Deep Learning Microscopy for Enhanced Digital Pathology

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4TH Digital Pathology & AI Congress
June 26th, 2018, New-York City, NY, USA

Rivenson, Y., et al., ACS Photonics (2018), DOI: 10.1021/acsphtotonics.8b00146
Deep convolutional neural network

- Deep convolutional neural network implement functions by solving an optimization problem. 
  \[ f(y) = O_n A_n \cdots O_2 A_2 \cdot O_1 A_1 y \]
- Optimized only once and remains fixed.
- Reconstruction performed in a single feed-forward step.

DEEP LEARNING COMPUTATIONAL MICROSCOPY

Deep learning computational microscopy

- Works with standard microscope hardware.
- Towards real time performance (we achieved inference time < 1sec)
- Do not apply image formation models.
**Supervised deep network training**

- 40×/0.95NA tissue section images matched to 100×/1.4NA tissue section images (brightfield microscopy).

**Cost function**

\[
l(\Theta) = MSE(Y^{HR}, f(Y^{LR}; \Theta)) + \lambda(\nabla f(Y^{LR}\Theta))^2
\]
• Filter size (throughout the network) – $3 \times 3$
  \[ \nu_{i,j}^{k,l} = \sum_{r} \sum_{p} \sum_{q} w_{i,j,r}^{p,q} \nu_{i-q,j}^{k+p,l+q} + b_{i,j} \]

• Activation function – Rectified Linear Unit - ReLU(x) = max(0, x)

• Number of learnable parameters ~ 230K

• Number of layers = 13

Convolutional filtering
Implementation details

- Preprocessing – before training, the low resolution and high resolution images were accurately registered.
- Training time ~ 4.5 hours (630 epochs) –
  - 9,536 patches (60×60 pixels) → (150×150 pixels).
- Inference time < 1 sec on a dual GPU laptop for a 40× objective field-of-view.

Resolution enhancement

- The image is enhanced while keeping the original field-of-view (>6-fold the field-of-view of the 100x objective).

Extended depth-of-field and cross-tissue

depth of field ≈ \( \lambda / NA^2 \)

• Trained on lung tissue, inferred on kidney tissue (same stain).
Network input - 40×/0.95NA
Network output ×2

1µm
Z-stack (100×/1.4NA): Δz = 0.4 μm
Z-stack (100×/1.4NA): Δz = 0.4μm
Cross tissue and cross staining

- Trained on lung tissue, inferred on breast tissue, with different stain.
Network input - 40×/0.95NA

2µm
×2 network output trained on H&E stained breast tissue
×2 network output trained on Masson’s trichrome stained lung tissue
Ground truth (100×/1.4NA)
Modulation transfer function estimation

- Network trained with lung tissue.
DEEP LEARNING ENHANCED MOBILE-PHONE MICROSCOPY

Resolution ~ 0.87µm (half pitch)
FOV ~ 1mm²
Challenges in mobile microscopy

• Main challenge: keep the design cost-effective, robust and portable.
  • Non-optimized, often battery powered illumination.
  • Spectral distortions.
  • SNR due to the pixel size.
  • Spatial aberrations.
  • Lack of mechanical stability.

Smartphone microscope

Benchtop microscope (20×/0.75NA)
Smartphone microscope
SSIM($U_1, U_2$) = \[
\frac{(2\mu_1\mu_2+c_1)(2\sigma_{1,2}+c_2)}{\mu_1^2+\mu_2^2+c_1(\sigma_1^2+\sigma_2^2+c_2)}; \mu_{1,2} = E[U_{1,2}]; \sigma^2_{1,2} = E[(U_{1,2} - \mu_{1,2})^2]; \sigma_{1,2} = E[(U_1 - \mu_1)(U_2 - \mu_2)]
\]

$c_1, c_2$: stabilization parameters

Structural similarity ~ 0.9
DEEP LEARNING ACHIEVES SUPER-RESOLUTION IN FLUORESCENCE MICROSCOPY

Fluorescence microscopy super-resolution

Network input (10×/0.4NA)  Network output (10×/0.4NA)  Ground truth (20×/0.75NA)
Quantification

Network input (100×/1.4NA, confocal)  Network output (100×/1.4NA, confocal)  Ground truth (100×/1.4NA, STED)
Quantification

Network input (100×/1.)

![Image of network input (100×/1.).]

![Histograms showing network input and output compared to ground truth.](Image)

- Network input (confocal)
- Network output (confocal)
- Ground truth (STED)

**Fig. 6 (version 1)**
DEEP LEARNING-BASED VIRTUAL HISTOLOGY STAINING USING AUTO-FLUORESCENCE OF LABEL-FREE TISSUE

**Virtual H&E staining (Salivary gland tissue)**

Contrast enhanced unstained tissue auto-fluorescent image

Unstained tissue auto-fluorescent image (network input)

H&E *virtually* stained tissue (network output)

H&E *chemically* stained tissue (brightfield)
Virtual Masson’s Trichrome staining (lung tissue)

Contrast enhanced unstained tissue auto-fluorescent image

Unstained tissue auto-fluorescent image

MT3 virtually stained tissue (network output)

MT3 chemically stained tissue (brightfield)
Virtual Jones’ silver staining (kidney tissue)

Contrast enhanced unstained tissue DAPI image

Unstained tissue DAPI image (network input)

H&E virtually stained tissue (network output)

Jones Silver stained tissue
Summary – enhanced microscopy

• Deep learning can substantially enhance microscopic images in terms of:
  • Spatial resolution
  • Field of view
  • Depth of field
  • Spectral distortions
  • Compression
  • Telemedicine
  • Towards real time performance
  • Virtual staining
  • Staining standardization

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Acknowledgment