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AND ARTIFICIAL INTELLIGENCE

Bio-Image Data Science

Robert Haase

GEFÖRDERT VOM



Exam Bio-image Data Science

- Day: July 15th 2025
- Time: 9:15
- Room: S 202
- Schillerstraße 6
04109 Leipzig



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Basics of Microscopy

Robert Haase



Robert Haase
@haesleinhuepf
BIDS Lecture 12/12
July 11th 2025



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Microscopy

- Common tool to answer biological questions



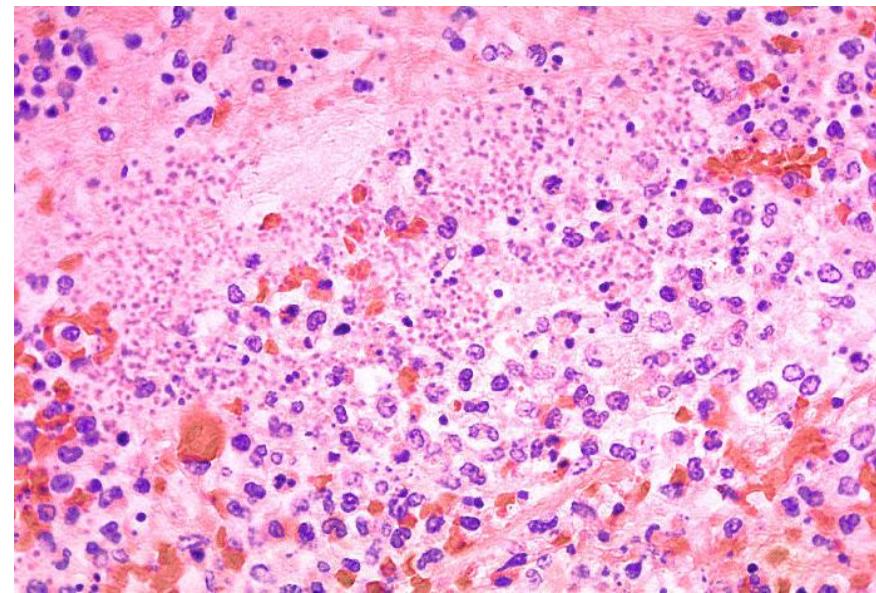
Transmitted light microscope

Fluorescence microscope

Electron microscope

Recap quiz

- This image is from a ...



Transmitted light microscope



Fluorescence microscope

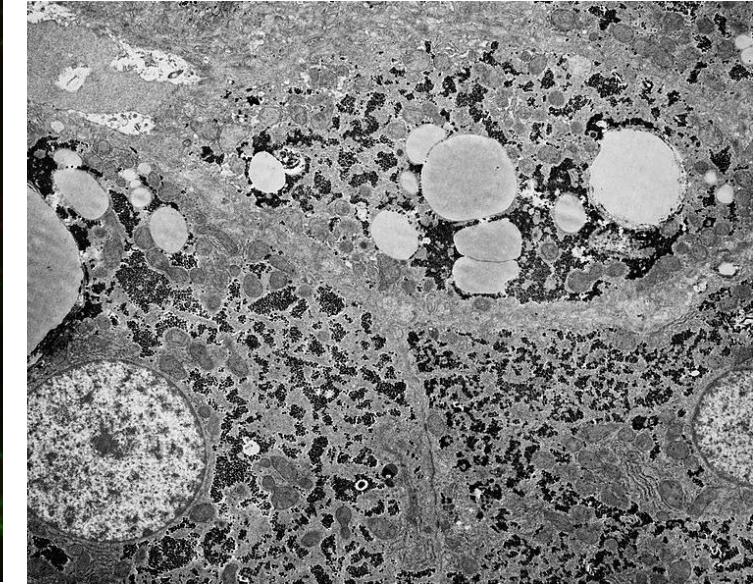
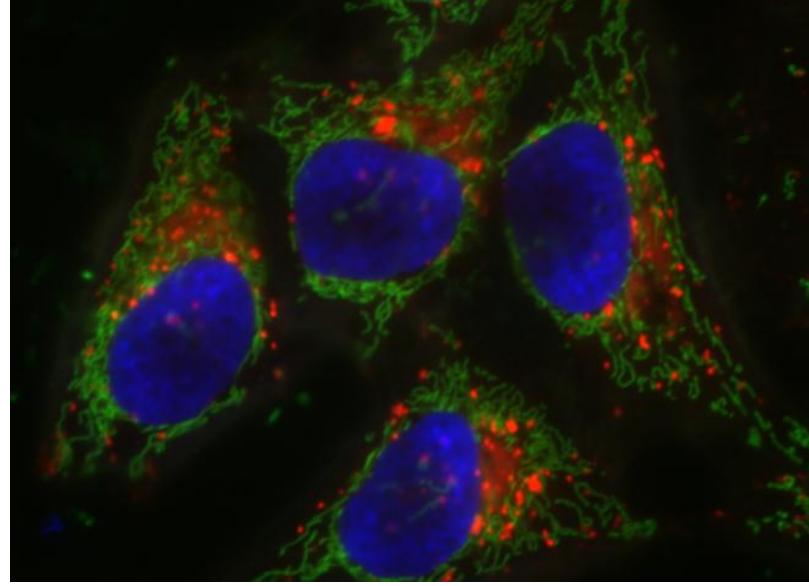
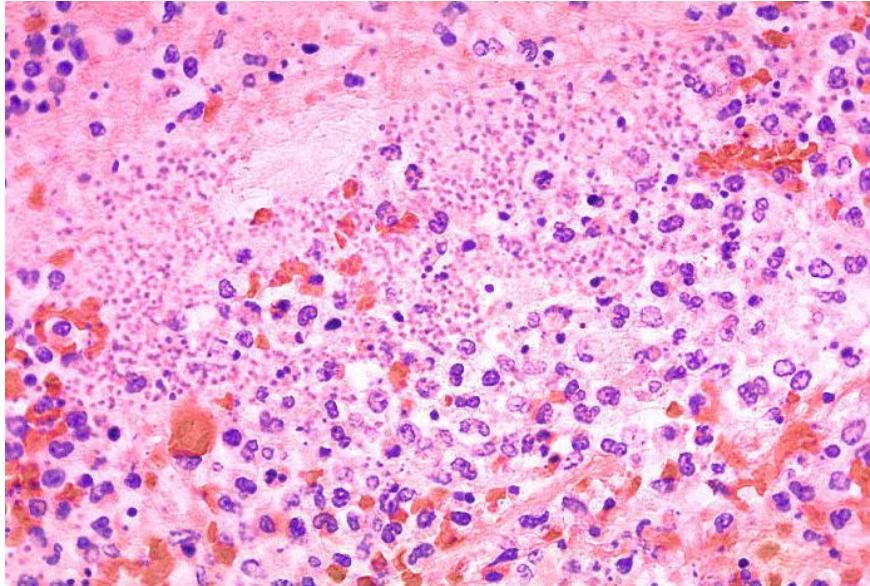


Electron microscope



Microscopy

- Common tool to answer biological questions



Transmitted light microscope

Fluorescence microscope

Electron microscope

Pixel size versus resolution

- What is the difference between pixel size and resolution?

Quiz

- Resolution in imaging describes...

Size of pixels
on a screen

Size of pixels
on a camera
chip

Maximum size
of objects in
relation to the
image

Minimum size
of objects that
can be
differentiated
in an image



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Introduction to Bioimage Analysis

Robert Haase



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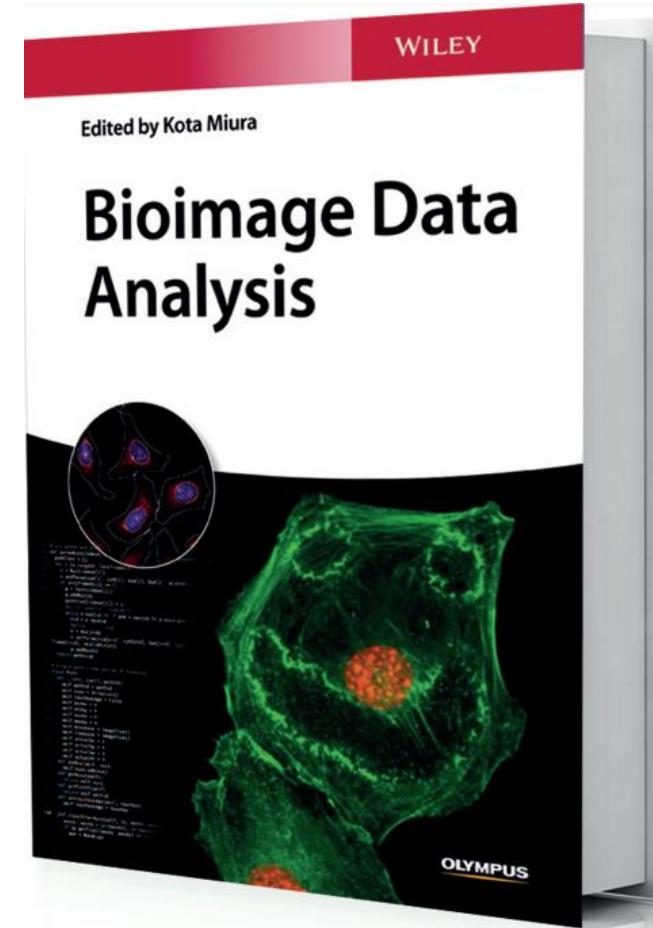
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Bioimage Analysis

- Kota Miura & Sébastien Tosi 2015:

In the light of this definition, image analysis, which is also called “computer vision,” aims at mimicking the way we see the world and how we identify its visible structures. Image analysis in biology does undeniably also hold this element, but more importantly, its main goal is to *measure* biological structures and phenomena in order to study and understand biological systems in a quantitative way.

To achieve this task, we in fact do not have to be bothered with similarity to the human recognition – we have more emphasis on the objectivity of quantitative measurement, rather than how that computer-based recognition becomes in agreement with human recognition. Therefore, in biology, image analysis is a process of identifying spatial distribution of biological components in images and measuring their characteristics to study their underlying mechanisms in an unbiased way. To underline this difference in the goals of image analysis in the two fields and to distinguish them from each other, we will now refer to image analysis in biology as *bioimage analysis*.



Quiz

- Enabling others to do your experiment is about ...

Repeatability

Reproducibility

Replicability

Reliability

Quiz

- Reproducibility can be achieved by

Writing documentation

Writing code

Providing example data

Recording Video tutorials

Bio-image analysis is supposed to be

- **Quantitative**
 - We derive numbers from images which describe physical properties of the observed sample.
- **Objective**
 - The measurement does not depend on who did it. The measurement is free of interpretation.
- **Reliable (trustworthy / validated)**
 - We are confident that the measurement is describing what it is supposed to describe.
- **Reproducible**
 - Enabling others to re-do the experiment. For this, documentation is crucial!
- **Replicability**
 - Others *do* execute the same analysis, potentially on other data, and see consistent results.
- **Repeatable**
 - We can do the same experiment twice under the *same conditions* and get the same measurements.



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Software Management

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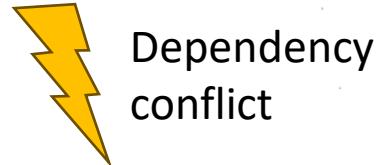
Quiz

conda install package_a

Depends on:
numpy<1.22.0

pip install package_b

Depends on:
numpy>=1.22.0



fails



passes



passes but...





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Microscopy Image Processing

Robert Haase

Reusing materials from Mauricio Rocha Martins (Norden lab,
MPI CBG); Dominic Waithe (Oxford University); Alex Bird, Dan
White (MPI CBG), Marcelo Leomil Zoccoler, TU Dresden

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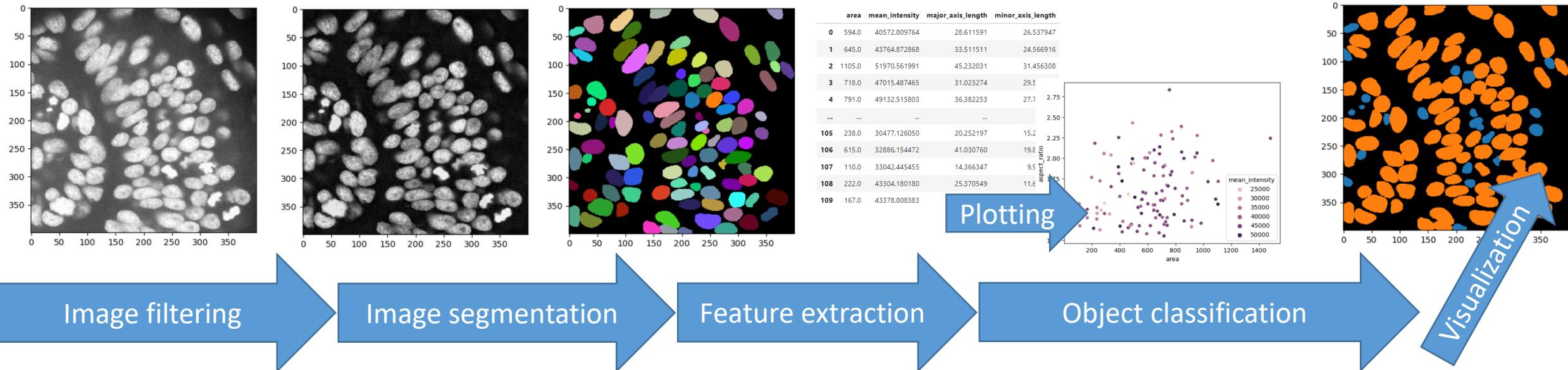
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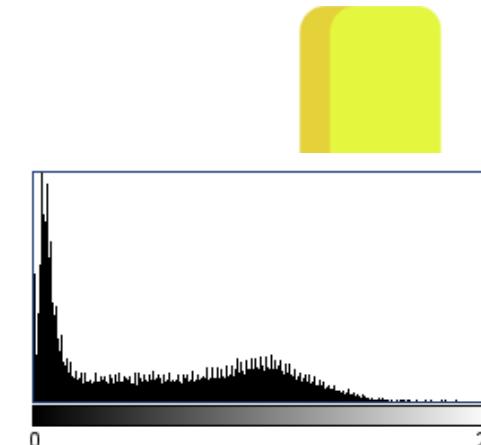
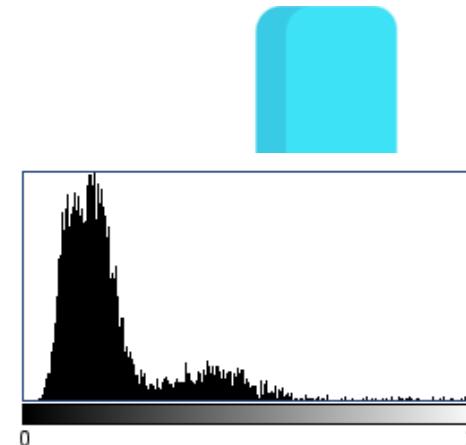
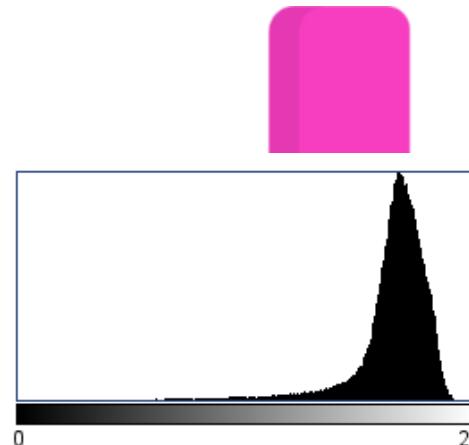
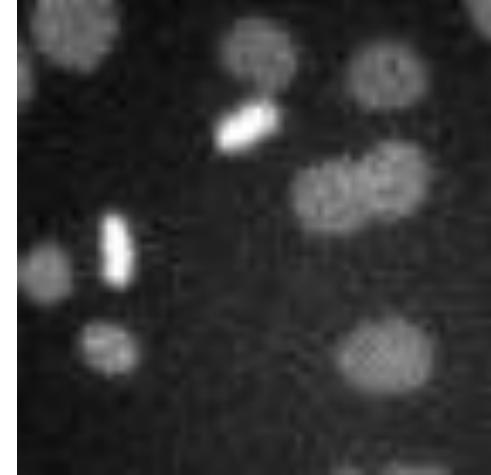
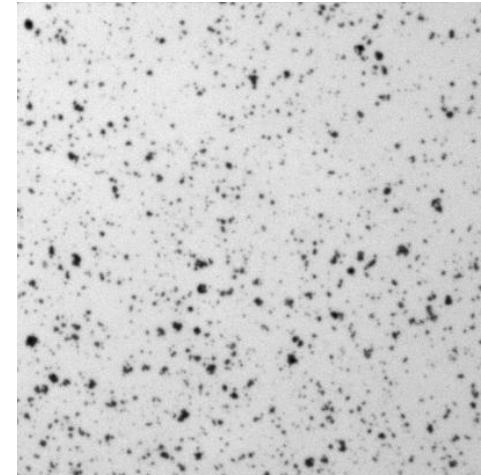
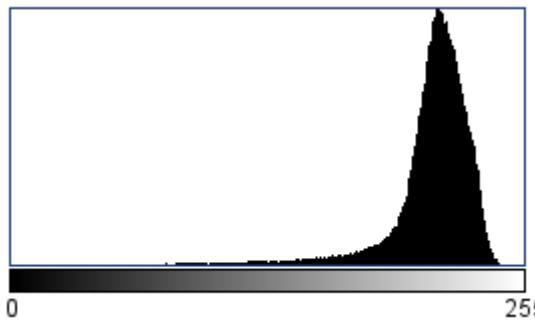
Lecture overview: Bio-image Analysis

- Image Data Analysis workflows
- Goal: Quantify observations, substantiate conclusions with numbers



Histograms

- To which image does this histogram belong to?



Quiz

- What is noise?

Quiz (recap)

- Which of the following is a band-pass filter?

Gaussian



Median



Top-hat

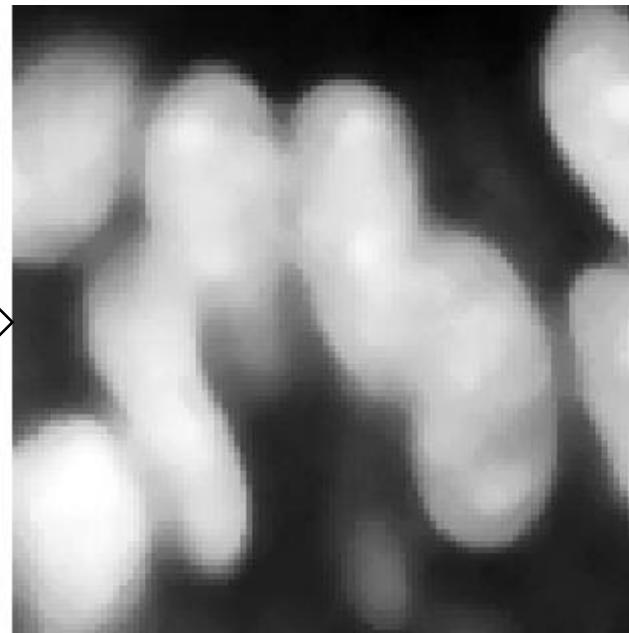


Difference
of Gaussian



Quiz: Noise removal

- The median filter is a ...



Linear
filter



Non-linear
filter

Quiz: What differentiates linear and non-linear image processing filters?

Quiz (recap)

- Which of the following is a denoising filter?

Gaussian



Median



Top-hat



Difference
of Gaussian





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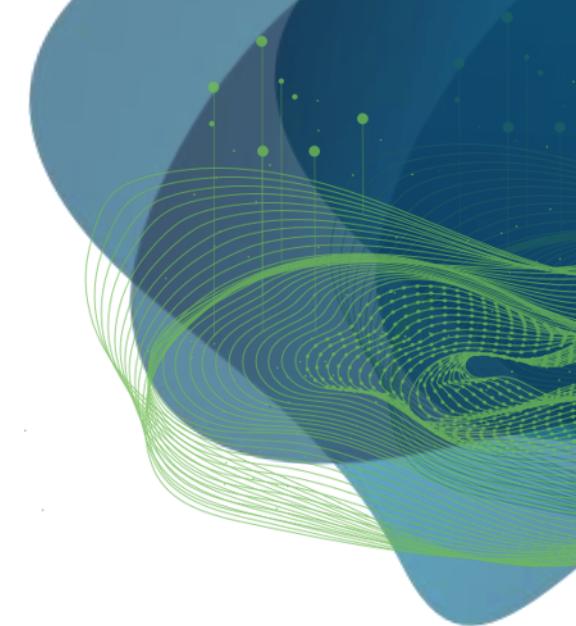


Image Processing: Morphological Operations

Robert Haase

With material from

Marcelo Leomil Zoccoler, Physic of Life, TU Dresden

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Microscopy Image Processing in Python

Robert Haase



Robert Haase
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BIDS Lecture 12/12
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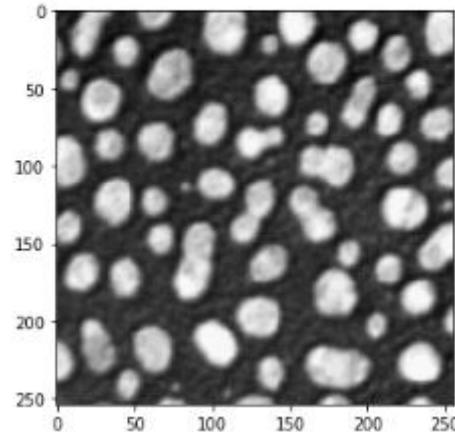
42

Cropping and resampling images

- Indexing and cropping *numpy-arrays* works like with python arrays.

```
imshow(image)
```

```
<matplotlib.image.AxesImage at 0x
```

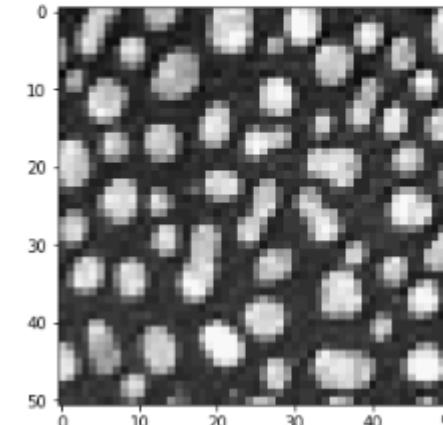


Original image

```
sampled_image = image[::5, ::5]
```

```
imshow(sampled_image)
```

```
<matplotlib.image.AxesImage at 0x
```

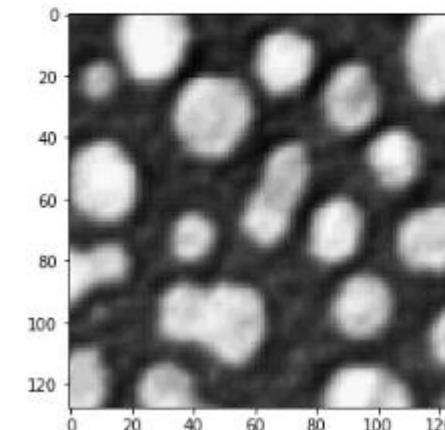


Sub-sampled image

```
cropped_image2 = image[0:128, 128:]
```

```
imshow(cropped_image2)
```

```
<matplotlib.image.AxesImage at 0x29e
```

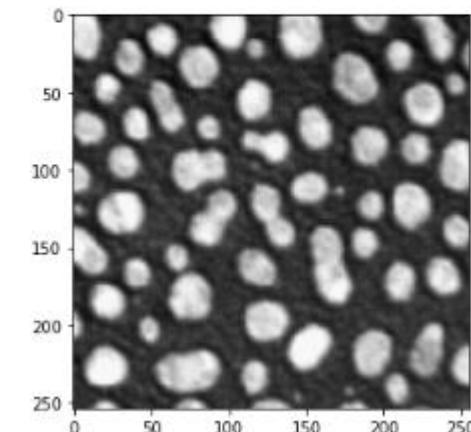


Cropped image

```
flipped_image = image[:, ::-1]
```

```
imshow(flipped_image)
```

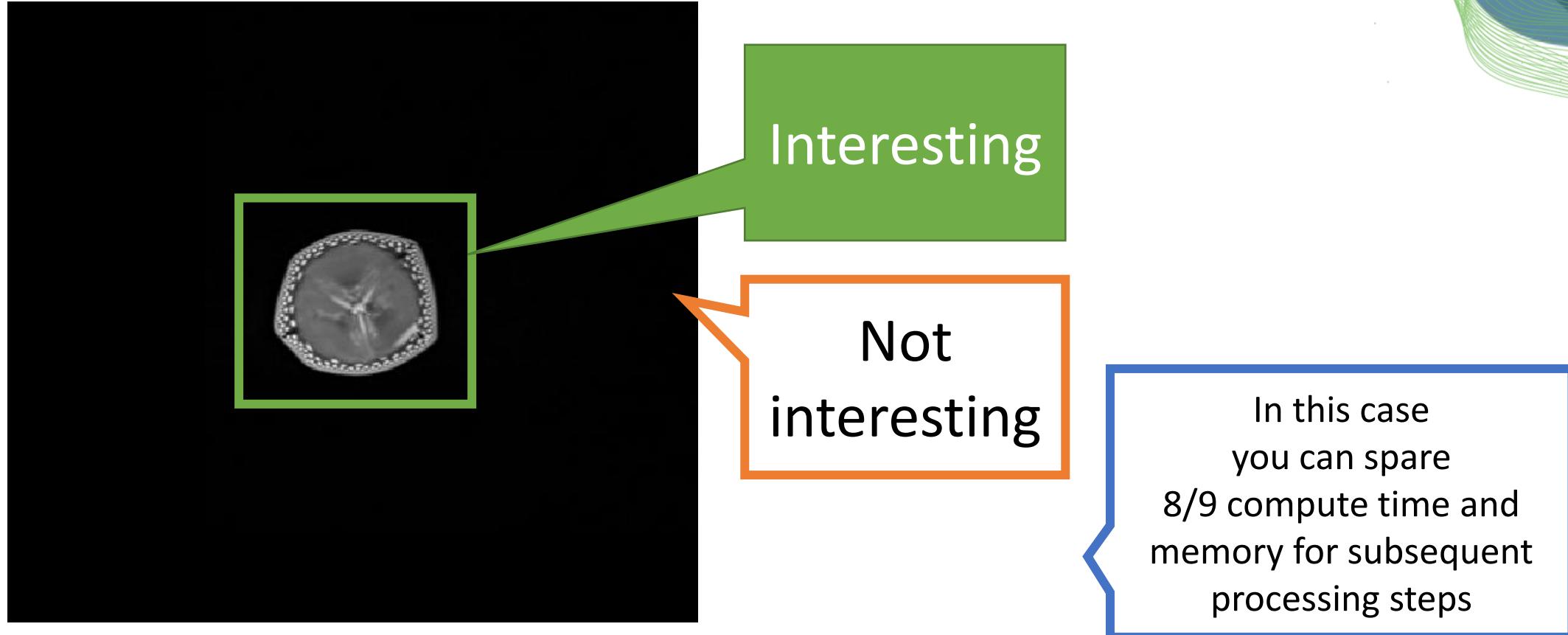
```
<matplotlib.image.AxesImage at 0x
```



Flipped image

Cropping and resampling images

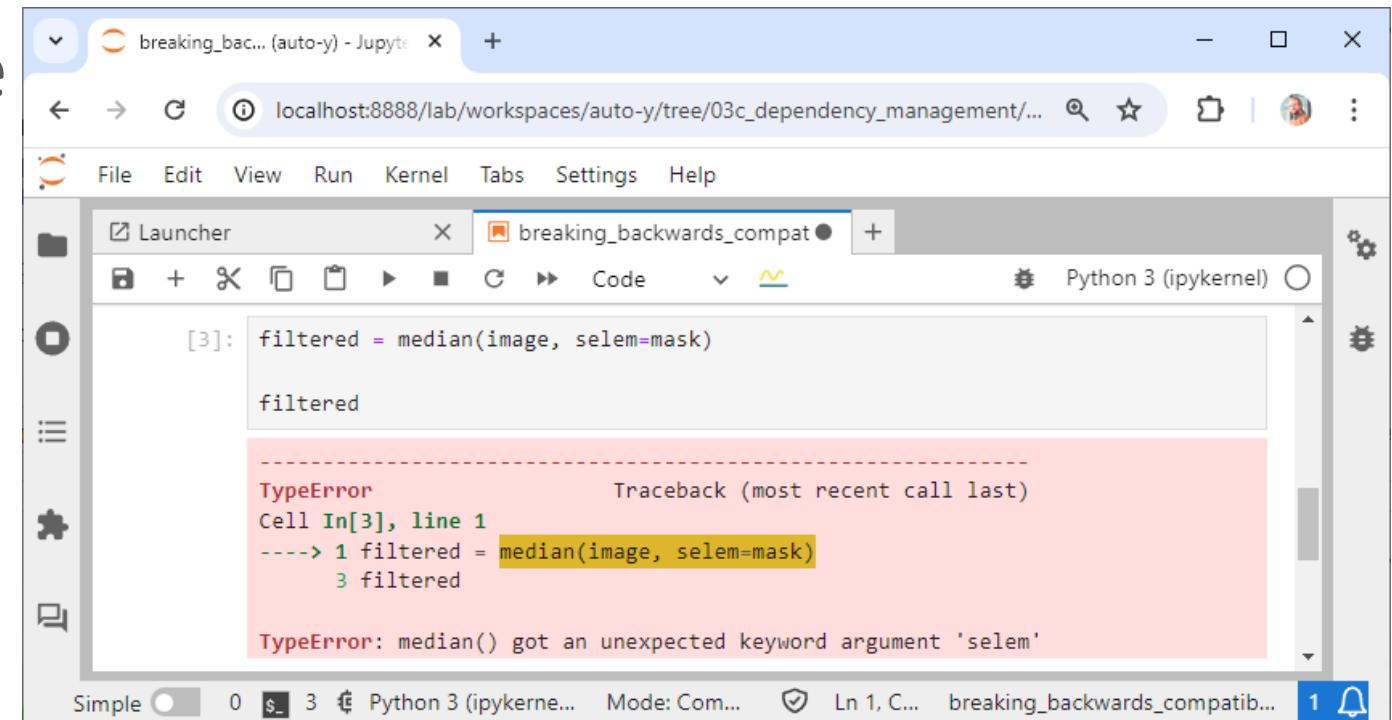
- Crop out the region you're interested in



Recap

How can one solve this problem?

- A) By modifying the code
- B) By not modifying the code



The screenshot shows a Jupyter Notebook interface with a single code cell containing the following Python code:

```
[3]: filtered = median(image, selem=mask)
```

The cell output shows the variable `filtered`. Below the cell, a red box highlights the error message:

TypeError Traceback (most recent call last)
Cell In[3], line 1
----> 1 filtered = median(image, selem=mask)
 3 filtered

TypeError: median() got an unexpected keyword argument 'selem'

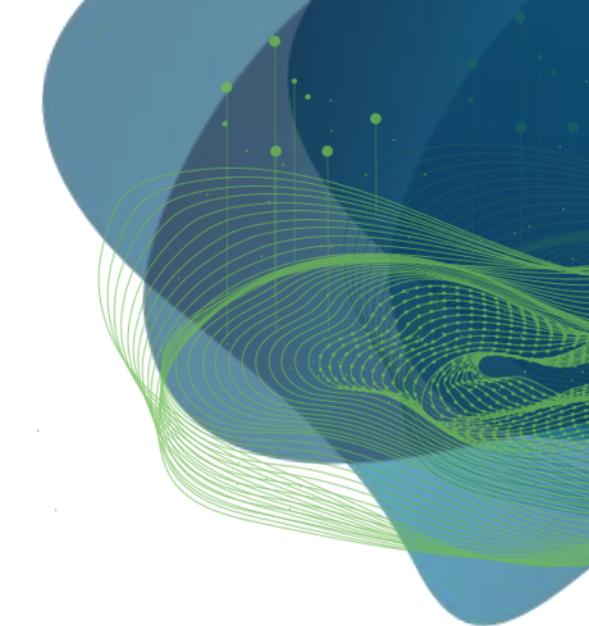


Image segmentation

Robert Haase

Using materials from Marcelo Leomil Zoccoler and Johannes Soltwedel,
PoL, TU Dresden

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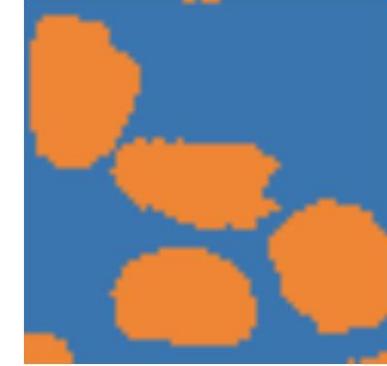
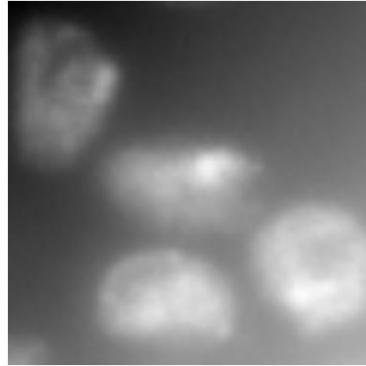


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Pixel-Classification

- Quiz: How is this task called?



Combinatorial
Segmentation



Semantic
Segmentation



Instance-
Segmentation

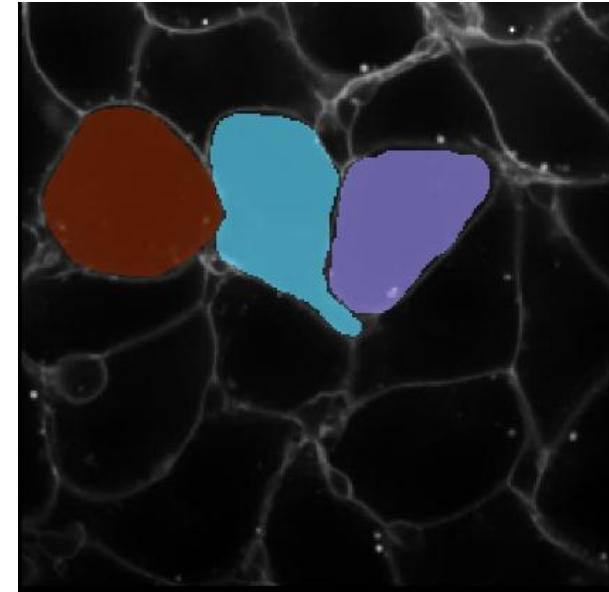


Connected-Component
Segmentation



Sparse Jaccard Index

This label image
shows a ...



Instance
segmentation



Semantic
segmentation



Sparse
semantic
segmentation

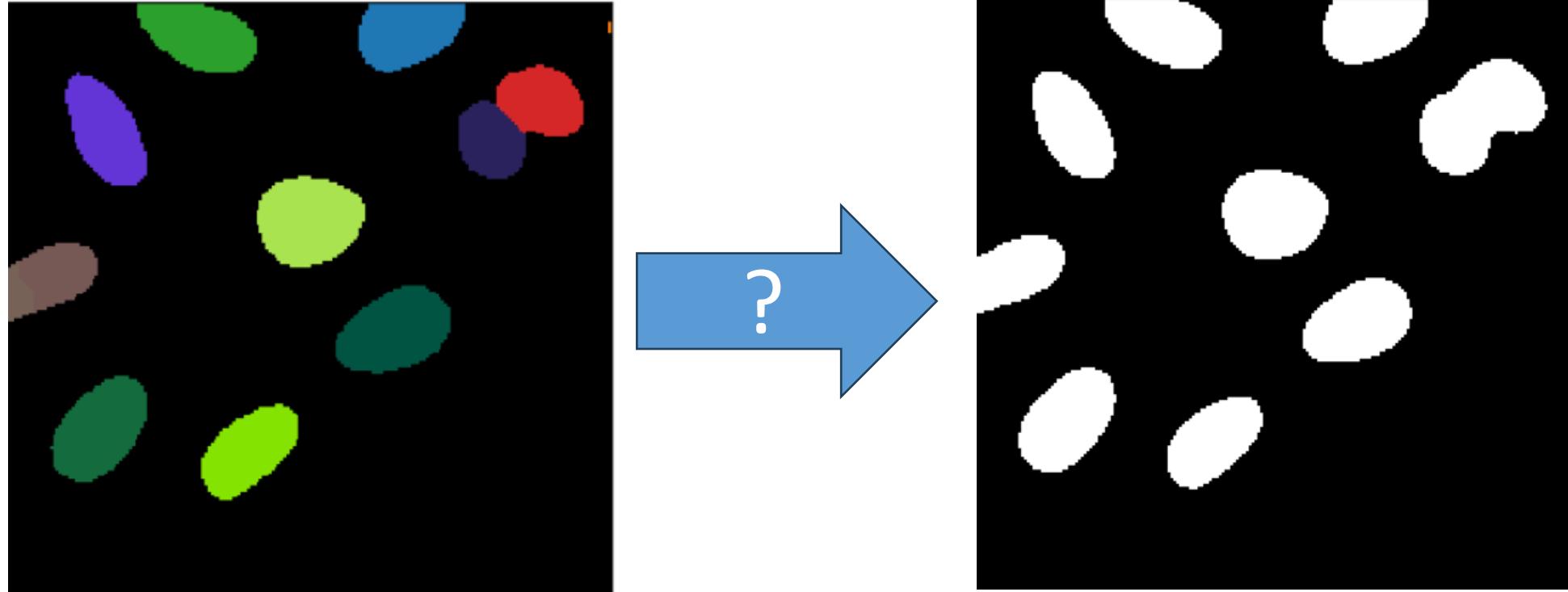


Sparse
instance
segmentation



Quiz

- How could I turn a label image into a binary image?



Interactive image processing using Napari

Robert Haase

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Chan
Zuckerberg
Initiative

[not relevant for the exam]



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Surface reconstruction

Robert Haase

Using materials from Alba Villaronga Luque and Jesse Veenvliet (MPI CBG Dresden), Marcelo Leomil Zoccoler, Johannes Soltwedel and Mara Lampert, PoL, TU Dresden

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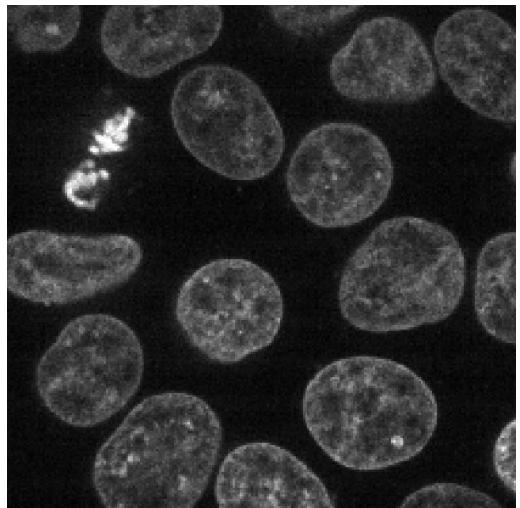


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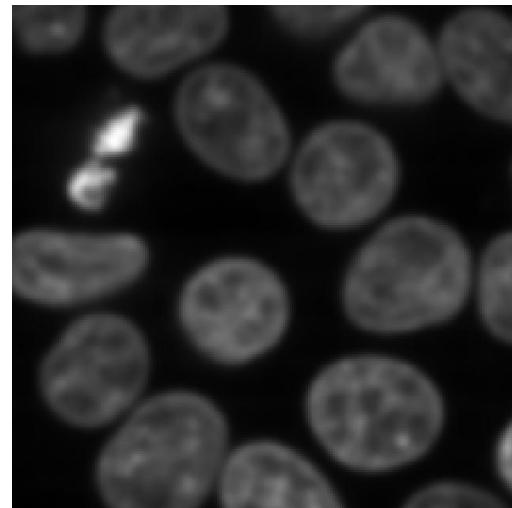
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Motivation: Surface reconstruction

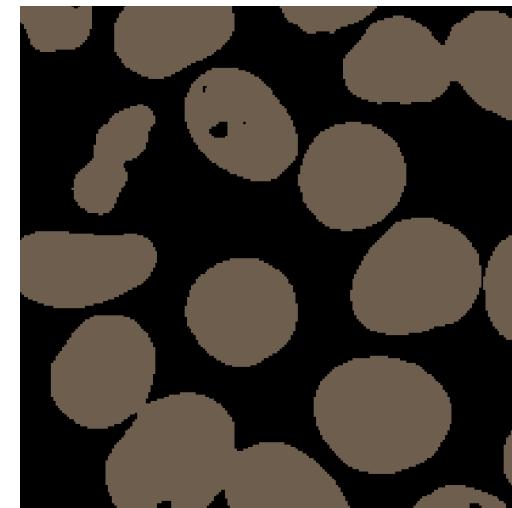
- Pixel and voxel arrays can be huge in memory
- Processing 3D arrays is time-consuming



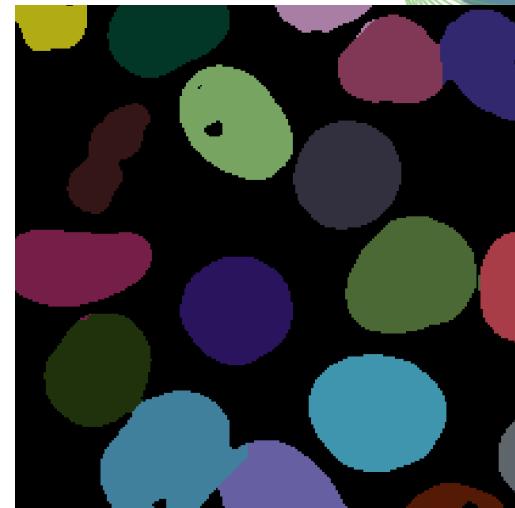
1024 x1024 x 100
16-bit image



1024 x1024 x 100
16-bit image



1024 x1024 x 100
8-bit image



1024 x1024 x 100
16-bit image

How much memory does
this workflow cost?

700 MB

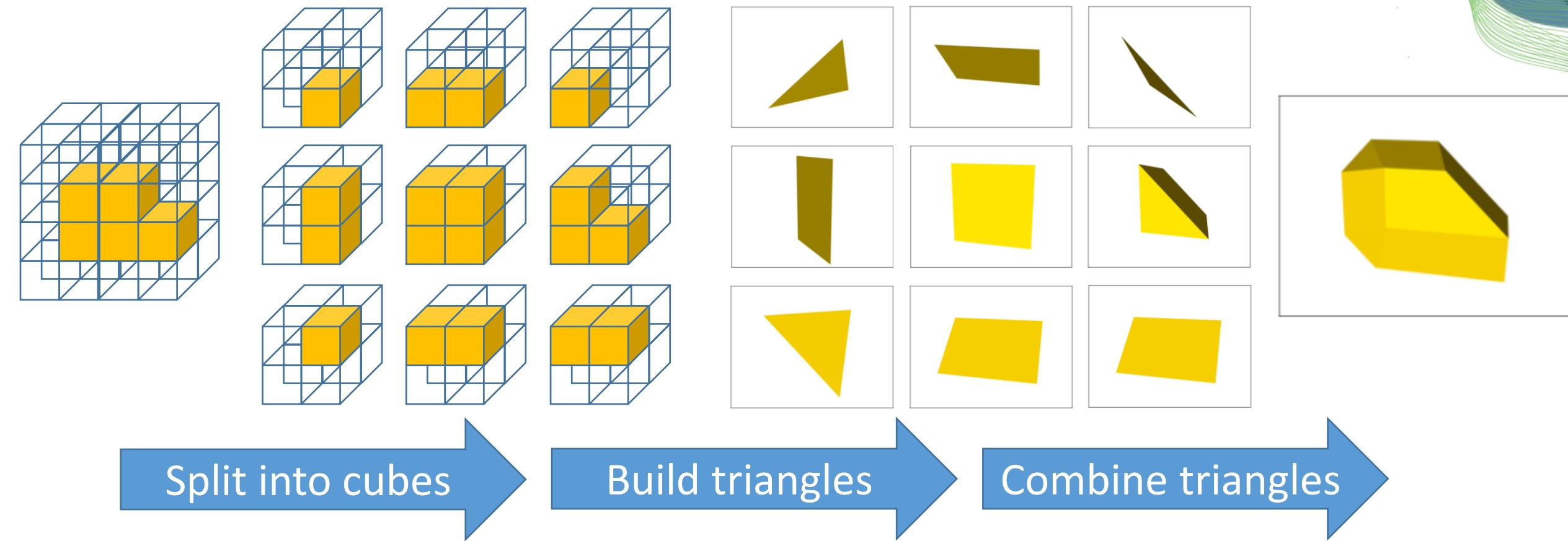
400 MB

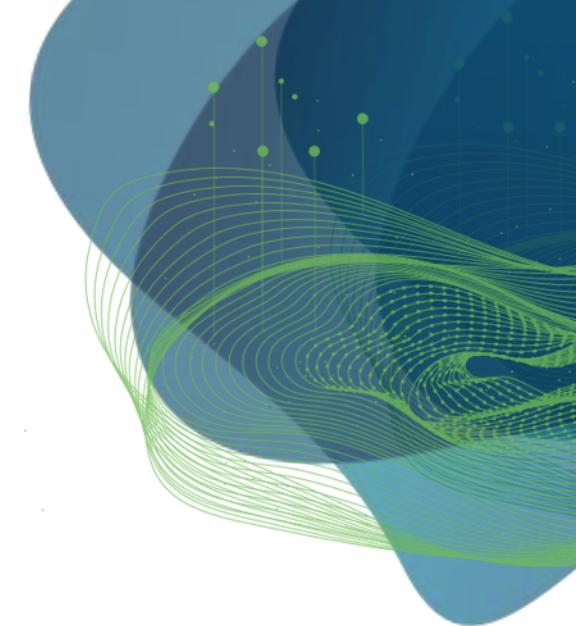
4 GB

7 GB

Marching cubes algorithm

- Starting point: 3D binary image
- Cuts the image in small cubes and iterates over them





Feature extraction

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Quiz

- “aspect ratio” is what kind of *feature*?

Feature image



Image
feature



Object
feature

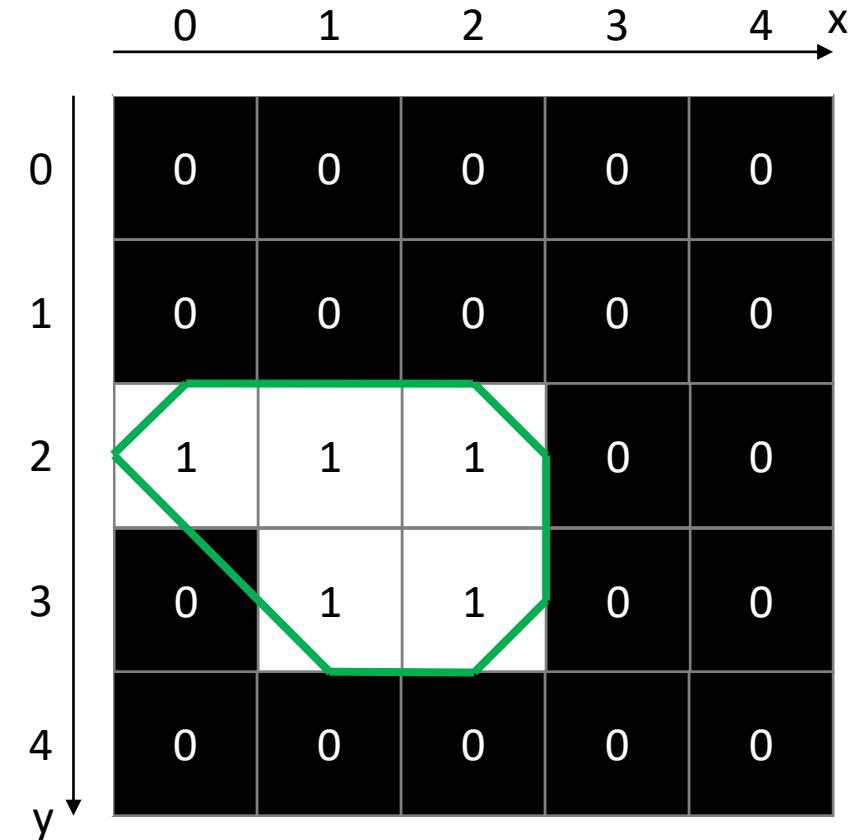
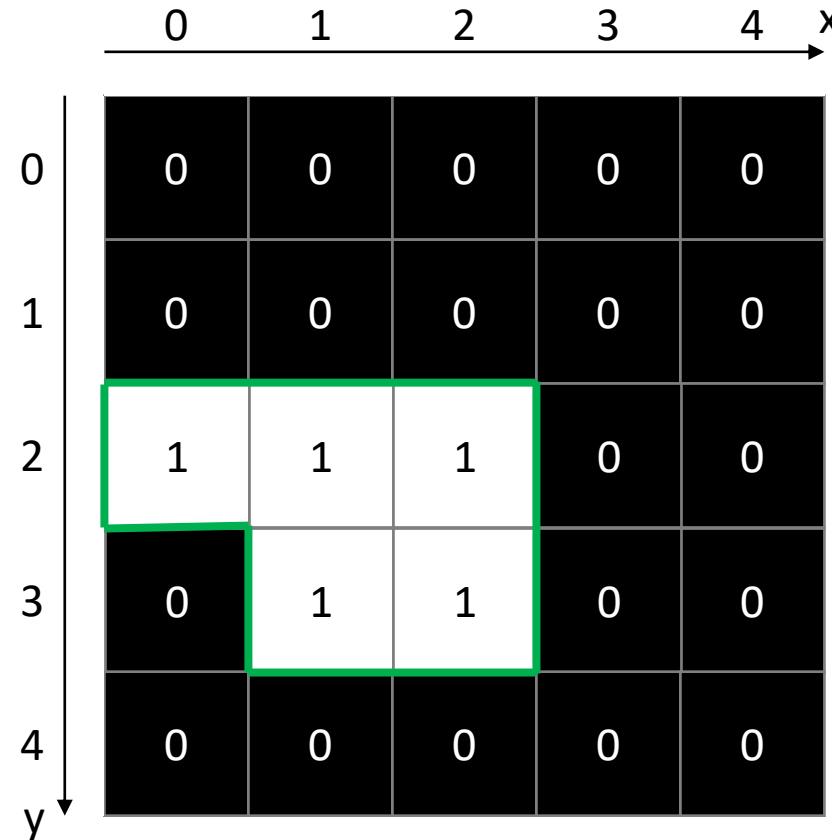


Feature
object



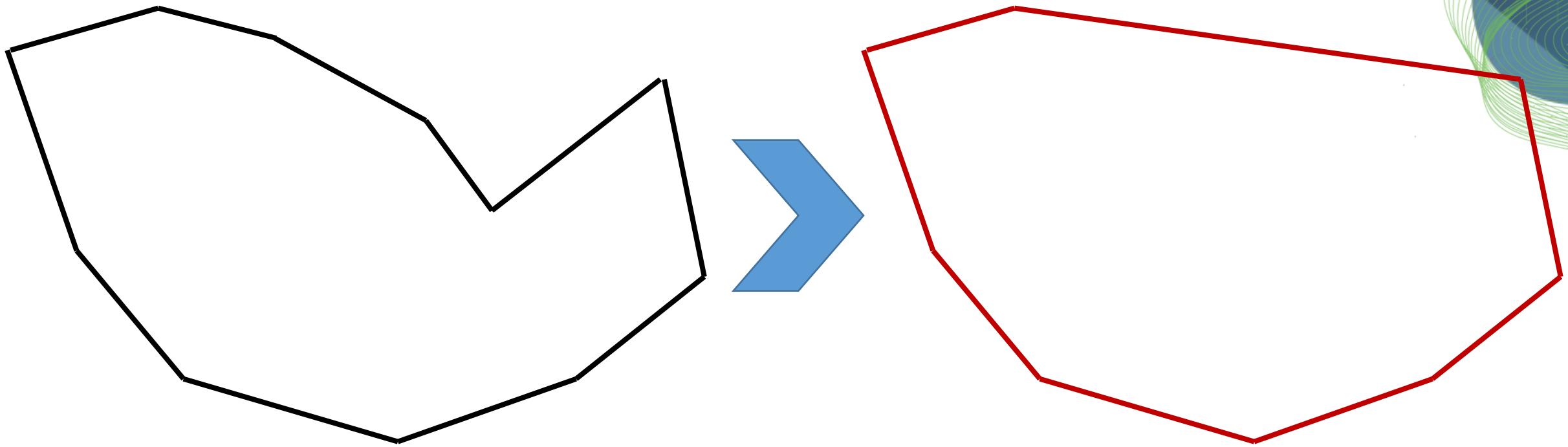
Perimeter

- Length of the outline around an object
- Depends on the actual implementation



Convex hull

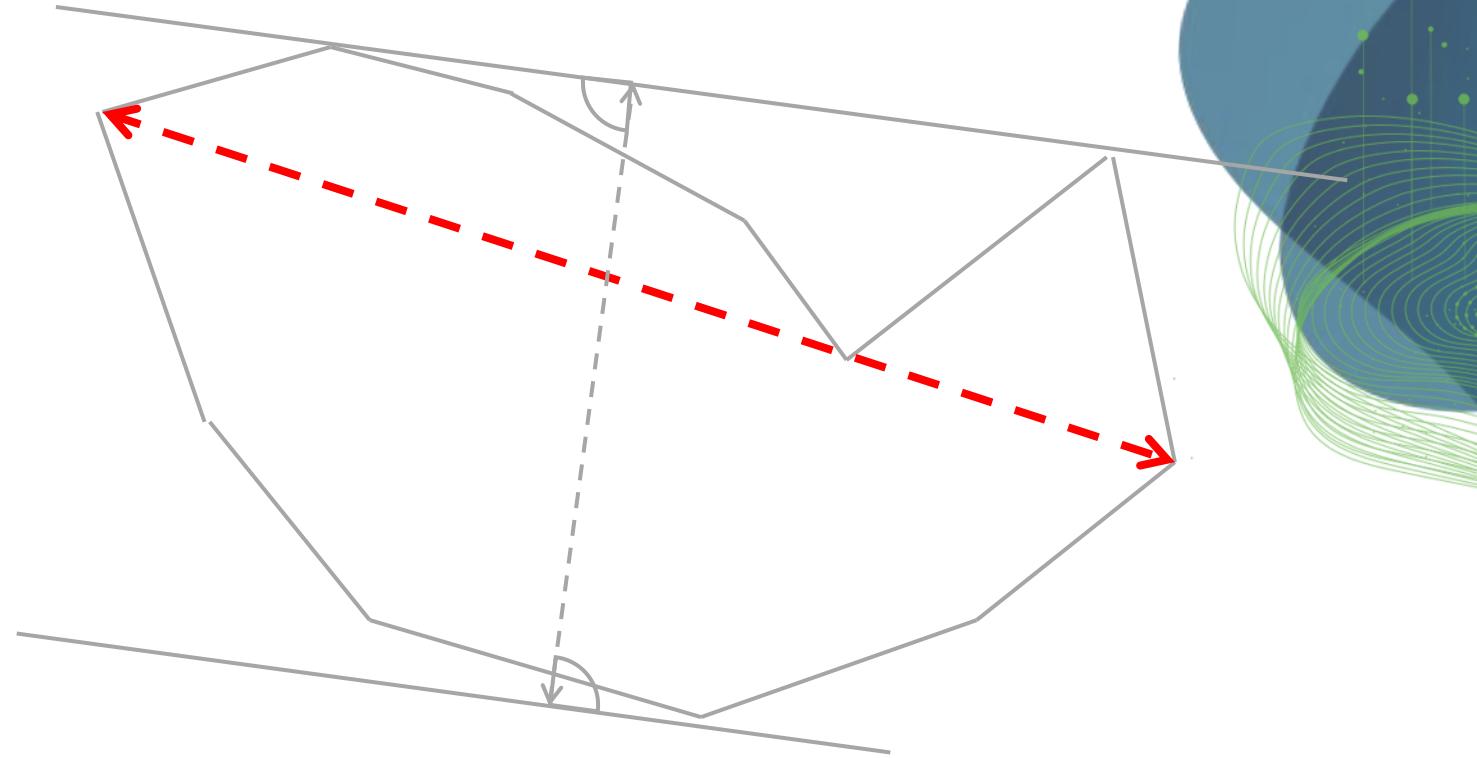
- By removing all concave corners of an object, we retrieve its **convex hull**.



$$solidity = \frac{A}{A_{convexHull}}$$

Quiz: Recap

How is this feature called?



Feret's diameter



Minimum Caliper



Minor Axis length



Major Axis length



Tiled & GPU-accelerated Image Processing

Robert Haase

Funded by



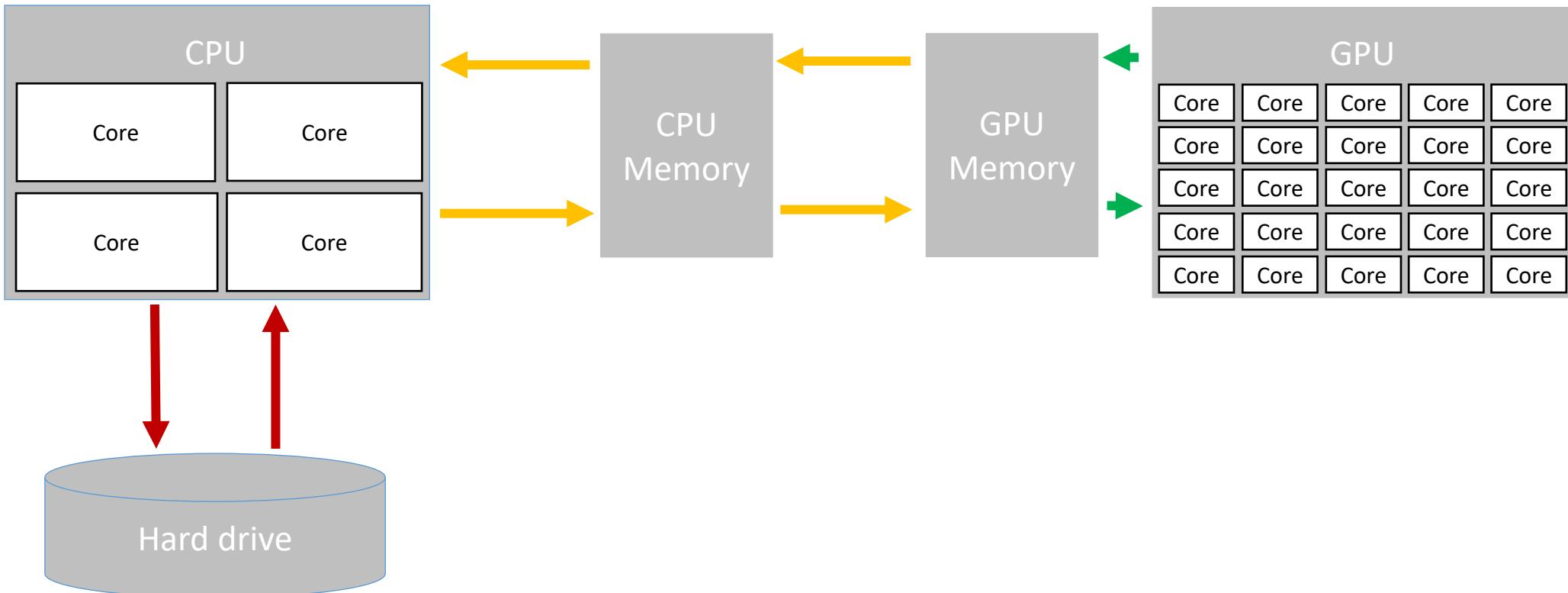
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GPUs allow real-time image processing

- GPUs are specialised in processing, very fast thanks to many cores and fast memory access



Tiled image processing

Robert Haase

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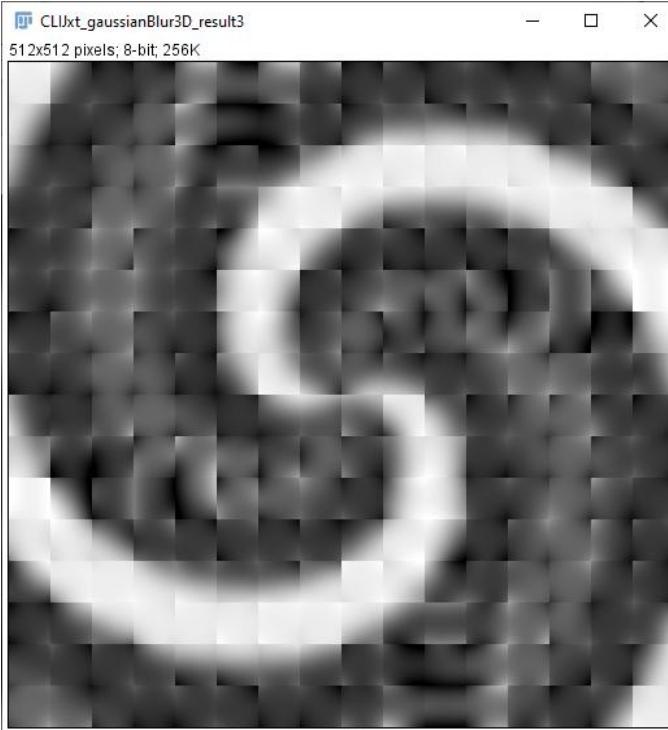


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Tiling

- Example: Gaussian blur (sigma = 20)
- Solution: Process with overlapping tiles (size + margin)

Margin: 0 pixels



Margin: 10 pixels



Optimal margin size depends
on algorithm and its
parameters

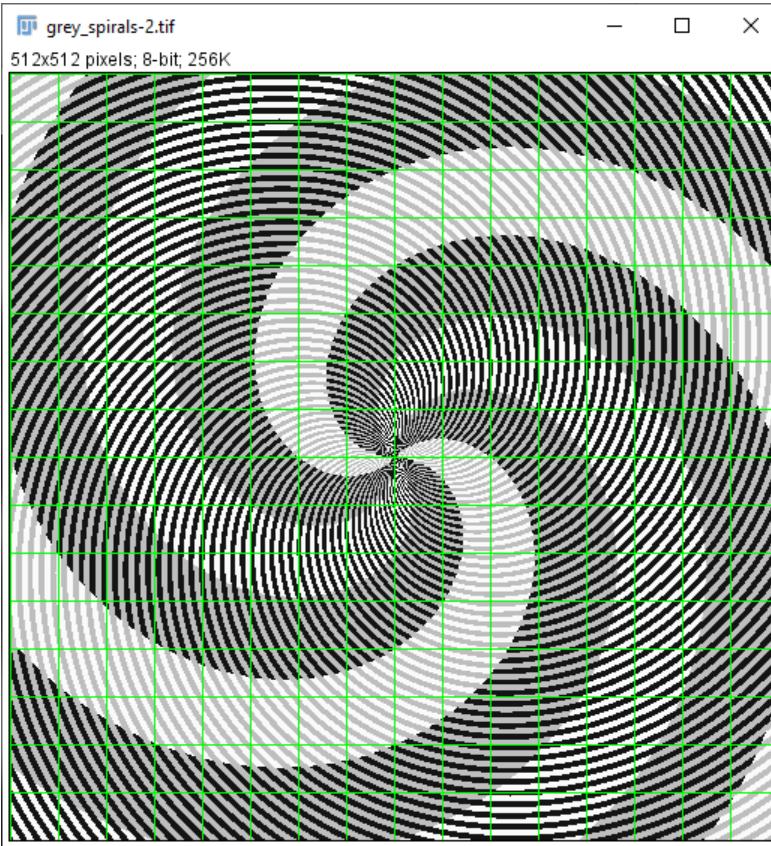
Margin: 20 pixels



Tiling

- Example: Gaussian blur (sigma = 20 pixels)
- Solution: Process with overlapping tiles (size + margin)

Computation time depends
on tile size and margin width



Margin: 20 pixels
Size: 5x original

Margin: 10 pixels
Size: 2.7x original

Tile
32x32 pixels

52x52 pixels

72x72 pixels



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Segmentation quality estimation

Robert Haase

Reusing materials from Lena Maier-Hein, Annika Reinke (DKFZ) et al.
and Martin Schätz (Charles Uni Prague)

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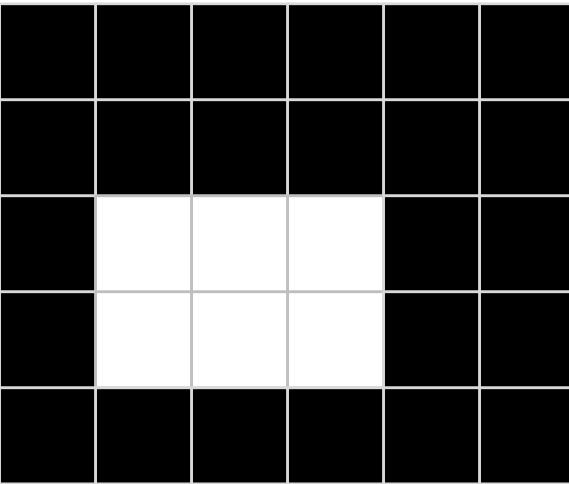
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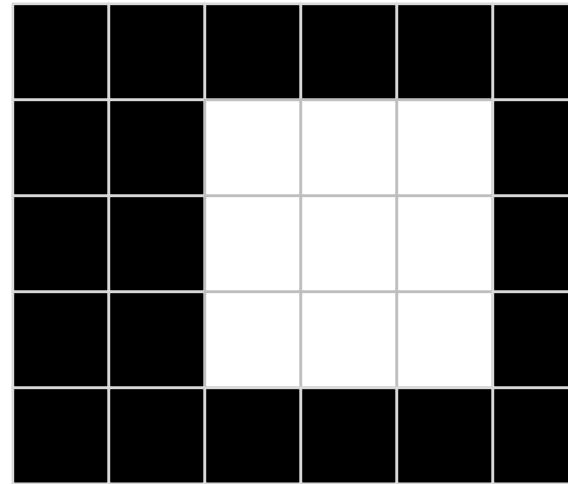
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Pixel-wise versus Object-wise evaluation

- Segmentation (binary)



- Ground truth (binary)



Quiz: What is the Jaccard Index
(Intersection over Union, IoU) of this
segmentation?

$$\begin{aligned}\text{IoU}(A,B) &= \frac{\text{diagonal hatched}}{\text{blue} + \text{orange} - \text{diagonal hatched}} \\ &= \frac{|A \cap B|}{|A| + |B| - |A \cap B|} = \frac{|A \cap B|}{|A \cup B|}\end{aligned}$$

Unsupervised Machine Learning for Bio-image Analysis

Robert Haase

Reusing materials from Johannes Soltwedel, Till Korten, Johannes Müller, Laura Žigutytė (TU Dresden), Ryan Savill (MPI-CBG), Matthias Täschner (ScaDS.AI/Uni Leipzig) and the Scikit-learn community.

Quiz

- An embedding is ...

N-dimensional
space



Result of
dimensionality
reduction



Result of
clustering



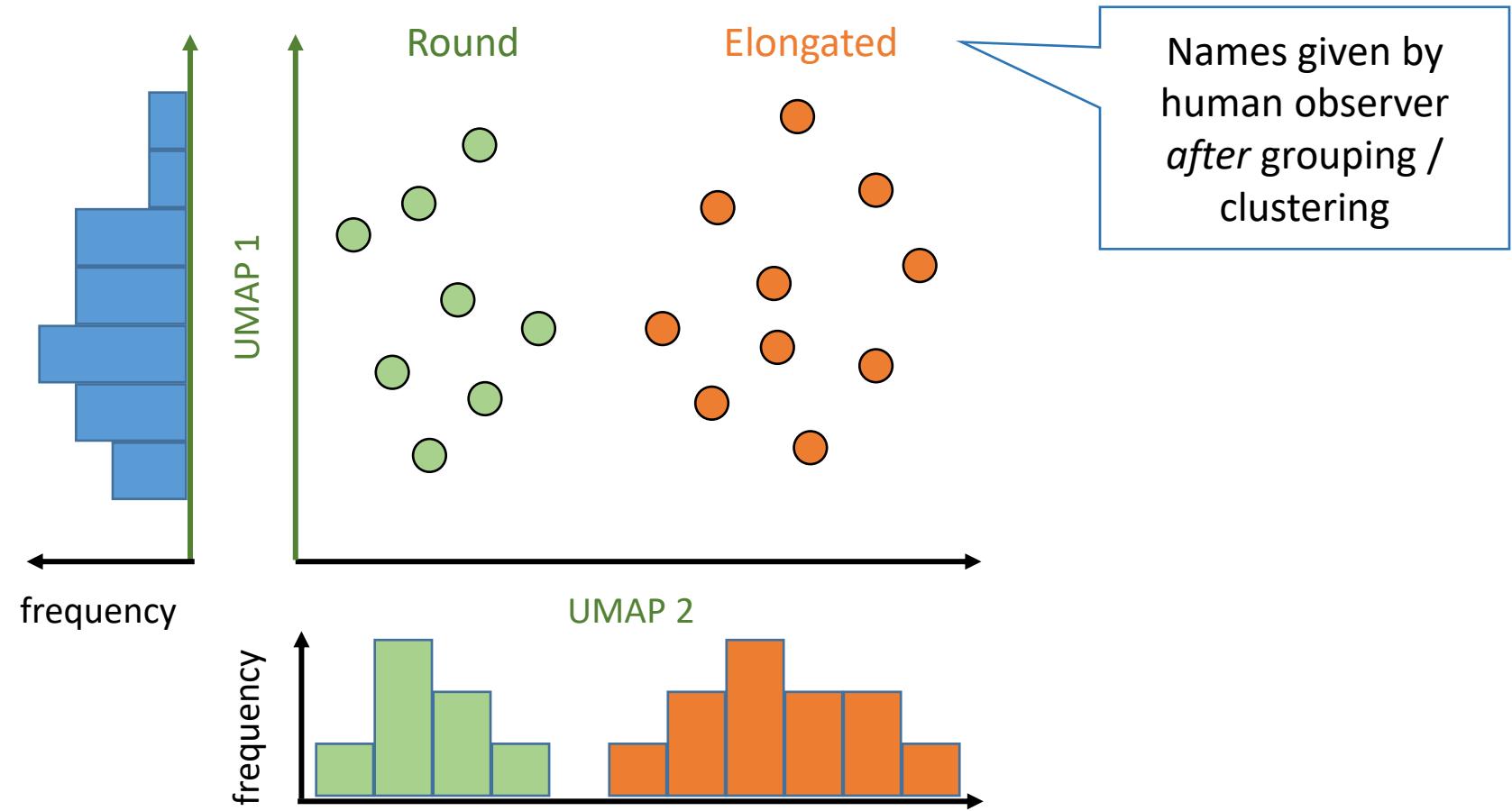
Result of model
training



Quiz: What is the difference between clustering and classification?

Clustering

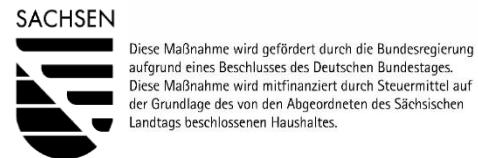
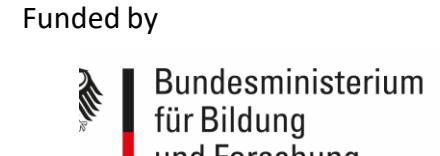
- Unsupervised machine learning may include grouping objects without given ground truth



Supervised and Unsupervised Machine Learning for Bio-image Analysis

Robert Haase

Reusing materials from Johannes Soltwedel, Till Korten, Johannes Müller, Laura Žigutytė (TU Dresden), Ryan Savill (MPI-CBG), Matthias Täschner (ScaDS.AI/Uni Leipzig) and the Scikit-learn community.



Quiz

- Supervised machine learning requires...

A human supervisor



Labeled data



A binary image



Dimensionality reduction



Deep Learning Basics

Robert Haase

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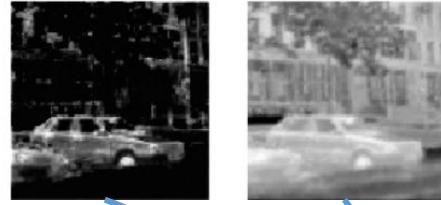


Diese Maßnahme wird gefördert durch die Bundesregierung
aufgrund eines Beschlusses des Deutschen Bundestages.
Diese Maßnahme wird mitfinanziert durch Steuermittel auf
der Grundlage des von den Abgeordneten des Sächsischen
Landtags beschlossenen Haushaltes.

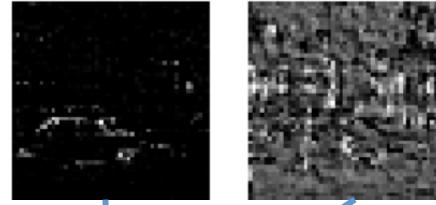
Quiz

- What is **this part of a DNN** typically called?

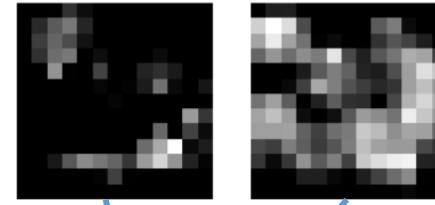
Layer 1 (256, 100, 100)



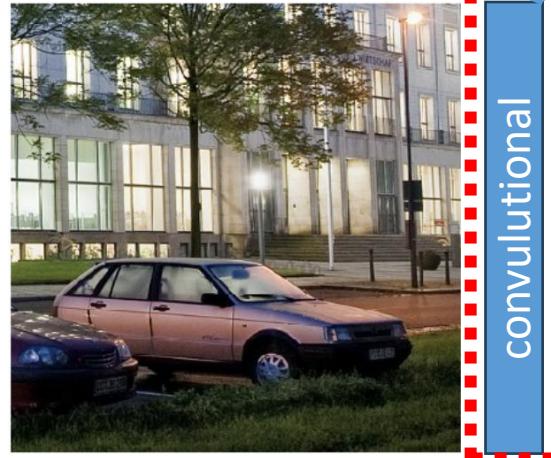
Layer 2 (512, 50, 50)



Layer 4 (2048, 13, 13)



400x400



convolutional

convolutional

conv.

conv.

- Beach wagon
- goldfish
- palace

Reducer



Increaser



Encoder



Decoder



Explainable Deep Learning

Robert Haase

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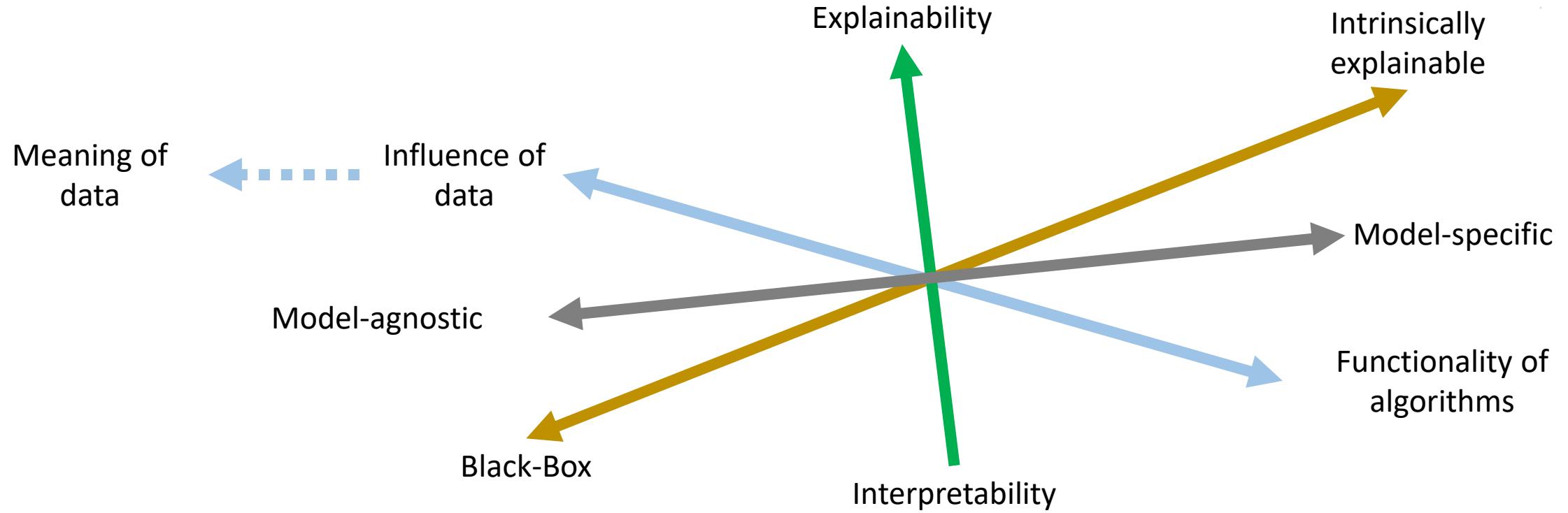
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Landtags beschlossenen Haushaltes.

Summary: Explainable AI

- Methods of XAI can be classified on different scales



Deep Learning for Microscopy Image Processing

Robert Haase

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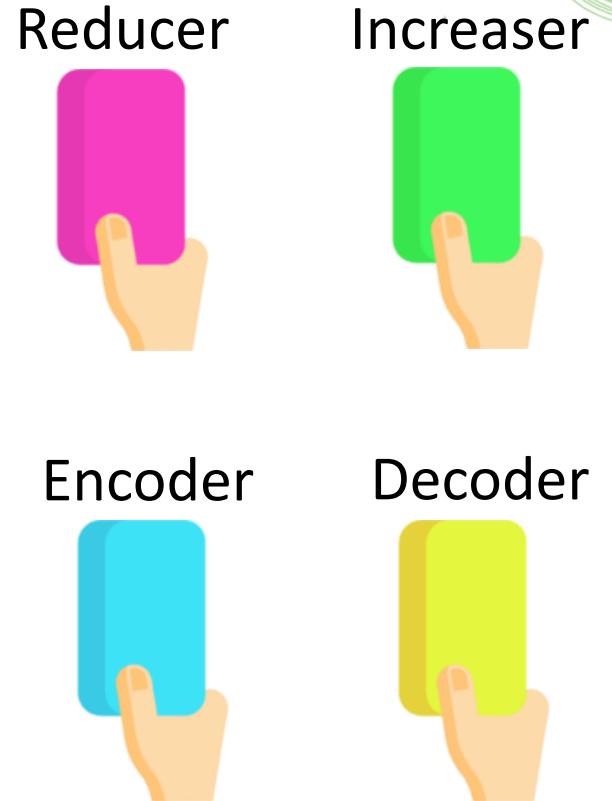
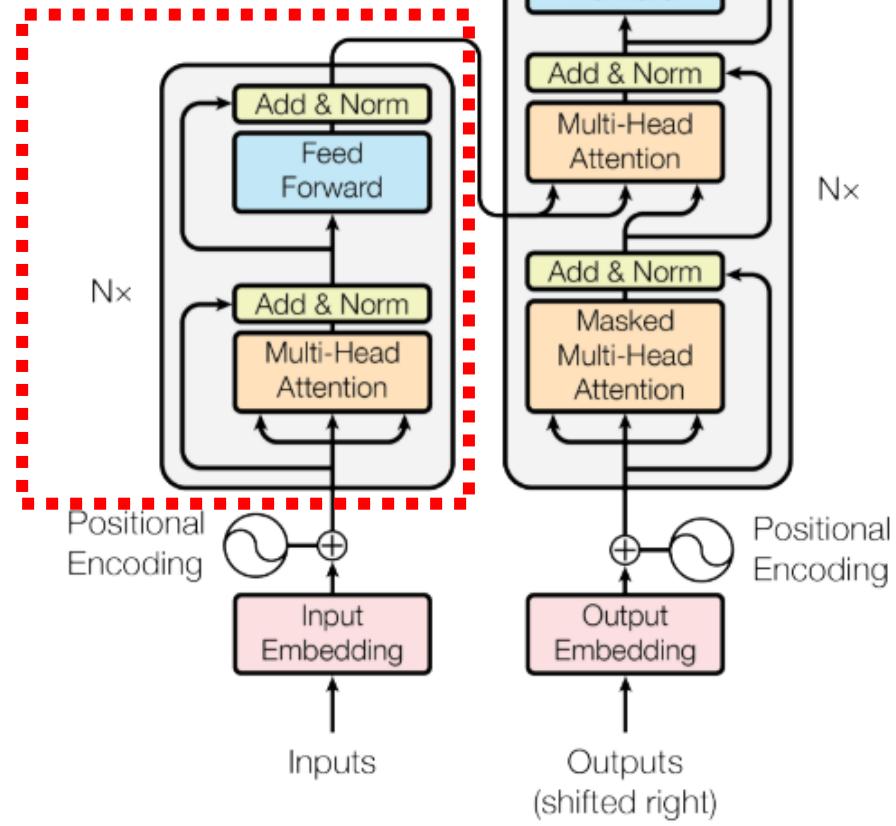
Quiz

- Which shape features may be compromised if we use StarDist for segmentation? Why?

Transformer neural networks

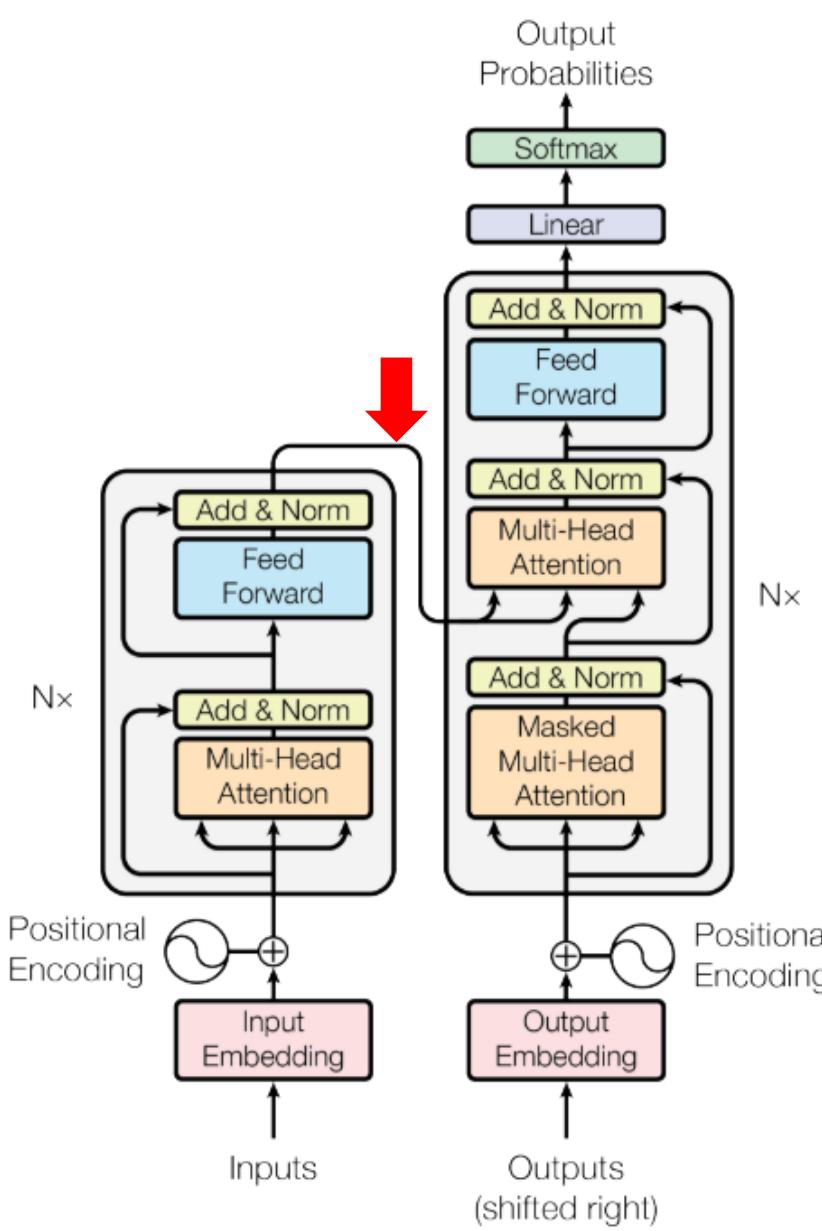
Quiz

- What is **this part of a LLM** typically called?



Quiz

- What is **this part of a LLM** typically called?



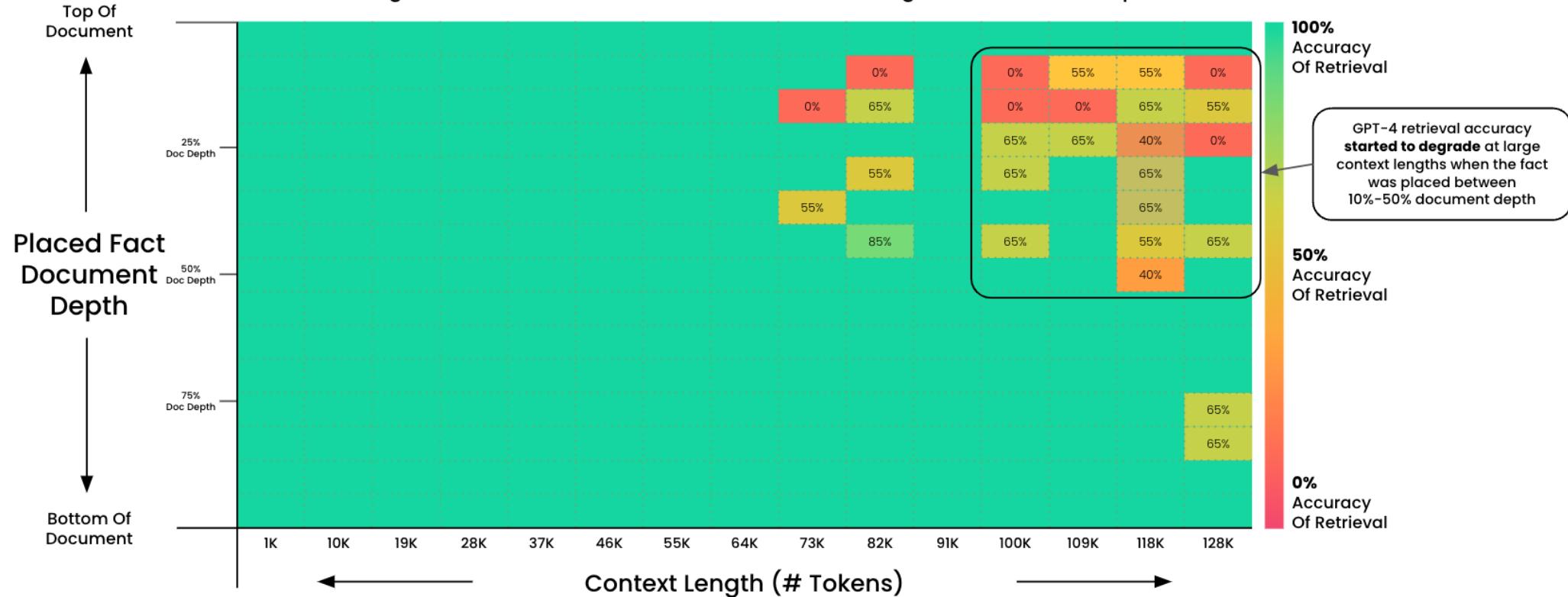
Prompt Engineering

Quiz: What is prompt engineering?

Prompt engineering

Pressure Testing GPT-4 128K via "Needle In A HayStack"

Asking GPT-4 To Do Fact Retrieval Across Context Lengths & Document Depth



Goal: Test GPT-4 Ability To Retrieve Information From Large Context Windows

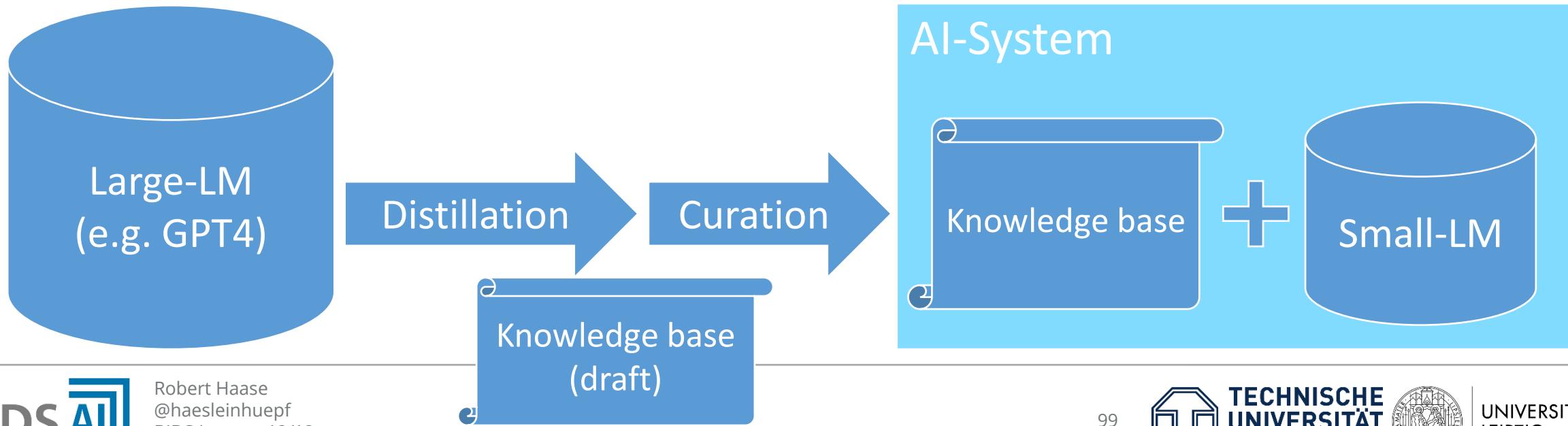
A fact was placed within a document. GPT-4 (1106-preview) was then asked to retrieve it. The output was evaluated for accuracy.

This test was run at 15 different document depths (top > bottom) and 15 different context lengths (1K > 128K tokens).

2x tests were run for larger contexts for a larger sample size.

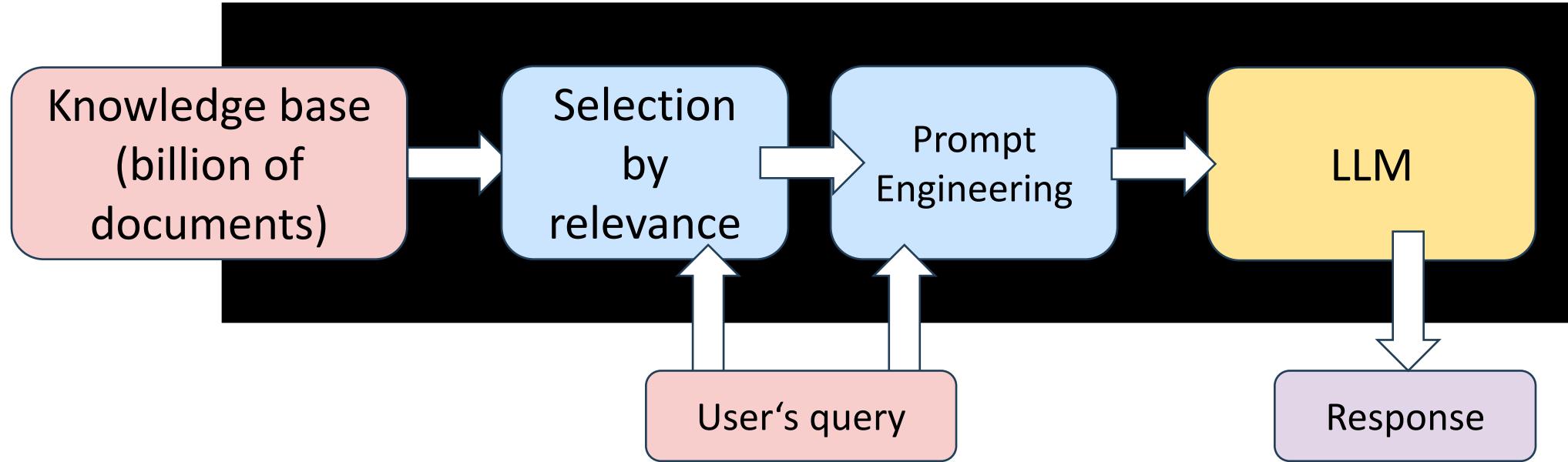
Knowledge distillation

- To focus a small AI-System / LLM on a specific domain with a high-quality knowledge base, one can distill it from a larger AI-System / model.
- Example: “Write 25 code snippets + explanations from the bio-image analysis domain. The code snippets should cover image denoising, segmentation, feature extraction, statistics, plotting, ...”



Quiz

- How is this prompt engineering technique / architecture called?



Reflection



Long-context
prompting



Retrieval
augmented
generation



Knowledge
distillation

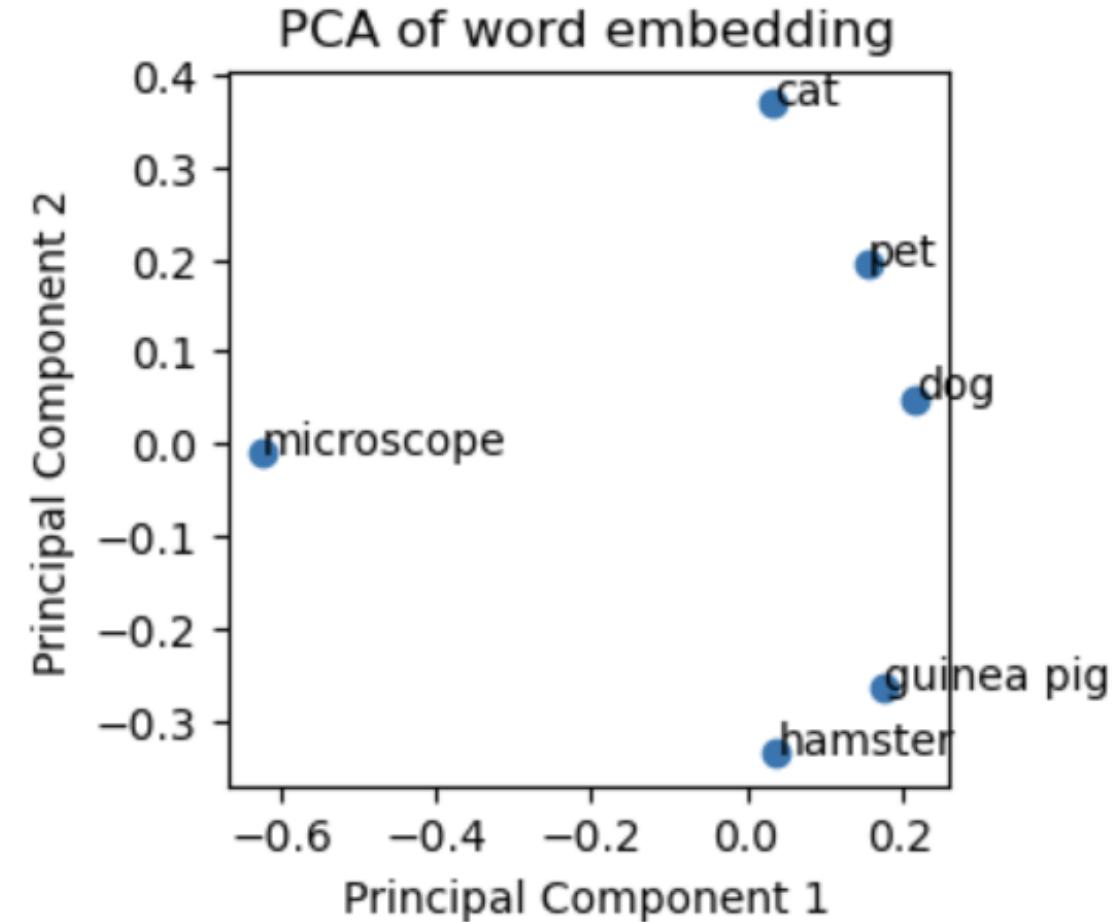
Retrieval-Augmented Generation (RAG)

Query encoder: Text-embeddings

- Abstract numeric representation of words or text [or images]

```
from llama_index.embeddings.huggingface import HuggingFaceEmbedding  
  
embed_model = HuggingFaceEmbedding(model_name="BAAI/bge-base-en-v1.5")  
  
vectors = embed_model._get_text_embedding("Hello world")  
  
len(vectors)
```

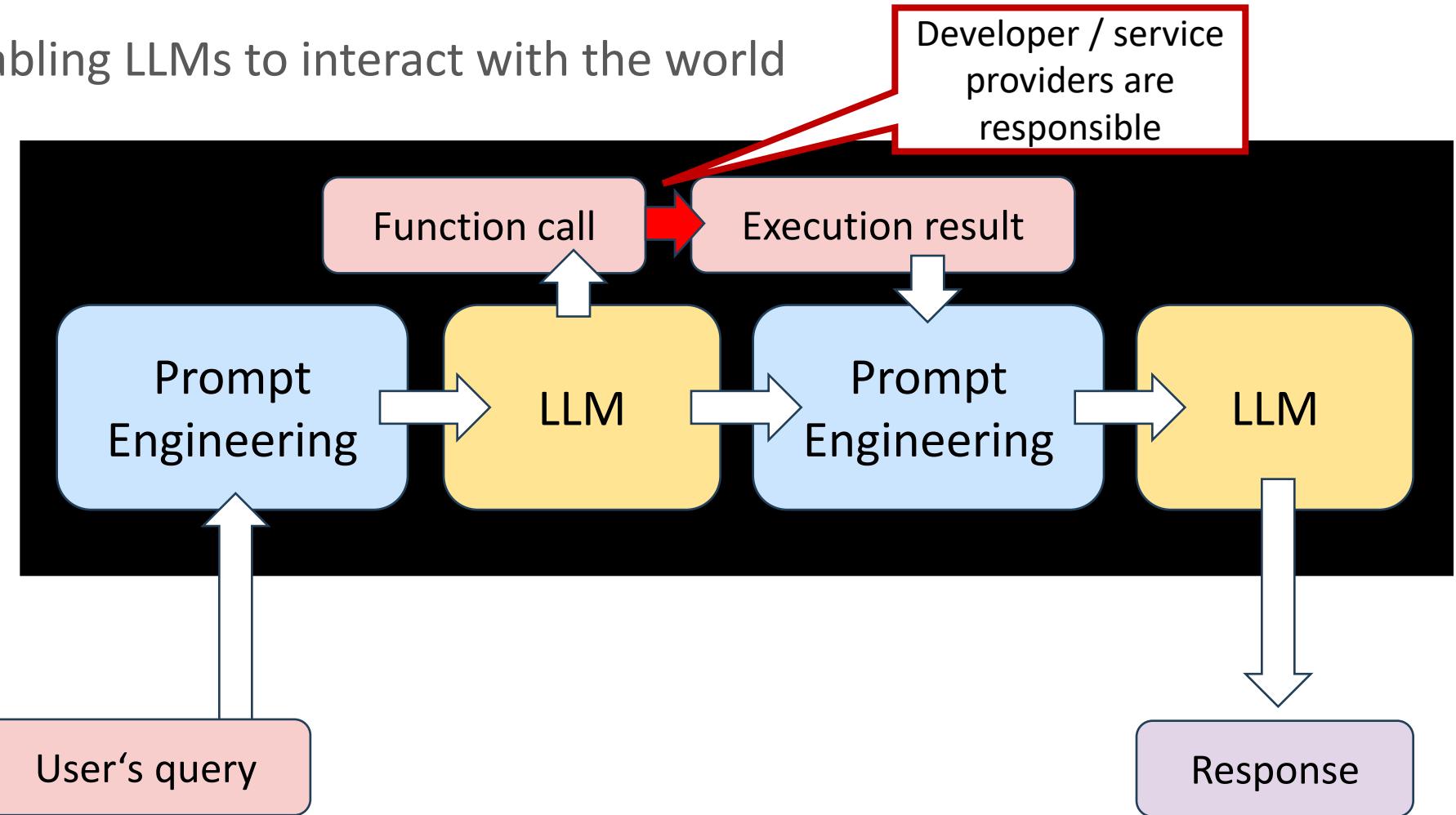
768



Function calling & AI Agents

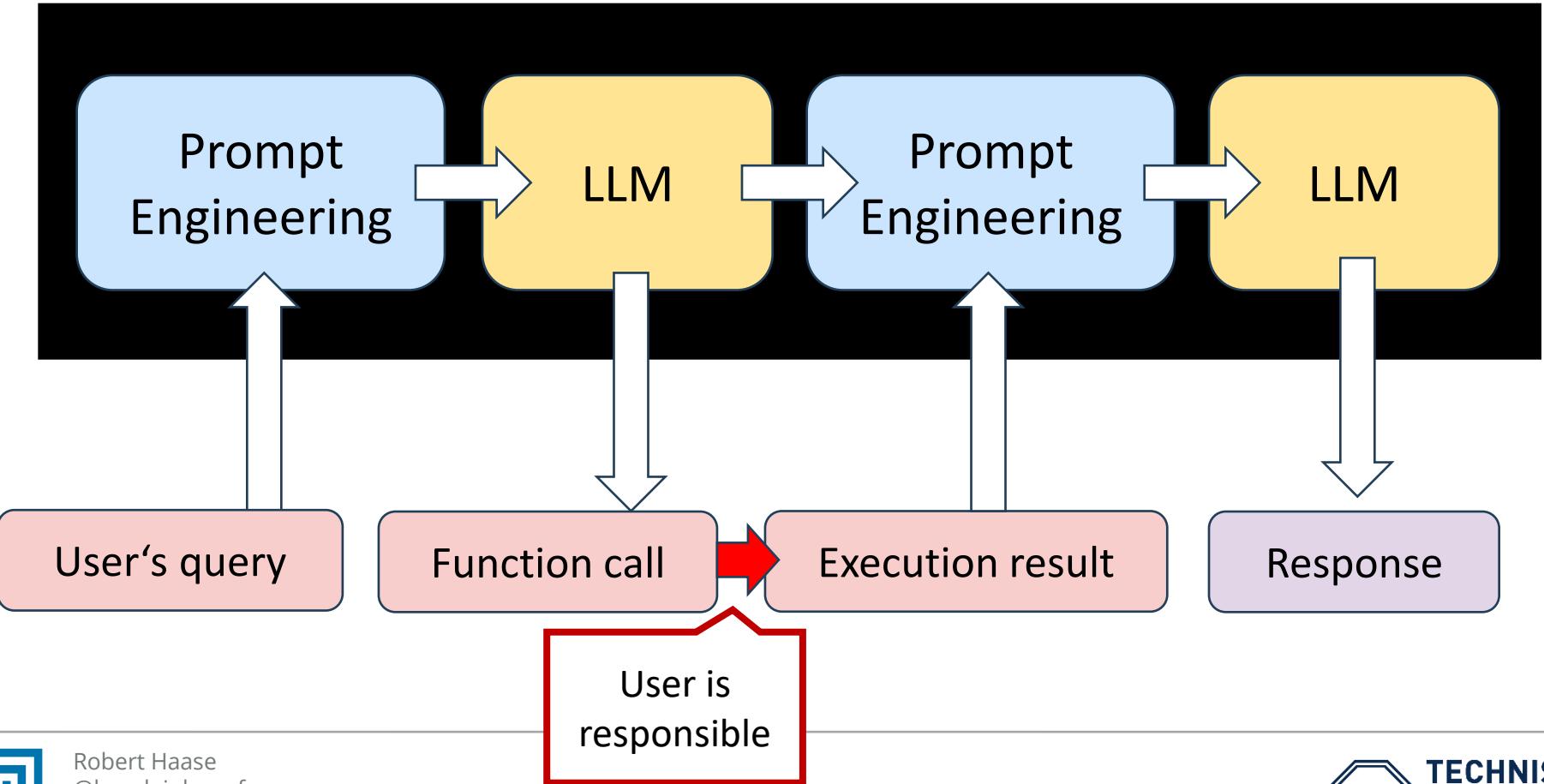
Function calling / “tool calling”

- Enabling LLMs to interact with the world



Function calling / “tool calling”

- Enabling LLMs to interact with the world



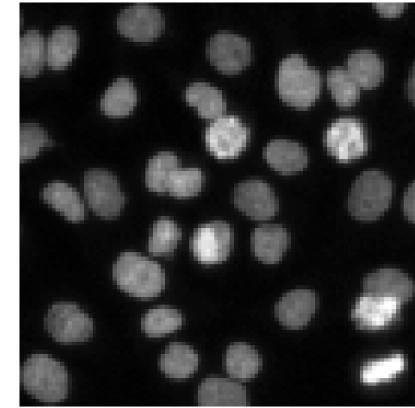
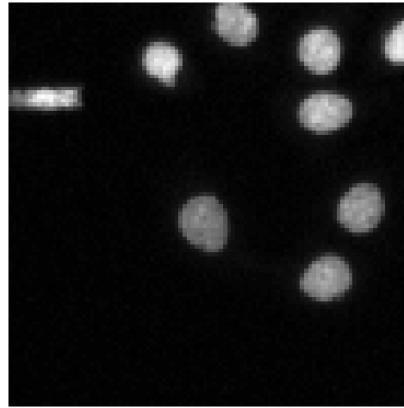
Multi-modal Language Models

Robert Haase

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Quiz

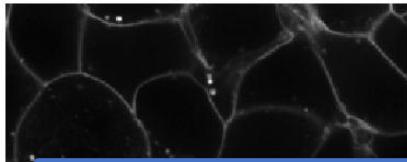
- You have two images and two segmentation algorithms. Assign the algorithms to the images. Explain your choice.



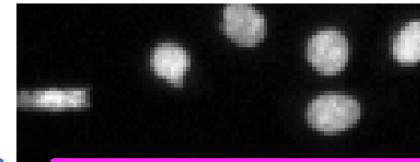
- Voronoi-Otsu-Labeling
- StarDist

VLMs guessing segmentation algorithms

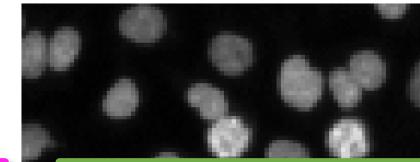
```
prompt = """You are a bioimage-analysis expert. You have a rule-book what algorithms to use for specific images.  
  
## Rules  
  
* If an image shows sparse objects such as nuclei, use Otsu-thresholding for segmenting them.  
* If an image shows dense, partially overlapping objects such as nuclei, use StarDist.  
* If an image shows large cell-like structures with bright membranes, use the Watershed algorithm.  
* In case of doubt, use CellPose.  
  
## The task  
  
What is the best image processing algorithm to segment this microscopy image?  
Answer the algorithm name only. No explanations.  
"""
```



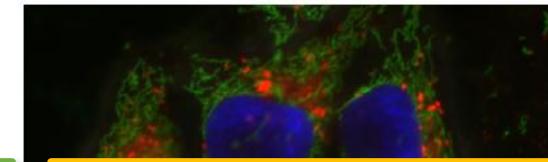
watershed



thresholding otsu
otsu thresholding



stardist
otsu thresholding



cellpose
stardist



DRESDEN LEIPZIG

CENTER FOR SCALABLE DATA ANALYTICS
AND ARTIFICIAL INTELLIGENCE

Research Data Management

Robert Haase

GEFÖRDERT VOM



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Quiz

Thinking of the FAIR principles for data management, which one is wrong?

Findable

Accessible

Inoperable

Reusable

Quiz

- What is the role of Github in the context of publishing open-source code?

Copyright holder



Author



Publisher



Licensee



Quiz

Can I build a commercial product on the basis of GPL-licensed code?

Yes



No



Do I have to release the code openly for this commercial product?

Yes



No



Quiz

It's ok to reuse this code if ...

haesleinhuepf / **imagej-run-async** Public

Code Issues Pull requests Actions Projects Wiki Security Insights Settings

master 1 branch 0 tags Go to file Add file Code

haesleinhuepf initial version 2f8c334 on 23 Jun 2019 1 commit

src/main/java/net/haeslein... initial version 3 years ago

.gitignore initial version 3 years ago

pom.xml initial version 3 years ago

Help people interested in this repository understand your project by adding a README. Add a README

About No description, website, or topics provided.

0 stars 1 watching 0 forks

Releases No releases published Create a new release



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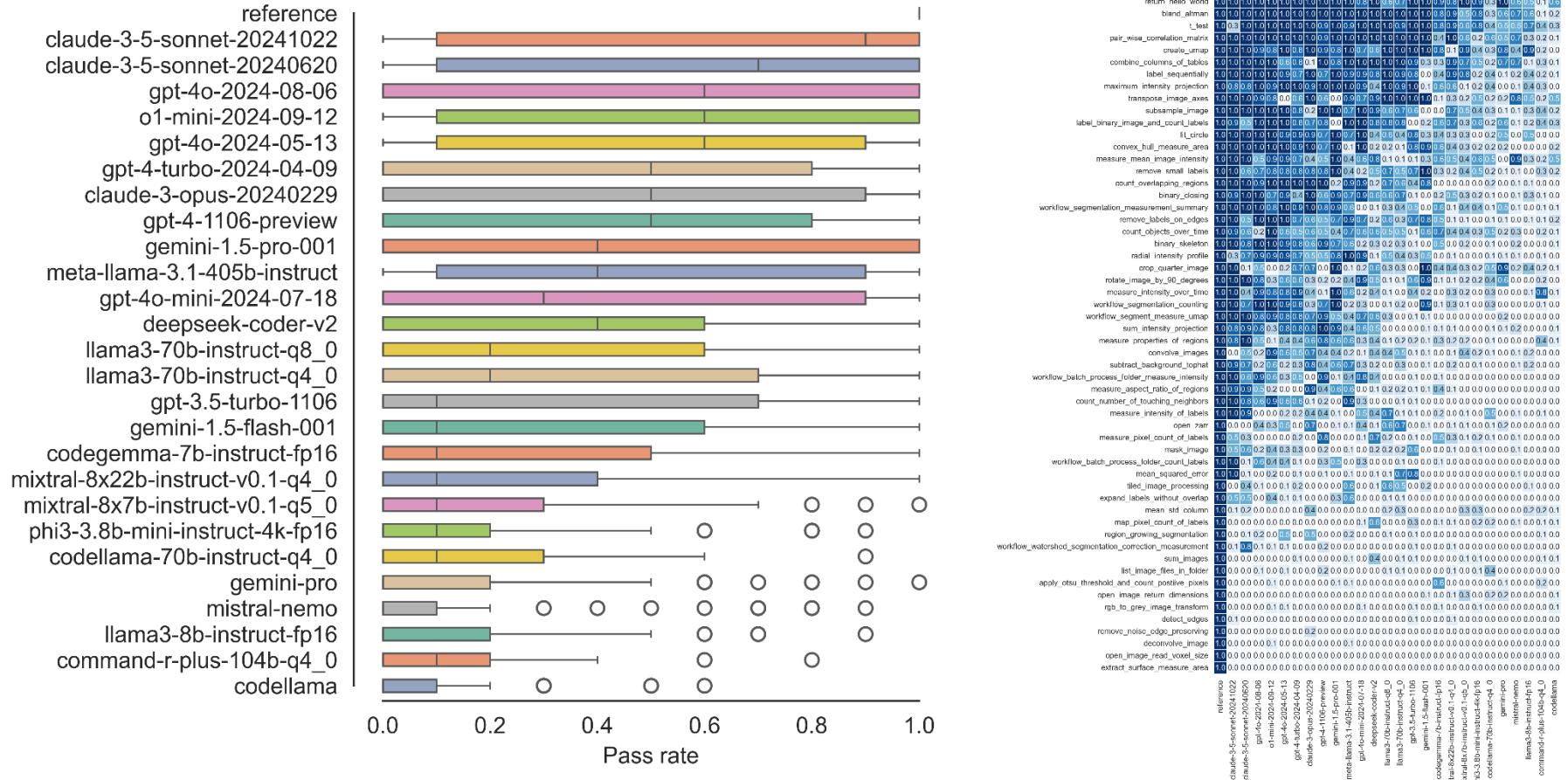
Copy the copyright statement

One more thing

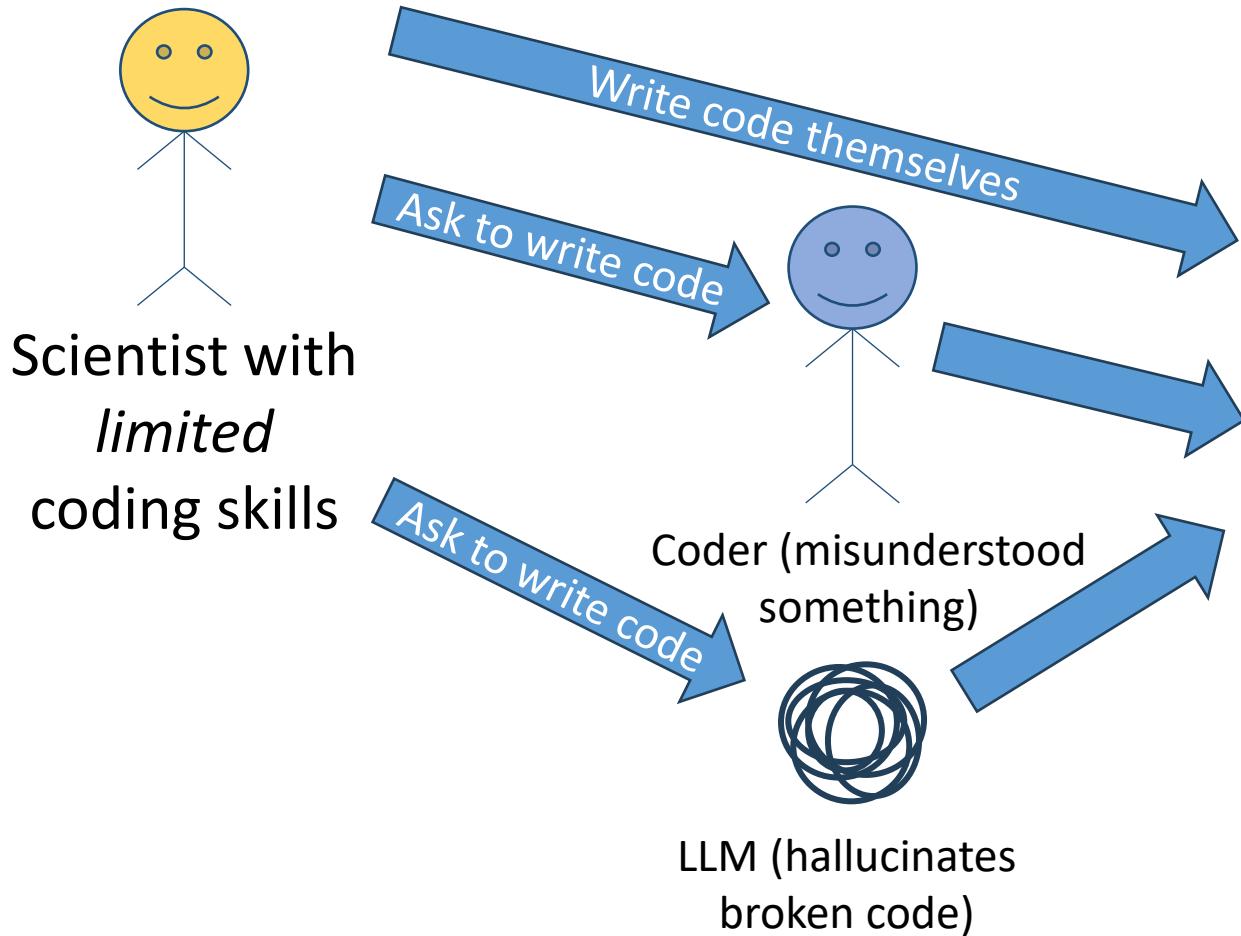
- (actually 2)

Potential Master thesis topic: AI-assisted bio-image analysis

- Benchmarking LLMs for BIA: 57 use-cases (yet), 26 LLMs (yet), n=10



Potential Master thesis topic: AI-assisted bio-image analysis



[1]: `import unprompted`

Cheap, fast

👉 Hi, this is *unprompted* 0.1.1 using gpt-4.1-nano under the hood. Following code cells and related output will be interpreted by AI to provide feedback and suggest improvements. If you want to keep code and/or its output private, do not use this tool or configure it to use a local LLM. Check the documentation for details. Also *unprompted* does mistakes. Treat its suggestions carefully.

[2]: `distance = 5`

`time = 3`

`speed = distance * time`

► 😊 unprompted feedback: Change to `speed = distance / time` to reflect the correct physical relationship.

Unprompted feedback may facilitate identifying issues in code early

Unprompted feedback from an AI-assistant cannot replace human peer-review

Suggested topic Bioimage analysis

Questions:

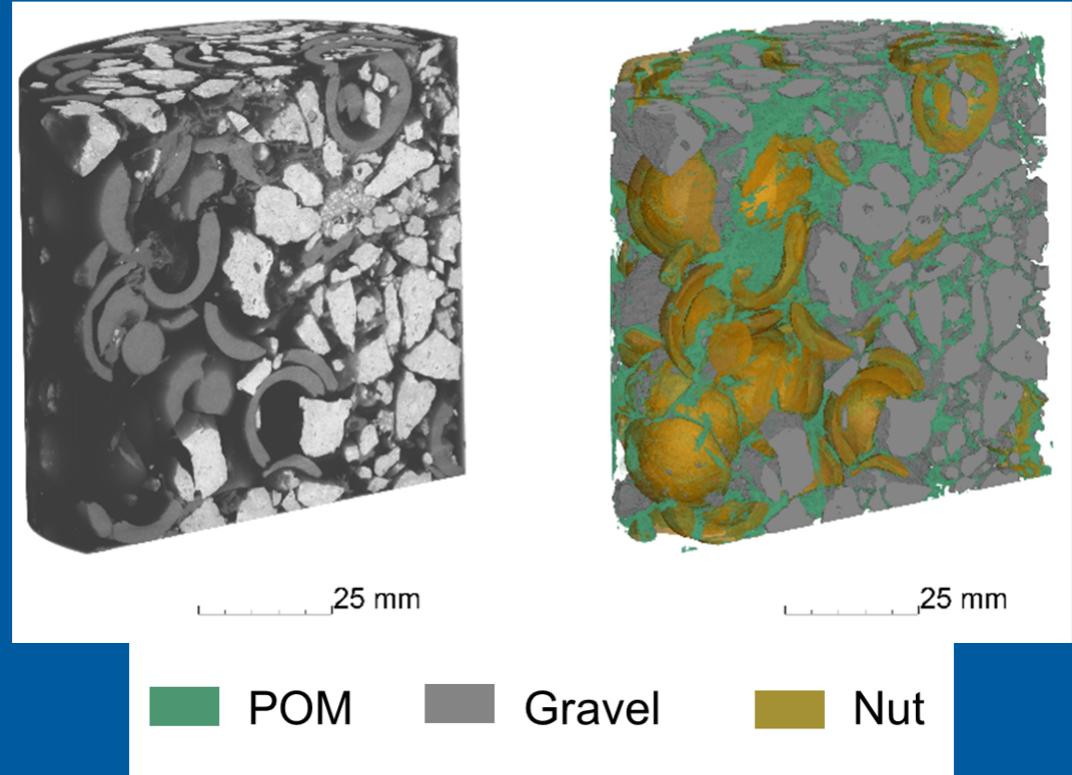
- Volume of defined material in the sample volume
- Surface area per material in the sample volume
- Porosity of the sample
- Orientation of the objects (especially nutshells in space) for later flow simulations

Optional:

- Preparation of the data set for 3D representation in the UFZ visualization center
- Tracking of the root structure and 3D visualization

Methodology:

- 3D X-ray computed tomography (3D X-ray µCT)
- 3D rendering numerical models
- Segmentation -> assignment of gray values to materials
- Comparison of segmentation and ML Object recognition methods (trainings data: pure material scans)



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