cell is shown in Fig.5.3. The pressure cell body is made of stainless steel. On the top, a thick sapphire plate is glued to the body for alignment and to release unabsorbed light. For the objective side, a steel plate with a hole and a glued thin cover glass is retained onto the body by a metallic ring. The space enclosed by the two plates and the steel body is the gas chamber. Gases can be flushed into the pressure cell through the steel pipes and retained in the cell by the gas valves. The optimal diameter of thin

glass window D is calculated using the following equation [115]:

$$D = 2t_g \sqrt{\frac{P_{edge}}{P_{cell} \times 0.75}} \quad (5.5)$$

where t_g is cover glass thickness, P_{edge} is the edge stress of the glass, estimated to be around 58.6 MPa [115], P_{cell} is the working pressure of the pressure cell, and the constant 0.75 is empirically determined [116]. The cover glass we used has an average thickness around 0.18 mm. From the above equation, we can have a working pressure up to 18 MPa if we use a 0.75 mm diameter optical window. For the experiments in supercritical xenon, we need a minimal working pressure of about 6 MPa. Then the maximal diameter of the sample glass window is calculated to be 1.3 mm. We can tune the size of the optical window by changing the hole size of the metallic plate according to the working pressure.

A cover glass (thickness in the range of 0.16-0.19 mm) with coated samples is glued to the home-built high-pressure cell with an optical window of 0.75 mm diameter (See scheme in Fig.5.3). There is also a temperature control on the cell, which allows temperature tuning from about 286 K to ambient temperature 296 K. For the measurements in the pressure cell, first we test the quality of our glued sample by applying high pressure nitrogen to the pressure cell. Once no damage of the sample slide and no gas leakage are found in about one hour, we flush the cell with pure xenon for a few seconds in order to get rid of dust or impurities in the cell. Then we increase or change the pressure of xenon by applying pressure to a piston in a cylinder where xenon is compressed. The high-pressure cell is mounted on a three dimensional scanning piezo stage, and gold particles are located with the photothermal microscope. An intensity-modulated 532 nm CW beam is used to heat the gold nanoparticles, and a CW beam at 815 nm is used as the probe beam for the photothermal detection. A fast photodiode and a lock-in amplifier are used to pick up the photothermal signal. A typical integration time is 10 ms per pixel, and a typical imaging area is $15 \times 15 \mu\text{m}^2$. We vary the pressure and temperature in the xenon cell, then scan the same area and record the photothermal images under same heating and probe powers. Post processing of images includes the subtraction of the background where there are no particles, fitting intensity of each spot with a two-dimensional Gaussian function and statistics such as mean value and distribution of the signals.

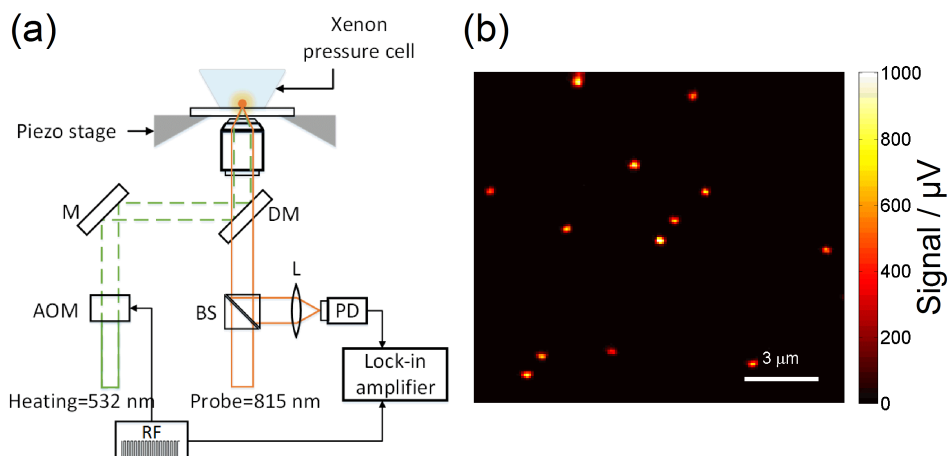


Figure 5.4: Experimental setup and the photothermal image of 20 nm gold nanoparticles on a cover glass in supercritical xenon. (a): scheme of the optical setup. AOM: acousto-optics modulator; DM: dichroic mirror; PD: photodiode; RF: signal generator. (b): photothermal image of 20 nm gold nanoparticles in supercritical xenon. The heating power used is $4 \mu\text{W}$ at 532 nm; the probe power is 15 mW at a wavelength of 815 nm. All the powers mentioned were measured at the back aperture of the objective. The integration time at each pixel is 10 ms, and the modulation frequency in this case is 50 kHz. The image has been background-corrected by subtracting the average signal over an area where there are no particles. The pressure and temperature in the xenon cell are 6.21 MPa and 287 K respectively.

5.4. Results and discussion

The scheme of the optical setup is shown in Fig.5.4 (a). Fig.5.4 (b) shows the typical photothermal image of 20 nm gold nanoparticles deposited on the glass in the supercritical xenon environment. With only about $4 \mu\text{W}$ heating power, 15 mW probe power, and 10 ms integration time, we got an averaged photothermal contrast as high as 0.8 mV against the background ($3.5 \mu\text{V}$, has been subtracted from Fig.5.4.b) for a 20 nm gold particle. Compared with the results formerly reported by our group [42], the photothermal signal normalized by heating and probe powers in supercritical xenon improves by at least a hundred times (note that former measurements were done in glycerol, alignment and modulation frequency are different). The signal to noise ratio (SNR) here is about 400. Normalized by powers and by the integration time, this ratio is about 20 times that in glycerol in Ref.[42]. The noise in the measurements could originate from the power fluctuation of probe intensity, photon noise (shot noise) in the probe due to the quantum nature of photons, and thermal noise in the photodetector. All of them could contribute to the total noise in the photothermal signal and could be "amplified" by the supercritical xenon. Thus, the gain in SNR is

less impressive than the gain in sensitivity (signal with respect to background). However, the big advantage of using supercritical xenon is that much less heating power is required because the photothermal contrast still remains good at low heating power. This will facilitate single-molecule detection because the photobleaching time of the molecules will be accordingly lengthened. The absorbed power in Fig.5.4 (b) is estimated to be around 4 nW, and the temperature rise of the gold particle surface to about 0.8 K.

Under the same powers, integration time, and modulation frequency, we vary the pressure and temperature of the xenon cell to check the signal dependence on these two parameters. We image the same area under each temperature and pressure conditions, and get the averaged photothermal signal in one image. Then we plot the averaged signal as a function of temperature and pressure in a two-dimensional color-coded map, as shown in Fig.5.5.

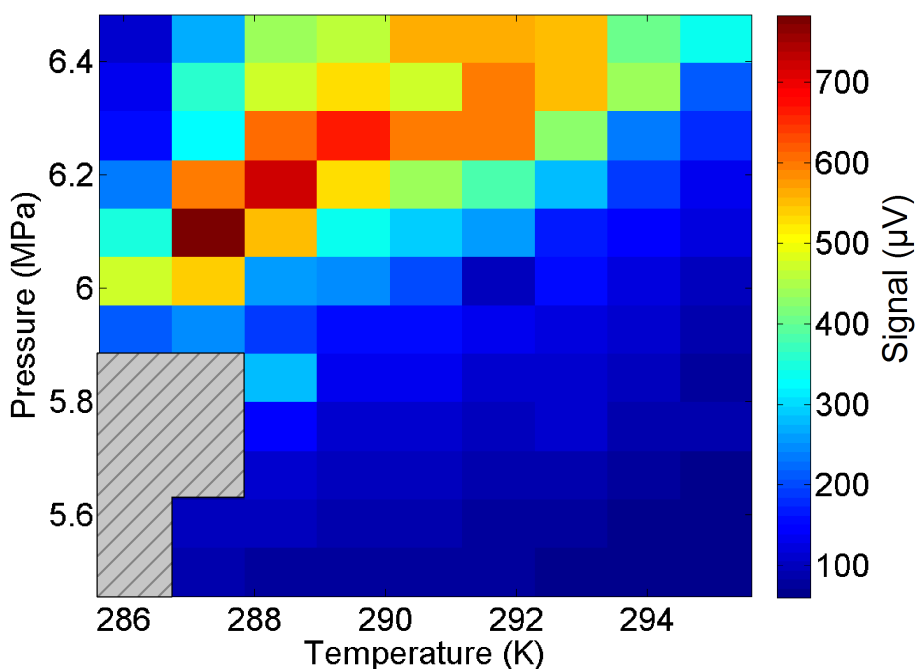


Figure 5.5: Photothermal signal of 20 nm gold particles in xenon under variable temperature and pressure of the xenon cell. Each pixel is the averaged signal over 13 particles in one image. The averaged signals have been corrected by subtracting the background. The heating beam (532 nm) is modulated at 50 kHz, the power at the back focal plane of the objective is 4 μ W; the probe power is 15 mW at 815 nm. The integration time is 10 ms. There are no data measured in the hatched region in the left corner from (286 K, 5.5 MPa) to (288 K, 5.8 MPa).

As expected, the photothermal signal indeed significantly increases when

the temperature and pressure of xenon approach to the critical point (5.842 MPa, 289.733 K). The maximal signal in the measurements is found under conditions around 6.1 MPa and 287 K, slightly different from the optimal conditions calculated with NIST data in the Section 5.2 (5.9 MPa, 290.6 K). The slight difference could be due to the following reasons. First, the local temperature of the gold nanoparticle is different from the temperature measured in the experiments (the pressure cell temperature), and the probe beam could also induce some heating of the gold nanoparticle because of the non-negligible absorption at the probe wavelength; Second, xenon purity in the cell might change slightly from measurement to measurement even though the cell is always flushed with xenon before each measurement.

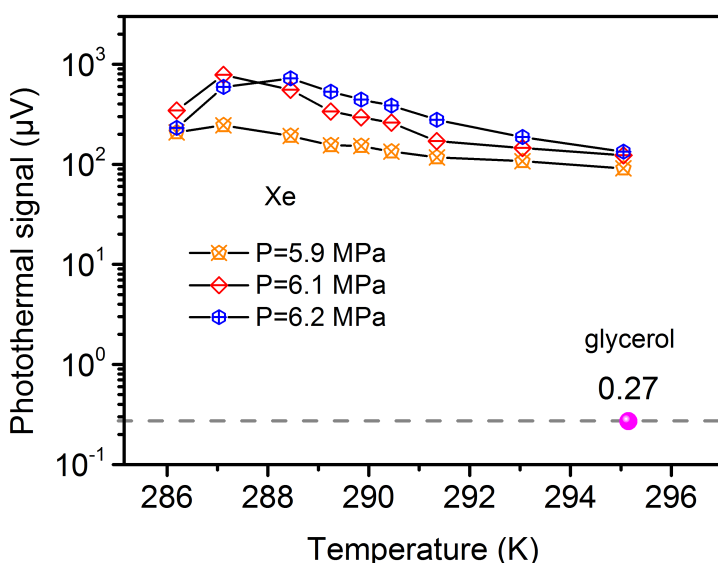


Figure 5.6: Three isobaric curves (in different colors and labels) showing the photothermal signal as a function of temperature of the xenon cell above the critical pressure. Orange circle with cross: at pressure 5.9 MPa; red rhombus: at pressure 6.1 MPa; blue circle with plus: at pressure 6.2 MPa. The pink dot in the right corner represents the typical normalized photothermal signal of 20 nm gold particles averaged over 11 particles when glycerol is used as the transducing medium at ambient conditions.

We cut a number of lines along the temperature axis in Fig.5.5 and get serials of isobaric curves of photothermal signals as a function of the xenon cell temperature, as shown in Fig.5.6. We can see that the signal drops by about a factor of 7 when temperature moves away from the optimal conditions to well above the critical point, even though xenon is in the supercritical state in both cases. We also indicate the photothermal signal of 20 nm gold particles

in glycerol under ambient conditions (295 K, 0.1 MPa), normalized to the same laser powers used in xenon. The maximum photothermal signal in supercritical xenon is three orders of magnitude higher than that in glycerol. Even at the tails of curves where temperature and pressure are away from optimal conditions, photothermal contrast is still much higher than in glycerol because of the relatively large compressibility of the supercritical fluid compared with normal liquids. We can also compare the data of our measurements with the results obtained from NIST data, and a good agreement is found between the two results. (for glycerol, $-dn/dT$ is $2.7 \times 10^{-4} \text{ K}^{-1}$, and thermal conductivity κ is $0.28 \text{ W m}^{-1} \text{ K}^{-1}$, the $(-dn/dT)/\kappa$ is 9.6×10^{-4} in S. I. units. The maximum enhancement number $(-dn/dT)/\kappa$ from the NIST data in Fig.5.2, is 1.8 in S. I. units. Then the ratio of enhancement factor between Xe and glycerol is $1.8/9.6 \times 10^{-4} = 1900$, of the same order of magnitude as we measure.)

We examine the modulation frequency dependence of the photothermal sig-

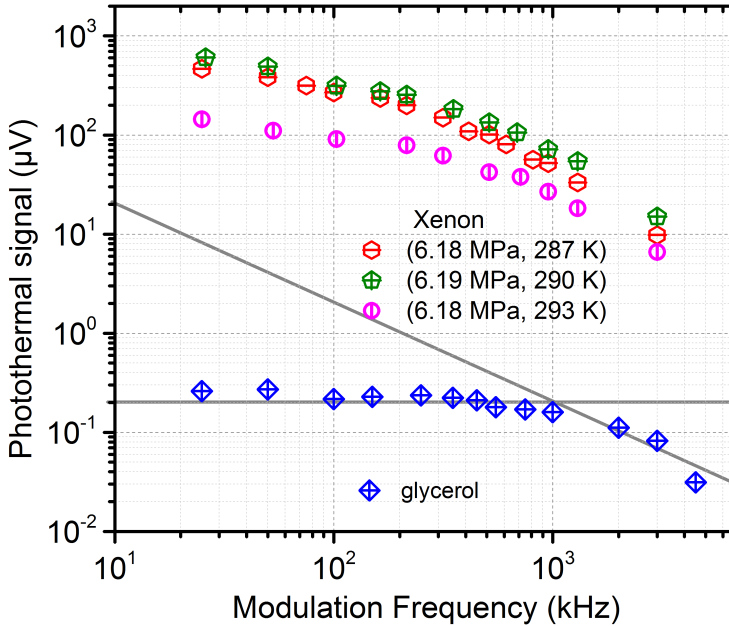


Figure 5.7: Modulation frequency dependence of photothermal signal of 20 nm gold nanoparticles in supercritical xenon and glycerol (blue diamonds). The three scatter plots represent three different temperatures of the xenon cell above critical pressure: (6.18 MPa, 287 K) red hexagons, (6.19 MPa, 290 K) green pentagons and (6.18 MPa, 293 K) pink circles. The heating power used is $4 \mu\text{W}$; the probe power is 15 mW ; integration time is 10 ms . Again, each point is the average photothermal signal over 13 particles in one image. The blue diamonds are the measurements in glycerol normalized to the same heating and powers as above. The cross point of two gray lines shows the cutoff frequency in glycerol.

nal in supercritical xenon and compare with the case of glycerol. The results are shown in Fig.5.7. The modulation frequency is tuned from 25 kHz to 2 MHz. In both cases of supercritical xenon and glycerol, the photothermal signals show a low-pass behavior, as reported previously in ref.[49]. The heat diffusion length r_{th} is related to the modulation frequency through the equation $r_{th} = \sqrt{2\kappa/C_p\Omega}$ [49], where κ is the thermal conductivity of the medium, C_p is the heat capacity per unit volume, and Ω is the modulation frequency of the heating beam.

At higher frequencies (higher than the corner frequency $\Omega_c = 2D(2\pi n/\lambda)^2$, where D is the thermal diffusivity, n is the refractive index, λ is the incident wavelength), the thermal diffusion length becomes much smaller than the focus waist of the probe beam, and the induced thermal lens is less efficient to scatter the probe beam. Thus the photothermal signal decreases as the modulation frequency increases. At lower modulation frequencies, the scattering object or thermal lens becomes much bigger than the point spread function of the probe beam, but as it efficiently scatters only in the probe focus, the signal saturates. Compared with the frequency dependence in glycerol with a corner frequency at round 1 MHz (see the crossing point of two grey lines in Fig.5.7), the photothermal signal in supercritical xenon does not show any clear corner frequency. Instead, it continues to increase with a lower slope as the modulation frequency decreases, similar to what has been observed in photothermal deflection measurements in supercritical xenon [117]. We assign the further increase of signal at lower frequency to "critical slowing down" effects in the phase transition when a substance approaches/crosses the critical point. The critical slowing down has been observed in many ferromagnetic systems [118]. These effects are related to the absence of clear length and time scales in critical fluids. Response functions such as the compressibility become infinite at the critical point, but only when averaging over long relaxation times. Similarly, the full extent of such fluctuations requires the participation of very long distances. Because of the finite modulation frequency and integration time of the measurements, we actually "catch" only a fraction of $-\partial n/\partial T$ of the fluctuations. The slower we modulate and the longer the integration times we use, the higher this fraction will be and the larger the critical enhancement effect. Based on this, one can, in principle, further increase the photothermal signal of gold nanoparticles in supercritical xenon by lowering the modulation frequency. However, at lower frequencies, all kinds of mechanical and laser intensity noises will become dominant, and the signal to noise ratio would degrade due to the increased noise level. Therefore, in the experiments, we aim for a trade-off between modulation frequency and signal-to-noise ratio in order to optimize measurements.

5.5. Conclusions

In this chapter, we experimentally demonstrated that the photothermal contrast of small gold nanoparticles (20 nm in diameter) can be greatly enhanced when supercritical xenon is used as the transducing medium for photothermal microscopy. We check the signal's dependence on xenon's temperature and pressure, and compare the signal in supercritical xenon with that in glycerol which is frequently used in our previous work. The signal in supercritical xenon is several orders of magnitude higher than that in glycerol near the critical point. The measured enhancement factor shows a qualitative agreement with the results calculated with NIST data. We also examine the modulation frequency dependence of the photothermal signal in supercritical xenon. It shows a continuous increase as the frequency decreases. A possible explanation is offered by the "critical slowing down" effect in a critical fluid. Both length and time scales diverge at the critical point. The enhanced photothermal contrast could be helpful in single-molecule detection, since much lower excitation powers can be used to study the absorption properties of molecules, while photo-bleaching is mitigated at lower power.

