A Real-Time Suite of Biological Cell Image Analysis Software for Computers, Smartphones, and Smart Glasses, Suitable for Resource-Constrained Computing

Alexandre Matov[1, †]

[1] DataSet Analysis LLC, 155 Jackson St, San Francisco, CA 94111

[†] Corresponding author:

email: matov@datasetanalysis.com

Key words: Real-Time Detection and Feature Tracking, On The Fly Image Analysis, Medical Software, Diagnostic Software, Resource-Constrained Computing, Multiplatform Software, Smartphones Software, Smart Glasses Software

ABSTRACT

Introduction: Methods for personalizing medical treatment are the focal point of contemporary clinical research. In cancer care, for instance, we can analyze the effects of therapies at the level of individual cells. Complete characterization of treatment efficacy and evaluation of why some individuals respond to specific regimens, whereas others do not, requires additional approaches to genetic sequencing at single time points. Methods for the continuous analysis of changes in phenotype, such as morphology and motion tracking of cellular proteins and organelles over time frames spanning the minute-hour scales, can provide important insight to patient treatment options. The integration of measurements of intracellular dynamics and the contribution of multiple genetic pathways in degenerative diseases is vital for the development of biomarkers for the early detection of pathogenesis and therapy efficacy.

Methods: We have developed a software suite (DataSet Tracker) for real-time analysis designed to run on computers, smartphones, and smart glasses hardware and suitable for resource-constrained, on-the-fly computing in microscopes without internet connectivity; a demo is available for viewing at datasetanalysis.com.

Results: Our objective is to present the community with an integrated, easy to use by all, tool for resolving the complex dynamics of the cytoskeletal meshworks, intracytoplasmic membranous networks, and vesicle trafficking. It is our goal to have this integrated tool approved for use in the clinical practice.

INTRODUCTION

The complex dynamics of cytoskeletal proteins make them a difficult subject of study without quantitative analytical tools. The *ex vivo* analysis of living patient-derived cells comprises of the evaluation of the response of cytoskeletal filaments and meshworks, intracytoplasmic membranous networks, and vesicle trafficking before and after drug treatment and requires motion tracking. Tracking is the process of finding object (feature) motion correspondence from one time-lapse frame to the next throughout a time-lapse sequence. The features can be fluorescently labeled proteins or imaged label-free using a microscopy technique that highlights the gray-scale intensity gradient to their immediate surroundings in the image. Methods that can reliably analyze the evolution of the morphology and localization of cellular proteins over hundreds of time-lapse frames will be very relevant for capturing the changes in the subcellular organization in disease and during treatment (*1-4*). Such methods allow physicians to compare visually and quantitatively the effects of treatment regimens and select the one most likely to be efficacious.

Alternatively, intercellular dynamic behavior can be analyzed with available computer vision robotics libraries, which deliver results in real-time. On-the-fly image analysis and instant quantitative feedback can significantly speed up clinical work and allow for the precise calibration of the imaging set-up and optimization of the drug regimens based on considerations of their effects on cell function. To improve on the ability to precisely calibrate the set-up, augmented reality artificial intelligence software can be added to existing live-cell microscopes, thus providing instant feedback during sample observation and image acquisition. The selection of drug dose and deducing its effects, depending on the dose administered, is a daunting task. During treatment of tubulin inhibitors, for instance, different microtubule (MT)-associated proteins are activated depending on the drug dose administered, and consequently, different resistance mechanisms may be triggered. The effects of MT inhibitors change nonlinearly with a dose increase. While a high dose of nocodazole depolymerizes MTs, a low dose,

which could lead to no side effects, increases polymerization rates (*5*). Also, while a high dose of paclitaxel stabilizes MTs, a low dose similarly increases MT polymerization rates (*3*).

Our work has been focused on the automated analysis of cytoskeletal dynamics, predominantly those of MTs (*6-8*) and filamentous actin (F-actin) (*9, 10*), to study the effects of tubulin, actin, and tropomyosin inhibitors and post-translational modifications as well as modulation of focal adhesions (FAs) (*11*) and ectopic activation of GSK3β (*12*). During pathogenesis, or as a result of drug treatment in disease, cells change their intracellular organization, rearrange their internal components as they grow, divide, and adapt mechanically to a hostile environment. These functions depend on protein filaments (the cytoskeleton and FAs), which provide the cell shape and its capacity for directed movement.

In this effort, we have developed a real-time computer vision software, which connects to the microscope‘s camera, processes multiple image frames per second (five frames per second for the example in Fig. 1 – see lower right corner of the image and Video 1, supplemental data), and instantly displays and stores statistical readouts in parallel to sample observation and image acquisition. Our software is optimized for resource-constrained computing and can be installed even on microscopes without internet connectivity. Our computational platform can provide high-content analyses and functional secondary screening of novel compounds that are in the process of approval, or at a pre-clinical stage of development, and putative combination therapies based on FDA-approved drugs. Importantly, dissecting the mechanisms of drug action with quantitative detail will allow the design of drugs that impede relapse and optimal dose regimens with minimal harmful side effects by carefully exploiting disease-specific aberrations.

The main discoveries presented in the manuscript are related to the ability to quantify and visualize, in real time, the changes in morphology and motion of cellular components as the cell adapts to a wide

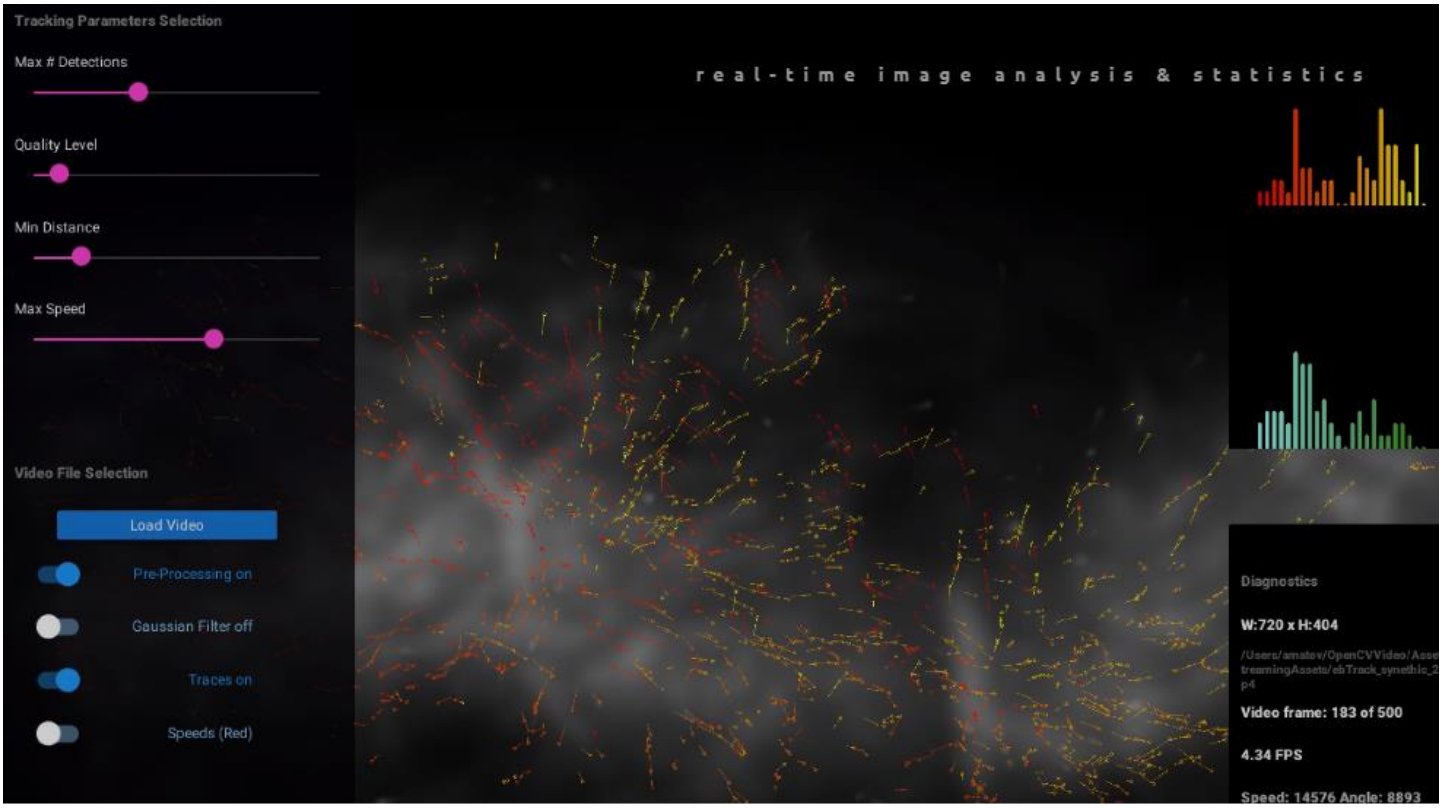

Figure 1. Tracking the motion of synthetic markers in time-lapse image series. The figure presents novel software developed by DataSet Analysis (a demo video of this package is available for viewing at datasetanalysis.com/synthetic-demo) with the gaming engine of Unity Technologies. The figure shows computer vision analysis of the motion of synthetic markers, which mimic live-cell fluorescent microscopy image sequences. Displacement vectors color-coding is used to show the angular direction as well as the speed of motion. A button selection allows changing the display preferences. On the figure, yellow vectors move to the right and are also shown in yellow within the right peak of the bi-modal histogram. Similarly, the vectors moving to the left are in red, both on the image overlay and within the left peak of the bi-modal histogram in the upper right corner of the screen. The second display option (not shown) changes the displacement vectors color-coding to showing different shades of green, depending on the speed. Observe on the unimodal histogram to the right showing that most of the particles move slower (the light-green peak to the left), while a few particles move very fast (the dark-green distribution tail on the right side). Real-time information on the frames per second analyzed, the average values for the speed and the angular vector orientations are displayed in the lower right corner of the screen. On the left side of the screen, there are sliders in the upper left corner, which allow to set the (i) the upper limit for the number of detected particles based on the a priori knowledge of the nature of the motion in the analyzed sample, (ii) the level of statistical significance for the particle selection step, i.e., the level of particle detection stringency, (iii) the minimum distance between particles, which is another parameter selection done based on a priori knowledge of the type of sample analyzed, and (iv) a cut-off for the particle search radius, which limits the maximal allowed displacement; this is another parameter, which is selected based on the knowledge of the sample. By providing sample-specific input to the tracking module, the parameters selection allows to limit the computational complexity, to minimize the tracking errors and to deliver the fastest analysis results. The blue buttons in the lower-left corner of the screen allow to change various aspects of the screen display in terms of showing segmentation or tracking results, single-segment tracks (between just two frames) or the aggregated trajectories and, as described above, the vector color-coding (angles in red/yellow vs. speeds in different shades of green). We will extend the current real-time 2D functionality to 3D analysis using AI algorithms.

range of biochemical and mechanical perturbations. We have investigated these changes in the context of the two main cytoskeletal proteins, tubulin and actin. Tubulin has been the target of numerous therapeutic approaches for six decades now. Most of these therapies have been, in essence, a “black box” approach, where a treatment with debilitating side effects is administered, without having the ability to anticipate whether it would affect the desired target.

With our approach, an instant quantitative evaluation of some of the more difficult to visually interpret effects of tubulin-targeting compounds can be performed while calibrating the imaging set-up and during image acquisition, which will facilitate deducing mechanistic explanations for the reasons behind the measured differences. Actin, for instance, is instrumented in wound healing as well as during tumor metastasis; it is also the most important protein in heart and other muscle function. Very few drugs currently modulate actin activity directly, albeit the abundant availability of anti-inflammatory medications which affect the COX pathway. It is the variability of patient response in genomics precision medicine that underscores the importance of utilizing quantitative imaging techniques in the clinic.

A plethora of the cellular proteins exhibit ambiguity in function and response to perturbations. There exists a high level of regional variability in the dynamics of polymer networks in living cells, which are considerably more interdigitated than initially perceived and reported in the literature. Drugs bind to proteins within these networks and affect their function. Consequentially, the modulation of MT and F-actin dynamics inevitably affects drug efficacy. Answers about the potential efficacy of a drug can, thus, be obtained by utilizing clinical software systems that measure, in great detail, the effects of a regimen on its cellular targets. Molecular manipulations of living patient-derived cells *ex vivo* can reveal which secondary mechanism would be activated after a particular intervention. Such clinical research can aid the discovery of an optimal drug selection for each disease, which is the motivation for this work.

This manuscript explores, for the first time, a real-time quantitative way, the changes occurring in the cell. All degenerative diseases are associated with impairment in intracellular trafficking. The highly dynamic organization and remodeling of the cellular cytoskeleton are dysfunctional in pathology and often lead to drug resistance. Successful analyses of the mechanism of drug action require statistical analysis of large-scale readouts of molecular interactions. Our objective has been to develop resources

for functional interrogation of drug response in a physiologically relevant system amenable to molecular manipulations and investigate personalized drug response *ex vivo*. We have developed image analysis software for automated motion tracking of labeled microtubules and filamentous actin – ClusterTrack (*1*) (for measurements of interphase cells), Instantaneous Flow Tracker (*2*) (for measurements of interdigitated flows in dividing cells, contractile filamentous actin meshworks in migrating epithelial cells and growth cones), and DataSet Tracker (for real-time optical flow feature tracking – this contribution), which can serve as the base module of an integrated platform of all existing and future algorithms for real-time cellular analysis. The computational assay we propose could successfully be applied to evaluate treatment strategies for any human organ.

REAL-TIME OPTICAL TRACKING

For the purposes of demonstrating the workings of our real-time analysis software, we have generated a synthetic video displaying the radial motion of fluorescent features originating from two distinct focal points (Fig. 1 and Video 1, supplemental data). In this contribution, we pre-process images making up the video and select features for motion tracking on-the-fly. For each feature type, we will extract a set of distinctive key features, identify descriptor vectors of the features and compute motion metrics to distinguish between drug-resistant and sensitive profiles in cells derived from patients and elucidate mechanisms of resistance. This quantitative analysis (Video 2, supplemental data) will, therefore, allow for the identification of the key signaling pathways involved in resistance, which may contribute to the personalization of the drug treatment regimen and the functional testing of novel compounds. Our approach will allow evaluating the relative contribution of different signaling pathways in drug response in disease cells originating from different organs and tissues. Our preliminary software development uses a 2D tracking strategy in which we utilize three steps performed by well-established algorithms implemented as real-time libraries by OpenCV (*13, 14*). In brief, images making up the video are pre-processed or de-noised by a specialized background subtraction method (*15, 16*). Next, a watershed-

based algorithm was used to select the features for tracking (*17*). Lastly, the Lucas-Kanade optical flow algorithm, which has been cited some 20,000 times, was used for motion tracking (*18*). The development environment we used was of the cross-platform game engine of Unity Technologies Inc. We compiled and tested our software on multiple platforms, such as Windows PC, Macintosh computer, iPhone smartphone, Android smartphone, and Microsoft HoloLens smart glasses.

A key novelty is that our computational platform outputs results in real-time, in parallel to image acquisition, which facilitates and speeds up research and clinical efforts by delivering instant visualization of data analysis and statistical outcomes. Such approach offers a clear improvement on the selection of imaging parameters and drug concentrations empirically by offering precise quantification of the dynamics of the underlying cellular processes.

REAL-TIME MOTION TRACKING WITH TRANSFORMERS

To extend the current functionality into obtaining the full trajectories, we will utilize the vectors obtained in each frame, together with the associated information on feature intensity and morphology, to generate embeddings (*19*) for a generative transformer network in which the tokens are the spatial coordinates of the features we track. This will allow training of the network to associate the most likely next feature in an image sequence, similar to the way large language models generate text. Further, we will retrain a transformer network with a new set of tokens - with lists of 2D or 3D coordinates rather than words and with trajectories (lists of linked coordinates) rather than sentences of human speech, i.e., we will retrain a large language model with motion trajectory data. We have generated several thousand time-lapse microscopy movies with about 2-10,000 trajectories consisting of about 3-20 features (either MT end-binding proteins or fluorescent speckles) linked in each trajectory (*1, 3-6, 8, 20-25*). These are about 60 million data points, but many more existing movies/data can be obtained from our academic collaborators. As we have pre-processed the data, the preliminary computer vision analysis has already

been done and the results have been validated and published, which will facilitate the training and benchmarking.

CONCLUSIONS

Until now, the accurate computational analysis of dynamic cytoskeletal structures was limited due to the lack of appropriate software tools. Since the entangled networks in the cell are fast evolving, rapidly changing their turnover rates and directions of motion, it has been a very tedious process for scientists using traditional tracking methods to resolve complex motion patterns. Our method, by not requiring any additional steps for the analysis but rather displaying the tracking vectors in real time, can aid in establishing a new level of insight into cellular processes and, thus, advance our understanding of the dynamic organization of the cytoskeleton, cell division and motility. In doing so, it is our goal to advance the field of drug development and contribute to improving patient care.

The potential clinical application of the analytics approach outlined in this manuscript pertains to anticipating drug resistance in cancer therapy and the treatment of neurodegeneration based on the microscopic evaluation of living patient cells *ex vivo*. Cell therapy is becoming the forefront of precision medicine, and it would be critical to be able to anticipate the mechanisms of action of the engineered immune cells. Natural killer cells and cytotoxic T cells employ different mechanisms to kill their targets, for instance by secreting cytotoxic lysosomes using the MT cytoskeleton for trafficking and release. There is also a plethora of effects induced in the target cells, most well-studied of which lead to apoptosis and ferroptosis, a recently identified potent, caspase-independent, mechanism of programmed cell death. The exact pathways activated during therapy can be pinpointed by measuring its effects on the target proteins in patient-derived living cells *ex vivo*.

It is conceivable that for different patients, with overall very similar genetic profiles, a distinct type of engineered immune cells will be required. Given the availability of high-quality fundamental research equipment for high resolution live-cell microscopy in most university hospitals, what we propose is to embed in these systems such real-time software for automated quantification and on-the-fly statistical analysis of intracellular behavior. Introducing this quantitative imaging method to the clinic will allow physicians to fine-tune, with a high level of certainty, an optimal treatment regimen for each patient.

The main concern regarding patient treatment, which motivated this work, has been that important differential effects of therapies on their cellular targets pertaining to drug efficacy cannot reliably be identified with genetic sequencing or pathology slides alone. There are differences in the mechanisms with which cells react to treatment, for example in regard to the immune system, which can only be appreciated by live-cell imaging methods. The effects of a physiologically relevant dose of a drug are not visible to the naked eye, looking through the microscope lens, because of the inability of our vision to detect gradient in pixel intensity when bright features are co-localized with dim features. In many instances, the cell biology imaging methods require quantification because of the overwhelming number of cellular proteins and components involved. For these reasons, we suggest real-time image analysis software as a tool to extract a quantitative readout of the underlying processes. A tool not only capable to anticipate drug resistance for existing regimens, but also to be applied in the identification of putative molecular targets during drug discovery. Quantifying with ease the morphological, localization and other dynamical changes in patient cells can certainly help clinical decisions and support timely changes in the original course of treatment by providing feedback and evidence for treatment efficacy, or the lack thereof, in real time. It will also advance our understanding of the regulation of the cells comprising our organs and tissues in normal physiology and in disease.

Non-invasive, for instance from urine samples, or minimally-invasive methods for obtaining patient cells allow to monitor disease progression and patient response to treatment throughout the course of a clinical trial. We can transduce cells and label proteins of interest overnight and, depending on the disease and drugs used, tests can be carried out as soon as a few hours after sample collection. Having the ability to test a number of drugs and combinations *ex vivo* will be critical in the treatment of pathologies for which there is no known cure. Once the specific impaired molecular mechanisms is identified for the particular patient, treatment options which correct the aberrations can be selected. This way, we can transform patient treatment.

MATERIALS AND METHODS

Image analysis

All image analysis programs for detection and tracking, and graphical representation of the results were developed in the cross-platform game engine Unity in C#. The computer code is available for download at: https://www.github.com/amatov/DataSetTracker. OpenCV for Unity requires a license from Enox Software.

Ethics declaration

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

No competing interests.

Funding

No funding.

Authors' contributions

A.M. conceived the project, wrote the computer vision code, prepared the figures, and wrote the manuscript.

ACKNOWLEDGEMENTS

I thank James Cumberbatch for his help with Unity and XCode.

SUPPLEMENTARY MATERIALS

Video 1 – Synthetic movie with moving bright features. https://vimeo.com/999588708/43e6111879

Video 2 – Overlay of Video 1 with vectors displaying the tracking results.

https://vimeo.com/999589508/d6dbcf35f5

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