The Quantitative Phase Imaging & Morphological Analytics Handbook

A Comprehensive Technical Guide for Cell Culture Research, Process Development, and Manufacturing Control

Date: March 18, 2026

Version: 1.1

Abstract

Quantitative image analysis bridges the gap between qualitative microscopic observations and robust, statistically viable biological data. This handbook integrates the complete technical documentation for Thrive Bioscience's imaging suite. By utilizing the Transport of Intensity Equation (TIE) alongside advanced optical modalities, Thrive captures and performs Quantitative Phase Imaging (QPI) on 5-Megapixel tiles in under two seconds per tile. These tiles are then seamlessly stitched to provide high-resolution, full-well imaging. This document details the extraction of biomass, 2D planar morphology, 3D topology, symmetry, and spatial density metrics.

Thrive Bioscience Documentation Team

Contents

1 Introduction: High-Speed QPI and Multi-Modal Imaging

In traditional microscopy, biologists often struggle to see live, unstained cells because they are transparent and have low amplitude contrast. Thrive Bioscience eliminates traditional bottlenecks by using the **Transport of Intensity Equation (TIE)** to recover phase information from axial brightfield Z-stacks.

1.1 Tile-Based Capture and Full-Well Stitching

The Thrive system captures and performs all QPI calculations on detailed **5-Megapixel tiles**. Once the rapid Z-stack captures and QPI transforms are complete for each tile, the software seamlessly stitches these images together to produce a continuous, ultra-high-resolution full-well image.

2 Unified Feature Extraction Engine

Thrive applies an exhaustive suite of morphological and topological algorithms directly to the QPI phase maps. This section details the core metric groups visualized in Figures 1–5.

2.1 Feature Group 1: Dry Mass and Optical Thickness

Mean, Median, and Max Values: The Max Optical Thickness pinpointing the absolute densest intracellular structure. **Optical Thickness Sigma (Complexity):** The variance of the dry mass density values across the cell; a sudden drop serves as an early warning sign of culture stress.

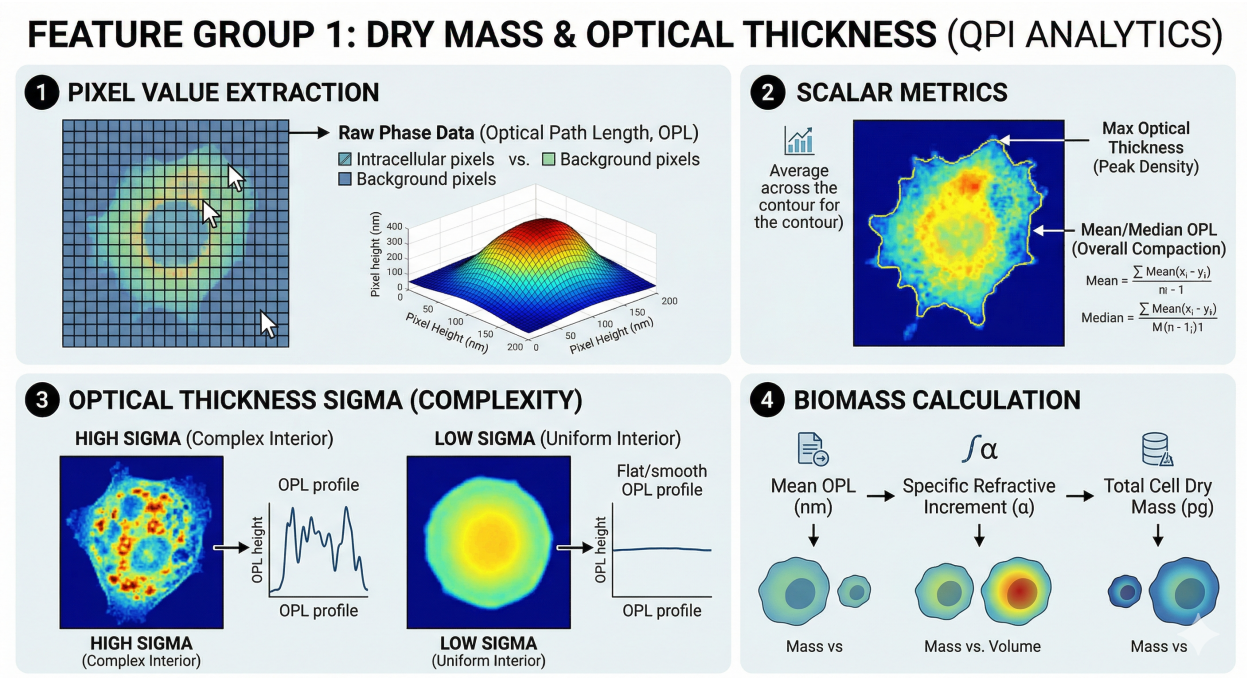

Figure 1: Feature Group 1: QPI Analytics Pipeline for Dry Mass and Optical Thickness Extraction.

2.2 Feature Group 2: 2D Planar Morphology

Area, Perimeter, and Centroid: Measures cell spreading, substrate adhesion, and tracks migration. **Feret Diameters:** Caliper metrics measure true physical extremes regardless of rotation. **Shape Form Factors:** Includes Circularity ($4\pi \times \text{Area}/\text{Perimeter}^2$) and Convexity.

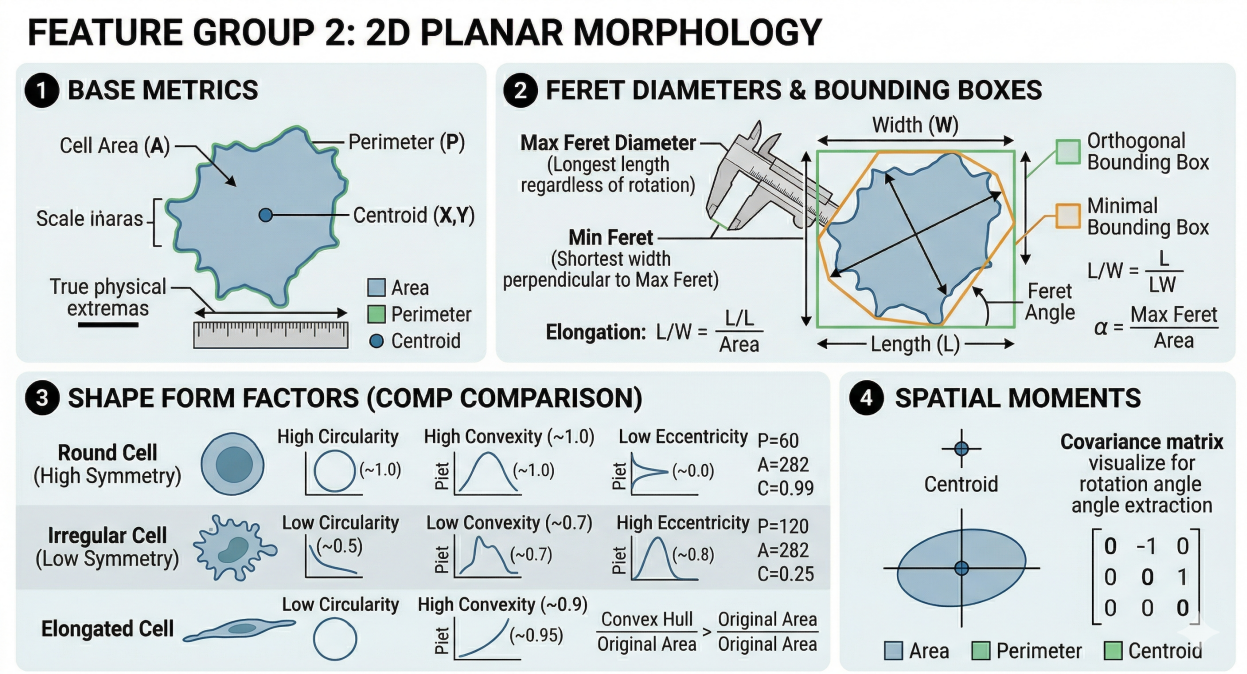

Figure 2: Feature Group 2: Planar Morphology Metrics for Geometric Characterization.

2.3 Feature Group 3: Symmetry and Polarization

Shape Symmetry (S_{shape})**:** Calculates the ratio of overlapping area after folding the mask. **Height Symmetry** (S_{height})**:** Compares mirrored pixel intensities to detect internal mass polarization long before physical deformation.

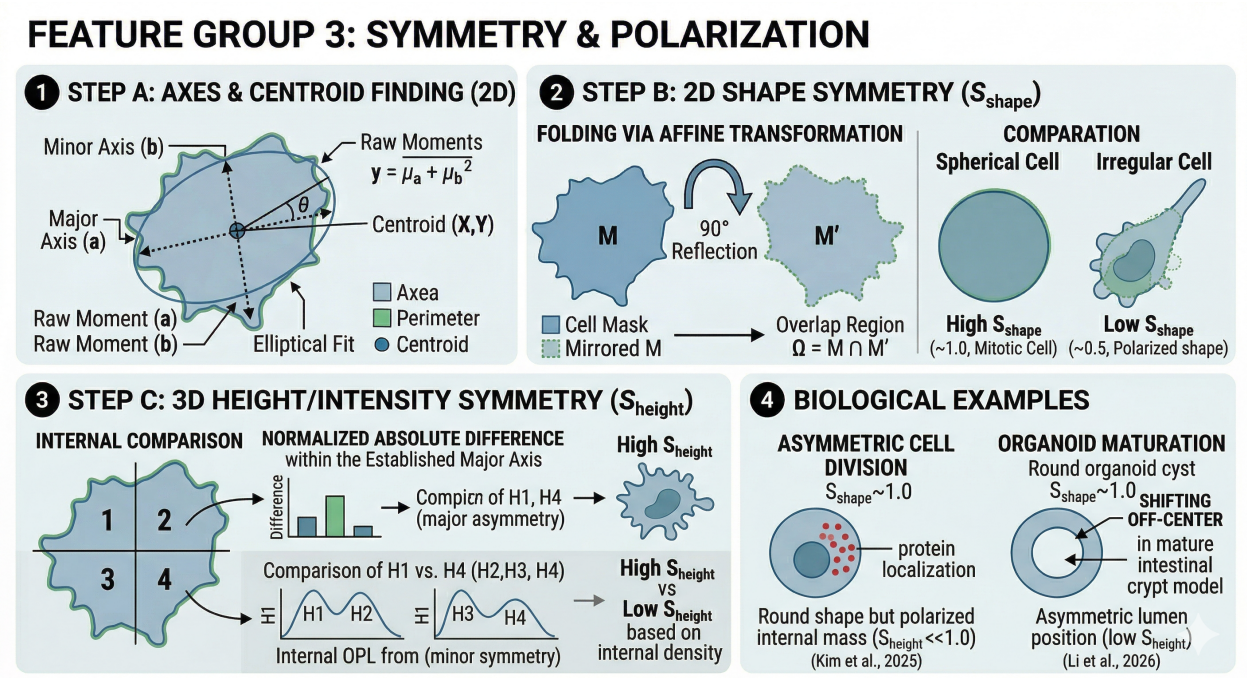

Figure 3: Feature Group 3: Symmetry & Internal Polarization Pipeline.

2.4 Feature Group 4: Surface Topology and Distribution

Brightness Ratio: Isolates the core from the periphery; a ratio > 1 indicates a proliferating edge but a sparse, necrotic center. **Central Convexity** evaluates apical curvature, while a central dip triggers the **"Is Global Volcano"** flag.

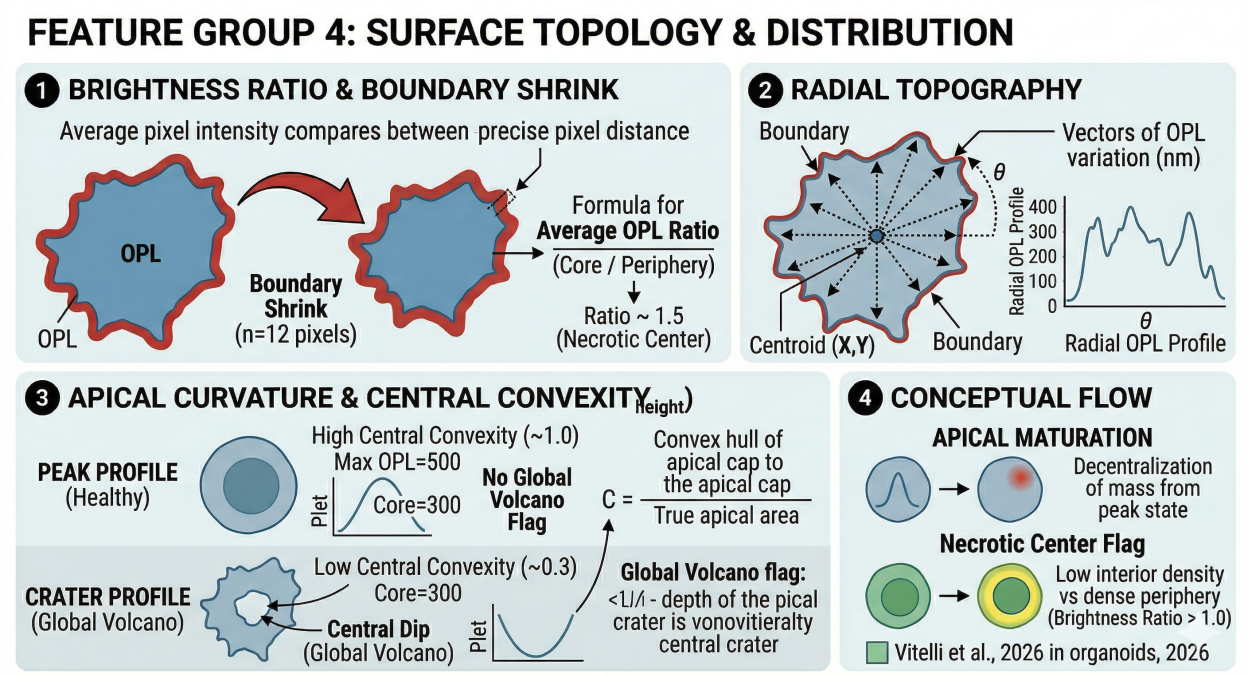

Figure 4: Feature Group 4: Surface Topology & Compaction Distribution.

2.5 Feature Group 5: Spatial Density Mapping

Detected centroids are used to generate a 32-bit Absolute Density Map. This Local Cell Crowding Score serves as a proxy for contact inhibition and G1-phase arrest.

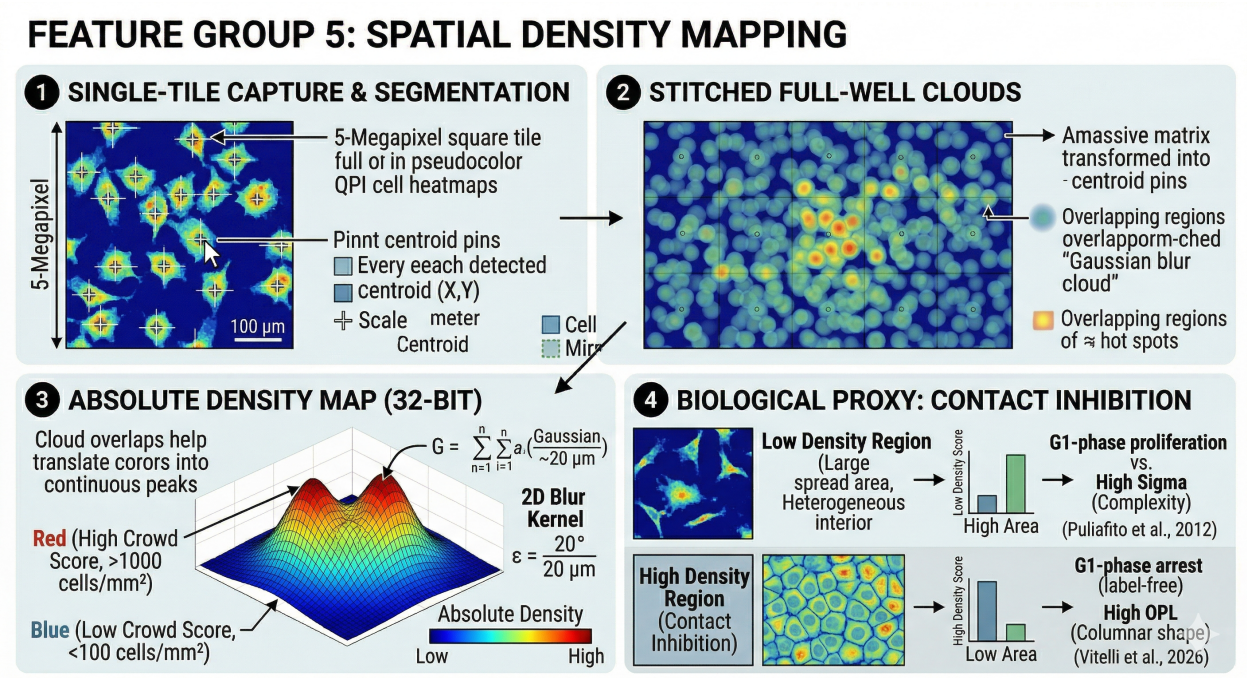

Figure 5: Feature Group 5: Spatial Density Mapping Pipeline.

A Appendix A: Literature-Inspired Applications

Note: This abridged list of applications was compiled by Gianna Vitelli at Emory University.

A.1 1. Differentiating Apoptosis from Lytic Cell Death

Vitelli tracked apoptosis by identifying early mass condensation (spike in **Max Optical Thickness**) followed by membrane blebbing [1]. Lytic cell death was tracked via a sudden drop in **Mean Optical Thickness**.

A.2 2. Macrophage Activation and Phagocytosis

Phenotypic shifts were measured by tracking increases in cell **Area** and **Perimeter** [2]. Filopodia extension was quantified via **Max Feret**, while internal lysosomal accumulation was measured via **Optical Thickness Sigma**.

A.3 3. 3D Organoid Morphology and Lumen Formation

Internal organoid maturation was mapped to Thrive features without fluorescent sectioning [3, 4]. By analyzing the **Brightness Ratio**, necrotic cores can be identified (Ratio > 1).

A.4 4. Quantifying Cellular Senescence

Senescent populations were identified by abnormally large **Area** combined with a **Brightness Ratio** approaching 1.0 [5].

A.5 5. Directional Migration and Cell Polarization

Highly polarized, migrating cells correspond to high **Eccentricity** and high **Shape Symmetry** [6]. **Height Symmetry** serves to detect early internal mass polarization.

A.6 6. Contact Inhibition and Density-Dependent Arrest

Vitelli applied the **Spatial Density Map** to predict G1 arrest label-free [7]. High density scores correlated with reduced **Area** but increased **Max Optical Thickness**.

References

[1] Kučera, et al. "The Quantitative-Phase Dynamics of Apoptosis and Lytic Cell Death." *Cell Death Discovery*, 2020.

[2] Pavillon, et al. "Noninvasive detection of macrophage activation." *PNAS*, 2018.

[3] Kim, et al. "Morphology-Preserving Holotomography." *arXiv*, 2025.

[4] Li, et al. "Scattering-enabled epi-quantitative phase imaging." *bioRxiv*, 2026.

[5] Hernandez-Segura, et al. "Hallmarks of Cellular Senescence." *Trends in Cell Biology*, 2018.

[6] Ridley, et al. "Cell Migration: Integrating Signals from Front to Back." *Science*, 2003.

[7] Puliafito, et al. "Collective and single cell behavior in epithelial contact inhibition." *PNAS*, 2012.