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Carina Barbosa Pereira
Janosch Kunczik
André Bleich
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Stefan Treue
Michael Czaplik

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Carina Barbosa Pereira,^{a,*} Janosch Kunczik,^a André Bleich,^b Christine Haeger,^b Fabian Kiessling,^c Thomas Thum,^d René Tolba,^e Ute Lindauer,^f Stefan Treue,^{g,h,i} and Michael Czaplik^a

^aRWTH Aachen University, Faculty of Medicine, Department of Anesthesiology, Aachen, Germany

^bInstitute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany

^cRWTH Aachen University, Faculty of Medicine, Institute for Experimental Molecular Imaging, Aachen, Germany

^dInstitute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany

^eRWTH Aachen University, Faculty of Medicine, Laboratory Animal Science, Aachen, Germany

^fRWTH Aachen University, Faculty of Medicine, Department of Neurosurgery, Aachen, Germany

^gUniversity of Goettingen, Faculty for Biology and Psychology, Goettingen, Germany

^hLeibniz ScienceCampus Primate Cognition, Goettingen, Germany

ⁱGerman Primate Center—Leibniz Institute for Primate Research, Cognitive Neuroscience Laboratory, Goettingen, Germany

Abstract. To refine animal research, vital signs, activity, stress, and pain must be monitored. In chronic studies, some measures can be assessed using telemetry sensors. Although this methodology provides high-precision data, an initial surgery for device implantation is necessary, potentially leading to stress, wound infections, and restriction of motion. Recently, camera systems have been adapted for animal research. We give an overview of parameters that can be assessed using imaging in the visible, near-infrared, and thermal spectrum of light. It focuses on heart activity, respiration, oxygen saturation, and motion, as well as on wound analysis. For each parameter, we offer recommendations on the minimum technical requirements of appropriate systems, regions of interest, and light conditions, among others. In general, these systems demonstrate great performance. For heart and respiratory rate, the error was <4 beats/min and 5 breaths/min. Furthermore, the systems are capable of tracking animals during different behavioral tasks. Finally, studies indicate that inhomogeneous temperature distribution around wounds might be an indicator of (pending) infections. In sum, camera-based techniques have several applications in animal research. As vital parameters are currently only assessed in sedated animals, the next step should be the integration of these modalities in home-cage monitoring. © The Authors. Published by SPIE under a Creative Commons Attribution 4.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.24.7.070601](https://doi.org/10.1117/1.JBO.24.7.070601)]

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1 Introduction

Animal studies have made a great contribution to medical advancement and play a vital role in current scientific work, as they replicate key aspects of human physiological and pathophysiological processes.^{1,2} They allow a higher understanding of various disease mechanisms as well as of genetic predisposition to certain diseases. Furthermore, they have been improving medication and treatments, allowing for a higher quality of life for people all over the world.³ In 2011, nearly 11.5 million animals were used for scientific purposes in the EU, of which 76.8% were rodents (including mice, rats, guinea-pigs, and hamsters), whereas only 0.67% were pigs, 0.25% were sheep, and 0.05% were primates.⁴ The remaining 22.23% correspond to other animal species (including birds, reptiles, and amphibians).⁴

In the last decades, the use of animals in medical and scientific research has been an issue of public concern and debate.³ In Europe, in 2010, the legal framework for responsible and ethically acceptable animal research was updated and brought to the same standard across all EU member states. The European Parliament passed Directive 2010/63/EU, which stresses the

3R principles of animal research, as proposed by Russel and Burch:^{5,6} replacement, reduction, and refinement. First, animal research shall be replaced with other methods, whenever possible. Second, the number of animals used for scientific purposes shall be reduced as much as possible. Third, the procedures shall be refined to avoid or minimize stress, and improve the well-being of the animals.

In order to comply with the principle of refinement, the animals must be kept under constant surveillance concerning their vital parameters [for instance heart rate (HR), respiratory rate (RR), and oxygen saturation], mobility/activity, wounds, and emotional state.⁷ In chronic animal experiments, vital signs are commonly assessed by implanting a telemetry sensor. Although this methodology provides high-precision data, its use is associated with several and critical drawbacks. First, an initial implantation surgery is required.⁸ According to Braga and Burmeister,⁹ animals require up to 5 to 7 days to recover their normal circadian rhythms. Second, the implanted device itself might cause distress and discomfort, particularly in small species.⁹ In addition, it has significant adverse physiological effects; e.g., increased volume in abdominal viscera can compromise the movement of the diaphragm, and, as a result, alter breathing patterns (depth and/or rhythm).⁹ Third, telemetry is a very expensive method; in addition to the initial acquisition costs, periodic expenses for telemeter refurbishment are still

*Address all correspondence to Carina Barbosa Pereira, E-mail: cbarbosapere@ukaachen.de

high. Fourth, the battery life of the transmitters is mostly warranted for six months of continuous use.¹⁰ In acute experiments with animals undergoing anesthesia, vital signs are assessed using electrocardiography (ECG) or photoplethysmography (PPG). Although accurate, they require the use of wires, which often hinder the researcher.¹¹ In contrast, other parameters such as locomotion capability or activity, emotional state, and pain are assessed subjectively.⁸ Finally, unwanted infections or surgical wound complications occur occasionally during animal trials. Those are often detected belatedly, since there are currently no technologies available to sensitively predict inflammation or infection.

Innovative monitoring modalities, which offer contactless and unobtrusive surveillance of laboratory animals, could refine and improve research procedures and help to reduce the number of research animals.^{7,12} In recent years, new monitoring applications based on camera-based systems [imaging in the visible, near-infrared (NIR), and thermal spectrum of light] have been developed for human medicine. Due to positive feedback, these techniques have been recently adapted for animal research. Infrared thermography (IRT) (long-wavelength infrared/thermal band of the electromagnetic spectrum, 7 to 14 μm) records heat emission without any harmful radiation or light source.¹³ It is used in various medical fields for measuring, e.g., HR and RR,^{13,14} thermal regulation,¹⁵ circulation, and perfusion dynamics.¹⁶ NIR and visible imaging systems have higher spatial resolution in relation to IRT. In addition to HR and RR, these technologies allow monitoring peripheral oxygen saturation.

Based on the drawbacks of the current monitoring modalities and considering the new directives on protection and welfare of animals, this review aims at giving an overview of new camera-based methods (visible, NIR, and IRT imaging) for unobtrusive and remote monitoring of research animals. This paper focuses not only on vital parameters, which can be estimated (such as heart activity, respiration, and oxygen saturation), but also on the assessment of motion activity and wound infection (elements analyzed frequently during animal experiments). For each parameter and species (mice, rats, pigs, and primates), an enumeration of the imaging systems and algorithms that can be applied is carried out. Additionally, this paper indicates the regions of interest (ROIs) to be used as well as camera properties and light conditions. Finally, for each application, previously published as well as preliminary results of our working groups are presented in order to demonstrate the potentiality of these techniques.

2 Animals, Materials, and Methods

2.1 Heart Activity

Heart activity can be monitored either in visible spectrum (VIS), NIR, or IRT imaging. These techniques are based on different principles and thus methods used to estimate HR. In VIS/NIR videos, this vital parameter can be extracted using one of the following approaches: motion-based (presented by Balkrishnan et al.¹⁷ and Li et al.¹⁸) or color-based (presented by Poh et al.¹⁹).

The former uses optical flow to measure the weak vibrations of the body caused by the beating heart. In short, feature points (around the ROI) are identified in the first frame of the video and tracked over time (applying, e.g., a template-based point tracker). Afterward, their trajectories are processed to extract the chest motion related to the cardiac function. This step consists in

filtering the trajectories of the feature points to remove noise outside the expected signal frequency range. Using blind source separation, based either on principal or on independent component analysis, the trajectories are decomposed into subsignals (principal components) to isolate the heart-related component. The principal component that most closely resembles the heart signal is selected; its main frequency in the frequency spectrum corresponds to the HR.¹⁸

The latter approach (color-based) uses color or brightness changes of the skin due to blood flow to quantify heart activity (it is an extension of the classical reflective PPG). Using this method, an ROI, enclosing the subjects' skin, must be identified in the first frame of the video and tracked over time for motion compensation. Thereafter, the ROI should be separated into the three RGB channels and spatially averaged to obtain the raw RGB traces (i.e., the mean color pixel intensity of the ROI). Blind source separation (independent component analysis) is also used in this approach in order to decompose the traces into three independent source signals. Finally, the component containing the heart signal is selected; the frequency with the highest power of the spectrum—within the operational frequency band—corresponds to the pulse frequency.¹⁹

Both approaches were primarily developed for human medicine and have recently been applied in animal research. Regarding thermal videos, only the motion-based approach can be applied. Table 1 presents a detailed overview of the imaging systems and their properties, applicable algorithms (whether color-based or motion-based), ROIs, and light conditions necessary to extract HR from four animal species: mice, rats, pigs, and primates. Some of the commercially available systems capable of detecting this vital parameter are listed in Table 2.

2.2 Respiration

Respiration can be assessed by measuring the displacement of the chest or abdomen using one of three imaging systems: VIS,²³ NIR,¹⁸ or IRT.²⁴ For this purpose, the motion based-approach, described in the previous section, can be used.

In thermal videos, temperature variation around the nostrils during the respiratory cycle also offers insights into this physiological process. This results from the inhalation of cold air from the environment and exhalation of warm air from the lungs.²⁵ In this approach, the nose (ROI) is segmented in the first frame of the thermal video. Afterward, a rough tracking overtime is performed to compensate motion. The filtered mean temperature of the ROI, which represents the respiratory waveform, is computed. Finally, the respiratory frequency can be extracted either by selecting the frequency with the highest power of the spectrum or using breath-to-breath estimation algorithms.

In addition to RR, these techniques allow us to monitor breathing patterns²⁶ (such as tachypnea, Kussmaul breathing, Cheyne–Stokes respiration, and apnea phases) and tidal volume.²³ As in the previous section on heart activity, respiration assessment with imaging systems has primarily focused on human medicine. In the last years, these new techniques have been translated to animal research.^{7,11,20,27} Table 3 presents a detailed overview of the imaging systems and their properties, relevant algorithms, ROIs, and light conditions necessary to extract the RR from four animal species: mice, rats, pigs, and primates. For this application, the cameras presented in Table 2 can be used. However, if the focus is only on respiration, cameras with lower frame rates and resolutions are an option as well, as the amplitude of the thorax movement is prominent and

Table 1 Detailed overview of the imaging systems and properties to extract HR from four animal species: mice, rats, pig, and primates. Algorithmic approach (color-based or motion-based), ROIs, frame rate, spatial and thermal resolution, as well as required light conditions are specified.

Technique approach	Visible		NIR		IRT
	Motion	Color	Motion	Color	Motion
Species	Mice	Mice	Mice	Mice	Pigs
	Rats	Rats	Rats	Rats	
	Pigs	Pigs	Pigs	Pigs	
	Primates	Primates	Primates	Primates	
ROI	Chest/abdomen: all species	Shaved chest/abdomen: mice, rats	Chest/abdomen: all species	Shaved chest/abdomen: mice, rats	Chest: pigs
	Head: primates	Tail: mice, rats ²⁰ Pigs: snout, chest, abdomen ²⁰ Face: primates ²²	Head: primates	Tail: mice, rats Pigs: snout, chest, abdomen ²¹ Face: primates ²²	
Illumination	Visible light	Visible light	Active illumination (visible/IR light) (Power: e.g., 9 W)	Active illumination 940 nm (power: e.g., 9 W)	Not required
Frame rate			Mice: 60 Hz Rats: 60 Hz Pigs: 30 Hz Primates: 30 Hz		
Resolution		All species: at least 1280 × 720 pixels			All species: at least 1024 × 768 pixels
Temperature resolution					0.03 K

Table 2 Examples of imaging systems.

Technique	Imaging systems
Visible	Mako G-223B (Allied Vision GmbH, Stadtroda, Germany) IDS UI-3270LE/GV-5240/UI-5240 color (IDS GmbH, Obersulm, Germany) Grasshopper 3 GS3-U3-51S5C-C (FLIR Systems, Wilsonville, Oregon) Chameleon 3 U3-13Y3C (FLIR Systems, Wilsonville, Oregon)
NIR	Manta G-223B NIR (Allied Vision GmbH, Stadtroda, Germany) IDS UI-3270LE/GV-5240/UI-5240 NIR (IDS GmbH, Obersulm, Germany) Grasshopper 3 GS3-U3-41C6NIR-C (FLIR Systems, Wilsonville, Oregon)
IRT	FLIR T1020 (FLIR Systems, Wilsonville, Oregon) VarioCAM head 800 (InfraTec GmbH, Dresden, Germany)

the frequency smaller than the HR. Two examples are the webcam Logitech C270HD (Logitech International S.A., Lausanne, Switzerland)—RGB, and the FLIR T640 (FLIR Systems, Wilsonville, Oregon)—IRT.

2.3 Oxygen Saturation Monitoring

In addition to heart activity and respiration, NIR imaging also permits the assessment of arterial oxygen saturation (SpO_2) in blood by adapting the principles of conventional pulse oximetry. As well-known, oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) have different absorption spectra, whereas HHb absorbs more light in the red band of the electromagnetic spectrum (600 to 750 nm), HbO_2 absorbs more light in the infrared band (850 to 1000 nm). Therefore, conventional pulse oximeters contain two LEDs of different wavelengths (usually red and infrared). By capturing a PPG at two suitable wavelengths, the relative concentrations of HbO_2 and HHb can be derived, and consequently, the SpO_2 . In fact, this can be defined as the ratio of HbO_2 to the total hemoglobin (sum of HbO_2 and HHb) as given by

$$SpO_2 = \frac{[HbO_2]}{[Hbb] + [HbO_2]} \times 100\%, \quad (1)$$

where $[HbO_2]$ and $[Hbb]$ are the concentration of oxyhemoglobin and deoxyhemoglobin, respectively.²⁸

Table 3 Detailed overview of the imaging systems and properties to extract RR from four animal species: mice, rats, pig, and primates. Algorithmic approach (color-based or motion-based), ROIs, frame rate, spatial and thermal resolution, as well as required light conditions are specified.

Technique approach	Visible/NIR	IRT	
	Motion	Motion	Color
Species	Mice	Mice ¹¹	Mice ⁷
	Rats	Rats ¹¹	Rats
	Pigs	Pigs	Pigs
	Primates	Primates	Primates
ROI	Chest: all species	Chest: all species ¹¹	Nostrils: all species
	Abdomen: all species	Abdomen: all species ¹¹	
	Back: all species	Back: all species	
Illumination	Visible light	Not required	Not required
Frame rate		Mice: 30 Hz	
		Rats: 30 Hz	
		Pigs: 10 Hz	
		Primates: 10 Hz	
Resolution	All species: at least 1280 × 720 pixels	All species: at least: 640 × 480 pixels	
Temperature resolution		0.03 K	

The measurement of SpO₂ using NIR cameras is again an extension of the classical pulse oximetry method. In addition to the monochromatic/RGB camera, a trigger-controlled wavelength LED-array is required. Alternatively, two or more cameras with different optical filters can be used. The application of NIR imaging for SpO₂ measurement was primarily tested on human subjects.^{29,30} In the last years, this technique has also been being used in animal research to measure cortical blood oxygenation.³¹ For this application, publications reported the use of monochromatic/RGB cameras (sensitive also in the infrared region of the electromagnetic spectrum) with a resolution of 1024 × 768 pixels or higher, a dynamic range of 12 bits, and a frame rate of 30 Hz (e.g., AD-080GE, Jai A/S, Denmark).^{28,31} The illumination unit should be composed of at least two LED arrays with two appropriate wavelengths (e.g., red and NIR). However, Shao et al.²⁸ demonstrated that the combination of orange/infrared LEDs is the best choice considering the PPG signal-to-noise ratio. Finally, the measuring system should include a control unit to trigger LEDs and camera, since the red/orange and infrared LEDs must flash alternately.^{28,31} It is important to note that SpO₂ assessment is based on the color-based approach (presented in Sec. 2.1). Thus to extract the signal of interest, a portion of skin or tissue must be visible and illuminated.

2.4 Motion and Activity

In addition to vital parameters, camera-based techniques are frequently used for monitoring of activity and exploratory behavior, mostly in rodents. As is well-known, movement and location of animals, e.g., during open field tests, are major markers for anxiety, stress, and pain.

Motion activity can be remotely assessed using one of three imaging systems: VIS, NIR, or IRT. Although all systems are qualified for this application, IRT has some additional advantages. First, this technique does not depend on any light source. In contrast, visible and NIR systems require a light source (whether artificial and natural light). This property plays a major role for circadian rhythm monitoring. Second, IRT identifies defecation and urination (which can also be affected by stress or fear) as hot (and later cold) spots. Third, animals can be easily released from the background, which is significantly colder (animals are hot spots in IRT videos). In addition, the color of the animals does not have any influence on the detection/segmentation algorithm. Despite all advantages, IRT is still an expensive technology in comparison with visible and NIR imaging.

For motion analysis, there are two main steps that may influence the performance of the system: the detection algorithm and the tracking algorithm. In fact, robust detection and tracking are the key factors of a reliable software. From tracking, several parameters can be assessed, including acceleration, position, velocity, total distance travelled, and measurement of the time spent in different regions of the open field, among others. Furthermore, for visualization of the exploratory behavior, motion heat maps can be estimated.

Regarding the measurement setup, the camera should be aligned perpendicularly (90 deg) to the cage or open field. When using an IRT camera, it should have a resolution of 1024 × 768 pixels or higher as well as a frame rate of at least 30 Hz (e.g., FLIR T1020, FLIR Systems, Wilsonville, Oregon; or VarioCAM head 800, InfraTec GmbH, Dresden, Germany). Visible or NIR cameras should have similar properties: ANY-maze 60516 (Dublin, Ireland), Logitech C270HD (Logitech International S.A., Lausanne, Switzerland) and Sony DCR-SR60 (Sony Corporation, Tokyo, Japan) are two examples used for these applications.³² Equally important is the calibration of the camera, which must be calibrated to remove lens distortion.

2.5 Wound Monitoring

The four cardinal symptoms of inflammation include rubor (redness), calor (heat), dolor (pain), tumor, and *functio laesa* (disturbance of function).³³ Unfortunately, these signs are hard to diagnose by visual inspection alone. Fur and thick skin complicate the diagnosis even further, because they hide rubor and tumor. Touching the wound area can be useful to detect calor, dolor, and tumor, but should be avoided since it can be painful for the animal and for hygienical reasons.

The usage of various cameras with subsequent image processing can contribute to a contactless monitoring of the cardinal symptoms (rubor, calor, and tumor) that are not sensitively assessable by existing (clinical) methods. IRT can display the temperature distribution around the wound area, which can be examined for homogeneity, size, and shape. A normally healing wound is expected to show a homogeneous warm area around the wound, with margins equidistant from the wound origin. The presence of localized hot areas or cold spots can

imply problems in the healing process. Visual- and depth-sensing cameras can be used as a replacement for manual visual inspection. Using computer algorithms for wound analysis, the results can be made quantifiable and more objective. Those cameras can be used to detect rubor (visual cameras) and tumor (depth cameras), e.g., by comparing successive images taken of a wound.

For wound monitoring, we use two classes of IRT cameras: high-end and entry level devices. All camera systems contain additional integrated visual cameras. The use of depth sensing camera technology is currently a subject of research. An exemplary entry-level IRT camera comprises a 160×120 pixel image resolution, a horizontal field of view (HFOV) of 50 deg, and a minimum object distance of 30 cm (e.g., FLIR One Pro, FLIR Systems, Wilsonville, Oregon). Thus a maximum resolution of ~ 6 pixel/cm can be achieved. Given the assumption that four pixels are enough, structures of around 0.7 cm can be represented. High-end cameras deliver the most flexibility, as they feature high image resolutions, e.g., 464×384 pixels, and

changeable lenses (e.g., FLIR E95, FLIR Systems, Wilsonville, Oregon). Resolutions of 22 pixels/cm and the detection of structures larger than 0.2 cm are easily possible.

3 Results

This section presents the principal findings from previous publications, as well as some of our own preliminary results from camera-based monitoring systems for laboratory animals.

3.1 Heart and Respiratory Rate

Tables 4 and 5 present the performance of the imaging systems for monitoring of HR and RR in different animal species. Since the application of such techniques in animal research is very recent, these tables cannot be filled completely since studies are still lacking. Visible imaging was the first system analyzed. To date, its feasibility has merely been tested in rodents (mice and rats) and nonhuman primates. For HR and RR, promising results were obtained, the root-mean-square errors (RMSEs)

Table 4 Accuracy of three imaging systems for assessment HR.

			Imaging systems		
			Visible	NIR	IRT
HR	Mice	<i>N</i>	6	—	—
		$\overline{HR}$	386 ± 41 beats/min	—	—
		$\overline{RMSE}$	1.4 ± 0.9 beats/min	—	—
		ROI, method	Thorax/abdomen, motion	—	—
		Reference	Kunczik et al. (2019) ³⁴	—	—
	Rats	<i>N</i>	6	—	—
		$\overline{HR}$	374 ± 46 beats/min	—	—
		$\overline{RMSE}$	1.3 ± 0.6 beats/min	—	—
		ROI, method	Thorax/abdomen, motion	—	—
		Reference	Kunczik et al. (2019) ³⁴	—	—
	Pigs	<i>N</i>	—	Unknown	17
		$\overline{HR}$	—	Unknown	131 ± 23 beats/min
		$\overline{\varepsilon}_{abs}$	—	Unknown	3.4 ± 3.0 beats/min
		ROI, method	—	Snout, color	Thorax, motion
		Observation	—	Good agreement	—
		Reference	—	Blanik et al. (2019) ²¹	Pereira et al. (2019) ³⁵
	Nonhuman primates	<i>N</i>	4	—	—
		$\overline{HR}$	140 beats/min	—	—
		$\overline{RMSE}$	3.8 ± 1.5 beats/min	—	—
		ROI, method	Face, color	—	—
		Reference	Unakafov et al. (2018) ²²	—	—

Note: $\overline{HR}$, mean heart rate; $\overline{RMSE}$, mean root-mean-square error; $\overline{\varepsilon}_{abs}$, mean absolute error.

Table 5 Accuracy of three imaging systems for assessment RR.

			Imaging systems		
			Visible	NIR	IRT
RR	Mice	N	6	—	5
		$\overline{RR}$	98 ± 35 breaths/ min	—	113 ± 30 breaths/ min
		$\overline{RMSE}$	1.4 ± 1.0 breaths/ min	—	—
		$\overline{\varepsilon}_{abs}$	—	—	5.3 ± 3.8 breaths/ min
		ROI, method	Thorax/abdomen, motion	—	Thorax/abdomen, motion
		Reference	Kunczik et al. (2019) ³⁴	—	Pereira et al. (2018) ¹¹
	Rats	N	6	—	5
		$\overline{RR}$	50 ± 7 breaths/ min	—	52 ± 7 breaths/ min
		$\overline{RMSE}$	0.3 ± 0.2 breaths/ min	—	0.4 ± 0.1 breaths/ min
		$\overline{\varepsilon}_{abs}$	—	—	0.1 ± 0.1 breaths/ min
		ROI, method	Thorax/abdomen, motion	—	Thorax/abdomen, motion
		Reference	Kunczik et al. (2019) ³⁴	—	Pereira et al. (2018) ¹¹
	Pigs	N	—	Unknown	17
		$\overline{RR}$	—	Unknown	31 ± 3 breaths/ min
		$\overline{\varepsilon}_{abs}$	—	Unknown	0.3 ± 0.5 breaths/ min
		ROI, method	—	Snout, motion	Thorax, motion
		Observation	—	Good agreement	—
		Reference	—	Blanik et al. (2019) ²¹	Pereira et al. (2019) ³⁵

Note: $\overline{RR}$, mean respiratory rate; $\overline{RMSE}$, mean root-mean-square error; $\overline{\varepsilon}_{abs}$, mean absolute error.

averaged <4 beats/ min and 1.5 breaths/min, respectively. Although in rodents vitals were derived using the motion of the thorax/abdomen, in primates HR was estimated by measuring variations in blood volume with consecutive changing skin color (color-based approach). Blanik et al.²¹ were, to the best of our knowledge, the only researchers studying the application of NIR imaging in animal research. In their study, the authors assessed HR and RR in the snout of pigs using the color-based approach and motion, respectively. Unfortunately, instead of quantitative data analysis (comparison with a gold standard method), only a qualitative analysis was carried out. According to the publication, a good agreement between NIR and the gold standard was obtained. Finally, regarding IRT, there are more studies applying this technique in animals, especially for assessment of RR.^{7,11,27} For the selected studies, the mean absolute error ($\overline{\varepsilon}_{abs}$) was <3.5 beats/ min and 5 breaths/min for HR and RR, respectively. Note that HR was only assessed in thermal videos of pigs. Figure 1(a) shows a Bland–Altman plot comparing the HR estimated with visual imaging—using the motion-based approach—and the gold standard (ECG). These results were presented by Kunczik et al.³⁴ As illustrated, a great agreement between ECG was obtained with the limits varying between -3.2 and 1.7 beats/min. Figure 1(b) illustrates the regions of the rodent (rat) used to extract HR; regions with a signal-to-

noise ratio smaller than a specific threshold were not used to compute this vital parameter.

Figure 2(a) shows, in turn, a Bland–Altman plot comparing the RR estimated with IRT and the gold standard (ECG-derived RR). In this example, a good agreement between both techniques is also visible, with the limits of agreement ranging from -0.8 and 0.6 breaths/min. Figure 2(b) illustrates the thermogram and the points used for motion tracking.

3.2 Oxygen Saturation

Van Gastel et al.³⁰ conducted a study with human subjects to compare two methods of camera-based SpO₂ extraction algorithms: the classical ratio-of-ratio (ROR) algorithm, used in pulse oximeters, and their own method (“APBV”), which improves the raw signal quality through signal fusion and *a priori* knowledge.³⁰ Four minutes of videos were recorded with static and moving subjects. The contactless SpO₂ was extracted from the subjects’ heads. A classical pulse oximeter was used as reference.

For the static experiment, the RMSE averaged 3% in case of the ROR algorithm and 2% for the APBV algorithm, respectively. The RMSE increased to 27% for the ROR algorithm, once free head movements were allowed. On the other hand, the APBV approach managed to contain an RMSE of 3% in this case.³⁰

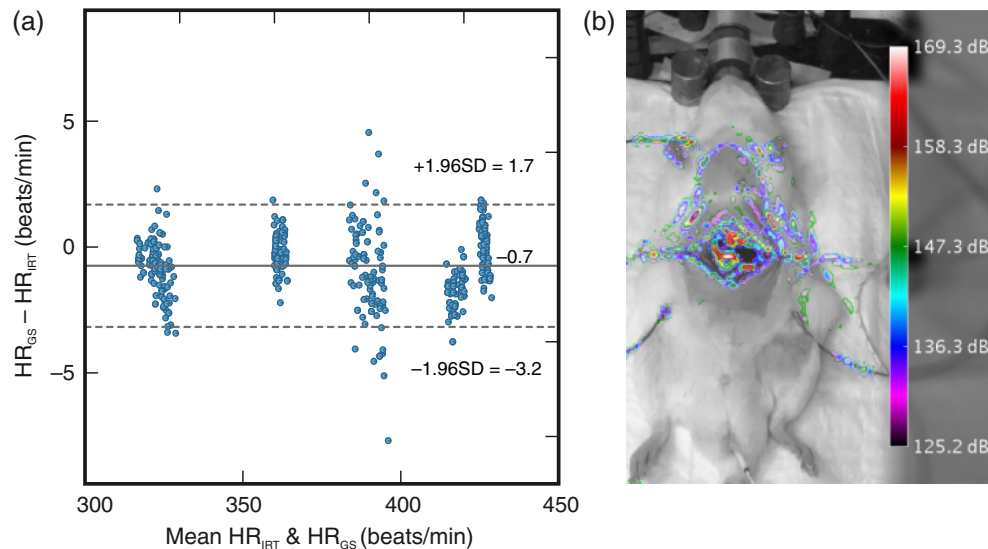


Fig. 1 (a) Bland–Altman plot comparing the HR estimated with visual imaging—using the motion-based approach—and the gold standard (ECG).³⁴ The limits of agreement range from -3.2 to 1.7 beats/min. The bias averages -0.7 beats/min. (b) Regions of the rat used to extract HR.

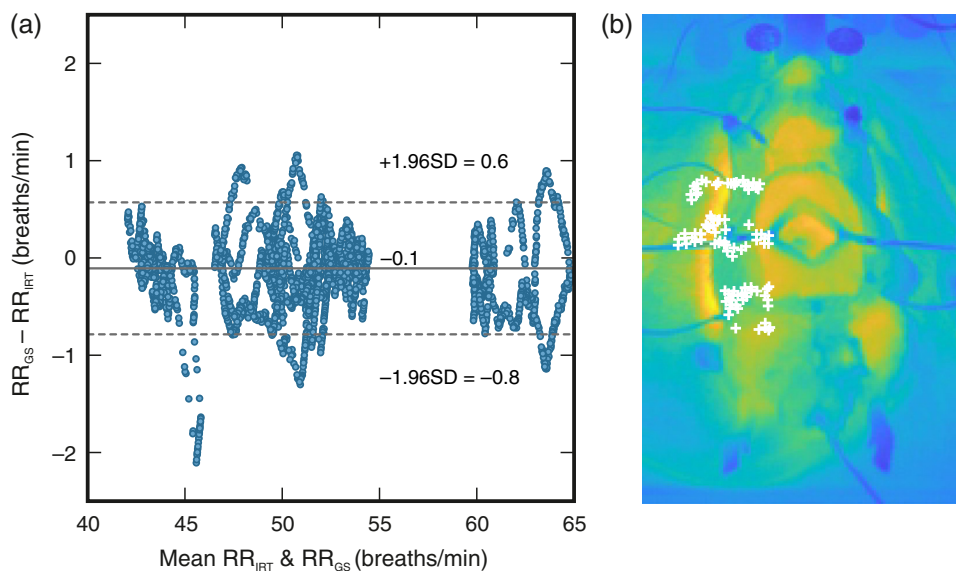


Fig. 2 (a) Bland–Altman plot comparing the RR estimated with IRT and the gold standard (ECG-derived RR).¹¹ The limits of agreement range from -0.8 to 0.6 breaths/min. The bias averages -0.1 breaths/min. (b) Thermogram of a rat and feature points used for motion tracking.

In a rat model, changes of cerebral blood flow and oxygen consumption were examined during a 16-s electrical forepaw stimulation. By thinning out the bone to translucency, a cranial window was prepared enabling to record image sequences. Although blood flow increased by about 15%, deoxyhemoglobin concentration dropped and oxyhemoglobin concentration rose by the same amount. Not only the sum level but spatially resolved blood flow and oxygenation maps could be obtained (Fig. 3).

3.3 Motion Activity

Several approaches for assessing motion activity have been published.^{11,32,36,37} In these studies, the results of the tracking

performance were evaluated qualitatively. They demonstrated the ability to accurately detect and track the animal during different behavioral tasks, including open field test, Y-maze task, and Barnes maze, among others. Note that the animal detection was performed automatically. Since automated identification is still a challenging task (especially when the animal and background have similar colors), in some processing algorithms, the position of the animal must be defined manually by the user. As demonstrated in Fig. 4(a), this task is simplified with IRT, as the animal is a hot spot in the thermal video (the background is much colder than the animal). Figure 4(b) shows a motion heat map, which is usually used to demonstrate the cumulative time spent in different parts of the arena; yellow denotes more time and blue, less or no time.

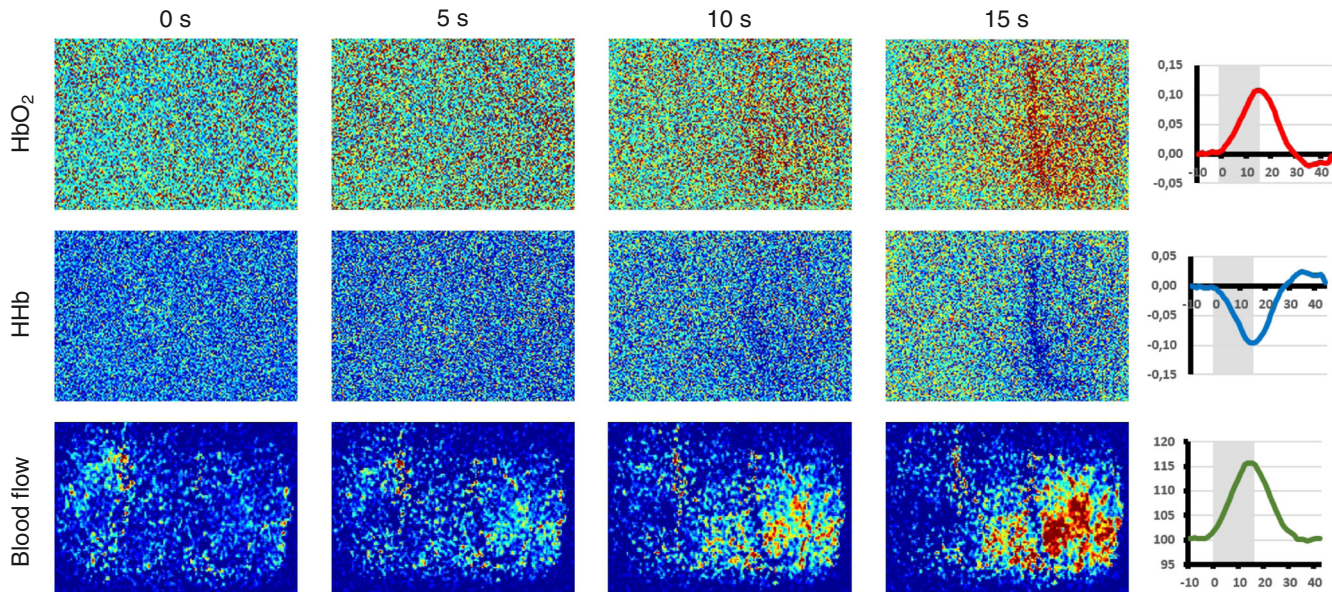


Fig. 3 Superficial tissue imaging system for combined RGB reflectometry and laser speckle contrast analysis [superficial tissue imaging system (STIS) provided by Kohl-Bareis and Steimers, Biomedical Optics Laboratory, Rheinahrampus Remagen, Germany]. Blood flow and blood oxygenation responses to 16-s electrical forepaw stimulation were recorded through a cranial window preparation (bone thinned to translucency) in isoflurane plus fentanyl anesthetized rats. Laser speckle contrast, HbO₂, and HHb-images were online calculated from raw images of speckle pattern and red, green, and blue LED-illumination images using STIS and stored at ~0.75 Hz (LabVIEW 2010, National Instruments Inc.) for later offline calculation of cortical blood flow from speckle contrast.

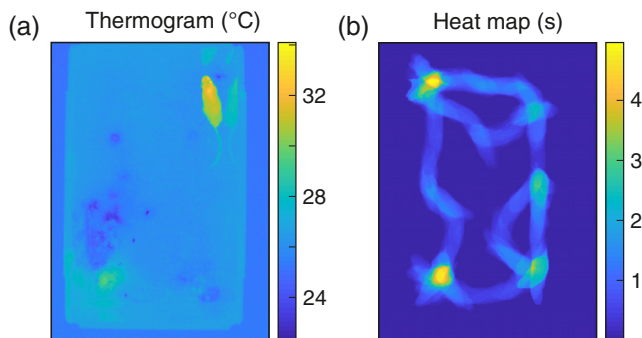


Fig. 4 (a) Thermogram of the open field arena. The animal is the hot spot in the image. (b) Motion heat map.¹¹

3.4 Wound Monitoring

Figure 5 shows the exemplary results for contactless wound monitoring. Both upper images show visual images of laparotomy wounds of two different, male Wistar rats. In addition, from the incision, no redness can be observed. The right wound image seems to show better healing, as the visible remainder of the incision is much smaller.

Below, the same images are presented with an IRT overlay, which shows the temperature distribution around the wound area in “false colors.” Here, Fig. 5(b) shows high-temperature fluctuations around the wound, whereas Fig. 5(a) presents a much more homogeneous temperature distribution. Also large cold spots can be observed in Fig. 5(b), which imply the secretion of exudate.

4 Discussion

The first steps toward the application of camera-based systems in medical research were taken in the late 90's. However, the big

boom in this area took place in the years around 2010, when different approaches for measurement of vital parameters (HR and RR) in humans were presented. More recently, these techniques have been adapted to animal research.

As presented in Tables 4 and 5, the performance of imaging systems has only been tested in four animal species (mice, rats, pigs, and nonhuman primates) to date. In general, a high agreement between video-based vital parameters and references was obtained. Surprisingly, these techniques were even capable of quantifying the cardiac activity in rodents. These species have high HRs, and also exhibit very little precordial excursion of the chest during respiration and heart activity.

Of the three techniques, NIR was the least explored. In contrast to Blanik et al.,²¹ who reported a good agreement between HR estimated with NIR and the gold standard (study on pigs), Unakafov et al.²² could not extract this parameter (HR) from NIR videos of nonhuman primates. However, this research group did not use active illumination (ideally 940 nm) during video acquisition. As a result, the environmental ambient illumination might have negatively affected the signal-to-noise ratio.

Except for one study, all video acquisitions were conducted on anesthetized animals. Although in the study of Unakafov et al.,²² the nonhuman primates were awake, to minimize facial motion, no reward was handed out during the video recording. The presence of motion in videos plays an instrumental role for the quality of the extracted signal and, consequently, for the estimation of vital parameters. To date, no approach that has been described in the literature is capable of compensating strong motion artefacts. Therefore, for long-term monitoring, new concepts or strategies must be developed to avoid measurement errors. For instance, the monitoring system should include a motion index. On the one hand, if the index decreases below a predefined threshold or is equal to zero, the animal is still and,

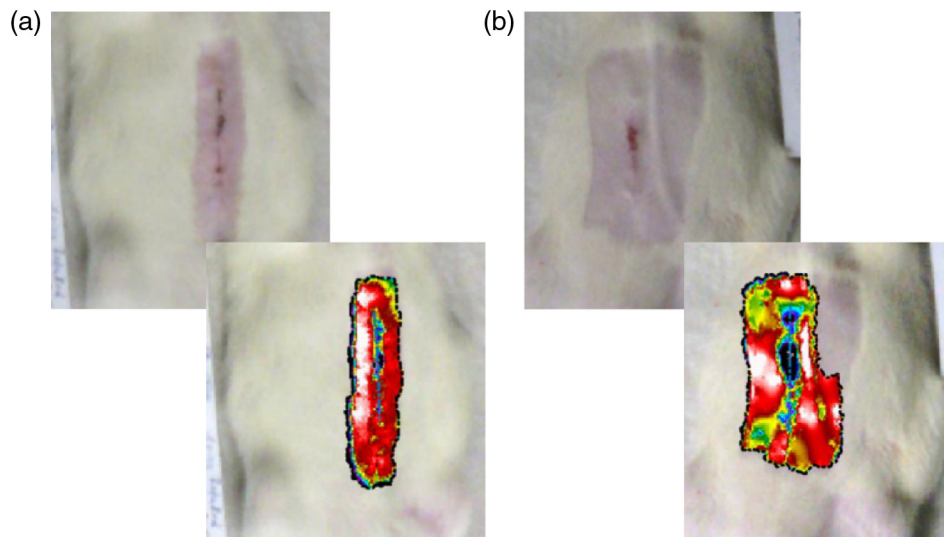


Fig. 5 (a) and (b) Images of two laparotomy wounds. The lower images present, in addition, an IRT overlay showing the temperature distribution around the wound area in “false colors.”

thus, the vitals might be assessed. On the other hand, if the motion index surpasses the threshold, it means that the animal is moving and no valid estimation can be performed.

In addition to movement, the chosen ROI also influences the outcome. Tables 1 and 3 show the best regions for each species. Although there are some differences between species, the thorax is the most common and reliable area for HR and RR measurement. For the color-based approach, the animal's skin must be visible to present the varying light absorption. Therefore, the animals usually need to be shaved.

The technical requirements of the cameras are further factors conditioning the performance of the camera-based systems. In addition to resolution and frame rate, the focal length of the lens must be taken into consideration, which strongly depends on the experimental setup. For assessment of vital parameters, the most important criterion to be met is that the ROI or the body of the animal should represent a large portion of the image. Thus the ideal focal length must result from the trade-off between the distance of the camera to the object and the area of the ROI (ideally, a long-focus lens for great distances between camera and subject; a normal/wide-angle lens for smaller distances). For applications such as open field tests, wide-angle lenses are recommended.

Finally, the strengths and weaknesses of the three optical channels (visible, NIR, and thermal) must be reported. When measuring respiration, all three techniques present similar outcomes, since the respiratory signal is very prominent. Thus the relative strengths and weaknesses of these three channels only rely on the costs and necessity of external light sources. However, there are other factors that should be considered with regard to monitoring HR. In contrast with visible and NIR imaging, IRT delivers images with less contrast and sharpness, since it relies on contrasting temperatures to clearly define objects. Thus this technique is less effective in situations where definition is expected, and the surface temperatures of objects are very uniform. This is the case of body temperature, which is relatively uniform, making it difficult to find body landmarks or tracking feature points, as the edges are not so “sharp.” Weak edges/contours might lead to inaccurate tracking of feature points, and consequently, reduce the signal-to-noise ratio or

even compromise the whole monitoring/measurement. This problem can be minimized by further improving detector sensitivity. RGB and NIR cameras present, in turn, better contrast, but are sensitive to illumination changes. In NIR, these variations can be significantly reduced and the signal quality enhanced by actively illuminating the subject at a narrow-bandwidth NIR (e.g., 940 nm) and by applying a matching bandpass filter on the camera. However, although in the visible spectrum, the intensity changes due to blood flow are very small compared to the absolute pixel intensity values; in NIR frequencies, the already weak pulsatile signal is additionally minimized. In this bandwidth, the hemoglobin absorption is significantly lower when compared with the absorption peak in the green range of the light spectrum. On the top of that, the sensitivity of camera sensors is decreased in the NIR frequencies, thus the strength of the pulse signal is much lower than in the visible spectrum. It is important to note that illumination variance is not an issue in the motion-based approach, thus there should not be any difference between visual and NIR imaging. To sum up, when selecting the imaging system as well as the algorithmic approach (either motion- or color-based), all previous factors should be weighed.

Remote PPG (color-based approach; contact-free monitoring of the optical absorption changes in the skin due to varying blood volume) triggers the extension of algorithms to include a new feature, assessment of oxygenation or SpO_2 (contact-free pulse oximetry). Van Gastel et al.³⁰ reported outstanding results; in their study, the RMSE between NIR and the gold standard averaged 2%. Although this parameter was not assessed in animal trials, a similar technique was used by Steimers et al.³¹ to quantify cortical HbO_2 and HHb changes. Note that HbO_2 and HHb are the parameters used to quantify SpO_2 (after a calibration). Their system was capable of detecting HbO_2 and HHb changes with high temporal and spatial resolutions during different conditions (including ischemic stroke and hypercapnia). In addition, this demonstrated to be highly sensitive.

Regarding motion activity, there are several commercial and noncommercial systems that permit us to track the animal and analyze its motion. In fact, to help other researchers, some groups (such as Patel et al.³²) have published, in combination

with the journal article, the source-code of the tracking and analysis software. These approaches were mainly developed for behavioral tasks such as open field tests, where the animal is always in the camera's field of view. Although the monitoring of animals in their home cage is widely desired (due to the circadian rhythms), this is still a challenging task. The motion of a single animal can be easily computed by comparing the background model against the current image or by establishing an image reference. In cages with more animals, the task is more complex when the aim is to analyze individual movements. Multitracking approaches are not capable of identifying similar/identical objects. In addition, occlusion is a major problem in imaging processing.

Close observation of wounds is necessary to detect inflammation in an early stage and to avoid systemic consequences such as a sepsis. Although laboratory parameters respond slowly and can be very unspecific, reliability of visual inspection depends on the observer's experience and the accessibility of wounds. In fact, the combination of cameras sensitive in infrared and visible spectrum is highly promising to derive new insights for wound pathophysiology. Moreover, image processing might lead to objective and comparable parameters and, thus, also to wound status scores. Although several morphological aspects have already been noted, clinical validation is pending and potential clinical benefits should be proven. However, a few studies already report the clinical application of IRT. In acute third-degree burn wounds, IRT has been capable of determining unsalvageable tissue, similar to widely used indocyanine green angiography.³⁸ In another study, elevation of temperature between wound region and healthy skin of 4°C to 5°C was noted, leading to correct differentiation between the patients suffering from infected wounds and the control group patients.³⁹

5 Conclusions

To date, severity assessment typically requires animal handling or even the application of invasive methods (e.g., implantation of transponders). But, for various application areas, contactless alternatives are available to acquire such information as respiration rate, HR, oxygenation, motion activity, and potential wound infection. Requirements on the appropriate camera system highly depend on the type of application. Thermal imaging or IRT takes advantage of being absolutely passive, meaning that no ambient light is required. In this context, it is noteworthy that, only movements and thermal effects (cooling or warming) are detectable, whereas color changes, e.g., caused by perfusion, are not. Imaging systems operating in the visible spectrum (or NIR) are relatively cheap and permit the analysis of movements as well as color changes. However, they require ambient light or even active illumination to improve the signal-to-noise ratio (especially when NIR is used).

Disclosures

M. C. is a co-founder and CEO of Docs in Clouds GmbH; J. K. and C. B. P. are employed by Docs in Clouds GmbH, providing applications for camera-based vital detection and telemedicine. T. T. is a shareholder of Cardior Pharmaceuticals GmbH.

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Carina Barbosa Pereira received her MSc degree in biomedical engineering from the University of Minho (Braga, Portugal) in 2011, and her Dr.-Ing. degree from the RWTH Aachen University (Aachen, Germany) in 2018. In the same year, she was appointed senior scientist at the Department of Anesthesiology of the RWTH Aachen University. Her research interests include infrared thermography, imaging and biosignal processing as well as feedback control in medicine.

Janosch Kunczik is an engineering scientist at the University Hospital of RWTH Aachen. He received his MSc degree in electrical engineering, focusing on system technology and automation, from RWTH Aachen University in 2017. After gaining in-depth experience in robust control for medical applications during his graduate studies, he is now focusing on contactless biomedical sensing techniques.

André Bleich heads the Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School. Besides, he also represents the animal welfare commissioner at his university. His major research interests include animal welfare, microbiome and gnotobiology, and animal models of inflammatory bowel disease, as well as infection models.

Christine Haeger studied biology at the University of Bremen, Germany (2000–2005), and completed a doctoral thesis on lung inflammation and immune responses at the Hannover Medical

School (MHH), Germany, obtaining a Dr. rer. nat. in 2008, followed by a postdoc focusing on vascular inflammation and regeneration, MHH (2009–2012). Since 2013, she has been a postdoc in the Institute for Laboratory Animal Science, MHH, focusing on murine models for inflammatory bowel disease. Since 2017, she has served as principal investigator within the DFG-funded research unit 2591: severity assessment in animal-based research.

Fabian Kiessling studied medicine in Heidelberg. Until 2008, he worked in the Departments of Radiology and Medical Physics in Radiology at the German Cancer Research Center. In parallel, he did his clinical training at Heidelberg University and received the board certification as radiologist. Since 2008 he has led the Institute for Experimental Molecular Imaging at RWTH Aachen University. He has authored over 300 publications and book chapters, edited three books, and received multiple research awards.

Thomas Thum studied medicine in Hannover, with state examination in 2001 and board examination for internal medicine/cardiology in 2009/2010. He received his PhD at the Imperial College, London (2008). He is a full professor and director of the Institute of Molecular and Translational Therapeutic Strategies at the Hannover Medical School, and visiting professor at the Imperial College. He has coauthored over 300 publications and is a distinguished reviewer, board member, patent holder, and founder.

René Tolba studied medicine in Bonn (Germany) and Pittsburgh (USA). In 2002, he received his PhD from the University of Bonn. From 1999 to 2007, he worked in the Department of Surgery at the University Hospital of Bonn. Nowadays, he holds a professorship at the RWTH Aachen University and is director of the Institute for Laboratory Animal Science. His research focuses on visceral organ transplantation and preservation as well as animal models for various disciplines.

Ute Lindauer completed the state examination in veterinary medicine in 1988 and obtained her doctorate in 1991 from Ludwig-Maximilians-University in Munich. She has worked as a postdoctoral fellow in Berlin in the Experimental Neurology Lab at University Hospital Charité—Universitätsmedizin Berlin in Ulrich Dimagli's group, where she finished her professional dissertation (habilitation) in experimental neurology in 2001. She worked as an associate professor at the Technical University in Munich, from 2009 to 2014, and is now an associate professor of translational neurosurgery and neurobiology at the Medical Faculty of RWTH Aachen University, Germany. Her research focuses on mechanisms of cerebrovascular regulation in health and disease.

Stefan Treue holds a professorship for cognitive neuroscience at the University of Goettingen, Germany and is director of the German Primate Center. He received his PhD in systems neuroscience in 1992 from MIT. His research interest is focused on the role of attention in cortical processing of visual information in primates (www.dpz.eu/cnl). Additionally he is interested in advancing the wellbeing of primates in research and is engaged in public outreach about animal research.

Michael Czaplik is working for the Department of Anesthesiology, University Hospital RWTH Aachen as senior consultant in anesthesiology. While heading the interdisciplinary section "Medical Technology," he has been leading diverse research projects combining medical and technical expertise since 2014. His main research focus includes camera-based monitoring modalities in terms of anesthesiology, as well as interoperability and telemedicine. Besides his academic affiliation, he is also cofounder and CEO of Docs in Clouds TeleCare GmbH.