Erik Meijering

Cell Segmentation: 50 Years Down the Road

Ever since the establishment of cell theory in the early 19th century, which recognized the cell as the fundamental building unit of life, biologists have sought to explain the underlying principles. Momentous discoveries were made over the course of many decades of research [1], but the quest to attain full understanding of cellular mechanisms and how to manipulate them to improve health continues to the present day, with bigger budgets, more minds, and more sophisticated tools than ever before. One of the tools to which a great deal of the progress in cell biology can be attributed is light microscopy [2]. The field has come a long way since Antoni van Leeuwenhoek's first steps in the 1670s toward improving and exploiting microscopic imaging for studying life at the cellular level. Not only do biologists today have a plethora of different, complementary microscopic imaging techniques at their disposal that enable them to visualize phenomena even way below the classical diffraction limit of light, advanced microscope systems also allow them to easily acquire very large numbers of images within just a matter of hours. The abundance, heterogeneity, dimensionality, and complexity of the data generated in modern imaging experiments rule out manual image management, processing, and analysis. Consequently, computerized techniques for performing these tasks have become of key importance for further progress in cell biology [3]–[6]. A central problem in many studies, and often regarded as the cornerstone of image analysis, is image segmentation. Specifically, since cellular morphology is an important phenotypic feature that is indicative of the physiological state of a cell, and since the cell contour is often required for subsequent analysis of intracellular processes (zooming in to nanoscale), or of cell sociology (zooming out to millimeter scale), the problem of cell segmentation has received increasing attention in past years [7]. Here we reflect on how the field has evolved over the years and how past developments can be expected to extrapolate into the future.

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A VERY BRIEF HISTORY OF CELL ANALYSIS

The first uses of computers for the analysis of cells date back more than half a century. Already in the mid-1950s, systems were developed to automate the classification of smears of exfoliated cells, with the ultimate aim to enable mass screening for cervical cancer. These systems applied thresholding-based decision rules to serial one-dimensional (1-D) microscopic line scans of a specimen [8]. The 1960s witnessed the first examples of automated processing of two-dimensional (2-D) images for the purpose of differential counting of white blood cells (leukocytes) according to their main classes based on simple colorimetric and morphological measurements [9]. Commercial systems for performing this routine clinical test hit the market around the mid-1970s and even contained multiple computer circuits to parallelize the tasks of analyzing the image of the previous cell, while grabbing the image of the present cell, and at the same time locating the next cell in the specimen [10]. These were also the times when the first computer-assisted microscopes were developed for tracing and morphological analysis of neuronal cells [11]. The advent of confocal microscope systems in the 1980s opened the door to three-dimensional (3-D) cell image analysis. But it was not until the 1990s, when computers became powerful enough to handle 3-D data, or even complex 2-D data such as in histopathology [12], that the image processing and computer vision communities really began to take up the challenge. Over the past decades, literature on the subject has grown exponentially, with more than half of the bulk of papers appearing after the year 2000. Published cell image analysis methods have already been the basis of numerous studies involving cell counting (numbers), the identification of cell types or cell phases (shapes), the quantification of cell migration and interaction (morphodynamics), cellular sociology (tissue-level organization), and intracellular structures (cell organization) [5].

COMMON CELL SEGMENTATION APPROACHES

Automated image segmentation for cell analysis is generally a difficult problem due to the large variability (different microscopes, stains, cell types, and cell densities; see Figure 1) and complexity of the data (possibly time-lapse, acquired at multiple wavelengths, using multiple microscopes, and containing large numbers of cells). Nevertheless, screening the literature published on the subject since 1960, we find that the vast majority of cell segmentation methods are based on only a few basic approaches. Here we highlight the most common ones. Approaches specific to neuron segmentation are excluded, as these usually address the quite different problem of tracing the extensive

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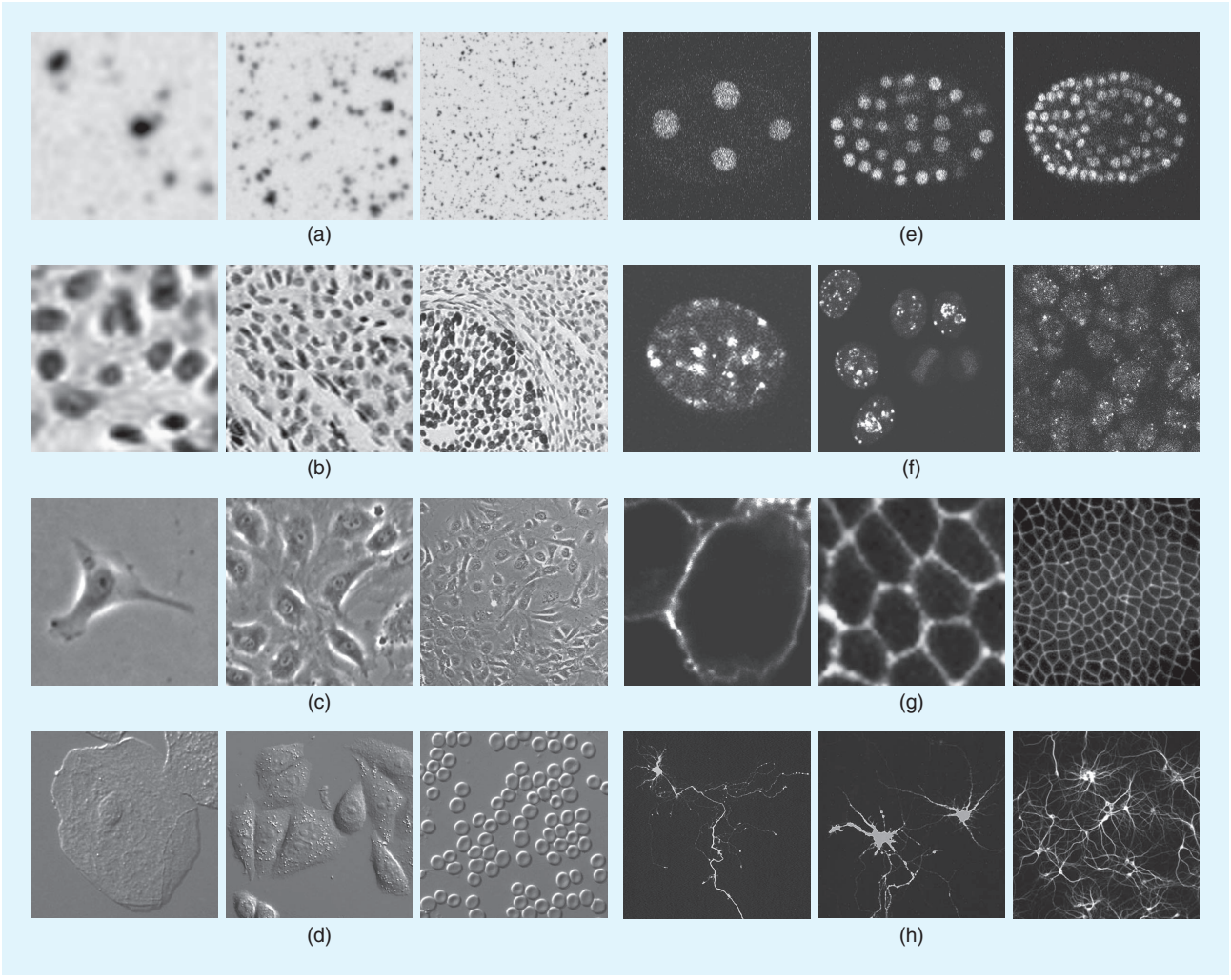


[FIG1] Cell images may vary widely, depending on the type of microscopy and staining used, as well as the cell type and cell density. This makes the development of a generally applicable cell segmentation method a huge challenge. Shown here are various illustrative examples of cell images (with cell density increasing from left to right), acquired using (a) and (b) bright-field microscopy, (c) phase-contrast microscopy, (d) differential interference contrast microscopy, and (e)–(h) fluorescence microscopy. In the latter case, the use of fluorescent dyes or proteins enables biologists to selectively label virtually any target of interest in the cell or nucleus, such as (e) the nucleoprotein histone involved in DNA folding, (f) DNA binding proteins such as Rad18 or Rad54, or (g) cell adhesion proteins such as E-cadherin. The resulting additional variability in cell appearance further complicates the development of generic cell segmentation methods. Finally, whereas most cells are fairly spherical, some classes of cells may show quite different shape. Especially, (h) neurons may extend far beyond the compact cell body.

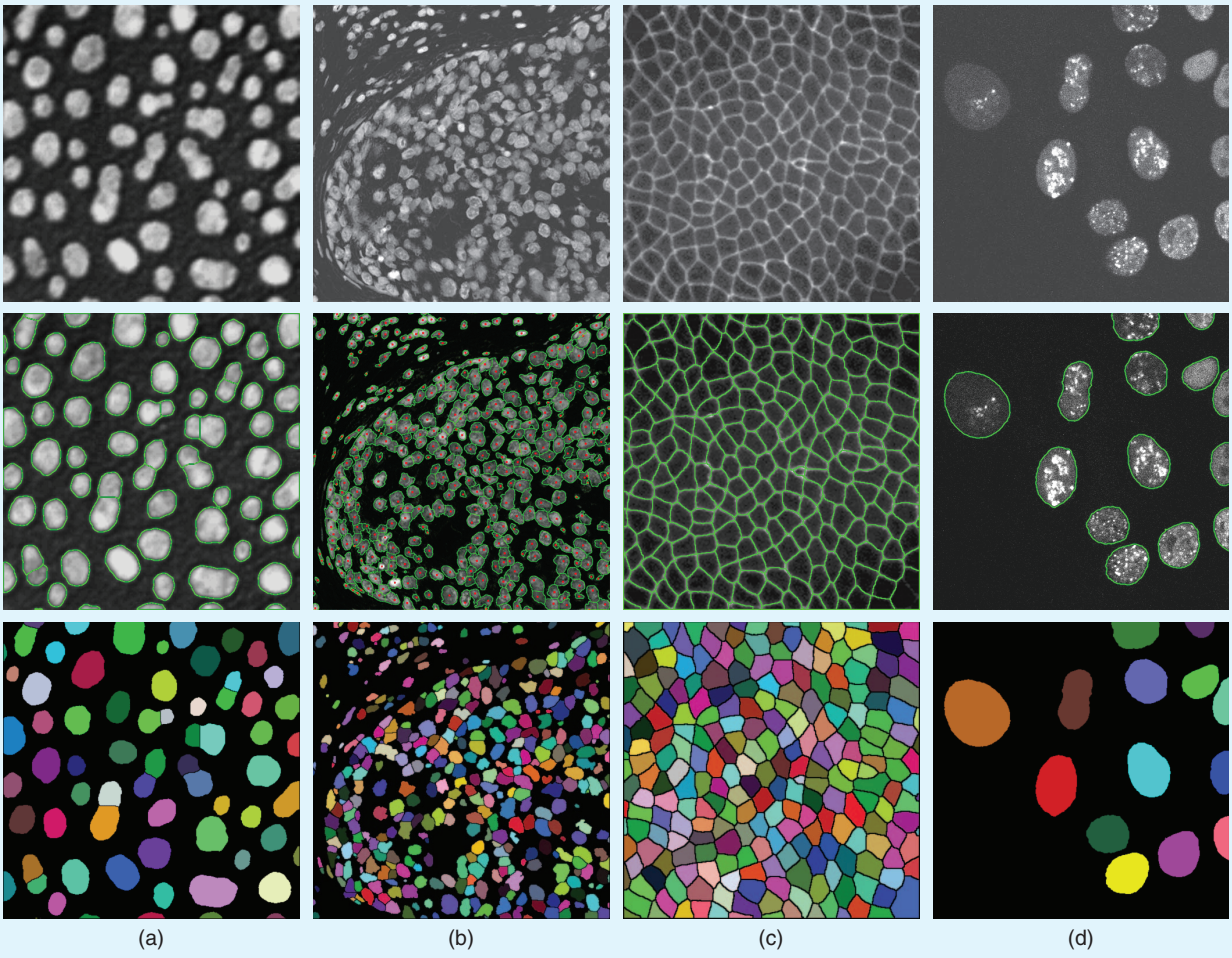
dendritic arborizations, and have been reviewed recently [11]. Generally applicable methods for image denoising prior to segmentation are also ignored.

INTENSITY THRESHOLDING

The first and, ironically, still one of the most predominant approaches to cell segmentation is intensity thresholding [13]. The underlying assumption is that cells have significantly and consistently different intensities than the background [Figure 2(a)], either globally, in which case a fixed threshold would suffice, or locally, which would require adaptive thresholding. Approaches to automated threshold selection are usually based on statistical analysis of the global or local image intensities using the histogram. However, in practice, the fundamental assumption is often violated, and thresholding alone produces poor segmentation results. If at all, most cell segmentation methods apply thresholding only as a first step in the pipeline.

FEATURE DETECTION

Rather than by their absolute intensities, cells may be segmented based on intensity derived features that can be easily detected using linear image filtering. For example, at low magnifications, cells resemble compact particles, and may be found using a blob detector such as the Gaussian or Laplacian-of-Gaussian filter [Figure 2(b)]. At higher magnifications, cells appear as larger regions, but if their shapes are relatively invariant, a dedicated filter template could be derived from the images. Alternatively, in more variable scenarios, edge detection (first-order differential filtering) or ridge detection (second-order differential filtering) is often used, followed by some linking procedure [13]. Similar to



[FIG2] Examples of cell image segmentation based on the discussed approaches. The columns show, respectively, the input images, the automatically found cell contours (overlaid in green), and the corresponding labeled cell regions (arbitrary colors). (a) Cells that are fairly well separated and clearly brighter than the background are easily segmented using thresholding. Binary ultimate erosion and reconstruction was used to split the few clumped cells. (b) Scenarios with higher cell densities and intensity variations require more sophisticated methods. The method used here involves graph-cuts-based binarization, Laplacian-of-Gaussian-based cell detection (see red dots), and marker-based clustering (images from [14] and used with permission). (c) Membrane-stained images are ideally suited for watershed-based segmentation. Grayscale morphological prefiltering was used both for background estimation (opening operation) and filling imperfectly stained segments (closing operation). (d) Studies of intracellular dynamic processes often result in images with significant intensity variations (in both space and time) and require robust cell segmentation and tracking methods. The method used here is based on level sets [15]. All of these methods were specifically designed for the given application and required careful parameter tuning.

thresholding, such filters alone usually do not produce definitive cell outlines, but may provide useful cues for subsequent steps in the pipeline.

MORPHOLOGICAL FILTERING

Another popular class of filters are those from the field of mathematical morphology. Being nonlinear, operators such as erosion, dilation, opening, and closing allow for the examination and manipulation of geometrical and topological properties of objects in images and are often used in connection with cell segmentation. More complicated filters can be easily constructed by combination or successive application of such operators. A distinction must be made between binary morphology and grayscale morphology [13]. The former is used mostly as a postprocessing step to polish coarse segmentations [Figure 2(a)] while the latter is used mostly as a preprocessing step to enhance or suppress specific image structures for segmentation [Figure 2(c)].

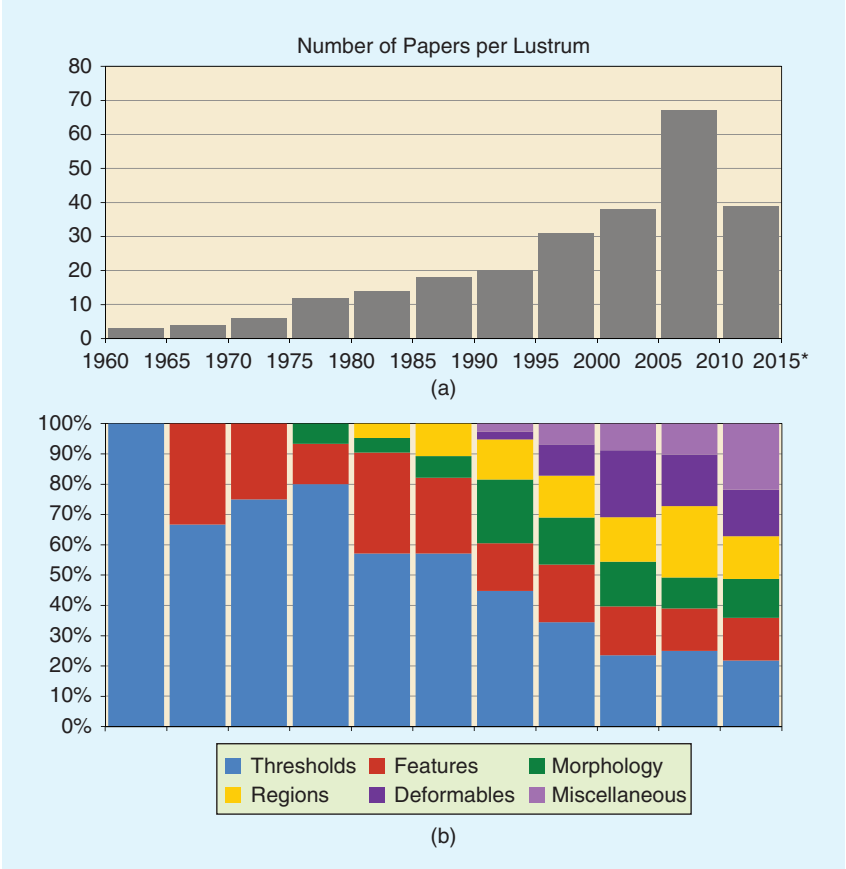
REGION ACCUMULATION

An alternative approach to cell segmentation is to start from selected seed points in the image and to iteratively add connected points to form labeled regions. The most straightforward implementation of this idea is ordinary region growing, which works per neighborhood layer of connected points and, when applied directly to the image, assumes (and suffers from) a similar image model as in the case of intensity thresholding. Hierarchical

split-and-merge schemes, operating per resolution layer and using some uniformity predicate, have also been used occasionally. Another example is the watershed transform [13], the main segmentation approach from mathematical morphology, which works per intensity layer and requires an edge enhanced image (gradient magnitude), as it is commonly desired to have the watershed lines at the edges [Figure 2(c)]. Though by far the most popular region accumulation approach, the watershed transform is infamous for producing oversegmentation and usually requires further processing.

DEFORMABLE MODEL FITTING

The final class of cell segmentation approaches mentioned here consists of procedures that fit a deformable model to the image data [Figure 2(d)]. Deformable models may be formulated either explicitly, as a parametric contour (2-D) or surface (3-D), or implicitly, as the zero-level of a function with dimensionality (nD) one higher than the image to be segmented. Level sets have the advantage that they naturally handle topological changes, such as cell division, and are therefore attractive for cell tracking [16]. Initialized by a first, coarse segmentation, a deformable model is iteratively evolved to minimize a predefined energy functional, which typically consists of image-based and shape-based terms, the latter of which allow the incorporation of prior knowledge to constrain the results. The energy terms need to be carefully designed to avoid erroneous segmentation.



[FIG3] Literature on cell image analysis shows an exponentially increasing interest in cell segmentation and the emergence of new approaches for this purpose. In total, 250 journal papers describing cell segmentation methods were analyzed for this article. Part (a) shows their time histogram using lustrum bins (as indicated by the asterisk, the last bin obviously contains only partial data, up to March 2012). The distribution is representative of that of the total numbers of papers published on the subject in the various periods. Part (b) shows the breakdown of published methods per lustrum into six main classes of approaches (explained in the main text): intensity thresholding (blue), feature detection (red), morphological filtering (green), region accumulation (yellow), deformable model fitting (violet), and miscellaneous approaches (magenta) that could not be classified as any of the former. Most methods use a combination of several approaches.

CELL SEGMENTATION APPROACHES ANALYZED

It is interesting to analyze the usage of the different approaches to cell segmentation over the years (see Figure 3 in relation to the following discussion). Intensity thresholding, being the conceptually simplest and computationally cheapest of all, was the first approach to be used since the 1960s. Soon after, it was realized that differential features (in particular, edges) could also be computed relatively easily and might provide useful information. The field of mathematical morphology started to develop around the same time, and its basic operators were first used in the late 1970s for refining the results of thresholding-based cell segmentation. During that same decade, all mentioned region accumulation approaches were conceived, and first examples of their usage in the context of cell image analysis appeared around the mid-1980s. Finally, the first deformable model fitting approaches for image segmentation were introduced in the second half of the 1980s and were first applied to cell images in the early 1990s. Since then, miscellaneous other segmentation approaches found their way into the field, with as yet limited usage. Examples include dynamic programming, graph cuts, active masks, support vector machines, tensor voting schemes, neural networks, Markov random fields, and other concepts.

Several observations follow from the analysis of the literature on cell segmentation in the past 50 years. First, most of the different approaches were originally developed for applications in other fields (computer vision, robotics, materials science, medical imaging), and were later adopted for cell segmentation. This is remarkable, given the unique and unparalleled challenges in cell image analysis, which should provoke the development of original ideas.

Second, even though new approaches are introduced once in a while, they seem to never fully replace old ones. Apparently, while none of them alone produces satisfactory results, they all continue to be useful to some extent. Third, as a consequence, methods proposed in recent times are rarely based on a single new concept, but are often merely new combinations of the discussed approaches, tailored to a specific application. Rather than converging to a robust, unified solution, it thus seems that the field is diverging, and by now almost as many cell segmentation methods have been developed as there exist cell analysis problems [7]. However, the explosion of technical papers in the past decade suggests that the performance and applicability of these methods remain limited, and more powerful methods will need to be developed.

THE FUTURE OF CELL SEGMENTATION?

Reflecting on the developments in the field in the past 50 years, one might wonder what to expect from the next 50 years of research. As early as 1966, researchers already exclaimed that "automation of the acquisition and interpretation of data in microscopy has been a focus of biomedical research for almost a decade," and they concluded that "many facets of the problem appear to be well within the grasp of present-day technology," leading them to anticipate that "modern large-capacity, high-speed data facilities at last provide the ability to manipulate the hitherto unmanageable quantities of optical information contained within all but the simplest images" [9]. On one hand, it may feel embarrassing to admit that today, half a century down the road, very similar remarks still apply. It seems as if from the very beginning, the "grasp of present-day technology" in the field of image analysis has held a firm position a few years ahead in the future, barely able to keep up with the rate of progress in its application areas. While microscopic imaging, biological experimentation, and computer hardware development all underwent major revolutions in the past decades, most cell image analysis methods are still based on textbook ingredients [13] from the early days. On the other hand, perhaps this is just a testimony to the fact that the ease with which humans can see things in images is very deceptive, and computerizing this capacity is actually a notoriously difficult problem. The provisional solution has thus far been to isolate applications and to develop a dedicated method for each. But the real challenge remains to design methods that are sufficiently generic to be easily trainable for a wide range of applications while consistently achieving high sensitivity and specificity in each case. Perhaps the rapidly increasing market share of alternative segmentation approaches (Figure 3) signifies the beginning of a new era. Important catalysts for the development of more powerful methods will be improved availability and testability. The technical literature is full of alleged great methods, which were claimed to beat all previous methods for a given application, but subsequently disappeared into oblivion because no one was able to use or reproduce them. Their availability in popular (open-source) image analysis platforms will alleviate this problem and should be increasingly enforced before publication. But even then, methods may be easily abused by others to "prove" superiority of their own methods. The organization of open challenges based on standardized test data and criteria should suppress this practice and can be expected to further accelerate progress in the field in the near future.

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AUTHOR

Erik Meijering (meijering@imagescience.org) is an associate professor of bioimage analysis at the Erasmus MC (University Medical Center Rotterdam) in The Netherlands. He received an M.Sc. degree (cum laude) in electrical engineering from Delft University of Technology (1996) and a Ph.D. degree in medical image analysis from Utrecht University (2000), both in The Netherlands. His research interests are in the areas of computer vision, image processing, and image analysis, with applications in cellular and molecular imaging. He published more than 60 peer-reviewed articles in this area. He is a Senior Member of the IEEE. He was an associate editor of *International Journal on Biomedical Imaging* (2006–2009) and *IEEE Transactions on Image Processing* (2008–2011). He has been an associate editor of *IEEE Transactions on Medical Imaging* since 2004.

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