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TITLE:

Magnetic Field Mapping Using Off-Axis Electron Holography in the Transmission Electron Microscope

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KEYWORDS:

Off-axis electron holography, transmission electron microscopy, electron optical phase, Lorentz microscopy, magnetic characterization, magnetic field mapping, Bloch-type skyrmions

SUMMARY:

Guidelines are presented for recording and interpreting off-axis electron holograms to provide quantitative images of magnetic fields in nanoscale materials and devices in the transmission electron microscope.

ABSTRACT:

Off-axis electron holography is a powerful technique that involves the formation of an interference pattern in a transmission electron microscope (TEM) by overlapping two parts of an electron wave, one of which has passed through a region of interest on a specimen and the other is a reference wave. The resulting off-axis electron hologram can be analyzed digitally to recover the phase difference between the two parts of the electron wave, which can then be interpreted to provide quantitative information about local variations in electrostatic potential and magnetic induction within and around the specimen. Off-axis electron holograms can be recorded while a specimen is subjected to external stimuli such as elevated or reduced temperature, voltage, or light. The protocol that is presented here describes the practical steps that are required to record, analyze, and interpret off-axis electron holograms, with a primary focus on the measurement of magnetic fields within and around nanoscale materials and devices. Presented here are the steps involved in the recording, analysis, and processing of off-axis electron holograms as well as the reconstruction and interpretation of phase images and visualization of the results. Also discussed are the need for optimization of the specimen geometry, the electron optical configuration of the microscope, and the electron hologram acquisition parameters as well as the need for the use of information from multiple holograms to extract the desired magnetic contributions from the recorded signal. The steps are illustrated through a study of specimens

of B20-type FeGe, which contain magnetic skyrmions and were prepared with focused ion beams (FIBs). Prospects for the future development of the technique are discussed.

INTRODUCTION:

Magnetic nanostructures are increasingly used in applications that include nanoscale logic, storage, and spintronic devices^{1–5}. A local understanding of the magnetic properties of the constituent materials requires the development of techniques for magnetic characterization with nanometer (nm) spatial resolution, both in projection and in three dimensions, ideally while the specimen is subjected to external stimuli such as elevated or reduced temperature, applied voltage, or light. Currently-available magnetic characterization techniques include magneto-optical Kerr effect microscopy, magnetic force microscopy, spin-polarized scanning tunneling microscopy, spin-polarized low-energy electron microscopy, X-ray magnetic circular dichroism, X-ray holography, and scanning transmission X-ray microscopy^{6–11}.

In transmission electron microscopy, magnetic characterization techniques include the Fresnel and Foucault modes of Lorentz microscopy, off-axis electron holography, differential phase contrast (DPC) imaging, and electron magnetic circular dichroism (EMCD)^{6,7,12–14}. The focus of this paper is on the technique of off-axis electron holography, which is capable of providing real-space quantitative measurements of magnetic fields inside and around nanoscale materials with sub-5-nm spatial resolution, both in projection and when combined with electron tomography in three dimensions^{13,14}.

In the TEM, a highly accelerated electron beam is passed through an electron-transparent (usually solid) specimen to provide access to its crystallographic, chemical, electronic, and/or magnetic structure with a spatial resolution that can reach the atomic scale. Typically, a thin (<100 nm) specimen is irradiated with electrons that are emitted from an electron gun and accelerated by 60–300 kV in a high-vacuum (<10⁻⁵ Pa) column. Electromagnetic lenses are used to focus electrons onto the specimen and subsequently onto one or more detectors. The electrons interact strongly with the atomic potentials in the specimen and with electromagnetic fields within and around it. Although this information is encoded in the electron wavefunction, an in-focus bright-field or dark-field TEM image records only variations in the intensity of electrons that reach a detector, while information about their phase shift is lost. This so-called “phase problem” is also encountered in X-ray and neutron experiments.

One of the techniques that allows the measurement of the phase shift of the electron wavefunction is off-axis electron holography. Further details about fundamental aspects of electron wave functions are available elsewhere¹⁵. The concept of electron holography was first proposed by Denis Gabor in 1948 to overcome limitations in the spatial resolution of electron microscopy due to aberrations of the primary imaging lens of the microscope¹⁶. The technique allows information about both the amplitude and the phase of an electron wave to be recorded. It has been readily available for commercial electron microscopes since the 1990s, in part, owing to developments in field emission gun technology. Although more than 20 variations of electron holography have been described, the most popular and versatile

type is currently the TEM mode of off-axis electron holography¹⁷ for electromagnetic field mapping with high spatial resolution^{18–23}.

The TEM mode of off-axis electron holography involves the formation of an interference pattern or hologram by overlapping two parts of an electron wave (**Figure 1A**), one of which has passed through a region of interest on the specimen and the other is a reference wave²⁴. The phase shift ϕ can be retrieved digitally from a recorded off-axis electron hologram and interpreted to provide quantitative information about local variations in the electrostatic potential and magnetic vector potential by using **Equation 1**²⁵.

$$\phi(x, y) = C_E \int_{-\infty}^{+\infty} V(x, y, z) dz - \frac{2\pi e}{h} \int_{-\infty}^{+\infty} A_z(x, y, z) dz, \quad (1)$$

where C_E is an interaction parameter that depends on the microscope accelerating voltage ($C_E = 6.53 \times 10^6 \text{ rad/(Vm)}$ at 300 kV), $V(x, y, z)$ is the electrostatic potential, $A_z(x, y, z)$ is the z component of the magnetic vector potential, z is parallel to the incident electron beam direction, e is an elementary unit of charge, and h is Planck's constant. The electrostatic and magnetic contributions to the phase shift can be separated, for example, by combining information from electron holograms recorded before and after turning the specimen over, from electron holograms recorded below and above the magnetic Curie temperature of the specimen, or from electron holograms recorded at different microscope accelerating voltages^{13,26}. Once the magnetic contribution to the phase shift ϕ_m (i.e., the second term on the right side of **Equation 1**) has been retrieved, the in-plane magnetic induction projected in the electron beam direction, B_p , can be obtained from its first derivatives by using **Equation 2**.

$$B_p = (B_{p,x}, B_{p,y}) = \frac{h}{2\pi e} \left(-\frac{d\phi_m}{dy}, \frac{d\phi_m}{dx} \right) \quad (2)$$

where $B_{p,x} = \int_{-\infty}^{+\infty} B_x(x, y, z) dz$ and $B_{p,y} = \int_{-\infty}^{+\infty} B_y(x, y, z) dz$. A magnetic induction map can then be displayed using contours and colors to provide a visual representation of the magnetic field of a thin film or nanostructure^{26–31}, as described below. Magnetic phase images and magnetic induction maps should always be interpreted with great care: first, because they represent two-dimensional projections of three-dimensional (3D) magnetic vector fields; second, because they are insensitive to out-of-plane components of the magnetic field B_z ; and third, because they combine information from magnetic fields that are present both inside and outside the specimen. Fortunately, it is now possible to recover 3D magnetic information from tomographic tilt series of magnetic phase images by using either backprojection-based^{32–37} or model-based^{38–40} reconstruction algorithms.

Transmission electron microscopic studies of the magnetic properties of materials are usually carried out with the specimen in magnetic-field-free conditions, i.e., after turning off the conventional microscope objective lens and using either a non-immersion Lorentz lens or the transfer lenses of an image aberration corrector as the primary imaging lens. The use of an additional specimen stage located between the condenser and objective lenses⁴¹ or a double objective lens system to cancel the magnetic field at the specimen position⁴² can also help achieve magnetic-field-free conditions. The recording of images with the specimen located in magnetic-field-free conditions is often referred to as Lorentz microscopy. Lorentz

transmission electron microscopy is a rapid technique to check the magnetic state of the specimen in the presence of external stimuli. However, it is usually only applied qualitatively and is not readily applicable to studies of magnetic fields in the smallest nanostructures, in part due to the presence of Fresnel fringes from local changes in specimen thickness. Depending on the specification of the microscope and the specimen of interest, a variety of different imaging, diffraction, or spectroscopy techniques (e.g., DPC imaging and EMCD) can be used to perform magnetic characterization in transmission electron microscopy.

Off-axis electron holography is often applied in combination with the simpler, albeit less quantitative, technique of Fresnel defocus imaging (i.e., the Fresnel mode of Lorentz microscopy), particularly for studies of magnetic domain walls. Just as for off-axis electron holography, the contrast in Fresnel defocus images arises from the refraction of electrons by the in-plane component of the magnetic field within and outside the specimen. To a first approximation, an in-plane magnetic field B_{xy} in a specimen of thickness t results in deflection of the incident electron beam by an angle $\vartheta = \frac{e\lambda}{h} B_{xy} t$, where λ is the (relativistic) electron wavelength. When using Fresnel imaging, the positions of magnetic domain walls are revealed as lines of dark or bright intensity in defocused bright-field images. Phase information can be recovered from such images by solving the transport-of-intensity equation⁴³. However, a lack of knowledge of boundary conditions at the edges of the field of view can result in errors in the reconstructed phase.

In contrast, when using the Foucault mode of Lorentz microscopy, an aperture is used to allow only electrons that have been deflected in a specific direction to contribute to image formation. It should be noted that DPC imaging in scanning transmission electron microscopy and the Fresnel mode of Lorentz microscopy record signals that are approximately proportional to the first and second derivatives of the phase shift of the electron wave, respectively. As a result, they can contain strong contributions from local changes in specimen thickness and composition, which can dominate magnetic contributions to the contrast^{6,7}. From an experimental perspective, the TEM mode of off-axis electron holography requires the use of an electrostatic biprism, which usually takes the form of a thin conductive wire that is positioned close to one of the conjugated image planes in the microscope. The application of a voltage to the biprism to overlap the object and reference electron waves (**Figure 1A**) results in the formation of an electron hologram, which can be recorded on a charge-coupled device (CCD) camera or a direct electron counting detector⁴⁴.

The condenser lens stigmator settings are typically adjusted to make the electron beam highly elliptical to maximize the lateral coherence of the beam in a direction perpendicular to the biprism, while retaining a sufficient number of electron counts. The region of interest on the specimen is positioned so that it covers part of the field of view, while a reference hologram is usually obtained from an adjacent region of vacuum or a region of thin clean support film. The experiments that are described below were carried out in an image-aberration-corrected TEM operated at 300 kV. This microscope has a large (11 mm) pole-piece gap and is equipped with two electron biprisms (**Figure 1B**). In these experiments, only one of the prisms was used to record electron holograms. The advantages of using multiple biprisms are described elsewhere^{45,46}. Fresnel defocus images and off-axis electron holograms were recorded using either a conventional 2k x 2k CCD camera or a 4k x 4k direct electron counting detector. Lorentz mode was set up by adjusting the objective lens to a small negative excitation to

187 achieve a magnetic-field-free environment at the specimen position by compensating for the
188 residual magnetic field of the objective and nearby lenses.

189
190 The first transfer lens of the image corrector unit was then used as a non-immersion imaging
191 lens. Specimens could be imaged either at remanence (in zero magnetic field) or in the
192 presence of a pre-calibrated magnetic field⁴⁷, which could be applied by exciting the
193 conventional microscope objective lens. The twin structure of the objective lens in this
194 microscope allows magnetic fields of approximately 200 mT to be applied in both negative
195 and positive vertical directions to study magnetization reversal processes in situ in the TEM
196 by tilting the specimen in the presence of an applied vertical magnetic field. Although in-plane
197 magnetic fields can, in principle, be applied using dedicated magnetizing specimen holders,
198 such a holder was not used in the present work.

201 **PROTOCOL:**

203 **1. Electron microscope alignment**

204
205 1.1. **Switch the microscope** (see **Table of Materials** for details and **Figure 1B** in the
206 representative results section) **to Lorentz mode by loading a dedicated alignment file.**

207
208 1.1.1. **Load the specimen (e.g., a micron-sized lamella attached to a 3-mm diameter Cu grid;**
209 **see Figure 1C in the representative results section for specimen preparation details; here, the**
210 **investigated material is skyrmion-hosting B20-type FeGe) into a TEM specimen holder** (see
211 **Table of Materials** for details).

212
213 **1.1.2. Perform standard preparation of the microscope (e.g., filling of the cold trap) and**
214 **alignment (e.g., electron beam shift, pivot points, rotation center, condenser lens**
215 **astigmatism, and eucentric height of the specimen).**

216
217 NOTE: Re-alignment of the microscope may be required following changes to the microscope
218 settings (imaging mode, objective lens current, gun lens, spot size), biprism, specimen
219 position, or temperature.

220
221 **1.2. Correct the two-fold astigmatism of the Lorentz lens using a thin amorphous region**
222 **on the specimen by monitoring the Fourier transform of such a region in real time using the**
223 **image recording and processing software that is used to control the detector.**

224
225 NOTE: If there is no amorphous region on the specimen, then a separate cross-grating or
226 amorphous carbon thin film can be used for this purpose. The specimen of interest then needs
227 to be loaded into the microscope after alignment and aberration correction have been
228 completed. This note also applies to step 1.3.

229
230 **1.3. If applicable, tune the image aberration corrector of the microscope using appropriate**
231 **software.**

NOTE: Higher-order aberration correction may not be required for electromagnetic field mapping with nm spatial resolution.

1.4. Adjust the magnification of the microscope for the desired field of view, ideally including a region of vacuum across at least 10% of the image.

1.5. Move the specimen away from the field of view.

1.5.1. Insert the biprism, and orient it with respect to the specimen (usually parallel to the specimen edge).

1.5.2. Apply a desired voltage to the biprism, typically at a rate of no more than 1 V/s to avoid damage.

1.6. Set up an elliptical electron beam condition by tuning the condenser lens astigmatism, and center the electron beam.

NOTE: To avoid damage, do not focus the beam onto the biprism.

1.7. Maximize the holographic interference fringe contrast by fine-tuning the gun and condenser lens astigmatism settings.

NOTE: The fringe contrast and spacing can be monitored on the fly using acquisition control software.

1.8. Wait for 15–30 min to allow the electron beam, microscope, biprism, and specimen to stabilize.

NOTE: The choice of magnification and biprism voltage depend on the desired field of view, spatial resolution, and signal-to-noise-ratio in the reconstructed phase.

1.9. Determine the magnification according to the size of the region of interest.

NOTE: A small vacuum region (~10% of the field of view) should ideally be included in the hologram.

1.10. Adjust the biprism voltage according to the desired overlap width and spatial resolution.

NOTE: The electron optical spatial resolution is at best two or three times the holographic interference fringe spacing, depending on the mask size used for reconstructing the hologram (see the representative results section for details). The magnification and biprism voltage may need to be optimized iteratively. In general, the greater the magnification or the lower the biprism voltage, the better are the fringe contrast and signal-to-noise ratio in the reconstructed phase, but the poorer is the spatial resolution.

2. Region of interest

280
281 **2.1.** Select the area on the specimen by moving the region of interest into the field of view.
282

283 NOTE: The region of interest should normally be close to the specimen edge (or to a region of
284 thin clean support film), as a reference wave is needed for interference with the object wave
285 that travels through the region of interest on the specimen, and a vacuum region (ideally
286 ~10% of the field of view) should be included in the hologram.
287

288 2.2. If required, adjust the specimen temperature using the temperature controller of the
289 TEM specimen holder. Cool the cold trap of the microscope before cooling the specimen.
290 Make sure that the column is in the best possible vacuum condition to avoid the deposition
291 of contamination or ice on the specimen.
292

293 NOTE: Additional waiting time may be needed for stabilization of the specimen temperature.
294

295 **3. Lorentz TEM (Fresnel defocus imaging)**

296
297 3.1. Remanent magnetic state of the specimen
298

299 3.1.1. For Fresnel defocus imaging, switch back to round beam illumination. If required,
300 move the biprism from the field of view.
301

302 NOTE: The condenser lens settings for round and elliptical beam illumination can typically be
303 saved and recalled using electron microscope control software.
304

305 3.1.2. Change the defocus of the Lorentz lens (e.g., in multiples of $\pm 200 \mu\text{m}$) to record Fresnel
306 defocus images. Control the acquisition of defocused images by using a script in the
307 microscope control software.
308

309 3.1.3. Set the desired exposure time, and record in-focus, underfocus, and overfocus images
310 using the camera control software (see **Figure 2** in the representative results section).
311

312 **3.2. Field evolution of the specimen**

313
314 3.2.1. Change the magnetic field applied to the specimen by tuning either the current of the
315 conventional microscope objective lens while remaining in Lorentz mode, or if applicable, the
316 current in the coils of a magnetizing TEM specimen holder.
317

318 3.2.2. Adjust the eucentric height of the specimen and the defocus.
319

320 3.2.3. Check the alignment of the microscope (see section 1.1).
321

322 3.2.4. Set the exposure time, and record in-focus, underfocus, and overfocus images using
323 the camera control software.
324

325 3.2.5. Change the applied magnetic field, specimen tilt, and/or temperature (using the
326 temperature controller for the TEM specimen holder), as required, to follow the magnetic

response of the specimen, as well as to select a suitable condition for off-axis electron holography.

4. Off-axis electron holography

4.1. Zero-field magnetic state of the specimen

4.1.1. Switch back to an elliptical beam condition, and bring the biprism to the center of the field of view.

4.1.1.1. Check the alignment of the specimen, biprism, and microscope.

4.1.1.2. Focus the specimen after waiting for 10–30 min for stable conditions.

4.1.2. Place the region of interest on the specimen within the field of view (see step 2.1).

4.1.3. Set the desired exposure time, and record single or multiple electron holograms of the specimen using the control software of the camera.

4.1.4. Translate the region of interest on the specimen from the field of view, and record single or multiple reference holograms using the control software of the camera.

NOTE: The exposure time and the number of holograms in steps 4.1.3 and 4.1.4 are typically chosen to be the same.

4.2. Field evolution of the specimen

4.2.1. Change the magnetic field applied to the specimen (see step 3.2.1).

4.2.2. Set up the elliptical beam condition, which typically depends on the objective lens setting.

4.2.3. Realign the specimen, biprism, and microscope (see step 1.6). Focus the specimen.

4.2.4. Place the region of interest within the field of view.

4.2.5. Set the desired exposure time, and record single or multiple electron holograms of the specimen using the control software of the camera.

4.2.6. Translate the region of interest on the specimen from the field of view, and record single or multiple reference holograms using the control software of the camera.

NOTE: The exposure time and the number of holograms in steps 4.2.5 and 4.2.6 are typically chosen to be the same.

4.2.7. Repeat the above steps for each desired value of applied field and/or applied voltage or specimen temperature, as well as before and after turning the specimen over.

4.2.7.1. Pay careful attention to the experimental design to record a dataset that can be used to separate the magnetic contribution to the phase from the electrostatic contribution.

4.2.7.2. Check whether changes in external stimuli result in instabilities of the microscope, beam, biprism, and/or specimen, and if an additional waiting time is then required to achieve stability.

4.2.7.3. Take particular care to design experiments that can be used to achieve a separation of the magnetic contribution to the phase from the electrostatic contribution.

NOTE: There are several ways to achieve this separation¹³. Here, the specimen temperature has been used to switch the specimen to a paramagnetic state and subsequently evaluate differences between phase images recorded at different specimen temperatures.

4.3. Process the recorded electron holograms digitally.

4.3.1. Reconstruct the electron holograms to calculate amplitude and phase images using commercial or home-written software.

NOTE: Normally, a Fourier-transform-based reconstruction approach is used (see the representative results section for details of hologram reconstruction).

4.3.2. Align the images in magnification, position and angle, and, if required, remove geometric distortions from them. Combine information from multiple phase images to separate the magnetic from the electrostatic contribution to the phase (see **Figure 3** in the representative results section for details).

REPRESENTATIVE RESULTS:

The results shown below are taken from a Lorentz microscopy and off-axis electron holography study of magnetic skyrmions in a single crystalline FeGe specimen.

TEM specimen preparation. Electron-transparent specimens of single crystalline B20-type FeGe were prepared for TEM examination using a dual-beam scanning electron microscope equipped with a Ga FIB, a micromanipulator, and gas injection systems. FIB milling was performed using 30 and 5 kV ion beams with currents between 6.5 nA and 47 pA. A lift-out method⁴⁸ was used to fabricate a lamella, which was attached to a Cu grid (**Figure 1C**). To reduce thickness variations from curtaining, amorphous C was deposited on the crystal before FIB milling. Remaining ion-beam-induced damage was reduced by using low-energy (<1 keV) Ar ion beam sputtering⁴⁹. The final specimen had approximate values of width, height, and thickness of 15, 10, and 0.1 μm , respectively.

Magnetic imaging–Lorentz microscopy. The magnetic state of the FeGe specimen, which is expected to follow the magnetic field vs temperature phase diagram, shown in **Figure 4A**, was

first studied using Lorentz microscopy by recording Fresnel defocus images, both at room temperature and at reduced temperature (below the Curie temperature of FeGe).

B20-type FeGe is paramagnetic at room temperature. Below a transition temperature of 278 K (i.e., the Curie temperature), different magnetic configurations can form, depending on the applied magnetic field⁵⁰. In the present study, images were recorded both at room temperature and at reduced temperature using a double-tilt, liquid-nitrogen-cooled TEM specimen holder. The specimen temperature was monitored and controlled using a temperature controller and the control software of the camera. Below the transition temperature, FeGe typically contains a helical magnetic structure in zero applied magnetic field. This magnetic texture produces maze-like lines of black and white contrast in Lorentz (Fresnel defocus) images of thin TEM specimens, as shown in **Figure 4B**.

Characteristic magnetic parameters of FeGe, such as its exchange constant, Dzyaloshinskii-Moriya interaction constant, and saturation magnetization M , determine the equilibrium period of the helical phase (~ 70 nm), as well as the critical field for magnetic saturation (320 mT). A lattice of Bloch-type skyrmions can be formed from the helical state by applying an out-of-plane magnetic field to the specimen using slight excitation of the objective lens. In the microscope used in the present study, an excitation of 6% provides a field of ~ 100 mT. The typical defocus values that are required to image a helical structure or a lattice of skyrmions are in the range of $300\ \mu\text{m}$ – $1\ \text{mm}$, depending on the TEM specimen thickness.

Figure 2 shows Fresnel defocus images of Bloch-type skyrmions recorded at a specimen temperature of 100 K in the presence of an out-of-plane magnetic field of 100 mT, which was applied using the conventional microscope objective lens. Cooling of the specimen in the presence of an applied magnetic field results in the formation of a regular close-packed lattice of Bloch-type skyrmions⁵¹. Depending on the sign of the defocus, the contrast of each skyrmion appears as an intensity maximum or minimum, as shown in **Figure 2A** and **Figure 2B**, respectively.

Magnetic imaging–Off-axis electron holography. Off-axis electron holograms of the FeGe lamella prepared with FIBs were recorded both at room temperature and below the critical temperature using the procedure described above.

Figure 3A shows an off-axis electron hologram recorded from B20-type FeGe at a specimen temperature of 200 K following cooling in the presence of a 100 mT applied magnetic field. A voltage of 120 V was applied to the biprism, resulting in a holographic interference fringe spacing of 2.69 nm and holographic interference fringe contrast of $\sim 25\%$.

Reconstruction of the amplitude and phase involved digitally selecting one of the side bands in the Fourier transform of the hologram (**Figure 3B**), masking everything outside a circular mask with a soft edge centered on the side band to zero, centering the masked side band in Fourier space, and calculating its inverse Fourier transform to provide a real-space complex wave image that contains both amplitude and phase information. The phase $\phi = \arctan(i/r)$ and amplitude $A = \sqrt{r^2 + i^2}$ of the real-space complex wavefunction were evaluated from its real part r and imaginary part i . The phase (**Figure 3C**) was initially evaluated modulo 2π and therefore contained phase discontinuities, which could be unwrapped using a suitable

algorithm to provide an unwrapped phase image (**Figure 3D**). Further details of the reconstruction procedure and corresponding open-source software can be found elsewhere^{52–54}.

A similar approach was used to record a reference hologram from vacuum alone. The reconstructed phase of the reference hologram was subtracted from that of the specimen hologram to remove phase artifacts associated with the imaging and recording system of the microscope. The specimen was then heated to room temperature, and both specimen holograms (**Figure 3E**) and vacuum reference holograms were recorded using the same procedure as that at reduced temperature. As FeGe is paramagnetic at room temperature, the electron optical phase shift originates purely from the electrostatic (mean inner potential) contribution to the phase. The difference between aligned phase images recorded at room temperature and reduced temperature (after correction using vacuum reference holograms) was used to provide the magnetic phase shift alone (**Figure 3F**). Subtraction of the phase images required sub-pixel alignment. The final magnetic phase image provides information about the in-plane component of the magnetic induction within and around the specimen integrated in the electron beam direction (see **Equation 2**).

A visual representation of the projected in-plane magnetic induction can be obtained by adding contours to the magnetic phase image (e.g., by evaluating the cosine of its chosen multiple). Its derivatives can also be used to generate colors, whose hue and intensity can be used to represent the direction and magnitude of the projected in-plane magnetic induction, respectively. **Figure 5A** shows a representative magnetic phase image of Bloch-type skyrmions in FeGe obtained from the off-axis electron holography results shown in **Figure 3**. A corresponding magnetic induction map is shown in **Figure 5B**.

A magnetic phase image can be analyzed further to determine the projected in-plane magnetization in the specimen using either a model-independent⁵⁵ or a model-based⁴⁰ algorithm. **Figure 5C** shows a map of in-plane magnetization determined from the magnetic phase image shown in **Figure 5A** using a model-based iterative reconstruction algorithm⁴⁰, together with an independent measurement of the TEM specimen thickness performed using electron energy-loss spectroscopy. The magnetization is shown in units of kA/m and reveals the hexagonal shapes of the skyrmions, which result from their close-packed arrangement. The skyrmion cores, where the spins are oriented parallel to the electron beam direction, have sizes of ~8 nm. The measured magnetization peaks at a value of ~135 kA/m, which is in good agreement with an averaged value that considers the presence of surface twists and non-magnetic damaged specimen surface layers⁵⁶. A similar approach can be used to study the evolution of the spin texture systematically as a function of applied magnetic field and temperature.

FIGURE AND TABLE LEGENDS:

Figure 1: Basic off-axis electron holography setup and example specimen geometry. (A) Schematic ray path for off-axis electron holography. **(B)** Photograph of the transmission electron microscope used in this study. This microscope is equipped with a field emission gun, an image aberration corrector, a Lorentz lens, and two electron biprisms and was operated at

300 kV. (C) Secondary electron scanning electron microscopic image of a TEM specimen of B20-type FeGe, prepared with FIBs, attached to a Cu TEM support grid (see **Figure 2B** for a TEM image of the specimen). Scale bar = 500 μm . Abbreviations: OA = open aperture; Ltz = Lorentz; SA = selected area; e^- = electron; TEM = transmission electron microscope.

Figure 2: Fresnel defocus images of Bloch-type skyrmions in B20-type FeGe. (A) Underfocus and (B) overfocus images of Bloch-type skyrmions in a FeGe lamella prepared with focused ion beams and recorded at a specimen temperature of 100 K in the presence of an out-of-plane magnetic field of 100 mT. The defocus values are $\pm 500 \mu\text{m}$. The wide bands of wavy dark contrast are crystalline bend contours arising from diffraction contrast. The insets show magnified regions of the images. Scale bars = 2 μm .

Figure 3: Reconstruction of an off-axis electron hologram. (A) Experimental off-axis electron hologram of a B20-type FeGe lamella recorded at a specimen temperature of 200 K in the presence of an applied out-of-plane magnetic field of 100 mT. The inset shows a magnified region of the hologram; scale bar = 200 nm. (B) Fourier transform of the hologram containing a center band, two side bands, and streaks originating from Fresnel fringes at the edges of the biprism. An enlargement of one of the side bands reveals spots associated with the ordered arrangement of skyrmions; scale bar = 0.4 nm^{-1} . (C) Wrapped phase image obtained by inverse Fourier transformation of one of the side bands; scale bar = 200 nm. (D) Unwrapped phase image; scale bar = 200 nm. (E) Unwrapped phase image of the same region recorded at room temperature in zero out-of-plane applied magnetic field; scale bar = 200 nm. (F) Part of a final magnetic phase image obtained by subtracting the total phase image recorded at room temperature from that recorded at 200 K, after aligning them with sub-pixel precision; scale bar = 200 nm.

Figure 4: Magnetic field vs temperature phase diagram of FeGe. (A) Phase diagram of B20-type FeGe showing magnetic states vs temperature. (B) Fresnel defocus image of a representative helical magnetic state in a FeGe lamella prepared with FIBs and recorded at a specimen temperature of 260 K in zero applied magnetic field. Scale bar = 2 μm . Abbreviations: PM = paramagnetic phase; T_c = Curie temperature; FIBs = focused ion beams.

Figure 5: Quantitative analysis of magnetic phase shift. (A) Magnetic phase shift ϕ_m of Bloch-type skyrmions in B20-type FeGe recorded at a specimen temperature of 200 K in the presence of an applied out-of-plane magnetic field of 100 mT. (B) Magnetic induction map created by displaying the cosine of a multiple of the magnetic phase image and adding colors generated from its derivatives. The phase contour spacing is $2\pi/20 \sim 0.314$ radians. (C) Projected in-plane magnetization obtained from the magnetic phase image shown in (A) using a model-based iterative reconstruction algorithm. Scale bars = 50 nm.

DISCUSSION:

Off-axis electron holography provides fully quantitative measurements of the magnetic properties of nanoscale materials with nm spatial resolution, either in projection or in three dimensions when combined with electron tomography. These advantages make the technique distinct from X-ray and neutron-based techniques for the high spatial resolution

characterization of magnetic nanostructures. However, care is required in the design and execution of experiments, as well as during data analysis. Some of the factors to be considered are mentioned here. First, magnetic materials are, in general, sensitive to ion-beam-sputtering-induced artifacts, which can result in the formation of defective, amorphous, and/or non-magnetic layers on the surface of a TEM specimen. They may also need to be stored in an inert gas atmosphere or vacuum to prevent oxidation. Furthermore, TEM specimens prepared with FIBs are small and delicate. Therefore, mechanical tools, such as tweezers, are generally not recommended. Instead, vacuum tweezers can be used to insert specimens into TEM specimen holders.

Second, conventional cooling TEM specimen holders, such as the one used in the present study, can introduce specimen drift due to changes in the temperature of the specimen cradle, screws, and heat-transporting wires. Specimen drift typically slows down to an acceptable rate over a timeframe of 10–40 min. Third, during a cooling experiment, electron-beam-induced charging can be present, especially when studying a specimen that contains insulating materials. Charging can introduce slowly varying contributions to the electrostatic contribution to a recorded phase image, which can vary with specimen temperature, as well as with electron beam illumination and specimen position. Sometimes, coating the specimen with a thin layer of C can help to reduce electron-beam-induced charging.

Fourth, magnetic imaging experiments performed in the TEM require an electron-transparent specimen. The preparation of such specimens is briefly described above in the representative results section. When planning, conducting, and interpreting an experiment, the shape of the specimen must be considered, as results obtained from “thin films” may be different from those obtained from bulk samples. For example, magnetic domains are often smaller and demagnetizing fields are stronger in TEM specimens than in bulk materials. Nevertheless, magnetic properties, such as saturation magnetization and domain wall width measured from thin TEM specimens, typically match values obtained from bulk materials. Fifth, the application of a perpendicular magnetic field to a specimen using the conventional microscope objective lens changes the image magnification and rotation, which should be corrected before digital image alignment. Slight misalignment between phase images can result in the misinterpretation of misalignment artifacts as magnetic contrast.

Looking toward the future, electron holographic tomography provides a route to the 3D reconstruction of magnetic fields and magnetization distributions in materials based on either backprojection-based³⁴ or model-based⁴⁰ tomographic reconstruction algorithms. Such experiments require the acquisition and processing of large numbers of images including subtraction of the mean inner potential contribution to the phase at each specimen tilt angle. Tomographic experiments are susceptible to changes to the sample during extended experiments, changes in dynamical diffraction as a function of specimen tilt angle, artifacts resulting from the acquisition of incomplete datasets, as well as the effects of misalignment and image distortion. The automation of workflows for data acquisition and analysis promises to overcome some of these problems. Other future experimental developments may include the design of sophisticated magnetizing coils and approaches for performing time-resolved off-axis electron holography experiments of magnetic switching experiments during the application of multiple external stimuli to specimens.

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DISCLOSURES:

The authors have nothing to disclose.

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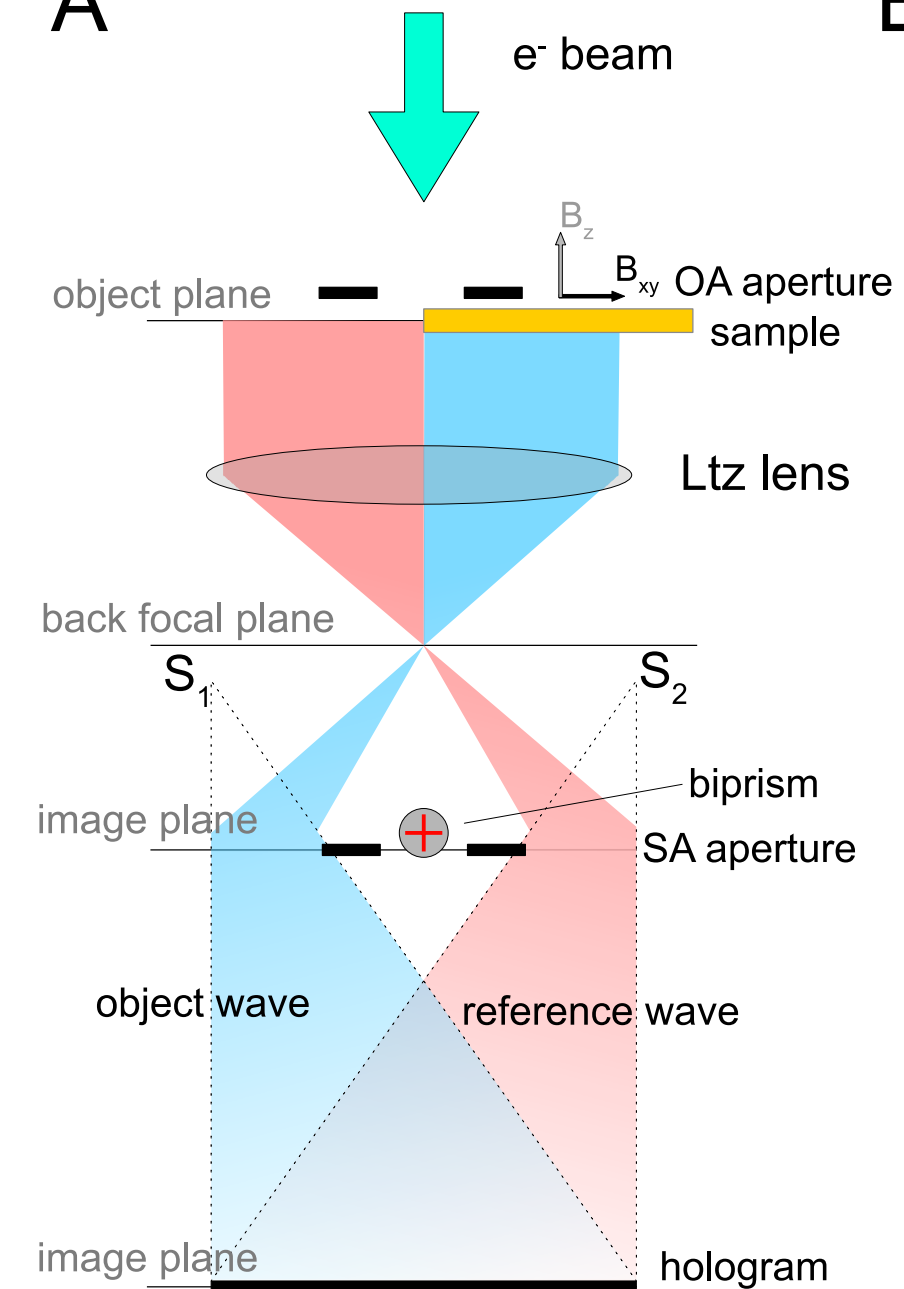
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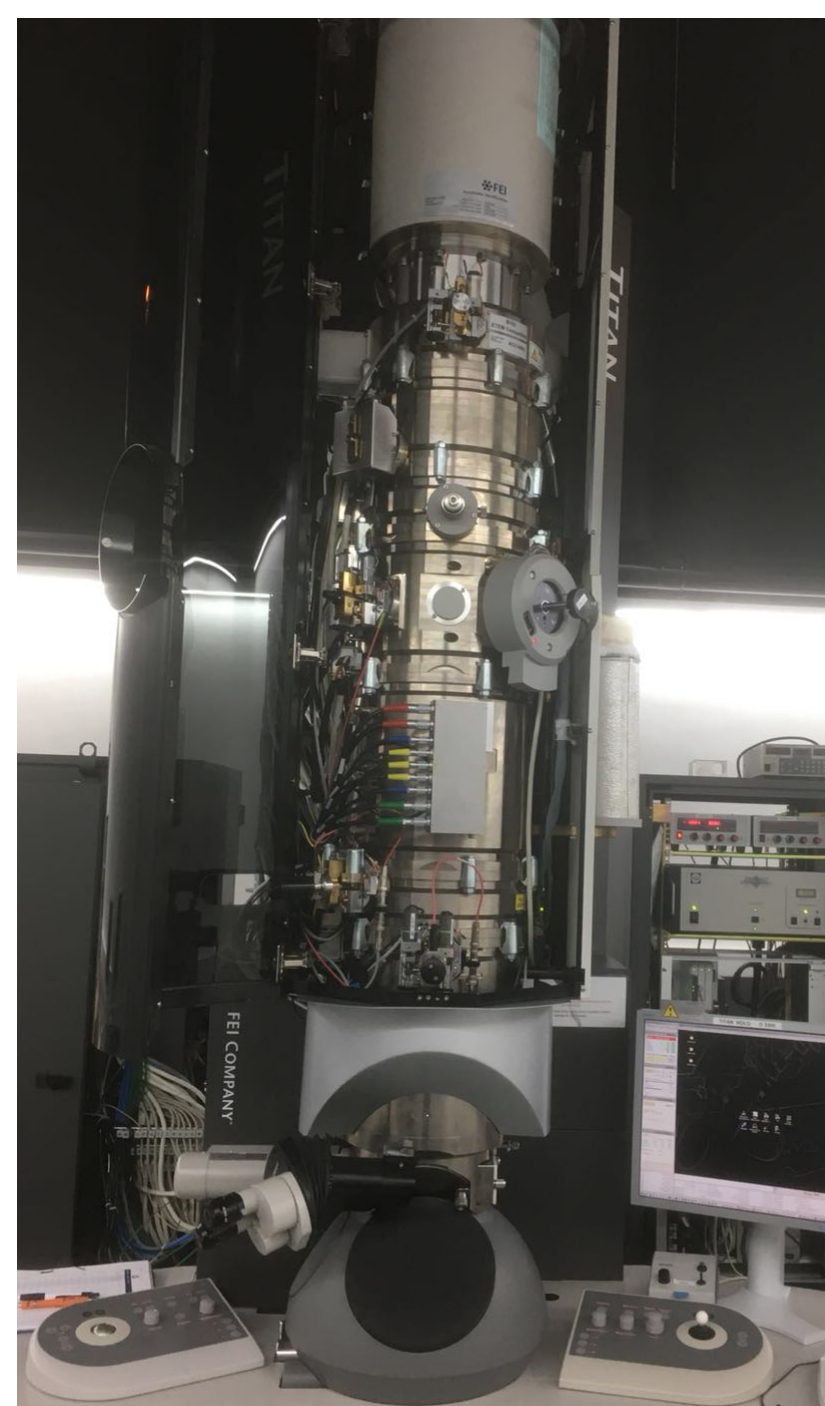
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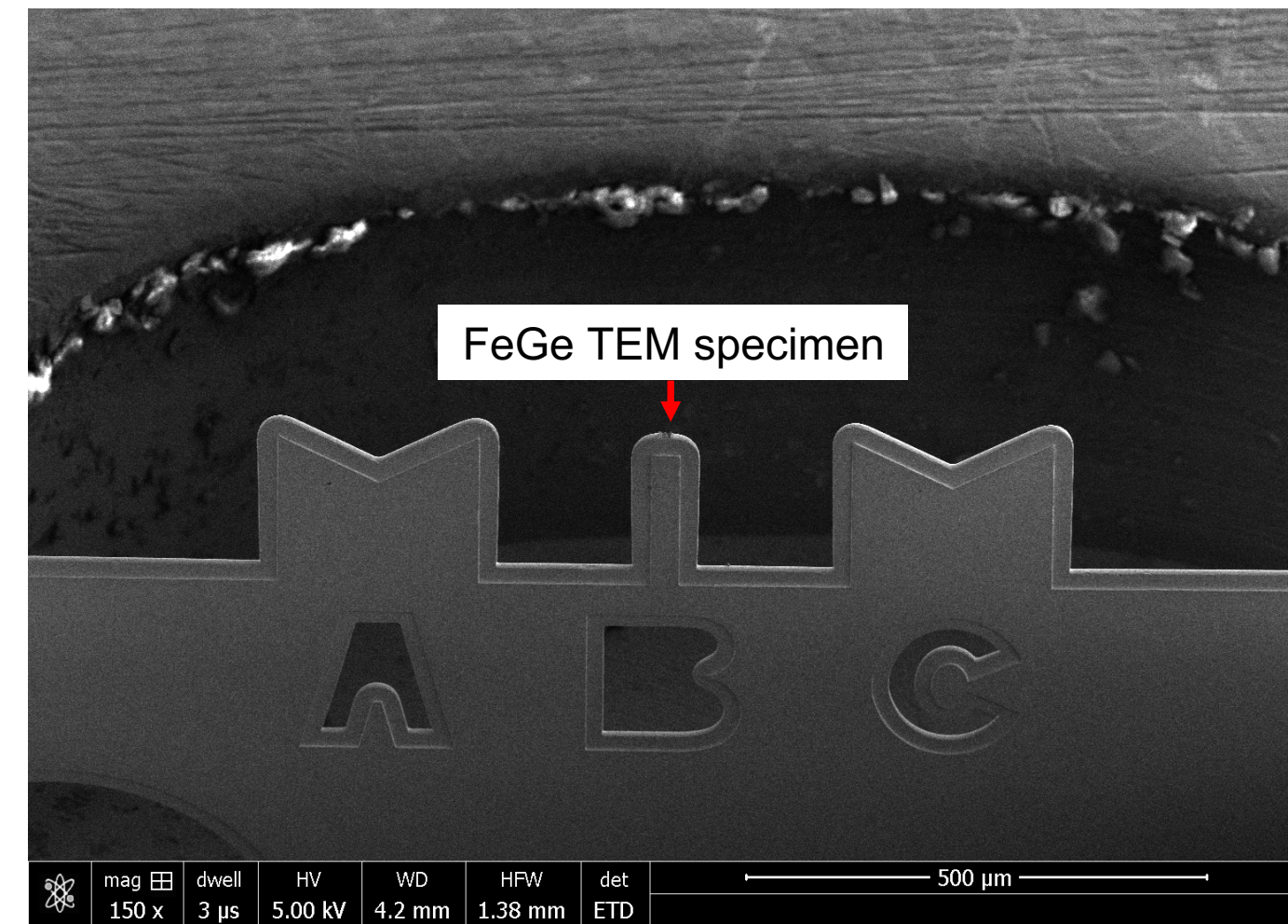
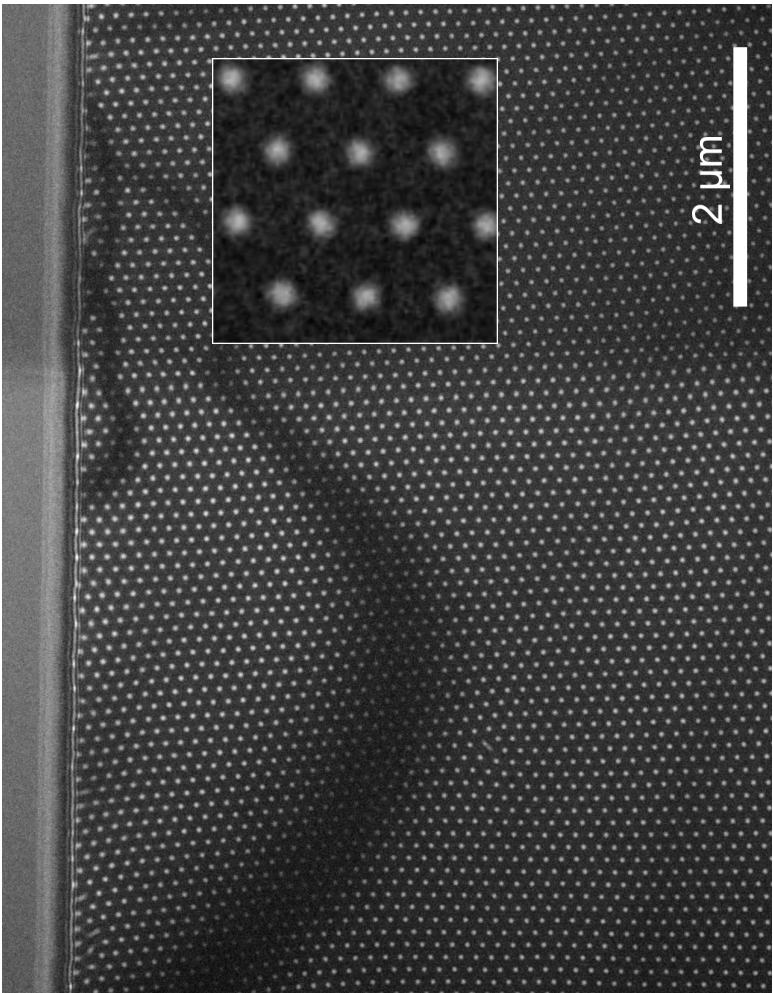
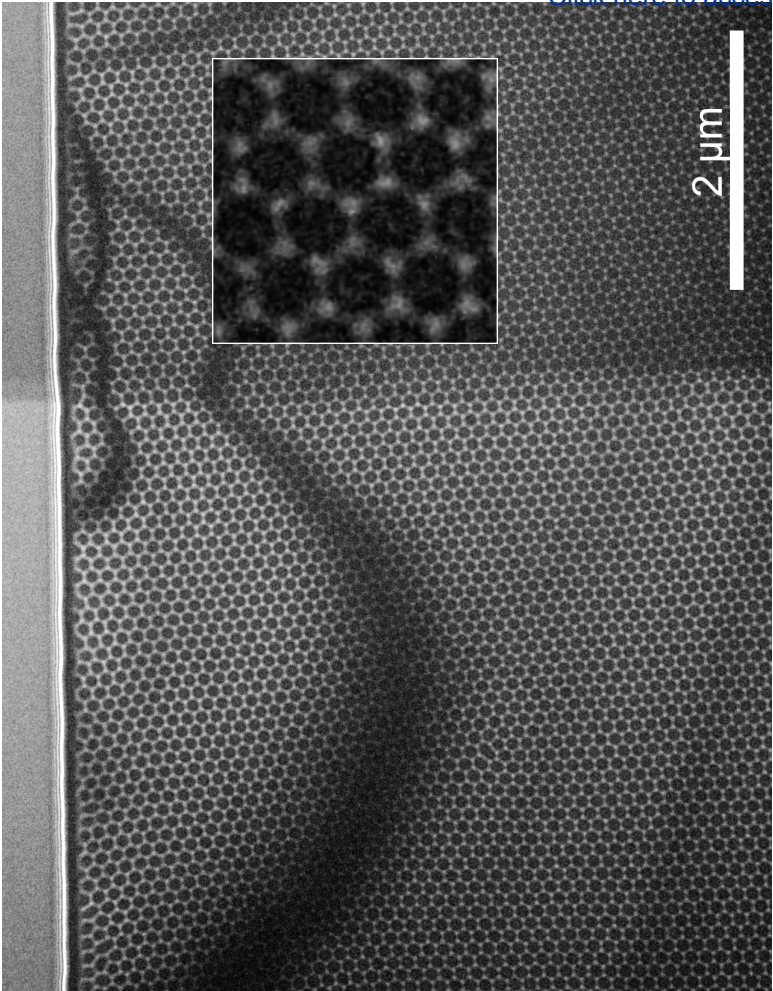


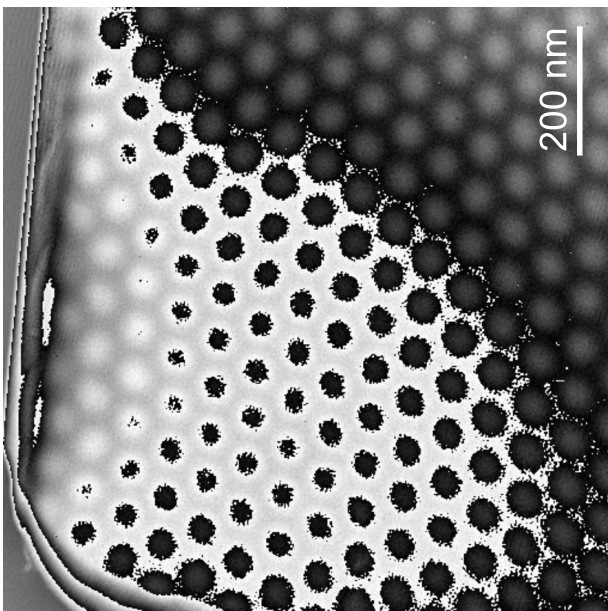
Figure 2



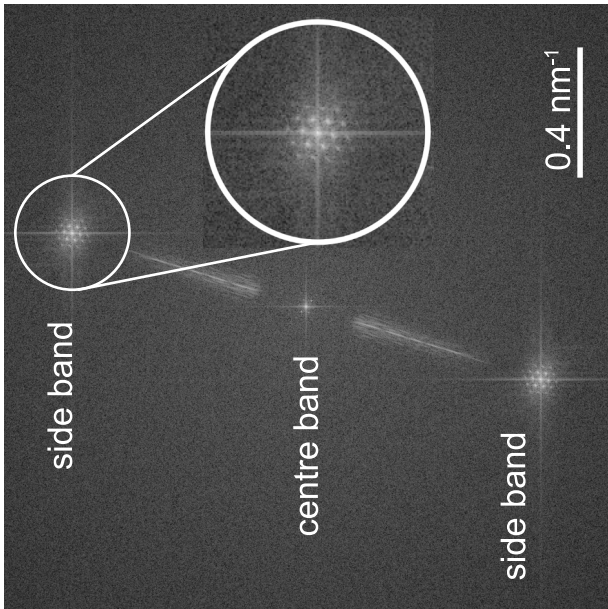
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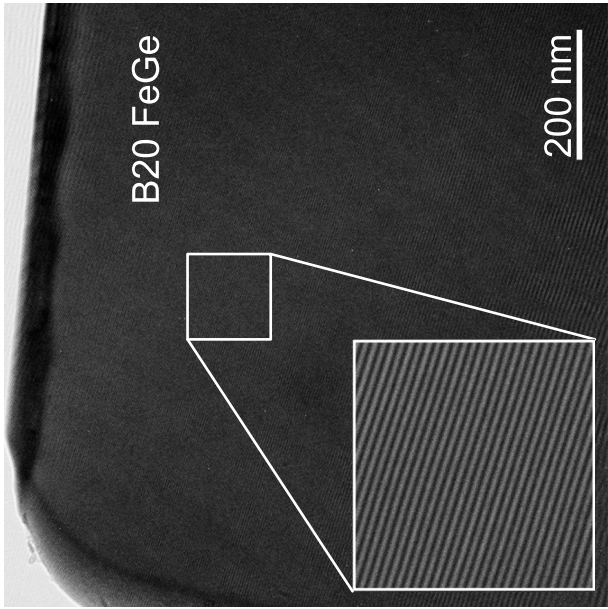
Figure 3



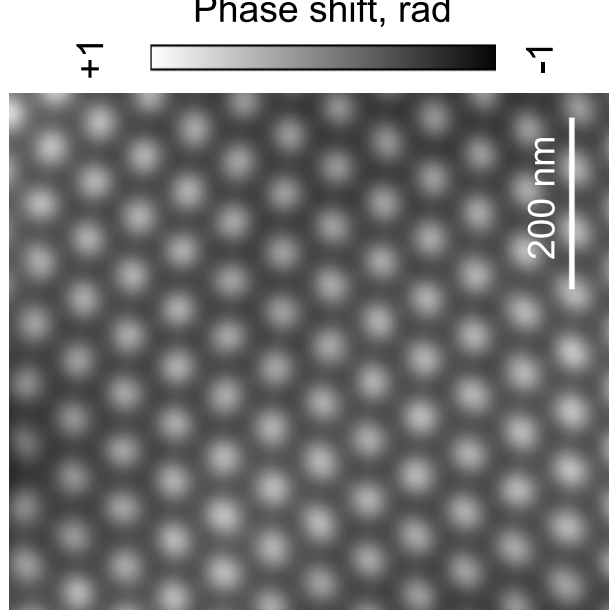
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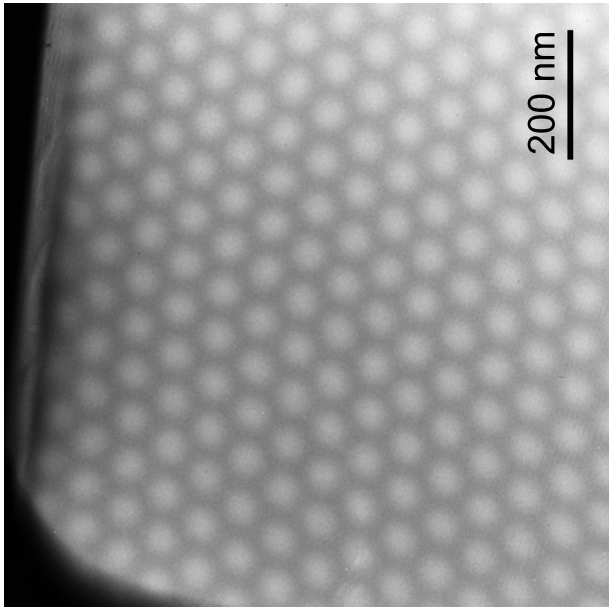
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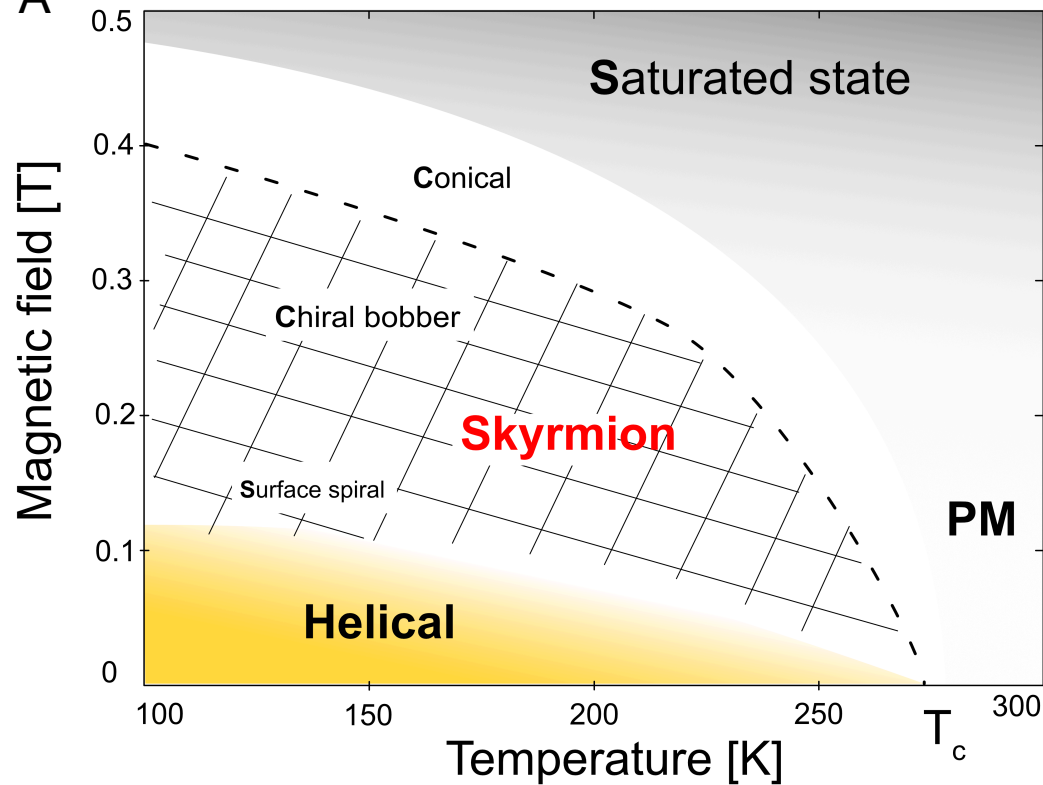
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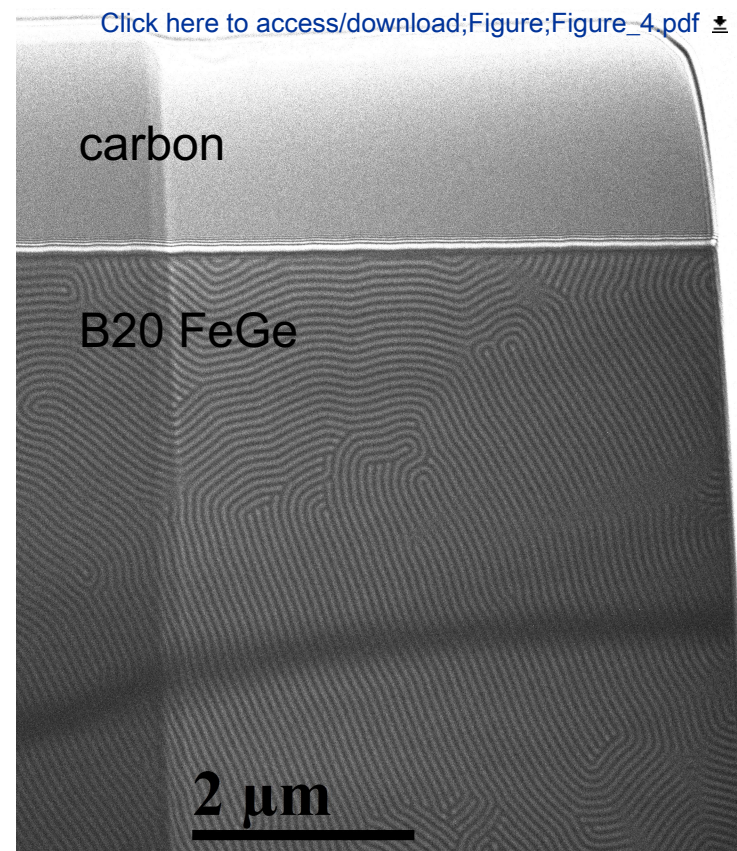
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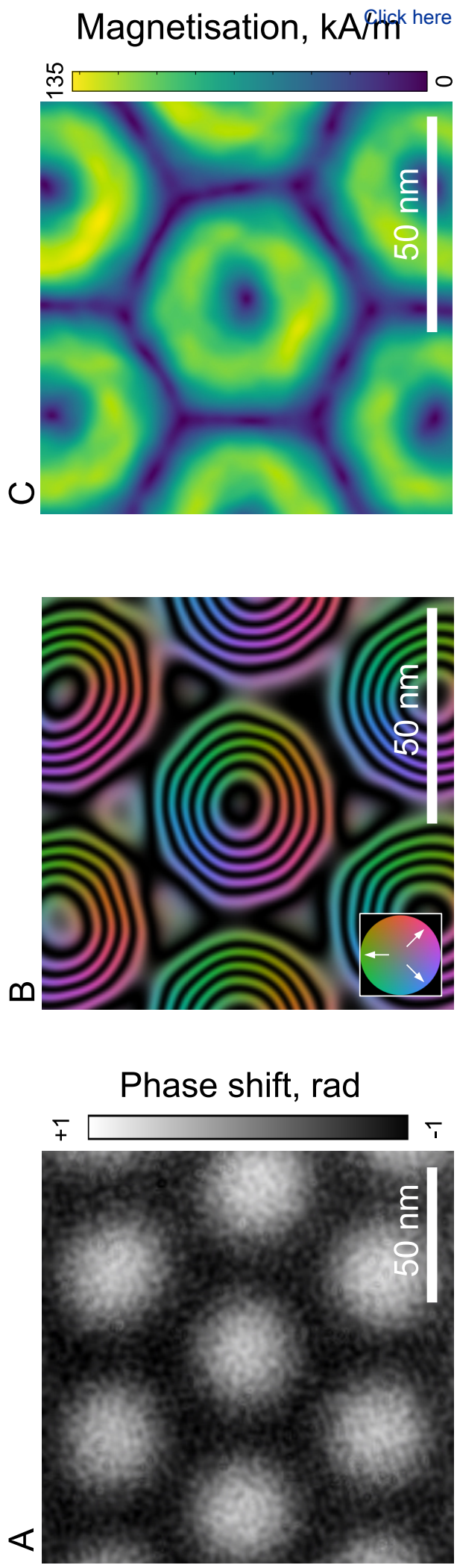
Figure 4

A



B





Name of material/ equipment	Company
Materials	
B20-type iron germanium single crystal	
Software	
Aberration correction software	CEOS
Gatan microscopy suite (GMS)	Gatan
Holoworks	Holowerk
Technical equipment	
Cu Omniprobe grid	Omniprobe
DC high voltage power supply	Fug Elektronik
Direct electron counting detector	Gatan
Double tilt liquid-nitrogen-cooled TEM specimen holder	Gatan
Focused ion beam scanning electron microscope	Thermo Fisher Scientific
Temperature controller for liquid-nitrogen-cooled TEM specimen holder	Gatan
Transmission electron microscope	Thermo Fisher Scientific

Catalog number	Comments/description
	Investigated material
2.21.49	Software for aberration corrections
3.41	Software for controlling Gatan K2 IS camera
6.0 beta	Software for hologram reconstruction
AGJ420	Support grid for TEM lamella
HCL 14M-1250	Biprism voltage supply and controller
GATAN K2 IS	Lorentz images and hologram acquisition
GATAN model 636	Specimen holder
FEI Helios NanoLab 460F1 FIB-SEM	Specimen preparation
GATAN model 1905	Specimen temperature controller
FEI Titan G2 60-300 FEG TEM	Lorentz microscopy and electron holography

DC High voltage HCL

14M-1250

Biprism voltage DC High voltage

HCL

14M-1250

Biprism voltage DC High voltage HCL

14M-1250

Biprism voltage supply and controller

November 1, 2020

Manuscript ID: JoVE61907

“Magnetic field mapping using off-axis electron holography in the transmission electron microscope”

Dear Dr. Vineeta Bajaj,

Thank you very much for handling our submission to the *Journal of Visualized Experiments*. We are very grateful to the referees for their comments, which have helped us to improve and clarify many aspects of our manuscript. Please find below our point-by-point response to the referees’ comments, as well as our revised manuscript, in which our changes have been highlighted.

We hope that you will find our response satisfactory and that the revised manuscript is suitable for publication in the *Journal of Visualized Experiments*.

With best regards,

Fengshan Zheng and András Kovács
on behalf of all of the co-authors

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points
3. Please provide an email address for each author.
4. Please provide at least 6 keywords or phrases.
5. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Holoworks, Gatan software, etc.
6. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “This protocol presents ...”
7. Throughout the manuscript equations are not displayed properly.
8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions

should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”

9. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? For this please include mechanical actions, button clicks in the software, knob turns in the instruments, command lines, scripts (as supplemental file) if any.

10. What is the specimen in this case? How is it prepared?

11. There is a 10-page limit for the Protocol, but there is a 3-page limit for filmable content. Please highlight 3 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

12. Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.

13. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

14. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations, please do not make points.

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods
- e) Any future applications of the technique

15. Please sort the materials table in alphabetical order.

Our reply:

We have revised the manuscript to satisfy the editorial comments.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The submitted manuscript is a paper on the protocol of off-axis electronic holography technique, a useful technique for quantitatively measuring magnetic field changes inside magnetic materials at high resolution.

This is useful information about practical experimental conditions for relevant field analysts. However, the protocol is not specific and Reviewer thinks it is not enough for analysts to produce consistent results.

Reviewer thinks that this manuscript needs a considerable revision to be published.

Major Concerns:

1. The protocol as a whole requires a detailed and quantitative explanation as much as possible.
2. In particular, detailed preparation is required for electronic hologram measurement conditions and the reconstruction process of the holograms.
3. Detailed procedures should also be presented in quantitative analysis of magnetic phase changes.

Our reply:

The protocol and discussion have been extended to provide further details about hologram measurement, reconstruction and quantitative analysis for magnetic phase measurement. The changes are highlighted in the revised manuscript. The descriptions are contained within the word limit of the journal. The authors work in a user facility that is open to universities and research institutions and are able to provide further assistance in off-axis electron holography measurement and analysis to scientists who are interested in this topic.

Reviewer #2:

Manuscript Summary:

In the current manuscript, Fengshan Zhang et al. give an impressive introduction for the off-axis electron holography of the transmission electron microscopy. Off-axis electron holography is a powerful technique in investigating the magnetic configurations of the specimen. There are various techniques in TEM for magnetic characterization such as Lorentz microscopy, off-axis electron holography, differential phase contrast (DPC) imaging and electron magnetic circular dichroism, and this short introduction mainly focused on the off-axis electron holography. Ideally, the off-axis electron holography can be used to reconstruct the 2d magnetization of the thin sample with a sub-5-nm spatial resolution, and the combination of electron tomography make it possible to reconstruct the magnetization in three dimensional. The examples of Off-axis electron holography and Lorentz TEM images are taken using FeGe which is a B20-type chiral magnet that can host magnetic skyrmions below the T_c . The corresponding Lorentz TEM images are shown in Figure 3 and the reconstruction of magnetic structure of FeGe is depicted in Figure 4. The manuscript is well written and I would like to recommend its publication after the following minor comments are addressed.

Minor Concerns:

- 1) All the equations are not properly displayed, please correct them.

Our reply:

The problem occurred during automated generation of the pdf version of the manuscript in the journal's submission system. We will do our best to correct it with the help of the editor.

2) In page 3 the concepts of Off-axis electron holography, Lorentz microscopy, Fresnel mode and Foucault mode are introduced, however, it's not straightforward for readers who are not familiar with the Lorentz mode to understand these concepts. So it would be better to introduce them in a more logical way.

Our reply:

We have added the following introductory paragraph on page 3:

"TEM studies of the magnetic properties of materials are usually carried out with the specimen in magnetic-field-free conditions, i.e., after turning off the conventional microscope objective lens and instead using either a non-immersion Lorentz lens or the transfer lenses of an image aberration corrector as the primary imaging lens. Magnetic-field-free conditions can also be achieved by using an additional specimen stage located between the condenser and objective lenses⁴¹ or by using a double objective lens system to cancel the magnetic field at the specimen position⁴². The recording of images with the specimen located in magnetic-field-free conditions is often referred to as Lorentz microscopy. Depending on the specification of the microscope and the specimen of interest, a variety of different imaging, diffraction or spectroscopy techniques (e.g., DPC imaging and EMCD) can be used to perform magnetic characterisation in the TEM."

3) TIE is also a common method to extract in-plane magnetization, I would be better to compare it with EH briefly in the manuscript.

Our reply:

A meaningful comparison between the recovery of phase information using off-axis electron holography and by applying the transport-of-intensity equation (TIE) to Fresnel defocus images would require a systematic discussion of the different analysis methods that are used in TIE, as well as comparisons of the advantages of each technique for studies of different samples. Such a comparison would not be possible within the word limit of the present manuscript and is arguably beyond its scope. We have therefore chosen to add the following sentence (on page 4), in order to refer the interested reader to a recent review of TIE:

"Phase information can be recovered from such images by solving the transport-of-intensity equation (TIE)⁴³. However, a lack of knowledge of boundary conditions at the edges of the field of view can result in errors in the reconstructed phase."

Reviewer #3:

The manuscript describes a protocol for off-axis electron holography in a transmission electron microscope, used in the so-called Lorentz configuration.

The holography experiment enables to reconstruct the phase of an electron wave, at nanometer spatial resolution, after passing through a sample. The reconstructed phase then

enables to map electrostatic and magnetic potential distributions, according to the Aharonov-Bohm equation, in the form of 2D projections perpendicular to the direction of the electron beam.

This is a classic and well-developed experiment. However, it still remains niche expertise in the community of electron microscopists. Therefore, if a JOVE article can help in encouraging more people to use this method, then this article is a very worthy effort.

However, the concept of JoVE is new to me so I am not sure how detailed this proposal needs to be at this stage. Therefore, my comments may be too soon and are planned to be addressed in the next stage.

The abstract notes that this article focuses on mapping of magnetic fields. It is fine to choose one aspect of electron holography. However, I think that the choice of B20-FeGe with skyrmions is too complicated for the objective of this article.

I would choose a soft magnet thin film, such as permalloy, which can form 90 degrees or 180 degrees Neel domain walls. Thus, the reconstructed phase would be a linear increase/decrease across the domain, from which the magnetic induction field can be quantitatively determined. This aspect is important for first time users to check that the calibration aspects of the experiment work.

Our reply:

Thank you very much for the suggestion. The study of magnetic domain structures and magnetic domain walls in thin films using TEM techniques has been the subject of several recent reviews, including those of McVitie et al., *Ultramicroscopy* 152 (2015) 57 on DPC imaging and Zweck, J. Phys: Cond. Matter 28 (2016) 403001. We deliberately chose to focus on Bloch-type skyrmions, as they illustrate how recorded magnetic contrast from a topical material can be interpreted quantitatively. This subject is also important because of the ease with which the recorded contrast can be misinterpreted, as discussed recently by Loudon et al., *Adv. Mater.* 31 (2019) 1806598 for studies of magnetic bubbles using Fresnel defocus imaging in Lorentz TEM. We therefore believe that our choice of Bloch-type skyrmions is valuable for scientists who are interested in studying modern and challenging aspects of magnetism quantitatively using electron microscopy.

Introduction:

The first paragraph reads as a general intro to specialized chapters on magnetic imaging or an article on the topic. I would start with a more general description of the electron wave and phase reconstruction by electron holography.

Our reply:

As this manuscript aims to show how off-axis electron holography experiments are performed from a practical perspective, we would like to keep the introduction short. Extensive descriptions of electron microscopy can be found in textbooks, for example in Chapters 7 and

8 in Transmission electron microscopy - Diffraction, imaging and spectrometry, edited by C B Carter and D B Williams, 2016. In order to help the reader, we have added the following paragraph to the introduction:

"In the TEM, a highly accelerated electron beam is passed through an electron-transparent (usually solid) specimen, in order to provide access to its crystallographic, chemical, electronic and/or magnetic structure with a spatial resolution that can reach the atomic scale. Typically, a thin (<100 nm) specimen is irradiated with electrons that are emitted from an electron gun and accelerated by 60-300 kV in a high vacuum (<10⁻⁵ Pa) column. Electromagnetic lenses are used to focus electrons onto the specimen and subsequently onto one or more detectors. The electrons interact strongly with the atomic potentials in the specimen and with electromagnetic fields within and around it. Although this information is encoded in the electron wavefunction, an in-focus bright-field or dark-field TEM image records only variations in the intensity of electrons that reach a detector, while information about their phase shift is lost. This so-called 'phase problem' is also encountered in X-ray and neutron experiments. One of the techniques that allows the phase shift of the electron wavefunction to be measured is off-axis electron holography. Further details about fundamental aspects of electron wave functions are available elsewhere (e.g., Chapters 7 and 8 in Ref¹⁵)."

Mathematical equations: There is some bug in the presentation of equations and mathematical symbols throughout the manuscript so I cannot comment if they are OK. This is a problem, as it is important to get the various derivatives correct, and can be confusing for first time users.

Our reply:

These errors arose from the submission system, which generates a PDF file automatically from a submitted Word file. We will work with the editors to ensure that the equations are typeset properly in the final version.

Some comments are general. Considering the purpose of JoVE, I do not see how the reader will be able to translate this to actual use, e.g.: "A magnetic induction map can then be displayed using contours and colours to provide a visual representation of the magnetic field of a thin film or nanostructure"

Our reply:

Our intention is to describe the possibilities of how our experimental results can be visualized, beyond the details of how to conduct the experiments. The representation of a magnetic phase image as a magnetic induction map is essential for understanding the projected in-plane magnetic field in a sample. We have endeavoured to describe briefly the steps that are required to create such magnetic induction maps, within the constraint of the word limit of the manuscript.

The following claim: "Off-axis electron holography is often applied in combination with the simpler, albeit less quantitative, technique of Lorentz microscopy, in particular for studies of the motion of magnetic domain walls."

A similar claim is in 3.2.5 in the protocol.

I guess that by Lorentz microscopy for tracking motion of domain walls, the author is referring to Fresnel-contrast. In that case, I do not agree that it is less quantitative, but rather a more subtle comparison of pros/cons of the methods. There are several papers that do various comparisons between various "2D forms of holography".

Our reply:

We were indeed referring to the Fresnel mode of Lorentz microscopy. We have added some introductory sentences and have removed the word "motion", as our intention was to discuss the imaging of magnetic domain walls. We have also added a sentence about the transport-of-intensity equation, which can be used to provide quantitative information from Fresnel defocus images. As the aim of this manuscript is to focus on off-axis electron holography, we have added references to other techniques where appropriate.

The use of Lorentz mode is necessary to reduce application of a magnetic field from the objective lens to the sample, but also it is necessary to use some form of magnetic field cancellation to reduce the EMI contribution from the surrounding area of the microscope. At least, it is important to measure this remnant field to understand its possible influence (typically, with no special care, a remnant field of several tens of Oe without additional treatment of demagnetization and field cancellation).

Our reply:

The magnetic field at the position of the sample in the microscope that was used in this manuscript was pre-calibrated using a Hall probe sensor. There is a small remnant magnetic field of ~20 Oe at the sample position when the objective lens is turned off. We use an excitation of -0.1% of the objective lens to reduce this field to close to zero. We have added the following sentences about Lorentz mode, including details of the magnetic field calibration, to page 4 of the manuscript:

"Lorentz mode was set up by adjusting the objective lens to a small negative excitation, in order to achieve a magnetic-field-free environment at the specimen position by compensating for the residual magnetic field of the objective and nearby lenses. The first transfer lens of the image corrector unit was then used as a non-immersion imaging lens. Specimens could be imaged either at remanence (in zero magnetic field) or in the presence of a pre-calibrated magnetic field⁴⁷, which could be applied by exciting the conventional microscope objective lens ... Although in-plane magnetic fields can in principle be applied using dedicated magnetizing specimen holders, such a holder was not used in the present work."

The author mentions two electron biprisms, but they do not explain the contribution of using two electron biprism for this experiment. What is the advantage over a microscope with one biprism?

Our reply:

We have added the following sentences:

“In the present experiments, only one of the prisms was used to record electron holograms. The advantages of using multiple biprisms are described elsewhere^{45,46}. ”

Protocol:

Several points are missing, in my opinion:

The relation between carrier frequency, hologram contrast and field of view. The author should discuss how to determine/measure these relations, and how to use this data to choose optimal conditions for a specific experiment.

Our reply:

In order to clarify the choice of magnification and biprism voltage, we have added the following paragraph to the end of the first part of the Protocol:

“The choice of magnification and biprism voltage depend on the desired field of view, spatial resolution and signal-to-noise-ratio in the reconstructed phase. First, determine the magnification according to the size of the region of interest. Be aware that a small vacuum region (~10% of the field of view) should ideally be included in the hologram. Second, adjust the biprism voltage according to the desired overlap width and spatial resolution. The electron optical spatial resolution is at best two or three times the holographic interference fringe spacing, depending on the mask size used for reconstructing the hologram (see the Representative results section for details). The magnification and biprism voltage may need to be optimized iteratively. In general, the greater the magnification or the lower biprism voltage, the better fringe contrast and signal-to-noise ratio in the reconstructed phase, but the poorer the spatial resolution.”

I am missing a discussion of a possible overlap of the sample wavefunction with itself and how to avoid it.

In that respect, the explored area of the specimen should cover most of the hologram field of view. In my opinion the authors should discuss this.

Our reply:

We usually try to keep 10% of the vacuum region in the field of view, first to ensure that the sample does not interfere with itself and second to have a region of vacuum that can be used to evaluate phase noise and unwanted artefacts, such as this associated with electron-beam-induced charging or phase ramps. Our criterion is mentioned in the following phrase in 2.1 of the Protocol: *“ideally ~10% of the field of view.”*

The author wrote about applying voltage to the biprism. I think that they need to mention the rate of increasing the voltage, to avoid damaging the biprism.

Our reply:

We have added the following phrase to 1.5 of the Protocol:

“typically at a rate of no more than 1 V/s to avoid damaging it”.

Discussion:

1. The preparation of a thin lamella from a bulk material already changes the entire magnetic configuration. Therefore, the research objective should be defined with respect to the sample geometry (thin sample, large demagnetization field).

Our reply:

We have added the following short section to the Discussion to highlight the influence of preparing a thin specimen on magnetic measurements performed in the TEM:

“Magnetic imaging experiments performed in the TEM require an electron-transparent specimen. The preparation of such specimens is briefly described above in the Representative results section. When planning, conducting and interpreting an experiment, the shape of the specimen must be considered, as results obtained from “thin films” may be different from those obtained from bulk samples. For example, magnetic domains are often smaller and demagnetizing fields are stronger in TEM specimens than in bulk materials. Nevertheless, magnetic properties such as saturation magnetization and domain wall width measured from thin TEM specimens typically match values known from bulk materials.”

3. I am not sure why electron beam induced charging is especially problematic during a cooling experiment?

Our reply:

Electron-beam-induced charging is present in many TEM experiments, as a result of the emission of secondary electrons from the specimen. If the specimen has poor conductivity, then the resulting positive charge cannot be compensated by ground currents. Below room temperature, the electrical conductivity often changes due to a lower thermal activation energy and a lower probability for electrons near the Fermi level to reach the conduction band.

Figures:

Fig. 1(a) - please mark the conjugate planes, as this is relevant for the position of the biprism. Also, it would be good to mark schematically the aperture positions, as this would help in orientating the reader with respect to conventional TEM.

Our reply:

The conjugate planes and aperture positions are marked in the new version of Fig. 1(a).

Fig. 1(b) is blurry, it should be replaced with a better resolution figure.

Our reply:

Figure 1(b) has been replaced by a higher quality image.

Fig 5 (c), the color of the radians bar is just with one color, the bar needs to be fixed. Some of the scale bars on the figures do not contain the length, if the authors put the length on some of the figures they should do it consistently on all figures .

Our reply:

The color bars in Fig. 1(a) and Fig. 1(c) have been fixed. The scale bars have also been changed, including those in Figs 4 and 5.

Reviewer #4:

Manuscript Summary:

Electron holography is a very powerful technique in the electron microscopy field. However, as contrasted with the general electron imaging techniques, electron holography is not widely used, this is due to its complication, especially interpretation of the hologram. This manuscript is very time and that group is one of the epicenters of electron holography (not too many in the world). Therefore, publication of this work can help the community to follow the steps as how to do that. I would suggest the publication of this work with the follow minor changes.

Minor Concerns:

1) Figure 1 A, the sample geometry looks peculiar, I would suggest rotate the sample, rather than with the corner at the center of the beam.

Our reply:

Figure 1 A has been improved according to the referee's suggestion.

2) A detailed step of capturing electron hologram should be given in step by step

Our reply:

The protocol has been expanded to include additional steps and descriptions about how to set up the electron beam illumination and the conditions for recording holograms.

3) Simulation and software package should be clearly demonstrated, this is high barrier for a lot of users.

Our reply:

We understand the reviewer's concern. In the past, custom-made software and commercial software packages were used. Several open source codes are now available. We have expanded the description and references to reconstruction procedures and open source codes, as follows:

"Further details of the reconstruction procedure and corresponding open-source software can be found elsewhere (e.g., in Refs⁵²⁻⁵⁴)."

4) A warning of wrong doing or common mistake or artifact should be important point of the paper as well.

Our reply:

We have added several warnings to the protocol that can help users to avoid mistakes.