Diatom classification via deep learning using raw holograms captured by a lenless holographic system

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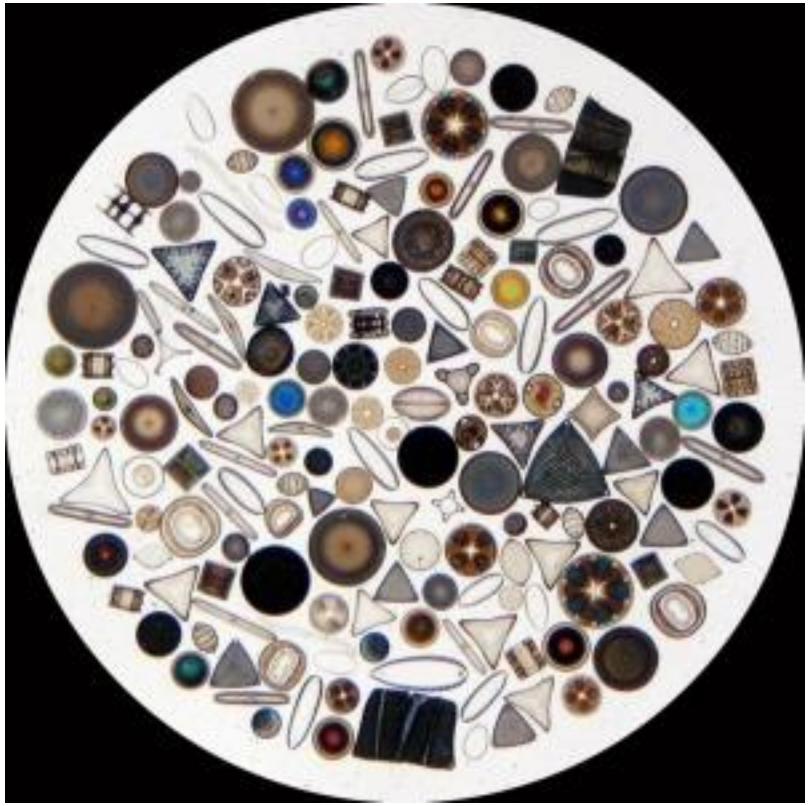
Monitoring of Diatoms is Crucial in Understanding the Overall Health of Aquatic Ecosystems

- **Diatoms** are microscopic algae that play a crucial **role** in **aquatic ecosystems**.
- Diatoms' **size** depends on the **species**, ranging from 2 to 200 µm.
- Diatoms are **sensitive to changes** in water quality, including nutrient levels, temperature, and pollution.
- Their **abundance** and **diversity** indicate the overall **health** of aquatic ecosystems.
- **Monitoring diatom** communities can help assess water quality and **identify** potential **environmental problems**.



https://underthecblog.org/2013/10/21/diatom-detectives/

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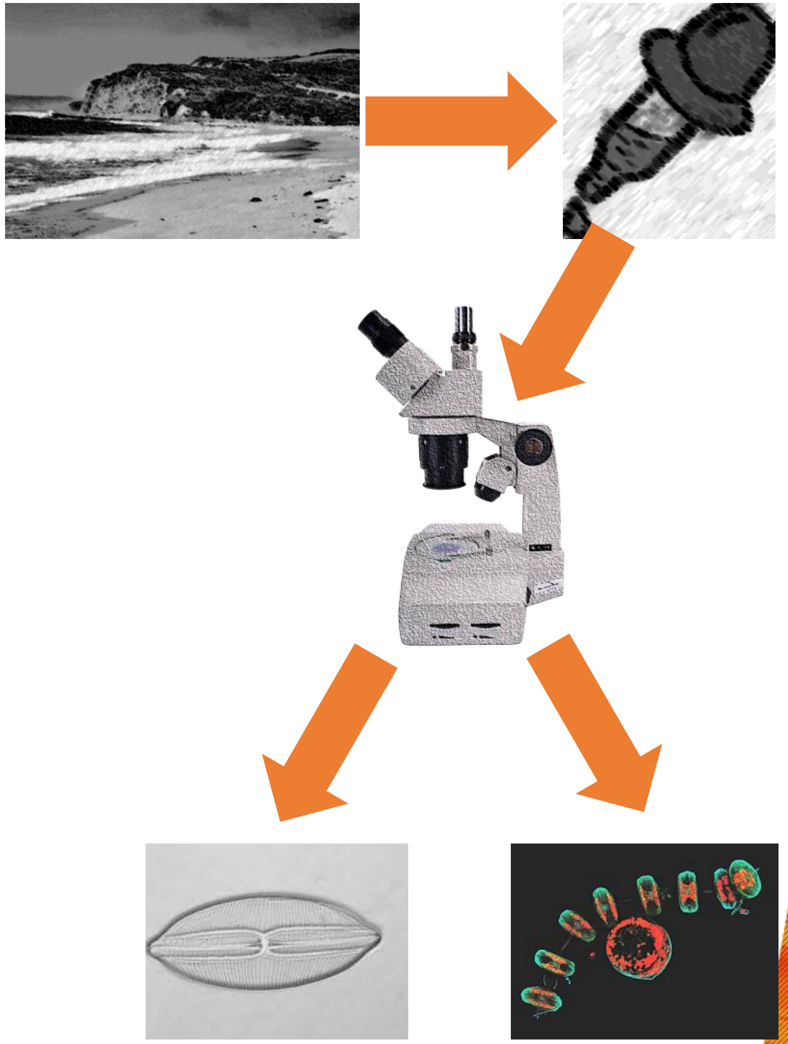
Diatoms are traditionally identified based on their morphology in a third-party lab

- **Conventional identification** of diatoms requires the **collection of water samples** from the target environment.
- Traditional **brightfield microscopy** is typically used to inspect the **morphological features** of diatoms.
- **Widefield fluorescence microscopy** has been used to study **live diatoms** and assess **their physiological status,** providing insights into the health and vitality of diatom populations.
- **Limitation: diatom cells** should be **mounted** on **microscope slides** for their analysis.



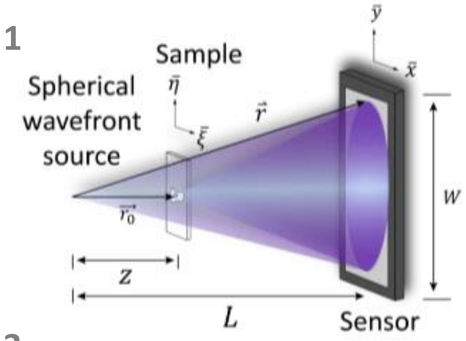
Digital Holographic Microscopy (DLHM) enables the study of diatoms

- **DLHM** systems **preserve** the **natural state** of the **diatoms**[1]**,** minimizing artifacts that may be introduced by sample preparation techniques.

- **DLHM systems** have been implemented in **submersible systems**[3]

- **Monitoring of micro-organisms** in potable water, aiming to reduce water-related diseases[2]

- **Quantitative measurements for biodiversity and ecosystem monitoring**[3]: plankton concentration, average size and size dispersion of individuals, particle size dispersion, water turbidity, suspension statistics.

1


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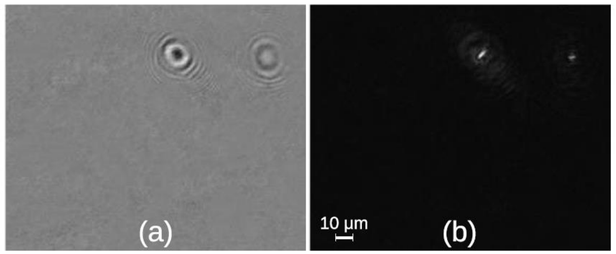

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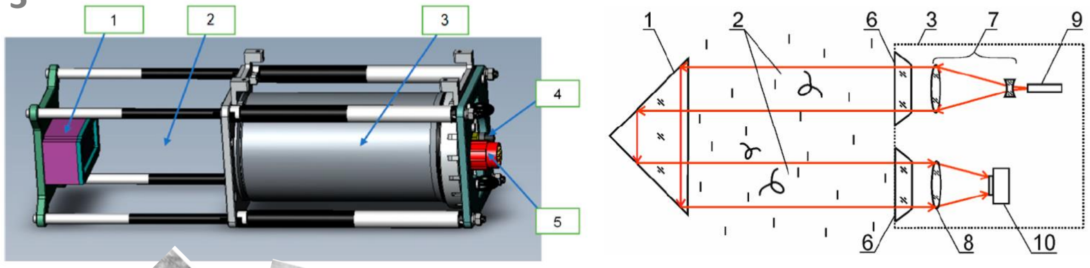

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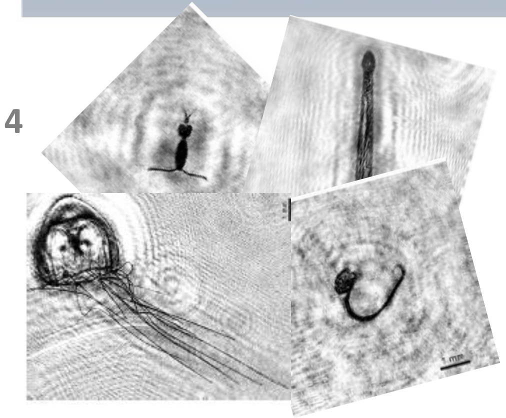

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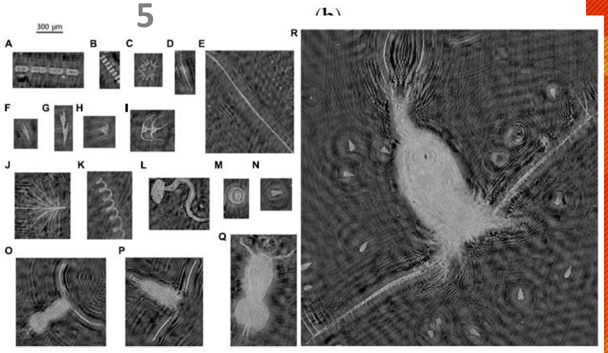

1. Credit to Maria Josef Lopera Acosta, Master dissertation, 2022.
2. Pitkaaho *et al.*, Digital Holography and 3D Imaging 2027, paper W2A.44 (2017)
3. Dyomin, *et. al.*, Sensors 21, 4863 (2021) & Dyomin *et al.*, Appl. Sci. 12, 11266 (2022) & Nayak *et al.*, Frontiers in Marine Science 7, 572146 (2021)
4. Schnitzler *et al.*, Marine Pollution Bulletin 163, 111950 (2021)).

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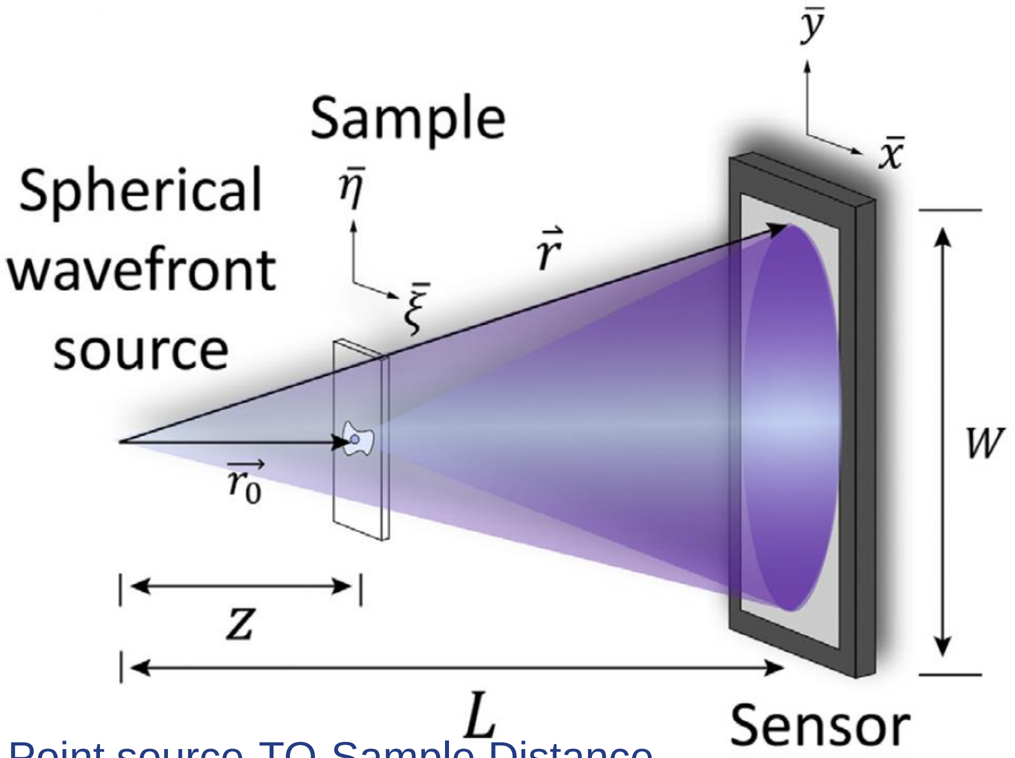
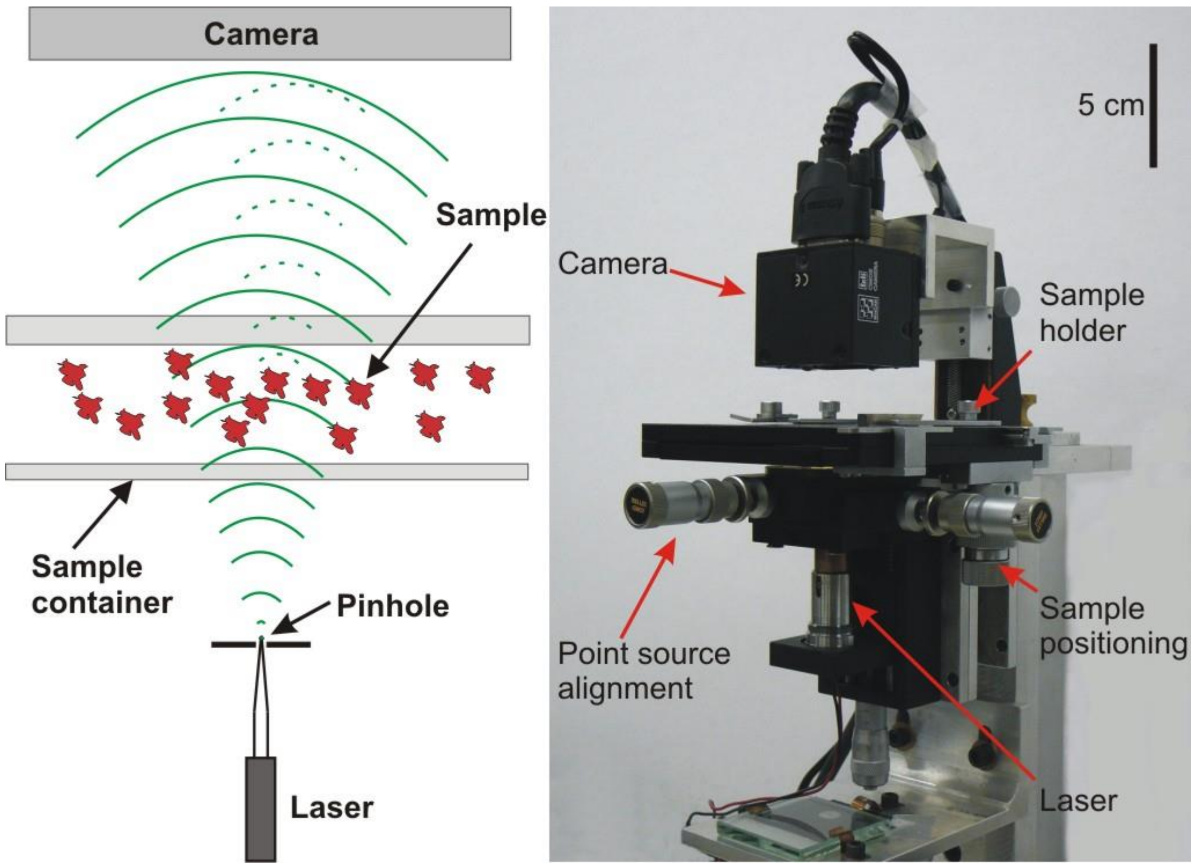
Principle of Digital Lensless Holographic Microscopy (DLHM)



z: Point source-TO-Sample Distance
L: Point source-TO-Sensor Distance
$M = z/L$: Lateral Magnification.
λ: Laser's wavelength
W: Sensor size ($M\,\Delta_{xy}$).
M: Number of pixels
Δ_{xy}: pixel size



Trujillo, Restrepo, and García-Sucerquia, U.S. patent 7620179 (2018).

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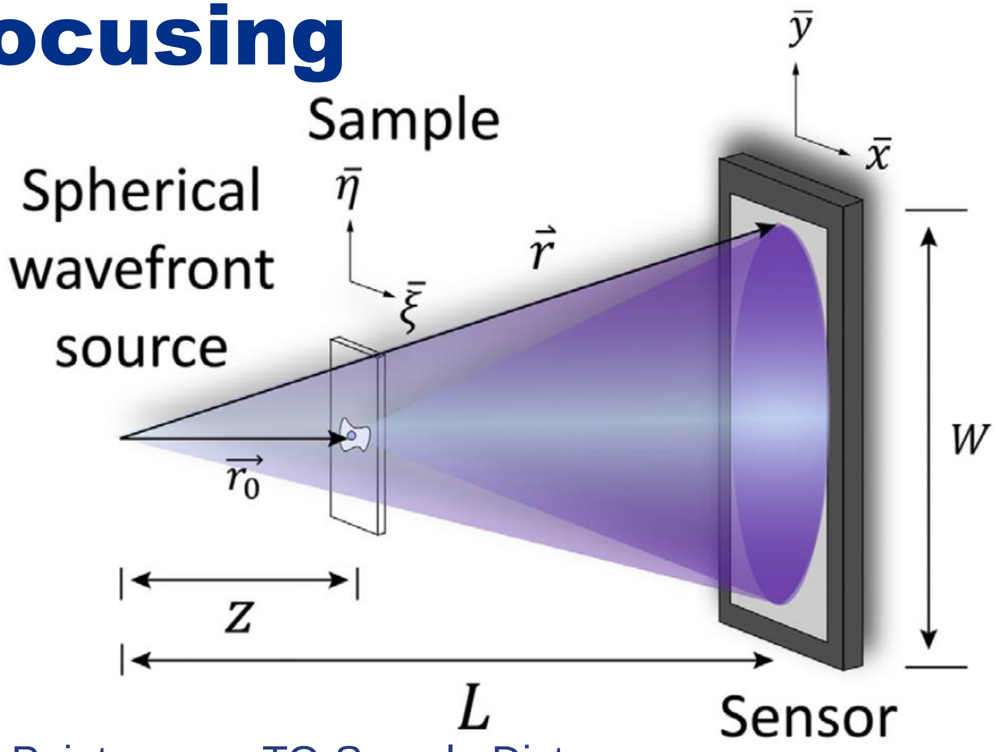
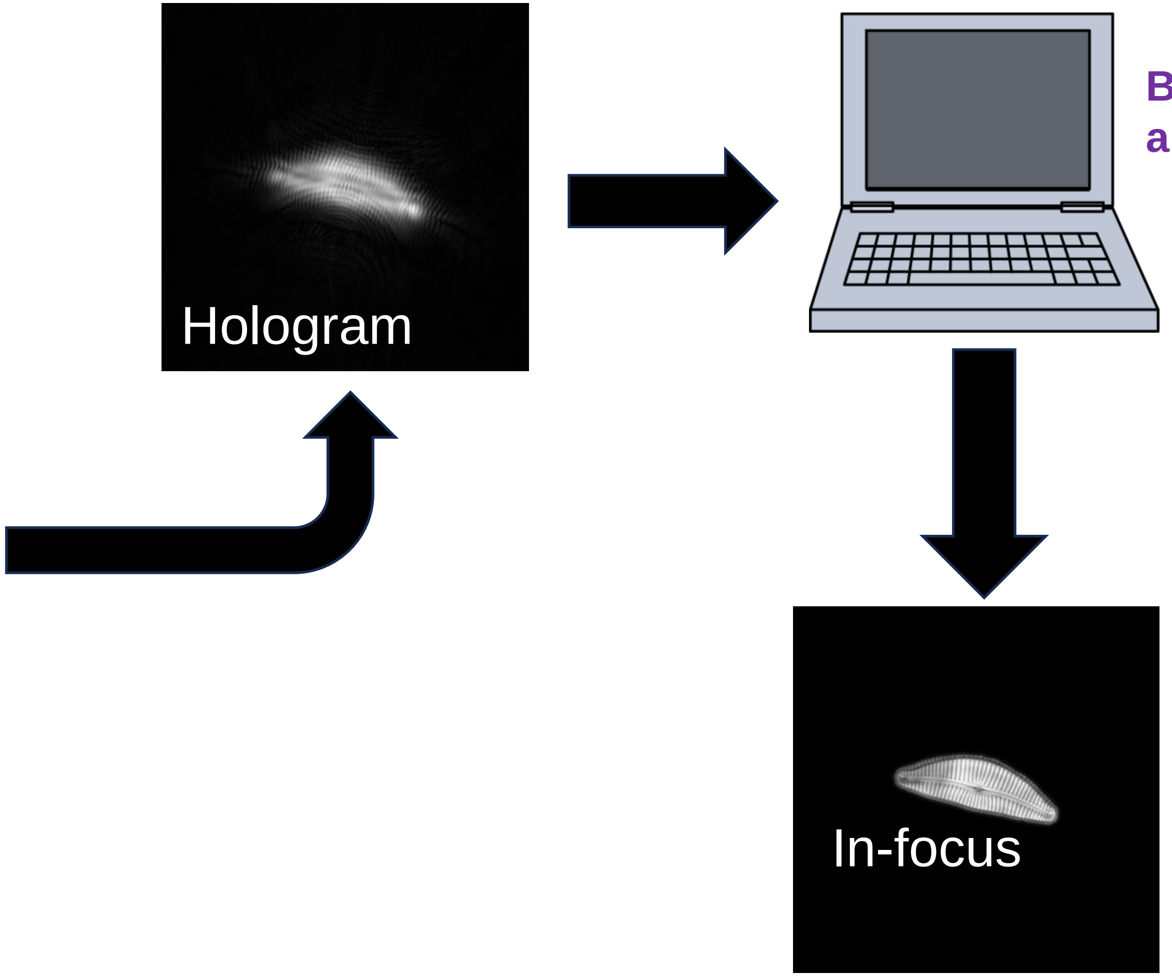
DLHM system records the diffraction pattern of a sample, requiring a computational method for focusing





z: Point source-TO-Sample Distance
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Δ_{xy}: pixel size

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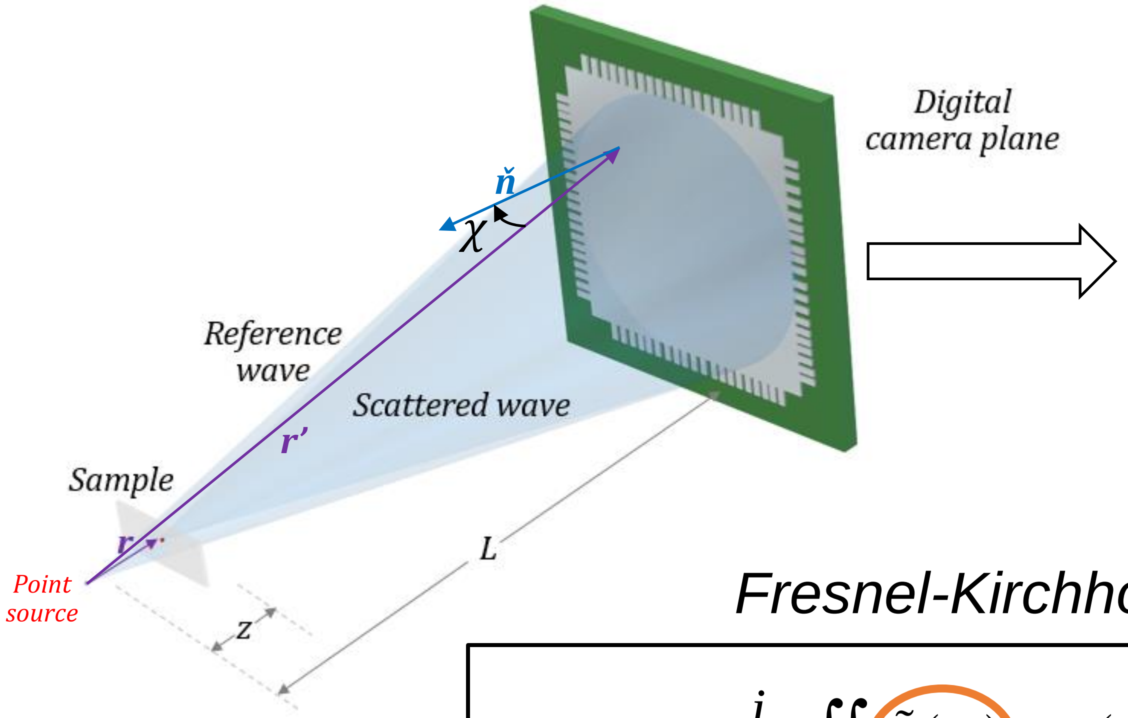
The backpropagation algorithm aims to solve the Fresnel-Kirchhoff diffraction formula



The acquired hologram is transferred to a PC for further processing

Fresnel-Kirchhoff diffraction formula[1]

$$A_{scat}(\mathbf{r}) = -\frac{i}{2\lambda}\iint_{Screen} \tilde{I}(\mathbf{r}')\, A_{ref}(\mathbf{r}')\frac{\exp\left[-ik\left|\mathbf{r}'-\mathbf{r}\right|\right]}{\left|\mathbf{r}'-\mathbf{r}\right|}(1+\cos\chi)\,dS_{\mathbf{r}'}$$

Contrast Hologram

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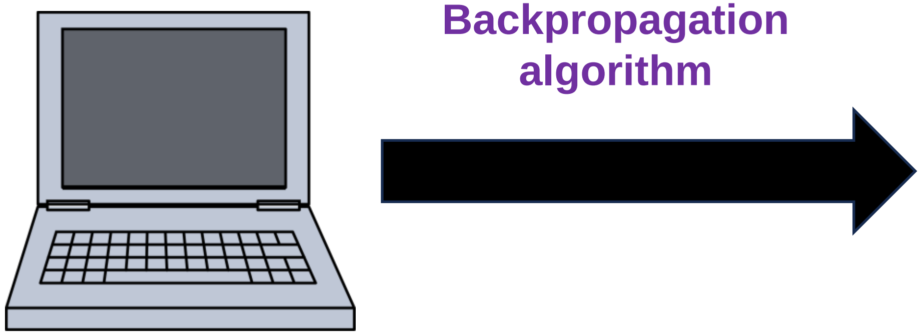
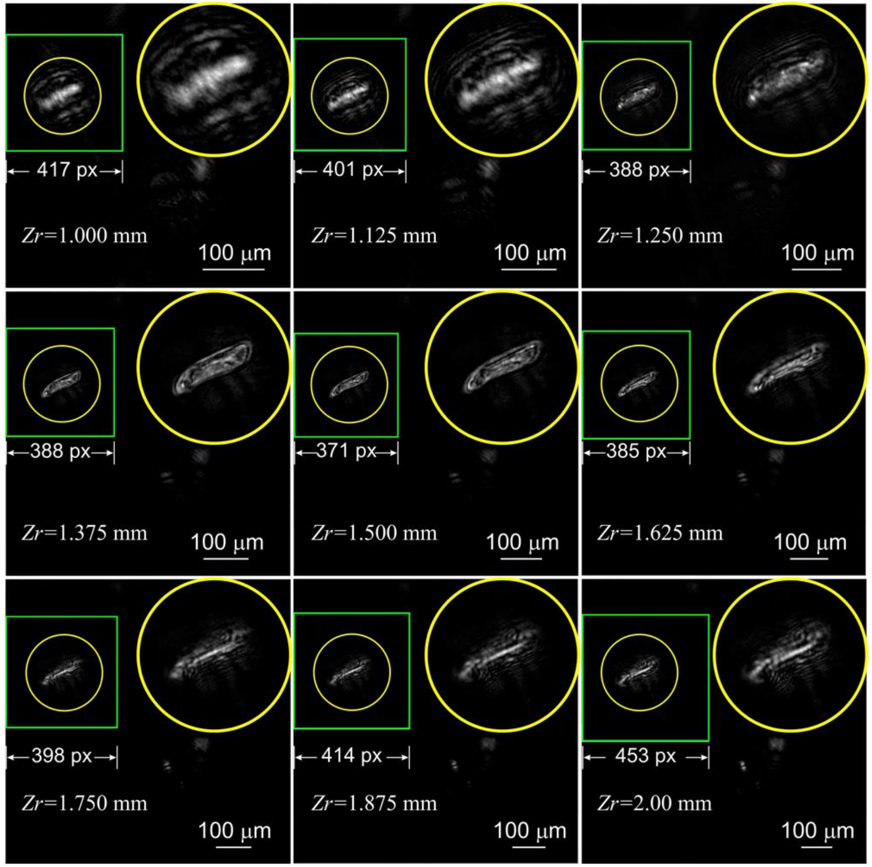
[1]Garcia-Sucerquia, et al., Appl. Opt. 45, 836–850 (2006).

The backpropagation algorithm is expensive in terms of computational complexity and processing time if one has not prior knowledge of the sample distance (z)





How many manual reconstructed images should one estimate to find the correct propagation distance?

Trujillo and Garcia-Sucerquia, Opt. Lett. 39, 2569–2572 (2014)

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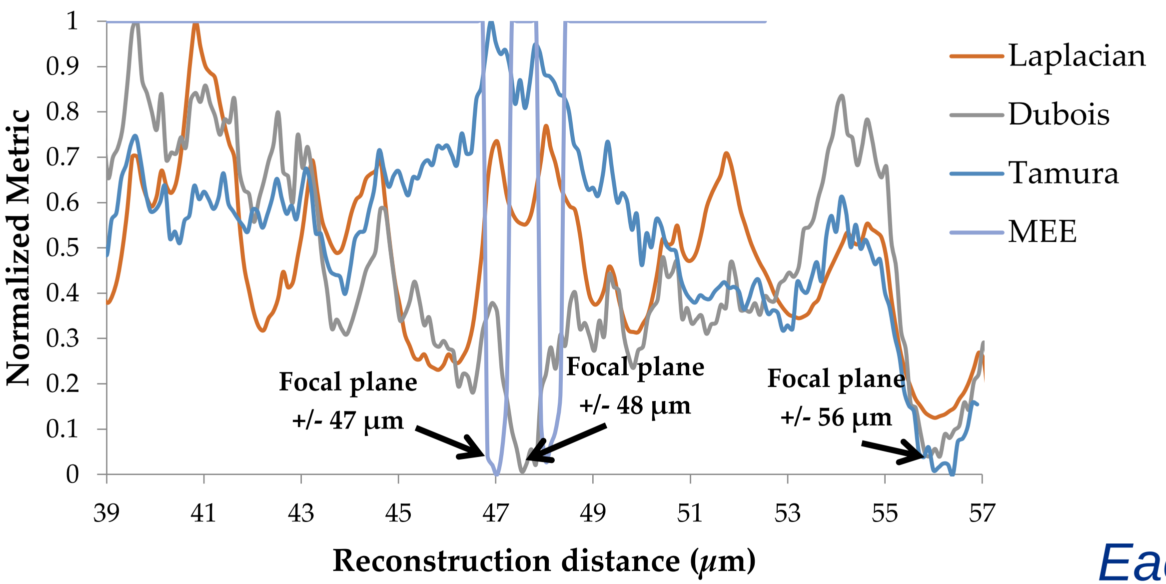
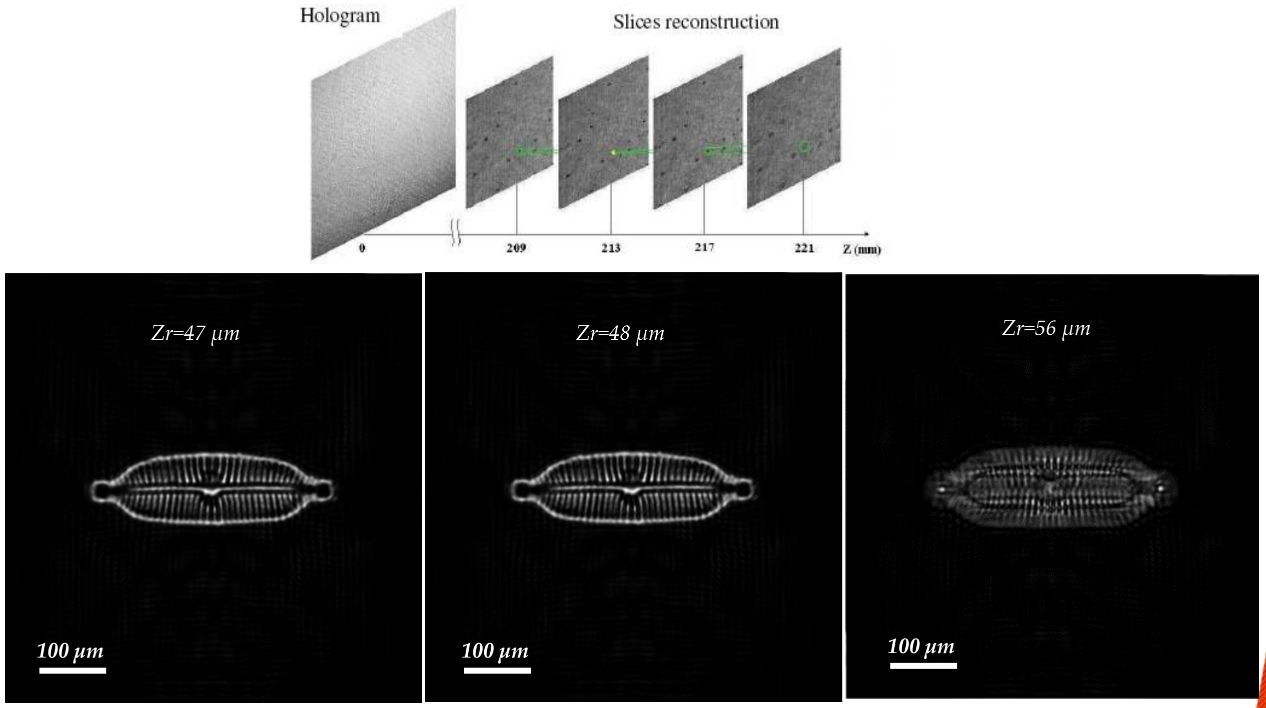
Although DLHM allows a numerical focusing, it is required to define a metric to reconstruct in-focus lensless holograms

MEE: Modified Enclosed Energy





Each metric provides a different reconstruction distance
The best metric changes with the sample[1]

[1]Trujillo and Garcia-Sucerquia, Opt. Lett. 39, 2569–2572 (2014)

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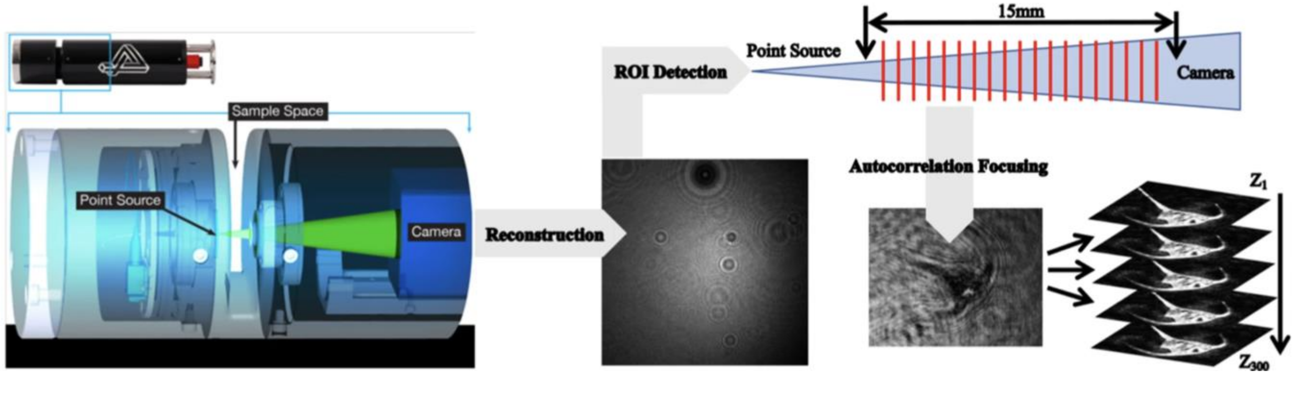
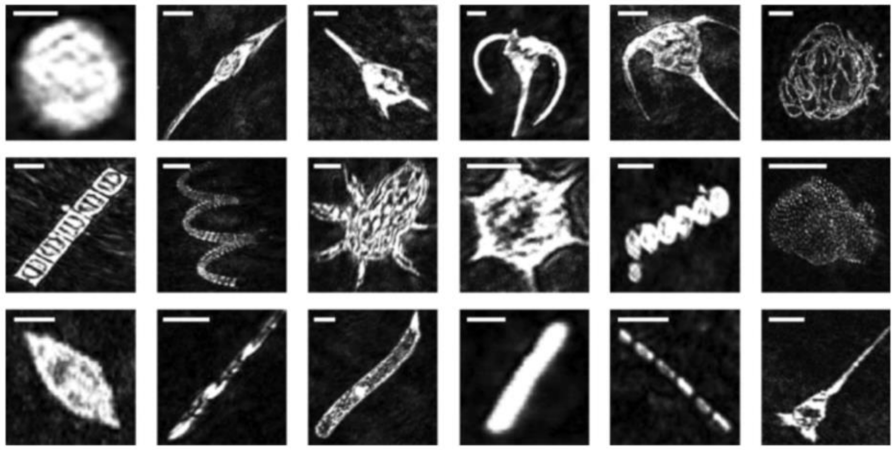
Diatoms have been able to classify with high accuracy using in-focus images from a DLHM system

DLHM system

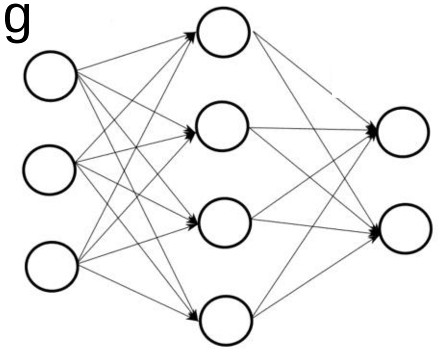


Reconstructed in-focus images



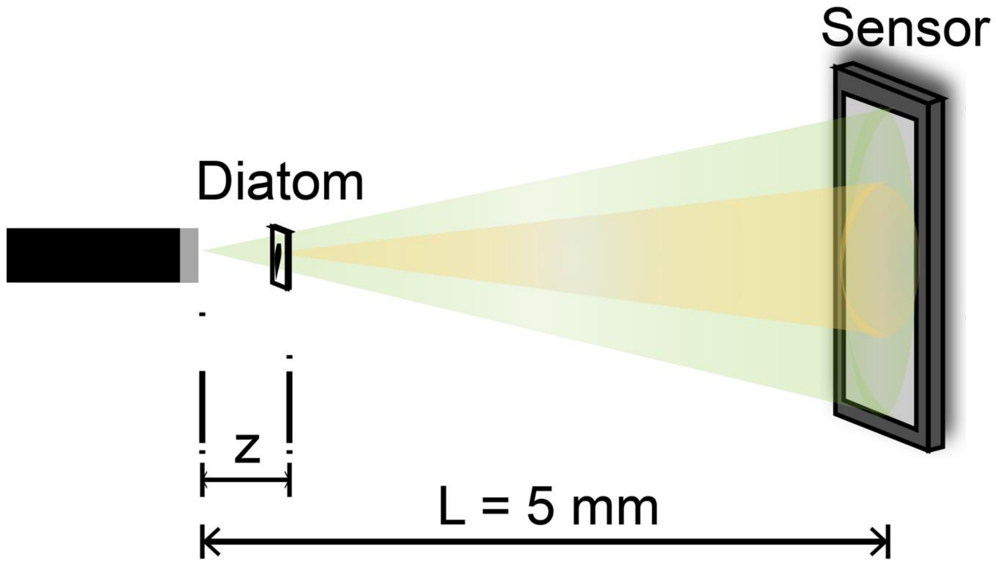
Model	Accuracy (%)
VGG16	88.2 ± 1.2
InceptionV3	82.2 ± 1.8
ResNet50V2	88.2 ± 1.1
Xception	90.1 ± 1.6

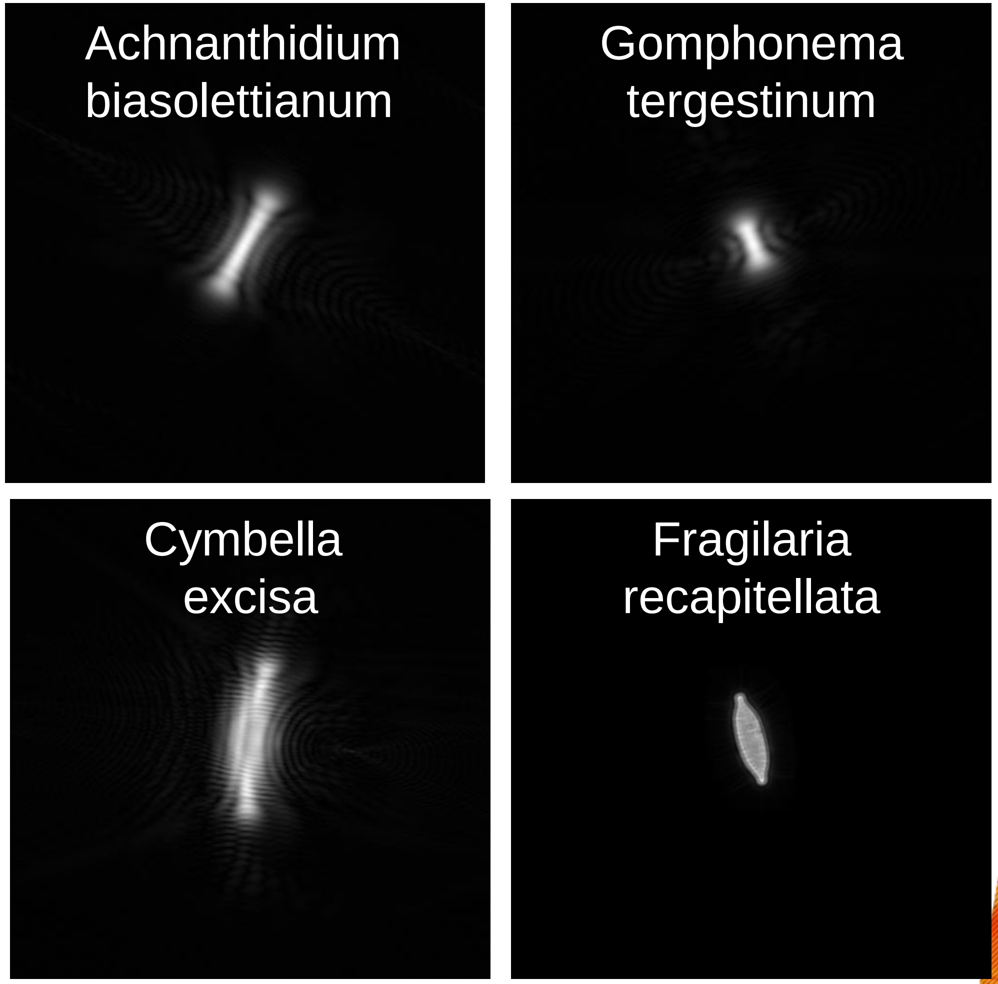
Deep Learning Model



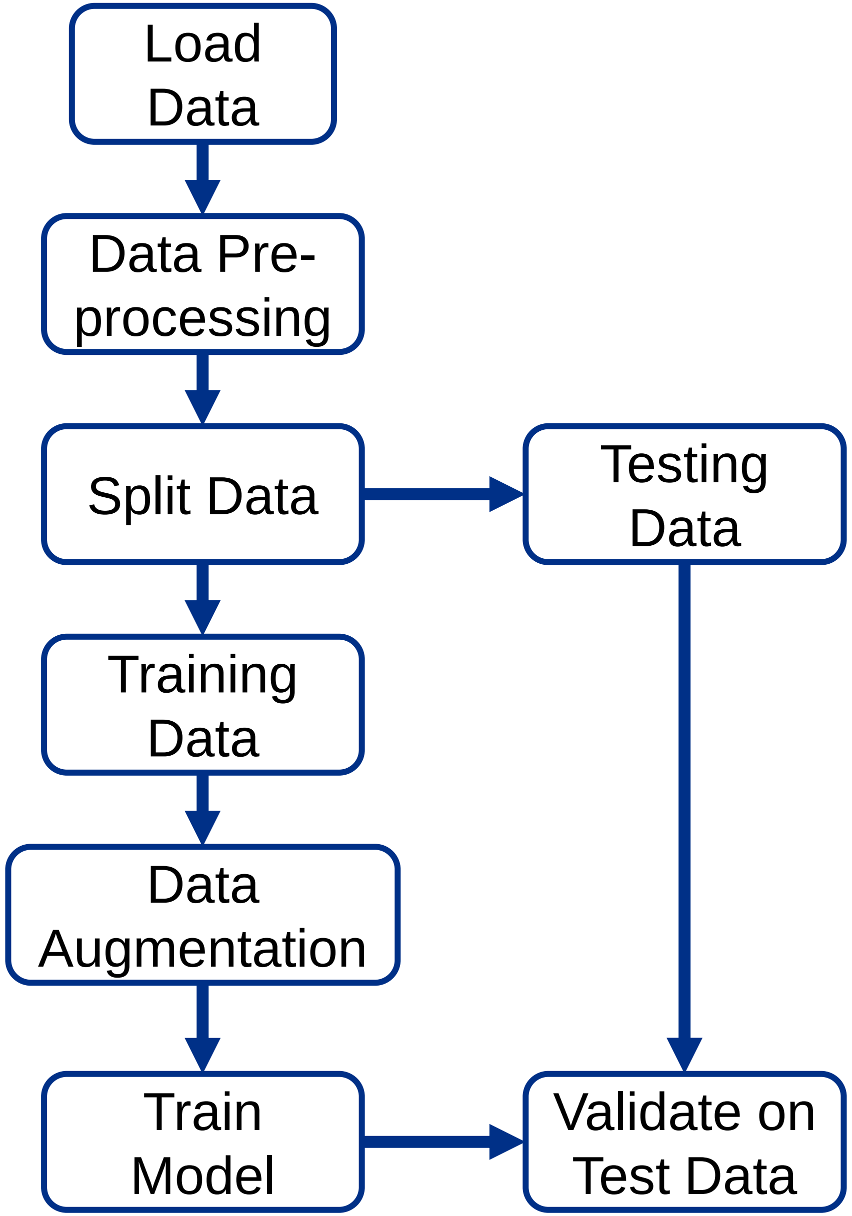
Results from MacNeil, Missan, Luo *et al. BMC Ecol Evo* 21, 123 (2021).

Research Question: Can we classify diatoms from the raw holograms? Do the raw holograms have enough classification information?





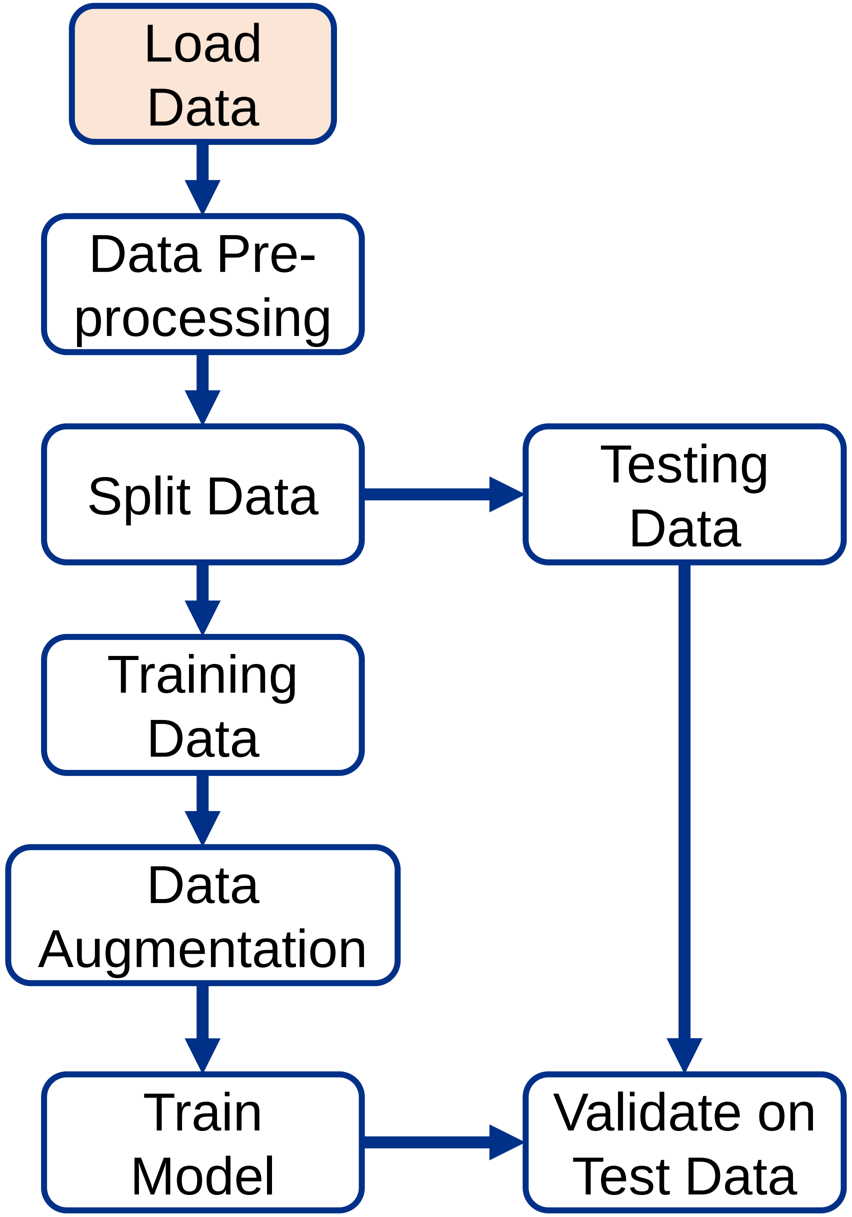
Research Study Framework



Loading the diatom dataset

- Dataset from a public dataset of segmented diatoms located in Turkey water.[1,2]
- The image size for each segmented diatom is 1583x1583.[2]
- We converted the color images to monochrome images.
- Our dataset contains **1,816 images** from **36 classes.**

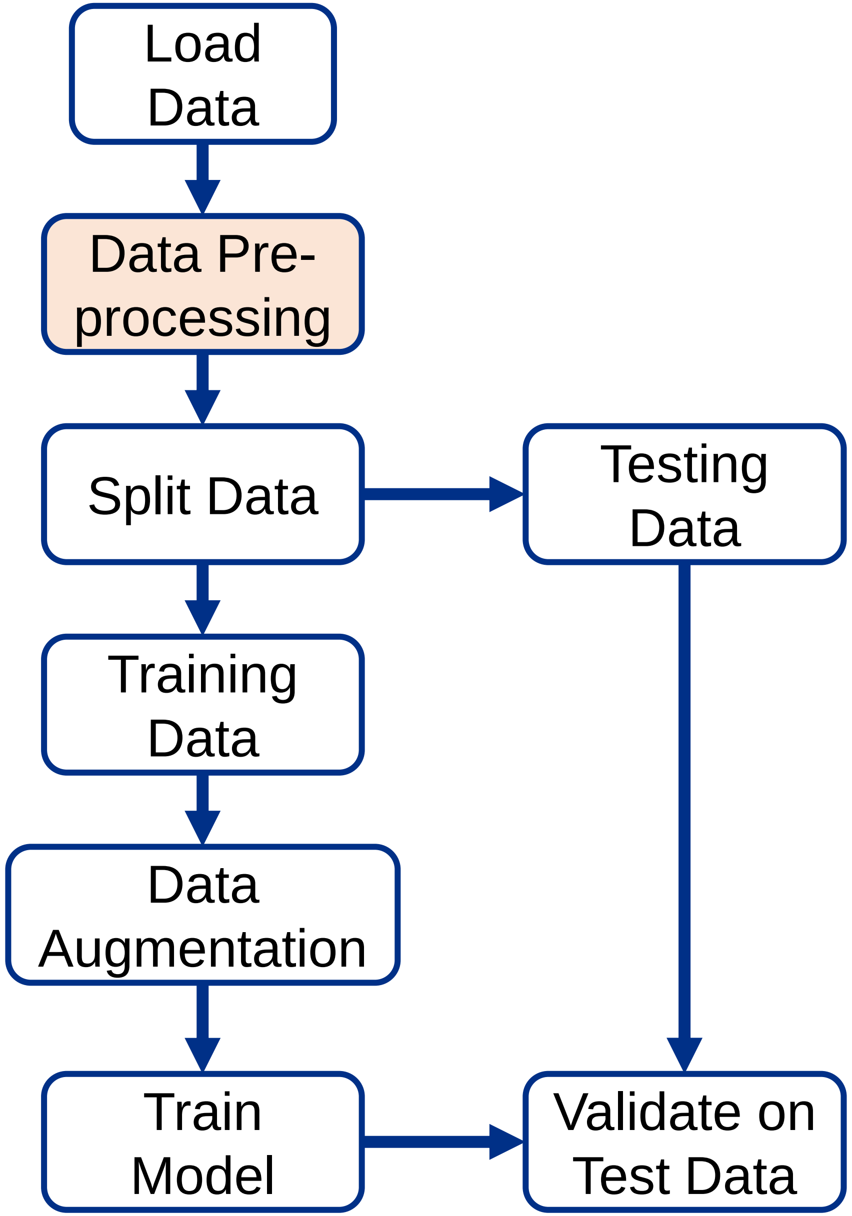
[1] Gunduz et al., Turkish J. E.E.C.S. 30 2268 (2022)
[2] Akinlar et al., Intern. J. of P.R. and A.I. 26 (2012)
[3] https://www.kaggle.com/huseyingunduz/diatom-dataset



Creating the DLHM dataset

- **Diatoms images** were transformed **into DLHM holograms** using the **Bluestein method**[1] and the **modified Angular Spectrum method**[2] depending on the distance *z* to properly emulate the location of the specimen within the inspection volume.
- Diatoms were considered amplitude cells.
- Raw holograms were simulated at 5 evenly spaced axial depths, ranging from z = 0.5 – 4.1 mm.
- DLHM configuration:
  - Source Wavelength = 528 nm
  - Sensor Width = 1583 x 1583
  - Sensor placed at L = 5 mm from the spherical point source

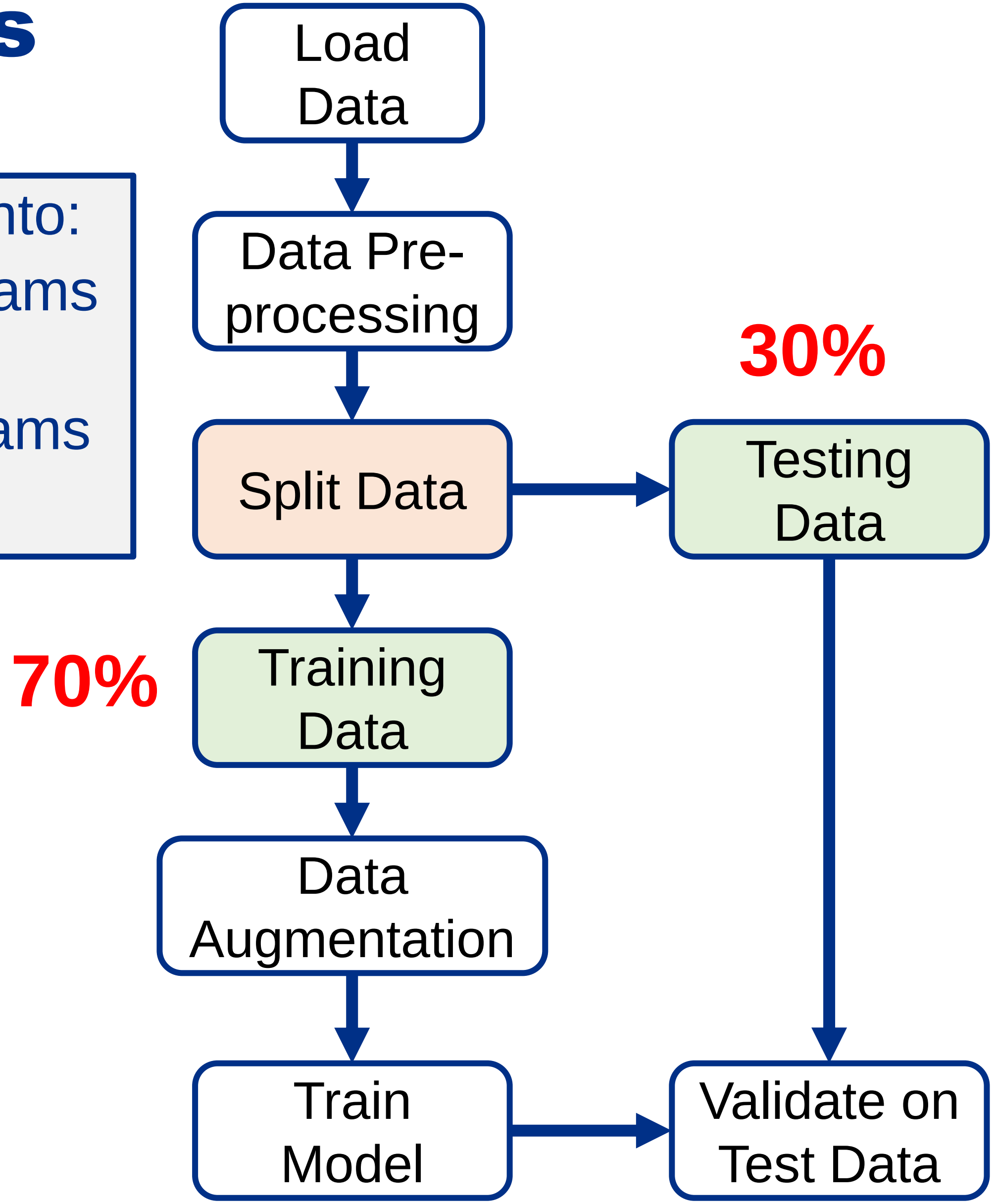
[1] Restrepo et al., Appl. Opt. 50, 1745-1752 (2011).
[2] Mendlovic et al., J Mod Opt. 44(2):407–414 (1997).



Training/Testing Datasets

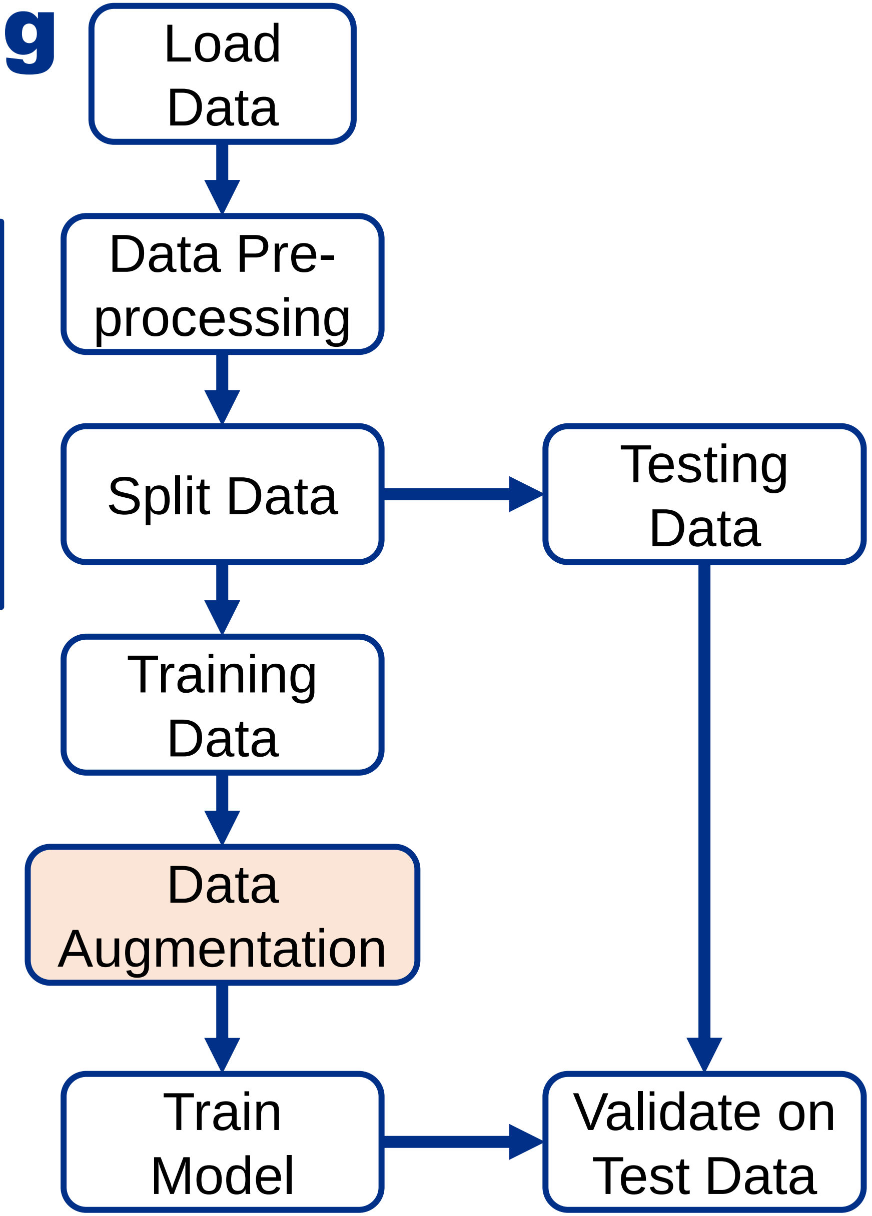
The total **9,080 holograms** are divided into:

- **Training** dataset with **6,356** holograms (= 1,816*5*0.7).
- **Testing** dataset with **2,724** holograms (= 1,816*5*0.3).



Further increased of training instances

Training dataset was **randomly flipped vertically and horizontally** to further increased the training dataset, improving generalization.



Training classification models

- **Selected** classification **models: AlexNet**, **VGG16**, and **ResNet18.**
- **Transfer learning** was **used** by taking previously MATLAB trained models and replacing their last learning layers, allowing the initial weight and bias learning rates to be higher.
- **Bayesian Optimization** was used to **select the best model hyperparameters.**
- **Hyperparameters Optimization:**

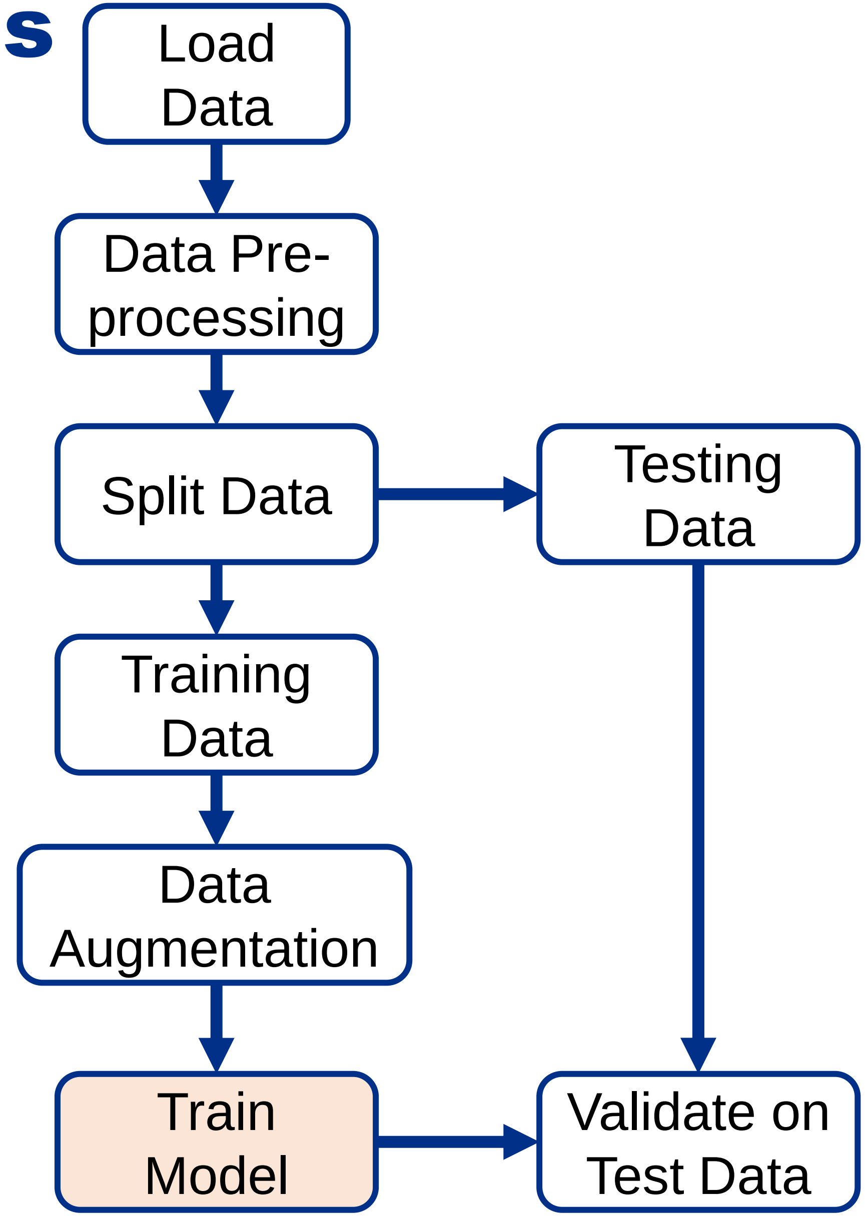
  Initial Learning Rate: [0.0001,0.01, 0.01]

  Epochs: [3,50,1]

  Batch Size: [16,128,16]

  Validation Frequency: [16,128, 16]

  Optimizer: adam, rmsprop and **sgdm**



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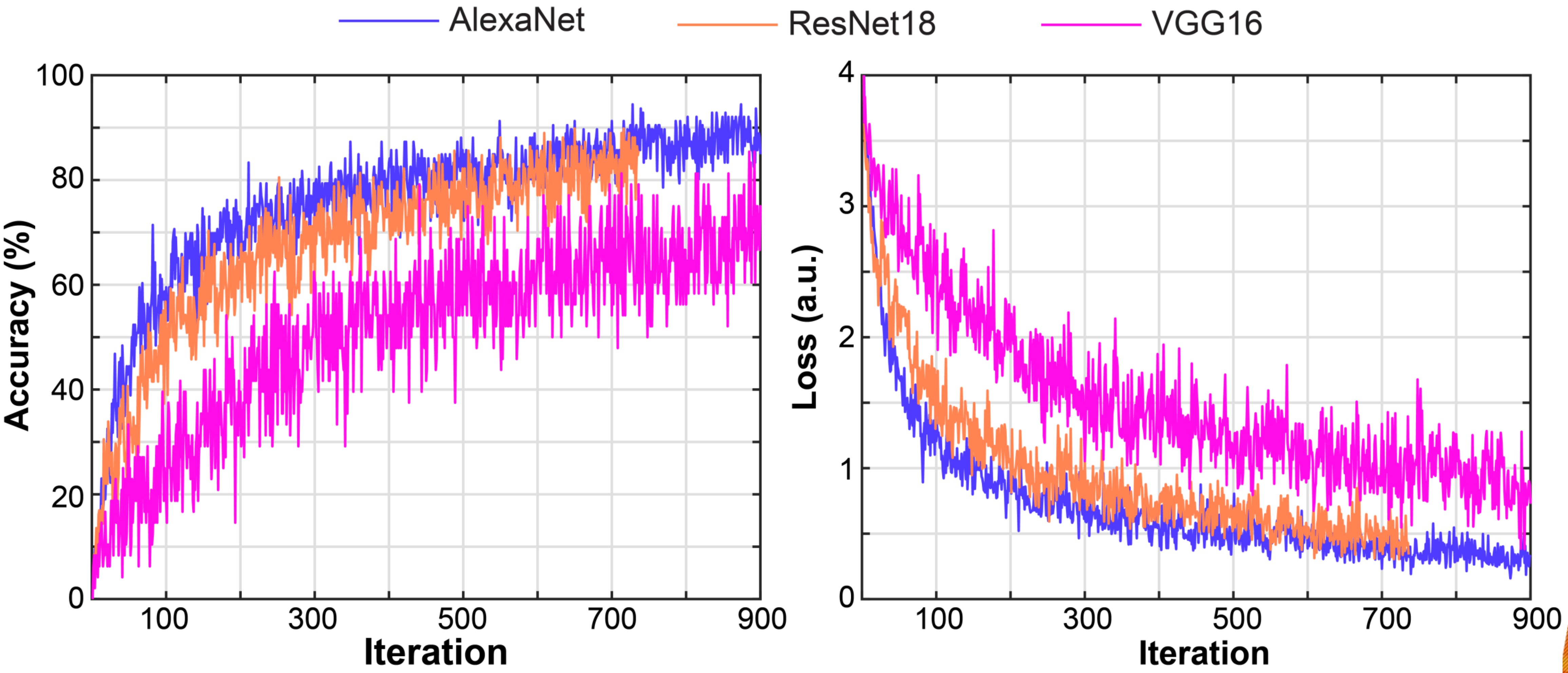
Healthy Trained Models for all the selected models



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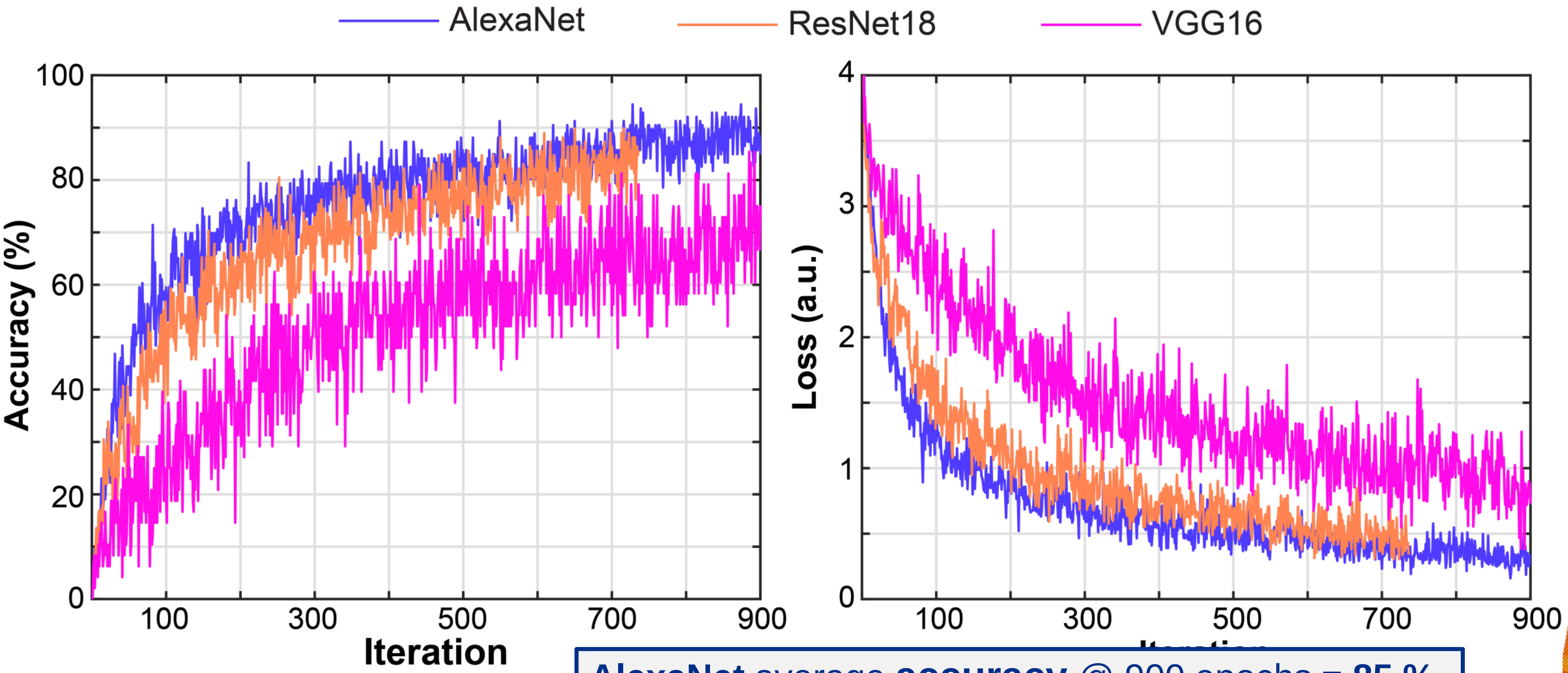
Healthy Trained Models for all the selected models



AlexaNet average **accuracy** @ 900 epochs = **85 %**
ResNet18 average **accuracy** @ 735 epochs = **85 %**
VGG16 average **accuracy** @ 900 epochs = **75 %**

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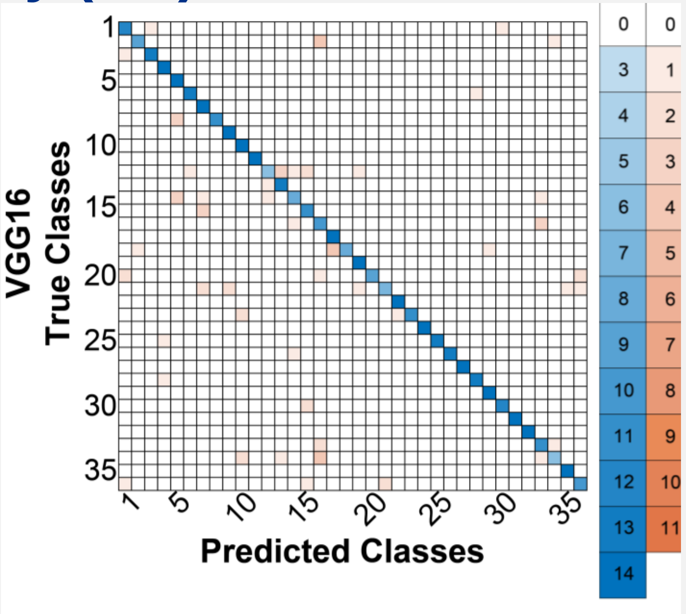
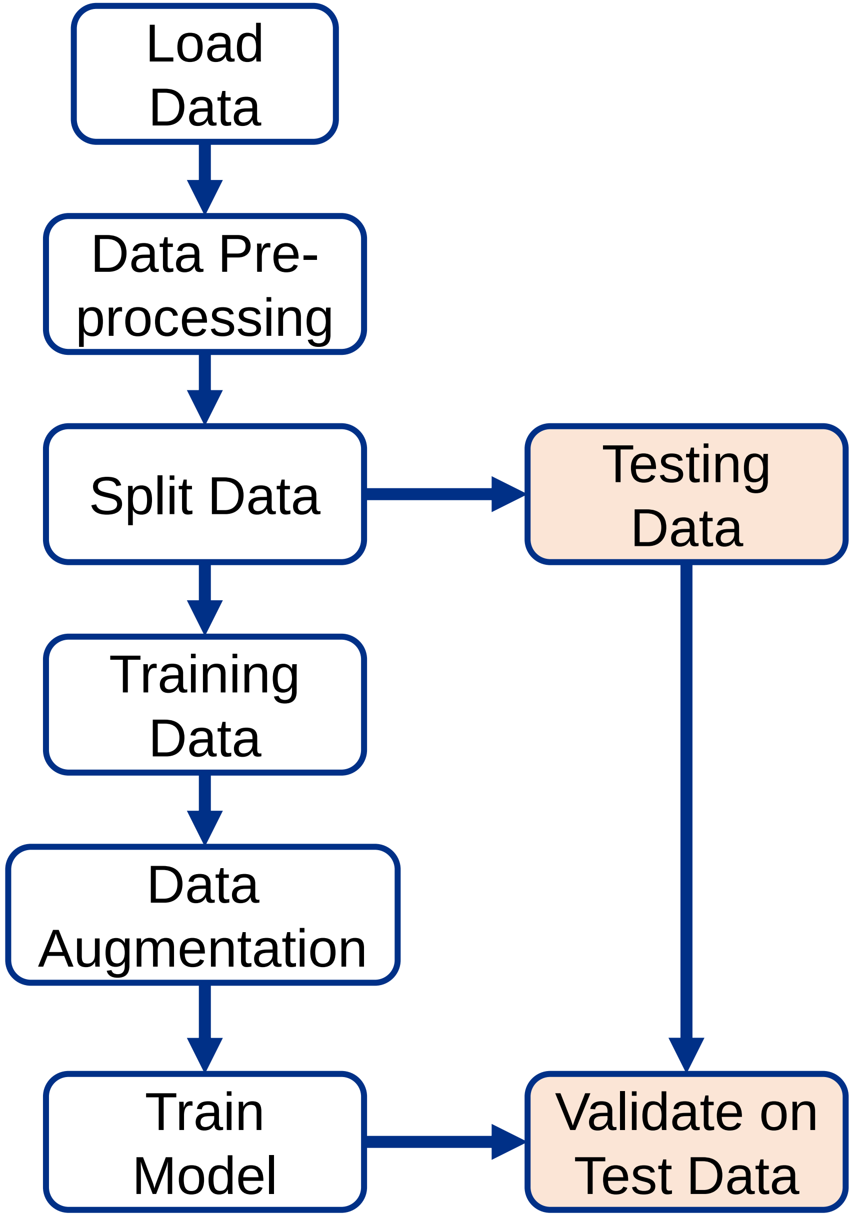
Testing the models

- **Models** are **validated** using the **unseen testing** dataset.
- Performance **metrics** used are the **accuracy (AC)** and **confusion matrix.**



$$AC(\%) = \frac{\text{Predicted True Values}}{\text{Total Values}}$$



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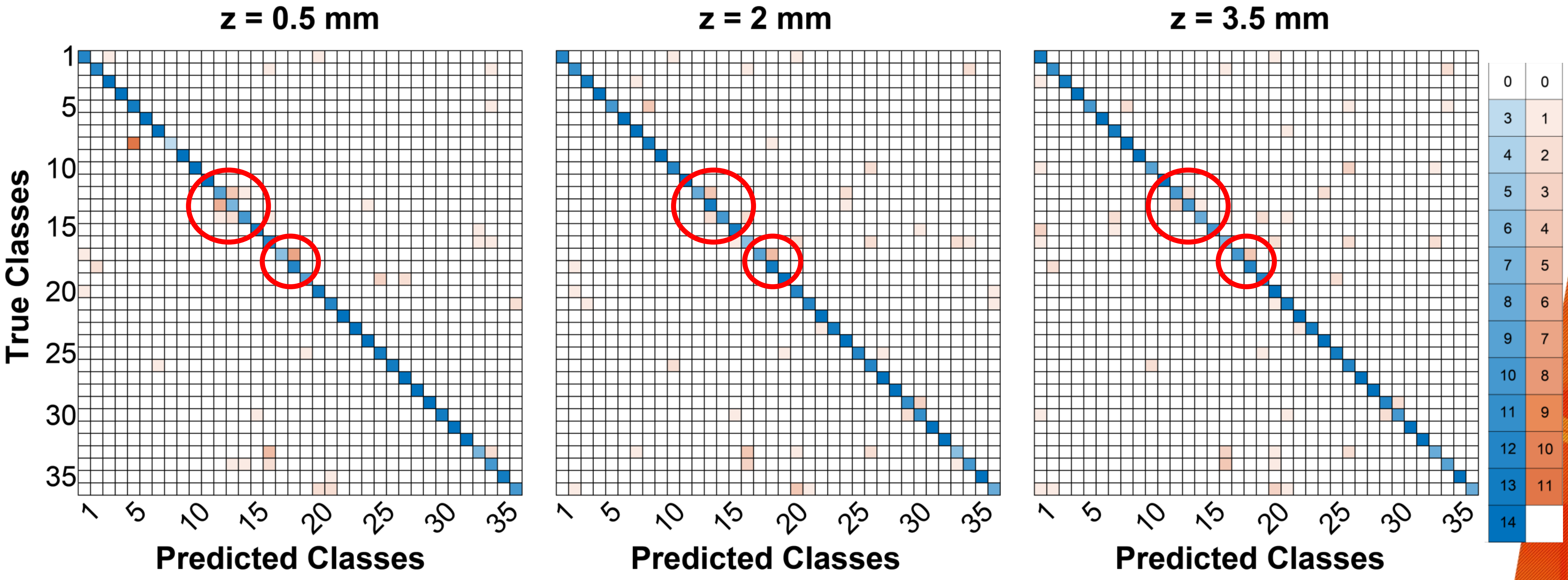
Can the trained AlexaNet model identify diatoms?



87.7 ± 15.4 %

87.2 ± 15.1 %

86.7 ± 14.5 %

The classification accuracy does not depend on the axial position of the diatom

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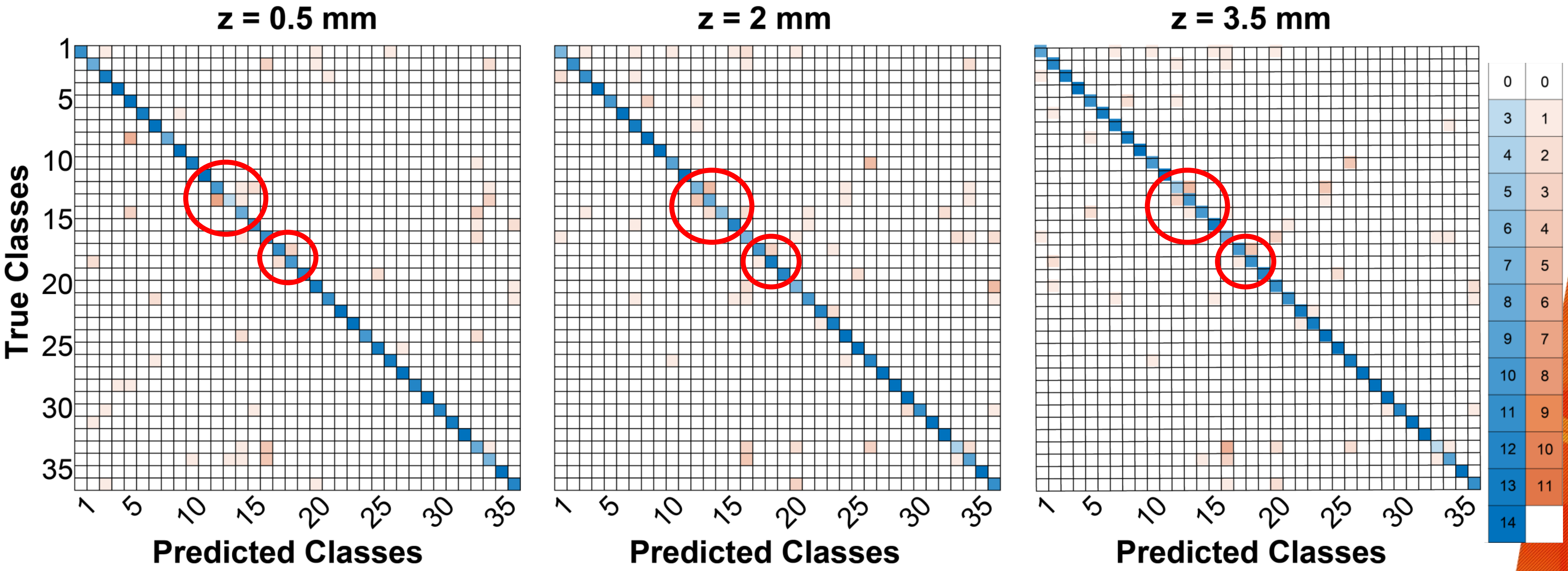
Can the trained ResNet18 model identify diatoms?



85.3 ± 17.2 % **83.4 ± 18.1 %** **84.8 ± 16.3 %**

The ResNet18 model has a lower performance than the AlexaNet one – lower accuracy values and higher standard deviation values.

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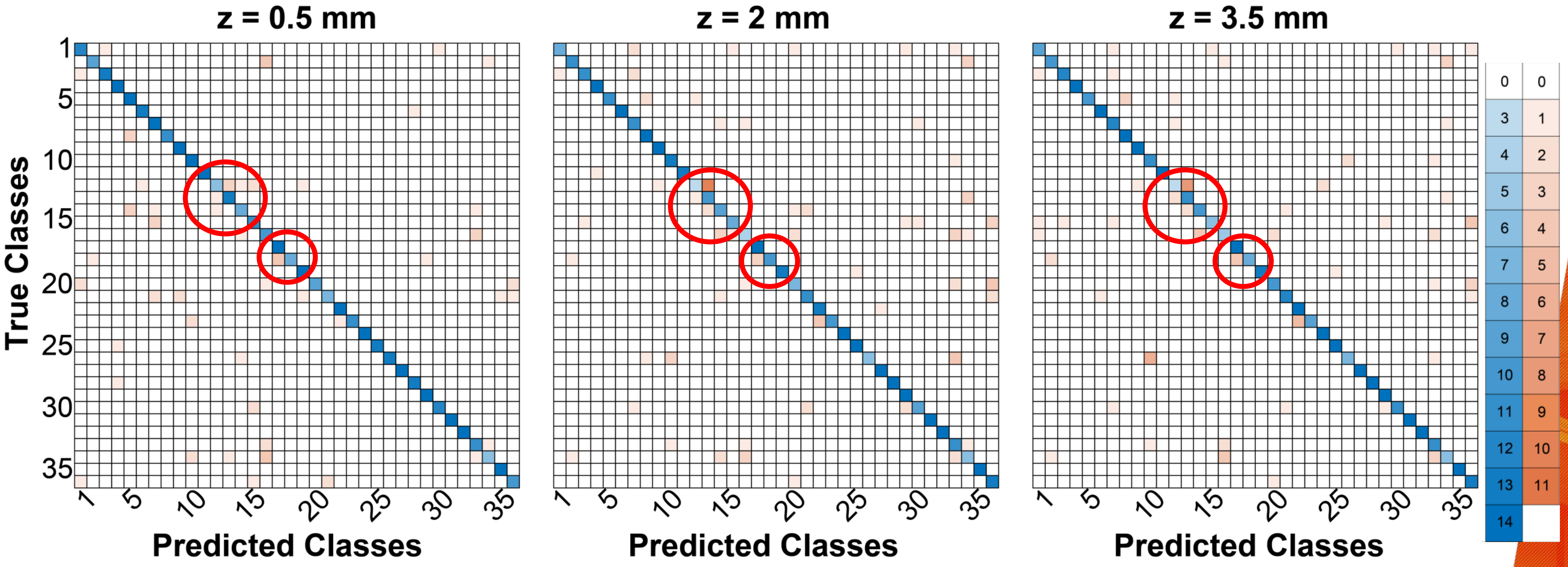
Can the trained VGG16 model identify diatoms?



86.5 ± 13.1 % | **81.8 ± 19.3 %** | **82.2 ± 18 %**

The VGG16 model provides the lowest accuracy to the raw holograms that were closer to the sensor.

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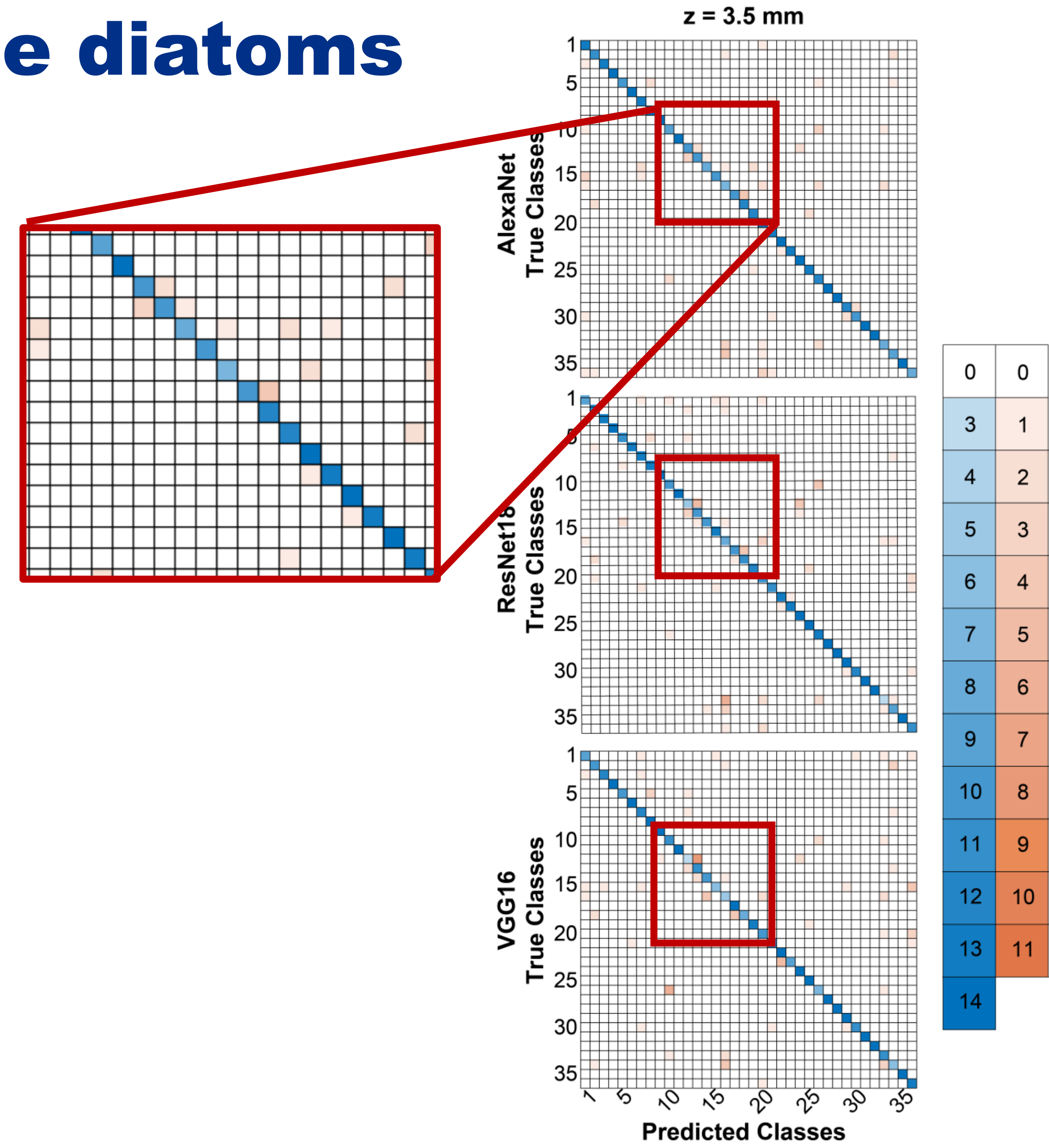
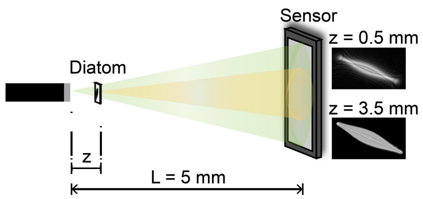
Challenges to recognize some diatoms

Models fail to predict

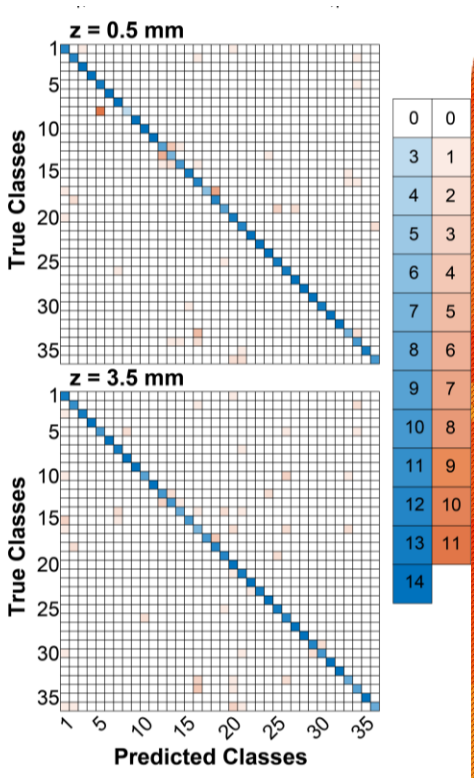
- The *Gomphonema drutelingense* diatom, prediciting it as Gomphonema micropus or *Navicula moskalii* diatom.
- The *Halamphora paraveneta* diatom, predicting as the *Halamphora veneta* diatom.
- The Nitzschia hantzschiana diatom, predicting as the *Achnanthidium biasolettianum* or *Nitzschia archibaldii* diatom.



Summary



- We have investigated the potential use of lensless imaging systems for underwater monitoring by identifying diatom species and their diversity
- We have shown the classification power of traditional classification models (i.e., AlexaNet, ResNet18, and VGG16) to predict diatom species using raw hologram recorded by a DLHM system.
- The AlexaNet model provides the highest accuracy, being independent of the axial position of the diatom.
- The mean accuracy of the AlexaNet model with raw holograms (87.7%, 87.2%, and 86.7%) is quite close to the one provided using reconstructed in-focus images (88.2%)[1].
- We have had difficulty identifying some diatoms, regardless of the models.
- **Future work:** analyze the classification accuracy for transparent diatoms and real experimental dataset



[1]MacNeil et al. BMC Ecol Evo 21, 123 (2021).

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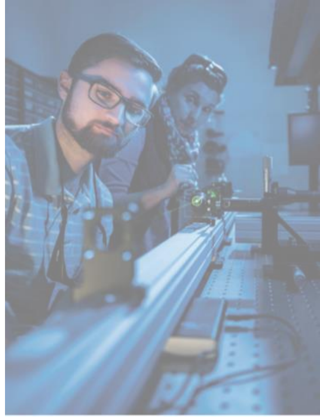
Acknowledgments



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