**MonoXtract — Installation & User Guide**

*Environment Setup, Configuration, and Workflow (Windows + Anaconda + MATLAB R2021b)*

This guide describes how to set up the runtime environment and use the MonoXtract software. Follow the steps below in order. If you are using a different MATLAB version or Python interpreter, adjust paths accordingly.

## Prerequisites

* MATLAB R2021b installed, with the Image Processing Toolbox.
* Anaconda (or Miniconda) installed.

## Environment Setup (Anaconda)

Create a new conda environment (Python 3.8 is recommended):

conda create -n your\_env\_name python=3.8

Activate the environment:

conda activate your\_env\_name

Install required Python packages in this environment:

pip install tifffile matplotlib scipy scikit-learn

Install PyTorch appropriate for your system (CPU/GPU) using the official instructions, or:

pip install torch

## Install MATLAB Engine for Python

Install the MATLAB Engine API for Python within the same conda environment:

1) Locate the Engine installer directory (example for R2021b on Windows):

cd "C:\Program Files\MATLAB\R2021b\extern\engines\python"

2) Run installation with the active environment’s Python:

python setup.py install

3) Verify installation from Python:

>>> import matlab.engine

(no error indicates the engine is available)

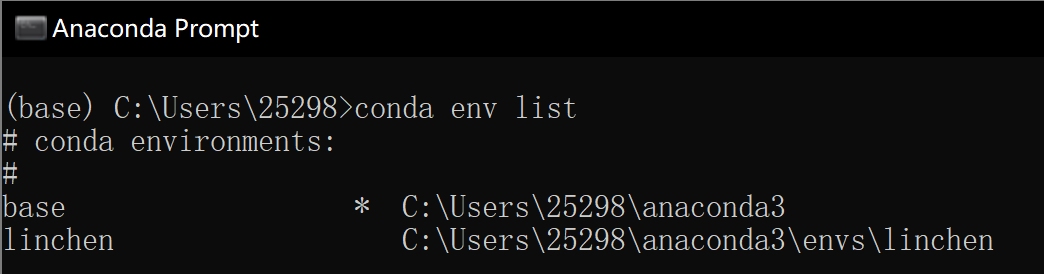
## Configure Interpreter Path

Open the file: config.json

Set the key "interpreter" to the absolute path of the Python executable inside your conda environment.

Tip: You can find environment locations via:

conda env list







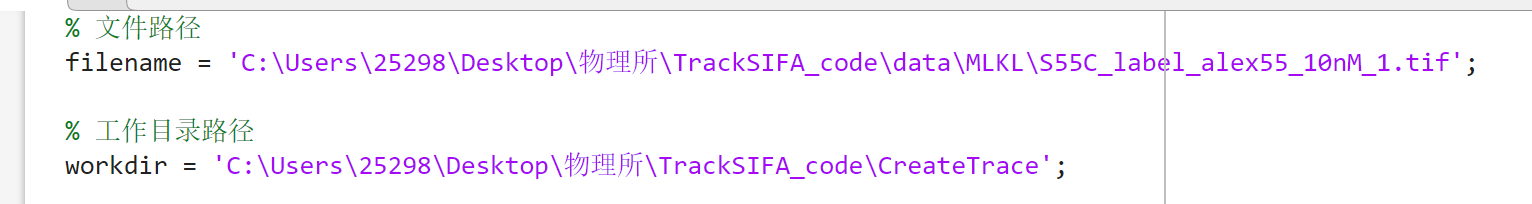
## Update Local Paths in MATLAB

Open the MATLAB function:

DeepSIFA\_main\_code\CreateTrace\LIN

Update the hard-coded paths inside this function to match your local directories.

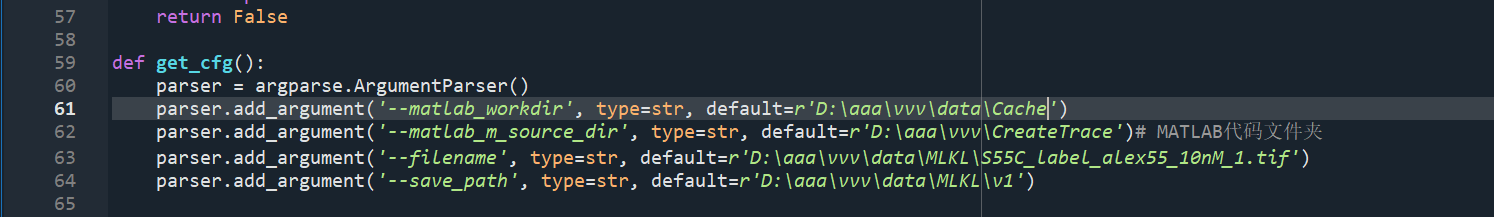
Then click the Run button in MATLAB to verify that the code executes properly.



## Update Local paths in sample.py

Open the sample.py:

Update 61-64 to match your local directories.



## Software Usage

Step 1 — Extract intensity traces

1) Launch: DLRunner.exe

2) Click: “Select TIF file” and choose the raw recording to analyze. The video files should be placed under:

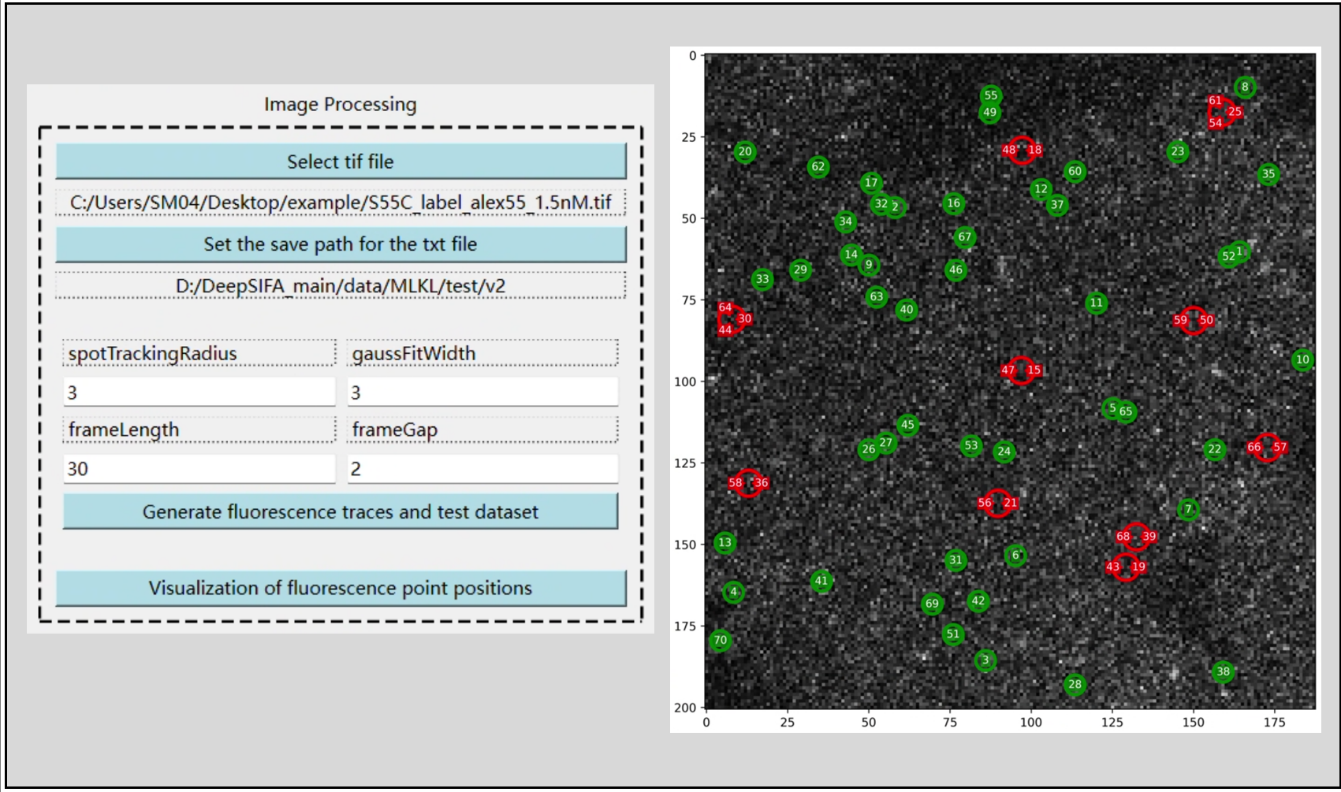
DeepSIFA\_main\_code\\data\\

3) Click: “Set the save path for the txt file” and choose an output directory. Recommended naming convention:

DeepSIFA\_main\_code\\data\\<ProteinName>\\v1

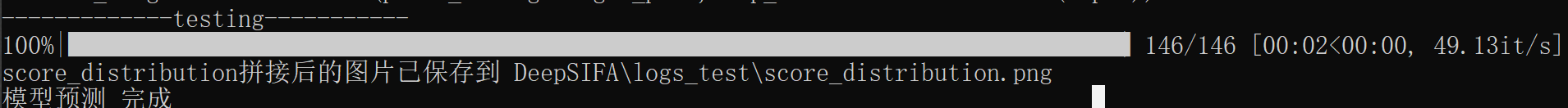
Examples: MLKL or LL37 as folder names; use v1, v2, … for versioning.

4) Keep the parameters at their default values unless you have a specific reason to adjust them.



Step 2 — Classify traces using deep learning

1. Click: “Select the data path that needs to be classified” and choose the directory containing the extracted traces.
2. Click: “Run deep-learning classification”. The console (black prompt) will display progress messages.



3) Click: “Display the probability distribution histogram of the test dataset” to visualize the probability distribution (classification scores histogram).

