Pathology & Diagnostics Newsletter

European Edition

reSOLUTION

- In vitro Fertilisation
- IMSI / ICSI
- Micromanipulation
- FISH

June
2008

SPECIAL EDITION

EDITORIAL

Dear Reader,
We are very happy to present this special edition of our European Pathology & Diagnostic Newsletter, dedicated to the fascinating world of Human Reproduction.

Leica Microsystems has always been a supporting part of this branch of Life Science, since the early days of IVF, with its first and famous M8 stereomicroscopes and still unrivalled Leitz high precision mechanical micromanipulators.

But Technology and Science have progressed tremendously in the last years and we are happy to still be partners of many scientists and institutes all over the world, developing continuously new techniques and methods to improve this wonderful and joyful part of Medical Sciences.

In this Newsletter we have combined articles and interviews with some of our European partners, with a brief overview of the complete range of solutions we can offer for every step from IVF to Prenatal Diagnostic.

A wide range of stereomicroscopes, inverted microscopic stations integrated with any type of micromanipulators, upright microscopes and dedicated Kariotyping & FISH Software, digital cameras: Leica Microsystems has the right solution for all your requirements.

In all Europe, our dedicated Clinical Microscopy team, supported by the European Product Manager Frédéric Ribay, is ready to answer all your questions, and find the right product for you.
Just click on www.leica-microsytems.com to find all our contacts in Europe and detailed information on our products.

We hope this Newsletter is of interest to you and are looking forward to your feedback.

Anja Schué,
Corporate Communications

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Zygote observation phase

After fecundation, the zygotes are observed to check the number, the size and the locations of the pronuclei. The stereomicroscope is the main optical tool for visualising the development of the embryos – from the zygote to the blastocyst state. In case of extended culture, the check will take place at D5 or D6 (D0 = Day of puncture). As the microscope is intensively used in this phase, ergonomic design, reliability, and ease of use are crucial as well as high image quality in terms of resolution and depth of field. In addition, compatibility with various accessories is essential, e.g. ergotube, choice of different optics and eyepieces, heating stage, and cameras to visualise the sample image on a screen.

Focus on the Oocyte

Stereomicroscopy in IVF applications

Since the world's first test tube baby nearly 30 years ago, reproduction medicine has become the only hope of having a child for many infertile couples, unless they decide to adopt. Several methods are available today, including intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI), besides the classic method of *in vitro* fertilisation (IVF). Yet the prospects of success of artificial insemination depend on a great number of factors. Ultimately, obtaining an embryo with higher implantation potential not only relies on optimal culture conditions and transfer technology, but also optimal selection and therefore quality of the oocyte. To select oocytes after puncture, and also to select blastocysts for implantation, stereomicroscopes are used, as their 3D imaging capability makes them ideal for these examinations.

Decoronisation or denudation phase

When the oocytes have been collected after the follicle puncture, the samples must be denuded, i.e. stripped of their granulosa cells. This delicate procedure requires a large working distance, so a high-quality stereomicroscope is needed. The free oocytes can then be observed and selected depending on their quality. If the ISCI (intracytoplasmatic sperm injection) technique is used, the oocytes are isolated in order to accomplish fecundation. For the classical IVF method, denudation takes place one day after puncture so that the pronuclei can be visualised. To perform a fecundation the collected oocytes are checked to determine their maturity and their morphological aspects.

Convincing quality

Dr Fernando Marina, Biologist at the CEFER Reproduction Institute, Barcelona, Spain:

"The high optical quality of the Leica Microsystems' stereomicroscopes has convinced us. Especially the zoom range from low to very high magnification helps us for selection and denudation of the oocytes after puncture. Today we are equipped with several Leica stereomicroscopes and a micromanipulation system."

The CEFER Reproduction Institute is one of the leading private, interdisciplinary medical centres for reproduction medicine in Spain. The Institute was set up in 1977 by Dr Simón Marina, who is the founder and president of the Spanish Andrology Association. The institute established the first human Semen Bank in Spain in 1977, and in 1993 the "Fundació pro donació d'òvuls" to help infertile women by providing them with donated eggs. The CEFER Reproduction Institute was the second in the world to develop and practice semen-washing techniques. These methods allow HIV-positive men to father children without infecting their female partners. The team consists of 80 specialists, including andrologists, gynaecologists, biologists, urologists and auxiliary staff.

More information on CEFER Reproduction Institute:
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New Dimension in Stereomicroscopy

The stereomicroscope for IVF applications

Stereomicroscopes have been used for many years in IVF, as they are a comfortable and easy to use tool in many steps of the proces.

A tool for many applications

Classical applications of stereomicroscopes are: oocytes observation, selection of oocytes in the follicular punction liquid, decoronisation, change of media, observation of catheter and as a tool to acquire digital images for reporting and publication.

The key features stereomicroscopes should have for use in IVF are:

- Excellent optical qualities, zoom range and depth of field to easily resolve details with deep 3D rendering
- Long working distance to allow comfortable positioning of cups, dishes & wells
- Transmitted light bases to allow different illumination techniques when observing, for example, the "zona pellucida"
- Best ergonomy to allow fast and fatigue-free work for long periods
- Possibility of adapting different types of heating stages
- Compatibility with laminar flow cabinets to ensure a sterile working environment

Leica Microsystems has always been a world-wide leader in stereomicroscopy, both in the industrial field and in life science. At the beginning of ICSI, the Wild M8 was the reference stereomicroscope for preparation and selection techniques and today Leica Microsystems has the most extensive and complete range of instruments and accessories. From educational stereomicroscopes, to the compact "S" series, up to the classical high-performance "MZ" series, the right solution is provided for all types of applications.

Top performance zoom optics, resolution and depth of field:

On top of the well known and complete "S" and "MZ" series Leica Microsytems has just launched a revolutionary "M"series: Leica M125, M165C and M205C stereomicroscopes. These microscopes unite top performance zoom optics and resolution to produce brilliant images with outstanding richness of detail. Used for the first time in the Leica M205C, FusionOptics™ (patent pending) takes advantage of a neurological phenomenon: The left beam path produces great depth of field, while the right beam path provides a high-resolution image. The human brain itself then combines the best information from both channels, using it to compose an image whose resolution and depth of field have never been achieved in any stereomicroscope before.
The Leica M205C is the world's first stereomicroscope with a fully apochromatically corrected 20.5:1 zoom. In the zoom range from 0.78x to 16x, the resolution increases continuously up to 1,050 lp/mm (planapochromat objective 2x). The exceptional performance of Leica M205 C is evident in its impressive maximum magnification of 1,280x. This new generation of objectives brings with it the absolutely largest working distances (planapochromat objective 1x:61.5 mm) in stereomicroscopy.

With the Leica M165C, the classic design principles for stereomicroscopes were stretched to the very limits of optics. With a zoom range of 16.5:1 and a maximum resolution of 906 lp/mm (planapochromat objective 2x), this is the most powerful stereomicroscope in its class. The Leica M125 stereomicroscope completes the M series. It offers fully apochromatically corrected 12.5:1 optics, a zoom range from 0.8x to 10x, and a maximum resolution of 862 lp/mm (2x planapochromatic objective).

Fig.1: The classical Greenough stereomicroscope Leica S6 E with 6.3:1 zoom and a standard magnification of 6.3x-40x has a comfortable 38° viewing angle. The optical design coupled with this ergonomic viewing position allows this microscope to be used for long periods without causing eye strain. Courtesy of: Dr. B. Keppi, Laboratoire de PMA, Beaumont, France

Fig.2: The high-performance stereomicroscope Leica MZ9.5 features an advantageous 9.5:1 zoom ratio and magnifications up to 480x. The high resolution up to 300 lp/mm, extremely high image contrast, and amazing sharpness offer the ultimate in image fidelity and data transfer for critical inspection applications.

Fig.3: The Leica M165 C with a zoom ratio of 16.5 :1 and a maximum resolution of 906 lp/mm (planapochromat objective 2x), is the most powerful stereomicroscope in its class.

Spermogram in IVF Labs

The first step to assisted reproduction

The spermogram is an essential preliminary step in IVF to identify infertility problems. It provides information on spermatozoa characteristics and quality. Sperms are collected in a sterile cup, and have to be checked within the next hour. This quantitative and qualitative examination is part of a series of analysis performed on ejaculated sperm.

The analysed criteria are: volume of sperm, spermatozoa concentration (sperm concentration is measured in millions of spermatozoa per millilitre), spermatozoa motility, morphology like abnormal shapes, leucocyte possibility (trace of infection). Other parameters, such as pH, viscosity, and biochemical compounds, are measured in the semen.

To perform this analysis an upright microscope with a special configuration is required. The microscope has to be equipped with a heating stage (37°C) and with three different objective magnifications (10x in brightfield for motility, 20x and 40x in phase contrast for concentration, morphology and leucocyte presence). The head of the sperm will appear like a small white sphere, while the flagellum will show up as a dark filament. When atypical morphology is present, the study of the fine morphology is done with an oil objective 100x on the spermatozoa stained with Papanicolaou and fixed.

As these microscopes are intensively used in IVF laboratories, an ergonomic, comfortable and robust optical device is needed. The Leica DM1000, DM2000 and DM2500 microscopes perfectly fulfill these needs. The Leica DM Microscope Series adapt well to the rigorous demands of routine laboratory work. For example, microscope operation is based on the natural movements of the user while viewing a specimen. The controls fit naturally and can be adapted to suit both the laboratory and in the user´s hand individual user's requirements.

Fig.2: The focus and stage can be adjusted with just one hand to allow faster, more efficient workflow.

Fig.3: No two hands are alike. And so the Leica DM Series enables every hand to rest on the focus knobs in complete comfort. The focus knobs can be precisely adjusted to fit the user's hand.

Fig.1: DM 1000-2000-2500, from routine sperm check to research analysis, a complete range for all contrasting methods and requirements.

Fig.4: Proper arrangement of the eyetubes promotes good posture while sitting at the microscope. The Leica DM Series offers a wide range of products to customise the eyetube settings.

“Beauty Contest” for Sperm

Morphological sperm selection increases chances of success in assisted reproduction

Sperm quality is frequently at fault when standard methods such as intra-uterine insemination (IUI), in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) do not lead to fertilisation of the oocyte. The relatively new method of intracytoplasmic morphologically selected sperm injection (IMSI) uses high-resolution microscopy to select sperm according to specific morphological criteria that do not occur in conjunction with DNA defects.

Since Louise Brown, the world’s first “test-tube baby”, first saw the light of day in 1978, over 3 million babies have been conceived with the aid of reproductive medicine. That number is currently increasing by 200,000 per year. The interdisciplinary Centre Médico-Chirurgical et Obstétrical (SIHCUS-CMCO) in Strasbourg is one of the leading public centres for reproductive medicine in France. Professor Stéphane Viville, head of the Department of Biology of Reproduction, and Dr. Christiane Wittemer, head of the laboratory for assisted reproduction, annually treat around 2,000 couples who would not otherwise be able to conceive. The SIHCUS-CMCO has been working with IMSI since 2005, and is one of three centres in France to do so.

Good looks matter

Studies have shown a positive correlation between the occurrence of defective DNA and abnormal morphology, especially intranuclear vacuoles, in sperm. The integrity of the nucleus (even shape, lack of vacuoles) is considered to be the most important parameter for successful injection. However, with the 200x–400x magnification typical of conventional ICSI, doctors cannot make out whether they have selected the best sperm in morphological and functional terms.

The Israeli biochemist and andrologist Professor Benjamin Bartoov, who has been studying the relationship between sperm morphology and successful fertilisation intensively for years, developed the IMSI method on the basis of ICSI four years ago. Using high-resolution optics for 6,000x–8,000x magnification, he was able to view and classify abnormal nuclei and other defects in sperm cells. With IMSI, real-time screening without dyes and the selection of sperm cells with the best possible morphological integrity became possible for the first time.

First-choice sperm had a maximum of one vacuole or several small ones covering less than 4% of the total area and the heads were of a smooth, symmetrical oval shape. Second-choice sperm had large vacuoles or misshapen nuclei. All other sperm with abnormalities at the head, connecting piece or tail were excluded if possible.

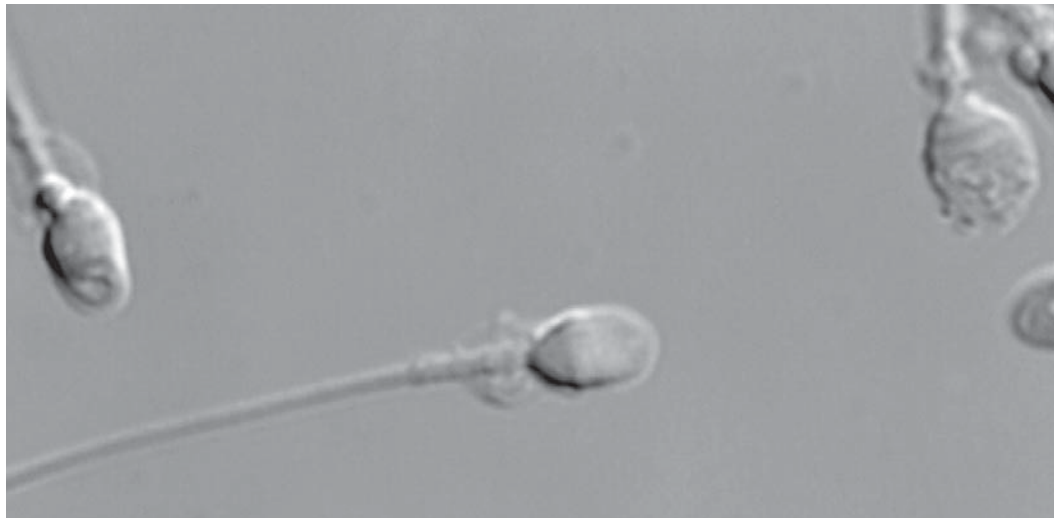

Fig. 1: Sperm cell suitable for IMSI without conspicuous morphology

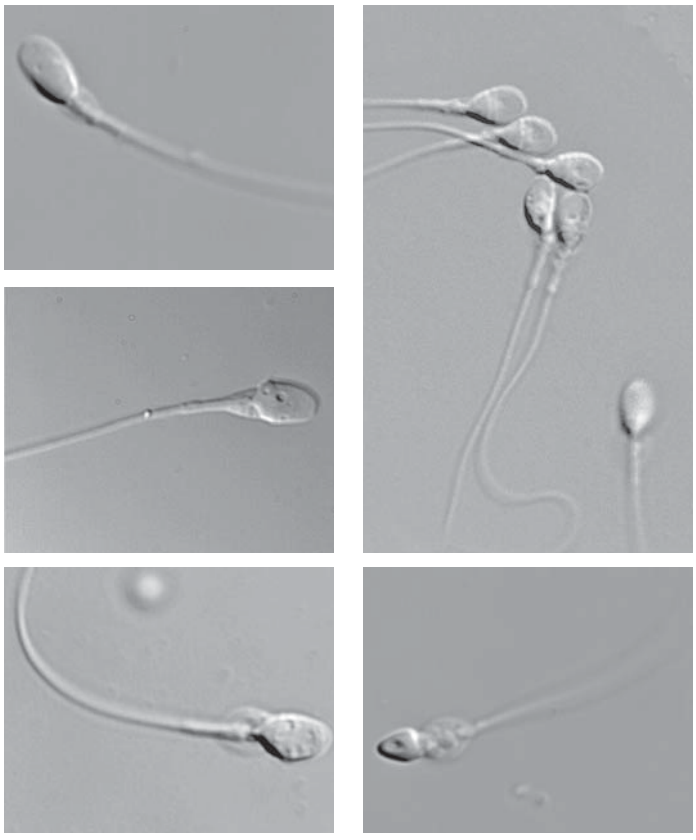

Fig. 2–6: Sperm cells with vacuoles, abnormally shaped head or connecting piece

Advantages of IMSI confirmed

To compare IMSI and IVF directly, Prof. Viville and Dr. Wittemer performed a study involving 55 couples for whom IUI treatments had failed. IVF or ICSI procedures were applied to groups of sibling oocytes. Per sample, they inspected at least 100 sperm cells at 12,500x magnification. In all, only 7.6% of all sperm were free of abnormalities. 64.9% had multiple intranuclear vacuoles or other defects.

The couples were divided into two groups: Group I (33 couples) with <8% normal sperm, and Group II (22 couples) with >8% normal sperm. In Group I, ICSI achieved a significantly higher fertilisation rate over IVF (Table 1). The success rate of ICSI was high in both groups, i.e. regardless of the share of normal sperm, resulting in 15 pregnancies. The study shows the negative influence of a large share of morphologically abnormal sperm on IVF fertilisation results. This impact of fine sperm morphology also confirms the predictable advantages of the IMSI method.

(n = 55)	Share of morphologically normal sperm	Fertilised oocytes	
		IVF	ICSI
Group I (n = 33)	<8%	38.8%	75.9%
Group II (n = 22)	>8%	65.7%	76.3%

Table 1: Influence of sperm morphology on fertilisation rate

Dr. Wittemer characterised the results to date as very promising. IMSI achieves higher pregnancy rates, the number of top-quality embryos is higher than with conventional ICSI, and fewer spontaneous abortions occur. SIHCUS-CMCO uses IMSI when conventional methods fail. The method is also the first choice in cases of pronounced sperm DNA fragmentation or severe teratozoospermia. The success rate justifies the higher time requirements for selection, which can take over two hours depending on the share of abnormal structures, as well as the exacting requirements with regard to the optical system.

Leica User Club

To address increasing interest in the IMSI method, Leica Microsystems has initiated a workgroup of Leica AM6000 users in cooperation with SIHCUS-CMCO in France. The group has nine further institutional members. In addition to regularly exchanging experiences, the participants are planning a multicentre study to systematically explore the relationships between morphological and functional sperm properties, fertilisation rates, embryo development and implantation. This will lead to an improved definition of indications and a further improvement of success rates. An image database and an iconographic atlas of sperm morphology is also being compiled. An important goal of these efforts is to establish detailed selection criteria for a standardised protocol.

Reference

This article was published in LaborPraxis, 10/2007
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Screening, Selection, Injection – All with a Single System

The Leica AM6000 Big S micromanipulation system

IMSI users need a system that offers both high resolution and flexible magnification. The high-resolution, automated Leica AM6000 Big S micromanipulation system is ideal for the purpose. The Big S configuration is compatible with all micromanipulators (Leica, AM6000, Narishige).

Users can not only screen, but also select and perform the injection with this user-friendly system. The fully automated, inverted Leica DMI6000 B research microscope features Vario-Zoom for continuous zooming to a magnification of 16,000x on the monitor. This level is sufficient to make out the fine morphology of intracellular structures in sperm in detail. The integrated electrical manipulators and most important microscope functions can be operated via the multifunction panel. The y-off function moves the manipulators in the x-direction only, simplifying injection. The z-limit function prevents downward motion and needle breakage. With its 100x IMM objective, the Leica AM6000 offers fully automatic differential interference contrast (DIC). A Leica Microsystems digital camera with a FireWire

Fig.: 1 Focus on the action: The microscope stage, objective turret, condenser, and manipulators form the heart of the Leica AM6000. They are perfectly attuned to one another for optimal software-supported interaction.

Fig.: 2 The multifunction panel controls manipulator and microscope functions such as focus, objective selection, and light intensity. As another special convenience, the magnification and contrast can be simultaneously stored and recalled on three freely programmable buttons.

The intelligent base – Leica DMI6000 B

The inverted Leica DMI6000 B digital microscope leaves nothing to be desired. The entire instrument is fully automated so that the user can concentrate on the experiment, and not on the microscope functions. The contrast and illumination manager take over all of the work when changing contrast – at the touch of a button – in transmitted light as well as in fluorescence. For detailed information, please request the Leica DMI4000 – 6000 B Series product brochure.

Leica AM6000 micromanipulators

Eppendorf's electrical micromanipulators are integrated to the complete system. In addition to proven functions such as "Home", "Clean", "Y-off", and "Limit", other functions are available that are unique to the Leica AM6000. The joystick, for example, can control the manipulators and the motorised microscope stage. Both components feature coarse and fine modes for fast travel and working with micron precision.

The key to enhanced efficiency – the multifunction panels

The entire system is controlled from multifunction panels that manage the most important microscope functions in addition to the full range of micromanipulation functions. This integrated system is your key to accelerated work processes and higher efficiency. In addition to all NK2-generation Eppendorf manipulator functions, the control panels feature focus hand wheels and function buttons to control brightness, the objective turret, and the magnification changer.

Contrast and resolution for every specimen – the new DIC

It's a familiar phenomenon when using DIC: Improved contrast results in lower resolution and vice versa. This effect increases when observing particularly thick or thin specimens. Leica offers special prism combinations for these cases: prism C for regular specimen thicknesses, C1 for especially thick, and C2 for especially thin specimens.

Take the optics into your own hands – the unique IMC

Leica Microsystems' optics experts have created an intermediate interpupillary interface. Now users have an effective, yet affordable modulation contrast solution with brightfield objectives.

Leica Big S – the unique solution for your qualified selection

System setup

- Leica DMI research microscope
- HCX PL FLUOTAR 100x/1.3 oil
- Leica DFC290 or Leica DFC360 digital camera
- PC Station with measurement and evaluation software

Advantages at a glance

- Excellent Interference contrast – Discover the fine structure of living sperm
  Superb objective HCX PL FLUOTAR 100x/1.3 oil – Perfect resolution and image correction
- Continuous increase and decrease of magnification without changing objectives –
  Keep your orientation and your sperm (edge of the drop)!
- Magnify sperm approx. 16,000 x on a 19" monitor – The right image ratio for easier and convenient evaluation
- Ergonomic and functional setup – Simple handling, all functions in range
- Leica digital cameras with high refreshing rate – Brilliant life image
- Leica evaluation software – Measure and qualify sperm morphology
- Adaptable to all Leica DMI microscopes and micromanipulator setups – Configure your station to your needs and taste
- Leica AM6000 Big S association – Workflow optimised system to control microscope and micromanipulator simultaneously
  ICSI and IMSI on one station

The Perfect Partner for Micromanipulation

The Leica DMI3000 B inverted microscope

Micromanipulation must be properly configured on a microscope system. Leica Microsystems offers an integrated solution consisting of a Leica DMI3000 B inverted microscope and Narishige micromanipulation equipment.

Featuring the largest number of transmitted light illumination options and a condenser with extra long working distance, the Leica DMI3000 B is ideal for all transmitted light contrast techniques. Leica Microsystems offers five manual condensers with different working distances for brightfield, phase contrast, darkfield, modulation contrast, polarisation or differential interference contrast (DIC)

High contrast and resolution for every specimen

It's a familiar phenomenon when using DIC: improved contrast results in lower resolution and vice versa when observing specimens that are unusually thick or thin. Leica Microsystems offers special prism combinations for viewing 'normally' thick, especially thick, and especially thin specimens.

Condensers for every contrast technique

Leica Microsystems has added a special condenser for micromanipulation with a free working distance of 40 mm to its wide selection of condensers. This condenser is suitable for conventional phase and modulation contrast as well as for IPH and IMC. A choice of five different condensers with different working distances (1, 23, 28, 40 and 70 mm) is available.

Variety of stages

The microscope can be configured with any one of a wide variety of. 3-plate cross stages and motorised stages A special development for the Leica DMI3000 B: an extra-narrow, 3-plate cross-stage for micromanipulation that provides ample room for accessories.

Integrated solution for micromanipulation applications

Leica DMI3000 B inverted microscope

- The micromanipulation stage with its slim design allows easy adaptation of micromanipulators.
- At 28 mm, the S28 condenser provides ample working distance for micromanipulation using all contrast techniques. If more working distance is required, the S40 condenser with 40 mm working distance is available for all contrast techniques except DIC; and the S70 condenser with 70 mm working distance is available for all contrast techniques except IMC/IPH.
- Two different transmitted light arms are available:
  – 12 V/100 W, which adapts to all Leica lamp houses
  – 12 V/30 W with integrated lamp house

Narishige micromanipulators

- Narishige's new micromanipulators feature a compact, rugged design for stability.
- The short distance to the tip of the pipette minimises vibration.
- The micromanipulators can be universally deployed on both sides.
- The hanging joystick provides a relaxed hand position and sensitive control.

Advanced Analysis Tools for Cytogeneticists

The Leica CW4000 CytoFISH

The Leica CW4000 CytoFISH provides fluorescence image acquisition and advanced analysis tools for cytogeneticists. It includes the ability to count FISH signals in interphase cells and to perform a ratio of the count of the number of two different colour FISH spots.

Leica CW4000 is fully compatible with the Leica Digital Microscopes Leica DM4000 – DM6000 B and Leica Digital FireWire Cameras. The user can capture combined brightfield and fluorescence images to view FISH images (Chromogenic In-Situ Hybridisation) and combined immunostaining with FISH experiments.

Leica CW4000 CytoFISH offers a range of easy to use tools to adjust the contrast, colour and definition of objects in the acquired images – these can be applied to either the full image or one or more areas of interest. The modular system includes a full range of Karyotyping, FISH, CGH and MFISH options, providing outstandingly accurate, detailed and high quality images and data.

IMPRINT

Publisher
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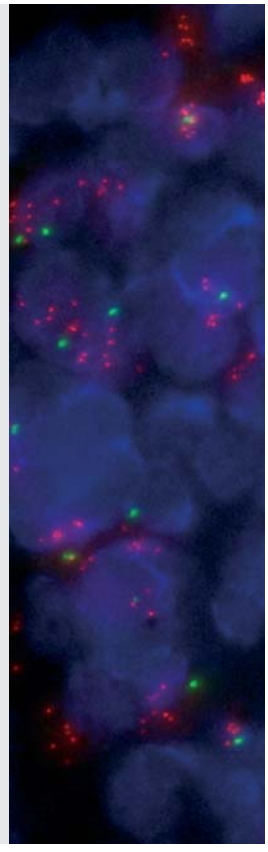
Leica Microsystems

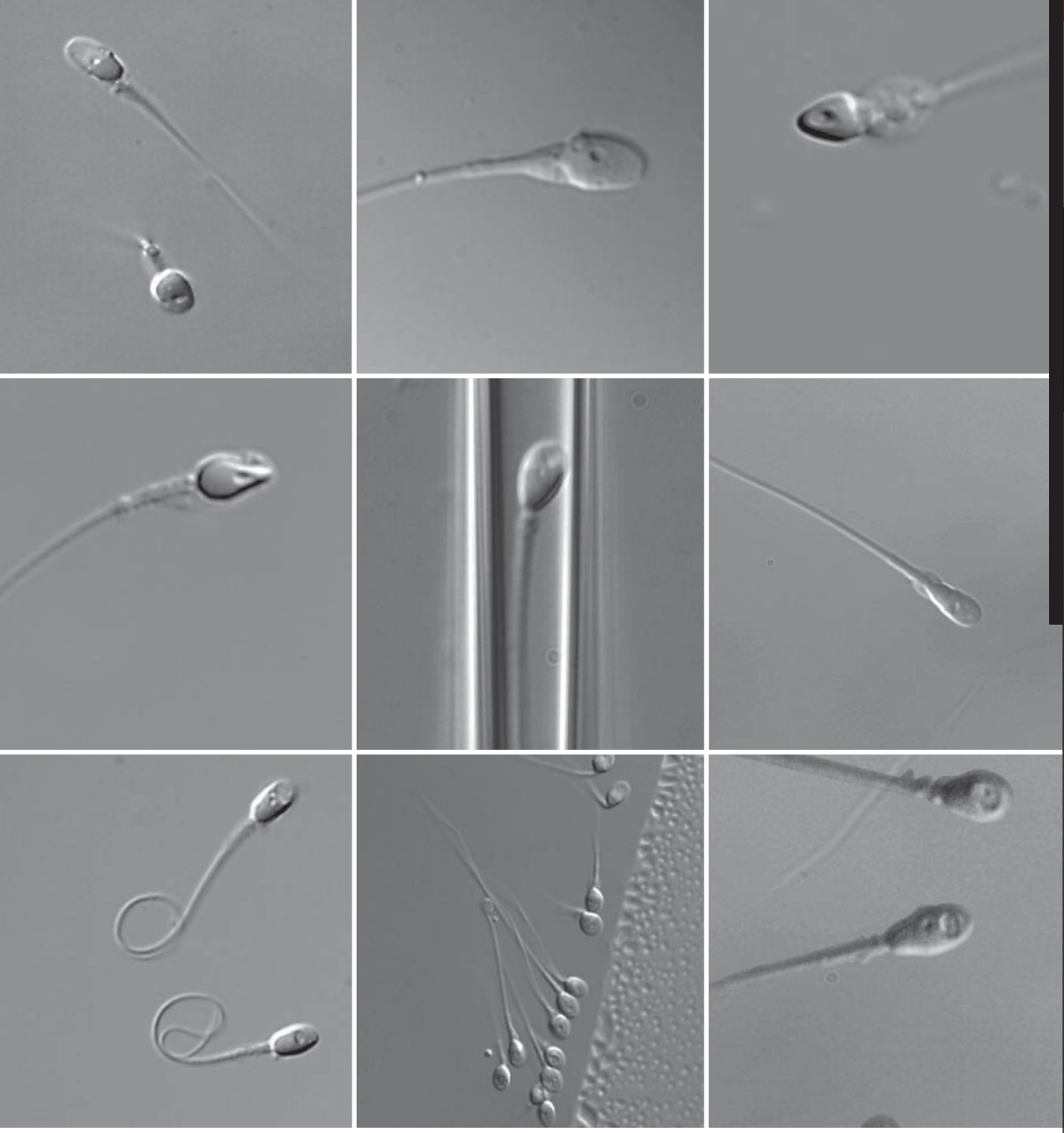
Printing Date
June 13, 2008

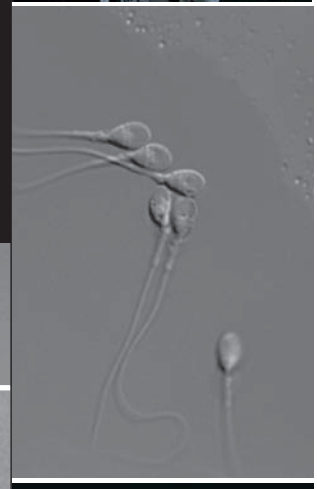
Order no.: **1924115**

The Leica CW4000 CytoFISH – advantages at a glance

- Acquisition facilities for up to 16 image planes, allowing the capture of standard FISH, MFISH and CGH images.
- The autoexposure facility can be used to provide a 'one touch' fully automatic capture sequence.
- Compatibility with Leica Digital FireWire Cameras (DFC). Either cooled or non-cooled cameras can be used, producing high resolution FISH images.
- Either capture a region of interest in the camera field of view, or capture using the entire CCD array for the highest spatial resolution. Binning can optionally be used to increase sensitivity.
- There is no need to threshold images to generate composite images - this removes the possibility of excluding faint 'real' signals from the image.
- Full compatibility with karyotyping applications to allow flexible karyotyping of 2, 3 or multicolour images.







Don‘t Let Them Zoom Off!

Zoom in – without losing the sperms from view and make your selection

- Evaluate sperm morphology with a magnification of up to 16,000x
- Perform both IMSI and ICSI on one single workstation

www.leica-microsystems.com/eu

Living up to Life

Leica
MICROSYSTEMS