

AD \_\_\_\_\_

Award Number: DAMD17-00-1-0159

TITLE: Screening for Breast Cancer Using Near-Field Infrared Spectroscopy of a single Strand of Hair

PRINCIPAL INVESTIGATOR: Shyamsunder Erramilli, Ph.D.  
Mi K. Hong, Ph.D.

CONTRACTING ORGANIZATION: Boston University  
Boston, Massachusetts 02215

REPORT DATE: December 2003

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040720 024

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY</b> (Leave blank)		<b>2. REPORT DATE</b> December 2003	<b>3. REPORT TYPE AND DATES COVERED</b> Final (1 Aug 00-30 Nov 03)	
<b>4. TITLE AND SUBTITLE</b> Screening for Breast Cancer Using Near-Field Infrared Spectroscopy of a single Strand of Hair			<b>5. FUNDING NUMBERS</b> DAMD17-00-1-0159	
<b>6. AUTHOR(S)</b> Shyamsunder Erramilli, Ph.D. Mi K. Hong, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Boston University Boston, Massachusetts 02215  E-Mail: shyam@bu.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited			<b>12b. DISTRIBUTION CODE</b>	
<b>13. ABSTRACT (Maximum 200 Words)</b> <p>This work was motivated by an Australian study that used synchrotron x-ray diffraction to identify changes in the structure of hair that may be linked to either the occurrence of breast cancer, or the increased predisposition to breast cancer because of the presence of a mutation of the <i>BRCA1</i> gene. In this study, we have successfully developed a new infrared method for the detection in a single strand of hair the presence of lipid deposits that were the putative cause of the observed x-ray patterns. Our method, which does not use synchrotron radiation, is based on a table-top infrared technique and provides an independent test of the proposed link between hair structure and breast cancer. Our tests show that we find the presence of lipids in healthy control patients as well. We performed independent x-ray studies in collaboration with researchers at Cornell University, who have confirmed our finding that the x-ray scattering patterns are observed in hair from healthy patients. Taken together our work suggests that (i) IR microscopy is promising, but (ii) the Australian study is wrong - a disappointing result for breast cancer.</p>				
<b>14. SUBJECT TERMS</b> Infrared spectroscopy; x-ray diffraction; hair			<b>15. NUMBER OF PAGES</b> 12	
			<b>16. PRICE CODE</b>	
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	6
Reportable Outcomes.....	7
Conclusions.....	11
References.....	12
Appendices.....	

## INTRODUCTION

This is the final report that describes the results of a research effort for performing high resolution infrared microscopy on a single strand of human hair. This work was motivated by synchrotron x-ray scattering experiments performed in Australia (James et al, 1999) that suggested that there are structural changes in hair samples taken from women who either have breast cancer, or have an increased genetic risk of the disease. The structural changes observed by x-ray scattering were attributed to lipid deposits, and it was suggested that the presence of lipid deposits in hair serve were correlated with breast cancer, or the predisposition for this disease. Because the technique was non-invasive, and was claimed to have a very degree of specificity and sensitivity, it appeared to provide a powerful screening tool for breast cancer.

Our proposal was to use infrared spectroscopy to search for the lipid deposits, as an alternative to synchrotron x-ray diffraction. Because infrared spectroscopy is uniquely capable of characterizing and identifying biomolecules like lipids from their spectral "fingerprints", this work had the potential for:

- (i) providing an independent test of the x-ray diffraction studies, and providing insight into the molecular basis for the structure and composition of human hair linked to the formation of breast cancer;
- (ii) leading to the development of a novel method of screening for early breast cancer based on a table-top high-resolution vibrational infrared microspectrometer.

The primary conclusions of our report may be summarized here as follows: (i) IR microspectroscopy is indeed a promising alternative table-top tool capable of identifying the presence of lipids in human hair; (ii) however, we find lipid deposits are present in control samples. The original x-ray work must be in error, and studies on single strands of human hair will not provide a viable screening technique for breast cancer. This is a disappointing negative result.

## BODY OF REPORT

A pilot study of 10 samples was used to test the following hypotheses : (i) Does high-resolution infrared spectroscopy have sufficient sensitivity and specificity to detect the presence of lipid domains in single strands of hair? (ii) Do the observed infrared signals correlate with x-ray diffraction data? (iii) Can IR spectroscopy of a single strand of hair be used for screening for breast cancer?

We have successfully shown that infrared spectroscopy does provide a cheap, table-top alternative method to synchrotron sources for the detection of lipid deposits. However, we find that the lipid signatures occur in hair from healthy control subjects as well. We have performed independent x-ray studies in collaboration with researchers from Cornell University. Their x-ray studies confirm that the diffuse ring, due to lipid deposits, reported by the original Australian report, is also present in healthy subjects, in agreement with our infrared studies. Taken together, as stated above, this report suggests that (i) IR microspectroscopy is a promising alternative table-top tool; (ii) However, the original x-ray work must be in error, and studies on single strands of human hair will not provide a viable screening technique for breast cancer. This is a disappointing negative result.

As described in our previous reports, we had proposed an extension in the Task list to perform x-ray diffraction studies on the same samples that were subjected to infrared spectroscopy. This was done, at no cost to the project, because there was some disagreement in the x-ray literature. The extension to the task list was approved. First we describe the disagreements reported by the x-ray community:

Briefly

1) James and her collaborators first reported the correlation between a certain diffuse ring in the small angle x-ray scattering from a single strand of hair using synchrotron radiation, and the incidence of breast cancer [James et al, 1999].

2) Several separate groups published criticisms (Meyer et al, 2000; Howell et al, 2000; Briki et al, 1999), showing data that appear to contradict the original James et al result.

3) Meyer and James (2001) reported a rebuttal, affirming the essential findings of the original James et al paper, and arguing the need for following a consistent protocol in acquiring x-ray data. What made this work noteworthy is that one of the authors (Meyer) was the lead author on one of the papers that contradicted the original result. In co-authoring the rebuttal, Meyer had changed his mind and appeared to support the original James et al finding.

4) A more recent paper (Laaziri et al, 2002) reported finding no correlation between x-ray studies and the incidence of breast cancer. This was criticized by James (2003) with another rebuttal has been published (Laaziri et al, 2003).

To summarize:

There is general agreement among all x-ray diffraction studies that the diffraction ring identified by James et al (1999) does occur in hair samples, and is related to lipid deposits.

There is a disagreement about whether these rings, due to lipid deposits, are linked to breast cancer. The relationship with lipids suggests that the original premise of our proposal – namely, that infrared microspectroscopy can offer an independent tool for studying the same structural changes – is now on very firm ground.

## Key Research Accomplishments

We outline here the research accomplishments associated with each of the approved tasks.

- Successful demonstration that infrared spectroscopy is capable of detecting the presence of lipids in hair, and therefore provides a table-top alternative to synchrotron x-ray source.
- Established that IR and x-ray diffraction studies of single strands of hair are consistent – both techniques suggest that the lipid peaks are not simply “yes-no” binary signatures, but rather form a continuum.
- Control subjects also show signatures of lipids in hair, in both IR and x-ray work, casting doubt on the original proposal of James et al (1999).

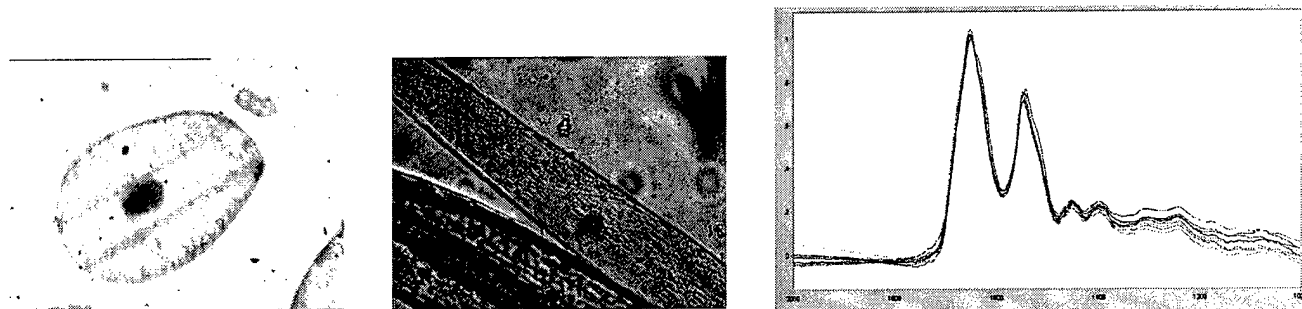
## Reportable Outcomes

### Task 1.

To obtain a high resolution infrared spectrum from a single strand of human hair.

This was accomplished successfully and spectral features characteristic of lipid deposits were identified. The same lipid deposits have been proposed as the putative source for the reported diffuse ring in synchrotron studies from the Australian group. High-resolution infrared studies were performed on cryomicrotomed sections of hair, placed on Zinc Selenide windows. Spectra were collected from several thousand points within a hair sample, with attention being paid to the protein amide regions, as well the CH stretch regions near 3 microns, where lipids are known to contribute significantly. This is very encouraging, because it suggests that infrared microspectroscopy is a table-top alternative to expensive synchrotron sources.

*Vibrational Infrared Microscopy of a single strand of human hair:* Sections of human hair were taken using a cryomicrotome, and transferred to an infrared transmitting calcium fluoride window. Fig 1 shows optical images combined with infrared spectroscopy on the same strand of hair, in both longitudinal and transverse sections. Both sections were analyzed because the x-ray diffraction studies suggested that lipid deposits that may be responsible for observed x-ray patterns may be randomly oriented.



**Figure 1:** (a) Cross section of single strand of human hair; (b) Longitudinal section of hair; (c) Infrared spectra obtained from the hair sample, showing the primary amide I and amide II bands (C. M. Smith).

One question that concerns infrared studies was whether routine hair treatment, such as shampooing has a significant effect on the infrared spectrum. Control subjects were requested to collect hair samples before and after shampooing, and then processed Hair samples were collected from control subjects. After collecting coarse resolution infrared spectra, an infrared map was generated, where spectra are collected from different locations.

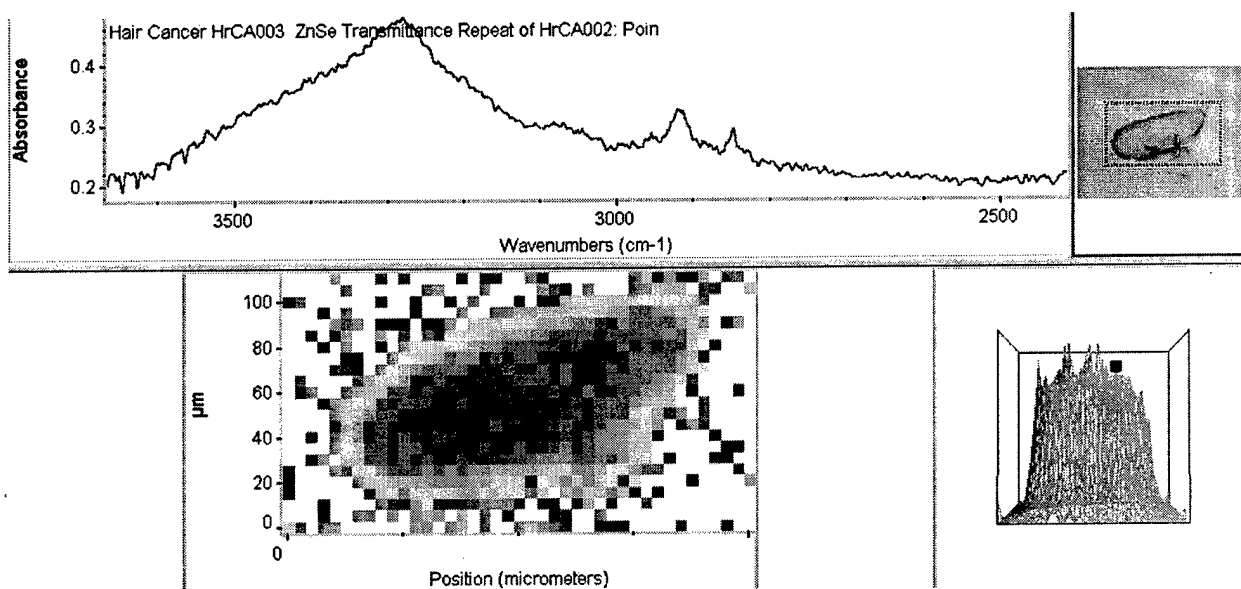


Figure 2. Infrared spectrum from one specific point (shown in the inset figure, top right), in the  $3000\text{ cm}^{-1}$  vibrational region. The small peaks are mostly due to lipid stretching vibrations. These features show significant variation from site to site in the hair sample.

### Task 2.

To obtain high resolution infrared spectra from single strands of human hair from a set of subjects in order to identify the presence of lipid deposits that may be correlated with increased risk of breast cancer.

This was accomplished successfully on a small sample (10) of patients from Dartmouth Hitchcock Lahey Medical Center. The review committee that commented on our first report also suggested that we take sections at several positions along the length of the hair – close to the root and further away. This was done, and there were differences noted in infrared peaks not associated with lipid bands. IR spectra of human hair are known to be sensitive to the presence of drugs, and are used in forensic analysis. Variations in additional (non-lipid) bands may be due to such additional factors, but we did not attempt a systematic study of such variations – the sample sizes were small, and the presence of lipid bands even in control samples suggested that the original hypothesis was in error. We feel that, while additional IR studies of the effect of drugs on hair may be of value, the correlation with breast cancer sought could not be established. In nearly every sample, we found infrared signatures of lipid deposits. Some of the peaks were due to aliphatic stretching frequencies, and were reported earlier. We found significant variation in the strength of the IR signatures, with no apparent correlation to the treatment protocol, or stage of disease (see Task 3 below).

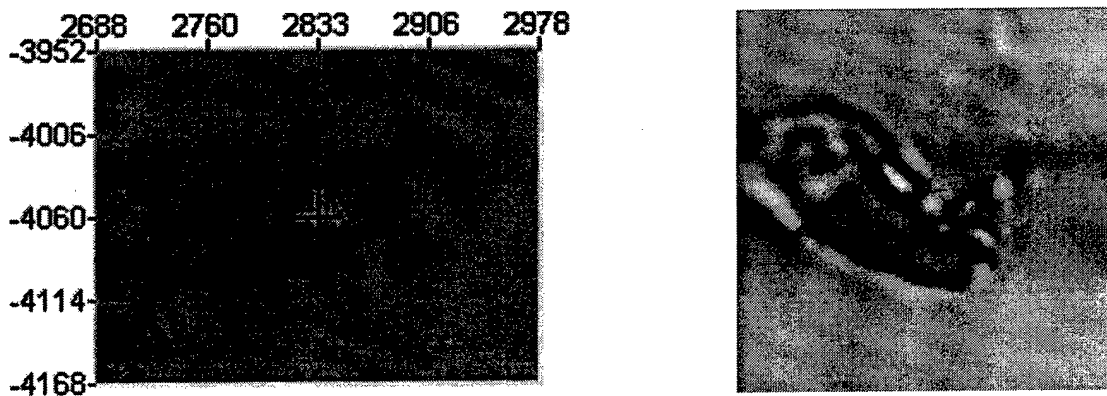


Figure 1: Comparison of optical image (left) and scanning near-field infrared (SNIM) image at  $\lambda = 10.6 \mu\text{m}$  of hair cross-section; near-field IR calibration standard (right) showing sub-wavelength spatial resolution ( $2 \mu\text{m}$ ).

Task 3. To perform concurrent analysis of the infrared spectra of hair obtained from cases and controls, in order to understand the biochemical basis for structural changes, and to test the potential of infrared microscopy as a screening tool.

Concurrent analysis was also performed on control hair samples, from healthy subjects. Again, the review committee had suggested for taking several sections along the length of the hair, close to the root and further away. We found that the same lipid signatures could be seen in the control samples as well. This was disconcerting, and was not consistent with the Australian study. So we undertook to perform x-ray diffraction studies, in collaboration with Prof Sol M. Gruner (Director, Cornell synchrotron x-ray source), who agreed to work at no cost to this project. The results of the x-ray work and infrared work can be summarized as follows:

- (i) The infrared work and x-ray data obtained at Cornell are both consistent – the control samples showed the diffuse lipid ring as well.
- (ii) There was considerable variation in the intensity of the x-ray ring and in the heights of the IR lipid peaks, but the strongest signals were found in the control samples. This work suggests that the strength of the lipid peaks is not a simple “yes-no” proposition, but rather a continuous variable that can span several decades in intensity. In one patient, the x-ray peak only was discernible after overnight exposure to x-rays from a table top source. To summarize: nearly every hair sample can reveal the presence of the diffuse lipid rings. While there is considerable variation in the strengths of the peaks found, some of the strongest peaks were found in healthy patients.

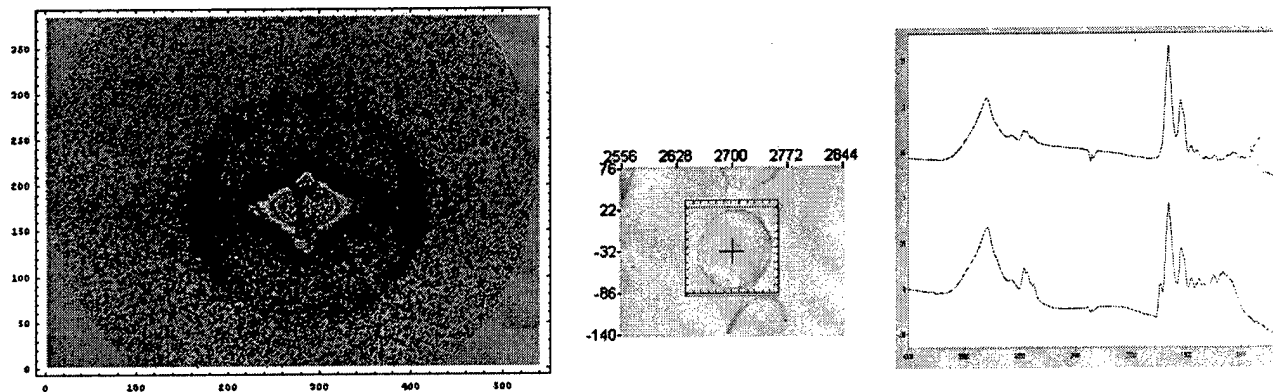


Figure 2 : Control samples show both the x-ray diffuse diffraction ring (left) and the associated infrared lipid peaks (right). Nearly every sample can show x-ray diffuse rings and lipid spectra, depending on source intensity/exposure. Lipid signatures show a continuum of intensity ranges rather than a simple binary "yes-no" correlation.

*Task 4.* To design a portable device that can be used for rapid screening (months 20-24), dependent on the outcome of Task 3.

Because the outcome of Task 3 was negative, we did not build a portable device. Instead the table-top device was used to conduct additional studies. A report was presented at the Era of Hope conference. We note that the presence of this peak in the controls, even if the number is small, already rules out the strong correlation proposed by the Australian group. We cannot, of course rule out a smaller correlation (given the small number of subjects studied). However, given the very high percentages required for any technique to be a useful screening tool, the studies already performed suggest that studies on a single strand of human will not be useful for breast cancer. The original Australian report claimed essentially 100% specificity. Both our x-ray work and infrared work even on the small number of samples suggest that this is clearly in error.

*Future research:* In view of the negative findings, we propose not to continue this line of research further. It is disappointing to researchers and (perhaps more importantly) to patient advocates of breast cancer research.

*Personnel:* Hair samples from patients were obtained by Dr. Peter Kaufman, M.D., Dartmouth Medical College. Another collaborator, Dr. Mary-Ann Mycek, of Dartmouth College, won appointment as Associate Professor at the University of Michigan. She is continuing research associated with the US Army Breast Cancer research program, on a different project of her own initiative. While this collaboration had a negative result, we are pleased that personnel associated with this project will be making valuable future contributions to this Congressionally mandated initiative. Initial studies reported were performed by C.M. Smith, under the supervision of the Principal Investigator and Prof. M. K. Hong at Boston University. We are pleased to say that Mr. Smith has now enrolled in Medical School (Albany Medical College), and we trust that the work on this project was responsible in part in

motivating him. Some of the IR work was performed by another medical student, Ji-Yeon Kim working in the laboratory at the Center for Photonics. We are pleased to report that Ji-Yeon Kim was admitted to Harvard Medical School and is undergoing training. X-ray work at Cornell is being performed by Dr. Peter Abbamonte, working with Prof. Sol Gruner (at no cost to this project).

### Conclusions

We have established that high-resolution infrared microscopy is capable of acquiring infrared spectra from both lipid and protein molecules from a single strand of human hair. We have extended the studies to perform x-ray diffraction studies, in collaboration with scientists at Cornell University.

The studies have shown that (i) infrared spectroscopy does indeed provide a table-top alternative to synchrotron studies for the detection of lipid deposits in hair. (ii) there are significant lipid signatures in both infrared and x-ray from *control* samples from healthy patients.

The observation of the lipid patterns in control samples is already sufficient to cast doubt on the original Australian proposal, that studies on a single strand of hair are not useful as a screening tool for breast cancer. This is disappointing.

Our technique works, and may provide a powerful new method for assessing biomarkers in other tissues, but the studies on hair fail – not because of a flaw in our technique, but because the original hypothesis appears to be wrong.

## REFERENCES

Briki, F. Busson, B., Salicru, B., Esteve, F., Doucet, J. *Nature* 1999; **400**:226.

Howell, A., Grossman, J.G., Cheung, K. C., Kanbi, L., Evans, D. G., Hasnain, S.S. *J. Med. Genet.* 2000; **37**:297-298.

Laaziri et al, *Phys. Med. Biol.* **47**: 1623-1632 (2002).

Laaziri et al, *Phys. Med. Biol.* **48**: L11-L13 (2003).

James, V. *Phys. Med. Biol.* **48**: L5-L9 (2003).

James , V., Kearsley, J., Irving, T., Amemiya, U., Cookson, D. *Nature* 1999; **398**:33-34.

Meyer, P., Goergl, R., Botz, J.W., Fratzl, P. *J. Natl Cancer Inst.* 2000; **92**: 1092-1093.

Meyer, P., and James, V., *J. Natl Cancer Inst.* 2001; **93**:873-875.