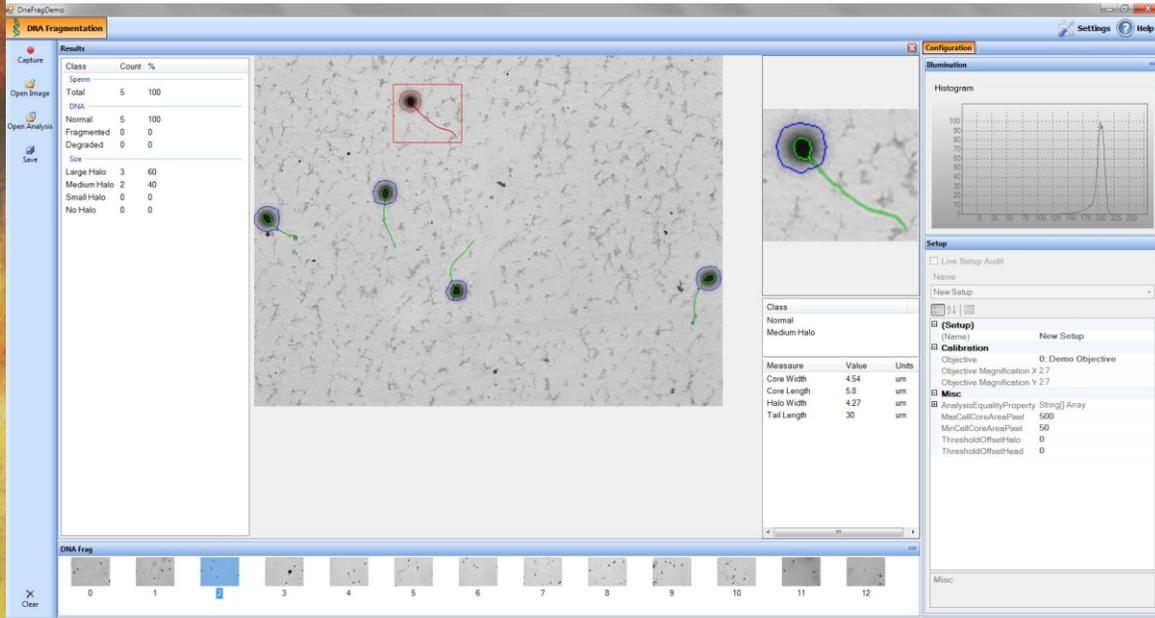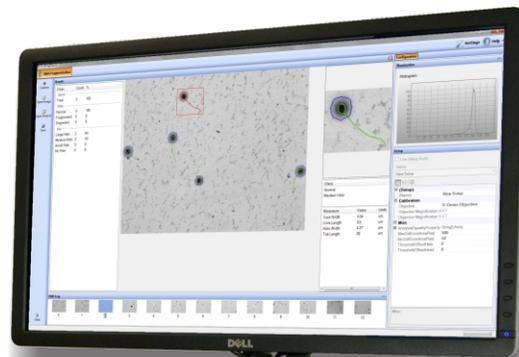# DNA Frag

Automated Sperm Chromatin Dispersion Measurement Software for IVOS II and CEROS II Systems

Compatible with the Halotech Halosperm® Assays

Innovations to Rely On

HAMILTON THORNE

For research use only in the USA

Principle of the Sperm Chromatin Dispersion Test

Intact unfixed spermatozoa (fresh, frozen/unthawed, diluted samples) are immersed in an inert agarose microgel on a pretreated slide. An initial acid treatment denatures DNA in those sperm cells with fragmented DNA. Following this, the lysing solution removes most of the nuclear proteins and, in the absence of massive DNA breakage, produces nucleoids with large halos of spreading DNA loops, emerging from a central core. However, the nucleoids from spermatozoa with fragmented DNA either do not show a dispersion halo or the halo is minimal.

In order to visualize the halos under bright field microscopy, prepared slides must be stained with a differential stain (included in Halopserm® G2 kit).

Sperm Classification

Sperm without fragmented DNA will show one of two states:

- Large halo: [H ⩾ C] the halo width is equal to or more than the minor diameter of the core
- Medium halo: [C > H > 1/3 C] the halo size is between the large and small criteria (less than the diameter of the minor core but greater than 1/3 the diameter of the minor core).
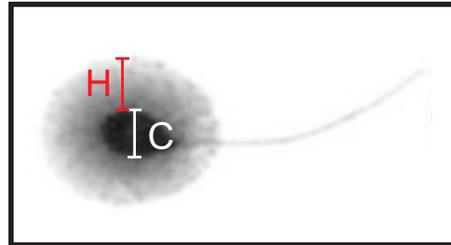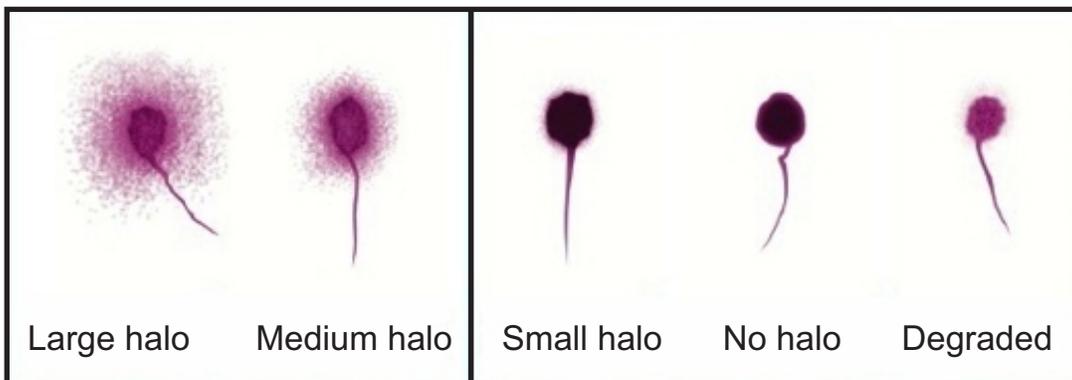
Sperm with fragmented DNA will show one of three states:

- Small halo: [H ⩽ 1/3 C] the halo width is equal to or less than 1/3 of the minor diameter of the core
- No halo: no halo is observed
- No halo and degraded: no halo is observed and an irregularly or weakly stained core is present

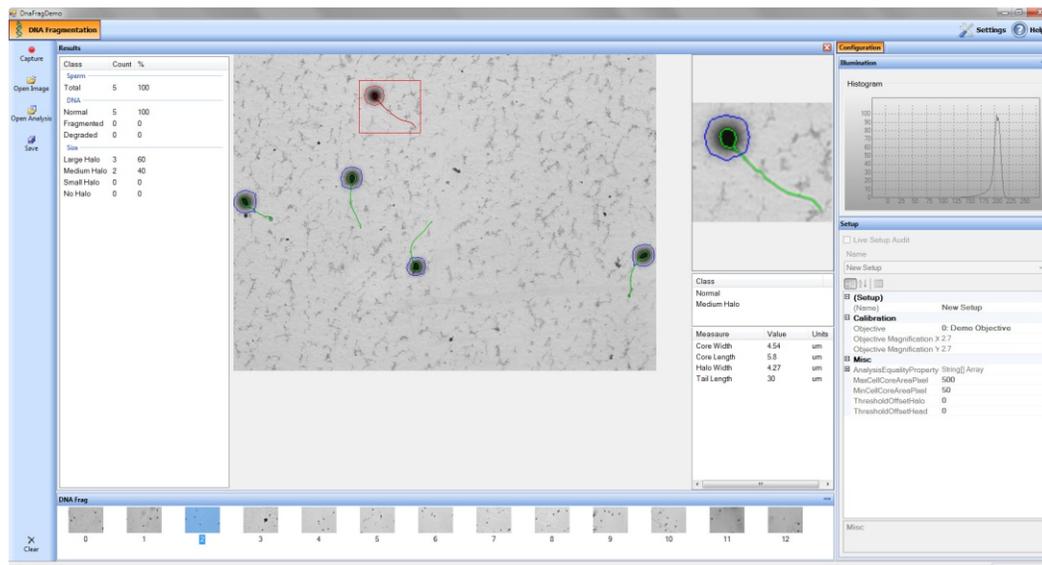| Non-fragmented		Fragmented		
Large halo	Medium halo	Small halo	No halo	Degraded

DNA Frag Software Overview

The DNA Fragmentation software is accessed from within the CASA II motility program. The interface of the DNA Fragmentation software is similar to the interface of motility software, with the image area centrally located, analysis controls along the top and left sides, image thumbnails along the bottom, and information and results to the right.

The software analyzes the prepared sperm samples and measures the size of the halo (large halo, medium halo, small halo, or no halo) and thus categorizes cells as Non-fragment, fragmented, or degraded. Results are shown cumulatively, per selected field (left) and per selected cell (right).

The DNA Fragmentation Software is designed to evaluate the level of DNA fragmentation of sperm smears prepared with the Halosperm® G2 test kit from Halotech® DNA (www.halotechdna.com), which is based on the Sperm Chromatin Dispersion (SCD) Test (Fernandez et al., J. Androl 24:59-66,2003; Fertil Steril 84:833-842,2005).