Introduction to the MinKNOW software

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MinKNOW

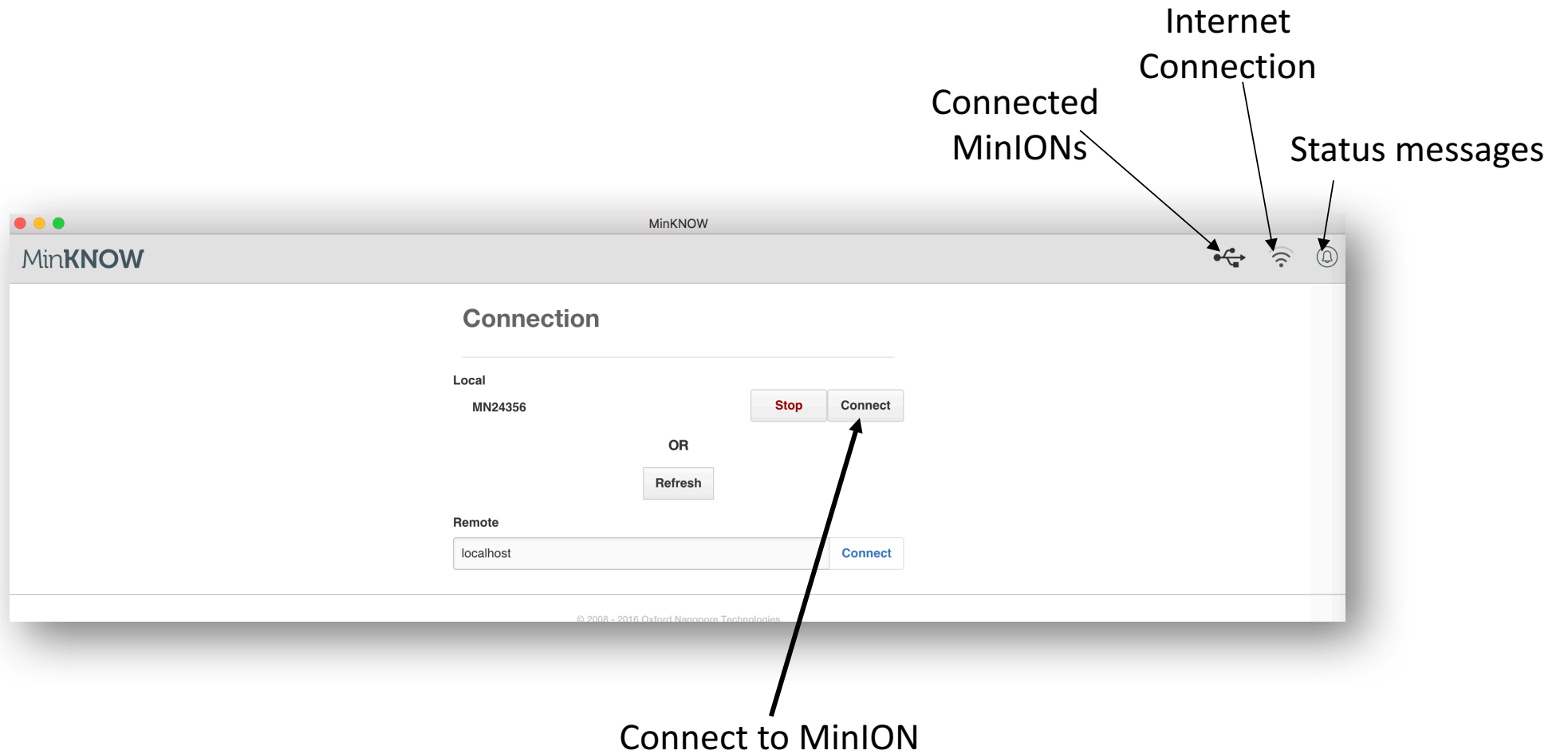Main Interface to your MinION sequencer to

- Conduct flow Cell QC

- Configure and monitor the sequencing run

- Perform life base calling

- Store the sequencing data

MinKNOW

- Free software

- Available only through the Oxford Nanopore Community

- Supports Windows, Mac and Linux

- Regularly updated

MinKNOW GUI

Main Menu

Main Menu

General Information

Sample / Experiment identifier

Flow Cell ID

Available memory

MinION Operation

MinION Operation

1. Configure Minion using the Test   Flow cell that comes with the MinION

2. Check your Flow Cell, i.e., how many pores are active

3. List of sequencing scripts for actual sequencing

4. Specific parameters for provided control experiment

Choose Operation

○ **Configuration Test Cell (CTC)**   ☐ *expert*
● **Platform Quality Control (QC)**
○ **Sequencing Run**
○ **Control Experiment**

Flow cell product code

● **FLO-MIN106** ○ **FLO-MIN107**

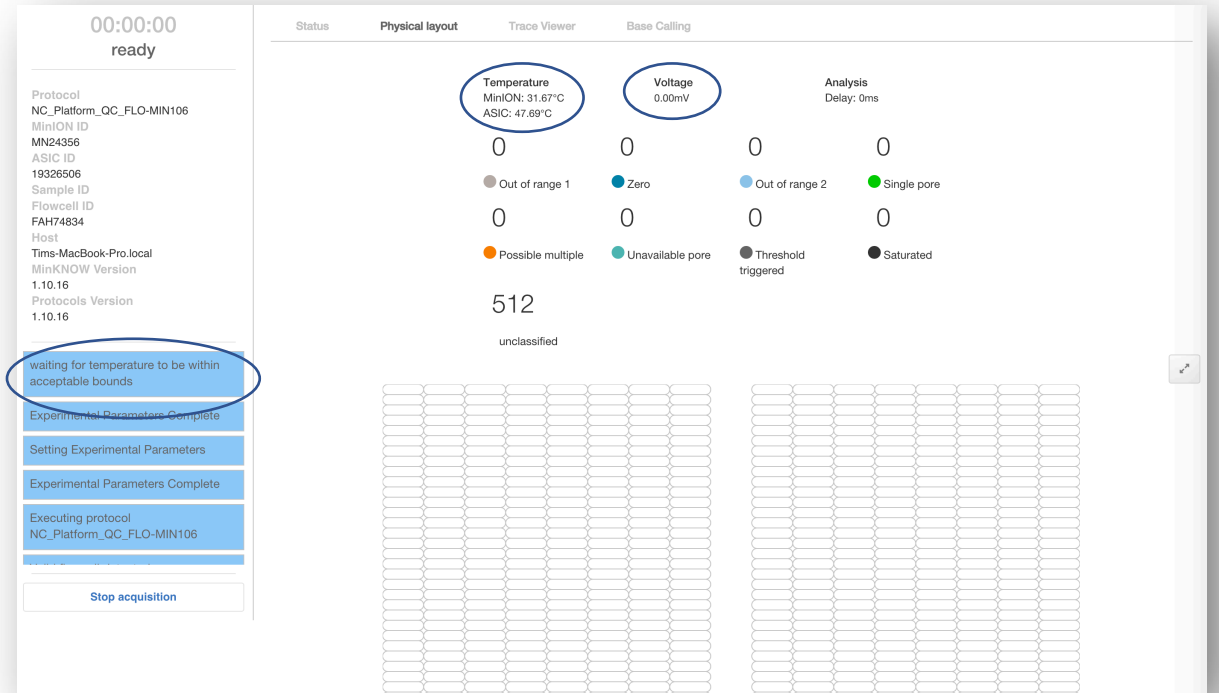There is 1 script choice:

NC_Platform_QC_FLO-MIN106 ⬍

Execute
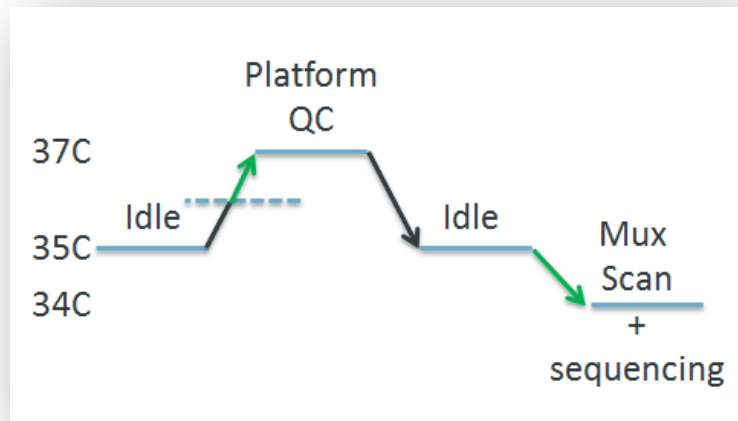
MinKNOW GUI – Flow Cell QC

Flow Cell QC

- Should be performed before every sequencing run

- Temperature has to be reached

- A series of current changes form positive (reversed) to negative -180mA to maximise number of free active pores
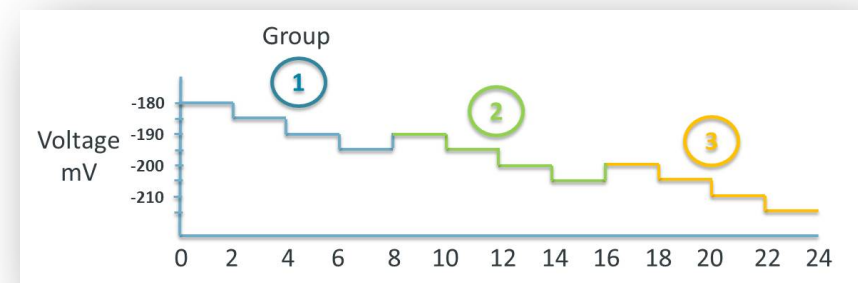
Flow Cell QC run

Temperature

- Higher temperature increase mobility of charged particles

Source: https://community.nanoporetech.com

Voltage

- Positive / reverse voltage to clear pores

- Over time current is decreased in 5mV steps to account for loss of potential (drift)

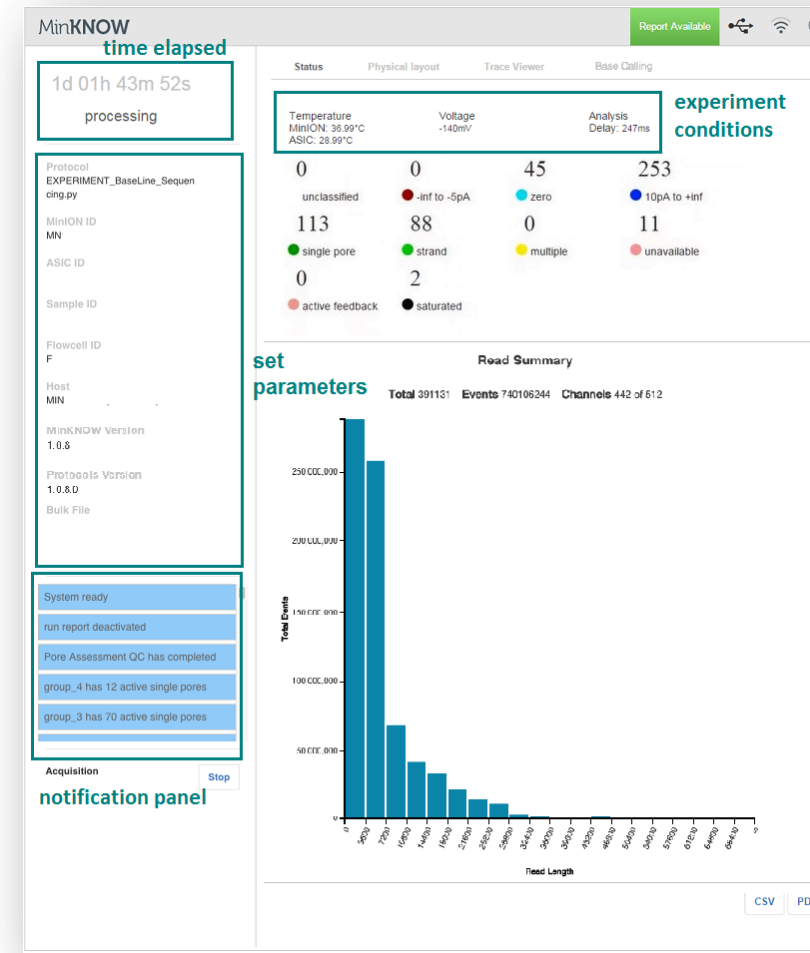Source: https://community.nanoporetech.com

MinKNOW GUI – Sequencing Run

MinION – Status Widget

- Sequencing yield overview

- Events ± nucleotides

  Per event ~1.7 (R9 Flow cells)

  nucleotides pass through one pore

Source: https://community.nanoporetech.com

MinION – Physical Layout Widget

Single pore	Open but empty pore
Strand	DNA translocating through pore, i.e., sequencing
Adapter	Pore sequencing unligated adapter oligo
Multiple	The current levels indicate that more than one pore is active
Active feedback	The current is flicked, e.g., to remove a stalled strand
Saturated	Current outside detector range and channel is switched of and won't be used for sequencing anymore

Source: https://community.nanoporetech.com
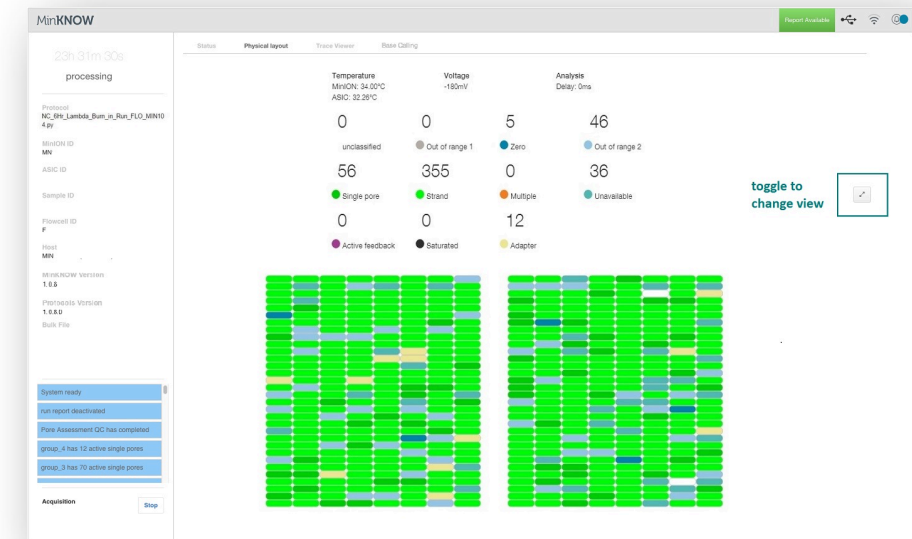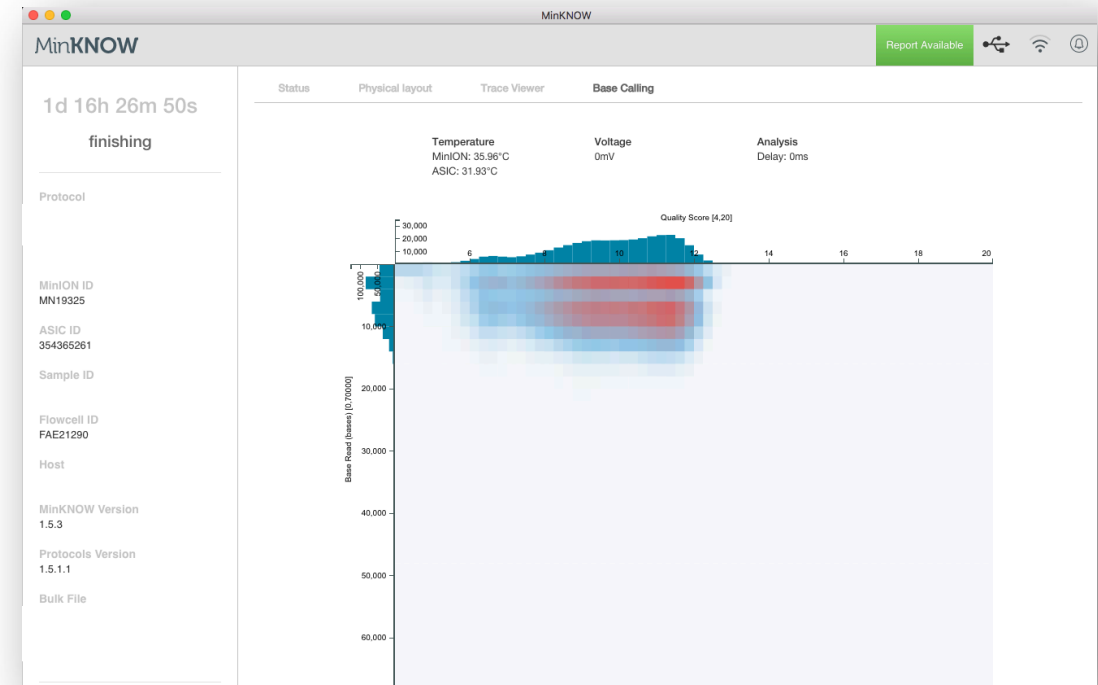
MinION – Trace Viewer Widget

- Visualisation of channnels

- Each channel is represented by one pixel in waterfall plot

Source: https://community.nanoporetech.com
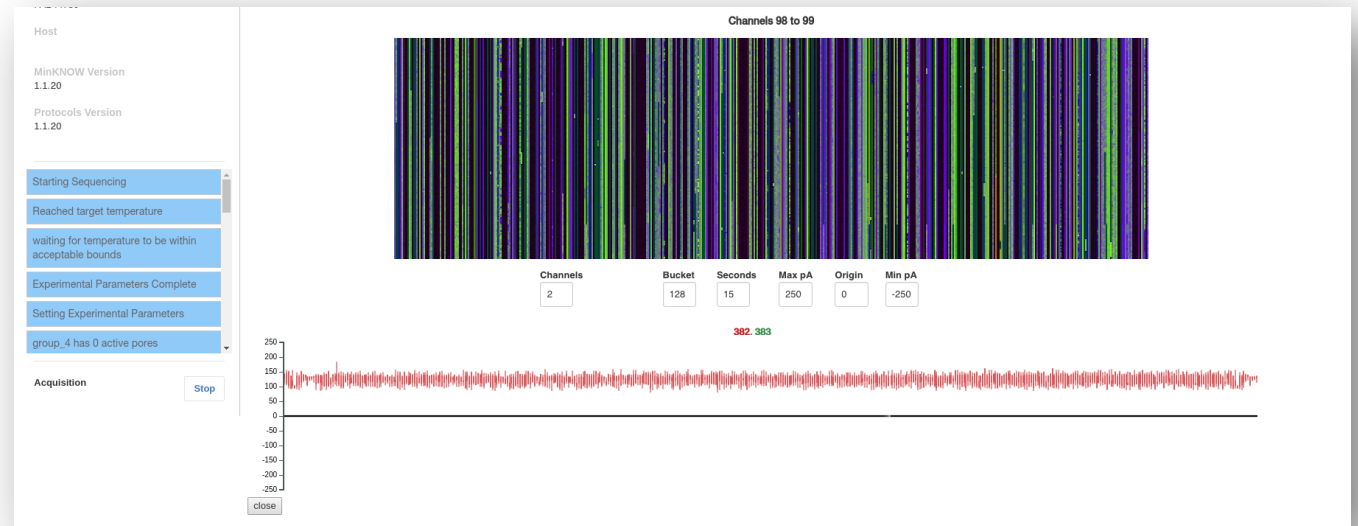
MinION – Base Calling Widget

- Overview over local base calling

- Plot showing quality score of base-called reads in relation to read length

Source: https://community.nanoporetech.com

Questions?