Evidential Uncertainty-guided Mitochondria Segmentation for 3D EM Images

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Abstract
Recent advances in deep learning have greatly improved the segmentation of mitochondria from Electron Microscopy (EM) images. However, suffering from variations in mitochondrial morphology, imaging conditions, and image noise, existing methods still exhibit high uncertainty in their predictions. Moreover, in view of our findings, predictions with high levels of uncertainty are often accompanied by inaccuracies such as ambiguous boundaries and amount of false positive segments. To deal with the above problems, we propose a novel approach for mitochondria segmentation in 3D EM images that leverages evidential uncertainty estimation, which for the first time integrates evidential uncertainty to enhance the performance of segmentation. To be more specific, our proposed method not only provides accurate segmentation results, but also estimates associated uncertainty. Then, the estimated uncertainty is used to help improve the segmentation performance by an uncertainty rectification module, which leverages uncertainty maps and multi-scale information to refine the segmentation. Extensive experiments conducted on four challenging benchmarks demonstrate the superiority of our proposed method over existing approaches.

1 Introduction
Positioned at the heart of cellular metabolism, mitochondria serve a key role in powering life through massive and varied metabolic functions (Annesley and Fisher 2019; Bock and Tait 2020). Thanks to the Electron microscopy (EM) technique, high-resolution images of mitochondria and other cellular structures are now available, making them a valuable resource for studying cellular biology and connectomics (Casser et al. 2020; Wei et al. 2020; Lucchi et al. 2011). The utilization of deep learning algorithms in mitochondria segmentation has shown significant progress, as demonstrated by state-of-the-art (SOTA) methods (Luo et al. 2021; Peng, Yi, and Yuan 2020; Peng and Yuan 2019; Yuan et al. 2021). Most of these techniques employ the U-Net (Ronneberger, Fischer, and Brox 2015) architecture or its variations (Casser et al. 2020; Mekuč et al. 2020) to address the unique challenges posed by EM image segmentation. Recently, transformer and self-attention (Franco-Barranco, Muñoz-Barrutia, and Arganda-Carreras 2022) have also shown advantages in mitochondria image segmentation (Franco-Barranco, Muñoz-Barrutia, and Arganda-Carreras 2022; Yuan et al. 2020, 2021).

Despite the advancements made by deep learning (DL) models, they remain plagued by considerable uncertainty within their predictions. This uncertainty originates from various sources (Guo et al. 2017), including out-of-domain inputs, data quality issues, and nuances of the training conditions. Within the EM images, this issue is exacerbated by the variations in data quality caused by artifacts or deformations during the image acquisition process. Consequently, DL models might produce overconfident but erroneous predictions. These predictions output by the current mitochondria segmentation framework impose limitations on the practical applicability of these models, particularly in 3D cell reconstruction and subsequent functional analysis. Therefore, it makes uncertainty estimation essential to prevent potentially disastrous decisions based on segmentation results. This brings three key questions: How to represent the uncertainty in EM image segmentation? What methodologies can be employed to accurately estimate this uncertainty? How can this uncertainty estimation be harnessed to enhance the segmentation performance?

For the representation of the uncertainty, there are two
main types of uncertainty in DL method (Kendall and Gal 2017): epistemic (data) uncertainty arising from the inherent randomness or variability in the data itself, and aleatoric (model) uncertainty arising from the limitations and lack of knowledge in the model to learn the data. This paper, along with many previous works, focuses on aleatoric uncertainty.

For the estimation of aleatoric uncertainty, three mainstream methods are now available: dropout-based (Gal and Ghahramani 2016; Mobiny et al. 2021), ensemble-based (Lakshminarayanan, Pritzel, and Blundell 2017), and evidential-based (Sensoy, Kaplan, and Kandemir 2018) methods. Among them, evidential-based methods, relying on the Dempster-Shafer Evidence Theory (Dempster 1968), have shown more robust results with lower computational costs (Zou et al. 2022) compared to the other two methods. Notably, while evidential-based methods have been used in segmenting natural and medical images, they have not been explored for EM images. In this paper, we intend to use evidential-based methods to estimate the aleatoric uncertainty for EM image segmentation.

Then, for the utilization of the estimated uncertainty, previous studies have explored various approaches, such as generating pseudo labels for unlabeled data (Peng, Yi, and Yuan 2020) and incorporating uncertainty-based weights to fuse predictions from diverse sources (Basir and Yuan 2007). However, in EM segmentation tasks, limited attention has been given to the potential of directly rectifying errors using uncertainty information. During our mitochondria segmentation experiments, as an example illustrated in Figure 1, we observe that the areas of high uncertainty are prone to erroneous segmentation predictions. By using the uncertainty estimation (d) to rectify the original probabilistic prediction (c), the performance of the rectified prediction (e) demonstrated a notable enhancement, with a 7.5% improvement in Dice and a 14.3% enhancement in mAP. Building upon these observations, we advocate harnessing the estimated uncertainty as a strategy to effectively rectify segmentation errors.

In this study, we introduce a novel segmentation method, named Evidential Uncertainty-guided Mitochondria Segmentation for 3D EM Images (EUMS-3D), which is illustrated in Figure 2. EUMS-3D enables both uncertainty estimation and segmentation rectification by taking advantage of evidential deep learning (Sensoy, Kaplan, and Kandemir 2018). Specifically, EUMS-3D initially predicts the probabilities of semantics (inner part of the objects) and boundaries for the mitochondria by the backbone network. Then, an Evidential Estimation Module (EEM) is incorporated to model the uncertainty at the voxel level for all probability predictions. Subsequently, these predictions are rectified through the attention mechanism-based Uncertainty Rectification Module (URM), which integrates the uncertainty information from EEM and the multi-scale information from the designed Feature Aggregation Module (FAM). Our experimental results demonstrate the effectiveness of incorporating evidential uncertainty estimation to enhance 3D mitochondria segmentation, as EUMS-3D outperforms existing methods on four benchmark datasets: MitoEM-R (Wei et al. 2020), MitoEM-H (Wei et al. 2020), Kasthuri++ (Casser et al. 2020), and Lucchi++ (Casser et al. 2020). Ablation studies further confirm the contributions of each designed module in improving segmentation performance.

In summary, the contributions of this paper are as follows.

- To our best knowledge, this is the first evidential uncertainty-guided 3D mitochondria segmentation network for EM images.
- The uncertainty rectification module is proposed to enhance the segmentation performance by leveraging estimations of associated uncertainty and incorporating multi-scale features using the attention mechanism.
- Effectiveness of our method is verified by extensive experiments on four challenging benchmarks and on different backbone models.

### 2 Related works

#### Mitochondria Segmentation

Recently, the field of 3D mitochondria segmentation has witnessed significant advancements. Numerous approaches have been proposed to tackle this challenging task. Traditional methods (Jorstad and Fua 2015; Lucchi, Li, and Fua 2013; Vazquez-Reina et al. 2011; Lucchi et al. 2012) often rely on manual or semi-automatic techniques, struggling with the complexity and variability of mitochondria structures in large-scale datasets. In response to these limitations, DL approaches have gained substantial attention. Convolutional neural networks and their variants have shown remarkable success in various image segmentation tasks. Recent studies have explored the adaptation and development of deep learning architectures for 3D mitochondria segmentation, including the use of U-Net (Ronneberger, Fischer, and Brox 2015), Mask R-CNN (Liu et al. 2018), and their 3D extensions. Additionally, advanced techniques such as attention mechanisms (Franco-Barranco, Muñoz-Barrutia, and Arganda-Carreras 2022), and generative adversarial networks (GANs) (Zhang et al. 2022) have also been investigated to enhance the accuracy and robustness of mitochondria segmentation.

#### Uncertainty estimation methods

Researchers have introduced a spectrum of uncertainty estimation techniques. These include Bayesian neural network (BNN) (Hinton and Van Camp 1993; MacKay 1992), ensemble-based (Lakshminarayanan, Pritzel, and Blundell 2017), dropout-based (Gal and Ghahramani 2016; Lakshminarayanan, Pritzel, and Blundell 2017), and evidential-based methods (Sensoy, Kaplan, and Kandemir 2018). Specifically, EUMS-3D initially predicts the probabilities of semantics (inner part of the objects) and boundaries for the mitochondria by the backbone network. Then, an Evidential Estimation Module (EEM) is incorporated to model the uncertainty at the voxel level for all probability predictions. Subsequently, these predictions are rectified through the attention mechanism-based Uncertainty Rectification Module (URM), which integrates the uncertainty information from EEM and the multi-scale information from the designed Feature Aggregation Module (FAM). Our experimental results demonstrate the effectiveness of incorporating evidential uncertainty estimation to enhance 3D mitochondria segmentation, as EUMS-3D outperforms existing methods on four benchmark datasets: MitoEM-R (Wei et al. 2020), MitoEM-H (Wei et al. 2020), Kasthuri++ (Casser et al. 2020), and Lucchi++ (Casser et al. 2020). Ablation studies further confirm the contributions of each designed module in improving segmentation performance.

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- Effectiveness of our method is verified by extensive experiments on four challenging benchmarks and on different backbone models.
Although been widely used, the ensemble-based methods require training multiple models, leading to high computational costs, and dropout-based methods may produce inconsistent outputs (Mobiny et al. 2021). Instead, the evidential-based method has shown more reliable performance in uncertainty estimation. Moreover, they demonstrate more robust results with lower computational costs compared to ensemble-based and dropout-based methods (Yager and Liu 2008). Although they have been utilized in natural and medical image segmentation tasks, their application in EM image segmentation, particularly for mitochondria segmentation, requires further investigation.

3 Method

Preliminary of Evidential Uncertainty Estimation

The evidential uncertainty estimation method (EDL) is a generalization of Bayesian theory to subjective probability. As Figure 2 illustrates, it assigns belief masses to each possible class label, and then the belief distribution of DST in the framework can be formalized as a Dirichlet distribution by Subjective Logic (SL) (Dempster 1968; Jøsang 2016).

Specifically, if we treat the segmentation as a voxel-wise $K$-class classification problem, for a voxel $i$, the EDL regards the classification task as giving a multinomial subjective opinion in a $K$-dimensional domain $\{1, \ldots, K\}$. The subjective opinion is expressed as a triplet $\omega = (b, u, a)$, where $b = \{b_1, \ldots, b_K\}$ is the belief mass, $u$ represents the uncertainty, and $a = \{a_1, \ldots, a_K\}$ is the base rate distribution. For any $k \in \{1, 2, \ldots, K\}$, the probability mass of a multinomial opinion is defined as $p_k = b_k + a_k u$. To enable the probability meaning of $p_k$, i.e., $\sum_k p_k = 1$, the base rate $a_k$ is typically set to $1/K$ and the subjective opinion is constrained by $u + \sum_k b_k = 1$. For a $K$-class setting, the probability mass $p = [p_1, p_2, \ldots, p_K]$ is assumed to follow a Dirichlet distribution parameterised by a $K$-dimensional Dirichlet strength vector $\alpha = \{\alpha_1, \ldots, \alpha_K\}$. The total strength of the Dirichlet is defined as $S = \sum_{k=1}^K \alpha_k$.

According to the evidence theory, the term evidence is introduced to describe the amount of supporting observations for classifying the voxel $i$ into a class. Let $e = \{e_1, \ldots, e_K\}$ be the evidence for $K$ classes, $e_k = \alpha_k - 1$. In this way, the Dirichlet evidence can be mapped to the subjective opinion by setting the following: $b_k = \frac{\alpha_k}{S}$, and $u = \frac{K}{S}$.

Therefore, we can see that if the evidence $e_k$ for the $k$-th class is predicted, the corresponding expected class probability can be rewritten as $p_k = \frac{\alpha_k}{S}$, and the predictive uncertainty $u$ can be determined after $\alpha_k$ is obtained.

Overview of the architecture

Here we introduce the evidential uncertainty-based mitochondria segmentation in 3D EM Images (EUMS-3D) method. As illustrated in Figure 2, EUMS-3D consists of four modules: the Backbone Network for feature learning, the Feature Aggregation Module (FAM) for multi-layer feature combining, the Evidential Estimation Module (EEM) for the uncertainty estimation, and the Uncertainty Rectification Module (URM) for rectifying predictions. In the following sections, we describe the four modules, respectively.

Backbone Network

Recently, U-Net and its variants (Siddique et al. 2021) have shown remarkable performance in segmenting biomedical images. Building on this success, transformer-based architectures, originally popularized in natural language processing (NLP) tasks, have emerged as promising alternatives. In our work, we have modified the skip-connected encoder-decoder architecture known as 3D UX-Net (Lee et al. 2023) as our backbone network to predict the probability of semantic and boundary for each input 3D patch simultaneously. As a result, the output of the backbone is the concatenation of the two probabilistic predictions, denoted as $P \in$...
 voluptuous ConvNet using hierarchical Transformers, demonstrates stable voxel segmentation performance across various challenging public datasets. It has proven its effectiveness in handling complex image segmentation tasks. It is essential to highlight that our framework is highly flexible, enabling designers to freely choose different backbones, such as Res-Unet3D (Li et al. 2022) and TransBTS (Lin et al. 2022), among others. This adaptability further enhances the versatility and applicability of our approach.

**Feature Aggregation Module**

Mitochondria in EM images are often with small size and ambiguous boundaries, necessitating details with higher resolution for enhanced differentiation. To effectively handle small objects while preserving the lightweight attribute of the system, we introduce the Feature Aggregation Module (FAM). FAM incorporates a multi-layer aggregation mechanism (Zheng et al. 2021), where the intermediate output of the decoder is concatenated together to produce a mask feature map \( f_{	ext{agg}}(x) \). To better leverage the information from the original resolution, after upsampling the mask feature map to the original resolution, we concatenate it with the original image and use another 3D convolution to fuse the information and generate the final mask.

**Evidential Estimation Module**

Based on the evidential uncertainty modeling method illustrated in Section 3.1, we facilitate the quantification of classification uncertainty by jointly modeling the probability maps \( P \) output by the backbone model. Figure 2 illustrates the process, where the output of the backbone network \( f(x) \) undergoes an activation function layer (softplus) to ensure non-negative values and gain the evidence. Subsequently, the subjective logic offers a belief mass function, enabling designers to freely choose different backbones, such as Res-Unet3D (Li et al. 2022) and TransBTS (Lin et al. 2022), among others. This adaptability further enhances the versatility and applicability of our approach.

**Uncertainty Rectification Module**

To refine the predictions \( P \), the Uncertainty Rectification Module (URM) leverages both the uncertainty estimation \( U \) and the aggregated multi-layer features \( f_{	ext{agg}}(x) \) as depicted in Figure 3. Since predictions with high uncertainty are prone to erroneous results, URM first divides the uncertainty maps \( U \) and probabilistic prediction \( P \) into certain and uncertain by a threshold \( \tau \). Then, for the voxel \( i \), two strategies are applied for the \( k \)-th class prediction \( p_{ik} \) to get the rectified prediction \( p_{ik}^{\text{rec}} \) by using the \( u_i \in U \), which is the uncertainty estimation of the voxel \( i \).

a) **Certain case** (\( u_i \leq \tau \)): the uncertainty \( u_i \) is regarded simply as a weight: \( p_{ik}^{\text{rec}} = p_{ik} \cdot (1 - u_i) \).

b) **Uncertain case** (\( u_i > \tau \)): when the prediction of the model is uncertain, mere reliance on the model’s self-contained prediction may not suffice for effective rectification. To address this challenge, an approach involves incorporating contextual information from the surrounding voxels to aid in decision-making, thereby preventing over-correction. To achieve this, the segmentation prediction is subjected to a convolutional operation with a kernel size of \( 7 \times 7 \times 7 \), resulting in \( P_{\text{conv}} \). \( p_{ik}^{\text{conv}} \) is the probability that the \( i \)-th voxel is classified into the \( k \)-th class output, which is then modulated by \( u_i \) as a weighting factor, leading to the refined prediction denoted as \( p_{ik}^{\text{rec}} = p_{ik}^{\text{conv}} \cdot (1 - u_i) \).

Furthermore, to mitigate potential overly arbitrary modifications caused by uncertainty estimates, we introduce a mask decoder that incorporates multi-scale information, adapted from (Cheng et al. 2022). As illustrated in Figure 3, the attention mechanism is applied to leverage the aggregated multi-layer features obtained from FAM. This process leads to the prediction of new uncertainty-aware masks, denoted as \( P_{\text{final}} \). Subsequently, we utilize the watershed (Shafarenko, Petrou, and Kittler 1997) algorithm to generate instance results from the uncertainty-aware masks.

**Loss Functions**

Three loss functions are utilized in voxel-wise manner. First, we use the EDL loss function modified from cross-entropy loss proposed in (Sensoy, Kaplan, and Kandemir 2018; Zou et al. 2022) for the outputs of EEM. For voxel \( i \), \( y_{ik} \) and \( p_{ik} \) are the label and predicted probability for class \( k \). \( \psi(\cdot) \) denotes the digamma function. \( S_i \) is the total strength of a Dirichlet distribution parameterized by \( \alpha_k \).

\[
\mathcal{L}_{\text{EDL}}^i = \frac{1}{K} \sum_{k=1}^{K} y_{ik}(\psi(S_i) - \psi(\alpha_{ik})). \tag{1}
\]

Second, the KL divergence loss function is introduced, where \( \Gamma(\cdot) \) is the gamma function. \( \bar{\alpha}_{ik} = y_{ik} + (1 - y_{ik}) \odot \alpha_{ik} \) denotes the adjusted parameters of the Dirichlet distribution, which aims to ensure that ground truth class evidence is not mistaken for 0.

\[
\mathcal{L}_{\text{KL}} = \log \bigg( \frac{\Gamma(\sum_{k=1}^{K} \bar{\alpha}_{ik})}{\Gamma(K) \sum_{k=1}^{K} \Gamma(\bar{\alpha}_{ik})} \bigg) + \sum_{k=1}^{K} (\bar{\alpha}_{ik} - 1) \psi(\bar{\alpha}_{ik}) - \psi(\sum_{k=1}^{K} \bar{\alpha}_{ik}) \bigg), \tag{2}
\]
### Experimental Results

**Evaluation on MitoEM.** The quantitative results highlight EUMS-3D (3D UX-Net) superior performance in precise mitochondria segmentation. Across both datasets, it shows the highest scores for both Dice and mAP, showcasing its excellent performance in accurately segmenting mitochondria. Specifically, it achieved approximately 84.5%/90.1% (Dice/mAP) on the human dataset and 89.0%/92.8% on the rat dataset, respectively. Notably, the comparison between Transformer-based and ConvNets-based methods indicates no significant difference in effect, while our EUMS-3D achieves SOTA performance. Several segmentation examples are presented in Figure 4, highlighting our method’s proficiency in capturing the morphology of mitochondria.

**Evaluation on Kasthuri++ and Lucchi++.** Considering the relatively smaller size of the two datasets, the segmentation boundary plays a critical role in influencing the scores. Remarkably, our method EUMS-3D (3D UX-Net) achieves a performance of 97.2% in Dice coefficient and 93.1% in mAP.
mAP on the Kasthuri++ dataset. Similarly, on the Lucchi++ dataset, the method achieves a Dice score of 93.7% and an mAP score of 95.3%, effectively closing the gap toward human-level benchmarks. The visual illustrations of segmentation instances, elucidated in Figure 4, reinforce and substantiate the prowess of our approach, adeptly and accurately refining the mitochondrial boundaries.

Exploring Uncertainty Estimation Methods

To further investigate the effectiveness of different uncertainty estimation methods, we compare the evidential-based method with two other methods, namely dropout (Mukhoti et al. 2021) and ensemble (Lakshminarayanan, Pritzel, and Blundell 2017) by replacing the uncertainty estimation module. All reported results are based on the 3D UXNet backbone model, and we use the two MitoEM datasets for evaluation. The Dice and mAP scores of the three methods are shown in Table 2. It indicates that the evidential uncertainty estimation method outperforms the other methods with at least 8% improvement in mAP for the two MitoEM datasets.

Besides, we compare the calibration performance across the three methods. As the estimation of uncertainty plays a key role in refining segmentations, it becomes imperative to possess a well-calibrated model. Such a model should

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**Table 2:** Performance of different uncertainty estimation modules on MitoEM-R and MitoEM-H datasets.

<table>
<thead>
<tr>
<th>Dataset / Metric</th>
<th>MitoEM-H</th>
<th>MitoEM-R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dice</td>
<td>mAP</td>
</tr>
<tr>
<td>dropout</td>
<td>0.835</td>
<td>0.873</td>
</tr>
<tr>
<td>ensemble</td>
<td>0.839</td>
<td>0.880</td>
</tr>
<tr>
<td>evidential</td>
<td><strong>0.845</strong></td>
<td><strong>0.901</strong></td>
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be confident in its predictions when being accurate, and be uncertain about inaccurate ones (Houlsby et al. 2011). The evaluation metrics are proposed in (Mobiny et al. 2021), including negative predictive value (NPV), true positive rate (TPR), and uncertainty accuracy (UA). Higher values indicate better calibration of the model, which was dependent on the uncertainty threshold. As shown in Figure 5, the evidential-based method has the highest scores across three metrics. This validates that the evidential-based method could be more effective to rectify the erroneous predictions.

### Ablation study

**Backbone Models.** We compare various backbone networks to investigate their influence on the efficacy of our methodology. The results demonstrate the improvements achieved by our approach across two MitoEM datasets when applied to different backbones, including two ConvNets-based (ResUnet3D, HIVE-Net) and three Transformer-based (TransBTS, SwinUNETR, 3D UXNet). The results in Table 1 and Figure 6 indicate the performance augmentation exhibited by all five original backbone models. Notably, the performance of SwinUNETR attained a commendable 5.6% increase in Dice score on the Lucchi+++ dataset. Additionally, the 3D UX-Net exhibited a noteworthy 6.6% improvement in Dice score on the MitoEM-H dataset. It should be highlighted that our proposed module is lightweight to gain performance enhancement by adding only 2.2M parameters.

**URM and FAM.** To investigate the effectiveness of the two modules, we conducted an ablation study by training the network without the two modules, denoted as w/o URM and w/o FAM. The quantitative results presented in Table 3 demonstrate that leveraging the URM with FAM can improve performance by effectively utilizing multi-scale information and uncertainty estimation to correct segmentation errors. Specifically, it shows an increment of 2.2% in mAP score across both datasets with the URM, and 1.4% in mAP with FAM. Several visualization results are shown in Figure 4, the uncertainty map has high sensitivity in contour areas. Particularly for regions with morphological similarities to mitochondrial structures that are not annotated in the ground truth, the model may make mistakes in these zones without URM. By using URM, the mistakes are effectively rectified. We also use dilated convolutions (dilation rate of 2) to replace 3D convolutions. It shows a slight decrease by using Dilated Conv compared to original 3D Conv. This marginal decrease may suggest that the broader receptive field potentially introduces additional noise.

**Parameter in URM.** In URM, we use a threshold $\tau$ to divide the uncertainty estimation and followed by the certain and uncertain strategies. To explore the influence of $\tau$, we train the model using various threshold values $\tau = 0, 0.25, 0.5, 0.75, 1$ on MitoEM-R dataset, respectively. Notably, when $\tau = 0$, URM treats all predictions as uncertain case, whereas $\tau = 1$ corresponds to certain case. The results in Table 4 demonstrate that the best performance is achieved when $\tau$ is set to 0.5, indicating that dividing the predictions by uncertainty can help improve segmentation performance.

### 5 Conclusion

This research, for the first time, presents a novel 3D instance segmentation method for trustworthy segmentation of mitochondrion on EM images. To our best knowledge, for the first time, we employ evidential-based uncertainty estimation and neighborhood information to modify segmentation outcomes and generate reliable fusion. We conduct comprehensive experiments on three benchmark datasets to validate the efficacy of our approach in improving segmentation results and uncertainty estimation.
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References


