生殖補助医療技術におけるICSI/IMSIと紡錘体観察

三宅範夫

ICSI/IMSI and Spindle Observation in Assisted Reproductive Technology†

Norio MIYAKE

生殖補助医療技術（ART: Assisted Reproductive Technology）の役割と，それを支える顕微鏡技術について紹介する．不妊症のカップルにとってARTは重要な選択肢であり，日本では2022年4月の不妊治療保険適用の開始以降，ARTによる新生児の割合が増加している．

ARTは，卵子や精子，受精胚を扱い妊娠を支援する医療技術であり，顕微鏡を使用する体外受精（IVF）や卵細胞質内精子注入法（顕微授精，ICSI），卵細胞質内形態選別精子注入法（IMSI）などを含んでいる．ニコンはARTの各ステップに対応する正立顕微鏡，実体顕微鏡，倒立顕微鏡を提供しており，ARTプロセスを支援している．例えば，正立顕微鏡は精子の運動性や形態の観察に適しており，実体顕微鏡は卵子や胚の立体的な観察を可能にする．一方，倒立顕微鏡はICSI/IMSIの際に精子や卵子の詳細な観察を行い，Nikon Advanced Modulation Contrast（NAMC）や微分干渉観察によりコントラストを向上させる．また，紡錘体観察では円偏光を利用し，卵子内の紡錘体の配置を把握することで受精率を高めている．

これらの光学技術は，ARTプロセスの効率化と精度向上に貢献しており，医療現場での負担軽減と治療成果の向上につながっている．ニコンはこれらの技術開発を通じて，社会的課題を解決するため取り組んでいる．

This article introduces the role of assisted reproductive technology (ART) and the microscopic techniques supporting it. ART is an important option for couples facing infertility. In Japan, since the implementation of insurance coverage for infertility treatments in April 2022, the proportion of newborns conceived through ART has been increasing. ART encompasses medical techniques that assist pregnancy by handling eggs, sperm, and embryos, including in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and intracytoplasmic morphologically selected sperm injection (IMSI), which rely on microscopes for precision.

Nikon provides specialized microscopes, including upright, stereo, and inverted microscopes, tailored for each step of the ART process. Upright microscopes are ideal for analyzing sperm motility and morphology, whereas stereo microscopes enable three-dimensional observation of eggs and embryos. Inverted microscopes facilitate detailed observation during ICSI and IMSI procedures and use technologies such as Nikon advanced modulation contrast and differential interference contrast to enhance image clarity. Furthermore, spindle observation using circular polarization helps to identify spindle positioning within the eggs, which improves the success rates of fertilization.

These optical technologies increase the efficiency and accuracy of ART processes, reduce the burden on medical professionals, and improve the treatment outcomes. Nikon contributes to addressing the societal challenges through these innovations.

Key words 生殖補助医療技術，体外受精，顕微授精，IMSI, 紡錘体観察
assisted reproductive technology, in-vitro fertilization, intracytoplasmic sperm injection, intracytoplasmic morphologically selected sperm injection, spindle observation

1 Introduction

Declining birthrates have become a serious challenge not only in Japan but also in many other countries. The continuing decline in birthrates has social and economic impacts, including a decrease in the working population, the collapse of social security systems, and stagnation of economic growth. Various factors have been cited as causes of the declining birthrate, including the economic burdens of child rearing and education, lifestyle changes resulting from the

† The products introduced in this article are available in many countries worldwide; however, the technical explanations provided here are based on Japanese regulations. Please note that the availability and primary intended use may vary depending on your region/country.

increased women's participation in the workforce, and a rise in infertility attributable to environmental and health-related issues. In particular, for couples who wish to have children but find natural conception difficult, assisted reproductive technology (ART) represents an important option. In Japan, the total fertility rate has been steadily declining over the past 40 years [1]. Meanwhile, the proportion of newborns conceived through ART has been increasing. Furthermore, since April 2022, infertility treatments have been covered by insurance, and the number of general infertility treatments, such as artificial insemination, as well as ART, such as in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), is expected to continue increasing in the future.

Throughout the history of assisted reproductive technology, Nikon has consistently collaborated with academia and medical institutions to develop groundbreaking products rooted in the needs of clinical practice. In recent years, aiming to reduce the workload of embryologists who support assisted reproductive technology, Nikon has been providing microscopes specialized for use in micro-insemination [2]. This paper presents an overview of ART and the microscopic techniques that support it.

2 Assisted Reproductive Technology (ART)

ART refers to medical technologies for treating infertility and supporting pregnancy and is a collective term encompassing all treatments and methods that involve handling human eggs and sperm, as well as the embryos fertilized from them, to achieve pregnancy. Even when the cause of infertility lies with the male partner, female partner, or both, pregnancy can be pursued through the use of appropriate techniques. A wide range of causes, including ovulatory disorders, tubal obstruction, endometriosis, reduced sperm quality, and immunological infertility, can be addressed. By employing IVF, ICSI, or intracytoplasmic morphologically selected sperm injection (IMSI)—all of which rely heavily on microscopy—it is possible to increase the likelihood of pregnancy even when sperm motility or count is low.

The main steps of ART are illustrated in Fig. 1. The ART cycle begins with the selection of sperm collected from the patient, followed by the selection of eggs, fertilization, and embryo culture, proceeding through various diagnostic and supportive processes until the embryos are returned to the patient's body for implantation.

The diameter of an egg is approximately 0.1 mm, while that of a sperm is approximately 0.05 mm. Given that the thickness of a single human hair is generally approximately

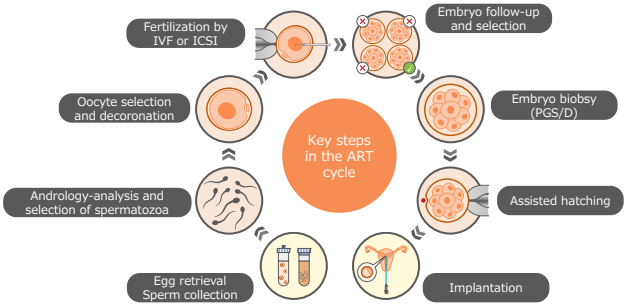

Fig. 1 ART cycle

0.05 mm to 0.1 mm, it is easy to visualize their size by considering them to be on a similar scale. In these main steps of ART, because extremely small eggs and sperm are handled, microscopes are employed in various workflows as tools to support observation.

3 Microscopes Utilized in the ART Cycle

From sperm collection and egg retrieval to fertilization and, ultimately, the selection of embryos for implantation, microscopic observation plays an extremely important role in ART. Nikon offers a full product lineup covering upright, stereo, and inverted microscopes, all of which are used in the major processes of ART. Here, the characteristics of the microscopes used are described along with the corresponding steps in the process.

3.1. Upright Microscope

In the "sperm analysis and selection" stage of the ART cycle, phase contrast observation using an upright microscope [3] is employed to determine whether the sperm are functioning properly (Fig. 2).

Fig. 2 Upright microscopes: ECLIPSE Si (left) and ECLIPSE Ci-L plus (right)

Phase contrast observation is a technique that imparts light–dark contrast to colorless and transparent specimens for visualization. It offers high detection sensitivity for thin specimens and is well-suited to observing sperm. Mean-

while, for thicker cells, the halo effect appears around their contours, making them difficult to observe; therefore, it is not suitable for observing eggs.

Evaluation parameters for sperm include motility, the numbers of motile and immotile sperm, sperm concentration, and morphology, all of which can be identified at relatively low magnification. The presence of abnormalities is considered to be associated with a reduced success rate of in-vitro fertilization.

3.2. Stereo Microscope

Equipped with independent zoom optical systems for the left and right eyes, a stereo microscope views an object from different angles on each side, producing differences (parallax) between the images perceived by the two eyes. The brain processes this parallax, enabling the perception of an object's depth and three-dimensional form. Because it allows three-dimensional observation without modifying the specimen, it is well suited for precision work. For this reason, stereo microscopes are used in "egg selection" and "embryo culture and selection" (Fig. 3). After retrieval, the eggs are examined, and those suitable for fertilization are selected. Mature eggs have a high potential for developing into normal embryos. Morphological characteristics such as egg size, morphology of the polar body, and thickness of the zona pellucida are important.

Fig. 3 Stereo microscopes: SMZ1270 (left) and SMZ800N (right)

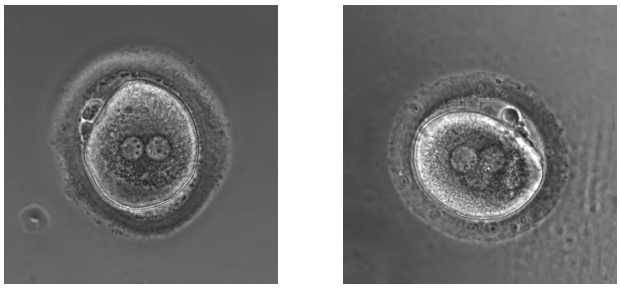

Fig. 4 Example of multinucleation: normal embryo with two pronuclei (left) and abnormal embryo with three pronuclei (right)

Image courtesy of Ronny Janssens, Centre for Reproductive Medicine, Brussels Free University, Belgium

The quality of an embryo is generally evaluated based on factors such as the number of cells at a specified time point, regularity of sizes and shapes, presence of multinucleation, and presence of vacuoles. Figure 4 shows examples of normal and abnormal embryos.

3.3. Inverted Microscope

In the ART cycle, fertilization is performed by either IVF or ICSI. Using an inverted microscope suitable for observing dish containers, ICSI, IMSI, and spindle observation are performed (Fig. 5).

Fig. 5 Inverted microscopes: ECLIPSE Ti2-I (left) and ECLIPSE Ti2-U (right)

ICSI is a method in which a sperm is injected into the cytoplasm of an egg using a manipulator and injector to achieve fertilization. The sperm is moved to the tip of the injector and injected into the egg at the focal plane. When the membrane of the egg is penetrated, a small amount of cytoplasm is aspirated and then re-injected into the egg together with the sperm (Fig. 6). In this process, a dedicated 20× or 40× objective lens is used for observation with modulation contrast [3], [4]. For example, observation can be performed using Nikon Advanced Modulation Contrast (NAMC) [5]. NAMC produces a light–dark contrast image with a three-dimensional appearance by adding shad-

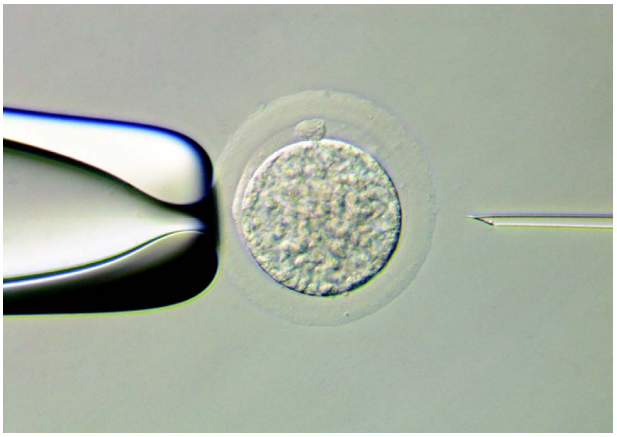

Fig. 6 Injection by ICSI

ing to colorless, transparent cells. Although the image quality is similar to that of differential interference contrast, which is described later (4.2.), it has the advantage of allowing observation even with plastic dishes.

The purpose of IMSI is to select the sperm with the highest likelihood of success in ICSI. In principle, it is the same as differential interference contrast observation [3], [6], allowing colorless and transparent cells to be visualized as three-dimensional images. Because it uses polarized light, observation cannot be performed with plastic dishes, as the polarization is disturbed. For this reason, sperm placed in a glass-bottom dish can be observed at high magnification, enabling the detection and selection of fine defects in organelles such as the sperm nucleus and vacuoles (Fig. 7). A 60× or 100× objective lens is used.

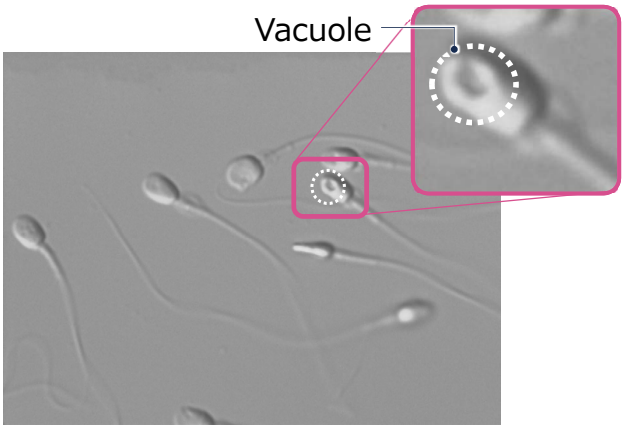

Fig. 7 IMSI with 100x objective lens
Image courtesy of Fujita Health University

The spindle plays a role in properly arranging chromosomes during cell division. Therefore, spindle observation is important for performing ICSI, as it allows determination of the spindle's position and shape within the egg. Because the spindle is a complex structure composed of microtubules, it exhibits optical anisotropy and birefringence, with refractive indices varying according to its orientation. This cannot be captured by phase contrast observation, modulation contrast observation, or differential interference contrast observation and can only be visualized using polarized light observation [6]. Furthermore, the spindle is not always present and typically appears near the first polar body of the egg (Fig. 8 (left)). In ICSI, to avoid damaging the spindle, the first polar body is positioned at the 12 or 6 o'clock orientation, and the injection is performed from the 3 o'clock direction. However, the spindle may sometimes appear away from the first polar body, making it important to confirm its location (Fig. 8 (right)).

Figure 9 shows an example configuration of an inverted

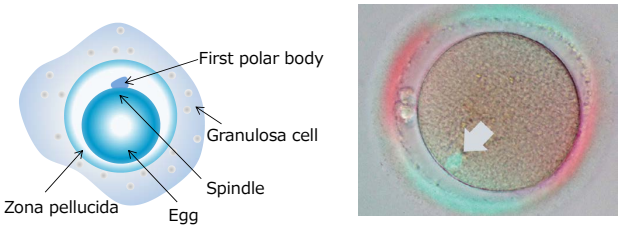

Image courtesy of: Reproduction Clinic Tokyo

Fig. 8 Schematic diagram of spindle and egg (left), example of spindle observation (right)

microscope set up inside a clean bench. In addition to the microscope, it is integrated with various equipment, including manipulators, injectors, a thermo plate for temperature control, and a laser hatching system for thinning the zona pellucida of embryos. Table 1 presents an example summarizing the objective lens magnifications on the objective revolver and corresponding tasks during operation of an inverted microscope. As summarized in Table 1, numerous microscope operations must be performed; however, because eggs experience stress once removed from the incubator, it is necessary to perform the procedures within a short period of time.

Fig. 9 Example setup of an inverted microscope

Table 1 Objective lens magnifications on the objective revolver and example tasks during operation of an inverted microscope

Objective	Example tasks
4x	Setting for injector, manipulator
10x	Oocyte selection and decoronation
20x	ICSI, Spindle observation
40x	ICSI, Spindle observation
60x or 100x	IMSI
Laser	Assisted hatching

4 Optical Principle of ICSI/IMSI and Spindle Observation

As shown in Fig. 10, the required optical components are

used in various combinations depending on the method used. This is because the properties of light used to achieve high-contrast observation of eggs and sperm differ for each method. The optical principles underlying each method are described in the following subsections.

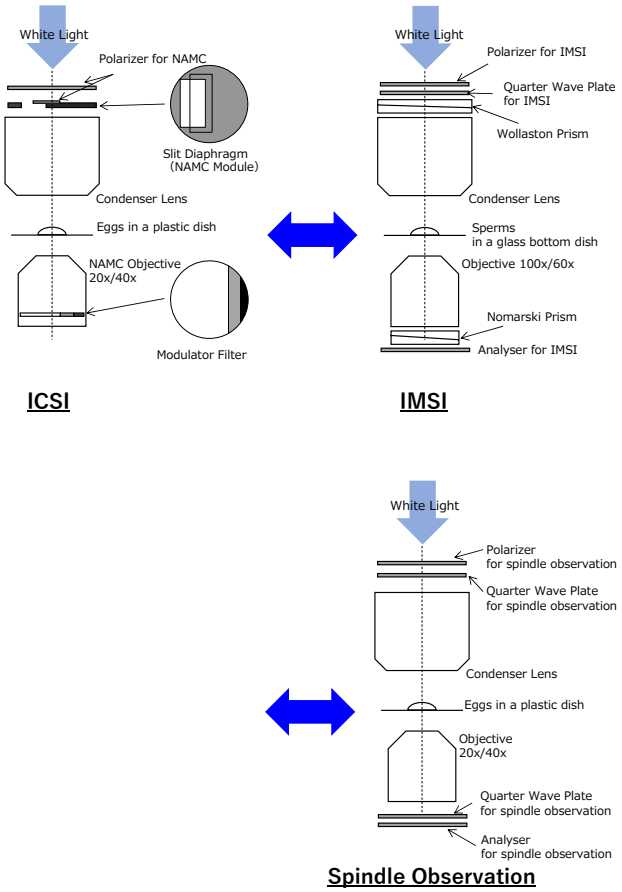

Fig. 10 Schematic diagram of the optical components required for each method

4.1. ICSI

Optically, this is a type of oblique illumination known as modulation contrast (referred to as NAMC by Nikon). The required optical components are a rotatable polarizer, slit aperture with an attached polarizer (NAMC module), and dedicated objective (NAMC objective) equipped with an internal modulation plate. The slit aperture is positioned at the front focal plane of the condenser, while the modulation plate is located at the rear focal plane of the dedicated objective (objective pupil; Fig. 11, left).

The outer edge of the slit in the NAMC module is adjusted to align with the boundary between regions a and b of the modulation plate in the NAMC objective. After passing through the NAMC module, the light rays enter the egg obliquely, are refracted, and then pass through regions a, b, and c of the modulation plate in the NAMC objective, which have different transmittances, thereby producing shading A, B, and C in the transmitted light (Fig. 11, right). Here, the transmittance of the modulation plate is 0% in region a, 100% in region c, and intermediate in region b. This shading gives the egg contrast, resulting in a three-dimensional image. By rotating the NAMC module, the orientation of the shading on the egg can be changed; however, the orientation of the modulation plate must also be aligned accordingly.

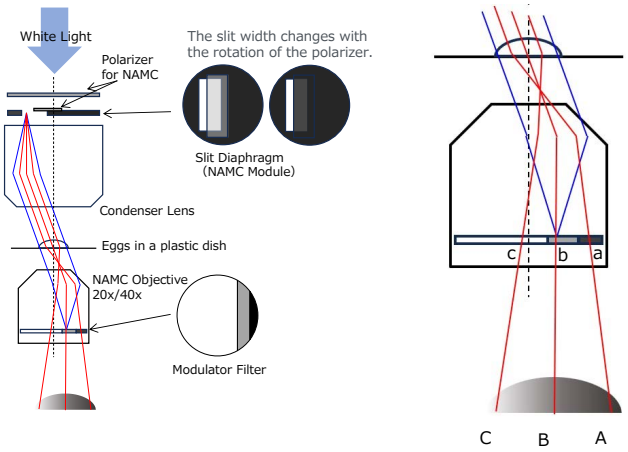

Fig. 11 Optical components and light path of NAMC (left) and reason for contrast formation (right)

By rotating the polarizer located above the condenser lens, the amount of transmitted light can be adjusted, and the slit width of the NAMC module can be changed. This changes the illumination angle of the light entering the egg obliquely, thereby allowing the contrast to be adjusted.

4.2. IMSI

Optically, this is differential interference contrast observation. On the condenser side of the optical path, a polarizer, $\lambda/4$ plate, and DIC prism are arranged in sequence, while on the objective side, a DIC prism and analyzer are positioned in that order.

As an initial adjustment step, only the polarizer and analyzer are placed in the optical path, and their transmission axes are set perpendicular to each other to achieve a crossed-Nicols condition. Subsequently, the DIC prisms corresponding to the condenser and objective lenses are inserted into the optical path (Fig. 12, left). The DIC prisms are positioned at the front focal plane of the condenser and rear focal plane of the objective lens (objective pupil). In Nikon systems, the polarizer and $\lambda/4$ plate are integrated, and the orientation of the polarizer's transmission axis is aligned with the fast axis of the $\lambda/4$ plate under the crossed-Nicols condition. Rotating the polarizer allows contrast adjustment for reasons described later.

When linearly polarized light enters the DIC prism on the condenser side, it is split into two linearly polarized beams

with orthogonal polarization directions, laterally displaced relative to each other in a direction perpendicular to the optical axis (Fig. 12, left). The lateral displacement between the two beams is called the shear amount and is designed to be smaller than the resolution of the objective lens. Figure 12 (upper right) shows the wavefronts of the two separated beams after passing through the sperm. Because a $\lambda/4$ plate is positioned on the condenser side, a retardation Δ_0 is imparted to the two beams. Figure 12 (lower right) shows the wavefronts of the two beams recombined after passing through the DIC prism on the objective side. It can be seen that retardations Δ_1 and Δ_2, corresponding to the surface profile of the sperm, are produced. From the shear amount and inclination of the sperm surface, the retardations Δ_1 and Δ_2 at each part of the sperm are determined and converted into interference colors corresponding to each retardation. This imparts shading to the sperm, thereby producing contrast. The background appears gray, corresponding to the interference color of the retardation Δ_0 produced by the $\lambda/4$ plate. By rotating the polarizer, the retardation Δ_0 can be finely adjusted, allowing the contrast to be varied.

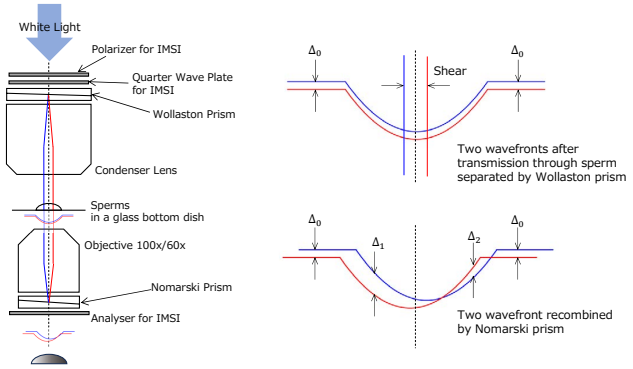

Fig. 12 Optical components and light path of DIC (left) and reason for contrast formation (right)

Because polarization must be maintained when light enters the DIC prism, observation cannot be performed using plastic containers. In addition, contrast can only be detected in the direction in which the shear is produced.

4.3. Spindle Observation

Optically, this observation is performed with polarized light, and Nikon employs circular polarization. The spindle, which exhibits optical anisotropy, is visualized with added coloration.

An optical element integrating a polarizer and $\lambda/4$ plate is placed on both the condenser side and objective side. The angle between the transmission axis of the polarizer and fast axis of the $\lambda/4$ plate is set to 45 degrees, producing circularly polarized illumination for the egg. After passing through the egg, the circularly polarized light is converted back into linearly polarized light by the $\lambda/4$ plate and polarizer on the objective side. At this point, the transmission axis of the polarizer is orthogonal to the linear polarization (Fig. 13, left).

Although the spindle can be observed with linear polarization, illumination with circular polarization offers the advantage of rendering the spindle in color (red or blue). The reason it appears red or blue is that the $\lambda/4$ plate does not function perfectly over the entire wavelength range of white light. In general, a $\lambda/4$ plate provides a retardation of one quarter of the wavelength at its reference wavelength (green in this case). However, at red and blue wavelengths, which deviate from the reference, the retardation differs from one quarter of the wavelength. As a result, at red and blue wavelengths, the light becomes elliptically polarized after passing through the $\lambda/4$ plate on the condenser side, and even after passing through the $\lambda/4$ plate on the objective side, it does not revert to perfectly linear polarization. Therefore, the light enters the final polarizer as elliptically polarized light, resulting in the residual presence of red and blue light (Fig. 13, right). In addition, by rotating the optical element on the condenser side, the major and minor axes of the elliptical polarization are interchanged, thereby changing the intensity of the red and blue light passing through the polarizer on the objective side and enabling adjustment of the coloration.

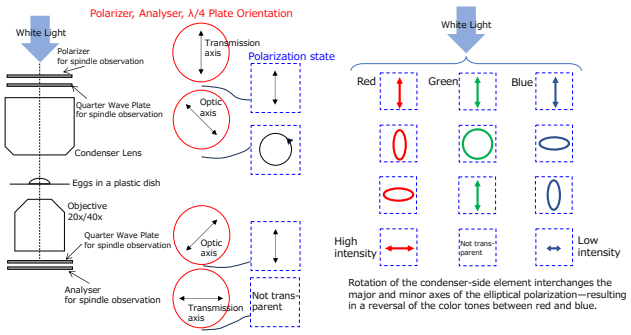

Fig. 13 Optical components and light path for spindle observation (left) and reason for red/blue coloration (right)

The use of circular polarization allows the spindle to be observed in red or blue, making it easier to locate even when the egg is rotated in the XY plane. With linear polarization, however, the spindle may appear white or black, or become invisible depending on the rotation of the egg, making it more time-consuming to locate and increasing the risk of overlooking it.

Because polarization must be maintained between the polarizer and $\lambda/4$ plate optical elements, observation cannot

be performed with plastic containers.

Table 2 summarizes the image characteristics, resolution, contrast, and applicable containers for each method described above.

Table 2 Optical characteristics of ICSI/IMSI and spindle observation

	ICSI	IMSI	Spindel observation
Microscopy	Modulation contrast (NAMC)	Differential interference contrast	Polarized light
Characteristics	Shading: 3D effect, direction adjustable	Shading: 3D effect	Birefringence: visible
Resolution	High (lower than DIC due to the slit diaphragm)	High	High
Contrast	High	High	High (Birefringence)
Contrast adjustment	Polarizer: rotation	Polarizer: rotation	N/A
Suitable sample	Sperm, Egg, Embryo	Sperm	Egg
Dish	Glass-bottom/Plastic	Glass-bottom (Plastic: not allowed)	Glass-bottom (Plastic: not allowed)

5 Conclusion

In Japan, where the declining birthrate is advancing, ART plays an important role. ART is a medical technology encompassing various processes, such as IVF and ICSI, that involve handling eggs and sperm to treat infertility and support pregnancy. Upright, stereo, and inverted microscopes essential to the ART cycle, along with their associated optical technologies, have been introduced. In particular, for ICSI/IMSI, which require precise manipulation with an inverted microscope, optical techniques such as modulation contrast (NAMC) and differential interference contrast are employed. In spindle observation, circular polarization is used to determine the spindle's position within the egg, and avoiding it during injection contributes to improved fertilization rates. We will continue to work toward advancing technological innovation in the medical field and provide new solutions to help address the societal issue of declining birthrates.

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三宅範夫 Norio MIYAKE
光学本部 第一設計部
1st Designing Department
Optical Engineering

三宅範夫
Norio MIYAKE