Publication-ready figures with OMERO

Workshop: **Bioimage data management and analysis with OMERO**

May 13th, 2024, Module 4

Trainers: Michele Bortolomeazzi, Riccardo Massei, **Christian Schmidt**

Programme

Module 1 (9 am - 10.15 am):
Basics of OMERO, data structuring and annotation
(Christian)

Module 2 (10.45 am - 12.45 pm):
OMERO and Fiji
(Michele)

Time for Lunch

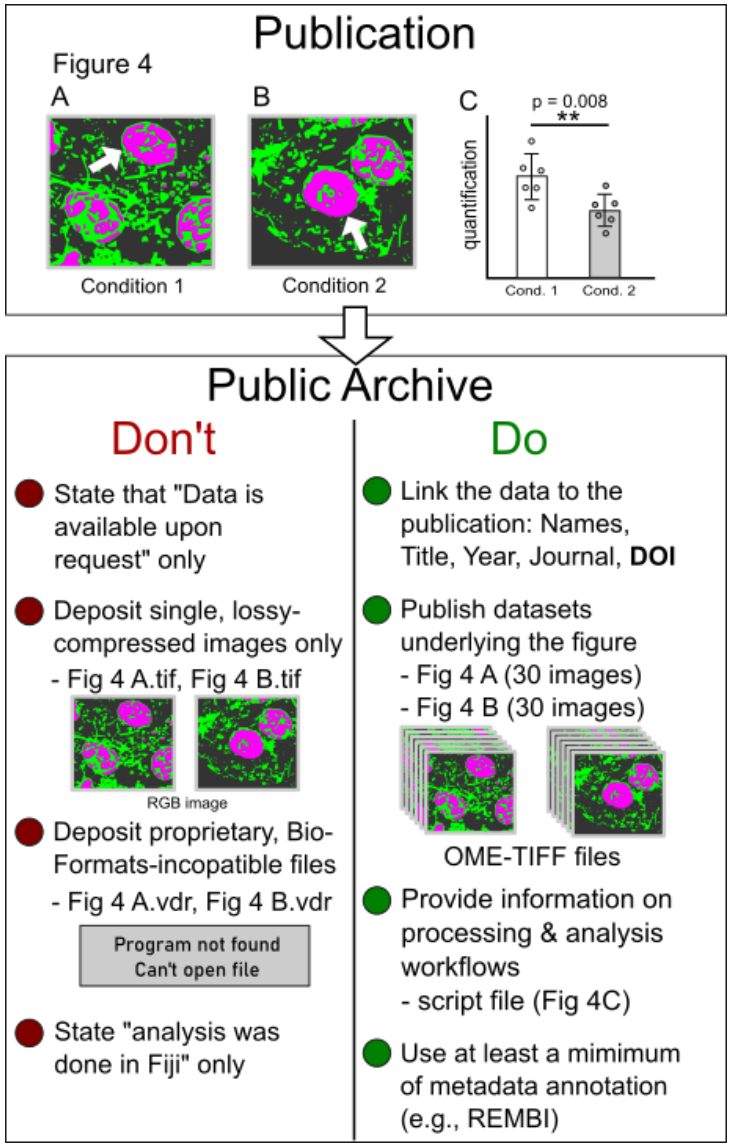
Module 3 (1.45 pm - 3.45 pm):
OMERO and Jupyter Notebooks
(Riccardo)

Module 4 (4.15 pm - 6. pm):
Publication-ready figures and data with OMERO
(Christian , Riccardo , Michele)

What makes a figure publication-ready?

- **Representation of the results**
  - Faithful
  - Complete
  - Allowed manipulations only
  - Easy to understand
  - Well described

- **Publishing the images?**
  - Not at all?
  - Only in the manuscript?
  - Public repositories:
    - BioImage Archive
    - Image Data Resource
    - others



https://gerbi-gmb.de/2023/05/31/introduction-to-image-data-repositories-and-public-archives/

Schmied, C., Nelson, M.S., Avilov, S. *et al.* Community-developed checklists for publishing images and image analyses. *Nat Methods* **21**, 170–181 (2024). https://doi.org/10.1038/s41592-023-01987-9

Focus for Module 4

Recommended Metadata for Biological Images (REMBI)

(Ontologies)

OMERO.figure

The following slides are taken from:
Fuchs, V. A. F., Schmidt, C., & Boissonnet, T. (2024, Mai 6). [Workshop] FAIR data handling for microscopy: Structured metadata annotation in OMERO. Zenodo. https://doi.org/10.5281/zenodo.11109616

REMBI provides guidelines for metadata for biological images

Metadata collected in 8 modules

See Sarkans et al., 2021,
https://doi.org/10.1038/s41592-021-01166-8

“Fig. 2: Different categories of metadata that are covered by REMBI.”

Original publication: https://doi.org/10.1038/s41592-021-01166-8
https://www.ebi.ac.uk/bioimage-archive/rembi-help-overview/

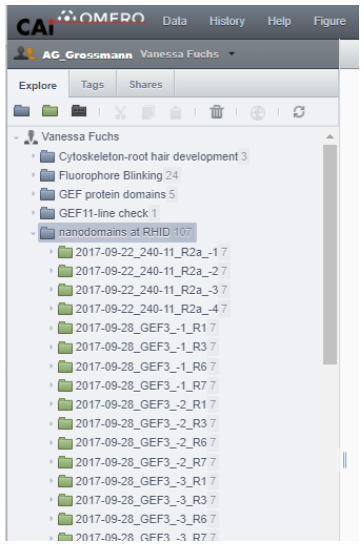
REMBI module 1: Study

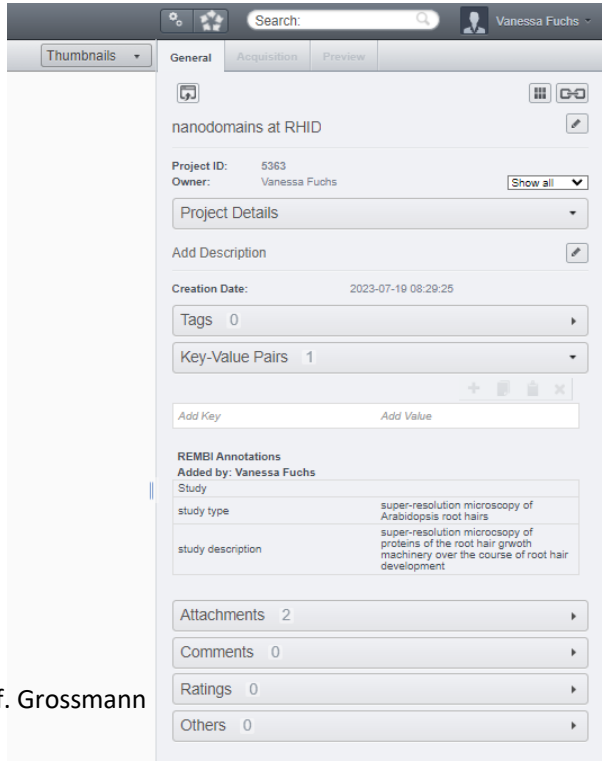
"**Study** is the highest level metadata, describing your project, including funding and publications."

Study			
(contains 1 or more	Study type	Type of the overall study, which may include	text, ontology
	Study description	Study description, e.g., title of published paper	text
	General dataset info	Authors, publications, licenses etc	misc.

Recommendation by I3D:bio:

Key-Value pairs in OMERO at the "Project"-level:





Data acquired in the lab of Prof. Grossmann
https://www.icib.hhu.de/

I3D:bio project: https://www.i3dbio.de/
Original publication: https://doi.org/10.1038/s41592-021-01166-8
https://www.ebi.ac.uk/bioimage-archive/rembi-help-overview/

REMBI module 2: Study component

Study Component acts as a container that helps you organise your data, based on experiment types or samples etc. A Study Component contains one or more of the following components: biosample, specimen, image acquisition, image correlation, image analysis (latter two are only required if relevant).

Study component			
(contains Image data	Imaging method	Technique used to acquire image data	ontology
	Study component description	Description specific to this image dataset	text

Recommendation by I3D:bio:

One per Dataset (Key-Value Pairs in OMERO at the Dataset-level)

I3D:bio

I3D:bio project: https://www.i3dbio.de/
Original publication: https://doi.org/10.1038/s41592-021-01166-8
https://www.ebi.ac.uk/bioimage-archive/rembi-help-overview/

REMBI module 3: Biosample

Biosample describes what you have imaged, for example: the species, the organism, a particular cell line, genetic background etc.

Biosample			
	Identity	Internal unique ID	
	Biological entity	What is being imaged	text and/or ontology entry (multiple possible)
	Organism	Species (multiple possible)	taxonomy
	Intrinsic variable	Intrinsic (e.g. genetic) alteration if applicable	text and/or ontology entry (multiple possible)
	Extrinsic variable	External biosample treatment (e.g. reagent) if applicable	text and/or ontology entry (multiple possible) or associated file
	Experimental variables	What is intentionally varied (e.g. time) between multiple entries in this study component	text and/or ontology entry (multiple possible)

Recommendation by I3D:bio:

One per Dataset (Key-Value Pairs in OMERO at the Dataset-level)

I3D:bio

I3D:bio project: https://www.i3dbio.de/
Original publication: https://doi.org/10.1038/s41592-021-01166-8
https://www.ebi.ac.uk/bioimage-archive/rembi-help-overview/

REMBI module 4: Specimen

Specimen metadata describes how your sample was prepared for imaging.

Specimen			
(linked to Biosample)	Experimental status	Test/ control	
	Location within Biosample	Plate/dish coordinate or tissue location	text or associated file
	Preparation method	Sample preparation protocol	text, file, ontology, or widget for specific method types
	Signal/contrast mechanism	How is the signal generated by this sample	text, ontology
	Channel - content	Specific specimen staining (e.g. IEM, DAB)	text
	Channel - biological entity	What molecule is stained	text, ontology entries

Recommendation by I3D:bio:
One per Dataset (Key-Value Pairs in OMERO at the Dataset-level)

I3D:bio project: https://www.i3dbio.de/
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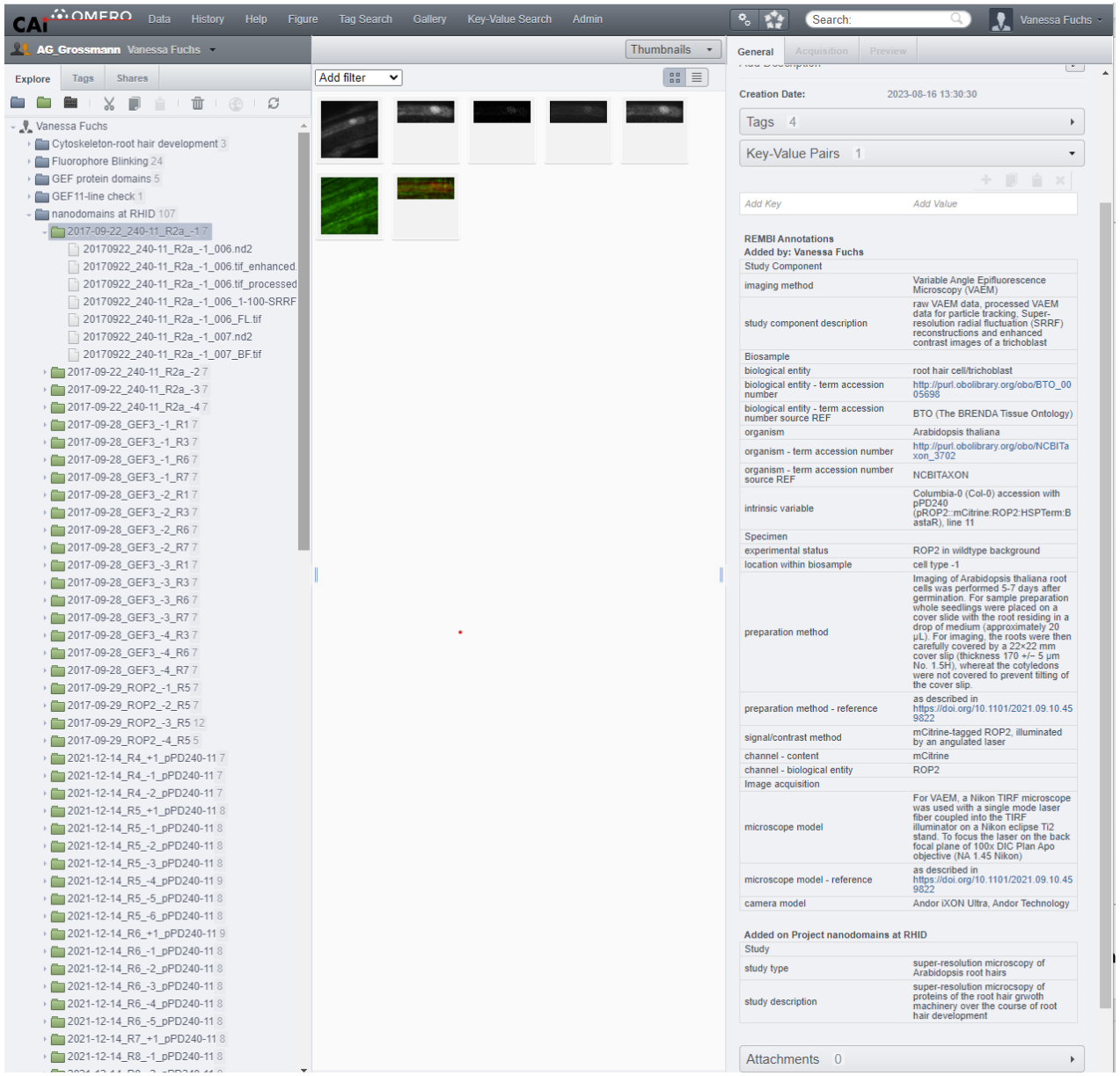
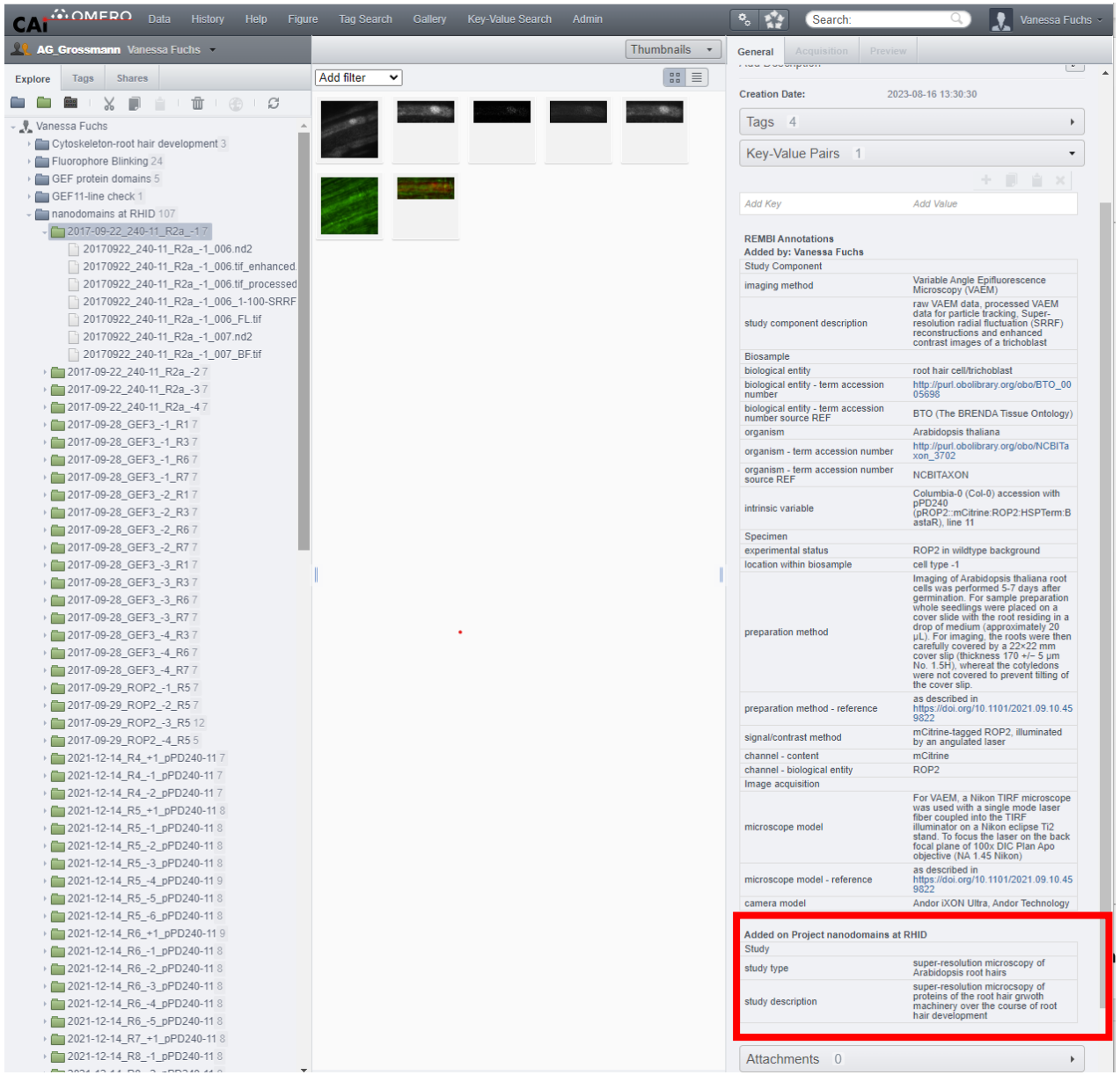
REMBI at CAi



Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

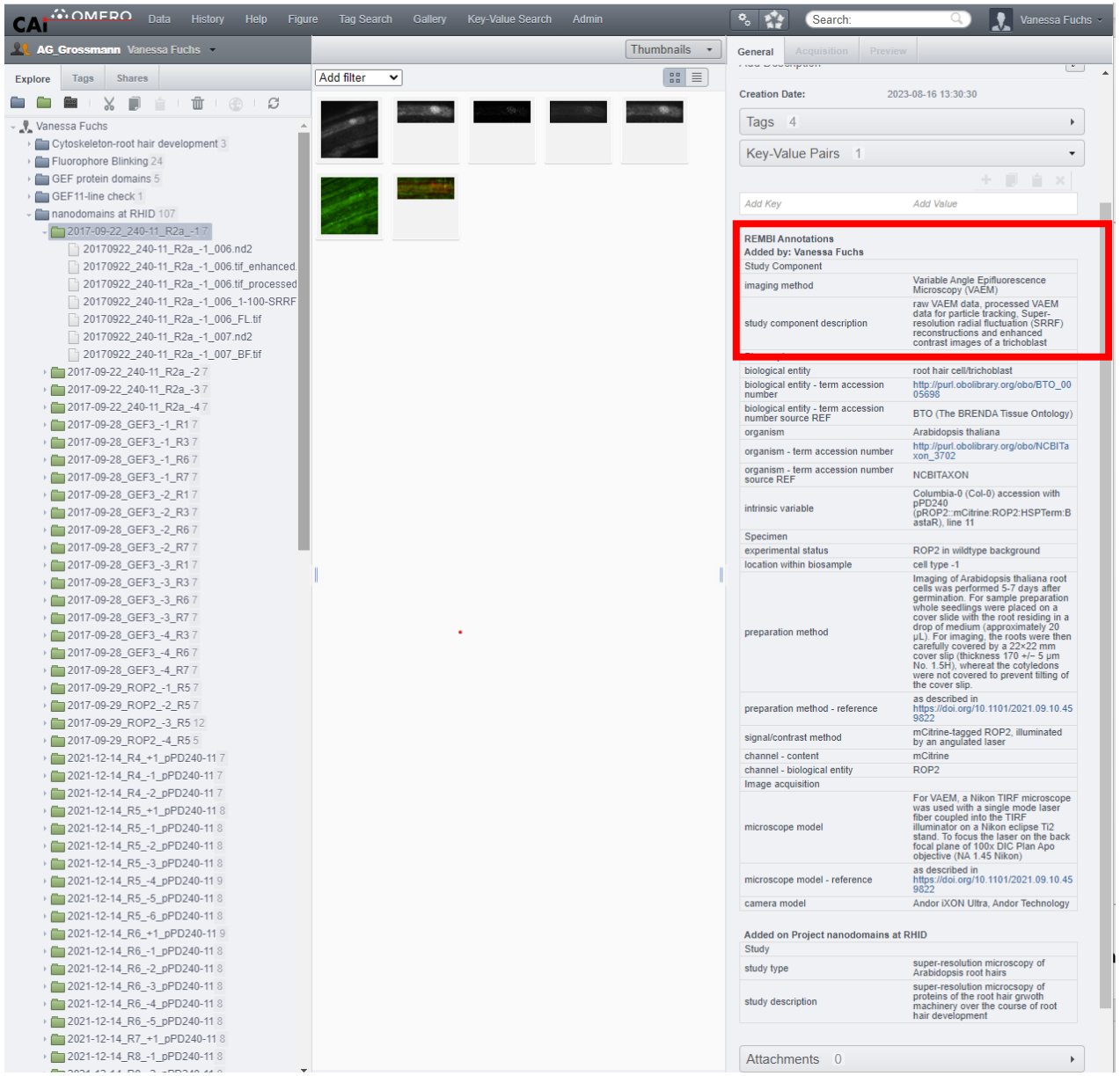
REMBI at CAi



Module1: Study – project level

Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

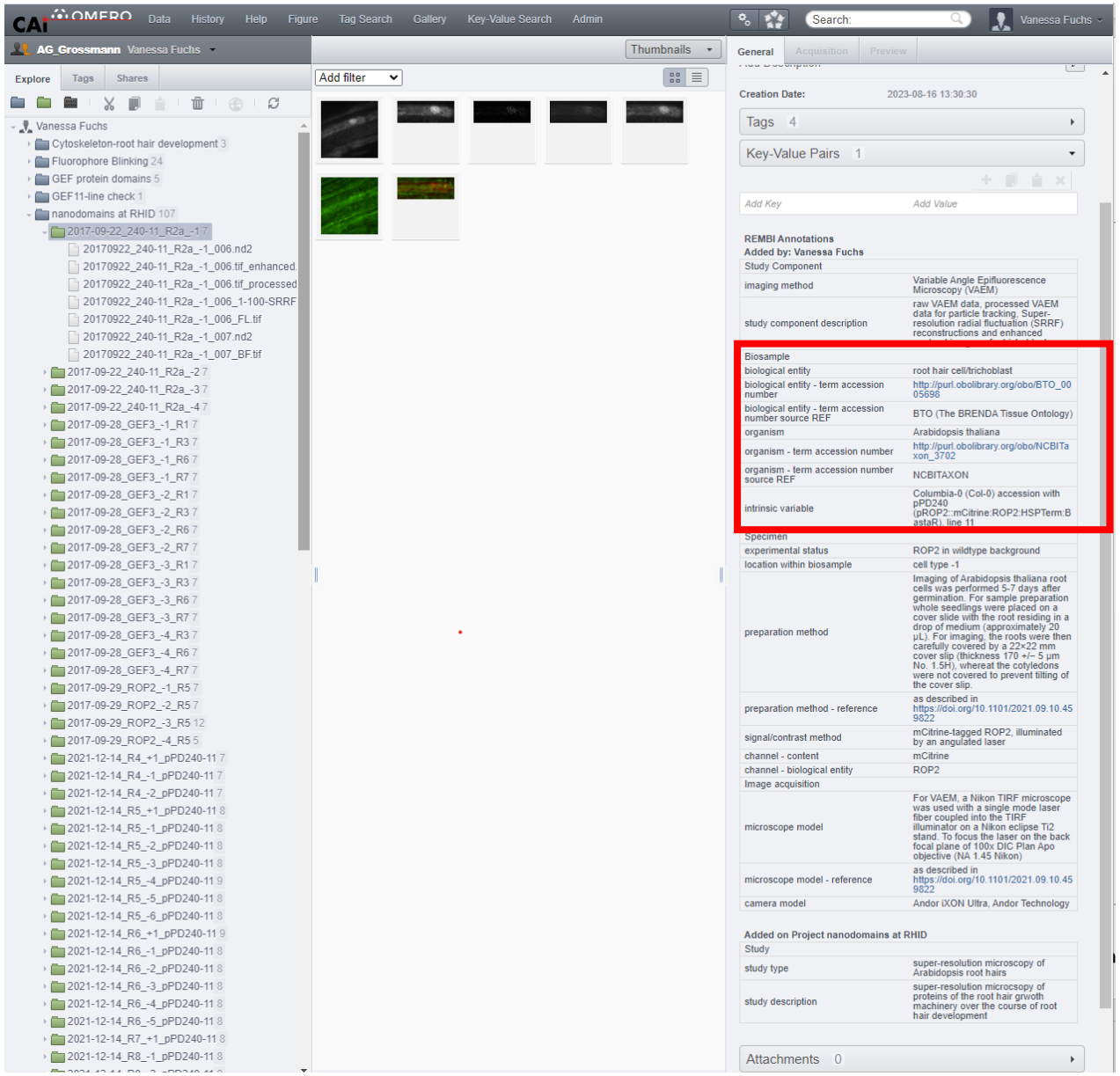
REMBI at CAi



Module2: Study component – dataset level

Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

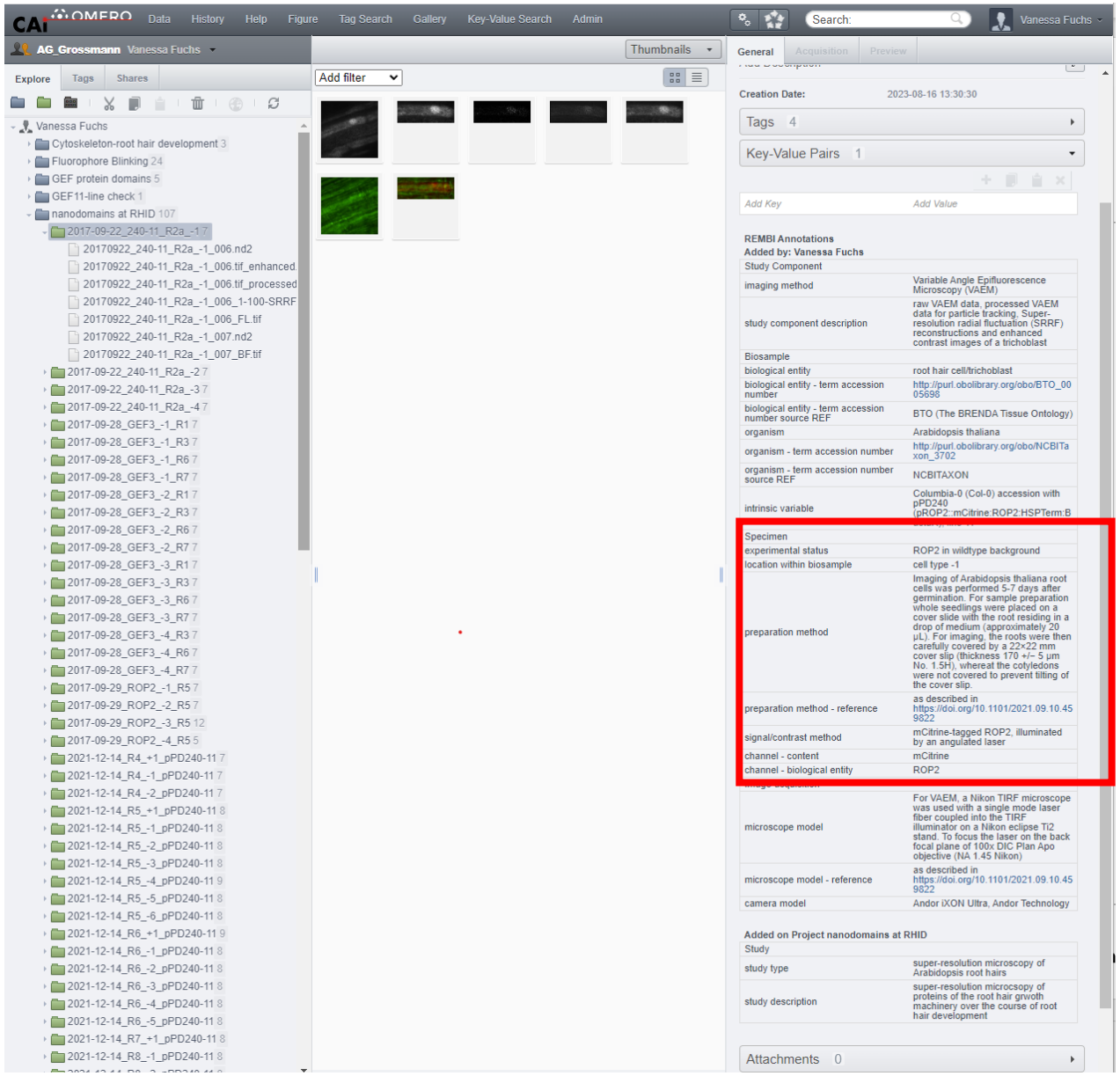
REMBI at CAi



Module 3: Biosample – dataset level

Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

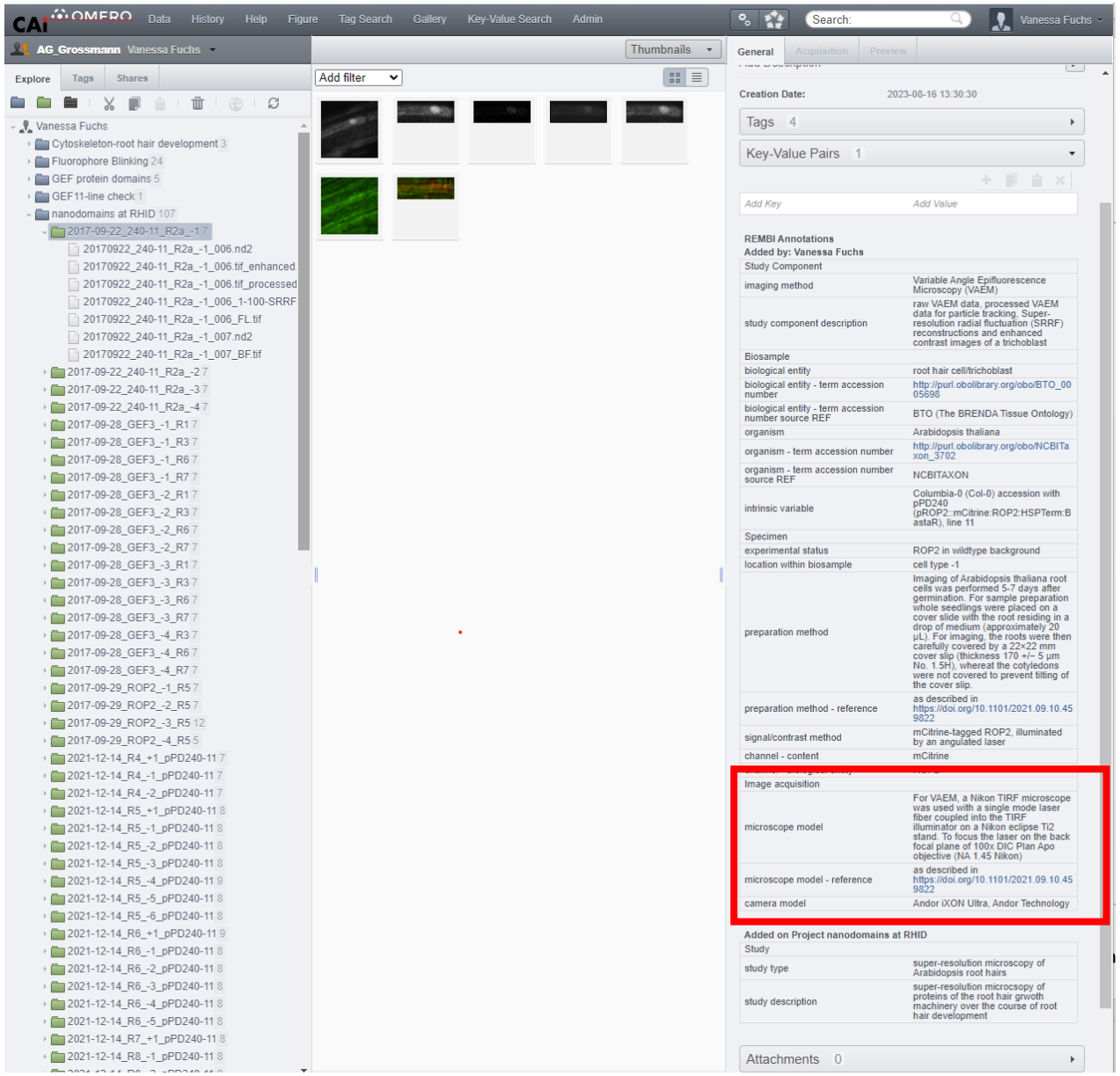
REMBI at CAi



Module 4: Specimen – dataset level

Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

REMBI at CAi



Module 5: Image acquisition – dataset level

Data acquired in the lab of Guido Grossmann
https://www.icib.hhu.de/

A brief glance at ontologies

What is an image?

Microsoft Bing search for „image definition“

1. a representation of the external form of a person or thing in art:
   "her work juxtaposed images from serious and popular art"

   Ähnlich: likeness | resemblance | depiction | portrayal | representation

2. the general impression that a person, organization, or product presents to the public:
   "she strives to project an image of youth"

   Ähnlich: persona | profile | face | identity | front | facade | mask | guise

3. a simile or metaphor:
   "he uses the image of a hole to describe emotional emptiness"

   Ähnlich: simile | metaphor | metonymy | figure of speech | trope

Asking a microscopist

https://en.wikipedia.org/wiki/Virtual_image

> In optics, the *image* of an object is defined as the collection of focus points of light rays coming from the object. A *real image* is the collection of focus points made by converging rays, while a **virtual image** is the collection of focus points made by extensions of diverging rays.

Asking a research software engineer…

https://docs.docker.com/guides/docker-concepts/the-basics/what-is-an-image/

> A container image is a standardized package that includes all of the files, binaries, libraries, and configurations to run a container.

Technical terms in science

Key: „cell type“ **Value:** „CD4+ T cell“

Key: „disease model“ **Value:** „Experimental Autoimmune Encephalomyelitis“

„cell type“ „type of cell“ „cell-type“ „cellular entity“ „cellular identity“

„CD4+ T cell“ „CD4-positive T-lymphocyte“ „naive, CD4-positive T cell“

„CD4-positive, alpha-beta T cell“ „Th0 cell“ „CD4+ T helper cell“

„Experimental Autoimmune Encephalomyelitis“ „EAE“ „Allergic Encephalomyelits“

How to avoid ambiguity?
How to describe the data objectively?
How to make the metadata machine-interpretable?

Ontologies

An **ontology** is a conceptual framework of how specific terms are used to represent *domain knowledge* in a (research) domain.

- Defines term attributes/properties, and relationships between the terms
- Terms with shared attributes are grouped into classes
- Terms in different ontologies are mapped to each other or adopted
- Can be extended over time with the evolving domain knowledge (i.e., an ontology is versioned)
- *Formalized*, i.e., ontologies can be expressed in ontology formats (machine-interpretable), e.g., OWL, SKOS, OBO

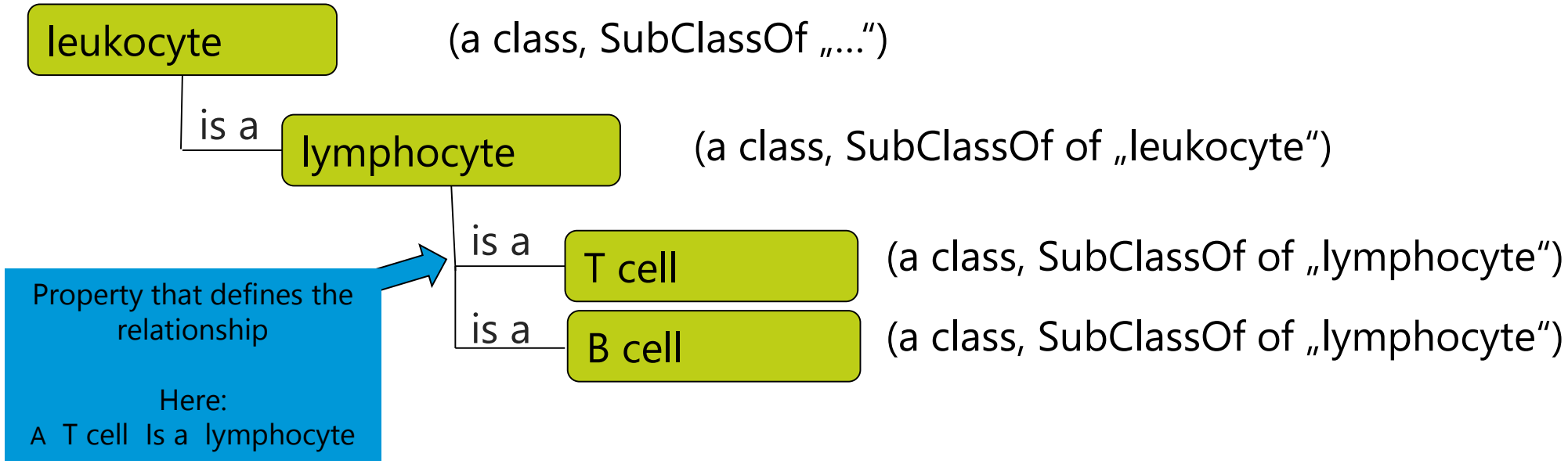
Examples of Ontologies:

- Experimental Factor Ontology (EFO) – curated by the EMBL EBI
- Biological Imaging Methods Ontology (FBbi) – curated by the Cell Image Library
- Cell Line Ontology (CLO) – community-based, curated at the University of Michigan

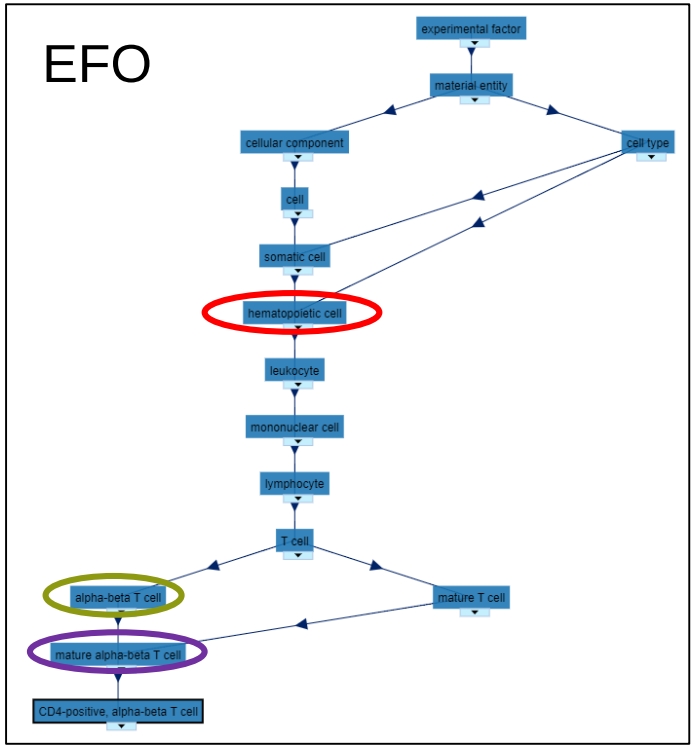
Examples of potentially useful ontologies

Different ontologies are designed to optimally ***represent their respective domain knowledge*** (for example, the relationship between terms)

This knowledge can be represented as a tree structure or „knowledge graph“. *Example:*



Advantage of using ontologies



A single Key-Value Pair can carry extended domain knowledge!

„CD4-positive, alpha-beta T cell“ following an ontology (here: EFO) includes more information from the domain knowledge formalized in the ontology (and cross-domain knowledge formalized by mapping):

- Is carrying a T cell receptor with αβ-chains
- Has completed thymic selection (i.e., is mature)
- Is a cell of the hematopoietic system
- etc…

Due to the ontology format, a computer can read the knowledge!

Examples of potentially useful ontologies

BAO	BioAssays Ontology
EDAM (EDAM BioImaging)	Ontology of bioscientific data analysis and data management
EFO	Experimental Factor Ontology
CLO	Cell Line Ontology
CL	Cell Ontology
GO	Gene Ontology
UBERON	Uber Anatomy Ontology
FBbi	Biological Imaging Methods Ontology
ChEBI	Chemical Entities of Biological Interest

Links resolve to https://www.go-fair.org/fair-principles/

Demonstration and Group Task

Try out different ontology lookup services:

Ontology Lookup Service	https://www.ebi.ac.uk/ols4/index
Semantic Lookup Service	https://semanticlookup.zbmed.de/ols/index
BioPortal Bioontology	https://bioportal.bioontology.org/
Ontobee	https://ontobee.org/

Recommendation for Ontologies in OMERO

- Where possible, use terms that are derived from a useful ontology
- How to indicate the Ontology-compliant term choice:

Key: Biological entity	**Value:** CD4-positive, alpha-beta T cell
Key: Biological entity Term Accession Number	**Value:** http://purl.obolibrary.org/obo/CL_0000624
Key: Biological entity Term Source REF	**Value:** http://www.ebi.ac.uk/efo/efo.owl *or* EFO

Why this style? Because it is already used by the ISA framework, hence, close to an annotation standard

- **Ontology compliance for *all* terms???**
  - Choose the essential keywords that represent your research
  - Identify a few ontologies that you can use sustainably

Exercise – Annotate data with REMBI

Option 1:
Use the training data (just make up the biological details behind it)

Option 2:
Work silently on your own data

Option 3:
Share with colleagues what you are working on and annotate a dataset together

Exercise – Bring structure to the data

OMERO.figure

(live demonstration)

Exercise – create figures

Create OMERO.figure:

KV-pair example

1. Select two examples at Week 1 and Week 2
2. Right click -> Open the images with OMERO.figure
3. in OMERO.figure, arrange your image in a grid
4. Adjust the contrasts and add the scale bar
5. In labels dropdown menu, select "key-value pair"
6. Add all the conditions you want to display
7. (you can do this for all four images at the same time)
8. Looking at the conditions of each image, you can rearrange them
9. Rearrange the labels per row columns to match your data
10. Adjust the page size in: File-> paper setup -> crop page around panel

Create OMERO.figure:

Multi-channel example

1. Select a z-stack from the example images
2. Duplicate the panel in OMERO.figure, one per channel + 1 for all channels
3. Set the colors of individual channels to gray level (except composite one)
4. Add scalebar
5. Add a label for the active channel, (channel separate labels)
6. copy paste the row of image, set the row to a different z-slice
7. Add a label for each row, showing the displayed z-slice depth
8. Adjust the page size in: File-> paper setup -> crop page around panel

Create OMERO.figure:

Zoomed view histology example

1. Select a histology image from the example images
2. Duplicate the panel in OMERO.figure, one per zoom level you want
3. For the zoomed version, in the preview, set the region
4. For the zoomed version, copy the coordinates (bottom of previey tvb)
5. Select the non-zoomed version
6. Go to Label tab, and under ROI, paste the coordinates
7. (if bug doesn't display ROI, save your figure and reload the page)

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https://www.i3dbio.de/

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- Support: Lena Krämer & Tom Boissonnet

Contact:

German Cancer Research Center (DKFZ), Heidelberg
Department Enabling Technology

Dr. Christian Schmidt, Project Coordinator

office@nfdi4bioimage.de

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