






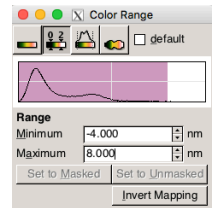


DNA processing in Gwyddion



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Flattening and masking:


-  Align rows using various methods → median of differences
-  Level data by mean plane subtraction
- Data Process → Level → Fix zero
-  Stretch colour range to part of data →
-  Explicitly set fixed colour range → Values: -4 – 8 nm
-  Mark grains by threshold → Instant updates ☒ → Threshold by height: 25% (*you need to set this manually to include DNA and other non-flat objects*)
-  Align rows using various methods → median → masking mode: exclude masked region
- Data Process → Level → Fix zero
- Remove mask (CTRL+K)
-  Filter → gaussian → 1.5 pixels (*to remove high frequency noise*) – optional
- File → Save as → filetype automatic by extension
 - .gwy – a gwyddion file with all your changes saved
 - .txt – an ascii file (what I use for python imports etc)
 - .tiff – standard images
 - Lateral scale → rulers (preferred), or, inset scale bar
 - Values → false colour ruler

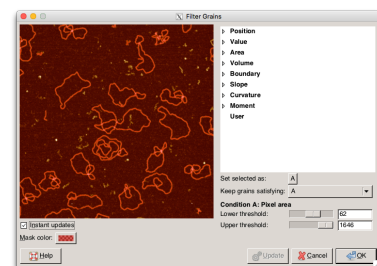


Tracing DNA:

-  Mark grains by threshold → Instant updates ☒ → Threshold by height: 25% (*you need to set this manually to include DNA and other non-flat objects*) redo this to ensure its perfect i.e. traces the DNA as contiguous lines, and doesn't include much background
-  Filter grains by their properties → pixel area → lower threshold: 62 (*just below the point where DNA starts being excluded, removes small objects*) → upper threshold: 1646 (*either max, or lower to remove large aggregates*)

Data process → grains → remove edge touching


-  Distributions of various grain characteristics → check required characteristics ☒
 - maximum value
 - mean value
 - pixel area
 - projected area
 - equivalent disc radius
 - area above half height
 - grain minimum basis volume
 - projected boundary length
 - minimum bounding size
 - maximum bounding size
 - mean radius



-  Export raw data → add informational comment header

Data process → mask → thin

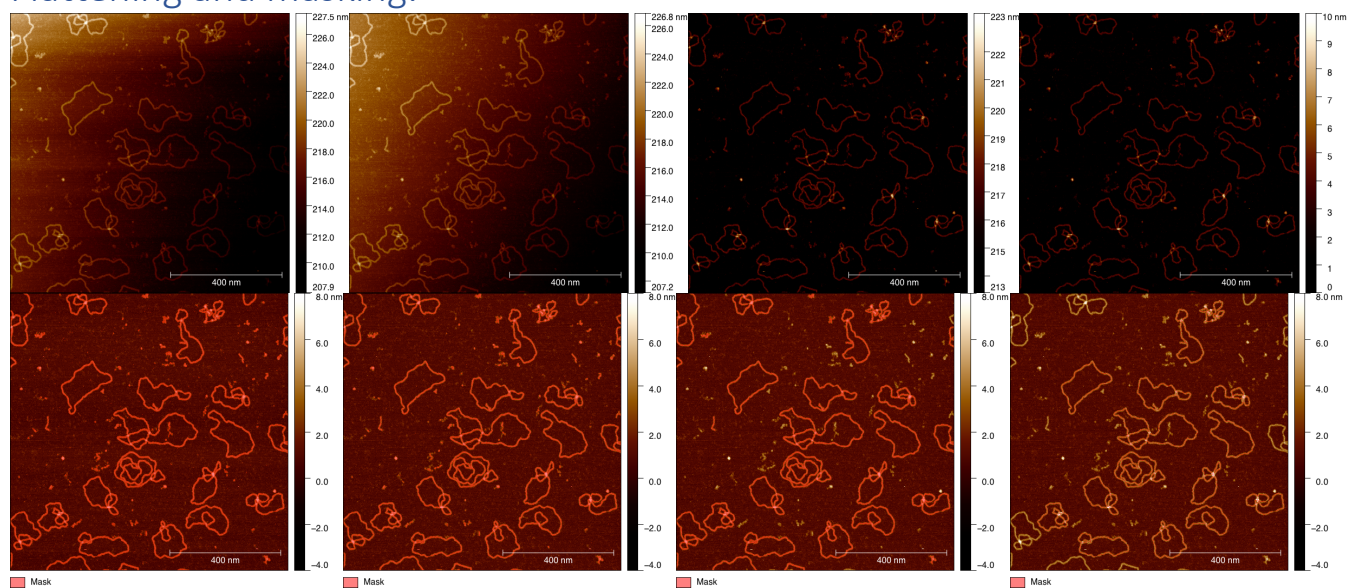
-  Export raw data → add informational comment header (*re-export for new thinned data set*)

-  Data process → mask → extract mask

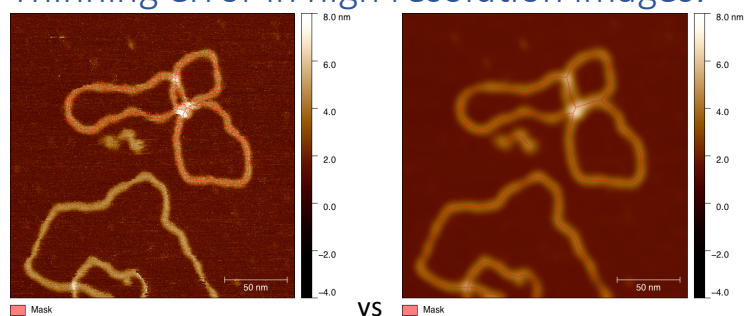
- This generates an image just the single pixel line – this should be perfect to export (possibly as an ascii)

In the case of thinning errors, may need to aggressively (10-20 pixel) gaussian filter before masking and thinning (*more likely in higher resolution images*)

Flattening and masking:



Thinning error in high resolution images:



Extracted mask:

