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Liquid scanning transmission electron microscopy: Nanoscale imaging in micrometers-thick liquids

Microscopie électronique à balayage en transmission en phase liquide : Imager à l'échelle du nanomètre à travers des films liquides de plusieurs micromètres d'épaisseur

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ABSTRACT

Scanning transmission electron microscopy (STEM) of specimens in liquid is possible using a microfluidic chamber with thin silicon nitride windows. This paper includes an analytic equation of the resolution as a function of the sample thickness and the vertical position of an object in the liquid. The equipment for STEM of liquid specimen is briefly described. STEM provides nanometer resolution in micrometer-thick liquid layers with relevance for both biological research and materials science. Using this technique, we investigated tagged proteins in whole eukaryotic cells, and gold nanoparticles in liquid with time-lapse image series. Possibly future applications are discussed.

RÉSUMÉ

La microscopie électronique à balayage en transmission (STEM) d'échantillons immergés dans un liquide est possible en utilisant une chambre microfluidique réalisée avec de fines fenêtres en nitrure de silicium. Cet article introduit d'abord une équation analytique permettant d'estimer la résolution spatiale accessible en fonction de l'épaisseur totale de l'échantillon et de la position de l'objet d'intérêt en son sein. Après une description brève de l'équipement utilisable, nous montrons comment cette approche STEM permet d'observer avec une résolution nanométrique des objets d'intérêt en biologie ou en science des matériaux, plongés dans une couche liquide de plusieurs micromètres d'épaisseur. Avec cette technique, nous avons étudié la distribution de protéines marquées dans des cellules eucaryotes complètes et celle dynamique de nanoparticules d'or dans un liquide au moyen de séries d'images résolues en temps. Enfin, nous proposons quelques grands axes pour de futures applications.

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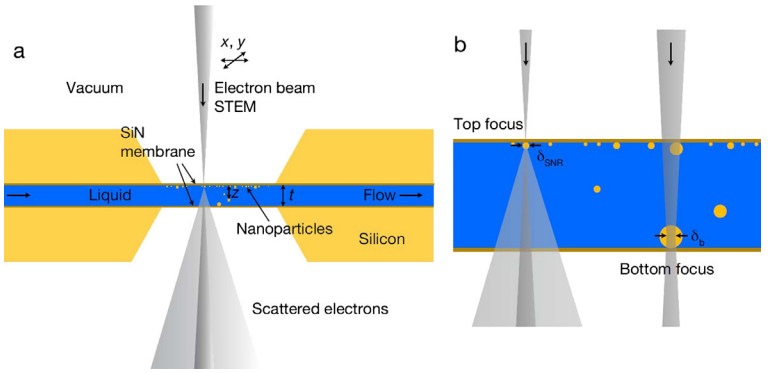

Fig. 1. (Color online.) Principle of scanning transmission electron microscopy (STEM) of specimens in liquid, termed Liquid STEM. (a) A microfluidic chamber is formed between two silicon nitride (SiN) membranes supported on silicon microchips. A sample, for example, containing nanoparticles is placed within a liquid layer of thickness t. The vertical distance from the beam entrance is referred to as z. Imaging is accomplished by scanning a focused electron beam over the sample. Elastically scattered and transmitted electrons are recorded with the annular dark field detector located beneath the sample. (b) Close-up of the impact of the focused electron beam with the sample for the case when focusing at the top layer of the liquid (left). The resolution δ_{SNR} is limited by noise and given by the diameter of the smallest visible object. The probe size of the focused electron beam may be smaller than δ_{SNR}. In the case when focusing at the bottom, scattering leads to a broadening of the electron beam (right). In the latter case, the resolution is limited by the size of the broadened electron probe δ_b.

1. Introduction

Electron microscopy traditionally studies solid samples in the vacuum chamber of the microscope. But for many research questions, it is desired to image specimens in a liquid environment, for example, for the study of colloidal assembly, catalyst particles, growth of nanomaterials, energy materials, and biological cells and macromolecules [1]. Already since the early days of electron microscopy, systems were discussed to study nanoparticles or biological cells in a wet environment [2,3]. Various systems for transmission electron microscopy (TEM) have been developed over the years [4,5], eventually leading to the achievement of atomic resolution in thin liquid layers [6–8]. A drawback of liquid-cell TEM remains that nanoscale resolution cannot be obtained for liquid thicknesses of more than several hundreds of nanometers on account of scattering of the electron beam in the liquid layer [1]. For several studies, it is required, however, to achieve high resolution in several micrometer thick liquid layers. Such thicknesses are needed, for example, for imaging eukaryotic cells, for imaging micrometer-sized objects relevant for energy storage and conversion, or to achieve a continuous liquid flow. One option to study more bulky samples in a liquid is to use environmental scanning electron microscopy (SEM) [9–11] or dedicated liquid specimen systems for SEM [12,13]. However, nanometer resolution can be reached only at or close to the surface or in very thin samples. A new approach was introduced combining scanning transmission electron microscopy (STEM) with the usage of silicon nitride (SiN) membranes as windows in a liquid compartment [14]. This so-called Liquid STEM approach can image nanoscale materials of high atomic number (Z) in low-Z liquids, resulting from the Z contrast of STEM [15–18]. For example, gold nanoparticles (AuNPs) can be imaged in a micrometer-thick liquid water layer within a microfluidic chamber [19,20], see Fig. 1. Additionally, liquid STEM can be used to study the distribution of a certain protein species in whole cells using AuNPs as specific labels [21], similar as proteins tagged with fluorescent labels are used for fluorescence microscopy [22], but then with a factor of 50-fold higher resolution. STEM of liquid specimens was also used in materials science, for example, to study the growth of Pb nanoparticles in solution with atomic resolution [23], to image the dynamic movement of AuNPs in liquid [24,25], and even electron energy loss spectroscopy in liquid layers has proven feasible [26,27]. In case STEM is used for thin liquid layers, lower Z materials are also visible with nanoscale resolution, for example macromolecular protein complexes [28]. In this paper we will provide the theoretical concepts behind Liquid STEM, describe the key components of the instruments, and discuss examples of its usage in both biology and materials science.

2. Theory

STEM produces (sub-)nanometer-resolution images by scanning a focused electron beam over a thin sample [15]. Electrons transmitted from the top through the sample and scattered outside the cone of the primary electron beam are detected with an annular dark field (ADF) detector [17]. The Z-contrast of STEM images is dominated by elastic scattering of the electrons at the core of the atoms in the specimen. The number of inelastic scattering events is actually higher for some samples, but they mainly involve small-angle scattering, and can often be neglected when calculating the signal in the ADF detector [17]. Yet, inelastic scattering needs to be taken into account mainly for beam broadening (discussed later in the text) and its influence on the specimen via radiation damage.

The number $N(t)$ of elastically scattered electrons in the ADF detector increases with the sample thickness t and decreases with the mean free path length $l(E, \beta, Z)$ of the electron in the sample [17,18]: