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Diffraction from a beam of laser-aligned proteins - for in-situ TEM ?.

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An electron diffraction camera is under construction which will allow diffraction patterns to be obtained from a beam of hydrated, laser-aligned proteins [1, 2]. Subsequently we hope to develop a version based on a water-jet holder for an environmental TEM to make use of higher accelerating voltages. The aim of the project is to determine the structure of proteins which cannot be crystallized. (A related chamber is under construction at the Advanced Light Source for similar work with X-ray diffraction). Figure 1 shows the arrangement, while figure 2 suggests an environmental TEM variant based on a side-entry holder. Cryoshields condense surplus ice. A mixture of water and proteins enters the vacuum through a submicron aperture at appropriate pressure to produce a monodispersed (single file) Rayleigh droplet stream, which freezes by evaporative cooling at 10^6 deg/second to form iceballs doped with proteins. Undoped iceball beams have been studied previously by electron diffraction [3]. Thermodynamic treatment will sublime surplus ice along a flight tube to leave a thin jacket of vitreous ice surrounding the molecules. Elliptically polarized laser light at one micron wavelength from a 100W fiber laser is used to align the molecules in the beam via the quadrupole induced dipole-moment interaction, as demonstrated in recent work on small molecule beams [4]. Diffraction data is acquired continuously, with many molecules located within the ten-micron diameter electron beam at any instant. The electron beam coherence width is chosen to be about equal to the molecular diameter, so that no interference occurs between different molecules (no speckle). After about 1 minute of acquisition, the data is read out, and the laser polarization is rotated to a new orientation. By acquiring diffraction date for many orientations, three-dimensional reciprocal space volume is filled. The phase problem will then be solved using iterative methods similar to those used recently to phase the diffraction pattern from a single nanotube [5], and for X-ray tomographic lensless imaging [6].

It is necessary for the molecular beam to pass through a gas-filled damping cell in order to damp oscillations of the molecules as they overshoot after entering the harmonic potential generated by the electric field of the laser light. The molecular motion in the damping cell is described by the theory of Brownian fluctuations for a mirror galvanometer. A passage through a 0.1mm long cell filled with nitrogen gas is sufficient, after which thermal fluctuations limit the resolution in the reconstructed charge density. For a protein modeled as an oblate spheroid of radii $a > b$ we find the resolution d [2]

$$d = \left(1 - \frac{b}{a}\right)\left(\frac{3\,a\,T}{3\mathbf{x}10^{8}\,I\,4\,\Box\,\Box}\right)^{1/2}$$

where a,b, and d are expressed in nm, I is the laser intensity in Watt/cm^2, T is the temperature in K and $\Box$ is an anisotropic shape factor. This indicates that a temperature of 20K is required for lysozyme at 0.7nm resolution, which is sufficient to see alpha helices (assuming a dielectric constant of 15 for hydrated material [7]). For TMV, which will be used initially, 1nm resolution should be possible at 320K. The liquid jet and diffraction camera are currently operating [8].

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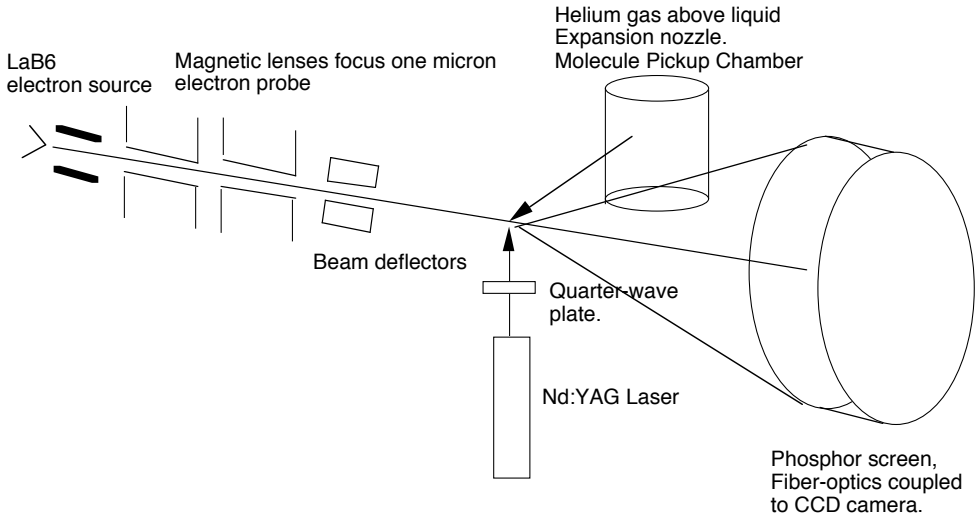

Figure 1. Orthogonal electron, laser and doped water-droplet beams for droplet diffraction.

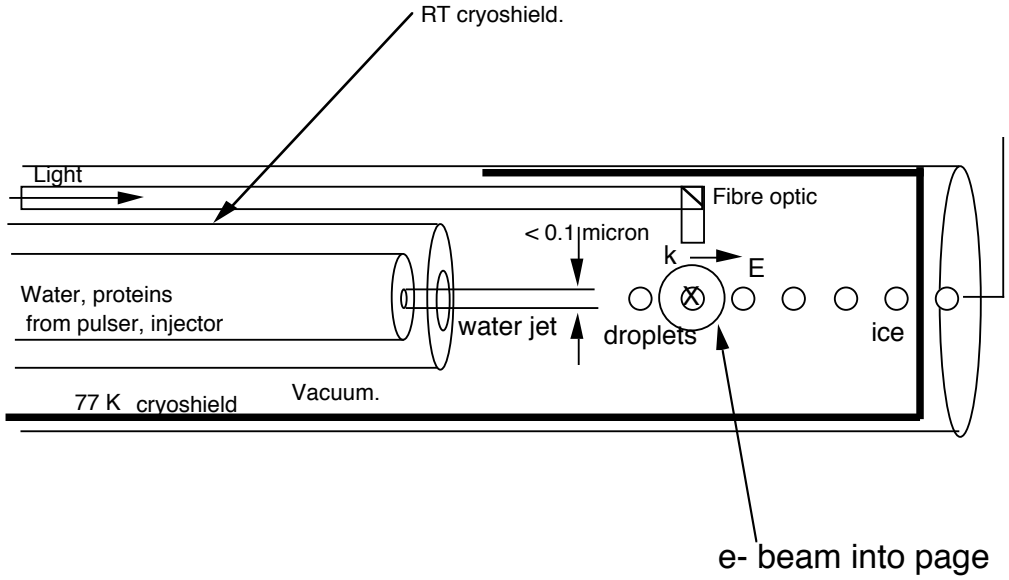

Figure 2. Suggested layout for environmental cell for droplet-beam diffraction in TEM.

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