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ExTemp: A computational and image-processing suite for extracting temporal barcodes

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Summary

We provide a package for fast and accurate extraction of temporal barcodes from a stack of microscopy images. This includes several steps such as object detection and tracking, 3D drift detection and correction, signal processing and denoising, and barcode extraction. A temporal barcode (Shah et al., 2019a) is defined as the intensity trace over time of an object of interest. In particular, the fluorescent signal comes from a dye-labeled fluorescent DNA, hence it is referred to as *temporal DNA barcodes*. To our knowledge, no other package can perform systematic extraction of time signals from microscopy images. The relevant application includes improved optical multiplexing and super-resolution imaging.

`ExTemp` is a set of MATLAB scripts that can act as a computer vision pipeline to extract relevant information from the raw image stack. There are several steps involved in extracting meaningful information from the recorded raw image stacks (Shah et al., 2019b). The first step includes data collection, *i.e.* recording a video (or an image stack) using a total internal reflection fluorescence (TIRF) microscope. Once we have the raw data (usually several gigabytes), we convert the proprietary Leica lif file to a mat file using the bfmatlab library. This can help us with the development of the programmable downstream MATLAB scripts as the raw data is now available in the supported format. The next step includes the estimation and correction of the lateral (x-axis, y-axis) and axial (z-axis) drift. For lateral drift correction, we use the redundant cross-correlation algorithm (Wang et al., 2014) and for axial drift correction, we subtract the mean pixel value of each frame.

Once the drift corrected data stack is available, our algorithm applies several signal processing filters (Shah et al., 2019b) to locate the localizations and find their centroid coordinates. After extracting the possible set of device coordinates, the temporal intensity time trace is generated assuming the point spread function of 3 X 3 pixels. Once we obtain the intensity time trace for each localization, the next step includes applying the wavelet filter. The filtered temporal barcodes are clustered in two or three states depending on the device using the unsupervised mean shift clustering technique to obtain a state chain. This state chain can be analyzed to extract parameters such as dark-time, on-time, double-blink, etc.

A pre-release version of `ExTemp` has been used in scientific publications to demonstrate the computational pipeline, signal processing (Shah et al., 2019b) and potential applications (Shah & Reif, 2018). The `ExTemp` package is implemented in MATLAB.

Author contributions

S.S. collected the data, wrote the paper and MATLAB scripts for the package. S.S. and A.D. designed fast algorithms and pipeline for the suite. J.R. supervised the study and reviewed the paper.

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