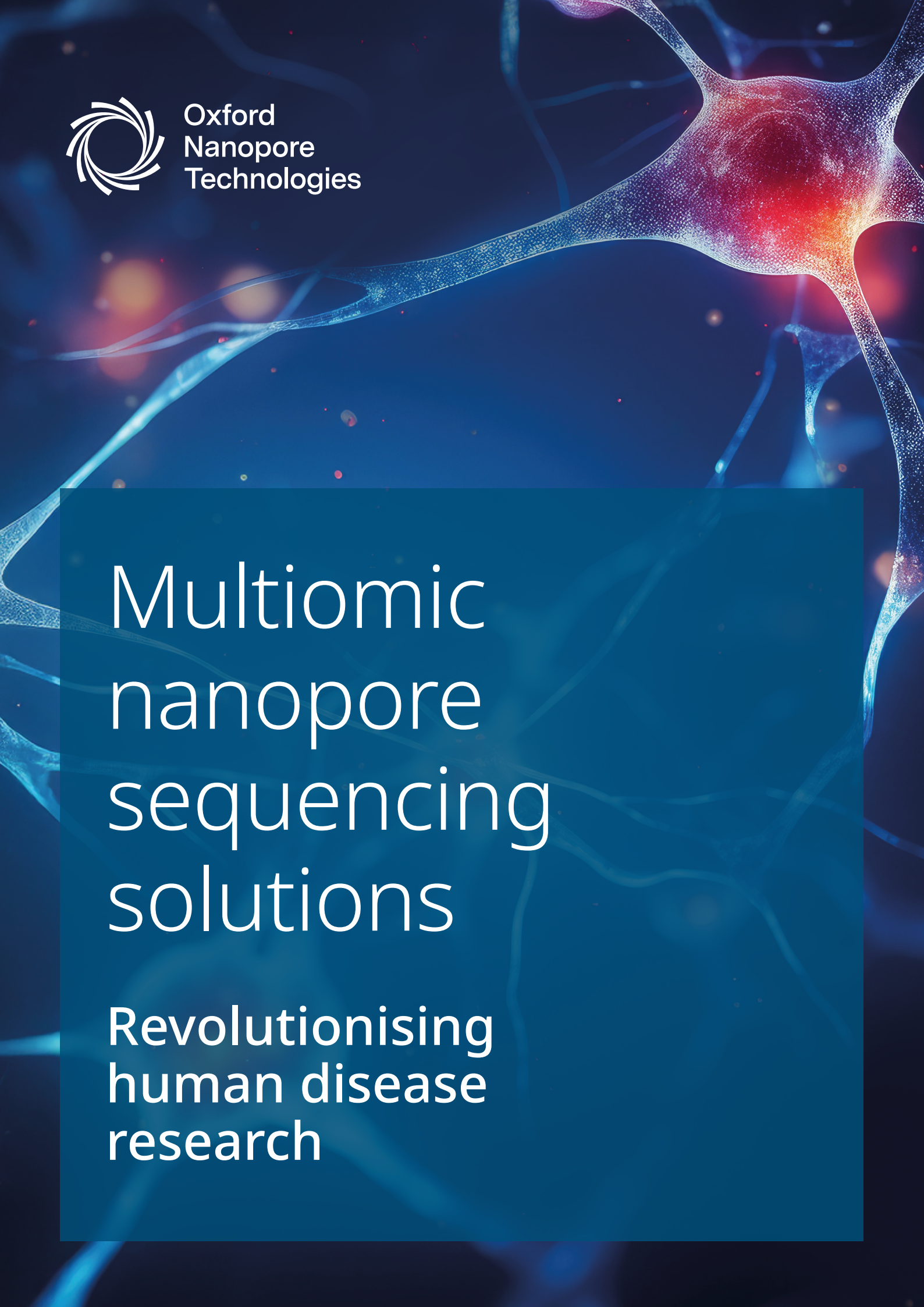


Multiomic nanopore sequencing solutions

Revolutionising human disease research

What you're missing matters

Resolving the mechanisms underpinning human diseases is vital to understand disease phenotypes, identify novel biomarkers, and enable drug discovery. Multiomic approaches — spanning genomics, bulk and single-cell transcriptomics, epigenetics, and proteomics — are crucial to this, providing data to help unravel complex pathways. In disease research, tissue samples are invaluable and scarce resources; maximising the information obtained from these samples is essential.

However, legacy multiomic methods require the use of multiple platforms and often involve complex workflows, lengthy turnaround times, and considerable costs. Even when combining data from multiple traditional technologies, valuable information is missed from precious research samples, leaving important biological mechanisms unresolved.

Ask bolder questions — make no compromises

Legacy technology

3 sequencing libraries

2+ sequencing platforms

3 separate data analyses & complex integration

Oxford Nanopore sequencing

1 sequencing library

1 sequencing run

1 analysis workflow

Ultra-rich data

Structural variation

SNVs

Methylation

Phasing

Ultra-rich data

Oxford Nanopore sequencing delivers...

Genomic and epigenomic data from a single dataset

- Directly analyse native DNA — no PCR required — to uncover genomic variants alongside methylation status in a single nanopore sequencing run
- Capture SNVs, SVs, repeat expansions, CNVs, and native epigenetic modifications, including 5mC and 5hmC, from a single library
- Phase data with confidence using long reads, for haplotype-level information
- Avoid amplification bias and access regions of the genome missed by traditional methods

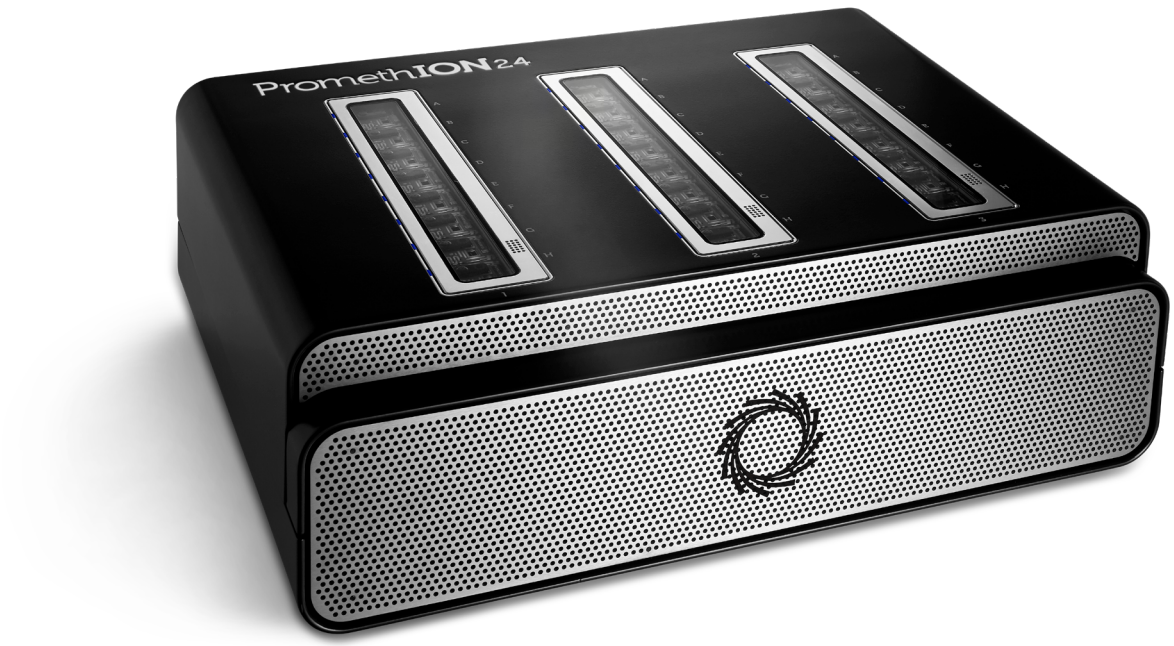
Powerful transcriptome and epitranscriptome analysis

- Go beyond gene-level analysis: gain isoform-level expression information with full-length transcript sequencing and identify fusions with ease using native RNA or cDNA sequencing
- With direct RNA sequencing, detect native RNA modifications including m^6A and characterise isoforms without PCR bias in a single sequencing run
- Measure poly-A tail length in cDNA or native RNA

High-resolution transcriptome analysis for single cells

- Analyse full-length transcripts from single cells to reveal biology hidden by short reads
- Shed light on cellular heterogeneity with a comprehensive view of isoform diversity, alternative splicing, expressed variants, and transcripts for nonsense-mediated decay
- Seamlessly integrate nanopore sequencing into your single-cell workflow — go from cDNA to sequencing-ready library in ~3 hours and perform cell barcode and UMI identification using our EPI2ME™ software

...from a single platform



Reveal more biology

Case study: immunology

T helper (Th) lymphocytes are modified by epigenetic mechanisms in response to signalling factors as part of the immune response. However, analysis of epigenetic modifications typically involves chemical conversion such as bisulfite treatment, which cannot distinguish 5mC from 5hmC and significantly damages DNA libraries. Goldsmith *et al.* used targeted, PCR-free Oxford Nanopore sequencing to directly profile methylation in Th cells[1]. They were able to efficiently detect both 5mC and 5hmC patterns and distinguish between Th cell subsets, illustrating a potential pathway to identify pathogenic subsets that may play an important role in disease.

> For detection of multiple modified bases ... most techniques require samples to be split, and different modified bases to be detected separately. In the present study, we took advantage of nanopore sequencing's ability to determine the 5mC and 5hmC simultaneously.

Goldsmith *et al.* bioRxiv (2023)[1]

Case study: neurology

Multiple sclerosis (MS) is a chronic neurodegenerative disease. Studies have identified that DNA methylation patterns may be associated with the pathogenesis of MS, suggesting their potential for future drug targets. Si *et al*. used Oxford Nanopore sequencing to directly detect methylation in brain samples from mice induced with experimental autoimmune encephalomyelitis (EAE), an animal model of MS[2]. Comparing to a control mouse group, they found 490 differentially methylated promoters. Several of these genes had been flagged as relevant in previous MS studies, but methylation data had not been available. With this methylation data and additional metabolomic analyses, they were able to identify 'a potential link between the dysregulation of promoter methylation and metabolome in the brain of EAE mice'.

> benefits of concurrent analysis of sequence identity, base modifications, real-time, and cost-effective generation of genome-wide data support the application of [nanopore sequencing] in studying the brain and spinal cord in different diseases

Si *et al*. J. Neuroimmunol. (2023)[2]

Case study: cancer

Single-cell sequencing is an important tool in acute myeloid leukaemia (AML) research, enabling the study of the impact of therapies on gene expression and identification of malignant and non-malignant cell populations. Penter *et al*. developed a targeted single-cell Oxford Nanopore sequencing method to analyse somatic nuclear mutations, isoform expression, and more in full-length transcripts[3]. Applying this method to AML bone marrow research samples, the team detected somatic mutations in the myeloid cell population — but also, unexpectedly, in erythroid and megakaryotic cell populations. Additional analysis revealed that these two cell compartments directly differentiate from leukaemic clones, prompting the researchers to define two new AML-derived expression clusters for transcriptome-based classification of AML. The authors concluded that their study demonstrates how 'a focus on myeloid progenitor populations for single cell genomic studies likely misses important AML subpopulations'.

> We present a long-read sequencing-based framework for integrative genotyping of single cell profiles that substantially improves the resolution of leukemia and immune cell phenotypes

Penter *et al.* Nat. Commun. (2024)[3]

Case study: cardiovascular disease

Cardiovascular disease is associated with abnormalities in human aortic smooth muscle cell (HASMC) plasticity. Aberrant splicing causes dysregulation of HASMCs and arterial contraction; however, analysis of this variation is limited with short-read sequencing, which cannot sequence full-length isoforms and relies heavily on reference transcripts, thereby missing novel transcripts. Wu *et al.* used Oxford Nanopore direct RNA sequencing to investigate alternative splicing events in HASMCs[4]. Of the full-length transcripts they identified, 75% were unannotated. This included CISD1-u, which was found to be involved in phenotypic switching in HASMCs, providing new insights into the mechanisms of cardiovascular disease.

> Long-read RNA-seq technology directly sequences full-length RNA transcripts, providing opportunities to precisely identify alternative splicing events

Wu *et al.* Commun. Biol. (2023)[4]

Why Oxford Nanopore for multiomic sequencing?

- Genomic, epigenomic, and transcriptomic data from a single platform
- Direct analysis of DNA and RNA — capture base modifications and previously hidden variants in regions inaccessible to traditional PCR-based methods
- Unrestricted read lengths, from short to ultra-long (>4 Mb achieved) — sequence full-length isoforms, reveal hidden structural variants, and phase haplotypes with ease
- Leave long, complex workflows behind — utilise simple, streamlined, and scalable end-to-end workflows, plus rapid turnaround times with fast library prep and real-time data streaming
- Scale to your needs with powerful, flexible sequencing devices — from multiplexed targeted sequencing to high-depth whole-genome and whole-transcriptome sequencing at the large cohort scale

Find out more about multiomic nanopore sequencing at nanoporetech.com/multiomics

References

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