PhD Program in Physics

Student: Mohammedmehdi Roushenas
Cycle: XXXVIII
Supervisors: Prof Alberto Diaspro, Dr. Marco Salerno

Provisional Research Project title:
High resolution microscopy imaging and characterization of chromatin patterns in human cells nuclei

Annual Report of Year 2

1. Research activity

Due to some delays in implementation of the Minflux localization microscopy laboratory, I have temporarily shifted to the Stellaris8 confocal microscope by Leica. Until now, the unavailability of samples of cell nuclei labelled with DNA fluorophores to be excited in the visible range available on that setup (440-800 nm wavelength) has made it impossible to obtain fluorescence microscopy images of nuclear DNA – and thus chromatin – in STED operation mode, which would allow for super-resolution below the diffraction limit. Therefore, I limited my activity to confocal mode imaging. Actually, I have used the STED laser of the system (775 nm wavelength) to excite fluorescence from nuclei labelled with standard fluorophores (Hoechst and DAPI) in 2-photon excitation (2PE) mode. By this approach, so far I have characterized 20 nuclei each for two different types of human cancer cells, namely HeLa and HepG2. From the obtained images, three useful parameters have been identified for extraction by processing and analysis, namely: chromatin condensation parameter [1] (here called shortly CP); radial distance of maximum condensation (shortly: condensation radius CR); and Fractal dimension (FD). The former two should describe chromatin condensation amount and its location with respect to the nucleus center, this latter information being probably associated with the balance between euchromatin - assumed to stay closer to the nuclear lamina - and heterochromatin - assumed to stay closer to the inner regions of nuclei. The third parameter (FD), which is associated with intricacy of the patterns, is widely used in this context [2-5] and could be correlated with both compactness (CP) and type of chromatin (CR).

One example of nucleus image, with respective treatment to obtain the three quantities of interest, is presented in Fig.1.

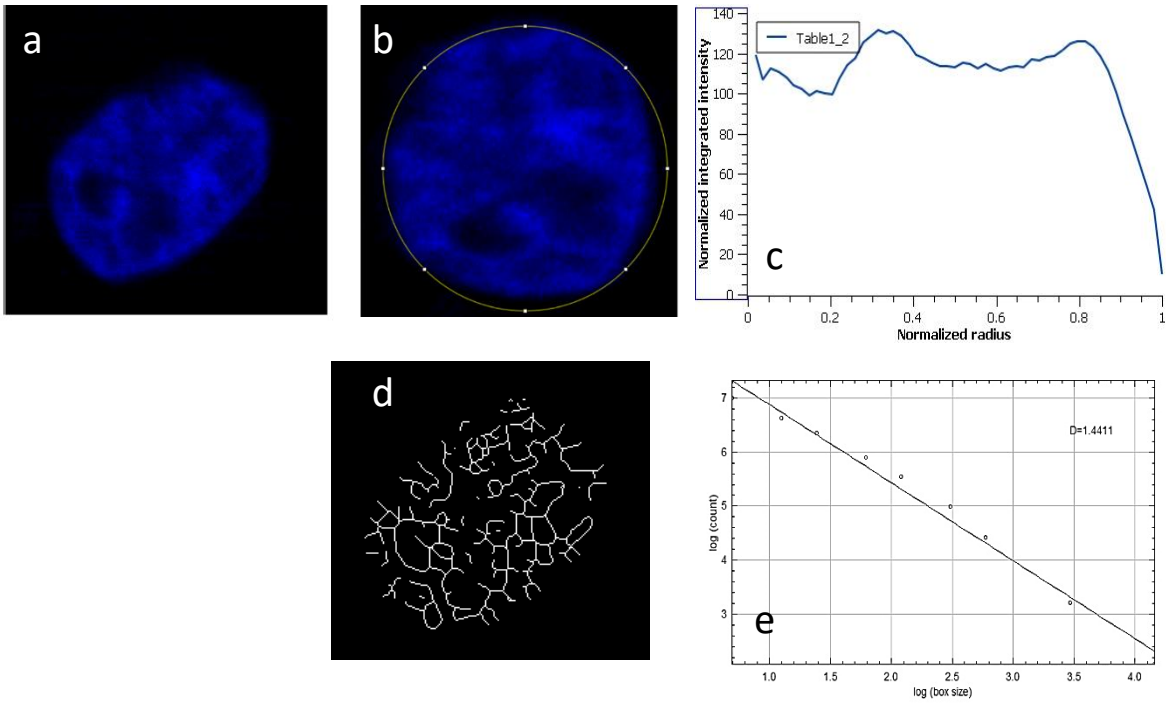

Fig.1: Example of a) confocal fluorescence microscope image of cell nucleus, with b) treatment made to extract the c) average (over all angles 0-360°, step 1°) normalized radial profile, from which CR is obtained; and d) skeletonized image with outer edge removed, from which CP is obtainbed; d) box-counting Log-Log plot of occupied boxes vs box size, from which FD is obtained.

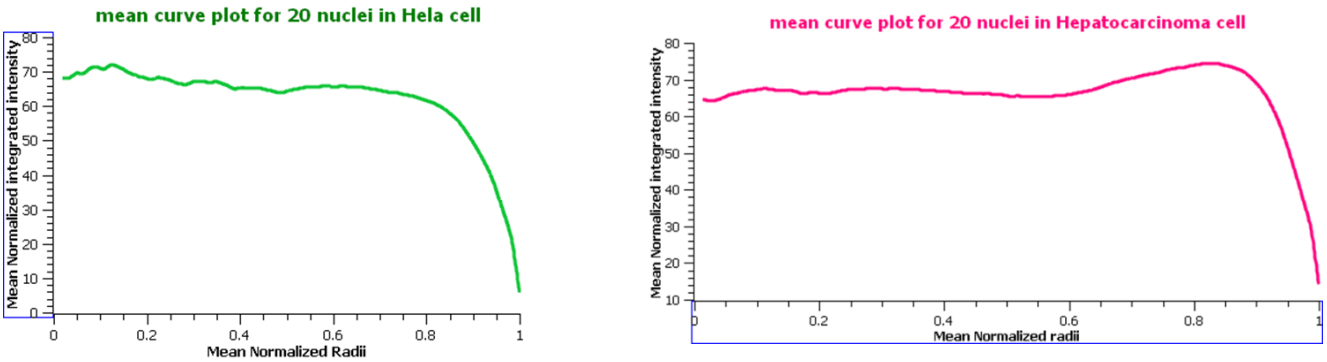

Fig.2: 2 mean curve plots for 20 nuclei in Hepatocarcinoma cell and also for 20 nuclei in in Hela cell

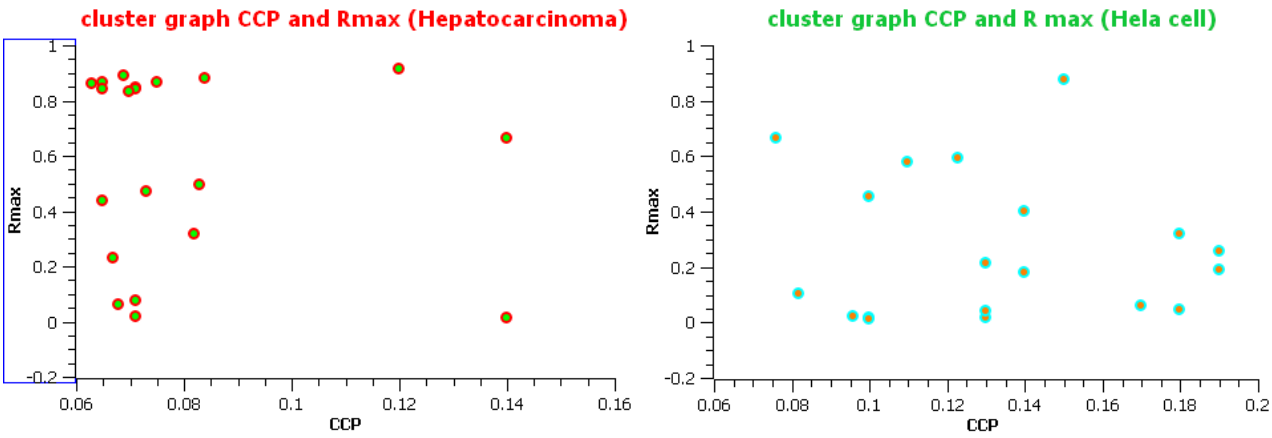

Fig .3: 2 cluster graphs of CCP and Rmax for 20 nuclei in Hepatocarcinoma cell and for 20 nuclei in Hela cell

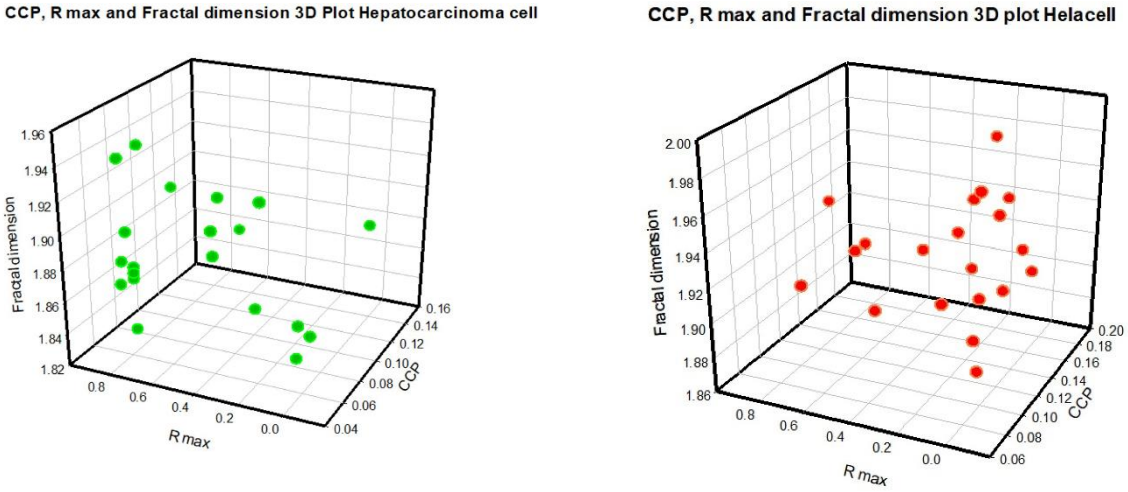

Fig.4: Data-points obtained in the 3D space of the identified parameters for the 20 nuclei of each type of cell investigated, namely a) HepG2 and b) HeLa.

If no difference is found, the radial plot "tool" is still open for identification of several significant parameters other than the main peak position, e.g.: intensity at center, intensity close to edge (at e.g. normalized radius 0.85), position of minimum, position of secondary maximum or minimum, etc.. Future availability of NucSpot Live 650 fluorophore, along with support by a new cell-culture biologist technician (the previous one has left), will make it possible to repeat the experiment in STED mode, gaining almost tenfold improvement in resolution and hopefully better insight into the target patterns.

2. Courses

taken during this year:

- Bioimaging: biology seen through the eyes of chemistry: Fabio De Moliner (Andrea Basso) (no exam – only certificated attendance - requested, 1 credit)

planned to be taken during the next year

- "Basics of applied statistics and probability", Prof. Simone Barani, DiMa.
- "Atomic force spectroscopy", Prof. Annalisa Relini, DiFi.

3. Exams of former course

- "Nanoscopy" (Prof. Diaspro), passed on 15/02/24 (2 credits)
- Presentation after the 2023 Erice School, passed on 20/03/24 (1 credit)
- COMULISglobe Training School Correlative Multimodal Imaging March 21st – 22nd, 2024 (1 credit)

4. Conferences, workshops, schools

- Focus on Microscopy 2024, 24-27 March, Genova, Itay.
- COMULISglobe Training School Correlative Multimodal Imaging March 21st – 22nd, 2024
- XXVII Congresso Nazionale SIBPA 2024, June 16-20, Genova, Italy.

5.Publications

• Minflux nanoscopy: a "brilliant" technique promising major breakthrough, Salerno et al., submitted to Microscopy Research and Technique, under review

6. References

1. Irianto et al. 2014, DOI: 10.1016/j.medengphy.2013.09.006
2. Mirny 2011, DOI: 10.1007/s10577-010-9177-0
3. Almassalha et al. 2017, DOI: 10.1038/srep41061
4. Yi et al. 2015, DOI: 10.1016/j.bpj.2015.10.014
5. Metze et al. 2019, DOI: 10.1080/14737159.2019.1597707

certificates

ATTESTATO DI PARTECIPAZIONE

SIBPA 2024
XXVII Congresso Nazionale
GENOVA 16-20 GIUGNO

Si attesta che

Mohammadmehdi Roushenas

ha partecipato al

"XXVII Congresso Nazionale SIBPA 2024"

tenutosi a Genova dal 16 al 20 Giugno 2024

La Segreteria Organizzativa

«ETTORE MAJORANA» FOUNDATION AND CENTRE FOR SCIENTIFIC CULTURE

Erice, 22 OTT. 2023

This is to certify that Mohammadmehdi Roushenas participated at the 48th Course of International School of Biophysics «Antonio Borsellino» on *"Memos for biophysics into the future: Lightness, quickness, exactitude, visibility, multiplicity, and consistency"* directed by Professors M. Dalla Serra, A. Diaspro and C. Viappiani, held in Erice from 16 to 22 October 2023.

Professor Alberto Diaspro
Director of the Course

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Correlated Multimodal Imaging in Life Sciences
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Chan Zuckerberg Initiative

COMULISglobe Training School
Correlative Multimodal Imaging

Certificate of Attendance

We hereby confirm that

Mohammadmehdi Roushenas

attended the COMULISglobe Training School in Genoa from March 21st – 22nd, 2024.

Lize Engelbrecht, COMULISglobe Training and Conference Management

Prof. Dr. Andreas Walter, Chair COMULISglobe

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