

High-Throughput Adult *Caenorhabditis elegans* Viability Monitoring System

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Hardware List

Table S1. Hardware List (Key Hardware)

ID	Component	Model	Supplier	Unit Price (USD)	Function Description
1	Temperature and Humidity Controllable Incubator	NPK-SPX-70BE	Sichuan Nopec Electromechanical Equipment Co., Ltd.	345.70	Provides a constant temperature environment of 18-25°C and supports PID control
2	Industrial Camera	IMX264	Shandong Botu Photoelectric Technology Co., Ltd.	270	2448×2048 pixel resolution, 60FPS frame rate
3	Collimated Light Source Panel	CRT-2FLD400400	Shenzhen Siaote Intelligent Technology Co., Ltd.	498	Provides parallel cold light to reduce image blurring
4	Bearing of Linear Guide Rail	HGH20CA	Lishui Hengli Automation Technology Co., Ltd.	2.8	Drives the camera array to move and realize batch imaging
5	Linear Guide Rail	HG20	Simon International Trading Co., Ltd.	100	

Legend: 1 Website:

https://www.alibaba.com/product-detail/Biochemical-Incubator-for-Mold-Bacteria-Microorganism_1601526992117.html?spm=a2700.galleryofferlist.normal_offer.d_image.613f13a0X6AeCI&priceId=d574cd91804d4435979d04e50a5d2356

2 Website:

https://www.alibaba.com/product-detail/Hikrobot-Area-Scan-Cameras-MV-CS050_1600978944292.html?spm=a2700.galleryofferlist.normal_offer.d_title.79d013a0jUPtKA&priceId=77bbd9970d394634816beb3dfae3f6f5

Detail: Key camera parameters include: Sensor model: Sony IMX264; Maximum effective pixels: 2448 × 2048 pixels @ 60 FPS; Pixel size: 3.45 × 3.45 μm; Target area size: 2/3"; Output image format: MJPG/YUY2'; Spectral response range: 380 ~ 1030 nm; Filter: Low-pass filter at 650 nm; Power supply during operation: USB 5V; Vision standard protocol: DirectShow interface; Acquisition modes: Continuous and software-triggered; Driver: Standard USB 2.0;

3 Website:

https://www.alibaba.com/product-detail/Automated-Intelligent-Industrial-Light-Source-Machine_1601269989534.html?spm=a2700.galleryofferlist.normal_offer.d_title.52d013a0ObWSI4&priceId=bcb4df3f663454cbc6094bbeb3d1145

4 Website:

https://chinese.alibaba.com/product-detail/Linear-Guide-Rail-Slider-Square-Block_1601577552212.html?spm=a2700.search_countrysitecn.normal_offer.d_title.4cf038d2PqAtvD

5 Website:

https://chinese.alibaba.com/product-detail/Hot-selling-CNC-Machine-Tool-Accessories_10000022636136.html?spm=a2700.search_countrysitecn.normal_offer.d_title.1838fa7adYEInO



Figure S1. Physical diagram of the incubator and computer. The incubator (left) was purchased commercially and served as the base for subsequent modifications (e.g., circuit adjustment, fan airflow redirection, and lighting replacement), providing a stable culture environment for *C. elegans*. The host computer (right) runs the data acquisition software developed based on C# and .NET framework, connecting to industrial cameras and the incubator's serial port via USB interfaces to realize automated imaging control and environmental parameter regulation.

Circuit and Structural Schematic Diagrams

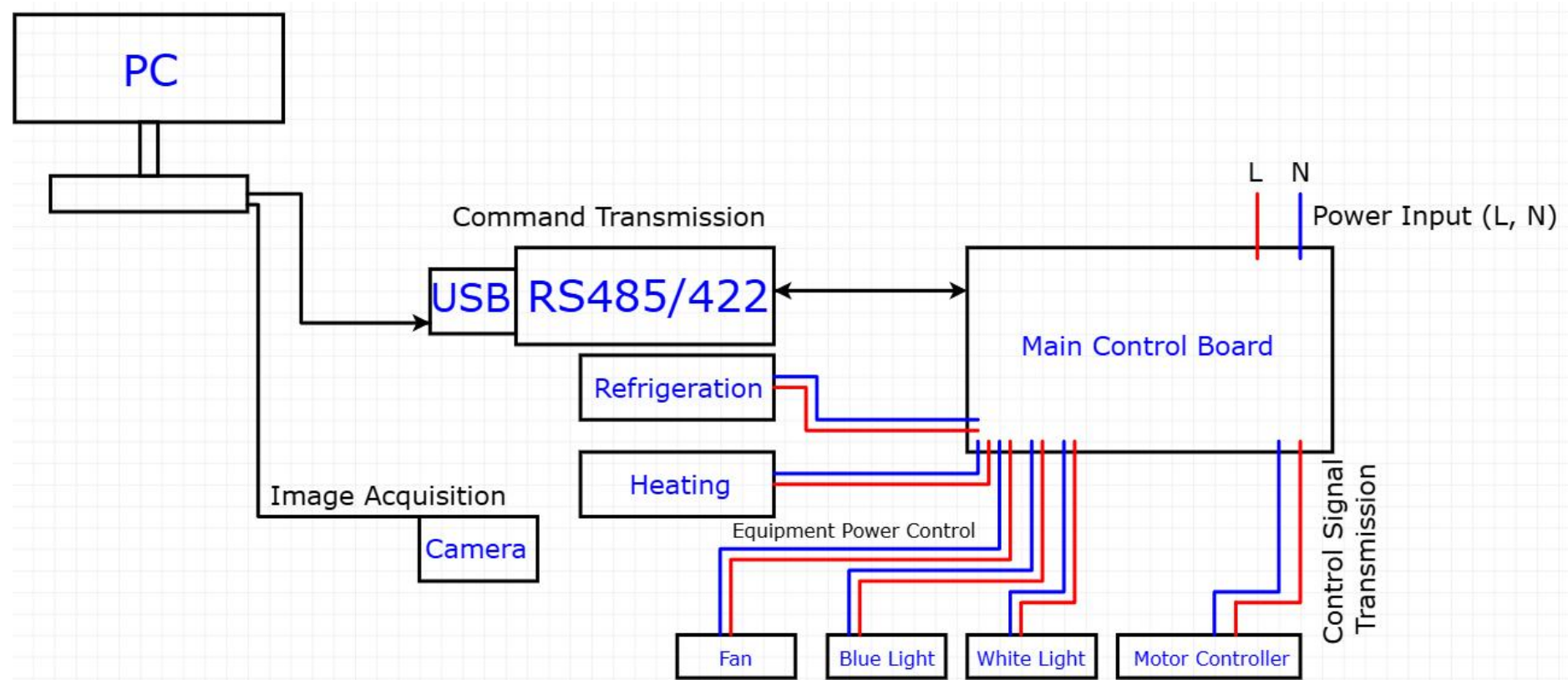


Figure S2. Circuit and Structural Schematic Diagrams. This diagram depicts the electrical connection framework of the system: The PC (host computer) fulfills two core functions: 1) Command transmission: It communicates with the Main Control Board via a USB-to-RS485/422 converter to send system operation instructions; 2) Image acquisition: It connects directly to the Camera to collect in-situ images of *C. elegans*. The Main Control Board (powered by external L/N input) regulates key functional modules through dedicated circuits: it controls the Refrigeration and Heating units to maintain stable environmental parameters in the incubator; manages the Fan for internal air circulation; switches the Blue Light/White Light (serving as imaging light sources); and links to the Motor Controller to drive the motion of the hardware platform.

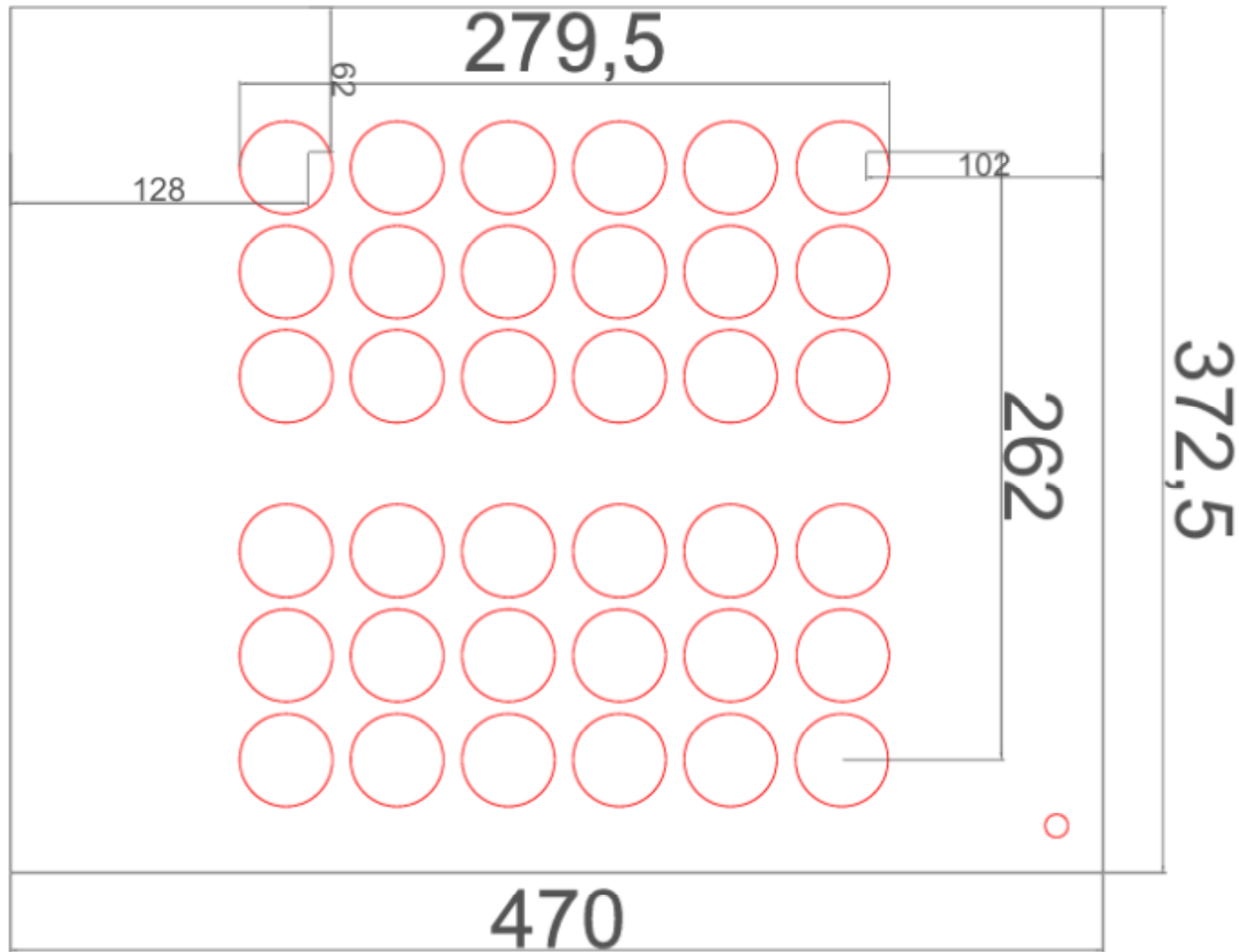


Figure S3. the diagram of the acrylic panel. This diagram depicts the structural layout of the perforated acrylic plate dedicated to holding Petri dishes in the

experimental system. The plate is designed with an array of 6 columns $\times$ 6 rows (36 total) circular holes; each hole is sized to stably accommodate one Petri dish. Key dimensions and positioning parameters are labeled: The horizontal span of the hole array is 279.5 mm, and its vertical height is 262 mm; The overall size of the acrylic plate is 470 mm $\times$ 372.5 mm; The distance from the left edge of the plate to the center of the first hole (horizontal direction) is 128 mm.

Table S2. Modbus Control Command Table

Action	Modbus Command	Memo
Turn Off All Light & Fan	A5 01 01 22 00 00 00 00 B5	/
Turn On Blue Light	A5 01 01 22 01 00 00 00 B5	/
Turn On White Light	A5 01 01 22 00 01 00 00 B5	/
Turn On Fan	A5 01 01 22 00 00 01 00 B5	/
Stop Linear Guide Rail	A5 01 03 22 00 00 00 00 B5	/
Move Linear Guide Rail	A5 01 03 22 01 01 01 18 B5	Move to 280 mm
Return to the Starting Point	A5 01 03 22 02 00 00 00 B5	/

Legend: This table lists the dedicated Modbus control commands (in hexadecimal format) corresponding to key operational actions of the system's hardware, including lighting control, fan operation, and linear guide rail movement. The "Action" column specifies the target operation; the "Modbus Command" column provides the hexadecimal instruction code for communication with the main control board; and the "Memo" column supplements additional information (e.g., movement distance for linear guide rail). These commands enable precise and automated control of hardware components, ensuring the coordinated operation of the imaging system, environmental regulation module, and mechanical motion subsystem during high-throughput monitoring experiments.

Software Information Description

The software system of the High-Throughput Adult *Caenorhabditis elegans* Viability Monitoring System serves as the core for coordinating hardware operations, processing imaging data, and realizing automated viability assessment. It consists of two mutually collaborative core subsystems—the **Image Automatic Acquisition Subsystem** and the ***C. elegans* Analysis Subsystem**—and supports full-process automation from image collection to data output. Both subsystems are developed using C# 9.0 based on the .NET 6.0 framework, ensuring high compatibility with Windows 10/11 Professional Edition and stable integration with industrial cameras, incubators, and linear guide rails. The modular architecture enables independent debugging of each subsystem while realizing seamless data interaction .

1. Image Automatic Acquisition Subsystem

This subsystem focuses on high-efficiency, stable imaging of *C. elegans* in 35 mm Petri dishes, supporting batch automated patrol imaging to lay a foundation for subsequent analysis.

1.1 Development Environment and Dependencies

- (1) **Programming Language:** C# 9.0
- (2) **IDE:** Microsoft Visual Studio 2022 (Community/Professional Edition)
- (3) **Target Framework:** .NET 6.0 (ensures cross-version compatibility and runtime stability)
- (4) **Mandatory Driver:** Hikvision Industrial Camera Driver (Version V[3.5.0.100](3.5.0.100)), compatible with the IMX264 industrial camera. The driver includes the Hikvision MVS (Machine Vision Software) development kit, which provides core APIs for camera parameter adjustment (e.g., exposure, gain) and image capture.

1.2 Core Functions and Implementation Logic

Hardware Connection and Initialization

- (1) Establishes communication with 6 industrial cameras via the MVS SDK. On startup, it automatically detects connected cameras and initializes default parameters: resolution (2448×2048 pixels), frame rate (60 FPS), exposure time (50 ms, adjustable by light intensity), and gain (1.0×, avoiding image noise from excessive gain).
- (2) Synchronizes control of the incubator's linear guide rail via RS485 serial communication. It sends movement commands to drive the camera array to pre-calibrated positions corresponding to each Petri dish, ensuring full coverage of the 6×6 Petri dish array (36 total).

Automated Image Acquisition Workflow

The subsystem supports two triggering modes: **user-defined scheduling** (e.g., once every 24 hours for lifespan monitoring) and **manual activation**. The workflow after triggering is as follows:

- (1) Activates the collimated light source panel via the incubator's control circuit to ensure uniform illumination;
- (2) Sends a "focus" command to each camera (pre-calibrated for 35 mm Petri dishes at a 15 cm working distance);
- (3) Starts parallel image capture across 6 cameras, saving images in JPEG format to a specified local directory (named by "camera number + time" to avoid confusion);
- (4) Turns off the light source and resets the camera array to the initial position via the linear guide rail.

Error Handling and Logging

A real-time error detection mechanism is embedded. If anomalies occur (e.g., camera disconnection, failed light source activation, linear guide rail movement deviation), the software pops up a warning message and records the error type, time, and location in a .txt log file (stored in the software installation directory) to facilitate troubleshooting (e.g., checking USB connections, verifying driver integrity).

2. *C. elegans* Analysis Subsystem

This subsystem processes acquired images to realize target detection, viability assessment, and data statistics, with a live-count accuracy of $\geq 95\%$ (taking manual counting as the gold standard).

2.1 Development Environment and Dependencies

- (1) **Programming Language & IDE:** Consistent with the Image Automatic Acquisition Subsystem (C# 9.0, Visual Studio 2022);
- (2) **Target Framework:** .NET 6.0;
- (3) **Key Libraries & Files:**
 - OpenCvSharp4 (Version 4.5.5.20220608): Used for image preprocessing (grayscale conversion, noise reduction) and post-detection morphological optimization;
 - ONNX Runtime (Version 1.14.0): Provides a runtime environment for the YOLOv5 model, supporting CPU/GPU inference (NVIDIA CUDA acceleration reduces detection time by 40% compared to CPU-only mode);
 - Pre-trained Model: `best.onnx` (generated via YOLOv5 training). Trained on 12,372 adult *C. elegans* images (including live/dead worms and background impurities: bubbles, rings, mildew), it supports 4 target categories and achieves an F1-score of 0.98 at a confidence threshold of 0.541.

2.2 Core Functions and Implementation Logic

Image Preprocessing

Loads JPEG images and optimizes them via OpenCvSharp4 to improve detection accuracy:

- (1) **Grayscale conversion:** Reduces data dimensionality and computational load by converting color images to grayscale;
- (2) **Contrast enhancement:** Uses histogram equalization to enhance the contrast between *C. elegans* and the background (critical for detecting worms with low color contrast against the culture medium).

YOLOv5-Based Two-Stage Target Detection

To reduce the miss rate from 1.75% to 0.8%, a two-stage detection strategy is adopted:

- (1) **First-stage Detection:** Identifies primary targets (*C. elegans*, bubbles, rings, mildew), marks bounding boxes and confidence scores, and filters out targets

with confidence <0.5 to reduce false positives;

- (2) **Filled-region Secondary Detection:** Fills the bounding boxes of first-stage detected targets with pure white (RGB: 255,255,255) to eliminate interference. It then performs a second round of detection on the filled image; for newly detected candidate regions, if the number of non-white pixels (representing potential worm tissue) exceeds 10, the region is added to the final target list.

Image Morphological Processing (Post-Detection Optimization)

- (1) **Erosion & Dilation:** Applies a 3×3 structural element to erode small noise regions (e.g., tiny mildew spots misclassified as worms) and dilate the edges of detected worm targets, ensuring complete preservation of worm morphology;
- (2) **Contour Extraction:** Extracts the contour of each worm to calculate morphological parameters, which serve as auxiliary indicators for viability assessment.

Data Output

Generates a structured analysis report (Excel/CSV format) containing the number of live/dead worms per Petri dish, detection time, and target confidence scores. The report is saved in the same directory as the acquired images, supporting direct import into GraphPad Prism for lifespan curve plotting.

3. Software Deployment and Configuration Guide

3.1 Deployment Steps

- (1) **Install Operating System and Dependencies:**

Install Windows 10/11 Professional Edition (64-bit) and enable .NET Framework 6.0 via "Control Panel > Programs > Turn Windows features on or off";

Install Hikvision Industrial Camera Driver (V[3.5.0.100](3.5.0.100)): Run Hikvision_MVS_Setup.exe, follow prompts to install, and restart the computer.

- (2) **Install Monitoring System Software:**

Download the installation package (provided by the corresponding author) and extract it to a local disk (recommended space ≥ 100 GB for image storage);

Double-click Setup.exe, select an installation directory (avoid system disk C:), and check "Create Desktop Shortcut".

(3) **Configure Pre-trained Model:**

Copy best.onnx to the ..\Assets\Weights\ subdirectory under the software installation path.

3.2 Hardware-Software Integration Testing

After deployment, perform a full-system test to ensure normal interaction:

- (1) Connect industrial cameras, linear guide rails, and the incubator control module to the computer via USB/RS485 cables;
- (2) Launch the Image Automatic Acquisition Subsystem, click "Hardware Detection", and confirm detection of 6 cameras and the linear guide rail;
- (3) Run "Test Acquisition" (select 1 Petri dish) and verify that light source activation, camera focusing, and image capture are completed within 2 minutes;
- (4) Import the test image into the *C. elegans* Analysis Subsystem, run "AnalysisAll", and confirm that the live/dead count error with manual counting is $\leq 5\%$.

4. Software Maintenance and Troubleshooting

4.1 Routine Maintenance

Data Backup: Back up acquired images and analysis reports to an external hard drive or cloud storage once a week to prevent data loss from disk failure.

4.2 Common Troubleshooting

Issue Description	Possible Cause	Solution
Industrial camera not detected by the acquisition subsystem	1. USB cable disconnected; 2. Driver not installed correctly; 3. Camera hardware failure	1. Reconnect the USB cable to a USB 3.0 port; 2. Reinstall the Hikvision driver and restart; 3. Replace with a spare IMX264 camera if hardware failure is confirmed

YOLOv5 model fails to load	1. <code>best.onnx</code> missing/corrupted; 2. ONNX Runtime not installed; 3. Incorrect model path	1. Re-copy <code>best.onnx</code> to the "Weights" directory; 2. Reinstall ONNX Runtime (V1.14.0) via the "Dependency Repair" tool
Excessive false positives (e.g., bubbles misclassified as worms)	1. Low light intensity leading to poor image quality; 2. Model overfitted to training data	1. Increase collimated light source intensity via the incubator control panel and re-acquire images; 2. Retrain the YOLOv5 model with additional bubble/mildew/rings samples (contact the corresponding author for the dataset template)

5. Software Availability

The full software package (including source code, installation files, and the `best.onnx` model) is available from the corresponding author **Qizhao Lin** (E-mail: 1575177305@qq.com) upon reasonable request, subject to institutional review to comply with ethical research protocols and intellectual property regulations.

Step-by-Step Implementation Protocol for High-Throughput Adult *Caenorhabditis elegans* Viability

Monitoring System Hardware

This protocol details the sequential assembly and modification steps for the hardware components listed, ensuring the system meets the requirements of stable environmental control, high-quality imaging, and automated batch detection. The total implementation time is estimated to be 8–10 hours (excluding component delivery time), and it is recommended that 2–3 operators collaborate to avoid damage to precision parts.

1. Pre-Implementation Preparation (1 Hour)

1.1 Component Inspection

Unpack all components according to the hardware list (Table 1) and verify the consistency of model, supplier information, and physical condition of each part:

For the Temperature and Humidity Controllable Incubator (Model: NPK-SPX-70BE): Check if the LCD display, PID control button, and air outlet are intact; confirm the internal dimensions (should be $\geq 65 \times 55 \times 100$ cm to accommodate subsequent components).

For the Industrial Camera (Model: IMX264): Connect it to a computer via a USB cable to test whether the image output (2448×2048 pixels, 60FPS) is normal; check if the 650 nm low-pass filter (standard accessory) is pre-installed.

For the Collimated Light Source Panel (Model: CRT-2FLD400400): Power it on to verify that the light output is uniform (no dark spots or uneven brightness); measure the light divergence angle (should be $< 5^\circ$ to meet the "parallel cold light" requirement).

For the Linear Guide Rail (Model: HG20) and Bearing (Model: HGH20CA): Confirm that the bearing slides smoothly on the guide rail without jamming; check if the mounting holes (matching the acrylic plate) are pre-drilled.

Prepare auxiliary tools and materials: Phillips screwdriver (size #2), hex wrench set (1.5–5 mm), acrylic plate (47 mm×372.5 mm, pre-cut with 6 camera mounting holes), USB 2.0 hub (8 ports, support 5V power supply), thermal insulation tape, and cable ties.

1.2 Safety Precautions

Disconnect the incubator from the power supply during circuit modification to avoid electric shock; use insulated tools (e.g., rubber-handled screwdrivers) when handling wires.

Place the linear guide rail and bearing on a clean, flat workbench (cover with a soft cloth) to prevent scratches on the precision sliding surface.

2. Incubator Modification (3–4 Hours)

2.1 Internal Structure Cleaning and Space Planning

Wipe the inner wall of the incubator with 75% ethanol to remove dust and residual contaminants; use thermal insulation tape to seal gaps around the air outlet (to prevent airflow from directly blowing the culture dishes later).

Mark the installation positions inside the incubator:

Linear Guide Rail Mounting Area: Mark two parallel lines (length 50 cm) on the left and right inner walls of the incubator (15 cm below the top) to ensure the guide rail is horizontally installed (use a level to calibrate, error $\leq 0.5^\circ$).

Collimated Light Source Placement Area: Mark a rectangular area (40 cm×40 cm) on the bottom inner wall of the incubator (centered) to match the size of the CRT-2FLD400400 light source panel.

2.2 Circuit Modification for Incubator Control

Remove the incubator's control panel (using a Phillips screwdriver) and install a custom circuit board (purchased separately or made by oneself, compatible with RS485/422 communication) and a USB-to-RS485/422 serial cable:

Connect the serial cable's USB port to the computer (for later software control) and the RS485 port to the incubator's internal fan, LED light, and linear guide rail motor terminals (refer to the incubator's circuit diagram provided by Sichuan Nopec Electromechanical Equipment Co., Ltd.).

Use cable ties to organize the wires (avoid contact with the incubator's heating tube to prevent wire aging) and reinstall the control panel.

Test the modified circuit: Power on the incubator, send commands via the computer (e.g., "turn on the fan," "adjust temperature to 20°C") through the serial communication software (e.g., PuTTY), and confirm that the incubator responds correctly (LCD display updates, fan runs normally).

3. Linear Guide Rail and Bearing Assembly (1.5–2 Hours)

3.1 Guide Rail Installation

Fix the Linear Guide Rail (HG20) to the pre-marked positions on the inner wall of the incubator using hex screws (M5×10 mm):

Align the guide rail's mounting holes with the marked positions; use a level to ensure the guide rail is horizontal (adjust by adding thin gaskets under the guide rail if necessary).

Tighten the screws sequentially (torque 2.5 N·m) to avoid deformation of the guide rail (over-tightening may cause jamming of the bearing).

3.2 Bearing and Camera Mounting Bracket Installation

Install the Bearing (HGH20CA) onto the HG20 guide rail:

Apply a thin layer of lubricating oil (provided with the bearing) to the guide rail's sliding surface; gently push the bearing onto the guide rail to confirm smooth sliding (no abnormal noise).

Fix the acrylic plate (camera mounting bracket) to the top of the bearing using M4×8 mm screws (6 mounting points, one for each industrial camera); use a ruler to ensure the acrylic plate is perpendicular to the guide rail (error $\leq 1^\circ$).

4. Imaging Module Installation (1.5–2 Hours)

4.1 Industrial Camera Installation

Mount the Industrial Camera (IMX264) onto the acrylic plate:

Align the camera's bottom mounting holes with the pre-drilled holes on the acrylic plate; fix the camera with M3×6 mm screws (torque 1.2 N·m, avoid damaging the camera's circuit board).

Connect each camera to the USB 2.0 hub (placed outside the incubator, connected via a USB extension cable through the incubator's wire hole); label each camera (e.g., "Camera 1–6") to avoid confusion during later software configuration.

4.2 Collimated Light Source Installation

Place the Collimated Light Source Panel (CRT-2FLD400400) in the pre-marked bottom area of the incubator:

Use double-sided adhesive tape (high-temperature resistant, $\geq 80^{\circ}\text{C}$) to fix the light source panel to the incubator's bottom wall (ensure the light-emitting surface is upward and free of obstructions).

Connect the light source's power cable to the incubator's internal power socket (12V DC); power on to test the light uniformity—place a white paper on the light source, and confirm that the paper is uniformly illuminated (no obvious brightness differences).

5. System Calibration and Functional Test (1 Hour)

5.1 Mechanical Calibration

Test the linear guide rail's movement: Send a "movement command" via the computer to control the bearing (with cameras) to slide along the HG20 guide rail (full stroke 50 cm); confirm that the movement speed is stable (adjustable via software, recommended speed 5 cm/s) and that there is no deviation in the stop position (error ≤ 2 mm).

5.2 Imaging Quality Test

Place a 35 mm *C. elegans* culture dish (containing 15–20 adult worms) on the collimated light source panel; control the industrial camera to take images:

Check the image resolution (2448×2048 pixels) and clarity—observe the worm's body edge (should be sharp, no blurring) and background noise (should be low, no light spots caused by non-parallel light).

Verify the temperature and humidity control: Set the incubator to 20°C and 60% RH; use a handheld thermometer-hygrometer to measure the temperature and humidity near the culture dish (error should be $\leq \pm 1^{\circ}\text{C}$ and $\pm 5\%$ RH, meeting PID control requirements).

5.3 Batch Detection Simulation

Simulate the high-throughput detection process: Place 6 culture dishes (one under each camera) in the incubator; run the automated imaging software (refer to the software configuration manual in Supplementary Materials):

Confirm that the system completes "guide rail movement → camera focusing → light source on → image acquisition" in 36–60 minutes.

Check the data output: Ensure the software automatically saves images (named by "camera number + time") and generates a preliminary count report (no missing or duplicate counts of worms).

6. Post-Implementation Maintenance Tips

Clean the collimated light source panel weekly (wipe with a lint-free cloth) to avoid dust accumulation affecting light uniformity.

Lubricate the linear guide rail and bearing monthly (use the special lubricating oil provided by Lishui Hengli Automation Technology Co., Ltd.) to maintain smooth movement.

Calibrate the incubator's temperature and humidity quarterly (using a standard calibrator) to ensure the 18–25°C constant temperature environment is stable.

Model Training Results and Metrics Explanations

This supplementary material presents the detailed results of the neural network model training for *Caenorhabditis elegans* detection, including key metrics (loss, precision, recall, F1-score, mAP) and evaluation curves. Each figure is accompanied by explanatory captions to help readers without specialized background in neural networks understand the results.

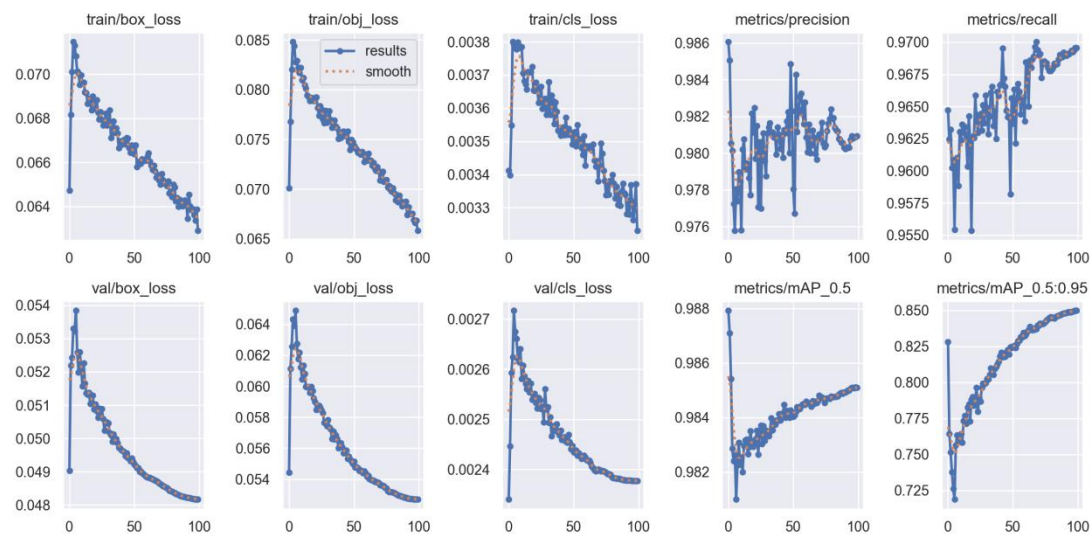


Figure S1. Training and Validation Metrics Curves. This figure shows the variation trends of key metrics during model training (x-axis: training epoch; y-axis: metric value). 1. Loss metrics (top row): box_loss (bounding box regression loss, reflecting the accuracy of target position prediction; lower values indicate better positioning), obj_loss (objectness loss, distinguishing between target and background; lower values indicate more accurate classification), cls_loss (classification loss, distinguishing between different classes such as *C. elegans* and background impurities; lower values indicate better classification performance). Both training and validation loss gradually decrease and stabilize, indicating no overfitting. 2. Performance metrics (bottom row): recall (proportion of true targets correctly detected), precision (proportion of detected targets that are true), mAP 0.5 (mean Average Precision at IoU=0.5, comprehensive evaluation of detection accuracy). All metrics show an upward trend and stabilize at high values (e.g., mAP 0.5 > 0.85), demonstrating the model's strong detection ability.

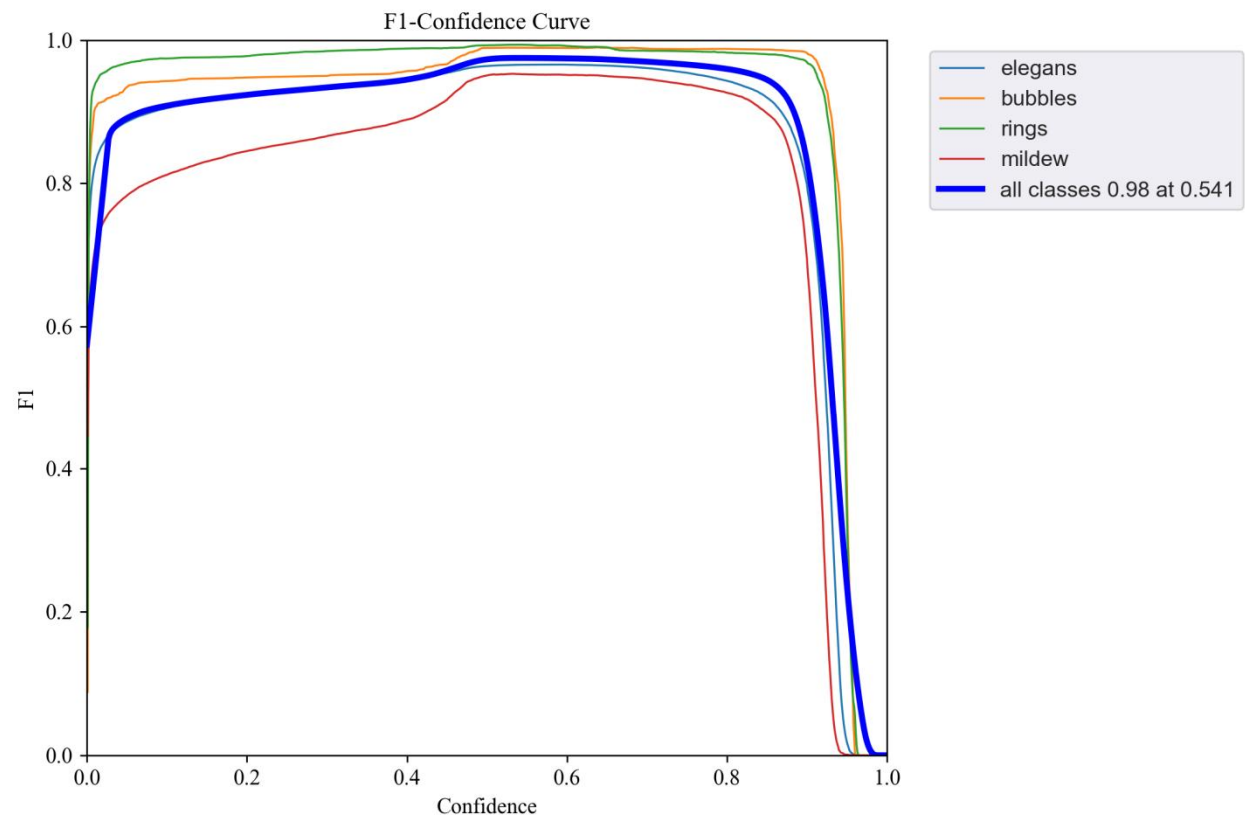


Figure S2. F1_curve. F1-score is a comprehensive metric combining precision and recall ($F1 = 2 \times (\text{precision} \times \text{recall}) / (\text{precision} + \text{recall})$), with a range of 0-1 (higher values indicate better balanced performance). The x-axis represents the confidence threshold (the minimum confidence value for the model to output a detection result), and the y-axis represents the F1-score. The curve shows the F1-score of each class (elegans, bubbles, rings, mildew) and all classes as the confidence threshold changes. For all classes, the maximum F1-score reaches 0.98 when the confidence threshold is set to 0.541, indicating that the model achieves optimal balanced performance at this threshold.

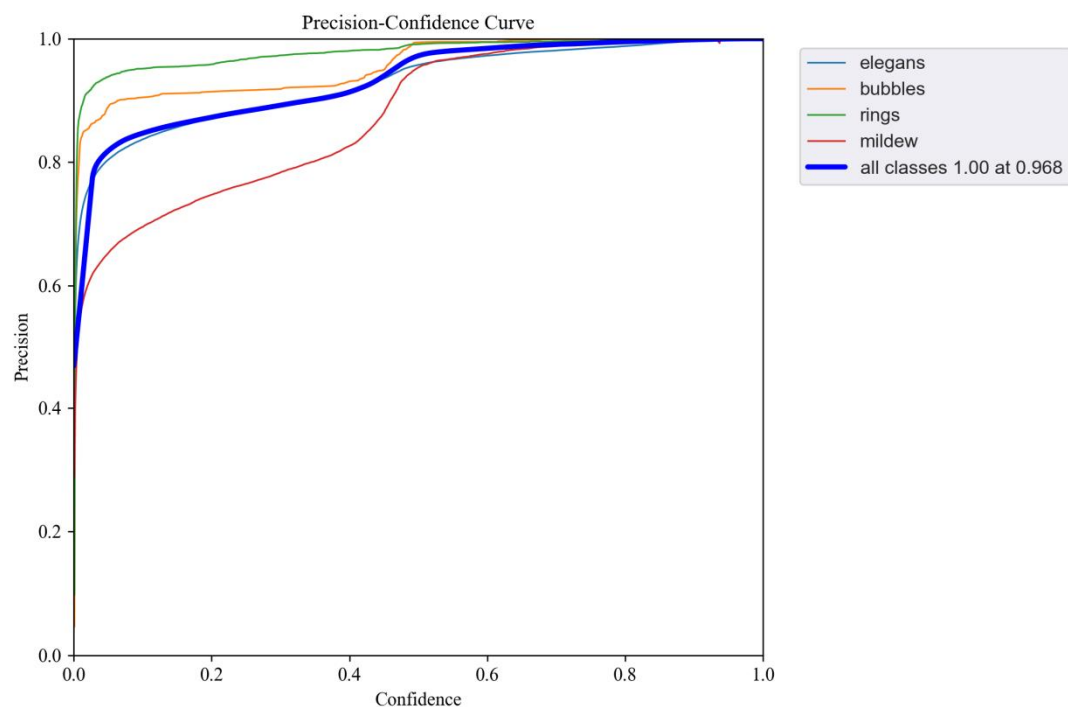


Figure S3. P_curve. Precision refers to the proportion of correctly detected targets among all detected results (Precision = true positives/(true positives + false positives)), reflecting the "accuracy" of detection results. The x-axis is the confidence threshold, and the y-axis is the precision. When the confidence threshold is set to 0.968, the precision of all classes reaches 1.00, meaning all results output by the model at this threshold are true targets (no false positives). This indicates the model's high reliability when using a high confidence threshold.

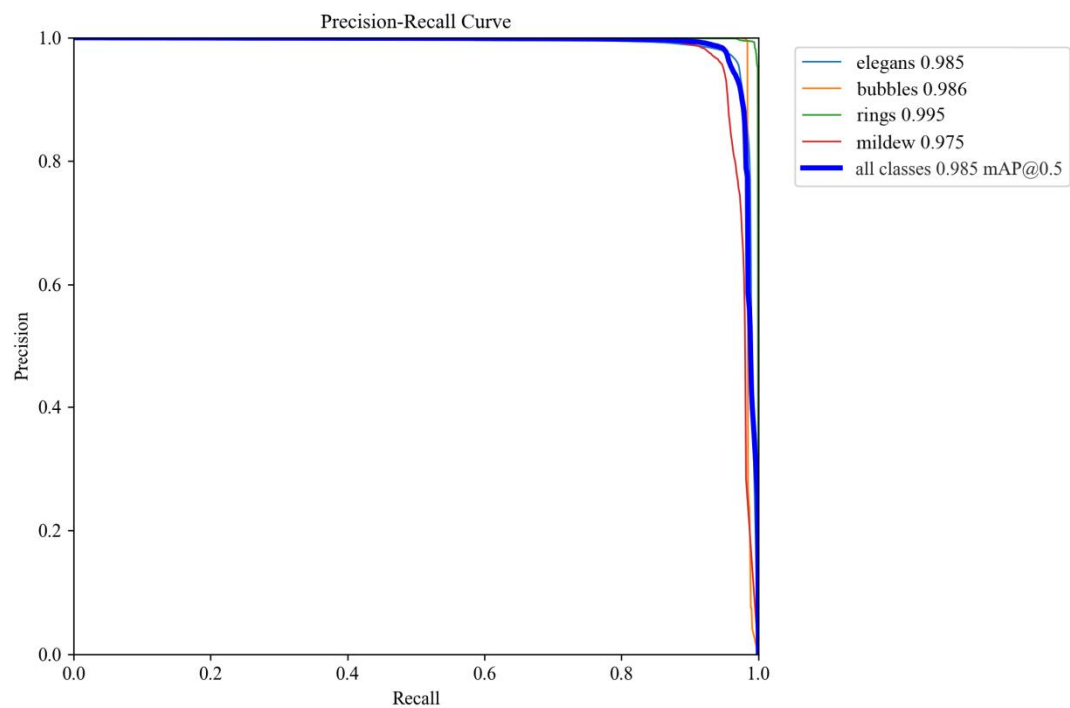


Figure S4. PR_curve. The PR curve plots precision (y-axis) against recall (x-axis), and the area under the curve (AUC) reflects the model' s detection performance (larger AUC indicates better performance). Each curve corresponds to a class (elegans, bubbles, rings, mildew), with all classes achieving high AUC values (e.g., elegans: 0.985, rings: 0.995). mAP@0.5 (mean Average Precision at IoU=0.5) is the average of AUC values across all classes, reaching 0.985 for this model. A high mAP@0.5 (>0.95) demonstrates the model' s excellent overall detection accuracy.

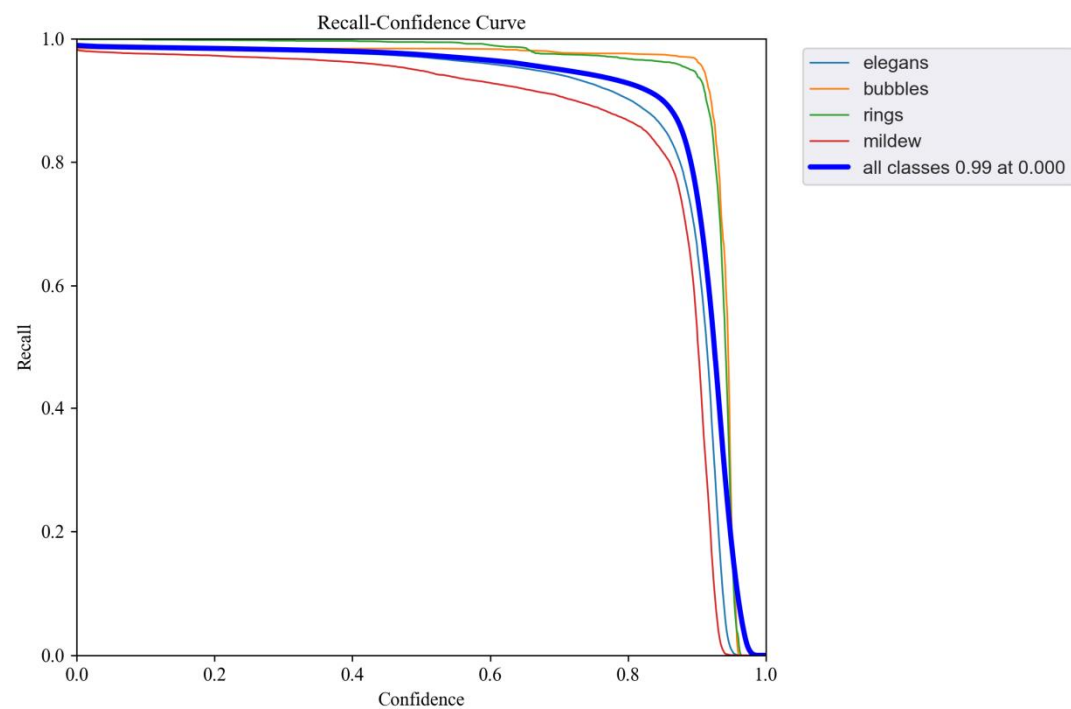


Figure S5. R_curve. Recall refers to the proportion of true targets that are correctly detected ($\text{Recall} = \text{true positives} / (\text{true positives} + \text{false negatives})$), reflecting the "completeness" of detection. The x-axis is the confidence threshold, and the y-axis is the recall. When the confidence threshold is set to 0.000 (i.e., all model predictions are retained), the recall of all classes reaches 0.99, meaning nearly all true targets are detected. As the threshold increases, recall decreases slightly, which is a normal trade-off between precision and recall.

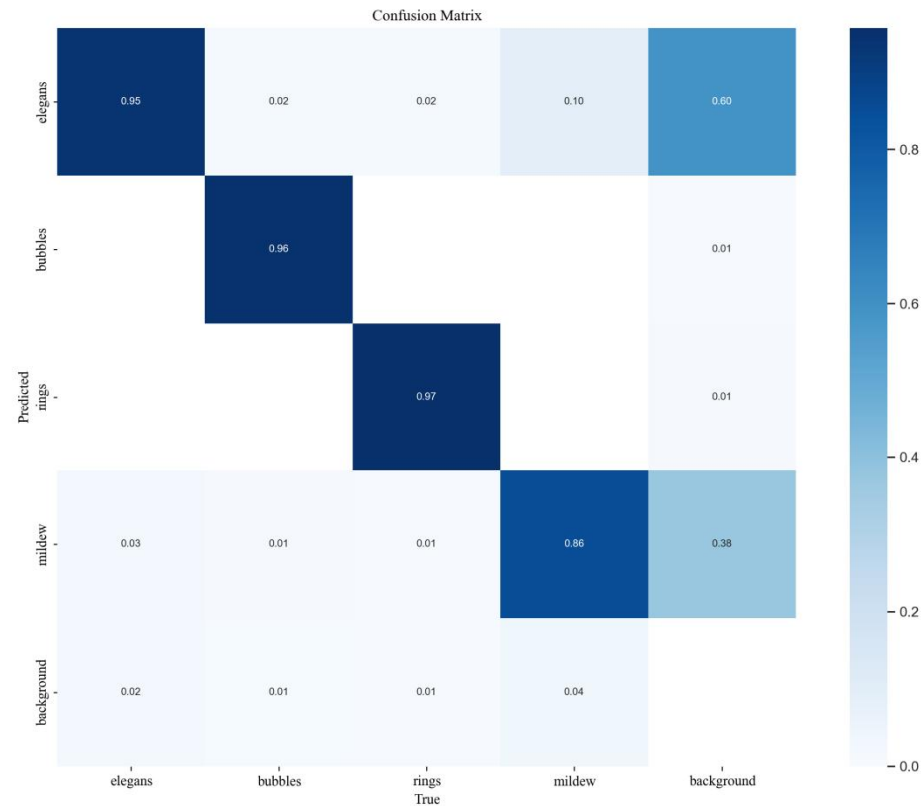


Figure S6. Confusion matrix of model training results. A confusion matrix shows the performance of the classification model by comparing predicted labels (x-axis) with true labels (y-axis). Each cell (i,j) represents the number of samples of true class i predicted as class j. The diagonal elements represent correctly classified samples, and off-diagonal elements represent misclassified samples. It can be seen from the matrix that the model achieves high classification accuracy for *C. elegans* and other classes (e.g., bubbles, rings, mildew), with few misclassifications. This indicates the model's strong ability to distinguish between the target (*C. elegans*) and background impurities.

Algorithm 1 Obtain *Caenorhabditis elegans* based on the YOLOv5 through two-stage detection

Input: Image paths of one petri *AllPath*;

Output: Coordinate list of *Caenorhabditis elegans* *Coordinate_list*;

```
1: AnnotationPaths, IndexOfMaxNumber  $\leftarrow$  GetAnnotationByYOLOv5ByTheBestPTFile(AllPath)
2: Coordinate_list  $\leftarrow$  GetCoordinate(AnnotationPaths, IndexOfMaxNumber)
3: Edited_image_path  $\leftarrow$  FilltheElegansRegionWithWhite(IndexOfMaxNumber, Coordinate_list)
4: New_annotation_paths  $\leftarrow$  GetNewAnnotationByYOLOv5ByTheBestPTFile(Edited_image_path)
5: New_coordinate_list  $\leftarrow$  GetNewCoordinate(New_annotation_paths, New_annotation_paths)
6: for (i  $\leftarrow$  0 to length(New_coordinate_list)-1 ) do
7:   Sub_Image  $\leftarrow$  CropBoxImageByBox(Edited_image_path, New_coordinate_list[i])
8:   Sum_White  $\leftarrow$  CountWhitePixel(Sub_Image)
9:   if (Sum_White > 9) then
10:    continue
11:   end if
12:   Coordinate_list  $\leftarrow$  Coordinate_list_appened(Coordinate_list, New_coordinate_list[i])
13: end for
```