crYOLO tutorial for picking using a "general model" without training:

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

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:

- <u>in between the following steps, please do NOT hit the "CLOSE" button when a step finishes. it would</u> <u>close crYOLO program. Instead, hit the "BACK" button to go back to the main menu.</u>
- "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.
 - hit START;
 - when finished, hit "BACK" (NOT "CLOSE").

ctions	General arguments Model o	ptions Denoising options	Training options	Validation config	uration Oth	er option	
cohfig train predict evaluation boxmanager	General arguments These arguments most often have to be changed when creating a config file for crYOLO						
	config_out_path Name of configuration file to create.		training data. sizes, use som	boxsize You should specify the same box size here as you used in your training data. If you train on several datasets with varying box sizes, use something like the average of the used box sizes. In case of the general model fill in your target box size.			
	config_cryolo.json	Brow	se				
	train_image_folder Path to the image folder contai field can only be left empty whe			_ containing the you ield can only be left			
		Brow	se			Browse	
	saved_weights_name Path for saving final weights.						
	cryolo_model.h5					Browse	

- "predict":
 - use the output from "config" for "conf" (*.json);
 - "weights": Browse and select the downloaded "gmodel_phosnet_202002_N63.h5";
 - "input": Browse and open the directory where the images are (CTFfind/job###/images/);
 - "output": Browse and open the newly created "cryolo_box".
 - 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.
 - hit "START". the program is very efficient, but may take a minute or two before it starts picking.
 - when finished, scroll down to the bottom of the output log window (before hitting the "BACK" button), and notice how many particles are picked.

Actions	Required arguments Optional arguments Fi	lament options	Deprecated/Experimental/Special			
config train	Required arguments These options are mandatory to run crYOLO prediction					
predict evaluation boxmanager	conf Path to the crYOLO configuration file.	weights Path to the trained model. It can either be a model that you trained from scratch, a refined model or a general model.				
	/mnt/data/crYOLO/config_cryolo.json	Browse	/mnt/data/crYOLO/cryolo_model.h5	Browse		
	input Path to one or multiple image folders / images (on in GUI).	ly directories	output Path to the output folder. All particle coordin there.	nates will be written		
	/mnt/data/crYOLO/full_data/	Browse	/mnt/data/crYOLO/output_boxes/ Brow			

- "boxmanager": this could be optional. it's used to verify the "picks".
 - "Image_dir": Browse to the folder where the images are (same as above);
 - "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.
 - "wildcard": leave this blank.
 - 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).
 - when done, close crYOLO.

crYOLO 1.6.0 The crYOLO particle	picking procedure!				8 8 8
Actions config train predict	General arguments General arguments Options to start the box manager.				
evaluation boxmanager	image_dir Path to image directory.		box_dir Path to box directory.		
	wildcard Wildcard for selecting specific images (e.g *	new_*.mrc)			

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:

- <u>Import</u>: 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".

RELION-3.1.1:/UTSW/EMData/Talos/arctica_benchmark/02012021 _ X						
File Jobs Schedules	Movies/mics Others Running					
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Subset selection 2D classification 3D initial model 3D classification 3D auto-refine 3D multi-body CTF refinement Bayesian polishing Mask creation Join star files Particle subtraction Post-processing Local resolution External		CtfFind/job068/tif/*.box ? Browse Particle coordinates (*.box \$? ?				
		Schedule Check command Continue!				
I/O view Job actions	Current: 069: Import/yolobox-1034/	Display:				
Finished jobs	Running jobs	Input to this job				
075: Class2D/073-074/ 074: Select/job074/ 073: Class2D/1034_box200/ 072: Extract/1034_box200/ 071: Class2D/1034/ 070: Extract/1034/ 069: Import/yolobox-1034/ 068: CtfFind/1034_gctf/ 067: MotionCorr/1034_bin2x 066: Import/tif_pix04415/ 065: PostProcess/055-064/	-gpu/	Output from this job 070: Extract/1034/ 072: Extract/1034_box200/				
064: MaskCreate/055/ 063: PostProcess/057-062/						
importing done!						

- <u>Extract</u>:

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- under I/O: chose the micrographs_ctf.star file from the CTFfind step;
- for "Input coordinates": Browse to the "Import" job you just finished, and there should be only one file available to be picked called "cords_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.
- the rest is simply Relion settings. you may use the Box size you found out earlier from boxmanager in crYOLO under the "extract" tab.
- when finished, you should be getting close to the number of particles picked by crYOLO.

RELION-3.1.1:/UTSW/EMData/Talos/arctica_benchmark/02012021 _ X						
File Jobs Schedules	I/O extra	act Helix Running				
Import Motion correction		micrograph STAR file:	l/job068/microgra	phs_ctf.star	Browse	
CTF estimation Manual picking		Input coordinates:	port/job069/coord	s suffix.box ?	Browse	
Auto-picking						
Particle extraction Subset selection		OR re-extract refined particles?	No	\$		
2D classification		Refined particles STAR file:			Browse	
3D initial model		eset the refined offsets to zero?		\$?		
3D classification 3D auto-refine	OR	: re-center refined coordinates?		\$?		
3D multi-body		Recenter on - X, Y, Z (pix):	0 0	0 ?	J	
CTF refinement						
Bayesian polishing Mask creation						
Join star files						
Particle subtraction						
Post-processing Local resolution						
External						
			Schedule o	Check command	Run!	
I/O view Job actions	Current	Give_alias_here	Display:		+	
Finished jobs		Running jobs	Input to	o this job		
075: Class2D/073-074/						
074: Select/job074/ 073: Class2D/1034 box200/						
072: Extract/1034_box200/						
071: Class2D/1034/						
070: Extract/1034/ 069: Import/yolobox-1034/		Scheduled jobs	Output	from this job		
068: CtfFind/1034_gctf/						
067: MotionCorr/1034_bin2x- 066: Import/tif_pix04415/	gpu/					
065: PostProcess/055-064/						
064: MaskCreate/055/						
063: PostProcess/057-062/						
staout will go nere; double-cli	CK THIS WIND	ow to open stdout in a separate wir	naow			
stderr will go here; double-clic	k this windo:	w to open stderr in a separate wind	dow			