Deep convolutional neural networks, U-net architecture in particular, allows fast segmentation of high-level features from microscopy images. With a reasonably large and accurate training set, the same code can be re-used without parameter or code tweaking for different experimental setups or even different organisms.

**Prerequisites for U-net:**

* Python (with Numpy and Skimage)
* Pytorch (CPU or CUDA version for GPU, check their website for installation)

I recommend using Anaconda Python environment to install packages, and Spyder IDE for running the code.

**How to segment images using U-net:**

1. Set Python path to folder ‘Unet’.
2. Open ‘segmentationRun.py’ in editor (e.g. Spyder).
3. Set parameters for a segmentation run:
   1. ‘exp\_dir’ is the path a folder with phase contrast images to be segmented (only .tif (or .tiff) images with a single channel; windows requires \\ in the folder path (e.g. ‘C:\\Work\\Test’).
   2. ‘file\_ending’ defines ending of the files (e.g. \*c1.tif) to be segmented from the folder.
   3. ‘saved\_model’ is the to be used for segmentation (e.g. Unet\_v1.pth).
      * Model are trained for different expected cell widths in pixels.
      * This is not a fixed width and model has a large range of actual widths, but it will help with cell clusters.
      * Try different models to choose the best one for the application.
   4. ‘win\_size’ is the size of smaller images in pixels (square) to which original image is split to (e.g. 512) in case you don’t have enough RAM. Set to 0 if no splitting.
   5. ‘use\_gpu’ - set to 1 if a GPU with CUDA is used for segmentation; for normal CPU based segmentation set to 0.
   6. ‘batch\_size’ is number of images segmented simultaneously (default 1). This is independent from splitting images above.
   7. Postprocessing parameters are applied after U-net segmentation to clean up the masks.
4. Run U-net segmentation by pressing F5 (or pressing green triangle in toolbar). Execution time per frame depends on image size, model and GPU/CPU.
5. Image masks for each cell will be saved to the same ‘exp\_dir’ folder with ending ‘\_mask.tif’