Making composite images from layered morphology 2D images

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2024-06-12

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1 Description

Composite images of CosMx™ Spatial Molecular Imager (SMI) fields of view (FOVs) can be useful when using open-sourced software such as squidpy and giotto. In this post, we describe the make_composite.py script, a developmental python script that creates such composite images from layered morphology 2D images that can be exported from the AtoMx™ Spatial Informatics Portal (SIP). Layered images are extracted from the 2D morphology TIF files and written in a file format selected by the user. The extracted images are converted to 8bit, and composite images are written from these 8bit images.

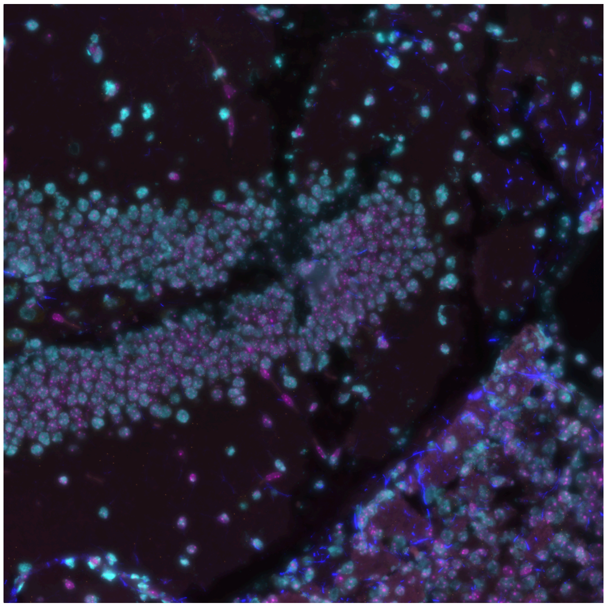


Figure 1: A composite image created using the make_composite.py script. This image represents all channels of a single field of view in the publicly available mouse coronal hemisphere FFPE dataset.

> Note
>
> make_composite.py is a development version. Use at your own risk.

2 Where to find the script?

The script and license can be found in the `assets/make-composite` folder of the repository.

3 Required libraries

The script requires the following libraries to be installed:

- Pillow (*e.g.*, `pip install pillow`)
- Numpy (*e.g.*, `pip install numpy`)

4 User inputs (required)

- `clipping` (int or float) - Histogram clipping percentage. This value is the percentage of the histogram to clip on the left and right side. The effect changes the contrast of the image. A higher percentage produces more contrast. The user needs to determine the appropriate percentage by testing on a subset of images. The same clipping value is applied to all images. Generally, setting the value between 1 and 3 is a good starting point. Specifying a clipping value of 0 will not alter the histogram. A value is required, there is no default. `clipping` is a required input.
- `user_format` (str) - File format to be written. Options are jpg, png, and tif. All output files will be written in the format specified by the user. `user_format` is a required input.

5 Additional inputs (changed within the script)

- Variable: `colors` = [‘cyan’, ‘red’, ‘yellow’, ‘blue’, ‘magenta’]. The variable is the composite color scheme (not a user input; changeable within the script). The colors are listed in order of channel number (channel 0 to channel 4). Example: Channel 0 is colored ‘cyan’,
- Variable: `compress_value` (set to 3). Lossless file compression value. Higher values produce smaller files at the expense of increased script execution time. The set value is a compromise between file size and execution time.

6 Output

- `raw` – The extracted tif files from the morphology 2D images will be saved in this folder. The file format will follow <fov_num>_ch<#>_raw.<user_format>. <fov_num> is the fov number,`ch<#>` is the channel number (from 0 to 4), and <user_format> is the specified file type format (see User inputs). Note: If the specified `user_format` is jpg, the raw files will be 8bit jpg files.

  Example

  `F001_ch0_raw.jpg (for user_format = jpg)`

- `8bit` - The images in the raw_folder are converted to 8bit and saved in this folder. Note: If the specified `user_format` is jpg, the 8bit files are identical to the raw files. The file format will follow <fov_num>_ch<#>_8bit.<user_format> except when `user_format` = jpg

  Example

```
F001_ch0_8bit.tif (for user_format = tif)
F001_ch0_raw.jpg (for user_format = jpg)
```

- `8bit_autocontrast` – Images in the 8bit folder are autocontrasted based on the user supplied clipping value. The file format will follow <fov_num>_ch<#>_8bit_autocontrast.<user_format>

  Example

  ```
  F001_ch0_8bit_autocontrast.png (for user_format = png)
  ```

- `composite` - Composite images created from the images in the `8bit` folder. The composite type is a screen composite. The file format will follow <fov_num>_composite.<user_format>

  Example

  ```
  F001_composite.jpg (for user_format = jpg)
  ```

- `composite_autocontrast` - Images in the composite_folder are autocontrasted based on the user specified clipping value. The file format will follow <fov_num>_composite_autocontrast.<user_f

  Example

  ```
  F001_composite_autocontrast.png (for user_format = png)
  ```

7 Usage

```
cd to/your/Morphology2D folder
python /path/to/your/make_composite.py # and follow the on-screen prompts
```

Regex pattern matching on 2D morphology file name format is implemented, however, only NanoString 2D morphology files should be present in the folder containing the make_composite script.

8 Example

The example dataset that we used was the mouse coronal hemisphere FFPE dataset that is available to download from NanoString's website here.

The Morphology2D folder is found within the CellStatsDir folder and has TIF files for each of the 130 FOVs.

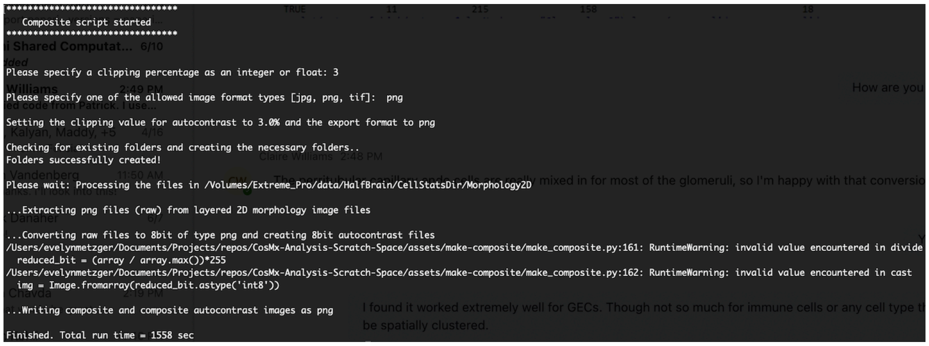
```
# In Terminal
cd /path/to/slide/CellStatsDir/Morphology2D
```

```
# In Terminal
tree -L 1
```

```
  20230406_205644_S1_C902_P99_N99_F001.TIF
  20230406_205644_S1_C902_P99_N99_F002.TIF
...
  20230406_205644_S1_C902_P99_N99_F129.TIF
  20230406_205644_S1_C902_P99_N99_F130.TIF
```

Once in the Morphology2D folder, simply run the script and follow the on-screen prompts (Figure 2).

```
# In Terminal
python /path/to/CosMx-Analysis-Scratch-Space/assets/make-composite/make_composite.py
```



```
********************************
  Composite script started
********************************

Please specify a clipping percentage as an integer or float: 3

Please specify one of the allowed image format types [jpg, png, tif]:  png

Setting the clipping value for autocontrast to 3.0% and the export format to png

Checking for existing folders and creating the necessary folders..
Folders successfully created!

Please wait: Processing the files in /Volumes/Extreme_Pro/data/HalfBrain/CellStatsDir/Morphology2D

...Extracting png files (raw) from layered 2D morphology image files

...Converting raw files to 8bit of type png and creating 8bit autocontrast files
/Users/evelynmetzger/Documents/Projects/repos/CosMx-Analysis-Scratch-Space/assets/make-composite/make_composite.py:161: RuntimeWarning: invalid value encountered in divide
  reduced_bit = (array / array.max())*255
/Users/evelynmetzger/Documents/Projects/repos/CosMx-Analysis-Scratch-Space/assets/make-composite/make_composite.py:162: RuntimeWarning: invalid value encountered in cast
  img = Image.fromarray(reduced_bit.astype('int8'))

...Writing composite and composite autocontrast images as png

Finished. Total run time = 1558 sec
```

Figure 2: Screenshot of standard output from terminal following script execution. In this example, I set the clipping percentage to 3 and the output to `png`. On a Macbook Pro M1, this took about 25 minutes to process 130 FOVs.

When complete, the structure of the Morphology2D folder should resemble this:

```
# In Terminal
tree -L 1
```

```
  20230406_205644_S1_C902_P99_N99_F001.TIF
  20230406_205644_S1_C902_P99_N99_F002.TIF
...
  20230406_205644_S1_C902_P99_N99_F129.TIF
  20230406_205644_S1_C902_P99_N99_F130.TIF
  8bit
  8bit_autocontrast
  composite
  composite_autocontrast
  raw
```

These composite images can now be imported into open-sourced software or explored further.