# A combined Expansion Microscopy and CIDS approach to Chromatin DNA study

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*We propose a method to improve the contrast and signal to noise ratio in our Circular Intensity Differential Scattering (CIDS) microscope by coupling it with Expansion Microscopy (ExM) in what we name ExCIDS.*
**Keywords**: Polarimetry, Optical Scanning Microscopy

## 1. Introduction

Circular Intensity Differential Scattering (CIDS) is a label-free polarization-based technique consisting in the measurement of the scattering component of Circular Dichroism (CD) [1] [2]. It corresponds to the $m_{03}$ element of the Mueller Matrix and it has been proven to be sensitive to structures of size down to $\lambda/20$ present, for instance, in chiral biopolymers such as Chromatin-DNA [1], explaining its potentiality in label-free microscopy imaging [3]. We have built a CIDS microscope setup that couples the CIDS modality with confocal fluorescence, demonstrating its effectiveness on fixed isolated nuclei of HEK cells [4].
In order to overcome artificially the diffraction limit and increasing the signal to noise ratio of this linear optics microscopy technique, we have coupled the acquisition of the CIDS signal with Expansion Microscopy (ExM) in what we have named ExCIDS. It consists in improving the resolution in biological samples by encasing them in a gel matrix and producing an isometric expansion of the features [5]. The digestion of biological structures caused by the expansion process maintains the structure of chromatin-DNA, increasing the S/N ratio for our technique while increasing the resolution. In Fig. 1 it can be seen that both the S/N ratio of the CIDS signal, as well as the correspondence of the CIDS signal to the fluorescence signal improve after expansion

## 2. Methods

We have created a CIDS microscope by implementing a Polarization States Generation (PSG) and Polarization States Analyzer (PSA) to a commercial confocal microscope. The light source is a Ti:Sa laser at 800nm. The PSG is composed of a linear polarizer and a Photoelastic Modulator (PEM) combined with a lock-in detection at the 50 kHz reference frequency, producing circular left and right polarization states in 20µs. The PSA is made of a Glan-Taylor polarizer that splits the light into two orthogonal polarization states, detected in two channels by Photodiodes. Fluorescence is simultaneously acquired in the backscattered configuration and it allows to pick up the fingerprint of the chromatin-DNA, by a DAPI-like labelling of the isolated cell, and to compare it to the polarization-based image.

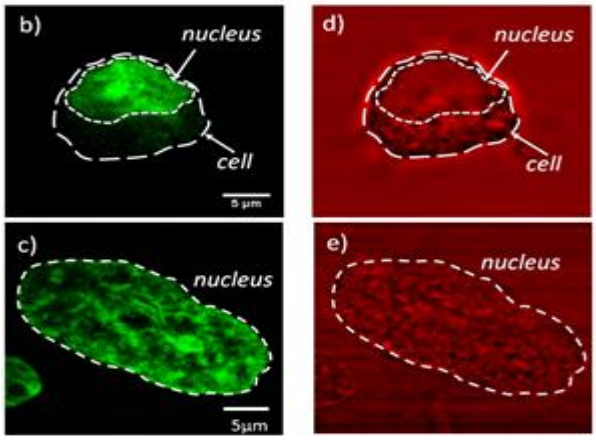

Fig. 1 Fixed Hek cells a), b) before and c), d) after expansion, imaged with a), c) Hoechst fluorescent marker and b), d) CIDS.

## 3. Conclusions

We have demonstrated the improvement in resolution and signal to noise ratio in ExCIDS over regular CIDS microscopy for isolated HEK cell nuclei.

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