ADOPTION OF REAL-TIME, LOW COST, PORTABLE WGS FOR POINT-OF-CARE DRUG SUSCEPTIBILITY AND ANTIMICROBIAL RESISTANCE TESTING

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Oxford NANOPORE Technologies

OXFORD NANOPORE

- Formed in 2005
- Substantial Intellectual Property portfolio
- Raised £351 million to date
- 300+ people in UK, US, Japan
- Shipped products to more than 55 countries

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REQUIREMENTS FOR PORTABLE CULTURE FREE COMPREHENSIVE DST

- **Complete Solution** – From sample to actionable report in a few hours.
- **Robust & Simple to Use** - In the hands of clinical teams in LMICs.
- **Low Cost** - No capital expense and low ongoing operating costs. No requirement for service.
- **High Sensitivity and Specificity** – Analysis of complex disease, co-infections and resistance. Mapping of highly-variable genomic regions and plasmids.

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WE ARE MOVING QUICKLY – SOME NUMBERS

THE NANOPORE

- Nanopores form holes in biological membranes
- Our membrane is a synthetic polymer, not a lipid bi-layer
- Very high electronic resistance and is more stable then a lipid bi-layer

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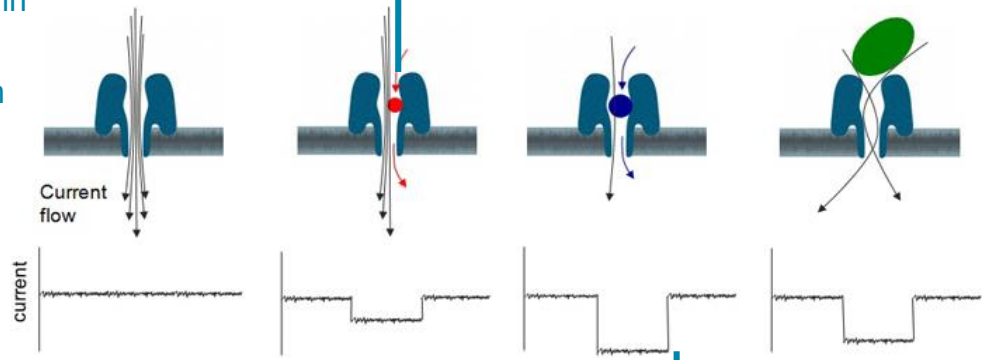
NANOPORE SENSING

1 Nanopore creates hole in membrane
Current passes through nanpore

2 As analyte passes through or near the nanopore, this created characteristic disruptions in the current



3 Current disruption is interpreted to understand the identity of the analyte

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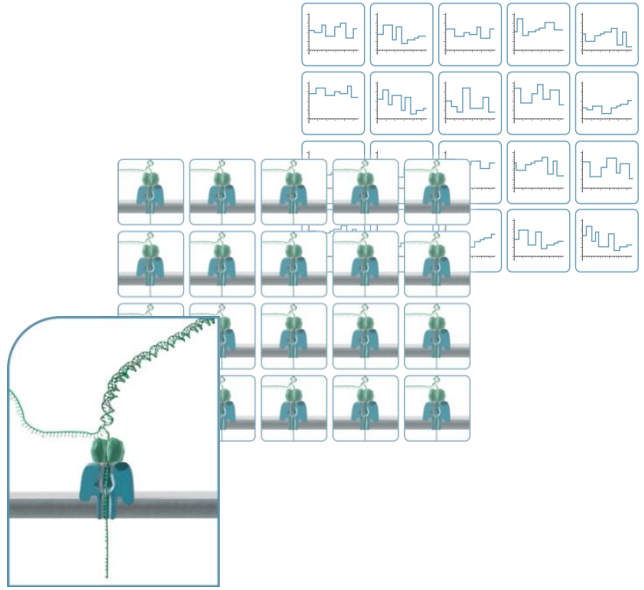
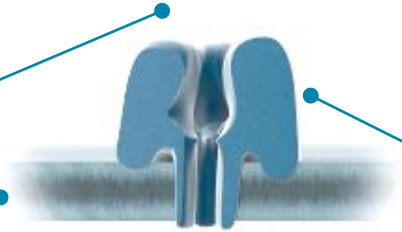
DNA SEQUENCING

How does it work?

Algorithm
(HMM, RNN etc...)





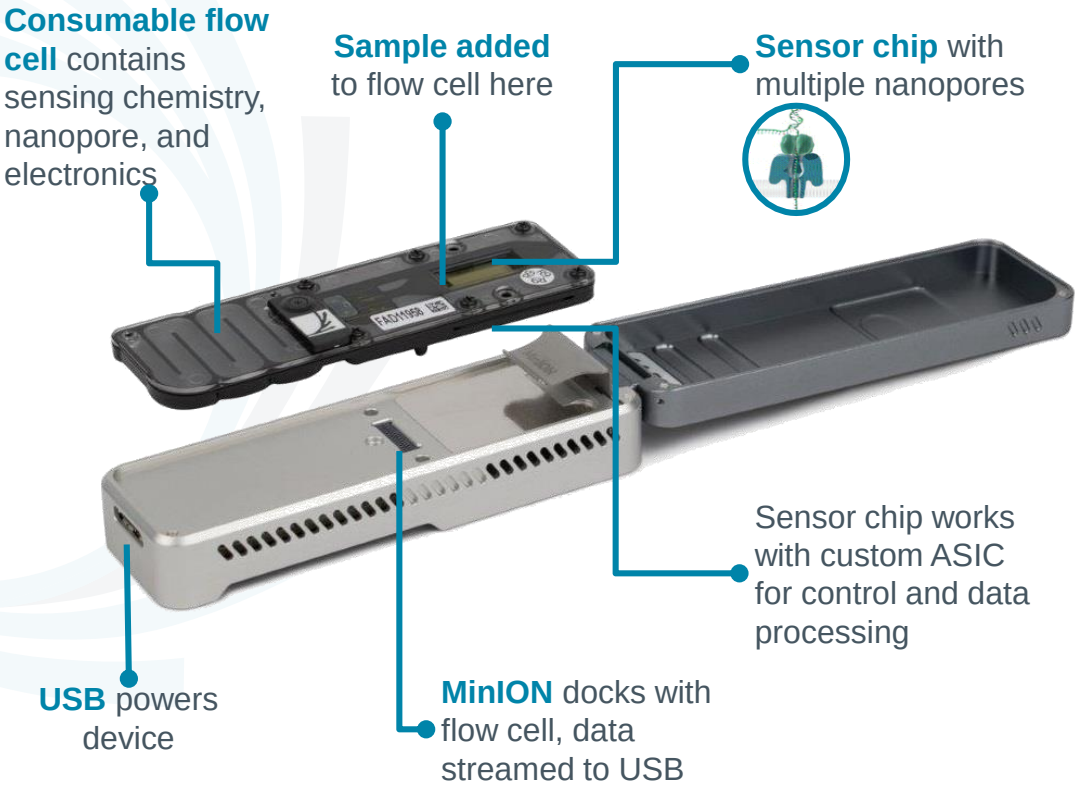
Motor
(E6, E7, E8)

Nanopore Reader
(R7, R8, R9, R10 etc...)

Membrane
(M9, M10 etc...)

Run Conditions
(Salt, fuel, script, temperature...)

MINION: PORTABLE DNA/RNA SEQUENCING



USB DEVICE AND FLOW CELL

Current flow cell version: R9.4

- 2048 wells
- 512 recording channels
- 4:1 multiplexing
- Can run at ~450 bps (bases per second per nanopore)

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FLONGLE
(“Flowcell Dongle”)

Adapts MkIB

- Smaller 128/256 channel SmidgION chip
  - Volume pricing
  - Targeted pull down
  - Panels
  - Dx version late 2017

Low cost, removable consumable docks with Flow Cell

MinION docks with flow cell, data streamed to USB

USB powers device

NANOPORE PRODUCTS: FULLY SCALABLE

Nanopore sensing:

- One core technology, fully scalable
- Real-time analysis ➔ on-demand sequencing
- Adaptable to DNA or RNA sequencing
- Ultra long reads (near-1Mb reported)
- Easy workflows, five minute library prep

SmidgION

In development

Smartphone sequencing

MinION

Commercially available

Portable, USB powered biological analysis

Up to 512 nanopore channels simultaneously

GridION

Commercially available

Five MinION Flow Cells and integrated computing

Available for sequencing as a service

PrometheION

Early access

High-throughput, high-sample number benchtop system

Modular: Up to 48 flow cells, each with up to 3,000 nanopore channels (total up to 144,000)

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SAME DAY DIAGNOSTIC AND SURVEILLANCE DATA FOR MULTI-DRUG RESISTANT TB

Iqbal et al. 2017

- Culture based methods take up to two months
- Demonstrated turnaround time of 8 hours, twice as fast as MiniSeq, for TB identification and drug susceptibility

Next steps

- 3 hour protocol
- Map the ‘Black Holes’ e.g. PE/PPE
- VNTR report

“Faster and more automated sample processing, as well as a cost reduction, is a clear necessity for global take up in low income settings. Achieving this would revolutionise the management of TB.”

http://jcm.asm.org/content/early/2017/03/02/JCM.02483-16.long

Derrick Crook and Zamin Iqbal (Oxford)

MINOR OPTIMISATIONS TO CURRENT NANOPORE WORKFLOW

DNA Extraction

< 1 hour

- Sputum inactivated
- Host cells removed
- Mtb cells mechanically disrupted
- DNA purification

Library Preparation

30 min to 2 hours

- Attach DNA adapters
- PCR amplification may not be required for high abundance Mtb
- Sequencing adapter addition

Minion Run

R-T Analysis → Presence / Absence (40 min) → R-T Analysis → Drug Resistance (40 min)

- Real-time analysis as sequencing data is generated.
- “Run Until” result is obtained – onboard control for defined LoD
- An Mtb-specific base caller can be trained to handle signal analysis better. This will increase accuracy, reduce the coverage needed to call a result.

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NANOPORE SUMMARY & NEXT STEPS

- **Portable, Robust, Low Cost**, **Real-time Analysis** with **Long Reads** and **Simple Sample to Report** workflow.
- Technology platform is ready – Nanopore & Oxford University – Refine and clinical accredit workflow.
- Collaborate with PHE – Clinical validation at field sites.

PARTNERSHIP NEEDED

- Requirements gathering, support & funding for clinical validation.
- Infrastructure for reporting and surveillance.
- Investigation of hyper-variable ‘unmappable’ regions.
- Acceptance and success – education, support, training.

THANKS FOR LISTENING!

Publications: nanoporetech.com/publications

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