

How using the wrong camera fucks up a project

Annabel Bantle, Toby Steven Waterstone, Christian Korfitz Mortensen

Abstract

Thou should not use thermal camera (Maybe something more scientific)

1. INTRODUCTION

Although there have been several studies, which have dealt with the occurrences within the capillary network, this area is not completely investigated. Especially not the phenomena of the oscillating changes of the vessel diameter of the capillaries. This phenomena, called vasomotion, which occurs in the microcirculatory system is an auto-regulation mechanism that optimizes blood distribution within the microcirculatory system. With better knowledge about the occurrences during vasomotion, it is possible to improve the treatment of intensive care patients. Particularly for sepsis patients, which are in a high risk of multi organ failure, might be the chance to prevent multi organ failure due to a lack of supply through the microcirculatory system in the organs.

Previous studies detected that the vasomotric blood flow is quantifiable as temperature micro oscillations in the frequency range of $0,005 - 0,05\text{Hz}$. Based on this, it must be possible to detect a difference in the mean spectrum temperature oscillations in the microcirculatory system, depending on the blood flow in the macrocirculatory system. Therefore a study of vasomotion in the peripheral circulation with infrared thermography is implemented. Thereby the temperature oscillations in the skin, which are used as an indicator for peripheral circulation, are measured by infrared imaging. The aim of this study was to investigate if there are changes in the vasomotric blood flow caused by partial occlusion of the blood supply. The restriction of the blood supply causes an ischemia, which leads to a lack of oxygen. This is used to image one effect of sepsis. This study lays the foundation for further experiments to elaborate the relationship between the pathological changes in the cardiovascular system and the microcirculation to draw conclusions out of the vasomotric blood flow to improve the treatment of patients with sepsis.

2. Method

2.1. Subjects

Four healthy subjects with age between 22 and 52 years (average age 30.5), three men and one female were recruited for this experiment. No subjects consumed caffeine or alcoholic beverages before experiment. All subject met inclusion and exclusion criteria and fully understood what the study involved.

2.2. Test setting

Subjects was placed in an upholstered adjustable dentist chair which allowed a comfortable sitting position. The hand placed on the armrest which had a vacuum pillow mounted for stability and a microfiber tissue for emissivity contrast. $37,5 \pm 1,0\text{ cm}$ over the hand the Xenics Gobi 640 $17\mu\text{m}$ GigE infrared camera is positioned with a tripod. The setup with camera, chair and computer can be seen on



Figure 1. Inductance of oscillation winding on amorphous magnetic core versus DC bias magnetic field

2.3. Data acquisition

2.4. Data processing

2.5. Statistical analysis

3. MATH

Before you begin to format your paper, first write and save the content as a separate text file. Keep your text and graphic files separate until after the text has been formatted and styled. Do not use hard tabs, and limit use of hard returns to only one return at the end of a paragraph. Do not add any kind of pagination anywhere in the paper. Do not number text heads—the template will do that for you.

Finally, complete content and organizational editing before formatting. Please take note of the following items when proofreading spelling and grammar:

3.1. Abbreviations and Acronyms

Define abbreviations and acronyms the first time they are used in the text, even after they have been defined in the abstract. Abbreviations such as IEEE, SI, MKS, CGS, sc, dc, and rms do not have to be defined. Do not use abbreviations in the title or heads unless they are unavoidable.

3.2. Units

- Use either SI (MKS) or CGS as primary units. (SI units are encouraged.) English units may be used as secondary units (in parentheses). An exception would be the use of English units as identifiers in trade, such as 3.5-inch disk drive.
- Avoid combining SI and CGS units, such as current in amperes and magnetic field in oersteds. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity that you use in an equation.
- Do not mix complete spellings and abbreviations of units: Wb/m² or webers per square meter, not webers/m². Spell out units when they appear in text: . . . a few henries, not . . . a few H.
- Use a zero before decimal points: 0.25, not .25. Use cm³, not cc. (bullet list)

3.3. Equations

The equations are an exception to the prescribed specifications of this template. You will need to determine whether or not your equation should be typed using either the Times New Roman or the Symbol font (please no other font). To create multileveled equations, it may be necessary to treat the equation as a graphic and insert it into the text after your paper is styled. Number equations consecutively. Equation numbers, within parentheses, are to position flush right, as in (1), using a right tab stop. To make your equations more compact, you may use the solidus (/), the exp function, or appropriate exponents. Italicize Roman symbols for quantities and variables, but not Greek symbols. Use a long dash rather than a hyphen for a minus sign. Punctuate equations with commas or periods when they are part of a sentence, as in

$$\alpha + \beta = \chi \quad (1)$$

Note that the equation is centered using a center tab stop. Be sure that the symbols in your equation have been defined before or immediately following the equation. Use (1), not Eq. (1) or equation (1), except at the beginning of a sentence: Equation (1) is . . .

3.4. Some Common Mistakes

- The word data is plural, not singular.
- The subscript for the permeability of vacuum ?0, and other common scientific constants, is zero with subscript formatting, not a lowercase letter o.
- In American English, commas, semi-colons, periods, question and exclamation marks are located within quotation marks only when a complete thought or name is cited, such as a title or full quotation. When quotation marks are used, instead of a bold or italic typeface, to highlight a word or phrase, punctuation should appear outside of the quotation marks. A parenthetical phrase or statement at the end of a sentence is punctuated outside of the closing parenthesis (like this). (A parenthetical sentence is punctuated within the parentheses.)
- A graph within a graph is an inset, not an insert. The word alternatively is preferred to the word alternately (unless you really mean something that alternates).
- Do not use the word essentially to mean approximately or effectively.

- In your paper title, if the words that uses can accurately replace the word using, capitalize the u; if not, keep using lower-cased.
- Be aware of the different meanings of the homophones affect and effect, complement and compliment, discreet and discrete, principal and principle.
- Do not confuse imply and infer.
- The prefix non is not a word; it should be joined to the word it modifies, usually without a hyphen.
- There is no period after the et in the Latin abbreviation et al..
- The abbreviation i.e. means that is, and the abbreviation e.g. means for example.

4. USING THE TEMPLATE

Use this sample document as your LaTeX source file to create your document. Save this file as **root.tex**. You have to make sure to use the cls file that came with this distribution. If you use a different style file, you cannot expect to get required margins. Note also that when you are creating your out PDF file, the source file is only part of the equation. *Your TeX → PDF filter determines the output file size. Even if you make all the specifications to output a letter file in the source - if you filter is set to produce A4, you will only get A4 output.*

It is impossible to account for all possible situation, one would encounter using TeX. If you are using multiple TeX files you must make sure that the “MAIN” source file is called root.tex - this is particularly important if your conference is using PaperPlaza’s built in TeX to PDF conversion tool.

4.1. Headings, etc

Text heads organize the topics on a relational, hierarchical basis. For example, the paper title is the primary text head because all subsequent material relates and elaborates on this one topic. If there are two or more sub-topics, the next level head (uppercase Roman numerals) should be used and, conversely, if there are not at least two sub-topics, then no subheads should be introduced. Styles named Heading 1, Heading 2, Heading 3, and Heading 4 are prescribed.

4.2. Figures and Tables

Positioning Figures and Tables: Place figures and tables at the top and bottom of columns. Avoid placing them in the middle of columns. Large figures and tables

may span across both columns. Figure captions should be below the figures; table heads should appear above the tables. Insert figures and tables after they are cited in the text. Use the abbreviation Fig. 1, even at the beginning of a sentence.

Table 1. An Example of a Table

One	Two
Three	Four

We suggest that you use a text box to insert a graphic (which is ideally a 300 dpi TIFF or EPS file, with all fonts embedded) because, in an document, this method is somewhat more stable than directly inserting a picture.

Figure 2. Inductance of oscillation winding on amorphous magnetic core versus DC bias magnetic field

Figure Labels: Use 8 point Times New Roman for Figure labels. Use words rather than symbols or abbreviations when writing Figure axis labels to avoid confusing the reader. As an example, write the quantity Magnetization, or Magnetization, M, not just M. If including units in the label, present them within parentheses. Do not label axes only with units. In the example, write Magnetization (A/m) or Magnetization A[m(1)], not just A/m. Do not label axes with a ratio of quantities and units. For example, write Temperature (K), not Temperature/K.

5. CONCLUSIONS

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

APPENDIX

Appendices should appear before the acknowledgment.

ACKNOWLEDGMENT

The preferred spelling of the word acknowledgment in America is without an e after the g. Avoid the stilted expression, One of us (R. B. G.) thanks . . .

Instead, try R. B. G. thanks. Put sponsor acknowledgments in the unnumbered footnote on the first page.

References are important to the reader; therefore, each citation must be complete and correct. If at all possible, references should be commonly available publications.

References

- [1] G. O. Young, Synthetic structure of industrial plastics (Book style with paper title and editor), in Plastics, 2nd ed. vol. 3, J. Peters, Ed. New York: McGraw-Hill, 1964, pp. 1564.
- [2] W.-K. Chen, Linear Networks and Systems (Book style). Belmont, CA: Wadsworth, 1993, pp. 123135.
- [3] H. Poor, An Introduction to Signal Detection and Estimation. New York: Springer-Verlag, 1985, ch. 4.
- [4] B. Smith, An approach to graphs of linear forms (Unpublished work style), unpublished.
- [5] E. H. Miller, A note on reflector arrays (Periodical styleAccepted for publication), IEEE Trans. Antennas Propagat., to be published.
- [6] J. Wang, Fundamentals of erbium-doped fiber amplifiers arrays (Periodical styleSubmitted for publication), IEEE J. Quantum Electron., submitted for publication.
- [7] C. J. Kaufman, Rocky Mountain Research Lab., Boulder, CO, private communication, May 1995.
- [8] Y. Yorozu, M. Hirano, K. Oka, and Y. Tagawa, Electron spectroscopy studies on magneto-optical media and plastic substrate interfaces(Translation Journals style), IEEE Transl. J. Magn.Jpn., vol. 2, Aug. 1987, pp. 740741 [Dig. 9th Annu. Conf. Magnetics Japan, 1982, p. 301].
- [9] M. Young, The Techincal Writers Handbook. Mill Valley, CA: University Science, 1989.
- [10] J. U. Duncombe, Infrared navigationPart I: An assessment of feasibility (Periodical style), IEEE Trans. Electron Devices, vol. ED-11, pp. 3439, Jan. 1959.
- [11] S. Chen, B. Mulgrew, and P. M. Grant, A clustering technique for digital communications channel equalization using radial basis function networks, IEEE Trans. Neural Networks, vol. 4, pp. 570578, July 1993.
- [12] R. W. Lucky, Automatic equalization for digital communication, Bell Syst. Tech. J., vol. 44, no. 4, pp. 547588, Apr. 1965.
- [13] S. P. Bingulac, On the compatibility of adaptive controllers (Published Conference Proceedings style), in Proc. 4th Annu. Allerton Conf. Circuits and Systems Theory, New York, 1994, pp. 816.
- [14] G. R. Faulhaber, Design of service systems with priority reservation, in Conf. Rec. 1995 IEEE Int. Conf. Communications, pp. 38.
- [15] W. D. Doyle, Magnetization reversal in films with bi-

- axial anisotropy, in 1987 Proc. INTERMAG Conf., pp. 2.2-12.2-6.
- [16] G. W. Juette and L. E. Zeffanella, Radio noise currents in short sections on bundle conductors (Presented Conference Paper style), presented at the IEEE Summer power Meeting, Dallas, TX, June 22-27, 1990, Paper 90 SM 690-0 PWRS.
 - [17] J. G. Kreifeldt, An analysis of surface-detected EMG as an amplitude-modulated noise, presented at the 1989 Int. Conf. Medicine and Biological Engineering, Chicago, IL.
 - [18] J. Williams, Narrow-band analyzer (Thesis or Dissertation style), Ph.D. dissertation, Dept. Elect. Eng., Harvard Univ., Cambridge, MA, 1993.
 - [19] N. Kawasaki, Parametric study of thermal and chemical nonequilibrium nozzle flow, M.S. thesis, Dept. Electron. Eng., Osaka Univ., Osaka, Japan, 1993.
 - [20] J. P. Wilkinson, Nonlinear resonant circuit devices (Patent style), U.S. Patent 3 624 12, July 16, 1990.



AALBORG UNIVERSITET
STUDENTERRAPPORT

Thermal imaging as method to study effect of induced ischemia on vasomotion activity

7. Semester project - Fall 2017

Group 7407



AALBORG UNIVERSITET
STUDENTERRAPPORT

7th Semester, Project

School of Medicine and Health

Biomedical Engineering and Informatics

Fredrik Bajers Vej 7A

9220 Aalborg

Title:

Thermal imaging as method of studying vasomotion

Theme:

Don't know

Project period:

P7, Fall 2017

01/09/2017 - 20/12/2017

Projektgruppe:

17gr7407

Synopsis

Students:

Annabel Bantle

Christian Korfitz Mortensen

Toby Steven Waterstone

To be written

Supervisors:

Lasse Østergaard

Carsten Dahl Mørch

Andrei Ciubotariu

Pages: ??

Appendix: ??

Handed in: 20/12/2017

Abstract

also needs to be written

Preface

Needs to be written

- Tell about us as group, the place we study, something about the study
- Thank the supervisors

Contents

Part I Background	1
1 Anatomy and Physiology	2
1.1 Macrocirculatory system	2
1.2 Microcirculatory system	3
2 Hemodynamics	6
2.1 Physiological Base	6
2.2 Physical Base	6
3 Vasomotion in disease	8
4 Methods of studying vasomotion	11
4.1 Thermal imaging	12
4.2 Laser Doppler flowmetry	13
4.3 Summarizing	13
5 Infrared Thermal Imaging	14
5.1 Measuring thermal energy	15
Part II Methods	18
6 Study setup	19
6.1 Subjects	19
6.2 Test setting	20
6.3 Software setting	21
7 Interpretation of data	22
7.1 Continous wavelet transformation	22
7.2 The paired t-test	24
Part III Data analysis	26
8 Preparation of the data	27
8.1 Dividing the data into frames	27
8.2 Regions of interest	28
8.3 Artifacts in temperature recording	30
8.4 Correction method	31
9 Data processing	35

9.1	Scalogram interpretation	35
9.2	Selecting ROIs for statistical test	37
9.3	Statistical approach	37
Part IV	Results	39
10	Results	40
10.1	Paired t-test	40
Part V	Discussion	43
11	Discussion	44
A	Protocol	47
B	Evaluation table	51

Part I

Background

1 | Anatomy and Physiology

The following chapter outlines the functions of the cardiovascular system and focuses on its microcirculatory part. Further the phenomena of vasomotion is illustrated.

1.1 Macrocirculatory system

The main function of the cardiovascular system is the blood supply of the whole body and the transportation of metabolites. The propulsion of this is the heart. It generates the systolic blood pressure through the strength of left ventricle. The pressure difference between the heart and the periphery emerging from there, ensures the blood flow. The blood flows from regions with high pressure, like the aorta, to regions with low pressure, like the periphery.[martini2012]

The heart supplies the body through the systemic and the pulmonary circuit with blood. Through these circuits the heart regulates the blood allocation with adjustment of stroke volume and heart frequency. The oxygen-rich blood accumulates in the left ventricle. From there the blood is pushed out through the aortic valve into the aorta and via the arteries spread into the whole body. The venous system returns the meanwhile low in oxygen blood back to the heart into the right atrium. From there the blood flows into the right ventricle and is pushed out through the pulmonary valve into the lung arteries. In the lungs gas exchange of the blood happens. Subsequent the oxygen-rich blood flows via the pulmonary veins back to the left heart to supply the body.[martini2012]

As mentioned, there are two types of vessels, arteries and veins. The difference between those two types of vessels is that arteries transport the blood away from the heart and veins solely transport blood to the heart. There are also some differences in the structure of arteries and veins. Arteries consist of three different layers, tunica interna, tunica media and tunica externa. The tunica interna consists of vascular endothelium, the tunica media consists of smooth muscle cells and elastic fibres, the tunica externa consists of connective tissue and also elastic fibres. Furthermore, there are two different types of arterial vessels. In arteries of the elastic type prevail the elastic fibres in the tunica media. This allows an abrupt extension of the vessel during the systole and ensuing constriction, due to this the blood is transported. This phenomena is called windkessel function. In arteries of the muscular type prevail the muscular fibres in the tunica media. This allows regulation of the lumen by constriction and dilatation, whereby the resistance and the blood flow in the organs is regulated.[martini2012]

Venous vessels are similarly structured like arterial vessels, however they are thinner and have also semilunar valves inside, to inhibit back flow inside the vessels. This system is supported by the skeletal muscles which help to hold up blood flow. The arterial and the venous vessel system are connected through the capillary system in the microcirculatory

system.[martini2012]

1.2 Microcirculatory system

The heart and larger arteries and veins are associated with the cardiovascular system, but those are only used for transportation of blood. Instead it is the capillaries, that permeate most tissues, that is responsible for the perfusion of tissue. These are the only vessels which permit exchange between the vessel and the surrounding interstitial fluids. Factors that affect tissue perfusion is cardiac output, peripheral resistance and blood pressure. Capillaries are made not of single individual fluid conductors like veins and arteries, but instead formed into capillary beds. Here they work as a interconnected network of vessels. As mentioned before the arteries decrease in size the further they expand into the peripheral system. The small arteries divide into arterioles which further divide into dozen of capillaries. The capillaries merge into a venule after the blood has been de-oxygenated. A capillary is divided into two segments, first the metarteriole and second the capillary. The blood flow between arterioles and venules can also be a direct connection, made by an arteriovenous anastomosis. This works as a bypass diverting blood flow around the capillary bed. An example of the structure of the capillary bed can be seen on fig. 1.1.[martini2012]

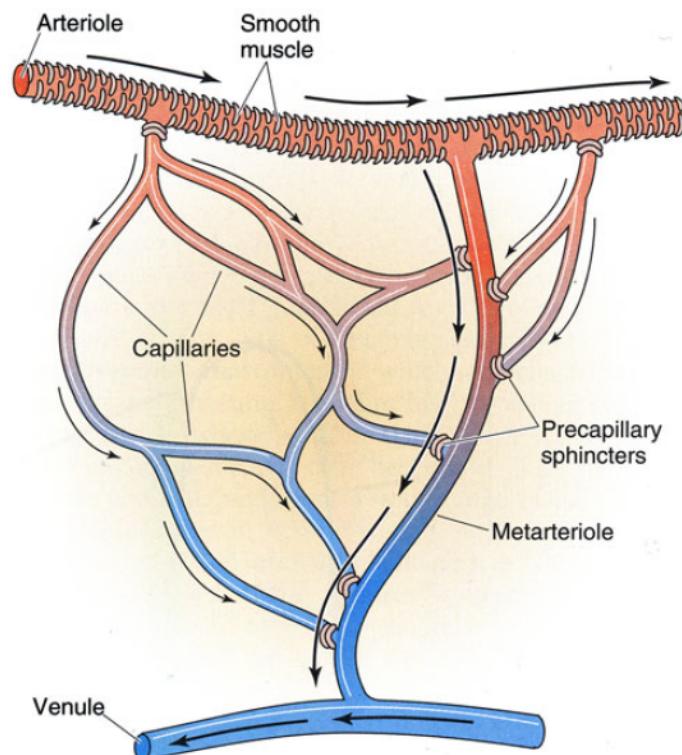


Figure 1.1: The basic structure of a capillary bed, with arteriole on the left side of the bed and a venule on the right[martini2012].

Each capillary entrance is controlled by a precapillary sphincter, which is composed of smooth muscle cells, that are able to contract or relax and thereby limit access of blood flow to certain capillaries. The blood flows relatively slow within the capillaries giving time for the two way exchange of nutrients and wastes. [martini2012]

1.2.1 Vasomotion

The flow within the capillaries varies. This is among other thing due to the earlier mentioned precapillary sphincters opening and closing. The opening and closing of sphincters is part of the autoregulation process performed at a local level, to control the blood flow. The vascular system does not contain blood enough for every vessel a capillary beds to be filled with blood. Therefore only 25% of the vessels in a capillary bed contains blood, and vessels activity needs to be well coordinated. Thermoregulation and control of nutrition balance are the primary functions of the microcirculatory system. Local changes in concentration of chemicals and interstitial fluids eg. dissolved oxygen concentrations in tissue modulates the vascular smooth muscles activity. Constriction and dilation of the vessel is thereby regulated by this periodic activity, also known as vasomotion. [martini2012, geyer2004]

Under normal circumstances cardiac output remains stable and the control of local blood flow happens through local peripheral resistance within local tissues. The regulation of cardiovascular activity is controlled by local homeostatic mechanism. These make sure that demands such as oxygen and nutrients are meet and wastes are disposed.[martini2012]

Physiological mechanism controlling vasomotion are not yet fully understood, but vascular smooth muscle activity has been shown to be roughly proportional to the tissue's metabolic demand for oxygen.[geyer2004] Studies also suggest that an increase in vasomotion activity enhances oxygen delivery[goldman2001]. Further have some factors that trigger homeostatic mechanism to alter the vasomotion been said to have an impact. Factors that trigger dilation is called vasodilators and can be some of the following:[martini2012, geyer2004]

- Decreased oxygen level or increased CO₂ level
- Lactic acid or other acids generated from tissue cells
- Nitric oxide NO released from endothelial cells
- Rising concentrations of potassium ions or hydrogen ions in the interstitial fluid
- Chemicals released during local inflammation
- Elevated local temperature

Chapter 1. Anatomy and Physiology

A vasodilation will result in increased oxygen, nutrients, buffers released to recreate homeostasis. Factors that stimulate constriction is called vasoconstrictors and can happen due to following:[**martini2012**]

- Damaged tissue
- Aggregating platelets

Furthermore mechanisms regulation vasomotion can be divided into three origins. These are endothelial/metabolic, myogenic and neurogenic. Endothelial regulation is based on registered O₂, CO₂, lactate, and H⁺ levels and from this releases nitric oxide as a vasodilator. Myogenic regulation senses strain and stress in vessels, which cause the smooth muscle to depolarize, contracting the vessels. Otherwise when the ion channels in the muscles close, the blood vessels relaxes leading to vasodilation. Neurogenic signals is said to come from the sympathetic nervous system where these promote vasoconstriction.[**segal2005, ince2005, nilsson2003**]

2 | Hemodynamics

Hemodynamics explains the movement or flow of blood. It is influenced by parameters like blood pressure, blood volume, cardiac output, blood composition, etc. It is possible to measure some of the hemodynamic parameters non-invasive, and also to calculate parameters.[martini2012, thiriet2008]

2.1 Physiological Base

The regulation of the blood pressure happens with baroreceptors in the walls of the big arteries in chest and neck area. These receptors register the changes of the elongation of the vessels and transmit this information to medulla oblongata. With the received pressure informations initiates the medulla oblongata, if necessary, regulatory measures. For the short-term regulation is the sympathetic responsible. Both, middle-term and long-term regulation, is made by the kidneys. For middle-term regulation messenger substance are released, which entail vasoconstriction. The long-term regulation occurs per pressure diuresis or reabsorption in the kidneys. It is possible to measure different blood pressures at different places in the cardiovascular system, for example the mean arterial pressure (*MAP*). The *MAP* increases in relation to the stroke volume and decreases when blood flows into the peripheral system.[martini2012, thiriet2008]

The cardiac output (*CO*) states the blood volume, which is pumped by the heart per time unit (*HR*). The calculation of the *CO* as follows.[martini2012]

$$CO = HR \times strokevolume \quad (2.1)$$

2.2 Physical Base

To consider the hemodynamics, it is possible to draw conclusions by analogy of physical laws. Especially of Ohm's law $R = \frac{U}{I}$ or rather $I = \frac{U}{R}$. A special case of Ohm's law constitutes Hagen-Poiseuille's law in the field of fluid dynamic and rheology. Hagen-Poiseuille's law describes the laminar flow of an homogeneous Newtonian fluid through a rigid pipe depending on characteristics of the fluid and of the pipe.[noordergraaf2011, thiriet2008]

Blood is an inhomogeneous suspension of liquid and corpuscular components, whose viscosity η depends on more factors than the temperature, and is consequently no Newtonian fluid. Nevertheless it is possible to draw conclusions by analogy out of Hagen-Poiseuille's law for the computation of the hemodynamics.[noordergraaf2011, thiriet2008]

$$\frac{V}{t} = \frac{r^4 \times \pi \times \Delta P}{8 \times \eta \times I} \quad (2.2)$$

Chapter 2. Hemodynamics

Here is the volume flow equivalent to the electrical current I and the pressure difference ΔP to the electric voltage U . Thus, the calculation of the resistance as follows.[**noordergraaf2011, thiriet2008**]

$$R = \frac{8 \times I \times \eta}{r^4} \quad (2.3)$$

Thereby volume flow increases 16 times and the resistance decreases 16 times for double radius r .[**noordergraaf2011, thiriet2008**]

3 | Vasomotion in disease

This chapter describes pathologic incidents in the cardiovascular system and organs during shock.

In general shock is characterized by hypoxia in tissues due to inadequate blood supply. The hypoxia during a shock leads to the deposition of metabolisms in organs what results in a increased risk of multi organ dysfunction. There are four different types of shocks: [lauridsen2015, vincent2013]

- **Hypovolaemic shock** is caused by a lack of volume. Either as a consequence of blood loss (hemorrhagic shock) or of water, plasma or electrolyte loss.
- **Cardiogenic shock** is caused by cardiac failure, for instance myocarditis, cardiomyopathy in final stage or acute myocardial infarction.
- **Obstructive shock** is caused by obstruction of blood flow, for instance pulmonary embolism, cardiac tamponade or tension pneumothorax
- **Distributive shock** covers, inter alia, septic shock, anaphylactic shock and neurogenic shock

Main cause for cardiogenic, hypovolaemic and obstructive shock is a decreased cardiac output without adapting the peripheral resistance. That leads to a lack of oxygen supply. Whereas the main cause for a distributive shock lies in a dysfunction of the peripheral areas in terms of reduced systemic vascular resistance as well as varied oxygen extraction. [vincent2013]

Shock affects alterations in the microcirculatory system what interferes the perfusion [maier2013]. The changes in the circulatory system are in the following part further elaborated with the aid of sepsis.

Sepsis

Sepsis is a condition, that develops on behalf of systemic inflammatory response syndrome (SIRS) with presence of an infection or bacteria in the tissue, within the body which triggers an immune response. This response often overburdens the immune system, to fight the inflammation or bacteria. Some of the normal macrohaemodynamics of sepsis are abnormal body temperature, abnormal heart rate, oxygen extraction and abnormal blood pressure.[pluta2010, kanta2014] Sepsis is often associated with three stages, sepsis, severe sepsis and septic shock. This is illustrated in figure 3.1.

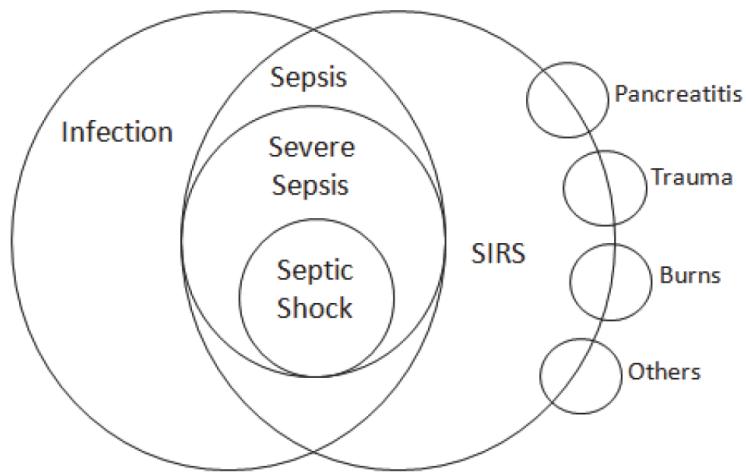


Figure 3.1: Relation between SIRS and infection. Showing the stages in sepsis and some of the causes of SIRS which include pancreatitis, trauma, burns etc.[**kanta2014**]

Stage 1: Sepsis

Under the stage of sepsis several things happen mainly at the capillary level, that leads to impaired homeostasis in the body. Bacteria which are responsible for causing irregularity are present in the blood and in the tissues around the vessels. Initially, when the body encounters an infection, white blood cells are recruited to release molecules which interact with the endothelial in the blood vessels will fight the infection. The interaction causes the vessels to dilate to increase the blood vessel diameter and permeability. The increased diameter slows down the blood flow, which causes a drop in blood pressure. Also the vessels permeability is increased. This reaction happens multiple places in the body where there is infected tissue present and will cause systemically vessel dilation. The characterization of sepsis is SIRS as a result of infection.[**baudouin2008, kanta2014**]

Stage 2: Severe sepsis

When the permeability of the vessels is increased, more fluid is in the tissue and the cells get less oxygen due to prolonged time for get to the demanding tissue. Also the endothelial of the vessels will get damaged when the white blood cells try to destroy the pathogens. This triggers coagulation and clotting is formed in the damaged areas in the blood vessels. At a point the damaged vessels leads to more leakage, because there will be a point where the coagulation cannot keep up. Organs will start to dysfunction at this stage. The characterization of severe sepsis is presence of sepsis with organ dysfunction and hypoperfusion is often included in this state. The mortality rate for patients with severe sepsis are about 25 to 30% [**baudouin2008, kanta2014**].

Stage 3: Septic shock

Septic shock occurs when the body has undergone sepsis for a greater duration of time. This stage is characterized by a condition with hypotension even after adequate fluid resuscitation is given. Because of lactic acidosis the cells are not getting a sufficient supply of oxygen and therefore the cells will begin to die. This can lead to a very dangerous state, where organs begin to fail because they get damaged to function. When multiple organs get damaged the state in septic shock reaches multiple organ failure also called multiple organ dysfunction syndrome (MODS)[**baudouin2008, kanta2014**]. The mortality for patients with septic shock are in the region of 40 to 70% [**kanta2014**].

4 | Methods of studying vasomotion

In the following chapter an introduction to different techniques of measuring vasomotion will be given. Here methods and applicability for measuring vasomotion will be presented, with main focus directed towards thermal imaging and important parameters using this technique.

For some time it has been the interest of researchers and health care clinicians to get a better understanding of the mechanisms that control and regulate local blood flow in the microcirculatory system[**sagaidachnyi2014, sagaidachnyi2017, geyer2004, liu2012**]. Visualization of the vessels in skin and the way these behave can be important for assessment of stages of sepsis as mentioned before in chapter 3, but also in peripheral vascular disease, the results of skin reconstructive surgery, wound and ulcer management.[**liu2012, kanta2014**] Spectral components of vasomotion seem to vary when influenced of some diseases. An example could be a decrease in amplitude of endothelial blood flow oscillations is assumed to be a biomarker for endothelial dysfunction. Endothelial dysfunction indicate cardiovascular disorders such as arterial hypertension and cardiac ischemia. An increased amplitude within the neurogenic frequency band is characterized by a decrease of vascular resistance and an increase of blood flow through the arteriovenous shunt.[**sagaidachnyi2017**]

For measuring regulation in the peripheral blood flow, it is assumed that these oscillating changes are the source of thermal waves propagating from microvessels toward the skin surface. Especially thermal imaging uses this concept.[**sagaidachnyi2017**] Furthermore a correlation between skin temperature in fingertips and blood flow oscillations has been found[**sagaidachnyi2014**]. When the thermal waves propagate from the vessels towards the skin surface they are prone to some attenuation. This is due to skin properties that function like a low-frequency filter.[**podtaev2008**] The magnitude of attenuation is directly proportional to the frequency. Therefore as illustrated in fig. 4.1, a higher frequency leads to a higher attenuation.

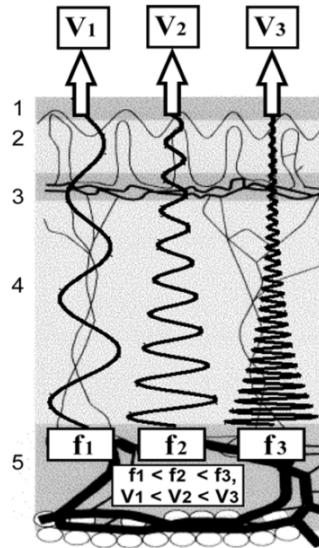


Figure 4.1: Graphical representation of amplitude dampening in three signals with different frequency.[[sagaidachnyi2014](#)]

There are multiple different techniques of measuring blood flow in the peripheral circulatory system. For example capillaroscopy, laser Doppler flowmetry (LDF), and thermal imaging. These have been used differently trying to quantify functional aspects of skin vasculature.[[liu2012](#)] Laser Doppler flowmetry is one of the most used[[geyer2004](#)] and thermal imaging being introduced as a new technique of measuring vasoregulation[[sagaidachnyi2014](#)].

4.1 Thermal imaging

In studies made by a Russian group by Sagaidachnyi et al. thermal imaging has been used to study vasomotion. In their studies they sought to get better understanding of the relationship between blood flow oscillations and temperature oscillations, and if it was possible to recreate the blood flow oscillation from temperature recording. Recordings of flow were done by Photoplethysmography and temperature of the skin by thermal imaging. The recordings were made on a small point of the fingertip. Through their work, five frequency bands were identified as vasomotion activity, and are following: endothelial (0.005–0.02 Hz), neurogenic (0.02–0.05 Hz), myogenic (0.05–0.15 Hz), respiratory origin (0.15–0.4 Hz) and cardiac origin (0.4–2.0 Hz).[[sagaidachnyi2017](#), [sagaidachnyi2014](#)] The choice of using thermal imaging to study vasomotion implies certain advantages. Mainly a larger sample area, but also a higher temporal, (up to 105 fps) and spatial (2048×1536 pixels) resolution. In addition it is also a non invasive way of measuring vasomotion.[[sagaidachnyi2017](#)]

4.2 Laser Doppler flowmetry

In an other study from Geyer et al. vasomotion is investigated trough the use of laser Doppler flowmetry as recording technique. In the study vasoregulation variables are sought quantified. LDF is a non invasive approach to measuring changes in vasomotion. The technique register changes in the depth of 1 mm, and works like Doppler ultrasound, utilizing the shift in frequency. Though instead of using ultrasonic waves, LDF uses light reflected from red blood cells. This study found the same frequency bands as Sagaidachnyi et al. with minimal difference. Data obtained were analyzed trough spectral analysis. Wavelet transform was used instead of the most used fourier analysis, because wavelet analysis offered better resolution to reveal characteristics in the low frequency area.[geyer2004] LDF uses a small sample area and the laser probe allows a sampling area as small as 1 mm³.[brothers2010]

4.3 Summarizing

Both Geyer et al. and Sagaidachnyi2017 et al. managed to show spectral components relating to vasomotion. The techniques both uses an non invasive approach, even though the methods are different when measuring red blood cell count compared to temperature. The use of thermal imaging as the method of measuring vasomotion offers interesting opportunities. Larger sampling area would allow interpretation and study of a more global tissue area. Along with the resolution of thermal imaging cameras, this makes thermal imaging the choice of measuring technique to be used in this study.

5 | Infrared Thermal Imaging

The following chapter will include an introduction to thermal imaging, where general concepts and physical principals will be explained. Furthermore it will be explained how a device measures infrared radiation.

5.0.1 Introduction to thermal imaging

Thermal imaging is a technique that utilizes infrared radiation emitted from nearly any objects. The existence of infrared radiation was first discovered in 1800 by Sir Frederick William Herschel. His experiments lead to the knowledge that there were a light spectrum beyond the visual spectrum humans are able to perceive. Any object above absolute zero emits energy-electromagnetic radiation depending on its temperature.[ignacio2017, optris2009]

Infrared radiation is also known as thermal radiation because of the relationship between temperature and infrared radiation. Temperature of the human body permits radiation in the infrared spectrum, but objects of much higher temperature are capable of emitting radiation in the visible and UV spectrum. This has to do with the difference between object and environmental temperature. If the temperature of these are relatively close to each other, the radiation emitted will be within infrared wavelengths. Infrared radiation has a wavelength from 769 nm to 1 mm. Objects emit more radiation in some region regions compared to others. Because of this is the infrared spectrum classified in the three regions, near, middle and far infrared. Near is between 769 nm and 2.5 μm , middle 2.5 μm to 50 μm and far 50 μm to 1 mm. The human body emits most radiation in the far infrared part, and most thermal cameras are build with this in mind. Near and middle cameras are used to measure gases.[ignacio2017]

Thermal imaging is commonly used to calculate surface temperatures. Two important concepts, heat and temperature emerge in the understanding of this. Temperature is a measure for the internal energy within an object and can be defined as the average kinetic energy of the object. Heat is the energy that passes from a warm object to a colder object. Warm objects will decrease in internal energy and cold objects will increase due to the temperature difference and therefore the heat transfer. In the human body, a constant temperature is kept, due to several factors and therefore the temperature will not decrease even though a heat transfer to the surrounding environment occurs. The environmental temperature do have an impact on how large the heat transfer gradient is. If a body is in a cold environment, the emitted heat will be greater than the absorbed. In the same way, if the environment is much warmer than 37°C, a greater absorption than emission will occur and the body will increase in temperature.[ignacio2017]

5.1 Measuring thermal energy

The theory of the black body is important to understand the absorption and emission of light relative to temperature. Because the theory of the black body is used to describe the laws of infrared radiation and its relationship to temperature. The black body is an ideal perfect emitter of infrared radiation because it absorbs all electromagnetic radiation permitted to it, and it emits the same amount of radiation as it absorbs, the absorption and emission are both equal to one. Spectral emissive power, also denoted E_λ is the energy emitted by a surface in relation to time and range of wavelength. Figure 5.1 shows an graphical illustration of spectral emissive power of the black body for specific wavelengths when the temperature changes. [ignacio2017]

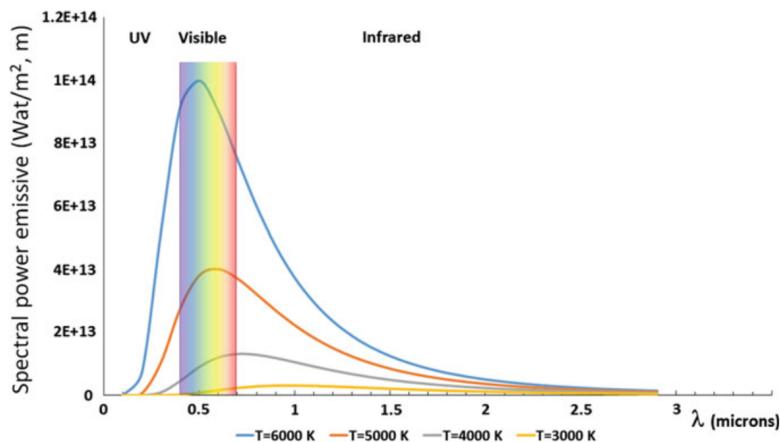


Figure 5.1: Spectral power emissive as a function of wavelength for different temperatures
[ignacio2017]

The knowledge of this principle helps in the understanding of how infrared radiation behaves, and how temperature affects the wavelength of the signal. The radiation from the human body which has a temperature at 37°C emits the maximum energy of $9.3\mu\text{m}$, which means that most of the radiation is in the far infrared spectrum.[ignacio2017]

Physical laws including Wien's displacement law and Stefan-Boltzmann's law are important for explaining how the infrared radiation behaves at different temperatures. [ignacio2017]

Wien's displacement law explains that the wavelength of the peak of the black body radiation curve decreases as the body temperature increases. This law can be used to describe different wavelengths according to the temperature of the black body which emits the radiation. Wien's law has the following equation:

$$\lambda_{max} = \frac{a}{T} \quad (5.1)$$

a has a value of $2.897 * 10^{-3} mK$ and denotes the Wien's displacement constant. T denotes the absolute temperature in kelvin. λ_{max} denotes the wavelength of emission peak with unit in meters.[ignacio2017]

Stefan-Boltzmann's law explains that small changes in temperature will lead to big changes in emissive power. This is seen in Stefan-Boltzmann's equation because it states that the total emissive power is proportional to the fourth power of the absolute temperature. [ignacio2017]

$$E = \varepsilon * \sigma * T^4 \quad (5.2)$$

In Stefan-Boltzmann's equation E denotes the total emissive power with unit W/m^2 . σ denotes the Stefan-Boltzmann's constant, and has a value of $5.67 * 10^{-8} W/m^{-2} K^{-4}$. T is the temperature in kelvin. ε denotes the emissivity and is normally not a part of the Stefan-Boltzmann's law, but part of the modified Stefan-Boltzmann's equation, because it is used for calculation of temperature in most thermal cameras.

Emissivity is different for all materials. Skin have an emissivity between 0.95 to 0.99, why these values typically are used when assessing the temperature of the skin of the human body with thermal imaging. This law is important when considering thermal imaging because the sensitivity when calculating the temperature from the emissive power is considerable. [ignacio2017]

Region of interest

Region of interest (RIO) is an important consideration when doing measurements with thermal imaging. One of the reasons why this is an important aspect is because it is the RIO that lays the foundation of the data that goes into the statistical analysis. A minimum of 25 pixels is recommended for the RIO to reduce error in the data. To get the best measurement the RIO should be filling most of the image to get the best thermal data and better resolution in the area you want to measure.[ignacio2017]¹

Thermal cameras

The radiation emitted from an object is focusing the RIO onto an array of detectors via a lens in the thermal camera. The array is also called the focal plane array and consists

¹Fixme Note: This is very badly explained. It it needed?

Chapter 5. Infrared Thermal Imaging

of typically $384 * 288$ to $1024 * 768$ single microbolometers.[**olbrycht2015**] The radiation emitted to the detectors generates an electric output proportional to the radiation. The output is then undergoing amplification before further signal processing that digitalizes the signal into pixels. This allows the final output signal to be viewed as a temperature for the object on an monitor, this is also illustrated on figure 5.2. [**optris2009**]

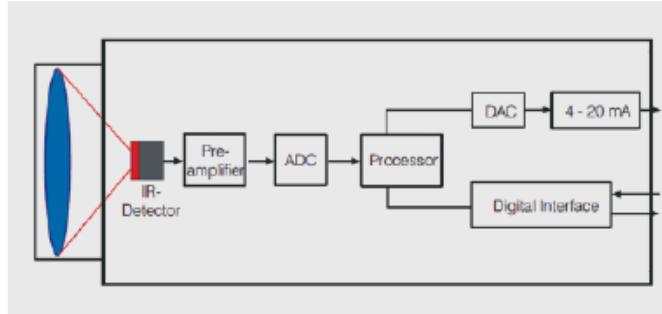


Figure 5.2: Simplified block diagram of an standard infrared camera.[**optris2009**]

The infrared radiation is made into an electrical signal in the camera, this data can be used in calculating the temperature for an object by knowing certain variables and putting these into Stefan Boltzmann's equation 5.2. The emissive power denoted E , is the radiation that the detector in the camera is getting. The variables σ are known and ε is specified for the object that is being measured, eg. the human skin with emissivity between 0.95 to 0.99. The temperature are then the only variable to calculate and this is done for each pixel in the image to make up the complete thermal image of the object. Each pixel will be representing one thermal data. [**ignacio2017**] ²

²Fixme Note: explain more about how camera works.

Part II

Methods

6 | Study setup

In this study the peripheral circulation is observed to investigate changes in microcirculation during partial occlusion of blood supply. Infrared imaging is used to measure the temperature changes in the skin of the hand, which is used as an indicator for peripheral circulation.

To see if there are changes in the microcirculatory system depending on flow to the observed area, the test is set in two conditions. The first measurement of the hand, is done with no intervention, and used as a control measurement. The second measurement of the hand is done during a partial occlusion of the blood supply. The partial occlusion of the arm leads to ischemia what leads to a lack of oxygen [martini2012]. Aim of the study is to investigate, if there are measurable changes in the micro temperature oscillations of the skin caused by hypoxia in case of shock. Therefore the induced ischemia is used as a way to mimic hypoxia due to shock. The reason to do a 50% restriction of blood flow, is due to the intend of creating ischemia without forcing to much discomfort on test subjects, over the 20 min occlusion period. Discomfort test was done prior to the start of the experiment.

By first taking the control measurement under normal conditions, the carry-over effect of occlusion is avoided. It enables taking both measurements of each subject straight successively, which should reduce inaccuracies within the setup of both experiments for each subject. The setting was assembled in the Regionshospital Nordjylland in Hjørring.

6.1 Subjects

Four healthy subjects within the age of 22 - 52, three male and one female were recruited for this experiment. The experiment is done as a pilot study, why it is presumed that the sample size of four is sufficient. The research focuses on assessing the microvascular system with the hand as a window on healthy subjects. Specific inclusion and exclusion criteria have been formed for this experiment:

Inclusion criteria

- Subjects must be in a normal healthy condition
- Subjects must have at least one hand to perform the measure on
- The cuff must be able to fit the arm circumference
- The subject must be able to sit still over the 45 min. recording period

Exclusion criteria

- Health conditions that set the subject in risk of injury when conducting the exper-

iment.

- Obesity to a greater extend
- Diseases that triggers tremors

6.2 Test setting

The subject will be placed in a upholstered chair with adjustable backrest, footrest and armrests, which allows a good positioning of the measured hand, while the subject remain in a relaxed position. Measurement will be carried out on the dominant hand. The hand is stabilized with a vacuum pillow which is covered by a micro fiber tissue to get a better background for the images. Microfiber has a low heat conduction [schacher2000]. That helps to identify the outlines of the hand on the thermal image, because the tissue is not conducting the temperature of the hand to a high extent. To provide a more comfortable position of the arm during the experiment the armrest of the adjustable chair is padded with some sheets under the vacuum pillow. A comfortable position in the chair is important, because the subject has to sit still and is not allowed to move during the test for at least 45 min. These precautions only counteract some small movement, and therefore it is important that the subject is focused on sitting still. $37,5 \pm 1,0$ cm over the hand the Gobi 640 $17\mu\text{m}$ GigE infrared camera (Xenics NV, Belgium) is positioned with a tripod. The setup with camera, chair and computer can be seen on figure 6.1.



Figure 6.1: The test setting at the Regionshospital Nordjylland.

The camera is via a Ethernet cable connected with a laptop, which is used to record the measurements with Xeneth 2.6 software. First cable connections between the camera, the laptop and the power supply have to be set. Afterwards the camera is turned on and has to warm up for about 15 min. During this the laptop should be started and the software for taking the measurements is set in operational readiness.

When the preparation of the test setting is done, the preparation for the subject can begin. At first the blood pressure of the subject is measured on the dominant arm. The blood pressure is measured three times while the subject is sitting relaxed on a chair. Mean systolic blood pressures is calculated. To get the total occlusion pressure (*TOP*) the mean has to be multiplied by 1.3. To reduce the blood flow in the arm to 50% during the measurement within the second condition, the arm is cuffed with 30% of the *TOP*.[\[mouser2017\]](#) The cuff is then affixed at the subjects dominant arm without tighten it, so that it is ready for the second part of the experiment. After that the subject can take place in the chair and the hand can be stabled with the vacuum pillow. The vacuum generator is attached to the pillow for giving the hand more stability. The lens focus has to be adjusted so the distance is taking in to consideration, to make sure the image is sharp.

When the camera is stable and the filename is modified according to the subject, the first measurement can be started for 20 min. During the whole experiment the subject is not allowed to move or speak to minimize movement bias. Directly after the first measurement the cuff on the arm of the subject is tightened with the calculated value. The pressure of the cuff should be observed during the whole measurement and if necessary adjusted.

To guide the conductors of the experiment, an experimental protocol has been formed and to be followed during the experiment. The experimental protocol can be seen in chapter A.

6.3 Software setting

To interface the Xenics camera, Xeneth 2.6 software was used and settings were controlled from here. Sampling rate for the thermal imaging camera was set to the lowest possible at $50 * \frac{1}{8} Hz$ or $6.25 Hz$. This should be sufficient according to the Nyquist theory [\[weik2001\]](#), when the frequencies of interest lies within $0.005 Hz - 2 Hz$ as presented in [??](#). Ambient temperature and room temperature was set to 25° and emissivity to 1.

7 | Interpretation of data

The following chapter will contain information about the techniques that is used for the data analysis. The wavelet transformation will be used for looking at features to be used for the statistics where the paired t-test will be the approach

7.1 Continuous wavelet transformation

Analysis of the collected data from the thermal camera will be done in the time frequency domain. For this the wavelet transformation will be used to look for specific frequency content in the data. Wavelet transformation is practical when looking for signals of lower frequencies compared to the normal Fourier analysis, because of the bigger resolution in the wavelet analysis. Another drawback of the Fourier transform is the loss of time information, which is preserved with the wavelet transformation. [geyer2004]

Both the discrete and the continuous wavelet transformation can be used, but the continuous wavelet transformation has better resolution, why this is being used for interpreting the data.[geyer2004]

The continuous wavelet transformation is using a variable sized region windowing technique. Long time intervals are used where low frequency information is computed and short time intervals are used where high frequency information is computed. This is represented in figure 7.1.

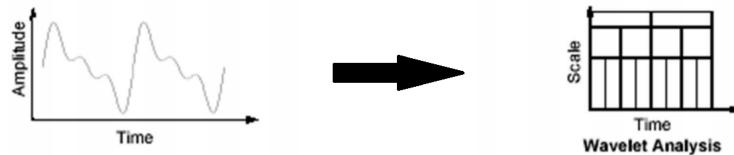


Figure 7.1: Signal to wavelet computation, modified from [Uvo1995]

The wavelet transform computes both the scale (s) and position (p) for the wavelet transform.

$$C(s, p) = \int_{-\infty}^{\infty} f(t)\Psi(s, p)dt \quad (7.1)$$

To achieve the continuous wavelet transformation, equation 7.1, the signal $f(t)$ is convoluted with the wavelet Ψ to get the wavelet coefficients for s and p .

Chapter 7. Interpretation of data

This is done by computing the wavelet of the signal which then is compared to the wavelet for a section at the beginning of the signal. Then C in equation 7.1 is calculated for the section of the signal. The wavelet is shifted to the right and repeated until the entire signal is covered. Then the wavelet is scaled and C is computed for the entire signal again for all scales. [Uvo1995]

Different wavelets can be used to compute the wavelet transformation. In Matlab the default is the Morlet wavelet.

The general form of the continuous wavelet transform is stated in equation 7.2:

$$W(t, s) = \int_{-\infty}^{\infty} \frac{1}{s^n} \psi * \left(\frac{\tau - t}{s}\right) x(\tau) d\tau \quad (7.2)$$

Where $\psi * \left(\frac{\tau - t}{s}\right)$ is the wavelet and $x(\tau)$ is the signal.[Uvo1995, Conraria2011]

The signal or function is put into the continuous wavelet transformation equation 7.2. The wavelet transform will give a scalogram as an output. In Matlab the function cwt can be used to compute the continuous wavelet transformation by inserting the signal and the sampling frequency. This will give a scolargram as shown in figure ???. [mathworks2017] The frequencies in Hz are shown along the y-axis if the sampling frequency is specified, else it will show the normalized frequency in cycles pr. sample. Along the x-axis is the time vector. The scolargram show the magnitude of the signal to show how the frequency in the signal is distributed. A scale to see the size of the magnitude is also implied. An example of a scalogram is given in figure ??

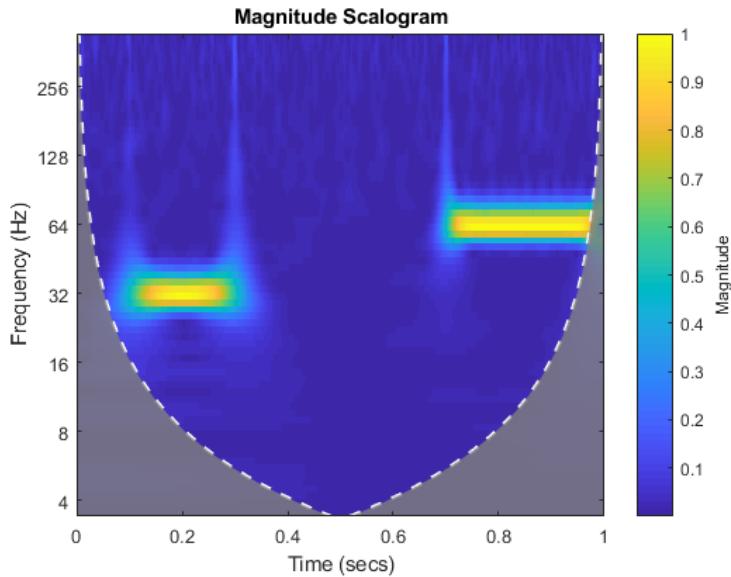


Figure 7.2: An example of a scolargram, showing frequency content at 32 Hz at time approx 0.2 sec and 64 Hz at approx 0.8 sec [mathworks2017]

The signal of the wavelet is typically of a finite length, which sets some limitations to the cwt, like the edge effect. The cone of influence (COI) shows the regions of the cwt where edge effects become significant. The gray zone are the areas where the edge effects become significant, which means that there will be some uncertainties to the cwt in this zone. [math]

7.2 The paired t-test

The paired t-test is used to check the difference of means of two conditional samples. This test is usually used to compare "before treatment" and "after treatment". The tested hypotheses are (where $\delta = \mu_1 - \mu_2$) [dodge2008]:

- $H_0 : \delta = 0$: No difference between uncuffed and cuffed arm is present
- $H_1 : \delta \neq 0$: Difference between uncuffed and cuffed arm is present

In this case, there is a relation between the microcirculatory and the macrocirculatory system shown, if the null hypothesis is rejected.

It is requisite that the sample size of the of both samples is identical.

Following some useful formulas [dodge2008]:

Chapter 7. Interpretation of data

- Difference within the subjects, with $i = 1, 2, \dots, n$

$$d_i = x_{i2} - x_{i1} \quad (7.3)$$

- Mean of the difference, with $i = 1, 2, \dots, n$

$$\bar{d} = \frac{1}{n} \sum d_i \quad (7.4)$$

- Standard deviation

$$s_d = \sqrt{\frac{\sum (d_i - \bar{d})^2}{n - 1}} \quad (7.5)$$

- Test variable

$$T = \frac{\bar{d}}{\frac{1}{\sqrt{n}} s_d} \quad (7.6)$$

- Degrees of freedom

$$n - 1 \quad (7.7)$$

- Decision rule for rejecting the null hypothesis

$$|T| > t_{n-1, \frac{\alpha}{2}} \quad (7.8)$$

The formulas 7.3 throughout 7.8 are used to calculate the required decision rule, for either rejecting or keeping the null hypothesis on behalf of the data.

Part III

Data analysis

8 | Preparation of the data

Before any analysis of the acquired data could begin, it was necessary to load and extract the content of the xvi files produced by the thermal camera.

8.1 Dividing the data into frames

Data acquired from the thermal camera using the Xeneth 2.6 software is saved as an xvi file¹. These files were loaded and read into Matlab R2017b as an uint16 vector file. Before the frames could be separated from the xvi file, the header in front of the files needed to be excluded. The header contained 307729 data points. With the header removed, the frame separation could be carried out. This was done by first calculating the size of one frame. When knowing that each frame would have the dimensions of 640x480 pixels, the size of one frame would correspond to 307200 data points for each frame. It should be noticed that each frame also contained a 16 bit header, so this should be added to the size of each frame. The number of frames was calculated by dividing the length of one frame by the entire length of the data file containing all frames, without the file header. By this calculation it was known how many frames the file contained. The data points for each frame were trimmed for its specific frame header and reshaped from a vector into a matrix and verified by showing the images. The images contain the pixel intensities of values from 0 to 65535, which corresponds to the size of the uint16 bit file.



Figure 8.1: Image of one frame from subject one after separation.

¹Fixme Note: what is xvi

8.2 Regions of interest

The regions of interest (ROI) were chosen as pixel locations in the image on specific places of the hand. ROI were selected on behalf of getting a full representation of the hand. Regions in the fingertips and nail folds are areas where it is easy to access the microcirculatory hemodynamics of the human body according to [**Iabichella2006**]. This region should therefore be expected to give some information on the changes in capillaries which can be an effect of vasomotion.

The original image on figure 8.1 is showing the hand but not with very good contrast. To easier choose ROI, the original image was converted to a gray scale image to improve the contrast in the image, this can be seen in figure 8.2.

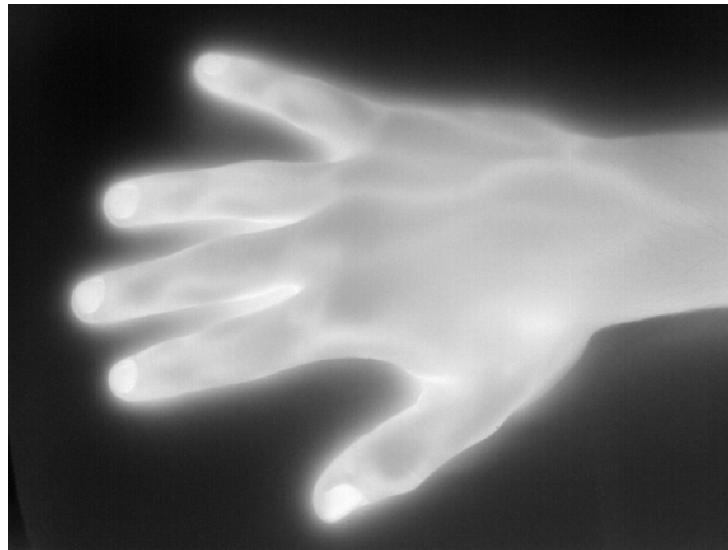


Figure 8.2: High contrast thermal image for subject one.

With the improved contrast, 28 ROI from the hand were chosen on the first image of the thermal image series, by finding the coordinates of the pixels on the image. The regions are illustrated on figure 8.3. The localization of the regions is originating from the fingertips and elongating down the hand to the beginning of the wrist. Each region gives an pixel intensity value from one pixel of the image. In the setup of this study one pixel corresponds to an area on the hand with a diameter of about $17\mu\text{m}$. Based on the fact, that the capillaries have an average diameter of $8\mu\text{m}$ [**martini2012**], it is sufficient to represent each region with just one pixel. Even the area represented by one pixel contains more than one capillary.

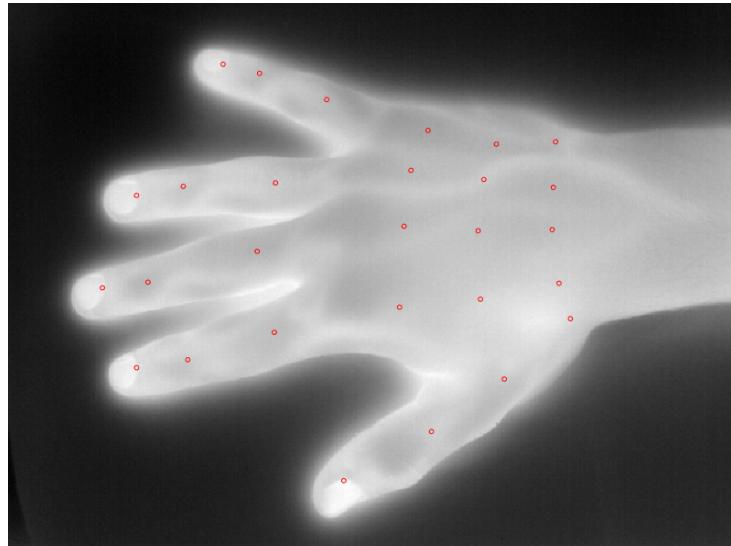


Figure 8.3: First thermal image of the thermal image series for subject 1, with ROI of interest plotted on 28 areas of the hand represented as red circles

The regions are fixed within the image matrix for the whole image series for each measurement, assuming that the subject was sitting still during the whole measurement. The regions also account for both the uncuffed and cuffed conditions for each subject, assuming that the position of the hand was at the same position in both conditions. Iterating over the image series saving ROI into a cell array with data points for each ROI, a vector for each of the 28 regions was made to give the pixel intensity variations over the whole measurement. An example of the pixel intensities for subject 1 is shown on figure figure 8.4.

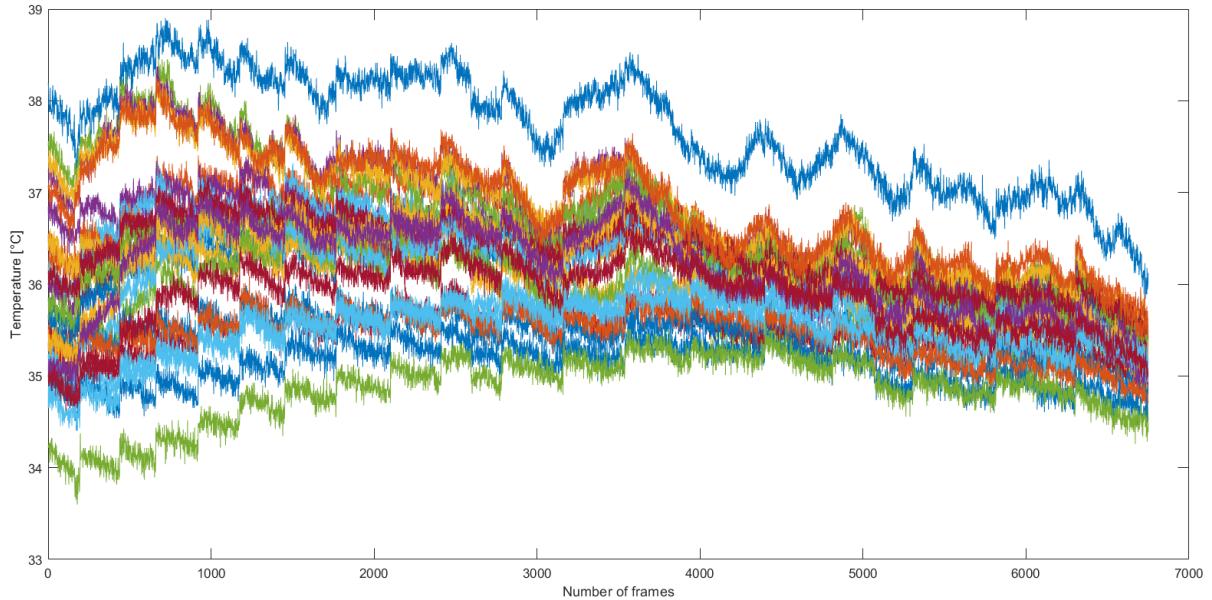


Figure 8.4: Pixel intensity for all 28 ROI, plottet from subject 1, for the entire thermal image series of a measurement.

8.3 Artifacts in temperature recording

As seen in figure 8.4 the data contain systematic changes which rule out a representation natural temperature variation. Since those changes occur at the same time in different locations of the thermal image, it is assumed that the greater shifts are due to a technological limit. Because of this assumption, it is chosen to further investigate the buildup of thermal cameras and look into other papers, to see if they had encountered similar difficulties.

8.3.1 Thermal pixel drift

In a study by Eriksen et al. where thermal imaging was assessed as use for measuring temperature of electrical systems, data recording showed similar behavior as recording in this study. They clearly state that two types of noise is present in their recording. One being white noise from the radiation detector and electronics, and another being a low frequency technical noise. To compensate for these artifacts, a moving average filter was applied.[[eriksen2014](#)] A moving average filter would not be an appropriate correction method, because of the risk of loosing the low frequent content of interest.

Thermal cameras are composed of a matrix of microbolometers as mentioned in section section 5.1. Each microbolometer is also known as a pixel detector for thermal radiation.[[olbrycht2015](#), [wolf2016](#)] Unfortunately it shows that these microbolometers are really sensitive to noise especially in uncooled cameras, where the internal tempera-

ture in not regulated. The noise is formed because each microbolometer has a different response to the same infrared excitation. Furthermore it is assumed by some, that this response changes linear[**olbrycht2015**]. This drift in each microbolometer in the focal plane array is also known as non-uniformity. To achieve radiometric precision the camera has to make a correction for this drift called non-uniformity correction. A common way to recalibrate bolometers is to move a shutter in between the lens and the focal plane array. The shutter has a uniform color which is used to create a new reference for all microbolometers.[**olbrycht2015, wolf2016**] These auto-adjustments might be what Eriksen et al. saw in their recordings.

Furthermore the drift component is increasing with increasing distance from the center of the thermal image. ²

8.4 Correction method

With the knowledge of what might be the source of the artifacts seen in the temperature recording, it is chosen to find a way to compensate and correct the recording. Jumps occur in each region of interest at the same time and are shown by a high difference of the values between two frames. The amount of the observed jumps varies between 0 and 20 in the recordings. Furthermore the appearance of the jumps is non-periodic and in each recording at different time points and interval.³

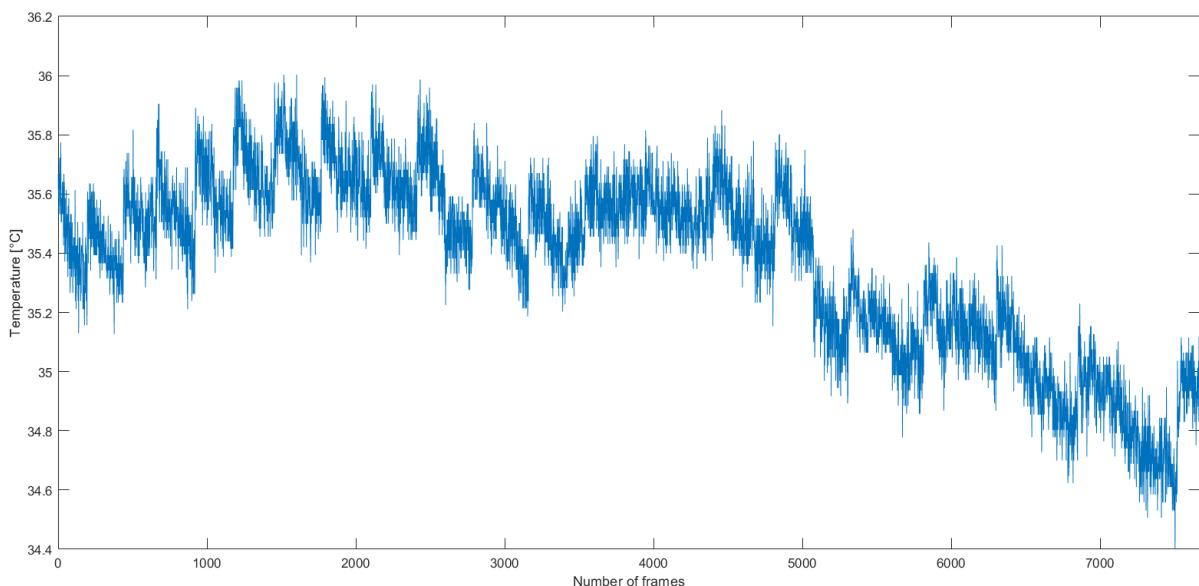


Figure 8.5: The original data of region 15 in the uncuffed recording of subject 1 including 20 jumps.

From the raw data magnitude of the scalogram show high magnitude peaks exactly at the

²**FiXme Note:** Reference needed.

³**FiXme Note:** show the one with no jumps also to see difference but it is shown later

same time points where the jumps are occurring, which can be seen in figure 9.1. Due to this falsifying of the magnitude, the results of the data analysis are also falsified.

Additionally there is also a drift occurring within each interval between two jumps, which hampers the correct data analysis, this can also be seen in figure ?? as the frequency band with higher magnitude. To reduce the drift component and the jumps in the signals, the two following correction methods have been compiled, whereby the second one has been implemented.

Method 1: Regression of first interval

The first implemented method is based on the assumption that the drift is equal in each interval. It is also assumed that the thermal camera has been calibrated just before the recording, so the first interval can be used as a reference to calculate the drift component. Therefore a linear regression for the first interval has been made. With the resultant slope m follows the calculation of the drift difference d within the first interval. Due to the assumption, that the drift difference is equal, the slope of the drift of each interval depends on the length of the interval. The slopes have been calculated with equation 8.1.

$$m = \frac{d}{\text{length(interval)}} \quad (8.1)$$

To compensate for the drift, a straight with the inverse slope and starting point in the first data point of the interval has been calculated. The middle points between the original data and the new calculated straight build the correction of the data signal. This correction only worked partially where several parts showed less drift and lower jumps. Through the method worked at some parts it still had a lot of weak points. Primarily because some jumps had been strengthened. Due to the outcome of this method, the assumption, that the drift is equal in each interval has been discarded.⁴

Method 2: Regression of each interval

The second implemented method is due to the failure of the first method based on the assumption that the drift is not equal in each interval. Out of the recorded data the exact drift is indeterminable. Hence a method which tries to fit the separate intervals together without the necessity of the awareness of the real drift component and avoids the suppression of the basic shape of the signal has been chosen. Therefore firstly the linear regressions for all intervals and the corresponding residuals are calculated. The idea is to move the end point of a regression line and the start point of the next regression line together. Thus the middle points between end and following start point are calculated. The alignment of the regression lines is changed, so that the start and end points of all the new created orientation straights fit the middle points, except the first and the last orientation straight. Both fit just one middle point. The start of the first orientation

⁴FiXme Note: figure missing

Chapter 8. Preparation of the data

straight is the start point of the regression line of the first interval and the end point of the last orientation straight is the end point of the regression line of the last interval.

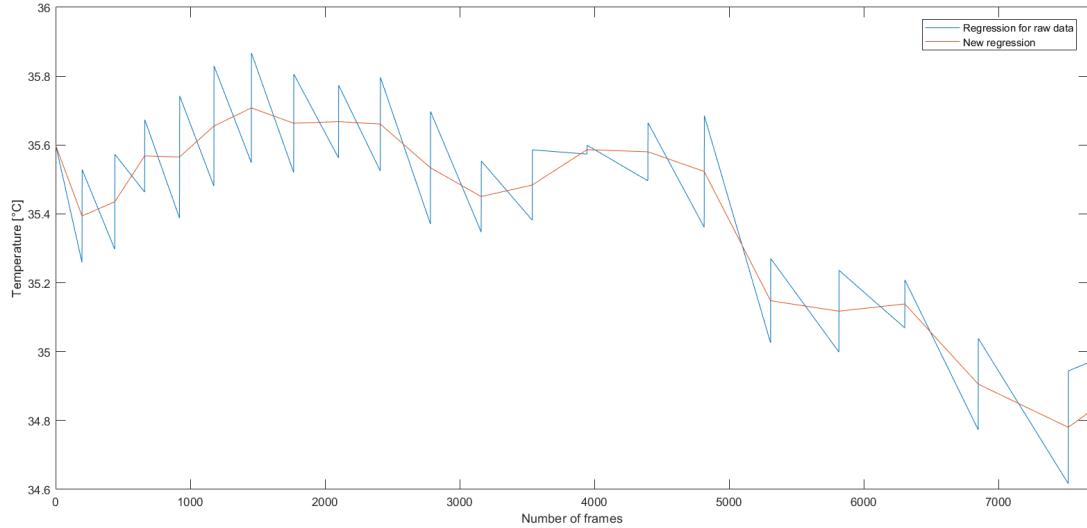


Figure 8.6: Connected regression lines of the original data of region 15 in the uncuffed recording of subject 1 in blue. New created orientation line of the same recording in the same region shown in red.

As shown in figure 8.6 the new orientation straight fits the regression lines together without suppressing the shape of the signal. Subsequently the residuals have been added to the new orientation straight, to sustain the ratio between the data points. Figure 8.7 shows the corrected signal wherein the jumps, separated intervals, have been connected and the jumps have been largely corrected.

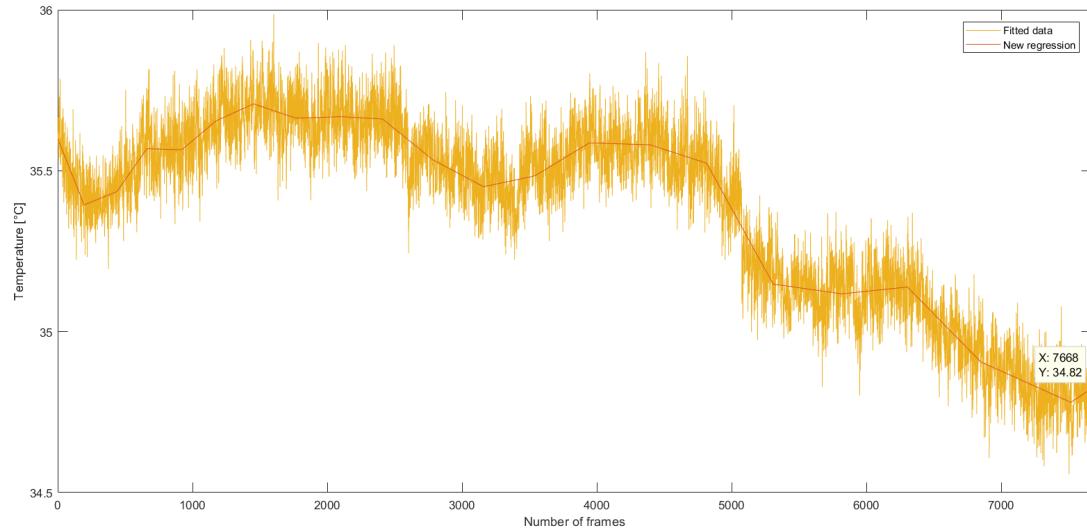


Figure 8.7: Orientation line based on the data of the uncuffed recording of subject 1 in region 15 shown in red. Corrected signal of the same data in yellow.

However, this method still has weak points. In figures 8.5 - 8.7 region of interest is located in the center of the thermal image. Regions which are located in the outer area of the thermal image show a few jumps after the correction which are bigger than before, illustrated on figure 8.8. These extended jumps are due to the fact that the thermal image is more unstable in the outer areas than in the center, thus the pixel drift is increasing with increasing distance to the center of the thermal image. That means that in the corrected data of ROIs located in the outer areas of the thermal image still artifacts occur.

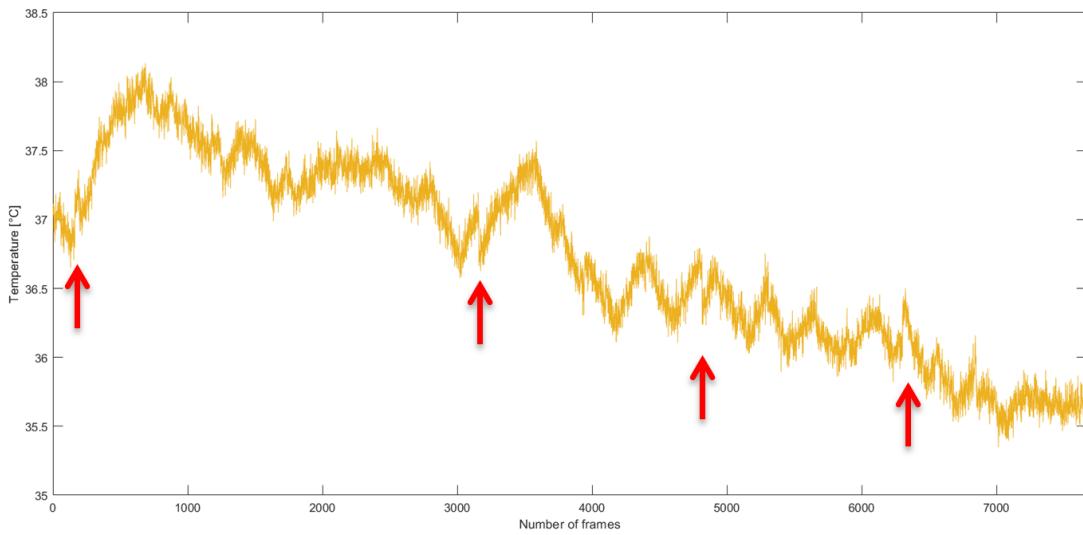


Figure 8.8: Corrected data of the uncuffed recording of subject 1 in region 23 shown in yellow. Red arrows show the jumps in this signal.

⁵FiXme Note: maybe show a total trace image before and after correction as comparison of correction

9 | Data processing

In this chapter the interpretation of the data from the corrected signal will be described. This interpretation will contribute to the values that will be used for the statistical test to see if the hypothesis will be rejected or not.

9.1 Scalogram interpretation

The data contains the time series of pixel intensities for each of the 28 ROI for each recording of a subject which the cwt is computed for after the signal has been corrected. A scalogram showing the time-frequency analysis content of the signal is given as output of the cwt. Frequencies of higher magnitude will show up with brighter colors, which can also be seen on the magnitude colorbar for comparison of the magnitude with related values. The frequency illustrated by the scalograms lies between 2.7370 Hz and 0.0031 Hz. This means, according to the literature, that bands of cardiac, respiratory, endothelial, myogenic and neurogenic is represented in the wavelet frequency span for the time frequency analysis. [geyer2004, sagaidachnyi2014]

The scalogram in figure 9.1 is representing the cwt of the raw signal for ROI 8 from subject 3.

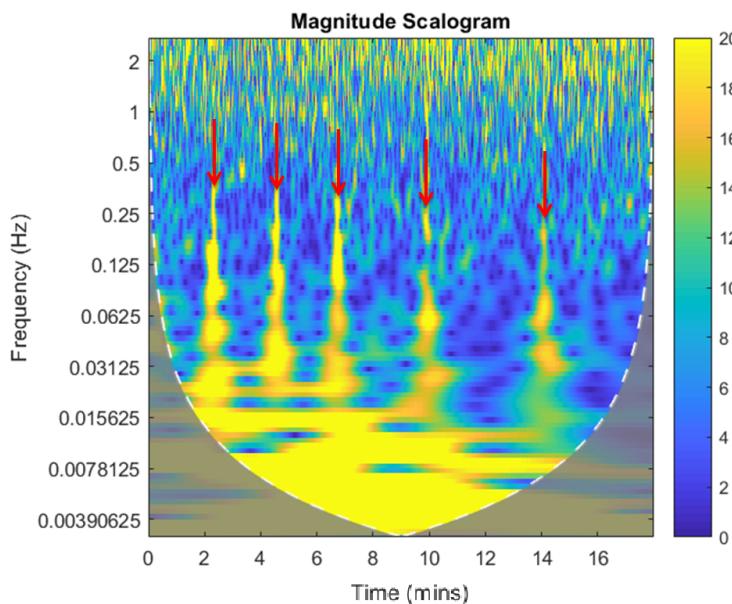


Figure 9.1: Scalogram from the raw data from region eight in the uncuffed recording of subject 3.

Looking at the scalogram from the raw signal in figure 9.1 the jumps can easily be seen as the high bright spikes which represent the jumps in the signal. Five jumps that are

indicated with the red arrows, are present in the scalogram at time points around 2.5, 4.5, 6.5, 10 and 14 minute time point in the signal. The jumps in the same signal shown in the time domain occur in the scalogram at the same time points. It is presumed that the corrected drift also is represented in the scalogram as low frequency content. Hereby different artifact components can be suspected to be included in the signal content.

The artifact components of the raw signal can be sorted into three categories:

- Uniform white noise artifacts
- Drift artifacts within each interval
- Jumps artifacts

Uniform white noise artifacts is characterized by having the same magnitude in all frequencies. This artifact will therefore not affect the signal of interest, because it will have a flat power spectral density throughout the bandwidth of the frequencies, because the signal of the white noise are independent and evenly distributed [hida2014]. The jumps in the signal is induces by the auto-adjustments from the camera described in 8.3. The drift artifacts can be seen as magnitudes in the scalogram as lower frequencies than the jumps. A frequency band occur at each jump, indicating that the drift is not uniform between intervals. The artifact components will be disturbing the presumed signal from the micro oscillations to get a representative cwt, because this signal can be hidden by the artifacts. The artifacts are not constant for each recording, why the correction of the signal has been implemented.

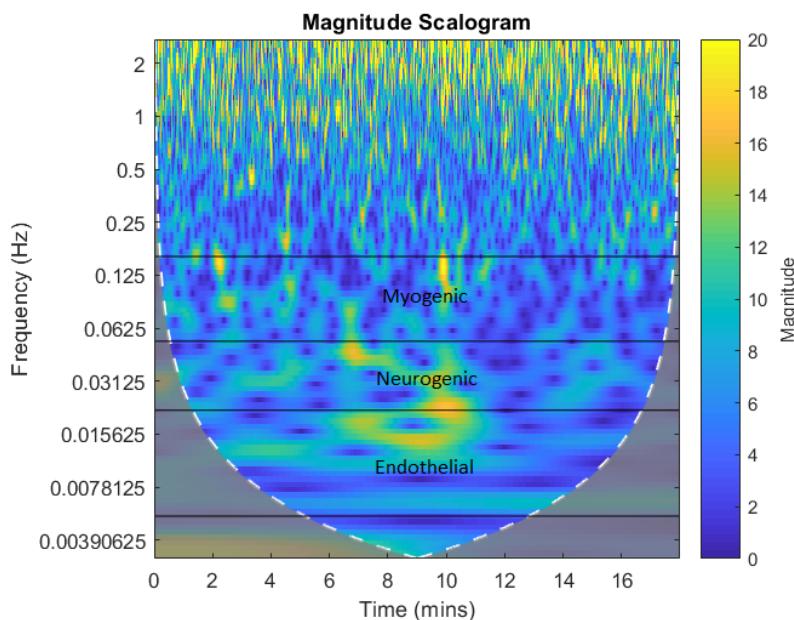


Figure 9.2: Scalogram from the corrected data of region 8 in the uncuffed recording of subject 3, where a dampening of the spikes and induced by the jumps has been achieved.

After the correction of the signal, the energy has been reduced in the areas induced from the jumps and the drift. The scalogram is left with less energy overall as seen in figure ??.

The three frequency bands are marked with red arrows in the scalogram seen in figure 9.2. To prepare the data for the statistical test, the average magnitude for each time period of each frequency band is first calculated by equation 9.1.

$$W(n_f) = \frac{1}{N} \sum_{n=1}^N W_n(n_f) \quad (9.1)$$

Where N denotes the total number of elements in the frequency band, W is the magnitude of the wavelet, n_f is the respective frame and n is the current element of the magnitude of the frequency band.

Then the mean of the average magnitude over the time period is calculated by equation 9.2

$$W_{mean} = \frac{1}{N_f} \sum_{n_f=1}^{N_f} W(n_f) \quad (9.2)$$

Where W_{mean} denotes the mean value of the frequency band over the time period and N_f is the total number of frames. This gives a single value for the specific frequency band to use in the statistical test for comparison between the two conditions.

9.2 Selecting ROIs for statistical test

After application of the correction method contain the signals of some ROIs still jumps what makes them for further data processing useless. Therefore each signal and its scalogram has been submitted a visual control. A table (Appendix) was used to evaluate each region in each subject within both conditions concerning the appearance of jumps after applied correction method and usability regarding further data processing.

With the aid of this evaluation table five ROIs have been chosen to use. The criterion for this selection was, that those ROIs showed in measurements good response to the correction method. All five ROIs are located in the back of the hand.

9.3 Statistical approach

To test if there is a statistical significant difference between the uncuffed and cuffed condition a paired t-test is applied on the outcome data from the scalograms. The approach builds on using the total mean of each frequency band, condensing these down to just one number for each frequency band for one ROI for one subject, the approach of the calculation is explained in 9.1. Before a paired t-test is computed for the data, a boxplot

is of the data is shown to get a visual representation of the data of the three frequency bands.

A paired t-test will be performed on all five regions, where uncuffed is the before condition and cuffed is the after condition. The outcome of the paired t-test will either be rejecting the h_0 hypothesis, that there is no difference between the two conditions, or not rejecting the h_0 hypothesis, which indicates that there is a difference.

The significance level of 0.005 is used the statistical test. This measures the likelihood of rejecting h_0 . The 0.005 significance level presumes that there is a 5% risk of concluding the sample mean to fall withing the critical region at the level of 0.005 and thereby reject h_0 . [zar2014]

Part IV

Results

10 | Results

In this chapter the results on behalf of the data analysis will be shown. A paired t-test of the mean magnitude for each frequency band specified in 4.1 is computed to test the likelihood of finding a difference between the uncuffed and cuffed conditions.

10.1 Paired t-test

The boxplots can be seen in figure 10.1 throughout figure 10.3. The boxplots show points for each mean magnitude for each subject ordered by color, a line is connecting the before and after condition to indicate if there has been an increase or decrease in magnitude.

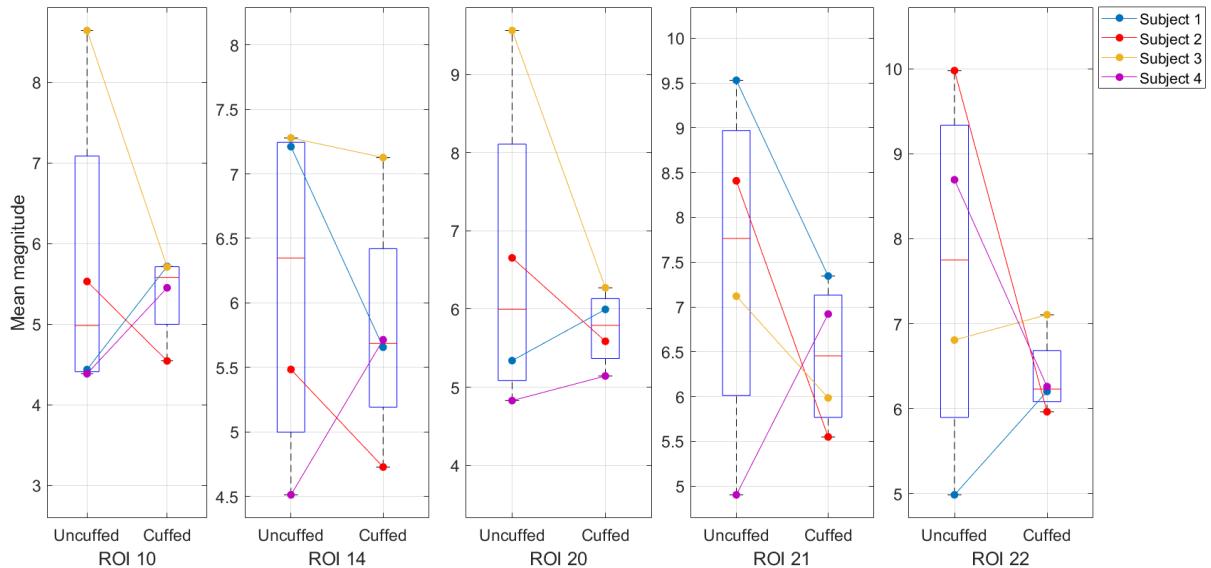


Figure 10.1: Boxplot for mean magnitude in the endothelial frequency band, for both conditions in five ROI.

Chapter 10. Results

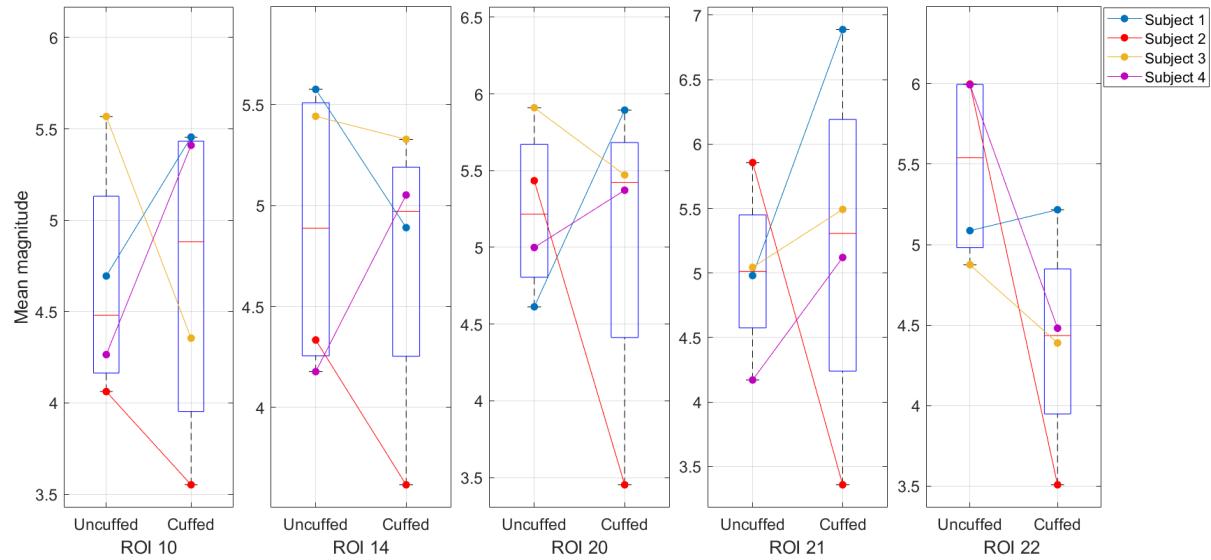


Figure 10.2: Boxplot for mean magnitude in the neurogenic frequency band, for both conditions in five ROI.

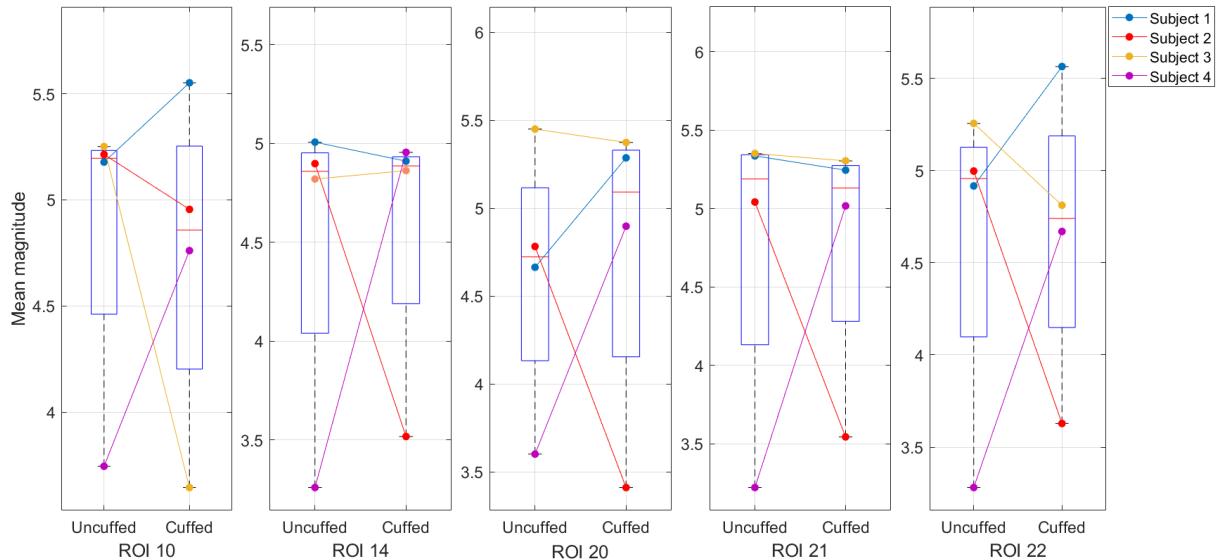


Figure 10.3: Boxplot for mean magnitude in the myogenic frequency band, for both conditions in five ROI.

The outcome of the paired t-test is a table showing the p-values, which is shown in 10.1.

	P-endo	P-myo	P-neuro
ROI 10	0.7116	0.8454	0.9389
ROI 14	0.6254	0.9237	0.6955
ROI 20	0.4141	0.9237	0.8004
ROI 21	0.4062	0.9564	0.8452
ROI 22	0.3826	0.9323	0.1552

Table 10.1: Table showing the p-values corresponding to specific ROI in correlation with frequency band.

As indicated in table 10.1, all of the tests show a significance level well above 0.005, which means that h_0 is not rejected, whereby there is no difference between the two conditions. When reflecting the p-values onto the boxplots the correlation between the p-values and the occurrence of a difference in the dataset can be seen. For instance the lowest p-value of 0.1552 indicates the greatest difference between means, this can also be seen in figure figure 10.2 for ROI 22.

Part V

Discussion

11 | Discussion

The present study used thermal imaging to detect if there are measurable changes in the micro temperature oscillations of skin depending on blood flow to the observed area. Whereby the temperature changes in the skin were used as indicator for vasomotoric activity. The means of the regarded frequency bands in both conditions resemble one another. Hence the results of the paired t-test of the regarded frequency band means show no clear significance. Likewise the qualitative comparison by mapping of t-values shows no significant difference.

The obvious assumption that there are no changes in the microcirculatory system or rather in vasomotion by 50% restriction of blood flow can be substantiated either by independence of the microcirculatory system or by incorrect occlusion of the arm.

The available amount of blood in the microcirculatory system is depending on the amount of blood the macrocirculatory system provides what means that the microcirculatory system is not independent. Consequently this explanation can be excluded.

Incorrect occlusion of the arm means the cuff which was used for occluding the arm during the second measurement is not able to provide sufficient restriction for affecting the microcirculatory system. Firstly it can be due to the shape of the cuff what is unlikely because a standard cuff which dose not differ from other cuffs was used. Secondly a wrong occlusion pressure could be applied. As it can be seen in TABLE, the blood pressure measurements of three subjects delivered high values around 140mmHg and around 150mmHg . Since those three subject were in different age and shape, the suspicion arises that the measured values are incorrect. But regarding the other subject's value which is in a low normal range around 100mmHg and the fact that the blood pressure measurements were conducted by an anesthesiologist with a blood pressure monitor used in clinical routine dash this suspicion. Even if the blood pressure monitor delivered wrongly high values, the outcome of the second measurement would not have been influenced negatively. A wrongly high occlusion pressure leads to a calculated occlusion pressure which is higher than needed to reach the intended restriction. At worst the restriction would have been higher than 50 % of the blood flow, what is just leading to a larger difference between both condition. This would have affected the outcome not negative.

The small amount of subjects must not be disregarded. With a sample size of four subjects applied statistical methods are not sufficiently meaningful. With a larger amount of subjects this study would get a more meaningful result. It might be possible that a larger amount of subjects provides a significant difference between both conditions. Also the gender distribution of the subjects was not equal. It might be better to use the same amount of male and female subjects. But regarding the measured data there cannot be seen a difference between female and male subjects.

Chapter 11. Discussion

Furthermore the missing randomization of the study design can be criticized. Though in the case of this study it was not possible to randomize which measurement is conducted first. If the condition with 50% restriction of the blood flow would have been carried out first, the data of the measurement under normal conditions would have been affected by a carry over effect.

However this study was used as a pilot study to investigate if thermal imaging can be used to detect vasomotoric activity. Also impaired by subsequent elaborated limitations it would not have been worthwhile to recruit a larger amount of subjects even though it was the first intention.

Regarding the previous studies, which were able to measure vasomotoric activity and the changes of the spectral components influenced of diseases, let assume that the results are due to a lack in the methodology of this study.

Those vasomotoric activities were measured in the fingertips. Whereas this study compared just areas on the back of the hand based on the assumption that vasomotoric activity can be measured equally in every area of hand's skin. The results lead to the suspicion that vasomotoric activity cannot be measured equally on the back of the hand and the fingertips.

The reason for not taking the fingertips into account is the uselessness of the data in these areas. Key reasons are the properties of the Gobi 640 17 μ m GigE camera (Xenics NV, Belgium). This uncooled thermal camera contains sensitive microbolometers which need from time to time a recalibration. Hence occurring jumps and the pixel drift within the intervals between the jumps make the data unusable. Therefore a correction method was implemented, which clearly affects parts of the signal.

The correction method is based on the assumption that the drift component in every interval is linear. This assumption might be wrong and a correction method that uses an other regression method or combines different regression methods adjust the artifacts in a better way.

Besides, the pixel drift increases with increasing distance to the center of the thermal image. Regarding the ROIs located in the outer areas of the thermal image the pixel drift cannot be completely compensated of the implemented correction method. As a result the ROIs located in the outer area of the thermal image were excluded from the data analysis.

Furthermore the smaller the observed area the closer the thermal camera has to be to this area. Thus the area represented by one pixel gets smaller. Within this study one pixel represents an area with a diameter around 417 μ m. Whereas previous studies observed smaller areas what means that the pixel represent a smaller area. It might be that previous studies detected vasomotoric activity by using smaller ROIs. With the use of a larger ROI, it might be that the amount of inverse dilating capillaries is equal. Due to

inverse dilating capillaries there might be no changes over time measurable because the frequency contents are occurring alternating in the different capillaries.

In addition, it is obvious that the noise content in the signal is significantly higher than in the temperature signals detected in previous studies. Comparing the signals the question arises if the noise overlaps or suppresses the frequency content of vasomotoric activity. Even though temperature changes over time in the skin were detected, it is uncertain, if this signal just represents the general skin temperature or also vasomotoric activity.

As outlined the Gobi 640 17 μ m GigE (Xenics NV, Belgium) thermal camera might be unsuitable in this setup. Besides, this was not the only limitation of the setup.

The subjects hands were stabilized by a vacuum pillow with the assumption the subjects were still, but it gives not sufficient support to inhibit all movements of a subject. Furthermore with the used setup exact same conditions for each subject cannot be granted. The position of the hand and the position of the camera varies marginally between the subjects due to missing markings. There was no possibility to influence the room temperature or to measure it and take it into consideration. So the room temperature was preset to 25°C for all subjects. Equally the software settings were preset and could not be influenced.

The presetting of the room temperature might have affected the data. This suspicion is enhanced by regarding subject 1's hand temperature which was 38°C. Also by regarding the hand temperature of 26°C of subjects 2 and 3, who had cold hands during the measurement, this suspicion is enhanced.

Despite the mentioned limitations of the setup there are some points which can be improved using the provided materials and given conditions. The software settings should be scrutinized and if necessary changed. Also the room temperature needs to be measured and taken into consideration by entering into the software setting. Since the thermal pixel drift is known the alleged best regions should be centered in the thermal image and the thermal camera should put closer to the observed areas. Moreover a markings for the placement of every component provide more stability of the setup between the subjects and increase the reproducibility. Nevertheless an improved setup could not compensate for limitations given by the used thermal camera. An optimal test setting requires a thermal camera of higher quality preferred a cooled camera. In addition a mechanism for better stabilization of the hands should be included. To create the same conditions for each subject, the room temperature should not vary and be adjustable.

A | Protocol

Experimental Protocol

Experiment

Study of temperature oscillations in the peripheral circulation with infrared thermography

Formalities

Date:	17.10.2017 and 18.10.2017
Place:	Regionshospital Nordjylland in Hjørring
Conducted by:	Toby Waterstone, Christian Mortensen, Annabel Bantle, Andrei Ciubotariu

Background

Aim:	The aim of the experiment is to measure vasomotion in the hand in two conditions
Type of study:	Quantitative research
Subjects:	<p>Number of subjects: 4</p> <p>Inclusion criteria:</p> <ul style="list-style-type: none">• Subjects should have at least one hand to perform the measure on• The cuff should be able to fit the arm circumference• The subject should be able to sit still for a greater extend of time• Health conditions that sets the subject in risk of injury when conducting the experiment like high blood pressure. <p>Exclusion criteria:</p> <ul style="list-style-type: none">• Age under 18 years old• Age over 60 years old• Obesity to a greater extend• Diseases that triggers tremors

Test Requirements

Materials:	Xenics Gobi 640 17µm GigE Infrared camera with power cord, Tripod, Cuff, Chair, Computer with recording software and power
------------	--

Experimental Protocol

	cord, Vacuum pillow, Vacuum pump, Stopwatch, Ethernet cable, Computer.
Setup:	
Preparation:	<ol style="list-style-type: none">1. The camera has to warm up for 15 min.2. During this laptop, software and all cable connections should be set in operational readiness.
Procedure:	<ol style="list-style-type: none">1. Systolic pressure is measured and mean is calculated2. Pressure to be used in cuff is calculated3. The cuff is affixed at the subjects dominant arm without tighten it.4. The subject can take place in the chair.5. The hand is put on the vacuum pillow.6. The vacuum pump is attached to the pillow.7. The camera needs to be positioned 37.5 cm over the hand with the focus adjusted.8. If the camera is stable, the first measurement can be started for exact 20 min.9. Save file as subject_number of subject.10. Tighten the cuff on the arm of the subject with XXX, without moving the subjects hand.11. The second measurement can be started for exact 20 min.12. Maintain same pressure for 20 min.13. Save file as subject_number of subject_cuff

Appendix A. Protocol

Table A.1: Table of blood pressure.

Subject number	1. systolic pressure	2. systolic pressure	3. systolic pressure	Mean pressure	30 % of TOP
1	141 mmHg	138 mmHg	137 mmHg	138.6 mmHg	54.08 mmHg
2	102 mmHg	102 mmHg	102 mmHg	102 mmHg	39.78 mmHg
3	155 mmHg	147 mmHg	146 mmHg	149.3 mmHg	58.24 mmHg
4	138 mmHg	145 mmHg	135 mmHg	139.3 mmHg	54.34 mmHg

B | Evaluation table

ROI	S1_U	S1_C	S2_U	S2_C	S3_U	S3_C	S4_U	S4_C
1	✗	✗	✓	✗	✗	✓	●	✗
2	✓	✓	✓	✓	✗	✓	✓	✓
3	✓	✓	✓	✓	✗	✓	✓	✓
4	✓	✓	✓	✓	✗	●	✓	✓
5	✗	✗	✓	✗	✗	✓	✓	✗
6	●	●	✓	✓	✗	✓	✓	✗
7	✓	✓	✓	✓	✗	✓	✓	✓
8	✓	✓	✓	✓	✓	✓	●	✓
9	✓	✓	✓	✓	●	✓	✓	✓
10	✓	✓	✓	✓	✓	✓	✓	✓
11	✗	✗	✓	✗	✗	✓	✓	✗
12	✓	✓	✓	✓	✗	✓	✓	●
13	✓	✓	✓	✓	✗	✓	✓	✓
14	✓	✓	✓	✓	✓	✓	✓	✓
15	✓	✓	✓	✓	✗	●	✓	✓
16	✓	✓	✓	✓	✗	●	✓	✓
17	●	✗	✓	✗	✗	✓	✓	✗
18	✓	✓	✓	✗	✗	✓	✓	●
19	✓	✓	✓	✓	✗	✓	✓	✓
20	✓	✓	✓	✓	✓	✓	✓	✓
21	✓	✓	✓	✓	✓	✓	✓	✓
22	✓	✓	✓	✓	✓	✓	✓	✓
23	●	✗	✓	✗	✗	✓	✓	✗
24	●	●	✓	✗	✗	✓	✓	✗
25	●	✓	✓	✓	✗	✓	✓	✓
26	✓	✓	✓	✓	●	✓	●	✓
27	✓	✓	✓	✓	✗	✓	●	✓
28	✓	✓	✓	✓	✗	✓	✓	✓

Legend

✓	ROI in this recording useful
●	ROI in this recording maybe useful
✗	ROI in this recording not useful
■	ROI used for data analysis
	ROI is improper for data analysis
■■	ROI cannot be used for data analysis