HARVARD
MEDICAL SCHOOL

Resolving chromosome 19 in super-resolution within single cells

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Abstract

Our laboratory has developed a suite of new tools derived from our Oligopaint FISH technology for visualizing genomes at a resolution of ≤20 nm, and I am using these tools trace whole chromosomes at the single cell level in a homolog-specific fashion. In particular, I will be visualizing the components of genomic organization ranging in size from single genes and loops to topologically associated domains (TADs), active and inactive subchromosomal regions (A/B-compartments), and even whole chromosomes. This study will enable me to query whether and, if so, how chromosome topology is correlated with genomic functions. For instance, are different transcriptional states associated with distinct chromosomal signatures?

The technologies I am using are HOPs, which uses SNPs to distinguish maternal and paternal chromosomes, and two super-resolution imaging strategies called OligoSTORM and OligoDNA-PAINT (Beliveau B. et al., 2015, Nat. Commun.), which combine Oligopaints with, respectively, stochastic optical reconstruction microscopy (STORM, Rust et al., 2006, Nat. Methods) and DNA-based point accumulation for imaging in nanoscale topography (DNA-PAINT, Jungmann R. et al., 2010, Nano Lett.). Most recently, OligoSTORM has been used to reveal that active, silenced, and Pc-repressed chromatin represent three different kinds of DNA packaging and that the boundaries between two flanking chromatin domains reflect the types of chromatin in those domains (Boettiger A. N. et al., 2016, Nature).

In summary, by further developing our technologies to visualize genomes and then applying them to visualize an entire human chromosome, I hope to make contributions to both the field of imaging technology and to our understanding of the relationship between genome function and the structure of genes and chromosomes.

Aims

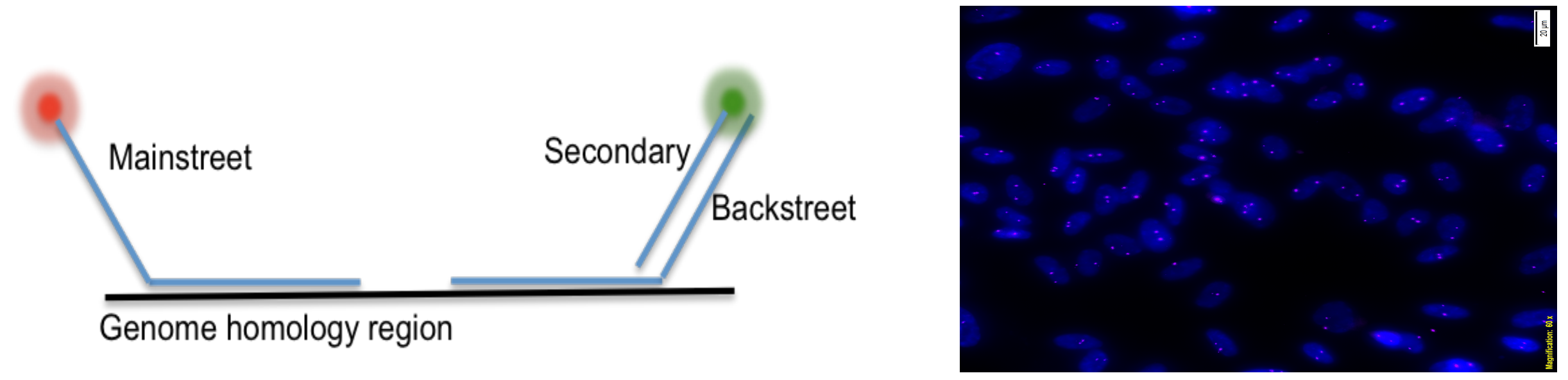
Aim 1: complete the first, to our knowledge, trace of an entire chromosome in super-resolution, visualizing one transcriptionally-associated compartment at a time, in order to resolve how a chromosome folds into a chromosomal territory.

Aim 2: conduct a multi-scale comparison of the conformations associated with active and inactive chromosomal regions in a variety of cell lines, examining loops, TADs, and compartments.

Addressing challenges in visualizing genomes

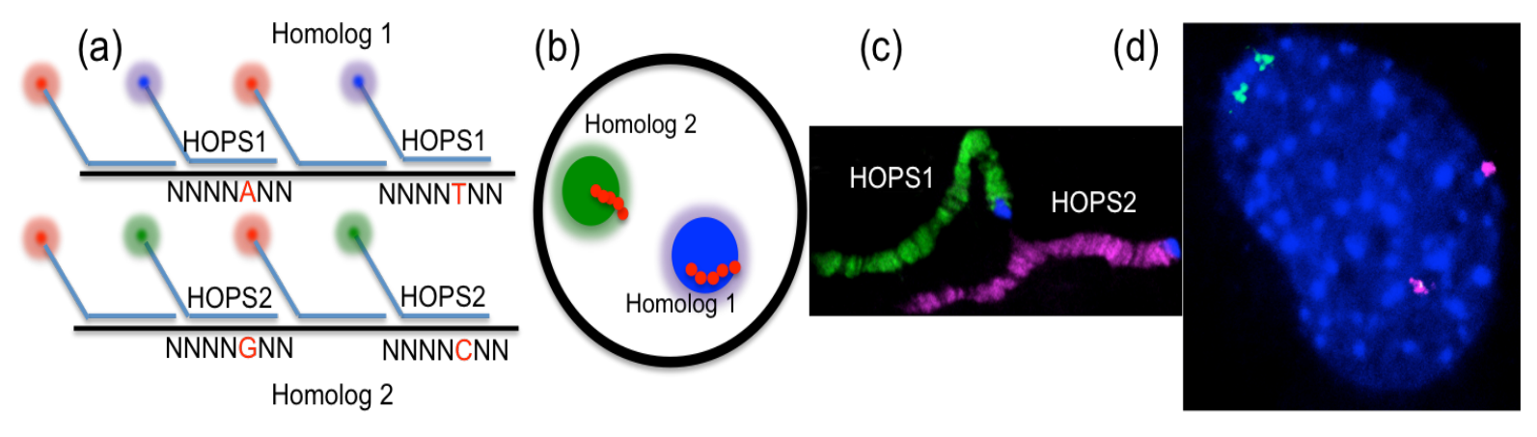
Our laboratory has made significant progress in addressing two major challenges to genome visualization, these being the labeling and resolution of such a dense and complex entity.

Utilizing Oligopaints to label genomes



Oligopaints are short ssDNA with a 42 nt region of genome homology, a ssDNA overhang (Mainstreet) that carries a fluorophore, (here, a red dye) and if desired, a second ssDNA overhang (Backstreet) and a dye-coupled secondary oligo for signal amplification. For instance, Oligopaints yields 2 spots, one per homolog, in >95% in IMR90 cells.

Homolog-specific Oligopaints (HOPs)

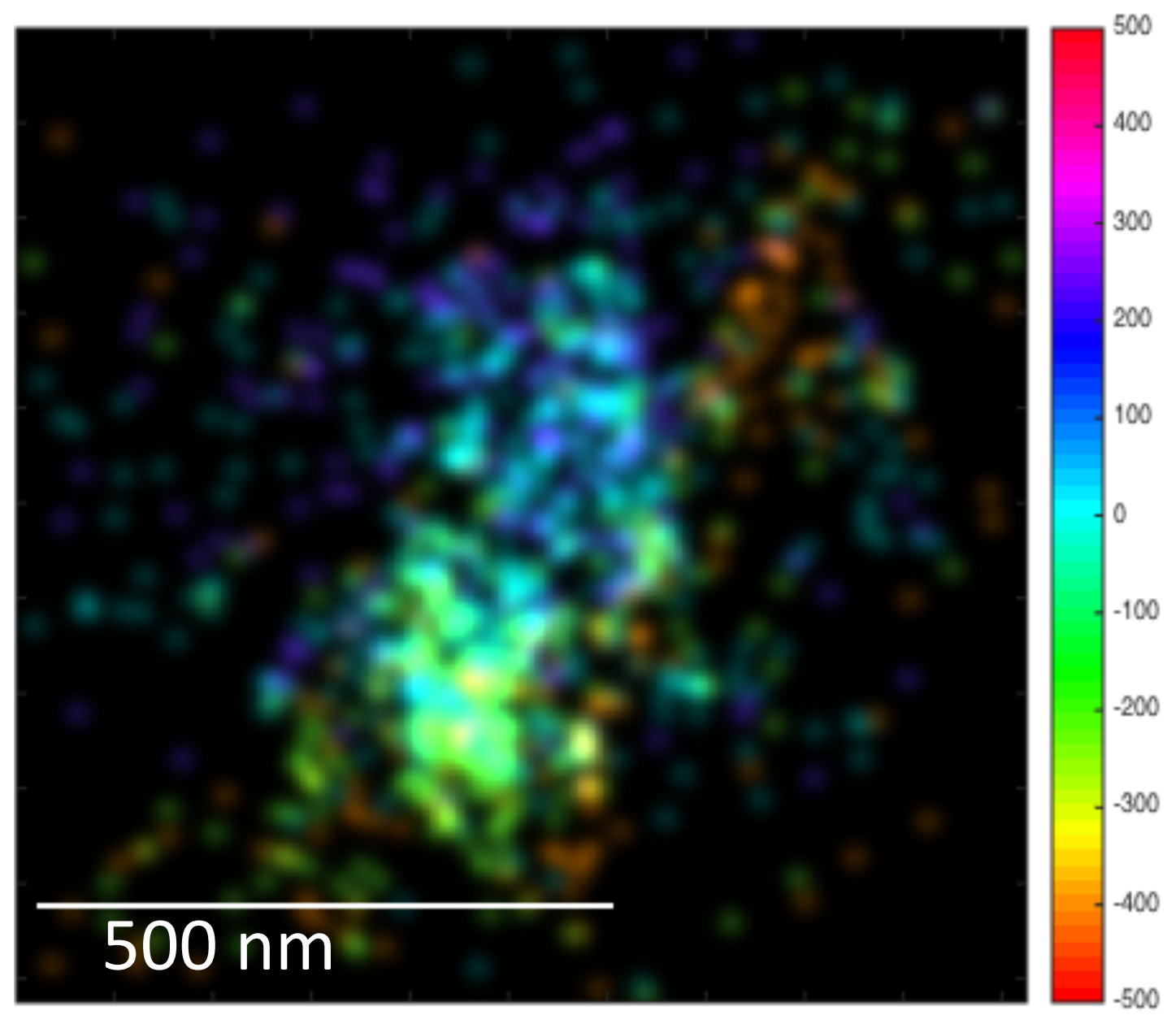


Homolog distinction of (c) polytene chromosome arms of a hybrid *Drosophila* salivary gland and (d) X-inactivation centers of a XXXX hybrid tetraploid mouse cell. Beliveau et al., 2015, Nat. Commun.

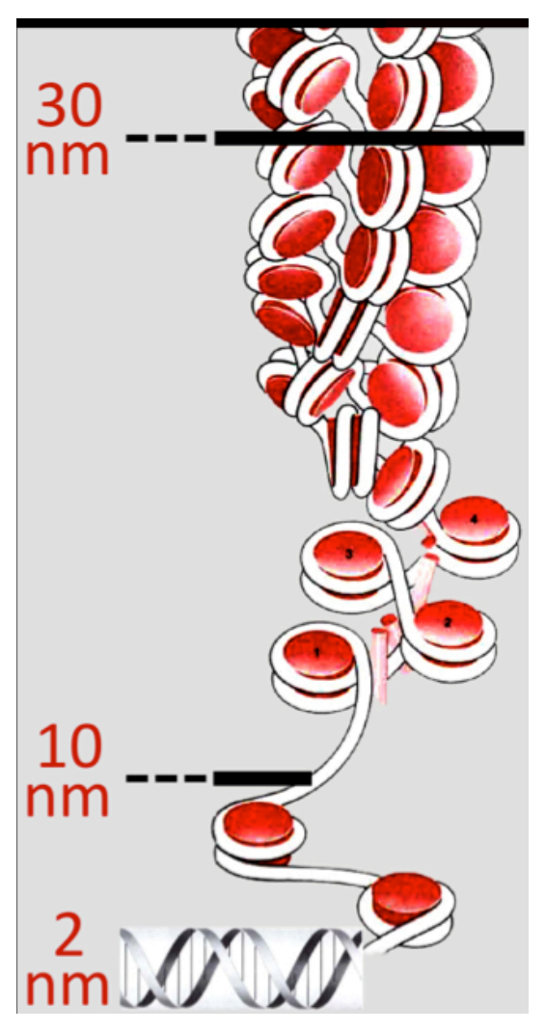
(a) HOPS are computationally designed to address SNPs (red bases) and thus discriminate different homologs. (b) A nucleus where two homologs are imaged through HOPS using conventional microscopy (green and blue), followed by OligoSTORM or OligoDNA-PAINT imaging to resolve the structure of the chromosomes.

Visualizing the genome at super-resolution

Here we demonstrate our cutting edge technology by utilizing OligoSTORM to visualize a single TAD (720 kb) of chromosome 19 at up to 20 nm resolution.



Cartoon illustrating dsDNA and nucleosome size. Kornberg, R. D., and A. Klug. 1981. Scientific American.



Research methodology and approach

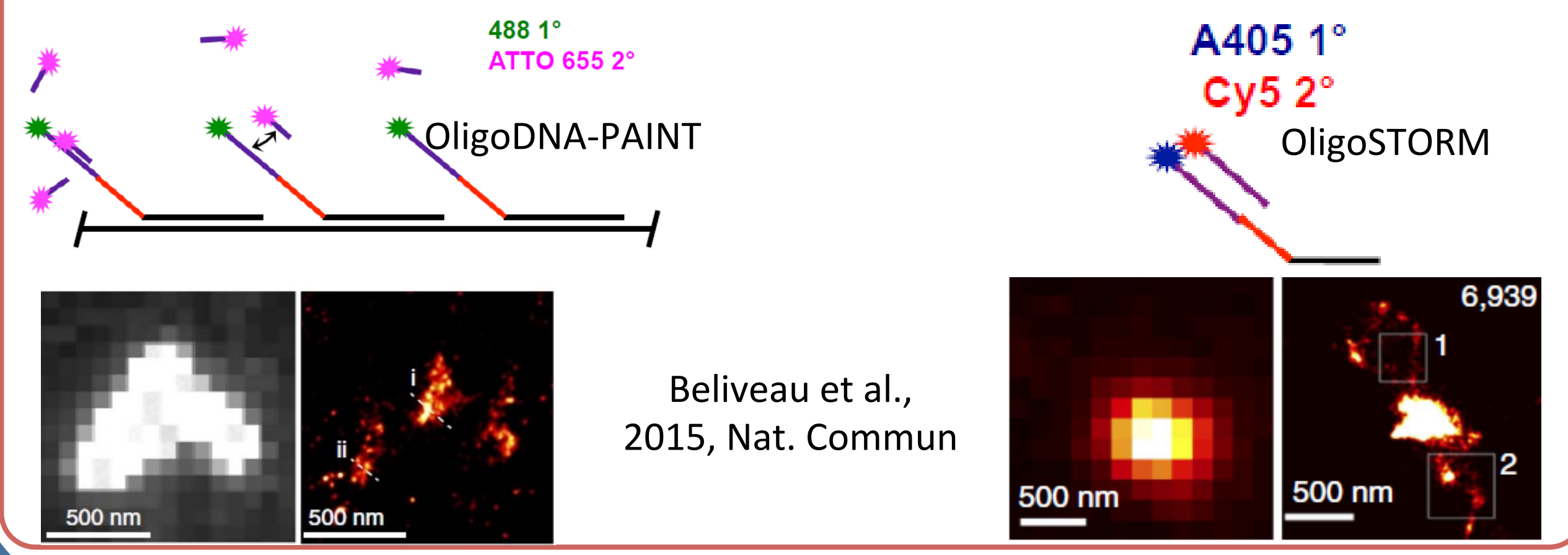
Choice of cell lines

Our choice of cell lines was guided by our: 1) intention to examine how our findings will compare with Hi-C, and, 2) desire to trace chromosomes in a homolog-specific manner. For example, we have chosen three human cell lines, one B-lymphoblast (GM12878), and two primary fibroblasts (IMR90 and PGP1F). GM12878 and IMR90 chromosome organization were well defined through Hi-C studies, providing us with the genomic coordinates of TADs and compartments, enabling us to target these regions by FISH. PGP1F offers additional values to this project; For instance, PGP1F is suitable for distinguishing maternal and paternal homologs, which will provide accurate and homolog-specific analyses of the relationship between chromosome folding and genome function, as PGP1F has been haplotyped and >99% phased (Lo C. et al., 2013, Genome Biol., K. Zhang, personal communication);

Choice of chromosome 19

I will focus on chromosome 19 for three reasons: a) Its length, ~60 Mb, is small enough to be traversed in <20 steps, and yet b) it is long enough to provide a good platform for refining our technology to enable tracing of even the longest human chromosome (~250 Mb). Finally, c) it contains several imprinted regions, wherein the maternal and paternal copies are differentially regulated, thus enabling comparisons of the active and repressed state of a single genomic sequence within the a single cell.

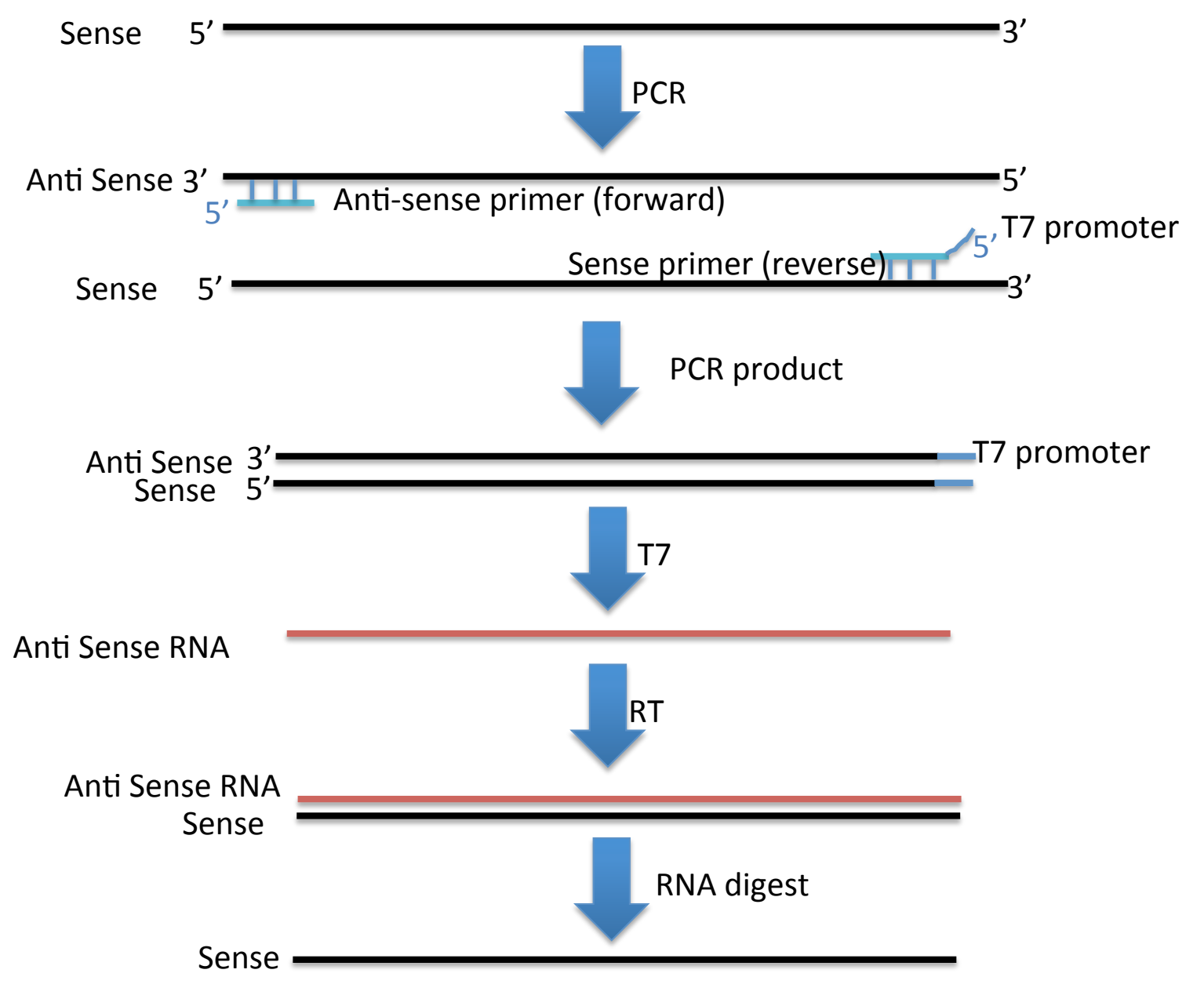
Choice of imaging method



Beliveau et al., 2015, Nat. Commun

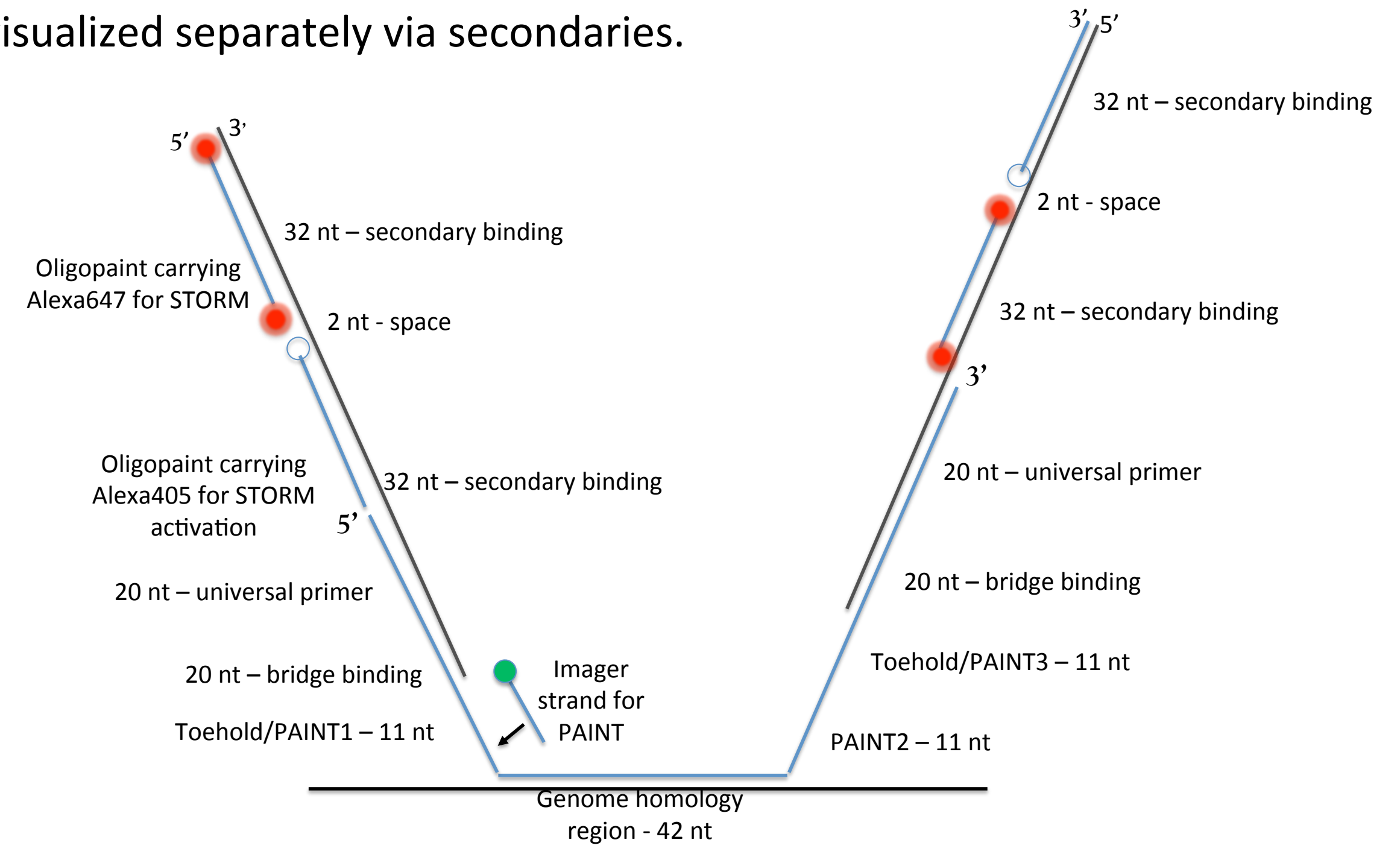
Oligopaints strategy

Oligopaints amplification



Oligopaints design

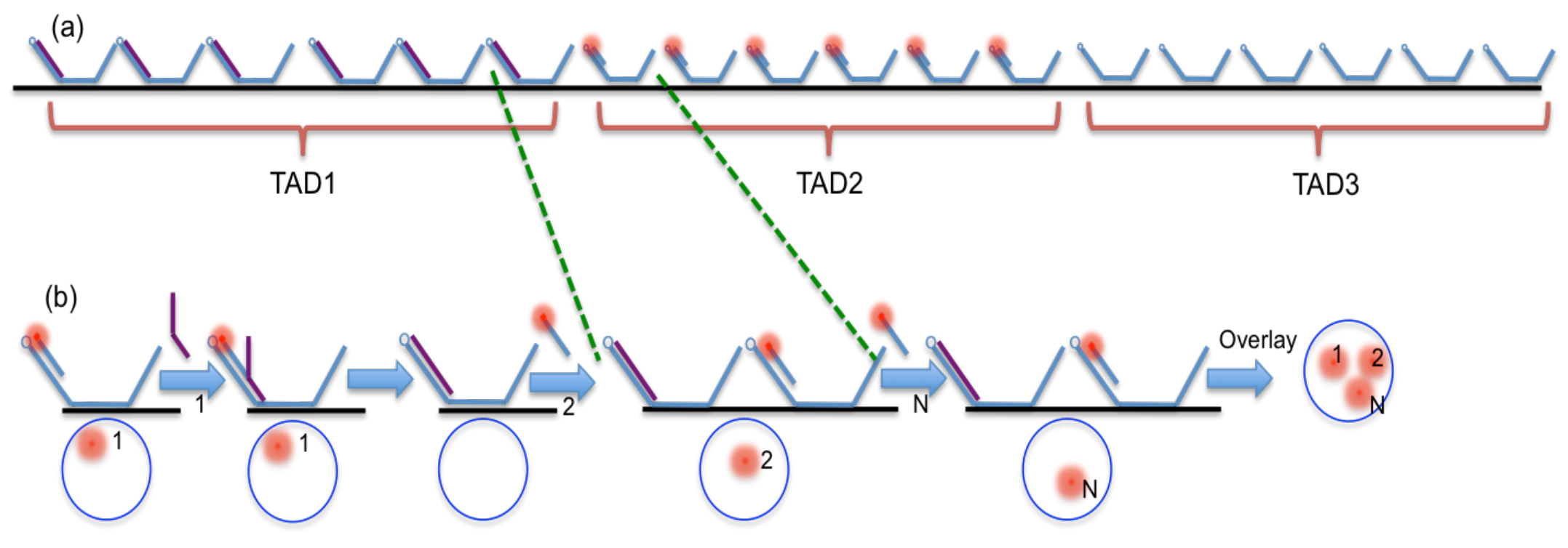
Each of our Oligopaints allows imaging through conventional microscopy, OligoSTORM and OligoDNA-PAINT. An entire Oligopaints library can be hybridized to the genome at once, and yet, allow each region to be visualized separately via secondaries.



Aim1: tracing chromosome 19

Tracing by sequential hybridization

I am honing the protocol for ChromoTracing such that I will ultimately be able to traverse the length of the chromosome in <20 steps, where each step corresponds to one round of hybridization, and each round uses multi-color imaging to label three compartments. To minimize sample degradation and constrain total imaging time to ~24 hours, I will bind all Oligopaint oligos (primary oligos) at once, after which I will image the chromosome, one step at a time, with rounds of OligoSTORM or OligoDNA-PAINT, taking care to remove signals from the previous round by either a) photobleaching the reporter fluorophores or b) using a “toehold” approach to exchange the fluorescent secondary oligo with an unlabeled secondary oligo. I aim to image a minimum of 20 nuclei, expecting each nucleus to display two chromosomal territories corresponding to the two homologs of chromosome 19. Finally, I will determine the parental origins of each homolog through the separate application of HOPs, wherein the Oligopaint probes are designed to target single nucleotide polymorphisms (SNPs) that are specific for either the maternal or paternal homolog.



Sequential hybridization. (a) All primary Oligopaints are hybridized to their target. TAD1 has already been imaged via STORM, hence the reporter dye has been removed through the toehold approach. TAD2 is being imaged, and TAD3 has not yet been addressed. (b) After N re-hybridization rounds I would overlay all spots to visualize chromosome 19.

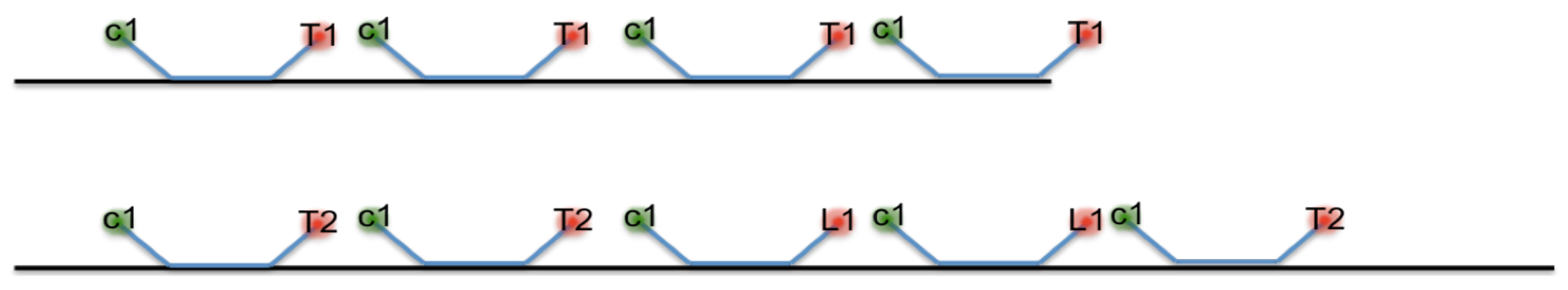
Assessing biological relevance

I will; a) compare the variation in folding between nuclei to that between homologs within a single nucleus; b) ascertain whether there are maternal- or paternal-specific patterns of folding, and c) collaborate with the laboratory of Leonid Mirny to determine whether there is a reproducible folding pattern for chromosome 19. I will also determine whether the patterns I detect are altered if compartment boundaries are mutated by CRISPR/cas9, or the expression levels of architectural proteins (e.g. CTCF and cohesin) are reduced by RNAi. This line of inquiry will lead to a better understanding of how an entire chromosome can be folded into an exquisitely functional unit.

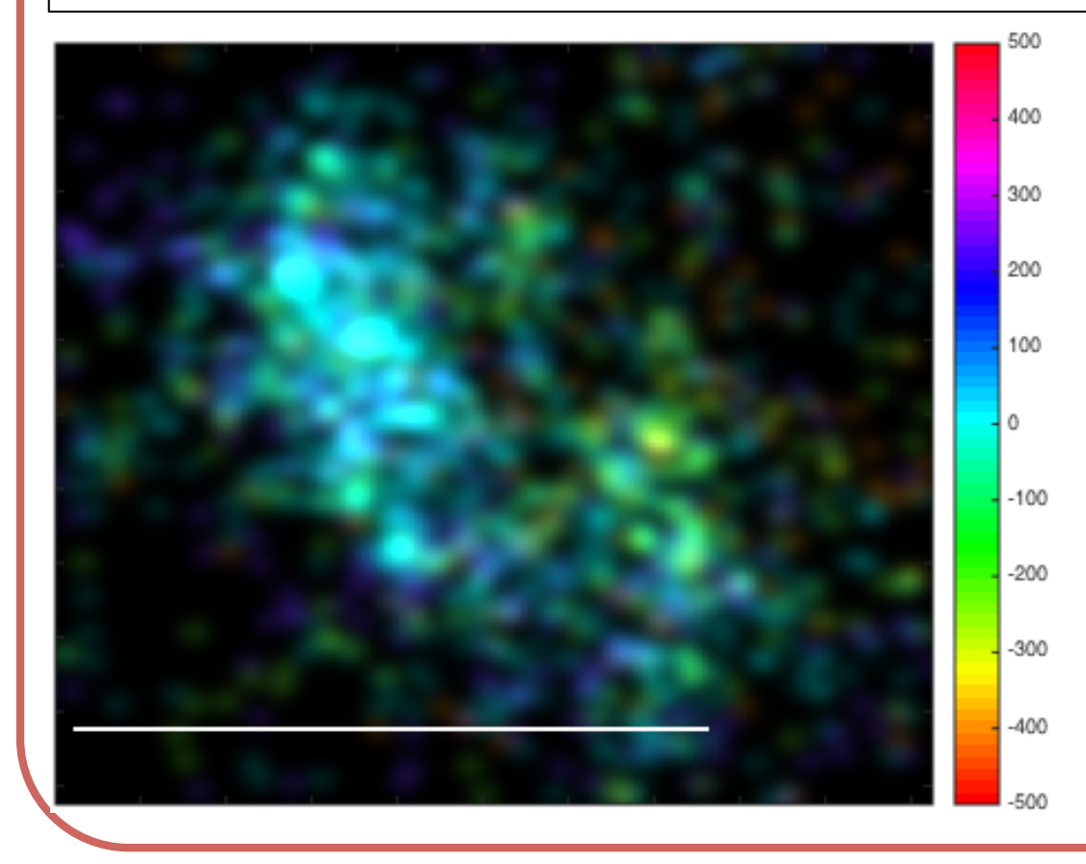
Aim2: exploring hierarchal genomic organization

Resolving sub-compartmental organization

Through super-resolution imaging, I am querying whether intracompartment organization is associated with the transcriptional state of an A or B compartment. Here, I will visualize loops, TADs (called by the Mirny lab), and imprinted regions by engineering barcodes into the Backstreet sequences and then targeting labeled secondary oligos to those barcodes.



For multi-scale imaging, I will use Mainstreets to visualize compartments, e.g. “c1”, for compartment 1, while Backstreets will be used for TADs, e.g. “T1” and “T2” and loops, e.g. “L1”.



Preliminary data: OligoSTORM image of a single TAD of 720 kb, where its long axis is about 250 nm, as inferred from the image.

Assessing biological relevance

I will; a) compare folding of TADs between A and B-compartments; b) trace loops of different cell types to compare cell type specific subTAD folding. Finally, throughout my entire study, I will probe cell-to-cell variability, thus going one step forward, from Hi-C studies, yielding an average chromosome conformation, to a distribution of conformations, while applying statistical tools to analyze the variability, e.g. utilizing the width of the distribution to assess genome structure stability.

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