Deep Learning based Localization for RNA Spatial Transcriptomics

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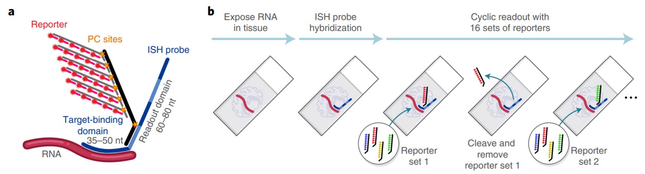
CONTEXT AND IMAGE COLLECTION

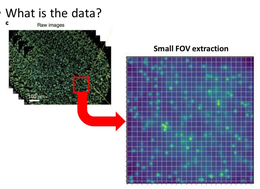
- Tissue samples are chemically treated with fluorophores that bind to specific RNA molecules. Fluorophores are imaged, creating the image data sets to be processed.
- Reporters bind to RNA nucleotide sequences. Fluorophores on the reporters emit light in imaging. Fluorophores are washed from the cells and replaced with the next set of emitters
- The fluorophores add up to create a 'barcode' that details which RNA molecule was bound to at that location. Bright spots represent bonded RNA, and their color determines type.
- For the duration of the project, we worked with provided images from the CosMx* platform.

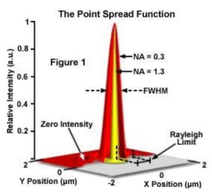


*CosMx : A single-cell imaging platform developed by NanoString which leverages cycle from mature cyclic fluorescent in situ hybridization (FISH) chemistry, to generate a high-resolution imaging readout for interactive data analysis and visualization software.

DATA and POINT SPREAD FUNCTION

• What is the data?

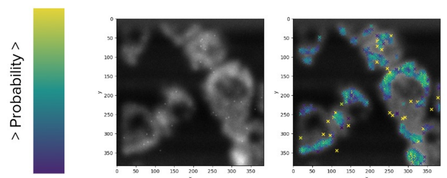




$$FWHM \approx \frac{\lambda}{2NA}$$

• Each small dot represents a target RNA molecule with a bonded fluorophore. By finding the exact position of each of these dots, we understand where the genes are.

INITIAL RESULTS and BACKGROUND ISSUE



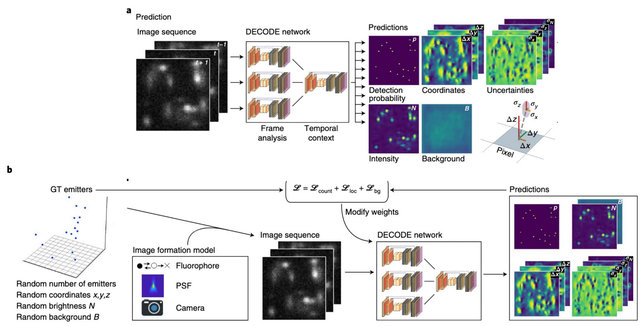
• Initially, we trained DECODE using the concept of simulator learning following the methodology mentioned originally within the DECODE architecture.

• Our fitting data had a noise component which yielded many false positives.

DECODE ARCHITECTURE

- DECODE is a deep learning-based architecture, built on U-Nets, used for single molecule localization microscopy (SMLM) i.e., detecting and localizing emitters at sub-pixel resolution. Notably, DECODE also predicts detection and localization uncertainties, which can be used to generate superior super-resolution reconstructions.

- Our loss function has three terms:
  - Count loss compares the true and detected number of emitters in the image
  - Localization loss that trains the network to localize the detected emitters and estimate the uncertainty of those emitters
  - Emitter brightness and optional background loss



TEMPORAL CONTEXT AND SIMULATOR LEARNING

- DECODE's temporal context module pools information across multiple (**three** in DECODE paper , **seven** for our data) frames, to improve both the detection accuracy and the localization error.

- Ground-truth data for supervised learning is not easily available for SMLM. Therefore, we train the DECODE network by generating a large amount of simulated data.

TRANSLATION FROM PYTORCH TO C#

- CosMX pipeline supports C#. To test real data on the pipeline and visualize model results, we need to convert the DECODE architecture to C#. To do this, DECODE models need to be run through a translation layer known as ONNX.

- ONNX Runtime is an open-source project that is designed to accelerate machine learning across a wide range of frameworks, operating systems, and hardware platforms. It enables acceleration of machine learning inferencing across all deployment targets using a single API.

- ONNX Runtime parses through DECODE models to identify optimizations and provides access to hardware acceleration. Exporting PyTorch models to ONNX also allows for Windows Machine Learning applications to be created in the desired C# language.

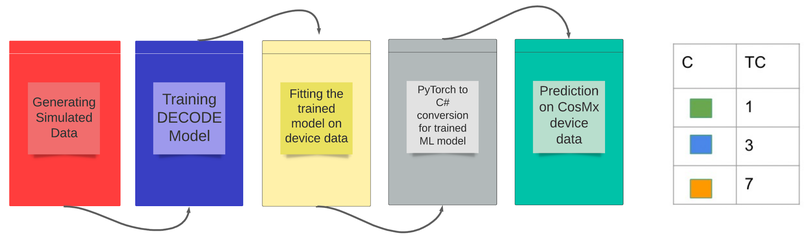
RESULTS

- We trained the DECODE with concept of simulator learning – simulated data being generated using specified camera parameters and PSF.

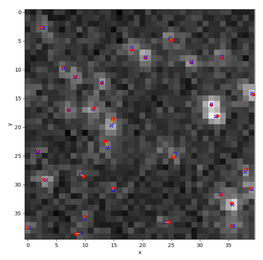
- We have trained the model for temporal context values of **one**, **three** and **seven** and plots below demonstrating these temporal contexts. The temporal context value of **seven** showed the best loss and F1 values for out test data.

- We were able to translate DECODE model into C# for use on NanoString imaging hardware, such as the CosMx system.

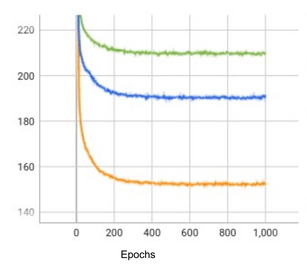
- We were able to find the work around for the background issue.



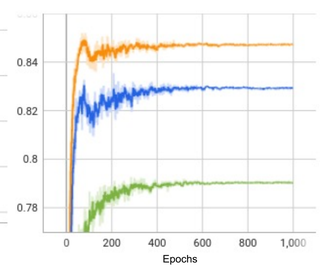
C	TC
	1
	3
	7



SIMULATED DATA



TRAINING LOSS VS EPOCHS



F1 VS EPOCHS

Future Work, References, and Acknowledgments

- Further improvements in DECODE network architecture.
- Translation of the 2D architecture to 3D architecture for 3D imaging.
- Researching changes in terms of network and loss for 3D architecture.

Nanostring Technologies: Jaemyeong Jung, Grant Tremal, Tushar Rane, Winnie Leung, Aster Wardhani, Mithra Korukonda, Dwayne Dunaway.

TA: Shruti Misra

[1] Speiser et. al. Deep learning enables fast and dense single-molecule localization with high accuracy. Nature Methods. 18. 10.1038/s41592-021-01236-x.
[2] He, Shanshan & Bhatt et. al. (2022). High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging. Nature Biotechnology. 40. 10.1038/s41587-022-01483-z.
[3] "I2K 2020 tutorial: DECODE for Single Molecule Localization Microscopy," YouTube, 04-Mar-2021. [Online]. Available: https://www.youtube.com/watch?v=zoWsj3FCUJs&ab_channel=JaneliaConferences%2CWorkshops%26Seminars. [Accessed: 13-Mar-2023].

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