Deep Learning based Method for Assessing the Phases of Estrus Cycle in H&E Stained Sections of Wistar Rat Uterus

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Introduction

- Determining the phase of the estrus cycle accurately and consistently helps in understanding interference of chemical entities with the female reproductive function of animals during pre-clinical studies. The approach of ascribing phases to the animals based on the evaluation of a synchronous system that includes the vagina and uterus is widely accepted. In addition to our novel work on Deep Learning-based estrus staging method using vaginal histopathology [1], an effort has been made to identify and quantify the morphological changes in the uterus across different phases of the estrus cycle.
- A Deep Learning-based [2] algorithm is proposed for an accurate identification of 4 phases, Proestrus, Estrus, Metestrus and Diestrus, of the estrus cycle using whole slide images (WSI) of conventional hematoxylin and eosin-stained sections of Wistar rat uterus.

Objectives

A. To generate a novel Deep Learning algorithm to determine all the stages of the estrus cycle in the WSI of H&E-stained paraffin embedded sections of Wistar rat uterus using a pre-defined morphological criteria.
B. To assess the performance of the proposed algorithm by correlating with the staging provided by the veterinary pathologist.

Materials

A. Leica SCN400 & Nanozoomer XR (Hamamatsu) scanners for image acquisition.
B. Training dataset of 60 H & E-stained WSI of Wistar rat uterus.
   - For the segmentation of various parameters, a total of 2600 patches of size 512x512 each, captured at 10x magnification were selected from the WSIs. The parameters like degenerated cell, mitotic figures, lining epithelial, and glands were labeled manually under the guidance of expert pathologists.
   - For the classification of the stages, the ground truth labels for training the Random Forest classifier was prepared as a consensus of the stage labels from three pathologists to tackle the inter-observer variability.
C. Test dataset of 100 H & E-stained WSI of Wistar rat uterus.
   - The ground truth labels for the validation were prepared in a similar manner as the training dataset described in B.

Methods

A. The pre-defined criteria [3] used to identify and classify the stages of the estrus cycle in uterus were:
   - Proestrus: Dilatation of uterine lumen (mild to severe); vacuolar degeneration of luminal epithelium and/or in glands – within normal limits to minimal; Thickness of luminal epithelium is usually low compared to luminal epithelium in the estrus and metestrus stage.
   - Estrus: Vacuolar degeneration/necrosis (mild to severe) in glands and/or luminal epithelium; Dilatation of uterine lumen - present or absent; Increase in thickness of luminal epithelium.
   - Metestrus: Vacuolar degeneration and increased mitosis in the glands and/or in luminal epithelium; Dilatation of uterine lumen - absent; Increase in thickness of luminal epithelium may be present.
   - Diestrus: Small, slit-like uterine lumen, presence (minimal) or absence of vacuolar degeneration in the glands and/or luminal epithelium; Thin luminal epithelium.

B. The proposed algorithm contains two modules:
   - For the segmentation task, the segmented parameters were Degenerated cells, Mitotic figures, Luminal epithelium, Glands, and Uterine lumen determined by training a variant of UNet-based [4] Deep Learning architecture (see Fig 1) on the tiles from the training dataset. Data augmentation was used to create a generalized model, which could accurately segment unseen data having different stain intensity.
   - For the classification task, a feature set of eight dimensions was prepared from the segmented output (see Fig 2) based on the quantity in terms of the percentage area with respect to the organ tissue. The features were used to train a Random Forest classifier that provided the output as one of the four stages of the estrus cycle.

C. The algorithm was validated on 100 WSI and the result is verified by the pathologist.

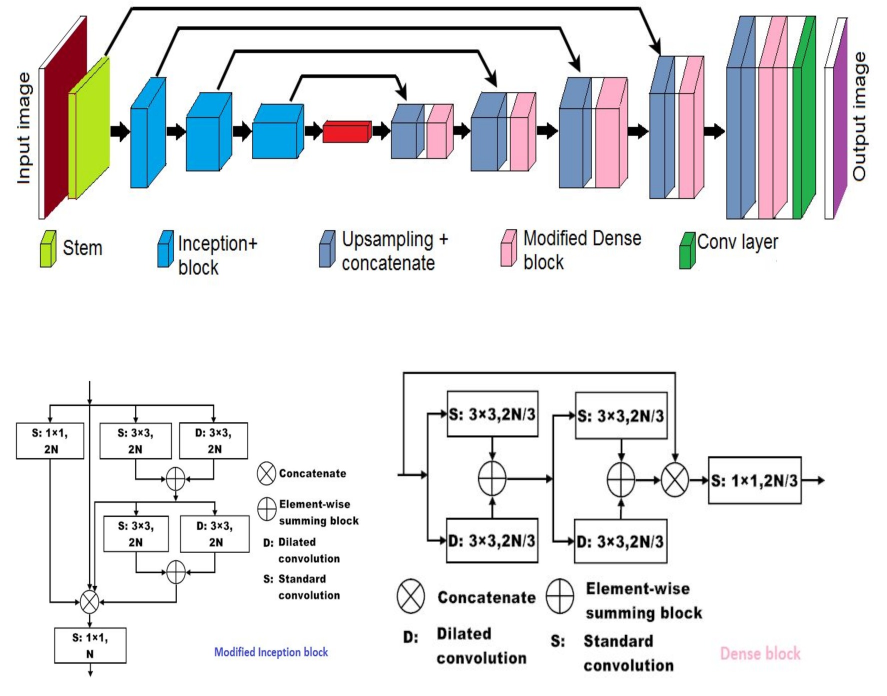

Fig 1. Segmentation Network Architecture

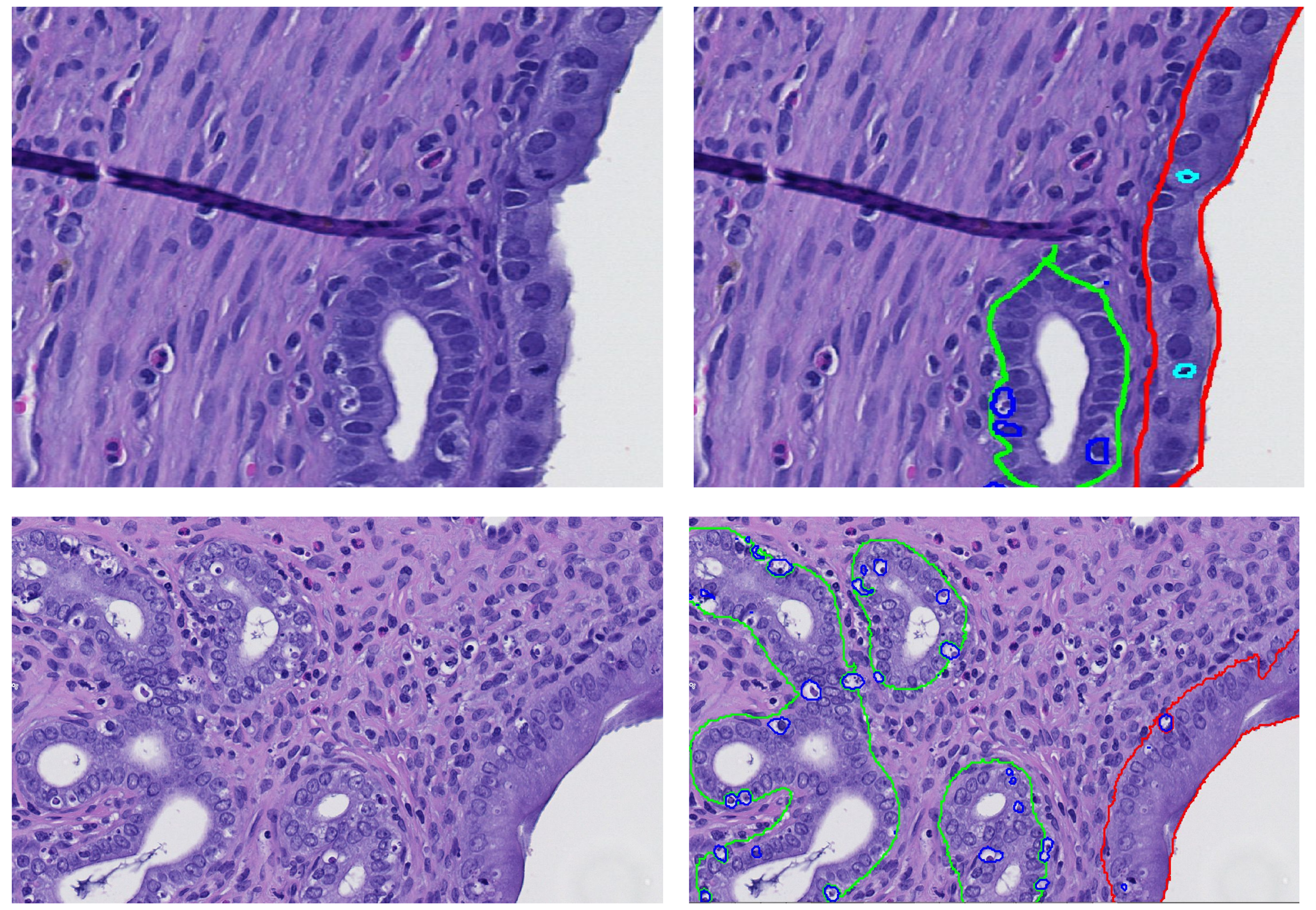

Fig 2 . H&E stained section, Wistar Rat Uterus, 20x, Segmentation Output samples : Red - Luminal epithelium, Green - Glands, Blue - Degenerated cells, Cyan - Mitotic figures

Results

A. The algorithm showed a mean sensitivity of 93.71% and specificity of 96.37%, over all the phases, when validated against the ground truth, which is the consensus of the labels from three different pathologists.
B. Table 1 shows the sensitivity and specificity obtained for the four stages.

Table 1

Stage	Sensitivity	Specificity
Proestrus	100%	96.34%
Estrus	94.10%	98.89%
Metestrus	84.61%	95.25%
Diestrus	96.15%	98%

Conclusion

The proposed algorithm provides an automated, accurate, and consistent method for the assessment of the phases of the estrus cycle in the WSI of H & E-stained sections of Wistar rat uterus that can act as a decision support system for the pathologist.

Future work

- Improving the sensitivity of the Metestrus stage by including more training samples because the duration of the Metestrus stage in Estrus cycle is very short, which results in less training samples.
- Combining the results of Estrus staging in the Uterus and Vagina [1] for comprehensive, accurate, and reproducible estrus cycle staging.

References

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