Signatures of irradiated cells from hyperspectral images

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Study plan

- Cultured cells procedure
    - SW1353 chondrosarcoma cell line
    - Cells exposed to ionizing radiation (x-rays, protons)
- Experimental images acquisition
    - Enhanced dark field microscopy
    - Hyperspectral images
- Images processing
    - Segmented nucleus area for cells irradiated with protons and nonirradiated
    - Hyperspectral decomposition
    - Code to compute parameters
- Single-pixel and global analysis
    - Specific spectral profiles at single-pixel level in an image
    - Geometric parameters
    - Statistical parameters on hyperspectral intervals

Motivation

- Nuclear medicine uses ionizing radiation for diagnosis, as well as for therapy.
- Frequently, radiotherapy is the first line of treatment for over 50% of cancer patients.
- Their advantages: local treatments aimed a specific area of the body and it can preserve the organ's function.
- Ionizing radiation can be used to kill cancer cells and shrink tumors because they damages the genes of cancer cells (Genes control how cells grow and divide). In these cases, cells can't grow and divide any more and after time they die.
- Different kind of investigations are used to highlight structural and morphological changes induced by ionizing radiation on the cell characteristics.
- The mechanism of changes inside cells under ionizing radiation is still unexplained.
- The main purpose of our investigation is to deliver a new tool to analyze cells nucleus and to highlight the differences between irradiated and nonirradiated samples.
- Enhanced darkfield microscopy (EDM) deliver information at nanometric scale about sample details using scattered radiation.
- Hyperspectral microscopy with resolution at pixel-level
- Pixel-level analysis – spectral profiles.
- Global analysis - geometric and statistical parameters
- Statistic information were computed on each spectral band grouped then on intervals.

Ionizing radiation

- We address the cytotoxic effects induced by **low-linear energy transfer (LET) (photons and protons)** and **high LET (protons)** irradiation of radioresistant SW1353 chondrosarcoma cells.
- The first change induced by ionizing radiation at single cell level is the formation of reactive oxygen species which conducts to oxidative stress, the alteration of the cell cycle, DNA damage, which eventually can lead to the tumor cells death.

- The X-ray irradiation was performed using **an XSTRAHL XRC 160 machine**, while protons irradiation was performed using **a TR-19 cyclotron**.
- The cells were irradiated at a **dose of maximum 4 Gy.**

Enhanced dark field microscopy

EDFM - new technique based on optical microscopy able to deliver information at nanometric scale about sample details using scattered radiation.

1. CytoViva High Resolution Adapter
2. CytoViva Dual Mode Fluorescence (DMF) Module
3. VNIR Hyperspectral Imager
4. Optical Microscope
5. Motorized Stage
6. 150W Halogen Light Source
7. Optical Camera
8. Dual Port
9. Computer

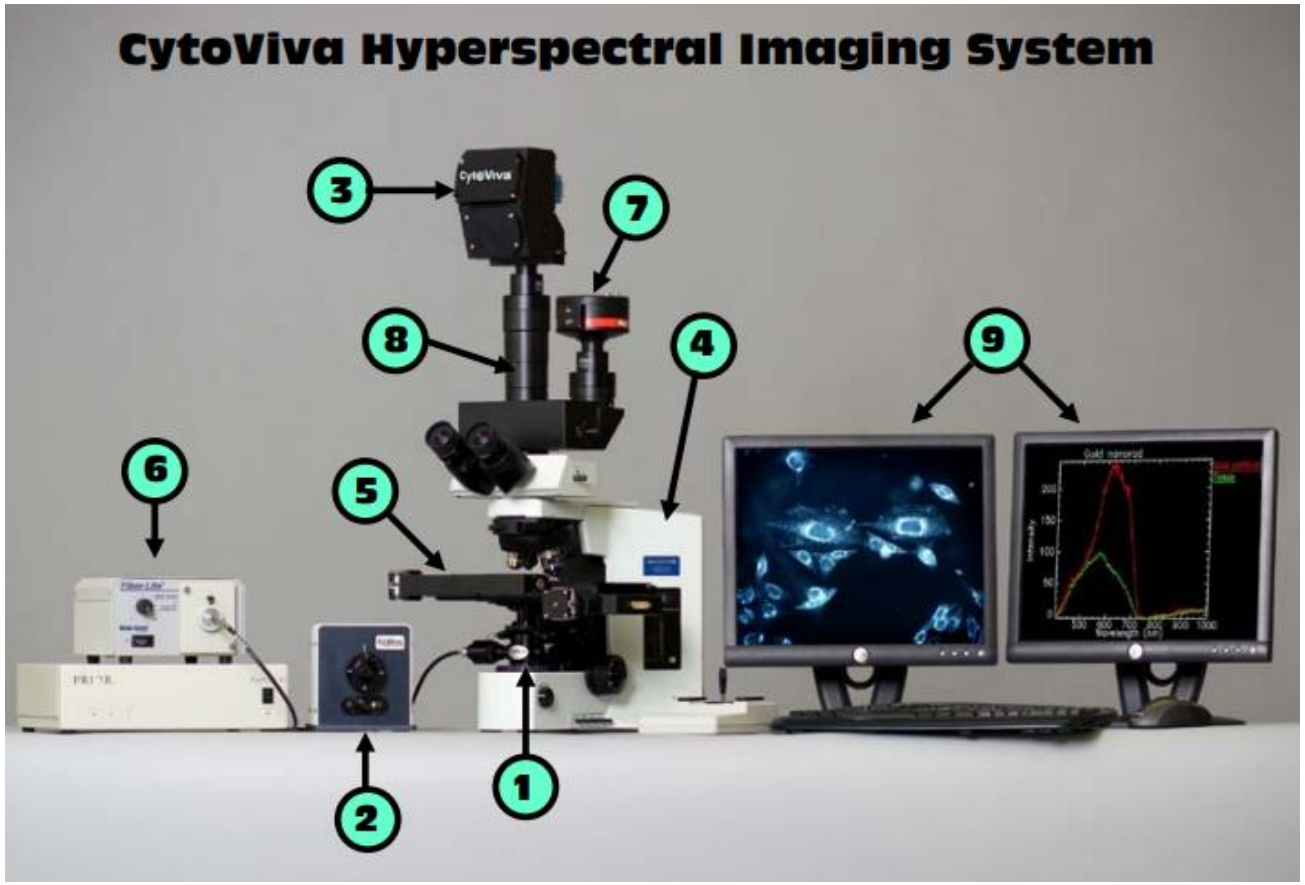

Fig.1 EDFM System

Hyperspectral images

- Experimental recording – optical fibre with liquid core, special shape of condenser, scanning technique.
- Each hyperspectral image can be decomposed into levels, each corresponding to a spectral band out of the **468 bands** recorded experimentally **(between 400 and 1000 nm).**

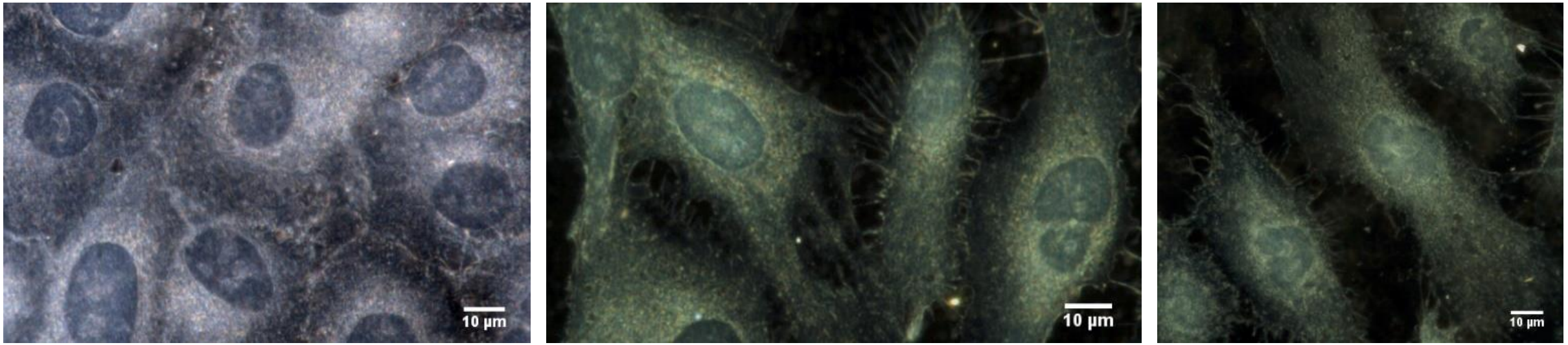

Fig.2 Experimental hyperspectral images with cells

a) nonirradiated, b) irradiated with protons, c) irradiated with X rays

Spectral single-pixel analysis

Differences are obvious:

- for **the irradiated cells** the spectral profiles contain one single peak around **450nm for nucleus and around 500nm for cytoplasm,**
- for **nonirradiated cells** the spectral profiles contain the peaks above, together with **peaks around 600nm and profiles with plateau.**

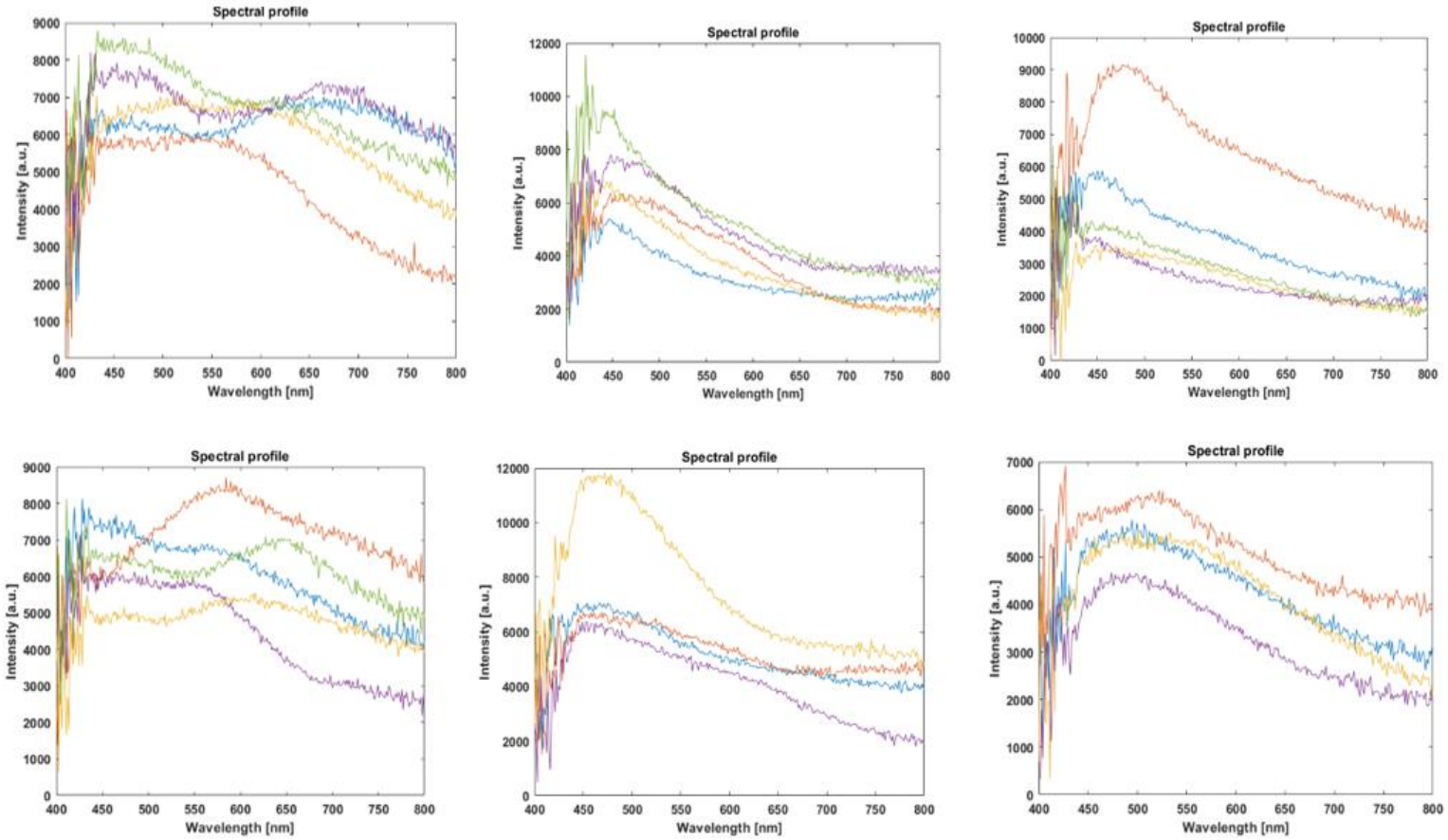

Fig. 3 Hyperspectral profiles from nucleus (top row) and cytoplasm (bottom row) areas for cells

a) nonirradiated, b) irradiated with protons, c) X ray irradiated.

Image processing

- **Segmentation:** The acquisition software ENVI generated **distinct contour of the nucleus** in each image.
- A binary mask is obtained for each nucleus, and by multiplying it with each spectral band, the corresponding ROI is separated.
- Taking the advantages of spectral images to be able to be decomposed into the bands allocated to each wavelength, we separated images corresponding to **the 468 wavelength values between 400 and 1000nm.**

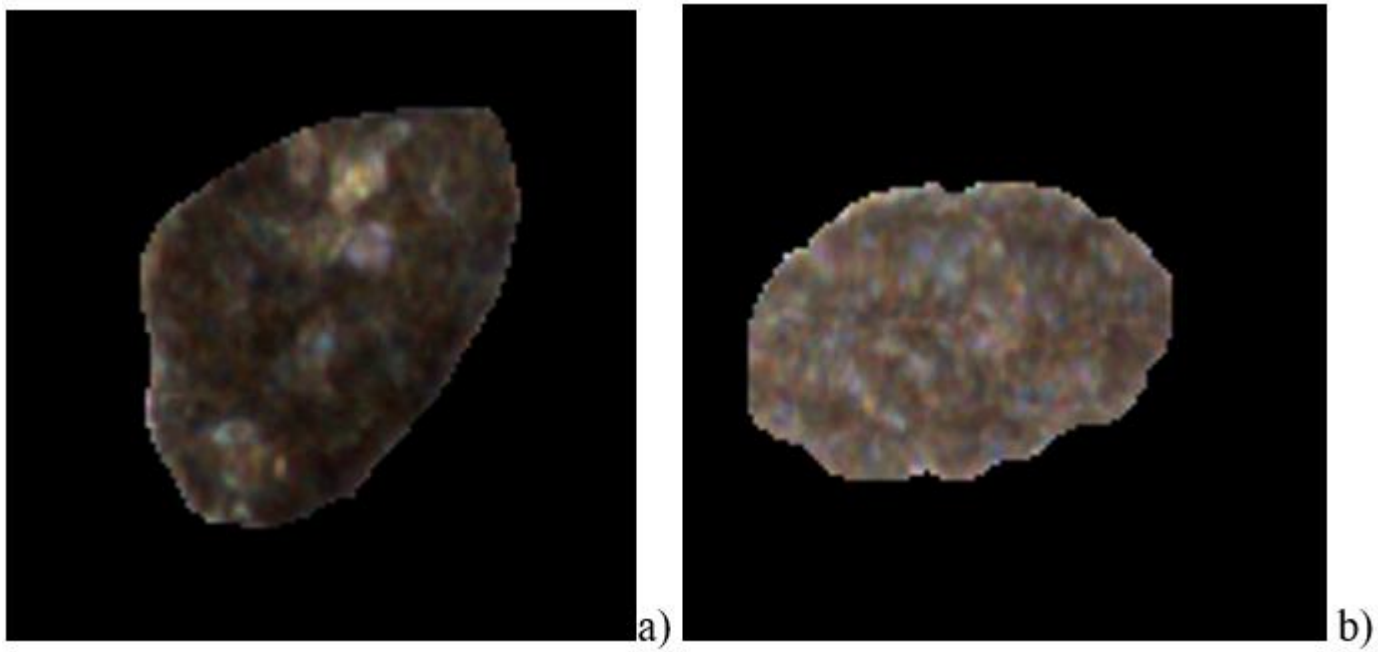

Fig. 4 Segmented nucleus area for
a) irradiated with protons, b) nonirradiated

- We grouped them in 5 intervals:
  - λ_{425} – 400-450 nm,
  - λ_{475} – 450-500 nm,
  - λ_{535} – 500-570 nm,
  - λ_{580} – 570-590 nm,
  - λ_{645} – 590-700 nm.

ROI – region of interest

Parameters - definitions

GEOMETRICAL PARAMETERS

- **Area (A), perimeter (P)** and **major axis (Ma)** were collected to assess and compare the size of irradiated and nonirradiated cells nuclei.
- **Solidity (Sol)** was computed as the ratio between the number of pixels of the ROI and the number of pixels of the corresponding convex hull.
- **Circularity (Ci)** gives us information about the edges of a shape whether it has smooth or corrugated edges.

$$\boldsymbol{Cir = \frac{4\pi \cdot A}{P^2}},$$

where A and P stands for area and perimeter, respectively.

- **Eccentricity (Ec)** has values close to 1 for elongated shapes and close to 0 for rounded shapes.

$$\boldsymbol{Ecc = \frac{r_{max} - r_{min}}{r_{max} + r_{min}}},$$

where r_{max}, r_{min} are the maximum and minimum radius of each focal of the ellipse with the ROI similar configuration.

Parameters - definitions

STATISTICAL PARAMETERS

- **Mean (*M*)** and **standard deviation (*Sd*)** which are usually considered.
- **Skewness *(S)*** parameter is a measure for the symmetry of a distribution. Its value can be positive, zero, negative, or undefined.
- **Kurtosis *(K)*** measures the extent to which a distribution contains outliers; it is unitless measure of a distribution shape and it only makes sense when comparing its values for several distributions.
- **Bimodality *(B)*** parameter is computed using the **skewness (S)** and **kurtosis (K)** values by the following formula:

$$\boldsymbol{B = \frac{S^2 + 1}{K + 3 \cdot \frac{(n-1)^2}{(n-2)(n-3)}}},$$

where n is the number of pixels corresponding to each cell examined and ROI the set of their values.

RESULTS: geometric parameters

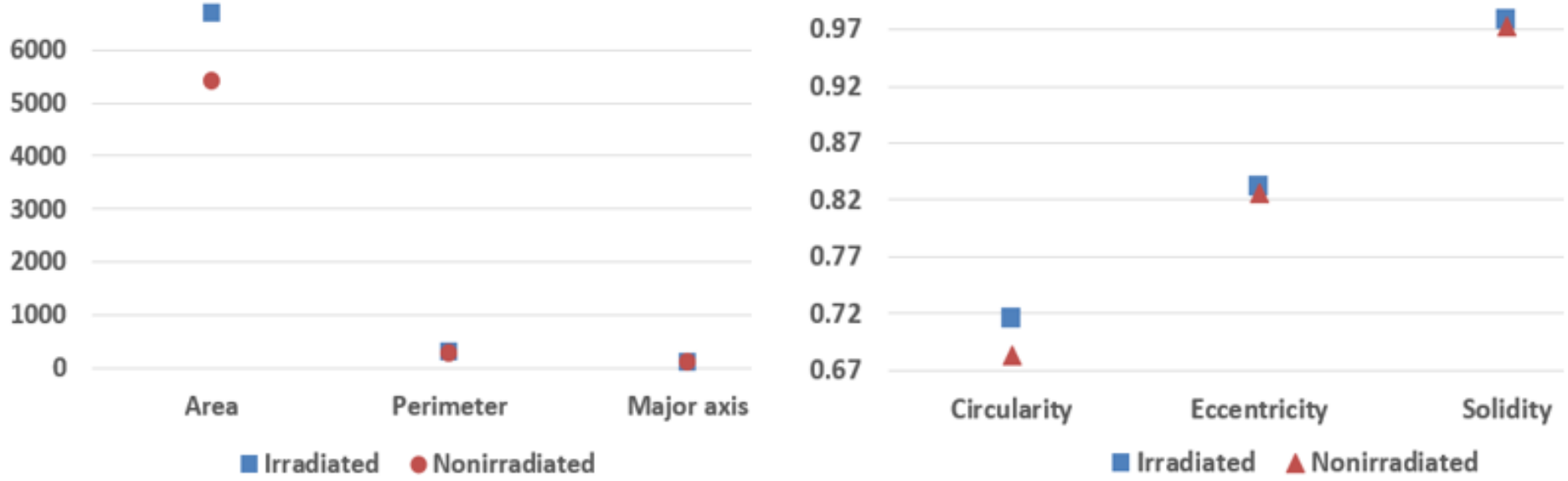

Fig. 5 Shape parameters of irradiated and nonirradiated cells nuclei from hyperspectral images

Shape parameters were computed and expressed in units of pixels:

- **Area - irradiated cells have a larger area than nonirradiated ones**, but not significantly.
- **Mean eccentricity -** almost **equal values** for the two types of cells, both having a relatively **high ellipticity**.
- **Mean circularity - higher values** for **irradiated cells nuclei**, which means that **their edges are more corrugated.**
- **Solidity** - values are **very close to 1**, so the **cells have no gaps or holes inside ROI**.

RESULTS: statistical parameters

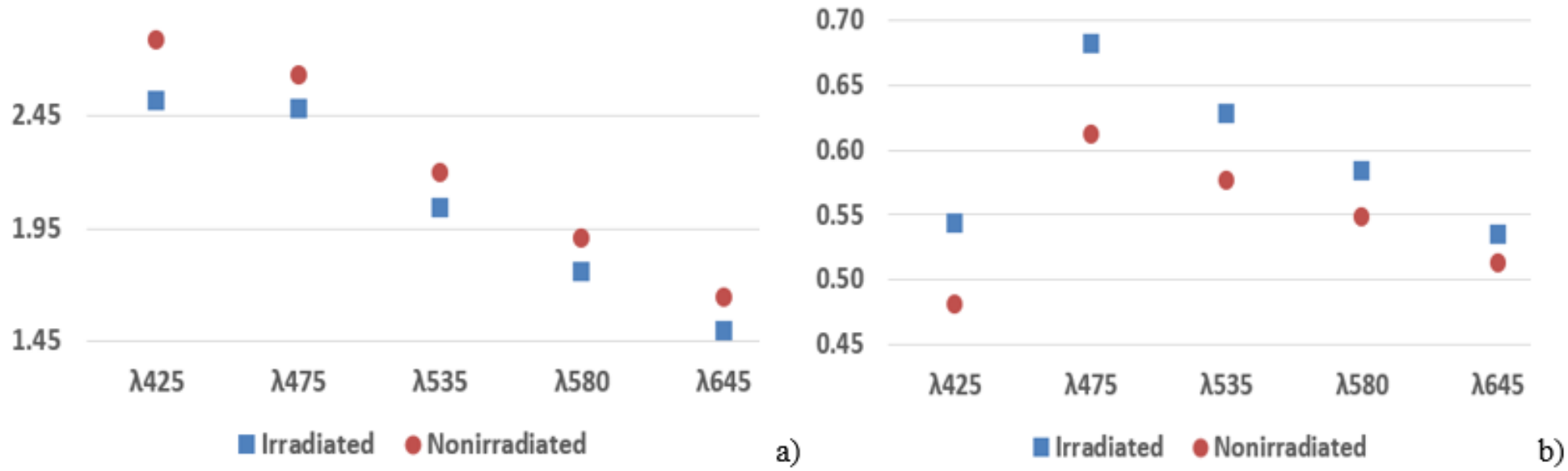

Fig. 6 a) Mean values and b) corresponding standard deviations on spectral intervals for irradiated and nonirradiated cells nuclei from hyperspectral images.

- **Smaller mean values** but **higher standard deviation** for irradiated cells - intensity values are distributed over larger intervals.
- It is interesting that spectral behaviour is similar for irradiated and nonirradiated nuclei:
  1) **intensity values** are high for interval centred on λ_{425} and decrease going towards λ_{645};
  2) the same **two spectral intervals** have **smaller standard deviation for both types of samples** (centred on λ_{425} and λ_{645}).

RESULTS: statistical parameters

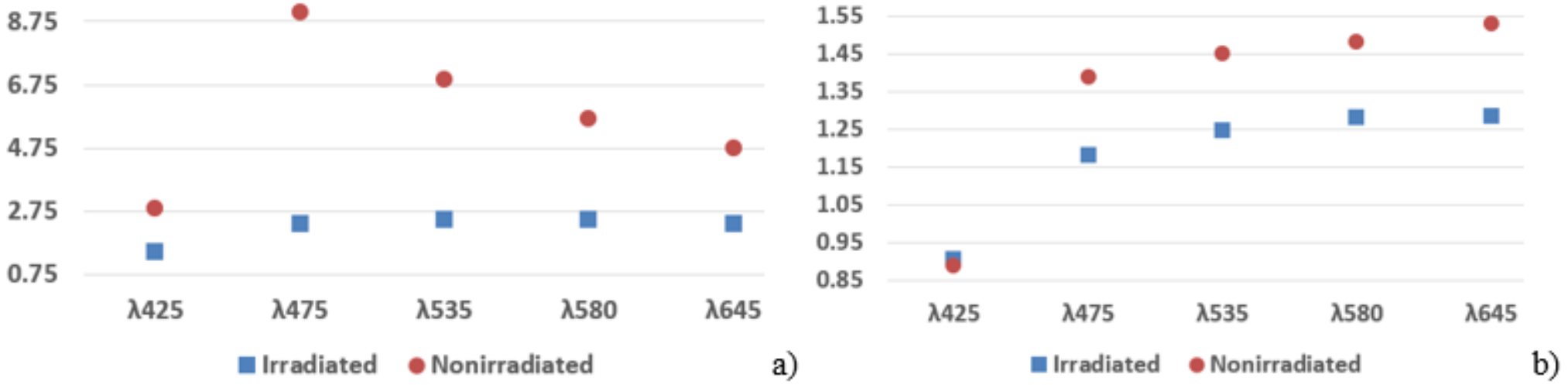

Fig. 7 a) Kurtosis and b) skewness on spectral intervals for irradiated and nonirradiated cells nuclei

Kurtosis is a statistic parameter that measures the extent to which a distribution contains outliers.

The values smaller than 3 - a more **flattened histogram** for irradiated cells than nonirradiated one.

The narrowest distribution is for the nuclei of nonirradiated cells for the spectral interval centred on the **wavelength 475 nm.**

Skewness parameter is a measure for the symmetry of a distribution.

The distributions are **moderately skewed** -wavelength of 425 nm, **tending towards highly skewed** - starting from **the wavelength of 475 nm** and **increasing** to spectral interval centred on the **wavelength of 645 nm.**

RESULTS: statistical parameters

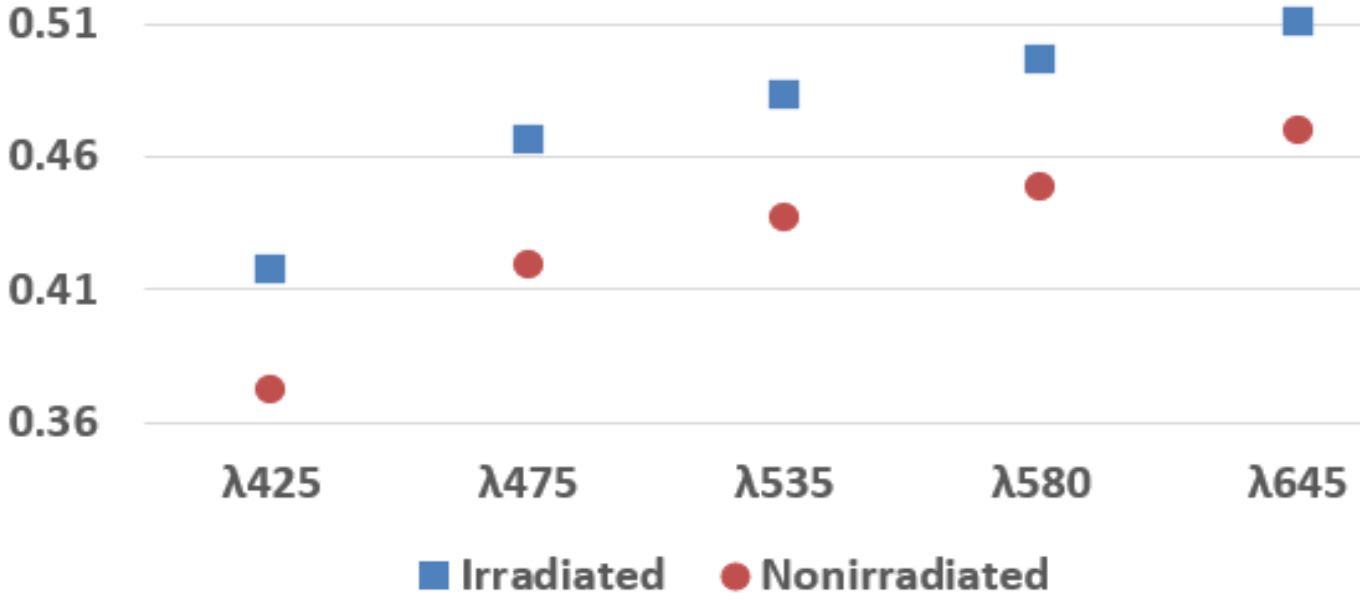

Fig. 7 Bimodality coefficients on spectral intervals for irradiated and nonirradiated cells nuclei

Bimodality coefficient measures if a distribution is a mixture of two normal distributions.

- all values are smaller than 0.55 - **bimodality was not demonstrated**
- **the variation of the bimodality coefficient** has **similar behavior for both types** of data for all spectral intervals analysed by us

CONCLUSIONS

- ✓ Our investigations were focused on two distinct directions - **to find global signatures for whole nucleus** and **punctual signatures at pixel-level.**
- ✓ Analysis at single-pixel level - **spectral profiles for nucleus and cytoplasm** - **spectral profiles from nonirradiated cells are more complex (they have more peaks).**
- ✓ Shape parameters **- similar values for both groups**, with small differences in circularity.
- ✓ Statistical analysis was done on HSI after they were decomposed on 468 spectral bands grouped in five spectral intervals centered on the wavelengths: **λ_{425}, λ_{475}, λ_{535}, λ_{580}, λ_{645}.** It is worth noting that both groups of cells (irradiated and non-irradiated) have **similar behaviour for all analysed spectral intervals.**
- ✓ We noticed **higher bimodality coefficient** and **standard deviations** for **irradiated nuclei.** The narrowest distribution was found for the nuclei of nonirradiated cells in the spectral interval centred on the wavelength of 475 nm.
- ✓ **Our analysis opens a new direction for investigating hyperspectral images by decomposing them into experimentally recorded spectral bands.**
- ✓ **Calculating the coefficients for determining the characteristic signature of each sample on these subimages can provide additional and more detailed information that can distinguish between spectral signatures of different types of samples.**

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Thank you for attention! ☺