Nano-particles Counting In Photonic Resonator Absorption Microscopy

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Outline

1. Introduction and Motivation

2. Object Counting Problem

3. Application of counting to particle counting
    3.1. Dataset
    3.2. Experiment
    3.3. Result

4. Discussion

Introduction and Motivation

miRNA - Cancer Biomarkers

- Expression found to correlate with cancer stage

Benefits of liquid biopsy detecting biomarkers:

- Non-invasive
- Early detection

Detection Challenges:

- Low concentrations
- Often vary by only one base from other miRNA

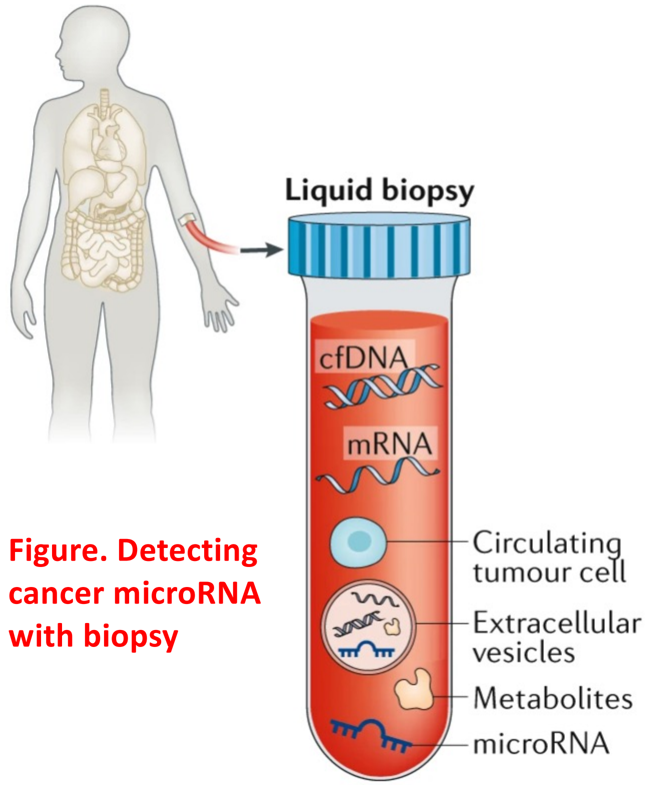


Figure. Detecting cancer microRNA with biopsy

Eur Urol. 2015 Jan; 67(1): 33–41.

Introduction: Photonic Resonator Absorption Microscopy (PRAM)

Principals of Operation

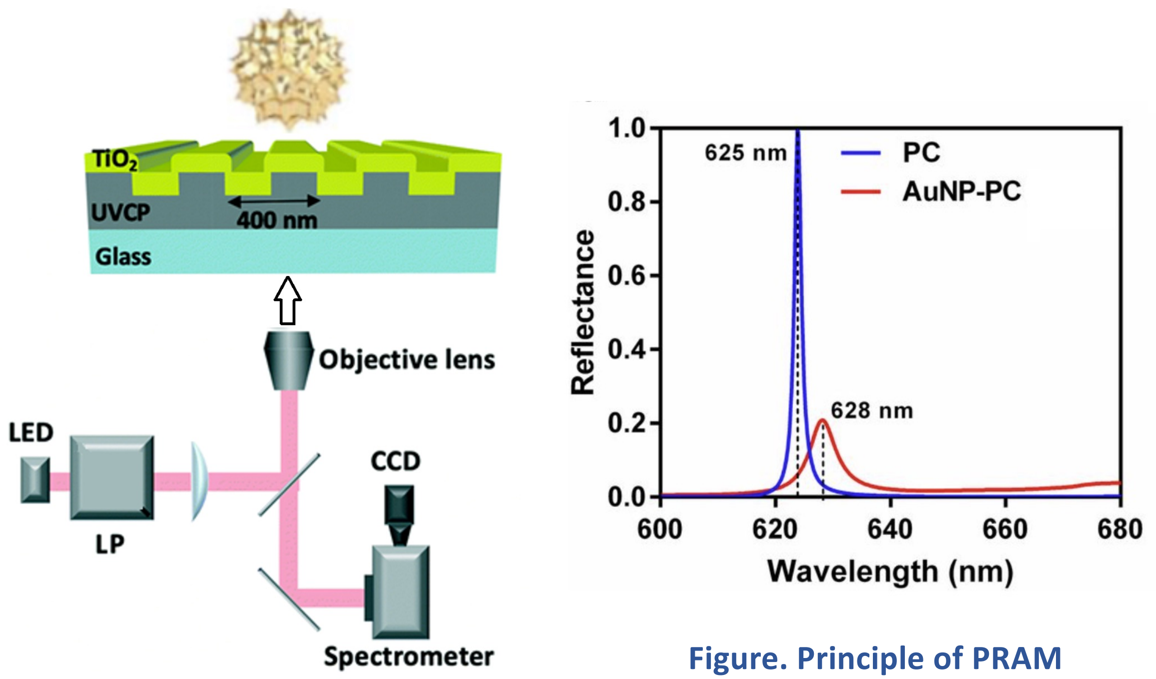
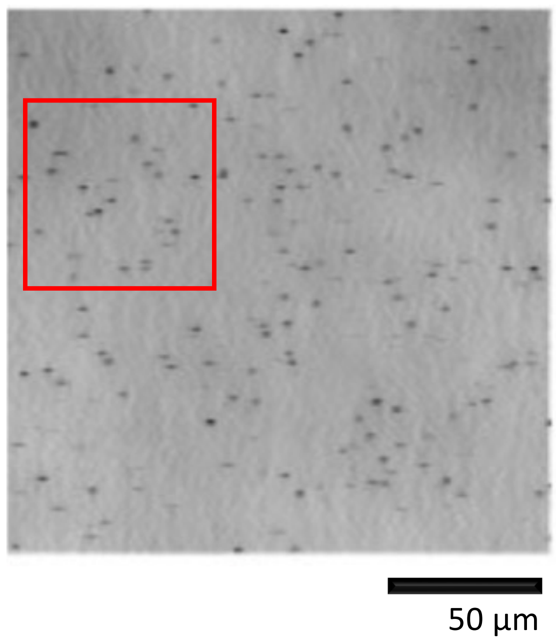


Figure. Principle of PRAM

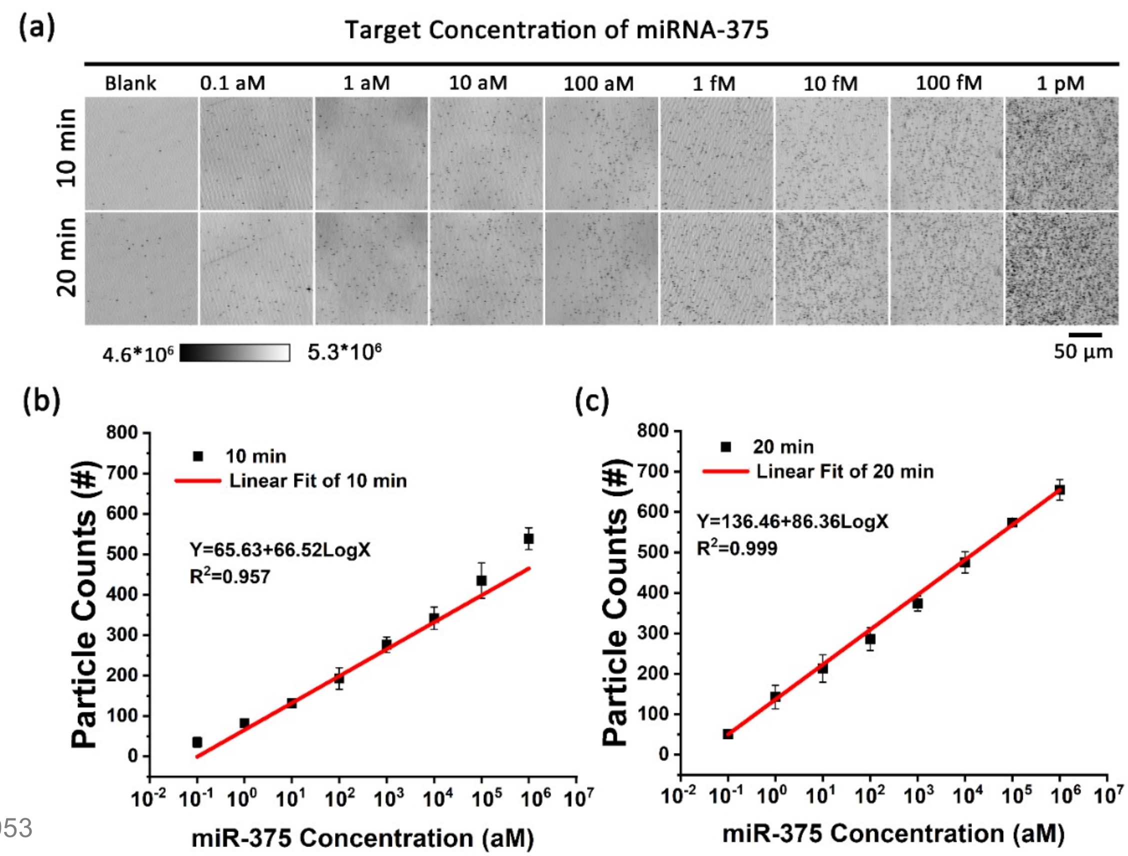


PNAS, 2019. Vol. 116, No. 39

*This project is in collaboration with Nanosensor Group UIUC. In this project, I mainly work on image processing of the images collected by the device.

Introduction and Motivation

The number of particles (blobs) in the light microscope images correlates with the concentration of target in solution.

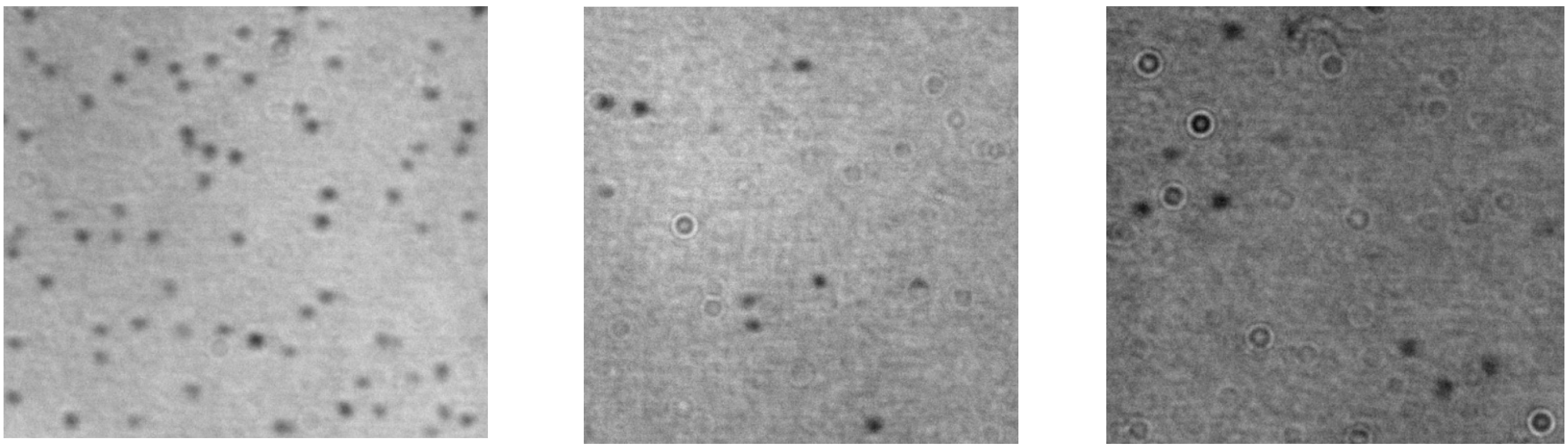


Lab on a Chip, 2019. 19.23, 3943-3953

Figure. Particle-concentration correlation

Introduction and Motivation

The question is: How can we quantify the particles (blobs) in the light microscope images?



15 μm

Count: ?

Count: ?

Count: ?

Figure. Example of PRAM images

Introduction and Motivation

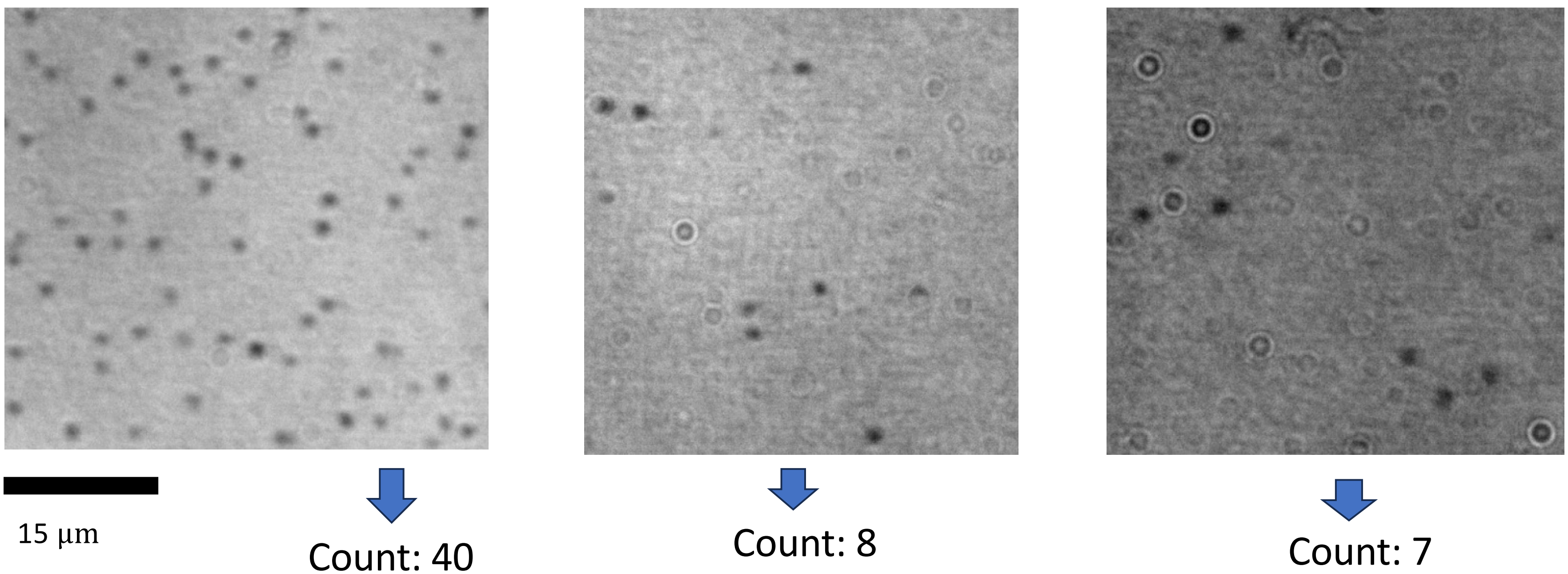


Figure. Example of PRAM images

Introduction and Motivation

Challenges in PRAM images:

Noise and Artifacts:
- Uneven illumination
- Dust particles, and stains

Low Contrast and Dynamic Range:
- Particle can be blended with background, resulting in low contrast

Blur and Distortion:
- Sample preparation techniques (diff assay have diff reflectance coefficient)
- Imaging conditions

Variability: Unevenness in morphology and shape of particles

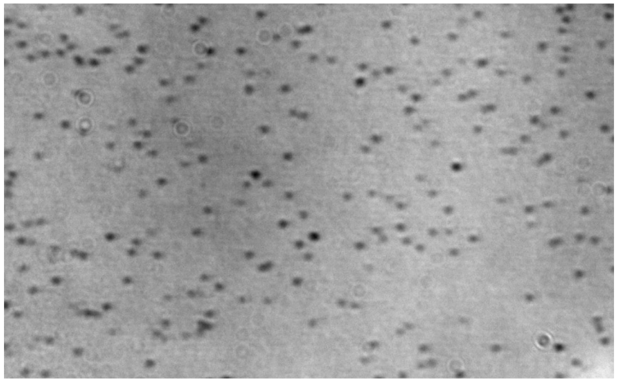


Figure. Example of uneven illumination

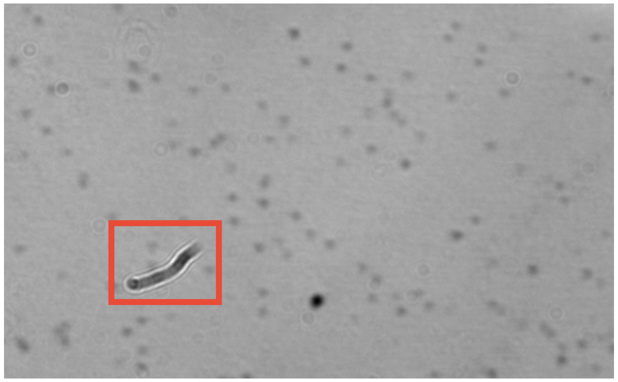


Figure. Example of artifacts

Experiment with The PRAM Image –Metrics for evaluation

Evaluation metrics:

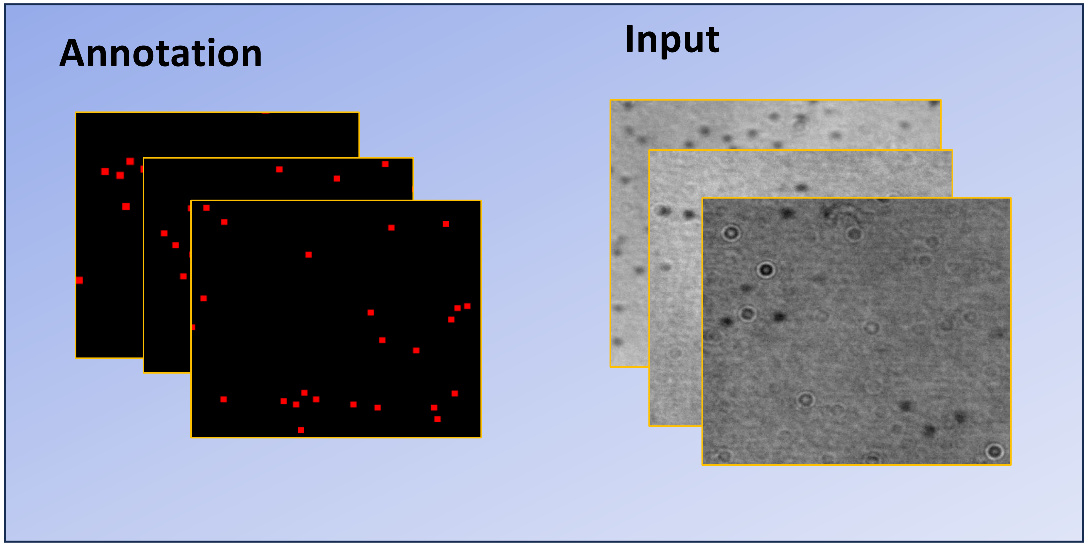
- **Mean squared error** (MSE)
- **Mean absolute error** (MAE)
- **Mean relative error** (MRE)
- C_i: true count of image *i*
- $\widehat{C}_i$: predicted count of image *i*
- N_t total images

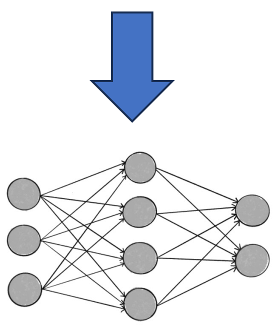
$$MSE = \sqrt{\frac{1}{N_t}\sum_{i=1}^{N_t}(C_i - \hat{C}_i)^2}$$

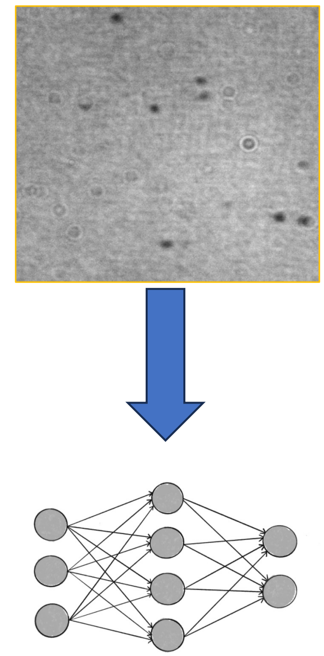
$$MAE = \frac{1}{N_t}\sum_{i=1}^{N_t}|C_i - \hat{C}_i|$$

$$MRE = \frac{1}{N_t}\sum_{i=1}^{N_t}\frac{|C_i - \hat{C}_i|}{C_i}$$

Application of counting to particle counting





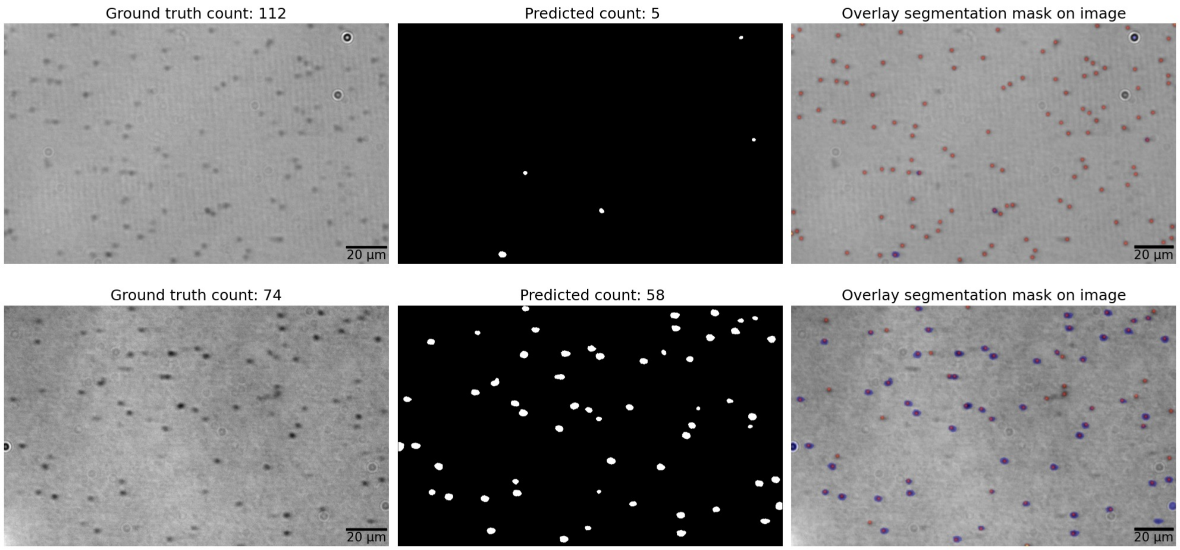


Count ?

Application of counting to particle counting

1. A simple threshold based method
2. Object detection based
3. Density based estimation

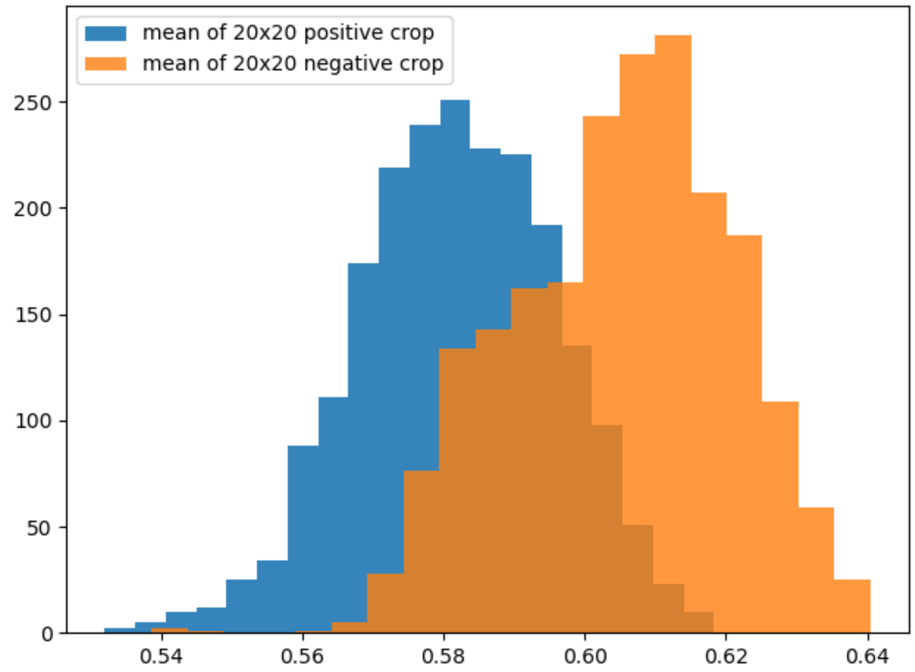
Application of counting to particle counting – threshold based



Using a threshold to classify each pixel into one of two classes

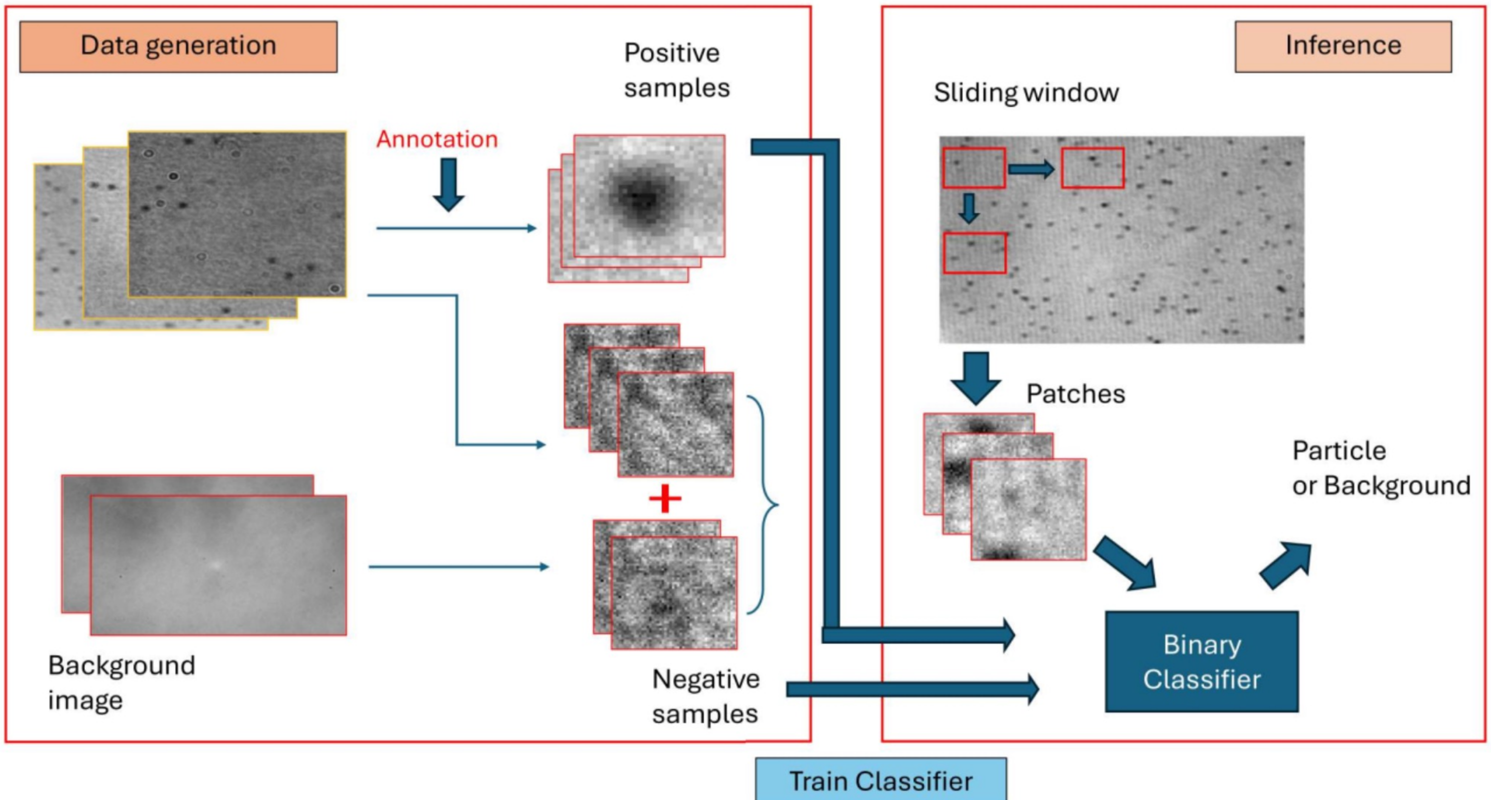
The computed MSE on a test dataset is approx. ~ 23

Application of counting to particle counting – threshold based



Visualization of the mean pixel intensity between the background and foreground of particle data

Application of counting to particle counting – detection based



Application of counting to particle counting – detection based

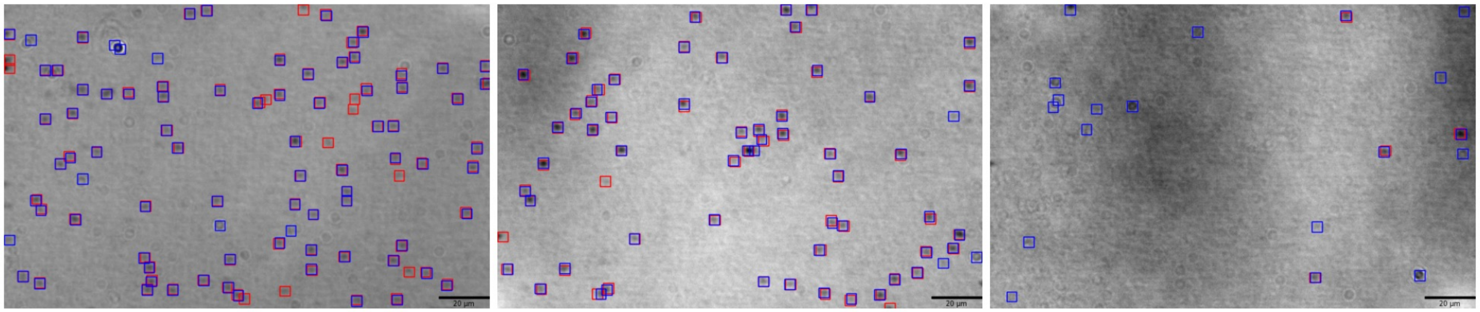
Proposal boxes generation:

Given: Image $\mathrm{X} \in R^{m \times n}$, we want to generate M anchor boxes (square boxes) as proposal regions for particle object detection.

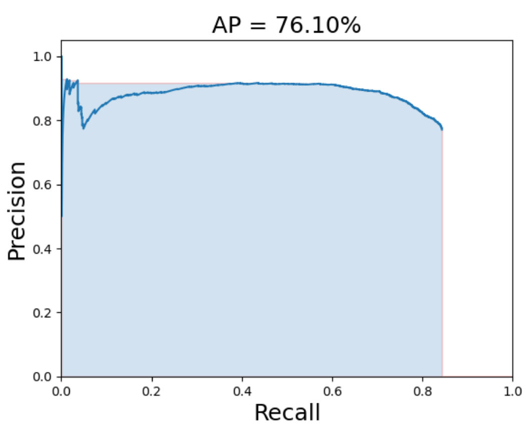
- Fixe a window size for boxes generation: Let w = 20, W = 2w = 40, stride $s = 20$.
- Generate anchors points: $\mathrm{A} = \{(x_t, y_t)\}_{t=1}^{M}$, x_t = w + k_ts, $k_t \in \left[1, \left\lfloor\frac{m-2w}{s}\right\rfloor\right]$; y_t = w + l_ts, $l_t \in \left[1, \left\lfloor\frac{n-2w}{s}\right\rfloor\right]$, $k_t, l_t \in \mathbb{Z}$.
- Proposal boxes are cropped from image: $B = \{b_i\}_{i=1}^{M}$

$$b_i = X[x_t - w: x_t + w; y_t - w: y_t + w]$$

Application of counting to particle counting – detection based



(a) Annotated boxes: 84; Predicted: 87 (b) Annotated boxes: 59; Predicted: 60 (c) Annotated boxes: 4; Predicted: 19



The experimental results show that a lot of background boxes are misclassified as the object (high false positive)

Application of counting to particle counting – density estimation

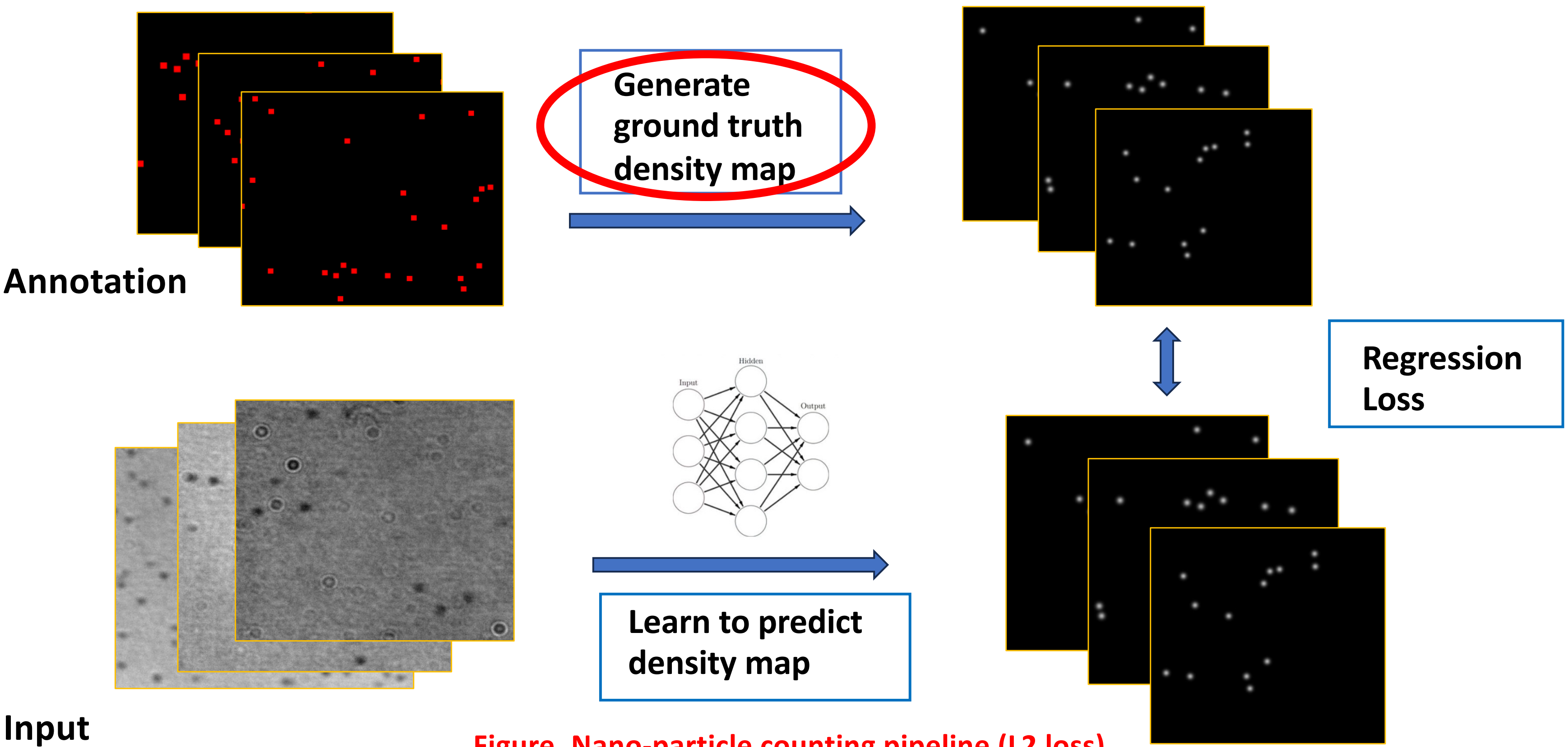


Figure. Nano-particle counting pipeline (L2 loss)

Application of counting to particle counting – density estimation

Ground truth generation:

- Set of ground truth pixels $\{x_m\}_{m=1}^{M}, x_m \in R^2, x_m = (i,j), i = 1, \dots H; j = 1, \dots W$
- Set of labeled pixels $\{z_n\}_{n=1}^{N}$
- Generate map $D^{gt}: R^{H \times W}$

$$D^{gt}(x_m) = \sum_{n=1}^{N} \mathcal{N}(x_m; z_n, \sigma^2 \mathrm{I}_{2\times 2}) = \sum_{n=1}^{N} \frac{1}{2\pi\sigma^2} \exp(-\frac{\|x_m - z_n\|^2}{2\sigma^2})$$

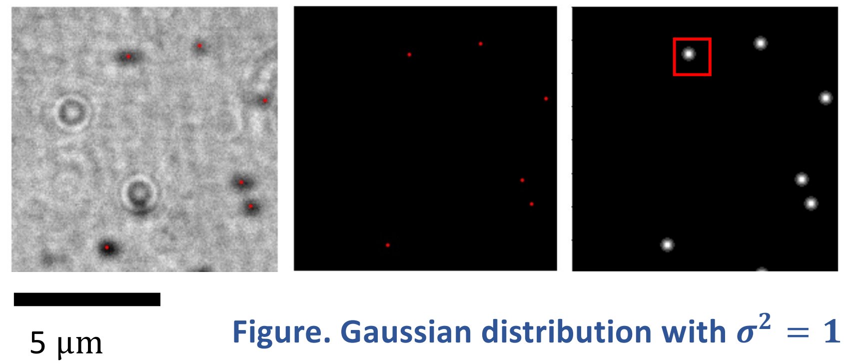
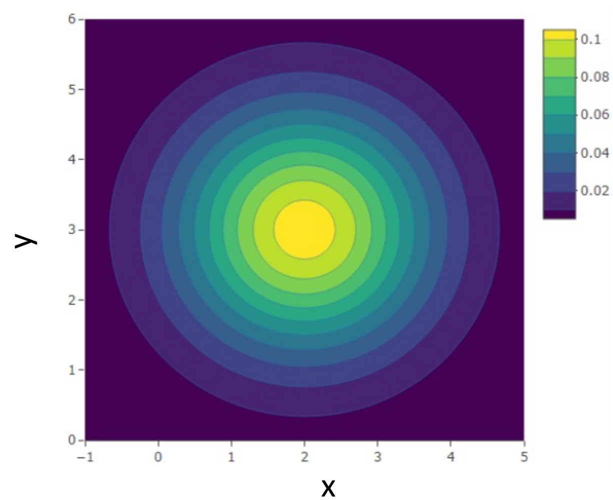


Figure. Gaussian distribution with $\sigma^2 = 1$



Application of counting to particle counting – density estimation

- D_i^{gt} is the generated map of image *i*, $D_i^{gt}: R^{H\times W}$
- D_i^{est} is the predicted density map of image *i*, $D_i^{est}: R^{H\times W}$
- N_t total images in the training set

The loss function is the mean squared (L2):

$$\mathcal{L}_{ms} = \frac{1}{N_t}\sum_{i=1}^{N_t}(D_i^{gt} - D_i^{est})^2$$

Problem Formulation for Particle Counting

U-Net for prediction map learning:

Encoder Pathway:

- Series of convolutional layers followed by max-pooling layers
- Progressively reduce the spatial dimensions; increasing the number of feature channels

Bottleneck:

- Consists of convolutional layers without max-pooling

Decoder Pathway:

- Consists of up-sampling layers followed by convolutional layers
- Skip connections

Final Layer:

- In image segmentation: softmax
- **In density estimation:** another convolution layer+ReLU

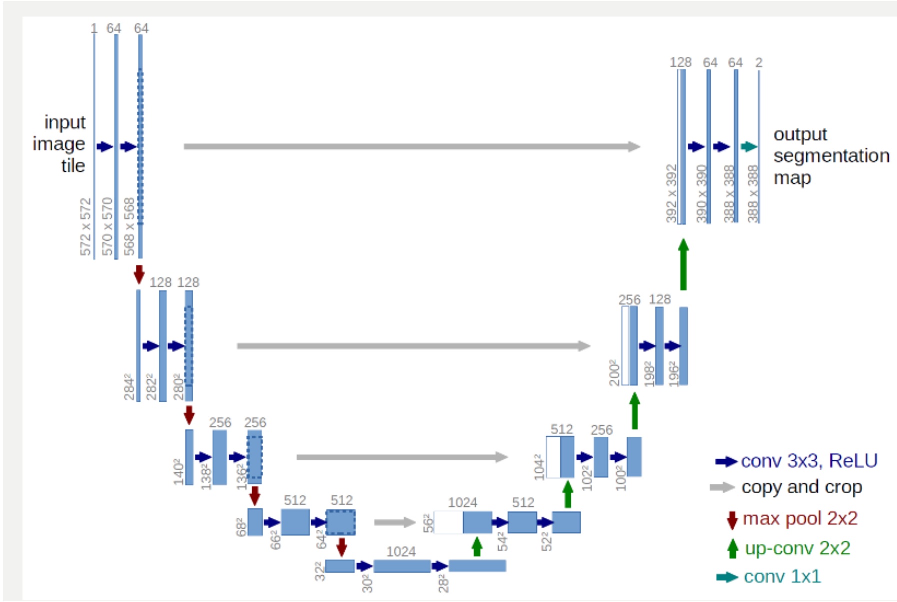


Figure 5. U-Net architecture

Ronneberger, O., Fischer, P., Brox, T. (2015). U-Net: Convolutional Networks for Biomedical Image Segmentation. Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015.

Experiment with The PRAM Image - Dataset

Data collection

Number of nano-particles per image: 1-272 particles per image
Resolution: 600x 960.

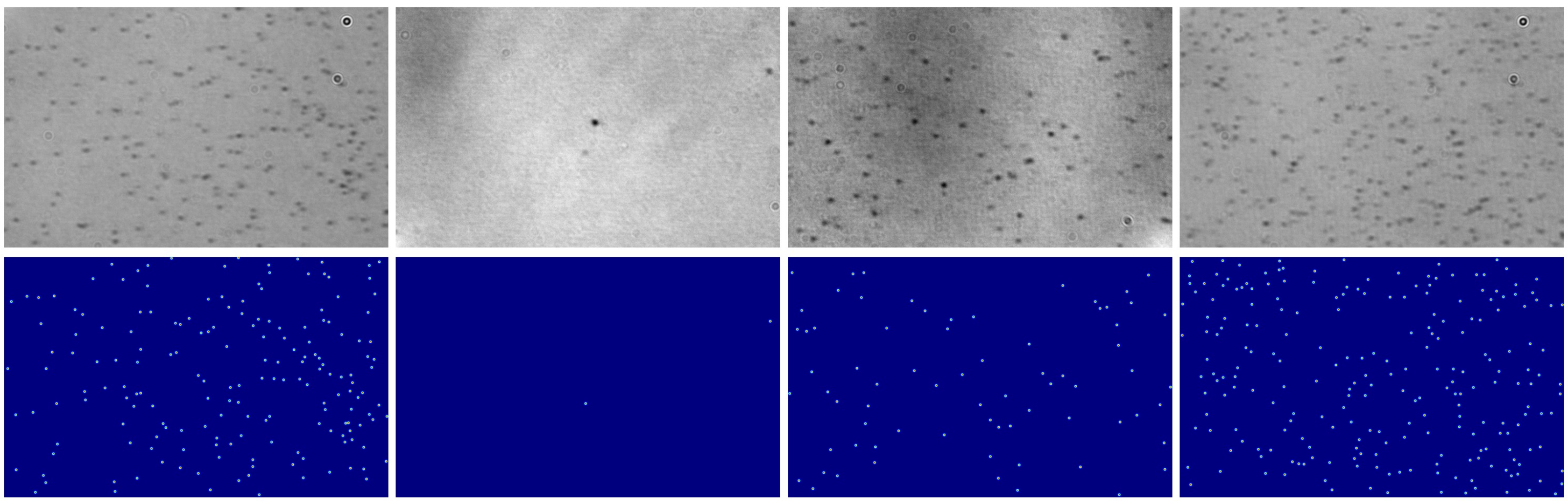


Figure. PRAM dataset raw image and annotated image

Experiment with The PRAM Image - Dataset

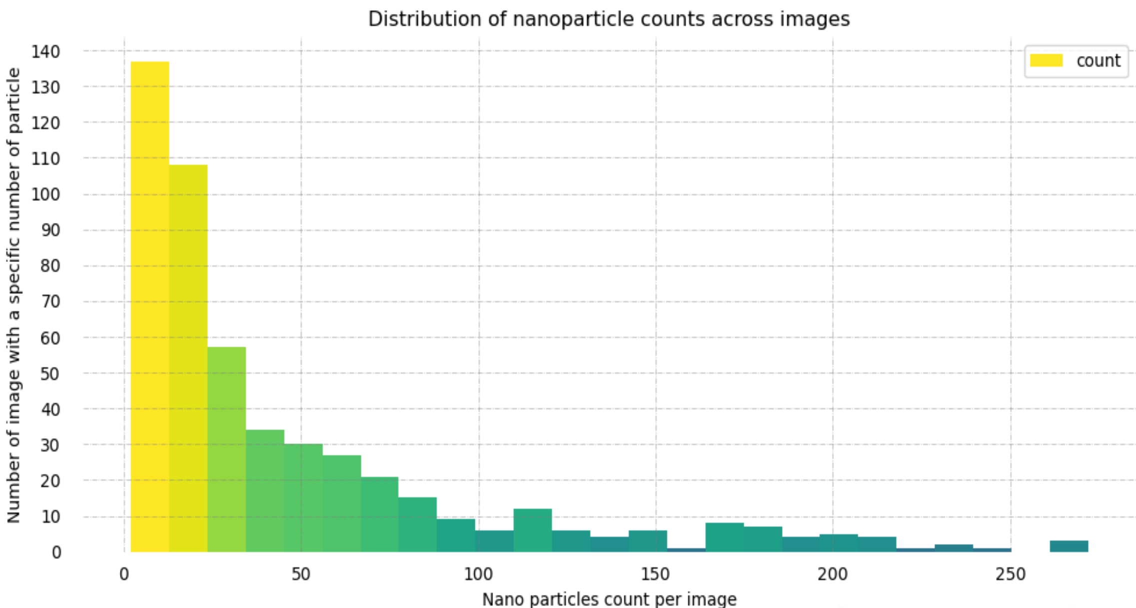


Figure. PRAM dataset statistics

Total image	Min annotation per image	Max annotation per image	Total annotation
508	2	272	23968

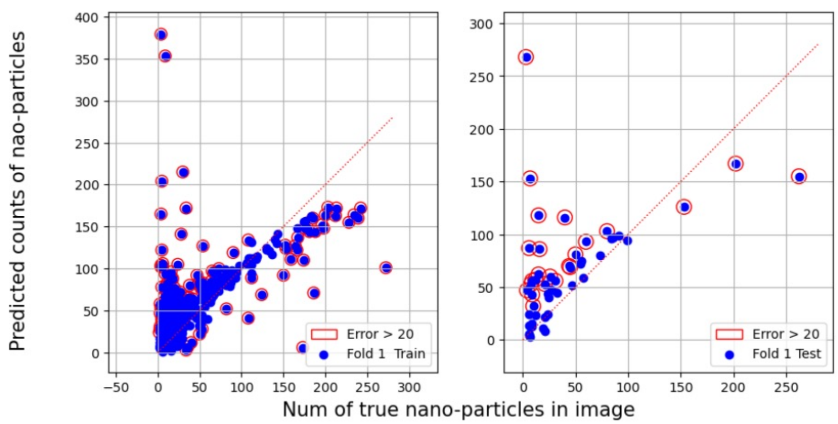
Results

Method	MSE	MAE	MRE	Run time (s)
Baseline	38.59	21.54	2.01	2.00
Detection then count	38.27	23.35	1.82	11.98
Deep learning based	11.19	6.4	0.14	5.92

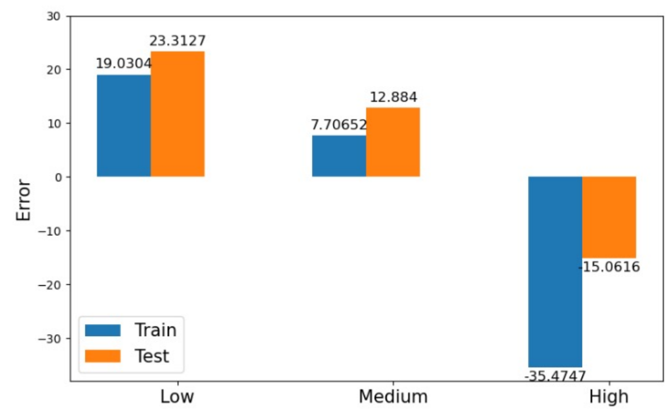
In general, the method of density based estimation performs best

Results

Detection then count approach



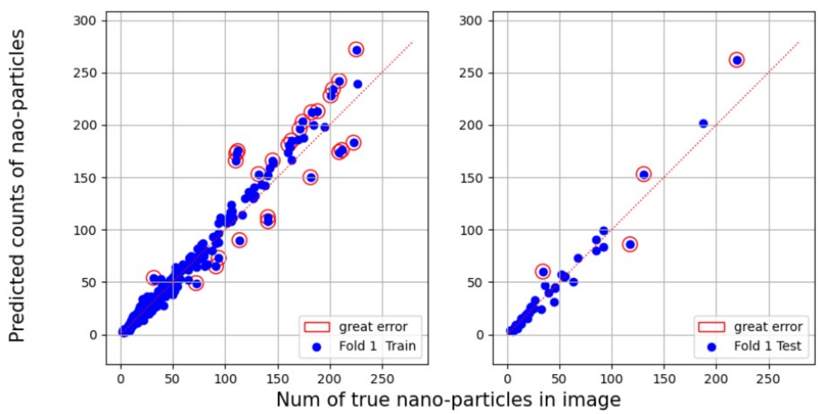
(a) True and predicted counts for train (left) and test (right) set



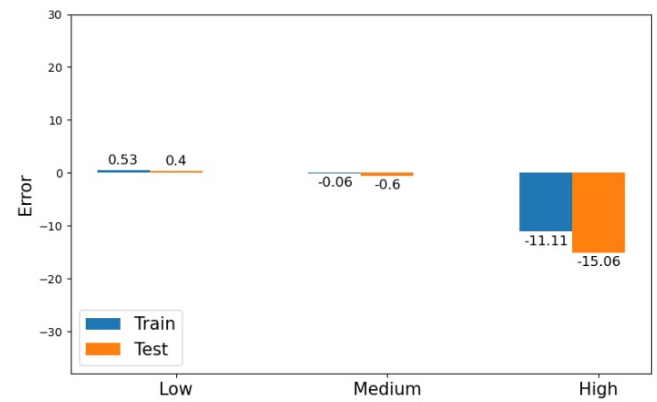
(b) Error of counting w.r.t. 3 bins of particle density for train (blue) and test (orange) set

Results

Density based count estimation approach



(a) True and predicted counts for train (left) and test (right) set



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Results

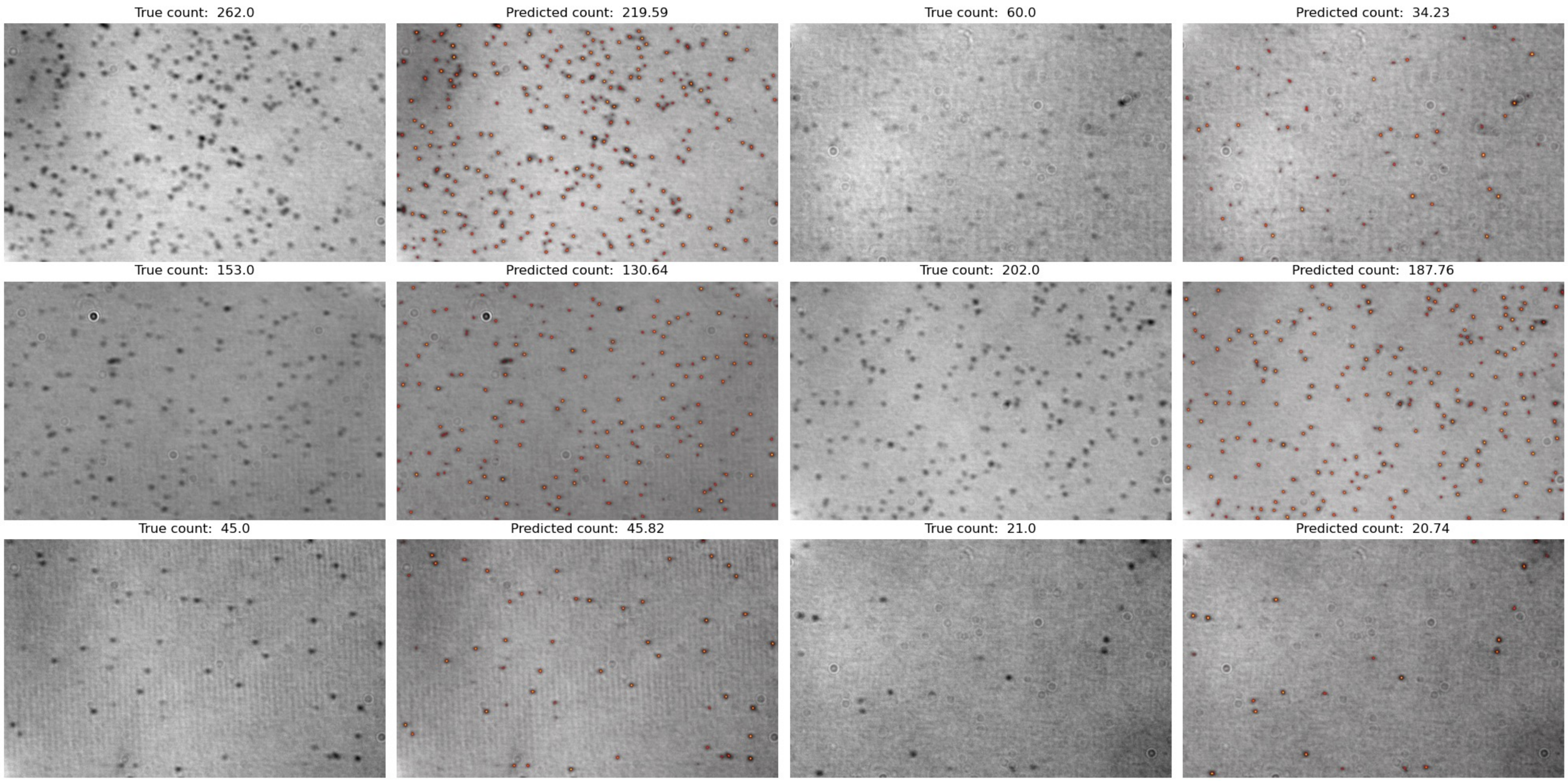

Figure. Count visualization

Summary

Conclusion:

- Detecting biomolecule cab be facilitated using PRAM and good particle counting algorithm
- Getting the accurate count is challenging, yet can benefit the detection pipeline
- This project aims to: introduce deep learning into the task of particle counting, experiments with different approaches and compare

Future works:

- Validate the performance on the dataset collected from PRAM
- Learning with fewer annotations, adaptation to new instances of PRAM images