

New Approach of Using Cryo-Soft X-ray Tomography for beam-sensitive materials

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Abstract

Cryo-soft X-ray tomography (cryo-SXT) is emerging as a promising characterisation technique for imaging nanomaterials in the solid state and in material science. This capability is demonstrated through high-resolution 2D and 3D imaging of nanomaterials, enabling analysis of particle size distributions, nanoscale topography, volumetric distribution, heterogeneity, and clustering behaviour, while providing non-destructive characterisation of newly synthesised structures. A novel, rapid synthesis method producing magnetite ($\text{Fe}_{2.8}\text{O}_4$) in water, in the tetragonal inverse-spinel $\text{Fe}_{2.8}\text{O}_4$ lattice, was achieved using KOH/ethanol at 80°C. Zerovalent iron $\text{Fe}(0)$ nanoparticles were synthesised using Dodecylamine (DDA) in a microwave reactor within 30 s. The face-centred cubic (FCC) crystal structure and phase purity were verified by powder X-ray diffraction (PXRD). The unit-cell parameter and crystal domain size were studied using Pawley refinements. $\text{Fe}(0)$ and $\text{Fe}_{2.8}\text{O}_4$ are beam-sensitive materials, and imaging using cryo-SXT was compared with conventional methods. Additionally, the size and shape of $\text{Fe}_{2.8}\text{O}_4$ and $\text{Fe}(0)$ nanoparticles were determined by scanning electron microscopy (SEM), high-resolution scanning transmission electron microscopy (HR-STEM), and simulation of cryo-SXT images using Fiji/ImageJ. Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), and cryo-SXT indicated the presence of a trace of DDA on the surface of the $\text{Fe}(0)$ nanoparticles. The results show that cryo-SXT has the potential to be used as a non-destructive characterisation method for a diverse range of beam-sensitive nanomaterials, overcoming previous limitations.

Introduction

Nanotechnology has emerged as a multidisciplinary field enabling the development of catalysts, energy storage systems, medical diagnostic uses, and technologies for environmental sustainability.[1] The use of iron-based nanostructures, including $\text{Fe}(0)$ and $\text{Fe}_{2.8}\text{O}_4$, has attracted significant interest due to their wide applications in agriculture and the medical sciences.[2] However, traditional synthesis methods in solid-state chemistry for metallic nanostructures need an excessive reaction time and consume high energy.[3, 4] In contrast to conventional hydrothermal/solvothermal processes, various rapid synthesis techniques (e.g. microwave and ultrasonic irradiation) have been developed in recent decades to overcome these limitations.[5–7] Unconventional methods can significantly reduce the reaction time by providing uniform heat through high-energy inputs that accelerate nucleation and crystal growth.[8, 9] Dissolved metallic ions can absorb and conduct microwave and ultrasonic radiation to form hot spots that reduce reaction time to a few minutes.[10] However, these techniques face challenges with scale-up due to the limited penetration depth in the solution. [8, 9] Sol–gel offers fine compositional control for iron-based nanostructures at relatively low processing temperatures; however, achieving crystallinity typically needs thermal treatment.[11]

A key requirement for progress in nanotechnology is the precise analysis and understanding of nanomaterials' properties, which rely on high-resolution techniques such as TEM and SEM.[12] However, some nanomaterials are beam-sensitive and can undergo structural and chemical changes during acquisition, affecting the reliability of measurements and data interpretation.[13] Beam-induced damage

effects, including radiolysis, bond-breaking, oxidation, atomic displacement, and phase transitions, pose significant challenges for the accurate analysis of materials, including perovskites, metal-organic frameworks (MOFs), and transition metal oxides (e.g., γ - Al_2O_3 and Fe_3O_4). [14–19] This leads to defects, structural damage and collapse, oxidation, and phase transitions, mainly occurring in oxygen-containing materials under electron-beam exposure. [20–22] High-energy electrons can displace lattice atoms and accelerate material degradation, primarily leading to structural collapse over prolonged exposure. [23, 24]

Cryogenic electron microscopy (cryo-EM) is an effective technique that can cool down the samples to cryogenic temperatures to mitigate radiolytic effects during beam exposure. [25, 26] For example, it maintains cryogenic conditions to protect samples, such as iron hydroxides, during extended analysis and beam exposure.[27] Meanwhile, cryo-FIB sample preparation helps prevent further oxidation and phase transitions when preparing specimens for TEM.[28] In situ environmental TEM (ETEM) allows for observing materials in real time under reactive conditions, which can help study phase transitions in oxygen-containing nanomaterials.[29]

SEM provides nanoscale morphology and composition via secondary/backscattered electrons using energy-dispersive spectroscopy (EDS).[30] However, many functional materials are dose-limited because low-keV electrons (1–30 kV) can trigger radiolysis, induced-field damage, heating, and charging, degrading the structure during imaging.[30, 31] For these beam-sensitive chemical environments, SEM can only be justified with strict mitigation, short illumination times, low total dose, and careful optimisation of acceleration voltage to improve analysis and interpretation reliability.[30]

The use of techniques such as 4D-STEM ptychography and exit-wave reconstruction (EWR) has enabled atomic-scale imaging with minimal electron dose.[17] These approaches have benefited metal oxides and perovskites, where capturing fine structural details is critical to understanding phase behaviour and electronic properties.[17] Despite further adjustments to minimise the damage, these techniques still face barriers in achieving consistent and defect-free characterisation for solid-state and nanomaterials. [21] A method for surface analysis is atomic force microscopy (AFM), a technique for assessing the topography, size, and shape of nanosheets; however, it struggles with nanoparticles smaller than 10 nm. [32, 33] Its finite tip size, typically around 5–8 nm, leads to convolution effects that overestimate nanoparticle dimensions.[34, 35]

Cryogenic soft X-ray tomography (cryo-SXT) offers an alternative approach for non-destructive three-dimensional imaging under cryogenic conditions. Its application to metallic and solid-state nanoparticles remains comparatively underexplored, particularly for materials susceptible to electron-beam-induced modification.

Herein, we firstly report an unprecedented rapid synthesis method for producing $\text{Fe}_{2.8}\text{O}_4$ nanoparticles at 80°C and using a microwave reactor to produce Fe(0) in 30 s at 110°C and subsequent beam-sensitive analysis. The FT-IR and TGA were used to identify the surface of the DDA adsorption on Fe(0)

nanoparticles. The neodymium magnet indicated magnetic behaviour of the synthesised Fe(0) nanostructures. The characterisation techniques in cryo-SXT included imaging nanomaterials, surface analysis, nanoparticles' size distribution, and 3D imaging by the Z-axis. The more detailed introduction to cryo-SXT on beamline B24 at Diamond Light Source (Oxfordshire), 3D resolution, and Fiji/ImageJ simulation and analysis of 2D/3D images, as shown in Fig. 1a-c. Cryo-SXT is compared with SEM and high-resolution HR-STEM to assess their differences and define the potential of cryo-SXT for analysing beam-sensitive nanomaterials. To the best of our knowledge, this is the first use of Beam 24 as an imaging method for the characterisation of metallic nanoparticles in solid-state and material sciences.

Experimental

Materials

Triiron dodecacarbonyl ($[\text{Fe}_3(\text{CO})_{12}]$, (Merck), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Sigma Aldrich), KOH (Fisher Scientific), Dodecylamine (DDA), (Sigma-Aldrich), ethanol (Sigma Aldrich), anhydrous acetone (CAS No. 67-64-1; $\geq 99.98\%$, max. 0.02% H_2O), and anhydrous acetone (CAS No. 67-64-1; $\geq 99.8\%$, max. 0.02% H_2O), were used as received without further purification. Deionized water (18 $\text{M}\Omega \cdot \text{cm}$) was obtained from a Simplicity UV ultrapure water system (Millipore).

Instruments and methods

PXRD was performed on an Anton Paar diffractometer using Co K α radiation ($\lambda = 1.7889 \text{ \AA}$). PXRD patterns were collected over a range of 5–100 $2\theta/^\circ$, with the powdered material pressed into a flat silicon holder. The analysis was carried out using an N_2 chamber at ambient temperature for the air-sensitive samples. The Fe(0) sample was loaded in the PXRD N_2 chamber in a glove box to minimise air interaction. Profile fitting of the PXRD patterns was carried out using GSAS-II (version 5806, Python 3.13.3 64-bit) to analyse the unit-cell parameters and crystal domain size in Fe(0) and $\text{Fe}_{2.8}\text{O}_4$. [36] The ICSD numbers for the simulated patterns of Fe(0) and $\text{Fe}_{2.8}\text{O}_4$ are 42967 and 35002, respectively.

SEM was performed on a Zeiss Gemini operating at 5 kV, providing a resolution of 1 nm at 1 kV and 0.6 nm at 3 kV. A silicon-conductive wafer chip from Agar Scientific Ltd was used to reduce surface charge on Fe(0) and $\text{Fe}_{2.8}\text{O}_4$. The Fe(0) nanoparticles were estimated to have been exposed to air for 1 minute during loading into the SEM instrument.

High-resolution scanning transmission electron microscopy (HR-STEM) measurements were carried out using a double aberration-corrected JEOL ARM200f TEM, operated at 200 kV. The Emitech Evaporator and graphite were used for carbon coating of Fe(0) and $\text{Fe}_{2.8}\text{O}_4$ nanomaterials. The size of the particles was determined based on the measurements of at least 100 particles. The sample was sonicated in anhydrous acetone for 5 min in an ultra-sound bath (500 W) to disperse the metallic nanoparticles, followed by immediate SEM and HR-STEM analysis, with an estimated air exposure time of 1 minute.

Cryo-soft X-ray tomography (cryo-SXT)

Samples were transferred to Diamond Light Source, beamline B24, under dry N₂ and mounted on the cryo-stage at – 170°C. Alignment used the visible-light cryo view (VLM) and X-ray overview mosaics in transmission mode. Tilt series were acquired with a plane-grating monochromator at 520 eV (water window; cff = 1.60) and at Fe-edge settings of 700 eV (pre-edge) and 710 eV (Fe L₃ -edge) for element-specific contrast. A zone-plate objective with a 40 nm outermost zone was used. The effective pixel size was 14.44 nm with a field of view of 14.2 µm × 14.1 µm. Tomography spanned – 70° to + 70° in 2° steps with 1 s exposures (1 frame per projection; detector binning = 1; 71 projections per tilt series). For coarse motions, the stage step sizes were set to 100 µm (X/Y), 10 µm (Z), and 5° (θ). Flat-field (reference) normalisation was applied before reconstruction. At 520 eV, the corresponding PGM geometry was grating pitch 85.4668°, mirror pitch 86.3177°, with translations of – 12.001 mm (grating) and – 34.000 mm (mirror). Fiji/ImageJ version v1.54t was used to analyse the 3D images as well as the particle size distributions.[37]

Synthesis methods

Fe(0) nanoparticles

The Fe(0) production used a microwave reactor, following this method.[38] The sample was recovered and washed three times with anhydrous acetone (15 mL) by centrifugation (6000 rpm for 3 min each time). The recovered sample was dried in the same plastic centrifuge tube under a gentle flow of N₂ at 19°C for 10 min. The final yield can not be accurately estimated because a part of the DDA is coated on the Fe(0) nanoparticles. In addition, this coating layer makes the Fe(0) nanoparticles non-flammable.[19]

Fe_{2.8}O₄ nanoparticles

FeCl₂·4H₂O (150 mg, 0.75 mmol) was dissolved in 12 mL of water in a 50 mL beaker, and 8 mL of ethanol was added to the solution, which was then heated to 80 °C. When the solution was at 80 °C, 8 mL of KOH (10 M) was added to the boiling solution, and the black Fe_{2.8}O₄ nanoparticles immediately appeared. Heating was stopped, and the sample was removed from the hotplate and left to cool to ambient temperature (19°C) for 5 minutes. The sample was washed three times with DI water and twice with acetone, and dried at 80 °C in a drying oven (in air). This sample should not be washed with ethanol as Fe_{2.8}O₄ reduces to Fe(0) during the drying process in the oven. The yield was 57.7 mg (99.12%).

Sample preparation for SEM, HR-STEM, and cryo-SXT

The sample preparation for SEM, HR-STEM, and cryo-SXT is similar, and 0.1 mg of Fe(0) and Fe_{2.8}O₄ were dispersed in 15 mL of anhydrous acetone (99.98%) for 5 min using a 500W ultra-sound bath. A few drops of liquid were transferred onto HR-STEM grids (S162AF1) for HR-STEM and cryo-SXT, as well as

onto the SEM's silicon wafer at 20 °C. For Fe(0), sample handling was performed in an inert atmosphere (a glove box under vacuum) to minimise oxidation; however, the Fe_{2.8}O₄ nanoparticles were prepared and mounted under ambient conditions.

Results and discussion

This study repeats that DDA can rapidly form Fe(0) nanomaterials from the dissolution of [Fe₃(CO)₁₂] in DMF and the use of a microwave reactor in 30s. According to previous reports, NaBH₄ is a common (and toxic) reducing agent in the Fe(0) production process at 25–30°C requiring a few minutes.[39, 40] Ultrasound-assisted synthesis is an unconventional method, conducted at 25–60°C for 15–20 minutes using NaBH₄ and NH₄OH as reductants. [41]

In this method, Fe_{2.8}O₄ is obtained only when FeCl₂·4H₂O is used as the precursor, and other compounds, such as Fe(NO₃)₃·9H₂O, can not produce Fe_{2.8}O₄. Regarding this method, when the KOH (10 M) was added to a mixture of ethanol and water solution at 80 °C, magnetic Fe_{2.8}O₄ was produced immediately. A study shows Fe_{2.8}O₄ synthesised in a continuous-flow reactor pressurised to 250 bar, using demineralised water preheated to 370°C, with reactions conducted between 340 and 440°C for about 30s.[42] Some classical co-precipitation methods yield phase-pure Fe_{2.8}O₄ only, synthesised when ferrous and ferric chlorides are mixed in strongly alkaline ethanolic water at 80 °C; ferric nitrate under identical conditions produces no spinel phase. [42] Conventional hydrothermal or solvothermal methods typically heat iron salts to 180–220°C for 6–24 h.[43, 44] The microwave reactor processes can reduce the reaction time to a few minutes; however, specialised heating is used at 100–200°C.[45] In contrast, this method produces Fe_{2.8}O₄ immediately at 80°C, providing a low-energy synthesis method compared to conventional benchmarks.

PXRD

As shown in Fig. 2a, the sharpness of the Bragg peaks in Fe(0) nanoparticles confirms the crystallinity and phase purity with an FCC structure and $Fm\bar{3}m$ space group with γ -phase. The Pawley refinement shows that the crystallite domain size and unit-cell parameter are 42.6 ($\pm$ 0.3) nm and $a = 3.5317$ ($\pm$ 0.1) Å, respectively, ($V = 44.054$ ($\pm$ 0.006) Å³). According to a previous study, the unit-cell parameter in the FCC structure of Fe(0) ranges from 3.447 to 3.615 Å.[46] The volume of the unit cell in Fe(0) shows that the crystal structure is FCC because in BCC it is around 25 Å³. In addition to the FCC structure in Fe(0), the γ -phase is found in austenite and forms above 920°C; it is not stable at lower temperatures.[47] Based on previous studies, the use of an amine-based stabiliser (DDA) can form the stable FCC structure and the γ -phase in Fe(0) at lower temperatures. [38, 48, 49]

The PXRD pattern of Fe_{2.8}O₄ in Fig. 2b confirms crystallinity and phase purity, as indicated by the broadness of the Bragg peaks. The crystal structure in Fe_{2.8}O₄ is a tetragonal inverse-spinel with the

$P4_32_12$ space group verified by the Pawley refinement. The unit cell parameters and volume are $a = b = 8.2847 (\pm 0.0029) \text{ \AA}$, $c = 7.8745 (\pm 0.0021) \text{ \AA}$, $V = 540.490 (\pm 0.497) \text{ \AA}^3$. The crystallite domain size is $11.12 (\pm 0.6) \text{ nm}$, as validated by the broadness of the Bragg peaks, attributed to rapid nucleation and crystal growth. The study by Andersen *et al.*, confirms that the intermediate stoichiometry between $\gamma\text{-Fe}_2\text{O}_3$ and Fe_3O_4 is confirmed by analysis of the Fe/O stoichiometry in Mössbauer spectrum analysis. [42] Notably, $\text{Fe}_3\text{O}_4/\gamma\text{-Fe}_2\text{O}_3$ share the same spinel-type crystal structure, and no evidence of the core-shell or heterogeneous phase being present in a non-stoichiometric Fe_xO_4 . [42] The Pawley refinement of our synthesis in $\text{Fe}_{2.8}\text{O}_4$ shows that the PXRD patterns are fitted well to the structure reported by Andersen *et al.* [42], see Fig. 2b.

Figure 2a,b: Pawley-refined PXRD patterns for the nanoparticles showing experimental data = black points, fitted pattern = red lines, and Bragg peak positions = blue, a) $\text{Fe}(0)$ produced by DDA at 19°C (FCC structure with $Fm\bar{3}m$ space group) with $42.6 (\pm 0.3) \text{ nm}$ crystal domain size, b) $\text{Fe}_{2.8}\text{O}_4$ produced by KOH at 80°C (tetragonally distorted inverse-spinel, space group $P4_32_12$) with $11.12 (\pm 0.6) \text{ nm}$ crystalline size.

Surface coating detection

a) FT-IR/TGA

The FT-IR spectrum of the $\text{Fe}(0)$ nanoparticles shows the characteristic vibrational modes of the functional groups in DDA (e.g., N-H, CH_2), as well as adsorbed oxygen and moisture on the surface, as shown in Fig. 3a. The stretching vibrational bands in the fingerprint region are observed at 670.98 and 824.54 cm^{-1} , assigned to Fe-O stretching vibrations and Fe-O-H bending and deformation modes, respectively. Previous reports indicate that the Fe-O stretching vibration appears at $606\text{--}610 \text{ cm}^{-1}$, while a characteristic band near 896 cm^{-1} is attributed to Fe-O-H bending modes. [50, 51] A bending vibration mode appears at 1042.97 cm^{-1} corresponding to C-N or N-H deformation modes associated with the surface-adsorbed DDA. The 1318.06 and 1385.40 cm^{-1} are attributed to CH_2 bending and C-N stretching motions of the aliphatic backbone of DDA. The band at 1614.50 cm^{-1} is indicative of N-H (from primary amine) bending modes in DDA, as previously reported; the -NH_2 bending mode is observed between 1629 and 1643 cm^{-1} . [38, 52–54] A broad absorption band between 3000 and 3500 cm^{-1} corresponds to the OH stretching mode (O-H) due to the surface adsorbed water.

As shown in Fig. 3b, there is a 67% weight loss between 0 and 2500°C , indicating the surface adsorption of DDA by $\text{Fe}(0)$ nanoparticles, which follows the FT-IR results. Therefore, the results confirm that DDA remains on the surface of $\text{Fe}(0)$ nanoparticles after synthesis.

This surface coating likely improves colloidal stability and prevents total oxidation, causing non-flammable metallic materials. According to the study by Peng *et al.*, oleylamine can form a dense protective shell, limiting oxygen interaction and improving the pyrophoric behaviour of zero-valent iron

nanoparticles.[55] A study showed the EDX could identify DDA on the surface of Fe(0) nanoparticles, which confirms the results of FT-IR and TGA in this study.[38]

a

Figure 3a,b: Fe(0) nanoparticles synthesised by DDA in DMF at 110 °C, a) The FT-IR spectroscopy of the DDA functional groups, and b) the TGA and mass loss of adsorbed DDA.

1. Surface layer detection by Cryo-SXT

As can be seen in Fig. 4a,b, a coating layer is present on the Fe(0) nanoparticles due to the contrast of those images between oxygen K-edge (water window) and iron L-edge (iron window). This shows that only the metal-rich centres remain bright, and the halo largely disappears. Cryo-SXT can be performed in the water window (284–543 eV) or at selected absorption edges; therefore, the projections were recorded at 700 eV (pre-edge) and 710 eV (Fe L₃-edge).[56, 57] This chemical contrast suppresses the carbonaceous matrix which allows simple thresholding to isolate the metal phase with minimal segmentation bias. [58] The contrast change in Fig. 4b is due to a low-intensity iron signal originating from a surface layer that may be coated by DDA.

As shown in Fig. 3a,b, FT-IR spectroscopy and TGA confirm that a trace of DDA covers the surface of Fe(0) nanoparticles due to the amine and carbon chain functional groups and weight loss. The Sirk *et al.* study shows that polyamines can form a surface layer on nanomaterials, which improves colloidal stability; however, amines have a low electron density and X-ray contrast relative to iron.[59] This study shows that amine-based stabilisers, including DDA, remain on the surface of metallic nanoparticles. [59]

Fe_{2.8}O₄ nanoparticles images in Fig. 4c,d show at the oxygen and iron absorption edges (water and iron window), and no lower contrast is observed in the image in Fig. 4d. This is due to the lack of a diffuse surface layer and a coating gradient around the Fe_{2.8}O₄ nanoparticles.

2 µm

Figure 4a-d: The surface analysis of Fe(0) and Fe_{2.8}O₄ nanomaterials measured by cryo-SXT, a,b) oxygen (water window) and iron edge (iron window) of Fe(0), and c,d) oxygen (water window) and iron edge (iron window) of Fe_{2.8}O₄.

SEM

At the Fe L-edge (700–710 eV), cryo-SXT presents clearer images compared to SEM on Fe(0) and Fe_{2.8}O₄ nanoparticles, as seen in Fig. 5a-d.

The SEM images of Fe(0) and Fe_{2.8}O₄ nanoparticles presented beam-induced surface charging and unclear images, features that were difficult to resolve at a scale below 100 nm.

The image quality of Fe(0) nanoparticles in Fig. 5a is low at a scale of 200 nm. After coating with carbon (with graphite), a shiny thin layer appears around the Fe(0) nanoparticles, likely due to surface oxidation as indicated by FT-IR, see Fig. 3a. The surface oxygen layer on the uncoated Fe(0) caused electron-beam interactions (charging or diffraction) in SEM; applying a coating layer could mitigate surface charge and enhance the signal-to-noise ratio, as seen in Fig. 5b. In contrast, cryo-SXT (which uses X-rays rather than electrons) produces high-quality images of uncoated Fe(0) with no beam-induced charging or damage (see Fig. 5c).

The charging effects of uncoated Fe_{2.8}O₄ nanoparticles are due to the strong electron-beam interactions with the rich oxygen-containing materials. Applying a conductive coating layer could increase the signal-to-noise ratio, as shown in Fig. 5d,e. A high-quality image in Fig. 5f shows ultrafine agglomerated Fe_{2.8}O₄ nanoparticles recorded by cryo-SXT, without bright halos or contrast mirroring. Flatabø *et al.* reported up to 15% apparent shrinkage in Au and Al nanostructures on insulating glass, attributing the error to probe deflection caused by surface charging during a 10 kV, 80 pA SEM scan.[60] For iron systems, particularly in the study by Sundararajan *et al.*, a modest electron dose thickened the native Fe-oxide shell, dramatically inflating nanoparticle size through diffusion-driven oxidation under the beam.[61] By developing and further adjusting cryo-SXT for solid-state analysis, higher resolution is expected as the voxel size decreases.

HR-STEM and Cryo-SXT

HR-TEM and cryo-SXT study on Fe(0) and Fe_{2.8}O₄ nanoparticles demonstrate that it doesn't show electron-beam interaction and damage, leading to high-quality images, as shown in Fig. 6a–f. The HR-STEM (HAADF) images at 20 and 5 nm show beam-induced interactions due to adsorbed oxygen and a DDA layer on the surface of Fe(0) nanoparticles (see Fig. 6a,b). As the Fe(0) is not coated in HR-STEM imaging, the oxygen surface layer undergoes beam interaction, as confirmed by FT-IR and SEM in Figs. 3a and 4b. In contrast, Fe L₃-edge (710 eV) cryo-SXT of uncoated Fe(0) at 2 μm produces a clear, high-quality image, as well as Fe(0) nanoparticle aggregates, see Fig. 6c.

A study on HR-STEM images of Fe(0) shows diffuse contrast and rapid structural evolution under the beam.[62] Collecting the results at the Fe L₃-absorption maximum (710 eV) yields strong element-specific contrast which allows a simple intensity threshold to isolate the metal phase with minimal segmentation bias.[63] The scale of HR-STEM for Fe_{2.8}O₄ nanoparticles ranges from 20 to 5 nm in Fig. 6d,e, indicating a significant beam interaction. Figure 6f shows a 2 μm-wide Fe L₃-edge cryo-SXT image of Fe_{2.8}O₄, which appears as ultrafine nanoparticles that aggregate across tomographic slices.

The early cryo-X-ray tomography studies by Shapiro *et al.*, achieved voxel sizes of 30–50 nm for iron oxides and even 8 nm resolution with advanced zone-plate optics, values sufficient to resolve 20–30 nm particles while surveying orders-of-magnitude larger volumes compared to HR-STEM or SEM.[64] This demonstrates the technique's ability to map particle aggregates in a non-destructive analysis.

a

Figure 6a-f: The beam-sensitive materials in HR-STEM and cryo-SXT analyses, Fe(0) nanoparticles in a) 20 nm, b) 5 nm, c) cryo-SXT, and Fe_{2.8}O₄ nanoparticles in d) 20 nm, e) 5 nm, and f) in cryo-SXT.

Size of Particles

Accurate size analysis in nanoparticles underpins phase-stability models, catalytic activity maps, and regulatory thresholds; however, the process is complicated by agglomeration, instrumentation limitations, and method-specific biases.

Figure 7a-b shows cryo-SXT mosaics with a 10 µm scale of the Fe(0) and Fe_{2.8}O₄ regions analysed with Fiji/ImageJ-derived size histograms. As shown in Fig. 7a,b, More than 80% of the 4400 Fe(0) counts are below 200 nm, and the modal class sits near 70–80 nm. As seen in Fig. 7c,d, the Fe_{2.8}O₄ histogram (7600 particles) tails off by 20 nm, indicating a monodisperse population of small nanoparticles. However, based on the voxel limitation, it is not possible to detect the size of nanoparticles under 20 nm. [65]

A similar study shows that voxel resolutions of 35–60 nm have already been validated against HR-STEM for the iron-oxide system, with diameter deviations of less than 10% observed when the particle span is ≥ 3 voxels.[66] The present modal diameters meet the 20–30 nm range reported for colloidal Fe₃O₄ prepared by controlled co-precipitation and the 22 ± 6 nm cores quantified inside cells by near-edge absorption transmission in cryo-SXT.[67]

The particle size distribution results are consistent with the broadness of the PXRD patterns for Fe(0) and Fe_{2.8}O₄ in Fig. 2a,b. The Fe(0) patterns indicate higher crystallinity, while the broader peaks of Fe_{2.8}O₄ show smaller crystallite sizes.

Topology 3D imaging

3D cryo-SXT is a form of X-ray computed tomography that non-destructively images materials in three dimensions with sub-micron resolution.[68] In practice, a cryo-SXT scan needs multiple 2D projections as the sample rotates, and the data are reconstructed into a 3D volume of X-ray absorption or phase contrast. [68]

In cryo-SXT 2D projection images, the X and Y axes represent the lateral dimensions of the sample, while the Z-axis represents the thickness and depth, which are compressed or overlapped into a single image. [69]

Based on the 3D images (top view, side view) in Fig. 8ab, the false-colour height maps represent relative intensity and reconstructed topographical variation within the tomographic volumes, with warmer colours indicating regions of increased projected thickness or density. Multiple viewing angles show the

nanoscale topography, volumetric distribution, height heterogeneity, clustering behaviour, and internal structural organisation in nanoparticles.

As shown in Fig. 8a, 2D cryo-SXT images at 710 eV (Fe L₃-edge) of Fe(0) nanoparticle aggregates are shown alongside corresponding 3D tomographic surface reconstructions generated from Fiji/ImageJ-based analysis and a tomographic rendering process. This highlights the particles that absorb the soft X-ray beam most strongly at 2-μm resolution (see the GIF file in the supporting document). The 3D reconstructions demonstrate that features appearing as compact or overlapping domains in 2D projections are highly heterogeneous surface topographies and variable aggregate thicknesses.

In contrast to Fe(0), the 2D cryo-SXT images of Fe_{2.8}O₄ in 710 eV (Fe L₃-edge) nanomaterials show more distributed nanoscale clustering, interconnected particulate domains, and broader spatial dispersion across the reconstructed volumes, see Fig. 8b (see the GIF file in the supporting document).

The AFM is a surface characterisation technique that can only scan in 2-axis point-by-point over each area.[70] The AFM tip has a limited radius and apparent lateral size, limiting its ability to detect nanoscale structures. [70] As Canet-Ferrer *et al.*, noted, AFM measurements are constrained by tip-sample convolution, leading to reduced lateral resolution. [71] In contrast, spatial resolution in cryo-SXT is determined by the integration of X-ray and optics rather than a mechanical probe, allowing it to approach 20–50 nm without widening effects. [72, 73] The future progress in 3D cryo-SXT could improve access to lower scales to provide more information about the surfaces of nanoparticles and likely the atomic d-spacing.

The 3D tomographic surface reconstruction in Fig. 9 identified a localised cavity on the surface of the Fe(0) crystal. It is simulated by the Fiji/ImageJ-based tomographic rendering, which provides x, y and z information by the 2 μm scale bar of the image. The cavity was estimated to be 2.70 μm in length and 2.00 μm in width. From the z-axis profile of the 3D reconstruction, the apparent cavity depth was 0.010 μm, assuming the volume of the estimated cavity in an ellipsoidal cavity geometry, which is approximately 0.028 μm³.

Conclusion

This study demonstrates that the use of cryo-SXT in beam 24 at Diamond Light Source can enhance the quality of characterisation, analysis and interpretation in solid-state and materials sciences. An unconventional synthesis method was used for the rapid formation of Fe(0) nanoparticles at 110°C in a microwave reactor. A novel, rapid, and low-energy method was developed to synthesise Fe_{2.8}O₄ magnetite at 80°C using a mixture of KOH/ethanol solution. To the best of our knowledge, this is the first time the beamline technique of cryo-SXT has been used as an imaging tool for beam-sensitive nanomaterials. This new approach enables analysis of nanomaterial shapes, topography, and surfaces without charge interactions or structural collapse under X-ray beam sources. The Fiji/ImageJ was used to analyse cryo-SXT images for size distribution and 3D image simulation. This 2D-to-3D imaging

approach reveals hidden nanoscale connectivity, aggregate thickness, and volumetric heterogeneity in iron-based nanomaterials that cannot be fully interpreted from 2D projection images alone. The cryo-SXT size-distribution analysis showed that over 80% of Fe(0) particles are below 200 nm, whereas most Fe_{2.8}O₄ nanoparticles are distributed at around 20 nm. Energy-selective cryo-SXT could distinguish the amine-coated layer on the Fe(0) nanoparticles, as confirmed by FT-IR and TGA. In the future, this approach could be developed for other beam-sensitive nanomaterials as cryo-SXT evolves, generating a new view window in the field of solid-state characterisation.

Declarations

Funding

Declaration

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Author Contribution

E.E.G.: Conceptualisation, Methodology, Validation, Investigation, Visualisation, Writing - Original Draft, and Writing - Review and Editing. Y.H.: Resources, Review, and Editing. F.S.: Investigation, 3D image refinement, Visualisation, Writing - Review and Editing, and Supervision. All authors reviewed and approved the manuscript.

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Data Availability

The data supporting this study are available from the University of Warwick WRAP repository at <https://wrap.warwick.ac.uk/id/eprint/194261/>.

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Figures

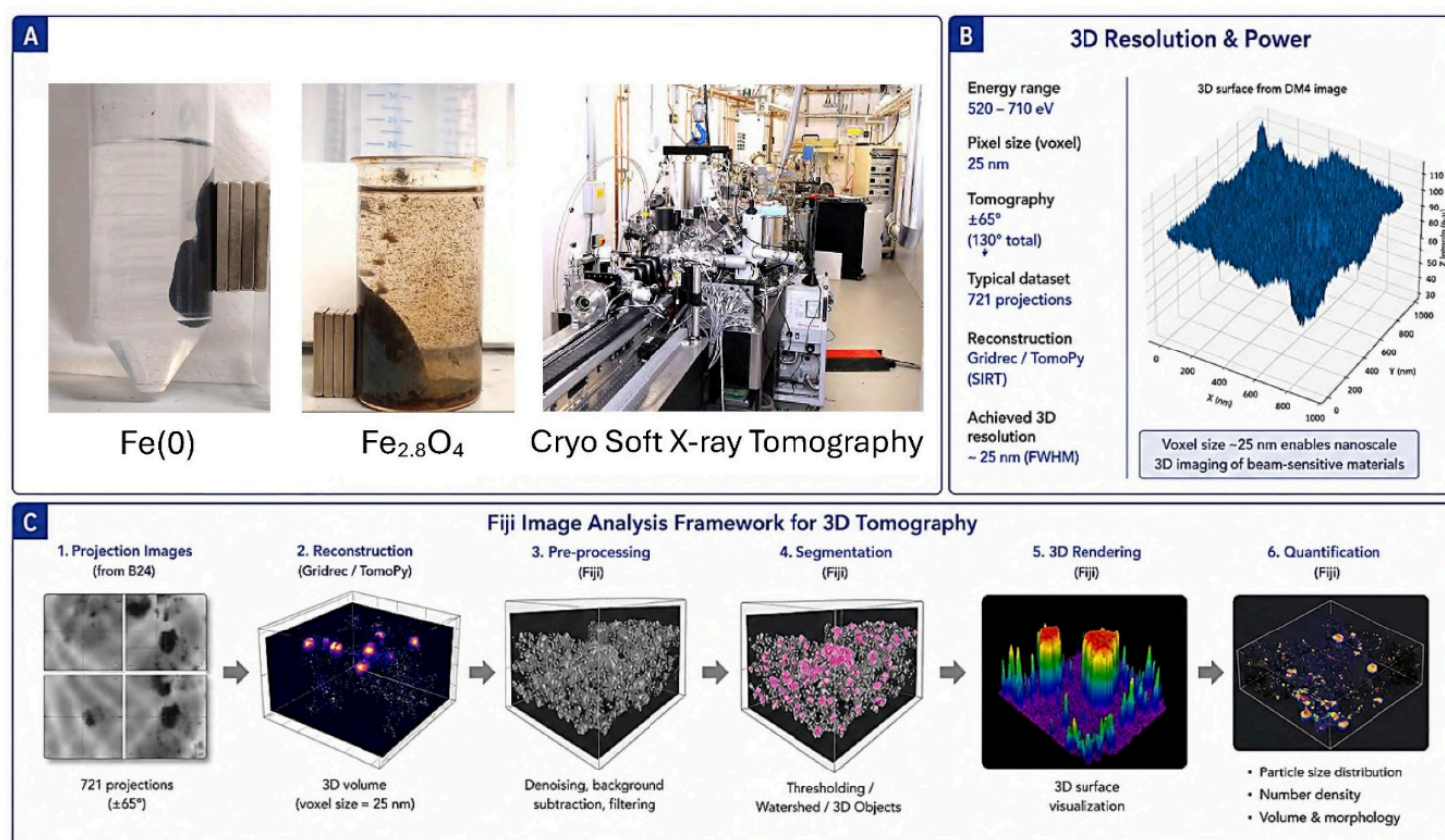


Figure 1

A-C: A) The magnetic properties of Fe(0), $\text{Fe}_{2.8}\text{O}_4$ nanoparticles have been tested by a neodymium magnet and imaged by Cryo-soft X-ray tomography. B) Cryo-soft X-ray tomography projection images are shown alongside corresponding 3D surface reconstructions generated using Fiji/ImageJ analysis. C) Fe-rich regions observed in the 2D projections correspond directly to enhanced volumetric structures within the reconstructed 3D datasets. Multiple viewing orientations reveal nanoscale heterogeneity in particle morphology, aggregation state, clustering behaviour, and spatial organisation that can not be fully

captured by 2D imaging alone. The reconstructed datasets demonstrate cryo-SXT's capability for non-destructive, volumetric imaging of beam-sensitive nanomaterials in their near-native state.

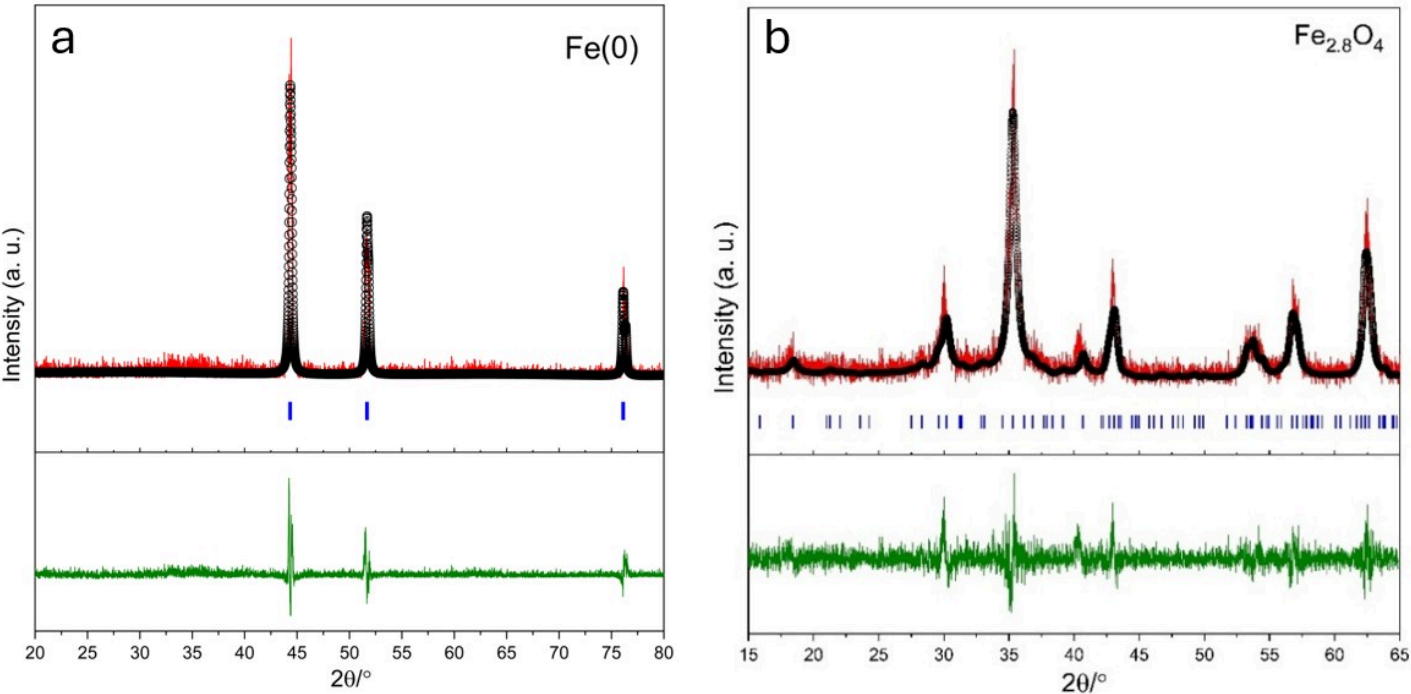


Figure 2

a,b: Pawley-refined PXRD patterns for the nanoparticles showing experimental data = black points, fitted pattern = red lines, and Bragg peak positions = blue, a) Fe(0) produced by DDA at 19 °C (FCC structure with Fmm space group) with 42.6 (± 0.3) nm crystal domain size, b) Fe_{2.8}O₄ produced by KOH at 80 °C (tetragonally distorted inverse-spinel, space group P4₃212) with 11.12 (± 0.6) nm crystalline size.

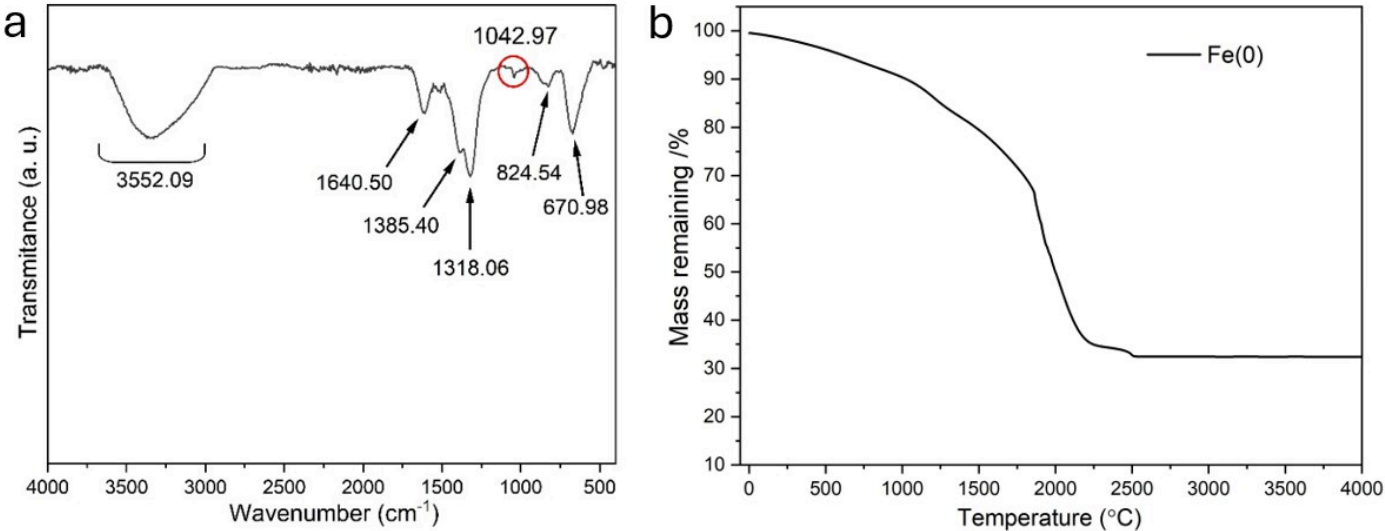


Figure 3

a,b: Fe(0) nanoparticles synthesised by DDA in DMF at 110 °C, a) The FT-IR spectroscopy of the DDA functional groups, and b) the TGA and mass loss of adsorbed DDA.

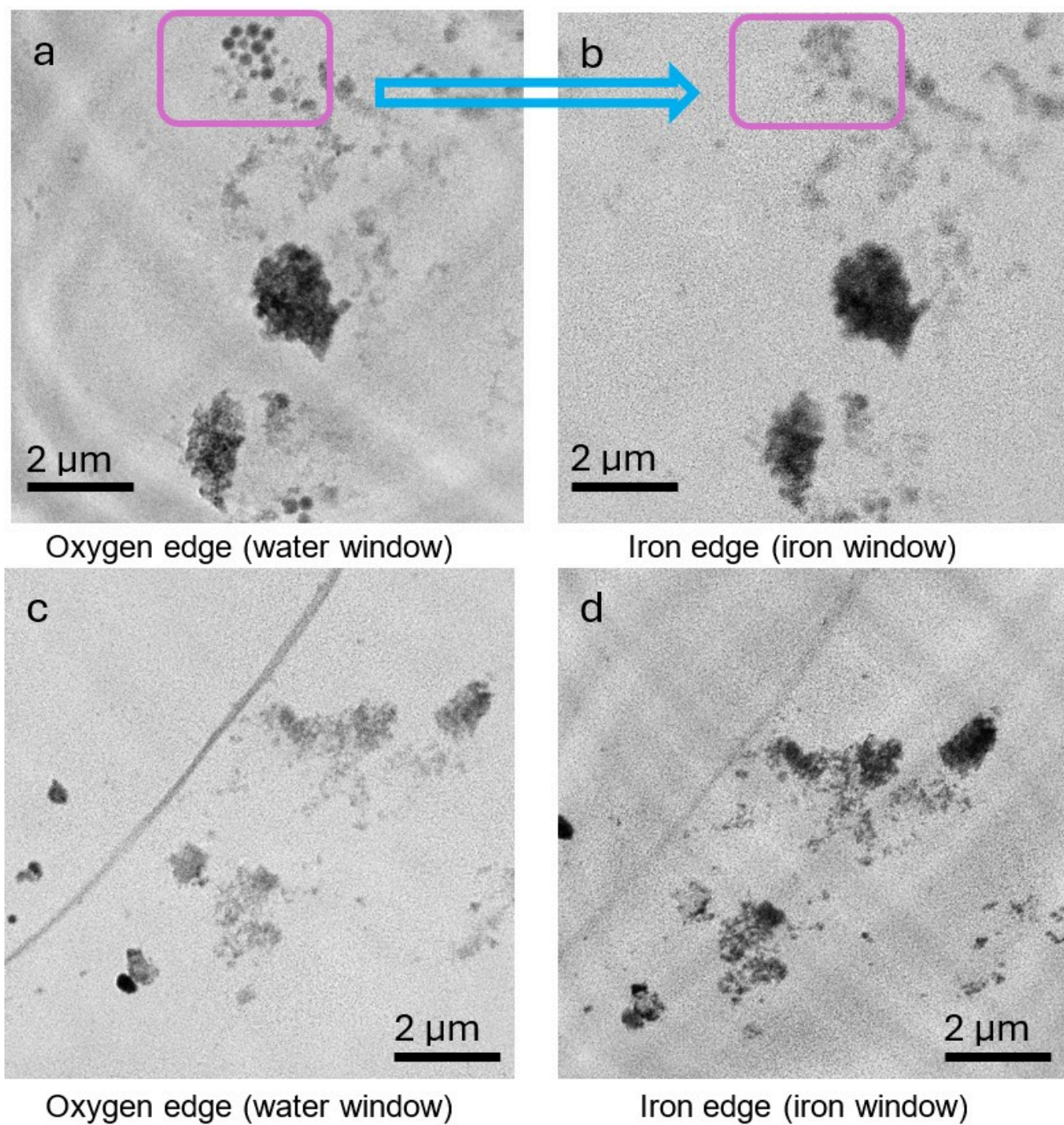


Figure 4

a-d: The surface analysis of Fe(0) and Fe_{2.8}O₄ nanomaterials measured by cryo-SXT, a,b) oxygen (water window) and iron edge (iron window) of Fe(0), and c,d) oxygen (water window) and iron edge (iron window) of Fe_{2.8}O₄.

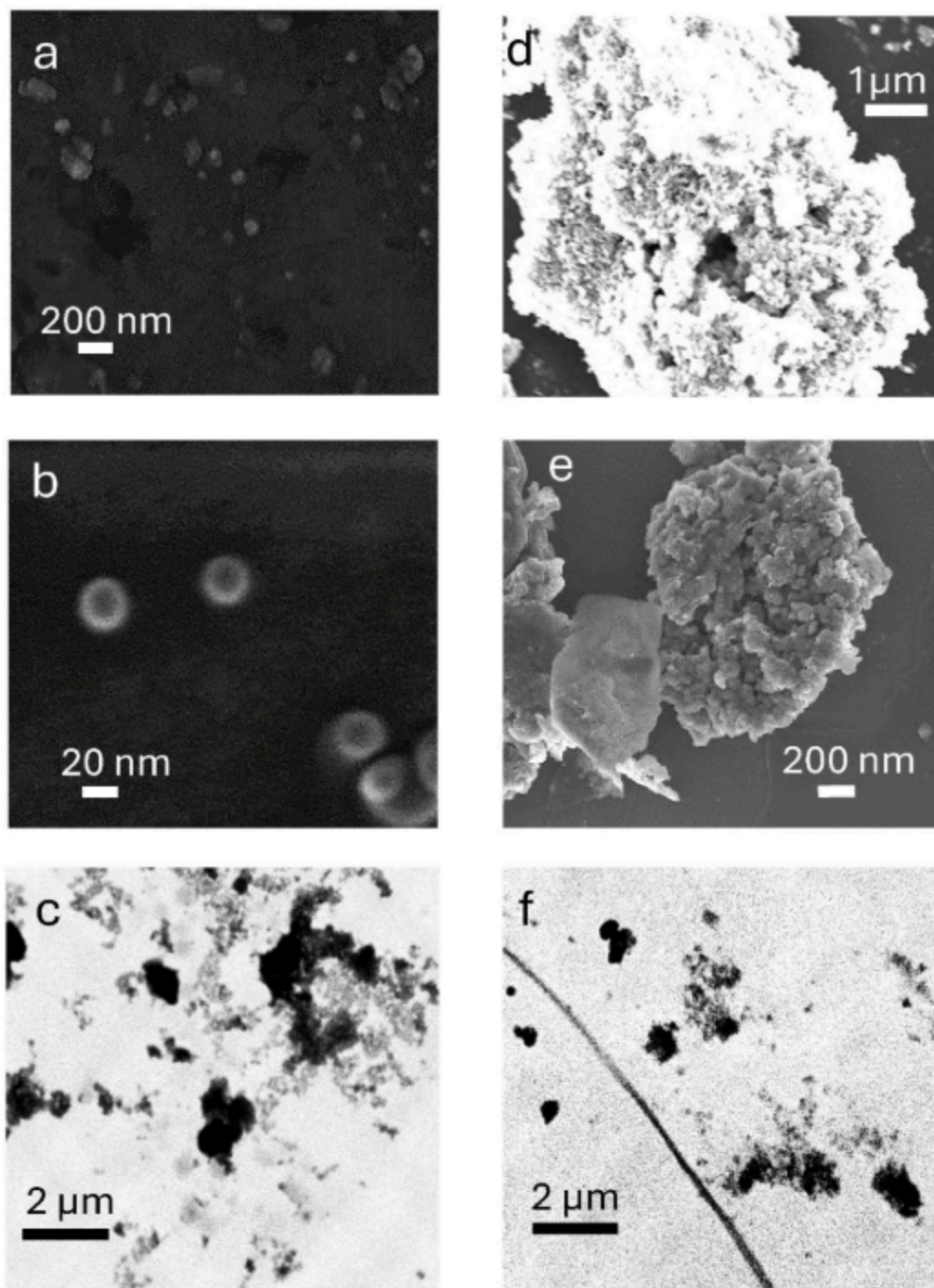


Figure 5

a-f: The beam-sensitive materials in SEM, a) Fe(0) nanoparticles without any adjustments, b) Fe(0) nanoparticles on a conductive silicon wafer with two times coatings by graphite, c) Fe_{2.8}O₄ nanoparticles in cryo-SXT, d) Fe_{2.8}O₄ on silicon wafer and without surface coating, e) on a conductive silicon wafer with three times coatings (by graphite), f) Fe_{2.8}O₄ nanoparticles in cryo-SXT.

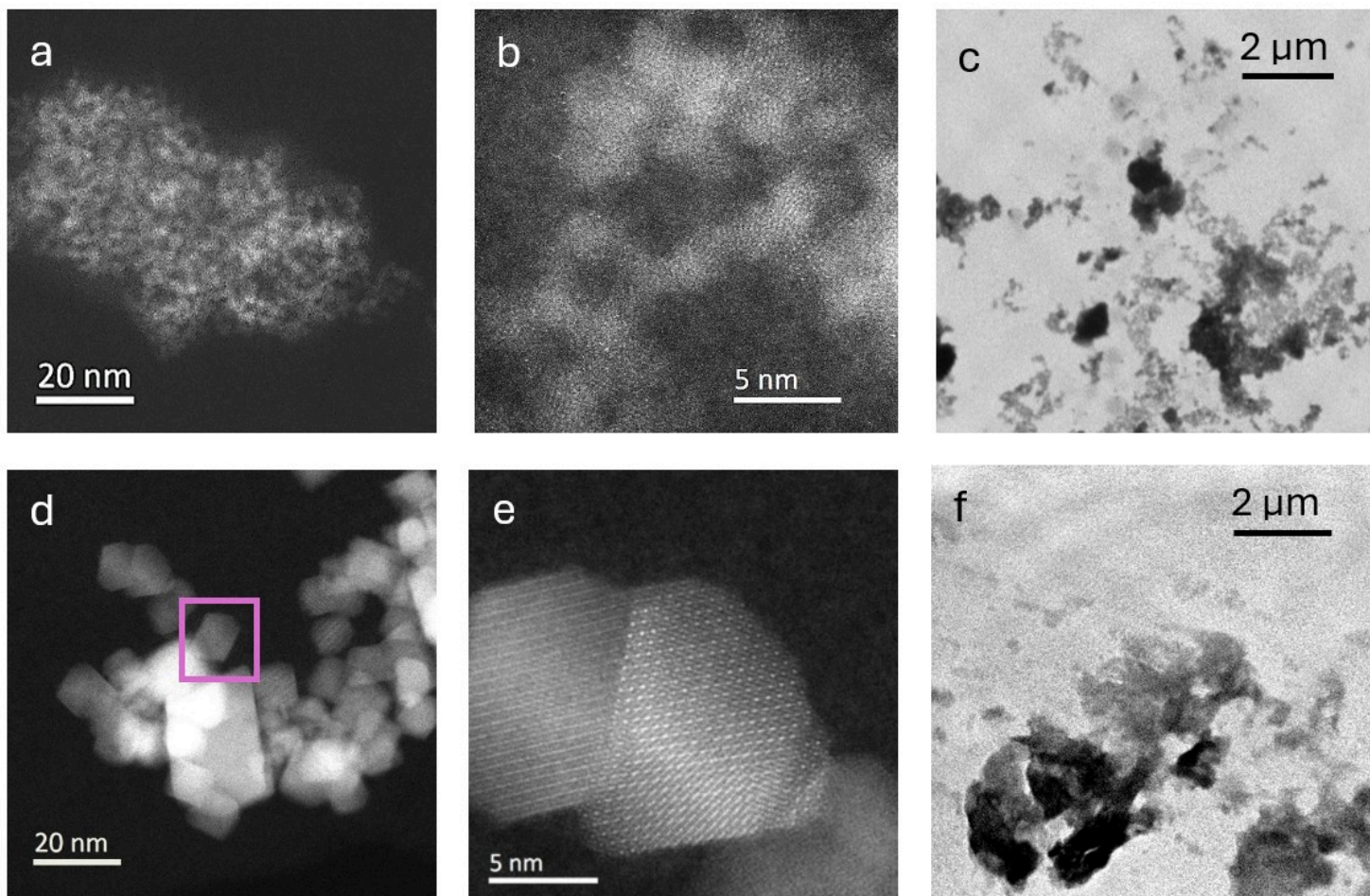
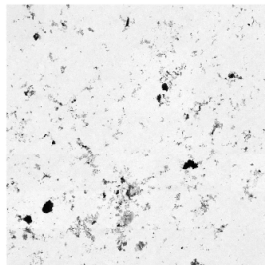


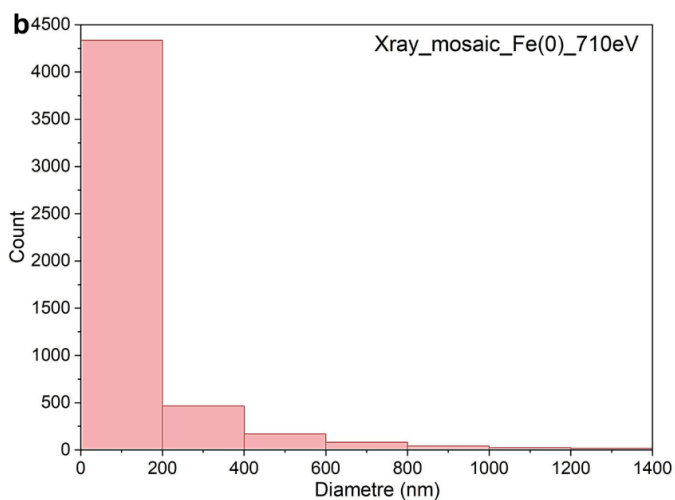
Figure 6

a-f: The beam-sensitive materials in HR-STEM and cryo-SXT analyses, Fe(0) nanoparticles in a) 20 nm, b) 5 nm, c) cryo-SXT, and Fe_{2.8}O₄ nanoparticles in d) 20 nm, e) 5 nm, and f) in cryo-SXT.

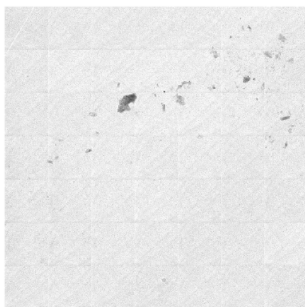
a Fe(0)



10 μ m



c Fe_{2.8}O₄



10 μ m

d

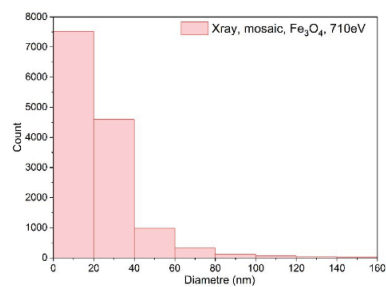


Figure 7

a-d: Stitched cryo-SXT mosaic images (10 μ m field of view) and corresponding particle-size histograms: (a,b) Fe(0) nanoparticles ($N = 4400$) and (c,d) Fe_{2.8}O₄ nanoparticles ($N = 7600$). Particle sizes were measured using ImageJ.

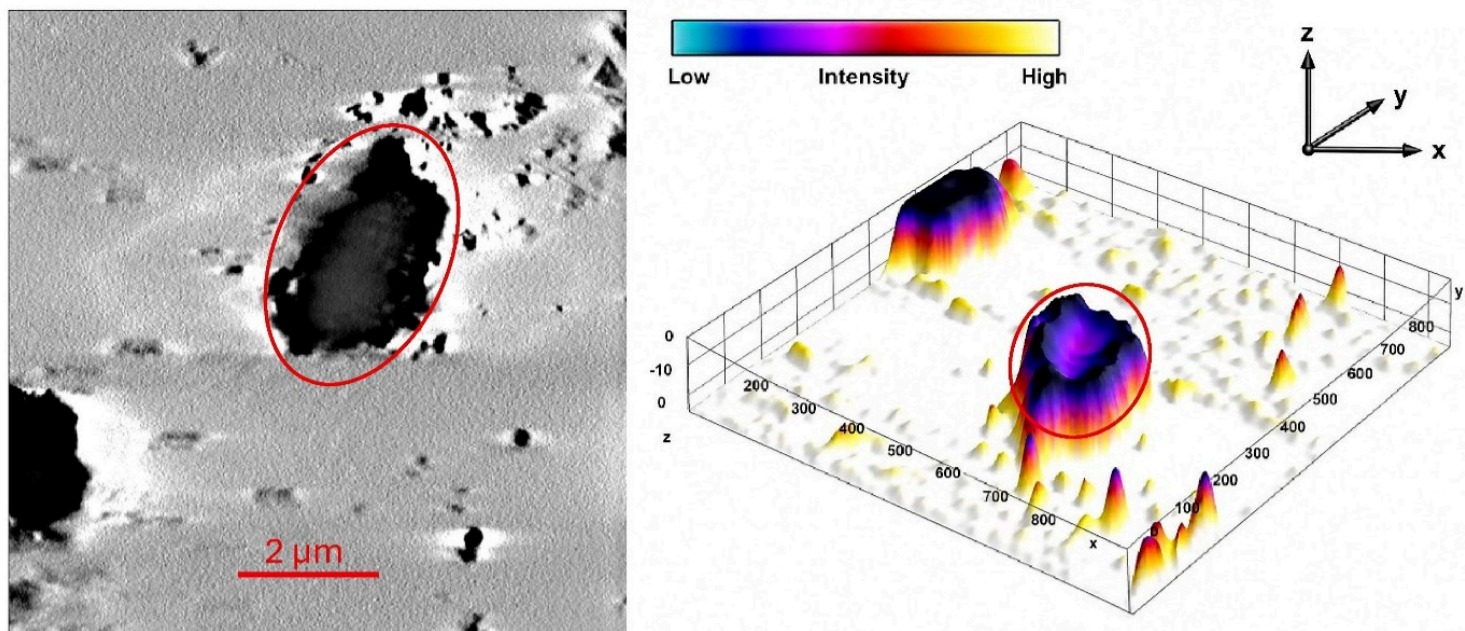


Figure 9

The topography of the 2D cryo-SXT images at 710 eV (Fe L3-edge) of Fe(0) crystals, and the size of the cavity can be estimated using Fiji/ImageJ ($V = \pi/6 \times L \times W \times D$). The 3D .GIF file has been attached to the supporting document (03_tomo_F4B_area4_WBP).