KLT picker 2.0 – user guide

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1 Downloading and running the KLT picker- MATLAB package (source code)

Running the KLT picker from its source code requires MATLAB to be installed on your system.

Download and extract the package from

`https://github.com/ShkolniskyLab/kltpicker2_matlab`

The extracted directory has the following structure:

KLTpicker_start	Main script
userManual	Documentation for the package
LICENSE	Package license
README	A short README file
matlab/	MATLAB source code
get10028	Script to download micrographs for the example of Section 3.
example/	Directory used in Section 3 to demonstrate the KLT picker

After downloading the package, start MATLAB in the directory of the KLTpicker package and run

```
KLTpicker_start
```

Using the KLT picker once started is described in Section 2.

2 Using the KLT picker

The following questions will appear, one by one

```
Enter full path of micrographs MRC file:
```

Type the path to the directory which contains the micrographs MRC files.

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`Enter full path of output directory:`

Type the path where the particles coordinate files will be saved.

`Enter the particle size in pixels:`

Type the particle diameter in pixels (more precisely, the diameter of the extracted box).

`pick all particles?(Y/N)? [Y]`

Type `Y` to pick all particles using the optimal threshold derived on the paper. If you type `N`, then the following question will appear

`How many particles to pick:`

Type the number of particles to pick in each micrograph.

`Pick noise images?(Y/N)? [N]`

Type `N` if you don't want to pick noise images. If you type `Y` then the following question will appear

`How many noise images to pick:`

Type the number of noise images to pick in each micrograph.

`Do you want to use ASOCEM for contamination removal?(Y/N)? [Y]`

Type `N` if you don't want to use ASOCEM for contamination removal. If you type `Y` then the following question will appear

`Do you want to save ASOCEM masks?(Y/N)? [N]`

Type `Y` if you want to save for each micrograph a binary mask (.mrc) which indicates the contaminated regions.

`Do you want to change ASOCEM default parameters?(Y/N)? [N]`

If you type `Y` then the following question will appear

`Enter ASOCEM downsampling image size (should be a positive number):`

Type the downsampling image size (the default parameter is 600).

`Enter ASOCEM covarience area size (should be a positive odd number)`

Type the covarience area size (the default parameter is 5).

`Do you want to use the GPU?(Y/N)? [Y]`

Type `Y` to use the GPUs found on your system, and `N` to not use them (CPU only).

The KLT picker will then start and will display progress notifications. The outputs are the coordinate files (box and star), a text file summarizing the picking process and the contamination masks (if the user chose to save them) at the output directory.

3 Example

In this section we demonstrate the use of the KLT picker on 5 micrographs of the EMPIAR-10028 data set (Plasmodium Falciparum 80S ribosome) [2] from the EMPIAR repository [1].

If MATLAB is installed, start MATLAB in the package directory and run

```
get10028 # downloading 5 micrographs to ./example/micrographs
directory.
```

If MATLAB is not installed, download the micrographs manually from

`https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10028/`

Start the KLT picker as described in Section 1. For example, if MATLAB is installed, type

```
KLTpicker_start
```

Once starting the KLT picker, enter the following input

```
Enter full path of micrographs MRC file:  ./example/micrographs/

Enter full path of output directory:  ./example/results

Output directory does not exist.  Create?(Y/N)? [Y] Y

Enter the particle size in pixels:  300

pick all particles?(Y/N)? [Y] Y

Pick noise images?(Y/N)? [N] N

Do you want to use ASOCEM for contamination removal?(Y/N)? [Y] Y

Do you want to save ASOCEM masks?(Y/N)? [N] N

Do you want to change ASOCEM default parameters?(Y/N)? [N] N

Do you want to use the GPU?(Y/N)? [Y] Y
```

Once the KLT picker has finished, it will display the massage: `Finished the picking successfully`. In order to display the picking results in EMAN [3], open a terminal in the package directory and create a new directory named `eman`, using the command

```
mkdir eman
```

Change directory to `eman` and enter the following commands one by one:

```
e2rawdata.py ../example/micrographs/*.mrc --invert --edgenorm
--xraypixel --ctfest --apix=1.0 --voltage=200.0 --cs=2.0 --ac=10.0
--threads=4 --defocusmin=0.6 --defocusmax=4.0
```

```
e2import.py
../example/results/pickedParticlesParticleSize300/box/*.box
--import_boxes --box_type=boxes
```

```
e2boxer.py --allmicrographs --boxsize=300 --ptclsize=300 --apix=1.0
--no_ctf --gui --threads=4
```

In order to display the picking results of the **first** micrograph in RELION [4], open the terminal in the package directory and create a new directory named `relion`, using the command

```
mkdir relion
```

Change directory to `relion` and enter the following command

```
relion_display --i ../example/micrographs/001.mrc --coords
../example/results/pickedParticlesParticleSize300/star/001.star
--scale 0.15 --particle_radius 150 --angpix 1 --lowpass 20 --pick
```

In order to display the picking results of the n'th micrograph change `001.mrc` and `001.star` in the above command to `00n.mrc` and `00n.star`.

4 Citation

If you use the KLT picker, please cite *KLT picker: Particle picking using data-driven optimal templates, Journal of Structural Biology, Accepted for publication.*. A preprint is available at `https://arxiv.org/abs/1912.06500`.

References

[1] Iudin, A., Korir, P., Salavert-Torres, J., Kleywegt, G., and Patwardhan, A. (2016). *EMPIAR: A public archive for raw electron microscopy image data.* Nature Methods, 13.

[2] Wong, Wilson and Bai, Xiao-chen and Brown, Alan and Fernandez, Israel S and Hanssen, Eric and Condron, Melanie and Tan, Yan Hong and Baum, Jake and Scheres, Sjors H W. (2014). *Cryo-EM structure of the Plasmodium falciparum 80S ribosome bound to the anti-protozoan drug emetine.* Wong et al. eLife ,3,e03080.

[3] G. Tang, L. Peng, P.R. Baldwin, D.S. Mann, W. Jiang, I. Rees & S.J. Ludtke. (2007). *EMAN2: an extensible image processing suite for electron microscopy.* Journal of structural biology, 157, 38-46.

[4] Scheres, Sjors HW. (2015). *Semi-automated selection of cryo-EM particles in RELION 1.3.* Journal of structural biology, 128, 114-122.