Supplementary Information

High-throughput label-free assessment of sperm DNA fragmentation index via intelligent morphological imaging

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Table S1. Conventional image-based feature parameters

Name	Parameter category	Symbol	Unit	Equation
Maximum phase	Phase parameter	f1	radian	—
Phase sum	Phase parameter	f2	radian	—
Average phase	Phase parameter	f3	radian	—
Phase variance	Phase parameter	f4	radian²	—
Vopd	Phase parameter	f5	radian · μm²	$\iint \phi(x,y)dxdy$
Mass	Phase parameter	f6	pg	$\frac{\lambda}{2\pi \cdot \alpha}\iint \phi(x,y)dxdy$
Average Mass	Phase parameter	f7	pg/μm²	$\frac{Mass}{Area}$
Area	Phase parameter	f8	μm²	—
Circumference	Phase parameter	f9	μm	—
Length	Phase parameter	f10	μm	—
Width	Phase parameter	f11	μm	—
Fold	Phase parameter	f12	—	$\frac{4\pi \cdot Area}{Circumference^2}$
Ellipticity	Phase parameter	f13	—	$\frac{Width}{Length}$
Phase median	Phase parameter	f14	radian	—
Phase kurtosis	Phase parameter	f15	—	—
Phase skewness	Phase parameter	f16	—	—
Energy	Phase parameter	f17	—	$\sum P(i,j)^2$
Head area	Intensity parameter	f18	μm²	—
Head circumference	Intensity parameter	f19	μm	—
Head length	Intensity parameter	f20	μm	—
Head width	Intensity parameter	f21	μm	—
Head ellipticity	Intensity parameter	f22	—	$\frac{Head\ width}{Head\ length}$
Head folds	Intensity parameter	f23	—	$\frac{4\pi \cdot Head\ area}{Head\ circumference^2}$
Head extensibility	Intensity parameter	f24	—	$\frac{Head\ length\ - Head\ width}{Head\ length\ + Head\ width}$
Head symmetry	Intensity parameter	f25	—	$\frac{\pi \cdot Head\ length \cdot Head\ width}{4 \cdot Head\ area}$
Head contour points	Intensity parameter	f26	—	—

Table S2. clinical routine semen parameters

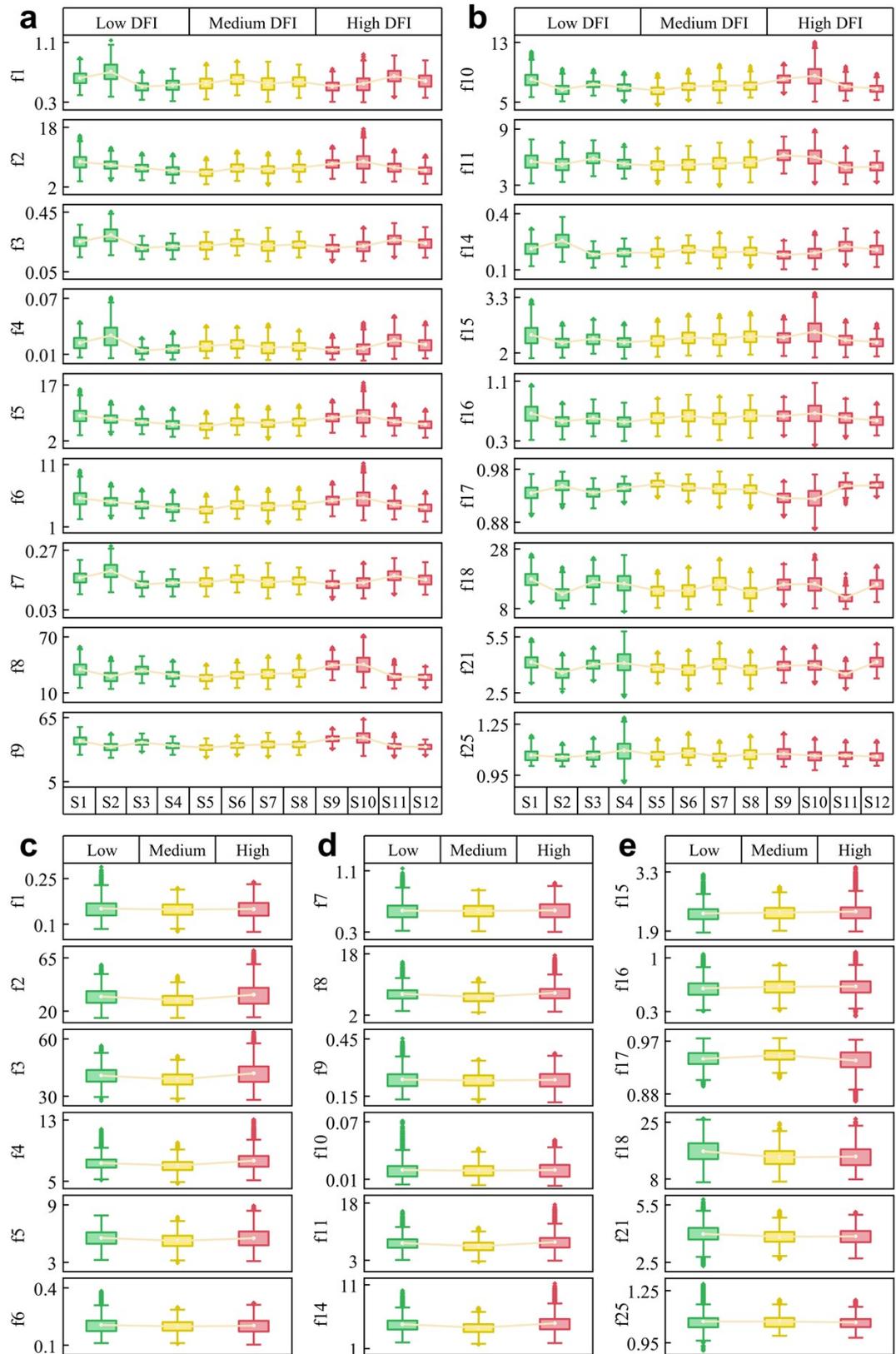| Name	Parameter category	Abbreviation	Unit
Semen volume	Clinical Parameter	SV	mL
Progressive motile sperm	Clinical Parameter	PR	%
Total motility	Clinical Parameter	PR+NP	%
Sperm concentration	Clinical Parameter	SC	10^6/mL
Total sperm count	Clinical Parameter	TSC	10^6/ejaculate
Non-progressive motile sperm	Clinical Parameter	NP	%
Slow progressive motile sperm	Clinical Parameter	SPR	%
Rapid progressive motile sperm	Clinical Parameter	RPR	%
Concentration of non-progressive motile sperm	Clinical Parameter	CNP	10^6/mL
Concentration of slow progressive motile sperm	Clinical Parameter	CSPR	10^6/mL
Concentration of rapid progressive motile sperm	Clinical Parameter	CRPR	10^6/mL
Straight-line velocity	Clinical Parameter	VSL	μm/s
Average path velocity	Clinical Parameter	VAP	μm/s
Curvilinear velocity	Clinical Parameter	VCL	μm/s
Linearity	Clinical Parameter	LIN	%
Wobble	Clinical Parameter	WOB	%
Straightness	Clinical Parameter	STR	%
Morphologically normal sperm	Clinical Parameter	MNS	%
Teratozoospermia Index	Clinical Parameter	TZI	—
Sperm Deformity Index	Clinical Parameter	SDI	—
Multiple Anomalies Index	Clinical Parameter	MAI	—
Sperm with excess residual cytoplasm	Clinical Parameter	ERC	%
Sperm with principal piece defects	Clinical Parameter	PPD	%
Sperm with midpiece defects	Clinical Parameter	MPD	%
Sperm with head defects	Clinical Parameter	HD	%

Supplementary Figure

Figure S1. Conventional image-based feature analysis of sperm images. a,b) Statistical

distributions of 18 conventional image-based features across 12 samples ordered by ascending DFI. c,d,e) Inter-group comparison of conventional image-based features among low, medium, and high DFI categories.
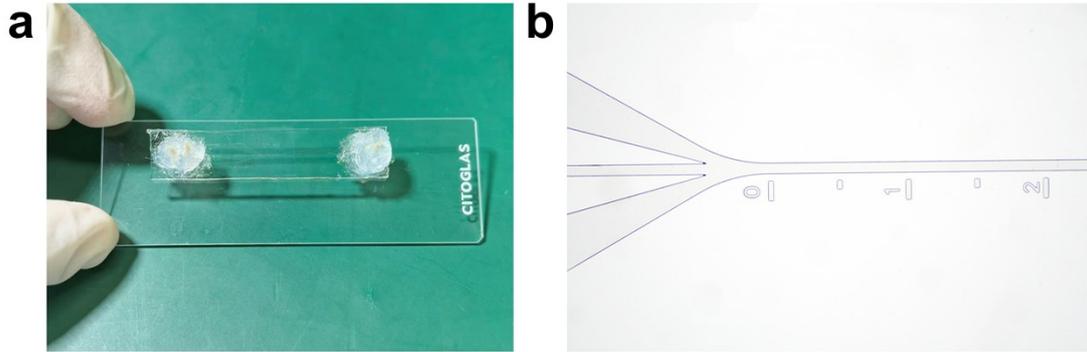
Figure S2. Appearance and internal structure of the microfluidic chip. a) Photograph of the microfluidic chip. b) Microscopic image of the internal channel structure of the microfluidic chip.