Exploring The Limits Of 2D Template Matching for Detecting Targets In Cellular Cryo-EM Images

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Accurately placing macromolecular assemblies in the cellular context is an important step in understanding their mechanistic role inside the cell. Template matching is a classic object detection method that finds patterns in an image that match a template by calculating a cross-correlation (CC) at each x, y location in the image. Recently, a 2D template matching (2DTM) approach was developed by our lab to locate targets such as ribosomes and viruses in cellular cryo-EM images with high positional and orientational accuracy, leading to a complete picture of local environments and molecular structure of a cell. For example, by modeling the relative 2DTM signal-to-noise ratios (SNRs), we showed that 2DTM could distinguish related molecular populations such as mature 60S and late nuclear 60S using the subcellular localization as prior or distinguish mixed populations of nuclear pre-60S when no prior is available.

Despite its success in detecting and discriminating related ribosome intermediates in cells, the current 2DTM approach suffers from several limitations. Firstly, 2DTM SNRs are proportional to the molecular weight of the target, limiting current detection to 300-400 kDa in samples with 150-200 nm ice thickness. Moreover, 2DTM has a high false negative rate, making it difficult to detect rare targets. Finally, multiple factors may affect the 2DTM SNRs, complicating their interpretation.

In this work, we explore the physical limits of 2DTM for detecting more challenging targets by modeling the 2DTM SNRs as mixed populations, thereby learning the distribution of signal and noise in cellular cryo-EM images. The 2DTM SNR at an x, y location is the maximum CC calculated at this location between the image and templates projected from different orientations. The fact that the SNR is defined as a maximum enables us to model its distribution using the extreme value theorem. We have developed a maximum-likelihood approach that models the 2DTM SNRs as a mixture of two populations, the background noise and the target signal, and applied this method to study the 2DTM outputs of in situ images of ribosome intermediates. We show that we could calculate the probability of individual pixels in the image belonging to noise or signal and provide an unbiased estimation of target molecule concentration in the cell based on the weights of two populations. By modeling both signal and noise in the image, the new method could provide a more reasonable detection threshold than the current 2DTM approach.