It is well known that cells change their cytoskeleton structure in order to move and to divide, as well as to respond to external stresses and maintain cell integrity. Even though much is known about the structural elements of the cytoskeleton and the motor proteins acting on these structures, the underlying physical processes are not well understood, nor is the manner in which the cell controls its mechanical properties. In this project, we proposed to address these questions by studying passive and active microrheology of in-vitro reconstituted cytoskeleton networks, which act as simple model systems for the cytoskeleton. These systems use a minimal set of proteins to reconstruct a cytoskeleton-like network. The physical, chemical, and materials properties of the resulting networks depend on the chosen set of proteins, thus allowing distinguishing their different roles.

Microrheology is an experimental method used to extract the mechanical properties of a fluid by analyzing the motion of a microscopic bead diffusing in it. Due to its simplicity of application, the economy of its sample size and its non-invasive nature, microrheology proves an ideal tool for probing mechanical properties of complex environments. Conventionally, microrheology is based on translating the diffusive motion of colloidal particles into the elastic properties of the diffusing medium by means of linear response and the fluctuation-dissipation theorem. However, non-linear processes such as stress transduction in cellular environments and strong shear in complex fluids are not accessible by this method. It has recently been suggested that by using active microrheology, where the tracer particles are driven, the non-linear nature of stress transmission in complex fluids can be measured. However, the interpretation of these experiments is not straightforward since the fluctuation-dissipation theorem no longer holds.

To study active microrheology of active in-vitro networks we had to develop three expertise: preparation of active networks and understanding of their formation dynamics, construction of optically based active microrheology setups, and implementation of passive and active microrheology analysis software and methodology. We have worked on all fronts during the past four years, collaborating with the group of Prof. Anne Bernheim-Grosswasser of Ben-Gurion University on actomyosin network synthesis and characterization, and with Prof. Haim Diamant from Tel Aviv University on the theoretical aspects of microrheology. One PhD students worked full time on the project, two PhD students and one M.Sc. student and one technician worked part time on setting up two different systems for active and passive microrheology experiments, and several undergraduate students participated in related colloidal synthesis.

During the past four years we achieved progress in all three directions:

Holographic optical tweezers combined with confocal microscopy - We built a one-of-its-kind holographic optical tweezers combined with confocal imaging, that allows for independent and simultaneous three-dimensional optical trapping and imaging. Combining imaging and control of multiple micron-scaled objects in three dimensions opens up new experimental possibilities such as the fabrication of colloidal-based photonic devices, 3D active microrheology, as well as high-throughput studies of single biological cell dynamics.

Actomyosin active networks - We performed a detailed study of the effect of constituent concentration on the morphology and dynamics of these gels as they undergo self-organization. We

showed that in contrary to other cytoskeletal motor proteins, such as kinesin, myosin II exhibits additional functionality in the self-assembly and self-organization of the cytoskeleton. We usede an *in-vitro* cytoskeletal model system to highlight the role of myosin II as a network nucleator, as an active bundling and cross-linking protein, and as an actin turnover regulating agent, all of which are required by the cell to control its morphology and dynamic remodeling in an adjustable manner. In addition, we were able to show how the concentration and size of the myosin II clusters affect network evolution, morphology and dynamics.

Microrheology of actin networks - While measuring with great care the correlated motion of tracer particles in entangled F-actin networks, we came across a new regime in the decay of the response of a complex fluid to a perturbation. At this intermediate regime the response decays as $1/r^3$ instead of the asymptotic decay of $1/r$. This behavior persists up to a very large distance of several microns away from the perturbation source. We have been able to account for this effect, and show that it is inherent to any complex fluid, and that it may appear at significantly large distances, over ten times the typical length-scale of the fluid (mesh size in our case), if the ratio between local and bulk viscosity is large. Deriving the response within the two-fluid model, we were able to compare experiment to theory, thereby measuring the dynamic correlation length of F-actin networks, as well as their bulk and local viscoelastic properties. In Fig. 1 the cross-over distance of many networks differing in mesh size ξ_s, and measured with different tracer particle sizes a, is plotted as a function of the ratio of local viscosity to bulk viscosity, H. By rescaling the cross-over length and presenting it as a function of the ratio ξ_s/a, all the experimental data collapses to the single master curve which was theoretically predicted.

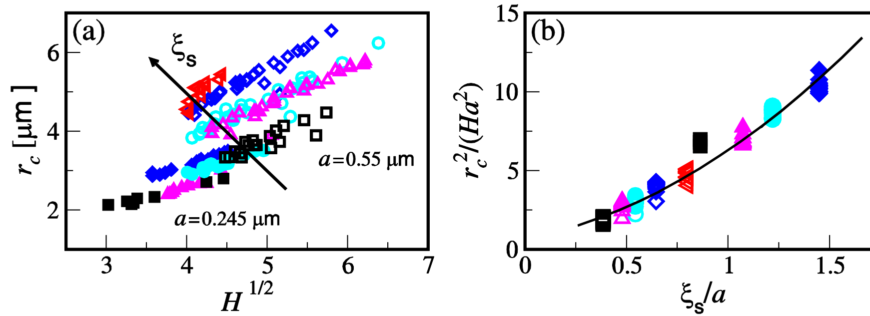

Fig 1: Crossover distance for all experiments. (a) For all conditions r_c is linear with $H^{1/2}$ the ratio of local to bulk viscosity, and increases with either mesh size, ξ_s, or particle size, a. (b) All experimental results fall on a master curve once r_c^2 is normalized by Ha^2 and presented as a function of ξ_s/a.

This work is significant for extending the characterization of any complex fluid, and as a new tool to characterize the structure of complex fluids. Moreover, there are cases in which the intermediate regime response is the only contribution to the fluid's response, for example, on a thin film near a rigid wall, or for fluids in a stiff porous medium. A particularly important system to consider is the biological cell, whose size is comparable to the crossover distance measured here. Cell viscoelasticity, therefore, should be reconsidered with the intermediate regime response in mind.