1. “We have recently updated our manuscript guidelines and now require between 6 and 12 key words per manuscript. Please add at least 1 more keyword.”

We added three key words, eman, iterative helical real space reconstruction, and single particle reconstruction

2. “Personal pronouns (we, you, our, etc.) should be used sparingly in most sections and not at all in the protocol section. Please reduce the use of personal pronouns throughout the manuscript. Please remove all instances of personal pronouns from the protocol.”

We removed all personal pronouns from the protocol and from the introduction and discussion sections.

3. “Please adjust the numbering of your protocol section to follow JoVE instructions for authors, 1. should be followed by 1.1) and then 1.1.1) and so on, and all steps should be lined up at the left margin with no indentations. Please use only numbers, not letters. Introductory text (such as the text you have at the very beginning of the protocol, before section I) does not need to be numbered.”

The steps of the procedure were renumbered in accordance with this request.

4. “Please add a space between each numbered step.”

Done

5. “Please update instances within the text where you refer to steps by number according to the updated numbering scheme.”

Such instances were changed to reflect the new numbering scheme.

6. “Please ensure that all steps in the protocol are written in the imperative tense (do this, mix that, etc.). For instance, "Sample preparation is performed as demonstrated in other JoVE articles." should be "Perform sample preparation as demonstrated?.."”

Changed any obvious instances of protocol steps which were not in the imperative tense.

7. “Introductory text segments and explanatory notes do not need to be written in the imperative tense. However this type of text should be used sparingly and the majority of the protocol section should contain action steps in the order they should be completed.”

Reduced explanatory notes.

8. “Your protocol currently exceeds the JoVE 3 page guideline and will be even longer when you add a space between each step. After revising your protocol as described above, please highlight (with yellow text background) less than 2.75 pages of text (including spaces between steps) to identify which portions of the protocol are most important to film; i.e. which steps should be visualized to best supplement the written section of the protocol. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader. Notes or comments that will not be filmed can be excluded from the highlighted portion. You may combine short steps that are related to shorten the protocol section. Ideally, each numbered step will contain 1-3 related actions. As a guideline, individual steps should not be longer than step 3 in section 1.”

The steps we intend to be filmed are now highlighted and correspond with screenwriter special request form.

9. “As you highlight please consider which steps will benefit most from being visualized and translate most appropriately to a video format. While a large portion of your protocol is computational, we ask that you include both experimental and computational methods in the video.”

See screenwriter special request form.

10. “For all steps that you will film, it is important that enough details are included so that a user can replicate the protocol and our script writer can plan the video properly. For instance "Dried grids are coated with carbon." How? Using what equipment / parameters? If this step is highlighted for filming please re-write it as a sequence of actions to be performed. Please ensure that all steps chosen to be filmed include enough detail for a user to properly replicate them.”

Modified some steps, including some of those we intend to film.

11. “This applies to both experimental and computational steps. Computational steps should be re-written as a sequence of distinct actions such as: open this program, select this window, click this option etc. For instance for than "Run hrefine program..." how is this done?”

See above.

12. “As you are considering the detail level of the protocol, please think about how you will demonstrate each step when you film your video, and the actions that are involved, and use these to guide the protocol text. Steps should be written with specific details for the example you will demonstrate rather than in vague or generic terms.”

Streamlined protocol to be more compliant with this request.

13. “After you have highlighted 2.75 pages of steps to film, please see the special request below. Please note that this request only applies to the steps you have highlighted. Please ensure that all desired revisions are made to the protocol section prior to completing this request.”

Done

14. “Please move the figure titles and captions from the individual figure files into the main text file. Please insert these at the end of the results section. Each figure should have a title and a brief description, and these should stand alone from the results text itself. There should not be any text in the figure files other than within the figures themselves. Please ensure that the protocol steps referred to in the figure captions use the correct numbers.”

Figure legends were removed from figures and placed in the appropriate location.

15. “If any of your figures are being reprinted from a previous work please obtain and provide the required permission. This is very important since you have requested expedited processing to be in press and not providing permission for reprints will delay processing your manuscript later on.”

No permissions need to be requested. Figures are original.

16. “JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript and use more generic terms to refer to materials and equipment. While information such as brand and company names is helpful to the user, these details should not be included in the manuscript text but should be included in the table of materials and equipment. Examples of commercial language in your manuscript include: Titan Krios microscope; software brand names (unless open source); etc.”

Removed trademarked names and described instruments in more general terms.

And 17. “In instances where the use of a specific type of material or equipment will directly affect the protocol outcome or parameters you may use the specific name when you introduce the equipment and then refer to it by more generic terms thereafter. No trademarks symbols can be published anywhere in the manuscript.”

Edited out use of trade names and applied more generic names to instruments.