Mapping chromosome positions with ‘sperm cartography’

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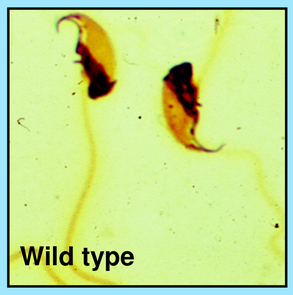
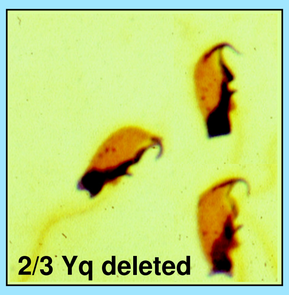
Mice have hook shaped sperm

Mice have distinctive hooked sperm. Deletions on the Y chromosome, as in our model with 2/3 Yq deletion (Yq-del) cause defects in sperm morphology and sex ratio skewing of offspring [1].

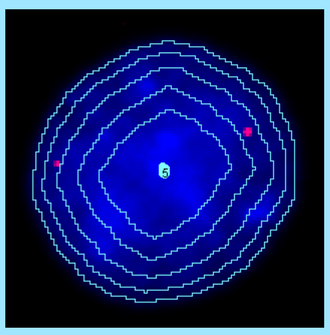
Nuclear organisation (chromosome position) is implicated in fertility issues in other species. Is this also true in mice? What effect does the deletion have on chromosome position?



Pronounced acrosomal hook



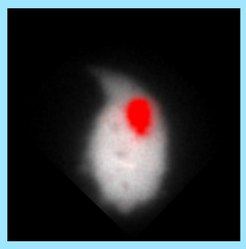
Fertile, flattened acrosome
60:40 sex ratio skew



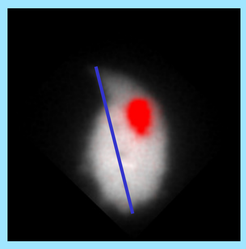
The problem: conventional shell based erosion analysis (left [2]) does not work well here – the sperm are asymmetric, and thus we want to know not just the distance of signals from the centre, but also whether the signals are towards the head or the tail, the hook side or the hump side.

We need an automated means to process FISH images, and determine the positions of signals within an asymmetric nucleus.

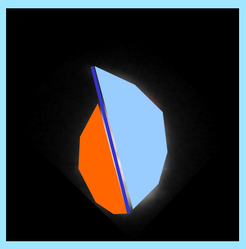
How we can orient sperm FISH images



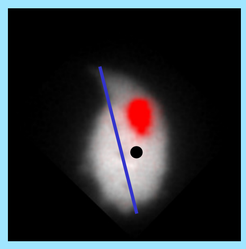
Sperm nucleus with **Yq paint**



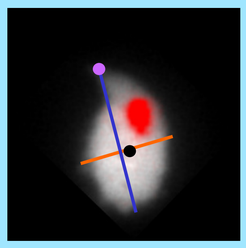
Longest diameter defines **head-tail** axis



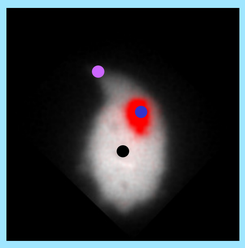
Larger area on **‘hump’ side** than on **‘hook’ side**: defines hook-hump axis



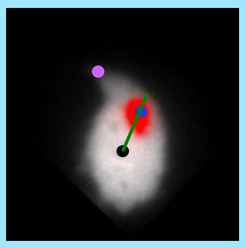
Find **centre of mass (CoM)** of sperm head



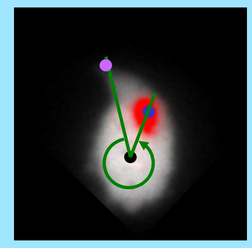
Draw **line** passing through **CoM**, orthogonal to **head-tail**. Longer half of **head-tail** identifies the **sperm head**.



Find **centre of mass** of the signal



Draw **line** from **sperm CoM** through **signal CoM** to nucleus border



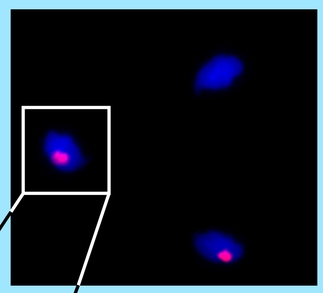
Measure:
1) **angle** from **sperm head**-**CoM**-**signal CoM**

2) distance of **signal CoM** from **sperm CoM**

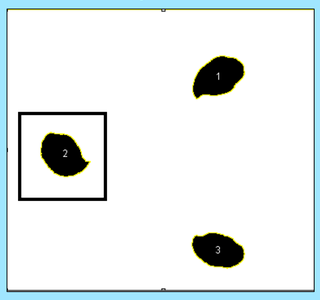
Automating the process

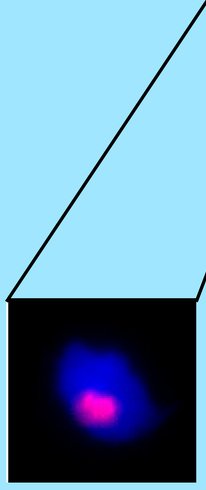
Detection - ImageJ

Original image

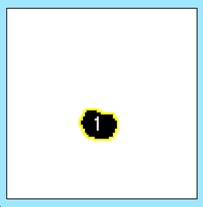


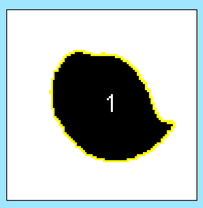
Identify nuclei





For each nucleus:

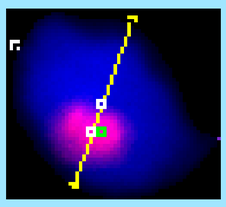




Split colour channels

Is there a clear signal?
Find centre of mass
Find area of signal

Centre of mass
Longest diameter
Borders of nucleus
Area of nucleus

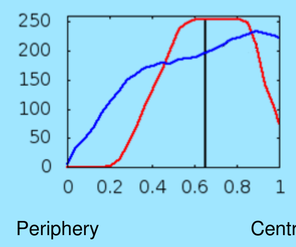


Calculate and log distances and angles as described above

Script to process ImageJ output:

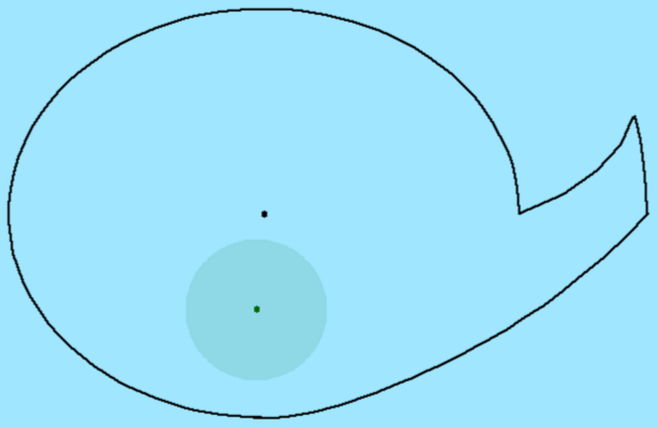
Use the signal density profiles to calculate the median signal position (50% signal on either side) from the nuclear centre to the nuclear periphery.

Split the data into 30° bins, calculate:
number of signals per bin
average signal position within each bin



Signal and DAPI intensity profiles

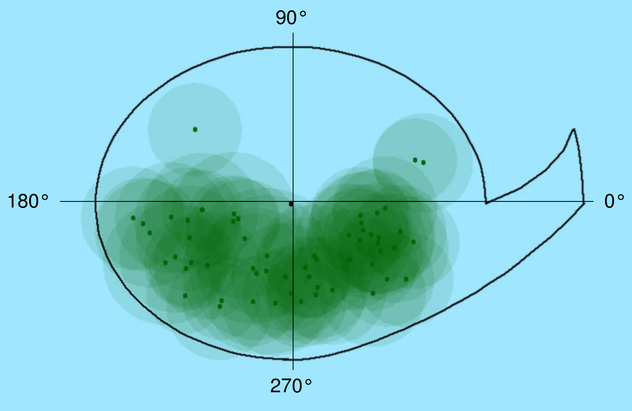
Analysis - Perl

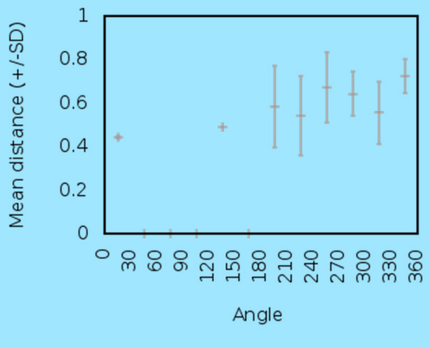


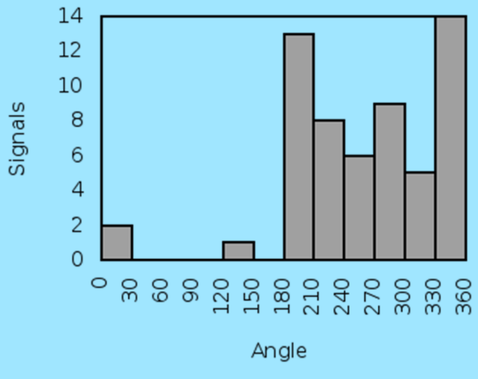
A circle with equal relative area to the original signal placed on a template at the calculated positions.

Results from many nuclei can now be overlaid to produce a standardised output.

Mapping the Y chromosome in Yq-del mice







Initial data suggest the Yq-del Y-chromosome adopts a position on the ‘hump’ side of the sperm, with territories roughly midway between the centre and periphery.

The distances seem to become more internal as the angle increases, suggesting the chromosome avoids the hook itself.

We can now map other loci in mouse sperm

We demonstrate the use of a new automated tool for investigating nuclear organisation in a cell type with an unusual nuclear shape, and have used it in initial work to show that the mouse Y to occupies a non-random position in the sperm nucleus.

With this method established, we will be able to compare sperm from wild type mice and Yq-del mice to determine whether the Yq-del phenotype includes abnormalities in nuclear organisation.

Further experiments will determine the positions of the other mouse chromosomes, and also to look at the levels of variation in position *between* individuals. Particularly, it will be interesting to find out which chromosome(s) occupy the hook itself; in other mammalian species, the X has been shown to lie in the first region of the sperm make contact with the egg, and the same may be true of mice.

References
[1] Conway SJ, Mahadevaiah SK, Darling SM, Capel B, Rattigan AM, Burgoyne PS. 1994. *Mamm Genome* **5**: 203–210.
[2] Skinner BM, Volker M, Ellis M, Griffin DK. 2009. *Cytogenet Genome Res* **126**: 156–164.
ImageJ: http://rsb.info.nih.gov/ij/ | Abramoff MD, Magelhaes PJ, Ram SJ. 2004. *Biophotonics Int* **11**: 36–42.