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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

6 Patrick Harrison*¹, Xuyang Zhou², Saurabh Mohan Das², Pierre Lhuissier¹, Christian H. Liebscher²,
7 Michael Herbig², Wolfgang Ludwig^{3,4}, Edgar F. Rauch¹

8

9 ¹ Univ. Grenoble Alpes, CNRS, Grenoble INP, SIMAP, F-38000 Grenoble, France

10 ² Max-Planck-Institut für Eisenforschung, Max-Planck-Strasse 1, 40237 Düsseldorf, Germany

11 ³ ESRF – The European Synchrotron, Grenoble, France

12 ⁴ MATEIS, INSA Lyon, UMR 5510 CNRS, 25 av Jean Capelle, 69621 Villeurbanne, France

13 *Corresponding author, email: patrick.harrison@simap.grenoble-inp.fr

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 (α -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

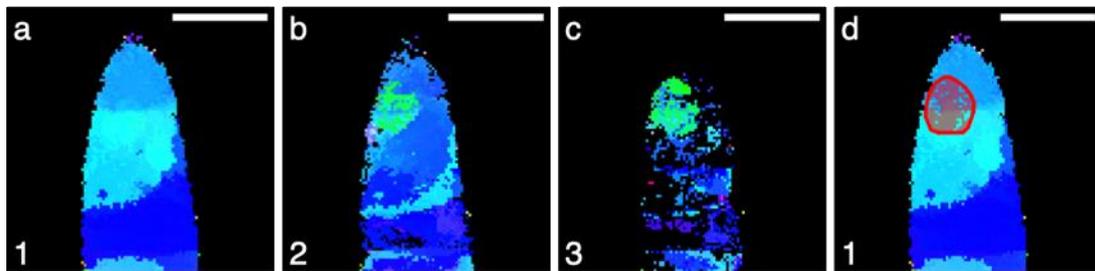
116 3 Processing

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 (Fe_3C , 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

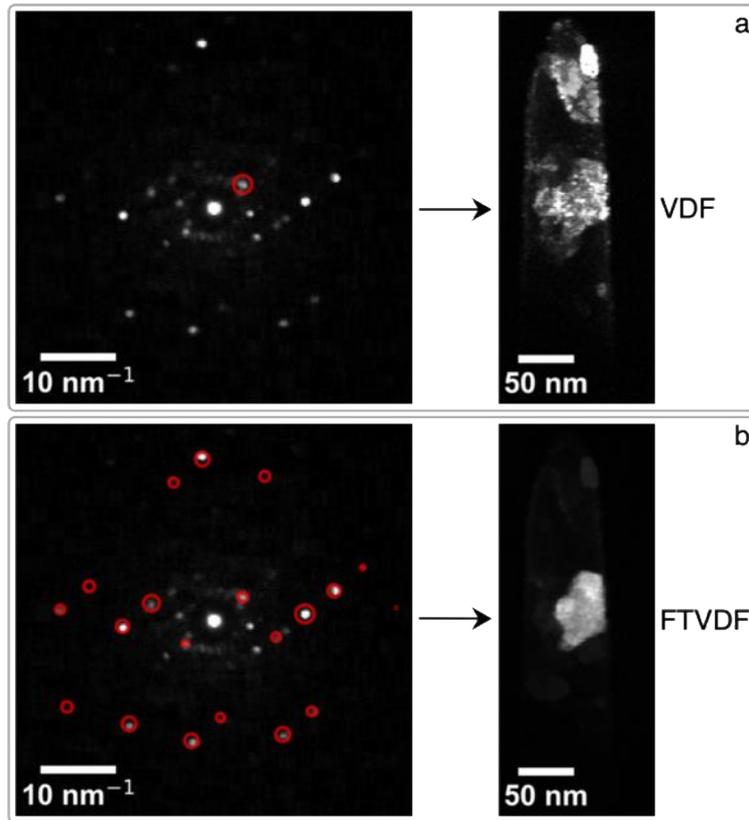
148 3.1 Frozen Template Virtual Dark Field

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

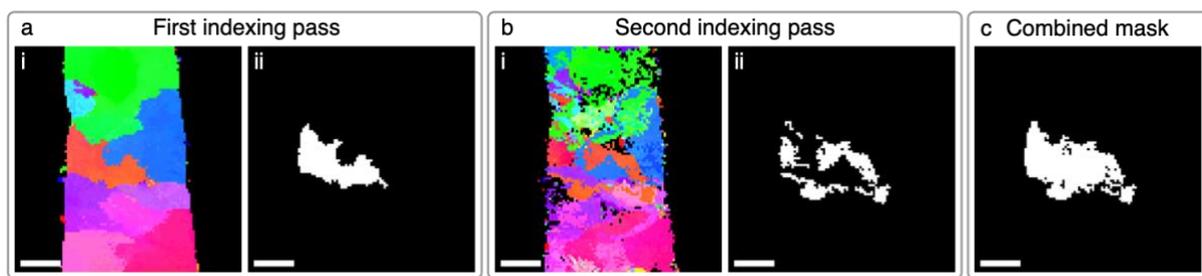
176

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°), 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

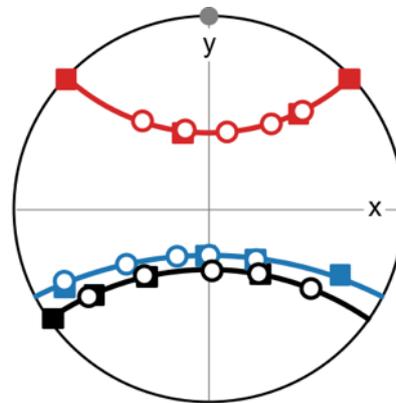
213

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° .

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

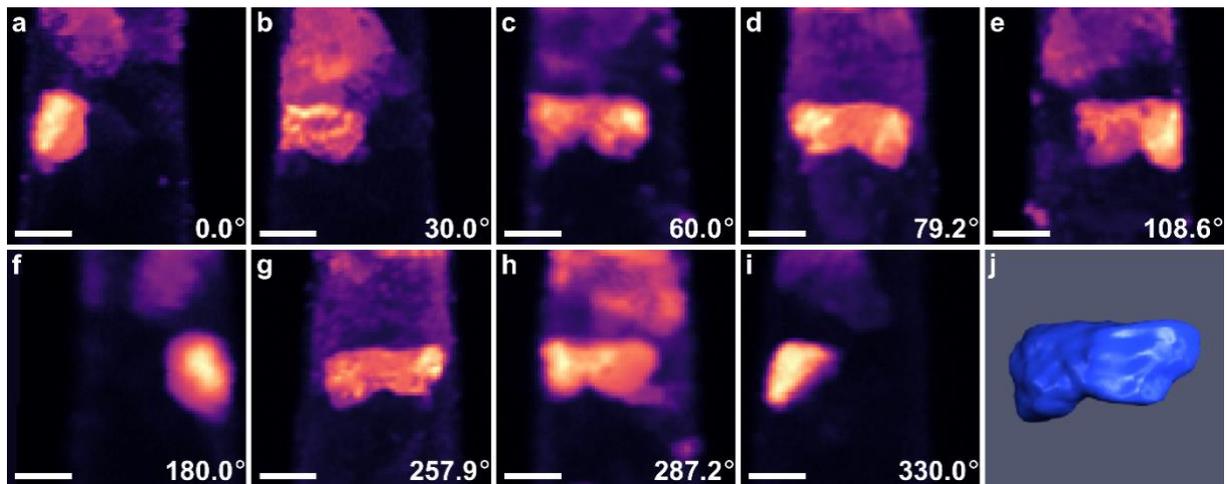
262

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° 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

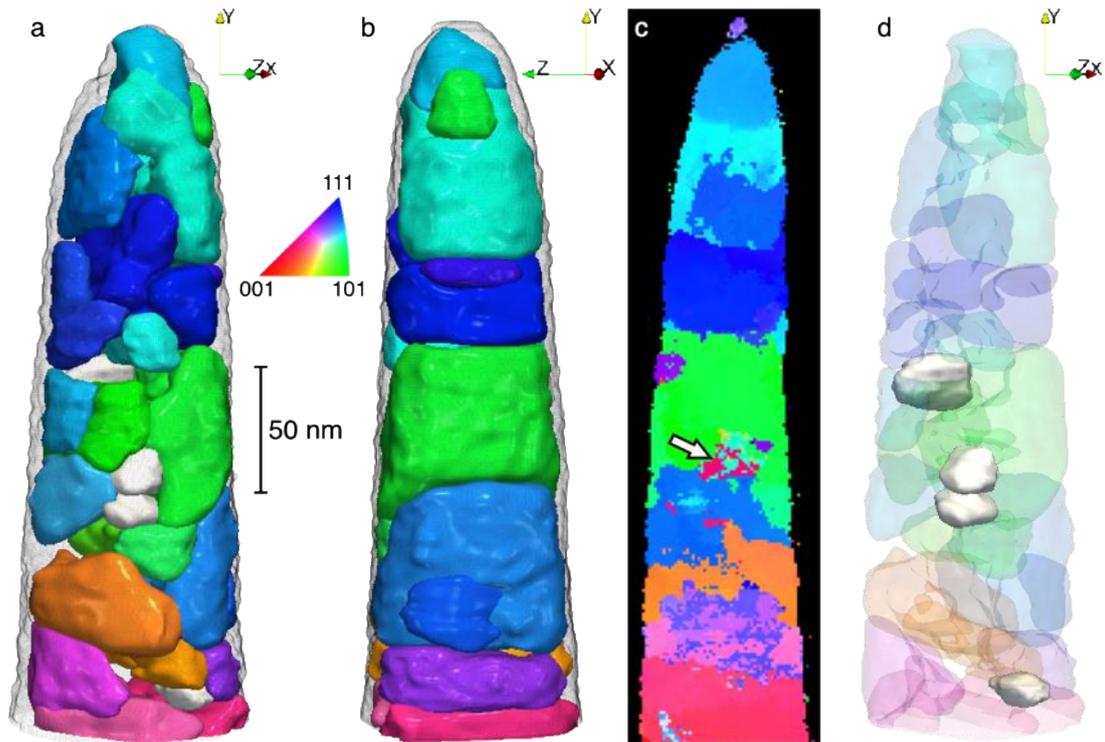
285 Figure 5: (a-i) FTVDF projections of an individual ferrite grain within the pearlitic steel tip. (j) 3D reconstructed isosurface
286 rendering of the grain. Scale bars are 25 nm.

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° and (b) 257.9° 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° 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, ~ 100 nm²) of unchanging orientation, and the
 325 same orientations were also calculated under the reverse projection dataset (0° and 180° 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

- 408 [1] M.A. Meyers, A. Mishra, D.J. Benson, Mechanical properties of nanocrystalline materials,
409 Progress in Materials Science. 51 (2006) 427–556.
410 <https://doi.org/10.1016/j.pmatsci.2005.08.003>.
- 411 [2] A. Borbély, A.R. Kaysser-Pyzalla, X-ray diffraction microscopy: emerging imaging techniques
412 for nondestructive analysis of crystalline materials from the millimetre down to the nanometre
413 scale, J Appl Crystallogr. 46 (2013) 295–296. <https://doi.org/10.1107/S0021889813004160>.
- 414 [3] J.V. Bernier, R.M. Suter, A.D. Rollett, J.D. Almer, High-Energy X-Ray Diffraction Microscopy
415 in Materials Science, Annu. Rev. Mater. Res. 50 (2020) 395–436.
416 <https://doi.org/10.1146/annurev-matsci-070616-124125>.
- 417 [4] H.F. Poulsen, Multi scale hard x-ray microscopy, Current Opinion in Solid State and Materials
418 Science. 24 (2020) 100820. <https://doi.org/10.1016/j.cossms.2020.100820>.
- 419 [5] Y. Hayashi, D. Setoyama, Y. Hirose, T. Yoshida, H. Kimura, Intragranular three-dimensional
420 stress tensor fields in plastically deformed polycrystals, Science. 366 (2019) 1492–1496.
421 <https://doi.org/10.1126/science.aax9167>.
- 422 [6] M. Calcagnotto, D. Ponge, E. Demir, D. Raabe, Orientation gradients and geometrically
423 necessary dislocations in ultrafine grained dual-phase steels studied by 2D and 3D EBSD,
424 Materials Science and Engineering: A. 527 (2010) 2738–2746.
425 <https://doi.org/10.1016/j.msea.2010.01.004>.
- 426 [7] D.J. Dingley, Orientation Imaging Microscopy for the Transmission Electron Microscope,
427 Microchim Acta. 155 (2006) 19–29. <https://doi.org/10.1007/s00604-006-0502-4>.
- 428 [8] G. Wu, S. Zaeferrer, Advances in TEM orientation microscopy by combination of dark-field
429 conical scanning and improved image matching, Ultramicroscopy. 109 (2009) 1317–1325.
430 <https://doi.org/10.1016/j.ultramic.2009.06.002>.
- 431 [9] J.M. Cowley, Applications of electron nanodiffraction, Micron. 35 (2004) 345–360.
432 <https://doi.org/10.1016/j.micron.2003.12.002>.
- 433 [10] A.S. Eggeman, Scanning transmission electron diffraction methods, Acta Crystallogr B Struct
434 Sci Cryst Eng Mater. 75 (2019) 475–484. <https://doi.org/10.1107/S2052520619006723>.
- 435 [11] R. Vincent, P.A. Midgley, Double conical beam-rocking system for measurement of integrated
436 electron diffraction intensities, Ultramicroscopy. 53 (1994) 271–282.
437 [https://doi.org/10.1016/0304-3991\(94\)90039-6](https://doi.org/10.1016/0304-3991(94)90039-6).
- 438 [12] P.A. Midgley, A.S. Eggeman, Precession electron diffraction – a topical review, IUCrJ. 2 (2015)
439 126–136. <https://doi.org/10.1107/S2052252514022283>.
- 440 [13] P. Oleynikov, S. Hovmöller, X.D. Zou, Precession electron diffraction: Observed and calculated
441 intensities, Ultramicroscopy. 107 (2007) 523–533.
442 <https://doi.org/10.1016/j.ultramic.2006.04.032>.
- 443 [14] E.F. Rauch, M. Véron, Automated crystal orientation and phase mapping in TEM, Materials
444 Characterization. 98 (2014) 1–9. <https://doi.org/10.1016/j.matchar.2014.08.010>.
- 445 [15] S. Nicolopoulos, D. Bultreys, E. Rauch, Precession coupled orientation/phase mapping on
446 nanomaterials with TEM Cs microscopes, Acta Crystallogr A Found Crystallogr. 68 (2012)
447 s104–s104. <https://doi.org/10.1107/S0108767312097978>.
- 448 [16] E.F. Rauch, M. Véron, Crystal Orientation Angular Resolution with Precession Electron
449 Diffraction, Microscopy and Microanalysis. 22 (2016) 500–501.
450 <https://doi.org/10.1017/S1431927616003354>.
- 451 [17] A. Valery, E.F. Rauch, A. Pofelski, L. Clement, F. Lorut, Dealing With Multiple Grains in TEM
452 Lamellae Thickness for Microstructure Analysis Using Scanning Precession Electron
453 Diffraction, Microscopy and Microanalysis. 21 (2015) 1243–1244.
454 <https://doi.org/10.1017/S143192761500700X>.
- 455 [18] E.F. Rauch, M. Véron, Virtual dark-field images reconstructed from electron diffraction patterns,
456 Eur. Phys. J. Appl. Phys. 66 (2014) 10701. <https://doi.org/10.1051/epjap/2014130556>.

- 457 [19] H.H. Liu, S. Schmidt, H.F. Poulsen, A. Godfrey, Z.Q. Liu, J.A. Sharon, X. Huang, Three-
458 Dimensional Orientation Mapping in the Transmission Electron Microscope, *Science*. 332
459 (2011) 833–834. <https://doi.org/10.1126/science.1202202>.
- 460 [20] A.S. Eggeman, R. Krakow, P.A. Midgley, Scanning precession electron tomography for three-
461 dimensional nanoscale orientation imaging and crystallographic analysis, *Nat Commun*. 6 (2015)
462 7267. <https://doi.org/10.1038/ncomms8267>.
- 463 [21] D. Kitamura, N. Ono, Efficient initialization for nonnegative matrix factorization based on
464 nonnegative independent component analysis, 2016 IEEE International Workshop on Acoustic
465 Signal Enhancement (IWAENC). (2016) 1–5. <https://doi.org/10.1109/IWAENC.2016.7602947>.
- 466 [22] T. Bergh, D.N. Johnstone, P. Crout, S. Høgås, P.A. Midgley, R. Holmestad, P.E. Vullum,
467 A.T.J.V. Helvoort, Nanocrystal segmentation in scanning precession electron diffraction data,
468 *Journal of Microscopy*. 279 (2020) 158–167. <https://doi.org/10.1111/jmi.12850>.
- 469 [23] Y. Meng, J.-M. Zuo, Three-dimensional nanostructure determination from a large diffraction
470 data set recorded using scanning electron nanodiffraction, *IUCrJ*. 3 (2016) 300–308.
471 <https://doi.org/10.1107/S205225251600943X>.
- 472 [24] P. Harrison, X. Zhou, S.M. Das, N. Viganò, P. Lhuissier, M. Herbig, W. Ludwig, E. Rauch,
473 Reconstructing grains in 3D through 4D Scanning Precession Electron Diffraction, *Microsc*
474 *Microanal*. 27 (2021) 2494–2495. <https://doi.org/10.1017/S1431927621008898>.
- 475 [25] M. Herbig, D. Raabe, Y.J. Li, P. Choi, S. Zaefferer, S. Goto, Atomic-Scale Quantification of
476 Grain Boundary Segregation in Nanocrystalline Material, *Phys. Rev. Lett*. 112 (2014) 126103.
477 <https://doi.org/10.1103/PhysRevLett.112.126103>.
- 478 [26] Y.J. Li, P. Choi, S. Goto, C. Borchers, D. Raabe, R. Kirchheim, Evolution of strength and
479 microstructure during annealing of heavily cold-drawn 6.3GPa hypereutectoid pearlitic steel
480 wire, *Acta Materialia*. 60 (2012) 4005–4016. <https://doi.org/10.1016/j.actamat.2012.03.006>.
- 481 [27] Y. Li, D. Raabe, M. Herbig, P.-P. Choi, S. Goto, A. Kostka, H. Yarita, C. Borchers, R.
482 Kirchheim, Segregation Stabilizes Nanocrystalline Bulk Steel with Near Theoretical Strength,
483 *Phys. Rev. Lett*. 113 (2014) 106104. <https://doi.org/10.1103/PhysRevLett.113.106104>.
- 484 [28] C.R. Harris, K.J. Millman, S.J. van der Walt, R. Gommers, P. Virtanen, D. Cournapeau, E.
485 Wieser, J. Taylor, S. Berg, N.J. Smith, R. Kern, M. Picus, S. Hoyer, M.H. van Kerkwijk, M.
486 Brett, A. Haldane, J.F. del Río, M. Wiebe, P. Peterson, P. Gérard-Marchant, K. Sheppard, T.
487 Reddy, W. Weckesser, H. Abbasi, C. Gohlke, T.E. Oliphant, Array programming with NumPy,
488 *Nature*. 585 (2020) 357–362. <https://doi.org/10.1038/s41586-020-2649-2>.
- 489 [29] SciPy 1.0 Contributors, P. Virtanen, R. Gommers, T.E. Oliphant, M. Haberland, T. Reddy, D.
490 Cournapeau, E. Burovski, P. Peterson, W. Weckesser, J. Bright, S.J. van der Walt, M. Brett, J.
491 Wilson, K.J. Millman, N. Mayorov, A.R.J. Nelson, E. Jones, R. Kern, E. Larson, C.J. Carey, Í.
492 Polat, Y. Feng, E.W. Moore, J. VanderPlas, D. Laxalde, J. Perktold, R. Cimrman, I. Henriksen,
493 E.A. Quintero, C.R. Harris, A.M. Archibald, A.H. Ribeiro, F. Pedregosa, P. van Mulbregt, SciPy
494 1.0: fundamental algorithms for scientific computing in Python, *Nat Methods*. 17 (2020) 261–
495 272. <https://doi.org/10.1038/s41592-019-0686-2>.
- 496 [30] J. D. N., M. B. H., C. P, M. P. A., E. A. S., Density-based clustering of crystal (mis)orientations
497 and the orix Python library, *Journal of Applied Crystallography*. 53 (2020) 1293–1298.
498 <https://doi.org/10.1107/S1600576720011103>.
- 499 [31] D.N. Mastrorarde, Fiducial Marker and Hybrid Alignment Methods for Single- and Double-axis
500 Tomography, in: J. Frank (Ed.), *Electron Tomography*, Springer New York, New York, NY,
501 2006: pp. 163–185. https://doi.org/10.1007/978-0-387-69008-7_6.
- 502 [32] C. Gammer, V. Burak Ozdol, C.H. Liebscher, A.M. Minor, Diffraction contrast imaging using
503 virtual apertures, *Ultramicroscopy*. 155 (2015) 1–10.
504 <https://doi.org/10.1016/j.ultramic.2015.03.015>.
- 505 [33] E.F. Rauch, P. Harrison, X. Zhou, M. Herbig, W. Ludwig, M. Véron, New Features in Crystal
506 Orientation and Phase Mapping for Transmission Electron Microscopy, *Symmetry*. 13 (2021)
507 1675. <https://doi.org/10.3390/sym13091675>.
- 508 [34] M. Gallagher-Jones, C. Ophus, K.C. Bustillo, D.R. Boyer, O. Panova, C. Glynn, C.-T. Zee, J.
509 Ciston, K.C. Mancina, A.M. Minor, J.A. Rodriguez, Nanoscale mosaicity revealed in peptide
510 microcrystals by scanning electron nanodiffraction, *Commun Biol*. 2 (2019) 26.
511 <https://doi.org/10.1038/s42003-018-0263-8>.

- 512 [35] B.H. Martineau, D.N. Johnstone, A.T.J. van Helvoort, P.A. Midgley, A.S. Eggeman,
513 Unsupervised machine learning applied to scanning precession electron diffraction data, *Adv*
514 *Struct Chem Imag.* 5 (2019) 3. <https://doi.org/10.1186/s40679-019-0063-3>.
- 515 [36] G.J.C. Carpenter, Image and Diffraction Pattern Rotations in the TEM, *Microsc. Today.* 20 (2012)
516 52–55. <https://doi.org/10.1017/S1551929512000697>.
- 517 [37] A. Morawiec, E. Bouzy, On the reliability of fully automatic indexing of electron diffraction
518 patterns obtained in a transmission electron microscope, *J Appl Cryst.* 39 (2006) 101–103.
519 <https://doi.org/10.1107/S0021889805032966>.
- 520 [38] D.F. Crouse, On implementing 2D rectangular assignment algorithms, *IEEE Transactions on*
521 *Aerospace and Electronic Systems.* 52 (2016) 1679–1696.
522 <https://doi.org/10.1109/TAES.2016.140952>.
- 523 [39] S. Hata, H. Furukawa, T. Gondo, D. Hiramami, N. Horii, K.-I. Ikeda, K. Kawamoto, K. Kimura,
524 S. Matsumura, M. Mitsuhashi, H. Miyazaki, S. Miyazaki, M.M. Murayama, H. Nakashima, H.
525 Saito, M. Sakamoto, S. Yamasaki, Electron tomography imaging methods with diffraction
526 contrast for materials research, *Microscopy.* 69 (2020) 141–155.
527 <https://doi.org/10.1093/jmicro/dfaa002>.
- 528 [40] P. Gilbert, Iterative methods for the three-dimensional reconstruction of an object from
529 projections, *Journal of Theoretical Biology.* 36 (1972) 105–117. [https://doi.org/10.1016/0022-](https://doi.org/10.1016/0022-5193(72)90180-4)
530 [5193\(72\)90180-4](https://doi.org/10.1016/0022-5193(72)90180-4).
- 531 [41] P.-Y. Tung, X. Zhou, D. Mayweg, L. Morsdorf, M. Herbig, Under-stoichiometric cementite in
532 decomposing binary Fe-C pearlite exposed to rolling contact fatigue, *Acta Materialia.* 216 (2021)
533 117144. <https://doi.org/10.1016/j.actamat.2021.117144>.
- 534 [42] B. Goris, T. Roelandts, K.J. Batenburg, H. Heidari Mezerji, S. Bals, Advanced reconstruction
535 algorithms for electron tomography: From comparison to combination, *Ultramicroscopy.* 127
536 (2013) 40–47. <https://doi.org/10.1016/j.ultramic.2012.07.003>.
- 537 [43] M. Herbig, A. Kumar, Removal of hydrocarbon contamination and oxide films from atom probe
538 specimens, *Microsc Res Tech.* 84 (2021) 291–297. <https://doi.org/10.1002/jemt.23587>.
- 539 [44] A.E. Ennos, The origin of specimen contamination in the electron microscope, *Br. J. Appl. Phys.*
540 4 (1953) 101–106. <https://doi.org/10.1088/0508-3443/4/4/302>.
- 541 [45] R.A. Crowther, D.J. DeRosier, A. Klug, The reconstruction of a three-dimensional structure
542 from projections and its application to electron microscopy, *Proc. R. Soc. Lond. A.* 317 (1970)
543 319–340. <https://doi.org/10.1098/rspa.1970.0119>.
- 544 [46] I. MacLaren, E. Frutos-Myro, D. McGrouther, S. McFadzean, J.K. Weiss, D. Cosart, J. Portillo,
545 A. Robins, S. Nicolopoulos, E. Nebot del Busto, R. Skogeby, A Comparison of a Direct Electron
546 Detector and a High-Speed Video Camera for a Scanning Precession Electron Diffraction Phase
547 and Orientation Mapping, *Microsc Microanal.* 26 (2020) 1110–1116.
548 <https://doi.org/10.1017/S1431927620024411>.
- 549 [47] D. Raabe, M. Herbig, S. Sandlöbes, Y. Li, D. Tytko, M. Kuzmina, D. Ponge, P.-P. Choi, Grain
550 boundary segregation engineering in metallic alloys: A pathway to the design of interfaces,
551 *Current Opinion in Solid State and Materials Science.* 18 (2014) 253–261.
552 <https://doi.org/10.1016/j.cossms.2014.06.002>.
- 553

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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

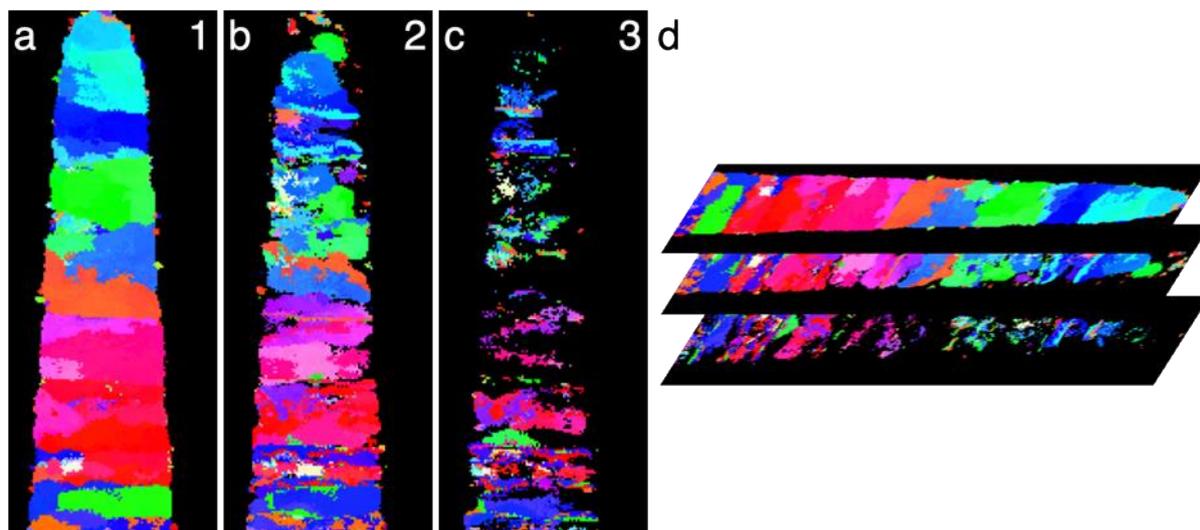
Patrick Harrison*¹, Xuyang Zhou², Saurabh Mohan Das², Pierre Lhuissier¹, Christian H. Liebscher²,
Michael Herbig², Wolfgang Ludwig^{3,4}, Edgar F. Rauch¹

¹ SIMAP Laboratory, CNRS-Grenoble INP, BP 46 101 rue de la Physique, 38402 Saint Martin d'Hères, France

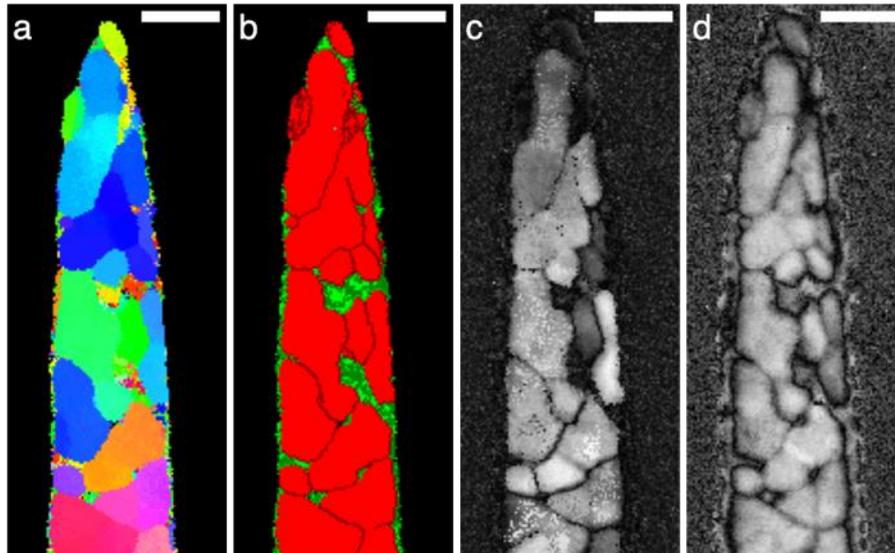
² Max-Planck-Institut für Eisenforschung, Max-Planck-Strasse 1, 40237 Düsseldorf, Germany

³ ESRF – The European Synchrotron, Grenoble, France

⁴ MATEIS, INSA Lyon, UMR 5510 CNRS, 25 av Jean Capelle, 69621 Villeurbanne, France



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.

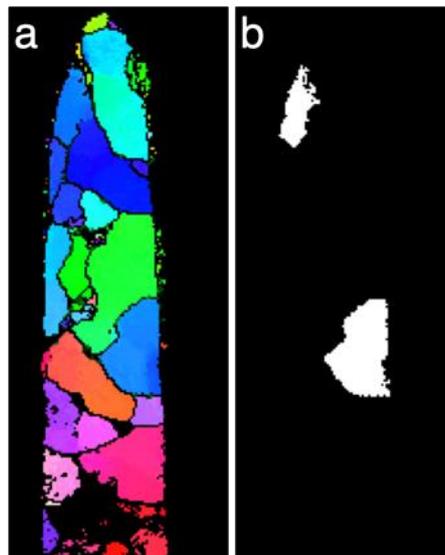


583

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

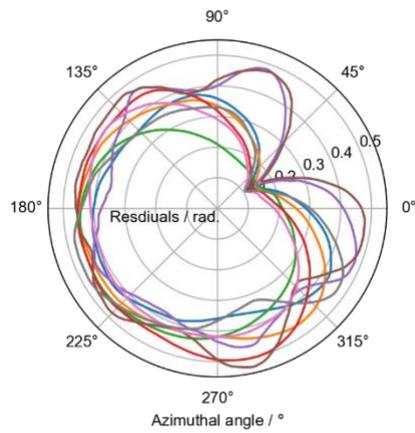
589



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

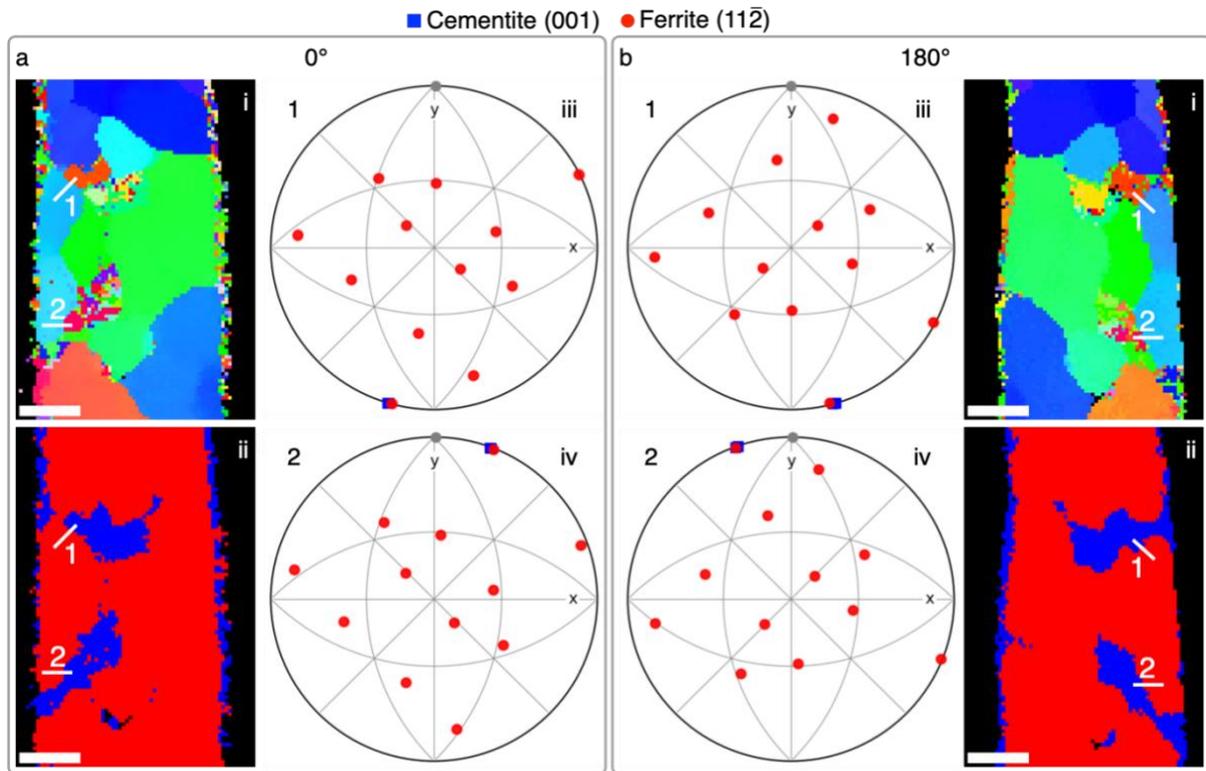
604 SI 5: Video. Pearlitic steel tip 3D rendering orbit.avi

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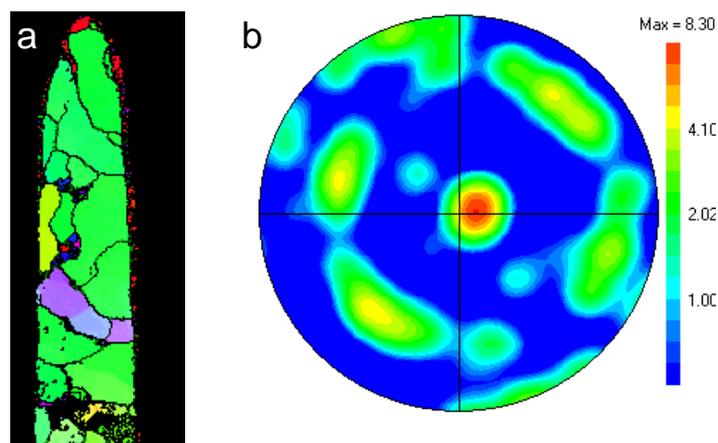
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609

610 SI 6: Cementite-ferrite orientation relationships observed in the pearlitic steel tip. The indexed diffraction patterns at 0° and
 611 180° (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° and (b) 180° 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.

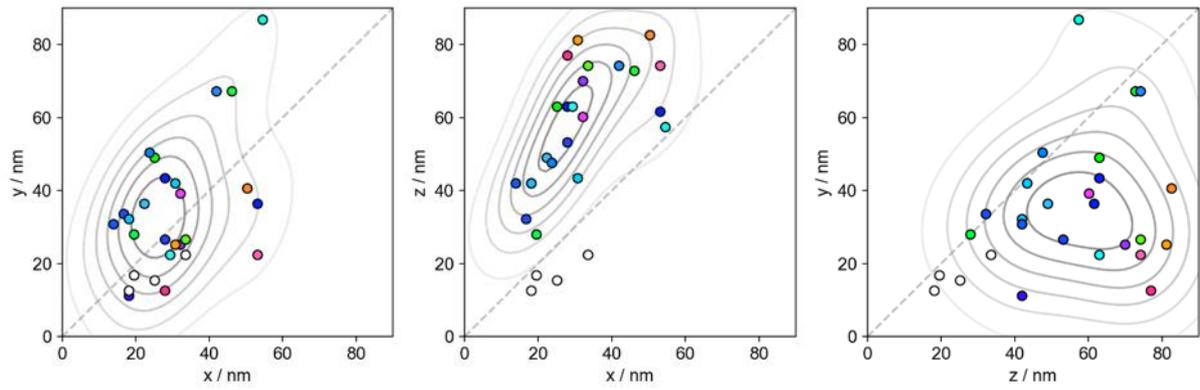
616



617

618 SI 7: (a) Orientation and grain boundary map for the 0° 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