

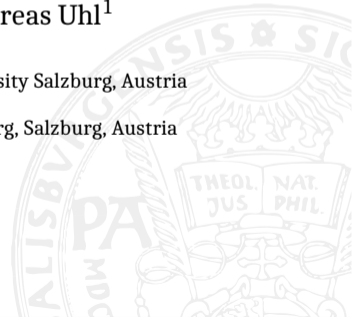
Towards Computational Sub-Diffraction Single Vesicle Detection in Fluorescence Microscopy

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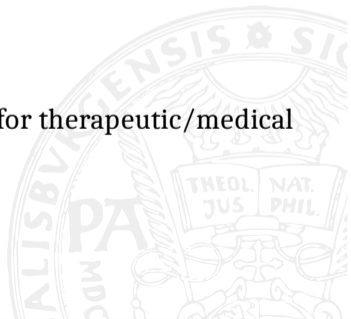
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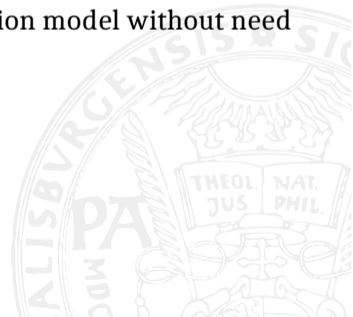
June 2, 2026



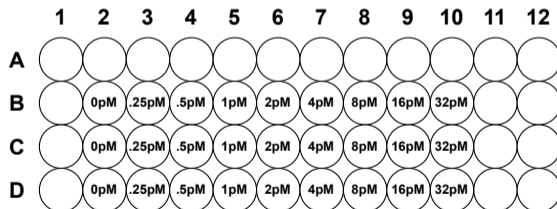
- EVs: small particles (few nm to hundreds of nm) released by cells
- carry diverse cellular materials and are decorated with specific proteins for target cell recognition
- can cross biological barriers while protecting their cargo
- bio-compatibility and targeting ability make them promising for therapeutic/medical delivery



- 1) Assess inter-annotator agreement for manually annotated data
- 2) Driven by result of 1), approach for sub-diffraction EV-detection model without need for ground truth



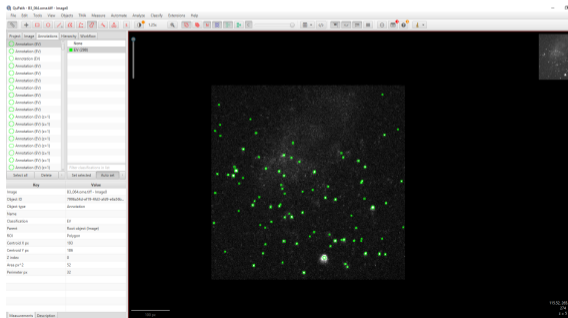
- lung tumor cells (A549 culture medium)
- vesicles isolated from milk
- 3 hours incubation
- 9 EV concentrations: 0 pM (control), 0.25 pM, 0.5 pM, 1 pM, 2 pM, 4 pM, 8 pM, 16 pM, 32 pM
- 16 photographs of size 2048×2048 pixel per well



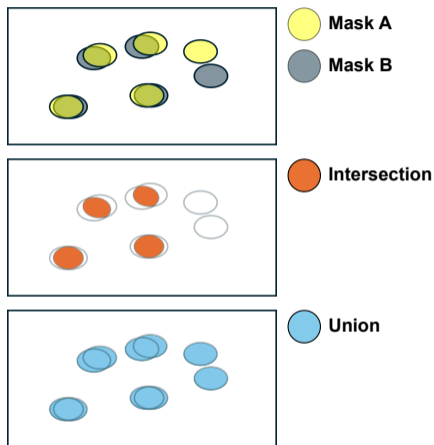
Empty rows E, F, G, H omitted for brevity

Methods | Annotation process

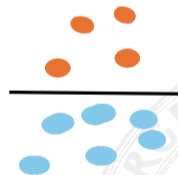
- Subset of 6 samples (512×512 pixel crops from 2 samples of concentration 0.25 pM, 2pM, 32pM)
- 4 Annotators (2 biology PhD students, 2 computer science PhD students)
- 2 annotation sessions per person
- Annotation tool: QuPath
- only CY5 channel used for annotation



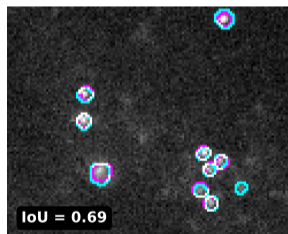
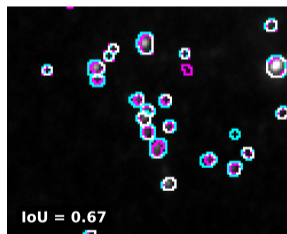
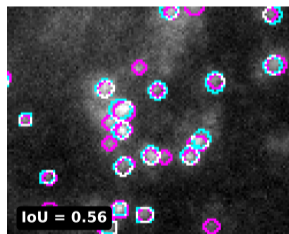
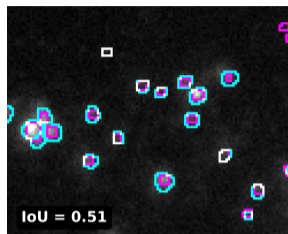
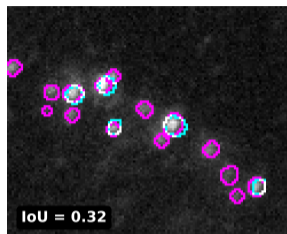
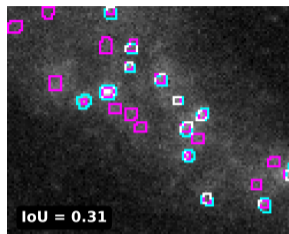
Methods | Evaluation metric: Foreground intersection over union



IoU =

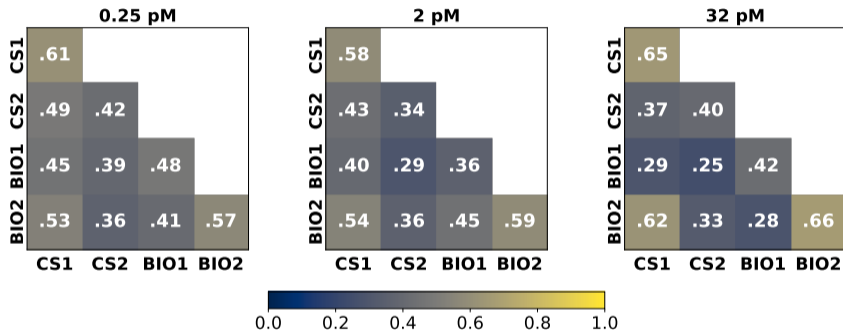


Methods | Evaluation metrics - examples for intuition

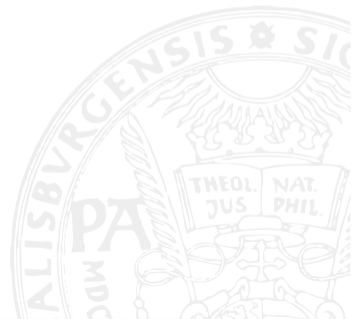


 Annotator 1  Annotator 2  Overlap

Results | Annotation Agreement

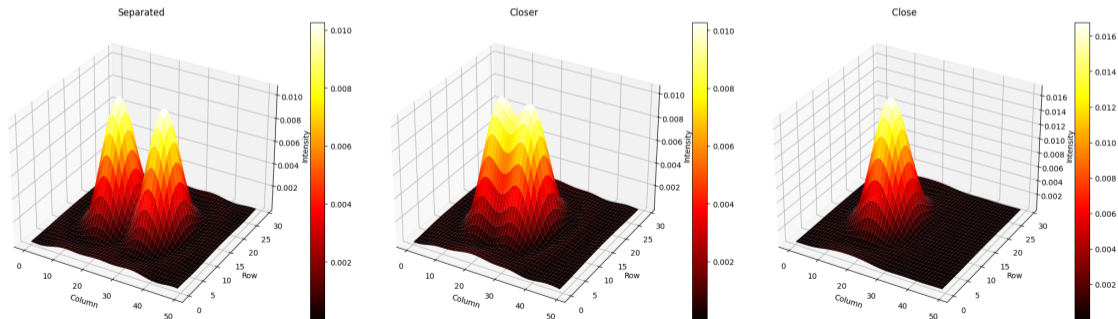


- Part II: Towards single EV-detection without ground truth

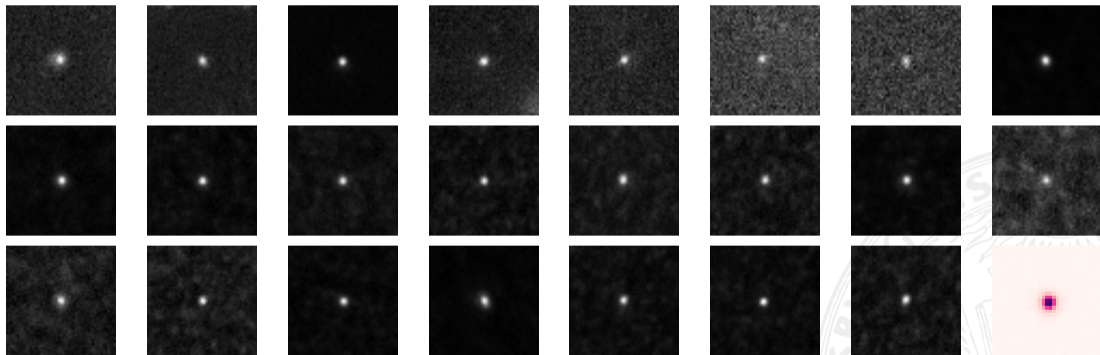


Methods | Problem: Diffraction

- optical microscopy suffers from resolution limit
→ point-source appears as cone
- EV sizes: few nanometers
- cone is known as point spread function PSF
- when single point light sources are close together, they appear as single, bigger cone (diffraction limit)



- Manual selection of suited spots for PSF estimation (lower right: resulting averaged PSF estimation)



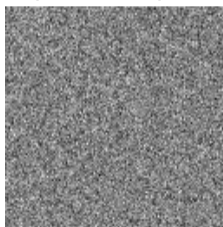
Random Pixels of varying intensity (simulated EVs)



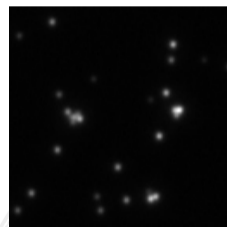
(convolution)



Background from real probe samples



Synthetic EV Image

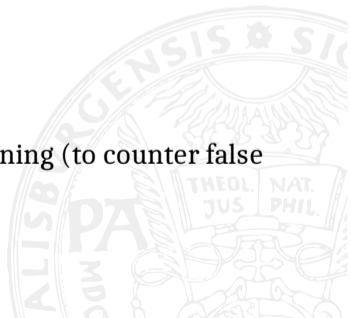


Synthetic images:

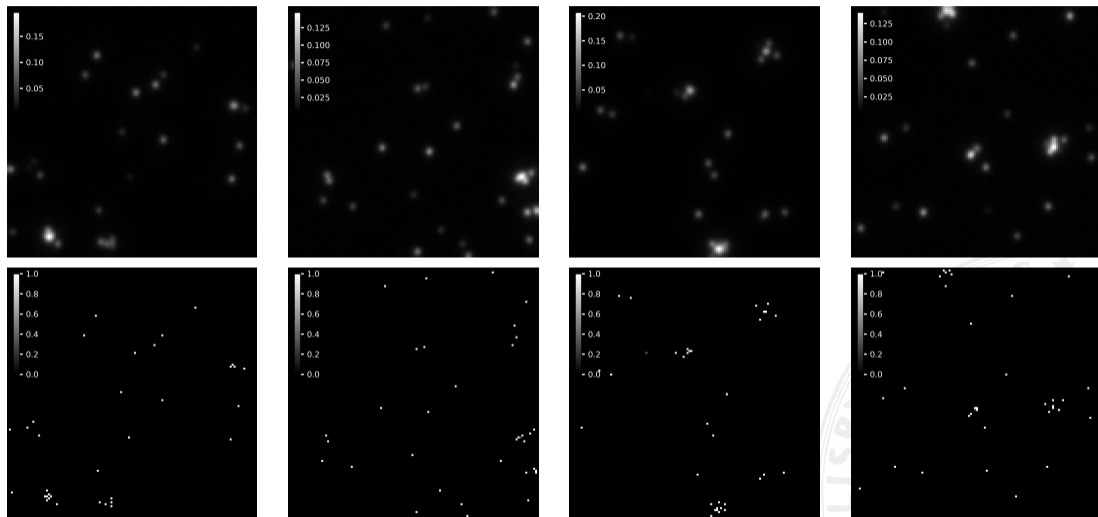
- Randomly place "EVs" with random intensity
- create three "EV hot spots" where EVs spawn preferably

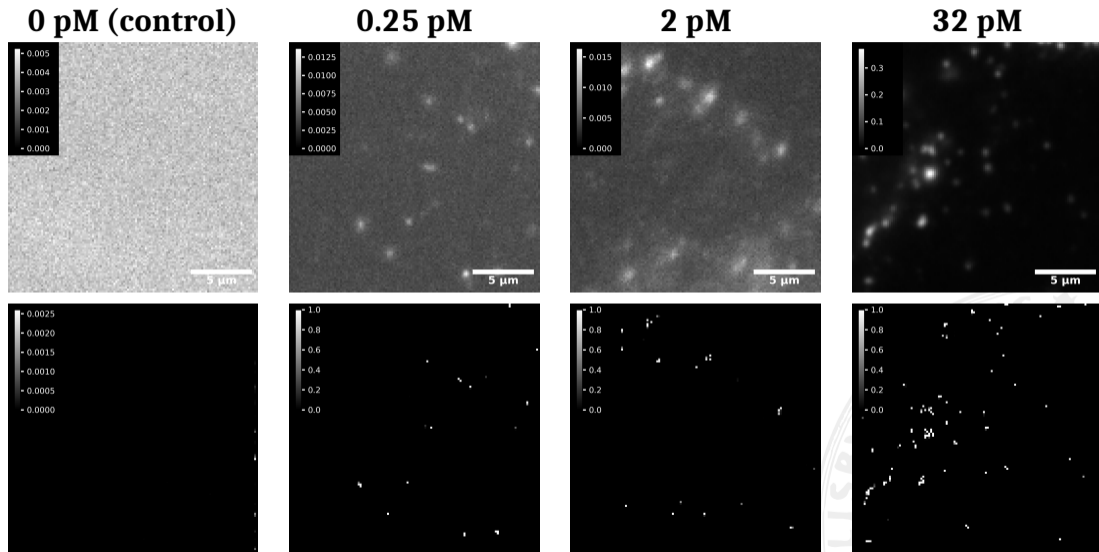
Segmentation Model:

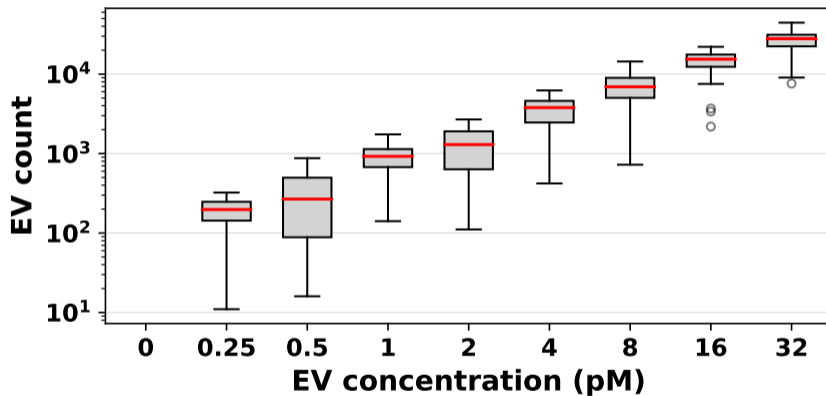
- U-Net with 4 Up-Downsampling blocks
- Dice (0.25) + Focal (0.75) loss
- 128×128 pixel inputs; Patchwise inference
- with 50% chance, only background (no EVs) was used for training (to counter false positives)



Results | Qualitative (model applied on synthetic data)

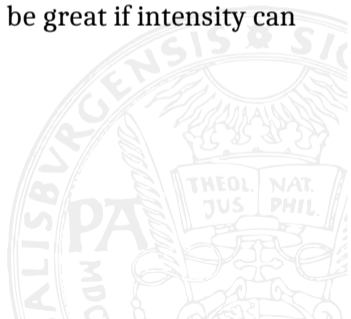






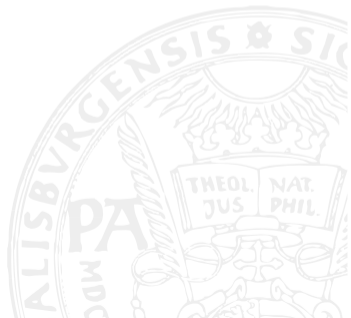
Limitations of this study

- EVs assumed to be of fixed size 1×1 pixel ($\sim 160\text{nm}$)
- model works only on 128×128 pixel patches
- EV existence predicted as binary value - in the future it would be great if intensity can be estimated
- model learned only 1 PSF (the one we measured)



This study did:

- show a discrepancy between annotators in manually generating ground for EV segmentation truth
- present a proof-of-concept approach to find single EVs
 - 1 via training on synthetic data (no ground truth needed)
 - 2 via computationally reversing diffraction artifacts



Q & A

