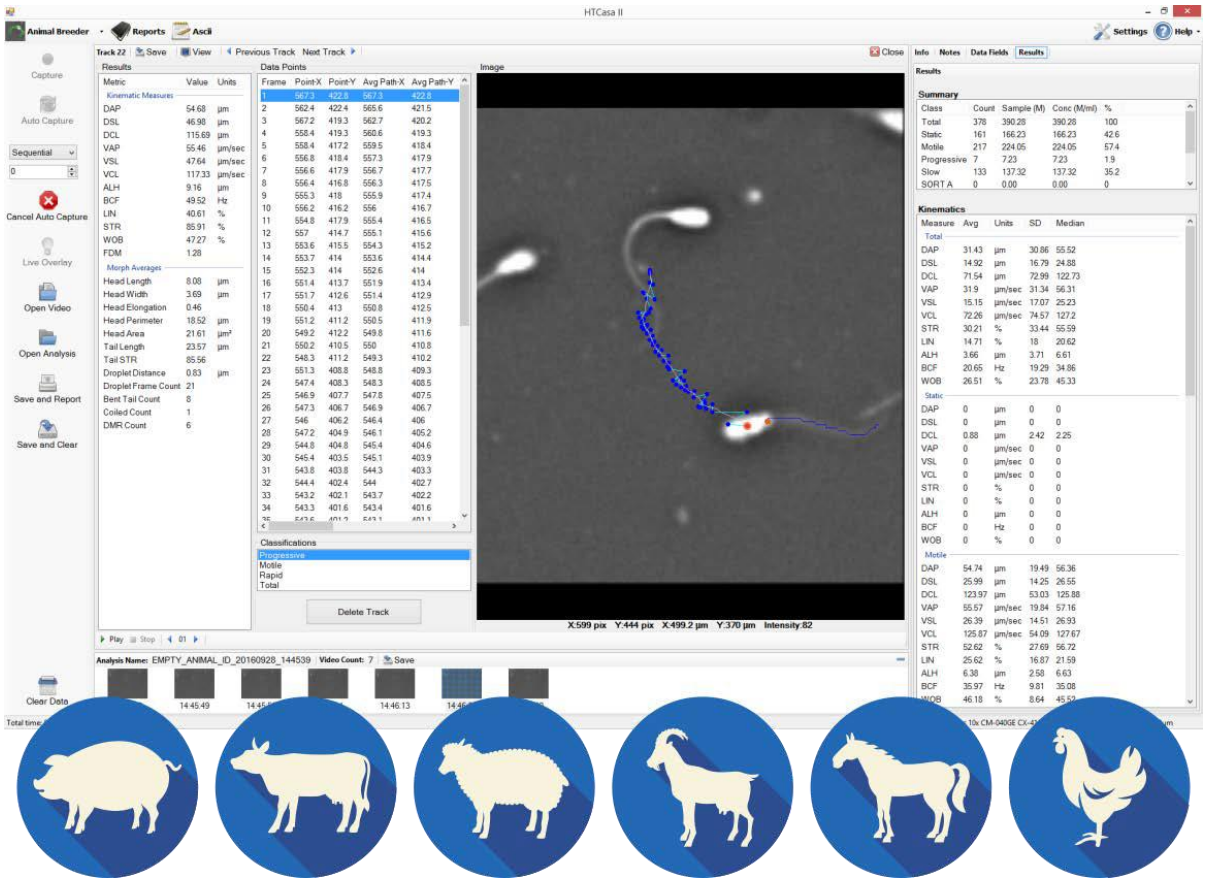
HT CASA II Software Manual for IVOS Pro System

Animal, Boar, and Equine Breeder
Version 2.1



SERIAL NUMBER

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HT CASA II Animal Breeder Software Instructions for Use

HT CASA II Animal Breeder Software Manual
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This product may not be available in all countries.

WARRANTY, SERVICE INFORMATION, RETURNS AND LIMITATION OF LIABILITY

QF-85-03-01-1 Rev . C
Effective Date: May 22, 2018

WARRANTY

HAMILTON THORNE INC. (HT) warrants that its products will be free from defects in workmanship or materials and perform in accordance with HT published specifications (or the specifications agreed to, in writing, by the Buyer and HT and made a part of the sales contract) for a period of one year from the date of installation. This warranty does not cover lost parts or security keys, and shall not apply to damage to the equipment resulting from abuse, negligence, accident or loss due to fire, flood, theft, power fluctuations or power failures, lightning strikes, temperatures or humidity outside of HT published Operating Environments, storage or use in a corrosive environment, off-label use, user-directed system changes such as incompatible computer systems, computer virus or malware induced system changes, use of non- HT approved software on HT provided computer systems, damage in transit or any other damages covered under the Distributor's or Final User's Insurance Policy. The warranty may be voided should the Buyer attempt any repairs, alterations or additions, including installation of third-party software, without prior written permission of HT. This warranty is not valid unless a completed Installation Checklist for the system is returned to HT within 30 days from the installation date.

SERVICE INFORMATION

During this warranty period, HT will, at no cost, repair or replace any defective equipment returned to HT. Transportation charges to return the equipment to HT will be prepaid by the sender. The shipping method and packaging are critical to the repair process. Consult HT before shipping. When the Buyer requests expedited shipping or special handling, the Buyer shall pay any associated charges.

HT provides Software Maintenance which includes updates to software such as patches and reliability enhancements during the warranty at no charge. The warranty does not include major software upgrades.

Backup all files before returning the equipment for repair or replacement. HT recommends that you have an external back- up system at all times to reconstruct lost or altered files, data or programs. **HT is not responsible for any loss of data**.

RETURNS

A Return Authorization Number must be obtained before returning any product to HT. Please call 1-800-323-0503 in the U.S., 1-978-921- 2050 outside of the U.S., your local distributor or email support@hamiltonthorne.com for this Return Authorization Number. When calling or contacting HT, please have the serial number of your system available.

LIMITATION OF LIABILITY

HT makes no other warranty, expressed or implied, and HT disclaims any implied warranty of merchantability or fitness for a particular purpose.

The Buyer and HT agree that the sole and exclusive remedies for breach of any warranty concerning the goods shall be repair or replacement of defective parts upon the terms above described or, at HT's discretion, refund of the purchase price. HT shall not be liable for contingent or consequential damages to persons or property, and its sole liability is as above set forth. Any action by the Buyer for any alleged breach of the warranty set forth herein shall be brought to the attention of HT by the Buyer within the warranty period, but not later than 30 days after the alleged breach.

This statement of warranty and limitations of liability is a complete and exclusive statement of all warranty and liability representations of HT. It may not be varied, supplemented, qualified or interpreted by any prior dealings between the parties or by any usage of the trade or upon the face or reverse of any form to which this is attached or a part of, nor may it be modified by any agent, employee or representative of HT unless such modification or representation is made in writing and signed by a duly authorized officer of HT.

Repairs and/or replacement under the terms of this warranty shall not extend the warranty life of the original equipment supplied. All repairs and service must be performed by HT service engineers or by an authorized representative.

For information about purchasing an additional Service Contract, please contact sales@hamiltonthorne.com

Securing and Protecting your Computerized Hamilton Thorne Systems

QF-75-04-01-1 Rev.E
Effective Date: 2021-06-03

Hamilton Thorne Inc. (HT) products are designed to operate with Microsoft Windows® operating systems. Therefore, as the owner of a Hamilton Thorne system, you have a great deal of freedom in determining how and whether to connect your system to a network, load other programs onto the computer, as well as manage and protect your data. In all cases, adhere to all regulatory standards which pertain to your environment such as risk management for IT networks and management of cybersecurity.

General Use:

First and foremost, this is medical/research equipment and needs to be treated as such. Overloading the system with unnecessary programs, exposing unprotected systems to the internet, inserting infected memory sticks and other similar actions, which routinely cause problems on personal computers, are also risks to your HT system. It is recommended that additions or modifications be limited to those required for proper operation of your HT system. It is strongly recommended that you backup your system regularly and prior to making any changes.

Data Backup:

It is recommended that important data be externally backed up frequently to a location such as an external hard drive or cloud backup service. Microsoft Windows™ includes backup utilities and there are also third-party backup and restore solutions available so that data you create, as well as the "state" of the system, can be backed up and used in emergency situations to restore the system to a previous operational state.

User Access:

Hamilton Thorne recommends controlling user access to systems. The following are some basic guidelines that are recommended;

a. Configure secure user account access using secure authentication via Windows OS controls;
b. Establish passcodes and authentication policies for all users;
c. Physical access should be limited to authorized personnel; and
d. Only system administrators should have access to critical system settings.

Virus and Malware Protection:

The use of an anti-virus, anti-malware program is strongly recommended. Windows Security Essentials and Windows Defender are compatible with HT systems and free to download from Microsoft or included with Windows. Hamilton Thorne may opt to pre-install anti-virus software on HT systems. You may opt to install your preferred anti-virus program at your discretion.

Hamilton Thorne recommends that anti-virus and anti-malware programs, as well as Windows OS security patches are kept up-to-date with the latest protection.

NOTE: It is not recommended to scan files created by HT software applications, as this can severely slow down system performance if anti-malware scanning is performed on large image or video files created by the HT software.

Networking:

Hamilton Thorne ships most of its systems "network capable." Hamilton Thorne is not responsible for networking the HT system to your network. To connect to a network, contact the system administrator for that network. The following are some basic guidelines that are recommended;

a. The HT system operation should be validated prior to network setup;
b. The system should be backed up/stored at this state. Only then should the networking steps be pursued;
c. Be aware that, although networking is the easiest way to share data, joining a domain can alter performance, especially if the HT system is trying to boot or run programs from a domain. HT programs require access to the local hardware and local hard drive;
d. A preferred method of network operation is to map a shared network drive that the HT programs can

access;

e. HT systems should be connected to a protected LAN only;

f. Limit device access to the internet for non-essential purposes;
g. The Windows firewall is typically enabled at HT to reject all incoming connections and should remain enabled for continued protection;
h. To prevent unauthorized use, data protection including passcodes are recommended; and
i. Limit and monitor any third-party access.

HT Software Program Installation and Operation Notes:

e. The following Legacy HT programs must be installed with and also run with administrator privileges, and also require that Windows User Account Control be set at a minimum level:
f. Analog Camera - HT CASA Versions V10, V12, V14;
g. Analog Camera - Dimensions Morphology; and
h. Analog or Digital Camera - HT Laser Version V5 (e.g., V5.09, V5.12, etc.).
i. Be aware that limiting these administrative privileges on Legacy systems, which have been configured by HT, will interfere with Legacy HT program operation.

Third-party Hardware and Software:

Ensure that third-party software, such as anti-virus programs or other application programs, do not directly conflict with the HT system operation and do not use excessive CPU resources during times which may interfere with HT system operation (e.g., do not run full system virus scans or live updates during the day). It is recommended to run full system scans on an after-hours automated schedule.

Installing third-party programs such as spreadsheet, database or PDF capabilities is possible on HT systems (e.g., Open Office, Microsoft Office). This is generally acceptable, but it is recommended to minimize the installation and use of additional programs to only what is required to run with the HT system.

Software Release Announcements (SRA):

When Hamilton Thorne releases new software, a Software Release Announcement (SRA) is generated and is included with the software distribution media. The SRA contains a summary of changes to the software which includes new features, changes to existing features, fixes and known issues. Please read the appropriate SRA before updating to a new version of Hamilton Thorne software

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Special Notations & Symbols Glossary

NOTE: **Indicates an important point to keep in mind.**

Indicates instructions that must be followed to ensure safe operation and performance.

Indicates risk of serious injury

ADMIN: **Indicates the users must have administrator privileges to perform a function.**

SYMBOL	INDICATION
	Manufactured by
	Consult instructions for use or consult electronic instructions for use.
	Date of manufacture
	HT CASA II software start up icon on desktop
	Settings access
	Country of manufacture
	Serial number
	Reference number (product part number)
	ASCII data access
	Report design and printing access

Conventions Used in This Manual

Some of the text in this manual uses special formatting to help indicate emphasis or keystrokes. The text conventions are as follows:

Table 1-1: Text Conventions

Convention	Example	Meaning
Bold	From the dropdown menu, select **Calibrate this image**	Bold text will indicate a menu item, control, function or setting
Bold with Arrows	Select **Patient > New** and enter patient information in the form fields.	Indicates menu selection and menu choice.

Chapter 1. Indications, Precautions & Warnings

1.1 Intended Use

CASA II Animal Breeder software is intended for animal use only to determine the concentration, velocity, and motility of sperm cells in a fixed-depth chamber. No diagnoses are performed by the CASA II software and no diagnostic interpretations are provided.

Not for clinical use.

CAUTION

1.2 Related Documentation

HT CASA II software is intended for use with IVOS Pro hardware systems. In addition to this document, one of the following hardware manuals will be provided:

- IVOS Pro Getting Started Manual

1.3 Guidance and Manufacturer's Declaration - Electromagnetic Emissions (IEC 61326-1:2020)

The HT CASA System is intended for use in the electromagnetic environment specified below. To ensureproper operation and not interfere with other equipment installed nearby, the device should only be placed inservice in the specified environment.

Emissions Test	Compliance	Electromagnetic Environment - Guidance
Conducted emissions. CISPR 11 Tested at 230VAC, 50 Hz	Group 1, Class A	
RF emissions CISPR 11 Tested at 230VAC, 50 Hz	Group 1, Class A	The HT CASA System RF emissions comply with test requirements and are not likely to cause interference in nearby electronic equipment.
IEC 61000-4-6 Voltage fluctuations/flicker Emissions Tested at 230VAC, 50 Hz	Complies	

1.4 Guidance and Manufacturer's Declaration - Electromagnetic Immunity (IEC 61326-1:2020)

The HT CASA System is intended for use in the electromagnetic environment specified below.

Immunity Test	Immunity Test	Compliance Level	Electromagnetic Environment - Guidance
IEC 61000-4-2 Electrostatic discharge (ESD)	± 2 kV/± 4 kV contact ± 2 kV/± 4 kV/± 8 kV air Tested at 240VAC, 60 Hz	± 2 kV/± 4 kV contact ± 2 kV/± 4 kV/± 8 kV air	Floors should be wood, concrete, or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
IEC 61000-4-4 Electrical fast transient/burst	0.5/1.0/2.0 kV for power ports Tested at 230 VAC, 50Hz	0.5/1.0/2.0 kV for power ports	Mains power quality should be that of a typical commercial or hospital environment.
IEC 61000-4-5 Surge	0.5/1.0 kV line(s) to line(s) 0.5/1.0/2.0 kV line(s) to earth Tested at 230 VAC, 50Hz	0.5/1.0 kV Differential Mode 0.5/1.0/2.0 kV Common Mode	Mains power quality should be that of a typical commercial or hospital environment.
IEC 61000-4-11	0 % during 1 cycle	0 % during 1 cycle	Mains power quality should be that of a typical

Voltage dips & interruptions	0 % during 10 cycles 70 % during 25 cycles 0 % during 250 cycles Tested at a rated voltage of 230VAC, 50 Hz.	0 % during 10 cycles 70 % during 25 cycles 0 % during 250 cycles	commercial or hospital environment. If the user of the HT CASA system requires continued operation during power mains interruptions, it is recommended that the HT CASA system be powered from an uninterruptible power supply or a battery.
IEC 61000-4-3 Radiated Immunity	10 V/m, 80 MHz to 1000 MHz 3 V/m, 1.4 GHz to 2.0 GHz 1 V/m, 2.0 GHz to 2.7 GHz Tested at 230VAC, 50 Hz	10 V/m 3 V/m 3 V/m	Portable and mobile RF communications equipment should be used no closer to any part of the HT CASA system including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter. Recommended separation distance d = 1.2 √P d = 1.2 √P 80MHz to 800 MHz d = 2.3 √P 800 MHz to 2.5 GHz where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and is the recommended separation distance in meters (m). Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey[a] should be less than the compliance level in each frequency range[b]. Interference may occur in the vicinity of equipment marked with the following symbol: 
IEC 61000-4-6 Conducted RF	3 Vrms, 0.15 MHz to 80 MHz Tested at 230VAC, 50 Hz	3 Vrms	

Note1: At 80 MHz and 800 MHz, the higher frequency range applies.
Note 2: These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects, and people

[a] Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the HT CASA system is used exceeds the applicable RF compliance level above, the HT CASA system should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as re-orienting or relocating the HTCASA system.

[b] Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.

WARNING

Use of accessories, including cables, other than those specified herein could result In increased electromagnetic emissions or decreased electromagnetic immunity of this equipment and result in improper operation.

To ensure proper operation and prevent degradation of performance, portable RF communications equipment (including peripherals such as antenna cables and external antennas) should be used no closer than 30 cm (12 inches) to any part of the HT CASA system, including cables.

1.5 Precautions

CAUTION

Not for clinical use.

Changing the Cell Travel Max from the standard value of 10 may alter proper operation.

To ensure proper classification, each new objective must be calibrated.

1.6 Warnings

WARNING

Use of accessories, including cables, other than those specified herein could result in increased electromagnetic emissions or decreased electromagnetic immunity of this equipment and result in improper operation.

To ensure proper operation and prevent degradation of performance, portable RF communications equipment (including peripherals such as antenna cables and external antennas) should be used no closer than 30 cm (12 inches) to any part of the HT CASA system, including cables.

Chapter 2. Overview of CASA II Software

2.1 Summary of Operation

A sperm sample is placed in a fixed depth chamber, examined microscopically and imaged with a digital camera. The HT CASA II software records a series of images (usually n = 30 to 45) of the field using a rate of 60 images per second. The sperm swim freely in the x-y direction, while z-motion along the optic axis is limited by the chamber ceiling and floor.

The concentration of sperm and their motility, velocity, motion, and morphometry parameters are derived for each sperm individually. The sperm tracks are classified and reported to the user according to their velocity and motion parameters.

2.2 CASA Methods, Definitions and Conventions

Image Acquisition

The consecutive series of field images is normally made under negative phase-contrast, giving bright sperm images on a dark background. Each field is composed of a specific number of sequential images or "frames." Identification of sperm by applying size and brightness limits is augmented using measurement of the head width/ length ratio (elongation) as a gate and counting only objects with appropriate elongation values. The centroid of the head is used to represent sperm head position. A most-probable-neighbor's assumption between frames allows accurate tracking of sperm provided the sperm concentration is not too high. If a high sperm concentration is present, sperm collisions or apparent collisions may invalidate the nearest-neighbor tracking assumptions and erroneous sperm tracks may result.

Effects of Collisions

If sperms coincide in position (or "collide") during acquisition, errors in tracking may result. These errors are minimal for concentration C ≤ 30 million/ml [M/ml] but become increasingly significant as the concentration is raised. Since the probability of collision increases with concentration, velocity, and the number of frames over which the sperm is tracked, collisions are minimized by using only the first ten frames to determine motile sperm count, static sperm count, and sperm motile fraction. All frames are used to determine the mean sperm velocity and motion parameters.

Counting Conventions

By convention, all sperm entering the field in the first 10 frames are counted during the acquisition. The corollary is that any sperm departing the field in the first 10 frames are not counted, in order to correctly determine concentration. Sperm entering the field after the first 10 frames are identified but are not counted, and analogously, sperm departing the field after the first 10 frames are still counted. In summary, only sperm present in the first 10 frames affect the counted summaries.

NOTE: *For samples presenting with high debris content, the count of static cells may be overestimated. A significant debris fraction, however, will not affect motile cell count or derived concentrations.*

Motion Parameter Definitions

Table 2-1: Motion Parameter Abbreviations and Definitions

Abbreviation	Parameter Name	Summary Description
VSL	**Straight Line Velocity**	The distance between the first and last points on the sperm track, divided by the elapsed time, gives VSL.
VCL	**Curvilinear Velocity**	The velocity along the sperm track is measured by summing the distance between the sperm head positions in each frame and dividing by the elapsed time.
VAP	**Average Path Velocity**	The average path is determined by smoothing the sperm head positions in a running average. The resultant path length is determined and divided by the elapsed time.
DSL	**Distance Straight Line**	The distance, in microns, traveled by the sperm along the VSL path.
DCL	**Distance Curvilinear**	The distance, in microns, traveled by the sperm along the VCL path.
DAP	**Distance Average Path**	The distance, in microns, traveled by the sperm along the VAP path.
ALH	**Amplitude of Lateral Head Displacement**	This is the maximum value of the approximately sinusoidal oscillation of the sperm head about the track. It is measured as the maximum distance between the actual sperm position and the corresponding average sperm position for all points over the track.
BCF	**Beat Cross Frequency**	The frequency with which the sperm head crosses the average path line during acquisition is the beat cross frequency.
LIN	**Linearity**	The ratio VSL/VCL in percent is a measure of track direction and is called the Linearity.
STR	**Straightness**	The ratio VSL/VAP in percent is a measure of track compactness and is denoted the Straightness.
WOB	**Wobble**	The ratio VAP/VCL in percent is denoted the Wobble.

How Motility is Determined

Motile, **Progressive**, **Slow** and **Static** cells are determined through application of kinematic parameters entered (see "Setups" on page 37).

Table 2-2: Kinematic Parameters and Functions

Kinematic Parameter	Function of Parameter
Progressive STR (STRP)	Minimum value of Straightness (STR) in percent required for a Track to be counted as Progressive.
Progressive VAP (VAPP)	Minimum value of Velocity of Average Path required for a track to be counted as Progressive.
Slow VAP (VAPS)	Maximum value of Average Path Velocity in micron/sec with which a Track can be counted as Slow.
Slow VSL (VSLS)	Maximum value of straight-line Velocity in micron/sec with which a Track can be counted as Slow.
Static Algorithm	Determines which algorithm is used to define Static Sperm. Select Length for default setting or Width_Mutliplier for custom setting. If Width_Multiplier is selected, the Static Width Multiplier (see below) parameter is used.
Static VAP (VAPSTATIC)	Maximum value of VAP for a sperm to be counted as Static.
Static VSL (VSLSTATIC)	Maximum Value of VSL for a sperm to be counted as Static.
Static Width Multiplier	Based on sperm head width, the maximum movement that a sperm head can make and still be classified as static (e.g., 0.50 is 50% of sperm head width).

Color-coded Categorization

On the Playback screen, sperm are assigned a specific color track based on the categorization shown below.

Table 2-3: Color-coded Sperm Categorizations

Category	Description	Color
Motile	A sperm that moves more than its head length from its original position during the acquisition.	Green Track
Progressive	A sperm moving with STR > STR_p AND VAP > $VAP_{p.}$	Cyan Track
Slow	A sperm moving with either VSL < VSL_{slow} **OR** VAP < $VAP_{slow.}$	Magenta Track
Static	A sperm moving with VSL < VSL_{static} **OR** VAP < $VAP_{static.}$	Red Dot
Border Crosser	Leaving the field during the first 10 frames.	Dark Blue Track
Late Track	Starts moving after first 10 frames ("Kicked").	Yellow Track
Late Entry	Entering the field after the first 10 frames.	Grey Track

Chapter 3. HTMotilityAdministrators Group

ADMIN: *Only Windows users with administration level access will be able to add and remove users assigned to the HTMotilityAdministrators Group.*

When new Windows users are created, they are automatically assigned to the BASIC users group. If the user does not require access to HT CASA II settings, then no further action is required.

If a user needs to be granted access to the HT CASA II settings, then they must be added to the HTMotilityAdministrators Group.

3.1 Create a New Windows User

1. Windows 11:
   a. Right-click on the Windows Start logo at the bottom left of the display.
   b. Click on Computer Management.
2. From the Computer Management window, select **Local Users and Groups / Groups**.
3. Right-click on User and select **New User**.

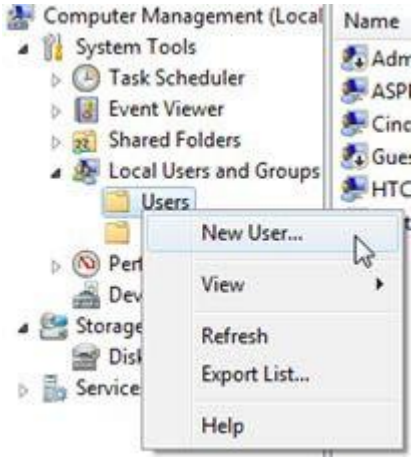


Figure 3-1: Add New Windows User

4. Enter the Username, Full Name, Description, Password and select required password options.
5. Select **Create**.

3.2 Add a Windows User to the HTMotilityAdministrators Group

1. Follow the steps previously outlined to open the Computer Management window.
2. From the Computer Management window, select **Local Users and Groups / Groups**.
3. Right-click on HTMotilityAdministrators and select **Add to Group**.

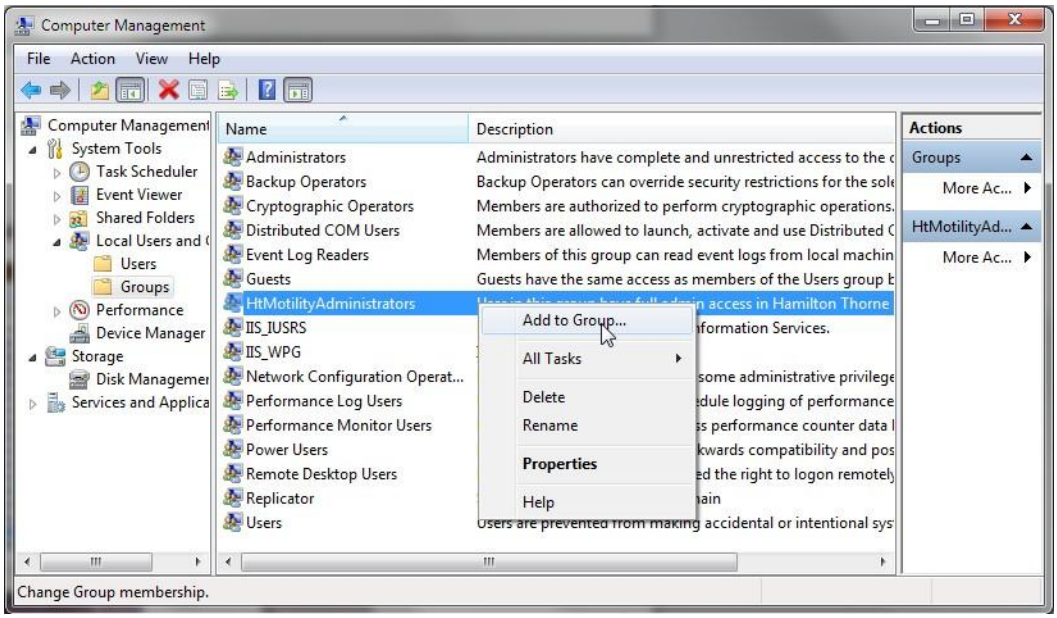


Figure 3-2: Add to Group

On the HTMotilityAdministrators Properties window, select **Add**. Make sure Users is listed in the Object Type and the Locations is set to the current IVOS Pro computer (the correct values should populate by default).

Type the name of the user to be added to the group and select **Check Names**. The entered name must exactly match an available user.

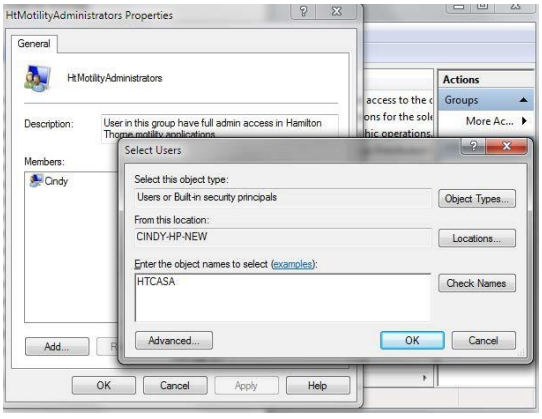


Figure 3-3: Select Users

4. If the system locates the correct user, select **OK** to add the user.
5. The added user will appear in the Group List. The user must log in for the addition to the group to become effective.

3.3 Remove a User from the HTMotilityAdministrators Group

1. Follow the steps previously outlined to open the Computer Management window.
2. From the Computer Management window, select **Local Users and Groups / Groups**. Follow the Steps above.
3. Right-click on HTMotilityAdministrators and select **Properties**.
4. Select the user to be removed from the list and select **Remove**.

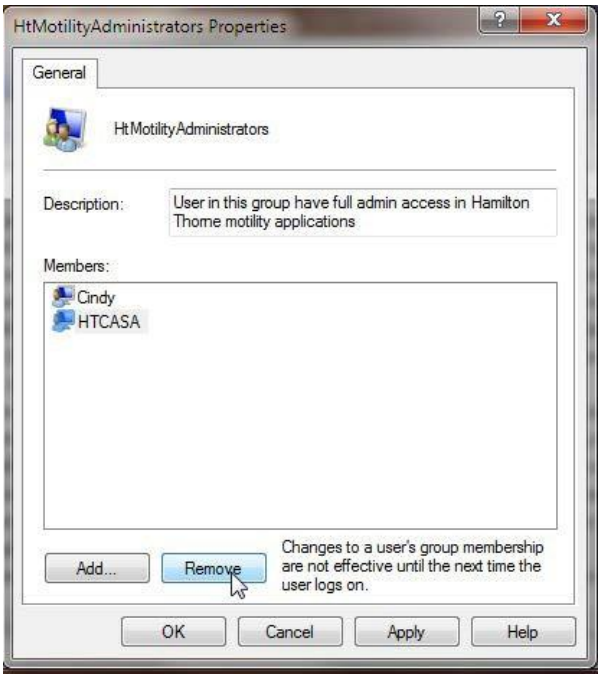


Figure 3-4: Remove User from Group

Chapter 4. Start Up and Software Overview

4.1 Log In and Software Start Up

Windows Log In

From the *Windows* log in screen, select your username and enter your password. Only *Windows* users belonging to the HTMotilityAdministrators Group (see “HTMotilityAdministrators Group” on page 8) will have full access to HT CASA II software settings. All other users will have limited access to settings.

HT CASA II Software Start Up

Locate the HT CASA II desktop icon and double-click to initiate the program.

4.2 HT CASA II Screen Layout and Controls

The HT CASA II software screen is comprised of the following main areas:

- **Main Menu**: top of the screen
- **Motility Sub-menu**: left side of screen
- **Motility Toolbar** and **Stage Toolbar**: below **Main Menu**
- **Info**, **Notes**, **Data Fields**, and **Results**: right side of screen
- **Image Area**: center of screen
- **Video Thumbnail Gallery**: below **Image Area**
- **Status Bar**: bottom of screen

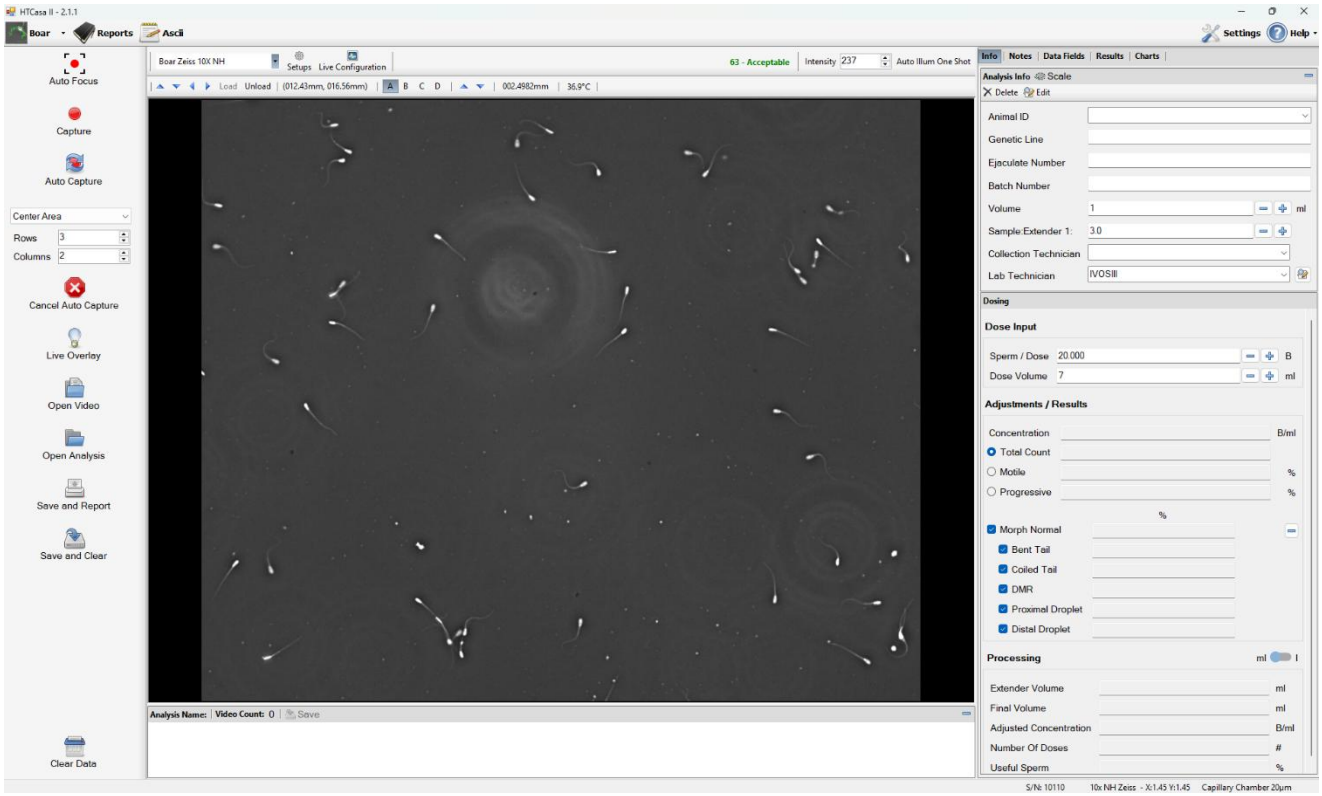


Figure 4-1: HT CASA II Screen

Main Menu

The **Main Menu** provides quick access to frequently used controls.

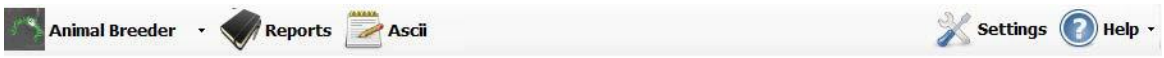


Figure 4-2: Main Menu

Table 4-1: Main Menu Items and Functions

Item	Function
HT CASA II Dropdown	Allows selection of the software program to run. Available programs may include motility, DNA Fragmentation or morphology options.
Reports	Provides access to the built-in reporting feature which includes creation of unlimited report formats.
ASCII	Permits data selection and output in ASCII format.
Settings	Provides access to the parameters governing analysis, camera settings, objective setup and general hardware and software settings.
Help	Provides access to database utilities and support options.

Motility Sub-menu

The **Motility Sub-menu** provides controls for capture, analysis and storage of data and the ability to open saved video images.

Table 4-2: Motility Sub-menu

Menu Item	Function
Capture	Initiates the image capture process for the current field.
Auto Capture	Initiates the image capture process with IVOS Pro stage automatically moving to capture the next field using either Sequential or Manual mode (see “Auto Capture” on page 22).
Cancel Auto Capture	Stops the Auto Capture process before all fields are analyzed. Results are discarded.
Live Overlay	Shows the current live image with overlays on sperm head (blue) and tail (red) to help in interactive adjustment of focus and illumination.
Open Video	Opens any single saved video file (.hmv format).
Open Analysis	Opens all videos stored in one analysis folder.
Save and Report	Saves current data to the database. Saves video files, prints/views reports, exports ASCII data to file, and clears data if enabled under Settings.
Save and Clear	Saves current data to the database. Saves videos if enabled under Settings. Automatically clears data.

Table 4-2: Motility Sub-menu

Menu Item	Function
Clear Data	Clears data and video thumbnails without saving.

Motility Toolbar

The **Motility Toolbar** is located below the **Main Menu** and provides access to setups and illumination controls.

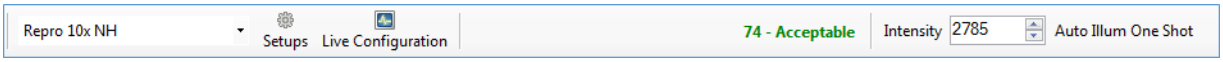


Figure 4-3: IVOS Pro Toolbar

Table 4-3: Motility Toolbar Items and Functions

Item	Function
Active Setup	Shows the setup being used in the analysis. Click the dropdown list to select the desired setup.
Setups	Opens the **Setup** dialog box directly (see "Setups" on page 37). The **Setup** provides access to the control parameters used for a particular analysis.
Live Configuration (Admin only)	Enables optimization of illumination and focusing of image and displays **Motility Setup Configuration** (see "Live Setup Configuration" on page 24).
Illumination Status	The Illumination status appears in green if it is **Acceptable** and in red if it is **Too High** or **Too Low**.
Intensity	Increases or decreases the level of selected illumination source
Auto Illum One Shot	Automatically adjusts the IVOS Pro illumination to the proper settings (see "Auto Illumination" on page 21).

Stage Toolbar

On IVOS Pro systems, the **Stage Toolbar**, which provides access to stage controls, is displayed beneath the **Motility Toolbar**.

Figure 4-4: IVOS Pro Stage Toolbar

Table 4-4: IVOS Pro Stage Toolbar Items and Functions

Item	Function
Jog Buttons	Jog In and Jog Out buttons to adjust stage position.
Stage Position	Shows the current position of the stage, in mm, from the front of the stage.
ABCD	Selects the chamber to view, based on Chamber Positions defined in setup.
Focus Buttons	Use these buttons to focus the sample on the stage.
Focus Position	Shows the current position of the stage focus (in mm).
Temperature	Shows the current stage temperature.

Info, Notes, Data Fields, Results, Charts

The right-hand side of the screen shows a tabbed panel for **Analysis Info**, **Notes** and **Data Fields** input and **Results** and **Charts** output (see “Info, Notes, Data Fields, Results, Charts” on page 47).

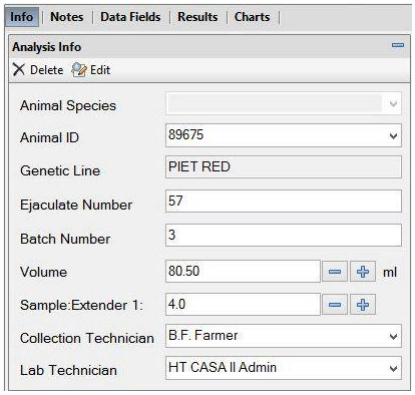


Figure 4-5: Info, Notes, Data Fields, Results and Charts

Image Area

The image area of the CASA II software shows the live image, playback image, zoomed image or saved video file.

Video Thumbnail Gallery

Once live fields are captured and analyzed, or video files are opened and analyzed, each file analyzed appears in the **Video Thumbnail Gallery** (see “Video Thumbnail Gallery” on page 54).

Status Bar

The status bar for the current analysis and selected setup appears along the bottom of the screen. This shows the analysis time, system serial number, the currently selected objective, its magnification values and the chamber type and depth.

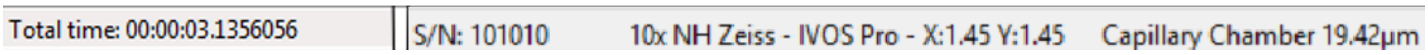


Figure 4-6: Status Bar

4.3 Using the Optional 3-way Footswitch

Figure 4-7: 3-way Footswitch

The optional 3-way foot switch consists of three (3) foot controls, programmed as follows:

- Center Pedal: **Capture**
- Left Pedal: **Save & Print** (requires that a **Default Report** is selected under **Settings > Reporting.** See “Reporting” on page 36)
- Right Pedal: **Save & Clear**

4.4 Keyboard Shortcuts

Several keyboard shortcuts are available to initiate typical functions. All shortcut keys must be pressed simultaneously.

- **Capture**: CTRL - ALT - SHIFT - 1
- **Save & Print**: CTRL - ALT - SHIFT - 2 (requires that a **Default Report** is selected under **Settings > Reporting.** See “Reporting” on page 36).
- **Save & Clear**: CTRL - ALT - SHIFT - 3
- **Live Overlay Toggle**: CTRL - ALT - SHIFT - 4

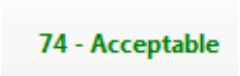
- **Clear Data**: CTRL - ALT - SHIFT - 5
- **Return to Main Screen**: CTRL - ALT - SHIFT - 6
- **Auto Capture**: CTRL - ALT - SHIFT -

Chapter 5. IVOS Pro Quick Start Analysis

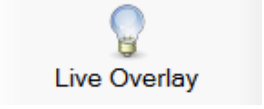
NOTE: *This section assumes that Live Setup Configuration and Analysis Settings and Parameters were previously set up by the HTMotility Administrator. It also assumes that the negative phase contrast is properly set up and the phase annulus aligned (see M-75-02-47 IVOS Pro Getting Started Manual).*

5.1 Performing a Standard Analysis

1. Confirm that the negative phase contrast microscope objective is used.
2. Confirm that the stage temperature is at the required temperature.
3. Select **Load** to access the IVOS Pro stage and place the loaded chamber on the stage. Select **Load** again to withdraw the stage.
4. Select the **Chamber Position** (A, B, C and D).
5. Select the appropriate setup from the dropdown list.
6. Check that an **Acceptable** illumination level is indicated in the **Motility Toolbar**. If not, click **Auto Illum One Shot** or increase or decrease the **Intensity** value.



7. Select **Live Overlay** on the **Motility Sub-menu**. This will show the live image with the blue head and red tail illumination overlays on the sperm cells.
8. Optimize the focus so that sperm show blue heads and long red tails (see Figure 6-1). In addition to the focus knob, focus may be adjusted using the **Focus** buttons on the **Stage Toolbar** or the mouse scroll wheel (when mouse is positioned over the image area).



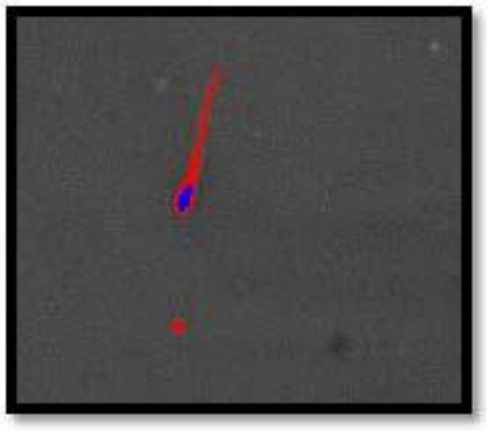
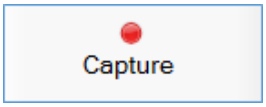


Figure 5-1: Sperm with Acceptable Focus

9. Click **Capture** on the **Motility Sub-menu** to capture the current field. Move the stage to a new field and repeat this process until the required number of fields are analyzed.



10. To check playback, select a **Video Thumbnail**.
11. The **Field Playback** screen appears showing the motile sperm labeled with color-coded tracks and the static sperm labeled with a red dot.

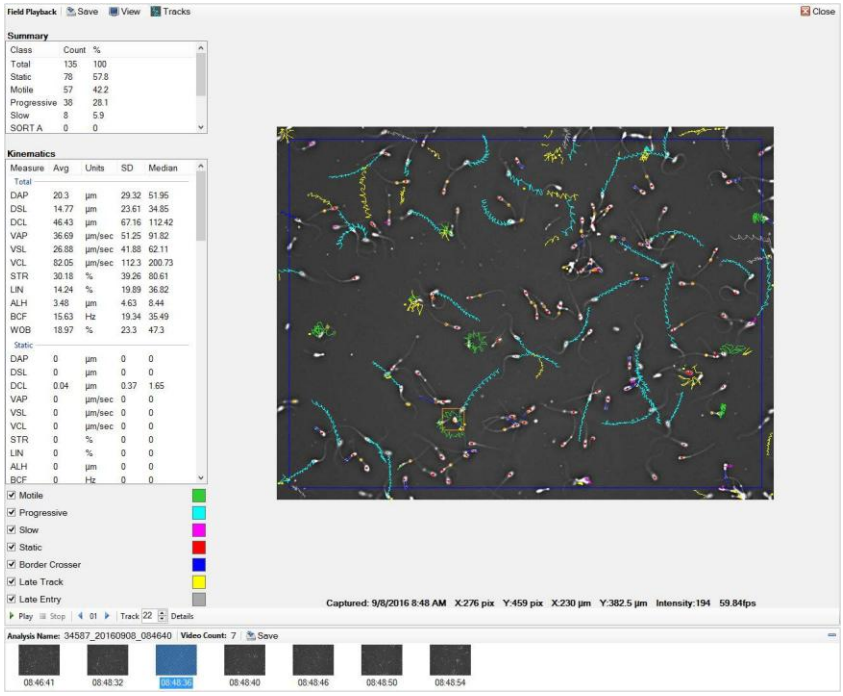
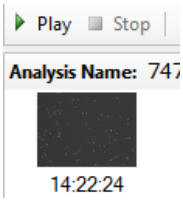


Figure 5-2: Playback Screen

12. Select **Play** located above the **Video Thumbnail** gallery on the left-hand lower corner of the screen. The sperm will move along their tracks.
13. Zoom on any sperm on the analyzed image by moving the cursor over it and clicking.
14. For more information on **Playback** and **Zoom** screens, see "Playback and Zoom Screens" on Page 50.
15. After the analysis has been performed, you may save the analysis results and associated field videos.



5.2 Auto Illumination

The **Auto Illum One Shot** feature on IVOS Pro helps the user set the illumination level for best sperm cell capture and tracking.

NOTE: *The Auto Illumination control may not always detect acceptable illumination and Photometer values each time it is selected. There will be instances where manual illumination adjustment is required.*

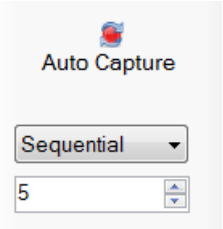
In general, **Auto Illum One Shot** works well for clean negative phase images without a high concentration of objects. However, there will be instances when **Auto Illumination** may not automatically detect acceptable illumination, even if a clean slide of normal concentration is used.

5.3 Auto Capture

There are three modes of **Auto Capture**: **Sequential**, **Manual and Center Area**.

Sequential Auto Capture

Sequential Auto Capture automatically captures the user-specified number of adjacent (sequential) fields when **Auto Capture** is selected.

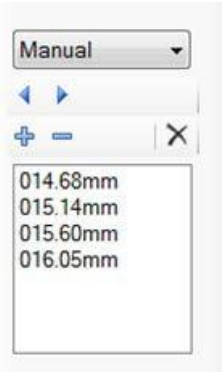


Select **Sequential** from the **Auto Capture** dropdown menu, enter the number of fields to be analyzed, position the chamber at the first field to be analyzed and select **Auto Capture**.

NOTE: *It is extremely important that the stage is positioned so that the automatic stage movement does not move the chamber out of view during analysis.*

Manual Auto Capture

Manual Auto Capture allows selection of specific fields for analysis. This allows the avoidance of any fields that may show air bubbles or a large clump of debris.

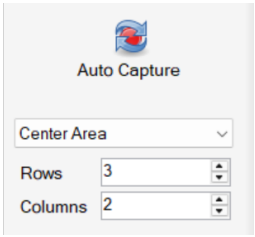


Select **Manual** from the **Auto Capture** dropdown, and using the **Jog buttons**, position the stage at the first field to be analyzed and select the **plus** . Continue selecting fields in this manner until an adequate number of fields is selected. To remove a field, highlight it with the mouse and select the **minus** . Select **Auto Capture** to begin the analysis.

To clear all fields from the list, select .

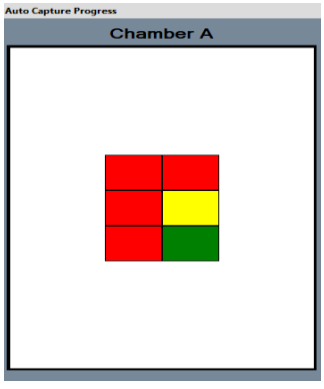
Center Area Capture

Center Area Auto Capture allows the user to create a grid of fields to be captured at the center of the chamber by defining a few **Rows** and **Columns**.



Select **Center Area** from the **Auto Capture** dropdown and enter your desired number of rows and columns.

Once **Auto Capture** is selected, the software will display an overlay of the chamber being captured. The white area represents the entire area of the chamber. The red fields represent the fields yet to be captured, yellow the field currently being captured, and green for fields that have completed capture.



Cancel Auto Capture

At any point during an **Auto Capture** analysis, the run may be canceled by selecting **Cancel Auto Capture**. All data captured during the current capture is discarded.

Chapter 6. Live Setup Configuration

ADMIN: *This feature is available to HT Administrative Users only.*

6.1 Live Setup Configuration - 10x Negative Phase Contrast

The **Live Setup Configuration** screen provides visual feedback on `the brightness levels of the live sample. The various settings are adjusted to optimize cell identification.

- Pixels with brightness above the **Minimum Head Brightness** and within the minimum and maximum **Head Size** and **Elongation** values are colored blue.
- Pixels with brightness between the **Minimum Head Brightness** and **Minimum Tail Brightness** are colored red.

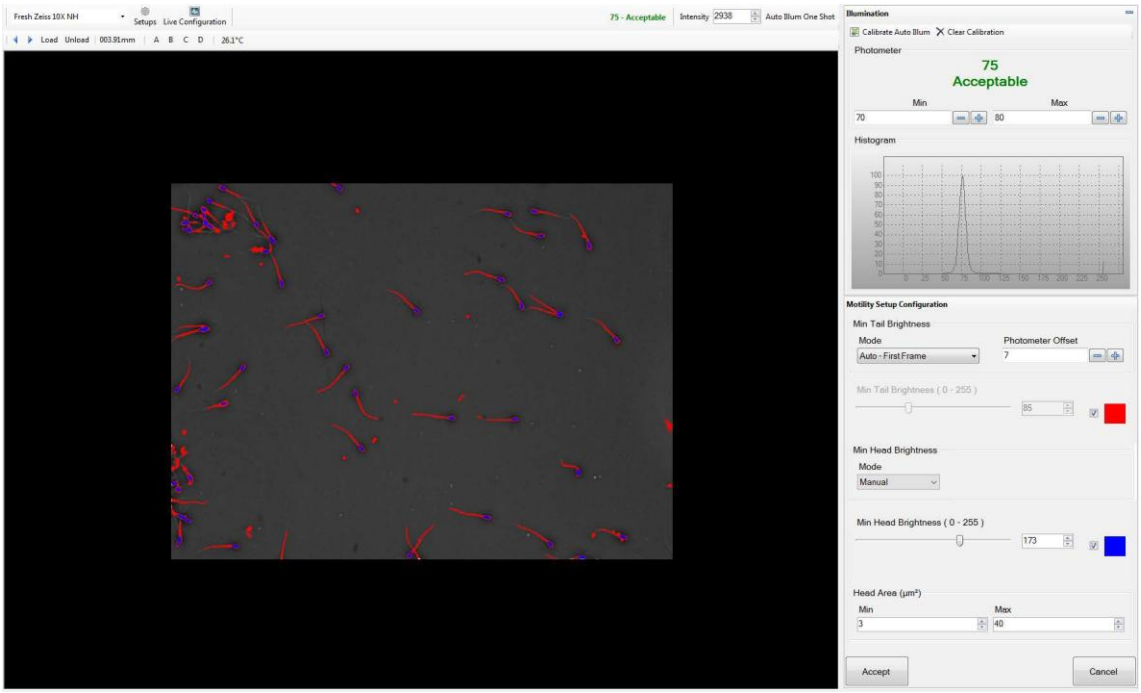


Figure 6-1: Live Setup Configuration Screen

The correct settings are essential for accurate identification of sperm. The goal is to achieve a sample image with blue sperm heads and red sperm tails (see Figure 6-1). Normally, the procedure to optimize the limiting controls on the **Live Setup Configuration** screen will only be necessary when the system is initially installed and should be performed by the system administrator.

NOTE: *All the settings on the **Live Configuration** screen are stored with the active analysis setup and may also be adjusted from the **Setup** screen (see "Setups" on page 34).*

Setting the Photometer

1. Place a sample on the IVOS Pro stage and focus the image.
2. Select **Live Setup Configuration** to open the screen (see Figure 6-1).
3. Under **Photometer**, set the **Min** and **Max** with a range of 10 (e.g., 60-70, 65-75, etc.). The **Min** and **Max** values will vary by species.
4. Adjust the illumination until an **Acceptable** illumination level is indicated above the histogram.
   - On IVOS Pro, use the **Auto Illum One Shot** feature or adjust using the **Intensity** control.
5. The histogram peak corresponds to the most frequent pixel brightness detected (e.g., close to the back- ground brightness).

Calibrate Auto Illum

The **Calibrate Auto Illum** feature pre-calibrates **Auto Illum One Shot**. When the **Calibrate Auto Illum** is enabled, the system calculates a plot of the **Photometer** versus illumination to determine the optimum illumination setting. This allows an extremely fast response when **Auto Illum One Shot** is selected.

Since **Calibrate Auto Illum** is configured on a per set up basis, the proper **Setup** must be selected and its associated objective in place. A good quality sample should be used to perform the calibration procedure.

Performing Calibrate Auto Illum

1. Select the **Setup** from the dropdown list.
2. Make sure the correct objective is in place.
3. Load a good quality sample on the IVOS stage and focus.
4. Open the **Live Configuration** screen.
5. Set the **Photometer** (see “Setting the Photometer” above).
6. Select **Calibrate Auto Illum** to begin the calibration process. During this process, a **Calibration in progress** indicator appears. In addition, the plot under Photometer will visibly adjust as the optimum illumination is determined.
7. Once complete, the calibrated illumination value will be saved with the current **Setup**.
8. To remove the calibrated value from the setup at any time, select **Clear Calibration**.

Setting the Minimum Tail Brightness (MTB)

Automatic Method

1. Ensure that the **Illumination** level is **Acceptable** as described above.
2. Under **Motility Configuration**, set the **Min Tail Brightness (MTB) Mode** to one of the following options:
   - **Auto - First Frame**: (recommended setting) an automatic adjustment of **MTB** will occur only in first frame of the field.
   - **Auto - All Frame**: an automatic adjustment of **MTB** will occur in every frame of the field.
3. Set the Photometer offset to 10 to start.
4. Using the **plus** or **minus** **-**, adjust the **Offset** value so that the tails are colored red along most of their length, but not so low that the background turns red. A typical **Photometer Offset** is between 8

and 14.

Manual Method

1. Ensure that the **Illumination** level is **Acceptable** as described above.
2. Under **Motility Configuration**, set the **Min Tail Brightness (MTB) Mode** to **Manual**.
3. Turn on the **MTB** toggle control so the red overlay will be visible.
4. Adjust the **MTB** value so that the red coloration extends down the sperm tail (typically in range 70 < **MTB** < 110). It may be necessary to refocus the image to maximize red tail length.
5. It is essential to set the **MTB** just above the setting where red patches appear on the background image. The red should be almost entirely confined to the sperm tails and the ring of fainter pixels around the blue sperm head. See Figure 6-2 for examples of correct and incorrect settings of **MTB**.

Setting the Minimum Head Brightness (MHB)

Automatic Method

1. Ensure that the **Illumination** level is **Acceptable** as described above.
2. Under **Motility Configuration**, set the **Min Head Brightness (MHB) Mode** to one of the following options:
   - **Auto - First Frame**: (recommended setting) an automatic adjustment of **MHB** will occur only in first frame of the field.
   - **Auto - All Frame**: an automatic adjustment of **MHB** will occur in every frame of the field.

Manual Method

1. Ensure that the **Illumination** level is **Acceptable** as described above.
2. Under **Motility Configuration**, set the **Min Head Brightness (MHB) Mode** to **Manual**.
3. Turn on the **MHB** toggle control so the blue overlay will be visible.
4. Set the **MHB** to 175. This will produce an image where the sperm heads are colored blue with no blue showing on the tails or in the background.
5. Fine-tune this value so that the blue color illuminates the head but does not progress down the midpiece or tail. See Figure 6-2 for examples of correct and incorrect settings of **MHB**.

NOTE: *If some heads flash between blue and red (or blue and white), this indicates an adjustment to the MHB or Head Area is required: typically, this would be to lower the Head Area Min or raise the Head Area Max.*

Image Comparisons: Varying MTB and MHB Settings

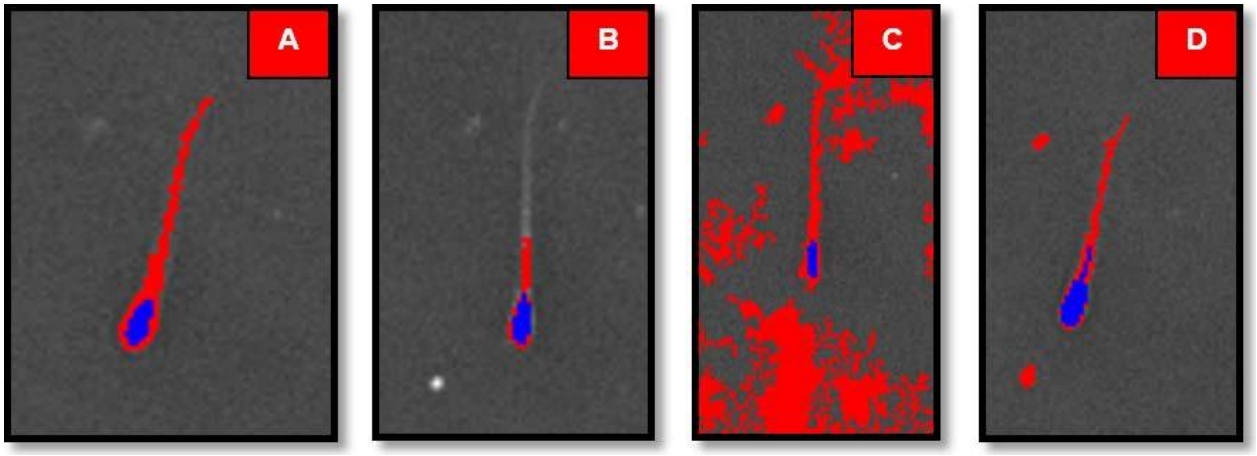


Figure 6-2: Image Examples of Minimum Tail and Head Brightness Settings

- Figure 6-2 (A): **Correct settings** - both **MTB** and **MHB** are properly set
- Figure 6-2 (B): **High Minimum Tail Brightness** - **MTB** is too high so tail is only partially detected
- Figure 6-2 (C): **Low Minimum Tail Brightness** - **MTB** is too low so many pixels in background are being detected
- Figure 6-2 (D): **Low Minimum Head Brightness** - **MHB** is too low so blue color travels down tail. **MTB** is correct on this image.

Static Cell Information

While not set on the **Live Setup Configuration** screen, three **Setup** parameters are used for accurate identification of static cells based on tail presence.

Table 6-1: Static Cell Tail Settings

Parameter	Definition	Recommended Setting
Static Tail Filter	True: Requires cells possess a tail to be labeled as static False: Tail not required for static determination.	True
Min Tail Length	Minimum length (µm) a tail must measure for the object to be classified as a static sperm.	8 – 14 µm
Tail Confidence	Percentage of captured frames that need to have a tail for the object to be considered as having a tail present.	20 - 30%

NOTE: *If Static Tail Filter is set to False, the remaining two parameter settings lose their function in identifying a static sperm cell*

Chapter 7. Settings Menu

ADMIN: *Settings are available to HTAdministrative Users only. Basic Users are only allowed access to the Camera Settings (see "eBus Video Settings" on page 39).*

The settings screens may be accessed by selecting **Settings** on the **Main Menu** or **Setups** on the **Motility Toolbar**.

The settings screens available are: **General**, **Notes**, **Reporting**, **Setups**, and **Sort**.

7.1 General

The **General Settings** screen provides access to **Analysis**, **Hardware**, **Info**, **Track**, and **Video Storage** controls.

Analysis Settings

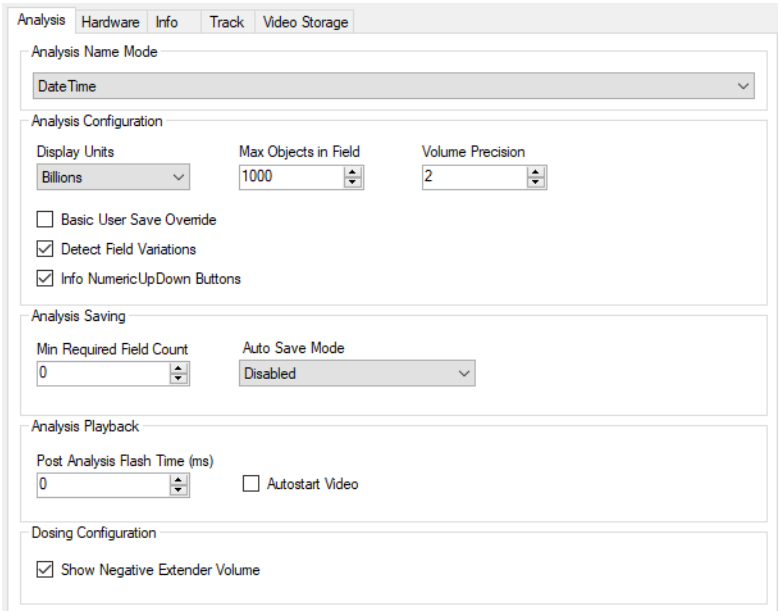


Figure 7-1: Analysis Settings

Analysis Name Mode

Analysis Name Mode allows you to select the naming convention of the captured video files. Three options are available:

- **DateTime**: Files will be saved with the following name format: YYYYMMDD_HHMMSS, with time based on 24-hour clock; for example, 20130405_160525.
- **Manual**: A dialog box prompt will appear to enter the file name.
- **SubjectID_DateTime**: The current **Boar ID/Animal ID** will append to the **DateTime** naming method. If no **Boar ID/Animal ID** is entered, a warning will appear before videos are saved.

When data is saved, a directory will be created using the selected naming method and individual files are saved within the directory. Sequential numbering will be added to the file name, beginning with 000.

Analysis Configuration

- **Display Units**: Allows selection of the units, as **Millions** or **Billions**, to be displayed in the analysis results.
- **Max Objects in Field**: Places a hard limit on the number of objects acceptable in each analysis field.
- **Volume Precision**: Allows setting of the maximum number of decimal points that may be used when entering **Volume** and **Dose Volume** on the **Info Panel.**
- **Basic User Save Override**: Enabling this control allows basic users to save analysis data even if the **Min Motile Percent**, **Min Progressive Percent,** or **Min Total Count** (see "Categories and Parameter Definitions" on page 35) are not met.
- **Detect Field Variations**: If enabled, **Detect Field Variations** detects if any individual field **Total Count** is > 2SD from the average of all fields analyzed and marks it with a red X on the thumbnail image. This alerts the user who can then decide to delete the field from the analysis.

- **Info Numeric Up Down Buttons**: When this is enabled, the Sample:Extender value under **Analysis Info** (see "Analysis Info" on page 44) may be adjusted using the **plus** or **minus** controls. If disabled, the icons are not visible and the value must be entered manually from the keyboard.

Analysis Saving

- **Min Required Field Count:** Defines the minimum number of fields that must be acquired for an acceptable analysis run.
- **Auto Save Mode**: Two **Auto Save Modes** are available, or it may be disabled.
  - **Save and Report**: results in the actions selected under **Reporting Actions** (see "ASCII Export" on page 33).
  - **Save and Clear**: results in the analysis results being saved and all data cleared. No **Reporting Actions** will occur.

  If **Auto Save** is enabled when using individual field capture, the analysis will be automatically saved once the **Min Required Field Count** is reached.

Analysis Playback

- **Post Analysis Flash Time (ms)**: Defines the amount of time, in milliseconds, that the playback screen will appear following analysis. The recommended starting value is 1000ms, so that the Playback screen appears on the monitor for 1 second. This should give the user enough time to see if there are any serious errors. This feature may be disabled by setting the value to 0.
- **Autostart Video**: Enables automatic video playback when a thumbnail video is opened from the gallery.

Dosing Configuration

- **Show Negative Extender Volume**: If checked, when the extender volume calculated is a negative value, it will be displayed on the **Processing** section of the **Dosing** panel.

Hardware Settings

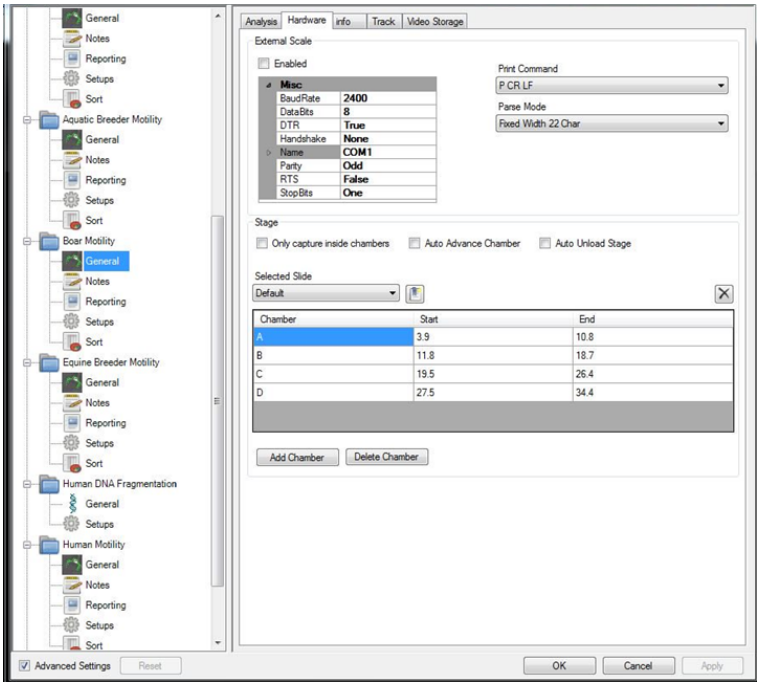


Figure 7-2: Hardware Settings (External Scale values for Ohaus-Pioneer Scale)

External Scale

Enabling the **External Scale** feature enables a **Scale** function on the **Analysis Info Panel**, which when selected, allows the weight of the sample to be automatically converted to the sample volume.

NOTE: *The ability to send commands and parse data has been tested and validated on the Ohaus-Pioneer and Sartorius scales. Refer to each respective scale manufacturer's instructions for use to determine correct settings.*

Stage

- **Selected Slide**: An unlimited number of slide configurations may be saved and selected.
  - To select a saved slide, choose it from the dropdown list.
  - To add a new slide, select the adjacent **New Slide** icon, enter a slide name, and enter the starting and ending chamber positions, in millimeters, for the first chamber (A).
  - To add a chamber to the selected slide, select **Add Chamber**. The **Start** and **End** values may be edited directly from the table.
  - To delete a chamber from the selected slide, select it in the table and select **Delete Chamber.**
  - To delete a slide, select it from the dropdown list and select .
- **Only Capture Inside Chambers**: If enabled, image capture is only allowed between the **Start** and **End** points of a chamber.
- **Auto Unload Stage**: If enabled, once an analysis is saved, the IVOS stage will automatically move out to allow a new sample to be added to the next analysis chamber on the slide.
- **Auto Advance Chamber:** If enabled, once data for Chamber A is saved, the stage automatically moves to Chamber B, and continues to Chamber C then Chamber D. This may be used in conjunction with **Auto Save**.

- **Auto Focus On Load/Advance –** This option will automatically perform an autofocus every time you load the stage or after auto advancing to a new chamber in conjunction with Auto Advance Chamber.
- **Auto Capture On Load/Advance –** This option will automatically start an auto capture upon loading the stage or auto advancing with Auto Advance Chamber. When combined with Auto Focus On Load/Advance, the system will autofocus first and then start auto capture.
- **Auto Focus On Chamber Move –** This option will perform an autofocus whenever the user manually moves between chambers using the stage hardware toolstrip chamber buttons. e.g. 'A', 'B', 'C', 'D'.
- **NOTE**: Auto Capture will not automatically perform an autofocus before capturing if any of these automatic autofocus options are enabled. This is to reduce wasting time by autofocusing the same field twice.

Info Settings

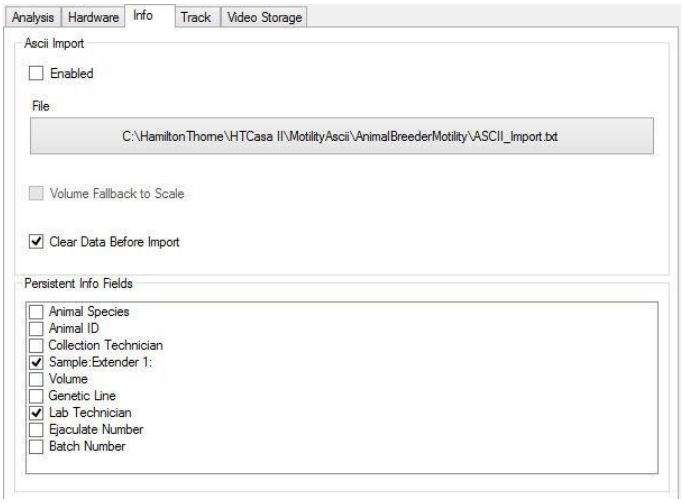


Figure 7-3: Info Settings

ASCII Import

Enabling **ASCII Import** allows external databases to transfer information to the CASA II software to automatically populate certain user-defined values. A properly formatted intermediate ASCII file is required. ASCII header values are specifically defined and only recognized fields will be imported. Missing or extraneous fields are ignored. If non-standard headers must be used, a mapping file needs to be created (see "Appendix B: ASCII Import Mapping File" on page 87).

For import into the Boar, Equine or Animal Breeder programs, Field Names must appear in the ASCII heading exactly as shown below:

Boar	Animal Breeder	Equine Breeder
AnimalId	AnimalSpecies	AnimalId
CollectionTech	AnimalId	CollectionTech
DilutionRatio	CollectionTech	DilutionRatio
EjaculateVolume	DilutionRatio	EjaculateVolume
GeneticLine	EjaculateVolume	GeneticLine

LabTech	GeneticLine	LabTech
EjaculateNumber	LabTech	EjaculateNumber
BatchNumber	EjaculateNumber	BatchNumber
SpermPerDose	BatchNumber	RequiredConcentration
DoseVolume	SpermPerDose	DoseVolume
	DoseVolume	
	UsableVolume	

The program accepts only one date record from the intermediate ASCII file. After importing, data is automatically cleared and only the headers remain.

- **Volume Fallback to Scale**: When enabled, the scale value is automatically read upon pressing the Import button even if no EjaculateVolume header exists. If this is disabled, the ASCII import file must contain an EjaculateVolume header in order to read the scale.
- **Clear Data Before Import**: When enabled, all previous analysis data will be cleared when new ASCII data is imported. If the data has not yet been saved, a warning will appear before data is cleared.

Persistent Info Fields

Select the checkbox next to the **Info Fields** to prevent data from being cleared on the **Info Panel** when the analysis data is cleared.

Track Settings

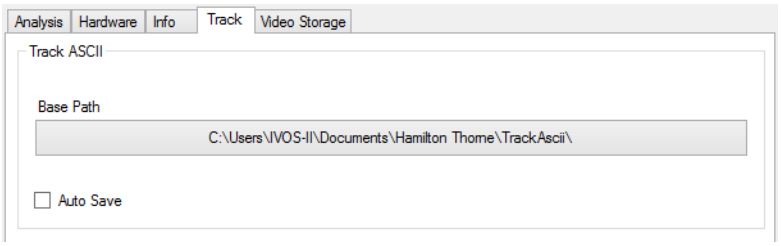


Figure 7-4: Track Settings

NOTE: *Export of track data to ASCII requires the optional Track II software.*

The **Track** feature provides the opportunity to save all track data to an ASCII file.

- Select the **Base Path** to which the data should be stored.
- Enable **Auto Save** to automatically save the track data when **Save and Report** or **Save and Clear** is selected.

Video Storage Settings

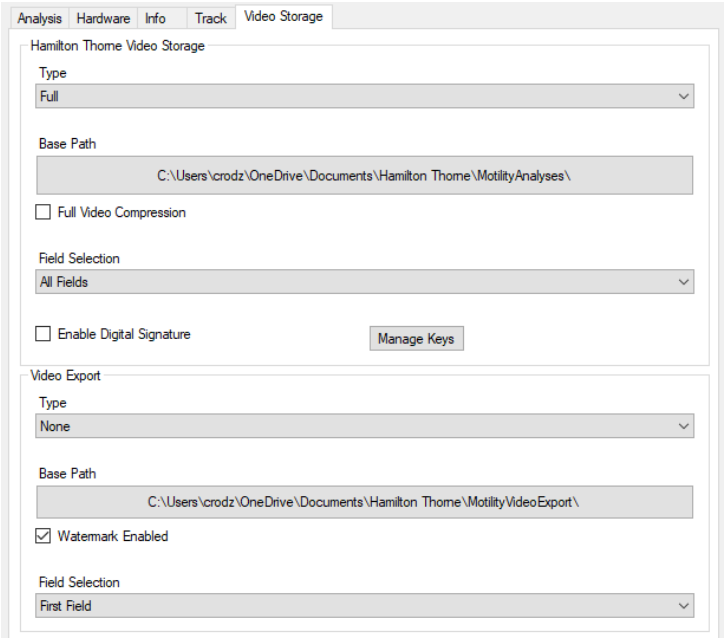


Figure 7-5: Video Storage Settings

Hamilton Thorne Video Storage

Type

- **None**: No video or still image is saved.
- **First frame with overlay**: The first frame of each field analyzed is saved as a TIFF file. The graphic overlay of the tracks is saved with the image.
- **Full**: All frames and all the analysis details are stored as a video file (.hmv). This option must be selected if you wish to recall and re-analyze video files.

Base Path

The **Base Path** is the directory to which the video or image files will be saved. Click the browse button to locate or change the directory. The path selected should be accessible to all users.

Full Video Compression

Checking **Full Video Compression** saves all video files using lossless compression. The default setting is unchecked (compression off).

Field Selection

Field Selection is only applicable if **Full Video** is selected under **Type**. Four options are available to determine which fields to save:

- All Fields
- Last Field
- First Field
- First and Last Field

NOTE: *If your facility requires complete analyses to be available for recall and reanalysis, All Fields MUST be selected.*

Enable Digital Signature

To store videos with an embedded **Digital Signature** of the current user, check the **Enable Digital Signature** box. The **Digital Signature** is generated by pairing a private key and a public key, stored by the operating system.

Manage Keys

The **Manage Keys** control provides access to **Refresh** or **Regenerate** the current user's key.

- **Refresh**: Re-reads the public key from the operating system.
- **Generate**: Deletes the existing key pair and creates a new private / public key pair.

Video Export

Type

- **None**: No *.mp4* file is saved
- **With Overlays**: An *.mp4* file is saved of all fields showing the graphic overlays.
- **Without Overlays**: An *.mp4* file is saved of all fields without the graphic overlays.

Base Path

- The **Base Path** is the directory to which the .mp4 video files will be saved. Click the browse button to locate or change the directory.

NOTE: *As of HT CASA II Software Version 11.1, videos are stored in .mp4 format instead of .avi format.*

Field Selection

Field Selection is only applicable if **Full Video** is selected under **Type**. Four options are available to determine which fields to save:

- All Fields
- Last Field
- First Field
- First and Last Field

Check **Watermark Enabled** to superimpose the Hamilton Thorne logo at the bottom left-hand corner of *.mp4* files (see "Field Save Options" on page 55).

7.2 Notes

The **Notes Panel** permits default text to be entered. This default text always appears on the **Notes Panel** and remain after data is cleared.

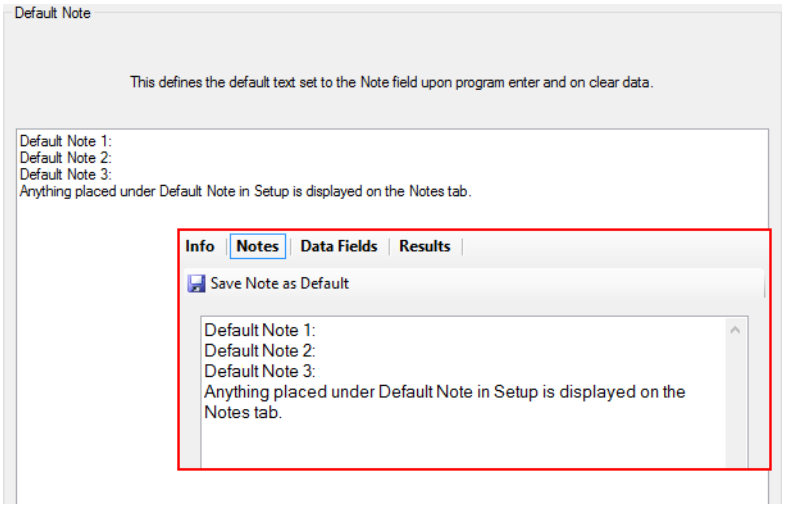


Figure 7-6: Default Note and Notes Tab

7.3 Reporting

Report Actions

Report Actions dictate what will automatically occur when **Save and Report** is selected. These include:

7.3.1 View Default Report
7.3.2 Print Default Report
7.3.3 Save Default Report
7.3.4 Export ASCII
7.3.5 Clear Data
7.3.6 Minimize Application

Any combination of actions may be enabled. If no actions are enabled, only the data will be saved.

Default Report

Default Report allows the selection of the report layout to be viewed, printed and/or saved when **Save and Report** is selected.

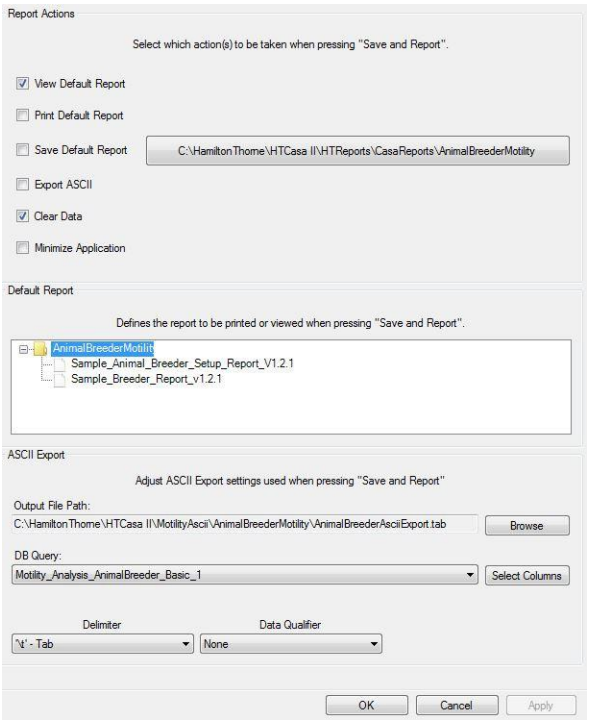


Figure 7-7: Reporting

ASCII Export

ASCII Export defines the settings to be used when **Export ASCII** is enabled and **Save and Report** is selected.

7.3.7 **Output File Path**: Indicates the output path and file name to which the ASCII file is saved. The default extension for ASCII files is *.tab*; however, this may be changed here.

7.3.8 **DB Query**: Allows the selection of data fields to be included in the ASCII file. Available data may be viewed and chosen by selecting **Select Columns**. Once data is saved to the file, the data fields cannot be changed.

7.3.9 **Delimiter**: Select the delimiter to be used in the ASCII file. Available options are comma, semicolon or tab (default setting).

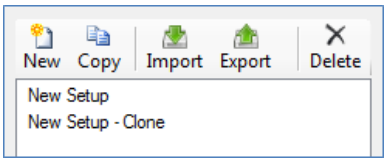
NOTE: *Even if only specific data fields are selected for export, all the analysis data is saved and is accessible from the ASCII function (see "ASCII" on page 81).*

7.4 Setups

The parameters configured under **Setup** optimize sperm identification and classification. All data must be cleared before changes may be made.

Working with Setups

The standard setup functions include:



7.4.1 **New**: Add a new setup to the list.

7.4.2 **Copy**: Creates a clone (copy) of the currently selected setup.

7.4.3 **Import**: Imports the setup values either from a saved video file (.hmv) or a previously exported setup file (.hms).

7.4.4 **Export**: Exports the currently selected setup to a file (.hms).

7.4.5 **Delete**: Deletes the currently selected setup.

To change the name of a setup, select the **Setup**, enter a new name in the parameter panel, select **Apply**, and click within the setup list to refresh the list.

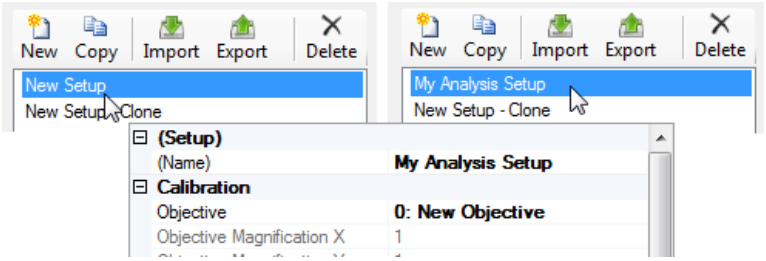


Figure 7-8: Changing a Setup Name

Setup Parameters

NOTE: *Each system is shipped with default setup parameters, which are to be considered as starting points only. It is the responsibility of the HTMotilityAdministrator to fine-tune the values for their facility.*

Select an analysis setup name to view the associated analysis parameters. The analysis parameters may be viewed Alphabetically or by Category using the sorting icon at the top.

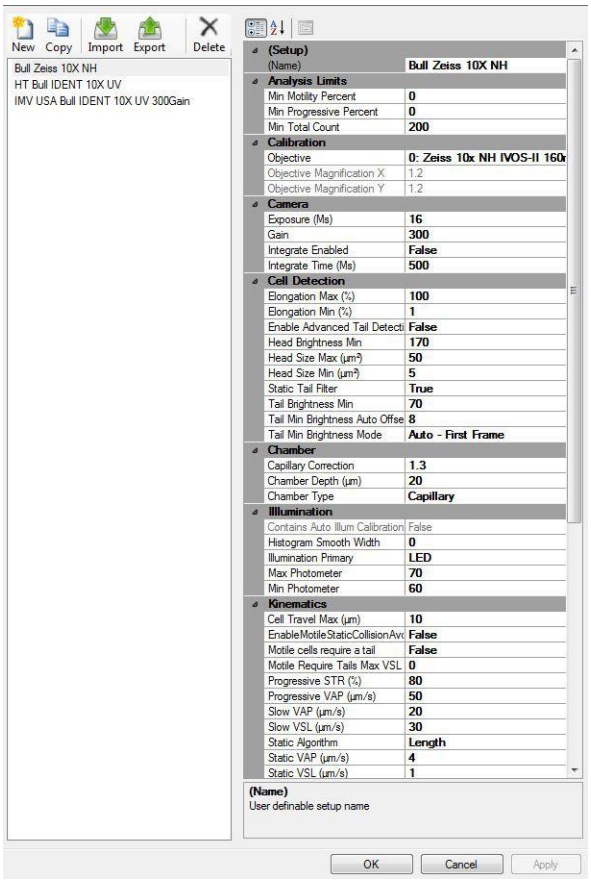


Figure 7-9: Setup Parameters

Categories and Parameter Definitions

(Setup)

7.4.6 **Name**: User-defined name of setup.

Analysis Limits

7.4.7 **Min Motility Percent**: Minimum required motility percent for an analysis when saving.

7.4.8 **Min Progressive Percent**: Minimum required progressive percent for an analysis when saving.

7.4.9 **Min Total Count**: Minimum required number of cells counted for an analysis when saving.

Calibration

7.4.10 **Objective**: Name/identifier of the objective used. Its calibration in microns/pixel is stored with the name.

7.4.11 **Objective Magnification X**: Objective magnification on the X axis (see “Magnification Calibration” on page 44).

7.4.12 **Objective Magnification Y**: Objective magnification on the Y axis (see “Magnification Calibration” on page 44).

Camera

7.4.13 **Exposure (ms)**: Typically set to 16 milliseconds for IVOS Pro

7.4.14 **Gain**: Camera gain for LED illumination.

Cell Detection

7.4.15 **Elongation Max (%)**: Maximum acceptable values for head elongation (ratio of head width to length).

7.4.16 **Elongation Min (%)**: Minimum acceptable values for head elongation (ratio of head width to length).

7.4.17 **Enable Advanced Tail Detection**: True/False - Set to true, enables higher quality morph analysis pre-processing with the potential to reduce analysis speed.

7.4.18 **Head Brightness Min**: Minimum acceptable brightness (scale 0-256) for a pixel to count as part of a sperm head.

7.4.19 **Head Size Max (µm²)**: Minimum acceptable head area in square microns. Used to exclude small debris.

7.4.20 **Head Size Min (µm²)**: Maximum acceptable head area in square microns.

7.4.21 **Large Object Min Area Micron**: Minimum area to be considered a large object in microns.

7.4.22 **Large Object Removal Enable**: True/False - Set to true, objects meeting the Large Object Min Area Micron will be removed from the analysis.

7.4.23 **Min Cell Brightness Auto Mode**: Manual; Auto - First Frame; Auto - All Frames. Enables auto or manual calculation of **Minimum Head Brightness** on the **Live Configuration** screen.

7.4.24 **Static Tail Filter:** True/False - Set to true, requires cells possess a tail to be labeled as a static sperm.

7.4.25 **Tail Brightness Min**: Minimum acceptable brightness (scale 0-256) of the sperm tail. Pixels with brightness below this number will not be included in sperm tail.

7.4.26 **Tail Min Brightness Auto Offset**: Tail Brightness Min will be set to a value equal to the photometer minus this offset value.

7.4.27 **Tail Min Brightness Auto Mode**: Manual; Auto - First Frame; Auto - All Frames. Enables auto or manual calculation of **Minimum Tail Brightness** on the **Live Configuration** screen.

Chamber

7.4.28 **Capillary Correction:** Value of the Segré–Silberberg correction to sperm concentration compensating for cell capillary segregation (typically 1.3 for 20 µm deep capillary load chambers).

7.4.29 **Chamber Depth**: Distance between the ceiling and floor of the analysis chamber.

7.4.30 **Chamber Type**: Capillary-load or Top-load.

Illumination Category

7.4.31 **Contains Auto Illum Calibration**: Returns true if this setup object contains an auto illumination calibration. It does not guarantee the calibration is optimal for current hardware configuration.

7.4.32 **Histogram Smooth Width**: Number of points histogram. 0 = smoothing disabled.

7.4.33 **Illumination Primary**:

7.4.33.1 **LED Red**, **LED Blue** or **LED Green**, plus illumination intensity setting.

7.4.34 **Max Photometer**: Maximum acceptable photometer value.

7.4.35 **Min Photometer**: Minimum acceptable photometer value.

Kinematics

7.4.36 **Cell Travel Max (µm)**: The maximum distance a cell can travel between video frames.

CAUTION *Changing the Cell Travel Max from the standard value of 10 may alter proper operation.*

7.4.37 **Enable Motile Static Collision Avoidance**: Enables algorithms to improve motile tracking when colliding with static objects.

7.4.38 **Motile Cells Require a Tail**: True/False - Set to true, cell requires tail to be counted as motile.

7.4.39 **Motiles Require Tails Max VSL**: Maximum VSL for cells to require tails to be considered Motile. Cells with higher VSL will not require tail to be considered Motile.

7.4.40 **Progressive STR (%)**: Minimum value (%) of Straightness for a track to be counted as progressive.

7.4.41 **Progressive VAP (µm/s)**: Minimum value (µm/s) of VAP for a track to be counted as progressive.

7.4.42 **Slow VAP (µm/s)**: Maximum value (µm/s) of VAP for a track to be counted as slow.

7.4.43 **Slow VSL (µm/s)**: Maximum value (µm/s) of VSL for a track to be counted as slow.

7.4.44 **Static Algorithm**: Length or Width_Multiplier - Determines which algorithm is used to define static sperm. Length is the default. Select Width_Multiplier for custom setting (see Static Width Multiplier below).

7.4.45 **Static VAP (µm/s)**: Maximum value (µm/s) of VAP for a track to be counted as static.

7.4.46 **Static VSL (µm/s)**: Maximum value (µm/s) of VSL for a track to be counted as static.

7.4.47 **Static Width Multiplier**: Based on sperm head width, the maximum movement that a sperm head can make and still be classified as static (e.g., 0.50 is 50% of sperm head width).

Morph

7.4.48 **Display Morph Results**: True/False - Determines if the Morph Results are displayed on the Dosing Panel.

7.4.49 **DMR Confidence (%)**: Fraction of frames required to confirm DMR (Distal Midpiece Reflex).

7.4.50 **DMR Droplet to tail end Max (µm)**: Max distance (µm) between tail end and droplet required to count cell as DMR.

7.4.51 **DMR Tail Length Max (µm)**: Max distance (µm) from base of head to apparent tail end for cell to be counted as DMR.

7.4.52 **DMR to Static**: True/False - set to true, cells tagged as DMR will automatically be classified as static.

7.4.53 **Droplet Confidence (%)**: Fraction of frames required to count as a droplet (proximal or distal).

7.4.54 **Droplet Distal Distance Min (µm)**: Minimum distance (µm) from base of head to the Distal Droplet.

7.4.55 **Droplet Proximal Head Length (µm)**: Minimum head length (µm) for sperm head and proximal

droplet combined.

7.4.56 **Min Tail Length (µm)**: Minimum length (µm) of a tail.

7.4.57 **Morph Normal Minimum Percent (%)**: Minimum percentage of cells required to pass morph normal

7.4.58 **Tail Bend Angle Averaging Length (µm)**: Length (µm) of tail that angle averaging occurs over when determining bent tail classification.

7.4.59 **Tail Bending Angle Rate Min (°/µm)**: Minimum degrees per micron the tail can bend to be classified as bent.

7.4.60 **Tail Bent Confidence (%)**: Fraction of frame required to count as bent tail.

7.4.61 **Tail Coiled Angle Min (°)**: Angle (°) of tail bend that must be exceeded to count as coiled.

7.4.62 **Tail Coiled Confidence (%)**: Fraction of frames required to count as coiled.

7.4.63 **Tail Confidence (%)**: Fraction of frames required that need to have detected a tail for the sperm to be classified as having a tail.

Stage

7.4.64 **Stage Temp (C)**: Required temperature (in Celsius) of stage. Setting the **Stage Temp** to 0°C turns off stage heating eventually registering the stage temperature to room temperature.

Video Capture

7.4.65 **Frame Capture Speed (Hz)**: Acquisition rate of video camera, normally 60 frames/sec (Hz).

7.4.66 **Frame Count**: Number of sequential images (frames) to be captured per field of analysis, typically 30-45.

7.4.67 **Imaging Type**: Negative Phase

Chapter 8. Optics Settings

8.1 eBUS Video Settings

The **Device** dropdown list indicates the name of the camera being used (typically JAI GOX 5102 M USB).

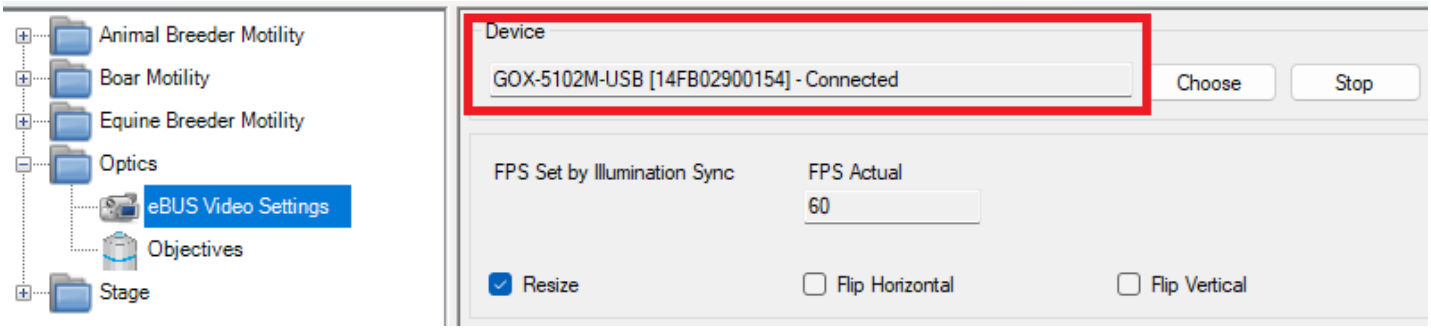


Figure 8-1: Optics Device Selection

8.2 Objectives

ADMIN: *This Objectives setting is available to HT Administrative Users only.*

Each objective to be used for analysis must be calibrated before use. This is required to calculate correct concentration and velocities with HT CASA II. Only objectives that have been named, calibrated and saved will be available for selection under **Setup**.

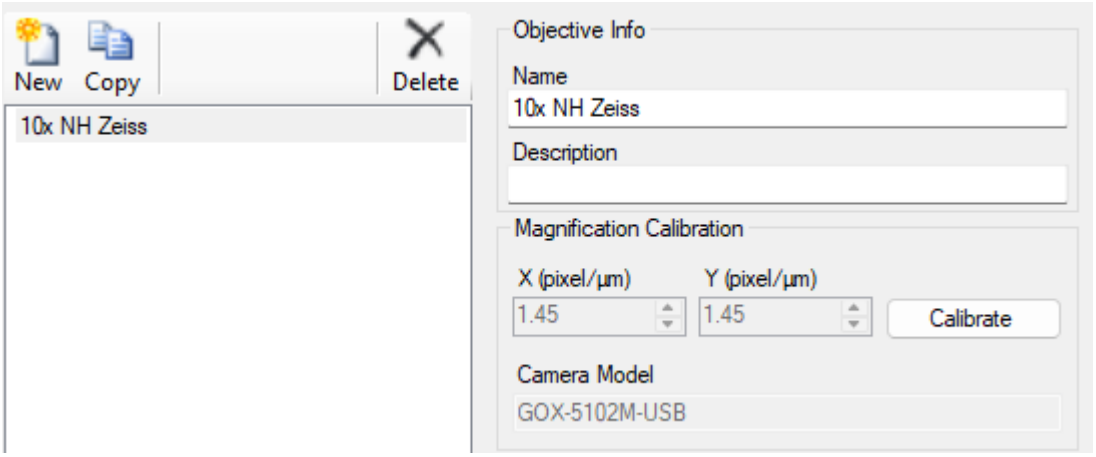


Figure 8-2: Objectives Setup

Working with Objectives

8.2.1 To select a previously calibrated objective, highlight the name in the list.

8.2.2 To add an objective, select **New**. An objective called **New Objective** appears in the list. Highlight **New Objective,** enter a name and description and select Apply.

8.2.3 To copy an existing objective, highlight the objective to be copied and select **Copy**.

8.2.4 To delete an existing objective, highlight the objective to be deleted and select **Delete**.

Magnification Calibration

Calibration of objective magnification scale is measured using a standard micrometer scale. The user adjusts the spacing between two yellow lines on the screen to ensure they are 100µm apart, on both the horizontal and vertical axes. When this is completed, the system will remember the current magnification calibration (units: pixels/micron).

CAUTION *To ensure proper classification, each new objective must be calibrated.*

Calibration Procedure

1. Position the objective to be calibrated.
2. Place the micrometer scale on the stage, locate the grid and focus.
3. On the **Objectives Panel**, create or select an objective and select **Calibrate**.
4. The live image area appears with yellow calibration lines overlaying the scale.

The position of the stage and calibration slide may be adjusted using the X, Y and Z controls on the Stage Panel (see Figure 9-3).

5. Once the scale is in view, adjust the positions of the yellow vertical calibration lines (X) using the mouse so they are 100 microns apart. The lines may be moved by clicking and dragging with the mouse. It is best to line them up with the edge of a scale line, as shown in Figure 9-4.
6. Click **Accept** to save the new calibration settings.
7. The horizontal lines (Y) appear.
8. Using the mouse, adjust the yellow horizontal calibration lines (Y) so that they are 100 microns apart.
9. Click **Accept** to save the new calibration.
10. The **X** and **Y** values represent the measured calibrations, shown as pixel/µm.

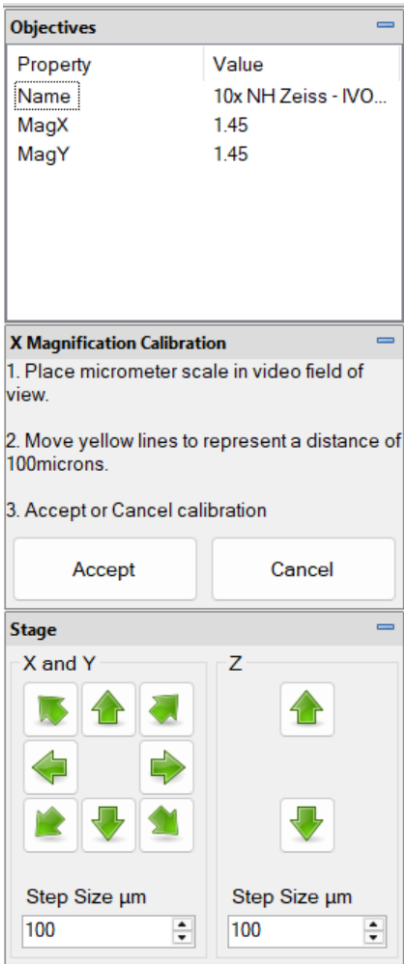


Figure 8-3 Objective Calibration Stage Controls

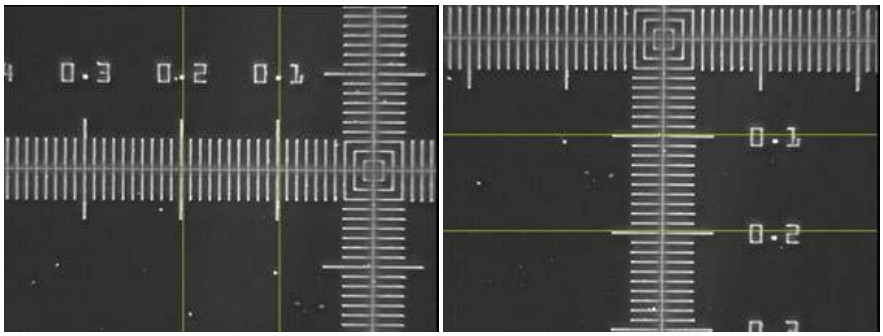


Figure 8-4: X and Y Objective Calibration using Micrometer Scale

Record these numbers, along with the type of objective used, in the following table. Any time this same objective is used in the future, the magnification can then be set by adjusting the numerical value - without having to use the grid.

The objectives supplied with the system have been measured in the factory and their magnifications are given in the table below.

Chapter 9. Stage Leveling

9.1 Stage Leveling

Stage leveling maps the slope of the stage to automatically apply a change in focus when moving the stage in the X and Y directions. This helps keep the sample in focus when moving from field to field.

- **Stage Leveling Enabled:** This check box enables or disables the stage leveling feature.
- **Calibrate Leveling:** This button starts the stage leveling calibration state.

Setup Stage Leveling

1. Insert Hamilton Thorne Alignment Slide (Part #508093)
2. Go to Settings.
3. Under “Stage”, select “Stage Leveling”

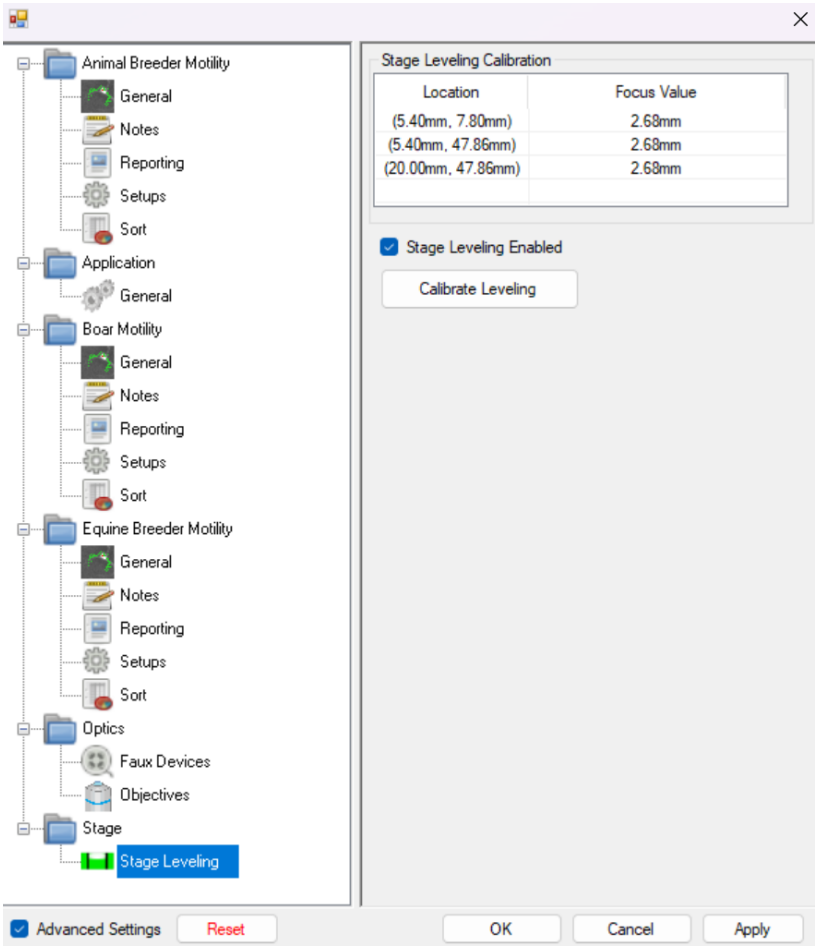


Figure 9-1: Stage Leveling Settings

4. Click “Calibrate Leveling” button.

5. Focus the image using focus knob or mouse wheel.
6. Click “OK” button.

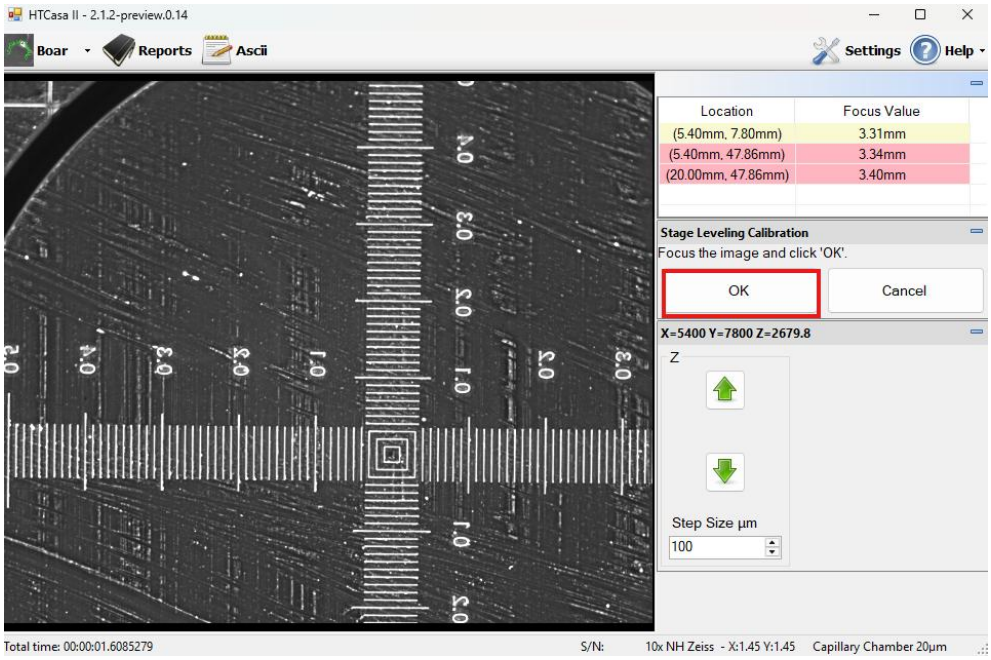


Figure 9-2: Stage Leveling Calibration

7. Repeat steps 5-6 for the remaining two locations.

Chapter 10. Info, Notes, Data Fields, Results, Charts

10.1 Info

Analysis Info

Information regarding the analysis is entered or imported under **Analysis Info**.

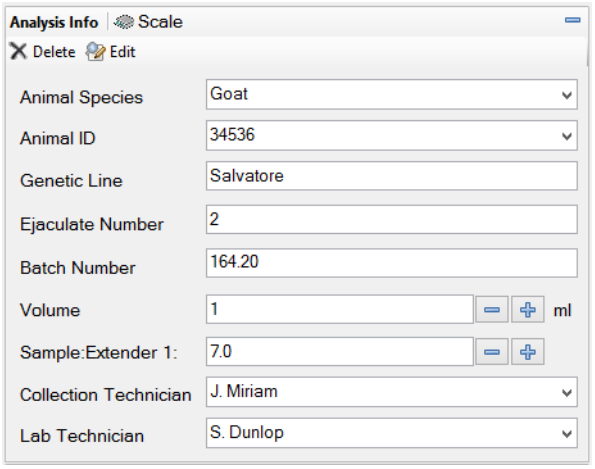


Figure 10-1: Analysis Info

- **Animal Species (Animal Breeder only)**: Enter the species of the animal.
- **Animal ID**: Enter a new **Animal ID** or select a **Animal ID** from the list. Stored IDs may be deleted or edited by selecting the appropriate control. Deleting a **Animal ID** from this list does not delete the animal or saved data from the database.
- **Genetic Line**: Enter the genetic line information, if applicable
- **Ejaculate Number**: Enter the Ejaculate Number, if applicable
- **Batch Number**: Enter the Batch Number, if applicable
- **Volume**: Enter the volume of the ejaculate in milliliters.
- **Sample: Extender:** The ratio of sample to extender. The only factor that is adjusted is the extender. The sample value always remains at 1. For example, if 2 ml or extender is added to 1 ml of sample, 2 is entered in the field. The value may be entered manually from the keyboard or by using the **plus** or **minus** . These controls may be enabled or disabled under **General Settings** (see "Analysis Settings" on page 24).
- **Collection Technician**: Enter or select the name of the person collecting the ejaculate
- **Lab Technician**: Enter or select the name of the person analyzing the sample.

Dosing

Dose Input (Insemination Dose)

The insemination dose is the required number of sperm per given volume. Both the **Sperm/Dose** and **Dose Volume** must be entered by the user.

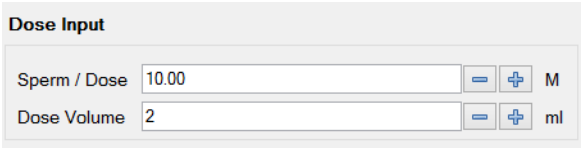


Figure 10-2: Dose Input

- **Sperm/Dose**: The number of total sperm required for each dose, given in millions (M) or billions.
- **Dose Volume**: The volume of the dose, in ml, required for insemination.
- **Usable Volume**: (Animal Breeder only) In instances where straws are used, a small amount of the initial dose (straw) volume may be lost due to the straw plug. The **Usable Volume** is the Dose Volume minus the lost volume.

Adjustments / Results

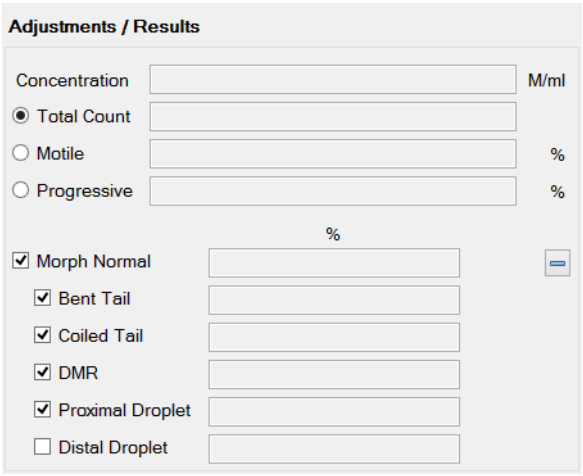


Figure 10-3: Adjustments / Results

Count Adjustment

The dilution calculation for the ejaculate may be based on the number of **Total**, **Motile** or **Progressive** sperm.

- **Total**: If **Total** count is selected, the required dilution is calculated with no adjustments.
- **Motile**: If **Motile** is selected, the required dilution is calculated from motile cells only. The outputs under **Processing** will then refer to the dilutions and numbers of doses available for motile sperm. This will reduce the dilution required and reduce the number of doses produced.
- **Progressive**: If **Progressive** is selected, the required dilution is calculated from progressive cells only. The outputs under **Processing** will then refer to the dilutions and numbers of doses available for progressive sperm. This will reduce the dilution required and reduce the number of doses produced.

Morphometry Adjustment

Morph Normal: The fraction of normal sperm may also be used to further adjust the required dilution and number of doses. The Morph Normal percentage shown is based on the number of normal sperm in the count adjustment population selected above. Click the box to enable Morph adjustment.

The **Morph Normal** results will show either a green **Pass** or a red **Fails** based on the **Morph Normal Minimum Percent (%)** entered under Setups (see "Setup Parameters" on page 35).

The user may enable or disable the following types of abnormalities to be used in determining the Morph Normal percent:

- *Bent Tail*: If the bending rate exceeds the Tail Bending Angle Rate (degrees/µm), the tail is counted as bent.
- *Coiled Tail*: If the tail bend exceeds 180º or more over its length, the tail is counted as Coiled.
- *DMR*: (Distal Midpiece Reflex) If the tail is bent in the distal region of the midpiece in the shape of the letter J, usually with a distal cytoplasmic droplet entrapped in the bend, it is counted as DMR.
- *Proximal Droplet*: If the combined droplet and head size exceeds the Droplet Proximal Head Length (µm), the sperm is counted as possessing a Proximal Droplet.
- *Distal Droplet*: If the droplet is located at a distance greater than the Droplet Distal Distance Min, the sperm is counted as possessing a Distal Droplet.

NOTE: *While some sperm will exhibit more than one defect, they will only be counted as abnormal once. Thus, the sum of the defects above does not add up to (100% - Morph Normal %).*

Processing

Figure 10-4: Processing

The results provided under **Processing** give the calculated volume of extender to add to the ejaculate (**Extender Volume**), the combined volume of the ejaculate and plus extender (**Final Volume**), the A**djusted Concentration** in the dose, and the **Number of Doses** available. *Useful Sperm* is the product of the motile or progressive cells (depending on which radio button is selected) and the **Normal** fraction, averaged over all fields. For Equine Breeders Software, the **Single Dose Ejaculate** and **Diluent Volume** are also calculated.

10.2 Notes

Any default text that was entered under **Settings > Notes** (see “Notes” on page 36) will appear on the **Notes Panel**.

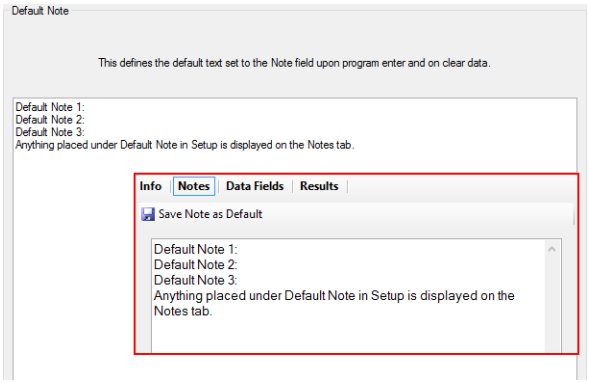


Figure 10-5: Default Note and Notes Panel

Additional text may also be entered directly on the **Notes Panel**. Selecting **Save Note as Default** will overwrite the previously saved default note text entered under **Settings > Notes**.

10.3 Data Fields

ADMIN: *Only HT Administrative Users may customize the Data Field labels.*

Located under **Data Fields** are 25 data input fields with customizable labels. To change the label text, click on the label text, enter a new descriptor in the text box, and select **OK**.

Figure 10-6: Customizing Data Field Labels

Checking **Persist** causes the input data to remain when the analysis data is cleared.

The system saves previously entered input values for customized data fields to allow quick selection by entering text or numbers in the data input field. As keystrokes are made, the available input values are dynamically filtered.

10.4 Results

The Results section shows both Summary and Kinematics results.

Summary

The **Summary** section displays the **Total**, **Static**, **Motile**, **Progressive**, and **Slow** cell counts, total cells in **Sample** (M), **Concentration** (M/ml or B/ml) and **Percentage**.

Summary

Class	Count	Sample (M)	Conc (M/ml)	%
Total	161	131.38	131.38	100
Static	37	30.19	30.19	23
Motile	124	101.18	101.18	77
Progressive	51	41.62	41.62	31.7
Slow	11	8.98	8.98	6.8

Figure 10-7: Summary Results

Kinematics

Average values, units, standard deviations, and median values of eleven motion parameters are provided for the three categories of **Motile**, **Progressive**, and **Slow** sperm. Definitions of motion parameters are provided in Table 2-1 on page 5.

Kinematics

Measure	Avg	Units	SD	Median
Motile				
DAP	41.4	µm	17.78	43.98
DSL	30.7	µm	14.9	30.12
DCL	74.78	µm	32.01	74.45
VAP	101.28	µm/sec	32.63	106.68
VSL	76.14	µm/sec	31.41	74.54
VCL	183.35	µm/sec	61.43	189.81
STR	74.19	%	17.1	77.6
LIN	42.18	%	14.38	42.9
ALH	7.28	µm	2.69	6.96
BCF	35.59	Hz	11.29	35.9
WOB	55.85	%	10.96	55.38
Progressive				
DAP	41.54	µm	16.64	41.25

Figure 10-8: Kinematics Results

10.5 Charts

Pie Charts are provided depicting **Percent of Total** (motile vs. static) and **Velocity Percent of Motile** (rapid progressive, medium and slow).

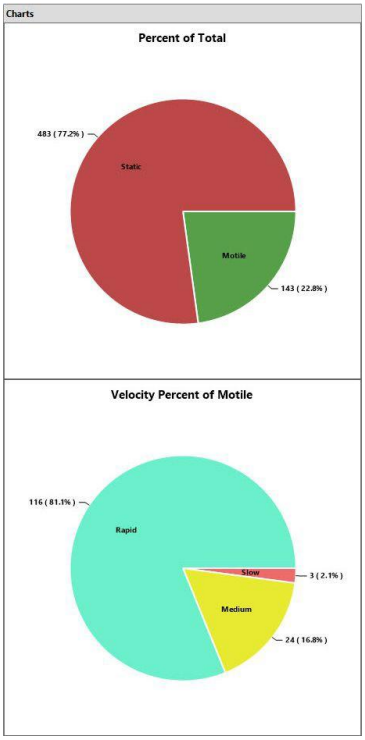


Figure 10-9: Charts

Chapter 11. Playback and Zoom Screens

NOTE: *The ability to save track ASCII data and delete tracks from the analysis requires the optional Tracks II software.*

11.1 Field Playback Screen

Click on a video in the thumbnail gallery to open the **Field Playback** screen. The **Field Playback** screen provides the ability to view the fields captured and analyzed, allowing a quality control check for proper acquisition of cells. The **Field Playback** screen shows the motile sperm identified by color-coded tracks and static sperm identified by a red dot, as shown in Figure 11-1. The **Field Playback** screen includes the following:

1. Image area showing video playback of selected field with graphic overlays
2. **Playback Image Status** bar
3. **Field Playback Menu** bar
4. Results for the currently selected field
5. Controls to show or hide track overlays
6. **Video Playback Control** bar
7. **Video Thumbnail Gallery**

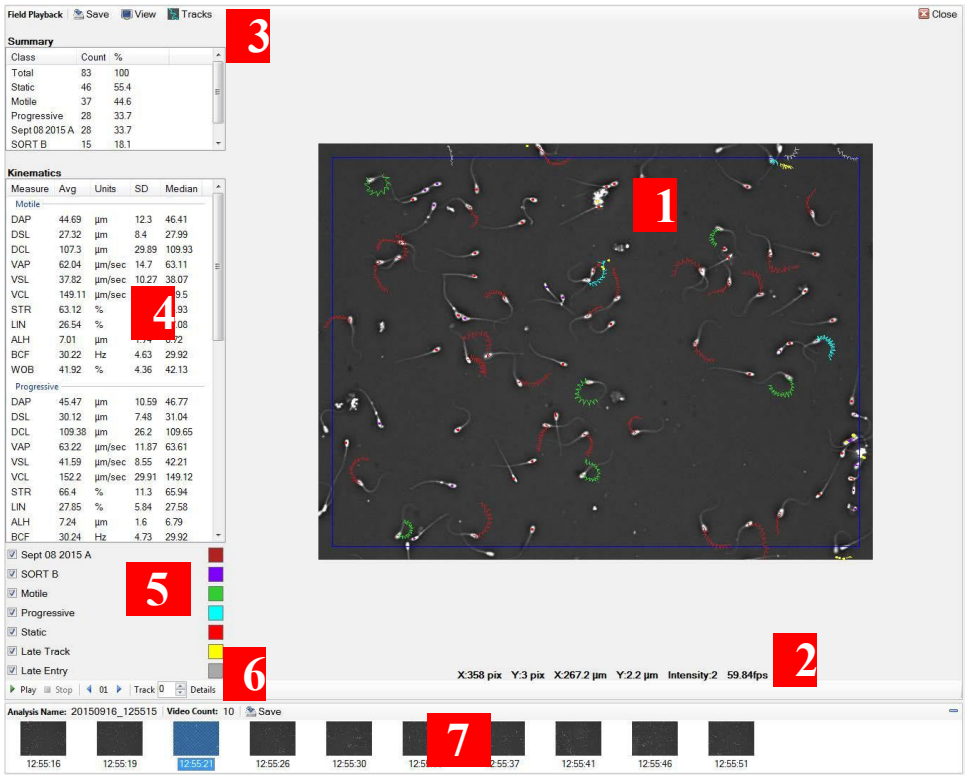


Figure 11-1: Field Playback Screen

Video Thumbnail Gallery

As fields are captured, they are displayed in the **Video Thumbnail Gallery**. In addition, the **Analysis Name** and number of captured fields (**Video Count**) is displayed.

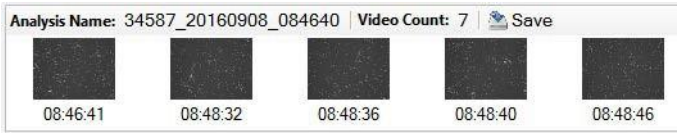


Figure 11-2: Video Thumbnail Gallery

- **View**: To view the **Field Playback**, click on the **Video Thumbnail**. If **Auto Start Video** is enabled (see "Analysis Saving" on page 30), the video will begin playing automatically when opened.
- **Delete**: To delete a captured field, right-click on the **Video Thumbnail** and select **Delete Video**. This will both delete the video and remove its associated data from the analysis results.
- **Save**: (Requires optional Track II software) To save track ASCII data for all fields analyzed, select **Save** located directly above the **Video Thumbnails** and choose to either save data for the **Total Cell** population or only the **Motile Cell** population. To set the location of the saved data, see "Track Settings" on page 29. To save ASCII data for a single field, see "Field Save Options" on page 52. To save ASCII data for a single cell, see "Zoomed Cell Save Options" on page 55.

If a thumbnail image is marked with a red X, this indicates that the individual field **Total Count** is > 2SD from the average of all fields analyzed. For this to occur, **Detect Field Variations** must be enabled on the **Analysis Setting** screen (see "Analysis Configuration" on page 25).

Video Playback

Locate the **Video Playback Control Bar** above the **Video Thumbnail Gallery**.

Figure 11-3: Video Playback Control Bar

The **Video Playback Control Bar** provides the following options:

- **Play**: Full playback of all frames captured.
- **Stop**: Stops the video from playing.
- **Frame by Frame Playback**: Use the horizontal arrows to scroll forward or back by one frame at a time.
- **Track**: Each identified track is assigned a number. Using the Track up and down arrows, change the currently selected a sperm (indicated by an orange square).
- **Detail**: Opens the **Zoomed Cell Track** screen (see "Zoomed Cell Playback Screen" on page 54) of the currently selected sperm.

Zoom and Pan Playback Field Image

When the mouse cursor is positioned over the field image, the image may be zoomed and panned.

- **To Zoom Image**: Position mouse cursor over the field and use the mouse scroll wheel to zoom in and out.

- **To Pan Image**: When the image is zoomed in beyond the maximum displayable size, the image may be panned to view different areas. With the mouse cursor over the image, press and hold the left mouse button and drag to reposition image view.

Playback Image Status Bar

Figure 11-4: Playback Status Bar

The **Playback Image Status Bar** provides the following details:

- Date and time captured
- **X** and **Y** coordinates in both pixels and µm of the image at the tip of the mouse cursor
- Pixel intensity of the image at the tip of the mouse cursor
- Actual frame rate (frames per second - fps) used for field capture

Field Save Options

Click **Save** on the **Field Playback Menu** bar to open the **Save** menu:

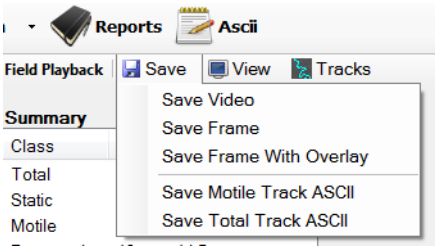


Figure 11-5: Field Playback Save Options

- **Save Video**: Select **Save Video** to save an *.mp4* file of the currently selected Field Playback, including track overlays. This file may be used as any standard *.mp4* file (e.g., import into PowerPoint presentations), but cannot be reanalyzed by the CASA II software.
- **Save Frame**: The currently displayed Playback frame is saved as a .tif file.
- **Save Frame with Overlays**: The currently displayed Playback frame and graphic overlays are saved as a .tif file.
- **Save Motile Track ASCII**: (Requires optional Track II software) Saves the ASCII data of all motile tracks in the currently selected Playback Field (see “Track Settings” on page 29).
- **Save Total Track ASCII**: (Requires optional Track II software) Saves the ASCII data of all tracks in the currently selected Playback Field (see “Track Settings” on page 29).

Field View Options

The color overlays on the playback image and the individual field results may be hidden or visible. From the **Field Playback Menu** bar, select **View**. Uncheck the options to hide the results or overlays.

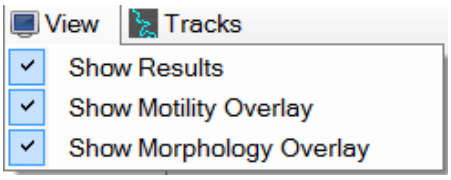


Figure 11-6: Field Playback Viewing Options

- **Show Results**: Displays the Summary and Kinematic results for selected field.
- **Show Motility Overlay**: Displays the color overlays on the **Playback** image and the Individual Track Overlay Controls.
- **Show Morphology Overlay**: Shows the presence of distal and proximal droplets and tail.

In addition, by using the individual track controls, the graphic overlays intended for specific cell categories may be hidden or made visible. For detailed definitions of the categories, Table 2-3 on page 6.

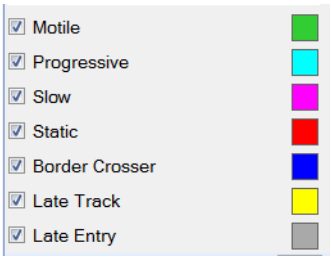
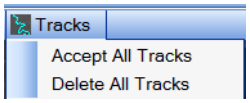


Figure 11-7: Individual Track Overlay Controls

NOTE: *Hiding progressive or slow cells moves the track into the Motile category and the track turns green.*

Only categories that have been identified on the field will appear in the list. For example, if no Border Crossers were identified in the field, that category will not appear in the list.

Tracks Options



(Requires optional Tracks II software) All tracks in a single field may be Accepted or Deleted by selecting the appropriate command, **Accept All Tracks** or **Delete All Tacks**, from the menu. The data for deleted tracks are removed from the analysis results and all deleted tracks are recategorized as **User Deleted**.

11.2 Zoomed Cell Playback Screen

To view the **Zoomed Cell Playback** screen, click on the track or select the track number from the **Video Playback Control Bar** and click **Detail**. The **Zoomed Cell Playback** screen includes:

1. Image area showing enlarged sperm cell with graphic overlays
2. **Zoomed Cell Menu** bar
3. Kinematic and morphometry results for the selected cell
4. **X-Y Data Points** of the sperm head center for each captured frame
5. **Video Playback Control** bar
6. **Classifications** of the selected cell
7. **Zoomed Cell Image Status Bar**

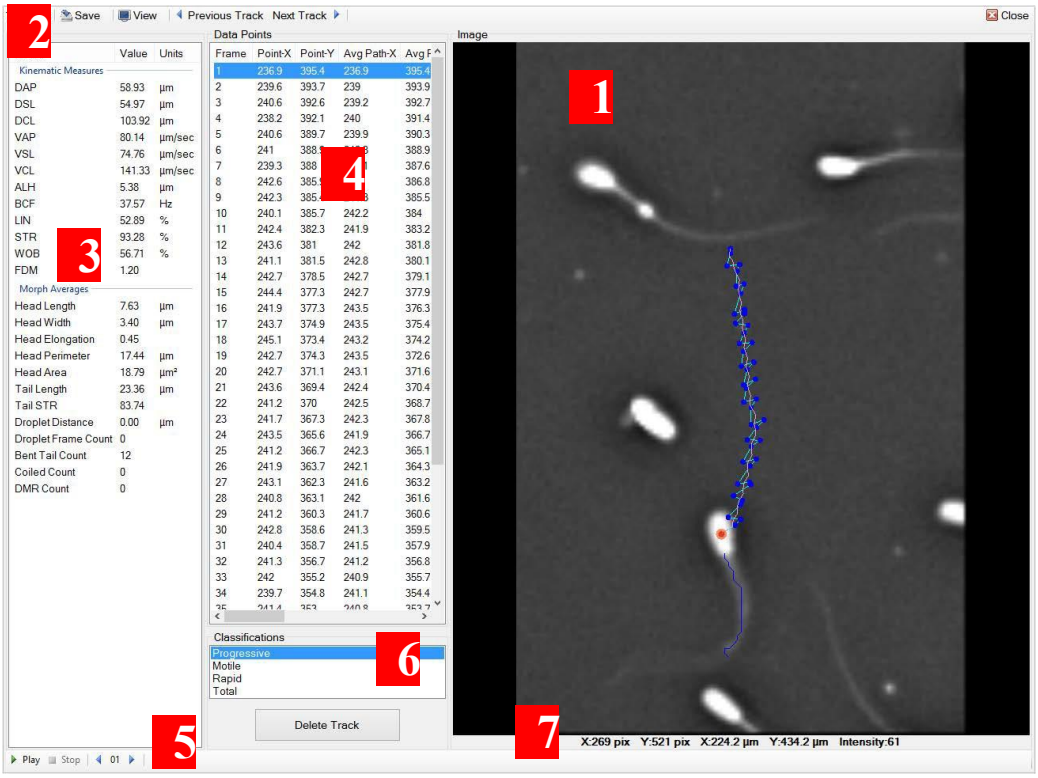


Figure 11-8: Zoomed Cell Playback Screen

Zoomed Cell Video Playback

Using the **Video Playback** controls, the video may be viewed in full or scrolled frame by frame. In addition, to jump to a specific frame, click on the **X-Y Data Points** or click on the sperm track overlay.

Zoomed Cell Save Options

Several Save options are available for individual sperm tracks.

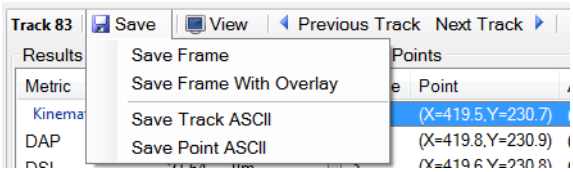
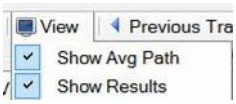


Figure 11-9: Individual Track Saving Options

- **Save Frame**: The currently displayed individual sperm track frame is saved as a .tif file.
- **Save Frame with Overlays**: The currently displayed individual sperm frame and graphic overlays are saved as a .tif file.
- **Save Track ASCII**: (Requires optional Track II software) Saves the kinematics data and X-Y Data Points for the currently selected individual track (see "Track Settings" on page 29).
- **Save Point ASCII**: (Requires optional Track II software) Saves only the X-Y Data Points for the currently selected individual track (see "Track Settings" on page 29).

Zoomed Cell View Options



- **Show Avg Path**: Displays the average cell path overlay (in purple) on the zoomed track.
- **Show Results**: Displays the Kinematic Measures, Morph Average, Data Points and Classification of the selected track.

Zoomed Cell Image Status Bar

- Date and time captured
- **X** and **Y** coordinates in both pixels and µm of the image at the tip of the mouse cursor
- Pixel **Intensity** of the image at the tip of the mouse cursor

Classification

The various classifications assigned to the selected cell are shown in the **Classification Panel**. This includes classifications such as **Total**, **Motile**, **Progressive**, **Slow**, **Late Track**, etc. This will also show any **Sort** categories into which the cell falls (see "Sort Option" on page 57).

Delete or Accept Single Track

(Requires optional Track II software) From the **Zoomed Cell Playback** screen, select **Delete Track** or **Accept Track** as needed to delete or accept a track. Deleted tracks are reclassified as **User Deleted** and the overlay track color turns white. The data for the deleted track is removed from the analysis results.

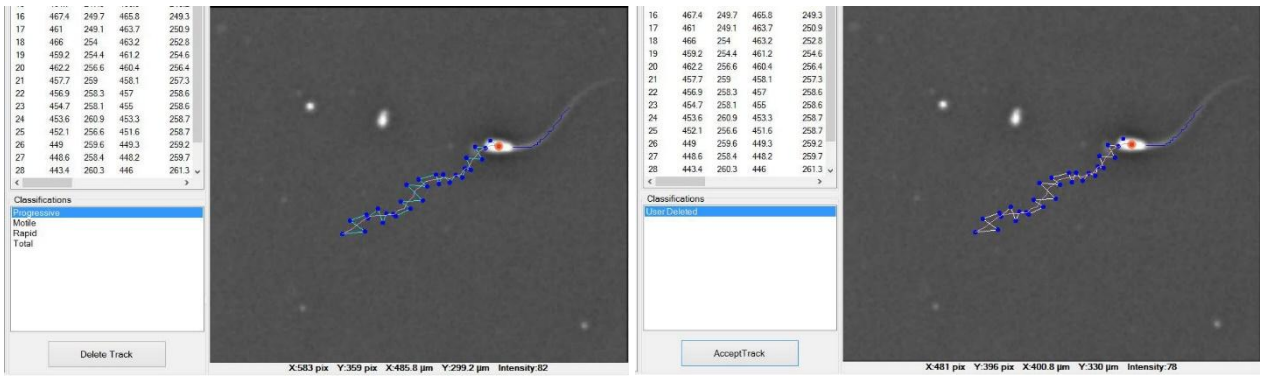


Figure 11-10: Delete Track / Accept Track

Chapter 12. Sort Option

The optional **Sort** software allows the ability to isolate a certain sub-population of cells (i.e., hyperactivated cells) for analysis.

Three independent **Sort** sets are available. For each analysis performed, the enabled **Sort** sets are applied separately to the cell population. The fraction of cells passing the sort criteria are calculated and presented under **Analysis Results Summary**.

12.1 Defining a Sort Set

ADMIN: *Only members of the HTMotilityAdministrators Group are able to define and enable Sort sets.*

From the Settings menus, select **Sort** to open the Sort definition screen.

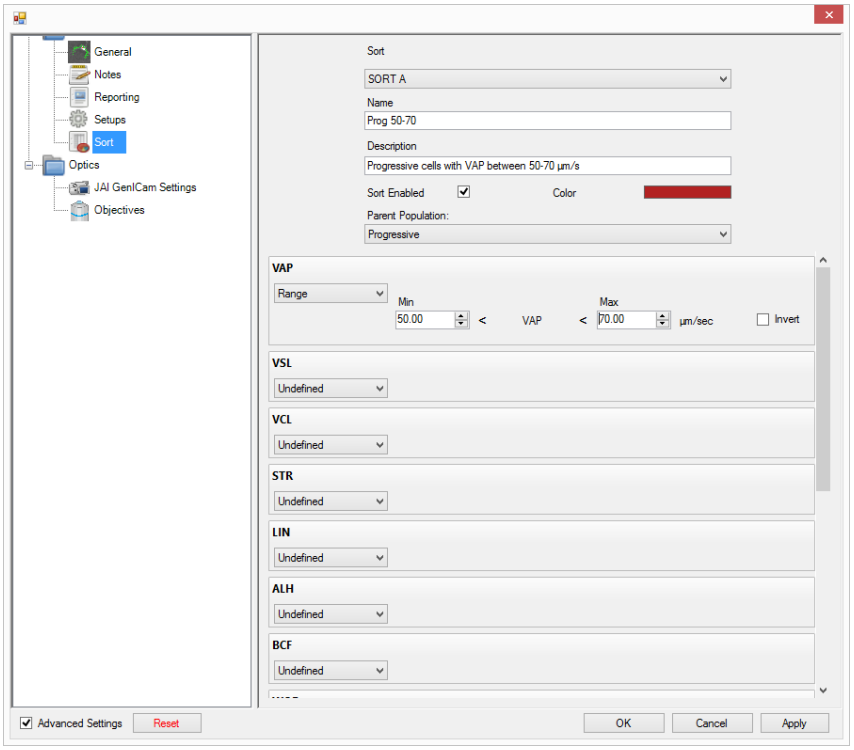


Figure 12-1: Sort Definition Screen

- **Sort Sets**: Three **Sort Sets** (A, B, and C) may be defined. Select one from the dropdown list.
- **Sort Name**: Enter a short name (e.g., Prog. 50-70) to identify the **Sort Set** on the various results and playback screens
- **Sort Description**: A more detailed description of the **Sort Set** may be added if needed
- **Sort Enabled**: The **Sort Set** must be enabled to appear on the results and playback screens.
- **Sort Color**: Select the color of the overlay that will identify cells included in the **Sort Set** on the **Playback** screen.
- **Parent Population**: Select the starting population of cells on which the enabled **Sort Definition** will be

applied. For example, if **Progressive** is selected, the cell must first be classified as **Progressive** to be considered for inclusion in the **Sort Results**. Cells that are classified as **Slow** would not be included. The available Parent Populations are:

- All Tracked Objects
- Total
- Motile
- Static
- Progressive
- Slow

Sort Criteria

The following kinematic and morphometric criteria may be used to Sort the cells.

Table 12-1: Available Sort Criteria

VAP	LIN	Bent Tail Count	Droplet Avg Distance	Head Avg Width
VSL	ALH	Coiled Tail Count	Tail Count	Head Avg Perimeter
VCL	BCF	FDM*	Tail Avg Length	Head Avg Length
STR	WOB	Droplet Count	Head Avg Area	Head Avg Elongation

**See "Fractal Dimension (FDM)" on page 64 for a detailed description.*

Setting the Sort Parameters

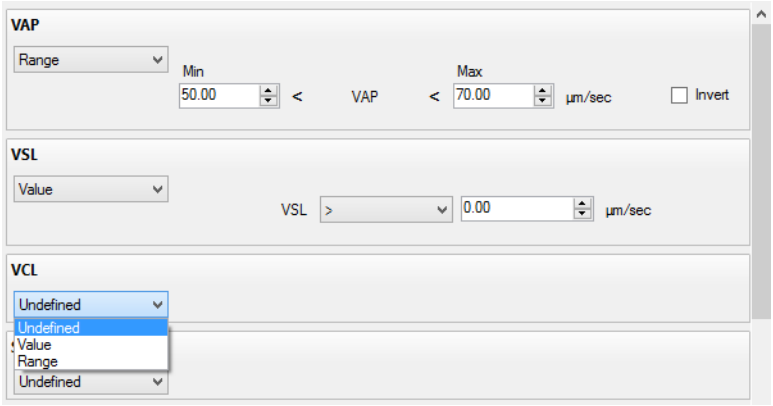
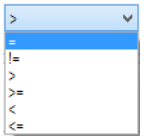


Figure 12-2: Setting the Sort Parameters

- If a parameter is not to be used in the selected Sort Set, leave the setting to **Undefined**.
- To enter a range in which the cell must fall inside of, select **Range** and enter the minimum and maximum numerical limits. If **Invert** is selected, the opposite logic will apply: the cell must fall outside the minimum and maximum values entered.
- Select **Value** to enter a specific value as a starting point, and then select the desired expression from the dropdown list.



NOTE: *The expression != means "does not equal".*

12.2 Sort Results

Summary & Kinematics Results

For each enabled **Sort Set**, the results for the cells that meet the **Sort Criteria** are displayed under **Results Summary and Kinematics** and identified by the **Sort Name**.

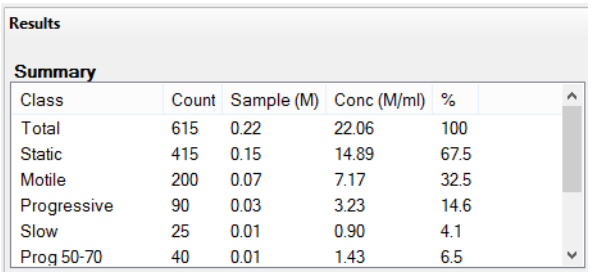


Figure 12-3: Sort Summary Results

Field Playback Screen

On the **Field Playback** screen, any cells falling within the enabled **Sort Set** are labeled with the corresponding color selected for that Sort Set. By deselecting the toggle box next to **Sort Set**, the original track classification colors may be viewed.

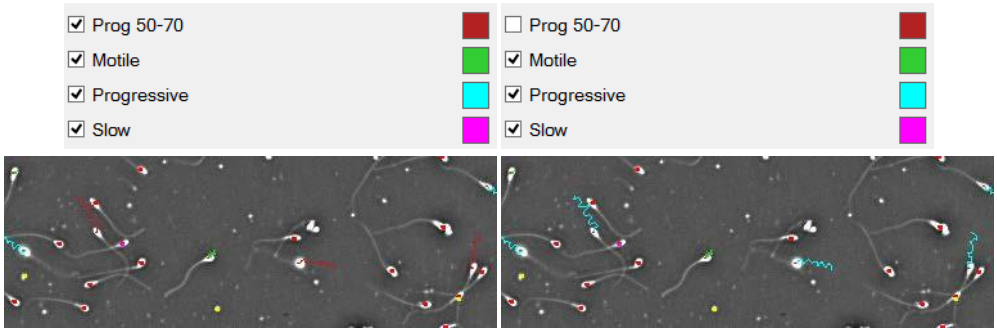


Figure 12-4: Sort Set Tracks on Field Playback

Zoomed Cell Playback

When viewing the **Zoomed Cell Playback**, the applicable **Sort Sets** are included under **Classifications**.

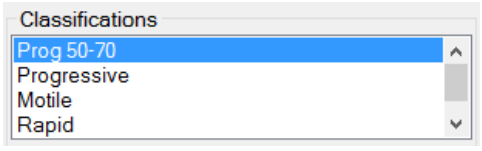


Figure 12-5: Zoomed Cell Classification: Sort Results

12.3 Fractal Dimension (FDM)

Fractal Dimension (FDM) is a specific algorithm used to detect hyperactivated sperm.

Fractal Dimension (FDM) for a track is:

$$\mathbf{FDM = log(n)/[log(n) + log\ (d/L)]}$$

where:d = maximum DSL (straight line distance) for a track L
= total DCL (curvilinear distance) for a track
n = number of points in a track -1 (one less than the number of points in a track)

If FDM > 1.3, the track is considered Fractal.

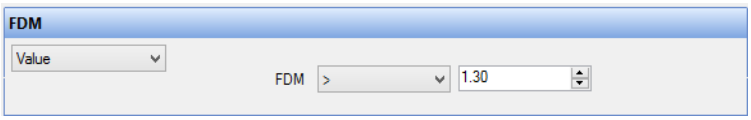


Figure 12-6: FDM Sort Parameter

FDM was initially developed for use in human sperm applications; however, it has also been applied to other species, such as ram sperm. For more information, please see:

Mortimer ST, Maxwell WM. Effect of medium on the kinematics of frozen-thawed ram spermatozoa. Reproduction. 2004 Feb;127(2):285-91. PubMed PMID: 15056794.

Mortimer ST. CASA--practical aspects. J Androl. 2000 Jul-Aug;21(4):515-24. Review. PubMed PMID: 10901437. (http://onlinelibrary.wiley.com/doi/10.1002/j.1939-4640.2000.tb02116.x/pdf)

Mortimer ST. A critical review of the physiological importance and analysis of sperm movement in mammals. Hum Reprod Update. 1997 Sep-Oct;3(5):403-39. Review. PubMed PMID: 9528908. (http:// humupd.oxfordjournals.org/content/3/5/403.full.pdf+html)

Mortimer ST, Swan MA, Mortimer D. Fractal analysis of capacitating human spermatozoa. Hum Reprod. 1996 May;11(5):1049-54. PubMed PMID: 8671389.

Chapter 13. Storage & Printout

13.1 Storing Still Images and Video Files

Automatic Storage

To automatically store images or videos when **Save and Report** or **Save and Clear** is clicked, the **Type, Base Path** and **Field Selection** must be specified under **Video Storage** (see "Video Storage Settings" on page 30).

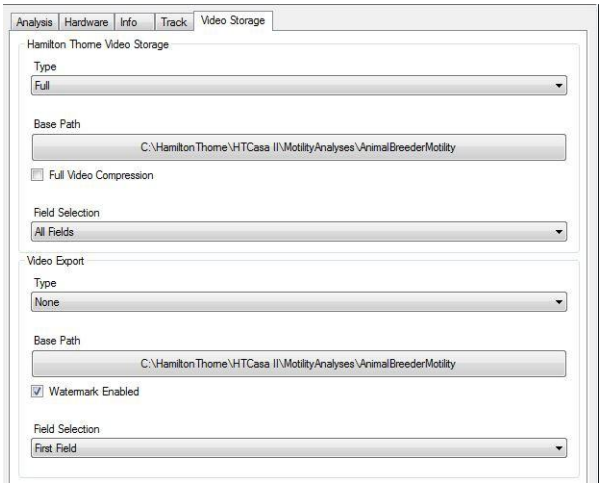


Figure 13-1: Video Storage

In addition, the **Analysis Name Mode** must be set (see "Analysis Name Mode" on page 24).

After an analysis, click **Save and Report** or **Save and Clear** to save the videos or images.

NOTE: *If SubjectID_DateTime is the selected Analysis Name Mode and an ID number is not entered on the Analysis Info Panel, the system will display a warning message when attempting to save.*

Manual Storage

- To manually save the current frame of an open **Field Playback** or **Zoomed Cell Track**, use the save button on the applicable screen (see "Field Save Options" on page 52 and see "Zoomed Cell Save Options" on page 55).
- To manually save an *.mp4* file, see "Field Save Options" on page 52.

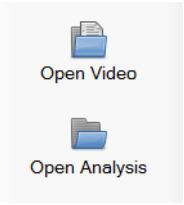
13.2 Viewing and Printing Reports

13.2.1 To automatically view and /or print reports when **Save and Report** is selected, the options may be enabled on the **Reporting** settings panel (see “Reporting” on page 32).

13.2.2 Reports may also be viewed or printed through **Reports** on the **Main Menu** (see “Reports” on page 65).

Chapter 14. Analysis from Stored Videos

If an analysis was stored in **Full** video format, the video can be retrieved and re-analyzed. The **Analysis Info** and **Analysis Parameters** used in the previous analysis are stored with the video.



1. On the **Motility sub-menu**, click **Open Video** to re-analyze one video or **Open Analysis** to re-analyze a complete analysis run.
2. Select a video file or folder from the list and click **Open**.

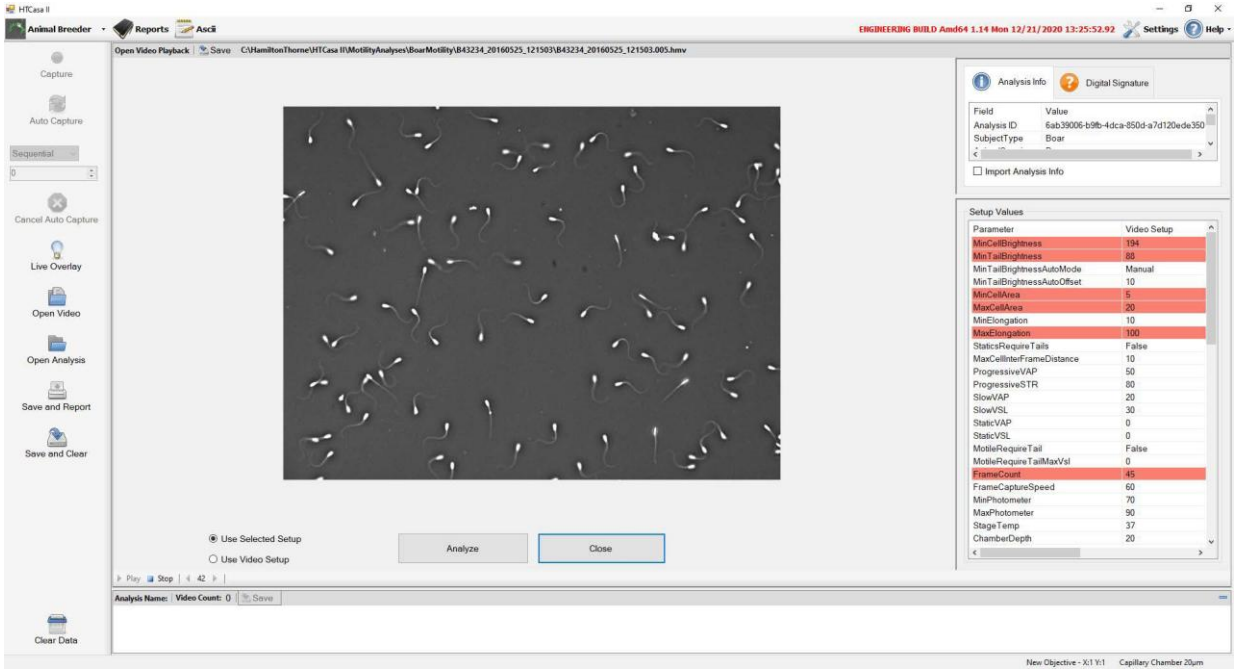


Figure 14-1: Open Saved Video or Analysis

a. The saved video will begin to play.
b. The path and name of the current video is displayed.
c. The **Analysis Info** (Animal ID or Boar ID, Genetic Line, Ejaculate Volume, etc.) saved with the video is displayed along with a checkbox option to automatically import the **Analysis Info** data.
d. If the video was saved with a **Digital Signature**, a green check mark appears on the **Digital Signature** tab and relevant details are displayed.

NOTE: *If the information in the video was tampered with and it affected the Digital Signature, the Green Check will be replaced by a Red X, to alert the user.*

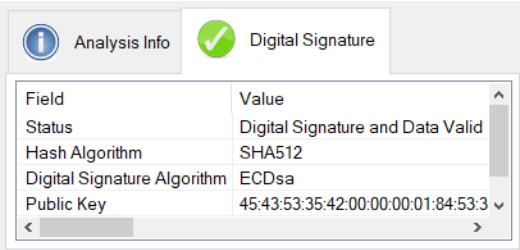


Figure 14-2: Video Digital Signature

e. The **Setup Values** dialog box appears showing motility settings saved with the video file and highlighting all motility options which differ between those in the video file and those of the currently selected setup.

f. Select either **Use Selected Setup** (to use new **Setup** values for reanalysis) or **Use Video Setup** (to use the same **Setup** values as original analysis) and click the **Analyze** icon to process the image.

g. Click **Close** to exit the screen without analyzing.

3. If **Use Video Setup** is enabled, selecting **Live Overlay** shows the illumination based on the values of the saved setup. If **Use Selected Setup** is enabled, selecting **Live Overlay** shows the **Motility Setup Configuration** controls (HT Administrative Users only), allowing adjustment of video parameter gates prior to analysis.

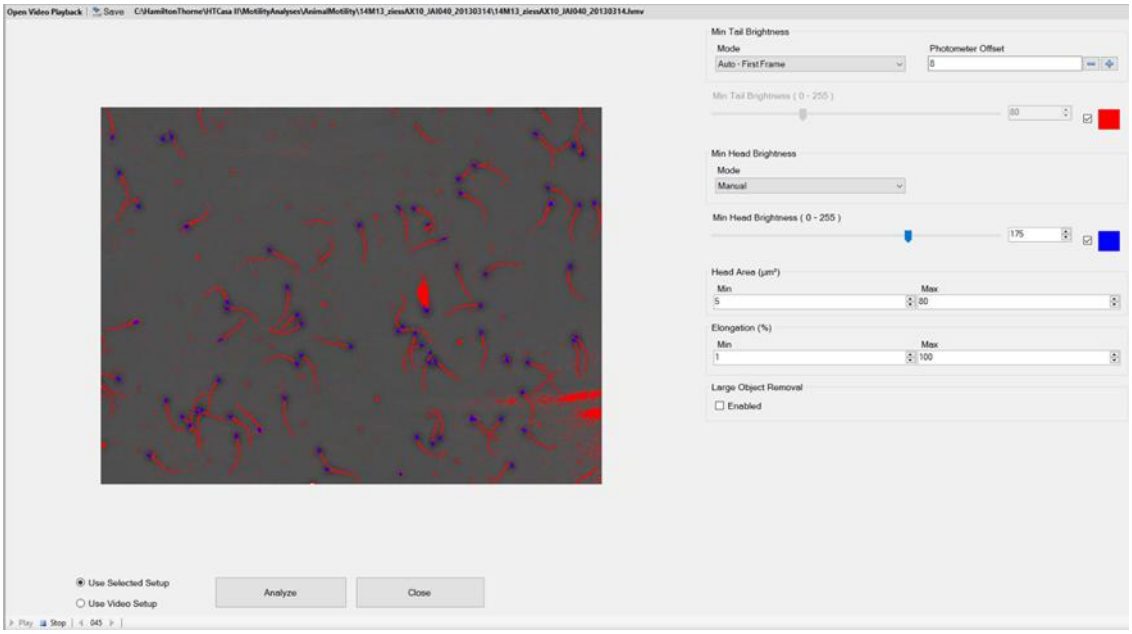


Figure 14-3: Use Selected Setup with Live Overlay

4. The analyzed video appears as a thumbnail on the main screen, which you can click to view the **Playback** screen.

5. Additional saved videos may be added to the analysis. The setup selected for the first video will apply to all subsequent videos added to the analysis run.

Chapter 15. Reports

Click the **Reports** icon on the **Main Menu** to open the **Reports** window. The **Reports** function allows the creation, copying, editing, viewing, printing or deleting of reports.

15.1 Report Template

Report Templates provides access to the design and customization of new or existing reports and the viewing of reports. Available functions are:

- **New Template**: Opens the **New Report** dialog box. Enter the name of the **New Report** and select **OK**.
- **New Folder**: Creates a new folder within **Report Templates Panel**.
- **Copy**: Opens a **New Report** dialog box. Enter a **New Report** name and select the report to be copied.
- **Delete**: Select the template to be deleted from the **Report Template Panel** and select **Delete**.
- **Design**: Select the template to be edited from the **Report Template Panel** and select **Design**.
- **View**: Select the report to view from the **Report Template Panel** and then select the **View** icon.

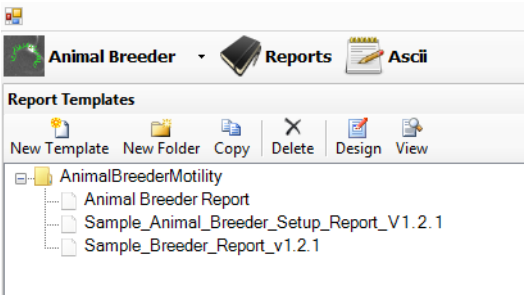


Figure 15-1: Report Templates Panel

15.2 Viewing Reports

Individual Reports

1. From the **Report Templates Panel**, select the report to be viewed and select **View**, or double-click the **Report Name**.
2. From the header section of the **Report Filter Panel**, initiate a query based on:
   a. All Animals
   b. Animal ID
   c. Genetic Line
   d. Lab Technician
   e. Batch Number
   f. Ejaculate Number
   g. Start and End Dates
3. Select **Refresh** to view a list of records meeting the search criteria.
4. Display data may be sorted by clicking on a column header.

5. Select a record within the resulting query table to view the report.

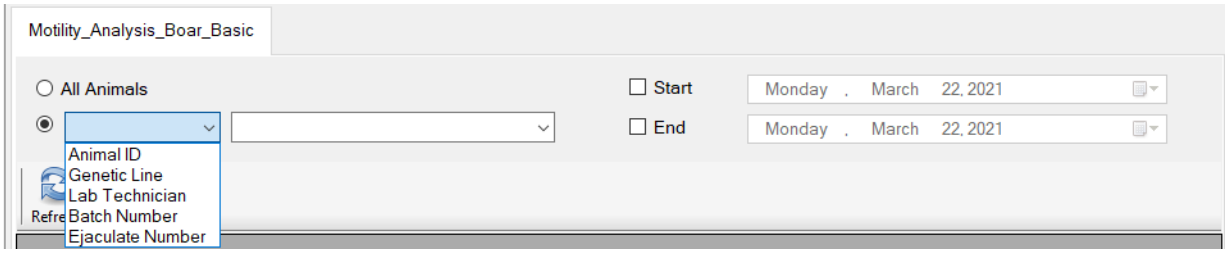


Figure 15-2: Report Filter Panel

Combined Reports

If viewing a combined report (based on two separate records), a second tab will appear. The desired records on both tabs must be selected before the report will appear. Combined reports must be designated during the design phase (see “Creating a Combined Report” on page 70).

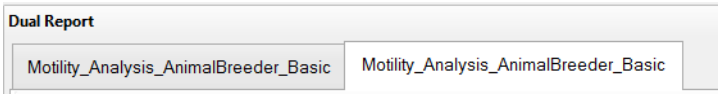


Figure 15-3: Combined Reports Query Tabs

View Reports Menu

The **View Reports** menu provides offers the following controls: **Save**, **Page Setup**, **Print** and **Fit Width**.

- **Save**: To save reports as shown with data, select **Save** from the menu bar. Reports may be saved in either TIFF or PDF format.
- **Page Setup**: Provides access to page settings for printing
- **Print**: Allows printing of the currently displayed report and data
- **Fit Width**: Adjusts the size of the report width to fit in the available window space.

15.3 Designing a Report

The **Design** option permits customization of the pre-designed forms or creation of entirely new forms. The user-friendly, “drag and drop” designer gives you complete control over the look and content of the report. Select **New Template** to open a blank report design window.

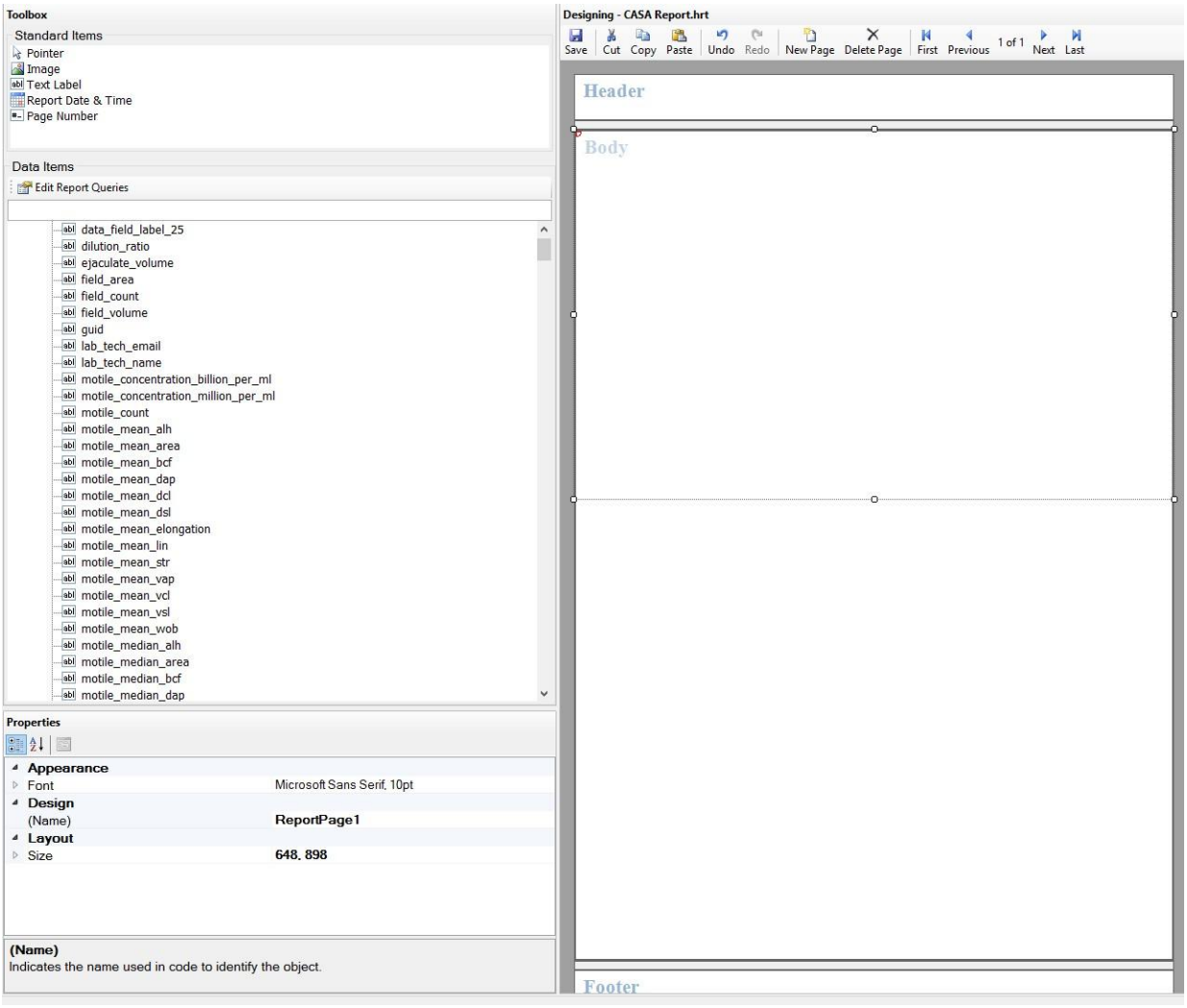


Figure 15-4: Blank Report Template

Design Area

The design area is divided into three parts, **Header**, **Body** and **Footer**. Items included in these sections are printed out as a report. Any input or output data may be added to the report. Free-form fields also allow the inclusion of non-analysis data such as contact information or company logo.

The menu items above the design area provides access to basic commands used in designing reports.

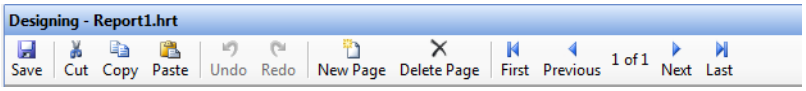


Figure 15-5: Report Design Menu

Adding Items to the Report

There are two methods for adding data fields to the report design area:

- Select the header, body or footer section of the report and then double-click on the **Standard** or **Data Item** to be added. The field appears at the upper-left corner of the selected section.
- Click on the item, hold the left mouse button and drag the field to the design area (Click Hold + Drag).

Adding an item only brings the placeholder for the actual variable. Labels for the variables must be created separately using the **Toolbox/Text Label** tool (see "Standard Items" on page 68).

Report Layout Aids

As fields are being dragged within the design area, alignment guidelines appear to help align and position the fields. If you select items and right-click, additional layout controls are available (i.e., center align, send to back, bring to front).

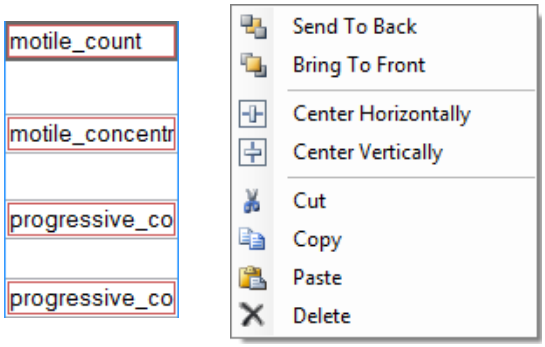


Figure 15-6: Report Layout Aids

Toolbox

The **Toolbox** is divided into **Standard Items** and **Data Items**, which are used to add data results to the report.

For detailed information on setting various properties for the **Standard** and **Data Items,** see "Properties" on page 69.

Standard Items

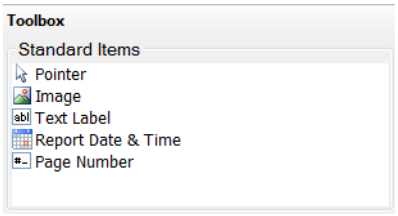


Figure 15-7: Standard Items

- **Pointer**: Standard mouse pointer
- **Image**: Insert an image into the report
- **Text Label**: Add a text label or text field

- **Report Date & Time**: Insert the date and time the report was generated
- **Page Number**: Insert a page number for multi-page reports

Data Items

The **Data Items** toolbox area consists of all data from the system's CASA database. These items can be click-dragged into the desired position on the report. In the actual report, the corresponding value of that variable will appear.

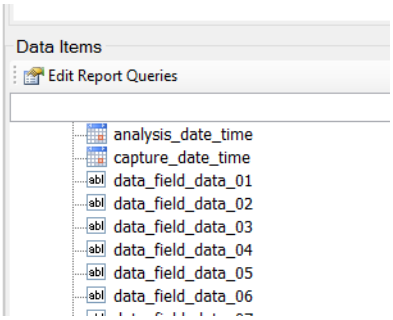


Figure 15-8: Data Items

Properties

Quick Properties Tool

When a **Standard** or **Data Item** is inserted into the report, the **Properties** may be set from the **Properties Toolbox** or the **Quick Properties Tool** located on each field box frame. This provides a short cut to enter parameters that will design the item (e.g., select image, formatting, alignment, etc.).

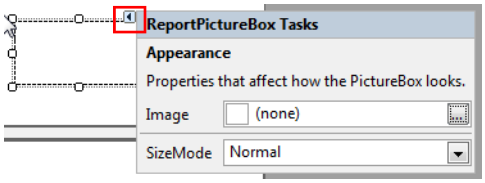


Figure 15-9: Quick Properties Tool

Properties Panel

The Properties toolbox area lets you format the report layout, **Standard Items** and **Data Items**. The variables listed under Properties will change depending on what is selected in the design window (see "Property Descriptions" on page 71).

Design View and Report Image

The image below shows the same report in the design view showing field placeholders and the report view showing actual data.

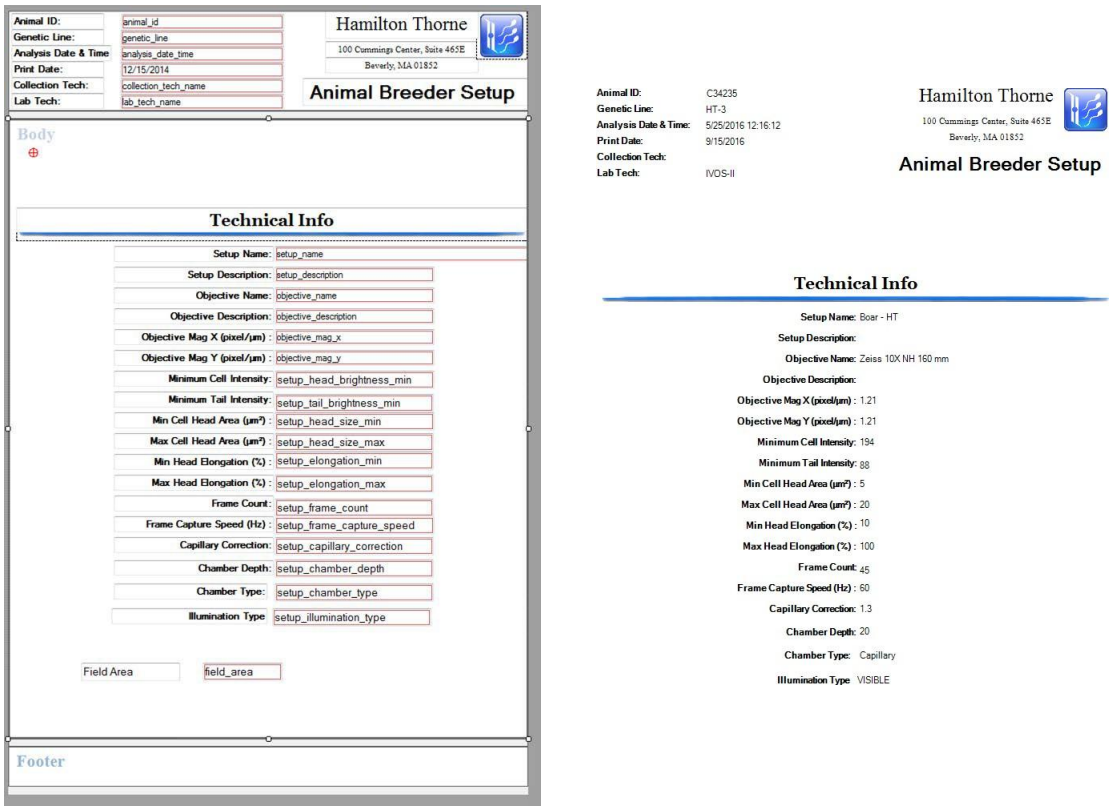


Figure 15-10: Design View and. Report Image

Creating a Combined Report

The Reports feature allows the creation of a report containing data from two separate motility data sets.

The report design for the first data set proceeds as detailed under “Designing a Report” on page 67.

To add a second data set to the report, select **Edit Report Queries** under **Data Items** to open the **Report Query Collection Editor**. Under **Available Queries**, select a second instance of the motility database and press **Add Query**.

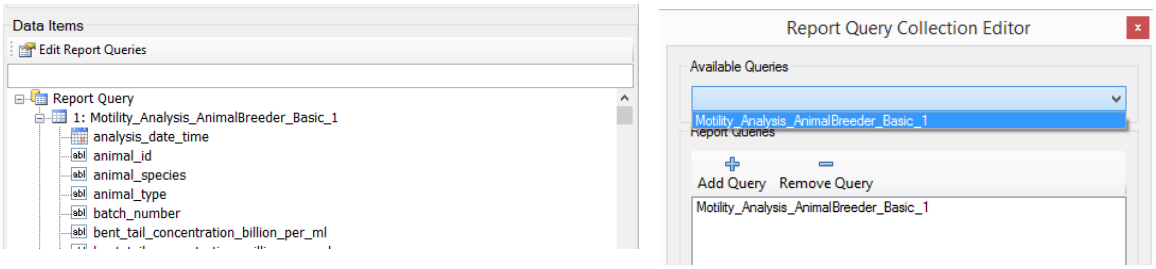


Figure 15-11: Add Query: Add Second Data Set to Report

The new query (2) will appear under **Data Items** and fields may be added to the report as with the first data set.

The data set (**Report Query 1** or **Report Query 2)** from which the *Data Item* is pulled can be confirmed by selecting opening the **Quick Properties** to view the **Data Path.**

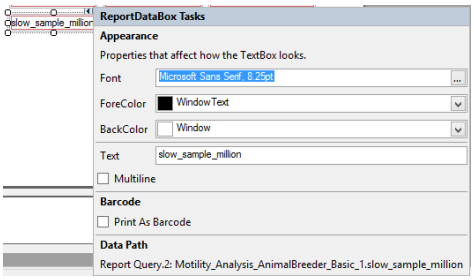


Figure 15-12: Quick Properties Data Path

To view previously designed combined reports, see "Combined Reports" on page 66.

15.4 Property Descriptions

General

Report Layout

To view the **Report Properties**, click on the "light gray" area between the header and body or body and footer in the design area.

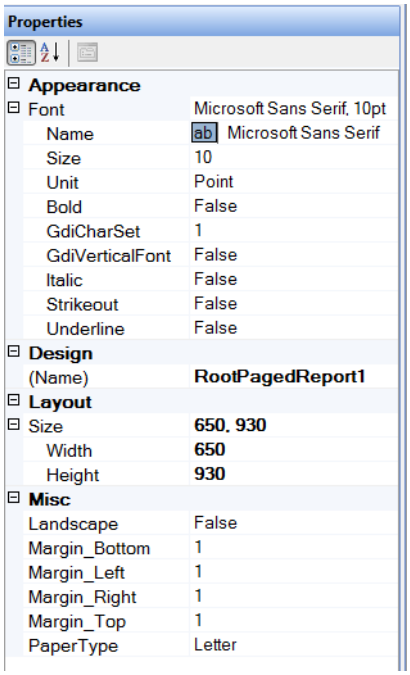


Figure 15-13: Report Properties

- **Appearance**
  - Font: Controls the typeface, size, style of the default font.
- **Design**
  - **(**Name**)**: The tag applied to the report. This can be left as is or changed.

- **Layout**
  - Size
    - *Width*: Report width based on the set margins.
    - *Height*: Report width based on the set margins.
- **Misc.**
  - *Landscape*: Determines paper orientation. Set to false to print standard portrait orientation.
  - *Margins*: Set desired margins.
  - *PaperType*: Select desired paper type (e.g., letter, legal, A3, A4, etc.).

Header, Body and Footer Properties

From within the design window, select either the header, body or footer section of the report. The default font and layout properties appear.

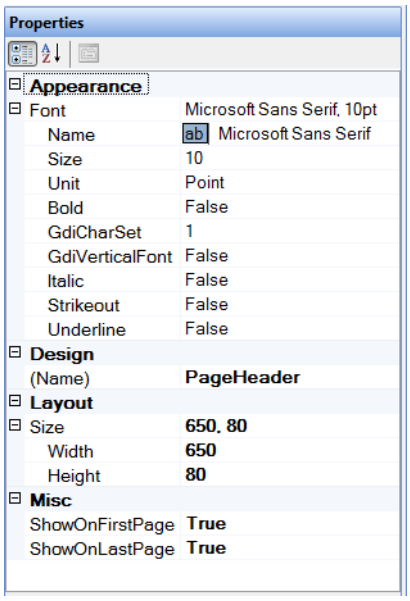


Figure 15-14: Header, Body, Footer Properties

- **Appearance**
  - *Font*: Controls the typeface, size, style of the default font.
- **Design**
  - (*Name*): The tag applied to the selected item. This can be left as is or changed.
- **Layout**
  - **Size**
    - *Width*: Based on the report width.
    - *Height*: Maximum header and footer height is 125. These may be adjusted by entering new values or by dragging the header or footer border. Body height is determined by report height minus the header and footer heights.
- **Misc** (header and footer properties only)
  - *ShowOnFirstPage*: Select true to show on first page.
  - *ShowOnLastPage*: Select true to show on last page.

Image Properties

Two types of images may be added to the report:

- Under **Standard Items**, the **Image** control allows the import of any compatible image.
- Under **Data Items**, inserting the image_path_first_field pulls an analysis image with graphic overlay automatically saved to the database file for each saved analysis.

When the image box is selected, the options under Properties reflect Image settings.

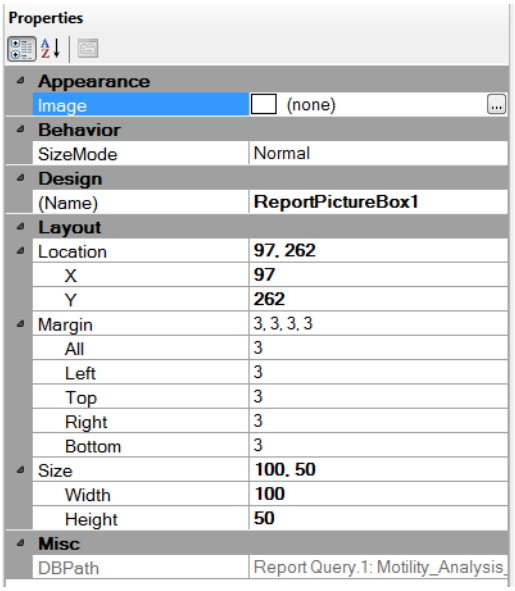


Figure 15-15: Image Properties

- **Appearance**
  - *Image*: Browse to locate the image file. Compatible image files are: .bmp, .jpg, .png, .gif, .ico, .emf and .wmf. Image files are embedded in the report template.
- **Behavior**
  - SizeMode
    - *Normal*: inserts image into box with no resizing or repositioning.
    - *StretchImage*: stretches image to fit the current image box (non-proportional).
    - *AutoSize*: adjusts the size of the image box to fit the image.
    - *CenterImage*: positions image to the center of the box.
    - *Zoom*: proportionally resizes image to fit the image box.
- **Design**
  - (*Name*): The tag applied to the selected item. This can be left as is or changed.
- **Layout**
  - *Location*: The X,Y location of the upper-left corner of the image box. This may be changed by adjusting the numerical value or by dragging the box to a new location.
  - *Margin*: The “snap-to” margin between the image box and other control boxes.
  - *Size*: The size of the image box. This may be changed by adjusting the numerical value or by dragging the box borders.

- **Misc.**
  - *DBPath*: This field is automatically populated when the **Data Item** image_path_first_field is added to the report.

Text Properties

The Text Properties apply to the following:

- Text Labels
- Report Date & Time
- Page #
- Data Items

Common text box properties are shown below:

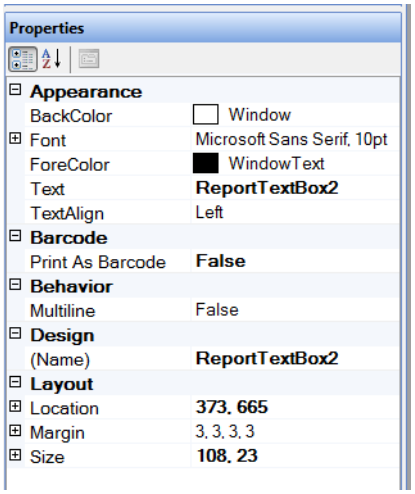


Figure 15-16: Text Properties

- **Appearance**
  - *BackColor*: The background color of the text box.
  - *Font*: Controls the typeface, size, style of the default font.
  - *ForeColor*: The color of the text.
  - *Text*: The text that appears in the text box in the design layout. For Text Labels, this will also appear on the final report. For Data Items, the text will be replaced with the correct value upon viewing report.
  - *Text Alig*n: Left, right, center.
- **Barcode**
  - *Print as Barcode*: To print the text as a barcode, set to true. To use standard font, set to false. When using barcodes, you must make sure the box is long enough to accept the complete code. This may take some trial and error.
- **Behavior**
  - *Multiline*: To extend printing to additional lines within the selected text box, set to true.
- **Design**
  - (*Name*): The tag applied to the selected item. This can be left as is or changed.

- **Layout**
  - *Location*: The X,Y location of the upper-left corner of the text box. This may be changed by adjusting the numerical value or by dragging the box to a new location.
  - *Margin*: The "snap-to" margin between the text box and other control boxes.
  - *Size*: The size of the text box. This may be changed by adjusting the numerical value or by dragging the box borders.

The **Report Date & Time Standard Item** and the **analysis_date_time Data Item** also have the following **Misc** options:

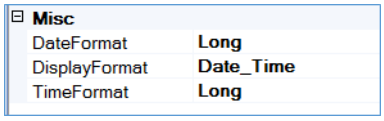


Figure 15-17: Date and Time Misc Formats

- **DateFormat**
  - Year_ Month_Day: 2025-08-28
  - Month_Day_Year: 08-28-2025
  - Day_Month_Year: 28-08-2025
  - Long: Monday, Aug 28, 2025
  - Short: 08/28/2025
- **DisplayFormat**
  - Date: Shows date only
  - Time: Shows time only
  - Date_Time: Shows date then time
  - Time_Date: Shows time then date
- **TimeFormat** (the outputs may vary based on geographic locale)
  - TwelveHour: 2:38:29 PM
  - Twentyfour Hour: 14:38:29
  - Short: 2:38 PM
  - Long:2:38:29 PM

NOTE: *The Short and Long date and time formats are set through the Windows operating system and may differ from the above.*

For **Data Items** with numerical output, one additional property is displayed:

- **Precision:** Number of decimal places to display for floating point data.

Chapter 16. ASCII

NOTE: *The file created by this mode of ASCII Export is not the same as the automatic ASCII Export file configured under General/Reporting/ASCII Export.*

The **ASCII Export** section provides the ability to manually select data fields for ASCII file output. All data stored from motility analyses is available for inclusion in these manually created ASCII Export files, regardless of the fields selected for automatic **ASCII Export** when **Save and Report** is selected. This allows you to filter data selectively and create multiple ASCII files based on the same data set.

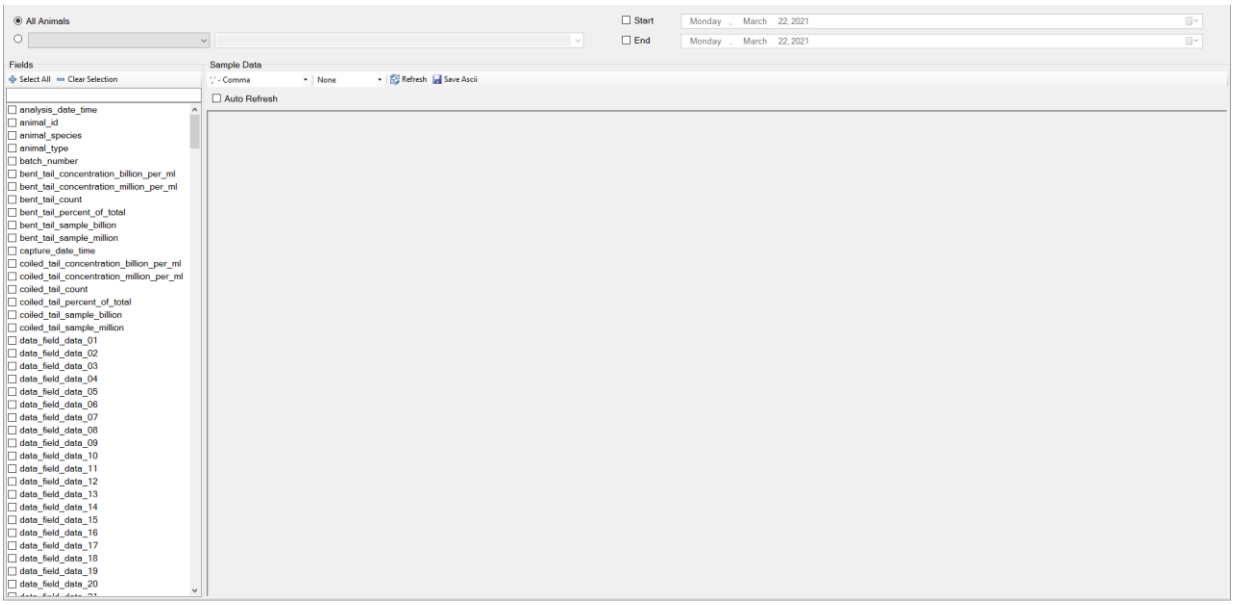


Figure 16-1: ASCII

1. From the header section of the **ASCII Filter Panel**, initiate a query based on any of the following filters:
   a. All Animals
   b. Animal ID
   c. Genetic Line
   d. Lab Technician
   e. Batch Number
   f. Ejaculate Number
   g. Start and End Dates
2. Select **Refresh** to view a list of records meeting the search criteria.
3. Under the Fields section are the list of variables available which may be included in the ASCII output.
   - **Select All**: Will check all the variables that will go in the report.
   - **Clear Selection**: Will clear all the checked variables.
   - Typing the desired variable in the Fields box will filter variables from the field.
   - Click the checkbox of variables to be included in the output.

- As these items are checked, a table will appear on the right side of the screen Design section listing their values under their output names.

4. Under **Sample Data**:
   - Click the dropdown list and select the type of delimiter to be used: **Comma**, **Semicolon**, **Space** or **Tab**.
   - Set the data qualifier to **None**, **Double Quote** or **Single Quote**.
   - Click **Refresh** to reload the current changes made to the sample data. To have the data refresh automatically, check the **Auto Refresh** box.
   - Click **Save ASCII** to save the ASCII table in the command line. The Save As dialog box appears, prompting you to provide a name and folder location to save the file. The file is saved in *.tab* format.

Chapter 17. Help Menu

The **Help** menu provides access to information on the HT CASA II software and database maintenance utilities.

17.1 About

- The **About** dialog box includes Version of the software.
- Click the **License Agreement** button to review and agree to Hamilton Thorne's standard end user license agreement (EULA).

NOTE: *If you do not agree to the terms and the license agreement you will not be able to use the program.*

- Click **Advanced**, to see more information on **Plugins** and **Options** of the software.

17.2 Database

Database provides access to the following **Backup**, **Restore** and **Optimize** database utilities.

Backup

The backup utility creates a copy of the motility database.

1. Select **Database > Backup** from the **Help** menu.
2. Select **Backup** from the **DB Maintenance** dialog box that appears.
3. Select a location, enter a file name for the backup file, and click **Save**. The file is saved with the extension *.backup*.
4. When the **Backup Complete** message appears, select **Exit**.

Restore

NOTE: *IMPORTANT: Any new data added to the CASA II database since the backup file was created will be lost if the database is restored from a backup file.*

1. Select **Database > Restore** from the Help menu.
2. A notice stating that the program must be exited appears. Select **Yes** to continue with the restore process or **No** to return to the program.
3. Select **Restore** from the DB Maintenance dialog.
4. Locate the *.backup* file from which to restore and click **Open**.
5. When the restore process is complete, the CASA II program will automatically restart.

Optimize

Database optimization occurs automatically each time the CASA II program is initiated; however, if the system is left running for an extended period of time, select **Database > Optimize** to make the database most efficient.

17.3 Save Support Package

In the event of an error, a detailed set of data may be saved and sent to Hamilton Thorne to help determine the cause of the problem. In addition to mandatory storage of the **Event Log** and **Application Settings**, **Report Templates** and **Database** may also be saved. All data is compressed into a single zip file.

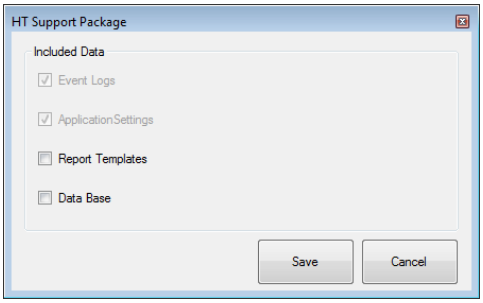


Figure 17-1: Save Support Package

Chapter 18. Remote Capture

The Remote Capture system is an optional workstation for the capture and storage of sperm motility videos (.hmv files). The .hmv video files include all the necessary calibration, setup, sample and patient information for future analysis on either an IVOS Pro.

18.1 Remote Capture Hardware

The Remote Capture system requires a negative phase contrast microscope, 10x NH objective, digital camera, computer and Remote Capture software. The hardware configuration is identical to the CEROS II analyzer. Please refer to the CEROS II hardware manual for setup instructions.

18.2 Remote Capture Software

The Remote Capture software interface is similar to that of the full version CASA II software; however, it provides only the ability to configure settings (HT Administrative Users only), add animal and sample information, capture videos and save .hmv motility files.

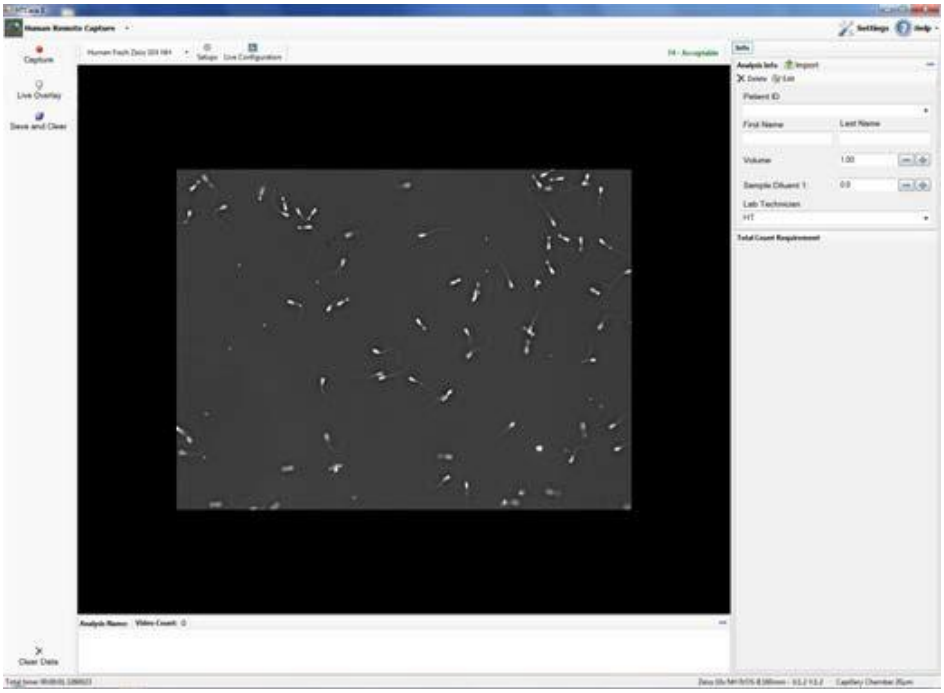


Figure 18-1: Remote Capture Software Interface

18.3 Required Settings

ADMIN: *Only HT Administrative Users are able to adjust settings.*

Optics

For detailed instructions on optical setup, see “Optics Settings” on page 39.

Under **Settings/Optics/eBus Video Settings**, select the installed camera from the device dropdown list.

Under **Settings/Optics/Objective**, enter the objective information and calibrate the 10x NH objective (see "Magnification Calibration" on page 40).

General Settings

For detailed instructions on **General Settings**, see "General" on page 24.

- Under **Analysis**, select the **Name Mode**. It is highly recommended that **SubjectID_DateTime** is used as the **Analysis Name Mode**. This will allow quickest location of video files for individual patients.
- Also under **Analysis**, set **Video Playback** options, **Display Units**, and **Maximum Number per Field**.
- Under **Hardware**, select **Enable Scale** if a scale will be used to measure ejaculate volume.
- Under **Info**, select **Enable ASCII Import** if sample information needs to be imported and enable any **Persistent Info Fields**.
- Under **Video Storage**, select **Full** as the **Video Type** and enter the **Base Path** for the saved video files.

Setups

For detailed instructions on **Setups** and setup parameters, see "Setups" on page 34.

For consistency with the main laboratory, it is recommended that the setup values used on the Remote Capture system are identical to those used on the IVOS Pro where the analysis will be performed.

- **Import**: If the main laboratory has provided exported setup files, use the Import feature to automatically add each setup to the Remote Capture system.

NOTE: *The objective will need to be confirmed and selected manually from the dropdown list, if needed.*

If the main system to be used for analysis is an IVOS Pro, the setting for Camera Exposure will need to be adjusted

- **New**: If the main laboratory has only provided a printout of the setup values, create a new setup and manually configure the parameters.

Live Configuration

For detailed instructions on setting the **Live Configuration** parameters, see "Live Setup Configuration Screen" on page 24. Follow instructions specific to the CEROS II analyzer.

- Under **Photometer**, set the **Min** and **Max** values.
- Adjust the illumination using the microscope control so an **Acceptable** illumination level is obtained.
- Turn on the toggle controls for the **Minimum Tail Brightness** (MTB) and **Minimum Head Brightness** (MHB) to view the color-coded overlay.
- Set the **MTB** either automatically or manually.
- Set the **MHB** so that only the sperm heads are colored blue.

18.4 Analysis Info

For detailed descriptions of **Analysis Info**, see "Info" on page 44.

18.4.1 Animal and sample information may be entered manually or imported from an ASCII file.
18.4.2 An **Animal ID** is required if the naming format selected is **SubjectId_DateTime**.
18.4.3 **Ejaculate Volume** is required to calculate total sample numbers.
18.4.4 **Extender:Diluent** ratio is required to determine accurate concentration numbers.

18.5 Capturing & Saving Video Files

1. Enter the **Analysis Information**.
2. Confirm that the negative phase contrast microscope objective is being used.
3. Place the chamber loaded with sample onto the heated microscope stage, position the chamber with the stage controls and focus the image.
4. Select the appropriate setup from the dropdown list.
5. Adjust the microscope illumination so that an **Acceptable Illumination** level is indicated in the **Motility Toolbar**.

Figure 18-2: Motility Toolbar: Acceptable Illumination

6. Select **Live Overlay** on the **Motility Submenu**. This will show the live image with the blue head and red tail illumination overlays on the sperm cells.
7. Optimize the microscope focus so that sperm show blue heads and long red tails as shown below.

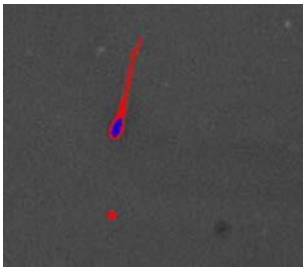


Figure 18-3: Live Overlay showing Proper Focus

8. Click **Capture** on the **Motility Submenu** to capture the current field. Move the stage to a new field and click Capture to analyze another field.
9. Repeat this process until the **Total Count Requirement** message indicates Total Count acceptable.

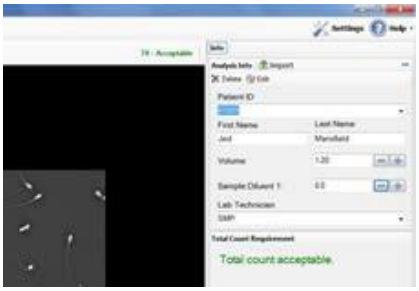


Figure 18-4: Total Count Acceptable

10. Click a video thumbnail to view the playback of the captured sequence. There will be no color-coded track overlay on the image.
11. To save the captured videos, select **Save and Clear**.

18.6 Motility Files

Locating Saved Video Files

The captured video files will be saved in the **Base Path** selected under **Settings > General > Video Storage**.

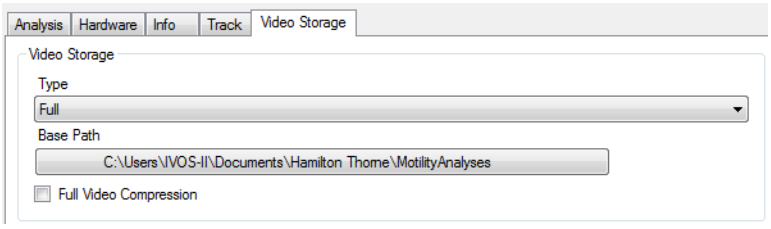


Figure 18-5: Video Storage

Video File Overview

A file folder based on the **Analysis Name Mode** (**DateTime**, **Manual**, or **SubjectID_DateTime**) is automatically created for each analysis and the captured video files are stored within the folder. Hamilton Thorne motility videos are given an .hmv extension.

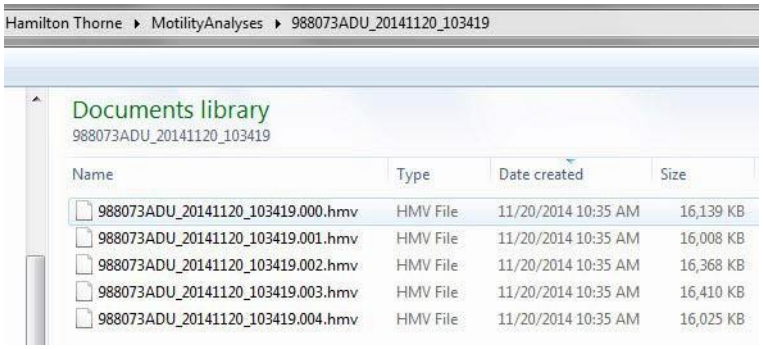


Figure 18-6: Saved Motility Files

In the previous image, the **SubjectID_DateTime Name Mode** was used to store the video files. A total of 5 videos (.hmv files) were saved for the analysis (000 to 004).

- **988073ADU**: Animal ID
- **20141120**: Date (YYYY/MM/DD)
- **103419**: Time (24-hour clock) of first image capture
- **.000 etc**.: Sequential file identification

The size of the video files depends on several factors, including the number of frames captured, the number of objects in the captured frames and if video compression is used

Appendix A. Localizing Language

ADMIN: *Only Windows users with administration level access will be able to install/uninstall language packages and add a new user.*

NOTE: *Maintain the "English" language user in case of need of support from Hamilton Thorne. Hamilton Thorne cannot provide support (such as remote access through TeamViewer) in other languages.*

A.1 Installing or Uninstalling a Display Language

DISCLAIMER: *HT CASA II software program only supports English, Spanish, French, Russian, German, Chinese, Japanese, and Portuguese languages. If other languages are installed and selected in Windows, the CASA II program will not display the unsupported language.*

NOTE: *Make sure the System is connected to a Network Connection.*

1. From the **Windows Control Panel**, select **Clock, Language and Region**.
2. Select **Language** and then select **Add a Language**.
3. Choose the language to add. If there are different dialects available, choose the preferred dialect, and select **Add**.
4. Select the **Options** link next to the newly selected language.
5. Select the **Download and Install Language Package** link.
6. The language package will appear in the list of installed languages.

A.2 Changing the Display Language

The HT CASA II Motility Software program includes language support for English, Spanish, French, Russian, German, Chinese (simplified), Japanese, Portuguese, and Vietnamese.

Systems are typically shipped configured for an English user. To use the system in a supported language other than English, you will need to log on to Windows as a user of that language.

For example, to log on as a French language user:

1. Create a new Windows user which will use French language.
2. Log on to Windows as the French User.
3. From the **Windows Control Panel**, select **Clock, Language and Region**.
4. Select **Language** and then select **Add a Language**.
5. Choose the language to add. If there are different dialects available, choose the preferred dialect, and select **Add**.
6. Select the **Options** link next to the newly selected language.
7. Select **Make this the primary language**.
8. Follow the prompt to **Log off now**.
9. When the French user logs back in, the French language will be displayed.

Appendix B. ASCII Import Mapping File

A per-species column map XML file can be defined. The XML file maps the ASCII header fields to the above application fields. This is only necessary when the import file cannot provide the below header field names.

Boar	**Animal Breeder**	**Equine Breeder**
AnimalId	AnimalSpecies	AnimalId
CollectionTech	AnimalId	CollectionTech
DilutionRatio	CollectionTech	DilutionRatio
EjaculateVolume	DilutionRatio	EjaculateVolume
GeneticLine	EjaculateVolume	GeneticLine
LabTech	GeneticLine	LabTech
EjaculateNumber	LabTech	EjaculateNumber
BatchNumber	EjaculateNumber	BatchNumber
SpermPerDose	BatchNumber	RequiredConcentration
DoseVolume	SpermPerDose	DoseVolume
	DoseVolume	
	UsableVolume	

B.1 XML File Name Convention

The file must be stored in the same folder as the HBS settings file. The default folder is C:\HamiltonThorne\HTCasa II\. The file name File name must be in the format: *AnalysisInfoAsciiImportColumnMap[Species].xml*

- AnalysisInfoAsciiImportColumnMapAnimalBreeder.xml
- AnalysisInfoAsciiImportColumnMapBoar.xml
- AnalysisInfoAsciiImportColumnMapEquine.xmll

XML files may be created using any standard text editor, such as Notepad or WordPad. Once the mapping file is created, the CASA II software application must be restarted.

B.2 XML File Format

- First line of file must be

  <?xml version="1.0"?>

- A **Dictionary** object must be defined

  <dictionary> </dictionary>

- A single column Map can be added as a dictionary item. Items are listed inside the **Dictionary** tags one after the other.
  - Item **Key** is the Column exactly as it will appear in the ASCII file.
  - Item **Value** is the Field Name exactly as it is expected in the application and defined above.

```
<item>
    <key>
        <string>Bull_Code</string>
    </key>
    <value>
        <string>AnimalId</string>
    </value>
</item>
```

B.3 Example Mapping File

```
<?xml version="1.0"?>
<dictionary>
    <item>
        <key>
            <string>Patient_Code</string>
        </key>
        <value>
            <string>PatientId</string>
        </value>
    </item>
    <item>
        <key>
            <string>Birth_Date</string>
        </key>
        <value>
            <string>DateOfBirth</string>
        </value>
    </item>
    <item>
        <key>
            <string>Physician</string>
        </key>
        <value>
            <string>PatientPhysician</string>
        </value>
    </item>
    <item>
        <key>
            <string>Dilution</string>
        </key>
        <value>
            <string>DilutionRatio</string>
        </value>
```

```
        </item>
    </dictionary>
```

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