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Using Power Doppler and Acoustic Resonance Imaging

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13. ABSTRACT (Maximum 200 Words) Intraductal breast carcinoma (DCIS) represents approximately one third of mammographically detected breast carcinoma. Currently, DCIS and benign breast microcalcifications can only be reliably be evaluated utilizing x-ray mammography. Our goal with our current project was to utilize breast sonography coupled with the technique of acoustic resonance to image and evaluate the breast microcalcifications in patients prior to biopsy. We have been successful in visualizing the calcifications utilizing sonography coupled with acoustic resonance. Our analysis, however, shows no statistically significant difference in resonance peaks between malignant and benign types of calcifications.				
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Introduction:

Technical advances in the field of mammography are resulting in higher detection rate of early breast cancers. Some of these technical advances include digital mammography, high frequency ultrasound transducers and breast magnetic resonance imaging (MRI). However, mammography is still the only reliable method of evaluating microcalcifications in the breast. Breast MRI, although has a sensitivity rate nearing 100% for invasive carcinoma, the reported sensitivity for DCIS has been reported to be as low as 40% (1-3). It is estimated that DCIS represents 20-30% of breast carcinomas detected on screening mammography (4) and has been steadily been rising in incidence since the 1970's (5). Since the 1970's the detection rate of ductal carcinoma in situ has steadily increased with the wider use of screening mammography. The detection rate for women less than 50 years of age was reported to be 2.3 cases per 100,000 women in the 1970's increasing to 6.2 cases per 100,000 women by the 1990's (6). More dramatically, in the group of women over the age of 50, the rate has increased from 14.3 to 54.6 per 100,000 (6) in the same time period. We have developed a technique utilizing acoustic resonance to visualize microcalcifications under ultrasound. The concept of acoustic resonance imaging (ARI) is based on the size of the microcalcifications and the binding strength with the surrounding tissues in which it is imbedded. When subjected to a wide frequency range, different sized particles will resonate at different frequencies given the same binding environment. By "tuning" into the appropriate frequency range, it would be possible to selectively visualize microcalcifications of varying sizes. Our goal in this project was to image breast microcalcifications utilizing sonography, which is readily available in breast imaging centers, coupled with acoustic resonance.

Progress Report Body:

The objective of the study was to utilize ultrasound to enhance the detection and evaluation of microcalcifications seen on mammography with power Doppler and acoustic resonance

Methods and Results:

A multi-disciplinary team of researchers at the Department of Radiology, University of Pennsylvania, carried out the proposed work.

Dr. Susan Weinstein is a radiologist specializing in mammography/women's imaging in the Dept. of Radiology. Dr. Weinstein was responsible for the imaging aspect. Dr. Sehgal Ph.D. has expertise in ultrasound imaging. Drs. Sehgal and Weinstein will coordinate the overall study, organize the experimental protocols and carry out computer image analyses. Dr. Weinstein and a research assistant performed color and power Doppler imaging using state of the art imaging equipment and the vibrator prototype. Histology was evaluated by, Dr. Carolyn Mies from the Department of Pathology. The research assistant, Sarah Kangas, was responsible for data management and programming needed for analysis.

At the writing of this report, we are currently at the end of our project.

Task 1: See report from 7/1/00-6/30-02.

Task 2: Patient recruitment period completed

Task 3: Patient recruitment period completed

Task 4: Patient recruitment period completed

Task 5: Data analysis completed

Patient Selection:

The patients presented to the Breast Imaging Section of the Hospital of the University of Pennsylvania on the day of their biopsy procedure. The patients recruited for the study had breast microcalcifications seen on mammography that are considered suspicious. On the day of the procedure, the patients were to undergo excisional biopsy in the operating suite by a surgeon or a percutaneous core needle biopsy in the Breast Imaging Section in order to obtain a tissue diagnosis. Prior to either of these conventional biopsy procedures the patients are recruited for the study.

We had anticipated to enroll about 80 women for the study. There was no specific age range target.

Upon arrival in the department, prior to the breast biopsy, either the radiologist or the research assistant, asked the women if they were interested in participating in a research project. The women were informed that a breast ultrasound would be performed using a small disc that emits vibrations in the sound wave range and that the entire procedure would take approximately 15 minutes. The women were informed that there are no known harmful side effects associated with the procedure but that they would not benefit directly from participating in the study. They were also informed that the results of the sonography would be correlated with the pathology results, and that their identity will not be revealed in any way in future publications. The patients were informed that they may decline to participate. All the elements of the consent form were

reviewed with the patient and a copy of the form was also provided to the patient. If the patient agreed to participate, the consent form was signed and the patient proceeded to have the breast sonography performed by the research assistant and/or radiologist. If the patient declined, she proceeded directly with her planned clinically performed breast biopsy.

Ultrasound Imaging:

Ultrasound and Doppler imaging will be performed using a state of the art, ATL3000 (ATL, Bothell, WA) scanner. This scanner is currently used a research scanner in our department in the laboratory of Dr. Seghal. Microcalcifications, as identified on x-ray mammography, will be imaged by acoustic resonance before tissue diagnosis. Breast sonography will include the conventional breast ultrasound coupled with a disc that has been designed to produce low frequency vibrations. A thin (5-mm) and lightweight (12.9 g) disc vibrator will be held on the breast by surgical tape near the site of calcification identified by mammography. Commercially used ultrasound gel will be used as a coupling medium. The disc vibrator emits vibrations in the sound wave spectrum ranging from 50 to 500 Hz. No side effects are known to be associated with such vibrations. The region of interest was imaged using power Doppler, color Doppler and B scan modes.

The patient then proceeded to have a clinically performed breast biopsy. Tissues from biopsy were processed under routine clinical protocol. The final histology results were obtained and the results correlated with the sonographic findings.

Image analysis:

The videotaped images were subsequently analyzed. Each scan takes approximately 2 minutes. At the video frame rate of 30 images per second, this generates

approximately $120 \times 30 = 3600$ images. An algorithm was developed to reduce the data by sorting the images on the basis of the frequency of the vibration. These images were subsequently used in a computer program developed in our laboratory to analyze the vibrational response of the microcalcifications. Analysis of color was performed for each image. For each image, computation was performed for mean color level (MCL), percent fractional area of color (FA), color weighted fractional area (WFA). To determine the MCL, the color palate on the image was read by the computer and divided equally on a scale of 0-100. With this scaling system, the computer constructed a look-up table for hue, saturation, and brightness values for the colors in the palette bar. Next the computer identified colored pixels in the image and using the look-up table assigned a color value to each pixel within the region of interest. The color level of the pixels in the region of interest was summed and divided by the number of color pixels to calculate the MCL. The percent fractional area of color (FA) was defined as the area covered by colored pixels divided by the area of region of interest, multiplied by 100%. Color weighted fractional area (WFA) was defined as the $(MCL \times FA)/100$, indicating the presence of net motion within the region of interest. Each parameter was plotted with respect to the frequency range.

Histology Evaluation:

Two representative sections from biopsy tissue samples will be examined for calcifications. The sections will then be fixed in 10% formalin, embedded in paraffin, and sectioned at $5 \mu m$ thickness in accordance with standard methods and stained with hematoxylin-eosin (H&E). Calcium phosphate is the predominant form of calcium seen in breast tissue and is

easily recognizable on standard H&E section. On the other hand, calcium oxalate, which can also be present in breast tissue, is particularly difficult to detect on the routine H&E stained sections. However, due to their bipyramidal shape, these crystals are birefringent and will be detected using polarized light.

Results:

Since the writing of our last report, we have completed patient accrual for the study as well as data analysis. Since the writing of the last report, we have recruited 53 additional patients. Eighty five patients with a total of 90 different groups of calcifications were evaluated. Our goal, in this project, was to recruit 80 patients total. Due to technical factors, data could not be obtained from 10 patients (12 clusters of calcifications). Additionally, in one patient, the clinical percutaneously biopsy was unsuccessful, therefore, the patient went for an excisional biopsy at another hospital. We do not have the patient's pathology results and she also was not included in the final data analysis. We have made significant progress in patient recruitment since the writing of our last annual report. Final analysis was performed on 75 patients with 78 groups of calcifications.

The age of the patients ranged from 38 to 83 years. The breakdown of the age of the patient population is shown in Figure 1 by the decade. The demographics of the patient population is shown in Figure 2. Out of the 78 different groups of calcifications analyzed, there were 22 cases of malignant calcifications and 56 cases of benign calcifications (Figure 3). Figure 4 shows the age of the patients relative to the pathology results.

First gray scale imaging was performed at the site of the microcalcifications. Next, the same area was scanned in Power Doppler mode in conjunction with acoustic resonance. The acoustic resonance device emitted low frequency vibrations in 10 Hz steps between 50-600 Hz. In the first nine patients, 100 Hz steps were utilized, and it was decided that the steps too large and a decision was made to utilize smaller incremental steps. The scanning was repeated using 3 different vibration amplitudes to determine variation and the frequency of response of the calcifications to the variation in the amplitude. All the images were stored on a videotape and used for quantitative analysis.

The mammogram from each patient was digitized at 300 DPI and stored in the database for future review. The ultrasound examination was performed using state of the art ATL 5000 ultrasound scanner. All imaging was performed with 12.5 MHz broadband transducer. The instrument resets determined from the studies conducted in the first year were used for all patients.

The acoustic resonance scans involving low frequency vibrations were tolerated by all the patients. We have had no adverse reactions reported at the writing of this final report. Average total scan time was approximately 15 minutes. Imaging was completed successfully in 75 patients with 78 clusters of calcifications. In 10 cases (12 clusters of calcifications), due to technical difficulties, data could not be successfully obtained.

We have completed quantitative analysis of images from all of the patients enrolled in the study to date. This involved digitizing the images from the videotape. The images of each patients were compiled in a single file and used for quantitative measurement of mean mean color level (MCL), percent area of color (FA) and color weighted fractional area (CWFA). The analysis was performed by selecting the entire

image as proposed in the application. However, the images show that structures other than calcifications, such as connective tissue are also enhanced by the acoustic resonance imaging.

Of the 75 patients (78 different groups of calcifications) studied, 56 cases (71.8 %) were benign and the remaining 22 cases (28.2 %) were malignant. Figure 5 shows an example of images obtained from a patient with malignant calcifications. The mammogram in 5a shows a cluster of calcifications. The biopsy proven malignant calcifications are denoted by the arrows. Figures 5b-d show 3 representative ultrasound images at 150 Hz, 330 Hz and 440 Hz. As the frequency of vibration increases, at 330 Hz, color pixels representing the calcifications are seen. In addition to the calcifications, enhancement of secondary structures associated with connective tissue are also observed (arrows) close to the skin in Figure 5d. The inset panel (Figure 5e) showing a graph represents change in image enhancement (CWFA) as a function of vibration frequency (Hz). The error bars on the data point represent +/- image deviation of the three measurements at three different scanning parameters. On all three different scans, the graph demonstrates a clear resonance peak at 420 Hz.

Figure 6 shows the results from a patient with biopsy proven benign calcifications. Figure 6a shows the mammographic image of the calcifications. There is a focal cluster of calcifications that is denoted by arrows. There is minimal enhancement seen at 120 Hz. However, there is increased enhancement seen at 320 Hz which continues to increase at 440 Hz. In this example, there is greater enhancement than would be expected from the mammographic area of interest. It is felt there is also enhancement of the secondary structure of the breast such as ligaments.

The peak position (P) and the width of the peak at half height (W) were measured for each patient. These values are summarized in Figures 7 and 8. In 19/22 (86.3 %) cases with malignant calcifications, enhancement was seen in the power Doppler mode and a peak in CWFA vs. frequency curve was observed. The mean $\pm$ standard deviation for the peak position (P) was 400 $\pm$ 79 Hz. The peak width (W) was 152 $\pm$ 58 Hz. In patients with benign calcifications, the image enhancement was observed in 47 (83.9 %) of 56 cases studied. Nine cases did not show any measurable enhancement. The mean $\pm$ standard deviation for the peak position and peak width were 400 $\pm$ 89 Hz and 144 $\pm$ 58 Hz respectively. There appears to be no statistically significant difference in the peak width and peak position between the benign and malignant calcifications.

Key Research Accomplishments:

- We have been able to visualize calcifications with power Doppler and acoustic resonance in patient studies in 66/78 different groups of calcifications. No significant enhancement could be seen in 12 cases (9 benign and 3 malignant cases).
- All the visualized calcifications demonstrated a resonance peak.
- There was no significant difference in the resonance peaks between the malignant and benign calcifications. We hope to further analyze the data to demonstrate that there indeed may be a statistically significant difference in the resonance peaks.

Reportable Outcomes:

Weinstein SP, Conant EF, Patton J, Seghal CM. Targeting and core biopsy of breast microcalcifications under ultrasound using acoustic resonance. Radiological Society of North America 1999. (see appendices)

Weinstein SP, Seghal C, Conant EF, Patton JA. Microcalcifications in Breast tissue Phantoms Visualized with Acoustic Resonance Coupled with Power Doppler US: Initial Observations. Radiology 2002;224:265-269. (see appendices)

Weinstein S, Seghal C. Detection of microcalcifications utilizing sonography coupled with power Doppler and acoustic resonance. Era of Hope, DoD Breast Cancer Research Program Meeting 1999.

Conclusions:

Our goal at the start of this project was to determine if we could successfully visualize calcifications using breast sonography coupled with acoustic resonance. We have been successful in accomplishing this goal in 66/78 groups of calcifications. We also have been successful in our recruiting process. In total, we were able to recruit 85 patients. Due to technical difficulties, we were able to complete the scans in 75 patients with 78 groups of calcifications.

Analysis of the data was then performed to see if there was a difference in resonance between malignant and benign calcifications. Our analysis reveals that there is no significant difference could be observed between the malignant and the benign calcifications. We again speculate, that the lack of differentiation between the two types of calcifications may be attributed to enhancement from the secondary structures such as ducts and connective tissues of the breast that would be seen with both benign and malignant calcifications. We believe that additional analysis of the available data may be helpful. We believe that with additional analysis, we are able to demonstrate that benign and malignant calcifications may indeed have different resonance peaks if we can select out the secondary enhancement. We have asked for a one year extension to further analyze the data.

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Appendices:

See attached

List of Personnel Supported by the Grant:

Susan Weinstein, MD – Principal investigator

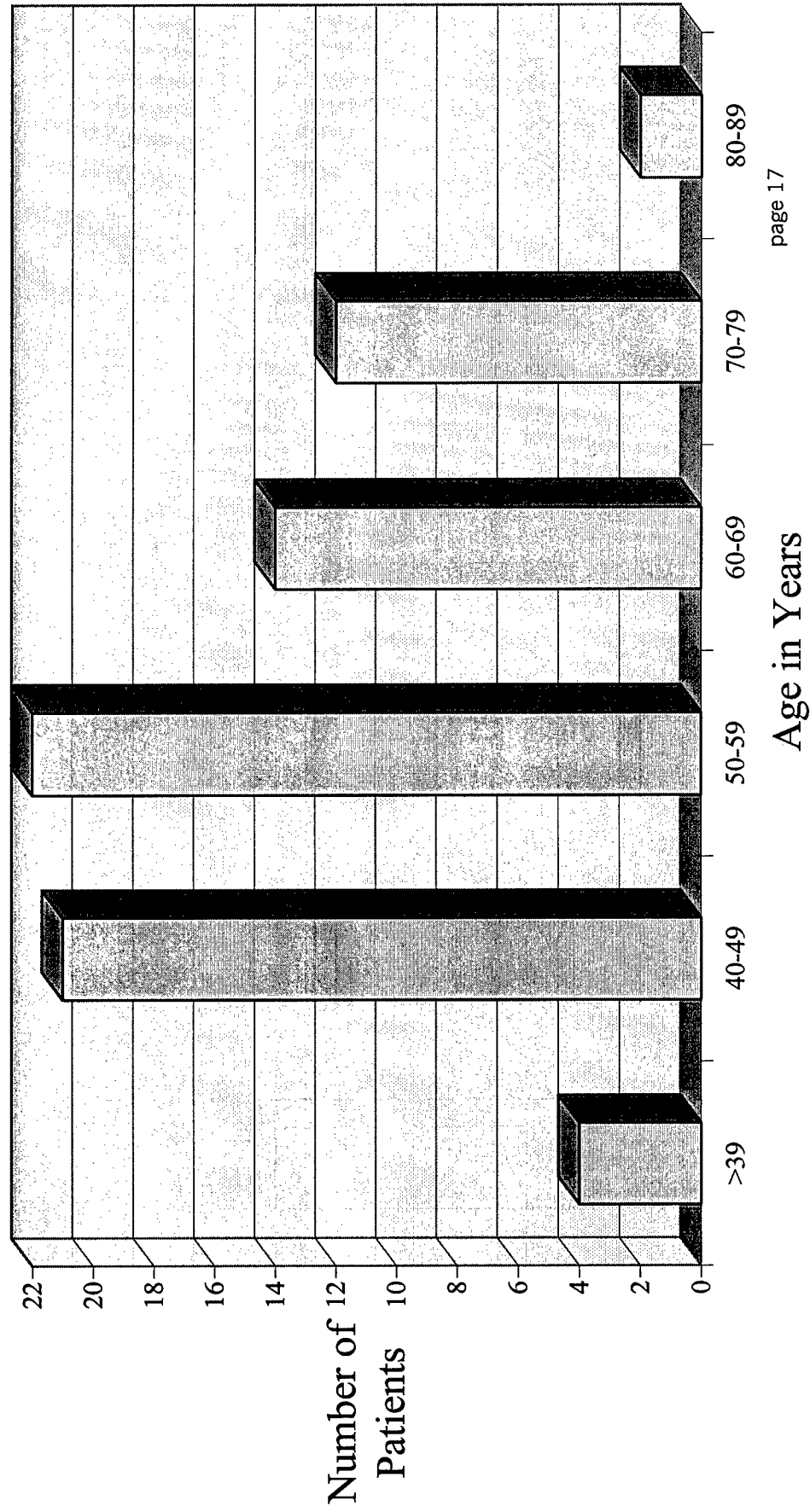
Chandra Seghal, Ph.D. – Co Principal investigator

Carolyn Mies – pathologist

Sarah Kangas – research assistant (Ms. Kangas replaced Jill Patton)

Age of the Patients

Fig 1



Demographics

Fig 2

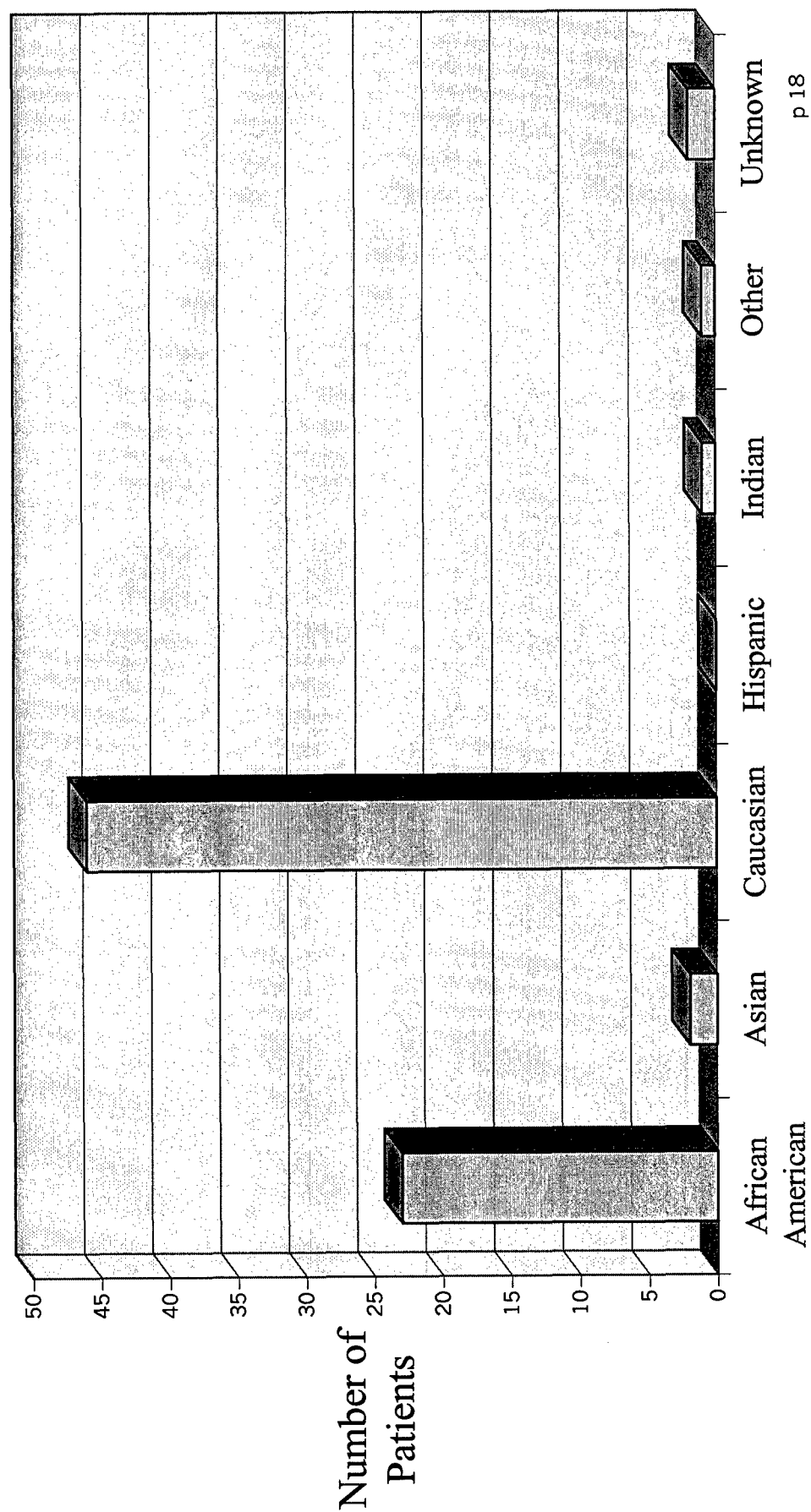


Fig 3

Benign vs. Malignant Calcifications

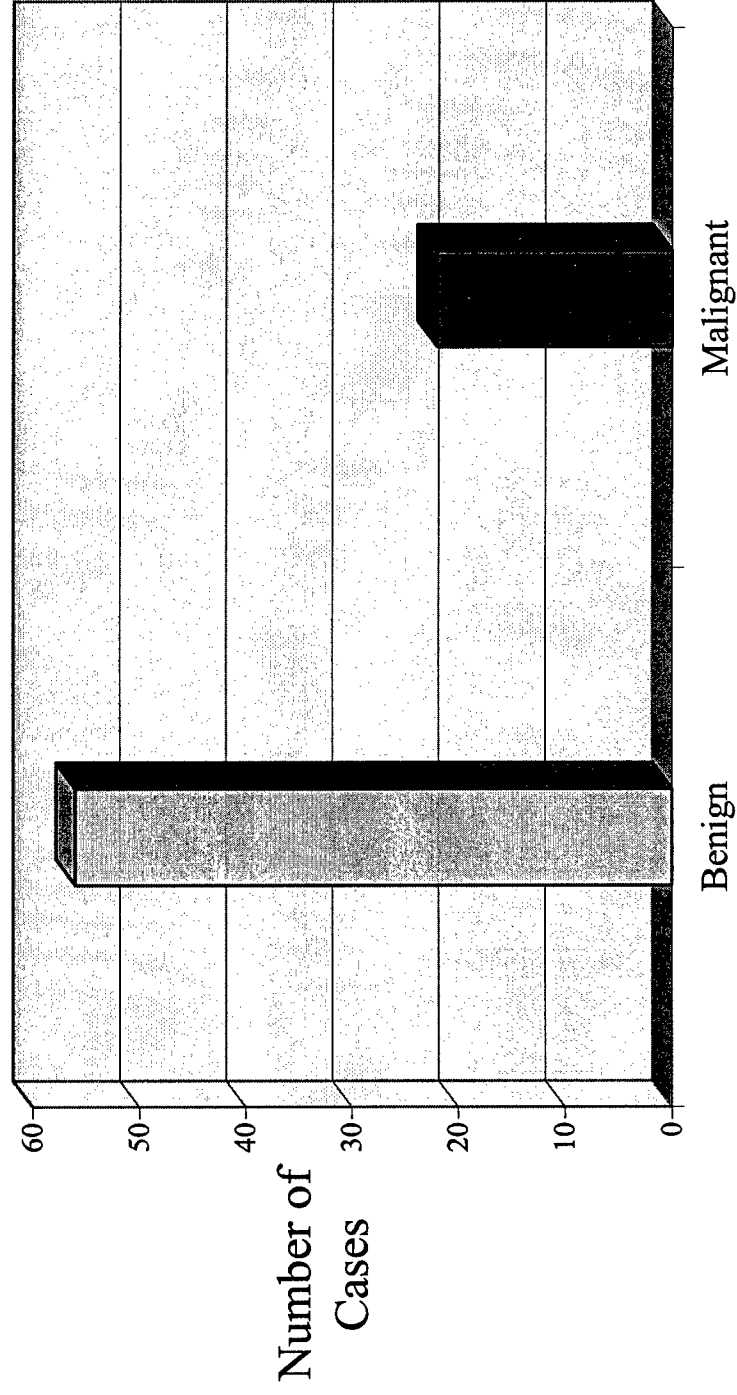


Fig 4

Age of the Patients vs. Pathology

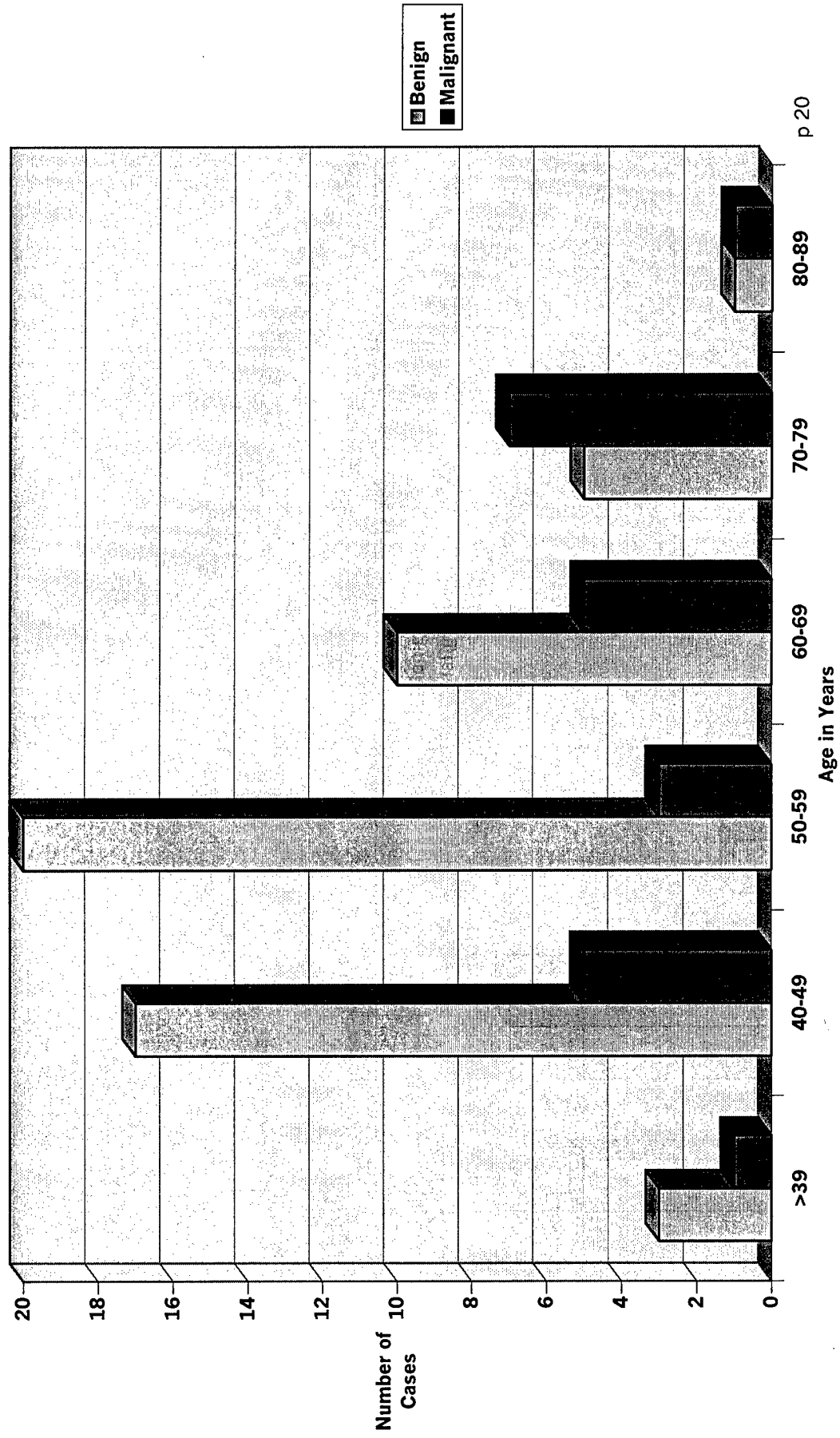
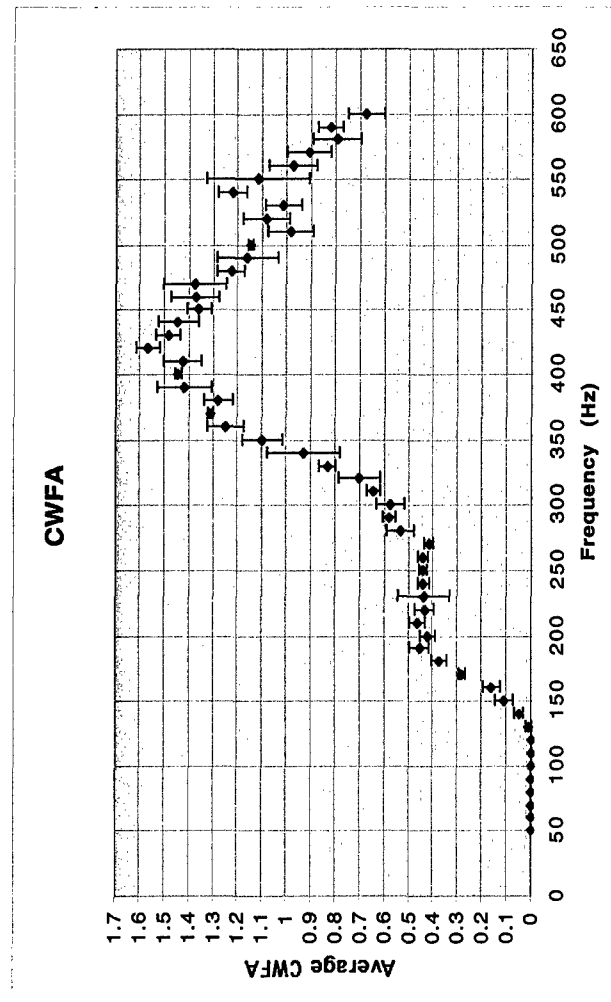
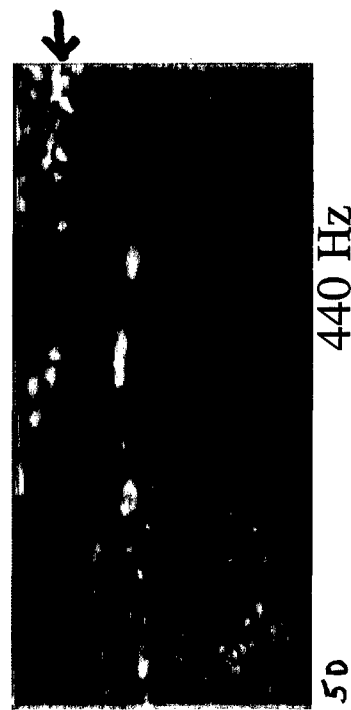
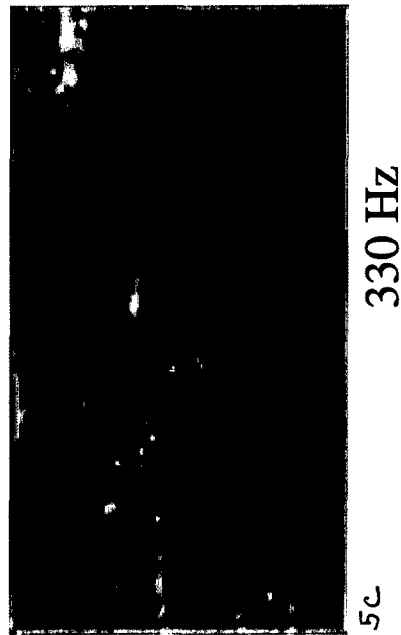
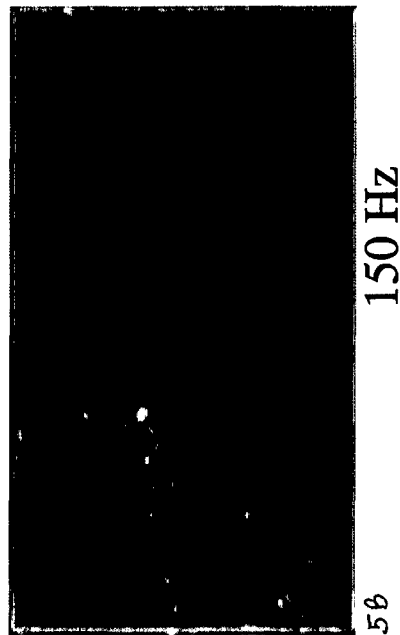
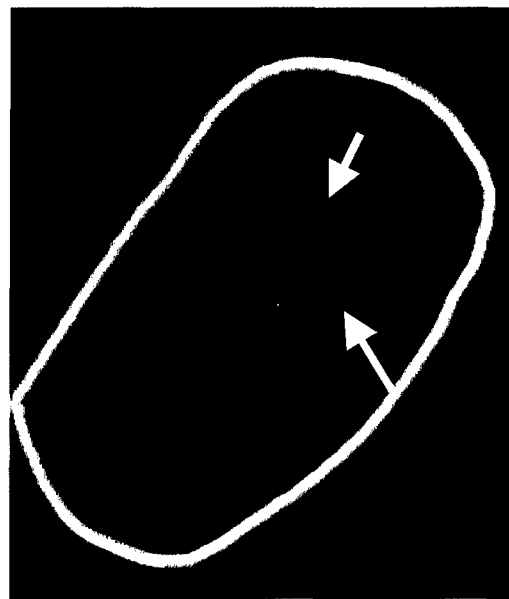


Figure 5

MALIGNANT CALCIFICATIONS



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Figure 6 BENIGN CALCIFICATIONS

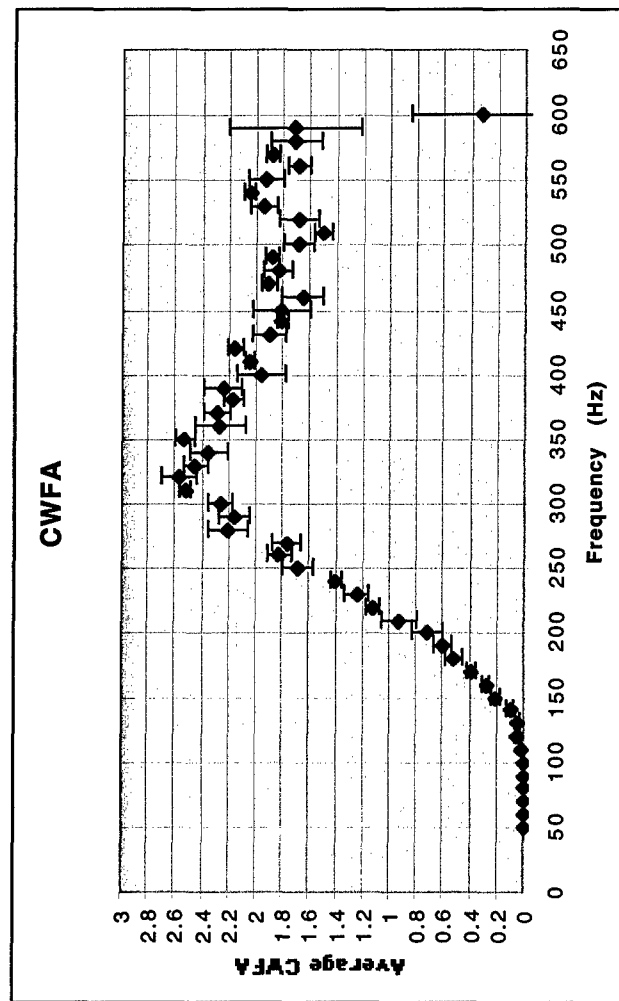
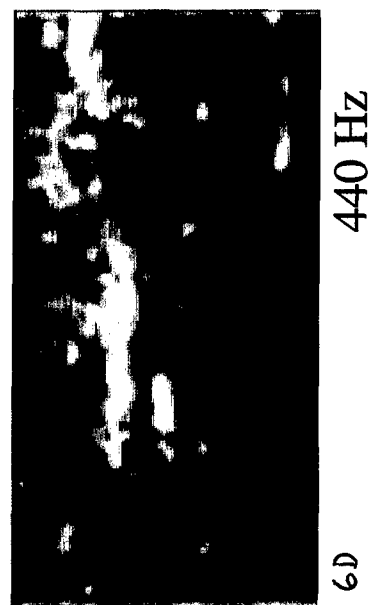
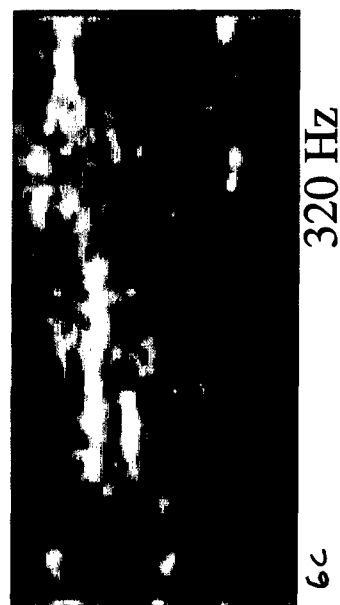
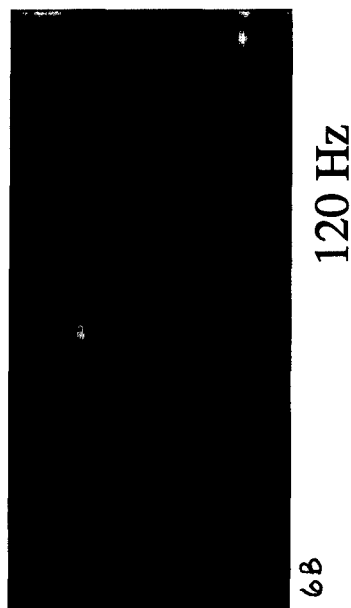
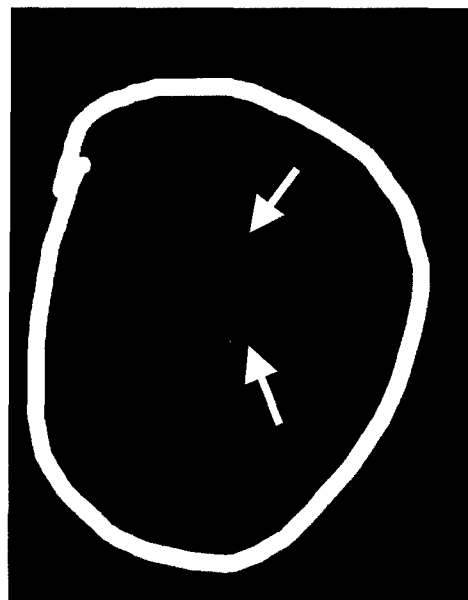


Fig 7

Case #	BENIGN		Case #	BENIGN	
	Peak Position P (Hz)	Peak Width W (Hz)		Peak Position P (Hz)	Peak Width W (Hz)
1	477	120	30	375	160
2	184	69	31	475	218
3	540	190	32	460	163
4	250	27	33	405	145
5	526	150	34	464	116
6	428	217	35	467	178
7	397	167	36	460	142
8	397	148	37	380	80
9	465	158	38	388	150
10	380	196	39	363	153
11	380	196	40	437	202
12	560	75	41	505	125
13	440	142	42	375	85
14	415	88	43	457	128
15	400	228	44	390	82
16	400	245	45	470	70
17	154	63	46	515	105
18	293	130	47	318	74
19	300	120	48	No Enhancement	
20	415	135	49	No Enhancement	
21	363	265	50	No Enhancement	
22	318	116	51	No Enhancement	
23	430	112	52	No Enhancement	
24	190	102	53	No Enhancement	
25	420	250	54	No Enhancement	
26	387	153	55	No Enhancement	
27	383	119	56	No Enhancement	
28	485	270			
29	342	122			
			Mean	400	144
			St. Dev.	89	58

Fig 8

Case #	MALIGNANT	
	Peak Position P (Hz)	Peak Width W (Hz)
1	493	178
2	460	158
3	500	100
4	400	100
5	353	102
6	285	99
7	423	275
8	490	185
9	241	78
10	277	120
11	450	208
12	450	120
13	425	203
14	375	126
15	385	98
16	370	243
17	318	103
18	400	200
19	500	200
20	No Enhancement	
21	No Enhancement	
22	No Enhancement	
Mean	400	152
St. Dev.	79	58

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Targeting and Core Biopsy of Breast Microcalcifications under Ultrasound Using Acoustic Resonance

S.P. Weinstein, MD, Philadelphia, PA • E.F. Conant, MD • J. Patton, BS, MS • C.M. Sehgal, PhD

PURPOSE: We have developed a novel method by which calcium particles can be brought to acoustic resonance and visualized with the aid of power Doppler ultrasound. The goal of this study is to demonstrate that breast microcalcifications detected with this method can be core biopsied under its guidance in gelatin phantom and tissue models.

METHOD AND MATERIALS: Our previous studies have demonstrated that breast microcalcifications may be detected by acoustic resonance imaging. Gelatin phantoms were made with calcium carbonate particles ($\approx 400-800\text{m}$). The particles were evaluated with power Doppler imaging while resonating the particles at 50-500 Hz. At maximum color visibility the particles were targeted and biopsied using a 14g core needle. The phantom and core samples were x-rayed to demonstrate that the area of color detection represented the calcium particles. The images were videotaped and analyzed quantitatively for mean color level (MCL), % area of color (FA) and color weighted fractional area (WFA). The experiments were repeated using turkey phantom models with embedded calcium particles.

RESULTS: The plot of MCL, FA, WFA vs. frequency peaked and fell in a bell shaped curve reaching maximum at $\approx 200-300$ Hz. At this frequency, optimal visualization of the particles was observed and enabled localization and biopsy of the microcalcifications under ultrasound guidance.

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CONCLUSIONS: Data using phantoms demonstrated that microcalcifications may be targeted under ultrasound with power Doppler and acoustic resonance allowing for dynamic ultrasound guided core biopsy.

**DETECTION OF MICROCALCIFICATIONS
UTILIZING SONOGRAPHY COUPLED WITH
POWER DOPPLER AND ACOUSTIC RESONANCE**

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Ductal carcinoma in situ (DCIS) represents the earliest form of breast cancer. Even though other imaging modalities may be utilized in the evaluation of breast abnormalities, currently, mammography is the only reliable method in detecting microcalcifications. Our goal with this project was to evaluate the efficacy of ultrasonography coupled with acoustic resonance imaging (ARI) in the evaluation of microcalcifications.

Gelatin phantoms and tissue phantoms, made from turkey breast, were imbedded with calcium carbonate particles. Patients, who were undergoing breast biopsy for microcalcifications, were recruited for the study. Imaging was performed using gray scale and power Doppler coupled with ARI. The phantoms and the patients were scanned in the B-scan mode and the power Doppler mode while the particles were resonated between 50-500 Hz. The visualized calcium carbonate particles in the phantoms were cored biopsied using a 14-gauge needle. The patients subsequently underwent needle localization and excisional biopsy or had stereotactic core needle biopsy of the microcalcifications.

The specimen radiographs from the core needle biopsy of the phantoms showed the calcium carbonate particles within the specimen indicating that the area detected on power Doppler and ARI correlated to the calcium particles. Thus far, we have sonographed 10 patients with benign microcalcifications and 8 patients with malignant microcalcifications. In all cases, significant enhancement was observed in the expected region of the calcifications between 200-300 Hz. There was also, however, enhancement of secondary structures associated with connective tissues of the breast.

We conclude that calcium carbonate particles can be detected using power Doppler sonography coupled with ARI in phantoms. Thus far in patients, enhancement may be seen in the region of the calcifications but there is also enhancement of surrounding secondary structures. The ability to visualize microcalcifications using sonography will enhance the detection and evaluation of the earliest form of breast cancer, DCIS, allowing for improved evaluation without exposing the patients to additional radiation.

Susan P. Weinstein, MD
Chandra Seghal, PhD
Emily F. Conant, MD
Jill A. Patton, MS

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Breast, US, 00.12981
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Author contributions:

Guarantors of integrity of entire study, all authors; study concepts and design, all authors; literature research, S.P.W.; experimental studies, all authors; data acquisition and analysis/interpretation, all authors; manuscript preparation, S.P.W.; manuscript definition of intellectual content, editing, revision/review, and final version approval, all authors.

Microcalcifications in Breast Tissue Phantoms Visualized with Acoustic Resonance Coupled with Power Doppler US: Initial Observations¹

Calcium carbonate particles embedded in gelatin and turkey breast tissues were visualized with acoustic resonance imaging and power Doppler ultrasonography. Sonography revealed that the region of color level detection corresponded to the location of the calcium carbonate particles. Correlation between color level detection and the location of the particles was confirmed on radiographs of the specimens obtained at core needle biopsy performed through the region of color level detection.

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It is estimated that ductal carcinoma in situ represents 20%–30% of breast carcinomas detected at screening mammography (1). Since the 1970s, the detection rate of ductal carcinoma in situ has steadily increased with the wider use of screening mammography. The detection rate for women younger than 50 years was 2.3 cases per 100,000 women in the 1970s and increased to 6.2 cases per 100,000 women by the 1990s (2). More dramatically, in the group of women older than 50 years, the rate has increased from 14.3 to 54.6 per 100,000 (2) in the same period.

Frequently, ductal carcinoma in situ manifests only as calcifications without an associated mass (3). Because ductal carcinoma in situ represents as much as half of mammographically depicted cancers (3), it would be efficacious to image microcalcifications with as many modalities as possible to allow flexibility in imaging-guided biopsy. Currently, even with all the technical advances in breast

imaging, including magnetic resonance imaging, scintigraphy, and ultrasonography (US), mammography is the only reliable method to help detect, characterize, and localize microcalcifications for biopsy.

We have developed a technique in which acoustic resonance is used to visualize microcalcifications. Acoustic resonance is used on the basis of the size of the microcalcifications and the binding strength with the surrounding tissues in which they are embedded. When subjected to a wide frequency range, particles of different sizes will resonate at different frequencies given the same binding environment. "Tuning" to the appropriate frequency range would make it possible to selectively visualize microcalcifications of varying sizes. The purpose of our study was to evaluate US coupled with acoustic resonance to demonstrate small calcified particles for targeting and biopsy.

Materials and Methods

Phantoms

Gelatin phantoms were constructed by using a mixture of water, gelatin, and glycerol with uniformly dispersed oil-lecithin emulsion (particle diameter, 0.2–2.0 μm). The suspension was placed in a mold and refrigerated until a solid state was reached. Calcium carbonate particles 400–800 μm in diameter were suspended in the gelatin phantom in an area 1.0–1.5 cm in diameter. The phantom was kept refrigerated until use, at which time it was removed from the mold. Ten phantoms were made.

Twelve tissue phantoms were made from boneless turkey breast. The turkey breast was dissected along the tissue planes, and 400–800- μm -diameter calcium carbonate particles mixed in acoustic cou-

pling gel were embedded carefully to avoid air trapping. The region of the calcium carbonate particles ranged from 1.5 to 2.0 cm in diameter

Imaging: US Coupled with Acoustic Resonance

Imaging was performed (S.P.W. or C.S., with the assistance of J.A.P.) by using gray-scale and power Doppler US coupled with acoustic resonance (Logic 700; GE Medical Systems, Milwaukee, Wis). A variable 10–13-MHz transducer was used. All the gelatin and tissue phantoms were scanned. The phantoms were imaged with B-mode US and power Doppler US, while the particles were resonated from 50 to 500 Hz. The frequency that corresponded to the maximum color level detection was noted.

The frequency used to excite the particles into resonance was emitted from a thin (5-mm) lightweight (13-g) piezoelectric speaker element with the capability to transmit low-frequency vibrations from 50 Hz to 2 kHz. The device was housed in a fiberglass case. The piezoelectric vibrator was designed for 8 Ω of impedance to be driven by an audio amplifier. The vibrator was specially designed by the biomedical medical instrumentation group at our university; they specialize in building custom instruments, and this vibrator was approved for human use by the university institutional review board. The disk was coupled with US gel (E-Z-Gel; E-Z-Em, Westbury, NY) and placed adjacent to the tissue to be scanned. The images were videotaped for analysis.

Image Analysis

The videotaped images were subsequently analyzed. Imaging time for each scan was approximately 2 minutes. At the video frame rate of 30 images per second, approximately 120 $\times$ 30, or 3,600, images were generated. An algorithm was developed to reduce the data by sorting the images on the basis of the frequency of the vibration. These images were subsequently used in a computer program developed in our laboratory to analyze the vibrational response of the microcalcifications.

Color was analyzed for each image: Mean color level, percentage of fractional area of color, and color-weighted fractional area were computed. To determine the mean color level, the color palate on the image was read by the computer and divided equally on a scale of 0–100. With this scaling system, the computer constructed a lookup table for hue, saturation,

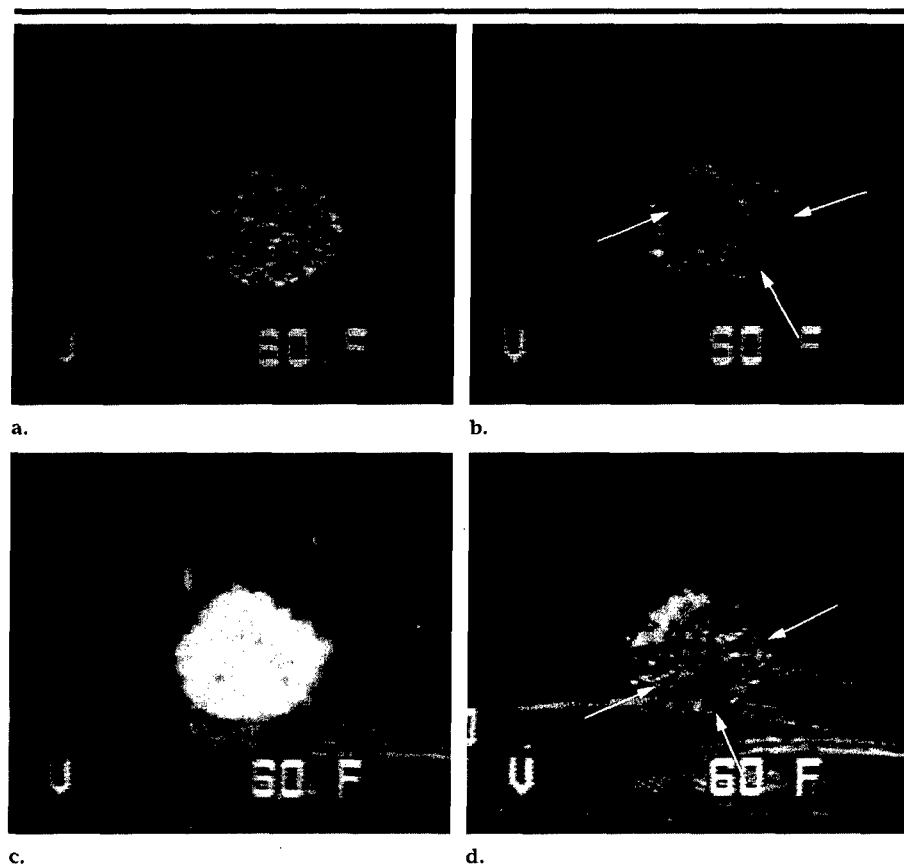


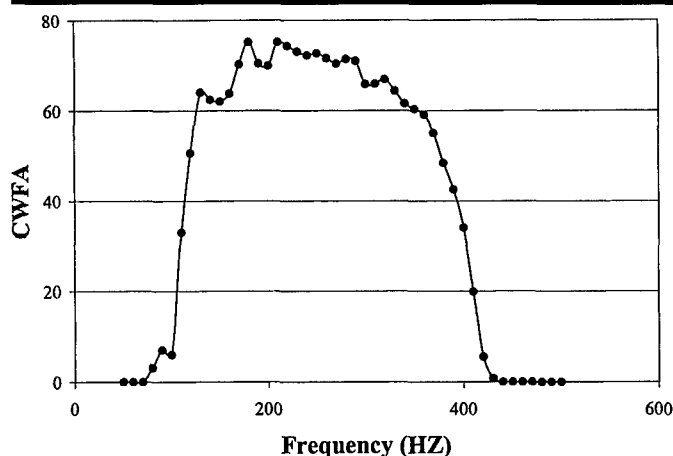
Figure 1. Color Doppler US images of gelatin phantoms evaluated with acoustic resonance and power Doppler US. Color is represented in gray scale. (a) Echogenic calcium carbonate particles are clearly visible in the gelatin phantom. (b) As the frequency is gradually increased to 100 Hz, the color pixels are seen as gray areas (arrows) in the region of the calcium carbonate particles. The pixels in this region were seen in color on the original images. (c) At 210 Hz, the entire region of the calcium carbonate particles is filled in with color pixels. (d) At 400 Hz, only a few color pixels (arrows) are seen.

and brightness values for the colors in the palette bar. Next, the computer identified colored pixels on the image and used the lookup table to assign a color value to each pixel in the region of interest. The color level of the pixels in the region of interest was summed and divided by the number of color pixels to calculate the mean color level. The percentage of fractional area of color was defined as the area covered by colored pixels divided by the area of the region of interest, multiplied by 100%. Color-weighted fractional area was defined as the mean color level multiplied by the percentage of fractional area of color, all divided by 100, and indicated the presence of net motion in the region of interest. Each parameter was plotted with respect to the frequency range.

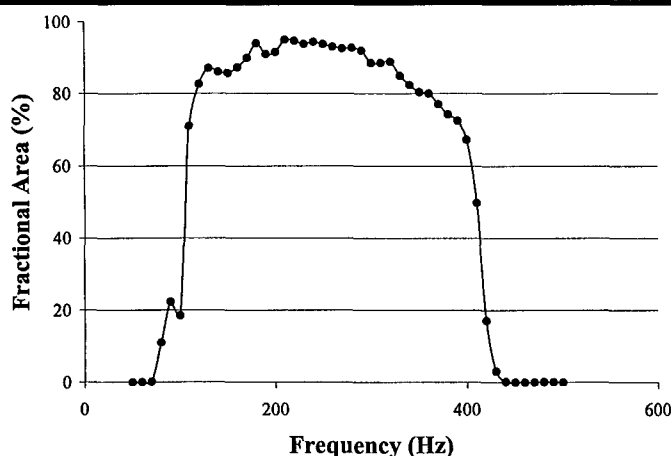
Biopsy

All the gelatin and tissue phantoms were scanned from 50 to 500 Hz. The

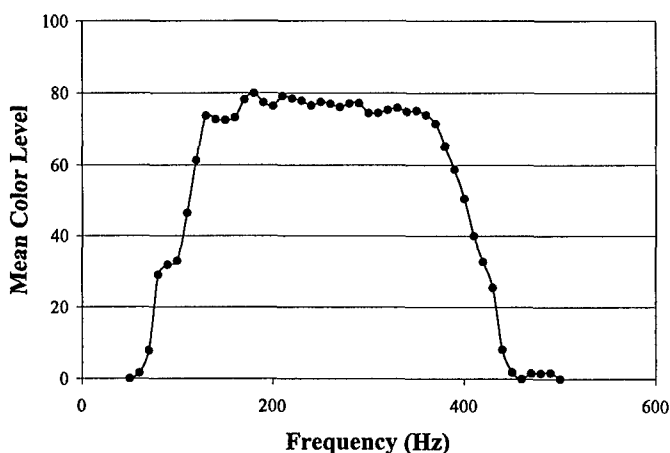
location where the maximum color level was detected and the corresponding frequency were noted. To demonstrate that the area of color level detection in the phantoms corresponded to the calcium carbonate particles, biopsy was performed through the region of color detection level. A 14-gauge disposable core biopsy needle (Monopty Biopsy Instrument; Bard, Covington, Ga) was used to perform biopsy in the region of interest during in real-time scanning by using power Doppler US coupled with acoustic resonance imaging. Approximately seven to 10 samples were obtained for each phantom. The core biopsy samples were placed in a core needle biopsy specimen container (Beekley, Bristol, Conn). Radiography of the specimens (alpha RT; Instrumentarium, Milwaukee, Wis) was performed at 22 kVp and 6 mAs, with magnification of 1.8, to confirm that the biopsy target, the region of depiction at



a.



b.



c.

Figure 2. Graphs plot (a) color-weighted fractional area (CWFA), (b) percentage of fractional area, and (c) mean color level versus frequency in the gelatin phantom. Detection levels at power Doppler US increase, then plateau, and then rapidly decrease relative to frequency.

Biopsy Results

The radiograph of a specimen is shown in Figure 4. All radiographs of the specimens depicted the calcium carbonate particles in the specimens.

Discussion

The concept of acoustic resonance imaging is relatively new. There is a large body of literature that reports various modes of US imaging to characterize motion and elasticity of soft tissues induced by external vibrations or due to natural motion (4–8). None of these authors deal with the issue of detection of microcalcifications, nor do they use the concept of acoustic resonance as a means to identify the properties of inhomogeneities in the tissue. Our goal was to be able to visualize the resonance motion of inhomogeneities relative to that of the surrounding tissue.

The 400–800- μ m calcium carbonate particles were excited into resonance when exposed to low-frequency vibrations from 50 to 500 Hz. The vibrations were of low amplitude, with no known harmful side effects. When the particles were in resonance and combined with power Doppler US, the calcium carbonate particles were expected to demonstrate a color level relative to the degree of resonance. At maximal resonance, there would be maximal color level detection. The frequency at which maximal resonance is demonstrated depends on the size of the microcalcifications and the binding properties between the tissue

power Doppler US, corresponded to the calcium carbonate particles.

Results

Gelatin Phantom

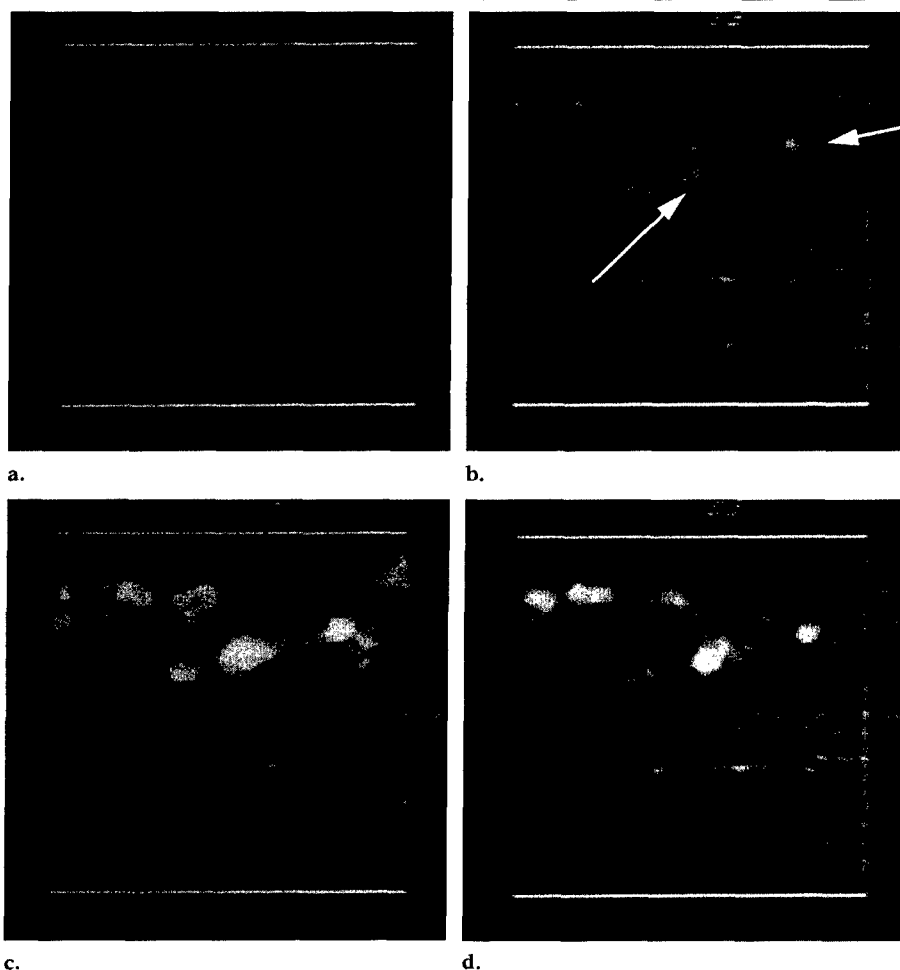
The calcium carbonate particles were readily visible as white particles in all the gelatin phantoms at B-mode US. Figure 1a shows one of the phantoms. The calcium carbonate particles were readily visible in all 10 specimens. With power Doppler US evaluation, a gradual increase in color level detection was seen in the region of the calcium carbonate particles (Fig 1b). The maximum detection level was reached and then plateaued at frequencies of 200–380 Hz (Fig 1c). This plateau was followed by a gradual decrease in the color level detection (Fig 1d). This trend in color level detection was seen in all the gelatin phantoms evaluated. As seen in Figure 1, there was a minimal color level detected outside the

region of the calcium carbonate particles. Graphs of mean color level, percentage of fractional area of color, and color-weighted fractional area versus frequency demonstrate the results seen on the static images in Figure 1, that is, a gradual increase in depiction level, followed by a decrease (Fig 2).

Turkey Phantom

The turkey phantom was also imaged with B-mode US and power Doppler US. In all 12 turkey phantoms, unlike in the gelatin phantoms, the calcium carbonate particles were not as readily visible at B-mode US before use of acoustic resonance imaging coupled with power Doppler US (Fig 3a). When the turkey phantom was imaged with power Doppler US coupled with acoustic resonance imaging, a gradual increase in color level detection was seen, a peak was reached, and a decrease followed (Fig 3b–3d). Similar findings in power Doppler detection were seen in all the turkey phantoms evaluated.

Figure 3. (a) US scan depicts a turkey breast phantom. Calcium carbonate particles are not as obvious as they are in the gelatin phantom. Color is represented in gray scale. (b–d) Power Doppler US scans coupled with acoustic resonance depict some color pixels in the region of the calcium carbonate particles (arrows) at 155 Hz (b); the number of color pixels continues to increase at 270 Hz (c); followed by a decrease at 355 Hz (d).

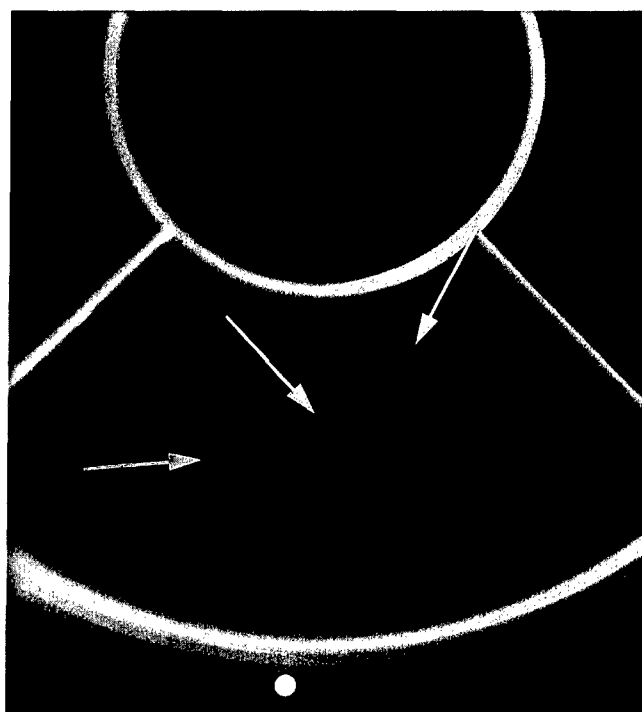


and the microcalcifications, which is demonstrated with the phantom models as shown in Figures 1 and 3. Graphs of the mean color level, percentage of fractional area of color, and color-weighted fractional area versus frequency demonstrate an increase in power Doppler US depiction level, followed by a plateau, then a decrease. The plateau, seen in the curves, may represent the particles of different sizes reaching resonance over a different range of frequencies.

In the simple gelatin phantom, the microcalcifications were visible without power Doppler US, which allowed direct correlation between the location of the particles and the area of color-level depiction. Unlike the homogeneous background of the gelatin phantom, the turkey phantom more closely approximates human breast tissue, with muscle striations and specular interfaces similar to the appearance of Cooper ligaments and other specular reflectors in human glandular tissue. Therefore, the location of the calcium carbonate particles is not obvious until power Doppler US coupled with acoustic resonance imaging is used. Core biopsy was performed in the area where color level was detected in both types of phantoms, and radiography of the specimens was performed. Findings on the radiographs of the specimens confirmed that the region of color level detection corresponded to the calcium carbonate particles.

There are multiple potential applications for this method. Once the microcalcifications are detected at US, biopsy could be performed with this dynamic imaging guidance. Results of various studies have demonstrated the cost-effectiveness of core needle biopsy compared with excisional biopsy (9–12) or US-guided core needle biopsy versus stereotactic core needle biopsy (13). With the ability to perform biopsy with vacuum assistance and US guidance, this method would allow performance of biopsy in calcifications by using US and possibly dynamic visualization of the extraction of calcifications, which would allow con-

Figure 4. Radiograph of a specimen depicts calcium carbonate particles (arrows) in the core biopsy samples.



firmation of targeting and, conceivably, a procedure time shorter than that for stereotactic core needle biopsy. Currently, sonographically guided biopsy is not always feasible because calcifications cannot always be definitively visualized with US alone.

We have shown that power Doppler US coupled with acoustic resonance imaging has the ability to demonstrate microcalcifications in phantom models. Future studies are needed to develop this technique for use in patients. US evaluation may be performed in women prior to conventional stereotactic or excisional biopsy. Correlation between particle size, peak resonance, binding properties, and pathologic results may provide beneficial information. Analysis of acoustic resonance properties may help determine the strength of adhesion between the calcium deposits and the surrounding tissues. Such forces are likely to be related to the molecular characteristics of the deposits and the mechanisms that cause calcifications to develop at specific sites in the tissues. Measurement of adhesive

forces with acoustic resonance, in conjunction with morphologic parameters detected at mammography, may help characterize calcifications.

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