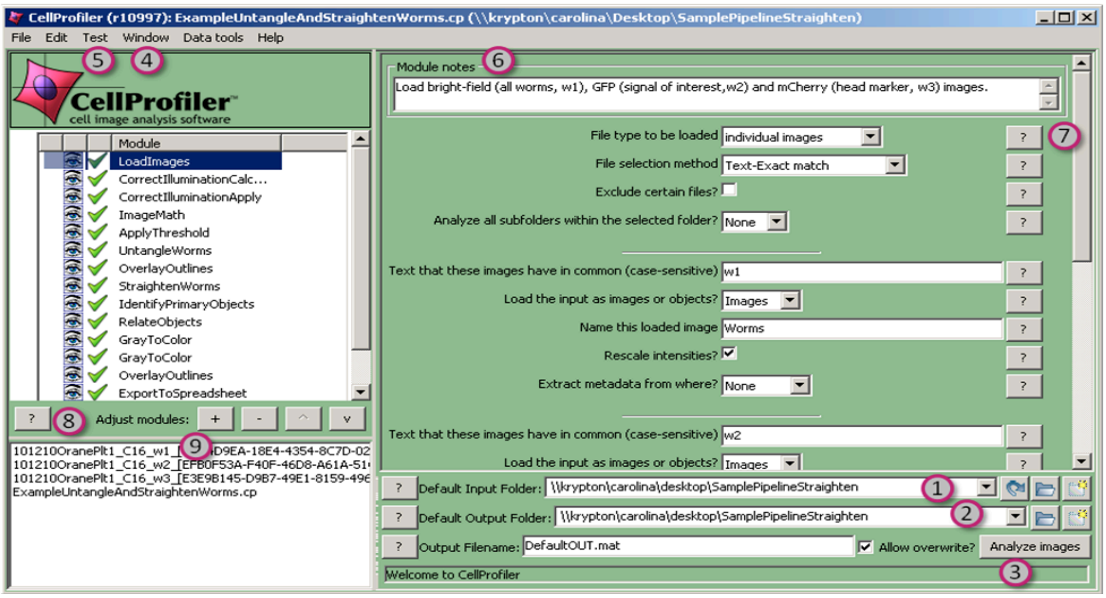
How to get started with the WormToolbox

The WormToolbox is a set of CellProfiler modules specific for worm analysis that are used in conjunction with standard modules of CellProfiler to analyze a set of images. A series of modules makes up a “pipeline”. In order to get you started, we provide a few example pipelines that use modules from the WormToolbox. After trying the example pipelines as described below you can adjust the modules within them to fit your own image data and experimental goals.

Image analysis

1. Download and install CellProfiler 2.0 (version r11710 or later) from Supplementary Software or www.cellprofiler.org.
2. Go to Supplementary Software or http://www.cellprofiler.org/examples.shtml and download **one** of the example pipelines for worm analysis. The instructions below are general, but the illustration refers to the example called ‘Straighten worms and extract intensity measurements using a low-resolution atlas’.
3. Start CellProfiler and set Default Input Folder (1) and Default Output Folder (2) to the folder where you have downloaded the sample pipeline (in the illustration below, ‘\\krypton\carolina\Desktop\SamplePipelineStraighten’).
4. Doubleclick the pipeline (.cp) file in the file display window (9) to load the pipeline. The modules of the pipeline now appear in the pipeline window.
5. You can now click Analyze images (3) to run the full pipeline on the example images contained in the folder, and a result-window will pop open for each module of the pipeline. Two csv files with extracted measurements will also appear in your output folder.
6. To get a better feeling for what each step in the pipeline does, first click on ‘Window’ (4) and ‘Close all open windows’. Then click on ‘Test’ (5) and ‘Start test run’. This allows you to step through the different modules by clicking the ‘Step’ button that appears below the pipeline. You can also choose to show or not show the result of a given module by clicking the ‘eye’ next to the module. The ‘Module notes’ (6) briefly describes what each module does.



7. If you want to run the pipeline on a larger example data set, go to http://www.broadinstitute.org/bbbc/ to download more example worm images and place them in your local folder. The full data set will be analyzed if you exit the test mode and click 'Analyze images'.
8. To adapt a pipeline to your own images, you can change the 'LoadImages' module to load your own data, and adjust the modules' settings guided by the help that will appear when you click '?' next to the different parameter settings (7) or below the pipeline (8). Click the '+' to add new modules to your pipeline if needed. The worm-specific modules are listed under 'WormToolbox'.
9. Large-scale, high-throughput experiments will likely require the use of CellProfiler's batch processing tools, which use a computing cluster to speed analysis. See http://cellprofiler.org/CPmanual/Help_Using%20CellProfiler_Other%20Features_Batch%20Processing.html. CellProfiler can also be run from the command prompt (without the user interface) if needed. For large datasets, we recommend using the Export To Database module in CellProfiler, which produces .csv files and export of extracted data to a database.
10. For further questions, please consult the CellProfiler manual and the user forum at http://cellprofiler.org/forum/.

Data analysis

Within CellProfiler: Once a pipeline has completed processing a set of images, some simple data analysis and visualization can be done within CellProfiler by adding modules in the "Data Tools" category to the pipeline (e.g., Display Scatterplot, Display Histogram, etc). You can also perform the same functions by using the Data Tools menu after a pipeline has completed running. One particularly useful module is Calculate Statistics, which can be added to a pipeline to calculate the Z' factor and other image assay quality statistics.

In a spreadsheet program (e. g. Excel): Measurements extracted by CellProfiler using example pipelines are saved in spreadsheet format by the Export to Spreadsheet module. The data may be explored using any software for handling of spreadsheets. Such software can be useful to rank-order samples based on the measurements of the phenotype of interest.

Using CellProfiler Analyst's visualization/analysis/exploration tools: This companion software for CellProfiler is also freely available at www.cellprofiler.org and can be used for the visualization and exploration of high-throughput data. One advantage compared to spreadsheet programs is that visualizations in CellProfiler Analyst are interactive and linked to the image data to allow visual confirmation of phenotypes. It also enables the visualization of quality-control results from a screen, and normalization of results to correct systematic errors (REF: http://JBX.sagepub.com/content/early/2011/09/27/1087057111420292.abstract).

Using CellProfiler Analyst's machine learning tools: The Classifier tool in CellProfiler Analyst can be used to train a classifier to recognize a phenotype of interest by dragging and dropping example worms. See CellProfiler Analyst's manual for a complete description of its usage.

Using database query tools: If exported to a database using the Export To Database module in CellProfiler, data can also be explored and analyzed with database query tools.