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Development of X-ray Holography for Biological Imaging

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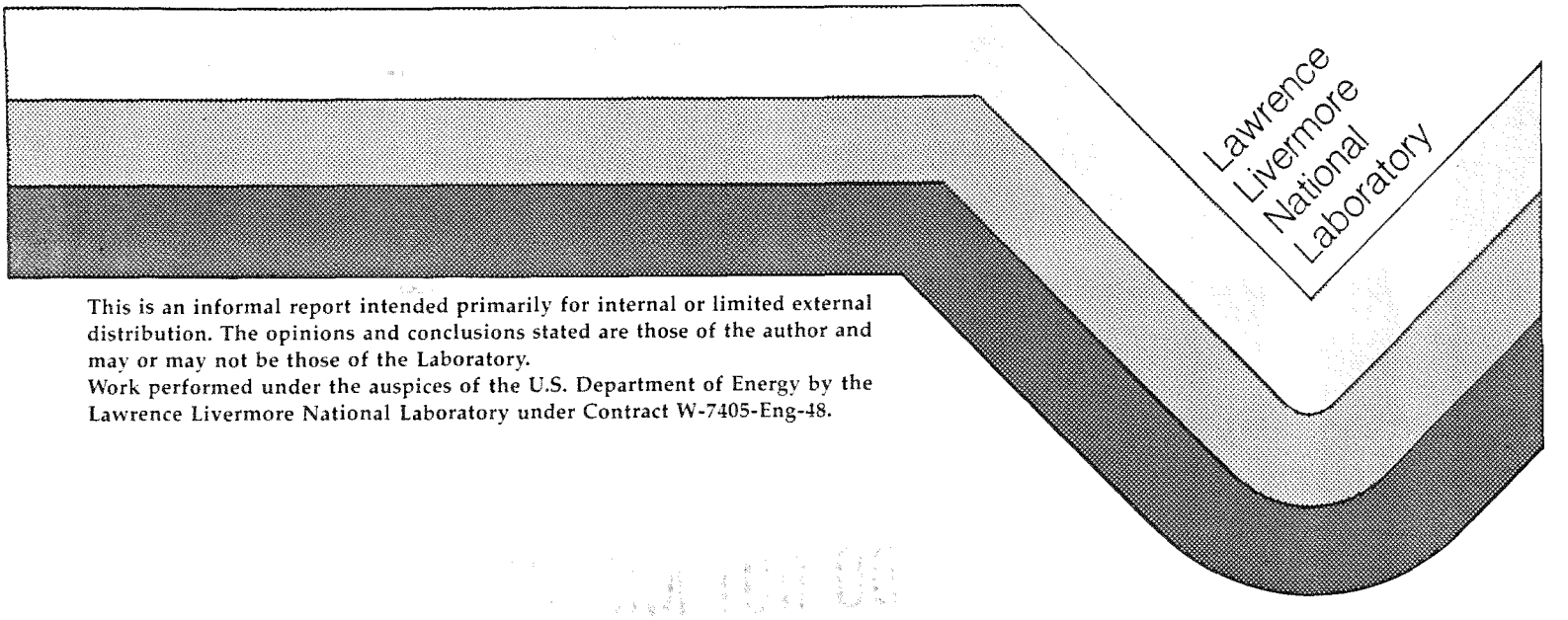

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Development of X-ray Holography for Biological Imaging

Much of modern biology is currently directed toward the analysis of the function of macromolecular structures such as chromatin-enzyme replication complexes, nuclear pore structures, structural protein complexes, and elements of the golgi apparatus. The physical size of these structures are smaller than the resolution limits of visible light microscopy (<2000Å). The emphasis on this size regime should increase steadily as the international genomics effort provides genetic information about the proteins that mediate cell function. Higher resolution imaging techniques such as electron microscopy can be used to provide general information in this regime, but only after the structures of interest have been made accessible to the probing electron beam and been prepared to withstand the harsh environment required for electron microscopy. This preparation involves thin sectioning or partial disassembly, fixation with electron dense cross linking agents, and dehydration. As a result of the sample preparation techniques required, the determination of the structure as it exists in a living cell and any changes resulting from environmental or genetic manipulation (needed to define structural function) may be difficult if not impossible to obtain. Newer technologies such as scanning tunneling microscopy also offer high resolution but require partial disassembly to allow probe access and only provide information of the structure surfaces. Clearly, higher resolution imaging techniques, applicable to the study of three dimensional macromolecular structures in living cells or in physiologically normal environments, are sorely needed by modern cell biologists.

To meet this growing requirement of high three dimensional spatial resolution, a research effort has been undertaken to develop x-ray holography as an imaging tool for structural biology studies. X-ray holography offers the potential for producing both three dimensional images (from the holographic imaging process) and high resolution (due to the short wavelength of the x-rays). This effort is fueled by the new developments in x-ray sources (high brightness, x-ray lasers) and in x-ray optics (high quality normal incidence mirrors).

The project to develop x-ray holography is multi-disciplinary in nature and involves the ICF Program, the Electrical Engineering Division, the Physics Department, and the Biomedical Sciences Division. The goal of this effort is to determine the feasibility of utilizing x-ray holography in biological structure function studies with resolutions of ~300Å and to demonstrate this imaging technology. The specific tasks to be accomplished in this effort are:

1. demonstrate that x-ray lasers have sufficient brightness and coherence to produce x-ray holograms

2. determine the optimal x-ray laser characteristics for x-ray holography of biological objects

3. develop and demonstrate x-ray lasers with these characteristics

4. develop and demonstrate numerical procedures for reconstructing and analyzing a three dimensional image from an x-ray hologram

5. determine the fundamental resolution limits due to finite x-ray laser brightness, x-ray damage to the biological structure, low x-ray contrast between complex biological structures within a cell, x-ray speckle produced in the coherent imaging process, and finite detector sensitivity and resolution

6. demonstrate high resolution, three dimensional x-ray holography in a series of experiments beginning with simple inanimate objects and progressing to complex structures within living cells.

Significant progress has been made in achieving these goals. X-ray holography has been accomplished using an x-ray laser for the first time. In these experiments a neon-like selenium laser with a wavelength of 200Å was used in a Gabor geometry to produce low resolution holograms of both 8 micron diameter carbon fibers and of three dimensional 10 micron gold stick figures. The spatial resolution achieved was limited by the x-ray film detector to approximately 4-5 microns. Image reconstruction was accomplished optically with a He-Ne visible light laser. These experiments demonstrated that:

1. x-ray lasers are highly reproducible in output and divergence
2. x-ray lasers have sufficient brightness and coherence to produce holograms on 200 psec timescales
3. multi-layer x-ray mirrors possess sufficient smoothness and flatness to be used in phase sensitive applications such as holography.

The optimal x-ray wavelength has been determined for holography of biological objects. This optimization was determined from an analysis of x-ray scattering from biological materials such as protein in water. The optimization was accomplished by maximizing the x-ray penetration, maximizing the elastic scattering and minimizing the x-ray absorption. The results indicate that the optimal wavelength is 45Å, just above the carbon K edge at 43.7Å. At this wavelength, the elastic scattering cross section is dominated by differences between the real parts of the indices of refraction of water and protein. The protein acts like a phase object. The imaginary part of the index of refraction of protein is at a minimum reducing the x-ray absorption. This result contradicts the early notion that the optimal wavelength regime for holography was in the "water window" (between the carbon and oxygen edges, 43.7-23.2Å). The difference results from a more complete treatment of the scattering process which correctly includes the boundary conditions imposed by the surrounding water.

The required x-ray laser output and pulse length can be determined if the elastic scattering cross-section and the x-ray absorption cross-section are known and the various losses in any realistic holography system are taken into account. Results from simulations of a holography systems indicate that interpretable holograms of simple protein structures in water can be produced with spatial resolutions of 300Å using only 120 microjoules of x-ray laser output energy at a wavelength of 45Å. The x-rays absorbed during the production of the hologram heat the object and cause hydrodynamic expansion. Limiting the x-ray laser pulse length to 50 ps or less results in the production of the hologram on a timescale short compared to the time necessary to compromise the spatial resolution.

The required x-ray laser energy can be reduced and the allowed pulselength increased in high contrast labels are attached to the biological structures of interest. This can be accomplished using well developed techniques such as immuno-gold labeling. With this labeling method gold micro-spheres are chemically attached to protein specific anti-bodies and micro-injected into the live cell. These gold spheres then attach themselves to predetermined structures in the cell allowing the x-ray contrast to be significantly increased. This results in the output energy requirement dropping to ~2 microjoules and the allowed pulselength being increased to well above a nanosecond.

The x-ray laser requirements stated above are well within the capability of x-ray lasers now under development. Existing x-ray lasers have pulse lengths under 200 psec and outputs above 1 milli-joule. Ni-like x-ray lasers at 50.3Å should be scalable to 45Å using tantalum. What is not understood is the x-ray laser coherence properties. Theoretical modeling of these lasers

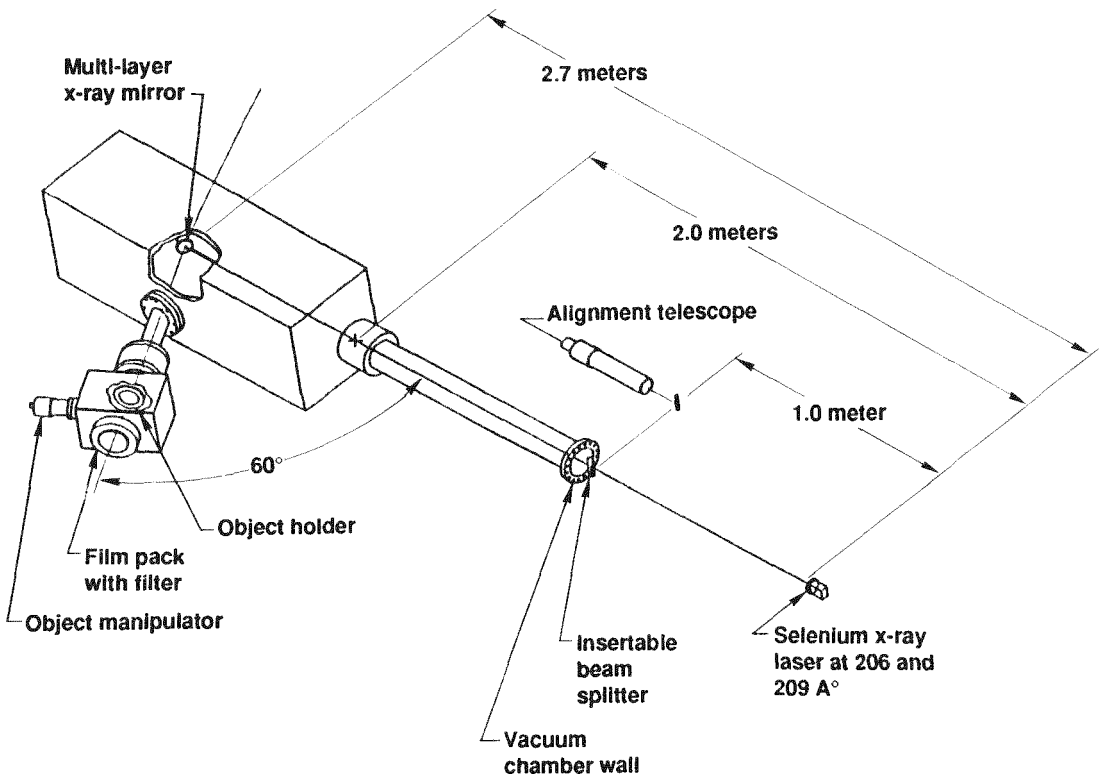
suggest that the longitudinal coherence length is ~300 microns and is more than enough for holography of biological objects. The spatial coherence is also expected to be very high due to the mode rejecting effects of refraction from the electron density gradients and from gain guiding in the x-ray laser. Experiments are planned for FY90 to determine the longitudinal and spatial coherence lengths of existing x-ray lasers. The longitudinal coherence length will be determined from a laser line width measurement. The spatial coherence length will be determined from the fringe patterns obtained from a double slit x-ray interferometer experiment. Following the measurement of the x-ray laser coherence properties, demonstrations of highly coherent 45Å x-ray lasers are planned at Nova.

The ability to produce x-ray holograms with resolutions of 300Å is insufficient to demonstrate the biological utility of x-ray holography. Interpretable three dimensional images must be reconstructed from the holograms. A three dimensional image reconstruction computer code based on back propagation techniques and including photon statistics has been developed for use in x-ray holography. This code is currently being tested using visible light holograms of simple objects. Final testing will be accomplished using visible light holograms of biological objects, whose known structure will be determined using optical sectioning microscopy. The use of biological objects allows holograms of objects with realistic complexity and contrast to be tested. Once fully tested, this code will be used with both simulated and real holograms to determine realistically achievable three dimensional spatial resolutions.

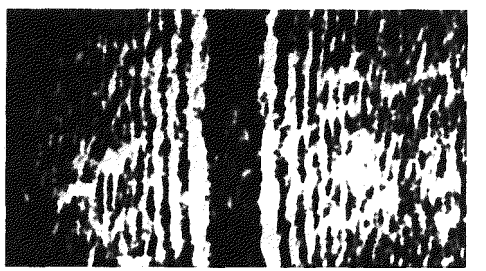
One result of the three dimensional image reconstruction development has been the determination that multiple simultaneous holographic views will be required to achieve depth resolutions comparable with transverse resolutions. The use of multiple views increases the required x-ray laser energy by approximately 10. It also reduces the resolution degrading effects of speckle and allows the incorporation of a prior three dimensional information which can be used to enhance the signal-to-noise ratio in the hologram through procedures such as algebraic reconstruction techniques.

High resolution holography experiments will begin following the completion of the image reconstruction code development and the demonstration of coherent x-ray laser operation at 45Å. Preliminary experiments will utilize gold labeled, dehydrated biological objects whose structure has been determined using electron microscopy. These will be followed by experiments using similar wet, gold labeled objects. Final experiments will utilize both gold labeled and unlabeled chromatin fibers in live cells. Immunofluoresence labeling will also be used to allow comparison with simultaneous visible light microscopy. These experiments should demonstrate the realistic utility of x-ray holography in biological structure studies.

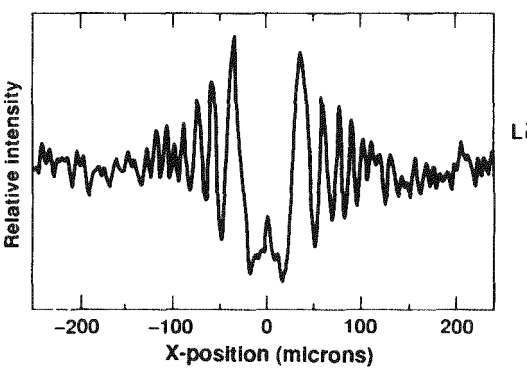
First demonstration of x-ray holography with an x-ray laser



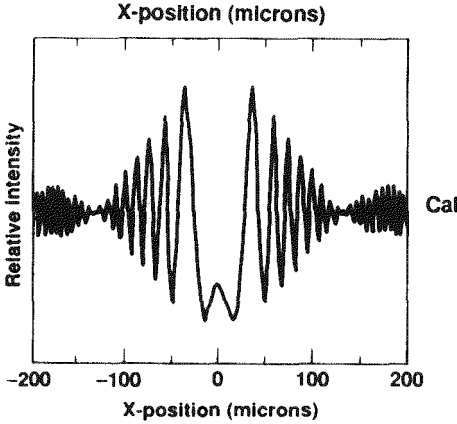
Experimental setup
(Gabor geometry)



Data



Line out
of
data



Calculation

Fringe patterns
(8μ wire)