

#USEPGP Hackathon! 20-22 July 2018



Match Phenotype with GenomeID/Name

Nathan, Olivia, Katie, Nancy, Brad, Matthew,
SarahE, SarahZ, SashaZ, Jie, Ahmet,
Ward, Keldin, Mike

PGP Hackathon!

20-Jul-2018 9 AM HMS, George Church

v.ht/PHNc

----- Reading ----- Writing ----- ArithmEL -----

.gov

.edu

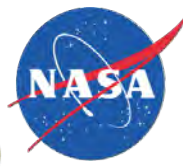
.org

.com

----- Reading ----- Writing ----- ArithmELSI -----



National Heart and Lung and Blood Institute



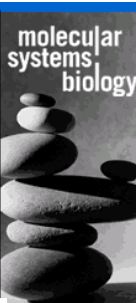
PersonalGenomes.org



Oppenheimer Foundation



Lipper Foundation



Hackathons

Openbsd.org/hackathons.html **First**

c99: Focused on Internet Protocol Security IPSec

Jun 4 - 6, 1999 Calgary, Alberta

10 developers

Funded by Theo de Raadt.

Open.nasa.gov/blog/worlds-Largest-hackathon

Apr 19, 2013 in 83 cities

8200 participants

83 consecutive hours of hacking

THANK YOU !

PersonalGenomes.org



Jeantine Lunshof

Jason Bobe,
Madeleine Ball, Pete
Estep, Mike Chou,
Alex Hoekstra



Sasha Zaranek, Tom
Clegg, Mike Chou,
Ward Vandewege,
Caryn Saitz, Celia
Walden



London:
Stephan Beck



Vienna:
Christoph Bock



Toronto:
Steve Scherer

PersonalGenomes.org & OpenHumans.org

Board of Directors



Misha Angrist, John
Cammack, Esther
Dyson, Juan
Enriquez



Michelle Meyer,
Ryan Phelan
Steve Keating
Dan Vorhaus, John
Halamka



Dana Lewis
James Turner
Chris Gorgolewski
Mad Ball (Director)

Brave New World: 1932 - 2018 - 2540

Aldous Huxley (Thomas, Andrew, Julian) 1932

Science and the Future, J. B. S. Haldane, 1924

Мы (We) Yevgeny Zamyatin, 1921

- **Genome Engineering**
- **Ectogenesis**
- **Neuroengineering**

Farahany et al. (2018) The ethics of experimenting with human brain tissue. Nature.

Aach J, Lunshof J, Iyer E, Church GM (2017) Addressing the ethical issues raised by synthetic human entities with embryo-like features. Elife

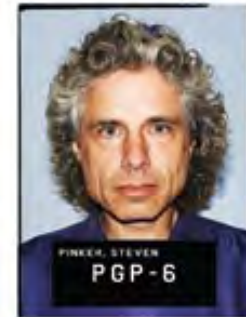
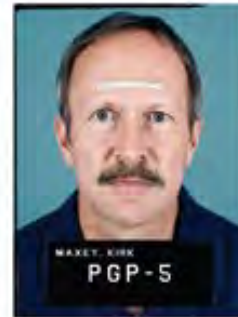
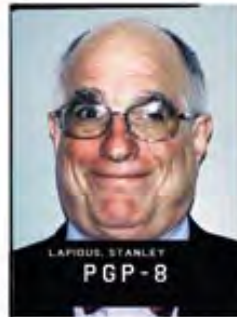
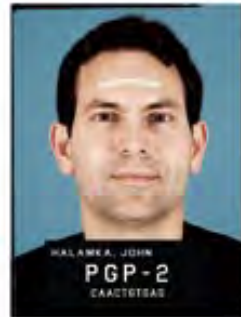
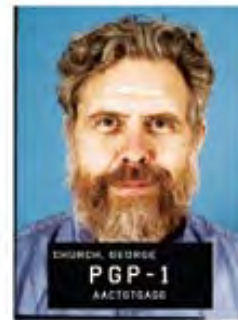
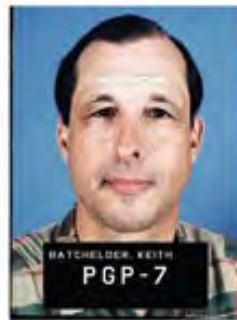
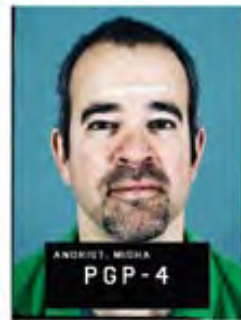
arep.med.harvard.edu/gmc/ELSI_gc.html & pgEd.org

30-Aug-2005 IRB Approval
28-Jun-2006 Personal Genetics Tour
Toronto, Ann Arbor, NYC ...

PGP#5



18-Jul-2007 Personal Genome Project Boston



27-Apr-2010 GET Cambridge MA



27-Apr-2011 GET Philadelphia



25-Apr-2012 Genomes Environments Traits (GET) Boston



25-Apr-2013 Genomes Environments Traits (GET) Boston (& Toronto)



29-Apr-2014 **GET** Labs Boston (Toronto, London)



3 May **2008** Korea

12 Sep **2015** PG-Palooza! Mind First Cambridge, MA

17-19 Sep **2015** GET Global, Vienna

25-26 Apr **2016** GET Expo Boston GETy Awards

3 Nov **2017** Fudan China

4-5 Jun **2018** CIFAR GETx Toronto

20-Jul-2018 PGP Hackathon !

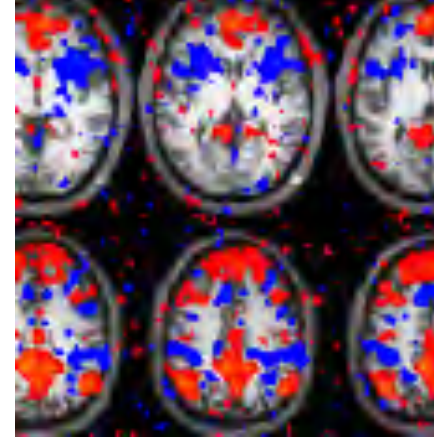
HMS Boston, MA



21-Jul-2018



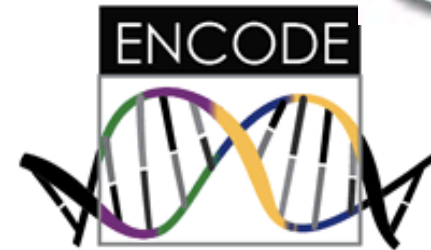
World's only fully open access human
Genomic, Environmental, Trait data & cells



NIST + FDA + PGP = Genomeinabottle.org
Standards.



NIH ENCyclopedia Of DNA Elements
11 isogenic cell types.



Critical Assessment of Genome Interpretation



US, Canada, UK, Austria, (China, Korea, India)

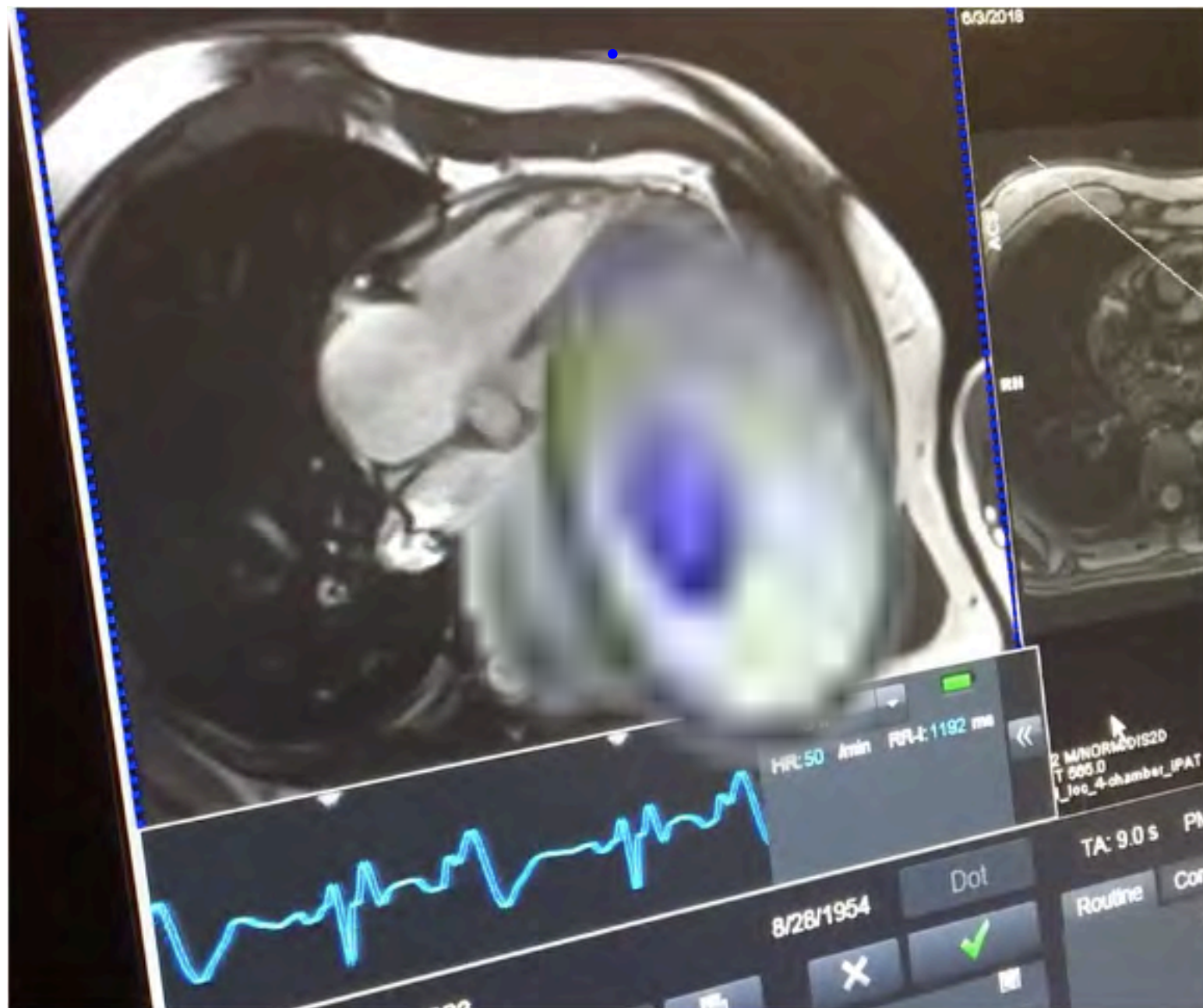
Parametrichuman.org

3-Jun-2018

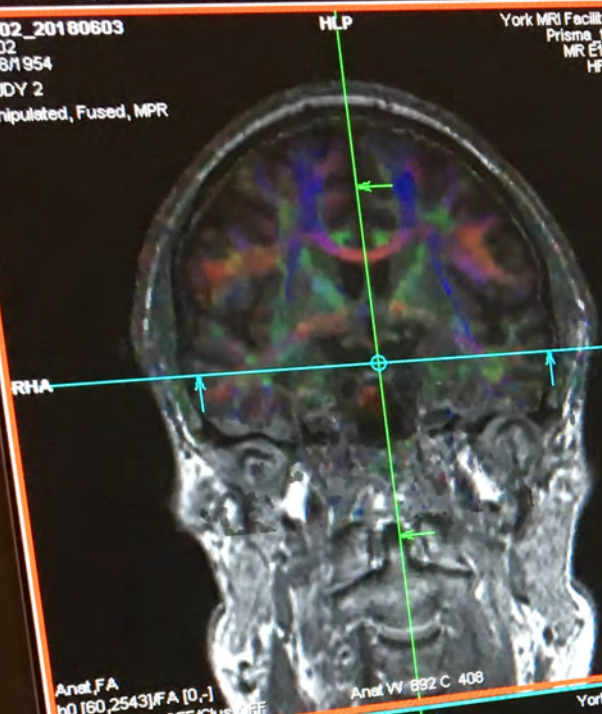


Azam Khan,
Autodesk
York MRI



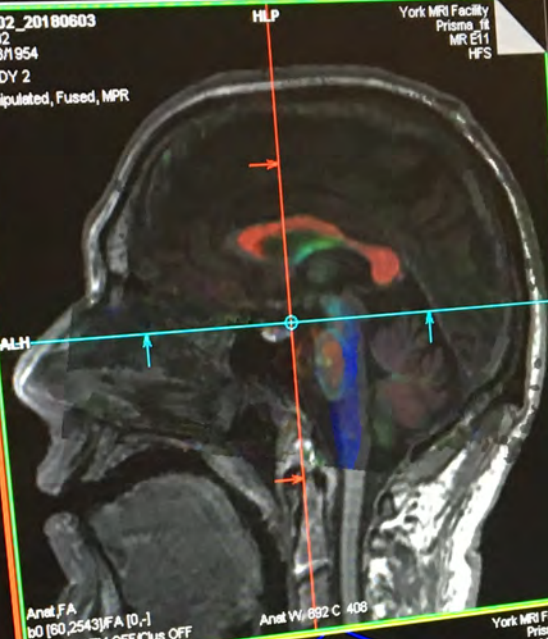


PHP02_20180603
PHP02
*8/28/1954
STUDY 2
Manipulated, Fused, MPR



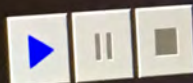
York MRI Facility
Prisma, ft
MR E11
HFS

PHP02_20180603
PHP02
*8/28/1954
STUDY 2
Manipulated, Fused, MPR



York MRI Facility
Prisma, ft
MR E11
HFS

PHP02_20180603
Study



PHP02_20180603
PHP02
*8/28/1954
STUDY 2
Manipulated, Fused, MPR



York MRI Facility
Prisma, ft
MR E11
HFS

PHP02_20180603
PHP02
*8/28/1954
STUDY 2
Manipulated, Fused, 3D



York MRI Facility
Prisma, ft
MR E11
HFS



Neuro 3D

6/3/2018 8:52:14 PM

Stimu=FL SAR=NM Diffusion Tensor images loaded successfully

PGP Research: Re-identification

Harvard Professor Re-Identifies Anonymous Volunteers In DNA Study
(Forbes, 25-Apr-2013) Latanya Sweeney



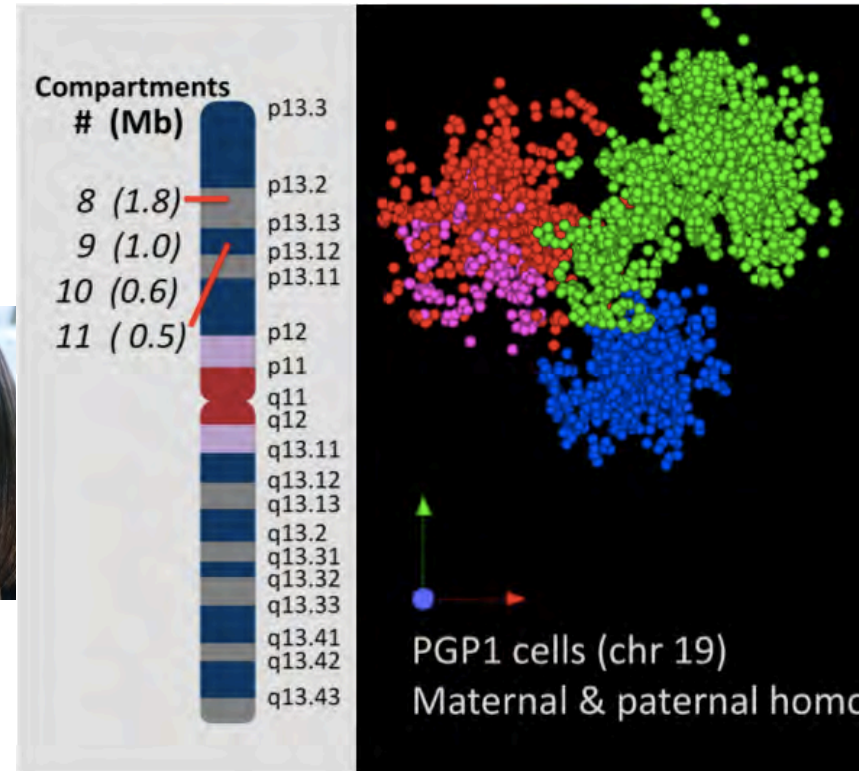
Scientists Discover How to Identify People from Anonymous Genomes
(Wired, 17-Jan-2013) Yaniv Erlich



PGP-Read: haplotyping & 3D structure

v.ht/fh76s

- 1A. Drmanac et al. (2010) Genome Sequencing Using Unchained Base Reads ... **Science**.
- 1B. Peters BA, et al. (2012) Accurate whole genome sequencing & haplotyping. **Nature**
- 1C. Lo et al (2013) On the design of clone-based haplotyping. **Genome Biology**
- 1D. Gore et al. (2011) Somatic coding mutations in human induced pluripotent stem cells. **Nature**
- 1E. Chu, et al. (2017) Ultraaccurate genome sequencing and haplotyping of single human cells. **PNAS**
- 1F. Half-siblings (on maternal side), public PGP95, PGP1 genome data.
- 1G. Lieberman, et al. (2018) HiC helps long-range assembly. [in prep]
- 1H. **Nir G**, Wu, CT, et al. (2018) Imaging [in prep]
- 1I. **Nguyen, et al.** PGP1-iPSC, FISSEQ & Oligopaints, 14 nm resolution, 3D structure, centromeres



PGP Write: Syn-Bio: TAL, CRISPR, TFs

- 2A. Mali P, Yang L, et al (2013) RNA-guided human genome engineering via Cas9. Science.
- 2B. Yang et al. (2013). Optimization of Scarless Human Stem Cell Genome Editing. Nucleic Acids Res.
- 2C. Yang et al. (2014) Targeted & genome-wide sequencing reveal single nucleotide variations impacting specificity of Cas9 in human stem cells. Nature Comm.
- 2D. Yang et al. (2014) CRISPR/Cas9-Directed Genome Editing of Cultured Cells. Curr Protoc Mol Biol.
- 2E. Busskamp et al. (2014) Rapid neurogenesis through transcriptional activation in stem cells. MSB
- 2F. Yankner B, et al. (2018). Inhibitory neurons [in prep]
- 2G. Khoshakhlagh P, Ng AHM, et al. (2018) Oligodendrocytes. [in prep]
- 2H. Ng, Khoshakhlagh et al. (2018) Programming cell fates [in prep]



First human sgRNA, libraries, stem cells

- 3A. **Chan, Chan, Lim, et al.** (2018) Multiplexed testing of pooled donor cells using SNPs. **Genome Med**
- 3B. Lee et al. (2015) FISSEQ RNA ...in intact cells and tissues. **Nature Protocols.**
- 3C. Lee et al. (2014) Highly multiplexed subcellular RNA sequencing in situ. **Science**
- 3D. Laserson et al. (2014) High-resolution antibody dynamics of vaccine-induced ... **PNAS**
- 3E. Zhang et al. (2009) Digital RNA Allelotyping **Nature Methods**
- 3F. Lee et al. (2009) ...Tissue-specific Regulatory Variants Using Personalized hiPSCs. **PLoS Genetics**



Rigel
Chan



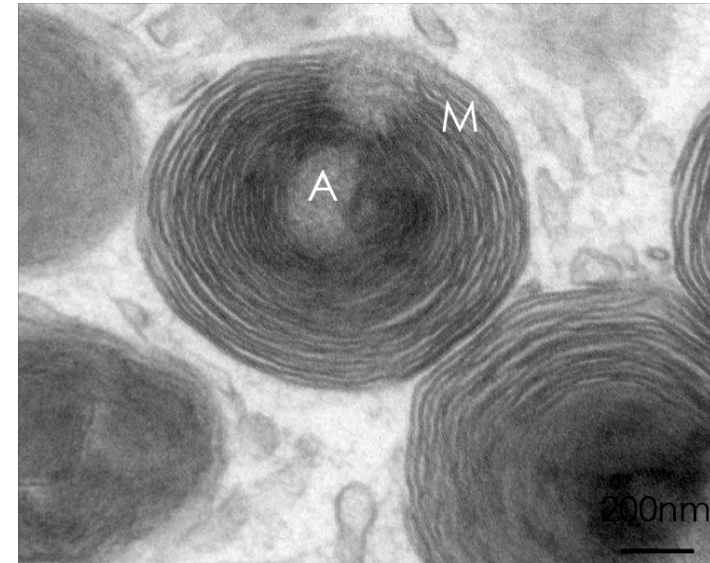
Elaine
Lim



Kai
Chan

(4) Sources PGP1 cells, epigenetically distinct isogenic PGP1 cells in ENCODE & Coriell. Homogeneity % without purification, unless specified.

- #1. primary fibroblasts, 100%
- #2. iPSCs (from #1 via retroviral Yamanaka factors), 100%
- #3. iPSCs (from #1 via Sendai-viral Yamanka factors) 100%
- #4. bipolar neurons (from #2 via NGN1/2) >95%
- #5. Lymphoblastoid cells (from primary B-cells at Coriell) 100%
- #6. Excitatory cortical neurons (from #4) >90-95%
- #7. Oligodendrocytes (from #4) >70%
- #8. Endothelial cells (from #4) >90%
- #9. Cardiomyocytes (from #4) >90%
- #10. Myocytes (from #4) >50%
- #11. Stromal cells (from #4) >90%
- #12. Astrocytes (from #4) >90%
- #13. Inhibitory neurons (from #4, Yankner lab)
- #14. Keratinocytes, primary
- #15. B-cells primary



\$3 billion



1990-2004

\$80 million



2007

\$350,000



2010

\$6,000



2013

(paid by the
patient)
\$999



2015

Veritas

Nebula

(paid to the
patient)

\$500



2019

Lowering costs
10-million-fold for more
equitable access.

Personal Genome → prevention/cure

John Lauerman: Scotoma/leg pain → JAK2 → Aspirin
gene





Enviromental monitoring

Neural reflex

Allergy

Viruses

Rhino, Corona, Influenza, Parainfluenza,
Respiratory-syncntial, Adeno,
Entero, Metapneumo

Bacteria

*Streptococcus, Haemophilus,
Chlamydophila, Mycoplasma*

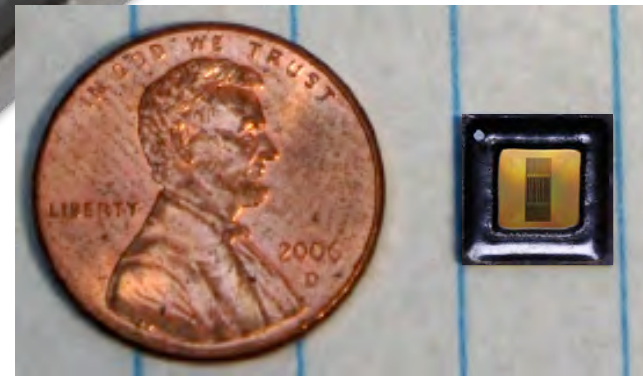
Fungi

*Histoplasma, Blastomyces, Cryptococcus,
Pneumocystis, Coccidioides*

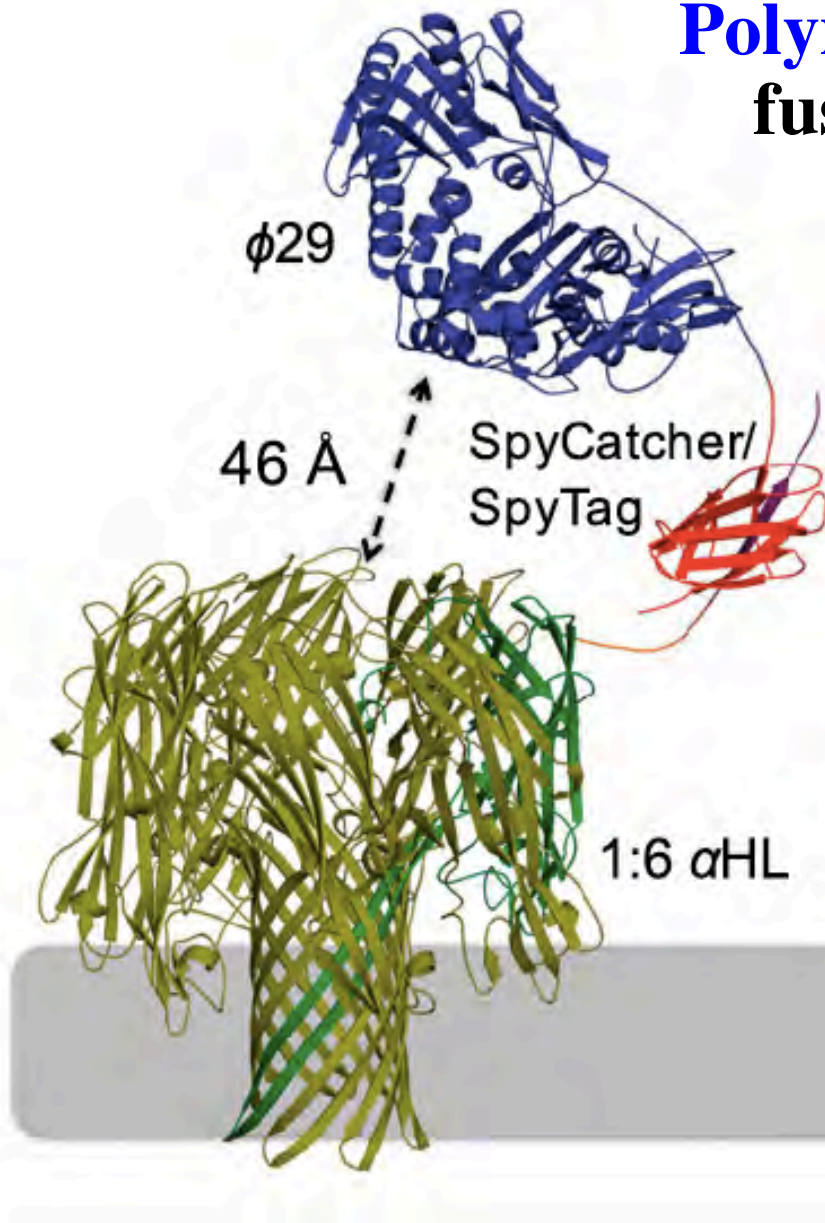
Wearable Sequencing Technologies (ONT)



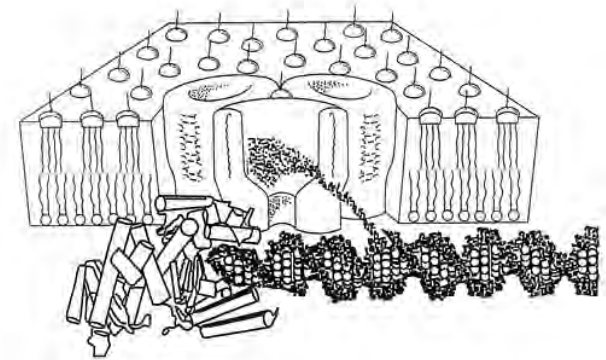
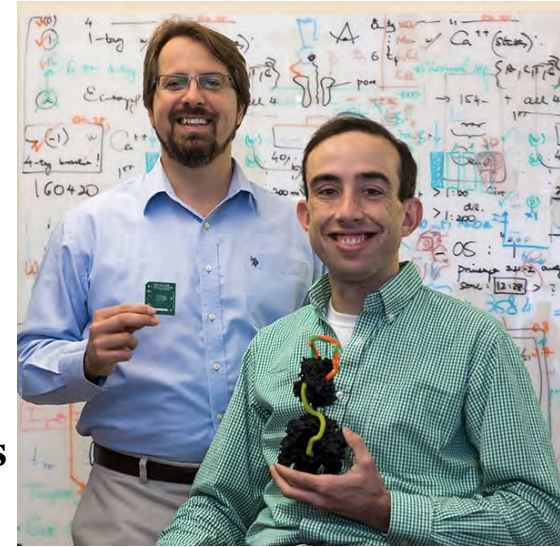
Genia-Roche



Polymerase + pore fusion protein



Mirko Palla
Ben Stranges



Sukanya
Punthambaker


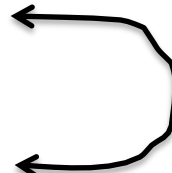



1989-98: US5795782
Church, Deamer,
Branton, Baldarelli,
Kasianowicz

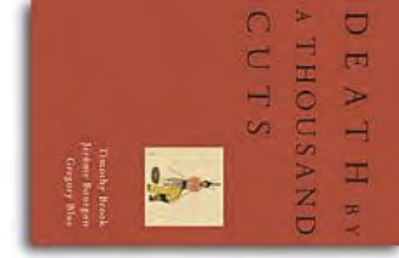
Human Genome Project Write: PGP Recoding

Make any cell resistant to all viruses

	U	C	A	G
U	189,379	171,196	132,715	117,458
U	216,388	196,012	161,579	135,489
U	86,682	139,095	5,404	9,518
U	143,507	50,249	4,328	134,648
C	147,569	198,345	123,609	49,921
C	212,802	225,420	168,062	115,976
C	79,488	192,119	140,427	68,859
C	437,308	81,354	387,120	129,331
A	175,259	147,134	190,114	139,465
A	220,634	203,602	206,688	220,505
A	82,922	167,136	278,169	133,268
A	236,510	66,200	353,825	131,616
G	121,302	204,091	246,943	117,456
G	155,761	311,996	278,549	247,607
G	78,882	178,106	336,665	183,190
G	305,878	84,501	451,726	182,999

U	Stop UAA	4,328	
C	Stop UAG	5,404	
A			
G	Leu UUR	230,189	
U	Leu CUY	360,371	
C	Leu CUR	516,796	
A			
G	Ser AGY	359,970	
U	Ser UCY	367,208	
C	Ser UCR	189,344	
A			
G	Arg AGR	264,884	
U	Arg CGA	68,859	
C	Arg CGG	129,331	
A			
G			

Editing Repeats!



Repeat	Mut	Species	Method	Reference
SSR (triplets)	?/36	Human	Cas9	Monteys..Mol Ther 2017
Telomeres	200/2k	Human	TERT	Ramunas .. FASEB J 2015
Centromeres	140/1M	Mus	Cas9	Adikusuma .. Mol Ther 2017
rDNA repeats	100/290	Fly	I-CreI	Paredes .. Genetics 2009
SINEs (Alu)	?/1M	Human	Spont	..
LINEs	400/20k	hiPSC	BE3	Smith .. (unpub)
ERVs	62/62	pig	Cas9	Yang .. Science 2015, 2017



HGP-Write & Test Why Organoids?

- Disease modeling
- Variants of Unknown Significance (VUS)
- Drug Safety & Efficacy
- Transplantation (e.g. UCART)

Need: Properly consented cells
Genome & Epigenome engineering
Genome sequencing before & after

HGP-Write & Test Why Organoids?

- Disease modeling
- Variants of Unknown Significance (VUS)
- Drug Safety & Efficacy
- Transplantation (e.g. UCART)

Need: Properly consented cells
Genome & Epigenome engineering
Genome sequencing before & after

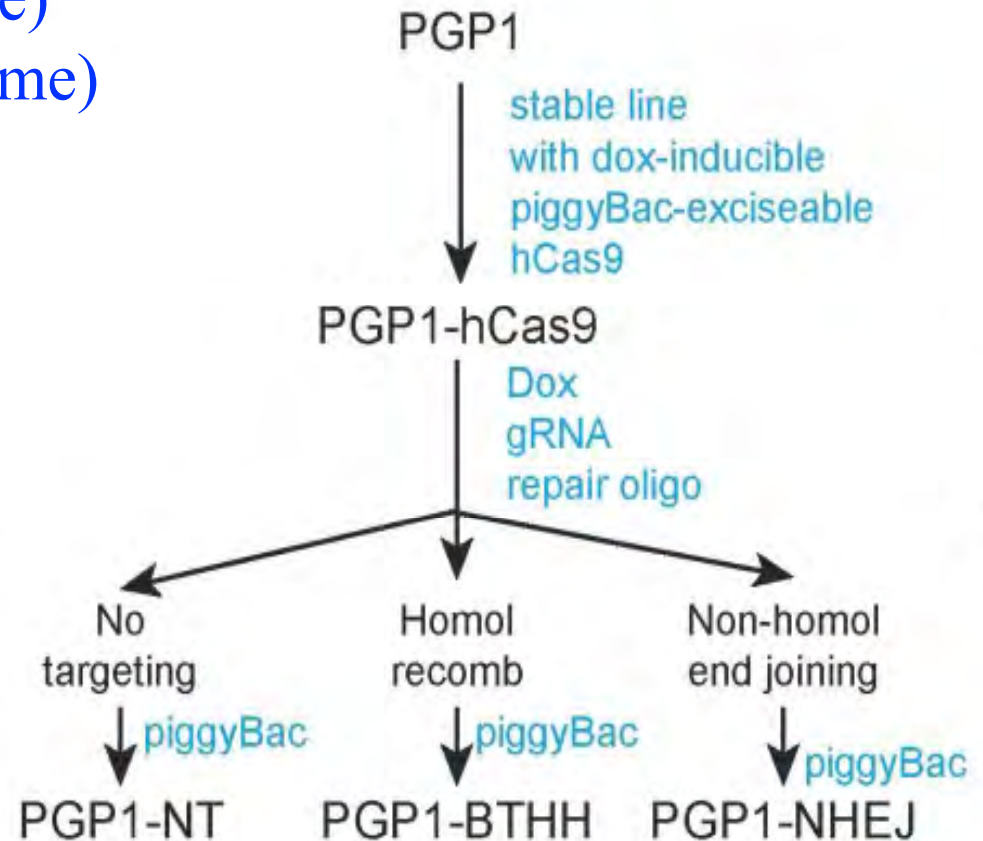
Patient mutations (VUS)

- clonal stem cells (genome)
- cardiomyocytes (epigenome)

Barth syndrome



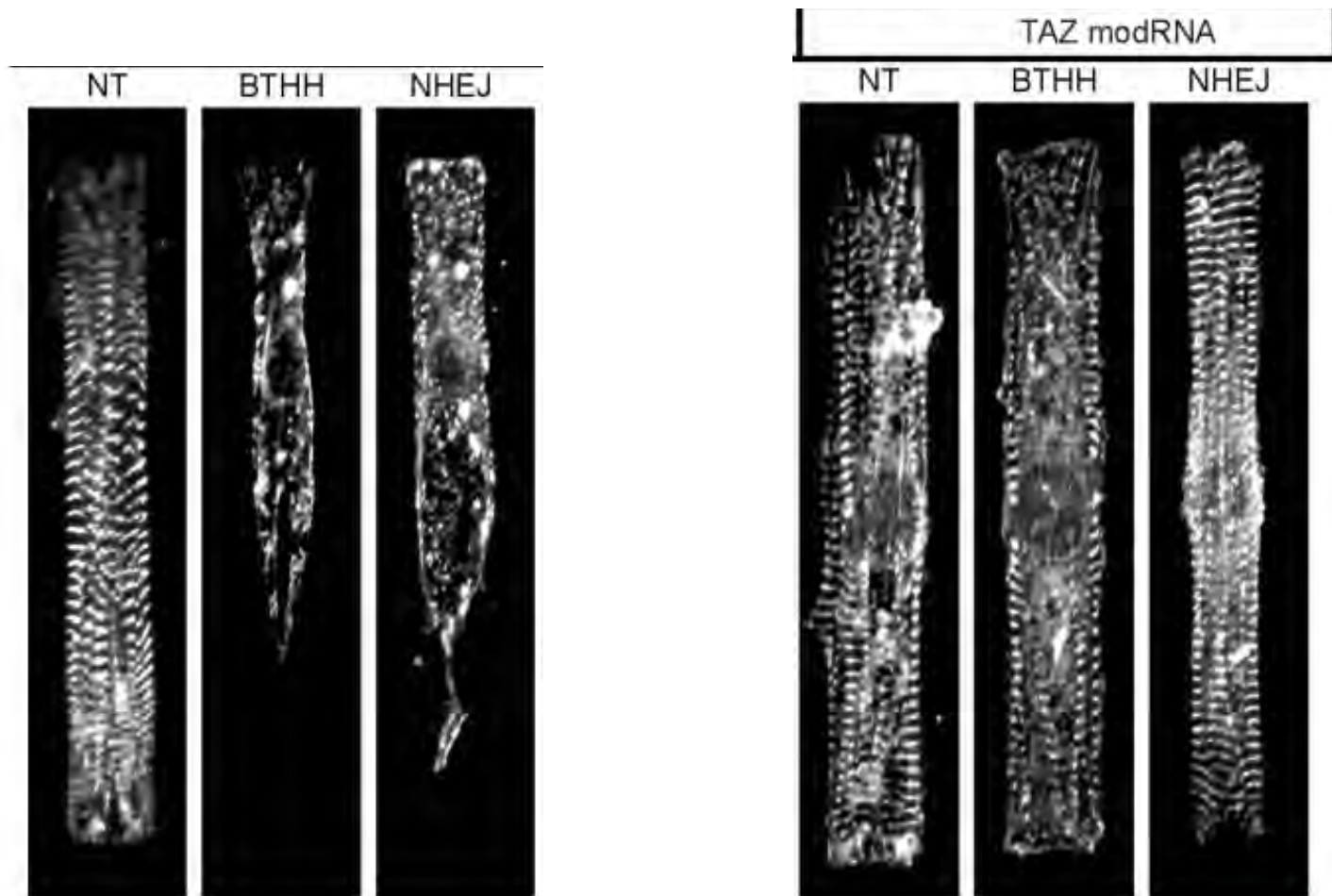
- Cardiomyopathy
- Defective cardiolipin composition
- TAZ mutation



PGP1-NT	AACCATG----- <u>G</u> GGACTGGGTG	0 bp
PGP1-BTHH	AACCATG-----GGACTGGGTG	-1 bp
PGP1-NHEJ	AACCATGagaagctaaccatgGGACTGGGTG	+8 bp

TAZ mutation is responsible for cardiomyocyte morphology abnormality (edited PGP iPSC)

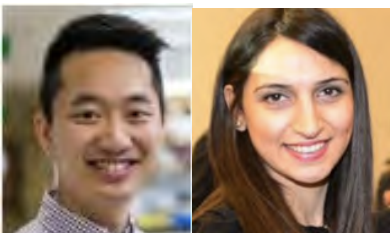
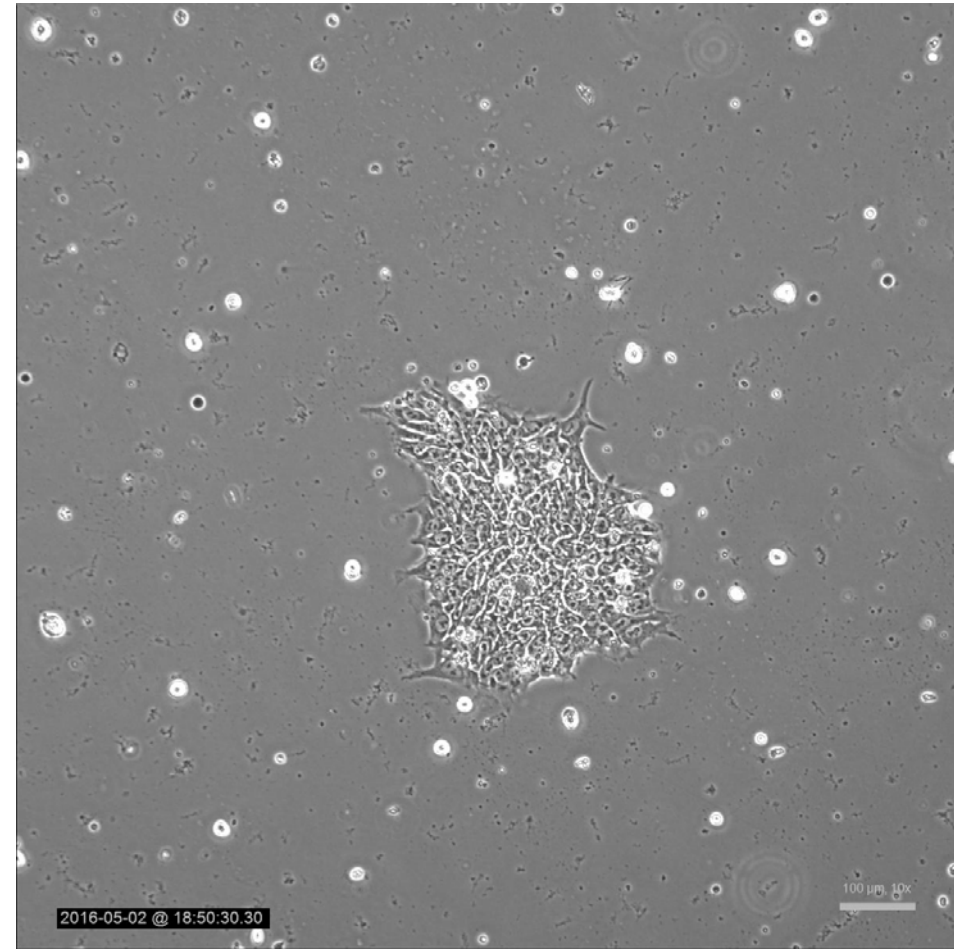
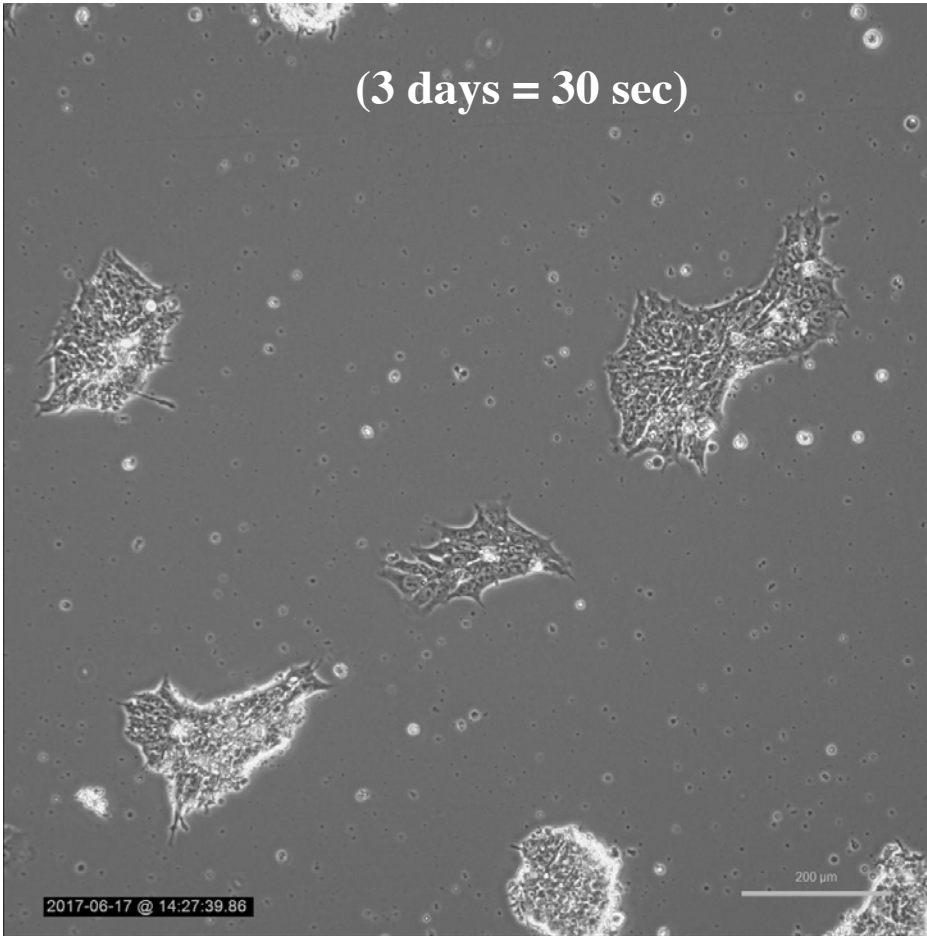
Reduced sarcomere organization -- complemented by mRNA



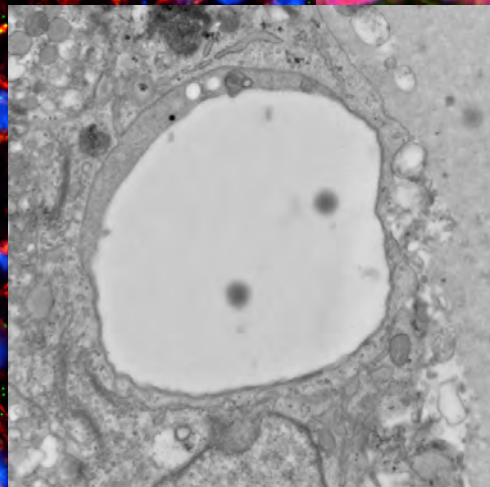
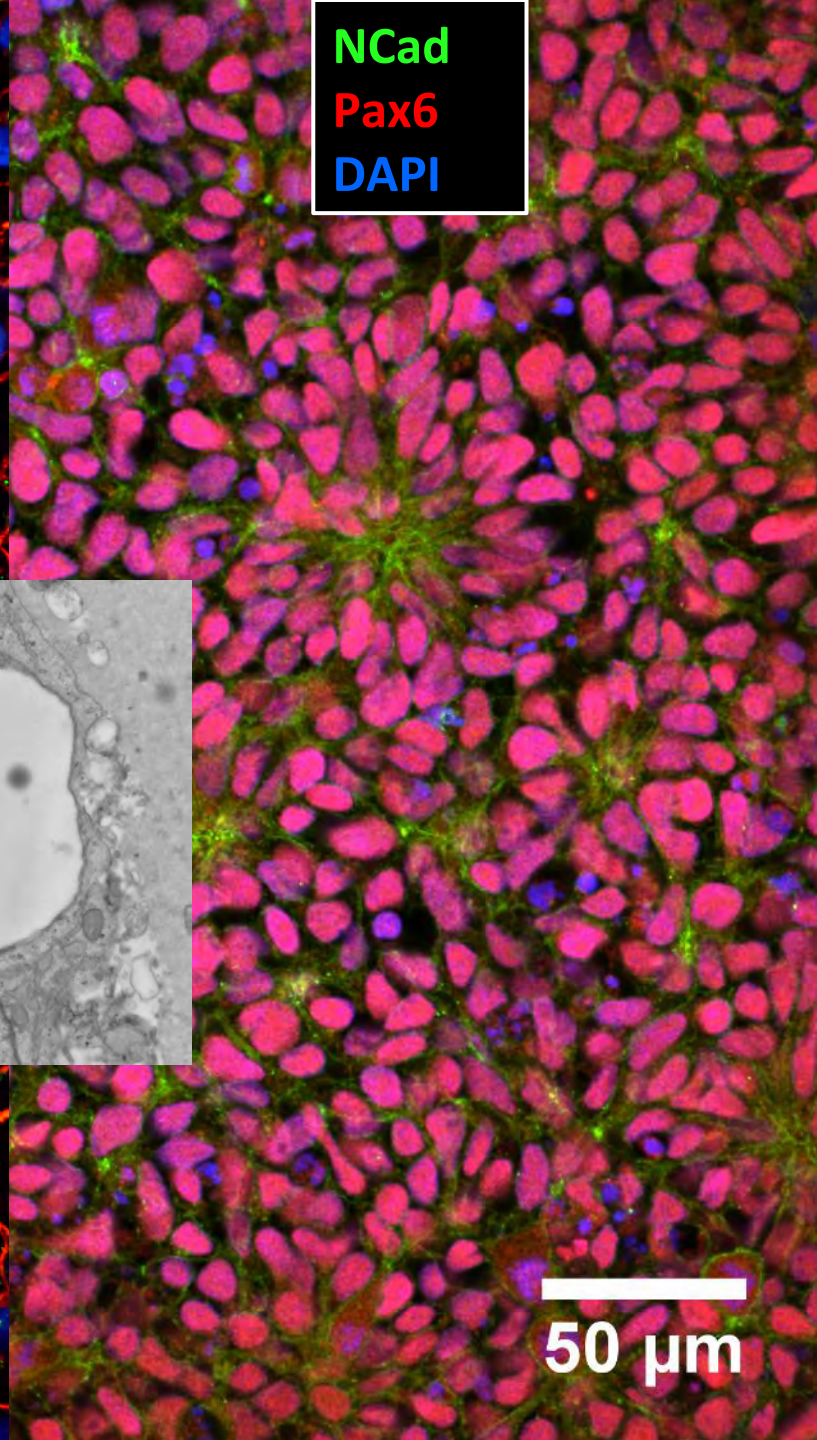
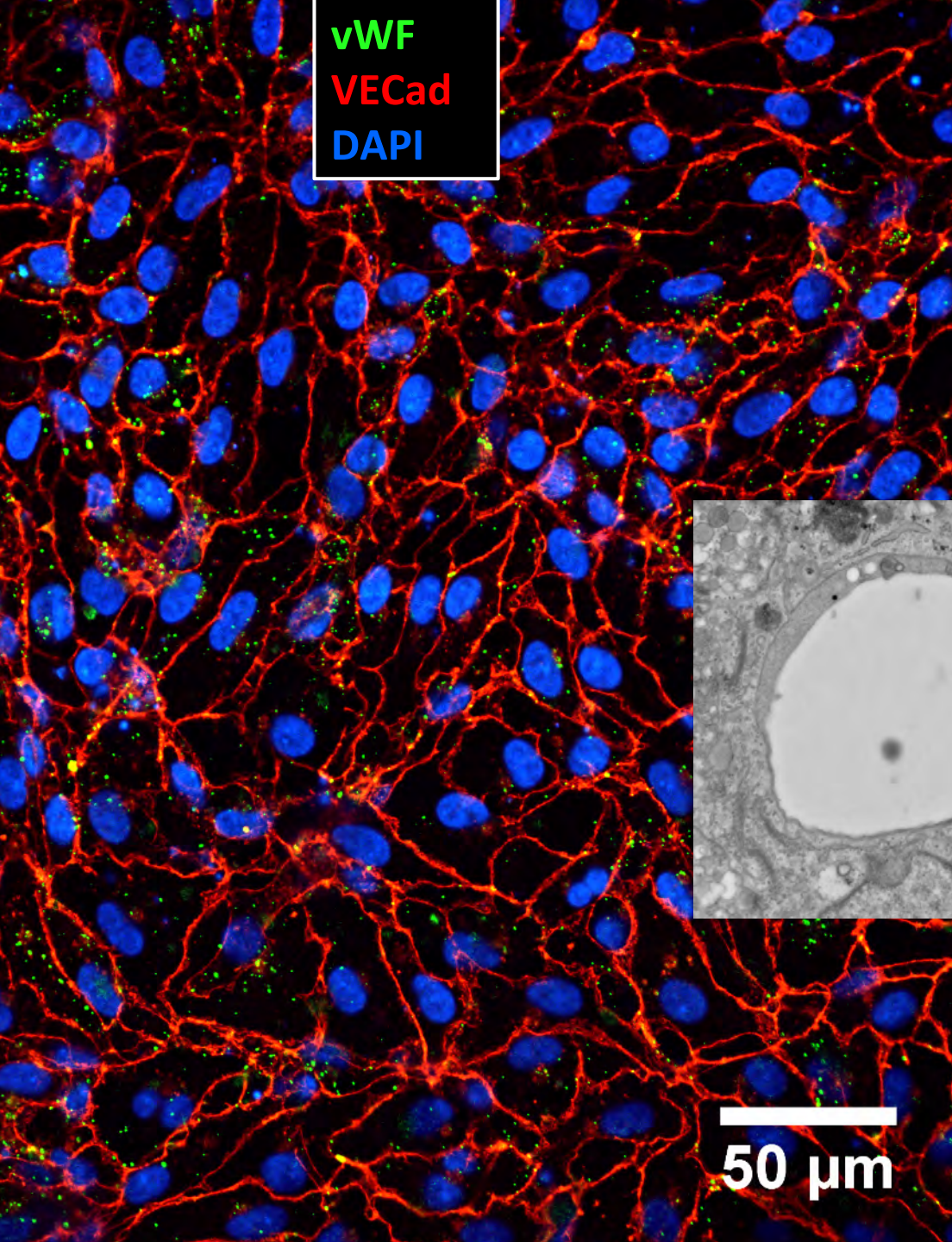
Stem cells to endothelia

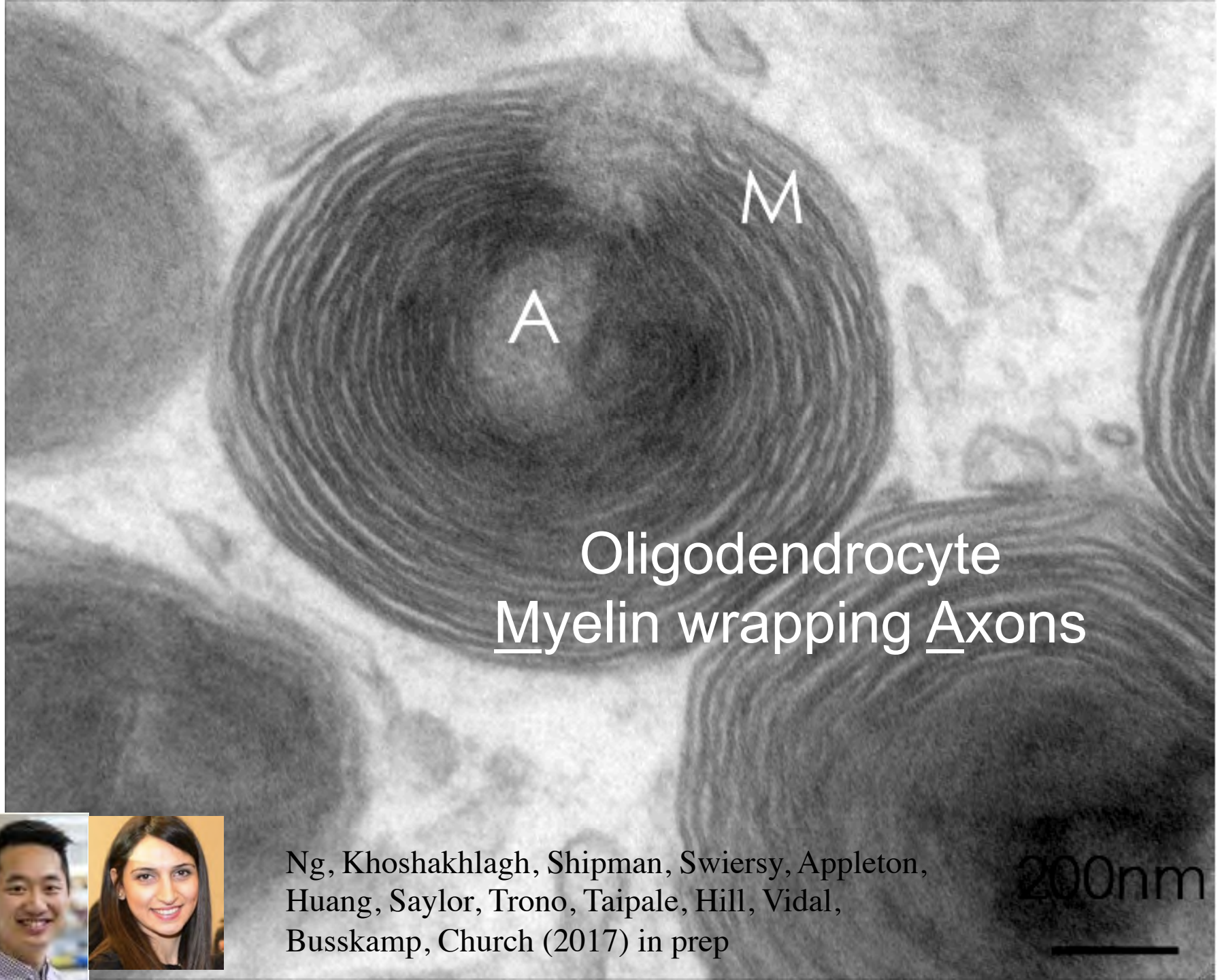
& bipolar excitatory neurons

(3 days = 30 sec)



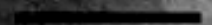
Ng, Khoshakhlagh, Shipman, Swiersy, Appleton, Huang, Saylor, Trono, Taipale, Hill, Vidal, Busskamp, Church (2017) in prep



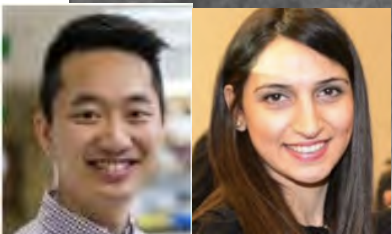


Oligodendrocyte Myelination Arounds

200nm

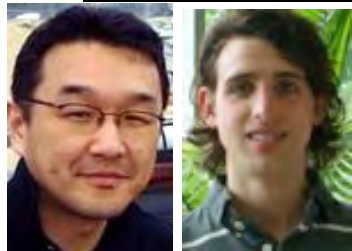
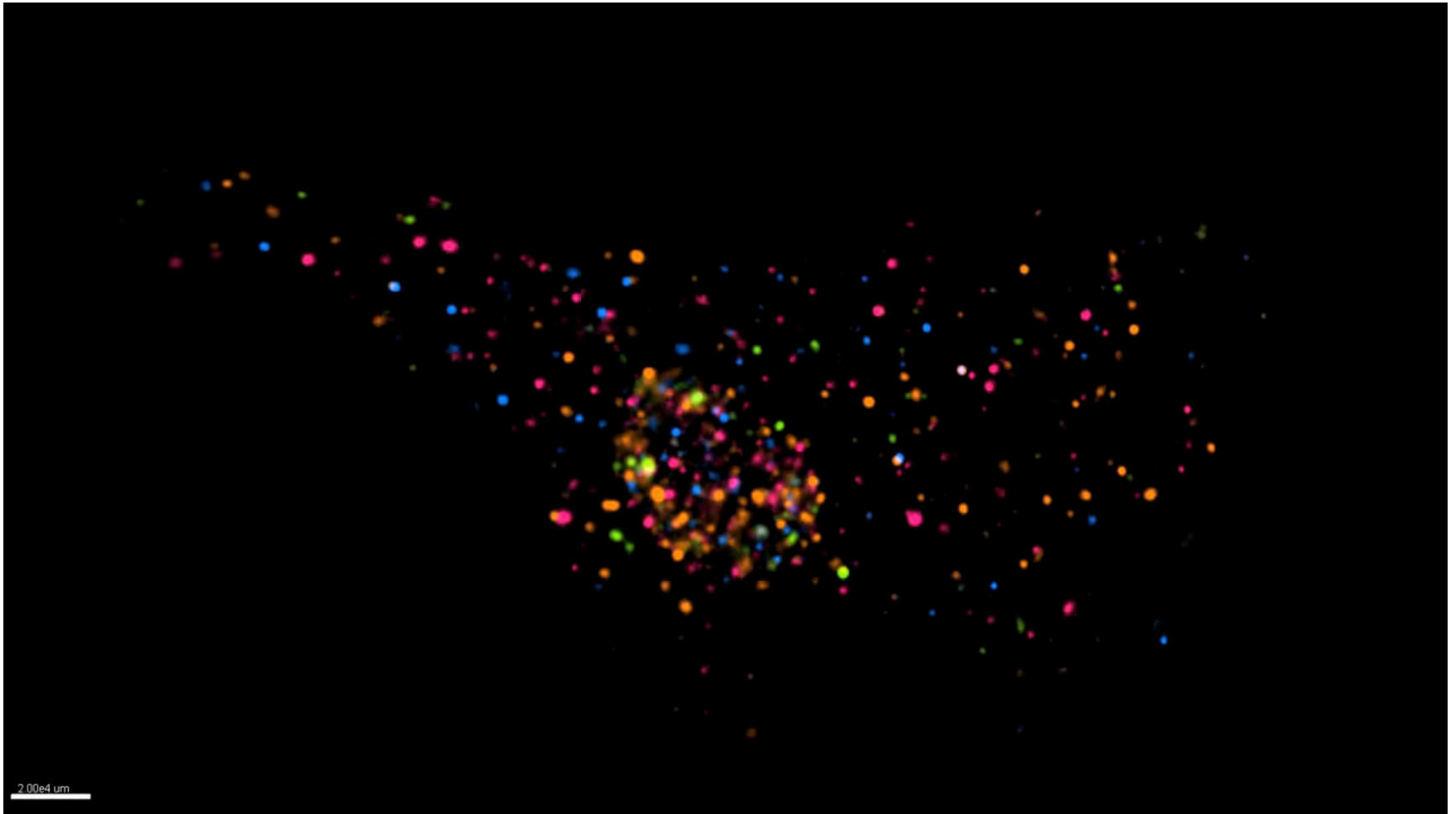


Ng, Khoshakhlagh, Shipman, Swiersy, Appleton,
Huang, Saylor, Trono, Taipale, Hill, Vidal,
Busskamp, Church (2017) in prep



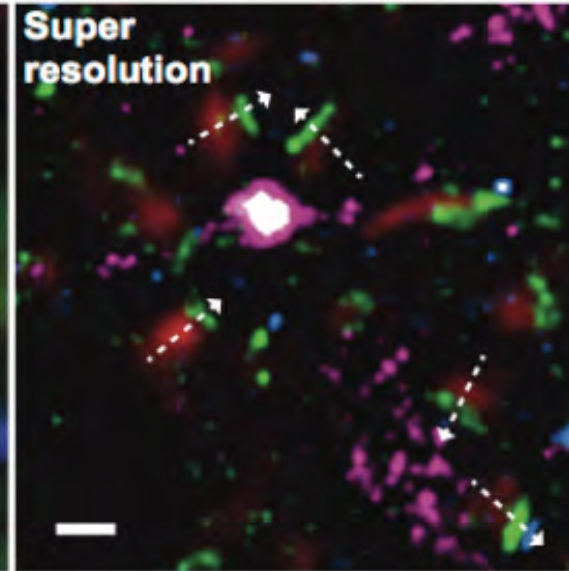
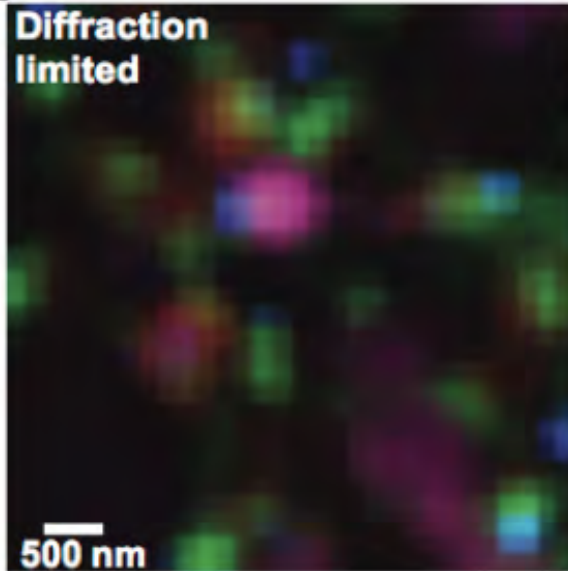
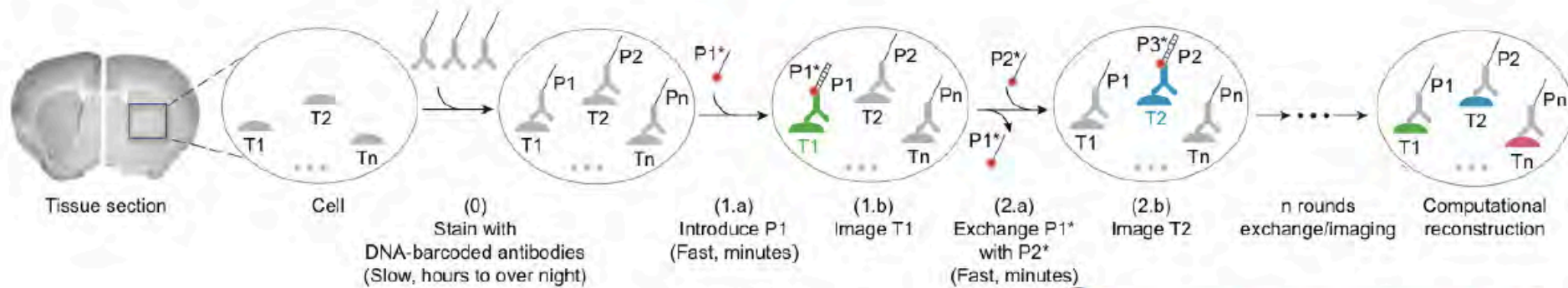
Fluorescent *in situ* multiplexing

Cell atlas: RNA, DNA, protein, lineage

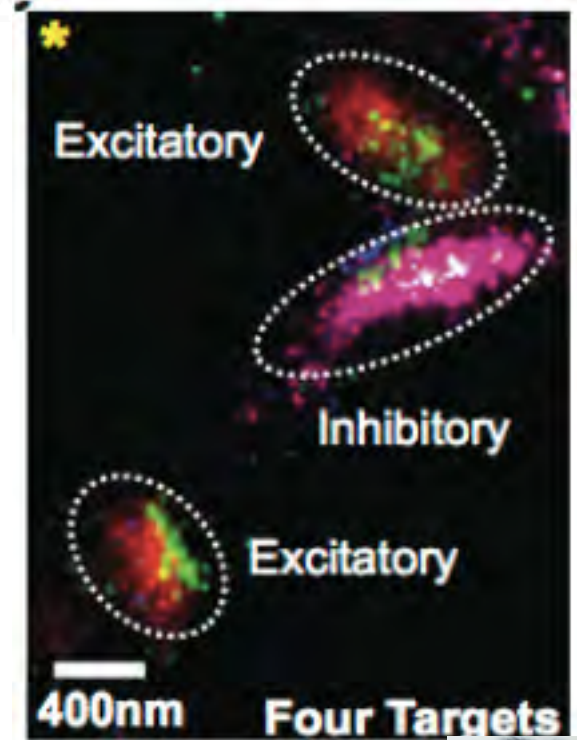


Lee, Daugharthy, .. Church (RNA)
Science 2014, Nature Protocols. 2015

Proteins: *in situ* multiplexing with DNA-Exchange



VGAT
 Gephyrin
 SynapsinI
 Bassoon



Wang, Woehrstein, Donoghue, Dai, Avendaño, Schackmann, Zoeller, Wang, Tillberg, Park, Lapan, Boyden, Brugge, Kaeser, Church, Agasti, Jungmann, Yin (2017)



Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling 2018

Guy Nir



Huy Nguyen



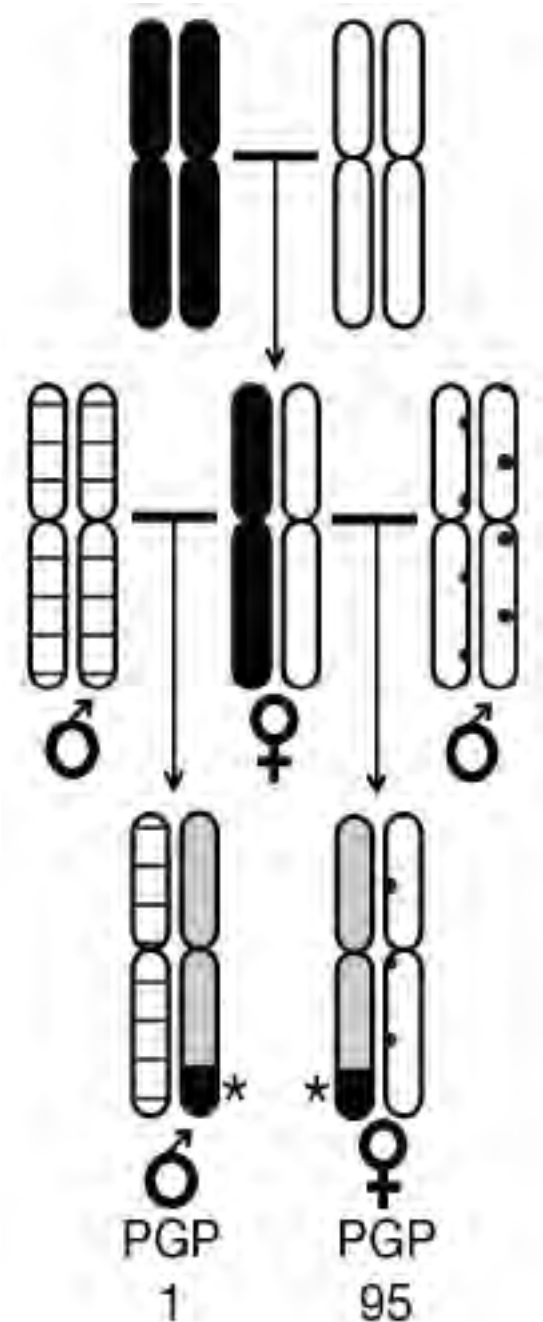
Shyamtanu Chatteraj



Brian Beliveau



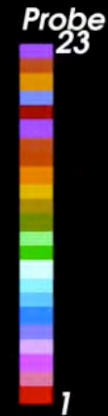
Ting Wu, Peng Yin, Erez Lieberman-Aiden, Marc Marti-Renom



19



Chr. 19 step sizes: 1300 to 400 kb
Chr. 5: all steps are 250 kb
Chr. 3: all steps are 500 kb

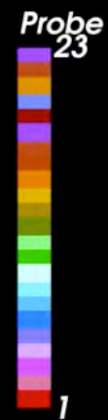


19



3 μ m

19

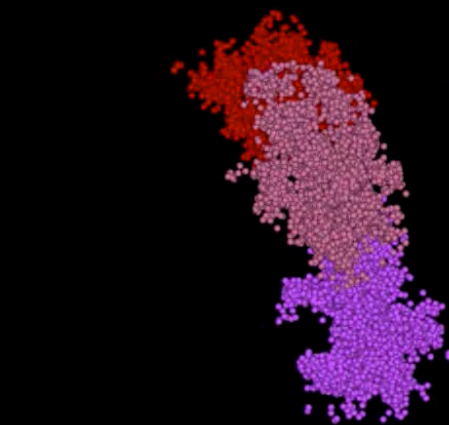


19



3 μm

19



Probe
23

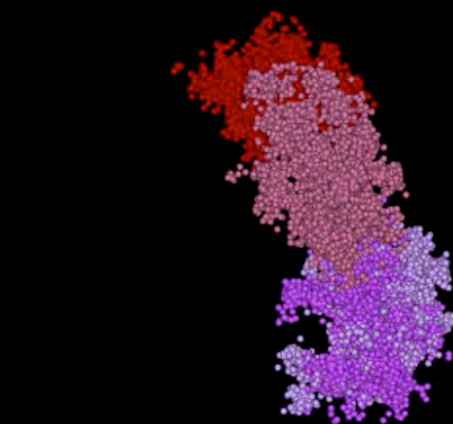


1

19

3 μm

19



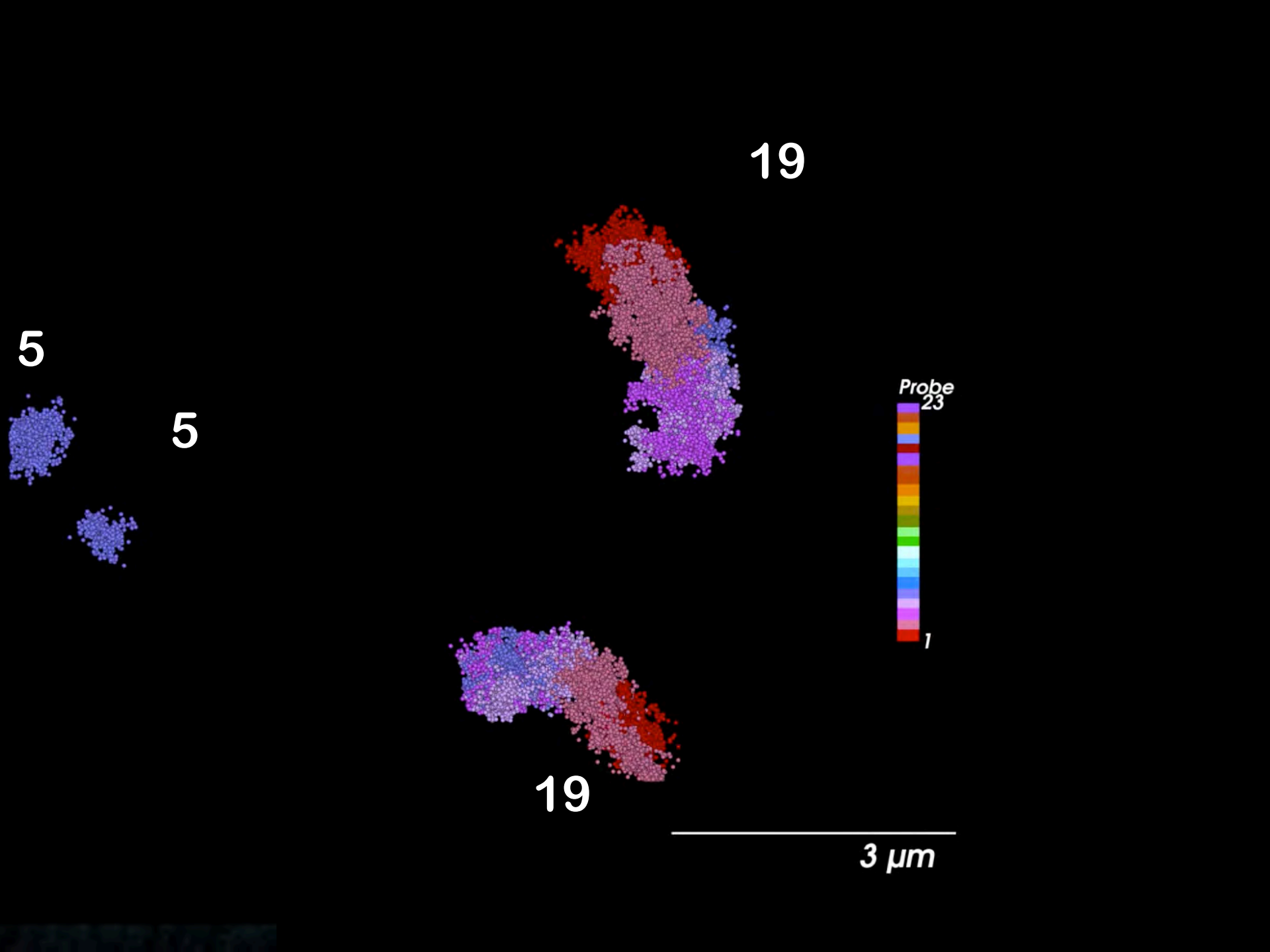
Probe
23

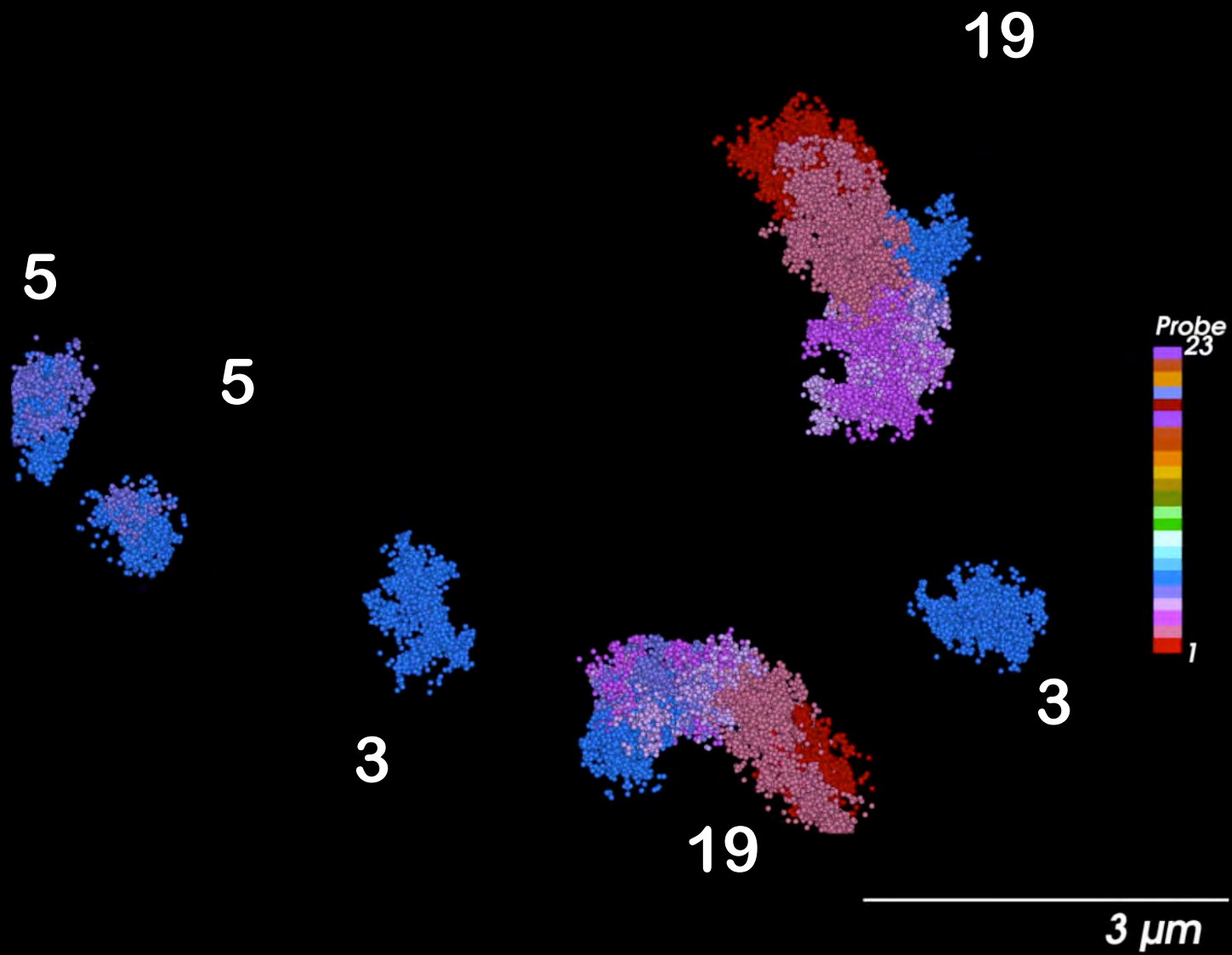


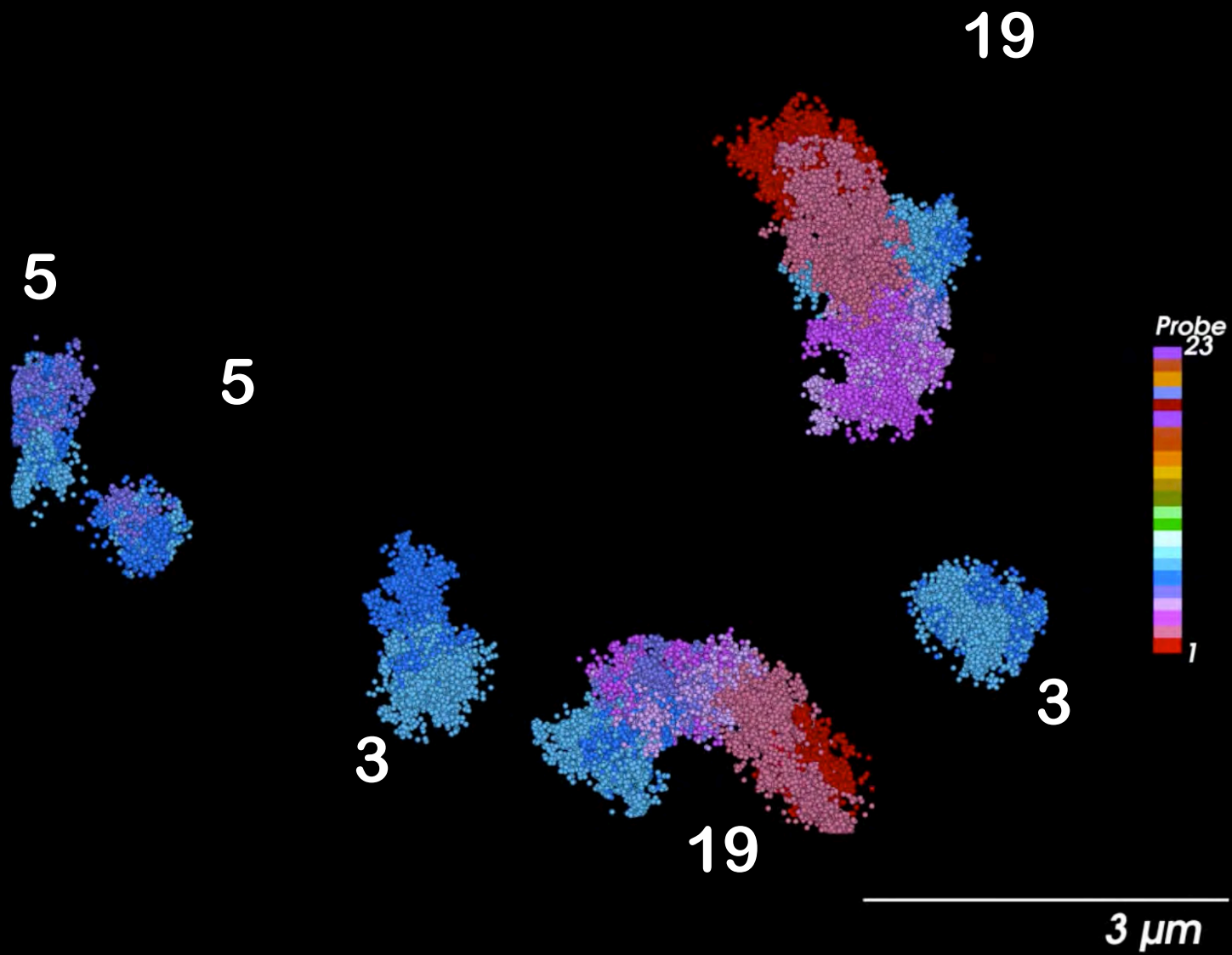
1

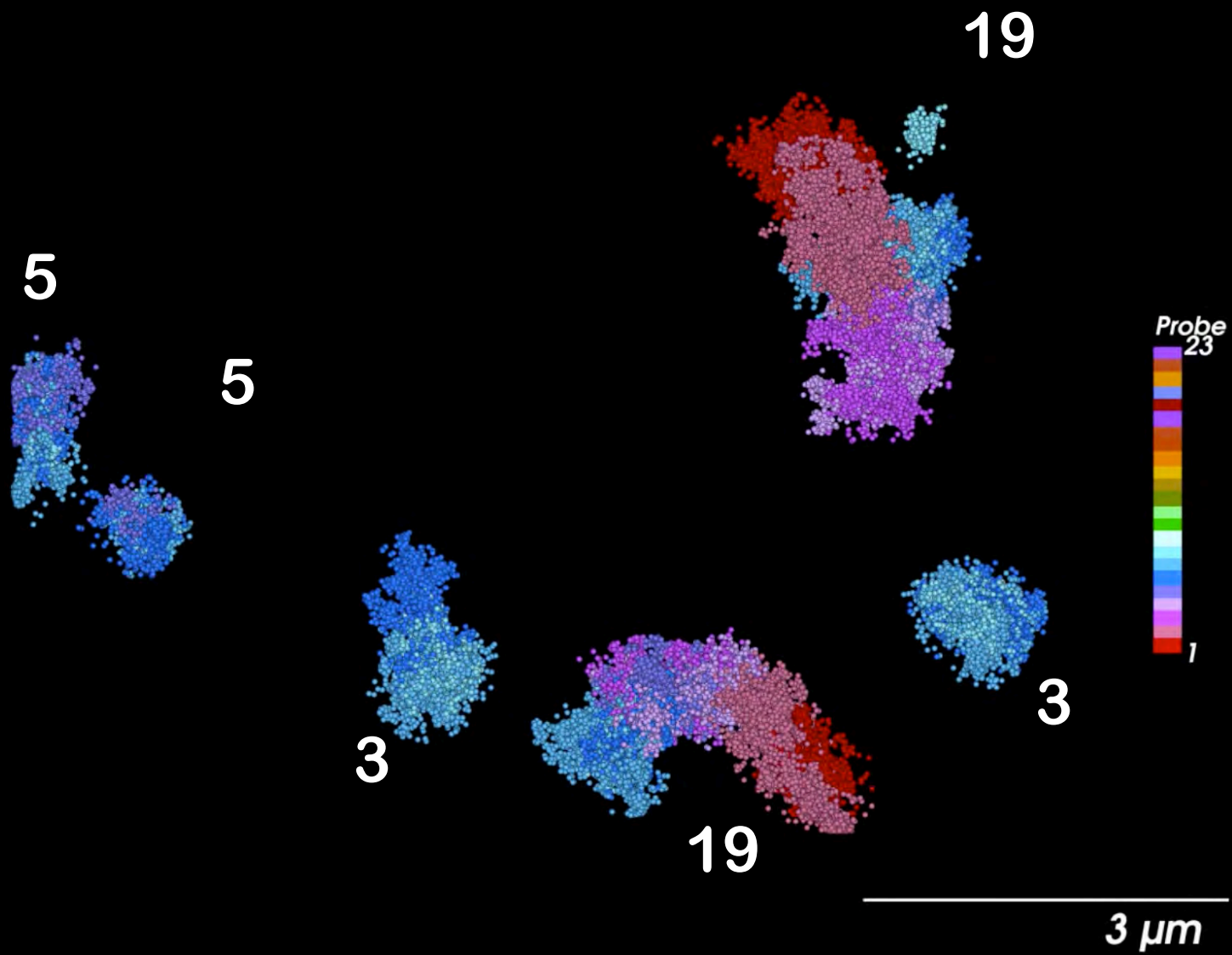
19

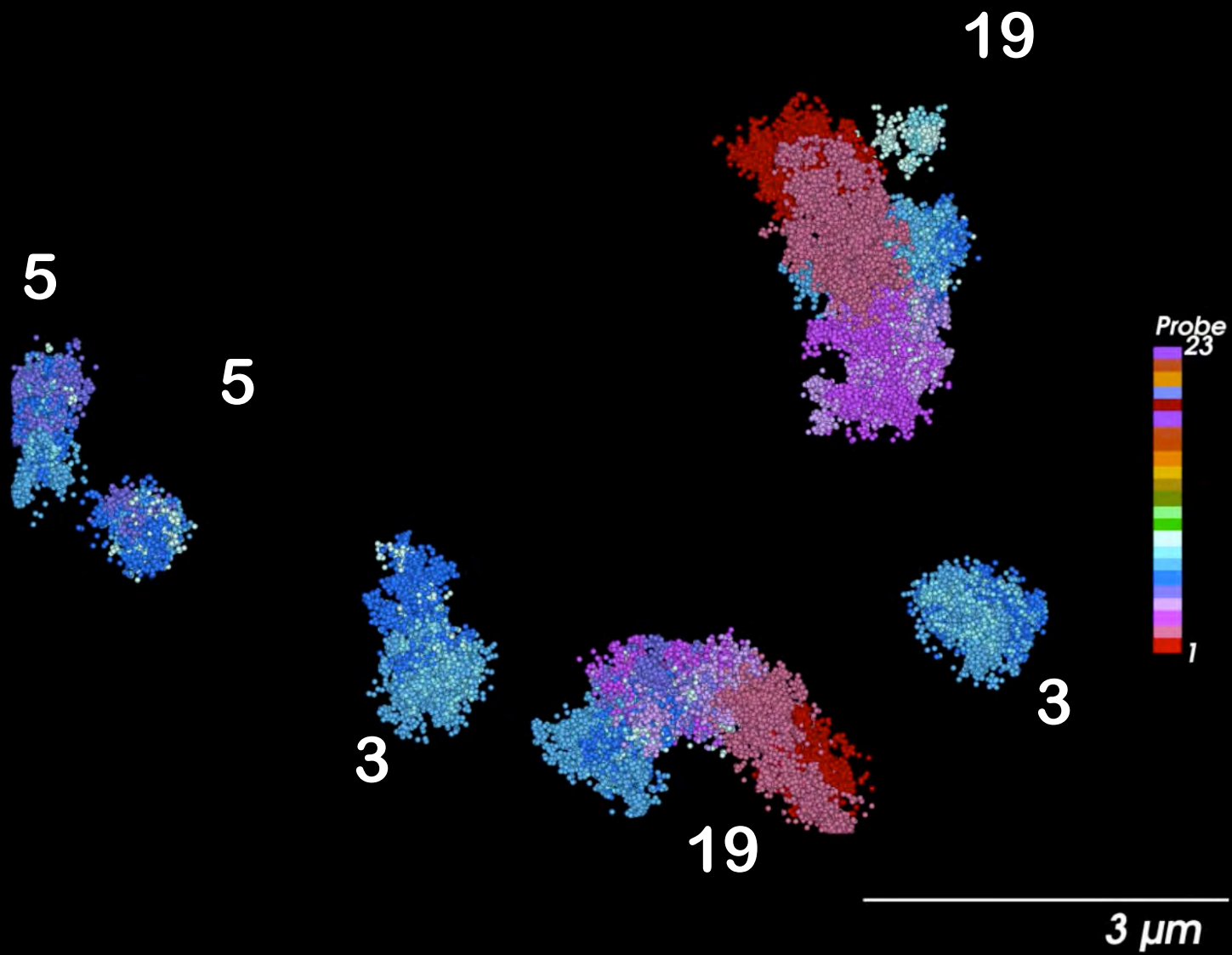
3 μm

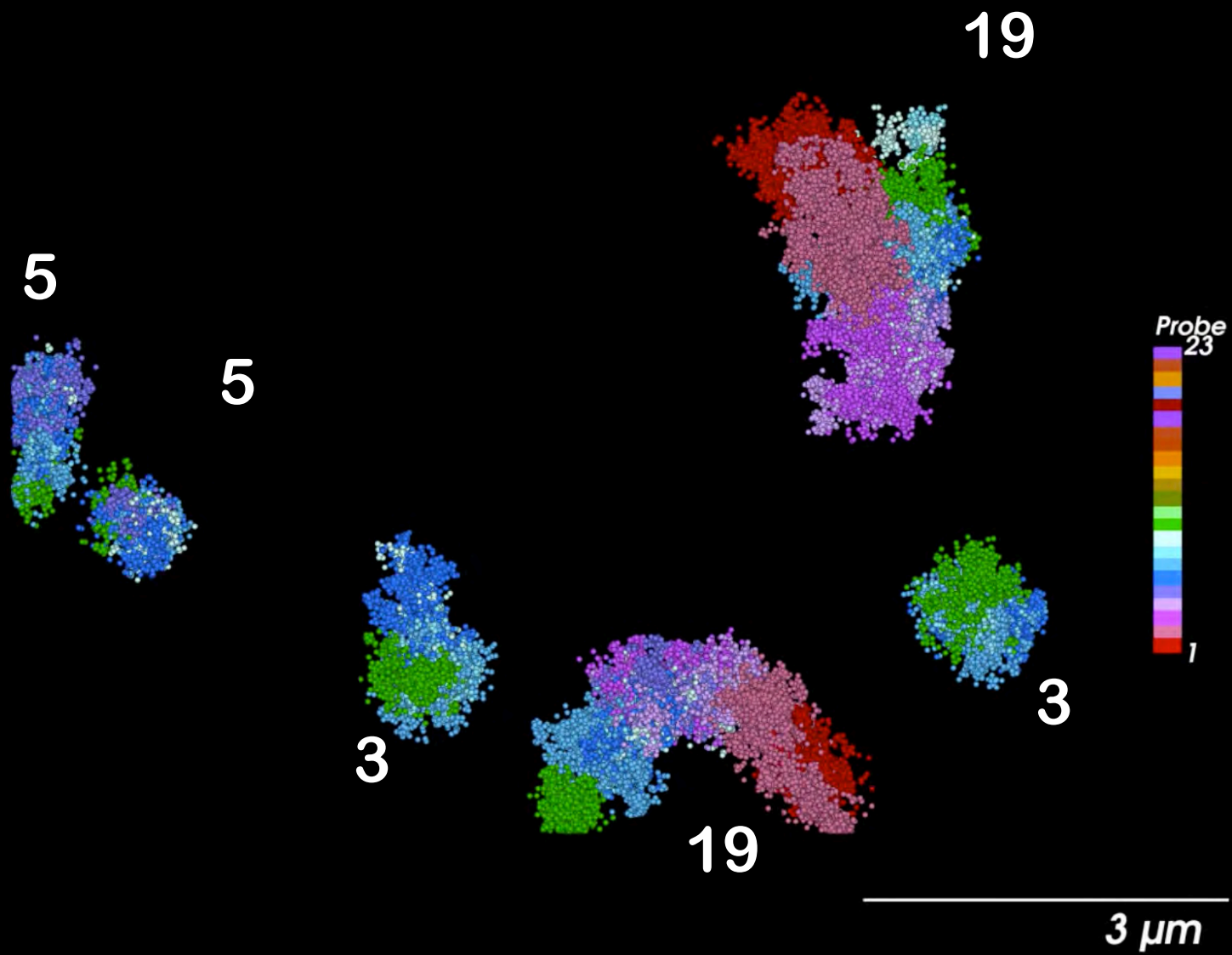


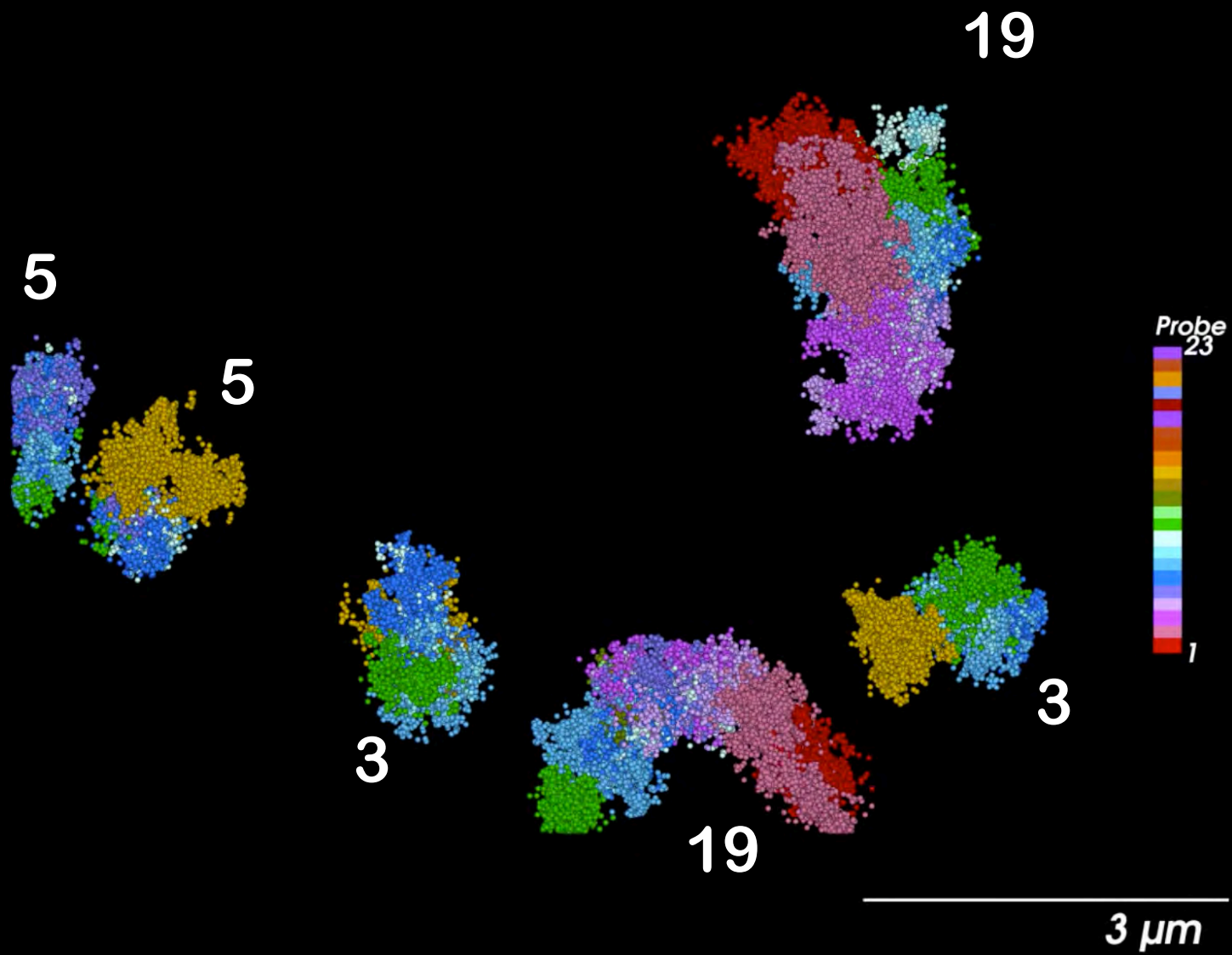


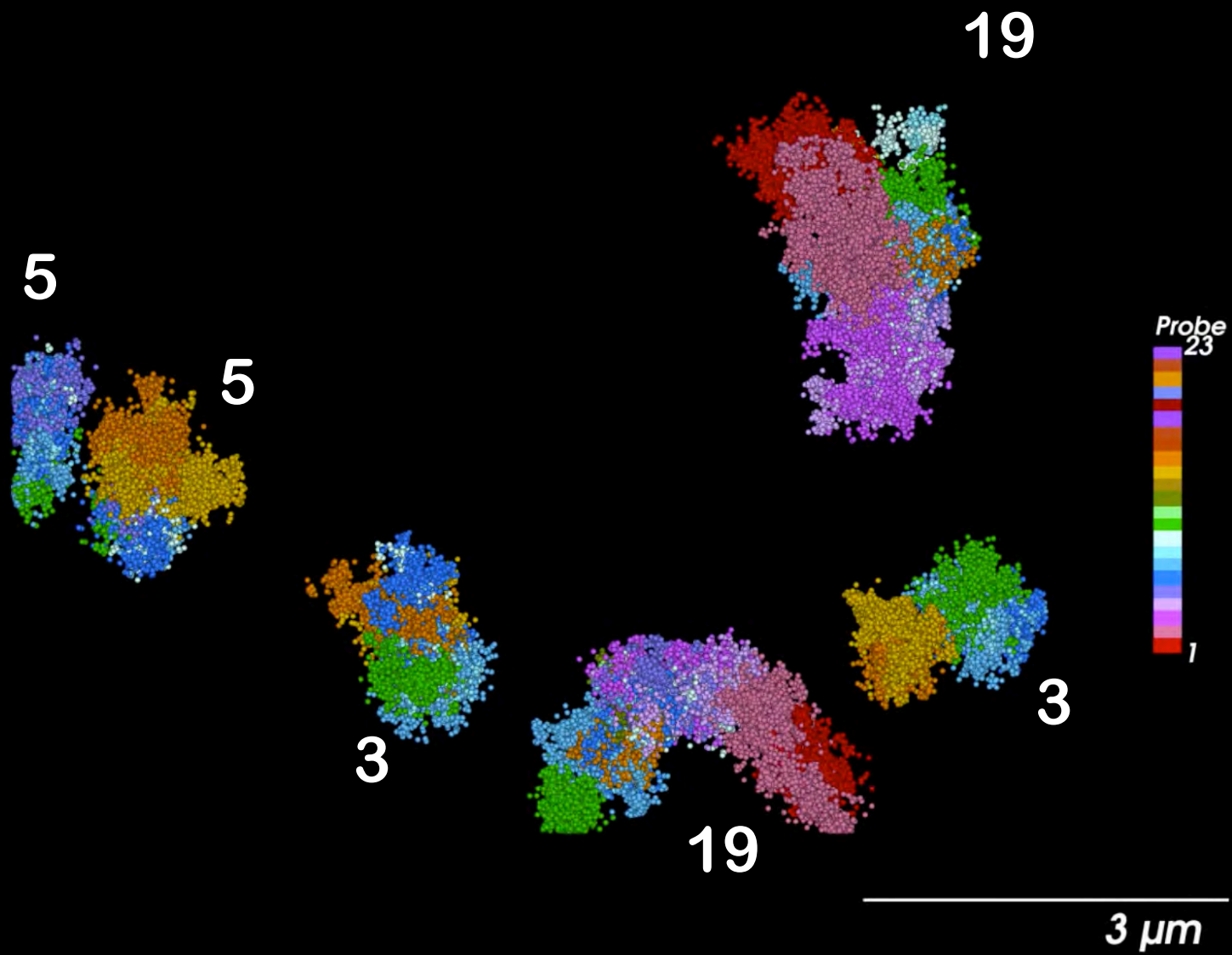


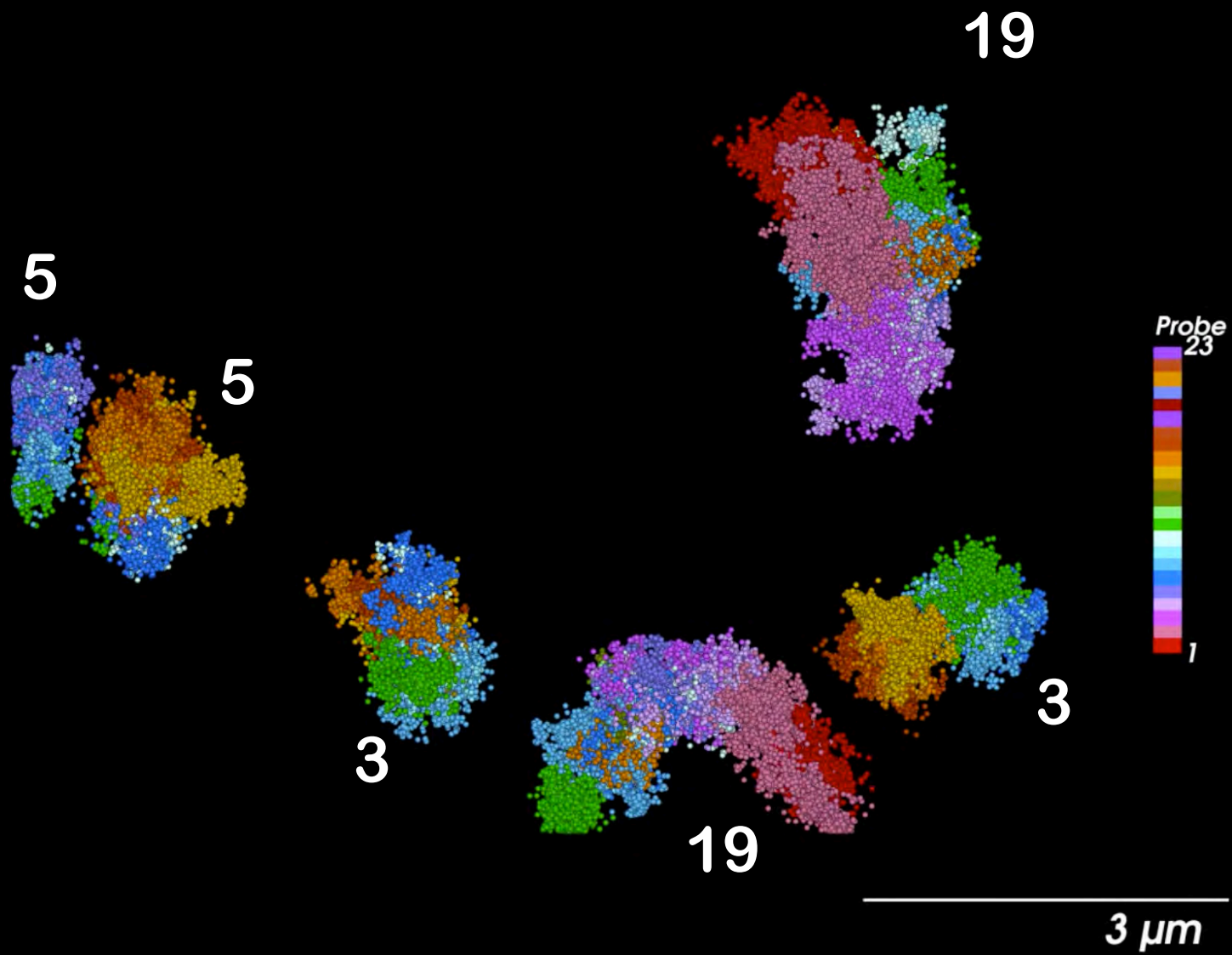


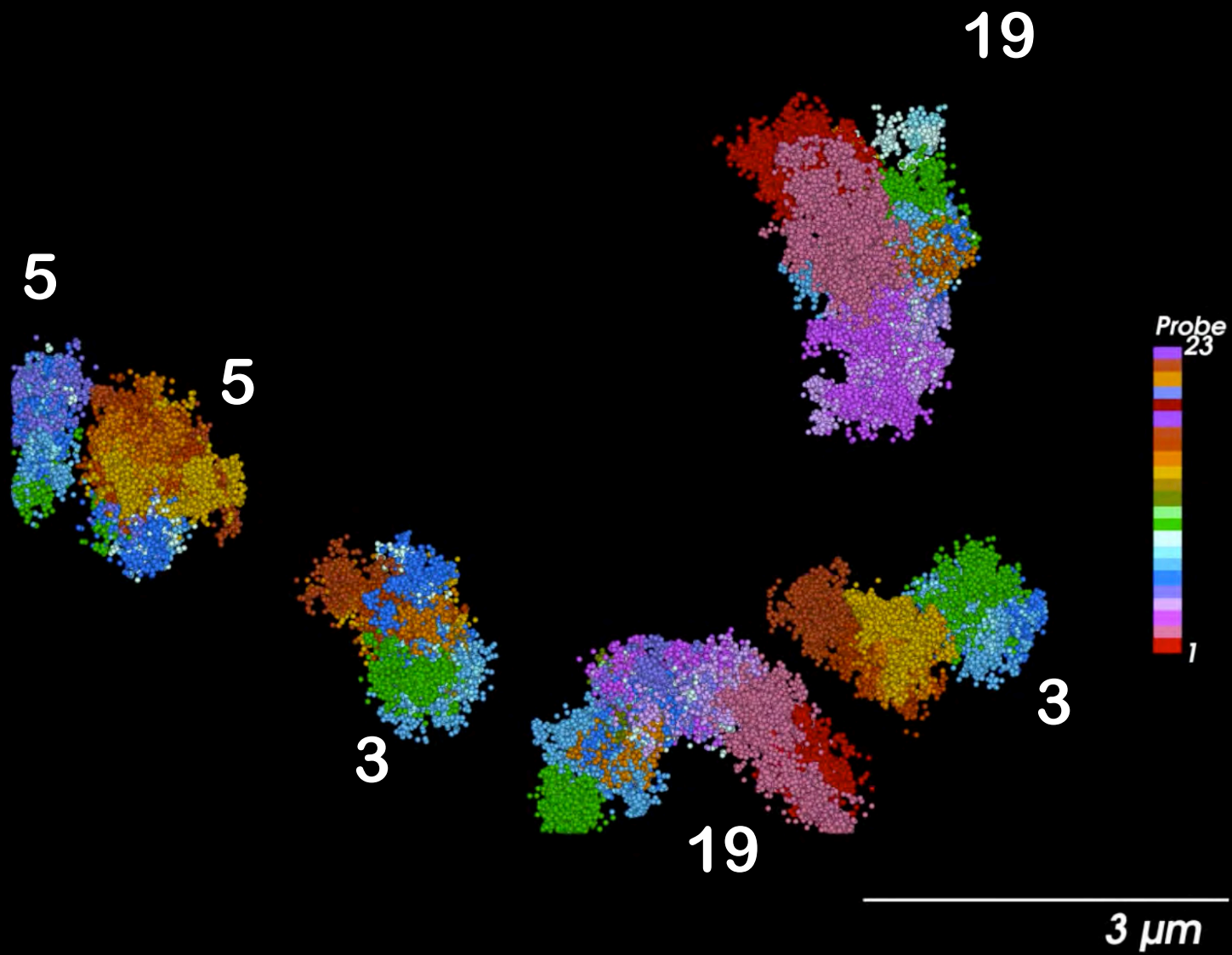


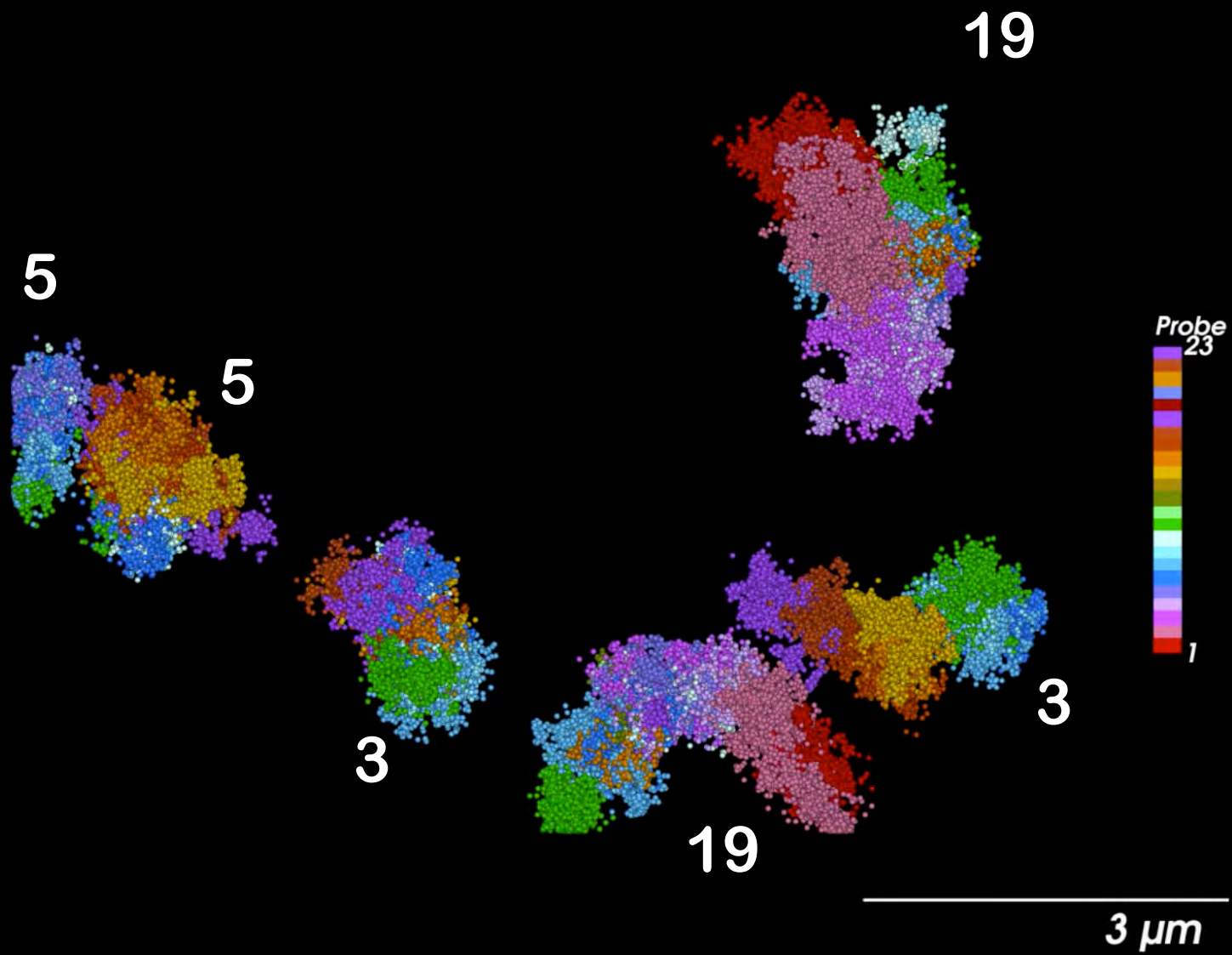


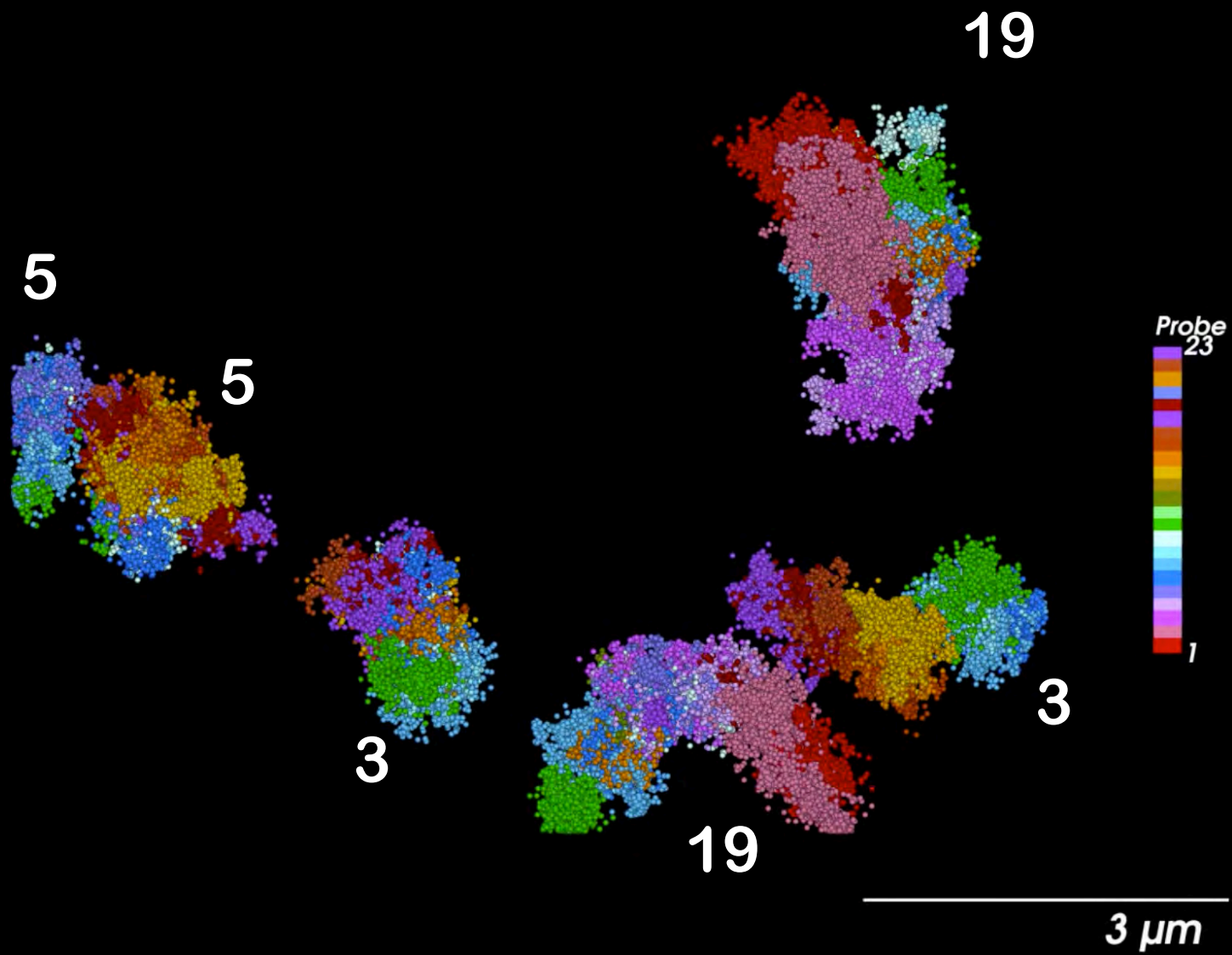


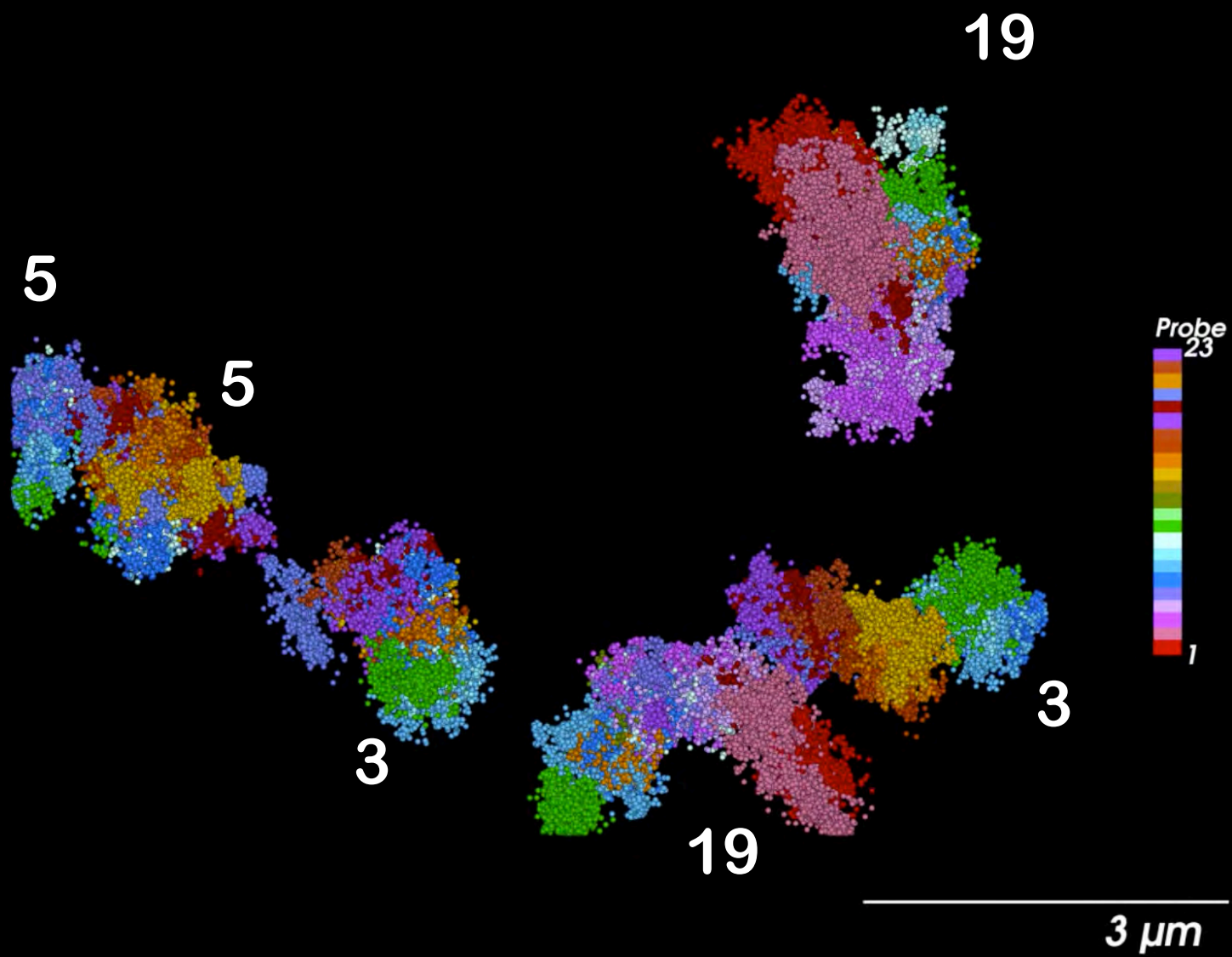


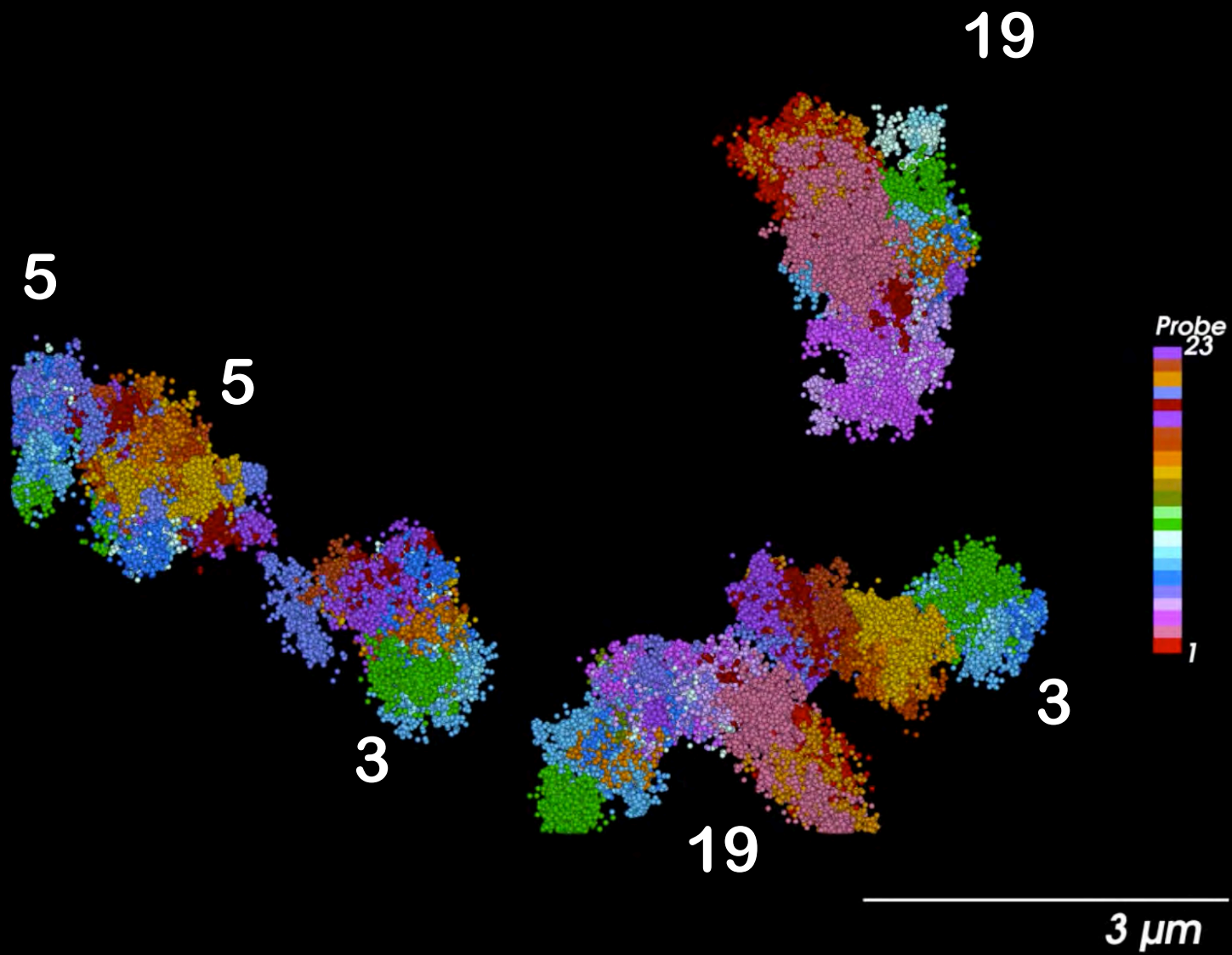


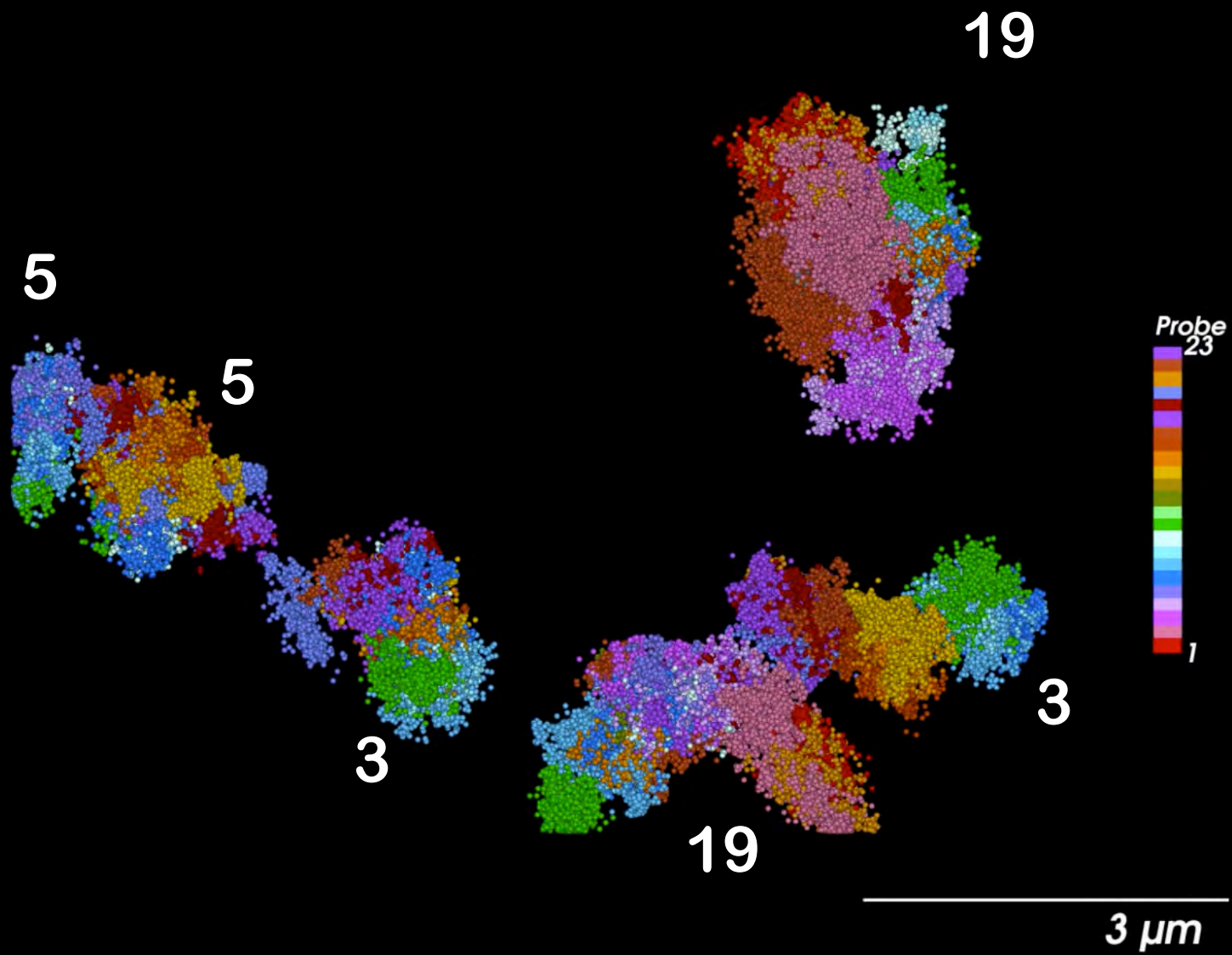




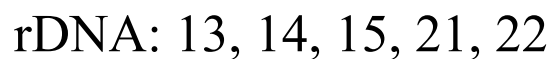








Gap Mbp	Chm #
4	X
34	Y
23	1
5	2
3	3
3	4
3	5
3	6
3	7
3	8
20	9
4	10
4	11
3	12
20	13
19	14
20	15
11	16
3	17
3	18
3	19
3	20
13	21
16	22



PI collaborators on PGP
NIH NHGRI CEGS
Center for Genomically Engineered Organs

Boyden, Church, Lewis, Wu



DARPA, NSF, DOE
HGP-Write Isaacs, Wang, Silver, Way



NIH Transformative Awards

Boyden, Church, Kording
Molecular Recording



Church, Yankner
Human Alzheimer's iPSC
Schizophrenia & Bipolar

