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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No data were collected de novo; the data collection strategy for the EMPIAR-10097 data set re-analyzed in this study is described in doi:10.1038/nmeth.4347
Data analysis	Warp 1.0.0, described in this study; cryoSPARC 0.65, doi:10.1038/nmeth.4169; RELION 2.1, doi:10.7554/eLife.18722; RELION 3.0, doi:10.7554/eLife.42166; InSilicoTEM, doi:10.1016/j.jsb.2013.05.008; TensorFlow 1.5 (https://tensorflow.org); MotionCor2 (doi:10.1038/nmeth.4193)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Figures 1 and S1 use exemplary data from EMPIAR-10078 (doi:10.6019/EMPIAR-10078). Figure 2 uses a cryo-EM image of RNA Pol II complexes, available from the authors upon request. Figure 3 and the benchmark section use data from EMPIAR-10097 (doi:10.6019/EMPIAR-10097) re-analyzed in this study. The refined maps shown in Figure 3a are available in Supplementary Data 1–4. The "Full Warp pipeline" map shown in Figure 3a has been deposited in EMDB as EMD-0025 (http://www.ebi.ac.uk/pdbe/entry/emdb/EMD-0025). Figure 4 and the benchmark section use data from EMPIAR-10061 (doi:10.6019/EMPIAR-10061) re-analyzed in this study, the 1.86 Å map shown in Figure 4a is available as Supplementary Data 5. Figure 5a uses a tomogram reconstructed from data from EMPIAR-10045 (doi:10.6019/EMPIAR-10045). Figure 6 and the benchmark section use data from EMPIAR-10045 and EMPIAR-10164 (doi:10.6019/EMPIAR-10164) re-analyzed in this study, the maps shown in Figure 6a and 6b are available in Supplementary Data 6 and 7, respectively. Figure S2 uses exemplary data from EMPIAR-10061. Figure S3 uses exemplary data from EMPIAR-10097 (doi:10.6019/EMPIAR-10097). Figure S5 uses in-house data, available upon request. Figure S6 uses exemplary data from EMPIAR-10078. Figure S7 uses exemplary data from (left) EMPIAR-10078, (center) in-house data available upon request, and (right) EMPIAR-10153 (doi:10.6019/EMPIAR-10153). Training data for BoxNet can be accessed through https://github.com/cramerlab/boxnet.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number and identity of the particles that went into each refined map were determined through 3D classification, as described under Methods, Benchmarking.
Data exclusions	The quality filters applied at the end of the Warp pre-processing pipeline are described under Methods, Benchmarking. Only data within the filter thresholds were used for map refinement. No manual data exclusion was performed.
Replication	No replication was performed as only one data set was available and all analysis algorithms were deterministic.
Randomization	During 3D map refinements, data were randomly split in 2 groups following the "gold standard" protocol (doi:10.1038/nmeth.2115) to enable resolution estimation through cross-validation (Fourier shell correlation). Randomization was not applicable to other parts of the study.
Blinding	No blinding was performed as data used for benchmarks were drawn from a pool of previously analyzed data sets (EMPIAR) to establish a direct comparison to the results of this study.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging