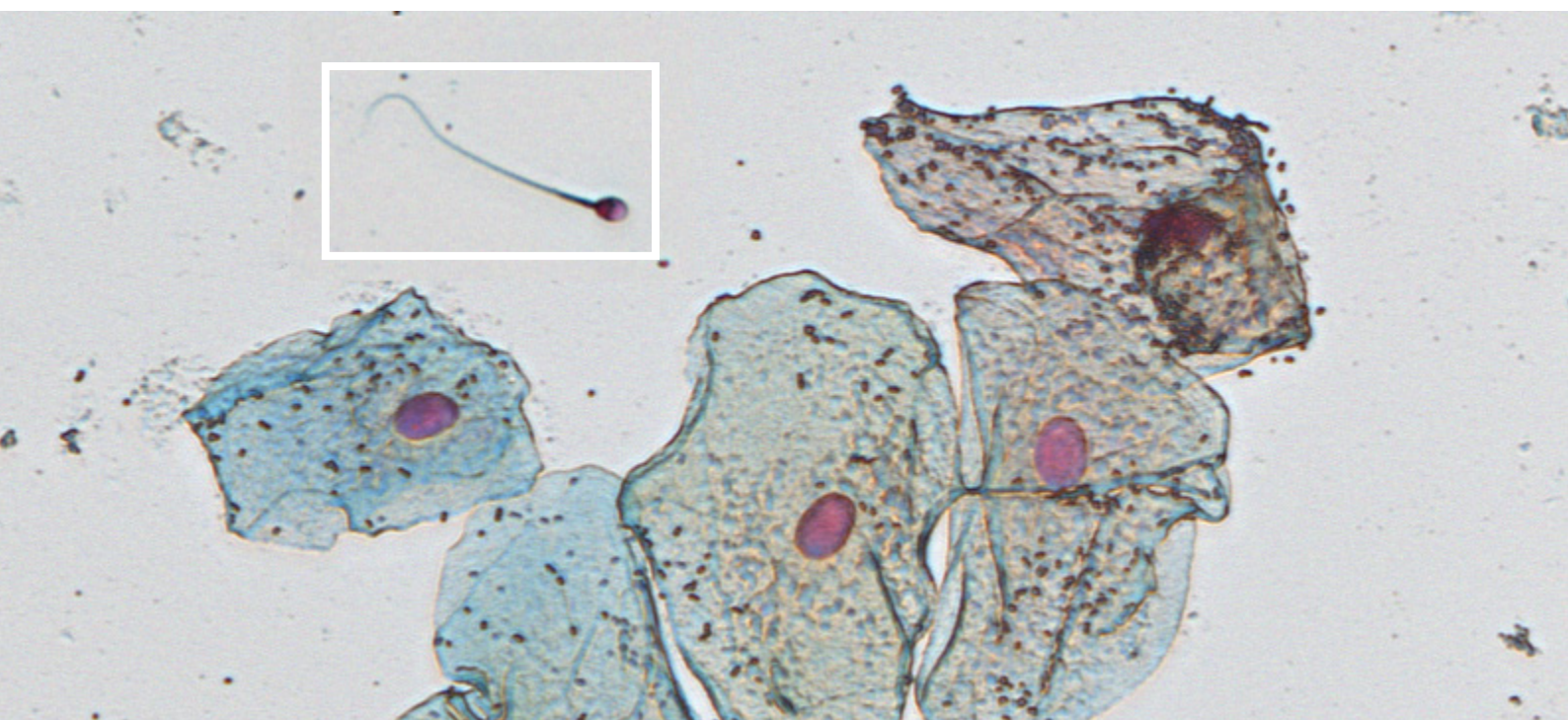


## Forensic sperm cell detection with artificial intelligence

ZEISS Axio Imager and Metafer with the Customization Package Sperm Detection



Seeing beyond

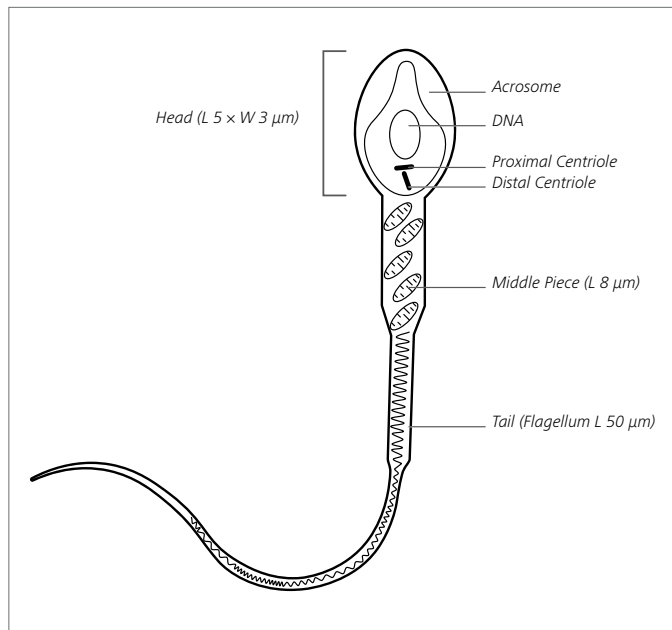
Date: November 2023

Sperm cells are a crucial piece of evidence for solving sexual crimes. The manual search for sperm cells in forensic samples with the help of microscopy is often labor-intensive and time-consuming. Most forensic labs have a serious problem: Thousands of sexual assault cases are on backlog and cannot be closed. Automated microscope-based imaging with artificial intelligence such as ZEISS Axio Imager and Metafer with the Customization Package Sperm Detection helps automating the search for sperm cells in forensic specimens and to significantly shorten turnaround times in specimen examination.

### Introduction

The presence of semen, either in the form of stains on objects or on swabs collected from victims after the crime is one of the most valuable pieces of forensic evidence in male sexual assault cases. Sperm traces can be obtained e.g., from the vagina, vulva, cervix, anus, skin, objects, or clothing. The microscopic detection of spermatozoa is a method of confirming the presence of semen in evidence.

Spermatozoa are identified by either the presence of intact sperm cells displaying a head, midpiece and tail, or sperm heads showing an acrosomal cap (Figure 1).



**Figure 1** Parts of sperm. They can be distinguished by different staining methods, e.g., Christmas Tree or Baecchi staining.

Usually, the traces are obtained by means of a swab and spread onto a microscope slide. There are different specific staining methods for sperm cells available, of which the most common are: "Christmas Tree" stain (Nuclear Fast Red/Picric Indigo Carmine) and the Baecchi stain (Acid Fuchsin/Methyl Blue). Both staining methods differentially stain heads and tails of the sperm.

### Typical staining methods for identification of spermatozoa in short:

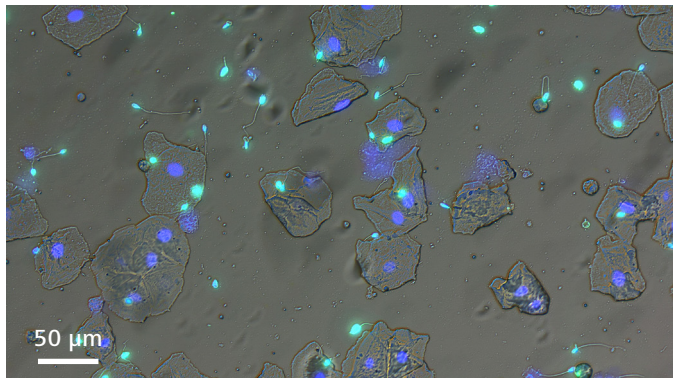
#### Christmas tree staining

Essentially, a Nuclear Fast Red stain solution is used to stain the heads of sperm red and the part of the tip, known as the acrosomal cap, pink. A Picro Indigo Carmine stain solution is used to stain the tails blue and green.

#### Baecchi staining

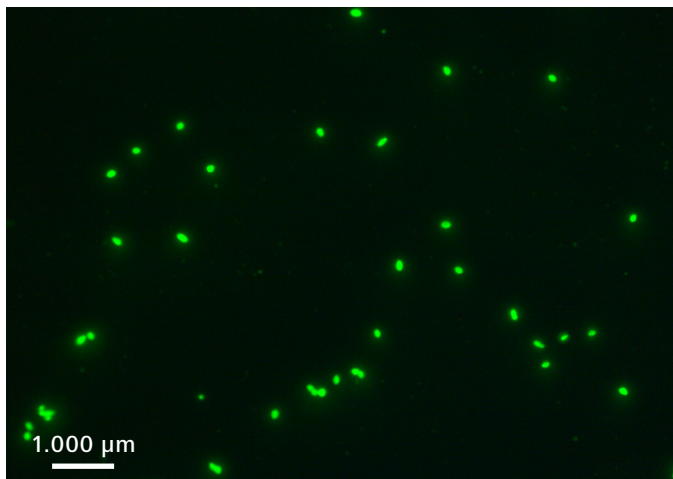
The Baecchi's staining reagent contains acid fuchsin and methylene blue, which stains the sperm head red and the tail blue, respectively.

While these routine stains are viewed in transmitted light, there are fluorescence antibody markers for sperm cells, such as Sperm HyLiter™, which the manufacturer claims are even specific for human spermatozoa and can distinguish them from cells of animal origin (Figure 2).



**Figure 2** Vaginal swab with fluorescent staining SPERM HY-LITER™. Acquired with ZEISS AxioScope 5.

They are used rather rarely for routine evaluation but can be helpful in confirming results obtained by classical methods.



**Figure 3** Staining of sperm with HY-Liter, green fluorescence, Alexa. Acquired with ZEISS Axio Scope.A1. Copyright of: Galantos Genetics, Martin Schatzl, Dr. Ulrike Schacker, Mainz, Germany

Generally, microscopy is used in forensics as a method to confirm screening tests such as the Acid Phosphatase Test and is widely considered the gold standard. However, the manual detection of sperm cells in a sample is a tedious and complex task, especially if the samples contain no or only low amounts of semen. As a result, many forensic laboratories worldwide report a backlog of cases that cannot be processed in a timely manner. According to a report of the U.S. Government Accountability Office published in 2019 for instance, the number of backlogged DNA analysis requests rose from about 91,000 to about 169,000 untested samples from 2011 to 2017 (GAO-19-216 \*1).

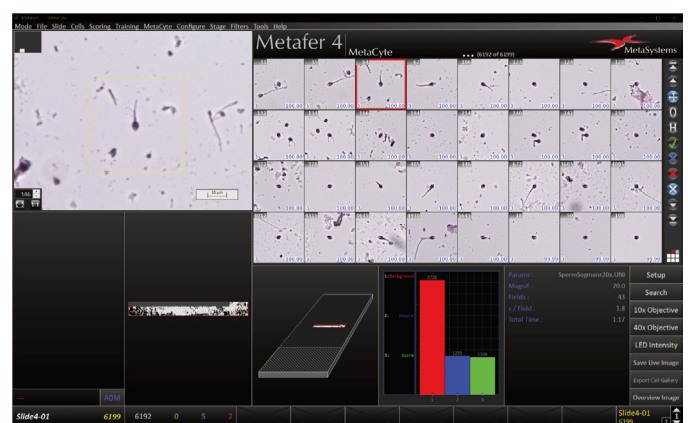
Automated sperm detection can therefore be one of the most efficient methods to quickly determine the presence of spermatozoa.

## Automated sperm detection with AI

Automation can play a key role to keep up with increasing sample amounts. MetaSystems (Altlusheim, Germany) has trained a deep neural network (DNN) on thousands of pre-classified data. Thus, the advantages of artificial intelligence have been harnessed for forensic sperm recognition. Metafer, a microscope-based scanning software with the Customization Package Sperm Detection, supports both Christmas Tree and Baecchi staining in brightfield (networks for other staining protocols are under development). Using machine learning methods, the software further scans fluorescently labeled samples, e.g., with the Sperm HyLiter™ Kit.

The technician can use normal routine specimens, such as with Christmas tree staining; it is not necessary to follow any special preparation protocols. Metafer takes the slides, images them, and uses its artificial intelligence to identify the location of sperms and sperm-like objects. The software controls the ZEISS Axio Imager microscope and the robotic slide exchanger, capable to digitize from 8 to 800 slides per run. The comprehensive image gallery shows all detected objects together with information on their position and their categorization. Immediately after the scan, the forensic expert has access to the results for review.

The artificial intelligence can distinguish between complete sperm with tails, sperm heads, artifacts, and blurred objects. During the review, these different classes can be evaluated separately in the gallery and confirmed or revised by the expert.



**Figure 4** Screenshot of Metafer software showing a gallery of found objects. The live image in the upper left corner displays the currently selected object in the gallery. The numbers in the gallery images indicate the respective assignment probability determined by the artificial intelligence.

## Success

Several forensic laboratories in Australia, Europe, and the USA are already taking advantage of the Metafer with the Customization Package Sperm Detection in their routine work. Customers report that their backlog has been significantly reduced or even eliminated within a few weeks of implementation. The time that

forensic experts must invest in evaluating specimens decreases noticeably, not only because the device works faster, but also because it scans unattended and thus produces results even at night or on days off. In addition, internal figures from various laboratories prove that the detection accuracy of the algorithm is at least as high as that of manual microscopy, but without being influenced by external factors (fatigue of the evaluator, lack of concentration). Several tests have shown that the instrument was able to correctly classify supposedly negative samples as positive.

### Secure documentation

Forensic results are sensitive data that, under certain circumstances, should also stand up in court. A fully comprehensive documentation of all images taken, all processing steps, and all results provides the security of being able to prove one's own findings at any time. Metafer encrypts and stores all data and images to ensure security, integrity, and traceability. The examiner benefits from the wide range of data display and compilation options and saves valuable time during documentation. Top-level data encryption, advanced user management, and audit trails ensure the highest data security standards. Paperless documentation of evidence enables collaboration between multiple experts, can be statistically evaluated, and generates court-ready documentation.



**Figure 5** A complete installation with the Metafer software. The hardware consists of an Axio Imager Z2 microscope, a motorized scanning stage, and MetaSystems' CoolCube4 cameras. The optional SlideFeeder x80 slide frame feeder is shown on the left.

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## About MetaSystems

MetaSystems, founded in 1986 near Heidelberg in Germany, specializes in the manufacturing of software for automated microscopy. The state-of-the-art scanning software Metafer is used in more than 100 countries all over the world for a wide variety of tasks. The focus is on routine testing, for example in clinical cytogenetics, toxicology, microbiology, and forensics.

In addition to its six subsidiaries, MetaSystems collaborates with a global network of partner companies. In many countries, these are local subsidiaries of ZEISS or ZEISS partners.

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The Customization Package Sperm Detection is a proposed workflow for the Metafer scanning software by MetaSystems. Metafer runs with the ZEISS Axio Imager motorized microscope.

Configuration:

- ZEISS Axio Imager motorized microscope
- MetaSystems CoolCube 4c ultra-high resolution, color digital camera
- White LED light source for the transmitted light path (e.g., ZEISS MicroLED)
- Modified Märzhäuser 8-slide scanning stage

For higher capacities, there is a robotic slide frame feeder (SlideFeeder x80) available, that allows to scale the installation from 80 slides to 800 slides per run. In addition, if fluorescence preparations are to be examined with the same installation, a monochrome camera (CoolCube 4c) and an RGB light source can be used for the transmitted light channel. Alternatively, a second, monochrome camera is supported.

The choice of objective depends on whether the user covers his specimens or not. The target magnification is 20x, so a ZEISS Epiplan-Neofluar 20x/0.5 objective, for example, can be used. Alternatively, a ZEISS N-Achroplan 63x/0.95 objective can also be installed for manual examination.

### Summary

Automating microscopy with AI to evaluate physical evidence from rape kits helps solve crimes faster and has the potential to reduce backlogs. The use of standardized parameters for image acquisition and evaluation increases the quality and significance of the results.

### **Cover photo**

Image from a forensic specimen stained with Nuclear Fast Red/Picric Indigo Carmine (“Christmas Trees”) staining. The red box indicates a sperm cell; the other objects are epithelial cells.

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