

Time-Lapse Microscopy of Living Cells: From Microcinematography to Sequential Image Digitalisation

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Keywords: Time-lapse microscopy, video microscopy, digitalized picture series, microcinematography, living cells

Abstract.

Dynamic nature of many cellular processes can only be scientifically valuable if studied in a suitable sequential format. Such a format is nowadays acquired with a combination of microscopy and image capturing devices. In this chapter, various analogous as well as digital possibilities are discussed in terms of their advantages and disadvantages for the particular study models.

Introduction

Dynamics is a basic attribute of life; none of its definitions must omit this characteristic feature. In basic biomedical research, capturing of dynamics (dynamic image capturing) is often employed for analysis of cell motility, organelle movement, cell growth and proliferation, assessment of the cell-xenobiotic interactions and in many other applications. Recording of dynamics and its analysis is also used in development and testing of new materials and medicines.

Considering the size of cells, it is obvious that the dynamics on cellular level may be studied only with help of microscopic techniques. Some of these techniques are feasible in this respect (optical and acoustic microscopy), others not (transmission or scanning electron microscopy). Various cellular activities proceed at different speed, with only some of them being slow enough to allow our visual analyzer to capture it and analyze. Other processes are too slow or too rapid. Technically, the problem may be solved by capturing a sequence of pictures of the given object on a recording medium. Traditionally, this medium represented 16 mm film, with the technique being called microcinematography. Microcinematographic recording was performed by normal speed (the speed of projection) or using higher speed (time magnifying glass) or lower speed (time-lapse), respectively. Time magnifying glass recording was used in studies of activities whose duration is measured in fragments of seconds or maximum seconds. Time-lapse approach is preferred in studies of activities lasting longer (up to days), and this type of capturing strategy in particular will be addressed in this work.

History

In the first half of 20th century a great many scientific films were prepared which successfully documented various biological activities while often leading to new discoveries (for example contact inhibition of movement and origin of multinucleated cells). Besides their scientific value, series of these films are used for teaching purposes, and that is why there were found institutions which professionally gather, store and utilize them. One such an example is IWF Wissen un Medien gGmbH (former Institut für den Wissenschaftlichen Film in Göttingen - see [http:// http://www.iwf.de/](http://www.iwf.de/)). With increasing interest in “micro world seen through camera” there were found scientific societies for microcinematography and international scientific meetings were organized. One of them was traditional “Cytocinema” which was organized by our

department since 1968 at regular basis every 3 years; the last meeting of this type took place in 2002 (<http://cyto.fxnet.cz/>). Recording on 16 mm film ensures high image quality; however, it is complicated by unavailability of black and white materials and by cost of color materials. From the point of view of contemporary science, the most serious disadvantage of this method is a long lasting procession, and thereby impossibility of actual changes (according to the results) in the experiment [1]. Moreover, impossibility of attachment of commentary to sequences and their rapid retrieval on demand further contributed to abandonment of this approach towards the end of 20th century.

In the eighties, 16 mm film as a recording medium was being gradually replaced with videotapes and classic cameras were exchanged for video cameras. At the end of eighties, the first special time-lapse videorecorders appeared in laboratories. In our laboratory, we used a videorecorder Mitsubishi HS-S5600E and HS-S8300E. With their help it is possible to capture (project) individual images as well as choose different capture frequencies (projections). The quality of recordings is based on technical parameters of VHS system. The great advantage of video recording is a possibility of immediate recording inspection. Furthermore, this recording has great capacity and therefore is cheap. On the other hand, rapid localization of chosen sequences is still laborious and a system of indexing has to be used to improve this time-consuming procedure. Another difficulty is associated with storage of tapes where a care has to be taken to avoid magnetic field and a number of copies are required as frequent use of recordings leads to their damage.

Since the nineties, it is possible to record microscopic image directly by means of digital cameras and store the final file in a digital form. This way of recording offers all advantages of computer-enhanced image analysis. The comparison of basic characteristics of all three systems is given in Table 1.

Table 1

Parameter	Film	Video	Digital recording
Image quality	high	low	medium
Speed of recording procession	slow	immediate	immediate
Storage of recordings	good	worse	good
Sequences retrieval	slow	slow	immediate
Analysis of individual frames (images)	possible	possible	possible
Resistance of recordings against use	medium	low	high
Choice of intervals during recording	unlimited	limited	limited
Capacity of recording	unlimited	unlimited	limited
Possibility of image description	None	(sound)	excellent
Cost of device	high	high	high
Cost of black and white recording	low	low	medium
Cost of color recording	high	low	medium

Present state

Nowadays it is possible to find two basic ways of recording dynamics of cellular changes in laboratories: video microscopy and digital microscopy.

Video microscopy is the combination of video technology (camera and image processor) and sophisticated microscopy (differential image contrast - DIC or phase-contrast microscopy). This technique allows very substantial enhancement in the gain of relevant information. The resulting resolution of video image is

sufficient enough to be indistinguishable from that of photographic film. The main advantages of this technique are:

- Direct display of recorded images
- Optional recording (from time-lapse to ultra speed)
- Digital contrast enhancement
- Recording at very low light levels (possibility of fluorescence emissions *in vivo*)
- Easy transfer of electronic images and their further processing

These technical innovations enable visualization of specimens invisible to the human eye (video-intensified microscopy, enhanced contrast microscopy, photon-counting cameras - see other chapters in this volume).

Digital microscopy makes it possible to record images in fully digital format. At present many firms offer software programs called image analyzers (LEICA Q550 IWB, Image-Pro ® Plus or LUCIA DI) which are tailored to support acquisition and analysis of individual frames (up to hundreds in various applications) but not bulky image series. To record and process such series, it is necessary to employ powerful devices which have only recently appeared on the market. One such a promising apparatus is digital Videodisc Recorder (LVR 4000AP) produced and marketed by Sony. Different technical strategy is used in special digital videorecorders (for example Mitsubishi DX-TL930). Despite their obvious technical advancement, their use in biomedical sciences is limited owing to the fact that they do not allow the conversion of acquired digital sequences into common formats and their procession in the computer. Therefore, in our laboratory we use a hybrid approach; i.e. recording of sequences with a time-lapse videorecorder and subsequent digitalization of the selected sequences.

Hybrid system – Digitalization of videosequences

This system is based on the original record of cellular behavior stored on magnetic videotape and acquired by time-lapse videorecorder. The resulting record is subsequently converted into digital format while taking advantage of the entire range of editing options. Finished digital files may be freely incorporated into presentation programs (Microsoft or Apple) or, and it is very important, they may be easily published in electronic Internet-based journals. The above-mentioned facts clearly demonstrate that future belongs to digital techniques in image analysis. Basic advantages of this approach include sufficiently high image quality, possibility of data transfer, flexible control of image quality, instant availability of all acquired images, electronic image databases comprising image descriptions and sharing images via Internet and electronic journals.

The hybrid approach employs the following steps:

- 1) Image capturing on magnetic videotape
- 2) Digitalization of recording - editing
- 3) Presentation
- 4) Storage

1) Video capturing

- it is required installed grabbing card = A/D converter
- it appears ideal to have direct signal transfer from camera; in this case the signal is digital (no noise)
- A/D converter is more expensive than fully digital interface (IEEE1394 – FireWire)
- sufficiently powerful hardware, in particular if the card does not contain hardware compression codec
- harmonization of card software (drivers – hardware platform)
- sufficient speed of disc subsystem (rapid E-IDE discs, RAID, eventually SCSI)
- special capturing software for time-lapse video (Ulead Media Studio)

- shareware utilities x complex dedicated software (VirtualDub)
- adequate compression codec for capturing of analogue video MPEG-4, MPEG-2, MPEG-1

2) *Digitalization and editing of video recording*

In this step, the most seminal question concerns suitable software. Apart from professionals' needs where different requirements are applied, amateur video standards are most satisfactorily met by Adobe Premiere 6.0 program. This software enables relatively rapid digital conversion of analogous recordings into digital format, their retrieval, editing and storage. Furthermore, cutting, various visual and sound effects plus descriptions of scenes are possible as well. Still, the entire process is rather system demanding as the size of single digital image at resolution 512 x 384 pixels is 384 KB, thus 5 minutes lasting videosequence (6,000 images) takes about 2.2 GB. To solve the problem of size, one has to think about compression. When it comes to compression, we have several possibilities of which we may choose a compression format, which is most suitable to our particular needs. Generally, the most common is MPEG (moving picture expert group) compression which exists in the following three formats:

	MPEG-1	MPEG-2	MPEG-4
year of introduction	1993	1995	1999
bit rate	1-1,5Mb/s	up to 100Mb/s	0,01-1Mb/s
image quality	sufficient	excellent	excellent
hardware requirements	150 MHz	300 MHz	400 MHz
size of film (120 min)	2CD	1DVD	1CD

- MPEG-4 offers higher quality, final files are smaller, but is hardware demanding
- MPEG-2 offers high quality, in particular for DVD and digital TV, resulting are bigger files
- MPEG-1 offers lower quality, but considerable compatibility; it is not demanding for hardware, resulting are bigger files

3) *Presentation of video sequences*

Digitalized video sequences may be stored on any transport medium; i.e. IoMega ZIP, CD-ROM, MO, DVD. Nevertheless, regarding various formats of coding it is required to accompany sequences with used codec. Sequences may be presented by means of specialized programs; for example ATI, WinDVD 2000 or PowerDVD.

4) *Storage of video sequences*

When choosing an optimal storing mode, the first question concerns again the type of compression program (codec). Each type of compression program offers different resolution (352x288, 640x480 and 720x576), bit rate (see above) and frame rate (typically 20-25 frames per second) and therefore careful selection has to be made to satisfy individual needs. Once the compression format is solved, we have to choose the medium where our recordings will be stored. Today we may select from CD (the average capacity is about 700 MB – approximately 120 minutes lasting recording), magneto optical mechanics (the capacity is in the order of GBs), magnetic devices (IoMega JAZ – the capacity is about 1GB) or DVD (the capacity is about 4.7 – 9.4 GB). Generally, the use of media with higher capacities is nowadays still limited because of their prices (magnetic devices) or prices of their mechanics (DVD burners). Moreover, still present complication is the existence of numerous mutually incompatible formats.

Common technical problems

The successful use of all the methods of time-lapse video microscopy presumes adequate handling of many common technical problems associated with long-time observation of living cells. These problems include maintenance of long-term constant temperature (37°C), anti-shock protection, maintenance of image sharpness and limiting the negative influence of light on cells. The special chapter represents the construction of the distinct cultivation chambers which on one hand enable the use of the modern microscopic techniques, and, on the other hand, allow the exchange of cultivation media during experiment. This part of problem is addressed elsewhere [2].

Publishing of results

Our research is oriented towards dynamic aspects of cell death. In our models we have experimentally proved that cells dying by apoptosis walk through various stages of which some are surprisingly dynamic (for instance so called blebbing stage) and these morphologically distinct periods are well correlating with other molecular changes such as the specific caspase-3 activation. The results appeared in the Internet journal *Frontiers in Biosciences* (<http://www.bioscience.org>) along with digitalized video sequences. Other sequences can be accessed at our departmental homepage (<http://www.biologie-lfhk.cz>). On the whole, publishing of digital sequences is only possible in fully electronic journals whose number to our knowledge is very low. Let it be hoped that in the future this new way of presenting scientific information will attract more interest from among publishers.

Future

In the area of time-lapse video microscopy the future will be no doubt of digital imaging due to the above-mentioned advantages. It is certain; however, that the future advancements in this field are strongly dependent on hardware and software development. This can be seen especially in fluorescence microscopy where the use of confocal microscopes, one-photon sensitive cameras and powerful software greatly reduced once serious limitations such as murkiness of images, photobleaching and phototoxicity. Combination of these approaches suggests that sometime in the future we will be able to track the fate of individual fluorescent-labeled molecules in the living cells much the same way as we observe morphology of dying cells in phase-contrast based video microscopy. Microscopy will then display not only morphological data but also biochemical and physiological information. On the other hand, no matter how enthusiastic are we about digital techniques we have to keep on our mind one basic rule: „The best digital images are still obtained by presenting the best possible optical image to the camera”. Therefore our goal should always be to prepare perfect digital images as a source of useful scientific information.

References

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