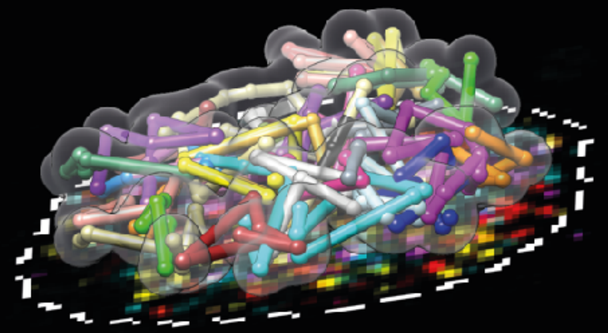
Chromosome tracing with OligoFISSEQ



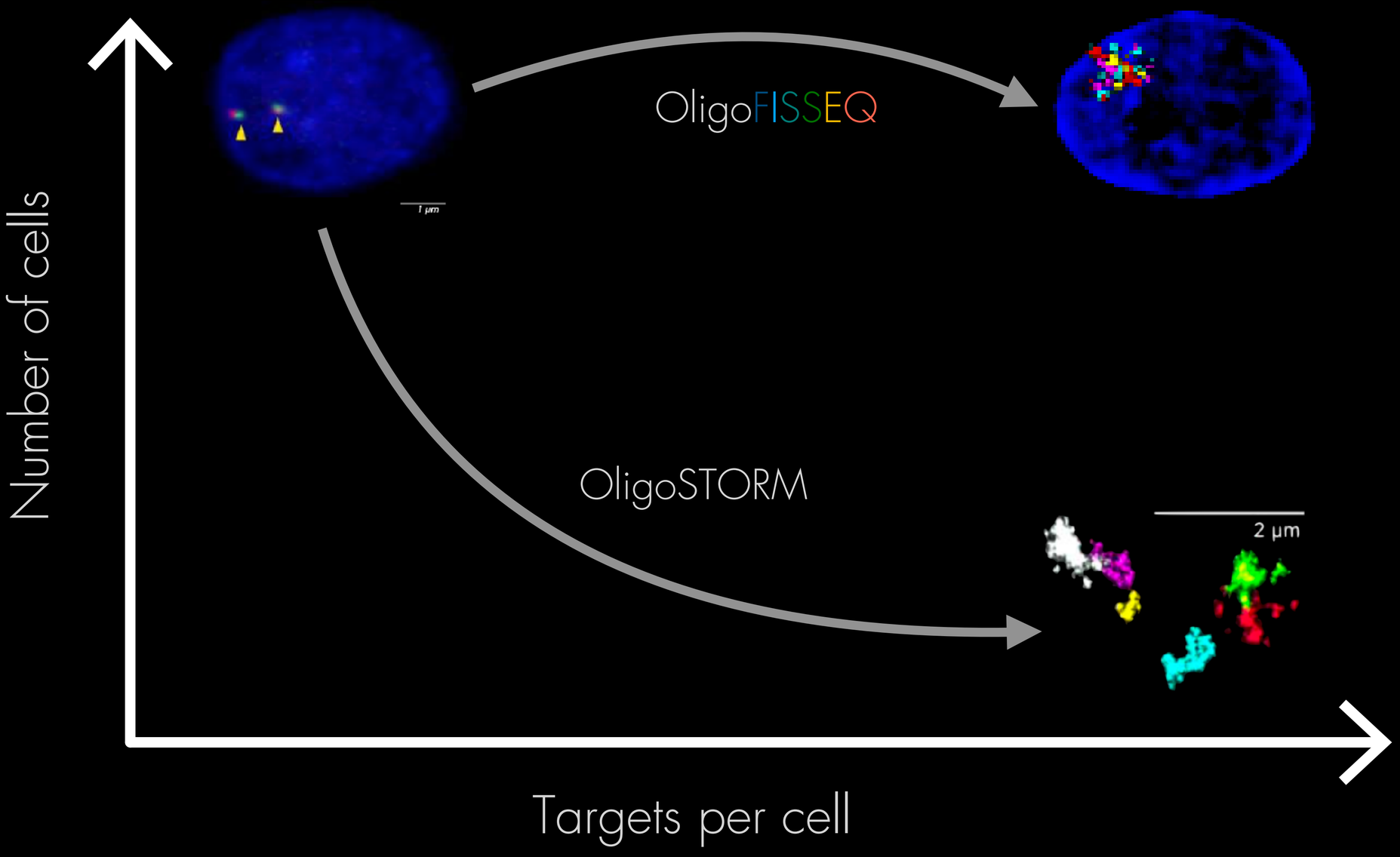
Marc A. Marti-Renom

CNAG-CRG · ICREA

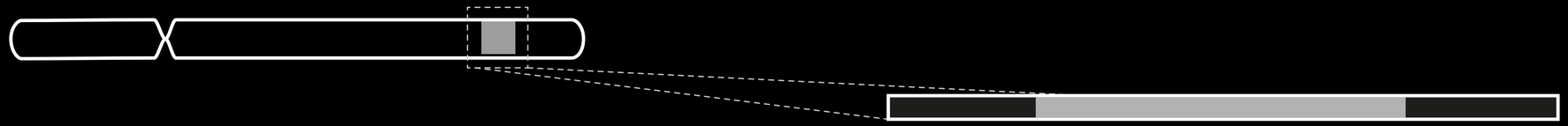
Huy Nguyen
Shyamtanu Chattoraj
David Castillo

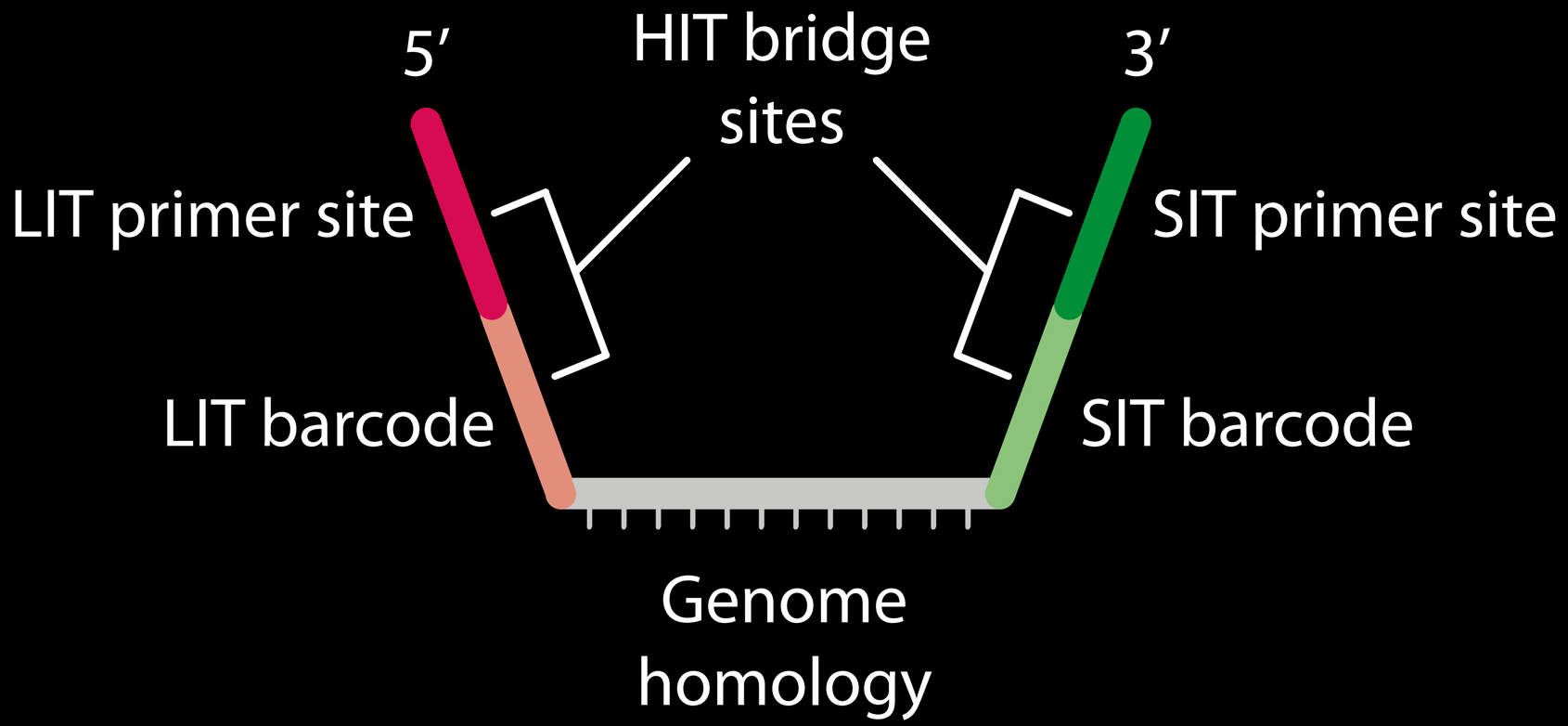
in collaboration with the Wu Lab (HMS)

Nature Methods (2020) 17 p822


Number of cells
OligoFISSEQ
OligoSTORM
2 µm
Targets per cell

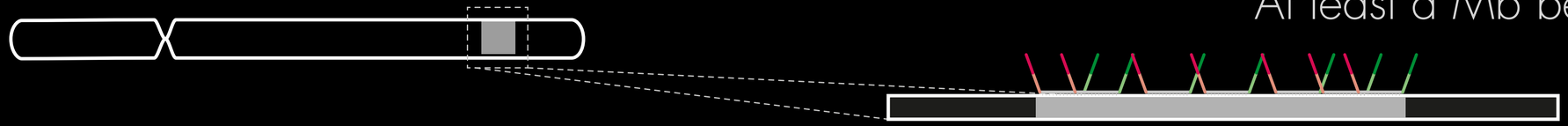
OligoFISSEQ



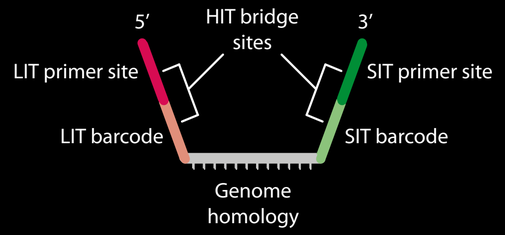


OligoFISSEQ

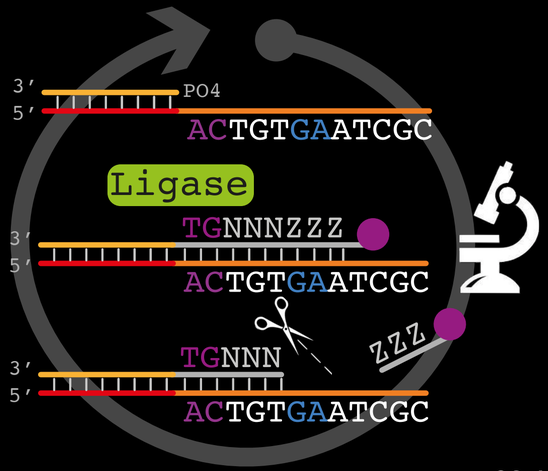
From tens of kb to Mb
Min. of few 100s oligos/target
At least a Mb between targets

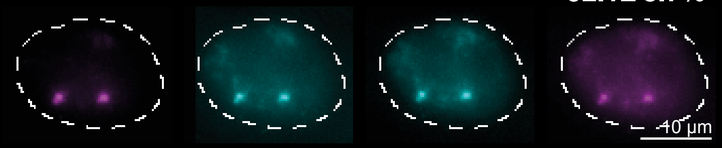


OligoFISSEQ

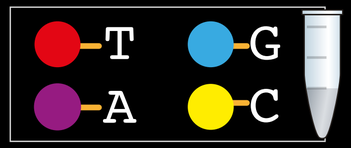


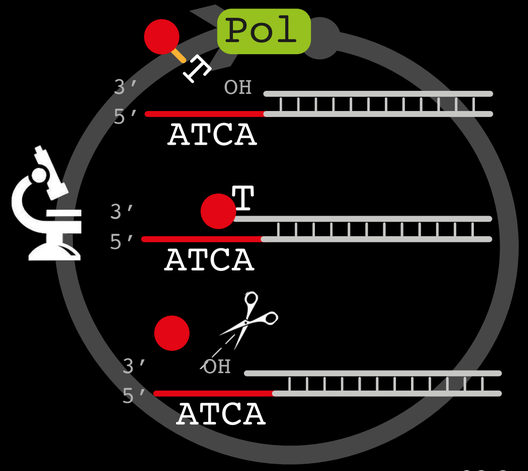
Ligation based Identification of Targets

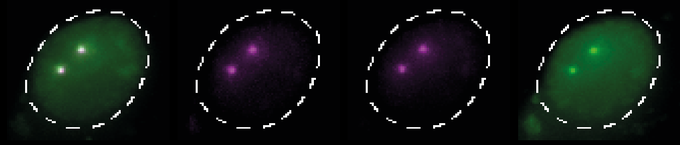




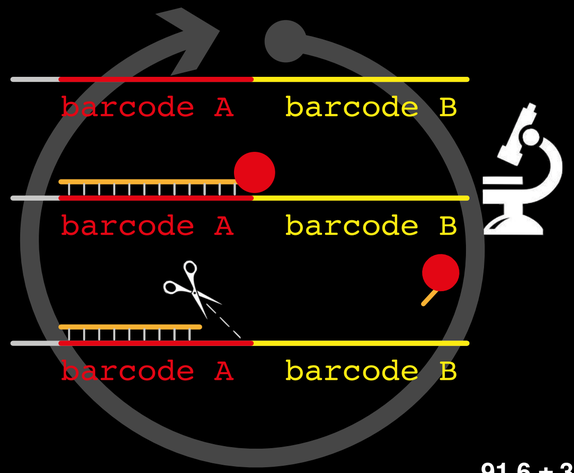
Synthesis based Identification of Targets

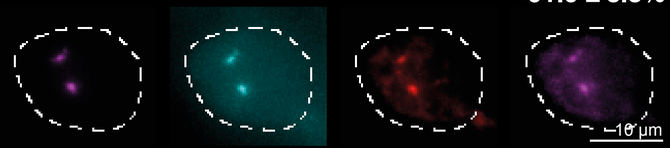






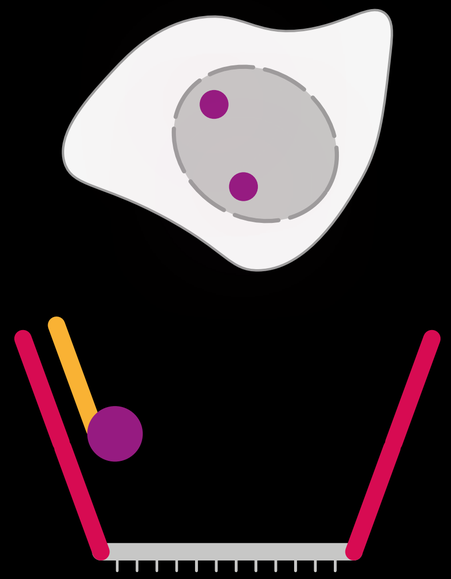
Hybridization based Identification of Targets





OligoFISSEQ scales exponentially!

Sequential hybridization

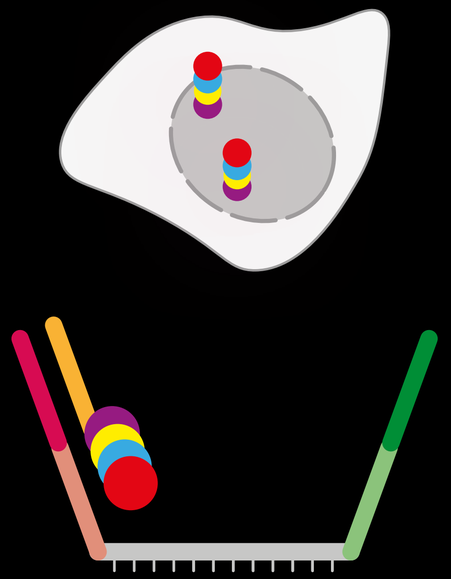


# of targets = F*N

F = # of fluorophores

N = # of seq. rounds

Barcode sequencing

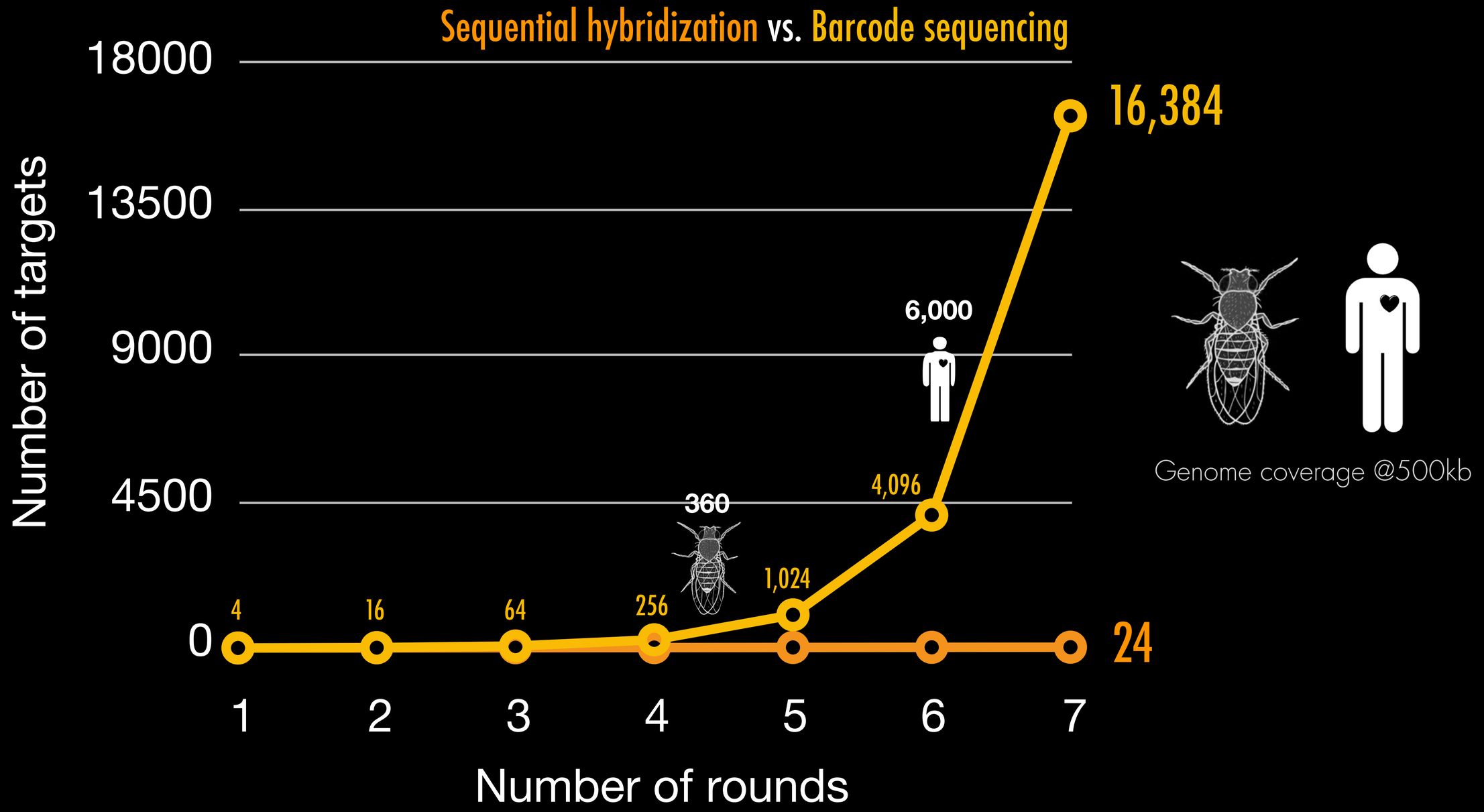


# of targets = F^N

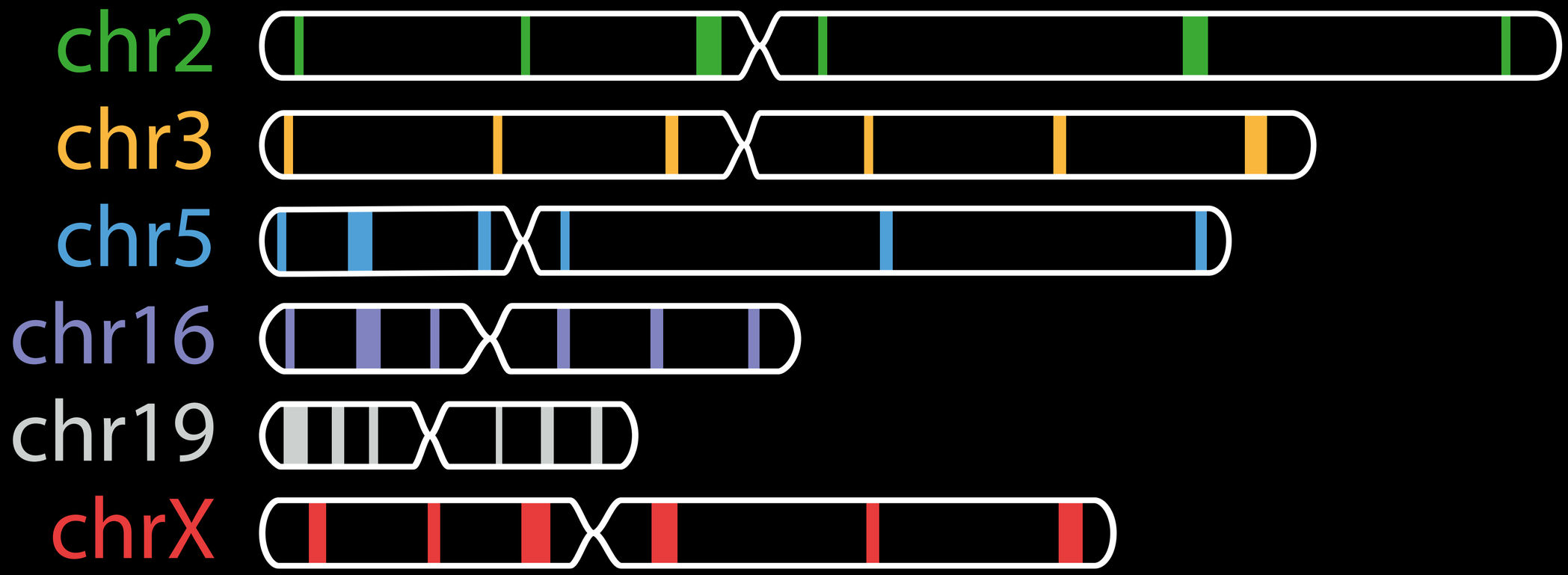
F = # of fluorophores

N = # of seq. rounds

OligoFISSEQ scales exponentially!

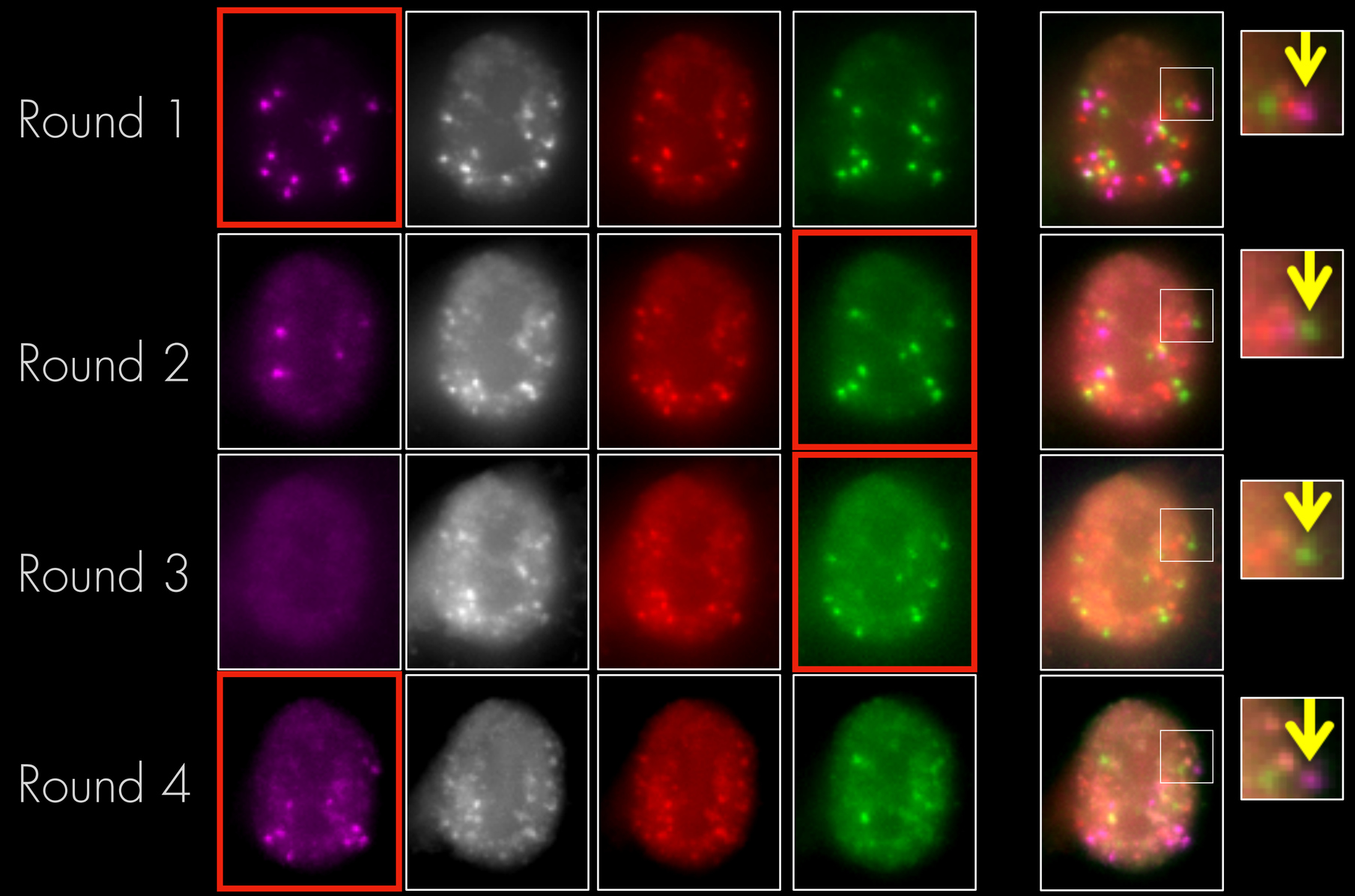


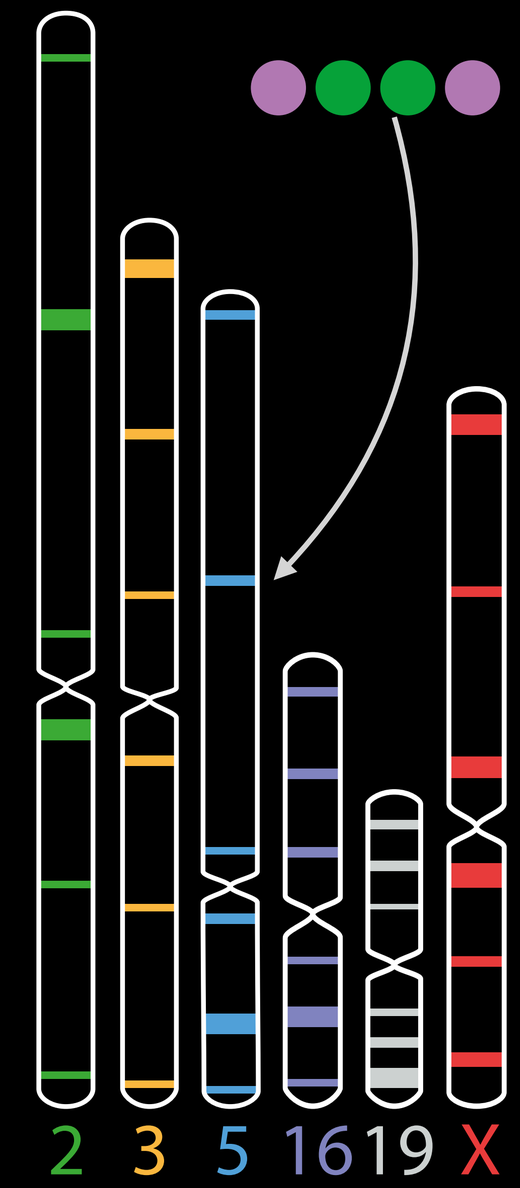
Proof-of-principle

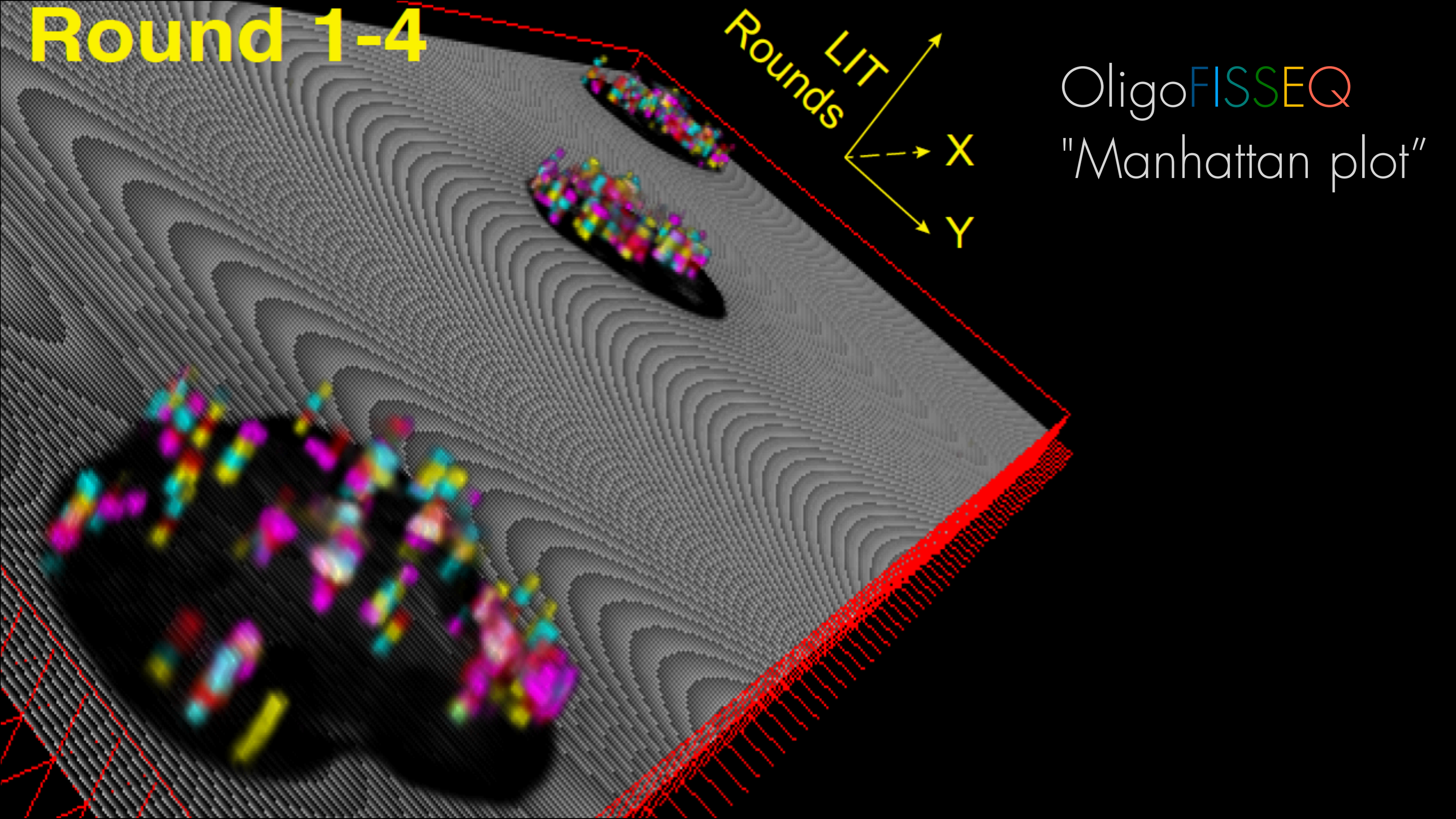


600kb-1Mb/target (876 kb average)
5,000 oligos/target
7-70Mb between targets

Detecting a given target



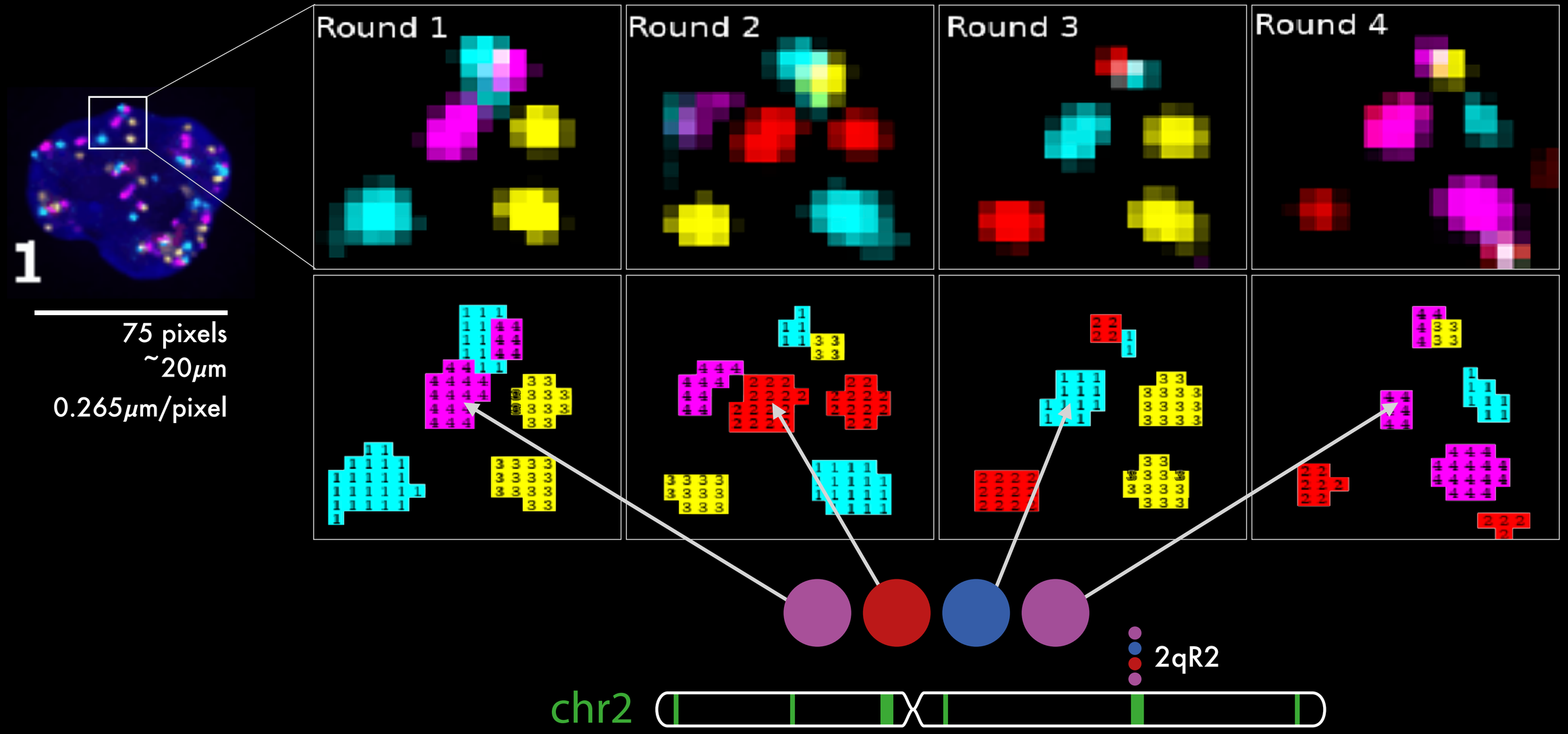




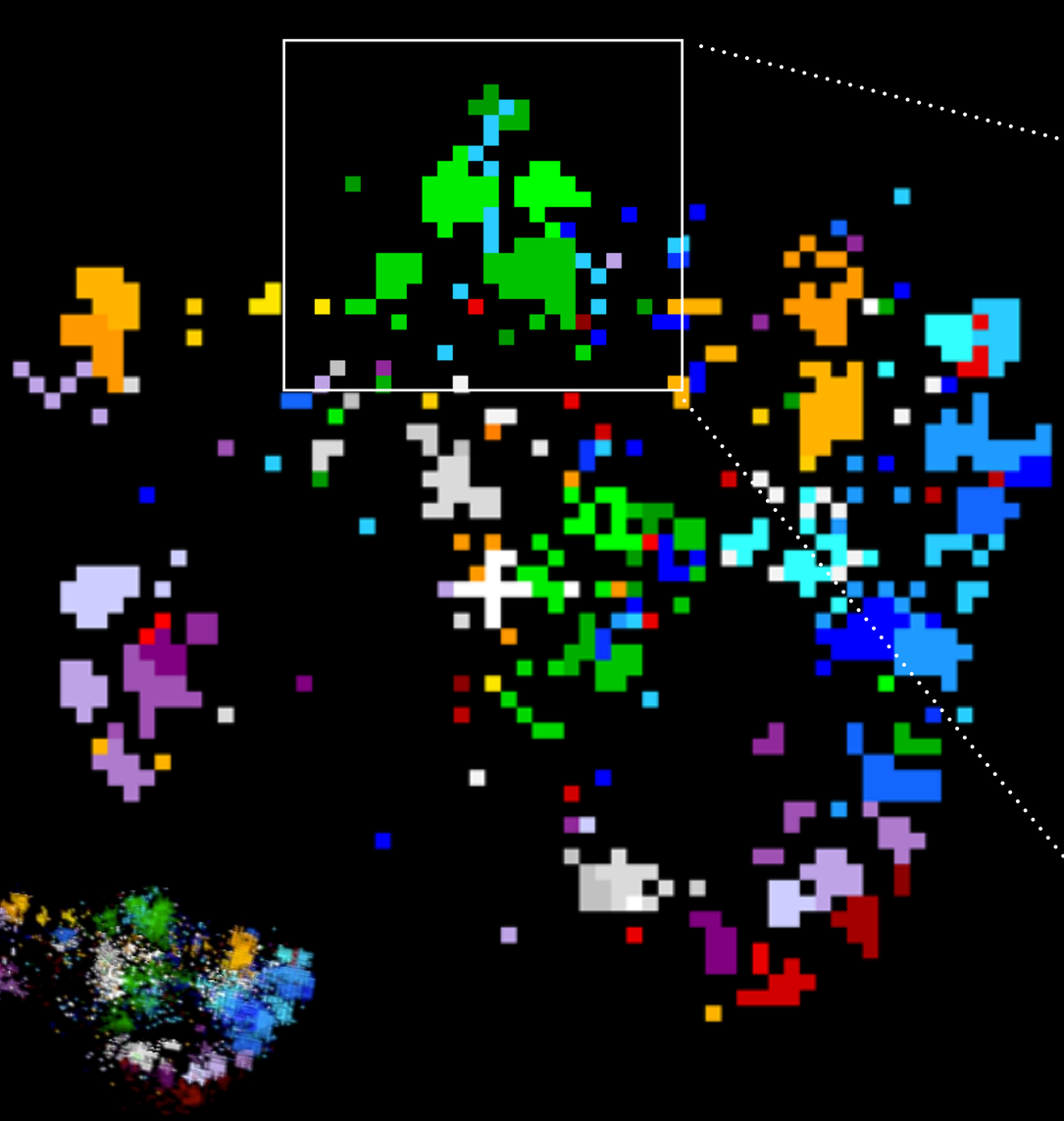
OligoFISSEQ
"Manhattan plot"

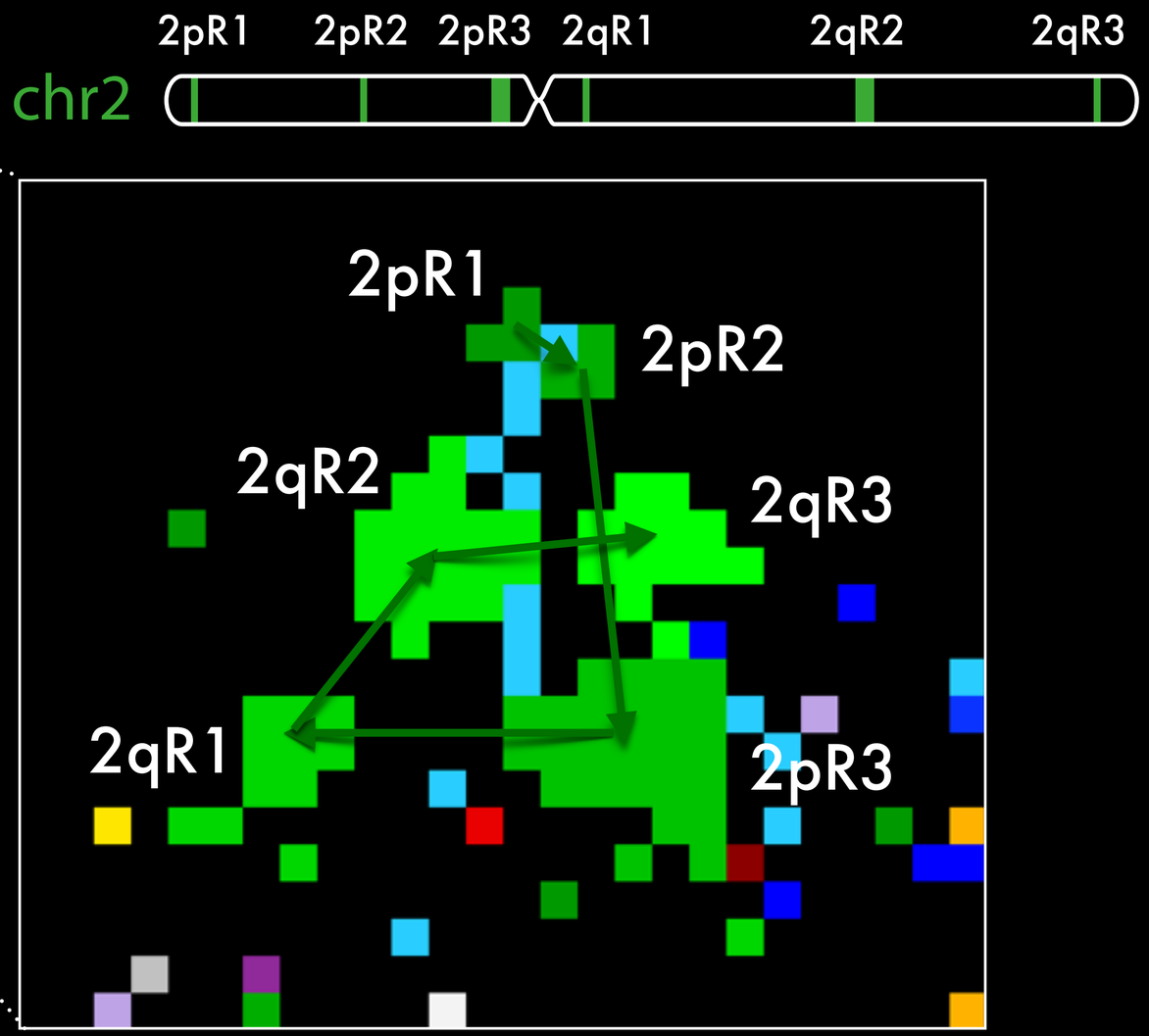
In OligoFISSEQ every pixel matters & make "patches"

4 rounds / 4 channels

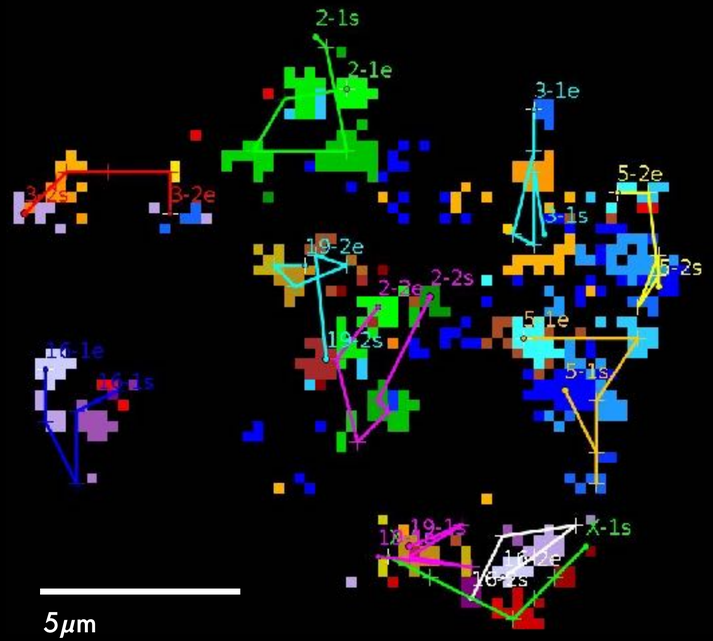


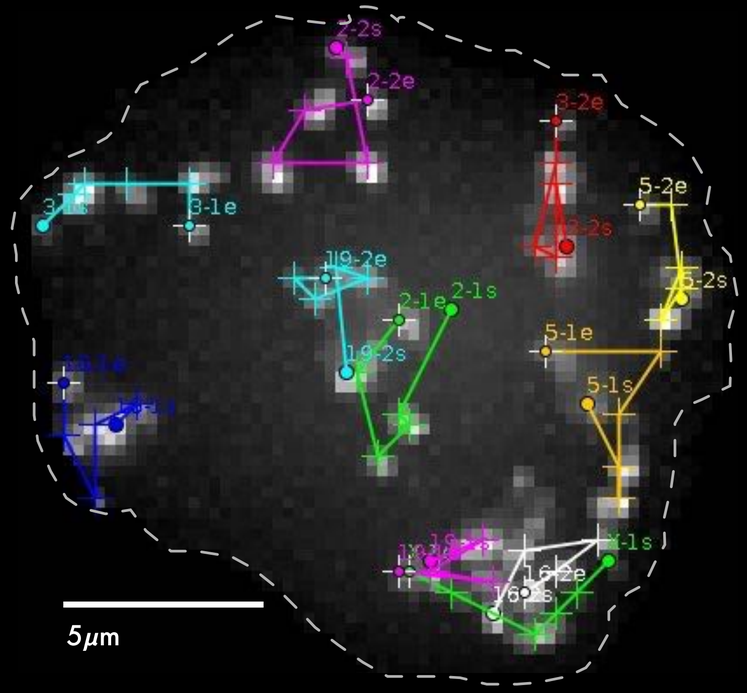
In OligoFISSEQ every pixel matters & make "patches"

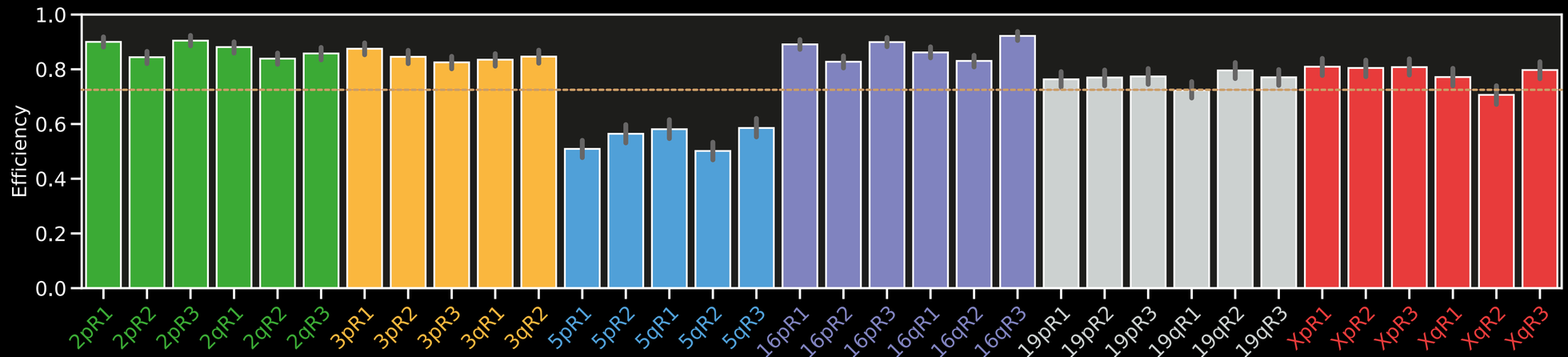




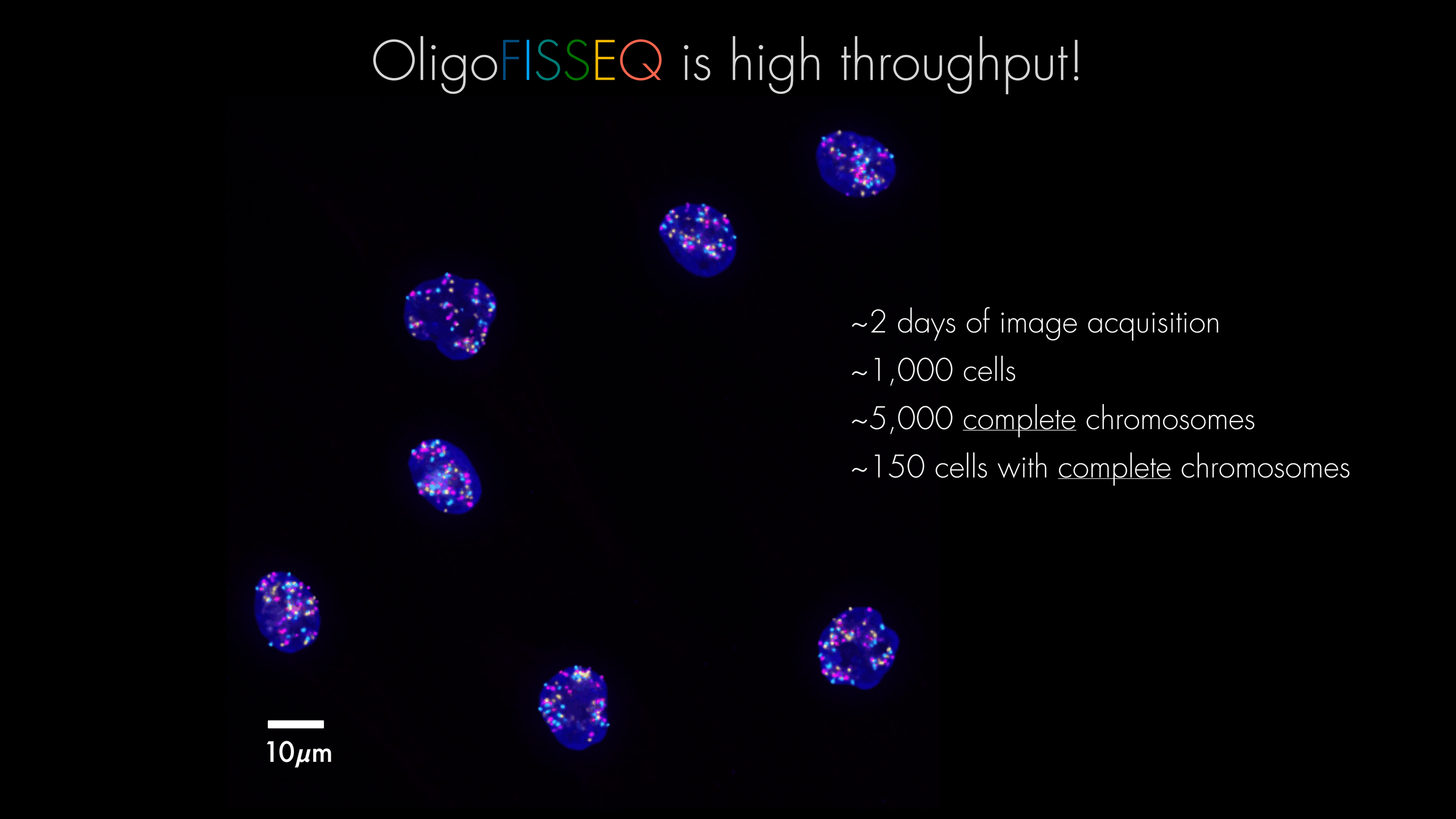
OligoFISSEQ barcode efficiency







OligoFISSEQ is high throughput!



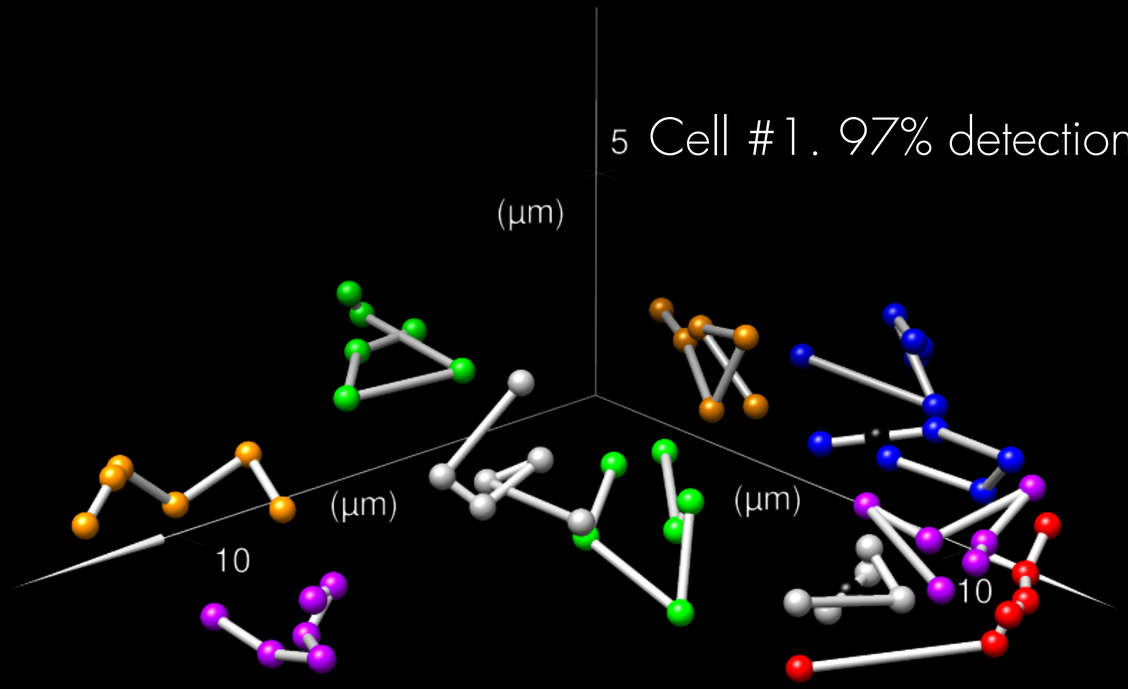
~2 days of image acquisition

~1,000 cells

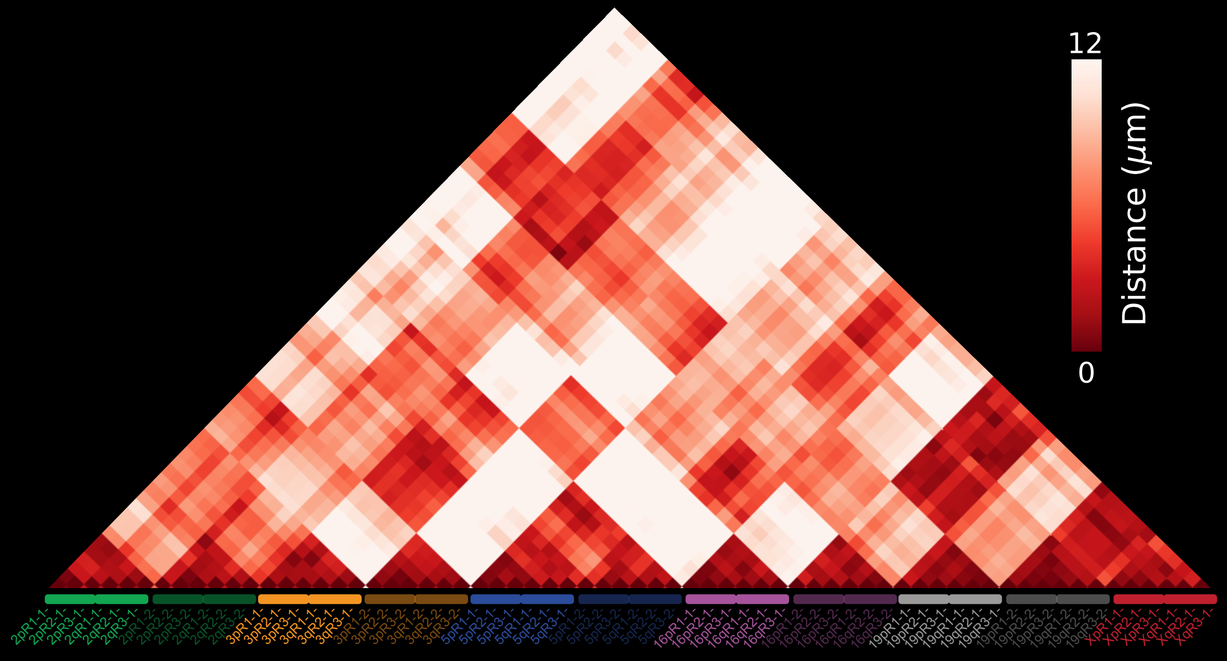
~5,000 complete chromosomes

~150 cells with complete chromosomes

Single cell homolog resolved tracing of chromosomes

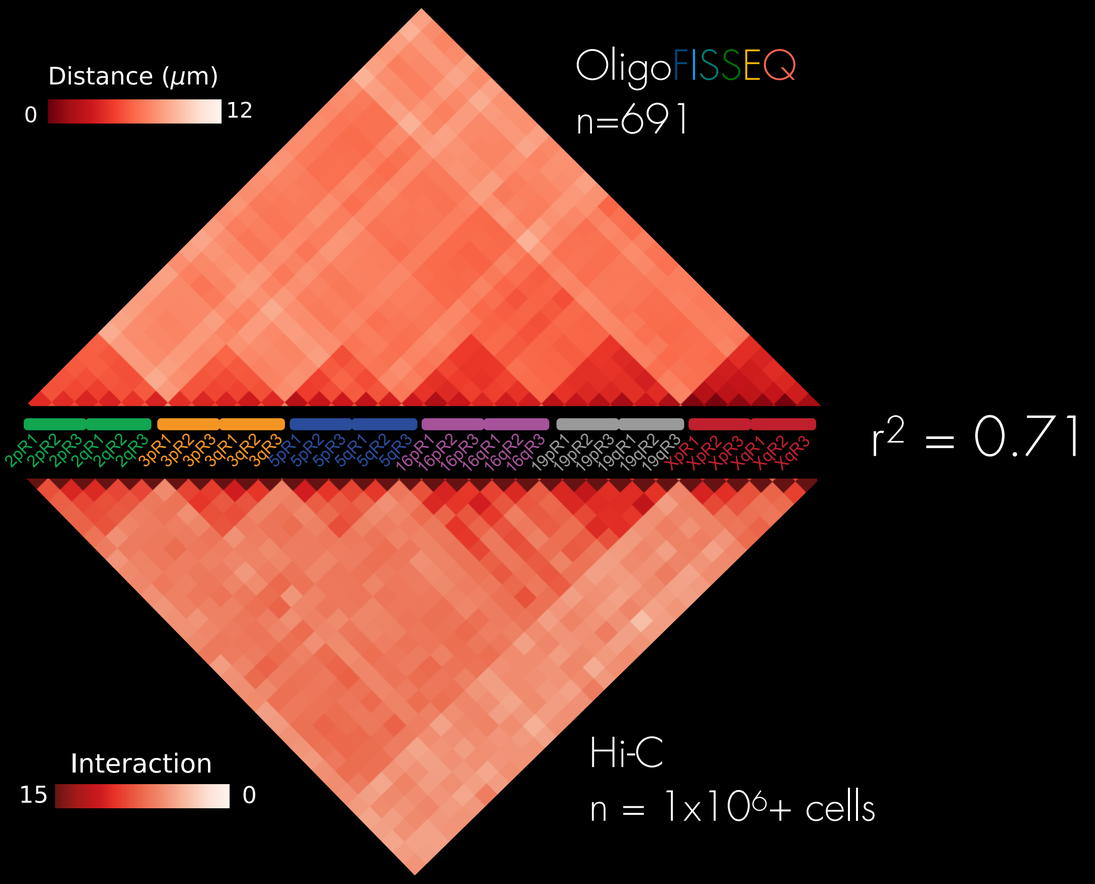


Cell #1. 97% detection

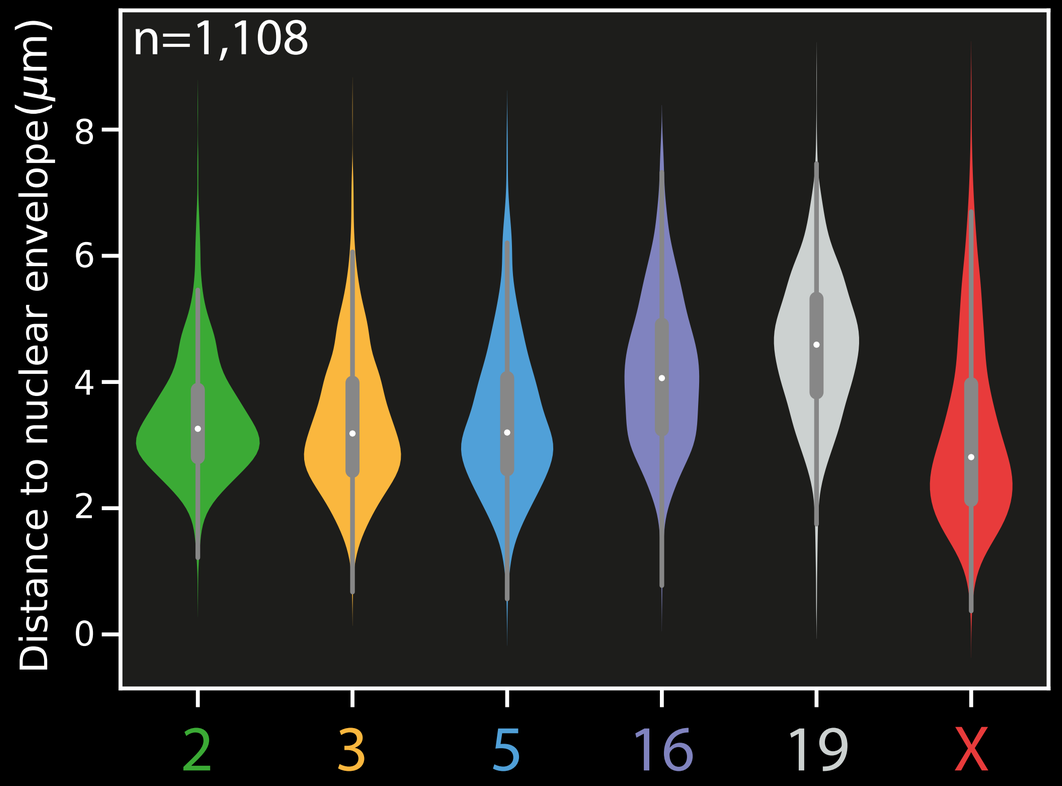


Do OligoFISSEQ tracing maps show known features?

Hi-C contact maps & Radial position of chromosomes



$r^2 = 0.71$

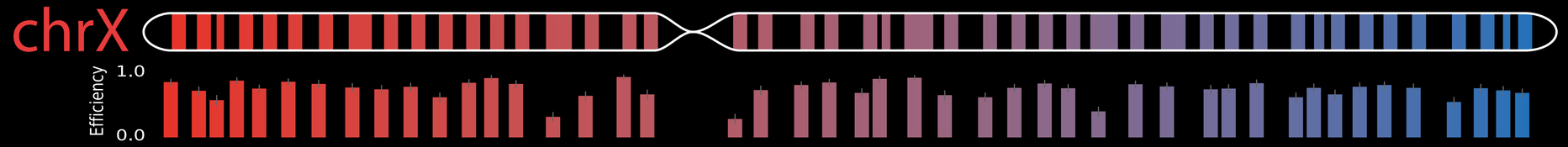


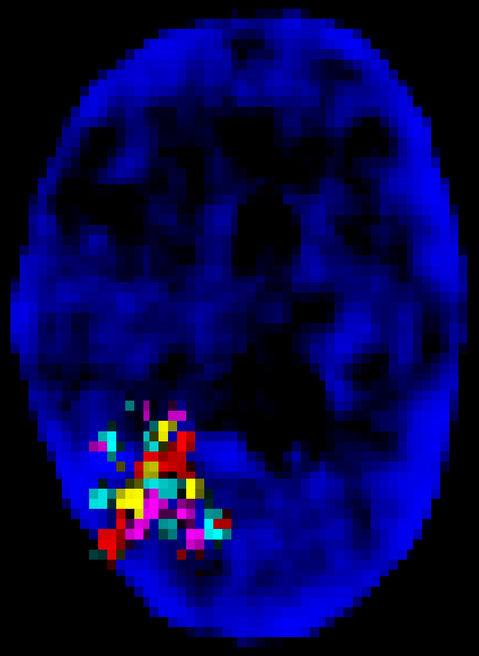
Are the chromosomes randomly located inside the nucleus?

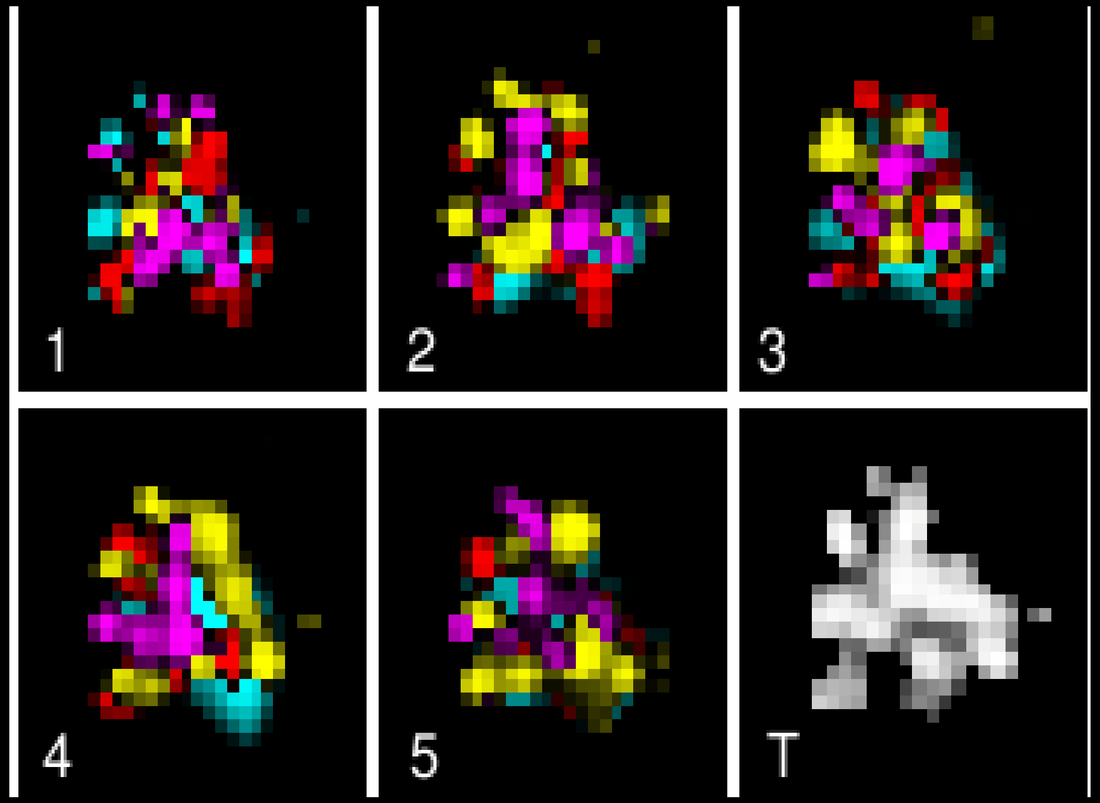
Are there preferred configurations in the cell population?

OligoFISSEQ tracing of (almost) entire chromosomes

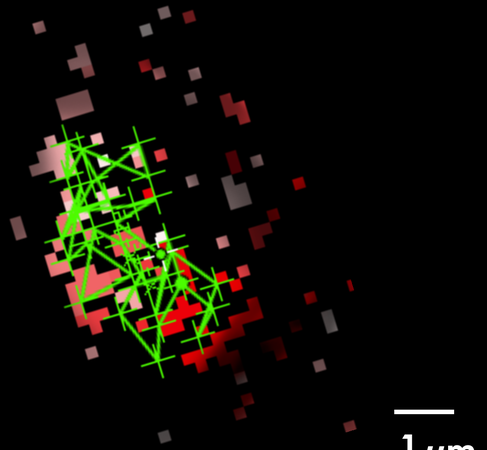
46 Plex in chromosome X





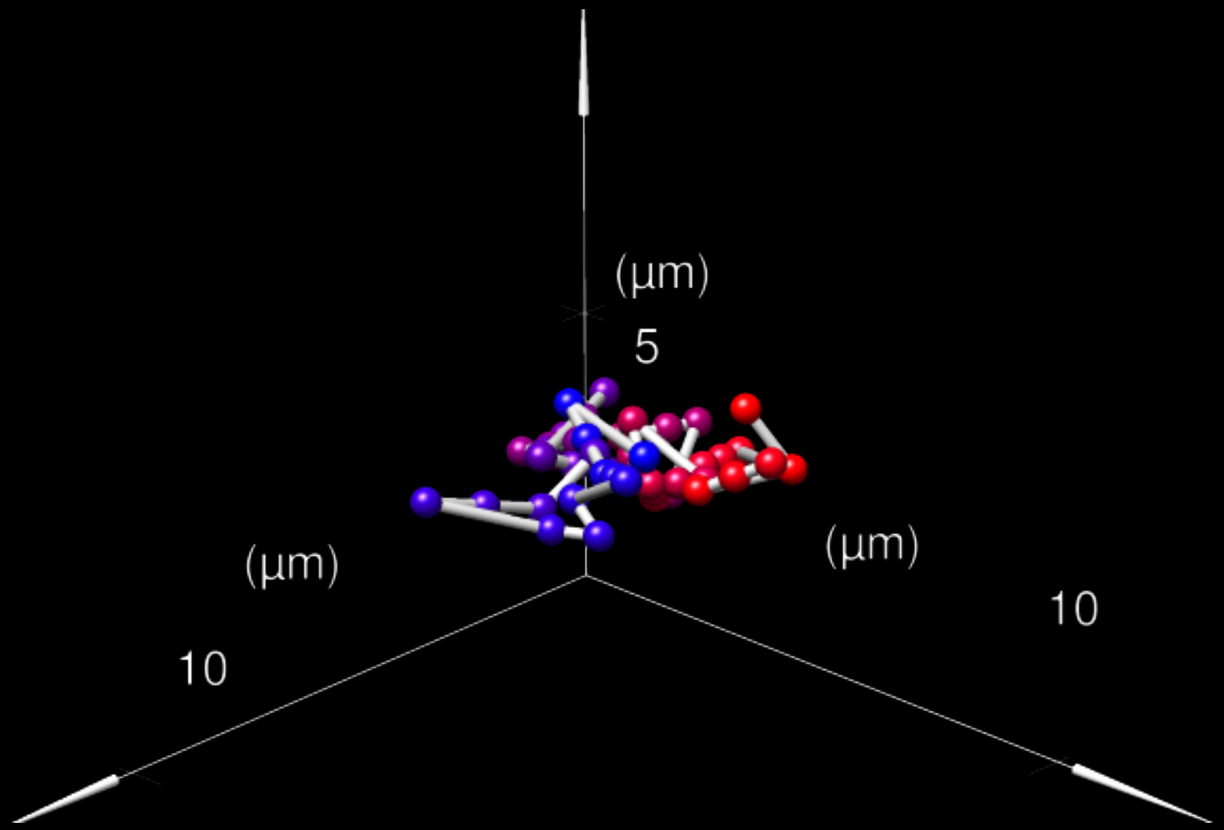


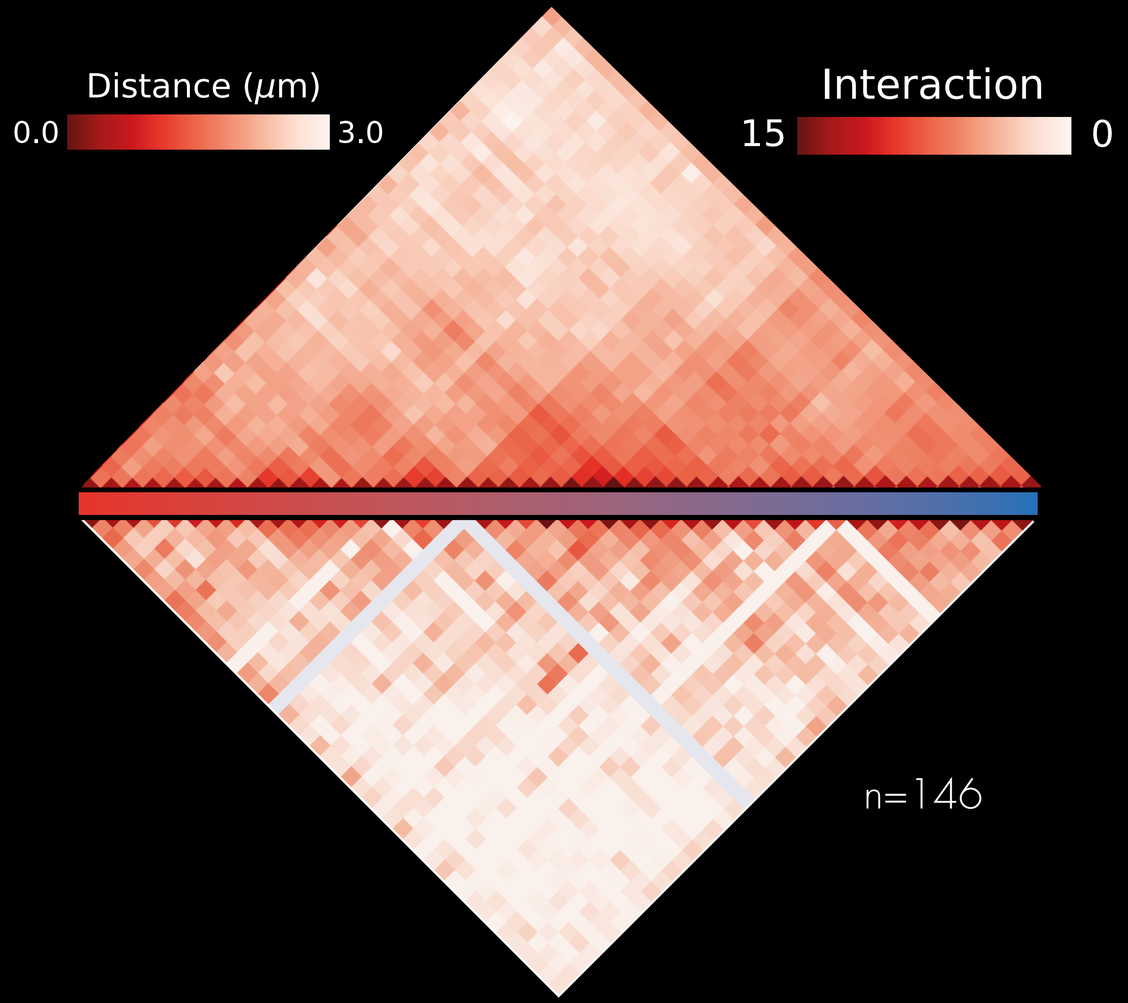
5 rounds
445 kb/probe
2,000 Oligopaints/probe
2 Mb between loci



OligoFISSEQ tracing of (almost) entire chromosomes

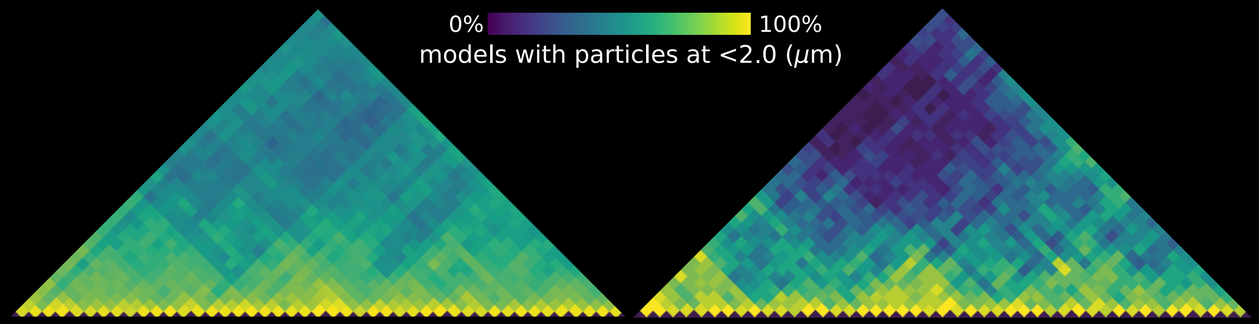
46 Plex in chromosome X





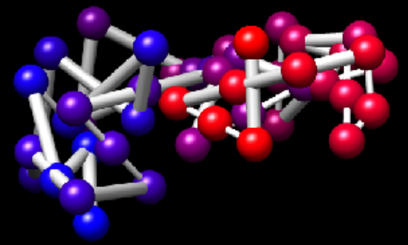
OligoFISSEQ tracing of (almost) entire chromosomes

46 Plex in chromosome X



Cluster 1 (n=156)

Cluster 2 (n=20)



OligoFISSEQ beyond chromosome tracing

OligoFISSEQ pipelined with OligoSTORM

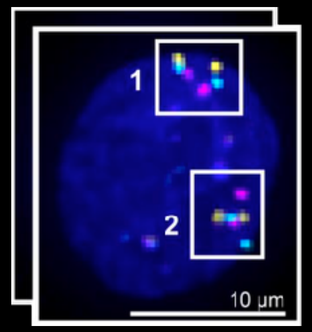
chr2

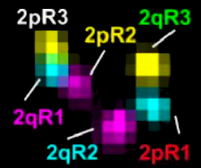
1 **OligoSTROM**
1 round
(2h/round)

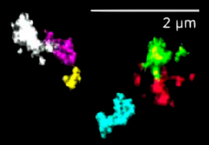
2 **OligoFISSEQ**
2 round
(3h/round)

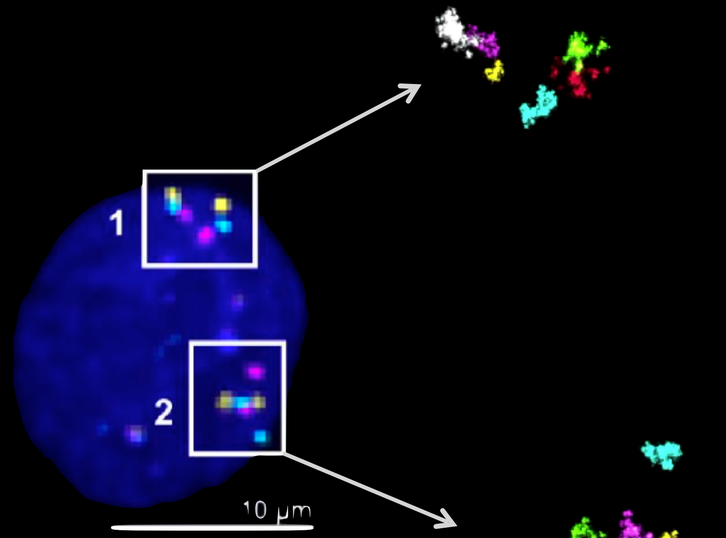
3 **Decoding**
OligoFISSEQ

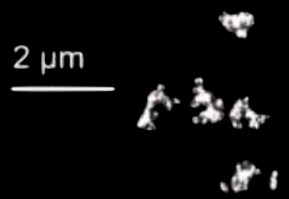
4 **Mapping**
OligoSTROM



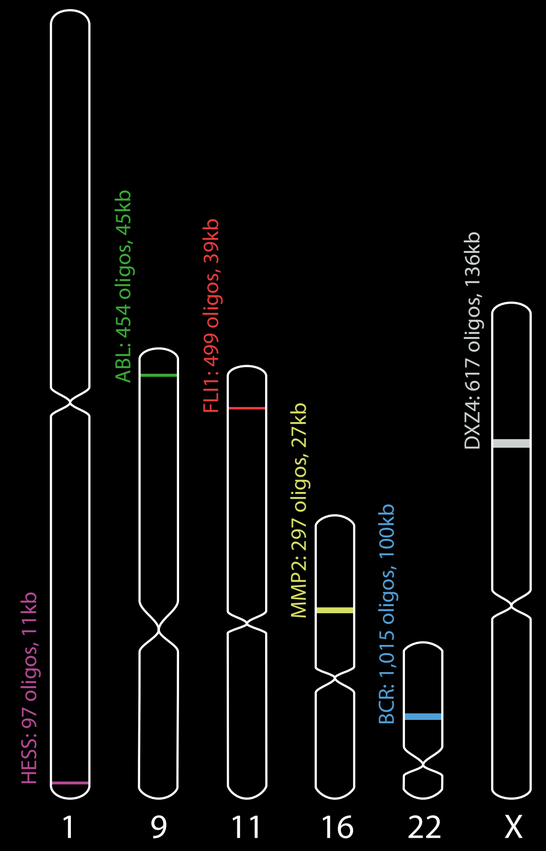


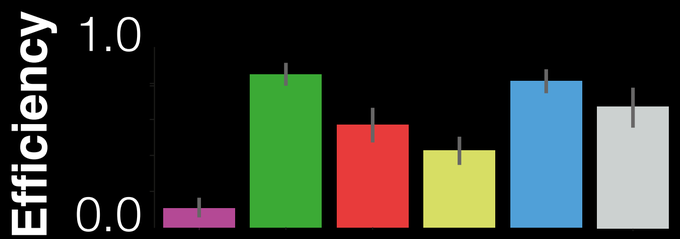


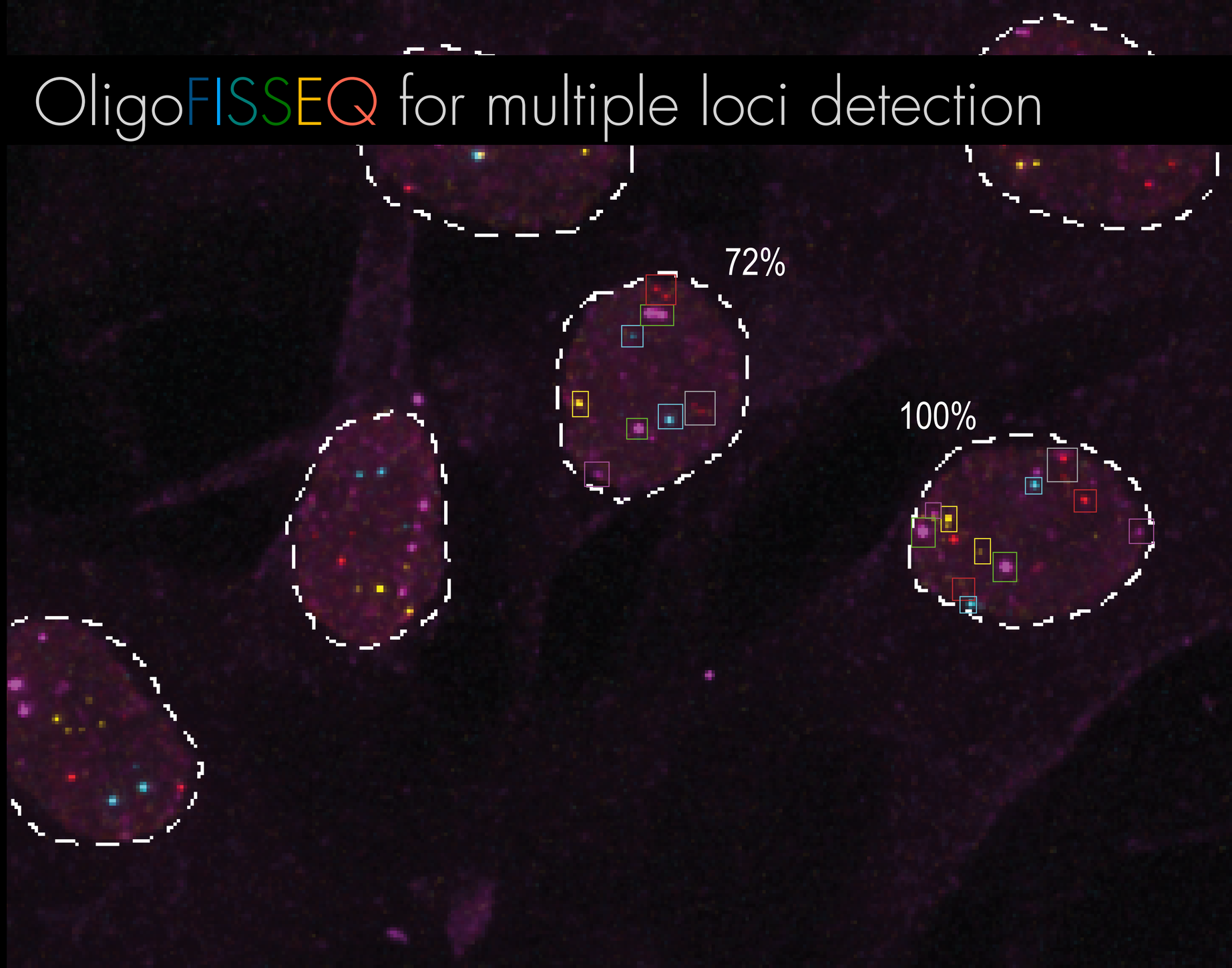




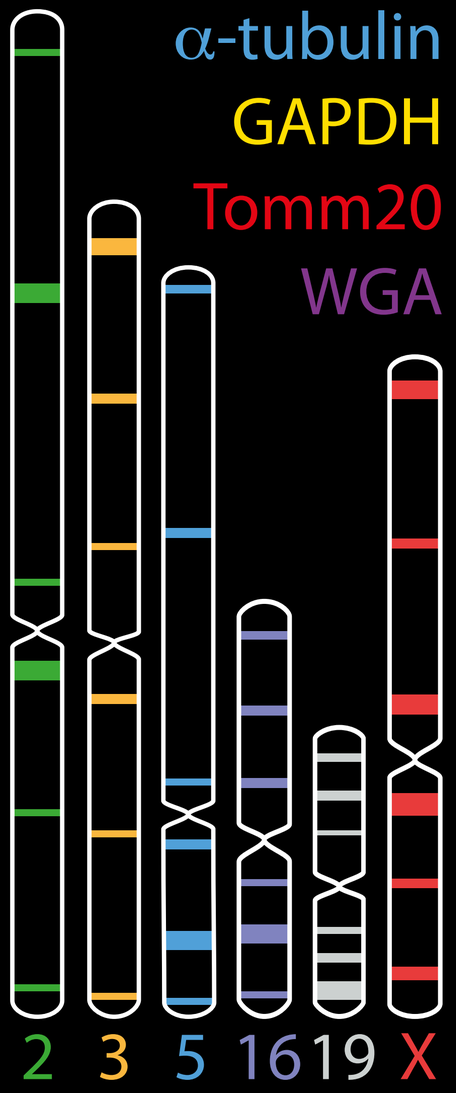
OligoFISSEQ for multiple loci detection



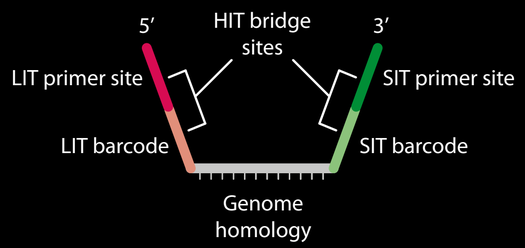




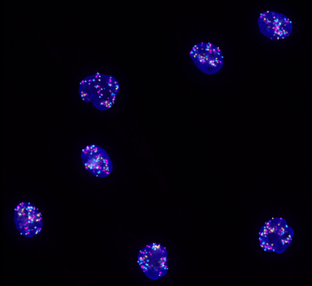
OligoFISSEQ + protein immunofluorescence



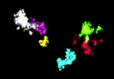
OligoFISSEQ



- Is a set of technologies for in-situ genome mapping
- Is highly versatile: mainstreet and backstreet



- Used with wide-field microscopy permits the analysis of thousands of cells.
- Identifies sub-clusters with specific conformational characteristics



- Can be pipelined with other approaches
  - OligoSTORM
  - Protein immunofluorescence
  - RNA…

http://marciuslab.org
http://3DGenomes.org

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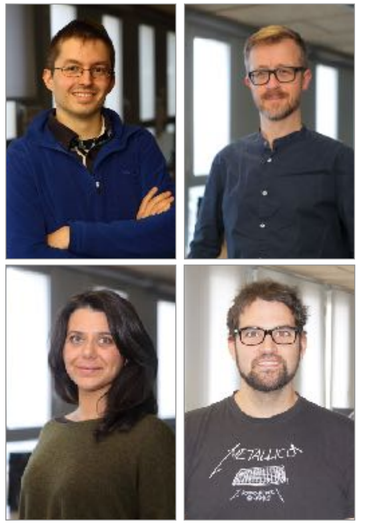

In collaboration with the Wu Lab — Ting Wu, Huy Nguyen & Shyamtanu Chattoraj

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