Research Paper

IR thermography-based monitoring of respiration phase without image segmentation

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GRAPHICAL ABSTRACT

Monitoring Respiration by IR thermography

HIGHLIGHTS

• A new algorithm for improving non-contact monitoring of respiration with IR thermography is established.
• Novel algorithm obviates the need for defining regions of interest, image segmentation and tracking of the nostril.
• Validation in a preclinical mouse model and human subjects confirm accurate, robust, user-friendly extraction of respiration phase.
• The novel approach facilitates wider application of IR thermography in biomedical and clinical research.

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ABSTRACT

Background: Respiratory rate is an essential parameter in biomedical research and clinical applications. Most respiration measurement techniques in preclinical animal models require surgical implantation of sensors. Current clinical measurement modalities typically involve attachment of sensors to the patient, causing discomfort. We have previously developed a non-contact approach to measuring respiration phase in head-restrained rodents using infrared (IR) thermography. While the non-invasive nature of IR thermography offers many advantages, it also bears the complexity of extracting respiration signals from videos. Previously reported algorithms involve image segmentation to identify the nose in IR videos and extract breathing-relevant pixels which is particularly challenging if the videos have low contrast or suffer from suboptimal focusing.

New method: To address this challenge, we developed a novel algorithm, which extracts respiration signals based on pixel time series, removing the need for nose-tracking and image segmentation.

Results & comparison with existing methods: We validated the algorithm by performing respiration measurements in head-restrained mice and in humans with IR thermography in parallel with established standard techniques. We find the algorithm reliably detects inhalation onsets with high temporal precision.

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1. Introduction

All mammals rely on the active ventilation of their lungs for pulmonary gas exchange. Coordinated muscle contractions control a suction-pump process which eventually delivers oxygen to and removes carbon dioxide from the bloodstream during inhalation and exhalation, respectively. Inhalation also mediates the transport of airborne odorant molecules to the olfactory receptors in the nasal epithelium, which is the first step in olfactory perception. During odorant sampling in the environment, inhalations are commonly structured into one or more brief pulses, also referred to as sniffing. Sniffing is an active sensing process that has a major influence on olfactory perception (Bensafi et al., 2003; Mainland and Sobel, 2006) and which is modulated in response to environmental factors and by emotional states (Bensafi et al., 2002; Ferdenzi et al., 2015, 2014). Olfactory dysfunction is also one of the earliest pathological symptoms in various neurodegenerative diseases (Godoy et al., 2015; Sobel et al., 2001). Respiration and sniffing behavior thus not only reflect a critical vital function, but are also crucial for assessing the physiological and psychological state of an organism.

A wide variety of methodologies have been established for monitoring respiration in biomedical research and in clinical settings. A particularly attractive approach involves the use of infrared cameras to enable non-contact, distant measurement. In this measurement modality, respiration rate is obtained based on imaging temperature differences related to inhalation and exhalation at the intranasal surface. Proof-of-principle demonstrations of nasal thermography have been performed in neonates and adult humans (Abbas et al., 2011; Chauvin et al., 2016; Cho et al., 2017; Fei and Pavlidis, 2010; Lewis et al., 2011; Pereira et al., 2015). Furthermore, we have recently adapted IR thermography for respiration monitoring in the most common preclinical experimental animal model, the mouse mus musculus. Specifically, we have established thermography for use in head-restrained animals (Esquivelzeta Rabell et al., 2017). This experimental approach facilitates combining precise stimulus control with monitoring neural activity and is widely used by researchers studying sensory systems including vision (Andermann et al., 2010), somatosensation (O’Connor et al., 2010) and olfaction (Rokni et al., 2014), as well as reward-based learning (Cohen et al., 2012) and decision-making (Allen et al., 2017) in rodents.

While the non-invasive nature of IR thermography undoubtedly offers advantages, it also bears the complication of extracting respiration signals from complex video data. Previously reported algorithms, including our own first-generation algorithm (Esquivelzeta Rabell et al., 2017), involve image segmentation to identify the nose in IR videos in order to extract breathing-relevant pixels. This step can be particularly challenging if the videos have low contrast or suffer from suboptimal focusing, compromising the overall robustness of the approach.

In this work, we have developed a novel algorithm to address this limitation. Signal extraction is based on the analysis of temporal pixel properties, rather than using image segmentation. We validated our approach by comparison to the invasive standard method in mice, which is based on monitoring respiration-related pressure changes in the nasal cavity. Compared to the previously published algorithm (Esquivelzeta Rabell et al., 2017), the new algorithm provides major improvements: a) it does not require manual parameter setting, b) it is about 10 times faster and c) it detects inhalation onsets with fewer errors. We have further applied the new algorithm to IR videos from freely breathing humans, taken while the subject was performing small head movements. Validation was performed by comparison to signals obtained from a piezo-electric belt.

2. Materials and methods

2.1. Mice

Experiments were performed in accordance with standards and rules of KU Leuven and national regulations, implementing European policies, including the EEC Council Directive 2010/63/UE. We used C57BL/6 males at postnatal ages of 2–6 months. Mice were housed on a 12 h dark/12 h light cycle (dark from 07:00 to 19:00).

2.2. Surgery

All surgeries were performed under aseptic conditions with animals under ketamine/medetomidine (60 mg kg$^{-1}$, and 0.5 mg kg$^{-1}$, intraperitoneal, respectively) anesthesia. Analgesia (ketoprofen, 5 mg kg$^{-1}$ intraperitoneally; buprenorphine, 0.1 mg kg$^{-1}$, intraperitoneally) was administered post-operatively.

2.3. Intranasal cannula implantation

To monitor intranasal pressure, a 7 mm long, 0.8 mm inner diameter × 1.6 mm outer diameter PTFE cannula (PTFE Tubing, item # EW-06407-41; Cole-Parmer) was implanted into the left nostril. An incision was made along the midline from the fur transitional area at the tip of the nose to caudal of the eyes using a scalpel. Then, a small hole was drilled with a carbide bur (Neoburr FG 1/4; Microcopy) in the bone overlying the nostril. The cannula was put on the bone such that it covers the hole, stabilized with luting agent (RelvyX Luting Cement; 3 M) and mounted using dental cement (Jet Denture Repair; Lang Dental). The cannula was capped using a steel plunger. The length of the plunger was chosen such that when inserted into the cannula, the distal end of the plunger would protrude ~200 μm from the distal end of the cannula.

2.4. Head plate implantation

Mice were implanted with a head plate for head fixation as described previously (Cohen et al., 2012). The scalp and fascia were removed, and a metal head plate was stabilized over the midline with luting agent (RelvyX Luting Cement; 3 M) and mounted using dental cement (Jet Denture Repair; Lang Dental).

2.5. Olfactometry

Animals were head-restrained inside a sound and light isolated box (75 cm$^3$) with constant exhaust to ensure rapid clearance of odorants and to prevent distraction of the animal. To test the performance of our method during sniffing, we presented novel and familiar odors to the mouse, as described previously (Esquivelzeta Rabell et al., 2017). Briefly, odors were delivered using a custom-made olfactometer. The flow rate was fixed to 500 ml min$^{-1}$. The olfactometer was controlled using custom-written scripts in LabVIEW (National Instruments).
2.6. Collection of thermal videos from mice

We imaged the wet nose of the mouse using a thermal camera (FLIR A325sc with 50 μm lens, 60 fps, 320 × 240 pixels). Fig. 1A shows an illustration of the setup. The camera was triggered using LabVIEW (National Instruments), which in turn triggered the recording in the camera software (FLIR ResearchIR MAX 4.30).

2.7. Measurement of intranasal pressure in mice

During recording sessions, the intranasal cannula was connected to a pressure sensor (MPXV7002GC6U, Freescale Semiconductor) with polymer tube (Tygon Lab Tubing, Non-DEHP, 1/16”ID x 1/8”OD, item # EW-06407-71; Cole-Parmer). The signal from the pressure sensor was sampled at 3 kHz and recorded with LabVIEW (National Instruments) using a data acquisition board (SCB-68A; National Instruments).

2.8. Data collection in human subjects

The experimental setup for imaging human respiration (Fig. 1B) was essentially the same as for the mouse, except that we did not use a close-up lens in the thermal camera. For validation purpose, we conducted measurements with a respiratory belt transducer containing a piezo-electric. Belt signals were sampled at 1 kHz. We collected 35 videos from 6 different human subjects, instructed to perform occasional head movements.

2.9. Extraction of inhalation onsets from mouse IR videos

The goal of the algorithm is to identify respiratory phase, specifically inhalation onsets, from raw pixel values. Importantly, due to movement of the subject, different pixels can contribute to the breathing signal in different frames. The respiration signal is contained in the raw signal values of pixels reflecting the nasal surface. Two major factors determine the temperature at the intranasal surface. On one hand, it is determined by the difference between radiation heat loss (natural convection) and passive warming of the body through the bloodstream. On the other hand, there is forced convection i.e. heating from exhalation or cooling due to inhalation. In addition, there is the temperature difference between inhaled air from the room (~20 °C) and body temperature exhaled air (~37 °C). As a result of the dynamic interplay of all factors, inhalation and exhalation appear as cooling and warming of the intranasal surface, respectively, (Fig. 1C). The highest temperature is observed in the nostrils at the exhalation offset which typically also corresponds to inhalation onset, unless the subject holds its breath. The lowest temperature occurs at the inhalation offset. Of note, even in the absence of exhalation, the intranasal surface undergoes passive warming through the bloodstream, although it occurs much slower than by forced convection. Therefore, extraction of inhalation onsets is inherently more unreliable than the extraction of exhalation onsets.

As a first step in the algorithm, all frames of a video are cropped around a central, rectangular region of interest (ROI) of 170 pixels x 210 pixels (height x width), large enough to cover the nose, even when it is moving. Then all frames are decomposed into pixel signals. For identification of inhalation onsets relative signal changes are sufficient. Therefore, we did not convert raw pixel signal values to absolute temperature values. Raw pixel values are given as arbitrary units (a.u.) throughout this article.

Inhalation onsets are characterized by rapid cooling of the nasal surface, which is reflected in a steep decrease in raw pixel values. The inhalation period terminates with the inhalation offset, which is also the coldest point during a respiration cycle. The overall approach of the algorithm is based on these two characteristics.

We first exclude non-varying pixels, using an empirically determined variance threshold. Due to nose movements, different pixels contribute to the signal during different frames and it is not straightforward to simply identify the signal pixel among the remaining raw traces (Fig. 2A). The key approach of our algorithm is to identify decreasing segments among the pixel signals, by applying empirically determined thresholds for minimum frame-to-frame decrease (32u). If there is a single frame (16.7 ms) between
two potential decrease segments, they are merged. Then we apply additional thresholds for minimum decrease amplitude (107u) and maximum decrease duration (10 frames/166.7 ms). All pixel signal values outside the decrease segments are set to NaN (not a number). True inhalations are characterized by multiple, overlapping decrease segments (Fig. 2B), because the wet nose is covered by several pixels. Hence, when there are only very few decrease segments in a frame (<1% of all decrease segments), i.e. too few pixels contribute, we remove these decrease segments. Moreover, if decrease segments are closer than 3 frames (50 ms) from each other, we remove one of them. These steps result in a final collection of decrease segments as depicted in Fig. 2B. We determine the inhalation onsets based on the onset of these decrease segments.

2.10. Extraction of inhalation onsets from human IR videos

We adjusted our algorithm to extract inhalation onsets from IR videos taken of a human subject. Fig. 1B shows the thermal images of the human nose in a respiratory cycle. As is the case in the IR videos obtained from mice, inhalation cools down the nostril surface, which gradually warms up again during exhalation. All frames of a video are cropped around a central, rectangular region of interest (ROI) of 170 pixels x 210 pixels (height x width), large enough to cover the nose, even when the head is moving. Then all frames are decomposed into pixel signals.

After excluding non-varying pixels, we directly construct an envelope around the signal using minimum pixel values, as shown in Fig. 5. Respiration frequencies in humans at rest are far lower (0.2–0.3 Hz) than in rodents (5–6 Hz). Therefore, we smooth the resulting signal using a moving average with window of 35 frames (583.3 ms), to increase robustness of the signals.

Next, we identify peaks and their corresponding pixels in the signal. Peaks are defined as a data sample which is larger than the two neighboring samples with minimum peak distance of 45 frames (750 ms). The minimum peak width and minimum peak height are set to 1 standard deviation. Identified peaks correspond to the onset of rapid cooling, which reflect potential inhalation onsets (Fig. 6A).

All analysis was performed using custom-written scripts in MATLAB (Mathworks). All scripts are available from the authors.

3. Results

3.1. Performance on mouse IR videos

For validation of the algorithm, we directly compared respiration measures derived from IR videos with standard recording techniques in mice and humans respectively. The most widely used method for measuring respiration in mice involves recording intranasal pressure through a cannula, which is chronically implanted into the nasal cavity. Hence, we recorded intranasal pressure as described previously (Reisert et al., 2014) while simultaneously recording 153 videos from 3 head-restrained mice. Examples of such recordings are shown in Fig. 3A. Inhalation onsets in the pressure signal are indicated by falling zero-crossings (Fig. 3A, black crosses). Inhalation onsets determined by the algorithm in IR videos are depicted in Fig. 3A (red circles).

To capture respiration during natural odor sampling behavior including during high frequency sniffing, we performed odor stimulation trials. In each trial, we first recorded baseline breathing for 5 s after which we introduced an odorant to the mouse for 2 s. We then continued to record breathing for another 5 s. We quantified the error rate of the algorithm by comparing the total number of inhalation onsets inh detected by either method in each trial. Trials contain false negative inhalation onsets if inhtrue > inh and false positive inhalation onsets when inhtrue < inh. The error rate for each trial was then calculated as the number of mismatches (false positive or false negative), divided by the total number of inhalations. The median error rate across all trials in our dataset was 2.8 % (±0.4 %, S.E.M.).

Next, we examined the temporal relationship between inhalation onsets detected by IR thermography and intranasal pressure. For each inhalation onset obtained by pressure measurement, we identified the next nearest inhalations derived from IR videos. The delay between such two inhalation onsets provides a measure of the temporal precision of inhalation detection. Delays were narrowly distributed with a peak at 13 ms (Fig. 3B) indicating that the algorithm extracts inhalations onsets with high temporal accuracy. The median absolute delay was 17 ms (±4 ms, S.E.M.). Inhalation onsets derived from IR thermography consistently lagged behind inhalation onsets determined from intranasal pressure (Fig. 3A,B). This is consistent with the respective underlying physical signals. Respiration-related changes of intranasal airflow which are captured by the pressure sensor, precede cooling and warming of the nasal surface. We did not find a strong bias of delays towards specific respiration frequencies (Pearson correlation coefficient = 0.05, p = 0.001), suggesting all respiration rates can be monitored with similar precision (Fig. 3C). Importantly, when comparing the distribution of breathing frequencies measured with both methods, we did not observe a specific bias towards detecting particular types of inhalations more readily than others (Fig. 3D,E).

To demonstrate the improvement of the new algorithm (referred to as v2.0) over the previously published version (referred to as v1.0, Esquivelzeta Rabell et al., 2017) we quantified the performance of both algorithms on the same dataset. Performance was tested against inhalation onsets extracted from intranasal pressure measurements. This comparison reveals that v2.0 extracts inhalation onsets with far fewer errors than v1.0 (Fig. 4C). This is particularly apparent in videos, which are poorly analyzed by v1.0 (animal 3 in Fig. 4C). According to the method described by Esquivelzeta Rabell et al., 2017, the behavioral session from animal...
3 would have been excluded from further analysis, but the algorithm v2.0 can faithfully extract inhalation onsets in those videos. Moreover, we find that v2.0 is about 10× faster than the previous version (Fig. 4D). Finally, it is important to note that v2.0 operates fully automatic. No parameters need to be set to appropriately enhance contrast and no manual confirmation of image segmentation is needed.

3.2. Performance on human IR videos

The validation of the signal extracted from human IR videos was performed in comparison with a piezo-electric thoracic belt. Each trial consisted of 30 s recording/imaging. We instructed human subjects to perform occasional head movements. Inhalation onsets determined by the two approaches corresponded well (Fig. 6A). The median error rate across all trials in our dataset was 6.2% (±1.8%, S.E.M.). Inhalation onsets derived from IR thermography lagged behind inhalation onsets extracted from the piezoelectric belt signal (Fig. 6A), in line with the fact that respiration-related thorax extension precedes cooling and warming of the nasal surface. The distribution of delays between inhalation onsets obtained with the two methods reveal a peak at 331 ms (Fig. 6B). The median absolute delay was 689 ms (±217 ms, S.E.M.). Consistent with the mouse dataset, we did not find a strong bias of delays towards specific respiration frequencies, however, we observed that the variance of the delays tends to be smaller at higher frequencies (Pearson correlation coefficient = −0.15, p < 0.002, Fig. 6C). This likely reflects higher detection accuracy at higher breathing rates. Importantly, when comparing the distribution of breathing frequencies measured with both methods in, we did not observe a specific bias towards detecting particular types of inhalations more readily than others (Fig. 6D,E).

4. Discussion

The development of the new algorithm for extracting inhalation onsets from IR videos described here provides a significant step forward in establishing IR thermography for chronic, non-contact monitoring of respiration in biomedical and clinical research. Our validation experiments in head-restrained mice demonstrate that IR thermography can be used to reliably monitor respiration in the same animal over long time periods in a simple, efficient way. Alternative non-invasive approaches for distant monitoring of respiration in head-restrained rodents have significant limitations. Placing a thermopile or thermocouple in front of the mouse’s nose is prone to frequent signal disruption due to
Fig. 4. Direct comparison between image segmentation-based algorithm (v1.0, Esquivelzeta Rabell et al., 2017) and the novel algorithm described here (v2.0). (A) Example frame highlighting pixels contributing to extracted signal according to v1.0 (shaded yellow circles). A circular region of interest (ROI) is drawn around the estimated center of the nostril. The signal is computed as the mean of the lowest 10% percentile of all pixel values in the ROI. (B) Same frame as in (A) but highlighting pixels contributing to signal according to the new algorithm (shaded yellow square). Here, pixels are identified based on their temporal signal properties, i.e. pixels are selected which mark the onset of decrease segments. (C) Error rates (mean ± S.E.M.) relative to ground-truth, pressure-based inhalation onsets for both algorithms in videos from three different animals ($n_{\text{animal}}$ = 3, $n_{\text{animal}2} = 5$, $n_{\text{animal}3} = 25$). Comparison of the mean error rate of v1.0 vs. v2.0 unveils superior performance of v2.0 (student’s t-test, significance level * $p<0.02$, ** $p<0.001$). (D) Runtime of old and new algorithms for 33 example videos (median per frame runtime $e\pm$ S.E.M., v1.0 vs. v2.0, student’s t-test, significance level *** $p<0.001$). The new algorithm is about 10 times faster. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Illustration of extraction of respiration signal from human IR video. (a) Raw pixel time series after exclusion of non-varying pixels and envelop (black).

nose motion, which precludes obtaining reliable measurements in awake, behaving mice. Whole-body plethysmography on the other hand is difficult to combine with odorant delivery and/or measuring neural activity.

The current standard practice for measuring respiration based on intranasal airflow, involves implanting a cannula in the nasal cavity. This procedure is invasive and requires surgical skills. Measurements also frequently fail after short time due to the aggregation of mucus or tissue regrowth. This poses a severe limitation for chronic long-term measurements in the same animal. Unilateral implantation of an intranasal cannula also introduces an asymmetry in the air flows and odor concentration between left and right nostrils, which likely affects olfactory perception. A recently developed approach involving implantation of a thermistor into a hollow space above the nasal cavity addresses several of these limitations (McAfee et al., 2016), but the requirement for surgery remains.

By removing the need for invasive surgery, IR thermography addresses the requirement for more refined animal experimentation according to the ‘3R’s’ principle (‘Refine, Reduce, Replace’), defined in the ethical framework for the use of animals. Moreover, IR thermography can be easily combined with other techniques to address the need for precise behavioral monitoring in head-restrained mice which is critical for linking neural dynamics to behavior.

A limitation of IR thermography in mice compared to intranasal pressure measurements is that it has a relatively low temporal resolution. The 60 Hz frame rate of the camera we used is sufficient to capture respiratory oscillations in mice, given they sniff at maximum frequencies up to 15 Hz. However, the temporal resolution of 16.7 ms is still relatively low. In fact, the resolution is even slightly lower due to the rolling shutter, which causes an additional delay of 16.7 ms between the first and last row of the image. As a consequence of the temporal resolution, the respiratory rate is discretized at high frequencies. Faster-frame-rate cameras with acquisition rate of 80 Hz are already available and further technological improvements can be expected.

Due to their lower breathing frequencies, monitoring of human respiration is not limited even by slow IR cameras. The validation also further demonstrates extraction of inhalation onsets from thermal videos taken of human subjects. While various algorithms have been developed for determining respiration rate from human thermal videos, to the best of our knowledge, this paper is the first to extract inhalation onsets.

Compared to the mouse validation, we observe much longer delays between IR thermography and the alternative measurement from the piezo-electric belt transducer in the human validation. This is likely related to the overall lower breathing frequencies in humans. In proportion to the length of a typical breathing cycle at rest of $4\text{~s}$ in humans (respiration frequency of 0.2–0.3 Hz) and 200 ms in mice, the observed delays of $13\text{~ms}$ and $331\text{~ms}$, respectively are very similar (6.5% vs. 8.3% of cycle), suggesting the algorithm achieves similar accuracy in both species. In fact, most errors result from the delays, when
Inhalations are already or not yet captured by one measurement modality.

In order to further improve signal extraction from IR videos, future versions of the algorithm might exploit the local signal correlation of pixels carrying the breathing signal, which might further constrain the identification of signal-relevant pixels.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jneumeth.2018.02.017.

References


