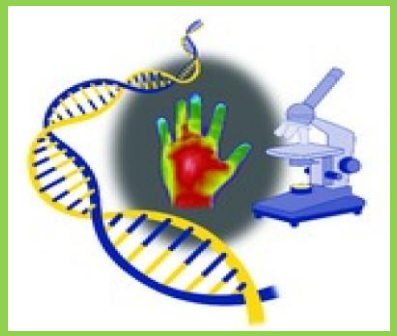


[1]The Catholic Univ. of America, Washington, DC

[2]The Burn Center, Washington Hospital, Medstar Health Institute

The Novel Application of a Spatial Frequency Domain Imaging to Determine Signature Spectral Differences Between Infected and Non-Infected Burn Wounds

Thu T.A Nguyen[1,2], Jessica C. Ramella-Roman[1,2], Lauren T. Moffatt[2], Rachel K. Truhan[2], Marion H. Jordan[2], and Jeffrey W. Shupp[1,2,*]

American Burn Association annual meeting

April 2012

Motivation

- Infected burn wounds present a problem
- Accurate and early diagnosis of burn wound infection is needed

- Still a challenge to scientist and physician

Motivation

- Patients suffering severe burns are at higher risk for local and systemic infections
- Complications of infection → morbidity and mortality
- Recently, emerging multi-drug resistant strains of bacteria have caused an unexpected rise in burn wound infections, sepsis, and associated death worldwide.

Motivation

- **MRSA** (**M**ethicillin-**R**esistant ***S****taphylococcus* ***a****ureus*) is one of them, also called **multidrug-resistant *Staphylococcus aureus***
- Each year, estimated 500,000 patients in American hospitals contract ***S. aureus*** infection [1]
- > 50% of hospital-associated and 30% of community-associated ***S. aureus*** isolates are MRSA [2]

[1] Bowersox John *et al.*, "Experimental Staph Vaccine Broadly Protective in Animal Studies," NIH. Archived from the original on 5 May 2007. Retrieved 28 July 2007.

[2] Patrick M.S *et al.*, "Secreted Virulence Factor Comparison Between Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus*, and its Relevance to Atopic Dermatitis," J Allergy Clin Immunol. , 125(1): 39, Jan 2010.

Motivation

- Because it's resistant to some commonly used antibiotics → TOUGH to TREAT
- **GOALS:** The early determination of burn wound infection →
  - help in early treatment, reduce systemic complications and minimize need for long-term intravenous antibiotics.

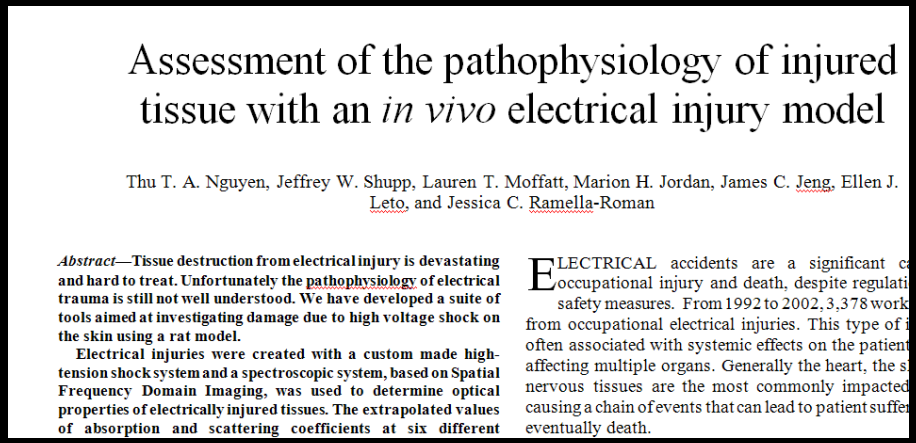
Motivation

- Quantitative wound cultures from biopsies are
  - gold standard to determine infection
  - invasive
- Needs
  - For non-invasive imaging techniques
  - Infection status determination of burn wounds in a controlled in vivo model

→ Propose: Spatial Frequency Domain Imaging (SFDI) technique

Previous work

- SFDI was applied to assess electrical injuries on rats suffering 1000VDC shock
- Some achievements



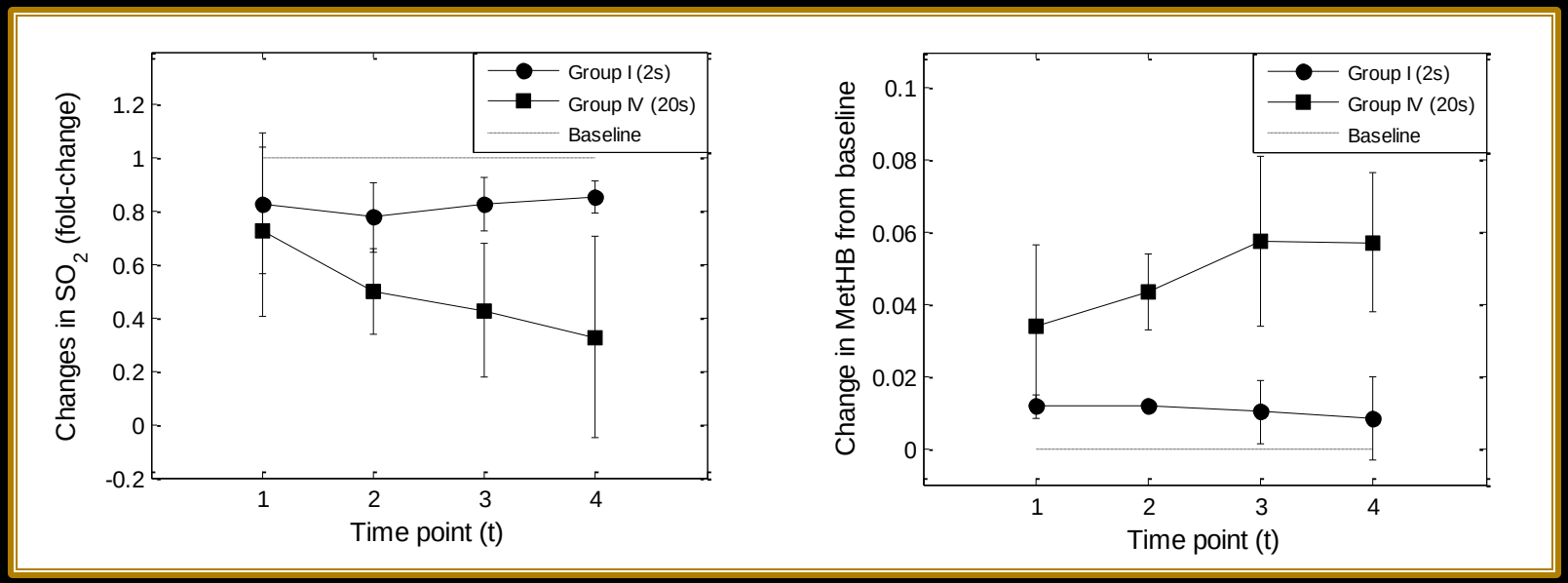
Assessment of the pathophysiology of injured tissue with an *in vivo* electrical injury model

Thu T. A. Nguyen, Jeffrey W. Shupp, Lauren T. Moffatt, Marion H. Jordan, James C. Jeng, Ellen J. Leto, and Jessica C. Ramella-Roman

Abstract—**Tissue destruction from electrical injury is devastating and hard to treat. Unfortunately the pathophysiology of electrical trauma is still not well understood. We have developed a suite of tools aimed at investigating damage due to high voltage shock on the skin using a rat model.**

Electrical injuries were created with a custom made high-tension shock system and a spectroscopic system, based on Spatial Frequency Domain Imaging, was used to determine optical properties of electrically injured tissues. The extrapolated values of absorption and scattering coefficients at six different

ELECTRICAL accidents are a significant c occupational injury and death, despite regulati safety measures. From 1992 to 2002, 3,378 work from occupational electrical injuries. This type of i often associated with systemic effects on the patient affecting multiple organs. Generally the heart, the s nervous tissues are the most commonly impacted causing a chain of events that can lead to patient suffe eventually death.



Experimental steps

- Thermal wounds (2 cm x 2 cm) were created on the dorsum of adult male Sprague-Dawley rats (n=6, 3 controls (non-infected), 3 infected).

- 1 day after, wounds of the infected group:
  → were inoculated with 200 µl of a vehicle containing 1 x 10^8 CFU *Staphylococcus aureus*.

During 10 days:

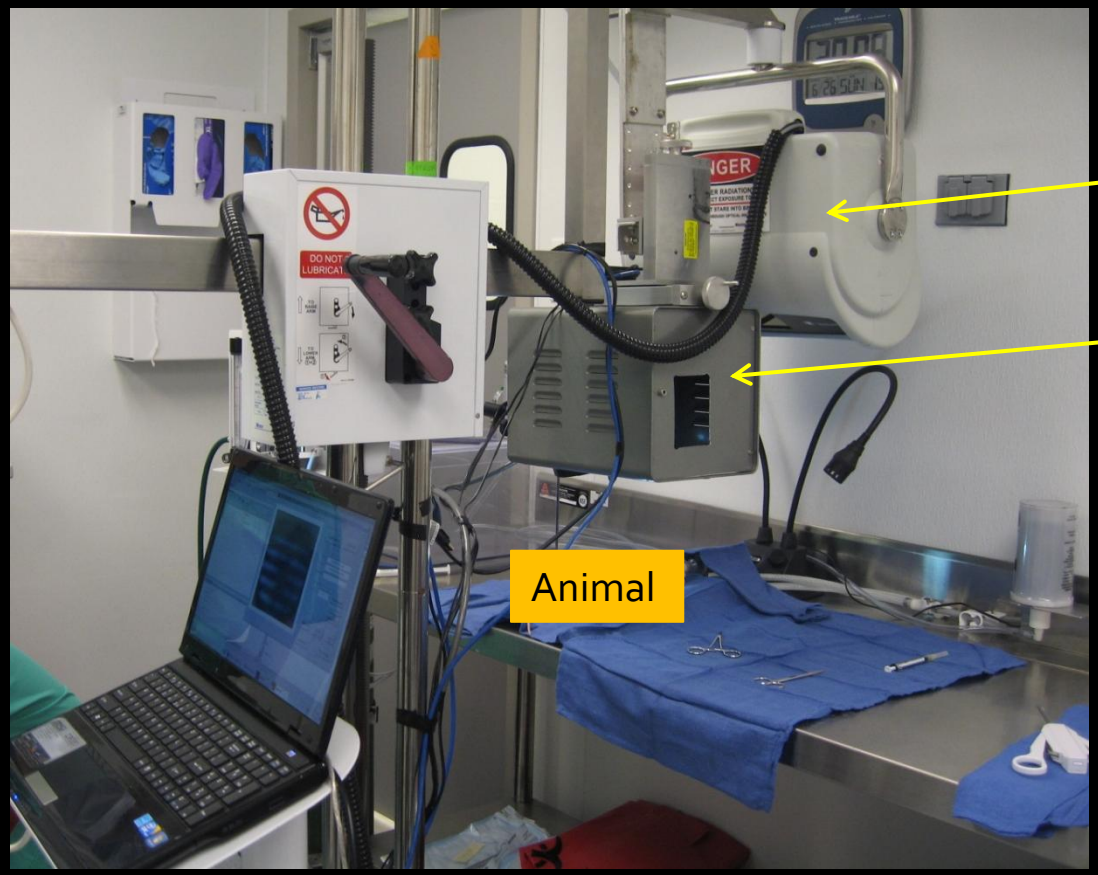
- Quantitative culture were measured
- Laser Doppler Imaging measured blood perfusion
- Spatial Frequency Domain Imaging system measure patho-physiological changes.

Spatial Frequency Domain Imaging

- SFDI
  - Is a novel and non-invasive imaging technique
  - Was first successfully utilized in 2009 [3]
- SFDI allows to calculate optical properties of biological tissues
  - Absorption Coefficient μ_a → How much light is absorbed in the skin
  - Reduced Scattering coefficient $\mu_{s'}$ → How much light is scattered in the skin
- μ_a can be used to calculate pathophysiological parameters:
  - So_2, Hemoglobin Volume Fraction, Met-hemoglobin Volume Fraction, Water content, Melanin Content , etc.

[3] Cuccia, D.J., et al., "Quantitation and mapping of tissue optical properties using modulated imaging," J Biomed Opt, 2009.

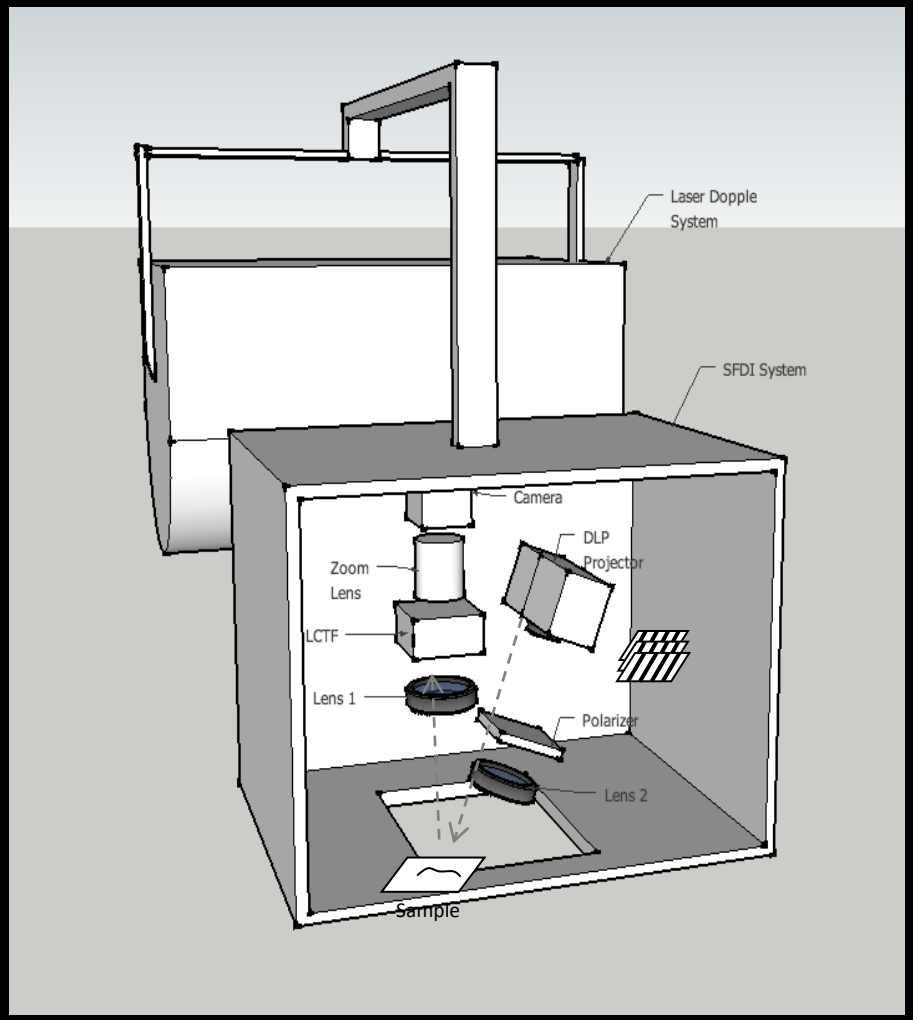
Instrumentation



LDI machine

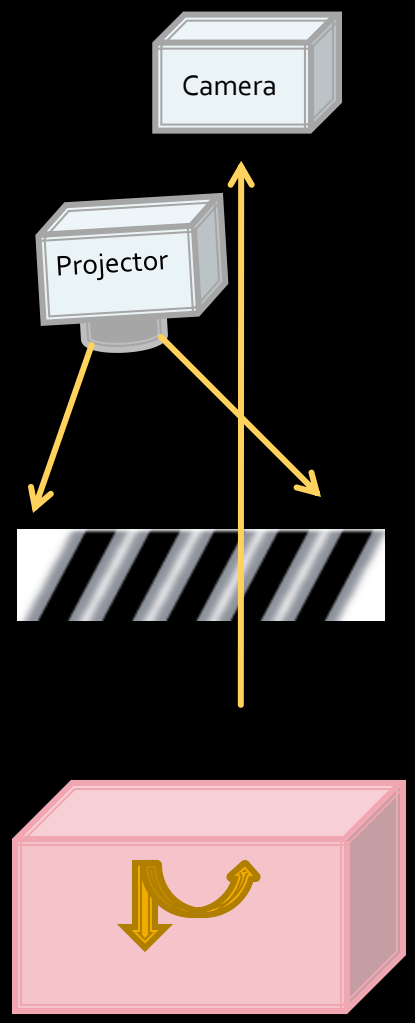
SFDI system

Instrumentation

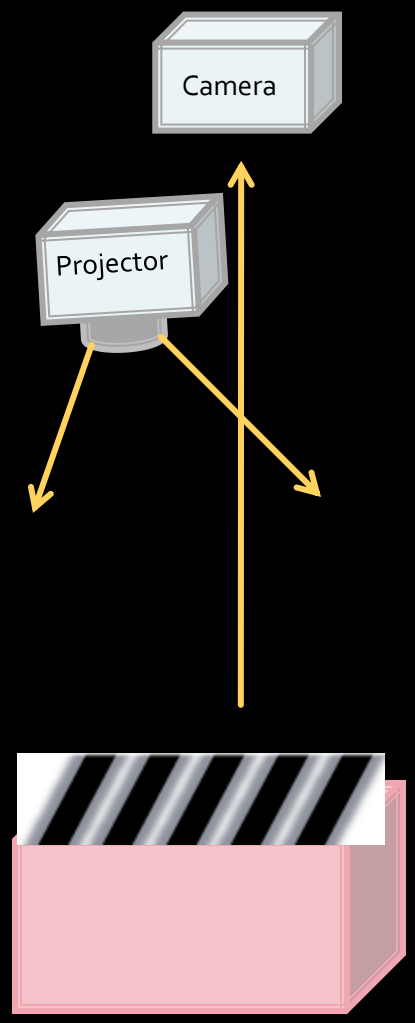


- DLP projector
  - To project fringes on a skin area, 1024 x 768 pixels
- Projection lens
  - To focus the light on the skin, *f*=50 mm
- Liquid Crystal Tunable Filter
  - To select desired wavelengths, 400 – 700 nm
- Imaging zoom lens
  - To focus reflected light to a camera, *f* = 200 mm
- Mono color camera
  - To image, 1024x1024 pixels
- 2 Crossed polarizers
  - To remove specular reflectance
- Whole SFDI system is controlled by a computer and the MATLAB programming software.

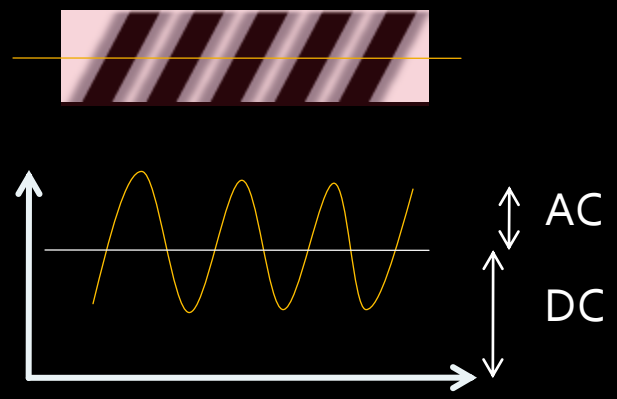
Principle of SFDI



Principle of SFDI



- The images captured by camera carry information of the tissue



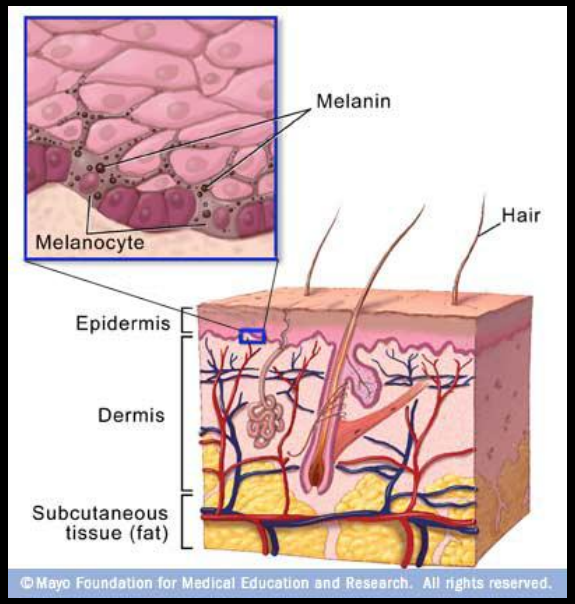
- DC and AC components indicate how much light is penetrated and scattered in the tissue
- These components are used to calculate optical properties (μ_a, $\mu_{s'}$) through Diffusion Equation

Calculate other parameters

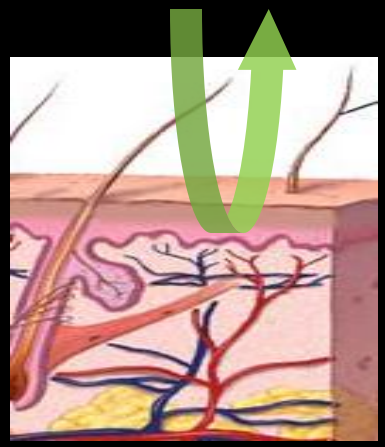
- Based on μ_a of each wound of at many wavelengths, we can calculate:
  - Oxygen Saturation (So_2)
  - Hemoglobin Volume Fraction (HB)
  - Methemoglobin Volume Fraction (MetHB)
  - Melanin Content (Mel)
  - Water Content (Water)

Calculate other parameters

- **Absorption coefficient μ_a of the skin : total absorption of instituting absorbers within the penetration depth of light**

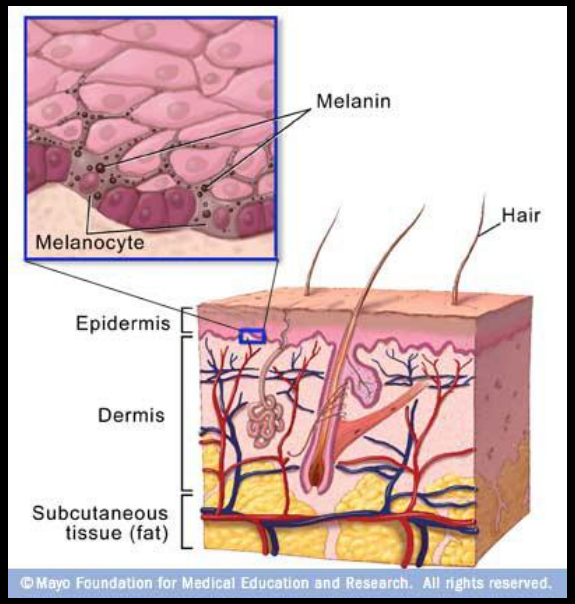


Short wavelengths



Calculate other parameters

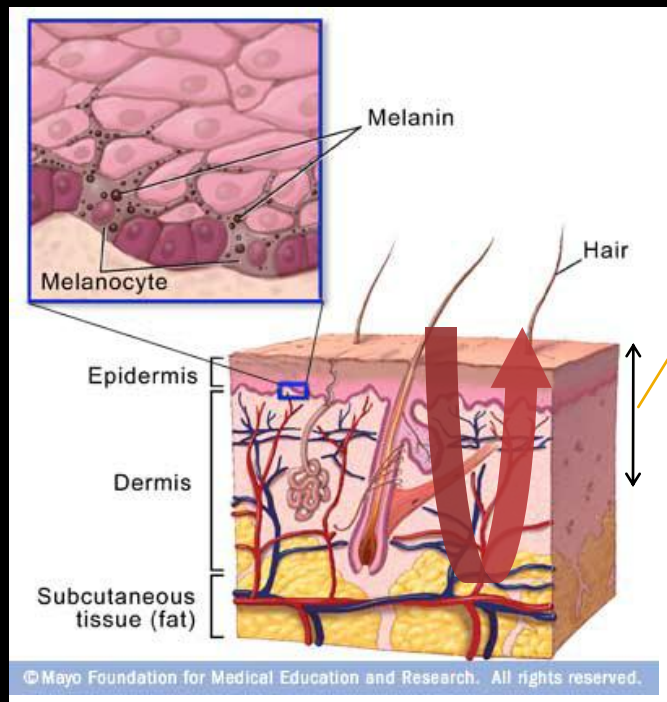
- Absorption coefficient μ_a of the skin : total absorption of instituting absorbers within the penetration depth of light



Long wavelengths

Calculate other parameters

- Absorption coefficient μ_a of the skin : total absorption of instituting absorbers within the penetration depth of light



L_{total}: total penetration depth of light into the skin

L_1: thickness of epidermal layer (1st layer)

L_2: penetration depth into dermal layer2nd layer
$= L_{total} - L_1$

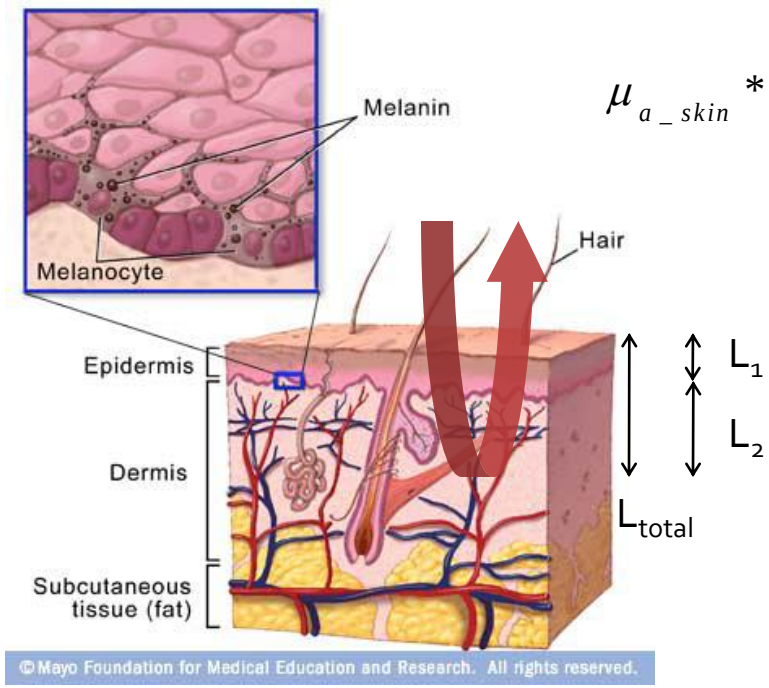
$$\mu_{a_skin} * L_{total} = (\mu_{a_layer1} * C_1) * L_1 + (\mu_{a_layer2} * C_2) * L_2$$

C_1 , μ_{a_layer1}: Concentration and Absorption coefficient of absorbers in Layer 1

C_2 , μ_{a_layer2}: Concentration and Absorption coefficient of absorbers in Layer 2

Calculate other parameters

- Main absorbers in 2 first layers of skin:
  - Layer 1 -epidermis: melanin
  - Layer 2 - dermis: oxy-hemoglobin, deoxy-hemoglobin, water, met-hemoglobin,..



$$\mu_{a_skin} * L_{total} = (\mu_{a_layer1} * C_1) * L_1 + (\mu_{a_layer2} * C_2) * L_2$$

$$\mu_{a_melanin} * C_{melanin}$$

$$\mu_{a_oxy_hemo} * \quad \mu_{a_deoxy_hemo} * \quad \mu_{a_water} * \quad \mu_{a_met-hemo} *$$

$$C_{oxy_hemo} \quad C_{deoxy_hemo} \quad C_{water} \quad C_{met-hemo}$$

Need to calculate

Calculate other parameters

- **Main absorbers in 2 first layers of skin:**
  - **Layer 1 -epidermis: melanin**
  - **Layer 2 - dermis: oxy-hemoglobin, deoxy-hemoglobin, water, met-hemoglobin,..**

$$\underbrace{\mu_{a_skin} * L_{total}}_{\text{Known inputs}} = function(\underbrace{C_1, C_{oxy-hemo}, C_{deoxy-hemo}, C_{water}, C_{met-hemo}}_{\text{Unknown outputs}})$$

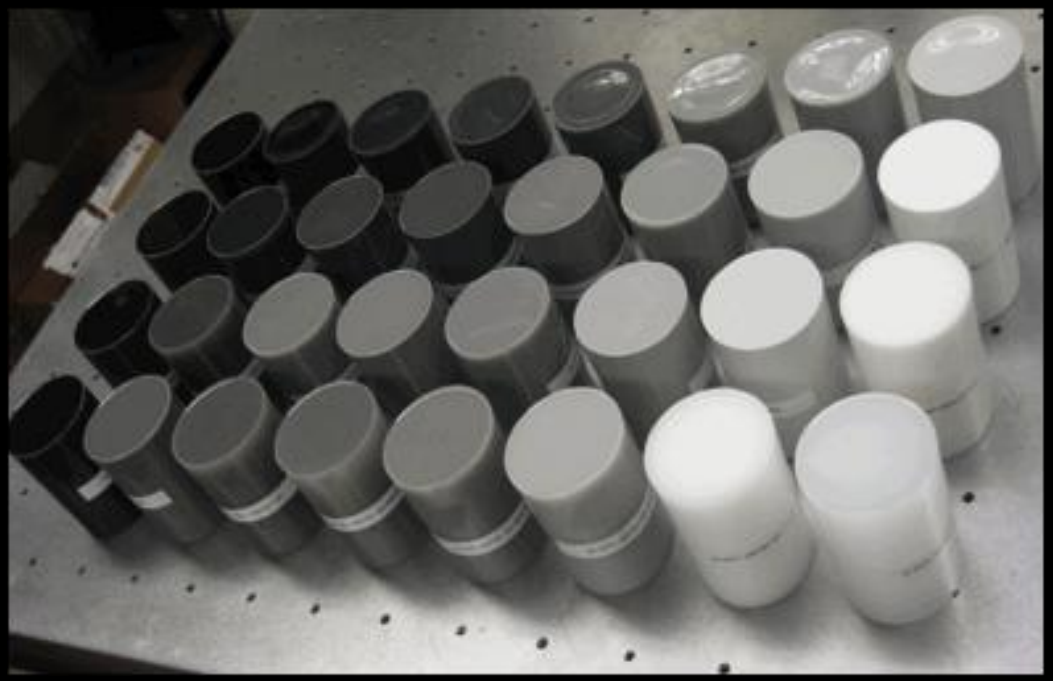
More Inputs

$$\left\{\begin{array}{ll} \mu_{a_skin} * L_{total} & at\ wavelength\ \lambda_1 \\ \mu_{a_skin} * L_{total} & at\ wavelength\ \lambda_2 \\ \ldots & \ldots \\ \mu_{a_skin} * L_{total} & at\ wavelength\ \lambda_n \end{array}\right\}$$

Solve for outputs

Calibration for SFDI system

- System was well calibrated on polyurethane diffusive phantoms mimicking tissue optical properties.



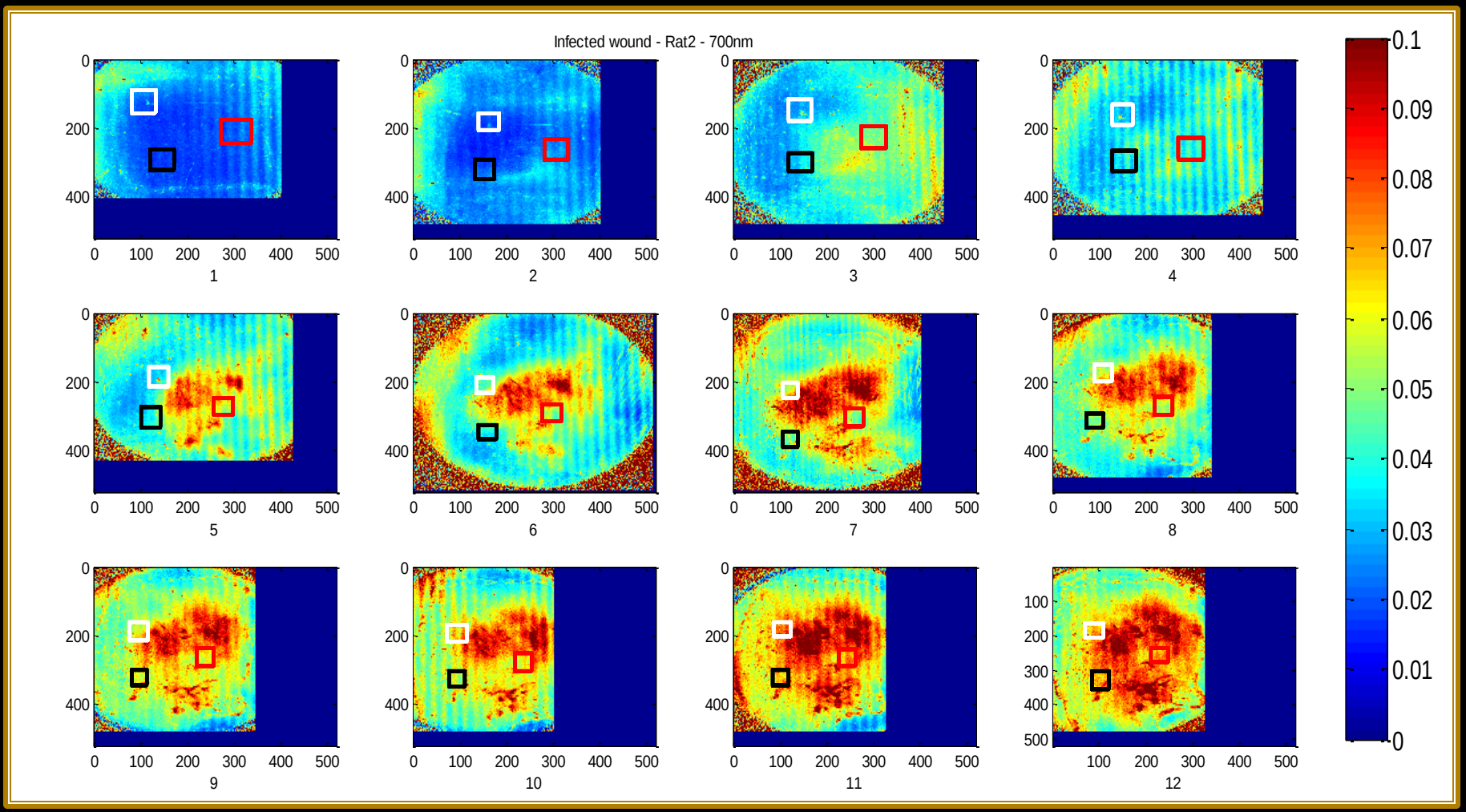
RESULTS

Results - μ_a & μ_s'

- Maps (1000 x 1000 pixels) of μ_a and μ_s' of each rat were reconstructed
  - at 12 time points (Pre-burn, Post-burn, Day1→ Day10)
  - and at 16 wavelengths (500-700 nm)

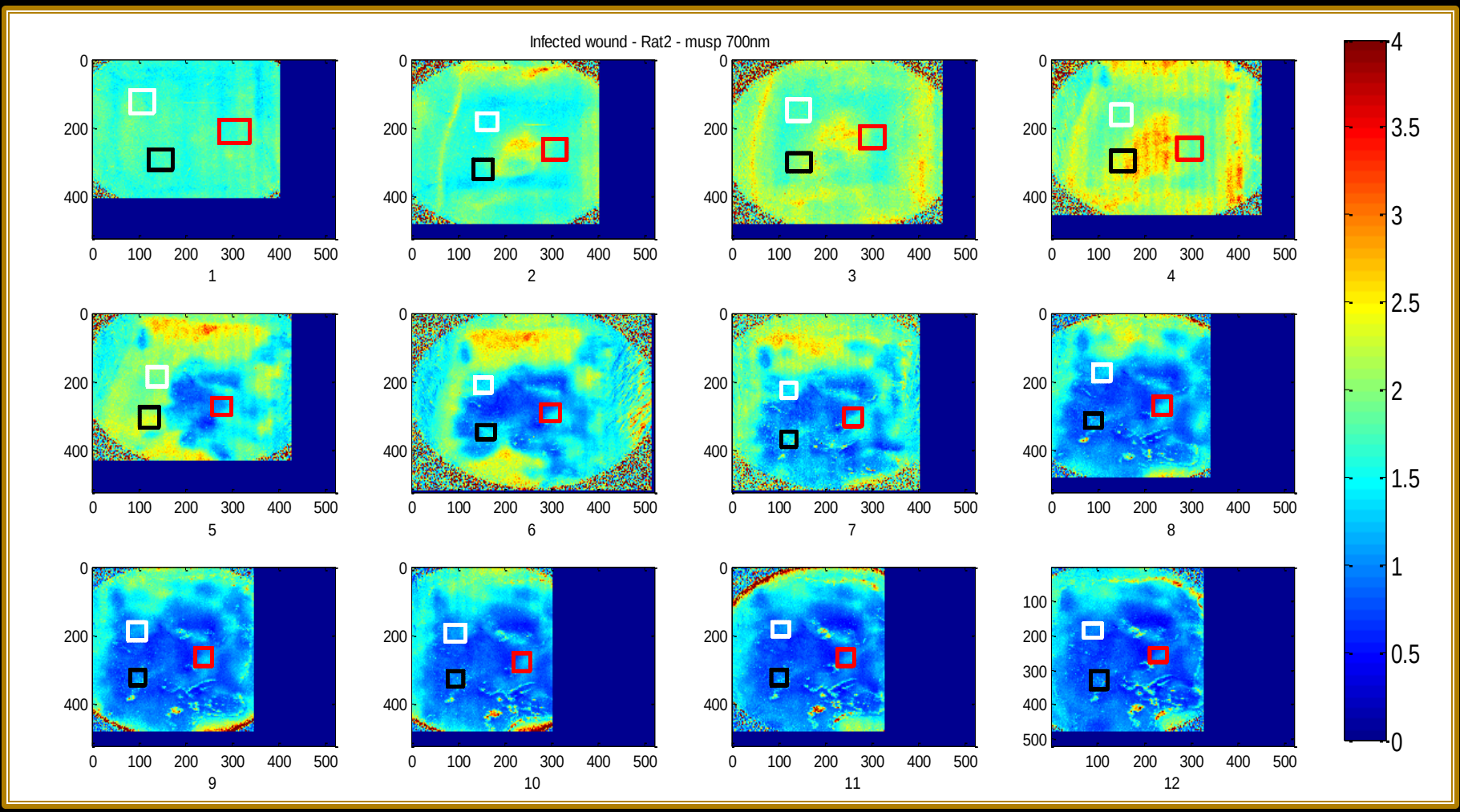
Results - μ_a & $\mu_{s'}$

- Maps of μ_a (mm^{-1}) of 1 rat in *Infected-group*, at 700nm, along 12 time points



Results - μ_a & $\mu_{s'}$

- Maps of μ'_s (mm^{-1}) of 1 rat in *Infected-group*, at 700nm, along 12 time points

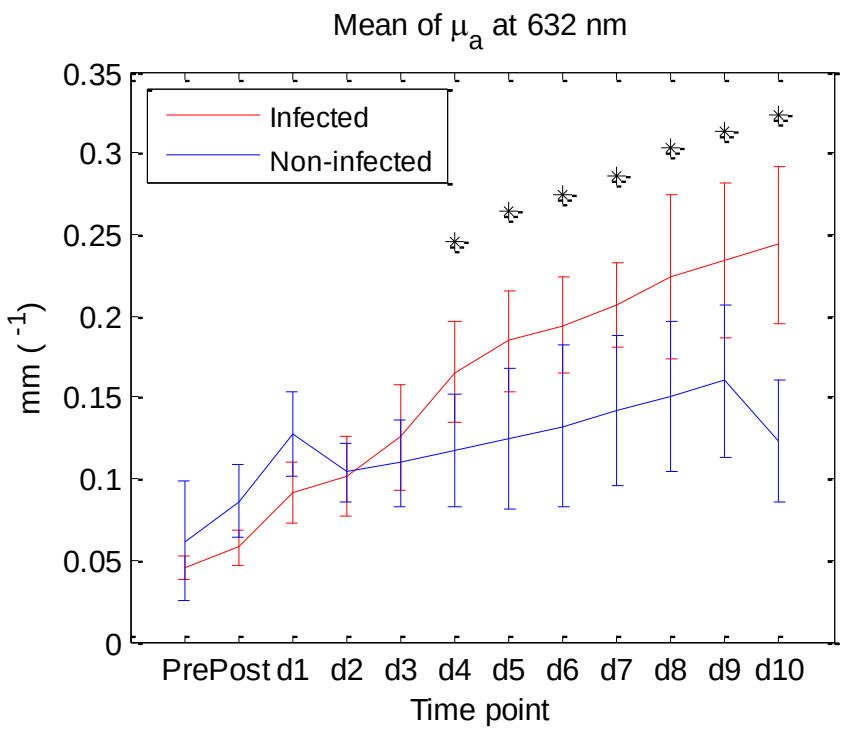


Results - μ_a & $\mu_{s'}$

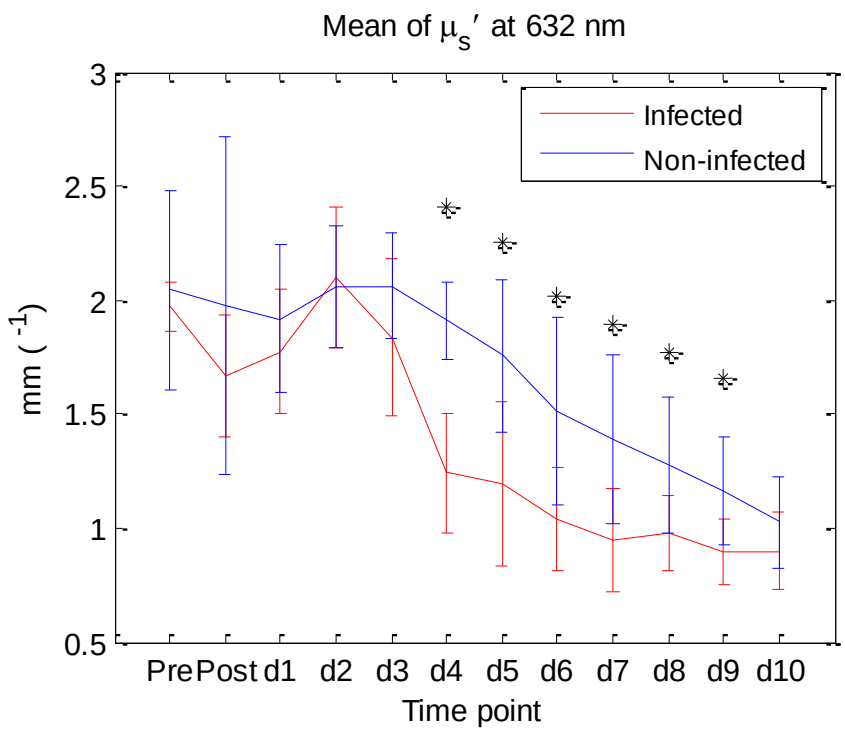
- 3 regions of interest on every optical properties map were selected to give a mean value
- Then, mean values of every 3 rats in the same group were calculated

Infected wounds and Non-infected wounds μ_a, and μ_s' at 632 nm

μ_a



μ_s'



P value from T-test

632	Pre Burn	Post burn	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
μ_a	0.22	0.00	0.02	0.82	0.40	0.02	0.00	0.01	0.00	0.02	0.02	0.00
μ'_s	0.66	0.27	0.26	0.73	0.07	0.00	0.01	0.03	0.03	0.03	0.01	0.28

Infected wounds and Non-infected wounds - μ_a, $\mu_{s'}$

- Short wavelengths (< 620nm) with shallow penetration into the skin (at the death layer) → do not show any separation.
- At longer wavelengths (620, 632, 650, and 700 nm) , separations are observed from day 4 with p value <0.05

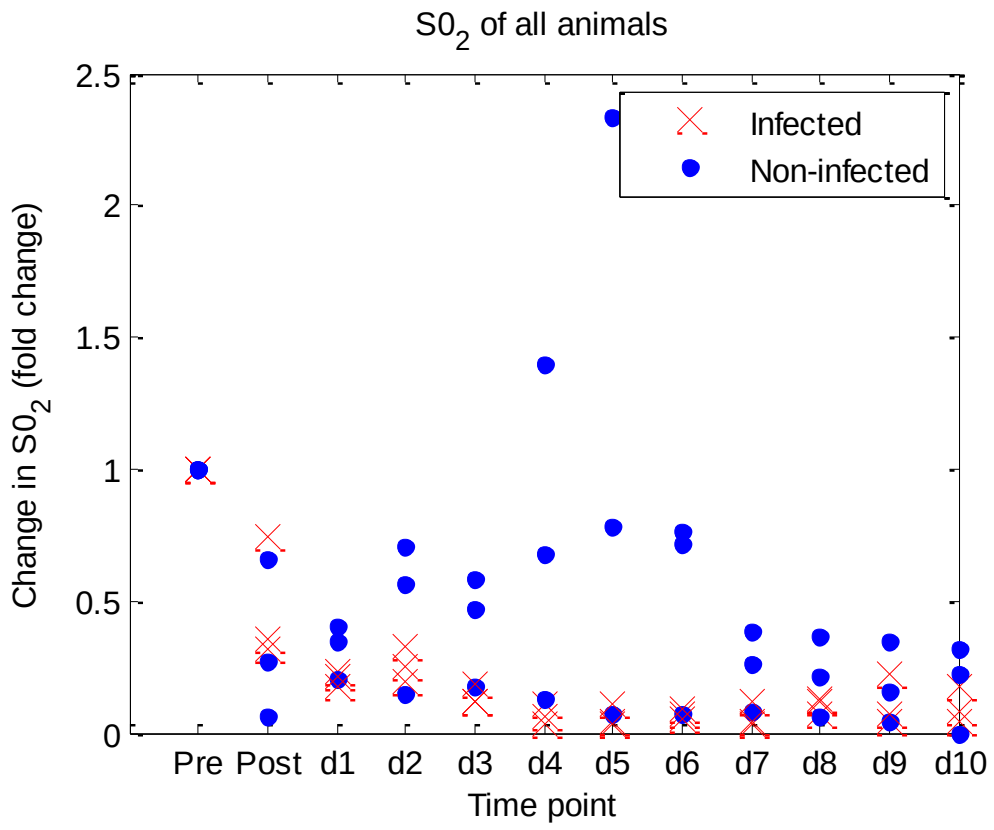
		Pre burn	Post burn	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
620nm	μ_a	0.21	0.01	0.02	0.71	0.29	0.02	0.01	0.02	0.01	0.02	0.02	0.00
	$\mu_{s'}$	0.62	0.33	0.26	0.98	0.15	0.00	0.01	0.03	0.02	0.05	0.03	0.25
632nm	μ_a	0.22	0.00	0.02	0.82	0.40	0.02	0.00	0.01	0.00	0.02	0.02	0.00
	$\mu_{s'}$	0.66	0.27	0.26	0.73	0.07	0.00	0.01	0.02	0.03	0.02	0.01	0.28
650nm	μ_a	0.34	0.02	0.01	0.80	0.23	0.04	0.01	0.02	0.01	0.02	0.01	0.00
	$\mu_{s'}$	0.45	0.32	0.38	0.71	0.04	0.00	0.01	0.03	0.03	0.02	0.01	0.25
700nm	μ_a	0.18	0.05	0.00	0.90	0.08	0.10	0.04	0.01	0.00	0.03	0.00	0.00
	$\mu_{s'}$	0.56	0.59	0.39	0.78	0.01	0.00	0.02	0.01	0.01	0.00	0.00	0.34

Results – pathophysiological parameters

- μ_a of each wound of at many wavelengths used, we can calculate:
  - Oxygen Saturation (So_2)
  - Hemoglobin Volume Fraction (HB)
  - Methemoglobin Volume Fraction (MetHB)
  - Melanin Content (Mel)
  - Water Content (Water)

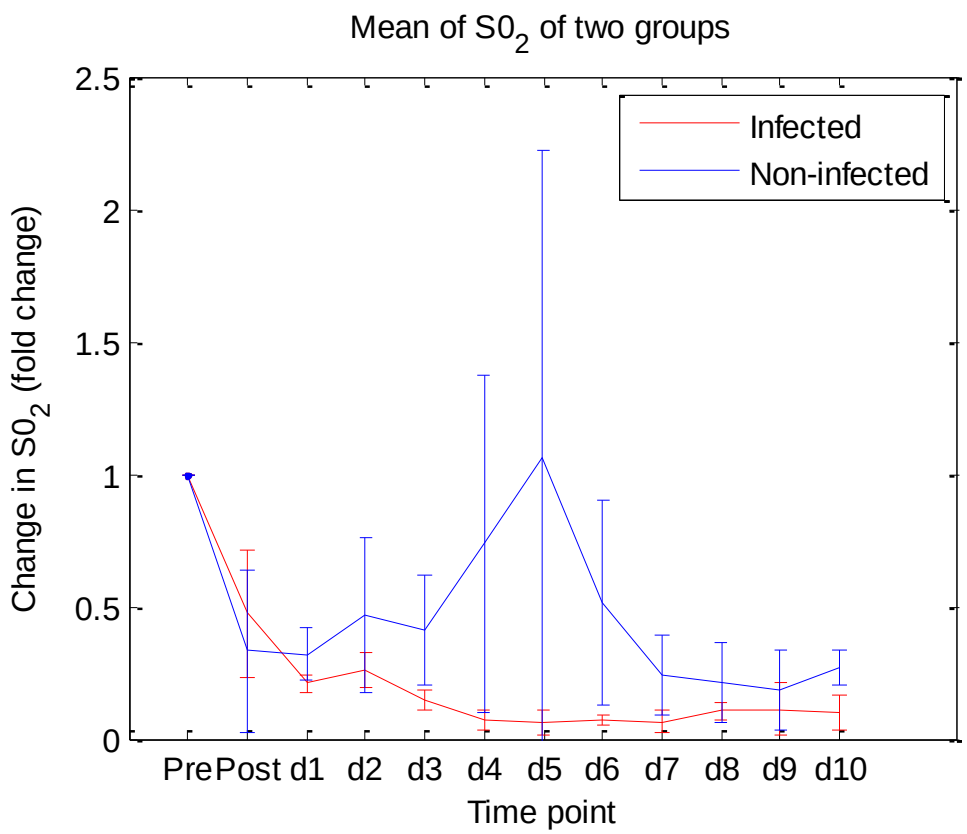
Results – SO_2

- SO_2



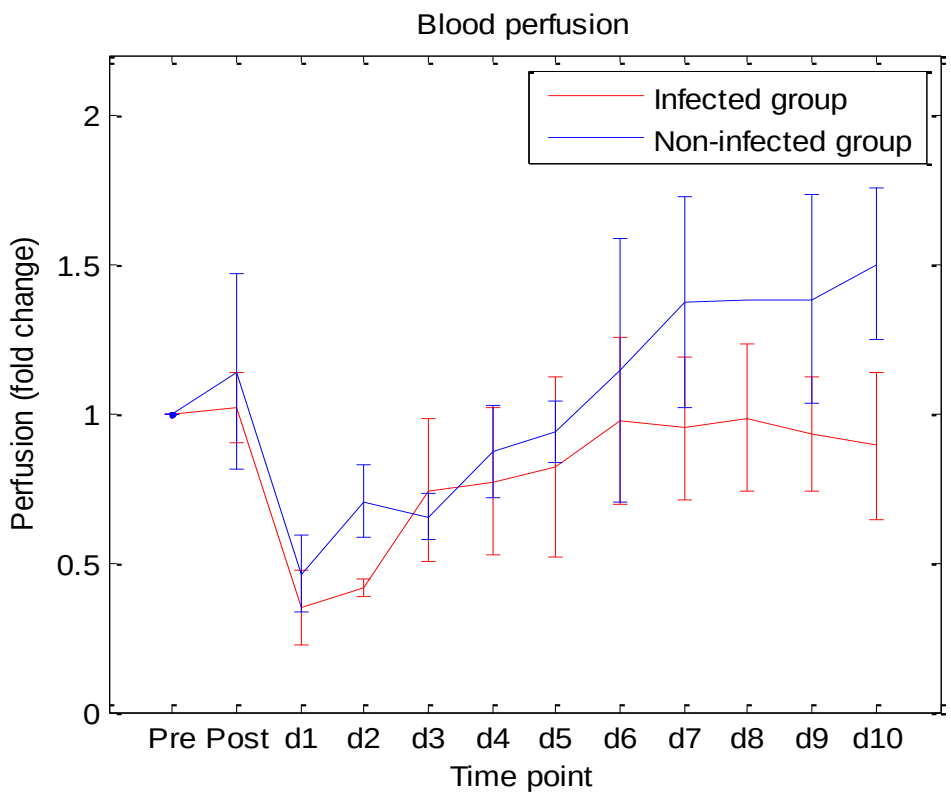
Results – SO_2

- SO_2 - mean values



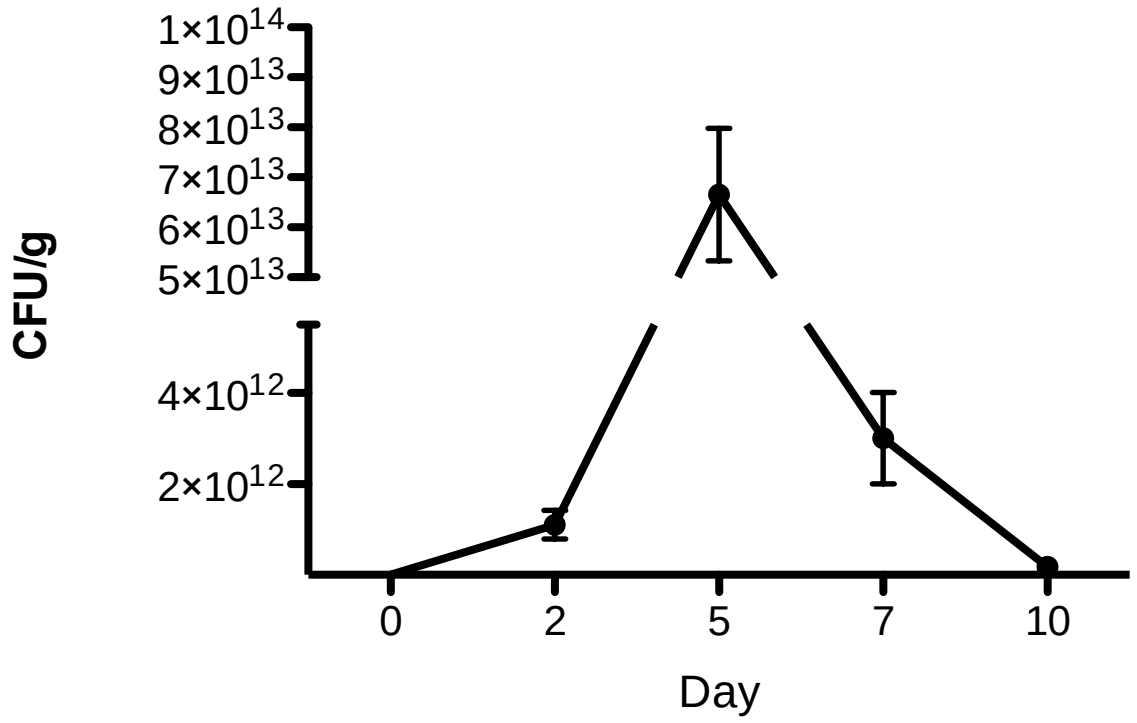
Results – Blood perfusion

- Blood perfusion was measured by a Laser Doppler Imaging machine



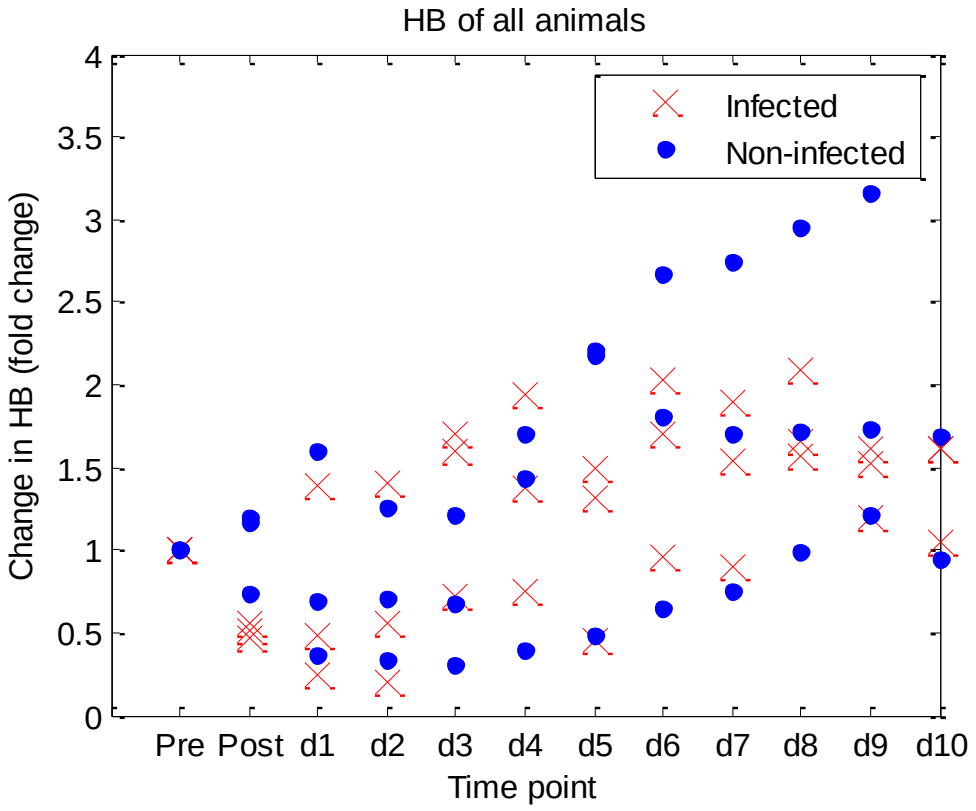
Results – Quantitative Cultures

- The highest culture was achieved at day 5



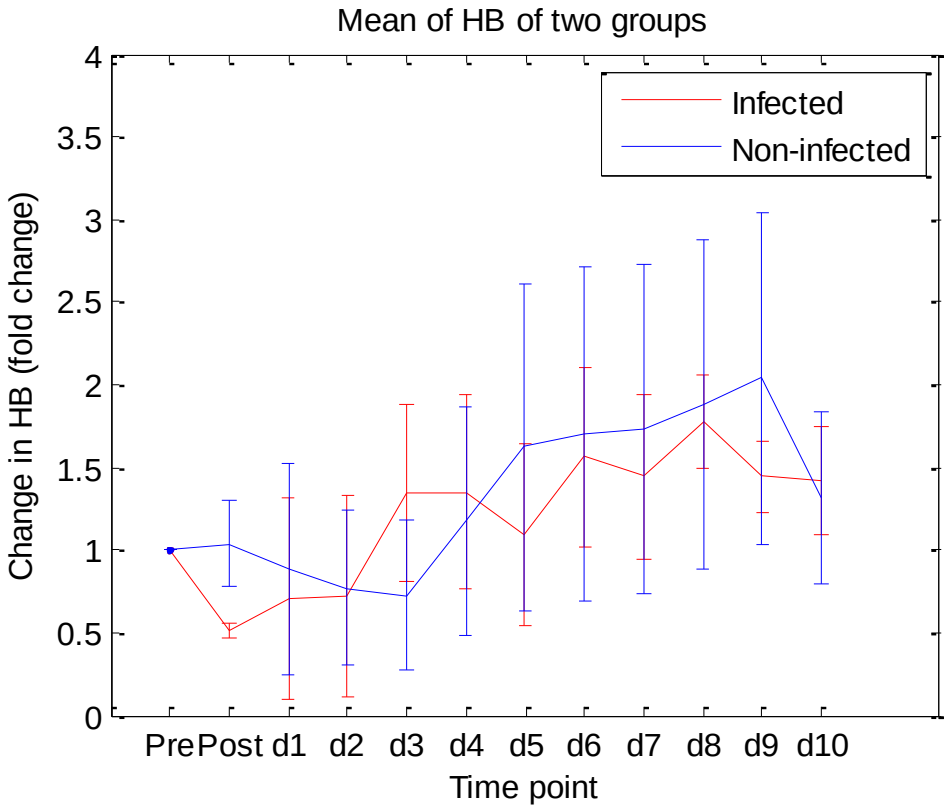
Results – Hemoglobin volume fraction

- Blood volume fraction (HB)



Results – Hemoglobin volume fraction

- Blood volume fraction (HB) - mean values



Conclusion

- A SFDI system was built and was integrated into LDI machine for infection wound study.
- Results of optical properties show differences between 2 groups (infected and control wounds) → a sign of infection
- Statistically significant differences in average (μ_a and μ_s') at 620-700 nm were observed between the two groups ($p<0.05$) by day 4 and persisted throughout the time course.
- SO_2 level of the non-infected group is higher than the infected group and comparable with blood perfusion and quantitative culture results.
- The values of Blood volume fraction of 2 groups are almost the same→ there is probably no changes in blood volume fraction in wounds before and after infections.

Discussion

- Further work
  - Observing other areas of wounds : eschar,..
  - Determining dose-response relationships

Acknowledgement

- Collaborators at the Burn Center, Washington hospital, Medstar Health Inst.
  - Marrion H. Jordan, MD, FACS
  - James C. Jeng, MD
  - Rachel K. Truhan
  - Matt. J. Mino
  - Neil A. Mauskar
  - Daniel Y. Jo
  - Pranay R. Randad
  - Nick Prindeze

THANK YOU

&

Have a great day !