Enclosure 3

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6. AUTHOR(S)

Professor William Happer

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Princeton University
Physics Department
Princeton, NJ 08544

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13. ABSTRACT (Maximum 200 words)

We have studied the physics underlying the production, accumulation, and cryogenic separation of hyperpolarized xenon-129. This research has led to a dramatically improved understanding of the physics behind nuclear relaxation in solid xenon and resolved long-standing discrepancies between theoretical predictions and experimental observation. The information gained from these experiments allowed us to produce greater quantities of hyperpolarized ^{129}Xe with higher nuclear polarization. This in turn permitted us to begin cutting-edge studies applying hyperpolarized ^{129}Xe nuclear magnetic resonance (NMR) spectroscopy to samples of biological interest. Specifically, we performed preliminary experiments which demonstrated that hyperpolarized ^{129}Xe spectroscopy may be useful in the diagnosis of atherosclerosis.

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Enclosure 1

(1) Foreword:

(2) Table of Contents:

(3) List of Appendixes, Illustrations, and Tables

Illustrations

(4) Statement of the problem studied

The goal of this project was twofold: (1) to improve yields of hyperpolarized ^{129}Xe and increase its nuclear polarization, and (2) to carry out exploratory experiments on the use of ^{129}Xe for medical diagnostics. Our research has allowed us to make great strides in explaining the relaxation of solid xenon, leading to a better understanding of polarization loss during the cryogenic distillation of hyperpolarized ^{129}Xe. By modifying the cryogenic separation procedure to account for these depolarizing effects, we were able to modify our xenon polarizer to produce larger volumes of more highly-polarized ^{129}Xe. With the resulting increase in signal, we could investigate the applications of hyperpolarized xenon-129 NMR spectroscopy to medical and biological research. We focused on contrasting the chemical shift of ^{129}Xe dissolved in healthy and in atherosclerotic blood vessels.

(5) Summary of the most important results

Experimental results[1] achieved in this lab demonstrate that the nuclear spin relaxation lifetime (T_1) of hyperpolarized xenon-129 is much shorter in the solid phase than had been expected. Previous theoretical calculations explained frozen xenon relaxation by invoking spin-flip interactions with lattice phonons, an effect which correctly predicts the 2.5-hour xenon T1 at liquid nitrogen temperatures. Our group made highly precise measurements of xenon relaxation times near melting and discovered that they are much shorter than anticipated (ranging from seconds to a few minutes), with the decrease being most dramatic at low magnetic field. We

have also generated a model which explains the relaxation of "warm" frozen xenon (solid xenon above 120K) as the result of vacancy diffusion in the crystal lattice. This model uses only previously measured quantities and yet fits these new data with impressive accuracy:

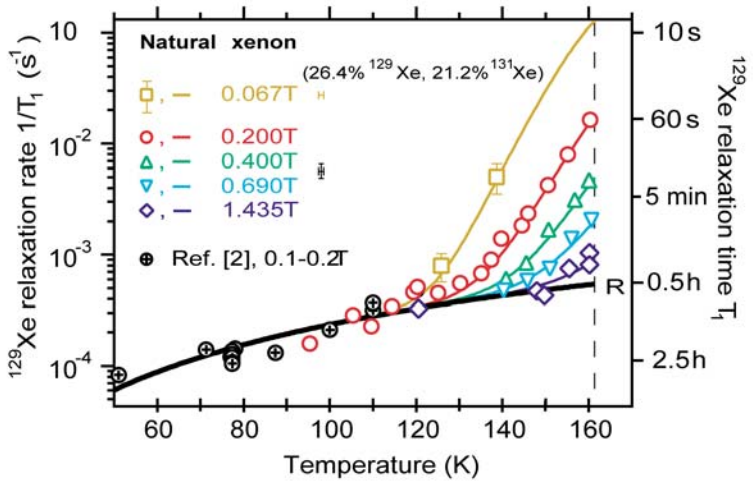


Figure 1: Relaxation rate of solid ^{129}Xe as a function of magnetic field and temperature. The previous theory (black line) was unable to account for enhanced T1 relaxation above 120 K and at low magnetic fields[2]. Our simulations (colored lines) predicted these relaxation rates very accurately. Figure taken from [1].

These relaxation data suggested two ways to conserve more polarization during cryogenic distillation: lower the collection temperature, or increase the magnetic field. The former tactic is very challenging to implement, since 77 K is the lower limit for the thermal bath (lest LN_2 condense in the cold finger) and also because the thermal conductivity of the xenon “snow" is so low. This leaves the option of increasing the holding magnetic field during solid xenon collection.

An experiment was performed to test the effectiveness of a stronger permanent magnet on the fraction of ^{129}Xe polarization retained during cryogenic separation. Xenon was polarized and collected in an accumulator held at 77K in a magnetic field which could be varied. The xenon was then thawed and flowed into an NMR spectrometer. By comparing the initial and final xenon polarizations, it was possible to measure the ^{129}Xe polarization retained during the cryogenic separation process. We found that increasing the field of the permanent magnet within the polarizer can improve the end polarization of the pure xenon by a factor of two or more. Typically, anywhere from seventy to eighty percent of the initial xenon polarization is lost when the xenon is frozen out in the 700-gauss field (previously the industry standard for commercial xenon polarizers), whereas the percentage lost in a 1.2-tesla field is only forty to fifty percent. It came as a welcome surprise that the simple measure of installing a new permanent magnet could enhance the xenon polarization (and thus the signal-to-noise ratio in NMR trials) by such a large amount. Moreover, we learned that most of the nuclear relaxation occurs in the very brief time it takes to thaw the xenon, and not throughout the cryogenic accumulation process:

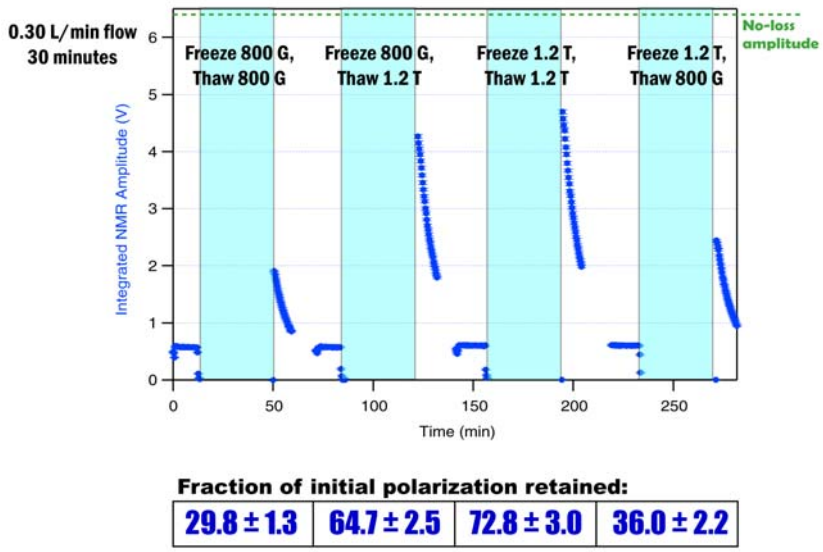


Figure 2: Relaxation of ^{129}Xe during the cryogenic accumulation process and subsequent thawing. Nearly 70% of the original xenon polarization is lost when the xenon is thawed in a moderate magnetic field of 800 Gauss; this loss is cut in half when the xenon is thawed in a magnetic field of 1.2 Tesla (12000 Gauss). From a talk presented at the 2004 DAMOP conference.

Armed with higher nuclear polarizations in the xenon gas, we switched our focus to the application of hyperpolarized ^{129}Xe NMR spectroscopy to disease diagnosis. Xenon is highly lipophilic and dissolves readily in most biological tissues. Moreover, the chemical shifts of dissolved xenon are extremely sensitive to its molecular environment. We exploited this sensitivity, combined with the high signal-to-noise ratios (SNR) available with hyperpolarized ^{129}Xe, to explore the structural differences between normal and diseased tissues of human aorta affected by atherosclerosis. This technique holds promise for clinical trials because inhaled xenon is absorbed into the bloodstream and then deposited into blood vessel walls, exactly where the symptoms of atherosclerosis are most manifest. Early results indicate that the chemical shift spectrum of xenon dissolved in diseased aorta tissue is qualitatively different from that of xenon in healthy tissue:

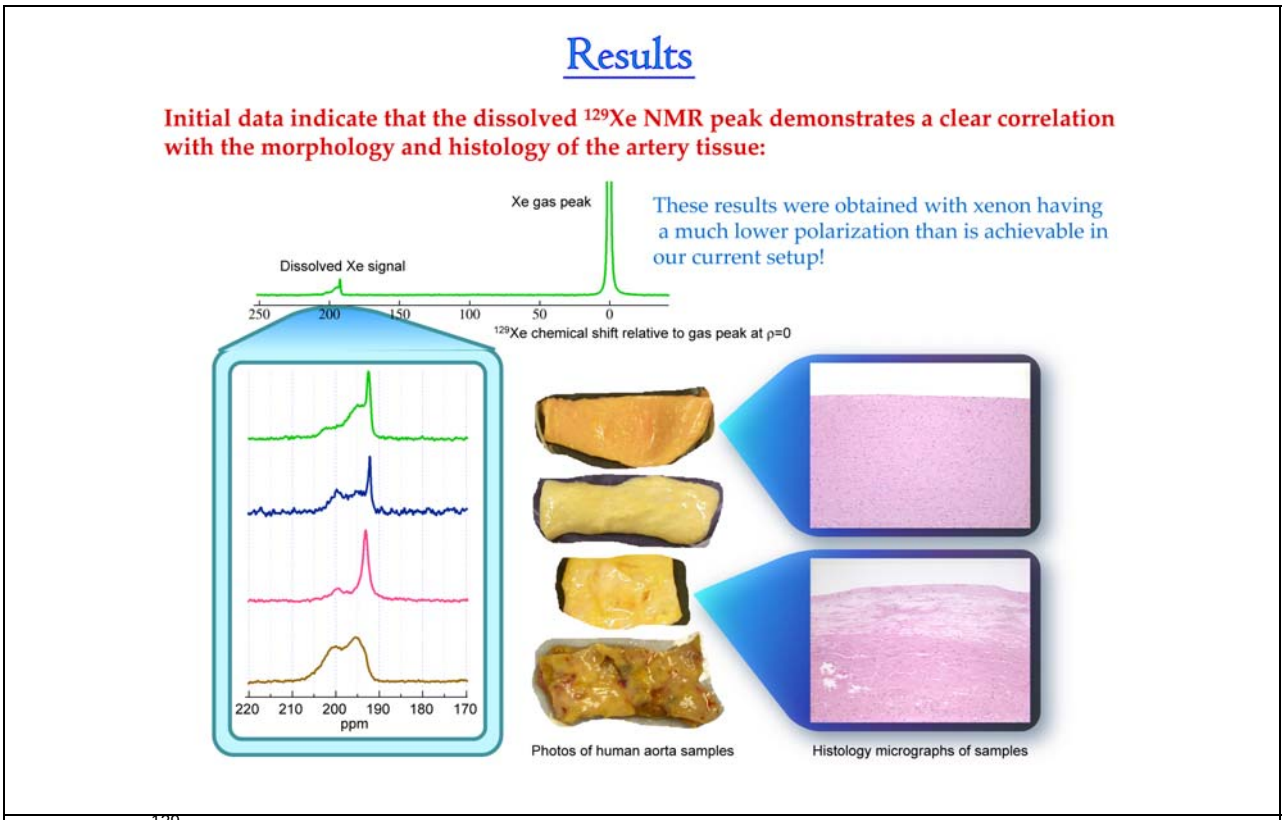


Figure 3: ^{129}Xe NMR spectra on ex vivo human aorta samples. A qualitative but evident correlation exists between the xenon NMR spectrum and the condition of the sample.

After these initial measurements we focused on improving the delivery of pure polarized xenon gas to the sample of interest. Based upon our knowledge of xenon gas relaxation rates as a function of magnetic field and magnetic field gradient [3], we designed an NMR probe which can store 100 cc's of xenon gas in the homogeneous high-field region of the NMR magnet bore, directly underneath the sample. Not only does this reduce relaxation in the gas-phase xenon; it also decreases the transfer distance to the sample and thus reduces additional mechanisms of relaxation (such as wall relaxation) which are incurred as the xenon flows from the storage volume to the NMR coil. The measured relaxation time of the gaseous ^{129}Xe in this container is over 2 hours, whereas the average experiment duration is less than 30 minutes. The experiment length is comparable to the time required to prepare an additional batch of xenon, so we could conceivably replenish the xenon as quickly as we deplete it and average *ad infinitum*, obtaining unprecedented signal-to-noise ratios for xenon spectroscopy.

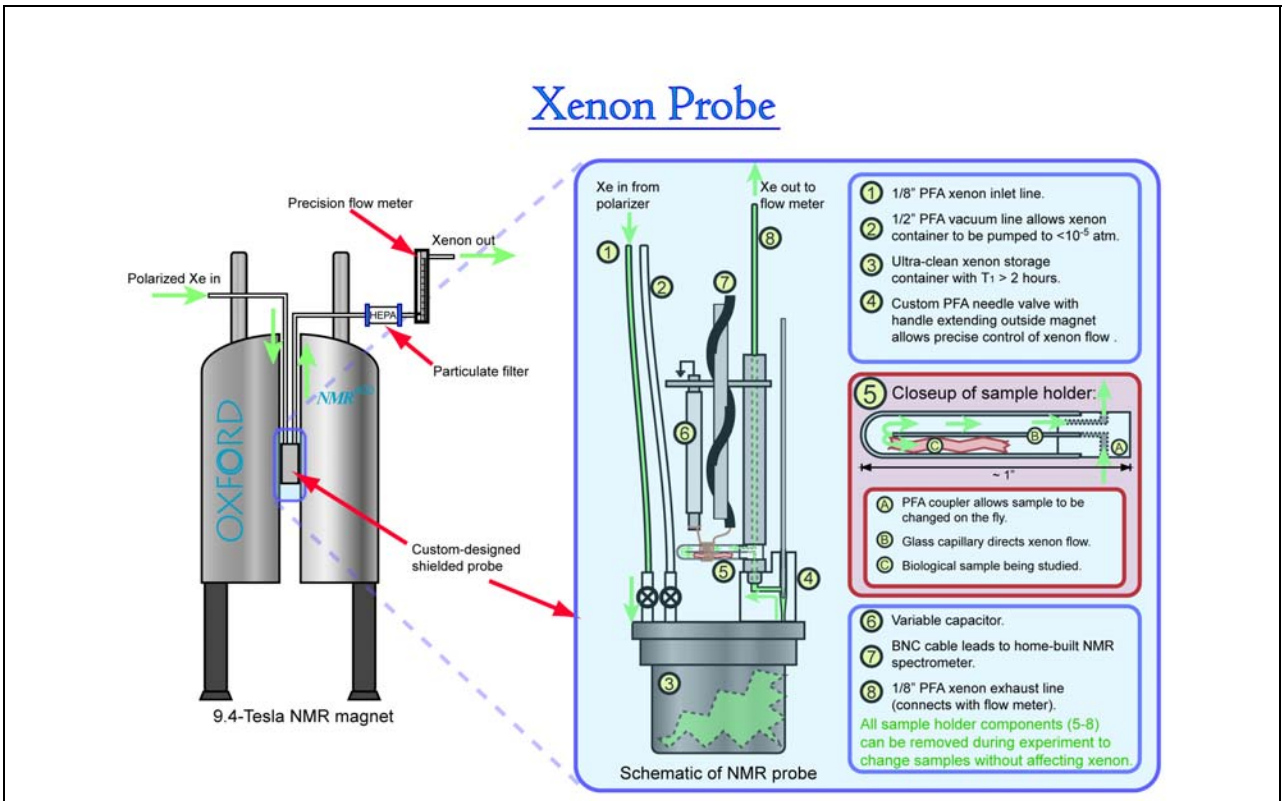
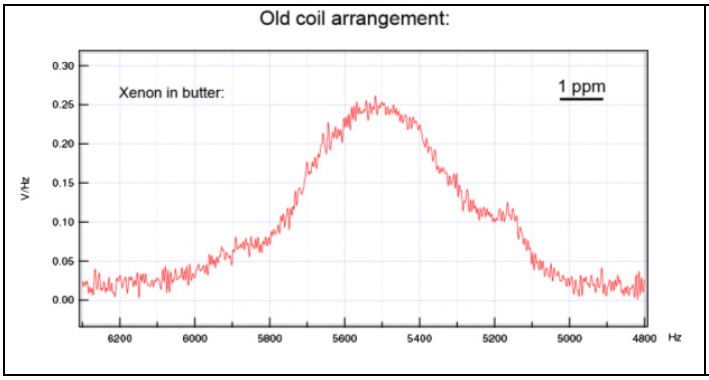


Figure 4: The ^{129}Xe NMR probe designed in these studies. Polarized xenon gas is held in a reservoir below the NMR coil, which can be removed from the probe independently from the gas container. This allows samples to be switched on the fly without depolarizing the remaining gas.

These advantages come at the cost of a larger inherent linewidth, since the internal geometry of the new NMR probe exacerbates the effects of magnetic susceptibility changes at material interfaces and thus enhances susceptibility-related field gradients within the NMR sample. We modeled these effects extensively and re-designed the probe in order to minimize these line-broadening gradients. The result was a dramatically narrowed xenon spectrum which allowed for identification of a heterogeneous lipid/water sample through hyperpolarized xenon NMR:



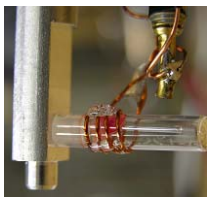
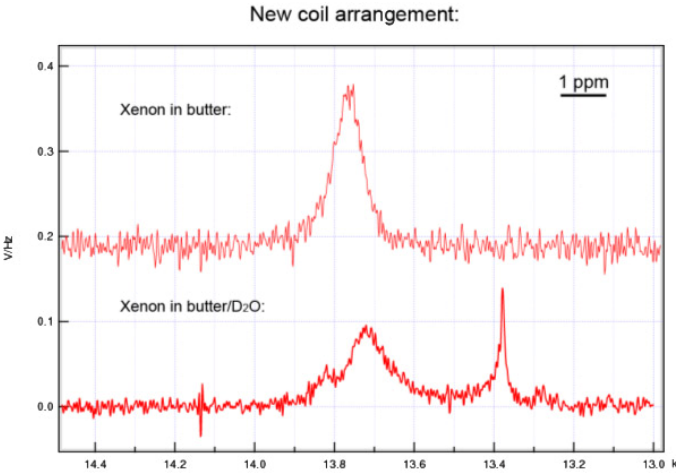


Fig 5: Original attempts to obtain ^{129}Xe chemical-shift spectra by blowing gas at a sample in a horizontal coil led to severe diamagnetic susceptibility broadening.



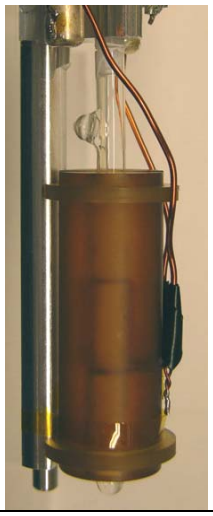


Fig 6: By using thinner coil wire in a vertical sample geometry and a susceptibility-matched coil enclosure, much narrower ^{129}Xe lines were achieved both in lipid/gas and lipid/D_2O phantom samples. In both cases a thin layer of lipid (dairy butter) was spread on the walls of 5-mm NMR tube. ^{129}Xe was either blown through the tube or bubbled through D_2O in the tube prior to NMR spectrum acquisition.

It is important to note that the technological achievements mentioned in this section are not limited in scope to biological samples, but that we now have the ability to perform high-resolution, long time-scale 129Xe NMR spectroscopy on any material.

Because of our expertise in the area of hyperpolarized xenon production and high-resolution ^{129}Xe NMR spectroscopy, several groups have sought our help in testing new applications of hyperpolarized xenon. Most recently, we collaborated with the Dmochowski group at the University of Pennsylvania to test new ^{129}Xe NMR biosensors for matrix metalloproteinase detection [4]. With our unique experimental setup we were able to perform high-resolution NMR spectroscopy and observe the change in ^{129}Xe chemical shift as matrix metalloproteinase was added to the biosensor. This is a very promising field of study in the near future, as such biosensors can be synthesized for a variety of compounds.

All these applications have benefited from our expertise in the production and detection of hyperpolarized xenon, and we expect that this area of research will be fruitful for many years to come.

[1] N. N. Kuzma, B. Patton, K. Raman, and W. Happer, Phys. Rev. Letters **88**, 147602 (2002).
[2] R. J. Fitzgerald, M. Gatzke, D. C. Fox, et al., Phys. Rev.B 59, 8795 (1999)
[3] G. D. Cates, D. J. White, T. R. Chien, et al., Phys. Rev. A **38**, 5092 (1988).
[4] Q. Wei, G. K. Seward, P. A. Hill, et al., Journal of the American Chemical Society 128, 13274 (2006).

(6) Listing of all publications and technical reports supported under this grant or contract.

(a) Papers published in peer-reviewed journals

1. Wei, Q.; Seward, G. K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; and Dmochowski, I. J. (2006). "Designing Xe-129 NMR biosensors for matrix metalloproteinase detection." Journal of the American Chemical Society **128**(40): 13274-13283.

2. Kuzma, N. N.; Babich, D.; and Happer, W. (2002). "Anisotropic nuclear spin relaxation in single-crystal xenon." Physical Review B **65**(13): 134301.

3. Kuzma, N. N.; Patton, B.; Raman, K.; and Happer, W. (2002). "Fast nuclear spin relaxation in hyperpolarized solid 129Xe." Physical Review Letters **88**(14): 147602.

4. Patton, B.; Kuzma, N. N.; and Happer, W. (2002). "Chemical shift of hyperpolarized 129Xe dissolved in liquid nitrogen." Physical Review B **65**(2): 020404.

(b) Papers published in non-peer-reviewed journals or in conference proceedings

None.

(c) Papers presented at meetings, but not published in conference proceedings

Talks presented at conferences:

1. “Progress in High-Field Pumping of Alkali Metal Nuclei”, presented by B. Patton at the 2006 DAMOP Meeting (the American Physical Society’s Division of Atomic, Molecular, and Optical Physics) May 16-20, 2006; Knoxville, TN

2. “NMR Studies of Phase Transitions in Alkali Metal Films on Glass Substrates”, presented by K. Ishikawa at the 2006 DAMOP Meeting, May 16-20, 2006; Knoxville, TN

3. “Reducing Relaxation of Hyperpolarized ^{129}Xe during Cryogenic Separation”, presented by B. Patton at the 2004 DAMOP Meeting, May 25-29, 2004; Tucson, AZ

4. “Hyperpolarized 129Xe NMR: Avoiding Spin Relaxation During Xenon Storage and Transfer”, presented by B. Patton at the 2003 DAMOP Meeting, May 21-24, 2003; Boulder, CO

5. “Hyperpolarized 129Xe NMR: Avoiding Spin Relaxation During Xenon Storage and Transfer”, presented by B. Patton at the 2003 APS March Meeting, Mar 3-7, 2003; Austin, TX

Posters presented at conferences:

1. "Hyperpolarized ^{129}Xe Spectroscopy in Heterogeneous Samples: Characterizing Mouse Models of Human Atherosclerosis"
   N. N. Kuzma and B. Patton
   presented at the 47th Experimental Nuclear Magnetic Resonance Conference (ENC), April 23-28, 2006; Asilomar, CA

2. "NMR Studies of Phase Transitions in Alkali Metal Films"
   B. Patton, K. Ishikawa, Y.-Y. Jau, and W. Happer
   presented at the 47th ENC, April 23-28, 2006; Asilomar, CA

3. "Using Hyperpolarized Xenon-129 NMR to Detect Atherosclerosis",
   B. Patton, N. N. Kuzma, I. E. Dimitrov, and N. V. Lisitza
   presented at the 2005 DAMOP Conference, May 18, 2005; Lincoln, NE

4. "Quantitative Spectroscopy of Atherosclerosis in Human Tissues with Hyperpolarized Xenon-129"
   B. Patton, N. N. Kuzma, I. E. Dimitrov, N. V. Lisitza, and W. Happer
   presented at the 46th ENC, April 14, 2005; Providence, RI

5. "Reducing the Relaxation of Frozen Hyperpolarized ^{129}Xe during Cryogenic Separation"
   B. Patton, N. N. Kuzma, and W. Happer
   Presented at the 45th ENC, April 18-23, 2004; Asilomar, CA

6. "High-field, high-density hyperpolarized xenon NMR of biological tissues"
   N. N. Kuzma, N. V. Lisitza, R. K. Mazitov, C. R. Pacheco, and B. Driehuys
   presented at the Gordon Conference on Magnetic Resonance, June 15 - 20, 2003; Newport, RI

7. "Hyperpolarized ^{129}Xe Spectroscopy at High Field: Avoiding Spin Relaxation During Xenon Storage and Transfer"
   B. Patton, N. N. Kuzma, N. V. Lisitza, and W. Happer
   presented at the 44th ENC, March 30 - April 4, 2003; Savannah, GA

8. "Xe-129 Spectroscopy in Medical Diagnostics: Identification of Spectral Features and Detection of the Disease".
   R. Mazitov, C. R. Pacheco, N. N. Kuzma, and B. Driehuys
   presented at the 44th ENC, March 30 - April 4, 2003; Savannah, GA

(d) Manuscripts submitted, but not published

None.

(e) Technical reports submitted to ARO

Do we have anything to put in this section?

(7) List of all participating scientific personnel showing any advanced degrees earned by them while employed on the project

Dr. William Happer, principal investigator

Brian Patton, graduate student

Nick Kuzma, postdoctoral researcher

(8) Report of Inventions (by title only)

(9) Bibliography

(10) Appendixes