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TECHNICAL REPORT

Combination of focused ion beam (FIB) and microtome by ultrathin slice preparation for transmission electron microscopy (TEM) observation

Yuchen Xu[1], Lixin Gu[1], Yang Li[2], Bing Mo[2] and Yangting Lin[1,3*]

Abstract

There are growing demands for integrated study of isotopes, trace elements and crystallography of micron-sized grains observed on polished sections or scattered on surface of sample holders. However, transmission electron microscopy (TEM) observation is largely restricted by the challenges associated with the preparation of ultrathin sections from given micron-sized grains after chemical and isotopic analyses. Here, we report a new method of combining focused ion beam (FIB) and microtome techniques. This method is demonstrated by the successful preparation of several ultrathin slices from a presolar graphite grain. The presolar graphite grain was a spherule with a diameter of 5 µm. It was found by nanoscale secondary ion mass spectrometry (NanoSIMS) mapping of a carbonaceous acid residue from the Qingzhen enstatite chondrite (EH3), deposited on a gold foil mount. The spherule showed isotope anomalies in C and Si, with $^{12}C/^{13}C = 99 \pm 2$ and $\delta^{29}Si/^{28}Si = 172 \pm 36‰$, and likely originated in an asymptotic branch giant (AGB) star. After NanoSIMS analysis, this spherule was transported and fixed on a pre-cut top surface of an epoxy stub, using FIB. The fixed spherule was then embedded entirely in resin, and cut into ~ 70 nm slices with a microtome. TEM observation of these ultrathin sections revealed a turbostratic structure free of any subgrains. The morphology, Raman spectrum, C and Si isotopic compositions, internal texture and crystallography of this graphite spherule suggest that it condensed from a carbon-rich outflow of an AGB star.

Keywords: Focused ion beam, Transmission electron microscopy, Microtome, Presolar grains, Graphite

Introduction

In order to understand geological and planetary processes, it is usually required to perform several coordinated analyses on the same sample. Due to advances in microbeam analytical techniques, it is now possible to measure a single micron-sized grain in situ with a field-emission scanning electron microscope (FESEM) equipped with cathodoluminescence (CL), energy-dispersive spectrometer (EDS) and electron backscatter diffraction (EBSD); a Raman spectrometer; an electron probe microanalyzer (EPMA); and a secondary ion mass spectroscopy (SIMS), in sequence. However, it is still a great challenge to obtain the crystallographic information from the same tiny grains after these microbeam analyses, which is often crucially important to constrain the geological or cosmochemical histories of the grains. The primary difficulty is to prepare the transmission electron microscopy (TEM) slices (≤ 100 nm) from a specific micron-sized grain on a polished section or deposited on surface of a sample mount. Three techniques have been used to prepare TEM slices: microtome, ion milling, and focused ion beam (FIB). Microtome methods are applicable to homogeneous powders, small diameter fibers, and most biomaterials. Ion milling allows TEM observations on the crystals located near the central hole thinned

*Correspondence: linyt@mail.iggcas.ac.cn
[1] Key Laboratory of Earth and Planetary Physics, Institute of Geology and Geophysics, Chinese Academy of Sciences, No. 19, Beituchengxilu, Chaoyang District, Beijing 100029, China
Full list of author information is available at the end of the article

by ion beam (Klaar and Hsu 1996; Müller and Krumeich 2000). However, neither method can be applied to precise cutting of a particular individual micron-sized grain. On the other hand, FIB has been becoming an available technique of preparing TEM slices from micron-sized minerals on polished sections, which is referred to as the "lift-out method" (e.g., Dobrzhinetskaya et al. 2003; Giannuzzi et al. 1998; Overwijk et al. 1993; Stroud et al. 2004; Zega et al. 2007). FIB technique can precisely cut out a micron-sized grain on polished section and then thin it to ~ 100 nm for TEM observation. However, the FIB lift-out method has also some disadvantages, e.g., amorphization of specimen surface and Ga^{+} ion implantation (Li et al. 2006; Tomus and Ng 2013). In addition, typically only one TEM foil can be prepared from each micron-sized grain.

Micron-sized presolar graphite grains are one typical case of grains deposited on surface of a sample mount that require coordinated SIMS and TEM. They are identified on the basis of their abnormal isotopic compositions, which together with internal structural information provide constraints on the nucleosynthesis processes and physical and chemical conditions of the formation in the mass outflows from stars (e.g., Bernatowicz et al. 1991; Croat et al. 2003, 2005, 2008; Zinner 2014). In the Qingzhen meteorite, the presolar graphite is only ~ 0.2% of carbonaceous acid-resistant residue, much lower than in other carbonaceous chondrites. Such low abundance, combined with small grain size, resulted in dense areas selected for NanoSIMS mapping and challenged the traditional sample transfer method by micromanipulator under optical microscope on an identified grain (Bernatowicz et al. 1991; Croat et al. 2003; Groopman et al. 2014). Therefore, we try to combine FIB and microtome techniques in order to prepare ultrathin sections.

Samples and instruments

A carbon-rich residue of Qingzhen (EH3) meteorite, separated by acid etching (Lin et al. 2002), was dispersed on a high pure gold foil mount. Presolar graphite grains were identified based on their anomalous carbon isotopic ratios measured by NanoSIMS analysis (Xu et al. 2016). They are spherical, 3–10 μm in size, and surrounded by amorphous carbonaceous residue with solar isotopic compositions. One of the presolar graphite spherules was selected for this study. This presolar graphite was labeled as Pink-55a, with a diameter of 5 μm after isotopic analysis (Xu et al. 2016). The grains morphology is referred to as the cauliflower-onion type (Xu et al. 2016). Raman spectra is representative of glassy-type with D/G = 1.13. It has a relatively high $^{12}C/^{13}C$ ratio (99 ± 2) with slight enrichment in ^{29}Si ($\delta^{29}Si/^{28}Si = 172 \pm 36$‰) but normal ^{30}Si ($\delta^{30}Si/^{28}Si = 69 \pm 81$‰), indicative of an asymptotic branch giant (AGB) origin.

The pick-up and transfer of the grain were conducted by FIB, with a Zeiss Auriga Compact dual beam instrument equipped with an Omniprobe AutoProbe 200 micromanipulator at the Institute of Geology and Geophysics, Chinese Academy of Sciences (IGGCAS). Ultrathin slices were prepared with a microtome RMC Power Tome XL at Institute of Geochemistry, Chinese Academy of Sciences, Guiyang. After slicing, crystallographic observations were carried out with two TEMs, a FEI Talos F200X at Suzhou Institute of Nano-tech and Nano-Bionics (SINANO), CAS, and a JEM 2100 at IGGCAS, both operated at an accelerating voltage of 200 kV.

Experiments

The TEM slices preparation process had two main steps, sample transfer with FIB and sections slicing with the microtome. Usually, the FIB operation took about 2 h, and the microtome sample preparing (the resin curing time not included) and cutting required 3–5 h.

Sample transfer

The spherule Pink-55a was picked up from the acid carbonaceous residue with a carbon fiber and then transferred onto a pre-prepared microtome resin stub (Fig. 1). The carbon fiber later served as a fiducial maker for locating the spherule in the subsequent microtome slicing and TEM observation.

Carbon fibers were dispersed onto a pre-cleaned aluminum mount with a pipette. The aluminum mount was rinsed previously with analytical reagent (AR) ethanol in an ultrasonic bath. An epoxy resin mount with a leading blockface area of about 200 × 200 μm^2 was prepared for placement of the graphite spherule. It was prepared by a pyramid-bottom-shaped capsule with resin, which was then pumped for 20 min to remove bubbles and cured in an oven at 125 °C for 10–12 h. The pyramid top of the resin base was then trimmed to form a blockface of ~ 200 × 200 μm^2. The whole resin base, from the blockface to the surface of the cylinder, was coated with gold for good conduction. The Au-coated resin base was fixed on a FIB sample holder with carbon tape to minimize charging in the FIB. The carbon fiber used in this study was ~ 8 μm in diameter, and it was welded on the tip (~ 1 μm in diameter) of a tungsten needle of the micromanipulator with FIB-deposited platinum (Pt) (Fig. 2a). The ion beam conditions for the Pt deposition were 30 kV and a beam current of 50 pA. The tip of the carbon fiber was trimmed with the ion beam to facilitate the pick-up of the graphite spherule.

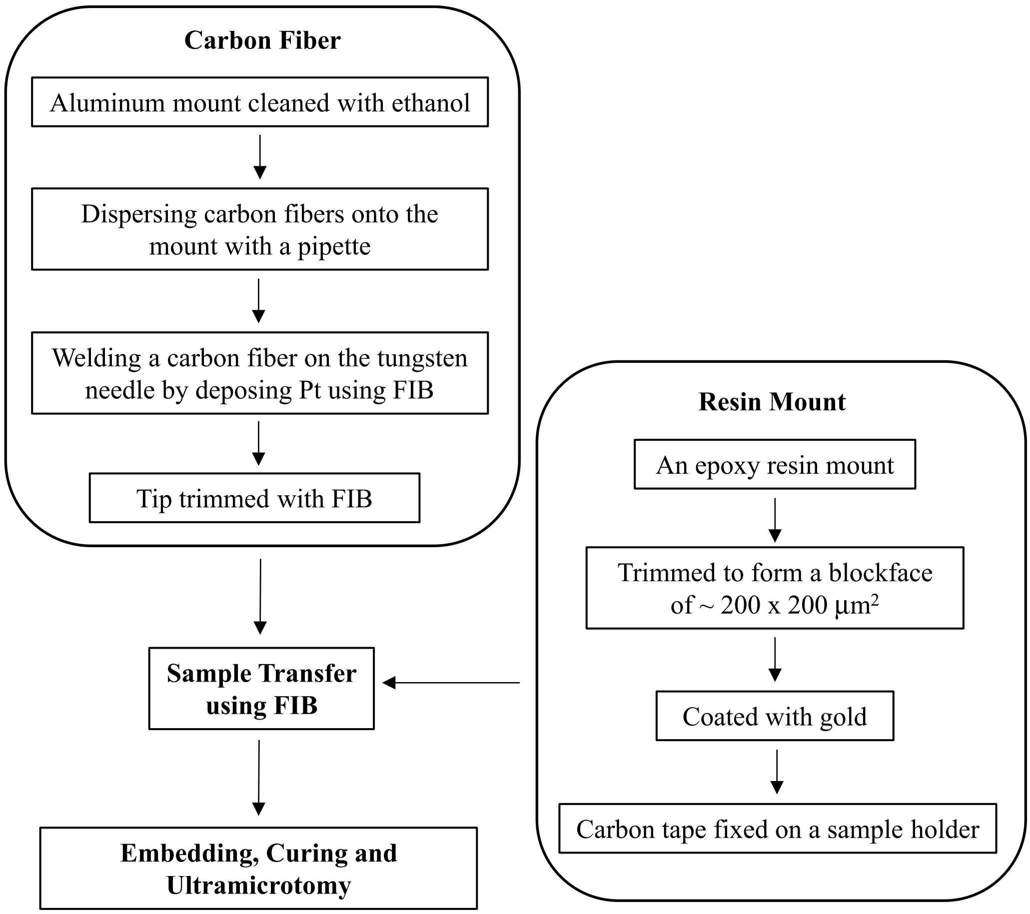

Fig. 1 The preparation procedure of the carbon fiber and resin base

The sharped carbon fiber was brought next to the graphite spherule Pink-55a and attached to it with a Pt weld. The spherule was then picked up from the surrounding amorphous carbonaceous residue (Fig. 2b) and moved to the top of the resin base (Fig. 2c). The sample stage was rotated slightly to be parallel to the carbon fiber. A small lump of Pt was deposited on the top surface of the resin base, and then, the carbon fiber was slowly lowered until it touched to the Pt lump. The sample was attached to the top of the resin by depositing Pt between them, and finally, the carbon fiber was cut free from the sample (Fig. 2d). After firmly attaching the spherule on the top of resin, the Au-coating in the region surrounding the sample was sputtered briefly with the FIB, in order to facilitate adhesion of a resin drop with the surface of the resin base, which was applied after the sample was removed out from the FIB.

Embedding, curing and ultramicrotomy

A drop of the same epoxy resin was placed over the graphite spherule attached to the face of the epoxy stub, with the aid of a micromanipulator under an optical microscope. Care was taken to ensure the sample was completely embedded in the resin drop. After curing for 18–24 h in an oven at 80 °C, the sample was ready to be sliced with the microtome.

The sample was centered in the microtome chuck with the sample face perpendicular to the screw axis. It was first sliced with a glass knife to remove the top resin until the sample close to the surface. The attached carbon fiber was helpful for locating the tiny spherule. Finally, the sample was sliced with a diamond knife, and a series of ultrathin sections were picked up with the holey-C-coated Cu TEM grids (Fig. 3a).

Results and discussion

TEM observations

The carbon fiber (~ 40–50 μm long), nearly 10 times of the graphite spherule, which makes it more efficient to locate the target section under TEM. The graphite slice is on one end of the carbon fiber (Fig. 3b–d), and it is easily identified by the presence of the deposited Pt

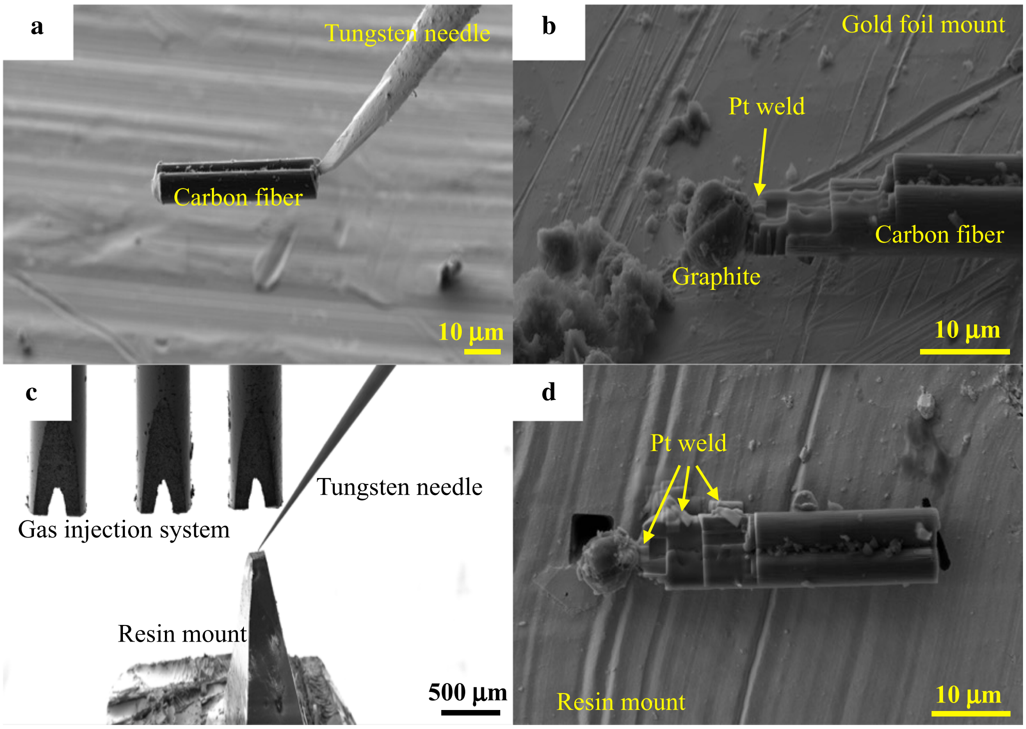

Fig. 2 The procedure of sample transferring with FIB. **a** A carbon fiber was welded on the tip of a tungsten needle. **b** A graphitic spherule was welded with a shaped carbon fiber. **c** Transferring onto a resin base. **d** Fixing on the top and cutting off the carbon fiber

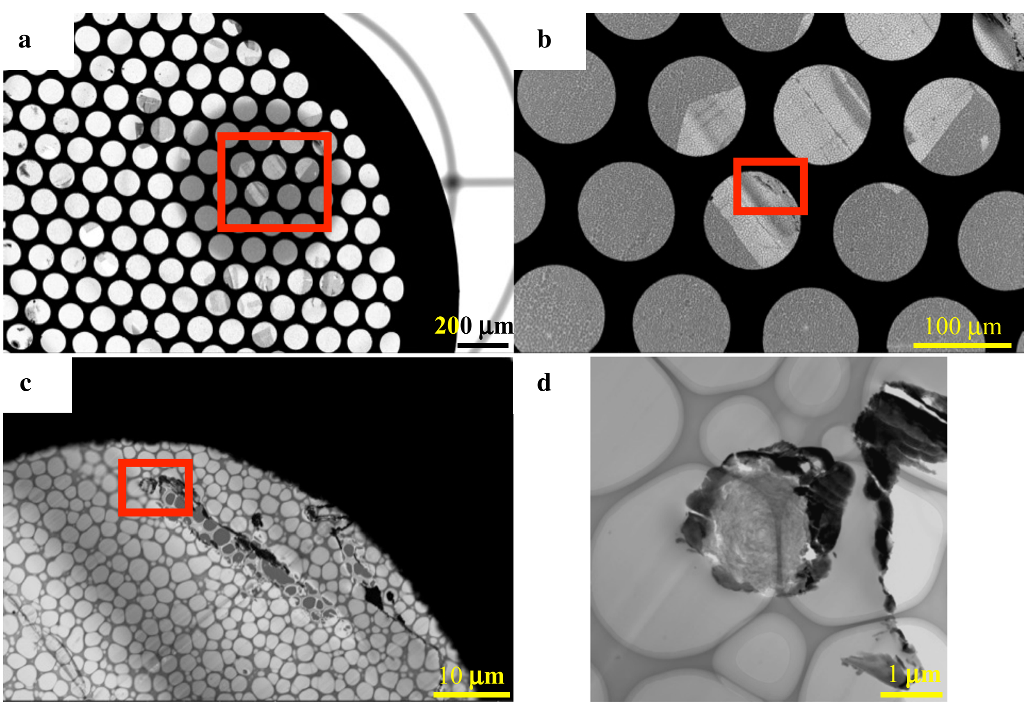

Fig. 3 TEM Observations. **a** Microtomed sections deposited on TEM grids. **b**, **c** A graphite found on one end of the carbon fiber. **d** TEM observations of the graphite

that appears black in the bright field (BF) TEM image (Fig. 3d).

As revealed in TEM, this spherule has a concentric layered structure (Fig. 4a). The electron diffraction patterns from both the center and the edge show a single continuous ring without diffraction spots (Fig. 4b). In the high-resolution TEM images, the layers are short in length, curved in shape, and discontinuous (Fig. 4c). These features demonstrate that the degree of graphitization is poor developed (Buseck and Huang 1985). This is consistent with the characteristics of its Raman spectrum, which is the type of glassy (D/G > 1) (Xu et al. 2016). Such turbostratic features suggest that this graphite spherule likely condensed in a gas–solid environment with a high abundance of carbon (Bernatowicz et al. 1996; Croat et al. 2005, 2008). Diffraction contrast imaging and EDS measurements reveal no other submicron-sized inclusions in the graphite spherule. However, lack of inclusions in AGB graphite grains is not unusual, since previous observations showed that some of AGB graphite grains from Murchison carbonaceous chondrite were also inclusion-free (Bernatowicz et al. 1991, 1996; Croat et al. 2005).

Advantages of FIB-microtome technique

The TEM observations of the prepared slices of the AGB graphite spherule demonstrate the success of the FIB-microtome technique. In comparison with traditional microtome and ion beam methods, the FIB-microtome technique has the following advantages: (1) many ultrathin sections can be obtained from a single micron-sized grain, whereas the typical FIB method can only produce one; (2) it extends the range of samples that can be microtomed to include individual micron-sized grains on polished sections with the help of FIB. It is a challenge to pick up a single micron-sized grain that adheres with many other grains, even using micromanipulator under optical microscope. Because of static charge, these grains could adhere any part of the needle of the micromanipulator. In addition, because of the very small size of the grains (≤ 5 μm), it is difficult to relocate such grains after they are embedded in resin. Generally, the traditional handling process with a micromanipulator under optical microscope can be successfully used to handle 10 s micrometer-sized grains, but has a higher risk of losing smaller grains. In contrast, we picked up the grain by welding it onto the tip of a carbon fiber by depositing Pt with FIB, and also firmly fixed it together with the carbon fiber onto the top of resin stub with Pt. This greatly reduced the risk of losing the unique grain during the process. In addition, the carbon fiber served as a fiducial marker, which was helpful in relocating the grain after it was embedded in resin for the microtomy, and during the subsequent TEM observation.

Although graphite is soft and ideal for microtome cutting, this technique can be applied to silicates and oxides. The hardness and brittleness of most silicates and oxides can cause materials fall out of the slices; however, these issues are not severe for grains < 10 μm.

Implications

TEM observations of the AGB graphite spherule Pink 55-a revealed its poor crystallization with turbostratic layering of contorted lattice planes and short-range layer continuity. This is consistent with the glassy-type Raman spectrum of the grain (Xu et al. 2016). The experimental results demonstrated that the FIB-microtome technique can be used to prepare TEM sections of micron-sized grains, including the presolar graphite spherules. Such

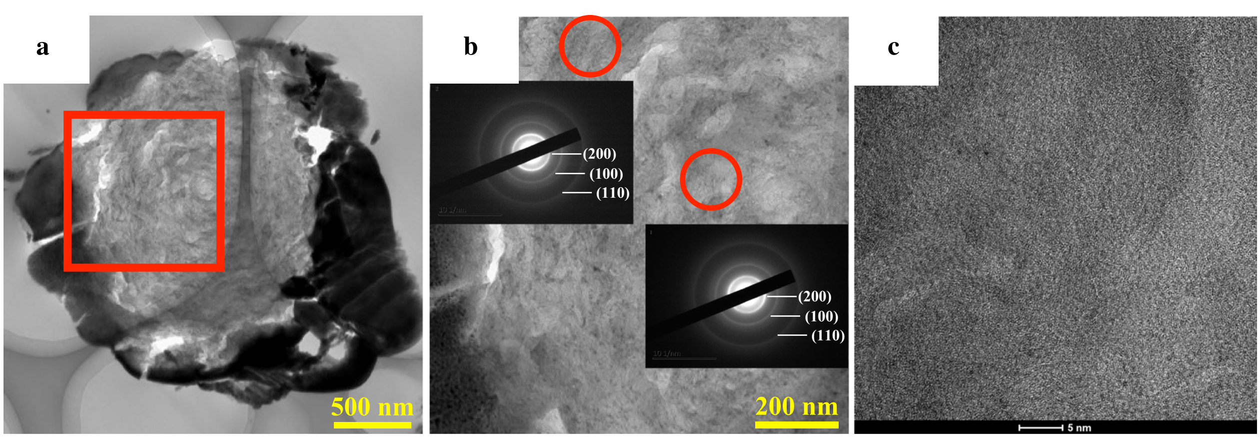

Fig. 4 Microstructure of the presolar graphite (Pink-55a). **a** Consisting of concentric layers. **b** The area with red box at high magnification. Electron diffraction pattern from the near red circle indicative of poor organization. **c** High-resolution TEM imaging. Turbostratic layering with contorted lattice planes and short-range layer continuity

TEM observations of the ultrathin slices of presolar grains can provide important constraints on the formation conditions and processing histories of the presolar grains from various stellar sources.

The China Lunar Exploration program, i.e., Chang'E 5, is planned to return lunar samples in 2019. Most of the returned lunar samples will be the fine-grained soil. In addition, the asteroid surface samples returned by future missions contain abundant micron-sized grains. In fact, the Itokawa asteroid samples returned by the Hayabusa mission of Japan are ~ 1500 grains with sizes of < 10–40 μm (Nakamura et al. 2011). The FIB-microtome technique can be a critical experimental method for analyzing the returned lunar and asteroid samples. This technique can also be applied to other rare and unique micron-sized grains of terrestrial rocks and meteorites.

Abbreviations
FIB: focused ion beam; TEM: transmission electron microscopy; NanoSIMS: nanoscale secondary ion mass spectrometry; AGB: asymptotic branch giant; EDS: energy-dispersive spectrometer.

Authors' contributions
YX designed the study, prepared and conduct experiments, interpreted the data and drafted the manuscript. LG helped to realize sample transferring with FIB. YL and BM performed important poles in resin base and microtome slicing. YL made substantial contributions to the conception and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Author details
[1] Key Laboratory of Earth and Planetary Physics, Institute of Geology and Geophysics, Chinese Academy of Sciences, No. 19, Beituchengxilu, Chaoyang District, Beijing 100029, China. [2] Center for Lunar and Planetary Sciences, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China. [3] University of Chinese Academy of Sciences, Beijing, China.

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Competing interests
The authors declare that they have no competing interests.

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Consent for publication
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Availability of data and materials
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References
Bernatowicz TJ, Amari S, Zinner E, Lewis RS (1991) Interstellar grains within interstellar grains. Astrophys J 373:L73–L76

Bernatowicz TJ, Cowsik R, Gibbons PC, Lodders K, Fegley B, Amari S, Lewis RS (1996) Constrains on stellar grain formation from presolar graphite in the Murchison meteorite. Astrophys J 472:760–782

Buseck PR, Huang BJ (1985) Conversion of carbonaceous material to graphite during metamorphism. Geochim Cosmochim Acta 49:2003–2016

Croat TK, Bernatowicz TJ, Amari S, Messenger S, Stadermann FJ (2003) Structural, chemical, and isotopic microanalytical investigations of graphite from supernovae. Geochim Cosmochim Acta 67:4705–4725

Croat TK, Stadermann FJ, Bernatowicz TJ (2005) Presolar graphite from AGB stars: microstructure and s-process enrichment. Astrophys J 631:976–987

Croat TK, Stadermann FJ, Bernatowicz TJ (2008) Correlated isotopic and microstructural studies of turbostratic presolar graphites from the Murchison meteorite. Meteorit Planet Sci 43:1516–1794

Dobrzhinetskaya LF, Green HW, Weschler M, Darus M, Wang YC, Massonne HJ, Stöckhert B (2003) Focused ion beam technique and transmission electron microscope studies of microdiamonds from the Saxonian Erzgebirge, Germany. Earth Planet Sci Lett 210:399–410

Giannuzzi LA, Drown JL, Brown SR, Irwin RB, Stevie FA (1998) Applications of the FIB lift-out technique for TEM specimen preparation. Microsc Res Tech 41:285–290

Groopman E, Nittler LR, Bernatowicz T, Zinner E (2014) NanoSIMS TEM, and XANES studies of a unique presolar supernova graphite grain. Astrophys J 790:9–22

Klaar HJ, Hsu FY (1996) A new preparation method for cross-sectional TEM specimens. Mater Charact 36:365–369

Li J, Malis T, Dionne S (2006) Recent advances in FIB-TEM specimen preparation techniques. Mater Charact 57:64–70

Lin YT, Amari S, Pravdivtseva O (2002) Presolar grains from the Qingzhen (EH3) meteorite. Astrophys J 575:257–263

Müller E, Krumeich F (2000) A simple and fast TEM preparation method utilizing the pre-orientation in plate-like, needle-shaped and tubular materials. Ultramicroscopy 84:143–147

Nakamura T, Noguchi T, Tanaka M, Zolensky ME, Kimura M, Tsuchiyama A, Nakato A, Ogami T, Ishida H, Uesugi M (2011) Itokawa dust particles: a direct link between S-type asteroids and ordinary chondrites. Science 333:1113–1116

Overwijk M, Van den Heuvel F, Bulle-Lieuwma C (1993) Novel scheme for the preparation of transmission electron microscopy specimens with a focused ion beam. J Vac Sci Technol B Microelectron Nanometer Struct Process Meas Phenom 11:2021–2024

Stroud RM, Nittler LR, Alexander CM (2004) Polymorphism in presolar Al_2O_3 grains from asymptotic giant branch stars. Science 305:1455–1457

Tomus D, Ng HP (2013) In situ lift-out dedicated techniques using FIB-SEM system for TEM specimen preparation. Micron 44:115–119

Xu Y, Lin Y, Zhang J, Hao J (2016) The first discovery of presolar graphite grains from the Qingzhen (EH3) meteorite. Astrophys J 825:111–122

Zega TJ, Nittler LR, Busemann H, Hoppe P, Stroud RM (2007) Coordinated isotopic and mineralogic analyses of planetary materials enabled by in situ lift-out with a focused ion beam scanning electron microscope. Meteorit Planet Sci 42:1373–1386

Zinner E (2014) Presolar Grains. In: Holland HD, Turekian KK (eds) Treatise on geochemistry, 2nd edn. Elsevier, Oxford, pp 181–213