ACE-M2 Progress and Preliminary results, Part 1

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(Dated: 31 July 2014)

We describe and comment on the progress of preparing, loading and launching samples for the Advanced Colloid Experiment (ACE-M2), in the Light Microscopy Module (LMM) within the Fluids Integration Rack (FIR) aboard the International Space Station (ISS). We have completed several rounds of experiments in June and July, 2014. Here we summarize our findings so far, primarily several operational developments and lessons learned, with scientific results still largely forthcoming.

CONTENTS

I. SCIENCE ON THE INTERNATIONAL SPACE STATION

A. Why tell our story?

There are lots of papers, reports, blogs and general writing on space, NASA, and science. So why write more? *Because very, very few people actually understand how science experiments are conducted, from start to finish, either in the lab, or onboard the on-orbit laboratory of the International Space Station (ISS).* My aim here to tell the story of how science actually works, for a broad audience:

- the general tax-paying public who wants to know more about what is going on in NASA

- NASA staff who may be deeply knowledgable about a small part of the process, but don't get a good overview—and who want to hear directly from the Principal Investigators (scientists)

- policy folks who want a deeper view of what we do, beyond what can be described in a report

- the astronaut crew on we work with, who want a direct line to what we are doing

I intend this to be a cross-breed of a blog, textbook, collection of essays and technical manual. Some of the posts will give the nitty-gritty *in medias res* narrative of what we are doing on a day-to-day basis. Other entries will summarize previous knowledge to give an historical context to the work we are doing. Still others may dive a little deeper into the technical details of the tools we are using, hardware and software, both to do the science and communicate its results. I hope to cover a broad range:

- The scientific background of experiments that precede and motivate ours

- What we are actually trying to understand in our current experiment

- How we prepare and characterize our experimental samples

- What instrumentation and laboratory apparatus we use, on the ground and in orbit

- The process of packaging and launching our samples to the ISS

- Conducting on-orbit experiments in collaboration with the astronaut crew

- The raw data and how we process it

- How our analysis leads to the scientific story we will tell through papers and presentations

- Writing a scientific paper

- Preparing a scientific talk (and possibly its presentation)

FIG. 1 Launchpad photo

B. Opening up the process of doing science

I aim to be maximally open throughout. This means posting raw data when feasible, the actual computer code we use for the analysis (and potentially giving a way for you all to play with the data and code yourself in an interactive environment—stay tuned), early drafts of the paper as we write it, and maybe our presentation drafts before they are delivered.

My commitment here is to make as much of our work accessible directly to anyone to tinker with: open-source software, open-access publications, but also free, open access to the raw data; even this website itself, hosted on Github, is completely open—not just the text, but the entire revision history, and the code that runs it. So please download, play around, make your own modifications, and let us know through the comments what you think and if you found something new that we might have missed. This is a different way of doing science that takes advantage of a lot of the new information-based technologies that were not available in years past, and the way I think science should be done. I will elaborate more on the motivation and tools in a later post.

C. Who am I?

I am a physicist at Harvard, currently serving in a staff position known as a post-doctoral fellow, meaning I continue to do research after receiving my PhD. I am funded by NASA directly through a research grant to my official employer, Harvard University (Cambridge, MA), which broadly-speaking supports the scientific aspects of our work. I also receive some additional support as a subcontractor to assist with the engineering aspects, helping to develop and test the hardware and software, of the experiment by working closely with the main NASA contractor, ZIN

Technologies (Cleveland, OH). More information on me and my work can be found on my webpage, peterlu.org. I act independently, and represent my own views here. Though I am employed by Harvard, they do not control (pretty much ever) what any of us say or do. NASA, similarly, gives a research grant and is not able (as much as they sometimes would like) to be able to control what its funding recipients can say. ZIN is contracted to engineer, deploy and run complex instrumentation and serves in a technical capacity, and does not appear to have much of a corporate message to spread beyond NASA.

I do anticipate some fraction of what I write to disagree with the perspectives of others; if 80% of the readers agree with me 80% of the time, then that to me is a healthy balance that still leaves room for new ideas to develop and be fleshed out in a congenial manner. If you think I am a little off-base, please leave some respectful comments and I will do my best to respond in a timely fashion (though note that I will moderate and delete anything offensive, off-topic, spam, or irrelevant).

Hopefully we have an interesting and informative journey, and do some great science along the way!

II. WHY ACE-M2?

We learned a tremendous amount from the series of BCAT experiments conducted aboard the ISS over the past decade. One of the challenges of macroscopic photography, however, is the limited ability to see small features. Observable structures have to be at least a few pixels wide, which means we can image features that have to be tens of microns across, or larger. The particles are a half-micron in diameter, which means that the early stages of phase-separation, or indeed any other process we wish to observe with colloids, are not visible with traditional photography. We see this in our data; we don't see much happening within the first (sometimes many) hours after sample mixing.

ACE allows us to put these same samples under the microscope, where we can comfortably get micron-level resolution, and if everything works out, significantly better. Scientifically, we are able to then watch the structures forming from a much earlier time, when they are smaller, which gives insight into the fundamental mechanisms driving these processes.

The microscope, however, is a new (to us, at least) facility, and its capabilities on orbit are not well-characterized for our purposes: can we get enough light to the sample to see the particles? What is the range of intensities that we can detect at the camera, with the various filters and dichroics in the system? Will the particles be visible? What is the image quality using different microscope objectives? These parameters all depend on our choice of sample, as well as the hardware on orbit, and the on-paper specifications from the manufacturers are often rather poor guides, as they apply primarily to pristine instruments in a clean,

ground-based, stable environment—not reflecting the real-world challenges of flying a vehicle in low-earth orbit.

Therefore, we are launching three categories of samples:

1. **Fluorescent dyes in solvent:** these samples establish that we are able to observe fluorescence, using the same fluorophores as are in the colloidal particles, but without any particles. This frees us from having to worry about, for instance, whether the particles are stable, or sediment out, or can be mixed.

2. **Suspensions of fluorescent colloidal particles in solvent:** these samples allow us to measure intensities of different particle concentrations, with the end-goal of being able to take a sample and identify its density (volume fraction) based on fluorecence intensity, a quantitative use of the microscope. They are also a test of whether the particles survive the launch and can be mixed, and are stable and bright.

3. **Mixtures of colloidal particles and polymers in solvent:** these samples should exhibit dynamic changes, undergoing the process of phase separation. We should see the formation of structures much as we saw in BCAT, but on a smaller length scale and starting much earlier, with higher-magnification microscopy.

III. SAMPLE COMPOSITION CHARTS

Here are the exact samples we delivered to NASA / ZIN, and are launching to the ISS. All of the samples have the same solvent: 18% cis-decalin, 22% tetralin and 60% tetrachloroethylene, where the percentages are based on masses (not volumes). We have two particle sizes—large (880 nm radius) and small (290 nm radius)—and all have the Cy3-MMA fluorescent dye. We use one polymer, a linear polystyrene with a nominal molecular weight of 11.4M; its concentration is expressed in mg of polymer per gram of solvent.

A. Fluorescent dyes

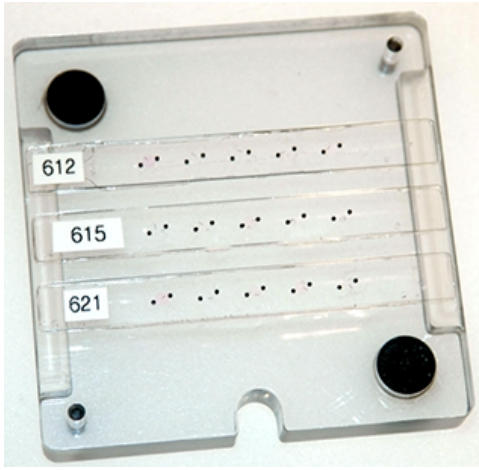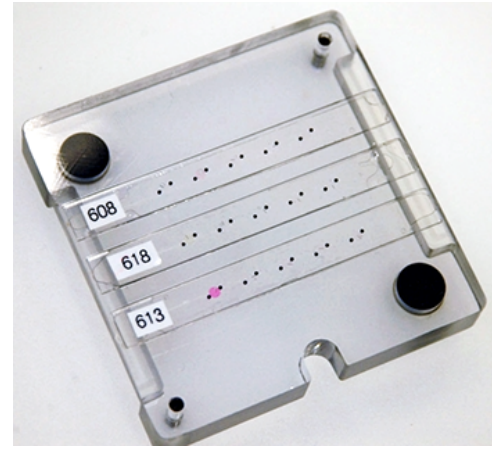| No.	Name	Dye	Conc.
1	plu_ACEM2_Cy3M1	Cy3-MMA	0.048%
2	plu_ACEM2_Cy3M4	Cy3-MMA	0.012%
3	plu_ACEM2_DiI1	DiI	0.062%
4	plu_ACEM2_DiI4	DiI	0.016%
5	plu_ACEM2_DiO1	DiO	0.041%
6	plu_ACEM2_DiO4	DiO	0.010%
7	plu_ACEM2_solv		0

FIG. 2 Sample platter 2104

FIG. 3 Sample platter 2105

B. Colloidal suspensions

No.	Name	Colloid size	ϕ
8	plu_ACEM2_lg_40p0	large	40.0%
9	plu_ACEM2_lg_30p0	large	30.0%
10	plu_ACEM2_lg_19p9	large	19.9%
11	plu_ACEM2_lg_10p7	large	10.7%
12	plu_ACEM2_lg_05p2	large	5.2%
13	plu_ACEM2_lg_00p4	large	0.4%
14	plu_ACEM2_sm_45p0	small	45.0%
15	plu_ACEM2_sm_29p9	small	29.9%
16	plu_ACEM2_sm_19p8	small	19.8%
17	plu_ACEM2_sm_10p1	small	10.1%
18	plu_ACEM2_sm_05p0	small	5.0%
19	plu_ACEM2_sm_00p5	small	0.5%

C. Colloid-polymer mixtures

No.	Name	Colloid size	ϕ	C_p
20	plu_ACEM2_sm_ps	small	15.0%	0.529 mg/g
21	plu_ACEM2_lg_ps	large	20.5%	0.153 mg/g
22	plu_ACEM2_lg_gel	large	22.0%	0.275 mg/g

IV. CHOOSING THE SAMPLE WELL LAYOUT

We have 30 wells in total, and 22 samples prepared. We distributed the samples throughout the strips in such a way that we would preserve the broadest range of scientific observations if we lost a well, or a strip; that is, we sought to avoid actively placing all of the same types of samples physically near each other, in case something went wrong.

The sample composition chart can be tedious to work with, because it's effectively just a list of numbers. So to fa-

cilitate keeping track of the different samples and their well locations, I created an icon-based chart. Each entry in the table has a square icon tile, containing all of the relevant information on each sample, which can be read at a quick glance.

A. Dye samples

Dye samples have the name of the dye written in the color of the dye solution (red and yellow), within the grey circle; the brightest colors represent the higher concentration (of two), and the faded / lower-saturation text occurs in samples with the lower concentrations.

B. Colloidal suspensions

Each sample has a pie chart showing the volume fraction (out of 100%) in bright red. The radius of the circle denotes the particle size. Samples with the larger (of two) particle diameters have circles that nearly fill the black squares, where the exact volume fraction is written in text *within* the pie chart. For samples with the smaller particle diameter, the pie chart is substantially smaller, and the text indicating the volume fraction resides *outside* the pie chart.

C. Colloid-polymer mixtures

These samples follow the same convention as for the colloidal suspensions; however, those samples with depletant polymer have a blue triangle at the lower-right of the square. For samples that are expected to phase separate in a thermodynamic process known as spinodal decomposition, the blue triangle is hatched. For samples that are supposed to kinetically arrest into a gel state, the blue triangle is solid.

Strip	Well 1	Well 2	Well 3	Well 4	Well 5
612	**4** DiI	**6** DiO	**11** 10.7%	**17** 10.1%	**20** 15.0%
615	**12** 5.2%	**9** 30.0%	**15** 29.9%	**10** 19.9%	**22** 22.0%
621	**1** Cy3-M	**7**	**8** 40.0%	**15** 29.9%	**20** 15.0%

TABLE I Sample platter 2104

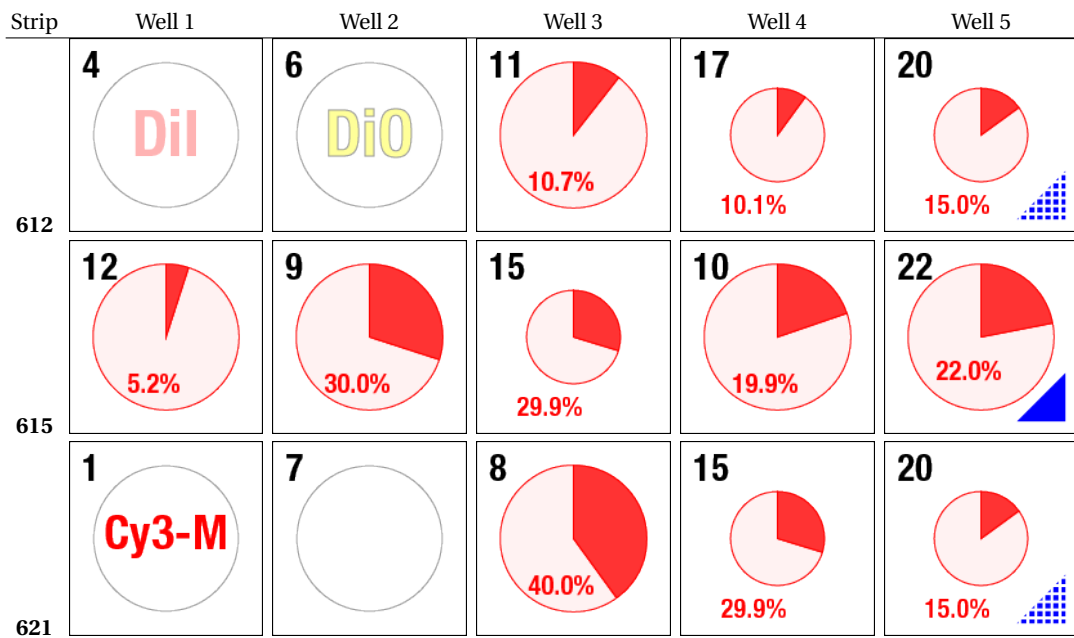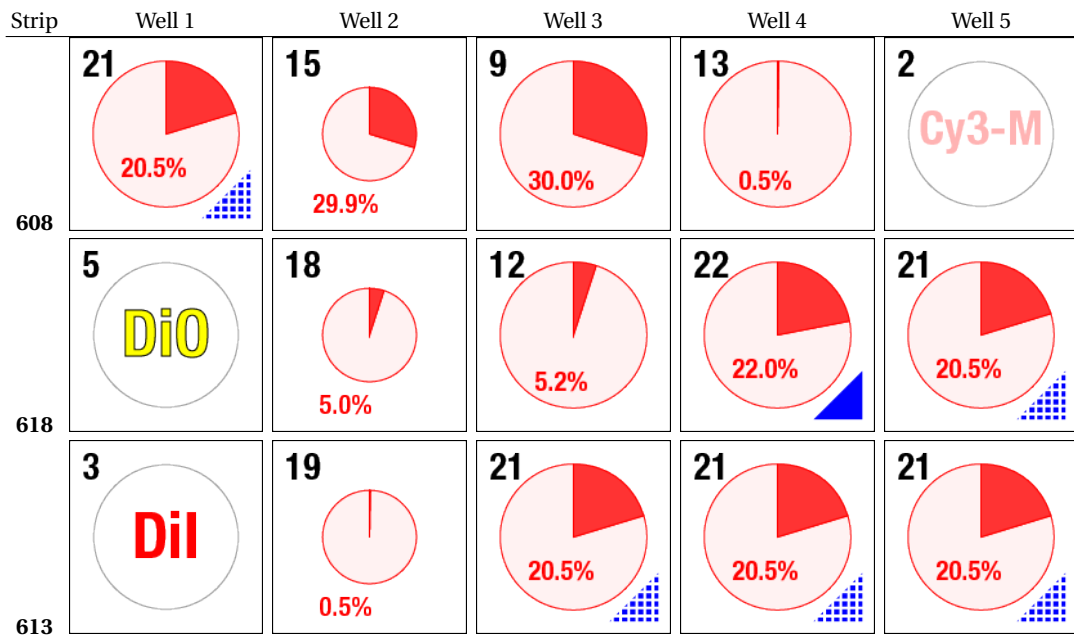| Strip	Well 1	Well 2	Well 3	Well 4	Well 5
608	**21** 20.5%	**15** 29.9%	**9** 30.0%	**13** 0.5%	**2** Cy3-M
618	**5** DiO	**18** 5.0%	**12** 5.2%	**22** 22.0%	**21** 20.5%
613	**3** DiI	**19** 0.5%	**21** 20.5%	**21** 20.5%	**21** 20.5%

TABLE II Sample platter 2105

V. FIRST DAY OF OPERATIONS

We have commenced the first image acquisition in the light microscopy module (LMM) as part of the ACE-M2 experiment. The LMM system is being controlled directly by the Payload Developer (PD), Lou Chesney, sitting at the bank of computer screens that run the control software:
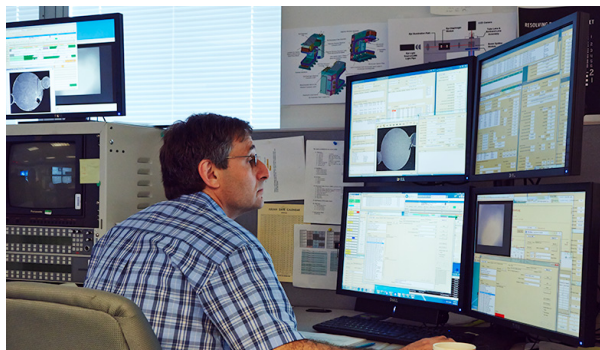

FIG. 4 Lou Chesney

Because the microscope is onboard the International Space Station, the communications between the commands Lou issues on the ground, and what happens on orbit depends on close coordination with the general communications with the ISS. In particular, there are frequent interruptions of communications and controls, depending on where in its flight path the Space Station is, relative to the geostationery satellites that relay its communications first to Marshall Space Flight Center (MSFC) in Huntsville, Alabama, then to the Telescience Center (TSC) here at NASA Glenn Research Center (GRC) here in Cleveland. The rack officer (RO) coordinates these communications, and integrates the specific timing of operations with the general ISS timeline; this morning, Jim Birchenough is serving in both this role, and as the data management officer (DMO)—overseeing the data downlink so we can live previews and downloaded images—and has his own bank of screens to monitor:
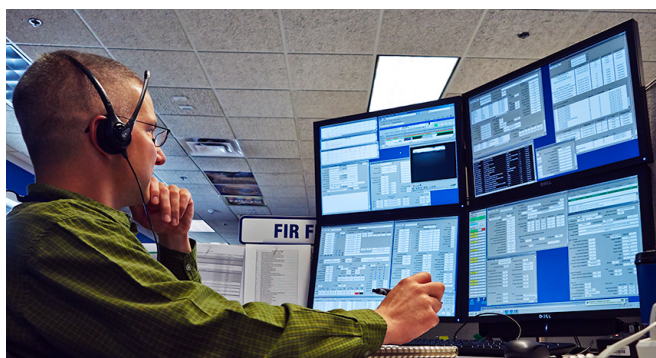
FIG. 5 James Birchenough

After a full 8-hour shift, Lou and James swapped positions with Tibor Lorik (PD) and Amber Krauss (RO / DMO):
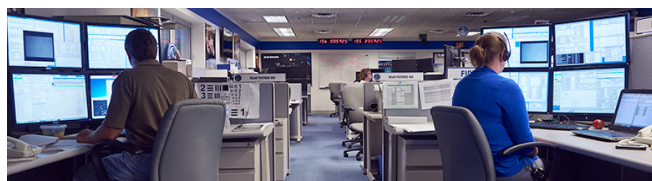
FIG. 6 Tibor Lorik and Amber Krauss

VI. FIRST IMAGES FROM THE LIGHT MICROSCOPY MODULE (LMM)

We have 15 samples in the holder in this set of experiments, and our first task is to image all of the samples with the lowest-magnification microscope objective lens (2.5x). This lens's magnification is ideal for the task, as it gives an image of each entire sample well. We first use brightfield transmission illumination (50/50 filter), where the intensity of the image is proportional to the amount of light passing through different parts of the sample. Thus, magnetic stir bar appears dark, as it transmits no light, and the glass appears bright, as most of the light passes through. Bubbles act as mini-lenses, and therefore are bright in the center and dark around their edges; the light from the edges is bent toward the center (or focused) by the bubble. These features are evident in an image of well number 5, containing a phase-separating sample, as shown in the image on the left.

The image on the right is the same sample and position, but collected in fluorescence mode, where the intensity is proportional to the number of (fluorescent particles). Interestingly, we observe higher concentrations of particles aggregating around the edges of the sample chamber, the stir bar and the bubbles. Neither the stir bar nor bubble contain any fluorescent particles, and like the background glass appear black.
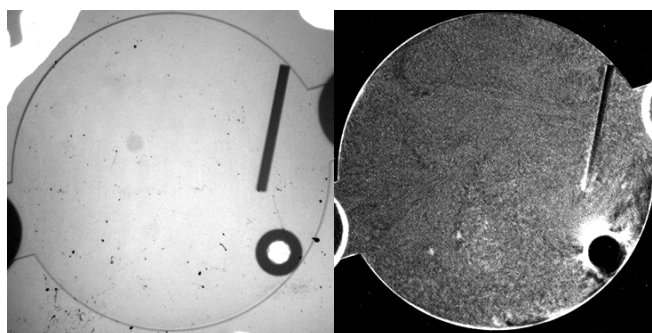
FIG. 7 well 5, 2.5x mag, brightfield transmission
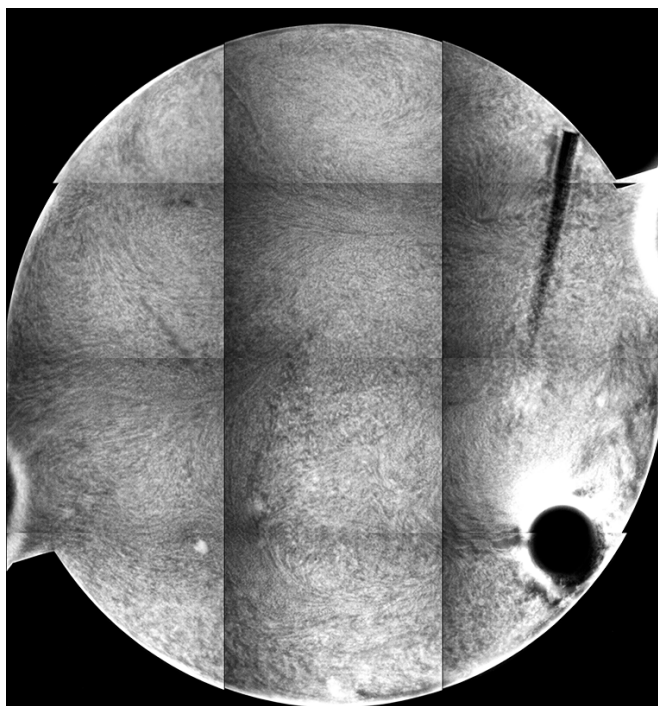
FIG. 8 well 5, 10x mag, fluorescence tiled composite

VII. COMPOSITING IMAGES TO GET A HIGHER-MAGNIFICATION VIEW OF THE ENTIRE SAMPLE

The 2.5x images are good to show the entire sample, but the resolution is limited. To get a higher-resolution view of the sample, we switch to a higher-magnification objective lens; however, the tradeoff is that the field of view is curtailed, and only part of the sample fits into the visible field of view. Fortunately, the LMM has an automated stage, so that the position of the sample visible to the microscope can be controlled remotely. As a result, we can take images in a tile-pattern that cover the entire sample:

The different fluorescence images are the tiled composite image taken at different depths from the cover slip inside the sample. If you look at the stir bar, you can see how different parts of the bar come into "focus" in the different images, giving an indicator of the depth within the sample from which the images are collected.

Each "tile" is a bit darker to the left, so that the rectangles representing each individual 10x image are easily seen. This is a result of uneven illumination; the samples are illuminated by light from a lamp, which does not cover the sample evenly (which might be ameliorated by changing internal microscope aperture settings).

But we have another way we might correct for this problem: a couple of our samples are fluorescent dye dispersed in a solvent inside identical sample wells—physically, the intensity of fluorescence should be completely even / isotropic, so any unevenness in the image should be caused

by the illumination. By taking images under identical conditions of both the phase-separating sample of interest, and of the even dye solution, we should be able to cancel the background and see the sample without the effects of the uneven lamp illumination. So we will be testing this in upcoming operations.

Meanwhile, next stage is to look at higher-magnification, where we seek to understand the details of small portions of the sample, and see the behavior of these colloid structures up close.

VIII. ACE-M3: QUANTITATIVE MICROSCOPY

Thus far, ACE-M2 has been a resounding success. Every one of the samples which we have launched appears to work as we expected: dyes are bright, colloidal suspensions show variations in intensity. But most importantly, we are able to watch quite clearly the early stages of phase separation, as described in previous posts. So far, so good; we have a great *qualitative* understanding of what is happening so far.

Upcoming experiments will look at the phase-separating samples in greater detail, with hope to quantify the rate of phase separation—something we have been able to do at larger lengthscales with the BCAT series of experiments, using photography and some image processing. But what we have never been able to figure out reliably is the concentration of particles in the two phases after phase separation. Unfortunately, the photos we take with the camera flash are not *linear* in relating intensity the image to particle concentration. Why? Imagine photographing clouds; you can't really tell how thick the cloud layer is just by its brightness; if a lower cloud passes in front of another one, even if the total cloud thickness doubles, the light (usually) doesn't fall by exactly half.

These concentrations after the samples phase separate are important for us to understand some fundamental physics, test existing theories in a new regime, and be able to place our samples in the context of other systems. And they cannot be measured on the ground, where sedimentation may take place—which will inherently change the measured concentrations. So being able to quantify the relationship between brightness in the microscope and particle density is extremely important for the science we want to explore, and something we were never quite able to do with the BCAT experiments.

ACE, however, uses fluorescence, a process that is linear. If you collect light from a sample well like we have on orbit, if the particle density is twice, the total brightness should also be twice. You might recall that we already launched a series of particle suspensions, which was intended to serve this purpose. We are looking at that data now, and hopefully that should work. But doing careful experiments means that we are always happy to run independent controls, cross-checks, and different systems to verify what we think we are seeing. A different method to quantify the brightness vs.

concentration relationship would therefore be very useful.

Fortunately, we have been given a great opportunity to send up more samples with the next round of ACE-M experiments, ACE-M3 which (at the moment) is set to launch in the fall aboard SpaceX-4. Preparing for this experiment involved a number of unique challenges:

1. We were only given the go-ahead a couple of weeks ago, after a bit of begging and pleading to squeeze us on board the next rocket! Fortunately, NASA and the great folks at ZIN agreed to make a little more space. So we had five new samples we could prepare—and only a few days to prepare them.

2. Because of the last-minute nature of the sample prep, we were restricted to the materials that were already approved as safe with a new set of sample cells (slightly different in construction from the ones we use on ACE-M2, but no time to update the loading procedures). But luckily, those materials include dyes that are the same colors as the particles, namely those based on fluorescein and rhodamine.

3. We need the samples we prepare to reflect similar optical behavior as the particles, suspensions and mixtures we already launched with ACE-M2. If they are too different, say involving different filters and lenses, then the results might not apply directly to the system whose physical behavior we want to quantify better on orbit.

A. Sample preparation and characterization

I chose the rhodamine dyes, for the reason that it is red / pink, and our particles are pink, so I figured it was a decent place to start. Two samples with the same fluorescence should be the same color in different lighting conditions. I then took some rhodamine dye and dispersed in water, and ended up with a very dark red color, like a red wine, and I could barely see through it. So it seems like it had way too much dye, and I started diluting it out by factors of 10. But if we want to control the dye concentration, how can you assess that? Unfortunately, weighing the dye out is not very accurate. I started with a few mL of water, but the amount of dye is only a couple of milligrams, and our balance only reads to 0.1 mg, so the error on the dye concentration is inherently no better than a few percent.

Moreover, how do we predict what the brightness will be in a fluorescence microscope? Obviously, we could use a fluorescence microscope, but if that is the instrument we are testing, we would prefer an independent way to measure the brightness. The best way would be to use a fluorometer, a specialized instrument that sends in a beam of light at a user-selectable wavelength (i.e. color), and then measures the amount of fluorescence emitted. Unfortunately, we don't have such an instrument, and the ones in the chemistry department are not that convenient.

1. UV-Vis absorption spectroscopy

We do, however, have a UV-Vis absorption spectrometer. This instrument sends light into the sample one color at a time, scanning over a large range of wavelengths, and records the amount of light passing through. These instruments are very carefully calibrated, and ours has a second reference light beam, so the data it outputs is a very careful, quantitative measure of absorbance. Why does this help? Because in order for light to be re-emitted as fluorescence, it must be absorbed at a lower wavelength. And by conservation of energy, doubling the fluorescence emission will lead to double absorption; it's also a linear relationship (barring some specific quantum mechanical effects which I don't expect to be sensitive to). So assessing the peak of the absorption gives us a way to characterize concentration.
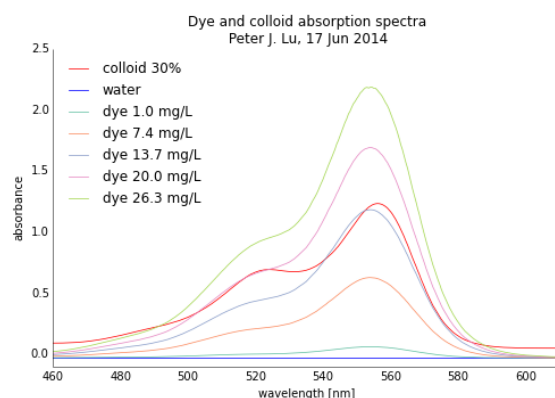
FIG. 9 UV-Vis spectra

First, I ran a sample of just plain distilled water. There should be no peaks in the absorption spectrum, and it should be basically be flat through the entire range of wavelengths (460 to 610 nm), as it is colorless—and therefore has no fluorescence—and transparent. Indeed, this is what I see, shown by the blue curve in the absorption spectra. I then ran the colloidal sample at 30% volume fraction, right in the middle of the range we have on orbit; its spectra has a nice peak at about 555 nm—which is why the samples appear pink: they absorb green light, and the absence of green light (e.g. on a color wheel) is pink / magenta.

Then I ran concentrations of the rhodamine dye; fortunately, the main peak in the absorption spectrum is very, very close to that of the particles, shown with the curves in the other colors. You can see how close the peaks are for the colloids (red) and the dye at a concentration of 13.7 mg/L. This is very, very fortunate, because it shows how the optical behavior of the dye is an excellent proxy for the spectral characteristics of our particles!

So by a bit of trial an error, I found the rough dye concentration corresponding to the brightness of our 30% colloid sample. Then with some careful sample prep, I spaced them

FIG. 10 Absorption vs. concentration

```
from pylab import *
import csv
import prettyplotlib as ppl

dye_concs_data = []
for row1 in csv.reader(open('peak_summary_130615
    ↪ .txt'), delimiter='\t'):
    dye_concs_data.append(row1)
header1 = dye_concs_data[0]
water_blank = dye_concs_data[1]
values1 = array(dye_concs_data[2:]).astype(np.
    ↪ float)
concs=values1[:,0]
window5nm = values1[:,2]
linfit = polyfit(concs, window5nm, 1)
fitrange =[0,30]

figure(figsize=(6,4))
for i in range (1,4):
    ppl.plot(concs,values1[:,i],'o')
ppl.plot(fitrange,polyval(linfit,fitrange),'-')
xlabel('concentration␣[mg/L]')
ylabel('absorbance')
title('Dye␣concentration-dependent␣absorption\n␣
    ↪ Peter␣J.␣Lu,␣17␣Jun␣2014')
xlim([0,30])
ylim([0,2.5])
lg= legend(['peak␣max','2␣nm␣window','5␣nm␣
    ↪ window'],'upper␣left')
lg.draw_frame(False)
savefig('dye_concs_abs_140617.pdf')
```

out linearly over the range of brightness that spans what I expect the colloids on orbit will (other colored lines in the plot), so we will learn about the camera and imaging system with parameters similar to what we will use for the colloids.
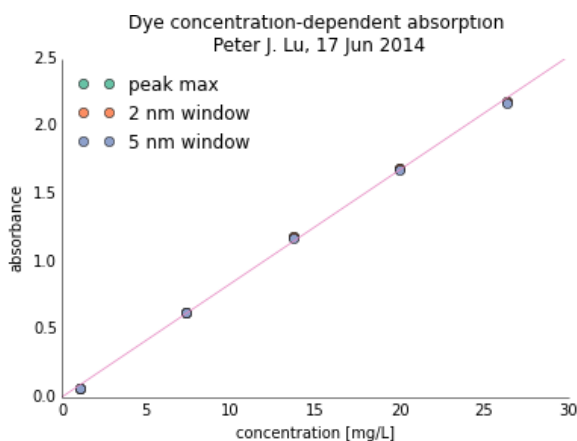
2. Samples to be launched

Here's the final list of samples we submitted to ZIN / NASA:

Sample	Dye concentration
plu_M3A	1.05 mg/L
plu_M3B	7.36 mg/L
plu_M3C	13.7 mg/L
plu_M3D	20.0 mg/L
plu_M3E	26.3 mg/L

B. Testing linearity

To check the linearity of the fluorescence intensity, I measured the height (absorbance) of each peak at 554 nm, and plotted it as a function of concentration (from the sample preparation). There are several ways to measure the peak: reading off the top value, or averaging over a few nm around the maximum. In all cases, the points fall on top of each other, as seen in the figure at right, where the different ways of measuring peak height are indistinguishable. And, critically, the points all fall along a straight line (purple in the figure), which extrapolates to very close to the origin. These data show very carefully that the relationship between absorbance—and therefore fluorescence—is linear with dye concentration, which have similar spectral characteristics to the colloidal particles we have launched.

This is exactly as we would hope for a good set of standard samples, to use to then explore and calibrate the imaging system properly, using a different chemistry while still

in the same physical realm (in terms of fluorescence intensity) that we are interested in for our colloidal samples. So we are very excited to launch these as a part of the ACE-M3 experiment!

Finally, here is the code from the second plot, with the linear fit to the raw data:

IX. SCIENCE DEPENDS ON FLIGHT OPS, WHICH CANNOT BE KNOWN AHEAD OF FLIGHT

A. Putting the cart before the horse

In the study of literature, there is a literary devive known as *hysteron proteron*, which colloquially is often termed "putting the cart before the horse." Alas, the way NASA has structured the way it does science experiments often has a similar flavor: requiring the answer to be known before the experiment is performed.

As NASA Principal Investigators (PIs), we are asked to specify requirements for instrumentation and apparatus that the engineers are then supposed to build. The problem is, the scientists often spec instrumentation based on what they can get in the laboratory on the ground, not necessarily what is practical or even possible to fly on ISS, with its myriad safety, operational, power, mass and mechanical shock requirements. Similarly, we are told to define scientific goals, not just a general overview of what we hope to accomplish, but specific criteria as to what would constitute

partial, satisfactory and complete scientific success.

But if we could define specifically what would be successful scientifically, then we would know the answer before asking the question—and there would be little point in doing the actual experiments!

Moreover, at present, the science office is a subdivision of payloads, which manage all of the hardware on the ISS. This is a completely different division than flight operations, the pilots who fly the station and manage its communication. So inevitably, if science or engineering requirements are defined from the viewpoint of only one division, it can conflict with what is going on elsewhere. This is, of course, not a specific problem to NASA, but instead symptomatic the challenges of working within any large organization.

B. Organizational divisions between science and flight-ops are problematic

From a PI standpoint, constraints from flight operations never enter the specification or definitions of the science experiments. We are told to specify what we need, which then the science / payloads office coordinates, and has to try to balance with all of the other experiments and payloads. They are tireless advocates for our cause, for which we are extremely grateful and have received very, very generous allocations of crew time and ISS resources. And through this process we have learned from the many iterations of the BCAT experiment is that it is invaluable to understand what everyone else's constraints are. Often times, we would specify that we needed two weeks to run our samples, but if the flight schedule only had 12 contiguous days available, we would not be able to use that time, and would have to wait until our nominal requirements (i.e. the two weeks) were available, even if that would not be possible for many months in the future. But from our standapoint, 12 days of data now could be worth a lot more than a "complete" set of data months later—especially because all kinds of things can go awry in the interim (samples may dry out, instruments may get broken, the Space Station itself may have an operational issue).

Alas, because the normal procedures do not involve science PIs in flight operations, these types of tradeoffs are not ordinarily presented to the PI, and instead are negotiated among several constituencies within NASA, who may not have the background to make the decisions that are best for the science. Conversely, unless the PIs have been involved in several successful flight experiments and have worked with the different NASA branches, it's unlikely that they understand the panoply of constraints that flight on orbit imposes, so it is hard for them to weigh the practical factors.

As a result, we do the best job we can to suggest what we *a priori* feel is a reasonable operational plan based on our best guesses as to how the apparatus will perform, with full awareness of the wisdom of Prussian (German) field Marshal Helmuth Karl Bernhard Graf von Moltke dictum that,

roughly translated, that "no battle plan survives first contact with the enemy."

X. ACE-M2 FIRST-ROUND FLIGHT OPS MAIN GOAL: DEFINE AND CHARACTERIZE OPERATIONAL CAPABILITIES

The main point of the first runs of the ACE-M2 experiment are to image all of the samples and see if we can actually observe them. If we can't image any sample in the microscope, then obviously there won't be much use for future operations. Fortunately, in general we were able to acquire good images of all of the samples, which I describe in greater detail in a later post.

Once this basic ability to image samples has been established, the main objective is to understand the capabilities of our system. This not only includes the physical aspects of the optical system, camera and other instrumentation, but the amount of time different operations actually take when a human is operating the LMM setup amidst all of the mayhem onboard the ISS itself.

A. Optical capabilities: exploring objectives

The LMM is equipped with several microscope objective lenses: 2.5x, 10x, 20x and 40x air, and the 63x and 100x oil-immersion objectives. There are several filter sets, though we use the Texas Red set for almost all of our samples, whose spectra we checked before launch so that they were compatible with the dyes we use in the particles. There is a single CCD camera, made by a company called QImaging, that uses the Sony ICX285 CCD chip that has been a workhorse for the entire industry for the first decade of this century, though more recently surpassed by much better imaging technologies.

1. 2.5x air: sample surveys

Using the lowest-magnification objective, the 2.5x air, we survey every well at the beginning of each full experiment, with both bright-field and fluorescence, to check on the status of the sample and see quite generally what is going on, i.e. the location and density of the particles. This objective lens has a very large depth of field, encompassing the entire sample well, giving us a very good overview of each sample. These images also form part of the safety checks at the beginning and end of each experiment, to make sure for example that the wells are all intact, not leaking or broken, etc.

2. 10x, 20x and 40x air: sample details

Perhaps the most important objectives are the medium-magnification air objectives. These allow us to zoom into particular areas in the sample, and get a more detailed view.

In general, the quality of the images appears to be best with the 10x, and good with the 20x and 40x.

3. 63x and 100x oil: highly disappointing

On paper, the 63x and the 100x should give the highest-quality views, because they at least double the *numerical aperture* of the air objectives; that is, they should have roughly double the resolution, so that features that are visible but blurry at 40x should be sharper and more clearly defined, with better contrast, with the 63x lens.

These two objectives require the addition of immersion oil, a droplet of which is added by the crew as the samples are loaded. We did a number of different tests, looking at the same areas with different objectives. Surprisingly—and not in a good way—the image quality is invariably worse with the oil-immersion, higher-magnification objectives, than with those that are air-based. This is backward, and should not happen, and demonstrates that something is wrong with the current configuration of the microscope on-orbit. Engineers at ZIN are working on this issue, so stay tuned.

But from a practical standpoint, there is no reason for the immediate future to incorporate any of the oil-immersion objectives. This has the collateral benefit that no oil then need be added by the crew members, or manually moved via the objective to different sample wells, giving greater flexibility to the imaging of different sample wells.

B. Timing individual operations

Once the objectives have been selected and we understand how they can best be deployed to give the images we want, the major task to understand how long different operations actually take:

- starting up the LMM inside the FIR rack

- loading the sample

- moving the microscope stage to the right positions on the samples

- establishing the distance between the objective lens and the sample coverslip

- adjusting imaging parameters on the camera to acquire good images

- saving and downlinking image data

- shutting down the rack

While we have estimates for how long these operations take on the ground, things are often quite different on orbit. And establishing the timing is *absolutely critical* for planning future operations, all of which must happen in the context of a competition for resources like crew time, machine time, operator time, power, etc.

C. Meshing with the ISS timeline and infrastructure: LOS

One of the major differences between doing experiments on the ground, in a well-controlled, isolated (university) research laboratory, and the ISS, is the number of other things happening at the same time. In our labs, there may be a few people walking in and out of the room, but generally things are still, power, light, temperature, gases and water are continuously available, and mechnically the building is stable in the absence of, say, high winds or a major rainstorm. Almost none of those factors is true on orbit; the ISS is a flight vehicle, and flies around the earth in orbit, traversing the planet every couple of hours.

The ISS communicates with the ground via a network of geostationary satellites. Unfortunately, this network does not cover the entire earth's surface, so that every few hours (and often more frequently than that), the Station loses communication, during events known as "Loss of Signal" or LOS. During this time, we cannot access our experiment at all, so either it is running something in an automated fashion that we can script ahead of time, or it must sit there until communications are re-acquired.

So not only is the total time relevant for each operation, but complex experiments must be broken down into a series of smaller sequential operations, either that execute remotely during an LOS event (ideal, because then we don't lose any time), or must be robust to being interrupted and (re)started again at a later time.

D. The human factor: reproducibility and robustness

Finally, the operators on the ground work very hard to collect our data, taking 8-hour shifts at the console controls. They have a wide range of scientific background, so that we must be very precise in specifying procedures for data collection and documentation. Moreover, the procedures have to be simple and reliable enough to be executed without error by an operator working all day, even as she gets more tired and might overlook something.

XI. MAJOR RESULTS FROM THE FIRST RUN: A FLIGHT-OPS PLAN FOR UPCOMING EXPERIMENTS

So the most important results from the first run are operations related, once we established we can image all of the samples. These procedures allow us to determine what to measure going forward, within the envelope of the resources that we have. After four 48-hour days to survey all of the samples, the remaining time will focus on the handful of colloid-polymer mixtures (samples 20, 21 and 22), and imaging them over time. We therefore expect to do the following for each sample, which based on the first-run operations will feasibly fit into the timelines we have requested:

1. Image all wells with 2.5x air objective. This is fast,

easy, simple and gives on overall status check for safety and scientific reasons.

2. Create full tiled images of the entire well with the 10x air objective. This is a fast operation that gives us a properly-detailed assessment of each sample of interest; thus far, this lens arguably gives us the best images.

3. Zoom into selective areas and image with the 20x and 40s air objectives. This allows us to focus in more on the evolution of microstructure, guided with the overall tilings from the 10x.

You will see this structure going forward for the observations of time-dynamics of these colloid-polymer mixtures. Note that this specific set of steps would *never* have been developed from entirely ground-based studies. Instead, we developed these procedures based on real experience aboard the ISS.

XII. ACE-M2 FIRST-ROUND FLIGHT OPERATIONS: MAJOR MILESTONES ACHIEVED

With ACE-M2, we sent up almost two dozen new, different samples, and did not know *a priori* if they would mix properly on orbit, how they would look in the microscope, or whether we could observe their dynamics with the instrumentation and constraints we have. After the first round of on-orbit flight operations, we have a number of significant milestones—that we can mix, load and image the samples; and that we now have flight operations procedures that will give us good data going forward, within the envelope of our resources. Over two 48-hour continuous runs (run 1 was 4-6 June 2014; run 2 was 18-20 June 2014), we were able to examine all of the samples (except for the well that broke). Because we looked at different racks over each of these runs, I am combining my discussion of the results treating this as one "round" of the experiment. I will describe a few of the results from the samples that we have thus far learned, in addition to everything else. As with most interesting science experiments, the answers beget new questions, which is why this is an exciting project to work on!

A. Samples can be mixed and imaged

As we now know from the first images, the mixing and optics are working well, so we can take images and see all of the samples well. This is a major success and demonstrates overall the samples are good, and that there is no obvious show-stopper preventing us from gathering good data from our samples.

For reasons that I will elaborate upon in a later post, we are still coming up with a system to label and organize all of the image data, so this summary of results will wait until that process is finished before I add the processed images.

B. Dye samples

We can image the fluorescence from all of our dye samples, using the Texas Red and FITC filters, as appropriate. We don't see any structures in these samples, so everything is as we expect. The field of illumination is not even, with sever vignetting near the edges for the lowest magnifications. These samples which we know to be spatially uniform might therefore provide a way to correct for inhomogeneous illumination.

C. Colloidal suspensions

We can observe different levels of brightness for different volume fractions in the simple particle suspensions. Ultimately, we want to be able to calibrate overall brightness with volume fraction, so these samples will hopefully provide a calibration mapping between image intensity and colloid volume fraction. This is critically important to the science, and a major advance of the ACE experiment over, say, previous BCAT iterations. We have always been interested in measuring the volume fraction after phase separation is complete, but cannot do so on the ground (because the phase separation process is different in microgravity, which is why we do the experiment in the first place!). Being able to measure that with ACE in our phase-separating samples would be a major advance.

1. Science is serendipitous

Even when a sample may "fail" by our pre-existing criteria, we can still learn interesting things, and turn that into a success by thinking along different lines. One of the colloidal suspension samples appears to have had a stir bar stuck in a dense suspension, very likely because the stir bar happened to be stuck in a place around which colloid sedimented densely, preventing the bar from being freed later on orbit. However, we know both the starting volume fraction of the sample throughout the whole sample well, and the fraction of that well occupied by dense colloid that sedimented. This allows us to estimate the volume fraction, which we expect to be near to the hard-sphere glass transition volume fraction of 58%. That would give us an additional volume fraction point on our calibration curve— one which is not possible to create otherwise, because you physically could not load a colloidal suspension at that density! Sometimes, even an initial failure can give you data you could not otherwise acquire!

D. Colloid-polymer mixtures

We have three colloid-polymer mixture sample classes. One is a phase-separating mixture of small particles and

polymer; another is a phase-separating one with large particles; and the third is an arrested gel (at least on earth) that involves large particles and polymer.

1. Phase separating samples

Initial observations indicate that the mixing is sufficient, and we can watch the evolution of structures in these samples over several days. The timescale is quite appropriate for the ACE experiment: samples change over hours or days, making useful the collection of similar sets of data over the course of days to weeks. They are not changing so fast that we will miss the activity during sample mixing and loading into the LMM; nor are they so slow that no activity is discernable during our runs. This is all very good news.

2. Gel sample

Moveover, after waiting several weeks, we see the formation of stable colloidal structure which may or may not be kinetically arrested; we are eagerly awaiting new data on this particular point.

XIII. ACE-M2 RUN 4: COMPARING TIME EVOLUTION AMONG WELLS WITH THE SAME SAMPLE

The main purpose of Run 4 for ACE-M2 is to observe the time evolution of phase separating samples in platter 2105, which has five wells all with sample 21 plu_ACEM2_lg_ps, the phase-separating colloid-polymer mixture using the larger particles. We know from the comprehensive survey in Run 2 that these samples visibly phase separate, so the goal is to look at the different wells and see if they all behave the same—as they should, given that the sample is identical. There may be some minor variations due to slightly different bubble size and/or mixing by the crew, but overall we expect similar evolution and structure.

A. Strips 608 and 613: samples A1, C3, C4 and C5

As we are standardizing on procedures, we first image the entire well with a 10x composite tiling at a depth of 80 microns, which for samples A1, C3, C4 and C5 are pretty much the same:

The wells look mostly well-mixed, with a small amount of phase separation visible as the texture in the images develop. But the feature size of the separation is similar in all wells, so this is suggests we are being consistent in loading, mixing and imaging.
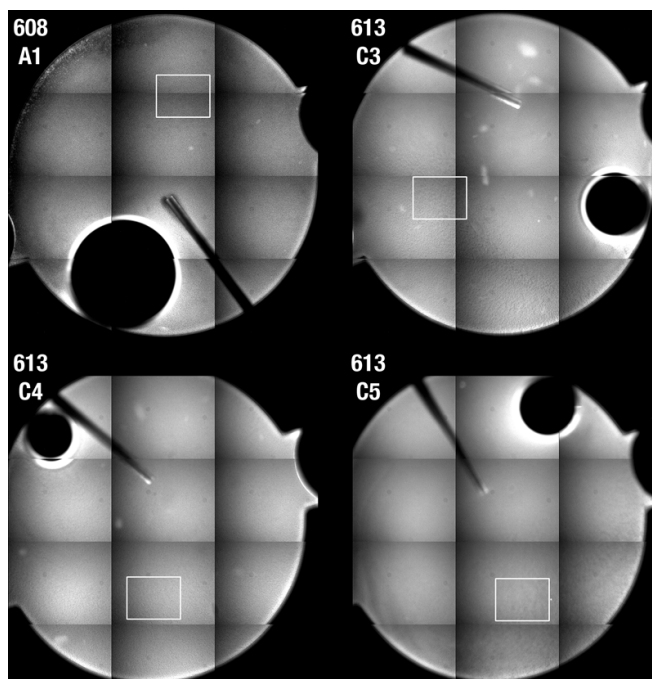

FIG. 11 four similar phase-separating samples A1, C3, C4 and C5

B. Strip 618: sample B5

By contrast, sample B5 is completely different. There is some structure forming, which is at a much larger length scale: uniformly far more coarse, throughout the sample well.

This differs significantly from the other four wells, and there is no obvious scientific reason that we should expect this. Hopefully we will be able to resolve this mystery with more data as it is collected.

XIV. DATA ORGANIZATION CANNOT BE DEFINED BEFORE THE EXPERIMENT RUNS

We have received hundreds of images from the first three runs, and the current fourth run, from ACE-M2 over the past few weeks. Yet I haven't been posting very much in the way of analysis. Why? It's not because I haven't received or looked at the image, but because of another fundamental challenge: how the data is organized and presented to us (as the PIs) from NASA and ZIN, who are running the experiment.

A. We don't know ahead of time what data we will take

NASA typically has an engineering culture, where goals are specified, often with mind-numbing specificity, and criteria for progress and success defined. This is fine when the task to be accomplished can actually be defined ahead
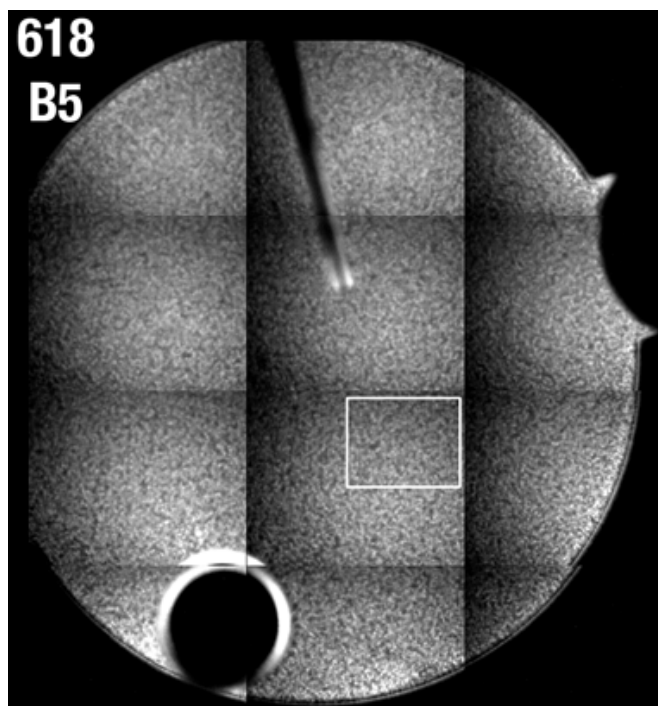
FIG. 12 different-looking phase-separating sample B5

of time, as in the design, engineering, fabrication, launch and operation of a piece of equipment. But this mentality is a less convenient fit for scientific experiments, where the main objective is to learn something *newe* about a scientific phenomenon. This is especially true in our little corner of soft-matter physics, where we do experiments quickly, change things rapidly in response to what we see, then do another experiment—much like the "fail quickly" mentality of Silicon Valley startups.

B. Lots and lots of potential data we could collect

To that end, we launched a wide range of samples of different kinds of materials: dyes, suspensions of colloids in solvent, and colloid-polymer mixtures that undergo phase separation and gelation. The LMM microscope has several objective lenses (2.5x, 10x, 20x, 40x, 63x, 100x) and several modes of imaging (bright-field, fluorescence) and the fluorescence mode has several filters to excite and detect at different wavelengths. And within each sample, there are several different locations that we could image, and a variety of depths. So there are thousands upon thousands of possible choices we can make to collect an image from the sample.

This tremendous flexibility makes ACE a potentially powerful experiment, but at the cost of simplicity. With BCAT, we had a single view (fill the frame with the sample), and only one sample at a time—which takes several weeks to run. The data involves a single time series of a few hun-

dred images, which get nicely zipped up and posted weekly by our extremely talented and hardworking point-woman at ZIN, Cathy Frey. Nonetheless, while the BCAT data is now easy to organize, it took several years and probably a dozen runs before we got our stride in what images to collect. With ACE, the problem is probably two orders of magnitude more complex!

C. Existing data structure therefore represents a best-guess by engineers years in advance

At the moment, there is a relatively byzantine file-folder structure that places images from data sets in a tree labeled by a number of things, including date, experiment number, and objective lens. We didn't know what samples we were going to launch until a few months before delivery, because we need always to keep the experiments as up-to-date as possible, to make sure we are using the most advanced sample preparation and to tie into the science that is actually interesting *now*. As convenient as it is for the bureacrats, specifying these things years in advance only guarantees that the results are not interesting to anyone.

By contrast, the instruments (e.g. the LMM) must undergo years of development and testing before launch, and simply cannot wait until we have samples ready, because of all of the constraints in engineering that operating on-board the ISS requires. So the engineers who build the hardware and design the software and protocols *cannot possibly know* what the actual experiments will be. This is the challenge of the long-latency structure of operating experiments in microgravity (and is absolutely not endemic to NASA; ESA has exactly the same issues, as do other agencies).

All of this is **absolutely not a criticism** of anyone or any part of the process. It's just a big challenge that we must overcome to be successful in doing real, dynamic, new science onboard the ISS. Nevertheless, this leads to an important truth:

The organization, transmission, storage and curation of data is a fundamental part of doing both proper engineering and actual new science onboard the ISS, which is almost never mentioned, and people never really think about.

It's basically assumed that, by some magical process, whatever data the PI wants will magically appear online, which they should hurry up and download, analyze, and get results back *this week* to send results back to everyone in the weekly science summary.

For any given week, I can tell you what the science summary is: we got data, and we are trying to figure out what we are looking at, and if it's correct. Jumping to any more rapid conclusions—or, worse yet, trying to spread the message in

public, to the press, or via social media—is courting cold-fusion disaster. Yes, the news cycles are quick, and people want an instant reaction or result spread immediately.

Unfortunately, the process of actually doing science is limited by human brainpower, and all evidence suggests that social media makes us if anything stupider. I certainly haven't become faster at fundamental understanding due to the advent of social media, though others may be more talented.

Nonetheless, the process of understanding what is happening quickly improves with experience. We had a good idea what we were seeing by the third round of BCAT phase-separation samples (though recent data from BCAT-KP is fundamentally different—therefore quite exciting—but then we are back to not know what is going on—and that's where the fun is). But ACE is a complex experiment where everything is new, and the main reason we launched so many different kinds of samples is that we didn't know what to expect!

And this is where the data organization is a big challenge, basically of the chicken-and-egg type. We don't know what images we are going to collect, so we can't specify how it should be organized. So we get what the engineers thought would work best, basically what is logical from a collection standpoint, which almost by definition is not going to be ideal for the science. So it is an enormous task to sort everything out, and validate the data: how do I know that an image I open up on my computer is from the right sample, with the combination of parameters (lenses, camera settings, position, depth, etc.)?

Ultimately, there needs to be a *combined, close effort between the science-based PIs and engineers (in concert with operations)* to define the right procedures for collecting, labeling, and transmiting the right data from ISS to NASA on the ground and ultimately to us in the lab who will analyze the data. To do this, we need first to understand the challenges of the existing systems, as well as the scientific requirements, then develop a proposal for moving forward. **And this cannot be done before actually acquiring and receiving significant data from ISS; there is no way to do this ahead of time!**

XV. LIMITATIONS OF THE CURRENT DATA ORGANIZATION

The current way data is collected and stored on LMM has several fundamental issues which must be resolved before the set of images collected onboard ISS becomes usable experimental data. Just as the raw voltage levels from each pixel of the camera need to be assembled into a digital image, those images must be assembled into well-organized, well-documented collections in order to be at all useful. And those images must be connected

A. Metadata is not stored with the images

The *metadata* of each image, including the camera settings (exposure, gain, black-level offset), microscope settings (objective lens, filter, illumination), sample location (sample well, and x-y-z position within it) are not stored in the image itself, for example in metadata fields within a TIF image (which could easily defined to contain this). The software that writes the image to disk does not incorporate any of this information with the image itself. So there is no "self-documentation" of each image, which represents a good practice in general, especially when we have so many images. Anything else quickly becomes prone to error.

B. Some information is stored in the directory

Right now, which sample well and platter, which can be mapped 1-to-1, then the objective, and the depth within the sample, are each stored in various directories / folders, within which the images for each day are sorted. There are additional levels of folders for the timing (seconds elapsed since experiment was run) and an experiment number defined by the operator.

C. When a number is assigned to an experiment, and what an experiment includes, varies significantly

As we are defining what data to collect, what amount corresponds to a new experiment number has varied both over time, and between operators. So data gets put into new experiments in some places in a way that is not the same as other, ostensibly identical data.

D. File names are basically all the same

This is a significant problem when it comes to data organization. A typical filename is `00001_00000.tif`, and in fact remains the same for different samples, and different days. Thus, if a file is moved out of its directory, all information is lost and it cannot be checked to see where it came from.

Moreover, when we look at the evolution of samples over time, having the same exact filename means I cannot simply drag images from the same place, objective, depth, and settings from successive days into the folder, but have to rename each one individually, without direct access by the operator who collected the images. This makes any form of automated analysis impossible, and even manual analysis is a challenge to even keep track of what images I am looking it.

Of course, there was no obvious way to specify how the engineers could have added the right information ahead of time, not knowing what samples would be, how we would look at them, etc. etc. Probably the best they could have done was to generate a random alphanumeric code as part

of each image's filename, which could then be later correlated, but that would not have been much of an improvement.

Instead, what we need is a comprehensive plan to organize the data we have collected, which involves close cooperation between us on the science / PI side, the engineers who designed the hardware and wrote the software, and the operators who actually collect the data. So here's the current proposal for how to move forward, which I and Louis Chestney (who wrote the software and collected a lot of great data) came up with:

XVI. HOW WE PLAN TO ORGANIZE THINGS

Unfortunately, there is no perfect, or even optimal way, to organize the data, involving thousands of images from dozens of amples on dozens of days of operations. We have a number of constraints, conceptual and practical, that have to be addressed.

There is always a tension between organizing the data in a way that is best for the subsequent analysis, and one that reflects the history of how it was collected. The problem is that the history can be haphazard, in our case jumping around to take different samples at different settings. And the analysis is never defined until basically it's done, and we don't want to have to do this process more than once.

Moreover, there is a limit to the number of characters that fit comfortably within a filename before some of the computer systems that handle the files get unhappy (thank you IBM and Microsoft from the 1980s for this horrid legacy).

So for the most common / redundant parts of the metadata, such as well, sample number, and objective, we use folders to hold that information. This is more for convenience and making it easy to check / validate the images, as well as consult historical records. For things that change each run that are important (e.g. sample position, date), we put that in the filename, which also serves to document . And for the less-important things (camera settings), we keep that information in a separate database of sorts.

A. Folder organization

The first set of folder hierarchies correspond to what sample well we are looking at, since that is the way the raw data is actually collected (i.e. at one well at a time). So the first three levels of directory structure [with examples] are :

1. Platter [`platter_2105`]

We have two platters (2104, 2105) in ACE-M2, and one platter with our samples (2109) in ACE-M3.

2. Strip [`strip_E_618`]

Each platter has three strips, so A-C (with their numbers) are contained in sample 2104, D-F are in 2105, and G is in 2109.

3. Well [`well_E4_s22`]

Each strip has five wells, numbered 1-5, and each well contains a sample (1-22 in ACE-M2, and probably 23-27 in ACE-M3).

With these levels, the directory for each well identifies its platter, strip and sample, all by number, which can be double-checked against the documentation, and gives a very quick reminder exactly what sample is being looked at. There may be path length limitations, in which case we might have to shorten these names.

Now, within this, there are two fundamental ways we could organize the data:

- by date, creating a new folder each day, and within that, subfolders for the different magnifications and positions.

- by objective lens, so that data from each magnification is stored closely, and then within that organize by date.

There are several advantages and disadvantages to each approach. Because we are looking at the evolution of samples over time, and we want to compare images collected under nearly-identical conditions over time, it makes most sense to group by all other parameters besides time first, and then have the images in the smallest subfolders differ only in time they were collected. This also facilitates the analysis, because the same operation applied to each image within a folder makes sense only when the images represent the same conditions and positions.

Therefore, within each well folder, we have several more levels of subfolder:

4. Objective lens / magnification [`10x`]

We image each sample with several objective lenses, and each sample has a different combination used. We have ultimately standardized the data collection operations to take 2.5x survey images, tile the full sample at 10x, and zoom into selected areas and collect tiled images at 20x and 40x magnification. The 2.5x images do not focus on a specific depth, so those images will sit in the 2p5x folder. Everything else must be more carefully specified, by how deep into the sample the images were collected.

5. Depth into the sample from the coverslip [z060um]

These will be the main folders that hold the data of interest. In particular, there should only be a single image from a single day (with the exception of near-identical duplication for redundancy). For the 2.5x, the image is of the whole sample chamber. For the 10x, there is a single composite of the entire well. For the 20x and 40x images, there are composites of smaller areas. Individual images that form the source of each composite can then be held in a subfolder, once for each day.

6. Date of data collection [179]

Everything NASA does uses the 3-digit numeric day of the year, which is convenient because it's short, sequential, and takes up on three characters.

Because the composites will be properly labeled, as long as the folder of the source images can be identified, these images need not have unique names (preferred but not necessary), since they will always be referenced to the uniquely-named composite images.

Therefore, the example path for the folder would be:

`/platter_2105/strip_E_618/`
`well_E4_s22/10x/z060um/`

B. Naming files

The next step is to define a file naming convention. We want to preserve as much information to identify uniquely the image in a transparent way, especially when it gets moved to a different folder (e.g. for analysis). To that end, the sample platter, strip, well, and sample should be included, in the most compact form minimizing characters but preserving the unique parts of the information (since the more complete info can be read from the directories).

- `p4` for platter 2104, `p5` for platter 2105, etc.

- `a` for strip A, `b` for strip B, etc.

- `1` for well 1 on that strip, `2` for well 2 on that strip, etc.

- `s01` for sample 1, `s02` for sample 2, etc.; two digits cover all samples

- `02x` for 2.5x magnification, `10x` for 10x magnification, etc.; two digit numbers

- `z060` for 60 micron depth relative to cover slip, `z100` for 100 micron depth, etc.; three digit numbers

- `xy1` for first composite xy-position, `xy2` for second, etc; `xyA` for position A of individual image within a

composite, `xyB` for position B, defined by the maps/tables. Composites are distinguished by the number, where individual images have a letter. No composite has more than 16 unique positions, so a single character is sufficient.

- `180` for 180th day of the year, etc. for other dates

- `15520` for experiment number 15520, to tie back into the log books.

So a complete filename and path in the example would be:

`/platter_2105/strip_E_618/well_E4_s22/10x/`
`z060um/p5e4s22_10x_z060_xy1_180_15520.tif`

This preserves a lot of information for each sample, and correlates it back to the date it was collected and the experiment number, which contains all of the imaging and position parameters. This way, if any image gets separated from its directory structure, all of the information can be reconstructed and recovered.

C. Practical implementation

Ultimately, we will have to set up a data store for both we as the scientific team, and ZIN as the engineers and operators, to have common access to move around and rename files appropriately. As you can imagine, this will take a significant amount of time and effort all around. This is an absolutely critical task that needs to be done before any data can be analyzed carefully. There may be some basic results which I present, but this organization is required before any full, systematic analysis can take place. And that is what we will be working on a lot going forward.

XVII. PROCESSING RAW IMAGES FROM STATION

We think that the samples we are looking at in the current run (the fourth) on ISS with ACE-M2 are undergoing phase separation and gelation, based on ground studies and preliminary images from orbit. To understand how these samples evolve over time, we perform nominally the same procedure—collecting images from the same positions, magnifications, depths, at similar settings—each day we run the experiment. In principle, then the images from successive days should have the same (or at least very close) brightness and contrast, and should overlay on top of each other exactly. To see whether this is happening in practice, here is the raw sequence of 10x composite images of sample 22 (platter 2105, strip B618, well 4) taken on days 2, 3, 5, 8, 11 and 15:

The position shifts slightly, and the brightness changes. Clearly, the images are not exactly aligned, and the motion distracts from being able to watch any evolution in the sample, as well as interfering with any automated analysis.
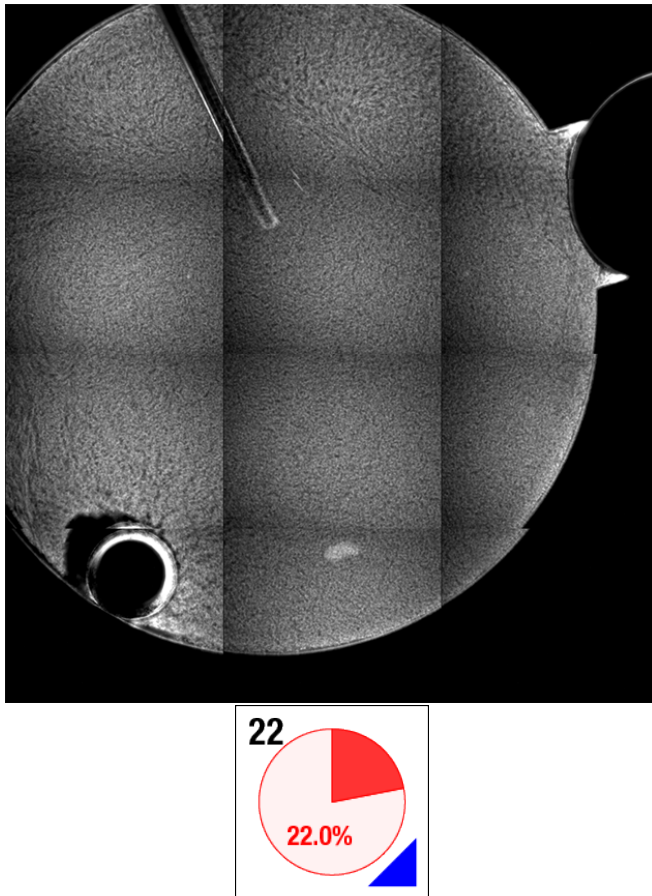
FIG. 13 10x composite, raw images

A. Pre-processing image data

To fix these issues, I will align the images within the time sequence, and match their brightnesses so that the intensity doesn't change so dramatically.

1. Aligning the images

The first major task is to align the images from subsequent days of data collection. I first rename the files, then in Photoshop CS6 import the set of images into separate layers in a composite image, with `File > Scripts > Load Files into Stack...`, enabling the option to `Attempt to Automatically Align Source Images`. This works pretty well for these images, where the overall shape of the well can guide the alignment algorithm.

This process is not fast, taking several seconds per image, but for just a handful of images, it works well enough. By contrast, for BCAT, where we have hundreds of frames, I create an image sequence movie in Adobe After Effects, and use the tracking features there to far more quickly do the alignment.

I then crop the images so that there are no blank borders, and save the file as a mult-layer `.psd` file.

2. Matching intensities

For color images, the Photoshop feature `Match Color...` is very good and extremely helpful. In our case, however, grayscale images do not have the same feature, and the option is unavailable. Instead, using a combination of `Levels` and "'Curves, I adjust each image to have the same mean (a little over 100 in 8-bit grayscale values) and standard deviation (about 60 8-bit grayscale levels).

3. Building the animation

In the `Timeline` palette, I `Create Frame Animation`, then in the drop-down menu select `Make Frames from Layers`. The great thing about this feature is that it otherwise doesn't affect anything about the image or its layers. The final step is to export to an animated `.gif` file, using the `Save for Web...` dialog box. There I reduce to final web resolution, and create the final file:
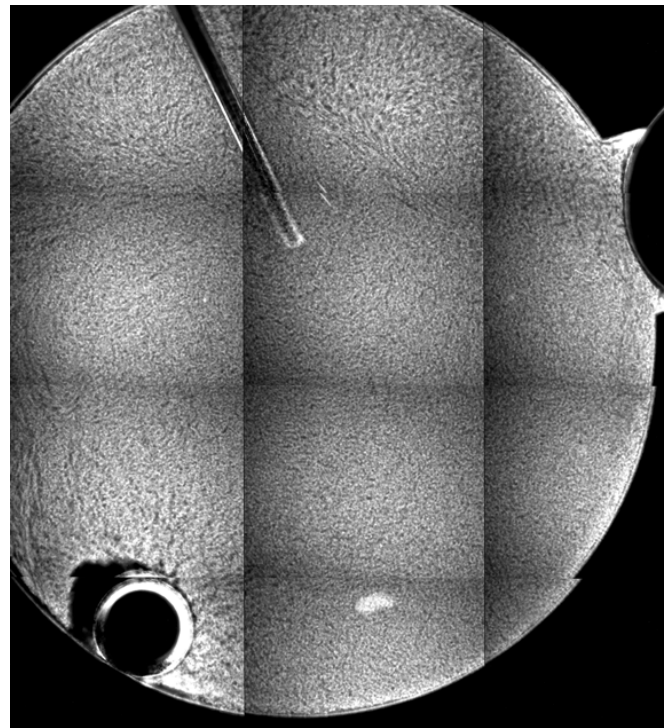

FIG. 14 10x composite, aligned images

B. Higher magnification

I apply the same procedure to the higher-magnification 40x composite. First, the raw data (just resized):
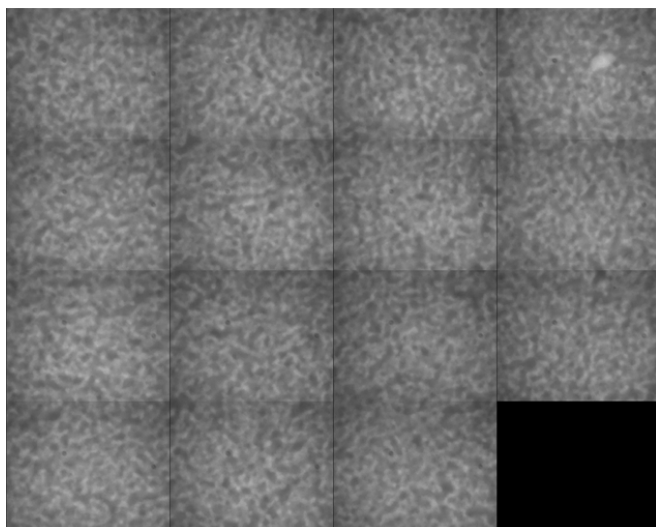

FIG. 15 40x composite, raw images

And then the corrected version, where the aligned im-

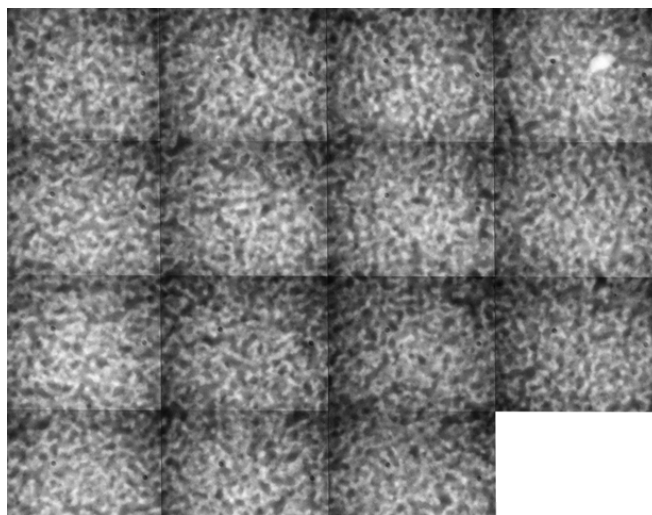ages have a mean of 128 and standard deviation of 40 8-bit grayscale levels:

FIG. 16 40x composite, aligned images

Several things are apparent from these sequences. First, the stage is not perfectly reproducible from a mechanical standpoint. The composites seem to move around a little bit, likely due to mechanical backlash in the stage. Second, intensities are not constant from day to day.