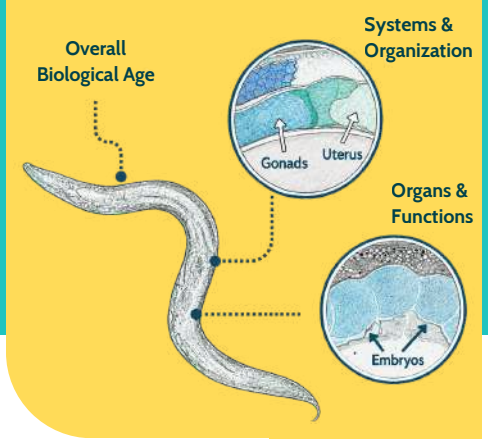
nagi bioscience

TECHNICAL NOTE

NAGI™ Biological Age : Phenotypic biological age measurements for healthy aging interventions.

A multi-dimensional biological age framework that moves beyond a single aging score

Multidimensional biological age assessment for healthy aging interventions



The goal of healthy aging research is not simply to extend lifespan but to increase healthspan, the proportion of life spent in good health and functional independence. While lifespan remains the most widely used endpoint in aging studies, it provides only a limited view of intervention efficacy. Compounds with similar effects on longevity can produce profoundly different physiological outcomes, while interventions that preserve health and function may have only modest effects on survival (Figure 1). Measuring healthy aging therefore requires quantitative approaches that capture the multidimensional physiological changes that occur throughout life.

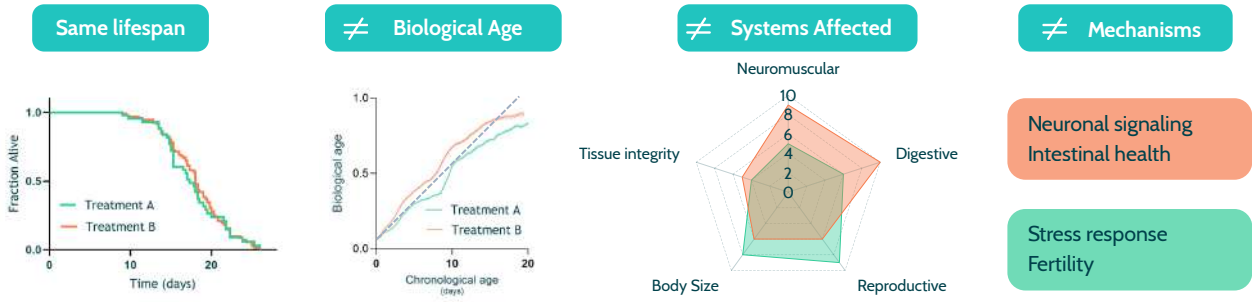


Figure 1. Example of how longitudinal functional phenotyping reveals how interventions shape aging beyond lifespan. The same lifespan can mask different biological aging trajectories, affected systems, and molecular mechanisms.

What is NAGI™ Biological Age?

NAGI™ Biological Age is an AI-powered phenotypic biological age assessment solution that enables longitudinal, non-invasive quantification of biological aging using *Caenorhabditis elegans* (*C. elegans*), a cornerstone organism in aging research. Built on the Nagi Bioscience SydLab™ One automated microfluidic imaging platform, NAGI™ Biological Age combines high-content brightfield imaging, state-of-the-art computer vision, and machine learning to generate an interpretable assessment of organismal health throughout life.

Unlike conventional aging assays that rely primarily on lifespan or single functional endpoints, NAGI™ Biological Age integrates more than 60 behavioral, morphological, and anatomical features into a multidimensional biological age model. Beyond providing an overall biological age estimate, the platform decomposes this prediction into biologically meaningful levels of interpretation, enabling researchers to identify which physiological systems drive the observed aging phenotype.

The analysis is organized into three complementary layers of biological insight:

- **Overall Biological Age**, providing an integrated measure of organismal aging.
- **Systems & Organization**, identifying the biological age contribution of major physiological systems, including neuromuscular function, digestive system, reproductive system, body size, and structural integrity.
- **Organs & Functions**, offering biological age measurements of individual organs and functional biomarkers that further dissect the biological age prediction.

This multiscale approach allows researchers not only to determine whether an intervention promotes healthy aging but also to understand how it reshapes the aging process. The result is a richer characterization of intervention efficacy, facilitating mechanism-of-action studies, compound prioritization, and the identification of tissue-specific responses that would otherwise remain hidden. Designed for nutrition, biotechnology, and pharmaceutical research, NAGI™ Biological Age provides an automated, scalable platform that transforms longitudinal phenotypic data into actionable insights for healthy aging discovery.

Three Layers of Biological Insight

Traditional biological age clocks typically generate a single prediction that summarizes the overall biological state of an organism (Meyer, D. H. & Schumacher, B. *Aging Cell*. 2021). Although complementary analyses of transcriptomic data can identify differentially expressed genes and conserved molecular pathways, such changes do not necessarily indicate meaningful effects on tissue or organismal function. By contrast, functional phenotypic readouts—such as improved movement or reduced muscle atrophy—directly capture physiologically relevant outcomes, providing a complementary and readily interpretable measure of biological aging.

NAGI™ Biological Age was designed to go beyond biological age prediction by providing an interpretable, hierarchical assessment of aging. The platform progressively decomposes the prediction into three complementary layers of biological information, allowing researchers to move from a global assessment of healthy aging to the specific physiological features underlying the observed phenotype.

Three Layers of Biological Insight

1st Layer – Overall Biological Age

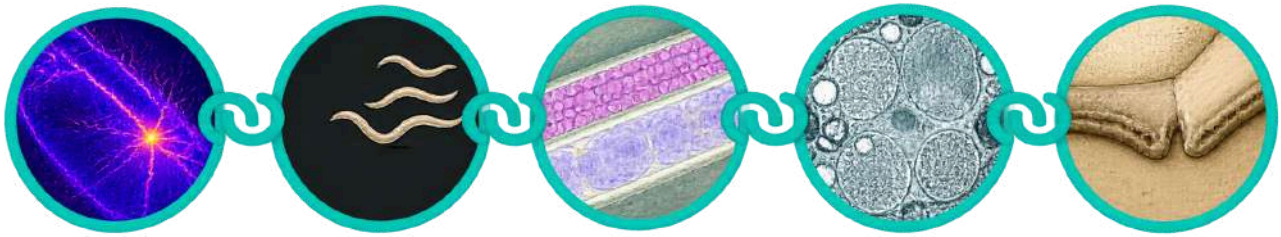
The first layer consists of an integrated biological age score generated by an artificial intelligence model trained on longitudinal phenotypic data collected throughout the lifespan of *C. elegans*. By simultaneously analyzing more than 60 behavioral, morphological, and anatomical biomarkers, the model estimates the overall biological state of each individual, providing a single quantitative endpoint that summarizes the cumulative effects of an intervention on healthy aging. NAGI™ Biological Age is expressed relative to the aging trajectory of the selected control condition. A biologically younger value indicates that the measured phenotype resembles an earlier stage of the control trajectory, whereas a biologically older value indicates resemblance to a later stage. These values describe phenotypic deviation from the reference trajectory and should not be interpreted as evidence of a specific molecular mechanism.

This global biological age serves as the primary indicator for comparing experimental conditions and rapidly identifying interventions that modify the aging trajectory.

2nd Layer – Systems & Organization Age

Once the overall biological age has been estimated, a second interpretable AI model quantifies how major physiological systems contribute to the final prediction. This intermediate level provides a functional interpretation of biological age by identifying which systems appear biologically younger or older relative to the organism as a whole.

NAGI™ Biological Age reports five major physiological domains:



Neuromuscular **Body Size** **Reproductive** **Digestive** **Structural integrity**

Modified Images from @WormAtlas

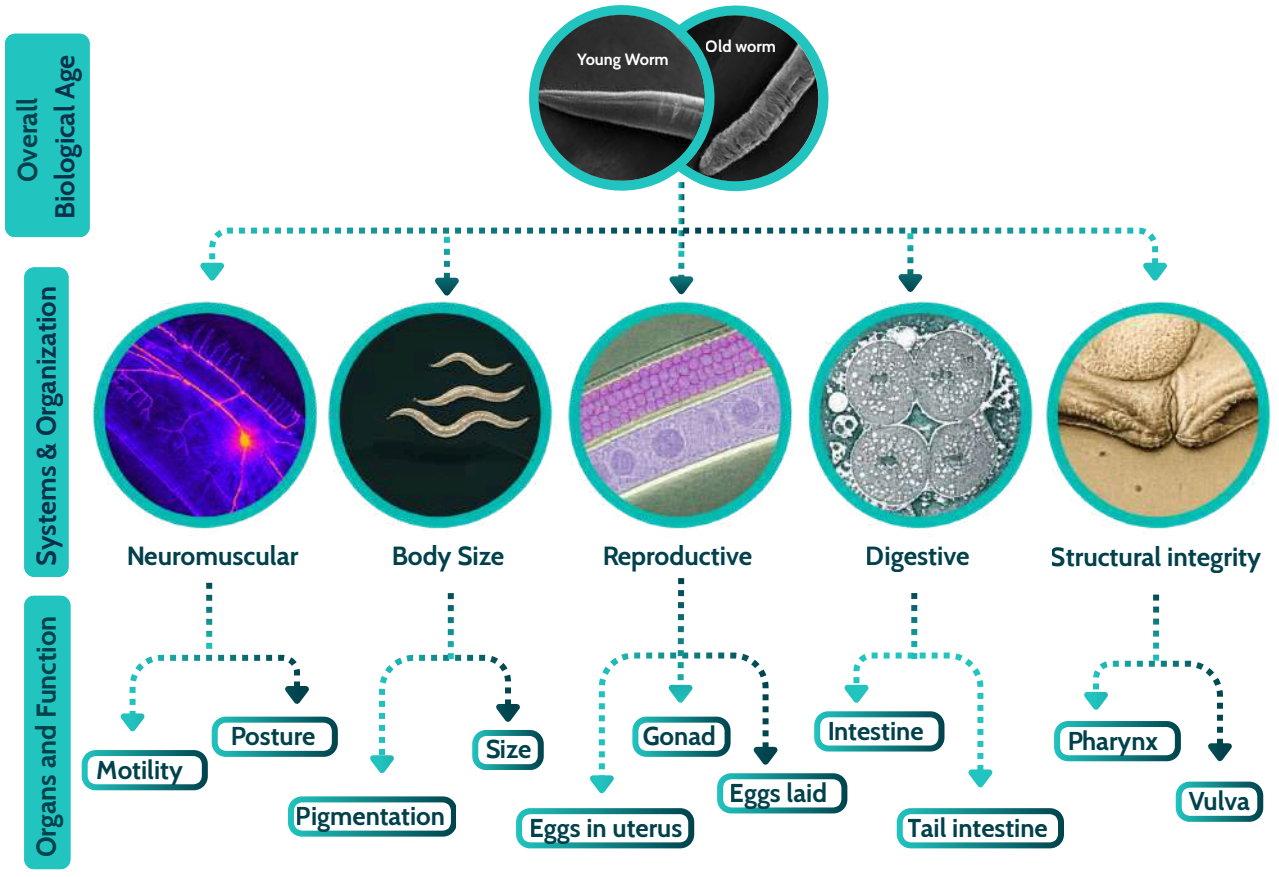
Explore the technology:
SydLab™ One Technical Note

3rd Layer – Organs & Functions Age

Each physiological system is further resolved into its underlying organs and functional biomarkers, providing the highest level of biological detail available within the phenotypic assessment (Figure 2).

This layer enables researchers to pinpoint the phenotypic feature group responsible for the observed changes in biological age and supports a more detailed interpretation of intervention-specific effects.

Organ-level outputs do not all represent the same type of biological information. For example, gonad, intestine, pharynx, and vulva scores are primarily derived from image-based morphological and appearance descriptors. Motility and eggs laid are functional readouts. "Eggs in uterus" quantifies the morphology and appearance of retained eggs rather than the morphology of the uterus itself. The biological meaning of each output must therefore be interpreted according to the measurements from which it is derived. For a detailed description, see the table in the appendix of this document (page 11).



Modified Images from @WormAtlas

Figure 2. Summary of NAGI™ Biological Age layers.

Importantly, the **Systems & Organization** and **Organs & Functions** layers are quantitative decompositions of the overall biological age. Each system and each organ contribute a fraction of the final biological age prediction, such that their combined contributions reconstruct the biological age of the whole organism. This hierarchical organization enables researchers to move from a single integrated biological age value to the physiological systems and individual functions that drive it. One organ may appear biologically younger while another appears older, and a system-level result may differ from individual organ-level results. Such apparent discordance is biologically plausible and should be interpreted using the underlying metrics, statistical significance, and complete aging trajectory.

Together, these three complementary layers transform biological age from a single predictive metric into an interpretable phenotypic framework that links organismal aging to physiological systems and individual functional biomarkers. This multiscale approach provides substantially richer biological information than conventional aging clocks while maintaining the simplicity of a single integrated biological age endpoint.

How NAGI™ Biological Age Works

NAGI™ Biological Age combines automated longitudinal phenotyping with artificial intelligence to transform complex image-derived phenotypic data into biologically interpretable aging metrics. From intervention exposure to the final biological report, the entire analytical workflow is standardized, automated, and designed to generate reproducible biological insights with minimal user intervention (Figure 3, page 9).

1 Longitudinal intervention monitoring

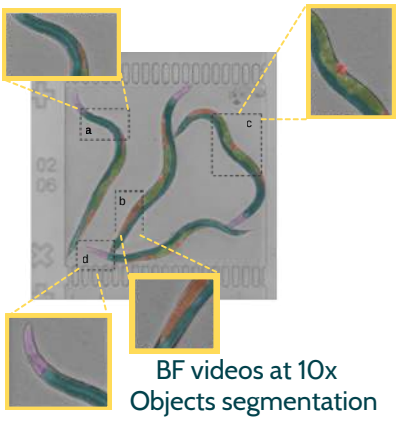
C. elegans from the L4 stage or from any subsequent time point in their lifespan are exposed to the intervention of interest within the SydLab™ One platform and maintained under tightly controlled environmental conditions throughout the experiment. If not otherwise specified, brightfield short videos (2 sec, 5 fps) are automatically acquired every 6 hours, allowing the same individuals to be monitored repeatedly across adulthood. Depending on the study objectives, experiments can be conducted over 6 to 15 days.

L4 stage *C. elegans* loading

NAGI™ Biological Age workflow

2 AI-powered image analysis

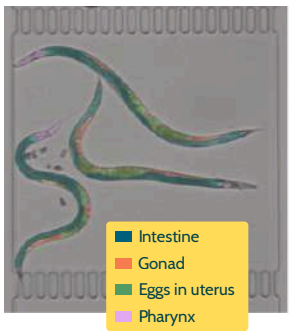
Brightfield (BF) videos of 2 seconds with 5 frames per second (8-bit, PNG) are acquired at 20°C for each of the chambers every 6 hours using a custom-made microscope. Each acquired video is processed using computer vision algorithms that automatically identify individual animals and segment the anatomical structures required for downstream phenotypic analysis. This fully automated approach enables high-content analysis without fluorescent labeling or manual image annotation.



BF videos at 10x
Objects segmentation

3 Phenotypic feature extraction

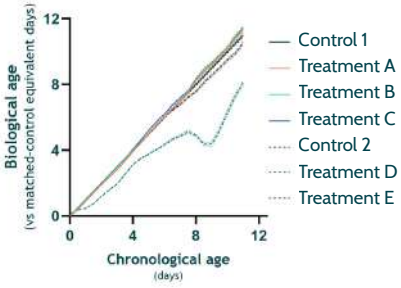
Following image segmentation, more than 60 quantitative phenotypic descriptors are extracted from the organisms and their anatomical structures. These measurements capture multiple aspects of organism physiology, including behavior, morphology, growth, reproduction, tissue morphology, and organ-specific characteristics.



60+ features extraction

4 Biological age prediction

The extracted phenotypic features are integrated by an artificial intelligence model trained on longitudinal aging trajectories to generate a quantitative biological age for each individual. Biological age values are automatically normalized relative to a user-defined control group, which is specified at the beginning of the analysis and serves as the biological reference for the experiment. This enables direct comparison of treatment groups by expressing biological age changes relative to the chosen control condition.



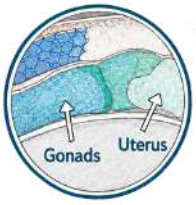
Biological Age Prediction over time

If required, users can redefine the reference control after the analysis has been completed, allowing NAGI™ Biological Age to automatically recalculate all biological age predictions and associated interpretations using the new baseline without requiring reprocessing of the experimental data.

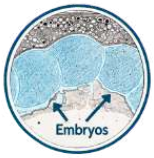
The contribution of individual features is age-dependent. For example, reproductive features are generally most informative during early adulthood, while motility and structural deterioration may become more informative later in life. The model interprets each feature in the context of its age-dependent control trajectory. For this reason, results from different time points should not be treated as directly interchangeable snapshots.

5 Multiscale biological interpretation

A second interpretable AI algorithm decomposes the biological age prediction into its physiological contributors, first identifying the relative contribution of each major biological system (Systems & Organization) and subsequently resolving these contributions into individual organs and functional (Organs & Functions) biomarkers. This hierarchical interpretation allows researchers to determine which physiological domains are driving the observed biological age.



Systems and Organisation



Organ & function

6 Automated biological report

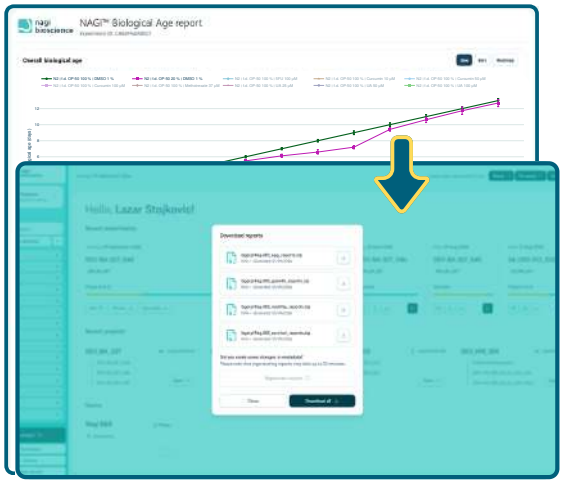
The final output combines biological age, systems and organization, organs and functions, longitudinal trajectories, and heatmaps into a comprehensive report that summarizes the biological response to the tested intervention. The resulting dataset provides both a high-level assessment of healthy aging and detailed phenotypic information for biological interpretation.

Longitudinal trajectories should be considered the primary basis for interpretation. A difference observed at a single time point may be transient, variable, or driven by the timing of a biological process. Interpretation should therefore consider the onset, direction, magnitude, duration, reproducibility, and statistical significance of the response.

NAGI™ Biological Age workflow

7 Output data and interpretation

After each experiment, NAGI™ Biological Age generates a comprehensive report that combines quantitative biological age assessment with interpretable phenotypic information. The results are excel files organized in a hierarchical manner (**biological age of the organism** > **systems and organization** > **organs and functions)** and allow researchers to evaluate intervention efficacy from the whole-organism level down to individual organs and functional biomarkers.



In addition to the summarized biological outputs, NAGI™ Biological Age provides access to the complete set of extracted phenotypic metrics used for biological age computation. These quantitative descriptors can be used for downstream statistical analyses, data exploration, or integration with complementary molecular datasets. A complete table describing all reported metrics, their biological meaning, and their corresponding physiological system is provided in the Appendix (page 13).

Summary of NAGI™ Biological Age workflow.

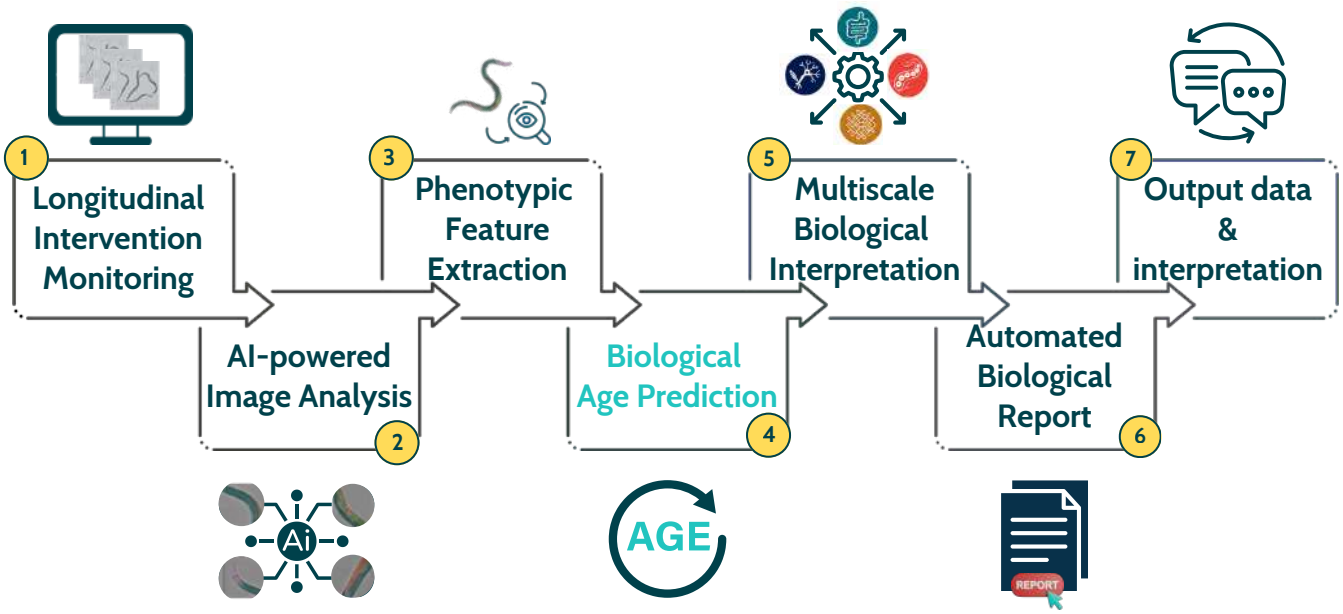


Figure 3. The 7 consecutive steps of the NAGI™ Biological Age workflow.

Orthogonal Biological Validation

To assess the biological performance of NAGI™ Biological Age, biological age predictions were compared with transcriptomic age estimates obtained from BiT age, a transcriptomic clock developed for *C. elegans* that provides a molecular benchmark for aging studies (1). The comparison included wild-type (N2), short-lived *daf-18* mutants, and long-lived *slcf-1* mutants at day 7.5, as well as calorie-restricted worms and worms treated with spermidine, a natural compound used in healthy aging, at day 5, enabling evaluation of whether phenotypic and molecular measures captured consistent responses to aging and longevity interventions.

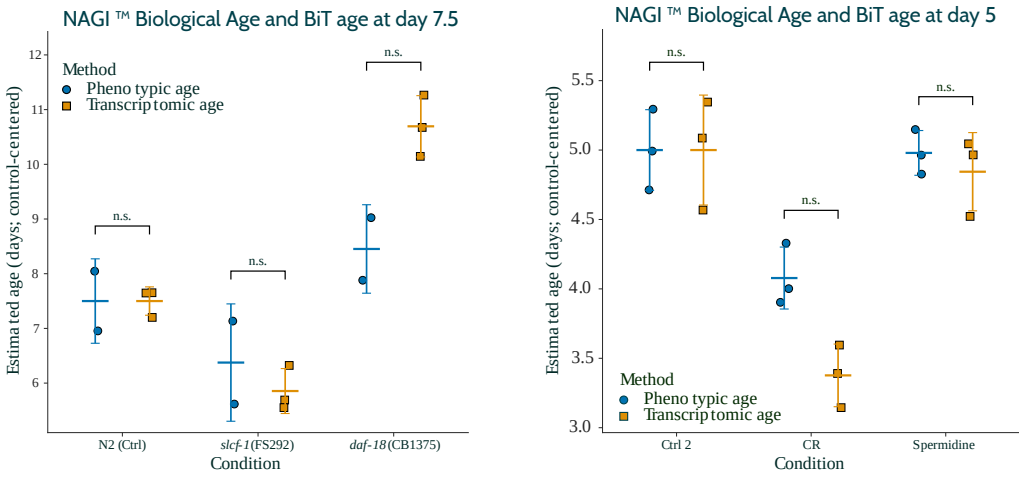


Figure 4. Orthogonal validation of NAGI™ Biological Age using transcriptomic age estimation. Boxplots show biological age estimates generated with NAGI™ Biological Age and BiT Age across genetic and intervention-based aging conditions. Wild-type worms, short-lived *daf-18* mutants, and long-lived *slcf-1* mutants were compared at day 7.5, while caloric-restricted worms and spermidine-treated worms were compared with their respective controls at day 5. Individual points at NAGI™ Biological Age represent pooled data from independent experiments. Statistical significance was assessed using a paired Wilcoxon test.

The observed agreement between phenotypic biological age and transcriptomic age demonstrates that NAGI™ Biological Age captures biologically meaningful aspects of organismal aging rather than simply recognizing image patterns associated with chronological time. Importantly, while transcriptomic approaches require destructive sampling at discrete time points, NAGI™ Biological Age provides the unique advantage of non-invasive longitudinal monitoring, enabling repeated measurements of the same individuals throughout the experiment.

This orthogonal validation provides confidence that the biological age reported by Biological Age represents a robust and biologically interpretable measure of healthy aging suitable for mechanistic studies and intervention screening.

Conclusion and Future Perspectives

NAGI™ Biological Age provides a multidimensional assessment of healthy aging by combining automated longitudinal phenotyping with interpretable artificial intelligence. Beyond estimating biological age, it identifies the physiological systems, organs, and functions driving the observed phenotype, enabling a deeper understanding of intervention efficacy than lifespan measurements alone.

By delivering reproducible, biologically interpretable outputs from non-invasive brightfield imaging, Biological Age supports healthy aging research across nutrition, biotechnology, and pharmaceutical applications.

Appendix Table

The age measurement for each layer is the interpretation of the AI algorithm of the reference features in the context of age-dependent trajectories.

Layer	Metric	Description	Biological interpretation	Example: control vs caloric restriction (CR)
1. Age of the whole organism	Biological Age	Integrated AI-derived estimate based on age-dependent behavioral, morphological, and anatomical features.	Overall phenotypic aging relative to the selected control trajectory.	A lower value under CR indicates that the integrated phenotype resembles a younger control state.
2. Systems & Organization	Neuromuscular	Integrated age estimate derived from motility and posture features.	Functional aging of movement, body bending, and neuromuscular coordination.	A younger score under CR reflect better-preserved movement or posture than the control at the same time point.
	Digestive	Integrated age estimate derived from intestine and tail-intestine morphology.	Age-related remodeling of intestinal structure.	A younger score under CR indicate better-preserved intestinal morphology.
	Reproductive	Integrated age estimate derived from gonad morphology, eggs in the uterus, and eggs laid.	Combined reproductive-organ morphology and reproductive activity.	CR may reduce early egg production but extend the reproductive period; interpretation depends on time point.
	Body Size	Integrated age estimate derived from whole-animal size and pigmentation.	Age- and intervention-related changes in growth, morphology, and appearance.	CR animals are smaller and of lighter color than controls.
	Structural Integrity	Integrated age estimate derived from pharyngeal and vulval morphology.	Age-associated structural deterioration or preservation in the segmented tissues.	A younger score under CR would suggest that these structures resemble younger controls.

Appendix Table

Layer	Metric	Description	Biological interpretation	Example: control vs caloric restriction (CR)
3. Organs & Functions	Motility	Quantification of movement using video-derived locomotor features.	Functional locomotor performance and its age-related decline.	Higher or better-preserved movement under CR supports delayed functional decline.
	Posture	Quantification of body shape patterns.	Neuromuscular coordination and age-related behavioral changes.	A more youthful posture profile under CR contribute to a younger neuromuscular score.
	Intestine	Assessment of intestinal morphology, size, texture, and appearance.	Intestinal atrophy, remodeling, and structural aging.	Reduced intestinal atrophy relative to control supports structural preservation.
	Tail Intestine	Assessment of posterior intestinal morphology and appearance.	Regional integrity and age-related remodeling of the posterior intestine.	A younger value would indicate that the tail-intestine phenotype more closely resembles younger controls.
	Gonad	Assessment of gonad size, shape, texture, and appearance.	Structural state of the gonad; not a direct measurement of fertility.	CR may produce a smaller, older-looking gonad because organ size is reduced, even if reproductive activity persists.
	Eggs in Uterus	Assessment and morphological characterization of the eggs as group, retained inside the uterus.	State of retained eggs in the uterus. Late-life detection may reflect prolonged reproductive activity or egg retention.	Eggs detected later under CR may suggest extended reproductive activity, not necessarily greater egg production.
	Eggs Laid	Assessment and timing of eggs deposited outside the animal.	Reproductive output and temporal egg-laying profile.	CR may reduce early output while extending egg laying across a longer period.
	Size	Whole-animal measurements such as body length and	Growth and body-size changes across adulthood.	CR animals are commonly smaller. This is a feature of younger organisms.
	Pigmentation	Assessment of whole-body image contrast features.	Age- or intervention-associated appearance changes.	A darker or lighter phenotype may generate a metabolic hypothesis, but lipid content requires orthogonal validation.
	Pharynx	Assessment of pharyngeal size, shape, texture, and appearance.	Structural aging of the feeding apparatus; not a direct measurement of pumping function.	A younger value indicate better-preserved morphology. Feeding activity should be confirmed through correlation with other metrics (i.e., size) and functionally if relevant.
	Vulva	Assessment of vulval morphology, texture, and appearance.	Structural integrity and age- or toxicity-associated abnormalities.	A younger value suggest fewer age-associated structural changes than control; marked abnormalities may also indicate toxicity.

Explore our multidimensional framework for healthy aging

Discover the capabilities of NAGI™ Biological Age

Let's connect

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Technical Note. September 2026.
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