

How to use CellProfiler for CyCIF experiment

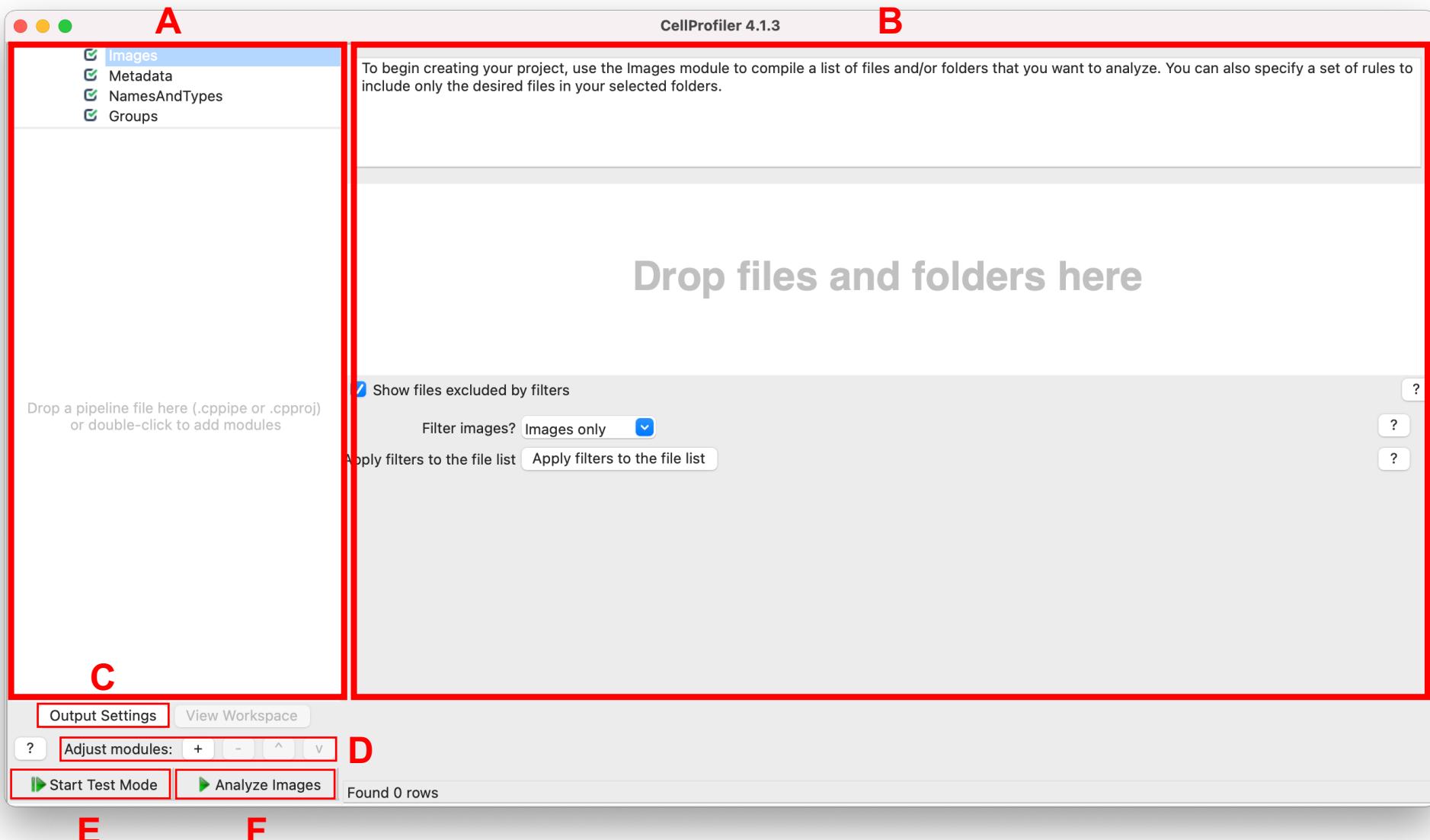
Heng

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Please note. This protocol supported CellProfiler version 4.3.1.
It will not be compatible with the other version**

See <https://cellprofiler.org/tutorials> for more tutorials.

Program Overview



- A. Module panel
- B. Setting for each module
- C. Set output folder
- D. Add/Remove module
- E. Test mode
- F. Run this pipeline

* All first 4 modules are needed for every pipeline.

In order to analyze the CyCIF experiment, we need to **run CellProfiler 2 times separately**.

- 1) Run “[**1_cycif_illumination_v4.3.1.cpproj**](#)” for collecting illumination using blank well images.
- 2) Run “[**2_cycif_segmentation_v4.3.1.cpproj**](#)” for image segmentation.

This example contains 2 cycle of images.

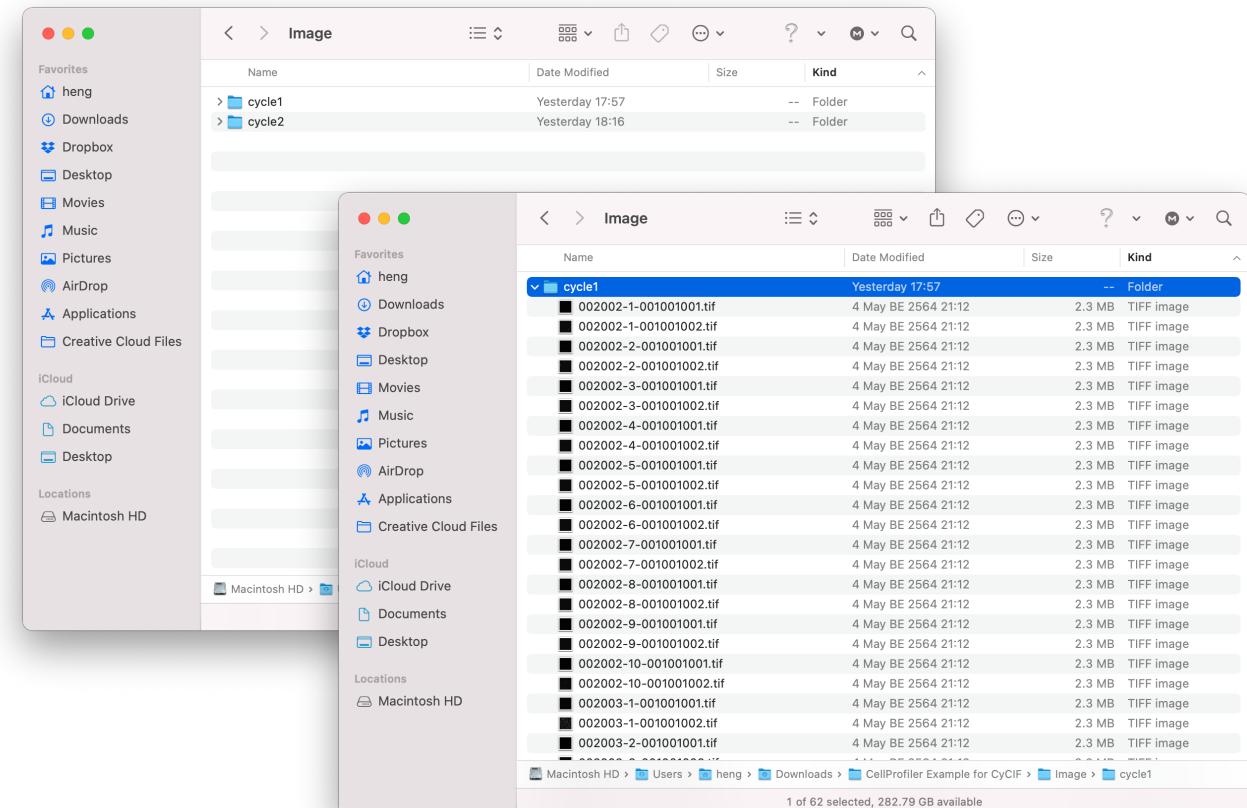
Cycle 1: Hoechst and YFP

Cycle 2: DAPI and EDU

Abbreviation

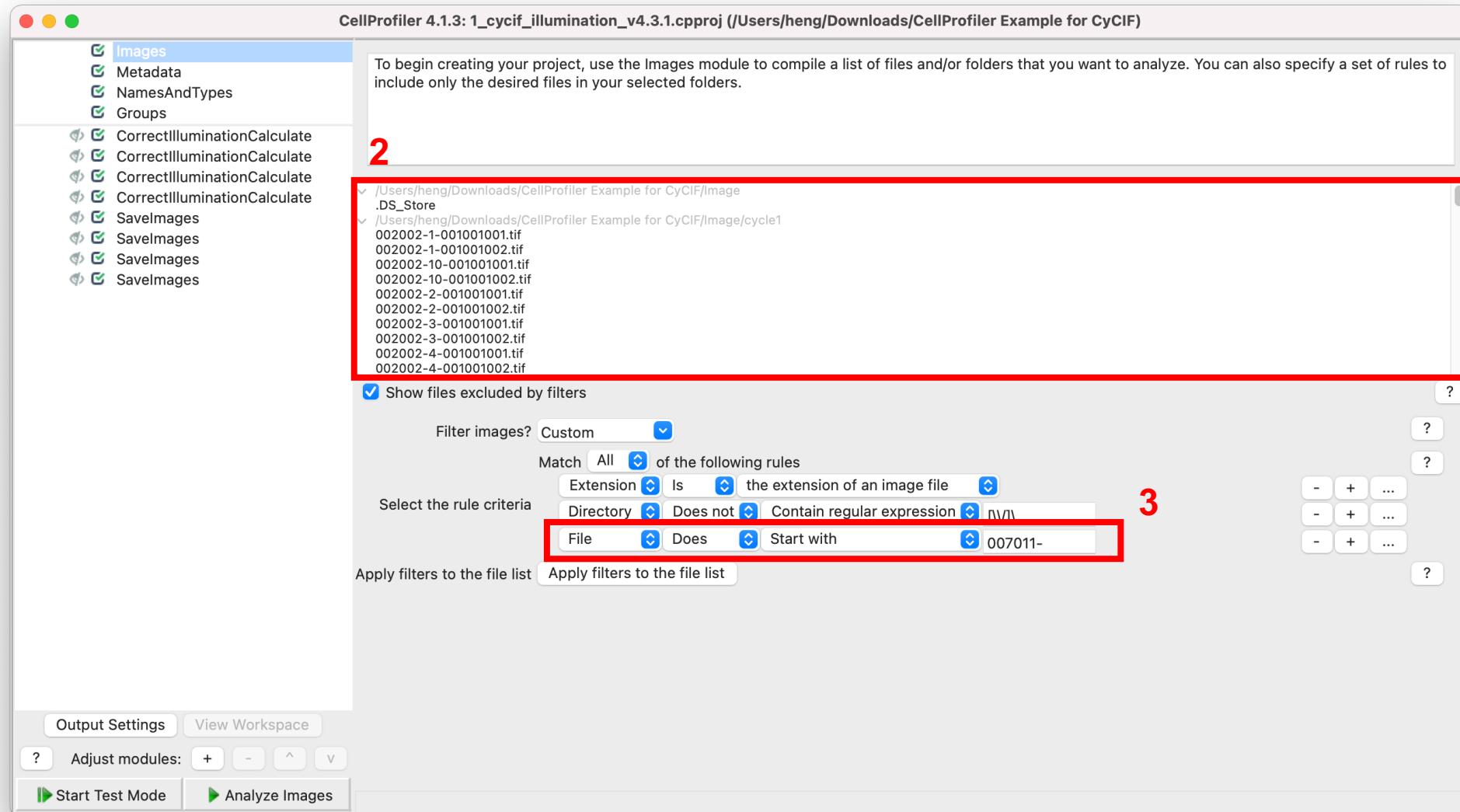
IllumCorr = Illumination Correction

ImSeg = Image Segmentation



1. Run “**IllumCorr**” pipeline

1. Open “[1_cycif_illumination_v4.3.1.cpproj](#)” pipeline.
2. Import images to the “**images**” module.
3. Set filter for ‘Blank well’ only (in this example, we use **G11** (metadata = **007011-** = **G11**) as a blank well).

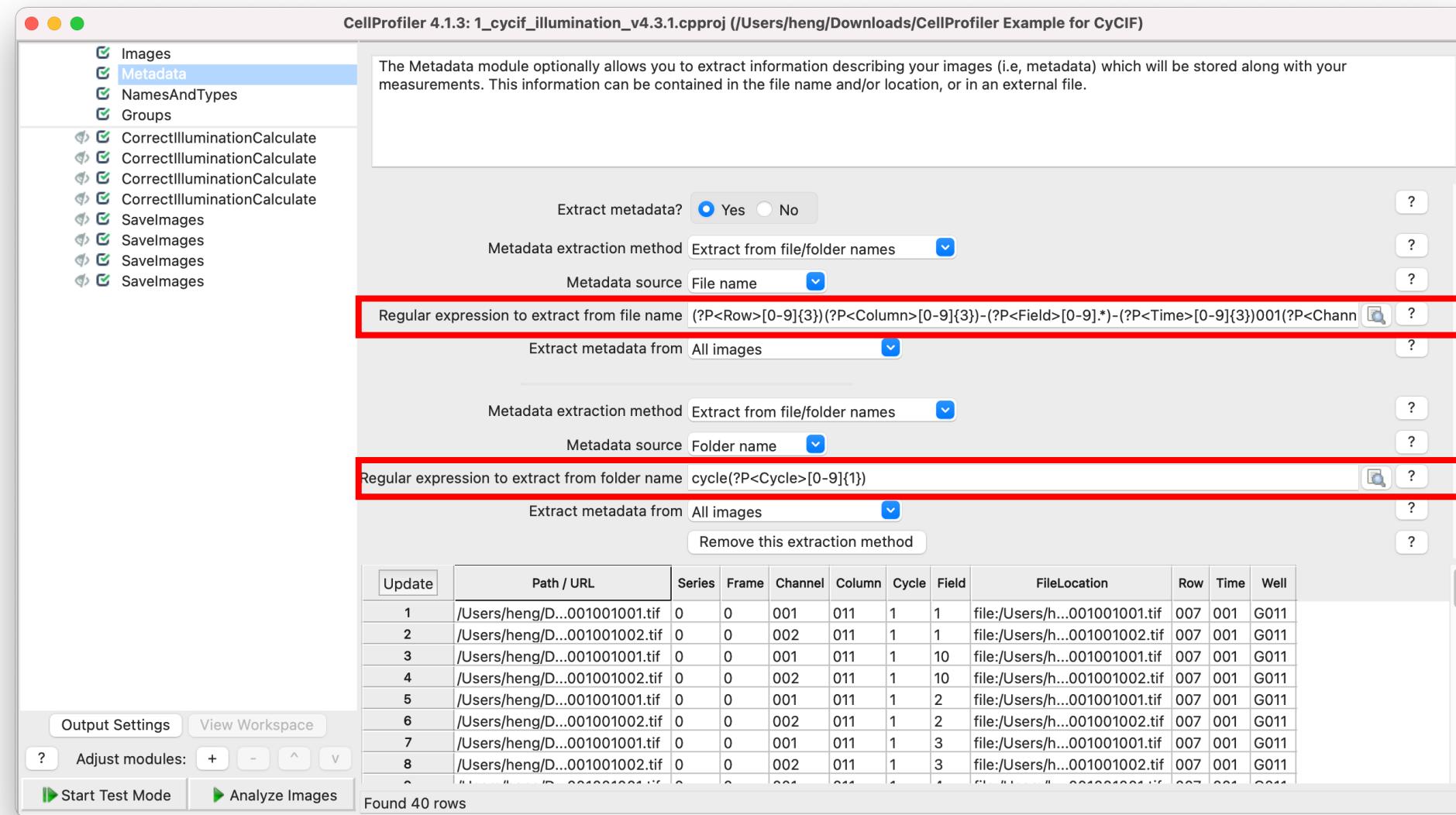


4. Select “Extract metadata” and set regular extraction for “file name” as following.

(?P<Row>[0-9]{3})(?P<Column>[0-9]{3})-(?P<Field>[0-9].*)-(?P<Time>[0-9]{3})001(?P<Channel>[0-9]+).*\$

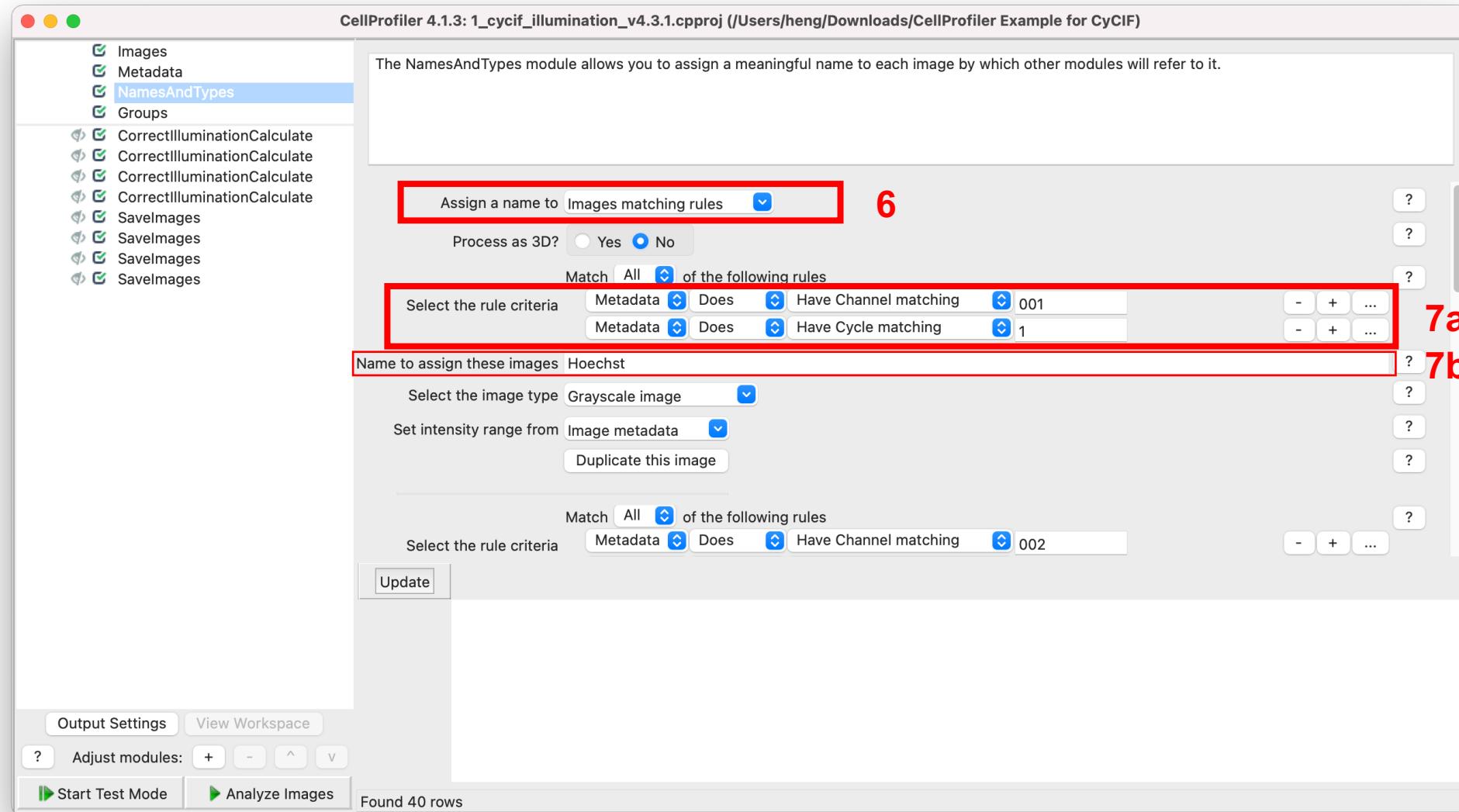
5. Set regular extraction for “folder name” as following.

cycle(?P<Cycle>[0-9]{1})



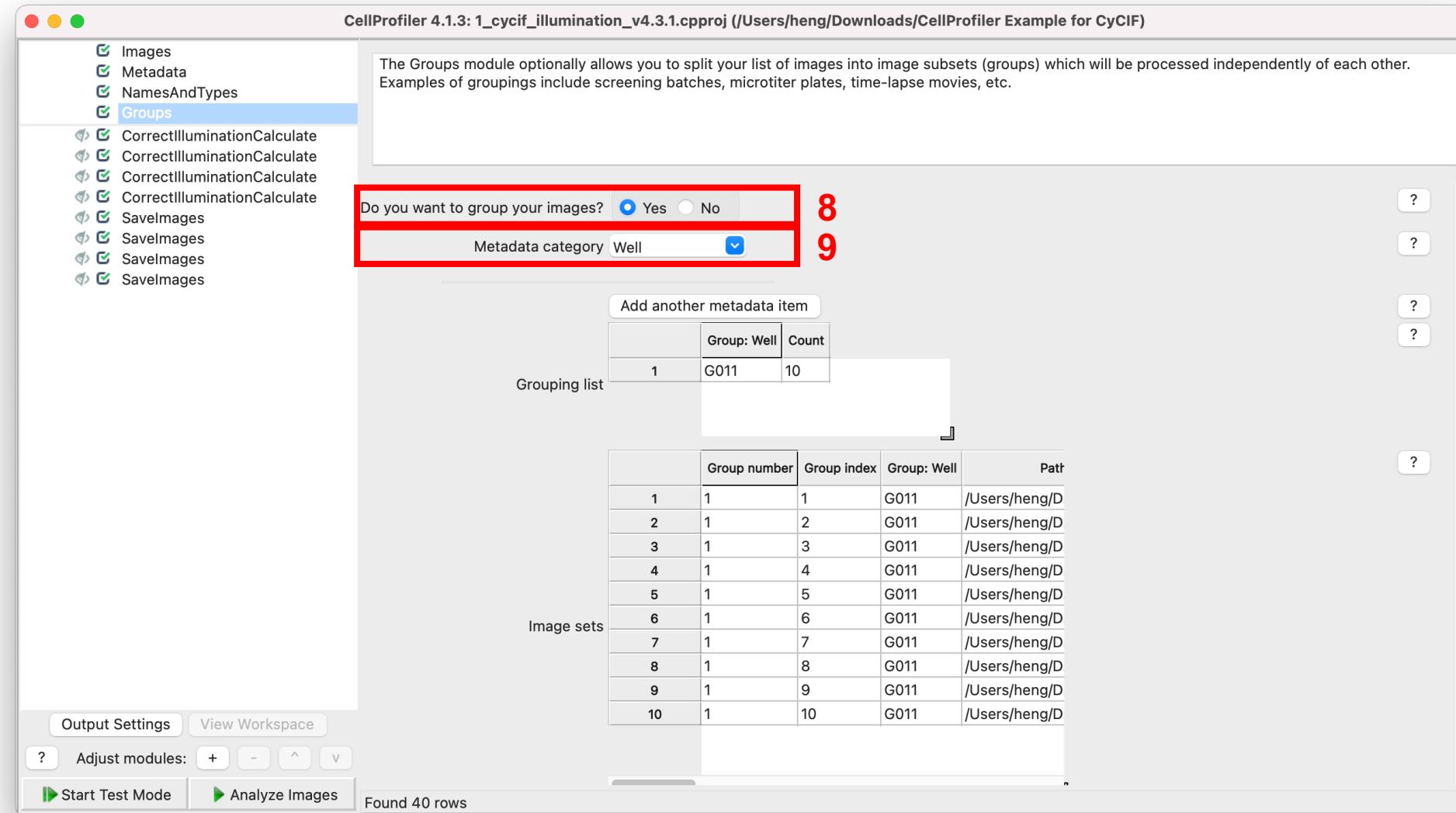
6. Select assign a name to “Image matching rules”.

7. Select the rule criteria (7a) and Name (7b) for all channels. (In this example, we have **2 cycles** and each cycle has **2 channels** so that is why we need to set 2 criterias.)



8. Select “Yes” to group your images.

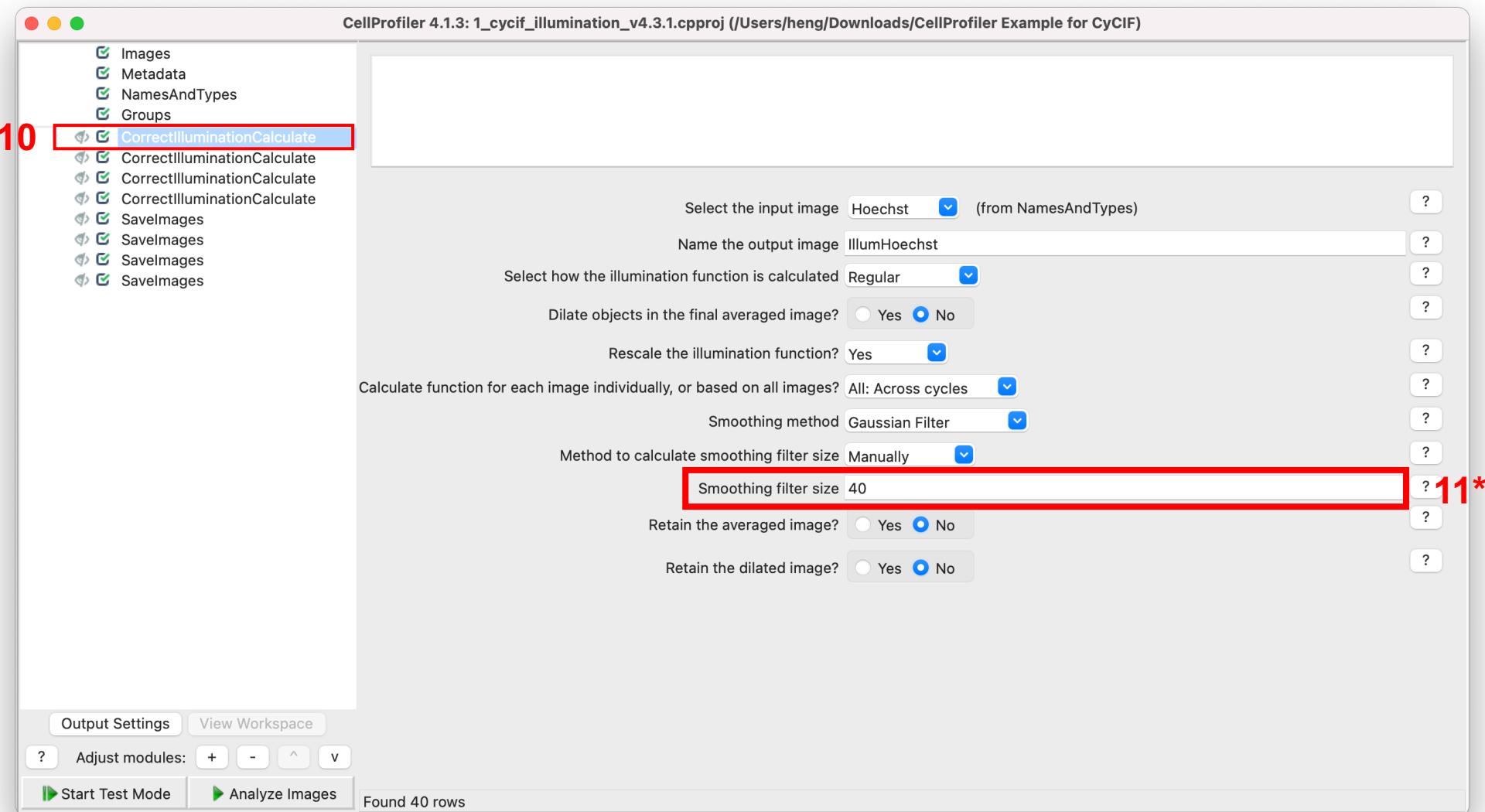
9. Select “Well” metadata category.



10. Import “CorrectIlluminationCalculate” module.

11. Set the details information as following. (Do this step for **all channels**.)

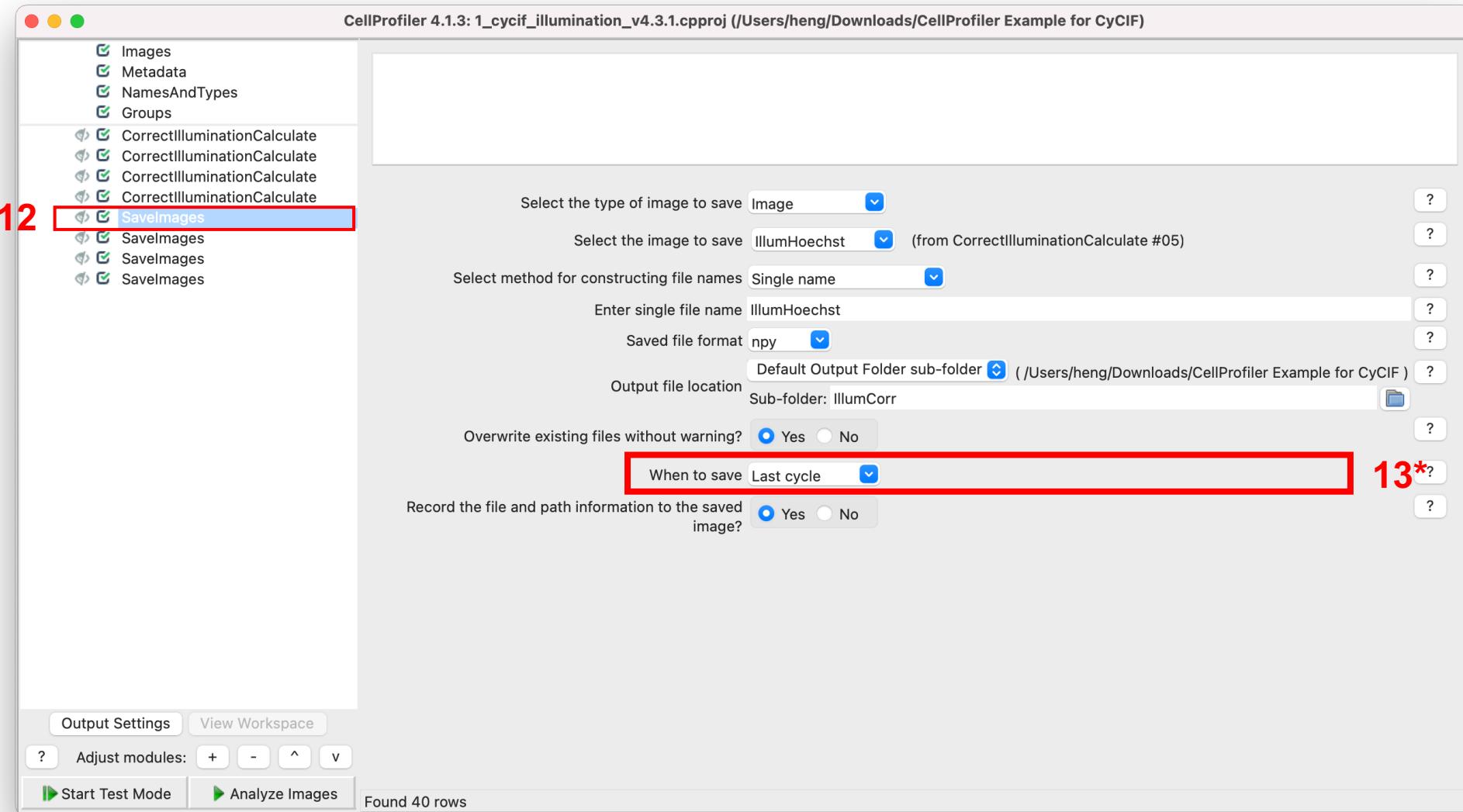
***This parameter must be optimized further in order to get better illumination background. (‘too low’ will get noisy background and ‘too high’ will not get anything)**



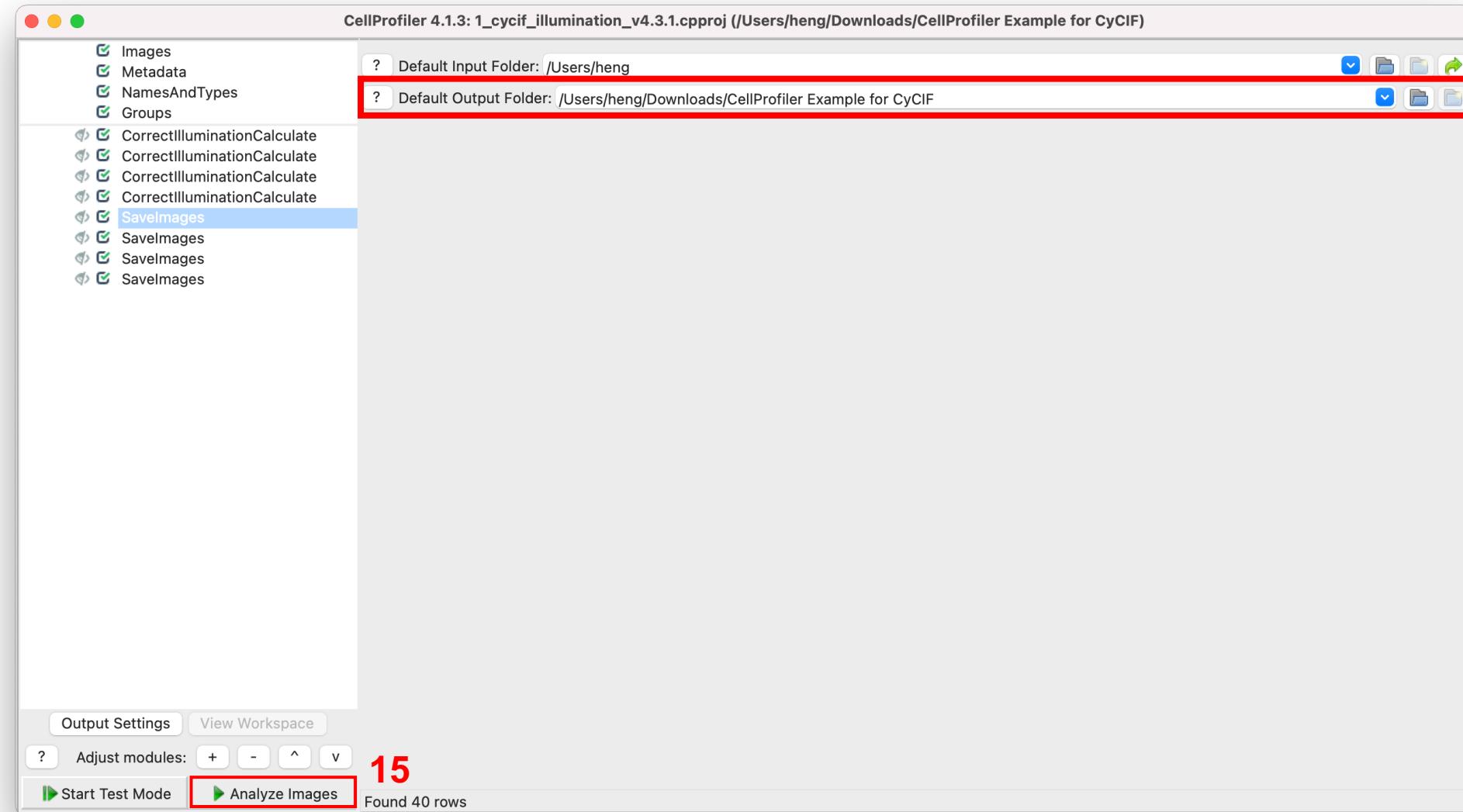
12. Import “SaveImages” module.

13. Set the details information as following. (Do this step for **all channels**.)

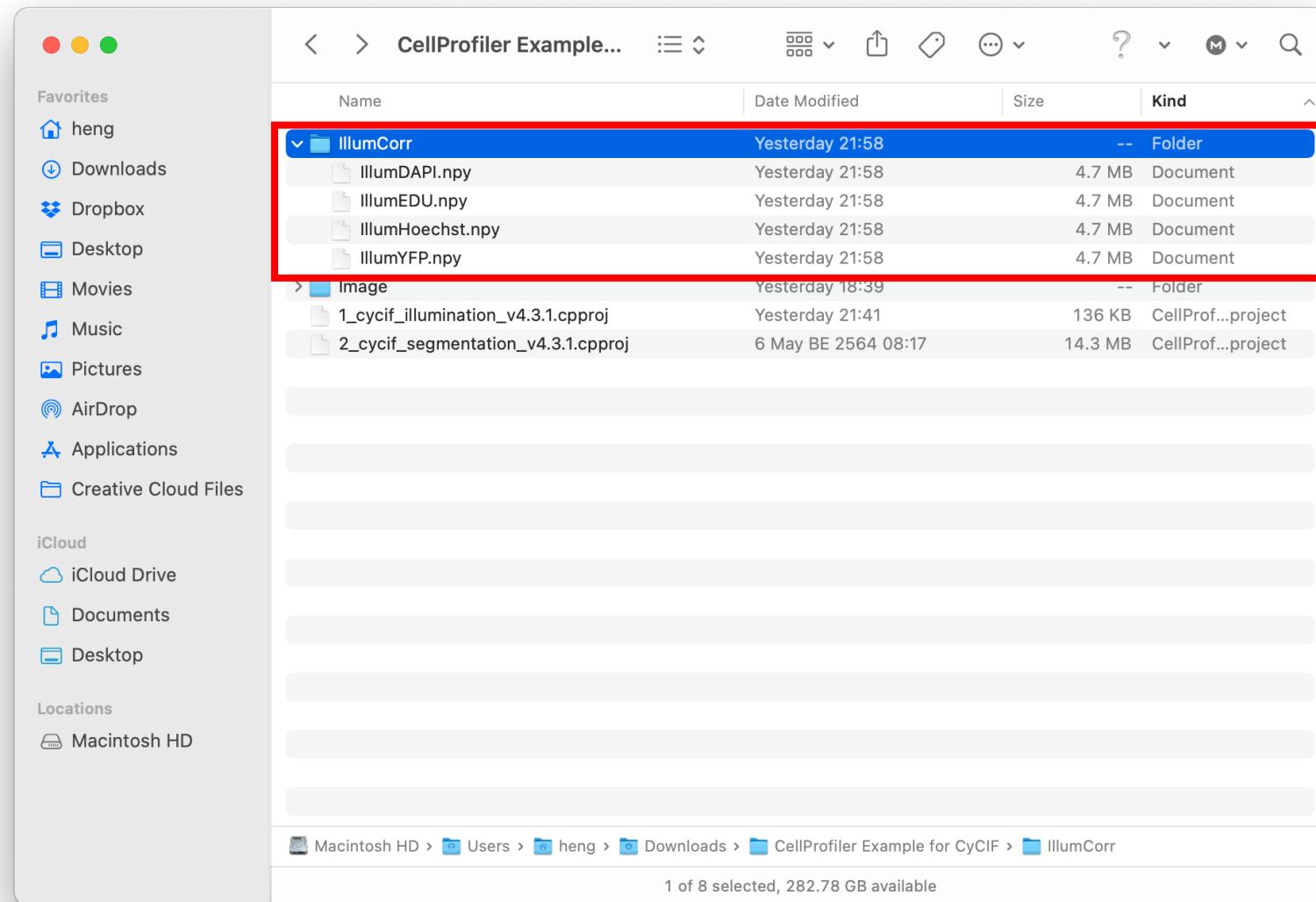
*This setting will save file in “Last cycle” of running.



14. Click “Output Settings” and select Output folder.



16. After “Analyze Images”, the illumination file will be saved in “IllumCorr” folder.



2. Run “**Segment**” pipeline

Segmented Pipeline Overview

*All parameters in each module can be able to adjust in order to get the best segmented protocol.

