Ultramicroscopy 140 (2014) 1–8

Contents lists available at ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

Time-resolved measurement of the three-dimensional motion of gold nanocrystals in water using diffracted electron tracking

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ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form
27 December 2013
Accepted 27 January 2014
Available online 5 February 2014

Keywords:
DET
Single molecular dynamics
Three-dimensional motion
Environmental cell
SEM
EBSD

ABSTRACT

We introduce diffracted electron tracking (DET), which combines two electron microscopy techniques, electron backscatter diffraction and the use of an environmental cell in a scanning electron microscope to measure changes in nanocrystal-orientation. The accuracy of DET was verified by measuring the motion of a flat gold crystal caused by the rotation or tilting of the specimen stage. DET was applied to measure the motion of semi-fixed gold nanocrystals in various environments. In addition to large motions induced in water environment, DET could detect small differences in the three-dimensional (3D) motion amplitude between vacuum environment and an Ar gas environment. DET promises to be a useful method for measuring the motion of single nanocrystals in various environments. This measuring technique may be used in a wide range of scientific fields; for example, DET may be a prospective method to track the single molecule dynamics of molecules labeled with gold nanocrystals.

1. Introduction

Recently, biophysical research has benefited from numerous analytical tools designed to measure single molecule dynamics [1]. Among the most commonly used techniques is laser optical microscopy, which measures the motion of fluorescently labeled single molecules [1]. Motion can also be quantified using a high-speed atomic force microscope (AFM) [2] or using diffracted X-ray tracking (DXT) methods [3,4]. Previously, our research group had applied DXT to the dynamics of single-protein molecules [4,5]. In this method, the rotation of a single nanocrystal, which is linked to a specific site in the molecule, is tracked using a time-resolved Laue diffraction technique. However, because DXT requires a very strong white X-ray source, in a large institution, competition for the use of the machine by many researchers is fierce. To alleviate this problem, we have developed a compact instrument that uses electron beams in place of X-rays. The nanocrystal-orientation is measured using electron backscatter diffraction (EBSD) instead of X-ray diffraction spots. We refer to this measuring technique as diffracted electron tracking (DET). DET can be performed with a commercially available scanning electron microscope (SEM) in a small laboratory setting and provides instantaneous experimental feedback on the measurement results. In DXT, the Laue diffraction from a gold nanocrystal appears as bright spots on a fluorescent screen. Conversely, in DET, the electron diffraction produces a widespread band pattern, termed an electron backscatter diffraction pattern (EBSP), which contains 3D information regarding the specimen movement. A further advantage of DET is that it allows *in situ* observation of a specimen. Because nanocrystals can be observed and identified directly using the secondary electron (SE) imaging mode of an SEM, an EBSP can be obtained from targeted gold nanocrystals of identical sizes (approximately 40–60 nm), excluding larger or aggregated gold nanocrystals.

In this study, the accuracy of DET was verified by tracking the motion of a flat gold crystal caused by the rotation and tilting of the SEM specimen stage. The motion of gold nanocrystals in various circumstances—in vacuum, in Ar gas and in water—was

Abbreviations: DET, Diffracted electron tracking; 3D, Three-dimensional; AFM, Atomic force microscope; DXT, Diffracted X-ray tracking; EC, Environmental cell; SEM, Scanning electron microscope; EBSD, Electron backscatter diffraction; EBSP, Electron backscatter diffraction pattern; SE, Secondary electron; TAC, Triacetyl cellulose; MOPS, 3-Morpholinopropanesulfonic acid; CHAPS, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate; CI, Confidence index; MSD, Mean-square displacement

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http://dx.doi.org/10.1016/j.ultramic.2014.01.012
0304-3991

measured using DET. DET is a promising method for single molecule observations.

2. Materials and methods

2.1. Flat gold crystal and verifying the performance of DET

A flat gold crystal was grown on a silica glass surface using a vacuum evaporation method [6]; only the evaporation time differed from the procedure used to grow the gold nanocrystals. The flat gold crystal was attached to a general specimen stub with conductive double-sided tape (Nissin EM Ltd.) and silver conductive paste (Silvest P-255; Tokuriki Chemical Research Ltd.). The SEM (JSM-7001F; JEOL Ltd.) specimen stage was rotated from 0° to 3° in the clockwise direction or tilted from 70° to 67° relative to the home angle in 0.2° steps, and the EBSP signal was collected at each step at the same crystallographic face. To detect the EBSP signal, we used a 30 kV accelerating voltage, an 87 pA beam current, and a 17 mm working distance. The lattice orientations were calculated in the same manner as for the gold nanocrystals, which is described in Section 2.4. Each measurement was performed for three different crystal faces.

2.2. Carbon sealing-film for the environmental cell (EC)

The sealing-film for an EC must be sufficiently thin to allow electron transmission and sufficiently tough to withstand the pressure differences in excess of atmospheric pressure. The development of the 20 nm thick carbon sealing-film used in this study has been previously described [7]. Briefly, a 1% (w/v) triacetyl cellulose (TAC) (Okenshoji Ltd.) solution was prepared by dissolving TAC in a mixed solvent that contained 90% (v/v) 1,2-dichroloethane and 10% (v/v) methanol. To fabricate the TAC thin film that was used as a support for the carbon film, a glass slide was soaked in the TAC solution and extracted at 2.5 mm/s. A 20 nm thick carbon film was deposited onto the TAC film on the glass slide using the vacuum evaporation method. The thickness of the carbon film was chosen as a compromise between electron permeability and the ability to withstand pressure. The carbon/TAC combination film was cut into 2×2 mm square pieces on the glass slide. The combination-film pieces were drawn from the glass slide onto the surface of a water bath by surface tension. Each piece of combination film was scooped up with a copper ring (diameter = 5 mm), dipped in water and placed on a special three-slit grid made of phosphor bronze (Fig. 3c), set on a piece of wet filter paper, which is described in detail in Section 3.3. After drying, the TAC film was dissolved at the slits of the grids by soaking the grids in acetone. The ability of the carbon sealing-film covering the slits to withstand pressure was evaluated using a standard pressure test. Once it was confirmed that the carbon film on each grid could withstand up to 1.3 times atmospheric pressure, an EC was sealed with the grid.

2.3. Specimen preparation for DET

Gold nanocrystals were grown on a cleaved NaCl (100) surface using the vacuum evaporation method [6]. The sealing-film on the three-slit grid (Fig. 3c) was processed with a mercapto-silane coupling reagent (KBM-803; Shin-Etsu Chemical Ltd.) using the vapor deposition method [8]. Briefly, 1 ml of mercapto-silane was evaporated to vapor pressure in a well-sealed container. The grids were left standing for 1 h in the mercapto-silane vapor. After incubation, the grids were heated in air at 95 °C for 5 min, after which they were stored under vacuum until they were used. To ensure that thiol group of the mercapto-silane would react with the gold nanocrystals, the grids were made hydrophilic by exposure to glow discharge (HDT-400; JEOL Ltd.). To prevent the gold nanocrystals from aggregating on the sealing-film, the side of the gold nanocrystals that had been in contact with the NaCl crystals was exposed to a thin buffer solution (50 mM MOPS–NaOH (pH 8.0), 50 mM CHAPS) layered onto the sealing-film for 10 min. After incubation, the remaining NaCl crystal was removed by soaking in distilled water. The EC that contained a water layer was assembled as follows. The three-slit grid with carbon sealing-film and gold nanocrystals was enclosed with 0.2 μl of degassed distilled water as shown in Fig. 3a and b. The EC that contained Ar gas was assembled in an Ar-gas-filled glove box with no water present. The vacuum EC was prepared by removing the O-ring that was used to seal the EC. Except for the vacuum EC, the sealing of the ECs was checked by observing the SEM images of the ECs (Fig. 4a).

2.4. Tracking the 3D motion of gold nanocrystals using DET

To detect the distribution of the gold nanocrystals, an SEM image was first observed with an SE detector by scanning the minimum necessary electron beam over the specimen. Each individual gold nanocrystal of suitable size (approximately 40–60 nm in diameter) was chosen from the SEM image, which was obtained at magnification of 50,000 (Fig. 4c). To detect the EBSP signal from a targeted gold nanocrystal, the electron beam was fixed at an edge point on the crystal for 2 s. The irradiation conditions were as follows: a 30 kV accelerating voltage, an 87 pA beam current and a 17 mm working distance at an angle of 70° relative to the normal direction of the specimen stage. The EBSD was projected on the phosphor-screen detector, and the produced EBSP was recorded with a CCD camera after being intensified with an image intensifier (V8070U-74; Hamamatsu Photonics Ltd.), which was operating at a shutter speed of 60 ms. Thus, 26 frames of time-resolved sequential EBSP images were obtained from each gold nanocrystal. The orientations of the gold nanocrystals were calculated from the EBSPs using OIM™ Data Collection version 5.31 and OIM™ Analysis version 5 software (EDAX; AMETEK, Inc.). Both software packages were used as directed by their instruction manuals and nearly automatically determined the orientations of the lattices of the gold nanocrystals. To eliminate the pseudo-orientations that appeared in the results because of low signal quality, we set the criterion that the confidence index (CI) value [9] must be greater than 0.2 (average of sequential 26 data). Moreover, the results that contained incorrect orientations were estimated by averaging the values of adjacent steps. To analyze the motion of the gold nanocrystals, the time-resolved lattice orientations in terms of Euler angles [9] were calculated using OIM™ Analysis.

The Euler angles indicate the rotational relationship between the specimen coordinate system C_s (X_s, Y_s, Z_s) (in which the specimen is spatially fixed) and the crystal coordinate system C_c (X_c, Y_c, Z_c) (in which the gold nanocrystal is fixed).

The Euler angles (ϕ_1, Φ, ϕ_2) are defined as follows. The C_s system can be made coincident with the C_c system by a three-phase rotational operation. First, C_s is rotated by ϕ_1 around the Z_s axis, which corresponds to the normal direction of the specimen stage, so that the X_s axis lies on the (X_c, Y_c) plane. Then, the resultant C_s is rotated by Φ around the new X_s axis to make the Z_s axis coincident with the Z_c axis, and finally, the resultant C_s is rotated by ϕ_2 around the new Z_s axis, namely, the Z_c axis, to make the C_s axis coincident with the C_c axis. Thus, in terms of the Euler angles, the orientation matrix g of the crystal can be given as follows:

EBSD is commonly used for the analysis of the crystal-orientation mapping of a polycrystalline material by electron beam scanning on the material surface. However, we used the EBSD approach to investigate the motion of the nanocrystals, not via spatial scanning, but via time-sequential beam irradiation on a fixed point of each nanocrystal. In this way, we were able to obtain the changes in the crystal-orientation, namely, the Euler angles, from the EBSP and the