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Intra-operative light-sheet fluorescence microscopy

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1 mm

Dual-color light-sheet microscopy of a human prostate core-needle biopsy



Motivation: pathology has remained unchanged for a century







Goal: non-destructive, slide-free, 'digital' pathology





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Advantages

Fast Digital Non-destructive Slide-free Wide-area



Methods: fluorescence microscopy for imaging human tissues



Surface microscopy

Volumetric microscopy



Fluorescence microscopy method

	MUSE	Structured-illumination	Confocal, Multiphoton	Light-sheet
Optical sectioning	UV light	Patterned demodulation	Physical pinhole	Selective plane illumination

MUSE - Levenson, UC Davis, Structured-illumination - Brown, Tulane, Confocal - Rajadhyaksha, MSKCC, Nonlinear, Fujimoto, MIT



Confocal laser scanning microscopy (CLSM)

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Optical-sectioning



A physical pinhole is used to reject out of focus scattered light.

*Slow due to the need for raster-scanning



Milind Rajadhyaksha, MKSCC (Skin) Abeytunge *et al.*, JBO (2011)



Multiphoton microscopy (MPM)

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Optical-sectioning



A physical pinhole is used to reject out of focus scattered light.

*Nonlinear process confines fluorescence excitation.



James Fujimoto, MIT (Breast) Tao *et al.*, PNAS (2014)



Structured-illumination microscopy (SIM)

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Optical-sectioning



Patterned light is demodulated to reject out of focus scattered light.

*Volumetric imaging not possible

PBS LED + ASL SCMOS

Quincy Brown, Tulane (Prostate, kidney) Schlichenmeyer *et al.*, Biom. Opt. Exp. (2014)



Microscopy with UV surface excitation (MUSE)

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Deep UV excitation light (<300 nm) is rapidly attenuated.

*Volumetric imaging not possible



Richard Levenson, UC Davis (unpublished)



Light-sheet microscopy (LSM)

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Optical-sectioning



Selective plane of illumination.

*Decouples illumination and collection paths

Light-sheet microscopy of human prostate tissue Radical prostatectomy Before surface Prostate slices extraction (3-5 mm thick) Excised prostate 1 cm Fresh prostate slice After surface 2 mm extraction

Jonathan Liu, University of Washington (Prostate, breast) Glaser *et al.*, Nature Biom. Eng. (2017)

1 cm



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Long depth of focus enables rapid 3D imaging

Conventional microscopy

Light-sheet microscopy





Experimental image of human prostate tissue

W

Challenge: light-sheet microscopy is not suited for human tissue

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Challenge

Designed for imaging smaller transparent samples. Human tissues are large + highly scattering.

Intraoperative histlogy: Lightning 3D histopathology, Nature Biomed. Eng. (2017)



Solution: "open-top" light-sheet microscopy

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Solution

Place all optics underneath the scanning plane, opposite the sample being imaged

Intraoperative histlogy: Lightning 3D histopathology, Nature Biomed. Eng. (2017)



Pathology-optimized light-sheet microscope

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Characterization of illumination light sheet

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Lateral resolution from USAF target

Axial resolution



Group 9 element 1 ~ 1.5 μ m resolution

^{*}H&E tissue section is 5 µm



Open-top light-sheet microscope demonstration

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Imaging of a fresh piece of human prostate tissue

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Glaser et al., Nature Biomed. Eng. (2017)

Fresh prostate slice



1 cm

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Acridine Orange



Imaging of a fresh piece of human prostate tissue

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Light-sheet microscopy of prostate tissue



Tilt, ~2 μ m/mm (slope = 0.2%)

Normal prostate glands



Prostate adenocarcinoma









Comparison study for N = 25 fresh human prostate samples

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Light-sheet microscopy Histology 2 92% sensitivity 92% specificity 12 11 \$ 10 10 16-14 13 13 20 21 21 20 17 23 24 25 23 22

1 cm



Imaging of a fresh piece of human breast tissue

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Fresh breast tissue



5 mm

H&E



5 mm



Glaser et al., Nature Biomed. Eng. (2017)

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Imaging of a fresh piece of human breast tissue

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Invasive ductal carcinoma with adjacent normal breast tissue



Adipose tissue



Benign breast lobules





Dual-color imaging of PpIX in a human brain tumor

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False-colored H&E imaging using light-sheet microscopy

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DRAQ5 and Eosin dual-channel fluorescent staining and imaging of a human prostate biopsy



Elfer et al., PLoS One, (2016), Giacomelli et al., PLoS One, (2016)



Dual-color 3D imaging of a human prostate core-needle biopsy





Dual-color 3D imaging of a human prostate core-needle biopsy

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Clearing and staining time: <1 hour in 60% TDE Imaging time: ~10 min **Tissue size**: ~0.1x0.1x2.2 cm Resolution: 1.25 µm/pixel

Stains DRAQ5 (nuclear) **Eosin** (cytoplasmic)





Overview of clinical applications at the University of Washington

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Real-time intraoperative guidance

• Breast, prostate, brain, skin ...





10 mm

3D pathology of biopsies

• Prostate, breast, kidney, lung, bone ...









Future directions at the University of Washington

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Summary of intra-operative fluorescence microscopy

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Surface microscopy

Volumetric microscopy



Fluorescence microscopy method

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