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# Reconstructing dual-phase nanometer scale grains within a pearlitic steel tip in 3D through 4D-scanning precession electron diffraction tomography and automated crystal orientation mapping

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1 Reconstructing Dual-Phase Nanometer Scale Grains within  
2 a Pearlitic Steel Tip in 3D through 4D-Scanning Precession  
3 Electron Diffraction Tomography and Automated Crystal  
4 Orientation Mapping

5

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8

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14

15 **Abstract**

16

17 The properties of polycrystalline materials are related to their microstructures and hence a complete  
18 description, including size, shape, and orientation of the grains, is necessary to understand the behaviour  
19 of materials. Here, we use Scanning Precession Electron Diffraction (SPED) in the Transmission  
20 Electron Microscope (TEM) combined with a tilt series to reconstruct individual grains in 3D within a  
21 polycrystalline dual-phase cold wire-drawn pearlitic steel sample. Nanoscale ferrite grains and

22 intragranular cementite particles were indexed using an Automated Crystallographic Orientation  
23 Mapping (ACOM) tool for each tilt dataset. The grain orientations were tracked through the tilt datasets  
24 and projections of the individual grains were reconstructed from the diffraction data using an orientation-  
25 specific Virtual Dark Field (VDF) approach for tomographic reconstruction. The algorithms used to  
26 process and reconstruct such datasets are presented. These algorithms represent an extension to the  
27 ACOM approach that may be straightforwardly applied to other multi-phase polycrystalline materials  
28 to enable 3D spatial and orientation reconstructions.

29

## 30 Keywords

31 Transmission Electron Microscopy (TEM), Scanning Precession Electron Diffraction (SPED),  
32 Automated Crystal Orientation Mapping (ACOM), Diffraction tomography, Pearlitic Steel, Nanoscale  
33 grains

34

## 35 1 Introduction

36

37 The microstructure of polycrystalline materials plays a role in determining the material properties such  
38 as ductility, hardness, and wear resistance [1]. Over the past 20 years, a variety of synchrotron X-ray  
39 based techniques have been developed to measure the 3-dimensional microstructure (see references [2,3]  
40 and references therein), however the spatial resolution is typically limited to  $\sim 1\mu\text{m}$ , and although this  
41 has been improved to  $\sim 100\text{nm}$  for recent variants [4,5], it remains insufficient to resolve nanoscale  
42 structures. Electron Backscatter Diffraction (EBSD) is commonly employed for surface orientation  
43 mapping and can be extended into three dimensions in combination with Focused Ion Beam (FIB)  
44 sectioning [6]. This technique, however, is destructive and the resolution is limited to tens of  
45 nanometers.

46 Scanning TEM (STEM) is a powerful tool for investigating nanomaterials and commonly achieves  
47 atomic resolution in imaging modes. TEM-based techniques have been developed to perform orientation  
48 mapping at the nanometer scale, notably the dark-field conical scanning [7,8] and scanning electron  
49 nanobeam diffraction techniques (NBD) [9,10]. In the latter, the electron beam is scanned over two  
50 dimensions across a specimen and a diffraction pattern is recorded at each probe position, resulting in  
51 4-dimensional data. Due to strong dynamical effects associated with electron diffraction this technique  
52 is commonly extended to Scanning Precession Electron Diffraction [11,12]. Here, the electron beam is  
53 precessed through a small-angle hollow cone which probes more lattice points in reciprocal space. This  
54 reduces the measured dynamical scattering effects by averaging the diffraction signal over many  
55 different beam directions [13]. The crystal orientations may then be calculated from the set of recorded  
56 diffraction patterns using Automated Crystal Orientation Mapping, which utilizes diffraction template  
57 matching and is a well-trusted strategy for orientation and phase mapping in TEM [14]. The combination  
58 of both ACOM and precession is commonly employed as the use of precession improves the orientation  
59 resolution of the indexing procedure [15,16]. The resulting 2-dimensional orientation maps provide  
60 information about the sample microstructure, however some ambiguity as to the true through-thickness  
61 positions of the identified grains remain. Techniques have been further developed to separate  
62 information resulting from overlapping grains in transmission [17] and to reconstruct images of the  
63 grains from the set of diffraction patterns, known as virtual reconstruction [18].

64 Both TEM orientation mapping techniques outlined above have been extended to 3-dimensions by  
65 different groups. Liu et al. [19] applied the dark-field conical scanning technique 3D-OMiTEM to  
66 reconstruct the shapes and orientations of an aluminum sample. Eggeman et al. [20] utilized SPED  
67 combined with a tilt series, called Scanning Precession Electron Tomography (SPET), to image  
68 precipitates in a Ni-based superalloy. Their processing technique used the unsupervised machine  
69 learning technique non-negative matrix factorization (NMF) to classify the diffraction patterns obtained  
70 from the different phases in the sample. Whilst the non-negativity constraint imposed by NMF promotes  
71 physical interpretation of the solution components, the algorithm is sensitive to initialization methods  
72 [21] and is ill-suited to gradually changing features such as materials containing local misorientations

73 [20,22]. Meng and Zuo [23] used the virtual dark field (VDF) reconstruction method to reconstruct  
74 grains from their diffraction intensities. VDF reconstructions were created by integrating Bragg  
75 reflections which highlighted the same grain in real space and subsequently combined to create a final  
76 image of each grain. Both NMF and VDF methods have the advantage that they do not require prior  
77 knowledge of the crystal structure and have recently been further compared [22]. The reconstructed  
78 images of the grains at different tilt angles from the diffraction data were used as projections for  
79 tomographic reconstruction in both cases.

80 Here, we reconstruct grains of a pearlitic steel tip in three dimensions from SPED data using an expanded  
81 VDF technique that incorporates knowledge of the crystal orientation and phase. Manual selection of  
82 the Bragg reflections and subsequent comparison of the grain projections is a subjective and time-  
83 consuming procedure, thus we automate the selection of VDF apertures by using orientation and phase  
84 selective diffraction templates calculated through template matching. Cementite particles were  
85 identified using the same algorithms in the predominantly ferrite sample and subsequently reconstructed  
86 [24]. The orientation maps calculated from the tilt series datasets were coupled and used to track the  
87 grains in 3D for subsequent fiducial alignment.

88

## 89 2 Experimental

90

91 A pearlitic steel wire with composition Fe-0.98C-0.31Mn-0.20Si-0.20Cr (wt.%) was cold-drawn to a  
92 true strain of 6.5 and annealed for 2 min at 400°C was used as the material system [25] to conduct the  
93 method development. The cold-drawing process leads to a microstructure of columnar ferrite grains and  
94 the annealing to slight grain coarsening to a diameter of ~30 nm perpendicular to the columnar axis, as  
95 well as to carbon grain boundary segregation and the formation of spherical cementite [26]. This  
96 microstructure is ideally suited to establish the measurement approach as the grain orientations can be  
97 measured without significant grain overlap along the columnar direction. Further, this material system

98 is of interest because of its outstanding strength [27] that is linked to the segregation of carbon to its  
99 grain boundaries [25].

100 SPED experiments were performed using a JEOL JEM-2200FS electron microscope operated at 200 kV  
101 with a spot size of 1 nm and with 0.5° precession angle supplied by a Digistar hardware unit  
102 (NanoMEGAS SPRL). Each SPED dataset was acquired with a step size of 1.4 nm over a scan frame  
103 comprising (255 x 276) pixels at a frame rate of 24 frames per second. A Fischione Instruments Model  
104 2050 On-Axis Rotation Tomography Holder was used to tilt the sample and SPED datasets were  
105 acquired at nine projection angles over 360°. At each applied tilt an Annular Dark Field (ADF) image  
106 was also acquired as a reference image, using a camera length of 25 cm. An Omega-type energy filter  
107 with a slit width equivalent of 30 eV was used to reduce the influence of inelastically scattered electrons  
108 and the diffraction patterns were recorded with a scintillator coupled CMOS (complementary metal-  
109 oxide-semiconductor) camera (TVIPS TemCam-XF416) at (512 x 512) pixels ((8 x 8) pixel binning).  
110 The data was processed using the ASTAR software package [14] (NanoMEGAS SPRL) and Python  
111 codes making use of the well-used NumPy [28] and SciPy [29] software stacks. ASTAR was used to  
112 calculate crystal orientations using both multi-indexing (5 indexing passes) and dual phase ( $\alpha$ -Fe and  
113 cementite) approaches. Further orientation calculations were performed using the orix [30] library.  
114 Fiducial alignment was performed as implemented in the IMOD [31] software.

115

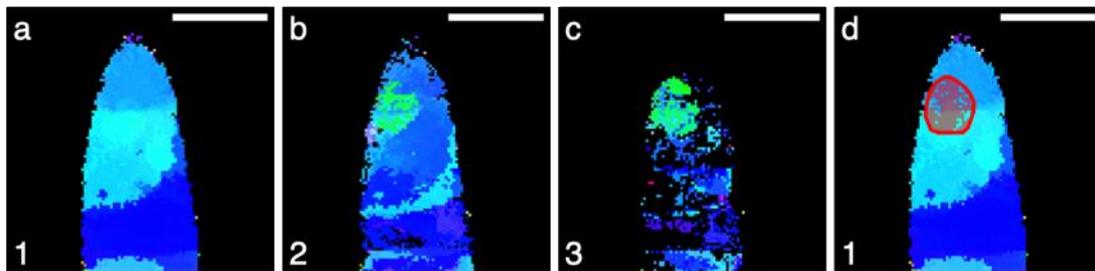
### 116 3 Processing

117

118 For polycrystalline materials such as the pearlitic steel tip presented here, an individual diffraction  
119 pattern may contain information constituted by many grains through the sample volume along the beam  
120 direction. The ACOM template matching algorithm will calculate the orientation from only the dominant  
121 grain within a given diffraction pattern despite this possibility. A multi-indexing strategy has been  
122 previously developed to characterize the information from overlapping grains [17]. Briefly, this involves  
123 iteratively calculating the optimum template for a given pattern multiple times. After each indexing pass,

124 the information within the diffraction pattern that was identified by optimum template is removed and  
125 the resulting 'reduced' pattern then undergoes another indexing pass to find the next dominant  
126 orientation. Performing this procedure for every diffraction pattern within the dataset results in multiple  
127 orientation maps which are combined into an orientation map stack as shown in SI 1. This procedure  
128 may be used to quantify grains and phases that would remain unidentified through a single indexing  
129 pass. An example is shown in Figure 1, which shows that the green grain near the sample apex would  
130 remain unidentified without the use of multi-indexing.

131



132

133 Figure 1: Multi-indexing reveals hidden grains in the pearlitic steel tip sample. (a-c) Orientation maps produced from the first  
134 three indexing passes, as indicated by the numbers inset. The grain with orientation close to (101) (green) is not recognized in  
135 the first indexing pass (a) but is identified in subsequent indexing passes (b-c). (d) The position of this grain overlaid in red on  
136 (a). The red line represents the convex hull of the grain and is shown for clarity. Black regions represent ignored values as  
137 determined by a lower threshold limit on the calculated correlation index. Colour map is the same as shown in Figure 6 and  
138 orientation maps were calculated from the 108.6° projection. Scale bars are 50 nm.

139

140 Pearlite is a dual-phase material consisting of lamellar ferrite (Fe, spacegroup 229, lattice parameter  
141  $a=b=c=2.87$  nm) and cementite ( $\text{Fe}_3\text{C}$ , spacegroup 62, lattice parameter  $a=5.09$  nm,  $b=6.75$  nm,  $c=4.52$   
142 nm), as such templates calculated from both phases were incorporated into the ACOM indexing strategy.  
143 The cementite phase was easily identified in the sample, as shown in SI 2, however the orientation  
144 reliability of the indexed cementite particles was low due to their small sizes and weak diffraction  
145 signals. As such the larger ferrite grains were used to align and process the datasets as discussed in the  
146 coming sections.

147

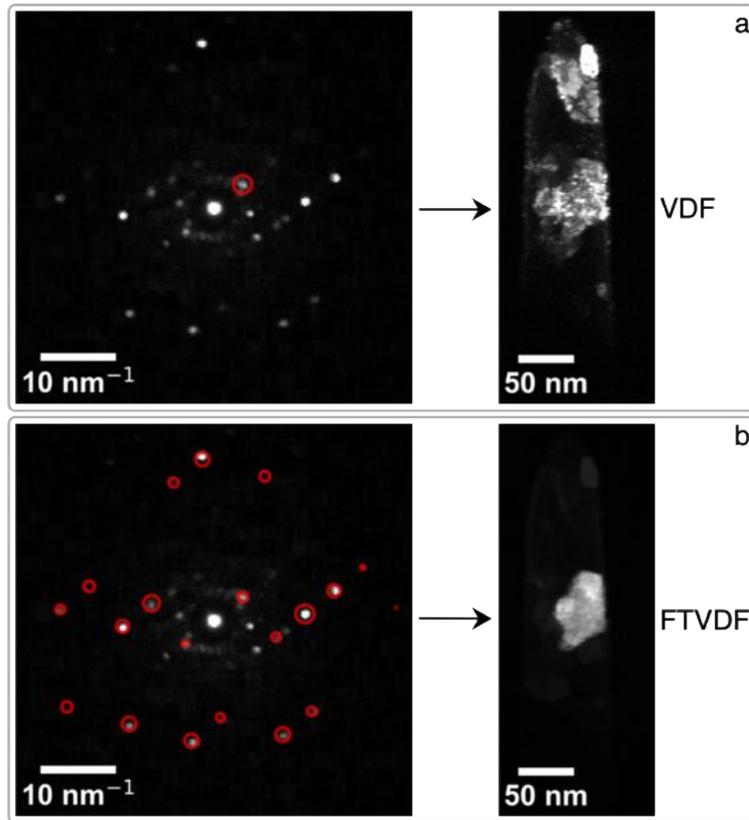
### 148 3.1 Frozen Template Virtual Dark Field

149

150 The recent rise in 4-dimensional scanning electron diffraction experiments has led to an increased  
151 importance of post-processing techniques. For scanning electron diffraction data this includes the use of  
152 virtual apertures [18,32] to highlight sample regions which possess a common diffracting signature. A  
153 virtual reconstruction is then performed by integrating the data contained in each diffraction pattern  
154 within the dataset underneath these virtual apertures. An example VDF reconstructed image is shown in  
155 Figure 2(a). In this case a Bragg reflection in the diffraction pattern was selected as a virtual aperture  
156 and the resulting VDF image highlights two individual grains.

157 An improved representation of the diffraction signature of a grain may be calculated using knowledge  
158 of the crystal orientation calculated with ACOM. Expanding on this, a full diffraction template, which  
159 is used in the ACOM indexing procedure, contains the predicted excited Bragg reflections. The projected  
160 positions of these Bragg reflections on the detector for a given crystal orientation are calculated and then  
161 used as virtual apertures. The resulting image is called a Frozen Template Virtual Dark Field (FTVDF)  
162 reconstruction [33] as the positions of the virtual apertures are fixed whilst integrating over the scanning  
163 pixels, despite the potentially varying orientation of the sample over the scan area. An example  
164 illustrating this calculation is shown in Figure 2(b), which shows that the reconstructed grain contour is  
165 much improved using FTVDF when compared to VDF as the calculation includes more of the diffracted  
166 beams, but also that the reconstruction is more specific to the crystal orientation from which the  
167 diffraction template was calculated.

168



169

170 Figure 2: Calculation of (a) Virtual Dark Field (VDF) and (b) Frozen Template Virtual Dark Field (FTVDF) reconstructed  
 171 images. The left column shows a selected experimental diffraction pattern. The virtual apertures used for the calculations in  
 172 both cases are shown as red circles with radii proportional to the weight of each aperture. The right column shows the resulting  
 173 reconstructed images.

174

### 175 3.2 Orientation Components

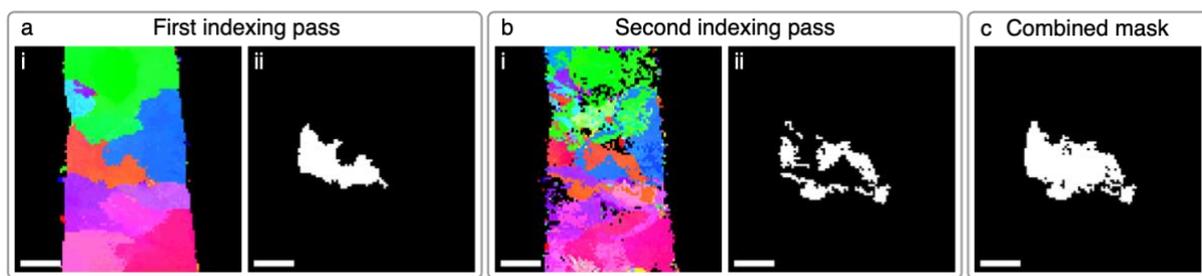
176

177 SPED datasets contain independent diffraction patterns which originate from the same grain and so a  
 178 natural processing step is to cluster these patterns. Various methods, including diffraction pattern-based  
 179 [22,34,35] and orientation-based [30,33] clustering, have been used to group and segment electron  
 180 diffraction data. Here, the latter approach is used and a given orientation map is segmented into regions  
 181 of similar orientation, which we call components. These components represent grains and are  
 182 constructed by first calculating the disorientation matrix, the smallest misorientation after taking into  
 183 account crystal symmetry operations, between all orientations within the orientation map and a given

184 orientation. Disorientation values smaller than a user-defined threshold, which should be larger than the  
185 observed intragranular orientation spread (nominally  $5^\circ$ ), are segmented and labelled as a unique  
186 component. The initial reference orientations are chosen as those with the highest correlation index and  
187 this procedure happens automatically over the entire multi-indexed orientation map until all data points  
188 have been processed. The result is a labelled segmented map of grain components.

189 The overall projection of a grain may be better elucidated by combining multiple calculated orientation  
190 maps produced by multi-indexing. An example of this is shown in Figure 3. After the first orientation  
191 indexing pass the orange region (grain) in Figure 3(a-i) produces the calculated component in Figure  
192 3(a-ii). However, the second indexing pass (Figure 3(b)) shows that this grain extends beyond its bounds  
193 as determined by the component calculated from the first pass. The combined component, shown in  
194 Figure 3(c), represents the projection of the grain more accurately. This observation is general to  
195 overlapping grains and inclined grain boundaries. The output from this procedure produces a binary  
196 mask of the grain, however an improved greyscale representation of the grain contour may be calculated  
197 using FTVDF which was introduced in 3.1 and will be discussed further in 3.4.

198



199

200 Figure 3: Schematic demonstrating the calculation of an individual component from a multiply-indexed orientation map.  
201 Orientation colour code is the same as shown in Figure 6. (i) Orientation maps and (ii) calculated components from the (a) first  
202 and (b) second indexing passes. Components were calculated using the same reference orientation in both (a) and (b). (c) The  
203 combined component (grain) mask calculated from (a(ii)) and (b(ii)). Scale bars are 25 nm.

204

205 The calculated components are then be filtered to remove erroneous data, such as few-pixel components  
206 which are typically noise originating from the indexing algorithm, and average statistics are calculated

207 for each component. The calculated components provide two benefits over full orientation maps: firstly,  
208 a reduction in data size from thousands of orientation data points to tens or hundreds of components  
209 and, secondly, calculation of the average grain orientations and centers of mass for each component,  
210 which may be used for orientation and real space alignment of the tilt series, respectively.

211

### 212 3.3 Orientation and Real Space Alignment

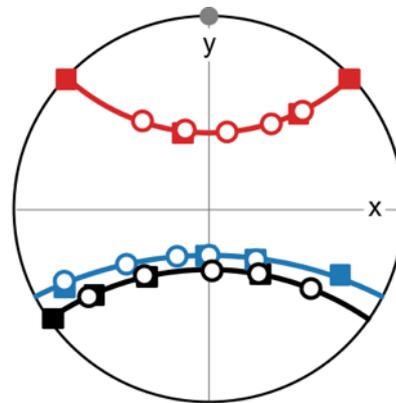
213

214 Whilst the rotation axis of the specimen holder in the electron microscope is known, this axis normally  
215 does not directly relate to the rotation of the diffraction pattern on the camera in the microscope, which  
216 is due to azimuthal rotation of the electron beam around the optical axis by the projector lenses. This  
217 rotation may be compensated in some microscopes or calibrated for a defined set of imaging parameters  
218 [36], however these calibrations may change over time. As a result, the rotation axis which couples the  
219 tilt series together must be calculated. An automatic approach to calculate this rotation vector (axis and  
220 angle) has been implemented.

221 The orientations  $o$  of components from tilt dataset  $i$  are coupled to the next tilt dataset  $i + 1$  by  $o_{i+1} =$   
222  $ro_i$  where  $r$  is the rotation vector. The angle of  $r$  is known as it is the applied tilt to the specimen,  
223 whereas its axis within the reference frame of the calculated orientations is not known. This axis,  
224 however, typically has no vertical component as the applied rotations are confined to the specimen x-y  
225 (azimuthal) plane of the microscope. The disorientation between the datasets after applying symmetry  
226 operations then follows as  $d = o_{i+1}(ro_i)^{-1}$ . For two given tilt series orientations, small disorientations  
227 will always be calculated due to uncertainties in the applied rotation and the calculated orientations from  
228 orientation indexing. There is also the possibility of pattern misindexing due to the 180° ambiguity  
229 problem [37] which creates further complications for orientation coupling between tilt datasets, however  
230 algorithms have been developed to overcome this [33] and are employed here. On top of this it is also  
231 possible that the sets of orientations between tilt datasets are incomplete due to differing fields of view.

232 The disorientation matrix  $d$  is therefore calculated between all component orientations in each tilt dataset  
233 and the total cost of this matrix is determined by the solution to the minimum cost assignment problem  
234 [38], which assumes that an orientation in the first tilt dataset  $o_{i,j}$  matches to only one orientation in the  
235 second tilt dataset  $o_{i+1,k}$ . The matching cost is therefore computed for many axis directions of  $r$   
236 contained within the azimuthal plane of the diffraction patterns, and the axis direction with the lowest  
237 associated cost is chosen as the rotation vector which subsequently undergoes further refinement. An  
238 example calculation for this procedure is shown SI 4. The orientation data is then corrected such that  
239 tilts in both the real space and orientation space data occur around the same axis, which is the y-axis for  
240 the data presented here. This information therefore allows a component to be coupled between tilt  
241 datasets and tracked throughout the tilt series, as demonstrated by the {100} pole figure in Figure 4 for  
242 the grain presented in Figure 5. The accuracy of this method can be estimated from the spread of the  
243 calculated rotation vectors for the same microscope conditions; for the dataset presented here, the  
244 standard deviation of the set of their azimuthal angles is  $0.4^\circ$ .

245



246

247 Figure 4: {100} pole figure of the ferrite grain presented in Figure 5 tracked throughout the tilt series. The measured component  
248 orientations at different tilts are shown as open circle and closed square datapoints for poles on the upper and lower hemispheres  
249 of the unit sphere, respectively. The corrected rotation axis is shown as a closed grey circle. The path of the calculated average  
250 orientation of the grain rotated around the rotation axis is shown as a line for each pole.

251

252 The tomographic reconstruction quality is limited by the alignment of the tilt series data amongst other  
253 factors, so it is necessary to calculate the proper alignment of the dataset. Initial coarse alignment and  
254 shear correction was performed by comparing the Virtual Bright Field (VBF) image reconstructed from  
255 each SPED dataset with the reference ADF image for each projection. Fiducial alignment algorithms  
256 have proven to be powerful tools for fine alignment [31] and are capable of linear and non-linear image  
257 distortion corrections which may be non-negligible in SPED datasets acquired over typical acquisition  
258 times of tens of minutes. The projected center of masses of each component were calculated for each tilt  
259 dataset and used as data points for real space fiducial fine alignment of the coarsely aligned SPED data.

260

### 261 3.4 Reconstruction

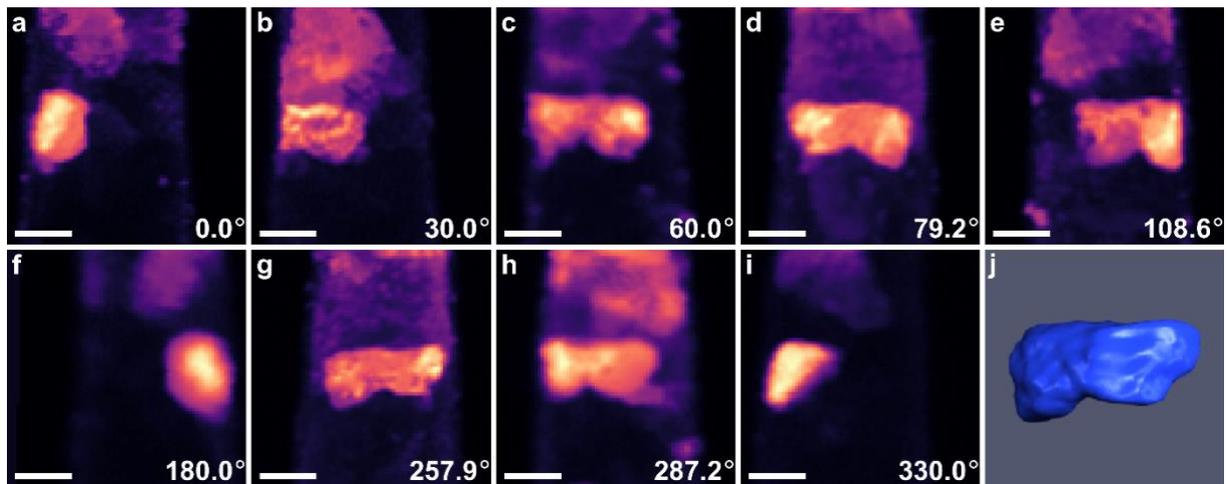
262

263 Projections of the individual grains were calculated using FTVDF, as shown in Figure 5. Not all grains  
264 could be unambiguously tracked through the dataset, as discussed above. In these cases a template  
265 created from an unambiguous orientation of a grain was rotated into the reference frames of the other  
266 tilt datasets and projections of the grain were computed using FTVDF from the simulated rotated  
267 template. The resulting images contain orientation-specific contrast of the grain of interest. It has been  
268 noticed that the grain contrast may be improved by refining the diffraction template on diffraction  
269 patterns originating from the highlighted grain of interest. Typically, the orientation of the refined  
270 template is around  $1^\circ$  from its initial orientation, which is an estimate of the total uncertainty of the  
271 calculated orientations and coupling rotations used in this work. As previously discussed, the low  
272 orientation reliability of the cementite phase means that it was not well-suited to FTVDF reconstruction.  
273 Cementite particles were identified using the calculated phase maps and VDF projections were  
274 calculated by manual selection of the Bragg reflections in the diffraction data.

275 The FTVDF intensities, reconstructed from diffracted beams, are affected by numerous factors,  
276 including dynamical diffraction, intragranular orientation gradients, and grain overlap. The effects of  
277 dynamical diffraction were reduced by beam precession during the experiment, whereas the latter two

278 effects are intrinsic to the sample and material studied. As a result, there are contrast fluctuations within  
279 each grain, which are visible in Figure 5, and, despite the use of precession, the FTVDF images do not  
280 exactly satisfy the projection criteria that the projected object intensity is proportional to the thickness  
281 of the object [39]. These effects, however, do not prevent a well-defined reconstruction of the grain  
282 contour.

283



284

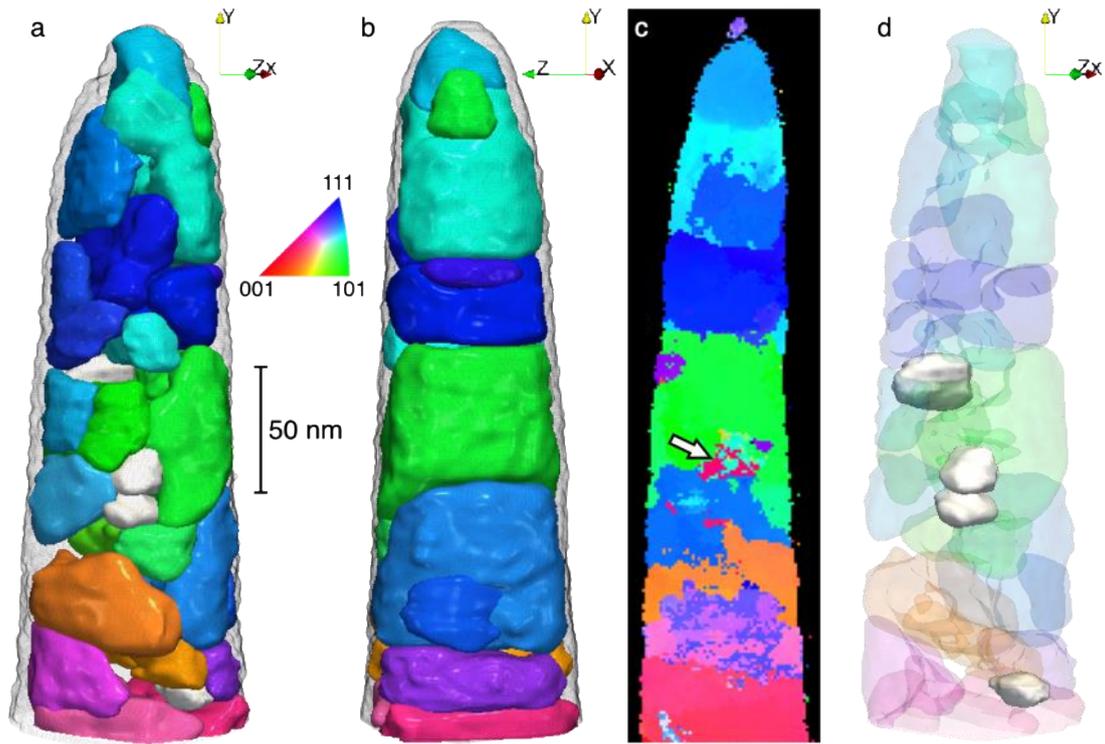
285 Figure 5: (a-i) FTVDF projections of an individual ferrite grain within the pearlitic steel tip. (j) 3D reconstructed isosurface  
286 rendering of the grain. Scale bars are 25 nm.

287

288 The total FTVDF intensity of each grain in each projection was normalized before tomographic  
289 reconstruction, under the assumption of a constant diffracting volume. Each grain was aligned using the  
290 global set of alignment parameters and reconstructed individually into a common volume using 25  
291 iterations of the Simultaneous Iterative Reconstruction Technique (SIRT) combined with a non-negative  
292 minimum constraint to promote physical solutions [40]. The resulting combined rendering is provided  
293 as a video in SI 5 and shown in Figure 6 for two different projection angles. Figure 6(b) clearly shows  
294 that the grains are columnar along the drawing direction (z-axis), as expected from the preparation  
295 technique. The calculated orientation map obtained under the same projection is also presented in Figure  
296 6(c). Comparison between Figure 6(b) and (c) demonstrates the extra information provided by 3D grain  
297 reconstruction. The positions and shapes of the grains are well defined in the 3D reconstruction whereas

298 in the 2D orientation map they are ambiguous in both two and three dimensions due to grain overlap.  
299 The hidden grain at the tip apex, discussed previously in section 3.2, is also absent from this orientation  
300 map, however its spatial position is well-defined in the 3D reconstruction. Furthermore, the cementite  
301 phase is identified within the sample (as depicted by the white arrow in Figure 6(c)), however the 3D  
302 reconstruction shows that these cementite particles are located on the far side of the sample and not in  
303 plane with other grains. These two artefacts arise from the dominant grain concept in the ACOM  
304 template matching procedure discussed previously. Further down the tip, the 2D orientation map shows  
305 regions of uncertain grain boundaries and shapes, for example the interface between the blue, orange,  
306 and purple grains which are oriented close to (111), (201), and (211), respectively. From the 2D  
307 orientation map it may be interpreted that these three grains are located on the same z-y plane (x-y plane  
308 of the 2D orientation map), however the 3D reconstruction disproves this notion and shows that the  
309 grains exist at different x-positions within the sample. The knowledge of the relative positions of these  
310 grains is critical to further analysis of their grain boundary interfaces.

311



312

313 Figure 6: 3D reconstruction of the pearlitic steel tip projected (a)  $30^\circ$  and (b)  $257.9^\circ$  from the columnar direction of the grains  
 314 (y-axis). Each grain is coloured by its average orientation projected along the length of the tip. Cementite particles are coloured  
 315 white. ADF reconstruction also shown as a black isosurface wiremesh in (a) and (b). (c) The 2D calculated orientation map  
 316 from the  $257.9^\circ$  projection. (d) The location of identified cementite particles within the tip, shown under the same projection  
 317 as (a). A Gaussian filter with standard deviation of 1.4 nm (1 voxel) was applied to the reconstructed volumes during rendering.

318

319 Figure 6(d) highlights the locations and shapes of cementite particles that could be reconstructed from  
 320 the dataset. The cementite particles are found at ferrite grain boundaries and have more spherical  
 321 geometries when compared to the elongated columnar ferrite grains, which has been shown to be due to  
 322 cementite growth during the annealing process [26]. Whilst the indexed cementite orientation is in  
 323 general difficult to determine from a single diffraction pattern, under certain projections the indexed  
 324 cementite displayed large, connected regions ( $>50$  pixels,  $\sim 100$  nm<sup>2</sup>) of unchanging orientation, and the  
 325 same orientations were also calculated under the reverse projection dataset ( $0^\circ$  and  $180^\circ$  in this case),  
 326 increasing the orientation reliability. Multiple planar  $(001)_\theta \parallel (11\bar{2})_\alpha$  relationships between cementite  
 327 particles and neighbouring ferrite grains were observed and are further detailed in SI 6. These represent

328 partial Bagaryatsky-type orientation relationships [41]. The lack of complete cementite-ferrite  
329 orientation relationship observed within the sample is likely due to the large amounts of plastic  
330 deformation within the sample due to the cold-drawing processing technique.

331 An ADF image was also recorded at each tilt position during the experiment. The ADF images were  
332 aligned using the cross-correlation technique and reconstructed using the SIRT algorithm with total  
333 variance minimization [42]. The resulting reconstruction is overlaid as a black mesh isosurface in Figure  
334 6(a-b). The ADF reconstruction is considered as the reference reconstruction for the sample as the effects  
335 of sample drift in each ADF image are much reduced, when compared to the SPED scans, due to  
336 approximately two orders of magnitude decrease in scan time. The reconstructed grains are well  
337 contained within the ADF reconstruction, as expected, demonstrating the accuracy of the alignment of  
338 this diffraction tomography reconstruction technique. From the diffraction data there appears to be a  
339 thin (~5 nm) amorphous region surrounding the tip, which may be surface oxide species or have  
340 originated from the FIB milling process [43]. These regions remain unreconstructed by the diffraction  
341 technique and are consistent with the missing layer in the grain reconstruction when compared to the  
342 ADF reconstruction.

## 343 4 Discussion

344

345 The 3D-SPED technique presented here has been combined with ACOM to reliably track grains through  
346 the tilt series datasets and reconstruct projections of the grains using FTVDF. This reconstruction route  
347 was performed semi-automatically for the ferrite grains; manual input was required to adjust the FTVDF  
348 templates when the grains could not be reliably tracked throughout the tilt series, to improve the FTVDF  
349 reconstruction contrast, and for the fiducial alignment. The two former points are partially related to the  
350 material choice in this study, as the ferrite grains within the sample contained local misorientations due  
351 to plastic deformation caused by the cold-drawing technique. The total amount of manual input is  
352 expected to be less for more regular polycrystalline samples and it is anticipated that further algorithmic  
353 work will also contribute to this end.

354 Semi-automatic FTVDF reconstruction of the cementite particles was not possible due to their low  
355 orientation reliability, small sizes, and generally weak Bragg reflections. In future works on this material  
356 the experimental conditions could be further optimized to improve their diffraction signal, for example  
357 by increasing the exposure time, however this would come with a trade-off of sample damage and carbon  
358 contamination [44] associated with long beam exposure times.

359 The resolution of a tomographic reconstruction follows the Crowther criterion [45] and will improve  
360 with an increasing number of projections. For typical ADF tomography each image may take a few  
361 seconds to acquire, however each SPED scan acquired in this work took approximately 40 minutes and  
362 this puts a significant constraint on the number of projections that can be reasonably acquired by the  
363 microscope operator within the duration of a typical microscope user session. Recent improvements in  
364 camera speed and sensitivity [46] will offset this limitation. Despite this, new information, including the  
365 3D grain position, size, and shape, are obtainable from the small number of scans (9) used in this work.  
366 This information cannot be properly resolved from a single 2D orientation map, nor from ADF  
367 tomography experiments due to the lack of observable contrast from differently oriented grains of the  
368 same phase.

369 The total reconstructed grain volume was calculated to be 78% of the reconstructed ADF volume as  
370 calculated by their convex hulls. Of this grain fraction 2% of the volume was identified as cementite,  
371 which would likely increase if all cementite particles in the sample could be reconstructed. The  
372 remaining unreconstructed volume apportioned to grain boundary regions, small grains, reconstruction  
373 errors, and ~10% of the unreconstructed volume is due to the unreconstructed amorphous layer, as  
374 discussed previously. Further analysis of the grain shapes is shown in SI 7 and confirms that the ferrite  
375 grains remain equiaxed perpendicular to the columnar axis and are also observed to have a  $\langle 110 \rangle$  texture  
376 along their columnar axis (z-axis), in agreement with previous studies on this material system [25].

377 The ACOM technique has previously been combined with Atom Probe Tomography (APT) to study the  
378 effect of the chemical composition of the grain boundaries on grain misorientation [25]. While the  
379 investigation of grain boundary segregation with the existing approach, which combines NBD, ACOM,  
380 and APT, was limited to materials with columnar grains [25]. Future work is imagined involving the

381 combination of the 3D-SPED approach presented here with APT to investigate equiaxed nanocrystalline  
382 materials and with that to a significantly larger quantity of grain boundaries.

383 The material choice used in this work was optimal for the method development as there was limited  
384 grain overlap along the columnar direction, however the methods presented here are applicable to other  
385 materials. Potential limiting factors for successful reconstruction arise for materials containing small  
386 grains and those containing many grains through their projection. In the former case the grains will  
387 typically have weak diffracting intensity, which would be unsuitable for ACOM and VDF  
388 reconstruction, and in the latter case the multi-indexing strategy is not well suited to identifying more  
389 than 3-4 grains in a single diffraction pattern.

390 In general, this new approach will enable the investigation of all materials science events that involve  
391 local chemistry and crystallography in greater depth. To give one example, the nucleation of a new phase  
392 at a defect is affected by the combination of the crystallographic character of the defect and the local  
393 composition, an effect used for segregation engineering [47]. Interpretation of such processes are only  
394 possible if the full 3D information about crystallography and composition are available.

395

## 396 5 Conclusions

397

398 Here, we have demonstrated the 3D reconstruction of grains within a dual-phase polycrystalline pearlitic  
399 steel tip. By extending the ACOM technique into 3-dimensions, the final reconstruction provides  
400 information about the grain orientation, phase, and shape, which constitutes a full set of information for  
401 materials science applications. The orientation-based algorithms presented here are straightforwardly  
402 applicable to other materials of known phase. Future applications of this analysis and reconstruction  
403 method are envisaged to study grain boundaries at the nanoscale and complementary chemical analyses  
404 using APT.

405

407

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- 553

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558 Humboldt Stiftung.

559

## 560 8 Competing Interests

561 The authors declare no competing interests.

562

## Supporting Information

### Reconstructing Dual-Phase Nanometer Scale Grains within a Pearlitic Steel Tip in 3D through 4D-Scanning Precession Electron Diffraction Tomography and Automated Crystal Orientation Mapping

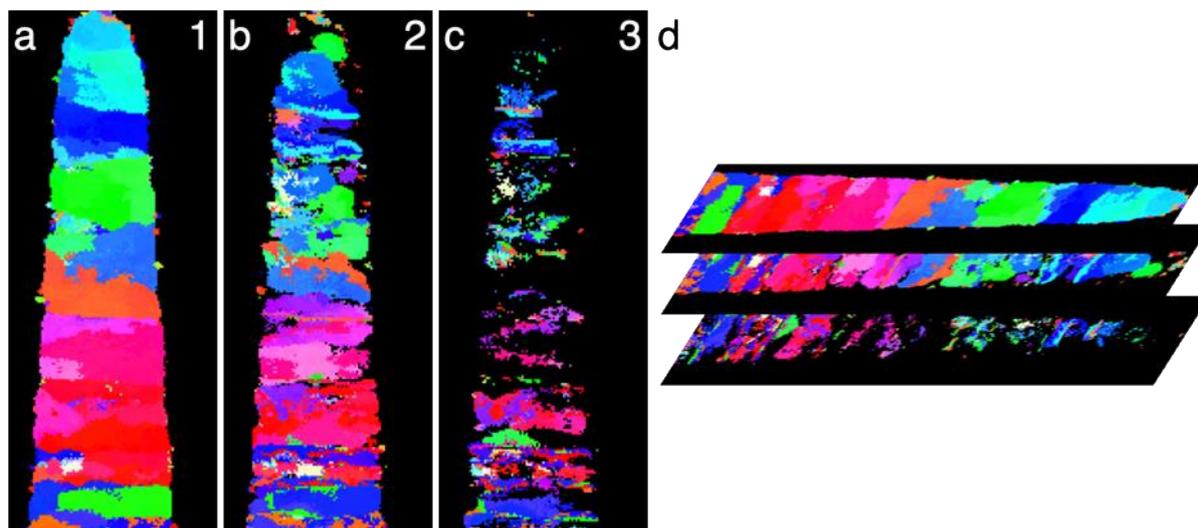
Patrick Harrison\*<sup>1</sup>, Xuyang Zhou<sup>2</sup>, Saurabh Mohan Das<sup>2</sup>, Pierre Lhuissier<sup>1</sup>, Christian H. Liebscher<sup>2</sup>,  
Michael Herbig<sup>2</sup>, Wolfgang Ludwig<sup>3,4</sup>, Edgar F. Rauch<sup>1</sup>

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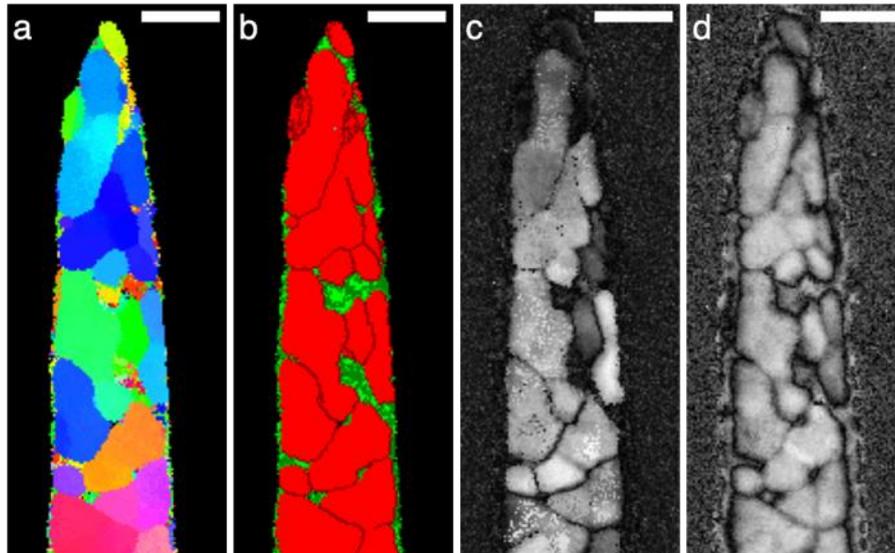
<sup>2</sup> Max-Planck-Institut für Eisenforschung, Max-Planck-Strasse 1, 40237 Düsseldorf, Germany

<sup>3</sup> ESRF – The European Synchrotron, Grenoble, France

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SI 1: Multi-indexing strategy example and strategy example taken from the 60° tilt dataset. (a-c) Raw orientation maps after 1, 2, and 3 indexing passes, respectively. Black pixels are masked by using a minimum threshold on the calculated correlation index. (d) These three maps (a-c) constitute a spatially aligned orientation map stack which is used for further processing, such as grain component calculation as discussed in section 3.2.

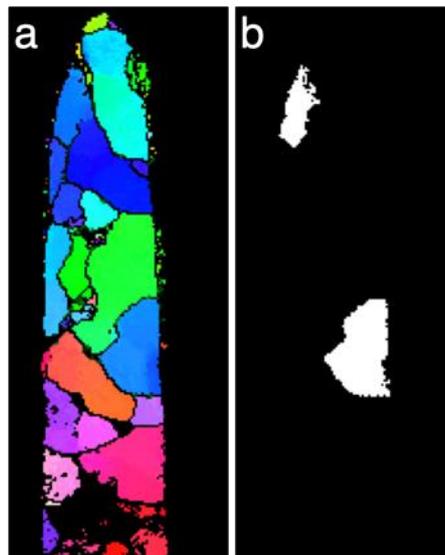


583

584 SI 2: (a) Calculated orientation map and (b) combined phase and grain boundary map for 180° projection. Ferrite and cementite  
 585 phases are shown as red and green, respectively, in (b). (c) Associated orientation and (d) phase reliability maps. These maps  
 586 combined show that the cementite phase is reliably indexed, but that the cementite orientation is not well defined. Note that the  
 587 black pixels represent masked regions in (a) and (b) as determined by an orientation index threshold. Scalebars are 50 nm.

588

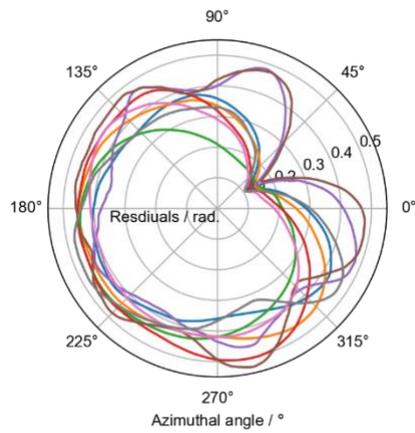
589



590

591 SI 3: Spatial separation of grain components. (a) Combined orientation and grain boundary map acquired from the 0° tilt dataset.  
 592 The multi-indexed orientation stack is reduced to components through orientation-based clustering. In each dataset there is the  
 593 possibility that spatially unconnected grains are assigned to the same component. This is the case as shown in (b), the orientation  
 594 clustering assigns the two white regions to the same component. In all cases the spatial information about each component is  
 595 used to separate any spatially unconnected regions with similar orientation into multiple separated components.

596



597

598 SI 4: Illustration of the calculation of the microscope rotation axis as described in the main text. The total coupled disorientation  
 599 between adjacent tilt datasets  $i$  and  $i + 1$  is calculated after rotating tilt dataset  $i$  around a rotation axis  $r$  contained within the  
 600 azimuthal plane. This residual disorientation is calculated for many directions of  $r$  positions around a full azimuthal rotation.  
 601 The total residual exhibits a strong minimum when the rotation axis is correctly defined. Each coloured line represents the  
 602 coupling residuals for different adjacent tilt datasets.

603

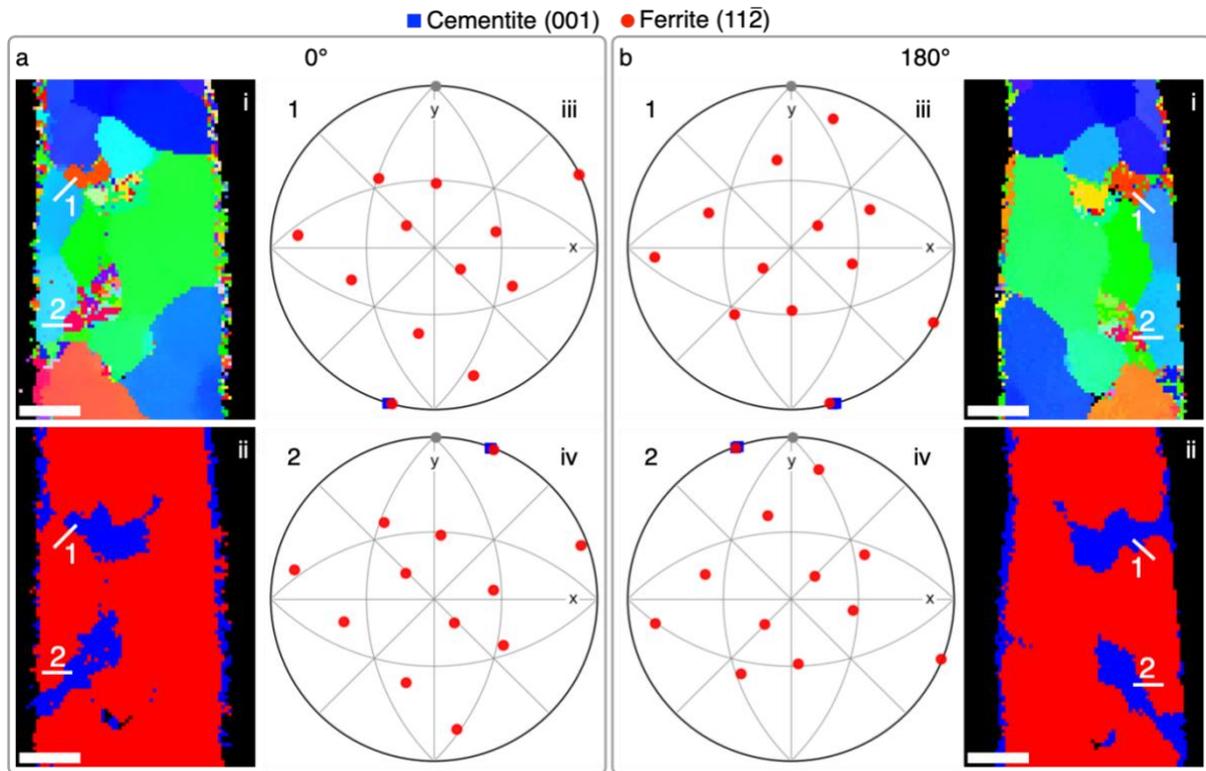
604 SI 5: Video. Pearlitic steel tip 3D rendering orbit.avi

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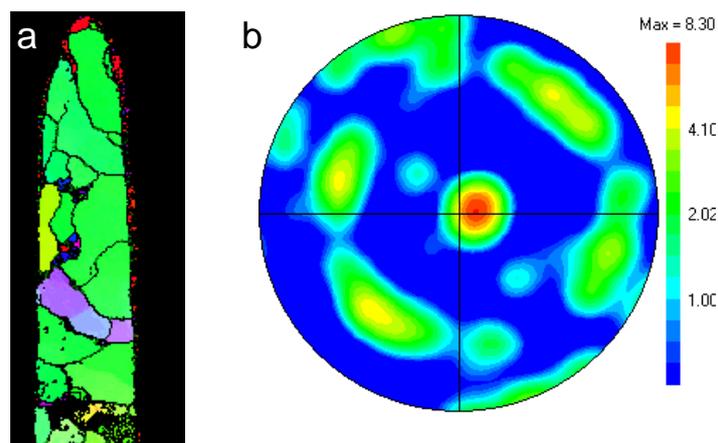
608



609

610 SI 6: Cementite-ferrite orientation relationships observed in the pearlitic steel tip. The indexed diffraction patterns at  $0^\circ$  and  
 611  $180^\circ$  (parallel to the drawing-direction) have the highest orientation reliabilities of all tilt datasets, this is due to increased grain  
 612 overlap away from the columnar direction. (i) Orientation map, (ii) phase map, (iii) and (iv) pole figures extracted from line  
 613 profiles 1 and 2, respectively, for the same grains under the (a)  $0^\circ$  and (b)  $180^\circ$  projection. In (ii-iv) the cementite and ferrite  
 614 phases are shown as blue and red, respectively. Cementite (001) poles and ferrite (112) poles are shown in (iii) and (iv) as blue  
 615 squares and red circles, respectively. Scale bars are 25 nm.

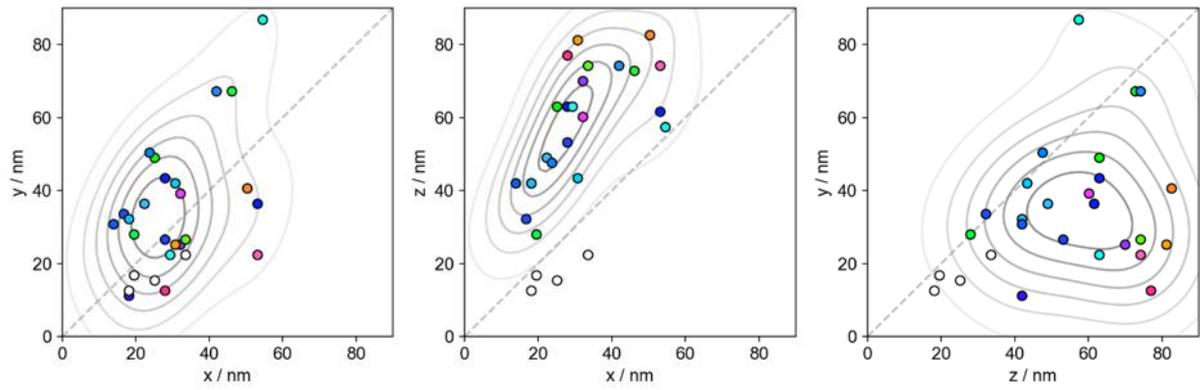
616



617

618 SI 7: (a) Orientation and grain boundary map for the  $0^\circ$  projection, which is parallel to the drawing direction (z-axis, Figure 6).  
 619 The ferrite grains have a  $\langle 110 \rangle$  texture along the drawing direction. Orientation colour codes are the same as in Figure 6. (b)  
 620  $\langle 110 \rangle$  pole figure calculated from (a) with colours mapped onto a log scale.

621



622

623 SI 8: Grain size distributions along all three pairs of axes (a) x-y, (b) x-z, and (c) y-z. Grain extents were calculated from their  
 624 convex hulls. Contour plots show the size distribution as calculated from the kernel density estimate of the ferrite grain size  
 625 distribution. The dashed line represents the expected trend for equiaxed grains. Datapoints are coloured by their orientation  
 626 colour codes as shown Figure 6. The ferrite grains remain equiaxed perpendicular to the drawing direction (z-axis, Figure 6)  
 627 and are elongated along the drawing direction.

628

629