Leading Edge
Essay

Cell

Computer Vision in Cell Biology

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Computer vision refers to the theory and implementation of artificial systems that extract information from images to understand their content. Although computers are widely used by cell biologists for visualization and measurement, interpretation of image content, i.e., the selection of events worth observing and the definition of what they mean in terms of cellular mechanisms, is mostly left to human intuition. This Essay attempts to outline roles computer vision may play and should play in image-based studies of cellular life.

Microscope images and especially time-lapse image sequences contain information about the dynamics of cells, the distribution of subcellular components, and the activity of molecules that is inaccessible to other techniques. Three parallel technical developments have fueled the unique role light microscopy plays today in the study of cells. First, bright and genetically encoded fluorescent probes allow us to follow the distribution and activity of several molecular species within a cell. Second, optimized optics and feedback-controlled microscope hardware permit efficient acquisition of large, high-quality image datasets. Third, rapid advances in electronic detector technology enable the recording of images with ever more sensitivity. Single-molecule detection is now routine, even in living cells.

Quite surprisingly, these innovations in microscopic imaging have not been paralleled by developments of image analysis tools. Here, it is essential to clarify the difference between image processing and image analysis. Image-processing tools transform the signal to emphasize a particular aspect of an image. Examples of image-processing methods include contrast and color enhancement, deconvolution, image registration, filtering, and even segmentation. None of these methods interpret the image content, although they may greatly facilitate that task. With the advent of the first digital images in the 1960s (Inoue and Spring, 1997), cell biologists thus began to use computer programs to manipulate their images in order to "better see what needs to be seen." However, the ultimate task of assigning meaning to image events has been left to the human observer. Even today the great majority of image-based studies in cell biology still rely on human visual inspection to build a model of the image content.

Computer Vision—The Science Concerned with Image Analysis

Computer vision is an application area of Artificial Intelligence (AI), broadly defined as the science and engineering of making machines intelligent. Accordingly, computer vision scientists are preoccupied with making machines that can see. Here, *see* means capable of extracting information from an image in order to solve a particular task or interpret the scene in a limited or broad sense. Computer vision systems are implemented in a wide range of industrial and scientific applications, including systems for the control of robots and autonomous vehicles, video surveillance and inspection, organization of image databases, object modeling, and human-machine interfaces. With all these applications the goal is to replace the human observer, at least in part, during the interpretation of image contents, including the decision of which image events are relevant to answering a particular question. The output of a computer vision program is an abstract representation—a model—of a particular aspect of the image or of the entire image content. Based on the model, robots plan their next actions, autonomous vehicles change speed or driving direction, fire alarms are initiated, images of maple leafs are searched on the internet, the morphology of a brain tumor is defined, or the graphics of video games are adjusted to the pose of the player. Translating the computer vision paradigm to microscopy in a cell biological study, a computer vision system will replace the cell biologist's staring at images for the purpose of describing a cellular process. This Essay focuses on the roles of computer vision in the interpretation of image data from light microscopy, although a similar essay could be written easily about the roles of computer vision in electron microscopy.

The function of a computer vision program as an autonomous image interpreter has significant implications for the structure and operation of the software. In contrast to image-processing programs, which run through a fixed sequence of algorithms independent of the input image, computer vision programs progressively adjust the strategy of visual information processing to the properties of the images and to the knowledge acquired in previous processing steps. The goal of a computer vision program is to learn iteratively, as opposed to linearly, about the image content. The complexity of the learning algorithm required will of course depend on the complexity of the analysis task or the variability between scenes. For example, if a cell biological experiment delivers highly reproducible images and the goal is only to count the number of cells expressing a particular protein, the program will be able to process the images by a fixed sequence of filtering and segmentation procedures. Although in strict terms such a program still solves an image-analysis problem, its algorithmic

structure does not differ much from, say, a deconvolution program where the procedures are independent of the particular image content. On the other hand, if an experiment delivers images containing several classes of cells with different dynamic properties—some known, some unknown, and some transient—and the goal is to generate a predictive model of the mixed cell population behavior, then the program will have to identify rules for how cell classes affect each other and how changes in the behavior of a particular cell class affect, for example, the chances for interaction between two cells. The program will have to distinguish meaningful from insignificant rules for building such a model. This requires an iterative learning process where the computer replaces human intuition in the search for representative patterns in the cell population behavior.

The distinction of image processing and image analysis entails different requirements for the developers of software. The programmer of an image-processing program does not need to know cell biology. The programmer of a computer vision program solving a cell biological problem, on the other hand, must be a cell biologist. Needless to say, both programmers must be familiar with the mathematics of image signal processing as the computer vision program will incorporate steps like image deconvolution, filtering, and segmentation. But the key challenge in developing a computer vision system for cell biology is to conceptualize in a programmable framework the parameters, rules, and models required to characterize cell biological function. This requires profound familiarity with the cellular mechanisms, the range of valid hypotheses, and the expected shifts in phenotypes that may distinguish one model from another. It often also requires familiarity with the molecular techniques for labeling and manipulating cells and with the microscopy itself, as the experimental design directly influences whether image data can be analyzed in terms of a particular question.

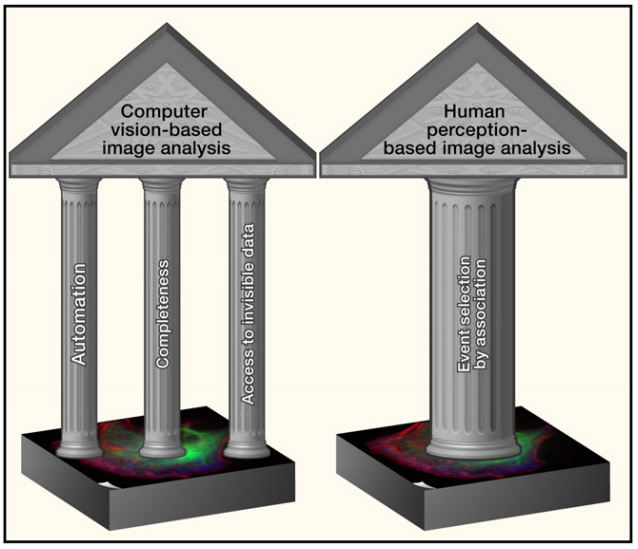

Figure 1. The Strengths of Computer Vision and Human Vision in Image Analysis

Computer vision rests on three pillars: it provides partial or complete automation of the analysis pipeline; it generates completeness in the data in that every image event fulfilling set selection criteria is considered by the analysis; and it can give access to processes underlying the image content that are not visible. Together, these pillars build a framework for solving complex image-analysis tasks that require integration of a large number of well-defined yet multidimensional and possibly indirectly accessed image events. Computer vision systems generate quantitative and reproducible models of image content. Human vision, in contrast, rests on one strong pillar, that is the association of observed image signals with previous visual experiences. Because the memory of visual scenes stored by the human brain is huge, the association strategy permits a fast and adaptive interpretation of ever-changing scenes that may consist of weakly defined image events, a performance currently unmatched by computer vision systems. However, human vision analysis results in a qualitative description of perceived image content that matches the best interpretation of the scene. The description may vary between individuals, it may be incomplete, and it may miss subtle but significant differences between distinct scenes.

Early and Recent Uses of Computer Vision in Cell Biological Studies

The first uses of computer vision systems in cellular analysis go back to the sixties, where research groups sought to replace human operator input in karyotyping (Gilbert, 1966; Castleman et al., 1976). The primary goal for eliminating visual inspection was to speed up the tedious processes of finding cells in mitosis, arranging the chromosome images into karyograms, and measuring and classifying features in the chromosome images for the purpose of detecting mutations and defects in the genome. Clearly, these systems qualified as image-analysis programs in that they involved several autonomous, intelligent decisions by the computer about the content of a karyogram. The accuracy of these decisions was deemed as *reasonably good* when compared to the classifications by a cytologist. Gilbert writes that "a majority of the misclassifications [by the computer program] occur in group III [of the karyogram], where the cytologist's identification will by no means be absolutely certain" (Gilbert, 1966), suggesting that the performance of computer and human vision were equivalent. Other early developments of computer vision systems served the purpose of counting or tracking cells (Farnoush, 1977; Lewandowska et al., 1979).

In their paper on neutrophil tracking, Howe et al. write: "Most methods of measuring neutrophil motility provide information mainly about the performance of a small proportion of the fastest moving cells. Application of a computer-linked image analysis technique provides a convenient, automated method of measuring the motility of the whole cell population. This makes it possible to test whether changes in motility represent a homogeneous alteration affecting all cells or a change in the numbers or performance of a subset of cells" (Howe et al., 1980). These sentences pointedly define two key contributions that computer vision systems can bring to image-based cell biology experiments, namely *automation* and *completeness* in extracting information from images (Figure 1).

Automation is an obvious gain from using computer vision. Many of us are all too familiar with the tedious clicking for weeks and months on images that were acquired in a few minutes. The demand for automated image analysis has rapidly increased with the overwhelming amount

of images that can be collected with high-throughput microscopy systems. Accordingly, many of the most recent developments of computer vision systems for cell biological studies have been driven by the need to identify phenotypes in cell morphology and subcellular protein distribution in large-scale pharmacological or genomic screens (Bakal et al., 2007; Carpenter et al., 2006; Collinet et al., 2010; Neumann et al., 2010; Perlman et al., 2004; Vitorino and Meyer, 2008). A common point between these studies is that the effects of cellular treatments can be robustly classified only by analysis of multiple image events, for example the spatial distribution, density, and colocalization of several protein markers. Acquiring multidimensional datasets from hundreds of thousands of images evidently exceeds the capacity even of teams of human operators. Computer vision systems have also been required to interpret data with an event density per image that overwhelms human analysis, such as single-cell tracking in developing tissues or following hundreds of thousands of subcellular molecular markers (Chen et al., 2009; Keller et al., 2008; Ponti et al., 2005). These applications will become increasingly important with the emergence of new imaging methods that monitor the molecular architecture and dynamics of entire tissues with light microscopic resolution (Micheva and Smith, 2007; Orth et al., 2011).

Completeness in data extraction is the second essential argument for using computer vision systems. Complete data are required first to characterize the full spectrum of heterogeneous behaviors and second to interpret the differential shifts of subpopulations of behaviors between experimental conditions. With incomplete data, what if the majority of selected events originate from a subpopulation of behaviors that do not change between experimental conditions? Conversely, what if the selection of events is focused on a few behaviors that do change, although most behaviors remain unaffected by an experimental shift? Images are probably more susceptible than any other kind of data used in cell biological investigation to incomplete analysis and thus biased interpretation. Although the dangers of image event selection are widely appreciated, until recently very few studies have tackled this upfront by quantifying the degree of heterogeneity in the event population. The standard is still to control heterogeneity by human selection of the important from the less important image events, unfortunately often without documenting the fraction of selected versus nonselected events. In contrast, several studies have demonstrated not only how the application of computer vision systems reduces the risk of biased interpretation in heterogeneous populations but how heterogeneity and differential cell responses can be harnessed to generate mechanistic insight into cellular pathways (Keren et al., 2008; Slack et al., 2008; Snijder et al., 2009). This approach can greatly complement molecular perturbation experiments, which in complex, nonlinear pathways tend to generate pleiotropic effects.

Complete measurements also allow the detection of exceedingly rare but mechanistically significant image events. For example, a computer vision system was employed to track the transient fusion and separation of cell-surface receptors, events that occurred for about 5 in 1,000 labeled receptors (Figure 2A, left) (Jaqaman et al., 2011). Despite the infrequency of the event, related to the need for substoichometric labeling to achieve single-molecule imaging conditions, the complete measurement of all visible receptors revealed a spatial pattern in receptor interactions that turned out to be essential for the signal transduction properties of the receptor. Hence, as Howe and colleagues projected in 1980, because of the completeness of data produced by computer vision systems, information is extracted from images that will likely escape the attention of a human observer.

Uses of Computer Vision to Access Invisible Information

The third and perhaps most exciting contribution that computer vision systems can make to cell biological research is to give access to image-based information that is inaccessible by eye (Figure 1). Although this seems paradoxical, computer vision programs can be directly coupled to mathematical models that describe the relation between hidden, invisible processes and measurable image events. Changes in the behavior of hidden processes are thus detectable as changes in the image. Among the first to promote this approach in cell biology were David Odde and colleagues, who coined the term *model convolution*. Their initial implementation of model convolution addressed metaphase kinetochore dynamics in the budding yeast *S. cerevisiae* (Sprague et al., 2003). Normal *S. cerevisiae* cells contain 16 pairs of sister chromatids that are segregated by a 1.5–2 μm long mitotic spindle. Given the small size of the spindle, individual kinetochores are not resolvable. Instead, upon bipolar attachment of all 16 sister chromatid pairs, the images of fluorescently labeled kinetochores merge into a blurred bilobed intensity distribution. Odde and colleagues showed that nevertheless they could infer kinetochore movements, which depend on the dynamics of kinetochore-associated microtubules. First, they modeled the hypothetical positions of all 32 kinetochores. Then, they synthesized a combined image of all kinetochores and estimated the parameters of microtubule dynamics so that the synthesized intensity distribution best matched the experimentally observed intensity distribution. Subsequently, the Odde lab also reported that the comparison between synthetic and measured images is sensitive enough to distinguish different models of the mechanism by which kinesin-5 motor proteins regulate metaphase chromosome congression (Gardner et al., 2008).

Similar model-image comparison strategies were used to estimate the parameters of interphase microtubule dynamics in mammalian cells (Shariff et al., 2010), the length distribution of microtubules in vertebrate meiotic spindles (Yang et al., 2007), and intracellular force fluctuations during epithelial cell migration (Ji et al., 2008) (Figure 2B). These examples compared model predictions and experimental data not at the level of synthetic and measured images but based on extracted image events: image texture (Shariff et al., 2010); single-molecule trajectories (Yang et al., 2007); and fluorescent speckle flow fields (Ji et al., 2008). Comparing model and experiment by image-derived events instead of the

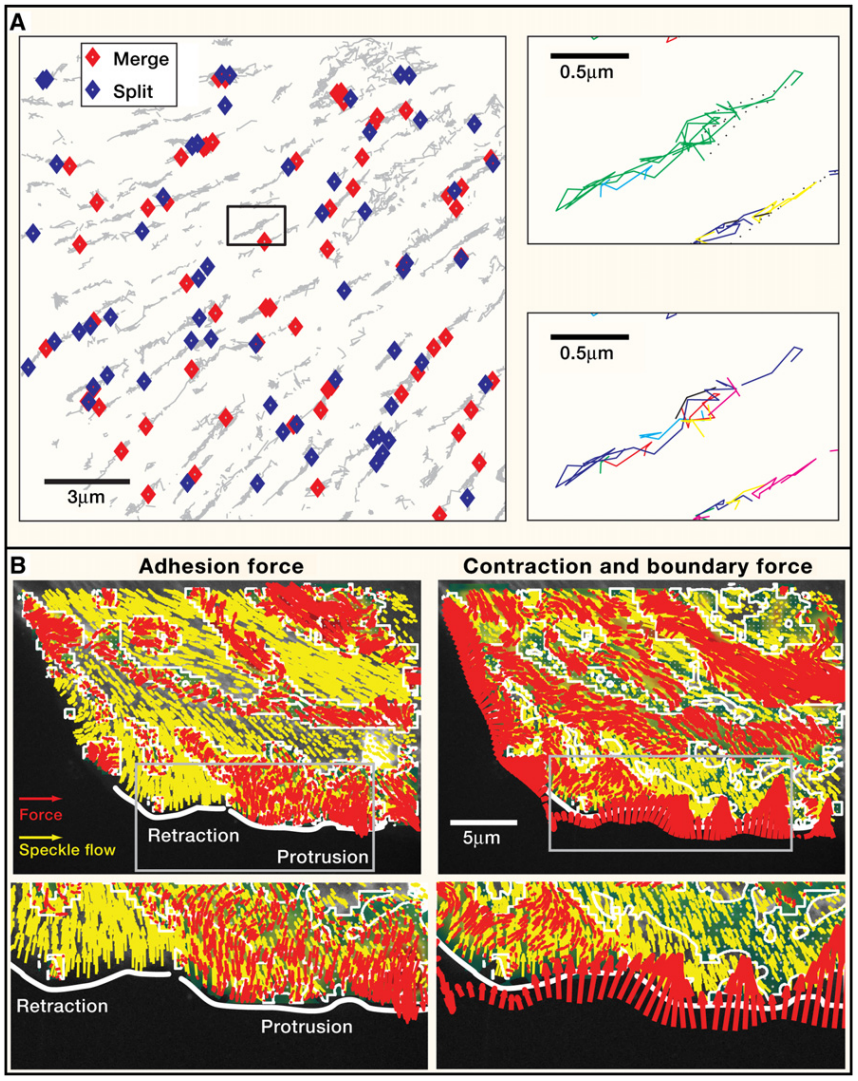

Figure 2. Computer Vision Provides Complete Data and Gives Access to Information Not Visible to the Human Eye

(A) Example of complete image data extraction. A computer vision algorithm was used to follow the dynamics of individual cell-surface receptors (as described in detail in Jaqaman et al., 2011). Left panel: By tracking all aspects of receptor behaviors, it was found that ~5 in 1,000 labeled receptor images display transient merge and split events with proximal receptor images. Although these events are exceedingly rare, they indicate transient interactions between receptors that turned out to be critical for signal transduction. Right panels: Comparison of the performance of a tracking method that integrates information in space and time to extract receptor trajectories (top) versus a method that tracks receptors time point by time point (bottom). The local image information accounted for by the second method results in broken trajectories (several tracks with different colors). Dotted line segments in top panel indicate where the computer extrapolated the trajectory over several time points without observation of the receptor image (temporary disappearance due to low signal to noise). Location of enlarged trajectory is indicated by the box in the left panel.

(B) Example of inferring invisible processes that are linked to visible image data. A computer tracking program was used to extract speckle flows of actin filaments. Yellow arrows show the direction and speed of actin filament movement in the cell. A continuum-mechanical model of the polymer network was implemented to estimate the forces, i.e., the invisible process, that must be exerted on the network to explain the visible speckle flow. The model further distinguishes adhesion (red arrows in left column) from contraction and boundary forces (red arrows in right column). The latter are forces counteracting actin polymerization at the cell edge. Boxes in top row indicate enlarged regions in bottom row. Images adopted from Ji et al. (2008).

original image generally enables better determination of free model parameters describing the hidden processes and increased robustness against image noise. Nevertheless, the key questions in recovering invisible information are the same with both approaches: How many alternative models in addition to the chosen one could accurately explain the experimental data? How much would the conclusions drawn by the alternative model differ from the conclusions drawn from the chosen model? Answering these questions requires a tight integration of model development with controlled variations in the experimental conditions in order to rule out as many alternative models as possible. Ultimately, it is essential that the remaining acceptable alternatives are documented and that it is shown that they would not fundamentally shift the conclusions. Only with these rigorous computational and experimental controls in place will it be possible to gain confidence in invisible information drawn from images.

The Association Paradigm—Why Human Vision Often Outperforms Computer Vision

Everyone who has programmed computer vision algorithms knows how hard it is to make a computer "see" the things our eye appears to recognize effortlessly. The reason for the ease with which the human vision system identifies relevant events in complex scenes is that our brain continually associates the observable image signals with our memory of previous visual experiences and our best interpretation of the scene (Figure 1): *Seeing is believing*. The size of this memory is enormous. The association of the measurable signal with the recollected image of a particular scene allows our brain to interpolate missing information due to low signals and occlusions, as well as to classify a wide range of different objects. Our brain also has the amazing ability to freely combine associations with different visual experiences and to integrate information from different spatial scales. To illustrate this with an example from microscopy, we can within fractions of a second recognize the overall organization of cells in a developing tissue although we have never seen that particular specimen and

at the same time identify, for instance, multinucleated, mitotic, and apoptotic cells among the many cell types present in the scene. Achieving the same classification with a computer vision system is a daunting task, mainly because it is nearly impossible to train a computer memory with the full spectrum of visual experiences that may be encountered in a microscope image.

The Integrator Paradigm—When Computer Vision Can Outperform Human Vision

The association between actual image signal and previous visual experiences bears also dangers and limitations. Foremost, when analyzing images by eye we have to be concerned that our focus of attention is directed to events that match our expectation: *Believing is seeing*. This may distort our interpretation if we do not take precautions to analyze images objectively. Moreover, every individual may have a slightly different focus, leading to nonreproducible data. On a more quantitative level, an association-based image analysis may blur the boundaries between similarly looking yet distinct image events, thus reducing the sensitivity with which subtle differences between images can be classified. In such cases, computer vision systems tend to outperform human vision. One of the first examples that made this point explicit in the cell biological literature came from Bob Murphy's work on subcellular location proteomics (Murphy et al., 2003). Although human and computer vision classification did equally well in classifying obvious location patterns of proteins like DNA, actin, or tubulin, computer vision outperformed human vision in classifying the patterns produced by the two Golgi-associated proteins Giantin and gpp-130 and the patterns produced by the lysosomal-associated membrane protein LAMP2 versus the transferrin receptor localizing in endosomes. The better performance by the computer vision system stems from the use of systematic and reproducible metrics to determine shifts in protein pattern and, more importantly, from the integration of information from a high-dimensional parameter space. The classification used by Murphy and colleagues relied on more than 100 parameters characterizing every aspect of the image texture produced by these labeled proteins. Even after eliminating redundancy, the parameter set used for final classification contained a few dozen parameters. For a human brain, the integration of so many image events at once is impossible. Numerous publications have followed Murphy's example and reported outstanding performances of computer classification of image data by virtue of integrating high-dimensional parameter sets (Perlman et al., 2004; Bakal et al., 2007; Collinet et al., 2010).

A second example where we found computer vision to outperform human vision was particle tracking. Presented with movies that contain thousands of particles moving between time points, a human observer tracks particles by toggling back and forth between consecutive frames to decide on correspondences between frames. Although we may consider a few particles in the immediate spatial neighborhood, and although we may toggle forward and backward two or three frames, in essence the assignment of correspondence is made for each particle individually and on the basis of particle configurations between two time points. In computer vision jargon, this is referred to as a greedy assignment. In contrast, a computer vision system can make assignments globally by integrating the positions of all particles over all time points. Reid was the first to introduce this data integration paradigm for particle tracking with the formalism of the Multi-Hypothesis-Tracking (MHT) algorithm (Reid, 1979). In brief, in the MHT framework all possible paths are constructed for all particles through all frames of a movie. Each path is attributed a cost that defines how likely the motion of a particle along the path is given the general expectation of particle behaviors in that particular dataset. For example, a path resulting in large velocity variations will be less likely, thus will get a higher cost, than a path with nearly constant velocity. From all the possible paths, the MHT algorithm then selects the largest set of mutually exclusive paths with overall minimal cost. This means that the tracking of an individual particle in one particular time point is accomplished by considering all of the past and future of that very particle as well as of all other particles in the movie. Although theoretically optimal, the MHT algorithm is impractical for image sequences of more than a few frames and with more than a hundred particles because of the combinatorial complexity of constructing all paths. In addition, the complexity grows further if the path construction has to account for temporary and/or permanent disappearance of particles and temporary particle occlusion. An entire field of computer vision is devoted to finding computationally affordable approximations to Reid's algorithms. Recent approximations designed to cope with the particularities of particle tracking in cell biology include those by Genovesio et al. (2006) and Jaqaman et al. (2008). How good these algorithms are is difficult to say, as human vision fails to provide a reliable reference for complex particle-tracking problems (Matov et al., 2010). Instead these algorithms had to be tested on simulated data. This provides merely a best case scenario analysis in that the definition of assignment costs relies on the same models of particle motion used for the simulation. Nevertheless, the gain in performance when using global information is obvious when one compares tracking solutions that assign particles per frame versus solutions that assign particles after integrating spatial and temporal information to settle on a particular link (Figure 2A, right).

The two examples from pattern recognition and particle tracking illustrate the key scenario wherein current computer vision systems bear significant advantages over human vision: When a decision requires integrated analysis of a large number of possible partial solutions in a well-defined but multidimensional space, the computer will fare better. I conjecture that the integrator paradigm holds with not only computer vision but broadly with all AI applications seeking to replace aspects of human brain power. Probably the best known victory the AI community has celebrated so far was the defeat of the world champion chess player Gary Kasparov by the IBM super computer Deep Blue. Similar to MHT's strategy for particle tracking, Deep Blue had the advantage over Kasparov in that it could compute for every move all possible configurations of future moves until termination of the game and then play according

to the globally best configuration. Clearly, the world is far from being taken over by intelligent machines; but, we should begin to accept that the human brain does not always set the performance bar in handling complex tasks. This includes the task of understanding images. No longer should we only see and believe; we should measure, measure completely, and integrate the various direct and indirect parameters that can be extracted from images to understand their content.

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