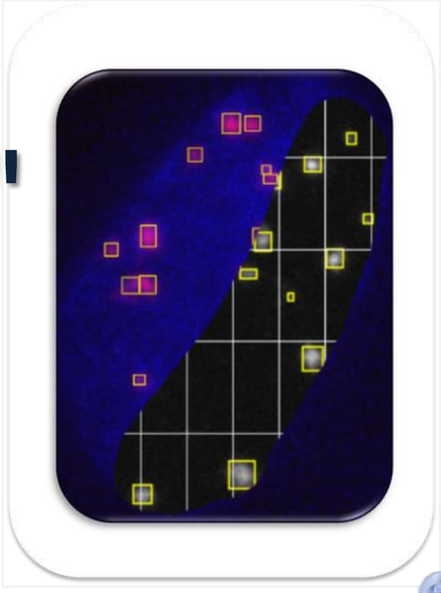
Development of the "MOSTLIT" Web Service for Radiation-Induced Foci Analysis



T.Zh. Bezhanyan[1,2], S. Shadmehri[1], O.I. Streltsova[1,2], M.Yu. Zuev[1], M.Yu. Bondarev[1,2], A. V. Boreiko[2,3], T. S. Hramko[2,3], A.K. Chgiasova[4], A.A. Osipov[5], N.Yu. Vorobeva[5], A.N. Osipov[5]

[1]Meshcheryakov Laboratory of Information Technologies, JINR Dubna, Russia
[2]Dubna State University, Dubna, Russia
[3]Laboratory of Radiation Biology, JINR , Dubna, Russia
[4]Emanuel Institute of Biochemical Physics, Russian Academy of Sciences
[5]Burnasyan Federal Medical Biophysical Center (SRC—FMBC), Moscow, Russia



MMCP' 2026
JINR, DUBNA

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Collaboration

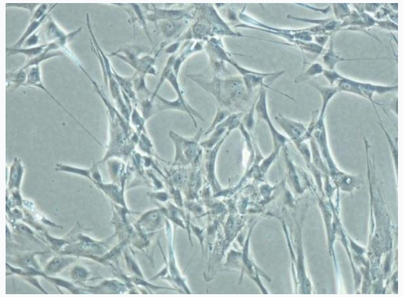
Burnasyan Federal Medical Biophysical Center

Meshcheryakov Laboratory of Information Technologies, JINR

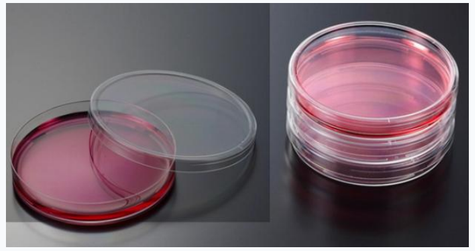
Laboratory of Radiation Biology, JINR

Materials

Human skin fibroblast culture cells



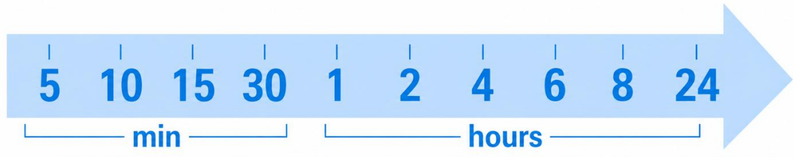
Ionizing radiation

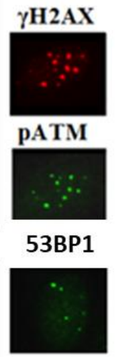


Burnasyan FMBC
X-ray biological unit RUB RUST M1

LRB JINR
γ-rays of Co^{60} Rokus - M

Immunocytochemical staining is used to visualize DNA DSBs



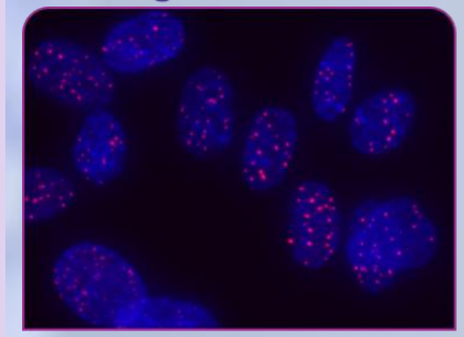


Cultured normal human skin fibroblasts were exposed to ionizing radiation. Following irradiation, the cells were fixed and stained using immunofluorescence methods at several time points after irradiation.

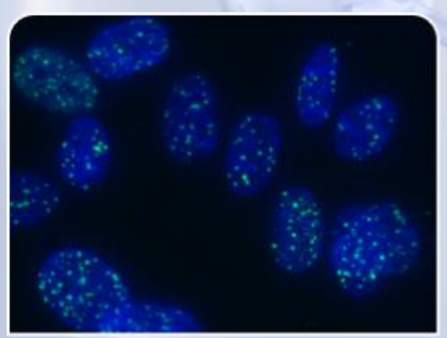
DNA DSBs are the most critical for cell survival and genomic stability. DNA DSBs were visualized using immunofluorescence staining for specific DNA damage-response proteins. These proteins accumulate at DNA damage sites and form microscopically visible radiation-induced foci.

Ionizing Radiation-Induced Foci (IRIF)

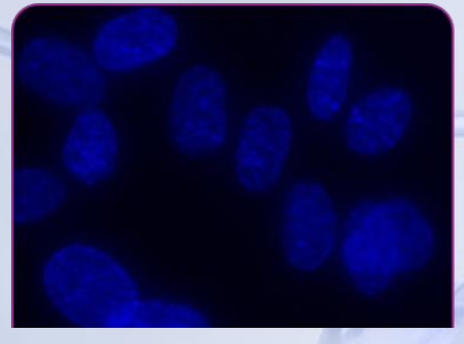
Input Data



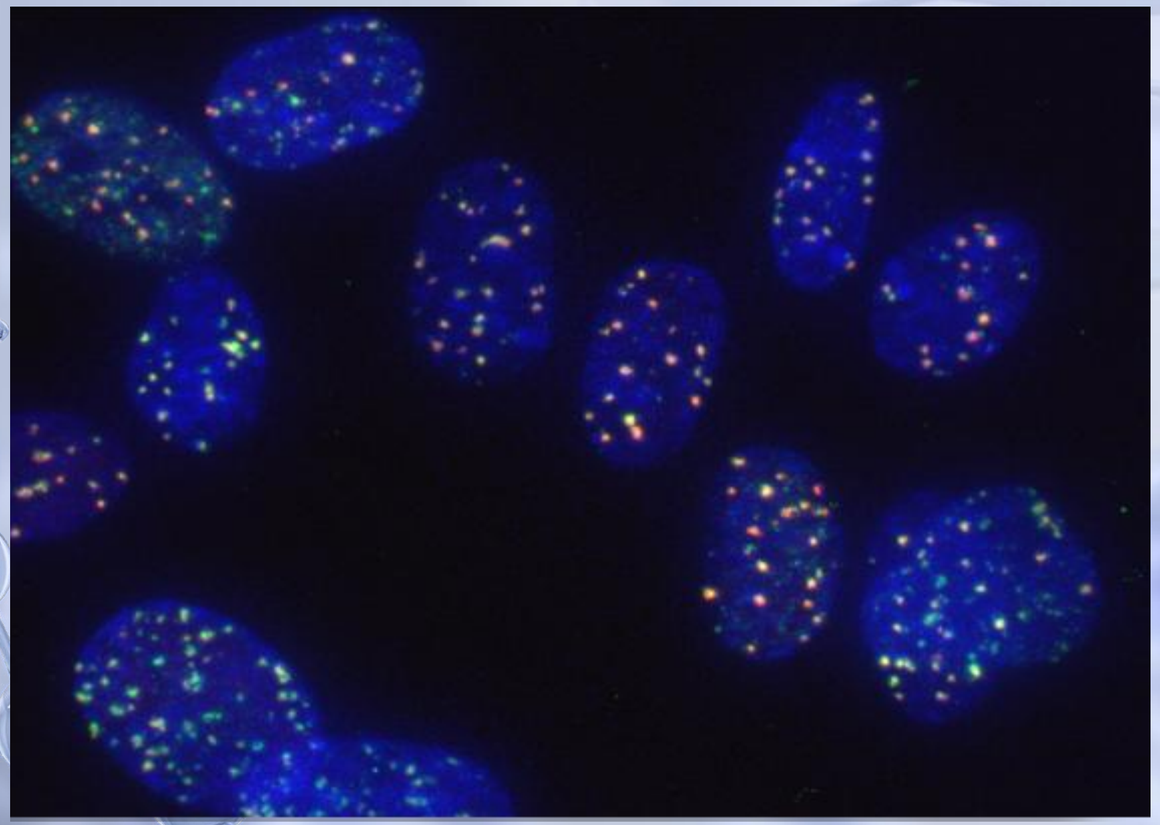
Cell Nuclei + 53BP1
DAPI + red foci



Cell Nuclei + γH2AX
DAPI + green foci



Cell Nuclei
DAPI



DNA Double-Strand Breaks

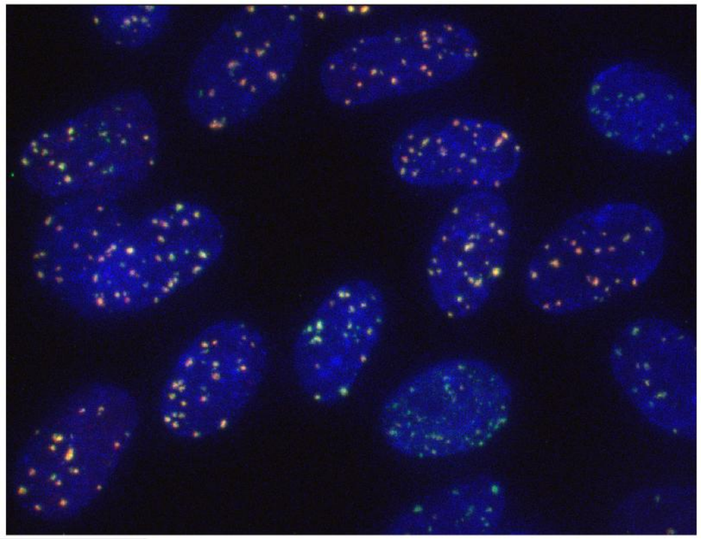


Exposure of living cells to ionizing radiation can produce many forms of DNA damage. One of the most crucial is a DNA double-strand break, If such damage is not repaired correctly, it may result in mutations, genomic instability, or cell death.

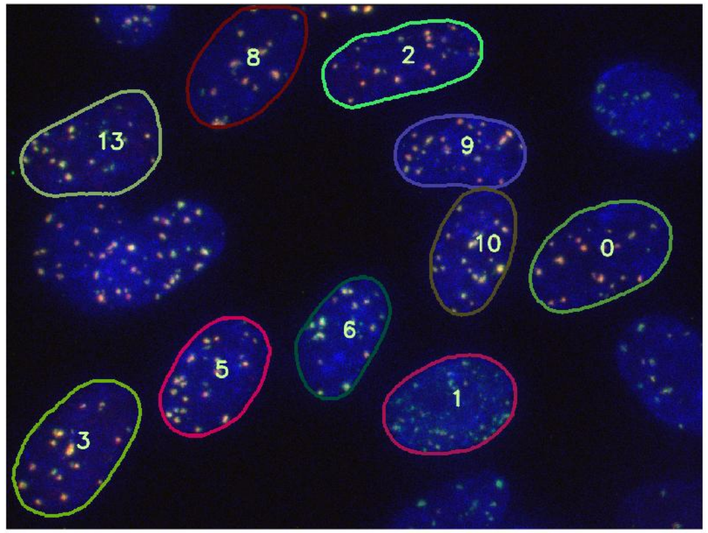
After a double-strand break occurs, several DNA damage-response proteins accumulate around the damaged region. These accumulations can be observed under a fluorescence microscope as small bright spots called **radiation-induced foci**.

Overview of the Pipeline

Upload the Image File

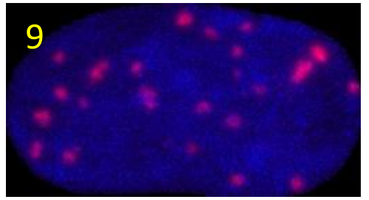


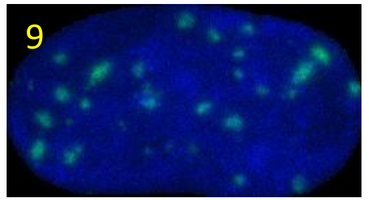
1: Detect cell nuclei



Marking the Cell Nuclei

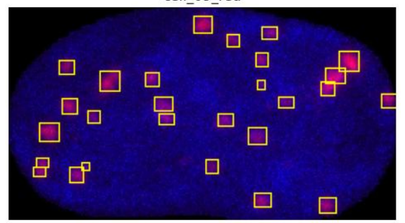
Extracting Cell Images



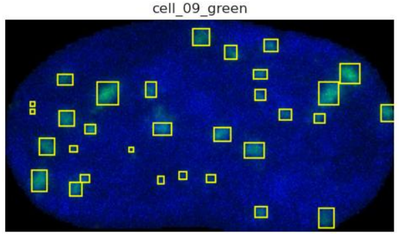


Marking the Foci per Cell

cell_09_red



cell_09_green

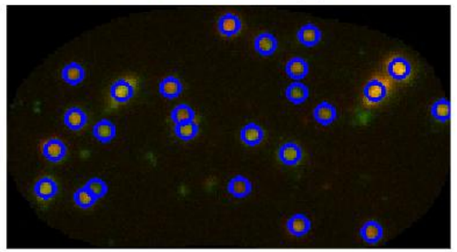


2: Detect foci

3: Calculate colocalization

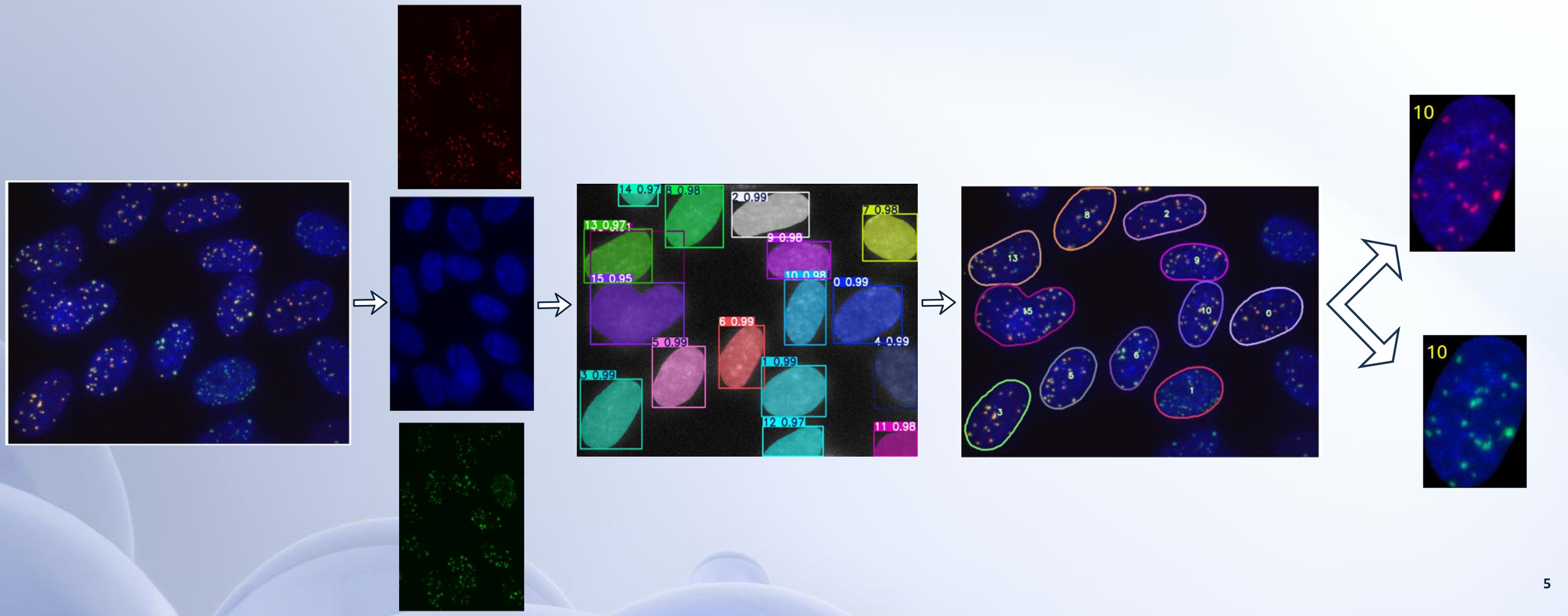
Marking Colocalization

Colocalized_09



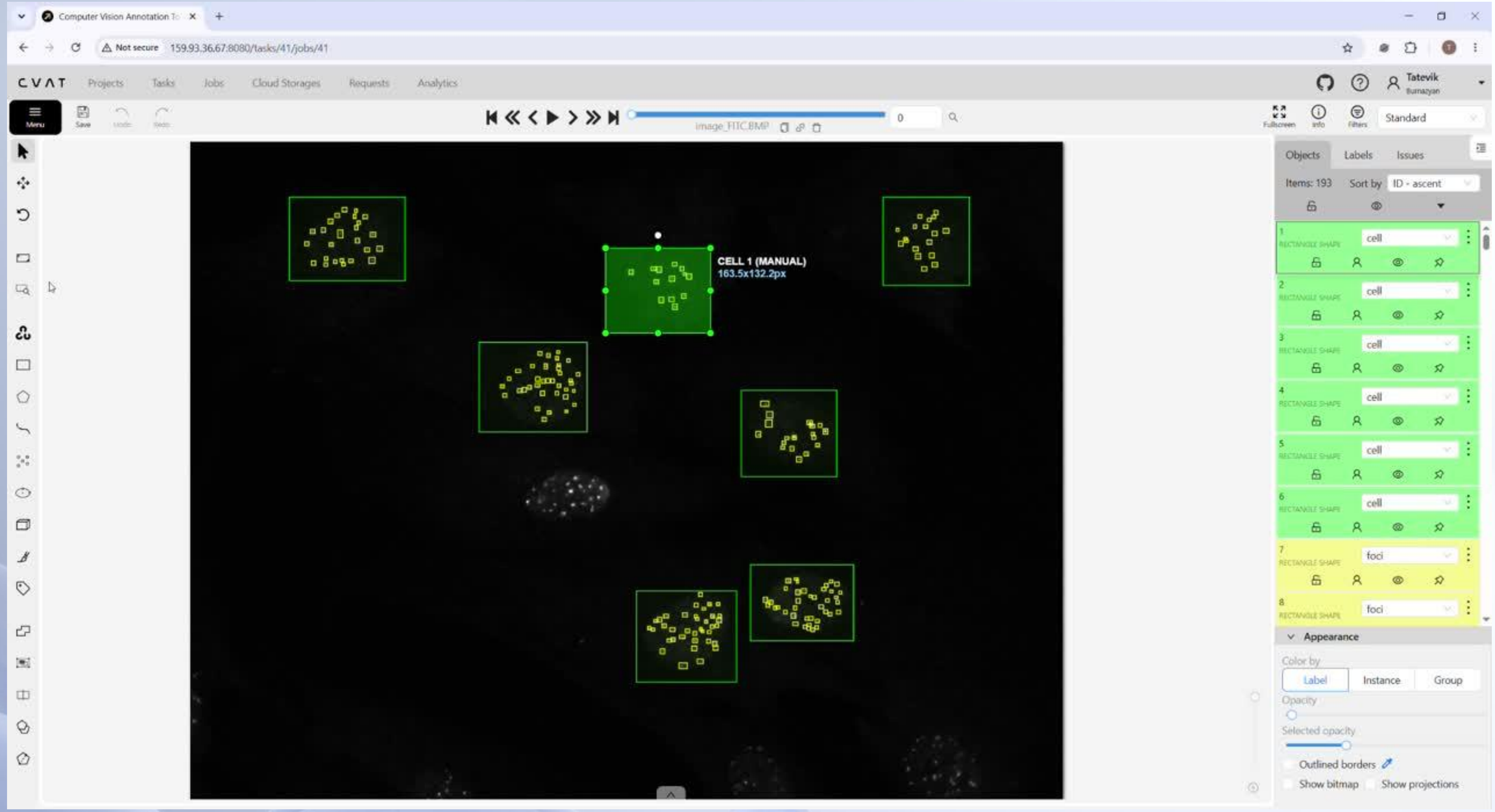
Cell Nuclei Detection - SAM2

Upload the Image File → Blue Channel → Cell Nuclei Detection Using SAM2 → Removing Boundary, Double-counted Objects & Artifacts → Cropped Cells per Channel



Data Annotation using CVAT

CVAT is installed on the ML/DL/HPC ecosystem of the HybriLIT Heterogeneous Computing Platform.

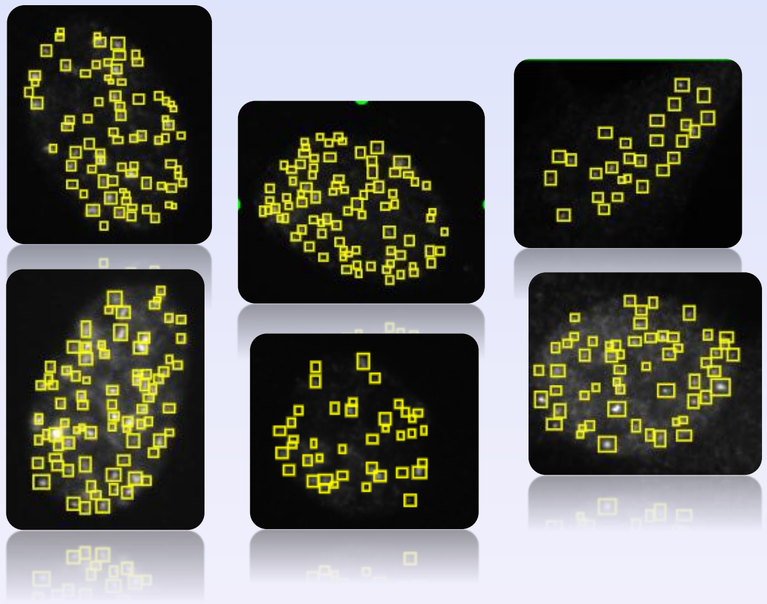


Annotation classes: cell and foci

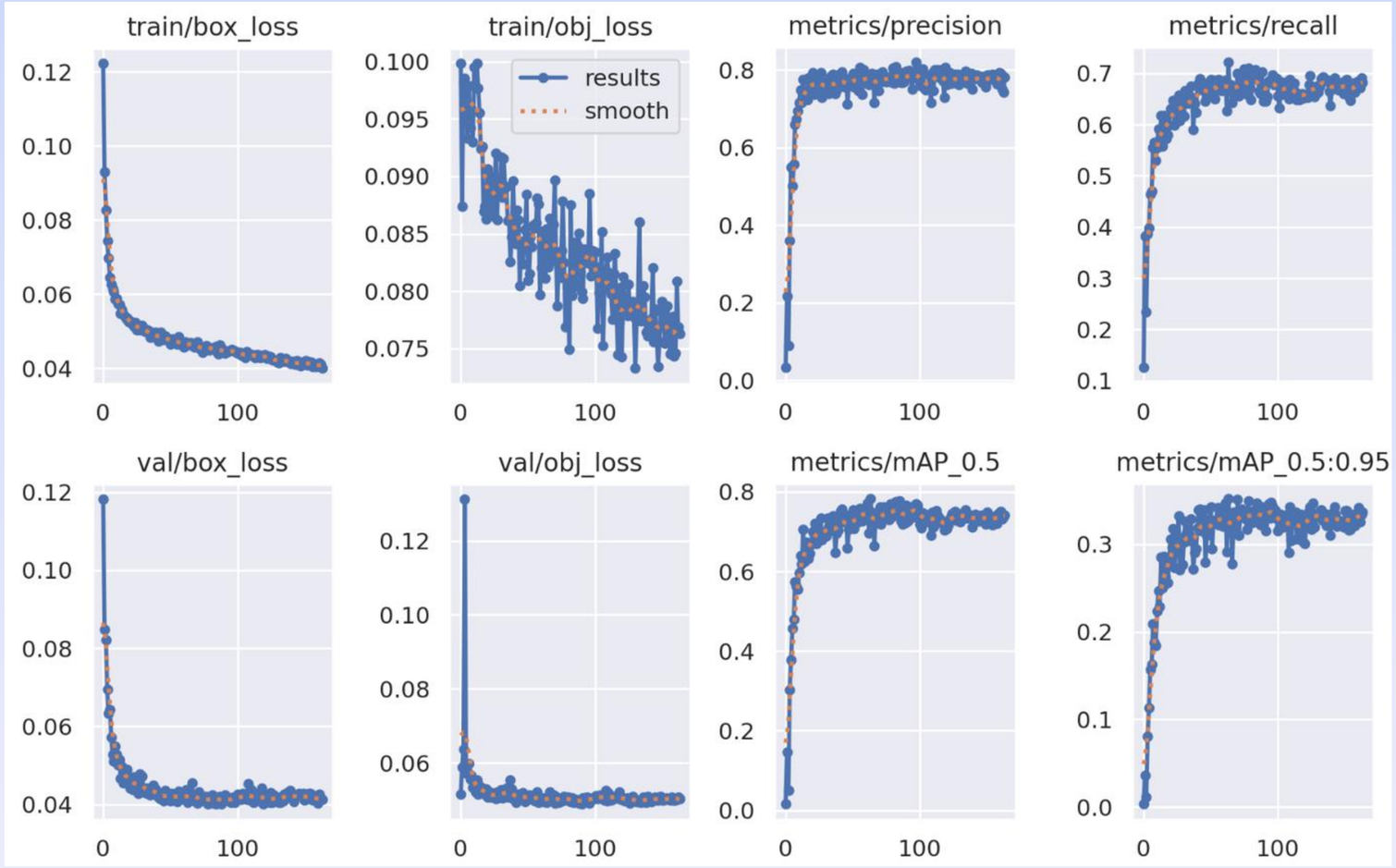
Exporting the dataset in YOLO format

YOLOv5 Performance Metrics

- ✓ Cropping the cells from images
- ✓ Reducing the dataset to a single class: foci

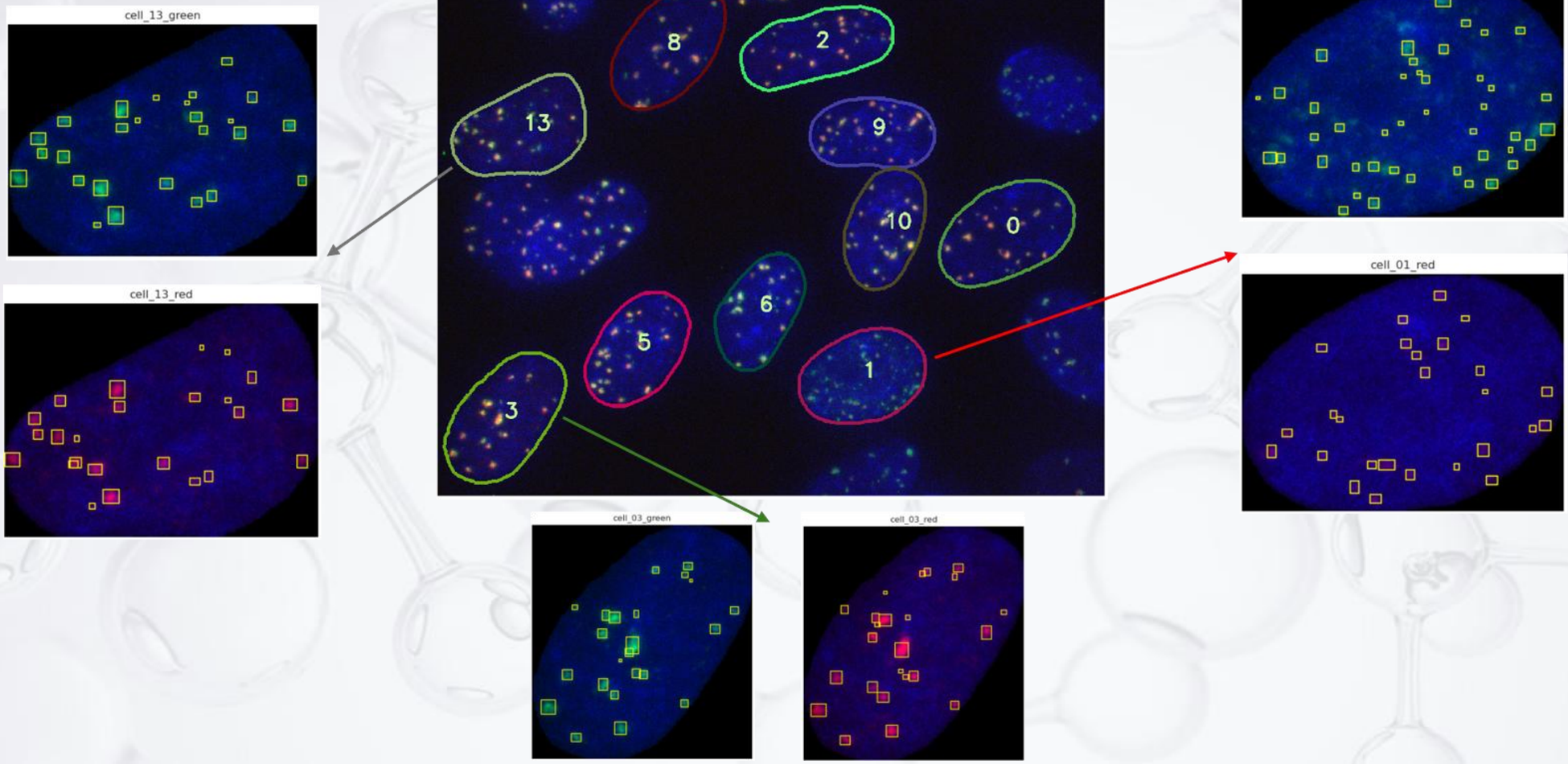


Training the YOLOv5 model



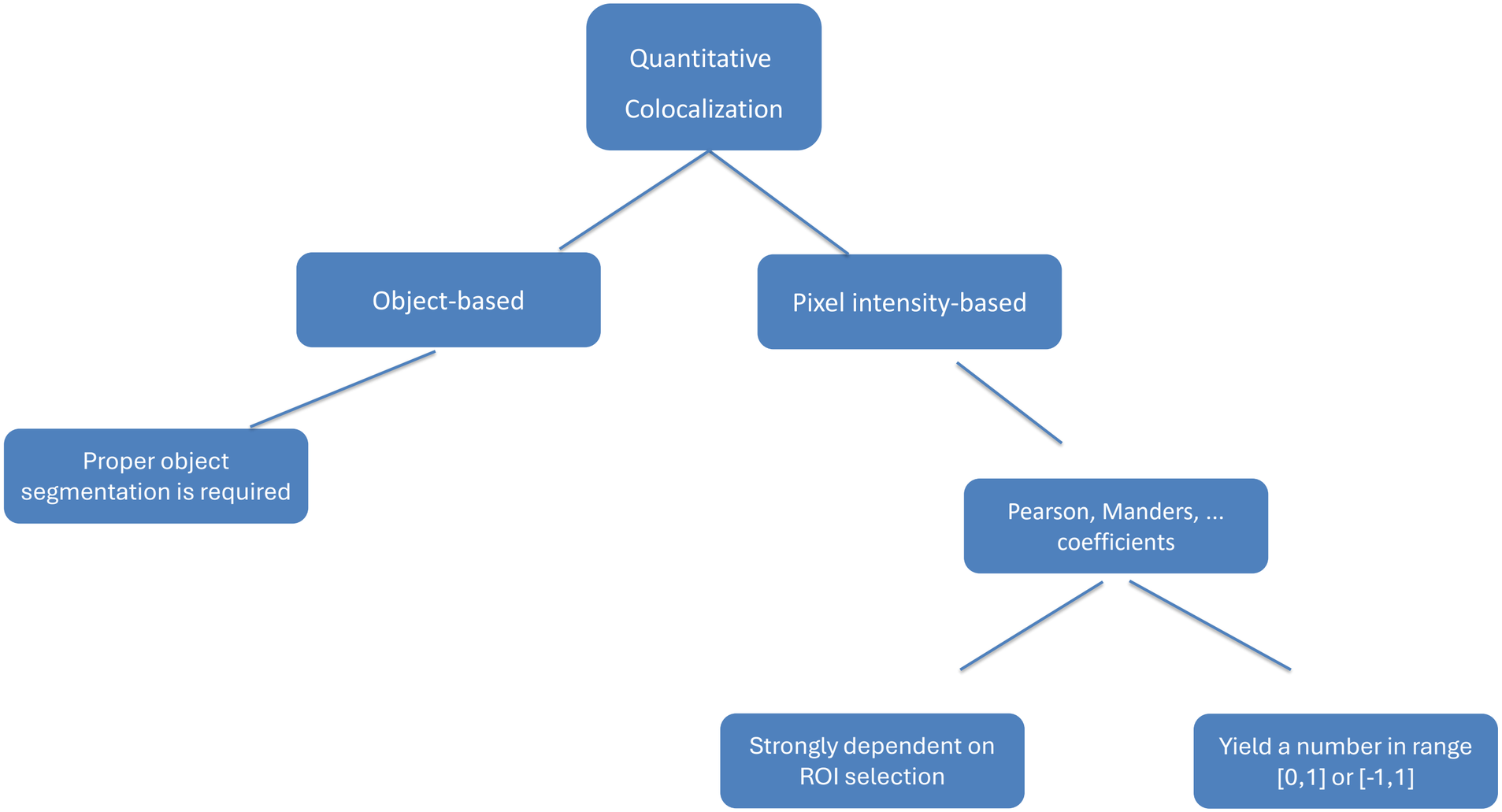
- Dataset: approximately 1,000 cropped cell nuclei
- Training/validation/test split: 70%/20%/10%
- Training epochs: 150

Foci Detected in Individual Nuclei Using YOLOv5



Co-localization

Overlap of the two signals



Object-based Colocalization

Colocalized objects are identified using thresholds for:

1. The number of overlapping pixels;
2. The distance between the centroids of neighboring objects

Optimal image processing is crucial to remove the background.

- Global image-based thresholding: CellProfiler[1]
- Local ROI-based thresholding: MatCol[2]

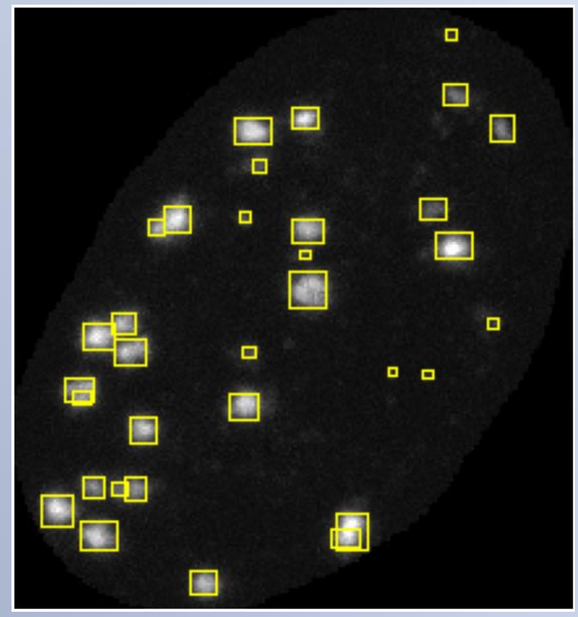
We apply local, ROI-based image processing before object-based colocalization analysis.

[1]Kamentsky, L. et al. *Bioinformatics* 27(8), p.1179 , 2011

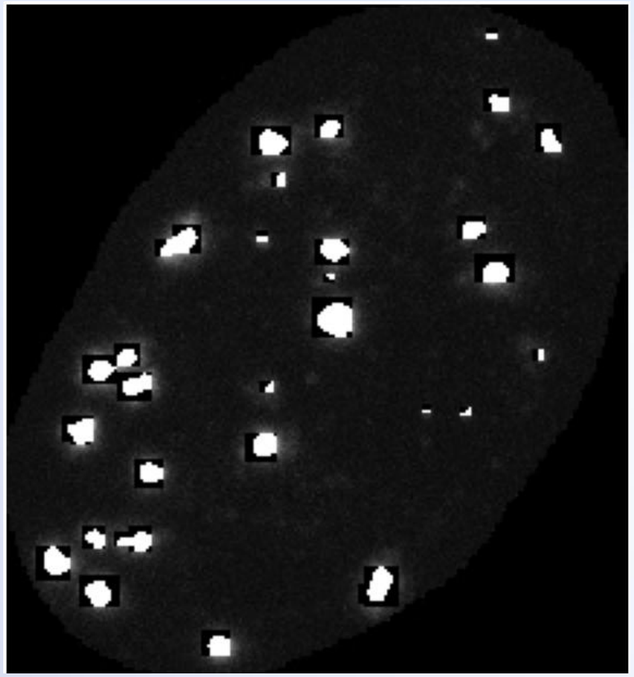
[2]Khushi, M. et al. *Scientific reports* 7(1), p. 8879 , 2017

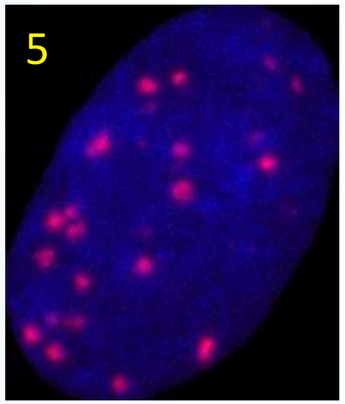
Object-based Colocalization

Detected Foci

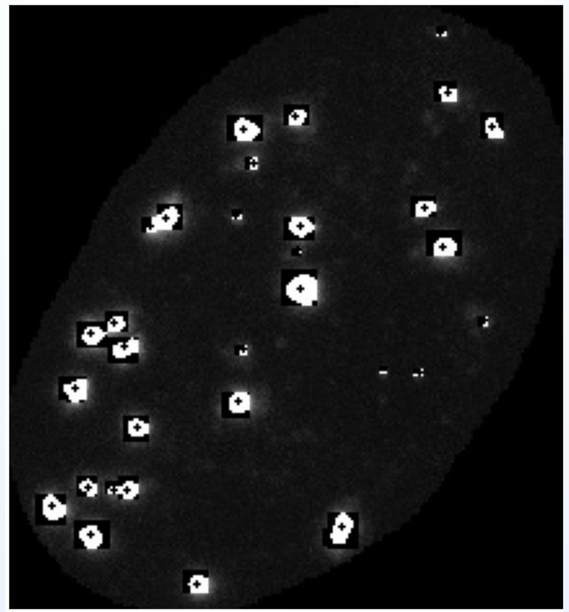


After Image Processing





with Centroids



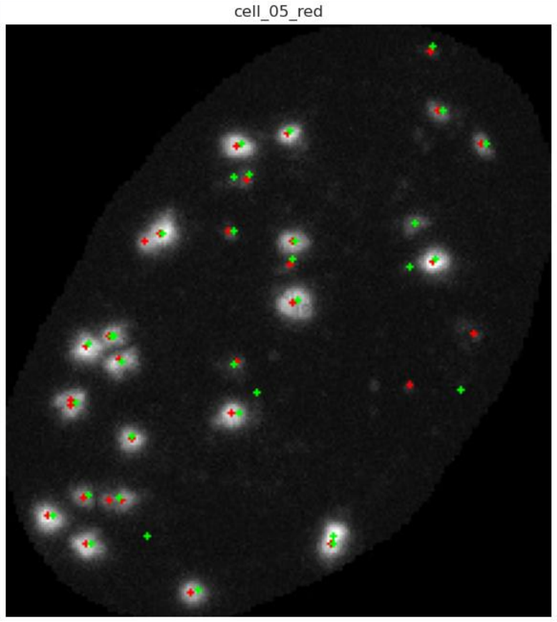
Local Image Processing on each foci:

- Gaussian Blur
- BINARY + OTSU

- Finding contours
- Calculating the moments M
- Calculating the centroid coordinates (C_x,C_y)

$$C_x = \frac{M_{10}}{M_{00}} \qquad C_y = \frac{M_{01}}{M_{00}}$$

Object-based Colocalization

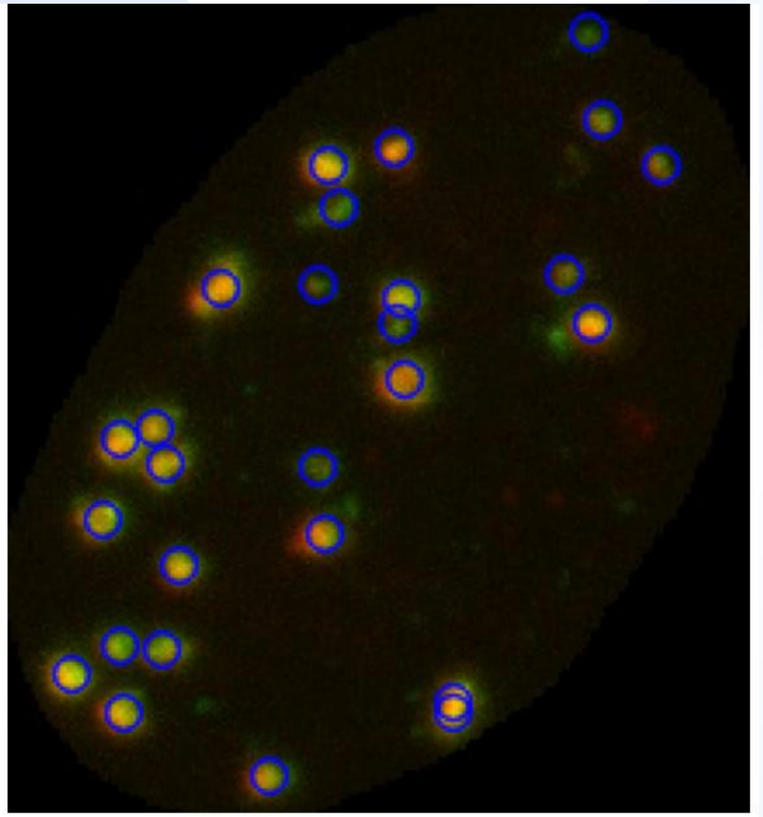


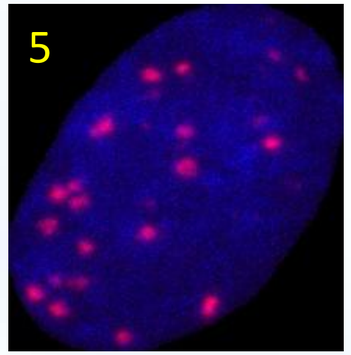
Co-localized Foci:
Two foci are considered colocalized when the distance between their centroids is less than or equal to the threshold distance d

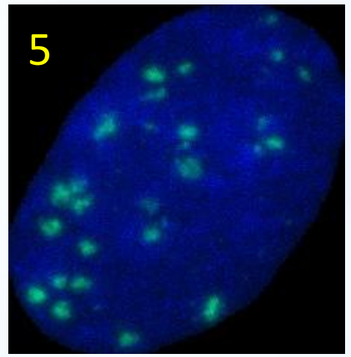
$$d = 0.61 \frac{\lambda}{NA}$$

NA : Numerical Aperture of the objective lens
λ : wavelength of the emitted light

Colocalized Foci







Pixel intensity-based Colocalization

Pearson's Correlation Coefficient:

Pearson Correlation Coefficient (PCC) quantifies the linear correlation between the intensities of two fluorescent signals. Values range from −1 to +1, where +1 indicates perfect positive correlation, 0 indicates no correlation, and −1 indicates perfect negative correlation.

$$\mathrm{PCC} = \frac{\sum_i \left(R_i - \overline{R}\right) \times \left(G_i - \overline{G}\right)}{\sqrt{\sum_i \left(R_i - \overline{R}\right)^2 \times \sum_i \left(G_i - \overline{G}\right)^2}}$$

Manders' Overlap Coefficient:

Manders' Overlap Coefficient (MOC) quantifies the degree of overlap between the intensity distributions of two fluorescence channels. Its values range from 0, indicating no overlap, to 1, indicating complete overlap.

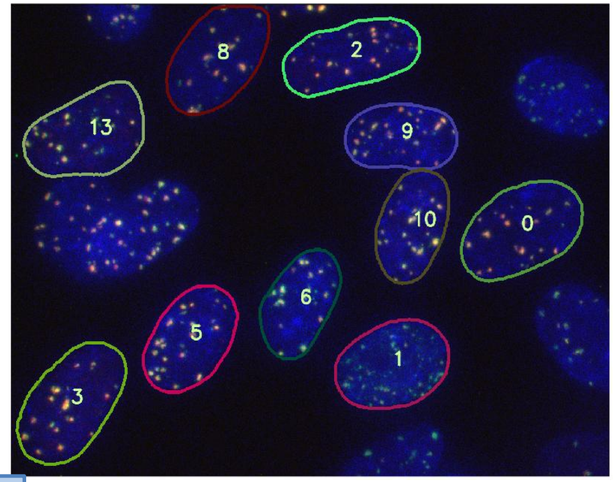
$$\mathrm{MOC} = \frac{\sum_i \left(R_i \times G_i\right)}{\sqrt{\sum_i R_i^2 \times \sum_i G_i^2}}$$

R_i and G_i intensity values of the red and green channels at pixel i

R̄ and Ḡ: mean intensities of the red and green channels within the ROI

Dunn, K. W. et al. *Am J Physiol Cell Physiol 300(4), p.C723, 2011*

Pixel intensity-based Colocalization

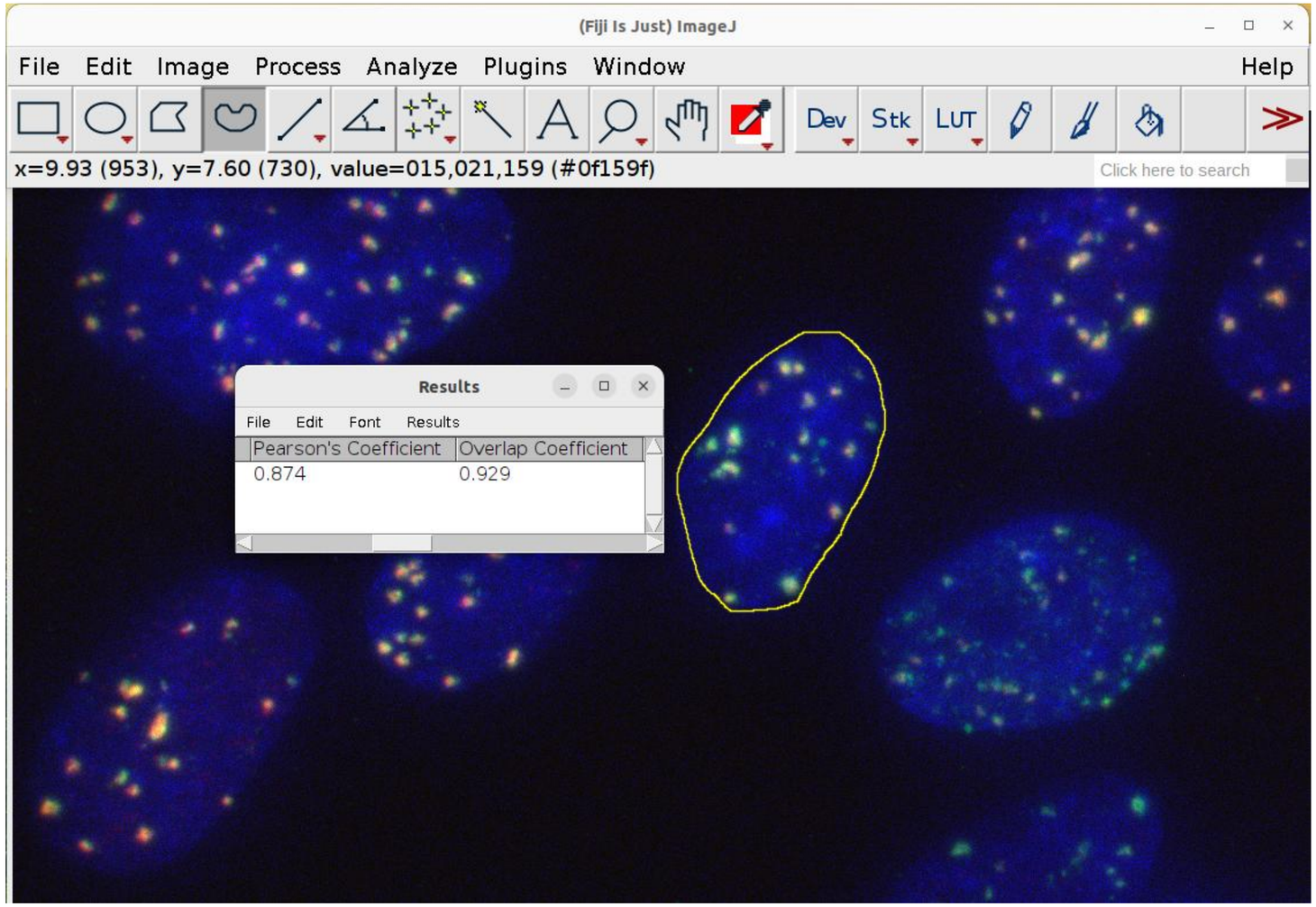


MOSTLIT

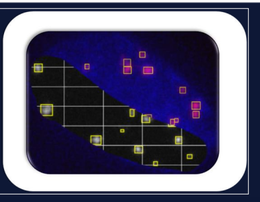
Cell Name	PCC	MOC
00	0.911	0.958
01	0.615	0.899
02	0.888	0.948
03	0.874	0.933
05	0.888	0.941
06	0.874	0.929
08	0.884	0.946
09	0.897	0.951
10	0.924	0.957
13	0.827	0.913

ROI : Individual cell nucleus

Fiji: BIOP JACoP plugin



Schindelin J. et al. Fiji: an open-source platform for biological-image analysis //Nature methods. – 2012. – Т. 9. – №. 7. – С. 676-682.



MOSTLIT PIPELINE

https://mostlit.jinr.ru/

1 IMAGE INPUT

2 **SAM2** CELL DETECTION

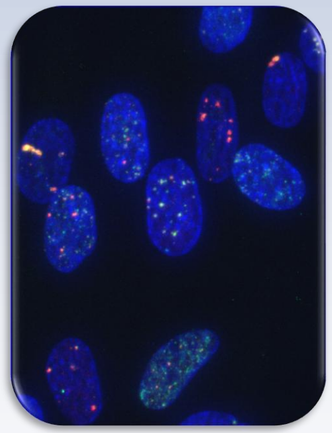
3 REMOVING OBJECTS

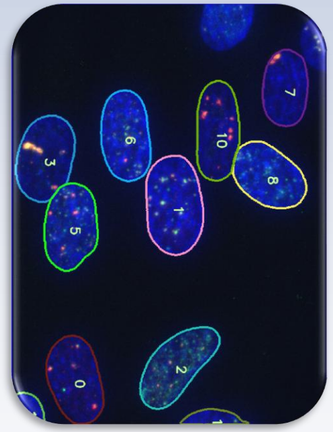
4 INTENSITY METRICS

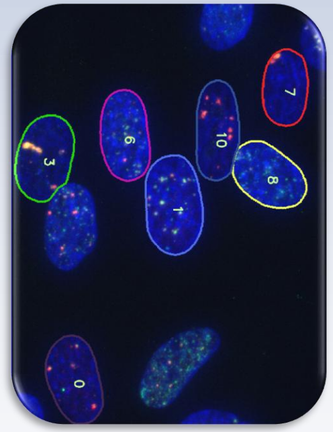
5 **YOLOv5** FOCI DETECTION

6 COLOCALIZATIO N ANALAYSIS

7 RESULTS

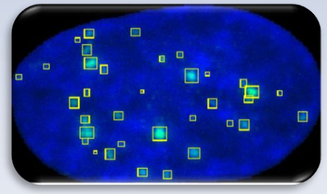


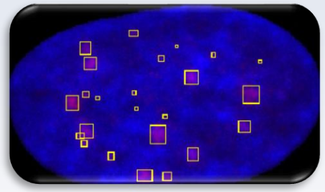


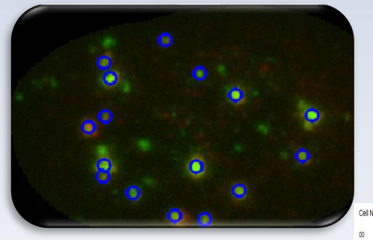


Intensity-based colocalization parameters:

- Pearson Correlation Coefficient (PCC)
- Manders' Overlap Coefficient (MOC)







Name	Average Number
Red Signal	17.7
Green Signal	28.0
Colocalization	10.9

Cell Name	PCC	MOC
00	0.551	0.834
01	0.775	0.912
03	0.942	0.940
06	0.597	0.884
07	0.921	0.915
08	0.619	0.927
10	0.821	0.841

Cell Name	Cell Area	Green Number	Green Area	Red Number	Red Area	Colocalization
00	40642	12	204	26	49	9
01	50147	35	886	24	1055	16
03	42003	18	789	10	1046	8
06	39339	46	764	31	499	22
07	30504	2	448	5	498	2
08	38220	54	853	11	134	8
10	38412	15	333	17	734	11

Uploading a fluorescence image	Detecting nuclei in the blue channel	Removing boundary and irrelevant nucleus	Calculating intensity-based colocalization metrics	Detecting foci in green and red channels	Performing object-based colocalization analysis	Generating per-nucleus and per-image results

Results

Numerical Data

Cell Name	Cell Area	Green: Number	Green: Area	Red: Number	Red: Area	Co-localization
00	40642	12	204	26	419	9
01	50147	35	886	24	1055	16
03	42023	18	789	10	1046	8
06	39339	46	794	31	499	22
07	30504	2	448	5	498	2
08	38220	54	853	11	134	8
10	38412	15	333	17	734	11

Average Metrics per image

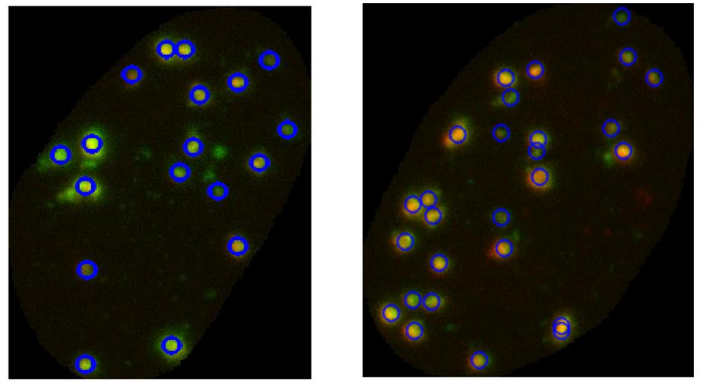
Name	Average Number
Red signal	17.7
Green signal	26.0
Colocalization	10.9

Intensity based colocalization per cell

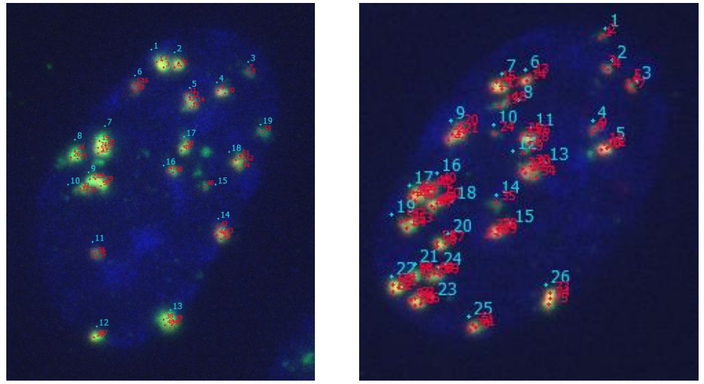
Cell Name	PCC	MOC
00	0.551	0.834
01	0.775	0.912
03	0.942	0.940
06	0.597	0.884
07	0.921	0.915
08	0.619	0.927
10	0.821	0.841

Representative Comparison of Object-Based Colocalization Results Obtained with MOSTLIT and Manual Counting

MOSTLIT



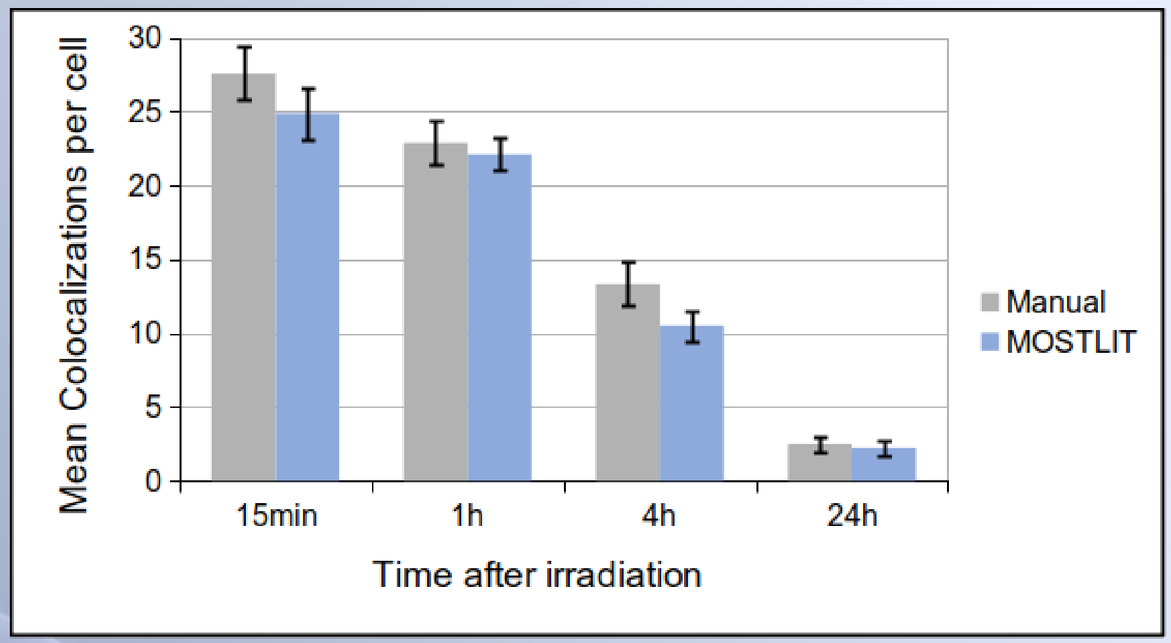
LRB manual counting



	Cell N1	Cell N2
MOSTLIT	18	28
LRB manual counting	19	27

Comparison of MOSTLIT with DARFI and Manual Counting

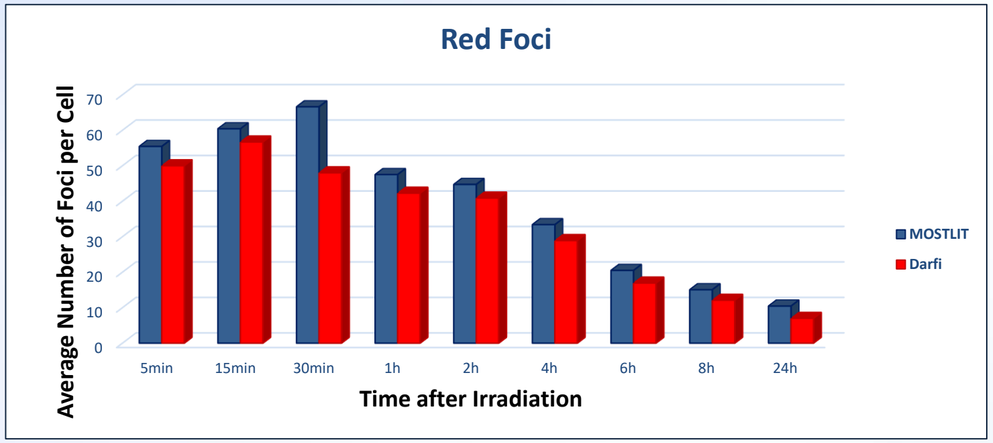
LRB
manual counting

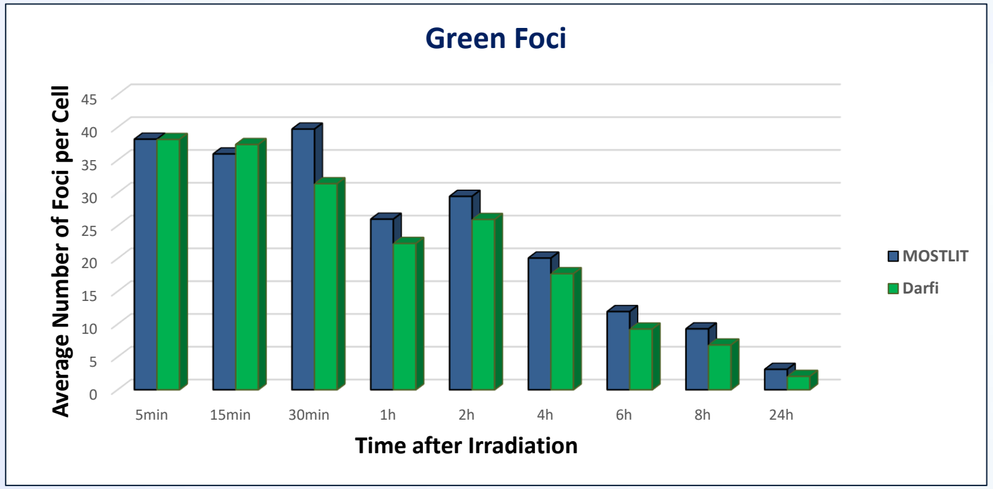


Kinetics of colocalized radiation-induced γH2AX (green) and 53BP1 (red) foci in normal human skin fibroblast nuclei after γ-irradiation with a dose of 1.25 Gy.
Colocalization was assessed in 79 cell nuclei.

Burnasyan FMBC
DARFI

http://github.com/varnivey/darfi





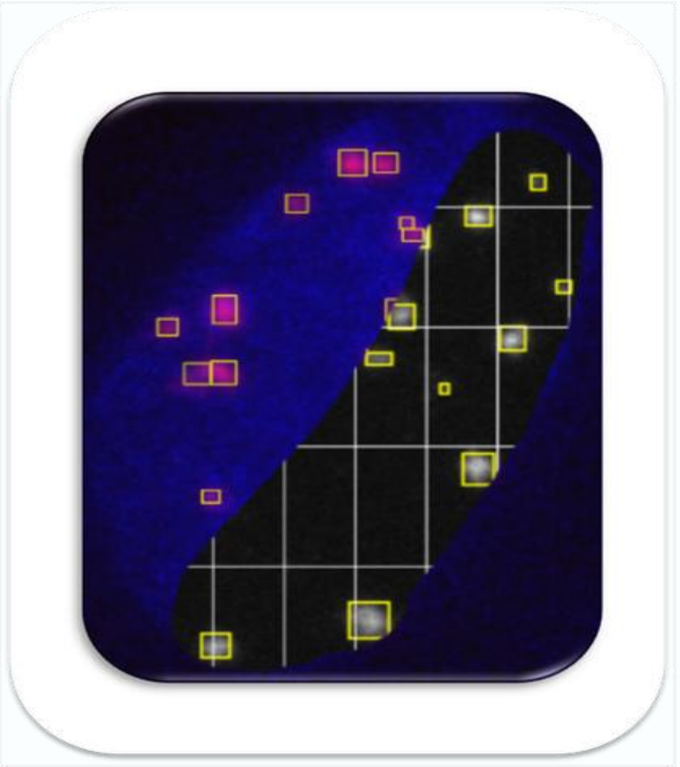
Kinetics of radiation-induced γH2AX (red) and pATM (green) foci in normal human skin fibroblast nuclei following 1 Gy γ-irradiation.
85 - 90 ROIs were analyzed at each time point.

Conclusion

- The MOSTLIT web service was developed for the automated detection and quantification of colocalization between two populations of radiation-induced foci.
  - A deep learning approach was developed to automatically detect and analyze of radiation-induced foci.
  - Colocalized foci are identified based on the distance between the centroids of neighboring objects in the two fluorescence channels.
  - Comparison with manual counting (LRB JINR) foci colocalization in normal human skin fibroblast nuclei demonstrated strong agreement between the methods.
  - Comparisons with DARFI (Burnasyan FMBC) showed close agreement, supporting the accuracy and utility of the method.
  - Intensity-based colocalization metrics, including PCC and MOC are calculated and demonstrated good agreement with the corresponding FIJI results.

MOSTLIT

Thanks for your attention



https://mostlit.jinr.ru/