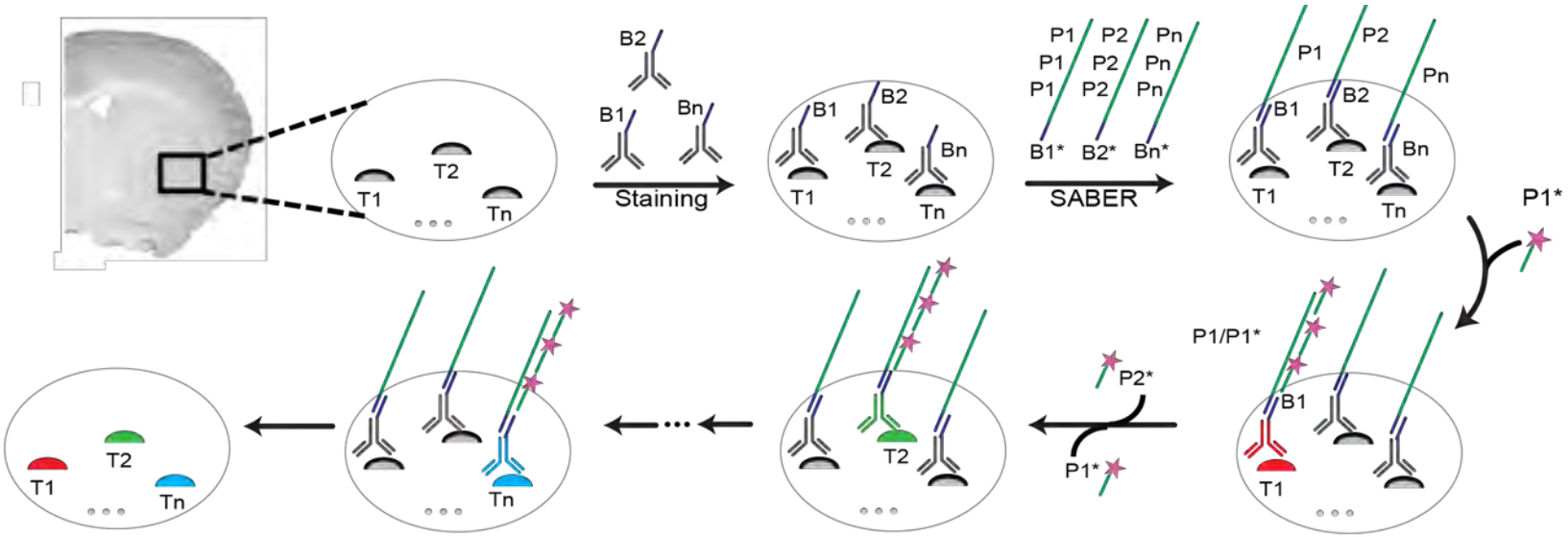
High-Throughput, Highly Multiplexed, In Situ Proteomic Imaging of Human Tissues

Harvard TTD

Peng Yin Lab

http://molecular.systems

SABER: DNA-reagent based method for highly multiplexed & amplified tissue imaging



1. Highly multiplexed (spectrally unlimited)
2. Amplified signal: high sensitivity and throughput
3. Applicable to proteins, RNA/DNA; FFPE and fresh-frozen tissues
4. Accessible: DNA-reagents based, compatible with common imaging platforms and workflow


Sinem Saka


Yu Wang


Josie Kishi

Kishi*, Beliveau*, Lapan*, West*, … Cepko+, Yin+ ***Nature Methods.*** 2019

Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

Project updates

- Immuno-SABER has been published in Nature Biotechnology

✓applied the method on fixed cryosections, whole mount preparations, FFPE human tissues
✓implemented simultaneous linear and branched amplification for tuning the signal level
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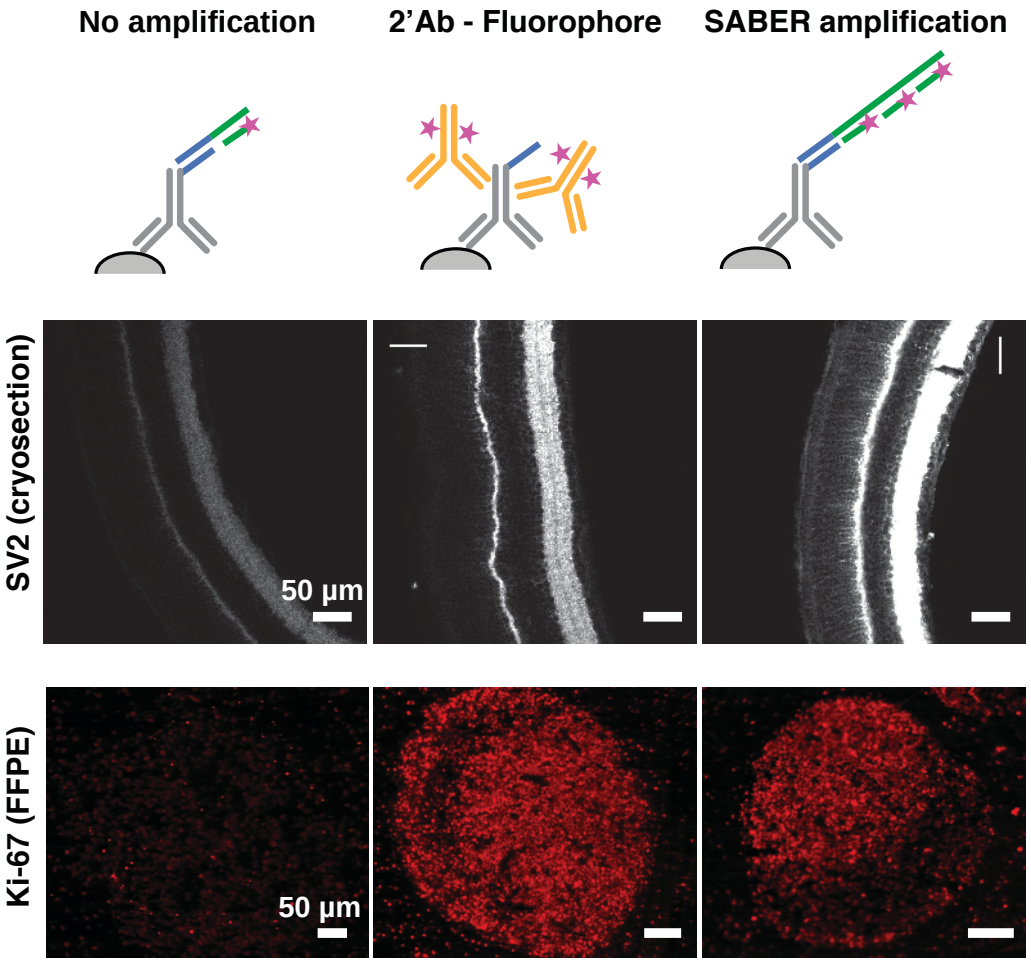
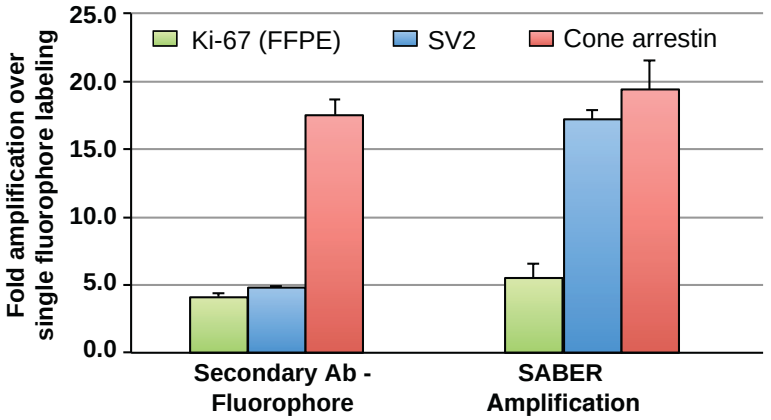
Kishi*, Beliveau*, Lapan*, West*, … Cepko+, Yin+ ***Nature Methods.*** 2019

Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

Immuno-SABER

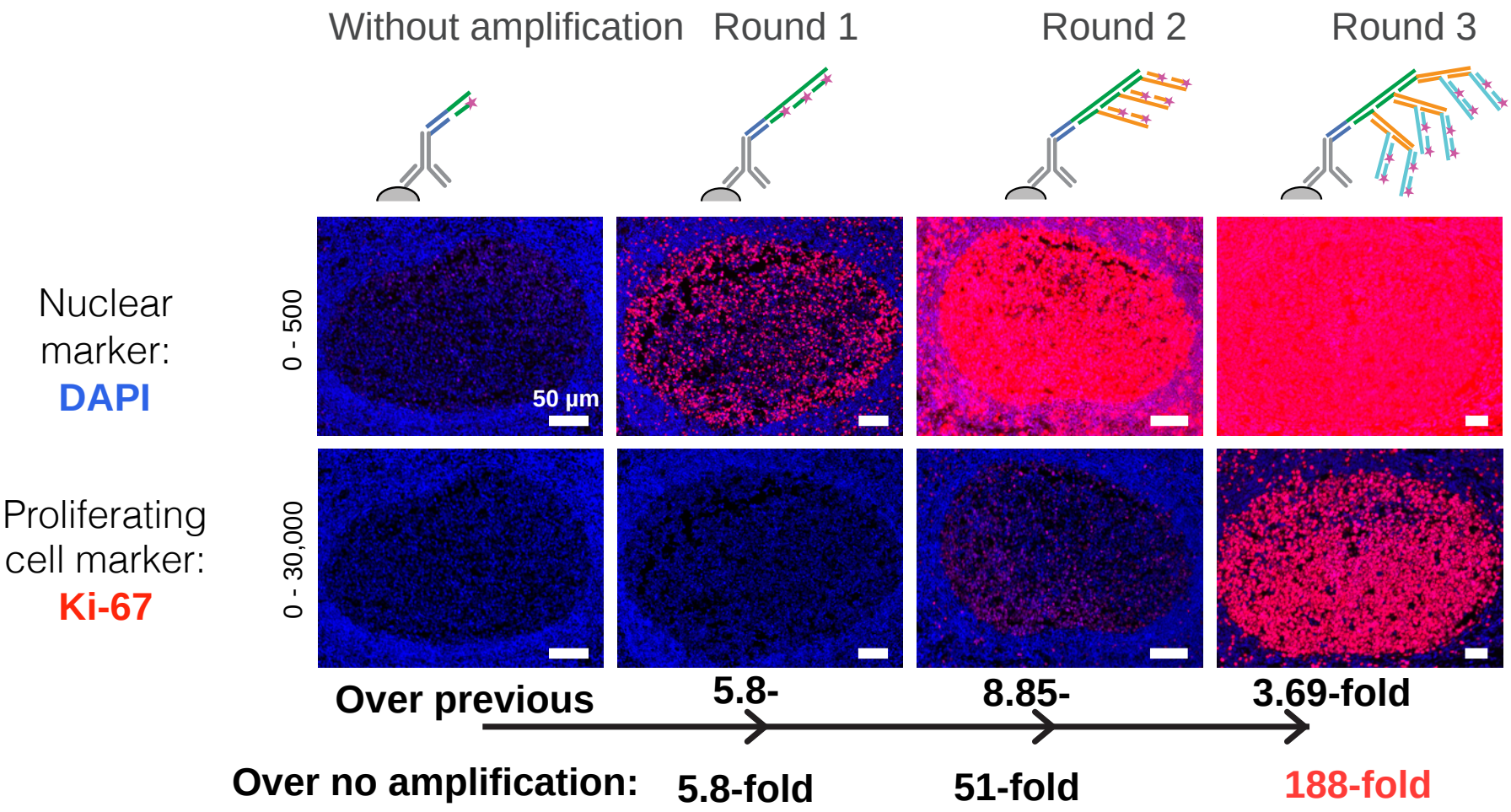
Quantification of SABER signal amplification in tissues



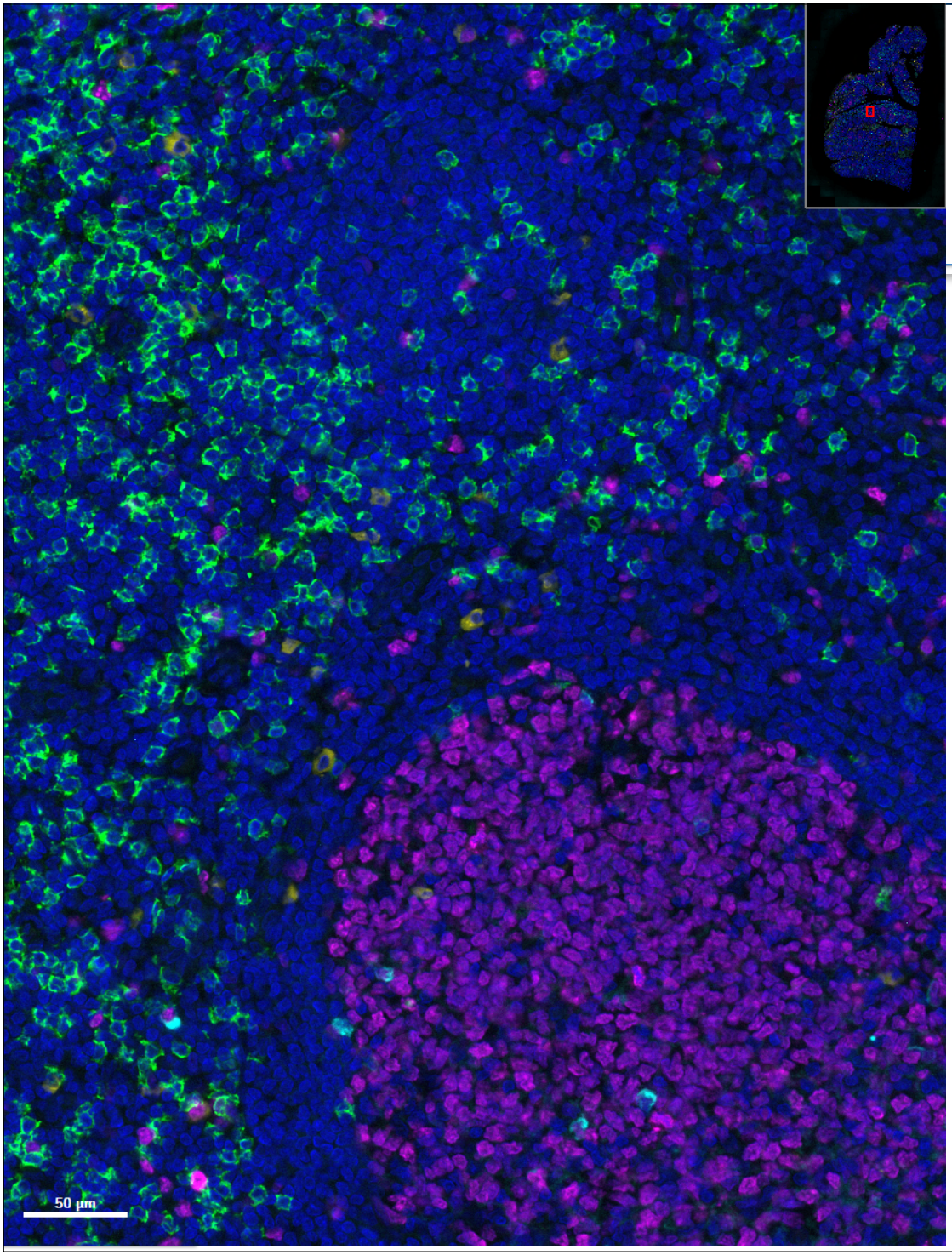


Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

Branching

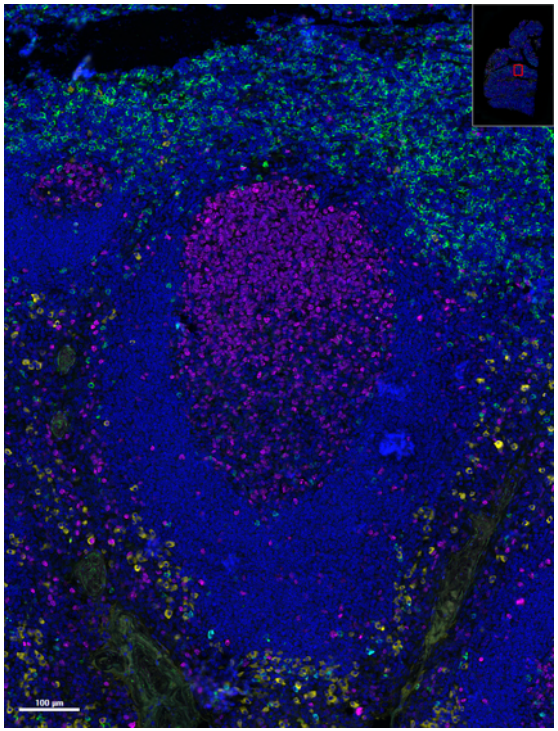


Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

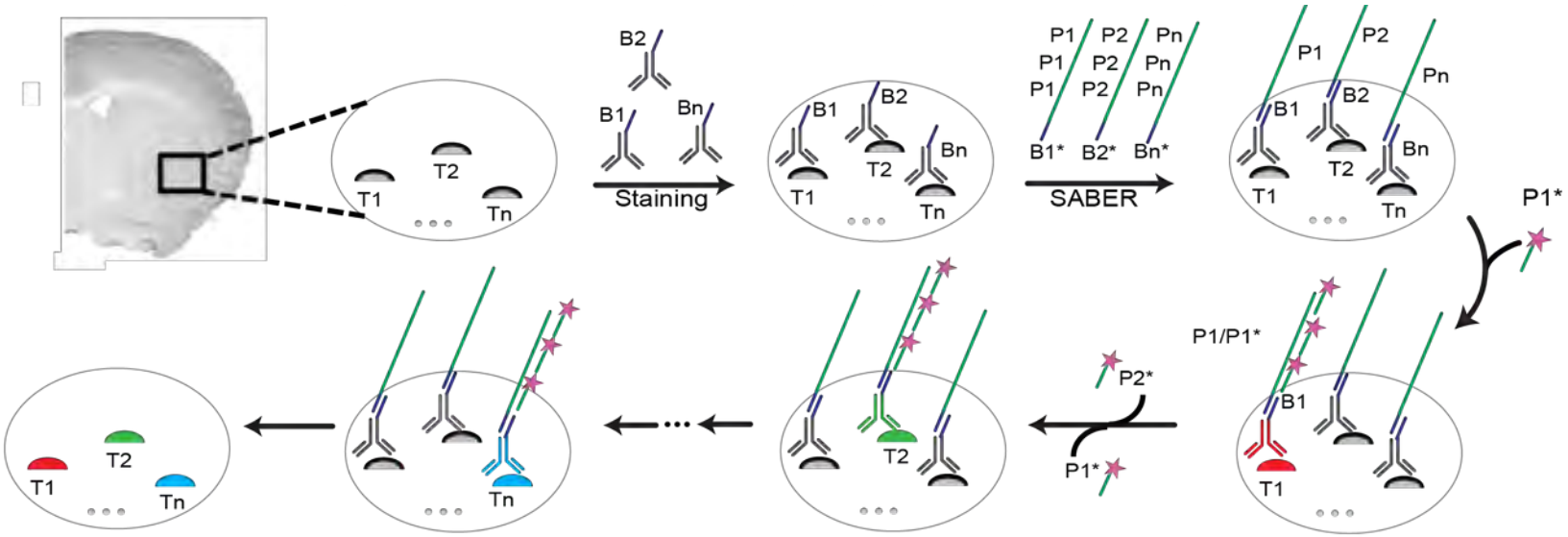


IgA **CD8a** **Ki67** **IgM**

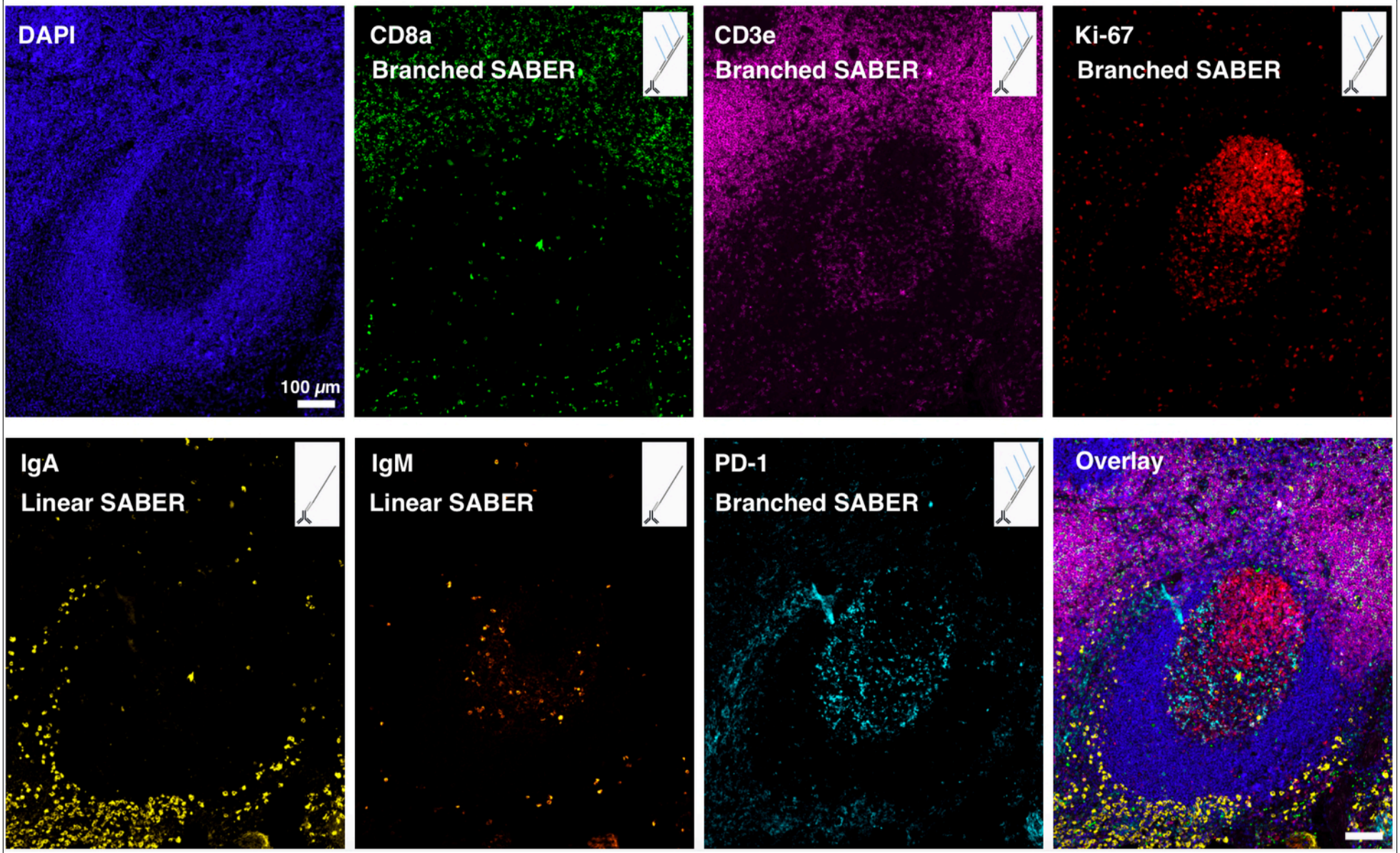
Fluorophore	Target	Exposure time
ATTO488	IgA	1.22 ms
ATTO565	CD8a (branched)	6.69 ms
Alexa647N	Ki67 (branched)	2.66 ms
Alexa750	IgM (Fab2)	18.8 ms



Exchange-SABER

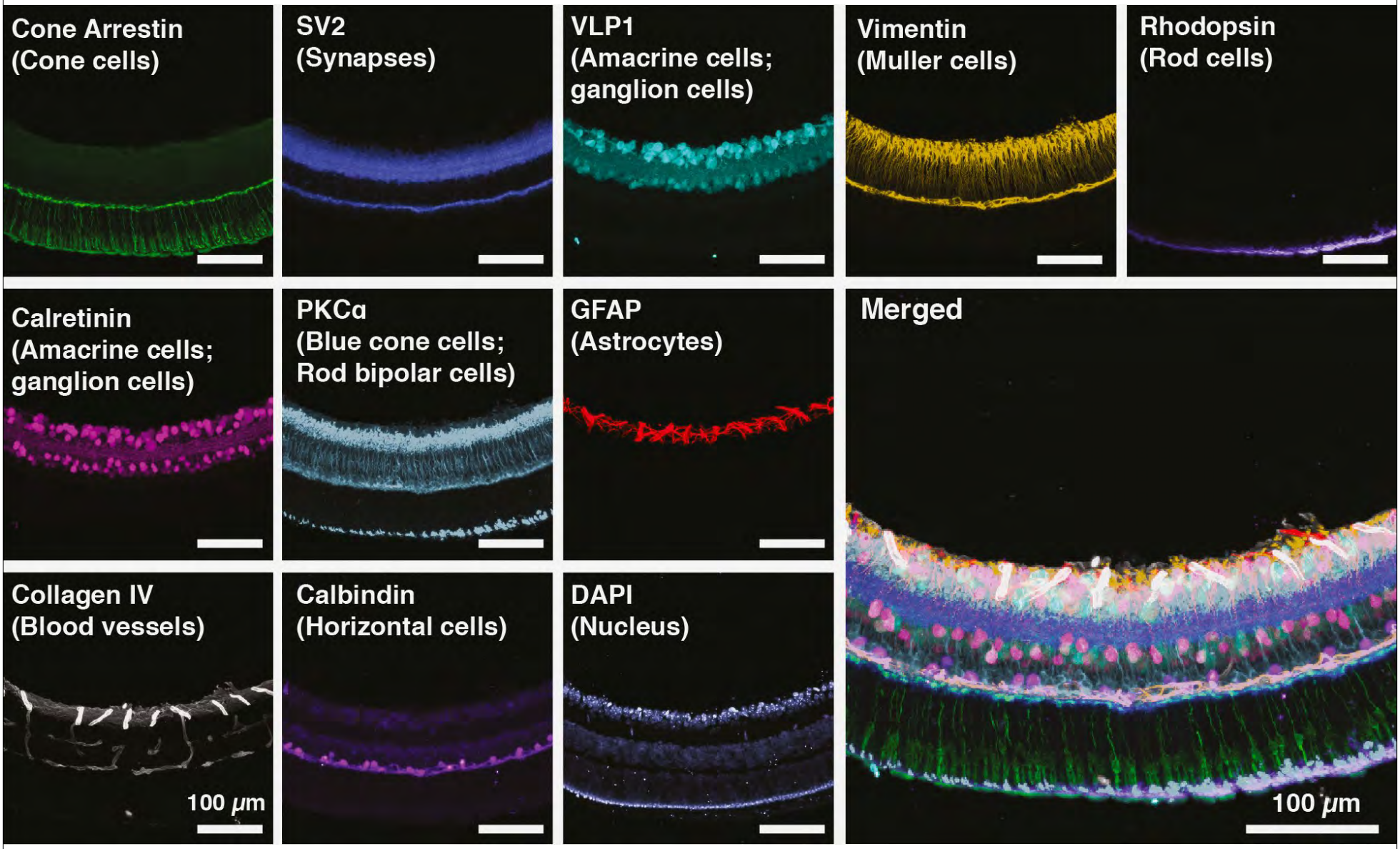


Exchange-SABER: 7-color imaging of FFPE human tonsils



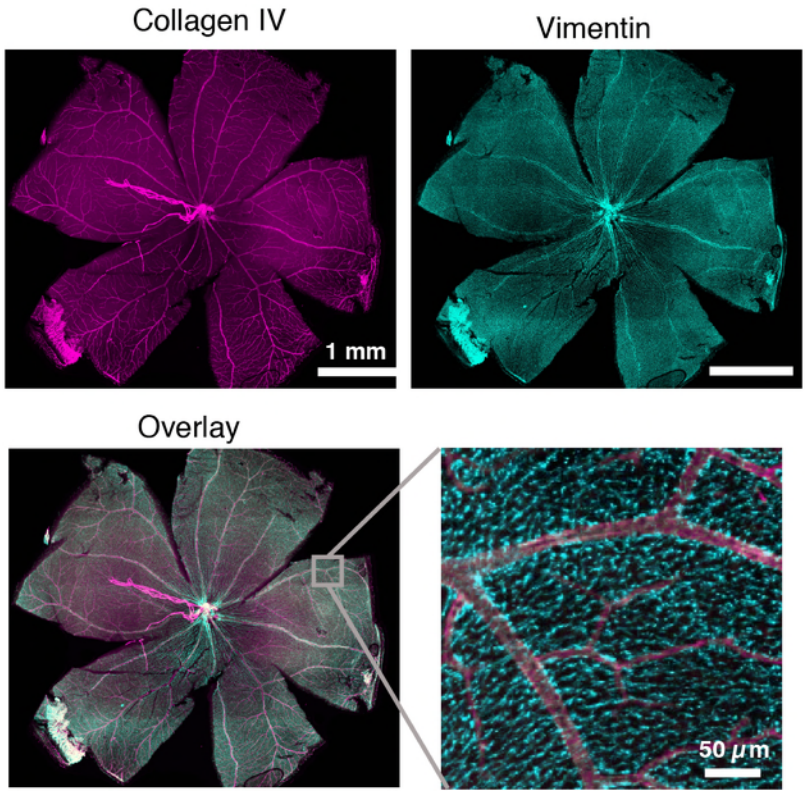
Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

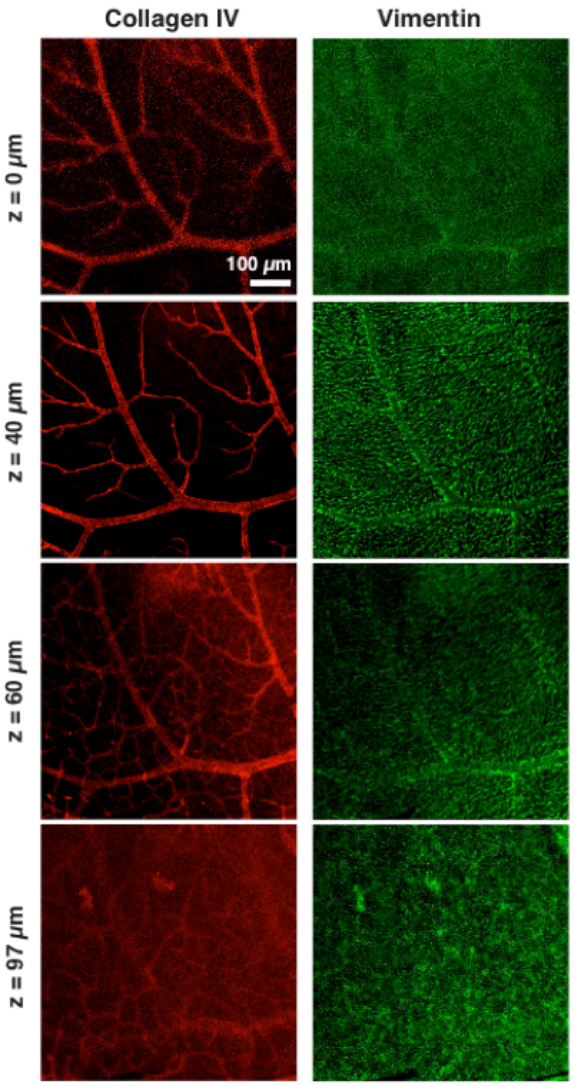
10-color imaging in mouse retina cryo-section



Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

Penetration of SABER concatemers into whole mount mouse retina

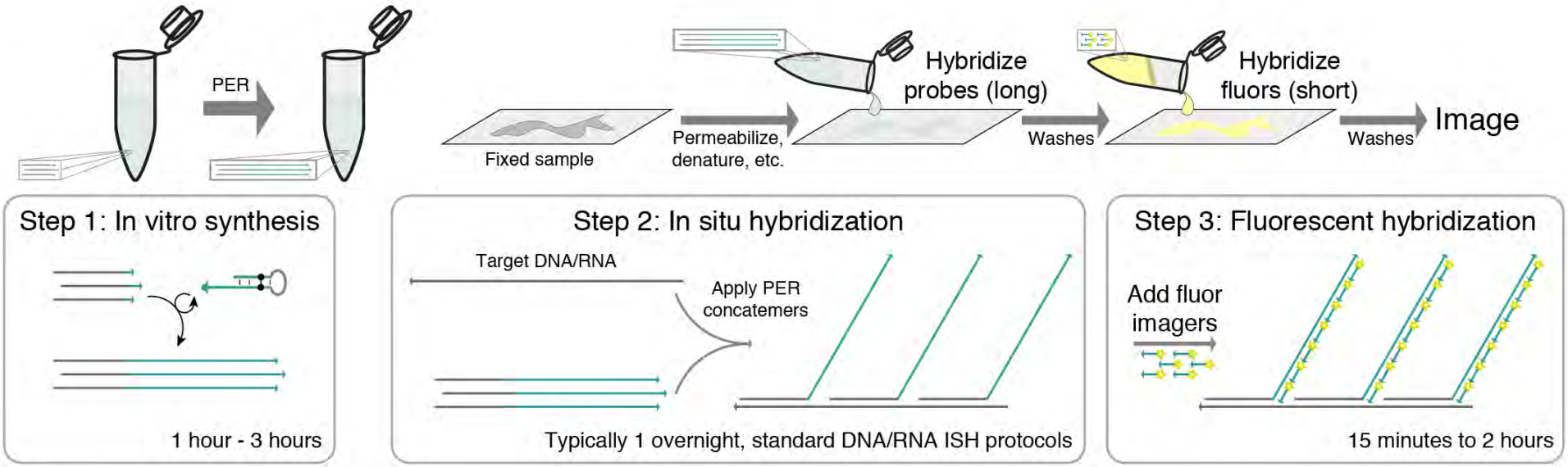




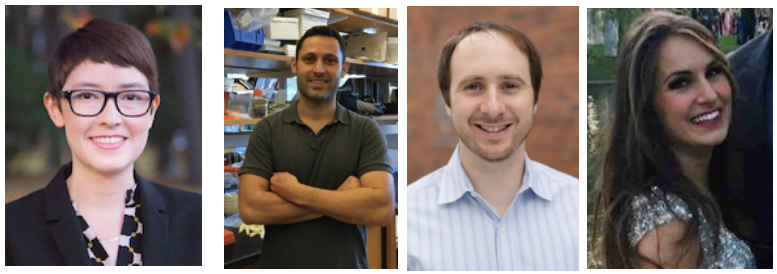
Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

SABER-FISH

We are using this concatemerization as a method for signal amplification



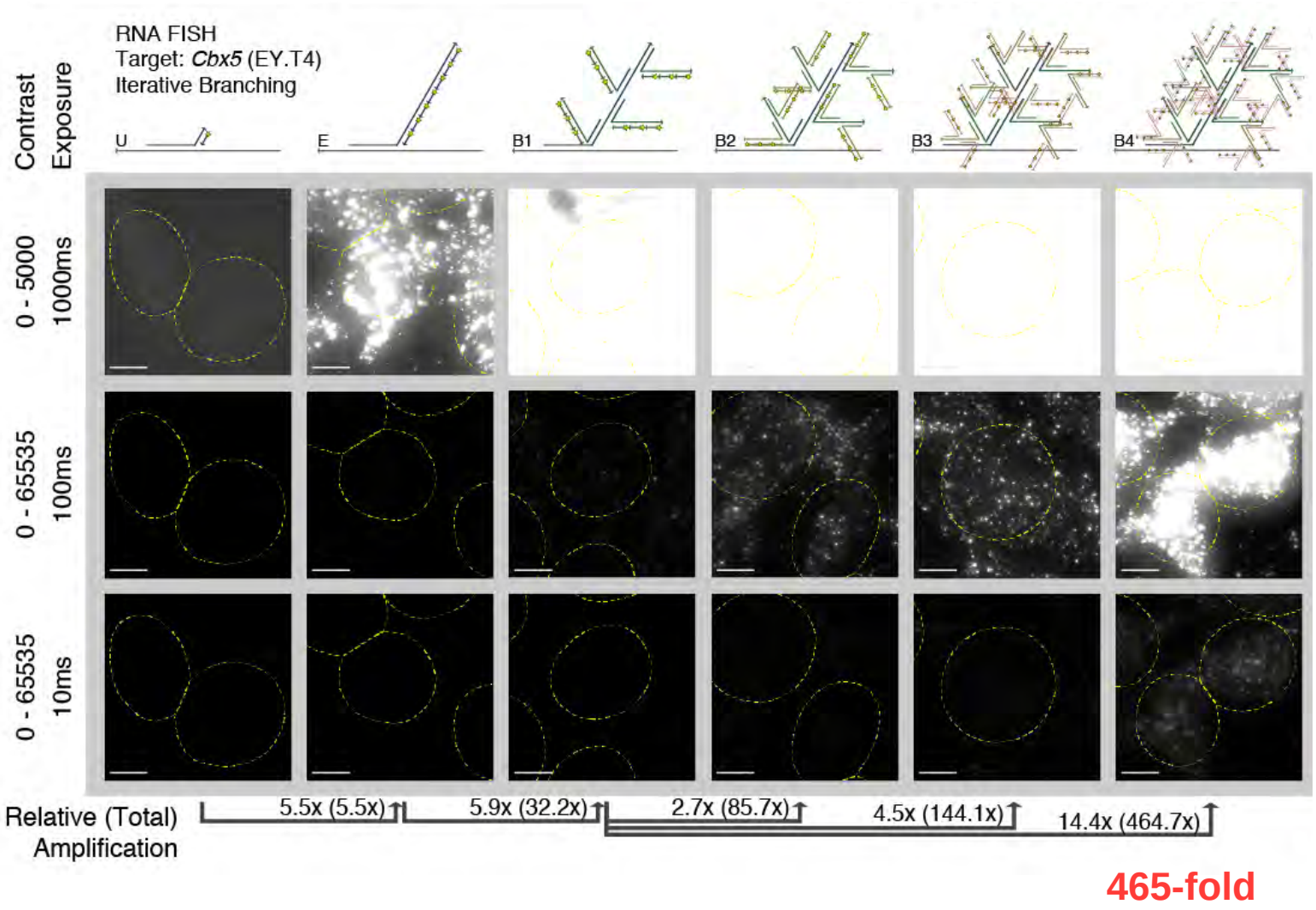
(Light) Signal Amplification By Exchange Reaction

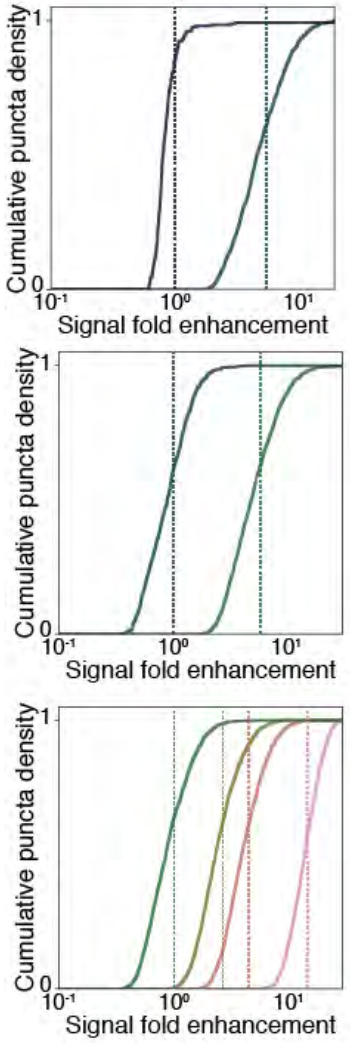


Jocelyn Kishi | Sylvain Lapan (Cepko lab) | Brian Beliveau | Emma West (Cepko lab)

Kishi*, Beliveau*, Lapan*, West*, … Cepko+, Yin+ **Nature Methods.** 2019

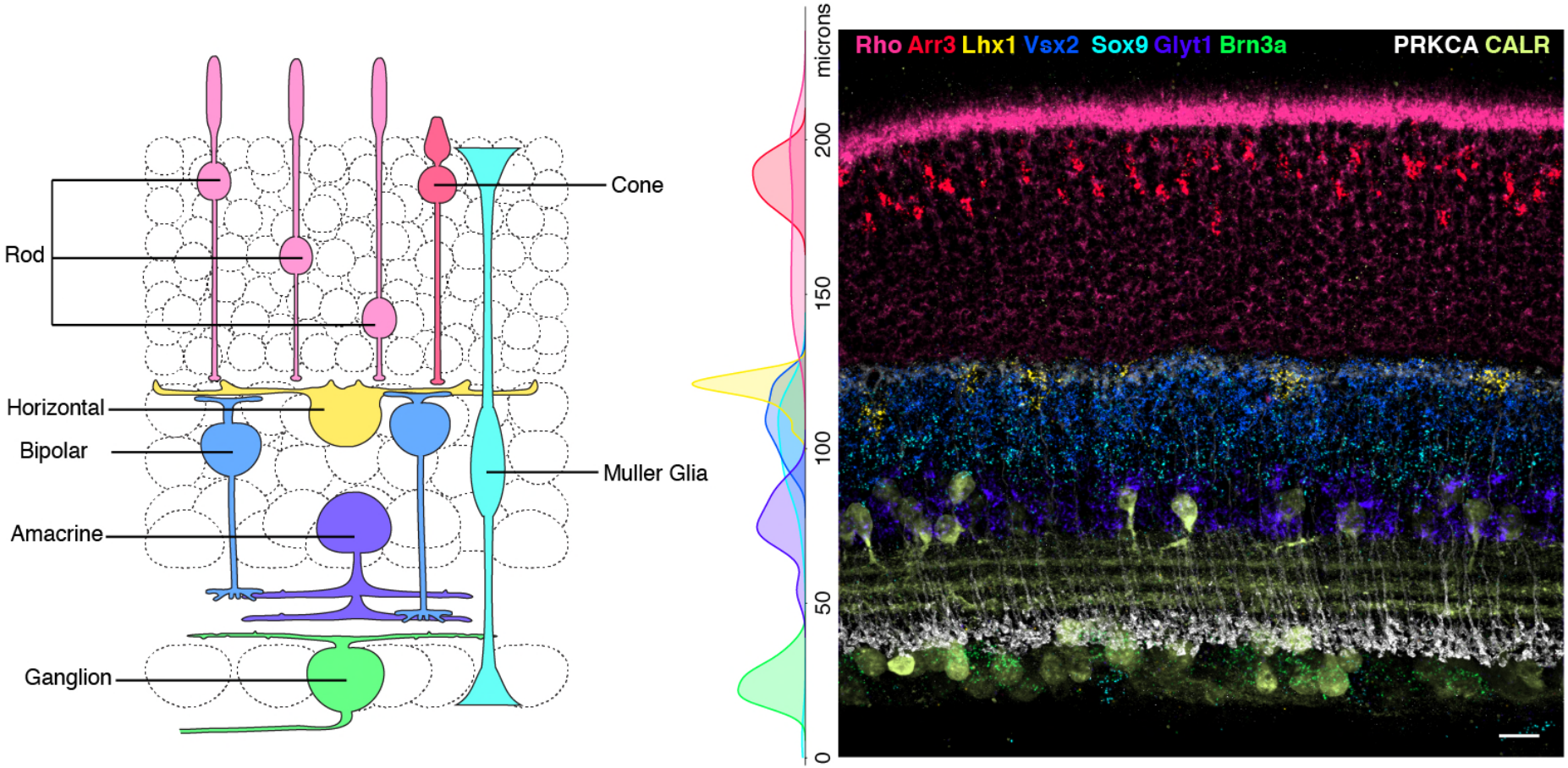
Branched SABER





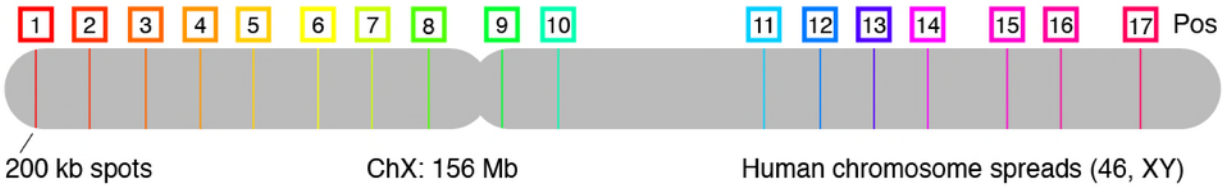
[2] Kishi*, Beliveau*, Lapan*, West*, et al. *Nature Methods.* 2019

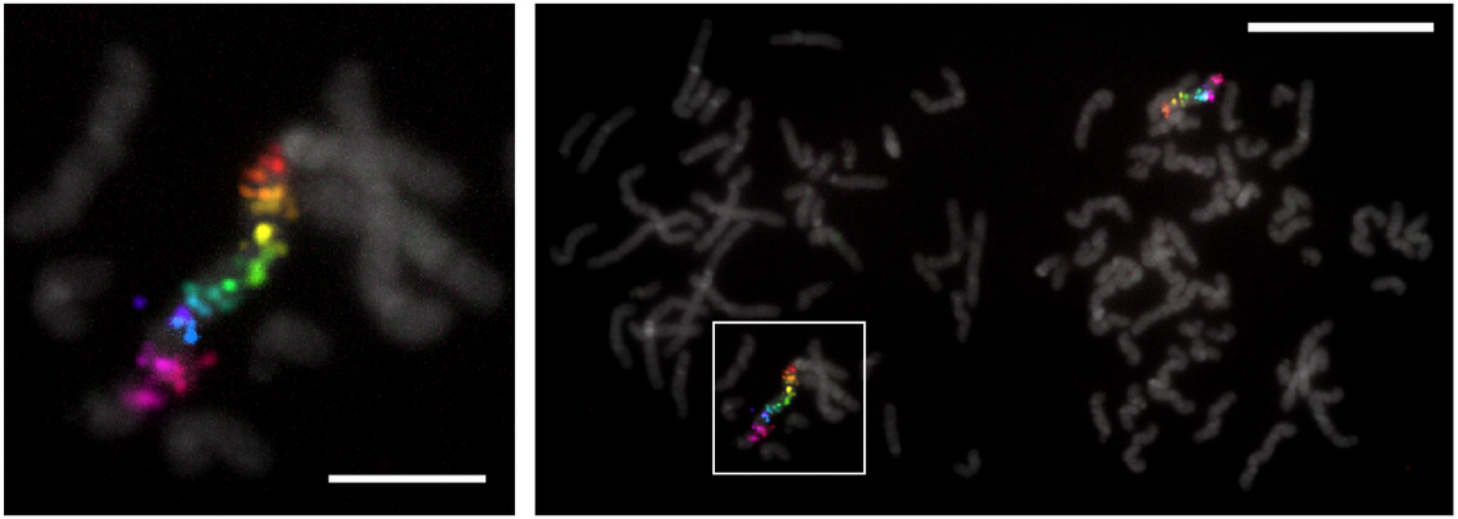
Exchange-SABER increases multiplexing level



Kishi*, Beliveau*, Lapan*, West*, … Cepko+, Yin+ **Nature Methods.** 2019

Exchange-SABER for DNA-FISH





17-plex simulatneou amplification

Kishi*, Beliveau*, Lapan*, West*, … Cepko+, Yin+ **Nature Methods.** 2019

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Saka*, Wang*, …., Yin. ***Nature Biotech,*** *2019*

http://saber.fish

http://immuno-saber.net

Next year deliverables

- Optimized protocols
  - Antibody conjugation,
  - Concatemer generation
  - SABER staining
- List of orthogonal antibody barcodes and amplifier sequences
- List of antibody clones that are validated (mostly immune markers for human FFPE tissues)
- 20-30 target multiplexed dataset for human tonsil FFPE's
- Publicly available software tools

Collaboration projects

Sample Type	Group (Institution)	Application	Status
Mouse lung tissues (FFPE or frozen)	Pehr Harbury & Tushar Desai (Stanford)	Combining SABER with active electrophoretic reagent delivery for volumetric multiplexed imaging	- exchanged protocols and know-how - cross-validating the methods
Human kidney tissues (FFPE or frozen)	Sanjay Jain (Uni Washington) & Kun Zhang (UCSD)	Comparative/ correlative analysis with SABER-FISH and scRNA-Seq	- testing SABER-FISH and Immuno-SABER on cryosections from Sanjay to validate the compatibility - designing FISH probes and preparing antibody-oligo conjugates
Mouse uterine tissues (frozen)	Julia Laskin (Purdue) & Kristin Burnum-Johnson (Pacific Northwest National Laboratory)	Comparative/ correlative analysis of SABER with imaging mass spectrometry	- planning the workflow details - settling on the targets based on Nano-DESI/ nanoPOTs results

Barriers:

- Distance between labs
- Lack of expertise with the tissue type, preparations, markers, protocols
- Time investment
- Difficulty to develop the project scope within the time and staff limitations

some cool science ... or tech ...

What can DNA do for imaging

1. SABER for signal amplification

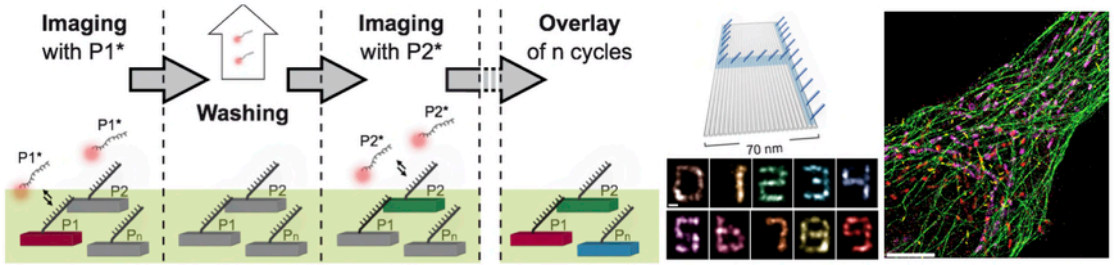
- Immuno-SABER: multiplexed & applied protein
- SABER-FISH: multiplexed & applied DNA/RNA

2. DNA-Exchange for rapid sequential multiplexing

- Exchange-PAINT (2014), super res
- DNA-Exchange-Imaging (2017), super+diff. res
- Exchange-SABER (2019), amplified
- Exchange-Expansion-SABER (2019), amplified & expanded

DNA-Exchange for rapid multiplexing

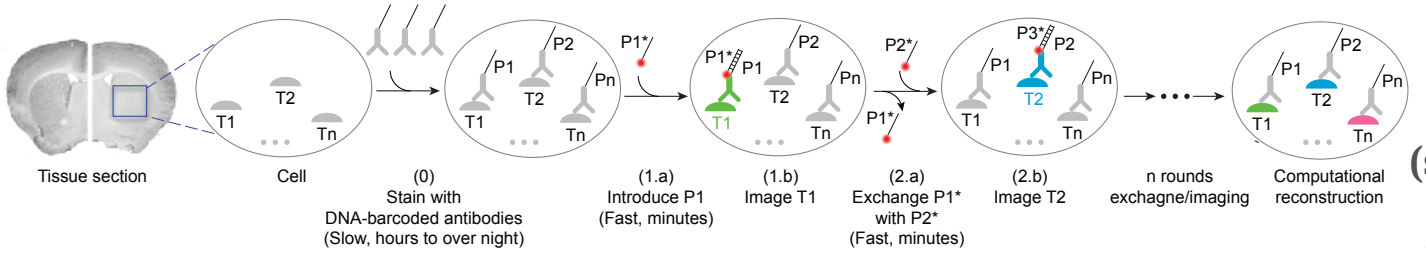
Exchange-PAINT (2014)



Super-res, 10 nm
Transient binding

Jungmann et al ***Nature Methods*** (2014)

DNA-Exchange-Imaging (2017)

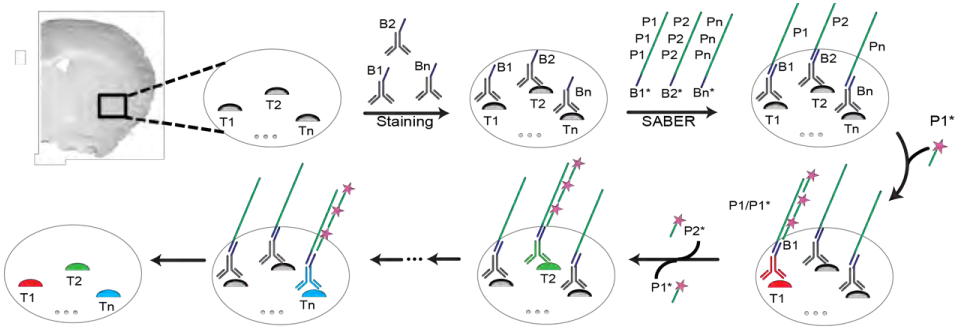


Super+standard res
(semi-)Stable binding

Exchange-STORM/SIM/STED
Exchange-Confocal

Agasti et al ***Chemical Sciences***, 2017, Wang et al ***Nano Letters***, 2017

Exchange-SABER (2019)



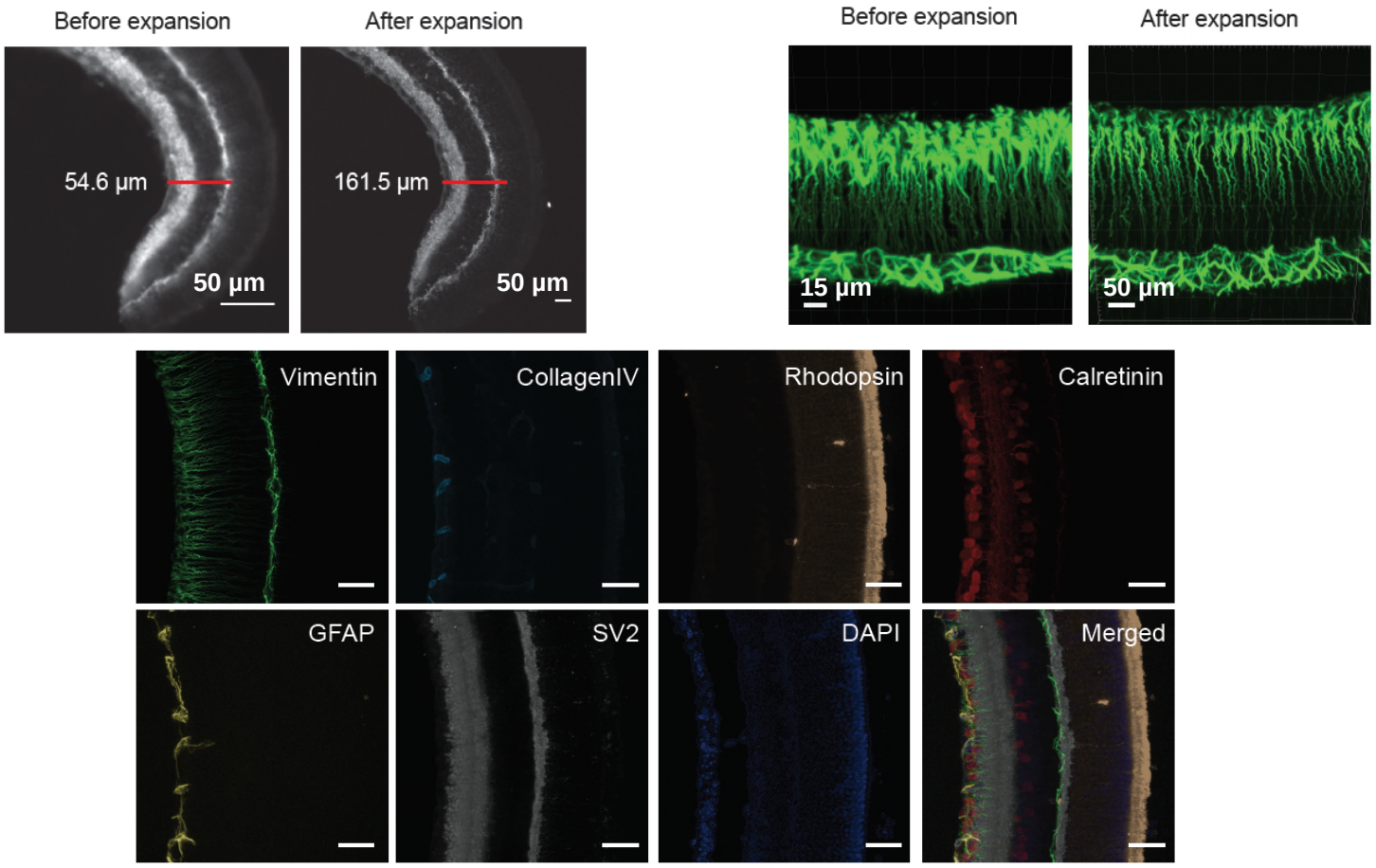
Amplified
Long concatamer

Kishi et al ***Nature Methods.*** 2019; Saka et al ***Nature Biotech*** 2019

DNA-Exchange for rapid multiplexing

Exchange - Expansion - SABER (E²-SABER)

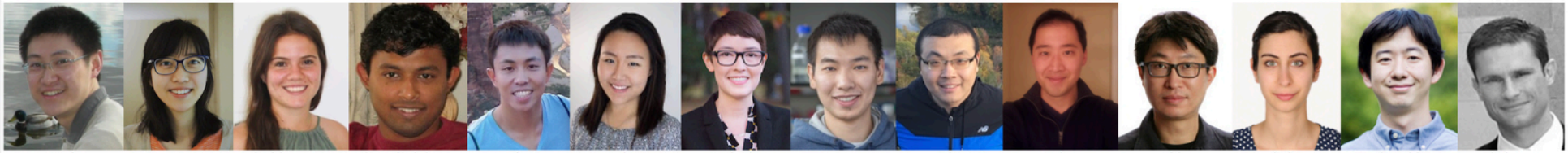
high multiplexing & resolution & throughput



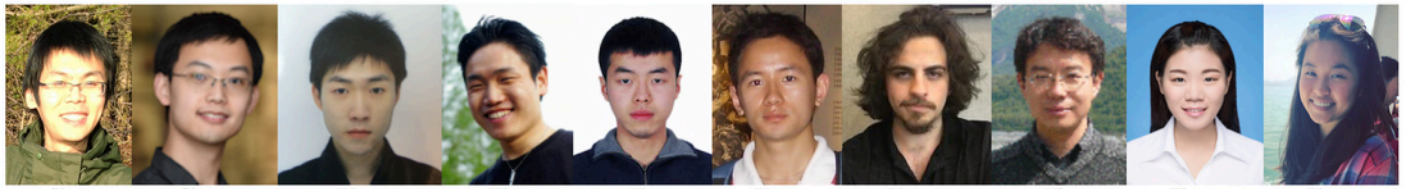
Saka, Wang et al ***Nature Biotech*** 2019

Molecular Systems Lab at Harvard

http://molecular.systems



Dai Fan Goldaracena Gopalkrishnan Jin Kim Kishi Lun Liu Liu Oh Saka Sasaki Schaus



Shen Sheng Wang Woo Xu Xuan Yaseen Yin Zhang Zheng

Alumni

Zhang (Asst. Prof. Rice Univ.)
Ke (Asst. Prof. GIT & Emory)
Wei (Asst. Prof. Tsinghua Univ.)
Jungmann (Group Leader MPI Biochem)
Green (Asst. Prof. ASU)
Agasti (Asst. Prof. JNCASR)
Hariadi (Asst. Prof. ASU)
Sun (Asst. Prof. Peking Univ.)
Kim (Asst. Prof. POSTECH Univ.)
Han (Asst. Prof. Beijing Univ. of Chinese Medicine)
Beliveau (Asst. Prof. Univ. of Washington)

Garreau, Chen, Manesse, Soundararajan,Wang, Ong, Myhrvold, Avendano Sadowski, Marblestone

Funding

Wyss Institute for Biologically Inspired Engineering

National Institute of Health
New Innovator Award
Transformative Research Award
Pioneer Award
HuBMAP Award
NIGMS, NIBIB, OD

National Science Foundation
Faculty CAREER Award
Expedition in Computing Award
CISE, NanoEngineering
CISE, Scalable NanoEngineering

Office of Naval Research
YIP Award
Biomat & Bionnaotech

Defense Advanced Research Projects Agency
Young Faculty Award
Living Foundries

Army Research Office
Biochemistry

Air Force Office of Scientific Research
Multidisciplinary University Research Initiative

Collaborators

Kwon An
Mark Bathe
Ed Boyden
Constance Cepko
George Church
Jim Collins
Kurt Gothelf
Ali Khademhosseini
Pascal Kaeser
Haitao Liu
Niles Pierce
William Shih
Pam Silver
Peter Sorger
Michael Strano
Hao Yan
Erik Winfree
Ting Wu

Disclosure

Ultivue, Inc.
(Co-Founder, Director)

NuProbe Global
(Co-Founder, Director)