

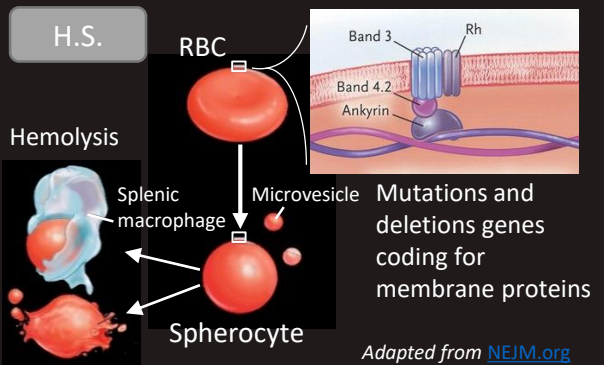
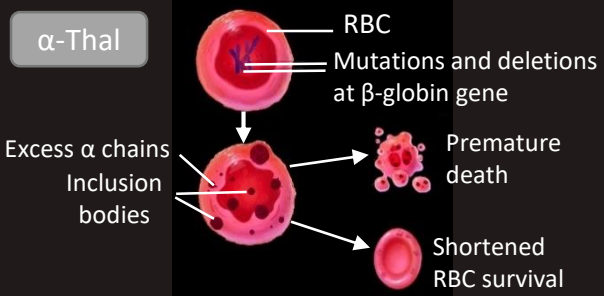
# Evaluating Confocal Microscopy as a Tool to Diagnose Red Blood Cell Diseases

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### INTRODUCTION

Traditional methods for diagnosis are insufficient for some erythropathologies, such as  **$\alpha$ -Thalassemia** ( $\alpha$ -Thal) and **Hereditary Spherocytosis** (H.S.)



**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.

**$\alpha$ -Thal** Subjects: 12 ♂, 5 ♀, ages 1-17 years

**Control**

**$\alpha$ -Thal severe**  
HbA2 homozygous mutation

**$\alpha$ -Thal minor**  
SEA heterozygous deletion

**Iron deficiencies**

Excitation: 405 nm  
Acquisition seq.: xy $\lambda$   
Range: 425-780 nm  
Objective: 63x (NA 1.4, oil)  
Hybrid detection

**H.S.** Subjects: 4 ♂, 4 ♀, ages 1-10 years

**Control**

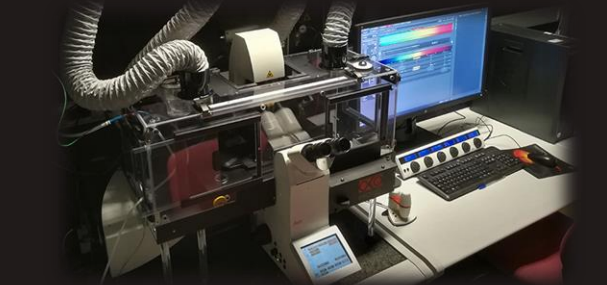
**H.S. moderate**

**H.S. moderate-severe**

**H.S. severe**

Hoechst 10mg/ml + CellMask

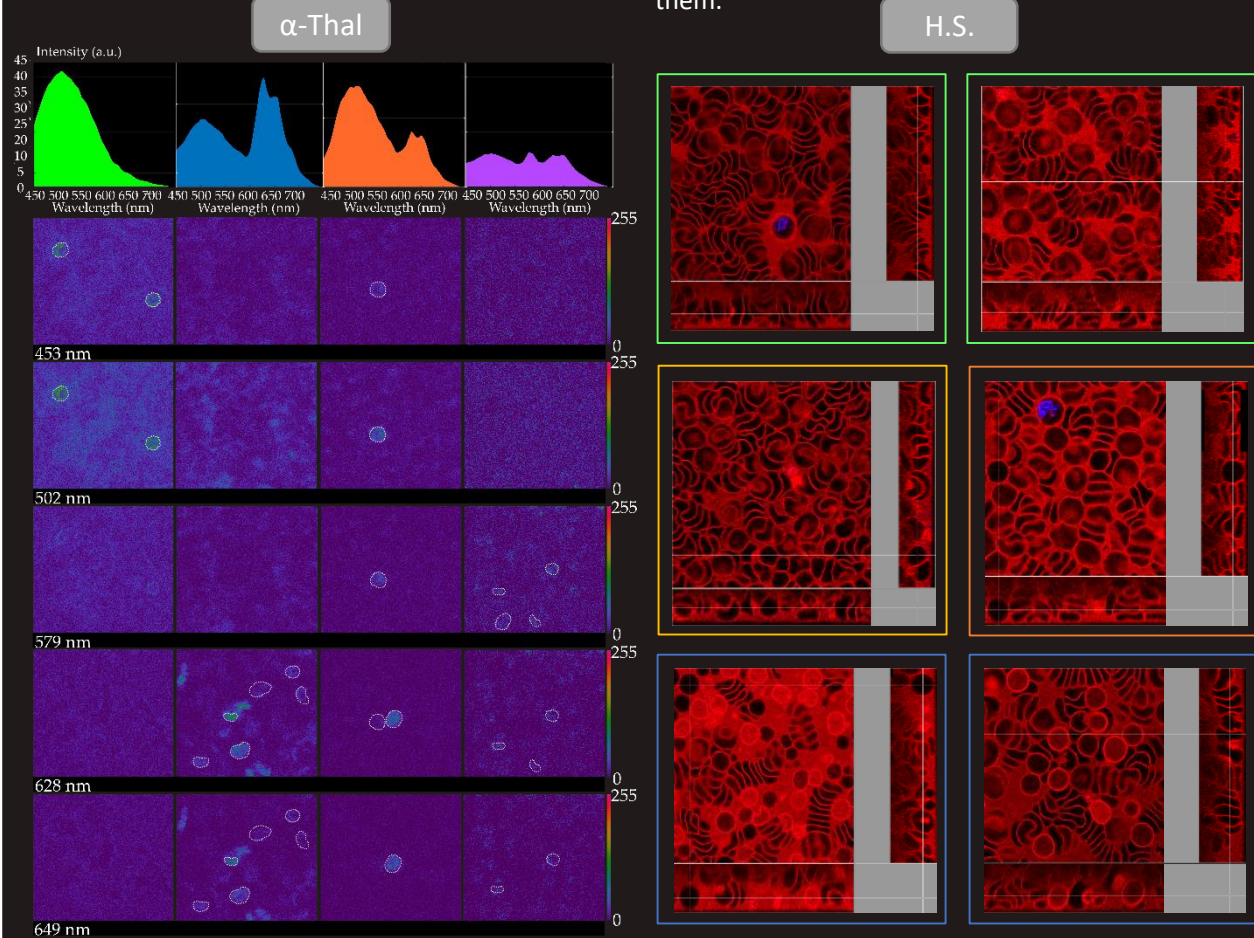
Excitation: 405, 660 nm  
Acquisition seq.: xy $\lambda_{1,2}$ z  
 $\lambda_1, \lambda_2 = 460-480, 680-700$  nm  
Objective: 63x (NA 1.4, oil)  
Hybrid detection



### RESULTS

**Autofluorescence:**  $\alpha$ -Thal severe  $\alpha$ -Thal minor and iron deficiencies present clear peaks  $\lambda_{em} = 628$  nm;  $\lambda_{em} = 649$  nm. **Controls** do not present them.

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



**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.