HEMATOLOGICAL MALIGNANCIES AND SOLID TUMOR RESEARCH

Genome variation common to cancer is too complex for low coverage whole genome sequencing. Complex rearrangements as well as highly repetitive regions of the genome present additional challenges for short- and long-read sequencing technologies.

Optical Genome Mapping (OGM) detects structural variations in an unbiased manner and at much higher sensitivities than sequencing-based technologies, and routinely at 5% variant allele fraction.

Extreme genomic rearrangements are hallmarks of cancer manifestation. Deciphering complex genomic structures requires a combination of short-read sequencing as well as long-range information with high coverage to unlock heterogeneity while providing a complete picture of the genome. To date, NGS applications in the clinic are limited to either low coverage across the whole genome, or high coverage exome sequencing that disregards 98% of the genome. The repetitive nature of the genome makes two-thirds largely inaccessible by short-read sequencing, and the short reads make elucidating the complex rearrangements seen in cancer impossible. The throughput of current long-read sequencing technologies is too limited to be realistically applied to cancer diagnostics or discovery. High cost and low throughput of long-read sequencing technologies, among other things, make cancer genomics intractable to those technologies.

Variant allele fraction detection

Coverage per sample

OGM uncovers large structural variations beyond what short- and long-read sequencing can see.

Insertions/ deletions

500 bp for diploid genomes

5 kbp for mosaic sample

Balanced and unbalanced translocations

Larger than 50 kbp

Inversions

Larger than 30 kbp

Duplications

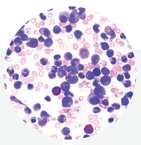
Larger than 30 kbp

The workflow is simple, and it starts with ultra-high molecular weight DNA isolation from blood, cells, tissue or tumor biopsies. A single enzymatic reaction places 500,000 fluorescent labels all throughout the genome at a specific sequence motif occurring approximately 20 times per 100 kbp in the human genome. The long, labeled DNA molecules are linearized in nanochannel arrays on a Saphyr chip® and imaged in an automated manner by the Saphyr® Instrument. Changes in patterning or spacing of the labels are detected automatically, genome-wide, to call all structural variants.

OGM CONSOLIDATES KARYOTYPING, FISH, AND CMA IN A SINGLE WORKFLOW

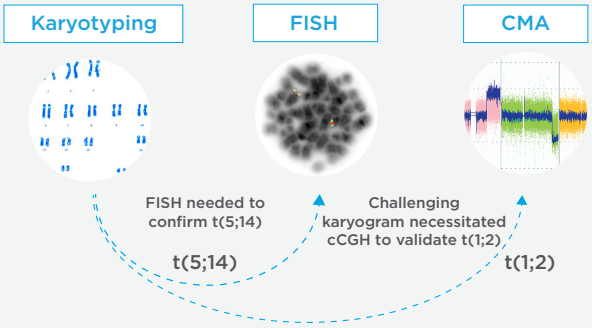
Identification of complex rearrangements in cancer often requires a combination of approaches, which are typically performed sequentially. This sequential approach manages costs, but increases the turnaround time and complicates the understanding of the genetic makeup of the sample. A leukemia sample presented a challenging karyogram, necessitating chromosomal microarray as a complement to validate t(1;2). Suspicion of a previously missed t(5;14) called for a FISH analysis. Results from karyotyping and FISH were inconclusive since the acceptor chromosome couldn't be clearly identified. The two events were captured by OGM in a single experiment and benign structural variants were automatically filtered out without complex pipelines.[1]

Challenging Leukemia Sample



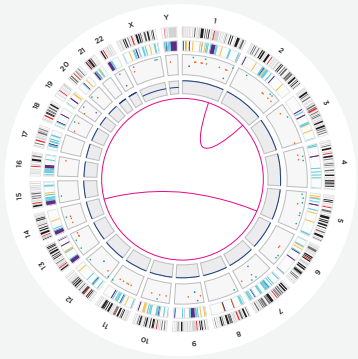
Current Methods

3 different platforms required to identify all events



OGM

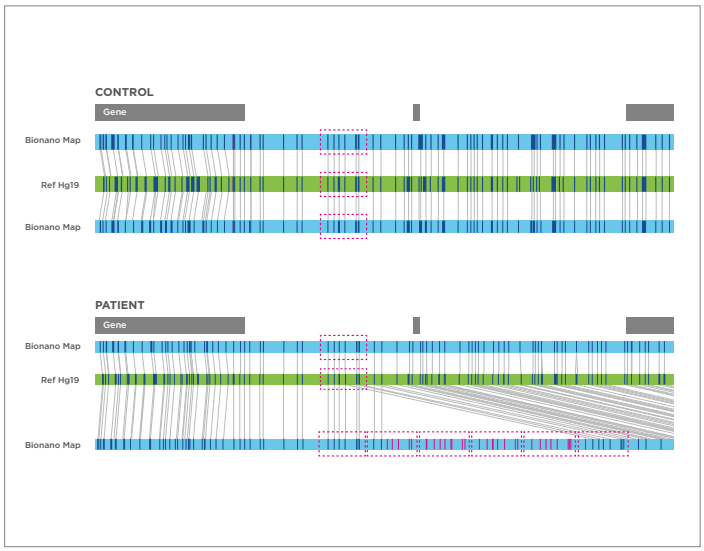
Consolidates 3 into 1 convenient workflow with all events identified



OGM IDENTIFIES VARIANTS IN AND AROUND GENES IMPLICATED IN CANCER THAT ARE MISSED BY SHORT- AND LONG-READ SEQUENCING

Because they are too long:

A case of familial cancer known to be linked to a particular gene was studied for a decade without any molecular evidence of events perturbing that gene. OGM identified a 38 kb cassette that was amplified in six tandem copies just upstream of this gene. Saphyr imaged single molecules spanning all 230 kbp of this tandem repeat, which allowed it to be observed directly, as opposed to inferred algorithmically, as is the case with other molecular methods.[2]

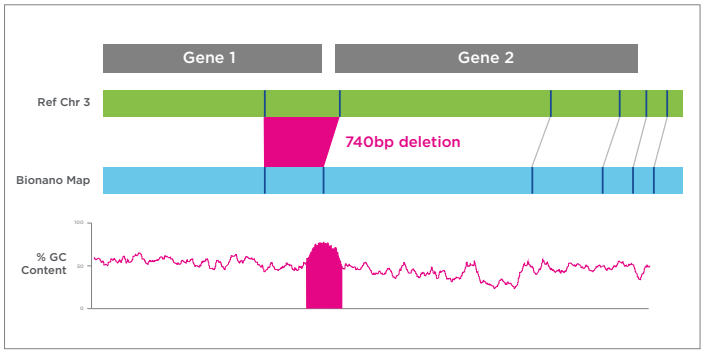


Because they are too rare:

The study of complex rearrangements in heterogenous cancer samples with long-read sequencing is done at the expense of coverage, throughput or cost. Reaching the low allelic fraction necessary to identify SV in biopsies requires deeper coverage than long-read sequencing can offer for a reasonable price. At 400X coverage depth in a human cancer sample, OGM can routinely detect variants at 5% allele fraction.

Because they are inaccessible by sequencing:

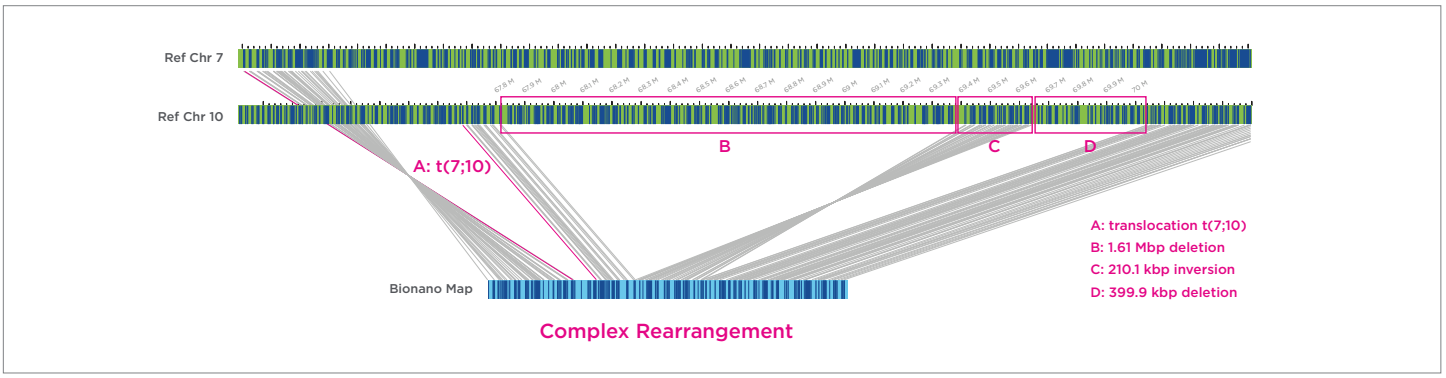
In a patient-derived model of uveal melanoma, no pathogenic variant or epigenetic event was found explaining the loss of expression of gene 1 using NGS, which is typically associated with this cancer. OGM identified a 740 bp deletion in the promoter of gene 1. This region was missed by sequencing due to the high GC content of that region.[3]



OGM IDENTIFIES MUTATION SIGNATURES AND BRINGS ORDER WITHOUT COMPLEX PIPELINES

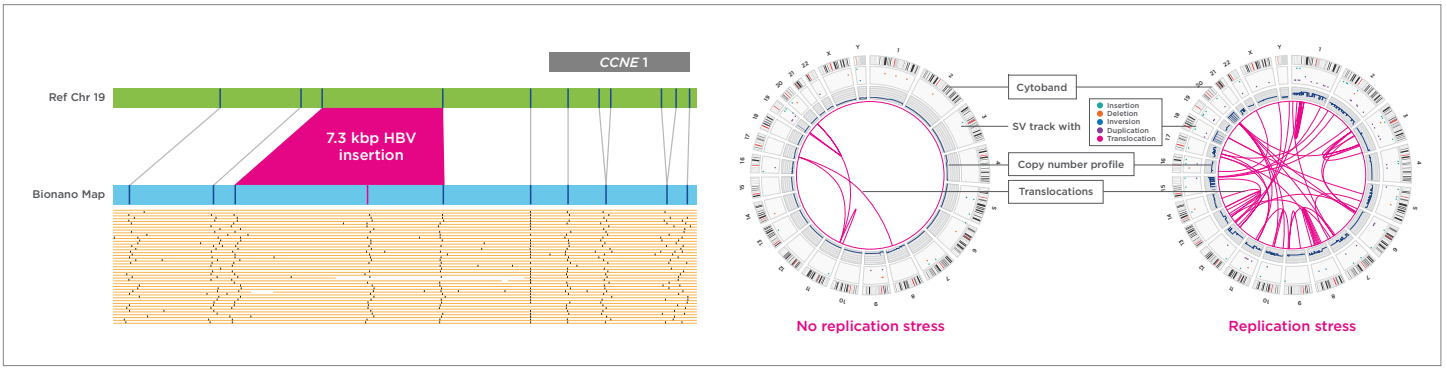
In highly rearranged cancer samples:

Cancer samples often display a high number of structural variants, and the limitations of the short-read length of NGS are particularly detrimental for correctly reconstructing these chained fusion events. In this patient-derived breast cancer cell line, a consensus map resulting from the alignment of dozens of molecules spanning the region allowed OGM to identify a succession of a translocation, deletions and an inversion missed by short- and long-read sequencing.



In samples where stratification is needed:

The ability to stratify patient samples based on mutational profiles usually requires extensive bioinformatic data curation downstream of sequencing. In a hepatocellular carcinoma study, our built-in pipeline automatically provides enough information to distinguish samples with or without a replication stress signature. The circos plot on the right in the image below shows replication stress resulting from a Hepatitis B virus insertion 9 kb upstream of the Cyclin E gene. Patients could be stratified based on the accumulation of DNA damage to determine if they are candidates for novel innovative therapies such as PARP inhibitors.[4]



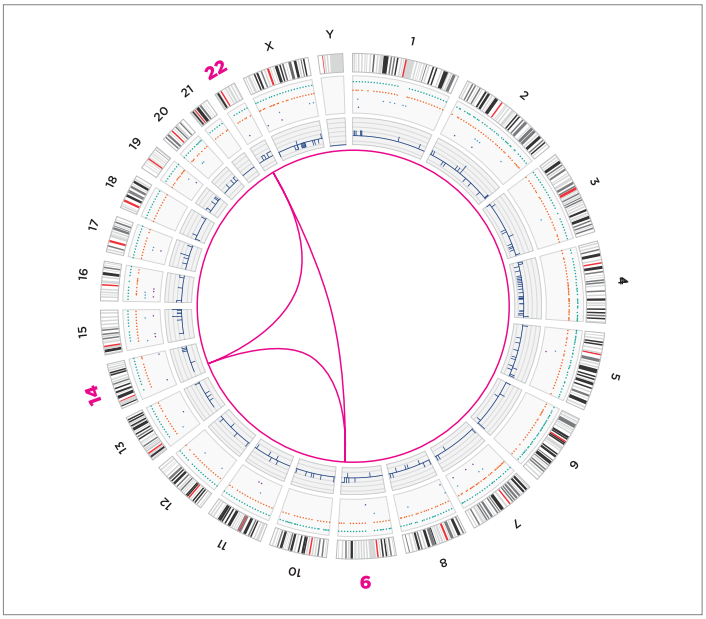
OGM IS A STREAMLINED TOOL FOR PERSONALIZED MEDICINE

Because it automatically identifies classical actionable fusions:

Rapid identification of *BCR-ABL1* translocations in Acute Lymphoblastic Leukemia is urgent for leveraging potential therapeutic options. Imatinib is a specific inhibitor of the fusion protein resulting from that translocation. OGM easily identifies complex and rare events such as this 3-way translocation of the Philadelphia chromosome t(9;22)(q34;q11).[5]

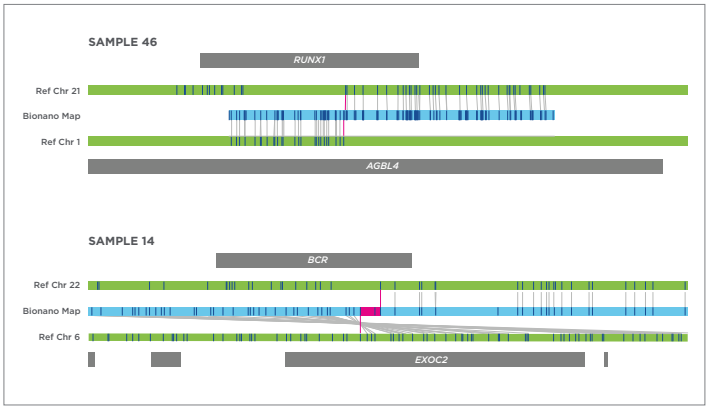
Because it identifies novel fusions:

In a study conducted on 48 leukemia samples, OGM identified 2178 rare structural variants, of which 95 were rare interchromosomal translocations, and 23 were unique calls containing potential gene fusions, leading to new avenues of research for drug development.[5]



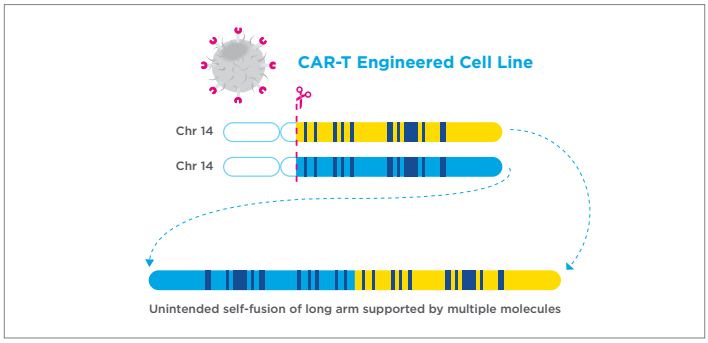
Because it easily identifies rare/new fusions of known genes:

In a large systematic comparative study between OGM and classical cytogenetics, OGM identified novel, non-recurrent fusions never reported before. In both cases shown here, one of the two fusion partners is well-known in leukemia as a gene fusion. These events were missed by classical cytogenetics, either because of their low allelic frequencies or because of the targeted FISH approach classically used in a diagnostics context.[5]



Because the ultrasensitive, automatic, genome-wide detection of rearrangements brings safety in CAR-T cells manufacturing:

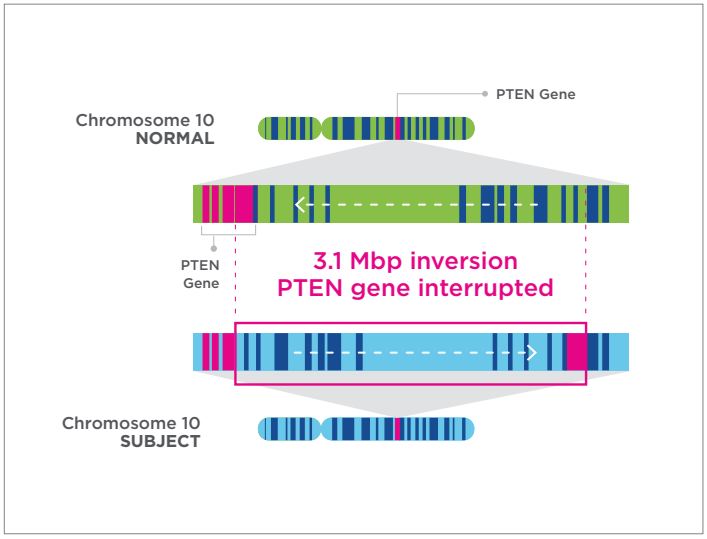
CAR-T cells are a powerful new therapeutic tool, but these engineered immune cells are often poorly characterized or remain unchecked for unwanted alterations. OGM can rapidly collect several thousand folds of coverage of a human genome, allowing for the detection of undesired rearrangements. Here, an unwanted inverted self-fusion of a large part of the long arm of chromosome 14 was detected in an engineered cell line, and several imaged molecules validated this fusion.



COMBINED WITH NGS, OGM PROVIDES A COMPREHENSIVE UNDERSTANDING OF CANCER EVENTS, FROM SNVS TO MULTI MEGA BASE PAIR STRUCTURAL VARIATIONS

To perform complete tumor profiling for cancer discovery:

In 12 leukemia genomes analyzed with OGM and whole-genome sequencing (WGS), dozens of genes were affected by multiple structural and single nucleotide variants. Most of these genes were not previously associated with leukemias or with cancer in general. The potential for novel biomarker discoveries, even in such a small sample size, is unexpectedly large. Many of the SVs were affecting regulatory sequences, absent from whole exome sequencing approaches. Intergenic mutations are a known mechanism for tumor escape from chemotherapy. TCGA data showed that expression differences in several of these novel biomarker genes are associated with outcomes and provide useful information for follow-up regarding response to chemotherapy. In the example shown here, a 3.1 Mbp inversion was detected by OGM breaking the *PTEN* gene, while a SNV was detected by WGS in the other *PTEN* allele. This compound heterozygous variant made this subject a candidate for trials with drugs attempting to rescue *PTEN* variants.[6]



To learn more about Bionano OGM applications for cancer research, visit bionanogenomics.com/cancer

References: 1. Hoischen A. Whole genome imaging to streamline cancer cytogenetics and identify novel rearrangements and biomarkers. ESHG Bionano workshop 2019. https://bionanogenomics.com/videos/eshg-2019-workshop-series/ 2. Bocklandt S. Whole genome imaging to streamline cancer cytogenetics and identify novel rearrangements and biomarkers. AMP Bionano workshop 2019. https://bionanogenomics.com/videos/amp-2019-series-dr-sven-bocklandt/ 3. Gentien D, Saberi-Ansari E, Servant N, et al. Multi-omics comparison of malignant and normal uveal melanocytes reveals novel molecular features of uveal melanoma. bioRxiv 2022.03.11.483767; doi: https://doi.org/10.1101/2022.03.11.483767 4. Péneau C, Imbeaud S, La Bella T, et al. Hepatitis B virus integrations promote local and distant oncogenic driver alterations in hepatocellular carcinoma. Gut. 2022;71(3):616-626. doi: 10.1136/gutjnl-2020-323153 5. Neveling K, Mantere T, Vermeulen S, et al. Next-generation cytogenetics: Comprehensive assessment of 52 hematological malignancy genomes by optical genome mapping. Am J Hum Genet. 2021;108(8):1423-1435. doi: 10.1016/j.ajhg.2021.06.001 6. Xu J, Song F, Schleicher E, et al. An integrated framework for genome analysis reveals numerous previously unrecognizable structural variants in leukemia patients' samples. bioRxiv 563270; doi: https://doi.org/10.1101/563270 7. Luebeck J, Coruh C, Dehkordi SR, et al. AmpliconReconstructor integrates NGS and optical mapping to resolve the complex structures of focal amplifications. Nat Commun. 2020;11(1):4374. Published 2020 Sep 1. doi: 10.1038/s41467-020-18099-z

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