

crYOLO tutorial for picking using a “general model” without training:

[https://sphire.mpg.de/wiki/doku.php?id=pipeline:window:cryolo:picking\\_general](https://sphire.mpg.de/wiki/doku.php?id=pipeline:window:cryolo:picking_general)

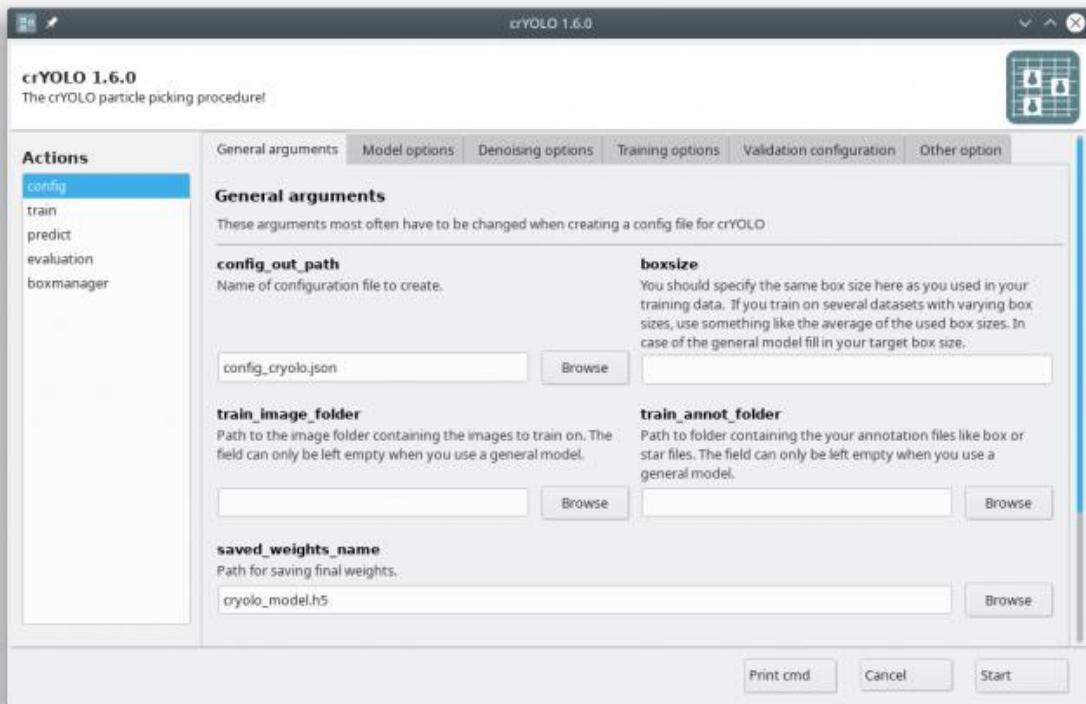
before running crYOLO, You will need to download the model.h5 file from crYOLO website:

[ftp://ftp.gwdg.de/pub/misc/sphire/crYOLO-GENERAL-MODELS/gmodel\\_phosnet\\_202002\\_N63.h5](ftp://ftp.gwdg.de/pub/misc/sphire/crYOLO-GENERAL-MODELS/gmodel_phosnet_202002_N63.h5)

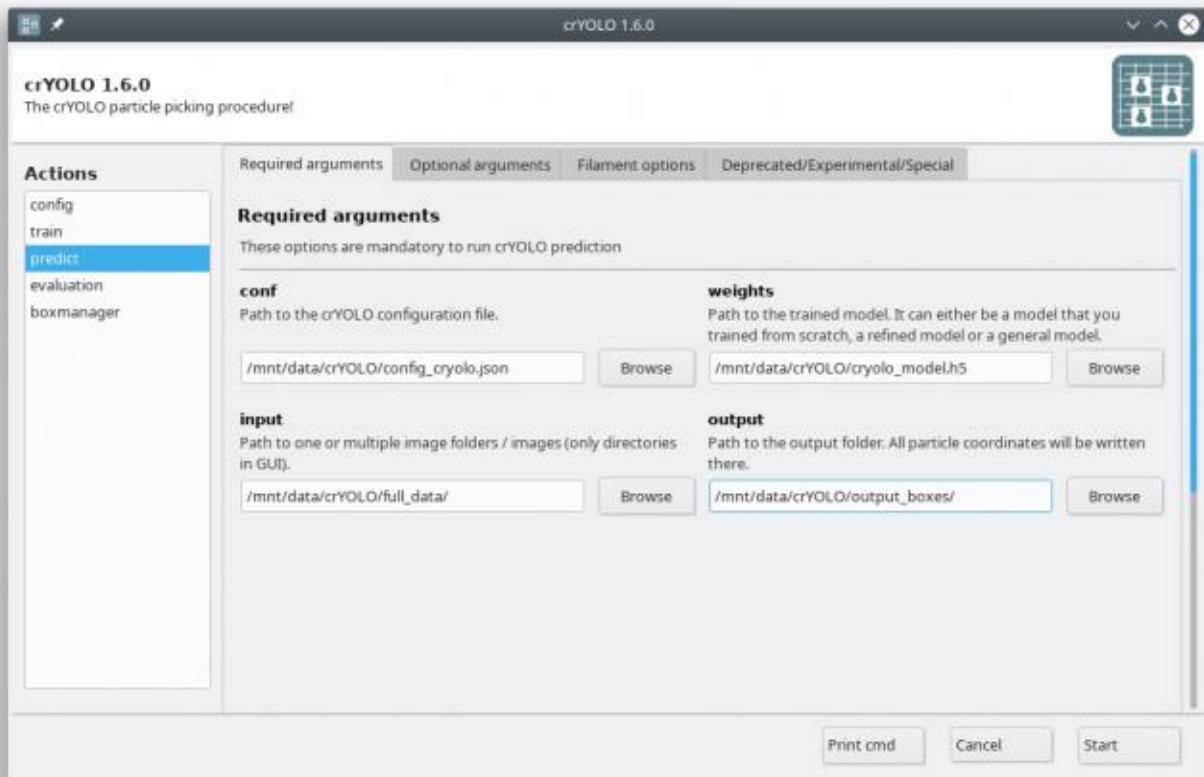
Though it's perfectly okay to run crYOLO with motion corrected images, it's recommended to run crYOLO after CTFfind step in Relion: make a folder (here called “cryolo\_box”) under your /Relion-working-directory/CTFfind/job###/cryolo\_box/, to store all the “boxes” or particle coordinates from crYOLO.

three major steps running crYOLO:

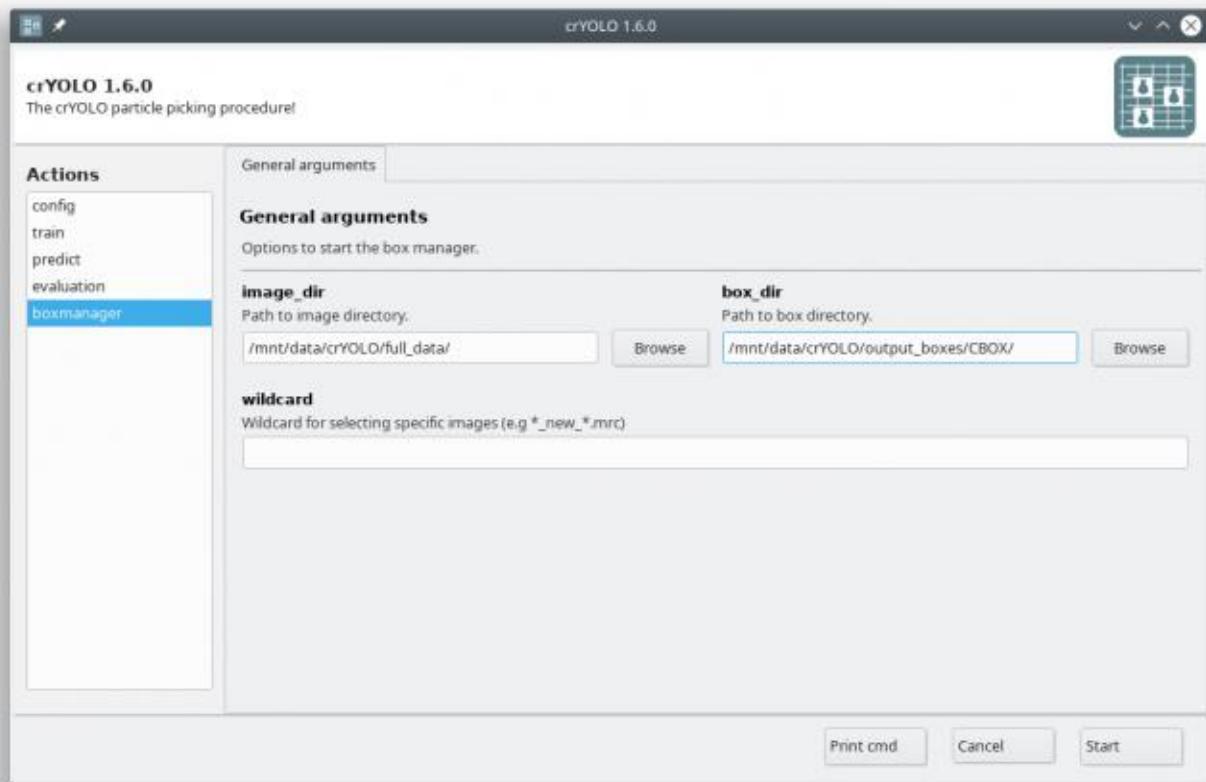
- **in between the following steps, please do NOT hit the “CLOSE” button when a step finishes. it would close crYOLO program. Instead, hit the “BACK” button to go back to the main menu.**
- “**config**”: the only thing one has to specify is a very generous estimate of the box size here. the output we'll import into Relion would be the coordinates of the “boxes”, and we will specify again the box size during particle extraction in Relion. One can make changes to this value later when running “boxmanager” in crYOLO. in our experience, this box size value really doesn't affect the particles picked or the number of particles, as long as one stays within 2x of the “real” size of the particle.
  - o hit START;
  - o when finished, hit “BACK” (NOT “CLOSE”).



- **“predict”:**
  - o use the output from “config” for “conf” (\*.json);
  - o “weights”: Browse and select the downloaded “gmodel\_phosnet\_202002\_N63.h5”;
  - o “input”: Browse and open the directory where the images are (CTFfind/job###/images/);
  - o “output”: Browse and open the newly created “cryolo\_box”.
  - o I rarely had to touch or change anything else under the other tabs. occasionally you may want to specify which GPU to run crYOLO on (by default it runs on GPU-0) under the “Optional arguments” tab. I’d recommend that you run this with a single GPU.
  - o hit “START”. the program is very efficient, but may take a minute or two before it starts picking.
  - o when finished, scroll down to the bottom of the output log window (before hitting the “BACK” button), and notice how many particles are picked.



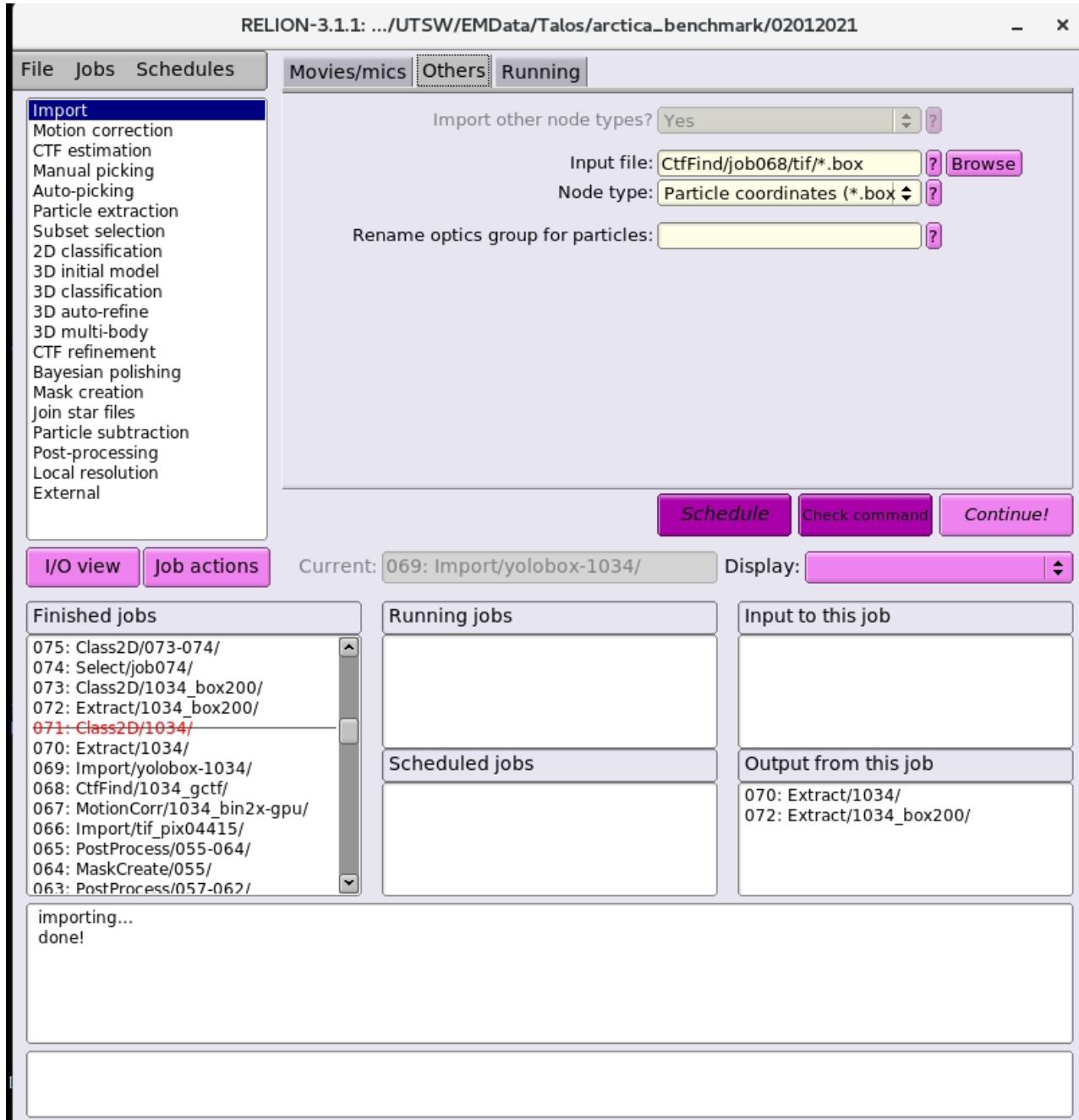
- “**boxmanager**”: this could be optional. it’s used to verify the “picks”.
  - o “Image\_dir”: Browse to the folder where the images are (same as above);
  - o “box\_dir”: under “cryolo\_box”, there would be 4 subfolders. I normally use the \*.box files under the “EMAN” folder. so Browse and open this subfolder here.
  - o “wildcard”: leave this blank.
  - o hit “START”. this would bring up a couple of windows and may take a minute. one can change the “box size” to see which value to be used in Relion’s extraction routine. if the image is hard to see, set the “Low pass filter cuf-off” to 0.49 (hit apply afterwards).
  - o when done, close crYOLO.



**this is important: make sure that you copy all the \*.box file from the /cryolo\_box/EMAN/ folder to the /CTFfind/job###/images/ folder. Please check and make sure that each \*.mrc image file has a corresponding \*.box file under this folder afterwards.**

Now go back to Relion:

- **Import:** run an import job, here select “NO” to movies in the main tab, go to the second tab “Others” and choose to import “particle coordinates (\*.box)”, and under “input file”, Browse and select one of the \*.box” file, and replace the filename with a wildcard (\*.box). Hit “start”.



- **Extract:**

- o under I/O: chose the micrographs\_ctf.star file from the CTFfind step;
- o for “Input coordinates”: Browse to the “Import” job you just finished, and there should be only one file available to be picked called “coords\_suffix.box”. select that one. If you forgot to copy the \*.box files over from the EMAN subfolder, you won’t be able to do this.
- o the rest is simply Relion settings. you may use the Box size you found out earlier from boxmanager in crYOLO under the “extract” tab.
- o when finished, you should be getting close to the number of particles picked by crYOLO.

