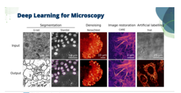
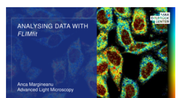
Query: How is AI used in bioimage analysis?
Approach A

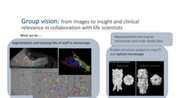
Query: How is AI used in bioimage analysis?
Approach B

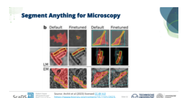
Query: What are the benefits of using machine learning in microscopy?
Approach A



Query: What are the benefits of using machine learning in microscopy?
Approach B

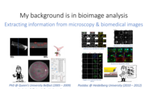




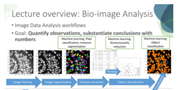


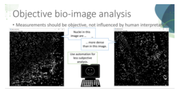
Query: What is bioimage analysis?
Approach A

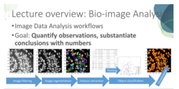
Query: What is bioimage analysis?
Approach B



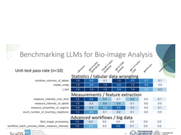
A plea to users of bioimage analysis software…

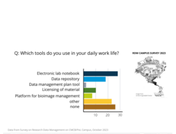




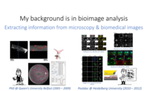


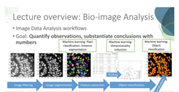
Query: What tools are commonly used for image analysis in bioimaging?
Approach A

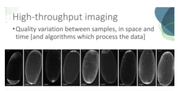


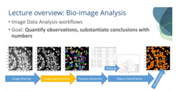


Query: What tools are commonly used for image analysis in bioimaging?
Approach B

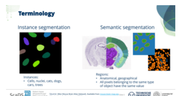


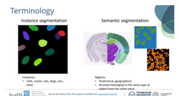


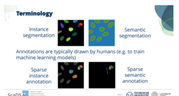


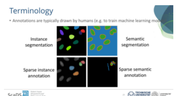


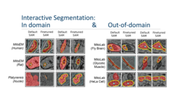
Query: What are different segmentation methods used for microscopy data analysis?
Approach A

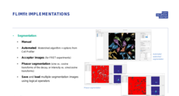


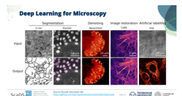




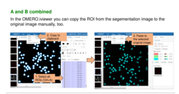


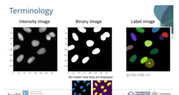


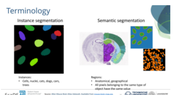


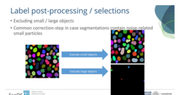


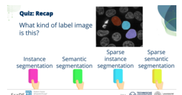
Query: What are different segmentation methods used for microscopy data analysis?
Approach B





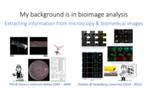




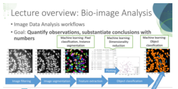


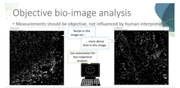
Query: What's the difference between bioinformatics and bioimage analysis?
Approach A

Query: What's the difference between bioinformatics and bioimage analysis?
Approach B

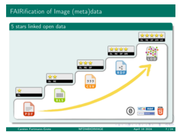


A plea to users of bioimage analysis software…

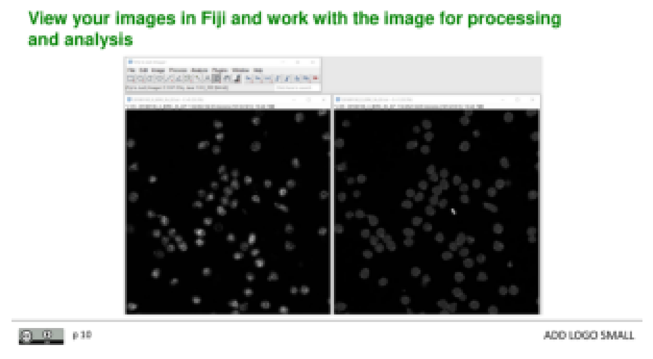


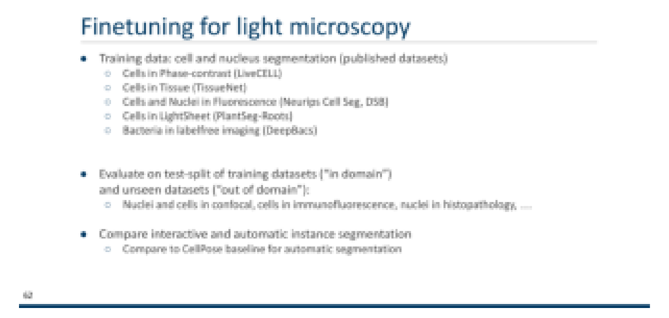


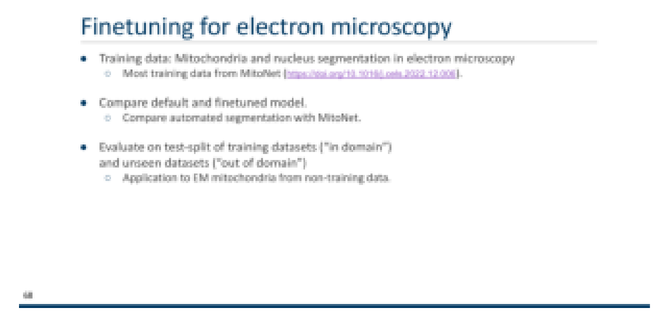
Query: How can I make my microscopy data FAIR?
Approach A

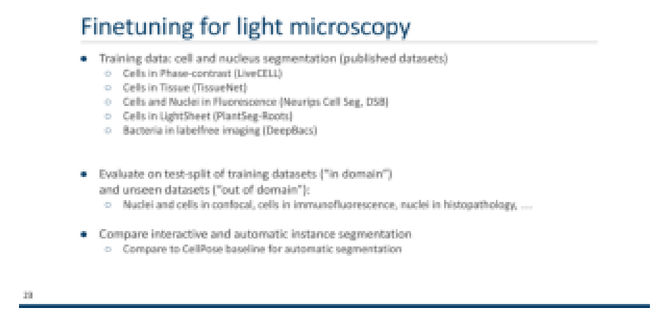


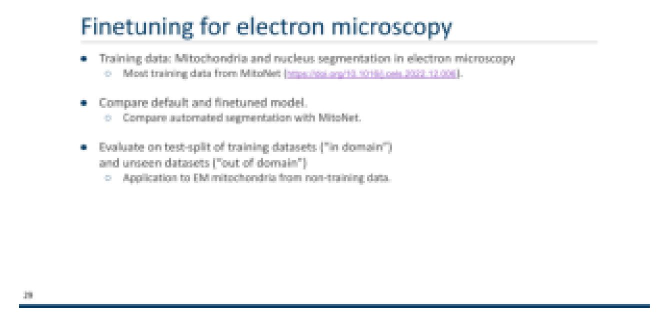
Query: How can I make my microscopy data FAIR?
Approach B

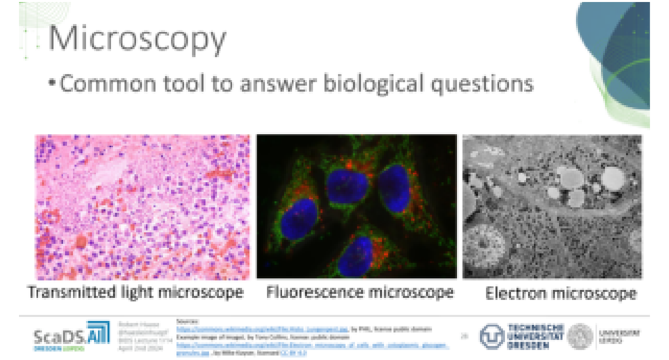


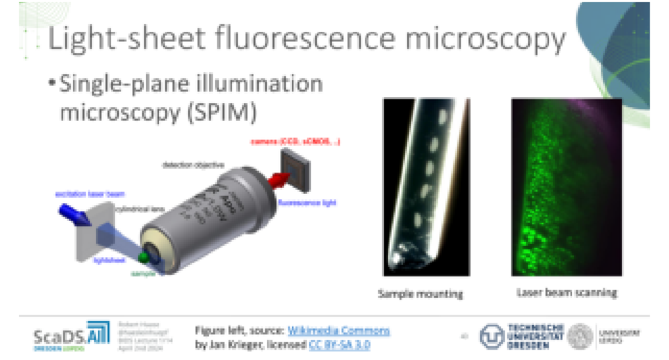


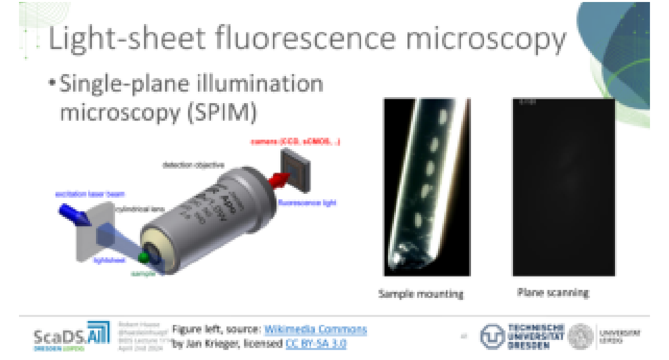


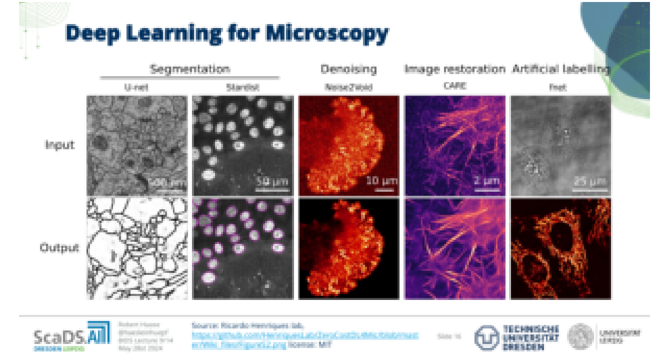






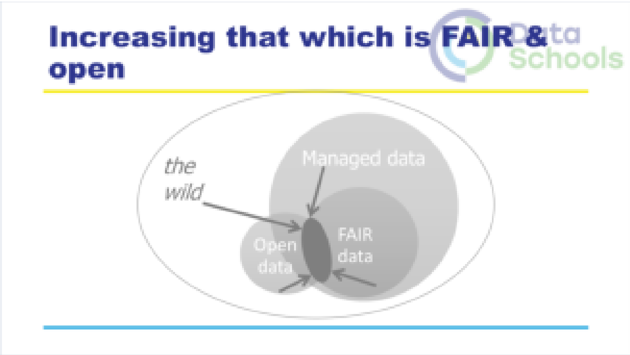


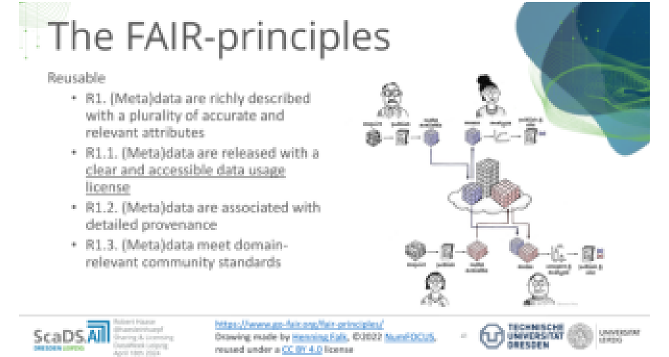


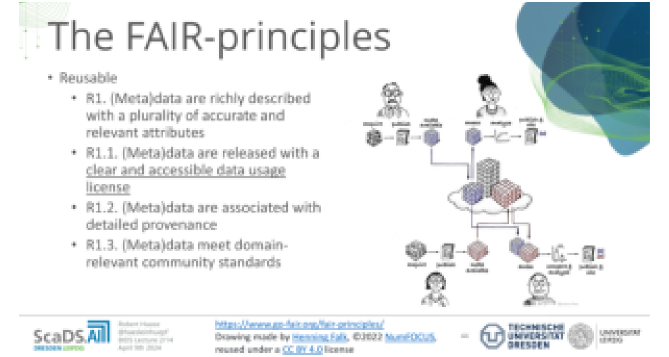


Query: What are the FAIR principles in data management?
Approach A

Query: What are the FAIR principles in data management?
Approach B



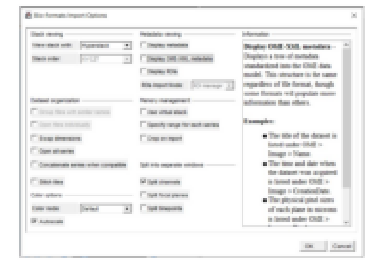




Query: What is Fiji and how is it used?
Approach A

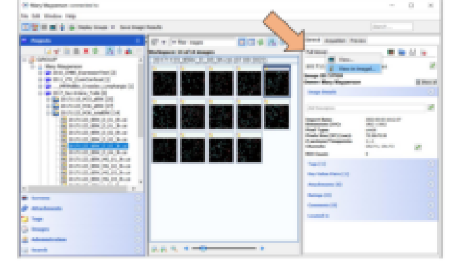
Choose settings for loading the image(s) in Fiji

Use your prefered settings to open the image(s) as required for your work



Select image(s) to open in Fiji (1/2)

1. Select image(s) from the file tree
2. Open in Fiji by clicking **Full Viewer** and then **View in ImageJ...**



OMERO-interoperable analysis tools

OMERO and image analysis software or platforms (examples)

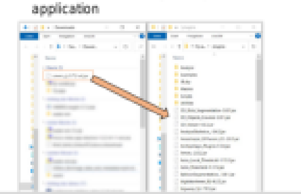
- Fiji (Chapter 8)
- QuPath https://github.com/qupath/qupath-extension-omero
- Galaxy Imaging https://usegalaxy-eu.github.io/posts/2020/11/23/OMERO-post/
- Napari https://www.napari-hub.org/plugins/napari-omero
- BioImage IT Prigent et al. Nat Methods (2022) doi: 10.1038/s41592-022-01642-9
- Jupyter Notebook & Cell Profiler: https://omero-guides.readthedocs.io/en/latest/cellprofiler/docs/cellprofiler.html

See more: https://omero-guides.readthedocs.io/en/latest/external_tools.html

Connect Fiji and OMERO (1/2)

Prerequisite:
You have downloaded Fiji (https://fiji.sc) and have access to the OMERO instance (direct or VPN)

1. Download the OMERO plugin for Fiji from the OME downloads website: https://www.openmicroscopy.org/omero/downloads
2. Move the *omero-ij-x.x.x-all.jar* file to the *Plugins* folder of your Fiji application



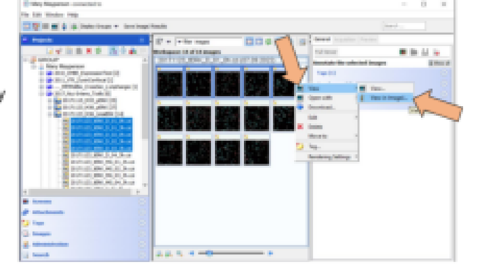
See the official OMERO guide: https://omero-guides.readthedocs.io/en/latest/fiji/docs/index.html

Select image(s) to open in Fiji (2/2)

OR

3. Open image(s) by right-click **View** and then **View in ImageJ...**

(or double-click)



Extended resources on using Fiji and OMERO

Official OMERO guide:
https://omero-guides.readthedocs.io/en/latest/fiji/docs/index.html

A workshop on image analysis with Fiji and OMERO:
https://learning.rc.virginia.edu/notes/fiji-omero/

Workshop recordings by the Open Microscopy Environment Consortium on YouTube, including scripting in Fiji:
https://www.youtube.com/watch?v=W5EDx3yKA_o

Image Analysis Lecture by Robert Haase (TU Dresden)

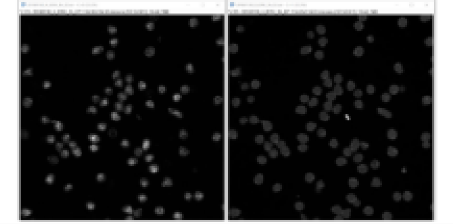
Help for Image Analysis or OMERO-related issues - Image.sc forum:
https://image.sc

Image processing in life-sciences

- State-of-the-art software for more than 20 years: ImageJ / Fiji

https://imagej.nih.gov/ij/ https://fiji.sc

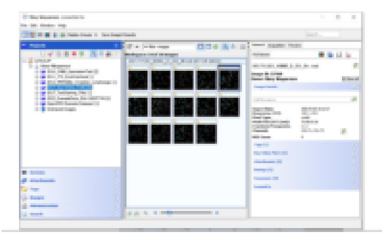
View your images in Fiji and work with the image for processing and analysis

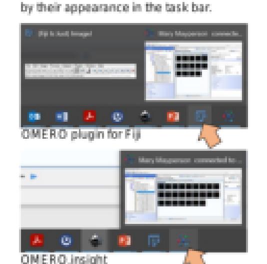


OMERO plugin for Fiji versus OMERO.insight

NOTE: The window looks similar to OMERO.insight, but it is a different application. For example, OMERO.insight has no **View in ImageJ** function nor allows **Save ImageJ Results**.

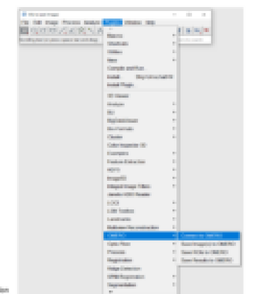
You can distinguish the applications by their appearance in the task bar.





Connect Fiji and OMERO (2/2)

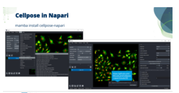
3. Open Fiji and go to *Plugins → OMERO → Connect to OMERO*
4. Log in to OMERO with your user credentials.



The Fiji OMERO plugin looks almost precisely like OMERO.insight, but is, in fact, part of the open Fiji application

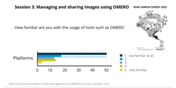
Query: What is Fiji and how is it used?
Approach B

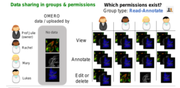
Query: What is Napari and when should I use it?
Approach A



Query: What is Napari and when should I use it?
Approach B

Query: How does OMERO help with data storage and sharing?
Approach A





Query: How does OMERO help with data storage and sharing?
Approach B

Advantages of using OMERO

- **Organize** your original image files in a central storage location
  - Unique IDs for images; data can be linked via IDs
  - View your images, add annotations, tags, and comments to your images
- **View & explore** images, and adjust, save and share visualization settings
  - Create publication-ready figures connected to original image files
- **Export** image data to different formats
- **Analyze** image data
  - Built-in analysis functions
  - Connect to popular image analysis software like Fiji or QuPath
- **Share** your data with colleagues
- and more…

Two ways of connecting to the OMERO server as a user

„**OMERO.insight**" client OR „**OMERO.web**" client
both clients offer similar functionalities

Desktop client
- Installed by the user on a local computer
- *Recommended for:*
  - Importing data into OMERO
  - Importing metadata

Browser-based web-client
- Installed by the IT department on the server, users access it with a browser
- *Recommended for:*
  - Visualizing data
  - Analyzing and managing data
  - Annotating data (e.g., with tags)
  - Running scripts
  - *It is not used to upload data!*

OMERO.web

The front-end application faced to the users

- Allows users to access and interact with their data in OMERO using a web-browser
  - Generates the HTML code behind the browser frontend based on its interaction with OMERO.server (via IP address and port)

OMERO.web

Which types of user groups exist in OMERO?

Prof Julie, Rachel, Mary, Lukas

- Uploaded data belongs to a specific user in a specific group
- In user groups, the users have different roles:
  - Group owner (one or more)
  - Group members
- The OMERO administrator has access to all groups
- Group owners can change group permission levels

Group permission levels

Private | Read-only
Read-Annotate | Read-Write

(Main) options to upload data into OMERO

Upload using **OMERO.importer**
- Is integrated into the **OMERO.insight** client
- Easy, graphical user interface

→ *Recommended for most users*

Persons with restricted admin rights can upload data for other users, too.

Upload using the **Command Line Interface (CLI)**
- See the OMERO guide for details: https://omero-guides.readthedocs.io/en/latest/upload/docs/import-cli.html

Upload data to OMERO using OMERO.importer 1/7

1) Open *OMERO.insight* on your computer
2) Connect to OMERO using your login credentials

(Note: If you haven't previously adjusted the settings, make sure to set the server settings correctly using the wrench icon)

Inspect the uploaded files in OMERO (here: using OMERO.web)

Data remains in the original file format but is presented to you by the software!

A multi-scene image | Preview thumbnails | Metadata

The original file is never changed by OMERO, even when you change rendering settings or metadata in OMERO.

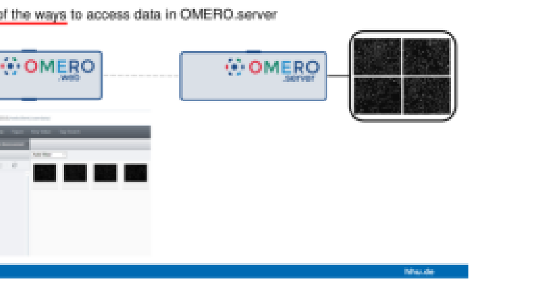
Important notes about Data Upload to OMERO

- Data is stored in *the original file formats* (raw data is conserved!)
- The storage location is not directly accessible for users via file explorers, only via OMERO (exception: „in-place import")
- The uploaded data is never changed or corrupted by OMERO
- The original data can be retrieved from OMERO if desired
- The storage location is on a backed-up server
- The available storage can be increased if necessary
- *Very* large data (e.g., whole slide images, light-sheet images) might require extra care – contact your facility/OMERO admin.

OMERO

hhu

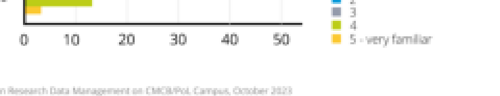
- OMERO.web is one of the ways to access data in OMERO.server



Session 3: Managing and sharing images using OMERO

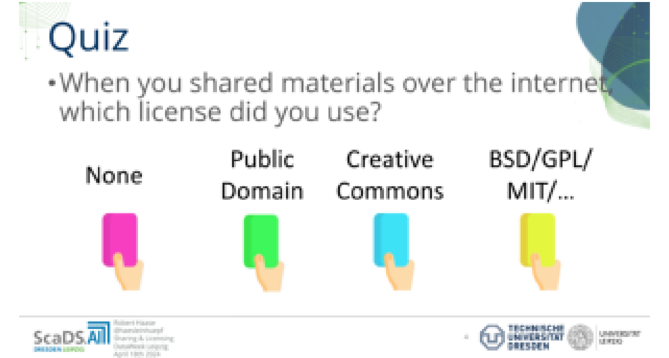
RDM CAMPUS SURVEY 2023

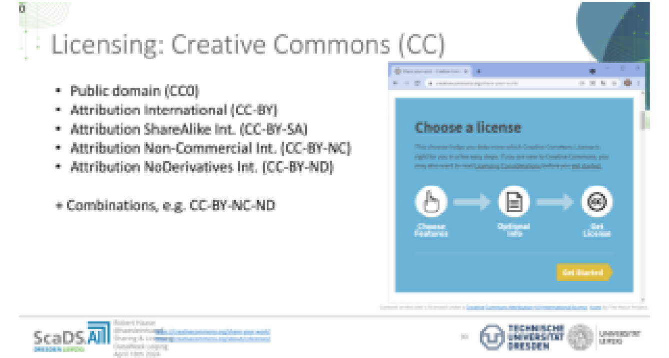
How familiar are you with the usage of tools such as OMERO

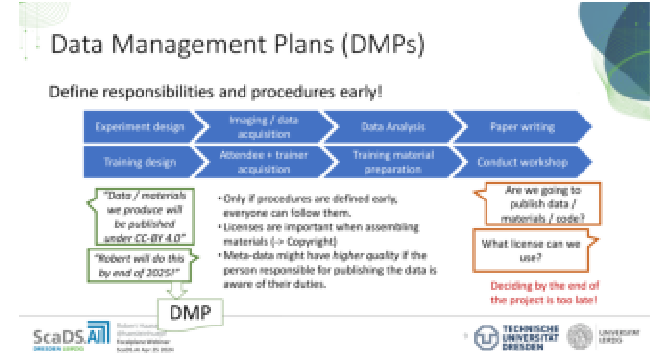


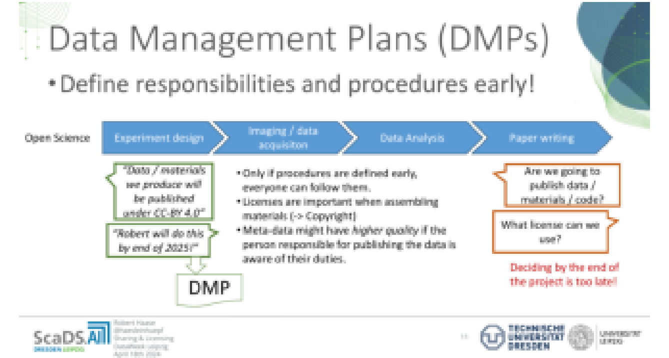
Data from Survey on Research Data Management on CMCB/PoL Campus, October 2023

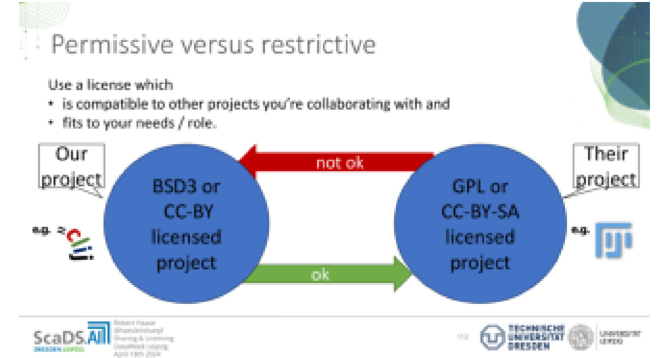
Query: What are different license types that can be used for sharing data?
Approach A

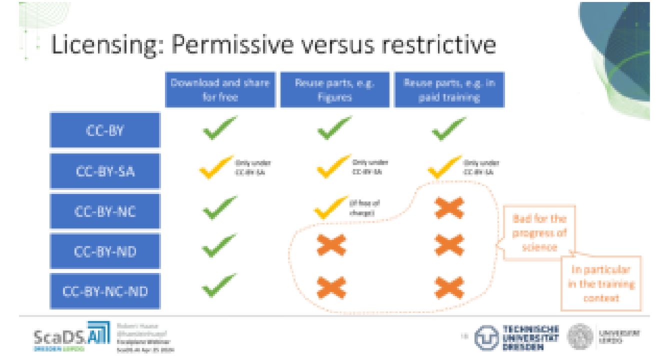


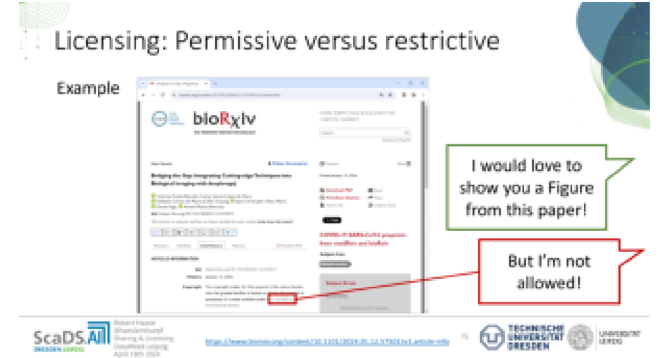


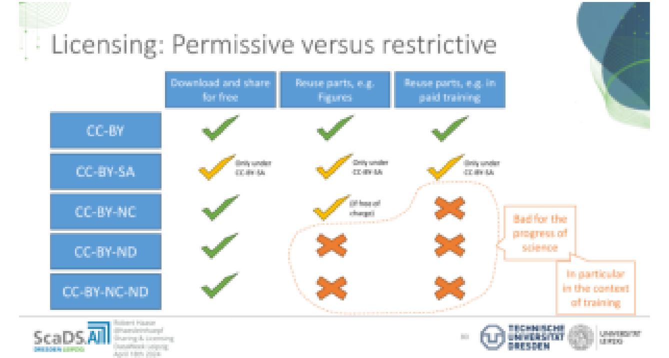


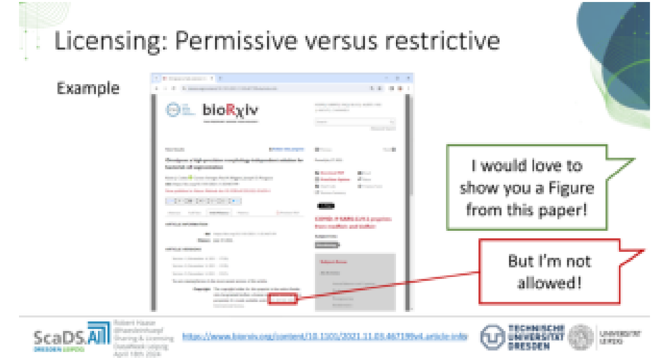












Query: What are different license types that can be used for sharing data?
Approach B

Query: What does open science mean for data sharing?
Approach A

Query: What does open science mean for data sharing?
Approach B

Query: Why is open source software important in scientific research?
Approach A

Query: Why is open source software important in scientific research?
Approach B

Query: How can I use Python for bioimage analysis?
Approach A

Query: How can I use Python for bioimage analysis?
Approach B

ChatGPT for analyzing scientific image data

ChatGPT in comparison with the Bio-image Analysis GPT

Analyzing images using LLMs / BiA-Bob

Use the %bob ... syntax to ask for small things.

```
%bob Load the blobs.tif image file and show it.
```

https://github.com/haesleinhuepf/bia-bob/blob/main/demo/basic_demo.ipynb

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Open or drag&drop a raw FLIM image or series of images

Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

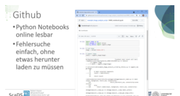
- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest

Chose a cluster of interest and further analyse the corresponding structures in your image

Cellpose in Python

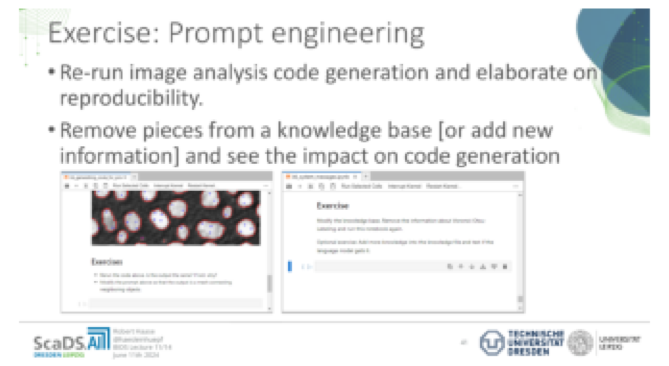
```
channels = [0,0] # This means we are processing single-channel greyscale images.

masks, flows, styles, diams = model.eval(image, diameter=None, channels=channels)
```

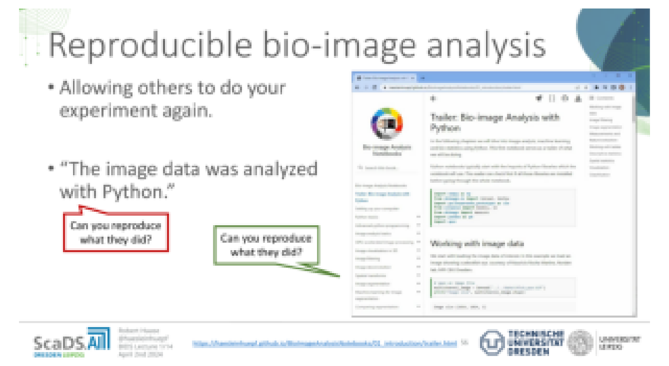
Query: What Python libraries are helpful for image processing?
Approach A

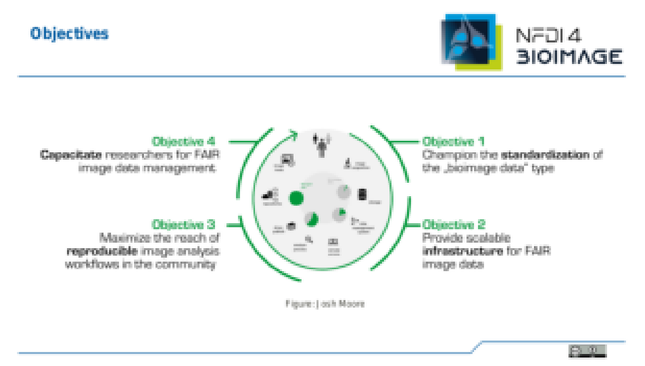


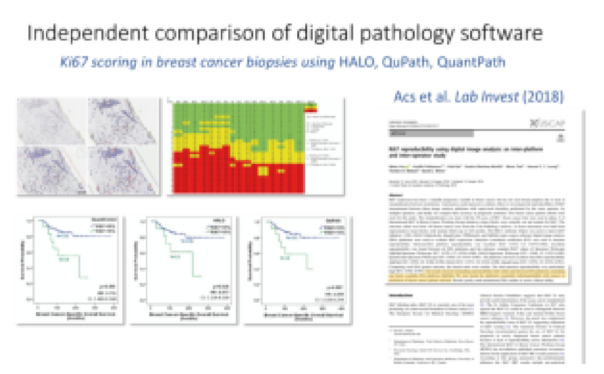
Query: What Python libraries are helpful for image processing?
Approach B

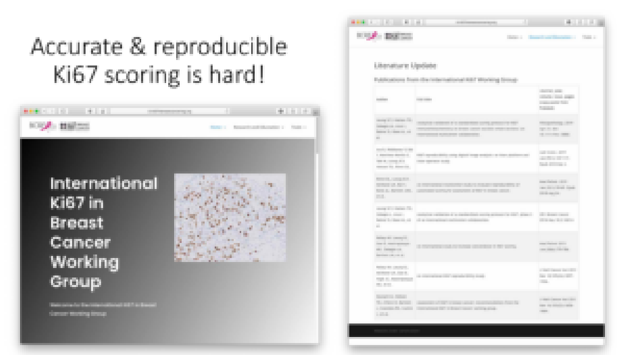
Query: How can I ensure reproducibility in my image analysis pipeline?
Approach A

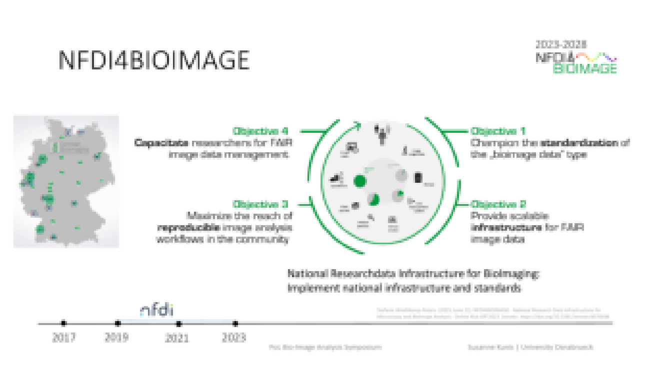


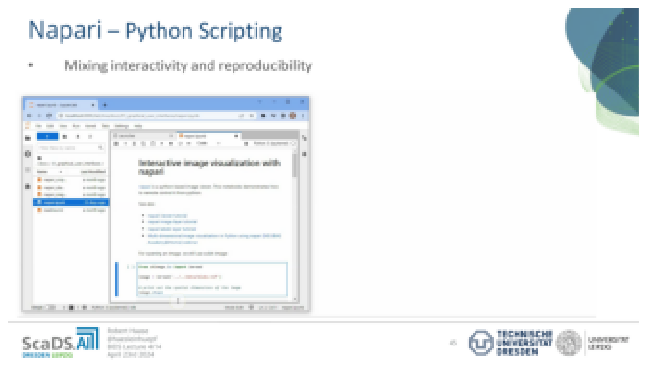


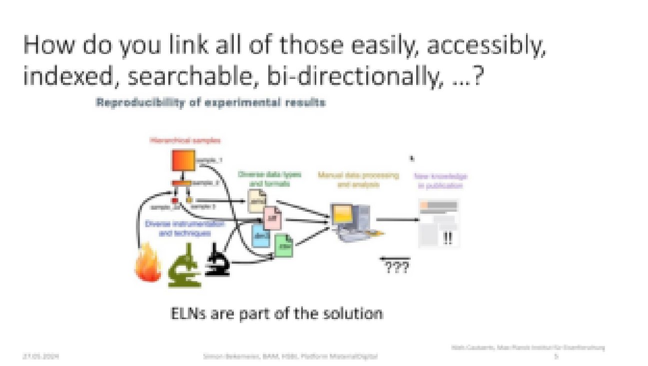


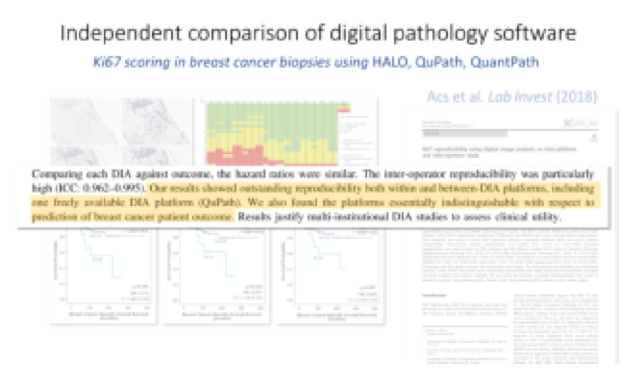




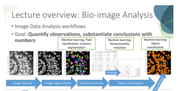


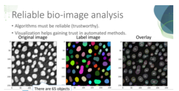






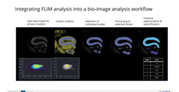
Query: How can I ensure reproducibility in my image analysis pipeline?
Approach B





Query: What are common steps in a reproducible bioimage analysis workflow?

Approach A



Integrating FLIM analysis into a bio-image analysis workflow

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations

Post-process your labels image for further analysis

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Identify and select reproducibly clusters in your dataset

Select the parameters used for clustering

Select the clustering method

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter

Identified clusters are visualized in the phasor plot and in the respective pixels of your image

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot

Define parameters such as a threshold and median to determine the g and s coordinates for the phasor plot by Fourier transformation

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations
- Perform instance segmentation
- Extract Features

Extract features of your identified objects

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest

Chose a cluster of interest and further analyse the corresponding structures in your image

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options

Change parameters of phasor plot visualization

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options

Adjust the settings for visualization

Integrating FLIM analysis into a bio-image analysis workflow

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

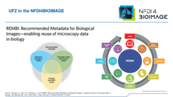
Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations
- Perform instance segmentation

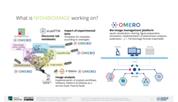
Identify objects of distinct lifetime properties in your image

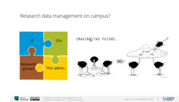
Query: What are common steps in a reproducible bioimage analysis workflow?
Approach B

A plea to users of bioimage analysis software...

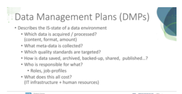


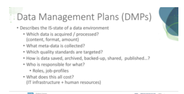
Query: What are the key parts of research data management?
Approach A



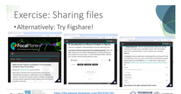


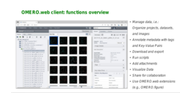
Query: What are the key parts of research data management?
Approach B





Query: How can I share my microscopy data with others?
Approach A





Query: How can I share my microscopy data with others?
Approach B