Optical genome mapping

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Summary

- Optical genome mapping produces digital genome maps that can identify structural variants from 500bp to those spanning millions of bases within the human genome. This could address the limitations of cytogenetics and next generation sequencing by using DNA fragments that are hundreds of thousands of bases in length
- Only one company is currently producing optical mapping consumables, equipment, and analysis software
- Optical genome mapping is gaining traction in haematological cancers, solid tumours and rare diseases, and aims to provide genetic diagnoses to patients and increased depth of information to clinicians
- The technique can be combined with next generation sequencing data to potentially provide the most in-depth analysis of the human genome so far achieved
- Optical genome mapping is not yet in widespread clinical use and needs further refinement in key areas such as turnaround time to improve clinical utility

Despite its name, optical genome mapping is not a DNA sequencing technology and information about distinct nucleotides is not produced. The output is more like a bird's eye view of the genome, revealing structural variants which can impact disease diagnostics, prognostics and treatment choices, and provide information on genetic variation within diseases. Genome scanning technologies such as optical genome mapping have found that the extent of structural variants and their impact on health and disease is much greater than previously thought.

Current standard of care tests use cytogenetic methods to examine structural variants including chromosomal banding analysis, fluorescent in-situ hybridisation and chromosomal microarrays. Each test has its limitations such as low resolution or throughput, necessitating multiple follow-on tests to identify or examine structural variants. Optical mapping aims to overcome these limitations and condense multiple tests into a single assay to improve structural variant resolution and reduce turnaround time and costs.

How does it work?

Optical mapping assesses large molecules of ultra-high molecular weight DNA that are hundreds of thousands of base pairs in length. Fluorescent labels are applied to motifs occurring regularly throughout the genome. This labelled DNA is stretched through nanochannels using electrical currents, which reveals the underlying genomic structure.

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Digital imaging then converts the data into genome maps that can be examined to identify structural variants involved in disease.

Figure 1: DNA strand stretched through nanochannels using electrical currents

Comparison to standard of care tests

Many comparison studies report 90-100% agreement between standard of care and optical genome mapping techniques and an overall improvement in resolution in haematological cancers.

The performance of optical genome mapping was assessed next to chromosomal banding analysis, fluorescent in-situ hybridisation and chromosomal microarray in chronic lymphocytic leukaemia. It identified more than 90% of known variants and identified novel structural information in known structural variants in 55% of patients [1] and reached 100% concordance rate when compared with standard of care tests for paediatric acute lymphoblastic leukemia. It was able to refine the original karyotypes by identifying cryptic structural variants (structural variants that fall below standard of care limits of detection) that correlated with clinical risk stratification indicators [2]. Another study found 100% concordance and reproducibility between sites, operators, and instruments when standard of care testing was compared with optical genome mapping in prenatal genetic testing [3].

Whole genome sequencing, which overcomes many limitations of cytogenetic tests and can identify variants down to individual base level, is increasingly being used in clinical services worldwide. While optical genome mapping cannot replace standard genome sequencing, it complements it by providing additional information that is difficult to extract from sequence data. Although there are few results directly comparing the two, in combination they could provide unrivalled clarity about the full range of genome variation.

As a research tool optical mapping can identify and map previously unexplored regions of the genome. In the largest genome mapping work of multiple populations optical genome mapping identified additional haplotypes in complex regions and confirmed the presence of population-specific structural variant patterns. Results of this nature can potentially feed into clinical workflows and drive additional, more targeted analyses to further benefit patients in the long term [4].

Considerations

There are several factors to consider in the development of optical genome mapping as a competitor or complementary genomics technology:

- **appropriate turnaround time**, from sampling to treatment decision, is crucial from patient and clinician perspectives, particularly in the face of aggressive, malignant disease. Optical genome mapping condenses multiple tests into one, aiming to reduce overall turnaround time. However, at this stage optical genome mapping results still require secondary confirmation
- **not requiring niche training** to perform key tests increases the opportunity to use a technology more widely. Bionano technology compresses multiple tests into one assay and provides interactive analysis software, so reliance on staff with specific skills is reduced

- **data that are easy to handle and analyse**, using tools that are accessible to more than just bioinformaticians, will increase the utility of a technology. Bionano's end-to-end pipeline provides customisable and interactive software, which is actively updated as the technology develops. However, consistent analysis standards for optical mapping data have not yet been developed
- **determining the full structural variant profile of a disease is costly.** Bionano protocols do not require specialist cell culture or DNA amplification. Bionano provide the reagents, machine, and analysis software as a rental package, avoiding the need for large purchases of expensive, specific equipment. Currently a single optical mapping run costs approximately 50% less than the equivalent whole genome sequence

As with standard genome technologies, it can be difficult to analyse certain regions of the genome using optical genome mapping and this can affect diagnostic accuracy. These include areas with the centromeres and telomeres of chromosomes, which are highly repetitive and not very well mapped in the human reference genome.

What patient populations could benefit?

Optical mapping currently has greatest utility as a clinical tool in areas such as cancer, especially blood cancers, and rare diseases.

Blood cancers are complex and heterogeneous diseases so there is a need to understand disease genotypes precisely to enable personalised and effective care. In a research setting, optical mapping has previously accurately resolved both known and unidentified complex standard variants in blood cancers, where standard of care tests have reached their limit of detection or poor resolution has caused test misinterpretation.

In other blood cancers, improved detection and visualisation of prognostic structural variants can be used for risk stratification of patients, inform treatment intensity, and monitor disease progression. This includes the up- or down-grading of risk scores, which may change disease management. Resolving cryptic structural variants can qualify patients for specific therapeutic strategies and may identify suitability for clinical trial enrolment. Optical genome mapping has detected complex structural variants in 'cytogenetically normal' patients, although the clinical impact of novel structural variants still needs evaluating. Many novel structural variants have been identified by optical genome mapping, which, although yet to be understood, could hold clinically relevant information.

Data from **solid tumours** is limited largely due to difficulties in extracting sufficient high quality ultra-high molecular weight-DNA from tumour tissues, since the fixative used on samples damages DNA. When combined with next generation sequencing, clinically relevant information has been extracted from lung squamous cell carcinomas, anaplastic thyroid carcinomas, and hepatocellular carcinomas. However, considerable validation in much larger cohorts and sample types is required.

The frequency and impact of structural variants is underestimated in **rare diseases** due to limits of detection and resolution imposed by current technologies. Optical genome mapping use in rare diseases is increasing and the improved resolution is proving beneficial. At this stage optical genome mapping is often used to resolve longstanding 'diagnostic odysseys' where patients do not have a genetic diagnosis, for example detecting a mosaic deletion that was missed by chromosomal microarray in a four-year-old with epilepsy [5].

Clinical practice

Whilst implementation of optical genome mapping into clinical practice is not yet widespread, there are steps being taken to investigate its integration into healthcare. The Leuven Centre for Human Genetics, Belgium, became one of the first centres in the world to

use optical genome mapping routinely in acute lymphoblastic leukemia and acute myeloid leukemia diagnostic workups. In 2023, GenQA, who provide external genomics quality assessments for the NHS, are launching a pilot assessment for optical genome mapping in haematological malignancies and rare diseases.

Pilot studies at various institutions have identified opportunities and challenges to be resolved for optical genome mapping to be used in routine clinical practice.

In 2021, two UK hospitals trialled optical genome mapping in their diagnostic laboratories, however, neither decided to incorporate it into their workflows. Low throughput was a barrier in delivering clinically useful turnaround times and services. In some instances, to compete with standard of care tests, optical mapping throughput needed to increase by two orders of magnitude. Sample preparation was complex with the result that test quality was strongly dependent on ultra-high molecular weight DNA quality. In another study, two genetics laboratory hubs in England carried out pilot projects using optical genome mapping in small cohorts of mainly haematological oncology patients, with positive outcomes. Using optical genome mapping on a case-by-case basis had benefit in these genetics laboratory hubs due to skilled worker shortages in standard of care testing, although throughput was still a limiting factor.

To help improve sample preparation, in 2022 Bionano partnered with laboratory robotics company Hamilton to develop the Long String VANTAGE, the world's first automated ultra-high molecular weight DNA isolation system.

Where is optical genome mapping heading?

Optical genome mapping has great promise as an advanced tool to complement existing next generation sequencing analyses. Currently, more development is needed to 'on the ground' logistics for clinical translation. Throughput is not yet sufficient for optical mapping to be a viable alternative to established techniques and there are no accepted standards or guidelines for data analysis meaning reproducibility is questionable. Targeted investment in solving the logistical barriers, but validation in much larger cohorts are required to support the use of optical genome mapping in routine clinical services.

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