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### Touch-free measurement of body temperature using close-up thermography of the ocular surface



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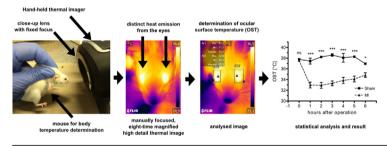
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#### GRAPHICAL ABSTRACT



#### ABSTRACT

In experimental animal research body temperature (BT) is measured for the objective determination of an animals' physiological condition. Invasive, probe-based measurements are stressful and can influence experimental outcome. Alternatively BT can be determined touch-free from the emitted heat of the organism at a single spot using infrared thermometers [1]. To get visual confirmation and find more appropriate surfaces for measurement a hand-held thermal imager was equipped with a self-made, cheap, 3D-printable close-up lens system that reproducibly creates eight-time magnified thermal images and improves sensitivity. This setup was

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*Abbreviations*: BT, Body temperature; OST, Ocular surface temperature; rOSTd, relative ocular surface temperature decline; MI, myocardial infarction; LAD, left anterior descending artery.

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used to establish ocular surface temperature (OST), representing the temperature of the brain-heart axis, as a touch-free alternative for measurement of BT in mice, rats, rabbits and humans.OST measurement after isoflurane exposure and myocardial infarction (MI) experiments in mice revealed high physiological relevance and sensitivity, the possibility to discriminate between MI and sham operations in one hour and even long-term outcome-predictive capabilities of OST after MI. Summarized here we present:

- Self-made close-up lens for thermal imaging cameras for eight-time magnification
- · Establishment of OST for touch-free determination of BT in rodents and humans
- Short- and long-term predictive capabilities of OST in experimental MI in mice.
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#### Method details

Body temperature (BT) is widely accepted and regularly used for objective determination of overall body function. In a broad spectrum of pathologic conditions such as sepsis but also in sterile inflammation BT rises or drops rapidly and represents an important prognostic indicator [2]. In experimental animal research BT is used for physiological surveillance but also to define humane endpoints e.g. to abort an experiment before the animal suffers excessively or dies. Such thresholds have been suggested for several disease models for example in viral or bacterial infection or shock [3–5]. BT is commonly measured in the rectum since temperature is well preserved in the rectum due to low heat diffusion and removal [6]. However, rapid temperature changes cannot be detected. As an alternative telemetric temperature surveillance by implanted probes was developed [7,8]. However, this procedure constitutes a considerable burden for the animal, requires a recovery phase and can influence the animal's physiology and experimental outcome.

To measure temperature non-invasively infrared radiation can be used that is naturally emitted from the surface of every object. Using a hand-held laser-spot guided infrared thermometer the chest surface temperature from mice was measured and correlated with rectal BT [11]. However this fast and flexible approach that reduces animal fixation time resulted in too low, non-physiological temperatures, probably due to the isolating fur at the measuring spot. Furthermore, analysis of a single defined spot (here: xiphoid process) on the chest surface seems questionable for reproducible temperature measurements, since it can be strongly influenced by the isolating fur and heat accumulation under the animal. Additionally it is unclear from the method description how a reproducible distance between thermometer and object is kept constant and how this can influence measurement results. This is crucial since thermal radiation is known to be influenced by environmental factors.

Compared to a single spot infrared thermometer, thermography generates images that can be analyzed on a per pixel basis as if thousands of infrared spot thermometers are used simultaneously. Using thermal imaging we were quickly able to identify the eyes as the most prominent and warmest surface on mice (Fig. 1). However the build-in optics did not allow close-up images from the mouse eyes. By using the thermal focus lens with fixed focus distance that is described here, we were able to create are reproducible distance to the object whenever a sharp image was achieved and so improved thermal sensitivity, which allowed quantification.

#### Materials and methods

#### Thermal-imaging and focus lens for close-up imaging

A factory-calibrated handheld thermal imaging camera (FLIR E8, FLIR Systems, Inc.) with 320 × 240 pixels (9 Hz refresh-rate) and a built-in screen for instant visual focus control was used for all thermal

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