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

Laura Rey-Barroso[1], Mónica Roldán[2,5], Francisco J. Burgos-Fernández[1], Susanna Gassiot[3,5], Anna Ruiz Llobet[4], Ignacio Isola[3,5] and Meritxell Vilaseca[1]

1. Centre for Sensors, Instruments and Systems Development, Technical University of Catalonia, Terrassa 08222, Spain

2. Unit of Confocal Microscopy, Service of Pathological Anatomy, Hospital Sant Joan de Déu, Esplugues de Llobregat 08950, Spain

3. Laboratory of Hematology, Service of Laboratory Diagnosis, Hospital Sant Joan de Déu, Esplugues de Llobregat 08950, Spain

4. Service of Pediatric Hematology, Hospital Sant Joan de Déu, Esplugues de Llobregat 08950, Spain

5. Institute of Pediatric Research, Hospital Sant Joan de Déu, Esplugues de Llobregat 08950, Spain

Hemoglobinopathies represent the most common single-gene defects in the world and pose a major public health problem. These defects can affect the synthesis of hemoglobin, the molecule in charge of oxygen transportation, quantitatively (thalassemia) or qualitatively (sickle cell disease). These affectations will terminally lead to the premature destruction of red blood cells (RBCs), nevertheless they have different etiologies and they need to be treated differently. Diagnosing a patient can sometimes be difficult due to the coexistence of several of such diseases and/or blood transfusions, and requires expensive and complex molecular tests. But alternatively, it is well-known that the spectral information of RBC autofluorescence and immunostaining can reveal important differences among these RBCs diseases [1–3]. The isolated assessment of cell shape has been proven to be insufficient. In these context, we conducted a first pilot study to analyze the spectral and morphological characteristics of healthy and diseased RBCs, under a spectral confocal microscope Leica TCS SP8 (Leica Microsystems GmbH, Mannheim, Germany). Specifically, fresh blood samples from 17 pediatric patients ages 1 to 17, some suffering from α-thalassemia and iron deficiency were evaluated. Autofluorescence was captured in the 425-790 nm range, using hybrid detection that provides with a higher sensitivity, a 63x (NA 1.4, oil) plan-apochromatic objective, and a diode laser line with emission at 405 nm for excitation. The autofluorescence signals captured showed spectral differences that can be used to discriminate among diseases and severity, especially at the 628 nm band as showed in Figure 1.

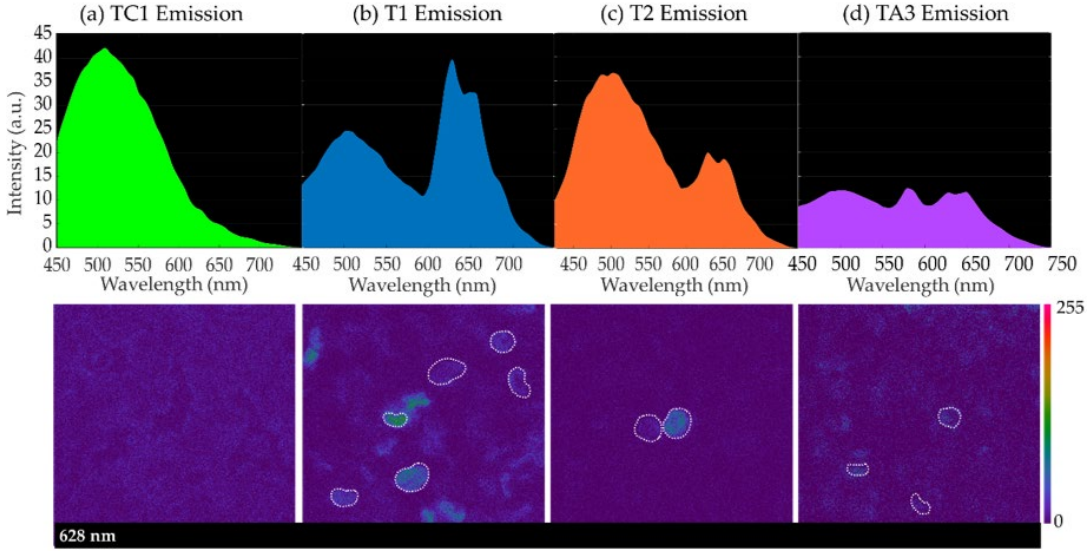

Fig. 1 Emission average curves (top) of a control (TC1), a severely diseased thalassemia patient (T1), a mildly diseased patient (T2) and a patient suffering from iron deficiency (TA3); and autofluorescence images at 628 nm (bottom).

Other RBC diseases are related to red cell membrane hereditary defects, that make the cell to present a smaller and rounder shape: these are known as hereditary spherocytosis. RBCs elasticity and deformability decreases and the mean corpuscular concentration of hemoglobin (CCMH) increases. Since they could also reveal different spectral traits and membrane structural differences, a second pilot study is being conducted by means of confocal microscopy and using specific antibodies to label cytoskeleton and cell membrane proteins.

References

[1] K. Khairy, J. Foo and J. Howard, "Shapes of Red Blood Cells: Comparison of 3D Confocal Images with the Bilayer-Couple Model," Cell. Mol. Bioeng. **1**, 173-181 (2008).

[2] L. Golan and D. Yelin, "Flow Cytometry using Spectrally Encoded Confocal Microscopy," Opt. Lett. **35**, 2218 (2010).

[3] A. Zeidan and D. Yelin, "Reflectance Confocal Microscopy of Red Blood Cells: Simulation and Experiment," Biomed. Opt. Express. **6**, 4335 (2015).