Developing an Oxygen Detection Device for a Microfluidic-based Hypoxia Chamber

Matthew Zanotelli, Chelsea Bledsoe, Karl Kabarowski, Evan Lange

Client: Professor Brenda Ogle, PhD

Advisor: Professor Randolph Ashton, PhD

Biomedical Engineering Design

University of Wisconsin – Madison

October 19th, 2012

Overview

1. Problem Statement
2. Background Information
3. Current Devices
4. Product Design Specifications
5. Design Alternatives
6. Design Matrix
7. Design Selection
8. Future Work
9. Acknowledgements and References
10. Questions

Problem Statement

- Need to understand impact of hypoxic stress on cells
- Use microfluidic devices to generate hypoxic environments
- Will be used to study:
    - Oxidative stress
    - Ischemia
    - Reactive oxygen species – mediated cellular pathways
- Previous semester’s work:
    - Produced a functioning microfluidic-based hypoxia chamber
- **This semester’s focus**:
    - Develop accurate oxygen detection mechanism for the device

Background

- Heart attacks [1]
  - Kill 600,000 people each year
  - Responsible for 1 in 4 deaths
  - Cardiac cell apoptosis = cell death
- Stem cell fusion to produce new cells
  - Proposed treatment for heat attack patients
- Cell Fusion more likely under hypoxic conditions [2]
- Hypoxic conditions mimicked in microfluidic devices

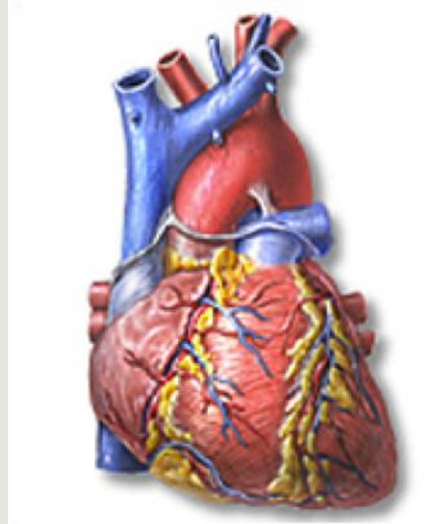

Figure 1. Image of the human heart [1].

Background

- Microfluidics
  - Micro-scale fluid mechanisms
  - Small devices with channels
  - Commonly used with cells
- Ogle Lab Device
  - One time use
  - Made of highly gas permeable poly(dimethylsiloxane) (PDMS)
- Oxygen Sensing/Detection
  - Fluorescent or Luminescent indicators
  - Light source to excite the dye
  - Brightness determines oxygen content

Figure 2. Master slide of microfluidic device developed.

Current Devices

- Commercial devices
  - None for oxygen detection in <u>microfluidic</u> devices
  - General oxygen detection devices
- Research institute devices
  - Oxygen detection methods for specific microfluidic devices
  - Designed specifically for those labs

Commercial Devices

- General oxygen detection
  - Thin-film sensors
    - Limited variety in luminescent material
    - Very high cost
  - Electrodes
    - Consume oxygen during detection
    - Poor accuracy

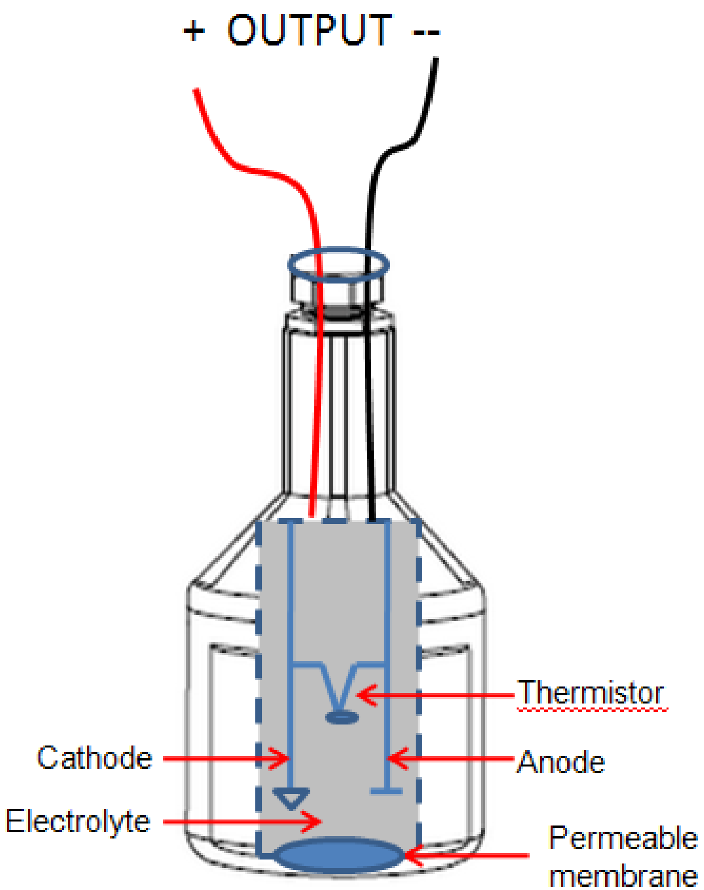

Figure 3. DO6400 Series Dissolved Oxygen Sensor with NI Wireless Sensor Networks (WSN) provided by National Instruments [3].

Research Devices

- Methods include:
  - Thin-film sensors
  - Micro/nanoparticles
  - Water soluble/macroparticles
- Various indicators utilized
- Detection methods:
  - Intensity
    - Fluorescence intensity proportional to concentration
  - Lifetime
    - Exponential decay rate of the fluorescence

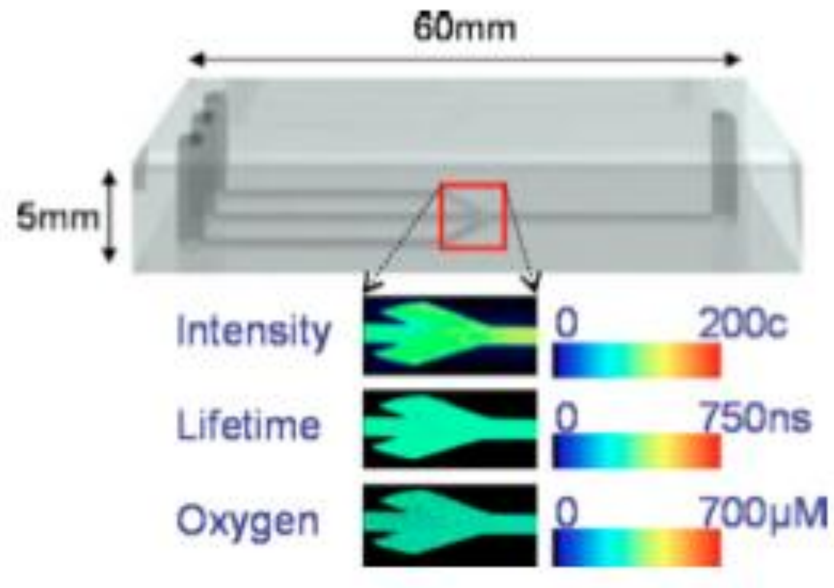

Figure 4. Illustration of fluorescence intensity and lifetime imaging in microfluidic devices using the method developed at University of Michigan [4].

Product Design Specifications

- *Performance Requirements*
  - Detect oxygen concentrations from 1% - 21% O_2
  - Ability to be used frequently with high level of repeatability
- *Accuracy and Reliability*

  Function within a range of +/- 2 to 3% oxygen concentration
- *Life in Service/Shelf Life*
  - Last through one experiment (no longer than two weeks)
- *Operation Environment*
  - Incubator environment (37°C and 5% CO_2)
  - Fluorescent exposure
- *Ergonomics*
- *Low cost*

Design Alternative 1: Thin-film Sensors

- Solution of indicator and encapsulation medium
- Fabricated by pipetting or spinning solution
- Placed directly above or below devices
- Successful with cell culture media
- Widely used already

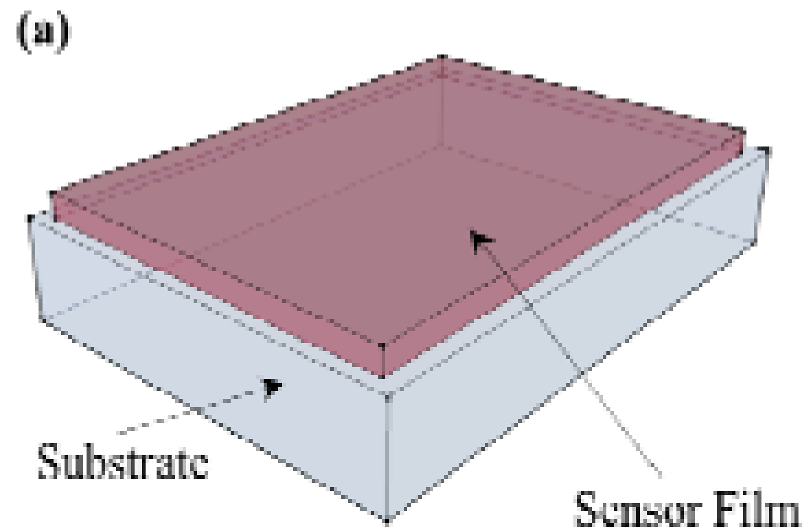

Figure 5. A single thin film sensor on a generic substrate [5].

2: Microparticles/Nanoparticles

- Encapsulated into polymer sensor
- Silica beads doped in indicator
- Added directly to thin films within channels
- High accuracy
- High cost
- Time-consuming

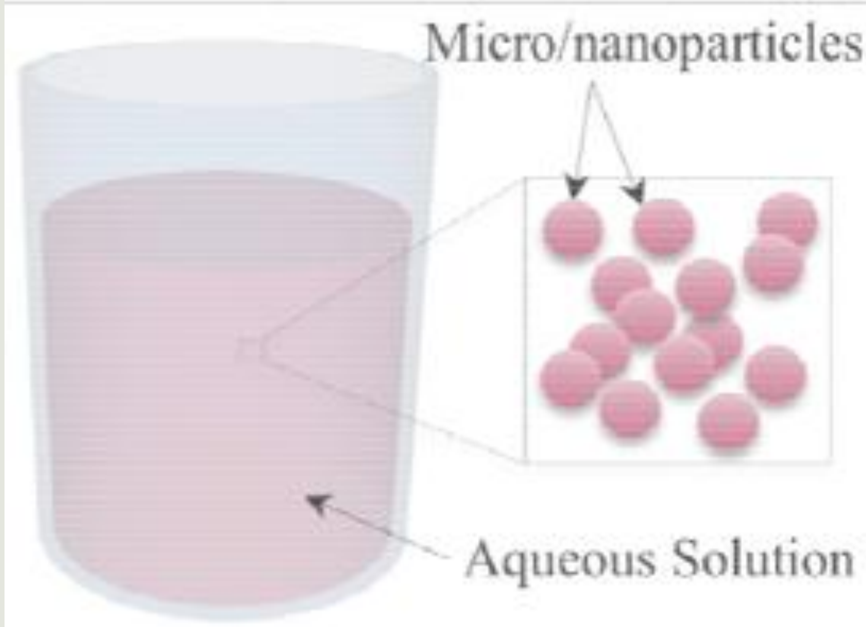

Figure 6. Diagram of micro/nanoparticle sensors suspended in aqueous media [5].

3: Water-soluble Macroparticles

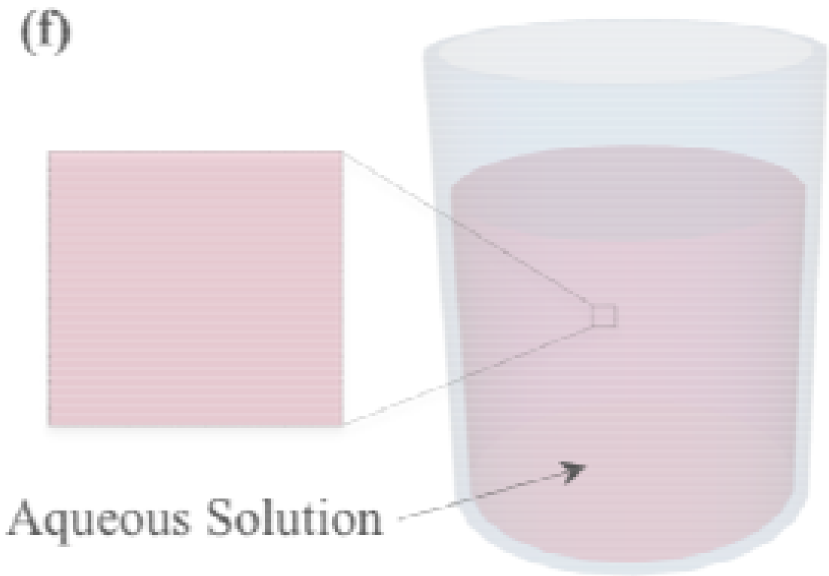

Figure 7. Diagram of water-soluble sensor compound dissolved in aqueous media [5]

- Higher cost
- Improved sensitivity
- Likely to interfere with environment
- Large potential leaching effects
- Time-consuming
- Versatile uses

Design Matrix – Sensor Format

Factors	Thin-Film	Micro/ Nanoparticles	Water-soluble Macroparticles
Accuracy (30)	4	5	2
Cost (25)	3	3	1
Ease of Use (20)	5	4	3
Ease of Assembly (15)	4	3	4
Biocompatibility (10)	5	4	2
Total Points (100)	**81**	**78**	**45**

Indicator Alternative 1: Ruthenium-based

- Very photostable
- Possible cytotoxic effects
  - After repeated excitation
- Lower sensitivity to oxygen
  - Not good for hypoxic conditions
- Used in thin films and nanoparticles

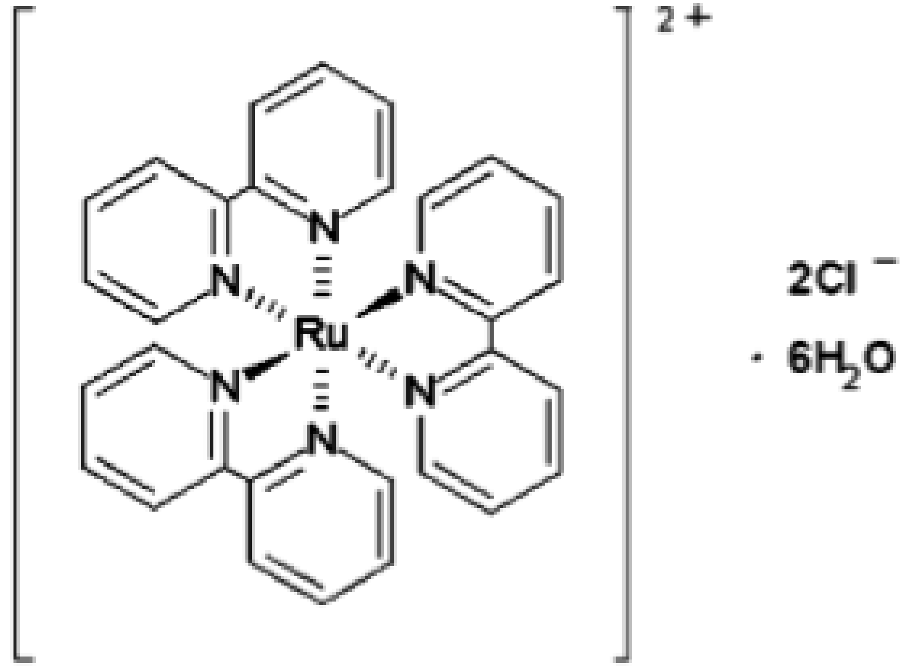

Figure 8. Tris(2,20-bipyridyl dichlororuthenium) hexahydrate, a common ruthenium compound used in optical oxygen sensors [6].

Indicator Alternative 2: Metalloporphyrin-based

- High sensitivity to oxygen
  - Applicable in low-oxygen environments
- Poor photostability
  - PtOEPK and PdOEPK have improved photostability
- No leaching effects

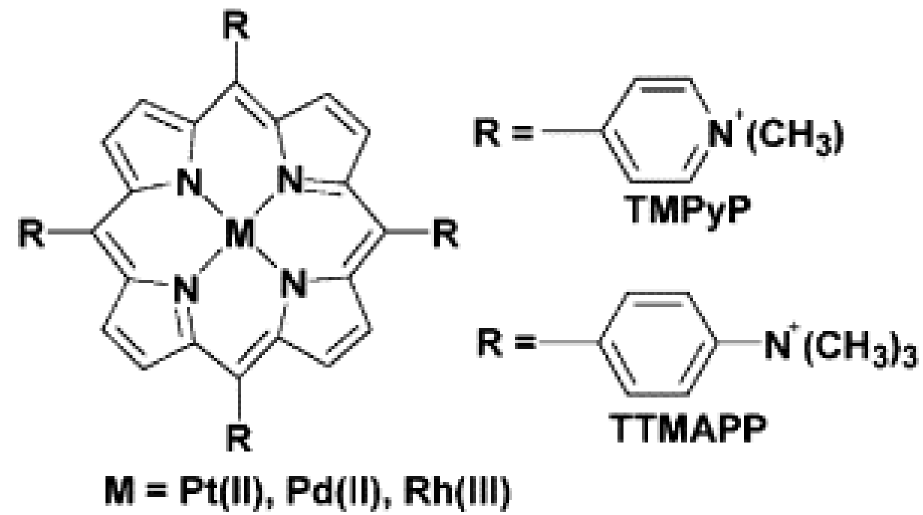

Figure 9. Structures of water-soluble cationic metalloporphyrins [7].

Design Matrix - Indicators

Factors	Ruthenium-based	Metalloporphyrin-based
Detection properties (25)	5	3
Sensitivity to oxygen (30)	2	5
Unquenched Lifetime (10)	2	4
Cost (25)	4	2
Biocompatibility (10)	3	5
Total Points (100)	**67**	**73**

Design Selection

- Metalloporphyrin-based indicator
  - PdOEPK or PtOEPK
- Used successfully in optical oxygen sensors
- Increases photostability [5]
- Phosphoresce rather than fluoresce
- Pd exhibits pro-oxidative actions and photo-oxidation [8]
  - Reduced electron density of porphyrin ring

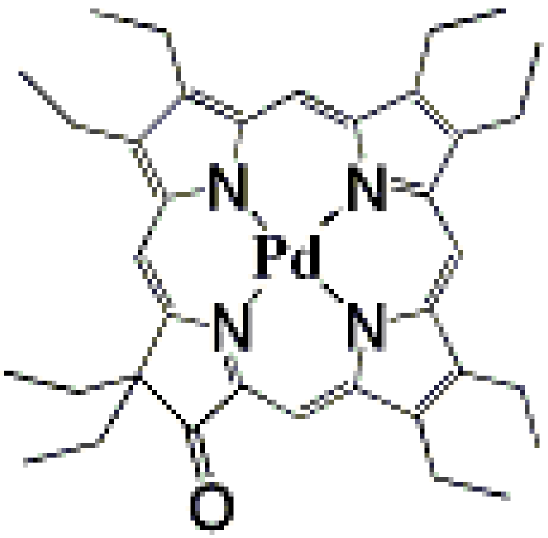

Figure 10. PdOEPK molecule [9].

Design Selection

- Thin film sensor
  - Manufacture with purchased chemicals
  - Made directly onto glass slides
- Encapsulation medium
  - Polystyrene

IMAGE KEY:

- PDMS
- PdOEPK in Polystyrene
- Glass Slide

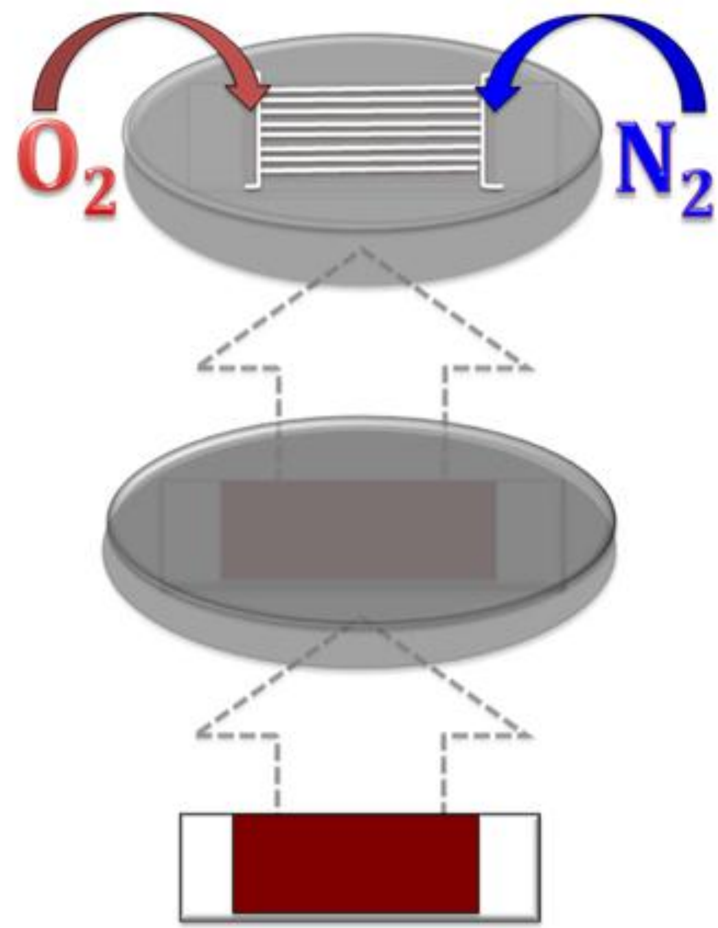

Figure 11. Thin-film oxygen sensor fabricated on a glass slide and placed beneath the microfluidic device for oxygen detection

Future Work

- Simplify oxygen detection system
- Disregard cell media
- Test oxygen sensor apart from the microfluidic device
- Create standardized curve of oxygen concentration

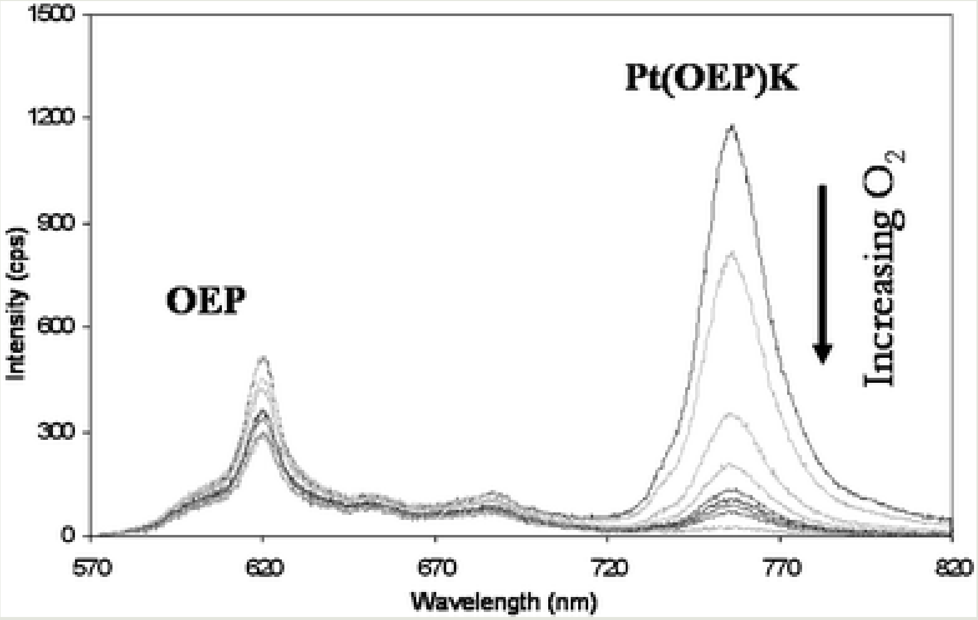

Figure 12. Example of a standardized curve for fiber optic oxygen sensing in various dissolved oxygen concentrations [10].

Acknowledgements

- Dr. Brenda Ogle
- Brian Freeman – Ogle Lab, graduate student
- Drew Birrenkott – Ogle Lab undergraduate student
- Dr. Randolph Ashton

THE UNIVERSITY of WISCONSIN MADISON

Questions

References

- [1] Centers for Disease Control and Prevention (CDC). 2012. “America’s Heart Disease Burden.” http://www.cdc.gov/heartdisease/facts.htm

- [2] Hu, Xinyang; Fraser, Jamie; Lu, Zhongyang; Ogle, Molly; Wang, Jian-An; Wei, Ling; Yu, Shan Ping. 2008. “Transplantation of Hypoxia-preconditioned Mesenchymal Stem Cells Improved Infarcted Heart Function via Enchanced Survival of Implanted Cells.” *The Journal of Thoracic and Cardiovascular Surgery*. Volume 135, Issue 4, p799-808.

- [3] http://www.ni.com/white-paper/9953/en

- [4] Sud D, Mehta G, Mehta K, Linderman J, Takayama S, Mycek M; Optical imaging in microfluidic bioreactors enables oxygen monitoring for continuous cell culture. J. Biomed. Opt. 0001;11(5):050504-050504-3.

- [5] Grist S.M., Chrostowski L., Cheung K.C. Optical Oxygen Sensors for Applications in Microfluidic Cell Culture. *Sensors*. 2010; 10(10):9286-9316.

- [6] Chang-Yen, D.A., Lvov, Y.M., McShane, M.J., Gale, B.K., "Electrostatic Self-Assembly of a Ruthenium-Based Oxygen-Sensitive Dye using Polyion-Dye Interpolyelectrolyte Formation," Sensors and Actuators B: Chemical, vol. 87, pp. 336-345, 2002. DOI: 10.1016/S0925-4005(02)00267-8

- [7] Victor V. Vasil’ev, Sergey M. BorisovOptical oxygensensorsbased on phosphorescent water-soluble platinum metals porphyrins immobilized in perfluorinated ion-exchange membraneSensors and Actuators B: Chemical, Volume 82, Issues 2–3, 28 February 2002, Pages 272–276http://dx.doi.org/10.1016/S0925-4005(01)01063-2

- [8] Amao, Y. and Okura, I. (2010), ChemInform Abstract: Optical Oxygen Sensor Devices Using Metalloporphyrins. ChemInform, 41: no. doi: 10.1002/chin.201021270

- [9] http://www.fluorophores.tugraz.at/substance/633

- [10] Kennedy, Robert; Kopelman, Raoul; Park, Edwin; Reid, Kendra; Tang, Wei. 2005. “Fiber Optic Sensors for the Detection of the Inter- and Intra-cellular Dissolved Oxygen.” *Journal of Materials Chemistry*. Volume 15. p2913-291