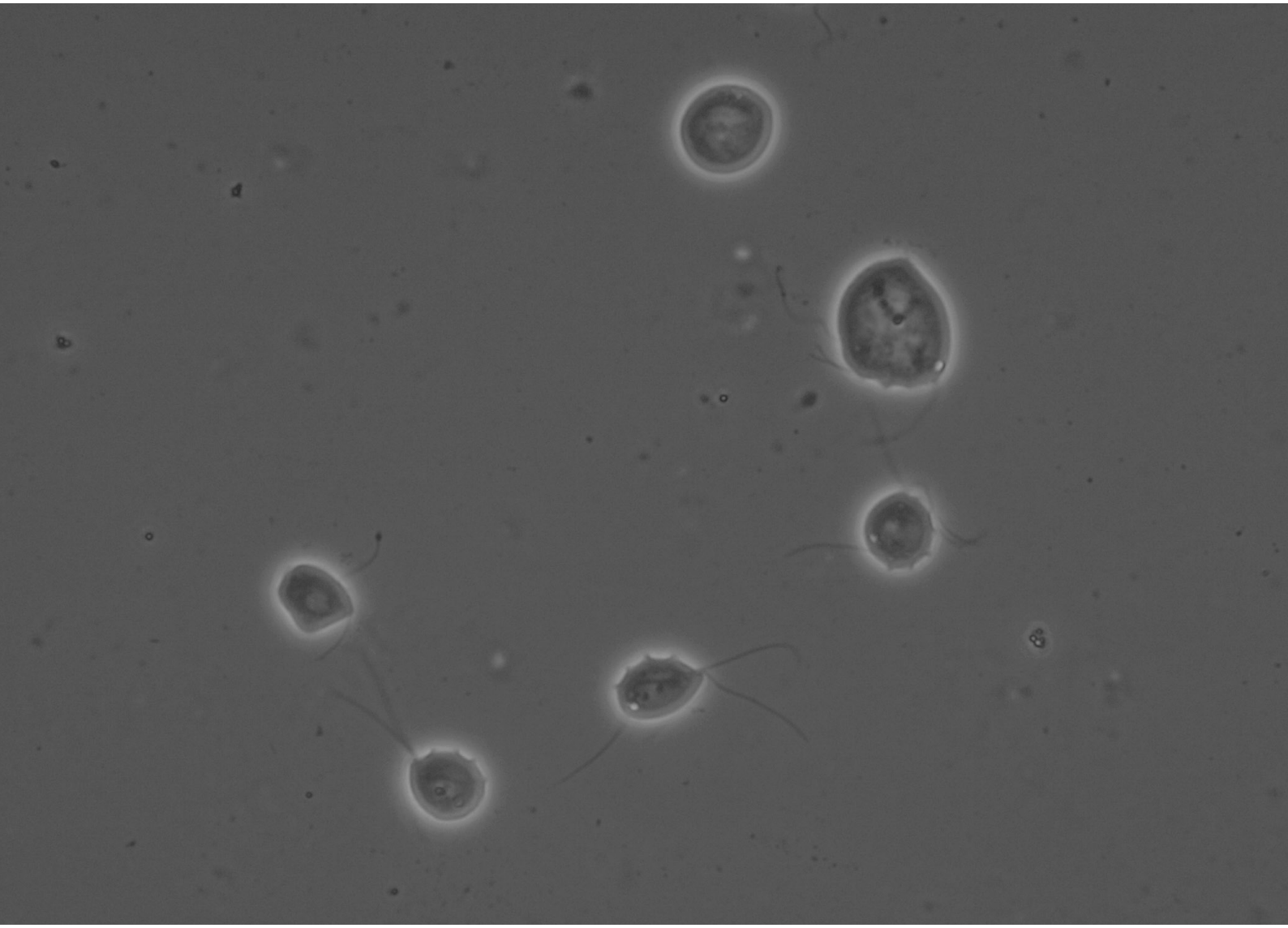
Application Note



ZEISS Axio Lab.A1

Microscopic Examination of Living Specimens of the Parasite *Trichomonas vaginalis*

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Microscopic Examination of Living Specimens of the Parasite *Trichomonas vaginalis*

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Things to Know about Trichomonads

Trichomonads are multiflagellated protozoans. As inhabitants of the urogenital tract, the oral cavity or digestive organs, they are present in a wide variety of life forms (including arthropods, mammals, and humans) (Fig. 1). In humans, the *Trichomonas vaginalis* flagellate is a parasite in the urogenital tract of both sexes which is transmitted primarily through unprotected sexual contact. Other means of infectious contact (such as hand towels) are very rare. Infection with *Trichomonas vaginalis* is the most common nonviral sexually transmittable infectious disease worldwide. WHO estimates (2008) indicate a worldwide total of up to around 280 million infections. Up to 20 % of infected pregnant women transmit the infection to their newborns perinatally. The incubation time lies between 5 and 25 days. The infection often remains unnoticed initially. It is asymptomatic in 10–30 % of women and up to 90 % of men. The infection of the urogenital tract in women (colpitis) is characterized by a change in the composition of vaginal secretion: vaginal discharge becomes strongly malodorous, mostly yellowish, and often frothy. The discharge contains many bacteria, pus cells, and trichomonads. Often the parasite then passes on to the urethra where it also causes infections which can be quite painful, particularly in women. In men, the infection typically passes asymptomatically. There are indications that an existing infection with *Trichomonas vaginalis* increases the risk of infection with other sexually transmitted diseases (such as HIV).

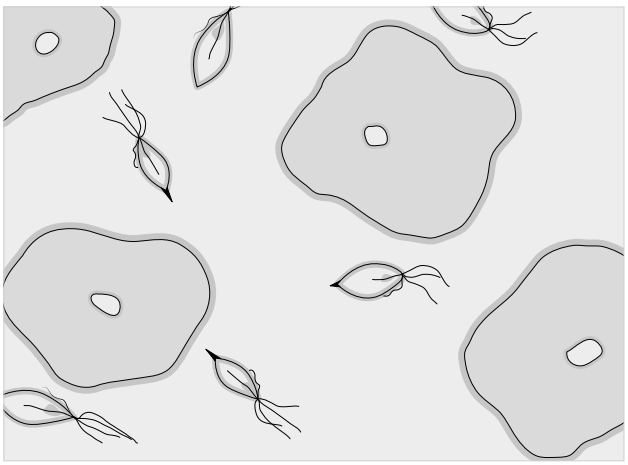

Figure 1 Trichomonas vaginalis *in vaginal secretion*

Source: Jenny J.: Phasenkontrastmikroskopie in der täglichen Praxis. Verlag Jenny u. Artusi, Schaffhausen, 1977.

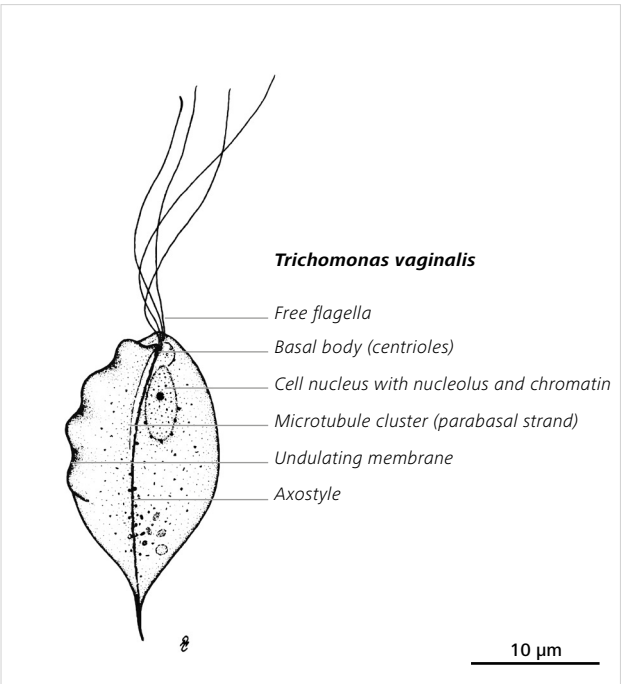

Figure 2 Trichomonas vaginalis *(schematic)*

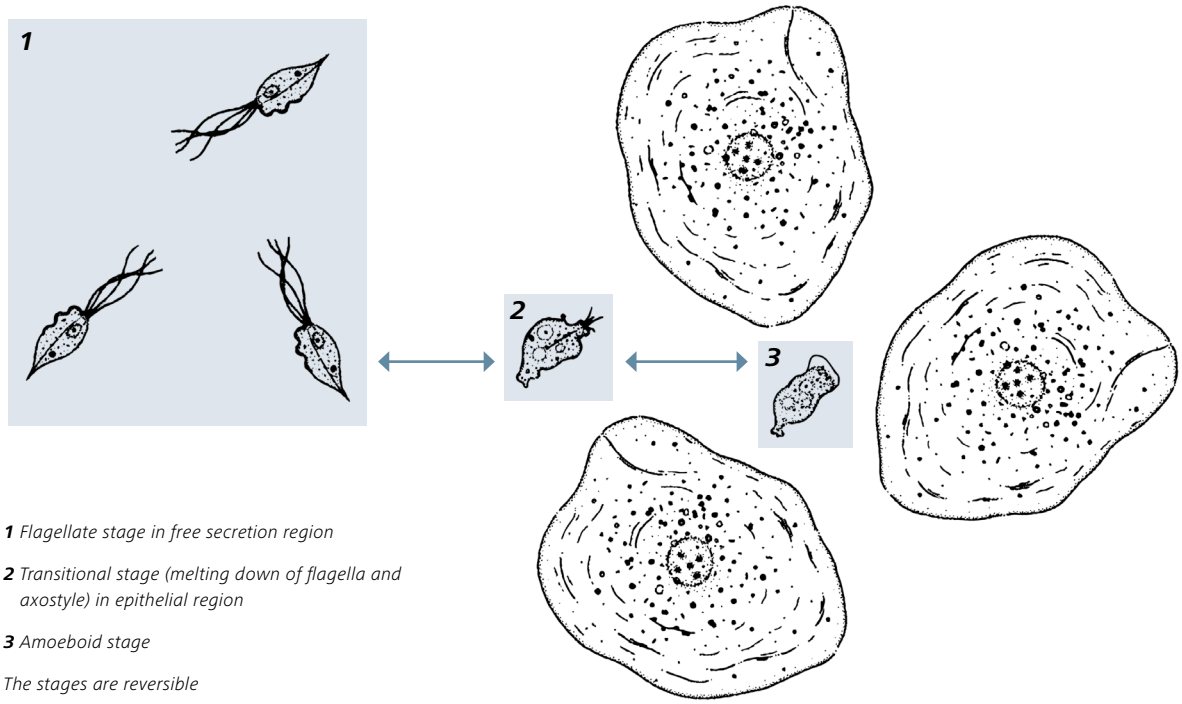

Figure 3 Trichomonas vaginalis *(flagellate and amoeboid stage)*

Both partners must be treated concurrently (orally and vaginally) for instance with nitroimidazol compounds such as *Clont™* or *Flagyl™*. Other options include derivatives of tinidazol and ornidazol such as *Fasigyn™* or *Tiberal™*.

Trichomonas vaginalis (length 5–30 µm, Figs. 2, 3) is a pear-shaped flagellate having five flagella. Four of the flagella are free. In living cells, they provide locomotion by making swimming movements (Fig. 4); at such times, they are oriented forwards. A further fifth flagellum also arises from the centriole. This flagellum runs closely along the cell body towards the posterior end. Surrounded by a protective membrane, it turns the membrane into an undulating membrane. Movement of the flagellum sets the membrane in vigorous, undulating motion (Fig. 5). Also arising from the centriole is a tapered axostyle. This provides mechanical stability to the flagellate within the thin secretion film. The axostyle is accompanied by a microtubule cluster (also called a para-basal strand). The ovoid cell nucleus is located in the anterior portion of the cell. Feeding occurs through the uptake of nutrients from the medium surrounding the cell (in this case, the vaginal fluid). The flagellates live anaerobically in an optimum pH environment of between 5.4 and 6.0.

They reproduce by cell division. The flagellated, freely swimming stages often attach themselves to the epithelial cells and, discarding their flagella, transform into amoeboid stages. These become polynuclear. The amoeboid stage can revert to the flagellated stage at any time (Fig. 3). Nonflagellated, rounded, permanent stages may occur; encysted permanent stages have not as yet been observed. Intermediate hosts are unknown.

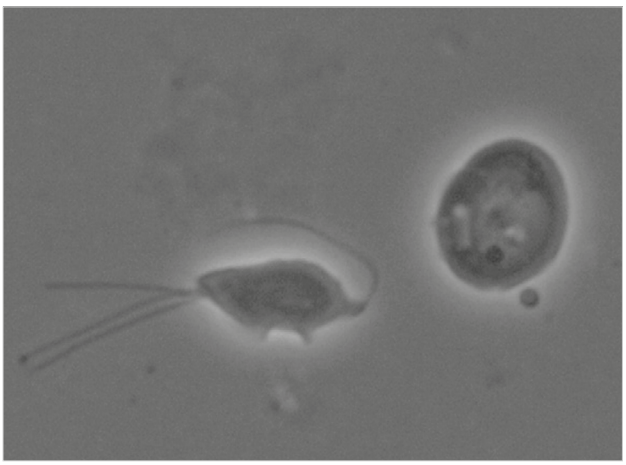

Figure 4 Trichomonas vaginalis *live image Phase contrast with the EC Plan-NEOFLUAR 40x/0.75 Ph2 objective*

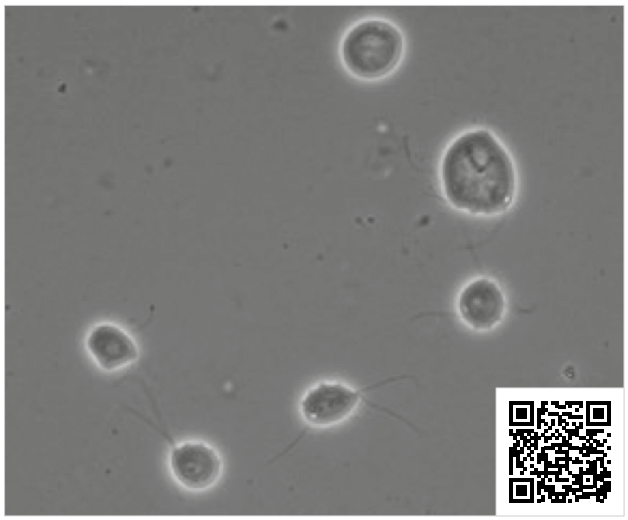

Figure 5 Trichomonas vaginalis *live image in phase contrast with the EC Plan-NEOFLUAR 40x/0.75 Ph2 objective*

Trichomonas vaginalis in practice

Trichomonads can be detected in living specimens using a light microscope. Phase contrast is the contrast method of choice (Figs. 4, 5).

ZEISS microscopes, with their ease of operation and exceptional image quality, make an important contribution to gynecological in-vitro examination.

The parasites can also be detected in a smear fixed with methanol using Giemsa, Wright-Giemsa, or Papanicolau stain (Fig. 6). It is inadvisable to use these methods due to the fixation artifacts which are created and the fact that unambiguous recognition of flagella with the microscope is difficult.

Preparation technique

Vaginal secretions are typically removed using cotton swabs (colposcopy). The secretion sample is dabbed into a not too large drop of saline solution which has been previously dripped onto the edge of a clean slide. This solution is then mixed again. The amount of sample is optimum when the eye can just make out the turbidity produced. Then the square cover slip is placed at the outer edge of the drop and held at an angle, then allowed to fall onto the drop quickly.

Any extraneous fluid which might appear at the edge of the cover slip is removed with absorbent paper. No fluid should now be present on the bottom surface of the slide.
The living sample with cover slip is now ready.

Absolute ethanol is used for cleaning and storing slides and cover slips. Slides and cover slips may be handled at their edges (using cover slip tweezers) and dried with lint-free cleaning paper (such as KIM WIPES).

Slides (76 × 26 mm) should be of the type "brilliant white, edged." The square cover slips (32 × 24 mm) must be 0.17 ± 0.01 mm thick. If cover slips which deviate from this thickness are used, images exhibit a clearly visible decrease in contrast due to spherical aberration. This phenomenon also occurs if the fluid layer in the slide is too thick.
By removing fluid at the edge of the slide through absorption, spherical aberration can be considerably reduced.

Microscopic equipment

The living sample can now be examined with the microscope. ZEISS Axio Lab.A1 with phase contrast condenser and LED illumination can make it possible (Fig. 7). The Axio Lab.A1 microscope has enough illumination reserves for all transmitted light microscopic methods (brightfield, darkfield, phase contrast, polarization contrast). It allows setting of Köhler illumination and is equipped with adjustable phase contrast annular filters Ph1, Ph2, and Ph3.

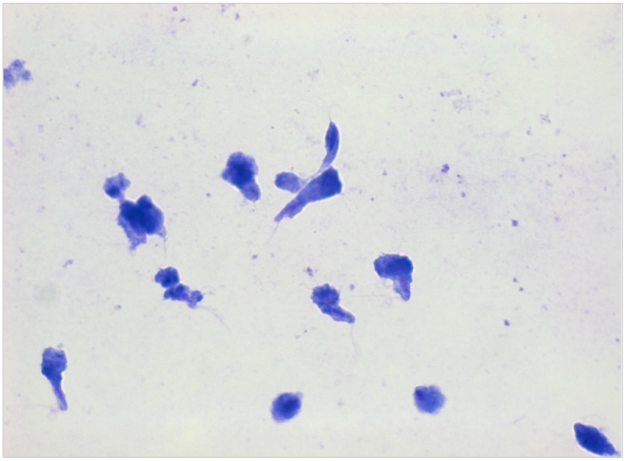

Figure 6 Trichomonas vaginalis, *prepared with GIEMSA stain. Brightfield with the EC Plan-NEOFLUAR 100x/1.30 Oil objective*

The microscope is equipped with the following objectives:

- A-Plan 10x/0.25 Ph1: This can be used to obtain an overview and more easily identify trichomonads in phase contrast.
- EC Plan-NEOFLUAR 40x/0.75 Ph2: This is the objective for investigating the stage of the flagellate and the movement of *Trichomonas vaginalis* in phase contrast.

To obtain special images of the fine structure of trichomonads in phase contrast, it is advisable to use an oil immersion EC Plan-NEOFLUAR 63x/1.25 Oil Ph3 or EC Plan-NEOFLUAR 100x/1.30 Ph3 objective. However, examination for a trichomonas infection does not require use of these objectives.

To increase contrast in phase contrast images it is urgently recommended to place an interference wideband green filter d = 32 × 4 mm (order No. 467803) over the light emission aperture.

Detection of *Trichomonas*

The locomotion of trichomonads in fresh vaginal secretions is by means of jerky swimming movements (Figs. 4, 5). These are achieved using the four free flagella, supported by the movements of the undulating membrane (Fig. 5). This is readily observable in phase contrast with the EC Plan-NEOFLUAR 40/0.75 Ph2 objective The cell nucleus of living stages is pear-shaped and tapered at the rear. No structures are visible inside the moving cells. In some flagellates, the tapered axostyle protrudes beyond the posterior end of the cell.

Air bubbles in the sample or droplets of oil from vaginal fluid are often round in shape. However, these can easily be distinguished from trichomonads by their lack of flagella and the difference in shape.

Liquid from the sample slowly evaporates in the course of microscopic examination. This is desirable as the sample thickness decreases more and more, thus making fine structures ever easier to identify.

Figure 7 *ZEISS Axio Lab.A1 for* Trichomonas *examination*
With condenser for brightfield, darkfield, and phase contrast. Equipped with objectives for phase contrast.

Dying stages become round in shape and gradually lose their flagella. The axostyle melts down (Fig. 4, cell at right).

With direct microscopic identification of living trichomonads in phase contrast, an infection with *Trichomonas vaginalis* can be detected.

The ZEISS Axio Lab.A1 microscope with phase contrast and the EC Plan-NEOFLUAR 40x/0.75 Ph2 objective makes it possible to detect trichomonas in less than one minute in a freshly prepared living sample (Fig. 7).

Specific diagnostic processes are determined by the doctor. The specialist doctor bears full responsibility for expert diagnosis.
Further detection methods may be required.

Not all products are available in every country. Use of products for medical diagnostic, therapeutic, or treatment purposes may be limited by local regulations.
Contact your local ZEISS representative for more information.

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