Unsupervised deep learning approach for automated annotation of cellular electron cryo-tomograms

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Electron cryo-tomography is the most widely applicable method for obtaining three-dimensional information from biological samples close to their native environment and at nanometer resolution. Owing to the complex content of these samples and the low signal-to-noise ratio of the reconstructions, interpretation of their three-dimensional content is not straight forward (1).

A relatively new class of machine learning approaches termed deep learning holds great promise to help with interpretation of electron cryo-tomograms. In contrast to other machine learning approaches that require careful construction of tailor-made data representations to yield good performance, the learning methods that underlie deep learning are fed with raw data and automatically discover the representations needed for detection or classification. The deep learning approach uses multiple levels of representations, each at a slightly more abstract level than the previous one. The layers of representations are not designed by human operators but are learned through general-purpose representation learning algorithm. These types of deep learning approaches were shown to be spectacularly successful in several application domains. In particular, deep convolutional neural networks have done exceedingly well in image recognition tasks. For example, before 2012 no computer algorithm was able to surpass the 25% mark in error rates for the ImageNet competition, which concerns classifying and locating different objects in a large set of natural images. With the introduction of convolutional neural networks in 2012, this rate dropped to 16% (2) and, after additional improvements, is now reduced to a few percent (3 - 5).

With this type of success in the imaging domain, it is not surprising that applications based on deep learning approaches started to appear for particle picking tasks in single-particle electron cryo-microscopy (6 - 8), neuron tracing in block face scanning electron microscopy reconstructions (9) and are beginning to trickle down to electron cryo-tomography (10).

However, all these algorithms are of the supervised learning category where the algorithms get trained with classifications performed by human operators. A potential issue with these supervised deep learning approaches is that they are inherently subject to human bias by being trained by humans. In fact, the severity of human bias can be magnified by deep learning algorithms, sometimes significantly and in quite unpredictable and unexpected ways (11, 12).

We are developing an unsupervised deep learning approach that overcomes these potential obstacles by using of correlative high-resolution fluorescence microscopy and cellular electron cryo-tomography instead of human classifications. We developed a method for accurately aligning the two modalities (13) and use an optimized variant of the watershed segmentation approach (14) on the aligned fluorescence images to classify the regions of interest. We then transfer that classification to the cryo-tomograms. These classifications are then used as input to a convolutional neural network and the deep learning algorithm commences in the usual way, using only the underlying cryo-tomography data in the process. Thus, after learning is completed, the resulting classifier can be used on cryo-tomograms that do not have a corresponding aligned fluorescence image. Funding for this work was provided by NIH.

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