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Cellular Imaging to Biometrics

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1 Introduction

At the most basic, biometrics can be best explained by breaking down the word: bio, as in biological, and metric as in measurement. That is to say, biometrics are associated with life measurement. They represent a set of metrics extracted from biological data using mathematical and statistical methods to quantify living organisms. When these metrics are unique, biometrics are particularly suited for identification through DNA, face, iris, fingerprint and signature recognition.

Cells are the fundamental building units of life. Since the establishment of this theory in early 19th century, scientists have been striving to study and understand the mechanisms underlying fundamental cellular and molecular functions. To obtain a better understanding of biological function of cell, cell imaging was pioneered in the first decade of the 20th century ([105], [48], [106], [1]). Since then, several microscopy methods have been developed that allow researchers to study cells with more precision and in an automated way. Consequently, heterogeneous, complex and large number of images are generated in modern cell imaging using the advanced microscope systems which require computerized techniques for quantitative and automatic analysis. These computational methods allow the measurement of the biometrics or characteristics of the cells, quantify cell migration and interaction (morphodynamics) and cells organization (intracellular structures) ([52], [47]).

In this book chapter, we present an overview of cellular imaging and how its biometrically describe the cells. Our book chapter is concise, it brings together different aspects of the cellular imaging from microscopy to cell biology and from image processing to genomics. It is novel to combine all those aspects and discuss them from biometrics angle. Our goal here is to gather and summarize some aspects of cellular imaging from the microscopy to the analysis of generated data. The rest of this chapter is structured as follows: the techniques of cellular imaging are presented in section 2, the generated data types and issues are presented in section 3, some specific applications of cellular imaging are presented in section 4, the biometric approaches of cellular images analysis are presented in section 5 and the unique biometry of the cell, the DNA in section 6. In the last section 7, we talk about the future of cell imaging and how past and actual technologies and developments can be expected to extrapolate into the future.

2 Cellular Imaging Techniques

Microscopic methodologies accepted as milestones in scientific world, have been employed in biomedical fields. Optical microscopic imaging, electron microscopic imaging, scanning tunnel microscopic imaging, fluorescence microscopic imaging and super-resolution fluorescence microscopic imaging are some of the many technique we will hold forth.

The optical microscopes, invention which dates back to 16th century, can be accepted as the oldest design for microscopes [2]. Two main components of the device are an objective lens which is used to amplify the object to be observed and eyepieces which are used to further magnify the projected image on the intermediate image plane. In this pipeline, the optical lenses determine the spatial resolution which is limited to $0.2 \mu m$ using the visible light with the wavelength in the range of $390 - 760 nm$. Optical microscopes can be grouped under different categories based on a variety of criteria. According to the lighting method they can be grouped under two categories, i.e. transmission and reflection microscope. In a transmission microscope, the light is transmitted through the specimen to the viewer from bottom to top, while in reflection microscope the specimen is illuminated from the top and is reflected from the specimen to the viewer. Optical microscopes can also be grouped according to the observation methods, i.e. bright field microscopes, dark field microscopes, phase difference microscopes, polarized light microscopes, interference microscopes, and fluorescent microscopes ([3], [4], [5] and [6]) where each can use transmission or reflection approach.

An important invention in 20th century was development of electron microscopy [7] where the magnification power of microscopic imaging was improved, i.e. spatial resolution reached up to $0.3 nm$, by using electron beam and magnetic lenses instead of the initial optical lenses. The magnification power is obtained by accelerating the electrons to very high speeds which yields to reduction in the optical wavelength. A disadvantage of the electron microscope is only non-living specimens can be observed since it works under the vacuum environment. Another invention in 20th was Scanning Tunnel Microscope (STM) [8]. The working scheme of this microscopic imaging was totally different, i.e., no lenses are employed but a probe is used. Voltage is added between the probe and the observed object and when the distance between the probe and the surface of the observed objects is sufficiently small, tunnel effect occurs, i.e., electrons pass through the object surface from the probe and weak electronic current occurs. Strength of the current changes according to the distance between the probe and the observed surface, thus measuring the current change yields to observe the surface morphology of the object. Spatial resolutions reach less than $0.001 nm$ at STM which provides to locate single atoms.

Fluorescence microscopy is a branch of optical microscopy that has been a very common approach for studying dynamic cellular events of live cell ([9], [10], [11]). While a conventional optical microscope uses visible light to illuminate and magnify the observed sample, a fluorescence microscope uses a much higher intensity light source for the same purpose. Once this high intensity light excites a fluorescent species in the observed sample, it emits a light of longer wavelength and magnified image of the observed sample is produced. Fluorescence microscopy provides better resolution than the conventional optical microscopy by increasing contrast between neighbouring structures in the cells and allows to label different structures with different colours. The working scheme of the fluorescence microscopy starts with illuminating the sample which are labelled by fluorophore with high energy light source (such as Mercury arc lamp) through the lens. Fluorophores that are attached to the sample absorbs the illumination light and emits lower energy light with longer wave-

length and the emitted fluorescent light is separated by a specialized filter that can be visualized.

Three types of fluorescence microscope systems exist: widefield, scanning confocal and spinning disk confocal systems, schematics of which are given in Figure 1, No single microscope system is suited to every experiment and compromises must be made depending on the experiment type. Therefore, three parameters are to be kept in consideration: the sensitivity of detection, the speed of acquisition and the viability of the specimen [10]. The whole sample is permanently illuminated and emitted fluorescence is detected simultaneously by eye or camera in widefield microscopy, while confocal systems illuminate and record only one focal spot at once. Therefore, the acquisition is much faster at widefield microscopy. On the other hand, since the confocal systems remove the out of focus light, the obtained resolution is higher. Scanning confocal microscopes use a pinhole to eliminate the out-of-focus light whereas the spinning disk confocal microscopes exploit a rotating array of micro lenses to focus illumination. Acquisition rate is higher at spinning disk confocal microscopes than scanning confocal microscopes since specimen is scanned by thousands of points of light in parallel.

With the best optics, the resolution of fluorescence microscopy is limited to 200 nm and in the following years many efforts are attained for super-resolution fluorescence microscopy [12], examples include but not limited to saturated structured-illumination microscopy (SSIM), stimulated emission depletion microscopy (STED), photoactivation localization microscopy (FPALM), photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). A very good review on basic principles and their applications in biology of these schemes can be found in [12]. As mentioned in [12], each technique has its own strengths and weaknesses and experimental designs are limited by the available fluorescence probes. For example, while STED employs traditional dyes and fluorescent proteins, PALM and STORM require special photoactivatable fluorophores. Moreover, while STED is more complicated and providing lower resolution (50 to 100 nm), PALM and STORM are technically easier and providing higher resolution (20 to 40 nm).

3 Data types and issues

In many cellular imaging applications, the signal level is weak especially in fluorescence microscopy where the fluorophore bleaches after exposure to light. Consequently, the number of detected photons is limited and the quality of image is degraded. To estimate the quality of the image, the signal-to-noise ratio (SNR) is considered: the more noisy the image it is, the lower the ratio of signal-to-noise [89]. The noise is not the only problem in cell-based imaging but also its complexity. The biological diversity increase the intra-class heterogeneity of cellular morphology. When studying the living cells using time-lapse microscopy, the number of cells could not be the same in all the time-series images, it could for example be multiplied after the cellular division. Designing algorithms which deal with the noise of the microscope and the intra-class variability of the cells is not trivial task and need a lot of expertise.

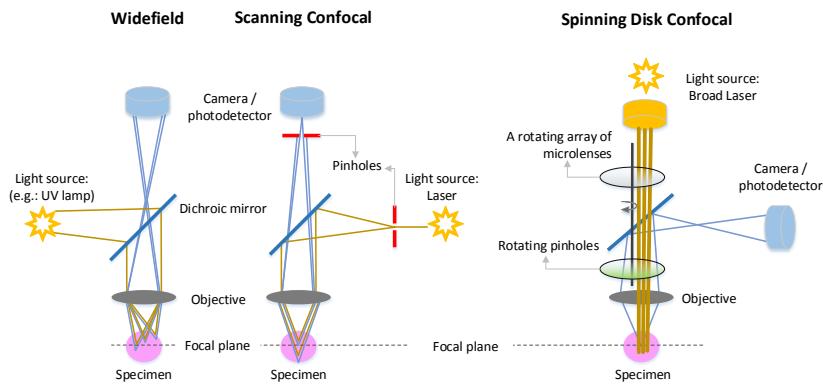


Fig. 1: Types of Fluorescence Microscope Systems. Schematics demonstrate three types of fluorescence microscope systems. On the left, widefield microscopy is depicted where the whole sample is permanently illuminated and emitted fluorescence is detected simultaneously. Scanning confocal microscopy that is depicted in the middle panel uses a pinhole to eliminate the out-of-focus light. Right panel depicts the spinning disk confocal microscope which exploits a rotating array of micro lenses to focus illumination.

Image acquisition techniques presented in Section 2 generate large image data sets especially time-series of large volume data (time-lapse) imposing high requirements on storage capacity [87]. Furthermore, it is not only difficult to archive such huge data sets but also to visualize them [88]. It is also challenging to process and extract information from those big data sets to provide much deeper insight in detailed cellular phenomena. Thanks to multi-core processors, programs are properly designed to take advantage of parallelism that they could be executed faster than their sequential counterparts. This multiprocessing architecture increase the overall speed for programs and accelerate the process of big data. Biomedical image processing is one of the fields that benefit from this technological progress. However, much remains to be done especially that the data volume growth is extraordinary.

The low ratio of signal-to-noise, biological diversity, abundance, big dimensionality, heterogeneity and complexity of the data generated in modern cell imaging experiments make the handling, storage, visualization and computing more difficult, more demanding, more costly and needs more scientific requirements and efforts.

4 Cell analysis applications

4.1 Imaging of biomolecules to study cell behaviour

Cells exhibit various shapes (round, tubular, branched, elongated, etc.) and have the ability to morph (constrict, dilate, retract, elongate, etc.) under different biological

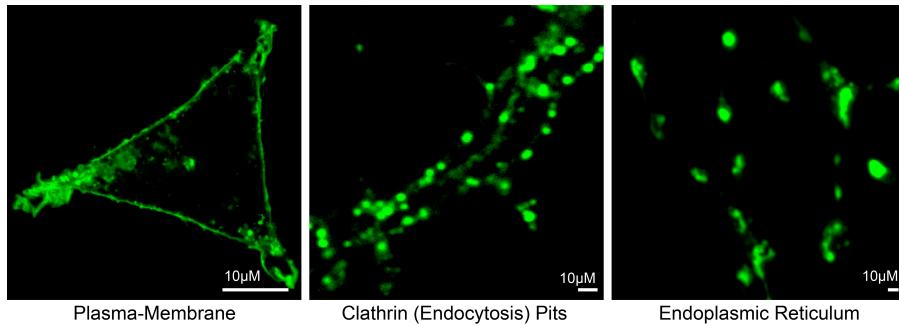


Fig. 2: Molecular Labelling in Cells. Different examples showing how molecular labelling could be achieved. On left, plasma membrane is identified using a pHluorin-tagged plasmid in HEK cells; middle panel identifies endocytosis pits using clathrin-mRFP plasmid in cultured hippocampal neurons; on right are mitochondria labelled using Mitotracker-green dye in live cultured hippocampal neurons.

conditions. This dynamic morphing of cells is controlled by the biomolecules present within a cell as well as external factors. Therefore, it is necessary to understand the underlying molecular processes that regulate the bulk behaviour of a given cell.

Several biomolecules such as lipids, proteins, carbohydrates, and/or nucleic acids are present within cells. Within a cell, these biomolecules form and are localized within different sub-cellular compartments; plasma membrane, endoplasmic reticulum, golgi bodies, cytosolic fluid, nucleus, etc. There are several tools and reagents to label either of these biomolecules and organelles. For example certain lipid types labeling using recombinant cholera-toxin B conjugated with a dye [25], mitochondria labeling using Mitotracker dyes [16], telomerase/proteins labeling using halo-tag technique ([18], [26]) has been achieved. Labeling of cellular proteins by antibodies, transfection and/or by transgenic expression is most commonly used to study the behavior of labeled proteins and the cells expressing them. Antibodies based labeling are primarily detected using organic fluorophores such as Alexa dyes. Transfection/transgenic expression involves conjugation and expression of protein of interest with a fluorescent protein such as GFP (Green Fluorescent Proteins). These fluorescent images like Fig. 2 are typically imaged using epi-fluorescence or confocal microscopes as described above.

Independent of the tool used to label cells (organelles or biomolecules), it can be argued that cell imaging is in fact (bio)-molecular imaging. The data extracted following image analysis can be used to not only obtain information about the biomolecules but also that of the cells ([27], [28], [29], [25]). When imaging is performed at cellular resolution ($1 \mu\text{m}$, e.g. using low magnification objectives), it is not possible to define the precise localization and / or composition of biomolecules. However, in this case, it is possible to separate between two closely spaced cells by segmentation (see Section 5.2). It is noteworthy that segmentation of individual cells is only possible when the staining is bright and uniform with high SNR. However, this is not often the case as biomolecules exist in multiple forms: free unbound (diffused labeling- segmen-

tation possible) and localized within certain sub-compartment (segregation does not separate individual cells but organelle). Further complexity may arise due to homomeric and / or heteromeric super-structure formation. Therefore, in order to study the localization of biomolecules, often, high-resolution ($100 \mu m$, e.g. high magnification confocal) imaging approaches are applied. 3D imaging allows nearly accurate localization of molecules within the cellular sub-compartments. When biomolecules exist as clusters (several molecules bound together), their localization within a given cellular compartment can be determined. For biomolecules that are free (single molecules) in the cytosol/membrane; a diffuse labeling like background is seen, making it difficult to quantify their precise localization and number as explained in next paragraph.

Even at high magnifications, confocal microscopes cannot separate between two individual biomolecules due to the diffraction limitation of light first noted by Ernst Abbe. This means that a microscope could not resolve two objects located closer than $\frac{\lambda}{2NA}$, where λ is the wavelength of light and NA is the numerical aperture of the imaging lens [13]. Visible light has a diffraction limit of 200-300nm and it is not possible to separate molecules that are within this distance. This limitation has been overcome by the development of super-resolution imaging techniques such as PALM / STORM /SIM as described in Section. 2 [30]. Following the advent of super-resolution imaging approaches, it has now become possible to understand precise molecular organization/dynamics within a cell that allows the modeling of the behavior of entire cell. Now it is possible to study cell shape dynamics [22], cytoskeletal dynamics [21], protein dynamics ([18]; [28]), cell-to-cell-contact [23] and many other biological events that define cell behavior. Super-resolution techniques also allow us to understand the physico-pathology of neurodegeneration, which implies studying the interaction of pathological proteins such as amyloid β and α -synuclein, with the plasma-membrane [107].

4.2 Cell and molecular imaging of neuronal cells and role of image processing

Studying the morphology of cells expressed in the central nervous system (brain) at high resolution has always remained a challenge for neuronal cell biologist and biophysicists. This is because of the highly complex architecture and dynamic morphology of neuronal cells ([20], [15], [19]) as shown in Figure 3 . Different cell-types within the brain are identified using cell-specific antibodies (neurons, astrocytes or microglia) or by transgenic expression of cell-specific fluorescent proteins. For simple cell counting, 3D, z-stacks can be obtained using low-resolution objectives (e.g. 10X). If the SNR is good, segmentation based algorithms can be used as cells appear round at low magnifications allowing cell counting. For neuronal morphology determination, imaging is performed at higher magnifications as discussed below.

It is increasingly becoming clear that neuronal cells and not round and within the brain they make hundreds and thousands of connections with other cells, and therefore scientists now are trying to identify these connections in order to understand the functioning of the brain. BRAIN Initiative [31], Allen Brain atlas [32], and Human Brain Project [33] are some of the sources providing information on brain cell connections. Extracting any information from these databases is impossible for man and

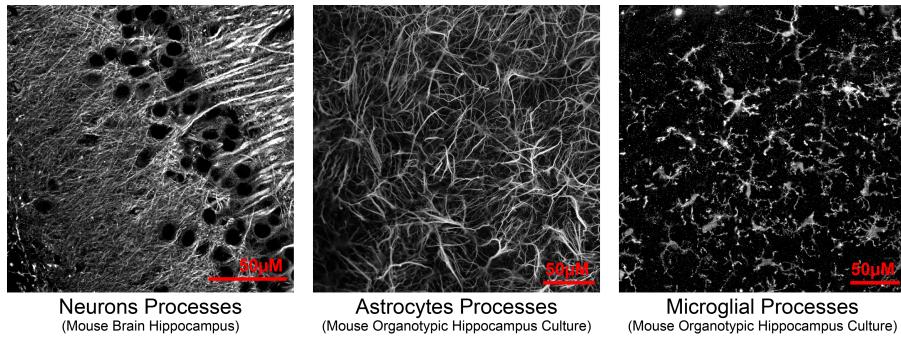


Fig. 3: Morphology of Complex Neuronal Cells. Representative examples showing the complex morphology of neuronal cells (single z-stack) in tissue, neurons are tightly packed within the brain of adult mice hippocampus (left). Middle and right panel depict the complex morphology of astrocytes and microglial cells. Both these latter cells are highly dynamic and respond to external stimulus such as neuroinflammation.

requires complex image processing algorithms. Using image processing, it is possible to model certain circuits and correlate with the function of cells involved. Albeit of these existing databases, the information about neuronal cells architecture and function is far from complete and requires the development of new tools and software to analyze huge data [20].

One focus has been to develop new methodologies that would allow the imaging of single cell at molecular resolution within an entire brain. This is because currently it is only possible to obtain z-stacks / 3D images at high cellular resolution in thick tissues using confocal microscopes (depth 100-200 μm) or two-photon microscopes (depth 100-200 μm). These distances are much shorter than the length covered by single neuronal processes. The depth limit in image acquisition is due to the fact that light is scattered by thick biological samples. This depth-limitation could be overcome by the use of tissue clearing approaches such as CLARITY [17] and iDISCO [24] that reduces the scattering of light through biological specimens, thereby allowing the imaging at higher resolution at greater depth ($> 1cm$) using light-sheet fluorescence microscopy. The potential of this approach was recently revealed in a study that used tissue clearing and antibody labeling protocol to analyze human embryo [14]. The volume of cellular and molecular information obtained by these studies is so high that they require the development of new computational tools to rapidly process such data.

4.3 Cell based HCS Applications

4.3.1 Introduction to High-content screening

A trade-off on resolution for scale is offered by high-content screening (HCS) cellular imaging technology popularly called phenotypic HCS. Cellular imaging with HCS is

a powerful tool for gaining systematic insights into biological processes through hundreds of image features (cell biometrics) at single-cell resolution on thousands of cells from various experimental conditions such as large chemical or natural substance libraries and their varying doses, or RNA interference studies. Typically, hundreds of low-level features such as texture, shape, intensity, intensity ratios of signals between sub-cellular compartments (e.g., cytoplasm, nucleus), spatial colocalization of signals etc are measured in the hope that together they represent a complex phenotype which is representative of the morphology induced by drug or genetic perturbation. Measuring biological markers in different cellular compartments indicate phenomenon like protein activation, translocation or colocalization. Phenotypic HCS is increasingly being adopted at all stages of drug discovery pipeline from compound screening and toxicity testing, to target identification and validation [34]. In academic research as well, imaging millions of cells with HCS is becoming routine for large scale perturbation screens of genome-wide cDNA expression systems and gene knock-down technology (siRNA interference) for understanding molecular mechanisms and pathways. Often, HCS set-ups is used to prioritise hypotheses testing which is followed up with experiments carried out by conventional higher resolution microscopy modalities for experimental validation.

HCS is at the cross-roads of multiple disciplines, combining molecular cell biology, robotic sample preparation, automated high-resolution microscopy and image and data analysis. Cell-based HCS enable screening large libraries of compounds or genetic material and investigating cellular events in relevant in-vitro biological context in living complete cells. This generates more accurate insights about complex, interdependent biological processes that was impossible with traditional cell extracts based screens. HCS systems come with various imaging modalities such as epi-fluorescence, confocal and phase contrast from different vendors.

Multichannel fluorescence microscopy, capturing differently labelled subcellular components and various proteins, can provide evidence to decipher mechanism of drug action or to understand gene functions. Phenotypic HCS rich and heterogeneous big data by imaging of various biological phenomena in millions of cells under different conditions generates. There is immense scope to exploit the rich high dimensional multi-parametric feature spaces modelling complex subcellular morphology to reveal novel chemical and biological knowledge about cellular pathways. The interested reader may refer to [35] for a nice introduction and for a recent review about the state of the art to [36].

4.3.2 HCS applications: drug discovery, biometrics and personalized medicine

Biometrics from cellular imaging has wide ranging applications from basic molecular biology to drug discovery, toxicity and personalized medicine. Cellular image based HCS have boosted the traditional target based drug discovery research in various ways and is noted as a more successful tool than traditional non-cellular-image-based approaches for discovery of small-molecule, first-in-class medicines [41]. Industry forecast expects cell-image based phenotypic tools to change greatly the pipeline of drug discovery research [42]. In toxicology studies in vitro phenotypic HCS assays of relevant cell models are used to predict in vivo toxicity of xenobiotics to humans

[43], which is attractive for preclinical trial safety of drugs. In fact, the overall trend is a push in the community to replace existing disease and toxicity models with more physiologically relevant assays such as 3D HCS cell cultures of differentiated stem cell or patient derived primary cell instead of immortalized cell lines [44]. Hence, fast and efficient 3D cell segmentation and feature extraction algorithms are required to support analysis of 3D HCS data.

Phenotypic HCS enables aggregating of single cell biometrics across millions of cells. Therefore, it is very attractive for studying population heterogeneity such as tumor cell populations where cellular micro-environment has been observed to influence variable response to drug therapy and development of resistant subpopulations. Such heterogeneity of subpopulation can only be identified by exploiting multi-parametric cellular morphology features based on multichannel fluorescence images which is a major advantage over traditional cell count based methods and can be used to optimize treatment planning in cancer therapeutics [45].

The subpopulation studies with phenotypic HCS can even be scaled up to human population context to study the variability in cellular morphology and dynamics arising out of genetic differences. The Human Genome Project and the advancements in pharmacogenomics, have brought to light the impact of genetic composition as a significant factor of drugs metabolism and efficacy, which can sometimes manifest as severe adverse drug reactions [46]. Cell-image based HCS aids personalized medicine approaches to customize and optimize treatment planning by establish the biometric identity of patients. Given the high-throughput nature of HCS, in-vitro screens on patient cells with large drug libraries and various dosage can simulate to some extent *in vivo* responses and help to identify drugs effecting desired responses and to minimize dangers of idiosyncratic responses.

Phenotypic HCS has the potential to completely revolutionize disease characterization and treatment planning. Alongside the benefits of phenotypic HCS come the technical hurdles arising from the complexity of highly multiplexed big data. The image and data processing for accurate inference drawing from high content, high dimensional data places huge challenges for morphological feature representation and machine learning and requires development of methods well adapted to phenotypic HCS data analysis.

5 Cell analysis approaches and softwares

It is now possible to quantify several biometrics like cell types or cell phases, cell interaction and morphodynamics and cell organization [47]. For that, we need computational approaches and algorithms to process the cell images in a stand-alone, similar and automatic way without tuning the different parameters of the algorithms. The development of smart automatic systems of cell processing were introduced in 1960s by using two-dimensional images for classifying white blood cells (leukocytes) in the base of color and morphology features ([52], [48]). Since the mid-1950s, a very large technological progress allows the development of new technologies like microscopy and the development of multiple computer circuits to parallelize the tasks. Consequently, computers became powerful enough to handle big data and to propose

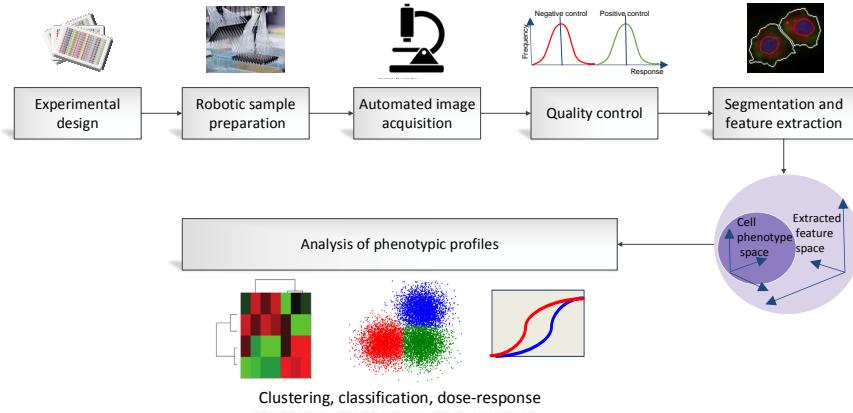


Fig. 4: High Content Screening Workflow. It starts with experimental design and distribution of controls access the many plates for normalization and quality control. Usually with large scale screens the cell and liquid handling is performed by well calibrated robotics. Images that satisfy stringent quality control criteria are then pre-processed, segmented for single cell identification and feature extraction. The resulting phenotypic feature matrices are finally used for classification, clustering and other analysis.

robust, efficient and smart computational methods to answer biological issues. These approaches concern the detection and the quantification, the tracking and the generation of images of cells. In the following sections, we present in a non-exhaustive way the approaches most invested in cell bioimaging field.

5.1 Spot detection

When the SNR is extremely low, especially in live cell imaging, cells may look like blobs or sub-resolution spots at low magnifications[50]. The spots are relatively blurred, small and compact, have no clear borders, sometimes touching or overlapping. Consequently, separating cells and delimiting them is very hard task and this is why researchers prefer to speak of detection rather than segmentation. Spot detection is a quite challenging task which has been well-studied in the literature. Several detectors are used for spot detection in fluorescence microscopy imaging [69] and Phase contrast microscopy imaging ([70], [71]). Those detectors can be divided into unsupervised and supervised methods. The unsupervised family of approaches describe and model the cell structures from unlabeled data, like the linear or morphological filtering [49], clustering [55], model fitting [54], the spatial-frequency detectors like wavelet [59]. The supervised family of approaches learn the cell structures from training data and then can predict using the generated model the new cell structured from test data, hence, it is a classification task. Different classifiers have been used for spot detection like AdaBoost or Fisher Discriminant Analysis [50]. In [50], a quantitative

comparison of spot detection methods in fluorescence microscopy was established by comparing seven unsupervised and two supervised methods. Synthetic images of three different types, as well as real images annotated manually by experts are used to perform experiments. The results of experiments suggest that for very low SNRs (≈ 2), the supervised approaches perform better than the unsupervised ones. At high SNRs (> 5), the difference in performance of all studied spot detectors becomes negligible.

5.2 Cell segmentation and quantification

The cell segmentation offer a better delimitation to the cell than the spot detection, on the other hand, it requires a better resolution and a lower SNR. The precise delimitation of the cells permits its quantification by the extraction of set of features. There had been lots of work done in cell segmentation with most of them being application specific [52]. For accurate cell segmentation, fundamental assumptions can vary based on which imaging method had been used, types of staining, cellular organization, and finally the relevant biological question at hand. The performance of segmentation methods depends on how high is the SNR, and how concise are the cell size and shape. Most of the segmentation method fails to perform consistently when there is large cell size and shape variation within the image and when there is significant overlap between cells specially in case of 2D images. Moreover, the task of accurate segmentation becomes even harder when cells are stained together with cytoplasm. More than that, it is usually hard to incorporate the prior knowledge of the biologists on the cell types. Therefore, semi-automated or semi supervised methods have been found to be more useful for biological studies.

Recently, deep neural networks have become very successful at various classification tasks in diverse domains including phenotypic HCS data. Empirical studies demonstrate deep learning based Convolutional Neural Networks (CNN) outperforms traditional machine learning methods such as random forests, SVMs [66] in accurate classification of single cell images. Deep learning, in contrast to traditional machine vision algorithms, presents the scope for extraction of complex, high-level abstractions of cell morphology by means of unsupervised feature learning. This is due to a hierarchical learning process in neural networks, where complex abstractions are formulated based on relatively simpler abstractions learnt in preceding levels of nodal layers. For example, in the context of face detection, the first layer may detect edges (abrupt intensity/ color change in image); the next may pool edges together to detect eyes or lips and subsequent layers may build features resembling complete faces. The main reason why deep learning outperforms traditional classifiers is due to superior feature representation learnt in the hierarchical model. The typical CNN alternates convolutional and pooling layers. The pooling layers merge semantically similar features with small variances of shift, intensity etc in the next higher layer creating an invariant feature map [39]. However, the training of deep CNN is a demanding task given the very large number of hyper-parameters to be learned, the intraclass heterogeneity of cellular morphology and lack of single cell labels. Chemical or genetic perturbations of cells can induce very subtle phenotypic changes (compared to identi-

fying cats versus bicycles), challenging even to domain experts. But variants of CNN have given promising results in the literature of histopathology images of cancer tissue in highlighting relevant differences in healthy and diseased cells ([81], [82]). Another rationale in favor of deep learning is the ability of HCS to generate thousands of cellular images. These large volumes of images can assist to scale up training data and has the potential to create good deep learning models.

5.3 Tracking

The tracking of cells concern the study of the dynamic processes of living cells at unprecedented spatial and temporal resolution and for that, we need to detect and follow single or multiple particles in a time series of images. The image processing pipeline of the tracking can be generally divided into two steps: the first step cover the spatial domain and concern the detection of the spots and the estimation of their coordinates in every frame of the image sequence, while the second step cover the temporal domain and concern the linking between the detected spots in the different frames using a set of criteria to form tracks. The first step of the pipeline was presented in earlier subsection on spot detection. Approaches of particles or spot linking ranged from simple nearest-neighbor to multiframe association, some include multiple tracking hypothesis with or without explicit use of motion models [53]. While previous studies are limited to either detection or linking and they are less objective in the sense that the evaluation is done by their original inventors and with their own data sets, in a recent study [53] a straightforward comparison of fourteen traditional and sophisticated methods of particle tracking based on an open competition was organized during ISBI 2012 conference. This comparison is unbiased since the methods are evaluated on a commonly defined data set and using commonly defined evaluation criteria. Chenouard et al. in [53] believe that there study is representative of the state of the art and in our knowledge it is the more recent one and the more objective. None of the evaluated approaches performed best across all scenarios and clear differences are revealed between the various approaches.

Lately, modern microscopes like PALM/STORM offer single particle tracking (e.g. SPT-PALM) options ([28], [108], [109], [110]). These methodologies allow simultaneous generation of thousands of short of trajectories. Due to high density and short length of trajectories, classical tracking methods are not ideal and new methods are being developed. For example one method is based on variational Bayesian treatment of hidden Markov models combining information from thousands of short single-molecule trajectories to extract diffusive states [111]. Another approach, multiple-target tracing, can be used to generate dynamic maps at high densities [112]. More recently, high-density SPT-PALM has been combined with statistical inference to map the diffusion and energy landscapes of membrane proteins across the cell surface for neuronal receptors ([108], [113]).

5.4 Generation of images with cell populations

It is often difficult to decide whether obtained segmentation and tracking results are correct or not. In effect, manual generation of ground truth data is a long and tedious task, it is imprecise and highly variable among different experts. To answer that, versatile tools capable of generating image sequences of simulated living cell populations are previously introduced. Besides, dynamic cellular processes are simulated like cell motion, cell division, and cell clustering up to tissue-level density. Recently, a review is introduced on simulation methods ranging from particles up to tissue synthesis and then they have discussed the validity checking of the presented simulators [62]. In fact, the most elaborated and complete simulators of time-lapse image sequences of synthetic fluorescence-stained cell populations are: for 2D images TRAgen [60] and for 3D images MitoGen [61]. Those two simulators and their implementations are freely available ([78],[79]). Both simulators simulate cell motility, shape and texture changes as well as cell divisions. To obtain realistic images, photobleaching, blur with real point spread function (PSF) and several types of noise are considered during the simulation.

5.5 Projection

In the microscopic image acquisition methods, it is common to take several images of the same object at different depth to capture entire part of object in focus. From those sets of images, it is important to make the optimum projection converting into one final 2D image comprising the most in focus pixels, there are some popular and simple methods such as maximum intensity projection, maximum variance projection, average intensity projection, etc. Those methods are implemented in public software like FIJI, Icy or Cellprofiler. None of these methods maintain the physical properties of the object. This issue was addressed for the first time in ([75], [76]) where they used wavelet analysis to compose a 2D image that captures the extended depth of field. Recently, in [77] they developed a projection technique that is locally consistent and follows the manifold of the object. This method classifies the pixels into foreground and background class and projects pixels from the manifold that goes through the estimated foreground.

5.6 Bioimage analysis Softwares

At present, many software tools are available for bioimage processing. The most famous and elaborated open source ones in the field are Icy [56], Fiji [58] and Cellprofiler [57]. For the paid softwares, Imaris [80] remains one of the most used softwares for microscopy image analysis and 3D visualization.

For data analysis and visualization of high content screening images, the HCS microscope vendors provide proprietary softwares, but alternatively there are open source platforms for image analysis and feature extraction - Cellprofiler [37], CellXplorer [38]. In fact, Cellprofiler offers far more variety of sophisticated image analysis

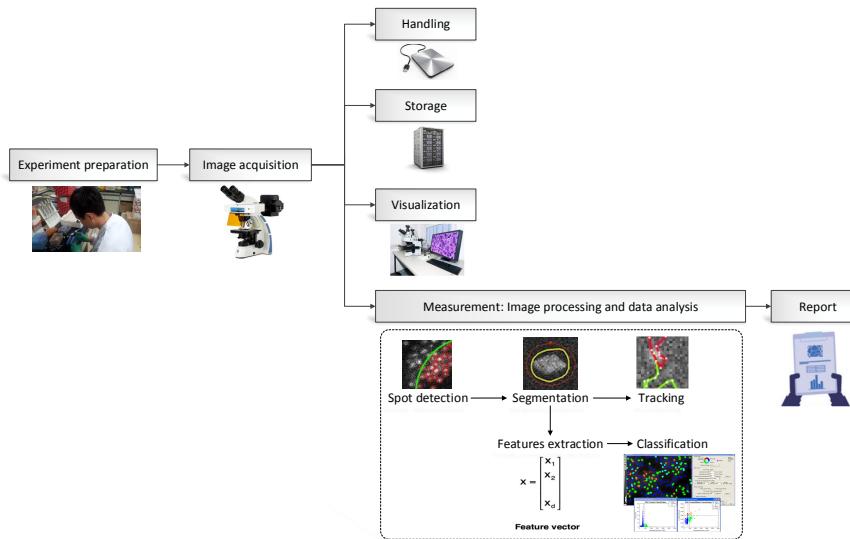


Fig. 5: Process Flow for Cellular Images Analysis starting with the biological samples preparation. Then, cellular images are acquired using one of the techniques described in section 2. The large volume of data impose high requirements on storage, handling and visualization (section 3). Several biometrics are extracted from the cell images and for that several cell analysis approaches and softwares are developed, more details are given in section 5.

algorithms compared to CellXplorer, but the latter provides plate layout visualization interface, quick quality control check options and ability to select and view images in multi-and-single channel by wells and by frames. For data pre-processing, adapted quality control and analysis there exists good packages in most scripting languages such as Bioconductor [34], HCS-analyzer [40].

6 Unique biometry of the cell: its DNA

6.0.1 DNA decryption and future perspectives in biometrics

On a molecular level, DNA can be compared to a database within each cell where vital information is stored and sequentially retrieved. From this storage system originate exhaustive instructions essential for various aspects of cell life. Most of our visible traits or phenotype are coded in the series of nucleotide base composing our DNA. The shape, the size, the growth and function of every cell that make up a living organism is compiled in its genes. These coding regions of DNA are transcribed into RNA molecules and later translated to build proteins specialized in a variety of biological processes. Proteins involved in pigments synthesis for example will determine

characteristics such as eye or hair color, others involved in hormonal pathways affect subtler traits such as stress resistance.

While physical and behavioral measurements provide enough information to accurately distinguish one individual from another, the analysis of genetic material opens access to a wider range of information going from genealogy to disease susceptibility. In humans, this genetic material or genome is made of 3.2 billion bases of DNA. Even though all humans have the same set of genes, small differences in DNA sequence, mostly single nucleotide polymorphisms (SNPs) account for the variety of physical features we observe making each individual unique. An increasing number of studies are using genome wide association approaches (GWAS) to correlate DNA polymorphisms with biometric features ([83], [84]). Influential genomic regions associated with facial structure, weight, height and common diseases have already been highlighted using such methods ([86], [85]). Whether an individual exhibits a trait or not might also depend on the abundance and panel of genes expressed in a subset of cells. Differential expression analyses are used to unveil alterations and abnormalities occurring in gene expression under given conditions. A variety of factors come into play when deciding what genes are switched on or off from genealogy to disease susceptibility. Most of our visible traits or phenotype are coded in our DNA. The shape, the size, the growth and function of every cell that make up a living organism is compiled in its genes. These codius affecting cells function and by extension that of the whole organism. The study of methylation through epigenetic approaches provides valuable insight into genes regulatory mechanisms. Understanding how genes are regulated and what stimuli affect critical genes expression is key to precisely harness the dynamics driving living organisms. With recent advances in sequencing technologies, we now have access to unprecedented volume of data to perform extensive studies on genomes (DNA), transcriptomes (RNA) and proteomes (proteins). Therefore, increasingly complex challenges occur requiring adapted computational and statistical tools to get the most out of produced biological data. On the long run, such advances will refine the way biomedical research operates, providing faster identification, more accurate diagnosing and personalized treatments of complex diseases.

6.0.2 DNA imaging & biometrics

DNA has a rich history of imaging. Although it is not known when DNA was first seen, the pioneering microscopist Antony van Leeuwenhoeck was likely the first person to see it when he visualized the nucleus at the beginning of the 18th century [90]. Chromosomes were first observed in the second half of the 19th century, thanks to the introduction of fixation and staining procedures in microscopy preparations [91]. Originally fascinated by chromosome movements, scientists soon started to count the chromosome number [92]. In 1959, when numerical abnormalities of the karyotype were linked to the Down [93], Turner [94] and Klinefelter [95] syndromes, the interest in quantifying the genetic material became even more relevant. In the same decade, a huge milestone was achieved when the double-helix structure of DNA was revealed using x-ray fibre diffraction [96], bringing unprecedented resolution to DNA imaging. Visualizing the DNA backbone itself allowed us to generate detailed mea-

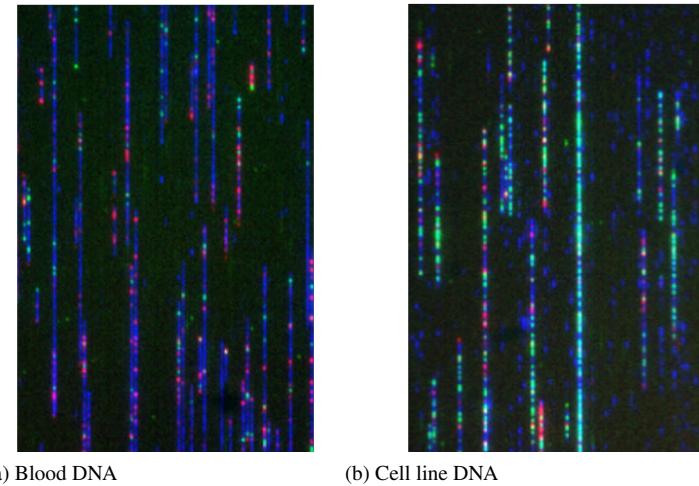


Fig. 6: Genomic DNA from both Primary Human Blood Cells (a) or a Human Lymphocyte Cell Line (b) was dual labeled with genetic labels (Nt.BspQI, red labels) and methylation sensitive labels (M.TaqI, green labels), and stretched and imaged on a nanochannel array chip. Representative images from both samples are presented here. DNA backbone, stained with YOYO-1 is displayed in blue. (Figure extracted from the paper of Grunwald A. et al [100] and used under permission.)

surements of DNA geometry. Since then, many technical advancements have been made. From electron and atomic-force microscopy to ultra-high-resolution methods, molecular imaging techniques have provided a massive amount of information about the DNA structure, its physical and chemical properties and DNA/ligand interactions. Thus, DNA has always been an intriguing object to image and quantify because it carries the genetic instructions of all living organisms. One of the breakthroughs offered by DNA analysis was human DNA biometrics, which is the identification of an individual thanks to their DNA characteristics.

Imaging DNA in the form of electrophoretic bands allows us to measure the length of a specific polymorphic locus that varies in size among the population. The combination of multiple polymorphic loci yields a unique allelic pattern for a given person, and sets up the basis for forensic DNA fingerprinting [97]. Technological advancements such as next generation sequencing (NGS) have allowed us to reach the ultimate step in DNA analysis giving us access to the entire human genome. However, because of the short length of NGS reads (few hundred bases), NGS cannot detect large ($> 1kb$) structural variants [98]. DNA imaging presents unique advantages to address this problem. For example, fluorescent labelling of long (i.e. megabase-size) DNA molecules at specific sequence motifs permits us to generate unique barcode patterns that can be revealed by high-throughput, single-molecule imaging in nanochannels arrays [99]. This generates optical maps that present several advantages

over NGS: first, they can detect all structural variants beyond the kilobase scale (these are especially meaningful in human cancers characterisation); second, they increase the sensitivity in haplotype resolution (finding allelic variants at any locus); third, they allow the study of difficult genomic regions (e.g. repetitive regions, GC-rich regions). Finally, by labelling different genomic features with different colours, we can extract several types of information from the same DNA molecule. As an example, methylation analysis was recently achieved with a hybrid genetic / epigenetic barcode and high-throughput imaging in nanochannels [100], see figure 6. Ultimately, DNA imaging has the unique power to highlight molecular features in their native genomic context thus revealing the unique biometric signature of a human being.

7 Cell imaging, which perspectives?

Cellular imaging and analysis, as seen in this chapter, is an interdisciplinary effort of researchers from multiple fields and disciplines: biology, robotics, physics, microscopy, computer vision and image processing, machine learning and statistics. The coordination and integration of expertise from multiple disciplines is fundamental in addressing the major challenges facing the study of biometry of the cell. In our experience, it is often difficult to put it into practise with challenges ranging from academic to administrative. We should provide good conditions, resources and change mentalities and structures to facilitate interdisciplinary research to achieve common interests and shared goals. This effort is critical to accelerate progress of cellular imaging and biometrics for knowledge discovery.

The naked-eye visualization is never sufficient to make scientific conclusions. Recent advances of robotic sample preparations and microscopy technologies enable capturing cellular images in unprecedented resolution and volume. This has created novel demands from computational and quantitative analysis of cellular imaging data. For example, the impetus for the re-emergence and success of the field of deep learning for microscopy images is partially driven by the advances in automation of data acquisition, generating huge amounts of training data.

The plethora of modern microscopy imaging modalities and diverse markers and resolution of image has opened up new challenges for interpretation of cell images. With huge volumes of data being generated, protocols for efficient storage and retrieval of this data is now a priority. There is also a necessity for such bioimages to be made open-source to allow computer vision scientists to design new methods to provide novel insights into biological phenomena. In spite of decades of efforts, it remain challenging to design generic methods to be easily trainable for different cell imaging applications without requiring parameters tuning or algorithm adaptation. Image analysis methods seem to be locked in a stalemate where new techniques are proposed by the hundreds every year and yet are only applicable to the specific data they were developed on. To accelerate development of more robust and generic methods, we should first of all enforce the availability and the testability of the open-source model (both data and softwares) even before publication and frequently organize open challenges and hackathons to integrate new methods into popular software platforms and compare their performance on standardized test data and criteria [101].

Some very laudable efforts to integrate existing methods into one platform for ease of access for biologists and reproducibility of results are Fiji/ImageJ [58], ICY [56], and CellProfiler [57]

Aside from pushing the boundary of the state of the art a paradigm shift is expected in the field of cellular biometrics. Progress of bioimage analysis methodologies will be driven by trends in biology, for example, the current push for studying disease models in 3D even with high-throughput imaging systems due to the more physiologically relevant context it represents ([102], [103]). There are also arguments for studying the dynamic cellular environment rather than static images which lead to the development organ on a chip models although at the beginning these technology is still low -throughput. Sophisticated image analysis algorithms are required to address the demands raised by such new developments. Finally, we are looking at bioimage research moving gradually into in-silico experimentation supported by augmented reality and artificial intelligence in the form visualization with holographic projections - where researchers can have a direct view into the 3D dynamic environments of cells, unconstrained by the complexities of markers and microscope lenses.

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