

Automated AI-assisted estrous cycle staging in rodent models of reproductive disorders

Authors

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Background & Aim

Assessment of estrous stage is a key endpoint in preclinical models involving the reproductive system. Traditionally, this relies on manual histopathological scoring subjecting to inter- and intra-observer variability, thus affecting reproducibility. This study aimed to validate GHOST (Gubra Histopathological Objective Scoring Technology), an automated image analysis pipeline for objective assessment of estrous staging.

Methods

Female C57BL/6J mice, 11 weeks of age, was kept in cages of three and a vaginal flush with sterile saline was performed early morning for 14 consecutive days. The sample was placed on a glass slide and stained with HE.

The analysis was done in three steps (Figure 3):

- First, individual samples are detected on the slide using CLAHE to enhance contrast and a Grounded SAM [1] text prompt for the pixel-wise detection.
- Next, 0.5 mm of the sample borders are removed to avoid edge artefacts, and each sample is divided into 224x224 image tiles, and each tile is encoded by a foundation model (Prov-GigaPath [2]).
- Finally, the encoded tiles, i.e. embeddings vectors, are passed on to a multiple-instance learning model (MADMIL [3]). The model outputs an estrous stage prediction alongside a visualisation of the highest- and lowest-attention tiles used during the prediction.

[1] Ren, Tianhe, et al. "Grounded SAM: Assembling open-world models for diverse visual tasks." arXiv preprint arXiv:2401.14159 (2024).

[2] Xu, H., Usuyama, N., Bagga, J. et al. A whole-slide foundation model for digital pathology from real-world data. Nature 630, 181–188 (2024).

[3] KeshvariKhojasteh, Hassan, Josien Pluim, and Mitko Veta. "Multi-head attention-based deep multiple instance learning." arXiv preprint arXiv:2404.05362 (2024).

1 The stages of the estrous cycle

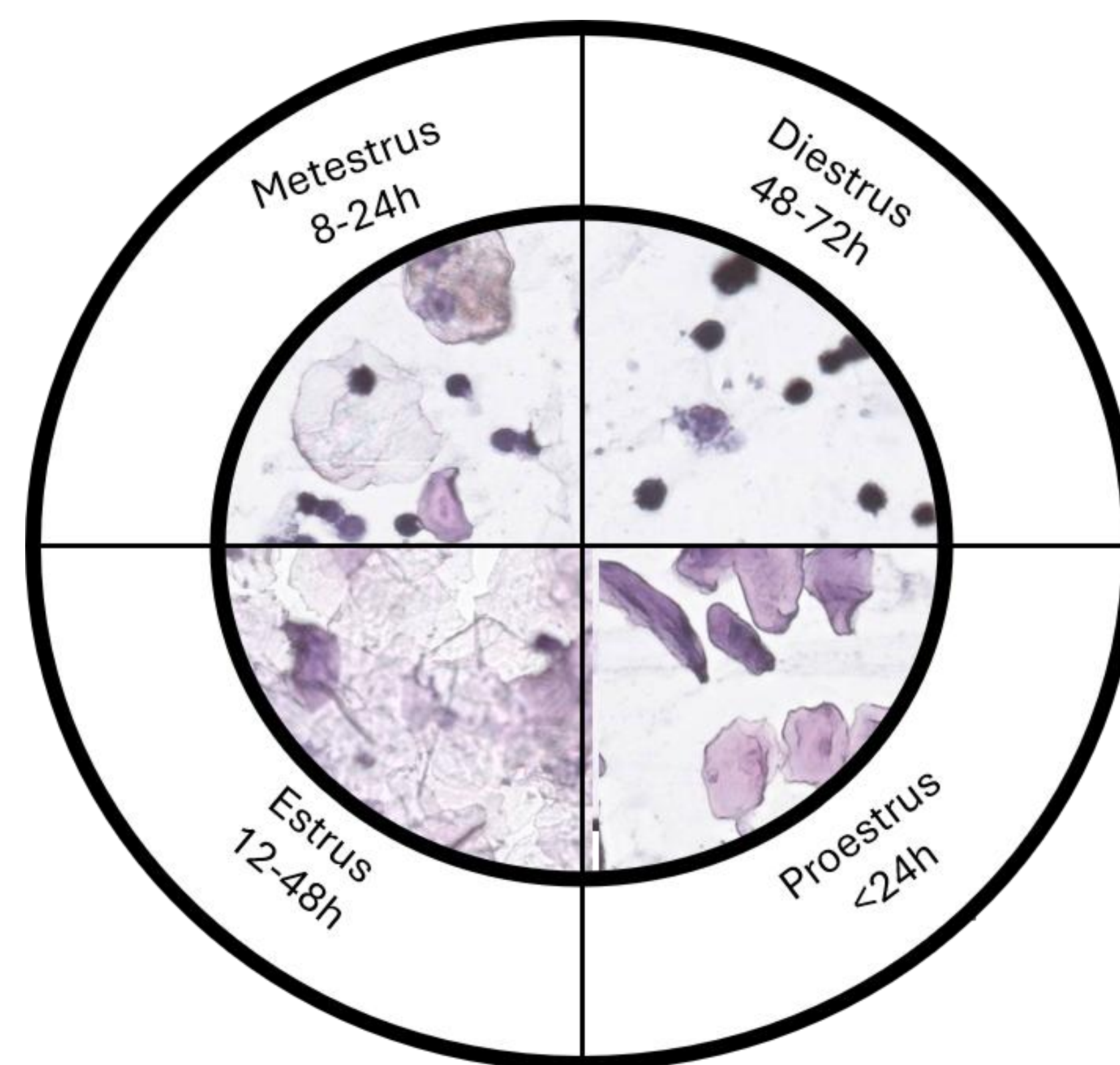


Figure 1. The stages of estrous cycle.

The estrous cycle consists of four stages: **proestrus**, **estrus**, **metestrus**, and **diestrus**. Each of the stages have a characteristically cytological appearance.

In **proestrus**, the cells are round to oval nucleated epithelial cells with a prominent nuclei and basophilic cytoplasm, while leukocytes are absent or rare. In **estrus**, there is mostly cornified epithelial cells, which are large, irregular, angular cells lacking nuclei. In **metestrus**, a mixed cell population is observed, including cornified epithelial cells, nucleated epithelial cells, and increasing numbers of leukocytes. In **diestrus**, there are mainly leukocytes, with only occasional epithelial cells present. There are fewer cells compared to the remaining stages and corresponds to the quiescent phase of the cycle.

2 Estrous monitoring of fertile female mice

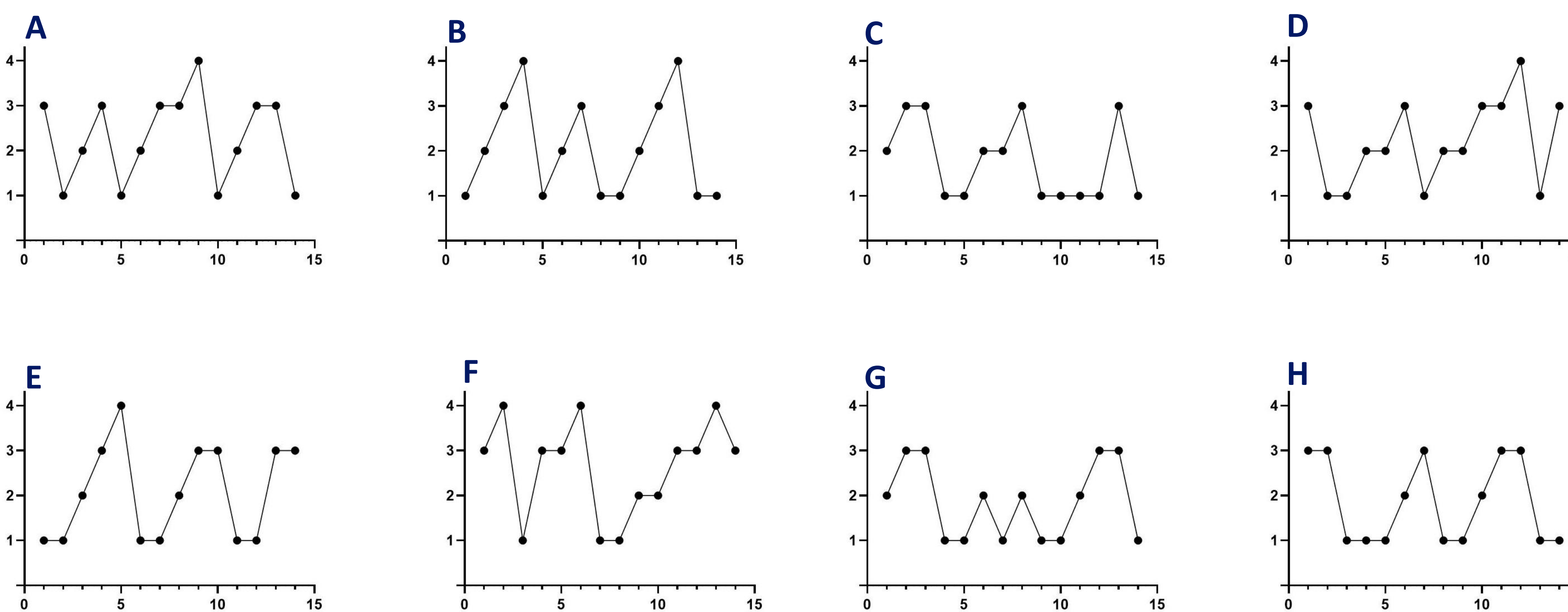


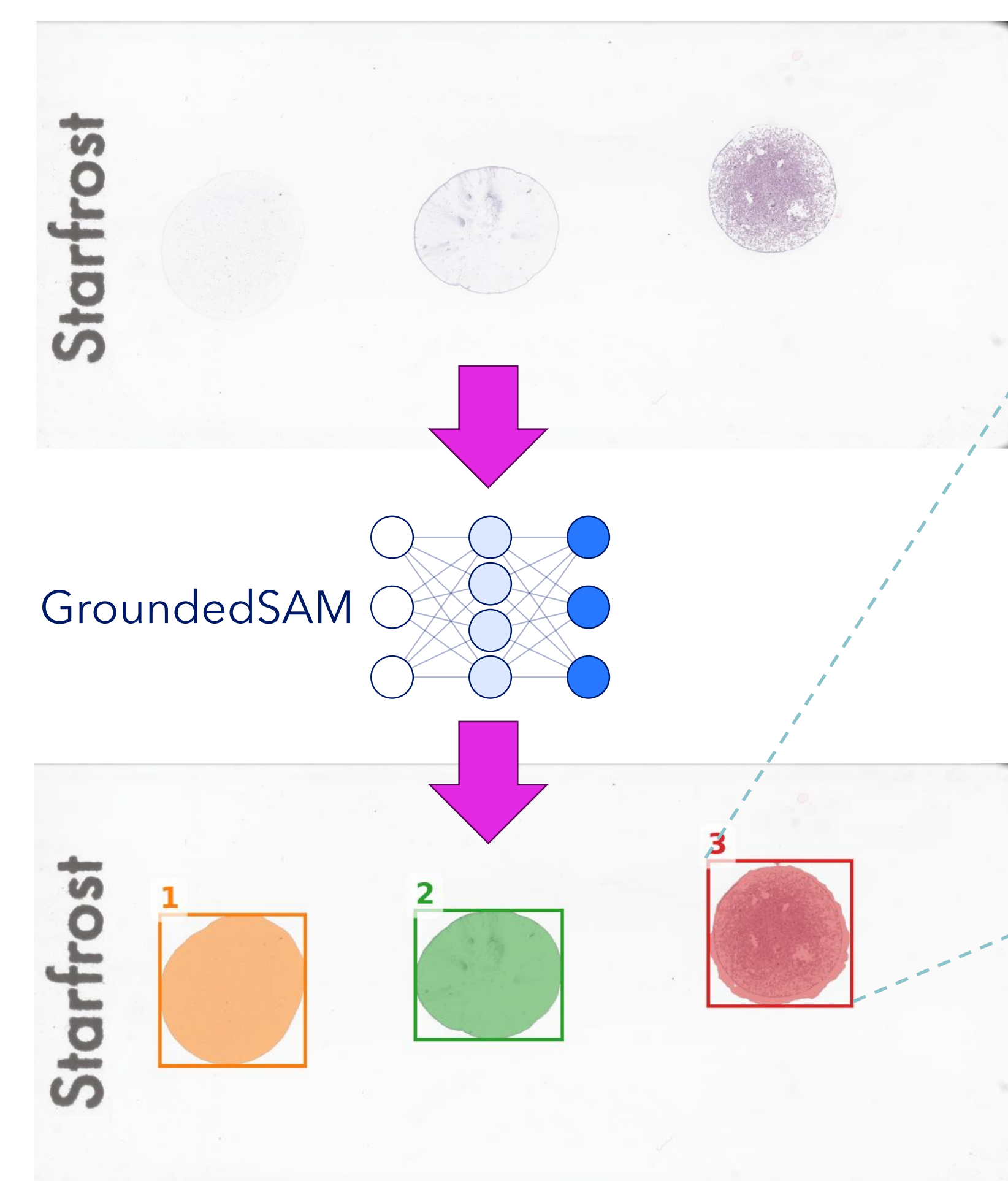
Figure 2. Estrous monitoring of fertile female mice. Diagram (A-H) showing estrous monitoring data scored with automated scoring from 8 mice in a 14-day period. Y-axis is 1: Diestrus. 2: proestrus. 3: Estrus. 4: Metestrus. X-axis is showing days.

Conclusion

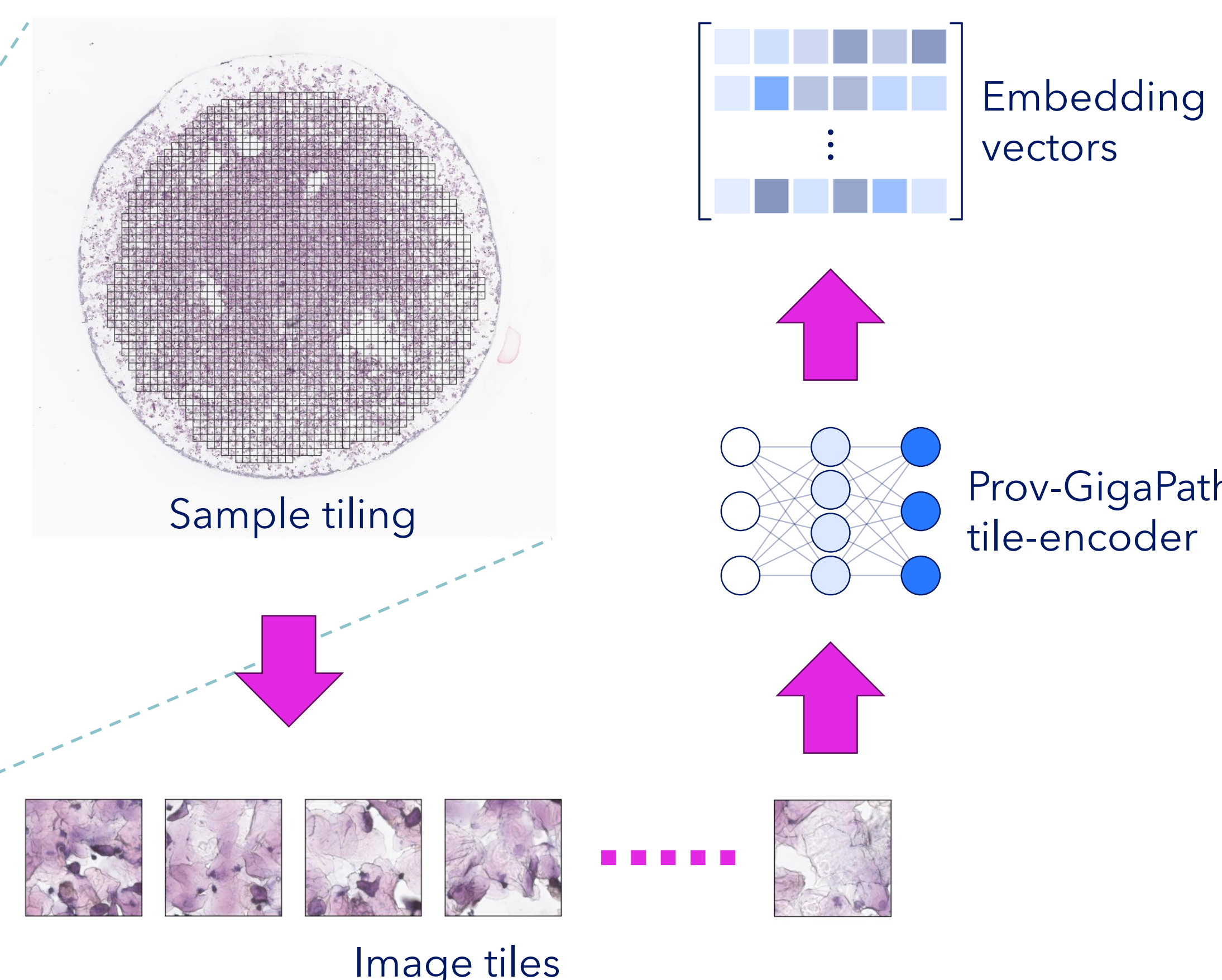
- + GHOST pipeline shows high overall agreement with manual scoring of estrous staging
- + Establishes GHOST as an accurate AI-based method for unbiased and automated estrous assessment
- + Applicable in preclinical studies for reproductive disorders

3 Workflow for staging estrous samples with automated scoring

A. Sample detection



B. Processing pipeline



C. Analysis output

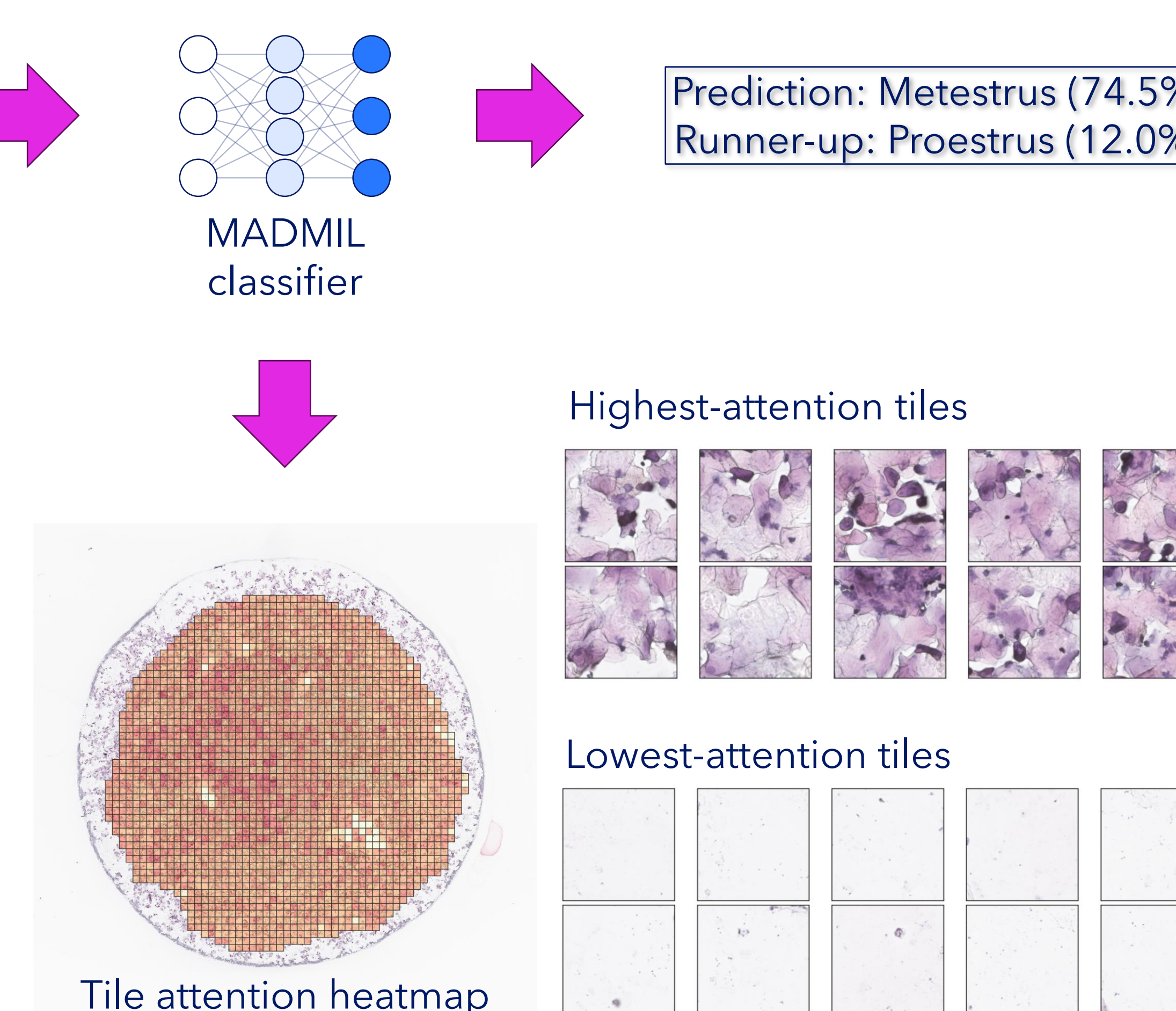


Figure 3. Workflow for scoring of estrous samples. A. Sample detection and segmentation. B. Tiling and encoding. C. Stage prediction

4 High agreement between AI and manual scores

		Predicted stage			
		Diestrus	Proestrus	Estrus	Metestrus
True stage	Diestrus	96.8	0.0	0.0	3.2
	Proestrus	27.8	66.7	0.0	5.6
	Estrus	0.0	10.3	86.2	3.4
	Metestrus	16.0	8.0	32.0	44.0

Confusion matrix (row-normalized)
Cohens Kappa = 0.73

Figure 4. Confusion matrix for agreement of scores. Automated estrous scores showed a high concordance with manual scoring with Cohens Kappa value of 0.73.

Most mis-classifications observed are in neighboring stages, which is expected due to the continuous nature of the staging, seen in Figure 1.