

# Chapter 2

## Video Bioinformatics Methods for Analyzing Cell Dynamics: A Survey

Nirmalya Ghosh

**Abstract** Understanding cellular and subcellular interrelations, spatiotemporal dynamic activities, and complex biological processes from quantitative microscopic video is an emerging field of research. Computational tools from established fields like computer vision, pattern recognition, and machine learning have immensely improved quantification at different stages—from image preprocessing and cell segmentation to cellular feature extraction and selection, classification into different phenotypes, and exploration of hidden content-based patterns in bioimaging databases. This book chapter reviews state of the art in all these stages and directs further research with references from the above-established fields, including key thrust areas like quantitative cell tracking, activity analysis, and cellular video summarization—for enhanced data mining and video bioinformatics.

### 2.1 Introduction

In the postgenomic era of computational biology, automatic and objective analysis of biomolecular, cellular, and proteomic activities is at the center stage of current bioinformatics research. Microscopes, the prime instrument for observing the cell and molecular world, have treaded a long path of revolution. Widefield microscopy with deconvolution, confocal scanning microscopy, and scanning disk confocal microscopy have facilitated observing cells and their activities and capturing static image and video data for precise and automated analysis, both in 2D and 3D [108, 131]—even closing the gap between live cell imaging and atomic resolution structures in cryo-electron tomography (3–8 nm) [127]. Green fluorescent protein (GFP) markers in the antibody have acted as illuminant in the molecular world to visualize cell activities and brought a new era in cell research [36]. Sometimes

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N. Ghosh (✉)

Department of Pediatrics, School of Medicine,  
Loma Linda University, Loma Linda, CA, USA  
e-mail: nirmalyaghosh11@gmail.com

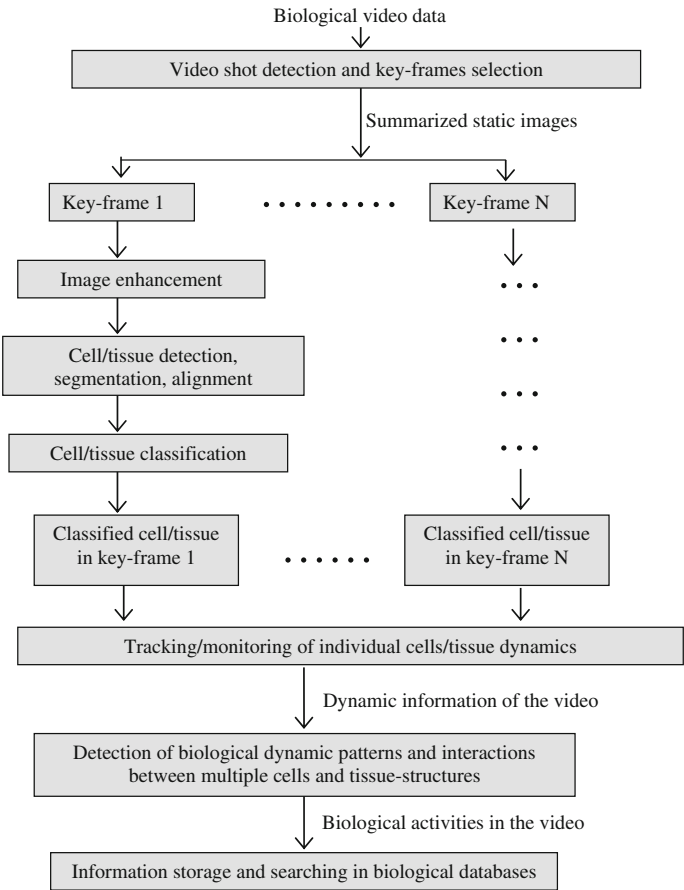
bright field defocused and/or stereo [131] microscopic imaging are utilized to analyze multiple cells at diverse depth with advantages of low phototoxicity and minimal sample preparation, though lower contrast poses difficulty in segmentation and tracking [80].

The challenge has now shifted from automatic capturing of the slow-varying cell activity in digital media to automated analysis of this vast amount of digital data being stored every day with minimum human interaction [17, 64, 96]. Even an expert cell biologist takes hours to preprocess the microscopic images or videos, analyze numerically the structure of cells, recognize them, recognize the cell activity, and come to a biological conclusion. Automated computational methods are absolute necessities to avoid human fatigue-related errors, to perform intensive data mining beyond human tractability and to make results objective and statistically comparable across international studies [22, 26]. Established techniques in computer vision, pattern recognition, and machine learning fields often come handy to rescue from this tremendous information boom in biology in the recent years [28, 36].

Video bioinformatics is a recently burgeoning field of computational biology that analyzes biological video and image data to automatically detect, quantify, and monitor complex biological phenomena—at molecular, cellular, and tissue levels, internal activities and their interactions, in healthy as well as in injured conditions, and with/without drugs and antibodies injected. A complex end-to-end video bioinformatics procedure generally requires multiple major steps as follows. (1) At first, reduction of computational complexity requires detecting video shots and extracting key frames based on biological activities. This makes established image processing techniques effectively applicable to the static key frames. (2) Images are then enhanced by filtering out noise. (3) Biological regions of interests (ROI) are automatically segmented out and aligned to models if necessary. (4) Different morphological, signal intensity, contrast, shape, and texture features are extracted for different biological objects. (5) Based on their discriminative powers, optimal sets of features are selected to recognize entities. (6) Segmented objects are then classified as different biological entities. (7) Multiple consecutive static images (key frames) are considered again to track entities over space and time and to identify biological activities for an individual entity. (8) Interactions between different entities are then automatically monitored using advanced video data mining techniques. (9) Image and video-based information is then stored in a structured and distributed database for availability and query over the Internet. (10) Machine learning techniques are applied to improve all previous procedures including content-based retrieval.

A big proportion of research is devoted to this marriage of quantitative microscopy, computer vision, and machine learning. A number of research groups have concentrated on processing static images of the cells and classifying them using pattern recognition techniques [11, 17, 20, 26, 31, 75, 83, 94, 106]. Major steps and associated tools that are involved in such complete high-content screening and analysis pipeline have been summarized in recent publications [36, 98, 105, 115, 120]. They derived numerical features from the 2D images and used feature-based classification of the biological molecules. Relatively less effort has been exerted for dynamics of

the cells and recognizing the cell activity. Only a small body of research has studied cell dynamics, migration, tracking, bacterial movement, and biological events over the 2D/3D microscopic videos [18, 39, 118, 123, 137, 143] but often lack in automated analysis of such dynamics. This chapter provides reviews of the computational tools for above ten steps that have been already applied in biology or demonstrated potential in mainstream computer vision and pattern recognition (CVPR) field of research for future biological applications. Broad conceptual diagram of a typical video bioinformatics system is summarized in Fig. 2.1. Instead of the mainstream biology, this review chapter is from the perspective of the computational methods applicable in biology.



**Fig. 2.1** Conceptual diagram of a typical video bioinformatics system

## 2.2 Salient Video Activity: Shot Detection and Key Frame Extraction

Cell activities are often very slow and corresponding videos often do not contain enough changes in visual information over a long sequence of frames. Hence to reduce computational complexity, images are sometimes captured periodically—i.e., low frames-per-second (fps) video [64, 118, 143] or periodically sampled from a high fps video [31, 119]. These methods are naïve counterpart of key frame selection that might ignore some salient quick and transient cell transformation information.

### 2.2.1 Shot Detection

Shot detection and key frame selection are two often-used techniques in video processing to reduce computational complexity without losing details, and more contextual in cell activity videos with in general slow dynamics with few quick transients. With low-cost digital storage, taking high-speed (30 fps) cell videos and detecting shots and key frames to trace salient transient cell activities is more practical. Although shot detection is now a relatively matured domain in computer vision, it is surprisingly unused by cell biology community. As cell videos often have fewer types of cells present in the same videos, established shot detection techniques from histograms might work well, e.g., global dissimilarity or temporal changes in different pixel-level features—color components [38], intensity [1, 144], luminance [112] and their distributions and combinations [1, 16], or regional features and likelihood ratios [29, 136] across consecutive video frames.

### 2.2.2 Key Frame Selection

Key frames are representative frames of a particular video shot, analyzing which one can safely summarize about the frames they represent. A set of key frames are generally selected such that these frames contain enough visual information and its change (dynamics) over the video sequence. The frames acquired periodically or heuristically [31] and analyzed by the cell biologists in the state-of-the-art systems are actually a naïve substitute of these key frames. Key frame selection is also a well-established domain in computer vision. Other than clustering-based techniques [139], most of the keyframing methods attempt to capture the temporal information flow with varying computational complexity—starting from simple first and last frame selection [85], periodic selection [113], after constant amount of change in visual content [19], by minimization of representational error (distortion) in the feature space [47], by iterative positioning of break points (like sub-shots) and key

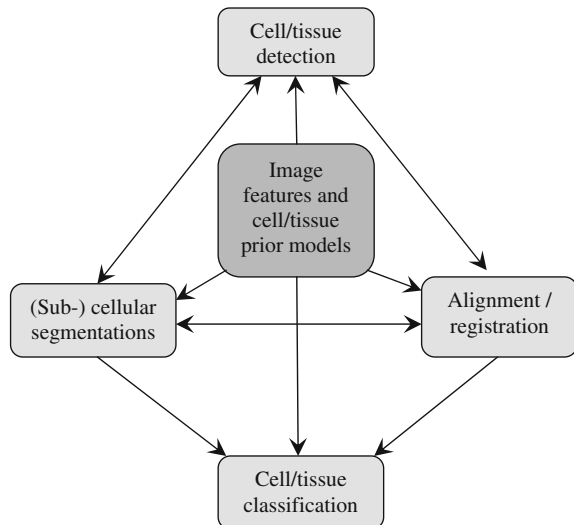
frames (one in each sub-shot) to minimize distortion [61], and by minima in motion feature trend [133]. Unlike these previous methods, sometimes psychoanalytical perception models might be used to automatically decide the number of key frames to be selected depending on change in visual content from the feature trends [40]. From cell video point of view, specifically with morphological transformation (morphogenesis), tracking geometric structures and keyframing based on salient differences [141] might be adopted. Based on complexity and application, similar methods can be envisaged in cellular videos to decide on which frames are to be analyzed to reduce computational burden.

Once the shots and corresponding key frames are decided, cell videos can be analyzed in the same way as single static bioimages as discussed in the following sections. Even for motion-based tracking the cells over the frames, key frames may reduce the computational burden by few orders, specifically for slowly changing cell videos.

### 2.3 Image Processing and Biological Object Detection

After denoising and preprocessing of static images (from keyframing), cellular and tissue region of interest (ROI) extraction mainly comprises of three stages: detection, segmentation, and alignment (sometimes called “registration”). All these stages are often interrelated, mutually supplementary in nature, and even sometimes inseparable, as depicted in Fig. 2.2. For the simplicity of understanding, they would be dealt separately in following subsections. All these stages directly depend on image features and prior biological models.

**Fig. 2.2** Interdependency of different low and midlevel image processing modules in bioinformatics



### 2.3.1 *Preprocessing and Noise Reduction*

Even after following recommended techniques for sample preparation and data acquisition [48, 108], noise in bioimages is ubiquitous and almost always requires preprocessing and denoising. Though few research works adopt simulated (flat) background without any explicit noise filtering [118], this is an unrealistic assumption for in vivo cell images and videos. During the conversion of patterns of light energy into electrical patterns in the recording device (e.g., CCD camera or photomultiplier tube) random noise is introduced [39]. Specifically because of the high-frequency noise, biological cell shapes lose sharpness and affect segmentation and overall analysis.

For any practical automated analysis of bioimaging data, reduction of random and speckle noises [11, 28, 115, 138] and variations in illumination (e.g., GFP) [123] are the first steps. Usual low-pass filters, besides reducing the high-frequency noise, also reduce the sharpness of the edge and contour features (as they are also high-frequency components of the image). Nonlinear filters (e.g., median filters) often resolve this problem [109, 114]. Sometimes sophisticated anisotropic diffusion filters are used that preserve local characteristics and image qualities [39]. The rationale of this method is that image areas containing structure and strong contrast between edges will have a higher variance than areas containing noise only. Hence diffusion algorithms remove noise from an image by modifying the image via partial differential equation. Homogeneous regions are handled by diffusion equation (heat equation) equivalent to Gaussian linear filters with varying kernel size. While anisotropic diffusion filter controls the diffusion process by an “edge-stopping function” that depends on local image features, e.g., magnitude of the edge gradient.

Speckle noise generally comes from small intracellular structures that can be reduced by model-based filtering, e.g., modeling cells as ellipse and removing outliers not fitting the model [138]. Sometimes nonlinear least-square-designed FIR filters are used to improve contrast between cell objects and fluid background as they are immersed and then histogram-based dynamic threshold is applied to deal with illumination variation due to fluorescence decay [49]. This contrast improvement might be more effective if some fluorescence decay model is applied [123]. A series of filters often assists in the overall preprocessing—e.g., histogram equalization [109] or auto-density filter increases the contrast, morphological filters (in sequence—dilation, histogram-based intensity threshold and erosion) reduce model-based outliers and finally median filter removes salt-&-pepper (random) noise [31].

In recent reviews [108, 127] of different preprocessing steps, potential methods and pitfalls are provided where starting from selection of particular microscope and acquisition parameters, preprocessing steps like flat field correction and background subtraction, intensity normalization, different Gaussian filtering techniques, and deconvolution strategies are discussed.

### 2.3.2 Segmentation

After preprocessing, generally explicit segmentation and feature extraction are required for classification. Rare exceptions are the data with no intercell occlusion [31], or where chromosome profiles are extracted using dominant points and variants [104]. Most of current quantitative microscopy data are from cells that are immersed in an in vitro biochemical solutions (beneath a coverslip) and imaged individually [31, 115] or in a nonoverlapping (i.e., without occlusion) situation [118]. Segmentation might be redundant for bioimages in such controlled environment [13]. With the assumption of small roughly uniform background, manual polygonal cropping and dynamic thresholds work well to identify cells [13]. For in vivo data with different cell types and intercell occlusion, these methods are too restrictive and explicit automated segmentation is an absolute necessity. Hence later researchers from Carnegie Mellon University (CMU) have adopted a seeded watershed algorithm for segmentation in 3D microscopic data where seed for each nucleus is created by filtering DNA channel output from the confocal scanning microscope and 93 % segmentation accuracy is reported [49].

An early review paper on interest of image processing in cell biology and immunology [109] proposes three ways of segmenting cells (illuminated by GFP): histogram-based bimodal segmentation, background subtraction, and (heuristic) threshold-based boundary following. Generating outlines of the biological structures, i.e., image segmentation is a challenging task and influences the subsequent analysis [53]. This work on analyzing anatomical tissues (conceptually quite similar to the cell and molecular images) proposes one 2D color image segmentation method. In the grayscale image, segmentation involves distribution of seed points in a microscopic image and generating a Voronoi diagram for these seeds. Gradually, this Voronoi diagram and its associated Delaunay triangulation are modified according to the intensity homogeneity. For the color images, this region-based approach is extended with sequential subdivisions of the bioimage, classifying the subdivisions for foreground (the cell or tissue), or background or both, until each subdivision is uniquely classified. Voronoi statistics (including HSV mean color intensities and their variances) of each subdivision are utilized to classify them. Seed points can be initialized manually or randomly. Then a continuous boundary of the cell or tissue is obtained by fitting splines. Although this procedure is tested for anatomical tissues, like segmenting the lungs, the procedure is generic enough for cell and molecular bioimages and can be extended in 3D using 3D Voronoi diagrams, of course with increased time complexity.

Another review paper [39] addresses a rather innovative way of segmentation in cell images. The method applies multiple levels of thresholds to form a confinement tree that systemizes the knowledge that at what level of threshold, which cells are merged to a single object. Then morphological filtering reconstructs grayscale images in various levels. Thus the method is adaptable to the analysis needs. They also address another edge-based segmentation operating on nonmaximum suppression algorithm and refining the contour by active contours (snakes) with energy

function associated with curves. Sometimes morphological operations and regional intensity gradients assist in segmentation. In an application of immunohistochemical stain counting for oesophageal cancer detection [60], the region of interest is first manually cropped, color image is converted to grayscale image, contrast is enhanced by histogram equalization, and morphological TopHat (and other) filtering) is performed for initial segmentation. Then watershed algorithm segment out the nuclei and gradient transform-based edge detection is performed. After two-stage watershed segmentation nuclei are detected.

In pioneering research of Euro-BioImaging group (<http://www.eurobioimaging.eu/>) in clinical wound-healing video, distinct textural difference between the wound and normal skin is mentioned [67, 77, 107, 118, 147], but for wound segmentation, histogram equalization (to improve contrast), edge detection, and modal threshold are utilized. It is rather surprising that no texture feature is utilized. In another video-based bacterial activity work [118], individual cells are segmented by seed-based region growing algorithm. But seed initialization process is not clear. And in presence of occlusion, which is not considered in this work, region growing procedure may perform poorly. In such cell videos, motion-based segmentation from tracking across frames [140] might help, specifically when the background (however complex it is) does not change too fast. One recent work on automated wound-healing quantification from time-lapsed cell motility video, cascaded SVM-based initial segmentation, and graph cut-based outlier rejection are applied on basic image features [143].

Cell population-based studies (in contrast to study on few cells in an image) sometimes provide more statistical power—specifically for phenotypic changes by drugs, compounds, or RNAi [64]. Centerline, seeded watershed, and level set approaches are common in such applications. Except for such rare cases [50, 64], multicell images are segmented into individual cells before any phenotyping (classification). Segmentation in cell images in presence of speckle noise (intracellular structures, like nucleus, mitochondria, etc.) are dealt systematically by the Lawrence Berkley National Laboratory (LBNL) research group [11] by model-based approach. In multicell images, they model the cells and intracellular structures as ellipses and mathematically demonstrate that, removing the speckle noise and interpolating the cell images accordingly can be done by finding solution to a Laplace equation. They call it “harmonic cut”. The cells touching one another are segregated by regularized centroid transform, where normal vectors generated from cell boundaries are clustered to delineate touching cells. This sophisticated method is a generic up to some extent as long as cells can be modeled as ellipses (with smooth quadratic splines). Similar approach has been utilized in model-based detection of cell boundaries and then seeded watershed separation of touching (but non-occluding) cells in the same image [115]. But in many cases, like data used by CMU [13], cells are of irregular shapes. Proper extension of the harmonic cut and regularized centroid transform method for these irregularities is yet to be tested. Recently, “tribes”-based global genetic algorithm is applied to segment cells with partial occlusion by part configuration and learning recurring patterns of specific geometric, topological, and appearance priors in a single type of cell in histology



and microscopic images [91]. Different cell shapes (without occlusion) are segmented out from defocused image stack of embryonic kidney cells (HEK 293T), where the best representative slice is first selected by a nonparametric information maximization of a Kolmogorov complexity measure, then active contours are initialized and expanded for level set segmentation [80]. A nice level set and active snake-based multilevel approach segment out core and membrane of cells from uncontrolled background [86]. Seeded watershed algorithm and level set approaches could successfully segment out *Drosophila* cells and nuclei and then tracked across time-lapsed frames to detect cell divisions and migration with and without drugs [64]. Interested reviewers are encouraged to read CVPR reviews [30] on fusion of different features like color, texture, motion, and shape and unified approach of level set segmentation for potential applications in cell images and videos.

Cellular and subcellular segmentation and colocalization in fluorescence microscopic images are still very relevant research areas [106]. Recently, Fuzzy C-means clustering is found better than baseline hard C-means clustering in segmenting single pap smear cells as well as separating their nuclei and cytoplasm for classification and abnormality detection [23]. In another work, for model-based segmentation of more-confluent (occluded) cell nuclei, predefined patterns in attributed graphs of connected Sobel edge primitives (in different orientations: top, bottom, right, left) are iteratively searched and reassigned as needed to localize nucleus boundaries and then region growing is performed to separate occluded nuclei [4]. Sometimes 2D segmentation results can enhance 3D segmentation from stacks of microscopic images of neuronal nuclei—and also correct some of the 2D under and over segmentation errors by connectivity and centroid clustering [59]. 3D watershed segmentation is the baseline for comparison in this work. In a neuron tracing research, morphological features at multiple levels and in different neuronal parts can successfully segment the entire neuronal cells [76]. Recent review papers [115, 127] critically discuss many such segmentation techniques along with associated advantages and disadvantages.

Texture-based segmentation is one area where future bioimaging research might gain momentum. Few nice reviews [6, 52, 103, 124, 145] summarize well-established texture descriptors that are applied in CVPR applications over decades, including texture-based feature space smoothing that preserves salient edges with supervised [126] or unsupervised methods [33], split-and-merge segmentation by facet models, and region adjacency graphs [72] using multiple resolution [99] texture information measures [100, 101], or region growing segmentation from gradients of textures [45] utilized as inter- and intraclass dissimilarity [130] for random walk [102, 103] or quadtree-based methods [121]—to name a few. Cellular and molecular images have distinct textures for different species and this can immensely enhance segmentation.

Sometimes pixel-, local-, or object-level relations (based in morphology, color, proximity, local texture, shape, and motion) can be represented graphically with objects as nodes and weighted links as strength of interrelations [116]. In such cases graph matching and partitioning methods like normalized graph cut [129] can

partition highly connected regions as clusters to segment image objects. Except for few exceptions [4, 143], graph-based segmentation methods are yet to be applied in cell images to their full potential.

### 2.3.3 *Object Alignment*

Though not very common, image registration—i.e., aligning the object with a template or model is sometimes required for better feature extraction, quantification, and analysis. This is performed either before the cell segmentation [18, 105] or after it [115]. Although segmentation and registration are dealt separately in most works, they are quite interrelated and mutually cooperative (see Fig. 2.2). For examples, in atlas-based segmentation methods (very common in medical imaging) data model alignment is a prior requirement, while segmented structures assist in landmark-based alignment of test object with the model. Specifically, in shape-based methods segmentation and registration are so similar that a new term “regmentation” is coined in medical image analysis [37]. Due to high variability of cellular and subcellular objects, object alignment is not always possible in a reliable manner and hence not informative for automated analysis. For protein structure alignment—where similar structures and partial resemblance are of importance—optimal paths and distances between atoms are successfully utilized in a graph-matching paradigm [117]. In database search, image registration is required for developing atlas or representative model from similar cellular datasets [96, 98] or for comparing with manually annotated reference images for local features (to overcome variations in sample preparation) before a multireference graph cut [22] or level set [21] does the nuclear segmentation. Registration might help in detecting eccentricity of a test data from the model and thus estimating abnormality for further analysis. Classic image registration algorithms in CVPR [148] or in medical imaging [93] might have immense potential in cellular image analysis [115], specifically when close-loop cooperation between segmentation and registration [37] are adopted in a deformable model approach [44].

## 2.4 Feature Extraction

A large proportion of the quantitative microscopic analysis research is done with “static” images of the cells and molecules. In cell classification, static features dominate, sometimes due to slow biological processes and sometimes to compromise with the computational burden. Three basic steps in static image analysis are (1) feature extraction, (2) feature selection, and (3) object (cell, biomolecules) classification. These steps are interdependent. Human perception of the cell images provides idea on type of classification strategy expected to perform better. The classifier type influences the selection of features, which in turn guides the image

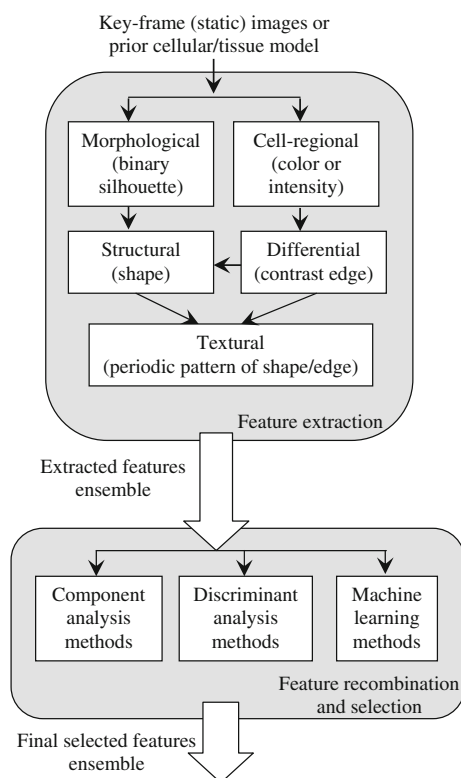
processing strategies. Hence the steps are quite overlapping. This chapter attempts to address these steps individually as far as possible for better understanding.

Image features that are signatures of the object of interest are extracted after the segmented image is processed through different types of image (morphological, textural, intensity-based) operators and filters. Sometimes image processing also covers the occlusion-handling strategy by interpolation or extrapolation of the cells. Image processing (just like corresponding features) can be classified into:

- Morphological (binary silhouette-based)
- Cell regional (color or intensity-based)
- Differential (or contrast edge-based) and Structural (or shape-based)
- Textural (structural periodicity-based)

Relation between these different types of features is summarized in Fig. 2.3. Current section describes the example of these image processing types applied in cell-imaging community, followed by some of the classical CVPR examples to inspire future research.

**Fig. 2.3** Different types of extraction, recombination, and selection methods for static image features and interrelations between them



### 2.4.1 *Morphological Features*

Morphological image processing techniques basically consider the binary silhouette (obtained from segmentation) of the cells or biological molecules and find different geometric properties [114]. These are probably the lowest level image processing techniques, yet sometimes very useful—especially as different types of cells and biomolecules generally have significantly different outer shapes (morphology).

After the sequence of the human genome is determined, next task is to determine genomic functionality. Proteins encoded by novel human cDNA clones cause morphological changes and/or protein localization at the cellular level which result in various cellular forms [122]. After histogram equalizations, first-order principle component analysis (PCA) of manually segmented sub-images is used as models. They consider 16-bit grayscale images of subcellular compartments like endoplasmic reticulum, Golgi complex, plasma membrane, mitochondrion, nucleolus, peroxisome, etc. Then morphological convolution of the model and original images are done to get the local maxima that are taken as the focal points.

CMU researchers have used an extensive morphological image processing and selected number of features [115]. Extensive list can be found in [82]. Some of the salient ones are: (1) number of subcellular objects per cell, (2) Euler number of the cell (i.e., number of objects minus number of holes per cell), (3) average pixel size, (4) average distance of objects to the center of fluorescence, (5) fraction of fluorescence not included in the objects, (6) fraction of the area of the convex hull not in the object, (7) eccentricity of the hull, (8) average length of the skeletons (or medial axis; found by morphological iterative thinning), (9) average ratio of the skeleton length to the area of the convex hull, (10) average fraction of the object pixels (or fluorescence) within skeleton, and (11) ratio of branch points to the skeleton length. Most of these 2D features are also extended for 3D scanning microscopic data [24, 115]. For images with multiple (same) cells, features like ratio of largest to smallest cells are also considered [50].

In the research of Euro-BioImaging group [118] simple morphological features are extracted for solving “correspondence problem” to track the bacterial cells in the cell motility videos for event detection. The features extracted for each segmented cells include spatial position in the frame (i.e., the centroid of the cell), its area, its length, and width (determined by PCA, along major and minor axes, respectively) and its orientation in the space. These morphological features are used to track and to detect orientation change over the frame sequence for bacterial “tumbling” and other behavioral response to the drugs applied. In a medical tissue diagnostic work from wound-healing video [107], they apply morphological cleaning of the wound area in the image, and compute application-specific morphological features like wound length and wound-area-per-unit-length. From the dynamic variation of the “wound-area-per-unit-length” feature, they decide the healing (or worsening) of the wound with time as drug is applied periodically. LBNL researchers utilize detailed morphometric analysis of TCGA glioblastoma multiforme for tumor categorization from hematoxylin and eosin (H&E) stained tissue where they compute

cellularity (density of cells), nuclear size, and morphological differences of nuclei [20].

In recent reviews [25, 105] different morphological operations in cell image analysis—from segmentation to characterization to abnormal cell identification—are nicely summarized where different cell morphological features are discussed including circularity, rectangularity, eccentricity (ratio of minor to major axis length), morphological texture from gray-level co-occurrence matrix: energy, uniformity, entropy, smoothness. These features are also extended to 3D morphology and deformable models [25].

### 2.4.2 *Color and Intensity Features*

Intensity value for grayscale cell images and color component values in different color spaces like red-green-blue (RGB), hue-saturation-value (HSV), or other application-specific combinations of them [114] sometimes have the unique region-based features to segment and classify cell and molecular objects in biochemical fluid—especially when salient portions of the cells are illuminated by GFP tags. Color decomposition might also reduce computational load, and might assist thresholding, refinement, and normalization of input image to the base image [20]. Grayscale intensity-based moment of inertia is successfully applied for chromosome slice estimation and profile extraction [104]. According to this work, shape profile is the moment of inertia of the normalized gray value distribution in each slice relative to the tangent to the longitudinal axis at the subdivision point of the slice. Similarly, different grayscale-based features such as brightness, histogram, and amplitude of a region assist in genomic classifications [122].

The prognosis of esophageal cancer patients is related to the portion of MIB-1 positively stained tumor nuclei. An image analysis system is developed on LEICA Image Processing and Analysis System to reduce the subjective, tedious, and inaccurate manual counting of nuclei staining [60]. It can analyze in 15 min. Proliferative activity of tumor is a useful parameter in understanding the behavior of tumor. Correlation between the proliferation activity and overall prognosis has been observed in some tumor. MIB-1 score by immunohistochemical method and stain counting is one affective process. Brown nuclear stain is regarded as cancerous cell and blue nuclear stain as normal cell. Intensity-based classification is performed in RGB space: brown nuclei by red-component-higher-than-blue-one and blue nuclei by the vice versa. Automated systems might suffer from variations in illumination and focusing problems, mainly due to dynamic nature of the protein molecules. Heuristic application-specific filtering [60], fluorescence decay models [123], or illumination-invariant color component-like saturation [53] might overcome such problems.

*Haematococcus pluvialis* (Chlorophyte) produces carotenoids that are utilized as color pigments and analyzing agents for different degenerative diseases in humans. *Haematococcus* has two distinct phases in its life cycle: green flagellated motile

phase and nonmotile nonflagellated cyst phase formed due to stress conditions. Automated evaluation of red component of imaged cells can give estimate of the carotenoid content without disrupting the cell wall. One work [56] adopts grayscale conversion, histogram equalization, and edge-based segmentation for ROI extraction. Then cell pigment percentage change is detected from hue component by three-layered artificial neural network (ANN) classifier that classifies into two classes: Chlorophyll and Carotenoid, for medical diagnostics.

Another work on semiautomated color segmentation method for anatomical tissue [53], considers mean a variance of color in different voronoi cells dividing the tissues in segmentation and classification of lungs like organs. They have converted RGB tissue images into HSV space and reported that saturation plays important role in distinguishing between biological tissue and cell structures. This coincides with the well-established fact in CVPR that saturation is relatively invariant to the illumination changes, and might be even better than explicit modeling of temporal decay of fluorescence strength (called “leaching effect” of GFP) [123]. Sometimes, to separate out an actual fluorescent tag from noise in low signal-to-noise ratio (SNR) data, cell spots are detected by intensity-based local maxima detection where a “spottiness”-value is computed to characterize the similarity of the intensity signal in the neighborhood of a local maximum with respect to the intensity signal of a theoretical spot [123]. This theoretical spot neighborhood has been modeled using the Gaussian point spread function. Gaussian filtering and interpolation of intensity features have been extensively used in bioimaging [11].

### 2.4.3 *Edge and Shape Features*

Edge and shape features are comparatively higher level features than the last two, as they have more uniqueness for object recognition (see Fig. 2.3). Naturally, in cell and biological specimen classification and analysis, edge and shape based features play significant role. Edges are the convolution output of the images from contrast differential operators (number of dimensions same as the data), e.g., Sobel, Roberts, Prewitt, and Canny edge detectors [114]. The edges are connected by boundary-following algorithms to get the contour of the cells/objects in 2D or 3D. These contours are low-level representation of shapes for cell classification.

CMU research group extracts number of edge/shape features from differential operators [82], both in 2D and 3D domain. Salient ones are: (1) fraction of pixels distributed along the edges, (2) measures of magnitude and directional homogeneity of the edges, (3) different Zernike moment features (computed by convolving with Zernike polynomials) to find similarity in shape between the cells and corresponding polynomial. They utilize all these features directly in ANN or other classifier module without trying to develop any shape models. Probably a middle level shape model can improve the classification, as is the case for number of computer vision applications [114].

In an early work [109], shape features are utilized to study human neutrophils exposed to chemotactic stimuli, to describe cell polarization and orientation and to identify chemotactic abnormalities in cells from heavily burnt patients. Information relevant to the mechanisms of adhesive interaction is extracted from the distribution of intercellular distances in cell–cell contact areas. This contact area estimation allows conceptual discrimination between “actual contact” (i.e., with intermembrane distance compatible with molecular interactions) and “apparent contact” (i.e., apparent membrane apposition with intermembrane distance of 50–100 nm). LBNL researchers estimate parameters of the elliptical model of individual cells as shape features [138] and extend their harmonic cut method for iterative tensor voting to refine ill-defined curvilinear structures and for perceptual regrouping of 3D boundaries to separate out touching cells [71]. Sometimes cell shapes are utilized indirectly to classify [31], where the hidden layer of a modular neural network (MNN) might compute the shape features internally, takes into account the shapes of the cell at different scales, and maps directly to different cell classes in the output layer of the MNN. Another work [104] utilizes shape or chromosome boundary and contour curvature to define the singularities (called dominant points) in the chromosome pattern. Longitudinal axes of the chromosome are found by fitting quadratic splines of the distribution of these dominant points and their variants. These axes act as the backbones of the grayscale intensity-based slice determination for extracting chromosome profiles.

In CVPR research shape and boundary-based features are one of the most successful ones for decades [3, 8]. These methods are nicely reviewed in [146] and can be broadly classified under four categories as follows. (1) Scalar boundary transformation techniques: for example, tangents represented as parametric (turning) function of arc lengths; shape centroid methods with polygonal approximation; radial distances [70]; Fourier (frequency) domain features or bending energy of the boundary; circular autoregressive shape models [65]; and central distance of the boundary from fixed length arc placed at different boundary locations. (2) Spatial boundary transformation techniques: for example, multilayered chain code for shapes at different resolution; syntactical coding of strings of primitive shape features; split-&-merge spline approximation with minimum error [27]; hierarchical scale-space representation from multiple-width Gaussian filters [5, 7]; and boundary decomposition by template contour template matching [68]. (3) Scalar global transformation techniques: for example, multiple order 2D or generalized polynomial moments of the silhouette; different shape matrices and vectors from polar raster, concentric circles or maximum shape radius; and granulometries, morphological covariance, geometric correlations, residuals [73]. (4) Spatial global transformation techniques: for example, medial axis, and r-symmetric axis transformations; and shape decomposition based on convex–concave boundary points and fuzzy likelihood. Among these, only a few methods like Fourier shape descriptors [115], spline approximation by iterative refinement of curvilinear structure to separate touching cells [71], elliptical cell shape models [11], Zernike polynomial moments for cellular matching [28], and shape decomposition for neuron tracing [76] are utilized in bioimaging research. Accuracy of these results needs to be evaluated more critically

in classification and image retrieval scenarios [3]. Scale-space shape models and features [5, 7, 8] from cellular and subcellular images might improve classification in multicell bioimaging data. One recent work [106] reports a user-friendly freely available software for colocalization in near real time (<1 min for 2D, <5 min for 3D) by segmentation and quantification of subcellular shapes (Squass).

#### 2.4.4 Texture Features

Shape features distinguish objects or cells from what is seen from the outside. Textures are the features of the cells as seen from inside. In cell images, different cells and biomolecules generally have distinct textures (in 2D patch or 3D surfaces) compared to the biochemical fluid (in vivo) or solution (in vitro) they are floating in. Similar is the case for anatomical tissues [107]. These textures are actually periodicity of similar patterns in visual spectrum and are also affected by biophysical and biochemical properties like viscosity, smoothness, fluorescence absorption, diffuseability, etc. Texture often characterizes the cell or solution when other surrounding conditions remain the same. Hence, cell-image analyzers also apply textures as primary features for cell classification [28, 122] and cell video understanding [118]. Among several texture descriptors utilized by CMU researchers for subcellular localization [28, 82], the key ones are:

- Haralick texture features [6]: These are computed as gray-level co-occurrence matrix (might be extended to color co-occurrence matrix for each components) and then averaged for rotational and translational invariance. Intrinsic statistics including angular second moment, contrast, correlation, sum of squares, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference variance, difference entropy, and information measures are often extracted as features.
- Gabor wavelet texture features: Spatio-intensity periodicity is extracted using Gabor kernel with different scales and orientation. Mean and standard deviation at different abstraction levels are considered as features. Non-orthogonal Gabor wavelets can capture the derivative information of the images.
- Daubechies four wavelet textures features: Cell images are decomposed up to level 10. The average energies of the three high-frequency images at each level are utilized as features. Scales and orientations provide textural fineness and relative arrangements.

Besides above, Low's textures and 15-element feature vector describing symmetries [114] might be utilized in subcellular localization. Recent bioimage analysis research starts to look back on some of the established texture descriptors [52, 124, 145] including Haralick's texture descriptor [6], local binary patterns [87], co-occurrence matrix-based grayscale textural features [122], and learning-based local binary patterns [46] in analysis and classification of 2D-Hela databases and recognition of abnormal smear cells in pap smear medical databases. Multicellular



textures are found to be excellent descriptors in monitoring wound-healing and cell-scattering assays in differential interference contrast (DIC) images [143]. There are inherent differences in tissue and cell textures, and many more such research efforts in bioimage analysis are expected in the near future.

## 2.5 Feature Recombination and Selection

Human vision can recognize different biological cells and their activities in bioimages and videos relatively easily due to complex vision perception experience cycles. But that is neither well understood nor yet implementable in computer programs. Human brain automatically selects best features and their different combinations to analyze the data effortlessly. Automated systems can at the best extract a very large number of low-level image features with the hope that no valuable information is lost, sometimes without knowing the actual usefulness of those features to classify (cells) and recognize (their activities). Cellular and biomolecular images/videos often capture irregular structures and unknown interdependent dynamics between them. To analyze them often several features are extracted [82, 115]. But more features mean exponential increase in computational time. This often leads to overlearned complex model that is good only for the seen (training) data. Minimum description length (MDL) principle in machine learning [79] suggests rather simple and generic representation that is valid for unseen (test) data also. Besides relevance in MDL, feature selection also leads to efficient learning of classifiers and data mining in growing complex databases [58]. Feature extraction itself is a dimensional reduction to avoid working with every pixels of the high-resolution bioimage. Selection of salient features based on their distinguishing power is the next step to avoid dimensional explosion [58, 81, 128, 134], sometimes followed by generating better (often complex) features by recombining simple features so that the second-level features fit the application better. Integrating feature from different domains—color, texture, motion, and shape—is gaining importance in recent image informatics [30, 52]. CMU computational bioimaging group is among the very few in bioimaging research to address this major issue in very systemic way [50, 115]. In cell classification domain, classifiers are sometimes limited by underdetermined classification boundaries due to limited number of available cell images in comparison to number of features considered. One solution is feature reduction either by feature recombination or by feature selection utilizing one or combination of algorithms, as summarized in Fig. 2.3 and exemplified as follows.

### 2.5.1 Component Analysis Methods

**Principal Component Analysis (PCA)** considers salient eigenvectors of the feature covariance matrix (in general much fewer than number of features) based on corresponding high eigenvalues (i.e., stronger basis vectors). The strongest eigenvectors (above a threshold) often define the linear transformation matrix [122]. This transformation provides weighted linear combinations of the original (sometimes normalized) features in least square error sense to fit the actual feature-set variation—but in a much lower dimensional feature space such that the classes are well separable (with less overlaps) [105].

**Nonlinear PCA (NLPCA)** is PCA, but with nonlinear transformation and combinations of the original features. From infinite possibilities to get such nonlinear transformations, one way is to learn it from a symmetric neural network [14, 35]. At the trained state, the first layer of the ANN structure converts the input original features to a linear combination and the second layer transforms them in a nonlinear way. Then they are inversely transformed back (first nonlinearly, and then linearly) to the output features, which are identical (or very close) to the input original features. Second layer outputs are the NLPCA-recombined features.

**Kernel PCA (KPCA)** adopts a nonlinear kernel function—like polynomial function, multilayer perceptron (MLP), radial basis function (RBF) or any other nonlinear function that first transforms the original feature space to a very high-dimensional feature space. Thus in a way, KPCA is feature extractor as well. Then linear PCA reduces the huge ensemble of features to very few recombined features compared to the original input features.

**Independent Component Analysis (ICA)** makes use of the fact that less the dependency among individual features for the acquired dataset, more mutually cooperative and precise the feature set is to describe the (biological) features of the (cell) image [105]. Criteria such as non-Gaussian nature are utilized to maximize the independence among recombined features (sometimes with both linear and nonlinear transformations) to cover larger area in potentially infinite dimensional feature space.

### 2.5.2 Discriminant Analysis Methods

**Classification or Decision Trees (DT)** is formed with individual original feature where the effective classification power of each feature is measured by entropy-based information gain and penalized for too much fragmenting of the data by a split information feature as defined by C4.5 algorithm [79]. Features with higher information gain separates different classes better and hence might be selected for classification.

**Fractal Dimensionality Reduction (FDR)** works on the principle that few features are often redundant because same information is shared by multiple

features, while few other features might be intrinsic because that data points are cohesive and better classified in the corresponding space. The fractal dimensionality of the data set, often represented by correlation fractal dimension, describes self-similarity of the data points and is a good approximation of the intrinsic dimensionality of the data. Correlation-based fractal dimensionality of the whole data set is computed first. Partial fractal dimensionality of a feature is measured in the same way, but without using that particular feature. Feature leading to minimum decrease in correlation is considered noise and hence not selected for further consideration. Thus iterative backward elimination is continued to reduce number of features.

**Linear Discriminant Analysis (LDA)** selects those features that separate the classes best (with least classification errors) with linear class boundaries. The criterion to be minimized is directly proportional to intraclass variations and inversely proportional to interclass distances between means [35]. Thus minimizing this measure class cohesiveness and as well as interclass separation can be increased. Feature set that minimizes this criterion is selected [23]. But the number of possible feature sets explodes with the feature dimensions.

**Stepwise Discriminant Analysis (SDA)** applies a (split-&-merge like) greedy search approach to solve computational explosion in LDA. It adopts same criterion as in LDA for the present feature set and computes F-statistics for each left-out feature to enter into and for each currently selected feature to exit from the current set. The feature with highest F-to-enter is added and the feature with lowest F-to-exit is eliminated in turn based on F-statistics computed in between. Thus forward selection and backward elimination are iterated till the criterion value stabilizes [49].

### 2.5.3 *Evolutionary Learning Methods*

**Genetic Algorithm (GA)** attempts to avoid the usual problem of entrapment in local minima in the feature-dimensional search space inflicting the greedy search algorithm of SDA. GA follows “survival of the fittest” rule from evolution theory and utilizes “mutational” randomness to come out of the local minima in the search of the global minima. It considers a string of bits (1: for feature being selected and 0: for feature being left out) as a species. Systematic variations are applied by “crossovers” and randomness by “mutations” among the current “chromosomes” to change the initial populations toward more fitted populations. Defining proper fitness function is critical for evaluation of intermediate populations and individual species. For feature selection, classification error often defines the fitness function such that reduced error means better feature sets. To bias the selection toward minimum possible sets of optimal feature, MDL constraint sometimes works in parallel with the classification errors [66, 79].

### 2.5.4 *Performance Analysis and Future Scope*

CMU work [115] in recognition of proteomic subcellular location patterns in cellular images reveals that understanding protein functionalities is facilitated by localization of subcellular compartments as they create unique biochemical environment for protein folding and other functionalities [84, 97]. To classify these patterns with less number of available static images, they start with host of features (discussed earlier) and then select features with evaluation by above-mentioned strategies for all ten major subcellular patterns in HeLa cells. Their results reveal that in this specific application, feature selection procedures (DT, FDR, LDA, SDA, and GA) perform better than feature recombination procedures (PCA, NLPKA, KPCA, and ICA). SDA performs the best with reduction of feature dimensionality by 0.46 factor while increasing the classification accuracy by 2.2 % [50]. GA-based method is the close second with reduction in dimensionality by 0.51 factor while increasing the accuracy by 2.3 %.

One possible future research direction for GA-based feature selection might be cascading a classifier like ANN to evaluate performance of the current set of features. ANN output is the value of the fitness function that is fed back to GA for crossover and mutation decisions to reach the fittest set of features in GA output. For feature recombination, novel ideas of synthetic feature generation adopting genetic programming (GP) methods might be successful as in CVPR applications [66]. In GP method, sequential image operators are represented by tree-like structure and crossover or mutation of branches (as in GA) leads to generation of novel synthetic (recombined) features, that might not make sense ordinarily but might define the best feature to define class boundaries in a lower dimensional feature space. CMU work [50] underscores the need of proper feature selection procedures before the classification stage. One recent review [120] discusses different applications of machine-learning techniques in cell biology—from preprocessing, detection, feature extraction, feature selection, supervised and unsupervised classification, performance optimization, and availability of such software packages for cell biologists.

## 2.6 **Cell Classification**

In static image bioinformatics, cellular or subcellular recognition or classification is often the ultimate goal. As infinite structural variations among cells and intercellular organelles and molecules are possible, high-throughput automated recognition of biological structures and distributions requires both robust image feature sets and accurate classifiers. The dynamic characteristics of the cells and biochemical activities make classification even more difficult. As an example, the most typical Golgi images are characterized by compact structure, while prior to mitotic cell division, Golgi complex undergoes fragmentation to reunite at the later stage in two offspring cells. This dynamic fragmentation in Golgi complex gives problem in

simple morphology-based structural classifications even for manual detections, while computed texture descriptors (imperceptible to human eyes) recognize them much better [90]. Similarly, in human protein atlas, manual annotations are corrected by automated classification in support vector machine (SVM) classifier followed by hierarchical clustering [63]. Sometimes, without explicit segmentation, morphologically preprocessed images themselves are fed to modular neural network [31]. Modularity of the hidden layer considers the image at different scales and for different regions. As the bioimage data used in this work have no cell-to-cell occlusion, the different regions of the images generally contain individual cells (i.e., segmentation is implicit) and provide good classification results.

CMU group applies several supervised and unsupervised classifiers to recognize subcellular localizations from multiple biological image sets [50, 115]. They list links to several proteomic databases that are freely available for comparison, but notify that unified framework for all expressed proteins in different cell types under many biological conditions is still a big challenge [90]. Their work reports recognition accuracy of 95 % for 2D and 98 % for 3D HeLa cell images. Comparison of results for each image from these classifiers permits estimation of the lower bound classification error rate for each subcellular pattern, which they interprets as to reflect the fraction of cells whose patterns are distorted by mitosis, cell death, or acquisition errors. They claim that sometimes automatic classification can outperform human visual classification. For easily confused endomembrane compartments (endoplasmic reticulum, Golgi, endosomes, lysosomes) pattern classification is improved by 5–15 % over the human classification accuracy [49]. Specifically, cell and organelle characteristics like Gabor texture features and Daubechies  $-4$  wavelet features cannot be measured visually for manual classification and that makes the difference in favor of statistical pattern classification strategies. They distinguish ten major eukaryotic subcellular location patterns in 2D microscopic images and eleven in 3D images, including few that are not discernible in human eyes.

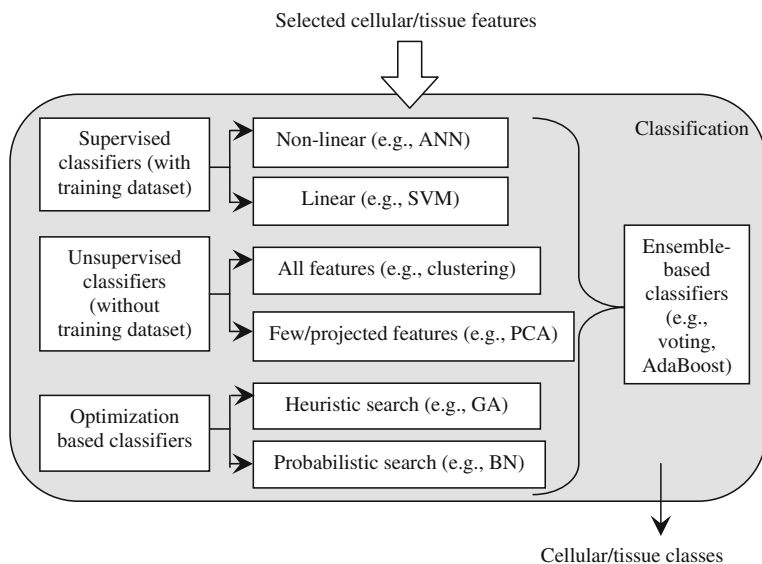
Some of the image-based cell molecular applications utilize simple histogram-based thresholds or seed-growing segmentation for classification. For the automated analysis of epithelial wound-healing process from time-lapsed image sets [107], simple region-based segmentation and seed-growing technique are used as the classification between lacerated wound and unaffected skin—although seed initialized method is not clear. Similar technique is also applied segmenting and classifying bacteria [118], tracking them individually, estimating spatiotemporal tracks and recognizing biological activity with and without application of drugs. When there are similar cellular or subcellular structures in a distinctly different background (in vitro solutions), segmentation itself is type of pixel-based classification, where geometrical models and intensity clustering are adopted in several works [11]. Sometimes location-based region adjacency graphs could distinguish luminal epithelial cells, stromal cells and nuclei from in vitro subcellular images [20, 22] or sparse features are learnt to classify tumors in histopathology [89]. When several different proteins are present for each location class, local features information from protein as well as reference marker images (acquired in parallel)

might be useful to correct classification [28]. In a recent work [23] on classification and abnormality detection in Pap smear cells, multiclass datasets (4 and 7 classes) are merged for 2-class problems—normal and abnormal—to compare performance. Five classifiers are tested—Bayesian classifier, LDA, K-nearest neighbor (KNN), ANN, and SVM—where ANN performed the best—with >95 % accuracy for multiclass and >97 % for 2-class problems.

Major pattern classification methodologies used by different bioimaging groups are depicted in conceptual diagram in Fig. 2.4 and briefly addressed below with pointers to references for necessary details. Interested readers might review recent survey papers on complete cell analysis systems that summarize different unsupervised, semi-supervised and supervised machine learning-based classifiers [115, 120].

### 2.6.1 Artificial Neural Network (ANN)

This are layered directed acyclic graph (DAG) imitating biological neural networks to transform input signals or features to output activations through linear or complex nonlinear functions. Complex nonlinear multi-input-multi-output (MIMO) mapping functions are learnt from a training dataset by supervised learning rules like back-error propagation, reinforcement learning, competitive learning, etc. [14]. This is generally applied when the mapping function might be considered as a black box and no further structural analysis is warranted. There are number of variants of



**Fig. 2.4** Major pattern recognition techniques utilized in cellular and tissue classification in biological datasets

ANN structures [12], although fully connected multilayered-perceptron (MLP) with sigmoid activation function and back-error propagation learning rule is the most common and successful [13]. In the feature-based supervised classification of cellular images, input features and correct output class labels (often visually classified) are utilized to tune the neuronal connection weights (only changeable parameters in ANN) over the training cycles and once trained can classify unseen (test) data [23, 25, 49, 56].

One work [31] adopts a modular neural network (MNN) trained with sets of confocal sections through cell lines fluorescently stained for markers of key intracellular structures. MNN is developed as three 2D layers of input. In the modular structure, MNN input layer obtains monocular pixel-level intensity values, hidden layer considers different sections of the cellular image at different resolutions/scale to capture the overlap and the structural relations and the output layer produces the classes like mitotic nucleus, nucleus, Golgi, etc. Training is done with standard back-error propagation with 67 % of the randomly sampled data. Key feature of MNN is capability to capture structure of the organelles in 2D.

### 2.6.2 *Support Vector Machines (SVM)*

Unlike ANN, which is a nonlinear classifier, SVM generally is a linear classifier that finds a hyperplane between the two classes that maximizes the distance between the plane itself and the data points from different classes closest to the hyperplane [125]. It is an example of margin-based classifier in pattern recognition. The plane is supported by multiple pairs of closest data points from different biological classes, where the plane is at equal distance from both the points in a particular pair. Actual feature dimension is mapped nonlinearly to a very large dimensional space with the hope that class overlaps can be nullified or reduced for better classifications with less error. General two-class SVMs are extended to multiclass applications by max-win, pairwise, and classifier DAG. SVMs can adopt different kernel functions for mapping different low-dimension to high-dimension, like linear, radial basis functions (RBF), exponential RBFs, polynomials, etc. One way to decide which one to select is to start with complex kernels and gradually reduce order of complexity and compare performance to stop at an optimum level for a particular application. In CMU work, SVMs with exponential RBF perform the best for image-based subcellular location classification [50, 63, 115]. SVM classifiers are very successful in several bioimaging applications [23, 25].

### 2.6.3 *Ensemble Methods*

To avoid the general problem of entrapment in local minima of the error surface during training (as in ANN) [35, 125], one way of robust classification is

considering multiple classifiers with different structures and parameters and fusing the results from these individual classifiers to get the final result. This is the basis of ensemble methods with many variants based on the technique of fusing the base classifiers [12]. AdaBoost learning starts with weak classifiers and gradually increases weights of those classifiers that have made wrong classification in last iteration. Thus more stress is exerted to learn to classify confusing examples. On the other hand, Bagging method tries to increase the training dataset by resampling with replacement and to learn base classifiers with different input sets. Results from them are combined to get the final result. Mixture of experts is another ensemble technique that follows divide-and-conquer strategy so that training set is partitioned by some similarity, classified by different base classifiers for different partitions (like, gating network) and then combined (e.g., by local expert network). Majority or consensus voting is one simple ensemble method that, married with Kaplan Meier test, can effectively perform tumor subtyping [20].

### 2.6.4 *Hierarchical Clustering*

When subcellular images cannot be classified by visual inspections (like in Golgi compartments, due to mitotic cell division), supervised learning is not possible. Clustering is an unsupervised learning technique to know the classes from unlabeled training data from proximity in the feature space. After proper normalization of different features (to give proper weights on different dimensions), proximity can be measured with any standard distance metric [35], like Euclidean distance or Mahalanobis distance using feature covariance matrices, etc. Individual classes can be formed with proximity and multiple discriminant analysis (MDA) of the clusters in feature space. K-means spectral clustering [25] is one such variant. CMU researchers adopt bottom-up approach to learn a hierarchical subcellular location tree (SLT) from clustering, where at first every organelle structure is taken as individual classes and then classes are iteratively merged by similarity (proximity and MDA) in layers to form a classification tree [115]. This type of hierarchical classification tree or SLT is a very high-level tool for cell molecular biology and can be applied in other medical diagnostic systems as well. A SLT is automatically developed from cellular and subcellular image sets [90, 115] where classes are merged correctly as expected from biological knowledge (like first to merge were giantin and gpp130 as both are the Golgi proteins). In protein regulatory networks, graphical connectivity-based hierarchical clustering is applied to classify cell lines and data mine parts of them to categorize whether it is “living” or “dying” [95]. The biggest problem with unsupervised learning is visual or manual validation of the classification results is not possible as there is no labeled training set.



### 2.6.5 PCA Subspace-Based Classifiers

Classifying cellular forms of proteins encoded by human cDNA clones is a primary step toward understanding the biological role of proteins and their coding genes. Classifier surface is successfully estimated in PCA subspace to classify protein structures with a novel framework I-GENFACE for protein localization [122]. Morphological, geometrical, and statistical features, such as brightness of a region of pixels, the object boundary, and co-occurrence matrix-based grayscale textural features, spot and line features, histogram, and amplitude features, etc. are extracted semiautomatically. Distance-based metric in PCA subspace is adopted to classify the protein forms and then the corresponding images. Classification accuracy achieved is approximately 90 % for seven subcellular classes.

### 2.6.6 Performance Comparison

Beside standard performance evaluation tools in CVPR [12, 35], sometimes application-specific criteria are defined in cell imaging [49].

(1) *Complexity of the decision boundaries that a classifier can generate* For a pair of Golgi proteins, giantin, and gpp130 (ones difficult to classify visually) and two most informative features derived by SDA-based feature selection (namely, fraction of fluorescence not in any object and convex hull eccentricity) CMU research illustrates the complexity on the 2D scatter plot. This is one way to check the complexity of the classifier needed, like the order of the activation function in ANN, or polynomial order in kernel-based SVM, etc. Although complex classifiers sometimes classify small dataset (with less variation) utilizing complex features, according to minimum description length (MDL) principle [79] these are overfitted classifiers as they lose generality for the dynamic organic environment.

(2) *Dependence of the classifier performance on the size of the training set* This is the capability to learn from limited training set and insensitiveness to the presence of outliers in the data. In cell molecular biology, complexity of the dynamic environment might demand multiparameter classifier that in turn needs larger training sets (to fit the parameters iteratively) which is not always available. CMU work shows [50] that with more training data classification accuracy improves, as expected. Even without access of the complete dataset, probabilistic active learning could model and discover biological response in gene datasets [88]. In this work, greedy merge structure learning iteratively combines distributions with the assumption that same conditions affect the unseen variants similarly. Outliers generally affect in incremental learning modes of different classifiers, like ANN and reduce accuracy.

(3) *Sensitivity of performance to the presence of uninformative features* All the features may not contribute cooperatively toward classification. CMU research claims that ANN perform better for 3D cell images than 2D images, which is

somewhat unexpected and underlines the importance of feature selection. They adopt SDA feature ranking based on information content and gradually add features according to high-to-low ranking to compare how the classifiers behave [50, 115]. This is a classical pattern recognition method to check how many features are adequate to work with a particular classifier applied to a particular task [35]. They also conclude that the ability of a classifier to adapt to more noisy features depends on the feature space itself.

Above indices are very general for any applications. CMU work also evaluates classifiers based on statistical paired t-test. They conclude that SVM with exponential RBF kernel performs most consistently for different subcellular location feature sets [50].

### ***2.6.7 Other Methods and Future Scope***

Evolutionary computation a key machine learning paradigm not yet utilized to its full potential in bioimaging. Methods like genetic algorithms (GA), genetic programming (GP) (see Sect. 2.5) and their variant like colony optimization, particle swarm optimization, “tribe”-based global GA, etc. fall in this class. Only few recent works report successful usage of such techniques for segmentation [91], feature selection, and classification [58]. Bayesian learning [57] is another area where very limited cell classification research is so far invested [23] but might lead to success—specifically as cellular localizations can easily be represented as cause-and-effect relations with the surrounding biological processes and injected drugs and antigens.

## **2.7 Dynamics and Tracking: Cell Activity Recognition**

Cellular processes are heterogenous and complex, yet very organized. For understanding molecular mechanisms at the systems level, like cell migration and signal transduction [137], complex spatiotemporal behaviors of cellular processes are needed to be datamined with objective computational tools. Cell migration, besides motion, involves shape changes (morphogenesis) and interactions at different levels, from single cell flagella-driven movement to bacterial swarming to collective stem cell migration toward chemoattractants. Estimated motion leads to preferred migratory paths as well as related shape deformations. Sometimes correlation between signaling events to spatial organization of the biological specimens might enhance understanding biological processes [105]. One recent work [18] proposes a complete pipeline of computational methods for analyzing cell migrations and dynamic events, starting from acquisition, registration, segmentation, and classification, and finally addressing cell tracking, event analysis, and interpretation. Changes in shape and topology are tracked and motion fields are computed. Another application oriented review [105] of developments and challenges in

automated analysis summarizes few examples of intracellular dynamics, cell tracking, and cellular events as follows: (1) Estimation and control of cell cycle state and its rate of change directly will link to cancer and DNA damage. (2) Very little is known on intricate dendrite branching pattern unique for each neuronal class. Tracking of dendrite arbors in 3D microscopy might help estimating dynamic relationship between dendrite growth and synaptogenesis. (3) Embryonic heart development and embryogenesis of Zebra fish could be monitored to enhance understanding and quantifying structural phenotypes in tissue.

The challenge of the postgenomic era is functional genomics, i.e., understanding how the genome is expressed to produce myriad cell phenotypes [11, 88, 94]. To utilize genomic information to understand the biology of complex organisms, one must understand the dynamics of phenotype generation and maintenance. Also cell signaling and extracellular microenvironment have a profound impact on cell phenotype. These interactions are the fundamental prerequisites to control cell cycles, DNA replication, transcription, metabolism, and signal transduction. All the biological events in the list require some kind of particle tracking and then classification of the dynamics. Signal transduction is believed to be performed by protein molecules passing across the cell membrane, carrying some electrochemical message to the target cell or organelle, where it initiates some biochemical event [119]. Hence tracing signals and analyzing their implications also require tracking over image sequences. Biochemical activities in the molecular (e.g., protein, DNA, RNA, genes etc.) and atomic levels (e.g., protein folding leads restructured form of the proteins followed by higher level activities like mitotic fragmentation) in intracellular compartments (e.g., mitochondria, Golgi bodies, nuclei, cytoplasm etc.) and intercellular signaling are one of the prime signs of life [97]. Higher level activities in living organism trace back to these molecular level biochemical activities [36].

One crucial point in bioinformatics is that the biochemical processes are often very slow, while few transient processes are very fast—e.g., red blood corpuscles (RBC) are dying in hundreds every minute, and new RBCs replace them to keep the equilibrium. After effective shot detection and key frame selection depending on rate of change of information content of the cellular video (see Sect. 2.2), cell tracking, and biological activity analysis can be efficiently performed only with those key frames (see conceptual diagram in Fig. 2.1).

Like RBC lifecycle, many other cellular events are transient in nature—including plant cell activities. In plant physiology research, cell dynamics analysis plays a significant role—from monitoring cell morphogenesis [62] to dynamic gene activity [78] for developmental processes. In one *in vivo* study of  $CA^{2+}$  in the pollen grain and papilla during pollination in *Arabidopsis* in fluorescence and ratiometric imaging, yellowameleon protein indicator is utilized to detect change in  $CA^{2+}$  dynamics over different phases of pollination [54]. Unfortunately, growth rate of pollen tube is measured manually by the rulers that, unlike in computational methods, reduces statistical reliability. Similarly, interactions between B and T cells are essential for most antibody responses, but the dynamics of these interactions are poorly understood [92]. By two-photon microscopy of intact lymph nodes, it is

demonstrated that upon exposure to antigen, B cells migrate with directional preference toward the B-zone-T-zone boundary in a CCR7-dependent manner. There are salient variations in velocity, speed, and duration of activity based on antigen doses. These findings provide evidence of lymphocyte chemotaxis *in vivo*, and can define similar dynamics associated with T cell-dependent antibody responses. Development of many vertebrate tissues involves long-range cell migrations that are often quantified from time-lapsed images and few samples of data. One work [132] utilizes two-photon laser scanning microscopy and quantitative analysis of four-dimensional cell migration data to investigate the movement of thymocytes through the cortex in real time. This work tracks the thymocytes over multiple frames of cell video, forms time-stamped spatiotemporal trajectories to classify into two classes of motility rates (higher and lower), and concludes that displacement from origin varies differently for these motility rates (lower motility follows linear rule, while higher ones follow quadratic rule). And these two distinct migratory behaviors within wild-type cortical thymocytes are analyzed for further higher level biological decisions. Cell activities like cell division, cell migration, protein signaling, and protein folding in biological videos should be computationally analyzed and classified into spatiotemporal processes to understand the dynamics behind them.

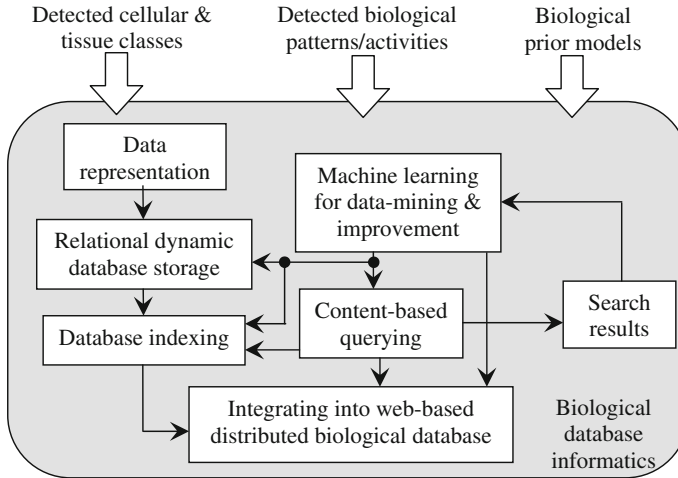
Euro-BioImaging consortium is one of the very few groups actually analyzing dynamics in cell videos. They analyze bacterial motility videos taken under light microscopy and *in vitro* solutions [118]. They record the trajectories of free-swimming bacteria of the species *Rhodobacter spheroides* under a variety of incubation conditions, and estimated trajectories of the rotations of these bacteria tethered to glass coverslips using an anti-flagellin antibody. The rapid rotations of helical flagella by proton-powered molecular rotary motors (embedded in the bacterial membrane) cause free-swimming bacteria to swim forward in curved trajectories. Brief reversal of the direction of rotation of the motors in a single bacterium induces its flagellar bundle to fly apart, causing the cells to undergo a “tumble”, leading to randomizations of the new direction of motion upon resumption of the normal rotational direction. Bacteria swimming up a concentration gradient of a chemoattractant (e.g., glucose) tumble less frequently than bacteria entering a hostile (e.g., acidic) environment. Euro-BioImaging group studies these bacterial responses to environmental stimuli by considering spatiotemporal trajectories of individual bacteria and the times and positions of bacterial events, such as tumbles and reverses. It would be impractical and intractable to undertake such detailed analysis and annotations of events manually. Image/video processing techniques are indispensable for such analysis. Hence they track multiple bacterial motions, form spatiotemporal traces (after smoothing and merging of tracks as in “boundary-tracking” algorithm in CVPR), and detect cell biological states like “swimming forward” (flagella rotating counterclockwise), tumbling (flagella rotating clockwise), and stopped. These state transitions are then mapped to different biochemical ambience and corresponding responses in terms of bacterial speed, rotational frequency, etc.

Automated monitoring of wound-healing assay in time-lapsed microscopic data is another application where cell motility dynamics might help clinically [118, 143].

Euro-BioImaging group performs textural segmentation and quantify healing by rate of change of dimensional spread with or without drug [118]. In a recent sophisticated work on wound-healing and cell-scattering data, cascade of SVMs performs initial classification of local patches, and then graph cut corrects and reclassifies [143]. Acceleration effect of Hepatocyte growth factor/scatter factor (HGF/SF) is utilized for monitoring. Few recent works in cellular biology point out important future application of CVPR strategies to understand cellular events. In one system pharmacological application [64], cell population is tracked by morphology, proximity, and specifically the motion fields obtained from particle filters and interacting multiple model filters. Morphological phenotypes help identification while SVM, factor analysis, and Gaussian mixture models classify the profiling types. All the above examples demonstrate how CVPR strategies can enhance the cellular activity analysis, and how understanding several other similar biological processes, upon computational analysis and exploratory data mining, can enrich our higher level knowledge (see Fig. 2.1). Different established object tracking [140] and structure from motion algorithms [12] could be adopted to analyze these applications.

## 2.8 Bioimaging Databases and Content-Based Retrieval

Vitality of living cells and their dynamic behaviors separate them from innate rigid objects [119]. Innate objects generally have same shapes (e.g., rigid objects like a car can change its 2D projection due to motion and viewpoint change, but always have same 3D structure [43]) or shape changes in few discrete predictable ways (e.g., flexible objects like a military fighter jet changes its 3D structure due to different missile attachments, wing positions, and other artillery manipulations; but definitely in a few discrete and predictable ways [32]). In case of living cells and their intracellular molecules, environmental effects are more complex and often not yet understood and cause the cell shapes to change in very unpredictable ways [17, 115]. Like a motile cilium changes its shape unpredictably to extend one part (like a leg), get hold (like a hand) and then shifts the body organisms toward that direction. Its shapes have sharp differences, yet it is a cilium [119]. Time-lapsed imagery demonstrates wide variations that are to be stored in a dynamic database for future search, research, and analysis. Additionally proper content-based image retrieval schemes are to be adopted so that mere shapes cannot misguide the retrieval [26, 88]. Even a single type of cell might have high variability as different biologists study it at different conditions [22]. Distributed databases and web-based querying facilities increase cooperative efforts of experts from different parts of the world [77]. But at the same time this underscores demanding requirements of necessary mapping between different nomenclatures and formats [147], content-based indexing strategies [26], and machine learning methods for improved retrieval [36, 96, 98, 120]. Besides phenotyping and understanding dynamics,



**Fig. 2.5** Bioinformatics modules in large-scale biological dynamic databases that are often distributed across the globe and datamined by complex content-based queries over the Internet

database might be useful for building atlas for model organisms or for 3D reconstruction of brain wiring from international data with wide variability [96].

Feature-based image representation, dynamic database maintenance, content-based indexing, content-based image retrieval (CBIR), and learning-based improvement of retrieval performance are relatively matured subfields in CVPR. Interrelations between these broad CVPR modules in bioinformatics context are summarized in Fig. 2.5. In cell biological image processing, only few works are reported in this direction, mostly for static images [22, 26, 88, 98] and rarely for cell dynamics in videos [77, 147]. These efforts are summarized below with relevant pointers to future research directions.

### 2.8.1 Data Representation

The first step of any database design is the determination of the scope of the data to be stored, i.e., together with their complete description, taking into account the targeted group of users and the applications of interest. To determine these generally biologists and microscopy experts develop a list of biological descriptors for data representation and database querying. These descriptors guide modules down the line including image processing, feature extraction, classification, database management, and structural query systems.

Organisms express their genomes in a cell-specific manner, resulting in a variety of cellular phenotypes or phenomes [22]. Mapping cell phenomes under a variety of experimental conditions is necessary in order to understand the responses of

organisms to stimuli. Representing such data requires an integrated view of experimental and informatics protocols. This is more critical when experimental procedure varies [22] or even nomenclature differs between research groups [147]. BioSig system developed in LBNL [20, 94] adopts a hierarchical data model to capture experimental variables and map them to light microscopic image collections and their computed representations (features) at different levels—sample tissues, cells, and organelles. At each layer, information content is represented with an attributed graph of cellular morphology, protein localization, and cellular organization in tissue or cell culture.

There are two kinds of information associated with visual objects (image or video): information about the object, called its metadata, and information contained within the object, called visual features [34, 67]. Metadata (such as the name of a protein) is alphanumeric and generally expressible as a schema of a relational or object-oriented database [147]. Visual features, in contrast, are mathematical properties of the image derived by computational tools from image processing, CVPR, or geometric routines discussed in earlier sections [11, 105, 115]. A database system that allows a user to search for objects based on contents quantified by above-mentioned features is said to support content-based image retrieval (CBIR).

Euro-BioImaging group [34, 147] utilizes following (quite generic) data representation covering wide information variability including the rare support to cell video data.

- General information: administration and organizations: submitter's name, title, funding, references, and contacts.
- General information: microscopic data: metadata on location, format, and size, channel and axes information (coordinate system), annotated figures.
- Biological data: details of the biological specimens (taxonomic information and parameters that depend on the type of specimen) and observable biological features.
- Experimental details: sample preparation: experiment, preparation steps, their biochemical and physical parameters, instruments used (e.g., for microinjection). Free-text comments for nonstandard information can be provided as well.
- Experimental details: data acquisition and instrumentation: for reproducibility, microscopic settings, and image-recording schemes are stored.
- Experimental details: image processing: ranging from simple enhancement to complex 3D reconstruction algorithmic information.

The need of unified data representation comes from the diversity of experimental procedures followed by biological researchers around the globe [22], like different application-specific microscopic systems are used: all kinds of light, electron and scanning probe microscopy with different resolutions. The biological targets are also diverse, ranging from entire organisms as observed by developmental biologists to the macromolecules studied by structural biologists [36, 98]. Datasets are also of quite different sizes ranging from less than 1 MB for many electron microscopic datasets to hundreds of MB for scanning microscopic videos in cellular dynamics contexts [67]. Dimensionality of the datasets also differs a lot [108].

Atomic force microscopy (AFM) images are two-dimensional. Three-dimensional density distributions are reconstructed from 2D electron microscopy data. Video microscopy generates 3D files with two spatial axes and one time axis. And confocal light microscopes can even record 3D datasets as a function of time. In addition complementary information may be stored in several channels in multi-labeling fluorescence microscopy [28]. Sometimes infrared high-spectral frequencies are utilized as an additional dimension for lung cancer tissue diagnosis [2]. Dynamic database should have flexibility to handle such multidimensional data. Even recent reviews on spatiotemporal dynamics of cellular processes inform that representation of behavioral knowledge in biological database is still a great challenge [137]. For distributed and web-based databases that is being accessed by hundreds of researchers around the globe with diverse datasets, unified data representation needs number of seemingly trivial information to be stored and incorporated in the relational database model [77].

### 2.8.2 *Database and Indexing*

Database indexing is an established field with enormous success in text-based systems. Success of these systems stands upon the general user-independent definitions or meanings of text-based database elements. Images and videos often contain richer information than textual explanations and informatics researchers work on content-based indexing and querying the image/video databases [10, 55]. There are also specialized databases like those with neuronal morphology [76]. Indexing and CBIR in relational database management systems (RDBMS) with multidimensional biological and cellular images are very challenging [26].

In biological databases, querying should be based on implicit information content, rather than by their textual annotations only. “Query-by-content” generally makes reference to those data modeling techniques in which user-defined functions aim at “understanding” the informational content of the datasets (at least to some extent) from the quantified descriptors (features). Euro-BioImaging consortium of multiple European nations is engaged in a pioneering effort (<http://www.eurobioimaging.eu/>) of developing one such web-based distributed database prototype [34, 77]. There are number of similar web-based biological databases, not necessarily image databases, like databases of sequences of nucleic acids (GenBank and EMBL Data Library) and those for protein sequences (SWISS-PROT and PIR). A digital neuronal database can help neuromorphological pattern analysis and brain atlas modeling (a significant paradigm shift) from advanced microscopy and image processing. A recent critical review discusses challenges in such applications—including dynamics, machine learning, and associated computations [98].

The complexity of stored information sometimes requires a unique database modeling tool, like Infomodeler used by the Euro-BioImaging group [67] to form a entity–relation (E-R) diagram with biological entities, their attributes, and relationships (like generic: “is related to”, aggregations: “is composed of”, and inheritance:



“is a” relations). Infomodeler provides database design in two abstraction levels. First, it allows an object-oriented approach (object role modeling; ORM) and second, it allows design in a logical model (E-R diagram). They also mention a denormalization step where redundancy is introduced to improve database performance. Among many entities already entered and several attributes defined among them, not all of them are relevant for a particular submission, since some of them depend on the microscopy technique or on the specimen [77]. As an example a commercial microscope can have number of optional laser beam, objective lens, and filter settings, only few of which are available at a particular location, and still fewer selective ones are actually used for a particular biological experiment. Hence to reduce the burden on the submitter, inheritance-based schemes are utilized just to specify the personal settings, and then the submission database fills out the rest with the default values (if entered earlier).

Euro-BioImaging database provides pointers and links to relevant databases at appropriate place, like SWISS-PROT protein database, EMBL Nucleotide database, protein data bank (PDB), etc. Their database comprises of three primary interfaces: submission interface, query interface, and visualization interface and two database modules: submission database and production database (they are independent to ensure security) [67]. Submission interface is the most complex one as, beside handling queries and incorporating results for visualization, it should also normalize incomplete datasets (by itself or by forcing the user to provide mandatory information) and interact integratively with the database in the background. The data is temporarily stored in submission database. Database curator modules then review the input, complete the unfilled format if necessary and migrate the data to production database. Query interface converts the submission into a structural query language (SQL) code with logical operations. Visualization interface handles the results from the SQL code converting to user-understandable forms to incorporate with the display page.

The backbone of any database system is the RDBMS. Due to high complexity of the cell video data in biological dynamics, and due to the semantic level queries preferred by the experts [26, 77], biological databases require Object-Relational Database Management System (ORDBMS), as it supports complex relationships between the biological entities [67, 147]. The complexity of the queries demands extension of the standard SQL for 3D data handling, named SQL-3. Queries are often needed to be modified for unified framework before actual database search.

### 2.8.3 *Content-Based Querying*

In contrast to other databases, the term “query-by-content” (QBC) is seldom used in the context of biological databases. However, some of the functionality implied by this term is in common usage in biological databases [34, 67, 77]. When a new gene sequence is searched for similar sequences in GenBank without using textual annotations, algorithms like Fast-All (FASTA) will provide a rank-ordered similar

gene sequence list. Besides textual descriptions, structural databases (e.g., PDB) store thousands of atomic resolution structures of proteins and nucleic acids with a list of coordinates of the atoms. In such databases, alongside keywords, queries might contain organisms, resolution, etc. as structural information and hence considered QBC. Searching 3D structural similarity could help discovering novel biologically active molecules and investigating the relationship between proteins' structures and their functions. Web-based QBC system by Euro-BioImaging group [34, 147] is one such protocol which searches for similar 3D structures of the macromolecules where similarity is measured in terms of features like 3D bounding size, multiscale shapes, channels of low density areas, internal cavity, and geometric symmetry. First two features are generic ones, while others are application-specific. Last type of features, although constrains the applicability and query space, makes the search space more dense with potential match and increases precision and accuracy. These are more relevant for database querying in terms of features like run lengths, velocities, and frequencies, and events like durations and patterns of bacterial tumbles, and correlated bacterial stops and reversals with changes in environmental conditions [118].

One of the most challenging issues is to choose an effective measure of structural resemblance (i.e., biological similarity) between two biological objects [26, 67, 77]. To align a pair of proteins, inter-atom distances of a 3D structure are often represented as 2D matrices and found useful for comparison since similar 3D structures have similar inter-residue distances. So, the problem of matching two proteins structures boils down to graph-matching problem where fundamental graph-partitioning and graph-matching methods [116, 129] can be applied to partition the proteins into smaller subgroups by forming hierarchical structural relations and quantifying matching percentages. One similar hierarchical graph cut method represents eXtended Markup Language (XML) data of complex protein regulatory networks as connected graphs, decomposes it spectrally into cohesive subnets at different abstraction levels and then data-mines for hidden cell motifs and cancerous activities [95]. Another graph-based similarity measure [117] applies combinatorial extension (CE) of the optimal path to find an optimal 3D alignment of two polypeptide chains and utilizes characteristics of local geometry (defined by vectors between C-alpha positions) of the compared peptides. In this web-based system, users submit complete or partial polypeptide chains in PDB format. Then, statistical results are returned along with the aligned sequence resulting from the structure alignment. Similar protocols are adopted for 3D searching in databases of small molecules to facilitate drug and pesticide discovery [84].

There are two different styles for providing examples or queries [10, 12]: (1) pictorial example (Virage Image Engine and NETRA system) and (2) feature value (like color, region area, texture, etc.) and expected percentage similarity as example (QBIC engine from IBM). In the first style of querying, features are first computed for the query example and the target images in the database and then it boils down to the second method. Distance metric is defined as a monotonically increasing function (e.g., weighted Euclidean measure) of these features to give a unique value and this metric should satisfy axioms of validity [79]. Generally CBIR

focuses more on 2D images, less on videos [118] and still lesser for 3D images. In a content-based 3D neuroradiologic image retrieval system [69], a multimedia database contains a number of multimodal images—namely magnetic resonance and computer tomography (MR/CT) images as well as patient information (patient's age, sex, symptom, etc.). With proper CBIR tool, such a system could help medical doctors to confirm diagnoses, as well as for exploring possible treatments by comparing the image with those stored in the medical knowledge databank.

### ***2.8.4 Learning in Content-Based Image Retrieval***

When a content-based retrieval system is applied to any specific domain it needs to answer two pivotal questions discussed earlier in details: (1) feature selection: of the extended list of features discussed in earlier sections, which computable features are sufficient to describe all images in the domain and (2) classification: what mathematical function should be used to find a measure of similarity between two objects. The second one poses more problems due to subjectivity of perceptual similarity among the observers. Two cells in two biological images can be decided as “similar” by one biologist due to their partwise structural similarity (e.g., they consists of a central cell body and cilia projections with the same pattern), while another biologist may classify them as different due to their functionalities. This dynamic nature of similarity measure makes CBIR more challenging. Machine learning strategies based on relevance feedback [12, 110] might help in such cases where the similarity measure (or even weights for combining different features) could be learned from user feedback (interactive manual inputs) regarding relevance of the result. This method learns the user query, structures the query and the search space to take into consideration more potential matches and incrementally improves the retrieval results over multiple iterations for the same user (see Fig. 2.5). Moreover, this method is extended for short-term learning from a single user and long-term learning from multiple users using the system several times to improve the overall retrieval performance [10]. Query-by-content in biological databases is yet to adopt this type of practical learning strategies.

### ***2.8.5 Distributed Databases and Web-Based Querying***

Distributed computation and web-based dissemination strategies are required [77] because of several necessary qualities of large dynamic databases: (1) flexibility to enter new data and update RDBMS continuously and incrementally with streaming data from users around the world; (2) integration of apparently diverse frameworks to analyze data and results including cross-database search in a seamless unified way; (3) fault tolerance of multiserver systems for computationally expensive

database manipulations that can be split into parallel and multithreaded modules. More is the complexity and abstractness of the data (like images, videos, cell activities), more is such requirements. For emerging field like bioinformatics, where hundreds of research groups are working globally on similar (but not exactly the same) biochemical processes, distributed database systems, web-based querying and platform-independent visualization tools are absolute necessities [77, 96]. These will give the researcher facilities to enrich the database, share their results with international community, statistically compare their results with existing knowledge and cooperatively work toward better results [36, 105].

A very nice overview of web database operation, implementation, scalability, interoperability, and future directions are discussed by the Euro-BioImaging group [77], including methods to cope up with mapping different names used for the same entities, integrating the data diversity, and updating the web database incrementally while the storage location, experimentation and associated parameters are continuously changing [147]. This consortium does a pioneering research in developing a web-based online biological database system. It describes the ongoing research on developing the dynamic database on an Informix Dynamic Server with Universal Data Option [67, 77]. This object-relational system allows handling complex data using features such as collection types, inheritance, and user-defined data types. Informix databases are used to provide additional functionality: the Web Integration Option enables World Wide Web (WWW) access to the database; the Video Foundation Blade handles video functionality. WWW facility provides the necessary structure for worldwide collaboration and information sharing and dissemination [34, 147]. Future scopes lie in incorporating new microscopy techniques, customizing WWW visualization interface that depends on user profile, and tighter interaction with collaborating databases [147]. Current biomolecular databases [20, 26] basically follow similar RDBMS structures, just from different providers.

Database over the web has to bear extra burden of providing simple interfaces for the (sometimes computer-naïve) biologists and at the same time ensure security and integrity of the distributed and dynamic database from intrusion and misleading submissions. Hence it is better to separate out submission module and actual database by a buffer database. The standard approach to connect with a database involves calling a CGI application (a program running on the web server) through calls from flat files containing HTML text [67, 94]. The alternative approach involves making a direct call to a database program with the page names and all relevant parameters. In both cases, SQL code is incorporated into standard HTML code. When the WWW browser requests the HTML file, the SQL code segment is extracted, passed to the RDBMS, and interpreted [147]. The result is formatted in standard HTML for visualization. Web pages are created dynamically, i.e., some template formats are modified based on user needs to create a specific web page. It also reduces the development time. Other domain-specific creation is stressed with user-defined web tags. Importantly, all the semantic web standards can still be combined and this generic framework can be extended to many other web database applications (e.g., medicine, arts) [77]. BioSig system developed in LBNL [94] also makes their

computational biology framework distributed and platform-independent using eXtended Markup Language (XML) protocol generated by biological experiment and handling those to reach bioinformatics decisions [20].

## 2.9 Future Research Directions

Many future research scopes are already discussed under individual sections followed by relevant pointers toward related works from CVPR and other research fields. To summarize, cell video analysis can be immensely enhanced by future research areas including: (1) cell video summarization methods [40], (2) texture [52] and graph cut-based segmentation [116], (3) close-loop cooperation between segmentation and object alignment with deformable models [37, 44], (4) synthesizing combinatorial features by genetic programming [66], (5) evolutionary learning in feature selection and classification [91, 120], (6) utilizing hyperspectral image features beyond currently applied frequency ranges [2, 9], (7) application of Bayesian classifiers [57], (8) improvement of performance even from small training dataset often encountered in bioimaging [10, 84, 88], (9) motion-based segmentation [15, 111, 135, 142, 143] and tracking [140] in cell videos, and (10) continuous learning by relevance feedback to improve database retrieval [10].

Learning enumerable phenotypes and distinguishing them requires parametric models that can capture cell and nuclear shapes as well as nonparametric models to capture complex shapes and relationships between them. Such generative models could be learned from static datasets [17]. Interesting future direction will be making those generative models dynamic—to capture temporal evolutions—possibly by dynamic Bayesian networks [57]. But unified framework of generative models to handle behavior of cells from diverse pedigree is still a very challenging task [84]—as model topology itself need to change within and across time. Morphogenesis and cell fragmentation complicate the Bayesian graphical analysis even more. Expectation maximization (EM) learning [35] cannot handle such flexibility to learn widely varying cell shapes, protein distributions within organelles and subcellular location patterns [97]. Recently an evolvable Bayesian graph has been proposed in incremental 3D model building application [43]. This generic probabilistic graphical model has flexibility [41] to represent unpredictable structural changes of the same cells, replication, and effects of drug or antigen applications over time. This framework also has potential [42] of modeling cellular behavior caused by different biochemical environments, analyzing interrelations among neighboring organelles in uncontrolled unpredictable environment, and even handling content-based video querying in complex database search engines.

## 2.10 Conclusions

Automated analysis of microscopic bioimages is making significant progresses with application of established tools and algorithms from computer vision, pattern recognition, and machine learning. Quantification and exploration of dynamic activities and evolving interrelations between them from cellular and biomolecular videos are the current key frontiers [36, 98, 105, 120, 137]. More cohesive and cooperative merger between computational and biological sciences [51] are expected to overcome these challenges toward achieving better understanding of the hidden patterns in living universe [74].

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