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Nano-dot markers for electron tomography formed by electron beam-induced deposition: Nanoparticle agglomerates application

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ABSTRACT

A method allowing fabrication of nano-dot markers for electron tomography was developed using an electron beam-induced deposition in an ordinary dual beam instrument (FIB and SEM) or an SEM. The electron beam deposited nano-dot markers are suitable for automatic alignment of tomographic series. The accuracy of the alignment was evaluated and the method was demonstrated on agglomerated nanoparticle samples using a rod-shaped sample with no missing wedge effect. Simulations were used to assess the effect of marker size on alignment accuracy.

1. Introduction

Electron tomography is a method employed in a transmission electron microscope (TEM) to reconstruct a three-dimensional (3D) volume from a series of images acquired at suitable tilt increments [1]. An accurate, preferably fully automated alignment of the individual images in the series is critical to obtain good quality 3D reconstruction of the sample. Fiducial markers, usually gold nanoparticles placed from a suspension at random positions within a sample, are often used for tomography of biological samples. For precise alignment, markers must be dispersed suitably over the region of the specimen that is to be observed while not interfering with the observed objects. However, it is difficult to obtain even dispersion because the colloidal gold nano-particles are usually dense materials [2,3]. If dense colloidal gold nano-particles are close to or within the area of interest of the specimen, they introduce artifacts into the reconstructed 3D images. In the case that no gold nano-particles actually exist, precise alignment cannot be carried out.

An alternative method for alignment uses landmarks in the sample itself for cross correlation allowing to obtain lateral shift of images, but the results can be operator dependent. The presented e-beam fabricated fiducial marker method allows to place the markers at desired locations near the region of interest. The method makes it possible to obtain, in addition to lateral shift, image rotation and tilt and azimuth angles. The applications shown below make use of a rod-shaped specimens prepared by focused ion beam (FIB) instrument allow obtain tomograms without missing-wedge [4]. The rod-shaped specimens are typically used in materials science where high resolution, resulting in small field of view, is usually required making it difficult to disperse colloidal gold nano-particles near the observing area while not interfering with the objects of interest. Very accurate alignment is desirable for such samples. The e-beam fabricated marker method can be applied to radiation sensitive, polymer and biological samples, since the area of interest is not irradiated during the fiducial marker fabrication step.

In a previous study, we used a helium-ion microscope (HIM) equipped with a tungsten carbonyl ($W(CO)_6$) gas injection system (GIS) to form tungsten nanodots [5,6]. Moreover, we demonstrated for the first time, the use of nano-dot markers with ~10 nm size on a ~100 nm-diameter rod-shaped specimen for aligning a TEM tomographic tilt series. To make the method accessible to a broad research community the previously demonstrated nano-dot marker fabrication in a HIM must be transferred to instruments that are more wide spread than HIM. In this paper we report

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fabrication of electron beam-fabricated tungsten nano-dots for automated alignment of electron tomographic data. We refer to the dots as “nano-dot markers”.

The formation of electron beam induced deposition of tungsten nano-dot markers was achieved using a standard scanning electron microscope (SEM) equipped with a tungsten carbonyl ($W(CO)_6$) gas injection system (GIS). We first describe the experimental set up, then discuss the fabrication of the nano-dot markers and demonstrate the application of the method to a sample of agglomerated TiO_2 nanoparticles and a sample of a regular array of silver nanoparticles with sub-5 nm diameter. Both the annular dark field scanning TEM (ADF STEM) and the bright field TEM (BFTEM) operation modes for tilt series acquisition were tested for suitability of automated nano-dot markers detection and alignment. Moreover, we investigate the effect of markers' shape on alignment accuracy using simulated tilt series with varied marker sizes.

2. Instrumentation

A Hitachi NB 5000 dual beam (FIB and (SEM)) instrument was used for sample preparation and for electron beam-induced deposition of nano-dot markers. For fabrication of the rod-shaped specimen we used 40 keV Ga ion beam. For deposition of the nano-dot markers we used 5 keV electron beam. The angle between SEM column and FIB column is 58°. Hitachi HF 3300, a 300 kV transmission electron microscope (TEM)/scanning TEM (STEM) equipped with a cold field emission electron gun (CFEG) was used for collecting tomographic tilt series. The data collection was assisted by the use of a Matlab™-based computer control system controlling the microscope and associated hardware [7]. The same micropillar holder was used for sample preparation in the NB 5000 dual beam instrument and HF 3300 TEM/STEM for data acquisition without the need for remounting a sample [8].

3. Fabrication and properties of e-beam deposited nano-dot markers

3.1. Experimental conditions

Initially, the electron beam-induced deposition of tungsten from $W(CO)_6$ was investigated using about 20 nm thick amorphous carbon substrate. The substrate was prepared by electron-beam evaporation of carbon onto a mica substrate. The carbon film was then floated on deionized water surface and picked onto a standard 200-mesh copper grid.

For high reproducibility of the fabrication, $W(CO)_6$ gas pressure and exposure time of electron beam was controlled. The timing diagram of the nano dot marker deposition is shown in Fig. 1. First, a $W(CO)_6$ precursor gas source was opened for a 10 s period (Fig. 1a) and the fabrication of the first marker in each row commenced within delay time of 0.5 s after the $W(CO)_6$ precursor gas supply was closed. While the gas source was opened the electron beam gun valve was closed in order to protect electron beam gun (Fig. 1b), it was opened after closing the gas source. Then, 10 markers were deposited subsequently (Fig. 1c). The $W(CO)_6$ precursor partial pressure decreased as schematically shown in Fig. 1d. Bright field STEM images acquired in the NB 5000 of the nano dots deposited with zero delay time between depositions at individual positions are shown in Fig. 2a (the first to last nano dots are shown from left to right). Each row was fabricated with the same dwell time per nano dot (5 s, 3 s, 2 s, 10 s and 20 s) indicated near the first marker of each row and shown in Fig. 2a. The measured electron beam current was about 0.5 nA. The dwell time per individual nano dot was between 2 s and 20 s corresponding

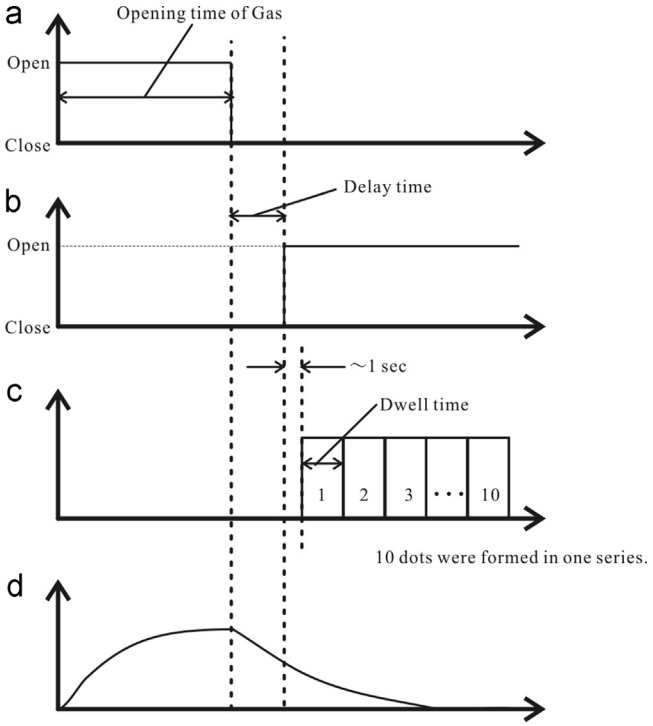

Fig. 1. Timing diagram of procedure for deposition of nano-dot markers. The *x*-axis is time from the initial opening of the gas precursor source while the *y* axis represents the status of various experimental variables. (a) Delivery of the $W(CO)_6$ gas precursor source. (b) Opening of the SEM column gun valve. (c) Exposure of the sample by the incident electron beam leading to deposition of nano dots. (d) $W(CO)_6$ gas precursor partial pressure in the chamber.

to exposure dose between 1 nC and 10 nC per nano dot. Similar experiments were performed to explore the effect of the precursor delivery time where the dwell time was kept constant and the opening time of the gas source was changed from 10 s to 30 s. The thickness of the uniform carbon film used in this experiment was estimated to be about 20 nm.

The image in Fig. 2b acquired at 7° tilt, i.e. 51° relative to the SEM column of the NB 5000, shows that WC_X was deposited on both top and bottom surfaces of the amorphous carbon film. This is of importance when electron beam deposited markers are used on samples dispersed on an amorphous carbon support. Due to beam broadening in the amorphous carbon, the WC_X deposit was broader on bottom (exit) surface of the carbon film. The results are further discussed in Section 3.2.

3.2. Results and discussion

It is known that the electron beam-induced deposition is capable of fabricating extremely small dots. Sizes down to 2 nm were demonstrated [9–11] when using organometallic precursors and 200 kV ultra high vacuum TEM. Similar results were demonstrated using $W(CO)_6$ precursor [12]. In our experiments we opted for the $W(CO)_6$ precursor leading to deposition of mixture of tungsten and carbon, WC_X [12]. The nano dot deposition is thought to be due to secondary electron induced decomposition of the precursor landing on the surface [12]. The smallest achievable size of the dots is thought to be limited by the secondary electrons emitted from the side of the growing nano dots leading to deposition of material on the side walls of the growing nano dots [13,14]. The size of the nano dots is also expected to be dependent on the amount of the precursor available for deposition and on the current and time of the electron beam exposure of the individual nano dots [8]. The partial pressure of the precursor can be