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Video microscopy: an old story with a bright biological future

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Abstract

Single-cell analysis is increasingly popular in the field of biology, enabling more precise analyses of heterogeneous phenomena, particularly in the fields of embryology and the study of different diseases. At the heart of this evolution is video microscopy, an ancient but revolutionary technique. From its first use on embryos, through the study of *C. Elegans*, with the development of algorithms for its automation, the history of video microscopy has been fascinating. Unfortunately, many unresolved issues remain, such as the sheer volume of data produced and the quality of the images taken. The aim of this review is to explore the past, present and future of this technique, which could become indispensable in recent decades, to understand cell fate and how diseases affect their destiny.

Keywords: Video microscopy, Biology, Microscopy, Cell cycle, Cancer, Neurology, History

Introduction

The word video-microscopy is a combination of the Latin word "*video*" standing for "*I see*" and "*mikros*" "*skopein*" in Greek, meaning "*little*" "*examination*". Over the past two decades, dynamic studies of live beings have gained popularity. The concept of video microscopy is part of this process: capturing videos or pictures of living beings in a defined amount of time.

What are the benefits of video microscopy? In contrast to the current methods, which are limited to the capture of a snapshot only at the end of the experiment, a camera allows pictures to be taken at defined intervals ranging from milliseconds to weeks. Video microscopy leads to a complete dynamic analysis of the treatment response. Moreover, the microscope is patient and methodical; pictures will be taken with the same watchfulness and desired details. Multiple clamps can be performed at the same time; for example, hundreds of cancer cells can be followed during a one-week period, or hundreds of Drosophila embryos can be imaged simultaneously [1].

The main purpose of video microscopy is to perform experiments with living beings on the microscope stage. For example, cells must be in conditions that promote their growth and normal function. Different types of video microscopes, such as confocal

microscopes, are now equipped with environmental culture chambers, thus ensuring that crucial conditions such as temperature, air conditions, pH, or humidity are not disrupted. This technique is noninvasive and permits continuous observation of live and living samples [1, 2].

On NCBI, more than 11,000 publications are linked to timelapse. With time, there are increasing applications of this technology in different fields. Owing to progress in computing and imaging techniques, analyzing the morphology and motility of cells is gaining importance. The first use of time-lapse video microscopy was in 1929, when the development of rabbit embryos was studied. This field of research is not limited to only animals: mice, hamsters, rabbits, zebrafish, *C. Elegans* and others have been used, including in humans [1]. The technique of video microscopy allows the characterization of embryo development in humans. This discovery had a significant effect on the evolution of in vitro fertilization. In fact, the most valuable aspect of this method is that it makes it possible to culture and monitor embryos without disturbing them, thus leading to a better selection of embryos with a greater chance of implantation success [3]. Another interesting characteristic of video microscopy is that it provides information on the position, motility, and shape of the cell. Indeed, during some events, such as morphogenesis, a failure or error in migration can result in living damage. Cell migration is also crucial for homeostasis, such as the immune response [2]. Moreover, analysis of in vitro motility is a useful tool for understanding the cellular response to a drug, for example. It is vital to analyze these parameters because changes in cell behavior can be promising indicators of drug response, cell development and differentiation during embryogenesis, or in vitro *fertilization* [4]. This principle is attractive for other research fields as well: *C. Elegans* models can be used not only to follow the different stages of larval development but also to visualize how proteins are moving, which is made possible by the transparency of the organism. Time-lapse imaging is an advantage in that these proteins cannot be overlooked [5]. For bacterial populations, antibiotic resistance is an ongoing issue caused mainly by the appearance of phenotypic variations in microbial populations. Single-cell analysis is necessary to understand the nature of bacterial resistance behaviors and to determine how these behaviors are affected by treatments to obtain interesting insights into this problem [6].

Finally, in medical biology, personalized oncology is the new future for treatment; it will replace "bulk therapy" in which there is no patient-specific treatment strategy. Single-cell analyses, such as sequencing or video microscopy, are used as more precise techniques for understanding the effects of drugs [7]. Moreover, video microscopy makes it possible to visualize the effects of drugs not only in vitro but also in vivo. It is possible to monitor cancer cell activity in live animals. Intravital video microscopy allows us to understand the metastatic process, nature (adherence, migration, vascularization), and timing of the proteins or RNAs implicated. An important benefit of this approach consists of a better understanding of the metastasis process [8].

However, video microscopy has many challenges. Biologists can be confronted with poor-quality images and low-contrast microscope images. In addition, expensive microscope equipment is necessary to perform state-of-the-art analyses. Even if a large variety of devices, software, and modalities exist, there are always limitations. For example, brightfield microscopy leads to a decrease in resolution and contrast, whereas

fluorescence microscopy requires the use of dyes that most of the time interfere with cell behavior. Finally, phase contrast microscopy is expensive, and a halo effect is sometimes visible [7]. In brief, there are always limitations for the human eye.

Another important limitation to consider is that the human analysis of video-microscopy images is time-consuming; for a long time-lapse (> 24 h), it can take more than two hours for the experimenter to analyze the video and several hours for data processing [9]. In fact, the main way to track cells is by manually marking each image of the video, which is not only a lengthy process but can also lead to errors. This motivated the increased research on the development of automated cell-tracking systems since the 90's [4, 10, 11]. In addition, video microscopy generates many data, so it is crucial to have the necessary drive space [1].

The main purpose of this review is to provide an overview of the video-microscopy technique. First, we will approach a historical perspective of microscopy and time-lapse microscopy, followed by a detailed section on the principles of video microscopy, and finally, the subject of image acquisition and analysis will be discussed.

Historical perspective

Early developments in microscopy

Microscopy, as it is known today, was initiated by a rather simple concept that was subjected to numerous improvements. As presented in Fig. 1, the earliest appearance of the magnification concept was an enlargement of letters using a globe of water by Seneca in the first century A.D. [12]. In 1284, this concept was used by Salvino D'Armate to design the original eyeglasses, which rapidly gained popularity [13]. A few years later, approximately 1595, Zacharias and Hans Janssen created the first compound microscope, which consisted of multiple lenses in a tube. The instrument was further developed by Galileo Galilei in 1609 with two lenses: the first lens collects light, whereas the second lens magnifies the image. It was not until 1625 that the instrument was named "*microscope*" by Giovanni Faber [14]. The word microscope evolved from the Greek words standing for "*small*" and "*to view*" [15]. The first observations recorded with a compound microscope were made in 1665 by Robert Hooke in *Micrographia*. Hooke analyzed seeds, plants, and the eye of a fly and used the term "cells" for the first time to describe the structure of cork [14]. While Hooke focused on multiple-lens apparatuses, Anthony van Leeuwenhoek explored the potential of single-lens microscopes and built over 500 of them [12]. His handmade instruments were quite simple, with a base, adjusting screws, and, in some instances, a system to control the illumination, which came from an external light source. The use of one lens rather than two lenses significantly improved the quality of the images by reducing the optical aberration that was observed at the time [14]. Some of his lenses had a magnification power of 300X and a resolution of 1.4 microns [12]. Leeuwenhoek used his microscopes to observe microorganisms such as bacterial cells that he described as "animalcules", and because of this discovery, he is known as the father of microbiology [16, 17].

In the following years, improvements were made to the resolution limit of the microscope. When more than one lens is used, spherical aberration disturbs the image, thus limiting the resolution of the microscope. In 1830, Joseph Jackson Lister reported that combining low-magnification lenses at precise distances from each other prevents

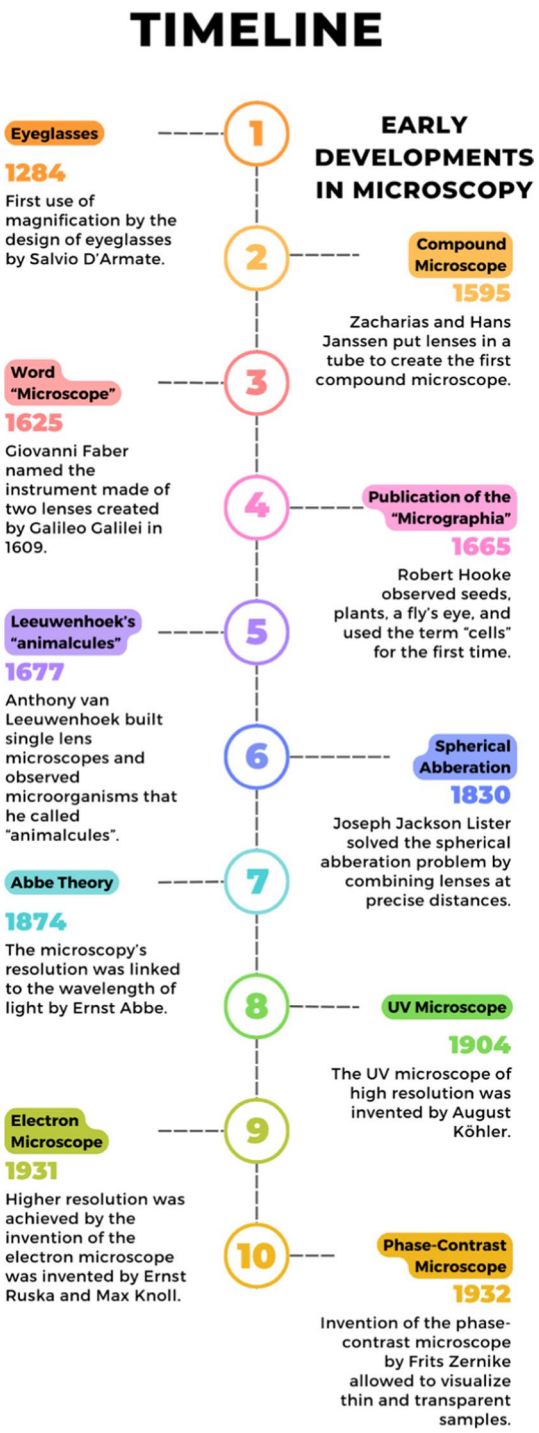

Fig. 1 Timeline representing the early developments in microscopy and important milestones from the first appearance of eyeglasses to phase-contrast microscopy [10–18]

spherical aberration caused by the subsequent lenses [12]. Forty years later, in 1874, Ernst Abbe published his theory that related the wavelength of light to the resolution of the microscope, which indicates that the resolution could only be smaller if a shorter wavelength was used. Therefore, in 1904, August Köhler made the first UV microscope

that overcame the classical light microscope because of the higher wavelengths used [18]. However, the greatest breakthrough came from the invention of the electron microscope in 1931 by Ernst Ruska and Max Knoll. Their original microscope had a magnification of 400x, and two years later, Ruska developed a more advanced model that surpassed the resolution of classical optical microscopes. Ruska analyzed the possible applications of this new microscope in 1937 with the help of Bodo von Borries and Helmut Ruska. Around the same time, the scanning electron microscope was designed by Manfred von Ardenne and was available commercially in 1938 [19]. Another crucial invention of the 1930 s was the phase-contrast microscope, which was developed by Frits Zernike. This new microscope has had a significant impact on biological research because of its ability to convert the light variations induced by a phase change into varying light amplitudes. This principle makes it possible to visualize transparent and thin samples such as living cells [20].

Milestones in video microscopy

Along with the development of microscopy, new technologies have been developed in the field of video imaging. Not only was it a significant discovery for recording and television, but it also allowed microscopy to become an even more powerful imaging tool. One important factor in the origins of video microscopy is the appearance of the closed-circuit television (CCTV) system. This technology, invented in 1927, uses video cameras to record and transmit images to a specific audience. The signal is thus not publicly available but is refined to viewers who were given permission; for example, CCTV is used in many stores for owners to discourage and potentially catch thieves or other criminals [21]. Scientifics started using CCTV to enhance the contrast of their microscopy images and to record time-lapse videos. It also facilitates classroom instruction and enables the use of a larger spectral range [22]. In the following years, different combinations of video imaging systems and microscopes were used, but crucial challenges were encountered, such as damage to living cells due to the use of UV light, insufficiently bright light sources, and a lack of image quality [22]. Some early apparatuses include a phase-contrast microscope used in conjunction with an RCA system, a flying-spot microscope, and a tandem-scanning reflected-light microscope [22]. The invention of image intensifier tubes during World War II made it possible to amplify incoming light and create a brighter image from a low light-level input image. These vacuum tubes can generate photoelectrons from light and increase their intensity to return a brighter image [23]. When applied to microscopy, the recurrent problem of a lack of bright light sources could be solved. In approximately 1970, Caspersson and his team developed a technique to image chromosomes via a camera combined with a fluorescence microscope [24]. Using a TV camera with a zoom lens and an amplifier, they made it possible to amplify the video signal to visualize the chromosome regions, which presented important variations in their fluorescence intensities. They project the photographic negatives of fluorescence-stained metaphases on a monitor and optimize the contrast depending on the chromosomes to analyze. The team also developed a similar system to analyze Giemsa-stained chromosomes and connected a TV camera directly to the microscope. This allowed us to produce a video of the chromosomes' localization but required high-quality optics that could be obtained with an ocular of

great zoom and an image intensifier tube. Both systems are time saving and overall more efficient than the previous karyotyping methods while allowing multiple people to look at the monitor simultaneously to conduct the analysis [24]. More recently, automated microscopy was developed for the detection and segmentation of biological structures such as cells. The algorithms and deep learning methods greatly simplified the analysis of microscopy and video-microscopy outputs in the field of hematology and in biomedical research [22, 25].

Evolution of imaging technologies in cell biology

Since the first cells were observed with a microscope, numerous techniques have been developed to image even smaller objects such as proteins and chromosomes. In 1936, the invention of a microscope photometer provided better contrast for the visualization of biological cells and tissues. A novel technique called microspectrophotometry was then developed by Caspersson and made it possible to quantify the concentration of specific substances in a sample. Given that cellular components absorb light at specific wavelengths, it is possible to use light absorption as a tool for chemical quantification. With this technique, Caspersson can identify nucleic acids and certain proteins in the nucleus and cytoplasm, respectively [16, 26]. A few years later, Evgenii Brumberg described the ultrachemiscope, which images biological samples using three different UV wavelengths and merges the images to obtain a "color transformation". This process produces an image in which nucleic acids, proteins, and pigments are shown and can be analyzed on the basis of their specific distribution [27]. During the same period, researchers such as Hans R used the principle of time-lapse imaging to visualize biological phenomena such as meiotic divisions. Spermatocytes and embryonic cells from *Tamalia coweni were studied* to investigate how chromosomes move during cellular division. Using a camera lucida, they recorded the distance between the daughter chromosomes over time and produced a graph describing the apparent movement [28].

In parallel, fluorescent dyes were discovered and developed for use in both cell biology and medicine. One of the most notorious dyes, fluorescein, was initially synthesized in 1871 by Adolf Ritter von Baeyer. This dye was used by Paul Ehrlich to study the relationship between intraocular pressure and aqueous humor production in the eye [29]. Ehrlich also applied a fluorescent dye to cell biology by staining bacteria, which led to a significant increase in contrast, thereby facilitating microscope imaging [18]. In 1893, August Köhler presented a novel illumination technique composed of a field and an aperture iris diaphragm in a microscopy setup. This would prevent the presence of an obscuring glare while providing a bright image [30]. Twenty years later, a new collaboration between Köhler and Zeiss Optical Works generated a microscope with ultraviolet illumination, which proved to be a significant advancement in microscopy [30]. The Zeiss factory pursued advancements in the field and presented its first fluorescence microscope in the early 1990 s. However, imaging organisms is still difficult because of their thickness and opacity. The issue was partially solved via incident light rather than transmitted light, an idea that was introduced by Ellinger and Hirt and which they called an intravital microscope. Their technique, which resembles modern fluorescence microscopy, involves the use of fluorescent dyes and ultraviolet

illumination to better visualize organisms [31]. Fluorescence microscopy was initially presented as a tool in pathology and histology by Herwig Hamperl, who also observed autofluorescence phenomena in plant tissues and different proteins. In collaboration with Max Haitinger, Hamperl is also at the origin of fluorescence staining used for fluorescence microscopy [16, 32]. Giemsa cellular staining made it possible for Halberstäedter and von Prowazek to identify trachoma inclusion bodies in the conjunctivae scrapings of baboons treated with trachoma secretions and thus demonstrated chlamydia pathogenicity [33, 34]. The potential of Giemsa staining has increased the scientific interest in fluorescence staining. In 1929, an intravital microscope composed of vertical UV illumination and a water immersion objective was commercialized by Zeiss. With such a microscope, it was possible to examine fluorescent dyes in the kidneys and liver of frogs and mice and to analyze their distribution; thus, it is a novel tool for the visualization of different living organs [16]. Another dye was also discovered that serves as a cell vitality indicator; the acridine orange dye presented by the Siegfried Strugger fluorescently stains cells green if they are alive and red when they are dead because of the difference in absorption capacity [35]. Immunofluorescence was presented by Albert H. Coons as a technique for the detection and visualization of mammalian antigens in biological samples when fluorescent conjugated antibodies are used. This approach is extensively used in virology and for the study of bacteria [36].

Currently, most microscopic methods rely on nonlinear optics, which include but are not limited to two-photon excitation fluorescence, harmonic generation, and stimulated light scattering [37]. Such processes were made possible via infrared pulsed lasers. Nonlinear optical microscopy and the related working wavelengths increased the penetration depth in biological samples and reduced the need for staining, which was, in some instances, detrimental to the samples. The technique can also be used for functional imaging since it allows for 3D sectioning, has a small focal volume and does not require any labels to observe samples. Some nonlinear optical processes include two-photon excited fluorescence, second- and third-harmonic generation, and coherent Raman scattering microscopy [38]. Second- and third-harmonic generation microscopy techniques are particularly relevant for the observation of biological samples because of their ability to construct 3D images in the absence of an out-of-focus background. However, the dependency on focal plane signal generation restricts the use of second harmonic generation for collagen fibers and striated muscle myosin. With third-harmonic generation, a greater number of structures can be observed because they can be induced from tissue interfaces and from colorants, which intensifies the signal [39]. For coherent Raman scattering microscopy, the use of two laser beams for sample excitation generates four coherent Raman phenomena, thus leading to a faster imaging speed [40]. Another technique relying on the use of a laser called multiphoton excitation microscopy is extensively used for the observation of tissue in vivo, especially in the field of neuroscience. The ultrafast pulsed laser in this technique provides great depth, speed, and versatility [41]. Biologists continue to make improvements and develop new laser sources, objectives, and molecular probes to further enhance the quality of biological imaging with microscopy [16].

Advanced video-microscopy techniques

Confocal microscopy

Described for the first time in 1953 but commercially available at the end of the 1980 s, confocal microscopy is different from optical microscopy; it has the particularity of taking optical sections with a low depth (400 nm). This makes it possible to obtain a series of images that can then be used to obtain a 3D representation of the object. To proceed with such modeling, the images are recomposed with a computer. Confocal microscopy can be performed using either reflected light or fluorescence. The illumination is made by a laser, which is condensed by a lens and then scanned in a pinhole beside the detector. This allows for the photons that are coming from the focal plane only to reach the pinhole. Thus, it is possible to obtain an optical slide of the focal plane only, and when proceeding to slides, a 3D representation of the observed object can be obtained [42, 43].

Multiphoton microscopy

Multiphoton microscopy is an imaging technique developed in 1990 that combines the techniques of fluorescence microscopy and two-photon absorption. It is used to image living tissues at depths of less than 1 mm. The main difference with fluorescence microscopy is the lower excitation light. The technique also results in fewer background signals. This method is also appreciated for its limited photobleaching effect [44–46].

Superresolution microscopy

The main limit of microscopy is the resolution, which implies that when a small sample is observed, the principal source of light is received as a distributed pattern. When many patterns overlap, the diffraction phenomenon makes it difficult to clearly observe two objects that are close together. Superresolution microscopes can overcome this limit and are thus very popular in biomedical research. However, their cost is high, and they can only be used with thin objects [47, 48].

Principles of video microscopy

Basics of light microscopy

All microscopes have the same main optical components, including an eyepiece, objectives, and an illumination source, which can also be referred to as a condenser [49]. Lenses are at the heart of the microscope components. Convex lenses are used to converge light and thus induce a magnification of an image. However, in microscopy, the convex lenses used are complex, as they should not only offer great magnification to allow for the observation of biological structures or other miniature objects but also account for aberrations that can reduce image quality. The objectives are described in terms of their magnification and numerical aperture (NA), which represents the lens focal length and is an influencing factor for the resulting resolution [50]. Different types of objectives exist, but the most widely used objectives are called achromatic objectives. Achromatic objectives converge light into a single focal point and are designed for axial chromatic aberration correction in blue and red

and spherical aberration in green. Such corrections can be sufficient in the majority of microscopy experiments, but in some cases, a higher degree of correction is necessary [51]. High-level chromatic correction can be achieved via apochromatic objectives, which are corrected in deep blue, blue, green, and red while also providing spherical aberration correction in deep blue, blue and green. These lenses with high numerical apertures are more expensive but are a better choice when observing samples in different colors [52]. A more economic choice can be to use fluorite objectives; their specially designed glass material gives them spherical aberration correction in up to three colors instead of one for achromatic objectives. Such objectives have improved contrast, image brightness, and resolving power [53].

In regard to sample illumination, numerous factors must be considered to optimize the technique used and ensure good image quality. Before deciding on an illumination method, the sample opacity, physical characteristics, composition, and sensitivity to heat or ultraviolet light must be established. For example, translucent samples should be visualized via transmitted illumination, such as brightfield, polarized, oblique or darkfield illumination [54]. One of the most widely used illumination techniques is Köhler illumination. In this technique, the light source is scrambled when it reaches the sample, which results in better control of the field size, power, and angle of the illumination. The overlying principle is that lenses produce parallel light rays from a lateral structure; thus, when the illumination source is placed on the focal point, the structure is scrambled. Köhler illumination greatly depends on the use of an aperture stop that controls the light power and can be adjusted depending on the sample to be observed [50].

Video-microscopy components

When proceeding to a video-microscopy experiment, the microscope of choice is used in combination with a video camera and a monitor. The microscope is first chosen and adjusted to optimize the visualization of the sample and obtain an image of great quality, which is then transferred to the image intensifier tube of a video camera. The input the camera receives is a 2D image; it must be processed to produce a video signal. The apparatus transforms the image into a linear train of high-frequency electrical impulses, most commonly referred to as a video signal. A monitor will receive this signal and will convert it back to its original form, i.e., a 2D image [22].

One of the key components of video microscopy is thus the video camera. There are a variety of video camera types to choose from, but one popular and reliable choice is the charge-coupled device (CCD) camera. This type of camera is known for its high resolution and its capacity to image samples when the light source intensity is limited. Furthermore, scientists particularly favor CCD cameras because they are associated with high resolution and are more sensitive than other cameras [55]. There are several drawbacks associated with those cameras, as their antinoise capacity is limited when the microscopy application requires a low frame acquisition time. They still represent a better choice than complementary metal oxide semiconductor (CMOS) cameras, which are associated with greater noise and do not provide the same level of image quality. CMOS cameras can still be used in some cases when noise removal is not crucial since they have high yield and low integration and are generally less expensive than CCD

cameras are [56]. Other types of microscopy cameras include monochrome and color cameras. For the latter, an optical filter will process the color data, which will then be interpolated by a color filter array that uses a demosaicing algorithm. Therefore, the colors are not measured directly, and the interpolation involves an inevitable margin of error. Furthermore, most color cameras use infrared filters to limit the presence of color aberrations, which in turn reduces the number of photons that can pass. When a monochrome camera is used instead of a color camera, the color filter array and the infrared filters are absent. This allows monochrome cameras to be less sensitive and have a broader spectrum of detection. Therefore, monochrome cameras work significantly better with low illumination and overall produce brighter images of higher resolution and do not have the margin of error that is introduced with the color filter array [57].

Finally, a digital image processor is required to increase the quality of the video-microscopy output. This type of processing is equivalent to darkroom transformations that are performed with analog images. Digital processing relies on algorithms based on a matrix of integers to reversibly remove the noise in images. This step is crucial, as even the most powerful microscopes can output images with noise, uneven backgrounds, artifacts, and many more unwanted defaults. Furthermore, with video microscopy, the camera can introduce additional imperfections in the image from the video signal processing, thus resulting in aliasing, camera noise, and poor contrast, among other things. Therefore, a digital image processor is imperative and must be fast, reliable, and efficient. Some digital image processing techniques include look-up tables, flat-field correction, background subtraction, image integration, convolution filters, and Fourier transforms [58].

Resolution and contrast

In any microscopy or video microscopy system, the most crucial parameters are the resolution and contrast obtained for the output images. It is imperative to understand the underlying principles of these two concepts to obtain high-quality images.

Resolution is described as a measurement of a microscope's capacity to distinguish two neighboring points. In other words, it is the level of detail that can be captured with optical systems [59]. The limit of resolution is, therefore, the smallest distance at which a microscope can detect two objects that are illuminated [60]. In microscopy, the resolution of the objectives is limited by diffraction because the angle at which light waves pass through the lenses impacts the apparent resolution. This is particularly true at high magnifications, where out-of-focus images might still be optically resolved even if they do not appear sharp [51].

In contrast, this concept refers to the ability of a microscopy system to differentiate a sample from the background. Contrast is also commonly known as the difference in light intensity between an image and background. While the way the sample interacts with the light is important to consider, the optical system also plays a crucial role in contrast enhancement. The level of contrast is affected by the aperture settings, grade of optical aberration, and detector, among other factors [61]. The user can adjust the contrast through several parameters. For example, the settings of the field and condenser aperture diaphragm can be optimized depending on the sample and application.

Other techniques to increase contrast include the use of an electronic camera, image processing, and sample staining [62].

The number of techniques, including video microscopy, significantly improved contrast adjustment. To name a few such techniques, differential interference contrast, polarized light, phase contrast, and darkfield microscopy have important impacts on the field [63]. One technique is called video-enhanced contrast microscopy. This advanced microscopy system makes use of a high-resolution high-light-level video camera and a light microscope with optimized settings that diverge from those of conventional systems. With digital image processing, including contrast enhancement and background subtraction, the output of video-enhanced contrast microscopy is a high-quality image. This allows individual microtubules to be visualized and to even image axonal transport in great contrast. For the first time, objects smaller than the resolution limit of the apparatus could be visualized [64].

Imaging modes

As previously mentioned, multiple imaging and illumination techniques exist because of their specific advantages. In this section, an overview of the main microscopy imaging modes is presented.

The simplest imaging mode, called brightfield, involves a compound light microscope and staining of the sample of interest. This technique is the one of choice for the study of biological samples when their motility behavior, morphology, or replication are of interest [65]. However, simple and efficient this technique is, its limitations are rather important to consider. First, staining of the samples for observation is crucial, and in some cases, it can be insufficient and impossible to have enough contrast for visualization. Furthermore, brightfield microscopy cannot be used when the background color is too similar to that of the sample or if the latter is transparent [66]. Brightfield imaging is still commonly used and sufficient for diverse applications in cellular and microbiology, bacteriology, and parasitology, among other fields. Negative and Gram staining are popular staining methods that are used in combination with brightfield microscopy [67].

On the other hand, the dark-field imaging mode is more complex and better at providing great contrast. The system is similar to a brightfield microscope setting, but the condenser is made differently; it includes a black disc to block the illuminating light. This results in the sample being illuminated by peripheral oblique light, thus enhancing the darkness of the background [49, 68]. Different types of condensers with varying diameter sizes are available. Furthermore, certain condensers are made specifically for dry or immersion objectives. Dark-field condensers must be used with objectives with a numerical aperture smaller than theirs. It is also possible to use central light rather than peripheral light with dark-field illumination; an image is generated from the light that is bent by the sample [68].

Phase-contrast microscopy is an imaging mode that was revolutionary as a nonstaining method. This technique is based on the destructive interference of light waves when they are out of phase at half a wavelength. This microscopy system thus works by speeding up the light that is directly shining on the sample to create a phase shift of half of the wavelength with deviated light. The image generated by this positive phase contrast

has darker features and details, whereas the background appears lighter [69]. The setup consists of a phase plate or phase rings and annular grooves that allow the diffracted light to be delayed. Together with the wavelength retardation caused by the specimen itself, the resulting light rays are in the optimal phase difference range to generate an image of great contrast [49].

The last imaging type that will be described in this section is fluorescence microscopy. Fluorescence microscopes are designed to isolate the emitted light, which enhances the contrast of the fluorescent structures with the background. To be more precise, filters are used to ensure that the reflected light has a higher wavelength than the excitation light. The excitation light rays are generated from multispectral light induced from an arc-discharge lamp and pass through an excitation filter. The resulting light is then reflected on a dichromatic mirror or a beam splitter, which allows the sample to be fully illuminated. The fluorescence of a sample causes light emission, which is redirected to the dichromatic mirror and passes through a barrier or emission filter. This ensures that only the wavelengths of interest keep obtaining the final image. Fluorescence microscopy is unique in that it is the only imaging mode in which the sample produces its own fluorescence, which is emitted spherically after excitation, independent of the angle at which the light source is directed [70].

Sample preparation

Cell culture techniques

During a time lapse, cells or other live beings must be under the same conditions that they would be under if they were in incubators. For this reason, an environmental chamber can be coupled to a video microscope. The first time-lapse incubator became commercially available in 2009 [71]. There are two types of chambers (Fig. 2); the first one is described as an "open chamber", which allows the user to put Petri or multiwell dishes. When the chamber area is accessible, manipulation of the cells is possible, which allows, for example, adding drugs or changing the medium. The second type is a "closed chamber" that provides better isolation from the environment and better control over the temperature. There are an increasing number of custom-made video microscope incubators that are being designed for specific purposes, and some private companies, such as *Incucyte, have* also commercialized different live-cell imaging and analysis systems [2, 72]. The main difference lies in the price of the available products, which is

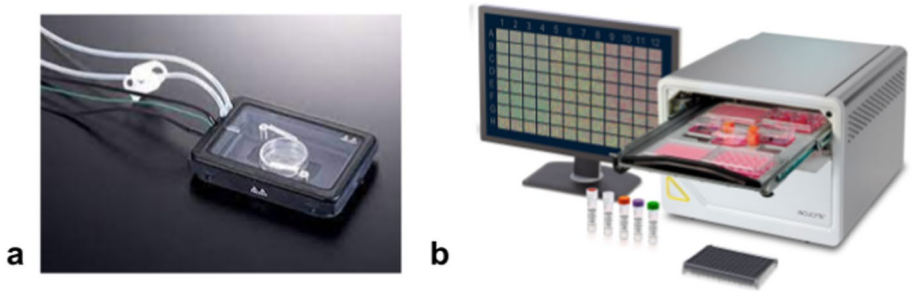

Fig. 2 Examples of different chambers; **a** INUB-GSI TOKAI HIT standard heating stage top incubator [77], **b** Sartorius' IncuCyte live-cell imaging [78]

directly linked to the related application. *The IncuCyte* is used principally for monitoring cell migration chemotaxis and is automated [73]. On the other hand, different models of time-lapse embryo monitoring systems exist; the first group consists of building an incubator environment around a previously available microscope. There is also a second group in which a time-lapse microscope system is placed in a standard incubator. Finally, the third group consists of a device with a specifically designed incubation environment and a generated microscope system inside it [74].

In all cases, live-cell chambers are coupled with a metal heating plate under thermistor control, and generally, a ring is placed around the microscope's optical lens. Not only are cells extremely sensitive to temperature variations, but microscopes are also highly sensitive. A shift in temperature can introduce unwanted movement in the optical path, causing a loss of focus [2].

Depending on the experimentation, there are different methods of image acquisition. In a long-term video-microscopy experiment, frames can be acquired every 10 min or over longer periods. In the short term, it is possible to take a maximum of 100 frames per second [75, 76].

Labeling and staining methods

One major problem in video microscopy is live sample labeling. Many biologists underestimate phototoxicity, which affects living organisms and can lead to death. Most of these deaths are caused by the production of reactive oxygen species (ROS). The deterioration of the samples is primarily the result of illumination overhead, which means that the fluorescence is not captured by the camera and still illuminates the samples [79]. One example of this phenomenon is the exposure of unlabeled green monkey kidney (Vero) cells for two to five minutes to blue light. Compared with cells exposed to direct sunlight, the authors reported a substantial reduction in cell proliferation with long exposure to blue light [80]. In general, fluorescent molecules absorb light energy and become excited. However, some of them can undergo photobleaching and generate ROS during the time-lapse process. These ROS can induce the production of catalases, peroxidases, or antioxidants [80]. This further delays the cell cycle. The high number of ROS produced in addition to the physiological ROS already present in cells cause enzymes to be overwhelmed, thus preventing the detoxification of ROS and causing cell death due to DNA damage. It is possible to measure the intensity of excitation with a power meter [80]. However, phototoxicity and photobleaching are two phases of this phenomenon; phototoxicity can affect organisms long before photobleaching is measurable [80]. To resolve this issue, two different solutions exist. The first consists of decreasing the power of the excitation light and compensating for it by increasing the exposure time. The second consists of decreasing the exposure time and increasing the power of the excitation light. This issue has been assessed by the development of fast-switching lamps (LED or not) and transitor-transitor logic circuits [79]. An example of a probe used for time lapse is SiR-DNA, a far-red fluorescent molecule that dyes DNA red and is well commercialized (Fig. 3). It is reported to be nontoxic, but some studies have shown that at concentrations less than 1 μM, SiR-DNA induces DNA damage responses and G2 arrest. This can be explained by the photochemical toxicity caused by

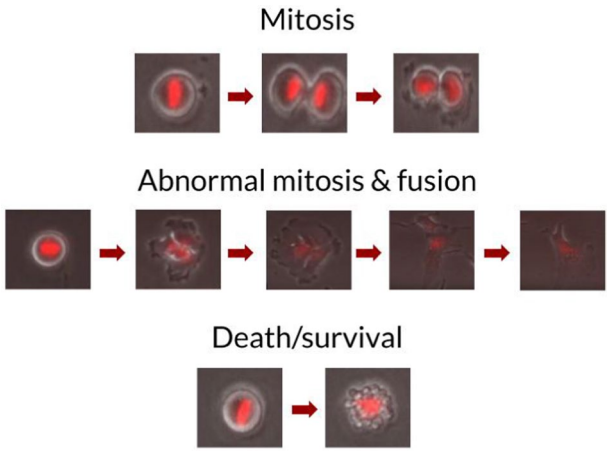

Fig. 3 An example of manipulations with Sir-DNA. Using this dye, we can identify different phenomena, such as mitosis, abnormal mitosis and fusion and cell death

the fluorescent excitation of molecules or by the DNA dying products that cause damage [81].

It is difficult to provide a general overview or protocol to prevent photo damage because it depends not only on the illumination but also on the nature of the sample and duration of the observation. Even the life cycle stage of a sample can affect the process; in fact, the early stages of cell development are more sensitive to illumination and damage [80]. Putting controls in total darkness without illumination is a good indicator of stress due to the illumination factor. Another phenomenon called "splash damage" is caused by excitation near the imaging field [80]. The majority of available cell-tracking systems are designed primarily for use with fluorescently labeled images [4]. However, time-lapse devices employ visible light imaging, which does not have a destructive effect on cells. This technique is a compromise between image quality and preservation of cell viability [2].

Image acquisition and analysis

Setting acquisition parameters

In a video-microscopy setting, the optimization of acquisition parameters is crucial for obtaining reliable high-quality data. The two main parameters that can be adjusted to better suit the experiment are the exposure time and the frame rate. The exposure time is defined as the number of photons that reach the camera detector and is time dependent; a longer exposure time is associated with a greater number of photons. This in turn intensifies the pixels, which results in a brighter image. The exposure time to be used must be adjusted depending on the camera choice and its associated dynamic range. The optimal exposure time requires some trial and error to be determined and is highly dependent on the signal or staining of the samples. In instances where the signal is not even, the exposure time must be set to obtain the best image possible, which might require some compromises. As a rule of thumb, a longer exposure time is better, but pixel saturation must be avoided. Luckily, most software shows the pixel intensity in red when it is saturated, thus making it possible to adjust the exposure time

so that most pixels are right below the saturation limit [82]. The second parameter that can be optimized depending on the experiment is the frame rate, which is related to the exposure time. The appropriate frame rate is determined by evaluating the speed at which the specimens move and thus how close each frame is to one another. However, high frame rates imply that the luminosity must be sufficiently high, thus limiting the exposure time [83].

Image processing software and image analysis

Once the video-microscopy experiment is complete and time-lapse images are obtained, processing software can be used to analyze the data quantitatively and qualitatively. Different analyses can be performed, but segmentation and cell tracking are among the most important methods and are discussed in this section. First, segmentation consists of the automatic detection of individual cells via a computer model. Most available methods rely on the intensity of the pixels and their distribution in the 2D space, but optimization of such techniques for a specific experiment is necessary and must be performed with extensive knowledge in code adaptation and image processing. The number of parameters, such as the threshold, can be subjective and affected by background noise, thus resulting in poor segmentation. Numerous algorithms have been developed and used in combination to overcome such issues [84]. Once segmentation is completed, the cells can be tracked to analyze their migration, cell cycle, and behavior. Video microscopy and cell migration analysis are crucial for the study of embryonic development, cell differentiation, the immune response, and tumor invasion. The plug-in Trackmate in Fiji (ImageJ) can perform both segmentation and tracking of cells [85]. While it is a powerful and user-friendly tool, it is not optimal for all types of images, and the output is not interactive, thus making it difficult and lengthy to analyze the results [85]. On the other hand, CellProfiler can also be used for tracking, but it does not provide a graphical user interface, thus making it difficult to verify the results while the images are being processed and to understand the effect of varying each parameter [85]. These software programs have difficulty using cancer cell lines when video microscopy is the tool used to study their comportment. For this reason, new software is being developed to facilitate cell segmentation, tracking, and other image analysis processes.

Computational advances and machine learning in image analysis

One major problem of time-lapse imaging is image analysis; analyzing the images composing videos can be time-consuming. To resolve this issue, the Cell Tracking Challenge was launched in 2012. The main goal of this challenge is to develop algorithms that can help biologists in their research. Moreover, with the share of robust cell segmentation and tracking algorithms, developers can evaluate their peers'algorithms. Since February 2017, the challenge has been opened for submissions that are ranked monthly and posted on the website, thus helping developers evaluate their algorithmic development. The first edition was dedicated to segmentation and tracking for time latches of labeled cells and nuclei in 2D and 3D. The second edition of the challenge in 2014 was dedicated to phase contrast in 2D and 3D developmental fluorescence data. Over the years, the cell tracking challenge has attracted increasing attention worldwide [86]. In 2023, the Cell Tracking Challenge published updates in

Nature Methods, presenting state-of-the-art methods and datasets [87]. Some other improvements in computational advances will be presented in this section as a sample of what the literature offers. As mentioned previously, the FIJI plug-in module TrackMate can be used for different experiments; for example, bacteria can be detected by this module, thus allowing the user to follow the total displacement of the bacteria [88]. A study compared 4 software programs for bacterial 2D time-lapse images: CellProfiler, SuperSegger-Omnipose, Deep Learning for Time-Lapse Analysis (DeLTA), and Feature Assisted Segementer Tracker (FAST). They discovered that deep-learning scripts performed the best. The main point to consider is how the software can perform segmentation of the bulk group of cells. Traditional segmentation involves watershed and thresholding adjustments, and both parameters are included with CellProfiler and FAST. For machine learning-based algorithms, DeLTA is trained on input images and corresponding annotations. Once the models are trained, they can generate annotations without the need for exterior input from the user [89].

Some scientists have also developed custom-made scripts for the following parameters. For example, a MATLAB code was designed for the measurement and analysis of cell morphology with *B. exovorus,* which allows the extraction of cell length and area [90]. In fact, an increasing number of homemade algorithms have been adapted to issues associated with biological research. Cancer Cell Tracker was developed to quantify the percentage of living myeloma cells and to identify the best therapeutic options for patients. In the age of precision medicine, this method has been revolutionary. It is standardized and easily reproducible; it makes documentation available to help participants use their algorithms [7]. Other researchers improved the analysis of virus time-lapse images by applying a superresolution algorithm, which resolved the issue of limited frame resolution. Their algorithm made it possible to detect and track the growth of viruses on individual plaques [91].

Application

Cell biology

Video microscopy can be used for live beings such as worms; it has been used since 1977 with the *C. Elegans* model [92]. Different fields of research use time–lapse microscopy, but this technique was initiated mainly in the field of cell biology [76]. It is used to provide information on the transport and localization of proteins and their involvement in cell fate. Unlike fixed-cell techniques, time-lapse microscopy allows dynamic analysis, in contrast to a "snapshot" of a precise moment [93].

However, the benefit of such a technique also comes with some challenges; it can be difficult to focus on cells that are suspended. Some researchers have used agar pads to force the cells to be suspended at a defined location [93]. Additionally, when studying cells or complex organisms, disorganized or intricate movements can complicate analysis, whether conducted by humans or automated systems. The longer the sequence, the greater the complexity. [94, 95].

As explained earlier, personalized medicine is an emerging domain that will surely be revolutionary. This represents the future for serious illnesses that used to be considered untreatable, such as cancers [96]. As an example, Sahu et al. used reflectance confocal microscopy to capture the real-time evolution of basal cell carcinoma and melanoma

in vivo in patients. Video microscopy is perfectly appropriate for such research, as an analysis of patient-derived cells significantly helps clinicians make decisions on optimal treatment options. Moreover, it can be applied at any time during the progression of the disease. In other words, video microscopy is reusable for each patient at each time point. Furthermore, this technique is complementary to the use of genomic or proteomic data. In this case, the main goal is not to identify prognostic markers but to determine the single-cell evolution of cells exposed to a particular treatment. In other words, video microscopy can define, in a heterogeneous cancer cell population derived from patient tumors, how cancer cells respond individually to a specific drug and/or drug combination and predict the best treatment for the patient. Finally, this technique is noninvasive [7].

Cell division and its dynamics

Mitosis is the principle of division, which is highly regulated during cell life to protect against aberrant genetic transmission of information [97]. In terms of cell division, several noteworthy examples emerge, such as divisions for the development of individuals. With the *C. In the Elegans* model, researchers were able to measure seam cell division and distal tip cell migration. Using video microscopy, they monitored and controlled the cell cycle of *C.elegans* during embryogenesis as well as gene expression [98, 99]. Moreover, in cell division, it is crucial to know the cell cycle and which stage the cell is in. With temporally multiplexed imaging, it is possible to analyze the relationships between kinase activities in a single-cell manner, thus making it possible to identify different cell cycle signals and signal transduction cascades. This technique is interesting because it allows us to understand the precise relationships between the signals. Temporally multiplex imaging is an inexpensive strategy for analyzing signals in living cells and understanding the temporal properties of the cell cycle. The authors showed a relationship between CDK2 and CDK4/6 in specific phases of the cell cycle. However, it is not suitable for the detection of fast-changing dynamics (less than 0.5 s), and the intense illumination that is required must be considered [100]. In the field of live-cell imaging and quantification of proteins involved in the cell cycle, Cyclin B1 was quantified after nocodazole treatment, and mechanical stress and aberrant mitoses were evaluated [101]. Finally, cell division can also be studied in the context of diseases; Aurora kinase A was assessed in HEK293 T cells for chemoresistance via fluorescence time lapse [102].

What about the dynamic inside the cells? Many phenomena are characterized by changes in organelles or the cytoskeleton. Video microscopy offers an opportunity to gain insight into such events and to understand the underlying dynamics [103]. Observation of organelles and the cytoskeleton allows the elucidation of several points, such as embryo development [104]. Conventionally, the behavior of DNA is observed by injecting mRNA, which makes it possible to visualize the gene via fluorescence. Currently, live-cell imaging allows the observation of DNA (chromosomes), microtubules or microfilaments by dying agents [104]. During mitosis, the position of the centrosome is crucial and is regulated by a number of factors, either cytoplasmic, nuclear or extracellular. High-resolution video microscopy revealed that the centrosome in RPE-1 cells is regulated only by nuclear signals [105]. Another possibility for observing

chromosomes is to use StayGold, a fluorescent protein that is currently used to visualize cell fusion or for other membrane-targeting applications [106]. Furthermore, it can be used to observe the dynamics of chromosomes. With its improvements, StayGold is now able to target mitotic chromosomes, movement of the Golgi apparatus, and repartitioning of actin in live-cell imaging [106]. For the nuclear envelope, the envelope disassembles during the prophase stage and reassembles at telophase. Images were taken every minute with a DeltaVision RT microscope. However, the authors determined that some elements are lacking in their system to perfectly mimic the formation of the nuclear envelope subdomain [107].

In another study, an analysis of live images of organelles after virus infection was performed. With a reporter fluorescent label, the subcellular parts, such as microtubules, lysosomes and mitochondria, are colored [108]. Furthermore, it is now possible to visualize the dynamics of F-actin and MyoF into the cytosol of a parasite via video microscopy. The authors showed that MyoF organizes actin in Toxoplasma to promote cargo movement [109].

Moreover, observation of the structure of the endoplasmic reticulum and the nuclear envelope was made possible via video microscopy. There is no doubt that these junctions are vital for cells, but little is known about their structures. To resolve this issue, the authors investigated the ultrastructure during the cell cycle via live-cell imaging and high-resolution 3D imaging [110].

Developmental biology

Embryonic development studies

A number of studies were conducted on *C.elegans*; one notable article published in 1983 by Sulson and al. showed the complex embryonic cell lineage of the nematode [111]. Additionally, in the 1970 s, *Fertility and Sterility* published the article "In Vitro *Fertilization of Rabbit Ova: Time Sequence of Events*", in which the author provided documentation on the timing of spermatozoa penetrating the oocyte, polar body extrusion, formation of pronuclei, and cell division. During the experiment, 16 pictures were taken every minute [112]. With the rapid advances in microscopy technology and, more specifically, video microscopy, embryonic development studies in live time have been conducted. It is a powerful tool for recording living embryonic cells over time. This technology has been used for the selection of single embryos.

One of the most important aspects of this technology is that it is noninvasive and thus allows researchers to follow the morphology and development of embryos postfertilization. Most importantly, the observation period from fertilization to transfer is uninterrupted. Previously, embryo monitoring before implantation involved multiple steps back and forth from the environment to evaluate different parameters (morphology and developmental progression, for example). With this method, embryologists end with a single picture a day and disturbed culture conditions, which led to detrimental impacts on the embryo. With video microscopy, embryologists have more detailed information, such as the timing of cell division and changes in embryo morphology. The multiple advantages of video microscopy and the data quality it provides allow the selected embryos to have greater implantation potential.

Previous studies have shown that time-lapse devices allow the identification of morphokinetic parameters such as the disappearance of pronuclei and the duration of the cell cycle, which are indicative of low implantation rates or a greater risk of blastocyst development. Similarly, aneuploidy (an abnormal number of chromosomes) is a major hurdle *in* in vitro fertilization (IVF). Most tests used to verify whether an embryo is aneuploid require the extraction of one or more embryonic cells via biopsy or are invasive, which frequently leads to the death of the embryo. However, video microscopy provides a noninvasive alternative; some of the morphokinetic parameters remarkable by video microscopy are used to identify ploidy. Interobserver and intraobserver variability in video-microscopy evaluations are extremely consistent and reproducible, in contrast to static embryo culture and assessment [1, 71]. Moreover, the availability of video-microscopic devices for human studies has facilitated the use of this technique [74]. Owing to the development of artificial intelligence (AI) analysis of time-lapse embryo development, spontaneous abortion of a selected embryo can be predicted with 77% accuracy [112].

Tissue morphogenesis

In the context of tissue morphogenesis, bones are an interesting subject. They are highly regulated in terms of microstructure owing to mechano-regulation. This principle was investigated via in vivo video microscopy [113]. Obviously, some video-microscopy techniques also exist for tissue morphogenesis imaging in embryology. In fact, neural tube closure was investigated because of its designation as the most common human birth defect. This study is based principally on previous research on Axolotl and Xenopus. Even if it is impossible to compare different species, numerous similarities could be observed [114]. The closure of the neural tube was visualized via tdTomato fluorescence in the two species previously mentioned as well as in mouse models [115]. Finally, the same technique can be applied to vegetal studies; time-lapse imaging was combined with mechanical measurements to map the age of the cell wall and determine its elasticity in *Marchantia polymorpha gemmae* and *Arabidopsis Thaliana*. Indeed, the different parts of the plant do not grow in an isotropic manner and have some preferences in terms of growth direction [116].

Neuroscience

Neuronal imaging and synaptic activity

Studies using video microscopy for neuronal imaging and synaptic activity are numerous. Transfection with fusion proteins such as Rasal1 is common, and depolarizing solutions are used. Pictures are generally taken in a short time to understand the dynamics of the cells. For example, Rasal1 and its translocation have been implicated in neuronal development [117]. In another study, neurons were used to assess neurite integrity, and pictures were taken every 2 h over a period of 6 h via an IncuCyte. The neurite length and number of branch points were calculated, and the data were used in the study of Alzheimer's disease [118]. On the other hand, it is also possible to explore neurons in living animals. Owing to their transparency, zebrafish can be used to observe the fluorescence of dyed neurons [119]. Two-photon

live imaging is also possible and was used to track individual cells in the mouse cortex. The focus of this study was to understand astrocyte-to-neuron conversion [120].

Microbiology

Microbial populations show many variations, which is why investigations of individual cell behavior have increased in interest. The heterogeneity of bacteria originates mainly at the level of gene expression. Currently, gene and protein dynamics are analyzed via transcriptome and/or proteome analysis. Unfortunately, such techniques do not allow the detection of gene variation throughout the lifetime. However, the apparition of video microscopy was a revolution and made it possible to overcome this issue [6]. A protein can be tagged, and its evolution in cells can be followed in real time, which further provides an analysis of the characteristics of the cell morphology. For example, it is possible to analyze the protein AKT in individual cells and to observe the motility of *P. aeruginosa* via the use of green fluorescent protein [121]. AKT is an important protein in cell division and replication. Another application made possible by video microscopy is the monitoring of prey–predator interactions between *B. exovorus* and its prey fate via time lapse. The research group who first studied this phenomenon presented new information about *B. exovorus* attacks and the role of the S-layer [90]. In the context of biofilms such as *P. aeruginosa* biofilms, 3D time–lapse confocal imaging makes it possible to investigate the diffusion of proteins such as lectins. It was shown that chitosan nanoparticle penetration was improved in the presence of DNAse with both *P. aeruginosa* and *Staphylococcus aureus* [122].

For viruses and viral particles, it is challenging to focus on cells, particularly on suspended cells. Some researchers have used agar pads to monitor the cells correctly and control their location. For example, it was used for human immunodeficiency virus type 1 [93]. Moreover, time-lapse imaging can be used for investigating the behavior of viral plaques, which is crucial for understanding their spatial dynamics [91].

Discussion

Technical limitations

One of the main drawbacks of video microscopy is sample visualization: dyeing the nucleus or organelles has a severe impact on biological processes. In addition, phototoxicity and photobleaching effects must be considered to prevent side effects that can impact living beings, which is of particular importance in reproductive biology [79].

On the other hand, currently used embryology video microscopy does not allow for embryo rotation, a phenomenon that is particularly important for understanding blastomeres [74]. Monitoring a population of heterogeneous cells is also a major hurdle in video microscopy. This is particularly true when a biopsy is taken from a cancer patient and cultured, even when a single cancer cell line is cultured. Studying these types of cells in vitro is still a great challenge.

Finally, the majority of the studies are made on transparent subjects, making it easier the visualization of the components or stains [123, 124].

Overcoming imaging artifacts

An artifact is an object that has undergone even the slightest transformation by humans; thus, it is distinct from any object whose modification is due to a natural phenomenon. Scientists have been trying to understand how to overcome artifacts for many years. An analysis of vascular networks revealed that representing 3D vascular networks is laborious and involves many artifacts. To resolve this issue, the authors created 3DVascNet, a deep learning-based software for automation and quantification [125]. Artifacts can also result from the inherent contractile movements of the lungs or heart. New software was developed to balance these effects [126]. In oncology, artifacts limit the ability of radiologists to analyze mammography; computer-aided diagnosis was created to overcome these difficulties [127]. When biological samples are imaged, the interaction between light and the object can affect the image formation process. Artifacts generally come from single-sided illumination, which makes stripes appear and contributes to the poor quality of the images. To reduce this effect, light-sheet fluorescence microscopy was developed with selective illumination of a single plane of a sample. However, sometimes, this path occludes the light sheet. A study presented a solution with a flexible multibeam light sheet [128]. Furthermore, imaging systems such as EVOS (produced by *ThermoScientific*) encounter some issues with image analysis. Some of these issues involve stitching artifacts that impact the efficiency of the analysis. To overcome these problems, scientists created cell counting pipelines from the images taken by EVOS. Illumination correction was automatically applied [129].

Future directions in video microscopy

Finally, what could be the future of video microscopy? However, the progression of this technique has occurred in recent years; some improvements are still needed. The integration of an automatic cell tracking system coupled with a video-microscope device could save time but would also reduce the subjectivity of the analysis.

Artificial intelligence, particularly deep learning and machine learning, offers new perspectives. However, as mentioned earlier, image artifacts can pose significant challenges. Moreover, progress is hindered by a general lack of understanding surrounding AI. Regular updates are essential to help biologists learn how to effectively integrate this tool into their daily work [130–133]. Improvements in artificial intelligence will make in silico experiments possible with computer simulations. Far surpassing the human capacity to generate experiences, as well as inter- and intra-experimental variability [134].

But for the moment, guidelines on the duration of illumination must be established to provide generalized protocols. Moreover, this technology benefits from being accessible to everyone at reduced costs to help in the linearization of time-lapse imaging [112].

With this aim in mind, we developed a new software named Toto-Cell that can provide quantifying and qualifying data of lime imaging information [76].

Conclusion

In conclusion, the advancement of single-cell analysis has grown exponentially in recent years. Alongside video microscopy, techniques such as genomic and proteomic sequencing at the single-cell level have emerged. Novel methods like seqFISH and MERFISH enable researchers to analyze the spatial distribution and copy number of RNA or DNA within cells on a larger scale [135–138].

Video microscopy has led to significant improvements in biological research, providing novel insights into cellular and subcellular processes. This review outlined the historical development of microscopy and the parallel evolution of video microscopy and emphasized the significance of this technique in various research fields. Furthermore, the challenges and limitations of this technique, such as phototoxicity, photobleaching, and difficulty in cell tracking, were mentioned, and several potential solutions were presented. Video microscopy opens the realm of possibilities, especially when combined with other imaging technologies, and provides answers to a variety of biological questions and for the study of many human diseases.

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LIR and KB wrote the manuscript, and EA supervised, reviewed and revised the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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