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ESSAY

ARTICLE SERIES: IMAGING

Cells on film – the past and future of cinemicroscopy

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ABSTRACT

Movie making is now a ubiquitous experimental tool that biologists use alongside more traditional techniques such as molecular biology and biochemistry. It is no longer just cell biologists, but scientists from many other disciplines, such as immunology and neuroscience, that utilise movies to dissect their processes of interest. When did filming become such a standard laboratory technique? Who developed the use of the movie as an experimental tool? The Wellcome Library has recently restored and digitized a number of original 16-mm films from two pioneering cinemicroscopists, Ronald Canti and Michael Abercrombie, which are now freely available to the scientific community. In light of these films, this Essay will give a brief history of the early cinemicroscopists and discuss what is driving the use of movies in the laboratory today.

Introduction

Most cell biologists these days are also cinematographers. Making movies of the cells, tissues and embryos that we study under a microscope is a regular occurrence in the laboratory. Like cinematographers working on any cinematic production, we are in charge of the technical aspects of filming. We choose the type of microscope and microscope lens, along with deciding on the lighting of our 'actors'. To be clear, there is no director in these productions; the cells are responsible for their own performance and we are only there to facilitate their storytelling. This approach is certainly not unique. Movie making is now commonplace in virtually all biological disciplines and is part of any experimentalist's toolkit. When did making movies of cells become such a standard technique? Who were the pioneers of 'cinema photomicrography' as it was originally known? A number of research groups in the UK played a central role in the early development of tissue culture and cinema photomicrography, with many working at the Strangeways Research Laboratory in Cambridge (Wilson, 2005). With the help of the Wellcome Library, films that had been sitting on a shelf in our laboratory from two Strangeway's cinemicroscopists (Fig. 1) have been digitized and are now freely available on their website for all to see.

Cinemicroscopy before cell culture

It is interesting that the very first use of motion picture photography was not for entertainment but for an experimental purpose, and some scientists have even claimed that "the motion picture originated in the biological laboratory" (Rosenberger, 1929a). In 1872, Eadweard Muybridge used a series of cameras with automatic shutters to document the stages of a horse's trot. This 'movie' allowed him to freeze time and visualize events that were difficult to see with the naked eye (e.g. that all four feet of the horse were for a moment off the ground while trotting) (A Horse's Motion Scientifically Determined, *Scientific American*, 1878). Very soon after the advent of motion picture cameras around the turn of the century, cinematographers turned their cameras on microscopic specimens. In 1891, Etienne Jules Marey, after perfecting his microchronophotographique technique, filmed red blood cells coursing through a capillary. The first microscopic movie of a living organism was of cheese mites in 1903 (see article by S. Pain, Histories: Microscopic stars of the silver screen, *New Scientist*, 2008), and in 1909 Jean Comandon who is thought to be a pioneer of cinematography for scientific purposes, filmed the syphilis bacteria at the Pasteur Institute in Paris (Microbes Caught in Action, *New York Times*, 31 October 1909). However, it was documentary film makers, such as Percy Smith and Francis Duncan (of 'cheese mite' fame) rather than scientists, that drove the development of early cinemicroscopy. Even Jean Comandon, who developed the technique as part of his doctoral thesis, subsequently went to work for the French film company Pathé Frères. Filming as an actual experimental technique took some time to take off in the laboratory. This was partly owing to the expense of the cameras and film stock, along with the difficulty of growing living cells in a dish [tissue culture was only discovered around 1907 (Harrison, 1907)].

Cinemicroscopy circa 1910–1950

Not long after the advent of cell culture, scientists turned their film cameras on mammalian cells. Alexis Carrel, for example, an early pioneer of mammalian cell culture, used his cinematograph to study the locomotion of fibroblasts and macrophages (Carrel and Ebeling, 1926). Subsequently, Carrel's technical assistant, Heinz Rosenberger (who actually produced Carrel's movies for publication) published a methods article in *Science* on the use of the microcinematographic apparatus, apparently trying to convince investigators "who have not yet realized the great possibilities of the motion-picture camera in research laboratories" (Rosenberger, 1929b). However, scientists did not need much convincing, as movie-making in the laboratory quickly moved beyond cell culture. In the late 1920s and early 1930s, the renowned embryologist Warren Lewis began experimenting with movies of embryos and published a seminal time-lapse study of developing rabbit eggs (Lewis and Gregory, 1929). However, despite some speculation that he was the first to produce such movies (Corner, 1967), some of the films from Ronald Canti (that are now on display at the Wellcome Library website) predate those of Lewis. Unfortunately, few in this day and age know anything about Canti's work (at the time of writing this Essay, he doesn't even have a Wikipedia entry), and yet he probably did more to legitimise the use of movie making as an experimental tool than any of the more widely known names in early cinemicroscopy. Canti, a pathologist who worked at St Bartholomew's Hospital in London, and later at the Strangeways

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Fig. 1. Early Strangeways Laboratory cinemicroscopists. Left panel, Ronald Canti. Right panel, Michael Abercrombie.

Laboratories, was a very early adopter of filming as an experimental tool (his first microscope and camera apparatus were assembled by him in his own home). His obituary in *Nature* in 1936 highlighted that this apparatus allowed him to generate a "world-famous cinema photomicrographic study" which was "shown in all continents of the world" (Obituary: Dr. R. G. Canti, *Nature*, 1936). His death was not just covered in scientific periodicals, but in the popular press as well (Fig. 2A), suggesting that he was indeed a preeminent scientist of his day. It is unfortunate that compared with his contemporaries, such as Lewis, he is no longer so renowned. With the help of the Wellcome Library, our hope is to remedy this.

The Wellcome Library has digitized a series of Canti's movies entitled 'The Cultivation of Living Tissue', which was sponsored by the British Empire Cancer Campaign, a predecessor of the cancer charity, Cancer Research UK. These films were on 16-mm film stock and transferred to this format in the early 1930s for wider distribution. However, the actual experiments from the movies are likely to be a decade older as they represent a highlight reel of what was going on at the Strangeways Laboratory (the British Film Institute holds the original 35-mm stock in their archive, which was released in the 1920s).

These three films represent some of the earliest movies of cultured cells. In Part 1 of the series, Canti highlights the new technique of tissue culture while also explaining some of the technical logistics of the experiment. Here, we see movies of migration of a number of cell types, such as macrophages, and an explanation of the intracellular structures that are visible under the microscope. It is particularly interesting to see how Canti marks time in his film. While time-lapse movies are common today (everyone has seen a nature documentary that uses time-lapse to show the slow growth of plants), this mode of filming would have been foreign in Canti's time. As a result it was important for him to try and convey a sense of the passage of time to his audience. He came up with an ingenious idea to simultaneously film an analog clock, which he displayed on the upper corner of the film (Fig. 2B,C). This allowed the audience to

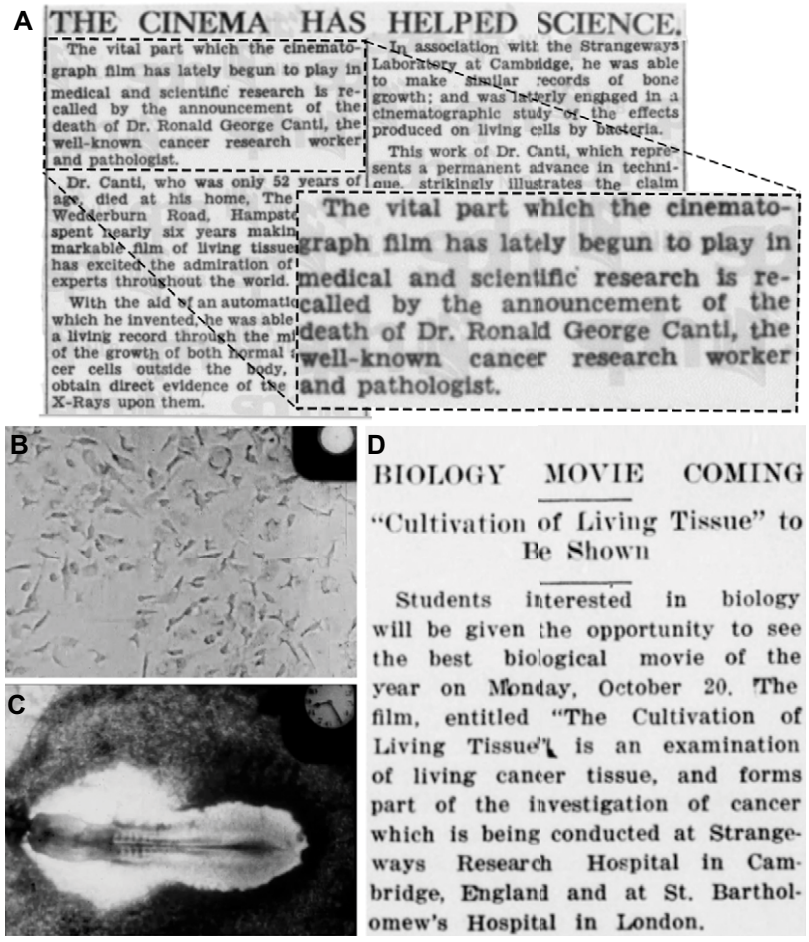

Fig. 2. The scientific life of Ronald Canti. (A) Obituary for Ronald Canti in a Singapore newspaper, *The Straits Times* (1 February, 1936) highlighting his contribution to cinemicroscopy. (B,C) Still images taken from 'The Cultivation of Living Tissue' showing the migration of cancer cells (B) and the embryonic development of the chick (C). Note the time stamp (i.e. the clock) in the upper right hand corner of the images. (D) Article in *The Oberlin Review* in 1930, a university newspaper, on the showing of the movie to biology students.

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see the minute hand frantically spinning around as the cells were migrating, which highlighted just how slow these cellular movements were.

All good movies have a sequel, and in Part 2, we see movies of living embryos (e.g. rabbit) as well as the growth of whole tissues, such as the thigh bone of a chick. These films are particularly impressive owing to the length of time required for each experiment. The thigh bone, for example, was grown for 2 weeks and it is not trivial to maintain the health of these cultures for such a long period of time, never mind the task of keeping the camera working and the microscope focused. Furthermore, 35-mm film stock was not cheap (the less expensive 16-mm format had not even been released at the time of Canti's initial experiments).

Finally, in Part 3 we see experiments that led to Canti's fame in his day. Canti was best known for studying the effects of radiation on cells and, as highlighted in his *Nature* obituary, his photomicrographs helped convince "more people of the efficacy of the radiation treatment of cancer than any other form of publication" (Obituary: Dr. R. G. Canti, *Nature*, 1936). In these films, we see the movement of normal and cancer cells before and after the addition of radium (the effect of the radium on cells as visualized by live imaging is quite stark).

Who saw 'The Cultivation of Living Tissue' in Canti's time? There are references to their display at scientific conferences around the world. However, they were not just aimed at a scientific audience. It is clear from the experimental explanations in the film and their wide distribution, that they were also meant for a lay audience as the films were likely a public engagement exercise [the British Empire Cancer Campaign regularly produced public engagement films (A. Saward, Wellcome film of the month: the fight against cancer, 2012). These films were widely displayed throughout the world, from a Friday evening discourse at the Royal Institution (The cultivation of living tissue cells, *Nature*, 1932), to university students at an American college in Ohio (Fig. 2D). Even the Prime Minister of the UK, James Ramsay MacDonald, received a private showing at No. 10 Downing Street (Cinematographic demonstration of living tissue cells, *British Medical Journal*, 1933).

Although Ronald Canti was not the first to make movies of a cell under a microscope, he certainly was one of the first to use it to address an experimental question (i.e. what are the effects of radiation on cells). He therefore went a long way in legitimizing the use of cinemicroscopy as a scientific tool, which paved the way for its common use in laboratories to this day. It is interesting to see, in Part 3 of his film, the original experimental apparatus that he developed for his studies, alongside his new imaging system that was purchased with the help of the British Empire Cancer Campaign; he received funding from a cancer charity – rather than a film company – to develop this new technique, which is probably the best evidence that Canti had driven its acceptance by the scientific community as a legitimate experimental tool.

The Michael Abercrombie years

The more recent of the films digitized by the Wellcome Library represent experiments performed in the 1950s and 1960s from the laboratory of a scientist who is well known to cell biologists. Michael Abercrombie is best known for his qualitative descriptions of cell migration as he spent his career studying the mechanisms behind cell movement. He was one of the first to hypothesize cell migration as a stepwise process, and his early description of cell movement forms the foundation for how scientists think about cell motility to this day (Danuser et al., 2013). However, Abercrombie should be equally well known for his quantitative approaches to studying cell motility; he was one of the first to use cinemicroscopy as a quantitative tool to extract statistically testable parameters (e.g. cell speed) (Abercrombie and Heaysman, 1953; Dunn and Jones, 1998) (Fig. 3). Until Abercrombie, films of cells were used in an experimental setting in a rather subjective way, and his rigorous quantitative approaches showed us the full extent of the information that could be gained from live imaging, which is largely why movie making is such a useful experimental tool. What is amazing about the films that are now in the Wellcome Library is their quality (Fig. 3). Although they were taken over half a century ago using standard 16-mm format, they still look amazingly clear and have a resolution that approaches what we currently achieve using our modern microscopes and cameras. It is also interesting that he added prefaces to each of the experiments in the movies (these films represent an edited compilation of different experiments), which gives us some information about the type of cell we are looking at or the temporal resolution of the timelapse imaging.

Abercrombie's movies can be viewed at the following URLs:

- http://www.youtube.com/watch?v=W3pMVTflyy4&list=UUaDJONKhVydHbOii9faPNMA
- http://www.youtube.com/watch?v=46LQ6SjzOAI&list=UUaDJONKhVydHbOii9faPNMA
- http://www.youtube.com/watch?v=w-V_eZFmvXA&list=UUaDJONKhVydHbOii9faPNMA
- http://www.youtube.com/watch?v=e05xGQT_3H4&list=UUaDJONKhVydHbOii9faPNMA
- http://www.youtube.com/watch?v=D2eKqkgpD7g&list=UUaDJONKhVydHbOii9faPNMA

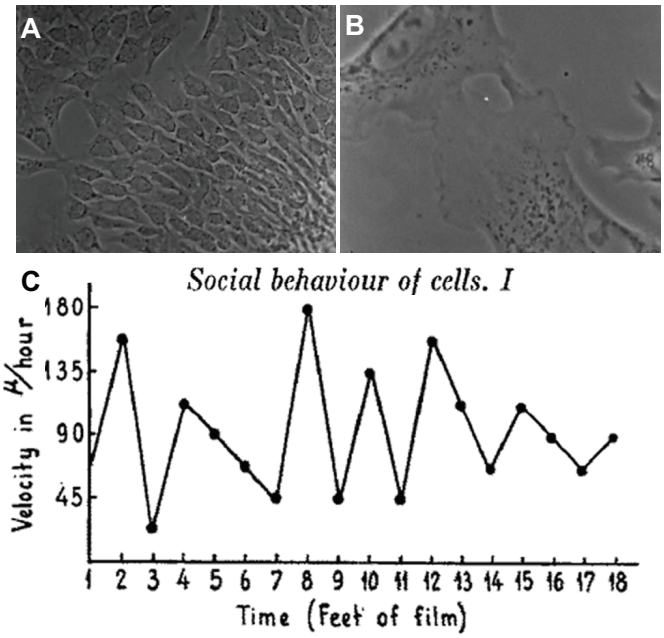

Fig. 3. Quantification of cellular behaviours performed by Michael Abercrombie. (A,B) Still images taken from Abercrombie's movie, 'Cells in Culture' showing two chick heart fibroblasts undergoing contact inhibition of locomotion (CIL) and (B) the 'monolayering' of fibroblasts (which he also assumed was driven by CIL). (C) Analysis of speed of an individually migrating chick heart fibroblast cell as a function of time (or foot of film) as reported in Abercrombie and Heaysman, 1953. This graph was reprinted from Abercrombie and Heaysman, 1953 with permission from Elsevier.

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The future of cinemicroscopy

Over the years there have been major advances in microscopy that have greatly benefited experimentation by movie-making: phase-contrast microscopy in 1932 (Zernike, 1955); the laser scanning confocal microscope in 1986 (Amos and White, 2003); and cloning of the green fluorescent protein of jellyfish in 1992 and the development of its derivatives (Giepmans et al., 2006; Prasher et al., 1992), to name just a few (for a historical perspective of microscopy developments see Dunn and Jones, 2004). Today, there are also new imaging modalities, such as multiphoton microscopy (Denk and Svoboda, 1997) and selective plane illumination microscopy (SPIM) (Keller et al., 2008; Krzic et al., 2012), which allow for live imaging deep inside living embryos and three-dimensional reconstruction of all the cells that make up the organism. At the other end of the imaging spectrum are super-resolution techniques that overcome the diffraction barrier of the microscope and enhance the imaging of subcellular structures [leading to the 2014 award of the Nobel Prize in Chemistry to Stefan Hell, William Moerner and Eric Betzig (see Van Noorden, Nobel for microscopy that reveals inner world of cells, *Nature*, 2014)]. Excitingly, many of these super-resolution techniques are compatible with live imaging (Kner et al., 2009; Manley et al., 2008; Westphal et al., 2008), which will certainly play a major role in the next generation of experimental filming.

However, making ever clearer or faster movies of whole tissues and cellular processes is not enough. The Nobel Prize winning scientist, Peter Medawar, was critical of the early microcinematographers and sceptical that their movies, while beautiful, could actually “solve biological problems” (Landecker, 2009; Medawar, 1986). It is the information pulled from these films that is arguably more important than any advance in the hardware behind the images.

Similar to Abercrombie’s novel analytical approach to investigate cellular behaviours, what is currently driving – and will continue to drive – the use of movie making in the laboratory are the quantitative techniques that scientist use to analyse their films. Computational post-processing of time-lapse movies has allowed for an amazing array of image analysis techniques. From image processing algorithms, which automatically segment cells and intracellular structures of interest, to tracking programs that subsequently record the position of these components over time, we can gather vast quantities of data that is impossible (or too time consuming) to do by eye (Danuser, 2011). Abercrombie’s original analysis of cell speed involved the arduous task of making a “series of enlarged prints of individual frames” at regular intervals (per “foot of film”) and manually quantifying cellular displacement (Abercrombie and Heaysman, 1953) (Fig. 3). Now, this can be accomplished in the blink of an eye using standard tracking algorithms (Fig. 4). However, these computer vision tools are not just replacing the human eye and our manual labour, but allowing us to ‘see’ things that would be otherwise impossible. For example, our knowledge of the dynamics of actin retrograde flow using speckle microscopy would not be possible without the help of computer vision algorithms (Danuser and Waterman-Storer, 2006).

Many of these movie analysis tools are now freely available and ready to use (with the help of a little computer programing knowledge), from automatic tracking of cells (Sacan et al., 2008) and microtubule motion (Applegate et al., 2011), to precise measurement of leading edge dynamics (Döbereiner et al., 2006). Filming now allows us to not only visualize events that are difficult to see in static images but also to subsequently deal with time as a variable in our experiments. With time as a measurable component, we do not only quantify the kinematics of events but can also infer the biochemical properties of intracellular proteins (Reits and Neefjes, 2001) and even measure complex physical parameters such as the forces that are occurring both within and between cells (Betz et al., 2011; Campàs et al., 2014; Mayer et al., 2010).

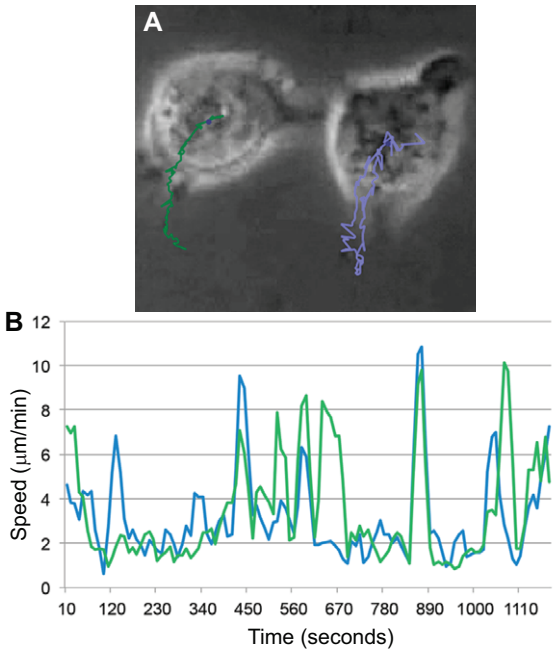

Fig. 4. Computational analysis of cell speed in Abercrombie’s movies. (A) A still image from a movie of migration of the S180 cancer cell line taken by Abercrombie. Cells were automatically tracked using Volocity imaging software. (B) Graph of cell speed over time of the cells tracked in A.

Many of the biological parameters mentioned above can only be gleaned from scientific filming, and as a direct result of these movies, great advances have been made in numerous fields. Furthermore, it is not just basic science that has benefited. Pharmaceutical research and development increasingly involves high-content phenotypic screening of compounds examining numerous cellular parameters by live imaging. This approach requires the use of an automated microscope placed within an environmental chamber allowing for high-throughput analysis of molecular and cell dynamics in thousands of cells placed within multi-well plates (a kinetic imaging platform in pharmaceutical jargon) (Carragher et al., 2012; Isherwood et al., 2011). Again, image processing algorithms are crucial in these screens as it would be impossible to analyse so many samples by eye. We have come a long way since measuring cell speed per foot of film. Early critics of scientific movie-making would eat their words; cinema photomicrography is here to stay as a quantitative experimental tool.

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Competing interests

The authors declare no competing interests.

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