Title: IMPROVED INVERTED LIGHT OPTICAL MICROSCOPE

Abstract: The present invention concerns an improved inverted light optical microscope, comprising at least one light source (1), apt to generate white light following at least one optical path having at least one first section (p1) and at least one second section (p2), the light being diverted from the first to the second section through first optical means (7), at least one supporting plane (4), apt to house at least one specimen (3) illuminable by the light generated by said at least one source (1), and at least one objective (5) being placed in succession along said at least one first section (p1) of said at least one optical path, second optical means (9), apt to allow an operator (10) to observe at least one image of at least one specimen (3), being placed at the end of said at least one second section (p2) of said at least one optical path, the microscope being characterised in that it comprises, after said at least one source (1) and before said at least one supporting plane (4), light polarising optical means (11) apt to allow light polarised according to a polarisation direction to pass.
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
IMPROVED INVERTED LIGHT OPTICAL MICROSCOPE

The present invention concerns an improved inverted light optical microscope, applicable in particular to the manipulation of cells and living cellular aggregates, as for instance in the micro-injection of sperms into the oocyte or ICSI (Intra Cytoplasmic Sperm Injection), that is reliable and efficient, allowing an extremely accurate observation of the ultrastructural and functional properties of cells and cellular aggregates and of their sub-cellular organelles, and an improved selection of cells and cellular aggregates to be manipulated.

In the following of the description, explicit reference will be made to the application of the microscope according to the invention to the ICSI manipulation technique. However, it should be understood that the microscope according to the invention may be applied to any type of observation or manipulation of cells, cellular aggregates, microorganisms, and living tissues, as for instance in always in-vivo monitoring of the embrional development by examining its protoplasma depth.

It is known that in manipulation techniques of cells, cellular aggregates, microorganisms, and living tissues the so-called inverted optical microscopes are used, which allow operating on cells and cellular aggregates immersed in solutions held in suitable small transparent containers in substantially natural conditions.

With reference to Figure 1, it may be observed that a conventional inverted light optical microscope comprises a light source 1 that illuminates, through a condenser 2, a specimen 3 placed on a supporting plane 4. Light follows a first section of an optical path, indicated in the Figure by the short dashes line p1, which, after the specimen 3, continues through an objective 5 followed by a first optical unit 6, preferably comprising an image forming lens and a transmission prism. After the first optical unit 6, light meets a mirror 7 that reflects it upwards along a second section of the optical path indicated by the short dashes line p2. The image of the specimen 3 forms on an image surface, intersecting such second section p2 at the point 8. Afterwards, light continues through a second optical unit 9 of magnification and observation (usually the so-called eyepiece), preferably comprising an image magnifying device for allowing an operator 10 to observe the magnified image of the specimen 3. In particular, the first optical unit 6 may comprise further optical devices, for instance devices apt to make the image of the
specimen be captured by a CCD camera for showing it on a suitable display.

However, conventional inverted light optical microscope presents some drawbacks.

In fact, it allows a rather reduced resolution of the observed images, generally ranging from 40x to 60x with conventional objectives, increaseable up to 100x through the use of special extremely expensive oil immersion objectives, which do not allow manipulation of living specimens observing the internal ultrastructure.

This may greatly limit the work of an operator who is observing cells and cellular aggregates.

In case of ICSI, observation by inverted light optical microscope allows discriminating the quality of sperms substantially only on the basis of their motility. However, the quality of sperms is revealed not only by their motility, but it is also defined by the state of their internal sub-cellular organelles. Therefore, through inverted light optical microscope, the operator is not capable to correctly discriminate sperms having comparable motility but different ultra-structural properties within the protoplasm.

Presently, it is possible to observe the interior of a cell, such as a sperm, through electron microscopes. However, the latter are not applicable to ICSI manipulation, since the specimen to be observed by the (both transmission and scanning) electron microscope must be suitably prepared in order to be placed in a vacuum chamber, consequently rendering all cells and cellular aggregates present within the specimen no longer living.

It is therefore an object of the present invention to provide an improved inverted light optical microscope, that is reliable and efficient, and that allows a highly accurate observation of the structural and functional properties of cells and cellular aggregates, and of their sub-cellular organelles, allowing as a consequence an improved selection of cells and cellular aggregates to be manipulated.

It is therefore specific subject matter of the present invention an improved inverted light optical microscope, comprising at least one light source, apt to generate white light following at least one optical path having at least one first section and at least one second section, the light being diverted from the first to the second section through first optical
means, at least one supporting plane, apt to house at least one specimen illuminable by the light generated by said at least one source, and at least one objective being placed in succession along said at least one first section of said at least one optical path, second optical means, apt to allow an operator to observe at least one image of at least one specimen, being placed at the end of said at least one second section of said at least one optical path, the microscope being characterised in that it comprises, after said at least one source and before said at least one supporting plane, light polarising optical means apt to allow light polarised according to a polarisation direction to pass.

Always according to the invention, said improved inverted light optical microscope may further comprise, along said at least one optical path after said at least one supporting plane, analysing optical means, oriented so as to be apt to allow light polarised according to a second polarisation direction, orthogonal to the polarisation direction of the polarising optical means, to pass.

Still according to the invention, the analysing optical means may be placed along said at least one first section of said at least one optical path.

Furthermore according to the invention, the analysing optical means may be placed along said at least one second section of said at least one optical path.

Always according to the invention, said improved inverted light optical microscope may further comprise, along said at least one optical path after the polarising optical means and before the analysing optical means, compensating optical means, oriented so as to be apt to introduce a phase difference between two light rays at the same frequency which are polarised according to respective orthogonal polarisation directions.

Still according to the invention, the compensating optical means may be placed along said at least one first section of said at least one optical path.

Furthermore according to the invention, the compensating optical means may be placed along said at least one second section of said at least one optical path.

Always according to the invention, said at least one supporting plane may be rotating round a rotational axis parallel to an axis of said at least one first section of said at least one optical path.
Still according to the invention, the second optical means may be apt to magnify said at least one image of at least one specimen.

Furthermore according to the invention, said improved inverted light optical microscope may further comprise, along said at least one optical path, transmission optical means.

Always according to the invention, said improved inverted light optical microscope may further comprise, along said at least one optical path, image forming optical means.

Still according to the invention, said improved inverted light optical microscope may further comprise, along said at least one optical path, optical means for capturing at least one image of at least one specimen and for displaying said at least one captured image on displaying means.

Furthermore according to the invention, said capturing optical means may comprise at least one CCD camera.

The present invention will now be described, by way of illustration and not by way of limitation, according to its preferred embodiments, by particularly referring to the Figures of the enclosed drawings, in which:

Figure 1 schematically shows a conventional inverted light optical microscope;

Figure 2 schematically shows a first embodiment of the inverted light optical microscope according to the invention; and

Figure 3 schematically shows a second embodiment of the inverted light optical microscope according to the invention.

In the Figures, alike elements are indicated by the same reference numbers.

Figure 2 shows a first embodiment of the improved inverted light microscope according to the invention. It comprises a light source 1, apt to generate white light, that illuminates, through a polariser 11 and a condenser 2 in cascade, a specimen 3 placed on a supporting plane 4.

Light follows the first section p1 of an optical path that passes, after the specimen 3, through an objective 5. After the objective 5, the microscope comprises a compensator 12 and an analyser 13. In particular, the analyser 13 is oriented so that the polarisation directions of the polariser 11 and of the analyser 13 (i.e. the vibrational directions of the light that is allowed to pass by the polariser 11 and by the analyser 13) are orthogonal
to each other. Afterwards, along the first section p1 of the optical path, a first optical unit 6 is present, preferably comprising an image forming lens and a transmission prism. After the first optical unit 6, light meets a mirror 7 that reflects it upwards along a second section p2 of the optical path. The image of the specimen 3 forms on an image surface intersecting such second section p2 at the point 8. Afterwards, light continues through a second optical unit 9 of magnification and observation, preferably comprising an image magnifying device for allowing an operator 10 to observe the magnified image of the specimen 3. In particular, the first optical unit 6 may comprise further optical devices, for instance devices apt to make the image of the specimen 3 be captured by a CCD camera for showing it on a suitable display.

The microscope according to the invention profits from the fact that the rod structure internal to sperms, when it is complete, is birefringent, and it has a stretching birefringence (nucleus, acrosome) and an intrinsic birefringence since it is a mixed body according to Wiener. Therefore, by observing the refractive characteristics of the sperms, it is possible to evaluate its both internal and superficial ultrastructural properties, in vivo and in motion.

In fact, when the white light generated by the light source 1, that is composed of waves having various frequencies within a wide spectrum, passes through the polariser 11, the latter lets only waves having the vibrational plane parallel to its polarisation direction pass. Therefore, light illuminating the specimen 3 vibrates only along this vibrational plane.

The birefringence of the elements immersed within the specimen 3, such as the sub-cellular organelles (i.e. the rods) of sperms immersed within a solution held in a container, makes polarised light illuminating one of such elements be subdivided into two rays vibrating perpendicularly to each other and propagating through such element at different velocities. When the two rays are recombined into a single ray by passing through the analyser 13, they interfere either destructively or constructively, depending on the phase difference produced by the birefringent element through which they have passed and on the frequency of the specifically considered light wave. In other words, when the same birefringent element is illuminated by polarised white light, the phase difference produced by it varies depending on the wave frequency (being part of the white light spectrum) that is considered.
The light that is observed after the analyser 13 is a coloured light, indicative of the birefringence properties of the specific crossed element within the specimen 3, and hence indicative of the structural properties of such element.

In the case when the birefringence of the elements immersed within the specimen 3 is not sufficient for generating interference colours after the analyser 13, the compensator 12 adds (or subtracts) a further phase difference between the two rays in which the wave passing through a birefringent element is split. In particular, the compensator 12 is oriented so that the vibrational directions of the light (which the compensator 12 allows to pass) are aligned at 45° between those mutually orthogonal of the polariser 11 and of the analyser 13. In this regard, the compensator 12 may also be placed before the specimen 3, provided that it is located between the polariser 11 and the analyser 13 along the optical path of the light.

Preferably, the condenser 2 is provided with a mechanical motion system so as to focus the light illuminating the specimen 3 on an adjustable height within the specimen 3.

Advantageously, the supporting plane 4 of the microscope of Figure 2 is rotating, so that the interference colours produced by the birefringence of an element immersed within the specimen 3 may change depending on the orientation of the same element modified by the operator 10 by making the plane 4 rotate.

Through the microscope of Figure 2, an operator 10, who carries out an ICSI manipulation by observing a specimen 3 of solution containing sperms, is capable to evaluate both their motility and the integrity of their internal rod structure, being therefore capable to select sperms of better quality.

Figure 3 shows a second embodiment of the microscope according to the invention, in which the compensator 12 and the analyser 13 are placed along the second section p2 of the optical path, i.e. after the mirror 7. In this case, the first optical unit 6 is preferably replaced with a first optical sub-unit 6A, comprising a transmission prism, placed along the first section p1 of the optical path, and with a second optical sub-unit 6B, comprising an image forming lens, placed along the second section p2 of the optical path after the analyser 13.
The preferred embodiments have been above described and some modifications of this invention have been suggested, but it should be understood that those skilled in the art can make other variations and changes, without so departing from the related scope of protection, as defined by the enclosed claims.
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CLAIMS

1. Improved inverted light optical microscope, comprising at least one light source (1), apt to generate white light following at least one optical path having at least one first section (p1) and at least one second section (p2), the light being diverted from the first to the second section through first optical means (7), at least one supporting plane (4), apt to house at least one specimen (3) illuminable by the light generated by said at least one source (1), and at least one objective (5) being placed in succession along said at least one first section (p1) of said at least one optical path, second optical means (9), apt to allow an operator (10) to observe at least one image of at least one specimen (3), being placed at the end of said at least one second section (p2) of said at least one optical path, the microscope being characterised in that it comprises, after said at least one source (1) and before said at least one supporting plane (4), light polarising optical means (11) apt to allow light polarised according to a polarisation direction to pass.

2. Improved inverted light optical microscope according to claim 1, characterised in that it further comprises, along said at least one optical path after said at least one supporting plane (4), analysing optical means (13), oriented so as to be apt to allow light polarised according to a second polarisation direction, orthogonal to the polarisation direction of the polarising optical means (11), to pass.

3. Improved inverted light optical microscope according to claim 2, characterised in that the analysing optical means (13) is placed along said at least one first section (p1) of said at least one optical path.

4. Improved inverted light optical microscope according to claim 2, characterised in that the analysing optical means (13) is placed along said at least one second section (p2) of said at least one optical path.

5. Improved inverted light optical microscope according to any one of claims 2 to 4, characterised in that it further comprises, along said at least one optical path after the polarising optical means (11) and before the analysing optical means (13), compensating optical means (12), oriented so as to be apt to introduce a phase difference between two light rays at the same frequency which are polarised according to respective orthogonal polarisation directions.

6. Improved inverted light optical microscope according to claims 4 and 5, characterised in that the compensating optical means (12)
is placed along said at least one first section (p1) of said at least one optical path.

7. Improved inverted light optical microscope according to claims 4 and 5, characterised in that the compensating optical means (12) is placed along said at least one second section (p2) of said at least one optical path.

8. Improved inverted light optical microscope according to any one of the preceding claims, characterised in that said at least one supporting plane (4) is rotating round a rotational axis parallel to an axis of said at least one first section (p1) of said at least one optical path.

9. Improved inverted light optical microscope according to any one of the preceding claims, characterised in that the second optical means (9) is apt to magnify said at least one image of at least one specimen (3).

10. Improved inverted light optical microscope according to any one of the preceding claims, characterised in that it further comprises, along said at least one optical path, transmission optical means (6, 6A).

11. Improved inverted light optical microscope according to any one of the preceding claims, characterised in that it further comprises, along said at least one optical path, image forming optical means (6, 6B).

12. Improved inverted light optical microscope according to any one of the preceding claims, characterised in that it further comprises, along said at least one optical path, optical means (6) for capturing at least one image of at least one specimen (3) and for displaying said at least one captured image on displaying means.

13. Improved inverted light optical microscope according to claim 12, characterised in that the said capturing optical means (6) comprises at least one CCD camera.
Fig. 1
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

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According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Date of the actual completion of the international search: 24 June 2005

Date of mailing of the international search report: 08/08/2005

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 Hl Pipoewijk Tel. (+31-70) 340-2040, Tx. 31 651 epi nl Fax: (+31-70) 340-3016

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