# CellProfiler Help: DeveloperInfo

Programming Notes for CellProfiler Developer's version

#### \*\*\* INTRODUCTION \*\*\*

CellProfiler - the easiest way is to modify an existing one. CellProfiler DataTools, and ImageTools folders looking for files. Simply put your new is modular: every module, image tool, and data tool is a single MATLAB m-file (extension = .m). Upon startup, CellProfiler scans its Modules, file in the proper folder and it will appear in the proper place. They You can write your own modules, image tools, and data tools for are automatically categorized, their help extracted, etc.

please do give it a try. Many beginners find this language easy to learn If you have never tried computer programming or have not used MATLAB, and the code for CellProfiler is heavily documented so that you can understand what each line does. It was designed so that biologists without programming experience could adapt it.

# \*\*\* HELP SECTIONS AT THE BEGINNING OF EACH MODULE AND TOOL \*\*\*

(technical description), and See also NameOfModule. The license/author information should be separated from the help lines with a blank line so tools and data tools (Help menu in the main CellProfiler window) as well as MATLAB's built in 'help' and 'doc' functions at the command line. It the convention of: Help for the XX module, Category (use an exact match the "Add module" window), Short description, purpose of the module, description of the settings and acceptable range for each, how it works of one of the categories so your module appears in the proper place in also be saved in tif format, using the same name as the module, and it will automatically be included in the pdf manual page as well. Follow The first unbroken block of lines will be extracted as help by GellProfiler's 'Help for this analysis module' button, Help for image will also be used to automatically generate a pdf manual page for the module. An example image demonstrating the function of the module can that it does not show up in the help displays.

# \*\*\* SETTINGS (CALLED 'VARIABLES' IN THE CODE) \*\*\*

equals sign will affect the ability of the variables to be read properly. Variables are automatically extracted from lines in a commented section near the beginning of each module. Even though they look like comments critical - indenting lines or changing the spaces before and after the they are critical for the functioning of the code. The syntax here is

\* The '%textVAR' lines contain the variable descriptions which are

text will wrap appropriately so it can be as long as desired, but it must be kept on a single line in the m-file (do not allow it to wrap).

\* Whether the variable is entered into an edit box, chosen from a popup menu, or selected using browse buttons is determined by %inputtypeVAR

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lines and the %textVAR lines. The options are:

- edit box (omit any %inputtypeVAR line for that variable number and use a %defaultVAR line to specify what text will appear in the box when the user first loads the module)
- lines, in the order you want them to appear, for each option that should - popup menu (use %inputtypeVAR = popupmenu and then use %choiceVAR appear in the popup menu)
- popupmenu custom (this allows the user to choose from choices but also - pathname box + browse button (omit the %inputtypeVAR line and instead popupmenu custom and then use %choiceVAR lines, in the order you want them to appear, for each option that should appear in the popup menu) to have the option of typing in a custom entry. Use %inputtypeVAR = use %pathnametextVAR - the default shown in the edit box will be a period; this default is currently not alterable)
- use %filenametextVAR the default shown in the edit box will be the text - filename box + browse button (omit the %inputtypeVAR line and instead "Do not use"; this default is currently not alterable)
- belong to. You will notice that many entries that the user types into the modules. This works by classifying certain types of variable entries as \* The %infotypeVAR lines specify the group that a particular entry will main window of CellProfiler are then available in popup menus in other follows:
- imagegroup indep: the user's entry will be added to the imagegroup, and will therefore appear in the list of selectable images for variables whose type is 'imagegroup'. Usually used in combination with an edit box; i.e. no %inputtype line.
  - imagegroup: will display the user's image entries. Usually used in combination with a popupmenu.
- objectgroup indep and objectgroup: Same idea as imagegroup, for passing along object names.
  - outlinegroup indep and outlinegroup: Same idea as imagegroup, for passing along outline names.
- datagroup indep and datagroup: Same idea as imagegroup, for passing along text/data names.
- gridgroup indep and gridgroup: Same idea as imagegroup, for passing along grid names.
- actually extracts the value that the user has entered in the main window of CellProfiler (which is stored in the handles structure) and saves it \* The line of actual code within each group of variable lines is what as a variable in the workspace of this module with a meaningful name.
- \* For CellProfiler to load modules and pipelines correctly, the order of %textVAR01 = Whatever text description you want to appear variable information should be as follows:

%defaultVAR01 = Whatever text you want to appear

(OR, %choiceVAR01 = Whatever text)

%infotypeVAR01 = imagegroup indep

3laBla = char(handles.Settings.VariableValues{CurrentModuleNum,1}); %inputtypeVAR01 = popupmenu

For cases in which the variable input is optional or your module should gnore the contents of the variable box, the standard placeholder text is 'Do not use." Please follow this naming convention whenever new modules

are created or modified.

In particular, when the input type is "popupmenu custom", the choiceVAR01 line should be after textVAR01. This order is necessary because the textVAR01 creates a VariableBox associated with a variable number. Also, the defaultVAR01 value will inadvertently overwrite saved settings when loading a saved pipeline if it is located after infotypeVAR01 or inputtypeVAR01.

When loading the settings of pipeline modules, CellProfiler tries to find handles.Settings.VariableValues(WoduleNums,i) from the list of handles.VariableBox(WoduleNums)(i),

for example,

the pipeline-specified 'Gaussian Filter' from the list of available Smoothing methods in the loaded module.

It is searched and set in CellProfiler.m, exactly starting

with this line of code:
PPos = find(strcmp(handles.Settings.VariableValues{WoduleNums,i},OptList));
You may want to add your own action code here when a certain setting is

found in a loaded module.

\* CellProfiler uses VariableRevisionNumbers to help programmers notify

users when something significant has changed about the variables. For example, if you have switched the position of two variables, loading a pipeline made with the old version of the module will not behave as expected when using the new version of the module, because the settings (variables) will be mixed up. The line should use this syntax:

"""VariableRevisionNumber = 1

If the module does not have this line, the VariableRevisionNumber is assumed to be 0. This number need only be incremented when a change made to the modules will affect a user's previously saved settings. There is a revision number at the end of the license info at the top of the m-file for our source-control revisions - this revision number does not affect the user's previously saved settings files and you can ignore it. However, a line with "% Revision: 5791 %" should be added to any new function, so that the vession-control system will find and update the number upon new commits.

\*\*\* STORING AND RETRIEVING DATA: THE HANDLES STRUCTURE \*\*\*

In CellProfiler (and MATLAB in general), each independent function (module) has its own workspace and is not able to 'see' variables produced by other modules. For data or images to be shared from one module to the next, they must be saved to what is called the 'handles structure'. This is a variable, whose class is 'structure', and whose name is handles. The contents of the handles structure can be printed out at the command line of MATLAB using the Tech Diagnosis button and typing "handles" (no quotes). The only variables present in the \*main\* handles structure are handles to figures and GUI elements. Everything else should be saved in one of the following substructures:

handles.Settings:

Everything in handles.Settings is stored when the user uses File > Save pipeline, and these data are loaded into CellProfiler when the user uses

File > Load pipeline. This substructure contains all necessary information to re-create a pipeline, including which modules were used (including variable revision numbers), their settings (variables), and the pixel size. Fields currently in handles.Settings: PixelSize, VariableValues, NumbersOfyariables, VariableInfoTypes, VariableRevisionNumbers, ModuleNames, SelectedOption.

\*\*\* N.B. handles.Settings.PixelSize is where you should retrieve the PixelSize if needed, not in handles.Preferences!

handles.Pipeline: This substructure is deleted at the beginning of the analysis run (see "Which substructures are deleted prior to an analysis run?" below). handles.Pipeline is for storing data which must be retrieved by other modules. This data can be overwritten as each image cycle is processed, or it can be generated once and then retrieved during every subsequent image set's processing, or it can be saved for each image set by saving it according to which image cycle is being analyzed, depending on how it will be used by other modules. Example fileds in handles.Pipeline: FileListDrigBlue, PathmameOrigBlue, OrigBlue (which contains the actual image). Whether the handles.Pipeline structure is stored in the output file or not depends on whether you are in Fast Mode (see Help? HelpFastMode or File > SetPreferences). See note below for the FileList..., Pathname..., and Filename... fields.

#### handles.Current:

This substructure contains information needed for the main CellProfiler window display and for the various modules and help files to function. It does not contain any module-specific data (which is in handles-Pipeline). Example fields in handles.Current: NumberOfModules, StartupDirectory, DefaultOutputtDirectory, DefaultImageDirectory, FilenamesInImageDir,

DataToolsFilenames, DataToolHelp, HelpFilenames, Help, NumberOfinageSets, SetBeingAnalyzed, SaveOutputHowOften, TimeStarted, CurrentModuleNumber, FigureNumberForModuleXX.

#### handles.Preferences:

Everything in handles. Preferences is stored in the file

CellProfilerPreferences.mat when the user uses File > Set Preferences. These preferences are loaded upon launching CellProfiler, or individual preferences files can be loaded using File > Load Preferences. Fields in handles.Preferences: PixelSize, DefaultModuleDirectory, Defaultungebirectory, DefaultImagebirectory, IntensityColorMap, LabelColorMap, StripPipeline, SkipErrors, PontSize.

The PixelSize, DefaultImageDirectory, and DefaultOutputDirectory fields can be changed for the current session by the user using edit boxes in the main CellProfiler vandow, which changes their values in handles.Settings or handles.Current. Therefore.

- \*\*\* N.B. handles.Settings.PixelSize is where you should retrieve the PixelSize if needed, not in handles.Preferences!
- \*\*\* N.B. handles.Current.DefaultImageDirectory is where you should retrieve the DefaultImageDirectory if needed, not in handles.Preferences!
  - retrace the DefaulthingSurrectory if medget, not in managerifications.
    \*\*\*\* N.B. handles.Current.DefaultOutputDirectory is where you should retrieve the DefaultOutputDirectory if needed, not in handles.Preferences!

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#### handles.Measurements:

is deleted at the beginning of the analysis run (see 'Which substructures analyzed and is therefore accessed by the data tools. This substructure are deleted prior to an analysis run?' below).

MeanAreaCells). Use the appropriate substructure to ensure that your data aggregate measurement based on individual object measurements (e.g. Image number for the entire image, which could come from one measurement from and Image measurements. Object measurements have one number for every object in the image (e.g. Object Area) and image measurements have one the entire image (e.g. Image TotalIntensity), or which could be an Note that two types of measurements are typically made: Object will be extracted properly.

and therefore each nucleus has a cytoplasm's number in the nucleus example, a nucleus might be associated with a particular cytoplasm measurement field which links the two. Or, for multiple speckles ModuleError, TimeElapsed, FileName\_IMAGEMAME, and PathName\_IMAGEMAME. See note below for fields having to do with module or Identify Secondary or Tertiary modules). Image measurements include a few standard fields: ModuleErrorFeatures, indicating which nucleus the speckle belongs to (see the Relate The relationships between objects can also be defined. For within a nucleus, each speckle will have a nucleus' number file and path names.

that record measurements must use the subfunction CPaddmeasurements. The Measurement storage was overhauled 2008-04-25 such that all modules

handles = CPaddmeasurements(handles,ObjectName,FeatureName,Data);

This will create this data structure:

handles.Measurements.ObjectName.FeatureName = Data

-ObjectName is a single string denoting the name of the object, or -FeatureName is a single string, with category and parameters simply "Image" for image measurements (optional) underscored, like this:

## Category\_SpecificFeatureName\_Parameters

your new category will be selectable (in the future, this will CPgetfeaturenamesfromnumbers, and all choiceVAR lists so that \* Category = Module name (e.g., AreaShape), or useful category, - Note: If you create a new category, be sure to add it to or nothing if there is no appropriate category (e.g., if - Note: Do not include the word "Measure" when naming. be a drop down menu) for modules that ask the user to feature name = ObjectCount there is no category). the list of categories below, as well as in

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choose a category.

- (e.g., Perimeter). Usually the module recording the measurement assigns this name, but a few modules allow the the module in the Help section. See MeasureObjectAreaShape CalculateRatios module allows the user to name the ratio). \* SpecificFeatureName = specific feature recorded by a module - Note: Be sure to list the Specific features measured by user to type in the name of the feature (e.g., the for an example.
- Muclei in two different images, blue and green). Primarily \* Parameters (optional) are used for modules that measure the used for Channel or scale of Texture. Multiple parameters MeasureObjectIntensity module can measure intensities for same objects in different ways (e.g. the can be separated by underscores.

(someday, CP will look at upstream modules and make dropdowns)

### Category List:

These reflect choiceVAR lists in many modules, with their necessary extra parameters:

No extra parameters: AreaShape, Math Image:

Imageintensity, Granularity, Children, Parent, AreaOccupied SizeScale:

SizeScale and Image:

Neighbors

Texture and RadialDistribution Not to include in choiceVAR lists:

Align, Ratio, ClassifyObjects, ClassifyObjsByTwoMeas, ModuleError, Crop (though Crop could be added to the Image group above if needed), DefinedGrid

When these categories are altered, please update the code in Ofgetfeaturenamesfromnumbers and any module that uses this subfunction.

Usage: CPjoinstrings('texture',42,'foo') => 'texture\_42\_foo' names from strings and integers. (If you are just joining Note: CPjoinstrings can be helpful in constructing feature strings, it is usually more convenient to join them directly with ['texture\_', stringvariable], etc.)

#### -Data is either:

- (a) Nx1 vector of numerical data, one number per object where there are N objects.
- (b) [], i.e., the empty matrix if the module did not measure any objects in this instance. YES, it is very important to pass the empty matrix through CPaddmeasurements even if no objects were
  - (c) A single string (only makes sense when the ObjectName = found or measured for a particular image.
- "Image")
- (d) In the future, we might add the capability to store Nx1 strings, i.e., one string for every object.

Be sure to consider whether measurements you are storing will overwrite

You can differentiate measurements by including something specific in the name (e.g. Intensity modules include the image name (e.g. Blue or Green) each other if more than one of the same module is placed in the pipeline. where new measures are appended to the end of an existing substructure in the substructure name). There are also several examples of modules (i.e. forming a new column). See Calculate Ratios.

## handles.Measurements: Order

by a more intelligent measurement selection system using context dependent statements within each function. In the future, this will be superceded Be certain that the order in which measurements are added correspond to the Feature & FeatureNumber in each function's Help section. This FeatureNumber will correspond to the order of CPaddmeasurements drop-down slectors.

Why are file names stored in several places in the handles

structure? The Load Images module creates all of the following:

- handles.Pipeline.FileListIMAGENAME
  - handles.Pipeline.Pathname
- handles.Measurements.Image.PathName\_IMAGENAME - handles.Pipeline.FilenameIMAGENAME
- handles.Measurements.Image.FileName\_IMAGENAME

The primary reason for the fields in the Measurements branch is that it allows the information to be exported easily. However, these

name and appends the frame number for every frame in the movie. This allows the names to be used in other modules such as SaveImages, fields are also used elsewhere, e.g., the SaveImages module. The FileList field is mainly useful for movies. For movies, the FileList field has the original name of the movie file and how many frames it contains. The Filenames field has the original movie file beginning of the run and contains all the images that will possibly be analyzed, whereas the Filename location is only populated as the which would otherwise over-write itself on every cycle using the original file name. The FileList location is created at the images cycle through.

When images are loaded from subdirectories, the information stored Suppose that N Then h.P.Pathname will be the string B; h.P.FilenameIMAGENAME image files are loaded from various subdirectories of B. Let Si be Let B be the base directory (either the default image directory or in the Pipeline and Measurements branches become subtly different. the subdirectory of the i-th file loaded, and let Fi be its file ... }; and h.M.I.FileName\_IMAGENAME will be a cell array { 'F1', h.M.I.PathName\_IMAGENAME will be a cell array { 'B/S1', 'B/S2' the directory specified as an option to LoadImages). will be a cell array { 'S1/F1', 'S2/F2', ... };

deleted at the beginning of the analysis run, whereas anything stored in retained from one analysis to the next. It is important to think about which of these data should be deleted at the end of an analysis run Anything stored in handles. Measurements or handles. Pipeline will be handles.Settings, handles.Preferences, and handles.Current will be Which substructures are deleted prior to an analysis run?

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because of the way MATLAB saves variables: For example, a user might process 12 image sets of nuclei which results in a set of 12 measurements ("TotalStainedArea") stored in handles.Measurements.Image. In addition, a if, in the second analysis run, the user runs only a module which depends repeatedly use the processed image of nuclei leftover from the last image remaining 8 measurements will still be present. So, the user will end up with 12 measurements from the 4 sets. Another potential problem is that on the output "SegmentedNuclei" but does not run a module that produces overwrite the first 4 measurements of the previous analysis, but the handles.Pipeline.SegmentedNuclei. Now, if the user uses a different "TotalStainedArea" to analyze 4 image sets, the 4 measurements will an image by that name, the module will run just fine: it will just module which happens to have the same measurement output name processed image of nuclei from the last image set is left in set, which was left in handles. Pipeline.

the guidata command can only store one variable at a time, be sure to use module to the next unless they are saved to the GUI first. This is done function which identifies the CellProfiler window to the module. Since in MATLAB by using the command guidata(gcbo,handles), where gcbo is a Any changes you make to the handles structure are not kept from one How do I save the handles structure in a GUI module? it on the handles structure only.

### \*\*\* IMAGE ANALYSIS \*\*\*

If you plan to use the same function in two different m-files (e.g. a module and a data tool, or two modules), it is helpful to write a CPsubfunction called by both m-files so that you have only one subfunction's code to maintain if any changes are necessary. Images loaded into CellProfiler are in the 0 to 1 range for consistency across modules. When retrieving images into your module, you can check the images for proper range, size, color/gray, etc using the CPretrieveimage subfunction.

We have used many MATLAB functions from the image processing toolbox. Currently, CellProfiler does not require any other toolboxes for processing. The 'drawnow' function allows figure windows to be updated and buttons to of breaks where the figure windows/buttons can be interacted with. This reasonable to remove most of these lines when running jobs on a cluster 'drawnow' function is sprinkled throughout the code so there are plenty does theoretically slow the computation somewhat, so it might be be pushed (like the pause, cancel, help, and view buttons). where speed is important.

### \*\*\* ERROR HANDLING \*\*\*

## \* In data tools & image tools:

CPerrordlg(['Image processing was canceled in the ', ModuleName,' module because your entry ', ValueX,' was invalid.']) return

\* In modules and CPsubfunctions (no need for "return"): error('Your error message here.')

#### Moto

Always try to make the subfunctions as less likely to have errors as possible. Whenever you can, have error checks in the calling function before the subfunction gets called. Since CPsubfunctions use error ("message"), you should try to nest any calls to them in a try/catch. Plus, this allows you to add more specific information to the error message (such as where in the calling function did the error additional information together with lasterr (which retrieves the last error message). In data tools and image tools CPerrordlg("message") and return is needed because they are usually called independently, and using error (message") would just stop execution, but would not prompt the user with the corresponding error message.

### \*\*\* DISPLAYING RESULTS \*\*\*

Each module checks whether its figure is open before calculating images that are for display only. This is done by examining all the figure handles for one whose handle is equal to the assigned figure number for this algorithm. If the figure is not open, everything between the "if" and "end" is ignored (to speed execution), so do not do any important calculations there. Otherwise an error message will be produced if the user has closed the window but you have attempted to access data that was supposed to be produced by this part of the code. This is especially you plan to save images which are normally produced for displays. If you plan to save images which are normally produced for displays only, the corresponding lines should be moved outside this if statement. Also, any additional utcontrols (popupmenus, pushbuttons) should be designed using the unit of pixels, since this is standard across platforms unlike other units such as inches and points.

STEP 1: Find the appropriate figure window. If it is closed, usually none of the remaining steps are performed.

ThisModuleFigureNumber = handles.Current.(['FigureNumberForWodule',CurrentModule]); if any(findobj == ThisModuleFigureNumber)

STEP 2: Activate the appropriate figure window so subsequent steps are performed inside this window:

CPfigure(handles,'Image',ThisModuleFigureNumber);

For figures that contain any images, choose 'Image', otherwise choose 'Text'. Timege' figures will have the RGB checkboxes which allow displaying individual channels, the InteractiveZoom and CellProfiler Image Tools menu items, and the Raw/Stretched intensity scale pulldown.

Note: unfortunately there is no convenient way right now to have more than one figure window per module. We work around this in the case of IdPrimAutomatic when run in "test mode", for example, by creating a new window with a special 'Tag' property that allows you to find it again in subsequent cycles. Having the 'Name' property of the figure window containing "cycle #" at the end allows CellProfiler to recognize it and

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list it in the Windows menu. Also note: In general, you should not change figure properties like this: CPfigure('Tag', 'My figure name')

...because it messes up the menus in the figure window. Use this instead: set(FigureHandle,'Tag','My figure name');

STEP 3: (only during starting image cycle) Make the figure the proper

if handles.Current.SetBeingAnalyzed == handles.Current.StartingImageSet CPresizefigure('', 'NarrowText', ThisModuleFigureNumber)

end
The figure is adjusted to fit the aspect ratio of the images, depending
on how many rows and columns of images should be displayed. The choices
are: OneByOne, TwoByOne, TwoByTwo, NarrowText. If a figure display is
unnecessary for the module, skip STEP 2 and here use:

if handles.Current.SetBeingAnalyzed == handles.Current.StartingImageSet
close(ThisModuleFigureNumber)

close(ThisModuleFigureNumbe end or simply use the subfunction: CPclosefigure(handles,CurrentModule) Note that in the above we do not use this: if handles.Current.SetBeingAnalyzed == 1

... because if the user has chosen the Restart module to resume analysis, the first image set being processed will not be #1, and yet we want the figure window to be sized properly.

STEP 4: Display your image:

ImageHandle = CPimagesc(Image, handles);

This CPinagesc displays the image and also embeds an image tool bar which will appear when you click on the displayed image. The handles are passed in so the user's preferences for font size and colormap are used.

\*\*\* DEBUGGING HINTS \*\*\*

\* Use breakpoints in MATLAB to stop your code at certain points and examine the intermediate results.

\* To temporarily show an image during debugging, add lines like this to your code, or type them at the command line of MATLAB:

CPfigure

CPimagesc(BlurredImage, [])

\* To temporarily save an intermediate image during debugging, try this: imwrite(BlurredImage, 'FileName.tif', 'FileFormat');

imwrite(BlurredImage, 'FileName.tif', 'FileFormat');
Note that you may have to alter the format of the image before saving. If the image is not saved correctly, for example, try adding the uint8 command:

imwrite(uint8(BlurredImage), 'FileName.tif', 'FileFormat');

\* To routinely save images produced by this module, see the help in the SaveImages module.

 $\star$  If you want to save images that are produced by other modules but that are not given an official name in the settings boxes for that module, fieldname = ['SomeDescription(optional)', ImgOrObjNameFromSettingsBox]; alter the code for the module to save those images to the handles handles.Pipeline.(fieldname) = ImageProducedBytheModule; handles.Pipeline.(fieldname) = SegmentedObjectImage; structure and then use the Save Images module. fieldname = ['Segmented', ObjectName]; The code should look like this: Example 1:

Example 2:

handles.Pipeline.(fieldname) = CroppedImage; fieldname = CroppedImageName;

For General help files:

visible. We are not using CPhelpdlg because using helpdlg instead allows the help to be accessed from the command line of MATLAB. The one line of code in each help file (helpdlg) is never run from inside CP anyway. We have one line of actual code in these files so that the help is

\*\*\* RUNNING CELLPROFILER WITHOUT THE GRAPHICAL USER INTERFACE \*\*\*

In order to run CellProfiler modules without the GUI you must have the following variables: handles.Settings.WoduleNames (for all modules in pipeline) handles.Settings.VariableValues (for all modules in pipeline) handles.Current.CurrentWoduleNumber (must be consistent with pipeline) handles.Current.NumberOfImageSets (set by LoadImages, so if it is run handles.Current.SetBeingAnalyzed (must be consistent with pipeline) first, you do not need to set it)

handles.Current.DefaultOutputDirectory

handles.Current.DefaultImageDirectory

handles.Current.NumberOfModules

handles.Preferences.IntensityColorMap (only used for display purposes) handles.Preferences.LabelColorMap (only used for display purposes) handles.Preferences.FontSize (only used for display purposes)

call CP subfunctions for many tasks. The CurrentModuleNumber needs to be variable values are called. In order to see what all of these variables look like, run a sample analysis and then go to File -> Tech Diagnosis. You will also need to have the CPsubfunctions folder, since our Modules set correctly for each module in the pipeline since this is how the This will let you manipulate the handles variable in MATLAB.

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## CellProfiler Help: FastMode

Fast mode can be set in File > Set preferences.

intermediate images and calculations for the most recent image cycle are If you uncheck the box you will run in diagnostic mode, where all the saved in the output file, which drastically increases the output file size. Check the box if you would instead like to run in normal (fast) mode, producing smaller output files.

See also the SpeedUpCellProfiler module.