Update on **OME-NGFF**

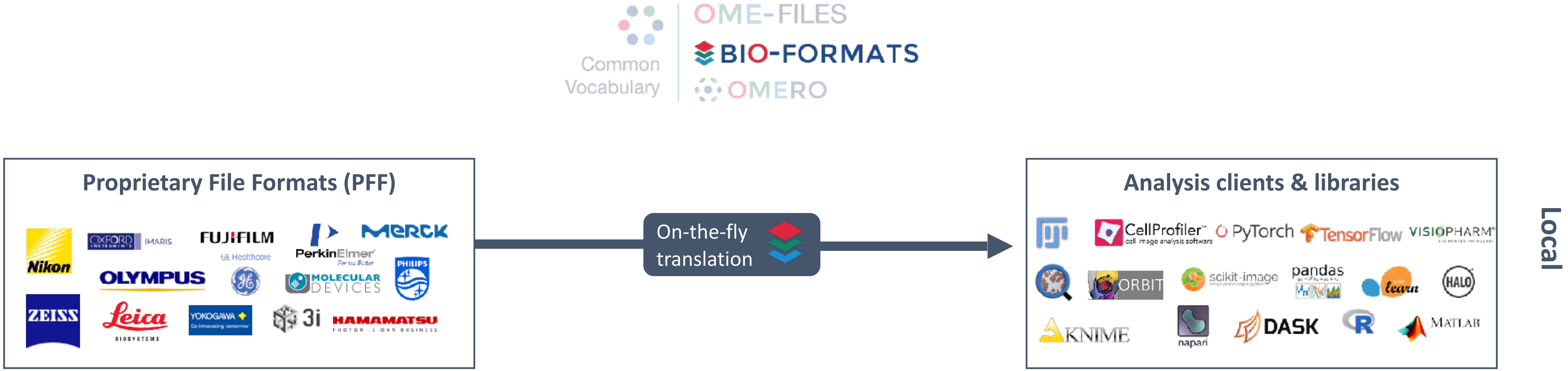
for developers, users, and other communities **2021-2024**

Josh Moore, Senior RDM Officer

German BioImaging, e.V. / Open Microscopy Environment

1. Questions
2. Status
3. Future

NGFF?



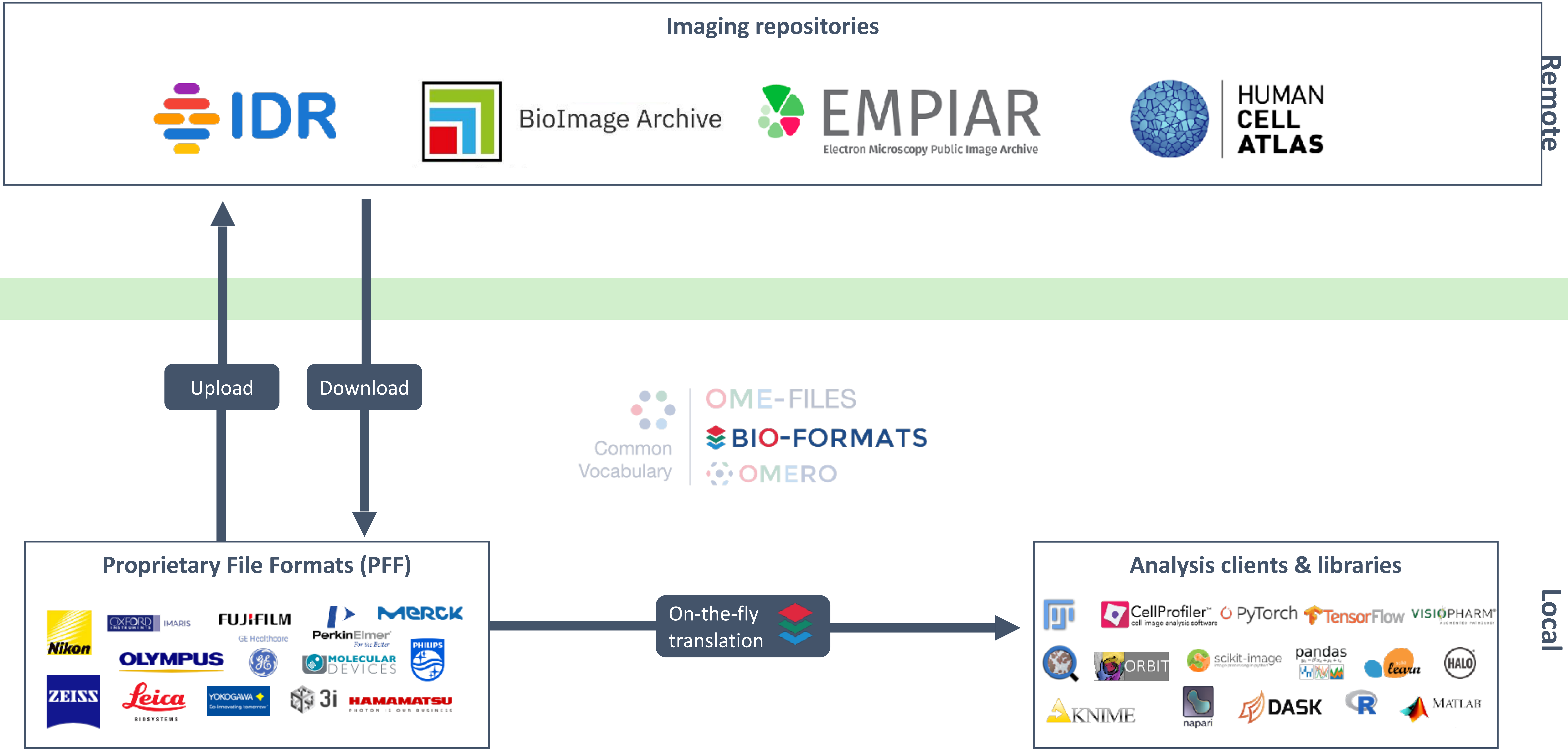
Common Vocabulary

Proprietary File Formats (PFF)

On-the-fly translation

Analysis clients & libraries

Local



Imaging repositories

Remote

Upload

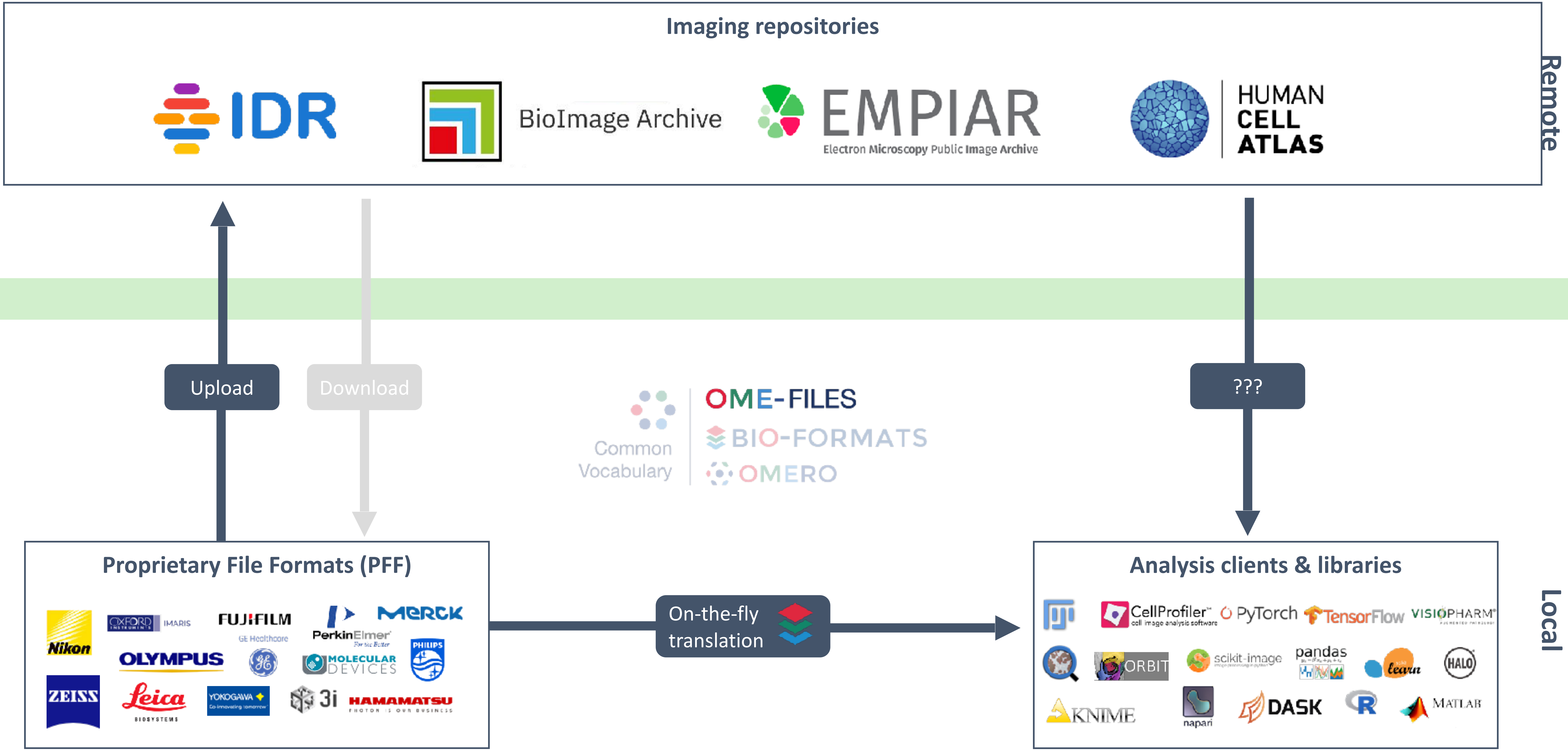
Download

Proprietary File Formats (PFF)

On-the-fly translation

Analysis clients & libraries

Local



Imaging repositories

Remote

Upload

Download

???

Common Vocabulary

OME-FILES

BIO-FORMATS

OMERO

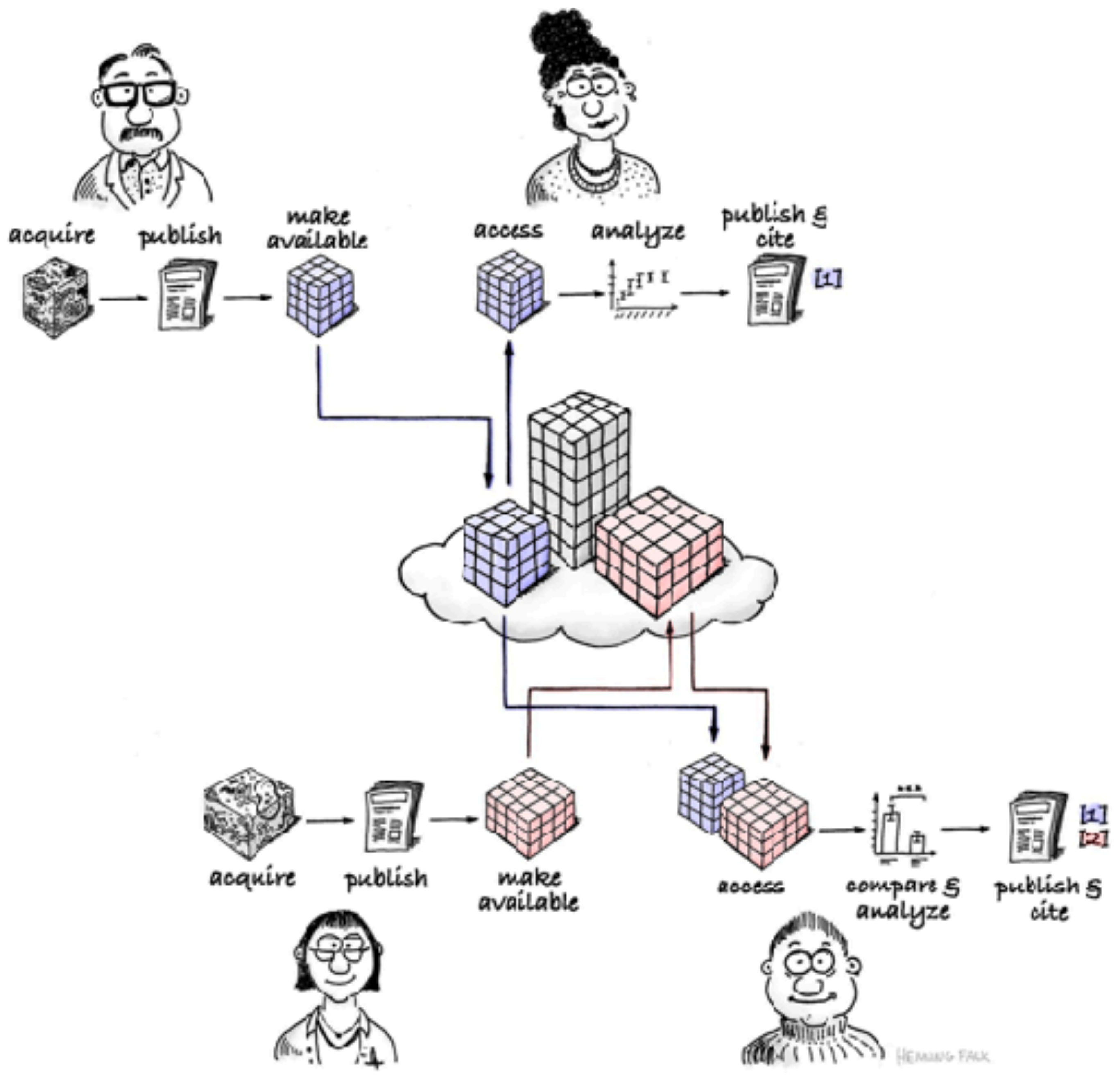
Proprietary File Formats (PFF)

On-the-fly translation

Analysis clients & libraries

Local

Another file format?



"FAIR re-use" by [@DrHenningFalk](),
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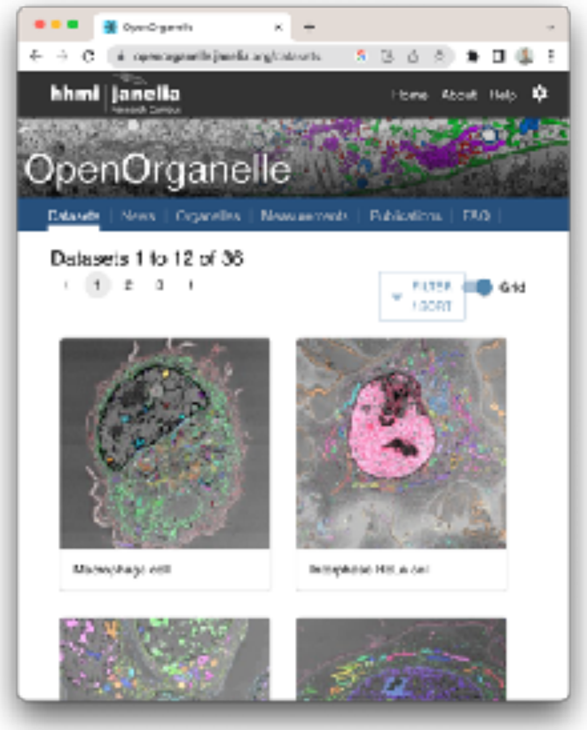
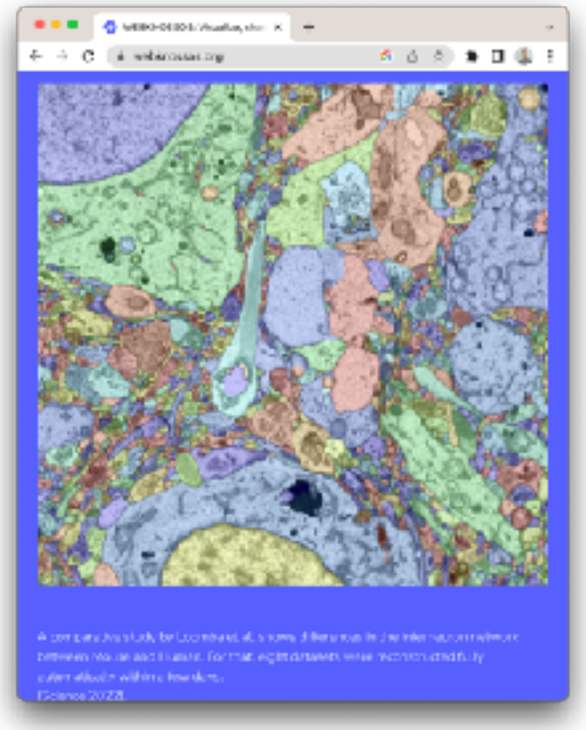
N5



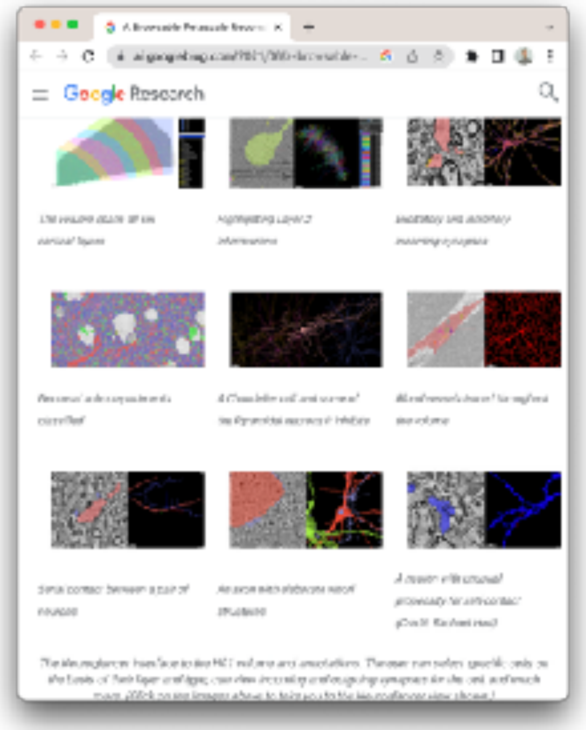
https://openorganelle.janelia.org/datasets

WKW



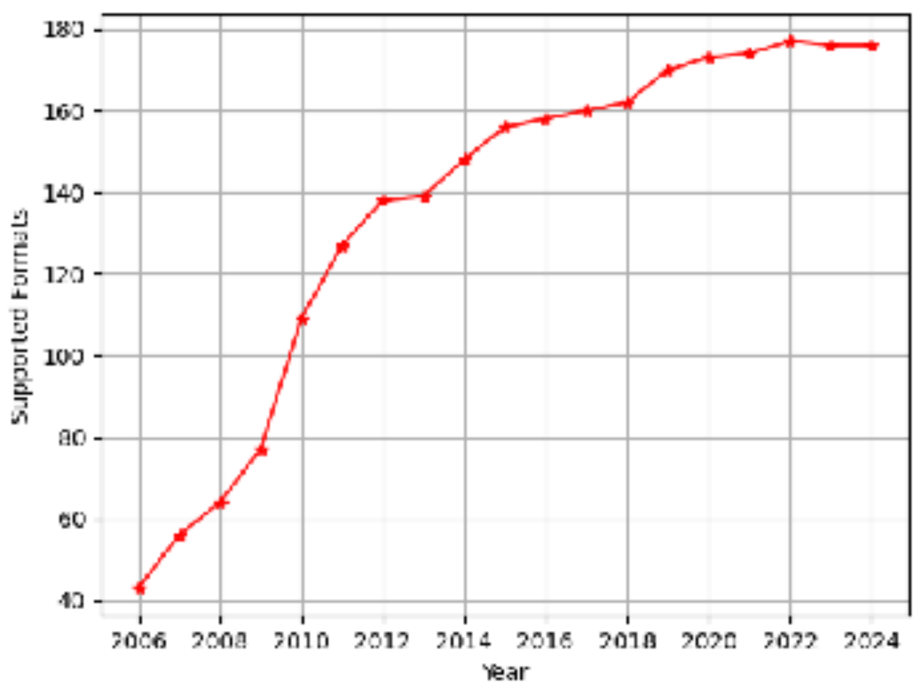
https://webknossos.org/

Precomputed



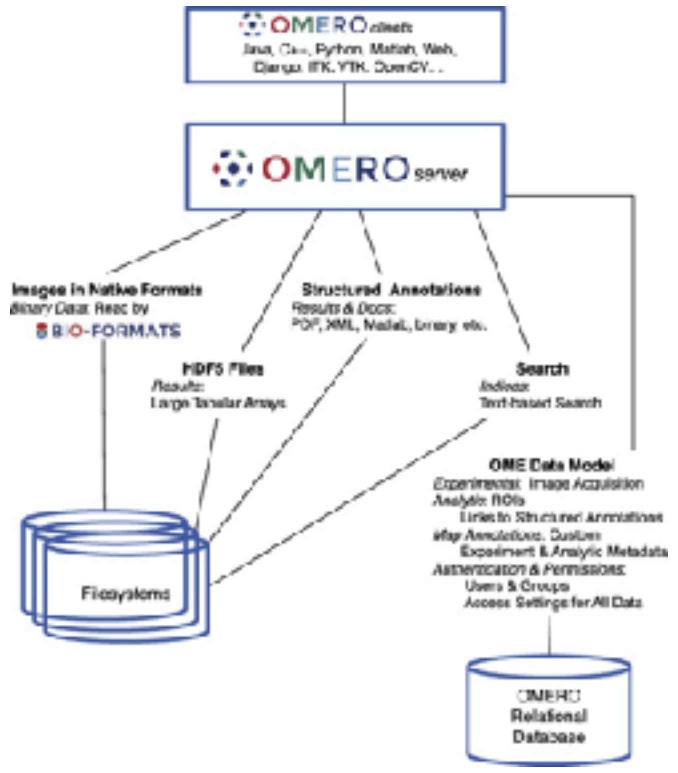
https://github.com/google/neuroglancer

Supported formats by year



https://bit.ly/bio-formats-list

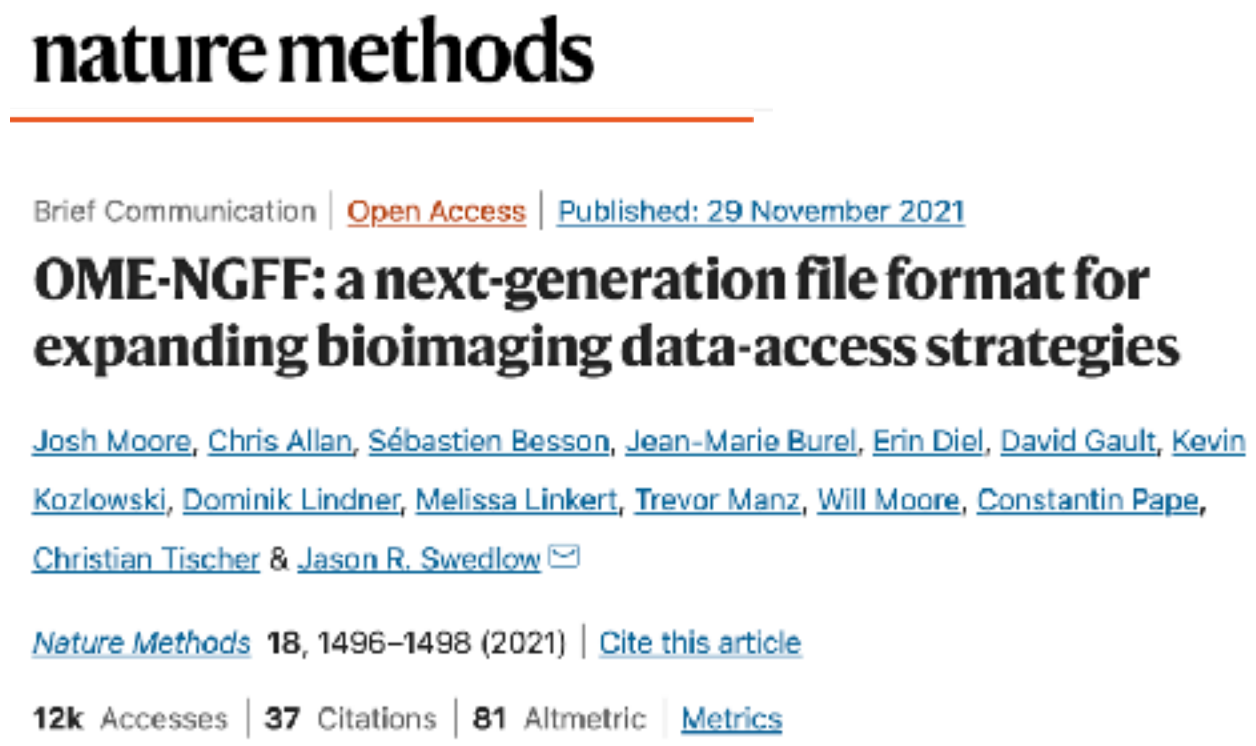
OMERO API



https://doi.org/10.1016/j.ymeth.2015.10.006

Is it "OME-NGFF" or "OME-Zarr"?

2021



nature methods

Brief Communication | Open Access | Published: 29 November 2021

OME-NGFF: a next-generation file format for expanding bioimaging data-access strategies

Josh Moore, Chris Allan, Sébastien Besson, Jean-Marie Burel, Erin Diel, David Gault, Kevin Kozlowski, Dominik Lindner, Melissa Linkert, Trevor Manz, Will Moore, Constantin Pape, Christian Tischer & Jason R. Swedlow ✉

Nature Methods 18, 1496–1498 (2021) | Cite this article

12k Accesses | **37** Citations | **81** Altmetric | Metrics

The specification and community process for improving our community format(s).

2023

Home > Histochemistry and Cell Biology > Article

OME-Zarr: a cloud-optimized bioimaging file format with international community support

Original Paper Open access Published: 10 July 2023

Volume 160, pages 223–251, (2023) Cite this article

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Histochemistry and Cell Biology

Aims and scope →

Submit manuscript →

Josh Moore ✉, Daniela Basurto-Lozada, Sébastien Besson, John Bogovic, Jordão Bragantini, Eva M. Brown, Jean-Marie Burel, Xavier Casas Moreno, Gustavo de Medeiros, Erin E. Diel, David Gault, Satrajit S. Ghosh, Ilan Gold, Yaroslav O. Halchenko, Matthew Hartley, Dave Horsfall, Mark S. Keller, Mark Kittisopikul, Gabor Kovacs, Aybüke Küpcü Yoldaş, Koji Kyoda, Albane le Tournoulx de la Villegeorges, Tong Li, Prisca Liberali, Dominik Lindner, Melissa Linkert, Joel Lüthi, Jeremy Maitin-Shepard, Trevor Manz, Luca Marconato, Matthew McCormick, Merlin Lange, Khaled Mohamed, William Moore, Nils Norlin, Wei Ouyang, Bugra Özdemir, Giovanni Palla, Constantin Pape, Lucas Pelkmans, Tobias Pietzsch, Stephan Preibisch, Martin Prete, Norman Rzepka, Sameeul Samee, Nicholas Schaub, Hythem Sidky, Ahmet Can Solak, David R. Stirling, Jonathan Striebel, Christian Tischer, Daniel Toloudis, Isaac Virshup, Petr Walczysko, Alan M. Watson, Erin Weisbart, Frances Wong, Kevin A. Yamauchi, Omer Bayraktar, Beth A. Cimini, Nils Gehlenborg, Muzlifah Haniffa, Nathan Hotaling, Shuichi Onami, Loic A. Royer, Stephan Saalfeld, Oliver Stegle, Fabian J. Theis & Jason R. Swedlow ^ Show fewer authors

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The cloud-optimized format that is being developed.

Is it “OME-NGFF” or “OME-Zarr”?

2021

nature methods

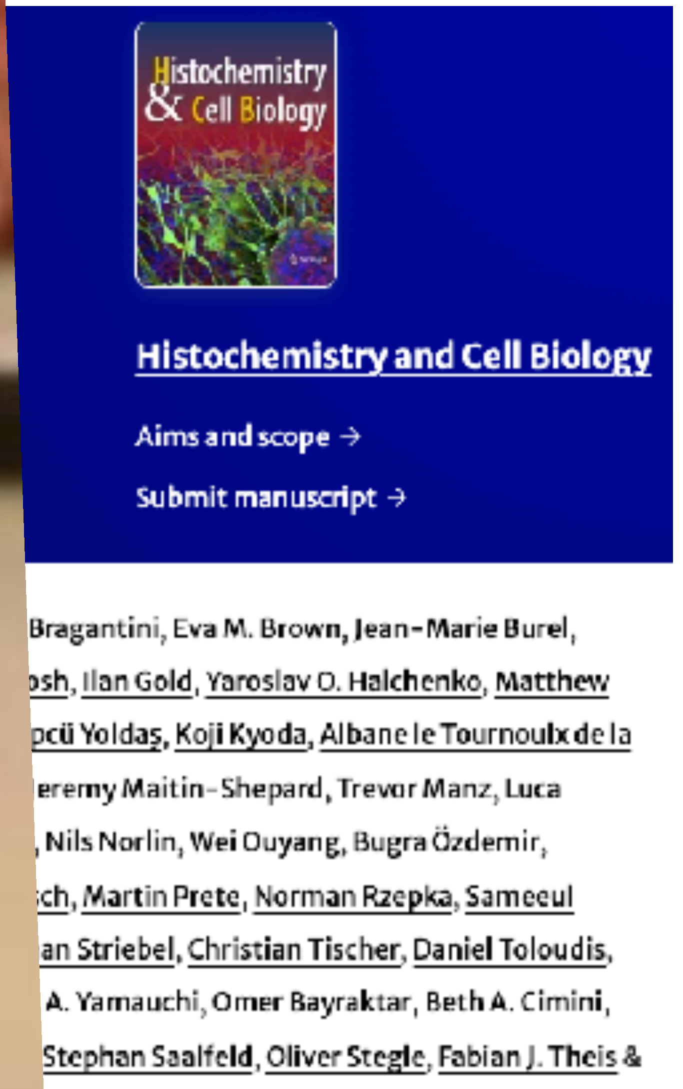
Brief Communication |

OME-NGFF: a
expanding bi

Josh Moore, Chris Allan,
Kozlowski, Dominik Lind
Christian Tischer & Jaso

Nature Methods **18**, 149

12k Accesses | **37** Cita



Histochemistry & Cell Biology

Histochemistry and Cell Biology

Aims and scope →

Submit manuscript →

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an Striebel, Christian Tischer, Daniel Toloudis,
A. Yamauchi, Omer Bayraktar, Beth A. Cimini,
Stephan Saalfeld, Oliver Stegle, Fabian J. Theis &

The specif … t that is
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https://forum.image.sc/t/is-there-an-ome-ngff-logo/73999/5

1. Questions
2. Status
3. Future

Publications

2021

nature methods

Brief Communication | Open Access | Published: 29 November 2021

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Nature Methods 18, 1496–1498 (2021) | Cite this article

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2022

Correspondence | Published: 11 May 2022

Viv: multiscale visualization of high-resolution multiplexed bioimaging data on the web

Trevor Manz, Ilan Gold, Nathan Heath Patterson, Chuck McCallum, Mark S. Keller, Bruce W. Herr II, Katy Börner, Jeffrey M. Spraggins & Nils Gehlenborg

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2023

Correspondence | Published: 10 February 2023

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2024

Brief Communication | Open access | Published: 20 March 2024

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Nature Methods (2024) | Cite this article

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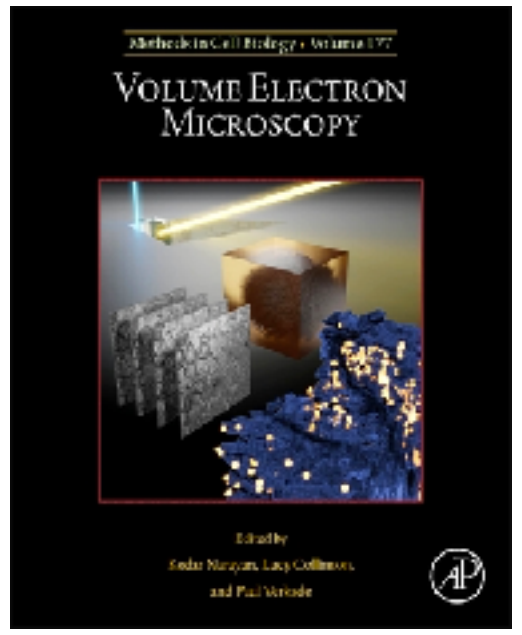


Methods in Cell Biology • Volume 177

VOLUME ELECTRON MICROSCOPY

Edited by Kedar, Lucy, and Paul

CHAPTER

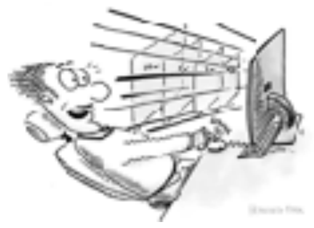
Toward scalable reuse of vEM data: OME-Zarr to the rescue

14

with John Bogovic and Norman

Adam's Desktop Distress

Bea Computes



Navid Zooms

Kit's Cluster Calamity

Clara's Workstation Woes

Qui Downloads

https://github.com/zarr-developers/zarr-illustrations-falk-2022/

TECHNOLOGY FEATURE | 02 October 2023

How open-source software could finally get the world's microscopes speaking the same language

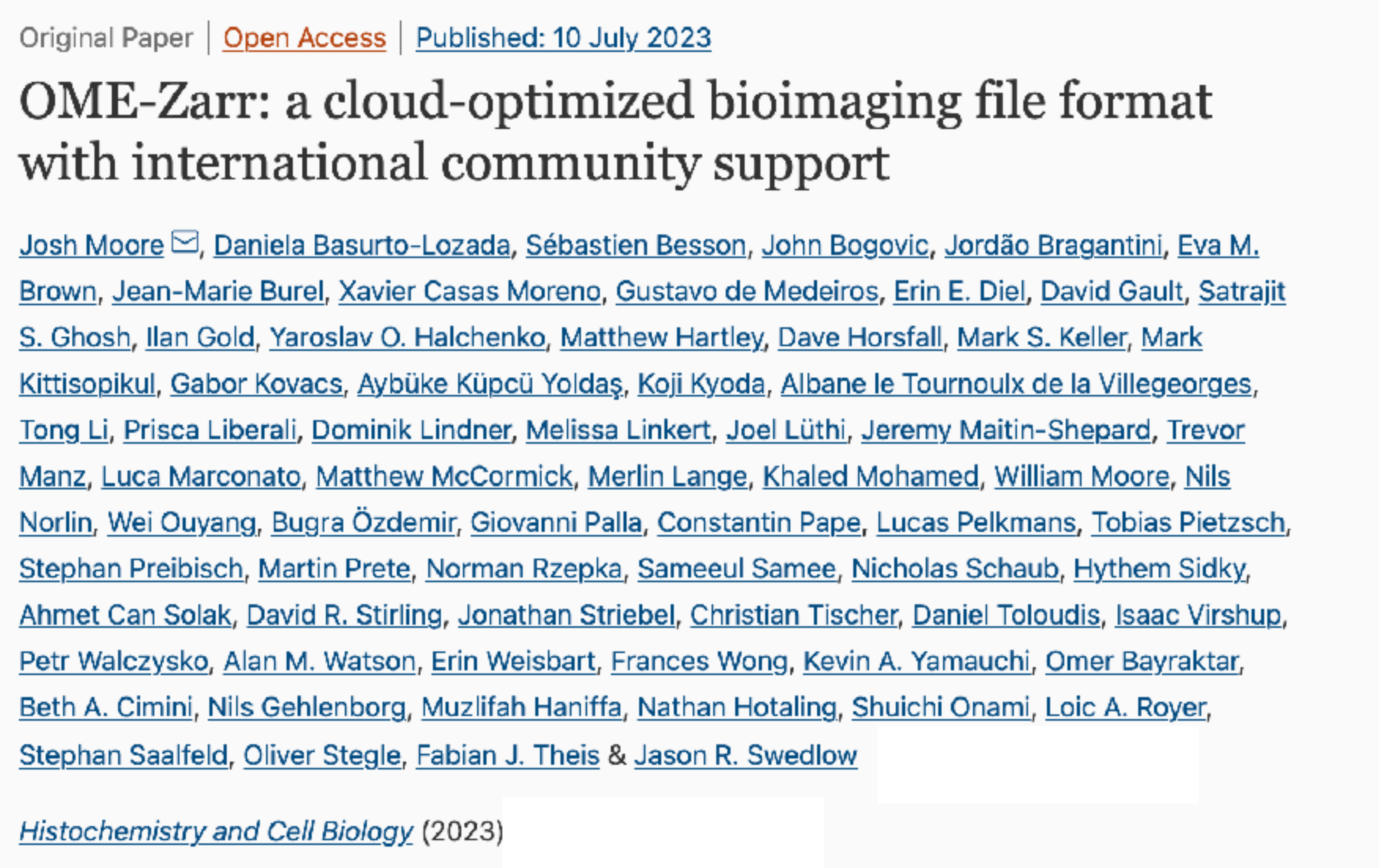
A plethora of standards mean shareable and verifiable microscopy data often get lost in translation. Biologists are working on a solution.

Michael Brooks

https://www.nature.com/articles/d41586-023-03064-9

https://news.ycombinator.com/item?id=37737318

State of the Community (2023)



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Histochemistry and Cell Biology (2023)

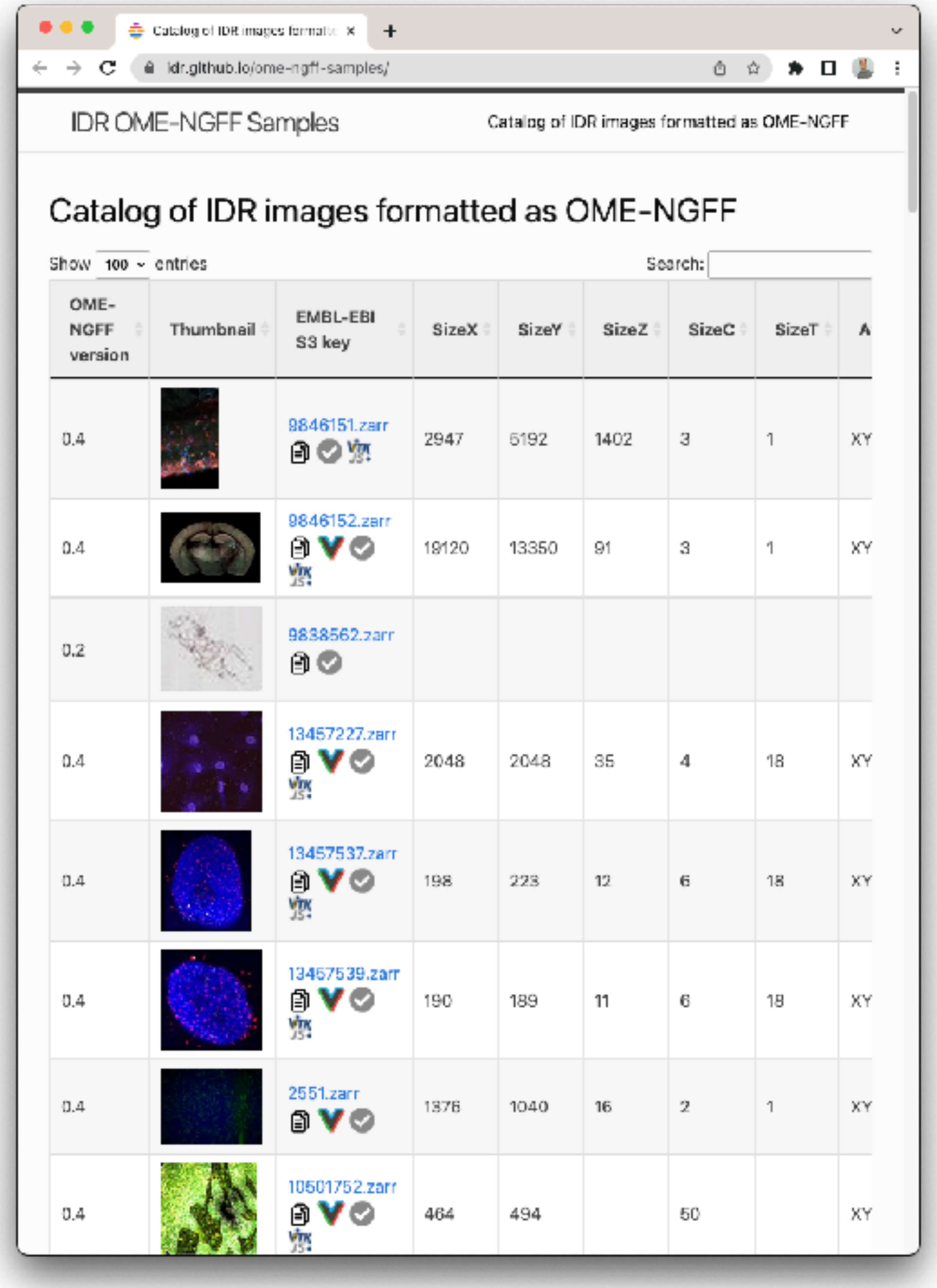
Special Issue of Histochemistry and Cell Biology
by Ben Giepmans, Katy Wolstencroft, Doug Taatjes

Countries	Authors	Institutions
CH	5	3
DE	12	7
JP	2	1
SE	3	2
UK	16	4
US	31	15
6	**69**	**32**

Shared Data

Catalog	Hosting	Zarr Files	Size
BIA Samples	EBI	90	200 GB
Cell Painting Gallery	AWS Open Data Program	136	20 TB
CZB-Zebrahub	czbiohub	5	1.2 TB
DANDI (identifiers.org,github)	AWS Open Data Program	3914	355 TB
Glencoe	Glencoe Software, Inc.	8	165 GB
IDR Samples	EBI	88	3 TB
MoBIE	EMBL-HD	21	2 TB
Neural Dynamics	AWS Open Data Program	90	200 TB
Sanger	Sanger, UK	10	1 TB
SpatialData	EMBL-HD	10	25 GB
SSBD	SSBD	12	196 GB
webKnossos	scalableminds GmbH	69	70 TB

https://ngff.openmicroscopy.org/data



IDR OME-NGFF Samples

Catalog of IDR images formatted as OME-NGFF

Catalog of IDR images formatted as OME-NGFF

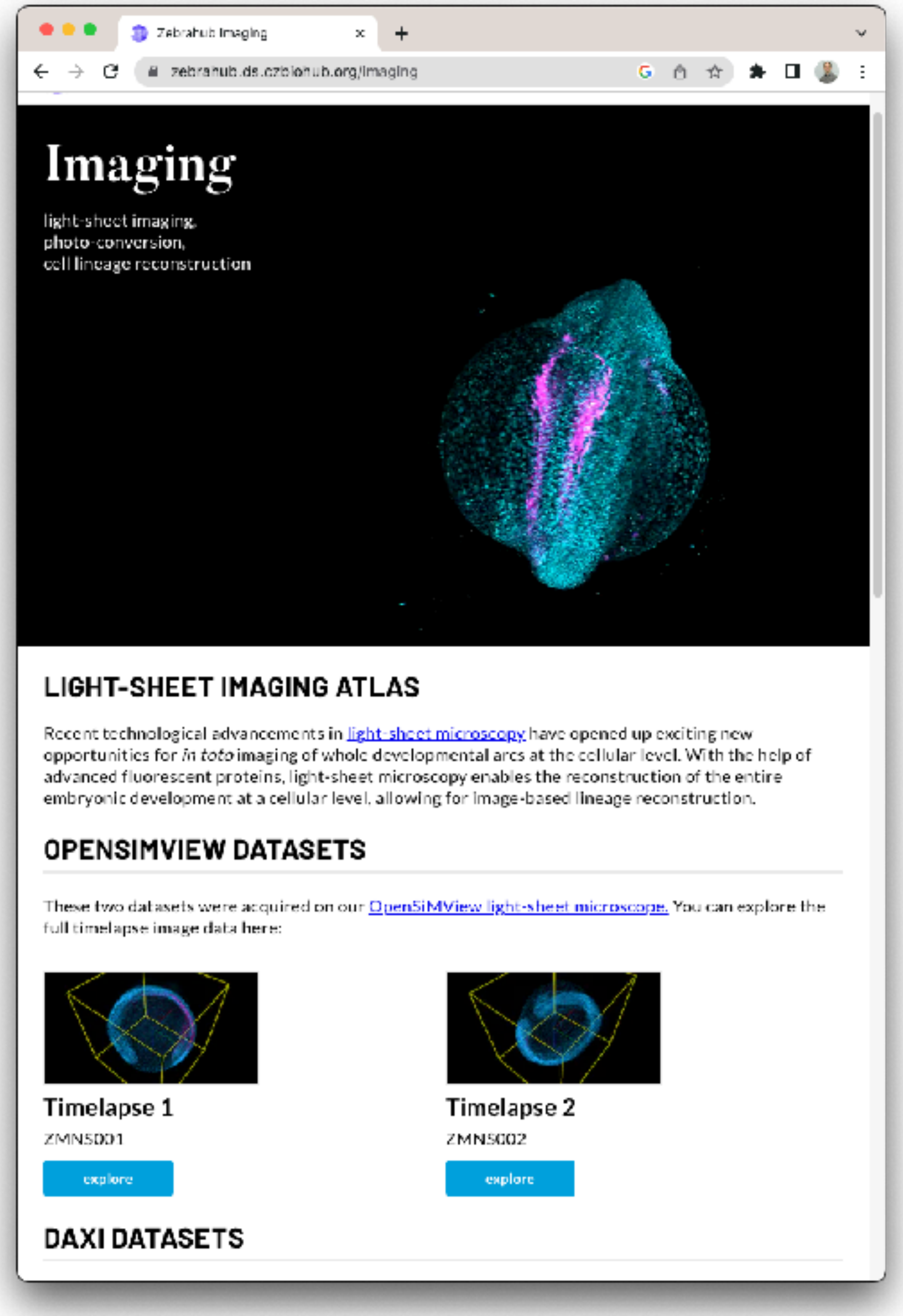
Show 100 entries

Search:

OME-NGFF version	Thumbnail	EMBL-EBI S3 key	SizeX	SizeY	SizeZ	SizeC	SizeT	A
0.4		9846151.zarr	2947	5192	1402	3	1	XY
0.4		9846152.zarr	19120	13350	91	3	1	XY
0.2		9838562.zarr						
0.4		13457227.zarr	2048	2048	35	4	18	XY
0.4		13457537.zarr	198	223	12	6	18	XY
0.4		13457539.zarr	190	189	11	6	18	XY
0.4		2551.zarr	1376	1040	16	2	1	XY
0.4		10501752.zarr	464	494		50		XY

https://idr.github.io/ome-ngff-samples

Shared Data



Zebrahub Imaging
zebrahub.ds.czbiohub.org/imaging

Imaging

light-sheet imaging,
photo-conversion,
cell lineage reconstruction

LIGHT-SHEET IMAGING ATLAS

Recent technological advancements in light-sheet microscopy have opened up exciting new opportunities for *in toto* imaging of whole developmental arcs at the cellular level. With the help of advanced fluorescent proteins, light-sheet microscopy enables the reconstruction of the entire embryonic development at a cellular level, allowing for image-based lineage reconstruction.

OPENSIMVIEW DATASETS

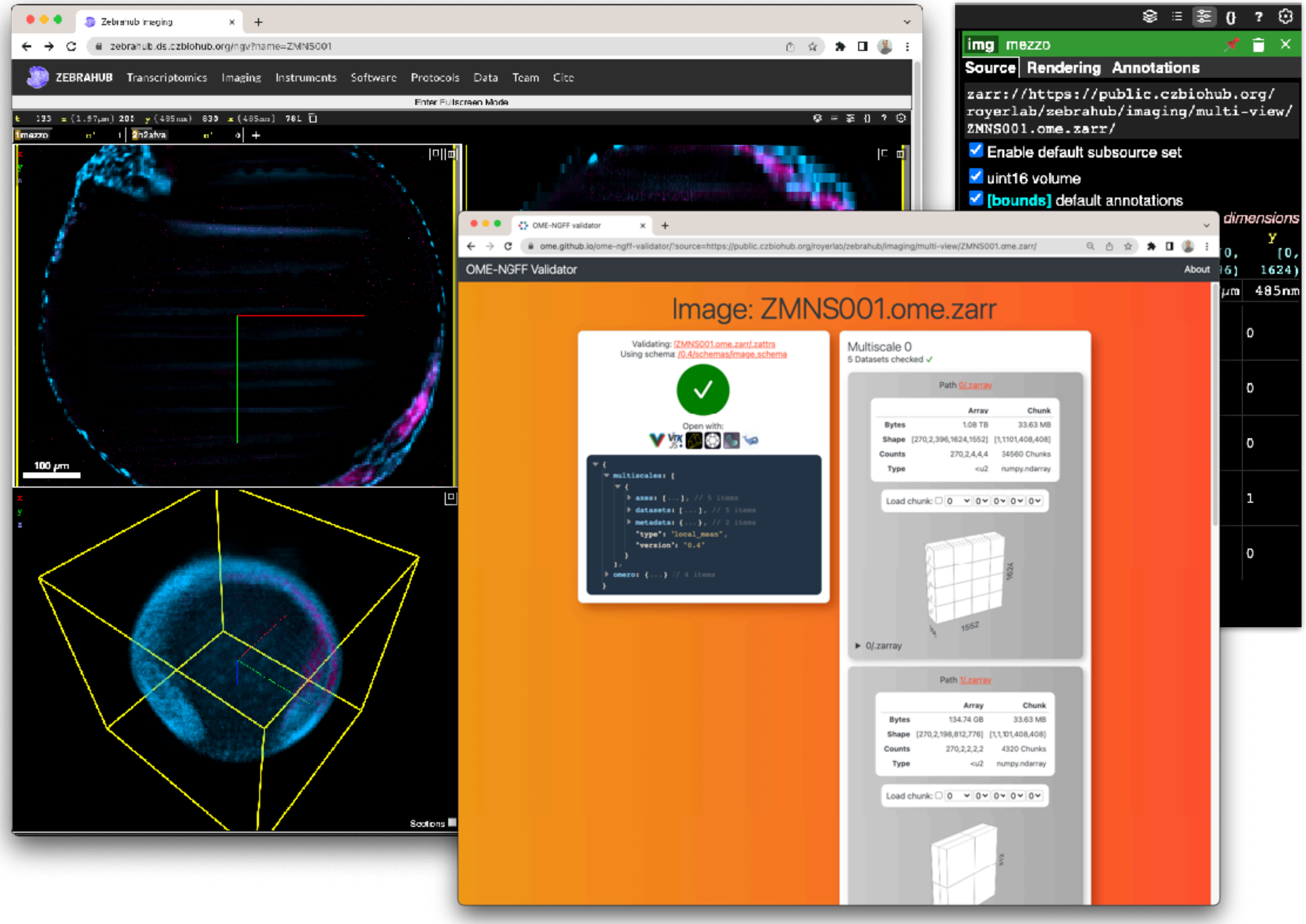
These two datasets were acquired on our OpenSiMView light-sheet microscope. You can explore the full timelapse image data here:

Timelapse 1
ZMNS001
explore

Timelapse 2
ZMNS002
explore

DAXI DATASETS

https://zebrahub.ds.czbiohub.org/



Zebrahub imaging
zebrahub.ds.czbiohub.org/ngv?name=ZMNS001

img mezzo

Source Rendering Annotations

`zarr://https://public.czbiohub.org/royerlab/zebrahub/imaging/multi-view/ZMNS001.ome.zarr/`

☑ Enable default subsource set
☑ uint16 volume
☑ [bounds] default annotations

OME-NGFF validator
ome.github.io/ome-ngff-validator/?source=https://public.czbiohub.org/royerlab/zebrahub/imaging/multi-view/ZMNS001.ome.zarr/

OME-NGFF Validator

About

Image: ZMNS001.ome.zarr

Validating: /ZMNS001.ome.zarr/.zattrs
Using schema: /0.4/schemas/image.schema

Open with:

```
{
  multiscales: [
    {
      axes: [...], // 5 items
      datasets: [...], // 5 items
      metadata: {...}, // 2 items
      "type": "local_mean",
      "version": "0.4"
    }
  ],
  omero: {...} // 4 items
}
```

Multiscale 0
5 Datasets checked ✓

Path 0/.zarray

	Array	Chunk
Bytes	1.08 TB	33.63 MB
Shape	[270,2,396,1624,1552]	[1,1,101,408,408]
Counts	270,2,4,4,4	34560 Chunks
Type	<u2	numpy.ndarray

Load chunk: ☐ 0 0 0 0 0

▸ 0/.zarray

Path 1/.zarray

	Array	Chunk
Bytes	134.74 GB	33.63 MB
Shape	[270,2,198,812,776]	[1,1,101,408,408]
Counts	270,2,2,2,2	4320 Chunks
Type	<u2	numpy.ndarray

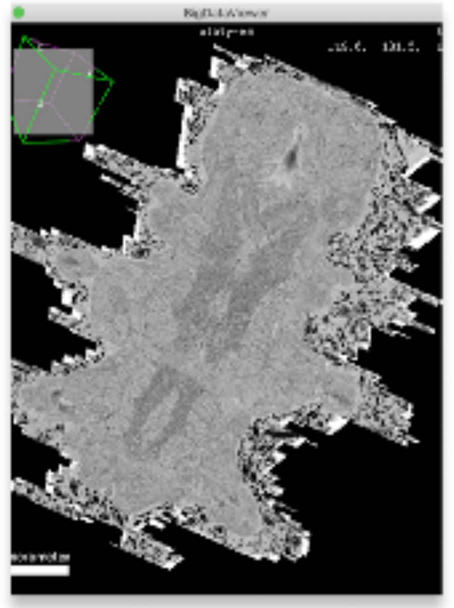
Load chunk: ☐ 0 0 0 0 0

https://ome.github.io/ome-ngff-validator/

Tools

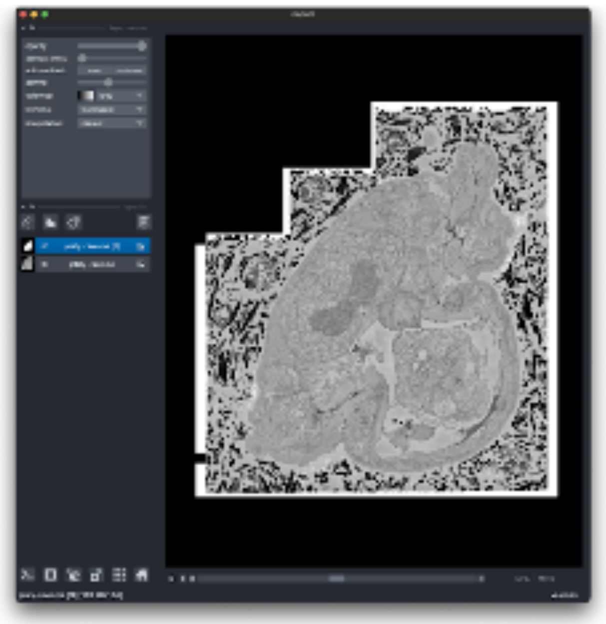
NFDI4BIOIMAGE

MoBIE



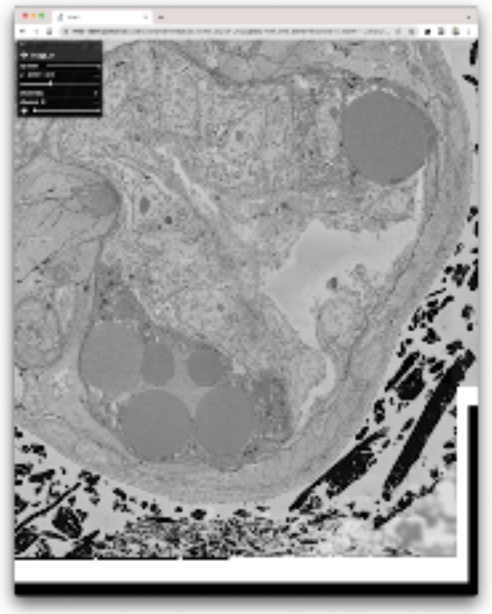
EMBL

napari-ome-zarr



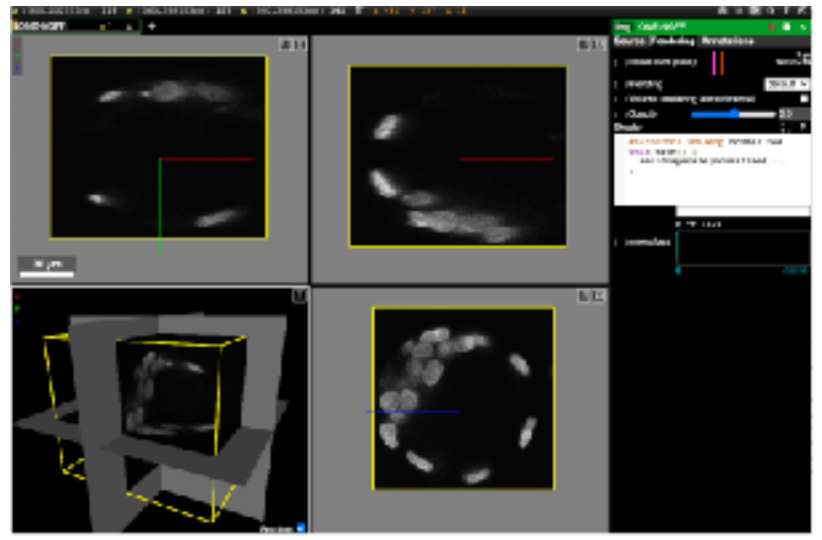
University of Dundee

Vizarr



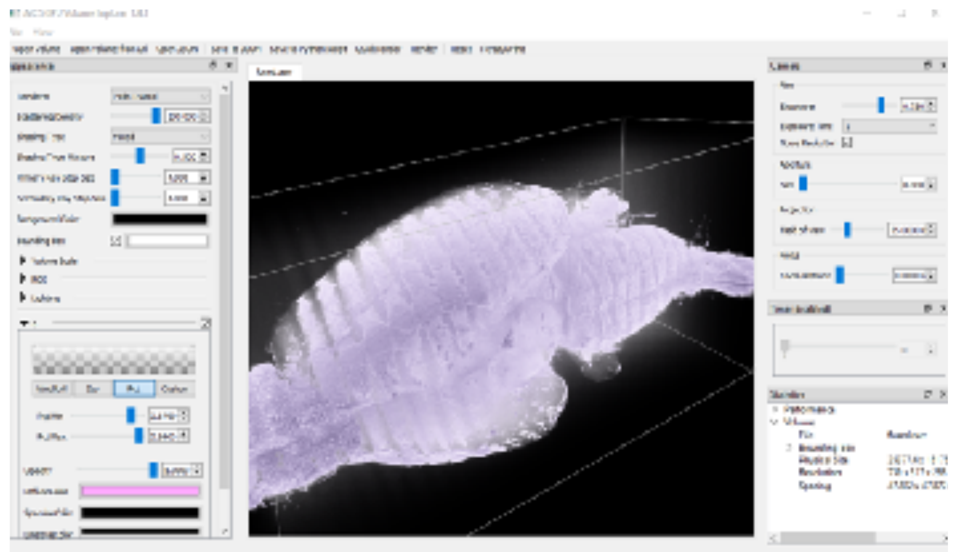
HARVARD MEDICAL SCHOOL

Neuroglancer



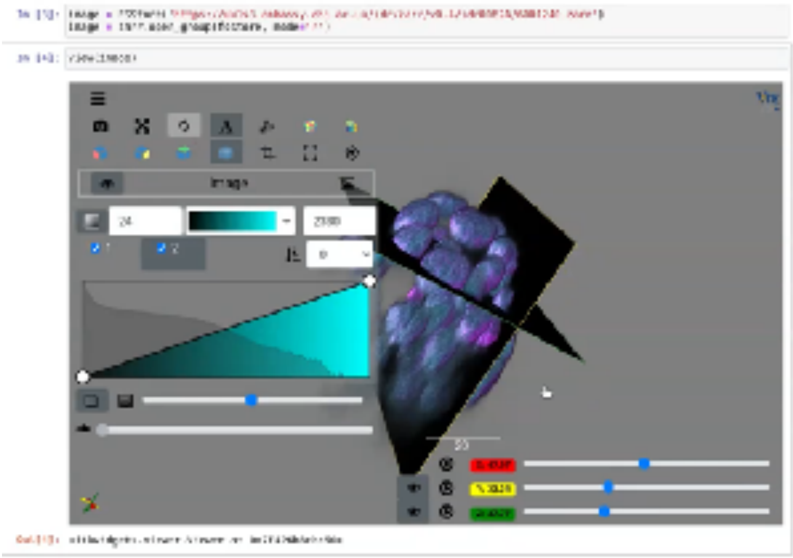
Google Research

AGAVE



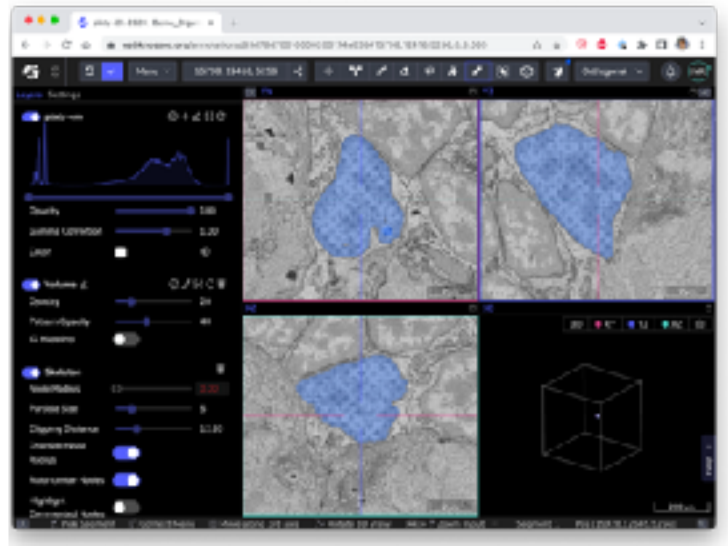
ALLEN INSTITUTE

ITKWidgets



kitware

webKnossos



scalableminds

…and the related libraries

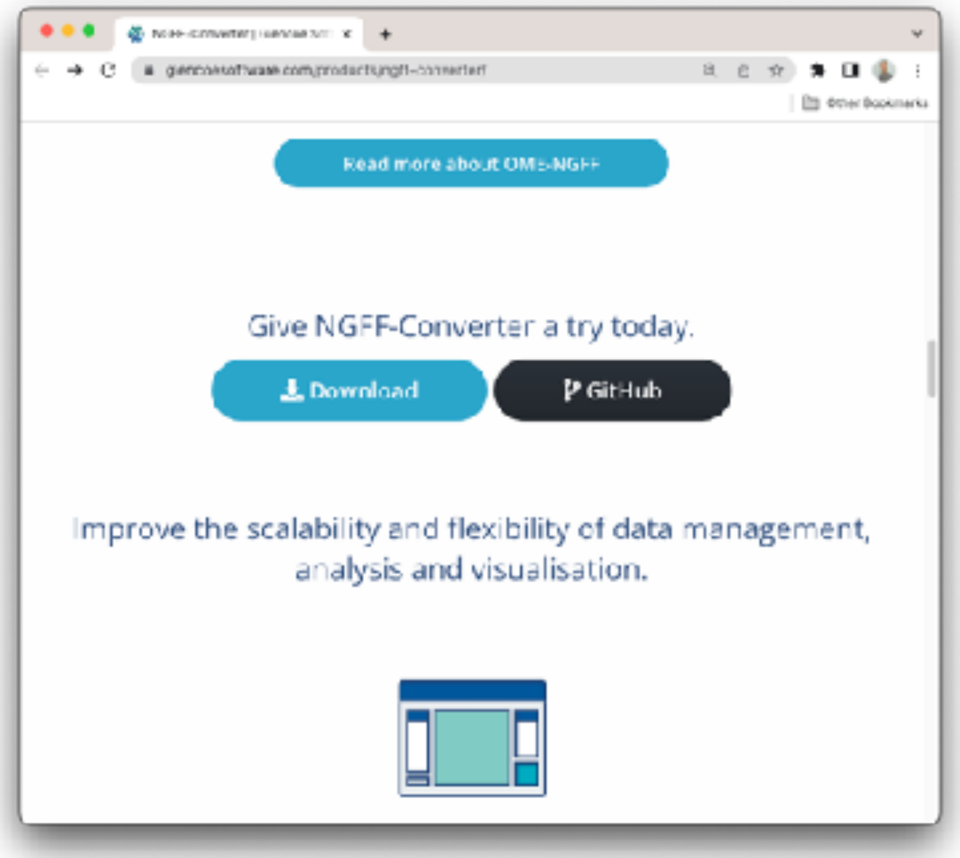
https://ngff.openmicroscopy.org/tools

NGFF Quickstart

An easy to use and open source converter for bioimaging data.

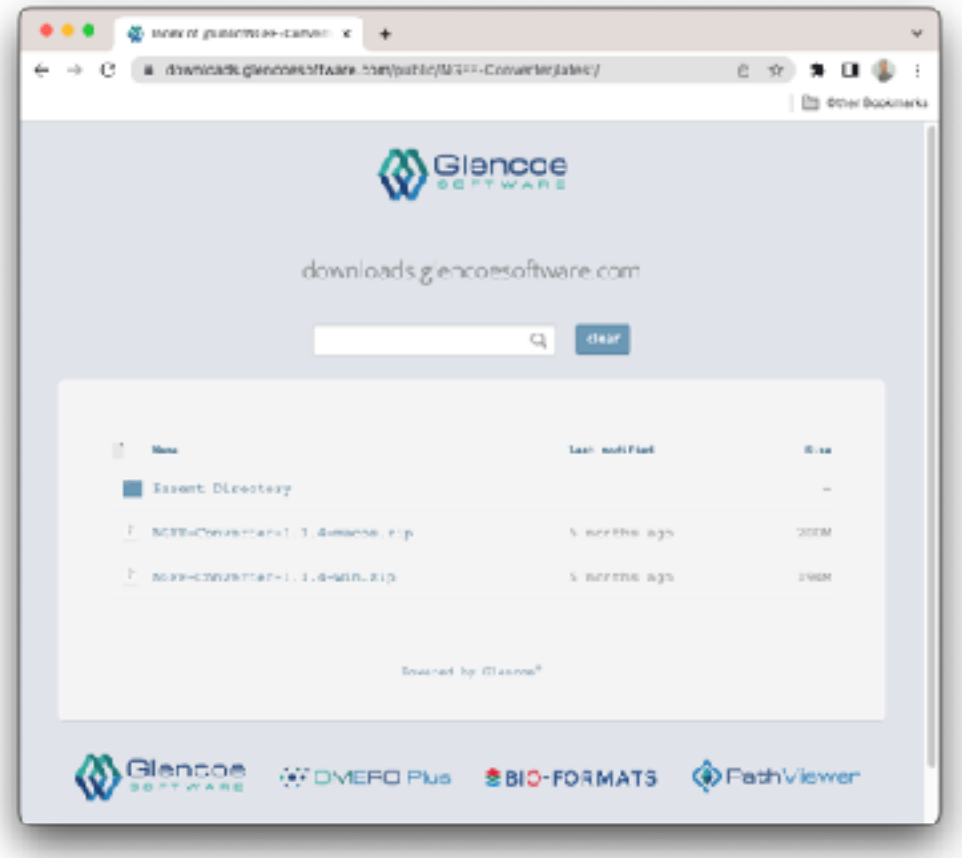
Read the latest press release on NGFF-Converter HERE.

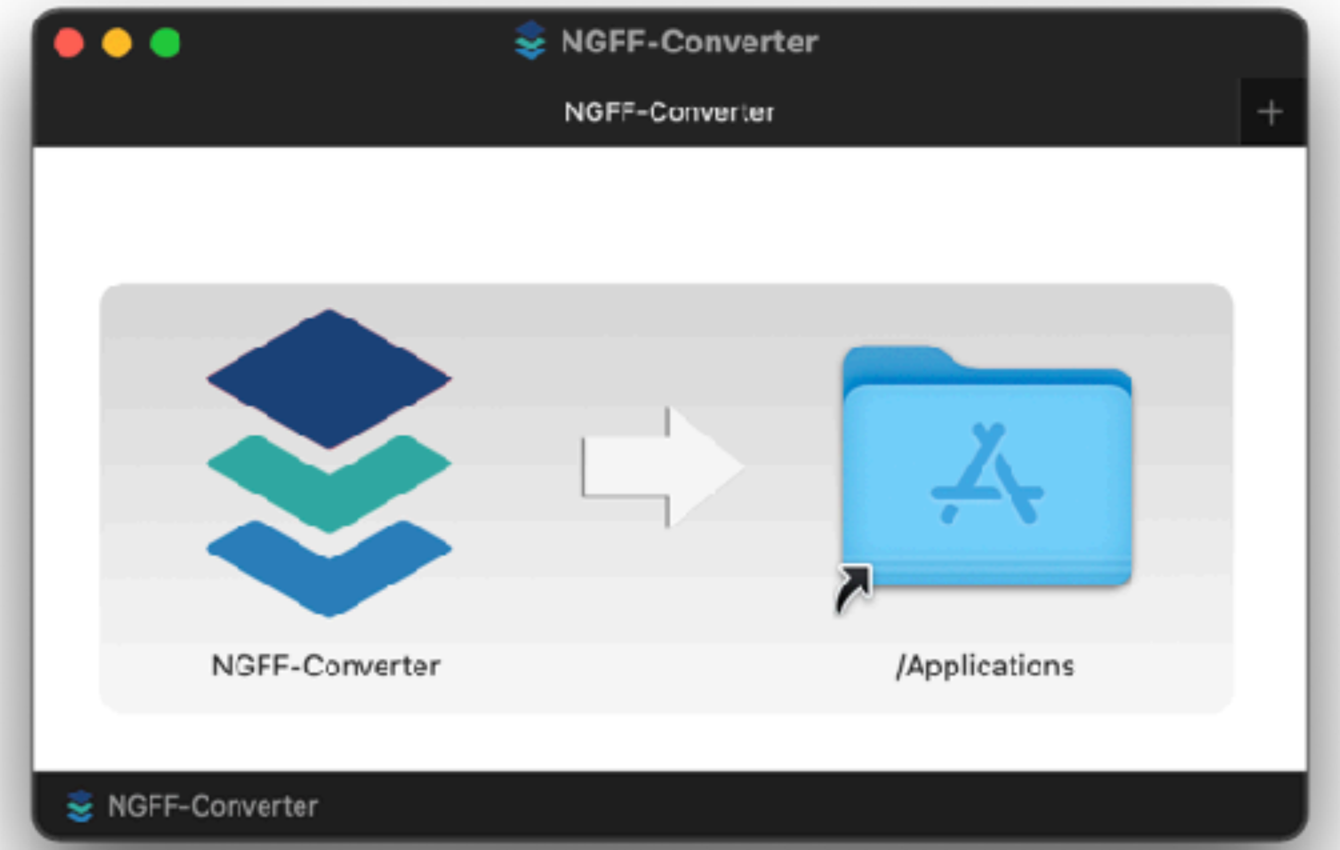
Learn more about using NGFF-Converter for streamlining and scaling your data workflows.



Give NGFF-Converter a try today.

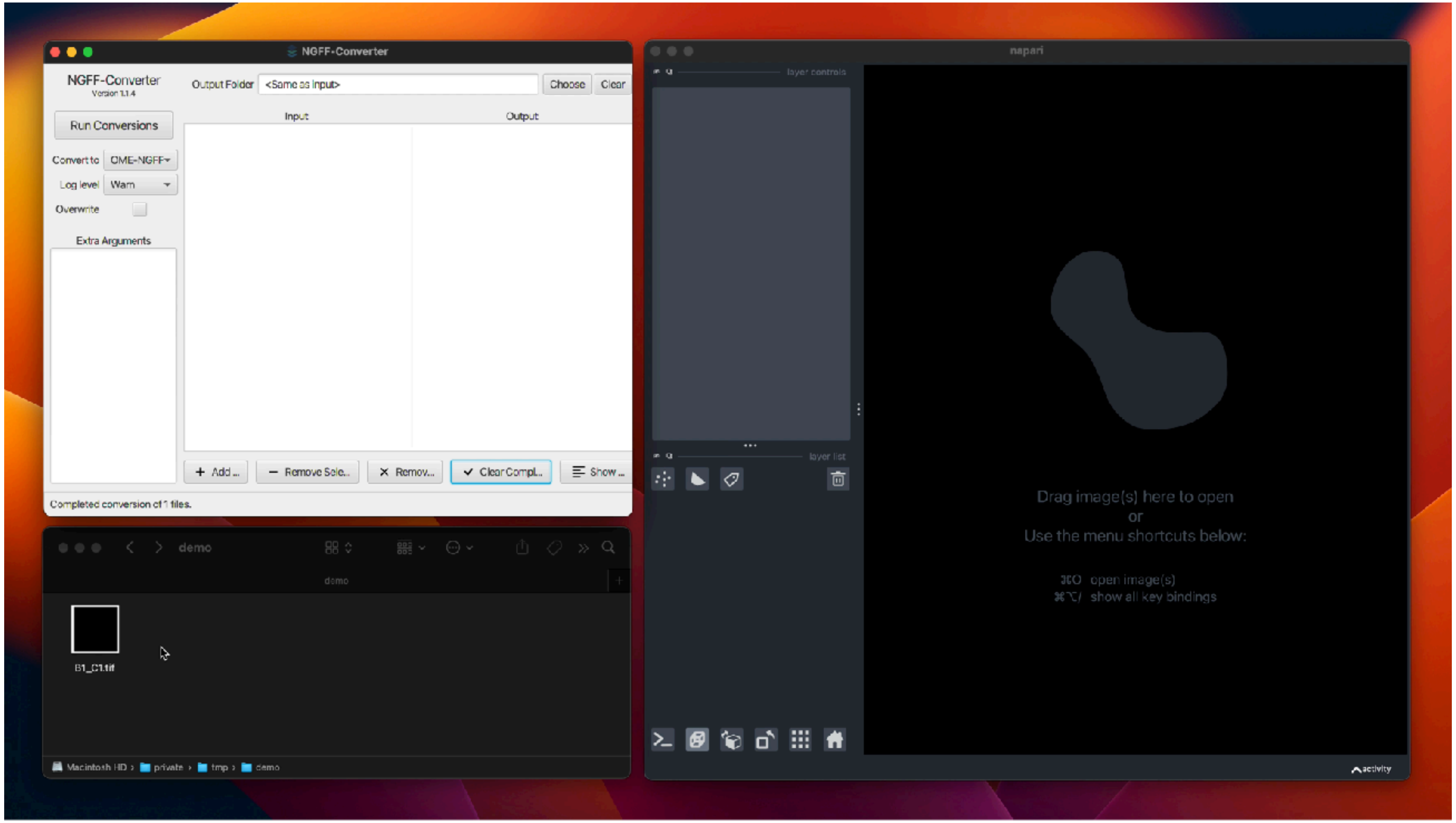
Improve the scalability and flexibility of data management, analysis and visualisation.





https://www.glencoesoftware.com/products/ngff-converter/

NGFF Quickstart



Output Folder: <Same as Input>

Convert to: OME-NGFF

Log level: Warn

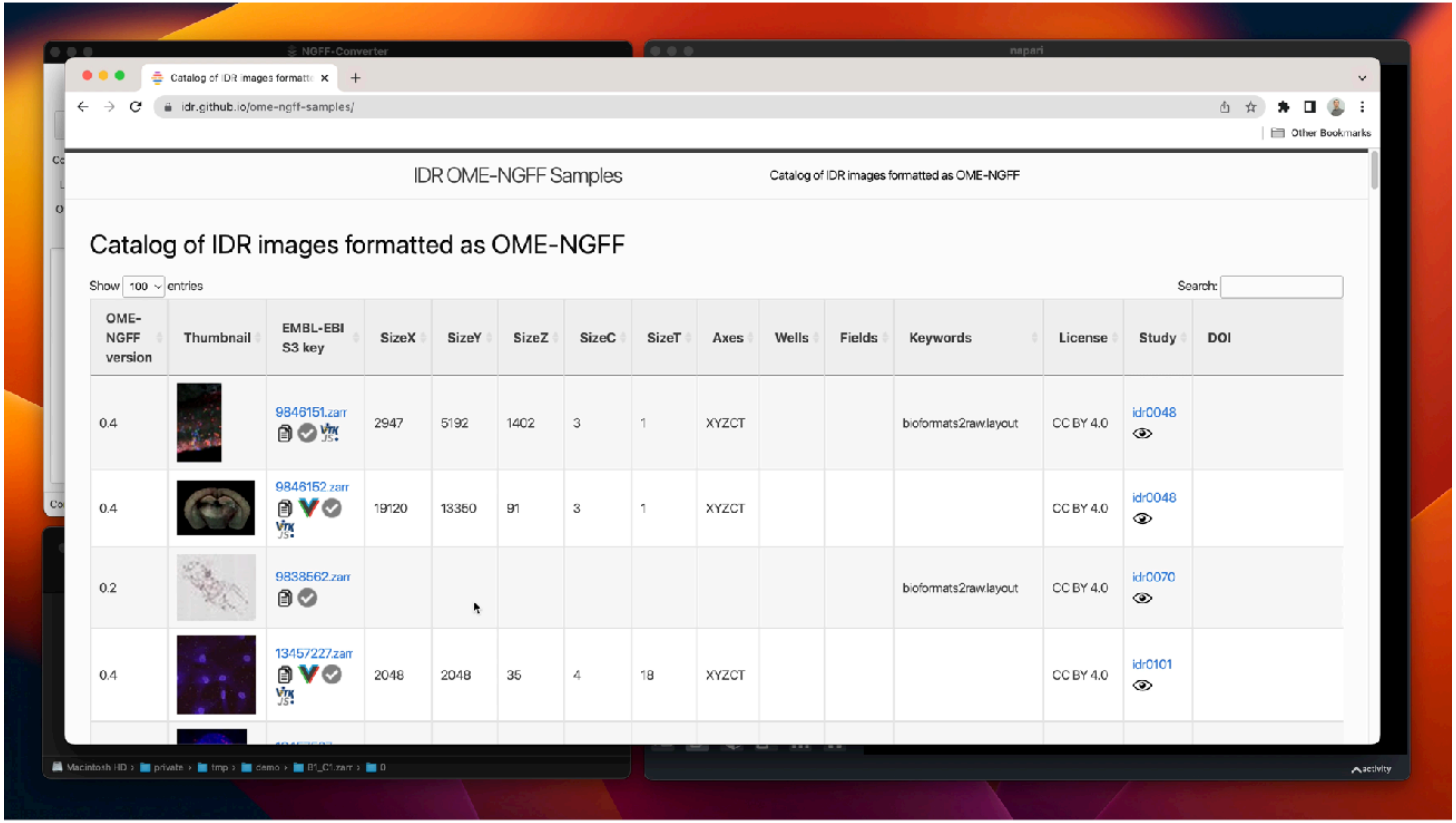
Overwrite ☐

Completed conversion of 1 files.

Drag image(s) here to open
or
Use the menu shortcuts below:

⌘O open image(s)
⌘⌥/ show all key bindings

NGFF Quickstart



IDR OME-NGFF Samples

Catalog of IDR images formatted as OME-NGFF

Catalog of IDR images formatted as OME-NGFF

Show 100 entries

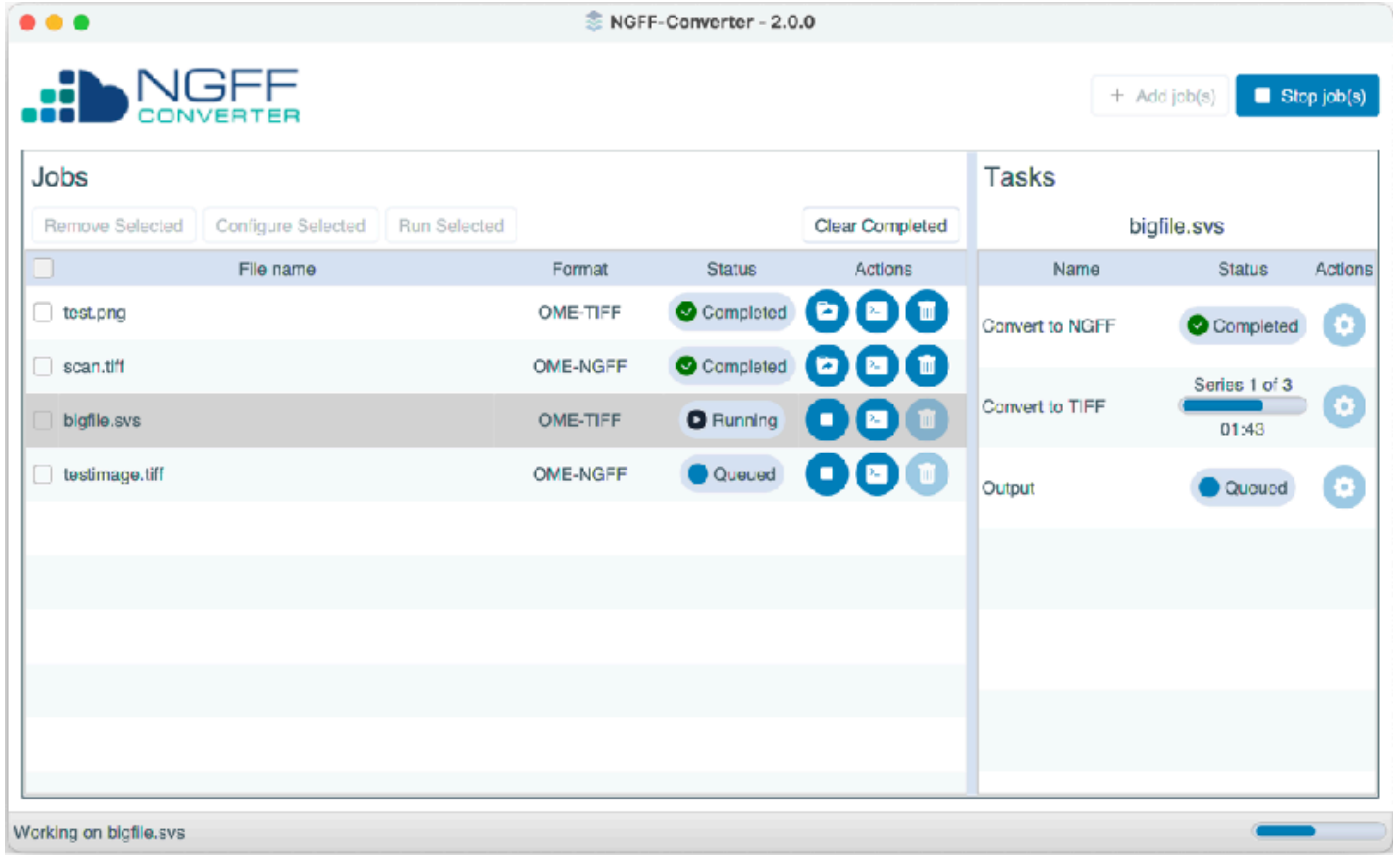
Search:

OME-NGFF version	Thumbnail	EMBL-EBI S3 key	SizeX	SizeY	SizeZ	SizeC	SizeT	Axes	Wells	Fields	Keywords	License	Study	DOI
0.4		9846151.zarr	2947	5192	1402	3	1	XYZCT			bioformats2raw.layout	CC BY 4.0	idr0048	
0.4		9846152.zarr	19120	13350	91	3	1	XYZCT				CC BY 4.0	idr0048	
0.2		9838562.zarr									bioformats2raw.layout	CC BY 4.0	idr0070	
0.4		13457227.zarr	2048	2048	35	4	18	XYZCT				CC BY 4.0	idr0101	

NGFF-Converter

Latest release: 2.0

- User interface redesigned and modernised.
- Conversion "Jobs" are now broken down and displayed to the user as a sequence of configurable "Tasks".
- Progress bars are now shown to the user during conversion.
- Conversion jobs can now be started/stopped individually.
- Settings panels are available for each underlying task, instead of requiring the user to supply command line arguments.
- It is now possible to import/export conversion settings and set user defaults.
- Job settings can now be configured in bulk.



☐	File name	Format	Status	Actions
☐	test.png	OME-TIFF	Completed	
☐	scan.tiff	OME-NGFF	Completed	
☐	bigfile.svs	OME-TIFF	Running	
☐	testimage.tiff	OME-NGFF	Queued	

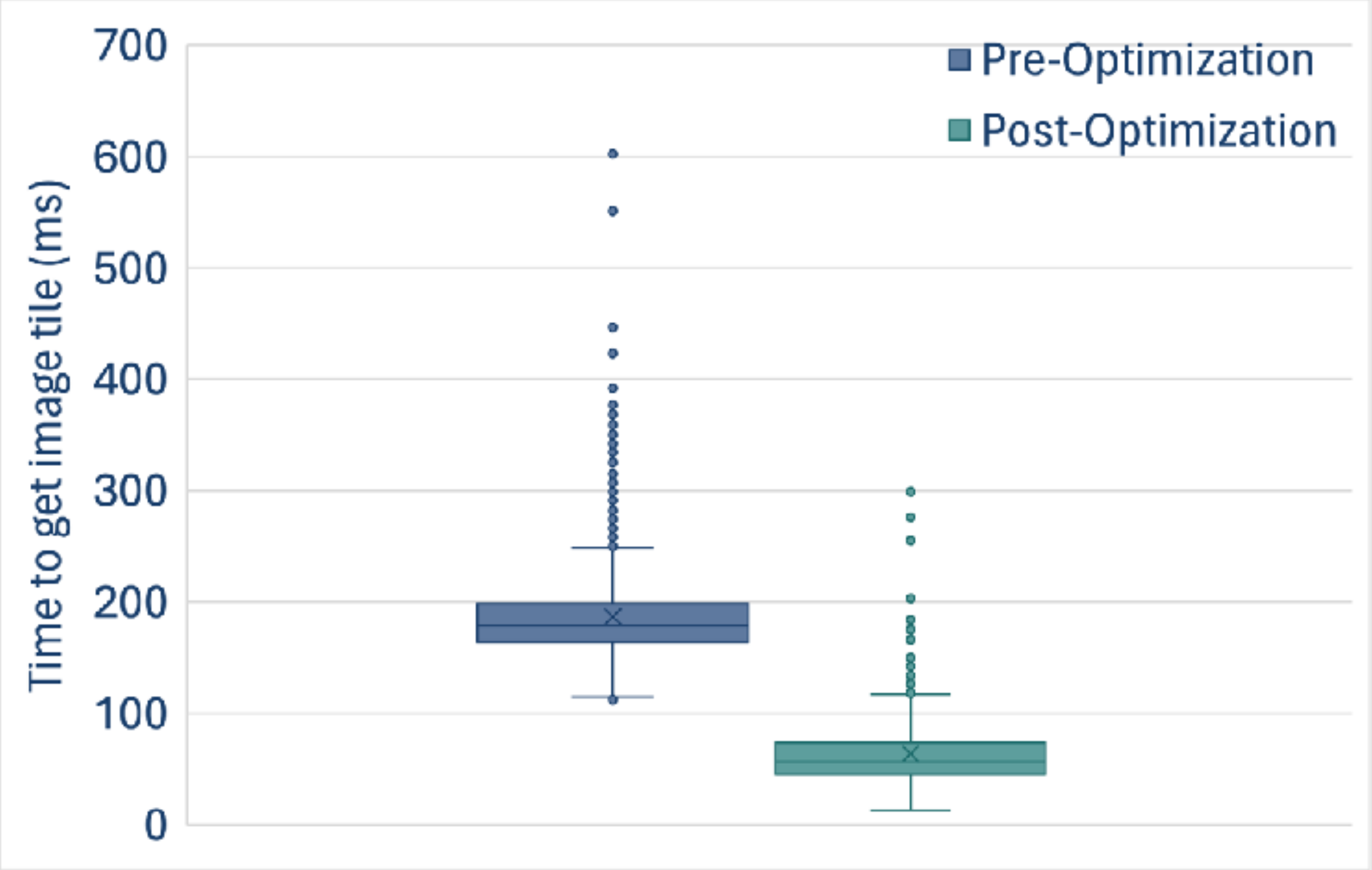
Name	Status	Actions
Convert to NGFF	Completed	
Convert to TIFF	Series 1 of 3 01:43	
Output	Queued	

Working on bigfile.svs

Glencoe
SOFTWARE

OME-Zarr Read Performance

- OMERO Plus implementation of OME-Zarr reading and rendering (omero-zarr-pixel-buffer) is now public.
- Optimization of third party libraries like s3fs to remove unnecessary overhead in the number of requests to S3
- Initial implementation of parallel channel requests completed for RGB data
- Future work will propose upstream changes in open source OMERO to allow for generalized parallel channel requests/reads



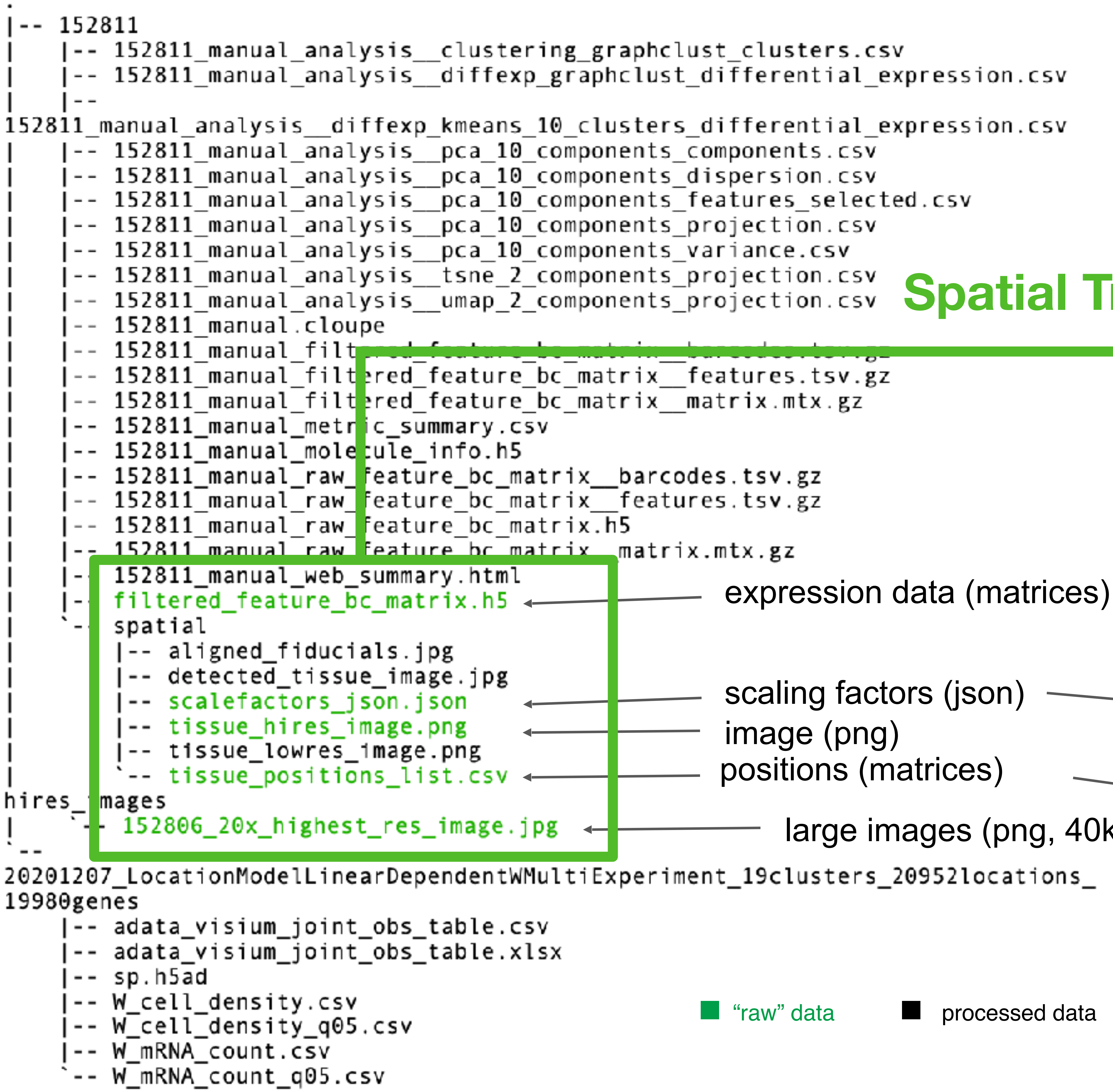
Communities

Spatial omics

Volume EM

Hyperspectral

Communities | **SpatialData**



```
.
|-- 152811
|    |-- 152811_manual_analysis__clustering_graphclust_clusters.csv
|    |-- 152811_manual_analysis__diffexp_graphclust_differential_expression.csv
|    |--
152811_manual_analysis__diffexp_kmeans_10_clusters_differential_expression.csv
|    |-- 152811_manual_analysis__pca_10_components_components.csv
|    |-- 152811_manual_analysis__pca_10_components_dispersion.csv
|    |-- 152811_manual_analysis__pca_10_components_features_selected.csv
|    |-- 152811_manual_analysis__pca_10_components_projection.csv
|    |-- 152811_manual_analysis__pca_10_components_variance.csv
|    |-- 152811_manual_analysis__tsne_2_components_projection.csv
|    |-- 152811_manual_analysis__umap_2_components_projection.csv
|    |-- 152811_manual.cloupe
|    |-- 152811_manual_filtered_feature_bc_matrix__barcodes.tsv.gz
|    |-- 152811_manual_filtered_feature_bc_matrix__features.tsv.gz
|    |-- 152811_manual_filtered_feature_bc_matrix__matrix.mtx.gz
|    |-- 152811_manual_metric_summary.csv
|    |-- 152811_manual_molecule_info.h5
|    |-- 152811_manual_raw_feature_bc_matrix__barcodes.tsv.gz
|    |-- 152811_manual_raw_feature_bc_matrix__features.tsv.gz
|    |-- 152811_manual_raw_feature_bc_matrix.h5
|    |-- 152811_manual_raw_feature_bc_matrix__matrix.mtx.gz
|    |-- 152811_manual_web_summary.html
|    |-- filtered_feature_bc_matrix.h5
|    `-- spatial
|         |-- aligned_fiducials.jpg
|         |-- detected_tissue_image.jpg
|         |-- scalefactors_json.json
|         |-- tissue_hires_image.png
|         |-- tissue_lowres_image.png
|         `-- tissue_positions_list.csv
hires_images
|    `-- 152806_20x_highest_res_image.jpg
`--
20201207_LocationModelLinearDependentWMultiExperiment_19clusters_20952locations_
19980genes
     |-- adata_visium_joint_obs_table.csv
     |-- adata_visium_joint_obs_table.xlsx
     |-- sp.h5ad
     |-- W_cell_density.csv
     |-- W_cell_density_q05.csv
     |-- W_mRNA_count.csv
     `-- W_mRNA_count_q05.csv
```

Spatial Transcriptomics

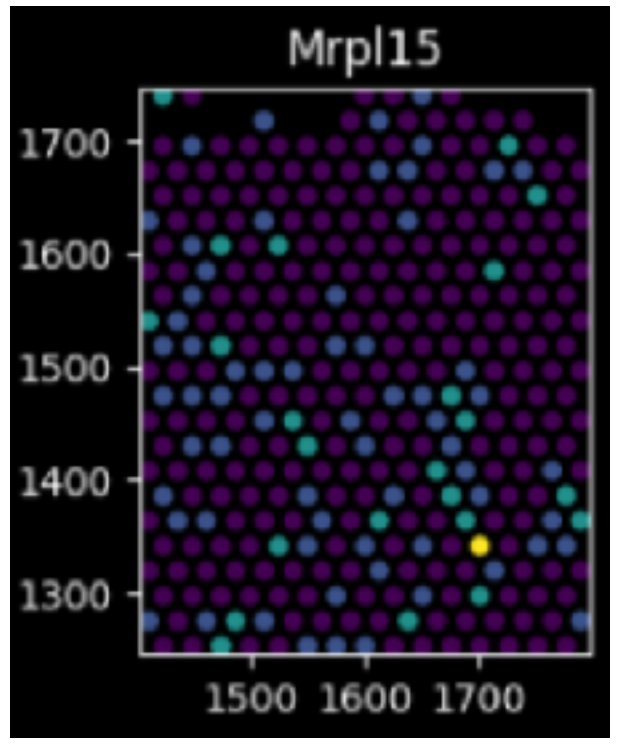
expression data (matrices)

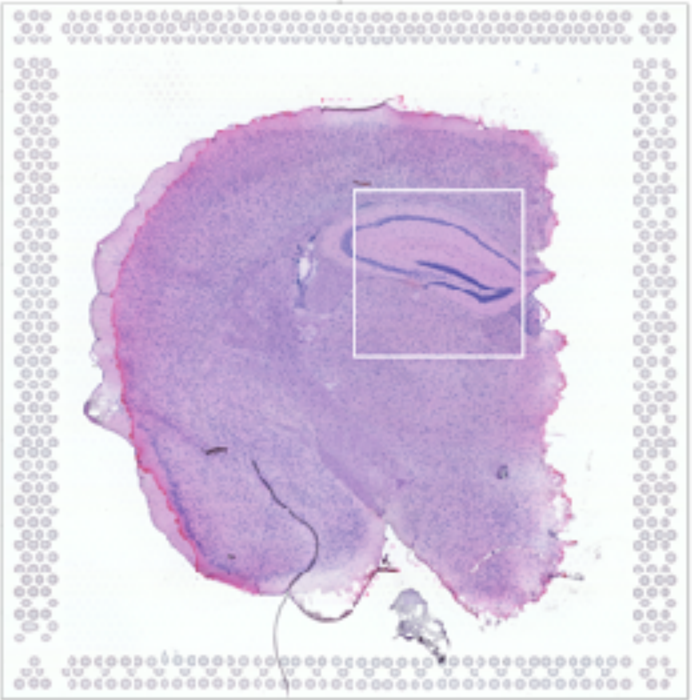
scaling factors (json)

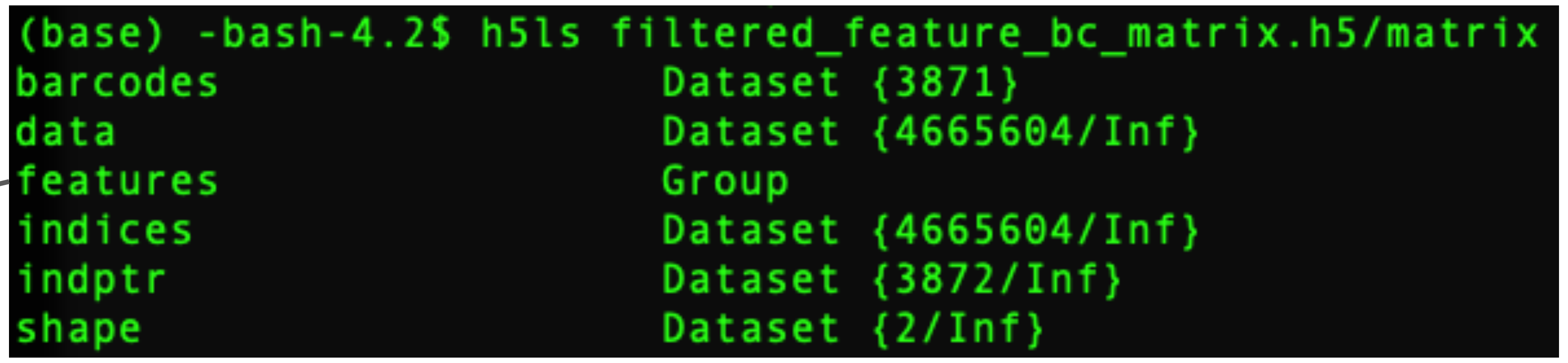
image (png)

positions (matrices)

large images (png, 40k x 40k pixels)







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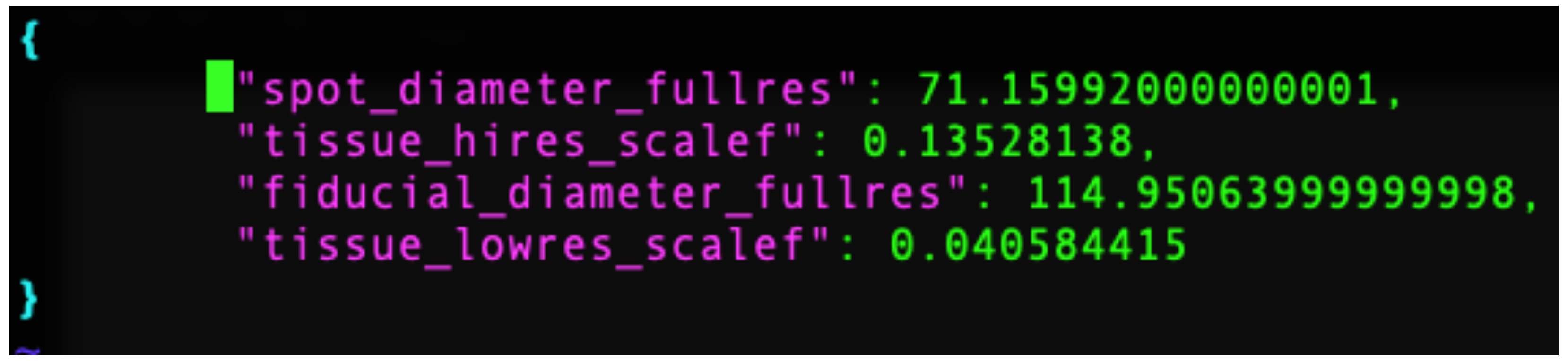
(base) -bash-4.2$ h5ls filtered_feature_bc_matrix.h5/matrix
barcodes                 Dataset {3871}
data                     Dataset {4665604/Inf}
features                 Group
indices                  Dataset {4665604/Inf}
indptr                   Dataset {3872/Inf}
shape                    Dataset {2/Inf}
```



```
{
       "spot_diameter_fullres": 71.15992000000001,
       "tissue_hires_scalef": 0.13528138,
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       "tissue_lowres_scalef": 0.040584415
}
```

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ACGCCTGACACGCGCT-1,0,0,0,4044,1229
TACCGATCCAACACTT-1,0,1,1,4139,1283
ATTAAAGCGGACGAGC-1,0,0,2,4044,1338
GATAAGGGACGATTAG-1,0,1,3,4139,1393
GTGCAAATCACCAATA-1,0,0,4,4044,1448
TGTTGGCTGGCGGAAG-1,0,1,5,4140,1502
GCATCCTCTCCTATTA-1,0,0,6,4045,1557
GCGAGGGACTGCTAGA-1,0,1,7,4140,1612
TGGTACCGGCACAGCC-1,0,0,8,4045,1667
GCGCGTTTAAATCGTA-1,0,1,9,4140,1721
```
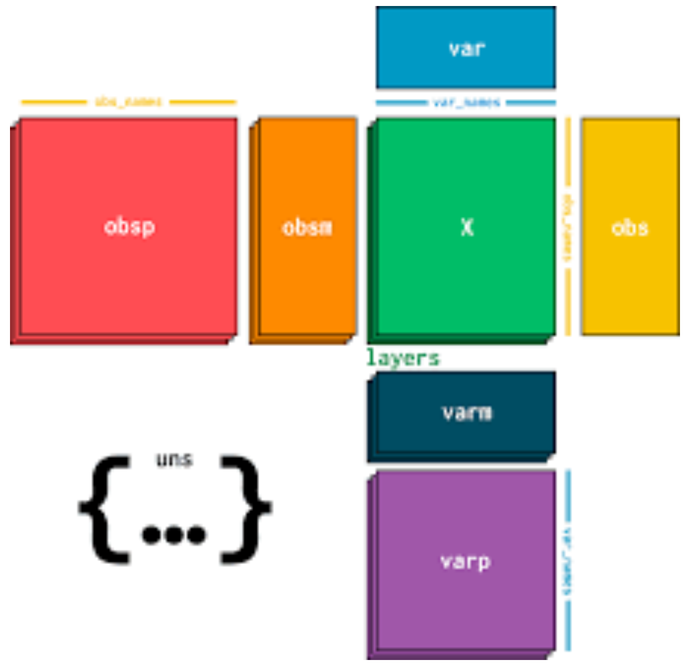

🟩 "raw" data ■ processed data

Slide courtesy: Luca Marconato (EMBL)

Communities | **SpatialData**

AnnData



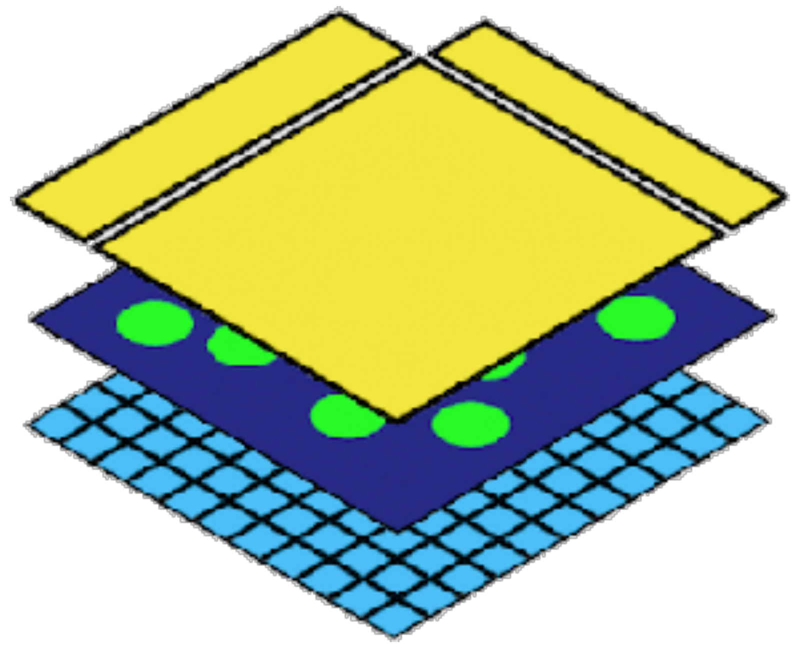
scverse

Single cell and spatial omics data analysis

squidpy

scanpy

SpatialData

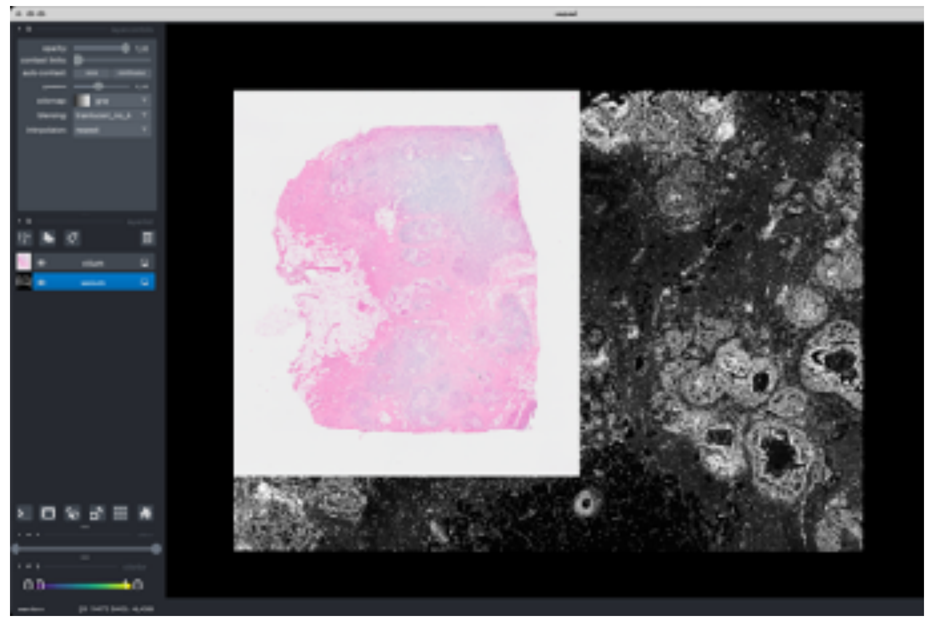


OME

Zarr

Very large images, standard cloud-native formats

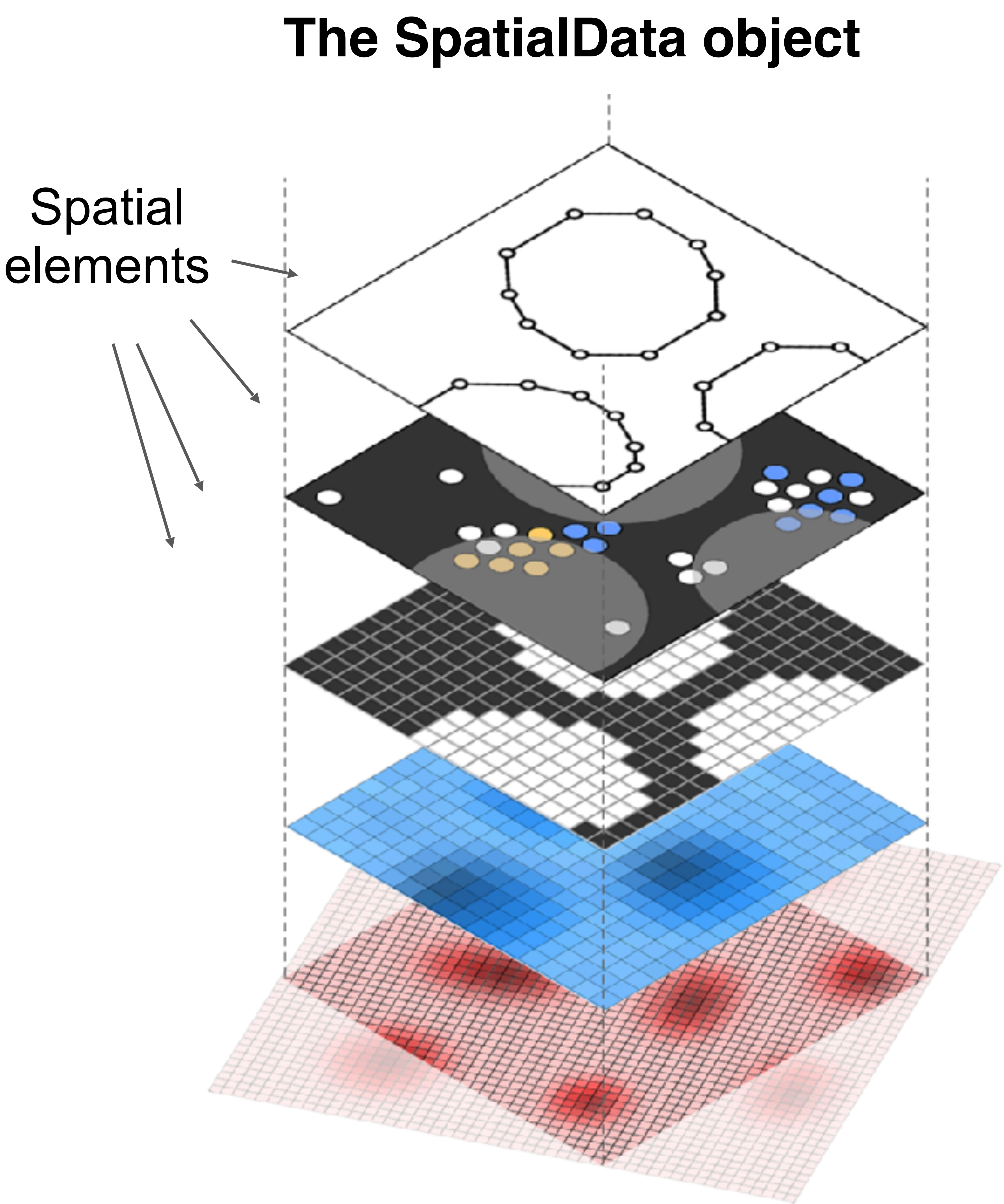
napari

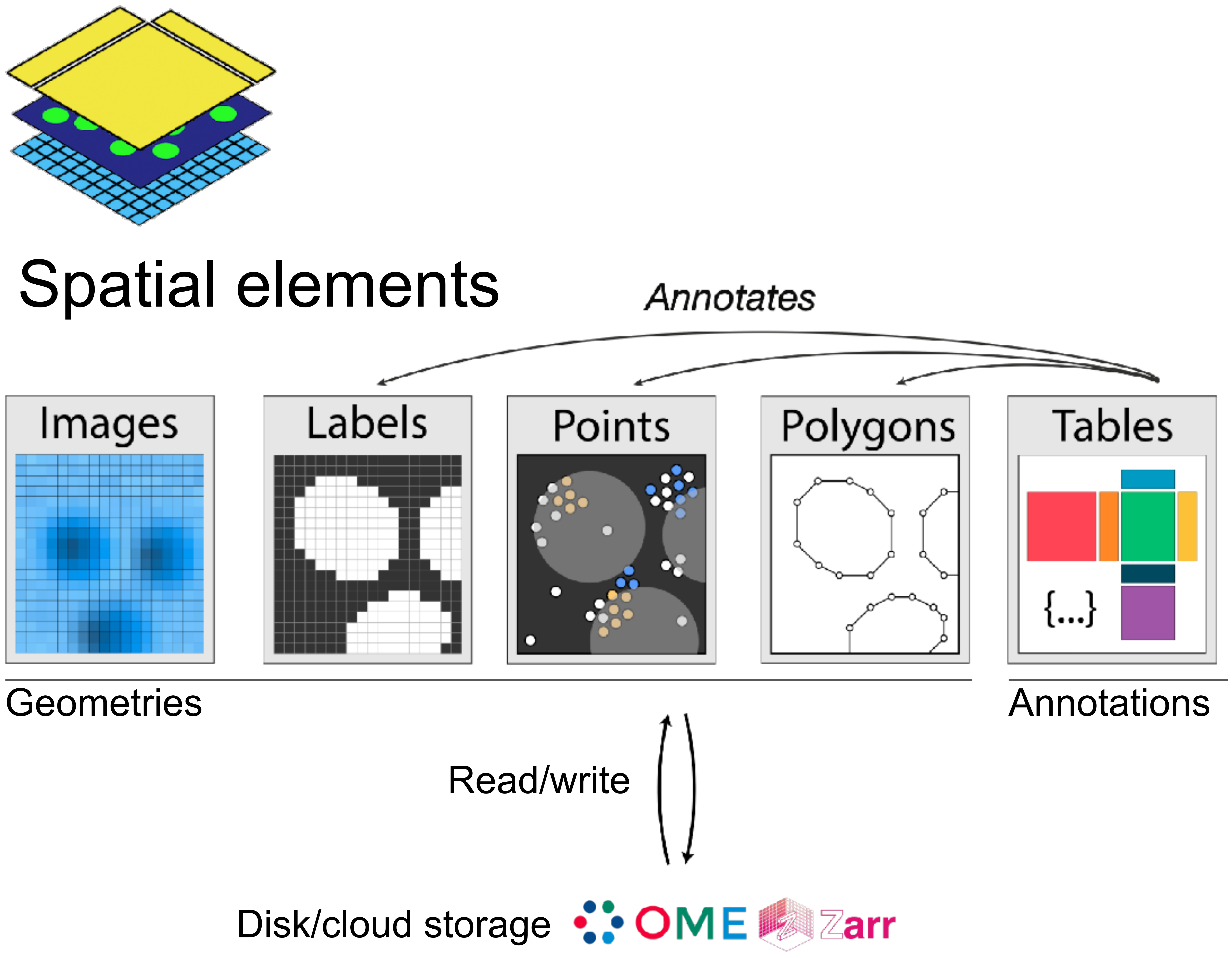


Interactive visualization and annotation

Slide courtesy: Luca Marconato (EMBL)

Communities | **SpatialData**

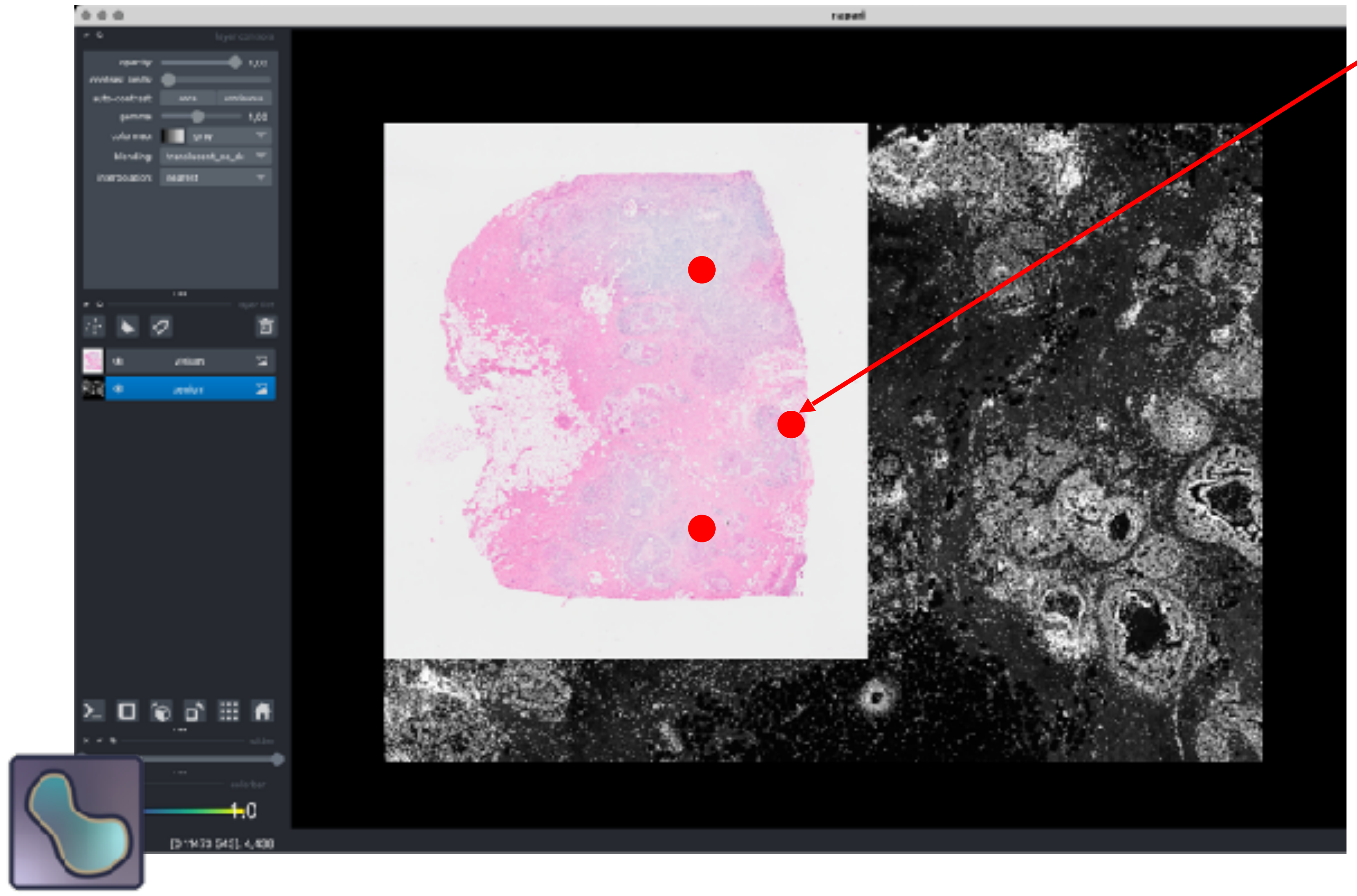




Slide courtesy: Luca Marconato (EMBL)

Communities | **SpatialData**

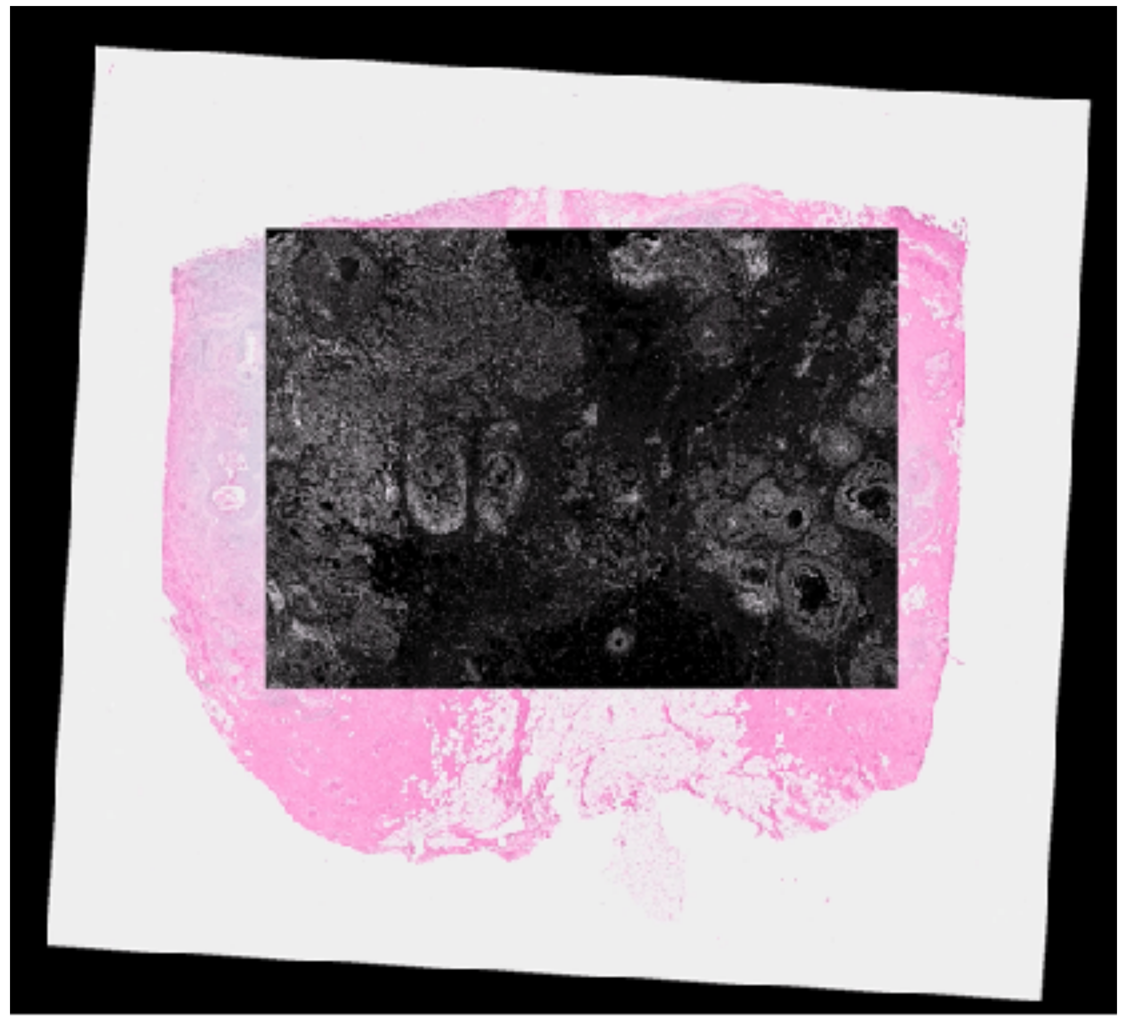
Before alignment (raw data)



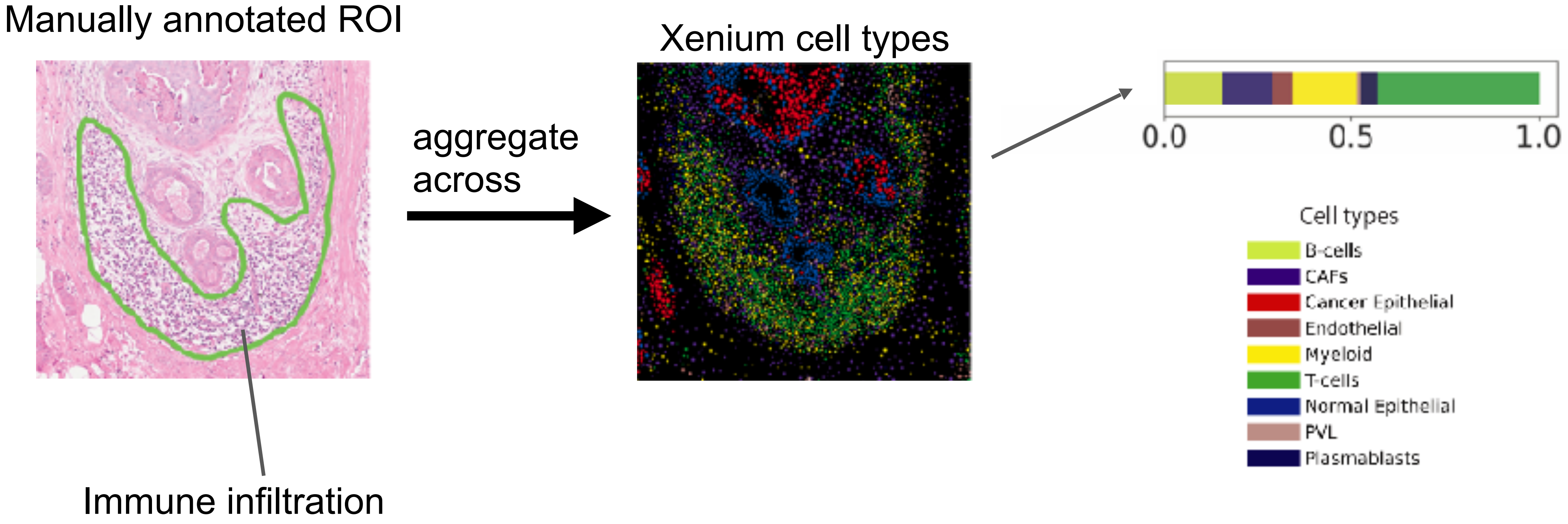
Landmark
points
annotations

After alignment

(Common Coordinate System)



Interactive annotation



Manually annotated ROI

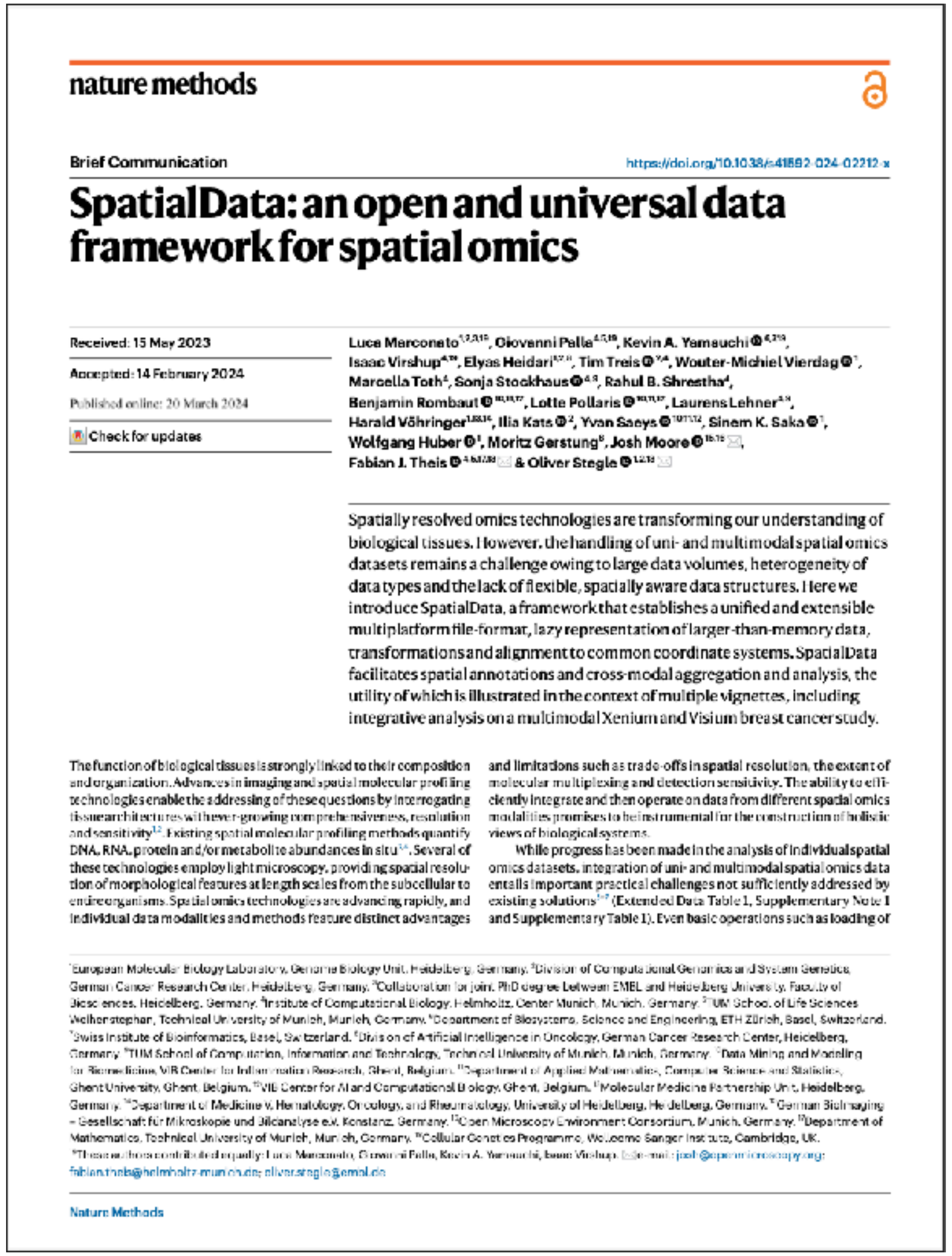
aggregate across

Xenium cell types

Immune infiltration

Slide courtesy: Luca Marconato (EMBL)

Communities | **SpatialData**



nature methods

Brief Communication https://doi.org/10.1038/s41592-024-02212-x

SpatialData: an open and universal data framework for spatial omics

Received: 15 May 2023

Accepted: 14 February 2024

Published online: 20 March 2024

Check for updates

Luca Marconato[1,2,3,19], Giovanni Palla[4,5,19], Kevin A. Yamauchi[6,7,19], Isaac Virshup[4,19], Elyas Heidari[1,7,8], Tim Treis[7,4], Wouter-Michiel Vierdag[1], Marcella Toth[4], Sonja Stockhaus[4,8], Rahul B. Shrestha[4], Benjamin Rombaut[10,11,12], Lotte Pollaris[10,11,12], Laurens Lehner[4,5], Harald Vöhringer[1,13,14], Ilia Kats[2], Yvan Saeys[10,11,12], Sinem K. Saka[1], Wolfgang Huber[1], Moritz Gerstung[8], Josh Moore[15,16] ✉, Fabian J. Theis[4,5,9,17,18] ✉ & Oliver Stegle[1,2,18] ✉

Spatially resolved omics technologies are transforming our understanding of biological tissues. However, the handling of uni- and multimodal spatial omics datasets remains a challenge owing to large data volumes, heterogeneity of data types and the lack of flexible, spatially aware data structures. Here we introduce SpatialData, a framework that establishes a unified and extensible multiplatform file-format, lazy representation of larger-than-memory data, transformations and alignment to common coordinate systems. SpatialData facilitates spatial annotations and cross-modal aggregation and analysis, the utility of which is illustrated in the context of multiple vignettes, including integrative analysis on a multimodal Xenium and Visium breast cancer study.

The function of biological tissues is strongly linked to their composition and organization. Advances in imaging and spatial molecular profiling technologies enable the addressing of these questions by interrogating tissue architectures with ever-growing comprehensiveness, resolution and sensitivity[1,2]. Existing spatial molecular profiling methods quantify DNA, RNA, protein and/or metabolite abundances in situ[3,4]. Several of these technologies employ light microscopy, providing spatial resolution of morphological features at length scales from the subcellular to entire organisms. Spatial omics technologies are advancing rapidly, and individual data modalities and methods feature distinct advantages and limitations such as trade-offs in spatial resolution, the extent of molecular multiplexing and detection sensitivity. The ability to efficiently integrate and then operate on data from different spatial omics modalities promises to be instrumental for the construction of holistic views of biological systems.

While progress has been made in the analysis of individual spatial omics datasets, integration of uni- and multimodal spatial omics data entails important practical challenges not sufficiently addressed by existing solutions[5–7] (Extended Data Table 1, Supplementary Note 1 and Supplementary Table 1). Even basic operations such as loading of

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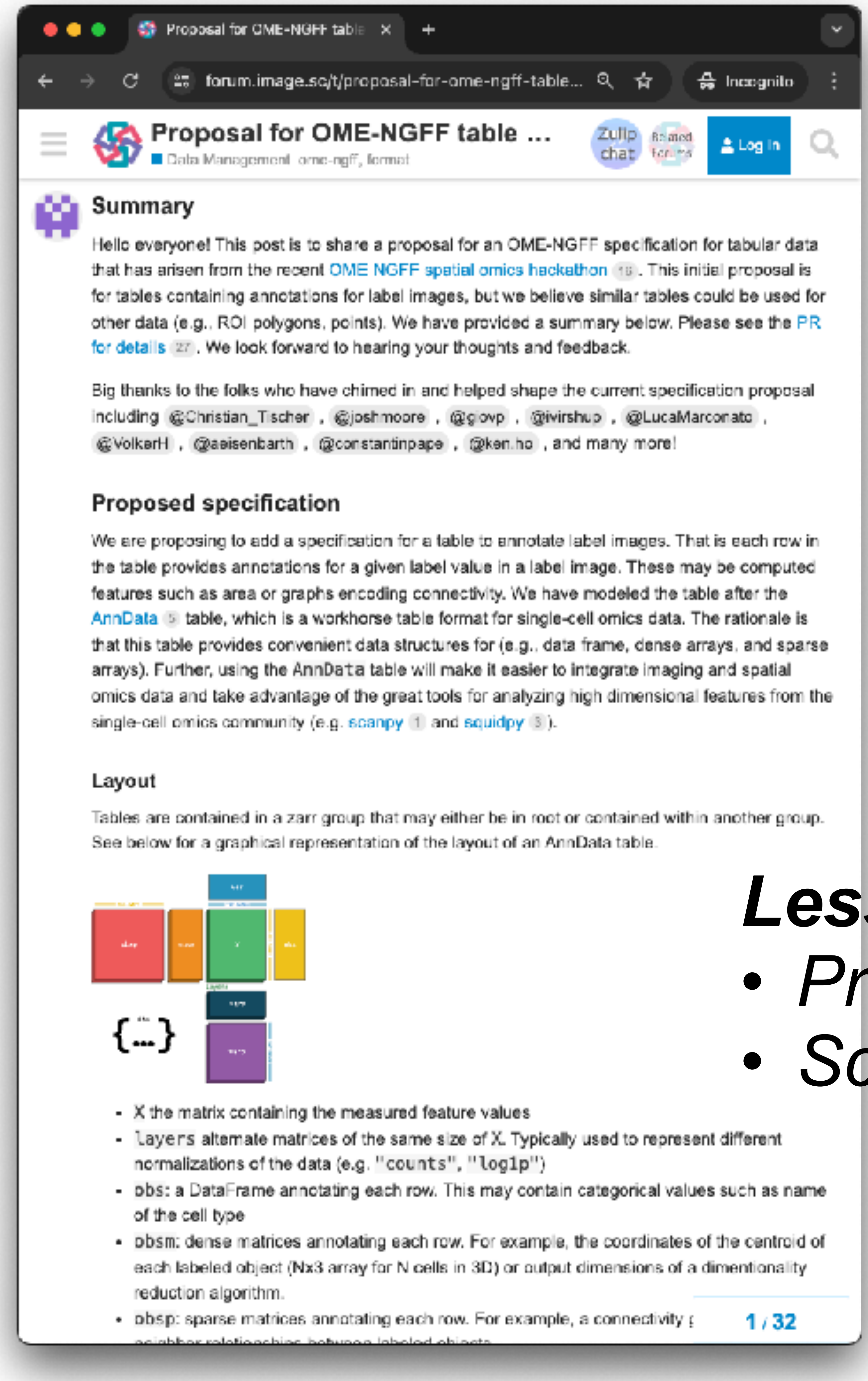
Nature Methods



Proposal for OME-NGFF table ...

Data Management ome-ngff, format

Summary

Hello everyone! This post is to share a proposal for an OME-NGFF specification for tabular data that has arisen from the recent OME NGFF spatial omics hackathon 18. This initial proposal is for tables containing annotations for label images, but we believe similar tables could be used for other data (e.g., ROI polygons, points). We have provided a summary below. Please see the PR for details 27. We look forward to hearing your thoughts and feedback.

Big thanks to the folks who have chimed in and helped shape the current specification proposal including @Christian_Tischer , @joshmoore , @giovp , @ivirshup , @LucaMarconato , @VolkerH , @aeisenbarth , @constantinpape , @ken.ho , and many more!

Proposed specification

We are proposing to add a specification for a table to annotate label images. That is each row in the table provides annotations for a given label value in a label image. These may be computed features such as area or graphs encoding connectivity. We have modeled the table after the AnnData 5 table, which is a workhorse table format for single-cell omics data. The rationale is that this table provides convenient data structures for (e.g., data frame, dense arrays, and sparse arrays). Further, using the `AnnData` table will make it easier to integrate imaging and spatial omics data and take advantage of the great tools for analyzing high dimensional features from the single-cell omics community (e.g. scanpy 1 and squidpy 3).

Layout

Tables are contained in a zarr group that may either be in root or contained within another group. See below for a graphical representation of the layout of an AnnData table.

- X the matrix containing the measured feature values
- `layers` alternate matrices of the same size of X. Typically used to represent different normalizations of the data (e.g. `"counts"`, `"log1p"`)
- `obs`: a DataFrame annotating each row. This may contain categorical values such as name of the cell type
- `obsm`: dense matrices annotating each row. For example, the coordinates of the centroid of each labeled object (Nx3 array for N cells in 3D) or output dimensions of a dimentionality reduction algorithm.
- `obsp`: sparse matrices annotating each row. For example, a connectivity

Lessons learned:

- *Process*
- *Scope*

https://doi.org/10.1038/s41592-024-02212-x

https://forum.image.sc/t/proposal-for-ome-ngff-table-specification/68908

Communities | **SpatialData & WebAtlas**

WebAtlas pipeline for integrated single cell and spatial transcriptomic data

Tong Li[1,*], David Horsfall[2,1,*], Daniela Basurto-Lozada[2,1,*], Kenny Roberts[1], Martin Prete[1],, John E G Lawrence[1], Peng He[1], Elisabeth Tuck[1], Josh Moore[3], Shila Ghazanfar[1,4], Sarah Teichmann[1], Muzlifah Haniffa[1,2,**], Omer Ali Bayraktar[1,**]

[1] *Wellcome Sanger Institute, Hinxton, UK*
[2] *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
[3] *German BioImaging – Gesellschaft für Mikroskopie und Bildanalyse e.V., Konstanz, Germany*
[4] *School of Mathematics and Statistics, The University of Sydney, Sydney, AU*
[5] *Charles Perkins Centre, The University of Sydney, Sydney, Australia.*

* These authors contributed equally to this work
** Co-last and co-corresponding: mh32@sanger.ac.uk and ob5@sanger.ac.uk

Abstract

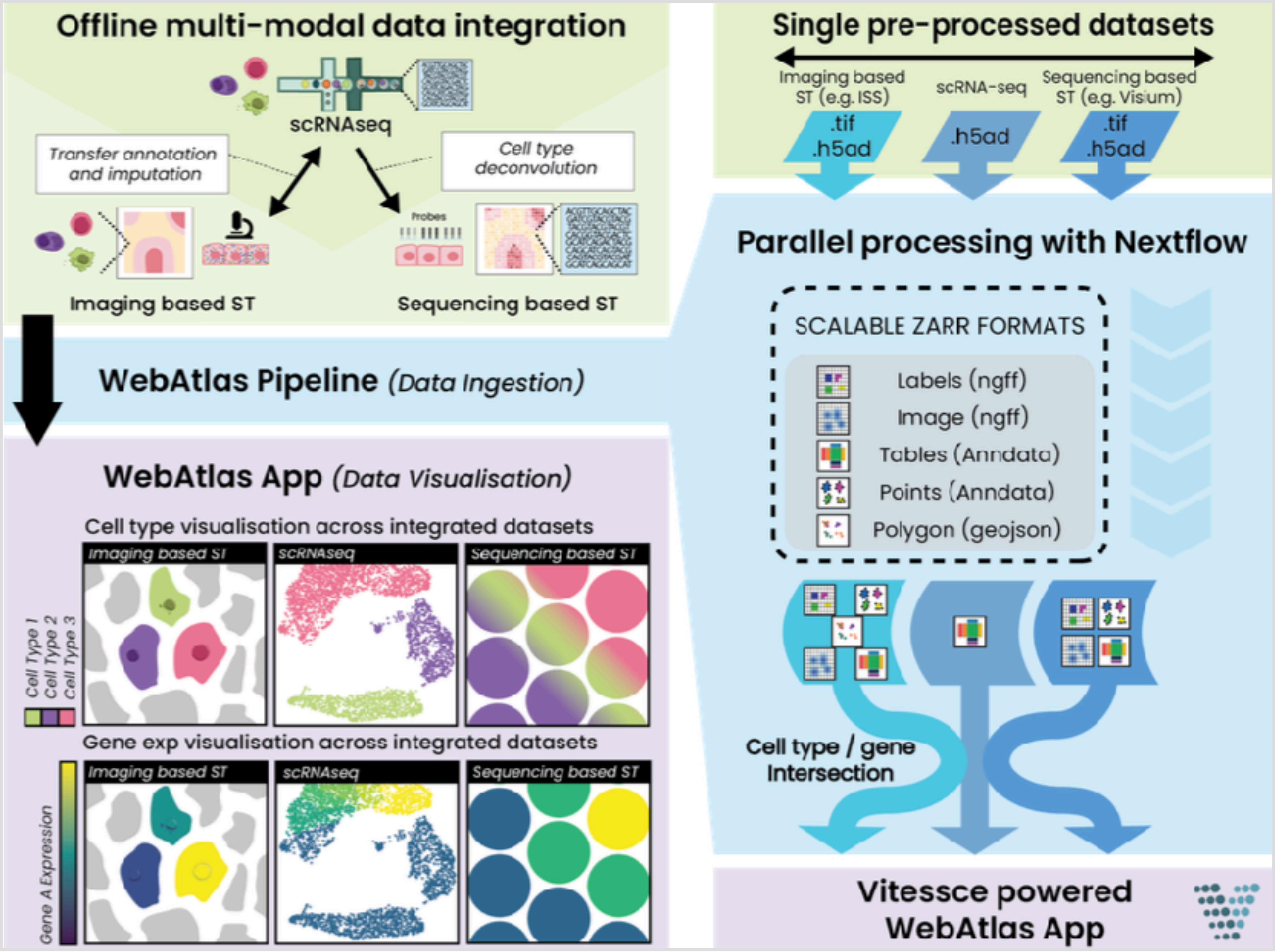
Single cell and spatial transcriptomics illuminate complementary features of tissues. However, online dissemination and exploration of integrated datasets is challenging due to the heterogeneity and scale of data. We introduce the WebAtlas pipeline for user-friendly sharing and interactive navigation of integrated datasets. WebAtlas unifies commonly used atlassing technologies into the cloud-optimised Zarr format and builds on Vitessce to enable remote data navigation. We showcase WebAtlas on the developing human lower limb to cross-query cell types and genes across single cell, sequencing- and imaging-based spatial transcriptomic data.

Main text

Single cell and spatial transcriptomics provide complementary tools for tissue atlassing. While single cell RNA-sequencing (scRNA-seq) profiles whole-transcriptomes of individual cells, it does not capture their spatial locations in tissues. Conversely, sequencing-based spatial transcriptomics (ST) can profile whole transcriptomes *in situ*, but widely used technologies such Visium and Slide-Seq do not provide single cell spatial resolution. Although alternative imaging-based ST methods such as In Situ Sequencing and MER-FISH provide true single cell resolution, they are limited to targeted gene panels of around 100 to 1000 genes[1].

Computational integration can harness the strengths of scRNA-seq and ST modalities to resolve cell types and impute transcriptomes of single cells *in situ*[2]. However, we lack user-friendly software solutions to disseminate and navigate integrated single cell and spatial transcriptomic datasets. First, scRNA-seq and ST data objects are often saved in non-unified sequencing and imaging file formats that perform poorly with web technologies[3-5]. Second, existing software platforms do not readily support simultaneous browsing of multiple integrated data modalities[3,5,6]. These limitations impede usable and interpretable access to a wealth of

1



bioRxiv

https://doi.org/10.1101/2023.05.19.541329

Communities | **SpatialData & WebAtlas**

Consumers

Napari
CZI Core Funding

WebAtlas
HCA WSSS Extension through 2023

BIA
EBI Core Funding

IDR
Wellcome into 2024

...

Formats Stack

SpatialData
CZI Single Cell - Data Insights 2023-2025

Languages: Javascript, Python, R

NGFF
NFDI4BIOIMAGE 2023-2028
CZI EOSS5 2022-2024

Languages: Java, Javascript, Python

Zarr
CZI EOSS4 2021-2024

Languages: C/C++, Java, Javascript, Julia, Python, R, Rust

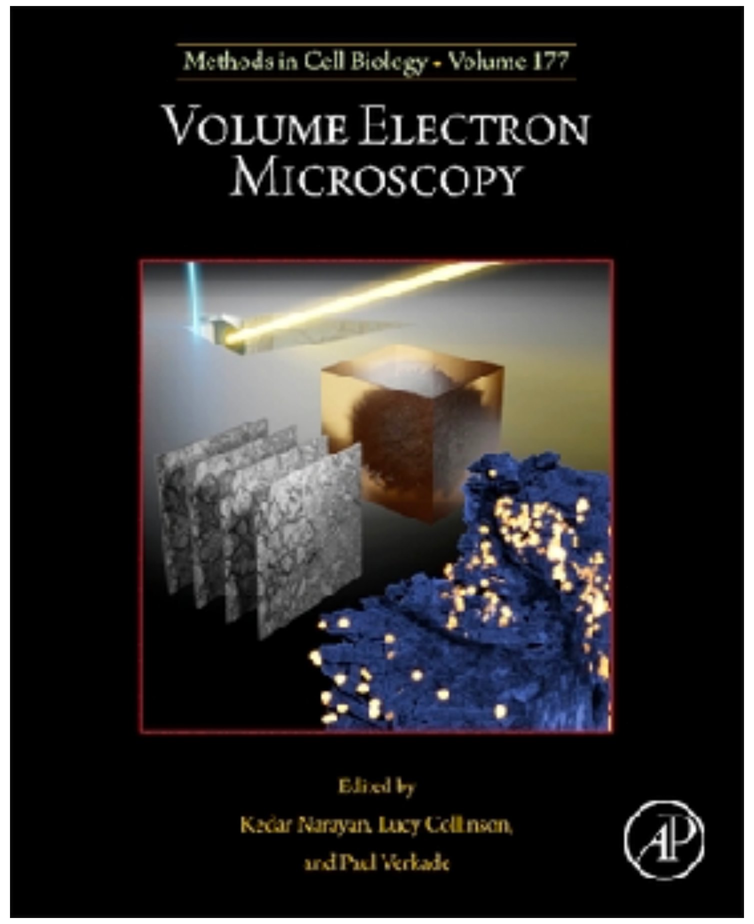
Development

EMBL
HELMHOLTZ
wellcome sanger institute

ALLEN INSTITUTE
CHAN ZUCKERBERG Biohub Network
BROAD INSTITUTE
HARVARD MEDICAL SCHOOL
hhmi | janelia Research Campus
kitware
MIT
RIKEN

Google Research
Microsoft
NASA

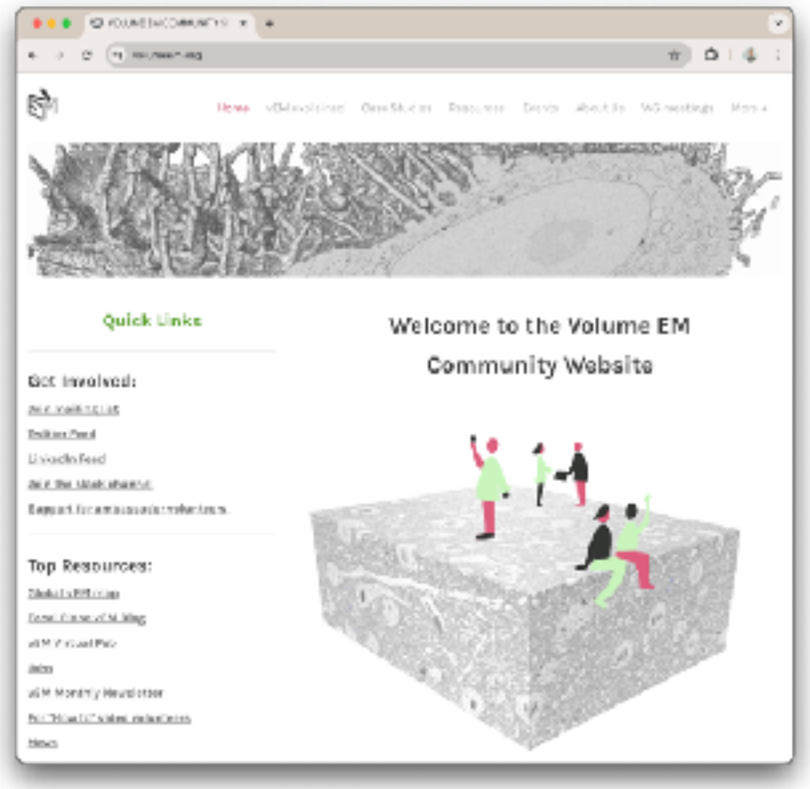
Communities | **VolumeEM**



Methods in Cell Biology • Volume 177

Volume Electron Microscopy

Edited by
Kedar Narayan, Lucy Collinson,
and Paul Verkade



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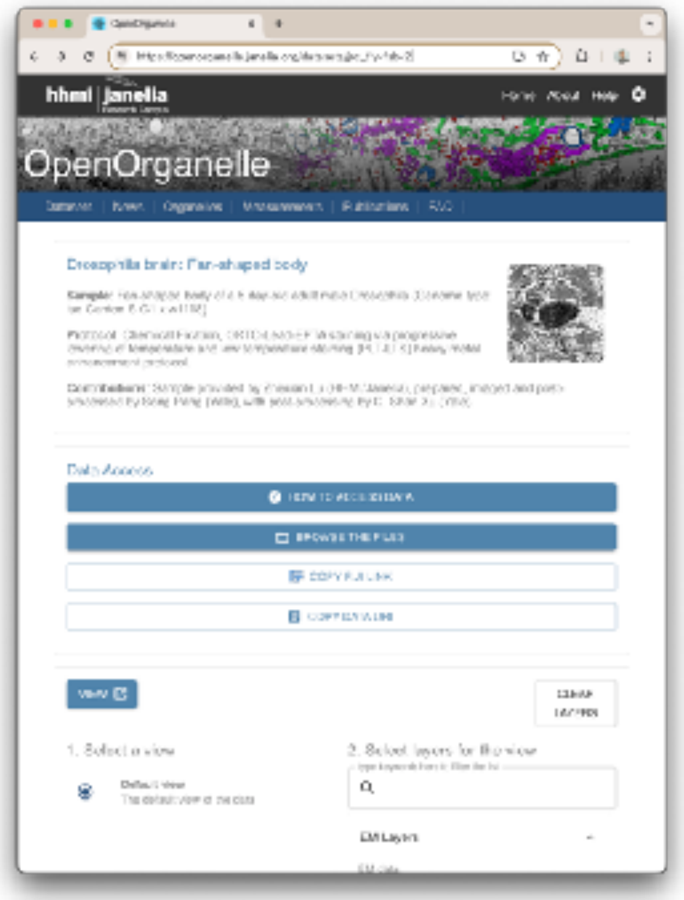
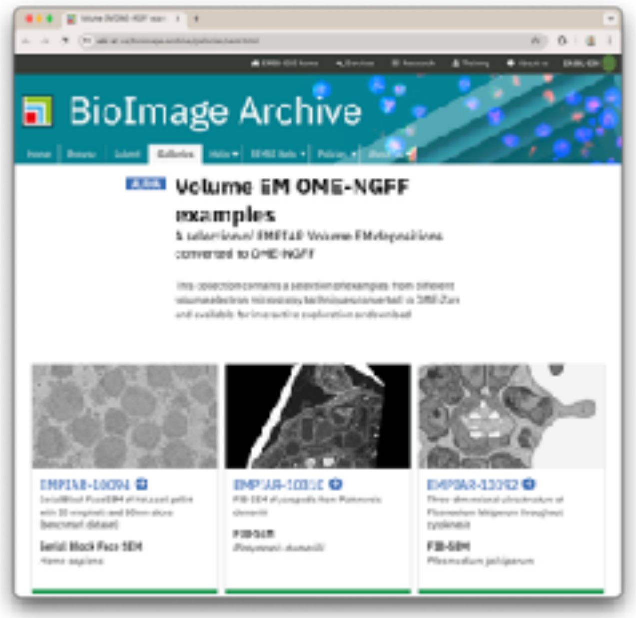


Drosophila brain: Fan-shaped body

https://openorganelle.janelia.org/datasets/jrc_fly-fsb-2

https://zarr.webknossos.org/

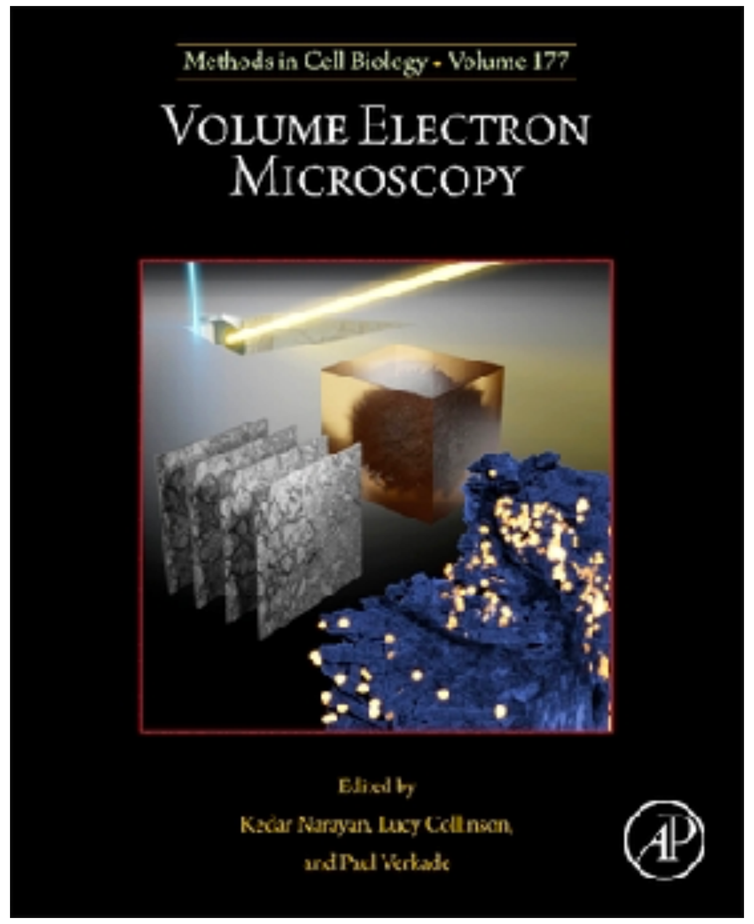


Volume EM OME-NGFF examples

https://www.ebi.ac.uk/bioimage-archive/galleries/vem.html

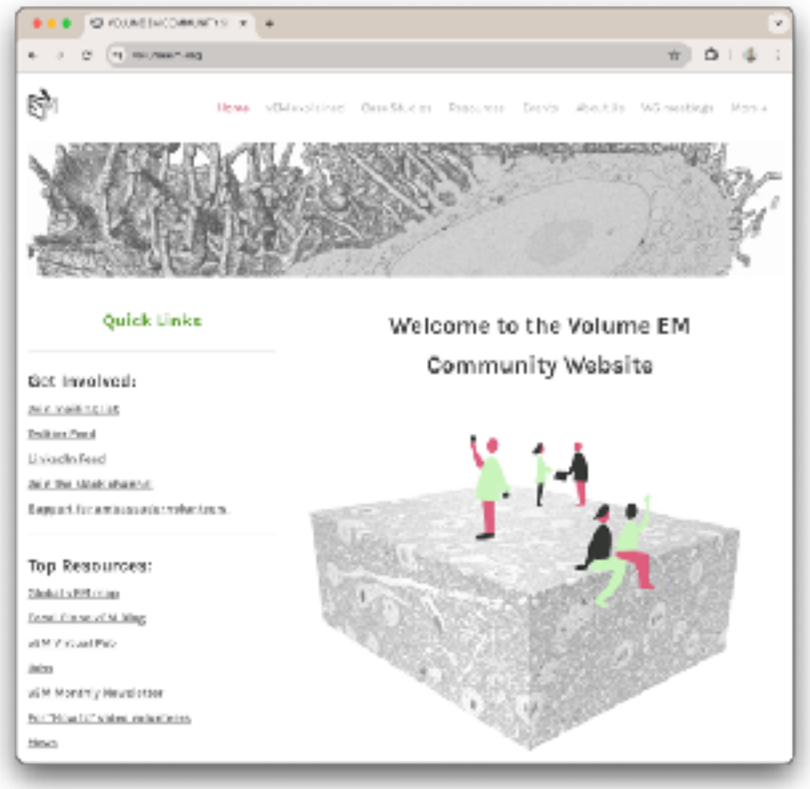
- Self-organizing community with critical use-case
- Inaugural Gordon Research Conference (2023)
- Discussions with companies

Communities | **VolumeEM**


Methods in Cell Biology • Volume 177

VOLUME ELECTRON MICROSCOPY

Edited by
Kedar Narayan, Lucy Collinson,
and Paul Verkade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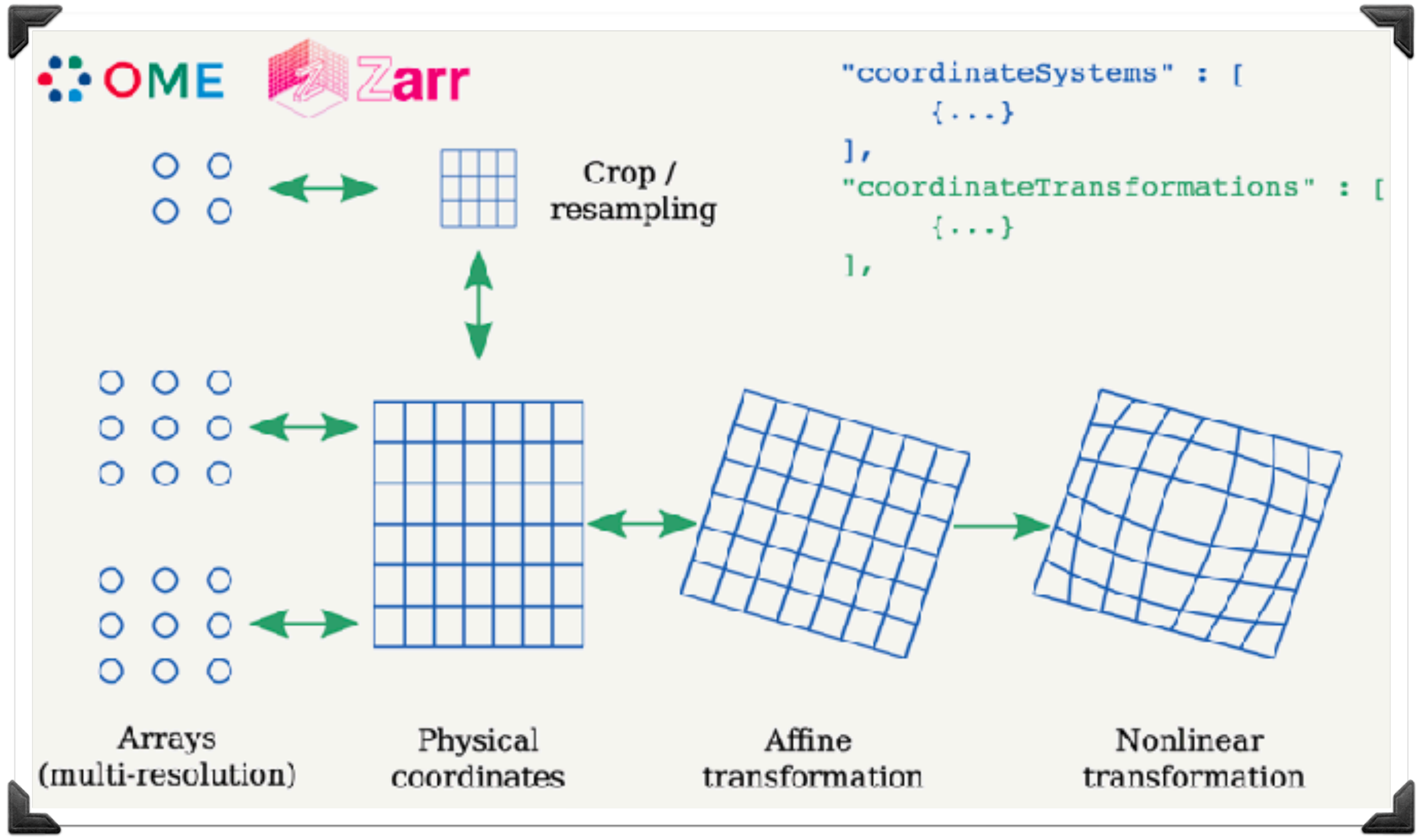
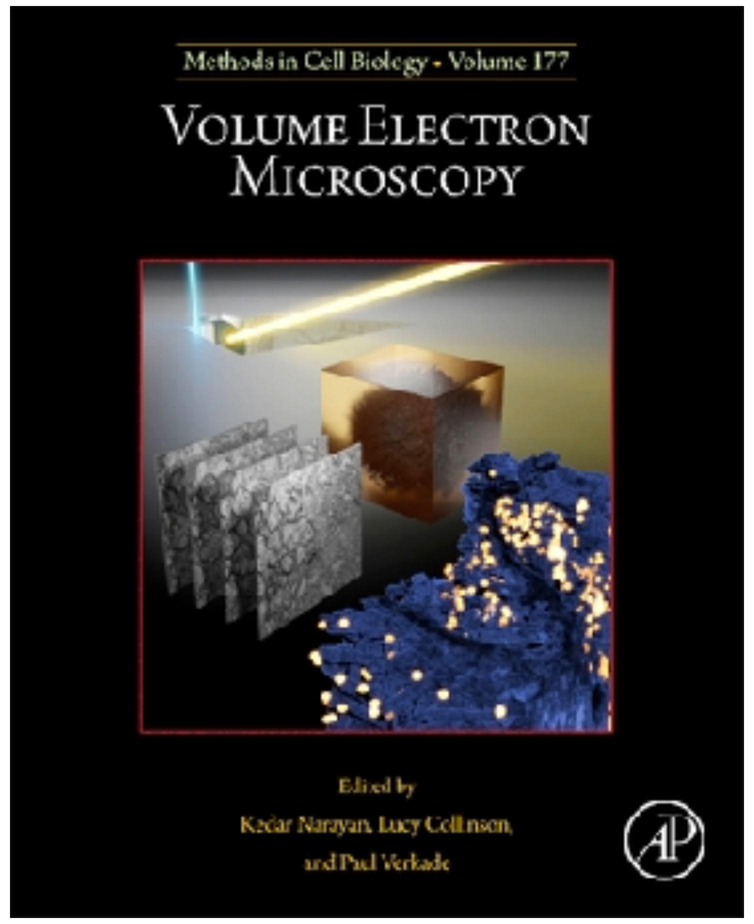


```
"coordinateSystems" : [
    {...}
],
"coordinateTransformations" : [
    {...}
],
```

Credit: **John Bogovic**

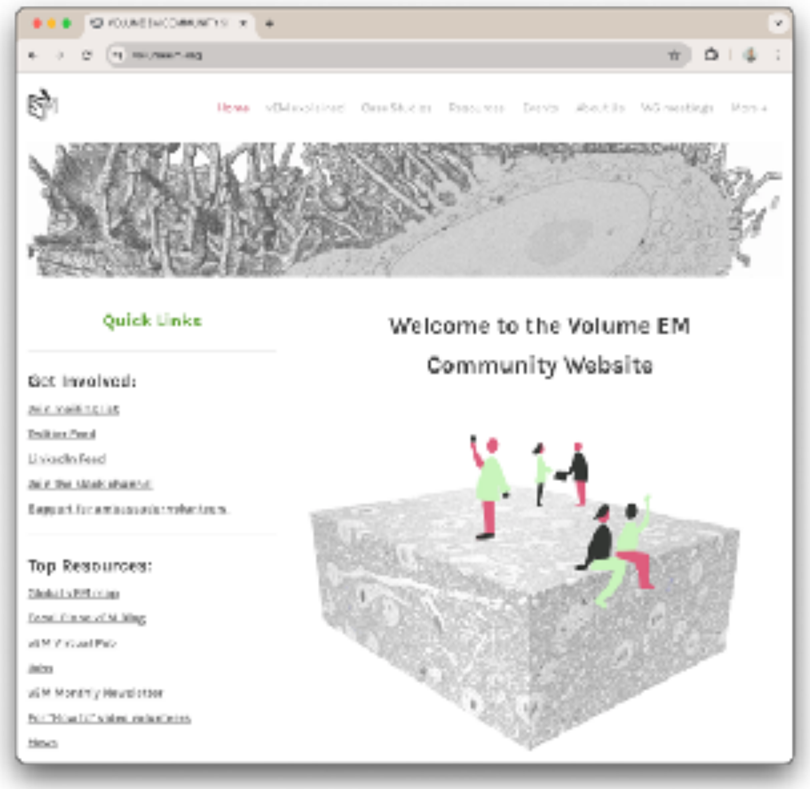
- Self-organizing community with critical use-case
- Inaugural Gordon Research Conference (2023)
- Discussions with companies

Communities | **VolumeEM**

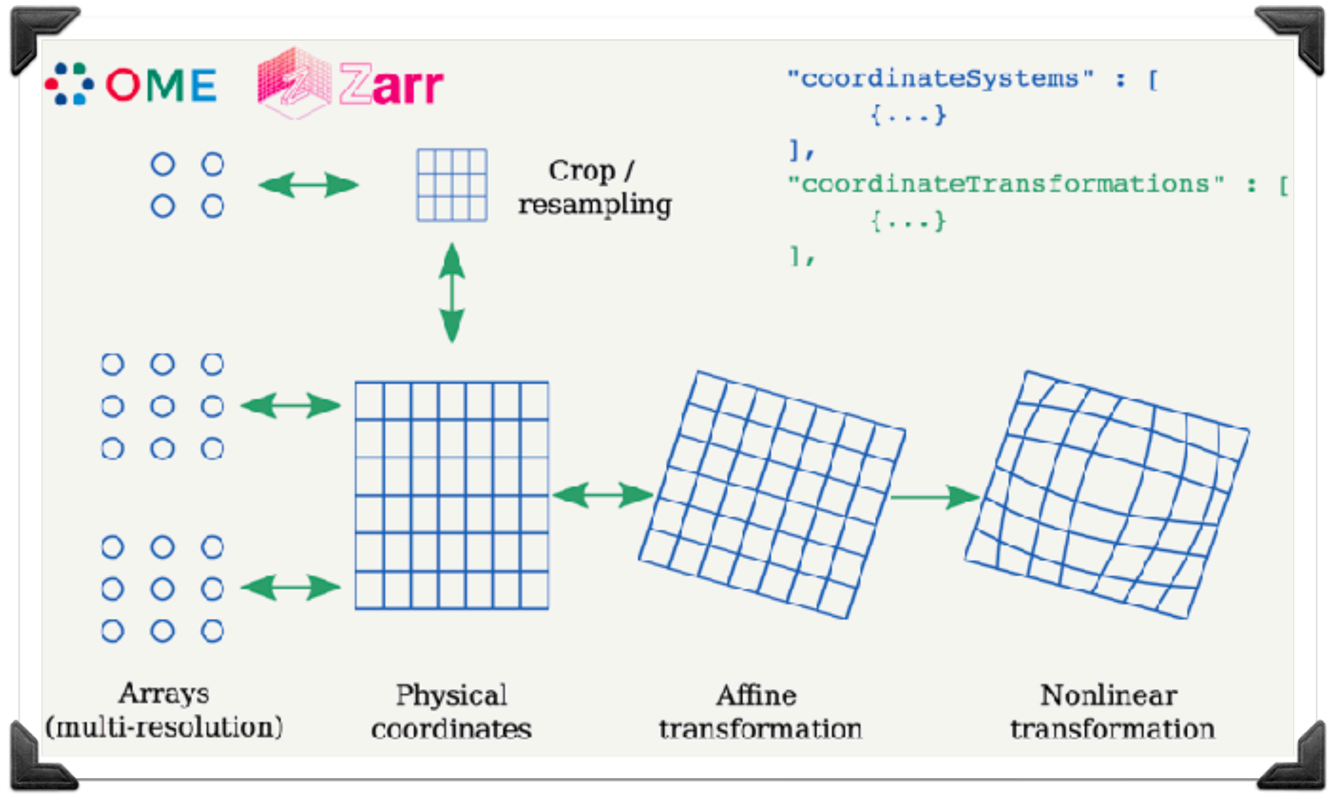

Methods in Cell Biology • Volume 177

VOLUME ELECTRON MICROSCOPY

Edited by
Kedar Narayan, Lucy Collinson,
and Paul Verkade

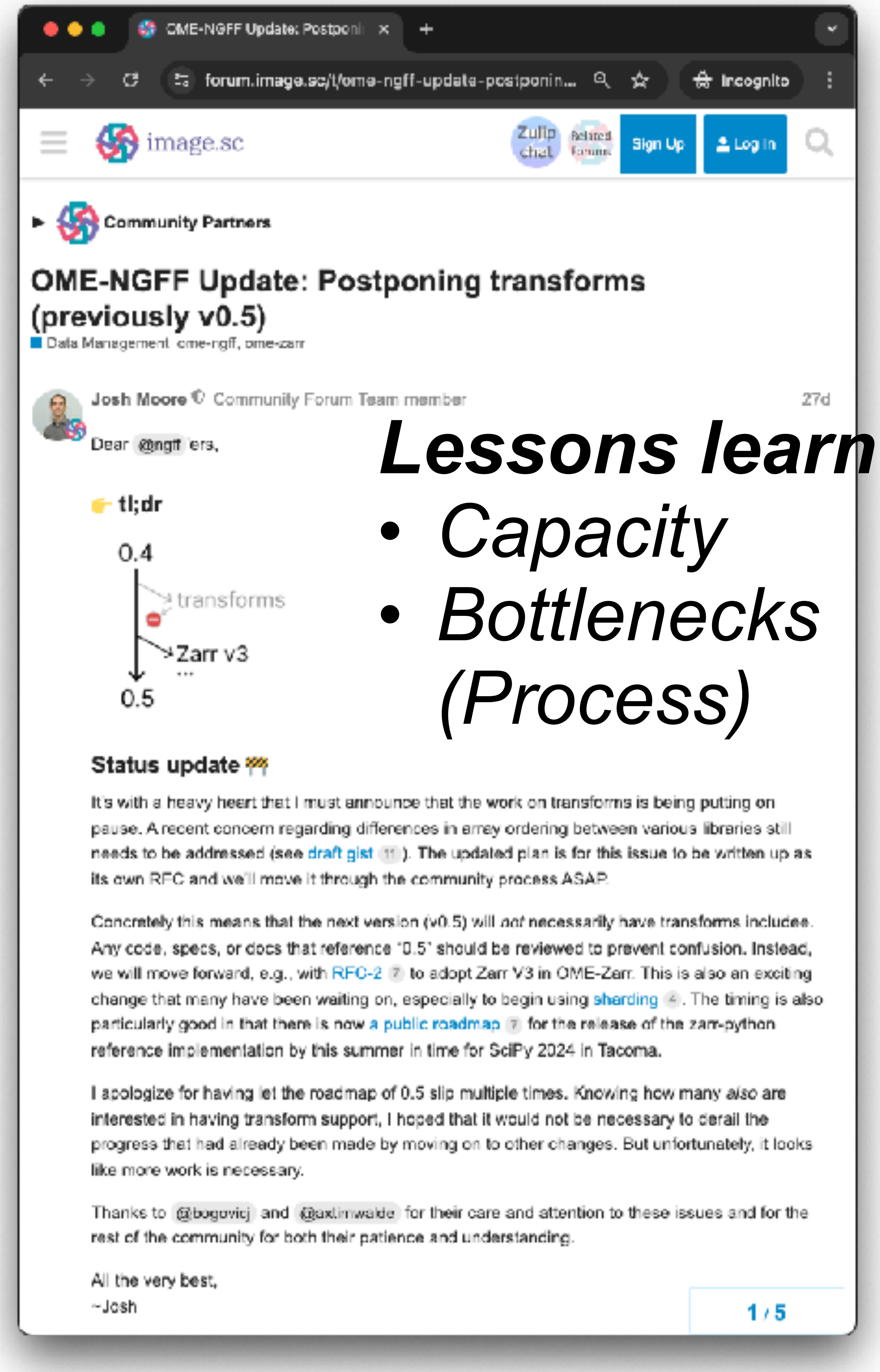


https://www.volumeem.org/



Credit: **John Bogovic**

- Self-organizing community with critical use-case
- Inaugural Gordon Research Conference (2023)
- Discussions with companies



OME-NGFF Update: Postponing transforms (previously v0.5)

Data Management ome-ngff, ome-zarr

Josh Moore Community Forum Team member 27d

Dear @ngff ers,

tl;dr

Status update

It's with a heavy heart that I must announce that the work on transforms is being putting on pause. A recent concern regarding differences in array ordering between various libraries still needs to be addressed (see draft gist 11). The updated plan is for this issue to be written up as its own RFC and we'll move it through the community process ASAP.

Concretely this means that the next version (v0.5) will *not* necessarily have transforms includee. Any code, specs, or docs that reference "0.5" should be reviewed to prevent confusion. Instead, we will move forward, e.g., with RFC-2 7 to adopt Zarr V3 in OME-Zarr. This is also an exciting change that many have been waiting on, especially to begin using sharding 4. The timing is also particularly good in that there is now a public roadmap 7 for the release of the zarr-python reference implementation by this summer in time for SciPy 2024 in Tacoma.

I apologize for having let the roadmap of 0.5 slip multiple times. Knowing how many *also* are interested in having transform support, I hoped that it would not be necessary to derail the progress that had already been made by moving on to other changes. But unfortunately, it looks like more work is necessary.

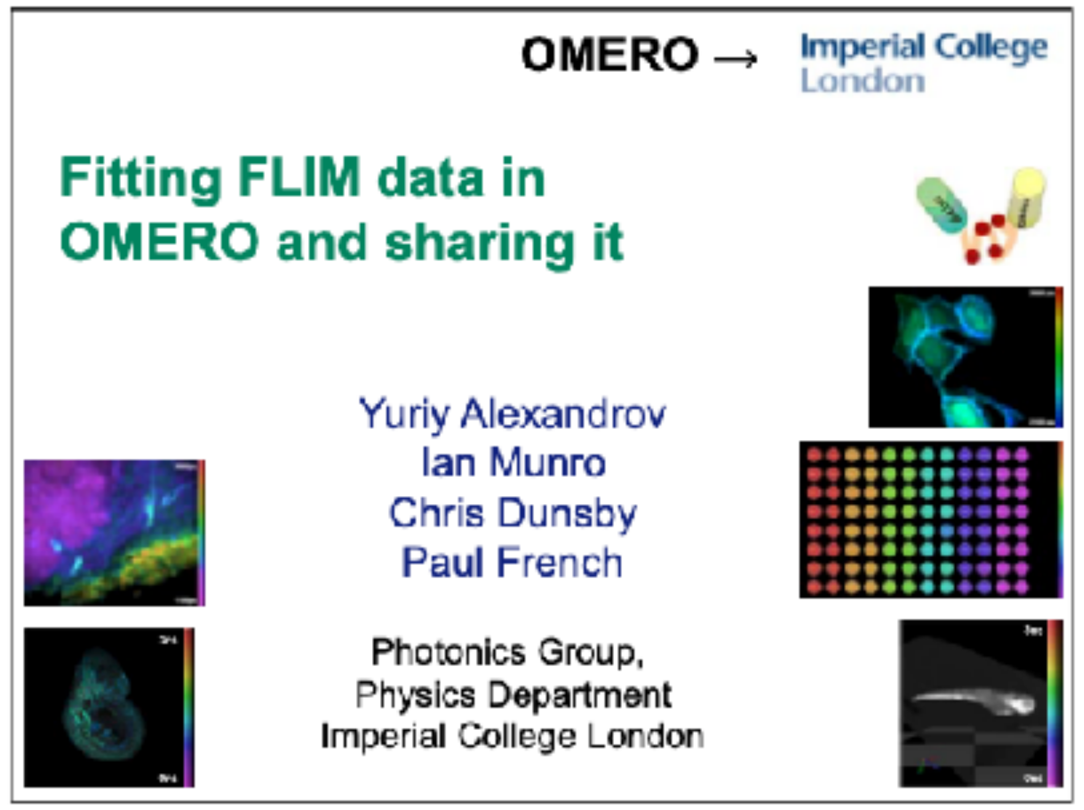
Thanks to @bogovicj and @axtimwalde for their care and attention to these issues and for the rest of the community for both their patience and understanding.

All the very best,
~Josh

Lessons learned:
- *Capacity*
- *Bottlenecks (Process)*

https://forum.image.sc/t/ome-ngff-update-postponing-transforms-previously-v0-5/95617

Communities | **Hyperspectral**

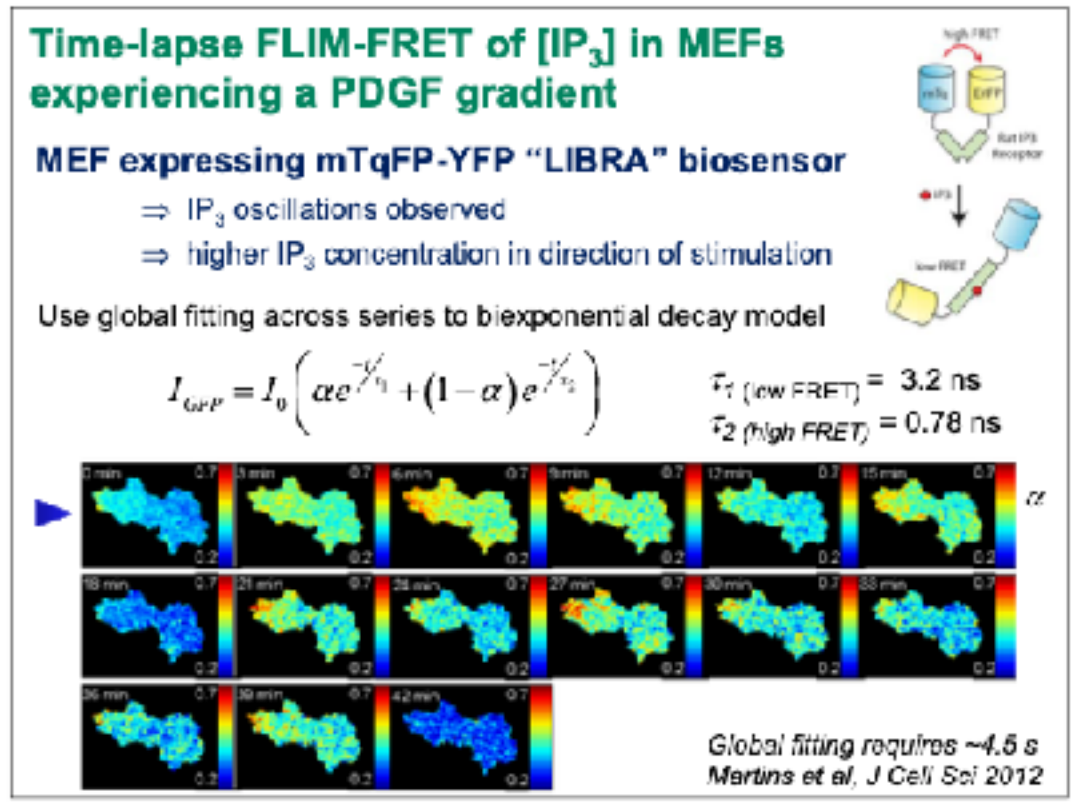


OMERO → Imperial College London

Fitting FLIM data in OMERO and sharing it

Yuriy Alexandrov
Ian Munro
Chris Dunsby
Paul French

Photonics Group,
Physics Department
Imperial College London



Time-lapse FLIM-FRET of [IP$_3$] in MEFs experiencing a PDGF gradient

MEF expressing mTqFP-YFP "LIBRA" biosensor

⇒ IP$_3$ oscillations observed

⇒ higher IP$_3$ concentration in direction of stimulation

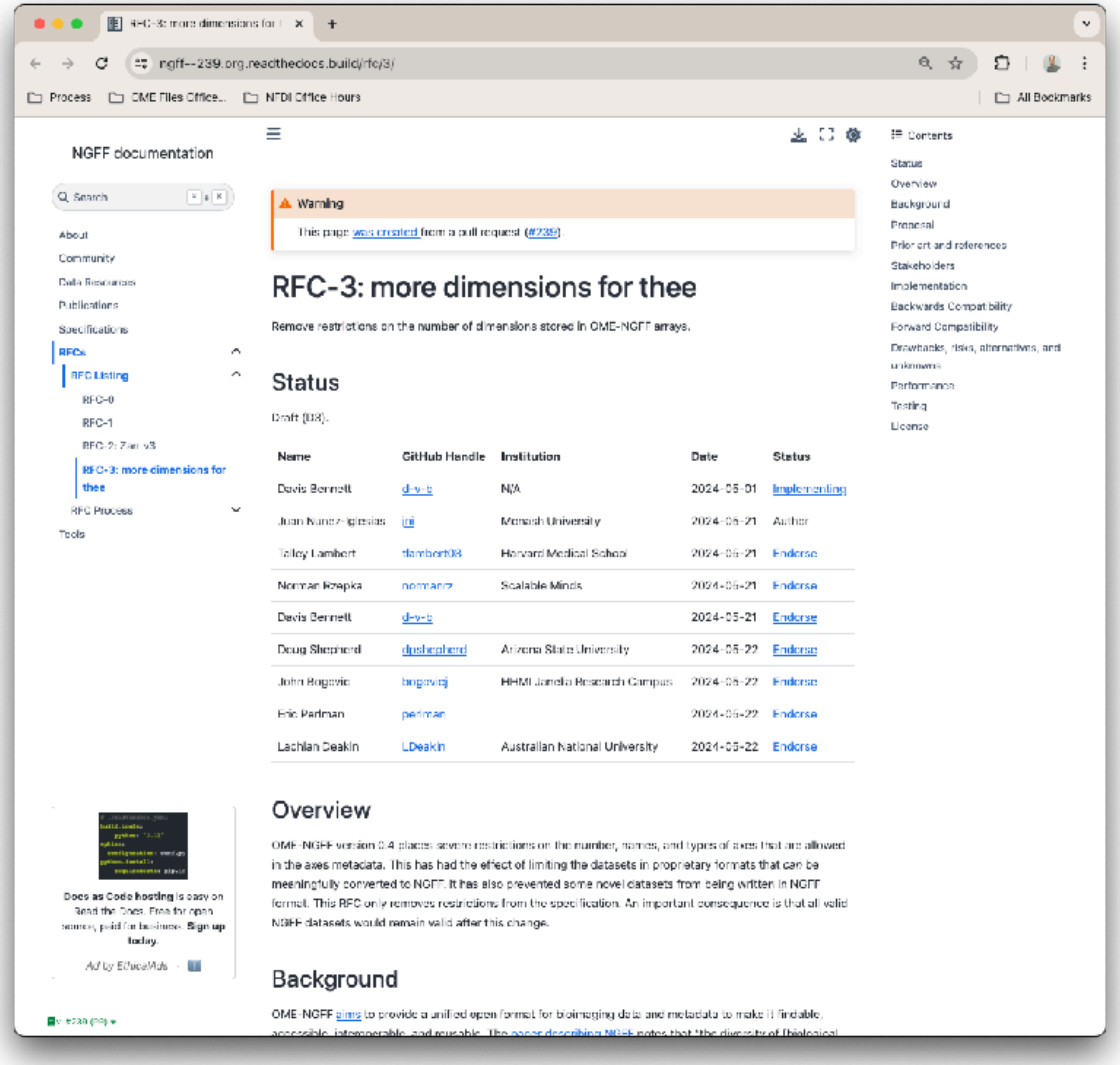
Use global fitting across series to biexponential decay model

$$I_{GFP} = I_0\left(\alpha e^{-t/\tau_1} + (1-\alpha)e^{-t/\tau_2}\right) \qquad \tau_1\ (\text{low FRET}) = 3.2\ \text{ns} \quad \tau_2\ (\text{high FRET}) = 0.78\ \text{ns}$$

Global fitting requires ~4.5 s
Martins et al. J Cell Sci 2012

https://bit.ly/Talk-3-OMEMeeting-2013-02-28

- Medical, FLIM, etc.
- Requires new dimensions
- Move beyond the 5D model
- Similar PR work now (RFC-3)



⚠ Warning

This page was created from a pull request (#239).

RFC-3: more dimensions for thee

Remove restrictions on the number of dimensions stored in OME-NGFF arrays.

Status

Draft (D3).

Name	GitHub Handle	Institution	Date	Status
Davis Bennett	d-v-b	N/A	2024-05-01	Implementing
Juan Nunez-Iglesias	jni	Monash University	2024-05-21	Author
Talley Lambert	tlambert03	Harvard Medical School	2024-05-21	Endorse
Norman Rzepka	normanrz	Scalable Minds	2024-05-21	Endorse
Davis Bennett	d-v-b		2024-05-21	Endorse
Doug Shepherd	dpshepherd	Arizona State University	2024-05-22	Endorse
John Bogovic	bogovicj	HHMI Janelia Research Campus	2024-05-22	Endorse
Eric Perlman	perlman		2024-05-22	Endorse
Lachlan Deakin	LDeakin	Australian National University	2024-05-22	Endorse

Overview

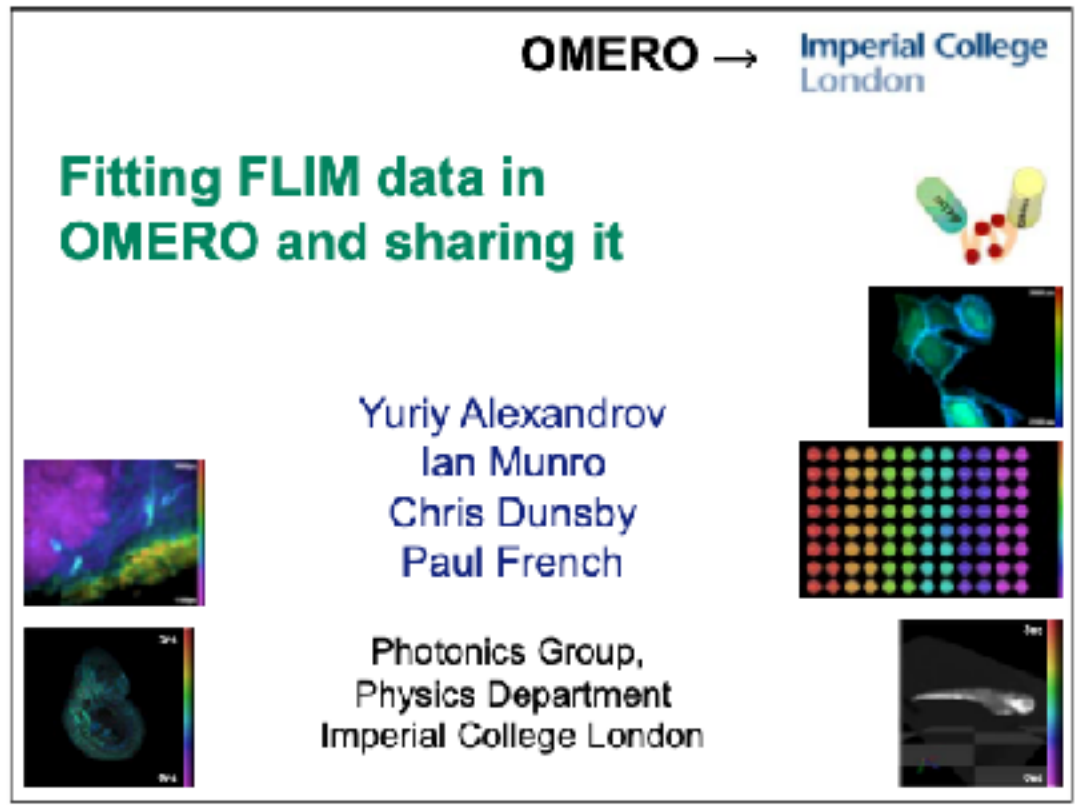
OME-NGFF version 0.4 places severe restrictions on the number, names, and types of axes that are allowed in the axes metadata. This has had the effect of limiting the datasets in proprietary formats that can be meaningfully converted to NGFF. It has also prevented some novel datasets from being written in NGFF format. This RFC only removes restrictions from the specification. An important consequence is that all valid NGFF datasets would remain valid after this change.

Background

OME-NGFF aims to provide a unified open format for bioimaging data and metadata to make it findable, accessible, interoperable, and reusable. The paper describing NGFF notes that "the diversity of

https://ngff--239.org.readthedocs.build/rfc/3/

Communities | **Hyperspectral** *et al.*



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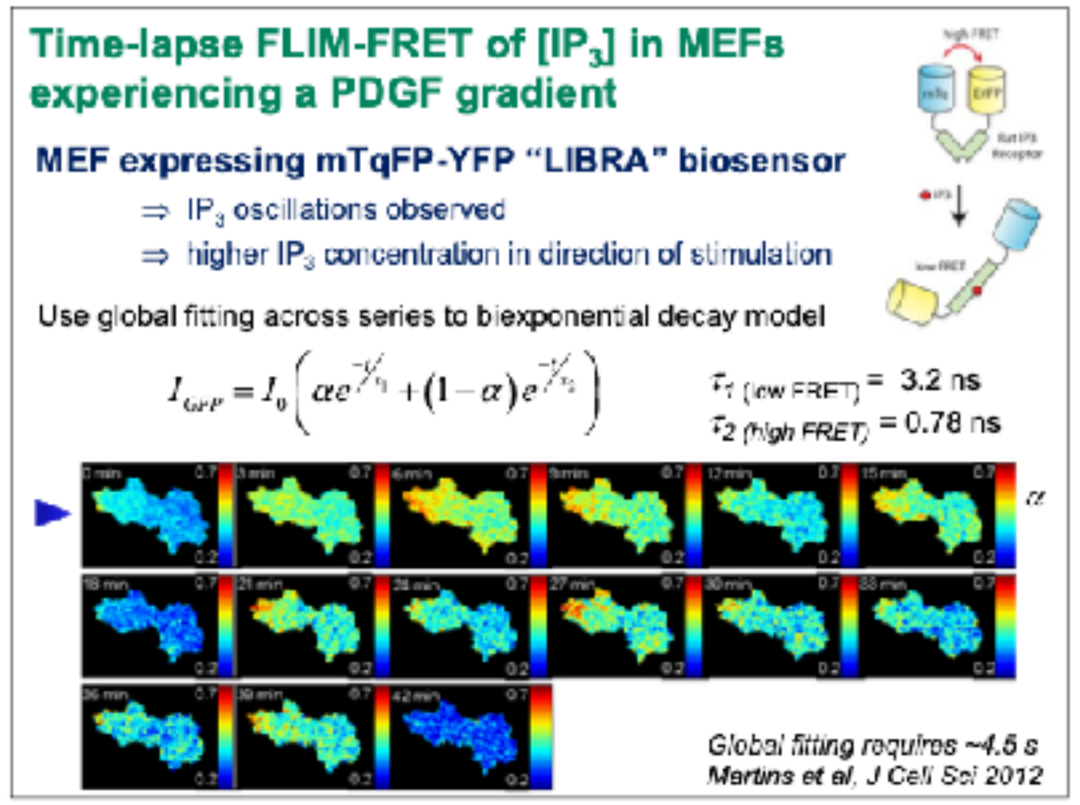

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- Requires new dimensions
- Move beyond the 5D model
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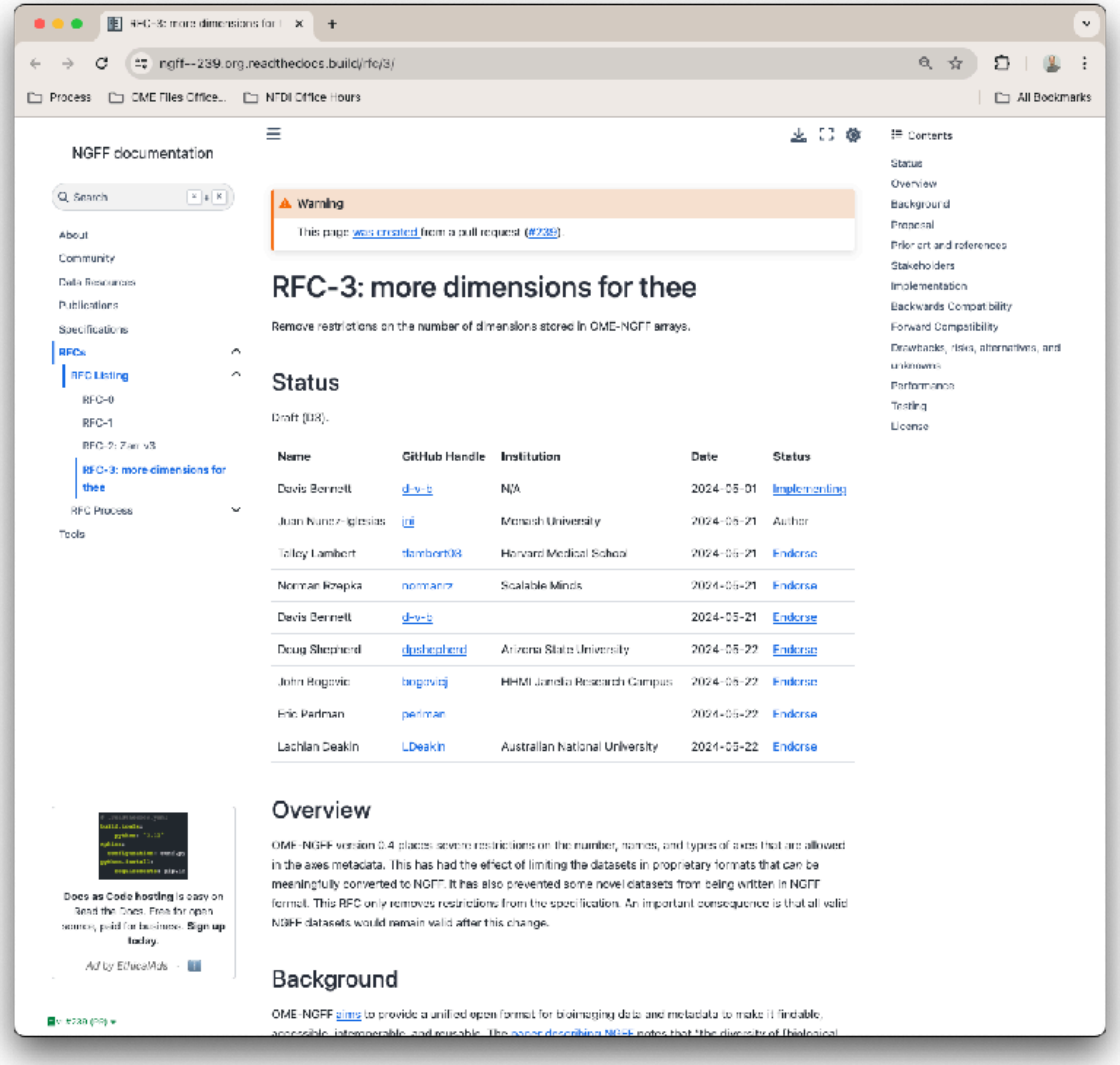
What's this?!



ngff--239.org.readthedocs.build/rfc/3/

NGFF documentation

⚠ Warning

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Davis Bennett	d-v-b	N/A	2024-05-01	Implementing
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Norman Rzepka	normanrz	Scalable Minds	2024-05-21	Endorse
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Background

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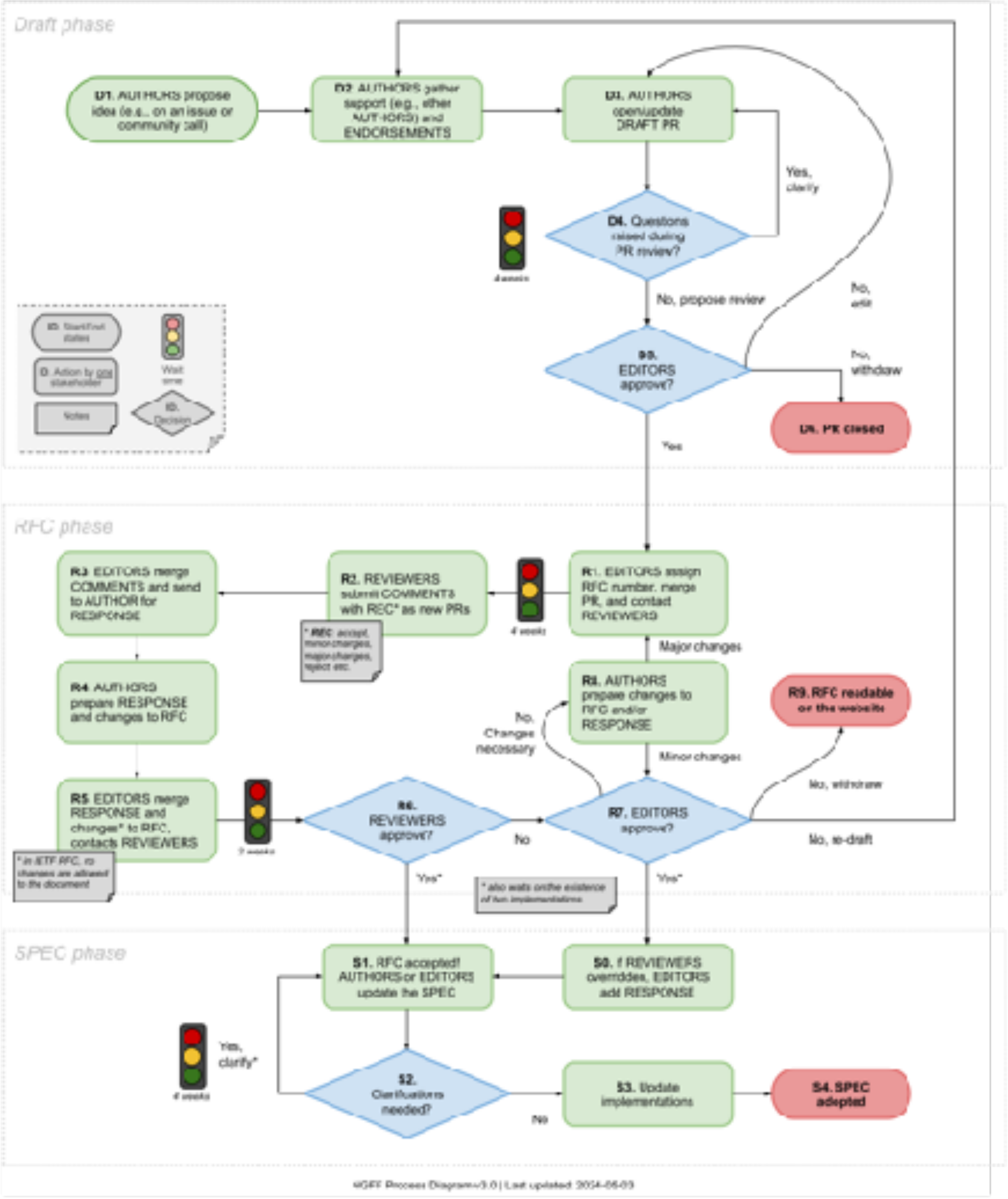
https://ngff--239.org.readthedocs.build/rfc/3/

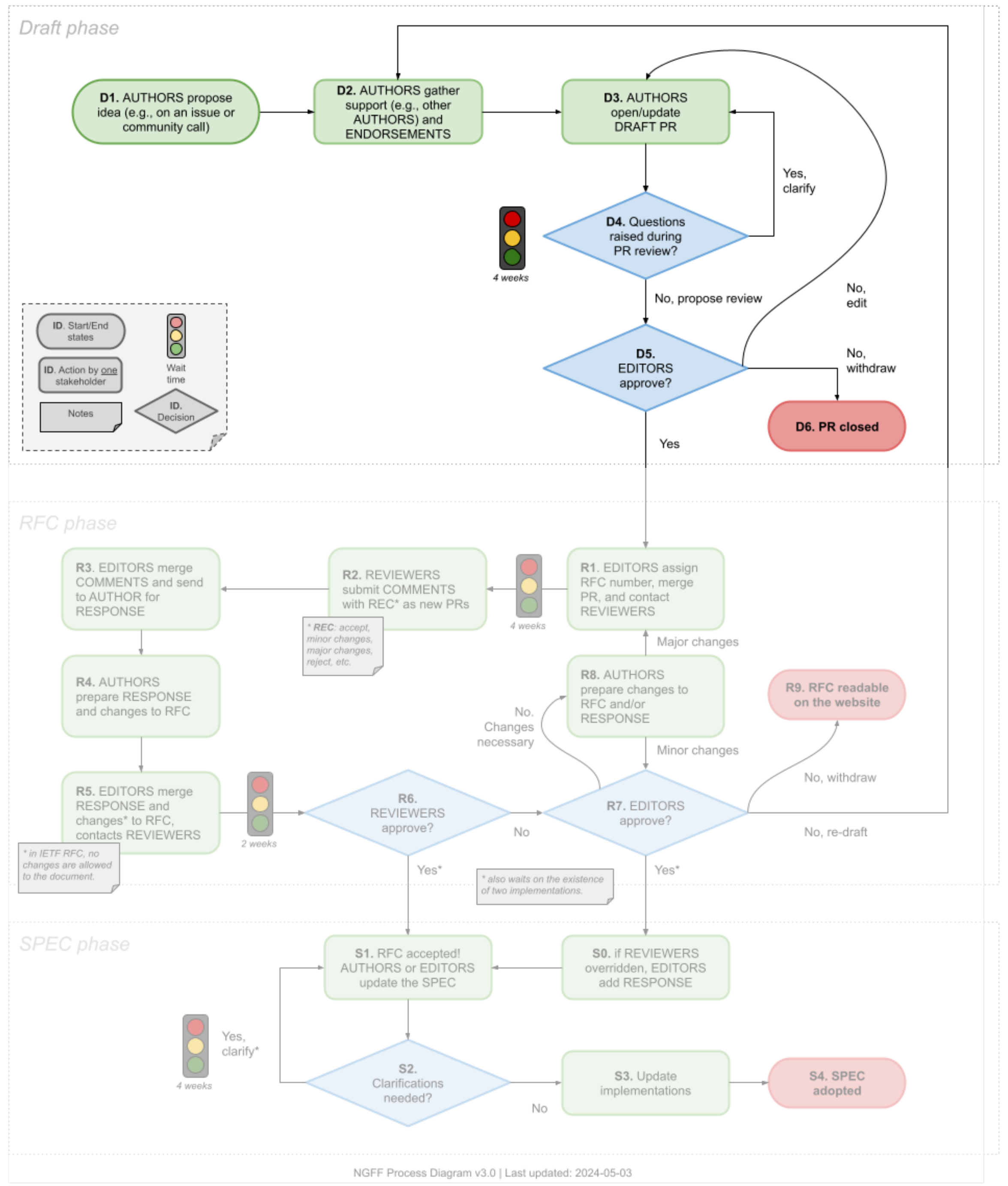
1. Questions
2. Status
3. Future

RFC-1 | **Request for Comments**



- RFC from IETF (*Internet Engineering Task Force*)
- *plus* Authors/Editors/Reviewers
- Keep things moving forward

RFC-1 | **Request for Comments**



Draft phase

D1. AUTHORS propose idea (e.g., on an issue or community call)

D2. AUTHORS gather support (e.g., other AUTHORS) and ENDORSEMENTS

D3. AUTHORS open/update DRAFT PR

D4. Questions raised during PR review?

4 weeks

Yes, clarify

No, propose review

D5. EDITORS approve?

No, edit

No, withdraw

D6. PR closed

Yes

ID. Start/End states

ID. Action by one stakeholder

Notes

Wait time

ID. Decision

RFC phase

R1. EDITORS assign RFC number, merge PR, and contact REVIEWERS

4 weeks

R2. REVIEWERS submit COMMENTS with REC* as new PRs

* ***REC***: accept, minor changes, major changes, reject, etc.

R3. EDITORS merge COMMENTS and send to AUTHOR for RESPONSE

R4. AUTHORS prepare RESPONSE and changes to RFC

R5. EDITORS merge RESPONSE and changes* to RFC, contacts REVIEWERS

* in IETF RFC, no changes are allowed to the document.

2 weeks

R6. REVIEWERS approve?

No

R7. EDITORS approve?

No. Changes necessary

R8. AUTHORS prepare changes to RFC and/or RESPONSE

Major changes

Minor changes

No, withdraw

R9. RFC readable on the website

No, re-draft

Yes*

* also waits on the existence of two implementations.

Yes*

SPEC phase

S0. if REVIEWERS overridden, EDITORS add RESPONSE

S1. RFC accepted! AUTHORS or EDITORS update the SPEC

S2. Clarifications needed?

Yes, clarify*

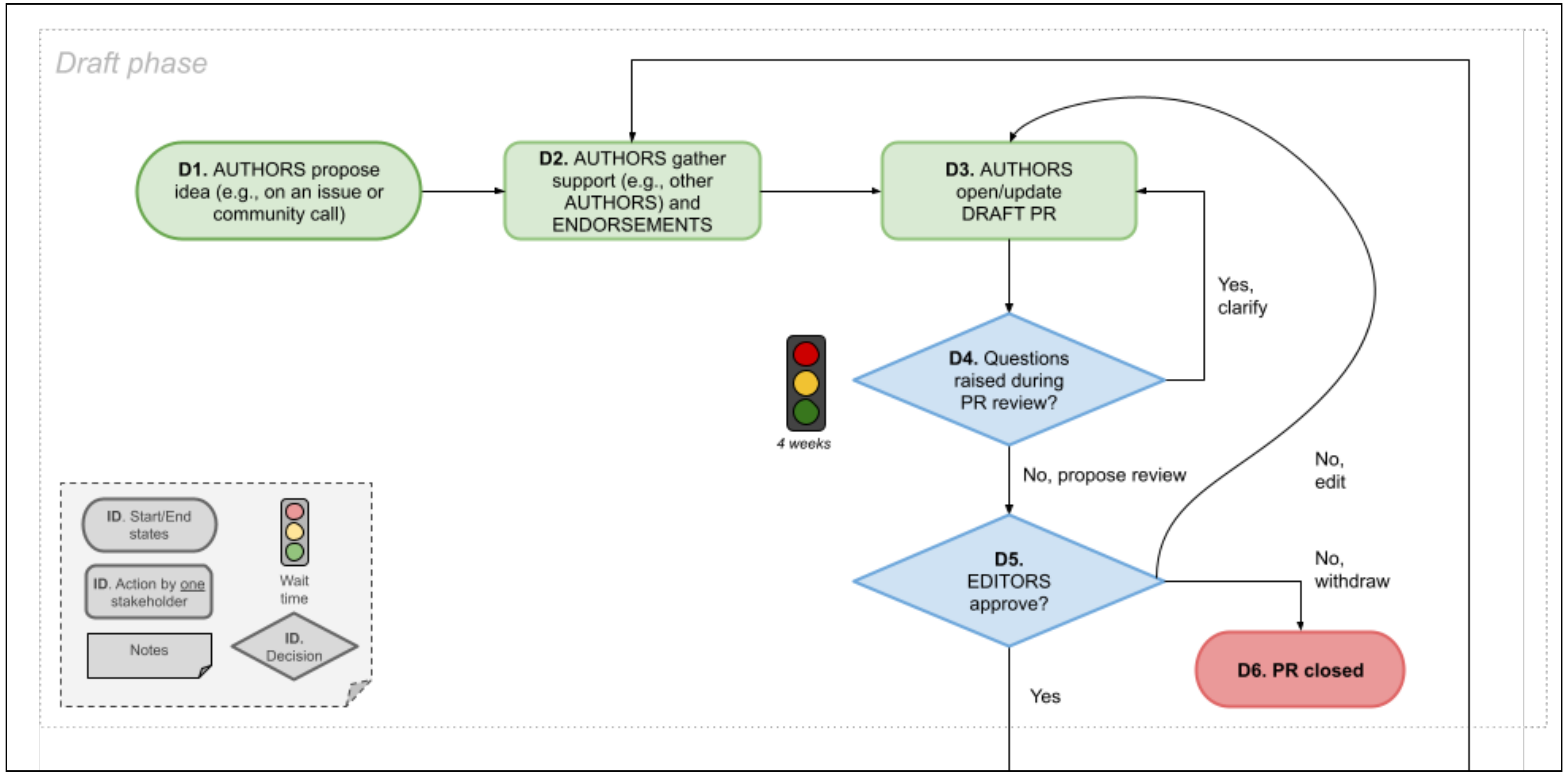
4 weeks

No

S3. Update implementations

S4. SPEC adopted

NGFF Process Diagram v3.0 | Last updated: 2024-05-03



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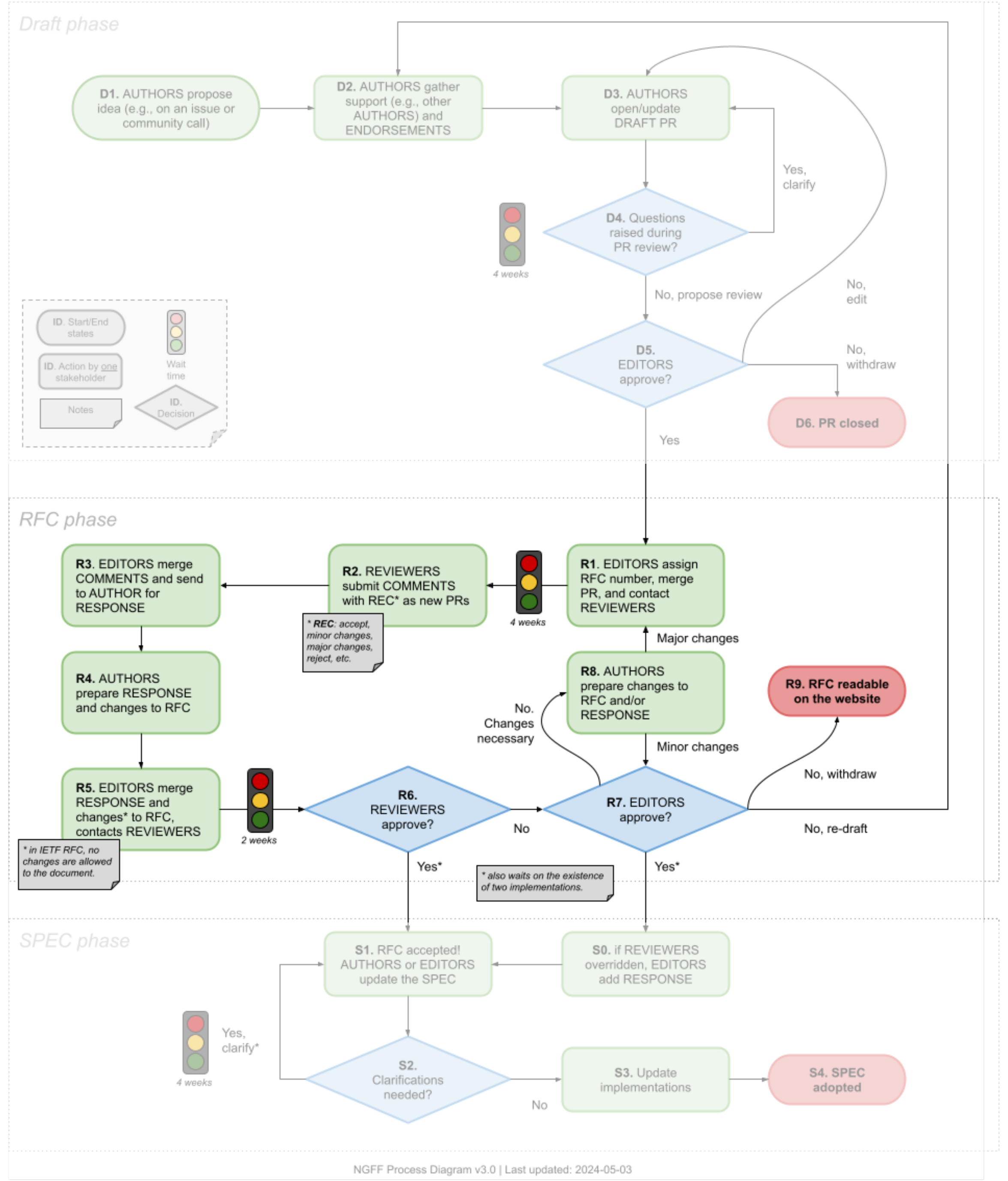
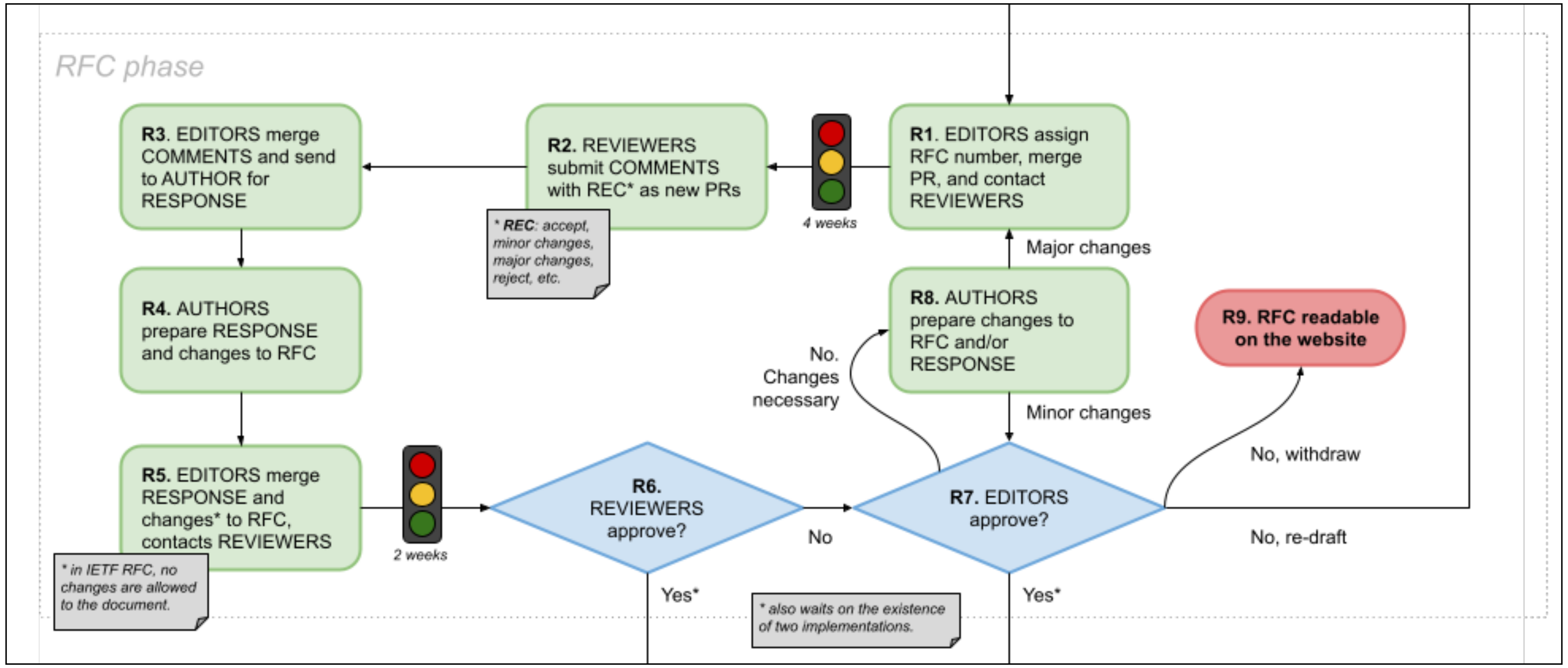
RFC-1 | **Request for Comments**


NGFF Process Diagram v3.0 | Last updated: 2024-05-03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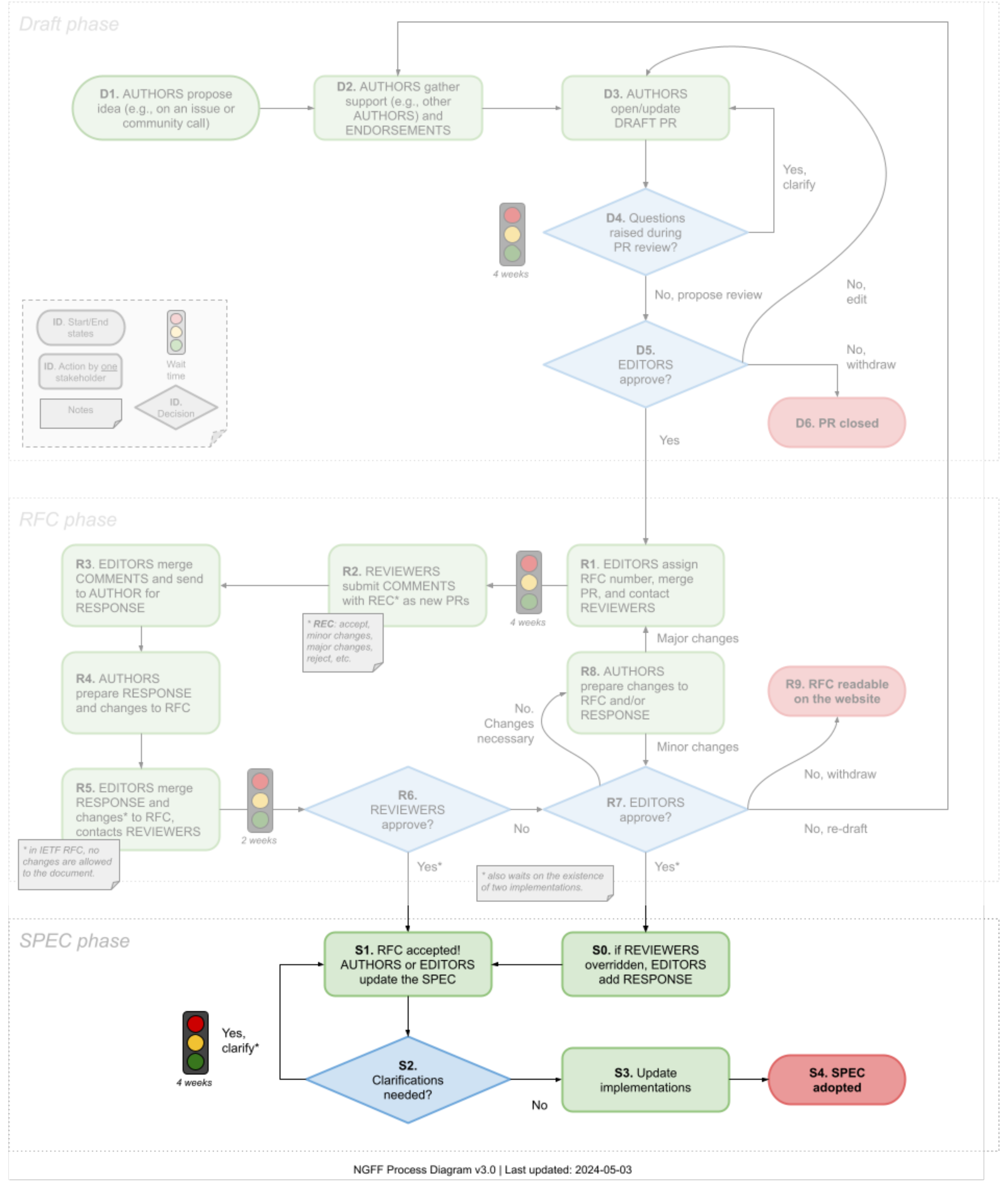
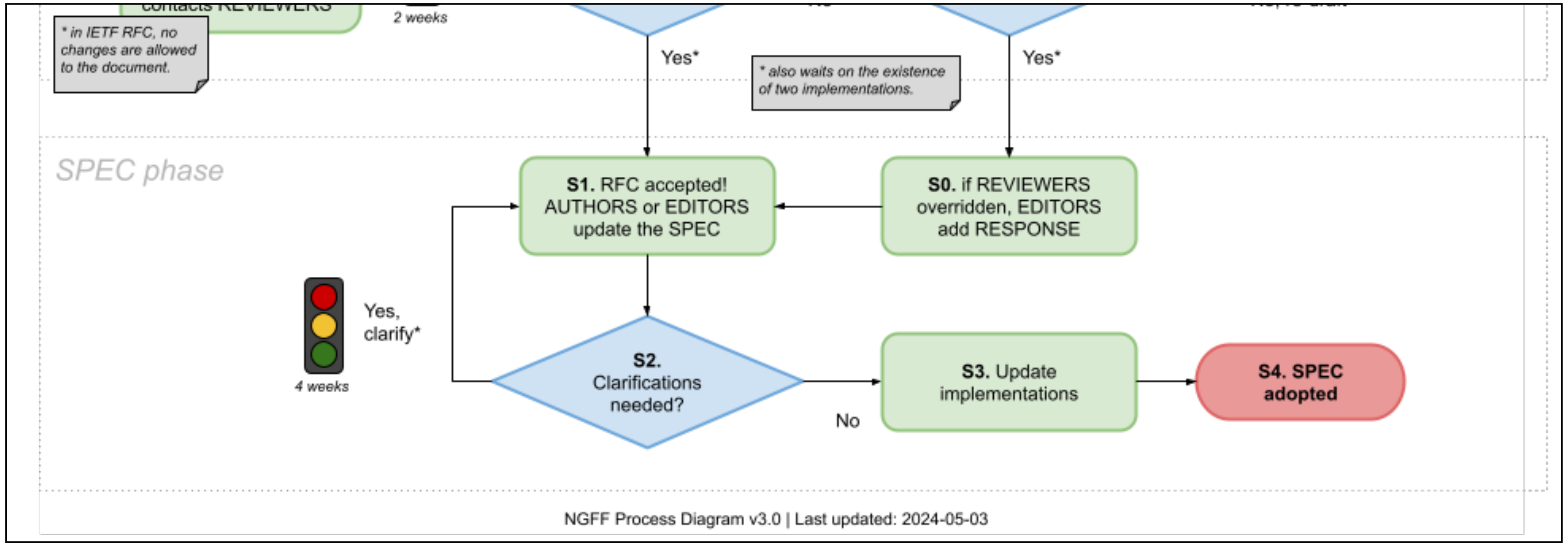
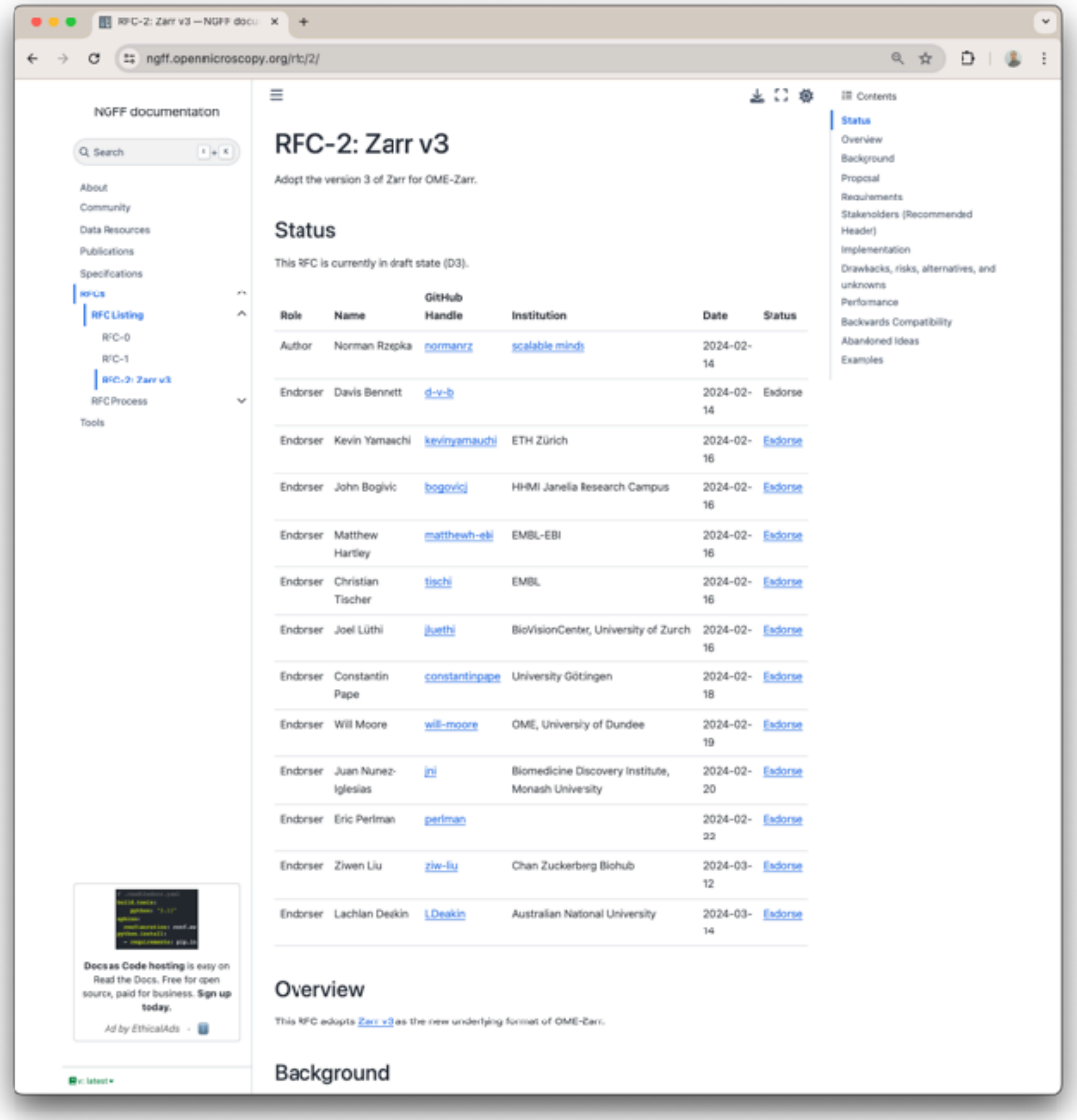
RFC-1 | **Request for Comments**





RFC-2 | **Zarr Update**



RFC-2: Zarr v3

Adopt the version 3 of Zarr for OME-Zarr.

Status

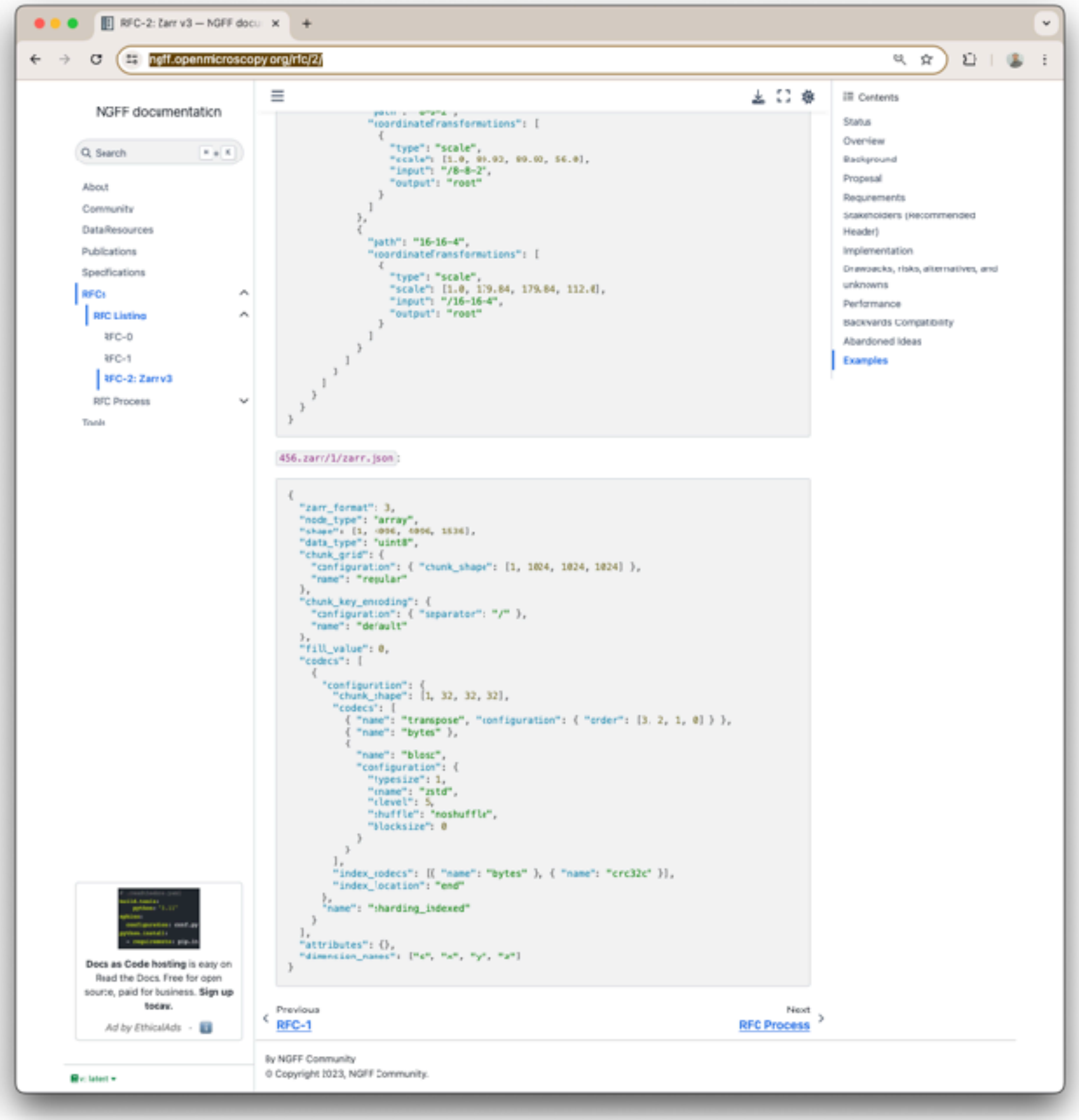
This RFC is currently in draft state (D3).

Role	Name	GitHub Handle	Institution	Date	Status
Author	Norman Rzepka	normanrz	scalable minds	2024-02-14	
Endorser	Davis Bennett	d-v-b		2024-02-14	Endorse
Endorser	Kevin Yamauchi	kevinyamauchi	ETH Zürich	2024-02-16	Endorse
Endorser	John Bogovic	bogovicj	HHMI Janelia Research Campus	2024-02-16	Endorse
Endorser	Matthew Hartley	matthewh-ebi	EMBL-EBI	2024-02-16	Endorse
Endorser	Christian Tischer	tischi	EMBL	2024-02-16	Endorse
Endorser	Joel Lüthi	jluethi	BioVisionCenter, University of Zurich	2024-02-16	Endorse
Endorser	Constantin Pape	constantinpape	University Göttingen	2024-02-18	Endorse
Endorser	Will Moore	will-moore	OME, University of Dundee	2024-02-19	Endorse
Endorser	Juan Nunez-Iglesias	jni	Biomedicine Discovery Institute, Monash University	2024-02-20	Endorse
Endorser	Eric Perlman	perlman		2024-02-22	Endorse
Endorser	Ziwen Liu	ziw-liu	Chan Zuckerberg Biohub	2024-03-12	Endorse
Endorser	Lachlan Deakin	LDeakin	Australian National University	2024-03-14	Endorse

Overview

This RFC adopts Zarr v3 as the new underlying format of OME-Zarr.

Background



```
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            {
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              "output": "root"
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              "output": "root"
            }
          ]
        }
      ]
    }
  ]
  }
 }
}
```

`456.zarr/1/zarr.json`:

```
{
  "zarr_format": 3,
  "node_type": "array",
  "shape": [1, 4096, 4096, 1536],
  "data_type": "uint8",
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    "name": "regular"
  },
  "chunk_key_encoding": {
    "configuration": { "separator": "/" },
    "name": "default"
  },
  "fill_value": 0,
  "codecs": [
    {
      "configuration": {
        "chunk_shape": [1, 32, 32, 32],
        "codecs": [
          { "name": "transpose", "configuration": { "order": [3, 2, 1, 0] } },
          { "name": "bytes" },
          {
            "name": "blosc",
            "configuration": {
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              "cname": "zstd",
              "clevel": 5,
              "shuffle": "noshuffle",
              "blocksize": 0
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          }
        ],
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        "index_location": "end"
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    }
  ],
  "attributes": {},
  "dimension_names": ["c", "x", "y", "z"]
}
```

Previous RFC-1

Next RFC Process

By NGFF Community

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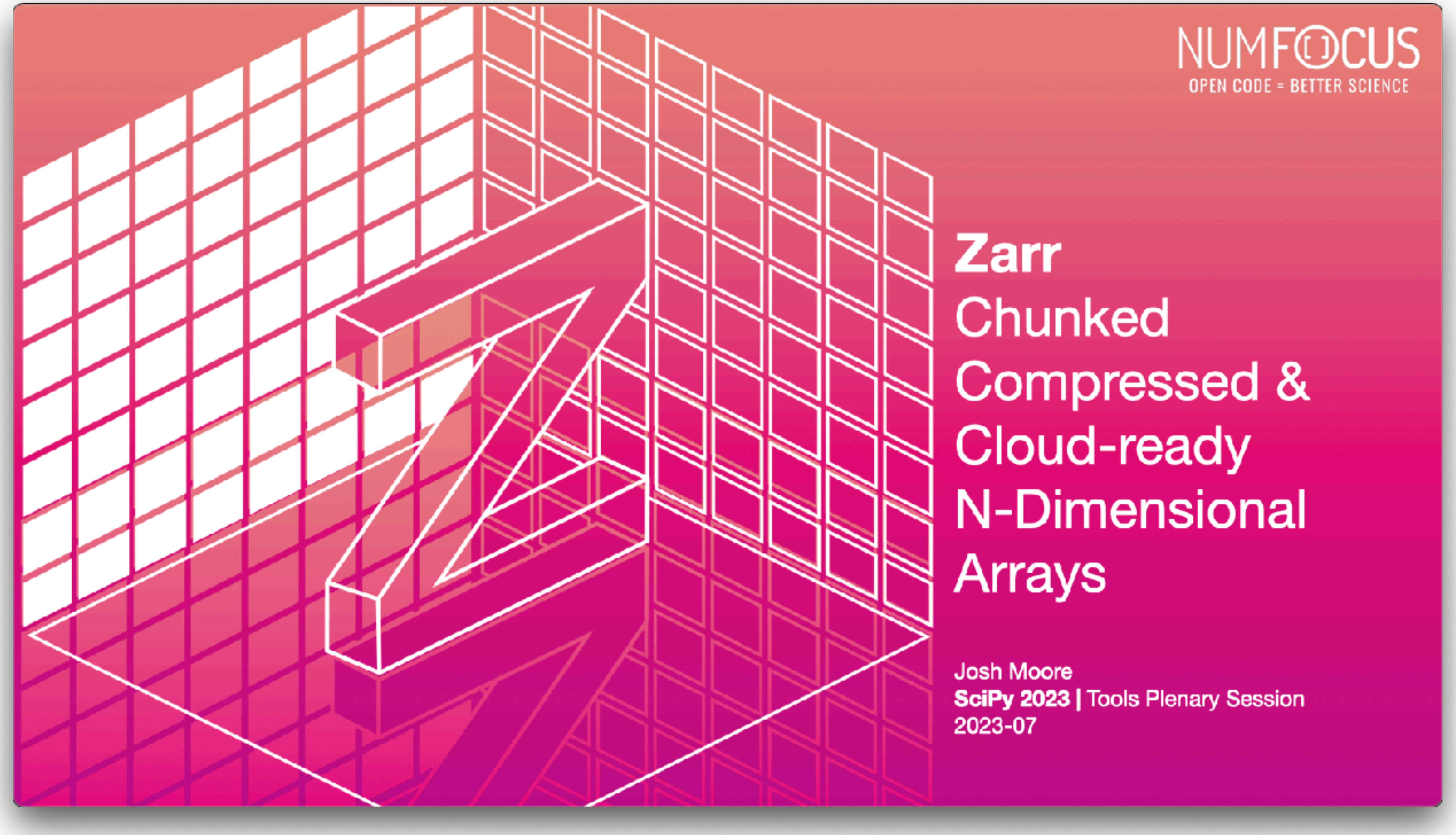
https://ngff.openmicroscopy.org/rfc/2/

RFC-2 | **Zarr Update**

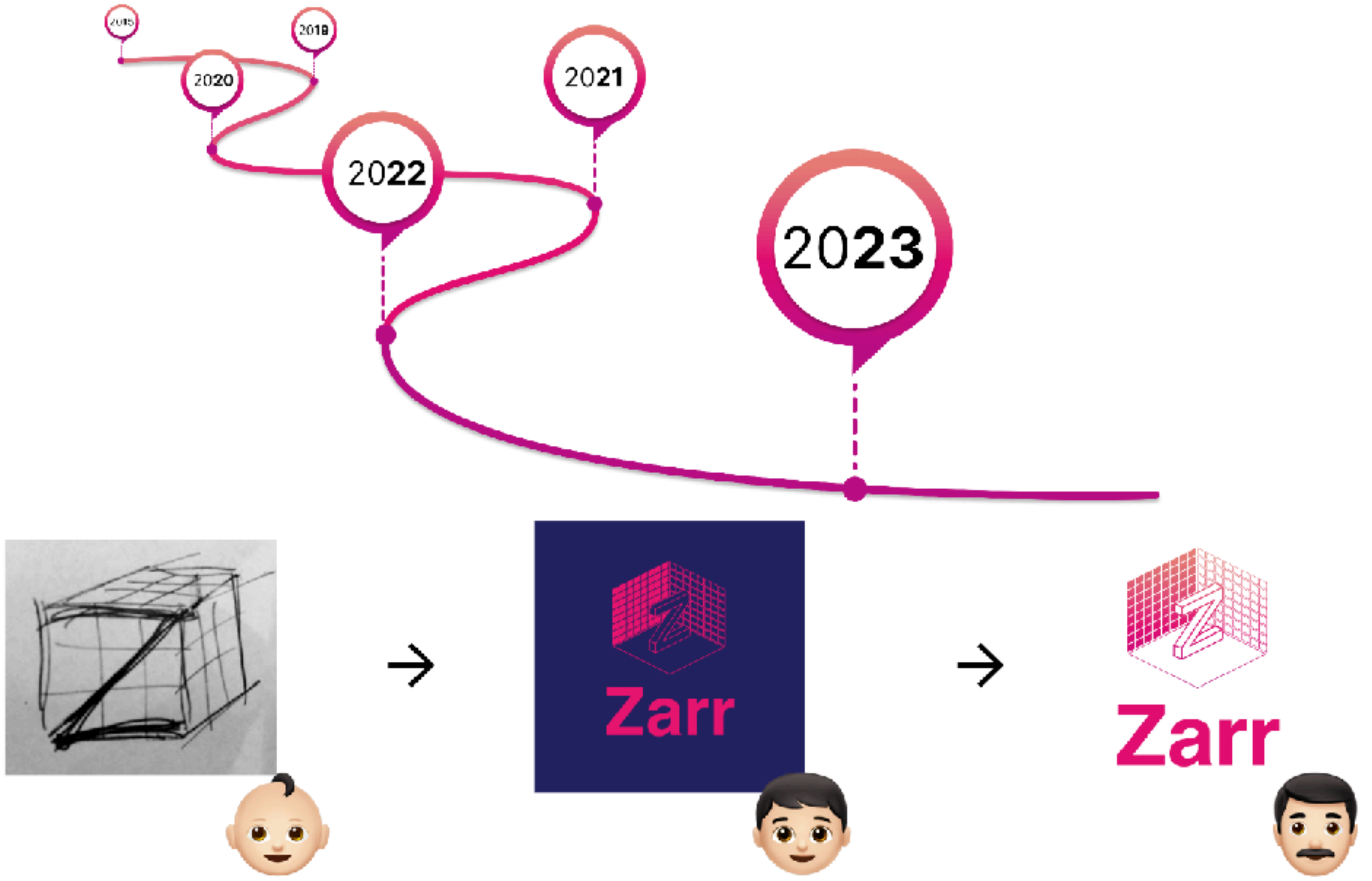


NUMFOCUS
OPEN CODE = BETTER SCIENCE

Zarr
Chunked
Compressed &
Cloud-ready
N-Dimensional
Arrays

Josh Moore
SciPy 2023 | Tools Plenary Session
2023-07

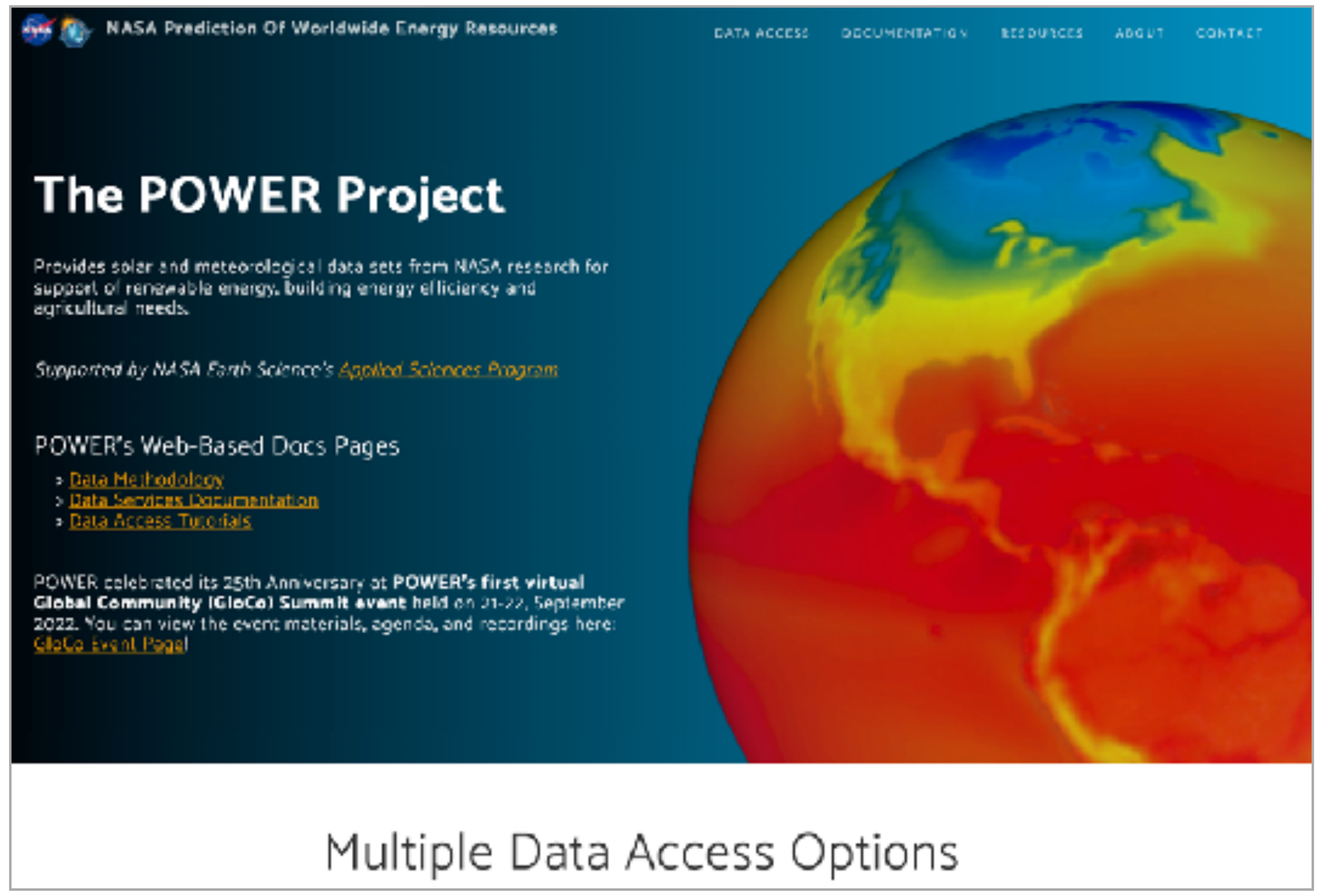
RFC-2 | **Zarr Update**



Slide courtesy: Sanket Verma (Zarr)

RFC-2 | **Zarr Update**

https://science.nasa.gov/open-science/transform-to-open-science



The POWER Project

Provides solar and meteorological data sets from NASA research for support of renewable energy, building energy efficiency and agricultural needs.

Supported by NASA Earth Science's Applied Sciences Program

POWER's Web-Based Docs Pages

- Data Methodology
- Data Services Documentation
- Data Access Tutorials

POWER celebrated its 25th Anniversary at **POWER's first virtual Global Community (GloCo) Summit event held** on 21-22, September 2022. You can view the event materials, agenda, and recordings here: GloCo Event Page!

Multiple Data Access Options

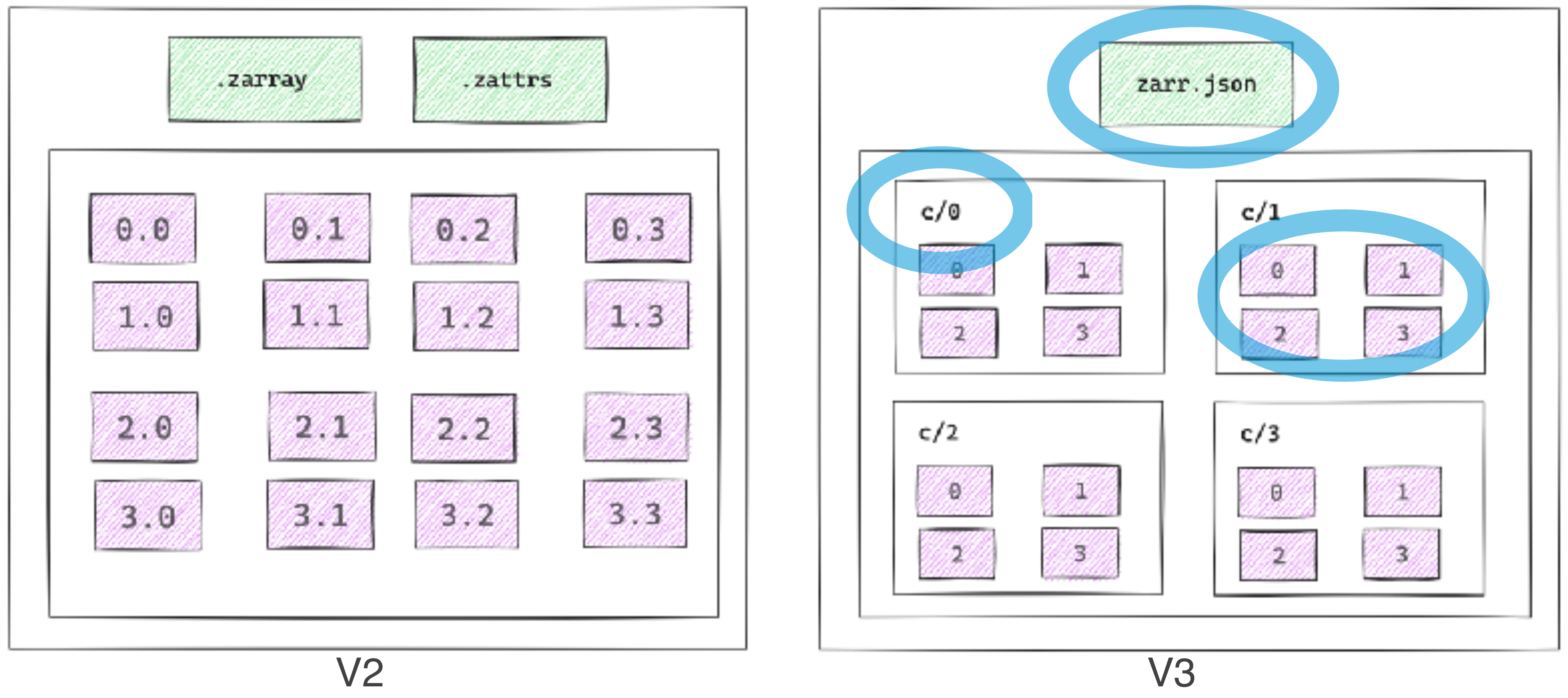
https://power.larc.nasa.gov/

Microsoft NASA PANGEO unidata

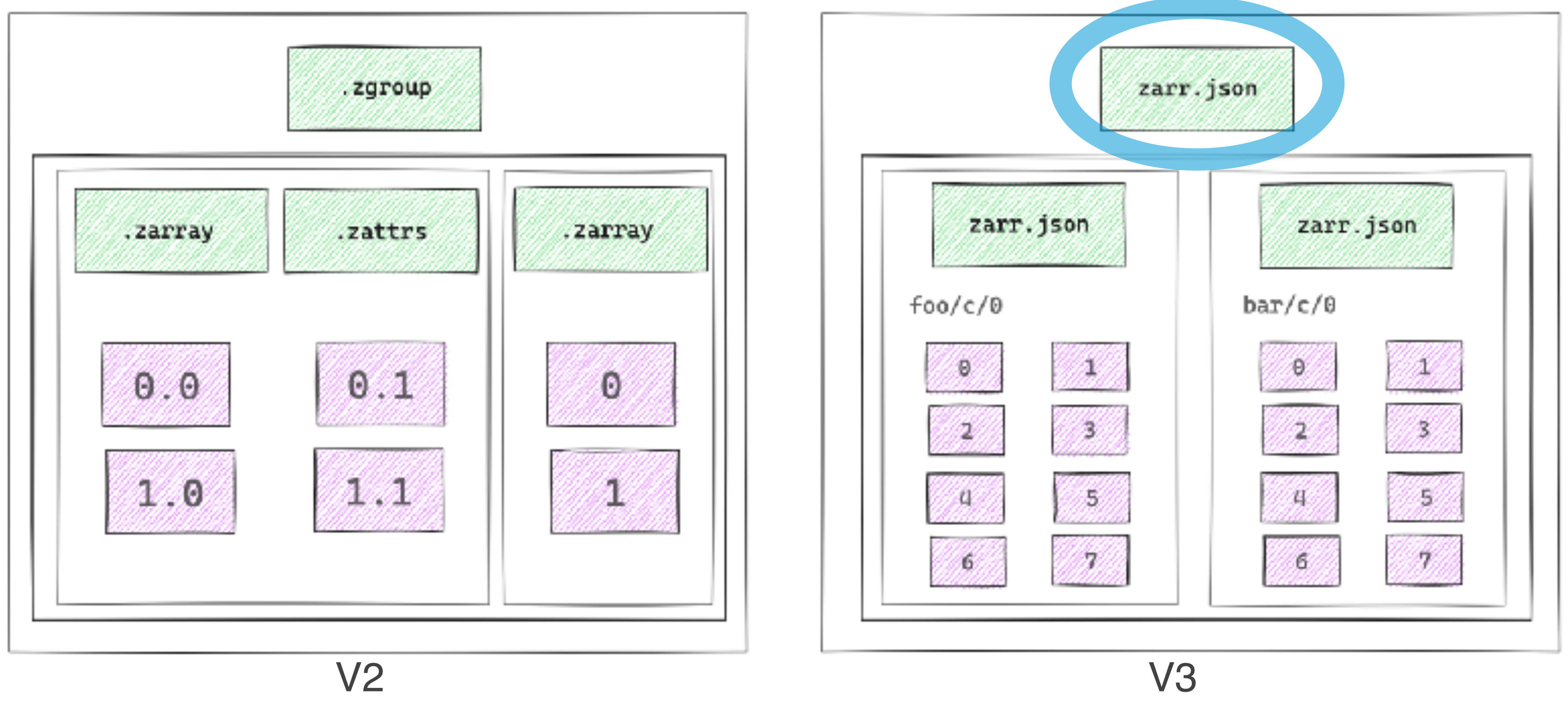
https://zarr.dev/adopters/

RFC-2 | **Zarr Update** (V3 Details)

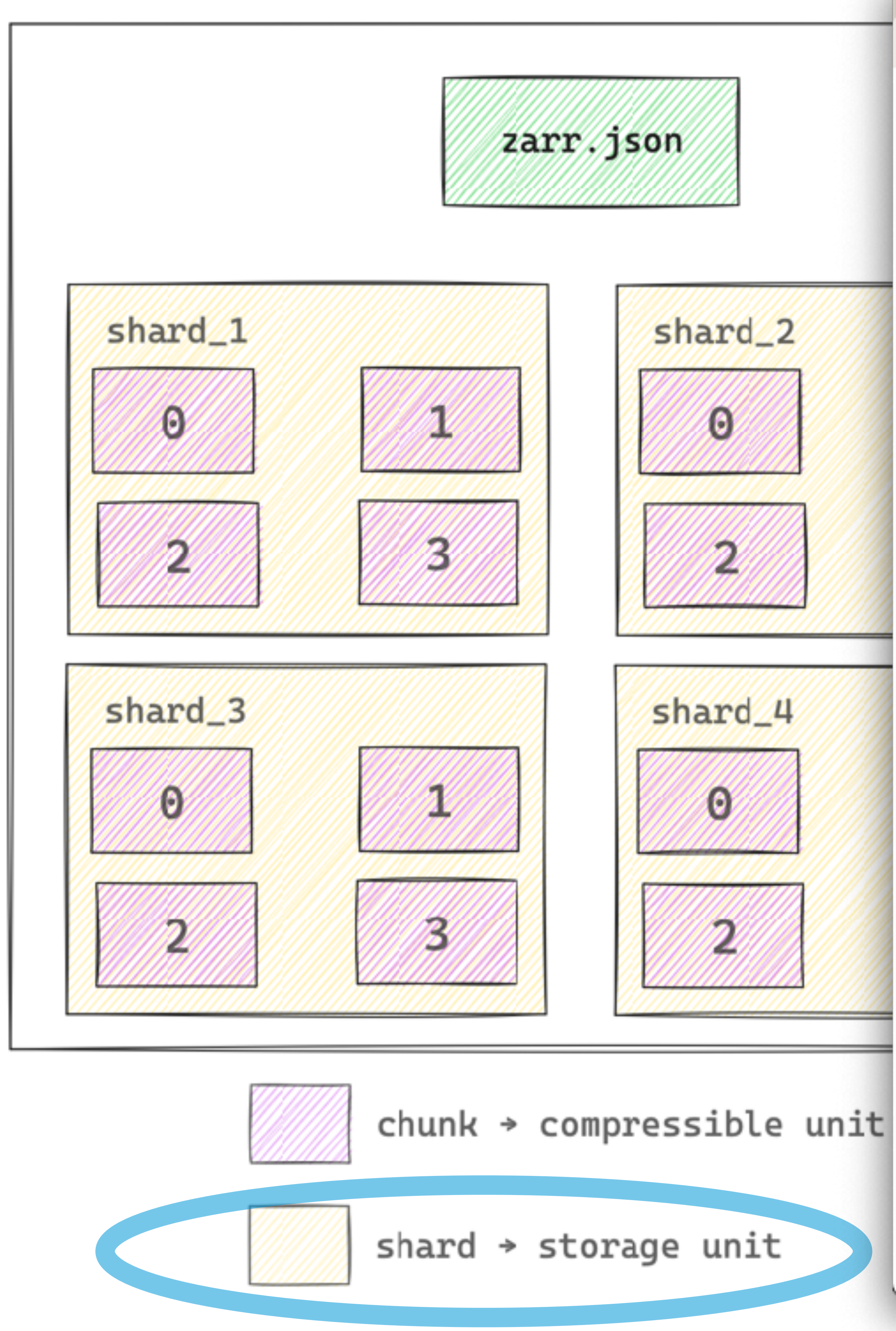
Arrays

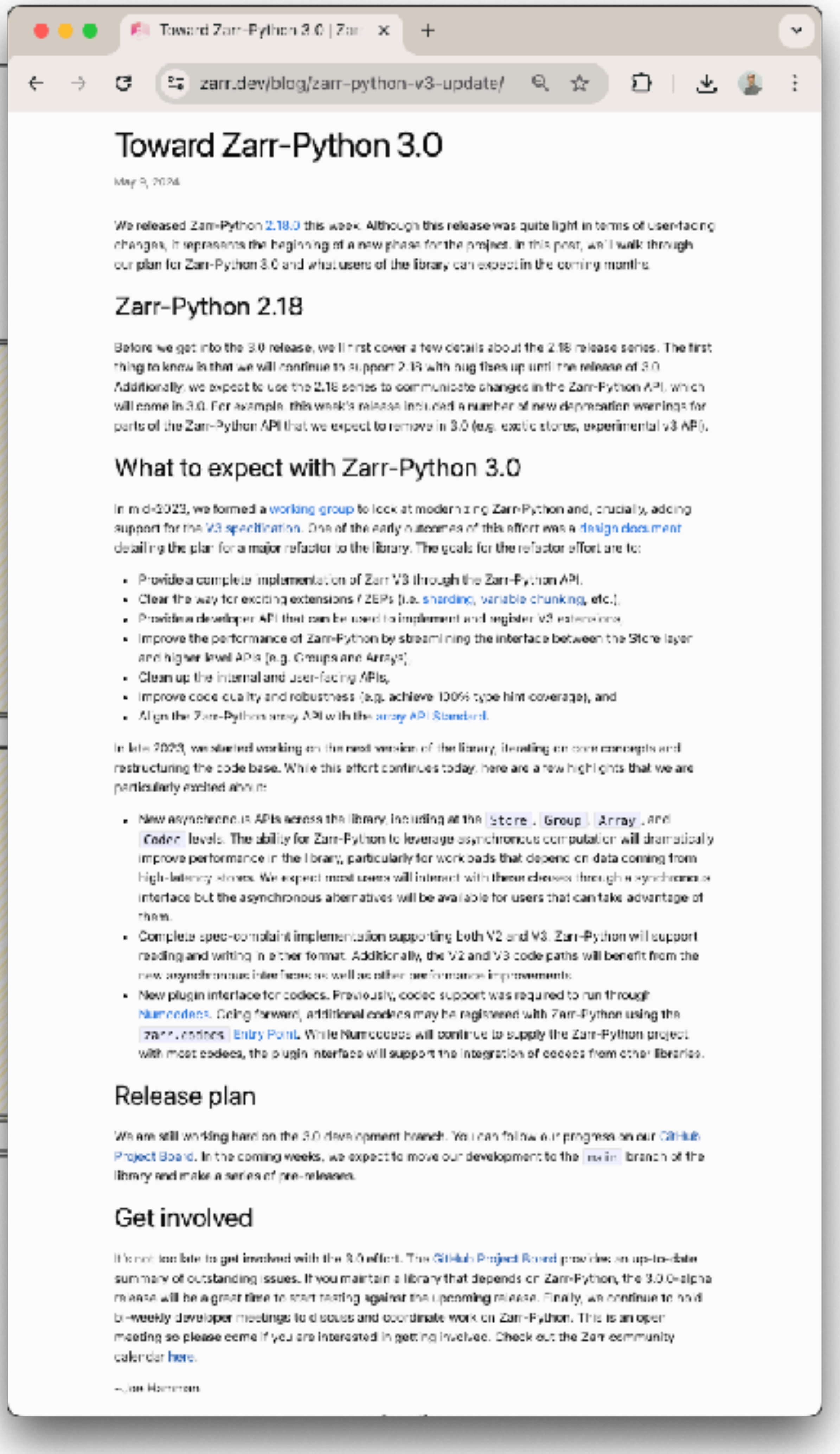


Groups



Sharding





Toward Zarr-Python 3.0

May 9, 2024

We released Zarr-Python 2.18.0 this week. Although this release was quite light in terms of user-facing changes, it represents the beginning of a new phase for the project. In this post, we'll walk through our plan for Zarr-Python 3.0 and what users of the library can expect in the coming months.

Zarr-Python 2.18

Before we get into the 3.0 release, we'll first cover a few details about the 2.18 release series. The first thing to know is that we will continue to support 2.18 with bug fixes up until the release of 3.0. Additionally, we expect to use the 2.18 series to communicate changes in the Zarr-Python API, which will come in 3.0. For example, this week's release included a number of new deprecation warnings for parts of the Zarr-Python API that we expect to remove in 3.0 (e.g. exotic stores, experimental v3 API).

What to expect with Zarr-Python 3.0

In mid-2023, we formed a working group to look at modernizing Zarr-Python and, crucially, adding support for the V3 specification. One of the early outcomes of this effort was a design document detailing the plan for a major refactor to the library. The goals for the refactor effort are to:

- Provide a complete implementation of Zarr V3 through the Zarr-Python API,
- Clear the way for exciting extensions / ZEPs (i.e. sharding, variable chunking, etc.),
- Provide a developer API that can be used to implement and register V3 extensions,
- Improve the performance of Zarr-Python by streamlining the interface between the Store layer and higher level APIs (e.g. Groups and Arrays),
- Clean up the internal and user-facing APIs,
- Improve code quality and robustness (e.g. achieve 100% type hint coverage), and
- Align the Zarr-Python array API with the array API Standard.

In late 2023, we started working on the next version of the library, iterating on core concepts and restructuring the code base. While this effort continues today, here are a few highlights that we are particularly excited about:

- New asynchronous APIs across the library, including at the `Store`, `Group`, `Array`, and `Codec` levels. The ability for Zarr-Python to leverage asynchronous computation will dramatically improve performance in the library, particularly for workloads that depend on data coming from high-latency stores. We expect most users will interact with these classes through a synchronous interface but the asynchronous alternatives will be available for users that can take advantage of them.
- Complete spec-compliant implementation supporting both V2 and V3. Zarr-Python will support reading and writing in either format. Additionally, the V2 and V3 code paths will benefit from the new asynchronous interfaces as well as other performance improvements.
- New plugin interface for codecs. Previously, codec support was required to run through Numcodecs. Going forward, additional codecs may be registered with Zarr-Python using the `zarr.codecs` Entry Point. While Numcodecs will continue to supply the Zarr-Python project with most codecs, the plugin interface will support the integration of codecs from other libraries.

Release plan

We are still working hard on the 3.0 development branch. You can follow our progress on our GitHub Project Board. In the coming weeks, we expect to move our development to the `main` branch of the library and make a series of pre-releases.

Get involved

It's not too late to get involved with the 3.0 effort. The GitHub Project Board provides an up-to-date summary of outstanding issues. If you maintain a library that depends on Zarr-Python, the 3.0-alpha release will be a great time to start testing against the upcoming release. Finally, we continue to hold bi-weekly developer meetings to discuss and coordinate work on Zarr-Python. This is an open meeting so please come if you are interested in getting involved. Check out the Zarr community calendar here.

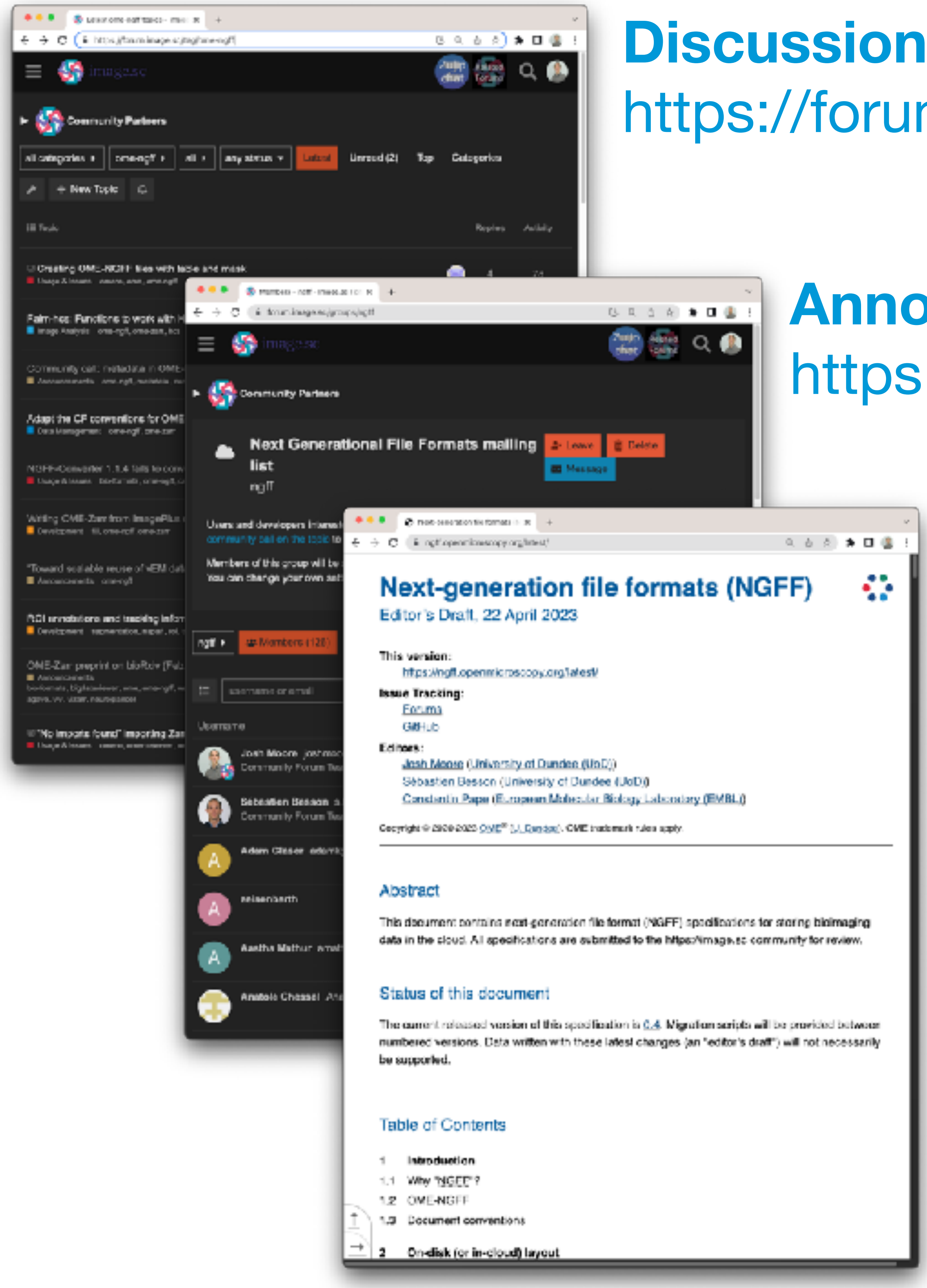
— Joe Hamman

https://zarr.dev/blog/zarr-python-v3-update/

Credit: Joe Hamman

Next steps

- Acquisition- & HPC-optimization
- Library & tool re-use
- Further language support (e.g. MATLAB & .NET)



Next-generation file formats (NGFF)

Editor's Draft, 22 April 2023

This version:
https://ngff.openmicroscopy.org/latest/

Issue Tracking:
Forums
GitHub

Editors:
Josh Moore (University of Dundee (UoD))
Sébastien Besson (University of Dundee (UoD))
Constantin Pape (European Molecular Biology Laboratory (EMBL))

Abstract

This document contains next-generation file format (NGFF) specifications for storing bioimaging data in the cloud. All specifications are submitted to the https://image.sc community for review.

Status of this document

The current released version of this specification is 0.4. Migration scripts will be provided between numbered versions. Data written with these latest changes (an "editor's draft") will not necessarily be supported.

Table of Contents

1 Introduction
1.1 Why "NGFF"?
1.2 OME-NGFF
1.3 Document conventions
2 On-disk (or in-cloud) layout

Discussion
https://forum.image.sc/tag/ome-ngff

Announcements
https://forum.image.sc/groups/ngff

Specification:
https://ngff.openmicroscopy.org/

Growing community but…

- Use more hands
- Adoption
- Financial commitment

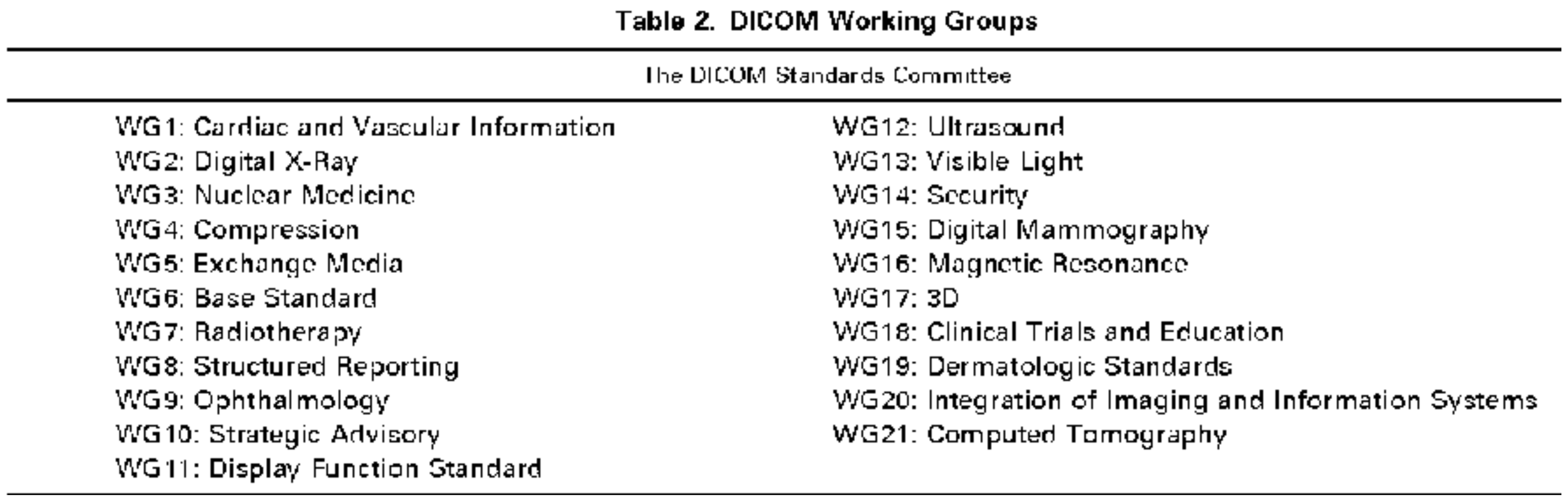


Table 2. DICOM Working Groups

The DICOM Standards Committee

WG1: Cardiac and Vascular Information
WG2: Digital X-Ray
WG3: Nuclear Medicine
WG4: Compression
WG5: Exchange Media
WG6: Base Standard
WG7: Radiotherapy
WG8: Structured Reporting
WG9: Ophthalmology
WG10: Strategic Advisory
WG11: Display Function Standard
WG12: Ultrasound
WG13: Visible Light
WG14: Security
WG15: Digital Mammography
WG16: Magnetic Resonance
WG17: 3D
WG18: Clinical Trials and Education
WG19: Dermatologic Standards
WG20: Integration of Imaging and Information Systems
WG21: Computed Tomography

Acknowledgments

NFDI4BIOIMAGE

University of Dundee
(2005-2022)

wellcome

BBSRC

Chan Zuckerberg Initiative

EOSC-Life

CORBEL

GLOBAL BIOIMAGING
growing collaboration

EURO-BIOIMAGING

German BioImaging
Gesellschaft für Mikroskopie und Bildanalyse

An OME contributor

Chan Zuckerberg Initiative

leap

nfdi
Nationale Forschungsdaten Infrastruktur

DFG Deutsche Forschungsgemeinschaft
German Research Foundation

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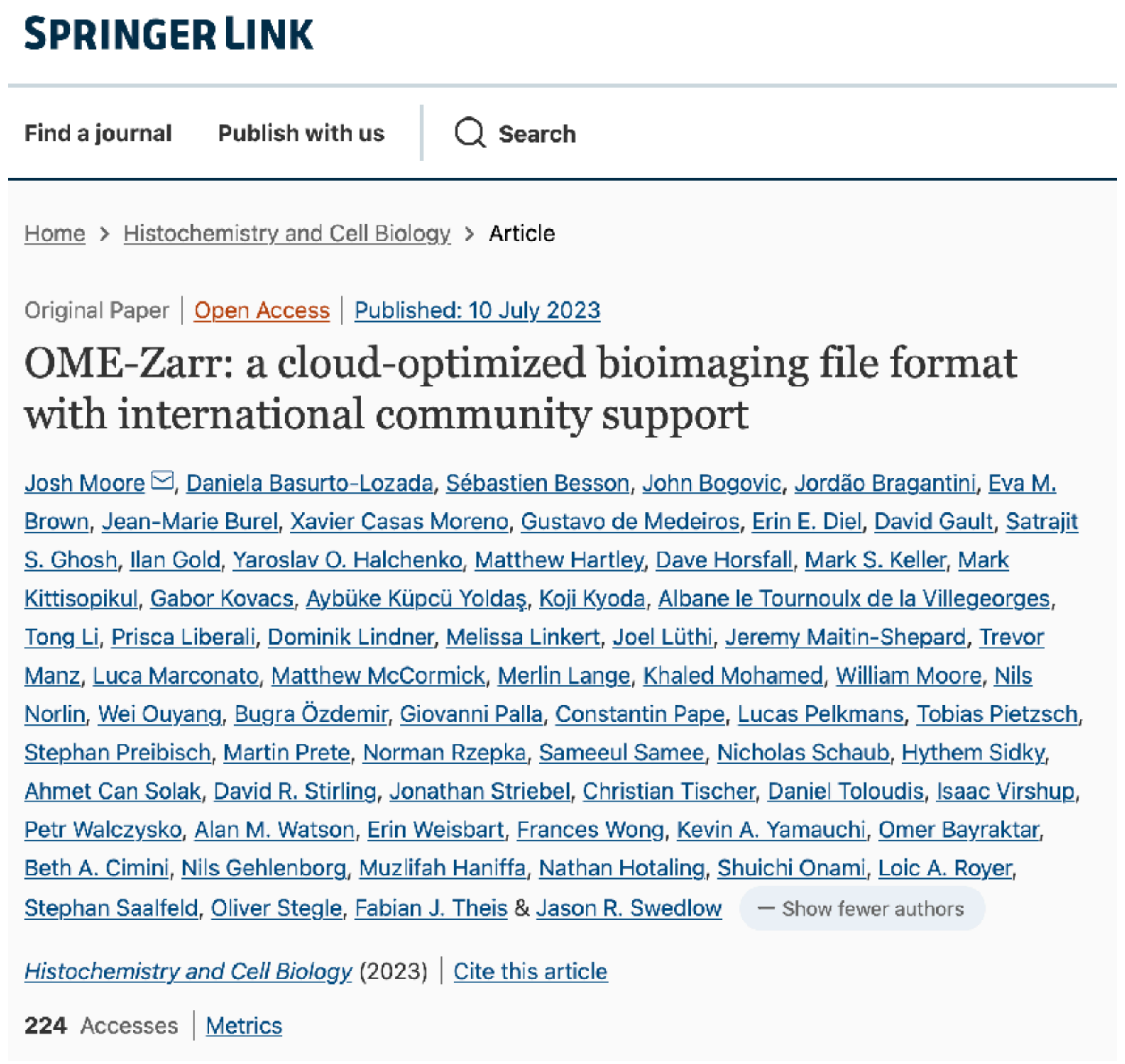

SPRINGER LINK

Original Paper | Open Access | Published: 10 July 2023

OME-Zarr: a cloud-optimized bioimaging file format with international community support

Josh Moore, Daniela Basurto-Lozada, Sébastien Besson, John Bogovic, Jordão Bragantini, Eva M. Brown, Jean-Marie Burel, Xavier Casas Moreno, Gustavo de Medeiros, Erin E. Diel, David Gault, Satrajit S. Ghosh, Ilan Gold, Yaroslav O. Halchenko, Matthew Hartley, Dave Horsfall, Mark S. Keller, Mark Kittisopikul, Gabor Kovacs, Aybüke Küpcü Yoldaş, Koji Kyoda, Albane le Tournoulx de la Villegeorges, Tong Li, Prisca Liberali, Dominik Lindner, Melissa Linkert, Joel Lüthi, Jeremy Maitin-Shepard, Trevor Manz, Luca Marconato, Matthew McCormick, Merlin Lange, Khaled Mohamed, William Moore, Nils Norlin, Wei Ouyang, Bugra Özdemir, Giovanni Palla, Constantin Pape, Lucas Pelkmans, Tobias Pietzsch, Stephan Preibisch, Martin Prete, Norman Rzepka, Sameeul Samee, Nicholas Schaub, Hythem Sidky, Ahmet Can Solak, David R. Stirling, Jonathan Striebel, Christian Tischer, Daniel Toloudis, Isaac Virshup, Petr Walczysko, Alan M. Watson, Erin Weisbart, Frances Wong, Kevin A. Yamauchi, Omer Bayraktar, Beth A. Cimini, Nils Gehlenborg, Muzlifah Haniffa, Nathan Hotaling, Shuichi Onami, Loic A. Royer, Stephan Saalfeld, Oliver Stegle, Fabian J. Theis & Jason R. Swedlow

Histochemistry and Cell Biology (2023) | Cite this article

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& openmicroscopy.org/about/#consortium

Slides: https://downloads.openmicroscopy.org/presentations/2024/Dundee/Day1/

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