Computational Deep Learning Microscopy

Presented by:

OSA Photonic Detection Technical Group

COMPUTATIONAL DEEP LEARNING MICROSCOPY WEBINAR

21 March 2019 • 14:00 EDT

OSA Photonic Detection Technical Group

Speaker: Prof. Yair Rivenson UCLA





Committee 2019



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About Us

The Photonic Detection technical group is part of the Photonics and Opto-Electronics Division of the Optical Society. This group focuses on the detection of photons as received from images, data links, and experimental spectroscopic studies to mention a few. Within its scope, the PD technical group is involved in the design, fabrication, and testing of single and arrayed detectors.

This group focuses on materials, architectures, and readout circuitry needed to transduce photons into electrical signals and further processing. This group's interests include: (1) the integration of lens, cold shields, and readout electronics into cameras, (2) research into higher efficiency, lower noise, and/or wavelength tunability, (3) techniques to mitigate noise and clutter sources that degrade detector performance, and (4) camera design, components, and circuitry.





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Optical Interaction Science

Photonics and Opto-Electronics + Fiber Optics Technology (PF) Integrated Optics (PI)

Laser Systems (PL)

Optical Communications (PC)

Photonic Detection (PD)



This group involves the detection of photons as received from images, data links, and experimental spectroscopic studies to mention a few. Within its scope, it is involved in the design, fabrication, testing of single and arrayed detectors. Detector materials, structures, and readout circuitry needed to translate photons into electrical signals are considered by this group. Also included in this group is the

integration of components such as lens, cold shields, and readout electronics into cameras. Research into higher efficiency, lower noise, and/or wavelength tunability is included here. Additionally, techniques to mitigate noise and clutter sources that degrade detector performance are within the purview of this group. In the imaging area, camera design, componentry, and circuitry are considered.

Announcer

Join the Photonic D Group for their ina Wednesday, 27 Apı

In this webinar, Dr. describe his recent speed quantum ke photonic integrate scalable quantum i processors based c networks.

Register for the W



Technical Group Activities

- **Special Sessions** at OSA conferences such as CLEO and OFC.
- ~4 Webinars for this year!
- Interactions with local sections and student chapters.
- Interactive community for bringing together researchers across interdisciplinary fields for tackling advances in photonic detection technologies.
- Example: Panel discussion on *Silicon Photonics for LiDAR and Other Applications* at OFC 2019 which had great turn-out and a lot of interest!





Computational Deep Learning Microscopy

Yair Rivenson

Electrical and Computer Engineering Department UCLA

OSA Webinar

March 21st, 2019

Optica 4, 1437-1443 (2017) Light Sci. Appl. 7, e17141 (2018) ACS Photonics (2018), DOI: 10.1021/acsphotonics.8b00146 Nat. Methods 16, 103 (2019) Nat. Biomed. Eng. 1 (2019). doi:10.1038/s41551-019-0362-y Light Sci. Appl. 8, 23 (2019) Light Sci. Appl. 8, 25 (2019)

Tradeoffs in microscopy



Computational microscopy

 Using a numerical model of the imaging system to computationally estimate the underlying object model.

Computational microscopy - inverse problems

- Reconstruction (dense prediction): $p(\mathbf{x}|\mathbf{y})$ $p(\mathbf{x}|\mathbf{y}) = \frac{p(\mathbf{y}|\mathbf{x})p(\mathbf{y})}{p(\mathbf{x})}$
- Leads to linear / non-linear estimators: $x_e = \arg \min_{x_e} ||y - Hx_e||_2^2 + \lambda \phi(x_e)$
- *H* forward operator, measurement model.
- $\phi(.)$ Prior information on the object (sparsity, non-negativity, support, ...).
- λ Regularization parameter.

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.

LeCun, Y., et al., . "Gradient-based learning applied to document recognition," Proceedings of the IEEE, Nov. 1998. LeCun, Y., Bengio, Y. & Hinton, G., "Deep learning," Nature 521, 436–444 (2015). Schmidhuber, J., "Deep learning in neural networks: An overview. Neural Netw.," 61, 85–117 (2015).

Deep learning microscopy



- Works with standard microscope hardware.
- Towards real time performance,
- Do not use forward models.

Supervised deep network training



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





Convolutional filtering

Filter size (throughout the network) -3×3

$$v_{i,j}^{k,l} = \sum_{r} \sum_{p} \sum_{q} w_{i,j,r}^{p,q} v_{i-q,j}^{k+p,l+q} + b_{i,j}$$

- Activation function Rectified Linear Unit -ReLU(x) = max(0,x)
- Number of learnable parameters ~ 230K

• Number of layers
$$= 13$$

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) \rightarrow (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).

Rivenson, Y., et al., "Deep learning microscopy," Optica 4, 1437-1443 (2017)

Extended depth-of-field and cross-tissue

depth of field $\approx \lambda / NA^2$

• Trained on lung tissue, inferred on kidney tissue (same stain).





Network output ×2

Traine tissue

Network input 40×/0.95NA



Z-stack



Z-stack (100×/1.4NA): $\Delta z = 0.4 \mu m$

• Traine tissue





Extended depth-of-field image (100×/1.4NA)

Traine tissue Network input

40×/0.95NA





ey

Z-stack 00×/1.4NA





Cross tissue and cross staining

• Trained on lung tissue, inferred on breast tissue, with different stain.



Modulation transfer function estimation

• Network trained with lung tissue.





DEEP LEARNING ENHANCED MOBILE-PHONE MICROSCOPY

Rivenson, Y., et al., "Deep learning enhanced mobile-phone microscopy," ACS Photonics (2018), DOI: 10.1021/acsphotonics.8b00146



Resolution ~ 0.87 μ m (half pitch) FOV ~ 1mm²

Challenges in mobile microscopy

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





Benchtop microscope (20×/0.75NA)





Elastic pyramid registration

Benchtop microscope (20×/0.75NA)



Elastic pyramid registration

Smartphone microscope



Elastic pyramid registration

Distortion aligned benchtop microscope image



Smartphone microscope

Network output

Benchtop microscope (20×/0.75NA)



Structural similarity ~ 0.9

 $SSIM(U_1, U_2) = \frac{(2\mu_1\mu_2 + c_1)(2\sigma_{1,2} + c_2)}{(\mu_1^2 + \mu_2^2 + c_1)(\sigma_1^2 + \sigma_2^2 + c_2)}; \mu_{1;2} = E[U_{1;2}]; \sigma_{1;2}^2 = E[(U_{1;2} - \mu_{1;2})^2]; \sigma_{1,2} = E[(U_1 - \mu_1)(U_2 - \mu_2)]; \sigma_{1,2} = E[(U_1 - \mu_1)(U_2 - \mu_2)];$



DEEP LEARNING ACHIEVES SUPER-RESOLUTION IN FLUORESCENCE MICROSCOPY

Nat. Methods 16, 103 (2019)

Super-resolution fluorescence microscopy



- Provide unprecedented access to the inner working of cells and various biological processes
- Often rely on relatively sophisticated optical setups and extensive computational processing of the image data.

Deep learning enables super-resolution



 Major super-resolution techniques introduce extensive photo-toxicity/damage to living samples.¹

Purposed deep-learning-based

approach:

- Network input:
 - Low-resolution image (i.e., captured with low-NA objective)
- Network output:
 - High-resolution image (i.e., captured with high-NA objective)
- Data-driven approach: does not rely on image formation models
- Extended depth-of-field & improved SNR

Training workflow of the neural network model


Fluorescence microscopy super-resolution



Fluorescence microscopy super-resolution



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Quantification



Quantification



Imaging SUM159 cells expressing eGFP labeled clathrin adaptor AP2: TIRF \rightarrow TIRF-SIM imaging



Error analysis with NanoJ-Squirrel toolbox¹

• Minimum differences was observed between the network output and the ground truth images.

Overlay of output and ground truth



Error map (input vs. output)

¹Culley, S. et al. Nat. Methods 15, 263–266 (2018).

Error map (input vs. ground truth)

Spatial frequency spectrum analysis



Spatially-varying PSFs measured by neural network



Network inferred image has extended depthof-field

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



Green and **Yellow** arrows point to features that demonstrate extended depth-of-focus effect.

Synthesized from a z-stack of 34 images with 0.3 µm spacing

Network inferred image has higher SNR



Generalization to new types of samples





DEEP LEARNING-BASED VIRTUAL HISTOLOGY STAINING USING AUTO-FLUORESCENCE OF LABEL-FREE TISSUE

Nat. Biomed. Eng. 1 (2019). doi:10.1038/s41551-019-0362-y

Histopathology

• Histopathology is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope.



Histological staining

• Histochemistry a technique that is used for the visualization of biological structures.



Histochemical staining drawbacks

- Laborious process
- Time consuming
- Expensive (reagents, training, personnel, monitoring)
- Doesn't support tissue preservation for advanced diagnosis
- Staining variation



https://doi.org/10.1186/1746-1596-6-S1-S15

Alternative contrasting methods



- 1. Tao, Y. K. et al. Assessment of breast pathologies using nonlinear microscopy. Proc. Natl. Acad. Sci. 111, 15304–15309
- 2. Fereidouni, F. et al. Microscopy with ultraviolet surface excitation for rapid slide-free histology. Nat. Biomed. Eng. 1, 957–966 (2017).
- 3. Tu, H. et al. Stain-free histopathology by programmable supercontinuum pulses. Nat. Photonics 10, 534–540 (2016).
- 4. Orringer, D. A. et al. Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy. Nat. Biomed. Eng. 1, 0027 (2017).

Interpretability



- 1. Tao, Y. K. et al. Assessment of breast pathologies using nonlinear microscopy. Proc. Natl. Acad. Sci. 111, 15304–15309
- 2. Fereidouni, F. et al. Microscopy with ultraviolet surface excitation for rapid slide-free histology. Nat. Biomed. Eng. 1, 957–966 (2017).
- 3. Orringer, D. A. et al. Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy. Nat. Biomed. Eng. 1, 0027 (2017).

Deep learning-based virtual staining using auto-fluorescence of label-free tissue



Rivenson Y., et al. Virtual histological staining of unlabelled tissue autofluorescence images via deep learning. Nat. Biomed. Eng. 1 (2019). doi:10.1038/s41551-019-0362-y 48

Virtual H&E staining (Salivary gland tissue)

Contrast enhanced unstained tissue DAPI image



Unstained salivary gland tissue DAPI image (network

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



H&E *histologically* stained

salivary gland tissue

Virtual Masson's Trichrome staining (lung tissue)

Contrast enhanced unstained tissue auto-fluorescent image



Unstained tissue autofluorescent image

MT3 *virtually* stained tissue (network output)



MT3 *chemically* stained tissue (brightfield)



Virtual Jones' silver staining (kidney tissue)



Blind assessment by pathologists

Serial number	Tissue,	Pathologist #	Histochemically / Virtually stained	Diagnosis				
	fixation,							
	stain							
1	Ovary,	1	VS	Adenocarcinoma				
	Frozen	2	VS	Borderline serous tumor				
	section,	3	HS	Mucinous adenocarcinoma				
	H&E	4	HS	Adenocarcinoma, endometrioid				
2	Ovary,	1	VS	Benign ovary				
	Frozen	2	VS	Benign ovary				
	section,	3	HS	Normal ovary with corpus luteal cyst				
	H&E	4	HS	Normal				
3	Salivary	1	VS	Benign salivary glands with mild chronic				
	Gland,			inflammation				
	FFPE,	2	VS	Benign parotid tissue				
	H&E	3	HS	Normal salivary gland				
		4	HS	No histopathologic abnormality				
8	Prostate,	1	HS	Prostatic adenocarcinoma 3+4				
	FFPE,	2	HS	Prostatic adenocarcinoma 4+3				
	H&E	3	VS	Prostatic adenocarcinoma, Gleason pattern 3+4				
		4	VS	HG-PIN with cribiforming vs carcinoma				
15	Thyroid,	1	VS	Papillary thyroid carcinoma				
	FFPE,	2	VS	Papillary thyroid ca				
	H&E	3	HS	Papillary thyroid carcinoma				
		4	HS	PTC				

• The analysis of 15 tissue section images by 4 board certified pathologists (who weren't aware of the virtual staining technique) demonstrates 100% non-major discordance, defined as no clinically significant difference in diagnosis between observers.

Stain quality assessment by pathologists

Tissue #	Pathologist 1			Pathologist 2			Pathologist 3				Average					
	ND	CD	EF	SQ	ND	CD	EF	SQ	ND	CD	EF	SQ	ND	CD	EF	SQ
1 – HS	3	2	1	1	4	4	3	4	1	1	1	3	2.67	2.33	1.67	2.67
1 - VS	3	3	3	3	3	3	2	3	2	2	3	3	2.67	2.67	2.67	3.00
2 – HS	3	2	4	4	4	4	3	4	1	2	2	2	2.67	2.67	3.00	3.33
2 - VS	3	3	4	4	4	3	3	3	2	2	3	3	3.00	2.67	3.33	3.33
3 – HS	3	3	2	2	3	3	4	3	1	1	1	1	2.33	2.33	2.33	2.00
3 - VS	3	2	1	1	3	3	1	4	1	1	1	1	2.33	2.00	1.00	2.00
4 – HS	3	2	4	4	3	4	4	4	1	2	1	2	2.33	2.67	3.00	3.33
4 - VS	3	3	4	4	4	3	4	4	2	2	3	3	3.00	2.67	3.67	3.67
5 - HS	3	3	4	4	3	3	2	1	1	3	2	2	2.33	3.00	2.67	2.33
5 - VS	3	2	3	3	3	3	4	2	2	1	3	3	2.67	2.00	3.33	2.67
6 – HS	3	2	3	3	4	4	4	3	2	2	2	2	3.00	2.67	3.00	2.67
6 - VS	3	3	4	3	4	3	4	3	1	1	1	1	2.67	2.33	3.00	2.33
7 – HS	3	3	4	4	3	4	4	3	2	1	2	2	2.67	2.67	3.33	3.00
7 - VS	3	2	3	3	4	4	4	3	2	2	3	3	3.00	2.67	3.33	3.00
8 – HS	3	3	4	4	4	4	4	3	1	1	1	1	2.67	2.67	3.00	2.67
8 - VS	3	2	4	4	4	3	4	4	2	2	3	2	3.00	2.33	3.67	3.33

nuclear detail (ND), cytoplasmic detail (CD) and extracellular fibrosis (EF) and overall stain (SQ) ; 4 = perfect, 3 = very good, 2 = acceptable, 1 = unacceptable

Staining standardization



Liver tissue section 2



Liver tissue section 3











Nat. Biomed. Eng. 1 (2019). doi:10.1038/s41551-019-0362-y

DEEP LEARNING BASED HOLOGRAPHIC IMAGE RECONSTRUCTION AND PHASE RECOVERY

Rivenson, Y.*, Zhang, Y.*, Gunaydin, H., Teng, D. & Ozcan, A. Phase recovery and holographic image reconstruction using deep learning in neural networks. Light Sci. Appl. 7, e17141 55

Coherent imaging systems

• Coherent illumination interaction with a specimen:

$$A_{out}(x, y) = A_0 a(x, y) e^{-j\phi(x, y)}$$

- The propagated wave complex field amplitude allows us to capture all the information about the specimen.
- Optoelectronic sensors are only sensitive to the intensity of light, i.e, phase information cannot be directly acquired.

Phase retrieval via measurement diversity



W. Luo, Y. Zhang, A. Feizi, Z. Gorocs, and A. Ozcan, "Pixel super-resolution using wavelength scanning," Light: Science & Applications (Nature Publishing Group) (2015)

W. Luo, A. Greenbaum, Y. Zhang, and A. Ozcan, "Synthetic aperture based on-chip microscopy," Light: Science & **Applications (Nature Publishing Group) (2015)**

A. Greenbaum, et al, "Wide-field Computational Imaging of Pathology Slides using Lensfree On-Chip Microscopy," Science Translational Medicine (AAAS) (2014) And many others...

Inference results – on chip holographic microscopy (Papanicolaou smear)



Inference results – on chip holographic microscopy (Breast tissue section)



Virtual staining through specimen optical path length



Virtual staining through specimen optical path length





Brightfield image of the H&E chemically stained skin tissue (20×/0.75NA)

Brightfield Holography



Light Sci. Appl. 8, 25 (2019)

Cross-modality deep learning brings brightfield microscopy contrast to holography



Cross-modality deep learning brings brightfield microscopy contrast to holography



3D PSF comparison using 1 µm beads



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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
 - System characterization
 - Virtual staining
 - Virtual propagation

Optica 4, 1437-1443 (2017) Light Sci. Appl. 7, e17141 (2018) ACS Photonics (2018), DOI: 10.1021/acsphotonics.8b00146 Nat. Methods 16, 103 (2019) Nat. Biomed. Eng. 1 (2019). doi:10.1038/s41551-019-0362-y Light Sci. Appl. 8, 23 (2019) Light Sci. Appl. 8, 25 (2019)







