Int. J. Morphol.,
44(3):829-841, 2026.

http://dx.doi.org/10.4067/s0717-95022026000300829

A Socratic–Platonic Dialogue on Quantitative Morphology and Stereological Inference

Un Diálogo Socrático-Platónico Sobre Morfología Cuantitativa e Inferencia Estereológica

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MANDARIM-DE-LACERDA, C. A. A socratic–platonic dialogue on quantitative morphology and stereological inference. *Int. J. Morphol.,44(3)*:829-841, 2026.

SUMMARY: This manuscript presents a conceptual and methodological synthesis of quantitative morphology and stereological inference, structured as a Socratic–Platonic dialogue between Socrates and Theaetetus. Through a progressive sequence of questions and answers, the dialogue contrasts descriptive morphology with quantitative approaches, emphasizing that measurement alone does not confer rigor unless embedded in an appropriate sampling design and statistical inference. Core themes include the interpretation of numerical data through their distributional properties, the treatment of digital images as quantitative entities, and the role of controlled image formation, particularly Köhler illumination, in ensuring reproducibility. The distinction between precision, accuracy, and systematic bias is examined, together with major sources of bias arising from tissue processing, sectioning artifacts, and improper inference from two-dimensional sections. Fundamental stereological principles are presented with emphasis on design-based approaches. Volume estimation by Cavalieri's principle is discussed as an example of inference independent of geometric assumptions. At the same time, the fractionator–disector method is highlighted as a robust estimator of object number independent of size, shape, or tissue volume. Model-based and design-based stereology are contrasted to underscore the shift from assumption-driven to probability-based inference. Image analysis is addressed as a tool that accelerates measurement but does not alter the definition of the experimental unit or the logic of inference. Across all examples, the manuscript argues that quantitative morphology is defined not by the accumulation of measurements but by the disciplined integration of measurement, probabilistic sampling, and statistical reasoning. When these elements form a coherent framework, morphology advances from descriptive observation to reproducible scientific knowledge.

KEY WORDS: Quantitative morphology; Design-based stereology; Bias and sampling; Image analysis; Fractionator–disector method.

INTRODUCTION

Plato's dialogue is a classical philosophical form in which knowledge is advanced through structured conversation rather than linear exposition (Plato. Theaetetus. In: Platonis Opera, ed. J. Burnet. Oxford: Oxford University Press). Typically, one interlocutor, most often Socrates, guides the discussion by posing questions that expose assumptions, reveal contradictions, and progressively refine concepts. Understanding emerges not from authoritative statements but from dialectical inquiry, in which claims are tested through reasoned exchange. This method emphasizes process over conclusion, inviting the reader to participate intellectually in the unfolding argument rather than passively receiving doctrine.

In Plato's works, dialogue serves both a pedagogical and an epistemological function: it mirrors the act of thinking itself, moving from opinion (doxa) toward justified knowledge (episteme). By dramatizing inquiry, Plato shows that rigorous reasoning depends on precise definitions, methodological awareness, and logical coherence. A paradigmatic example is Theaetetus, in which Socrates examines competing definitions of knowledge through systematic questioning, without arriving at a final dogmatic answer. This open-ended structure underscores Plato's conviction that genuine understanding emerges through disciplined inquiry rather than rhetorical persuasion.

Two supporting documents—a Best-Practice Checklist for Quantitative Morphology Studies and Table 1—are provided to guide methodological decisions and ensure transparent inference.

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Received: 2026-01-21 Accepted: 2026-04-26

Best-Practice Checklist for Quantitative Morphology Studies

A. Study Design and Biological Question

Is the primary outcome clearly defined as a planar (2-D) or spatial (3-D) parameter?
Does the biological interpretation require absolute quantities (volume, surface, length, number) or only relative/planar comparisons?
Is the reference space (organ, region, compartment) explicitly defined?
Is the chosen quantitative approach (morphometry or stereology) justified based on the dimensionality of the parameter?

B. Method Selection: Morphometry vs. Stereology

Morphometry used only for strictly planar or relative measurements without 3-D inference
Stereology selected for volume, surface, length, or number estimation
Design-based stereology is preferred when object size, shape, or distribution is heterogeneous
Tissue isotropy or anisotropy evaluated prior to sectioning strategy
Appropriate section orientation method applied (IUR, vertical sections, orientator), when required

C. Sampling Strategy

Biological experimental units clearly defined (e.g., animal, specimen, patient)
Distinction made between biological replication and technical replication
Sampling hierarchy explicitly described (sections per specimen, fields per section)
Random or systematic random sampling applied at all relevant levels
Pilot study performed to estimate variability and guide sampling density
Emphasis placed on increasing the number of biological units rather than excessive within-unit counts (“do more, less well”)

D. Digital Image Acquisition

Microscope type, objective magnification, and numerical aperture reported
Each objective is independently calibrated using a stage micrometer or equivalent
Pixel size explicitly stated and consistent across all images
Illumination, exposure, and detector settings were kept constant between groups
Lossless image formats (e.g., TIFF or RAW) used for quantitative analysis
Bit depth reported (preferably ≥12-bit for intensity-based analyses)
No post-acquisition rescaling or mixing of images with different resolutions

E. Image Analysis

Software name and version reported
Calibration verified within the analysis software
Thresholding and segmentation criteria predefined and documented
Region-of-interest selection standardized across observers and groups
Inter- and/or intra-observer variability assessed when manual steps are involved
Automated or AI-based pipelines validated against established quantitative methods

F. Stereological Implementation (if applicable)

Reference volume explicitly defined and measured
Appropriate unbiased probes selected (points, lines, frames, disectors)
Counting rules (forbidden lines, inclusion/exclusion criteria) clearly stated
Section thickness, disector height, and guard zones reported
Adequate number of points/intercepts achieved based on pilot estimates
Estimates expressed as densities and, when appropriate, converted to total quantities using the reference volume

G. Statistical Analysis

Nature of variables identified (continuous vs. discrete)
Data distribution assessed and reported
Parametric tests are used only when assumptions are reasonably met
Nonparametric tests are justified when used
Sample size rationale or power considerations described
Effect sizes and confidence intervals reported alongside P values
Error bars clearly defined (SD, SE, or confidence interval)

H. Reporting and Reproducibility

All methodological choices explicitly justified
All acquisition, analysis, and sampling parameters are transparently reported
Limitations related to method choice and potential sources of bias acknowledged
Terminology used consistently (morphometry, model-based stereology, design-based stereology)

Table I. Decision Guide for Choosing Morphometry or Stereology (Practical rule of thumb: If the parameter of interest exists inherently in three-dimensional space (volume, surface, length, number), stereology is required. Morphometry should be restricted to planar measurements and relative comparisons that do not imply three-dimensional inference.

Primary question	Nature of the parameter	Tissue characteristics	Risk of bias if morphometry is used	Recommended approach	Rationale
Do you want to measure lengths, diameters, or areas directly on sections?	Two-dimensional, planar	Isotropic or consistently oriented	Moderate (orientation and reference-point dependent)	Morphometry	Direct measurements are adequate when interpretation remains strictly planar and reference points are well defined.
Do you want to compare relative changes using ratios or percentages (e.g., area fraction)?	Relative, dimensionless	Comparable across groups	Low to moderate restore reproducibility	Morphometry or point-based stereology	Relative parameters can be reliably obtained if acquisition and sampling are consistent.
Do you want to estimate the total volume of an organ or structure?	Three-dimensional, absolute	Any tissue type	High (section thickness and deformation effects)	Stereology (Cavalieri principle)	Volume is a 3-D quantity and requires systematic random sampling across sections.
Do you want to estimate the number of cells, vessels, or particles?	Discrete, three-dimensional	Any tissue type	Very high (profile counting, loss of caps)	Design-based stereology (disector/fractionator)	Only stereology provides unbiased estimates of object number without assumptions on size or shape.
Do you want to estimate surface area (e.g., membranes, interfaces)?	Three-dimensional surface	Requires orientation control	Very high	Stereology (Sv with IUR or vertical sections)	Surface estimates are highly sensitive to section orientation and require unbiased probes.
Do you want to estimate total length (e.g., capillaries, fibers)?	One-dimensional in 3-D space	Often anisotropic	High	Stereology (Lv)	Length density cannot be inferred reliably from planar measurements.
Is the tissue anisotropic (e.g., skeletal muscle, stratified epithelia)?	Direction-dependent	Strong orientation effects	Very high	Stereology with IUR or vertical sections	Morphometric measures vary with section orientation, leading to systematic bias.
Is this an exploratory or pilot study with limited resources?	Preliminary	Any	Acceptable if clearly stated	Morphometry (with caution)	Suitable for hypothesis generation, not definitive quantification.
Is the study intended for mechanistic, translational, or clinical inference?	Quantitative, inferential	Any	Unacceptable	Design-based stereology	High rigor and unbiased estimation are required for biological and clinical interpretation.

Characters

Socrates (Athens, c. 469–399 BCE) – representing methodological scrutiny and logical discipline.

Theaetetus (Athens, c. 417-369 BCE) – representing mathematical training and empirical practice.

1. PROLOGUE (Fig. 1)

Theaetetus: The work under consideration presents extensive imaging, numerical measurements, and formal statistical analyses. Within current scientific practice, such elements are often regarded as sufficient indicators of rigor.

Socrates: Such regard is understandable, yet it is incomplete. When equivalent tissues, prepared and observed under ostensibly similar conditions, yield discordant conclusions, the discord cannot be attributed solely to nature. In such cases, the source of divergence lies in the mode of observation and in the logic by which observations are transformed into conclusions.

Theaetetus: Then rigor does not arise automatically from quantity, but from governance of method.

Socrates: Precisely so. Figure 1 establishes this distinction. Qualitative morphology persuades through appearance and analogy, whereas quantitative morphology aspires to knowledge through explicit rules of observation, sampling, and inference. Where such rules are absent, numerical description merely substitutes numerals for adjectives, without altering the epistemic status of the claim.

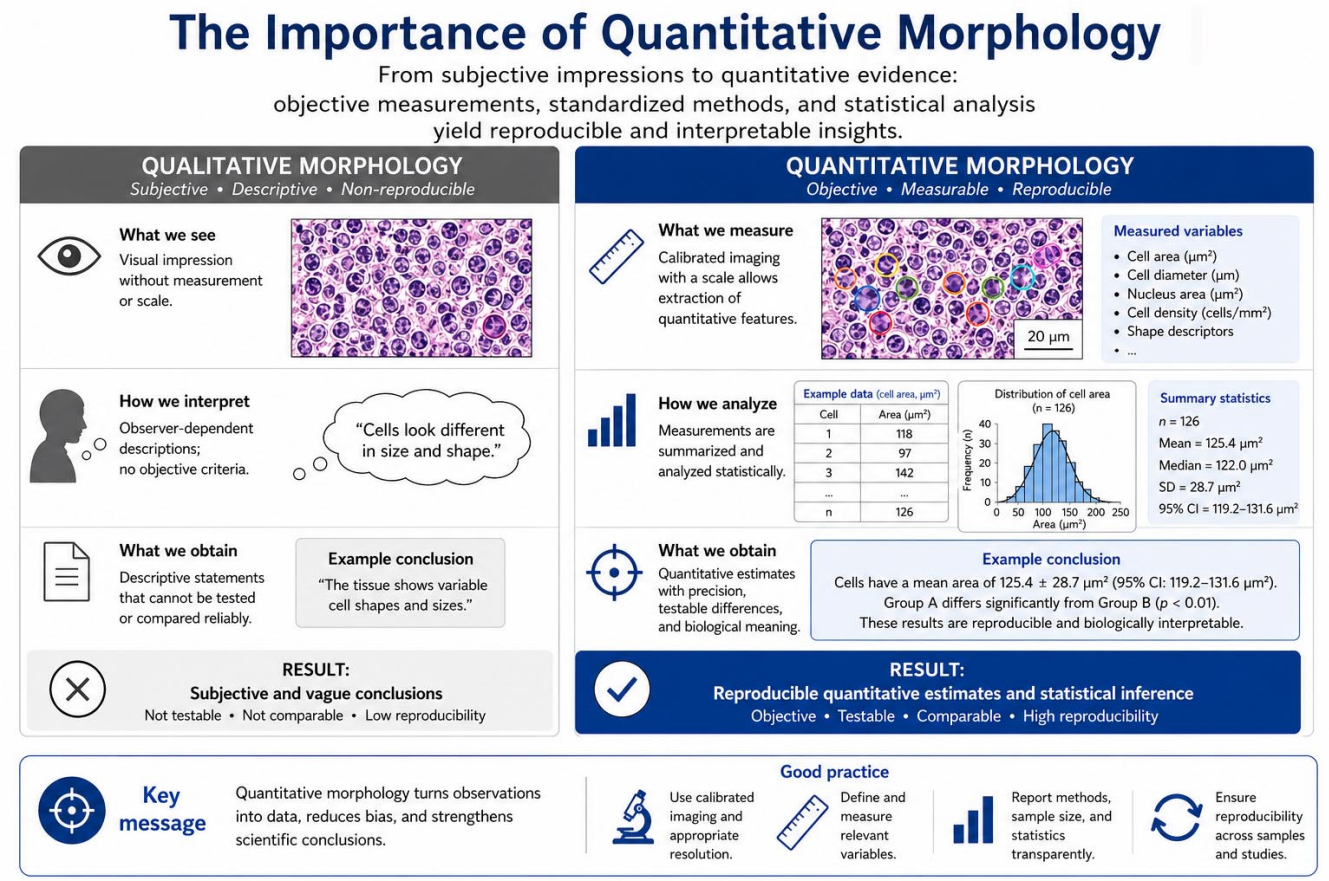

Fig. 1. Rationale for quantitative morphology. This figure contrasts qualitative and quantitative approaches to morphology. The qualitative side represents observer-dependent visual interpretation without standardized criteria, whereas the quantitative side depicts a structured workflow based on calibrated measurement, defined variables, and statistical inference. The figure emphasizes that scientific validity arises from controlled measurement, probabilistic sampling, and coherent analytical reasoning rather than from the accumulation of observations.

2. ON NUMBER AND STATISTICAL FORM (Fig. 2)

Socrates: Measurement yields numbers, yet numbers do not possess meaning in isolation.

Theaetetus: Meaning arises from their organization.

Socrates: Indeed. Figure 2 illustrates that numerical values may be symmetrically distributed, skewed, multimodal, or uniform. Each form carries distinct implications for inference. To apply a statistical test without prior examination of the distribution is to assume structure rather than to discover it.

Theaetetus: Then statistical rigor begins before hypothesis testing.

Socrates: It begins with form. Only when the distribution is understood can dispersion, central tendency, and comparison acquire legitimate meaning. Otherwise, numerical precision masks conceptual error. Statistical validity begins with verification of distributional assumptions.

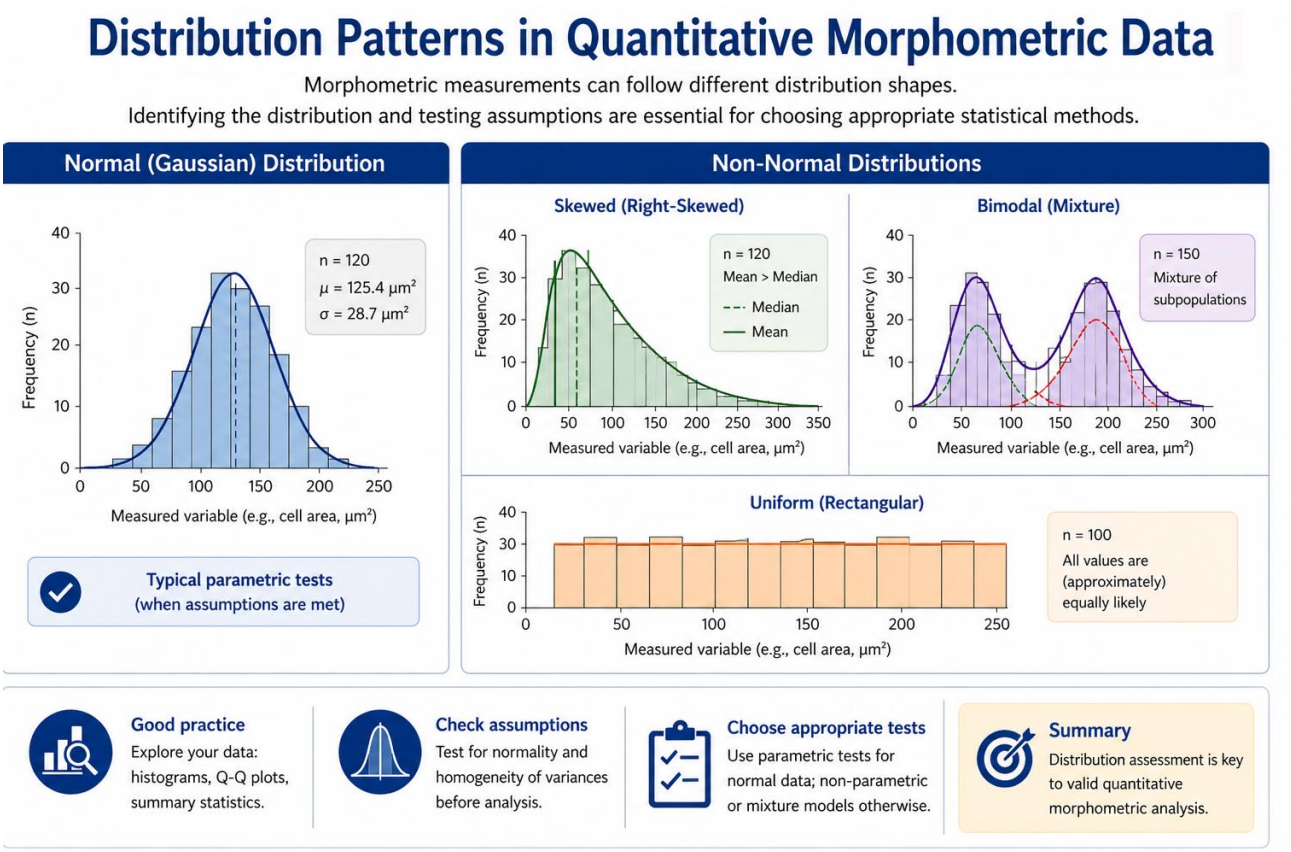

Fig. 2. Distribution patterns in quantitative morphometric data. This figure illustrates common distributional forms in morphometric datasets, including symmetric (Gaussian), skewed, bimodal, and uniform distributions. The shape of the distribution determines how to interpret the central tendency and dispersion, and whether parametric assumptions are valid. Statistical inference, therefore, depends on prior verification of distributional properties rather than on numerical summaries alone.

3. ON IMAGES AS NUMERICAL ENTITIES (Fig. 3)

Socrates: From what source do quantitative measurements most commonly arise in morphology?

Theaetetus: From images acquired by optical or digital systems.

Socrates: Then an image must be examined not as a picture, but as a numerical construct.

Theaetetus: Each pixel represents a discrete intensity value.

Socrates: Figure 3 addresses a common confusion. Spatial resolution determines the smallest resolvable feature and thus the image's information content. Dots per inch, by contrast, concern only reproduction. Quantitative analysis cannot recover information absent at acquisition, regardless of visual refinement.

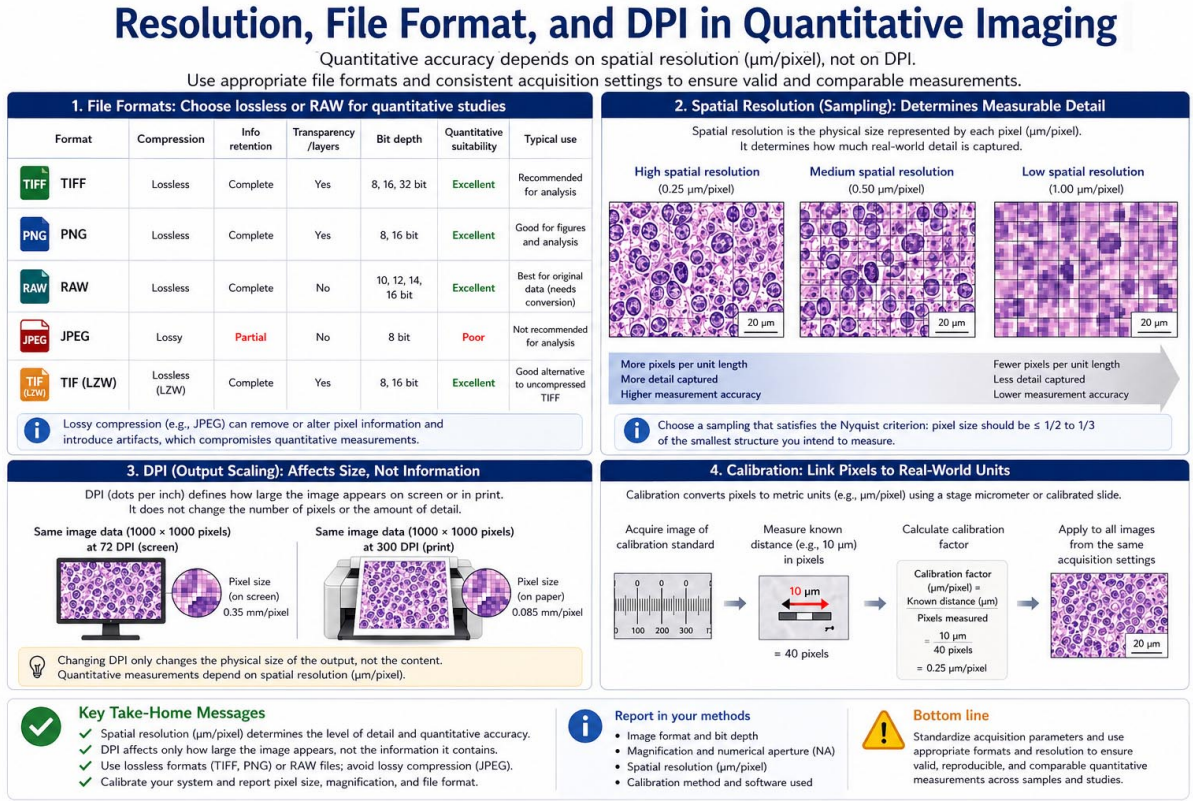

Fig. 3. Spatial resolution and image data in quantitative imaging. This figure distinguishes spatial resolution from display parameters. Spatial resolution (µm/pixel) defines the smallest measurable feature and thus the quantitative information content of the image. In contrast, dots per inch (DPI) affect only display or print scaling and do not alter underlying data. Lossless formats preserve pixel information, ensuring that measurements reflect acquired data rather than compression artifacts.

4. ON ILLUMINATION AND EPISTEMIC CONTROL (Fig. 4)

Socrates: Does adequate resolution alone guarantee faithful representation?

Theaetetus: It does not. Illumination must be uniform.

Socrates: Then illumination is a condition of knowledge rather than an aesthetic preference.

Theaetetus: Köhler illumination provides such control.

Socrates: Figure 4 demonstrates that uneven illumination generates intensity gradients indistinguishable from biological variation. In such circumstances, measurement records the optical artifact rather than the structure. Reproducibility, therefore, begins at image formation, not at numerical analysis. Such control is achieved through Köhler illumination, which standardizes the illumination field and regulates the effective numerical aperture, ensuring reproducible imaging conditions.

5. ON MEASUREMENT AND THE LIMITS OF DESCRIPTION (Fig. 5)

Socrates: With illumination controlled, measurement proceeds.

Theaetetus: Linear dimensions and areas may be obtained with high precision through calibrated videomicroscopy. Calibration, however, is not implicit. Pixel dimensions must be converted into metric units using a reference standard, as illustrated in Figure 5; otherwise, measurements lack physical meaning.

Socrates: Yet Figure 5 recalls a fundamental limitation. A histological section reveals only an intersection between a plane and a three-dimensional object.

Theaetetus: Thus, morphometry describes sectional appearances.

Socrates: Precisely. Description, even when precise, does not constitute an inference about the object as a whole. Confusion between these levels transforms measurement accuracy into interpretation error.

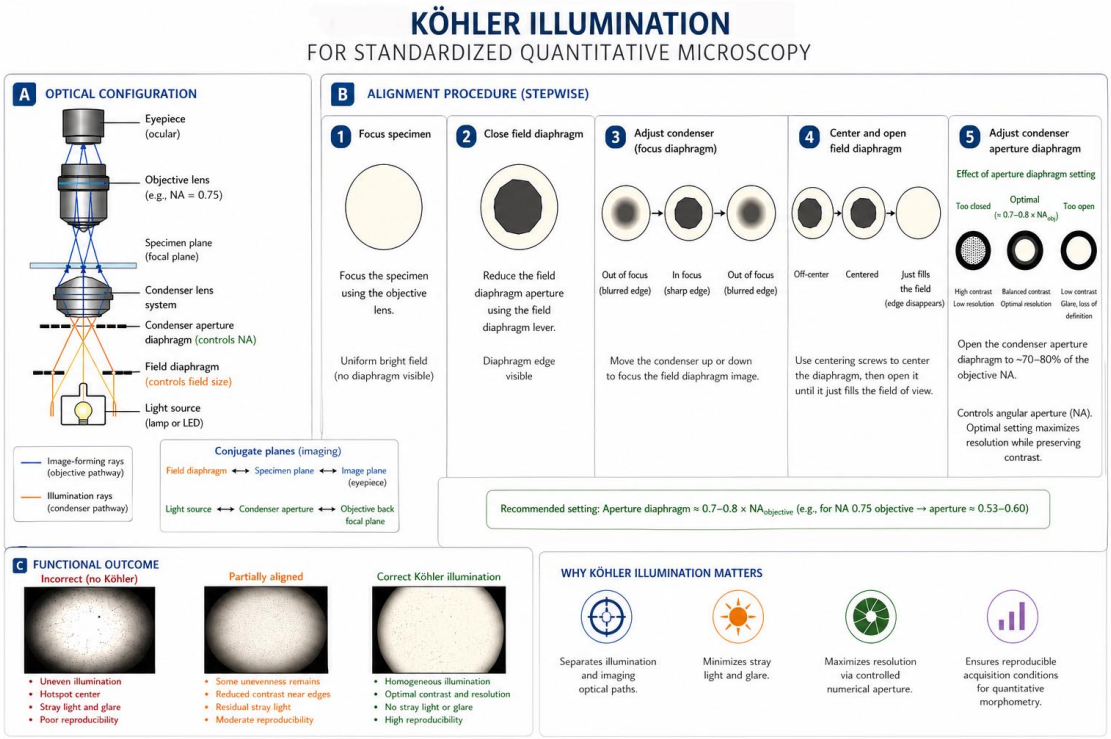

Fig. 4. Köhler illumination for standardized microscopy. This figure summarizes the principles and outcome of Köhler illumination. Proper alignment produces uniform illumination, stabilizes contrast, and minimizes optical artifacts. By controlling condenser aperture and illumination geometry, Köhler illumination ensures that intensity variations arise from specimen properties rather than imaging inconsistencies, thereby enabling reproducible quantitative measurements.

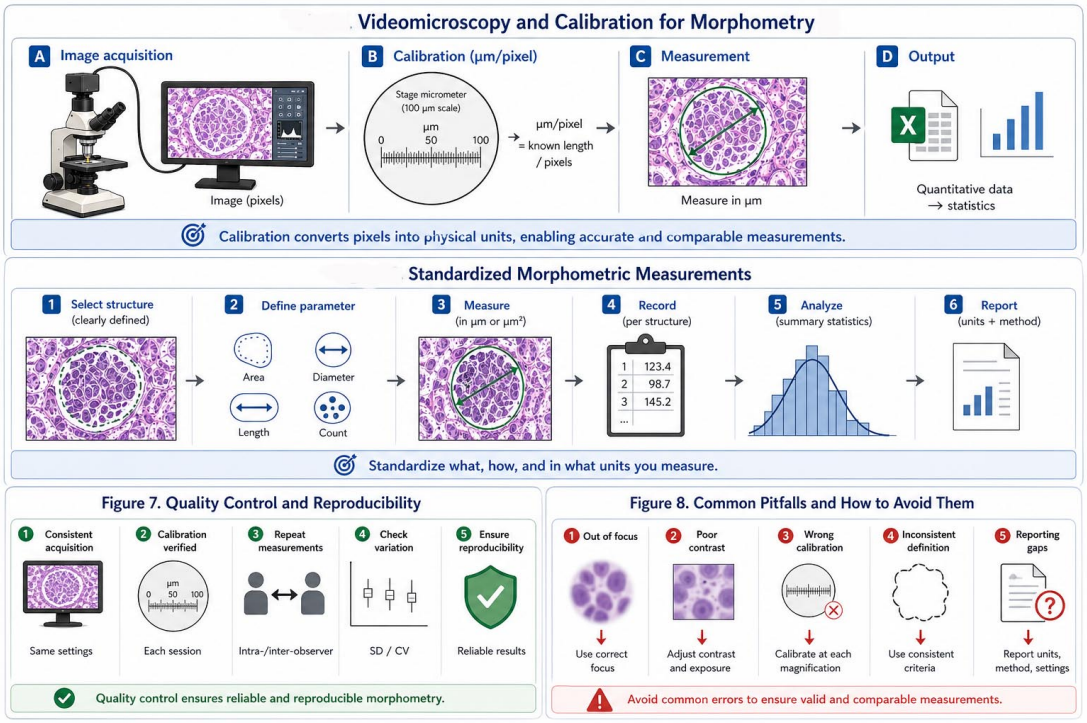

Fig. 5. Calibration in videomicroscopy. This figure illustrates the conversion of pixel-based measurements into physical units using a reference standard. A known reference distance is measured in pixels to establish a calibration factor, which is then applied to all measurements acquired under identical imaging conditions. This procedure ensures that lengths, areas, and derived quantities are expressed in consistent and traceable metric units.

6. ON PRECISION, ACCURACY, AND SYSTEMATIC BIAS (Fig. 6)

Socrates: Repetition reduces variability.

Theaetetus: Yet variability is not synonymous with error.

Socrates: Figure 6 illustrates this distinction. Precision concerns dispersion, whereas accuracy concerns correspondence with truth. Systematic bias persists irrespective of repetition. Only an appropriate sampling design and unbiased estimators can remove such error.

7. ON TISSUE TRANSFORMATION DURING PROCESSING (Fig. 7)

Socrates: Does biological tissue preserve its original dimensions throughout histological preparation?

Theaetetus: Fixation, dehydration, and embedding induce shrinkage and deformation.

Socrates: Figure 7 shows that such transformations are neither negligible nor uniform.

Theaetetus: Then, the measured dimensions no longer correspond to the original structure.

Socrates: Indeed. These changes lead to systematic underestimation of dimensions and derived quantities if uncorrected. Internal consistency does not imply validity.

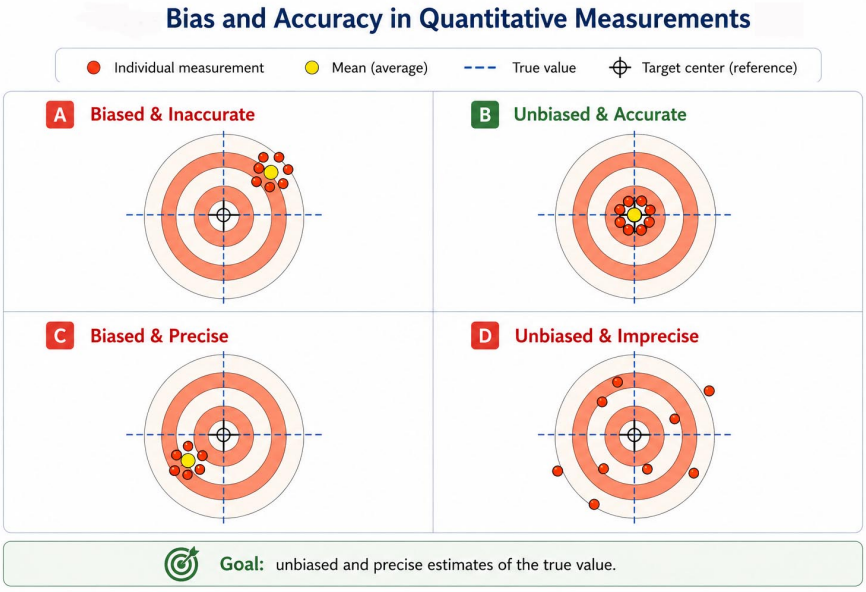

Fig. 6. Precision, accuracy, and bias in quantitative measurements. This figure distinguishes precision, accuracy, and bias using target diagrams. Accuracy refers to how close the mean measurement is to the true value, whereas precision reflects the variability among repeated measurements. Bias represents a systematic deviation from the true value and is not reduced by repetition. Reliable quantitative inference requires both high precision and the absence of bias. Key sources of bias and their control are summarized in Table 1.

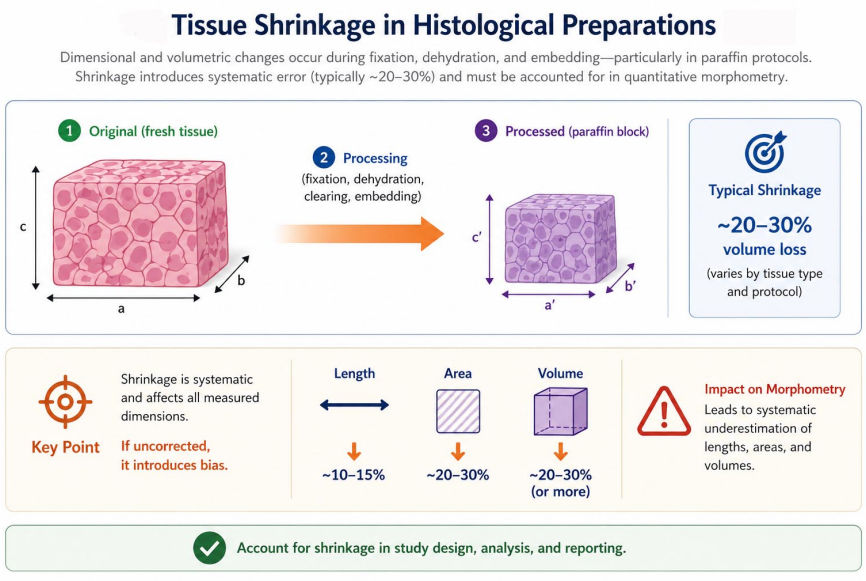

Fig. 7. Tissue shrinkage during histological processing. This figure depicts dimensional changes induced by tissue processing. Comparison between original and processed specimens highlights volumetric shrinkage, typically ranging from 20–30%, depending on protocol and tissue type. Such changes lead to systematic underestimation of size and volume if not properly accounted for, even when measurements are internally consistent.

8. ON SECTIONING AND DIMENSIONAL INFERENCE (Fig. 8)

Socrates: What dimensional information is directly accessible in routine histology?

Theaetetus: Two-dimensional information.

Socrates: Yet biological structures occupy three-dimensional space.

Theaetetus: A conceptual gap, therefore, exists between observation and inference.

Socrates: Figure 8 demonstrates that section thickness, compression, and orientation intervene in this gap. Without explicit probabilistic rules, inference across dimensions becomes conjectural.

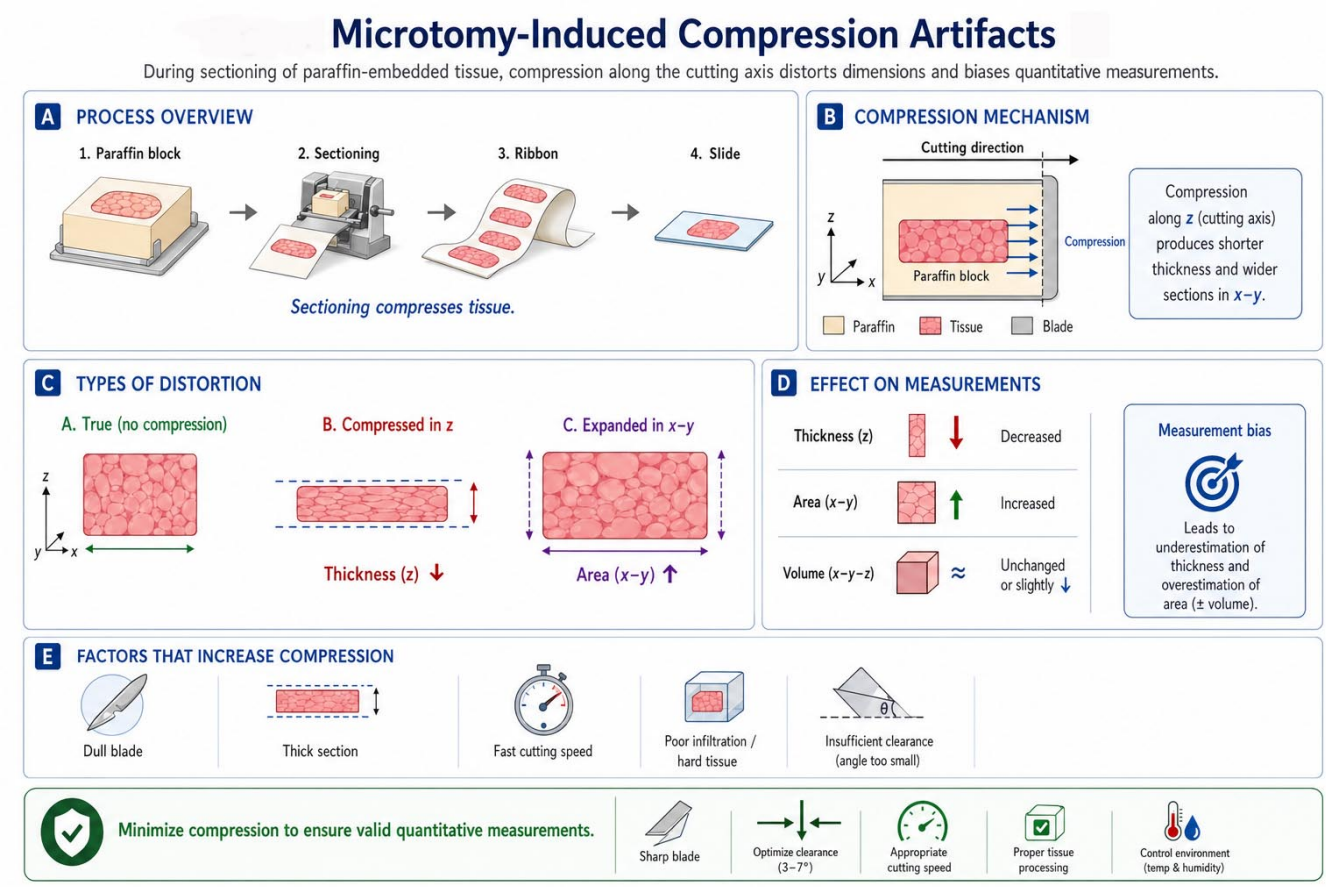

Fig. 8. Microtomy-induced compression artifacts. This figure illustrates distortions introduced during sectioning. Mechanical compression reduces section thickness while expanding dimensions in the plane of the section, resulting in anisotropic deformation. These alterations affect measured area and thickness and can bias derived volumetric estimates, especially when isotropy cannot be assumed. Practical recommendations for minimizing these artifacts are outlined in the Best-Practice Checklist.

9. ON VOLUME WITHOUT GEOMETRIC ASSUMPTION (Figs. 9 & 10)

Socrates: Volume frequently underlies biological interpretation.

Theaetetus: Displacement methods provide direct estimates for suitable specimens.

Socrates: Figure 9 illustrates this approach. However, Figure 10 introduces a principle of greater generality. Cavalieri demonstrated that volume emerges from systematic sampling of areas, independent of shape.

Theaetetus: Probability thus replaces geometry as the foundation.

Socrates: Exactly so. When sampling is systematic and known, shape assumptions become unnecessary.

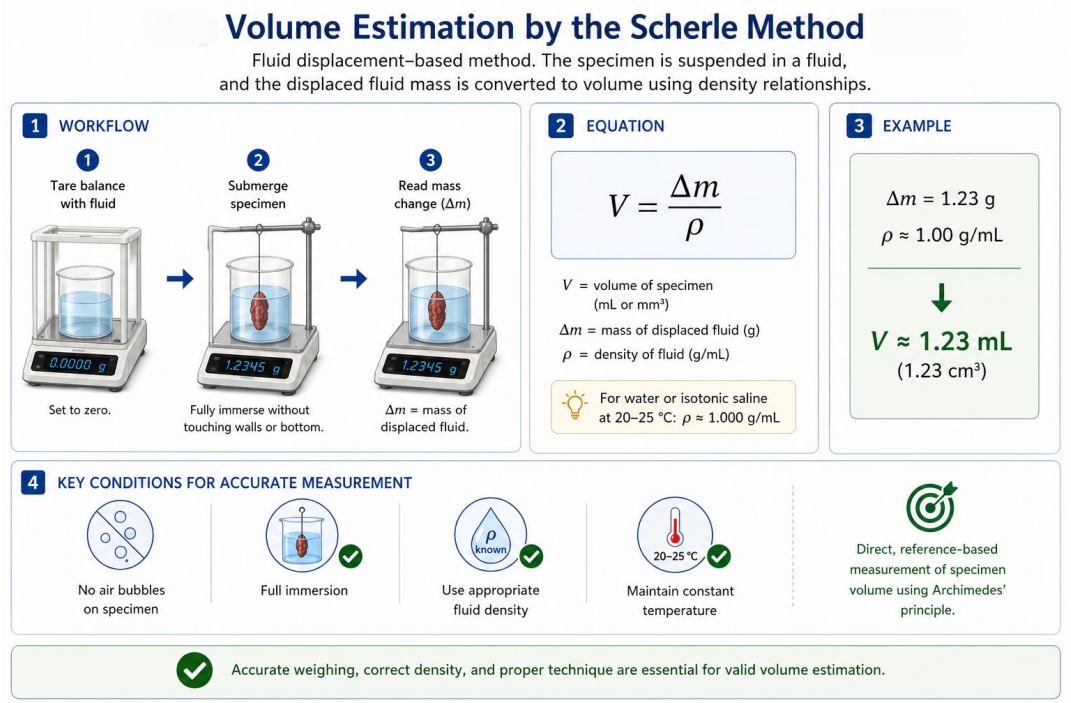

Fig. 9. Volume estimation by fluid displacement (Scherle method).This figure shows volume estimation based on fluid displacement. When a specimen is immersed, it displaces a volume of fluid equal to its own volume. The increase in measured mass corresponds to the displaced fluid and allows the calculation of the specimen volume when the fluid density is known. Accurate estimation requires complete immersion and the absence of air bubbles.

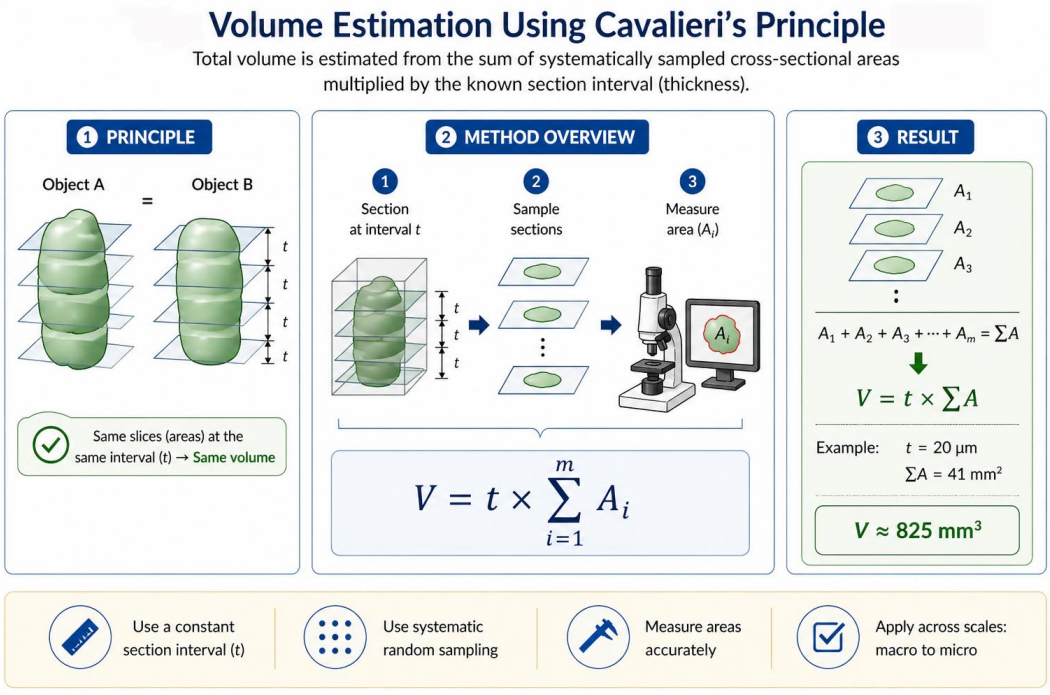

Fig. 10. Volume estimation using Cavalieri's principle. This figure illustrates volume estimation from systematically sampled sections. The object is sectioned at a known interval, and the areas of sampled sections are measured and summed. Total volume is obtained by multiplying the section interval by the sum of the sampled areas. When systematic random sampling is applied, the estimate is unbiased and independent of object shape.

10. ON IMAGE ANALYSIS AND THE MEANING OF n (Fig. 11)

Socrates: Automated image analysis accelerates classification and counting.

Theaetetus: Speed, however, does not redefine replication.

Socrates: Figure 11 shows that automated analysis involves segmentation and classification steps that generate quantitative outputs; however, these do not redefine the experimental unit.

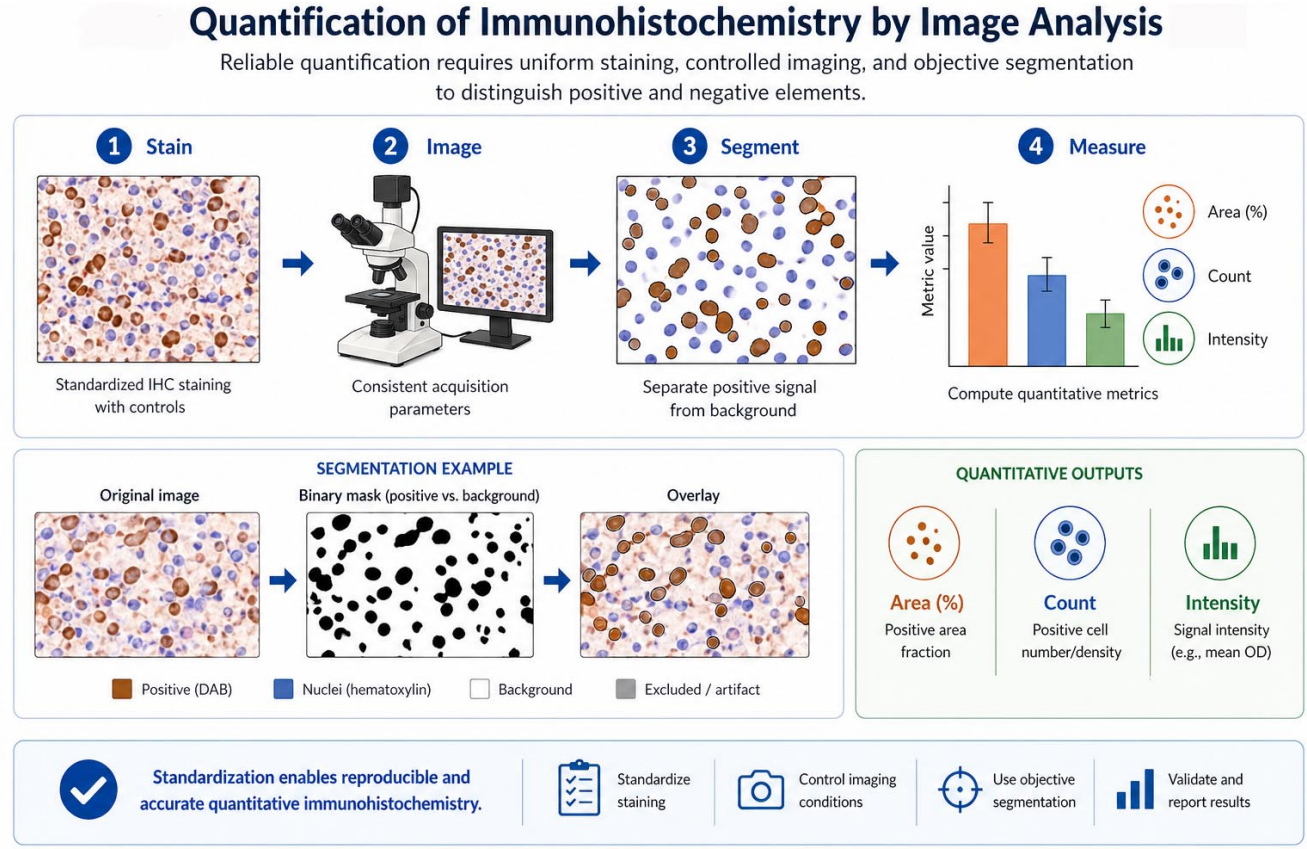

Fig. 11. Quantification of immunohistochemistry by image analysis. This figure presents the workflow of image-based quantification. After standardized staining and image acquisition, segmentation separates the signal from the background, enabling the extraction of quantitative measures such as area fraction, object counts, or intensity. The figure emphasizes that results depend on consistent acquisition and validated segmentation, and that the experimental unit remains the biological replicate rather than the number of detected elements. Segmentation accuracy must be validated to ensure reliable quantification.

11. ON ASSUMPTIONS AND STEREOLOGICAL DESIGN (Fig. 12)

Socrates: Certain quantitative methods presume specific geometric properties.

Theaetetus: Such presumptions characterize model-based stereology.

Socrates: Figure 12 contrasts this with design-based stereology, in which randomness and isotropy replace geometric belief. Inference is grounded in sampling design rather than conjecture about form. Design-based stereology replaces geometric inference with probability-based estimation.

12. ON COUNTING EXISTENCE RATHER THAN APPEARANCE (Fig. 13)

Socrates: Why do larger objects appear more frequently in sections?

Theaetetus: Because their probability of intersection is greater.

Socrates: Figure 13 shows that the disector resolves this illusion by counting existence rather than profiles. Combined with the fractionator, the total number is obtained without reference to volume, size, or shape.

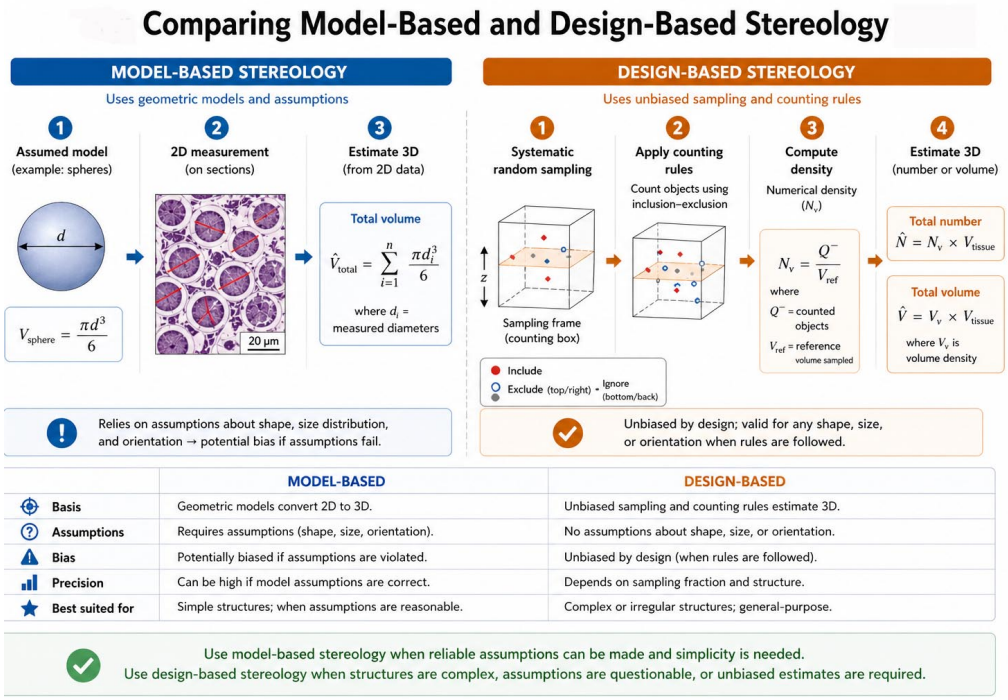

Fig. 12. Model-based versus design-based stereology. This figure contrasts two approaches to three-dimensional inference. Model-based stereology estimates parameters using geometric assumptions about object shape, whereas design-based stereology relies on systematic random sampling and unbiased counting rules. The latter avoids assumptions about size, shape, and orientation, yielding more robust, generally valid estimates. Design considerations are further systematized in Table I.

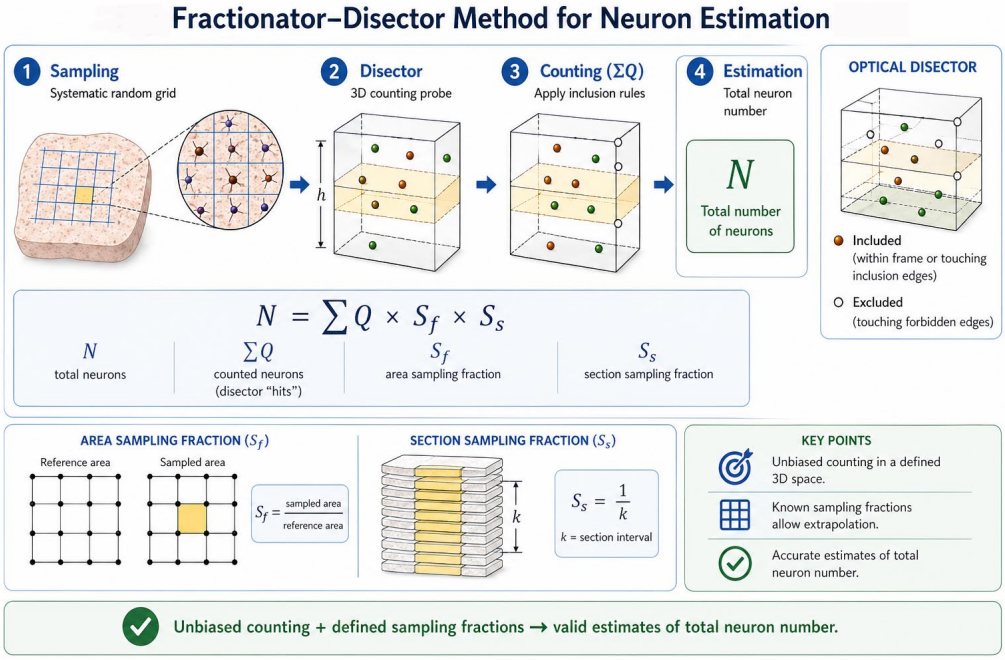

Fig. 13. Estimation of neuron number using the fractionator–disector method. This figure illustrates an unbiased estimation of total cell number using systematic random sampling combined with optical disector counting. Objects are counted within a defined three-dimensional sampling volume using inclusion and exclusion rules. The total number is obtained by scaling the counted objects by the inverse of the sampling fractions, yielding estimates that are independent of tissue volume, shrinkage, cell size, or shape.

EPILOGUE

Theaetetus: Quantitative morphology thus rests not on arithmetic alone, but on disciplined inference.

Socrates: Where observation is governed by design and inference by probability, morphology advances toward knowledge. Where these conditions are absent, numbers merely lend authority to opinion.

Final Message:

Where measurement ends, and reasoning begins, science either finds its foundation or reveals its limits.

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RESUMEN: Este manuscrito presenta una síntesis conceptual y metodológica de la morfología cuantitativa y la inferencia estereológica, estructurada como un diálogo socrático–platónico entre Sócrates y Teeteto. A través de una secuencia progresiva de preguntas y respuestas, el diálogo contrapone la morfología descriptiva a los enfoques cuantitativos, subrayando que la medición por sí sola no confiere rigor si no está integrada en un diseño de muestreo adecuado y en un marco de inferencia estadística. Entre los temas centrales se incluyen la interpretación de los datos numéricos a partir de sus propiedades de distribución, el tratamiento de las imágenes digitales como entidades cuantitativas y el control de la formación de la imagen—en particular la iluminación de Köhler—para garantizar la reproducibilidad. Se examina la distinción entre precisión, exactitud y sesgo sistemático, así como las principales fuentes de sesgo derivadas del procesamiento tisular, los artefactos de seccionamiento y la inferencia inadecuada a partir de cortes bidimensionales. Se presentan los principios fundamentales de la estereología, con énfasis en los enfoques basados en el diseño. La estimación del volumen mediante el principio de Cavalieri se analiza como un ejemplo de inferencia independiente de supuestos geométricos, mientras que el método fraccionador–disector se destaca como un estimador robusto del número de objetos, independiente del tamaño, la forma o el volumen tisular. Se comparan la estereología basada en modelos y la basada en el diseño para subrayar la transición hacia una inferencia fundamentada en el muestreo probabilístico. El análisis de imágenes se aborda como una herramienta que acelera la medición, pero no modifica la definición de la unidad experimental ni la lógica de la inferencia. En conjunto, el manuscrito sostiene que la morfología cuantitativa no se define por la acumulación de mediciones, sino por la integración disciplinada de la medición, el muestreo probabilístico y el razonamiento estadístico. Cuando estos elementos conforman un marco coherente, la morfología progresa desde la observación descriptiva hacia el conocimiento científico reproducible.

PALABRAS CLAVE: Morfología cuantitativa; Estereología basada en el diseño; Sesgo y muestreo; Análisis de imágenes; Método fraccionador–disector.

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