Electronic Supplementary Information

High-throughput sperm DNA analysis at the single-cell and population levels

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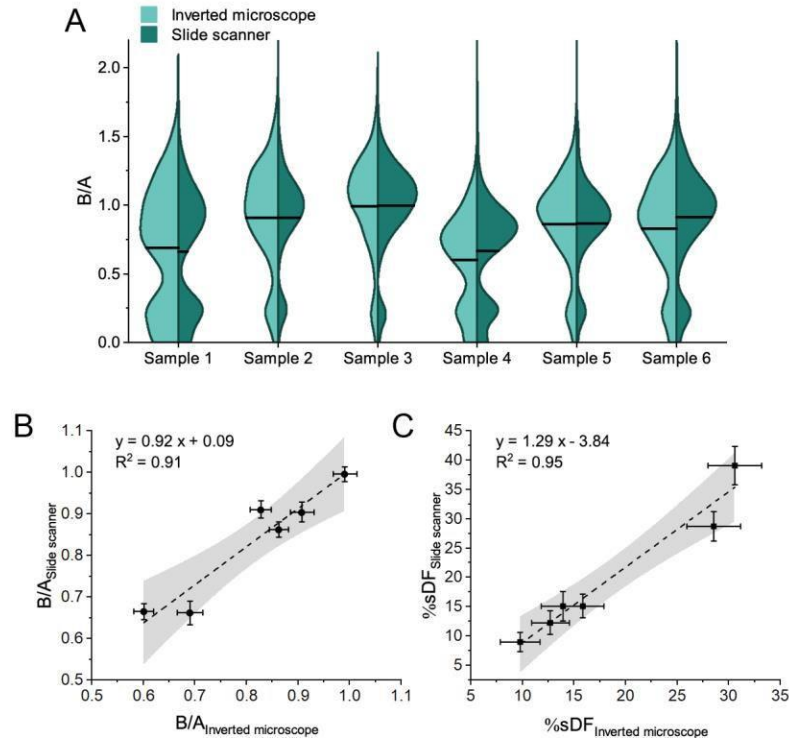*Emails: reza.nosrati@monash.edu, sinton@mie.utoronto.ca

Figure S1 Automated analysis of images obtained using the inverted microscope and slide scanner. (A) Violin plots of sperm B/A measued by the developed software from images taken with the inverted mictoscope against the slide scanner images. (B) Mean B/A and (C) %sDF of the six samples obtained from images taken with the inverted microscope and the slide scanner. Error bars indicate the 95% confidence intervals.
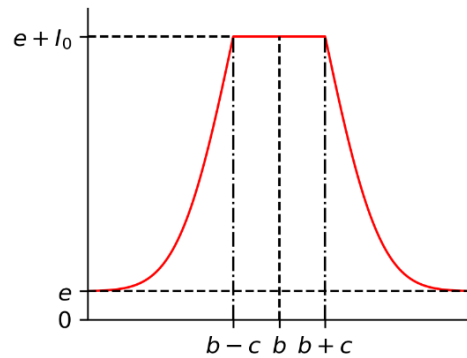

Figure S2 A sample representation of the used diffusion equation for the DNA halo profile fitting.
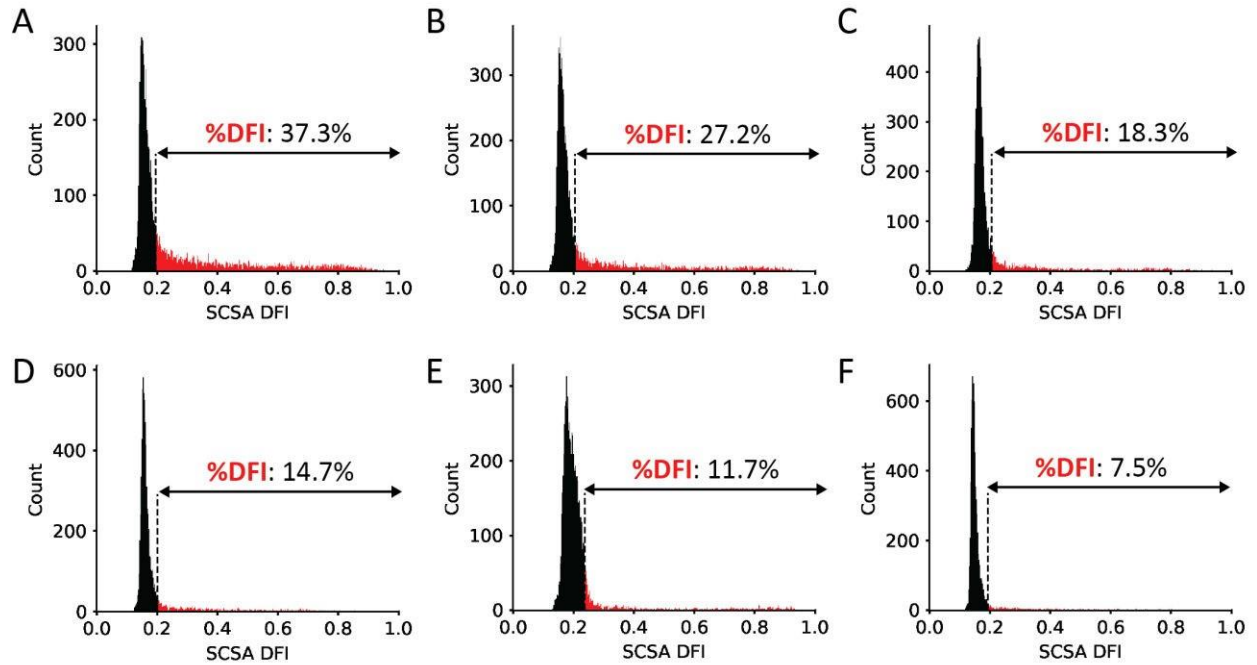
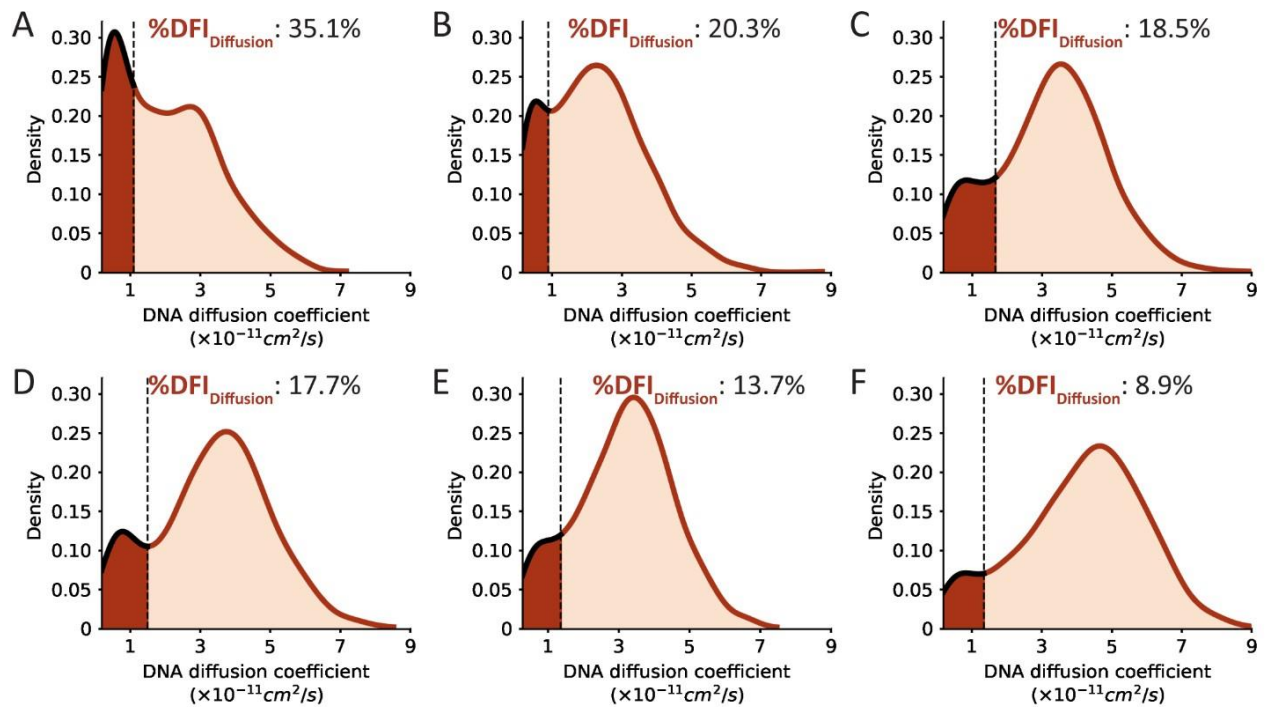**Figure S3** SCSA DFI histogram for six different human semen samples.

Figure S4 The probability density function of sperm DNA diffusion coefficient (D_{DNA}) for six different human semen samples.
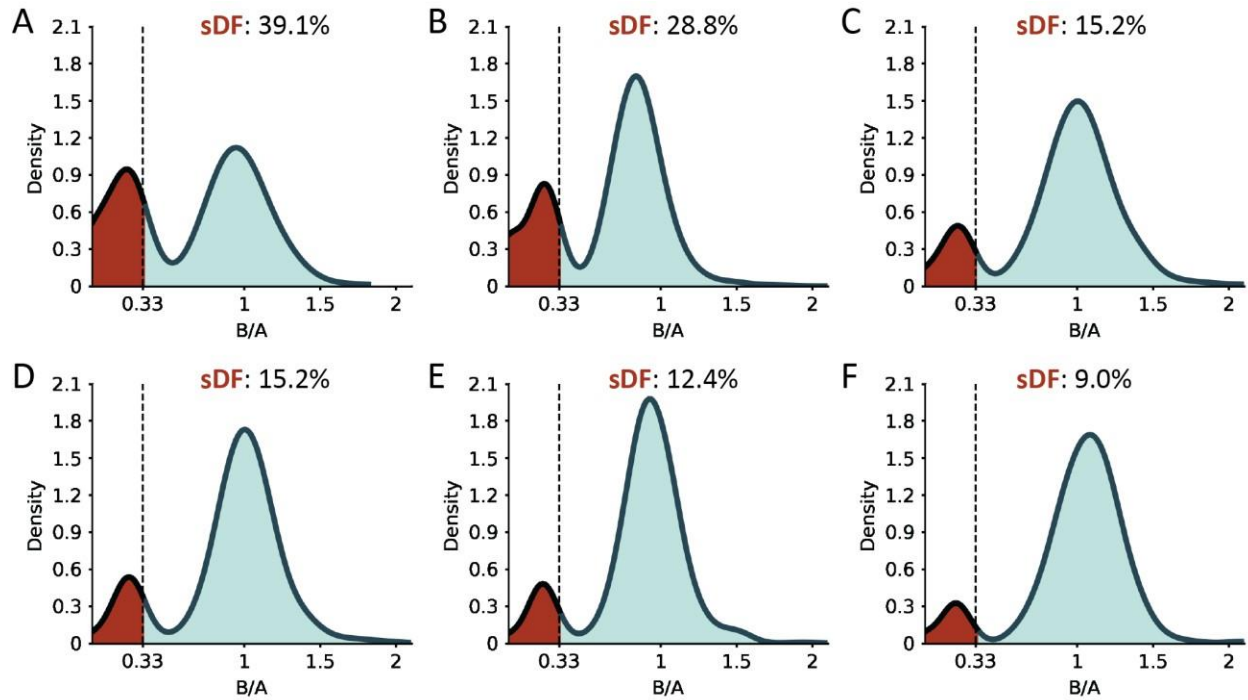
Figure S5 The probablity density function of sperm normalized halo thickness (B/A) for six different human semen samples.
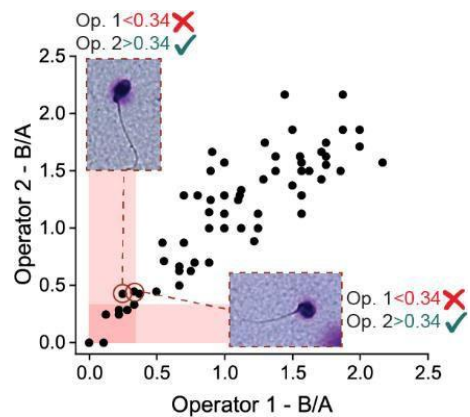

Figure S6 The measured B/A of sperm cells by two operators showing a significant variation between operators that can significantly affect the final %sDF measurement (% of sperm with B/A<0.34).
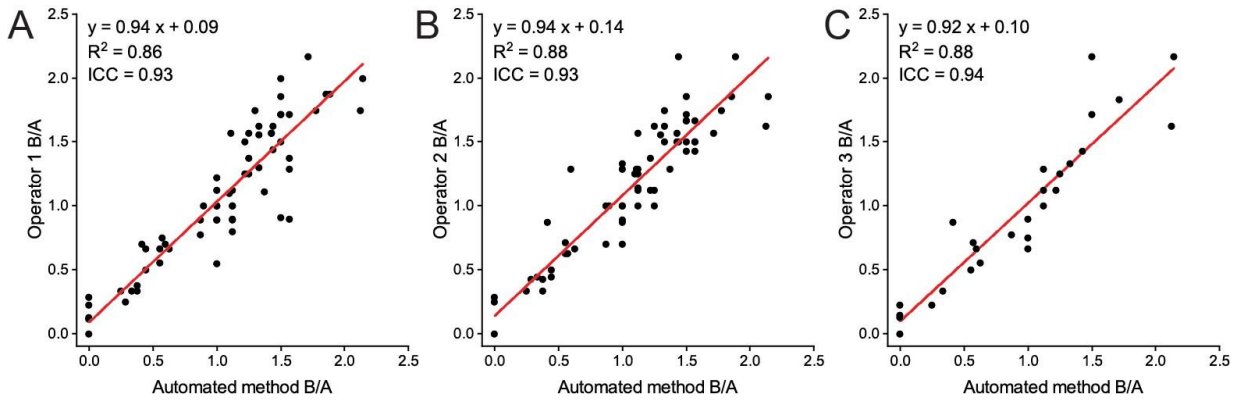
Figure S7 The measured B/A values by the opeartors against the results of the automated method.
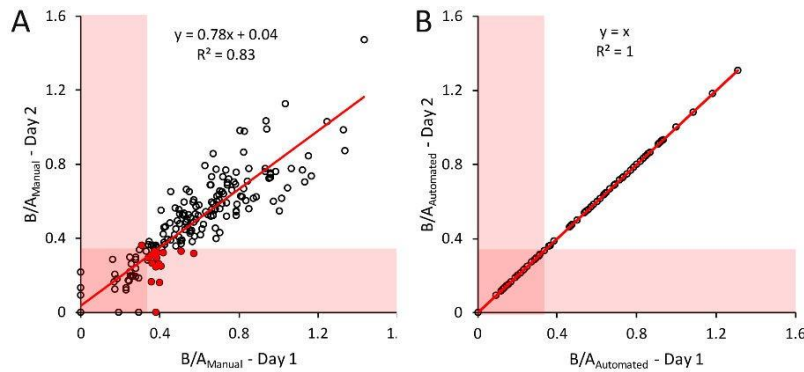

Figure S8 Duplicate measurement of 203 sperm cells. (A) Comparison of measurement results obtained by the same operator on two different days. Red dots represent sperm cells that shifted between fragmented and non-fragmented DNA as a result of repeated measurements. (B) Results obtained by the developed software on two different days.

Table S1 Results of a duplicate measurement performed by an operator and the developed automated approach. (N=203 sperm cells).

Manual method		Day 2	
		Fragmented	Non-fragmented
Day 1	Fragmented	49	1
	Non-fragmented	16	137

Automated method		Day 2	
		Fragmented	Non-fragmented
Day 1	Fragmented	64	0
	Non-fragmented	0	139