

Abstract of “Microcavitation as a Neural Cell Damage Mechanism in an *In Vitro* Model of Blast Traumatic Brain Injury”

by Jonathan B. Estrada, Ph.D., Brown University, May 2017.

Blast traumatic brain injury is a serious type of injury in the armed forces and considered the signature injury of the wars in Iraq and Afghanistan. While compressive hydrostatic pressures resulting from blast waves have been investigated, negative pressures resulting from the blast wave and wave-brain tissue interaction have the potential to nucleate bubbles in the nearly-incompressible tissue, leading to a separate damage mechanism of inertial cavitation. The specific relation between the mechanics of inertial cavitation and primary cell injury, to date, has not been investigated in detail. While inertial cavitation damage to free surfaces has been well-studied in the field of fluid dynamics, bubble interactions within 3D neural cultures and hydrogels are in their infancy, particularly experimentally. With the goal of quantifying the specific effects of inertial microcavitation as the proposed cellular injury mechanism in blast traumatic brain injury, presented here is an experimental approach of initiating inertial microcavitation in primary dissociated 3D neural cultures coupled with a new analysis technique for computing the critical local strains and stresses around cells that are found to induce cellular injury and mechanical disruption. Bubble dynamics during injury are quantified using high-speed imaging and custom image processing algorithms, while laser scanning confocal microscope image stacks of neurons before and after cavitation injury correlate bubble kinematics to fragmentation and disruption of cytoskeletal proteins. To get the high-rate constitutive behavior of the surrounding matrix, and subsequently spatiotemporal strains, strain rates, and stresses on the cells during cavitation, an approach called inertial microcavitation-based ultra-high-strain rate rheometry (IMUR) is presented as a new theoretical-numerical technique to assess local rheological properties of soft materials.

Microcavitation as a Neural Cell Damage Mechanism in an
In Vitro Model of Blast Traumatic Brain Injury

by

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DEDICATED TO MY FAMILY,
AND TO THOSE WATCHING OVER ME.

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Chapter 1

Introduction

1.1 Cavitation mechanics in soft and biological materials

Foundational work on pressures induced by collapse of a single cavitation bubble in an incompressible, infinite liquid was presented exactly one century ago by Lord Rayleigh [1], paving the way for extensions by Plesset [2] to incorporate pressure gradients, Keller and Miksis [3] to incorporate local finite wave speeds, and Prosperetti [4] to gas behavior more sophisticated than the polytropic gas assumption, including heat transfer. Motivated primarily by cavitation-related damage of, for example, underwater blast pressures and damage induced to underwater propellers during use, the early literature focused heavily on cavitation in fluid systems, with later modifications to fluid constitutive properties by considering viscoelastic models e.g. by Fogler and Goddard [5], Tanasawa and Yang [6], Shima et al. [7], and others.

With the recent but increasingly widespread usage of many focused energy techniques in biology and medicine including diagnostic ultrasound [8–10], therapeutic ultrasound for cancer treatment [11], ultrasound litho/histotripsy [12–14], and laser ablation techniques [15,16], a more thorough understanding of the potential biological effects of inertial cavitation became increasingly necessary. Improvements on inertial cavitation dynamics theory for biological applications were made by Yang and Church [17] to incorporate a solid viscoelastic model

intended to mimic biological tissue behavior, and Kreider et al. [18] to incorporate water vapor mass transfer over the bubble wall, among many others. Various approaches, ranging from computational fluid dynamics to experimental ultrasound array platforms and laser-induced cavitation setups have been designed, investigating a wide range of phenomena.

However, predictive cellular models in response to the mechanics of cavitation-based cell injury have been limited. Cavitation studies by Rau et al. [16, 19] were among the first in connecting bubble expansion to resulting damage zone information, but primarily focused on the complex problem of bubble collapse inducing fluid shear on cell-coated 2D surfaces. The specific effects of mechanical strains induced by spherical cavitation bubbles to date has remained conspicuously absent from the literature. Decoupling strain from fluid shear allows for comparison of the relative damage each induces on cells, and would provide critically important information to the modeling community, such as strain, strain rate, and stress damage criteria.

1.2 Blast traumatic brain injury

In the United States, over 1.7 million people annually suffer some form of traumatic brain injury (TBI) that requires hospitalization [20], which results in significant public health and economic burden [21]. Not only is TBI the leading cause of mortality and disability among people under 45 years of age in the United States [22], but even mild TBI prevalence is a risk factor for a host of other diseases, such as Alzheimer’s disease [23, 24], chronic traumatic encephalopathy/dementia [25, 26], post-traumatic stress disorder and depression [27, 28], among others.

TBI results from either, or some combination of, direct or indirect loading to the head, and results in disruption of normal brain function [29]. While mild blunt TBI represents the prevalent TBI classification in civilians, indirect, explosive blasts have become one of the more serious causes of TBI in the armed forces and have necessitated a separate class of so-called blast TBI, which is largely considered the signature injury of the wars in Iraq

and Afghanistan [30]. According to the Defense Veterans Brain Injury Center, between 10-20% of troops engaged in active combat in Iraq and Afghanistan for four months or more have reported symptoms of blast TBI (e.g. loss of consciousness, altered mental status) caused by improvised explosive devices [31]. The recent observation and consideration of blast injury as a distinct TBI mechanism in these modern wars could be explained in part by improvements in helmets and battle armor, which have stopped shrapnel from inducing penetrating TBI while perhaps not being optimized for protection against blast [32].

The mechanism by which blast injury interacts with the body as a whole is complex and multifaceted. While this work focuses on the effects of primary blast TBI, other blast TBI classifications are listed in Table 1.1. The wave inducing primary blast injury consists of a

Table 1.1: Brain injury mechanism classification, adapted from [37].

Blast TBI Classification	
1. Primary	Blast overpressure; internal transmission, reflection, refraction of stress waves Gas- and liquid-filled organs and structures at risk for damage
2. Secondary	Shrapnel or objects moved by blast impacting the head
3. Tertiary	Blast force causing the victim to strike surrounding objects or surfaces
4. Quaternary	Effects of smoke, environmental debris, infection, or other environmental considerations

shock wave, or overpressure, followed by a subsequent low-pressure region, described mathematically by the Friedlander equation [33]. Unlike impacts or collisions commonly attributed to primary blunt trauma, primary blast injury is distinct due to this rapid overpressure that travels in excess of the sound speed and for the duration of just milliseconds [34]. Environmental boundary effects during primary blast propagation, such as reflections of the usually-spherical wave with the ground, walls, or other surfaces, can amplify overpressures locally, i.e. by Mach stem formation [35]. Air-filled structures in the body, such as the inner ear, intestines, sinus cavities and lungs are at particular risk and are commonly damaged (e.g. tympanic rupture, blast lung) during the primary blast pressure transmission and reflection [36].

While the danger of high-amplitude pressure wave interaction with air-filled structures is

intuitive from a physical standpoint, many studies have focused on characterizing clinical symptoms of blast injury [32, 37–40] and found positive correlation with traumatic brain injury. The commonality of blast-induced neurotrauma suggests additional mechanisms of injury to the central nervous system during primary injury [41]. Primary injury to the brain and spinal cord are a result of the shock wave in addition to pressure damage caused by gas embolism, which can, in turn, cause tissue ischemia and/or infarction. Direct effects of the blast pressure wave transmission, reflection, and refraction inside the brain are similarly considered primary injury [37]. The exact mechanism by which the blast wave damages brain tissue and cells, however, is not yet known. The most characterized pathophysiological mechanisms associated with blunt TBI are diffuse neuronal injury (including diffuse axonal injury, or DAI), tissue damage, and contusion due to coup/contrecoup injury [42]. These mechanisms are also seen in moderate or more severe blast TBI, but are often combined with secondary and tertiary effects as well [40], which makes delineation from primary blast TBI challenging.

Separating the effects of primary versus secondary or tertiary traumatic brain injury to focus on effects at the cellular level thus can benefit from *in vitro* isolation, which has proven promising and repeatable for blunt TBI reproduction as reviewed over many studies by Morrison III [43]. Experimentally, animal models are commonly used for investigation of aspects of many different types of TBI as described at length in Xiong et al. [44], but while pathophysiological mechanisms can be identified, further tests for neuroprotective drugs have universally failed to date, and considerable limitations (e.g. physiology, live injury assessment, comorbidity with other injuries) still remain. To this end, a variety of *in vitro* surrogates have been employed, ranging from acute [45, 46] to organotypic [47–49], dissociated primary cell culture [50–52], and immortalized cell line [53, 54] platforms. Acute and tissue-based models are systems most physiologically representative of the *in vivo* brain, but face significant challenges with long-term culturing and sample-to-sample reproducibility. In contrast, primary cell and immortalized cell cultures require employment of alternate 3D extracellular matrices to gain physiological relevance [55]. However, simplifying the experimental platform to 3D cell-hydrogel models has considerable advantages, particularly the

ability to control mechanical loading conditions to create, for example, shear [51], biaxial stretch [56], or uniaxial compression [57]. Furthermore, the alteration of apoptotic machinery and intracellular signaling cascades for apoptosis in immortalized cell lines [43] makes primary dissociated cells preferable in injury models.

While loading conditions mimicking those found in blunt TBI are reproducible via conventional mechanical systems, experimentally reproducing the mechanics of blast injury at the cellular level is a significant challenge in large part due to recreation of blast-associated high strain rates. Experimental apparatus, for this reason, is commonly limited to blast wave generation with shock or blast tubes, which may produce more complex loading conditions than currently completely understood [41,58]. To fully understand and characterize the injury mechanism, it is important to try and isolate the principal damaging agent of the blast wave. Increasing evidence points to collapsing inertial cavitation bubbles as a potential key blast damage mechanism [59,60], as opposed to the wave overpressure. At critically low pressures from any type of loading condition, cavitation injury can be induced in tissue [61,62]. Inertial (sometimes called temporary) cavitation is a well-known injury mechanism in ballistic or penetrating trauma, in which projectiles at high speeds leave low-pressure regions in their wakes, creating large radial deformations which are particularly dangerous and damaging in the confined space of the brain [63]. Various observations in tissue affected by blast injury such as astroglial scarring foci at large tissue interfaces [64,65], small hemorrhages, and chromatolytic changes in white matter neurons [66] suggest the possibility of damage from a small bubble collapse mechanism. The methods by which local pressures become critically low, and thus microcavitation is hypothesized to occur, are schematically shown in Fig. 1.1. The blast front, which consists of a high pressure front and underpressure tail, first contacts the skull, inducing a low pressure region which induces cavitation at the contrecoup region of the brain (Fig. 1.1A). Furthermore, impedance mismatch (Fig. 1.1B) and wave transmission, reflection, and refraction contributing to local constructive interference (Fig. 1.1C) are hypothesized mechanisms for shock induction of cavitation. While bubble growth represents one potential damage-inducing mechanism of

cavitation, collapse, particularly in the case of asymmetric local properties (e.g. inhomogeneous matrix, boundaries), may contribute to damage as well due to jet-formation from asymmetric bubble collapse.

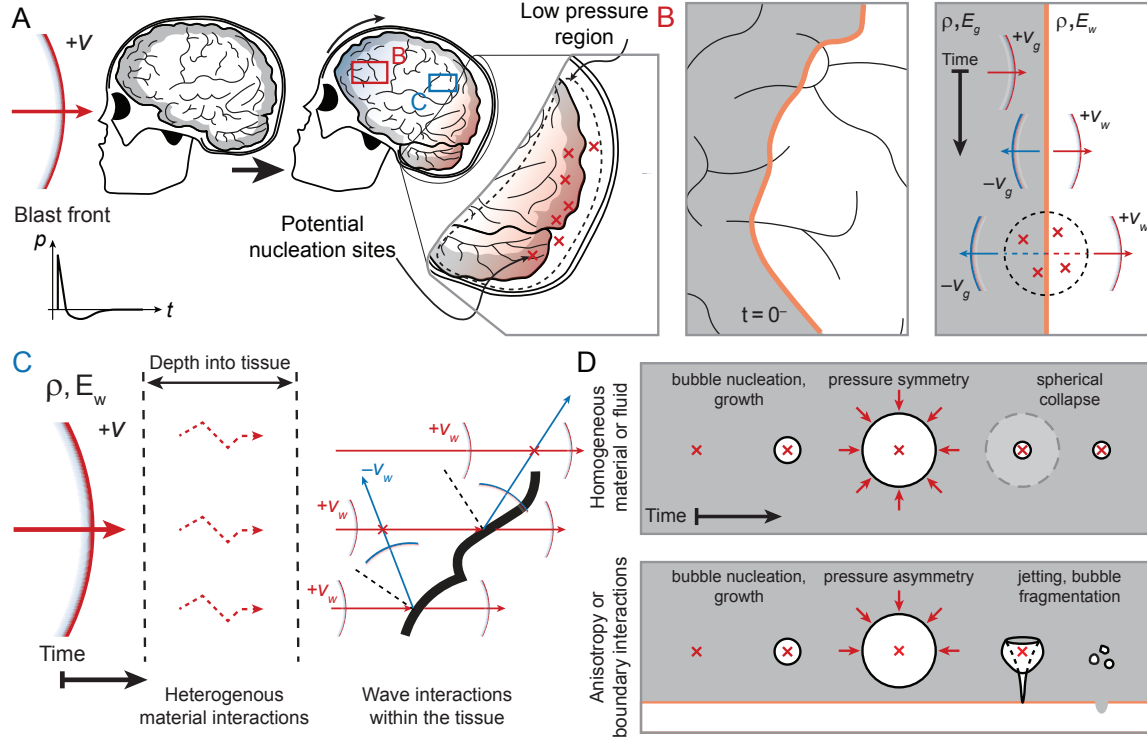


Figure 1.1: Hypothesized mechanisms of cavitation nucleation and interaction in the brain during blast primary injury. (A) The blast front, which consists of a high pressure front and underpressure tail, first contacts the skull, inducing a low pressure region which induces cavitation at the contrecoup region of the brain. (B) At interfaces between regions of differing mechanical properties (e.g. gray and white matter), acoustic mismatch introduces wave transmission and reflection, creating local low pressure regions. (C) Constructive wave interference within a tissue of uniform mechanical properties can develop from interfaces, e.g. wrinkles and folds. (D) Bubble dynamics depend on the local environment, and both bubble expansion and collapse represent potential damage mechanisms.

1.3 Accomplishments

The purpose of this dissertation is thus to present a simplified model of dissociated primary neural cells in a Type-I collagen substrate that can be used to study inertial microcavitation as a primary cell injury mechanism.

Chapter 2 provides the framework for a length-independent inertial microcavitation-based

ultra-high-strain rate rheometry technique based on a theoretical-numerical model of spherically symmetric bubble collapse dynamics in hydrogels with limited compressibility. An experimental platform of generating single-bubble inertial microcavitation with one pulse of a 6 ns Q-switched Nd:YAG laser, with capability of pre- and post-cavitation laser scanning confocal imaging, is presented. Experimental validation of the inertial microcavitation rheometry technique is performed for polyacrylamide gels and water. Further mechanical quantities of interest are highlighted, including strain, strain rate, and stress fields as a function of position and time in the material.

Chapter 3 utilizes the theory developed in the proceeding chapter in a 3D Type-I collagen model of dissociated primary neural cells. Cells are subjected to single microcavitation bubble events and observed before and after using laser scanning confocal microscopy. New image differencing algorithms are developed for quantitative determination of primary, mechanical cell injury and disruption zones, which are in turn correlated with induced mechanical strain information from the measured kinematics to provide qualitative injury strain thresholds. Higher-resolution confocal imaging of live intracellular esterase activity and post-fixation F-Actin suggests a range of critical radii for different types of primary cellular component damage. These radii of interest are then correlated mechanically with strains, strain rates, and stresses determined from inertial microcavitation rheometry fitting.

Chapter 4 summarizes the findings and suggests interesting further avenues for exploration. For completeness, Appendix A describes a scheme for analyzing the kinematic response of cells on their local environment by a mean deformation approach. Appendix B describes a teaching tool for quantitative evaluation of user-supplied images for digital image and volume correlation.

Chapter 2

Cavitation in viscoelastic materials

Note: A version of this chapter is in preparation for submission to the Journal of the Mechanics and Physics of Solids. Data and figures have been used with all co-authors' consent.

Estrada JB, Barajas C, Henann DL, Johnsen E, Franck C (2017). Inertial microcavitation-based ultra-high-strain rate rheometry.

2.1 Introduction

The ability to make accurate predictions about how mechanical forces affect cellular and tissue response depends in large part on the material properties of the underlying tissue through which these forces propagate. Accurate mechanical characterization of soft matter, including hydrogels and tissues, relies on the availability of appropriate experimental techniques that can address the highly compliant nature, length, and time-dependent characteristics of these materials.

Classical tension-compression, as well as plate-and-cone rheometer devices provide a good approach for estimating the bulk, isotropic material properties [67–71], yet their interpretation of the data is somewhat limited to the overall macroscale response of the material at hand. Alternatively, small-scale techniques such as microindentation, nanoindentation, and

atomic force microscopy allow for interrogation of the local, rather than bulk elastic [72,73], and poro/viscoelastic properties [71,74]. This makes these techniques attractive for probing tissue properties at the size scale of cells and tissues. However, due to the intrinsic inhomogeneous loading conditions, accurate determination of material properties beyond the linear elastic regime requires more sophisticated contact mechanics models to be employed beyond the classical Hertz and Johnson-Kendall-Roberts (JKR) theories, which are not always readily available [75,76].

Recently, several promising *in situ* microrheology approaches including optical trapping [77], confocal rheology [78,79], and pressure-induced cavitation [80–82] have emerged for providing new detail of the intrinsic 3D mechanical properties inside polymeric materials. In particular, pressure-induced cavitation rheology as pioneered by Crosby et al. is a powerful, compact testing platform for determining the local mechanical properties of soft materials and tissues across material length scales by controlling the cavitation bubble radius [80–82]. In this approach, air or immiscible liquid injection via syringe pump reaches a critical pressure, which either induces cavitation or fracture depending on material composition and stiffness.

While measurements via these techniques have contributed much to our current understanding of the mechanical, or constitutive, properties of hydrogels, tissues, and soft matter, almost all of them have focused on the quasi-equilibrium response of the material at low- to medium-level strain rates ($10^{-4} < \dot{\epsilon} < 10^2 \text{ s}^{-1}$). Extension of the mentioned techniques to faster loading rates is often challenged by limitation in the employed hardware or necessity for more sophisticated theoretical frameworks [83] accounting for internal wave propagation and inertial effects.

With increasing demand for understanding soft matter behavior at higher loading rates (i.e., strain rates $> 10^3 \text{ s}^{-1}$) motivated by application in laser- and ultrasound-based surgical procedures [14,15,84] and impact injuries [85,86,100], significant demand exists for experimental techniques capable of physically characterizing the tissue response at those rates. For example, injury prediction during traumatic brain injuries and collateral tissue

damage estimates during lithotripsy procedures require a quantitative understanding of the relevant tissue at strain rates in excess of $\dot{\epsilon} > 10^3 \text{ s}^{-1}$.

Established techniques for traditional high-speed mechanical characterization of polymeric and less compliant materials, including Split-Hopkinson (Kolsky) bar and pressure-shear testing, have been met with challenges when used for soft materials due to the high compliance, low impedance, and slow shear wave speeds in these materials [87]. These challenges are compounded further by the lack of readily available theoretical frameworks that allow rapid interpretation of the dynamic, inertia-dominated data at hand.

To overcome these significant challenges and to address the need for a high-strain rate characterization technique, we present a robust, minimally-intrusive, local microrheology approach for characterizing the material constitutive properties at high (10^3 s^{-1}) to ultra-high (10^8 s^{-1}) strain rates. Using a spatially-focused pulsed laser we are able to generate inertial cavitation bubbles in a variety of hydrogels of varying stiffness at pre-defined depth of fields. Analogous to the quasistatic, inertial-free cavitation rheology, the bubble dynamics within the interrogated material are a direct function of the material mechanical properties. Recording the spatiotemporally resolved bubble dynamics via high-speed videography, one can determine the constitutive material model via a standard least squares fitting approach to an appropriate theoretical-numerical cavitation framework model, which we developed for this particular purpose. Our mathematical inertial cavitation framework is built upon previously developed single-bubble cavitation studies [3, 4, 88–93] in order to provide accurate estimates of the non-linear, finite deformation viscoelastic material properties of the tissue or hydrogel.

To demonstrate the capability and validity of this new inertial microcavitation ultra-high-strain rate rheometry (IMUR) characterization technique, we mechanically characterize soft and stiff polyacrylamide gels as a benchmark case. Lastly, we show that once the material properties are obtained, one can use the theoretical framework to predict the spatiotemporal evolution of cavitation stresses and strains, which should be of significant interest in understanding the physical forces experienced by tissues in laser eye surgery

[16], ultrasound surgical methods in cancer treatment [97], lithotripsy [98], and impact deformations in the brain [99,100].

2.1.1 Inertial Microcavitation Ultra-high-strain rate Rheometry

Analogous to other rheometry techniques, the inertial microcavitation ultra-high-strain rate rheometry (IMUR) method correlates the evolution of the bubble pressure field and its associated material stresses with the resulting kinematics, namely the change in bubble radius over time, which is recorded via high-speed photography (Fig. 2.1A). In essence, one can think of this technique as a force-controlled tribology method, in which a certain amount of energy is added into the material system to start the cavitation process as shown in Fig. 2.1B and 2.2. Although cavitation initiation requires the creation of a pressure differential across the bubble wall, various means have been devised to accomplish it, whose underlying physics need to be properly reflected in the governing mass, momentum and energy equations.

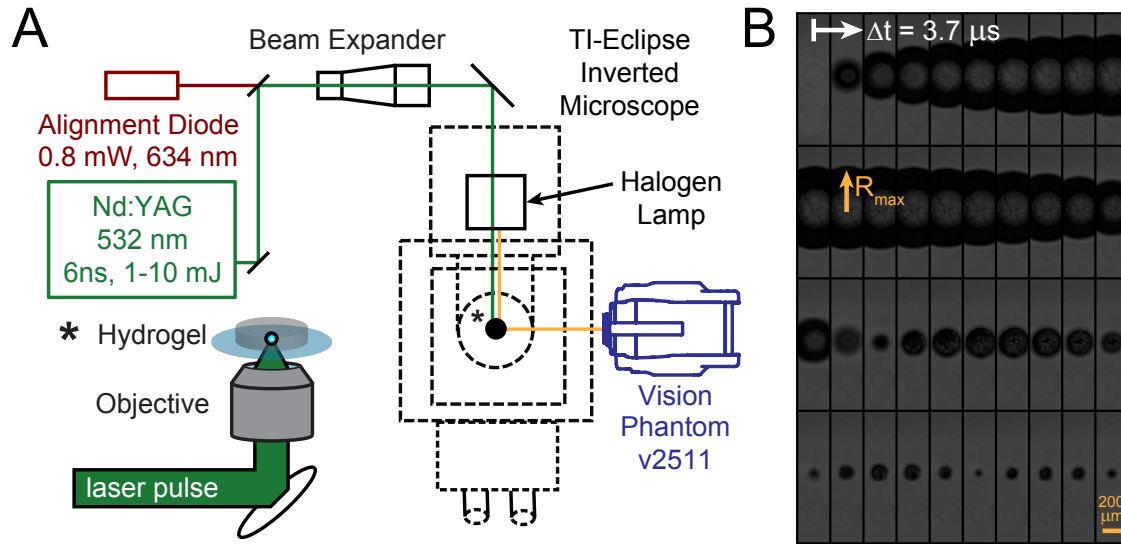


Figure 2.1: Experimental setup and schematic of inertial cavitation rheometry. (A) A single 6 ns, Q-switched 532 nm Nd:YAG laser pulse of 1-10 mJ passes through a beam expander to fill the back aperture of an objective mounted into an inverted TI-Eclipse microscope, and (inset, star) converges into a cylindrical hydrogel sample. Bright-field illumination is supplied by a condensed halogen lamp. (B) Bubble growth, collapse, and subsequent oscillation are imaged using a Phantom v2511 high speed camera (Vision Research, Wayne, NJ). Image size 512x128 px, filmed at 270,000 fps. Scale bar, $200 \mu m$.

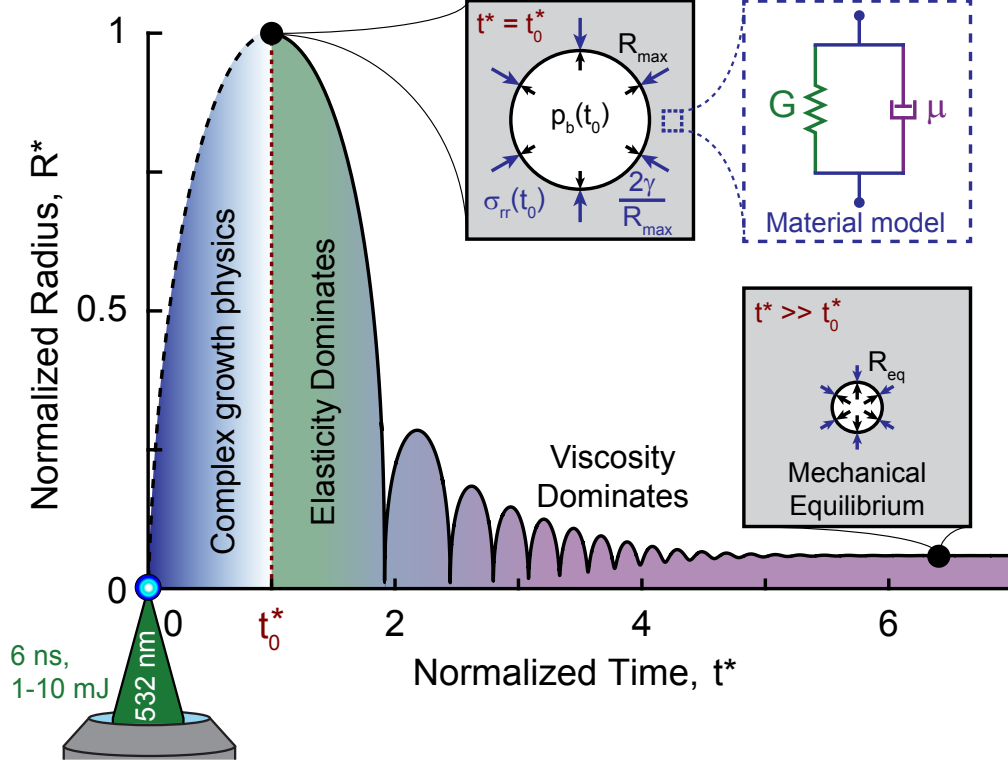


Figure 2.2: Schematic of the timeline after inertial cavitation bubble nucleation. After initial sonoluminescence and plasma formation, bubble growth is caused by plasma recombination and vapor and non-condensable gas volume expansion, and reaches thermodynamic equilibrium. Modeling of the bubble dynamics is begun at the time of maximum bubble radius, t_0 . The peak bubble size builds large elastic stresses and surface tension in the surrounding material, which drive primary bubble collapse. Later, lower-amplitude bubble oscillation is dominated by material viscous effects, finally reaching mechanical equilibrium.

In this study, experimental inertial cavitation is generated via single pulses of a frequency-doubled Q-switched Nd:YAG 532 nm laser as shown in Fig. 2.1A. Shortly after bubble nucleation, sonoluminescence occurs, signifying initial plasma formation and chemical reactions at the laser focal point (Fig. 2.2). As the bubble rapidly expands, plasma recombines into the gas inside the bubble and the system reaches thermodynamic, though not mechanical, equilibrium. While bubble dynamics are recorded experimentally during and after growth (Fig. 2.1B), we do not consider the laser-induced process of material rupture in our numerical model as the physics during inertial cavitation are relatively complex and, at present, only incompletely understood [93].

Instead, we construct our theoretical framework beginning at the time of the peak bubble

radius. Fig. 2.2 shows a schematic of the initial ($t = t_0$, $R = R_{\max}$) and reference ($t \gg t_0$, $R = R_{\text{eq}}$) states of the model. At $t = t_0$, the surrounding hydrogel material is out of dynamic equilibrium, with surface tension and material stress greater than the internal bubble pressure. At later times $t \gg t_0$, the material is assumed to be in mechanical equilibrium with the gas inside the bubble. A widely-accepted, general approach for estimating bubble pressure inside hydrogel- or tissue-based soft matter systems is to treat the bubble as a two-phase mixture of condensible water vapor and non-condensable gas (for which condensation rate is much slower than the timescales of the bubble oscillations) [92].

While Fig. 2.2 provides a schematic of the typical oscillatory single bubble cavitation behavior, it is important to note that the amplitude, frequency and decay of the bubble radius oscillations is directly affected by the underlying viscoelastic material properties. Connecting the characteristic oscillation to material response is the determining goal of the presented IMUR method. If the material behaves viscoelastically, the initially large elastic stress component determines the rate of primary collapse, while the overall decay envelope is dominated by viscous dissipation.

2.2 Theoretical Cavitation Framework

We construct the cavitation boundary value problem with the following key assumptions. First, since the bubble oscillations remain largely spherical over the course of the cavitation event (Fig. 2.1B) we assume spherical symmetry, where the bubble has an equilibrium radius R_0 , which has expanded due to an instantaneous change in pressure. The referential coordinate in the surrounding material is denoted by $r_0 \geq R_0$. The bubble wall is given as a function of time by $R(R_0, t)$, while any material point $\mathbf{x}_0 = r_0 \mathbf{e}_r$ in the reference configuration gets mapped to a point $\mathbf{x} = r \mathbf{e}_r$ in the deformed material configuration. The surrounding medium has far-field ambient pressure p_∞ and temperature T_∞ , as well as material constitutive properties to be defined later. Next, given the high water content of many biological tissues and hydrogels and fast cavitation dynamics, we assume no water flux with respect to the material (i.e. the hydrogels remain undrained) in the near field

Table 2.1: Glossary of symbols for the cavitation framework.

Symbol	Definition
γ	Surface tension of the surrounding medium
κ	Polytropic index of the gas inside the bubble
λ	Stretch ratio
μ	Dynamic viscosity component of the surrounding medium
ρ	Density of the surrounding medium
ρ_m	Density of the gas mixture inside the bubble
$\boldsymbol{\sigma}$	Cauchy stress tensor
$\mathbf{B}$	Left Cauchy-Green tensor
c	Longitudinal pressure wave speed in surrounding medium
D	Diffusion coefficient of vapor into non-condensable gas
$\mathbf{E}$	Logarithmic (Hencky) strain tensor
$\mathbf{F}$	Deformation gradient tensor
G	Shear modulus of the surrounding medium
$\mathbf{I}$	Identity tensor
K	Bubble mixture thermal conductivity
p_0	Ambient pressure (atmospheric)
p_b	Pressure inside the bubble
p_R	Total pressure on the time-evolving bubble wall
p_v	Vapor pressure due to temperature inside the bubble
p_∞	Pressure at infinity (atmospheric plus hydrostatic)
q_v	Mass flux of vapor from the gas into the material
$r(t)$	Radial position at time t
r_0	Radial position at time $t = 0$
$R(t)$	Bubble wall position at time t
R_0	Original radius before expansion, equivalently R_{eq}
R_{max}	Maximum bubble radius
$\mathcal{R}$	Gas mixture gas constant
$\mathbf{s}$	Deviatoric component of Cauchy stress
S	Stress integral term
t	Time
$T(r)$	Temperature of the gas mixture at position $r < R$

and thus the material to be locally incompressible. Finally, due to a lack of time-resolved measurements very close to the collapse of the bubble, we approximate the cavitation bubble dynamics by the general low-Mach number approach [17], which neglects higher Mach number effects [93]. To derive the bubble motion as a function of time and pressure terms, we start with the general conservation of mass and conservation of linear momentum:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) = 0 \quad (2.1)$$

$$\rho \mathbf{a} = \rho \mathbf{b} + \nabla \cdot \boldsymbol{\sigma}, \quad (2.2)$$

where ρ is the density of the medium, $\mathbf{v}$ is the velocity of a material point, $\mathbf{a}$ is the acceleration of a material point, $\mathbf{b}$ represents the body force acting on the medium, and $\boldsymbol{\sigma}$ is the Cauchy stress tensor. Incorporating incompressibility and assuming that body forces acting on the system are small, the conservation equations reduce to

$$\nabla \cdot \mathbf{v} = 0 \quad (2.3)$$

$$\rho \mathbf{a} = -\nabla p + \nabla \cdot \mathbf{s}, \quad (2.4)$$

where the Cauchy stress tensor $\boldsymbol{\sigma}$ is decomposed into its hydrostatic pressure p and deviatoric stress components $\mathbf{s}$ as

$$\boldsymbol{\sigma} = \mathbf{s} - p\mathbf{I}. \quad (2.5)$$

Incorporating spherical symmetry into equations (2.3) and (2.4) yields

$$\frac{\partial v_r}{\partial r} + \frac{2v_r}{r} = 0 \quad (2.6)$$

$$\rho \left(\frac{\partial v_r}{\partial t} + v_r \frac{\partial v_r}{\partial r} \right) = -\frac{\partial p}{\partial r} + \frac{\partial s_{rr}}{\partial r} + \frac{2}{r} (s_{rr} - s_{\theta\theta}), \quad (2.7)$$

where v_r represents the radial velocity, $\frac{\partial p}{\partial r}$ is the radial pressure gradient and s_{rr} and $s_{\theta\theta}$ represent the radial and hoop components of the deviatoric stress tensor $\mathbf{s}$. Solving (2.6) and expressing the radial velocity at the bubble wall in terms of the bubble radius, i.e., $v_r(R) = \dot{R}$, allows us to establish the following relationship between the radial velocity v_r ,

the bubble radius R , and the spatial coordinate r via

$$v_r = \dot{R} \frac{R^2}{r^2}. \quad (2.8)$$

Substituting (2.8) into (2.7) allows us to recast the momentum Eq. (2.7) in terms of the bubble radius R as

$$\rho \left(\frac{R^2 \ddot{R} + 2R \dot{R}^2}{r^2} - \frac{2R^4 \dot{R}^2}{r^5} \right) = -\frac{\partial p}{\partial r} + \frac{\partial s_{rr}}{\partial r} + \frac{2}{r} (s_{rr} - s_{\theta\theta}). \quad (2.9)$$

Keller et al. [3, 88, 89] used a formulation combining the incompressible near field behavior and the far-field wave propagation solutions in order to capture the effects of acoustic radiation as an energy transfer mechanism, which is important for large bubble amplitudes [3]. Matching these solutions asymptotically gives an approximate form for the bubble radius motion in the entire field to first order in the Mach number.

2.2.1 Near-field solution

Since the spherically symmetric velocity field is irrotational, the velocity can be written as a velocity potential function ϕ , where $\nabla\phi = v_r \mathbf{e}_r$. The conservation of mass can be written in terms of ϕ as

$$\nabla^2 \phi = 0. \quad (2.10)$$

In the local regions near the bubble wall (i.e. where $r = O(R)$) the expansion and compression of the bubble dominate the dynamics. We can use the integral of the balance of linear momentum to get the local behavior of the material. Integrating (2.9) from R to r gives the near-field pressure p_r^{nf} as

$$p_r^{\text{nf}} = p_R - \rho \left(R \ddot{R} + \frac{3}{2} \dot{R}^2 - \frac{(R^2 \dot{R})'}{r} + \frac{R^4 \dot{R}^2}{2r^4} \right) + s_{rr} \Big|_R^r + 2 \int_R^r \frac{s_{rr} - s_{\theta\theta}}{\hat{r}} d\hat{r}, \quad (2.11)$$

where $\dot{}$ is equivalent to the time derivative $\cdot$.

2.2.2 Far-field solution

The far-field solution accounts for damping via acoustic radiation and requires relaxation of the incompressible assumption. The far-field conservation of mass can be treated as the potential form of the acoustic wave equation (arising from the combination of conservation of mass and balance of linear momentum), written generally to first order as

$$\nabla^2 \phi - \frac{1}{c^2} \frac{\partial^2 \phi}{\partial t^2} = 0, \quad (2.12)$$

where c is the finite longitudinal pressure wave speed in the material. The pressure and density changes, stress terms, and convection terms decay to zero in the far field (e.g. r of $O(ct)$). The general solution to the acoustic wave equation in the far-field is

$$\phi^{\text{ff}} = \frac{1}{r} \left[\psi_1 \left(1 - \frac{r}{c} \right) + \psi_2 \left(1 + \frac{r}{c} \right) \right], \quad (2.13)$$

where the wave equations ψ_1 and ψ_2 represent the characteristic outgoing and incident waves, respectively. With the other terms in the far-field being small, the resulting expression for far-field pressure is

$$p_r^{\text{ff}} = p_0 - \rho \frac{\partial \phi^{\text{ff}}}{\partial t}, \quad (2.14)$$

where p_0 is the static pressure (i.e. atmospheric).

2.2.3 Matching near- and far-field solutions

The near- and far-field solutions are then superimposed to give a complete form for the bubble dynamics that takes into account both the dynamics near the bubble and the outgoing acoustic wave. We equate the boundary conditions of mass flow and pressure in the respective intermediate zones of the two solutions. For the near-field solution, the intermediate

zone is at $r \rightarrow \infty$, and for the far-field solution, the intermediate zone is at $r \rightarrow 0$.

$$4\pi r^2 \rho v_r^{\text{nf}} \Big|_{r \rightarrow \infty} = 4\pi r^2 \rho v_r^{\text{ff}} \Big|_{r \rightarrow 0} \quad (2.15)$$

$$p_r^{\text{nf}} \Big|_{r \rightarrow \infty} = p_r^{\text{ff}} \Big|_{r \rightarrow 0}. \quad (2.16)$$

From substituting (2.8) into (2.15), the velocity fields of both solutions are equal. Combining (2.11) and (2.14) into (2.16) and using that the stresses far from the bubble decay to zero gives

$$p_R - \rho \left(R\ddot{R} + \frac{3}{2} \dot{R}^2 \right) - s_{rr} \Big|_R + S = p_0 - \rho \frac{\partial \phi^{\text{ff}}}{\partial t} \Big|_{r \rightarrow 0}, \quad (2.17)$$

where the stress integral, S , is defined as

$$S = 2 \int_R^\infty \frac{s_{rr} - s_{\theta\theta}}{r} dr. \quad (2.18)$$

The pressure at the bubble wall p_R is the boundary condition given by the sum of terms contributed by surface tension, γ , gas pressure in the bubble, p_b , and material stress at the wall,

$$p_R = p_b - \frac{2\gamma}{R} + s_{rr} \Big|_R. \quad (2.19)$$

The potential term $\partial \phi^{\text{ff}} / \partial t \Big|_{r \rightarrow 0}$ in Eq. 2.13 can be simplified further using L'Hopital's rule and Eq. 2.8,

$$\frac{\partial \phi^{\text{ff}}}{\partial t} \Big|_{r \rightarrow 0} = \frac{1}{c} \left(2\psi_2'' + (R^2 \dot{R})'' \right). \quad (2.20)$$

While taking $(R^2 \dot{R})''$ induces a third time derivative of R , this term is small compared to the longitudinal wave speed c as observed by Prosperetti [91] and can instead be kept as second order in time by manipulation of Eq. (2.17),

$$R\ddot{R} + \frac{3}{2} \dot{R}^2 = \frac{1}{\rho} (p_R - p_\infty) \quad (2.21)$$

where

$$p_\infty = p_0 + s_{rr} \Big|_R - S - \frac{2\rho}{c} \frac{\partial^2 \psi_2}{\partial t^2}. \quad (2.22)$$

Rewriting Eq. (2.21) in terms of $R^2\dot{R}$ yields

$$\frac{1}{R}(R^2\dot{R})' - \frac{1}{2}\frac{(R^2\dot{R})^2}{R^4} = \frac{p_R - p_\infty}{\rho} \quad (2.23)$$

$$(R^2\dot{R})' = R\left(\frac{1}{2}\dot{R}^2 + \frac{p_R - p_\infty}{\rho}\right) \quad (2.24)$$

Finally, taking another derivative of (2.24), substituting into (2.20), and rewriting (2.17) yields the following relations, found by Keller and Miksis [3]

$$\left(1 - \frac{\dot{R}}{c}\right) R\ddot{R} + \frac{3}{2}\left(1 - \frac{\dot{R}}{3c}\right) \dot{R}^2 = \left(1 + \frac{\dot{R}}{c} + \frac{R}{c} \frac{d}{dt}\right) \frac{p_R - p_\infty}{\rho}. \quad (2.25)$$

The acoustic wave term in the pressure at infinity p_∞ can be considered the driving pressure supplied experimentally, e.g. if ultrasound is used. For our chosen initial conditions, rather than drive the bubble using the acoustic wave, we choose to begin the problem at lowest internal bubble pressure, or maximum radius. With these initial conditions, the acoustic solution term can be neglected (i.e. by that time it is far away from the bubble) and we can write the pressure differential as

$$p_R - p_\infty = p_b - \frac{2\gamma}{R} + S - p_0. \quad (2.26)$$

2.2.4 Bubble pressure

As the bubble increases and decreases in size, pressure is altered due to volume changes, temperature, and mass transfer effects. To improve the accuracy from a single-component polytropic (ideal-gas) assumption, the bubble is assumed to consist of both water vapor, which may condense at the bubble wall, and non-condensable gas obeying polytropic behavior. A commonly-used assumption is to take the pressure in the bubble as uniform [3, 4, 90], which has furthermore been numerically validated in [93] for Mach numbers $M_c = \dot{R}/c$ below 0.3.

As in [4, 94], to get the expression for heat conduction from the bubble into the material we

start with the balance of energy for the spherically-symmetric bubble assuming Fourier's law for heat conduction,

$$\rho_m C_p \frac{\partial T}{\partial t} + \frac{T}{\rho_m} \left(\frac{\partial \rho_m}{\partial T} \right)_p \dot{p}_b = \frac{1}{r^2} \frac{\partial}{\partial r} \left(K r^2 \frac{\partial T}{\partial r} \right), \quad (2.27)$$

where ρ_m is the density of vapor/non-condensable gas mixture, C_p is the heat capacity at constant pressure, $T(r, t)$ is the instantaneous temperature field in the bubble, and $K(T) = AT + B$ is the bubble mixture thermal conductivity as a function of two empirical constants A and B . We can write the spherical conservation of mass of the gas as

$$\frac{\partial \rho_m}{\partial t} + \rho_m \frac{\partial U}{\partial r} = 0 \quad (2.28)$$

where U is the position-dependent gas mixture velocity, multiply by $C_p T$, and combine with Eq. (2.27) to get

$$\frac{d}{dt} (\rho_m C_p T) + \frac{T}{\rho_m} \left(\frac{\partial \rho_m}{\partial T} \right)_p \dot{p}_b + \rho_m C_p T \frac{\partial U}{\partial r} = \frac{1}{r^2} \frac{\partial}{\partial r} \left(K r^2 \frac{\partial T}{\partial r} \right), \quad (2.29)$$

where $\frac{d}{dt} = \frac{\partial}{\partial t} + U \frac{\partial}{\partial r}$ is the convective time derivative. We can use the ideal gas assumption for constant specific heat,

$$\rho_m C_p T = \frac{\kappa}{\kappa - 1} p_b, \quad \frac{T}{\rho_m} \left(\frac{\partial \rho_m}{\partial T} \right)_p = -1, \quad (2.30)$$

where κ is the polytropic constant, which upon replacing with Eq. 2.29 and dividing by p_b gives Substitution of Eq. 2.30 into Eq. 2.29 and integration for U with respect to r gives the bubble gas velocity as

$$U = \frac{1}{\kappa p_b} \left[(\kappa - 1) K \frac{\partial T}{\partial r} - \frac{r \dot{p}_b}{3} \right], \quad (2.31)$$

where the gas velocity at the bubble wall is given by $U(r = R) = \dot{R}$. The energy equation

2.27 can be used explicitly with substitution of Eq. 2.30,

$$\frac{\kappa}{\kappa - 1} \frac{p_b}{T} \left(\frac{\partial T}{\partial t} + U \frac{\partial T}{\partial r} \right) - \dot{p}_b = \frac{1}{r^2} \frac{\partial}{\partial r} \left(K r^2 \frac{\partial T}{\partial r} \right). \quad (2.32)$$

Spherical symmetry and the vapor mass flux also introduce boundary conditions for heat transfer of

$$\left. \frac{\partial T}{\partial r} \right|_{r=0} = 0, \quad L_v q_v = -K \left. \frac{\partial T}{\partial r} \right|_R, \quad (2.33)$$

where L_v is the latent heat of vaporization/condensation. The material is assumed to be highly thermally conductive with temperature T_∞ everywhere, which imposes a boundary condition of $T_R = T_\infty$ at the bubble wall.

As pertains to mass transfer, we assume that non-condensable gas is unable to transfer via diffusion into the surrounding material, while condensation is allowed for water vapor. The non-condensable gas is assumed to be air, which has a low solubility in water ($C_g \sim 10^{-5}$) and will not diffuse under the very fast timescales in inertial microcavitation. As in [18], mass diffusion for vapor is then given by the conservation of mass as

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial r} = \frac{D}{\rho_m r^2} \frac{\partial}{\partial r} \left(\rho_m r^2 \frac{\partial C}{\partial r} \right), \quad (2.34)$$

where $C = \rho_v / \rho_m$ is the relative concentration of water vapor in the bubble, ρ_v is the density of water vapor, D is the diffusion coefficient of water vapor into non-condensable gas, and u the modified position-dependent velocity of the water vapor, given by

$$u = U + \frac{\mathcal{R}_v - \mathcal{R}_g}{\mathcal{R}} \left(D \frac{\partial C}{\partial r} \right), \quad (2.35)$$

where $\mathcal{R}_g$ is the gas constant for non-condensable gas and the mixture gas constant is given by $\mathcal{R} = C\mathcal{R}_v + (1 - C)\mathcal{R}_g$. The boundary conditions for diffusion due to symmetry and at the wall are

$$\left. \frac{\partial C}{\partial r} \right|_{r=0} = 0, \quad \rho_R C_R = \frac{p_{v,\text{sat}}(T_R)}{\mathcal{R}_v T_R}, \quad (2.36)$$

where the second boundary condition arises from treating phase change at the boundary

as an equilibrium process. The saturated vapor pressure at the bubble wall is given by $p_{v,\text{sat}}(T_R) = p_{\text{ref}} \exp(-T_{\text{ref}}/T_R)$, where p_{ref} and T_{ref} are empirically determined. Assuming the gas mixture obeys the ideal gas law, the concentration C and mixture density ρ_m are related by

$$C = \frac{1}{\mathcal{R}_v - \mathcal{R}_g} \left(\frac{p_b}{\rho_m T} - \mathcal{R}_g \right). \quad (2.37)$$

Finally, the vapor mass flux is given from Fick's first law as

$$q_v = D \frac{\rho_{m,R}}{1 - C_R} \frac{\partial C}{\partial r} \Big|_R. \quad (2.38)$$

Combining Eqs. (2.31) and (2.35) with the boundary conditions for heat and mass transfer, and solving for the resulting pressure at the wall, the final expression for time-dependent bubble pressure becomes

$$\dot{p}_b = \frac{3}{R} \left[-\kappa p_b \dot{R} + (\kappa - 1) K_R \frac{\partial T}{\partial r} \Big|_R + \kappa \mathcal{R}_v T_R q_v \right], \quad (2.39)$$

where the subscript R denotes variables evaluated at the interface, or $r = R$.

Eqs. (2.25) (with pressures given by (2.26) and (2.39)), (2.32), (2.34), and (2.37) comprise the set of two nonlinear ordinary differential equations and two partial differential equations solved in the numerical model [95], with one remaining equation arising from the assumed viscoelastic material model.

2.3 Viscoelasticity

The present goal for the material model is to get an expression for the stress integral S . To do this, we begin by defining simple material models.

2.3.1 Finite deformation Kelvin-Voigt model

The Neo-Hookean Kelvin-Voigt model consists of a Neo-Hookean spring with shear modulus G in parallel with a linear Mises dashpot with viscosity μ . Taking the sum of Cauchy stresses

in the parallel elastic and viscous branches as the total stress and equating the deformation gradient tensors in each branch, the total deformation gradient $\mathbf{F}$ in the material is given for the Kelvin-Voigt model as

$$\mathbf{F} = \mathbf{F}^e = \mathbf{F}^v. \quad (2.40)$$

Recasting $\mathbf{F}$ in spherical coordinates undergoing radial expansion and contraction yields

$$\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{x}_0} = \begin{bmatrix} \frac{\partial r}{\partial r_0} & 0 & 0 \\ 0 & \frac{r}{r_0} & 0 \\ 0 & 0 & \frac{r}{r_0} \end{bmatrix}. \quad (2.41)$$

Consistent with the fluid formulation, assuming the near field to be incompressible ensures that the Jacobian of the deformation gradient tensor $J = \det(\mathbf{F}) = 1$. This in turn both relates the deformation gradient tensor components and, when solved at the bubble wall, gives the useful form for r ,

$$\frac{\partial r}{\partial r_0} = \left(\frac{r_0}{r}\right)^2 \rightarrow r(r_0, t) = (r_0^3 + R(t)^3 - R_0^3)^{1/3}. \quad (2.42)$$

The Cauchy stress $\boldsymbol{\sigma}$ in the Kelvin-Voigt material model is

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}^e + \boldsymbol{\sigma}^v = \mathbf{s}^e + \mathbf{s}^v - p\mathbf{I}. \quad (2.43)$$

The hydrostatic pressure p is given in terms of the Cauchy stress $\boldsymbol{\sigma}$ as

$$p = -\frac{1}{3}\text{tr}(\boldsymbol{\sigma}). \quad (2.44)$$

Since the stress terms are isolated into purely viscous and purely elastic stresses, they are readily decoupled. The viscous Cauchy stress is given in terms of the deformation gradient tensor or velocity $\mathbf{v}$ as

$$\mathbf{s}^v = \mu \left(\dot{\mathbf{F}}\mathbf{F}^{-1} + \mathbf{F}^{-T}\dot{\mathbf{F}}^T \right) = \mu \left(\nabla \mathbf{v} + (\nabla \mathbf{v})^T \right). \quad (2.45)$$

which can be written in terms of stress components as

$$s_{rr}^v = -4\mu \frac{R^2 \dot{R}}{r^3}, \quad s_{\theta\theta}^v = 2\mu \frac{R^2 \dot{R}}{r^3}. \quad (2.46)$$

To relate elastic stress to deformation of the material, we take the Helmholtz free energy, ψ^e , of the Neo-Hookean spring to be

$$\psi^e(\mathbf{F}) = \frac{G}{2} (\text{tr}(\mathbf{B}) - 3), \quad (2.47)$$

where $\mathbf{B} = \mathbf{F}\mathbf{F}^T$ is the left Cauchy-Green tensor. The deviatoric stress in the elastic branch is related to the Helmholtz free energy function as

$$\mathbf{s}^e = \text{dev} \left(\frac{\partial \psi^e(\mathbf{F})}{\partial \mathbf{F}} \mathbf{F}^T \right) \quad (2.48)$$

$$= G \text{dev}(\mathbf{B}). \quad (2.49)$$

Rewriting the deviatoric stress components in terms of the radial position in the material,

$$s_{rr}^e = -2s_{\theta\theta}^e = \frac{2G}{3} \left[\left(\frac{r_0}{r} \right)^4 - \left(\frac{r}{r_0} \right)^2 \right]. \quad (2.50)$$

The stress integral S from Eq. (2.18) is then evaluated as

$$S = 2G \int_R^\infty \left(\frac{r_0^4}{r^5} + \frac{r}{r_0^2} \right) dr - 12\mu \int_R^\infty \frac{R^2 \dot{R}}{r^4} dr, \quad (2.51)$$

which when recasting into a form using the stretch ratio $\lambda = r/r_0$ as in [96] and using the variable substitution of

$$d\lambda = \frac{1}{r_0} \left(1 - \frac{r^3}{r_0^3} \right) dr, \quad (2.52)$$

yields

$$S = 2G \int_{R/R_0}^1 (\lambda^{-5} + \lambda^{-2}) d\lambda - \frac{4\mu\dot{R}}{R} \quad (2.53)$$

$$= \frac{G}{2} \left(5 - \frac{R_0^4}{R^4} - 4 \frac{R_0}{R} \right) - \frac{4\mu\dot{R}}{R}. \quad (2.54)$$

Substituting S into the Keller-Miksis equation then gives the final pressure for the Neo-Hookean Kelvin-Voigt model as

$$p_R - p_\infty = p_b - p_0 - \frac{2\gamma}{R} - \frac{G}{2} \left(5 - \frac{R_0^4}{R^4} - 4 \frac{R_0}{R} \right) - \frac{4\mu\dot{R}}{R}. \quad (2.55)$$

2.3.2 Standard Non-Linear Solid model

Similar to the analysis done for the Kelvin-Voigt model, we can get an expression for the stress integral S in the three-element model using finite deformation continuum mechanics. The model is defined with a principal Neo-Hookean spring of shear modulus G in parallel with a Maxwell branch containing a Hencky spring of shear modulus G_1 and linear Mises dashpot of viscosity μ in series, and will be referred to henceforth as the Standard Non-Linear Solid (SNS) model. As before, we define the deformation gradient tensor $\mathbf{F}$ of the material undergoing a spherically-symmetric transformation using Eq. (2.41). We again assume the material to be incompressible as in Eq. (2.42), and for convenience, rewrite the ratio of deformed to undeformed coordinate as the stretch ratio $\lambda = r/r_0$, which allows us to write $\mathbf{F}$ as:

$$\mathbf{F} = \frac{\partial \mathbf{x}}{\partial \mathbf{x}_0} = \begin{bmatrix} \left(\frac{r_0}{r}\right)^2 & 0 & 0 \\ 0 & \frac{r}{r_0} & 0 \\ 0 & 0 & \frac{r}{r_0} \end{bmatrix} = \begin{bmatrix} \lambda^{-2} & 0 & 0 \\ 0 & \lambda & 0 \\ 0 & 0 & \lambda \end{bmatrix}. \quad (2.56)$$

We can write the following relations between the deformations in the equilibrium elastic branch (with the principal spring) and the non-equilibrium Maxwell branch,

$$\mathbf{F} = \mathbf{F}^{\text{eq}} = \mathbf{F}^{\text{neq}}. \quad (2.57)$$

The deformation gradient tensors in the non-equilibrium Maxwell branch elastic spring and viscous dashpot elements are composed as

$$\mathbf{F}^{\text{neq}} = \mathbf{F}^e \mathbf{F}^v = \begin{bmatrix} (\lambda^e)^{-2} & 0 & 0 \\ 0 & \lambda^e & 0 \\ 0 & 0 & \lambda^e \end{bmatrix} \begin{bmatrix} (\lambda^v)^{-2} & 0 & 0 \\ 0 & \lambda^v & 0 \\ 0 & 0 & \lambda^v \end{bmatrix}. \quad (2.58)$$

Eqs. (2.56) and (2.58) together produce the useful stretch ratio relation,

$$\lambda = \lambda^e \lambda^v \quad (2.59)$$

The total Cauchy stress $\boldsymbol{\sigma}$ in the material is comprised of the stresses in the equilibrium and non-equilibrium branches, which are again separated into its deviatoric and hydrostatic parts,

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}^{\text{eq}} + \boldsymbol{\sigma}^{\text{neq}} = \mathbf{s}^{\text{eq}} + \mathbf{s}^{\text{neq}} - p\mathbf{I}. \quad (2.60)$$

It is also useful to define the Cauchy stress deviator for the entire material,

$$\mathbf{s} = \mathbf{s}^{\text{eq}} + \mathbf{s}^{\text{neq}}, \quad (2.61)$$

The stress in the equilibrium branch is again assumed to be described by the Helmholtz free energy function of a hyperelastic Neo-Hookean material,

$$\psi^{\text{eq}}(\mathbf{F}) = \frac{G}{2} (\text{tr}(\mathbf{B}) - 3), \quad (2.62)$$

which in turn yields the deviatoric stress tensor in the equilibrium branch,

$$\mathbf{s}^{\text{eq}} = \text{dev} \left(\frac{\partial \psi^{\text{eq}}(\mathbf{F})}{\partial \mathbf{F}} \mathbf{F}^T \right) \quad (2.63)$$

$$= G \text{dev}(\mathbf{B}), \quad (2.64)$$

expressed in matrix form as

$$\mathbf{s}^{\text{eq}} = \begin{bmatrix} 2G/3 & 0 & 0 \\ 0 & -G/3 & 0 \\ 0 & 0 & -G/3 \end{bmatrix} (\lambda^{-4} - \lambda^2). \quad (2.65)$$

The free energy function for the non-equilibrium Hencky spring is given as

$$\psi^e(\mathbf{E}^e) = G_1 |\text{dev}(\mathbf{E}^e)|^2, \quad (2.66)$$

where $\mathbf{E}^e = \ln(\mathbf{U}^e)$ is the Hencky (logarithmic) strain and $\mathbf{U}^e$ is the stretch tensor from the right polar decomposition of the deformation gradient tensor, $\mathbf{F}^e = \mathbf{R}^e \mathbf{U}^e$, where $\mathbf{R}^e$ is the rotation tensor. In the case of spherical symmetry, there is no rotation ($\mathbf{R}^e = \mathbf{I}$, or $\mathbf{F}^e = \mathbf{U}^e$). The work-conjugate stress to the Hencky strain is the Mandel stress, defined as

$$\mathbf{M}^{\text{neq}} = \frac{\partial \psi^e}{\partial \mathbf{E}^e} = 2G_1 \text{dev}(\mathbf{E}^e). \quad (2.67)$$

As $\mathbf{M}^{\text{neq}}$ is purely deviatoric, we can convert it directly to the deviatoric Cauchy stress in the non-equilibrium branch via

$$\mathbf{s}^{\text{neq}} = \mathbf{R}^e \mathbf{M}^{\text{neq}} (\mathbf{R}^e)^T = \mathbf{M}^{\text{neq}}. \quad (2.68)$$

The Hencky strain can be written in terms of stretch ratios

$$\mathbf{E}^e = \begin{bmatrix} -2 \ln \lambda^e & 0 & 0 \\ 0 & \ln \lambda^e & 0 \\ 0 & 0 & \ln \lambda^e \end{bmatrix}, \quad (2.69)$$

and is also clearly deviatoric. Eq. (2.67) therefore simplifies to $\mathbf{s}^{\text{neq}} = 2G_1 \mathbf{E}^e$. The time-dependent Mandel stress-strain relation for the viscous Mises dashpot is defined in terms

of the stretch rate tensor, $\mathbf{D}^v = \dot{\mathbf{F}}^v(\mathbf{F}^v)^{-1}$ as

$$\mathbf{M}^{\text{neq}} = 2\mu\mathbf{D}^v. \quad (2.70)$$

Equating the Mandel stresses yields the relation

$$\mathbf{D}^v = \frac{G_1}{\mu}\mathbf{E}^e, \quad (2.71)$$

which can be expressed in terms of the stretch ratios as

$$\dot{\lambda}^v = \frac{G_1}{\mu} \ln \lambda^e \cdot \lambda^v. \quad (2.72)$$

Combining with (2.59) and replacing the viscous stretches yields

$$\frac{\dot{\lambda}}{\lambda} - \frac{\dot{\lambda}^e}{\lambda^e} = \frac{G_1}{\mu} \ln(\lambda^e). \quad (2.73)$$

We can write these in terms of the radial stress deviator components, $s_{rr}^{\text{neq}} = -4G_1 \ln(\lambda^e)$ and $\dot{s}_{rr}^{\text{neq}} = -4G_1 \dot{\lambda}^e / \lambda^e$,

$$\frac{\dot{\lambda}}{\lambda} + \frac{\dot{s}_{rr}^{\text{neq}}}{4G_1} = \frac{G_1}{\mu} \left(-\frac{s_{rr}^{\text{neq}}}{4G_1} \right), \quad (2.74)$$

which upon rearranging, gives

$$\frac{\mu}{G_1} \dot{s}_{rr}^{\text{neq}} + s_{rr}^{\text{neq}} = -\frac{4\mu\dot{\lambda}}{\lambda}. \quad (2.75)$$

Finally, combining with Eqs. (2.61) and (2.65) gives a differential equation for s_{rr} ,

$$\frac{\mu}{G_1} \dot{s}_{rr} + s_{rr} = \frac{2G}{3} \left[\lambda^{-4} - \lambda^2 + \frac{\mu}{G_1} (-4\lambda^{-5} - 2\lambda) \dot{\lambda} \right] - \frac{4\mu\dot{\lambda}}{\lambda}, \quad (2.76)$$

or equivalently expressed in terms of r and r_0 as

$$\frac{\mu}{G_1} \dot{s}_{rr} + s_{rr} = \frac{2G}{3} \left(\frac{r_0^4}{r^4} - \frac{r^2}{r_0^2} \right) - \frac{4\mu\dot{r}}{r_0} \left[1 + \frac{G}{3G_1} \left(2\frac{r_0^4}{r^4} + \frac{r^2}{r_0^2} \right) \right]. \quad (2.77)$$

The present goal is to adjust Eq. (2.77) to give an expression for the stress integral S , which can be done by the same method as in Warnez and Johnsen [101]. Utilizing the incompressible relation of $r(r_0, t) = (r_0^3 + R^3 - R_0^3)^{1/3}$ to replace all r_0 terms, proceeding to divide by r (to allow integral convergence), and solving via integrating factors gives the desired form of the stress ordinary differential equation,

$$\begin{aligned} \frac{\mu}{G_1} \dot{S} + S = & -\frac{G}{2} \left(5 - \frac{R_0^4}{R^4} - 4 \frac{R_0}{R} \right) \\ & - \frac{4\mu\dot{R}}{R} \left[1 + \frac{G}{3G_1} \frac{R^3}{R^3 - R_0^3} \left(\frac{3}{14} + \frac{R_0}{R} - \frac{3R_0^4}{2R^4} + \frac{2R_0^7}{7R^7} \right) \right]. \end{aligned} \quad (2.78)$$

Eq. (2.78) then becomes the additional fifth equation of the system of ordinary (ODE) and partial (PDE) differential equations solved to determine bubble pressure, radius, temperature, and vapor concentration as a function of time.

2.3.3 Non-dimensionalization

Before marching the four governing equations in time, it is more convenient to solve the numerical model non-dimensionally. To do so, we choose to non-dimensionalize by the maximum bubble radius $R_{\max}$, atmospheric pressure p_0 , material density ρ , and far-field temperature T_∞ as in [95]. From these parameters, we can construct a characteristic velocity $v_c = \sqrt{p_0/\rho}$. We can then define dimensionless groups as shown in Table 2.3.3. In order to simplify the expression for energy inside the bubble, the temperature field inside the bubble is non-dimensionalized as

$$\theta = \frac{1}{K_\infty} \int_1^T K(T_\infty \hat{\theta}) d\hat{\theta} \quad (2.79)$$

With all of the non-dimensional groups defined, we can rewrite the four governing equations,

$$\begin{aligned} \left(1 - \frac{\dot{R}^*}{c^*} \right) R^* \ddot{R}^* + \frac{3}{2} \left(1 - \frac{\dot{R}^*}{3c^*} \right) \dot{R}^{*2} = & \left(1 + \frac{\dot{R}^*}{c^*} + \frac{R^*}{c^*} \frac{d}{dt^*} \right) \\ & \times \left[p_b^* - 1 - \frac{1}{\text{We} R^*} + S^* \right] \end{aligned} \quad (2.80)$$

$$\dot{p}_b^* = \frac{3}{R^*} \left[-\kappa p_b^* \dot{R}^* + \frac{(\kappa - 1)\chi}{R^*} \frac{\partial \theta}{\partial y} \Big|_w + \frac{\kappa \mathcal{R}_v}{\mathcal{R}_w} \frac{\text{Fo}}{T_w R^*} \frac{p_b^*}{1 - C_w} \frac{\partial C}{\partial y} \Big|_w \right] \quad (2.81)$$

Table 2.2: Non-dimensional parameters.

Dimensional Parameter	Dimensionless Parameter	Name
c	$c^* = c/v_c$	Material wave speed
K	$K^* = K/K_\infty$	Mixture thermal conductivity
p_b	$p_b^* = p_b/p_0$	Bubble pressure
R	$R^* = R/R_{\max}$	Bubble wall radius
r	$y = r/R$	Radial coordinate
S	$S^* = S/p_0$	Stress integral
t	$t^* = tR_{\max}/v_c$	Time
γ	$We = p_0 R_{\max}/(2\gamma)$	Weber number
μ	$Re = \rho v_c R_{\max}/\mu$	Reynolds number
G	$Ca = p_0/G$	Cauchy number
D	$Fo = D/(v_c R_{\max})$	Fourier number
$T_\infty K_\infty$	$\chi = T_\infty K_\infty/(p_0 R_{\max} v_c)$	Lockhart-Martinelli number
G_1/μ	$De = G_1 v_c/(\mu R_{\max})$	Deborah number

$$\frac{\kappa}{\kappa - 1} \frac{p_b^*}{T_w K^*} \left(\frac{\partial \theta}{\partial t^*} + \frac{U^* - y \dot{R}^*}{R^*} \frac{\partial \theta}{\partial y} \right) - \dot{p}_b^* = \frac{\chi}{R^{*2}} [\nabla^2 \theta]_y \quad (2.82)$$

$$\frac{\partial C}{\partial t^*} + \frac{u^* - y \dot{R}^*}{R} \frac{\partial C}{\partial y} = \frac{Fo}{R^{*2}} \frac{[\nabla \cdot (\rho_m \nabla C)]_y}{\rho_m}, \quad (2.83)$$

where

$$U^* = \frac{1}{\kappa p_b^*} \left[\frac{(\kappa - 1)\chi}{R^*} \frac{\partial \theta}{\partial y} - \frac{\dot{p}_b^* R^*}{3} y \right] \quad (2.84)$$

$$u^* = U^* + \frac{Fo}{R^*} \frac{\mathcal{R}_v - \mathcal{R}_g}{\mathcal{R}} \frac{\partial C}{\partial y}, \quad (2.85)$$

and the subscript w denotes solving for the respective variable at the bubble wall, or where $y = 1$. The stress integral, inherent to the choice of material, is given for the single parameter Neo-Hookean model as

$$S^* = -\frac{1}{2Ca} \left(5 - 4 \frac{R_{eq}}{R} - \frac{R_{eq}^4}{R^4} \right) \quad (2.86)$$

and for the single parameter viscous fluid model as

$$S^* = -\frac{4\dot{R}^*}{Re R^*}. \quad (2.87)$$

For the Neo-Hookean Kelvin-Voigt model, the stress integral is given as the sum of the

one-parameter elastic and viscous stresses, or by

$$S^* = -\frac{1}{2\text{Ca}} \left(5 - 4\frac{R_{\text{eq}}}{R} - \frac{R_{\text{eq}}^4}{R^4} \right) - \frac{4\dot{R}^*}{\text{Re}R^*}. \quad (2.88)$$

For the case of the standard non-linear solid model, an additional non-dimensional ODE for stress arises,

$$\begin{aligned} \dot{S}^* = & -\frac{S^*}{\text{De}} - \frac{1}{2\text{CaDe}} \left[5 - \frac{1}{\lambda_R^4} - \frac{4}{\lambda_R} \right] - \frac{4\dot{R}^*}{R^*} \frac{1}{\text{ReDe}} \\ & - \frac{4\dot{R}^*}{R^*} \frac{1}{\text{Ca}} \left[\frac{\lambda_R^3}{\lambda_R^3 - 1} \left(\frac{3}{14} + \frac{1}{\lambda_R} - \frac{3}{2\lambda_R^4} + \frac{2}{7\lambda_R^7} \right) \right], \end{aligned} \quad (2.89)$$

where $\lambda_R = R/R_{\text{eq}}$ represents the radial stretch ratio evaluated at the bubble wall. Other potential user-defined functions for the stress integral can be found in [101].

2.3.4 Numerical method and model parameters

All ODEs and PDEs are non-dimensionalized and incremented using a fifth-order explicit Dormand-Prince Runge-Kutta method [102] with adaptive step-size control by the method presented in [95]. At each time substep, the PDEs are discretized on a mesh of $N + 1$ equidistant points in normalized r -space [4] inside the bubble and solved using the second-order central difference method. Interface conditions are set by the respective boundary conditions at the bubble wall. Material properties for the surrounding medium used in this study are shown in Table 2.3.4 and discussed further in [95].

Table 2.3: Material parameters in the study.

Property	Value	Property	Value
A	$5.3 \times 10^{-5} \text{ W/mK}^2$	B	$1.17 \times 10^{-2} \text{ W/mK}$
D	$24.2 \times 10^{-6} \text{ m}^2/\text{s}$	K_R	0.55 W/m
T_{ref}	5200 K	γ	$5.6 \times 10^{-2} \text{ N/m}$
p_0	101.3 kPa	T_{∞}	298.15 K
ρ	1060 kg/m^3	C_p	4.18 kJ/kgK
L	$2.264 \times 10^6 \text{ J/kg}$	κ	1.4
p_{ref}	$1.17 \times 10^8 \text{ kPa}$	c	1430 m/s

2.3.5 Determining non-linear, viscoelastic material parameters

To illustrate the practical workflow of the IMUR technique we characterize the non-linear, high-strain rate material properties of two different stiffness polyacrylamide gels. The extensive quasistatic characterization literature for polyacrylamide gels makes it an ideal benchmarking soft matter material for our IMUR methodology. Eqs. (2.80-2.83) are used to determine the constitutive properties of the underlying material by computing the radial and hoop stresses at every time increment. Since calculations of the material stresses involve invoking a particular material constitutive model [103], we designed a user-adjustable workflow space in which the iteration procedure begins with a simple fluid-like material model (i.e., a Newtonian fluid) followed by several simple material models of increasing complexity, i.e., non-linear Kelvin-Voigt and the non-linear standard solid material model. To start the IMUR procedure (Fig. 2.8), the experimental bubble wall position values for the collapse and subsequent oscillation, $R(t)$, are found via straightforward image processing of the experimental high-speed image time-lapse series. From the experimental $R(t)$ curve, two important input fitting parameters are extracted: the maximum radius, $R_{\max}$ at simulation time $t = 0$, and the equilibrium radius, R_{eq} for $t \rightarrow \infty$. As the experimental radius data is discrete, the value for $R_{\max}$ and peak location to ensure $R(0) = R_{\max}$ is found by fitting a fourth-order polynomial to $R(t)$ near the maximum bubble radius. Values of R_{eq} are estimated as the median of $R(t \gg 0)$ after oscillations have ceased.

For ease of the reader we reproduce the pressure differential expression with elastic and viscous terms for the non-linear Kelvin-Voigt model as in Eq. (2.55),

$$p_R - p_\infty = p_b - p_0 - \frac{2\gamma}{R} - \frac{G}{2} \left(5 - \frac{R_0^4}{R^4} - 4 \frac{R_0}{R} \right) - \frac{4\mu\dot{R}}{R}. \quad (2.90)$$

The initial bubble internal pressure $p_{b,0}$ is then determined via the experimental radius ratio $R_{\text{eq}}/R_{\max}$ by taking the pressure differential ($p_R - p_\infty$) to be zero for a bubble size of R_{eq} and then solving for the pressure for an expanded bubble of size $R_{\max}$. After the initial bubble pressure, the final free fitting parameters in the entire model are material parameters, e.g. shear modulus G and viscosity μ , and setting either to zero gives the

respective results for the purely viscous and purely elastic cases.

A standard least squares fitting routine of the simulation to the first three empirical peaks is employed to yield estimates for material properties of the purely viscous and purely elastic one-parameter models. If the fits do not capture amplitude decay or oscillation frequency sufficiently, the non-linear Kelvin-Voigt model may be used to fit the experimental $R(t)$ data by performing a two-parameter least squares fit over both G and μ . By employing standard convergence criteria (e.g., L_2 -norm) the number of constitutive parameters can be increased as necessary to provide the most robust material model fit (Fig. 2.8).

It is important to note that while these particular material models provide a good estimate for many homogeneous and isotropic materials, the overall approach is general enough to allow inclusion of any user-specific free energy function to account for spherically-symmetric anisotropy or material inhomogeneities.

2.4 Materials and Methods

2.4.1 Polyacrylamide preparation

Base 25 mm glass coverslips were hydrophilically functionalized using previously-developed protocols [104]. Two cylindrical polyacrylamide hydrogel samples were polymerized in custom Delrin molds with initial 15 mm diameter and 3 mm height at concentrations 5/0.03% and 10/0.06% acrylamide/bisacrylamide, respectively, for 45 minutes. Polyacrylamide gels were submerged completely in deionized water for 24 hours before inducing cavitation and uniaxial compression testing. Final dimensions of each gel post-swelling were about 16 mm in diameter and 8 mm in height.

2.4.2 Quasistatic characterization of polyacrylamide gels

After polyacrylamide gels were allowed to swell for 24 hours, they were characterized mechanically in a custom unconfined uniaxial compression device consisting of a centrally-positioned linear actuator (Series A, Ultramotion, Cutchogue, NY) attached to a force transducer (LCFA-25, Omega Engineering, Stamford, CT). A spherical tip attached to the transducer was brought into contact with a 15 mm glass coverslip placed on top surface of the gel to ensure uniform uniaxial compression. Strain rates were chosen to be between 10^{-2} – 10^{-4} s^{-1} . Consistent with previous studies, tested gels exhibited Neo-Hookean material behavior [104] with quasistatic shear moduli to be $G_1^q = 2.97 \pm 0.06$ kPa and $G_2^q = 0.461 \pm 0.004$ kPa (Fig. 2.3), for the stiff and soft gels respectively.

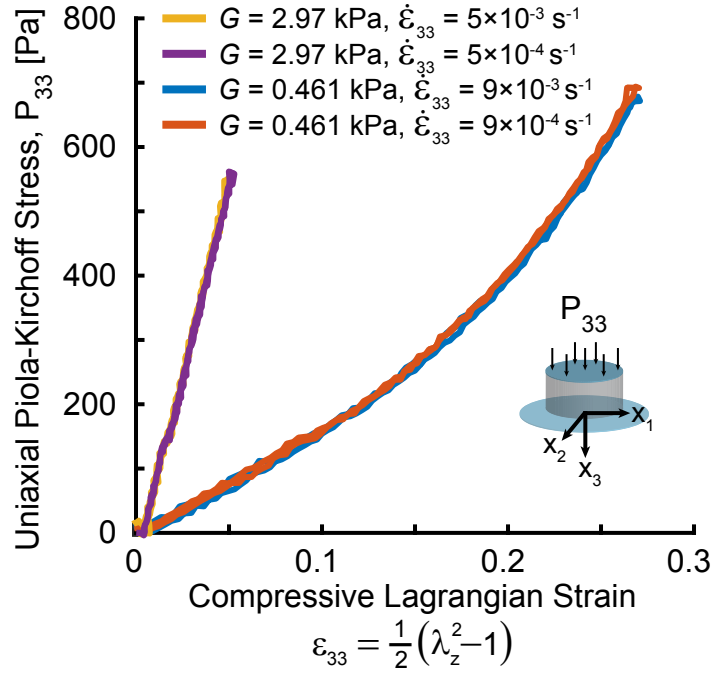


Figure 2.3: Quasistatic characterization of polyacrylamide samples. Two strain rates were tested for each gel. Experiments were fit to Neo-Hookean free energy potentials, yielding quasistatic shear moduli of $G_1^q = 2.97 \pm 0.06$ kPa and $G_2^q = 0.461 \pm 0.004$ kPa, for the stiff and soft gels, respectively.

2.4.3 Bubble generation and imaging

Single microcavitation bubbles were generated via one pulse of a user-adjustable 1-50 mJ, frequency-doubled Q-switched 532 nm Nd:YAG laser (Continuum, San Jose, CA), shown schematically in Fig. 2.1A. Laser pulse energies were kept approximately equal over all tests. Pulses were aligned into the back port of a Nikon Ti-Eclipse inverted microscope (Nikon Instruments, Tokyo, Japan) and reflected into the imaging objective using a 532 nm notch dichroic mirror (Semrock, Rochester, NY), and were expanded to fill the back aperture of the imaging objective using a variable beam expander (Thorlabs, Newton, NJ). Upon passing through the objective, pulses converged at the image plane as validated by continuous exposure alignment of a 635 nm laser diode (LDM635; Thorlabs, Newton, NJ). Microcavitation events were recorded with a Phantom v2511 high-speed CMOS camera (Vision Research, Wayne, NJ) using bright-field illumination with a halogen lamp. Samples were imaged in 25mm cylindrical Chamlide magnetic chambers (Live Cell Instrument, Seoul, South Korea). For all tests, a 10 $\times$ /0.3 NA Plan Fluor objective (Nikon Instruments) was used to both focus and image the microcavitation events. Camera settings were chosen to give maximum combined spatial (512 $\times$ 128 px) and temporal resolution (270,000 fps) in all tests. Exposure time was set to 2 μ s, and each video was chosen to be 101 total frames, with the first frame in each video designated as a reference frame before arrival of the laser pulse. Cavitation events were initiated at a z-height of 1 mm above the top surface of the bottom glass coverslip and x-y positions of at least $5R_{\text{max}}$ away from any previously-used location or edge of the gel to sufficiently avoid boundary effects. Twenty cavitation tests per sample were performed on each polyacrylamide gel and a water control.

2.4.4 Image processing

High-speed video frames were analyzed to determine the bubble radius at every time point using a custom image processing algorithm written in Matlab (Natick, MA). Using the first video reference image acquired immediately before bubble nucleation, differential images are calculated for all time points. Differential images were thresholded as a function of the

image noise floor and converted into a convex hull. Bubble surface points were then fit for their centroid and radius using a circle fit algorithm developed by Taubin [105], shown in Fig 2.4.

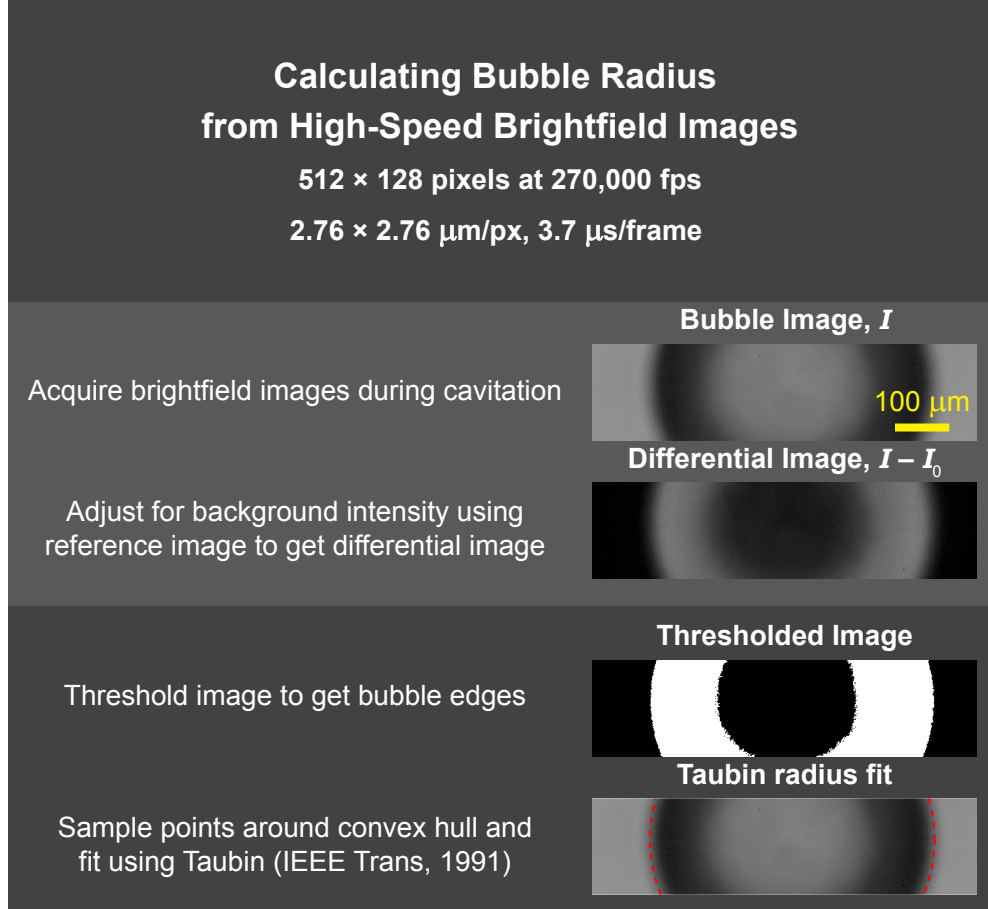


Figure 2.4: Image processing procedure for calculating bubble radius from high-speed brightfield images.

2.4.5 Experimental precision assessment

The maximum bubble radius for each tested polyacrylamide gel varied moderately, with $R_{\text{max}}^{(1)} = 388 \pm 35 \mu\text{m}$ and $R_{\text{max}}^{(2)} = 430 \pm 17 \mu\text{m}$ for the stiffer and softer gels, respectively. Non-dimensionalization of the bubble dynamics highlights material-specific similarities for both concentrations of polyacrylamide and water as shown in Fig. 2.5.

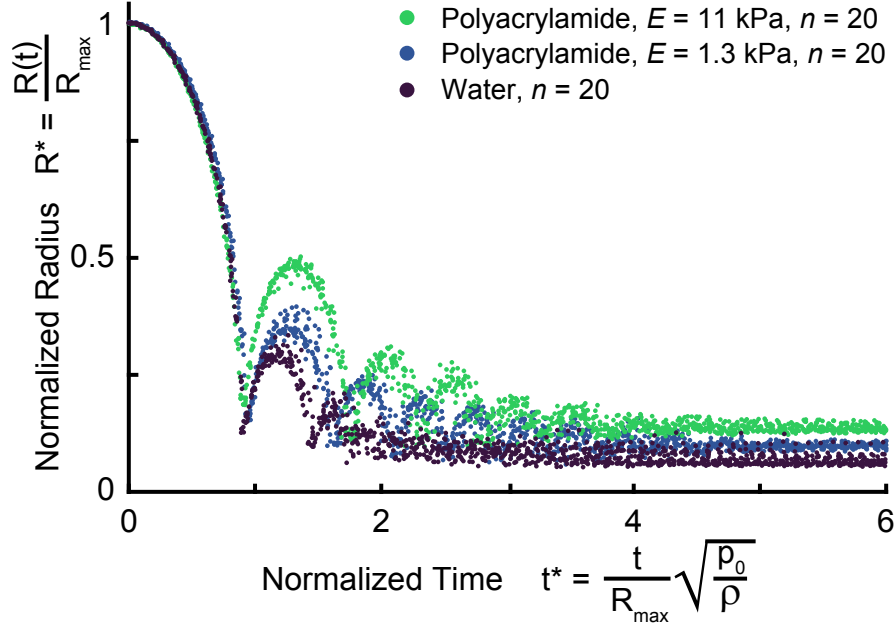


Figure 2.5: Normalized bubble radius vs. time for water and both tested concentrations of polyacrylamide. For all material platforms, dynamics coalesced into distinct bands.

2.4.6 Least squares error and mean values

Least squares error (LSE) is defined discretely by minimizing the perpendicular offset between experimental data points and the numerical simulation. Minimizing the discrete least squares error in the first three peaks gives the best estimate of the viscoelastic properties of the gel. To determine mean viscoelastic material properties (denoted as $\bar{x}$) and standard deviation σ , fits performed to the first collapse over $n = 20$ experiments were weighted by the goodness of least squares fit and summed,

$$\bar{x} = \frac{\sum_i \frac{x_i}{LSE_i}}{\sum_i \frac{1}{LSE_i}}, \quad (2.91)$$

$$\bar{\sigma} = \sqrt{\frac{\sum_i \frac{(x_i - \bar{x})^2}{LSE_i}}{\sum_i \frac{1}{LSE_i} - \frac{\sum_i 1/LSE_i^2}{\sum_i 1/LSE_i}}}, \quad (2.92)$$

where i represents the simulation index.

2.5 Results

2.5.1 Water validation

A water control at 20°C was used to validate the IMUR procedure. Least squares error was calculated between the first three full peaks of experimental data and a sweep over viscosity (range $10^{-5} \leq \mu \leq 0.1$ Pa·s) employing the Newtonian fluid model as shown in Fig. 2.6. The resulting least squares error profile over tested viscosity has a broad minimum due to the weak dependence of bubble dynamics on viscosities lower than 10^{-3} Pa·s. Simulations run for a fixed viscosity of 0.001 Pa·s were performed for $n = 7$ experiments in which the equilibrium bubble radius was stable and are shown overlaid on all water experimental data in Fig. 2.7 and show good agreement with experimental data.

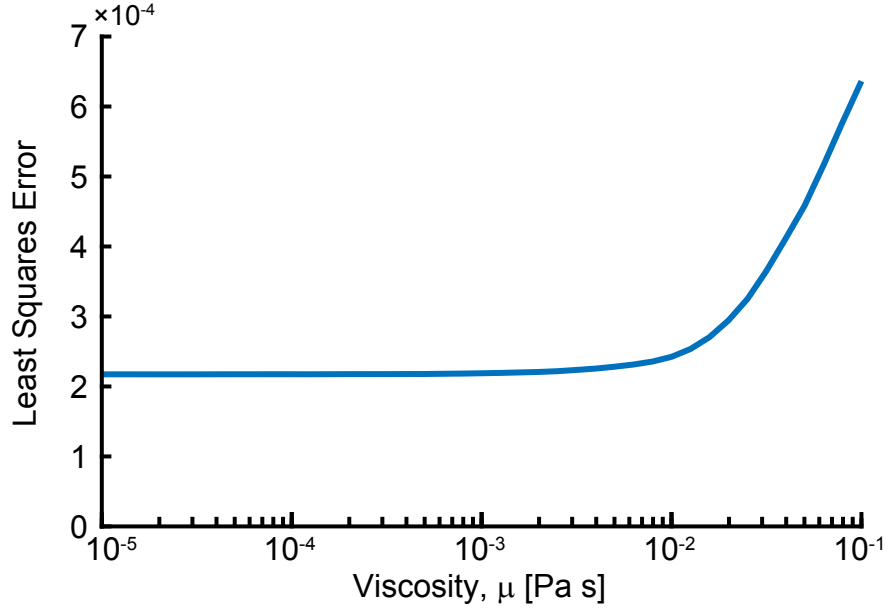


Figure 2.6: Total least squares error for $n = 7$ water experiments fit individually to numerical simulations with prescribed viscosity. As viscous effects decrease, the relative difference in error becomes small.

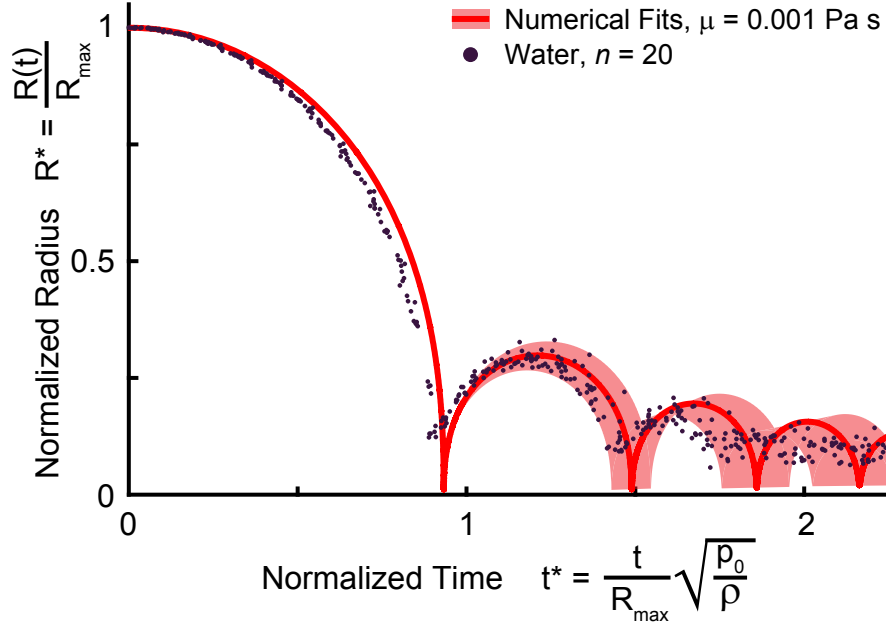


Figure 2.7: Normalized radius vs. time for water experiments ($n = 20$) and numerical fits ($n = 7$) using $\mu = 0.001$ Pa·s. Median simulation dynamics and upper and lower bounds are shown as an envelope.

2.5.2 Using IMUR for characterizing the high-strain rate behavior of polyacrylamide gels

To validate and demonstrate the versatility of the developed IMUR technique we characterized the mechanical behavior of two different stiffness polyacrylamide gels (quasistatic shear moduli of $G_1^q = 2.97 \pm 0.06$ kPa; $G_2^q = 0.461 \pm 0.004$ kPa) following the same general characterization procedure shown in Fig. 2.8.

We began by least-square fitting the experimentally obtained $R(t)$ (orange squares) data against the best fit numerical solution for a purely Newtonian fluid (magenta, Fig. 2.9A). Shown is a representative data set from a single stiff polyacrylamide experiment. The dot-dashed and solid lines indicate the lower and upper bound of the fitted dynamic viscosity, respectively, which ranged from inviscid to 0.32 Pa·s, with the shaded area providing values in between those bounds. As can be seen from Fig. 2.9A changes in viscosity strongly influence the temporal amplitude decay but fail to match the experimental data set without the additional elastic term.

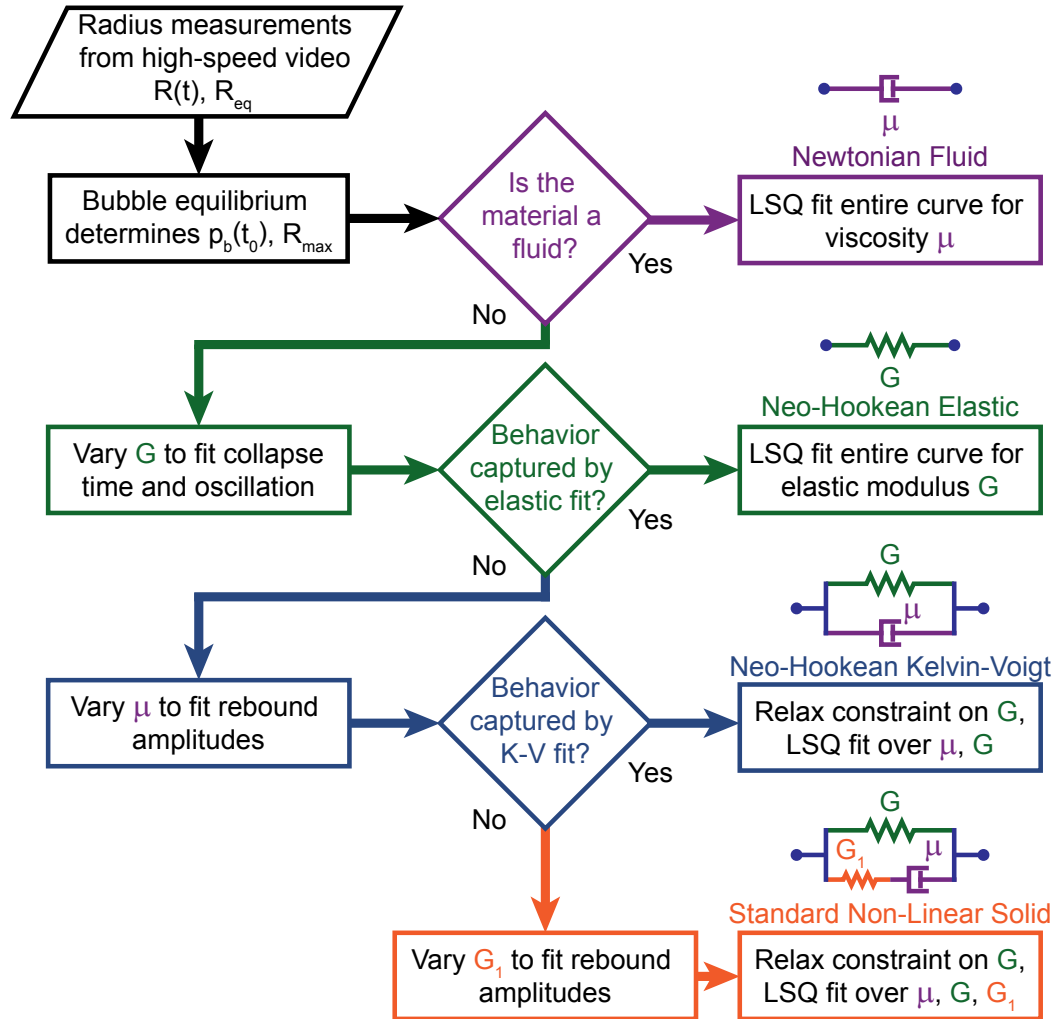


Figure 2.8: Flowchart for IMUR rheometry. Experimental input consists of radius data acquired from high-speed video. Bubble equilibrium radius R_{eq} and maximum expansion radius R_{max} determine the initial gas pressure in the bubble to start the simulation. Material parameters are then chosen to match the oscillation behavior.

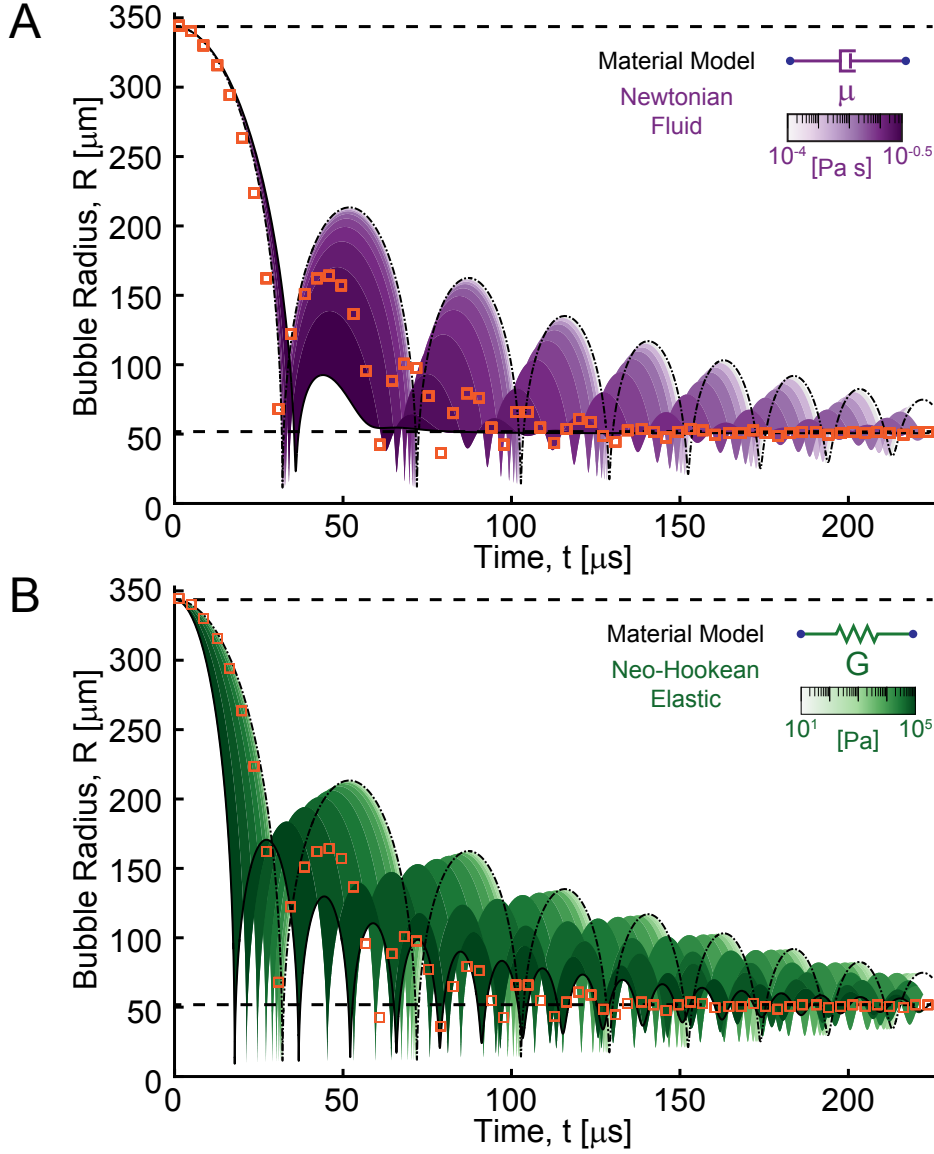


Figure 2.9: Roles of shear modulus and dynamic viscosity in numerical fit. For both plots, experimental data from a representative stiff polyacrylamide inertial cavitation event (orange squares) are overlaid on a one-parameter sweep using (A) a Newtonian Fluid model and (B) a Neo-Hookean elastic model. With increasing viscosity, resulting rebound amplitudes are suppressed, while initial collapse time is relatively unchanged. With increasing shear modulus, resulting rebound frequency increases and initial collapse time correspondingly decreases. Dashed-dotted line represents the inviscid, zero-elastic fluid case. For both plots, top and bottom horizontal dashed black lines denote $R_{\max}$ and R_{eq} .

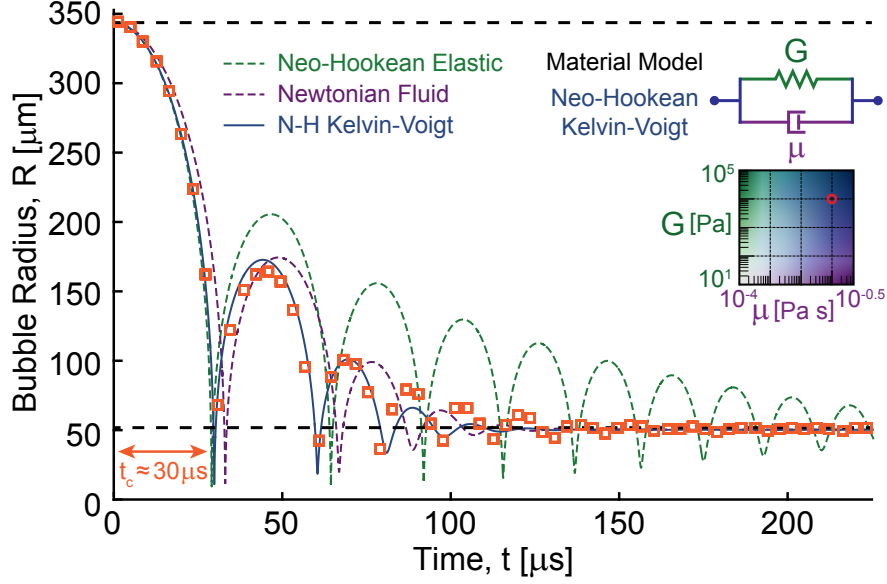


Figure 2.10: Least squares fit of the Kelvin-Voigt model (blue) to the first three peaks of the cavitation event with values $G = 10$ kPa and $\mu = 0.089$ Pa·s, shown with single-parameter Newtonian fluid ($\mu = 0.089$ Pa·s, dashed purple) and Neo-Hookean elastic ($G = 10$ kPa, dashed green) to highlight viscous and elastic contributions. Orange squares denote experimental data from a representative stiff polyacrylamide inertial cavitation event, and top and bottom horizontal dashed black lines denote $R_{\max}$ and R_{eq} . Characteristic time of collapse t_c was approximately $30 \mu\text{s}$.

Furthermore, the purely Newtonian fluid material model is incapable of matching the frequency response of oscillations in the experimental data set. This outcome is not surprising as polyacrylamide is expected to behave strongly elastically at the prescribed polymerization conditions.

Given that the fluid description of polyacrylamide was not able to capture the mechanical behavior, and as such is not classified as a fluid, we move on to prescribing a purely elastic, Neo-Hookean material model to the polyacrylamide gel and repeat the least squares fitting procedure (Fig. 2.8 and Fig. 2.9B). Similar to Fig. 2.9A the lower (zero-elastic) and upper (100 kPa) bounds of the fitted shear modulus range of the incompressible Neo-Hookean free energy function are given by the dot-dashed and solid lines, respectively. In stark contrast to the effect of viscosity in Fig. 2.9A, increasing the elasticity of the material leads to significant decreases in the initial collapse time and increases in the overall oscillation frequency with comparatively little effect on amplitude attenuation. However, as is apparent from Fig.

2.9B, a purely elastic description of the interrogated gels similarly fails in providing a good match to the experimental data, which motivates the modification of the free energy function to account for both elastic and viscous contribution according to Fig. 2.8.

Combining elasticity and viscosity into the material model, Fig. 2.10 shows the best fit least-squares results of the non-linear Kelvin-Voigt model (described in the previous section) to the experimental data, along with the individual contributions from the purely elastic (Fig. 2.9B) and purely viscous (Fig. 2.9A) constitutive models. As can be seen from Fig. 2.10, the inclusion of both elastic ($G = 10$ kPa) and viscous ($\mu = 0.089$ Pa·s) elements matches the experimental data well. To ensure that the non-linear Kelvin-Voigt model provided the most accurate fit to the experimental data for polyacrylamide gels tested, we also characterized the same gels using a standard non-linear solid (Fig. 2.8), which failed to produce any significant increase in accuracy compared to the simpler Kelvin-Voigt model.

To produce the final estimate for the Kelvin-Voigt material parameters, the shear modulus and material viscosities were averaged over $n = 20$ experiments for each of the two different polyacrylamide concentrations. However, in order to better account for variability between experiments, the total estimate on G and μ were appropriately weighted by the goodness of their least squares fit using Eqs. 2.91-2.92, producing the shear moduli of $G_1 = 7.69 \pm 1.12$ kPa and $G_2 = 2.12 \pm 0.70$ kPa and viscosities of $\mu_1 = 0.101 \pm 0.023$ Pa·s and $\mu_2 = 0.118 \pm 0.014$ kPa·s, for the two gels respectively.

While the material model for polyacrylamide was chosen to be Neo-Hookean Kelvin-Voigt as per the flowchart shown in Fig. 2.8, the standard non-linear solid model was also explored as a potential option for polyacrylamide. The resulting least squares error profile suggests a high value for G_1 as the optimal fit, as shown in Fig. 2.11. Notably, in the limit of $G_1 \rightarrow \infty$, or more precisely, as the Maxwell branch relaxation time μ/G_1 becomes much smaller than the characteristic collapse time t_c , the standard non-linear solid model converges to the Neo-Hookean Kelvin-Voigt model, suggesting validity of our model choice.

The inclusion of another spring constant G_1 has important effects on the resulting bubble dynamics. When values of G_1 are on the order of G (i.e. when μ/G_1 approaches the

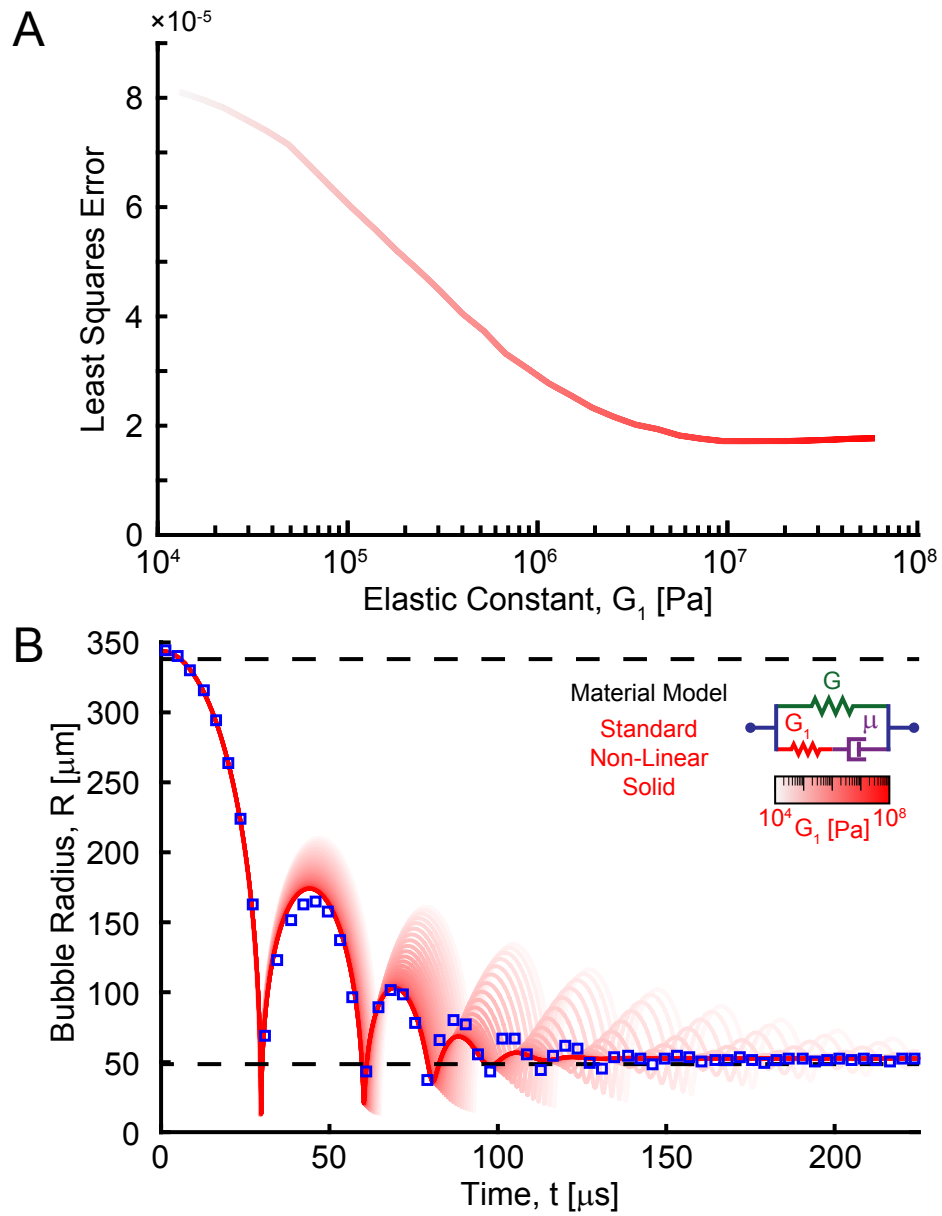


Figure 2.11: Standard non-linear solid model calculations. (A) Least squares error between and (B) bubble dynamics for a representative stiff polyacrylamide test and a one parameter sweep over second spring constant G_1 , using $G = 10$ kPa and $\mu = 0.089$ Pa·s.

characteristic collapse time), oscillation amplitudes are increased. Additionally, the relative ratios between subsequent peak amplitudes (e.g. $R_{\max}^{(1)}/R_{\max}^{(2)}$, $R_{\max}^{(2)}/R_{\max}^{(3)}$) increase as $G_1 \rightarrow G$. As our numerical fits even for the two-parameter Neo-Hookean Kelvin-Voigt model under-predicted later peaks, we found that the increasing influence of an added spring increased least squares error for the tested polyacrylamide gels as shown in Fig. 2.11A. Lowest least squares error was found as $G_1 \rightarrow \infty$ as shown in Fig. 2.11B, which confirmed our choice of the two-parameter model.

2.6 Discussion

Inertial microcavitation-based ultra-high strain rate rheometry is a robust, minimally-intrusive, local microrheology approach for characterizing the material constitutive properties at high (10^3 s^{-1}) to ultra-high (10^8 s^{-1}) strain rates. By combining simple, high-speed videography with an appropriate theoretical cavitation framework, the IMUR technique is suitable for reliably characterizing the material properties of soft matter using the classical case study of polyacrylamide. Specifically, the material behavior of polyacrylamide undergoing large, finite deformations ($E_{rr} > 0.05$) at strain rates of up to 10^8 s^{-1} is well-captured by a non-linear Kelvin-Voigt model, which extends the traditional quasistatic Neo-Hookean description of polyacrylamide to include a dynamic material viscosity on the order of $10^{-1} \text{ Pa}\cdot\text{s}$. There are several interesting observations when comparing the quasistatic vs. dynamic material response of polyacrylamide. First is the mentioned addition of an extra viscosity term under application of highly dynamic loads, which is necessary to properly capture the cavitation material response (Fig. 2.10). Provided the relatively low viscosity even at very high shearing rates, the contribution of viscous stresses during a quasistatic response is generally orders of magnitude lower [106] and is generally neglected, which reduces the non-linear Kelvin-Voigt model to the standard Neo-Hookean material model. Second, comparison of the magnitudes of the quasistatic and dynamic shear moduli showed the shear moduli obtained during cavitation to be stiffer than their quasistatic counterparts. The observed stiffening effect is likely that various physical relaxation mechanisms do not have adequate time to relax at high rate and behave as additional spring components as

compared to true equilibrium testing, illustrated schematically in Fig. 2.12. Another possibility is the occurrence of a restructuring of the bis-acrylamide cross-linked structure due to the large expansion and contraction strains. However, detailed analysis of the physical origin of the observed stiffening behavior of the material is beyond the scope of this work.

Interestingly, the relative ratio between the gel stiffnesses was significantly lower at ultra-high strain rates, with $G_1/G_2 = 3.62$ and $G_1^q/G_2^q = 6.46$, suggesting that the stiffness scaling of the incorporation levels between bis and acrylamide monomer as was established under quasistatic loading is not extendable into the fast dynamic loading regime. Thus, use of quasistatic tests cannot be assumed to adequately replace testing for ultra-high strain rate material response.

2.6.1 Material stresses and strains due to cavitation

Once the characterization of the material properties is achieved, the solution to equations (2.80-2.85) can be utilized to closely examine the stress and strain evolution of the material during collapse, subsequent rebounds, and oscillation as both a function of time and position. Such an analysis allows one to build an understanding of the associated strains, strain rates and stresses with cavitation in soft matter. While there are many state variables that can be plotted against space and time, we purposefully focus on the most relevant quantities to describe the stress-strain field in the surrounding material. Shown in Fig. 2.13 are the strains, strain rates, and stresses at the bubble wall as a function of time for representative numerical bubble dynamics solutions obtained by least squares fitting one test of each of the two different concentrations of polyacrylamide gels. Radial Hencky strain, $E_{rr} = -2\ln(r/r_0)$, evaluated at the bubble wall (Fig. 2.13A) is finite ($\gg 0.05$) over the first ~ 5 oscillations of the bubble, with large peak values in both initial radial compression and subsequent radial tension at collapse.

The primary collapse event occurs extremely quickly ($\sim 1 \mu\text{s}$) producing peak strain rates at the bubble wall on the order of 10^7 - 10^8 s^{-1} (Fig. 2.13B). Strain rates also change rapidly as the bubble oscillates between the expansion and collapse phases with an exponential

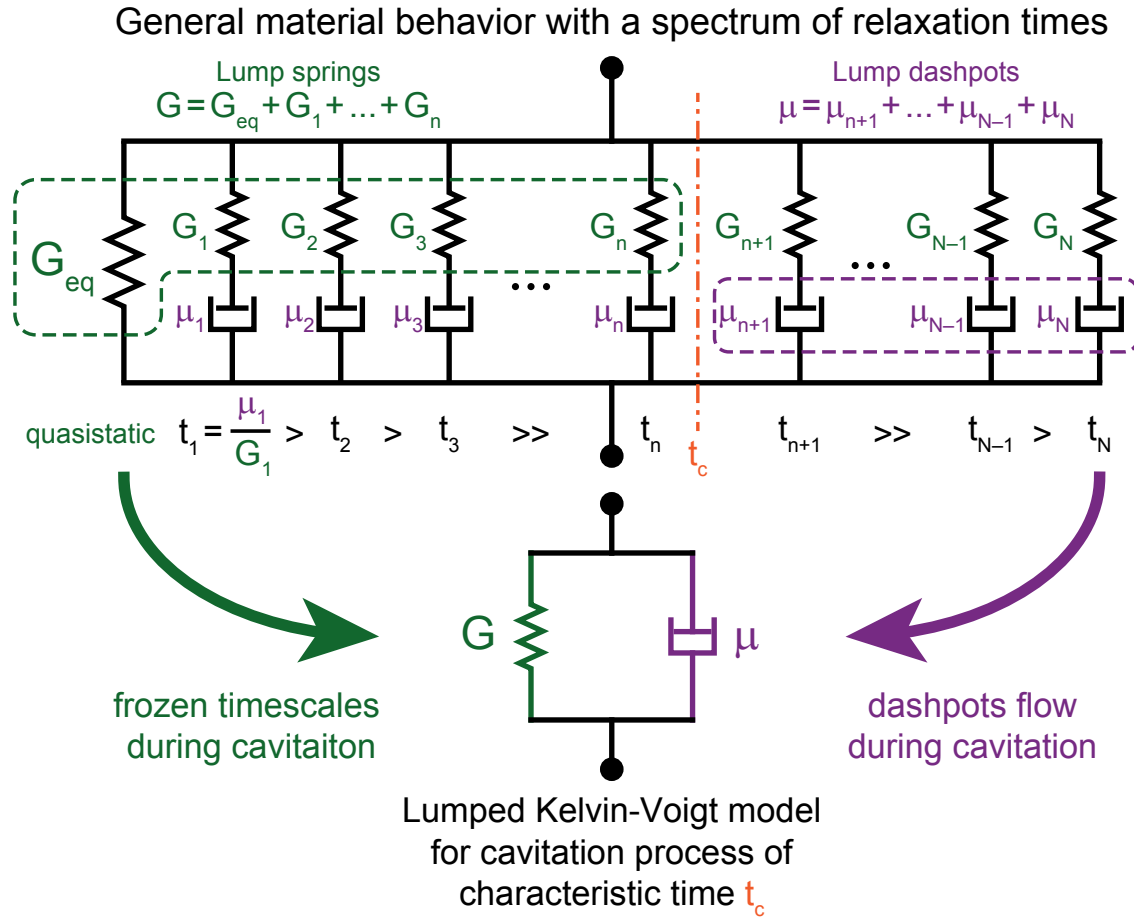


Figure 2.12: General material behavior with a spectrum of relaxation times. A viscoelastic material modeled by a spectrum of N Maxwell branches with different relaxation times can be lumped into elastic and viscous parts when probed at a certain frequency, or characteristic time t_c . While branches of long relaxation time do not have time to relax during cavitation and appear frozen, branches of short relaxation time flow easily. The lumped Kelvin-Voigt model shear modulus G and viscosity μ are then combinations of the springs and dashpots for branches with longer and shorter relaxation times, respectively.

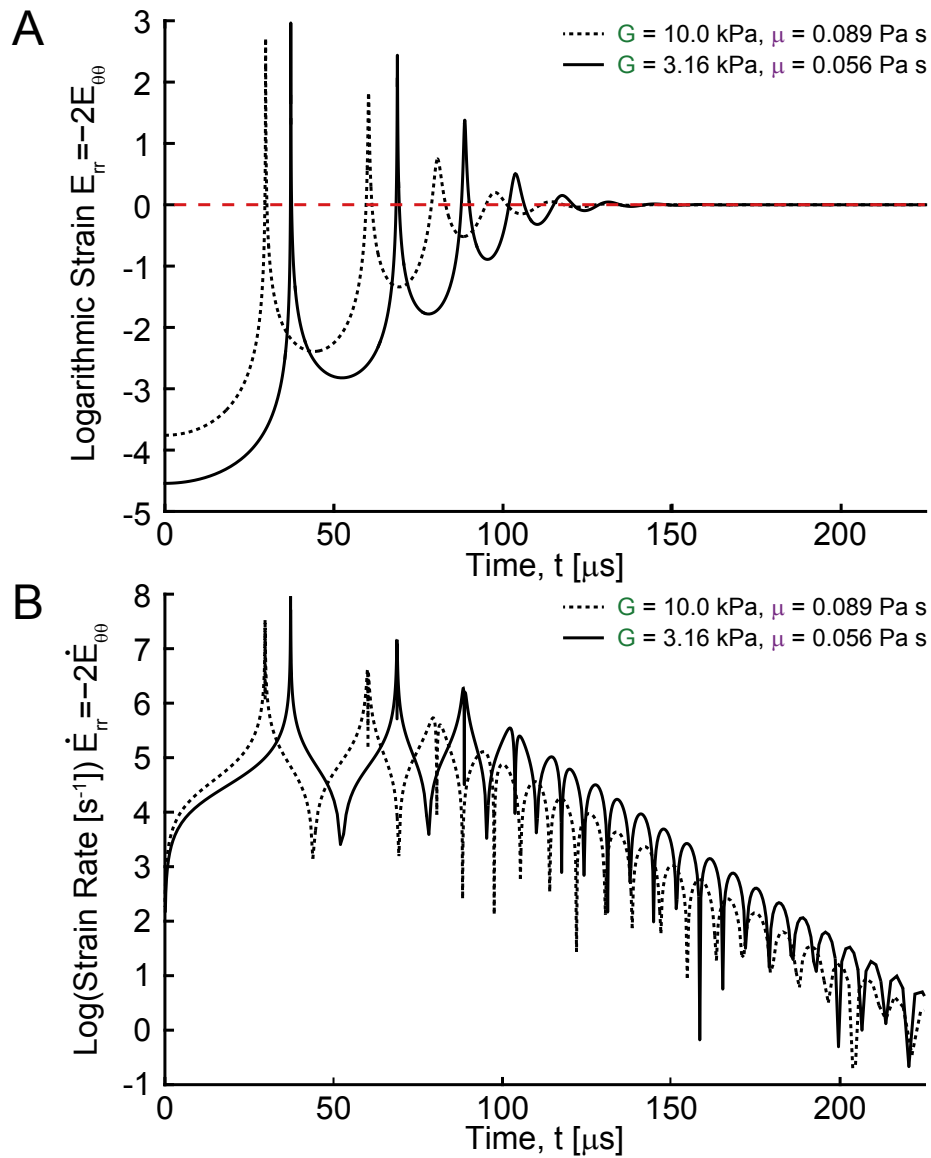


Figure 2.13: Material strains and stresses at the bubble wall during inertial microcavitation. (A) Plot of the logarithmic radial strain over time for numerical fits of two different stiffnesses of polyacrylamide. Red dashed line, zero strain; positive values of radial strain signify tension while negative values signify compression. (B) Semi-log plot of logarithmic strain rate magnitude over time.

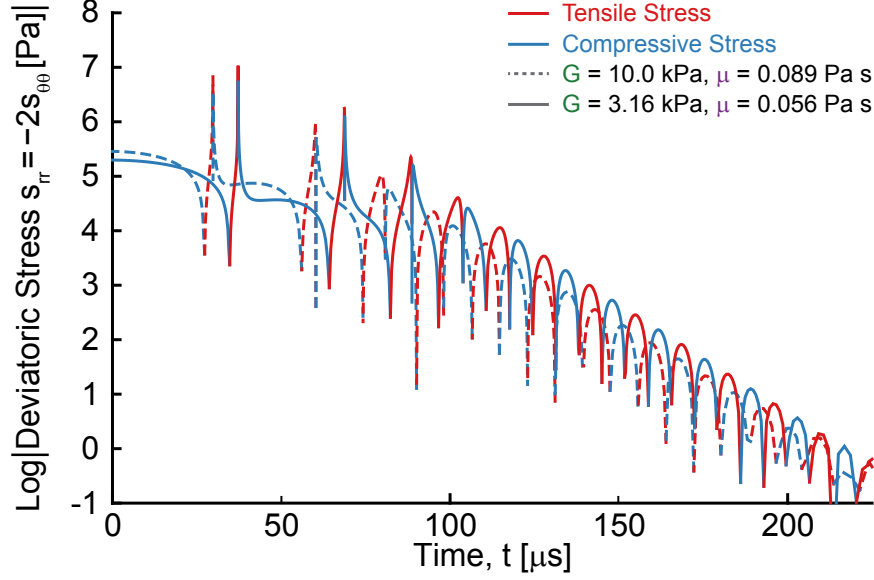


Figure 2.14: Semi-log plot of deviatoric Cauchy stress magnitude at the bubble wall over time. Linear decrease of peak magnitudes suggests an exponential decay envelope. Red portions of each curve signify tensile radial stress, while blue portions signify compressive stress.

decay as suggested by the linear decrease of peaks as a function of time on the semi-log plot (Fig. 2.13B). The radial stresses, shown in Fig. 2.14, also decay exponentially with time but exhibit a behavior distinctly different from the strains during the peak strain rates of primary collapse. The presence of material viscosity breaks the temporal symmetry in the stress response when compared to the symmetric Hencky strain profile.

In addition to temporal quantities at the bubble wall, field quantities in the material can be calculated at specific timepoints. For example, at peak radial compression and tension, we can examine strain and stress quantities as a function of radial position. This type of information is often useful in developing scaling laws or damage criteria as a function of the underlying material properties or microstructure [107,108]. Fig. 2.15A shows a plot relating the reference configuration (equilibrium) radial coordinate to the deformed radial coordinate during the peak radial expansion (red) and peak radial compression (blue). The dashed line signifies the no-deformation case where the deformed position equals the equilibrium position, which when approached asymptotically, signifies the small strain, linear viscoelastic regime.

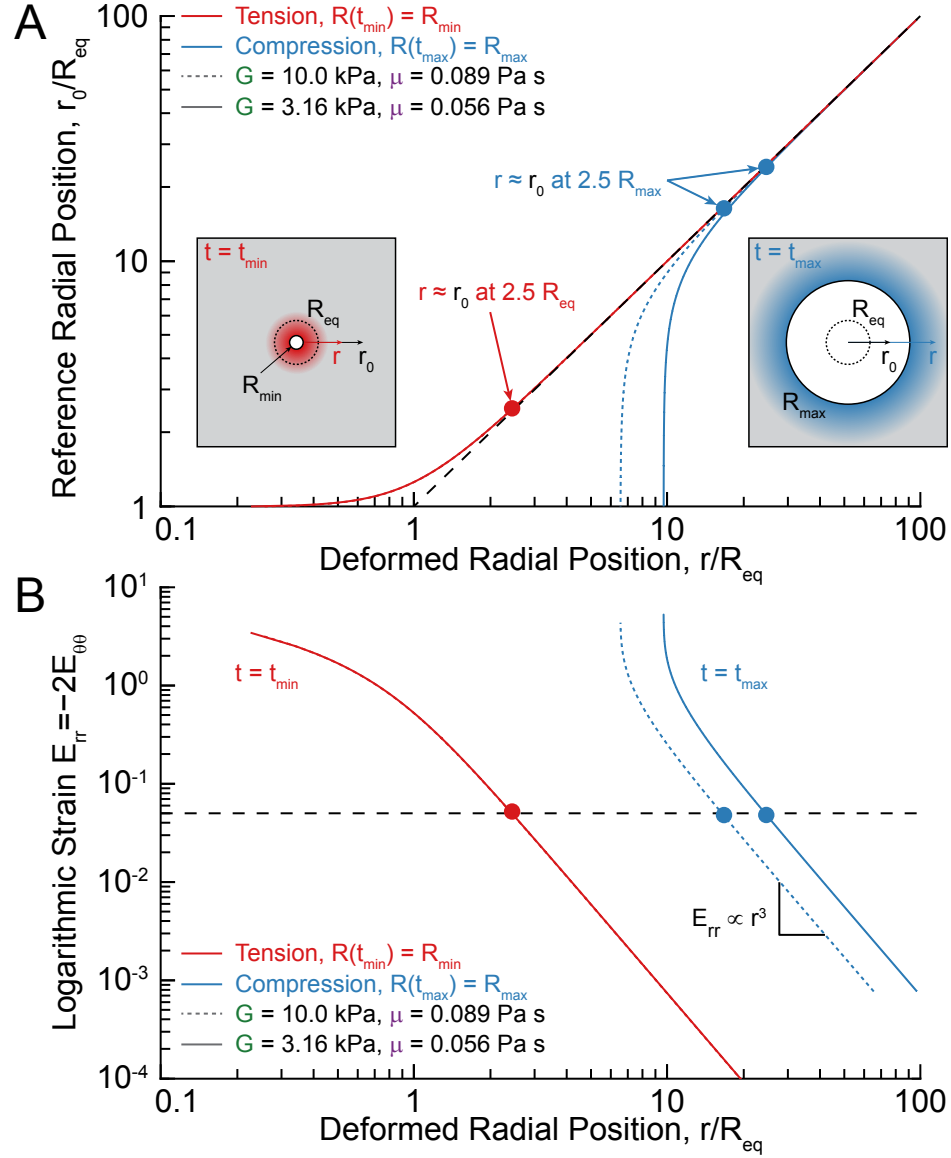


Figure 2.15: (A) Plot of normalized reference position (with zero deformation, black dashed line) and (B) logarithmic radial strain for the cases of maximum tensile (red) and compressive (blue) strain in the material. Marked are points where the reference and deformed coordinate are below the small-strain threshold defined as 0.05, at locations of $2.5 R_{eq}$ for tension and $2.5 R_{max}$ for compression. Strain values decay as $O(R^3)$, which agrees with the 3D inclusion solution.

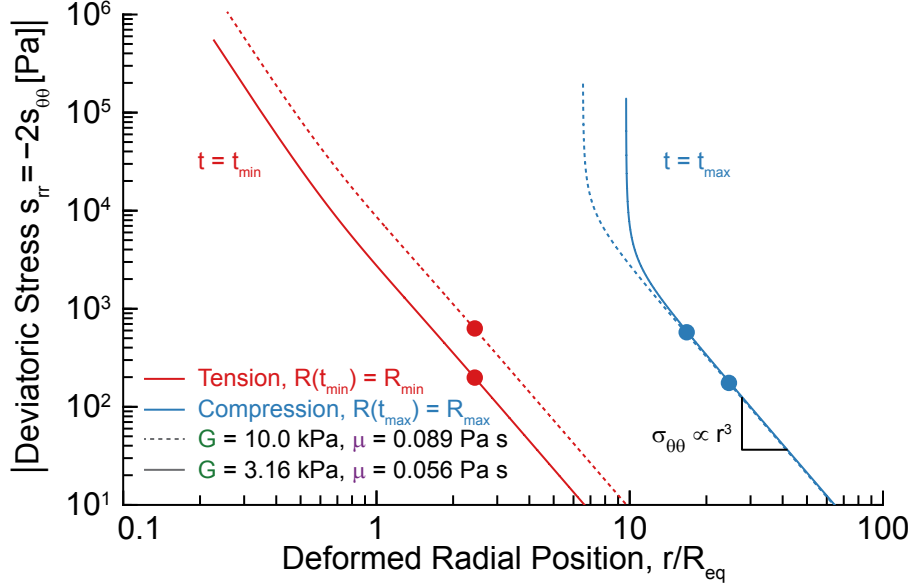


Figure 2.16: Deviatoric Cauchy stress versus deformed position for the cases of maximum tensile (red) and compressive (blue) strain in the material. Marked are points where the reference and deformed coordinate are below the small-strain threshold defined as 0.05, at locations of $2.5R_{eq}$ for tension and $2.5R_{max}$ for compression. Strain and stress values decay as $O(R^3)$, which agrees with the 3D inclusion solution.

Given the strongly inhomogeneous loading nature of cavitation, it is useful to establish a convergence length, i.e., a non-dimensional distance beyond which the solution to equations (2.25-2.39) can be linearized, to pave the way for developing simpler, analytically-closed form solutions under the assumption that stresses and strains in the immediate vicinity of the bubble do not need to be fully resolved. As an example, the positions where strains become small, e.g. less than 0.05 (see Fig. 2.15B), are found to be $2.5R_{eq}$ in radial tension (bubble collapse) and $2.5R_{max}$ in radial compression (maximum bubble expansion), which is consistent with the convergence length determined in Fig. 2.15A. The relative asymmetry between these two scales highlights the difference in degree of localization for radial tension and compression. While the states of maximum tension and compression produce comparable strain magnitudes, compression induced by bubble expansion in particular creates a finite strain region over a larger material volume. This may be of interest, for example, in determining strain damage criteria for hydrogel systems such as in [96].

The corresponding radial stresses as a function of position are shown in Fig. 2.16. Both the

far-field strains and stresses decrease as $O(r^3)$, which agree well with the classical analytical linear-elastic inclusion solution of [109].

2.6.2 Current limitations

In its current configuration, the IMUR technique has several important limitations. First, due to the intrinsic assumption of spherical symmetry, only $R(t)$ data that remains spherically symmetric during the cavitation event will produce accurate material property estimates. In homogeneous materials, this can typically be ensured by cavitating 2-3 maximum bubble diameters away from boundaries that feature significant impedance changes. Properly reconstructing and analyzing non-symmetric three-dimensional cavitation dynamics remains challenging both from a high-speed imaging and reconstruction standpoint as well as from a numerical perspective as the momentum and conservation of mass equations would have to be solved in 3D, which is computationally challenging. Second, the current mathematical framework assumes all of the to-be-employed material models to be incompressible in the near field, which is an adequate assumption for most tissues and hydrogels but may not be appropriate for foams and other highly compressible materials. Inclusion of compressible material models is possible, however the literature is still in its infancy when it comes to non-linear, highly compressible material descriptions. Third, while the current approach does not require independent measures of bubble pressure or temperatures, mainly due to the fairly simple constitutive models employed, future investigations of more complex materials might require the addition of independent measurements of pressure and/or temperature to ensure solution uniqueness. Fourth, the current theoretical treatment of cavitation only considers bubble dynamics within the low Mach number regime, which may not be sufficiently accurate close to the bubble collapse phase, where shock waves may be produced. For polyacrylamide, the highest Mach numbers recorded in simulations were approximately 0.4 at the collapse, however, for lower viscosity materials, the Mach number may approach or exceed 1. While inclusion of the higher Mach number region is possible and well-documented in the literature, the current imaging system does not have enough temporal resolution ($< 1 \mu s$) to fully resolve such detail. Finally, since the bubble lifetime is

directly proportional to the maximum bubble radius, proper temporal resolution becomes increasingly challenging for bubble sizes $10\ \mu\text{m}$ and below. We anticipate that many of these challenges will be overcome and addressed in future studies that will build upon the experimental and theoretical framework established herein.

2.7 Conclusion

IMUR represents a new approach for determining the viscoelastic material properties of soft matter at high ($10^3\ \text{s}^{-1}$) to ultra-high ($10^8\ \text{s}^{-1}$) strain rates via inertial cavitation that requires only a single pulsed laser and a high-speed imaging platform, which can be easily integrated into commercially available inverted or upright light microscopes. By combining high-speed video of bubble oscillation with an appropriate theoretical model of cavitation-bubble collapse, we demonstrate the ability to discern mechanical properties and field variables at previously undocumented strain rates in materials as soft as a few kPa. Specifically, we show that the material behavior of polyacrylamide undergoing large, finite deformations ($|E_{rr}| > 0.05$) at strain rates of up to $10^8\ \text{s}^{-1}$ is well-captured by a non-linear Kelvin-Voigt model, which extends the traditional quasistatic Neo-Hookean description of polyacrylamide to include a dynamic material viscosity on the order of $10^{-1}\ \text{Pa}\cdot\text{s}$.

Since cavitation induction is achieved via spot focusing of a single laser pulse, this technique is minimally invasive and can characterize material properties in three dimensions at length scales of single microns to millimeters. As such this technique shows great promise for use of the interrogation of *in vivo* materials as well as for samples of small size, odd shape, or high compliance, which may otherwise not be easily mountable in conventional dynamic testing apparatus. The suitability of this inertial cavitation rheology technique is demonstrated and benchmarked using the well-characterized hydrogel polyacrylamide and shows that, under high-strain rate loading, stress contributions due to viscosity effects need to be included alongside the well-known Neo-Hookean elastic nature of polyacrylamide. Furthermore, this technique has excellent precision and repeatability in its measurements, particularly when compared to other local soft matter tribology methods including nano-

and microindentation.

In sum, IMUR may fill the void of a simple and robust microrheology technique to determine soft matter and tissue properties at high to ultra-high strain rates, as well as provide a high-fidelity platform for understanding inhomogeneous cavitation stresses and strains as arise in many natural, biological, and clinical applications.

Chapter 3

Cavitation-Induced Neural Cell Injury

Note: A version of this chapter is in preparation for submission to Nature Communications. Data and figures have been used with all co-authors' consent.

Estrada JB, Cramer III HC, Scimone MT, Franck C. Inertial microcavitation as a neural cell damage mechanism in an *in vitro* study of traumatic brain injury.

3.1 Introduction

In order to get a full understanding of the mechanics of blast or cavitation at a cellular level, there is a distinct need for a ground-up approach, in which mechanical interactions are well-understood and experimentally repeatable. Given the complex mechanical interactions of blast loading with whole-brain or tissue slice systems and observations in blunt TBI showing manifestation of primary injury on a cellular level (e.g. DAI), this chapter aims to approach the problem by focusing on the specific mechanism of microcavitation, as opposed to interaction with the full blast wave, for primary dissociated neural 3D cell cultures.

The benefit to focusing on cavitation without inducing blast is twofold: experimentally,

pulsed laser-induced cavitation is an established method for inducing reproducible dynamics and cell injury in 3D cultures [16] which can be integrated with a conventional microscope setup, and physically, it simplifies the resulting mechanics to a tractable, spherically-symmetric bubble problem that can be modeled analytically. The combination of laser scanning confocal microscopy of the reference and injury states and high-speed imaging during pulsed-laser-induced cavitation injury allows for a high degree of control over the injury mechanics and provides necessary insight into critical strains for primary cell injury. Furthermore, confocal reflectance imaging validates the implicit continuum assumption of the material at tested length scales, as shown in Fig 3.1A by showing that mature cells are well-bound in the hydrogel, and were validated for strain continuity by comparison to the analytical Eshelby displacement solution for an inclusion [57].

Understanding the relation between mechanical deformations and failure on a cellular level requires knowledge of the underlying cytoskeletal structure. The dissociated neural cells are comprised of neurons, astrocytes, oligodendrocytes, and neural stem/progenitor cells as discussed in Dingle and Boutin et al. [110]. Astrocytes and neurons, the principal two cell types in the cortex, are generally comprised of a cell body, or soma, and projections [111]. In neurons, there exists a primary projection, called the axon, with an associated axonal terminal for signal transmission. These components are supported by a complex cytoskeleton comprised of many structural proteins including microtubules, tau protein, and filamentous actin (F-Actin) as shown schematically in Fig. 3.1B. F-Actin, also known as microfilaments, serves different roles depending on cell type. In oligodendrocytes, F-Actin primarily concentrates at the leading edges of cell extensions, or projections, and serves as stress fibers during remodeling and myelination [112]. In contrast, mature astrocytes and neurons each have well-defined cytoskeletal F-Actin structures, but are supplemented by different proteins (e.g. glial fibrillary acidic protein for astrocytes [113] and class III β -tubulin for neurons). Rather than distinguish the difference between each cell type-specific damage while keeping the goal of understanding primary microcavitation injury suggests use of fluorescent probes for common biological or cytoskeletal components. Live-staining for intracellular esterase activity and post-fixation staining for F-Actin, both of which are non-cell-specific, can be

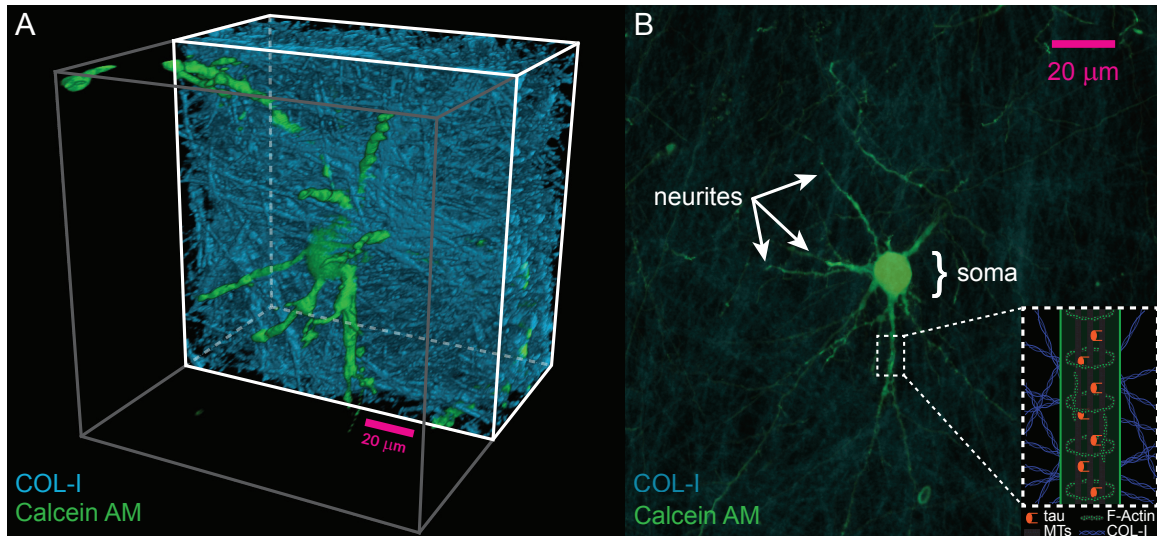


Figure 3.1: Reflectance imaging shows cells to be well-encapsulated in collagen matrices. (A) Calcein AM-stained cortical neuron (green) shown with a cropped volume of collagen fibers (blue). Scale bar, 20 μm . (B) Cortical neuron terminology and projection schematic. Distal from the cell body, or soma, are projections, or neurites. Large projection cytostructure is comprised primarily of microtubule bundles stabilized by tau protein and filamentous actin rings.

used to determine morphological and cytoskeletal information, respectively.

3.2 Methods

3.2.1 Isolation of primary neural cells

All procedures were performed in full accordance with the Institutional Animal Care and Use Committee of Brown University. Unless otherwise stated, reagents were acquired from ThermoFisher (Waltham, MA). Primary neural cells were isolated from postnatal day 0-1 Sprague-Dawley rats (Charles River, Wilmington, MA). Pups were rapidly decapitated, and the cerebral hemispheres were removed and placed in a cold solution of Hibernate A (HA; Brain Bits LLC, Springfield, IL) + 2% B-27 serum-free supplement (50 $\times$) + 0.25% GlutaMAX (100 $\times$), ventral side down. A cut was made around the lateral side of the brain, just above the entorhinal and perirhinal cortices, followed by separation at the corpus callosum. The excess tissue was removed from the cerebral cortex and the cortices were minced (~ 0.5 mm) and digested in a solution of Hibernate A – CaCl₂ (Brain Bits LLC) +

Papain (2 mg/mL; Worthington, Biochemical Corporation, Lakewood, NJ) for 30 minutes at 37°C with gentle agitation every 5 minutes. An equal volume of pre-warmed HA/B-27 was added to the HA-CaCl₂/papain solution and centrifuged at 150g for 5 minutes. After removal of the supernatant, the dissociated tissue was re-suspended by gentle mechanical trituration in cortical complete media (Neurobasal-A medium + 1% Penicillin/Streptomycin + 0.25% GlutaMAX 100×), allowed to settle for 1-2 minutes, and passed through a sterile cell strainer (40 μ m mesh size) to filter large tissue debris. The suspension was again centrifuged at 150g for 5 minutes and counted with a hemocytometer (Hausser Scientific, Horsham, PA). Supernatant was removed, replaced with 3 mL cortical complete media, and cells were re-suspended by gentle mechanical trituration. Cell solution was diluted with cortical complete media to 10⁷ cells/mL prior to preparation of 3D collagen gels to yield a final seeding density of 3,750 cells/mm³ [57, 110, 114].

3.2.2 Preparation of 3D collagen hydrogels

Type I Collagen (rat tail; Dow Corning, Tewksbury, MA) hydrogels were made to a concentration of 2.2 mg/mL according to the manufacturer’s standard protocol. 10× Phosphate-buffered Saline (PBS) was mixed with 1 M NaOH (Sigma-Aldrich, St. Louis, MO). Type I collagen stock solution (3.3–3.7 mg/mL) in 0.02 M acetic acid was added to the PBS/NaOH solution, vortexed, and kept on ice. Neuron-cell media suspension was then added to the collagen mixture to get a collagen concentration of 2.2 mg/mL and neuron density of 3,750 cells/mm³, and tested for pH with a pH spear (Oakton Instruments, Vernon Hills, IL) with a target of 8.4. Collagen gel composition is shown in Table 3.1.

Table 3.1: Concentrations for 3D Type-I Collagen hydrogels.

Component	10× PBS	1 M NaOH	Stock Collagen-I	Cell Suspension
Volume %	10	1.4	56.2	32.4

Cell-collagen gels were seeded at 50 μ L volumes directly into 48-well #1.5 glass-bottomed plates (MatTek, Ashland, MA), as shown in Fig. 3.2. Dimensions of the samples were

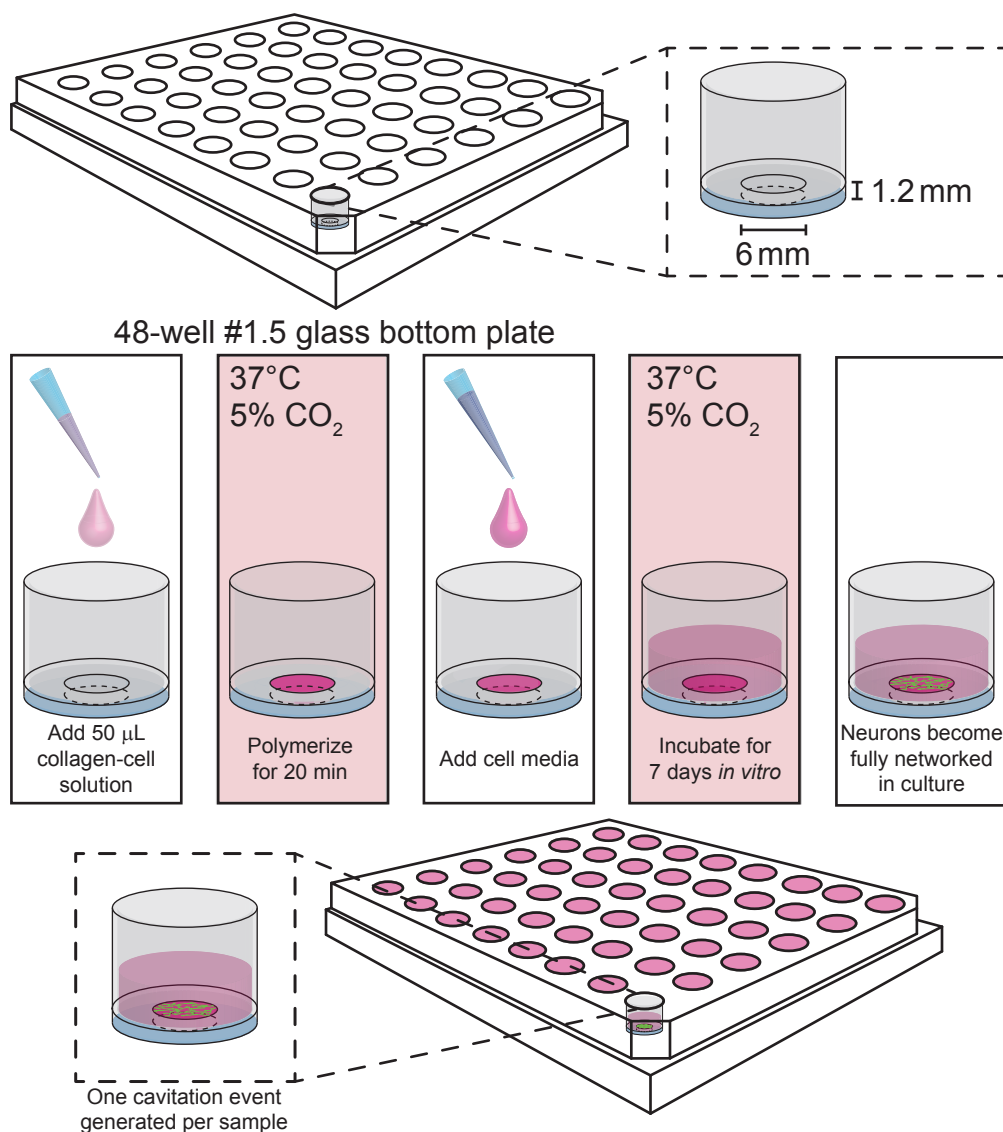


Figure 3.2: Schematic of a #1.5 glass bottom 48-well plate for high throughput sample testing. Each well is filled with 50 μL of collagen-neural cell solution and allowed to polymerize for 20 minutes at 37°C in a 5% CO_2 humidified incubator. Cortical complete media is added to each well after polymerization and plates are then incubated for 7 days *in vitro*, after which neurons show a high degree of network connectivity. Cavitation events are generated no more than once per gel, at the geometric midplane of the gels.

approximately 6 mm in diameter and 1.2 mm in height. Gels were polymerized in a humidified incubator at 37°C in 5% CO₂ for approximately 20 minutes after which 300 μ L of cortical complete media is added to each well after polymerization. Each plate of samples was incubated at 37°C, 5% CO₂ for 7 days *in vitro*, after which neurons showed a high degree of network connectivity.

3.2.3 Fluorescent staining

Live-cell fluorescence

Cell morphology was assessed before and after microcavitation via cell-permeant Calcein Acetoxymethyl (AM). Carboxylic acids modified by AM ester groups are non-polar, which facilitates transversal of the cell membranes. In living cells, colorless Calcein AM is converted into green fluorescent calcein by hydrolysis of the acetoxymethyl ester group by intracellular esterases. Cleavage of the ester groups additionally polarizes the molecule, ensuring high retention in live cells [115]. Cell cultures were incubated with 200 μ L of pre-warmed HA+2% B-27 buffer with Calcein AM (20 μ M) for 1 hr to allow for sufficient penetration into the gel, after which media was replaced with naïve pre-warmed HA+B-27 buffer. Samples not exhibiting healthy, fully-connected morphology were discarded from further data processing.

Cell death was assessed in control volumes of cells using Ethidium Homodimer-1 (EthD-1), a high-affinity nucleic acid stain which fluoresces upon binding to DNA and is impermeant until both cell membrane and nuclear poration occurs [115]. Control cultures were incubated with 200 μ L of pre-warmed HA+2% B-27 buffer containing both Calcein AM (20 μ M) and Eth-D1 (10 μ M) for 1 hr, after which media was replaced with naïve pre-warmed HA+B-27 buffer.

Fixation and Cytoskeletal Probes

neural cell cultures were fixed post-live-cell fluorescent imaging using a solution of 4% v/v paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) + 8% w/v sucrose in 1×

PBS for approximately 2 hours. Samples were subsequently washed with $1\times$ PBS + 0.1% Triton $\times 100$ three times for half hour increments and stored at 4°C .

Phalloidin is a cyclic peptide phallotoxin, derived from the *Amanita phalloides*, or death cap mushroom, which selectively binds to F-Actin in permeated cells. Coupled with the fluorescent dye tetramethylrhodamine (TRITC; ActinRed 555 ReadyProbes, Invitrogen), TRITC-phalloidin is photostable and provides insight into the state of the cytoskeleton before or after cavitation damage. Fixed samples were incubated in $1\times$ PBS with TRITC-phalloidin (1 drop per $300\ \mu\text{L}$ in each well) for 1.5 hours to ensure adequate diffusion to the center of each gel. Samples were then washed twice with $1\times$ PBS to remove any excess or unbound stain.

3.2.4 Confocal microscopy and imaging

For all live-cell experiments and controls, three-dimensional image stacks of cells immediately before and at timepoints after laser-induced microcavitation were acquired using a Nikon A-1 laser scanning confocal system, mounted on a Ti-Eclipse inverted optical microscope controlled by NIS-elements software (Nikon, Tokyo, Japan). To maintain physiological conditions, a custom-made environmental chamber was built around the microscope and thermally-controlled at 37°C with a closed-loop Air-Therm heater (World Precision Instruments, Sarasota, FL). Samples in 48-well glass-bottomed plates were secured using a well plate holder (Ti-SH-W; Nikon), which was allowed to thermally equilibrate for 2-3 hours before imaging.

Before imaging, the bottom coverslip was located in each sample using the Nikon Perfect Focus System (PFS), which uses a near-infrared 870 nm LED coupled with a CCD line sensor to define the bottom of the glass coverslip as the system reference plane. The PFS focal plane was defined as the top surface of the bottom coverslip. All cavitation events were performed $600\ \mu\text{m}$ above the top of the glass coverslip, or approximately at the mid-plane of the gels.

3.2.5 Laser-induced microcavitation

As described in section 2.4.3, experimental inertial cavitation is generated via single pulses of a 6 ns frequency-doubled 532 nm Q-switched Nd:YAG laser (Continuum, San Jose, CA) through a Plan Fluor 20 $\times$ /0.5 NA objective (Nikon). The beam was redirected into the back aperture of the objective by a 532 nm notch dichroic reflector (Semrock, Rochester, NY), and expanded to fill the back aperture of the imaging objective using a variable beam expander (Thorlabs, Newton, NJ). Upon passing through the objective, pulses converged at the image plane as validated by a continuous exposure alignment of a 635 nm laser diode (LDM635; Thorlabs, Newton, NJ) and were imaged using bright-field illumination with a halogen lamp at 270,000 fps. The condenser aperture was reduced as much as possible without overly reducing image intensity to minimize effects of non-parallel light interacting with the bubble. As each 3D collagen gel had dimensions of 3 mm radius and 1.2 mm height, and to reduce potential biological effects of cellular death factors and mechanical effects of introduced boundaries, only one cavitation event was generated in each non-control sample at the approximate middle z-plane of 600 μm .

3.2.6 Image acquisition

Prior to laser-induced microcavitation, reference laser scanning confocal image stacks of 1024 $\times$ 1024 $\times$ 11 px (1.24 $\times$ 1.24 μm spacing in x-y, 20 μm spacing in z) images of Calcein AM-stained neural cells were taken using a 10 $\times$ /0.3 NA Plan Fluor objective (Nikon) with a confocal pinhole of 4.0 Airy units, centered at 600 μm above the top surface of the bottom coverslip. Excitation of Calcein AM was achieved with a 488 nm Coherent Obis optically-pumped solid state laser. Rather than create a full 3D reconstruction, total volume size and laser settings were chosen to ensure minimal effects from phototoxicity, resulting in a pseudo-3D confocal volume stack.

For laser-induced microcavitation, both the PFS mirror was shifted out of and the 532 notch dichroic mirror was rotated into the beam path. A 20 $\times$ /0.5 NA Plan Fluor objective was used for pulsed laser focusing. The pulsed laser was then manually triggered along with

the high speed camera (color Phantom v2511; Vision Research, Wayne, NJ). Samples were imaged, cavitated, and imaged in sets of 6-8 to reduce the time delay between cavitation and post-imaging. After completing a set of samples, the dichroic was removed and the 10 $\times$ /0.3 NA Plan Fluor objective was repositioned to acquire a post-cavitation stack of images, again with dimensions 1024 $\times$ 1024 $\times$ 11 px (1.24 $\times$ 1.24 μ m in x-y, 20 μ m spacing in z). One final confocal image stack was taken for each sample at a time point up to 10 hours post-cavitation, after which the entire plate was fixed with paraformaldehyde solution, washed three times with 1 $\times$ PBS, and stored at 4°C.

Laser scanning confocal imaging of cell structures labeled with TRITC-Phalloidin was performed using a 25 $\times$ /1.1 NA Apochromat water immersion objective (Nikon). Excitation of TRITC-Phalloidin was achieved with a 561 nm He-Ne diode laser. Confocal image stacks of 512 $\times$ 512 $\times$ 201 px (1.00 $\times$ 1.00 $\times$ 1.00 μ m spacing) were taken post-fixation for structural regions of interest, with individual cells imaged in higher detail at 75 $\times$ with volume size of 512 $\times$ 512 $\times$ 123 px (0.33 $\times$ 0.33 $\times$ 0.33 μ m spacing).

3.3 Results

3.3.1 Image processing – high speed video

The general processing procedure for acquiring the maximum bubble radius and equilibrium radius from high speed video is outlined in Algorithm 1. Each time-series of images consisted of 101 frames of 512 $\times$ 128 px. Imaging speed for all events was set at 270,000 fps (3.7 μ s/frame) and a calibration image over multiple boxes of a hemocytometer etched grid was taken to set the μ m/px ratio for each image time-series ($\sim$ 1.38 μ m/px). The first image in each time-series was designed as a reference image I_0 of the gel before arrival of the laser pulse. For subsequent images of the bubble I_t , differential images $D = I_t - I_0$ were constructed, normalized, and thresholded to create binary masks. For the data set and common imaging parameters (e.g. light intensity, aperture settings, objective), a threshold value of 0.4 was found to provide universally good fit agreement. The built-in function

Input : Color high-speed bubble <code>.tif</code> stacks, 512×128 px and 100 time steps I_t plus one reference image before laser pulse, I_0 Output: Maximum bubble radius $R_{\max}$ and equilibrium radius R_{eq} <pre> 1 Convert all images to grayscale, <code>rgb2gray</code>; 2 for all clear images after reference, $t > 0$ do 3 Evaluate differential images ($D = I_t - I_0$); 4 Normalize and threshold to make binary mask, <code>ceil($\hat{D}$-thresh)</code>; 5 Determine connected objects, <code>bwconncomp</code>; 6 if bubble is split into two parts by the image walls then 7 Combine two largest connected objects into one; 8 end 9 Take convex hull of bubble object; 10 Remove points on boundary of image; 11 Fit circle using Taubin method, [R_t, centroid] = <code>CircleFitByTaubin</code>(convex hull points) 12 end // Oscillations universally ceased after about $250\mu\text{s}$ 13 $R_{\text{eq}} = \text{median}(R_t)$ for $t > 250\mu\text{s}$; 14 $R_{\max} = \text{maximum of the quadratic fit of peak bubble growth and collapse};$ </pre>

Algorithm 1: Calculating $R_{\max}$ and R_{eq} from high-speed bubble images

`bwconncomp` (Image Processing Toolbox, Matlab) was then used to find the largest connected object, in this case, the bubble. In cases where bubble sizes were large with respect to the image, split connected regions were recombined into single objects. A convex hull was made around the bubble object excluding points on the image boundaries. Points along the convex hull were then fit using a generalized eigenvector fitting method developed by Taubin [105] for the specific case of a circle. Bubble images were overlaid with the circle fit outline and manually inspected to ensure accurate fitting. The equilibrium radius was defined as the median radius after the oscillation amplitude of the bubble became small, universally after $\sim 250 \mu\text{s}$. The maximum radius was determined by a fourth-order fit of the first peak, or in cases of too few data points for unique fourth-order fitting, as the maximum measured radius for any time frame.

As derived in Chapter 2, the Hencky (or logarithmic) strain in an isotropic continuum with initial bubble of size R_{eq} undergoing a rapid, spherically-symmetric deformation to a

maximum bubble size $R_{\max}$ is given by,

$$\mathbf{E} = \begin{bmatrix} -2 \ln \left(\frac{r}{r_0} \right) & 0 & 0 \\ 0 & \ln \left(\frac{r}{r_0} \right) & 0 \\ 0 & 0 & \ln \left(\frac{r}{r_0} \right) \end{bmatrix}, \quad (3.1)$$

where $r(t)$ (such that $R(t) < r(t) < \infty$) is the position in the deformed material and r_0 (such that $R_{\text{eq}} < r_0 < \infty$) is a material position in the reference material. The peak radial strain magnitude in the material occurs at the bubble wall at the time of maximum bubble expansion, and is given in component form as

$$\max(E_{rr}) = -2 \ln \left(\frac{R_{\max}}{R_{\text{eq}}} \right). \quad (3.2)$$

Interestingly, the equilibrium bubble radius scaled directly with maximum bubble radius, as shown in Fig. 3.3. This suggests that not only is the ratio $R_{\max}/R_{\text{eq}}$ constant, but furthermore that the maximum strain is approximately the same for all bubbles, which agrees with experimental data as shown in Fig. 3.4.

3.3.2 Image processing – mechanical disruption and fragmentation radius

The general processing procedure for determining the mechanical disruption radius from the reference and post-injury confocal image stacks of cells stained with Calcein AM is outlined in Algorithm 2 and shown as a schematic in Fig. 3.5.

Each z-image in a confocal image stack I^z was first processed by an initial median filter with a 3×3 kernel (denoted henceforth as $\mathcal{M}_3$) and gamma shifted by a factor of 0.7,

$$I^z = (\mathcal{M}_3(I^z))^{0.7}. \quad (3.3)$$

Due in part to drift, switching imaging optics, and to the cavitation event itself, some rigid translation occurred between each image before and after cavitation. Registering image drift is a common part of image differencing techniques to get accurate results [116]. For

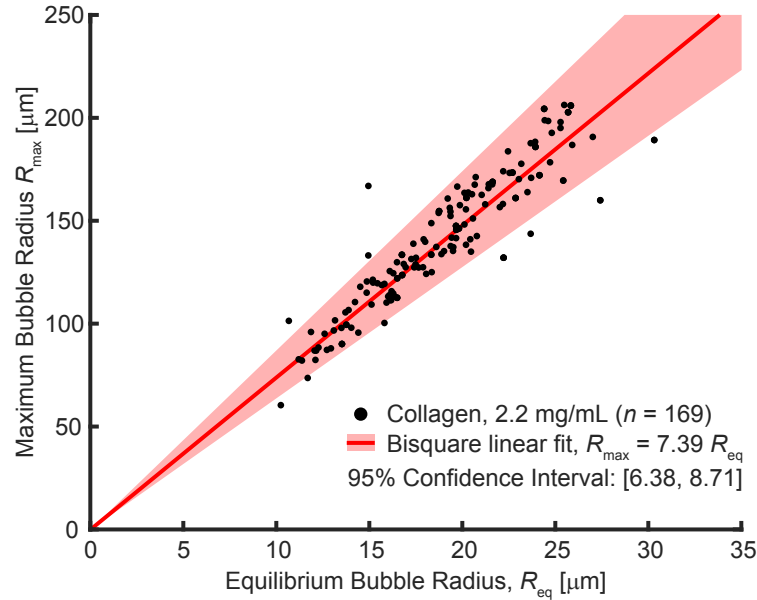


Figure 3.3: Maximum bubble radius vs. equilibrium bubble radius for collagen-cell gels over all bubbles ($n = 169$) ranging from about 70-220 μm . Tests in which final bubble radius appeared to fit improperly or was fragmented into multiple adjacent bubbles were manually excluded. A bisquare direct fit resulted in $R_{\max} = 7.39 R_{\text{eq}}$, with a 95% confidence interval of [6.38, 8.71].

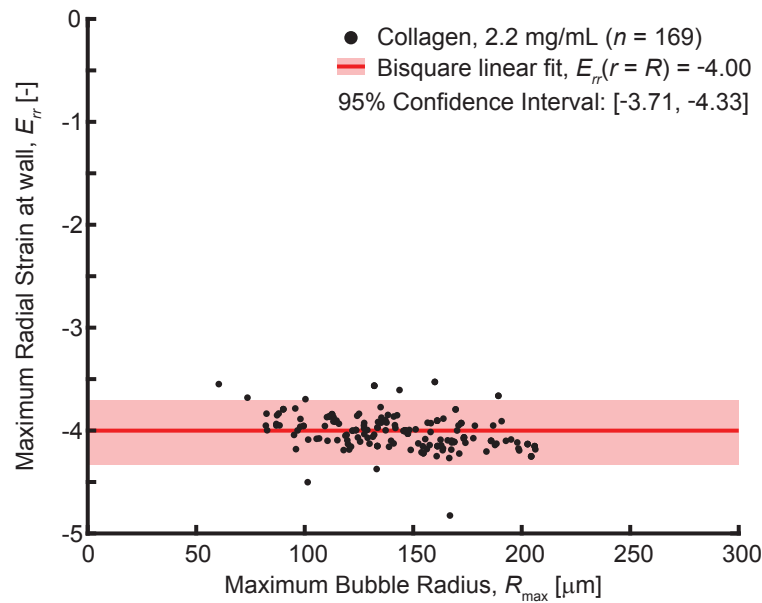


Figure 3.4: Maximum radial strain at bubble wall versus maximum bubble radius for collagen-cell gels over all bubbles ($n = 169$). A bisquare constant fit resulted in $\max(E_{rr}) = -4.00$, with a 95% confidence interval of [-3.71, -4.33].

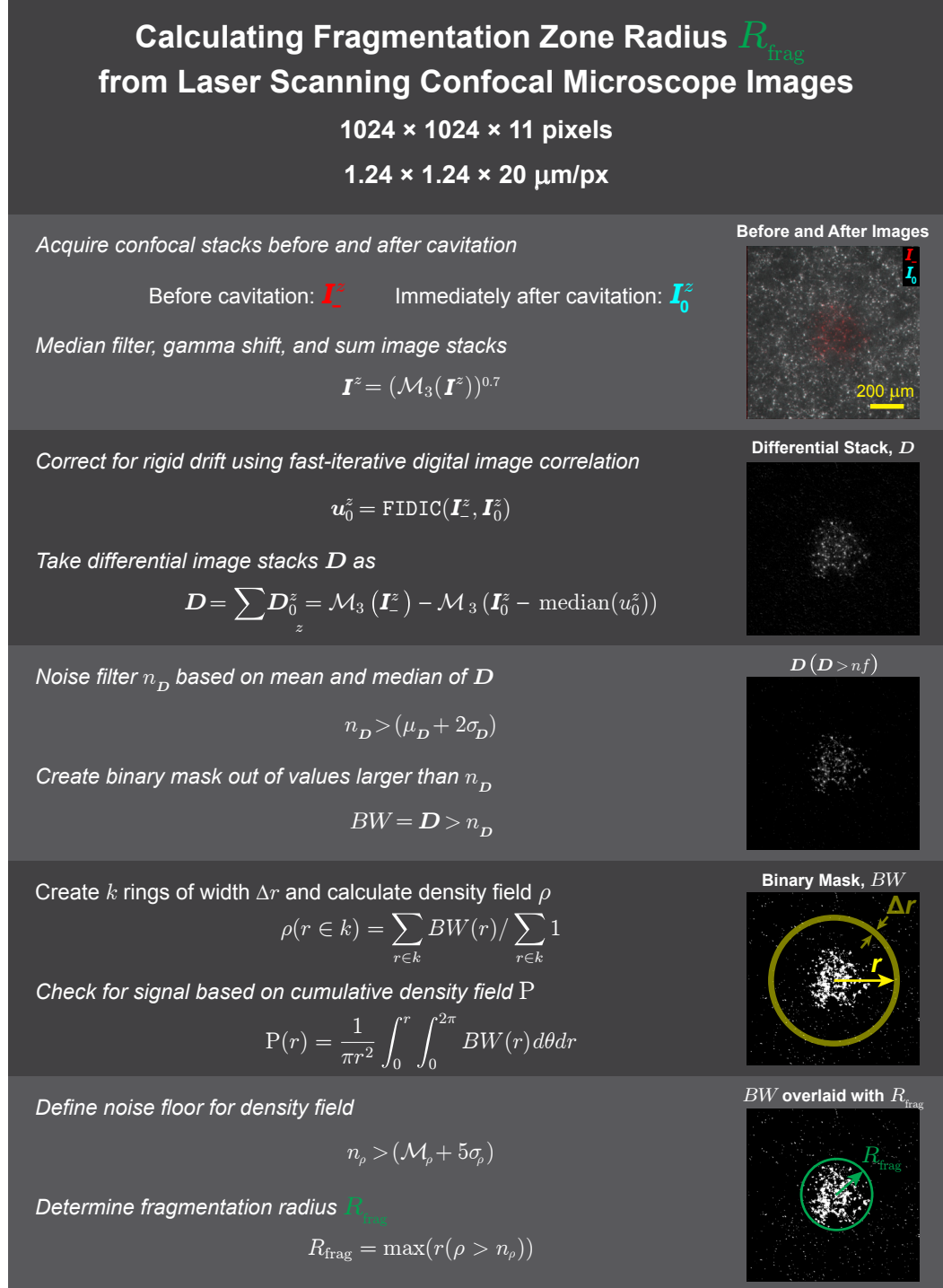


Figure 3.5: Image processing procedure for laser scanning confocal image stacks.

this study, reducing background noise effects is particularly important, and as such the rigid motion drift was corrected by running a fast-iterative digital image correlation method to get drift to sub-pixel accuracy. The fast-iterative image correlation algorithm was developed originally in 3D by Franck et al. [117], improved in Bar-Kochba et al. [118], and adapted for 2D by Alex Landauer. The median displacement was taken as the median of the top left quadrant of the images to reduce the non-rigid motion effect of the damage region. Corresponding images in the reference configuration I_-^z were then compared to displacement median-shifted images in the damaged configuration at times immediately after cavitation and at later times t . To further reduce the effect of salt-and-pepper noise, these images were median-filtered again and put through a custom image differencing algorithm. The differential images D_0^z were determined as

$$D_0^z = \mathcal{M}_3(I_-^z) - \mathcal{M}_3(I_0^z - \text{median}(\mathbf{u}_0^z)). \quad (3.4)$$

The differential image sum $\mathbf{D}$ was then defined as the median filter of the sum of all stack images D^z , and thresholded. A binary mask BW was constructed as the values in $\mathbf{D}$ above the threshold. The binary mask represents all pixels that significantly drop in intensity between the undamaged and damaged images, and physically represents local ceasing of intracellular esterase activity. A distance transform matrix $\mathbf{r}$, given by

$$\mathbf{r}(x, y) = \sqrt{(x - x_c)^2 + (y - y_c)^2}, \quad (3.5)$$

of distances away from the known bubble center location (x_c, y_c) set the coordinates of the binary mask. From the center of the bubble at $r = 0$, it is convenient to take the relative density difference as a radial measure of damage, that is, the high density of fluorescent intensity loss concentrated in the bubble region is considered the damaged zone. Intensity loss density ρ was determined by defining $\Delta\mathbf{r}$ -width concentric rings with index k , summing the lost pixels (where $BW = 1$), and normalizing by the number of pixels in each ring,

$$\rho(\mathbf{r} \in k) = \frac{\sum_{\mathbf{r} \in k} BW(\mathbf{r})}{\sum_{\mathbf{r} \in k} 1} \quad (3.6)$$

The noise floor n_ρ is user-adjustable, but was conservatively taken as 5 standard deviations above the median far-field density in this study,

$$n_\rho = \text{median}(\rho(\mathbf{r})) + 5\sigma_\rho. \quad (3.7)$$

The density of intensity values was then plotted for all tests as a function of radius. The radius of mechanical disruption or large-scale fragmentation of the plasma membrane immediately after cavitation, R_{frag} , was then defined as the maximal radial location at which the pixel density meets the noise floor, as shown in Fig. 3.6.

A radial cumulative intensity plot $P(r)$ is additionally generated for each image to determine the difference between a localized damage intensity volume and a homogeneous uniform damage control or negative noise distribution control,

$$P(r) = \frac{1}{\pi r^2} \int_0^r \int_0^{2\pi} BW(\mathbf{r}) d\theta dr. \quad (3.8)$$

For the particular case of a step intensity density change of ρ over a uniform noise floor density of ρ_n , the cumulative intensity $P(r)$ can be expressed mathematically as

$$P(0 < r < a) = \frac{\rho_n r^2 + (\rho - \rho_n) \left[H(R_{\text{frag}} - r) r^2 + H(r - R_{\text{frag}}) R_{\text{frag}}^2 \right]}{\rho_n a^2 + (\rho - \rho_n) R_{\text{frag}}^2}, \quad (3.9)$$

where a is the maximum radius and nearest edge of the image (to eliminate edge counting effects at corners), H is the Heaviside function, and R_{frag} is the radius at which the density shifts from $\rho + \rho_n$ to the noise floor density ρ_n . The function $P(r)$ for a step density shift is split-continuous and non-differentiable at R_{frag} , at which the two quadratic portions meet. For the particular case where the density step $\rho = 0$, or equivalently, where the disrupted area density is uniform over the entire image, $P(r)$ simplifies to

$$P_n(0 < r < a) = \frac{\rho_n}{\rho_n} \frac{r^2}{a^2} = \frac{r^2}{a^2}. \quad (3.10)$$

The behavior of the constant density (red dashed) and step density (blue dashed) analytical

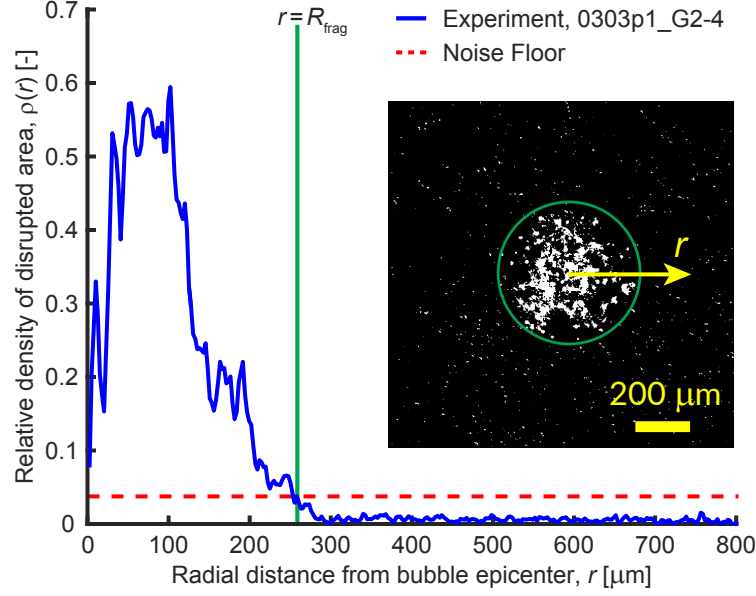


Figure 3.6: Relative density of disrupted, or fragmented, cell area versus distance from bubble epicenter. Representative image intensity density is calculated from a binary mask of neural cell damage due to cavitation over rings concentrically centered on the bubble epicenter (blue). The fragmentation radius (green) is defined as the maximum location where the density exceeds the noise floor (red dotted line) and shown overlaid on the binary mask (right, inset).

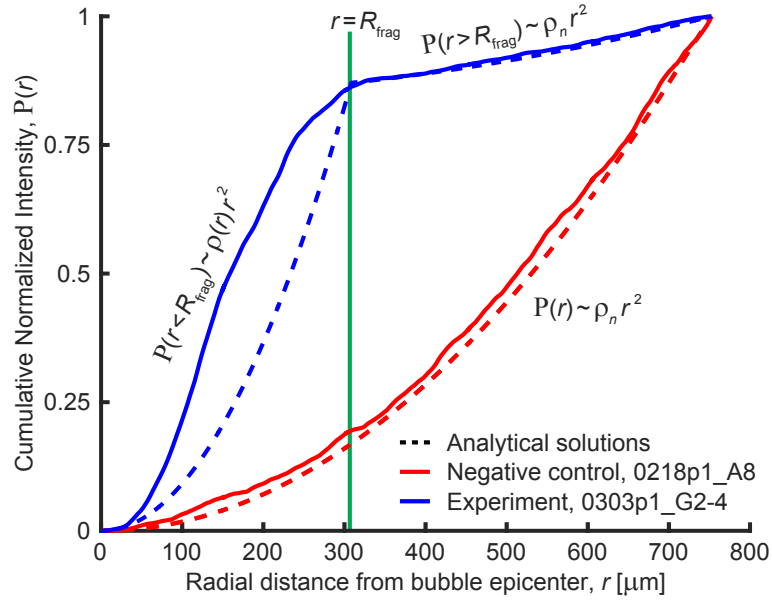


Figure 3.7: Cumulative normalized intensity versus radial distance from bubble epicenter for analytical and representative experimental results. For the case of uniform density, the analytical cumulative solution is quadratic (red dashed). For the case of a circular area of higher density inside an image with lower density, the solution is a split-continuous bi-quadratic function (blue dashed). Experimental results matched these two types of solutions well, with controls following the uniform solution (representative test, solid red) and injured samples (volume from Fig. 3.6, solid blue) following the split-density solution.

functions is shown in Fig. 3.7.

Both negative (undamaged) and positive (e.g. induced phototoxicity or membrane poration) controls differ from the respective reference images uniformly, leading to quadratic behavior in $P(r)$. Examples of a negative control (red solid line) and a representative cavitation event (blue solid line) are shown in Fig. 3.7.

As a goodness of fit metric, the root-mean-square (RMS) difference $\langle P \rangle$ between each cumulative intensity plot and the baseline uniform noise distribution was determined for each test, and found to be $\langle P_{n,0} \rangle = 0.0233 \pm 0.0119$ for sham samples immediately post-cavitation and $\langle P_{n,t} \rangle = 0.0256 \pm 0.0104$ for sham samples at later timepoints. The RMS threshold used for including a cavitation event in the usable data set was conservatively set at 0.1, or about twice the maximum RMS value for any individual control sample. For comparison, RMS values of injured samples were commonly between 0.2 and 0.5.

Input : Laser scanning confocal stacks of $1024 \times 1024 \times 11$ ($1.24 \times 1.24 \times 20 \mu\text{m}$) images from before ($I_-^z$) and immediately after (I_0^z) cavitation injury, laser position (x_c, y_c) Output: Mechanical fragmentation radius R_{frag} immediately post-cavitation 1 Median filter and gamma shift image stacks, $I^z = (\mathcal{M}_3(I^z))^{0.7}$; 2 for $z = 1$ to height of stack do 3 $\mathbf{u}_0^z = \text{FIDIC}(I_-^z, I_0^z)$; 4 $D_0^z = \mathcal{M}_3(I_-^z) - \mathcal{M}_3(I_0^z - \text{median}(\mathbf{u}_0^z))$; 5 end 6 $\mathbf{D}_0 = \sum_z D_0^z$; 7 Make binary mask using threshold $BW_0 = \mathbf{D}_0 > (\mu_{\mathbf{D}_0} + 2\sigma_{\mathbf{D}_0})$; 8 Define radial transform matrix $\mathbf{r}(x, y) = \sqrt{(x - x_c)^2 + (y - y_c)^2}$; // Create density field ρ by discretizing into k bands of $\Delta\mathbf{r} = 2\mu\text{m}$ 9 Define density noise floor $n_\rho = \text{median}(\rho) + 5\sigma_\rho$; 10 for ring index $k = 1$ to edge of image do 11 $\rho(\mathbf{r} \in k) = \sum_{\mathbf{r} \in k} BW_0(\mathbf{r}) / \sum_{\mathbf{r} \in k} \text{ones}(\mathbf{r})$; 12 end 13 $R_{\text{frag}} = \max(\mathbf{r}(\rho_0 > n_\rho))$;

Algorithm 2: Calculating R_{frag} from laser scanning confocal image stacks.

3.4 Discussion

3.4.1 Comparison of length scale metrics

Comparison of the maximum bubble radius $R_{\max}$ (or more generally, the spherical bubble dynamics $R(t)$) and the resulting damage zone R_{frag} allows correlation of the mechanics to failure regions, i.e. large mechanical disruption of cellular components. To confirm the uniformity of collagen behavior between sample batches, $R_{\max}$ and R_{frag} were first compared per batch of samples, and the ratio of $R_{\max}$ to R_{frag} for each plate was found not to vary significantly with the full data set via one-way ANOVA. The distributions of $R_{\max}$ (blue)

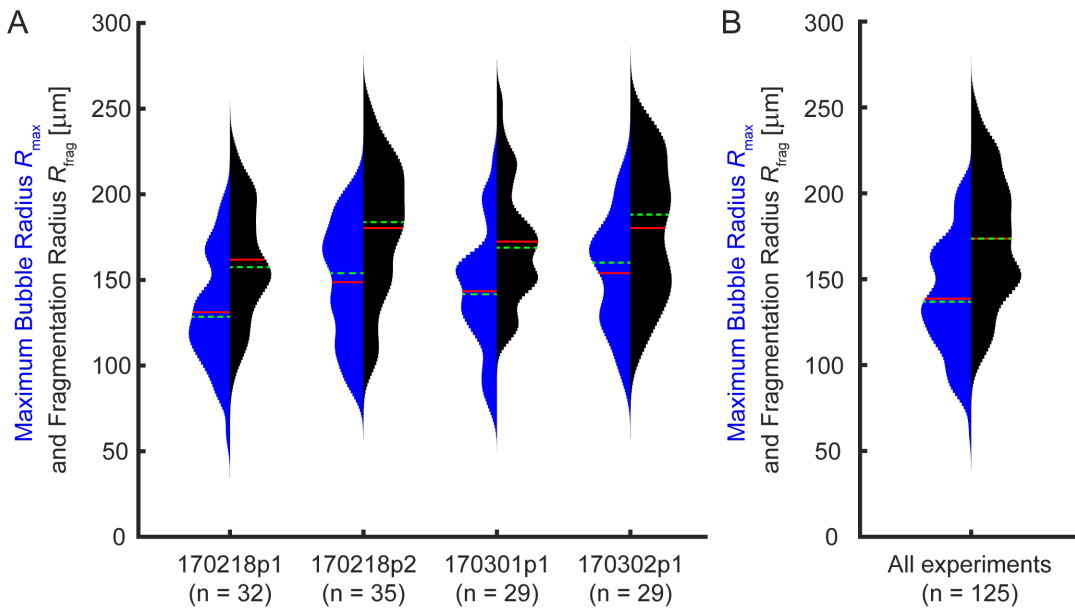


Figure 3.8: Maximum bubble radius (blue) and fragmentation radius (black) violin plots across (A) 48-well plates and (B) combined for all included experiments. Green dotted line, median. Red solid line, mean. Distributions appear similar for maximum bubble radius and fragmentation radius, suggesting a direct scaling between the two.

and R_{frag} (black) for each experimental set are displayed as violin plots in Fig. 3.8A and combined over all plates in Fig. 3.8B. While the maximum bubble radius is an independent variable and as a result need not be distributed normally, there was dependence of the fragmentation radius on the maximum bubble size as can be seen by the similar distribution profiles over each plate and over all tests. The degree of dependence was determined by direct comparison of R_{frag} and $R_{\max}$ over all tests, as shown in Figure 3.9A as a plot and

3.9B as a boxplot.

3.4.2 Calculation of critical primary injury strain

As before with the comparison of maximum and equilibrium bubble radii, the direct linear scaling between the two suggests a strain equivalence; that is, there exists a critical strain at which major plasma membrane injury/damage occurs. Using the strain relation of

$$E_{rr} = -2 \ln \left(\frac{r}{r_0} \right) = -2E_{\theta\theta}. \quad (3.11)$$

we can find the corresponding strain at locations of cell failure, i.e. the fragmentation radius R_{frag} . Direct comparison of the before and after images of cell damage validated the assumption that during the timescale (i.e. minutes) between cavitation and post-damage imaging the material relaxes approximately into the reference configuration, with any resulting permanently damaged zone smaller than the maximum inflated bubble radius. Thus, fragmented cells in the post-damage quasi-reference configuration of $t \rightarrow \infty$ are located at a radial coordinate $r_0 = R_{\text{frag}}$. The radial deformation mapping equation 2.42, reproduced here for the reader, is given by,

$$r(r_0, t) = (r_0^3 + R(t)^3 - R_0^3)^{1/3}. \quad (3.12)$$

During cavitation, the maximum strain occurs at the peak expansion radius R_{max} . We can then combine the location of known damage with the maximum strain during cavitation,

$$E_{rr}(r_0 = R_{\text{frag}}) = -2 \ln \left(\frac{\sqrt[3]{R_{\text{max}}^3 - R_{\text{eq}}^3 + R_{\text{frag}}^3}}{R_{\text{frag}}} \right). \quad (3.13)$$

We can formulate the deformation-based primary strain injury criteria $\bar{E}_c$ by assuming first that failure is just a function of the Hencky strain, e.g.

$$f(\mathbf{E}) = \bar{E}_c. \quad (3.14)$$

If we assume the failure criterion for cells is isotropic, the function f depends only on the principal invariants of $\mathbf{E}$, namely $I_1 = \text{tr}(\mathbf{E})$ (which is zero due to incompressibility), $I_2 = E_{ij}E_{ij}$, and $I_3 = \det(\mathbf{E})$, implying

$$f(I_2, I_3) = \bar{E}_c. \quad (3.15)$$

It is important to note that cells may be affected differently in compression and tension; various studies have imposed different types of quasistatic deformation or moderate strain-rate deformation fields [43, 51, 57] and found different types of resulting cytoskeletal and plasmalemma damage. As the case of ultra-high-rate tensile versus compressive deformation on neural cells has not yet been well characterized, we can remove reliance of $\bar{E}_c$ on I_3 , which encodes the difference between compressive and tensile deformation. Sole dependence on I_2 allows us to conveniently formulate the injury criterion $\bar{E}_c$ using the equivalent von Mises strain $\bar{E}$, written generally as

$$\bar{E} = \sqrt{\frac{2}{3}E_{ij}E_{ij}}, \quad (3.16)$$

and in the case of purely deviatoric strain as in our incompressible cavitation expansion, as

$$\bar{E} = \sqrt{\frac{2}{3} \left[(E_{rr})^2 + 2 \left(-\frac{1}{2}E_{rr} \right)^2 \right]} = |E_{rr}|. \quad (3.17)$$

For the particular case where the equilibrium bubble radius and cell fragmentation radius vary directly with the maximum bubble radius, the critical von Mises strain for cell fragmentation is constant over maximum bubble radius. Assuming $R_{\max} = a_1 R_{\text{eq}} = a_2 R_{\text{frag}}$, we can get the von Mises strain by combining Eqs. 3.13 and 3.17,

$$\bar{E}_c = |E_{rr}| (r_0 = R_{\text{frag}}) = \left| 2 \ln \left(a_2 \sqrt[3]{1 - a_1^{-3} + a_2^{-3}} \right) \right|. \quad (3.18)$$

The implications of Eq. 3.18 are twofold; the direct linear relations between the fragmentation radius, maximum bubble radius, and equilibrium radius suggest that, if the inertial

cavitation bubble dynamics are known to be repeatable, only measurement of the fragmentation zone and equilibrium bubble radius are necessary for determining the critical strain, which is of interest in systems without the high frame rates necessary to accurately determine the maximum bubble radius. Alternatively, knowing a critical strain value and the typical bubble dynamics of a tissue would be of direct interest to clinical applications in which fragmentation can either be predicted or measured. As a further note, this can be extended to different empirically-determined critical radii of interest R_c by replacing the primary injury radius relation $R_{\max} = a_2 R_{\text{frag}}$ with a different linear relationship $R_{\max} = a_c R_c$.

As a note, we make the implicit assumption that the continuum model holds for cells, i.e. that cells experience deformation exactly as the matrix. The continuum assumption for mapping strain on cells in this case relies on two ingredients: continuity of the matrix and displacement continuity across the cell-extracellular matrix interface. This need not be true for porous, fibrous matrices, where cells experience strains based on local fiber geometry. However, reflectance imaging of cells bound in our system shows good cellular encapsulation as shown in Fig. 3.1 suggesting continuum behavior at bubble scales, while displacement continuity was validated under quasistatic loading experimentally via digital volume correlation [57] and compared to the analytical Eshelby inclusion solution [119].

Using the empirical scaling values of $R_{\max} = 0.83 R_{\text{frag}} = 7.39 R_{\text{eq}}$ in Eq. 3.18, we found the critical von Mises strain $\bar{E}_c$ for primary injury to be 0.3. The critical strain was additionally determined pointwise for all tests to determine metric robustness, as shown in Fig. 3.10. Over all tests ($n = 125$), the mean value of critical Mises strain for primary injury was determined to be 0.30 with 25% and 75% confidence values of 0.26 and 0.35, respectively.

3.4.3 Morphology dependence on maximum strain during cavitation

Although global fragmentation injury was assessed via image differencing, more detailed morphological information such as projection thinning, blebbing, and retraction immediately after injury can only be determined with higher spatial resolution, as shown in Fig. 3.11. Increased resolution of the fragmentation zone was achieved over original resolution

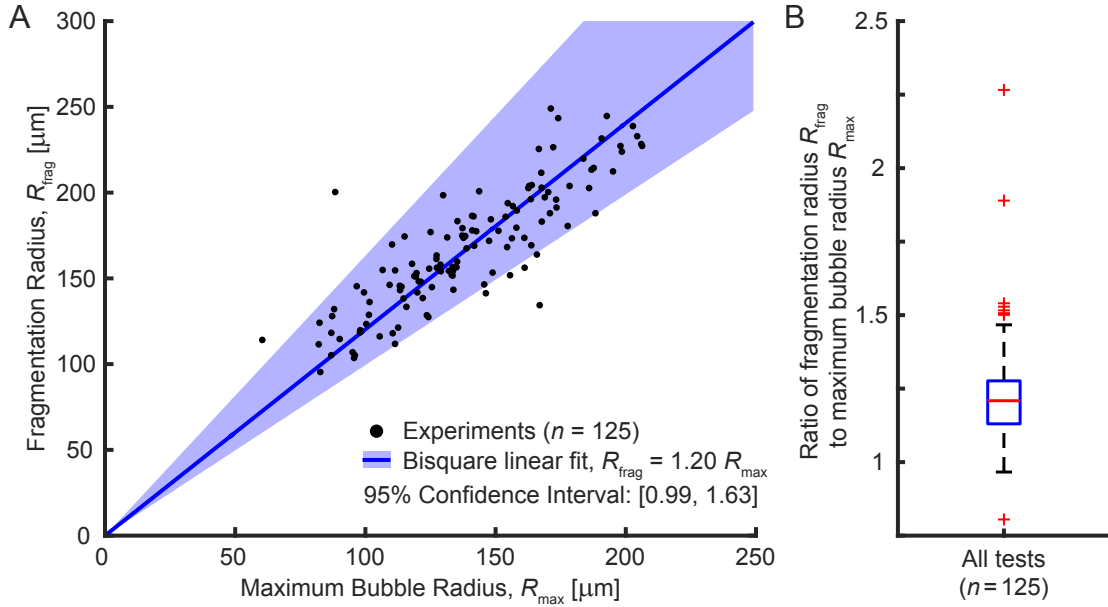


Figure 3.9: Fragmentation radius vs. maximum bubble radius over all experiments. (A) Bisquare linear fit (blue) with 95% confidence interval showing a direct relationship between fragmentation radius and maximum bubble radius, $R_{\text{frag}} = 1.20 R_{\max}$. (B) Pointwise ratio of R_{frag} and $R_{\max}$ for all included tests ($n=125$). Mean value (red line), 1.21. 25th and 75th percentiles: [1.13, 1.28].

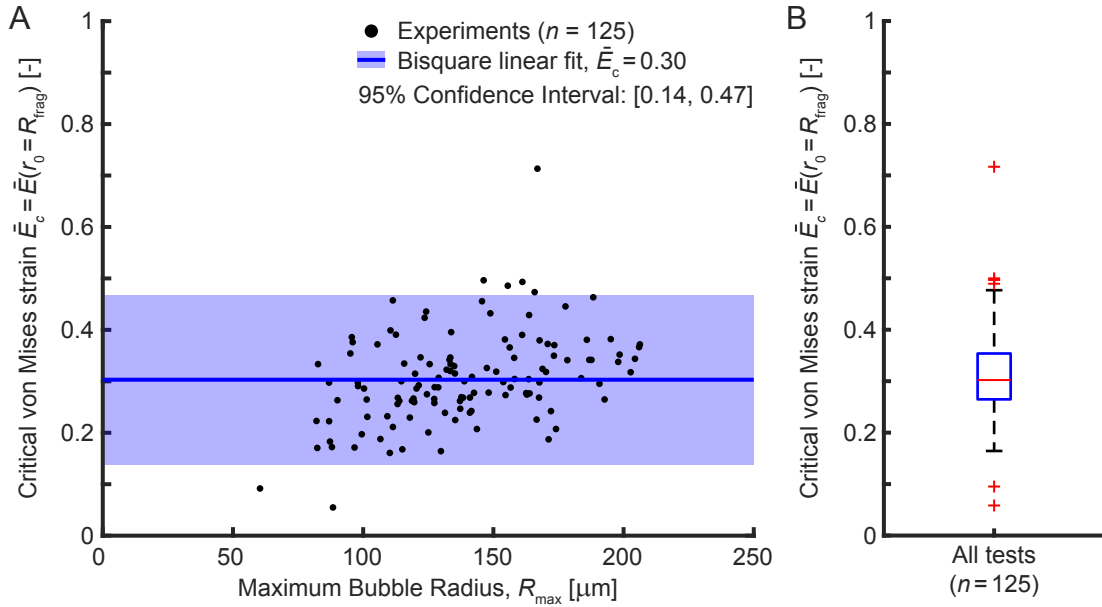


Figure 3.10: von Mises strain at the fragmentation radius as a function of maximum bubble radius shown as (A) a scatterplot with a bisquare linear fit of a single constant value of 0.30 and 95% confidence interval of [0.14, 0.47] and (B) as a boxplot over all tests ($n = 125$). Mean value (red line), 0.30. 25th and 75th percentiles: [0.26, 0.35].

images (Fig. 3.11A) in limited cases by using a $25\times/1.1\text{NA}$ Apochromat objective (Nikon) (Fig. 3.11B). Higher-resolution imaging near the fragmentation zone showed stark regions of differing morphology. Inside the original circular fragmentation zone with radius R_{frag} , there exists a smaller zone in which all cellular components, including cell projections and somas, are completely fragmented. Outside of this zone but inside the original fragmentation radius, somatic damage is severe but incomplete while the majority of projections are fragmented. Interestingly, an additional region outside the fragmentation radius R_{frag} exhibits some projection damage as well, including bleb formation and focal swellings. However, far enough away from the bubble epicenter, the cells show uniform mild morphological changes, such as occasional small focal swellings.

Further analysis on cytostructural components was performed through immunostaining. Fig. 3.12A shows a maximum projection of a subset of the stack of images used for Figs. 3.5-3.7 overlaid with the calculated fragmentation radius from image differencing, R_{frag} . The same volume was located after fixation and staining with TRITC-Phalloidin by manual registration of far-field neuron locations. High-resolution confocal image stacks of TRITC-Phalloidin (Fig. 3.12B) show the cellular cytostructure near the fragmentation zone (green circle). Identical to observations of damaged live cells stained for intracellular esterase activity, there existed a region centered at the bubble epicenter and inside the image-based fragmentation radius in which F-Actin was highly disrupted. In a middle region inside the fragmentation radius but outside the inner high-disruption zone, cells that exhibited considerably decreased intracellular esterase activity interestingly had large portions of their F-Actin network intact immediately after injury, suggesting plasma membrane damage without complete cytoskeletal damage. Additional imaging of cells near the boundary of the middle region and high-disruption zone (Fig. 3.12B, red dotted line) strongly suggested a critical strain effect, as cell projections inside are blunted.

The existence of multiple critical strain values for different kinds of fast 3D mechanical disruption or fragmentation has not been extensively studied in the literature, in part due to lack of control over the mechanics of the problem (e.g. formation of bubble clouds rather than single bubbles [120]), restriction to 2D platforms, inertia in mechanical actuation, or

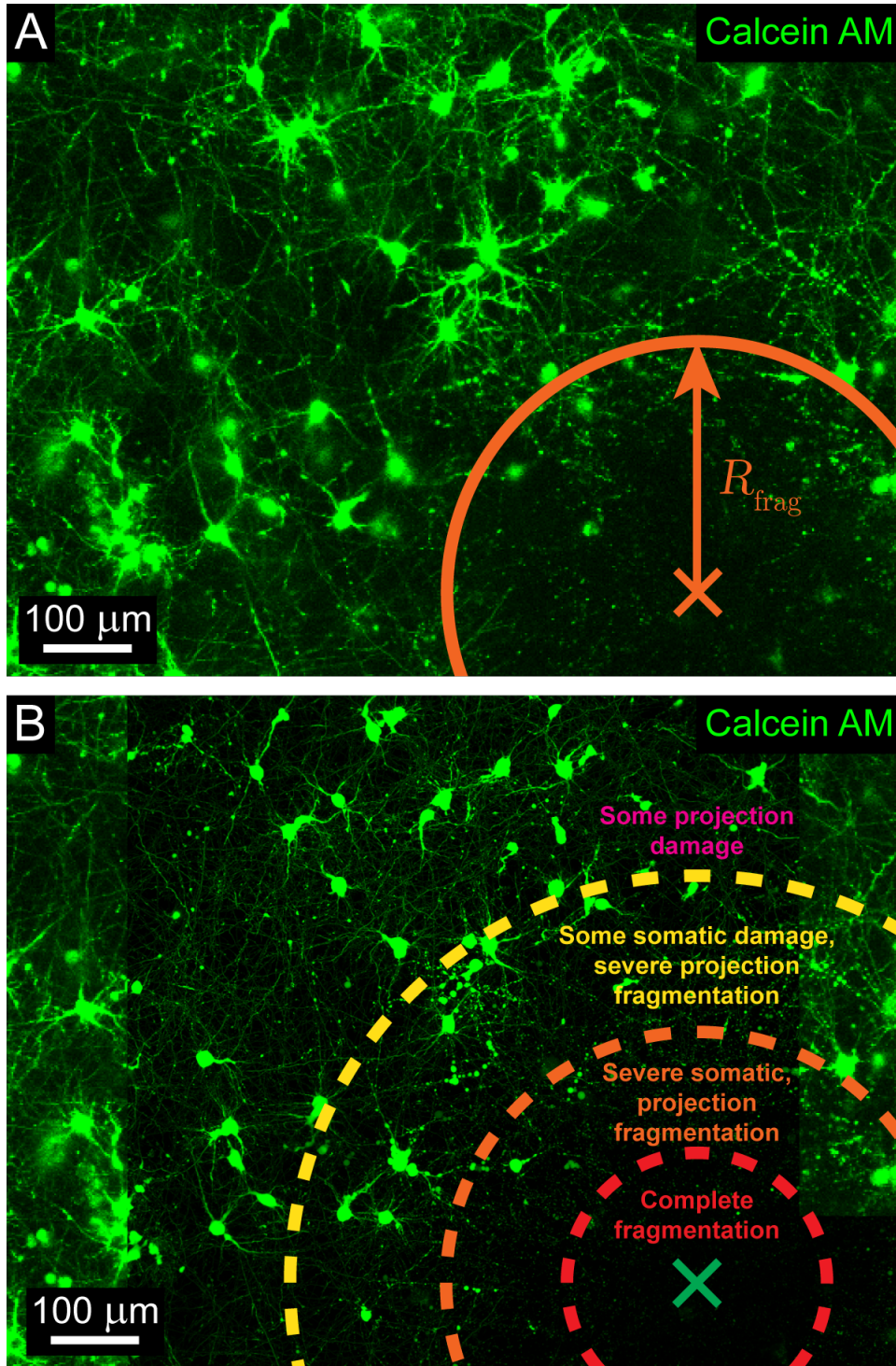


Figure 3.11: Post-damage injury morphology inspection at improved resolution. (A) Maximum projection of three laser confocal images of neural cells stained with Calcein AM, spaced at $20\ \mu\text{m}$, using a $10\times/0.3$ Plan Fluor objective. Fragmentation radius denoted in orange. (B) Maximum projection image of the same bounded volume, spaced at $0.3\ \mu\text{m}$ and imaged through a $25\times/1.1$ NA Apochromat objective. The increase in morphological detail, while unsuitable for long-term live confocal imaging, can be used to discern regions exhibiting particular damage morphologies (dashed lines), such as projection fragmentation and blunting and somatic damage.

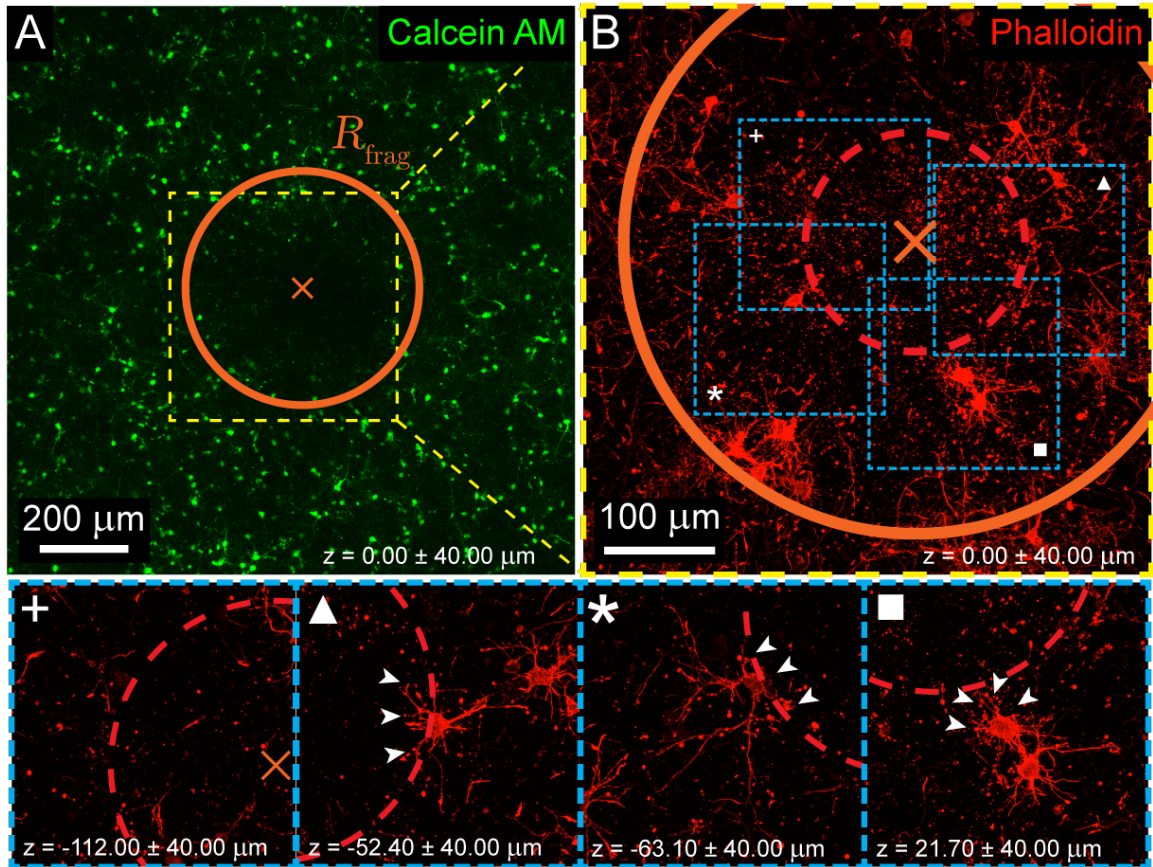


Figure 3.12: F-Actin morphology in the fragmentation zone. (A) Live-stained volumes are used to calculate the fragmentation radius (solid orange), after which the sample is fixed and stained with immunofluorescent probes. (B) The same volume in A stained with TRITC-Phalloidin. Cells inside the fragmentation radius exhibit damage as a function of distance away from the bubble center (orange x). Inside the complete fragmentation zone (red dashed), no projections remain intact. Abruptly outside the complete fragmentation radius, small cell components such as projections are damaged (white arrows) while some larger structures remain intact.

a combination of these issues. Knowledge of full kinematic fields and mechanical disruption length scales allows for direct correlation between the two. Shown in Fig. 3.13 is the von Mises strain induced by the bubble ($n = 125$) at maximum expansion as a function of radial coordinate in the reference configuration, r_0 , overlaid with the live-cell fragmentation results ($n = 125$) and estimates of radii for F-Actin fragmentation and severe projection damage. Multiple interesting features can be discerned from Fig. 3.13. First, the strain as a function of normalized radius at maximum bubble expansion was nearly identical across all tests, and was within 0.2% of the mean for all tests. Variation in the strain at the fragmentation radius was therefore primarily due to variation in $R_{\max}$, with $\bar{E}_{\text{frag}} = 0.30 \pm 0.05$ for the median and upper/lower quartile values. Estimates of strain criteria for other damage morphologies, such as projection membrane fragmentation (yellow dashed, Fig. 3.11B) and F-Actin fragmentation (red dashed, Fig. 3.12B) are also shown overlaid on the von Mises strain vs. position in the reference configuration r_0 and were found to be approximately $\bar{E} = 0.08$ and $\bar{E} = 1.6$, respectively. While it should be noted that these values only represent those found for one test and are not necessarily quantitatively representative, the highly non-linear field allows for isolation of different failure mechanisms in a way many other methods, (e.g. membrane stretching [48], compression [57], shear [51]) cannot achieve. Blebbed morphologies between the value of $\bar{E} = 0.08$ and 0.30 are consistent with observations in Bar-Kochba et al. [57] in which neurons embedded in Type I collagen were subjected to far-field uniaxial compression of $E_{zz} = 0.3$ (equivalent von Mises strain $\bar{E} = 0.25$) at a rate of 75 s^{-1} . However, blebs were observed immediately post-cavitation as primary injury, while the compression study found bleb formation as a secondary injury response after a mean of 8 hours, suggesting a further dependence of injury modality on strain rate.

3.4.4 Estimation of material strain rates and stresses

Further correlation of mechanics to cell observations can be acquired by incorporating inertial microcavitation-based ultra high strain rate rheometry (IMUR) as described in the preceding chapter. IMUR analysis of collagen is useful for a variety of reasons. First, given

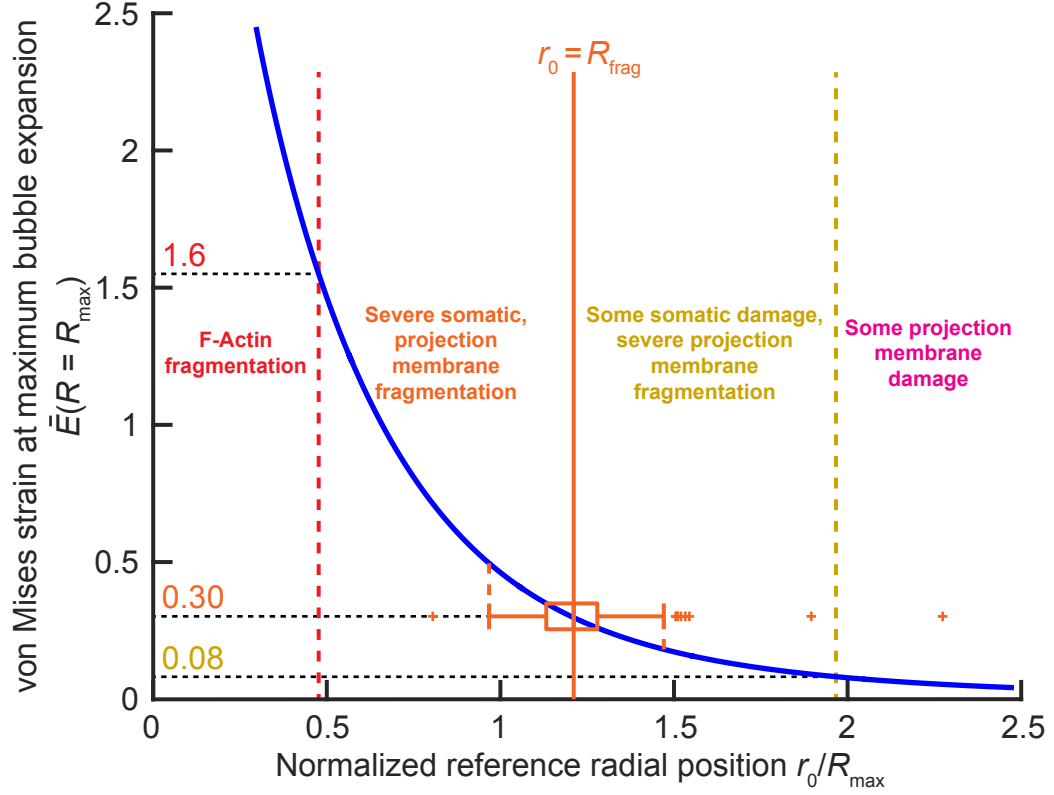


Figure 3.13: Equivalent von Mises strain vs. reference radial coordinate for all tests ($n = 125$). Strain fields normalized to maximum bubble radius were consistent over all tests and universally within 0.2% of mean values. Median fragmentation radius ($R_{\text{frag}} = 1.21$; green line) with boxplot highlighting critical fragmentation strain values of $\bar{E}_{\text{frag}} = 0.30 \pm 0.05$ for the median and upper/lower quartile values ($n = 125$). Single observation strain criteria estimates for projection membrane fragmentation (yellow dashed) and F-Actin fragmentation (red dashed) are approximately $\bar{E} = 0.08$ and $\bar{E} = 1.6$, respectively.

the limited temporal resolution of bubble dynamics available due to high-speed imaging frame rates, combining a physical model allows for finer resolution of timesteps to better estimate events near the bubble collapse as well as strain rate information. Second, while strain components or effective strain are convenient mechanical metrics for cell damage that can be compared over various studies in the literature, whether failure criteria for neural cells are truly strain- or stress-based is presently unknown. IMUR, based on solving the coupled pressure-dynamics equations in the system, also provides stress estimates on material during the cavitation event. Though cells experience stress locally through focal adhesions and by being constrained by fibers, stress on the surrounding material at a continuum level could still provide useful insight in modeling, for example.

A representative fit of the bubble dynamics from the same volume presented in Figs. 3.5 and 3.12 is shown in Fig. 3.14. From this fit, various interesting observations arise. First, the best-fit model overpredicted the first rebound peak. This could be due to the assumed linear Mises dashpot term, which may be better-represented by, for example, shear-thinning viscosity, which has been previously observed in collagen solutions [121,122]. Second, fits were not improved by incorporation of the three-element standard non-linear solid model. The resulting fit constants for shear modulus and viscosity were $G = 7.08$ kPa and $\mu = 0.044$ Pa s, which suggests behavior that is elastically similar but less viscous than the previously tested polyacrylamide samples.

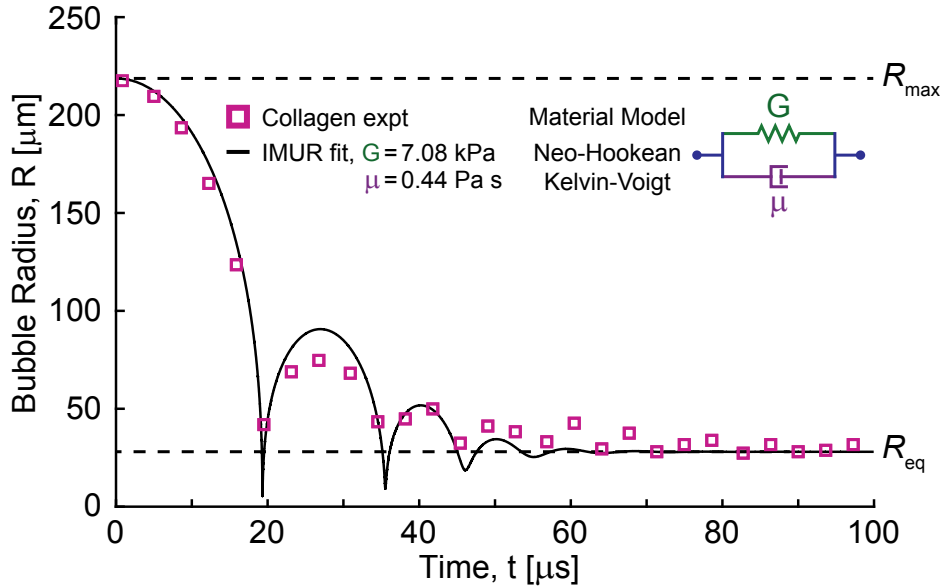


Figure 3.14: Inertial microcavitation rheometry fit for the bubble dynamics in the same volume from Figs. 3.5 and 3.12. The Neo-Hookean Kelvin-Voigt model was found to best reproduce features of bubble dynamics. Resulting best-fit parameters using the previously described least-squares method were a shear modulus of $G = 7.08$ kPa and viscosity of $\mu = 0.044$ Pa s.

As discussed at length in the preceding chapter, IMUR not only determines material properties, but also the mechanical field variables. With known radii of interest such as those highlighted in Fig. 3.13, we can pinpoint not just critical strain values, but also the entire temporal behavior of the strain and stress fields with their associated derivatives. Fig. 3.15 highlights strains and strain rates at the marked locations from Fig. 3.13 as compared to the bubble wall (black). Notably, the marked spike in radial strain (Fig. 3.15A) that

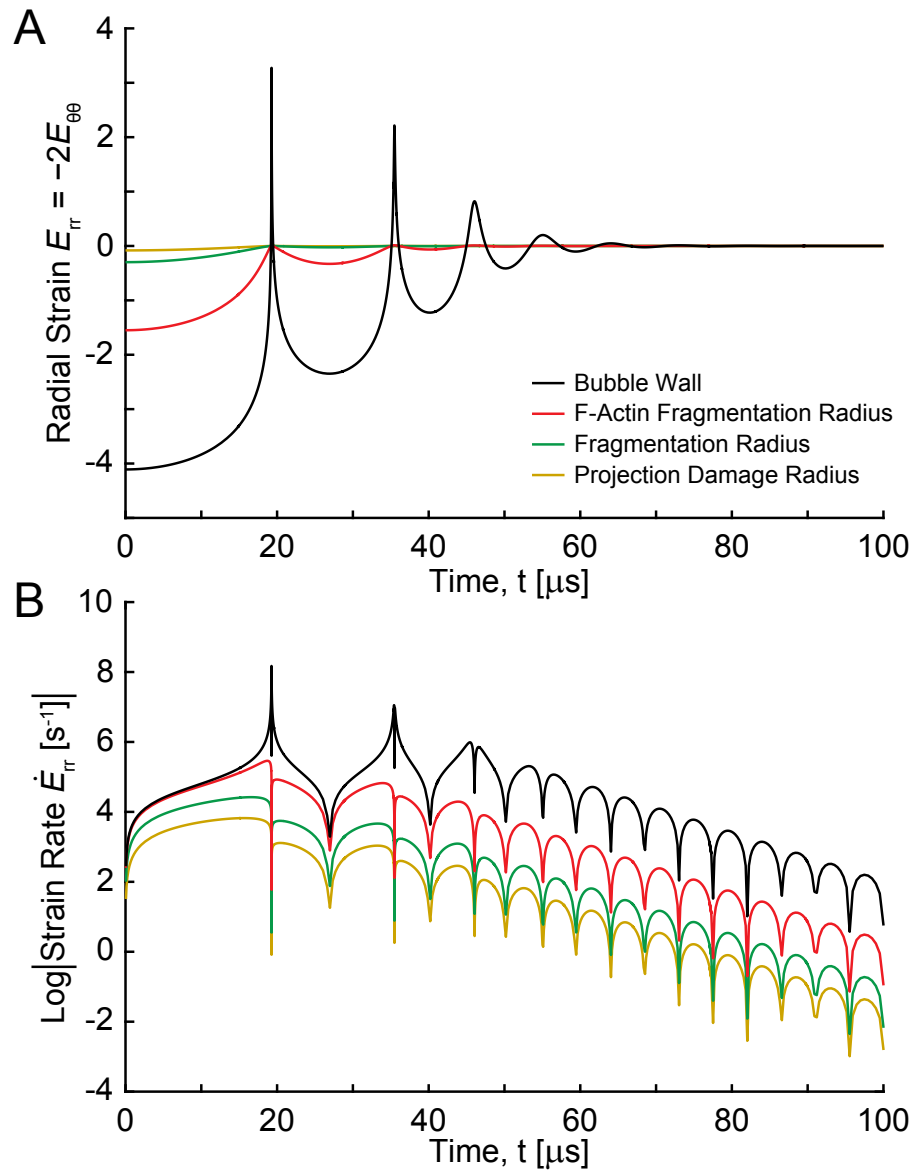


Figure 3.15: (A) Radial strains and (B) strain rates vs. time in 3D neural cultures at different radii of interest. Strain and strain rate fields were calculated from the least squares fit from inertial microcavitation rheometry. Highlighted are dynamics at the bubble wall (black), F-Actin fragmentation radius (red), image-calculated fragmentation radius (green), and projection damage radius (yellow). While the bubble wall experiences high tensile radial strains during collapse, locations farther away predominantly experience compressive radial strain. Similarly, strain rate behavior near the bubble wall exhibits a marked increase at collapse, while between F-Actin fragmentation and membraic fragmentation the increase of strain rate near collapse becomes minimal.

occurred at the bubble near collapse did not occur at the radii of interest, as corroborated by the small strain region threshold of about $2.5R_{eq}$ in tension (Fig. 2.15). However, the strain rates just before collapse (Fig. 3.15B) were the largest experienced during the entire microcavitation event in all cases. While strain rates at the bubble wall exhibited a marked spike due to the abrupt strain magnitude change, leading to a peak strain rate magnitude of $1.5 \times 10^8 \text{ s}^{-1}$, the peak strain rates at the other radii of interest also fell within the ultra-high-strain rate regime. Magnitudes of maximum strain rate near collapse were $\dot{E}_{rr} = [2.9 \times 10^5, 2.6 \times 10^4, 6.6 \times 10^3] \text{ s}^{-1}$ for the F-actin fragmentation, membrane fragmentation, and projection disruption radii, respectively. There are two major implications of coupling between the strain and strain rates. First, maximum expansion points of the bubble oscillation correspond to times at which the bubble wall velocity is zero, which stands in stark contrast to many testing techniques in which the strain peak and strain rate peak coincide [51,57]. This technique may thus offer a means of decoupling the effect of high strain and high strain rate testing. Second, the non-linear field in both strain and strain rate may induce different damage mechanisms in viscoelastic components of the cytoskeleton, e.g. microtubule breakage vs. tau phosphorylation [123]. The increased strain rate before collapse near the F-Actin fragmentation radius, compared to the relatively modest increase for the membrane fragmentation radius, may suggest a high strain rate-dependent mechanism, but would require independent low-strain rate testing via non-inertial cavitation rheometry [80–82].

Deviatoric stresses at the radii of interest are similarly interesting. At the bubble wall, a rapid rise in deviatoric stress occurred due to viscous effects, specifically, the high velocity during collapse. However, as distances increasingly far from the bubble wall, the viscous effects were less present, leading to less asymmetry in the total stress response. While the deviatoric stress spike just before collapse at the F-Actin fragmentation radius was not as pronounced as that near the bubble wall, the brief increase could play a role in the failure mechanism. To get a critical stress value for each radius of interest, we can convert the radial stress values into effective von Mises stress values for the cavitation problem by combining

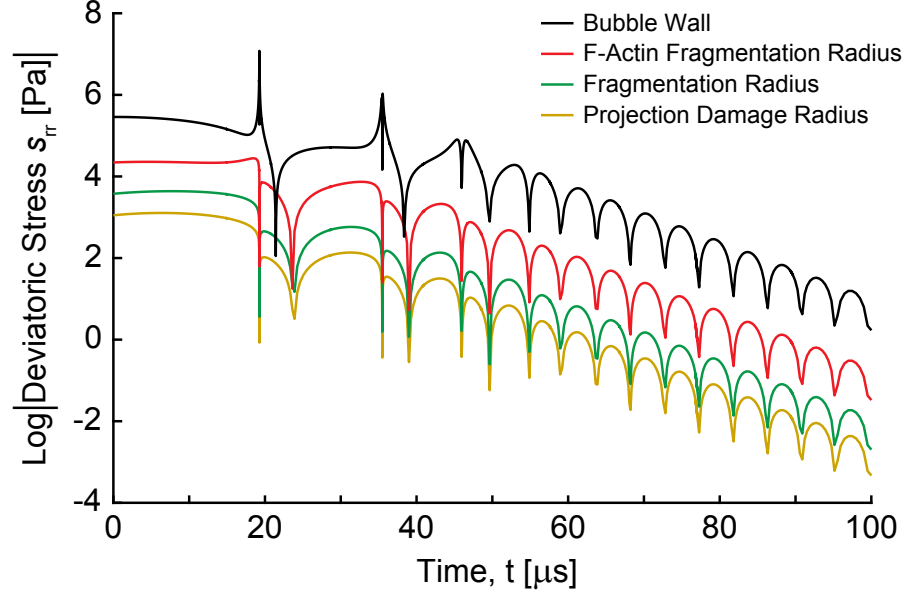


Figure 3.16: Radial stress vs. time in 3D neural cultures at different radii of interest. Shear modulus ($G = 7.08$ kPa) and viscosity ($\mu = 0.044$ Pa s) were calculated from the least squares fit from inertial microcavitation rheometry. Highlighted are stresses at the bubble wall (black), F-Actin fragmentation radius (red), image-calculated fragmentation radius (green), and projection damage radius (yellow).

the definition with Eq. 2.50,

$$\bar{s} = \sqrt{\frac{3}{2} s_{ij} s_{ij}} = \sqrt{\frac{3}{2} \left[s_{rr}^2 + 2 \left(-\frac{1}{2} s_{rr} \right)^2 \right]}, \quad (3.19)$$

$$\bar{s} = \frac{3}{2} |s_{rr}|. \quad (3.20)$$

The maximum effective stresses at each radius were found to be $\bar{s} = [18 \text{ MPa}, 42 \text{ kPa}, 6.5 \text{ kPa}, 1.9 \text{ kPa}]$ at the bubble wall and F-actin fragmentation, membrane fragmentation, and projection disruption radii, respectively.

3.4.5 Current limitations

In its current configuration, the pulsed-laser microcavitation generation method has several important limitations. As mentioned in the previous chapter, due to the intrinsic assumption of spherical symmetry, only $R(t)$ data that remains spherically symmetric during the

cavitation event will produce accurate radial information for scaling. Spherical asymmetry is particularly relevant in a non-ideally homogeneous matrix such as collagen, in which jetting can and did occur in some tests and the equilibrium bubble was not always simply-connected. While the method to mediate these effects was to run many tests and exclude those that exhibited asymmetry, this may not be viable in sample-limited tests, perhaps such as those using tissue. Further computational analysis and 3D bubble reconstruction methods must be developed to image full-field dynamics at high speeds. Alternatively, while material displacement and strain field measurements are commonly performed with digital image/volume correlation [117, 124] or particle tracking schemes, single plane light intensity requirements for high speed imaging introduce geometric constraints due to additional optics and/or speckle pattern challenges.

3.5 Future Work

The pulsed laser-induced microcavitation setup can be extended into many different directions and readily scaled up in terms of material and/or biological complexity. For example, the tissue surrogate platform utilizes Type-I collagen, which while proven effective in sustaining neural cells has limited physiological relevance as a replacement for brain extracellular matrix. Introducing more physiologically relevant surrogate materials, which if homogeneous on the scale of bubble dynamics, is a natural next step for determining if the cell failure criterion is strain- or stress-driven.

The present study focused on the immediate effects of primary injury to determine a fragmentation radius and failure strain criterion for neural cells. Particularly at regions near critical fragmentation radii, finer failure strain detail could be readily acquired by cell imaging and segmentation by the same method as in Bar-Kochba et al. [57] and overlaying with strain fields produced by bubble dynamics. Understanding and observation of the exact structural failure mechanism of the cytoskeleton can be achieved by immunostaining and high-resolution imaging of microtubules, phosphorylated tau protein, glial fibrillary acidic protein, among others.

Injury pathology remains of considerable interest, and live-cell imaging could be used to monitor neuronal deterioration e.g. due to secondary axotomy or neurotomy. Astroglial response to primary injury could be of interest and would only require image differentiation between astrocytes and neurons. Akin to post-fixation F-Actin visualization, immunofluorescence staining for specific cytostructural proteins such as microtubules and phosphorylated tau would be of direct interest for understanding internal cytoskeletal damage at high strains and strain rates.

Chapter 4

Conclusion and Future Directions

4.1 Summary

The aim of this dissertation is twofold – first, to develop and validate a experimental platform for inducing inertial cavitation in a hydrogel system and second, to demonstrate the platform’s capability in characterization of cell failure criteria in an *in vitro* model representing the cavitation damage mechanism in blast traumatic brain injury.

4.1.1 Inertial microcavitation-based ultra high strain rate rheometry

In chapter 2, an experimental platform for generating single spherically-symmetric inertial microcavitation bubble events via single pulses of a Q-switched Nd:YAG laser was presented. A spherically-symmetric continuum model of bubble dynamics in a viscoelastic hydrogel system was presented for finite deformations to numerically fit, or refine, the measured inertial cavitation dynamics. This technique, named inertial microcavitation-based ultra-high-strain rate rheometry (IMUR) was validated using water and polyacrylamide as material models. IMUR represents a new approach for determining the viscoelastic material properties of soft matter at high (10^3 s^{-1}) to ultra-high (10^8 s^{-1}) strain rates via inertial cavitation that requires only a single pulsed laser and a high-speed imaging platform, which can be easily integrated into commercially available inverted or upright light microscopes,

and has broad application to characterization of soft matter, including hydrogels and tissues, irrespective of tested length scale.

Further sophistication such as more detailed mass and heat transfer physics, including the process of bubble growth, and user-adjusted elastic and viscous components are natural extensions of the model. Introduction of compressibility effects, asymmetry in the deformation field, and material inhomogeneity are additional interesting, and necessary, directions toward an eventual full understanding of jetting dynamics in soft materials.

One potential interesting quantification option is incorporation of digital volume correlation on gels embedded with fiducial markers. Preliminary results from embedding collagen gels with fluorescent beads (see Appendix A for method details) showed promise in understanding remnant damage to the material after microcavitation as shown in Fig. 4.1. While the confocal imaging platform is limited to about $300\ \mu\text{m}$ volumes of fluorescent beads for adequate signal detection, displacement between the reference and damaged volumes show highly localized, asymmetric permanent damage in a radius of about the same size as the F-Actin damage zone. While this a preliminary result, the similarity in F-Actin damage and permanent deformation scaling certainly warrants further investigation, and recent work by Movahed et al. [96] could provide a direction for modeling damage regions.

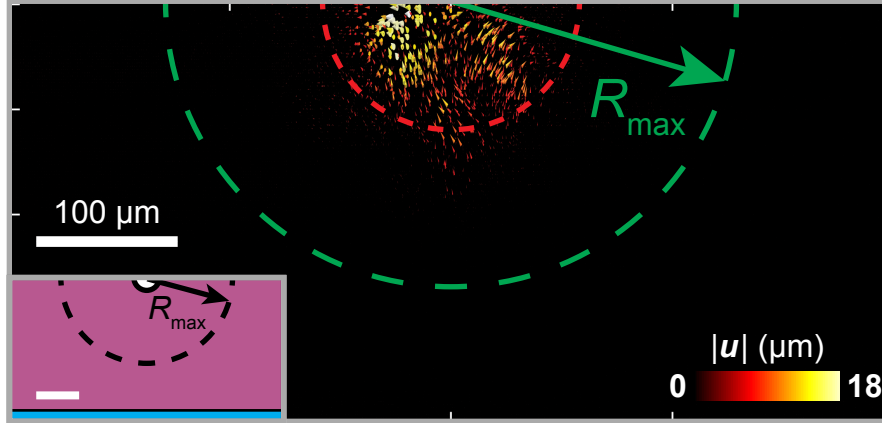


Figure 4.1: Cone plot highlighting local displacement due to microcavitation. Fluorescent beads and digital volume correlation were used to determine permanent deformation in the volume. Beads within about $0.75R_{\text{max}}$ remained relatively unmoved after cavitation. Large permanent deformation was found inside the red dotted line, which is the F-Actin fragmentation zone from Fig. 3.13.

4.1.2 Cavitation-induced neural cell injury

In chapter 3, the pulsed-laser experimental platform was used to generate single inertial microcavitation events in 3D primary dissociated cortical cell and Type-I collagen cultures. Laser scanning confocal images taken before and after injury and high-speed brightfield imaging of bubble dynamics during injury characterize cells morphologically as well as the reference and damaged configuration mechanically. New image processing techniques were created to correlate morphological fragmentation with known microcavitation-induced deformation strain. Furthermore, immediately after injury there are regions in which cells do not exhibit intracellular esterase activity but are either completely or partially structurally fragmented as determined by TRITC-phalloidin staining of F-Actin. These zones, which are all concentric with the original bubble, strongly suggest critical mechanical failure phenomena for fragmentation of cellular components. Major fragmentation of the plasma membrane was found at a median effective von Mises strain of 0.3, while complete fragmentation of the F-Actin network was estimated to occur at an effective strain of about five times higher.

While this work provides a foundation for correlating cavitation bubble dynamics to cell injury, there are a host of interesting future directions for more detailed investigation. These directions largely fall into three principal categories: cytostructural analysis/quantification, injury development and pathology, and secondary cellular response. Structural analysis can be readily done with immunohistochemistry combined with high-resolution imaging. Particular features of interest include determining whether ultra high strain rate injury induces phosphorylation of tau proteins or, as is believed, breakage of microtubules [123]. Certain cytoskeletal components could be more prone to damage, as suggested by the estimated lower strain threshold of about 0.08 for mechanical disruption of projections.

Injury pathology and development understanding remains the ultimate goal of traumatic brain injury research. Despite considerable efforts towards understanding of traumatic brain injury in general and blast in particular, our understanding of pathology, distinction between primary and secondary injury, and further correlation of damage to neurological disorder are

in their infancy. *In vitro* models can be used to bridge the *in vivo* knowledge gap [43] but at present require a compromise between biological and mechanical complexity to completely understand blast injury. Biological compromise impacts physiological relevance, particularly in assessing secondary injury effects. Future models should consider multi-cell culture in specific ratios and assessment of electrophysiological response to better represent *in vivo* conditions. Additional considerations of secondary injury include tracking release of cellular death factors, monitoring excitotoxic response, and understanding what role organelles play in the secondary injury pathway [125]. Furthermore, imaging modalities must consider the effects of live-staining and phototoxicity over time, and where applicable, use less damaging imaging options such as multiphoton microscopy. However, this study represents one small step among many towards understanding specific mechanisms of damage – in this case, the specific role microcavitation bubble expansion plays as an induced injury mechanism on cortical cells in 3D culture – and may provide some insight to the role of high strain rate mechanics in blast injury.

Appendix A

Mean Deformation Metrics for Quantifying 3D Cell-Matrix Interactions

A version of this chapter is published in the *Proceedings of the National Academy of Sciences* with Eyal Bar-Kochba and David A. Stout as first co-authors. Published data has been included in the section with co-authors' consent.

Estrada JB, Bar-Kochba E, Stout DA, Toyjanova J, Kesari H, Reichner JS, Franck C (2016). Mean deformation metrics for quantifying 3D cell-matrix interactions without requiring information about matrix material properties. *Proc Natl Acad Sci USA* 113(11), 2898-2903.

Abstract

Mechanobiology relates cellular processes to mechanical signals, such as determining the effect of variations in matrix stiffness with cell tractions. Cell traction recorded via traction force microscopy (TFM) commonly takes place on materials such as polyacrylamide- and polyethylene glycol-based gels, but such experiments remain limited in physiological relevance. This is so because cells natively migrate within complex tissue microenvironments

that are spatially heterogeneous and hierarchical, yet TFM requires determination of the matrix constitutive law (stress-strain relationship), which is not always readily available. In addition, the current achievable displacement resolution limits the accuracy of TFM for relatively small cells. To overcome these limitations and increase the physiological relevance of in vitro experimental design, we present a new approach and a set of associated biomechanical signatures that are based purely on measurements of the matrix displacements without requiring any knowledge of its constitutive laws. We show that our mean deformation metrics (MDM) approach can provide significant biophysical information without the need to explicitly determine cell tractions. In the process of demonstrating the use of our MDM approach we succeeded in expanding the capability of our displacement measurement technique such that it can now measure the 3D deformations around relatively small cells (~ 10 micrometers), such as neutrophils. Furthermore, we also report previously unseen deformation patterns generated by motile neutrophils in 3D collagen gels.

A.1 Introduction

Mechanical cues within the cellular microenvironment regulate numerous fundamental functions including cell adhesion, deformation and generation of traction [126–131]. Analysis of cellular force generation, and its role in regulating homeostasis across a variety of cellular phenotypes and experimental platforms, has received much attention over the last three decades [132–138]. Experimental quantification of cellular forces has produced several cell traction measurement techniques ranging from surface wrinkle detection and flexure of micro-pillars to traction force microscopy (TFM) [137, 139–145]. In TFM, measured cell-induced displacements are converted into tractions using various mathematical frameworks [139, 140, 142, 143, 146, 147]. Both two- and three-dimensional TFM techniques have steadily increased in sophistication and now feature high-spatial displacement resolution and advanced computational formalisms to connect this displacement information to complex material constitutive laws [142, 148, 149].

In order to successfully perform TFM it is critical to know the stress-strain constitutive

behavior of the matrix surrounding the cell. While many TFM substrates feature relatively simple artificial gel constructs, such as polyacrylamide and polyethylene glycol, these are unpenetrable by cells and obviate measures obtained while cells are in a three-dimensional setting (as would be the case within a bodily tissue). Most physiologically-important matrices have complicated, hierarchical microstructures and tend to be spatially heterogeneous. In addition, many cells apply significant forces leading to large matrix deformations which require more complicated, nonlinear constitutive laws for accurate calculation [142, 149–153]. Moreover, to generate a quantitative map of tractions exerted by a moving cell, the mechanical properties of the surrounding matrix must be resolved at the cellular level. Taken together, significant experimental challenges exist for accurately deducing the micro-constitutive laws for many physiologically realistic matrices, and by extension, the ability to map tractions onto small cells is lacking. Furthermore, and perhaps most importantly, many cells are known to actively remodel the matrix as they move through it. As a result, the constitutive laws are constantly evolving, making a one-time measurement of the matrix material properties insufficient for accurate determination of cell tractions in real time.

To circumvent this fundamental hurdle of requiring a material’s constitutive law, we present an alternate, kinematics-based quantification method that correlates cellular deformations to biological function. Based solely on the displacement field in the surrounding matrix, we define tensor-valued mean deformation metrics that quantify the overall shape change of the cell (e.g., contractility, mean volume change, and rotation). Because of its kinematic nature, the mean deformation metric approach does not aim to provide any information about the cell tractions. Rather, it should be viewed as a distinct, immediately-applicable methodology complimentary to TFM for investigations in which the material properties of the extracellular matrix are unknown.

Thus, the mean deformation metric approach provides a significant advantage to laboratories and investigators that may not have the means to conduct sophisticated material characterization measurements at cellular length scales. It is also well-suited for studies using primary cells for which fluorescent transfection approaches, such as in the use of

FRET-based force sensors [154], are not applicable.

We present the formulation of the mean deformation metrics (MDM) and validate our technique using well-established analytical solutions demonstrating our technique is accurate to within one percent. Next, we apply our MDM approach to analyze neutrophil-generated collagen matrix displacement fields obtained using confocal microscopy and Fast Iterative Digital Volume Correlation (FIDVC) [118]. In the process of demonstrating the MDM approach we solved the challenging problem of resolving local displacements around neutrophils (which are only 10 μm in size) with sub-micron resolution, and subsequently revealed previously unknown deformation patterns during the migration of neutrophils through a 3D collagen matrix.

A.2 Methods

Note: Experiments were performed by David A. Stout and Jennet Toyjanova. Data processing and analysis was performed and co-developed with Eyal Bar-Kochba.

A.2.1 3D collagen gel preparation for neutrophil chemotaxis

Neutrophils were isolated from healthy human volunteers using previously established protocols [155]. Neutrophil chemotaxis was induced via a custom-built three-well planar chemokine diffusion system (Fig. A.1). 3D collagen (type I, rat tail; BD Biosciences) matrices were prepared at a 2.2 mg/mL concentration with the addition of 6% (w/v) of 0.5 μm yellow-green carboxylate-modified microspheres (Life Technologies). Vybrant DiI (Life Technologies) stained cells were suspended in Leibovitz's L-15 (phenol free; Life Technologies) medium with 2 mg/mL glucose added for a final concentration of 10^6 cells/mL, and then added to the collagen matrix solution. The experimental system consisted of a rectangular three-well setup (Fig. A.1); the chemokine was pipetted into the left well and diffused through the collagen gel into the right sink. The diffusion system was fabricated

out of polydimethylsiloxane (PDMS; Dow Corning) with dimensions $10 \times 4 \times 6$ mm. Hydrophilic circular coverslips were used as the base of the mold. Two square hydrophobic coverslips were cut into 4×6 mm rectangles using a high-precision diamond-scribing tool (Lunzer). The rectangular coverslips were inserted into slots, spaced 10 mm apart, and separated the source and sink well on either side of the collagen gel. Planar diffusion began upon removal of the hydrophobic coverslips. The collagen solution was injected into the PDMS mold and then incubated for 25-30 min at 37°C and 5% CO_2 for polymerization. The bottom of the PDMS mold was coated with a thin layer of vacuum grease, pressed onto the circular coverslip, and placed into a Chambridge chamber (Live Cell Instrument Inc.) for live cell confocal imaging.

A.2.2 Determination of diffusivity in collagen

The planar interfaces between the wells generate a planar moving diffusion front. Validation of the diffusion kinetics is compared to the analytical diffusion equation for normalized concentration $\hat{C}$ at position x and time t given by

$$\hat{C}(x, t) = \left[\frac{x}{L} \left(\frac{C_1}{C_0} - 1 \right) \right] + \frac{2}{\pi} \sum_{n=1}^{\infty} \cos(n\pi) \sin\left(n\pi \frac{x}{L}\right) \exp\left[-\left(\frac{n\pi}{L}\right)^2 Dt\right], \quad (\text{A.1})$$

where C_0 and C_1 are the chemokine concentration at the inlet (Fig. A.1A left) and outlet (Fig. A.1A right), respectively. The diffusivity of the collagen gel is characterized by D , and L is the total length of the collagen gel (Fig. A.1A).

To estimate the diffusivity of fMLF through collagen, a fluorescent dye of similar molecular weight, Rhodamine (Sigma-Aldrich), was tested in the planar diffusion system. 2D Confocal images were recorded every two seconds at the mid plane of the gel, and normalized by the maximum intensity. Following the application of a 3×3 pixel mean filter to mitigate noise, the intensity of central points along the diffusion gradient axis were fit to Eq. (A.1) using least-squares regression (Fig. A.1D). For 2.2 mg/mL collagen gel, the diffusion coefficient was $1.28 \pm 0.35 \times 10^{-6} \text{ cm}^2/\text{s}$. It should be noted that, generally, the Fourier series in (A.1) allows for an infinite number of modes, i.e. $n \rightarrow \infty$, yet we did not see any significant

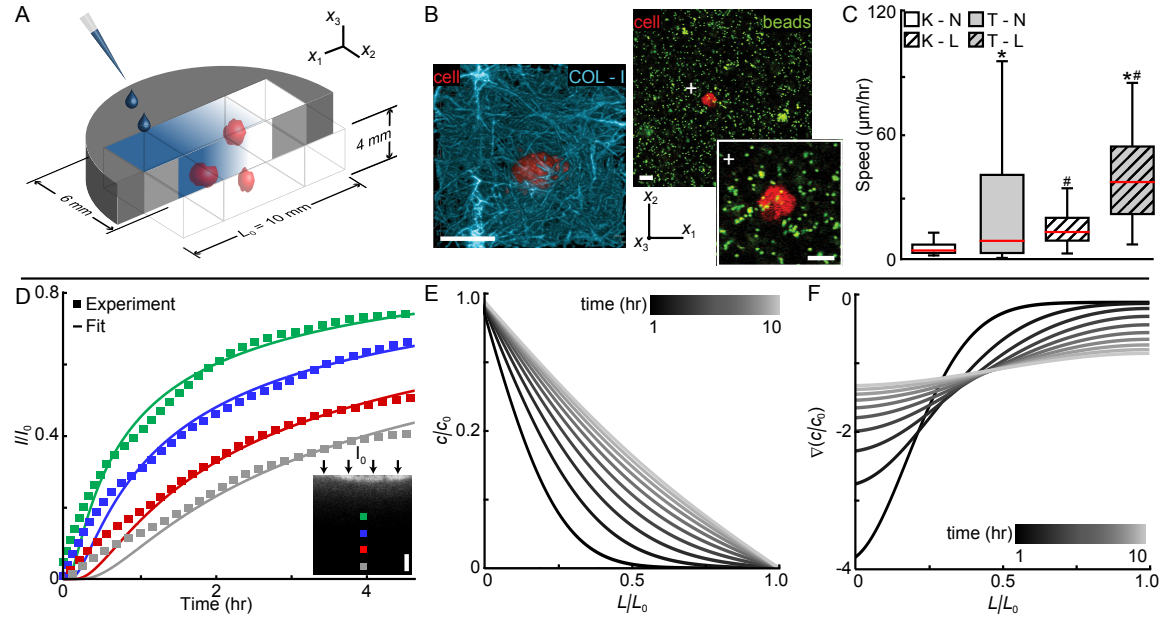


Figure A.1: Planar fMLF gradient diffusion system. (A) Schematic of the 3D diffusion system where the fMLF chemoattractant is introduced into the front well (blue) to induce neutrophil (red) chemotaxis against the planar diffusion gradient. The direction of the diffusion gradient is indicated by the shaded fMLF volume (blue to white), which is aligned with the x_1 -axis of the imaging coordinate system. (B) Volume rendering of a DiI-stained neutrophil (red) embedded in a fibrillar type I collagen matrix (cyan) imaged with reflectance confocal microscopy (left). Confocal micrograph of a neutrophil (red) migrating in a corresponding 3D collagen matrix containing randomly distributed 0.5 μm fluorescent microspheres (green) used to measure cell-induced matrix displacements. Scale bar, 10 μm . (C) Boxplot of neutrophil speed for all time points and tested conditions; K - N (solid white, $n = 30$), T - N (solid grey, $n = 48$), K - L (hatched white, $n = 44$), and T - L (grey hatched grey, $n = 57$). Red lines: medians, top and bottom boxes: upper and lower quartiles, whiskers: maximum and minimum values. $p < 0.05$ by Mann-Whitney U test across corresponding (*) chemokinesis and chemotaxis groups, (#) naïve and LPS activated groups. (D) Plot showing least-squares fit (solid) using Eq. (A.1) to the experimentally measured (square) normalized rhodamine dye intensity I/I_0 at increasing depths (green, blue, red, grey) in the collagen matrix. Inset shows a confocal micrograph of the intensity of the Rhodamine dye diffusion front at the beginning of the experiment. Scale bar, 200 μm . (E) Normalized chemoattractant concentration $\hat{C} = c/c_0$ and (F) concentration gradient in the diffusion system as a function of normalized chamber length L/L_0 as predicted by Eq. (A.1).

improvement in our fitting results for $n > 100$. Once D was determined, (A.1) can be utilized to predict the local chemokine concentration and concentration gradient at any point $\hat{C}(x, t)$ within the collagen gels (Fig. A.1E-F).

A.2.3 Neutrophil preparation

Neutrophils were isolated from healthy human volunteers by collection into ethylenediaminetetraacetic acid-containing Vacutainer tubes (BD Biosciences). Histopaque (Sigma-Aldrich) was used for the initial cell separation followed by gravity sedimentation through 3% Dextran (Sigma-Aldrich). Contaminating erythrocytes were removed by hypotonic lysis, yielding a neutrophil isolation purity of more than 95%. Neutrophils were suspended in Hanks Balanced Salt Solution (HBSS without Ca^{2+} and Mg^{2+} , Life Technologies) on ice until use in the experiments. Institutional Review Board approval was obtained from the Rhode Island Hospital's Committee on Protection of Human Subjects to allow donation of venous blood from normal, healthy donors via isolation of peripheral blood neutrophils. Informed consent was obtained in accordance with the Declaration of Helsinki. Human neutrophils in HBSS (-/-) at a concentration from $1 - 2 \times 10^6$ cells/mL were incubated with membrane dye DiI at a final concentration of 2 μM for 25 min at room temperature. After 25 min, the cells were centrifuged for 4 minutes at 700 revolutions per minute and re-suspended in Leibovitz's L-15 medium with 2 mg/mL glucose added for a final concentration of 10^6 cells/mL.

A.2.4 Chemotaxis, chemokinesis, and LPS activation

For naïve neutrophil chemotaxis experiments, both wells contained Leibovitz's L-15 with 2 mg/mL glucose. The left well also contained 100 μL of the chemoattractant, Formyl-Met-Leu-Phe (fMLF; Sigma-Aldrich), for a final concentration of 1 μM (Fig. A.1A). Lipopolysaccharide (LPS) activation was initiated through the addition of 100 ng/mL ultrapure LPS (LPS-EB, InvivoGen, San Diego, CA). Live cell imaging took place 5 minutes after the introduction of either chemokine, or chemokine plus activation solution for each respective experiment. Chemokinesis experiments were performed by adding 1 μM fMLF to both wells

in addition to adding 1 μM fMLF into the collagen mixture recipe prior to polymerization to establish a uniform chemokine concentration profile across the sample prior to imaging.

A.2.5 Microscopy and live cell imaging

Three-dimensional image stacks were acquired using a Nikon A-1 confocal system mounted on a Ti-Eclipse inverted optical microscope controlled by NIS-Elements Nikon Software. A Plan Fluor 40 $\times$ air objective (NA = 0.6; Nikon) mounted on a piezo objective positioner was used for all cell experiments, which allowed imaging speeds of 30 frames per second using a resonant scanner. Yellow-green fluorescent microspheres (0.5 μm ; Life Technologies) were dispersed throughout the collagen matrix and excited with an Argon (488 nm) laser. DiI stained cells were excited with a red HeNe diode (561 nm) laser. Confocal image stacks of $512 \times 512 \times 128$ voxels ($108 \times 108 \times 38 \mu\text{m}^3$) were recorded every 2 to 3 min with a z-step of 0.30 μm . To ensure physiological imaging conditions within the imaging chamber, temperature was controlled at 37 $^\circ\text{C}$ as previously described [142, 155]. For reflectance microscopy, an Apochromat 40 $\times$ water immersion objective (NA = 1.15, Nikon) with z-step = 0.25 μm was used to obtain typical imaging volumes of $167 \times 167 \times 81$ voxels ($52 \times 52 \times 20 \mu\text{m}^3$).

A.2.6 Calculating discretized cell surfaces

The 3D cellular surface boundary was determined from volumetric images of the fluorescently labeled cell membrane. A median filter with a $5 \times 5 \times 5$ window was applied to the raw volumetric image to reduce noise. Following a contrast adjustment where 1% of intensity values get saturated, a binary image was generated by setting all intensity values less than 50% of the maximum intensity of the image to 0 (black) and all others to 1 (white). The final binary mask of the cell was generated by removing all but the largest connected component. The triangular surface mesh of the binary mask was computed using the marching cubes algorithm [156] and then smoothed using the algorithm developed in Taubin [157].

A.2.7 Measurement of cell-induced displacements

Cell-induced full-field displacements were measured using the method described in Toyjanova et al. [142]. Three-dimensional time-lapse images of fluorescent beads embedded in the collagen matrix were captured using laser scanning confocal microscopy (LSCM). The incremental motion, or displacements, of the fluorescent beads was tracked between time points using a new fast iterative digital volume correlation (FIDVC) algorithm [118].

A.2.8 Interpolation of cell-induced displacements onto the cell surface

Matrix displacements are mapped onto the triangulation of the cell surface ∂V via a linear surface element interpolation scheme. Each area element has an associated surface normal, $\mathbf{n}$. Surface displacements are decoupled into surface normal $\mathbf{u}_\perp$ and surface tangential $\mathbf{u}_\parallel$ components by the following relations:

$$\mathbf{u}_\perp = (\mathbf{u} \cdot \mathbf{n}) \mathbf{n} \quad (\text{A.2})$$

$$\mathbf{u}_\parallel = \mathbf{u} - \mathbf{u}_\perp \quad (\text{A.3})$$

A.2.9 Calculating streamlines

A system of directed curves, which we refer to as streamlines, is plotted using Tecplot. The streamlines show the manner in which $\mathbf{u}_\perp$ varies over the cell surface. They lie on the cell surface and are constructed such that the local tangents to them at any point on the cell surface are equal to $\mathbf{u}_\perp$ at that point. More precisely, if $\mathbf{x}_S(s)$ is the parametric equation for a streamline, where s is the arc-length of the line, then

$$\frac{d\mathbf{x}_S(s)}{ds} = \mathbf{u}_\perp(\mathbf{x}_S(s), t) \quad (\text{A.4})$$

where t is time. Therefore, for each discrete time point there will be a distinct set of streamlines. Our definition of streamlines is different from the standard definition of streamlines used in the fluid mechanics literature, in which the material velocity field appears in the

place of tangential displacement field, $\mathbf{u}_\perp(\mathbf{x}_S)$, in (A.4). Despite the difference, we adopted the name ‘streamlines’ since we believe that the difference between definition (A.4) and the standard definition of streamlines is minor.

A.2.10 Finite deformations on the cell surface

The spatial gradient of the local surface displacement $\nabla \mathbf{u}$ is calculated numerically via convolution with the optimal-tap $9 \times 9 \times 9$ filter to minimize sampling aliasing errors as described in Farid [158]. The tensor $\nabla \mathbf{u}$ can be split into the symmetric, or stretch, and skew, or rotation components. The normalized magnitudes of the skew and symmetric components of $\nabla \mathbf{u}$ then signify the relative amounts of stretch and rotation of each element of the cell surface, ∂V . Strain metrics are also calculated from the displacement gradient. Small strain $\boldsymbol{\epsilon}$ and Lagrangian strain $\mathbf{E}$ are defined as

$$\boldsymbol{\epsilon} = \frac{1}{2} \left(\nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) \quad (\text{A.5})$$

$$\mathbf{E} = \frac{1}{2} \left(\nabla \mathbf{u} + (\nabla \mathbf{u})^T + (\nabla \mathbf{u})^T \nabla \mathbf{u} \right), \quad (\text{A.6})$$

where $\boldsymbol{\epsilon}$ neglects the higher-order effects of large deformations, or where $\|\nabla \mathbf{u}\| > 0.1$. Neutrophils exhibited large deformation gradients (Fig. A.2A,D,G,H), suggesting the use of Lagrangian strain. Mean eigenvalues of Lagrangian strain $\langle E_i \rangle$, analogous to contractility values $\langle \lambda_i \rangle$, also show isochoric deformation of neutrophils (Fig. A.2I).

A.3 Mean Deformation Metrics

The motion and the deformation of the cell and the surrounding matrix are described using the framework of continuum mechanics as

$$\mathbf{x}(\mathbf{X}, t) = \mathbf{u}(\mathbf{X}, t) + \mathbf{X}, \quad (\text{A.7})$$

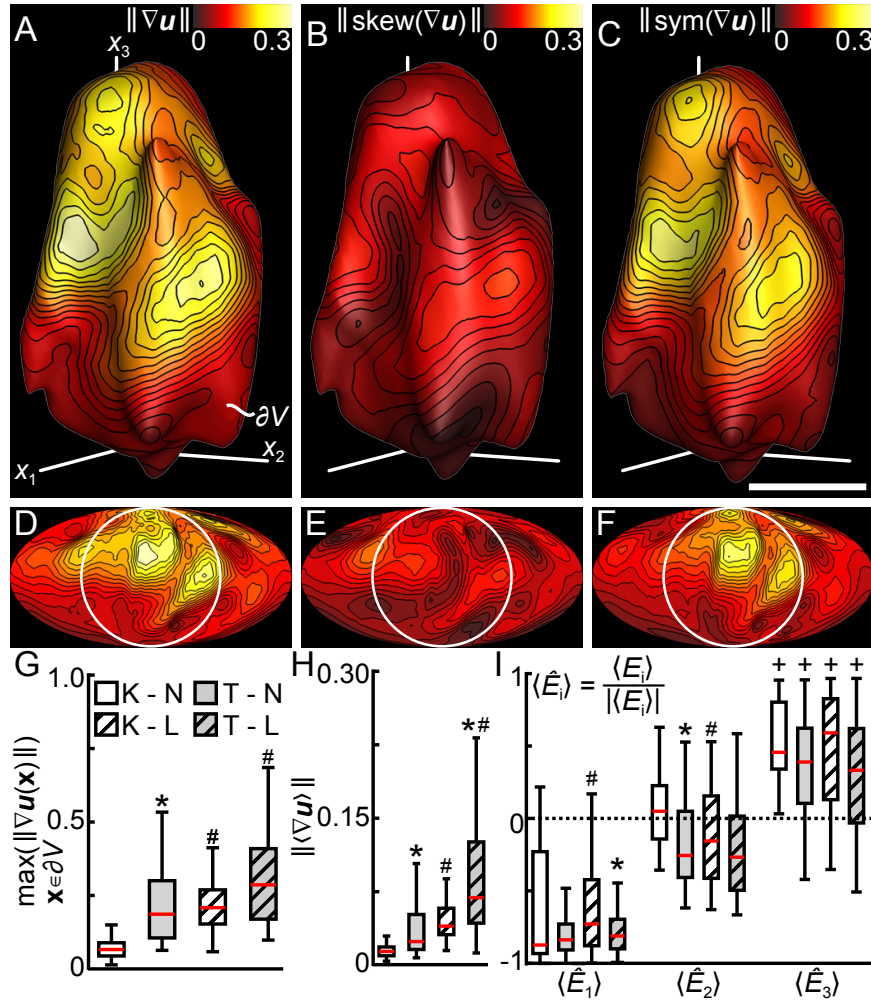


Figure A.2: Measurement of surface displacement gradients exerted by a migrating neutrophil. (A) Contour plot of the displacement gradient Frobenius ($L_{2,2}$) norm $\|\nabla \mathbf{u}\|$ on the surface ∂V (grey), color coded by magnitude. (B–C) Contour plot of the skew norm $\|\text{skew}(\nabla \mathbf{u})\|$ (rotational) and symmetric norm $\|\text{sym}(\nabla \mathbf{u})\|$ (stretch) parts of $\nabla \mathbf{u}$, color coded by magnitude. Scale bar, 5 μm . (D–F) Mollweide projection of corresponding surface data in A–C onto a 2D plane. Data interior to the solid circle (white) is located on the front hemisphere ($x_1 >$ cell centroid on ∂V) while data outside lies on the back hemisphere ($x_1 <$ cell centroid on ∂V) of the cell. Boxplot of (G) the maximum value of $\nabla \mathbf{u}$ on ∂V and (H) mean displacement gradient $\|\langle \nabla \mathbf{u} \rangle\|$ for all time points and tested condition, K – N (solid white, $n = 30$), T – N (solid grey, $n = 48$), K – L (hatched white, $n = 44$), and T – L (grey hatched grey, $n = 57$). Red lines: medians, top and bottom boxes: upper and lower quartiles, whiskers: maximum and minimum values. Boxplot of the three mean principal strain values normalized by its associated Frobenius norm $\langle \hat{E}_i \rangle$, calculated from the mean Lagrangian strain tensor $\langle \mathbf{E} \rangle$. Negative values of $\langle \hat{E}_i \rangle$ signify mean contraction while positive values of $\langle \hat{E}_i \rangle$ are mean expansion of the cell along the associated principal axis. $p < 0.05$ by Mann-Whitney U test across corresponding (*) chemokinesis and chemotaxis groups, (#) naïve and LPS activated groups, and (+) $\hat{E}_1$, $\hat{E}_2$, $\hat{E}_3$.

where $\mathbf{X}$ is the particle's position vector in the cell's initial or reference configuration, $\mathbf{x}$ is its position vector at time t , and $\mathbf{u}$ is the vector-valued displacement field.

The shape and size changes (strain measures) of infinitesimal material volume, surface, and line elements containing the particle $\mathbf{X}$ can be determined using the deformation gradient tensor

$$\mathbf{F}(\mathbf{X}, t) = \nabla \mathbf{x}(\mathbf{X}, t), \quad (\text{A.8})$$

where the operator ∇ gives the gradient of a vector-valued function with respect to $\mathbf{X}$. Considering the highly heterogeneous and complicated nature of the cell it is unlikely that $\mathbf{F}$ will be well-defined at each of the cell's material points. Consequently, we define a mean deformation gradient tensor, $\langle \mathbf{F} \rangle$, for the entire cell as

$$\langle \mathbf{F} \rangle := \frac{1}{\text{vol}(V_0)} \int_{V_0} \mathbf{F} \, dV, \quad (\text{A.9})$$

where dV is an infinitesimal volume element of the cell in its reference configuration V_0 and $\text{vol}(V_0)$ is the volume of V_0 . We believe that $\langle \mathbf{F} \rangle$, which is defined for the entire cell, is more meaningful than $\mathbf{F}$, which is defined pointwise. Mean deformation gradient tensors, defined in a way similar to ours, were used by Hill [159] in the development of theories of effective mechanical behavior of heterogeneous media, such as polycrystals and composites.

The quantity $\langle \mathbf{F} \rangle$ is a very useful metric for understanding the cell's overall deformation. An important advantage of using $\langle \mathbf{F} \rangle$ is that it can be computed solely from the displacements of the cell's surface points. Specifically, it can be shown using the divergence theorem that

$$\langle \mathbf{F} \rangle = \frac{1}{\text{vol}(V_0)} \int_{\partial V_0} \mathbf{x} \otimes \mathbf{n} \, dA, \quad (\text{A.10})$$

where ∂V_0 is the boundary of V_0 , dA is an infinitesimal element of ∂V_0 , $\mathbf{n}$ is the unit vector normal to dA , and the symbol $\otimes$ denotes the dyadic product. For computing $\langle \mathbf{F} \rangle$ using (A.10) we approximate the position vectors of the cell's surface points using the position vectors of their nearby matrix points.

Analogous to $\langle \mathbf{F} \rangle$ we define the mean displacement gradient $\langle \nabla \mathbf{u} \rangle$ as

$$\langle \nabla \mathbf{u} \rangle := \frac{1}{\text{vol}(V_0)} \int_{V_0} \nabla \mathbf{u} dV, \quad (\text{A.11})$$

$$\langle \nabla \mathbf{u} \rangle = \frac{1}{\text{vol}(V_0)} \int_{\partial V_0} \mathbf{u} \otimes \mathbf{n} dA. \quad (\text{A.12})$$

It follows from (A.9) and (A.11) that the mean deformation and displacement gradients are related as

$$\langle \mathbf{F} \rangle = \mathbf{I} + \langle \nabla \mathbf{u} \rangle. \quad (\text{A.13})$$

Once $\langle \mathbf{F} \rangle$ is determined, standard continuum mechanics calculations provide the mean compressibility, contractility, and rotation of the cell. The determinant of $\langle \mathbf{F} \rangle$, written as $\det \langle \mathbf{F} \rangle = \langle J \rangle$ is a measure of the mean compressibility, or volume change ratio, of the cell. Using the polar decomposition theorem $\langle \mathbf{F} \rangle$ can be written as $\langle \mathbf{F} \rangle = \langle \mathbf{R} \rangle \langle \mathbf{U} \rangle$, where $\langle \mathbf{R} \rangle$ is a proper orthogonal tensor and $\langle \mathbf{U} \rangle$ is a symmetric, positive-definite tensor. The tensors $\langle \mathbf{R} \rangle$ and $\langle \mathbf{U} \rangle$ can be termed the mean rotation and mean right stretch tensor, respectively.

The contractility (stretch or compression) of the cell in these primary directions is given by the eigenvalues of $\langle \mathbf{U} \rangle$, denoted as $\langle \lambda_i \rangle$, $i = 1, 2, 3$. The mean rotation of the cell $\langle \theta \rangle$ is computed as

$$\cos \langle \theta \rangle = \frac{\text{tr}(\langle \mathbf{R} \rangle) - 1}{2}. \quad (\text{A.14})$$

where $\text{tr}(\langle \mathbf{R} \rangle)$ is the trace of the mean rotation tensor $\langle \mathbf{R} \rangle$. The cumulative rotation $\langle \Theta \rangle$ is then defined as the time-integral of mean rotation, or

$$\langle \Theta \rangle = \int_t |\langle \theta(\tau) \rangle| d\tau. \quad (\text{A.15})$$

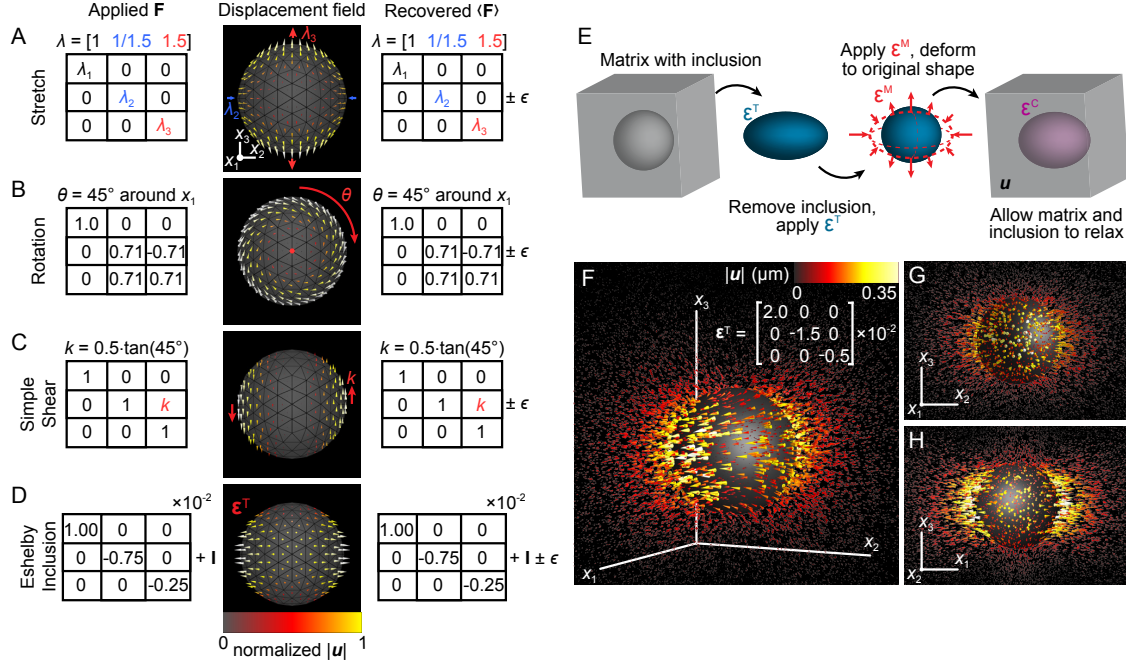


Figure A.3: Validation of mean deformation metrics on a spherical cell (grey) undergoing (A) stretch with three stretch ratios λ_1 , λ_2 , λ_3 , (B) rotation with angle θ around axis x_1 , (C) simple shear with magnitude k , (D) an Eshelby transformation strain ϵ_T . (left) Applied deformation gradient $\mathbf{F}$. (middle) Vector cone plot of the displacement fields interpolated onto the surface of the sphere for A–D, color coded by normalized magnitude $|\mathbf{u}|$. (right) Results for the recovered mean deformation gradient $\langle \mathbf{F} \rangle$ for A–D with associated error, (ϵ ; A–C, less than 10^{-12} due to numerical error; D, less than 10^{-5} due to small strain assumption). Calculation of full-field matrix displacements using the analytical Eshelby solution for an inclusion undergoing an eigenstrain. (E) Schematic illustrating the classical Eshelby inclusion problem, where an initially-spherical inclusion (grey) in an infinite matrix undergoes an eigenstrain ϵ_T and induces a known analytical displacement field $\mathbf{u}$ and confinement strain ϵ_C external to its boundary. (F) Vector cone plot of the solution of the matrix displacements calculated analytically from the inclusion eigenstrain ϵ_T . For visualization purposes, the displacement field is interpolated onto random locations as vector cones color-coded by magnitude $|\mathbf{u}|$. (G) $x_2 - x_3$ and (H) $x_1 - x_3$ plane views of the solution in F.

A.4 Results and Discussion

A.4.1 Validation

Validation of the mean deformation metrics was performed using four classical mechanics examples of well-known analytical forms: (A) simple stretch, (B) axial rotation, (C) simple shear, and (D) the Eshelby inclusion solution (Fig. A.3A-D). The Eshelby inclusion problem is a convenient analytical comparison to cells embedded in a 3D matrix. It considers an infinite, isotropic, linear-elastic extracellular matrix with Poisson's ratio ν and Young's Modulus E . The matrix is initially stress-free, with all displacement and strain values initially at zero. A single spherical cell, or inclusion, in the matrix then undergoes a transformation strain (or eigenstrain) ϵ_T , which is resisted by the matrix outside the region. Because the sphere is embedded in the material, strain ϵ_M and displacement $\mathbf{u}$ fields are induced in the matrix. The final strain field in the inclusion is given by the sum of the transformation and matrix strain, ϵ_C (Fig. A.3E-H). The strain field ϵ_C inside and on the surface of the inclusion is constant, while the displacement is found by taking the dot product of ϵ_C with the position vector $\mathbf{x}$. The displacement field outside the inclusion $\mathbf{u}$ has a closed-form solution given by [109,119,160] and is a function only of the inclusion radius (32 voxels), transformation strain ϵ_T , spatial position $\mathbf{x}$, and Poisson's ratio (chosen as $\nu = 0.2$). For each case, the mean deformation gradient tensor, $\langle \mathbf{F} \rangle$ was calculated from the analytical displacement field and surface normals using Eqs. (A.12) and (A.13). The test surface for the deformation was a sphere, meshed using recursive subdivision starting from an icosahedron. For cases A-C the number of subdivisions was 2 resulting in 320 discrete triangles, and for case D the number of subdivisions was 4, resulting in 5120 discrete surface triangles. Error from the analytical solution was on the order of 10^{-12} for cases A-C due to numerical error, and on the order of 10^{-5} for the Eshelby inclusion solution, D, predominantly due to the assumption of small-strain conditions.

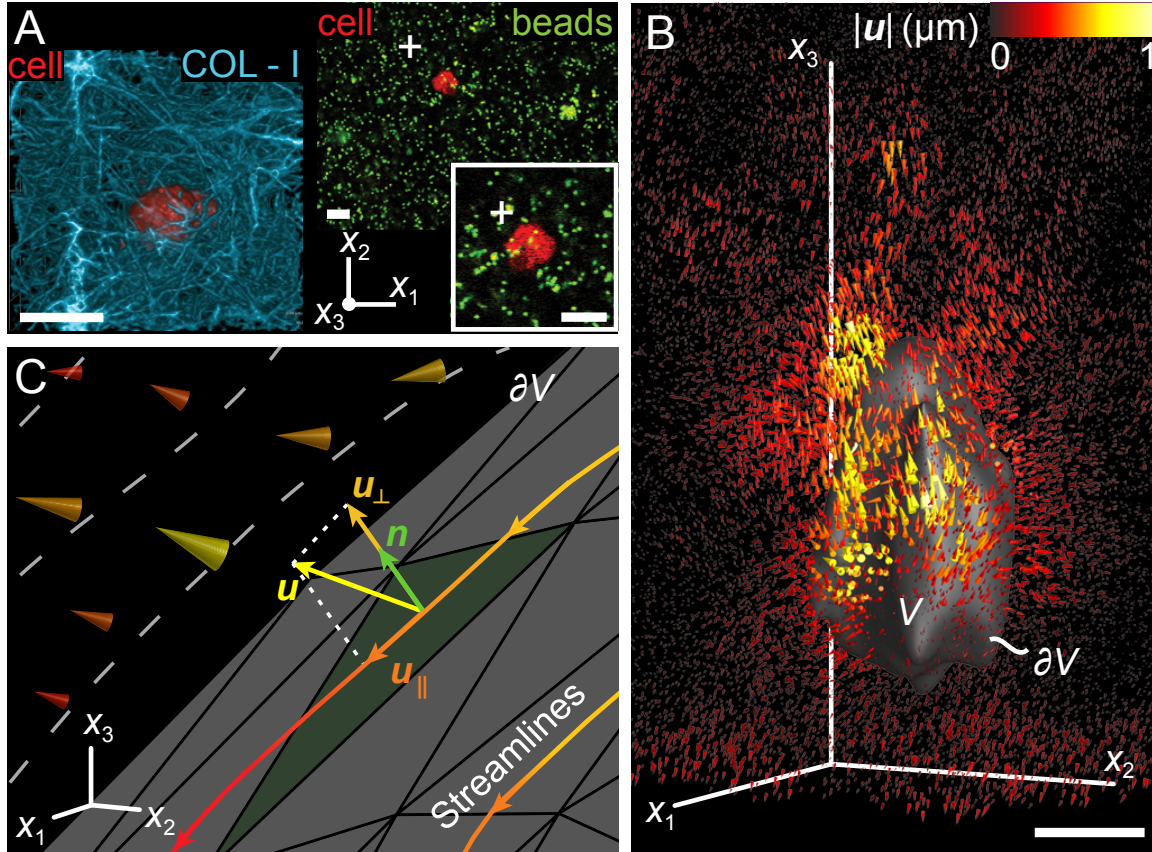


Figure A.4: Cell-induced hydrogel deformations and calculation of surface displacements. (A) Volume rendering of a DiI-stained neutrophil (red) embedded in a fibrillar Type I collagen matrix (cyan) imaged with reflectance confocal microscopy (left). Confocal micrograph of a neutrophil (red) migrating in a 3D Collagen matrix containing randomly distributed 0.5 μm fluorescent microspheres (green) used to measure cell-induced displacement fields. Scale bar, 10 μm . (B) Vector cone plot of the induced 3D displacement field measured by fast iterative digital volume correlation (FIDVC) surrounding a chemotactic neutrophil (grey) with volume V and surface ∂V . Scale bar, 5 μm . (C) Schematic outlining the interpolation of the 3D displacement field to ∂V to produce the surface displacements u . The surface normal n (green) is used to calculate the corresponding normal $u_{\perp}$ and tangential $u_{\parallel}$ component of u with respect to the cell.

A.4.2 Application of the MDM approach for the directional contractility, compressibility, and rotation of cells

We resolve the spatiotemporal surface features of cell-induced matrix deformations of a small, migrating human neutrophil ($<10\ \mu\text{m}$) in a fibrous 3D collagen matrix. Specifically, we encapsulate primary human neutrophils in a reconstituted type I collagen gel of final concentration of $2.2\ \text{mg/mL}$ resulting in the fibrillar microstructure shown (Fig. A.4A). The collagen fibers are visualized in blue via confocal reflectance microscopy and the neutrophil is visualized via the membrane stain DiI (Life Technologies) in red. Simultaneous injection of $0.5\ \mu\text{m}$ carboxylate-modified green fluorescent spherical particles (Fig. A.4A, right panel) during collagen polymerization serves as means for tracking the 3D matrix deformations imparted by the locomoting cell. To determine the cell-induced 3D displacement field, we employ our previously developed fast iterative digital volume correlation (FIDVC) algorithm [118]. This algorithm converts the motion of fiduciary markers obtained from time-lapse confocal image volumes into a 3D displacement field of matrix deformations around the neutrophil (Fig. A.4B). Full-field displacement can then be mapped to the rendered cell surface via interpolation (Fig. A.4C).

Our FIDVC algorithm accurately measures large material deformations, which we find significant during neutrophil locomotion in collagen since the local displacement gradient, $\nabla\mathbf{u}$, is non-negligible (Fig. A.2A-G). The FIDVC algorithm spatially refines the correlation windows, therefore allowing the capture of significantly higher spatial resolutions while increasing signal-to-noise and computational efficiency compared to our previous DVC method [117]. The displacement sensitivity in our study was $0.04, 0.06, 0.18\ \mu\text{m}$ (x_1, x_2 , and x_3 respectively), with a chosen spatial grid resolution set to eight voxels (~ 1.7 for x_1 and x_2 , and $2.4\ \mu\text{m}$ for x_3). The typical computation time on a GPU-equipped personal computer to determine the 3D cellular deformation field between two successive $512 \times 512 \times 128$ voxel volumes is approximately one to two minutes. While the FIDVC natively provides a detailed full-field map of the entire matrix displacements in the vicinity of the cell, many practical applications require simpler biomechanical classification metrics. In

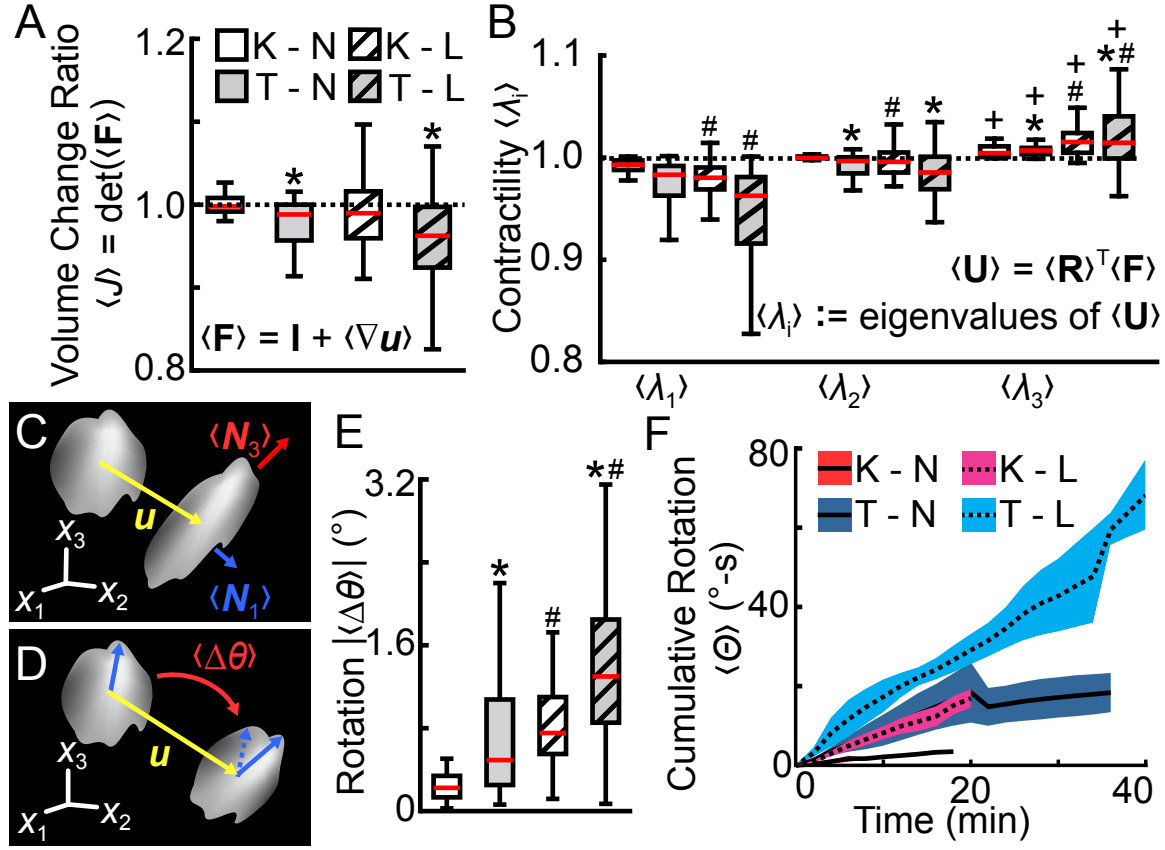


Figure A.5: Mean deformation metrics for neutrophils calculated from the mean deformation gradient tensor $\langle \mathbf{F} \rangle$. (A) Boxplot of the mean volume change ratio $\langle J \rangle$ of the cell (dotted line, constant volume), defined as the determinant of $\langle \mathbf{F} \rangle$ for all time points of the tested conditions; K - N, chemokinesis and naïve (solid white, $n = 30$); T - N, chemotaxis and naïve (solid grey, $n = 48$); K - L, chemokinesis and LPS-activated (hatched white, $n = 44$); and T - L, chemotaxis and LPS-activated (hatched grey, $n = 57$). Red lines: medians, top and bottom boxes: upper and lower quartiles, whiskers: maximum and minimum values. (B) Boxplot of the three mean contractility values $\langle \lambda_i \rangle$ calculated from the right Cauchy-Green stretch tensor $\langle \mathbf{U} \rangle$. Values of $\langle \lambda_i \rangle$ below 1 signify mean contraction while values of $\langle \lambda_i \rangle$ above 1 are mean expansion of the cell along the associated principal axis. (C) Schematic illustrating minimum $\langle \lambda_1 \rangle$ and maximum $\langle \lambda_3 \rangle$ principal stretches exerted by the cell with corresponding eigenvectors $\langle \mathbf{N}_1 \rangle$ and $\langle \mathbf{N}_3 \rangle$. (D) Schematic illustrating mean cell rotation angle $\langle \theta \rangle$ and (E) corresponding boxplot for all time points and conditions. (F) Integrated $\langle \theta \rangle$ for each cell over its migration time t for all tested conditions; K - N (solid red, $n = 4$ cells), T - N (solid blue, $n = 3$ cells), K - L (dashed magenta, $n = 5$ cells), T - L (dashed cyan, $n = 3$ cells). Middle line: mean, shaded area: standard deviation of integrals evaluated at the specified time point. $p < 0.05$ by Mann-Whitney U test across corresponding (*) chemokinesis and chemotaxis groups, (#) naïve and LPS-activated groups, and (+) across $\langle \lambda_i \rangle$ in B.

traditional TFM studies, these include simple scalar quantities such as root-mean-squared tractions, total forces, strain energies and contractility measures such as the trace of the dipole tensor [132, 140, 143, 161]. However, computation of these metrics requires the constitutive behavior of the matrix. The MDM approach, on the other hand, can provide biomechanical metrics without the use of the matrix constitutive law or having to solve a large system of equations.

All the biomechanical metrics in the MDM approach are defined solely using the displacement data from the cell-matrix boundary and the geometry of the cell-matrix boundary, which allows it to maintain an inherently low measurement noise floor significantly smaller than current state-of-the-art cell-matrix quantification methodologies [140, 142, 143, 162].

We determined $\langle \mathbf{F} \rangle$ from Eq. (A.13) for naïve (N) and lipopolysaccharide (LPS)-treated (L) neutrophils undergoing either chemokinesis (K) or chemotaxis (T). The mean compressibility $\langle J \rangle$ for the different phenotypes is shown in Fig. A.5A. Principal directions in which the cell stretches/contracts are given by the eigenvectors $\langle \mathbf{N}_i \rangle$ of $\langle \mathbf{U} \rangle$, whereas cell contractility in these directions is given by the eigenvalues of $\langle \mathbf{U} \rangle$, $\langle \lambda_i \rangle$, where $i = 1, 2, 3$. The mean contractilities $\langle \lambda_i \rangle$ are plotted for the different neutrophil phenotypes in Fig. A.5B. Examination of the contractility in conjunction with the cell compressibility reveals that neutrophils behave isochorically, contracting along two of the principal directions $\langle \mathbf{N}_1 \rangle$ and $\langle \mathbf{N}_2 \rangle$ while extending along the third axis $\langle \mathbf{N}_3 \rangle$ (Fig. A.5B and Fig. A.2I). Such behavior is indicative of a contractile, integrin-dependent mode of motility as opposed to a pushing, or integrin-independent mechanism [163]. This is consistent with recent findings showing that integrins continue to play a significant regulatory role for traction generation and motility under confinement in the presence of ligands [155]. Using the simple mean deformation metrics we detect statistically significant differences between naïve and LPS-activated human neutrophils. LPS-activated neutrophils demonstrate a significant increase in overall contractility (Fig. A.5B, and Fig. A.2G) and a trend towards a greater capacity to undergo changes in volume (Fig. A.5A).

The mean rotation $\langle \theta \rangle$ of the different types of cells is shown in Fig. A.5E-F. The figure

shows, for the first time, that neutrophils in the presence of LPS undergo significant rotatory motion as quantified by a marked increase in the average rotation angle (Fig. A.5E) and cumulative rotation (Fig. A.5F). We find statistically significant increases across all evaluated biomechanical metrics (Fig. A.5A-F) for both chemokinesis (K) and chemotaxis (T).

The capability to deduce cell mean compressibility, directional cell contractility, and cell rotations from displacement information is an important feature of the MDM approach. The information contained in these mean metrics is challenging to capture by current TFM methodologies or 3D image reconstruction algorithms.

A.4.3 Resolving local displacements around neutrophils with sub-micron resolution using FIDVC

Full-field vector maps of displacement fields obtained from FIDVC calculations during neutrophil migration through collagen show complex distributions (Fig. A.4B). Areas of large displacement (yellow) occur in close proximity to the cell surface ∂V (grey) and quickly decay in the far-field. To clearly interpret surface localization of the displacement field, we interpolate $\mathbf{u}$ onto a discretized triangular mesh structure of the cell surface with normal, $\mathbf{n}$ (Fig. A.7B), and decouple the surface normal $\mathbf{u}_\perp$ and tangential $\mathbf{u}_\parallel$ components (Fig. A.7C-D). Tangential displacements are represented with streamlines to assist in visualizing deformation patterns. The classical inclusion problem by Eshelby [119] was used as quantitative validation of the full-field displacement interpolation procedure, matching the analytical solution to within 10^{-3} percent error (Fig. A.3 and Fig. A.6).

The surface displacement field (Fig. A.7A) varies spatially across the entire surface of the cell and is visually viewpoint-dependent, complicating feature analysis for the viewer. To address this issue, we employ the homolographic Mollweide mapping technique to cast 3D surface data into viewpoint-independent 2D contour plots (Fig. A.7E-G). For illustration, the Earth's topological data (Fig. A.7B) is mapped from a spherical projection of the neutrophil surface into a familiar 2D globe map (Fig. A.7E). The Mollweide projection, used

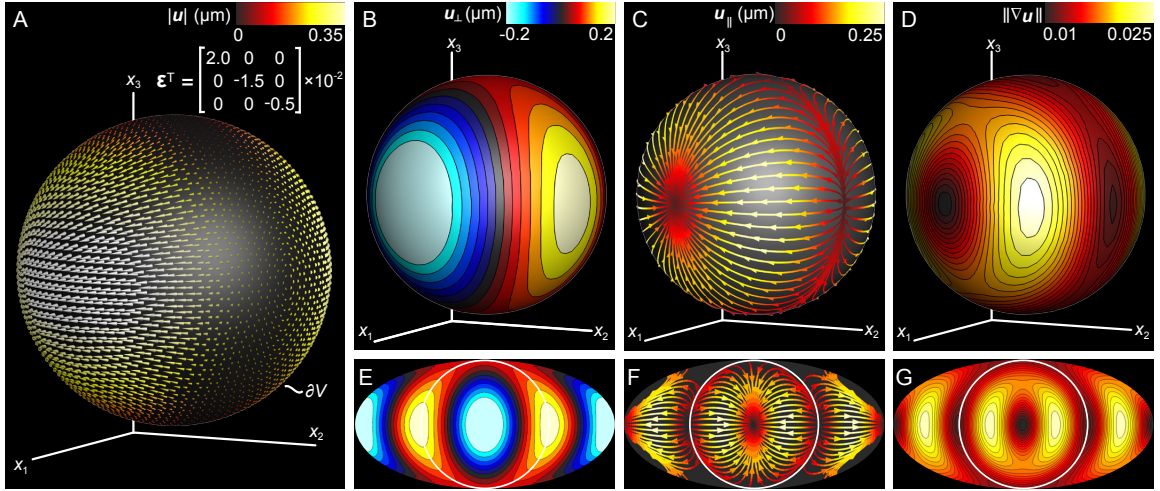


Figure A.6: Calculation of surface displacements using the analytical solution of Eshelby outside an inclusion undergoing an eigenstrain ϵ_T . (A) Cone plot of the displacement field interpolated onto the surface ∂V (grey) of the inclusion, color coded by magnitude $|\mathbf{u}|$. (B) Contour plot of the normal displacement component $u_{\perp}$ with respect to ∂V , color coded by direction and magnitude (red is outward and blue is inward normal surface displacement). (C) Streamline plot of the tangential displacement component $u_{\parallel}$ along ∂V , color coded by magnitude and arrow heads are pointed along the vector field. (D) Contour plot of the displacement gradient Frobenius ($L_{2,2}$) norm $\|\nabla \mathbf{u}\|$ on ∂V , color coded by magnitude. (E–G) Mollweide projection of corresponding surface data in B–D onto a 2D plane. Data interior to the solid circle (white) is located on the front hemisphere ($x_1 >$ inclusion centroid on ∂V) while data outside lies on the back hemisphere ($x_1 <$ inclusion centroid on ∂V) of the inclusion.

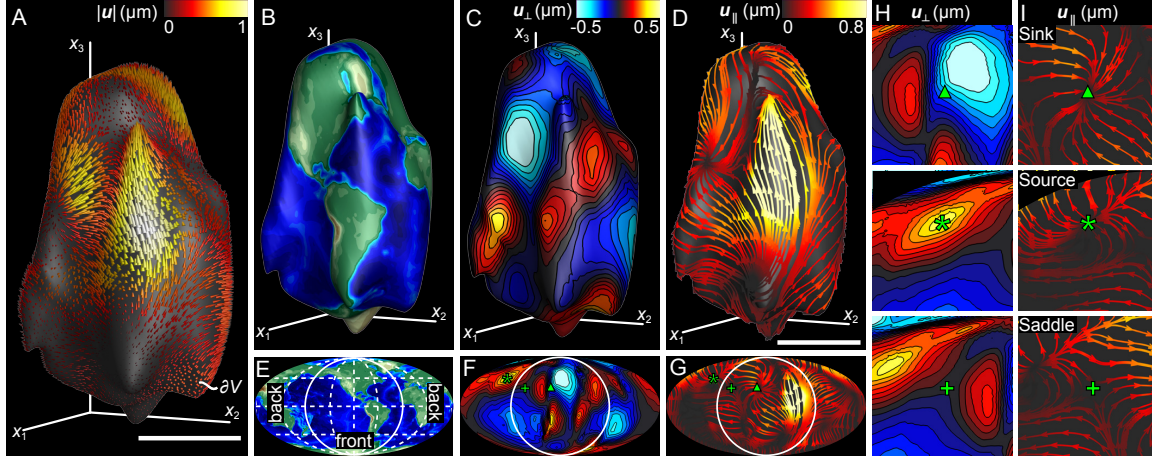


Figure A.7: Measurement of 3D surface displacements exerted by a chemotactic neutrophil and its associated homographic 2D projections. (A) Vector cone plot of the 3D displacement field interpolated onto the surface ∂V (grey) of the neutrophil, color coded by magnitude $|\mathbf{u}|$. (B) Elevation data of the Earth projected onto ∂V to illustrate the spatial position of surface data. (C) Contour plot of the normal component of $\mathbf{u}$ ($\mathbf{u}_\perp$) with respect to ∂V , color-coded by direction and magnitude (red is outward and blue is inward normal surface displacement). (D) Streamline plot of the tangential displacement component $\mathbf{u}_\parallel$ along ∂V , color-coded by magnitude with arrowheads pointing along the vector field. Scale bar, $5 \mu\text{m}$. (E) Mollweide mapping of the spherical projection of Earth's elevation data in B to allow for user-friendly visualization of all 3D data onto a plane. Dashed grid lines represent spacing of 45° along latitude and longitude lines. Data interior to the solid white circle is located on the front hemisphere ($x_1 > \text{cell centroid on } \partial V$) while data outside lies on the back hemisphere ($x_1 < \text{cell centroid on } \partial V$) of the cell. (F–G) Mapped contour plot of $\mathbf{u}_\perp$ in C and streamline plot of $\mathbf{u}_\parallel$ in D using the Mollweide projection in E. (H–I) Magnified view of F and G highlighting sink-like (top, green triangle), source-like (middle, green star), and saddle-like (bottom, green plus) features of $\mathbf{u}_\parallel$ and $\mathbf{u}_\perp$.

commonly in cartography, is a homolographic (iso-area) technique for mapping a spherical surface to an ellipse. Mapping was performed by first converting the position vectors from the centroid of each cell $\mathbf{x} - \mathbf{x}_C$ in ∂V to spherical coordinates (r, θ, ϕ) . The projection then transforms the (θ, ϕ) values to normalized map coordinates (x^M, y^M) by:

$$x^M = \sqrt{2} \sin \beta \quad (\text{A.16})$$

$$y^M = \frac{2\sqrt{2}}{\pi} \theta \cos \beta \quad (\text{A.17})$$

$$2\beta + \sin(2\beta) = \pi \sin \phi \quad (\text{A.18})$$

Corresponding surface and mapping locations are calculated using the `mfwdtran` function in the Matlab Mapping Toolbox and interpolated using the `scatteredInterpolant` function to improve contour visualization. Surface streamlines are mapped similarly from 3D to 2D using the Mollweide projection. We apply the same Mollweide mapping to the decoupled surface normal and tangential displacements (Fig. A.7C-D) on the neutrophil surface to visualize the complex local out-of-plane and in-plane deformations in convenient 2D contour maps (Fig. A.7F-G).

Detailed examination of specific regions along the cell surface reveal previously undocumented deformation structures reminiscent of sink, source, and saddle point flow structures (Fig. A.7H-I) commonly found in moving fluids. To our knowledge, these are the first observations of such well-defined deformation structures within the cell-generated displacement fields of a moving leukocyte.

A.4.4 Quantification of boundary-induced mean deformation error

Determining the true shape of any given cell using conventional fluorescent labels is challenging, and in most cases, including ours, leads to an inevitable loss of some structural detail (e.g., thin pseudopodia, protrusions, etc). Since our MDM technique requires cell boundary information, some error will be introduced by using a degenerate cell shape when

compared to the true cell shape. To estimate the error introduced in the mean deformation gradient $\langle \mathbf{F}_{\partial V} \rangle$ that is associated with the lack of a fully resolved cell boundary, we compute the error between the actual, fluorescent-based and two heavily degenerated cell shapes: (a) a minimum circumscribed ellipsoid around the cell, and (b) a minimum circumscribed sphere around the cell, shown schematically in Fig. A.8A. The error e between the cell surface ∂V and minimum circumscribed shape surface $\partial V'$ is defined as:

$$e = \frac{\|\langle \mathbf{F}_{\partial V'} \rangle - \langle \mathbf{F}_{\partial V} \rangle\|}{\|\langle \mathbf{F}_{\partial V} \rangle\|}, \quad (\text{A.19})$$

where $\|\star\|$ represents the Frobenius ($L_{2,2}$) norm. The results are shown graphically in Fig. A.8B, which provides the reader with an upper error bound estimate introduced in our MDM approach if the cell boundary cannot be resolved in full detail.

A.4.5 Comparison of the biophysical metrics provided by the Mean Deformation Metrics approach and Traction Force Microscopy

Fig. A.9 provides a general overview of the various biophysical metrics that can be determined from either Traction Force Microscopy (TFM) or the Mean Deformation Metrics (MDM) approach. Both techniques rely on the spatial resolution of the 3D cell-induced matrix displacement field, $\mathbf{u}(\mathbf{x}, t)$, which is typically computed either by cross-correlation (e.g., DVC, PIV) or single particle tracking techniques [164]). For the MDM approach, the only additional information required to compute the final mean deformation metrics is the discretized cell surface, commonly given by fluorescent labeling of the cell. In contrast, TFM requires both the discretized cell boundary (∂V) and material properties of the encompassing matrix. Reconstruction of the cellular traction fields can be achieved in three different ways, the first one requiring computation of the displacement gradient field, $\nabla \mathbf{u}$, the second making use of an inverse finite element analysis, and the third, if the material can be assumed to be linearly elastic, homogeneous and isotropic, utilizing the 3D Green's tensor. If the material properties are known, TFM provides the most comprehensive approach of characterizing cellular deformation fields, as both the displacements and tractions

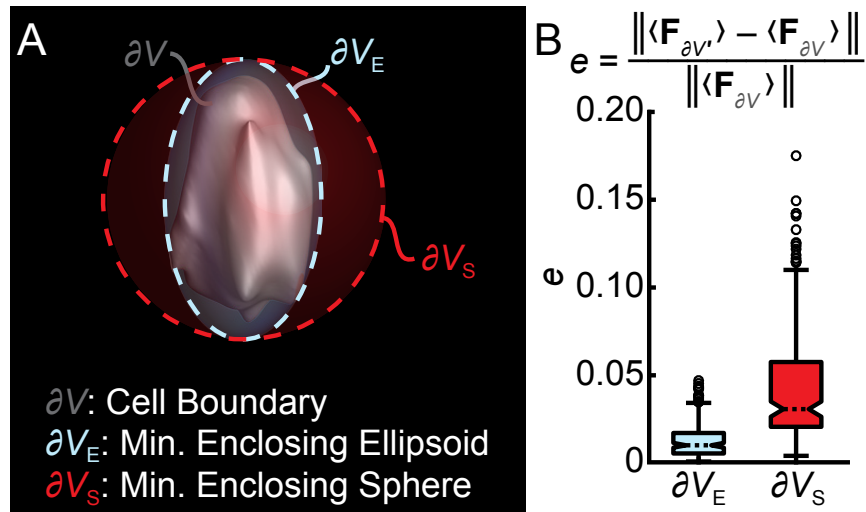


Figure A.8: Calculation of mean deformation error associated with estimating the cell boundary. (A) Actual cell boundary ∂V , minimum enclosing ellipsoid ∂V_E , and minimum enclosing sphere ∂V_S . (B) Error e in the norm of the mean deformation gradient tensor averaged over all cells and conditions ($n = 179$) of the cell boundary and minimum circumscribed ellipsoid and sphere. Horizontal lines indicate the 5th, 25th, 50th (dotted), 75th, and 95th percentiles. Notch indicates standard deviation.

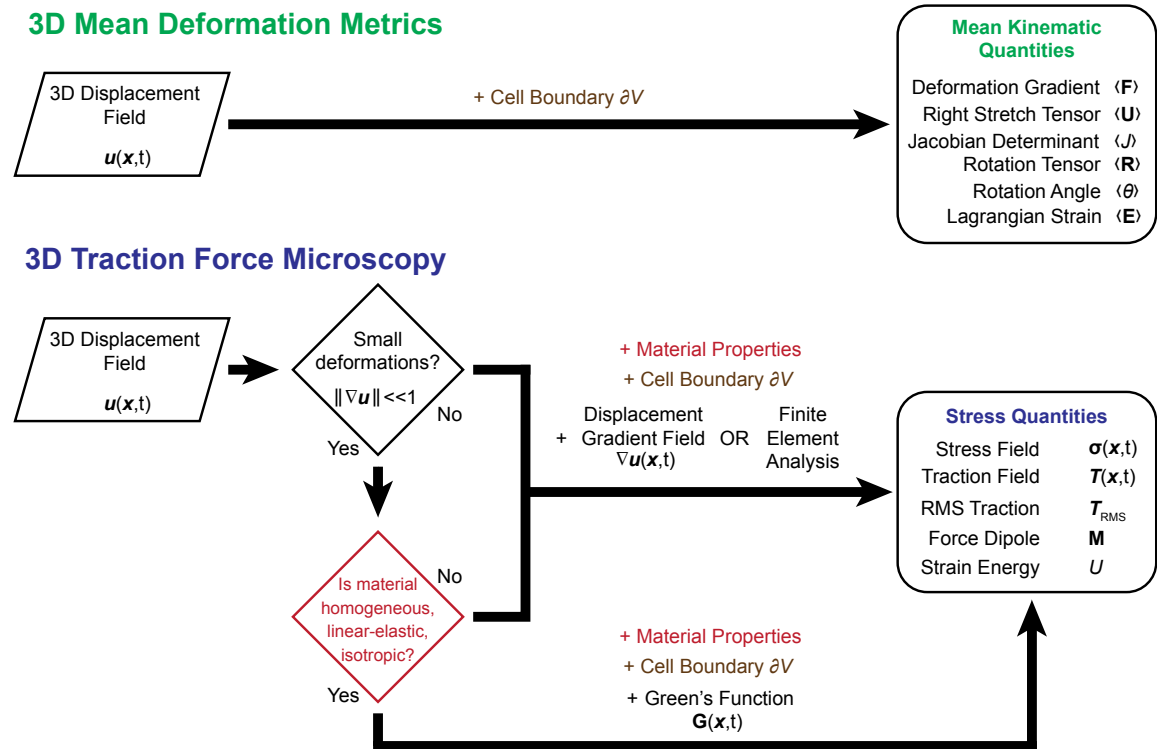


Figure A.9: Comparison of the biophysical metrics provided by the Mean Deformation Metrics approach and Traction Force Microscopy.

can be computed.

A.5 Conclusion

We present a new approach for quantifying cell-matrix interactions in mechanically complex microenvironments, such as 3D collagen gels, based purely on kinematic measurements. Similar to traditional TFM, we establish a set of new mean kinematic metrics to describe significant phenotypical differences between naïve and LPS-activated human neutrophils. Since our technique does not require any knowledge of the mechanical properties of the surrounding matrix, it can be applied to virtually any complex tissue including fibrous networks, multilayer scaffolds, and any transparent living tissue. This includes investigations in which cells actively remodel the matrix during the time window of observation.

The main limitation of the MDM approach, owing to its kinematic nature, is the inability to discern matrix stiffness effects which can only be calculated when the mechanical properties of the matrix are known. In cases where the mechanical properties are known, the FIDVC-derived displacement data can either be quantified using the presented MDM approach or be directly converted to traction data using e.g. our 3D Viscoelastic Traction Force Microscopy algorithm (see MDM - TFM comparison chart, Fig. A.9) [149].

In parallel to the MDM approach, we resolved spatially varying deformation features across cells about one order of magnitude smaller than what had been shown possible previously. By employing 3D surface and cartographic projection methods, we were able to reveal previously unknown 3D cell-matrix displacement patterns, such as source- and sink-type patterns. It is known that any continuous field defined on a 2-sphere will show features that are similar to the source and sink patterns that we observed on the neutrophil cell surfaces. However, we believe that the number of source and sink patterns and their relative spatial arrangement could contain biologically significant information, such as information about the cell's motility and adhesion mechanisms.

Appendix B

Speckle Pattern Evaluation for Digital Image and Volume Correlation Techniques

We acknowledge Soonsung Hong for the development of the original FFT-based DIC algorithm, Eyal Bar-Kochba for technical assistance with the preparation of this manuscript, and the broader mechanics community for the valuable and positive feedback in regards to the DIC and DVC GUI developments.

Abstract

Digital Image Correlation (DIC) and Digital Volume Correlation (DVC) are powerful means of resolving local kinematic descriptions of material deformation fields across a variety of material and testing platforms. Their spatial resolution, sensitivity, and accuracy depend in large part on the quality of the intrinsic material speckle pattern. Traditional evaluation of speckle pattern quality, or subset intensity distribution, relies on a set of well-characterized experimental measurements including rigid-body translation and rotation. In order to provide a significantly faster quantitative evaluation process on whether a particular speckle

pattern is suitable for DIC or DVC purposes, we present a simple, intuitive DIC and DVC speckle pattern graphical user interface tool programmed in Matlab. This tool assesses the DIC and DVC robustness of user-supplied speckle patterns via a two-step procedure: The first step involves warping the specific image according to a set of analytically prescribed deformation functions. The second step involves correlating the analytically warped and reference image pairs to recover the prescribed displacement field and its quantitative comparison to the prescribed warping function. Since the accuracy and precision of the recovered solution depend on the characteristics of the intensity distributions encoded in the image, this approach allows for a simple, yet effective, quantification procedure of the correlation suitability in the supplied image speckle pattern. In short, this procedure allows for fast and quantitative evaluation of the quality and suitability of a given speckle pattern to be used in DIC and DVC applications without the need of performing time-consuming experimental measurements. As such, we hope that this free tool will benefit anyone interested in performing DIC- or DVC-based kinematic measurements.

B.1 Introduction

The last three decades have shown a tremendous increase in the use of non-contact experimental imaging techniques due to the wider accessibility of high-resolution cameras and low-cost computing power [165–167]. Digital image correlation (DIC) and its volumetric extension, digital volume correlation (DVC) have provided significant insight into the deformation behavior of various material classes (e.g., from ceramics to hydrogels) across a broad range of length and time scales [155, 168–177]. In particular, experimental applications of DIC and DVC for resolving spatially-varying displacement and strain fields have spurred the development of various levels of sophistication in built-in motion capturing algorithms [118, 172, 178–181]. Equally exciting are recent endeavors to provide free, open-source access of digital image and volume correlation code packages to a broad spectrum of end users [118, 182–185].

Successful correlation of image subsets, or estimation of the kinematic motion fields between

successive images, relies in large part on the particular subset intensity profiles and their gradients, known as speckle patterns [165, 167]. Denser, more gray-valued speckle patterns generally correlate better, providing higher spatial displacement resolution and sensitivity compared to repeat patterns or large areas of constant intensity [118, 167, 177, 179, 186–188]. Since many materials employed in DIC and DVC investigations do not inherently possess high-quality speckle patterns, researchers have introduced various levels of sophistication in fabricating high-quality DIC and DVC speckle patterns. As such, almost every DIC or DVC experiment begins with a set of simple speckle pattern characterization tests to benchmark the maximum displacement resolution sensitivity at hand. These tests typically involve simple rigid body motions, such as rigid-body translation or rotation. The goal is to quantitatively compare the imposed motion with the recovered motion estimates from DIC or DVC. Although the experiments themselves are relatively straightforward, depending on the material in use, significant amounts of time and effort might be spent on generating reproducible and appropriate speckle patterns, resulting in a large number of required samples and evaluation tests.

In order to significantly reduce the material and time burden associated with this procedure, this paper presents an intuitive, open-source, Matlab-based interface for characterizing and optimizing a particular material speckle pattern. The purpose of our tool is to replace the traditionally experimentally-run calibration tests with a set of software-based quantitative simulations. The working principle is schematically illustrated in Fig. B.1. Here, the user-supplied reference image undergoes a series of simulated deformations to produce a set of analytically warped images (top row Fig. B.1). Each image pair, consisting of the reference (true) and synthetically-deformed (warped) image are then cross-correlated to obtain the full-field motion estimate between the images (middle row Fig. B.1). Finally, the recovered displacement fields for each deformation scenario are represented as displacement contour maps, and the recovery error is computed (bottom row Fig. B.1). The set of standard displacement warping functions, which are described in detail in the next section, include rigid body translation, uniaxial tension, pure shear, and the resulting displacement field due to application of a point force acting on an elastic half-space.

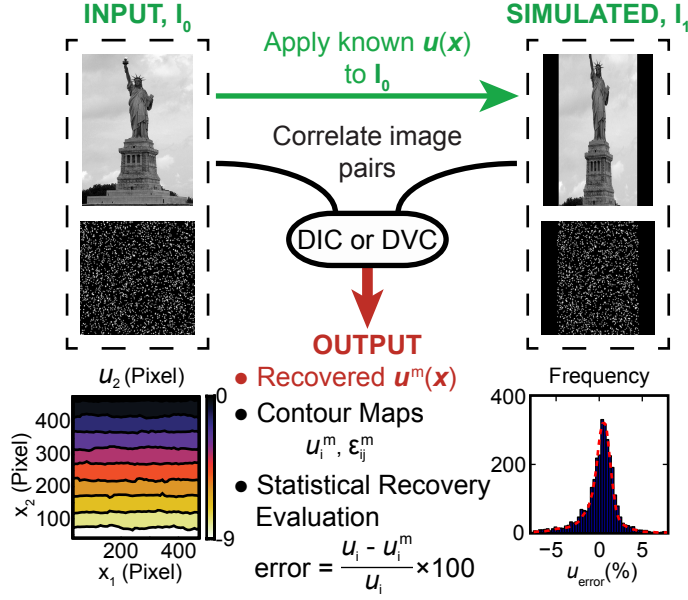


Figure B.1: Schematic overview of the Digital Image (DIC) and Volume Correlation (DVC) simulators.

B.2 Methods

The graphical user interface (GUI) for each DIC and DVC simulator provides simple and intuitive access for evaluating the speckle pattern quality in the user-specific image. Fig. B.2 provides a snapshot of the DIC GUI. Since the DVC GUI is similarly structured in comparison to the DIC interface, with the main difference being the addition of the third (x_3, u_3) dimension, the reader should be able to extrapolate any descriptions of the DIC interface onto the DVC GUI, which we purposefully omit here for the sake of brevity.

B.2.1 The Graphical User Interface (GUI)

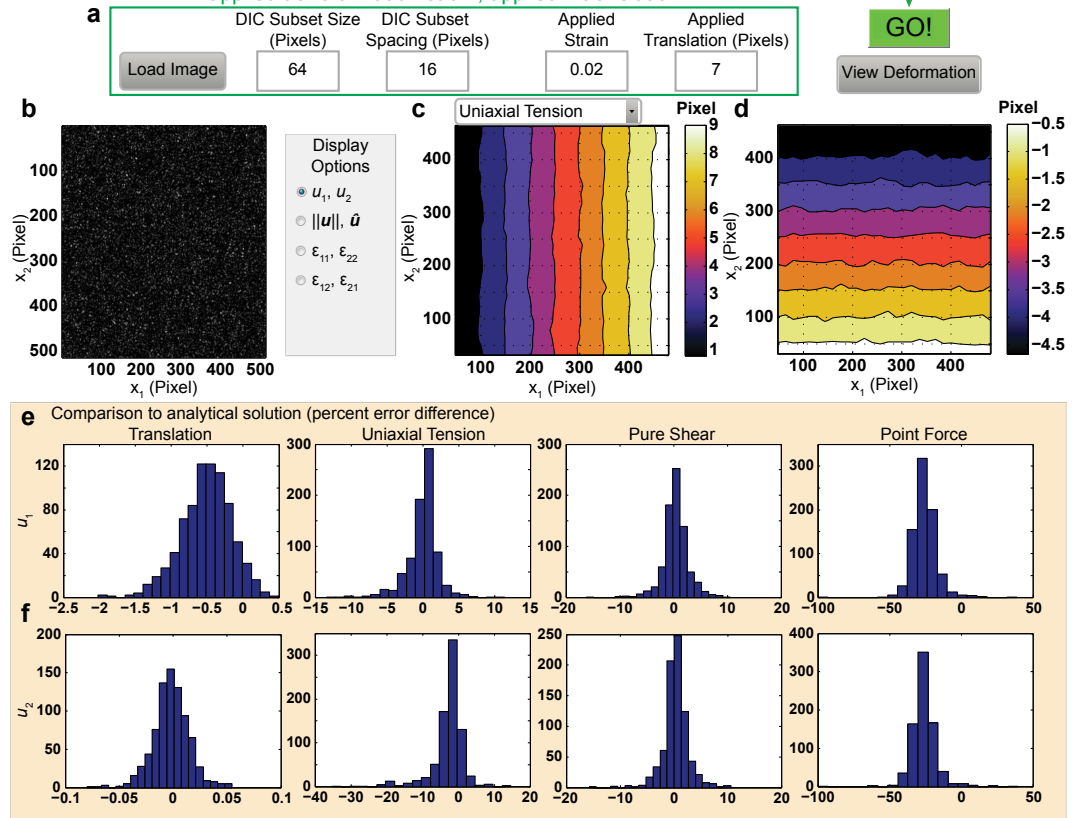
The DIC GUI is compartmentalized into three (top, middle, bottom) organizational rows (Fig. B.2a, b-d, and e-f, respectively). The input parameters in Fig. B.2a allow the user to upload an image in .tif, .jpg, .bmp, or .mat format, and to select the typical DIC algorithm parameters, such as interrogation (subset) window size, w , and spacing, d . The subset size, w , specifies the amount of intensity information to be correlated in each subset, which in turn determines the highest resolvable spatial frequency via DIC. The subset spacing, d ,

DIC Simulator Interface

INPUT PARAMETERS: Image, subset size, subset spacing, applied transformation strain, applied x-translation

EXECUTE DIC

GO!



OUTPUT DISPLACEMENT ESTIMATORS:

- Displacement and strain contour plots
- Residual error histograms of simulated vs. recovered deformations

Figure B.2: Screenshot of the Matlab-based DIC Simulator Graphical User Interface (GUI), with (a) input parameters, (b) input image, and output (c-d) contour maps and (e-f) residual deformation recovery error histograms.

determines the spatial resolution of the resulting DIC displacement grid. Although small values of d lead to high spatial grid resolution, the maximally resolvable spatial feature size (i.e. the highest intrinsic spatial frequency) depends primarily on w , not d [189–192]. Computationally, increasing d and/or decreasing w leads to a significant decrease in processing time. Due to practical limitations, most correlation studies employ subset window sizes ranging from 32-64 pixels, as a compromise between the amount of resolvable spatial frequency content and a robust image motion estimate. Further information regarding the effect of correlation subset window size and spacing on the final DIC or DVC resolutions can be found elsewhere [118, 167, 185].

Next, the user specifies the nominally applied transformation strain, ϵ_t , which is used to scale the relative amount of introduced image warping into the uploaded user image. It is important to note that the featured DIC and DVC simulator algorithms do not include higher order shape functions and thus it is strongly recommended to limit the level of transformation strain, ϵ_t , to within 10%. The next text box allows the user to specify the amount of horizontal pixel translation of the reference image. Again, since the formalism in the DIC and DVC algorithms utilize the same Eulerian cross-correlation frames, maximum pixel shifts should be limited to stay within half of the subset size ($\frac{w}{2}$) to maintain robust correlation. Once all text fields within the top row of the GUI have been appropriately populated, the **GO** button executes the simulator. After the simulator is executed, the user may select **View Deformation** to display and save both reference and deformed image pairs as well as the displacement component fields for each deformation case. Options to save resulting image deformation and simulated displacement data include as pictures in **.tif** format and numerically into variables as **imagedata.mat** and **dispdata.mat** files in the working directory.

The middle row features graphical outputs of the uploaded image by the user (Fig. B.2b), and user-selected color contour plots of the recovered displacement or strain fields using DIC (Fig. B.2c-d). Color contour plot data use a modified CMR map suitable for both black-and-white and color viewing [193]. The user-specified output variables are selected from a predefined radio button menu and displayed in pixels with intensities rendered

as color-coded contour maps. Possible graphical contour outputs include the horizontal (u_1) and vertical (u_2) displacements, as well as individual components of the infinitesimal strain tensor, ϵ_{ij} . The bottom row of the GUI represents a series of histograms quantifying distributions of the DIC displacement recovery error, which is defined as:

$$\text{Recovery Error (\%)} = \frac{u_i - u_{im}}{u_i} \cdot 100, \quad (\text{B.1})$$

where u_i and u_{im} represent the analytically-imposed and DIC-recovered (measured) displacements, respectively. Each histogram represents the recovery error for a given applied deformation mode (e.g., uniaxial tension) per given displacement (u_1 vs. u_2). The purpose of plotting recovery error is twofold. First, visual inspection of the error distribution provides quantitative information about the speckle-dependent DIC accuracy and precision, represented by the mean and the spread of the distribution, respectively, of the recovered solutions. Second, the amount of outliers provides information as to how much of the speckle intensity patterns are suitable for DIC or DVC purposes, i.e. a significant amount of outliers with respect to the total population of data points indicates that large portions of the image are not suitable for DIC use. In many images such areas correspond to areas of constant intensity over which the intensity gradient is zero, leading to poor cross-correlation [167,186]. As part of the downloadable simulator package (franck.engin.brown.edu) we supply the user with a high quality speckle image that exhibits very good DIC displacement recovery properties, which provides a reference for the statistical evaluation procedure for the unfamiliar user.

B.2.2 Simulated deformation fields

As shown in Fig. B.1 the first step in the image evaluation procedure begins by mapping the intensity values from the reference image to a synthetically deformed image of equal size using Matlab's built-in `interp3` and `interp2` functions for DVC and DIC, respectively. The mapping procedure in the DIC simulator is performed using the following four displacement

field functions:

$$u_i = u_0 \cdot \delta_{i1}, \quad (\text{B.2})$$

$$u_i = \epsilon_t[(x_1 - x_{10})\delta_{i1} - \nu(x_2 - x_{20})\delta_{i2}], \quad (\text{B.3})$$

$$u_i = \epsilon_t[(x_1 - x_{10})\delta_{i2} + (x_2 - x_{20})\delta_{i1}], \quad (\text{B.4})$$

$$u_i = \frac{1 + \nu}{2\pi E} \left\{ \frac{2(1 - \nu)r + x_3}{r(r + x_3)} P_i + \frac{2r(\nu r + x_3) + x_3^2}{r^3(r + x_3)^2} x_i \cdot (x_i P_i) \right\}, \quad (\text{B.5})$$

where ϵ_t is the applied transformation strain, δ_{ij} is the Kroneker delta, $r = \sqrt{x_i x_i}$ with $i = 1, 2$ (planar motion), and each $\mathbf{u}(\mathbf{x})$ describes either rigid-body motion (Eq. B.2), uniaxial tension (Eq. B.3), pure shear (Eq. B.4), or a point force, $\mathbf{P}$, acting tangent to an elastic halfspace (Eq. B.5) [194]. The Poisson's ratio, ν , is set to 0.45 for all deformation cases. The ratio of the material-normalized point force components in all directions P_i to the Young's Modulus E in Eq. (B.5) is taken to be the transformation strain ϵ_t , or $P_i/E = \epsilon_t(\delta_{i1} + \delta_{i2})$. The original (reference) image and the synthetically deformed image constitute a respective deformation image pair, which is analyzed using DIC. The volumetric DVC simulator employs 3D extensions of the displacement functions given by Eqs. (B.2) - (B.4), with the exception that the 2D Boussinesq problem is replaced by a point force acting in an infinite medium [109], for which the displacement components are given by

$$u_i = \frac{1 + \nu}{8\pi E(1 - \nu)r} \left\{ \frac{x_k x_i}{r^2} P_k + (3 - 4\nu)P_i \right\}, \quad (\text{B.6})$$

where $i = 1, 2, 3$ (3D motion). The Poisson's ratio is identical to the value used in Eq. (B.5), and the magnitude of the material-normalized point force, $\mathbf{P}/E$, is $P_i/E = \epsilon_t \delta_{i3}$.

B.2.3 Digital Image and Volume Correlation algorithm

Both DIC and DVC simulators make use of our previously developed Fast Fourier Transform (FFT) based correlation algorithm [117]. The analytical formulation behind both algorithms

is identical and can be written in typical cross-correlation form,

$$c(\mathbf{u}) = \int_{\Omega_x} f(\mathbf{x})g(\mathbf{x} + \mathbf{u})d\Omega_x, \quad (\text{B.7})$$

where $c(\mathbf{u})$ is the cross-correlation coefficient and $f(\mathbf{x})$ and $g(\mathbf{x})$ denote the image intensities in the reference and deformed image subsets, Ω_x . The displacement vector, $\mathbf{u}(\mathbf{x})$, between the two subsets is determined by the $\text{argmax}(c(\mathbf{u}))$. The main difference between the code structures of the DVC and DIC algorithms is that DVC uses cubic subsets and volumetric images in 3D voxel space, whereas the DIC uses square subsets in 2D pixel space. Both algorithms feature subpixel resolution capability [117]. This type of DIC and DVC formulation presents the simplest implementation and lowest order motion reconstruction – with 2 and 3 degrees of freedom (DoF) in DIC and DVC, respectively – based on rigid-body translation of individual subsets. Since the goal of the overall simulator is to provide the end user a general, zeroth order estimate about the DIC/DVC quality of their speckle pattern, we believe that the use of such a simple, yet fast motion algorithm is justified. If the user requires higher fidelity motion reconstruction we suggest utilization of already available higher order DIC and DVC algorithms [118, 166, 180, 185].

B.3 Results

To gain a better understanding of the typical output generated by either DIC or DVC simulator, Fig. B.3 provides a graphical example. As described in detail in the previous section, two user-specified images (Fig. B.3a, f) are uploaded and deformed according to Eqs. (B.2)-(B.5). For our illustration purposes here, we focus on uniaxial tension (Fig. B.3 b-c and g-h), and the applied point force (Fig. B.3 d-e and i-j) serving both as examples of uniform strain and concentrated strain deformation cases. First examining the results from the uniaxial tension deformation mode, it is clear that the image with the more speckle-like features provides the most accurate reconstruction (Fig. B.3b) of the uniaxially applied motion. This is highlighted by the linearly varying contour plot of the u_2 (vertical) displacement field. Similarly, the recovered displacement error is normally distributed with

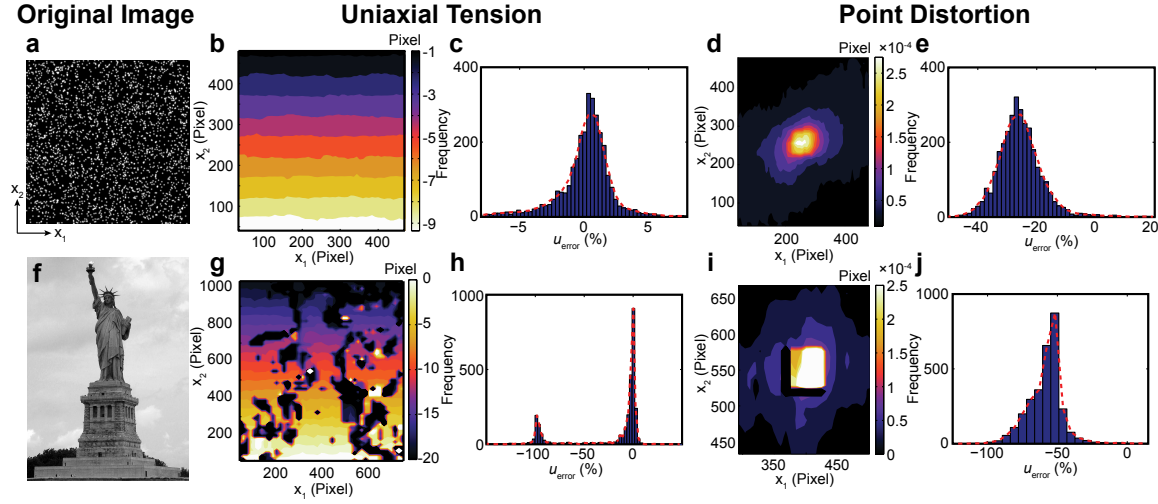


Figure B.3: Example (a, f) input images and output displacement contour maps and residual error histograms in (b-c, g-h) uniaxial tension and (d-e, i-j) for a point force.

a mean, μ , of -0.154% and standard deviation, σ , of 2.39% (Fig. B.3c).

When comparing the displacement contour results of u_2 recovered from the Statue of Liberty image to the image possessing a well-defined speckle intensity pattern, the Statue of Liberty image is able to provide reasonable displacement estimates in areas of varying grayscale intensities but to a much lesser extent in areas of similar intensity magnitudes. Local areas of constant intensity lead to poor correlation due to a vanishing local intensity gradient [167], which is highlighted by the degraded displacement information shown in Fig. B.3g. General uniaxial displacement behavior is observed in the contour image, and whereas some areas within the image correlate well and provide the proper displacement reconstructions, others produce significant error in their estimate. The considerable error is reflected in the cumulative recovery error distribution (Fig. B.3h), which shows a bimodal error distribution. One of the peaks is centered around a mean value close to zero ($-2.92 \pm 30.9\%$) corresponding to the areas in the images of well-defined grayscale intensity gradients, whereas the other, smaller, peak is centered close to 100% error representing the areas of poor correlation in the image.

The displacement recovery results from the analytical point force solution applied to both

the speckled and Statue of Liberty images display a higher degree of error due to under-resolved displacement gradients near the point force. Here, the spatial differences between the recovered displacement fields in Figs. B.3d and B.3i are primarily due to the differences in the intensity distribution of the image-respective subsets. Additional error is introduced by the inability of the simple DIC algorithm to properly resolve the local displacement gradients near the applied point force. This explains why the speckled image carries a displacement recovery error of $-26.4 \pm 8.48\%$ (Fig. B.3e) albeit the high quality subset textures. Upgrading the DIC algorithm to include higher order shape functions will significantly reduce this error estimate by resolving higher spatial frequency content in the displacement fields [180,185]. Nevertheless, the recovered displacement contour image (Fig. B.3e) portrays the qualitatively correct shape and distribution of the analytically imposed displacement field. The Statue of Liberty image, on the other hand, contains various vertical and horizontal features of similar intensities, which lead to poor cross-correlation and the resulting square-like shape of the point force displacement solution with a recovery error of $-62.3 \pm 30.7\%$ (Fig. B.3j), which are significantly more degenerate in comparison to the high-quality speckle image results (Fig. B.3e).

B.4 Conclusions

In this manuscript we present a simple graphical user interface (GUI) for the evaluation of image intensity (speckle) patterns for use in DIC or DVC applications. Using the GUI the user first uploads an image, which then undergoes a series of analytically imposed deformation functions, which are subsequently recovered via DIC or DVC. Computation of the displacement recovery error (Eq. B.1) provides a quantitative assessment of the correlation-appropriateness of the intrinsic image speckle patterns (Fig. B.3). Our simulator tool allows for a simple, fast and effective speckle pattern evaluation procedure, which traditionally is handled using a series of time- and/or material-consuming experimental measurements. Furthermore, while the current GUI version supports four different displacement function as DIC and DVC benchmark tests, it is relatively straightforward to incorporate additional or any user-specific analytical displacement functions within the Matlab-based framework

of the GUI itself. This should provide the end-user with significant flexibility. When using the GUI to evaluate the quality of the speckle pattern at hand, there are several current limitations that should be noted. First, for images at resolutions below 512×512 pixels (DIC) or $512 \times 512 \times 96$ voxels (DVC) only small deformations ($<10\%$) should be applied due to increasing accumulation of interpolation bias during the image warping process. This is so because the current image warping process relies on linearly interpolating the image intensities from the reference into the deformed configuration. If the original image is of low resolution (e.g., 128×128 pixels²) and the user applies large strains ($>10\%$), a significant amount of interpolation is required to map the image intensities from the reference into the deformed configuration producing significant amounts of interpolation bias, or image artifacts. Second, simulation of finite deformations ($>10\%$) should also generally be avoided since the current DIC and DVC formalism only capture rigid-body translation (2, 3 DoF in DIC, DVC respectively) at the subset level. Large deformation simulations would require upgrading the current DIC and DVC algorithms, which can be achieved using already existing packages [118,166,180,185] but is beyond the current work. Last, should the user decide to proceed with using DIC or DVC for his or her research purposes, we strongly recommend using more sophisticated and hence accurate DIC and DVC algorithms, which are freely available from our website (franck.engin.brown.edu) and elsewhere [118,182–185].

In conclusion, the presented speckle pattern evaluation GUI provides a simple and effective methodology for quantitatively determining whether a certain image or volume stack is suited for DIC or DVC purposes.

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