Hello and welcome to CellProfiler.

In this short movie, I will demonstrate the basics of using our software, from starting CellProfiler, opening images, changing directories, loading, creating, and saving pipelines, and where to go if you need help.

To begin using CellProfiler, you start it just like you do any program. By double clicking the Main file, or running the executable from the command line, whichever you prefer.

The best way to learn how to use CellProfiler is to load an example pipeline from our webpage an give it a try. All the images and pipelines I'll be using in this demo can be downloaded from our webpage: www.cellprofiler.org.

First, let's setup our default directories, so CellProfiler will know where the images to be analyzed are located and where the output files will be placed once the analyses are complete. The edit boxes for the image and output folders are located at the lower right in the main window. For this demo, I'll be using a set of human cell data.

Once I changed the image directory, you can see that on the lower left, all the files in the input directory are now listed. From here, you can take a look at any of the image files in the list box by double clicking on it.

A new window will appear showing the image you selected and file name at the top. At the top right is the popup box which lets you view the data as stretched so that the brightest values is white and the darkest value is black, or raw, which lets you see the data as required originally. If this were a color image, there would also be RG and B check boxes to the right to look at each of the color channels separately or in combination.

Now, at this point, we're ready to load our pipeline for this particular dataset. A pipeline is a sequential set of individual imaging analysis modules. Each module performs a specific task, so by stringing them together in order, you have the capability of automatically analyzing your dataset.

To load a pipeline, you can go to File->Load Pipeline in the main menu of CellProfiler. Each pipeline is a MATLAB file that ends with the extension .mat. You can also load the pipeline by double clicking on the pipeline file in a directory listing.

You can see the individual modules in a pipeline in a list box to the left. As you click on each module, the corresponding parameters that can be adjusted are shown on the right. Each module's executed in order, starting from the top and working downwards. Now, to execute the loaded pipeline, click on Analyze Images down at the bottom right.

As each module executes, a window opens so you can examine the performance of the module, its input, as well as its output. Also note that there's a status window that shows the progress of the pipeline execution.

Ok, now let's get started on creating a pipeline of your own.

First, I'm going to clear the windows and then clear the pipeline so I can start from scratch. A pipeline is constructed by placing modules together in order. From this demo, we're going to make a very basic pipeline. We're going to load in an image of a nuclear stain, identify the nuclei in the image, measure the shape an intensity of the nuclei, and then export the results.

To add a module, click the plus button at the bottom of the module list.

Now, typically the first module you want to put into place is the Load Images module. This module specific the identify of the images that you want to analyze. As we saw earlier, the first file in the directory was of a nuclear stain. The Load Images modules allows you to specify and load files for a particular text identifier in the name. In this case, the nuclear stain is a Tif file that ends with the letters d0.tif. So, I'll specify that I'm looking for matching text, and tell the module that I'm looking for files that have d0.tif in the name.

Once the image is loaded, the image needs to be identified with a specific name within CellProfiler, so we'll leave the name at the default — Origblue — for now. Now we're going to add a module for identifying objects. The module is called IdentifyAPrimaryAutomatic which will identify the nuclei based on a greyscale values in the image.

For this module, there's a whole host of options that you can adjust in order to optimize identification. But we're going to leave them at their default values for now. However, if you'd like more information on a settings that this module or any other module offers, you can always access the help by highlighting the module and then clicking on the question mark button below the module listing.

Now, in the IdentifyPrimaryAutomatic module, the identified objects, the nuclei, are given a name, Nuclei, in CellProfiler. We're going to use these objects as input for the measurement modules.

So we're gonna add two more modules. One to measure the florescent intensity of each nucleus, and one to measure the nuclear shape.

For the ObjectIntensity module, we need to tell it what image we want to measure the intensity from. here it defaults to OrigBlue because it's the only image that was loaded, but if we loaded other images with the load images module, they would appear in this popup box as well. Then we want tell the module that we want to measure the intensity from the nuclei objects that were identified in a previous module. Likewise, in the ObjectAreaShapeModule, we tell that modules to measure the shape features from the nuclei objects.

Keep in mind that we are not limited to just these modules — there are many other modules to choose from to measure a variety of features, and they operate in much the

same way.

Lastly, we'd like to export the results to a spreadsheet. So, we're going to add the module ExportToSpreadsheet, and tell it to export the measurements associated with the Nuclei objects obtained by the previous modules.

As you probably have noticed, modules are added to the end of the pipeline but you can adjust their order in the main window by selecting the module or modules and using the move up and move down buttons. I also added the ExportToExcel module twice, but in this case, I only need one. If you want to remove a module altogether, highlight the module and press the minus button in order to delete it.

Now we're ready to run our custom-built pipeline. Like last time, we click the Analyze Images button in order to run it. During a run, you have the option to cancel at any time using the status window.

We can see that the pipeline seemed to execute successfully. In the output directory there is now a file containing all the settings, plus an Excel file with the measurements just made.

Finally, we can save the pipeline we just created. Let's keep the settings we've used, even though the pipeline is not ready for use. Also, if a colleague wants to recreate your results, all you need to do is give them the pipeline file, along with the input images.

So to review, in this demo we learned how to open CellProfiler, change input and output directories, load pipelines or create your own using the individual modules, how to run a pipeline to export the results, and finally, how to save the pipeline for later use.

If you have any questions, you can always refer to the documentation or post on the online forum at forum.cellprofiler.org.

This concludes our demo on getting started with CellProfiler.