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Informal Report

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as a Contrast Agent**

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Dual-energy subtraction imaging utilizing indium as a contrast agent

September-October 1996

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Abstract

The purpose of our current work is to establish the minimum detection limit of indium contrast agent using dual-energy subtraction imaging above and below indium K-edge. Experiments were performed on the X12 and X17B2 beamlines at the National Synchrotron Light Source using the same method but with two different set-ups. Experiments were first carried out on InCl_3 solutions, then on V79 Chinese hamster cells and on BALB/c mice excised tumors, labeled with indium. For each experiment, several layers of Lucite were placed in front of the phantom to ensure a 43 mm thickness, close to that of a mammography examination. Results were the same on X12 and X17B2. As expected, indium-free materials disappeared on subtracted images (water, steel reference and screw). Indium samples were easily distinguishable for the following concentrations: 10-5-2-1 mg/cm². Smaller concentrations were not clearly distinguishable and we were unable to see cell samples and tumors. To conclude, the lowest concentration we can image is around 1 mg/cm². These results agree with theoretical results. Such results also suggest that indium concentration in both cells and tumors is lower than 0.5 mg/cm². Since the current detection is close to optimum, we conclude that dual energy subtraction imaging using indium to label tumors cells and tumors is not possible unless the indium uptake is increased by more than an order of magnitude.

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Fig. 1 The indium K absorption edge as a function of energy

Introduction

The K-edge subtraction imaging technique has been recently developed at the BNL synchrotron light source (NSLS) and applied to coronary angiography and bronchography by using iodine and xenon as contrast agents, respectively [1]. The subtraction of the image above the K-edge from the image below the K-edge, after contrast agent administration, provides a contrast agent map free of underlying tissue and bone structures.

In cancer research, porphyrins were used to label tumor tissues as they provide a differential uptake between tumor cells and normal cells [2]. It seems promising to link these porphyrins to particular compounds such as contrast agents or drugs to improve diagnosis or therapy respectively. Some of these compounds have been recently developed at the BNL Medical Department, including boronated porphyrins for boron neutron capture

therapy experiments [3-4] and deoxyuridine labeled with iodine for photoactivation therapy [5].

We decided to carry out imaging experiments with indium-labeled porphyrin (In-BOPP) at the indium K-edge (sitting at 27.94 keV) to study the potential of such a technique for breast cancer detection, which has been a program at NSLS's medical beamline these last few years [6-7]. For this particular disease, early diagnosis is the best guarantee for a good prognosis. This kind of technology could really be useful in current screening programs for detection of small lesions resulting in fewer false positives and/or negatives due to the heterogeneity of breast tissue. The purpose of this work is to establish the minimum detection limits of this technique based on indium K-edge imaging. The imaging parameters are constrained by the dose allowed in mammography (around 1 mSv).

Theoretical considerations

The K-edge

The synchrotron radiation sources have sufficient X-ray intensity so that crystal monochromators can be used to provide x-rays with a very narrow energy width. It allows images to be taken above and below the K-edge of particular elements such as iodine (33.17 keV), xenon (34.56 keV), gadolinium (50 keV) and in our case, indium (27.94 keV). Fig. 1 illustrates the abrupt discontinuity of the indium absorption coefficient at the K absorption edge. The beam with the energy above the K-edge will have attenuation information due to the components forming the sample and the indium, whereas the beam below the K-edge energy will have less indium absorption.

Fig. 2 A schematic representation of the K-edge experiment featuring N_b as the number of photons below and N_a as the number of photons above.

Dual energy subtraction

For dual energy subtraction above and below the indium K-edge, assuming that human tissue is made of water, samples labeled with indium can be represented as in Fig.2. The number of photons N_i coming from the incident monochromatic beam go through the sample and reach the detector. By performing a logarithmic subtraction between the two signals obtained above (N_a) and below (N_b) the K-edge, a map of indium concentration is obtained (= an image of the labeled tissue).

Assuming that the water thickness is t_w and the indium thickness is t_{in} , the number of photons received by the detector at energies above (indicated by superscript 1) and below (indicated by superscript 2) the indium K-edge are :

$$N_a = N_i e^{-\mu_{in}^a t_{in}} e^{-\mu_w^a t_w} \quad (1)$$

$$N_b = N_i e^{-\mu_{in}^b t_{in}} e^{-\mu_w^b t_w} \quad (2)$$

where μ_{in} and μ_w are the absorption coefficients of the indium and water.

The indium thickness t_{in} can be obtained from Eqs.1 and 2;

$$t_{in} = \frac{-\mu_w^a \log\left(\frac{N_b}{N_0}\right) + \mu_w^b \log\left(\frac{N_a}{N_0}\right)}{\mu_{in}^b \mu_w^a - \mu_{in}^a \mu_w^b} \quad (3)$$

The noise, assuming Poisson, statistics is:

$$\sigma_{t_{in}} = \sigma(t_{in}) = \sqrt{\frac{(\mu_w^a)^2 \frac{1}{N_b} + (\mu_w^b)^2 \frac{1}{N_a}}{(\mu_{in}^b \mu_w^a - \mu_{in}^a \mu_w^b)^2}} \quad (4)$$

Fig. 3 Indium concentration (mg/cm²) as a function of pixel size (cm)

And the SNR is $\frac{t_{in}}{\sigma_{t_{in}}}$.

For the specific problem of mammography imaging with indium contrast agents, the SNRs were calculated assuming zero electronic noise and 100% detector efficiency. The dependence of SNR on the indium concentration and resolution (pixel size) is shown in Fig.3 assuming a total skin entry dose of 100 mrem for images above and below the indium K edge and a subject of 42 mm water equivalent thickness. Fig.3 shows that for a pixel size of 0.02 cm typically desired for tumor detection in mammography, for a SNR of 3, the minimum indium concentration is around 0.5 mg/cm².

Dosimetry

Our experiments were carried out on phantoms with the radiation doses limited to less than the allowed dose in mammography. It has to be stressed that we must take into account two exposures with roughly equal doses (below and above the K-edge) for each image. Exposures were calculated using the following method. The exposure dose was measured by an ion chamber placed in front of the phantom. The current from the ion chamber was amplified by a Keithley amplifier (with the gain set at 10⁸) and the output voltage was converted into counts/sec by means of a voltage-to-frequency converter that is connected to a scaler. Knowing the current I of the ion chamber, the relationship between the incident photon number/sec, N_i , and the current I is the following :

$$N_i = \frac{I\omega}{Ee(1 - e^{-\mu d})}$$

where ω is the ionization energy for the gas in the chamber (argon at atmospheric pressure), E is the beam energy (keV), e is the electron charge, μ is the absorption coefficient of the gas and d is the thickness of the ionization chamber.

For each image, the total photon number N_{tot} per cm² for a translation length h (cm), a beam width w (cm) and a translation time ΔT (s) is :

$$N_{tot} = \frac{N_i \Delta T}{wh} = \frac{N_i}{sw}$$

where s (cm/s) is the scan speed. Thus, it is possible to control the exposure by controlling the speed. There is, however, a limit that is the maximum speed available and this means that the use of Lucite and aluminum filters

may be necessary to attenuate the beam. The surface entry dose exposure D is calculated by :

$$D = \mu_e N_{tot} E$$

where μ_e is the energy absorption coefficient of water.

Materials and methods

Experiments were carried out using two different beamlines (X17B2 and X12 hutches) but with the same digital subtraction method and similar imaging set-ups.

Set up of the beam at X17B2

The X17B2 set-up was primarily used for angiography and bronchography by respectively using iodine and xenon as contrast agents [1,2]. We moved it for the first time to the energy of the indium K-edge: the Bragg angle of the [1,1,1] silicon bent crystal monochromator is 8.1° at indium K-edge compared to 6.8° at iodine K-edge. This forced all beamline components to be raised by amounts proportional to their distance from the monochromator crystal. The detector was raised 18 cm above the position used for iodine imaging. In order to allow the fan beams to exit the monochromator's beryllium window, the tank stand was raised by 1.58 mm and the monochromator crystal was lowered by 1.58 mm. As described in Fig. 4, this system simultaneously provides two beams bracketing the chosen K-edge energy, and the above and below images are acquired at the same time. Both beams are about 13 cm wide and cross at the sample position with a vertical angle between them of about 1 mrad. The sample moved vertically through the cross-over point of the two beams. Each line of data was taken

Fig. 4 A schematic representation of X17B2 set-up

in 4 milliseconds with a 600 element dual array silicon detector.

Set up of the beam at X12

The experimental set-up is shown in Fig. 5. The white beam (4 mm high, 25 mm wide) was provided by the X12 bending magnet. The set-up, that was not under vacuum, began downstream of a water-cooled beryllium window. A 1 mm aluminum filter and a 0.25 mm copper filter were put at that position. Then, a tungsten slit provided an adjustable (white) beam according to the need of the experiment (normally 0.2 mm high, 25 mm wide). This beam was then incident on our monochromator made of two independent crystals mounted on two different motorized stages. The first crystal was flat and positioned to provide a diffracted beam at the desired energy. This diffracted beam was diffracted again in passing through the second crystal. This crystal was bent in order to provide a reflection bandwidth much larger than that provided by perfect unbent crystals. Bending the second crystal also allows the beam energy to be tuned above and below the indium K-edge by changing the Bragg angle of the first crystal only. The beam passing through the first crystal was stopped by a 0.3 mm lead beam stop. The monochromatized beam passed through the ionization chamber to reach the sample. The ionization chamber was filled with argon and used to measure the exposure to the phantom (50 mm deep, HV= 1 kV, Gain= 10^8 V/A, VF= 10^5 Hz/V). The image plate detector was placed 10 cm downstream of the sample. The detector and phantoms were fixed on the same support and were moved vertically by a stepper motor driver to perform the scan (scan speed = 8mm/s). The above and below images were acquired on the same image plate by moving the sample horizontally between the two scans. Lead slits were put in front of the ion chamber and the image plate to reduce scattering noise on the image. Images were recorded on a Fuji ST3 PSP image plate which was read with a Fuji BAS2000 image plate reader. Typical reading parameters were a sensitivity of 400 and latitude of 4. The plates were read out as a 2048x2560 matrix (0.1 mm/pixel).

Fig. 5 A schematic representation of X12 set-up

As fluorescence spectrometry is more sensitive to trace quantities of elements than radiography, the same set up was then adapted to fluorescence experiments in order to measure smaller indium concentrations. To differentiate the indium K fluorescence x-rays from the scattered incident beam, the monochromator was tuned to give an incident beam of 30 keV. Spectra were acquired with a germanium detector (CANBERRA GL0210R) and measured by using PCA software. The detector was equipped with an 8 mm diameter collimator, positioned at around 140 mm from the sample and calibrated with a copper foil. Spectra were acquired for 300 s with a 5 mm beam width to cover the chosen slice of the Eppendorf tube, containing the sample. Experiments were performed at 2.5 mm from the tip of the tube for solutions and tumors. For cells, experiments were performed at three heights respectively corresponding to cell medium, cell pellet and remaining indium clusters (localized at the tip of the tube).

Samples

Three kinds of samples were used for these experiments : saline solutions, tumor cells, tumor tissues. Saline solutions were labeled with several

concentrations of InCl_3 . Both cells and tumors were labeled with Indium-BOPP synthesized at BNL according to a protocol previously described by Kahl et al (1992) [8]. A home made phantom was both used on X17B2 and X12 for all the experiments (solutions, cells and tumors). For each experiment, several layers of Lucite were placed in front of the phantom to ensure a 43 mm thickness close to the standard thickness in a mammography examination. A piece of indium foil (100 mm thick) was embedded in the phantom for each experiment to determine the indium edge.

Cells

Chinese hamster V79 cells were grown as monolayers in two different flasks with Dulbecco's modified Eagle's growth medium (GIBCO). The first flask was used as a control sample whereas the second flask cells were labeled with indium. The day before an experiment, In-BOPP was dissolved in the cell medium and the tube was incubated at 37°C until the experiment. Before the experiment, cells were fed with In-labeled medium for 10 minutes. Then, the In-labeled medium was removed and after five PBS washings, cells were fed by normal medium for 4 hours. Subsequent to PBS washing, trypsinization and harvesting, cells of both flasks were counted two hours before the experiment and the number of control and labeled cells were respectively 20×10^6 and 35×10^6 . The original harvest tube was centrifuged into a pellet (1500 rpm at 20°C for 10 min) and most of the supernatant was removed. Then cells were mixed again and centrifuged in an Eppendorf tube (4 mm diameter, 30 mm high), to obtain a quite small pellet.

Tumors

Experiments were carried out using the KHJJ murine mammary carcinoma on a BALB/c mouse as described previously [9]. In-BOPP was administered as three 0.5 ml injections per day over two days.

Results

Imaging results

The results obtained on the images were identical on X12 (Fig. 6) and X17B2 (Fig.7). The absorption was stronger on images above the edge than below the edge for the indium labeled part of the phantom, whereas other components do not exhibit differences between above and below. As expected, indium-free materials disappeared on subtracted images (water, steel reference and screw). Indium samples were easily distinguishable for

the following concentrations : 10-5-2-1 mg/cm². It has to be stressed that the indium foil presented the same aspect above and below because of saturation of the image plate. The smallest concentration we were able to see was 1 mg/cm² both on X12 and X17. All the other concentrations under this threshold were not clearly distinguishable on the subtracted image. These results agreed with the theory previously described. Fig.3 showed that for a pixel size of 0.2 mm, for a SNR of 3, the minimum indium concentration was around 0.5 mg/cm².

A quantitative comparison between experiment and theory was done to determine the viability of our experiment. The indium thickness t_{in} for each sample on the subtracted image given by the previous formula (Eqs.3) can be simplified assuming that the water absorption is the same above and below the indium K-edge:

Fig. 6. Results obtained on X12 from left to right: phantom configuration with several InCl₃ concentrations in mg/cm², image obtained above the indium K-edge, image obtained below the indium K-edge, subtracted image.

Fig. 7 Results obtained on X17B2

Fig. 8 Correlation between experimental and theoretical results

$$t_{in}(\mu_{in}^b - \mu_{in}^a) = \log\left(\frac{N_a}{N_0}\right) - \log\left(\frac{N_b}{N_0}\right)$$

and:

$$\rho t_{in}\left(\frac{\mu_{in}^b - \mu_{in}^a}{\rho}\right) = \rho t_{in}\left(\frac{\Delta\mu}{\rho}\right) = \log\left(\frac{N_a}{N_0}\right) - \log\left(\frac{N_b}{N_0}\right)$$

where ρ is the indium density (g/cm^3). The left part of the equation is calculated using the known indium concentration whereas the right part is given by the detector or image plate measurement. For each concentration imaged, the image measurement was corrected by the background to compare to the theoretical result. The correlation between theoretical and experimental results is illustrated by Fig. 8. The increased scatter in the data near rt is probably due to the error in the dilution for small amounts of Indium. The same experiments were carried out with cell cultures and excised tumor tissues in Eppendorf tubes, in the same way as for the previous phantoms. Unfortunately we were unable to see any cell samples

on tumors on the subtracted images. Such a result highlights the fact that the indium concentrations in both cells and tumors was probably lower than 1 mg/cm^2 .

Fluorescence results

The results previously described seem to show that indium labeled biological samples are under the threshold we can see, less than 1 mg/cm^2 . It was thus interesting to carry out a fluorescence experiment in order to know the concentration range of these indium labeled biological samples. Fluorescence is a very sensitive technique which should allow detection of smaller indium concentrations. Fig. 9 shows a typical fluorescence spectrum obtained by using the germanium detector. The several peaks are localized as expected according to x-ray emission line energies. The indium $K\alpha_1$ peak is sitting at 24.210 keV . Around 10 keV , we can see lead peaks from the shielding ($\text{Pb } L_{\gamma_1}=14.76 \text{ keV}$). The biggest peak sitting at 30 keV comes from the scattering of the incident monochromatic beam.

Fig. 9 Typical fluorescence spectrum acquired with the germanium detector : lead, indium and the primary beam peak are respectively sitting at 14.76 keV (L_{γ_1}), 24.21 keV ($K\alpha_1$) and 30 keV

Fig.10 shows the number of counts obtained as a function of indium concentration for several InCl_3 phantoms of different concentrations, chosen under the threshold of imaging of less than 1 mg/ml. The number of counts, expressed as a function of InCl_3 concentration is linear. The same experiment carried out on several tubes containing cells and tissues showed that the indium concentration was less than 0.02 mg/ml for excised tumor samples, between 0.02 and 0.1 mg/ml for cell samples. There was some indium at the bottom of the tubes. The concentration measured between 0.1 and 0.5 mg/ml.

Fig. 10 The fluorescence results obtained are expressed in normalized counts as a function of the indium concentration contained in the several phantoms.

Discussion

All the experiments carried out on InCl_3 samples clearly demonstrates that the lowest indium concentration we can image is 1 mg/cm², using typical mammography dose and phantom thickness. These results were obtained on both beamlines used and fit well with theoretical results.

The results obtained with the biological samples were more disappointing. All the biological samples labeled with indium were less than 1 mg/cm².

Using fluorescence, it was shown that the indium concentration ranged below 0.1 mg/ml. The difference between the smallest concentration we are able to see by imaging and fluorescence is a factor of 10. Right now, the indium uptake is too small to provide useful images.

The experiment helped us to point out several problems with the compound, especially the presence of clusters remaining at the tip of the Eppendorf tubes after several rinsing. This may mean that the indium uptake was not optimal. The medical team is now working on the compound to improve this particular point.

Conclusion

The lowest concentration we have reached for imaging both on X17B2 and X12 is around 1 mg/cm². These results agree with theoretical considerations. The fluorescence spectroscopy results also suggest that indium concentration in both cells and tumors is lower than 0.5 mg/cm². Right now, the only way to improve the capability of imaging the cells is to the indium uptake by the cells. The physics limits are reached. Performing images above and below the K-edge of particular elements still remains an attractive technique, especially for a contrast agent such as gadolinium which seems to be a promising element.

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