Machine intelligence-driven classification of cancer patients-derived extracellular vesicles using fluorescence correlation spectroscopy: results from a pilot study

Abicumaran Uthamacumaran1,2 · Mohamed Abdouh3,4 · Kinshuk Sengupta5 · Zu-hua Gao6 · Stefano Forte7 · Thupten Tsering3 · Julia V. Burnier3,8,9 · Goffredo Arena10,11,12

Received: 4 July 2022 / Accepted: 22 November 2022 / Published online: 16 December 2022
© The Author(s), under exclusive licence to Springer-Verlag London Ltd., part of Springer Nature 2022

Abstract
Patient-derived extracellular vesicles (EVs) that contains a complex biological cargo is a valuable source of liquid-biopsy diagnostics to aid in early detection, cancer screening, and precision nanotherapeutics. In this study, we predicted that coupling cancer patient blood-derived EVs to time-resolved spectroscopy and artificial intelligence (AI) could provide a robust cancer screening and follow-up tools. In our pilot study, fluorescence correlation spectroscopy (FCS) measurements were taken on 24 blood samples-derived EVs. Blood samples were obtained from 15 cancer patients (presenting five different types of cancers), and nine healthy controls (including patients with benign lesions). EVs samples were labeled with PKH67 dye. The obtained FCS autocorrelation spectra were processed into power spectra using the fast Fourier transform algorithm. The processed power spectra were subjected to various machine learning algorithms to distinguish cancer spectra from healthy control spectra. The performance of AdaBoost Random Forest (RF) classifier, support vector machine, and multilayer perceptron were tested on selected frequencies in the $N = 118$ power spectra. The RF classifier exhibited the highest classification accuracy and performance metrics in distinguishing the FCS power spectra of cancer patients from those of healthy controls. Further, neural computing via an image convolutional neural network (CNN), ResNet network, and a quantum CNN were assessed on the power spectral images as additional validation tools. All image-based CNNs exhibited a nearly equal classification performance and reasonably high sensitivity and specificity scores. Our pilot study demonstrates that AI-algorithms coupled to time-resolved FCS power spectra can accurately and differentially classify the complex patient-derived EVs from different cancer samples of distinct tissue subtypes. As such, our findings hold promise in the diagnostic and prognostic screening in clinical medicine.

Keywords Cancer · Patient serum · Extracellular vesicles (EVs) · Liquid biopsy · Diagnostics · Spectroscopy · Machine learning · Artificial intelligence · Precision nanomedicine · Systems oncology

1 Introduction
Cancers globally remain amidst the leading-cause of disease-related mortality. Conventional therapies may be successful for certain subtypes of the disease, while others are complex adaptive systems progressing to clinically aggressive stages causing a paramount disease burden. Further, the long-term health complications and side effects, successfully treated patients must live with, must be emphasized. Within this pilot study, in efforts to advance precision oncology and patient-centered clinical medicine, we explored the application of artificial intelligence (AI) in tackling one of the greatest challenges in preventive and diagnostic medicine: early cancer detection and prognostic screening. Cancer biomarker discovery was pioneered by Gold and Freedman [1] with their recognition of the first tumor marker, Carcinoembryonic Antigen (CEA), which remains to date the most used clinically relevant, blood-based cancer screening and diagnostic in patient-care. Their co-discovery of the tumor-specific antigen gave birth to the field of precision immuno-oncology. Since then, significant progress has been made in the art of diagnostic medicine with the emergence of liquid biopsies and longitudinal blood monitoring. Liquid
biopsies are enriched with a complex variety of clinically relevant information which can be exploited for robust automated biomarker discovery in cancer screening. Some of the rich sources of these markers include differential methylation signatures of cell-free circulating tumor DNA, cell-free RNA/microRNAs, circulating tumor cells (CTCs) (including quiescent/dormant cells), immune cells (and their population densities), immune cells-secreted signals and cytokines, and extracellular vesicles (EVs) [2–6]. Among all of them, EVs are emerging as a promising clinical candidate for robust, financially cheap, quick, and noninvasive liquid-biopsy characterization of cancer dynamics, clinical screening, disease progression monitoring, and patient-therapy management [2, 7].

Early detection of cancer presents an interdisciplinary complex problem in systems medicine. The longitudinal analysis of patient-derived tumor biopsy sequencing and molecular cytogenetics may be inaccessible to patients, due to their invasiveness and financial barriers. Further, there are limited antigen/biomarker tests and clinically relevant blood-immune monitoring methods for complex adaptive cancers, such as aggressive brain tumors. Extracting CTCs or dormant cancer cells from disseminated tumors may be limited to only certain cancers at later stages of tumor progression. Then, the question arises: How do we sensitively detect cancers within patients at their early stages? How can we noninvasively perform longitudinal monitoring of therapy response in cancer patients? Medical physics applications such as CT/MRI-based imaging modalities, or the more painful lumbar punctures, are often limited to the detection of lesions with the presence of a minimal detectable size, the detection/profiling of later stages of disease progression, can be painfully invasive to patients, and present limitations. To reconcile the complex problem of early-stage cancer detection and screening, herein, we exploit complex systems physics and machine intelligence-driven pattern analysis in characterizing the time-resolved spectroscopic signals from patient sera-derived EVs.

Complex systems theory is the interdisciplinary study of quantifying the self-organized patterns and collective (emergent) behaviors in many-body nonlinear systems (i.e., complex systems) and processes (i.e., dynamical systems), by merging tools from intelligent systems/AI, statistical physics, information theory, and nonlinear dynamics. EVs dynamics are complex systems. The field of EVs is rapidly evolving and different categories are now being recognized including exosomes, microvesicles, ectosomes, apoptotic bodies, etc. EVs are nanoscopic lipid-bound entities found in different bioliquids such as blood sera. Notably, they transmit intercellular information and regulate many physiological and pathological processes, such as controlling cancer cellular cybernetics. Rose Johnstone initiated the field of EVs physiology when she first characterized them through electron microscopy (EM) imaging of reticulocytes [8–10]. Our study will mainly seclude to the isolation of EVs from patient blood samples. Cells-secreted EVs are one of the primary cybernetic control systems mediating intercellular communication in physiological conditions. In vivo patient EVs dynamics exhibit many complex adaptive features, including but not limited to the horizontal transfer of malignant traits, phenotypic reprogramming of distant tissue microenvironments into premetastatic niches, transcriptional and metabolic rewiring of cellular states, intracellular cargo transport, immune system control, regulating the phenotypic plasticity of cancer (stem) cells, conferring phenotypic heterogeneity in tumor microenvironments (TMEs), immunomodulation of tumor ecosystems, and promoting therapy resistance [11–16]. EVs are also emerging as cell fate reprogramming nanotechnologies in precision nanomedicine. For instance, the EVs derived from cancer stem cells can form complex cell–cell communication networks which promote and dynamically remodel an immunosuppressive TME, and thereby confer therapy resistance in tumor ecosystems [17]. Patient blood-derived EVs provide a rich repertoire of complex information dynamics, due to the heterogeneity emerging from their multicellular origins, and their adaptive signals in response to their environmental perturbations. EVs are also emerging as patient-compatible, personalized nanotherapeutics and targeted drug delivery vehicles [18]. Further, it remains questioned whether in vitro reconstitutions of these complex systems may exhibit collective dynamics and emergent behavioral patterns due to their aggregate interactions [19].

The application of AI is increasingly becoming prominent for pattern discovery in applications of precision medicine, ranging from automated multimodal drug discovery to blood/sera screening for complex disease markers. Precision medicine is now shifting toward the use of applied intelligent systems, and in specific, statistical machine learning (ML) algorithms-driven pattern discovery in disease monitoring/screening. Statistical ML algorithms, including state-of-the-art neural computing methods such as Deep Learning artificial neural networks, have been validated as robust tools for classification tasks/problems. There exists many examples of such types of works using AI and ML algorithms in the emerging field of liquid-biopsy-based cancer biomarker discovery [19–22]. In specific to AI applications to EVs profiling, a recent study demonstrated the merging of ResNet, a residual neural network-Deep Learning algorithm, and Surface-enhanced Raman spectroscopic (SERS) characterization of liquid-biopsy-derived EVs could yield > 90% sensitivity and accuracy in cancer detection [20]. These findings strongly suggest the pairing of liquid-biopsy-derived
cancer EVs with AI may pave a sensitive early-stage and prognostic detection of cancers in clinical medicine [20].

In extension to these findings, in our previous study we discovered that simple ML algorithms such as Random Forest (RF) classifiers and decision trees show high statistical accuracy in distinguishing the complex cancer patients-derived EVs Raman and FT-IR vibrational spectra from those of healthy patients [19]. Our study remains the first of such pilot studies to demonstrate the applicability of RF classifier, and similar ML algorithms, on patient serum-derived EVs’ vibrational spectra [19]. While advanced Raman techniques such as SERS and Raman imaging, and an increased patient size with a diverse cancer subtypes stages are required to further advance the clinical relevance of our previous findings, a fundamental limitation of such spectroscopic methods remains the lack of time-series analysis of the EVs temporal behaviors and features. In general, time-resolved spectroscopic techniques are under-investigated in the quantitative analysis of disease-driven complex systems, such as patient-derived EVs dynamics. As such, we wanted to explore time-resolved spectroscopies in AI-assisted cancer EVs characterization from patient liquid biopsies. In contrast to the previous study mentioned above, the current study uses a novel spectroscopy technique (i.e., FCS) and novel set of applied neural computing algorithms including Deep Learning networks. Therefore, it offers novel promising applications in applied intelligence/neural computing in the cancer detection problem.

Fluorescence Correlation Spectroscopy (FCS) is one such time-resolved technique in which we measure temporal fluctuations in fluorescently labeled particles or chemical agents within a system to quantify its behavioral dynamics [23]. In this study, we demonstrate the first-time applicability of FCS in distinguishing cancer patient-derived EVs from healthy patients. Within simple chemical systems, we assume the fluctuations to follow Brownian motion although complex systems can exhibit collective (aggregate), emergent behaviors [19]. Traditionally, the technique is used to quantify chemical characteristics of the system such as the diffusion coefficients, chemical kinetic rate constant, and molecular concentrations. Further, FCS allows the monitoring of ligand-macromolecule interactions with live-cell imaging at a single-molecular detection sensitivity [24]. As such, FCS provides a light-matter interaction interface to quantify complex systems dynamics, such as the chemical flow patterns of diffusive, molecular systems. A schematic of a generic FCS apparatus is shown in Fig. 1. Herein, we exploit this technique to quantify temporal features in nanoscopic complex systems such as patient-derived EVs systems.

At equilibrium, the fluorescent molecules undergo diffusive/flow processes within an illuminated opening/cavity (i.e., the focal volume), under the FCS microscope, giving rise to fluorescence intensity fluctuations over time. An autocorrelation function $G(\tau)$ is obtained as a function of the fluorescence decay time $\tau$, to quantify the average duration of the fluctuations. The autocorrelation function is given by:

$$G(\tau) = \frac{\delta F(t)\delta F(t+\tau)}{\langle F^2 \rangle}$$

where in $\delta F(t)$ denotes the fluctuations in the measured fluorescence $F$ from the average fluorescence $\langle F \rangle$ [23, 24]. The emitted intensity fluctuations are detected by the excited laser beam from the FCS apparatus, wherein the intensity is proportional to the number of fluorescently labeled EVs molecules in the confocal volume (illuminated region). The flow dynamics and interactions of the EVs system, via diffusion, reaction, or other collective dynamics, causes the fluctuations to emerge [25]. While traditional approaches to FCS analysis relied on extracting chemical and physical parameters from the autocorrelation function, we exploit herein complex systems tools, namely, FFT-power spectral analysis, multifractal analysis, and AI as complex feature extraction and classification approaches in the characterization of these time-resolved spectra. There remain a few studies which have used FCS to quantify EVs dynamics in healthy cellular systems. However, our study remains to date the first FCS application in cancer patients-derived EVs dynamics and demonstration of its clinical relevance to personalized nanomedicine.

FCS has been shown as a promising tool to quantify and visualize the EVs dynamics at the single-vesicle level of healthy cellular systems to elucidate cell to cell communication networks [26]. FCS techniques in combination with other molecular translation techniques have been implemented in the profiling of EVs surface proteins in relation to their diffusion times of antibody-vesicle interactions [18]. Wyss et al. [27] used ultrafiltration and size-exclusion chromatography, as purification techniques to isolate EVs secreted by mammalian cells and used fluorescence fluctuation analysis by FCS to investigate their biophysical properties, such as diffusion times, in relation to EVs size distribution. However, there may be more optimal techniques such as nanoparticle tracking analysis (NTA) or dynamic light scattering (DLS) better suited for such size-exclusion analyses [11]. We predicted that the
temporal fluctuations of patient-derived EVs within the FCS confocal volume may provide insights into their temporal behaviors and collective dynamics, which remain presently unreported in disease systems. Our pilot study demonstrates for the first time that FCS fluctuations could provide clinically meaningful insights into EVs dynamics and has the potential to accurately detect cancer EVs and be used in liquid biopsies. In contrast to our previous findings in Uthamacumaran et al. [19], we employed a novel technique FCS in the spectroscopic and machine intelligence-driven characterization of patient-derived EVs. Unlike our previous study, we also exploited a novel set of feature extraction algorithms such as power spectral analysis (in frequency/Fourier transform space) and spectral image classification algorithms within the Deep Learning neural networks framework.

2 Methods

2.1 Blood collection and serum preparation

Patients for the current study were recruited form the department of General Surgery at the Royal Victoria Hospital and St-Mary’s Hospital (Montreal, Canada) and underwent a written and informed consent for blood collection in accordance with protocols approved by the Ethics Committee of the McGill University Health Centre.
Blood samples were collected from both healthy individuals and patients who presented to our clinic for a follow-up or those that underwent resection of primary cancer Table 1. Blood samples (2–5 ml) were collected from a peripheral vein in vacutainer tubes (Becton Dickinson) containing clot-activation additive and a barrier gel to isolate serum. Blood samples were incubated for 60 min at room temperature to allow clotting and were subsequently centrifuged at 1500 g for 15 min. The serum was collected, and a second centrifugation was performed on the serum at 2000 g for 10 min, to clear it from any contaminating cells. Serum samples were aliquoted and stored at −80°C until further use.

### 2.2 Extracellular vesicles (EVs) isolation from serum samples

Serum samples were diluted in phosphate-buffered saline (PBS) at 1/10 dilution and were subjected to a series of sequential differential centrifugation steps. Samples were centrifuged at 500 g for 10 min to remove any contaminating cells, followed by centrifugation at 2000 g for 20 min to remove cell debris. Supernatants were passed through a 0.2 μm syringe filter (Corning), transferred to 26.3 ml polycarbonate tubes (# 355618; Beckman Coulter), and centrifuged at 16,500 g for 20 min at 4°C to remove apoptotic bodies and cell debris. Supernatants were transferred to new 26.3 ml polycarbonate tubes and ultracentrifuged at 120,000 g (40,000 rpm) for 70 min at 4°C using a 70 Ti rotor in Optima XE ultracentrifuge machine (Beckman Coulter). The crude EVs pellets were washed with PBS at 120,000 g for 70 min at 4°C, resuspended in 500 μl PBS, and stored in −80°C until further use.

### 2.3 Extracellular vesicles (EVs) labeling

Isolated EVs were labeled with PKH67 green, fluorescent probe according to the manufacturer’s instructions (Sigma). Briefly, EVs were resuspended in Diluent C and mixed with equal volume of the stain solution (4 ul PKH 67 in 1 ml Diluent C) for 5 min. The reaction was stopped by adding 2 ml of 2% bovine serum albumin or fetal bovine serum. Samples were passed through Exosome Spin Columns (MW 3000) (Thermo Fisher Scientific) to purify labeled EVs from unbound PKH67 dye. Samples were then centrifuged at 120,000 g for 70 min at 4°C. Labeled EVs pellets were resuspended in PBS for subsequent FCS.
analyses. For machine calibration, two controls were run in parallel: aliquots of PBS and diluted samples of PKH67 (10⁻⁸ M).

### 2.4 Fluorescence correlation spectroscopy (FCS)

The FCS system from McGill University’s ABIF (Advanced Bioimaging Facility) was used for our experiment. Fluorescence correlation spectroscopy measurements were taken at room temperature on a commercial Zeiss LSM 780 laser scanning confocal microscope with an inverted AxioObserver Z1 stand and operated with Zen 2012 SP5 FP3 software including an FCS module (Zeiss) Fig. 1. We used a continuous wave 25 mW 488 nm argon laser and a 40X C-APOCHROMAT NA= 1.2 W Korr UV–VIS-IR water immersion objective, with the correction collar adjusted for 0.17 mm cover glass at 23 °C. Before each measurement session, a blank measurement was made with PBS for calibration. Samples were diluted in PBS in a Mattek 35 mm petri dish with a 14 mm microwell and a No 1.5 cover glass, and measurements were taken by focusing roughly 5 μm above the surface of the cover glass in the center of the field of view. Laser intensity was controlled with an acousto-optic tunable filter set to 2% transmittance, the pinhole was set to 34 μm (as software recommended for 1 airy unit for this emission range and objective choice) and a 488 nm main beam splitter was used to separate excitation light from emission light. Raw photon counts were measured on a spectral detector with a range of 499–691 nm, and autocorrelation was calculated on the fly. Count rate binning was 1 ms, correlator binning time was 0.2 μs, and acquisition time was 30 s per run. Binned counts and calculated autocorrelation values were exported as ConfoCor3 fcs files and processed offline.

Note: Use the R-language ImportFCS code to obtain the autocorrelation spectra from raw FCS intensity counts (See GitHub link for Data and Codes Instructions).

### 2.5 Power spectra

The FCS autocorrelation spectra obtained from the ImportFCS code discussed above were further processed using the fast Fourier transform (FFT) using OriginPro v 8.5. The autocorrelation spectra data tables were inputted into the OriginPro software, and following, FFT analysis was performed using the Analysis function → Signal Processing → FFT. The function outputs various tables and graphs, and we exported the power spectra (Frequency (Hz) vs. Power (dB)) as shown in Fig. 2. The power (dB) measurements for each 118 FCS autocorrelation spectra obtained from the n = 24 patient samples were extracted, as 1D vectors, and subjected to Scikit-learn ML classification algorithms with their respective binary patient labels (Healthy vs. Control). Further, the power spectral images as shown in Fig. 2B, D, for healthy and cancer patient samples, respectively, were obtained for the 118 spectra, and subjected to classification by various Deep Learning neural networks, as discussed below.

### 2.6 Scikit-learn statistical ML classifiers

Machine Learning analysis was performed using binary classifiers from the Scikit-learn python library [28]. We adopted the same set of ML classifiers shown to well-perform in the pattern discovery of complex EVs vibrational spectra in our previous study [19]. The AdaBoost classifier was used as an ensemble learner to enhance the predictive performance of the Random Forest (RF) classifier. The cross-validation was set to tenfold (CV = 10). All shown results used both an 80:20 and a 50:50 training: testing split as classification conditions. Lower test splitting (i.e., 80:20 or 70:30) resulted in higher ML performance and hence, the 50:50 test size was used throughout our study for stringent machine intelligence pattern analysis. ML classification was performed on all N = 118 complete FCS power spectra of n = 24 patients. All sample data and codes are provided in the GitHub link repository (See Data and Code Availability Section). The hyperparameters of the ML classifiers were tuned as follows: The following frequencies were identified as the most optimal frequencies at which the patients’ FCS power spectral classification were best distinguished by all ML classifiers: F1 = 0 Hz, F2 = 0.237, F3 = 1.896 Hz, F4 = 2.60699 Hz, F5 = 2.72549 Hz. These frequencies were identified by manual brute-force searching and by visual inference of the power spectra for regions where prominent power fluctuations were observed distinguishing the two patient groups.

#### 2.7 Support vector machines (SVM)

Class sklearn.svm.SVC (C = 1.0, break_ties = False, cache_size = 200, class_weight = None, coef0 = 0.0, decision_function_shape = ’ovr’, degree = 3, gamma = ’scale’, kernel = ’linear’, max_iter = -1, probability = False, random_state = None, shrinking = True, tol = 0.001, verbose = False).

#### 2.8 Random forest (RF) classifier

The performance was constrained to a selected set of optimal frequencies (determined using SVM’s optimal performance). The hyperparameter tuning for the RF classifier was: RandomForestClassifier(max_depth = 6, max_features = ’sqrt’, min_samples_leaf = 3, min_samples_split = 10, n_estimators = 50).
2.9 Multilayer perceptron (MLP)

The hyperparameters for the MLP algorithm were tuned as follows:
```
class sklearn.neural_network.MLPClassifier(hidden_layer_sizes=(30, 30, 30), activation='relu', *, solver='adam', alpha=0.01, batch_size='auto', learning_rate='constant', learning_rate_init=0.001, power_t=0.5, max_iter=200, shuffle=True, random_state=None, tol=0.0001, verbose=False, warm_start=False, momentum=0.9, nesterovs_momentum=True, early_stopping=False, validation_fraction=0.1, beta_1=0.9, beta_2=0.999, epsilon=1e-08, n_iter_no_change=10, max_fun=15000).
```

2.10 Convolutional neural networks

The following is convolutional neural networks (Deep Learning algorithms) performed on the \( N = 118 \) FCS power spectral images obtained from the patient samples, as explained above.

2.11 Image CNN

The power spectra images were reshaped to uniform sizes using IMAGE_SHAPE = (224, 224) and an 80:20 training: Testing validation was used. Hyperparameter tuning was performed as follows:
```
optimizer = tf.keras.optimizers.Adam(lr = 1e-2)
model.compile( optimizer = optimizer, loss = 'categorical_crossentropy', metrics = ['acc']).
```

The training and cross-validation was set to epochs = 50, verbose = 1.
```
model = tf.keras.Sequential([hub.KerasLayer('https://tfhub.dev/google/tf2-preview/mobilenet_v2/feature_vector/4', output_shape=[1280], trainable=False), tf.keras.layers.Dropout(0.4), tf.keras.layers.Dense(train_generator.num_classes, activation = 'softmax')])
model.build([None, 224, 224, 3]).
```
2.12 ResNet image classification

Resnet models 101, 18, and 34 were attempted and all yielded nearly identical performance results. The model number was insensitive to our results. The hyperparameters were set as follows: bs = 64 (batch size): if your GPU is running out of memory, set a smaller batch size, i.e., 16 sz = 224 (image size), learn.fit_one_cycle (10, max_lr = slice(1e-3, 1e-3)), 80% for training and 20% for validation, and learn = cnn_learner (data, models. resnet101, metrics = accuracy). Further details of the ResNet hyperparameter tuning are provided in Table 2.

2.13 Quantum neural network

The study further involves benchmarking Quantum Convolutional Neural Network (QNN) algorithm [29] for comparing performance with classical convolutional neural networks (CNNs) used above. An 80:20 and 60:40 training: testing split were used as validation sizes on the N = 118 spectral images, as shown in the confusion matrices in Fig. 4C, D, respectively. The QNN hyperparameters are provided in Table 3. The workflow for the QNN algorithm was as follows:

1. Input raw data using Keras
2. Filtering the dataset to only 3 and 6 s
3. Down scales the images to fit in a quantum hardware.
4. Treating and removing contradictory examples
5. Convert binary images to Cirq circuits
6. Convert the Cirq circuits to a TensorFlow quantum circuit

2.14 Pre-processing/downscaling

OpenCV library was leveraged for morphological transformations, the method was employed majorly for handling noise and detection of intensity collisions. Further Image denoising and scaling using Python-OpenCV library was implemented to the entire dataset for standardization.

2.15 Circuit design approach

A two layered circuit (qubit = 3) was designed for the datasets with hinge loss as loss function and ADAM (adaptive learning rate optimization) optimizer instead of stochastic gradient descent-based optimizer being computationally inexpensive and easy to implement.

2.16 Linear and nonlinear feature extraction

The FCS power spectra data files were imported as a csv file containing the frequency as the first column, and subsequent columns corresponded to the Power (dB) measurements of each patient FCS power spectrum. The following feature selection methods were used to quantify additional spectral features which may be useful in downstream pattern analysis or prospective studies.

2.17 Principal component analysis (PCA)

PCA was performed as a linear dimensionality reduction on the N = 118 FCS power spectra using the Scikit-learn package in Google Colab (See GitHub link for code).

2.18 Nonlinearity dimensionality reduction

Diffusion Map and Isomap were used as nonlinear dimensionality reduction algorithms to observe whether any nonlinear features could help distinguish the healthy and cancer patients-derived power spectra. The Python codes for both algorithms are provided as Jupyter notebooks in the GitHub link.
2.19 Multifractal analysis

Multifractal analysis was used as a spectral feature extraction method to assess fractal dynamics in the time-series data. The Holder exponent was calculated using OriginPro, by taking the log–log plot of the power spectra and using a linear fit analysis on the log–log plot to estimate the slope $\alpha$ (i.e., the Holder exponent). The Hurst exponent was also computed using the MATLAB Wavelet Transform Modulus Maxima (WTMM) package using the $[dh1, hl, cp1, tauq1] = dwtlead (Power)$ function, where Power corresponds to the imported 1D vector (column) with the power (dB) measurements for a single power spectral sample. A table must be made with the computed Hurst exponent for all $N = 118$ samples.

3 Results and discussion

The results of our pilot study demonstrate for the first time that AI-driven FCS pattern analysis has the potential to become an accurate and automated diagnostic cancer screening tool that can be integrated in liquid biopsies and clinical precision oncology. Further, the data science tools, and medical informatics approaches used herein, namely, the combination of FCS power spectral analysis and statistical machine learning algorithms are innovative methods in liquid-biopsy cancer EVs characterization and cancer biomarker discovery. As shown in Fig. 2, the autocorrelation FCS spectra of healthy patient EVs sample and cancer patient EVs sample are shown in Fig. 2A, C, respectively. The characteristic inverted S-like autocorrelation curve is observed in both cases. There are prominent fluctuations seen in the tail ends of the curve. However, we predicted that using the Fast Fourier transform (FFT) to obtain the power fluctuations of the autocorrelation function in frequency space would provide a more robust screening tool to distinguish healthy patients’ spectra from cancer patients’ spectra. As such, the corresponding FFT-Power spectra for healthy and cancer patient EVs samples are shown in Fig. 2B, D, respectively. A power decay is observed in the fluctuations over the frequency range. We predicted machine learning algorithms, including binary classifiers and spectral image-based convolutional neural networks (CNNs) may be capable of better spotting patterns and signatures distinguishing the two patient groups using the processed power spectra.

Representative data are displayed from patient 417 (healthy) and patient 431 (cancer affected).

In Fig. 3, we see the statistical performances of various Scikit-learn ML classifiers on the processed FCS power spectra. All confusion matrices shown in Fig. 3 were subjected to a 50:50 training: testing validation split, for more stringent testing conditions. Their performances were found to be of higher accuracy with lower training sizes. The testing was also constrained to the power (dB) values at five selected frequencies, found to be the most optimal set of values for the performance of the SVM and RF classifiers. In Fig. 3A, the performance of a hyperparameter-tuned multilayer perceptron (MLP) neural network with 30 layers is shown as a confusion matrix. The classification accuracy was found to be 0.73 (i.e., 73%) with a tenfold cross-validation (CV) score of 61.33 ± 11.85%. The sensitivity was found to be 0.33 and the specificity was found to be 0.875, using the confusion matrix using the online confusion matrix calculator (See Data and Code Availability Section). The average f1-score, an additional measure of the ML’s accuracy, was found to be 0.61 and 0.71, for the healthy and cancer groups, respectively. The parametrization of our algorithms and their hyperparameter tuning was largely adopted from the ML classifiers trained/used in our previous study on the intelligent systems-based liquid-biopsy characterization of vibrational spectral patterns (in EVs).

In Fig. 3B, C, we see the cross-validation learning curve and confusion matrix for the ML performance of the AdaBoost Random Forest (RF) Classifier. The classification accuracy was found to be 0.9091, with a mean-square error of 0.09. The precision scores were found to be 0.92 and 0.91 for the healthy and cancer spectra, respectively, while the f1 scores were found to be 0.88 and 0.91, respectively. The sensitivity and specificity of the RF performance were 0.733 and 0.975, respectively. The RF classifier performed near the classification accuracy of our image-based CNNs when the complete power spectra were subjected to classification (i.e., near 80% accuracy) (data not shown). We found that its optimal performance is obtained by constraining the algorithm toward the five selected frequencies which optimized the SVM performance. Amidst all tested ML classifiers, the RF demonstrated the highest accuracy, sensitivity, and specificity. Further testing with larger patient-sample cohorts is required to validate its clinical potential.

In Fig. 3D, the performance of the Support Vector Machine (SVM) classifier is shown. The classification accuracy was found to be 0.618 with a tenfold cross-validation score of 69.33 ± 7.42%. The average f1-scores were 0.55 and 0.60 for the healthy and cancer groups, respectively. The sensitivity and specificity were found to be 0.30 and 0.80, respectively. SVM showed the poorest classification performance in terms of accuracy, amidst the three ML binary classifiers, as further explained by the poor linear separability seen in PCA analysis (See Fig. 5F). SVM uses a hyperplane to linearly separate data points from the two patient groups into two separate classes, and
hence, we suggest that such linear separability is a poor metric for classifying such complex spectral data.

(A) Multilayer Perceptron: Average precision for control group was 0.64 and for cancer group was 0.70. The average recall was 0.60 and 0.73, while the f1-scores were 0.61 and 0.71, respectively. The classification accuracy was 0.73. The tenfold CV score was 61.33 ± 11.85%. (B) and (C) random forest classifier: of a test set of 55 sample spectra out of 105 power spectra, Accuracy: 90.91%, MSE: 0.0909, CV = tenfold, cross-validation score was found to be 56\%.

To further validate our findings from the ML binary classifiers, we exploited image-based AI algorithms, namely CNNs on the FCS power spectra images. As shown in Fig. 4A, a Tensorflow image CNN’s performance is shown in the confusion matrix. The classification accuracy was 0.826, with a tenfold CV score of 0.74. The f1-score was found to be 0.875, whereas the sensitivity and specificity were 1.00 and 0.56, respectively. Although a perfect sensitivity is obtained, the accuracy and specificity are not as optimal and hence, such classification results should be interpreted with caution. In Fig. 4B, the cross-validation and learning curve for the Image CNN in Fig. 4A is shown. As seen, with increasing training steps, the validation curve (in orange) stabilizes to a near 0.74 CV accuracy score. Figure 4C, D displays the confusion matrices for the performance of a Quantum CNN adopted from Sengupta and Srivastava [29], with a training: testing validation sizes of 80:20 and 60:40, respectively. In Fig. 4C, the classification accuracy was found to be 0.833, while the f1-score was determined to be 0.882. The precision score was 0.938, while the sensitivity and specificity were both found to be 0.833, matching with the classification accuracy. In Fig. 4D, with the 60:40 validation size, the classification accuracy and f1-score were obtained as 0.78 and 0.864 m.
respectively. The precision score, sensitivity, and specificity were found to be 0.854, 0.875, and 0.400, respectively. As shown, while the QNN results seem to be of a reasonable classification performance with a lower validation size, when more stringent conditions are applied, there is a loss in accuracy and specificity. As such, the results remain inconclusive and require a larger patient cohort for clinical validation. Lastly, Fig. 4E shows the learning curve for the ResNet 34 CNN, which obtained equivalent results as those obtained for the Image CNN in Fig. 4A (i.e., the classification accuracy for the ResNet was 82.6%). The optimized hyperparameters $n$ for the ResNet network are provided in Table 2. Based on this preliminary evidence, we can conclude that the classical CNNs and the QNN perform very similarly on our dataset with near 80% classification accuracy. The tuned hyperparameters for the QNN are provided in Table 3. Given the complexity of the power spectra, we suggest these findings support the concept that CNNs be used as a cross-validation tool along with the RF classifier discussed above, in larger patient cohort screening in prospective studies.

Finally, we explored some feature extraction algorithms to determine whether certain dimensionality reduction algorithms or multifractal characteristics of the complex FCS spectra can be used to distinguish the patient groups in prospective ML analyses. As shown in Fig. 5A, Diffusion Map shows a clear separation between the two patient groups’ power spectra. In contrast, Fig. 5B, shows that Isomap, a local multi-dimensional scaling without the local optima, performs poorly in separating the two patient groups. Figure 5C, D displays two types of multifractal analyses, the Holder exponents (log–log plot scaling determined by the linear best-fit/correlation for the power spectra) and the Hurst index computed using the wavelet-based WTMM algorithm, are also poor classifiers of the two patient groups. Thus, no unique multifractal feature could distinguish the patient spectra, as further supported by the poor classification performance of the Hurst index scores of the two patient groups for the $N = 118$ spectra, using the AdaBoost RF classifier shown in Fig. 5E. Lastly, Fig. 5F shows that there is no linear separability in the power spectra by the linear dimensionality reduction.
algorithm PCA. PCA was used as a linear feature extraction method to verify whether the fluorescence fluctuations can be classified as linearly separable signatures of the heterogeneous EVs of both patient groups (i.e., healthy vs. cancer). However, PCA was unable to find such classification due to the complexity of the spectral/intensity fluctuations, and thus necessitating machine learning approaches. As shown in Fig. 5A, only Diffusion map, a nonlinear dimensionality reduction seems to separate the EVs features into their distinct binary classifications assigned. Prospective studies when extended to larger patient samples should exploit Diffusion maps as a feature analysis algorithm to potentially increase the classification accuracy and sensitivity/specificity metrics.

These preliminary tests of feature extractors show that Diffusion Map may hold potential in optimizing the image-based CNNs or ML classifiers in prospective studies due to their clearer separability of the two patient groups’ power spectra. Further, there remains a vast amount of nonlinear feature extraction methods which were not tested in our pilot study, including but not limited to, graph spectral clustering algorithms, spectral algorithmic complexity estimates, Gaussian processes, nonlinear neighborhood component analysis, and multivariate information-theoretic measures. We strongly suggest the testing of these nonlinear feature extraction methods in prospective analyses with larger patient cohorts. Prospective studies should also extend our applied neural computing methods and algorithms to other liquid-biopsy cancer biomarkers such as epigenetic markers (e.g., single-molecule profiling of plasma circulating nucleosomes (i.e., histone marks), methylation profiles of circulating tumor/cell-free DNA, etc.), proteins, metabolites, and immune-signals/cytokines [30].

4 Limitations

As shown by the classification results and their cross-validation scores, we should always keep in mind the limitations of these ML tools. Further, there are limitations in the Deep Learning frameworks of the CNNs used as well. For instance, adding a little random noise to CNN can largely fool its image classification. Flipping an image that was not
in training, can also overthrow the algorithm’s classification to false discovery (i.e., false positives). To overcome these barriers, a larger patient-sample size is fundamentally required to validate the clinical benefits and relevance of our study.

Usually, Deep learning approaches are data hungry methods and using only 118 samples typically is not enough to obtain a clinically meaningful good predictive performance. However, our pilot study sets the initial framework to extend these applied intelligent systems in patient-derived liquid-biopsy characterization in computational precision medicine. The results should be treated as that of an interdisciplinary pilot study pioneering the coupling of FCS spectra, AI, and EVs-based cancer screening with high accuracy and promising results as seen in the RF’s performance. A plausible explanation for the 90% classification accuracy in the RF classifier and not higher performance could be that benign mass patients were categorized as healthy for the ML training and assessment. Thus, given the vast heterogeneity and complexity of the tumor samples we analyzed within our pilot study of \( n = 24 \) patients, with their distinct cell of origin/tissue subtypes, we can safely agree that our results warrant further analysis given its high statistical performance metrics for some algorithms like the RF classifier and the CNNs. The quantum machine learning showed near equal accuracy with the CNN, and hence, we conclude there were no additional advantages provided by Quantum machine learning. Given that Quantum optimized hardware and resources are needed for such quantum machine learning, as far as CNN-like algorithms are concerned, our study suggests prospective studies with larger cohorts of patients for clinically relevant assessments could simply adhere to the use of classical CNNs for validation of our pilot results. For instance, the quantum ML results obtained in our pilot study employed FPGA (field programmable gate array) with a 256 GB RAM memory system. However, CPU or GPU systems can also be compatible with the Quantum AI codes. Cloud server systems are available for overcoming the computational memory resources.

Further, it should be noted that in contrast to the data-driven statistical machine learning algorithms we have utilized herein, there are various model-driven AI approaches better suited for complex feature analyses and forecasting patterns from the temporal features of complex time-series datasets not investigated herein. Some examples of such algorithms include recurrent neural networks such as liquid neural networks and Hopfield neural networks. There are certain biochemical limitations which were screened for during the FCS measurements, such as the clustering or clumping of EVs. The presence of such large aggregates/clumps was screened by the emergence of large spikes in the fluctuation intensity spectra (i.e., FCS counts) during the measurements. They could be additionally filtered manually by selecting time-windows omitting their presence, which was not needed in our case due to the careful analysis by the ABIF technician. Future studies should also investigate time-resolved spectroscopies with label-free (unstained) EVs.

4.1 Novelty and impact

Our innovative study shows for the first time that combining machine learning techniques/intelligent systems, and in specific, neural computing with FCS) could help provide accurate and automated early cancer detection, precision diagnostics, and prognostic cancer screening. While our previous study in Uthamacumaran et al. [19] investigated vibrational spectroscopies with static snapshots of cancer EVs vibrational dynamics, the presented study investigated the collective temporal dynamics and aggregate behaviors in fluid conditions by means of time-resolved spectroscopy. Our novel approach also consists of innovative informatics techniques such as FCS power spectral fluctuations analysis. Further, our pilot study remains the first application of neural networks/computing in characterizing cancer EVs in FCS spectra. Our intelligent systems have potential applications to improving precision diagnostics, cancer prevention, and clinical therapy/disease monitoring in patient-centered medicine.

4.2 Prospective studies and applications

As mentioned, in our previous study, we have already explored vibrational spectroscopies such as Raman and FT-IR. Herein, we explored for the first time the use of FCS, a time-resolved spectroscopy technique, to quantify, characterize and distinguish cancer patient-derived EVs from healthy patients-derived EVs. Future studies should further expand on our findings with larger cohorts of patients including cancers of distinct tissue subtypes and stage/grades. Further, there remains many other spectroscopic methods which can be coupled to patient-derived EVs and AI, including mass spectrometry techniques, SERS, Terahertz spectroscopy, and high-energy spectroscopies, to name a few. There may be other types of time-resolved spectroscopy, or the above-mentioned spectroscopies can be adapted to time-resolved methods (e.g., Raman time-lapse imaging).

As mentioned, in contrast to the data-driven methods exploited herein, causal inference models like RNNs, namely, liquid cybernetics (i.e., liquid neural networks), LSTM, or reservoir computing, and Hopfield neural networks, and neuro-symbolic computation methods should be exploited in future large-scale time-series analyses when dealing with more than hundreds of patients [31, 32]. These
initiatives could be useful in the automated scientific/pattern discovery of complex patients-derived EVs spectra and the molecular fingerprinting of the time-series EVs power fluctuations in the future of personalized/precision cancer nanomedicine. We have strictly focused our analyses on statistical ML-based classification. Further analyses should extend to physics and model-driven AI approaches for causal discovery, prediction, and forecasting, as discussed above. Further, in contrast to the binary classifiers used herein, future studies should investigate fuzzy systems and applied soft computing methods which treat the input values as continuous variables between 0 (healthy/control) and 1 (cancer). Prospective studies should also explore the applicability of Quantum Random Forest classifiers or Quantum Decision Trees (an ensemble of which becomes the RF) on the power spectra. Domain-free and model-independent feature selection algorithms optimized for minimal loss of algorithmic complexity should also be employed in prospective pattern analyses.

To conclude, our experiments are part of different pilot studies performed in the field of early cancer detection and liquid-biopsy classification of patient-derived EVs, driven by AI systems. Our innovative study demonstrates for the first time the interdisciplinary application of combined FCS and machine intelligence in characterizing patients-derived EVs as circulating cancer biomarkers. Our findings show that the coupling of ML algorithms with spectroscopy in the characterization of EVs provides a noninvasive, quick, efficient, accurate, and automated/intelligent diagnostic screening for cancer patients. Our pilot study warrants further advancement of the presented pairing of time-resolved spectroscopic techniques and machine intelligence in the characterization of cancer patients-derived EVs. The presented approach may help in disease prevention and therapy management by serving as a candidate for noninvasive, automated diagnostic, and prognostic blood-based clinical screening. Our findings suggest such applied intelligence may bear fruits in the progression of computational medicine, systems oncology, and (diagnostic) precision nanomedicine.

Appendix

Quantum ml circuit
The circuit from the training samples in the first iteration of the 2-layer circuit, reproduced from Sengupta and Srivastava [29]. For further details of the QNN, refer to the citation.

Acknowledgements
We are grateful to Ayat Salman for her assistance with the Ethical Committee approvals. Fluorescence correlation spectroscopy measurements were taken by Joel Ryan at the McGill Advanced BioImaging Facility (ABIF, RRID: SCR_017697).

Funding
This work was financially supported by Giuseppe Monticciolo and the Morris and Bella Fainman Family Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data and code availability
All codes and sample datasets obtained in this experiment are made available in the GitHub link below. GitHub link: https://github.com/Abicumaran/FCS_EVClassification. Multifractal analysis: WTMM toolbox guidelines in MATLAB to extract Hurst scaling exponent: https://www.mathworks.com/help/wavelet/ug/multifractal-analysis.html. https://onlineconfusionmatrix.com/ (to calculate the sensitivity and specificity from the confusion matrices).

Declarations

Conflict of interest
The authors declare no conflict of interest.
Ethical approval Patients were recruited in accordance with an approved ethics protocols by the Ethics Committee of the McGill University Health Centre (MP-37-2018-3916 and SDR-10-057). Patients signed consents were obtained before enrollment in the study.

Preprint An earlier version of the article is available at the Arxiv Preprint server in the following link: https://arxiv.org/abs/2202.00495.

References

1. Gold P, Freedman S (1965) Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 121(3):439–462. https://doi.org/10.1084/jem.121.3.439
2. Zhou B, Xu K, Zheng X et al (2020) Application of exosomes as liquid biopsy in clinical diagnosis. Sig Transduct Target Ther 5:144. https://doi.org/10.1038/s41392-020-00258-9
3. Alix-Panabière C, Pantel K (2013) Circulating tumor cells: liquid biopsy of cancer. Clin Chem 59(1):110–118. https://doi.org/10.1373/clinchem.2012.194258
4. Han X, Wang J, Sun Y (2017) Circulating tumor DNA as biomarkers for cancer detection. Genomics Proteomics Bioinformatics 15(2):59–72. https://doi.org/10.1016/j.gpb.2016.12.004
5. Bronkhorst AJ, Ungerer V, Holdenrieder S (2019) The emerging role of cell-free DNA as a molecular marker for cancer management. Biomol Detect Quant 17:100087. https://doi.org/10.1016/j.bdq.2019.100087
6. Sui J, Wu X, Wang C et al (2021) Discovery and validation of methylation signatures in blood-based circulating tumor cell-free DNA in early detection of colorectal carcinoma: a case–control study. Clin Epigenet 13:26. https://doi.org/10.1186/s13148-020-00985-4
7. Zhao Z, Fan J, Hsu Y-M, Lyon CJ, Ning B, Hu TY (2019) Extracellular vesicles as cancer liquid biopsies: from discovery, validation, to clinical application. Lab Chip 19(7):1114–1140. https://doi.org/10.1039/c8lc01123k
8. Johnstone RM (2005) Revisiting the road to the discovery of exosomes. Blood Cells Mol Dis 34(3):214–219. https://doi.org/10.1016/j.bcmd.2005.03.002
9. Pan B-T, Johnstone RM (1983) Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell 33:967–976
10. Pan B-T, Teng K, Wu C, Adam M, Johnstone RM (1985) Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 101:943–948
11. Szatean R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, Baran J (2017) The methods of choice for extracellular vesicles (EVs) characterization. Int J Mol Sci 18:1153. https://doi.org/10.3390/ijms18061153
12. Abdouh M, Zhou S, Arena V, Arena M, Lazaris A, Onerrheim R, Metrakos P, Arena GO (2014) Transfer of malignant trait to immortalized human cells following exposure to human cancer serum. J Exp Clin Cancer Res 33:86
13. Abdouh M, Hamam D, Arena V, Arena M, Alamri H, Arena GO (2016) Novel blood test to predict neoplastic activity in healthy patients and metastatic recurrence after primary tumor resection. J Circ Biomark. https://doi.org/10.1177/1849454416663661
14. Abdouh M, Tsering T, Burnier JV, de Alba Graue PG, Arena G, Burnier MN (2020) Horizontal transfer of malignant traits via blood-derived extracellular vesicles of uveal melanoma patients. Invest Ophthalmol Vis Sci 61(7):2835
15. Arena GO, Arena V, Arena M, Abdouh M (2017) Transfer of malignant traits as opposed to migration of cells: a novel concept to explain metastatic disease. Med Hypotheses 100:82–86
16. Shoa S et al (2017) Reprogramming malignant cancer cells toward a benign phenotype following exposure to human embryonic stem cell microenvironment. PLoS ONE 12(1):e0169899
17. Su C, Zhang J, Yarden Y et al (2021) The key roles of cancer stem cell-derived extracellular vesicles. Sig Transduct Target Ther 6:109. https://doi.org/10.1038/s41392-021-00499-2
18. Fu X, Song Y, Masad A, Nini K, DeRouchey JE, Richards CI (2020) High-throughput fluorescence correlation spectroscopy enables analysis of surface components of cell-derived vesicles. Anal Bioanal Chem 412(11):2589–2597. https://doi.org/10.1007/s00216-020-02485-z
19. Uthamacumar A, Elouatiuk S, Abdouh M et al (2022) Machine learning characterization of cancer patients-derived extracellular vesicles using vibrational spectroscopies: results from a pilot study. Appl Intell. https://doi.org/10.1007/s10489-022-03203-1
20. Shin H, Oh S, Hong S, Kang M, Kang D, Yi YG, Choi BH, Kang KW, Jeong H, Park Y, Hong S, Kim HK, Choi Y (2020) Early-stage lung cancer diagnosis by deep learning-based spectroscopic analysis of circulating exosomes. ACS Nano 14(5):5435–5444. https://doi.org/10.1021/acsnano.9b09119
21. Park J et al (2017) Exosome classification by Pattern analysis of surface-enhanced Raman spectroscopy data for lung cancer. Anal Chem 89(12):6695–6701
22. Shin H et al (2018) Correlation between Cancerous Exosomes and Protein Markers Based on Surface-Enhanced Raman Spectroscopy (SERS) and Principal Component Analysis (PCA). ACS Sensors 3(12):2637–2643
23. Ries J, Schwille P (2001) Fluorescence correlation spectroscopy. Springer series in chemical physics, vol. 65. https://doi.org/10.1007/978-3-642-59542-4
24. Thompson NL (2002) Fluorescence correlation spectroscopy. In: Lakowicz JR (eds) Topics in fluorescence spectroscopy, vol I. Springer, Boston. https://doi.org/10.1007/0-306-47057-8_6
25. Elson EL, Magde D (1974) Fluorescence correlation spectroscopy. I. Conceptual basis and theory. Biopolym: Orig Res Biopolym 13(1):1–27. https://doi.org/10.1002/bip.1974.360130102
26. Corso G, Heusermann W, Trojer D, Görgens A, Steib E, Voshol J, Graff A, Genoud C, Lee Y, Hean J, Nordin JZ, Wiklander O, El Andaloussi S, Meisner-Kober N (2019) Systematic characterization of extracellular vesicle sorting domains and quantification at the single molecule—single vesicle level by fluorescence correlation spectroscopy and single particle imaging. J Extracell Vesicles 8(1):1663043. https://doi.org/10.1080/20013078.2019.1663043
27. Wyss R, Grasso L, Wolf C, Grosse W, Demurtas D, Vogel H (2014) Molecular and dimensional profiling of highly purified extracellular vesicles by fluorescence fluctuation spectroscopy. Anal Chem 86(15):7229–7233. https://doi.org/10.1021/ac501801m
28. Pedregosa F et al (2011) Scikit-learn: machine learning in python. JMLR 12:2825–2830
29. Sengupta K, Srivastava PR (2021) Quantum algorithm for quicker clinical prognostic analysis: an application and experimental study using CT scan images of COVID-19 patients. BMC Med Inform Decis Mak 21:227. https://doi.org/10.1186/s12911-021-01588-6

30. Fedyuk V, Erez N, Furth N, Beresh O, Andreishcheva E, Shinde A, Jones D, Zakai BB, Mavor Y, Peretz T, Hubert A (2022) Multiplexed single-molecule epigenetic analysis of plasma-isolated nucleosomes for cancer diagnostics. BioRxiv. https://doi.org/10.1101/2021.11.01.466724v2.full

31. Maass W et al (2002) Real-time computing without stable states: a new framework for neural computation based on perturbations. Neural Comput 14:2531–2560

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Abicumaran Uthamacumaran1,2 • Mohamed Abdouh3,4 • Kinshuk Sengupta5 • Zu-hua Gao6 • Stefano Forte7 • Thupten Tsering3 • Julia V. Burnier3,8,9 • Goffredo Arena10,11,12

✉ Abicumaran Uthamacumaran
a_utham@live.concordia.ca
✉ Goffredo Arena
goffredoarena@gmail.com

1 Department of Physics (Alumni), Concordia University, Montreal, QC, Canada
2 McGill Genome Center (Majewski Lab), 740 Dr Penfield Ave, Montreal, QC H3A 0G1, Canada
3 Cancer Research Program, Research Institute of the McGill University Health Centre, 1001 Decarie Boulevard, Montreal, QC H4A 3J1, Canada
4 The Henry C. Witelson Ocular Pathology Laboratory, McGill University, Montreal, QC, Canada
5 Microsoft Research and Development, New Delhi, India
6 Department of Pathology, University of British Columbia, G105-2211 Wesbrook Mall, Vancouver, BC, Canada
7 IOM Ricera, Via Penninazzo 11, 95029 Viagrande, Italy
8 Gerald Bronfman Department of Oncology, McGill University, Montreal, QC, Canada
9 Experimental Pathology Unit, Department of Pathology, McGill University, Montreal, QC, Canada
10 Istituto Mediterraneo Di Oncologia, Viagrande, Italy
11 Department of Surgery, McGill University, Montreal, QC, Canada
12 Fondazione Gemelli-Giglio, Contrada Pollastra, Cefalù, Italy