ABSTRACT

Modern digital high-speed film systems are able to capture sequences by extremely high frame rates – over 100,000 fr/s. The equipment costs for such systems are high but the operational costs are practically zero. An important advantage of digital versus analogue high-speed films is the possibility to automatically “digitize” defined markers. The contrast and brightness of the sequences can be modulated in wide ranges; even at very high frame rates, a film can therefore be shot at relatively low light intensity. The construction and sensitivity of modern charge-coupled device (CCD) detectors allow filming with relatively high resolution at over 10,000 fr/s. The only remaining limiting factor for increasing the frame rate is the light level to which the animals are tolerant and still behave “normally” during the experiments. The present study uses film sequences from feeding events and defensive responses in animals, but also films of pistol shots, to demonstrate that choosing an adequate frame rate is crucial for any kinematical analysis.

Keywords: digital, high-speed, film, frame rate, animal behaviour

Introduction

Studying animal performance is an object of classical branches of the biological sciences like biophysics, bionics, functional morphology, physiology, as well as of some new disciplines like neuromechanics (see (7)). The methods used to study animal locomotion, feeding and ventilation are either invasive – like electromyography (EMG; for overview see (2); (3); (5)) and goniometry (for overview see (6)) – or non-invasive, like cinematography, cineradiography, and approaches using electronic transducers, digital particle image velocimetry (see (4)) as well as several other methods.

EMG is an important tool in investigations on the neuromotor control of different musculoskeletal complexes. Its use, however, is limited by a variety of constructional and operational challenges (see (1)). Another important factor constraining the application of electromyography and goniometry is that these surgical procedures often conflict with animal welfare policies.

Among all non-invasive methods mentioned above, high-speed cinematography has the brightest scope of application. The other functional techniques require very expensive equipment as well as highly specific laboratory conditions. Using analogue high-speed cameras (on 8, 16, 35 mm cine films) or high-speed video cameras, rapid behaviours can be observed (see (9)). The analogue systems, however, have a limited frame rate (up to 1000 fr/s), relatively low photosensitivity and restricted compatibility with different optical gears. Important advantages of the video systems compared to cinematography are the lower costs of the light-sensitive medium and the longer recording time. For further computer-based analysis, the images from the high-speed videos have to be “digitized”, which is a complicated and above all a time-consuming process.

At the end of the 20th century, first experiments with digital high-speed film systems were made. The single-frame resolution of the oldest systems was much lower than that of cine and video. Modern digital high-speed cameras have resolutions of over 1 megapixel at 2000 fr/s, which allows a precise kinematical analysis even of small structures. The brightness and contrast of the digital film can be adjusted in large ranges, making filming with a high frame rate possible even at moderate light intensity. Various software products permit automatically “digitizing” the film sequences.

In the present study we demonstrate the wide application range of digital high-speed filming systems in analysing rapid
movements. We comment on the negative impact of the high light levels needed to obtain high-speed sequences on animal behaviour. Our main goal is to emphasise the importance of estimating the appropriate frame rate for filming movements with different velocity.

Materials and methods

High-speed film sequences were obtained from feeding events in two fish species, the pike livebearer *Belonesox belizanus* (2000 fr/s) and the stonefish *Synanceia verrucosa* (3000 fr/s). Feeding events were also filmed in a third species, the goldfish *Carassius gibelius forma auratus*, at a frame rate of 6000 fr/s. Defensive responses in bryozoans were filmed at 500 fr/s using over-head light (in *Paludicella articulata*) and at 2000 fr/s using transmitted illumination (in *Plumatella emarginata*). To discuss the challenges of analysing motions in machines vs. living animals, we have documented the shutter kinematics in a pistol (“Walther CP99compact”), filming at 250, 2000 and 18 000 fr/s.

All film sequences were recorded with a “Photron Fastcam-X 1024 PCI” digital high-speed camera (Photron limited; Tokyo, Japan). Horizontal (on the X-axis) and vertical (Y-axis) coordinates of relevant landmarks were recorded frame by frame using “SIMI-MatchiX” (SIMI Reality Motion Systems; Unterschleißheim, Germany). For filming in macro regime, a highly light-sensitive objective AF Zoom - Nikkor 24-85 mm. (f/2,8-4D IF) was used. For illumination we used two “Dedocool Coolh” tungsten light heads with 2 x 250 W (ELC), supplied by a “Dedocool COOLT3” transformer control unit (Dedo Weigert Film GmbH; München, Germany). The colour temperature of the system can be adjusted in the range of 3000 to 3400 Kelvin. Due to the cooling systems integrated in the light heads, the object temperature 30 cm ahead of the light source front collecting lens is about 42 °C at an intensity of 1 million lux. For filming bryozoans the high speed camera was mounted over a C-mount adapter to either a “Wild M420” dissection microscope or a “Leitz Labovert” inverted microscope.

Results and Discussion

Modern digital technologies provide an opportunity to increase the frame rate by high-speed filming of over 100 000 fr/s. In studying animal behaviour and performance, filming with such a frame rate is an exception (see (8)) because a single frame’s resolution is restricted to several thousand pixels. The light intensity required for such a frame rate would be more then 5 million lux.

For filming at 10,000 f/s, the shutter speed at a medium sensitivity of 400 ASA and lens aperture of 8 is about 1/50 000 s. In this case, a light intensity of more than 2 million lux would be needed. Using cold light prevents overheating of the investigated objects. The main problem is that the animals are practically blind at such light levels. When filming feeding behaviour in species that rely completely on optical feedback in prey capture, the light intensity must be kept as low as possible. In filming *B. belizanus*, we used the “set up position” of the Dedocool transformer control unit because the light sources were positioned 70 cm away from the object. The lens aperture was almost open at maximum (4), which negatively impacts the depth of field. Only about 60 % of the sequences at 2000 fr/s were free of unsharpness (see Fig. 1). At 3000 fr/s the shooting of an in focus film was a matter of coincidence. The use of more intensive light blocked the predator response to the offered prey.

![Fig. 1. Selected frame from prey capture event in *Belonesox belizanus* (2000 fr/s). Note the limited depth of field due to the wide lens aperture.](image-url)

Species like the goldfish are also very sensitive to increased light levels. Nonetheless, after training, the animals used in our experiments fed even at light intensity of over 1 million lux (Fig. 2). The lights were positioned 30 cm from the experimental aquarium (16 x 7 x 16 cm) at a working voltage of 24 V. At a frame rate of 6000 fr/s and lens aperture of 11, we attained 100 % image sharpness. Every single film was suitable for further kinematical analysis. Such a success rate is very important in experiments in which the measured values are tested and compared statistically.

For those animals that are relatively insensitive to light, the light source can be positioned very close to the object (Fig. 3). For animals with transparent bodies, the use of transmitted light is more appropriate than incident light, especially when the investigated object is in water (compare Fig. 3a, b to Fig. 3c, d).
The examples given above demonstrate some of the problems involved in filming animals at high speed of over 1000 fr/s. A compromise has to be made between the quality of the sequences and the light level at which the animals still behave “normally”. In some cases obtaining a “perfect” sequence is simply not possible. **Fig. 4** represents a feeding event in *S. verrucosa*. The blurs in frames 4b and 4c show that the frame rate of 3000 fr/s is insufficient for filming such fast movements. The landmarks cannot be matched correctly because the exact position of some morphological structures can be only presumed.

Thus, choosing the right filming frame rate is crucial for reliability in any motion analysis. By comparing the same behaviour filmed at high and low frame rates, the kinematical pattern graphics may show great differences. If we compare the motion diagrams of the shutter unit of the “Walther CP 99c” pistol shots filmed at 250, 2000 and 18 000 fr/s (see **Fig. 6**), it is clear that even the highly stereotypical movements of a metal mechanism can be interpreted incorrectly when the frame rate is too low. We calculated that the velocity of the shutter pullback is about 1.8 m/s – a frame rate of 250 fr/s would be completely insufficient. On all frames on **Fig. 5** (except the first and the last one), the exact position of the

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**Fig. 2.** Selected frames from prey ingestion event in *Carassius gibelius* (6000 fr/s). The animals were trained to feed under extremely high light levels. Even small structures can be recognized and matched in details. a, start of “jaw opening”; b, “expansive phase”; c, the prey is sucked into the oral cavity during a static “peak gape” phase; d, “compressive phase”; terminology modified after (3); Time in seconds; p, prey.

**Fig. 3.** Defensive behaviour in bryozoans: a - b. polypide retraction in *Paludicella articulata* (500 fr/s); note that even at moderate frame rates the incident light is inappropriate for filming transparent animals; c - d. polypide retraction in *Plumatella emarginata* (2000 fr/s), the field depth is notably increased by transmitted illumination. Time in seconds.

**Fig. 4.** Selected frames from feeding event in *Synanceia verrucosa* (3000 fr/s) – sometimes it simply does not work perfectly. The frame rate is too low to capture a blur-free film sequence. Note the out-of-focus mouth contours in frames 3b and 3c. Time in seconds.

**Fig. 5.** Film sequence of a “Walther CP 99compact” pistol shooting BBs (250 fr/s). All frames except 5a and 5f are corrupt. The shutter movements are relatively slow, but the frame rate is still completely insufficient for kinematical analysis. Time in seconds.
shutter cannot be defined. Thus, the motion analysis software was unable to match the round white spots used as markers. Even after manually “digitizing”, the position of the dots was not recognisable because the markers resembled bars or lines. The graphic from the 250 fr/s sequence represented on Fig. 6 is a product of subjective estimation and does not represent the kinematical patterns correctly.

**Fig. 6.** Shutter kinematic profiles from three “Walther CP 99compact” shooting events. The circles represent a kinematic profile from a film sequence captured at 250 fr/s; the crests represent a kinematic profile from a film sequence captured at 2000 fr/s; the black line represent a kinematic profile from a film sequence captured at 18 000 fr/s. Note the differences in the diagram peaks.

**Conclusions**

In conclusion, obtaining optimal results in motion analysis based on high-speed filming requires the coherence between light intensity, lens aperture, frame rate and frame resolution. In and of themselves, the technical advantages of modern film equipment and the application of tracking software cannot compensate for errors in the theoretical basis or for failures in the realisation and execution of an experiment.

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