

**Supplementary Table 1**

<b>Task</b>	<b>Program name</b>	<b># Cores</b>	<b>Walltime (min)</b>	<b>Iterations</b>
<b>Raw data import</b>	e2import.py		1	
<b>Tomographic reconstruction</b>	e2tomogram.py <sup>†</sup>	12	9	2,1,1,1
<b>Reference-based particle picking</b>	e2spt_tempmatch.py		7	
<b>CTF correction</b>	e2spt_tomocf.py		2	
<b>Subtomogram extraction</b>	e2spt_extract.py <sup>†</sup>	1	31	
<b>Initial model generation</b>	e2spt_sgd.py <sup>†</sup>	12	41	3
<b>Subtomogram refinement</b>	e2spt_refine.py <sup>†</sup>	12	181	3
<b>Subtilt refinement</b>	e2spt_tiltrefine.py <sup>†*</sup>	96	308	6

**Supplementary Table 1.** Example program runtimes. Parallelism: \* = MPI, † = Thread.

Except for the final subtilt refinement all steps were performed on a single 12 core workstation. The final tilt refinement was run on 4x24 core nodes on a Linux cluster.

**Supplementary Table 2**

	<b>EMAN2</b>	<b>IMOD/PEET</b>	<b>pyTOM</b>	<b>Relion</b>	<b>emClarity</b>
<b>Tilt series alignment</b>	Yes	Yes	No	No	No
<b>Tomogram reconstruction</b>	Yes	Yes	Yes	No	No
<b>CTF correction</b>	Per-particle-per-tilt	Strip-wise	No	Per-particle 3D	3D back projection
<b>Particle selection</b>	Manual, template matching, neural networks	Manual, template matching	Template matching	No	Template matching
<b>Initial model generation</b>	Yes	No	No	No	No
<b>Subtomogram alignment</b>	Yes	Yes	Yes	Yes	Yes
<b>Subtilt alignment</b>	Yes	No	No	No	Yes (requires IMOD)

**Supplementary Table 2.** Comparison of features among different software packages for CryoET data processing.