Motor-driven advection competes with crowding to drive spatiotemporally heterogeneous transport in cytoskeleton composites

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The cytoskeleton—a composite network of biopolymers, molecular motors, and associated binding proteins—is a paradigmatic example of active matter. Particle transport through the cytoskeleton can range from anomalous and heterogeneous subdiffusion to superdiffusion and advection. Yet, recapitulating and understanding these properties—ubiquitous to the cytoskeleton and other out-of-equilibrium soft matter systems—remains challenging. Here, we combine light sheet microscopy with differential dynamic microscopy and single-particle tracking to elucidate anomalous and advective transport in actomyosin-microtubule composites. We show that particles exhibit multi-mode transport that transitions from pronounced subdiffusion to superdiffusion at tunable crossover timescales. Surprisingly, while higher actomyosin content increases the range of timescales over which transport is superdiffusive, it also markedly increases the degree of subdiffusion at short timescales and generally slows transport. Corresponding displacement distributions display unique combinations of non-Gaussianity, asymmetry, and non-zero modes, indicative of directed advection coupled with caged diffusion and hopping. At larger spatiotemporal scales, particles in active composites exhibit superdiffusive dynamics with scaling exponents that are robust to changing actomyosin fractions, in contrast to normal, yet faster, diffusion in networks without actomyosin. Our specific results shed important new light on the interplay between non-equilibrium processes, crowding and heterogeneity in active cytoskeletal systems. More generally, our approach is broadly applicable to active matter systems to elucidate transport and dynamics across scales.

KEYWORDS
fluorescence microscopy, myosin, single-particle tracking, differential dynamic microscopy, cytoskeleton, actin, microtubule
1 Introduction

The cytoplasm is a crowded, heterogeneous, out-of-equilibrium material through which macromolecules and vesicles traverse to perform critical cellular processes such as mitosis, endocytosis, migration, and regeneration [1–4]. Macromolecules and particles diffusing through the cytoplasm and other similar materials have been shown to exhibit widely varying and poorly understood anomalous transport properties that deviate significantly from normal Brownian diffusion. In particular, the mean-squared displacement, MSD, often does not scale linearly with lag time, $\Delta t$, but is instead better described by $\text{MSD} = \Delta t^{\alpha}$ where $\alpha < 1$ or $\alpha > 1$ for subdiffusion or superdiffusion, respectively. The distributions of displacements (i.e., van Hove distributions) also often deviate from Gaussianity and can display exponential tails at large displacements [5–8]. The cytoskeleton–an active composite of filamentous proteins including actin, microtubules, and intermediate filaments, along with their associated motor proteins—plays a key role in these observed anomalous transport properties [9–11]. Such anomalous transport phenomena are not just observed in cytoskeleton, but are ubiquitous in numerous other active and crowded soft matter systems, making their characterization and understanding of broad interest.

In steady-state, the thermal transport of particles through in vitro cytoskeletal systems exhibit varying degrees of subdiffusion and non-Gaussianity depending on the types and concentrations of filaments and crosslinking proteins [5, 6, 12, 13]. For example, single-particle tracking (SPT) of particles in composites of entangled actin filaments and microtubules, has revealed increasing degrees of subdiffusion ($\alpha$ decreasing from $-0.95$ to $-0.58$) as the molar ratio of semiflexible actin filaments to rigid microtubules increased [6]. The corresponding SPT van Hove distributions were reported to be non-Gaussian, displaying larger than expected probabilities for very small and large displacements, indicative of particles being caged in the filament mesh and hopping between cages.

Different dynamic microscopy (DDM), which uses Fourier-space analysis to measure the timescales over which particle density fluctuations decay, has also been used to measure transport and quantify anomalous characteristics over larger spatiotemporal scales compared to SPT [5, 14, 15].

![FIGURE 1](image-url)

Combining light sheet microscopy with real-space single-particle tracking (SPT) and reciprocal-space differential dynamic microscopy (DDM) to characterize particle transport in active cytoskeletal composites. (A) We create composites of co-entangled microtubules (blue) and actin filaments (purple) driven out-of-equilibrium by myosin II minifilaments (green). We track the motion of embedded 1 μm beads (red) in composites with varying molar fractions of actomyosin, which we denote by the fraction of actin comprising the combined molar concentration of actin and tubulin (5.8 μM): $\phi_A = 0.25, 0.5, 0.75, 1$. In all cases, the molar ratio of myosin to actin is fixed at 0.08. (B) Schematic of the light-sheet microscope we use for data collection, which provides the necessary optical sectioning to capture dynamics in dense three-dimensional samples. (C) Example frame from time-series of 1 μm beads embedded in a cytoskeleton composite, used to characterize particle transport in active crowded systems. (D) Cartoon of expected mean-squared displacements (MSD) of embedded particles versus lag time $\Delta t$, which we compute via single-particle tracking (SPT) and fit to a power law $\text{MSD}^{\alpha}$ to determine the extent to which particles exhibit normal Brownian diffusion ($\alpha = 1$, blue), subdiffusion ($\alpha < 1$, red), or superdiffusion ($\alpha > 1$, green). (E) Cartoon of van Hove distribution $G$ of $x$- and $y$-direction particle displacements $\Delta d = \Delta x$ $\Delta y$ for a given lag time $\Delta t$ computed from SPT trajectories. The distribution shown is described by a sum of a Gaussian and exponential function $G(r, \Delta t) = A e^{-r^2/2\Delta t} + B e^{-r^2/\tau^2}$, as is often seen in crowded and confined systems and those that display heterogeneous transport. (F) Cartoon of expected characteristic decorrelation times $\tau(q)$ as a function of wave number $q$, which we compute by fitting the image structure function computed from DDM analysis. We determine the scaling exponent $\beta$ from the power-law $\tau(q) \sim q^{-\beta}$ to determine if transport is diffusive ($\beta = 2$, blue), subdiffusive ($\beta > 2$, green), or ballistic ($\beta = 1$, red).
DDM analysis of a time-series of images provides a characteristic decay time $\tau$ as a function of the wave vector $q$ which typically follows power-law scaling $\tau(q) \sim q^{-\beta}$ [15, 16], with $\beta$ relating to the anomalous scaling exponent $\alpha$ via $\beta = 2/\alpha$. Specifically, $\beta = 2$, $>2$, <2 and 1 correspond to diffusive, subdiffusive, superdiffusive, and ballistic motion (Figure 1F). DDM analysis of actin-microtubule composites corroborated the SPT results described above, with subdiffusive $\beta$ values tracking with $\alpha$ values [5, 6]. However, we note that while the relation $\beta = 2/\alpha$ can be used to directly compare dynamics measured via SPT and DDM, $\alpha$ derived from this relation may not match that determined from SPT, in particular for systems that display multiple dynamic regimes at different spatiotemporal scales.

Similar SPT and DDM experiments demonstrated that crosslinking of actin and/or microtubules introduced bi-phasic transport with the subdiffusive scaling exponents dropping from $\alpha = 0.5$–0.7 to $\alpha = 0.25$–0.4 (depending on crosslinker type) after $\Delta t = 3$ s, due to strong caging and reduced thermal fluctuations of filaments. At the same time, van Hove distributions were well fit to a sum of a Gaussian and exponential, and the non-Gaussianity parameter increased, indicating enhanced heterogeneity [5, 6, 8, 12, 17].

Numerous studies have also investigated transport in non-equilibrium cytoskeleton networks, in which activity is introduced via motor proteins, such as actin-associated myosin II and microtubule-associated kinesin [2, 10, 18–21]. These studies have shown evidence of vesicle movement strongly tracking with actin movement, microtubule-dependent flow, and the simultaneous presence of subdiffusive and ballistic transport dynamics. While the majority of these active matter studies have been on systems of either actin or microtubules, recent studies have used DDM and optical tweezers microrheology to characterize the dynamics of actin-microtubule composites pushed out-of-equilibrium by myosin II minifilaments straining actin filaments [14, 22, 23]. These studies showed that active actin-microtubule composites exhibited ballistic-like ($\alpha = 2$) contractile motion, rather than randomly-oriented diffusion or subdiffusion, with speeds that increased with increasing fraction of actin in the composites, due to increased composite flexibility [14, 23]. Myosin-driven contractile motion and restructuring was also reported to increase viscoelastic moduli and relaxation timescales and induce clustering and increased heterogeneity of the initially uniform mesh [22].

However, particle transport through active actin-microtubule composites—likely dictated by the complex combination of active processes, crowding, and interactions between mechanically distinct filaments—has remained largely unexplored. The majority of studies that have examined the combined effect of activity and crowding have been in vivo [1, 24–29], where a large number of confounding variables that are difficult to tune make teasing out the effect of each contribution and mechanism highly non-trivial.

Here, we take advantage of the tunability of in vitro reconstituted cytoskeleton composites to systematically investigate the coupled effects of non-equilibrium activity, crowding, and heterogeneity on particle transport. We combine fluorescence light sheet microscopy (fLSM) with single-particle tracking (SPT) and differential dynamic microscopy (DDM) to examine the anomalous transport of micron-sized particles within active composites of myosin II minifilaments, actin filaments, and microtubules with varying molar fractions of actin and tubulin (Figure 1A). We leverage the optical sectioning and low excitation power of fLSM (Figure 1B) to capture particle trajectories with a temporal resolution of 0.1 s for durations up to 400 s (Figure 1C). Using both SPT and DDM provides transport characterization over a broad spatiotemporal range that spans $10^{-1}$–$10^2$ s and $10^{-1}$–$10^{-14}$ μm. From measured SPT trajectories, we compute ensemble-averaged MSDs and associated anomalous scaling exponents $\alpha$ (Figure 1D), as well as corresponding distributions of particle displacements, i.e., van Hove distributions (Figure 1E), for varying lag times $\Delta t$. To expand the spatiotemporal range of our measurements and provide an independent measure of transport characteristics, we use DDM to analyze particle density fluctuations in Fourier space, and evaluate the power-law dependence of decorrelation times $\tau$ on wave vector $q$, i.e., $\tau(q) \sim q^{-\beta}$ (Figure 1F).

2 Materials and methods

2.1 Protein preparation

We reconstitute rabbit skeletal actin (Cytoskeleton, Inc. AKL99) to 2 mg/ml in 5 mM Tris–HCl (pH 8.0), 0.2 mM CaCl$_2$, 0.2 mM ATP, 5% (w/v) sucrose, and 1% (w/v) dextran; porcine brain tubulin (Cytoskeleton T240) to 5 mg/ml in 80 mM PIPES (pH 6.9), 2 mM MgCl$_2$, 0.5 mM EGTA, and 1 mM GTP; and rabbit skeletal myosin II (Cytoskeleton MY02) to 10 mg/ml in 25 mM PIPES (pH 7.0), 1.25 M KCl, 2.5% sucrose, 0.5% dextran, and 1 mM DTT. We flash freeze all proteins in single-use aliquots and store at -80°C. We reconstitute the UV-sensitive myosin II inhibitor, (-)-blebbistatin (Sigma B0560) in anhydrous DMSO and store at -20°C for up to 6 months. Immediately prior to experiments, we remove enzymatically dead myosin II from aliquots using centrifugation clarification, as previously described [14, 22].

2.2 Composite network assembly

We prepare actin-microtubule composites by mixing actin monomers, tubulin dimers and a trace amount of 1 μm diameter carboxylated microspheres (Polysciences) in PEM-100 (100 mM PIPES, 2 mM MgCl$_2$, and 2 mM EGTA), 0.1% Tween-20, 1 mM ATP, and 1 mM GTP, and incubating at 37°C for 30 min to allow
for polymerization of actin filaments and microtubules. We coat microspheres (beads) with AlexaFluor594 BSA (Invitrogen) to visualize the particles and prevent nonspecific interactions with the composite [30, 31]. We fix the combined molar concentration of actin and tubulin to c = cA + cT = 5.8 µM and the ratio of myosin to actin to R = 0.08, and vary the molar fraction of actin in the composite (cA/c = ϕA) from ϕA = 0 to 1 in 0.25 increments (Figure 1A). To stabilize actin filaments and microtubules, we add an equimolar ratio of phalloidin to actin and a saturating concentration of Taxol (5 µM) [32, 33]. We add an oxygen scavenging system (45 µg/ml glucose, 0.005% β-mercaptoethanol, 43 µg/ml glucose oxidase, 7 µg/ml catalase) to inhibit photobleaching, and add 50 µM blebbistatin to control actomyosin activity.

2.3 Sample preparation and imaging

We pipet prepared composites into capillary tubing with an inner diameter of x=800 µm and length of y=10 mm, then seal with epoxy. Microspheres are imaged using a custom-built flSM with a 10× 0.25 NA Nikon Plan N excitation objective, a 20× 1.0 NA Olympus XLUMPlanFLN detection objective, and an Andor Zyla 4.2 CMOS camera [5]. A 561 nm laser is formed into a sheet to image the microspheres, while a collimated 405 nm laser is used to deactivate the blebbistatin, thereby activating actomyosin activity. Each acquisition location is at least 1 mm away from the previous one to ensure that there is no myosin activity when the image acquisition begins. For SPT, we collect ≥15 time-series consisting of ≥2000 frames, each with a 1000×300 pixel (194×58 µm²) field of view (FOV), at 10 frames per second (fps). For DDM, we collect ≥3 time-series of ≥4000 frames, each with a 768×266 pixel (149×52 µm²) FOV, at 10 fps. The x and y axes of each FOV are aligned with the cross-section and length of the capillary sample chamber, respectively.

2.4 Single-particle tracking

We use the Python package Trackpy [34] to track particle trajectories and measure the x- and y- displacements (Δx, Δy) of the beads as a function of lag times Δt. From the particle displacements, we use a custom-written Python script to calculate the time-averaged mean-squared displacement of the ensemble, \( MSD(\Delta t) = \frac{1}{2} \left( \langle \Delta x(\Delta t) \rangle^2 + \langle \Delta y(\Delta t) \rangle^2 \right) \), from which we compute anomalous scaling exponents, \( a \), via MSD –Δt with each Δt range in which we observe a single power-law. We compute MSDs for lag times Δt ≤ 180 s, but limit our power-law fitting to Δt ≤ 40 s for ϕA = 0 and Δt ≤ 100 s for ϕA = 0.75 and 1, as noise from low statistics dominates the data at larger lag times for these composites.

Additionally, we compute van Hove probability distributions of particle displacements, \( G(\Delta d, \Delta t) \) (Figure 1E), where \( \Delta d = \Delta x \cup \Delta y \), for 10 different lag times that span Δt = 0.1 s to 15 s. Following previous works [5–7], we fit each distribution for a given lag time to a sum of a Gaussian and exponential function: \( G(\Delta d) = A \exp(-\Delta d^2/2\sigma^2) + B \exp(-\Delta d/\lambda) \), where \( A \) is the amplitude of the Gaussian term, \( \sigma^2 \) is the variance, \( B \) is the amplitude of the exponential term, and \( \lambda \) is the exponential decay constant.

2.5 Differential dynamic microscopy

We obtain the image structure function \( D(q, \Delta t) \), where \( q \) is the magnitude of the wave vector, following our previously described methods [12, 35]. We fit each image structure function, or DDM matrix, to the following function:

\[
D(q, \Delta t) = A(q) \left[ 1 - \exp \left( -\frac{(\Delta d/\tau(q))^q}{\Delta t} \right) \right] + B(q)
\]

where \( \tau(q) \) is the density fluctuation decay time, \( q \) is the stretching exponent, \( A \) is the amplitude, and \( B \) is the background [5, 6]. In practice, we fit each \( D(q, \Delta t) \) out to \( \Delta t = 100 \) s, beyond which noise due to low statistics dominates the signal. From the fits, we determine the \( q \)-dependent decay time \( \tau(q) \), which is a measure of the timescale over which particle density fluctuations decorrelate over a given lengthscale \( l = 2\pi/q \). By fitting \( \tau(q) \) to a power-law (i.e., \( \tau(q) = \tau^q \)) we determine the dominant mode of transport, with \( \beta = 2, >2, and <2 \), indicating normal Brownian diffusion, subdiffusion and superdiffusion, respectively. We fit \( \tau(q) \) for \( q = 1 − 4 \mu m^{-1} \), with the (i) upper and (ii) lower bounds set by the (i) optical resolution of our setup and (ii) finite image size and \( \Delta t \) range over which we fit \( D(q, \Delta t) \), respectively. These effects lead to (i) unphysical upticks in \( \tau(q) \) curves for \( q > 4 \mu m^{-1} \) and (ii) \( q \)-independent plateaus for \( q < 1 \mu m^{-1} \) (Supplementary Figure S1).

We also examine the stretching exponent \( \gamma(q) \) that we extract from fitting \( D(q, \Delta t) \) as another transport metric, with \( \gamma(q) < 1 \) indicative of confined and heterogeneous dynamics [5, 36–38] and \( \gamma(q) > 1 \) indicative of active ballistic-like motion [14, 39–41]. Finally, we evaluate the \( q \)-dependence of \( \gamma(q) \) to ensure that the expected insensitivity to \( q \), necessary for unequivocal determination and interpretation of the scaling exponent \( \beta \), holds for all cases (Supplementary Figure S2).

3 Results and discussion

To elucidate the combined effects of non-equilibrium activity and steric hindrance on particle transport in crowded active matter, we leverage the tunability of reconstituted cytoskeleton composites [42] and the power of coupling real-space (SPT) and
reciprocal space (DDM) transport analysis, to robustly characterize particle transport as a function of active substrate content. Specifically, we tune the composition of actomyosin-microtubule composites to display a wide range of transport characteristics by varying the molar fraction of actomyosin, which we denote by the molar actin fraction $\phi_A$, keeping the myosin molarity fixed at 8% of $\phi_A$ (Figure 1A, Methods).

In Figure 2A, we plot the ensemble-averaged MSD as a function of lag time $\Delta t$ for particles diffusing in composites of varying $\phi_A$. While $\phi_A = 0$ (no actomyosin) exhibits subdiffusive transport across the entire $\Delta t$ range, with $\alpha = 0.67$, all $\phi_A > 0$ composites display multi-phasic transport which is subdiffusive ($\alpha_1 < 1$) at short $\Delta t$ and superdiffusive ($\alpha_2 > 1$) at long $\Delta t$. To more clearly show the transition from subdiffusion to superdiffusion, we plot $\text{MSD}/\Delta t$ versus $\Delta t$ (Figure 2B), which is a horizontal line for normal Brownian diffusion with the $\Delta t$-independent magnitude proportional to the diffusion coefficient. Positive and negative slopes correspond to superdiffusion and subdiffusion, respectively, with $\text{MSD}/\Delta t \sim \Delta t^{\alpha_1}$ indicating ballistic motion. Corresponding $\text{MSD}/\Delta t$

**FIGURE 2**

Actomyosin activity in actin-microtubule composites endows multi-phasic particle transport marked by pronounced subdiffusion at short lag times and superdiffusion at long lag times. (A) Mean-squared displacements (MSD) plotted versus lag time $\Delta t$ for myosin-driven actin-microtubule composites with molar actin fractions of $\phi_A = 0$ (blue), 0.25 (gold), 0.50 (green), 0.75 (red), and 1 (purple). Fits of the data to $\text{MSD} \sim \Delta t^\alpha$ over different $\Delta t$ ranges (shown in (B)) determine the anomalous scaling exponents $\alpha_i$ that describe the dynamics (see Figure 1). (B) Mean-squared displacements scaled by lag time ($\text{MSD}/\Delta t$) plotted versus lag time $\Delta t$ delineate regions of subdiffusion (negative slopes) and superdiffusion (positive slopes). Color coding is according to the legend in (A). Black lines indicate fits to $\text{MSD} \sim \Delta t^\alpha$ over the short ($\Delta t < \Delta t_1$), long ($\Delta t > \Delta t_2$) and intermediate ($\Delta t_1 < \Delta t < \Delta t_2$) time regimes where each curve is well-fit by a single power law. (C) Data shown in B plotted versus actin fraction $\phi_A$, with all MSD/$\Delta t$ values for each $\phi_A$ plotted along the same vertical, with the gradient indicating increasing $\Delta t$ from light to dark. The magnitude of each data point is proportional to a transport rate, with higher values indicating faster motion. (D) Lag time at which each composite transitions out of initial subdiffusive transport ($\Delta t_1$) and transitions into superdiffusivity ($\Delta t_2$). (E) Anomalous scaling exponents $\alpha_i$ derived from fits shown in (B) for $\Delta t < \Delta t_1$ ($i = 1$), $\Delta t > \Delta t_2$ ($i = 2$), and ($\Delta t_1 < \Delta t < \Delta t_2$) ($i = 1, 2$). Dashed line at $\alpha = 1$ represents scaling indicative of normal Brownian diffusion. Values above and below the line indicate superdiffusion and subdiffusion, respectively. Note that only $\phi_A = 0.25$ and 0.5 composites exhibit an intermediate $\alpha_{1,2}$ regime. For both (C) and (D) error bars indicate standard error of the mean. Color-coding in all subfigures matches the legend in (A).
magnitudes are proportional to \(\Delta t\)-dependent transport coefficients. While all \(\phi_j > 0\) composites exhibit similar general trends with \(\Delta t\), the lag time at which the dynamics transition from subdiffusive to superdiffusive, along with the degree to which \(a_1\) and \(a_2\) deviate from 1, depend non-trivially on \(\phi_A\). Moreover, as clearly seen in Figure 2C, while \(a\) values for active composites (\(\phi_A > 0\)) transition to superdiffusive at longer lag times, the magnitudes of the transport coefficients remain nearly an order of magnitude smaller than that of the inactive network (\(\phi_A = 0\)) at any given \(\Delta t\).

To evaluate the \(\phi_A\)-dependence of the multi-phasic behavior, we first compute the lag times at which composites transition out of initial subdiffusive scaling, denoted as \(\Delta t_1\), and transition into superdiffusive scaling, denoted as \(\Delta t_2\). We note that the MSFs for \(\phi_A = 0.75\) and 1 both exhibit a continuous transition from initial subdiffusion (quantified by \(a_1\)) to a final superdiffusive regime (quantified by \(a_2\)), seen as a smooth concave-up curve between the two power-law regimes. Conversely, MSFs for \(\phi_A = 0.25\) and 0.5 exhibit an extended power-law regime with weakly subdiffusive scaling exponent \(a_{12}\). To quantify \(\Delta t_1\), we compute the largest lag time for which linear regression of log MSD versus log \(\Delta t\) over the range \(\Delta t \in [0.1 s, \Delta t_1]\) yields \(R^2 > 0.99\). We define \(\Delta t_2\) as the lag time at which MSD/\(\Delta t\) exhibits a local minimum (i.e. where \(a\) crosses over from <1 to >1) (Figure 2C). We find that both timescales decrease with increasing \(\phi_A\) as does the separation between the timescales (\(\Delta t_2 - \Delta t_1\)), suggesting that the rate of particle motion mediated by directed motor-driven network dynamics increases with increasing \(\phi_A\). In other words, the time it takes for the active dynamics to be ‘felt’ by the particles, driving them out of their confined subdiffusive motion, decreases with increasing \(\phi_A\).

To understand this phenomenon, we consider that active ballistic transport would only be detectable at timescales in which the network motion can move a bead more than the minimum resolvable displacement: \(\Delta t_A \approx (100 nm)/(network\ speed)\). Using reported speed values of \(v \approx 2.2 - 85 nm/s\) for similar myosin-driven composites [23], we compute \(\Delta t_A \approx (100 nm)/v = 1 - 50s\), aligning with our \(\Delta t_1\) and \(\Delta t_2\) values, and thus corroborating that the deviation from subdiffusion and transition to superdiffusion is due to myosin-driven ballistic motion. Moreover, the previously reported speeds generally decreased with decreasing \(\phi_A\), such that \(\Delta t_A\) should increase as \(\phi_A\) decreases, just as we see in Figure 2B.

To determine the extent to which motor-driven transport and confinement contribute to the particle dynamics, we next evaluate the anomalous scaling exponents in the short, intermediate, and long \(\Delta t\) regimes by performing power-law fits to the MSFs in each regime (Figures 2B,E). Surprisingly, the scaling exponents in the \(\Delta t < \Delta t_1\) regime for all active composites (\(\phi_A > 0\)) are markedly smaller (more subdiffusive) than the inactive composite (\(\phi_A = 0\)), with \(\phi_A\) dependent values of \(a_1 = 0.29 - 0.37\) compared to \(a_1 = 0.68\) for the \(\phi_A = 0\) network. To understand the decrease in \(a_1\) with increasing \(\phi_A\) for the active composites, as well as the unexpected ~2-fold reduction in \(a_1\) for active composites, we turn to previous studies [5, 6], that reported that, in the absence of any crosslinking, steady-state actin-microtubule composites exhibit subdiffusion with scaling exponents that decrease from \(\alpha = 0.82\) to \(\alpha = 0.56\) as \(\phi_A\) increases from 0 to 1. This monotonic ~30% decrease with increasing \(\phi_A\), similar to the ~25% decrease we observe with increasing \(\phi_A\), was suggested to arise from increased composite mobility that entrains the bead motion as rigid microtubules are replaced with semiflexible actin filaments [6, 12]. This mobility is paired with a decreasing mesh size as \(\phi_A\) increases, which, in turn, increases composite viscoelasticity and particle confinement, both of which contribute to decreasing \(\alpha\) [6].

To understand the lower \(\alpha_1\) values we measure, compared to those previously reported for steady-state composites, we look to previous studies on \(\phi_A = 0.5\) actin-microtubule composites with varying types of static crosslinking. In these studies, subdiffusion is much more extreme (\(\alpha = 0.33\)) when actin filaments are crosslinked to each other compared to no crosslinking (\(\alpha = 0.64\)) [5]. Taken together, our results suggest that the ~2-fold reduction in \(\alpha_1\) between \(\phi_A = 0\) and \(\phi_A > 0\) composites likely arises from myosin motors acting as static crosslinkers on timescales shorter than the timescale over which they can actively translate the composite. As described above, myosin acting as a static crosslinker for \(\Delta t < \Delta t_1\) is consistent with previously reported speeds for myosin-driven composites [5, 6], as well as reported actomyosin turnover rates [24]. The weak decrease in \(\alpha_1\) with increasing \(\phi_A\) likely arises from the decreasing mesh size and increasing mobility of the network as \(\phi_A\) increases [43], as described above.

Examining the long-time regime, \(\Delta t > \Delta t_2\), our results show that the scaling exponent \(\alpha_1\) is largely insensitive to \(\phi_A\) for all composites (i.e., \(0 < \phi_A < 1\)) with an average value of \(\alpha_2 = 1.5\), only modestly lower than \(\alpha_2 = 1.8\) measured for \(\phi_A = 1\). However, the lag time at which superdiffusive \(\alpha_2\) scaling emerges is an order of magnitude larger for \(\phi_A = 0.25\) and 0.5 composites compared to \(\phi_A > 0.5\). Moreover, \(\phi_A = 0.25\) and 0.5 composites display nearly identical intermediate subdiffusive scaling regimes, absent for \(\phi_A > 0.5\), with an average scaling exponent of \(\alpha_{12} = 0.83 \pm 0.2\). Taken together, these results suggest that the timescale over which motor-driven dynamics dominates particle transport scales with the fraction of active substrate [23], while the superdiffusive signature of the active transport is controlled by the motorsubstrate ratio (which we hold fixed across composites).

To shed further light on the mechanisms underlying the anomalous transport shown in Figure 2, we compute van Hove distributions \(G(\Delta d, \Delta t)\), where \(\Delta d = \Delta x \cup \Delta y\), for two decades of lag times (\(\Delta t = 0.1 - 15 s\)) (Figure 3A). From the distributions, we first compute anomalous scaling exponents \(\alpha\), to corroborate our MSD analysis, by recalling that the full width at half maximum, FWHM, for a Gaussian distribution scales with...
Asymmetric non-Gaussian van Hove distributions reveal a combination of heterogeneous subdiffusion and advective transport of particles in active composites. (A) van Hove distributions $G(\Delta d, \Delta t)$ of particle displacements $\Delta d = \Delta \xi_1 + \Delta \xi_2$, measured via SPT, for lag times $\Delta t = 0.1, 0.2, 0.5, 1, 2, 3, 5, 10, 15$ s denoted by the color gradient going from light to dark for increasing $\Delta t$. Each panel corresponds to a different composite demarked by their $\phi_A$ value with color-coding as in Figure 2. (B) The square of the full width at half-maximum ($FWHM^2$) versus lag time $\Delta t$ for each composite shown in (A). Solid lines are fits to $FWHM^2 \sim \Delta t^\alpha$. For $\phi_A > 0$ composites we fit short ($\Delta t \leq 1$ s) and long ($\Delta t \geq 1$ s) lag time regimes separately. (C) The scaling exponents $\alpha$ as functions of $\phi_A$ determined from the fits shown in B, where $\alpha_1$ (stars) and $\alpha_2$ (triangles) correspond...
Conversely, informed by the multi-phasic short (0 for each active composite to separate power-law functions over FWHM (Figures 3B,C, and reported for steady-state actin-microtubule composites [6], Δ power-law, with of treating all MSD of displacements. This non-Gaussianity, seen in other crowded complex, with asymmetries and peaks at FWHM for steady-state actin-microtubule composites [5, 7]. In G(diam, Δt) for φi = 0 fits well to a single power-law, with α = 0.7, nearly indistinguishable from that computed from the MSD, across the entire Δt range. Conversely, informed by the multi-phasic MSD scaling we observe for active composites (Figure 2C), we fit FWHM for each active composite to separate power-law functions over short (0.15 s < Δt < 1 s) and long (1 s s < Δt < 15 s) lag times, relative to the average Δt, we determine from MSD fits.

Further, similar to the φi dependence of αi and α2 values determined from MSDs, the scaling exponents determined from FWHM, increase with increasing φi, with αi (for Δt < 1 s) increasing from ~0.62 to ~0.85, similar to values reported for steady-state actin-microtubule composites [6], and α2 (for Δt > 1 s) spanning from subdiffusive to superdiffusive. Higher α2 values compared to those determined from MSDs are likely due to the lower Δt resolution in FWHM fitting and the single Δt value used to divide the two regimes. Likewise, the lower α2 values for φi = 0.25 and 0.5 compared to those measured from MSDs are a result of treating all Δt > ΔtΔ data as obeying a single power-law rather than separate αiΔ and α2 scaling.

While our analysis described above assumes Gaussian distributions, Figure 3A shows that nearly all distributions have distinct non-Gaussian features similar to those reported for steady-state actin-microtubule composites [5, 7]. In particular, G(Δd, Δt) distributions for the inactive network (φi = 0) exhibit pronounced exponential tails at large displacements. This non-Gaussianity, seen in other crowded and confined soft matter systems [7], is a signature of heterogeneous transport and can also indicate caging and hopping between cages.

The distributions for active composites are even more complex, with asymmetries and peaks at Δd ≠ 0 (Figure 3A), not readily predictable from our MSD analysis. The first interesting feature we investigate is the non-zero mode value Δdpeak that increases in magnitude with increasing Δt, indicating directed ballistic-like motion, thereby corroborating our superdiffusive scaling exponents. Perhaps less intuitive is the robust asymmetry between the ‘leading (+) edge’ and ‘trailing (-) edge’ of each distribution, which we define by splitting each distribution about its peak, Δdpeak. Specifically, the leading edge is the part of the distribution that has displacements of the same sign as Δxpeak and greater in magnitude, while the remaining part is the trailing edge (Figure 3D). We observe that for most distributions the leading edge appears more Gaussian-like while the trailing edge exhibits pronounced large-displacement ‘tails’. To broadly quantify this asymmetry, we evaluate the half-width at half-maximum (FWHM) for the leading (+) and trailing (-) edges of each distribution and compute the percentage increase in FWHM for the trailing versus leading edge: ΔsFWHM = (FWHM – FWHM) / FWHM. (Figure 3E).

We find that ΔsFWHM is positive for all active composites and increases with increasing φi, demonstrating that the asymmetry is a direct result of active composite dynamics which contribute more to the transport as the actomyosin content increases.

We also note that the asymmetry shown in Figure 3A is always in the negative direction (to the left). To determine the directionality of this asymmetry we evaluate the distributions of Δx and Δy values separately (Supplementary Figure S3), which show that the asymmetry for G(Δy, Δt) is more pronounced and negative compared to the smaller positive anisotropy seen in G(Δx, Δt). This bias is likely due to the geometry of the sample chamber that has a y-direction length that is 10× the x-direction width of the chamber.

To determine if this directionality is a reproducible microscale bias or is an ensemble result of averaging over trials with positive and negative anisotropies, we evaluate the van Hove distributions for each trial of the φi = 1 case (that exhibits the most pronounced asymmetry) (Supplementary Figure S4). We observe that nearly all G(Δx, Δt) and G(Δy, Δt) distributions skew to +x and −y, respectively, with G(Δy, Δt) asymmetries being more pronounced, such that G(Δd, Δt) skews in the negative direction. This systematic effect corroborates our understanding that the directional bias is due to the sample chamber geometry that provides more freedom in the y-direction for the composite to restructure and flow.

To more quantitatively characterize the rich transport phenomena revealed in Figure 3, we first fit each G(Δd, Δt) to a sum of a Gaussian and an exponential (see Methods), as done for steady-state cytoskeleton composites [5–7]. Figure 4A compares the distributions and their fits for all composite formulations at Δt = 0.3 s (top panel) and 10 s (bottom panel),
and Figure 4B displays zoom-ins of the corresponding leading and trailing edges. As shown, while this sum describes the inactive network distributions reasonably well, it overestimates leading edge displacements and underestimates trailing edge displacements of the active networks (Figure 4B). This asymmetry suggests that the leading edges are more Gaussian-like and the trailing edges are more exponential-like. To account for this asymmetry, we fit each half of each distribution separately to a one-sided sum of a Gaussian and exponential and evaluate the relative contributions from the Gaussian and exponential terms. As detailed in the Methods, we denote the amplitude of the Gaussian term and exponential term as $A$ and $B$, respectively, such that their relative contributions are $a = A/(A + B)$ and $b = B/(A + B)$.

As shown in Figures 4C,D, in which $a$ and $b$ are normalized by the corresponding $\phi_A = 0$ value and plotted for each $\phi_A$, active composites are more Gaussian-like ($a(\phi_A = 0) > 1$) and less exponential ($b(\phi_A = 0) < 1$) than the inactive system for both leading and trailing edges. This result suggests that the active processes that induce contraction and flow of the composites likewise reduce transport heterogeneity and intermittent hopping, possibly by promoting mixing and advection. Consistent with this interpretation is the observation that the Gaussian contribution $a$ increases with increasing $\phi_A$ and is consistently larger for the leading edge, which consists of displacements oriented with the direction of the myosin-driven composite motion (Figure 4C).

Conversely, the increased contribution from the exponential term for the trailing edge, implies that displacements comprising these exponential tails are dominated by heterogeneous transport modes such as hopping between heterogeneously distributed cages [6, 7]. To better understand this effect, we recall that
FIGURE 5
DDM analysis reveals ballistic-like transport of particles entrained in active composites at mesoscopic spatiotemporal scales. (A) Sample image structure functions $D(q, \Delta t)$ for composites with actin fractions $\phi_A$ indicated in the legend. All curves shown are evaluated at $q = 3.92 \mu m^{-1}$, and solid black lines are fits to the data to determine corresponding $q$-dependent decay times $\tau(q)$ and stretching exponents $\gamma$, as described in Methods. (B) Decay times $\tau(q)$ for each composite shown in (A). Dashed and dotted black lines show scaling $\tau(q) \sim q^{-\beta}$ for ballistic ($\beta = 1$) and diffusive ($\beta = 2$). (Continued)
particle displacements comprising the trailing edge are lagging behind the bulk translational motion of the composite. As the composite moves and restructures, a fraction of the particles remain caged in the moving composite and thus move along with it, corresponding to displacements comprising the leading edge, whereas a fraction of the particles are squeezed out or hop out of composite cages and into new ‘trailing’ cages. It is also likely that as the composite contracts and forms more heterogeneous structures and larger open voids that characteristic ‘hopping’ displacements, as well as displacements withing cages, may increase and become more heterogeneous, thereby enhancing exponential tails.

To expand the range of length and time scales over which we can probe the non-equilibrium transport, and provide an independent measure of the transport rate, we complement our real-space SPT analysis with Fourier-space DDM analysis, as described in the Methods and previously [5, 6, 14]. Briefly, we compute the image structure function $D(q, \Delta t)$ of the Fourier transform of image differences as a function of wavevector $q$ and lag time $\Delta t$. From fits of $D(q, \Delta t)$ to a function with a stretched exponential term (see Methods, Figure 5A), we determine the $q$-dependent characteristic decay time $\tau(q)$ and stretching exponent $\gamma(q)$ for each composite (Figure 5), which characterize the dynamics. $\tau(q)$ typically exhibits power-law scaling $\tau(q) \propto q^{-\beta}$ where $\beta$ is related to the anomalous scaling exponent $\alpha$ via $\beta = 2/\alpha$, such that $\beta > 2$, $\beta = 2$, $\beta < 2$, and $\beta = 1$ correspond to, respectively, subdiffusive, normal diffusive, superdiffusive, and ballistic motion. Stretching exponents $\gamma(q)$ are typically independent of $q$ (see Supplementary Figure S2), with $q$-averaged values of $\gamma = 1$ for normal Brownian motion, $\gamma < 1$ for crowded and confined systems [15, 40] and $\gamma > 1$ for active transport [15, 45].

As shown in Figure 5B, $\tau(q)$ curves for all active composites follow scaling indicative of superdiffusive or ballistic transport while the $\phi_A = 0$ system more closely follows diffusive scaling. Further, $\tau(q)$ for $\phi_A = 0$ is an order of magnitude lower than for all active composites, indicating that particle transport is faster for the inactive composite, in line with our results shown in Figure 2C, despite the displacements exhibiting diffusive rather than ballistic-like motion. This effect can be more clearly seen in Figure 5D, which displays the $q$-dependent distribution of $\tau(q)^{-1}$ values, a measure of dynamic decorrelation rates, for each $\phi_A$. As shown, $\tau^{-1}$ values for $\phi_A = 0$ are an order of magnitude larger than those for $\phi_A > 0$ composites. Figure 5D also shows that decorrelation rates in active composites increase modestly with increasing $\phi_A$, suggesting that transport is dictated primarily by active restructurings and flow, rather than crowding and confinement, which increases as actomyosin content increases. The lack of subdiffusive scaling or crossovers from sub-to super-diffusive dynamics for active composites (as our SPT analysis shows) can be understood as arising from the larger length and time scales DDM probes. Namely, DDM spans length scales of $l = 2\pi q = 1.6 - 6.28 \mu m$ and timescales of $\tau = 20 - 100 s$ (Figure 5A) compared to the $0.1 - 1.5 \mu m$ and $0.1 - 100 s$ length and timescales accessible to SPT. As we describe in Methods, the lower bound on measurable length scales (upper bound on $q$) for DDM is set by the optical resolution limit of our setup, which is circumvented in SPT by using sub-pixel localization algorithms. At the same time, SPT is bounded at larger spatiotemporal scales by the duration of individual bead trajectories. The different scales that DDM and SPT are able to accurately probe is exactly the reason we use them both to fully characterize the dynamics of our system.

To better visualize differences in $\tau(q)$ scaling between composites we plot $\tau(q) \times q^2$ normalized by $\tau(q_{max}) \times (q_{max})^2$ (Figure 5C). Diffusive transport manifests as a horizontal line, as we see for $\phi_A = 0$, while ballistic-like motion follows a power-law scaling of 1, which roughly describes the $\phi_A > 0$ curves. To quantify the DDM scaling exponent $\beta$ that describes the dynamics, we fit each $\tau(q)$ curve to a power-law (i.e., $\tau(q) \propto q^{\beta}$) (Figures 5CE). For the active composites, we restrict our fitting range to $q > 1.5 \mu m^{-1}$, in which a single power-law is observed. For smaller $q$ values (larger length and time scales), we note that $\phi_A = 0.25$ and 1 composites exhibit roughly ballistic motion whereas $\phi_A = 0.5$ and 0.75 exhibit roughly diffusive dynamics (Figure 5C) [15, 16]. However, we restrict further quantification and interpretation of this small-$q$ regime, as it comprises relatively few data points and low statistics. Over the range that we fit our data, we find that $\beta \approx 2.02$ for the inactive composite, indicative of diffusive dynamics, whereas active composites exhibit near-ballistic values of $\beta = 1.03 - 1.26$, which are largely independent of $\phi_A$, similar to our $\alpha_2$ values measured via SPT (Figure 2E). To directly compare $\beta$ values to the anomalous scaling exponents $\alpha$ that we determine from SPT
However, our inactive system (evidence of this return due to the relatively slow active motors induce ballistic-like contraction, restructuring and concharacteristics have direct implications in key cellular processes. For instance, the competition between motor-driven active dynamics, crowding and connectivity—all of which increase with increasing actomyosin content. These intriguing transport characteristics have direct implications in key cellular processes. For instance, the competition between motor-driven active dynamics, crowding and connectivity—all of which increase with increasing actomyosin content. These intriguing transport characteristics have direct implications in key cellular processes. For instance, the competition between motor-driven active dynamics, crowding and connectivity—all of which increase with increasing actomyosin content. These intriguing transport characteristics have direct implications in key cellular processes. 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cytokinesis, polarization and mechano-sensing [23]. Moreover, our robust measurement and analysis toolbox and tunable active matter platform, along with the complex transport phenomena we present, are broadly applicable to a wide range of active matter and biomimetic systems of current intense investigation.

Data availability statement

The datasets generated and analyzed for this manuscript are available from the corresponding author on reasonable request.

Author contributions

JS analyzed and interpreted the data and wrote the paper; JG collected and helped analyze the data; SK and BL helped analyze data and prepare figures; RM conceived and guided the project, developed analysis code, helped analyze and help interpret the data, and helped write the paper; RR-A conceived and guided the project, interpreted the data, and wrote the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphy.2022.1055441/full#supplementary-material

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