Semi-automated mRNA counting in single cells

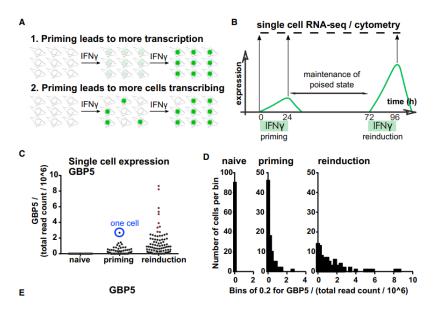
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Summary

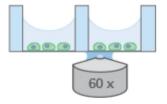
- ▶ 10×10 grid acquisition of DAPI, GAPDH, GBP5 in Hela cells on #1.5 chambered coverglass
- ► Cell segmentation using UNET implementation in PyTorch
- Spot detection using LoG filter
- Spot calling using Fisher information filter
- Kinetic models.. (TBD)

Single-cell RNA-seq identifies GBP5 as a memorized gene



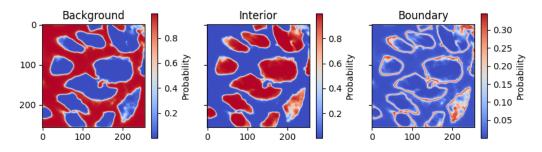
Siwek et al. Activation of Clustered IFNg Target Genes.... Molecular Cell 2020

Imaging approach



Training a semantic segmentation with cross-entropy loss (log loss)

 $100\ 256 \times 256$ images, 80 train $+\ 20$ validation,



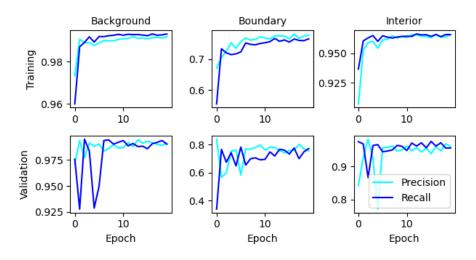
We train a 3-class semantic segmentation model with cross-entropy loss:

$$\mathcal{L} = \sum_{i,j} \log p_{ij}(\tilde{x}) = \sum_{i,j} \log \frac{\exp(-s_{ij}(\tilde{x}))}{\sum_{x \in \chi} \exp(-s_{ij}(\tilde{x}))}$$

 p_{ij} is the probability the model assigns a pixel to the true class $\tilde{x} \in \{a, b, c\}$

Training on HeLa GAPDH channel

Learning rate $\eta = 0.01$, Batch-size B = 5 (32 train iterations, 8 validation)



Spot calling using Fisher information filter

Results

