Autofocusing optical microscope using artificial neural network for large-area, high-magnification scanning

University of Delaware Spring 2022 CHEG/CISC 867-015
Final Presentation

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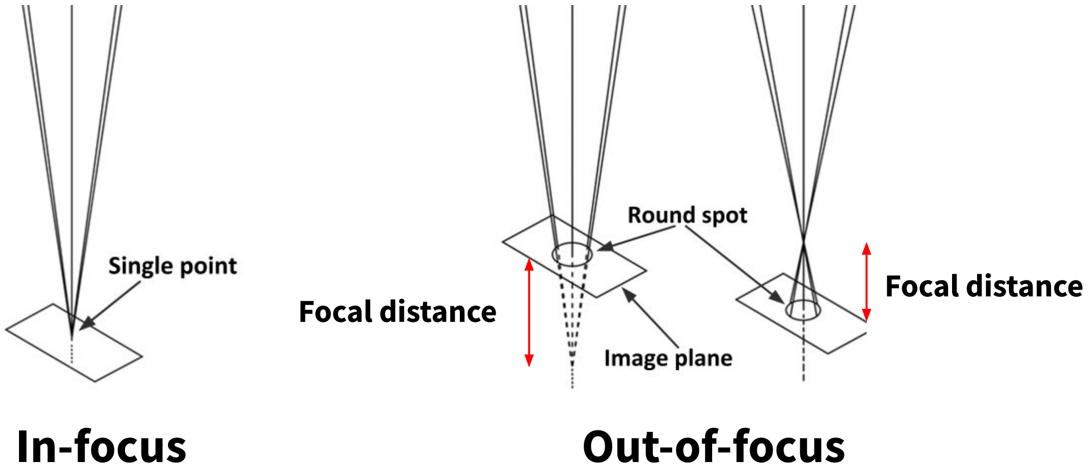
UNIVERSITY OF DELAWARE

Motivation

- **Microscopy imaging** can provide structural information important for elucidating structure-property relationships of soft materials.

- Delicate control of a microscope to quickly move to the **focal plane** is essential for timely, high quality, highly magnified images over a large area.



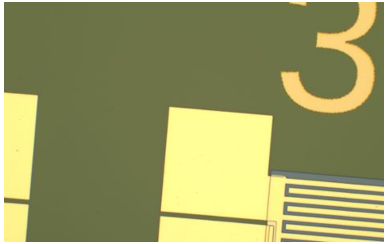
Lu, Y., et al., 2018. *AIP Advances*, *8*(1), p.015124.

Project Aim

Develop a machine learning workflow to predict the **distance to the focal plane** of microscopy images based on their sharpness and contrast

In-focus

Focal Distance 0 µm

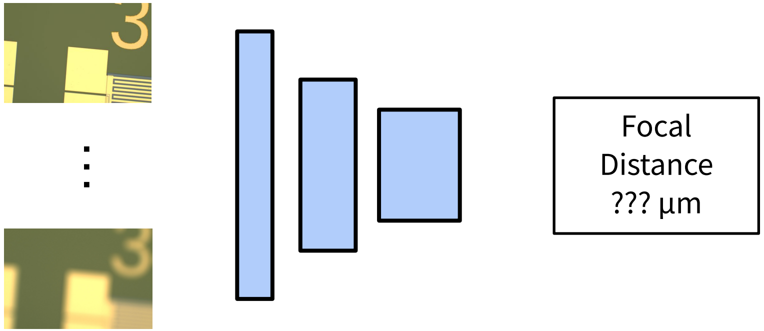


Out-of-focus

Focal Distance 43 µm

Level 1 images of metal patterns courtesy of Dr. Houk Jang

Labeled Data | **ML Model** | **Predictions**

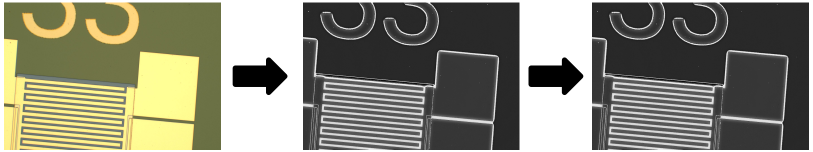


Computational Resources

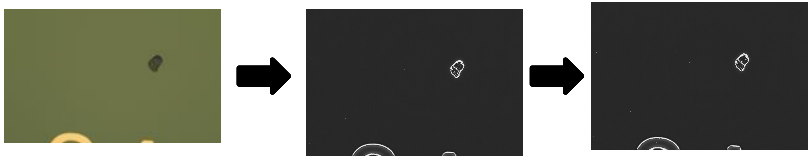
- Google Colaboratory Platform
- 2.30GHz Intel Xeon CPU
- Nvidia K80 GPU
- Python 3.7
- TensorFlow 2.8
- Scikit-learn 1.0

Schematic of the machine learning workflow

Image edge detection → Clean image selection



Raw Image | Filtered | **Clean**



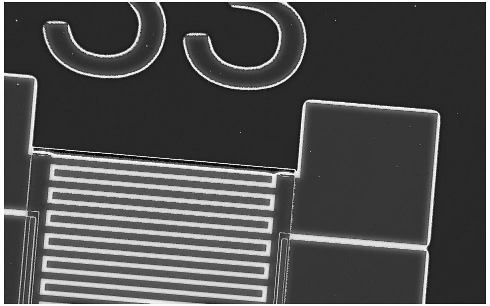
Raw Image | Filtered | **Noisy**

Clean image stack

In and out-of-focus images **above** 0.10 **edge area percentage threshold** at one location

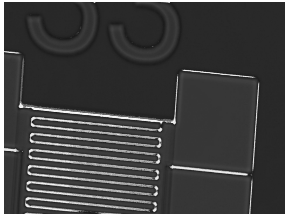
In-focus

Focal distance: 0 µm

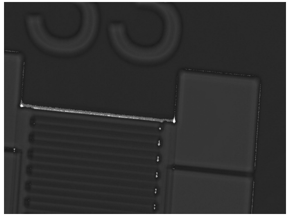


Out-of-focus

11 µm



15 µm



26 µm

-31 µm

-36 µm

-38 µm

-42 µm

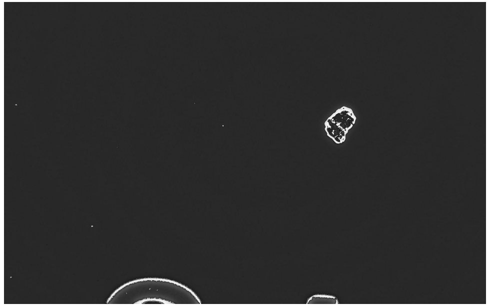
-49 µm

Noisy image stack

In and out-of-focus images **below** 0.10 **edge area percentage threshold** at one location

In-focus

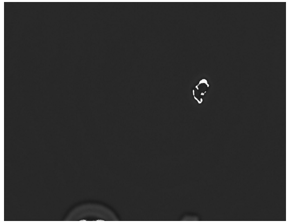
Focal distance: 0 µm



Out-of-focus

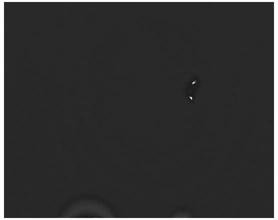
9 µm

-10 µm

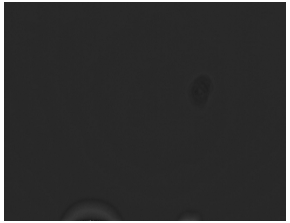


17 µm

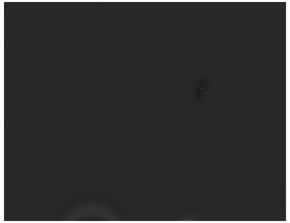
-18 µm



19 µm



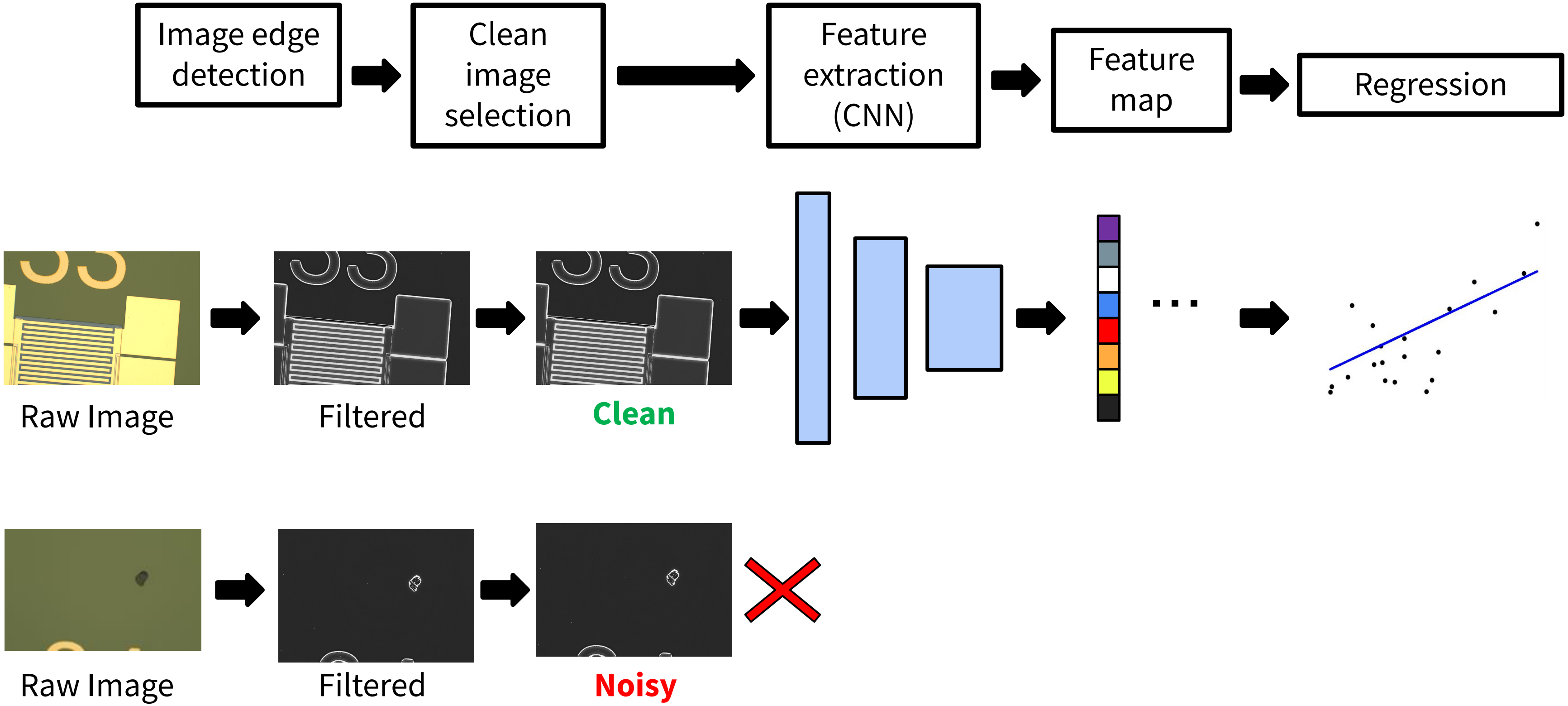
-28 µm



-31 µm

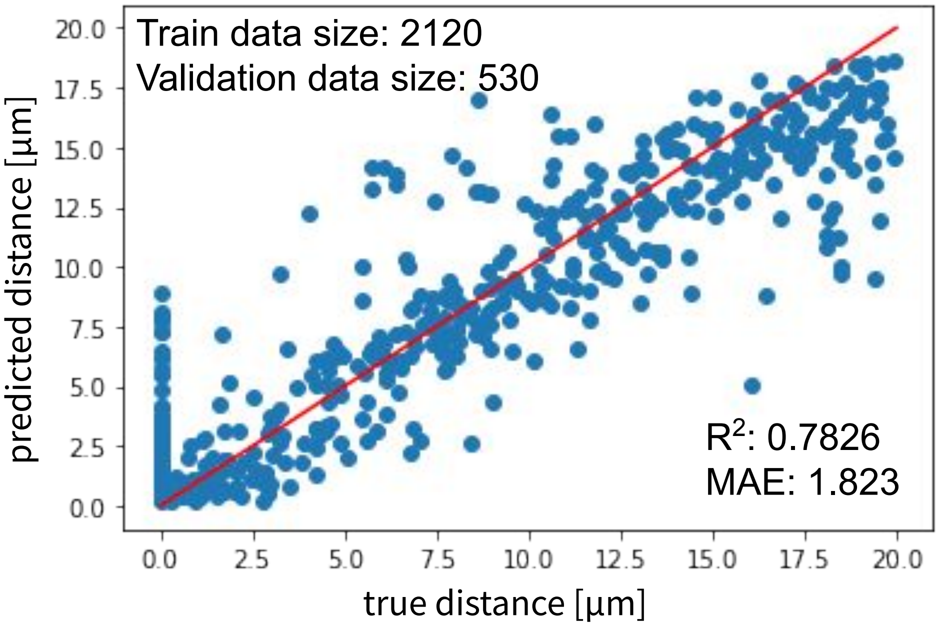
-38 µm

Schematic of the machine learning workflow

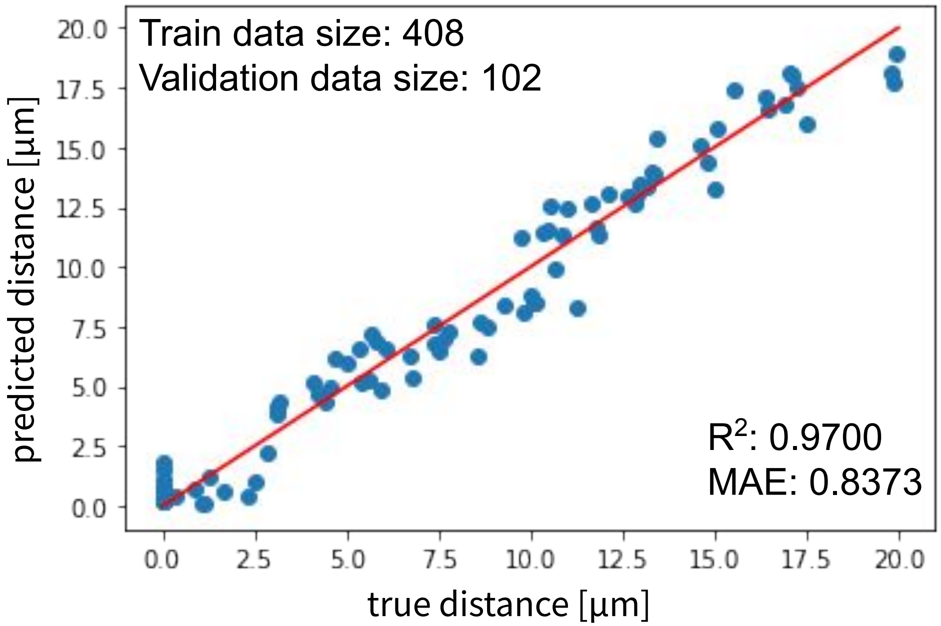


Applying threshold improves regressor task performance

No threshold



Edge area threshold: 0.10

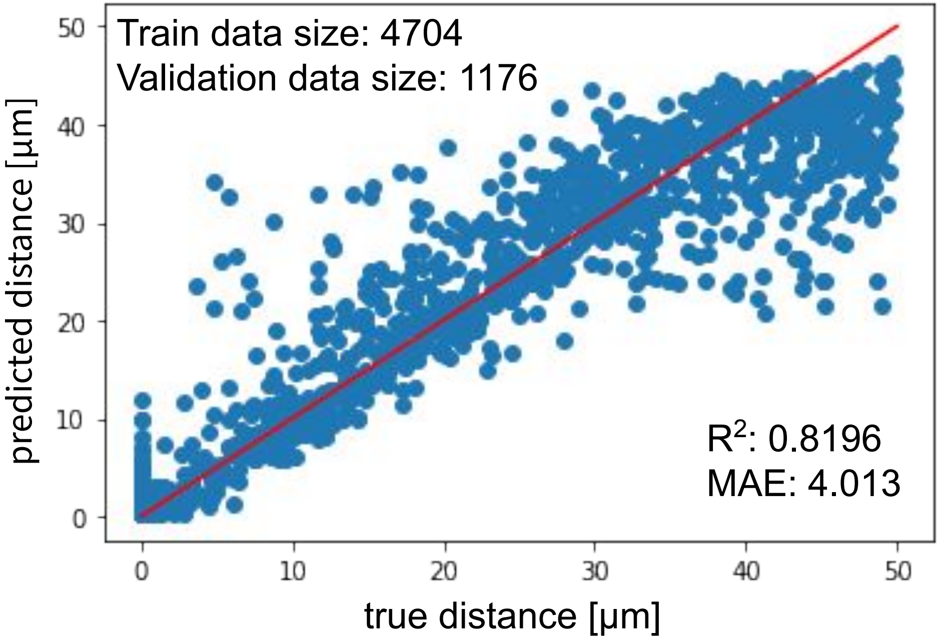


random forest regressor
focal distance capped at 20 µm

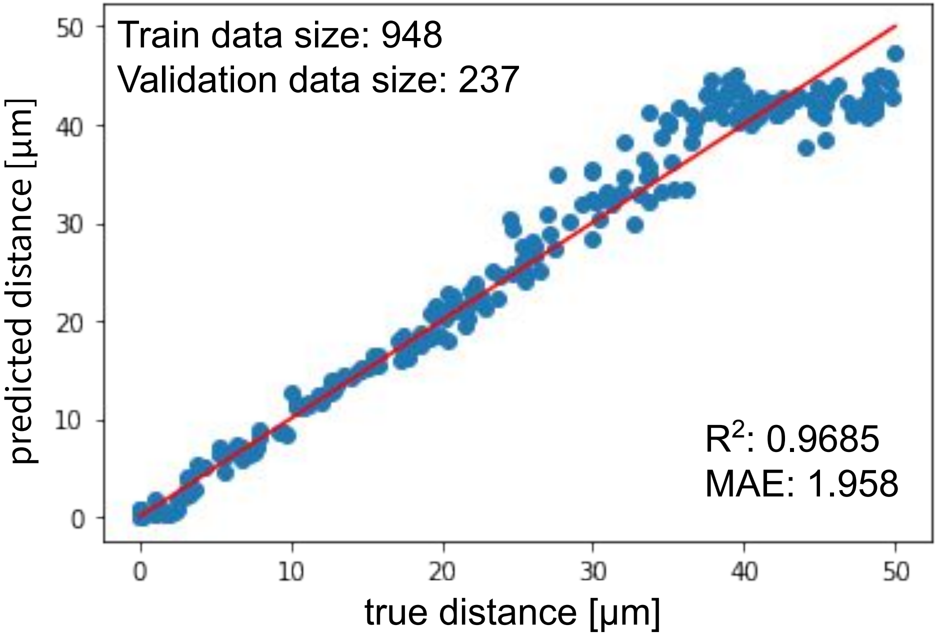
Points plotted in figures are validation data

Applying threshold improves regressor task performance

No threshold



Edge area threshold: 0.10



random forest regressor
focal distance capped at 50 μm

Points plotted in figures are validation data

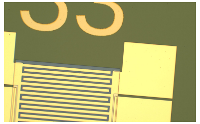
Schematic of the cross-validation workflow

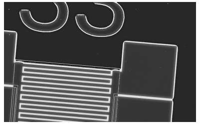
Image edge detection

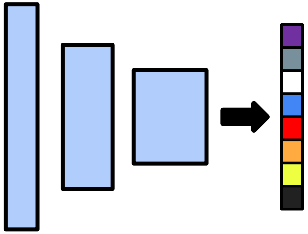
Clean image selection

Feature extraction (CNN)

K-fold cross-validation





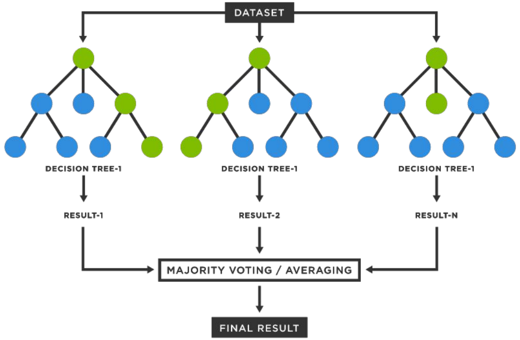


Ridge Linear regressor

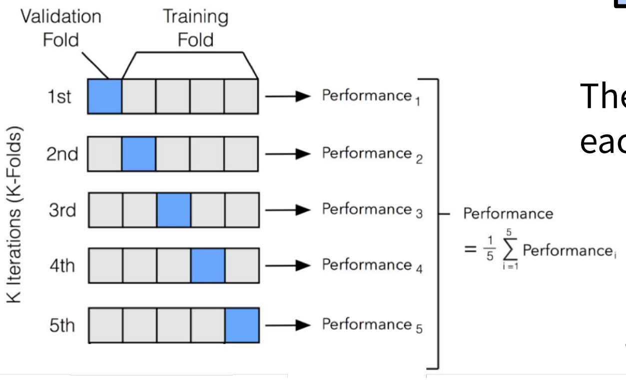
$$\sum_{i=1}^{n}(y_i - \sum_{j=1}^{p} x_{ij}\beta_j)^2 + \lambda \sum_{j=1}^{p} \beta_j^2$$

5.1 - Ridge Regression | STAT 897D (psu.edu)

Random forest regressor



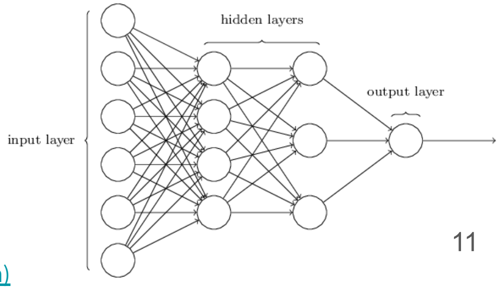
What is a Random Forest? | TIBCO Software



The clean images are shuffled 20 times, each perform a 5-fold cross-validation.

Modification: within the train / val fold, always use a set number for train / val.

Multilayer perceptron regressor

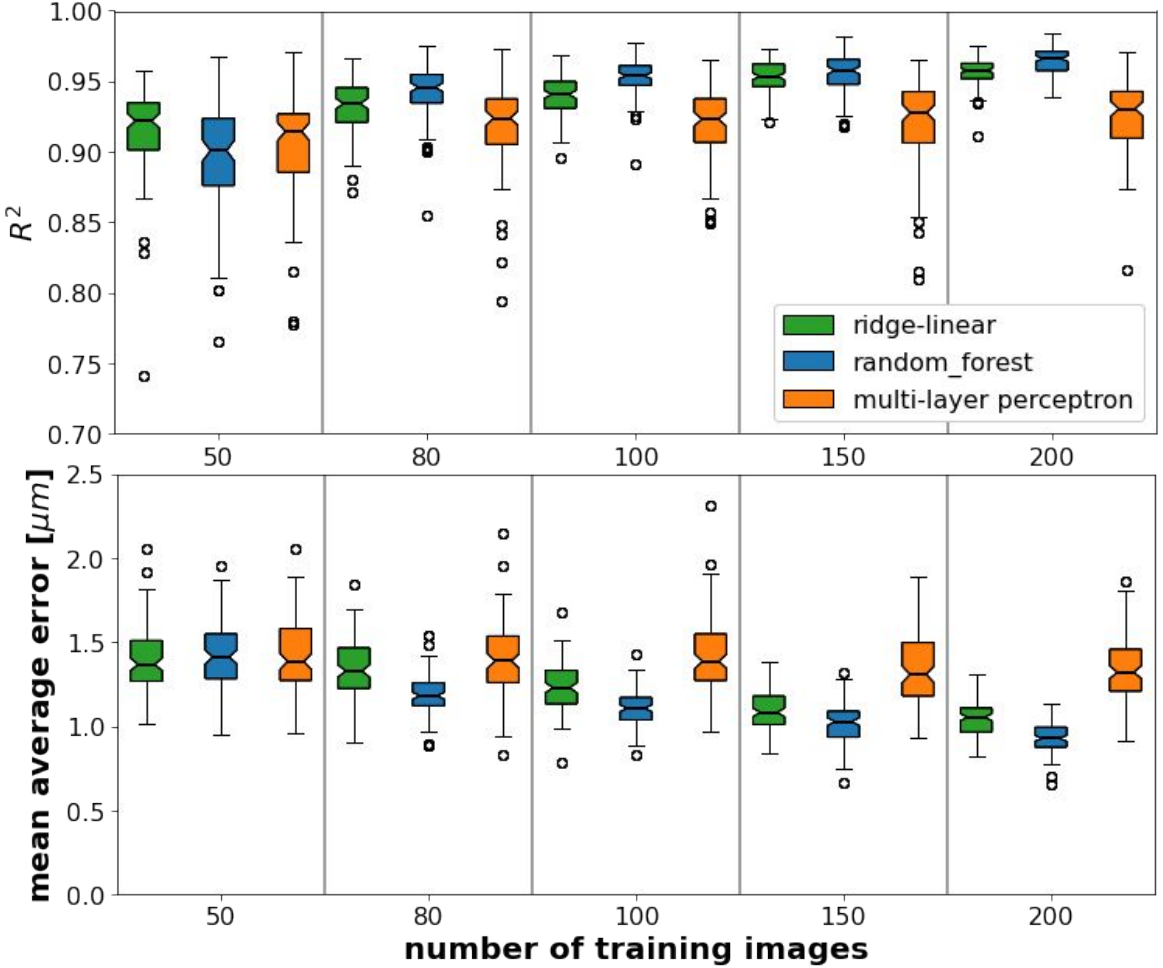


model_selection (ethen8181.github.io)

rcassani/mlp-example: Code for a simple MLP (Multi-Layer Perceptron) (github.com)

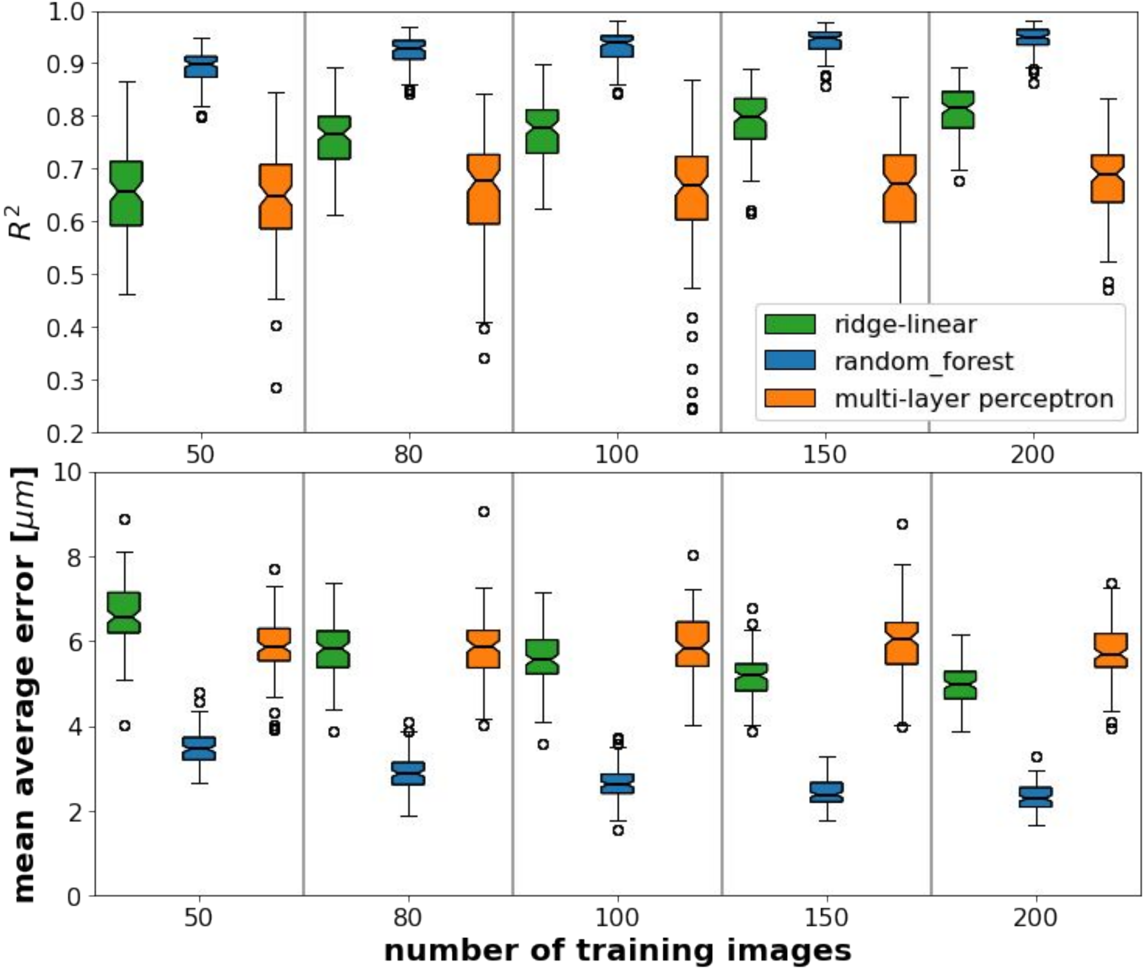
Focal distance capped at 20 µm, edge area threshold 0.10

Number of validation data is 50.
Each boxplot indicates median of 100 cross-validation trials



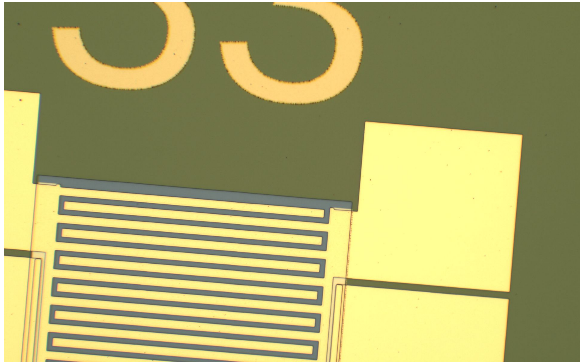
Focal distance capped at 50 µm, edge area threshold 0.10

Number of validation data is 50.
Each boxplot indicates median of 100 cross-validation trials

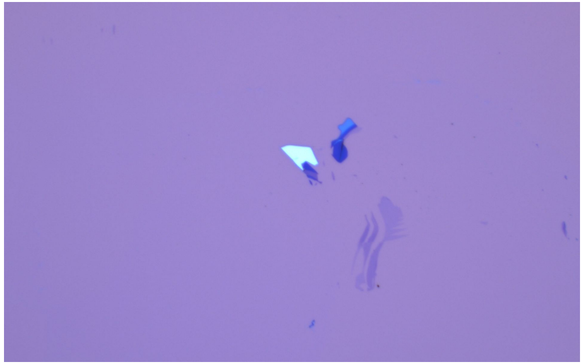


Differences between level 1 and level 2 images

Level 1 metal patterns



Level 2 mono- or multi- layers of graphite



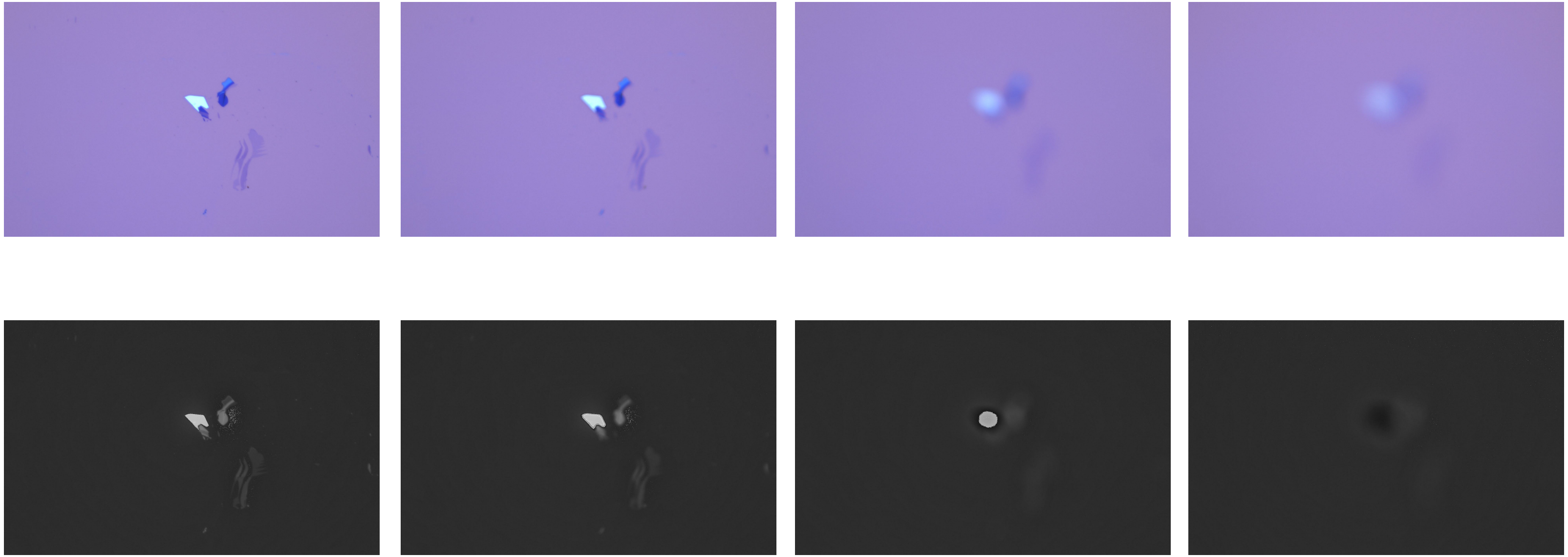
- Level 1 images contain objects of a single color whereas level 2 images contain objects of different colors.
- Level 1 images contain patterns giving large edge area whereas level 2 images contain objects with little edge area.

Level 2 images of graphite thin layers

Focal distance: [μm] | 0 μm | 10 μm | 36 μm | -47 μm

Before edge detection

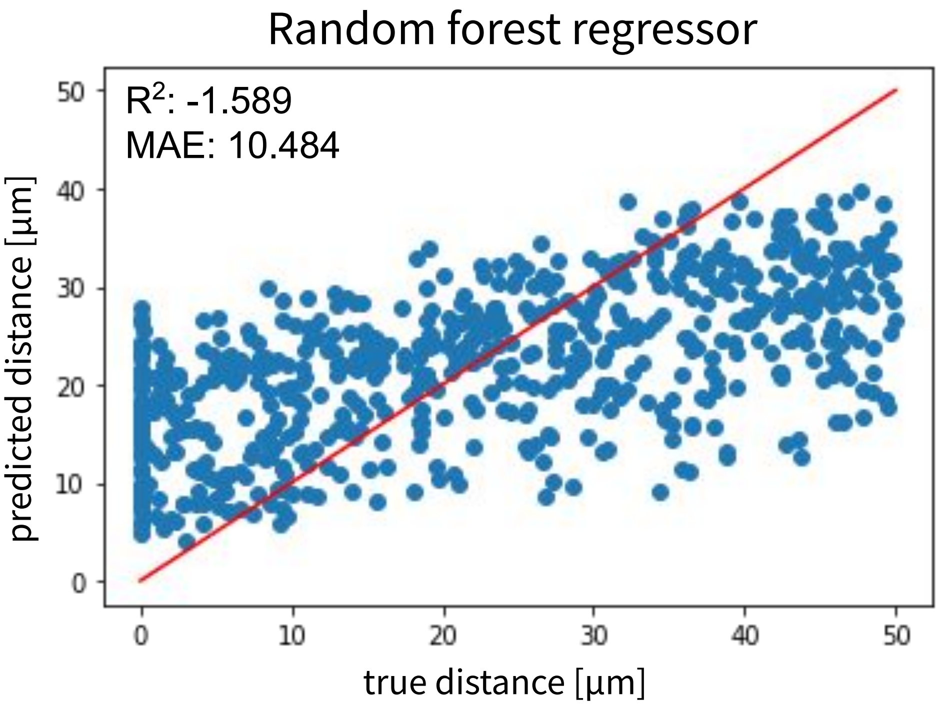
After edge detection

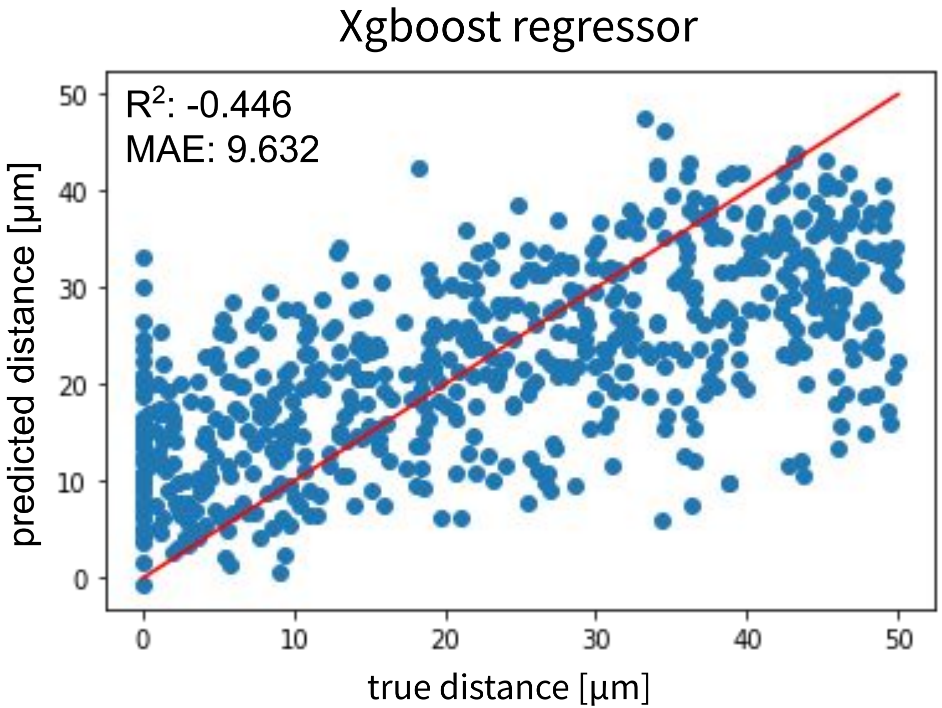


Images courtesy of Dr. Houk Jang

8829 training images, 663 test images
(selected by our industry mentor).

Focal distances of level 2 images are harder to predict





test data points are plotted in the figures.

Conclusions:

- We have developed a ML workflow that predicts the focal distance of microscopy images containing metal patterns with >0.90 R^2 value.
- Emphasis of our work is on preprocessing and feature extraction of microscopy images.

Future directions:

- Improve performance on images containing graphite layer -- applying multiple thresholds for detection of objects of different class (different colors).
- Potential application of our machine learning workflow: images with large edge area (interdigitating objects with the background).
- Develop application programming interface (API) that the microscope can integrate with to adjust the focal distance based on the prediction of our ML workflow in real-time.

Data and code available:

evanmacbride/microscope-autofocus: Autofocusing microscopy images with ML (github.com)

Member contributions:

- Shizhao Lu conceptualized the ML workflow and trained models on level 1 and level 2 images in Google Colab notebooks;
- Evan MacBride tested, adapted the machine learning workflow to Python scripts and prepared the workflow notebook for GitHub.