Nuclei and glands instance segmentation in histology images: a narrative review

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Abstract
Examination of tissue biopsy and quantification of the various characteristics of cellular processes are clinical benchmarks in cancer diagnosis. Nuclei and glands instance segmentation greatly assists the high-throughput quantification of cellular process and accurate appraisal of tissue biopsy. It subsequently makes a significant improvement to the computational pathology process for cancer diagnosis, treatment planning, and survival analysis. Recent advancements in the field of computer vision have automated the manual, laborious, and time-consuming histopathological analysis process. Automated image analysis of histopathological images for cells and tissues to trace the entirety of the ultrastructures, has been an active area of research in medical informatics for decades. The developments in computers, microscopy hardware, and the availability of large-scale public datasets have further fastened the development in this field. And the realization that scientific and diagnostic pathology calls for fresh ways to undertake, automated image analysis of histopathological images has captivated contemporary attention. In this survey, 126 papers illustrating the AI-based methods for nuclei and glands instance segmentation published in the last five years (2017–2022) are deeply analyzed, and the limitations of current approaches and the open challenges are discussed. Moreover, the potential future research direction is presented, and the contribution of state-of-the-art methods is summarized. Further, a generalized summary of publicly available datasets and detailed insights on the grand challenges illustrating the top-performing methods specific to each challenge is also provided. Besides, we intended to give the reader the current state of existing research and pointers to the future directions in developing methods that can be used in clinical practice enabling improved diagnosis, grading, prognosis, and treatment planning of cancer. To the best of our knowledge, no previous work has reviewed the instance segmentation in histology images focusing on nuclei and glands instance segmentation.

Keywords Histology · Nuclei · Glands · Instance segmentation · Survey · Digital pathology · Cancer treatment planning

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1 Introduction

In the last few decades, computational pathology has catalyzed advancements in clinical diagnosis, expedited the development of new interactive models for pathology education, and paved the way for an incredible rise in whole slide image analysis tools. It has revolutionized the entire tissue specimen analysis process for pathologists. From manually analyzing thousands of tissue slides via microscope requiring specialized doctors to automatic digital slide generation via scanning and using AI-based deep learning techniques has spawned fatal disease diagnoses such as cancer using image analysis. In digital pathology nuclei and gland, instance segmentation in the whole slide images is of pivotal value for abnormality assessment. It plays a key role in histology image analyses whether it be the identification of major chronic diseases including tumor localization through segmentation or classification as benign or malignant. Glands are often considered one of the main histology structures present in most organs as the primary mechanism for protein and carbohydrate secretion. However, it has been observed that adenocarcinomas, regarded as the most severe type of cancer, originating from glandular epithelium as malignant tumors. In Fig. 1, the left patch shows a histology image of a colon tissue stained with routinely used Haematoxylin and Eosin technique while the right patch is an individual gland of a colon tissue with substructures. This makes understanding glands morphology a pivotal step for assigning the degree of malignancy of major adenocarcinomas e.g in the breast, colon, lung, and prostate. Thus, accurate gland instance segmentation is considered a necessary step for obtaining valid morphology information.

Accurate segmentation and classification are of crucial importance during the examination of crisscrossing cellular events such as wound healing process (Oswal et al. 2013), cancer grading i.e. cancer grade assignment on the basis of variation in nuclei shapes, size, texture, and orientation. Similarly, Nuclei quantification is given strong prognostic significance during biomarker determination for immune tumor infiltrate quantification and other various pathological assessments as studied by Jung et al. (2019) Quachtran et al. (2018), and Höfener et al. (2018).

The expeditious transition of classical histology to computational pathology has revolutionized the entire image analysis workflow. A significant spike has been observed in research in computational pathology domain (Ahmedt-Aristizabal et al. 2021) in last few years compared to other medical fields including i.e neuroscience, cardiology, radiology, ophthalmology, and dermatology. This spike is mainly caused by the induction of many grand challenges of histopathological medical Imaging i.e. NuCLS Amgad et al. (2021), BACH Aresta et al. (2019), MoNuSeg Kumar et al. (2020) and CoNIC Graham et al. (2021) being introduced during last few years. This transformation, from manual reading

![Fig. 1](image) Left: An image of a colon tissue, right: an individual gland colon tissue with substructures

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and annotation of thousands of slides to the digitization of the biopsy process, has led to a rise in the need for predictive analysis (Mela and Liu 2021).

Various Med Image Anal studies regard the segmentation of cell nuclei as a preliminary step for extracting meaningful biological insights. Research studies including protein localization, moving population track, phenotype classification, profiling treatments, and many more are considered reliable candidates for the identification of single cells microscopy images. It also serves as a prerequisite for CAD systems in computational pathology, where features such as nuclear pleomorphism and cytology of the nucleus can assist in making a prognosis as discussed by Graham and Rajpoot (2018).

However, nuclei and glands instance segmentation cannot be regarded as a simple task for nonexperts in pathology labs. Despite continuous advancements in learning algorithms, nuclei segmentation is still an extremely challenging task because of blurred nuclei boundaries, differences in size and shape highlighted by Vahadane et al. (2021), uneven staining, pathological changes on pathological images, morphological abnormalities (Sirinukunwattana et al. 2016) and substantial color variations described by Rashmi et al. (2021). Similarly, varying morphology of glands at different histology grades, and different intrinsic features of glands WSIs pose a major challenge during segmentation of instances. Firstly, applying a mathematical shape model for instance segmentation gets difficult due to shapes heterogeneity. Figure 2 shows structural variations of nuclei in different organs. Secondly, granule-filled cytoplasm cause nucleus extrusion to flat shapes as compared to oval or round structures in normal cases mentioned by Xu et al. (2017). Thirdly, cellular matrix variations result in anisochromasia thus resulting in additive noise in the background compared to normal intensity gradients.

Improper staining often results in similarity in nuclei to the cytoplasm or background colors thus yielding blurred boundaries (Zhao et al. 2021; Fraz et al. 2018). Moreover, the occurrence of several overlapping nuclei in whole slide images causes further difficulty in object segmentation. The major challenge in model development is varying types of nuclei e.g., nerve cell nuclei are typically in triangular shape (Lin et al. 2021), while

![Fig. 2 A catalogue of morphological variation in nuclei at 20x magnification for different organs.](image)

(a) Pancreas  (b) Esophagus  (c) Adrenal Gland  (d) Cervix  (e) Bile duct  (f) Liver  (g) Oral  (h) Colon
glial and oligodendrocytes nuclei are usually round in appearance but the latter one has light rings, astrocytes have an oval shape, endothelial cells are usually slender in structure (Taneja et al. 2018), while malignant tumor cells have irregularly shaped nuclei (Hussain et al. 2020). The developed model is supposed to be robust enough to detect all these kinds of nuclei without any mislabeling. Digital image quality variation, background clutter, and image artifacts are some other extremely important opens problem in this domain. Nuclei curvature variation also impacts detection since normally in pathology images, nuclei contour points curvature changes smoothly (Roy et al. 2021; Parvaiz et al. 2022). However, a single contour having two or more touching or occluding nuclei results in large curvature change at touching points. Already existing automated Med Image Anal tools use classical segmentation including active contour models, and watershed or thresholding techniques for nuclei instances identification (Rashid et al. 2020). These tools need configuration with respect to each data to accurately analyze distinct microscopic modalities including scales and experimental variation, thus leading to an evident requirement of technological domain expertise for accurate algorithm selection and parameter adjustment. Still, for proficient ones too, this choice can be daunting, considering that every year numerous papers are published, presenting new research techniques for gland and nucleus instance segmentation. Even after examining under controlled experimental conditions, no single technique can be generalized for segmenting all microscopy images correctly, since classical machine learning algorithms are either sensitive to technical artifacts or often fails in adapting to biological sample heterogeneity (Rasool et al. 2021). Altogether, this situation yields slows the pace of research and at times inhibits research laboratories from adopting newer image analysis technologies owing to the time and expertise required. Many computational methods have been proposed for the task of automated nuclei and glands instance segmentation and broadly these can be divided into two major categories: handcrafted feature extraction approaches and deep learning-based approaches. The handcrafted category majorly targets heuristic methods including K-means clustering, adaptive thresholding i.e., local thresholding method for unevenly illuminated images, active contour model, watershed, graph-cuts, and other morphological operations while the second approach particularly uses different variants of deep learning-based techniques primarily based on neural networks (Nawshad et al. 2021).

1.1 Motivation of the review

Cell nuclei segmentation in whole slide images is often considered as the stepping stone towards whole slide image analysis in any biomedical and biological applications (Schmidt et al. 2018). The quality of chronic disease diagnosis, survival prediction, phenotype classification, feature extraction, and cell tracking highly depends on the segmentation accuracy of instances. It gives us the challenging opportunity to study tissue and cellular phenotyping and to draw biological conclusions on a large scale. In recent years, challenges organized specifically for nuclei and gland instance segmentation has brought significant improvement, including public accessibility of large, annotated segmentation dataset and 2-D architectures extension for 3-D nuclei images. As compared to natural objects, nuclei detection and instance segmentation seem easier and simpler due to homogeneous properties in representation. However, despite research on nuclei detection and segmentation topic for decades, still there is no publicly available model supporting nuclei instance segmentation and detection across whole slide images (WSIs) from various labs generated under different conditions. As described by Xu et al. (2019) till date, even today, this
debate towards a generalized architecture or a benchmark solution for all types of nuclei image segmentation is alive. Before the arrival of convolutional neural networks, conventional nuclei segmentation methods were based on either geographical or statistical image features for seed generation. A major domain shift from handcrafted feature segmentation technique (Narotamo et al. 2020) to using CNN variants for feature extraction has also been observed. This review has been conducted with an aim to provide a comprehensive overview of techniques being used in the last 5 years for nuclei and glands instance segmentation tasks as well as identification of the most used approach amongst all as a common interactive method for instance segmentation.

1.2 Scope of the review

For this survey, we have targeted 126 research papers published in top conferences and journals for the span of the last 5 years ranging from 2017 to 2022. For evaluating the advantages and drawbacks of each segmented technique a critical review is compiled having existing deep learning computational approaches for nucleus instance segmentation, focusing on both hand-crafted morphological feature-based techniques as well as deep neural network-based methodologies (Heaton 2018; Lin et al. 2020). We provide comprehensive coverage of the major publicly available datasets being used for the task of nuclei and glands instance segmentation, an extensive summary of grand challenges held globally for the task of instance segmentation as well as classification including Data Science BOWL Challenge (DSB–2018), MoNuSeg, MoNuSAC, GlaS, and CoNIC. There are review articles available in the kindred domain (Angel Arul Jothi and Mary Anita Rajam 2017; Li et al. 2022), but this is the first review paper that comprehensively covers the most recent approaches being developed for the task of both nuclei and glands instance segmentation. Through this critical analysis, we aim to provide a recapitulation of nuclei and gland instance segmentation techniques for fatal disease diagnosis via integrating automated tools and complex semantic networks. We have discussed some of the existing challenges being faced during analysis, including varying staining impacts, insufficient data causing overfitting, disparate nuclei, and glands structure, and model specificity to a single image set. Also, we have highlighted key challenges as well as major problems along with outlining future directions. Finally, we present potential future possibilities in generalizing this pivotal task.

1.3 Comparison with other reviews

A survey published in 2019 by Hayakawa et al. (2021) on computational nuclei segmentation methods in digital pathology discusses major challenges faced in digital pathology and nuclei segmentation due to staining variations during slide preparation and image modality as well as morphological differences in nuclei. Majors’ categorization was based on pre, and post-processing and techniques discussed seeds detection, color normalization, thresholding, watershed, active contour models, graph cut, and means. It is like our study in terms of key elements (nuclei instances) and partially for techniques (deep learning and machine learning). However, 82 papers were reviewed in their study while we review 126 papers. Another recent survey in 2022 by Hollandi et al. (2022) for nucleus segmentation. They have provided an overview of currently available datasets and annotation tools for training and testing models. Further pre- and post-processing techniques and challenges related to each are briefly described followed by insights into nuclei segmentation available.
automated tools and methods covering both classical approaches as well as deep neural-based models. For catering issues faced in 3D WSI processing, a set of most successful methods yielding promising results are discussed as well. This review related to our study in terms of context (nuclei segmentation) however, it was majorly focused on automated tool kits available for segmentation with a summary of methods used while we will be focusing on a generalization of techniques adopted in the span of these 5 years with respect to challenges held and datasets used. Another related review article, written by Ben Hamida et al. (2021) reviewed papers that deal with histopathological image analysis of colon cancer using deep learning methods. They thoroughly discussed a general methodology for histopathological image analysis and the challenges related to it. They categorized the literature into patch-level classification and pixel-level annotation of colon cancer. However, our paper has included the papers targeting instance segmentation of nuclei and glands in context of the cancer types listed in Table 2.

For comparative analysis of our review with Hayakawa et al. (2021) and Hollandi et al. (2022). We have compared the main points in Fig. 3.

2 Survey methodology

In this section, we have discussed the paper selection criteria for the articles included and reviewed in this paper. We have also demonstrated the distribution of the articles according to publication databases (journals or conferences), types (nuclei, gland), impact factors, and techniques for segmentation (handcrafted feature extraction, deep neural network learning). We have categorized the included papers based on the techniques adopted for the feature extraction and their technical contributions:

1. Hand crafted segmentation techniques
2. Deep learning based segmentation
   - Encoder decoder based segmentation
   - Region based segmentation
   - Adversarial models based segmentation
   - Attention-based segmentation

2.1 Study selection

We have mentioned quantitative measures of articles searched and included for review through preferred reporting items for systematic review and meta-analysis (PRISMA) criteria. Figure 4 shows papers selection summary.

We have searched research papers on Springer, Science Direct, IEEE Xplore, and lastly Google Scholar. From applying different search queries, we have found 261 articles, amongst these 9 were duplicates and are removed. Out of the remaining 256 articles, 106 were excluded based on title or abstract. Since they were not fulfilling the criteria of this review, in some of those articles segmentation of cells was discussed instead of nuclei or glands, while the rest of the papers were based on general histology image segmentation. After final full-text screening further 46 articles were eliminated for not addressing nuclei or glands segmentation directly. In the PRISMA we have represented papers selection distribution for the final 126 included papers. Our PICO question for this study was:
Comparison of all new techniques devised during the last 5 years for nuclei instance segmentation and their efficiency in evaluation for various datasets.

2.2 Data extraction methods

We targeted top journals and conferences for extracting from different platforms including Springer, Science Direct, and IEEE Explore for extracting research articles. We extracted deep learning, machine learning, and handcrafted-based feature extraction approaches on nuclei and glands instance segmentation articles published between 2017 and 2022. This specific time frame is chosen due to rapid advancement in techniques proposed for segmentation during this specific tenure and domain shift from classical towards deep network learning. Also, histology image analysis held many grand challenges from 2017 to 2022.
We used an oriented search string by combining different keywords with the logical operators ‘AND’, and ‘OR’ to get the relevant papers. Following are the search terms being used for research papers selection.

- Nuclei segmentation, Nuclei detection, Gland segmentation, Nuclei instance segmentation, Gland instance segmentation
- H&E, Whole slide imaging, Pathology, Histopathology

The inclusion and exclusion criteria for papers is represented in Table 1. Initially, papers were selected based on their titles, further abstract, conclusion and methodology is considered for selection where titles did not match inclusion exclusion criteria. We collected the keywords from all the reviewed article included in our review and generated the word cloud which is shown in Fig. 5. As our primary objective in this review is to summarize the techniques used for nuclei and gland segmentation, this tag cloud majorly highlights (pathology, histology, deep learning, nucleus, gland, segmentation, cancer detection, and classification) terms.
In this section, we have represented the distribution of papers across various journals, conferences, impact factors, and nuclei or gland types. The major aim of these division statistics is to give an eagle eye view of published research work, during the last 5 years and the amount of variation.

Figure 6 shows reviewed articles distribution in the last few years. It can be analyzed from the bar chart that the literature for nuclei and glands instance segmentation has seen a spike throughout the years from 2017 to 2022 since the rise of medical imaging competitions for better diagnostic and prognostic techniques.

Figure 7 shows Impact Factor wise papers distribution. Vertical axis shows the count of papers for each Impact Factor while horizontal axis shows impact factors.

The distribution of papers per source is shown below in Fig. 8. From the figure count of nuclei and glands based medical image segmentation articles taken from various top

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**Table 1** Inclusion exclusion criteria

| Inclusion criteria                                                                 | Exclusion criteria                                                                 |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Articles published during 2017-2022                                               | Articles that were not targeting nuclei instance segmentation directly            |
| Paper describing detailed techniques and parameters                               | Articles based on general medical image segmentation                              |
| Articles having nuclei segmentation, nuclei instance and gland segmentation keywords in title | Articles that are merely book chapters and not part of any conference journal     |
| Papers describing datasets                                                        | Papers published in conferences other than mentioned one                         |

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**Fig. 5** A visual depiction of most frequently used keywords in the reviewed articles

**2.3 Papers distribution**

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journals and conferences can be depicted. Each bubble represents the number of papers taken from a specific journal or a specific conference.
3 Datasets

The dataset reviewed in this study covers a wide range of image sets featuring prominent open-source image databases from the PanNuke, Multi-Organ Nucleus Segmentation and Classification (MoNuSAC), CryoNuSeg, Lizard to small private datasets thus enhancing the diversity of research study. Major tissues included in datasets include the breast, liver, bladder, colon, stomach, lung, kidney, prostate, cervix, gland, and brain. Table 2 summarizes all the details of the given datasets including their source.

3.1 Nuclei datasets

3.1.1 CoNSeP

Colorectal Nuclear Segmentation and Phenotypes Dataset (CoNSeP) is one of the first fully annotated open source datasets that enabled the development of models for simultaneous nuclear segmentation and classification approaches in Computational Pathology. It consists of 41 Haematoxylin and Eosin (H&E) images of size 1000 × 1000 px from colorectal adenocarcinoma (CRA) Whole slide images (WSIs) (Yen et al. 2020).
3.1.2 Kumar

Kumar dataset contains 30 H&E stained image tiles of 1000 × 1000 px size from seven different organs including breast, liver, kidney, prostate, bladder, colon and stomach acquired from The Cancer Genome Atlas (TCGA) database at 40 × magnification. Boundaries of each nuclei within that image are fully annotated.

3.1.3 PanNuke

PanNuke (Gamper et al. 2019) (An Open Pan-Cancer Histology Dataset for Nuclei Instance Segmentation and Classification) is the biggest and the most diverse semi-automated dataset created to date for the nucleus instance segmentation task. Its nuclei boundaries have been created automatically and further validation is performed by experienced pathologists. This is formulated by formalin-fixed and paraffin-embedded samples having about 200,000 nuclei spanning over 19 different organs comprising 481 visual fields forming patches of size 256 x 256 at different magnifications. These patches are further randomly distributed into training, testing, and validation subsets. It is of significant clinical importance because of its clinical importance specifically with respect to tissue phenotyping and technical significance as well.

3.1.4 MoNuSAC

The multi-organ nucleus segmentation and classification (MoNuSAC) (Verma et al. 2021) is a large and diverse dataset having boundary annotations for each nucleus along with class labels. Previously released public datasets for nucleus segmentation were either not having multiple organ data or are not curated to the level of individual nuclei. It comprises over 46,000 hand-annotated nuclei from 71 patients spanning over 31 hospitals and four organ cell types including epithelial, macrophages, lymphocytes, and neutrophils (Fig. 9).

3.1.5 Lizard

Lizard (Graham et al. 2021) (A Large-Scale Dataset for Colonic Nuclear Instance Segmentation and Classification) is the largest known dataset available for nuclei segmentation and classification in digital pathology having nearly half a million annotated nuclei along with their class label, consists of whole slide image regions of colon tissue having six different dataset sources including GlaS, CRAG, CoNSeP, Digest Path, PanNuke, and TCGA at 20× magnification. Its fully annotated the dataset for about 495,179 nuclei. We provide the nuclear class label for epithelial cells, connective tissue cells, lymphocytes, plasma cells, neutrophils, and eosinophils.

3.1.6 CryoNuSeg

CryoNuSeg (Mahbod et al. 2021) is the first fully annotated H&E stained dataset formulated by frozen samples (FS) derived images and the images of the organs used in dataset compilation have not been a part of any prior dataset. It comprises 30 digitized H&E-stained images derived from 10 different organs. Tissue types include the gland,
larynx, adrenal, lymph nodes, pancreas, skin, pleura, mediastinum, thyroid gland, thymus, and testes. It can be used as a standalone benchmark dataset or in combination with other publicly available datasets for training supervised machine learning and deep learning models.
Table 2  Nuclei and glands segmentation datasets in public domain

| S# | Dataset | # Nuclei | Label type           | Magnification | # Organs | Organs                      | Source        |
|----|---------|----------|----------------------|---------------|----------|-----------------------------|---------------|
| 1  | CoNSeP  | 24319    | Instance + Classification | 40x           | 1        | Colon                       | UHCW²         |
| 2  | Kumar   | 21623    | Instance              | 40 x          | 7        | Breast, Liver, Kidney, Prostate, Bladder, Colon, Stomach | TCGA¹         |
| 3  | MoNuSAC | 46909    | Instance + Classification | 40x           | 4        | Breast, Kidney, Lung, Prostate | UHCW²         |
| 4  | Lizard  | 495,179  | Instance + Classification | 20x           | 1        | Colon                       | TCGA¹         |
| 5  | GlaS    | n/a      | Instance              | 20x           | 2        | Colon, Prostate              | n/a           |
| 6  | CRAG    | n/a      | Instance              | 20x           | 1        | Colon                       | UHCW²         |
| 7  | PanNuke | 205,343  | Instance + Classification | 40x           | 19       | Adrenal, Bile-duct, Bladder, Breast, Colon, Cervix, Esophagus, Head, Neck, Kidney, Liver, Lung, Ovarian, Pancreas, Prostate, Skin, Testis, Stomach, Thyroid, Uterus | TCGA¹         |
| 8  | CryoNuSeg | 7596     | Instance + Classification | 40x           | 10       | Adrenal gland, Larynx, Lymph node, Mediastinum, Pancreas, Pleura, Skin, Testis, Thymus, Thyroid gland | TCGA¹         |

¹The Cancer Genome Atlas
² University Hospital Coventry & Warwickshire
3.2 Glands datasets

3.2.1 GlaS

Gland Segmentation Challenge dataset (GlaS) (Sirinukunwattana et al. 2017) is first used as part of MICCAI-2015. This data is extracted from 16 Haematoxylin and Eosin H&E-stained Whole slide images (WSIs) scanned through MIRAX MIDI Slide Scanner pixel resolution at 20x magnification. It consists of a total of 165 images out of which 85 are used as training (48 malignant and 37 benign) and 80 test images (43 malignant and 37 benign). The size of the images is 775 x 522 pixels each having associated instance-segmented ground truths highlighting glands boundaries align with accurate lumen annotations for glands.

3.2.2 CRAG

Colorectal Adenocarcinoma Glands (CRAG) dataset is primarily comprised of gland images. It consists of colon adenocarcinomas usually referred to as the colorectal gland (CRAG) dataset developed by University Hospital Coventry and Warwickshire (UHCW) NHS Trust Coventry UK. It is composed of 213 H&E stained CRA images from 38 whole slide images (WSIs) scanned by a VL120 scanner at 20× objective magnification and are mostly of size 1512 × 1512 px along with instance level ground truth. Training and testing images are 173 and 40 specifically with varying cancer grades (Alemi Koohbanani et al. 2020).

Figure 10 shows the percentage occurrence of nuclei and glands publicly available datasets used in the reviewed articles. The graph shows that most of the nuclei instance segmentation papers have used MoNuSeg, ISBI, MICCAI challenge, and Kumar covering 18%, 10%, 8%, and 8% respectively. Similarly, glands instance segmentation articles
have used Warwick-QU, CRAG, and Glas datasets comprising over 8%, 8%, and 6% distribution respectively. Similarly, Fig. 11 shows datasets usage frequency from various sources in the reviewed articles.

Figure 12 depicts the most frequently used tissue slides for the task of nuclei and glands segmentation within publicly available datasets or via privately generated whole slide images. The plot shows that most of the research has been done on breast, kidney, and colon datasets with a count of 28, 27, and 25 simultaneously out of the total review. Similarly, the prostate, liver, and bladder have also been utilized for research purposes. Finally, the cervix, neck, and skin are the least used ones with occurrence in 8, 6, and 3 papers.

4 Grand challenges

Grand Challenges have always been an effective approach towards crowdsourcing the development of best performant algorithms as well as pointing out new research directions. Despite a lot of effectiveness towards techniques enhancement and facilitating innovation, these competitions also suffer from a set of limitations. Most of the time validations of the resulting algorithms have not been typically performed independently by the algorithm developers which later leads to technical bias techniques not reproducible for a general setup. This lack of algorithms validation also poses great doubt towards generalization capability to cater to underlying critical issues, rather than merely fine-tuning competition design setups. Figure 13 shows data collection statistics from various data sources, the number of organs, and the types used in each grand challenge. Table 3 shows the dataset details that are released for grand challenges.

Fig. 11 Tree map representing frequency of datasets and its sources
Fig. 12 Most frequently used tissue slides in research articles

Fig. 13 The bar chart illustrates precise summary of data collection, tissue and nuclei counts specific to dataset launched for above mentioned grand challenges. Here data sources counts of various distinct hospitals, universities and databases referred for data collection. Similarly, Tissue type shows distinct variety of tissues in each dataset i.e. kidney, breast, colon. Lastly, Nuclei type count is depicting variation of nuclei kinds specific to each dataset
4.1 Nuclei segmentation challenges

4.1.1 MoNuSeg

Multi-Organ Nucleus Segmentation Challenge was organized for reducing the time to develop and validate visual biomarkers for new whole slide image datasets. Preprocessing techniques used by participants include color normalization being used by most participants while Unit Variance, Range Standardization, and Histogram Equalization have also been used by some of the participants. Similarly, Segmentation techniques used includes U-Net Ronneberger et al. (2015), Mask-RCNN He et al. (2017), FCN Long et al. (2015), FPN Lin et al. (2017), PANet, ResNet He et al. (2016), VGG-Net Simonyan and Zisserman (2014), DenseNet Iandola et al. (2014) and Distance Map. Cross Entropy and Dice Loss are the most used loss functions.

4.1.2 MoNuSAC

Multi-Organ Nuclei Segmentation and Classification Challenge has been organized with an aim of detecting, segmenting, and classifying different types of nuclei around the tumor matrix and it holds special importance in characterizing the tumor micro-environment for cancer research and prognostication thus freeing up pathologist’s time for other major tasks. This also helps in reducing the chances of errors being caused while doing this task manually. For this challenge, a dataset having 46000 nuclei of 4 different organ and types have been used. Majority of participant have used following techniques for achieving better results including pre-processing by color normalization, data augmentation, major CNN architectures used includes U-Net Ronneberger et al. (2015), FPN Lin et al. (2017), FCN Long et al. (2015), HoVer-Net Graham et al. (2019), DenseNet Iandola et al. (2014), VGG-Net Simonyan and Zisserman (2014) and Efficient-Net Tan and Le (2019) followed by watershed, morphological operations and thresholding techniques for post-processing to fatigue and subjectivity.

4.1.3 Data science bowl 2018

Around 3890 teams all over the globe participated in this first-ever challenge targeting nuclei segmentation. Top participants succeeded in developing a deep algorithm that can

### Table 3  Nuclei and gland competition statistics

| S# | Reference                        | Year | Challenge | Participants | Organ | Type | No. of objects |
|----|----------------------------------|------|-----------|--------------|-------|------|----------------|
| 1  | Verma et al. (2021)              | 2020 | MoNuSAC   | 170          | 4     | Nuclei | 46000          |
| 2  | Caicedo et al. (2019)            | 2018 | DS-Bowl   | 17929        | 1     | Nuclei | 37333          |
| 3  | Kumar et al. (2020)              | 2018 | MoNuSeg   | 80           | 7     | Nuclei | 21623          |
| 4  | Graham et al. (2021)             | 2022 | CoNIC     | 520          | 1     | Nuclei | 431913         |
| 5  | Sirinukunwattana et al. (2017)   | 2015 | Glas      | 200          | 1     | Gland  | 52             |

1 The Cancer Genome Atlas
2 University Hospital Coventry & Warwickshire
be applied to any 2-D image Caicedo et al. (2019). For this competition around 37,333 manually annotated nuclei from a set of 841 images have been generated after 30 different experiments for varying samples. Most participants have used deep convolutional neural networks (DNN’s) based techniques due to their better results for various microscopy images as well as pathology problems. Different variants of CNN architectures have been designed for accurate image segmentation and improving accuracy. Techniques used by most participants include an ensemble of U-Net Ronneberger et al. (2015), fully convolutional neural network Long et al. (2015), Mask-RCNN (Region-based CNN) model He et al. (2017) and feature pyramid network (FPN) Lin et al. (2017).

4.2 Gland segmentation challenge

4.2.1 GlaS

Gland Segmentation in Colon Histology Images (GlaS) Sirinukunwattana et al. (2017) challenge has been organized by MICCAI-2015. The major objective of this competition was to solve gland segmentation problems in Haematoxylin and Eosin (H&E) stained images. Algorithms developed by participants were applied to both colonic carcinomas and benign tissues. Around 165 images derived from 16 H&E stained histology slides of stage T3 or T42 colorectal adenocarcinoma have been used for this competition. Best performing algorithms in this competition have used Fully Convolution Neural Network(FCN) Long et al. (2015), Contour based U-Net Ronneberger et al. (2015), Object-NET, MSER based techniques, as well as data augmentation and pre-trained models, have been utilized as well.

5 Nuclei and glands instance segmentation methodologies in histology images

Nuclei and Glands Segmentation instance segmentation task basically gives information about distinguishing class, location, number of objects, and contours in an image. Considering the need and applications of nuclei and gland segmentation many automated algorithms have been designed for this task. These segmentation approaches can be categorized into two major categories including conventional segmentation methods and deep learning-based methods. Traditional segmentation methods primarily target handcrafted image features i.e., the variation, gradient, distribution, and other color features including following techniques such as thresholding followed by morphological opening or closing operations, active contour models, graph-based techniques, deformable methods, marker-controlled watershed segmentation, and their other variants alongside multitudes of other pre- and post-processing step addition for achieving segmentation results. Techniques based on deep learning from their ability to learn shape and color variations can achieve better accuracies. In the last few years, deep neural networks (DNNs) have rapidly dominated the field of image segmentation and classification. Using the recognizing ability of neural networks, fully convolutional neural networks improve the efficiency of semantic segmentation. Widely speaking, deep learning-based nuclei segmentation frameworks can be divided into two major categories. Firstly, CNN-based nuclei detection has deformable models. In this approach, deep neural networks are applied for generating a probability map of nuclei centroid while for post-processing clustering, watershed or active contour-based techniques...
are sued for nuclei boundary segmentation, and algorithms like the watershed transform, clustering, and active contour are utilized to post-process the boundary of nuclei. The second majorly used technique is FCN-based pixel-wise end-end segmentation. It comprises an encoder decoder-based architecture including fully convolutional layers embedded with a refining technique i.e., watershed transform and conditional random field (CRF).

5.1 Nuclei instance segmentation

Nuclei instance segmentation plays a major role in the automatic diagnosis of cancer and medical image processing and analysis. Plenty of research has been done to efficiently segment out nuclei and gland instances. In this section, we have reviewed selected articles segregated primarily into two categories i.e., handcrafted, and deep learning-based feature extraction techniques. Table 4 enlist the details of the reviewed articles along with highlighting top performing techniques for each category.

5.1.1 Handcrafted features extraction methods

Variability in size, shape, textural, and tissue appearance make the detection of cell nuclei exigent, which is vital for the automatic analysis of digital pathology slides. The analysis of pathology slides is crucial in the quantification of the phenotypic information contained in tissue sections. An approach is proposed by Brieu and Schmidt (2017) that dealt with the variability in size by treating this detection problem as local maxima detection on the center probabilistic map, where a nuclear surface area map with a-priori knowledge on the size of the object of interest used for detecting local maxima. This method exhibited good quantitative displays. Xu et al. (2016) presented a generalized Laplacian of Gaussian (gLoG) filter-based automatic technique for automatic nuclei detection in digital pathology slides. gLoG filters with different scales and orientations are first piled up and then a set of response maps is obtained by performing a convolution operation on the contender image using directional gLoG kernels. Further, they used the mean shift algorithm to detect and cluster the local maxima of the response map based on special closeness. In each group, the point with the maximum response is selected as the nucleus. The proposed technique is evaluated on two datasets, showing a good performance in nuclei detection. Rojas-Moraleda et al. (2017) introduced an abstract simplicial homology approach based on the principles of persistent homology to address the problem of cell nuclei segmentation which identify the salient region in the image that exhibits a pattern of persistence, by dealing with the persistence of disconnected sets. This topological image representation reduced the dependency of the segmentation task on the variation of color or texture. Images of liver tissue acquired from histology sections are used to determine the efficiency of the approach. The proposed method recognized hepatocyte and non-parenchymal cell nuclei combined, with an accuracy of 84.6%, and hepatocyte cell nuclei alone with an accuracy of 86.2%. Gautam et al. (2017) proposed contrast-based adaptable versions of mean-shift and SLIC algorithms for the segmentation in pap smear images. This algorithm is then followed by intensity-weighted adaptable thresholding. The proposed model evaluated using Herlev dataset, achieved effective performance on images having inconsistent contrast in comparison with the state-of-the-art clustering-based method. Similar, accelerated acquisition Diffusion filter-based K-means clustering approach is used by Battistella et al. (2016) for segmentation on thalamic brain anatomy dataset, model reported 84% accuracy. Reljin et al. (2017) presented an inverse multifractal analysis (IMFA) for the segmentation of
| S# | Reference | Year | Organ | Dataset | Feature extraction method | Performance |
|----|-----------|------|-------|---------|---------------------------|-------------|
| 1  | Rojas-Moraleda et al. (2017) | 2017 | Liver | Liver dataset | Topological features based approach | 51% F1 Score |
| 2  | Gautam et al. (2017) | 2017 | Cervix | Herlev dataset | SLIC, mean shift clustering, adaptive thresholding | 70% Accuracy |
| 3  | Reljin et al. (2017) | 2017 | Fish images | Institute of Pathology, University of Bern, Switzerland | Matrix of holders exponent thresholding | 90% Accuracy |
| 4  | Brieu and Schmidt (2017) | 2017 | Breast | 30 H&E images | Local maxima detection | 75% F1 Score |
| 5  | Xu et al. (2016) | 2017 | Skin, Breast | Skin histology images dataset | gLoG kernel, mean shift clustering | 91% F1 Score |
| 6  | Battistella et al. (2016) | 2017 | Brain thalamus | Thalamic Brain Anatomy dataset | ODFs, K-Means clustering | 84% Dice Score |
| 7  | Shahul Hameed et al. (2017) | 2017 | Breast | OSCC | Entropy, Thresholding, Watershed, GLCM | 96% DSC |
| 8  | Song et al. (2017) | 2017 | Kidney | Farsight dataset, TCGA | LoG filter, Morphological operation, Regression model | 70% DSC |
| 9  | Saha et al. (2018) | 2018 | Cervix | ISBI-2014 Cervical cytology dataset | LoG filter, Morphological operation, Regression model | 95% DSC |
| 10 | Lee and Lee (2017) | 2018 | breast | BBBC006v1, U20S | LoG filter, Thresholding, Ellipse fitting | 95% DSC |
| 11 | Kostrykin et al. (2018) | 2018 | Flourescence microscopy images | NIH3T3, ISBI-2013 | Second order optimization | 75% F1- Score |
| 12 | Li et al. (2018) | 2018 | Liver | Hepatocellular carcinoma (HCC) | Mean shift clustering | 92% Accuracy |
| 13 | Quachtran et al. (2018) | 2018 | Flourescence confocal microscopy images | CLARITY Dataset | Iterative radial voting | 64% Dice Score |
| 14 | Semedo et al. (2018) | 2018 | Thalamus | Thalamic nuclei dataset | Local fibre orientation segmentation | 70% F1- Score |
| 15 | Guo et al. (2018) | 2018 | breast | Floursecene Microscopy Dataset | Curvature point detection, Ellipse fitting | 69% Accuracy |
| 16 | Karthick et al. (2019) | 2019 | Thalamus, Brain | Private dataset | Wavelet decomposition, Random forest classifier | 75% Accuracy |
| #  | Reference                        | Year | Organ                      | Dataset | Feature extraction method                                      | Performance   |
|----|----------------------------------|------|----------------------------|---------|---------------------------------------------------------------|---------------|
| 17 | Song et al. (2019)               | 2019 | Breast, Prostate, Kidney, Liver, Stomach, Bladder | Kumar, KIRC, Farsight | Decision trees, ISBI-2015                                     | 78% Dice Score |
| 18 | Song et al. (2021)               | 2021 | Cervical cytology          |         | Depth first search strategy, Decision trees                   | 95% F1 Score  |
| 19 | Dongyao et al. (2021)            | 2021 | Breast, Cervix            |         | Watershed, GVF snake model, Convex detection, Ellipse fitting, Convex edge, Morphological operation | 87% Dice Score |
| 20 | Zhao et al. (2021)               | 2021 | Cervix                    |         | 18 Whole slide cervical cell images                            | 87% Dice Score |
| 21 | Ramirez Guatemala-Sanchez et al. (2021) | 2021 | Breast                    | BreakHis database, 18 Whole slide cervical cell images         | 87% Dice Score |
| 22 | Roy et al. (2021)                | 2021 | Bladder, Stomach, Colorectal, Breast, Kidney, Liver, Prostate | Kumar, KMC MAHE (80 H&E stained images) | Thresholding, Energy maximization function | 87% Dice Score |
nuclei in fluorescence in-situ hybridization (FISH) images. At first, the matrix of the Holder exponent from the blue channel of the FISH image, along with one-by-one conformity with the RGB image is determined. The proposed semi-automatic method, initially apply predefined hard thresholding to perform segmentation from the matrix of Holder exponents then segmentation is refined by changing the threshold because of user evaluation. The evaluation of the IMFA segmentation method carried out over 100 clinical cases, showed the benefits of the proposed method compared to already reported methods. Zhang and Li (2017) presented a four-stepped method for robustly and efficiently segmenting overlapped nuclei. These steps are contour extraction, concave point detection, contour segment grouping, and ellipse fitting. Contour extraction algorithms are used for the estimation of the level of the image blurriness, which determines parameters in the following steps for increasing the segmentation accuracy of the blurry nuclei. Different algorithms are proposed for extracting obvious and unobvious concave from the contender points. Kostrykin et al. (2018) proposed a second-order optimization technique for intensity-based segmentation on fluorescence microscopy images. It targeted only below threshold haematoxylin intensity-based areas while all higher areas of intensity were in the background. It resulted in 75% accurate segmentation results on NIH3T3 and ISBI-2013 datasets. Shahul Hameed et al. (2017) adopted a gray-level co-occurrence matrix based cross entropy thresholding approach via taking a histogram of the input image for minimizing entropy for optimum threshold value assignment followed by post-processing segmented objects via watershed transform this enhanced segmentation DSC score to 96%. However, on grayscale images, this thresholding-based segmentation resulted in a poor DSC score which is improved via preprocessing. Similarly, Quachtran et al. (2018) also worked on fluorescence image instance segmentation using iterative radial voting techniques, however, their result in accuracy is slightly fewer 64% dice score compared to the optimization-based approach. Lee and Lee (2017) identified the contender nuclei seeds as extrema in a Laplacian-of-Gaussian space. Similarly, non-nuclei seeds are removed from clusters acquired by ellipse fitting. Local and global thresholding is combined to define regions of interest. shape and roughness of shared boundaries connected nuclei is modeled for repeatedly merging and splitting these regions. The model shows success in splitting boundaries of connected nuclei and recognizing the nucleus region. Saha et al. (2018) detect and segment nuclei by merging over-segmented SLIC superpixel regions using a novel image consolidating technique based on pairwise special contrast and image gradient contour evaluation, from cervical cytology images in ISBI-2014 dataset. First overlapping cervical cytology image segmentation is used for the evaluation of the proposed framework. The framework surpasses the performance of state-of-the-art detection and segmentation algorithms. Guo et al. (2018) proposed an algorithm for segmentation of overlapped nuclei. The algorithm identifies contenders with point pair connections and evaluates abutted point connections with a contrive ellipse fitting quality criterion. After the establishment of the connection relationship, the ensuing dividing paths are recovered by following the path of certain eigenvalues from the image hessian in a contrived searching space. Qualitative and quantitative evaluation carried out on 560 image patches from two classes shows the promising results of the algorithm. Semedo et al. (2018) proposed an algorithm for the segmentation of the thalamic nuclei, a central part of the nerve propagating the impulses between subcortical regions and the cerebral cortex. The algorithm is dependent on thalamic nuclei priors and local fiber orientation. Thalamus connectivity-based parcellation methods are used for the validation of the algorithm. The algorithm successfully segmented the anatomical plausible thalamic nuclei. Li et al. (2018) segmented hepatocellular carcinoma (HCC) nuclei by
using structure convolutional extreme learning machine (SC-ELM) and case-based shape template (CBST). First pathology images are globally segmented where each connected region is treated as a nucleus clump. Then contour refinement of nucleus clumps is done through a probability model of three energy functions. And at last, a combination of the CBST method and pixel-based classification is used for the obscure boundary inference. This method, evaluated on 127 liver pathology images shows a good result in comparison with other related work. Song et al. (2021) designed a representation learning decision trees based sparse coding ensembles network where fast Intra-decoders and Inter-Encoder are used for enhancing connectivity patterns as well as iterative regression, via decision tree-based ensemble mappings through regularization, pruning and random sampling for increasing model generalization. Song et al. (2019) proposed an improved multi-layer sparse convolution model (ML-BSC) where a handcrafted approach is utilized for robust feature extraction and optimized computation via integrating discriminative probability-based decision tree ensemble for enhancing classification performance. Luna et al. (2019) addressed the problem of pinpoint boundary delineation of adjacent nuclei by introducing a novel deep neural network. The network makes the prediction about the instances that whether they are from individual grouped nuclei. It uses the decision-making with the Siamese network for learning the relation between the two adjacent instances and the surrounding features of the adjacent nuclei. The network further predicts the class and their overlapping dice score through a decoding network improving classification accuracy. The network exhibit significant improvement in cell separation accuracy. A joint contour-based boundary extraction method is used by Kurmi and Chaurasia (2021) via 3 stages cascaded network for slides pre-processing, nuclei point extraction and region refining via canny edge detector, and composite nuclei segmentation through contour estimation. Rashmi et al. (2021) developed an unsupervised model for segmenting the nuclei from breast histology images based on Chan-Vase model. It pre-processes images via color normalization for discriminating foreground instances and background, followed by multi-channel learning based on color features for efficient segmentation. Song et al. (2017) used a combination of the watershed and GVF snake model for nuclei separation from the background followed by Convex hull detection and concave point detection for the splitting of overlapped nuclei and yielded 91% precise results (Dongyao et al. 2021). Lapierre-Landry et al. (2021) proposed a regression-based joint V-Net and watershed-based approach in 2021 for 3-D microscopy nuclei segmentation on embryonic heart dataset having high cell density, with an accuracy of segmenting 1000 nuclei centroids in under a minute. In SEENS (Zhao et al. 2021) mathematical morphology operators are integrated with the selective search for segmenting nuclei from cervical images while eliminating non-nuclei regions and avoiding repeated segmentation. The canny edge operator is used for extracting edge information for enhancing nuclei edge selection precision. Ramirez Guatemala-Sanchez et al. (2021) focused on morphological transformations and adaptive intensity adjustments for segmentation. Karthick et al. (2019) used wavelet decomposition technique followed by random forest classifier for nuclei segmentation on thalamus dataset and further post-processed segmentation output for refinement reporting 75% Accurate segmentation results. Roy et al. (2021) proposed an energy maximization function for nuclei instance segmentation. They segmented cell nuclei via contrast adaptive technique followed by intensity adaptive weighted thresholding and energy maximization segmentation on the Kumar dataset resulting in 87% dice accuracy.

**Critical analysis** Summarizing all handcrafted nuclei instance segmentation techniques, we conclude that majority of these techniques are proposed for the separation of touching, overlapped, and clustered nuclei instances. These techniques work best in case of slightly
touching nuclei instances separation, however, it does not work out in case of numerous such instances. Overall, the topological features-based segmentation approach, iterative radial voting-based instance identification, and curvature point detection based on ellipse fitting criteria yielded poor results. In comparison to this regression model based on Laplacian of Gaussian (LoG) applied with morphological operations (Saha et al. 2018) or thresholding, ellipse fitting (Lee and Lee 2017) produced up to the mark results having 95% DSc on ISBI-14 and BBBC006v1 datasets. The major limitations observed in LoG and other handcrafted techniques are computational cost, extreme sensitivity towards staining and gray intensity variation, and segmentation performance limited to detecting nearly rounded instances (Song et al. 2017, 2019). Similarly, models proposed in Kowal et al. (2019) and Fu et al. (2017) yielded accurate results during smooth nuclei contours variation; however, in the case of multiple overlapped and touching nuclei contours, their touching points on the contour resulted in larger curvature change and poor performance. Other frequently used methods include meaning shift clustering yielding better results on various datasets. Amongst all feature extraction methods Shahul Hameed et al. (2017), a global entropy thresholding-based segmentation technique, outperformed all other state-of-art handcrafted feature extraction methods for nuclei instance segmentation by achieving MAD of 47.8% DC of 96.7% and accuracy of 97%.

5.1.2 Deep learning based methods

Region based nuclei localization The regional proposal-based segmentation approach was initially designed for natural image segmentation. However, in the last few years, they have proved extremely influential for other domains as well and are highly adapted for medical image segmentation too especially in nuclei and gland segmentation tasks. The main idea behind proposal base architecture is detecting regions according to the variations in similarity metrics and color differences, followed by classification for regions having a high probability of object existence, they are often regarded as a region-wise prediction as well. Mask base region proposal network also called Mask-RCNN comprising of CNN as a base for feature extraction and a region proposal network for suggestions about object regions which are further used for the prediction of binary masks. Mask-RCNN along with U-Net has been widely used in various nuclei segmentation tasks in the past few years. Table 5 is showing the details of the articles reviewed. In 2018 Liu et al. (2018) combined (Mask-RCNN) and fully connected conditional random field (LFCCRF) for segmenting cervical nuclei via localizing nuclei boundaries through multi-scale feature maps through regional proposals followed by enhancing segmentation by passing spatial information to LFCCRF for further refinement. However, the accuracy of this model required further enhancement. Vuola et al. (2019) comparatively analyzed both the regional proposal and U-Net segmentation techniques and ensembled architecture. For circular-shaped medium-sized nuclei, the Mask-RCNN model gave excellent results, but its performance degraded in the case of elliptical shapes however ensembled model resulted in overall accuracy enhancement. Baykal et al. (2020) used object detection models including Faster-RCNN, region-based convolution neural network, and Single Shot Detection model (SSD) for the first time for pathology image nuclei detection. However, SSD results for detection were not up to the mark while Faster-RCNN along with ResNet yielded the best results. A similarity learning-based approach is used by Sun et al. (2021), they introduced an embedding layer for building networks and training through the embedding loss function. These networks were able to learn distinguishing features based on similarity score which is further used for instance
Table 5 Nuclei instance segmentation via two stage patch extraction techniques

| S# | Reference          | Year | Organ                                   | Dataset                     | Technique               | Performance |
|----|--------------------|------|-----------------------------------------|-----------------------------|-------------------------|-------------|
| 1  | Liu et al. (2018)  | 2018 | Pap smear images                        | Herlev, BNS, MOD            | Mask-RCNN, CRF         | 84% F1      |
| 2  | Naylor et al. (2019)| 2019 | Bladder, Stomach, Colorectal, Breast, Kidney, Prostate | IIT                         | U-Net, FCN, Mask-RCNN  | 81% F1      |
| 3  | Sun et al. (2021)  | 2019 | Breast, Kidney, Liver, Prostate, Bladder, Colon | MoNuSeg                     | Mask-RCNN, R-CNN, ResNet | 85% F1      |
| 4  | Feng et al. (2019) | 2019 | Breast, Kidney, Liver, Prostate, Bladder, Colon | DAPI, TNBC                  | Mask-RCNN              | 0.54 AJI    |
| 5  | Vuola et al. (2019)| 2019 | Colon                                   | Fluorescence Images         | Mask-RCNN, U-Net       | 72% DSc     |
| 6  | Jung et al. (2019) | 2019 | Breast, Kidney                          | BNS, MOD                    | Mask-RCNN              | 86% F1      |
| 7  | Baykal et al. (2020)| 2020 | Lungs                                   | Pleural Effusion Cytology Images | Faster-RCNN, SSD       | 98% F1      |
| 8  | Liang et al. (2022)| 2022 | **Breast, Kidney, Lung, Prostate, Bladder, Colon** | **DSB-2018, MoNuSeg**       | **Mask-RCNN, FPN**     | **84% F1**  |
| 9  | Qu et al. (2020)   | 2020 | Lung, Multi organ                       | Lung Cancer (LC), Multi-Organ (MO) | SR-CNN, FPN           | **88% F1**  |
| 10 | Greenwald et al. (2022)| 2021 | Immune, Breast, Colon, Esophagus        | Vectra, MIBI-TOF, CyCIF, CODEX | ResNet, Faeature Pyramid | **84% F1** |

The bold text is used to highlight the methodologies which attained highest quantitative performance.
classification. A unique module incorporating guided anchoring into regional proposals is used in Feng et al. (2019) for candidate proposals generation along with a new branch for regressing intersection over union (IoU) between ground truths and detection boxes for bounding box localization. They also passed FBS to soft non-maxima suppression for true positive box preservation. Qu et al. (2020) came up with a novel weakly supervised segmentation framework based on partially annotated data. This two-level architecture’s first level uses a semi-supervised method for a detection model to learn from partially labeled data. The model is initially trained using an extended Gaussian mask. The model is then boosted, and false positives are reduced using unlabeled regions and self-training with background propagation. The second level of the architecture uses the detected nuclei regions to train a weakly supervised segmentation model. After that, two types of labels i.e., Voronoi Labels, and Cluster Labels are derived from the detected points to train a convolutional neural network with cross-entropy loss. The experimental results demonstrated on Lung Cancer (LC) dataset and Multi-Organ (MO) dataset, have shown performance supremacy by achieving F1 scores of 0.8879 and 0.8282 respectively. Greenwald et al. (2022) created a deep learning-based segmentation model named Mesmer to address the issue of cell segmentation in tissue imaging data through large-scale data annotation. Mesmer consists of two subnetworks PanopticNet architecture and Tissue Net. PanopticNet uses ResNet50 as the backbone that is connected to a feature pyramid. To provide predictions of the same size as the input image, two semantic segmentation heads are added to the feature pyramid. TissueNet is constructed using an image collection with more than 1 million paired whole-cell and nuclear annotations for tissue images from nine organs and six imaging platforms. Mesmer automated the previously challenging key cellular features extraction task such as subcellular localization of protein signal. Mesmer can also be used for harnessing cell lineage information in multifold datasets. The proposed pipeline achieved an F1 Score of 84% for immune cells.

Critical analysis

In general performance, comparison concludes that deep learning-based networks produced better segmentation results with fewer mistakes compared to handcrafted architectures. One of the major strengths of LFCCRF (Liu et al. 2018) is better accuracy not just for normal but abnormal nuclei as well, while the results produced by Baykal et al. (2020) although had better F1 score and results of some of them are highly accurate too, however, it’s standard deviation is very low. Overall, the solution of Liang et al. (2022) was found significantly better than all other previous state-of-the-art region proposal-based patch extraction techniques for instance segmentation of nuclei. It yielded the best performance results for generalization and other metrics on two major publicly available datasets including DSB-2018 and MoNuSeg.

Encoder decoder based segmentation techniques

Convolutional neural networks have always been the most used techniques for vision problems and have achieved great success in detection and segmentation tasks in medical image analysis. However, in deep neural network-based learning it was observed that CNNs despite having the ability to learn major features via layer-wise propagation at times results in lost spatial information. Compared to simple deep neural networks, fully convolutional neural networks (FCN) based nuclei segmentation frameworks were found more efficient in nuclei and gland segmentation in histology images. For instance, while classical CNNs work by classifying individual pixels via a sliding window approach, FCN allows upscaling classical features hence segmenting images in a single pass. For resolving shortcomings of CNN during segmentation, encoder-decoder-based approaches were proposed. It works on the principle of downsampling first for better feature learning followed by upsampling for final segmentation. In this architecture during downsampling network reduces activation size through a feed-forward
neural network, while in upsampling deconvolution network or unpooling operations are primarily used for regaining lost information. The Table 6 summarizes details about encoder-decoder based segmentation techniques. In 2017, Zhang et al. (2017) used FCN for segmenting the whole cell into cytoplasm, background, and nuclei probabilistic maps. It captured nuclei deep features precisely however accurate boundaries localization for segmented results was still an issue. As a resolution, graph-based segmentation is incorporated on FCN segmented features thus improving nucleus boundaries detection via probability map. Similar encoder-decoder architecture for nuclei instances segmentation is proposed by Graham and Rajpoot (2018). They used a down sampling operation for strong gradient features while in backpropagation residual blocks and max pooling operators are used, similarly for upsampling deconvolution operators were incorporated for fine image rebuilding. Cellpose is another generalized, deep learning-based segmentation algorithm proposed by Stringer et al. (2020) for precise nuclei segmentation. First annotation masks are transformed to vector flow representation and fed to a neural network that predicted nuclei x and y gradients, computed through spatial gradients pointing towards the center of the instance. The second output head yields pixel classification output and all three prediction maps are combined into a flow field (Javed et al. 2020).

A new stain-aware auxiliary loss function resulted in better performance by targeting only low hematoxylin intensity-based areas within nuclei and high-intensity areas in the background. Xu et al. (2019) proposed a joint segmentation detection module using U-Net and SSD for reducing segmentation inaccuracies of previous approaches. It dynamically integrated nuclei location output and semantic segmentation results for a joint performance boost. A deep regression distance-based approach by Naylor et al. (2019) resolved the segmentation issues faced for closed-touching nuclei. In this architecture pre-trained weights of VGG-16 and training results of three different models including FCN, Mask-RCNN and U-Net were compared for the prediction of an unnormalized intra nuclear distance map. Despite having up-to-the-mark accuracy, high computational complexity and generalization inability were still major challenges of this approach. On the other hand stage, wise segmentation approach was used by Qu et al. (2019) and Kowal and Korbicz (2019). Two similar SU-Net architectures were utilized in this approach where the first stage aimed at segmenting nuclei regions, while the second stage was designed for segmenting overlapping nuclei regions (Dogar et al. 2023). Finally for instance segmentation results output of both stages were merged. This multi-stage learning approach outperformed all previously existing state-of-the-art approaches.

A shape priorized regularized network architecture is proposed by Tofighi et al. (2019). In this technique, initial learnable layers learn from prior information (generated edge map through raw input image and predefined shapes) via fixed processing and perform nuclei detection consistent to the boundary.

Cui et al. (2019) proposed a model for simultaneously predicting nuclei and their contours at the same time via a nuclei boundary prediction model using attention-based segmentation and fast non-parameter dilation-based post-processing after contour-based segmentation via fully convolutional neural network.

Narotamo et al. (2021) devised a model that initially divides the image into equal-sized overlapping patches and segments nuclei and boundary map by a fully convolutional neural network (FCN). Detecting nuclei boundary in each patch enabled splitting touched and overlapped nuclei thus improving accuracy. Han et al. (2020) focused on an unsupervised learning approach via generating 3D nuclei data using Recycle-GAN along with Hausdorff distance loss for nuclei shape preservation. For the segmentation and classification of synthetically generated data, a 3D CNN is employed. Similarly, the 3D nuclei segmentation
| # | Reference | Year | Organ | Dataset | Technique | Performance |
|---|-----------|------|-------|---------|-----------|-------------|
| 1 | Zhang et al. (2017) | 2017 | Cervix | Herlev | FCN | Zijdenbos similarity 92% |
| 2 | Ho et al. (2017) | 2017 | Kidney | Rat kidney dataset | FCN | 83% F1 Score |
| 3 | Pan et al. (2016) | 2017 | Breast | David Rim's dataset | SegNet, Watershed | 80% Dice Score |
| 4 | Fu et al. (2017) | 2017 | Kidney | CPM-17 | U-conv, Deformable convolution | 93% Zijdenbos similarity |
| 5 | Graham and Rajpoot (2018) | 2018 | Breast | Ketel | FCN, Pnp | 82% F1 Score |
| 6 | Ho et al. (2018) | 2018 | Breast | David Rimm's dataset | FCN & PngNet | 80% F1 Score |
| 7 | Höfener et al. (2018) | 2018 | Breast | U-20S | U-conv + deformable convolution | 93% Zijdenbos similarity |
| 8 | Naylor et al. (2017) | 2017 | Breast, Kidney | U-20S | FCN, Bayesian Inference | 93% Zijdenbos similarity |
| 9 | Zhao et al. (2019) | 2019 | Breast | University Hospital Poland | OS-NET | 80% F1 Score |
| 10 | Tofighi et al. (2019) | 2019 | Breast | Kumar, TNBC | CNN, seed candidate selection | 94% F1 Score |
| 11 | Qingbo et al. (2019) | 2019 | Breast | sare, TNBC | Stacked U-Net | 80% F1 Score |
| 12 | Lee et al. (2019) | 2019 | Breast | Fluorescence Image Dataset | OS-NET | 70% Dice Score |
| 13 | Zeng et al. (2019) | 2019 | Breast | TCGA | Residual Inception U-Net | 84% F1 Score |
| 14 | Vuola et al. (2019) | 2019 | Colon | MOD, BCD | SSD + U-Net | 91% Dice Score |
| 15 | Xu et al. (2019) | 2019 | Breast | Kumar, TNBC | GlaS, CRAG, MoNuSeg | 80% F1 Score |
| 16 | Li et al. (2019) | 2019 | Breast | U-Net, Distance Map, Fusion Module | ASU-Net | 76% F1 Score |
| 17 | Pan et al. (2019) | 2019 | Breast, Kidney, Liver, Prostate | U-Net, Distance Map, Fusion Module | ASU-Net | 87% F1 Score |
| 18 | Yoo et al. (2019) | 2019 | Breast, Kidney, Liver, Prostate | U-Net, Distance Map, Fusion Module | ASU-Net | 60% Accuracy |
| S# | Reference                  | Year | Organ                                    | Dataset                        | Technique                  | Performance  |
|----|----------------------------|------|------------------------------------------|-------------------------------|----------------------------|--------------|
| 22 | Graham et al. (2019)       | 2019 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | CoNSeP, Kumar               | U-Net                      | 80% Dice Score |
| 23 | Qu et al. (2019)           | 2019 | Lungs                                    | Lung Cancer Dataset          | FCN + U-Net                | 88% F1 Score  |
| 24 | Kong et al. (2020)         | 2019 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | Kumar, TNBC                 | Stacked U-Net              | 80% F1 Score  |
| 25 | Long (2020)                | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | DSB-2018                     | U-Net&                     | 62% Accuracy  |
| 26 | Zhao et al. (2020)         | 2020 | Bladder, Stomach, Breast, Kidney, Colorectal adenocarcinomas, Prostate | MoNuSeg, CoNSeP, CPM       | U-Net&                     | 83% Dice Score |
| 27 | Kowal et al. (2019)        | 2020 | Breast                                   | David Rims dataset           | FCN + watershed            | 90% Accuracy  |
| 28 | Qu et al. (2020)           | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | Lung cancer dataset, MOD    | Bayesian CNN                | 83% F1 Score  |
| 29 | Cheng and Qu (2020)        | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | DSB-2018                     | UNet, FPN&                 | 77% Accuracy  |
| 30 | Xie et al. (2020)          | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                     | Res-UNet                   | 65% accuracy  |
| 31 | Qu et al. (2019)           | 2020 | Lungs, Prostate                          | Lung Cancer, Herlev          | U-Net, K-Means             | 75% F1 Score  |
| 32 | Yang et al. (2020)         | 2020 | Cervix                                   | Lung Cancer Dataset, U20S    | FPN, Encoder decoder       | 97% F1 Score  |
| 33 | Baykal Kablan et al. (2020)| 2020 | Breast, Lung                             | Kidney dataset               | FCN, SegNet, U-Net         | 95% Dice Score |
| 34 | Han et al. (2020)          | 2020 | Kidney                                   | Kidney dataset               | U-Net, Recycle-GAN         | 82% F1 Score  |
| 35 | Hussain et al. (2020)      | 2020 | breast                                   | Smear dataset                | FCN                        | 96% ZSI       |
| 36 | Xie et al. (2019)          | 2020 | Breast, Prostate, Kidney, Stomach        | Kumar, MICCAI-2017          | Marker controlled watershed| 87% F1 Score  |
| 37 | Narotamo et al. (2021)     | 2021 | Retina                                   | Mouse-Retina Dataset         | U-Net                      | 63% Score     |
| 38 | Huang et al. (2021)        | 2021 | Deep sea archea                          | Fluorescence microscopy images| Encoder decoder            | 70% F1 Score  |
Table 6 (continued)

| S# | Reference                      | Year | Organ                                      | Dataset                  | Technique                                      | Performance     |
|----|--------------------------------|------|--------------------------------------------|--------------------------|-----------------------------------------------|-----------------|
| 39 | Vahadane et al. (2021)         | 2021 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | CPM-17, Kumar, CoNSeP   | U-Net, Attention skip module                  | 81% Dice Score  |
| 40 | Wan et al. (2020)              | 2021 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg, TNBC           | U-Net, Internal, Co-variance Shift            | 95% F1 Score    |
| 41 | Kim et al. (2020)              | 2020 | Brain                                      | Diffusion weighted MRIs | DCN-Net                                      | 87% Dice Score  |
| 42 | Wang et al. (2020)             | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                 | Bending loss, U-Net                          | 83% Dice Score  |
| 43 | Valkonen et al. (2020)         | 2021 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                 | VGG, Transfer learning, FCN                   | 77% F1 Score    |
| 44 | Mandal et al. (2021)           | 2021 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | Fluorescence image dataset | U2OS                                         | 84% F1 Score    |
| 45 | Lapierre-Landry et al. (2021)  | 2021 | Heart                                      | Heart Dataset           | V-NET, Centroid calculation                  | 95% F1 Score    |
| 46 | Braga et al. (2021)            | 2021 | Cervical cytology images                   | ISBI-2014, Herlev        | Multi scale narrow band level set algorithm  | 85% Dice Score  |
| 47 | Kiran et al. (2022)            | 2022 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg, TNBC, CryoNuSeg | Distance map, Skip Connection, U-Net         | 90% F1 Score    |
| 48 | Javed et al. (2021)            | 2021 | Bladder, Stomach, Colorectal adenocarcinomas, Breast, Kidney, Liver, Prostate | 100 H&E stained CRC histology, CoNSeP, PanNuke | Correlation filter                           | 85% Dice Score  |
| 49 | Ahmad et al. (2017)            | 2017 | Large Intestine                            | 100 H&E Colorectal adenocarcinomas | Correlation filter                           | 84% F1 Score    |
| 50 | Alemi Koohbanani et al. (2019) | 2019 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                 | Spa-Net                                      | 82% F1 Score    |
| 51 | Zhou et al. (2019)             | 2019 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                 | CIA-Net                                      | 84% F1 Score    |
| 52 | Stringer et al. (2020)         | 2021 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg, DSB-18         | U-Net                                        | 91% AP          |
| S# | Reference                      | Year | Organ                        | Dataset               | Technique | Performance |
|----|--------------------------------|------|------------------------------|-----------------------|-----------|-------------|
| 53 | Lee and Jeong (2020)           | 2020 | Bladder, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg, BBBC038v1, and EM | U-Net     | 92% mDice   |

The bold text is used to highlight the methodologies which attained highest quantitative performance.
method has been proposed by Ho et al. (2017, 2018) and Guan et al. (2021) via distance transform, adaptive histogram equalization, and a 3-D convolution neural network for the classification CNN by searching nuclei centers and spatial & channel attention module for nuclei segmentation are proposed in 3D encoder-decoder model trained on initially generated synthetic volumes. An enhanced approach for resolving overfitting issues faced during multi-resolution feature extraction of conventional U-Net and enhancing convergence performance Majdi et al. (2020), Zeng et al. (2019) and Xie et al. (2020) proposed a residual U-Net based technique for segmentation where they incorporated batch normalization and drop out layers in architecture with the addition of scale-wise triplet learning and count ranking for vanishing gradient and exploding loss risks mitigation during segmentation. Mandal et al. (2021) proposed a Y-shaped model where a novel forked decoder is tied to segmentation and deblurring, this additional element in the model resulted in fine-tuning output for normal as well as blurred out-of-focus images. A similar technique is proposed by Aatresh et al. (2021) with a slight enhancement of dimension-wise convolution combined with atrous spatial pyramid pooling for improved encoder-decoder architecture and multi-task learning based on nuclei region and boundaries extraction. A similar architecture is proposed by Pan et al. (2019) and Dabass et al. (2021) where along with features learning, the diminishing gradient issue is alleviated as well. For resolving problems faced by the model during small objects features capturing a dual encoder architecture are proposed by Narotamo et al. (2021). They utilized prior feature information in the encoding network and input feature maps are utilized in attention skip modules for a segmentation performance boost. Vahadane et al. (2021) used a post-processing approach for separating touching nuclei and semantic segmentation of nuclei. Firstly, objects are thresholded followed by boundary map subtraction for nuclei instance map segregation, then an instance-based energy map is generated for a pixel to background distance calculation. Erosion is used for marker generation which is then passed to the watershed along with a distance map for final processing.

Sparse reconstruction based deconvolution technique is used in Pan et al. (2016), Naylor et al. (2017), Kim et al. (2020), Qu et al. (2019) and Long (2020). They used dilated dense block with an exponential increase in dilation rate for encoding cascaded multi-stage neural networks information which is further trained via gradient descent technique and an attention score map is used here as a regularization factor thus avoiding binding and interposed nuclei overlap. Image level and instance level alignment based on domain shift minimization approach is proposed by Wang et al. (2021) where INA initially extracted instance features through nuclei locations via a temporal ensembling based Nuclei Localization (TENL) module, this resulted in automatic candidate nuclei location generation. Huang et al. (2021) proposed a shared decoder path instead of the conventional two-path decoder technique used by Qingbo et al. (2019) thus increasing the recognizability range generated through the new half path served as a natural proxy for curriculum learning model. Baykal Kablan et al. (2020) designed a SegNet-based architecture, which is one of the best models for problems dealing with image segmentation tasks due to its property of direct information transfer instead of convolution.

To utilize contour-specific features to improve the quality of the entire segmentation BES-Net (Oda et al. 2018) and CIA-Net(Zhou et al. 2019) used their correlation and further introduced uni-directional and bi-directional information transmission, respectively, which means one decoder obtains extra features from the other one. But there are some downsides of these approaches too, firstly they used a shared encoder for both tasks thus underestimating essential differences between tasks in feature learning; for example, the boundaries which separates two overlapping instances cannot be directly inferred from the

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semantic segmentation because it considered them as one and adopted complex post-processing rules, making a variation of post-processing hyper-parameters a sensitive task.

For accurately segmenting overlapping and cluttered nuclei Wang et al. (2020) proposed a bending loss regularized network using a multi-task learning approach inspired by HoVer-Net architecture by Graham et al. (2019). It introduced generalization by training decoders for 3 streams including nuclei distance map prediction, overlapped nuclei distance map, and instance segmentation via using high penalty bending loss for large curvature contour points and low penalty for small curvature for simultaneous concave or convex transformation. While in HoVer-Net 3 branches were nuclei pixel branch (for pixels separation from background), hover branch in which nuclei horizontal and vertical distances concerning the center of masses is used for separating clustered nuclei thus resulting in accurate segmentation, especially for cases having a large number of overlapping nuclei segmented instances and lastly nuclei classification where segmented instances were further passed to a dedicated upsampling branch for type classification. HoVer-Net was also the first ever technique to achieve both segmentation and classification via the same network. Zhao et al. (2020) also used similar 3-branch architecture having RGB branch, segmentation branch, and hematoxylin branch simultaneously. In this technique RGB branch was used for raw features extraction for the segmentation task, the H branch for H&E aware feature extraction for the task of nuclei contour detection task, and the segmentation branch fused both RGB and Haematoxylin contour features for final results prediction. Braga et al. (2021) also used contour-based boundaries detection and encoder decoder-based learning approach.

Li et al. (2019) improved shortcomings of previous regression-based segmentation and devised a multi-task learning fusion-based approach for improving glioma nuclei segmentation accuracy via boundary and region information they adopted a U-Net-based encoder decoder where paired upsampling paths are used for boundary classification and touching nuclei separation while in other path regression model is used for nuclei region distance map prediction approach. These layers are further fused for final segmentation. In 2017 Fu et al. (2017) utilized watershed for cell nuclei quantification in parallel with CNN-based segmentation. Luna et al. (2019) used an encoder-decoder-based approach where contextual information is captured via harnessing multi-level CNN capabilities and features concatenation along with up-sampled features using skip connections followed by post-processing via morphological operations. This model detected malignant cases accurately but failed in the case of some benign test samples due to erosion during the noise removal process. A modification to this approach was proposed in the region proposal and encoder-decoder-based joint nuclei detection and segmentation technique given by Cheng and Qu (2020) where both shallow and deep layers are merged via skip connections for accurate detection of nuclei locations using bounding boxes and detected patches are further passed to U-Net for segmentation. Vuola et al. (2019) gave a comparative analysis of both regional proposal and U-Net model-based nuclei segmentation as well as ensembled architecture. For circular-shaped medium-sized nuclei, the Mask-RCNN model gave excellent results but its performance degraded in the case of elliptical shapes. It had some trouble in approximating bounding boxes for the nuclei while U-Net performed better in such cases, similarly, it was also found that an ensembled model resulted in overall accuracy enhancement. A novel kernelized correlation filter is used for nuclei detection in Ahmad et al. (2017) due to its dependence on a small training dataset as well as interpretable and computationally efficient nature as compared to other deep learning-based models and can give the state-of-the-art results. A similar approach is used in Javed et al. (2021) where the spatial structure is incorporated via constructing a dense graph based on different deep features across
different nuclei components then a correlation filter is used for discriminating nuclear and non-nuclear regions. Similarly, Salvi and Molinari (2018) used watershed segmentation in a multi-scale framework after progressive weighted mean detection for object detection and area-based correction for aligning over or unsegmented objects. In this technique, watershed transform is specifically used for nuclei detection purposes. In Xie et al. (2019), Kowal et al. (2019) color de-convolution is used for image preprocessing for H&E stain highlighting which are then trained on deep CNN followed by marker controlled seeded watershed technique for splitting touching nuclei but this process especially semantic segmentation used in CNN results in a computationally expensive model and performance degraded in case of highly overlapped nuclei further resulted in over-segmentation as well.

A multi scale proposal free spatially aware network is proposed by Alemi Koohbanani et al. (2019). The architecture comprised of dual-headed outputs yielding nuclei pixel-wise segmentation and centroid detection maps which are further fed to a single-headed SpaNet and it predicted nuclei instance positional information. Finally, spectral clustering is applied on the last spatial output which utilized the initial generated nuclei mask and gaussian detection maps for nuclei cluster identification.

An ensembling based improved architecture is proposed by Zhao et al. (2019) and Kong et al. (2020) having a U shaped ensembled convolution network as backbone with dense blocks for effectively transferring features information alongside overcoming vanishing gradient problem of prior architectures. Another feature added to this model was deformable convolution for dealing with nuclei of different sizes and irregular shapes thus enhancing model flexibility. A point annotation-based architecture is presented by Yoo et al. (2019) as PseudoEdgeNet. In this a guided segmentation network is trained for recognizing nuclei edges without prior annotations through multi-scale pyramidal model and backbone ResNet in Kiran et al. (2022). Another similar one-click approach for quick annotation collection is proposed by Alemi Koohbanani et al. (2020) where they used a single click for precise annotation for single as well as for structures comprising multiple nuclei such as glands. In 2021, Valkonen et al. (2020) proposed a supervised transfer learning model via applying unsupervised domain adaptation for model generalization on seen as well as unseen labeled images data and achieved 77% accuracy. Another transfer learning and logistic regression-based approach is used by Li et al. (2019) via unsupervised sparse auto encoder (SSAE) and case based post-processing module (CPM) technique. They extracted high-level features via transfer learning followed by logistic regression classifier(LRC) on extracted features and fine-tuning via CPM. Lee and Jeong (2020) introduced a cell segmentation framework that only uses a small number of scribbling annotations instead of full segmentation labels in a weekly supervised manner. The basic idea is to combine label filtering and pseudo-labeling to get accurate labels from week supervision. For this, pseudo labels are enhanced by iteratively averaging the predictions leveraging the consistency of prediction. The baseline network used in the proposed network was U-Net with the ResNet-50 encoder. The performance of the proposed method is evaluated on three different cell image datasets i.e. MoNuSeg, BBBC038v1, and EM, and achieved mDice score of 0.6408, 0.8426, and 0.9208 respectively.

**Critical analysis** Majority of current studies on nuclei instance segmentation in deep learning depends on encoder decoder frameworks primarily U-Net networks and makes slight changes to the base network structure according to the variation in data and structural variation of instances, however it also observed that some of the non-network morphological factors are also playing an important role towards the improvement of nuclei segmentation results. However one major limitation found in proposed FCN based techniques including (Hussain et al. 2020) is higher computational time complexity compared
to Ho et al. (2017) and other U-Net and Mask-RCNN models thus demanding more sophisticated software and hardware platforms. Internal co-variance shift based U-Net architecture proposed by Wan et al. (2020) in 2021, stood out as a top performing model due to its generalized network across diverse set of images, experimental inputs variation for a heavily imbalanced dataset with 80% of training images it yielded an accuracy of 95%. Similarly, FPN based encoder decoder architecture proposed by Yang et al. (2020) outperformed previous state of the art result with 97% F1-Score. Similarly, HoVer-Net (Graham et al. 2019) can be classified as a universal architecture amongst all U-Net based nuclei segmentation techniques due to its generic nature and diverse segmentation ability yielding up to the mark results on majority of nuclei datasets.

Adversarial models based segmentation Generative Adversarial Networks (GANs) were first introduced by Goodfellow et al. (2014) in 2014 with the basic idea of generating synthetic images by mimicking the content of actual training datasets as described by Skandarani et al. (2021). With the introduction of GAN-based models in several image processing tasks it was found that approaches relying on Generative Adversarial Networks have exhibited the capability of reducing the large annotated dataset requirements, thus reducing the potential barrier of automated image analysis in several medical imaging modalities as reviewed by Tschuchnig et al. (2020). Table 7 summarize details about adversarial models based segmentation techniques. In the field of computational pathology recent GAN-based developments have not only improved measures but have also enabled novel applications. Thus tasks relying on supervised techniques can now be performed via unsupervised techniques. In 2018, Zhang et al. (2018) proposed GAN based nuclei segmentation model as a dual contour enhanced adversarial network. In this approach contour highlighted and distance transformed masks are incorporated via an adversarial network for improving cell nuclei segmentation. This approach outperformed the previous state-of-the-art models on the MICCAI-2017 dataset, however, this model was generalizable. Mahmood et al. (2020) proposed an improved model in 2020, via utilizing conditional GANs-based training for nuclei segmentation on both real as well as synthetic data thus ensuring spatial consistency compared to conventional CNNs. A data augmentation-based approach proposed by Pandey et al. (2020) employed multi-GANs for improving the performance of conventional segmentation approaches, one for generating a synthesized mask which is incorporated into the second GAN for performing conditional generation of the synthesized image. Han et al. (2020) focused on an unsupervised learning approach via generating 3D data using Recycle-GAN along with Hausdorff distance loss for nuclei shape preservation. For the segmentation and classification of synthetically generated data, a 3D CNN is employed. Yao et al. (2021) generated Scaffold-A549 a very first synthetically generated nucleus segmentation dataset for training on varying density nuclei in 3D cell culture via the recycle-GAN approach. Xing et al. (2021) proposed an adversarial two-directional domain adaptive method for nuclei detection on multiple modalities. Specifically, this method learns via a deep regression model through the source to target and target to source image translation for each nuclei.

Critical analysis Limited availability of labeled nuclei datasets in medical imaging domain has now doubt incremented use of GANs for synthetic data generation in nuclei and glands detection as well. Amongst all GANs based reviewed techniques this has been observed that models using cycle-GANs for generation followed by segmentation have yielded quite low performance i.e. 50% on Fluorescence Image data as mentioned by Yao et al. (2021) and 71% on DAPI dataset trained and validated by Xing et al. (2021). Similarly, Multi-GAN based nuclei instance segmentation models performed better and yielded upto the mark results as studied by Pandey et al. (2020), and Han et al. (2020). However,
| S# | Year | Reference            | Organ                                      | Dataset         | Technique  | Performance   |
|----|------|----------------------|--------------------------------------------|-----------------|------------|---------------|
| 1  | 2018 | Zhang et al. (2018)  | Head, Neck, Squamous cell, tumors          | MICCAI-2017     | GAN        | 70% F1 Score  |
| 2  | 2020 | Pandey et al. (2020) | Bladder, Colon, Stomach, Breast, Kidney, Liver, Pancreas, Colorectal | DSB-2018        | Multi-GAN  | 82% Dice score |
| 3  | 2020 | Mahmood et al. (2020)| Bladder, Colon, Stomach, Breast, Kidney, Liver | TCGA            | cGAN       | 86% F1 Score  |
| 4  | 2020 | Han et al. (2020)    | Kidney                                     | Kidney dataset  | Recycle-GAN| 82% F1 Score  |
| 5  | 2021 | Yao et al. (2021)    | Lungs                                      | 3D Fluorescence image data | Cycle GAN  | 50% Dice Score |
| 6  | 2021 | Xing et al. (2021)   | Colon                                      | DAPI, TMI       | Cycle GAN  | 71% F1 Score  |
conditional GANs based nuclei instance segmentation technique by Mahmood et al. (2020) outperformed all other techniques mentioned above. This can also be concluded that despite being the wide spread use of GANs in different image processing techniques they are notoriously difficult to train.

**Attention based sequential models**  Attention mechanisms can be regarded as one of the hottest areas of deep learning research since last few years, originating primarily for natural language processing and now yielding excellent results in computer vision domain as well. They work exactly on the principle of human eye, while viewing a scene in form of partial glimpses and paying enhanced attention to parts relevant to the context. In this way it not only focuses on selected regions but also concludes object interpretations at that particular point thus improving visual structure understanding. It explores global contextual information via building associations amongst features using attention mechanism, alongside adaptively aggregating long range contextual details, thus improving feature learning for accurate object segmentation. It can be considered as a tool for fair divisioning of allocated resources according to the quantity of information carried by signal. In most models its often used on top of higher level contextual information representing layer for better adaption amongst objects. Table 8 summarize details about attention based nuclei instance sequential models technique.

For medical image segmentation, first specific attention based model for nuclei segmentation task has been proposed in 2019 by Zhang et al. (2019). They designed a binary tree network with two path fusion attention module via concatenating both low and high feature information followed by convolution for generating fused feature similar to a binary tree structure. A joint attention model based on Neural Architecture Spatial and channel weighting effect is proposed by Liu et al. (2020) using NAS search strategy for attention module automation with the addition of multiple attention module architectures searching within same network. A self supervised attention based nuclei segmentation approach is devised by Sahasrabudhe et al. (2020) from the assumption that nuclei texture and size could yield slide magnification thus generating self-supervised signal points for nuclei localization. For resolving prior noise issues a weakly supervised learning model is proposed by Guo et al. (2021) using nuclear centroid annotations for segmentation via generating boundaries and super pixel masks as ground truth labels. Salvi and Molinari (2018) worked on weakly supervised learning approach for mitigating high quality annotated datasets requirement for training. They trained nuclei segmentation module via nuclei centroid annotation which were used for generating boundary and masks as ground truth label for segmentation and further performance enhancement is done through mask guided attention auxiliary network.

For further refinement in previous approaches an encoder decoder based architecture and spatial channel joint attention module for abnormal nuclei detection is proposed by Ma et al. (2020) via using attention based merging technique for merging extracted varying and fixed features generated via R-CNN and fixed proposal module. Efficient feature extraction based approach is focused by Vahadane et al. (2021) and Lal et al. (2021) using 3 block architecture including residual bottleneck and attention decoder blocks. Robust residual blocks yielded high level object semantic maps while object localization is performed via attention module, thus improving accuracy. Zhao et al. (2021) employed a post processing pipeline for final segmentation from attention segmented image. They used a combination of morphological operations (binary opening and closing) for coarse and fine object enhancements followed by distance transform and Gaussian blurring for local maxima identification and in the end watershed is used for final results. Han et al. (2022) presented a generalized meta multi-task learning (Meta-MTL) for nuclei segmentation that relies less
Table 8  Attention based nuclei segmentation

| S# | Reference                  | Year | Organ                        | Dataset                              | Technique                                      | Performance                      |
|----|----------------------------|------|------------------------------|--------------------------------------|------------------------------------------------|----------------------------------|
| 1  | Lal et al. (2021)          | 2021 | Liver, multiple              | KMC Liver, Kumar Dataset             | Attention Mechanism, Encoder Decoder           | 70% Dice Score                  |
| 2  | Liu et al. (2020)          | 2020 | Bladder, Colon, Stomach, Breast, Kidney, Liver, Prostate | MoNuSAC                              | Neural architecture based Spatial & Channel Joint Attention Module | 86% F1 Score                  |
| 3  | Aatresh et al. (2021)      | 2021 | Kidney, Breast               | TNBC                                 | SegNet based attention module                 | 92% F1 Score                  |
| 4  | Ma et al. (2020)           | 2020 | Cervix                       | Herlev Dataset                       | SE-FPM based Self-attention                   | 92% F1 Score                  |
| 5  | Sahasrabudhe et al. (2020) | 2020 | Bladder, Colon, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg                              | Self-Supervised attention module              | 86% F1 Score, 0.53 AJI          |
| 6  | Vahadane et al. (2021)     | 2021 | Bladder, Colon, Stomach, Breast, Kidney, Liver, Prostate | CPM-17, CoNSeP, Kumar               | U-Net, Attention, skip module                 | 81% Dice Score                  |
| 7  | Zhang et al. (2019)        | 2019 | Cervix                       | ISBI-2014 Dataset                    | ResNext, Two path fusion binary tree           | 90% F1 Score                  |
| 8  | Gunesli et al. (2020)      | 2020 | Colon                        | Pathology Department Hacettepe University | FCN                                           | 94% F1 Score                  |
| 9  | Guo et al. (2021)          | 2021 | Bladder, Colon, Stomach, Breast, Kidney, Liver, Prostate | MoNuSeg, TNBC                        | Mask guided attention network                 | 83% F1 Score                  |
| 10 | Zhao et al. (2021)         | 2021 | Bladder, Colon, Stomach, Breast, Kidney, Liver, Prostate | ISBI-2014 Dataset, BNS, MoNuSeg       | U-Net, Attention                             | 72% AJI                        |
| 23 | Han et al. (2022)          | 2022 | Bladder, Stomach, Colorectal, Breast, Kidney, Liver, Prostate | MoNuSeg, CPM-17                      | U-Net, Attention                             | 87% Dice Score                  |
on data and is trainable on fewer data samples. They use contour-aware multi-task learning as an inner model. The multi-task learning model uses U-Net as the backbone for the segmentation task as well as the detection task. The outer of this segmentation model is optimized using model-agnostic meta-learning. Feature communication across both tasks is conducted using a feature fusion and interaction block (FFIB). A contour attention module is used in the decoding phase to further enhance the information in the segmentation task. The model performance is evaluated on two publicly available datasets i.e., MoNuSeg and CPM-17. For 100% dependency on data annotation, the model achieved a dice score of 82% and 87% respectively.

**Critical analysis** In attention based nuclei instance segmentation techniques we conclude that models based on simple attention learning modules resulted in lesser performance accuracy such as encoder decoder based attention mechanism proposed by Lal et al. (2021) and Zhao et al. (2021) having 70% accurate results compared to other advanced attention techniques such as neural architecture based spatial and channel joint attention module by Liu et al. (2020), self supervised attention module as discussed in Sahasrabudhe et al. (2020) and mask guided attention mechanism in Gunesli et al. (2020) yielding performance accuracies from 84 to 86%. Similarly, this has also been observed that ResNext based two path fusion binary tree based attention mechanism technique on ISBI-14 dataset by Zhang et al. (2019), SE-FPM self attention based Feature pyramid module performance on Herlev dataset Ma et al. (2020) and SegNet based attention guided architecture proposed by Aatresh et al. (2021) gave better nuclei instance segmentation results compared to others with a performance accuracy from 90 to 94% F1- Score and stood as top performing attention based instance segmentation techniques.

### 5.2 Glands instance segmentation methods

#### 5.2.1 Handcrafted features extraction

For medical image analysis, traditional handcrafted feature based techniques are more prevalent for segmentation compared to learning based approaches. Table 9 summarize details about features extraction techniques for glands instance segmentation. Classical methods depends heavily on image features including its color, shape and texture primarily. Similarly, for instance segmentation of natural images mostly pipeline comprises of object detection and masking. In 2017, Zarei et al. (2017) proposed a gland segmentation pipeline via integrating classical techniques. Firstly, digitized histology images are constructed using sixteen light wavelengths followed by RGB construction by Principal Component Analysis (PCA). For glands segmentation unsupervised clustering is applied and utilized morphological cleaning operation for small objects removal and then eroded processed gray scale image for further segmentation. Despite being novelty of this technique, generalizability and dataset size variation was the major loop hole. Similarly, Wang et al. (2017) used contour based energy minimization technique for efficiently segmenting glands instances from background. Two level sets are utilized including energy based and region based models. Model divided image into 2 parts, glands with lumens and glands without lumens. Glands with lumens were localized and segmented via edge-based level set. Similarly, glands without lumens stromal were segmented via region based technique. For output prediction results of both these models are combined together. Manivannan et al. (2018) combined both traditional handcrafted multi scale features, with features learned through deep convolutional network trained for mapping images to respective
| S # | Reference                  | Year | Organ   | Dataset          | Technique                                                                 | Performance                  |
|-----|----------------------------|------|---------|------------------|---------------------------------------------------------------------------|------------------------------|
| 1   | Zarei et al. (2017)        | 2017 | Prostate| TMA              | PCA, Clustering, Morphological Operation                                   | 80% Dice Score              |
| 2   | Xu et al. (2017)           | 2017 | Colon   | Warwick QU       | 3-channel Conv-Net, FCN, HED edge detection                               | 83% F1 Score                |
| 3   | Wang et al. (2017)         | 2017 | Endometrial | West China Hospital | Level set edge based energy minimization & region                        | 75% Dice Score              |
| 4   | Manivannan et al. (2018)   | 2018 | Colon   | Warwick QU       | FCN & SVM classifier                                                      | 89% Dice Score              |
| 5   | Rezaei et al. (2019)       | 2019 | Colon   | Warwick QU       | FCN & LinkNet & Local binary pattern                                       | 82% Dice Score              |
| 6   | Graham et al. (2019)       | 2019 | Colon   | GlaS             | FCN & Atrous Spatial Pyramid Pooling                                      | 94% F1 Score                |
| 7   | Gunesli et al. (2020)      | 2020 | Prostate| Pathology Dept, Hacettepe University | Attention Boost, Iterative Multistage Learning                             | 95% F1 Score                |
| 8   | Mei et al. (2020)          | 2020 | Colon   | Warwick QU       | CRF, Morphological Operations, CNN                                        | 79% F1 Score                |
| 9   | Yan et al. (2020)          | 2020 | Liver bone | CRAG             | Weighted Matrix Adversarial Loss                                          | NA                          |
| 10  | Ding et al. (2020)         | 2020 | Colon, Liver | CRAG Warwick QU | TCC-MSFCN Network                                                         | 91% F1 Score                |
| 11  | Xie et al. (2020)          | 2020 | Colon   | CRAG, GlaS       | Pairwise Relational Module, S-Net                                          | 87% Dice Score              |
| 12  | Rastogi et al. (2021)      | 2021 | Colon   | Warwick QU       | U-Net                                                                     | 85% F1                      |
| 13  | Wen et al. (2021)          | 2021 | Colon, Prostate | GlaS dataset, CRAG | Gabor Filter, Attention Module                                             | 83% F1 Score                |
| 14  | Salvi et al. (2021)        | 2021 | Prostate gland | GlaS dataset, CRAG | RINGS, ACM                                                                | 90% Dice Score              |
| 15  | Dabass et al. (2021)       | 2021 | Colon   | GlaS, CRAG       | U-Net, Attention Mechanism                                               | 93% F1 Score                |

The bold text is used to highlight the methodologies which attained highest quantitative performance.
segmentation output. They used structured learning approach for capturing structural information of image e.g (location between glands and neighbouring glands identification as separate instance). These were then used for training support vector machine classifier and is further combined and post processed for segmentation output. Rezaei et al. (2019) preprocessed input data via invariant local binary pattern based classical features processing, extracted features were further passed to LinkNet for glands segmentation training.

5.2.2 Deep learning based glands instance segmentation

Deep neural networks are highly effective at learning distinct features and discriminating generalizable priors from large scale medical image datasets thus making them an essential part of modern AI based systems and yielding excellent resulting on medical imaging tasks. Major advancements in CNN’s has been primarily fueled by novel architecture designs, improved optimization algorithms, open source libraries and availability of GPUs for heavy computation. In 2017 Xu et al. (2017) proposed neural networks glands segmentation algorithm based on image-image prediction model using deep multi channel model for automatic fusion of complex multi-channel information based on regional, local and boundary pixel features. This model reduced heavy feature designs issue due to the use of CNNs. Similarly, alternate channels resulted in better feature learning resulting in better performance for training single scale objects. However, problem with multi stage learning was still there. For incorporating multi scale objects segmentation a minimal information dilated network is proposed by Graham et al. (2019) where for segmenting varying sized glands, maximal information is retained during feature extraction on which atrous spatial pyramid pooling is applied. Final level evaluation of keeping or discarding predictions is done through object level uncertainty score. Considering the computational cost issues in prior approaches a cost efficient and adaptive multi stage attention based learning module is designed by Gunesli et al. (2020) for adaptively learning hard to learn pixels at each stage on given image data without any prior preprocessing via multi stage boosting network for in parallel adaptive learning and pixels correction at the same time by adjusting loss weight for each predicted pixel. A unique boundary adjustment loss function is used for paying focused attention to pixels nearer to boundaries.

Previously designed glands segmentation methods used several deep learning features and auxiliary contour prediction output maps for modeling segmentation tasks. However, they fail to capture complex structural variations in gland images thus resulting in contour imbalance problem due to limited performance. To overcome these problems, Mei et al. (2020) proposed a dense contour-imbalance aware (DCIA) framework including convolutional neural network (DenseNet) and focal loss (FL). In this technique features generated via DenseNet were explored for “optimal” image representation and focal loss function mitigated the contour imbalance problem in the training stage. Finally, for fine tuning predicted confidence maps, post-processing is done via morphological operations and convolutional conditional random fields (ConvCRFs). A single deep learning shape adversarial domain adaptation model for accurate segmentation of glands is proposed by Yan et al. (2020) where a segment level shape similarity measure is used for curve similarity calculation between each annotated boundary and corresponding boundary segment detection, images down sampled at multiple scales were integrated for context enabled global as well as local features training. Ding et al. (2020) proposed a multi scale fully convolutional network and three class classification (TCC-MSFCN) framework for better segmentation approximation. Multi scale architecture extracted varying receptive field features
corresponding to object size. Similarly, for global information loss computation, separate high-resolution branch is included in model. Finally, for accurate segmentation of touching glands, three-class classification based on edge pixels is applied. Rastogi et al. (2021) proposed an encoder decoder based module for better capturing of contextual information via harnessing multi level CNN capabilities as well as features concatenation and features upsampling via skip connections and harnessed the exceptional power of neural networks for capturing contextual information and features concatenation via upsampling. For fine tuning raw predicted samples are processed using morphological opening and closing operators yielding 85% accuracy. Xie et al. (2020) proposed a pairwise relations learning module for enhancing image representation ability by exploiting the semantic consistency between image pairs and transferring learned features to S-Net an encoder decoder based network for improving segmentation. Salvi et al. (2021) proposed a rapid gland identification module for prostate gland segmentation via similar multi-channel algorithm. However, in this approach both traditional and deep learning techniques are exploited and fused together via a hybrid instance segmentation strategy based on stroma detection for accurate detection and delineation of target gland contours. Dabass et al. (2021) with a slight enhancement of dimension wise convolution combined with atrous spatial pyramid pooling for improved encoder decoder architecture and multi task learning based on region and boundaries extraction along with feature learning, diminishing gradient issue is alleviated as well. Similarly, Wen et al. (2021) proposed Gabor encoder based module for texture enabled feature learning through cascaded squeeze parsing to Bi-Attention mechanism for capturing both channel and spatial information at multiple scales followed by class balancing.

Critical analysis

In this section deep learning based glands instance segmentation proposed techniques are covered where a large number of the authors have trained and tested on CRAG and GlaS datasets while a few of them have yielded experimental results on Prostate glands as well. One of the major limitation observed in majority of hand-crafted segmentation techniques is lack of reliability and robustness. Majority of the groups focused on detection of void space inside gland and using color information followed by actual image processing technique, however they failed to detect small sized glands and partially filled ones as well. Moreover performance degraded in case of color variation as well (Zarei et al. 2017; Wang et al. 2017). Amongst all glands segmentation reviewed techniques we found that FCN based Atrous spatial pyramid pooling technique by Graham et al. (2019) outperformed all previous state of the art proposed minimally tuned classical algorithms through a large margin, producing better segmentation outputs over all coverage thresholds.

6 Discussion and future prospects

This is the first study that reviewed both nuclei and glands instance segmentation techniques evolved during last 5 years for multiple organs included but not limited to liver, kidney, prostate, bladder, colon, stomach, lung, and brain cancer. This survey covers both handcrafted features extraction methods as well as deep convolutional neural networks based techniques emerged during this time span. Overall, This has been observed that deep learning methods outperformed all traditional segmentation methods. Best performing model was attention based U-Net techniques covering both local as well as global features mapping and multi scale refinement. Region proposal based Mask-RCNN accuracy also reported upto the mark accuracy with different optimization parameters and loss functions.
For MoNuSeg dataset, Mask-RCNN models performance was found even better than U-Net. Transfer learning based feature pre-training also yielded better results and model trained independent of tissue type for nuclei and glands instance segmentation respectively. This review summarises major evolution and advancements adopted by researchers in model designing for the task of instance segmentation. Overall, In histology whole slide image analysis, base techniques provide novel architectures for clinical workflows automation via automatic WSI feature extraction that serves a pivotal role in diagnosis, treatment, and survival prediction of various lethal diseases. Till date, advancements in digital pathology has automated complete histology cancer grading process, mitosis detection, cancer sub-typing, tumor classification & segmentation.

This all has been made possible via deep learning-based networks that enabled training possible for large scale highly varying whole slide images at contrasting magnifications. Rapid advancements in the field of oncology will lead novel innovations and insights in tumor nuclei instance segmentation and feature extraction thus yielding better cancer treatment selection methods. One such way would be AI-driven diagnostic techniques leveraging deep leaning architectures.

Encoder Decoder based U-Net techniques gave excellent results for biomedical images segmentation. Despite its great performance model struggles during classification of closely touching instances. Symmetrical network architectures further possess an opportunity to modify model structure and improve performance accuracy. Since, initial U-Net architecture followed a typical CNN based structure, having repetitive convolution, activation, and pooling layers for feature maps calculation.

However, with the rising complexity of data, training DCNNs resulted in further network architecture advancement and leads towards other novel networks including HoVer-Net Graham et al. (2019), SegNet Aatresh et al. (2021), ResUNet Majdi et al. (2020), ResNet Zeng et al. (2019), PR-Net Xie et al. (2020), ResNext Zhang et al. (2019), OS-Net Lee et al. (2019) and various others. These comparatively new approaches have embarked excellent state-of-the-art instance segmentation results with better feature extraction thus minimizing vanishing gradient problem, and improving network convergence. Performance boost primarily resulted by modification from base network architecture to modified architectures, either through replacement of initial skip connection layers with longer ones or by layers addition in base U-Net model for deeper neural network. Symmetrical network architectures further possess an opportunity to modify initial network structure and improve performance accuracy.

Figure 14 shows cumulative techniques occurrence in research articles reviewed for this paper.

Multistage learning architectures have attained huge success in domain of medical imaging especially segmentation tasks. However information loss at middle layers along with inconsistent features learning is the major common drawback of these architectures. Another primary limitations observed is often times they leads to a redundant information usage when similar low level feature are repeatedly extracted several times at multiple scales.

Similarly, whole slide images are usually regarded as texture images while fixed encoder decoder based architecture of U-Net does not serve as an appropriate model for texture based learning models because of its inability to extract features at different scales and orientations. It has been observed that malignant tumors have a particularly higher growth rate compared to benign and greater density as well causing nuclei overlapping or occlusion during squeezing operation. One important challenge is sufficient networks training to yield good generalizations for hard-to-learn pixels. A typical group of such hard-to-learn
pixels are boundaries between instances. In spite of the huge success of FCNs trained on very large datasets, training becomes difficult when small quantities of annotated data are available and when pixels of background and foreground classes are highly imbalanced. In such cases, without further adjustments, network tend to yield poor generalizations for pixels of a minority class as well as for hard-to-learn pixels. Similarly, in some approaches for long-range, features dependencies were not modeled efficiently thus leading to non-optimal discriminative representation of features.

Two stage learning based Mask-RCNN network is proposed for instance level annotations generation. In first stage it proposes class probability maps while its CNN backbone provides input feature map which is then fed in both regression as well as classification layer. Regression layer predicts region proposals while classification layer predicts object existence probability within the region proposal. Similarly, second stage of Mask-RCNN utilized generated region proposals for object classification, bounding box prediction and final segmentation.

Region of interest (RoI) alignment was introduced with Mask-RCNN. It incorporated bilinear interpolation for floating point values calculation from sampling points. It resulted in reduced computational time, by taking RoI defined feature maps and its scaling to fixed sized patches. Mask-RCNN variants output bounding box coordinates, objects classification and segmentation map for each object instance.

Although, R-CNN, Faster-RCNN and its other variants have achieved good performance in general target detection tasks by combining semantic segmentation and target detection, but their performance in histology tasks such as abnormal cell detection was still not as per expectations causing issues like over and under segmentation which later yielded incorrect estimation of the nuclear density, size and morphology (Fraz et al. 2020). Another primary limitation of the region proposal based methods is their
difficulty in merging instances prediction while processing neighbouring tiles e.g. in a condition when a nuclei sub segment at boundary is assigned a label this thing needs to be ensured that rest of the nuclei part present in neighbouring patch should be assigned same label.

Similarly, deep learning models also lacks theoretical understanding thus leading to degraded performance when limited training data is provided (Shaban et al. 2020).

Pre-deep learning techniques performed exceptionally well for small amount of data where training and testing inputs were taken from single feature space. Handcrafted segmentation techniques primarily used in majority of the approaches were intensity based thresholding, morphological operations including erosion & dilation, marker controlled watershed transform, active contour models, with machine learning classifiers such as RVM (Tipping 1999), SVM (Zhang 2012), KNN (Guo et al. 2003) clustering and other supervised classification methods.

Thresholding and morphological transformations are usually regarded as basic image processing operations but for nuclei having complex image background, segmentation via these operations gets difficult since thresholding techniques such as Otsu usually works with the assumption of nuclei having distinct intensity and thus fails in most cases where tumor cells display high level of variability in chromatin appearance (Shaban et al. 2019). Watershed performance was fine given target locations but in all other scenarios shows poor performance, since it considers a relatively homogeneous nuclei or gland appearance and a pre requisite of initially detected seed points before segmentation (Bashir et al. 2020).

Active contour models or level Sets deals with change in spatial temporal relations (i.e combination of image and shape features inside nuclei) but fails in handling boundary events and alike watershed this technique is also dependent on initialization of good seed points. Thus despite being in use for long, there are still problems in segmentation results of these techniques because they are based on simple assumption about the nucleus appearance characteristics including low intensity or circular borders which may not be true in all cases (e.g. some nuclei exhibits high intensity and irregular shapes (Zhang et al. 2017) thus yielding low accuracy or poor precision. Similarly, clustering and graph-based methods have been used too, but their computationally expensive nature and shallow feature learning consideration makes them a misfit for training large scale data (Graham and Rajpoot 2018). Similarly, Radiating gradient vector flow (RGVF) snake and graph cuts techniques were used for fine segmentation but these do not involve inherent shape constraints for the segmented boundary, thus resulting in irregular nucleus boundary.

For sparse data conditions, transfer learning techniques can be utilized using pre-trained weights of a comparatively larger model for training similar smaller dataset networks given the domain similarity checks.

A training limitation in the majority of datasets is the small amount of available data for learning, validation, and further testing due to the limited number of open source datasets. Similarly, data collection and preprocessing stages during sample collections such as manual H&E staining or varying slide scanners at times result in color inconsistency (Semedo et al. 2018) and unclear nuclear boundaries and this drawback later affects the entire model training and testing stages. Future work involves expanding tissue types and the number of images included in the dataset. Adversarial learning-based generative adversarial networks (GANs) serve as dual learning techniques that first generate synthetic whole slide images followed by a segmentation network for data training. GANs are considered a promising way of data synthesis, that generates H&E stained synthetic histology images yielding ground truths from learned features from training data.
7 Conclusion

Segmentation of nuclei and glands instances from histology images is the focus of attention because of its vitality in biomedical and biological applications. Automated glands and nuclei segmentation are fundamental to chronic disease diagnosis, survival prediction, phenotype classification, feature extraction, and cell tracking. It also has significance in cancer diagnosis, grading, and analysis as they are highly dependent on the quality of nuclei segmentation. Despite the significant advancement in automated segmentation, the separation of large clusters of nuclei, irregular glands structure, and outlining their boundaries with high precision and speed is still considered a challenge. Apart from that, researchers are still lacking a generalized benchmark solution for all types of nuclei and glands instance segmentation from distinct histology images under various conditions. Due to the emergence of Convolutional neural networks (CNNs) the classical hand-crafted feature extraction techniques are replaced by CNN. This review article covers the most recent approaches of the last five years for nuclei and glands instance segmentation, along with major publicly available datasets and a summary of the grand challenges held specific to this task. The review compiled deep learning computational approaches, and hand-crafted morphological feature-based approaches for evaluating the advantages and drawbacks of each segmented technique. The hindrances in the nuclei instance segmentation such as varying staining impacts, insufficient data causing overfitting, disparate nuclei and glands structure, and models specificity to a single image set are discussed.

Data availability  Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Conflict of interest  The authors declare no conflict of interest.

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