

## Supplementary Information

### Virtual organelle self-coding for fluorescence imaging via adversarial learning

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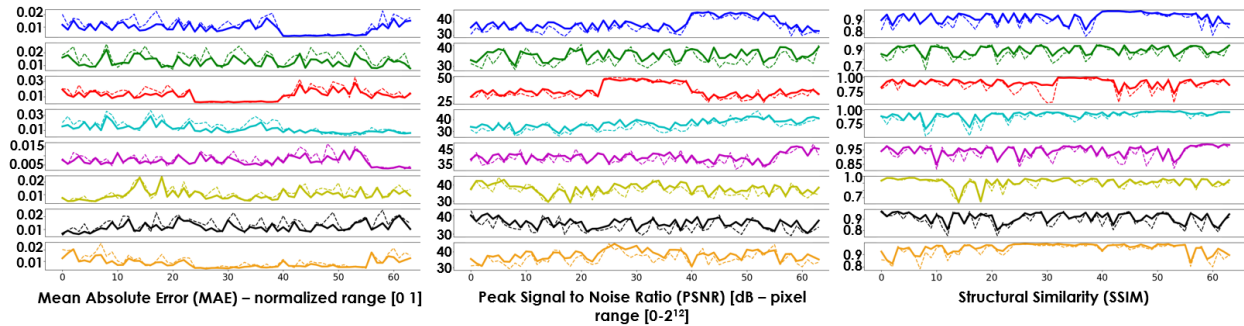
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## Methods

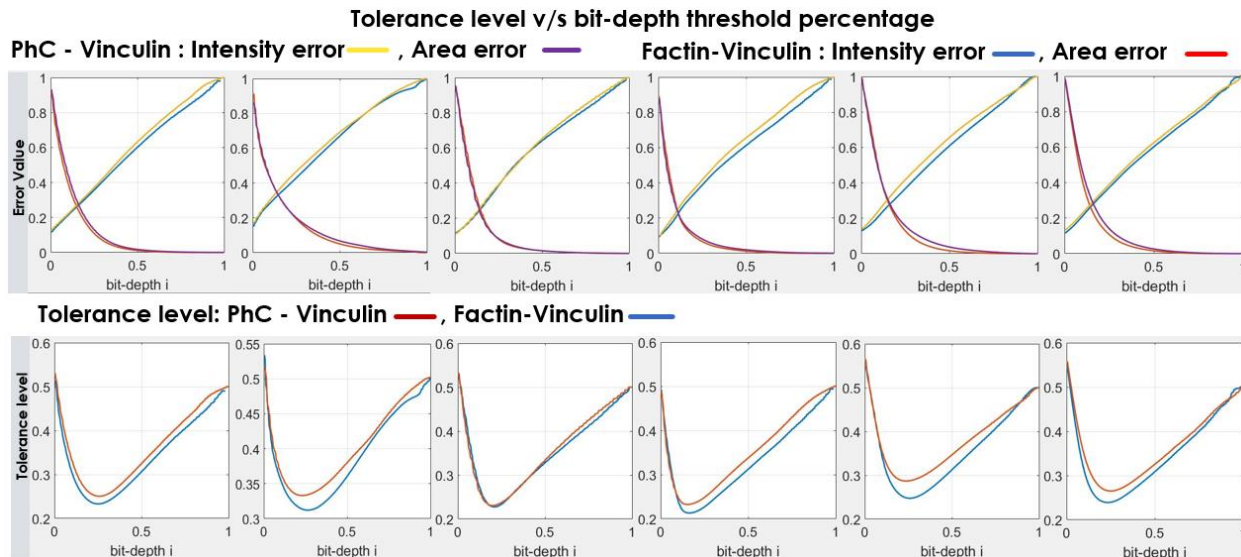
### Computational pipeline with CellProfiler:

Original pipeline could be found at [20], modified version that we used can be found at: [https://github.com/32nguyen/SupplementaryMethod\\_CellProfiler](https://github.com/32nguyen/SupplementaryMethod_CellProfiler)

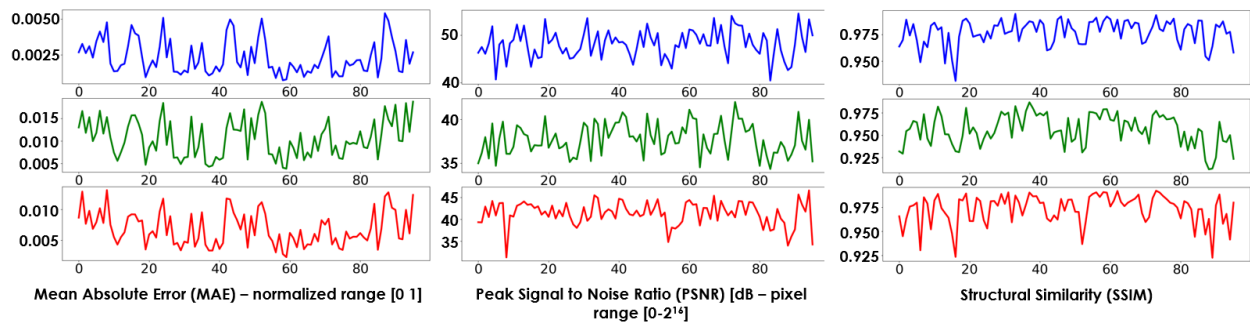
Python codes to extract the feature data automatically if not familiar with MySQL. Example of feature measurement is also provided to observation. Original dataset could be downloaded at <https://data.broadinstitute.org/bbbc/BBBC025/>,



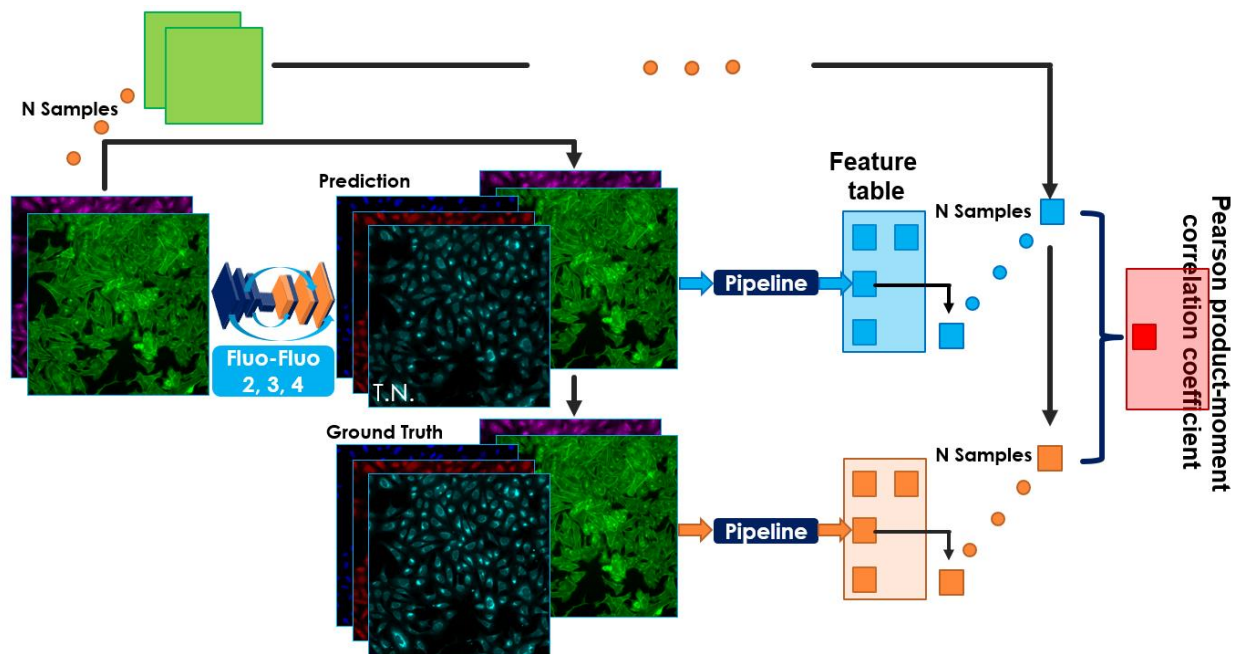
**Supplemental Fig. 1:** MAE, PSNR, and SSIM on 64 predicted images vs. focused ground truth images per z depth (-10, -8, -6, -4, 4, 6, 8 and 10 $\mu$ m, top to bottom) with AF model's prediction, dash line is out-focus image v/s ground truth, solid line is predicted image v/s ground truth.



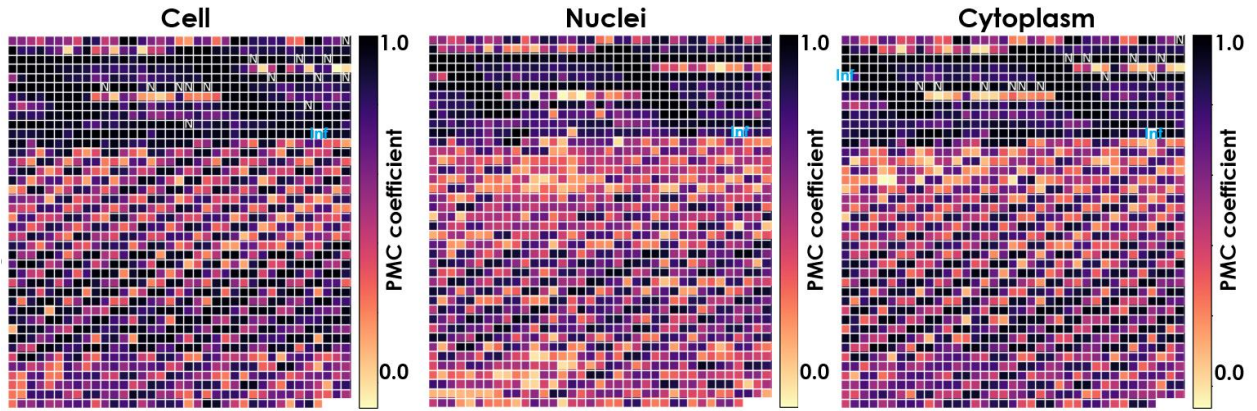
**Supplemental Fig. 2:** Tolerance level (y-axes) v/s bit-depth threshold percentage (x-axes) computed on 6 testing images (left to right). The first row is the intensity error ( $IE$ ) versus area fraction of intensity-based segmentation error ( $SE$ ) of both PhC-Fluo 2 and Fluo-Fluo 1 models. The second row is the summation of equally-weighted  $IE$  and  $SE$  ( $\beta_1=\beta_2=0.5$ ) of both PhC-Fluo 2 and Fluo-Fluo 1 models.



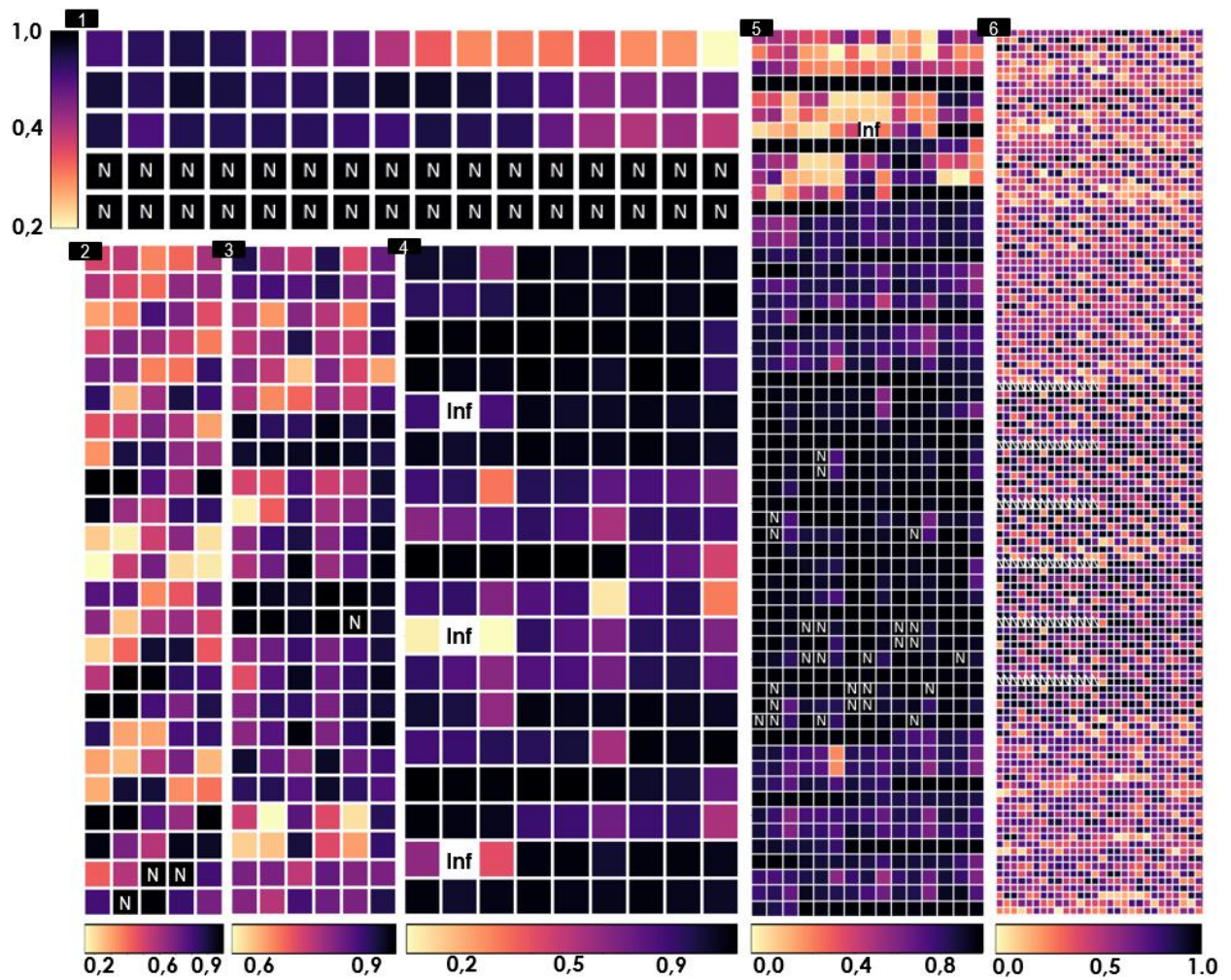
**Supplemental Fig. 3:** MAE, PSNR, SSIM on 96 predicted nucleus DAPI/Hoechst, Endoplasmic reticulum and Mitochondria images vs. corresponding ground truth fluorescence images in testing dataset of U2OS-PS. Top to bottom are for DAPI/Hoechst, Endoplasmic reticulum, and Mitochondria, respectively.



**Supplemental Fig. 4:** Procedure for Pearson product-moment correlation coefficient extracted from feature measurement table. Each feature type in table from original (5) channels and hybrid-virtual (2+3) channels crossing  $N$  samples (each sample is an image which has 5 channels) to compute the Pearson product-moment correlation coefficient.



**Supplemental Fig. 5:** Pearson product-moment correlation coefficient of each feature measurement across 96 images on testing data between original (5) channels and hybrid-virtual (2+3) channels. “N” marks the correlation coefficients of features measured only on 2-channel input images in both cases (Golgi apparatus + F-actin) which results perfect correlations. “Inf” marks un-resolved correlation due to 0-division in measurements. Those features are distributed across 3 compartments: Cell, nuclei, and cytoplasm [20] (see Supplement - Methods for feature’s organization and Supplement Fig. 6 for full feature measurements).



**Supplemental Fig. 6:** Pearson product-moment correlation coefficient of each feature measurement across 96 samples on testing data between original 5 channels and 2+3 channels (2 of inputs and 3 of predictions). “N” marks the correlation coefficients of features measured only on 2-channel input images in both cases (Golgi apparatus + F-actin) which results perfect correlations. “Inf” marks un-resolved correlation due to 0-division in measurements. Following #1-6, PMC coefficient is following 6 feature groups: Granularity, correlation, radial distribution, size-shape, intensity, and texture. Correlation coefficient grids were re-organized as the last axis index changing fastest.