CLEO/EUROPE EQEC 2021 Posters Session

Evaluating Confocal Microscopy as a Tool to Diagnose Red Blood Cell Diseases

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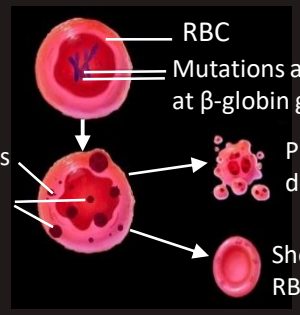
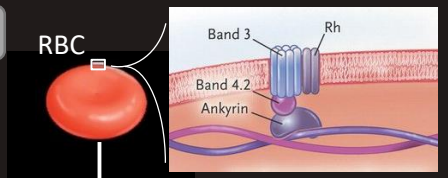
INTRODUCTION

Traditional methods for diagnosis are insufficient for some erythropathologies, such as **α-Thalasemia** (α-Thal) and **Hereditary Spherocytosis** (H.S.)

α-Thal



H.S.



Mutations and deletions genes coding for membrane proteins

Adapted from NEJM.org

GOAL: Analyse the spectral and morphological characteristics of healthy and diseased **red blood cells** (RBCs) by means of **Confocal Laser Scanning Microscopy** (CLSM).

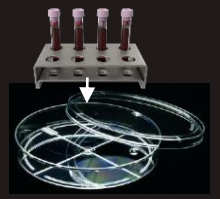
METHODS

Fresh blood samples +EDTA were loaded to adherent Petri dishes to perform **CLSM**. H.S. samples were also added Hoechst and CellMask.

α-Thal

Subjects: 12♂ 5♀, ages 1-17 years

- Control
- α-Thal severe — HbA2 homozygous mutation
- α-Thal minor — SEA heterozygous deletion
- Iron deficiencies

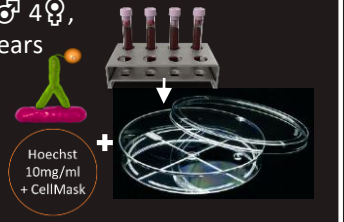


- Excitation: 405 nm
- Acquisition seq.: **xyλ**
- Range: 425-780 nm
- Objective: 63x (NA 1.4, oil)
- Hybrid detection

H.S.

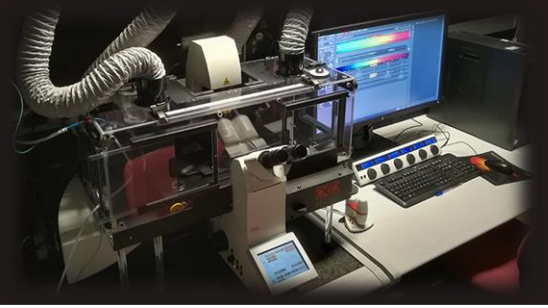
Subjects: 4♂ 4♀, ages 1-10 years

- Control
- H.S. moderate
- H.S. moderate-severe
- H.S. severe



- Excitation: 405, 660 nm
- Acquisition seq.: $\mathbf{xy\lambda_{1,2}z}$
- λ_1 , λ_2 = 460-480, 680-700 nm
- Objective: 63x (NA 1.4, oil)
- Hybrid detection

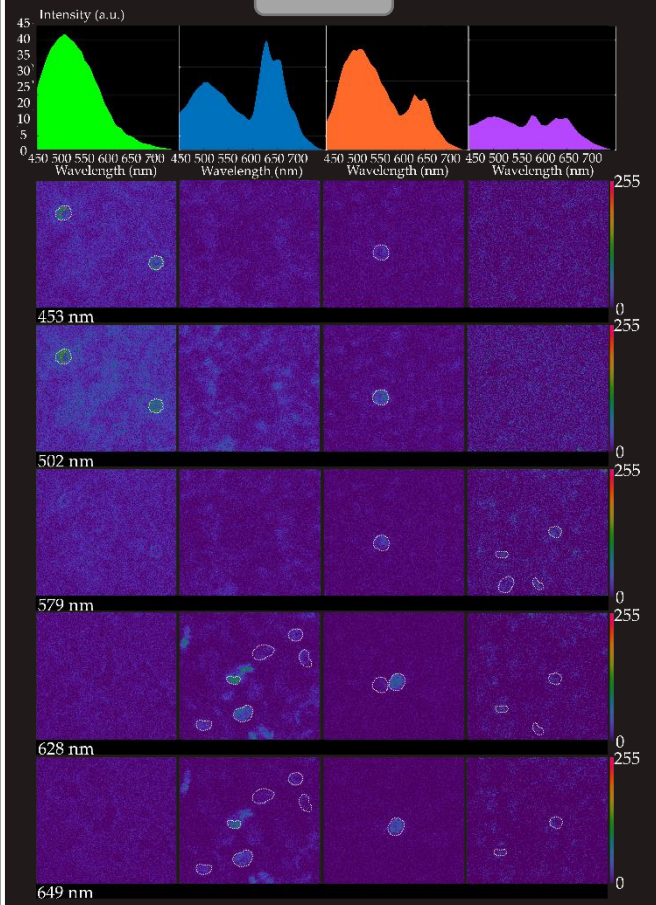
Leica TCS SP8 STED 3x



RESULTS

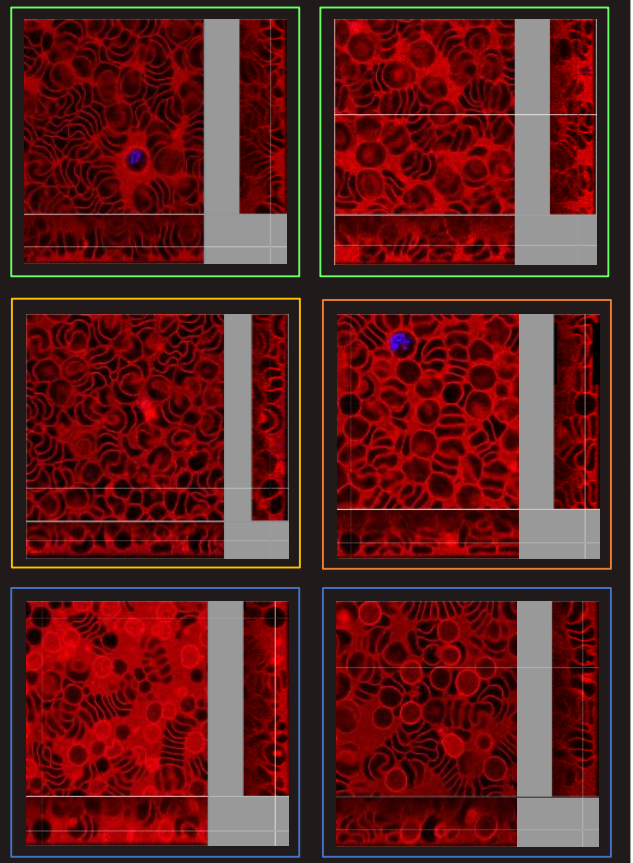
Autofluorescence: α-Thal severe α-Thal minor and iron deficiencies present clear peaks λ_{em} = 628 nm; λ_{em} = 649 nm. Controls do not present them.

α-Thal



Morphology: H.S. moderate and H.S. moderate-severe present some spherocytes. H.S. severe presents many spherocytes. Controls do not present them.

H.S.



CONCLUSIONS: CLSM showed to be a powerful diagnostic tool that could reveal spectral and morphological traits of RBCs that might go unnoticed by other techniques.