APPLICATION NOTE

Inverted microscope ECLIPSE Ti2-I

The Ti2-I inverted microscope empowers microinsemination with sophisticated optical performance and operability

Intracytoplasmic sperm injection (ICSI) is a microinsemination technique in which sperm cells are manually injected into egg cells. These oocytes can be visualized with NAMC (Nikon Advanced Modulation Contrast), which allows observation of transparent samples with relief-like contrast. In addition, Nikon's spindle observation system displays oocyte meiotic spindles in red or blue, helping to reduce the chance of them being mistaken for other intracellular structures or foreign matter. The spindle is consistently highlighted regardless of XY orientation. Another technique called intracytoplasmic morphologically-selected sperm injection (IMSI) requires a high magnification objective lens and Differential Interference Contrast (DIC) to select high-quality sperm based on morphology. Thus, the microscope plays a very important role in microinsemination. However, fluidly switching between bright field, NAMC, spindle observation, and DIC (IMSI) with a manual microscope is complicated and gaining proficiency takes time.

In this application note, we introduce the ECLIPSE Ti2-I inverted microscope, which can easily switch between bright field, NAMC, spindle observation and DIC.

Keywords : relief contrast, microinsemination, Polarization, Differential Interference Contrast (DIC) , ICSI, IMSI, spindle

Spindle observation

The meiotic spindle plays an essential role in the normal distribution of chromosomes during meiosis. Because spindles are not always visible in all oocytes, they are a useful indicator of oocyte quality and maturity. The spindle is often localized just below the polar body, but the polar body may be moved by pipetting or the like, so this localization is not always maintained. Thus, it is very important to observe the shape and position of the spindle to perform microinsemination without damage ([1], [2]).

However, the meiotic spindle cannot be seen with the typical contrast method for oocyte observation (Fig. 1).

Therefore, polarization is needed to observe the spindle. Nikon's Spindle Observation system illuminates the sample with circularly polarized light, which takes on a red or blue tinge as it passes through the spindle (Fig. 2). This technique works for any oocyte XY orientation (Fig. 2a, blue arrow and Fig. 2b). In addition, the red and blue colors change every 90 degrees, which can be shifted based on personal preference by turning the polarizer at the top of the microscope in the transmitted lightpath (Fig. 2c).

[1] Relationship between pre-ICSI meiotic spindle angle, ovarian reserve, gonadotropin stimulation, and pregnancy outcomes. *J Assist Reprod Genet*. 2017 May; 34(5): 609–615. Alina M. Mahfoudh *et al*.

[2] Egg maturity assessment prior to ICSI prevents premature fertilization of late-maturing oocytes. *J Assist Reprod Genet*. 2019 Mar; 36(3): 445–452. Zuzana Holubcová *et al*.

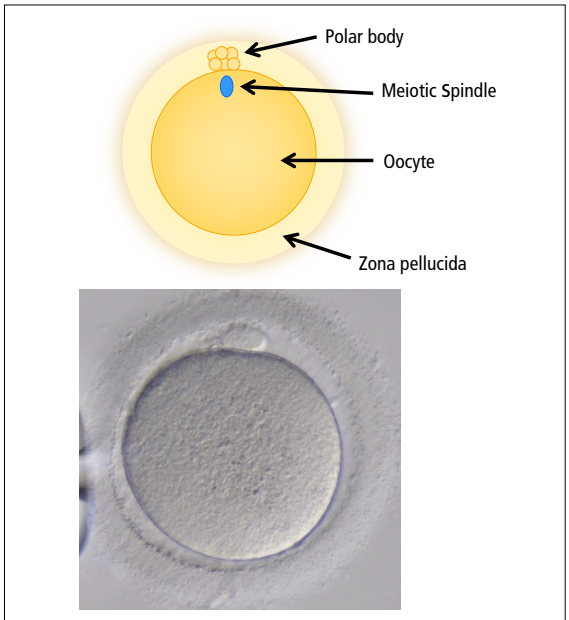


Fig. 1. Overview and NAMC image of the oocyte

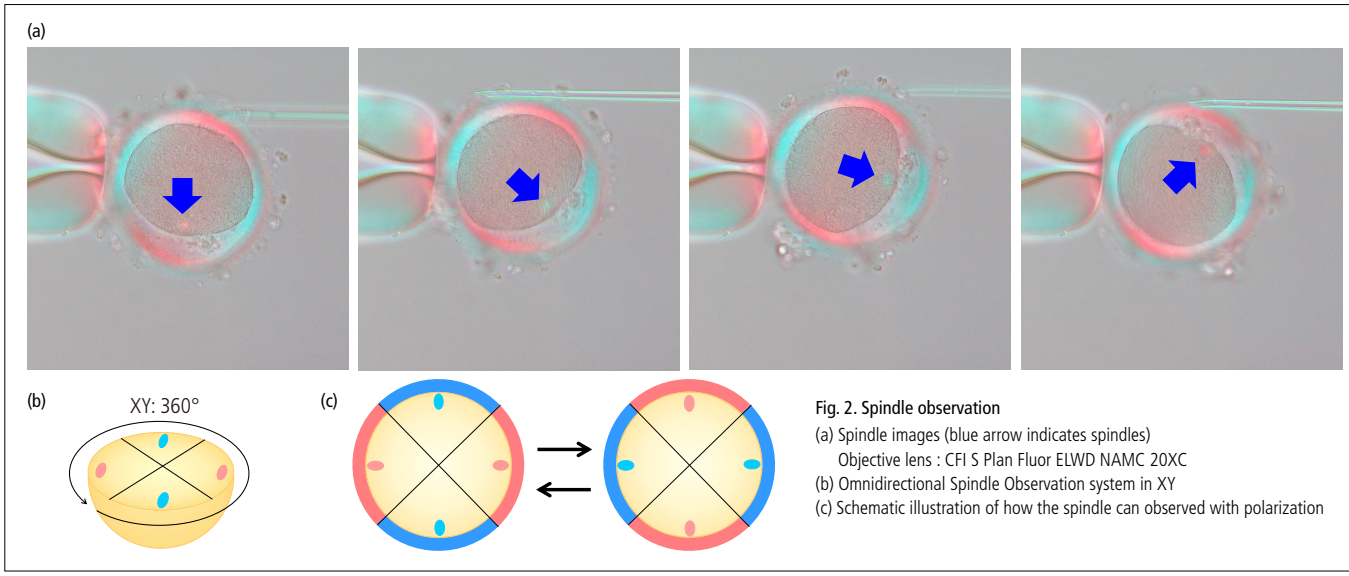


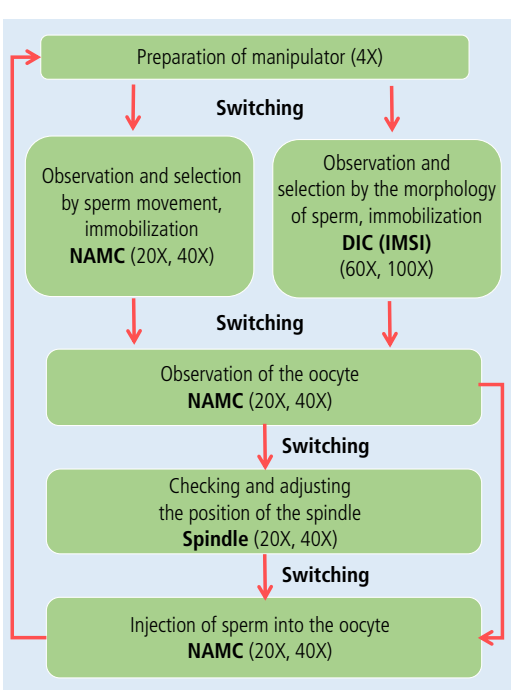
Fig. 2. Spindle observation
(a) Spindle images (blue arrow indicates spindles)
Objective lens : CFI S Plan Fluor ELWD NAMC 20XC
(b) Omnidirectional Spindle Observation system in XY
(c) Schematic illustration of how the spindle can observed with polarization

Automated observation mode and contrast technique switching

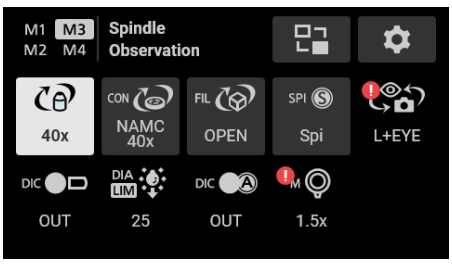
The Ti2-I is mainly operated using the front panel and physical buttons of the microscope body, which also indicate lightpath components. It does not require a remote controller, and can be easily operated by hand while visually confirming that the correct components and settings are in place for each particular observation mode or contrast technique while performing a typical microinsemination workflow (Fig. 3).

Contrast technique settings can be registered and automatically selected using the front panel of the microscope: Brightfield (Fig. 3 upper right: "Initial Preparation" in the front panel and button), NAMC (Fig. 3 upper right: "Oocyte Observation" in the front panel and button), spindle observation (Fig. 3 upper right: "Spindle Observation" in the front panel and button) and DIC (Fig. 3 upper right: "IMSI" in the front panel and button). Once registered, the mode, objective lens, optical modules, etc. will automatically engage with one click (lower right of Fig. 3). The microscope is easy to operate even when wearing gloves or looking through binoculars. An alert function indicates when a component is in a different state than when it was set (Fig. 3, lower left). The alert shown in Fig. 3 indicates that the optical path and intermediate magnification have changed.

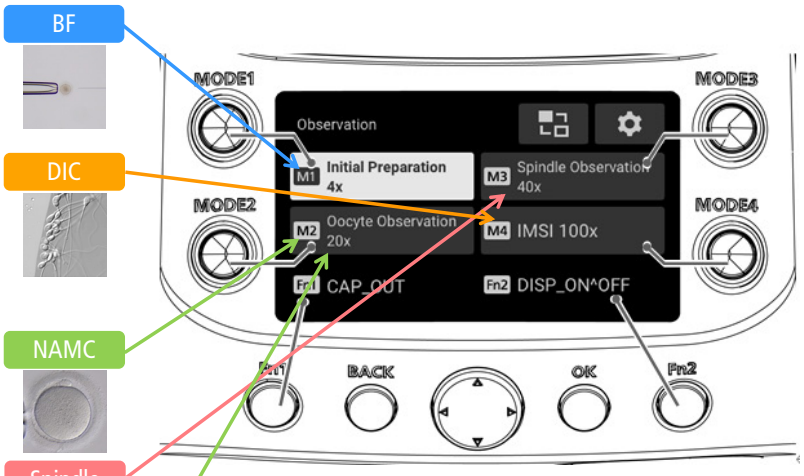
Furthermore, a light intensity management (LIM) function automatically adjusts the light intensity to maintain a constant brightness even when changing contrast techniques.



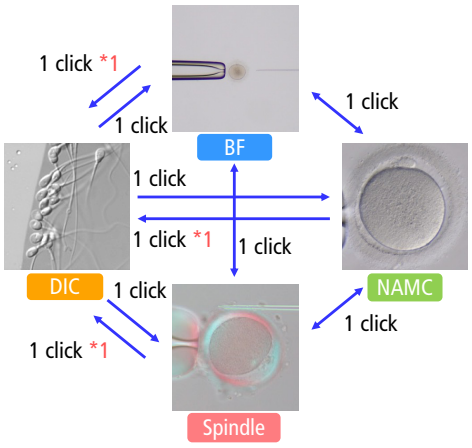
Workflow



Alert display



Front panel and button



Switching observation methods

*1: Only when switching to IMSI will the anti-collision warning appear and the MOVE button must be pressed.
But if the warning is canceled, you can operate with one click.

Fig. 3. An example microinsemination workflow and Ti2-I operation/display panel

Summary

Microinsemination involves a combination of various observation methods such as brightfield, NAMC, DIC and polarization. However, switching between these techniques is cumbersome, even on conventional microscopes with electric motors.

With Ti2-I, by registering the observation method as a selectable mode, the registered state can be reproduced with one click to quickly and consistently switch between contrast techniques and observation modes.

Ti2-I supports the simplification and streamlining of complex microinsemination workflows.

Product Information

Inverted microscope ECLIPSE Ti2-I

The inverted microscope ECLIPSE Ti2-I supports microinsemination.

By registering the observation methods used in microinsemination, such as bright field, NAMC (for ICSI), DIC (for IMSI) and polarization (for the spindle), as modes, it is possible to reproduce the registered conditions with one click.

The operation and confirmation functions are mounted on the front of the main body, making it possible to perform all tasks with fewer eye and hand movements.

Click here for detailed product information.