A Rheo-Optical Compression Assay for Viscoelastic Characterization of Cellular Spheroids

R. Ferraro [a,b], S. Guido [a,b] & S. Caserta [a,b]

[a] Università degli Studi di Napoli Federico II, Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale, P.le V. Tecchio 80, 80125 Napoli, Italy

[b] CEINGE Biotecnologie Avanzate Franco Salvatore, Via Gaetano Salvatore, 486, 80131 Napoli, Italy

E-mail: steguido@unina.it

Mechanical characterization of cellular spheroids plays a pivotal role in 3D biology and cancer research. However, traditional approaches often require complex instrumentation and specialized expertise, limiting their widespread adoption. To address these challenges, we present a novel, cost-effective, and user-friendly rheo-optical compression assay that enhances accessibility to mechanobiological studies.

Our method employs standard microscopy glass coverslips to apply controlled compressive loads to spheroids cultured in conventional multiwell plates. Real-time visualization and quantification of spheroid deformation under mechanical stress is obtained by exploiting the optical capabilities of standard laboratory microscopes and smartphones equipped with simple magnifying lenses. This straightforward setup significantly lowers technical barriers, making mechanical testing feasible for a wide range of biomedical laboratories.

We validated the technique using agarose gel particles, showing excellent agreement with results from standard rheological instruments. The assay was then applied to spheroids derived from different cell lines, including the non-tumoral NIH/3T3 and the tumoral PANC-1. Through transient rheo-optical compression tests and application of the Burger model, we captured their nonlinear viscoelastic behaviour, extracting key parameters such as the storage (E') and loss (E'') moduli across a range of frequencies. These measurements effectively discriminated between the mechanical signatures of different cell types. Integrating this rheo-optical assay into standard cell culture workflows offers a powerful tool for rapid, quantitative mechanical profiling of spheroids, paving the way for broader mechanobiological and oncological investigations.

Keywords: *Cell spheroids, rheo-optics, viscoelasticity*

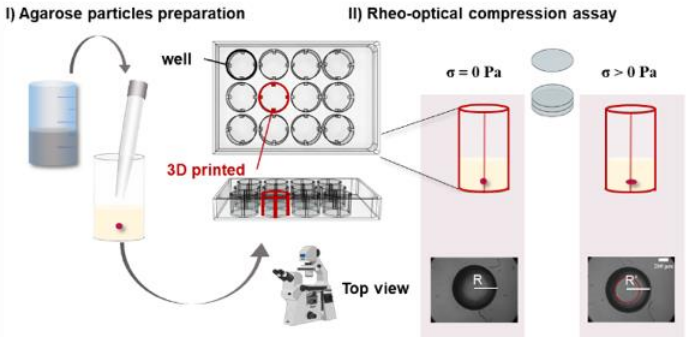

Figure 1. Our rheo-optical compression assay scheme.