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IMAGE ANALYSIS OF COLLOIDAL SYSTEMS FOR PAIR AND TRIPLET
CORRELATIONS

By

Brianna Price

B.S. University of Louisville, 2020

A Thesis

Submitted to the faculty of the

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A Thesis Approved on

April 9, 2021

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ABSTRACT

IMAGE ANALYSIS OF COLLOIDAL SYSTEMS FOR PAIR AND TRIPLET
CORRELATIONS

Brianna L. Price

March 29, 2021

Interactions among colloidal particles drive stability of suspensions or the assembly of particles into larger superstructures such as agglomerates or crystals. Typically, colloidal interactions are modeled using pairwise interaction potential energies which consider the interactions between pairs of particles. However, additional particles can modulate pair potentials, leading models to predict incorrect behavior. To analyze the effects of three-body interactions on the structuring of colloidal systems, pair and triplet correlation functions are calculated from published experimental data. These correlation functions are then translated into empirical pair and triplet potentials of mean force, which are then used to model systems more accurately by considering triplet energies. To extract data from images and videos and translate them into accurate potentials requires significant effort. Assisting the derivation of empirical potentials from microscopic media, analysis programs have been written and tested on published data and shared publicly via GitHub to be used and improved freely.

TABLE OF CONTENTS

ABSTRACT	iv
LIST OF FIGURES	vi
I. INTRODUCTION	1
i. Overview	1
ii. Colloids	4
iii. Purpose of Research	5
iv. Literature Review	6
II. THEORY	10
III. METHODS	16
IV. RESULTS AND DISCUSSION	20
V. CONCLUSION	40
REFERENCES	42
APPENDIX	45
APPENDIX A: VIDEO ANALYSIS CODE	45
APPENDIX B: IMAGE ANALYSIS CODE	45

LIST OF FIGURES

1. Contour plots of electrostatic potentials	9
2. Double layer charge on a colloid pair	11
3. Code flow path	16
4. Located frame 12 of colloidal videos from systems in Table 2	22
5. Particle location in colloidal videos.....	24
6. Pair distance correlation in Count vs. Pixel Distance	26
7. Pair free energy.....	28
8. Triplet distance correlation	31
9. Triplet triangle distance labels.....	32
10. Triangle distribution.....	34
11. Triplet free energy	36
12. Particle trajectories.....	39

I. INTRODUCTION

i. Overview

This thesis examines the interactions between colloidal particles. DLVO theory is the most common model used to rationalize colloidal behavior (Trefalt and Borkovec 2014). There are two main interactions between particles that are described by DLVO theory. The two main forces between particles are the Van der Waals attractive forces and electrostatic double layer repulsive forces. The electrostatic double layer forces can be described as two layers of charges on a colloid. Take an example of a positively charged colloidal particle. The double layer arises due to the positive charge of colloids attracting electrons carried by negative ions to form a barrier around the colloid. These negative charges repel each other, leading particles to disfavor being within certain distances of one another. However, DLVO theory only consider the interactions between two particles and does not account for how a third particle would affect the interparticle forces. In fact, according to other theories, such as Poisson-Boltzmann, a third charged particle should change the local electric field around two interacting particles, leading to shortcomings in DLVO theory (Denton 2010).

Given sufficient data, interaction potentials near equilibrium can be derived from images of colloidal systems (Adair 2001). It is known that the

probability of finding a specific structure is related to its free energy. Therefore, analyzing images or videos of colloidal systems can lead to empirical interaction potentials. Typically, analysis begins with a radial distribution function which is also known as the pair correlation function. The pair correlation function describes the local density over a bulk density for concentric discrete circles or spheres centered around particles. Pair correlation functions can be inverted to potentials of mean force, which can be thought of as free energies. Assuming that the potential of mean force is dominated by the interaction potential, it becomes simple to approximate pair interaction potentials among well-ordered colloidal particles at equilibrium, provided enough data.

Similar analysis can be undertaken for triplet correlation functions (Pallewela, Ploetz et al. 2018) . To account for the third particle's interaction, a triplet distance was found by adding the distances between three particles and was then plotted in a histogram to find the average total distance between three particles. The distances between the three particles were then used to create a distribution plot. This plot, inspired by the contour diagrams that can be seen in *"Three-particle correlation functions of quasi-two-dimensional one-component and binary colloid suspension"*, held one of the distances steady at a certain pixel distance range, and then the other two lengths of the triplet triangle were iterated over their possible distances, with their intersection point being plotted as pixel location (Ho, Lin et al. 2006). This plot was then used to find the number of triangles in the system, which when divided by the change in volume of the system, gave the triplet correlation function. This correlation function was then

plugged into the free energy equation to give the triplet free energy. In combination with the pair energy, this triplet energy in theory provides a better descriptor/predictor of particle interactions and structuring. In fact, it has been shown that by considering three-body interactions, which are commonly on the same order of magnitude as two-body interactions, predicted colloidal phase diagrams shift significantly (Hynninen, Dijkstra et al. 2003).

In addition to the free energies calculated from colloidal structures, trajectories of the particles and their mean squared displacement were also calculated. The mean square displacement can be used to characterize particle diffusion and viscosity.

Potentials of mean force tell us where the particles in the system “want” to be with respect to each other. The dips in the graphs are where the particles want to be and can be attributed to dominant Van der Waals forces in the system as that distance, and any peaks in the graphs are distances where the particles do not want to be indicating that electrostatic forces are stronger at that distance. The dips and peaks in the triplet free energy plot can typically be seen at pixel distances of approximately three times the distance of the pair free energy plot. This is due to the addition of the three lengths between the triplet particles being added together. This difference shows that when a third colloids interparticle forces are being included in the free energy, the distances where the particles likely are located increases, mainly due to the extra repulsive electrostatic forces.

All of the above-described analysis has been combined into a code designed for the easy input of colloidal images or videos to determine the

interparticle properties. While the code involving the pair correlations, the trajectory and the mean square displacement were all sourced from online sites, the code for the triplet correlations was created for this project. This new code was combined with the previously written code and posted on GitHub to allow for the easy access and analysis of colloidal systems by future researchers.

ii. Colloids

A colloid is defined as a particle with a linear dimension between 1 μm to 1 nm (Hiemenz 1997), and are found “dispersed throughout a continuous medium” (He 2014). Colloids, or colloidal suspensions, are comprised of two phases, and are often found as particles in the solid phase found in a liquid medium. However, they can be found in any combination of the three phases. Table 1 below contains examples of the different phase combinations of colloidal solutions.

Table 1

“Descriptive Names Used to Designate Two-Phase Colloidal Systems.”
Reproduced from (Hiemenz 1997)

Continuous Phase	Dispersed Phase	Descriptive Names
Gas	Liquid	Fog, mist, aerosol
Gas	Solid	Smoke, aerosol
Liquid	Gas	Foam
Liquid	Liquid	Emulsion
Liquid	Solid	Sol, colloidal solution, gel, suspension
Solid	Gas	Solid Foam
Solid	Liquid	Gel, solid emulsion
Solid	Solid	Alloy

Due to the varied phases of colloids, they can be used in many applications. In the environment “colloids play an important role in the transfer of nutrients and pollutants in the environment over short and long distances” (Shand 2011). Colloids are also found in industrial and consumer products like toothpaste, lotion, lubricants, and coatings. The colloids in these cases are used as thickening agents to achieve “desirable flow properties” as well as stabilization to “prevent phase separation” (Lower 2020). Colloids are also found in food products like milk, ice cream, eggs, and whipped cream (Lower 2020).

A key feature of the colloidal systems is that “the contact area between particles and dispersing medium is large” causing the suspension to be affected by interparticle interactions (He 2014). These interparticle interactions have an effect on the properties of the materials. These affects include stability, shelf life, behavior, and the rheology of the colloidal system (He 2014).

iii. Purpose of Research

The purpose of this research is to aid in the analysis of various videos and images of colloidal suspensions by finding the pair and triplet correlations of a given system. A Python code has been created which will determine the radial distribution, pair distances, triplet distances, and displacement of these colloidal systems. These analyses serve to better understand how colloids are moving, how much they are moving, and in what density they are found.

iv. Literature Review

Several experimental colloidal structures were collected from literature to develop and test analysis programs. Colloids have a history of being studied for their versatility in many fields. A popular field of study is how colloidal systems move and react to various stimuli. One study focuses on how colloids assemble in concurrent electric and magnetic fields (Bharti, Kogler et al. 2016). This study analyzes how colloids form into chains and then 2-dimensional crystals with increasing exposure to electromagnetic fields. Another study looks at freeform colloidal assembly, or “centimeter-scale, free-standing colloidal structures” (Tan, Beroz et al. 2018). Structures made prior to this study were formed by “planar confinement to the evaporative assembly process” to achieve large area films for electronic purposes, and by the confinement of colloids within liquid droplets leading to assembly upon evaporation to yield spherical clusters for optical applications (Tan, Beroz et al. 2018). However, with these techniques, the geometry is limited to planar shapes. Tan and coworkers examine a newer fabrication technique called direct ink writing. This technique can build “miniature antennae, light weight composites, batteries, and many other functional structure from metallic, dielectric, polymer, and biomaterial inks” (Tan, Beroz et al. 2018). The researchers adjust this technique and combine it with the advantages of evaporative colloidal assembly to create a technique call direct-write colloidal assembly. This approach achieves local control of the particle organization and the global control of the shape of the structure (Tan, Beroz et al. 2018).

A type of structure that can be formed by colloids are micromotors. These are “tiny engines that move in fluids, powered by chemical reactions or by external energy in the form of light, heat, ultrasound, or magnetic induction” (Wang, Duan et al. 2015). In chemical reactions the micromotors can create “local concentration gradients by consuming reactants and generating products”. These chemical gradients can cause particle interactions, both long-range and short-range.

These colloid patterns are dependent on the interactions between the colloids. Different interactions affect what structures the colloids will prefer. Interactions that are responsible for colloidal assembly and stability can be described by equations based on the DLVO theory, with varying degrees of accuracy.

DLVO theory describes the Van der Waals and electrostatic forces that are present within a colloid system. The theory is dependent on the assumption that the electrostatic forces are acting in a double layer with the Van der Waals forces being independent, meaning both forces are acting simultaneously. Due to this the forces can be superimposed at each interacting distance between two colloids. DLVO theory is therefore a good estimate of the intersurface forces in surface separations. However, this theory is only accurate for surface separations down to about 5 nm (Adair 2001).

In the system, fluctuations in the ionic strength will control the range of the double layer interactions, whereas the Van der Waals forces do not react to the ionic strength. The Van der Waals attraction is a negative inverse power law

function of the system separation, and the double layer force will decay exponentially with the separation and depends on the ionic strength of system. The combination of these two forces will give the total interaction energy at a particular distance (Adair 2001).

While DLVO is a good approximation of the forces in a colloidal system it does have some weaknesses. One large weakness is the neglect of adding a third particles forces into the equation. When a third particle interacts with a pair, its Van der Waals and double layer force will interact with existing pair of colloids at close distances. A better way to describe the forces in a colloidal system is described by Brunner and coworkers in “*Direct Measurement of Three-Body Interactions amongst Charged Colloids*” (Brunner, Dobnikar et al. 2004). Brunner describes how to determine the forces in a colloidal system by looking at the three particle interactions. This theory is based largely on this formalism. Figure 1 shows the electrostatic potentials between a pair and a triplet (Dobnikar, Brunner et al. 2018). In Figure 1a the colloids are $2.5 \mu m$ apart, and in Figure 1b, the pair is still $2.5 \mu m$, but the third particle is only $1.6 \mu m$ apart from each of the pair particles. The color scale in Figure 1 shows the strength of the electric field between the particles, and it can be seen that as a third particle is added the electric field of the third begin impinging on the electric field of the pair. This electric field impinging can be even more pronounced at longer screening lengths or in crowded systems.

Figure 1- Contour plots of electrostatic potentials

- (a) Difference between the full electrostatic potential for two particles and the superposition of two one-particle potentials. The distance between the particles is $r = 2.5 \mu m$.
- (b) Difference between the full electrostatic potential for three particles and the superposition of three two-particle potentials. The distance between particle 1 and 2 is $r = 2.5 \mu m$ and the position of the third particle is given by $d = 1.6 \mu m$. The color scales are in units of $k_B T$.

II. THEORY

The potential energy within a colloidal system plays a large role in what patterns colloids form and how well they assemble. There are two main potential energy forces that effect the colloidal systems. The first force is the Van der Waals forces. The Van der Waals forces for two spheres will attract the particles together. For two spheres of equal radius the Van der Waals potential can be determined by Equation 1 where a is the radius of the spheres and D is the separation distance of the two particles, the total interaction energy is V_{VDW} , and A_H is the Hamaker constant (Liang, Hilal et al. 2007).

$$V_{VDW}(D) = -\frac{A_H}{6} \left[\frac{2a^2}{D^2+4aD} + \frac{2a^2}{(D+2a)^2} + \ln \left(1 - \frac{4a^2}{(D+2a)^2} \right) \right] \quad (1)$$

The attractive forces between the two objects can then be determined by Equation 2 (Liang, Hilal et al. 2007).

$$F_{VDW} = -\frac{dV_{VDW}}{dD} \quad (2)$$

The second potential energy that needs to be considered comes from electrostatics. These forces are the result of a double layer of charged forces. Take a negatively charged colloid as an example. Due to their negative charge, the colloids will attract positive charges, which creates a double charge layer. This double charge layer can be seen in Figure 2. This double charge layer will cause the particles to repel each other, with the force being dependent on distance (Liang, Hilal et al. 2007). In Figure 2, the black lines represent the repulsive forces on the particles, the blue plus signs represent the layer of positive charges surrounding the colloids, and the red minus signs represent the negative charges of the colloids.

Figure 2- Double layer charge on a colloid pair

The electrostatic potential energy can be determined by Equation 3, where a is the particle diameter, h is the surface-surface separation between the particles, κ is the Debye-Hückel reciprocal length, n_{∞} is the bulk density of the ions and γ is the reduced surface potential and is given in Equation 4 (Liang, Hilal et al. 2007).

$$V_{elec} = \frac{128\pi a_1 a_2 n_\infty kT}{(a_1 + a_2) \kappa^2} \gamma_1 \gamma_2 \exp(-\kappa h) \quad (3)$$

Equation 4 is only valid when both $\kappa a > 5$ and $h \ll a$ (Liang, Hilal et al. 2007).

$$\gamma = \tanh\left(\frac{ze\psi}{4kT}\right) \quad (4)$$

The force generated from the electrostatic potential can be found in Equation 5 where $S_\beta(D)$ is the surface area of the spherical cell around the particle, n^0 is the ion number concentration, k is the Boltzmann's constant, T is the absolute temperature, z is the valence of the ions, e is the elementary electronic charge, and $\psi_\beta(D)$ is the potential at the surface of the spherical cell (Liang, Hilal et al. 2007).

$$F_{elec}(D) = \frac{1}{3} S_\beta(D) n^0 kT \left(\cosh\left(\frac{ze\psi_\beta(D)}{kT}\right) - 1 \right) \quad (5)$$

DLVO theory is based on the combination of the potential energies of the Van der Waals forces and the electrostatic forces. Equation 6 shows the total potential energy (Liang, Hilal et al. 2007).

$$V_T = V_{VDW} + V_{elec} \quad (6)$$

These potentials only consider the pair-interactions, however, and do not consider a third particle's interaction with the pair. Poisson-Boltzmann theory predicts that a third particle will attenuate the electrostatic and Van der Waals interactions of a pair of particles it approaches (Denton 2010). This attenuation can be on the same order of magnitude as pair potentials, especially in systems

that are crowded and systems with long screening lengths. Three body interaction potentials have been described by a modified Yukawa potential that is a function of the sum of the distances between three particles (Doma and El-Gendy 2015). Alternatively, Tersoff potentials have been used as in popular colloidal dynamics simulators such as HOOMD-blue (Anderson, Glaser et al. 2020). Yet, these potentials can be derived empirically rather than relying on theories which are applicable over narrow ranges of conditions.

The empirical potentials for the total interaction energy is given by Equation 7 where $u_{pair}(r_{ij})$ is the pair-potential between particles i and j and u_{123} is the three-body interaction potential (Dobnikar, Brunner et al. 2018).

$$U(r, d) = u_{pair}(r_{12}) + u_{pair}(r_{13}) + u_{pair}(r_{23}) + u_{123}(r_{12}, r_{13}, r_{23}) \quad (7)$$

The three-body interaction potential can therefore be found from the total interaction energy minus all pair potential energies. The triple distribution function is defined as $g^{(3)}$ and the triplet correlation can be characterized by the ratio between the triplet distribution function and its approximated form based on the Kirkwood superposition approximation (KSA) given in Equation 8 (Zahn, Maret et al. 2003).

$$g_{SA}^{(3)} = g(r_{12})g(r_{13})g(r_{23}) \quad (8)$$

The ratio from Equation 8 leads to the triplet correlation function denoted by $G(r_1, r_2, r_3)$ and can be seen in Equation 9. All the pair correlations in $g^{(3)}$ are included in $g_{SA}^{(123)}$ and the “extent of the intrinsic correlations due to the

simultaneous presence of a triplet of particles at positions r_{12}, r_{13}, r_{23} is quantified through the function G , which thus defines the local structure of the fluid beyond that expressed by the pair-correlation functions” (Zahn, Maret et al. 2003).

$$g^{(3)} = g_{SA}^{(3)} G \quad (9)$$

Equation 10 is then combined with Equation 9 to produce Equation 11, with $w^{(2)}$ and $w^{(3)}$ being the two- and three-particle potential of mean force, respectively and $\beta = \frac{1}{kT}$ where k is the Boltzmann constant, and T is temperature (Zahn, Maret et al. 2003). In Equation 11 $w^{(3)}(r_1, r_2, r_3)$ is the potential of mean force for the triplet, and the rest of the equation is the combination of the pair potentials of mean force with the triplet correlation.

$$\beta w^{(m)} = -\ln g^{(m)} \quad (10)$$

$$w^{(3)}(r_1, r_2, r_3) = w^{(2)}(r_1) + w^{(2)}(r_2) + w^{(2)}(r_3) - \frac{\ln G}{\beta} \quad (11)$$

Equation 11 shows that $-\ln G \equiv \beta \Delta w^{(3)}$ “plays the role of a three-body potential, and measures the energy of three correlated particles relative to the energy of superposed correlated pair of particles” (Zahn, Maret et al. 2003) .

Another way to describe $g^{(3)}$ can be seen in Equation 12. In this equation n is the “average number of triangles per configuration” (Krumhansl and Wang 1972). This means that n is the average of the number of triangles drawn between the possible colloidal three particles where r_{12} is the distance between points 1 and 2, r_{13} is the distance between points 1 and 3, and r_{23} is the distance

between points 2 and 3. ΔV is the change in volume and can be seen in Equation 13 (Krumhansl and Wang 1972).

$$g^{(3)}(r_{12}, r_{13}, r_{23}) = \frac{n(r_{12}, r_{13}, r_{23})}{\Delta V(r_{12}, r_{13}, r_{23})} \quad (12)$$

$$\Delta V(r_{12}, r_{13}, r_{23}) = 8\pi^2 r_{12} r_{13} r_{23} \Delta r_{12} \Delta r_{13} \Delta r_{23} \quad (13)$$

From Equation 12 and 13, the free energy of the triplet can then be determined and is found in Equation 14 where R is the ideal gas constant, T is the temperature in Kelvin, ΔG is the Gibbs free energy, and $g_{ref}^{(3)}$ is the reference triplet correlation function value to which all portions of the triplet correlation are compared. Note, that ΔG can be shifted up or down arbitrarily, because the relative free energy of two states is the important quantity for discretizing states. Typically, interactions at long distances are assumed to be zero by convention.

$$\Delta G = -RT * \ln \left(\frac{g^{(3)}}{g_{ref}^{(3)}} \right) \quad (14)$$

III. METHODS

In this study, a variety of videos and images of colloidal particles were accessed from literature sources to create a code to analyze properties of the colloidal systems. The base coding language used for analysis was Python 3.7. The finalized code was uploaded to GitHub for open use in further colloidal analysis.

Figure 3- Code flow path

Figure 3 shows the order of analysis. To begin, the particles in each video or image had to be uploaded. Particles had to be identified and tracked from frame to frame. To process the videos, a package called PIMS, or Python Image Sequence, was used. A specific feature of PIMS called pipeline was used to convert the video frames to greyscale, which then allows for the processing of the videos' frames (Contributors 2015-2020). PIMS was also used to import images for analysis.

To locate distinct particles and track their movements a package called Trackpy was used. "Trackpy is a package for finding blob-like features in video, tracking them through time, and analyzing their trajectories. It implements and extends the widely-used Crocker-Grier algorithm in Python" (Allan, Keim et al. 2010-2014). Trackpy was used to detect the particles by detecting particle brightness compared to the pixel brightness of the background, and then linked those located particles to a set of coordinates. The particles radii, while known from their given resources, is not needed due to location being based on pixel brightness.

It is noted that there is error associated with Trackpy's linking algorithm. Particle tracking works best at small length scales and are not well suited for studying large length-scale dynamics (Savin and Doyle 2005). Factors like signal noise and particle diameter effect the particle certainty, and can effect physical interpretation, with the scale being dependent on the particulars of the system (trackpy 2010). It should also be noted that a high statistical accuracy is

dependent on a large amount of data and a low amount of data, as in this project, leads to a lower accuracy (Savin and Doyle 2005).

After the particles were linked to their coordinates, distances were found between pairs of colloids and between colloidal triplets. The particle pair distances were found using a function called *cdist* which calculates the Euclidian distance between two sets of points. These distances were plotted in a histogram to show the correlations between distances. In the pair correlation, the count of the pair combinations was plotted versus the distance in pixels. Ultimately, distance histograms were converted into more familiar pair distribution functions. Triplet distances were found from the sum of three pair distances. These total distances, which represent the perimeter of a triangle with the three particles at the vertices, were plotted in a histogram to view the correlation of the perimeters. After finding the pair distances, the pair free energy was found using a prewritten code found on stackoverflow.com and can be viewed in Appendix A (Black 2019), where it was then normalized and plotted versus pixel distance. The triplet distances were also used to find the free energy by using Equations 8, 9, and 10. The total triplet distances were separated into separate variables, where the triangles were calculated by using the intersection points of two circles. The triangles are the distances between three particles and are then totaled to find the number of triangles. These triangles were then divided by Equation 13, where Δr_{12} , Δr_{13} , and Δr_{23} are the histogram bin widths. This $g^{(3)}(r_{12}, r_{13}, r_{23})$ from Equation 8 was then plugged into Equation 9, where $g_{ref}^{(3)}$ is equal to one, and the

free energy was then shifted to center on zero, to better view the changes in potential. The full code can be viewed in Appendix A.

The pair free energy and the triplet free energy were both calculated to determine the differences in the free energies when the interactions between two colloids are considered versus the interactions when a third particle is considered. The plots shown represent $w^{(3)}(r_1, r_2, r_3)$ and $w^{(2)}(r_1) = w^{(2)}(r_1) = w^{(2)}(r_1)$ from Equation 11.

IV. RESULTS AND DISCUSSION

The code outputs images to aid in the analysis of input colloidal system images or videos. Table 2 shows the different systems analyzed using the code with each system being represented by its alphabetical notation throughout.

Table 2

Published experimental colloidal systems analyzed using an analysis program

System	Description	Citation
a	Paramagnetic colloidal particles with diameter of $d = 2.8 \mu m$, dispersed in water and enclosed within a cell of thickness $h < 2d$	(Massana-Cid, Ortiz-Ambroz et al. 2020)
b	Colloidal gas-liquid-gas transition: gas to liquid	(Timonen, Demirors et al. 2016)
c	Colloidal gas-liquid-gas transition: liquid to gas	(Timonen, Demirors et al. 2016)
d	Concurrent electric and magnetic fields	(Bharti, Kogler et al. 2016)
e	Particle array before 2mA dc current	(Gong and Wu 2017)

The first image seen using the analysis program is a reproduction of a given frame set on an x-axis and y-axis. Figure 4 shows the frame image for several colloidal systems. These frames have differing amounts of order and visibility, showing that

a wide sample of colloidal types can be analyzed using the code. Figure 4a is a frame from an experimental microscopic video that observes paramagnetic colloidal particles with a diameter of $d = 2.8 \mu m$, dispersed in water and enclosed within a cell of thickness $h < 2d$ (Massana-Cid, Ortiz-Ambriz et al. 2020). In this study, additional videos show the colloids in a precessing field with a frequency of 1 Hz and 20 Hz, but the video selection in Figure 4a, contains the colloids in the absence of an external field. Figure 4b and 4c shows Frame 12 from an experiment showing the effects of electromagnetic tweezing on $1.5 \mu m$ silica colloids resting on a glass substrate. These silica colloids initially form a dilute 2D colloidal gas, and when this gas is approached by magnetic tweezers and the magnetic field is turned on, the gas is condensed into a 2D liquid, and then the field is turned off the liquid is then evaporated back into a gas (Timonen, Demirors et al. 2016). The video from Figure 4b shows the field in the on position, and Figure 4c shows the field in the off position. Figure 4d shows an experiment that views the collapse of colloidal networks with particle diameters of $5.7 \pm 0.2 \mu m$ into 2D crystals, in the time before the particles begin to converge and before the application of prolonged electric and magnetic fields (Bharti, Kogler et al. 2016). Figure 4e experiment viewing the nonclose-packed colloidal array before being submitted to a 2.0 mA dc current. The polystyrene microspheres are $2 \mu m$ in diameter with sulfonate groups, suspended in aqueous solution, and placed on negatively charge ITO glass slides (Gong and Wu 2017).

Figure 4- Located frame 12 of colloidal videos from systems in Table 2

The next image received from the code can be seen in Figure 5 which shows all the colloids located and plotted on top of the images seen in Figure 4. These located particles are linked to a set of coordinates and then those coordinates are plotted and shown as red dots on top of a still image for each video run. Figure 5a through Figure 5e corresponds with the correlating images in Figure 4a. Each video set needed a specific manual tuning within the code to ensure correct particle location. We hope to automate the tuning at some point in the future. Some error is introduced by imperfect particle identification. Equation 19 shows the code used to locate the particles in each video.

Each of the factors within Equation 15 can be changed to affect the particle selection. The first parameter to change is “21”. This is the diameter parameter and is input as an odd number based on Trackpy’s coding. The next parameter is the minmass which is the minimum integrated brightness. This brightness factor can help to eliminate spurious features and can be any float number. The next factor deals with the darkness of the particles. If the particles are darker than the background, then *invert = True*, but if the particles are lighter than the background *invert = False*. The following feature is the percentile and filters particles further by excluding particles than are darker than pixels within the chosen percentile. And finally, the last factor is the separation which set the minimum separation between features (Allan, Keim et al. 2010-2014). The images in Figure 5 are a result of Equation 15.

Figure 5- Particle location in colloidal videos

```
tp.locate(processed_video[12],21,minmass = 500,invert = True, (15)
percentile = 75,separation = 5)
```

The next set of images shows the correlation between the distance between two particles and can be seen in Figure 6. The x-axis shows the distance in pixels, and the y-axis shows the number of particles. The pair distance correlation plot shows the most common distance between two particles. Looking at Figure 6a, we can see that the most common distances between the pairs are between 200 and 300 pixels. This is not necessarily because there is an especially large attraction at this distance, but instead because the graph has not been normalized for the relative area of the bins. Larger bins represent a larger space and will therefore have a larger population. The decay of the histogram at large distances is caused by edge effects from searching for particles past the image boundaries. Figure 6e is the most telling of the histograms. The curve is jagged, indicating specific enrichment and depletion of neighboring particles at certain distances. When compared to Figure 5, it is obvious that Figure 6e shows the most ordering of the five videos tested.

These pair distance histograms can be converted into pair correlation functions by dividing the histogram counts by an expectation value derived from the differential area of a specific radius and the global density of particles. Subsequently, pair correlations give rise to a pair potential of mean force, which is considered to be a free energy through taking the natural logarithm of the function and multiplying by a factor of RT , where R is the ideal gas constant, and T is temperature. This pair free energy can be seen plotted versus pixel distance in Figure 6. When looking at the free energy plot, you can

Figure 6- Pair distance correlation in Count vs. Pixel Distance

determine what distance the particles prefer. The dips in the graph show where the particles likely will be located, and the peaks show where the particles likely will not be located.

Figure 7a contains significant noise and no real energy well where there is a distinct location that the colloids want to be. This may be due to particle vibration as well as low particle ordering. Figure 7b and Figure 6c also have significant noise with and no real energy well, with a dip at 10 pixels for each which can be expected due to the low ordering in systems b and c. Figure 7d has lots of noise, but a general increase in free energy as distance increases. This noise is likely due to the highly amorphous structure of the system and the general increase can be attributed to the applied electric and magnetic fields to the system. Figure 7e shows a pair free energy plot with the main dips at 35 pixels and 70 pixels. While the distance here is given in pixels, if wanted, the distances can be scaled if you know the distance scale per pixel, or scaled to the number of particle diameters. The structure of the graph has peaks and dips at descending amplitudes. The larger the amplitude of the peaks and dips, the more likely they will be or won't be at that given distance. This means that for Figure 7e, the particles are very likely to be found at 35 pixels and 70 pixels, and very likely to not be at 50 pixels and 90 pixels. The free energy plot for Figure 7e is more structured than that of Figure 7a through Figure 7d mainly due to the structures of the given systems. The system in Figure 7e is highly structured, as where the other systems are more amorphous with low structuring. Structured colloids are in stable equilibrium, and therefore interaction potentials show extended ordering.

Figure 7- Pair free energy

The next sets of images come from novel code, created to view the triplet distances, the triplet triangle distribution, and the triplet free energy. Most analyses on colloidal particles focus mainly on the pair interactions and ignore triplet interactions. There is widely and easily available code to analyze the pair interactions between colloids on sources like GitHub or even built into certain packages like SciPy, but when looking for code on triplet interactions, the sources are very limited, and if found are difficult to use, or incompatible with Windows systems. So, the code created for the triplet interactions was created based off theory and has been uploaded to GitHub for future use by anyone working on colloidal interactions.

Figure 8 shows the triplet distance correlations of all the previous systems. The triplet distance correlation is the sum of the distances between three particles and is then iterated for all the possible triplet possibilities in the given systems. These distances are then plotted in a histogram and shows the number of triplet combinations that are present at each pixel distance. Figure 8a looks very similar to Figure 6a but with the peak shifted to the right. This is due to the fact that instead of a single distance between two particles, there are three distances added up per triplet combination. For Figure 6a the peak is at around 170 pixels, and in Figure 8a the peak is at around 500 pixels, which is about three times the distance of the pair correlation, which makes sense. There are similar features in Figure 8b through Figure 8e. Figure 6b has two peaks at 90 and 350 pixels and Figure 8b has peaks at about 250 pixels and 820 pixels, which are both approximately triple the distances in pair correlation. Figure 8b

has two peaks which is consistent with the pair correlation plot due to the particle distribution. Figure 6c has a peak around 150 pixels and Figure 8c has a peak at 550 pixels which is again close to triple that of the pair correlation. Figure 6d has a peak at about 275 pixels, and Figure 8d has a peak at about 900 pixels. Figure 6e has a peak at 250 pixels and Figure 8e has a peak at 850 pixels. While all these peaks are not exactly triple the distance of the pair distances, they are close enough, with the difference being attributed to the different permutation of triplet combinations.

From these triplet distance correlations, the triplet free energy is found, $w^{(3)}(r_1, r_2, r_3)$. To do this first the number of triplet triangles must be found. For each triangle, three distances are found, r , s and t . A sample image of these distances can be seen in Figure 9. From these distances, length r is set to a range of 40 to 60 pixels, which can be varied based on a user's choice, and then the point between length s and t is plotted using Equation 16 and Equation 17. Equation 16 is the x-coordinate, and Equation 17 is the y-coordinate. These equations account for the r distance centering on the origin point of (0,0). Equation 16 and Equation 17 are then iterated through a loop for differing distances of r , s , and t . These coordinates are then plotted in a graph. These triangle graphs can be seen in Figure 10.

$$x = \frac{t^2 - s^2}{-2r} \quad (16)$$

$$y = \sqrt{s^2 - \left(x + \left(\frac{r}{2}\right)\right)^2} \quad (17)$$

Figure 8-Triplet distance correlation

Figure 9- Triplet triangle distance labels

Figure 10 shows the triangle distribution graphs for systems a through e. For all systems there is a higher concentration of points on the positive x side of the graph. This can be attributed to a positive r , as well as geometry restraints due to the positive r . When considering the limits of the lengths of s and t , there are less options of points being concentrated on the left side of the graph if r is positive. The different spans on the graphs can be attributed to the pixel span chosen based on the number of pixels between four to five different colloids.

Figures 10a through 10d all have a relatively uniform distribution of points without few signs of patterning. This uniform pattern is due to a low structure in the colloids. Figure 10e however has distinct waves where particles want to be. This is due to the highly ordered structure of the particles. The colloids all have distinct locations at a set distance apart, leading to the more structured distribution. This distribution plot is similar to contour diagrams seen in “*Three-particle correlation functions of quasi-two-dimensional one-component and binary colloid suspensions*” (Ho, Lin et al. 2006). The contour diagrams set different

properties in the system constant while varying the other properties. This is similar to the distribution plot created where one distance of the triplet is set to a certain range, and the other distances are varied.

The next step to finding the triplet free energy was to find the triplet correlation function. The triplet correlation function was found through Equation 12 and Equation 13. To begin, different bins had to be created, to place particles in different distance. The number of bins was set to 100 but can be varied based on computational power or preference. The bin width was then found and can be seen in Equation 18, where $bwidth$ is the bin width, $bmax$ is the bin maximum, $bmin$ is the bin minimum, and b is the number of bins.

$$bwidth = \frac{bmax - bmin}{b} \quad (18)$$

Equation 13 was found by taking the distances r , s , and t and plugging them in for r_{12} , r_{13} , and r_{23} respectively, and Δr_{12} , Δr_{13} , and Δr_{23} were input as the bin width, as they were the changes in the lengths, which is what the bin width is. Equation 12 was found by using Equation 19, where the floor of a value is when a value is rounded down to the nearest whole number, and n is the number of triangles in the system. Equation 19 was then divided by Equation 13 to find Equation 12, the triplet correlation function.

$$n = floor\left(\frac{((r+s+t)-bmin)}{bwidth}\right) \quad (19)$$

Figure 10- Triangle distribution

After the triplet correlation function was found after iterating over all colloids, the triplet correlation values were plugged into Equation 14. Equation 14 had the ideal gas constant value $R = 8.314 \frac{J}{mol-K}$, and the temperature $T = 300 K$. The temperature value can be varied if the temperature of the colloidal system is known, but 300 K was chosen as a standard temperature. The free energy found in Equation 14 was then plotted versus pixel distance, and shifted down the x-axis so that the free energy fluctuated around zero J/mol. This was done to mimic standard free energy plots that are seen in various literature. As the free energy is a relative number, the values can be shifted down without changing the meaning of the data. Figure 11 shows the plots of the triplet free energy for each of the previous systems. Figure 11a through Figure 11d are all noisy, with many dips and peaks, with no real energy well of where the particles either want to be or don't want to be. The lack of structuring in these plots is not surprising when considering the lack of structure in Figures 10a through 10d. This, like the pair free energy can be attributed to the relatively low structure seen the systems a, b, c, and d. No apparent three-body potential emerges. Figure 11e however, has a more structured format due to the more structured overall system, and shows that the free energy of the system does have a dependence on the triplet versus the pair. Looking at Figure 7e, the two main distances of attraction are at 35 pixels and 60 pixels, and Figure 11e has to main attraction distances of 110 pixels and 135 pixels, which are both approximate triple the distance of the pair. This means that the most common triplet triangle perimeter is three times the most common pair distance, which makes sense.

Figure 11- Triplet free energy

There are two additional features in the code that while previously coded within the Trackpy walkthrough ((Allan, Keim et al. 2010-2014) are able to show the plotted trajectories of the particles and fits the data to a line of best fit to find the mean squared displacement of the system of a video. Unfortunately, these features can only be used for videos and not a singular image as there is no movement to track or view the properties of. The trajectories are plotted per particle and show where each particle has been throughout the duration of the video. The mean square displacement can tell certain properties of the system. When the mean square displacement is printed out, two variables are given. The first variable, n , which is the power-law exponent of the system. The Trackpy walkthrough mentions that $n = 1$ for water, which is a viscous material (Allan, Keim et al. 2010-2014). The other variable give is A which is equal to $4D$ where D is the particles diffusivity. Equation 24 shows the equation to find the diffusivity for a 2-dimensional system, where k is Boltzmann constant, μ is viscosity, a is particle radius, and T is temperature. To find the diffusivity of a 3-dimensional system the 4 in the denominator is exchanged for a 6. This equation can be solved to find the system viscosity if all the variables of the system are known.

$$D = \frac{kT}{4\pi\mu a} \quad (24)$$

Figure 12 shows the trajectories for all the systems, and Table 3, shows the power-law exponent and A for each system from the mean square displacement plots as recorded from the Trackpy code. This is important, because before the mean square displacement was found, the properties

calculated from the system were purely structural properties. The mean square displacement, however, gives the dynamic properties of the system.

Table 3

Mean square displacement values for given systems as given by Trackpy code

System	n	$A \left(\frac{pixels^2}{frame} \right)$
(a)	1.032197	0.402554
(b)	0.953256	9.494735
(c)	0.531606	0.413112
(d)	1.436957	2.513255
(e)	0.371155	0.31939

Figure 12- Particle trajectories

V. CONCLUSION

Using the empirically derived two and three body potentials from this work, colloidal phase transitions, stable crystal structures, and phase stability can be more accurately predicted than by using DLVO theory alone. We foresee these empirical potentials and others derived from new images using our software being useful for designing new colloids and colloidal structures through simulation methods like molecular dynamics and Monte Carlo. We also envision future inverse design problems being simplified by finding working empirical potentials that create specific colloidal structures. In a sense, rather than designing the particles, we can design the potentials and then build the particles to match the potentials and verify the potentials through microscopic videos.

The strengths of the analysis code for this project includes its ability to import both avi and mp4 videos for analysis, the combination of pair and triplet analyses in the same program to find structural properties such as the pair and triplet potential mean forces, as well as the ability to find dynamic properties of the inputted system given that the particle diameters are known as well as the system temperature. Another strength is that new or existing experimental data can be used in the code. Running colloidal systems, one's own is not required to determine the potential and structural properties of a system. Some weaknesses

of the program include the length of time needed to compute certain properties such as the production of the triplet distance and distribution plots, a separate set of code is needed if looking to find the structural properties of a single image instead of a video compilation of images, and the fact that the code only works for 2-dimensional systems and other code would need to be added to allow for 3-dimensional analysis. The last main weakness is that the program includes edge effects in the analysis. At the edges of the frames, the correlations are not as effective due to edge effects of the system causing particles to react with less particles than those in the center of the frame. To adjust for edge effects the volume component in the triplet correlation would have to be adjusted. A new way to calculate the volume would have to be derived.

Properties in this code that are novel revolve around the triplet correlations. When researching code for this project, it was found that there is no easily available package to compute the triplet distances and the triplet potential mean force of a colloidal system. Packages like SciPy have functions that will compute the potential mean force of a pair, but not a triplet. This work uses published triplet free energy equations to create potential mean force plots for the triplets in a system as well as to create a distribution plot of the particles.

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APPENDIX

APPENDIX A: VIDEO ANALYSIS CODE

Attached is a link to the code for the video analysis of colloidal systems posted on GitHub to allow for free use by researchers.

https://github.com/blpric03/Pair-and-Triplet-Correlations-and-Free-energies-for-Colloidal-Systems/blob/026d7eb8973066f6fc4db308f09cb6b9443fac38/Colloids_video_analysis.ipynb

APPENDIX B: IMAGE ANALYSIS CODE

Attached is a link to the code for the image analysis of colloidal systems posted on GitHub to allow for free use by researchers.

https://github.com/blpric03/Pair-and-Triplet-Correlations-and-Free-energies-for-Colloidal-Systems/blob/684713e923f2e172f830a1f73e6889e58d054079/Colloids_image_analysis.ipynb