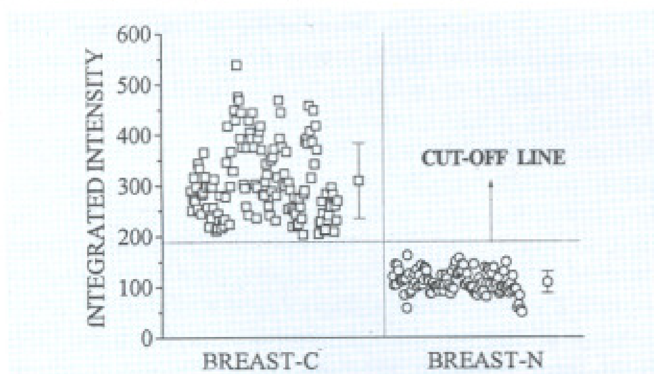
LASER PROGRAMME

Laser induced fluorescence diagnosis of cancer

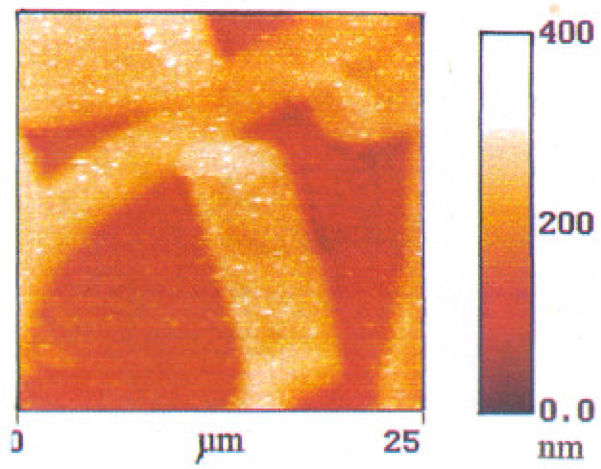
Laser induced fluorescence spectroscopy of human tissues is being actively pursued for sensitive, in situ, near real time diagnosis of cancer (see CAT Newsletter June '95). Recent work carried out at CAT on breast tissue samples has shown that with the use of 337 nm N_2 laser excitation the cancerous breast tissue sites are significantly more fluorescent compared to the normal and the benign tumor tissue sites. Therefore, the use of fluorescence intensity alone as the discrimination parameter could provide very good discrimination between the cancerous, normal and the benign tumor tissues. Sensitivity and specificity values of ~100 % were achieved in this in-vitro study involving 65 patients with breast tumor. Further, the studies carried out at CAT showed that the discrimination results were remarkably better with 337 nm excitation as compared with the use of 300 nm excitation being used in a prototype commercial system being developed elsewhere. Possible reasons for the improved discrimination with 337 nm excitation have been identified. Studies have also been carried out on the cancer of oral cavity. In contrast to breast malignancy, malignant tissue in the oral cavity was characterized by a significant reduction in the fluorescence intensity. In a study, involving 50 patients with cancer of oral cavity, the discrimination algorithm developed to quantify the observed differences in autofluorescence spectra could provide sensitivity and specificity of ~95% for discriminating malignant tissue from the normal tissue.

X-ray contact microscopic imaging using laser produced plasmas

Intense x-ray emission from laser produced plasma has been used for single shot x-ray contact microscopic imaging of physical and biological microstructures. This basically involves making a contact shadowgram of a sample placed on a photo-resist coated surface, by exposing it to a high brightness, short duration (~nsec) burst of soft x-rays from a point source like laser produced plasma. After exposure, the latent image of unit magnification in the photoresist is chemically developed to yield a relief pattern. Since the



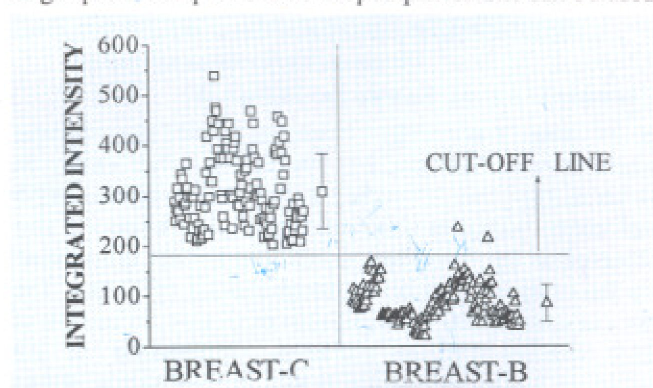
Scatter plot for the spectrally integrated intensity of cancerous (BREAST-C) and adjoining normal (BREAST-N) breast tissues.



Atomic Force Microscopy scan of x-ray image of yeast cells

etching depth at a given point on the photoresist depends on the x-ray dose received, the recorded image represents a two-dimensional topographical map of integrated x-ray attenuation of the sample. Spatial resolution in the range of 50 to 200 nm, governed by diffraction and penumbral blurring, can be obtained depending on the x-ray source size and the sample thickness.

Soft x-ray source was produced by focussing 10 J, 28 nS (FWHM) Nd:glass laser pulses on a planar copper target to a spot diameter of ~130 µm at a laser intensity of $2x10^{12}$ W/cm^2. Imaging of fine copper grid and free-standing gold transmission grating microstructures was done on a ERP-40 photoresist coated silicon wafer to characterize the spatial resolution and the x-ray dose requirement. A spatial resolution of 195 nm was observed from the image of a 10µm thick copper mesh in a single shot x-ray exposure of ~10mJ/cm^2 (for hν $\geq$ 0.8KeV). Further, experiments on imaging of biological samples were carried out in collaboration with P N Lebedev Physics Institute, Moscow. X-ray images of yeast and fungus cells were recorded with an estimated resolution of ~120 nm. Figure above shows an Atomic Force Microscopy picture of the image of the yeast cells alongwith a height profile distribution. Important role of x-ray imaging is noted from the high contrast and features observed in such images which are not revealed when the sample is viewed under an optical microscope. Moreover, height profile map of the developed photoresist can be used



Scatter plot for the spectrally integrated intensity of cancerous (BREAST-C) and benign (BREAST-B) breast tissues.

to obtain the x-ray dose distribution on photoresist and thereby distribution of optical density of the sample in x-ray spectral region. Work on extending this technique to stereoscopic imaging using two x-ray sources in different spectral regions is in progress.

Measurement of absorption coefficient of CO_2 laser windows

Single-mode line-tunable CO_2 lasers are used extensively for pumping molecular gas lasers and for spectroscopic applications. The operational characteristics of these lasers are significantly influenced by the undesirable absorption in intracavity components, such as the windows on a gas discharge section. Knowledge of loss introduced by the intracavity windows at wavelength of laser operation is therefore often essential. Although a number of methods are available for this purpose, each has its limitations. For example, measurement of the typically low absorption coefficient ($\sim 10^{-3}$ cm^{-1}) of laser-grade window material with a dual-beam infrared spectrophotometer requires a sample thickness of the order of a few centimeters to ensure a measurable transmission change of ~1%, whereas the window thickness typically used in continuous-wave CO_2 lasers is a few millimeters. A new technique recently demonstrated at CAT allows measurement of absorption co-efficients of the order of $\sim 10^{-3}$ cm^{-1} in windows a few millimeter thick.

This technique involves measurement of the output power of a single mode CW CO_2 laser as a function of its cavity length. This is conveniently achieved by a change in the applied voltage to a piezoelectronic transducer (PZT) on which one of the cavity mirrors is mounted. The curve of the output power versus cavity length shows hysteresis. This has earlier been shown to arise due to heating of the intracavity optical components in the presence of the laser beam and the consequent changes in the resonator path length. Since the laser heating induced change in path length (ΔL) depends on the absorption co-efficient of the window, knowing ΔL, absorption co-efficient can be determined. The change in the resonator path length arising due to laser induced heating of a given component and hence its absorption coefficient can be determined by measuring the hysteresis in the power-tuning curve both without and with the insertion of this component in the cavity and by ensuring that the laser operates at the same power level in both cases. The latter can be conveniently achieved by a small adjustment of discharge current. The technique has been used to measure absorption co-efficient of 3mm thick ZnSe and KCl windows. The measured values were found to be in reasonable aggrement with the values obtained by other methods.

Cover photograph *shows An industrial model of high power transverse flow CW CO_2 laser, developed and built at CAT. It gives 3.5 kW laser power and is coupled with a CNC work - table (3m x 1m span).*

Susceptibility Bridge

AC-susceptometers are widely used to study the magnetic response of magnetic materials and superconductors. This apparatus is particularly popular because starting from relatively fast non-destructive testing of new materials to elaborate study of magnetic properties as a function of temperature, frequency and applied field (both driving ac-field and dc-bias field) can be performed with a single apparatus. Such a facility with variable temperature (80K<T<300K), variable frequency (1Hz <f < 10KHz), sample environment and also with the capability of higher harmonic susceptibility measurement has been built at CAT.

Operation of an ac-susceptometer is based on detection of emf induced by an alternating field in a pick-up coil that contains the magnetic or superconducting sample. Pick-up coil system used in ac-susceptometer at CAT is formed by two axially symmetric oppositely wound coils mounted in series. A primary coil concentric with two secondaries carries a current that generates alternating field. Ideally in absence of a sample, the induced emf is zero. When a sample is introduced in one of the secondaries, an imbalance in the induced emf is created, which is proportional to susceptibility of the sample. This imbalance is measured directly by synchronous detection using a lock-in amplifier. In addition, the lock-in detection, is particularly useful while working in a noisy (electro-magnetic) environment.

To increase sensitivity of the apparatus further, a home made mutual inductance bridge, which can compensate imbalance in induced-emf, is incorporated. In this configuration, lock-in amplifier is used as a null detector.

The mutual inductance bridge can balance resistive and inductive part of the signal separately using a variable resistor and a variable mutual inductor. Instead of using a commercial variable mutual inductance (which is a costly item), in present bridge variable mutual inductance is simulated using an electronic circuit comprising a standard fixed inductance and operational amplifiers. Sensitivity of the order of 5×10^{-6} emu has been achieved in low frequency range and with some care it should not be difficult to extend it to 10^{-7} emu range. At present the apparatus is working regularly in the temperature range 80K<T<300K and frequency range 1Hz<f<1KHz. Work is in progress to extend the temperature range down to 4.2 K and frequency range to 100 KHz.

With help of a sophisticated lock-in amplifier (SR830) this apparatus is also used to measure non-linear magnetic response of various materials in the form of higher harmonic susceptibility.

This apparatus has been developed in steps. Since early 1997 it has been automated (using a PC) for synchronous signal detection using a lock-in amplifier. A user-friendly software is developed in 'C' language for this purpose. The effort is now focused in building an intelligent mutual inductance bridge which can be controlled by a computer, so that the apparatus can be completely automated even in its highest sensitivity range.

The apparatus has been used during recent past in non-destructive testing of new giant magneto-resistance materials