

Next Gen File Formats - Part II

a.k.a. Getting your hands-dirty with open-formats

a.k.a. Formats that make people happy

Materials for the workshop are available at

<https://downloads.openmicroscopy.org/presentations/2020/Dundee/Workshops/NGFF>

Software versions used for this workshop:

- conda: 4.8.3
- bioformats2raw: 0.2.2
- raw2ometiff: 0.2.4
- ome-zarr: 0.0.6
- napari: 0.3.1

Summary

⇒ [Installation instructions](#) (Best if done **before** the workshop)

⇒ [Review overall workflow](#)

⇒ [Glencoe's pipeline for whole-slide imaging including bioformats2raw](#)

- Standard conversion examples
- Zarr conversion examples

⇒ [Investigating the raw intermediate format for public data](#)

- Description of the format

⇒ [Towards an ome-zarr library with an example napari plugin](#)

Programme

Installation instructions

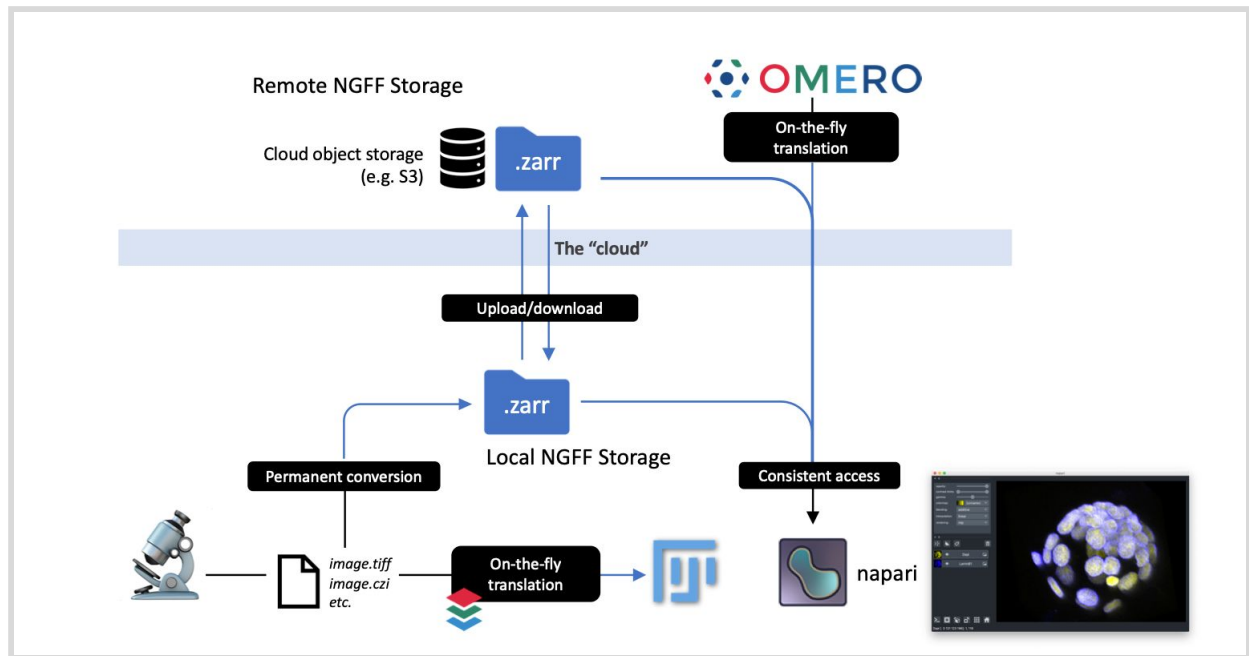
Easiest way to install the above tools is by creating a conda environment using the yaml file from [ome-zarr-guide](#) :

```
channels:  
  - ome  
  - conda-forge  
  - defaults  
dependencies:  
  - python=3.6  
  - bfutils=6.3.1  
  - bioformats2raw=0.2.2  
  - raw2ometiff=0.2.4  
  - blosc  
  - napari  
  - zarr  
  - dask  
  - s3fs  
  - pip  
  - pip:  
    - ome-zarr
```

1. Follow the [conda documentation](#) if you have not previously installed conda.
2. Place the above in a file named "environment.yml" and run:
`conda env create -n demo -f environment.yml`

Alternatively, you can manually download [bioformats2raw](#) and [raw2ometiff](#), and install the necessary Python libraries into your (virtual-)environment of choice, e.g. with `pip install ome-zarr[napari]`

Workflows



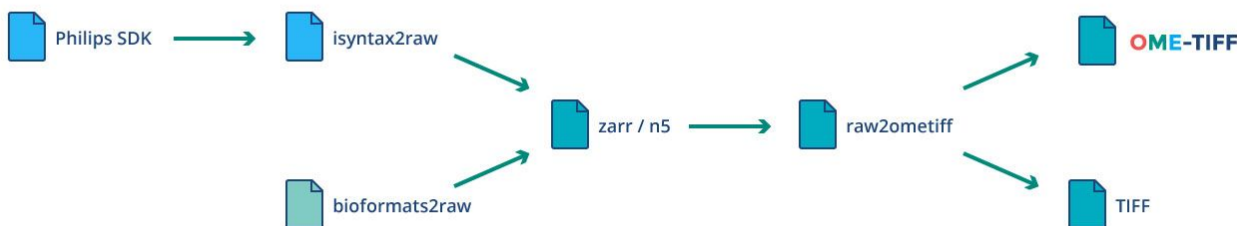
Slide 8 from https://downloads.openmicroscopy.org/presentations/2020/Dundee/main-talks/OME2020_NGFF_Moore.pdf

Writing pyramidal OME-TIFFs from the CLI

Links used during this section:

- <https://forum.image.sc/t/converting-whole-slide-images-to-ome-tiff-a-new-workflow/32110>
- <https://www.glencoesoftware.com/blog/2019/12/09/converting-whole-slide-images-to-OME-TIFF.html>
- <https://github.com/glencoesoftware/bioformats2raw/releases>
- <https://github.com/glencoesoftware/raw2ometiff/releases>
- <https://anaconda.org/ome/bioformats2raw>
- <https://github.com/joshmoore/ome-guide-zarr/blob/master/notebooks/conversion.ipynb>

Converting Whole Slide Images to OME-TIFF: The Solution



```
Basics
The two basic commands are bioformats2raw and raw2ometiff. Together they provide a pipeline to scalably convert large images into OME-TIFF. The primary caveat is that they require twice the storage for the conversion.

In [1]: !time
!bioformats2raw --help
Missing required parameters: <inputPath>, <outputPath>
Usage: <main class> [--debug] [--extra-readers=<extraReaders>[, <extraReaders>...]]...
      [--additional-scale-format-string-args=<additionalScaleFormatStringArgsCsv>] [--compression-type=<compressionType>]
      [--compression-parameter=<compressionParameter>]
      [--dimension-order=<dimensionOrder>]
      [--file-type=<fileType>] [--h=<tileHeight>]
      [--max_cached_tiles=<maxCachedTiles>]
      [--max_workers=<maxWorkers>] [--pyramid-name=<pyramidName>]
      [--r=<pyramidResolutions>]
      [--scale-format-string=<scaleFormatString>]
      [--tileWidth] <inputPath> <outputPath>
      file to convert
      <inputPath> path to the input pyramid directory
      <outputPath> path to the output pyramid directory
      --additional-scale-format-string-args=<additionalScaleFormatStringArgsCsv> Additional format string argument CSV file (without header row). Arguments will be added to the end of the scale format string mapping the at the corresponding CSV row index. It is expected that the CSV file contain exactly the same number of rows as the input file has series
      -c, --compression=<compressionType> Compression type for n5 (blosc, bzip2, gzip, lz4, raw, xxz; default: blosc)
      --compression-parameter=<compressionParameter> Integer parameter for chosen compression (see https://github.com/zaiaelab/n5/blob/master/README.md)
      --debug Turn on debug logging
      --dimension-order=<dimensionOrder> Override the input file dimension order in the output file
      XYZCT, XYZTC, XYCTZ, XYCTZ, XYTCZ, XYTCZ
      --extra-readers=<extraReaders>[, <extraReaders>...] Generate set of readers to include (default:
      [None])
```

```
OME Zarr format
Another option provided by bioformats2raw is --file_type which produces Zarr output rather than N5 as the intermediate format. If we additionally pass the --dimension-order argument, then the intermediate result can be used directly by the ome-zarr library.

In [2]: !time
!bioformats2raw a.fake /tmp/output-3 --file_type=zarr --dimension-order=XYZCT
2020-05-20 12:44:19,680 [main] INFO c.g.bioformats2raw.Converter - Output will be incompatible with raw2ometiff (pyramidName: data.zarr, scaleFormatString: 4d/4d)
2020-05-20 12:44:20,201 [main] INFO loci.formats.ImageReader - FakeReader initializing a fake
2020-05-20 12:44:20,324 [main] INFO c.g.bioformats2raw.Converter - Using 2 pyramid resolutions
2020-05-20 12:44:20,325 [main] INFO c.g.bioformats2raw.Converter - Preparing to write pyramid sizeX 512 (tileWidth: 1024) sizeY 512 (tileWidth: 1024) imageCount 1
2020-05-20 12:44:20,573 [main] WARN c.g.bioformats2raw.Converter - Reducing active tileWidth to 512
2020-05-20 12:44:20,573 [main] WARN c.g.bioformats2raw.Converter - Reducing active tileHeight to 512
2020-05-20 12:44:20,587 [pool-1-thread-1] INFO c.g.bioformats2raw.Converter - requesting tile to write at [0, 0, 0, 0] to /0/0
2020-05-20 12:44:20,598 [pool-1-thread-1] INFO c.g.bioformats2raw.Converter - tile read complete 1/1
2020-05-20 12:44:20,598 [pool-1-thread-1] INFO org.perf4j.TimingLogger - start[1589971460605] time[10] tag[getFile]
2020-05-20 12:44:20,601 [pool-1-thread-1] INFO c.g.bioformats2raw.Converter - successfully wrote at [0, 0, 0, 0] to /0/0
2020-05-20 12:44:20,601 [pool-1-thread-1] INFO c.g.bioformats2raw.Converter - Successfully processed tile; resolution=0 plane=0 xx=0 yy=0 width=512 height=512
2020-05-20 12:44:20,601 [main] WARN c.g.bioformats2raw.Converter - Reducing active tileWidth to 256
2020-05-20 12:44:20,601 [main] WARN c.g.bioformats2raw.Converter - Reducing active tileHeight to 256
2020-05-20 12:44:20,605 [pool-1-thread-2] INFO c.g.bioformats2raw.Converter - requesting tile to write at [0, 0, 0, 0] to /0/1
2020-05-20 12:44:20,612 [pool-1-thread-2] INFO org.perf4j.TimingLogger - start[1589971460605] time[6] tag[getTileDownsampled]
2020-05-20 12:44:20,612 [pool-1-thread-2] INFO c.g.bioformats2raw.Converter - tile read complete 1/1
2020-05-20 12:44:20,612 [pool-1-thread-2] INFO org.perf4j.TimingLogger - start[1589971460605] time[7] tag[getFile]
2020-05-20 12:44:20,613 [pool-1-thread-2] INFO c.g.bioformats2raw.Converter - successfully wrote at [0, 0, 0, 0] to /0/1
2020-05-20 12:44:20,614 [pool-1-thread-2] INFO c.g.bioformats2raw.Converter - Successfully processed tile; resolution=1 plane=0 xx=0 yy=0 width=256 height=256
```

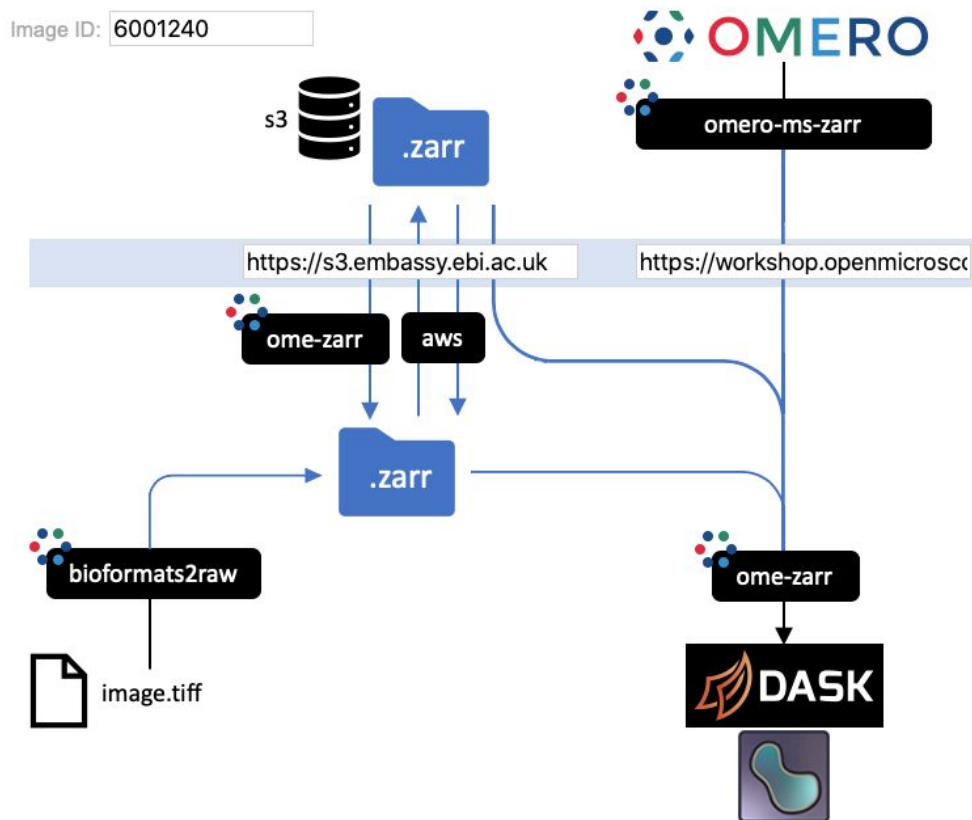
Conversion examples ([launch](#))

OME Zarr examples ([launch](#))

Using the raw (i.e. Zarr) format for public data

Links used during this section:

- <https://github.com/ome/omero-guide-python/blob/master/notebooks/zarr-public-s3-multiscale.ipynb>
- <https://github.com/joshmoore/ome-guide-zarr/blob/master/notebooks/zarr.ipynb>
- https://downloads.openmicroscopy.org/presentations/2020/Dundee/zarr_diagram/
- e.g. ome_zarr info <https://s3.embassy.ebi.ac.uk/idr/zarr/v0.1/6001240.zarr>
- e.g. napari <https://s3.embassy.ebi.ac.uk/idr/zarr/v0.1/9822151.zarr>



Access workflows ([diagram](#))

```
$ ome_zarr download https://s3.embassy.ebi.ac.uk/idr/zarr/v0.1/6001240.zarr
$ ome_zarr info 6001240.zarr

{'multiscales': [{'datasets': [{'path': '0'}, {'path': '1'}], 'version': '0.1'}], 'omero':
{'channels': [{'active': True, 'coefficient': 1, 'color': '0000FF', 'family': 'linear',
'inverted': False, 'label': 'LaminB1', 'window': {'end': 1500, 'max': 65535, 'min': 0,
'start': 0}}, {'active': True, 'coefficient': 1, 'color': 'FFFF00', 'family': 'linear',
'inverted': False, 'label': 'Dapi', 'window': {'end': 1500, 'max': 65535, 'min': 0, 'start':
0}}], 'id': 6001240, 'name': 'B1_C1.tif', 'rdefs': {'defaultT': 0, 'defaultZ': 118, 'model':
'color'}, 'version': '0.1'}}
...
```

(* If you plan on logging in: [OMERO.server "workshop" credentials](#))

Binary layout of an image

Each image is currently stored as a separate Zarr fileset named according to its OMERO ID:

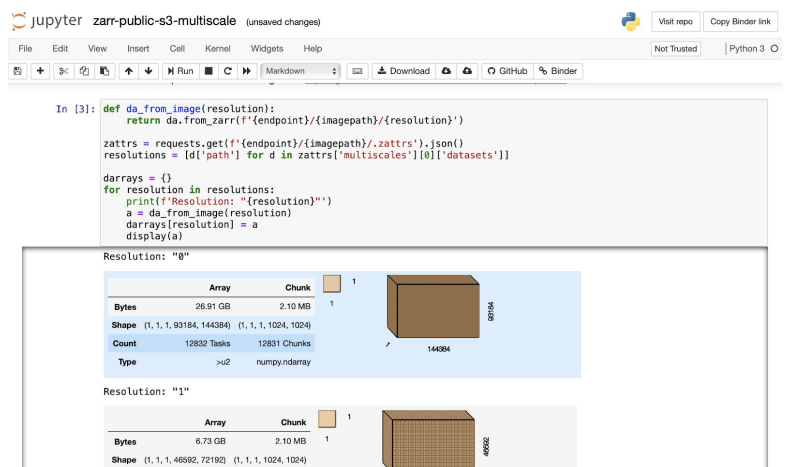
```
. # Root folder, potentially in S3,
├── 123.zarr # with a flat list of images by image ID.
├── 456.zarr #
│   ├── .zattrs # Group level metadata, including OMERO metadata.
│   ├── .zgroup # Each image is a Zarr group with multiscale metadata.
│   └── 0 # Each multiscale level is stored as a separate Zarr array.
│       ├── .zarray #
│       ├── 0.0.0.0.0 # Chunks are stored with the flat directory layout.
│       └── t.c.z.y.x # All image arrays are 5-dimensional
# with dimension order (t, c, z, y, x).
```

OMERO metadata

The OMERO metadata providing rendering settings and channel annotations is available under the “omero” key within the Zarr attributes (.zattrs) of the top-level group.

```
"id": 1, # ID in OMERO
"name": "example.tif", # Name as shown in the UI
"channels": [ # Array matching the c dimension size
  {
    "active": true,
    "color": "0000FF",
    "label": "LaminB1",
    "window": {
      "end": 1500,
      "max": 65535,
      "min": 0,
      "start": 0
    }
  }
],
"rdefs": {
  "defaultT": 0, # First time point to show the user
  "defaultZ": 118, # First Z section to show the user
  "model": "color" # "color" or "greyscale"
}
```

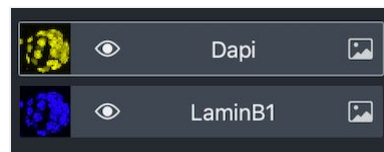
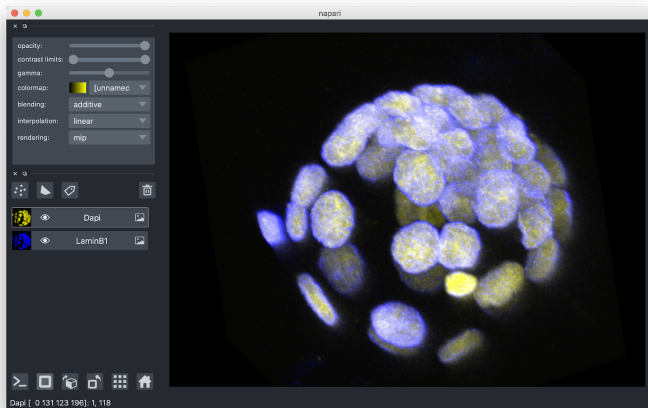
A notebook is available on mybinder.org to further explain the layout of the file format: [zarr-public-s3-multiscale.ipynb](https://mybinder.org/v2/omegacat/zarr-public-s3-multiscale)



ome-zarr plugin for napari plugin

Links used during this section:

- <https://github.com/ome/ome-zarr-py>
- <http://pypi.org/project/ome-zarr/>



```
$ pip install napari==0.3.1 ome-zarr==0.0.3 # numcodecs may need to come from conda
$ napari https://s3.embassy.ebi.ac.uk/idr/zarr/v0.1/6001240.zarr

# or in Python:
with napari.gui_qt():
    viewer = napari.Viewer()
    viewer.open('https://s3.embassy.ebi.ac.uk/idr/zarr/v0.1/6001240.zarr/')
```