

**DETERMINING AN APPROPRIATE METHOD TO SIMULATE PUMP SHEAR  
ON THE DIATOM *NITZSCHIA* SP. AND A METHODOLOGY TO QUANTIFY  
THE EFFECTS**

A Thesis

by

JARRETT WAYNE LASSIG

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Approved by:

Chair of Committee,	Ronald E. Lacey
Committee Members,	Raghupathy Karthikeyan
	Heath J. Mills
Head of Department,	Steve Searcy

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## ABSTRACT

When cultivated properly in bioreactors, microalgae have been found to produce vast amounts of biomass. In the case of diatom cultivation where the organisms will fall out of suspension quite easily, paddle wheels or pumps are the primary means to maintain the necessary velocity in the raceway. This study will focus on the potentially harmful shear stress these devices may impart onto the organisms.

The system used to impart shear stress to a diatom culture was a cone and plate viscometer. Cells were counted using a fluorescein diacetate staining method with a fluorescent and brightfield microscope. Under the white light all cells were visible while only the healthy cells were visible under fluorescent light.

The sample was exposed to shear stress with the cone and plate viscometer at 6 Pascals for 10 minutes and compared against a non-sheared sample. For each sample, 5 pairs of white and fluorescent light images were captured, counted, and averaged. A non-sheared sample was paired with a sheared sample to calculate the decrease in cell viability. The slope was calculated from the plot of shear stress and cell viability for 9 strains. In each case shear stress resulted in a significant decrease in cell viability; however, there was no statistical difference between strains.

While effective, this method would be impractical for a commercial algae cultivation facility as the viscometer in this study costs approximately \$100,000. Therefore, tests were performed to determine if a rotary mixer could be substituted for the viscometer. The hypothesis was that the cell damage was a product of shear stress

and exposure time. For the viscometer test, the shear exposure was 3600 Pa s. Two rotational mixer tests were performed, one at 1250 RPM for 7 hours and one at 313 RPM for 28 hours, providing the same 3600 Pa s shear exposure. After staining, cell viability decreased 35.62% and 11.07% in the 1250 RPM and 313 RPM test, respectively. This difference was significant compared to the 6.04% decrease in the viscometer test. The increased cell damage was attributed to turbulence in the mixer tests and the basis for further study.

## **DEDICATION**

To

My Family

## ACKNOWLEDGEMENTS

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## **NOMENCLATURE**

ANOVA	Analysis of Variance Test
LS Means	Least Square Means
OD	Optical Density
RPM	Revolutions per Minute
SSD	Shear Stress Duration

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# CHAPTER I

## INTRODUCTION

Photosynthesis is the most life-supporting process on the planet. Microalgae are photoautotrophs that use carbon dioxide as a carbon source and light as an energy source (Chen, 1996). When cultivated properly in bioreactors, microalgae have been found to produce vast amounts of biomass and up to 80 percent dry weight oil depending on the species (Christi, 2007). Oswald (1988) extensively studied the engineering aspects behind microalgae growth, primarily the design of the system to keep the algae in suspension in the media in open raceways. If the algae fall out of suspension, they will accumulate on the bottom and not grow at peak performance since the effective exposed surface area is much lower because of self shading (Bartok and Mason, 1958). Additionally, the culture must be mixed to prevent light bleaching (exposure to too much light), which is harmful or even fatal to the algae. Therefore, the media must be continually moved at a certain velocity through the use of paddle wheels or pumps. Potentially damaging shear is introduced into the system from these agitation devices. As systems get larger, the feasibility of paddle wheels decreases, making pumps the primary source of agitation; but, pumps induce much more shear than the paddle wheels. If tangential velocity is an indicator of shear, the paddle wheels are moving at most only 1.5 feet per second since that is the velocity of the culture in a raceway. A pump impeller will have a considerably higher tangential velocity to keep the fluid moving at the same speed as a paddle wheel. Shear caused by pumps has been shown to have

measurable effects on the growth of certain algal strains, though few studies have been performed to scale the effects from a laboratory to a commercial facility (Gudin and Chaumont, 1991; Barbosa et al., 2003).

Another consideration about pumps is economic in nature. According to modeling of a 32 hectare facility by Justin Stepp at Texas A&M University (Stepp, 2011), capital costs for electric mixers with sumps was estimated to be \$144,550. An estimated of operating costs at this facility included \$15,781.25 per hectare per year in electricity for the mixers. These values are considerable in the production of microalgae, and in the case of diatoms could hinder growth or even cause total death. Therefore, in order to keep the algae in suspension, the mixers not only would be costing money to install and operate, but could be lowering production by harming the algae.

Additionally, an operator of an algae facility needs to know if a different species will grow in his existing infrastructure before attempting large scale production, or risk wasting a considerable amount of time and money. For instance, if a facility had been growing one species (i.e. a green algae which is more robust) and then would like to switch over to a diatom that has been shown to produce more oil, the facility could potentially face problems growing this silica-walled diatom. Therefore, the operators of this facility need a quick, accurate model to determine if diatoms will grow using an existing setup.

Diatoms have a higher density than other algal strains because of the silica cell wall which causes diatoms to settle out of suspension quickly when removed from agitation. This settling could be beneficial to harvesting, but conversely requires more

agitation during cultivation. Therefore, the design of a system to cultivate diatoms must keep the culture stirred while not imparting too much potentially damaging shear stress. As algae gains more interest as a fuel source, research on these effects is vital. This research focuses on the effects of determining an appropriate methodology to simulate large-scale pump shear on the diatom *Nitzschia sp.* and an appropriate methodology to quantify the effects of shear on diatoms.

## CHAPTER II

### LITERATURE REVIEW

Shear from pumps is a primary reason why large scale, outdoor algal growth does not follow the same growth kinetics as at laboratory scale (Gudin and Chaumont, 1991). Midler and Finn (1966) showed that excessive agitation was harmful to the algal species *Protozoa*. Thomas and Gibson (1990) ranked microalgal groups in order from most shear tolerant to least as the following: green algae, blue-green algae, diatoms, and lastly dinoflagellates. Dinoflagellates being most susceptible to shear, and one cause of red tide, have made this microalgal group the focus of most studies; diatoms have received much less attention.

Limited experimental work over the last 20 years has attempted to quantify the effects of pumps on microalgae. These studies usually involved only one or two species, but in each case, shear was found to be detrimental to the growth of the algae. Jaouen et al. (1998) used a circulation system to continuously pump five liters of algae media through a variety of pumps. *Tetraselmis suecica* was used as it has four fragile flagella. The number of passes through each type of pump was compared to the percentage loss in motility as measured using a microscope. They concluded that centrifugal or rotary vane positive displacement pumps caused a loss of motility while eccentric rotor pumps or peristaltic pumps did not. Sobczuk et al. (2005) used a vertical mixer with multiple impellers up the shaft to shear two algae species, a pennate diatom and a red algae. Measurements of the steady state biomass concentration were used to quantify damage.

The tip speed of the impeller was used to calculate shear values and it was determined that bubbles created more shear force than the impeller. For the pennate diatom, any tip speed above  $1.56 \text{ m s}^{-1}$  was found to be detrimental. Mitsuhashi et al. (1994) used a double-rotating cylinder setup to generate Couette shear flow on the green algae *Spirulina platensis* and *Chlorella*, but their research focused primarily on benefits of agitation on photosynthesis and subsequent algal growth.

Determining cell damage or loss of motility has proven to be a challenging problem. Vandanjon et al. (1998) utilized a Nageotte cell to count cells with a microscope. Cells were considered damaged if they had lost any inner organelles or if the frustule had been broken. Cell damage can also be determined through a staining procedure. When oil producing algae become stressed, they will produce oil droplets, which can be seen under a microscope when stains are used (Cooper et al., 2010). For instance when stained with Nile Red the pennate diatoms cultivated at Texas A&M will show the cell in red and any oil droplets present in yellow; these oil droplets are signs that the culture is under stress. During cultivation minimal stress is desired, hence few oil droplets should be present. The appearance of additional oil droplets after inducing shear stress may be useful for determining cell health as these droplets may show a culture which is stressed and not growing optimally. Another stain, SYBR Green, has been used to show DNA in algal cells and to identify undesirable bacteria and other species in a growing culture (Vitova et al., 2005). This stain would show any piece of a cell with DNA, possibly providing a means to check for damaged cells.



Michels et al. (2010) reported a procedure where a concentric cylinder on a viscometer was used to expose diatoms to known amounts of shear stress and cell damage was quantified using a staining procedure utilizing fluorescein diacetate (FDA) to stain only the healthy cells. A stock solution of 46 mg of FDA stain mixed with 10 mL acetone was added to an algae solution at a ratio of 10  $\mu$ l of FDA solution to 1 ml of algae culture and incubated for 20 minutes with periodic stirring. Only healthy cells would absorb the stain, thereby providing a means to differentiate between healthy and nonhealthy cells by comparing white light images to fluorescent images under a fluorescent microscope. The total number of cells was counted under white light while only the healthy cells were apparent to be counted under the fluorescent light. Michels et al. (2010) also determined that it was not the shear rate that caused damage but the shear stress imparted on the algae that caused cell damage.

Scaling of shear methods from the laboratory to large systems is a known problem. Most work that has attempted scaling involves air bubble systems. Camacho et al. (2001) concluded that the microalgae *Phaeodactylum tricornutum* was damaged by small bubbles bursting at the surface of the fluid in a laboratory-scale bubble column. But, Camacho et al. also determined the hydrodynamic conditions that did not cause damage in the laboratory could not be scaled up to a pilot scale. Barbosa et al. (2003) revisited Camacho's work and concluded that bubbles rising and bursting in a laboratory-scale bubble column was not what caused cell damage; rather, the formation of the bubbles at the sparger was primarily responsible. From these studies, bubble formation may be the problem when attempting to scale any shear method from

laboratory scale to large-scale where laminar turbulence is generated. These scaling problems have resulted in very little large-scale shear research which is necessary to make algae, especially more shear-sensitive species like diatoms, a viable option for large-scale fuel production.

## **CHAPTER III**

### **OBJECTIVES**

The goal of this research was to determine shear effects on silica-walled diatoms for large scale production of algal biofuels. This research will be used to make informed decisions about survivability of diatoms in certain growth environments, primarily the effects of the pumps used to keep the diatoms in suspension. The specific objectives of the research were:

1. Develop an appropriate shear test apparatus to replicate commercial pumps
2. Determine an appropriate means of quantifying cell damage/growth
3. Provide quantifiable, repeatable data pertaining to the effect of pump shear on algal growth for several algal strains grown at Texas A&M
4. Develop a scaled testing protocol to replicate pump shear testing for other algal strains in order to predict the effects in large scale growth

## CHAPTER IV

### METHODS AND TESTING PROCEDURE DEVELOPMENT

From the literature, the simplest method to impart shear was based on tip speed in a rotational mixer as shown by Sobczuk et al. (2005) which most closely resembles a mixer or pump used in large scale facilities. An initial test utilized a Cole Parmer laboratory mixer (Model 50006-00, Canada) with a 30mm diameter straight impeller shown in Figure 1.



*Figure 1: Cole Parmer 30mm diameter straight impeller used in the laboratory mixer for initial shear testing*

A tip speed of  $1.56 \text{ m s}^{-1}$  was used as a baseline (Sobczuk et al., 2005) and the test was run side by side with a control beaker grown on a magnetic stir plate with a stir bar. Each 600mL beaker was inoculated at a 3:1 media to algae ratio to a volume of 400mL. The mixer was set to maximum speed of 2500 revolutions per minute (rpm) with the impeller 15 mm off the bottom of the beaker and run continuously. The

following equation was used to calculate  $3.93 \text{ m s}^{-1}$  as the tip speed of the impeller at 2500 rpm:

$$\text{Tip Speed} = (\pi * \text{RPM} * \text{Dia.}) / 60 \quad (1)$$

where:

Tip Speed is given in meters per second

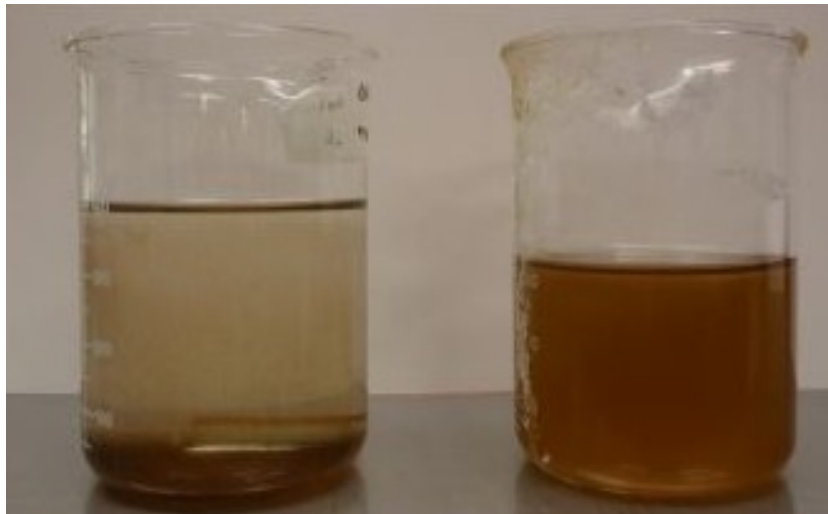
RPM = revolutions per minute of the impeller shaft

Dia. = diameter (meters)

The test duration was 114 hours in a twenty degree C incubator in front of two 15-watt growth lights. Undiluted optical densities were taken at one inch of depth in both beakers at the start, immediately after stirring was stopped 114 hours later, and after 10 minutes of settling. Immediately after stirring stopped, the sheared beaker was much darker and microscopy images using a Nikon Eclipse TS-100-F (Nikon Instruments Inc., Japan) showed many fragments of cells. After 10 minutes of settling, the diatoms in the control beaker settled out as expected while the sheared beaker remained the same; the same was seen after 2 hours of settling. Figure 2 shows the test setup. Figure 3 shows the beakers 2 hours after stirring was stopped; the sheared sample is on the right and the control is on the left.



*Figure 2. Test setup for initial shear testing of diatoms.*



*Figure 3. Results of the initial shear testing of diatoms two hours after mixing was ended. The sheared sample is on the right and the control on the left. The lack of settling in the sheared sample suggests that the diatoms have been extensively damaged and are no longer intact organisms.*

The same experimental apparatus was used to test the shear effects at 1250rpm for the same duration. Optical density measurements were taken initially, immediately after shearing stopped, and after one hour of settling at one inch of depth in the flask. This rotational speed equated to a tip speed of  $1.96 \text{ m s}^{-1}$  which was still higher than the threshold for damage Sobczuk (2005) found for a pennate diatom. As expected, the mixing on this test was not as turbulent, but results similar to the 2500 rpm test were found. The optical density measurements for both tests are given in Table 1 and showed an increase from the initial to final value for each sample (the "Initial to Final Change" column) as optical density would be expected to increase for growing cultures. In both tests there was significantly higher changes in the sheared samples; but when looking at the change after a period of settling ("Final to Final+time Change"), the optical density of the sheared samples did not change compared to the unsheared samples. The control samples settled as expected for diatoms without agitation as evidenced by the lower optical density value after a settling period. This indicated an unhealthy culture in the sheared samples where the optical density had much less change. The 2500 rpm test exhibited almost no change, most likely because of the cells being broken and mixed. For an unknown reason, the 1250 rpm test increased in optical density during the settling period. The microscope images captured by a Nikon Eclipse TS-100-F (Nikon Instruments Inc., Japan) showed fragments of cells in the sheared samples which suggested that the optical density readings did not provide an accurate measure of the shear effects.

*Table 1: Optical density measurements taken for the small laboratory mixer testing at two rotational speeds. Each sample increased in optical density meaning increased growth. But, after agitation was removed the sheared samples did not settle as diatoms should (as evidenced by the "Final+time" measurement) compared to the unsheared diatoms where the optical density dropped significantly.*

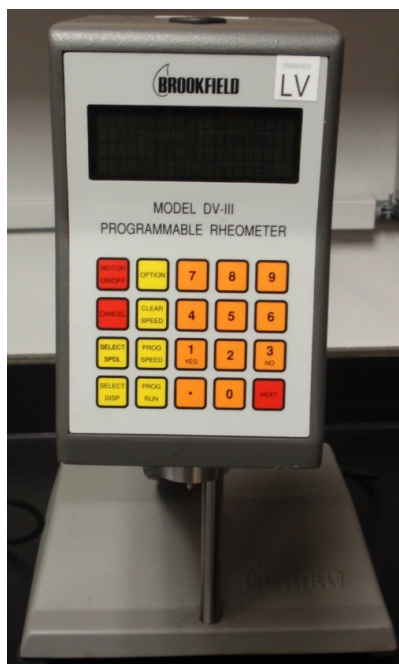
		Optical Density Measurements						
		RPM	Initial	Final	Initial to Final Change	Final + 10 Mins.	Final + 1hr	Final to Final+time Change
<b>Test 1</b>	Sheared	2500	0.045	0.177	<b>0.132</b>	0.171	N/A	<b>-0.006</b>
	Control	0	0.026	0.084	<b>0.058</b>	0.035	N/A	<b>-0.049</b>
<b>Test 2</b>	Sheared	1250	0.171	0.375	<b>0.204</b>	N/A	0.405	<b>0.03</b>
	Control	0	0.201	0.232	<b>0.031</b>	N/A	0.137	<b>-0.095</b>

Consequently, the mixer would successfully apply a shear force to diatoms and excessive shear was seen to be detrimental to diatom health. However, measurement of optical density was determined not to be a viable method for assessing culture health. In general, higher optical density indicates higher cell counts, which represents a healthy culturing environment. But, as seen in this test, the culture, which was clearly damaged based on settling and microscopy images, had significantly higher optical density readings. It was hypothesized that the higher optical density readings were a result of all the broken cell pieces in suspension in the culture. Therefore, another method to quantify cell damage in diatoms was needed.

In order to impart a more controlled amount of shear to the diatom and media, a more sophisticated viscometer was needed. The method reported by Michels et al. (2010) was used as the starting point for this study. Seawater is a Newtonian fluid;

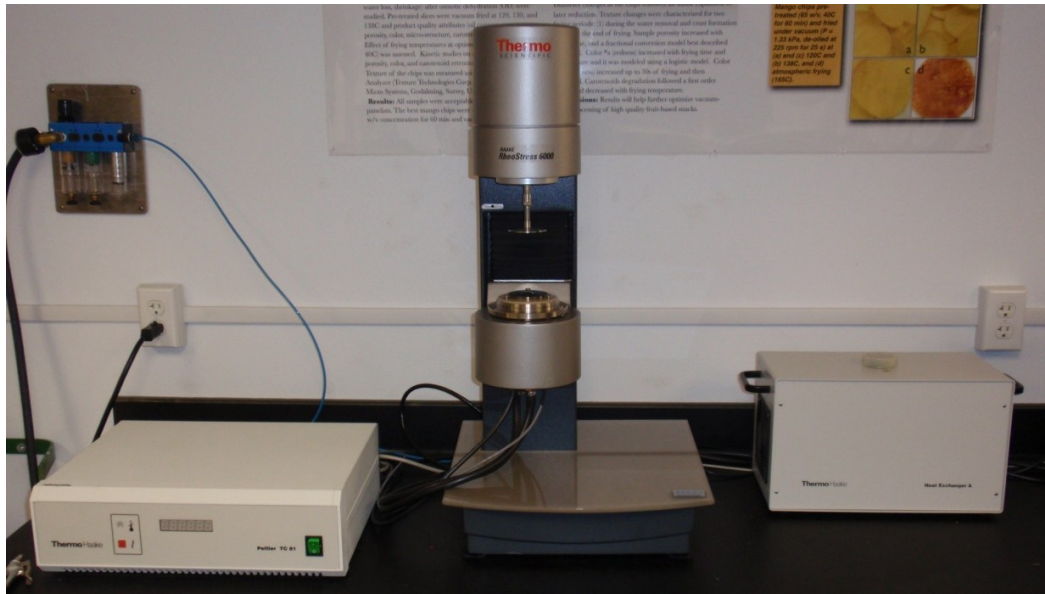


consequently the shear rate and shear stress are linearly related through a multiplier, the fluid viscosity. If the viscosity of the diatoms suspended in media could be proven similar to seawater, this simple relationship could be used to determine shear stress for a known shear rate. A Brookfield Engineering LV Programmable Rheometer (Model DV-III, Massachusetts), pictured in Figure 4, with a concentric cylinder setup was used to determine that the diatom and media suspension was Newtonian. The LV model viscometer is most commonly used for fluids with a viscosity similar to water as higher viscosity fluids overload the torque range, resulting in inaccurate measurements.

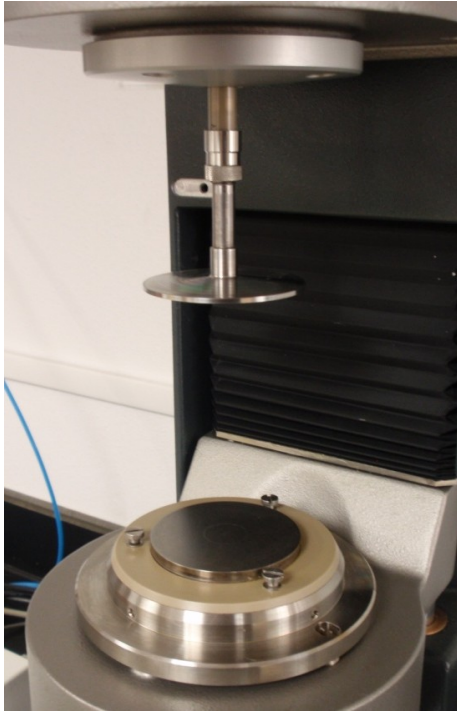


*Figure 4: Brookfield Engineering DV-III LV Programmable Rheometer which was the first rheometer used to impart shear onto the algae. In order to reach the necessary stress levels, the maximum allowable torque for this model was surpassed.*

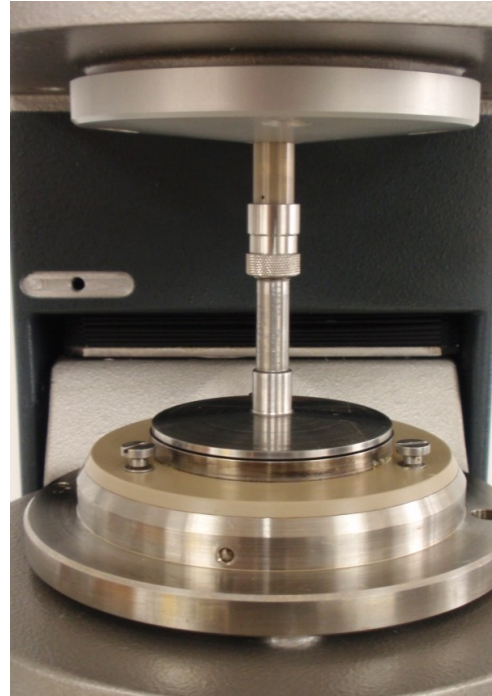
However, the Brookfield viscometer was not able to reach the necessary shear stress. With the torque at 100%, the shear stress did not exceed one Pascal (Pa), whereas Michels et al. (2010) reported that values up to 10 Pascals were desired. Michels et al. (2010) increased the shear stress in the media, a Newtonian fluid, by increasing the viscosity using a gum as a thickener. Adding gum made the fluid non-Newtonian, which would not model the real-life application where the viscosity would remain Newtonian. Thus it was decided to increase shear stress through use of a different rotational viscometer, a Thermo Scientific High Tech Rheometer (Haake RheoStress 6000, Canada) using a cone and plate attachment as seen in Figure 5 below. The viscometer used a 60mm diameter plate with a one-degree angle as the cone on a constant temperature plate shown in Figure 6. This viscometer allowed for much higher shear stress values to be obtained as well as permitting the user to specify a constant shear stress value. One milliliter of algae culture was applied to the center of the lower plate. When the probe reached the set gap, the fluid filled the space between the plates while surface tension prevented media from leaking out the edges, seen in Figure 7. The temperature controller was set to a constant 23° C and the viscometer software was programmed to maintain a constant shear stress for a fixed amount of time.



*Figure 5: Thermo Scientific Haake RheoStress 6000 rheometer which utilized a 60mm cone and plate setup to impart the desired shear stress onto a one milliliter sample of algae.*



*Figure 6: The 60mm cone and plate setup on the Haake Rheostress 6000. The upper part (the cone) had 1 degree of angle and the bottom (the plate) was held at a constant 23 degrees C.*



*Figure 7: The cone and plate setup during testing. Notice the very small gap between the cone and plate which was filled with algae fluid during testing.*

The first objective using this viscometer was to confirm that the viscosity of the fluid could be assumed as that of seawater. To test this, the healthiest looking of the diatom species was selected (LBK009) and the sample was subjected to a shear rate ramping test up to  $1000 \text{ s}^{-1}$  and back down to zero. A graph of the test results is shown in Figure 8. The data followed a linear pattern, as expected in a Newtonian fluid. The slope of the line gives the viscosity,  $0.0011 \text{ Pa s}$  or  $1.1 \text{ centipoise (cP)}$ . Viscosity values for seawater range from  $1.02 \text{ cP}$  to  $1.38 \text{ cP}$  at  $20^\circ\text{C}$  depending on the salinity (Isdale et

al., 1971); therefore the viscosity of the algae culture could be assumed to be similar to seawater.

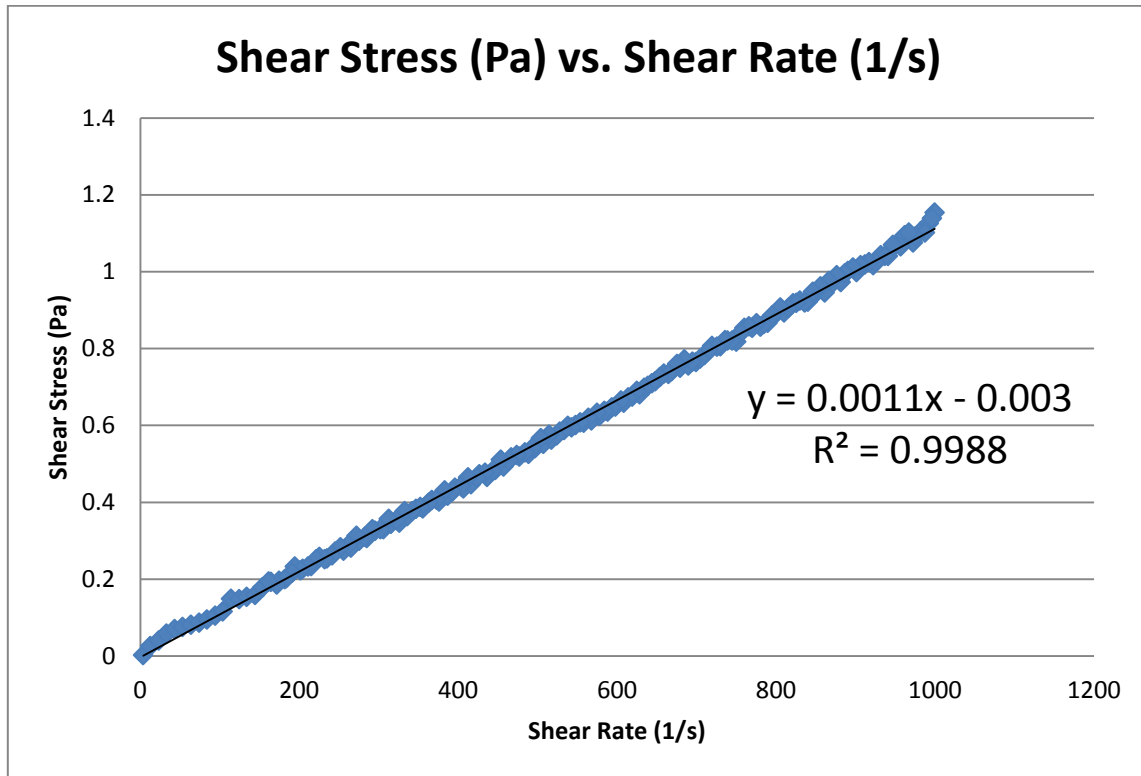
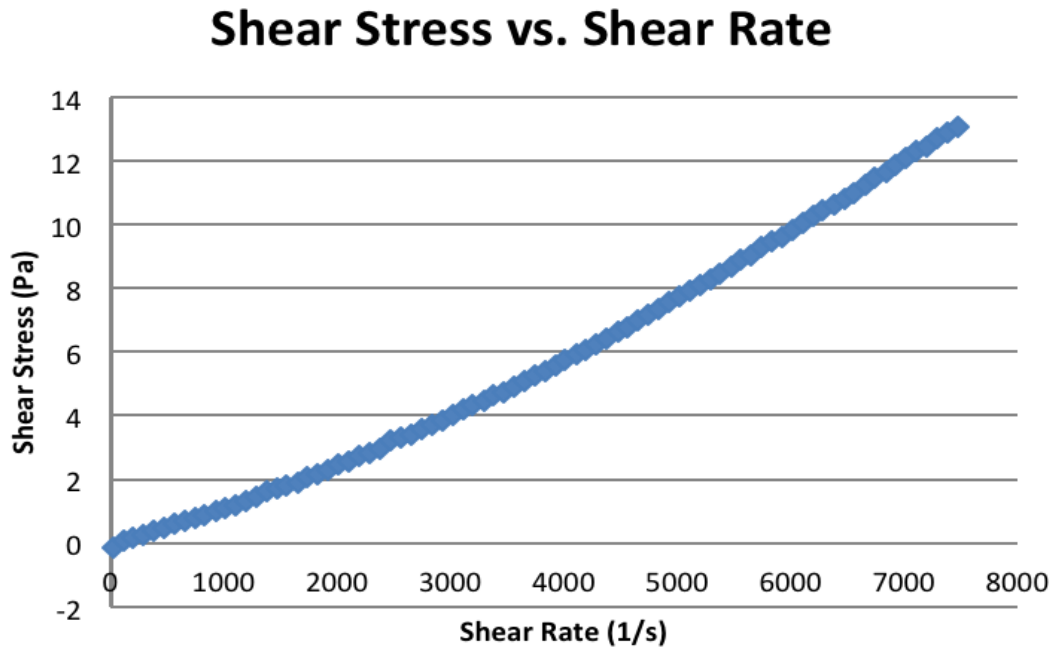


Figure 8. Shear stress (Pa) versus shear rate (1/s) ramping test for a diatom (LBK009) and growth media at 23°C to determine the diatom media behaved as a Newtonian fluid.

A subsequent test was performed in order to reach shear stress values near 10 Pa, which was the identified target value. Results are shown in Figure 9. Shear stress values of up to 10 Pa were achieved at a shear rate of 6000 s<sup>-1</sup>. Therefore, this method was deemed a viable option for imparting shear stress to the diatom samples.



*Figure 9. Shear stress (Pa) versus shear rate (1/s) ramping test up to 7000 s<sup>-1</sup> for a diatom (LBK009) and growth media at 23°C.*

From the initial mixer test, optical density did not provide an accurate representation of the growth or damage caused by shear stress. One option that was considered to determine health was, after imparting shear, to continue growing the sheared sample and compare against an unsheared sample. Two methods of comparison could be used; a visual color test or a cell count. Healthy cultures of these pennate diatoms have a dark brown color that turns yellowish as the culture becomes unhealthy. However; quantifying “yellowish” is a challenging task. Since the determination of a color change is inherently subjective, this was determined to be an unsatisfactory indicator of cell viability.

The second method would be to count cells under the Nikon Eclipse TS-100-F (Nikon Instruments Inc., Japan) microscope and see if the cell counts increase at the same rate for the sheared and unshered cultures. These methods for calculating damage would require a sample size of at least 100 ml to be sheared. However, the small sample size of 1 ml when using the cone and plate viscometer made this method impossible.

Using an FDA formulation and procedure (Michels et al., 2010), 7.5  $\mu$ l of stain were added to 1 ml of diatoms and media. Following the application of the stain, five random sites on a microscope slide were selected and photographed under the Nikon Eclipse TS-100-F (Nikon Instruments Inc., Japan) microscope. The number of cells at each site were counted under white light (brightfield) and then under fluorescent light (stained). Both images were taken at 20x magnification at the same location. The images were saved and each cell or group of cells were counted and labeled. Each image was also labeled with the image file name, the species, the shear stress level, the date of the test, and the total number of cells on the image. The brightfield image was counted and labeled first; the labels were copied onto the fluorescent image, corrected for any changes, and recounted. This procedure was repeated six times per each sample of diatom culture and the five best images were chosen based on clarity of the images. The average cell viability was calculated by adding up the total number of healthy cells (stained total) and dividing by the total number of cells (brightfield total).

A rubric for counting healthy and unhealthy cells was developed. Large groups of cells were avoided when capturing images since these groups made counting individual cells inaccurate or impossible because of clarity. In the event that a group of

cells could not be counted well in either image, these cells were not included in the count. If for instance a group of cells was countable under fluorescent light but not under white light, the group was ignored on both images. These cells were labeled with a red number. In order to give some insight into the counting procedure, the counting process for the sample images in Figure 10 followed this process:

- In circle A, the white light cell count was 4 while the fluorescent light count was 3. In this case, the second from the top cell was not counted in the fluorescent image because it was lighter than its neighbor cells.
- In circle B, the cell wall of most of the cells on the fluorescent image were visible, but many of the cells had the entire inside stained, indicating some difference between the cells; hence, the cells which were only “outlined” by the stain were assumed not as healthy and were subsequently not counted.
- In circle C, one of the cells on the fluorescent image was undetectable (or was extremely faint) indicating it was an unhealthy cell.
- In circle D on the white image, a cell is clearly there. On the fluorescent image, it almost appears the cell is not there; however, upon closer inspection it is visible, but the cell wall did not stain, suggesting that the cell was unhealthy and therefore uncountable.

The same process was used for all the images.



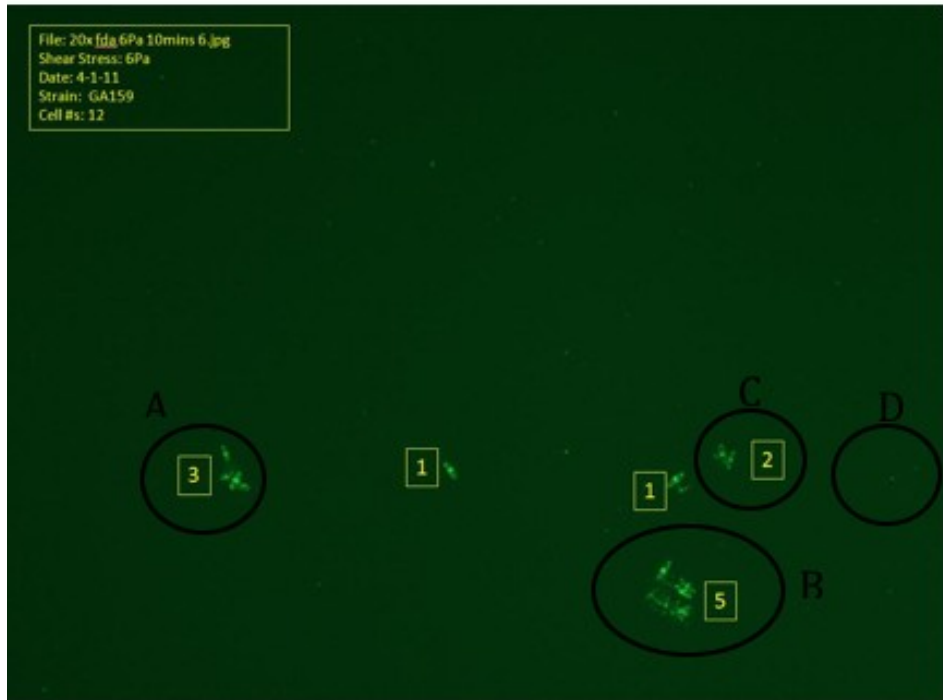
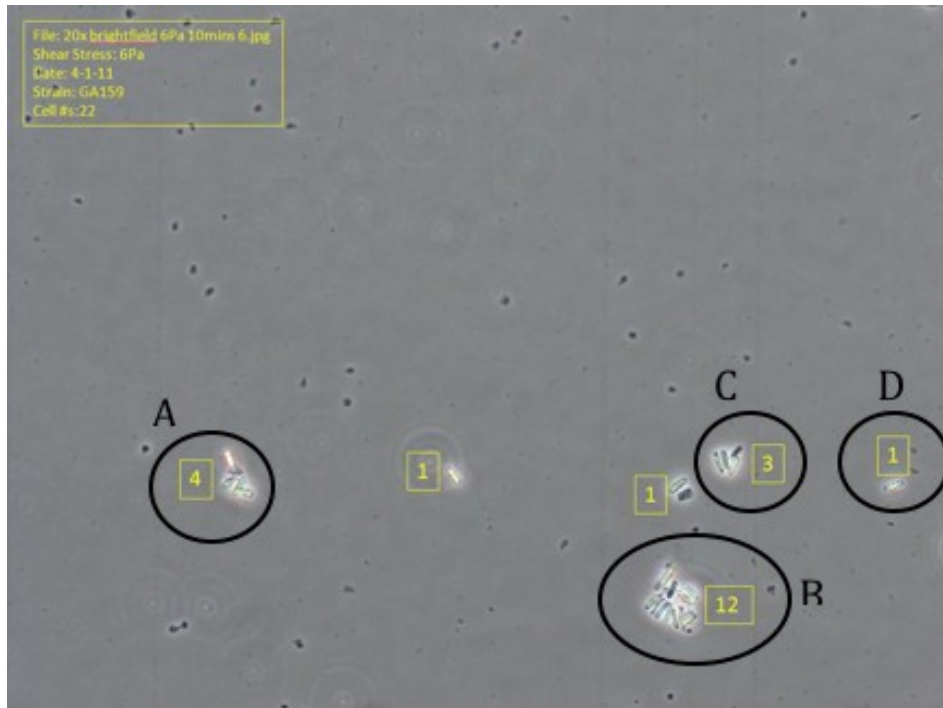


Figure 10. An example of corresponding images taken under brightfield (upper) and fluorescent (lower) light with labels and notations for data acquisition. The fraction of viable diatoms is the ration of the number of cells in the fluorescent image divided by those in the brightfield image.

With the method development complete, the diatom LBK009 was used for the first round of tests at 1, 2, 4, and 6 Pa of shear stress for 10 minutes and 20 minutes each. Ten minutes was selected as the minimum test time based on literature findings that after 8 minutes the majority of cell viability decrease had occurred (Michels et al., 2010). A general downward trend can still be seen in both the 10 and 20 minute tests with increased shear stress in Figure 11; as expected the 10 minute test had higher cell viability.

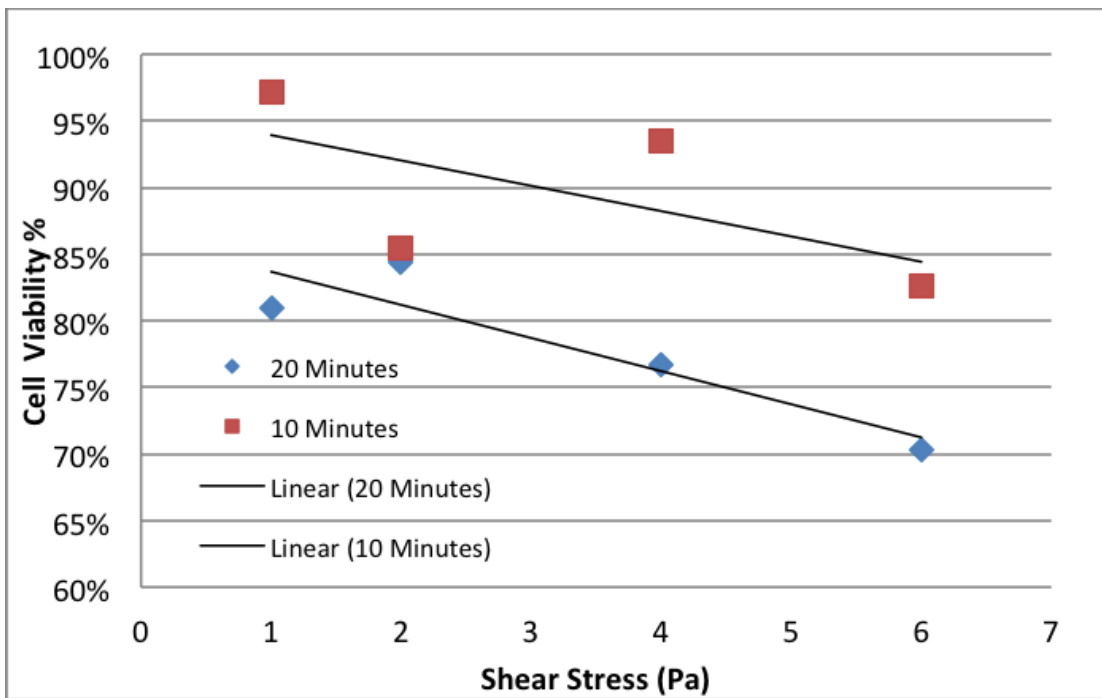


Figure 11. Graph of cell viability as determined by FDA stain and image analysis versus shear stress (Pa) for 10 and 20 minutes for diatom LBK009.

Additionally, a shear duration test was performed at both 4 and 6 Pa constant stress and samples were collected at intervals from 0 to 60 minutes. Data was collected

as before to calculate a cell viability percentage. These results are shown in Figure 12. This pattern showed that longer shear duration increased the percent of damage along a power law function.

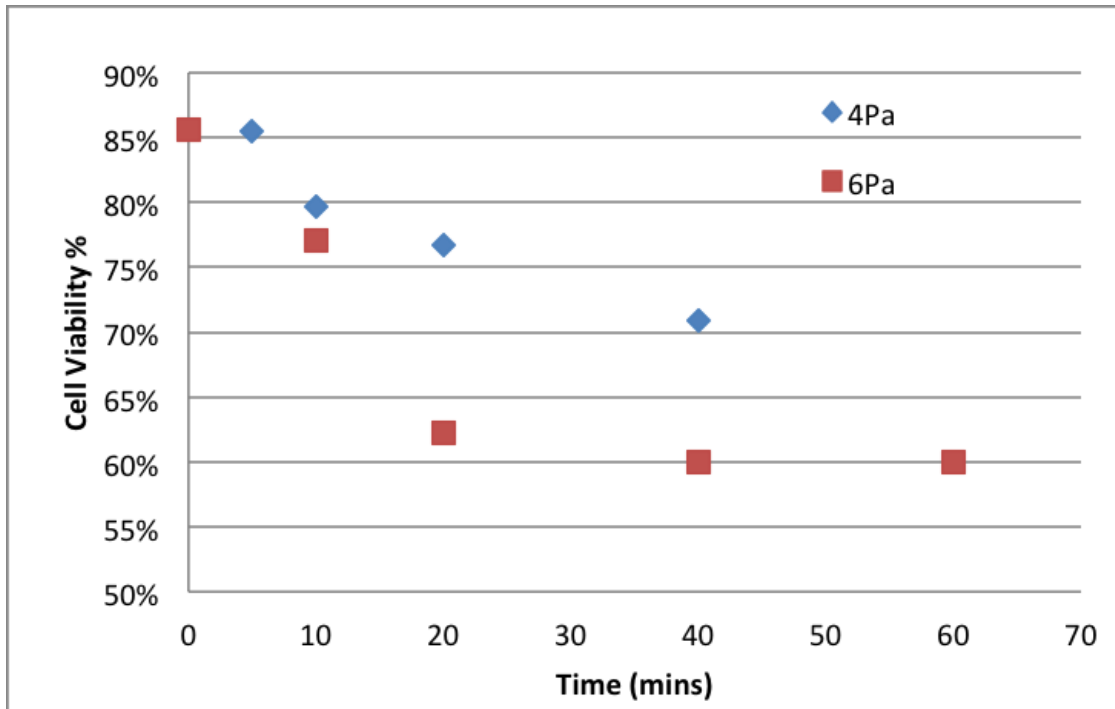


Figure 12. Graph of cell viability versus shear time at 4 and 6 Pa shear stress for diatom LBK009.

In a subsequent test, GA159 was chosen and the same set of tests were performed; 10 minute duration tests at 1, 2, 4, and 6 Pa shear stress. This was performed twice on different days and a 0, 4, and 6 Pa test on a third day. Data are shown in Figure 13. Little cell damage was seen at stress lower than four Pa.

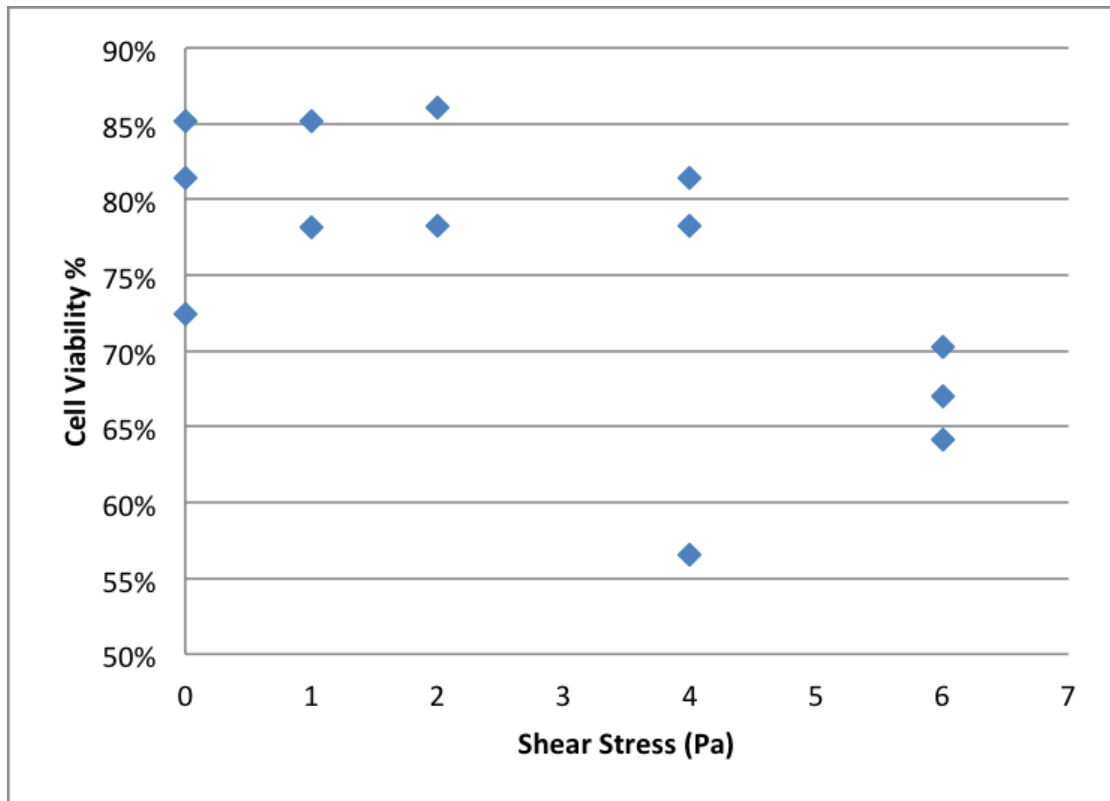
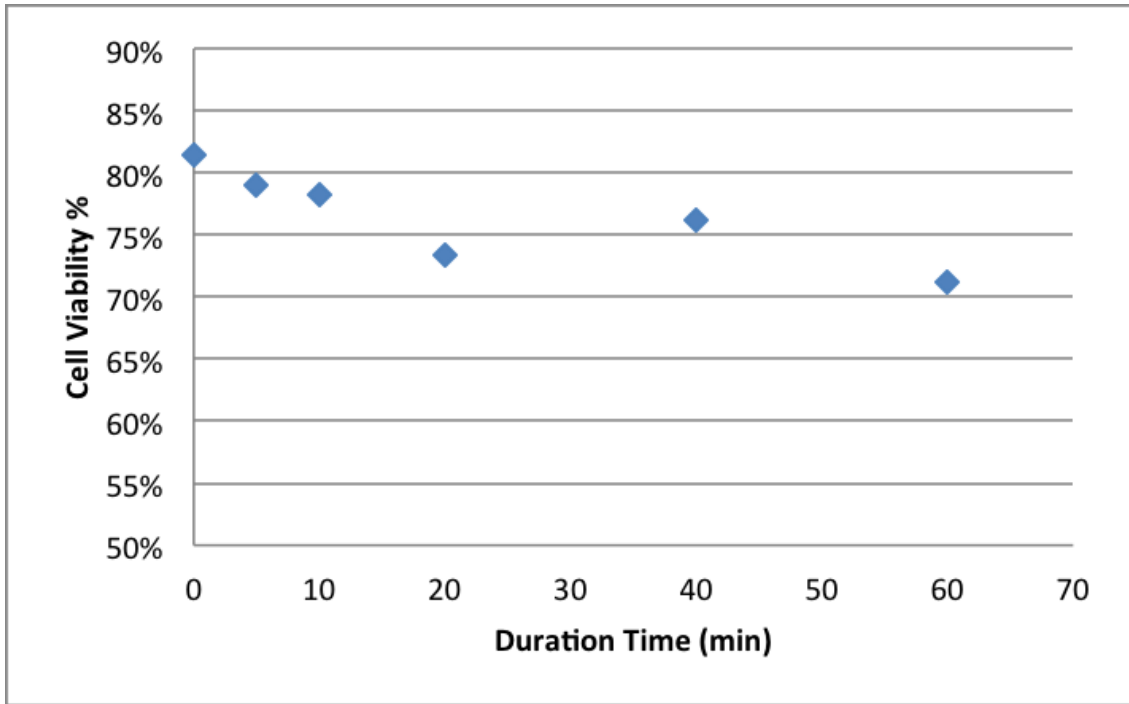


Figure 13. Graph of cell viability versus shear stress at a duration of 10 minutes for diatom GA159.

Additionally, a test at constant 4 Pa was run from 0 to 60 minutes of duration on GA159 and the results were similar to LBK009 as shown in Figure 14. The results from testing LBK009 and GA159 were used to establish the data collection protocol for the balance of the diatoms. This figure validated Michels' (2010) method of shearing for 10 minutes as there was a sharp drop off in cell viability percentage out to around 10 minutes at which point the rate of decrease leveled out. For all further tests 10 minutes was deemed a satisfactory shear duration.



*Figure 14. Graph of cell viability versus shear duration at 4 Pa shear stress of the diatom GA159.*

## CHAPTER V

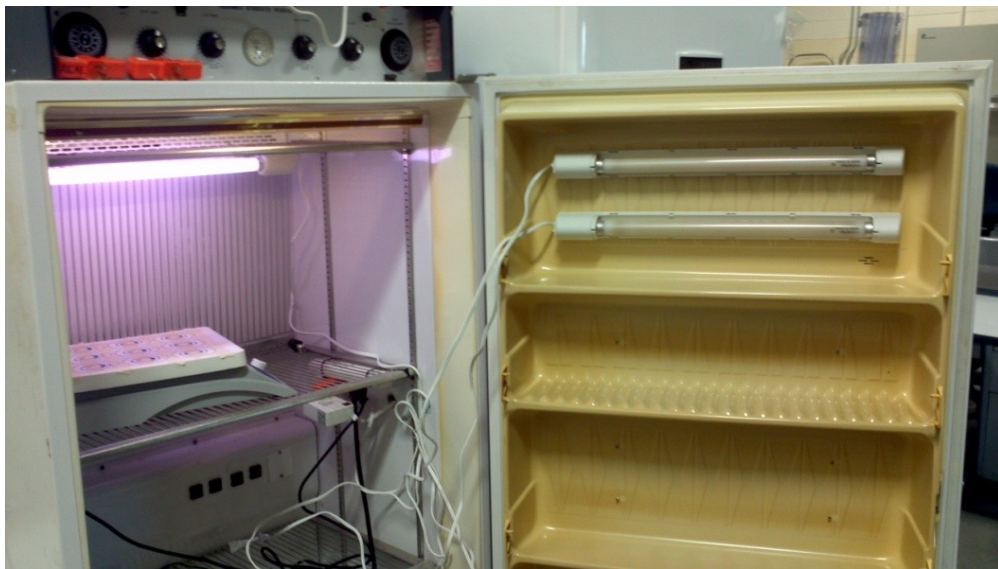
### SHEAR STRESS DATA COLLECTION FOR MULTIPLE STRAINS

The testing methodology as developed previously required a Thermo Scientific High Tech Rheometer with a 60mm cone and plate attachment (Haake RheoStress 6000, Canada) to impart six Pascals of shear stress onto each diatom sample for ten minutes. Three samples of each diatom were sheared and each paired with an unsheared sample of the same diatom. Both the sheared and unsheared samples were stained with a stock solution of fluorescein diacetate (46 mg FDA to 10mL acetone) at a rate of 7.5  $\mu$ L FDA stock solution to 1mL of diatom sample and incubated for 20 minutes, stirring gently periodically. The sheared sample was then compared to an unsheared sample under a fluorescent microscope (Eclipse TS-100-F, Nikon Instruments Inc., Japan) to determine the change in cell viability. All the cells were visible under white light while only the healthy cells showed under fluorescent light, allowing a cell viability percentage to be calculated for each pair of samples for each diatom.

Funding for this research required shear testing on multiple diatom strains. These strains were isolated by General Atomics and Texas AgriLife Research. Fifty milliliter samples of each diatom were delivered to College Station and started in 100 ml of media in a 125 ml flask as shown in Figure 15. Each flask was placed in an incubator at constant 22°C in front of three 15 watt growth lights on a magnetic stir plate, shown in Figure 16.



*Figure 15: Pennate diatoms growing in 125mL flasks on a multi-position stir plate in the incubator.*



*Figure 16: Image of the incubator setup used to cultivate diatoms. Note the two growth lights in the door and the one above the multi-position stir plate.*

Samples of two strains were collected in a centrifuge tube each morning of testing. Three samples of each strain were exposed to shear on the Haake RheoStress and collected in individual centrifuge tubes. These samples were then stained in the afternoon along with three unsheared samples (these came from the centrifuge tube collected that morning) of both strains. This resulted in twelve individual samples to have the cells counted under the microscope.

Triplicate samples of eleven diatom species were subjected to no shear and 6 Pa of shear stress for 10 minutes. Five random brightfield and fluorescent microscope images from each sample were analyzed and cell viability determined. The number of healthy cells were counted on each sample under white and fluorescent light and recorded. An average cell viability percentage was calculated by adding each of the fluorescent image counts and dividing by the summation of the white light image counts. A sheared and unsheared sample were paired to calculate a cell viability decrease percentage. Shear testing was performed on eleven species. Two strains on multiple trials would not fluoresce under fluorescent light either before or after shearing, making comparisons impossible. Table 2 shows the average total cells, the average live cells, and the average decreases in cell viability for the remaining nine species.



Table 2: Average data collected with standard deviation for nine species tested at 0 and 6 Pascal shear stress levels. The total cell count was all the cells viewed under white light and the live cells were those that appeared under the fluorescent light as well.

Species	Stress Level	Avg. Total Cells	Avg. Live Cells	Sites Counted	Avg. Viability %	Avg. Decrease
GA159	0Pa	23.33±9.29	18.60±7.41	15	79.68%	12.56%±9.51%
	6Pa	23.18±10.18	15.65±7.75	17	67.13%	
LBK002	0Pa	33.67±10.98	30.07±9.95	15	89.35%	7.10%±1.72%
	6Pa	32.40±17.63	26.47±14.20	15	82.25%	
LBK009	0Pa	40.19±11.77	31.94±9.96	16	79.49%	6.53%±5.84%
	6Pa	42.13±19.54	31.00±16.17	15	72.97%	
LBK011	0Pa	18.73±7.24	12.67±6.37	15	67.97%	11.13%±3.93%
	6Pa	18.20±5.67	10.27±4.13	15	56.83%	
LBK016	0Pa	21.53±6.23	18.40±5.72	15	85.51%	2.95%±1.24%
	6Pa	20.93±5.42	17.33±5.35	15	82.56%	
LBK017	0Pa	18.00±6.01	15.29±5.41	14	84.91%	6.04%±1.82%
	6Pa	30.31±7.30	23.88±5.38	16	78.88%	
LBK018	0Pa	17.67±7.15	14.80±7.06	15	84.03%	14.36%±9.04%
	6Pa	20.27±8.87	14.33±8.44	15	69.67%	
LBK021	0Pa	14.13±4.88	11.00±4.77	16	77.97%	1.30%±0.44%
	6Pa	23.73±6.91	18.20±6.42	15	76.68%	
LBK023	0Pa	13.53±6.83	11.13±6.57	15	80.37%	10.53%±5.47%
	6Pa	18.75±8.58	13.06±6.60	16	69.83%	

From the data, the most shear stress resistant species appeared to be LBK021, LBK016, LBK017, and LBK002 based on the lowest average cell viability decrease, though the small sample size likely prevented any statistical conclusion to differentiate between species.

The data collected from each set of images (brightfield and fluorescent) were used to calculate the slope between 0 Pa and 60 Pa; the slope represents the rate of loss

of cell viability with increasing shear stress. The results for nine of the species are shown in Table 3.

*Table 3. Calculated slope data from shear stress versus cell viability for nine diatom species considered in this research. Slope was calculated as the loss in viability between 0 Pa and 60 Pa.*

<b>Species</b>	<b>Trial 1</b>	<b>Trial 2</b>	<b>Trial 3</b>
GA159	-0.0350	-0.0241	-0.0037
LBK002	-0.0101	-0.0102	-0.0151
LBK009	-0.0015	-0.0102	-0.0209
LBK011	-0.0225	-0.0110	-0.0222
LBK016	-0.0062	-0.0061	-0.0025
LBK017	-0.0134	-0.0095	-0.0074
LBK018	-0.0071	-0.0287	-0.0361
LBK021	-0.0028	-0.0022	-0.0014
LBK023	-0.0076	-0.0194	-0.0256

The slope representing the decrease in viability for each species was statistically compared using a one-way ANOVA in a commercial software package (Design Expert, Stat-Ease, Inc.). The results of this comparison are shown in Table 4. There was no overall significant difference between the nine species tested ( $p = 0.1042$ ) and an LSMeans Differences Tukey HSD test indicated that there were no significant differences between any of the diatoms.

Table 4: ANOVA as developed by Design Expert to show there were no significant differences in cell damage amongst the species as evidenced by the p-value of 0.1042.

Response	1	Slope			
ANOVA for selected factorial model					
Analysis of variance table [Classical sum of squares - Type II]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1.289E-003	8	1.611E-004	2.01	0.1042 not significant
A-Species	1.289E-003	8	1.611E-004	2.01	0.1042
Pure Error	1.443E-003	18	8.016E-005		
Cor Total	2.732E-003	26			

As seen in the early tests with 10 and 20 minutes, the longer duration at the same shear stress level caused a downward shift in the curve. Thus, both the duration and shear stress generated must be considered in selecting a pump and designing a raceway. In order to take both of these factors into consideration a shear stress-duration unit of Pa-min was postulated to facilitate scale-up of these results to commercial mixers. For instance, at 6 Pa for 10 min, the diatom was subjected to 60 Pa-min of continuous shear stress. At commercial scale, the growth time will be days but each cell will only be exposed to shear briefly as it circulates around the raceway. The Texas AgriLife Research facility in Pecos, Texas, has a sloped pond which flows at  $1.5 \text{ ft s}^{-1}$ ; fluid movement is accomplished by a rotational horizontal mixer in a sump at one end providing the necessary lift. The flow length is about 270 feet. This means that it will take one algae cell about 180 seconds to complete a lap, thereby completing 483 laps per day. Making the assumption that the cell is in a turbulent zone in the sump of the pond for 1.5 seconds, each cell would be exposed to 725 seconds of shear daily, or 0.8% of the day. The tests performed in this laboratory scale study were continuously exposed to

shear; the period of rest in the raceway may provide the algae a chance to recover, thus reducing the amount of damage sustained especially when considering these diatoms double in growth every couple days. This concept of resting between shear events is one aspect that should be further developed in order to accurately model a large scale scenario.

At the conclusion of these tests, several observations were made. First, counting the cells manually under the microscope was both time consuming and tedious. The methodology appeared to work, but in order to make more definite conclusions many more replications of the tests are required. In order to provide more data, the cell counting method should be automated. For example, to test one species one time at no shear and 6 Pa required shearing 3 samples. At ten minutes each, shearing the samples happened quickly. But, to determine health, six samples were stained, and each sample had to wait 20 minutes after staining before images could be captured and the actual image capturing procedure took about 15 minutes per sample. Therefore, a triplicate test of a species at a constant shear duration and shear stress took about 2 hours to capture 36 images. Because of the time to shear and capture images of the cells, only two species in triplicate could be tested per day. Manually counting and labeling each image took considerable time, especially on dense cultures where there were many cells on the image.

Diatoms proved very difficult to grow consistently. For about six months the primary focus was getting the diatoms to grow, which eventually became possible though still inconsistent. One week the cultures would show every sign of excellent

growth, then for no apparent reason slow down growth or even die. This made collecting repeatable data difficult as cell health before shearing appeared to have an effect on shear damage. In order to have a true repeat of the shear study, the health and growth pattern of the species needed to be consistent, which was difficult to achieve as well as quantify. For this research, ideally instead of only a triplicate sample one day, triplicate samples would be taken multiple times over a longer period of time. But, with the inconsistent growth, a sample taken one day and a sample taken a week later would react differently to shear stress if the culture looked less healthy at the time of the second sample. This inconsistent growth is why there are limited data.

Because of the limited data collected, an assumption was required in order to proceed from the cone and plate viscometer to a scale-up shear apparatus. This assumption was the shear stress value and the duration of shear was equally related to the cell damage, allowing the shear stress duration unit of Pascal-seconds (or Pascal-minutes) to be developed. This assumption appears to hold except at extremes of either factor. But, this assumption should be validated by additional testing, which would be fairly straightforward except for the counting cells method and inconsistent growth of the diatoms. To validate this assumption, many replications of the tests would be required requiring cell counts of thousands of images. Under the current manual counting method, this is time consuming; an automated system would be required.

## **CHAPTER VI**

### **SCALE UP TESTING**

The results of the viscometer tests showed shear is detrimental to health of diatoms; this method is effective if a researcher has access to an expensive piece of equipment not normally used for cultivating algae. Therefore, most algal cultivation facilities will not have this instrument, nor have any reason to purchase one. This brings about the need for a more cost effective method to impart shear. The rotational mixer used earlier seemed the most cost-effective method. This resulted in a revisit of the mixer concept, but with the intent of replicating the viscometer.

The pattern for testing with the viscometer was no shear and a high value of shear stress for a set duration, in this case 6 Pa for 10 minutes. The hypothesis was that the cell damage was a product of shear stress and exposure time; therefore, shear exposure to the same conditions would have similar results. This gave a relation of 60 Pa min or 3600 Pa s working under the assumption of equal significance of shear stress value and shear duration on cell damage. In order to replicate this same relationship with a rotational mixer, the following formula relating shear stress and shear rate was used to calculate shear stress duration (SSD):

$$SSD = \mu \gamma d \quad (2)$$

where:

SSD = shear stress duration (Pa s)

$\mu$  = dynamic viscosity (Pa s)

$\gamma$  = shear rate ( $s^{-1}$ )

d = shear duration (s)

Prior to the shear testing, diatoms were cultivated in the incubator at 22<sup>0</sup>C in Erlenmeyer flasks. Initial samples of three diatom strains came from the Texas AgriLife Research center in Lubbock, Texas. Fifty milliliter samples of each diatom were collected and shipped overnight to College Station, Texas. Each diatom was cultivated in a 125 mL flask with a magnetic stir bar on a stir plate in the incubator. Growth light in the incubator consisted of three 15-watt growth lights set on 12 hours of dark and 12 hours of light daily. Ten percent media was added daily until the flask was filled at which time the flask was split into two 125 mL flasks. This process was repeated until two 250 mL flasks were filled to be combined into a 500 mL flask to be used under the mixer. Of the three diatoms started for this test, only LBK017 survived past the first week. The mixer was placed in front of two 15-watt growth lights in the same incubator as scale up, thereby keeping the diatoms in front of similar light and at the same temperature as they were previously incubated. The impeller was lowered to one inch from the bottom of the flask and set at the desired rotational speed.

The original mixer test at 2500 rpm resulted in extensive cell damage. But, one question left unanswered was what actually caused the damage: turbulence or diatoms being struck by the impeller. The cone and plate viscometer kept laminar flow eliminating turbulence as a factor; the only shear stress was a result of the cone and plate setup with no air bubbles. Since a linear relationship between shear stress level and duration had been assumed, any rotational speed could be selected and a corresponding duration to result in 3600 Pa s. The next mixer test was 1250 rpm, half the maximum rotational speed. Shear rate for a rotational mixer follows the following formula:

$$\gamma = (2\pi * \text{RPM}) / 60 \quad (3)$$

where:

$$\gamma = \text{shear rate (s}^{-1}\text{)}$$

RPM = Revolutions per minute

Formula 3 was used to calculate the shear rate of the rotational mixer. Using this shear rate as the shear rate value in formula 2, this required a shear duration of 7 hours. Samples were taken before and after shearing and analyzed in the same way as the viscometer tests using the fluorescein diacetate stain and the microscope. Upon first visual inspection, the diatoms created a large amount of foam and the diatoms which had a dark brown color before mixing (indicating healthy cells) turned to a lighter color after completion of the test as seen in figures 17 and 18.





*Figure 17: Diatom culture prior to shearing. Note the dark brown color indicating a healthy culture.*



*Figure 18: Diatom culture after shearing at 1250rpm for 7 hours. Notice the lighter color and slight yellow tint and the thick layer of foam on top.*

Healthy diatoms settle very quickly, but this flask did not settle at all, very similar to the test at 2500 rpm. The corresponding tip speed when running the mixer at 1250 rpm was  $1.96 \text{ m s}^{-1}$ . Based on the literature, this value was about 25 percent higher than the threshold of  $1.56 \text{ m s}^{-1}$ , which resulted in damage to a pennate diatom (Sobczuk et al., 2005). As expected, the microscope images showed an average of 35.62% decrease in viable cells.

Under the assumption that duration and shear stress have equal effect on cell viability, it was anticipated that the percent of damage from this test should be similar to that of the viscometer for the same species. But, this was not the case as the viscometer

had only an average of 6.04% damage compared to the 35.62% seen with the rotational mixer. One potential explanation for this difference is turbulence where the air bubbles collided with the cells to cause damage. In the viscometer, only fluid was present between the plates and the flow was kept laminar, so there were no bubbles to collide with the cells. Because of the mechanics of a mixer, air is pulled down into the fluid creating turbulent flow.

This flask was put back in the incubator for 5 days to see if the diatoms would recover. As seen in Figure 19, 5 days later the diatoms were still flocculated and not settling well. Many diatoms were also floating in the bubbles at the top of the flask indicating an unhealthy culture.



*Figure 19: Picture of the diatoms sheared at 1250rpm for 7 hours one week after shearing. The diatoms were returned to the incubator for continued growth. Note the bubbles and all of the dark dead cells on top; the culture still would not settle out much.*

In order to test the idea of turbulence, the mixer test was repeated but at a quarter of the rotational speed for 4 times the duration. The parameters of this test were 313 rpm or  $0.492 \text{ m s}^{-1}$  for 28 hours. The same setup was used as the 1250 rpm test. At the completion of the test, several differences were immediately obvious. First, there was little to no foam at the top and the color of the flask was almost identical to the initial dark color. Secondly, almost the entire culture settled quickly with very few clumps being present as the culture would do before shearing. When looking at the microscope images, the percentage of healthy cells decreased by 11.77%. This value is much closer to the 6.04% shown with the viscometer.

Between the three tests with LBK017 (2 in the rotational mixer and 1 test in the viscometer), there were significant differences in the damage percentages. The slope representing the decrease in viability for each species was statistically compared using a one-way ANOVA in a commercial software package (Design Expert, Stat-Ease, Inc.) shown in Table 5. The LSMeans Differences Tukey HSD test indicated that there were significant differences ( $p < 0.0001$ ) between each method. Working under the assumption that duration and shear stress value are directly and equally related to the damage, some other explanation must account for the differences in the damage percentages. The explanation that seems most logical is turbulence; the slower rotational speed of the mixer induced shear in a fashion similar to that of the viscometer.

Table 5: ANOVA comparing the slope of cell damage for the three shear tests of LBK017. The model was significant, indicating significant differences between the shear methods.

Response 1		Slope				
ANOVA for selected factorial model						
Analysis of variance table [Classical sum of squares - Type II]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.140E-008	2	5.700E-009	99.59	< 0.0001	significant
A-Type	1.140E-008	2	5.700E-009	99.59	< 0.0001	
Pure Error	3.434E-010	6	5.724E-011			
Cor Total	1.174E-008	8				

## CHAPTER VII

### CONCLUSIONS

The silica cell walls of diatoms have raised concerns about survivability in a high shear stress environment, such as when pumping in a raceway. In order to test the effects of shear stress on diatom health, an appropriate methodology for inducing shear needed to be developed. Various methods had been used in the past to accomplish this goal, but few geared toward large scale production of microalgae. The easiest method from the literature was to use a rotational viscometer to impart a known shear stress onto the algae and determine cell health using fluorescein diacetate stain under a fluorescent microscope. This was the method used to test nine pennate diatoms being grown by Texas A&M University. Shear stress was found to be detrimental to diatom health for all nine of the strains tested. The value of shear stress and the duration of shear were both found to impact the cell viability percentage in an assumed linear relationship. This relationship allowed a unit of Pascal-seconds to be developed.

Previous tests were completed using an expensive rotational viscometer which would not be found in a commercial algal cultivation laboratory. An inexpensive laboratory mixer was then tested to see if results similar to the viscometer were achieved. This mixer used an impeller, which looks similar to a mixer style pump found in commercial scale facilities. This mixer showed more damage at similar Pascal-second levels, most likely due to turbulence generated. The rotational viscometer operated under completely laminar flow, while the mixer was not. The turbulence generated

caused bubble formation that has been shown to be harmful to algae (Barbosa et al., 2003). A second test with the mixer at slower speed for longer duration to keep the same shear stress duration factor showed less damage than at the faster speed for shorter duration. The difference in damage seen between the rotational viscometer and the two tests with the mixer may be attributed to bubble formation. In a commercial system desiring to grow diatoms, a large enough pump must be chosen to minimize turbulence.

Since shear stress was found to be detrimental to these diatoms, the methods used to transport fluid must be carefully considered in a large scale production facility. Pumps must be checked for a low enough shear stress value to not cause cell damage; the pump availability may ultimately determine the size of a facility that will successfully cultivate diatoms. Pump selection requires a small-scale test to model the large pump using a simple, cost effective method. The viscometer used in this research, while providing accurate data, is quite costly and not a required piece of equipment at a commercial algal cultivation lab. For these reasons, the laboratory mixer idea was developed to model large-scale commercial mixers in a quick, cost effective way thus allowing a grower to determine if his large-scale setup will cultivate diatoms successfully.

Diatoms proved very difficult to grow; this may be attributed to the setup available or a lack of knowledge. In any case, cultivating a sample of diatoms to large enough volume for testing purposes proved challenging. Additionally, the cultures seemed to fluctuate in health quite rapidly. For example, one week the culture would appear healthy and a week later look unhealthy or even dead. Sometimes this change

would occur within days. The inconsistent growth and rapid changes made collecting repeatable shear data difficult; ideally multiple samples would have been sheared over a period of time to give repeatable results. Since the cultures would change quickly, repeatable experiments became difficult. To adjust for this variability, the three samples were taken the same day for data collection. This rapid change in the diatoms also ruled out letting samples continue to grow after a period of shear and measuring subsequent growth or damage since any changes may not actually have been a result of shear but simply a random decline.

The relationship developed between the viscometer and the rotational lab mixer utilized a linear assumption between shear stress value and the duration of shear. In order to validate this assumption, many more sheared samples would be required. Because of manual cell counting and the time required to stain samples along with the difficulty growing healthy diatoms, only a finite number of samples were tested. In an ideal situation, a much greater number of samples would be needed and impossible to assess without a more automated system of determining cell health.

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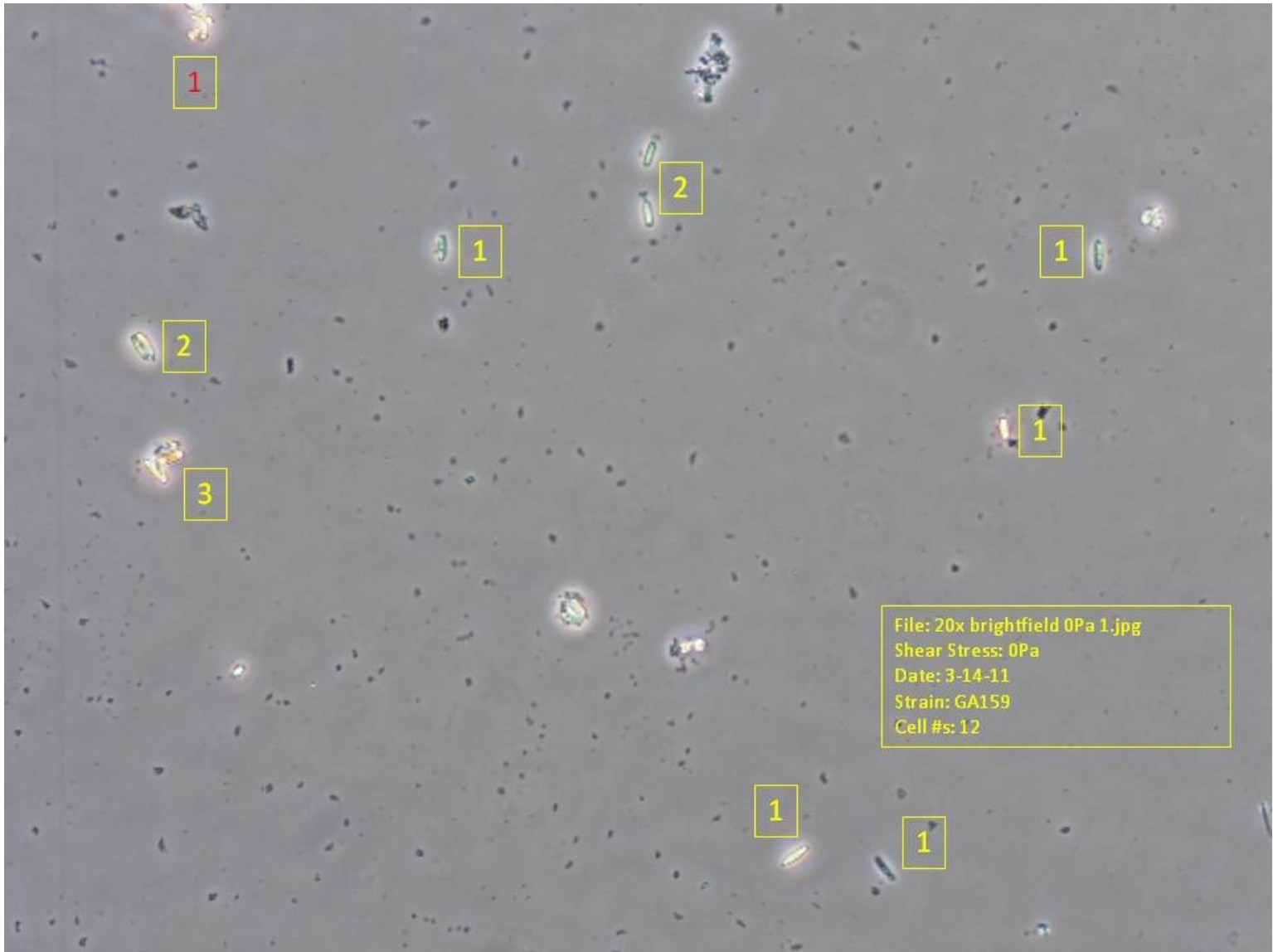
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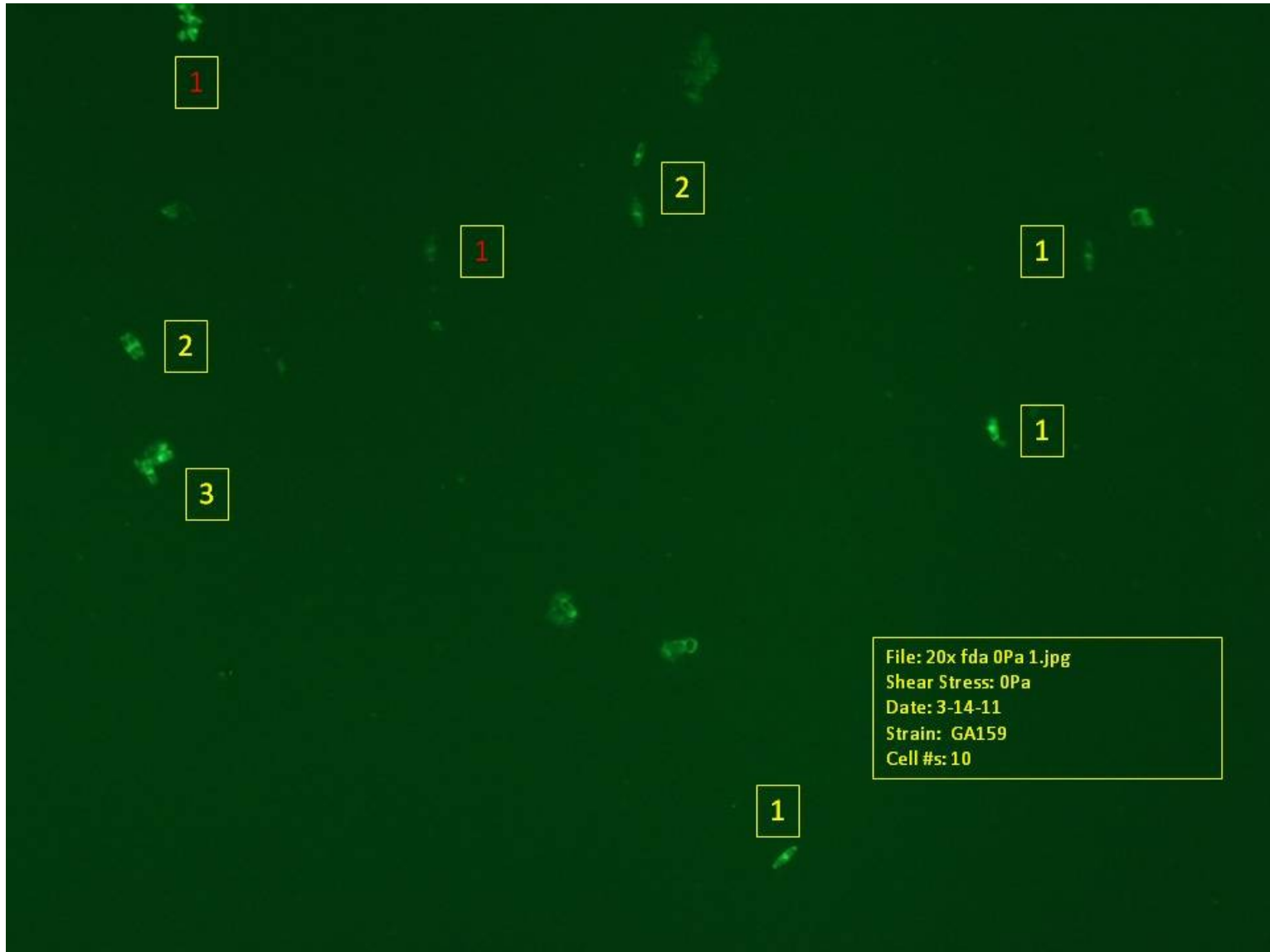
## APPENDIX A

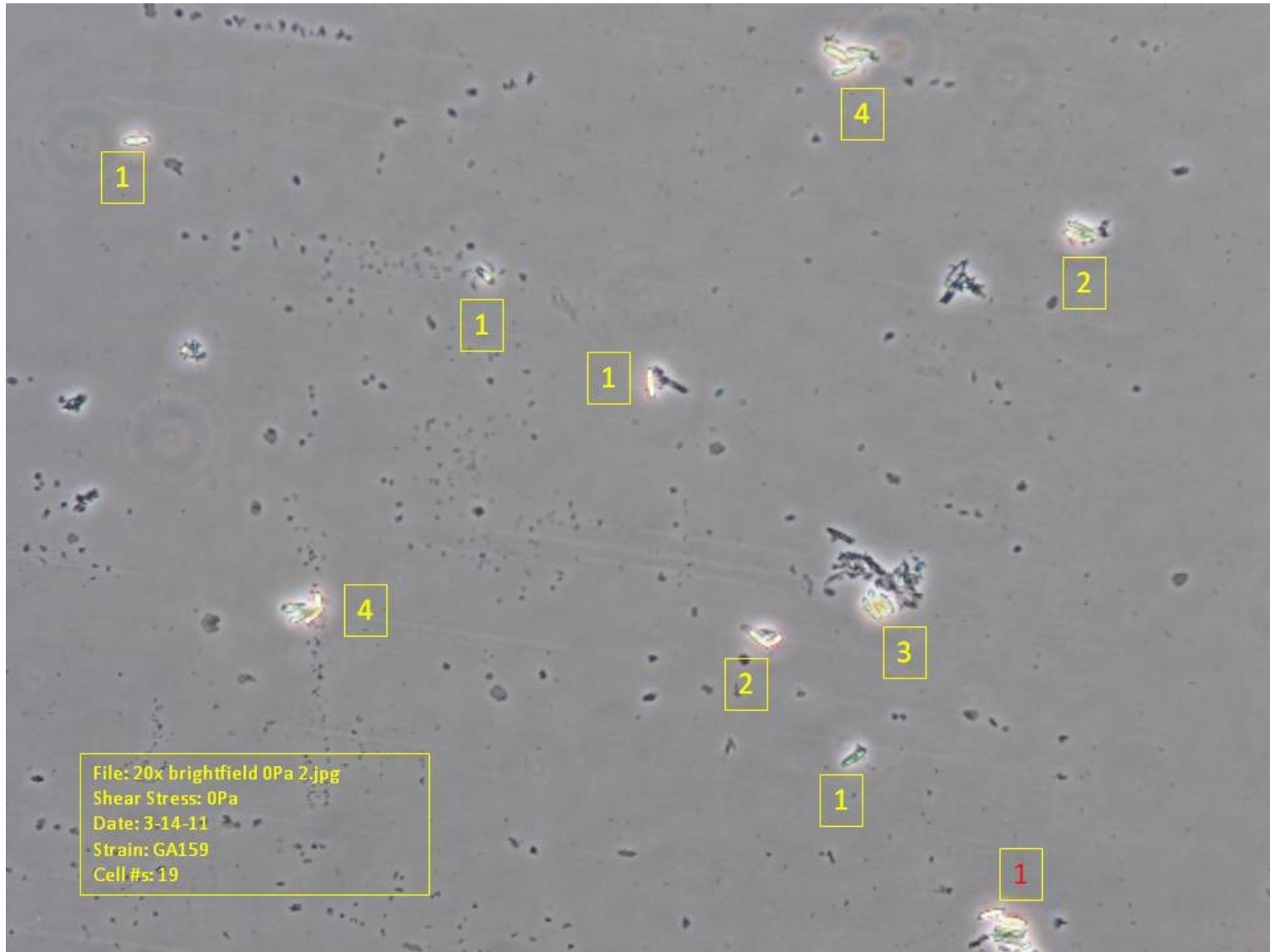
The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as GA159, at no shear stress and six Pascals of shear stress . This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	12	10	19	17	25	19	22	19	23	21			85.15%	20.98%	-0.03497
	6	19	12	28	14	26	16	25	18	22	17			64.17%		
Rep 2	0	18	15	32	25	9	9	46	37			35	28	81.43%	14.45%	-0.02408
	6	5	4	18	11	25	18	13	9	28	20	17	9	66.98%		
Rep 3	0	22	16			20	12	27	21	15	11	25	19	72.48%	2.83%	-0.00472
	6	29	25	15	12	53	38	31	19	18	11	22	12	69.64%		
														<b>Avg.</b>	<b>12.75%</b>	
														<b>Std. Dev.</b>	<b>9.19%</b>	

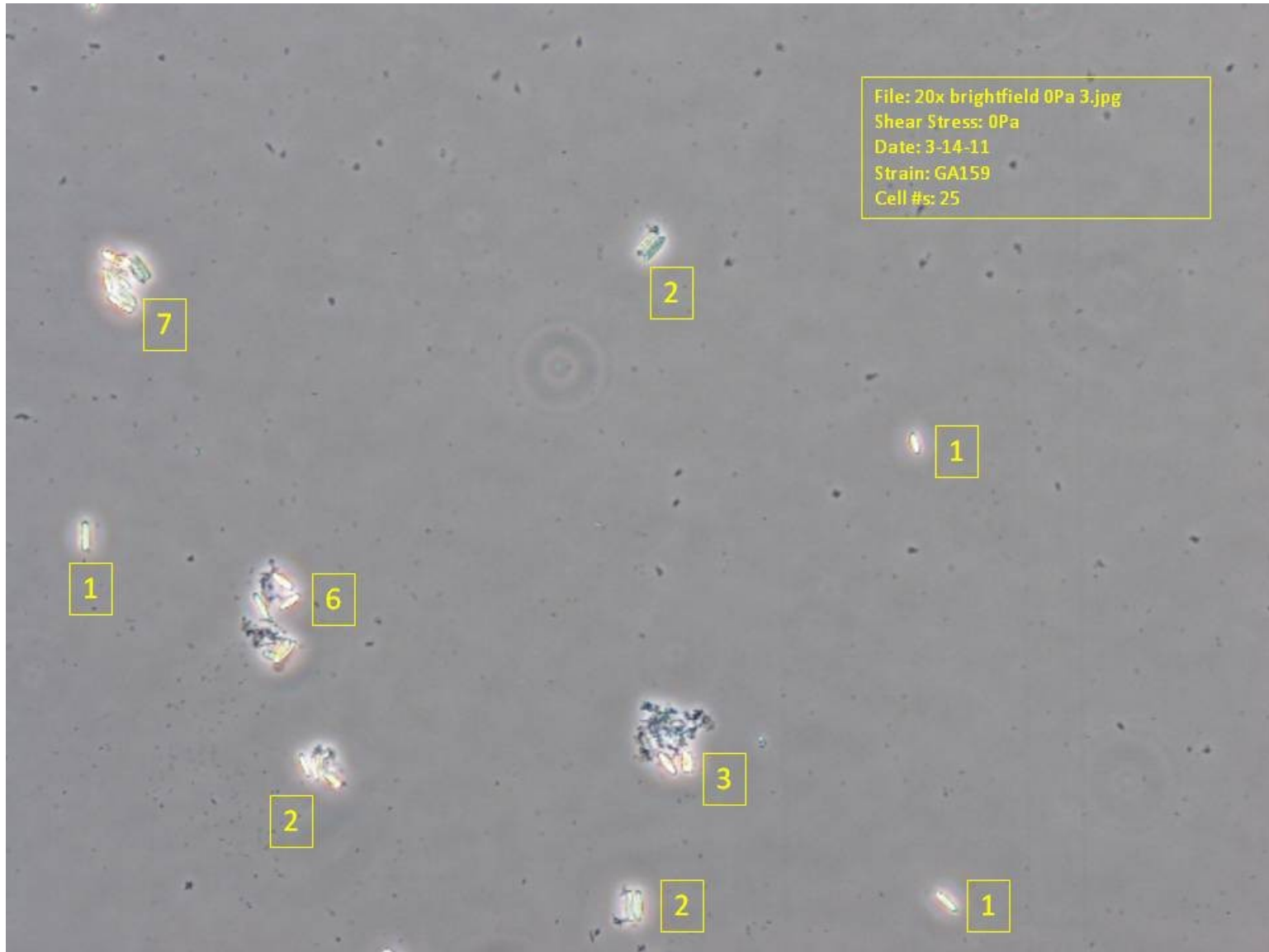
The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.

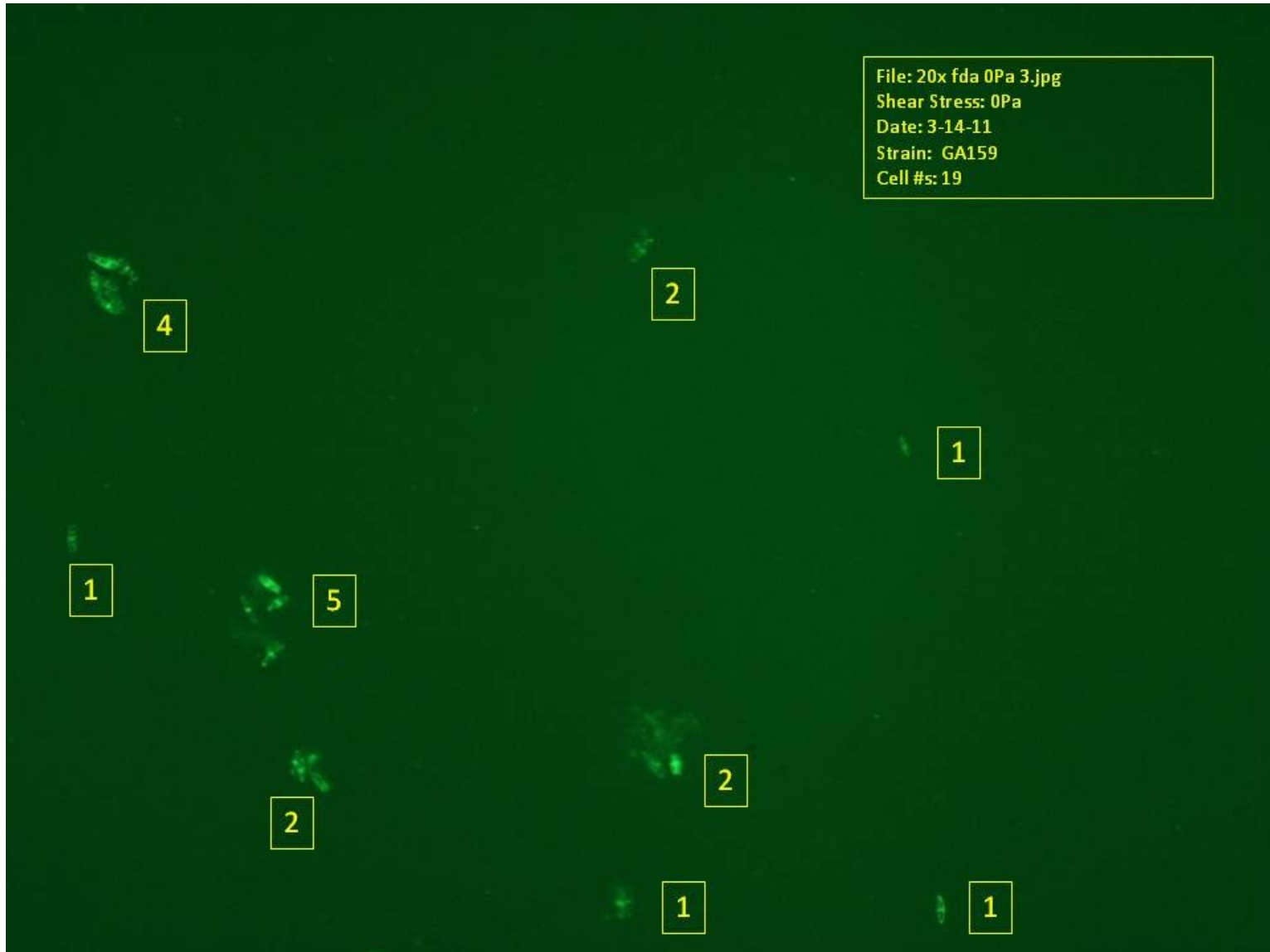




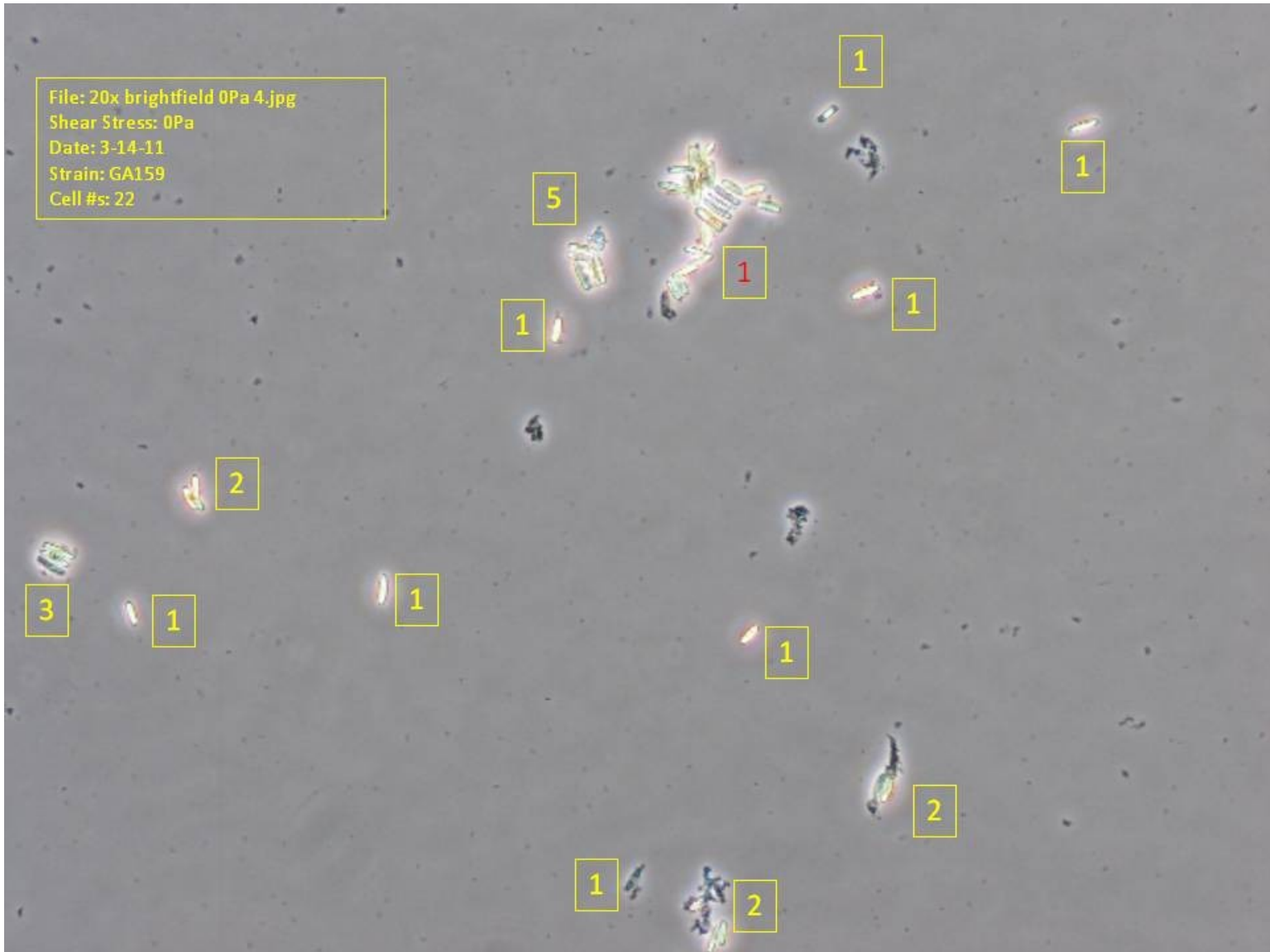


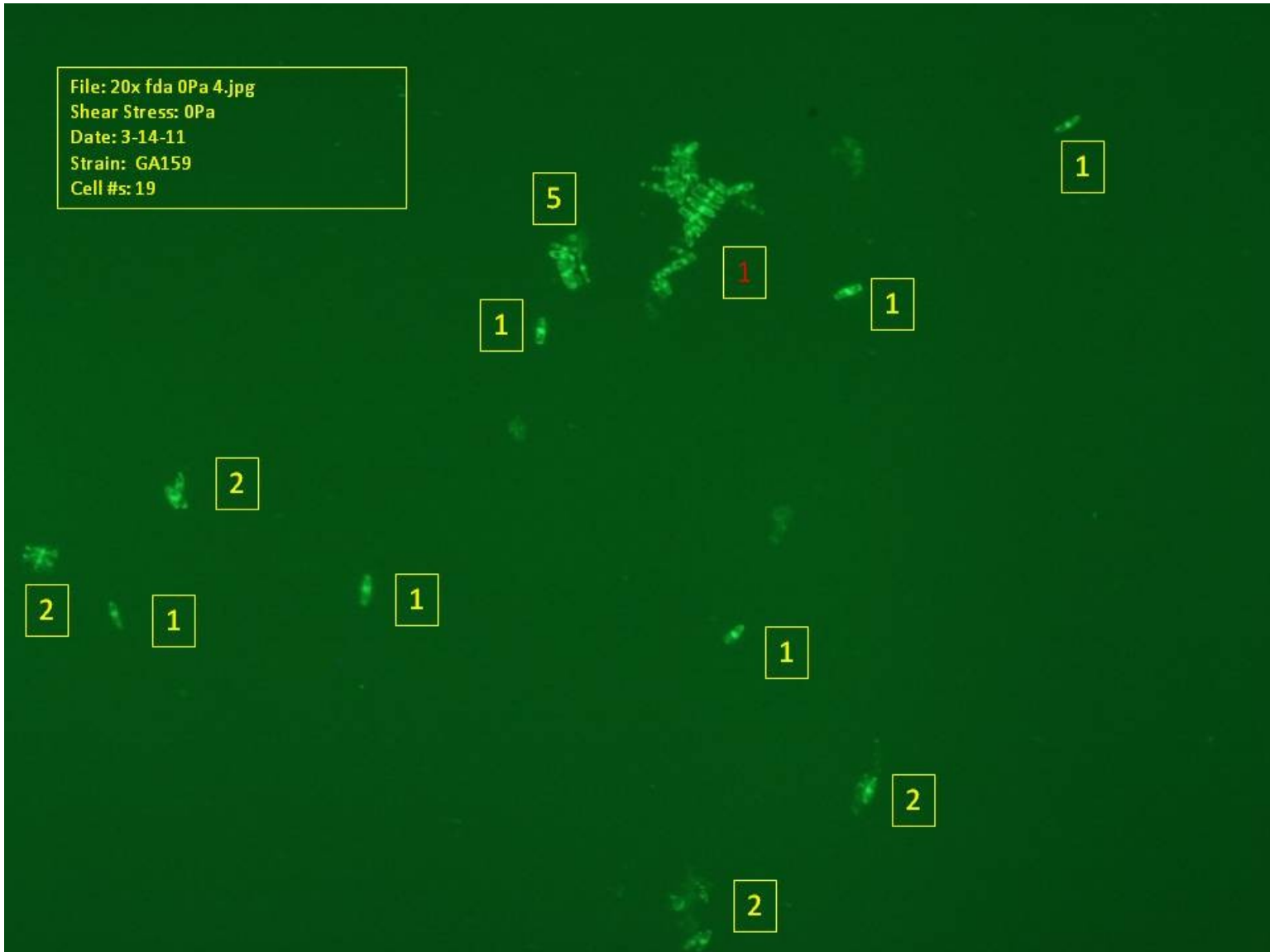


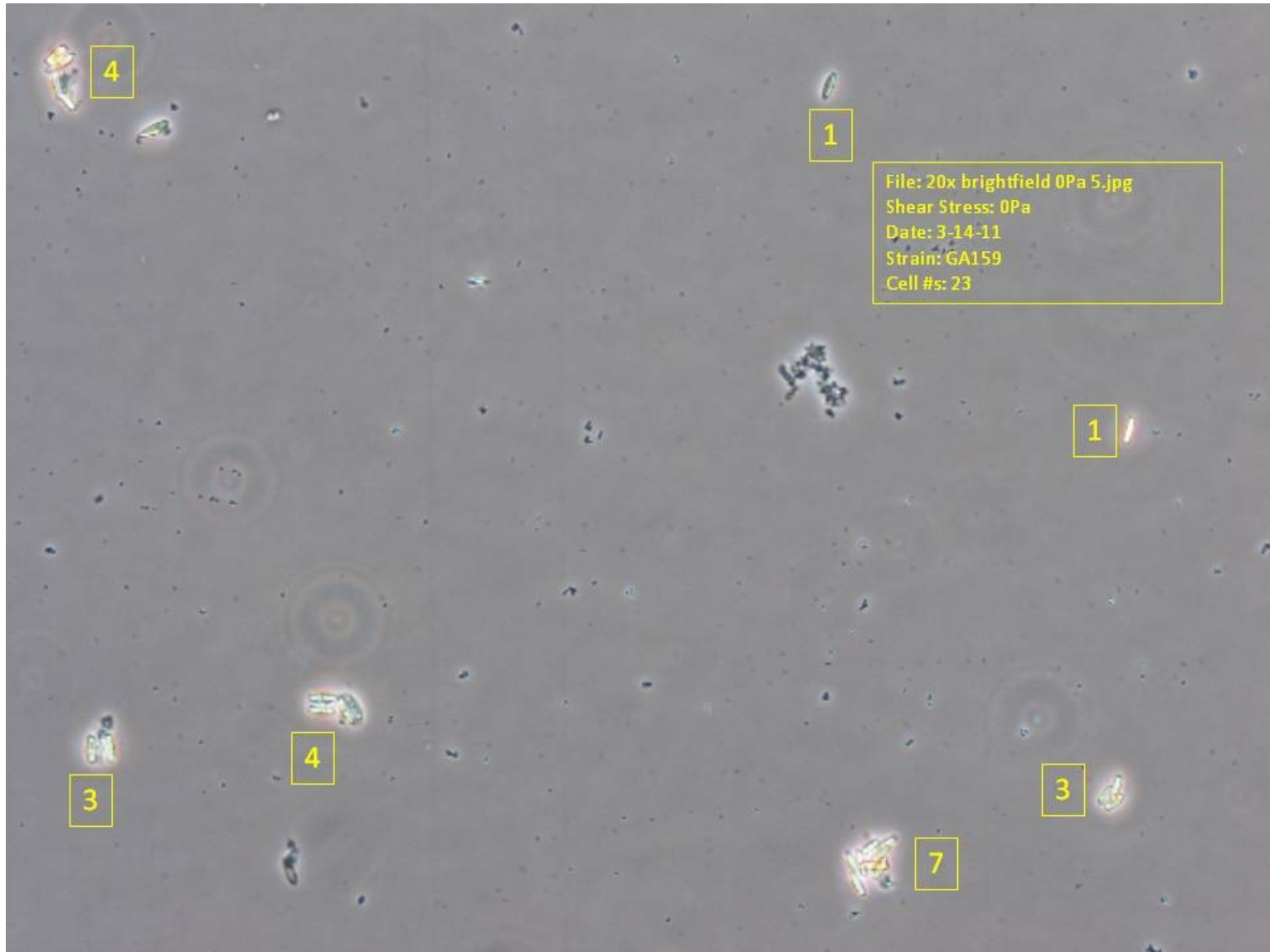


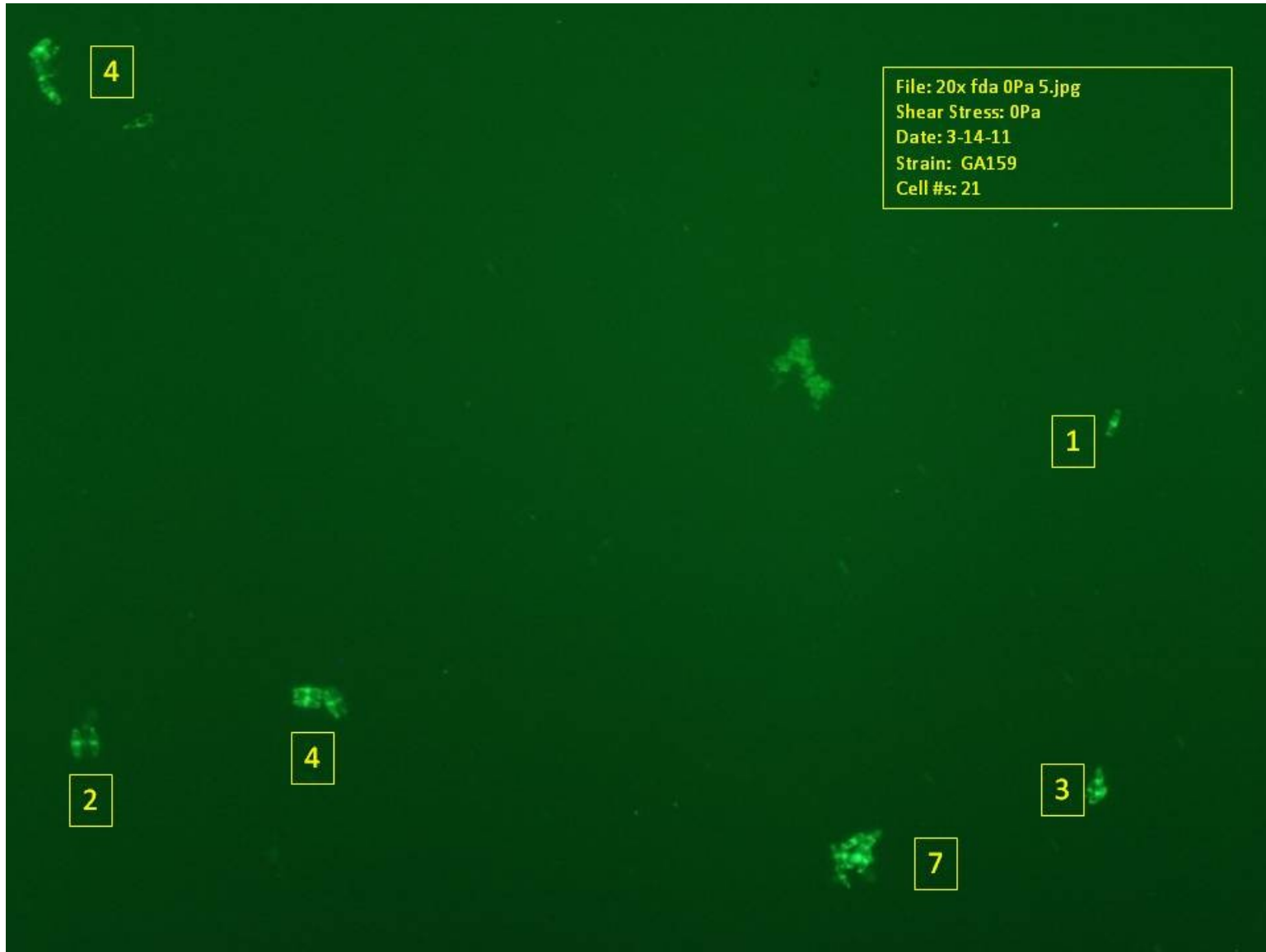


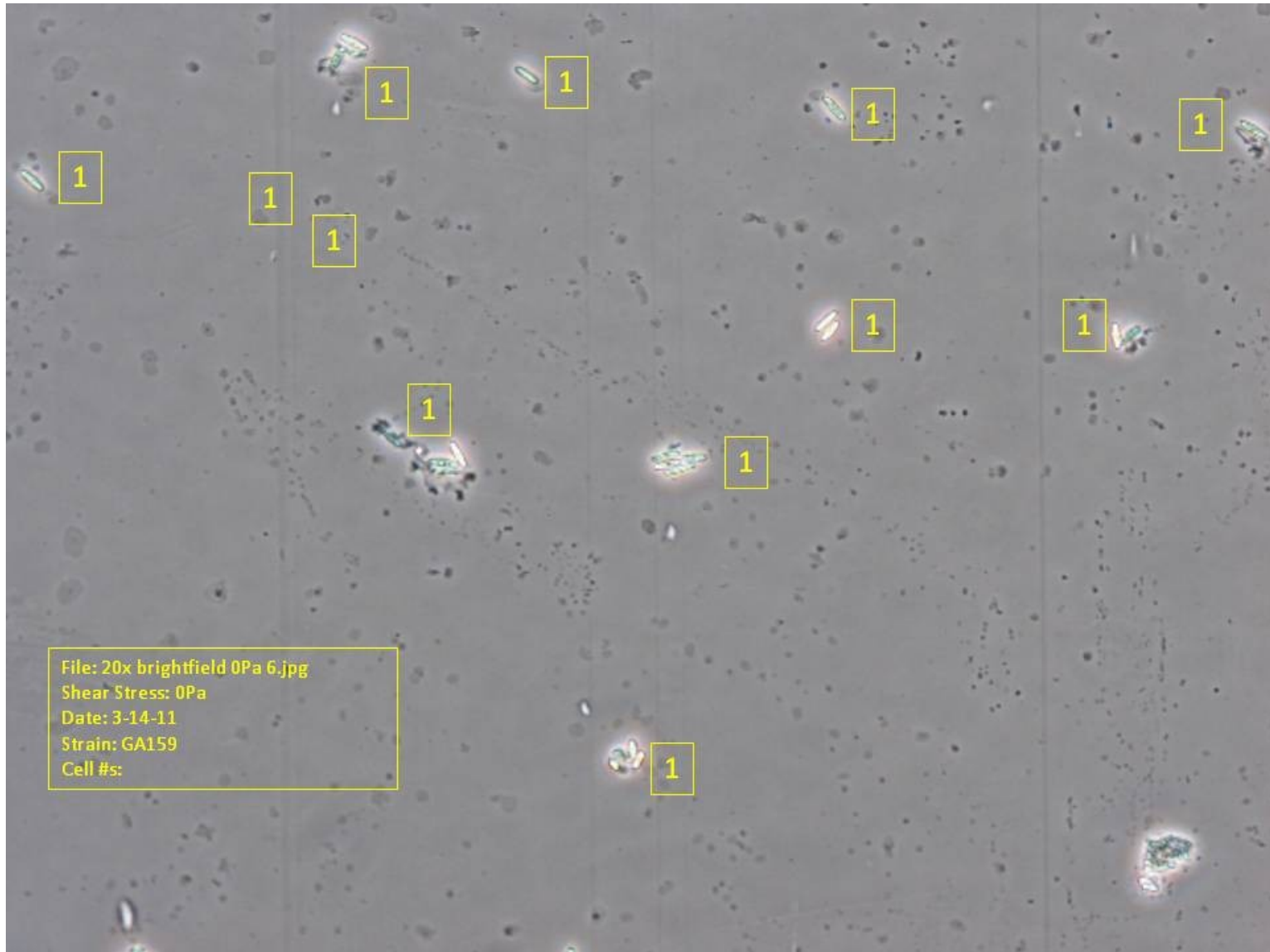


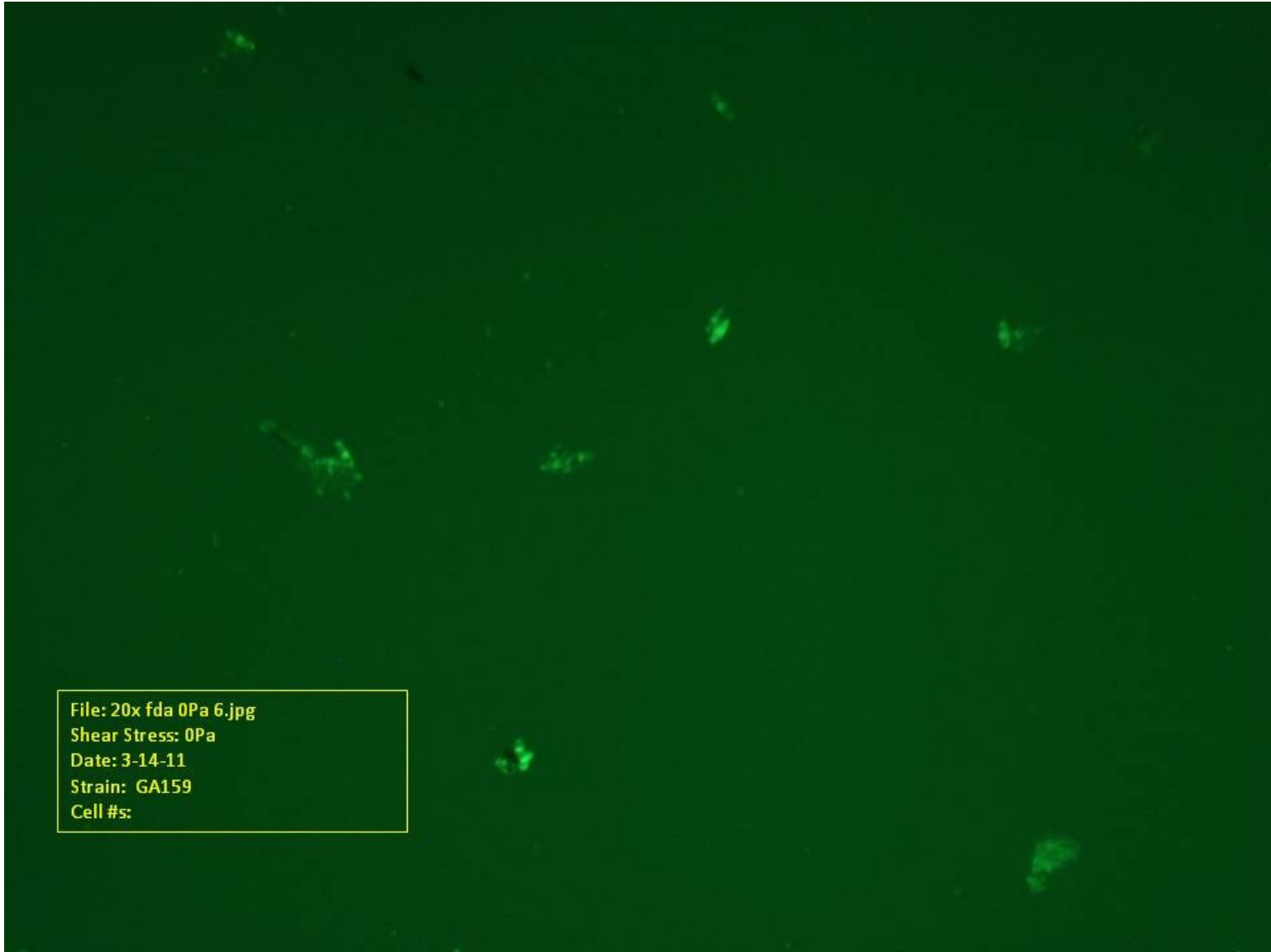


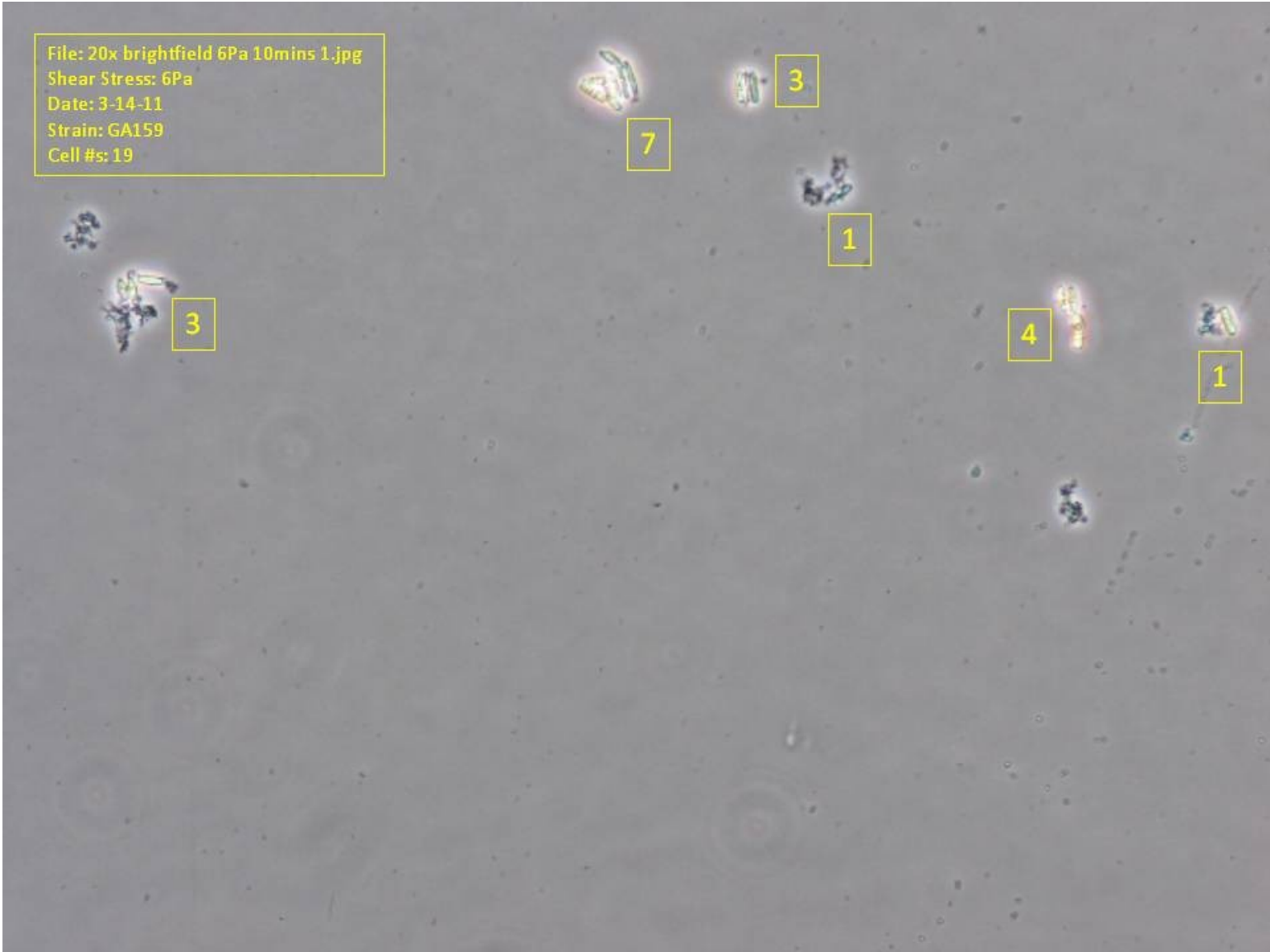








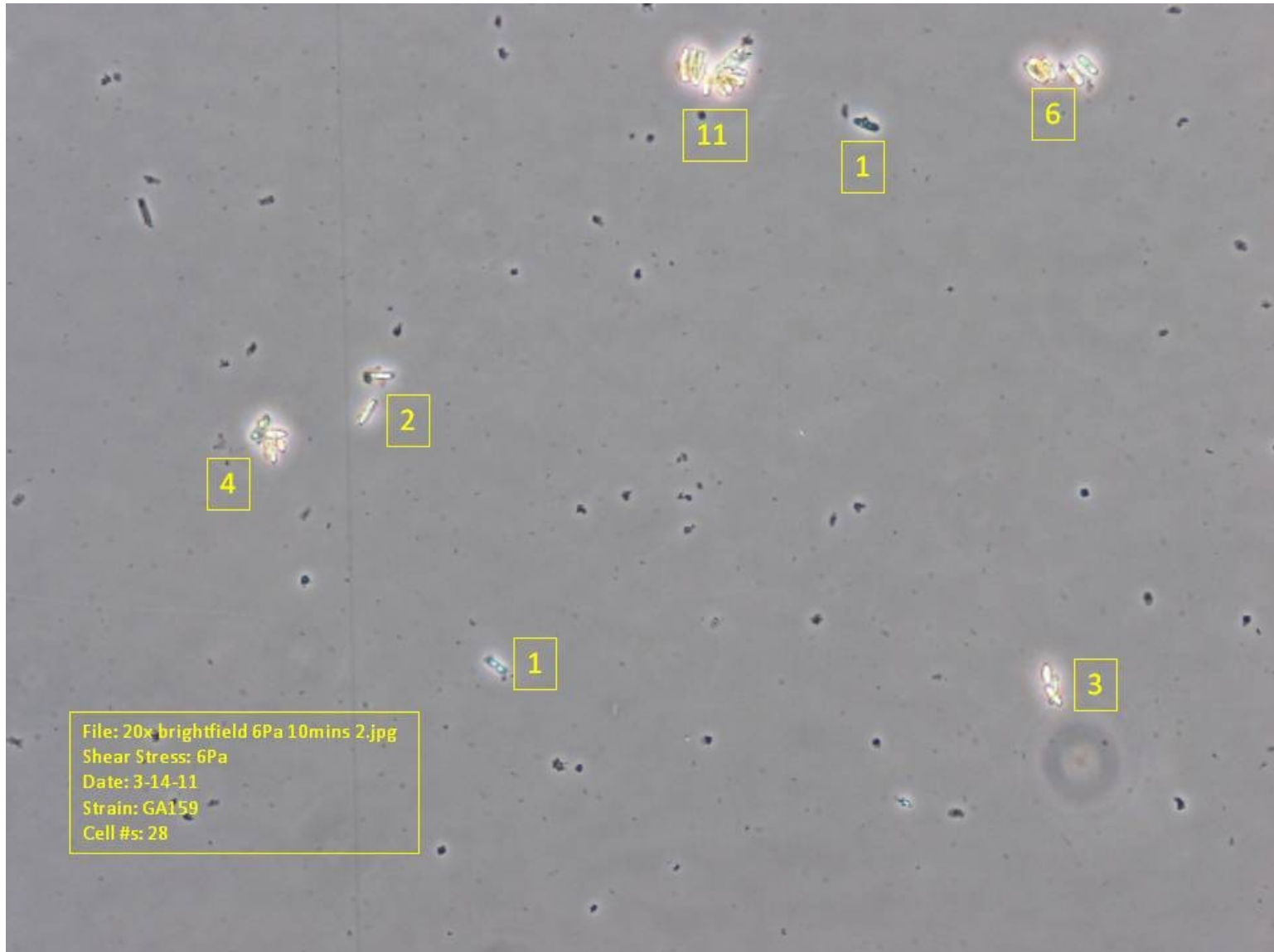




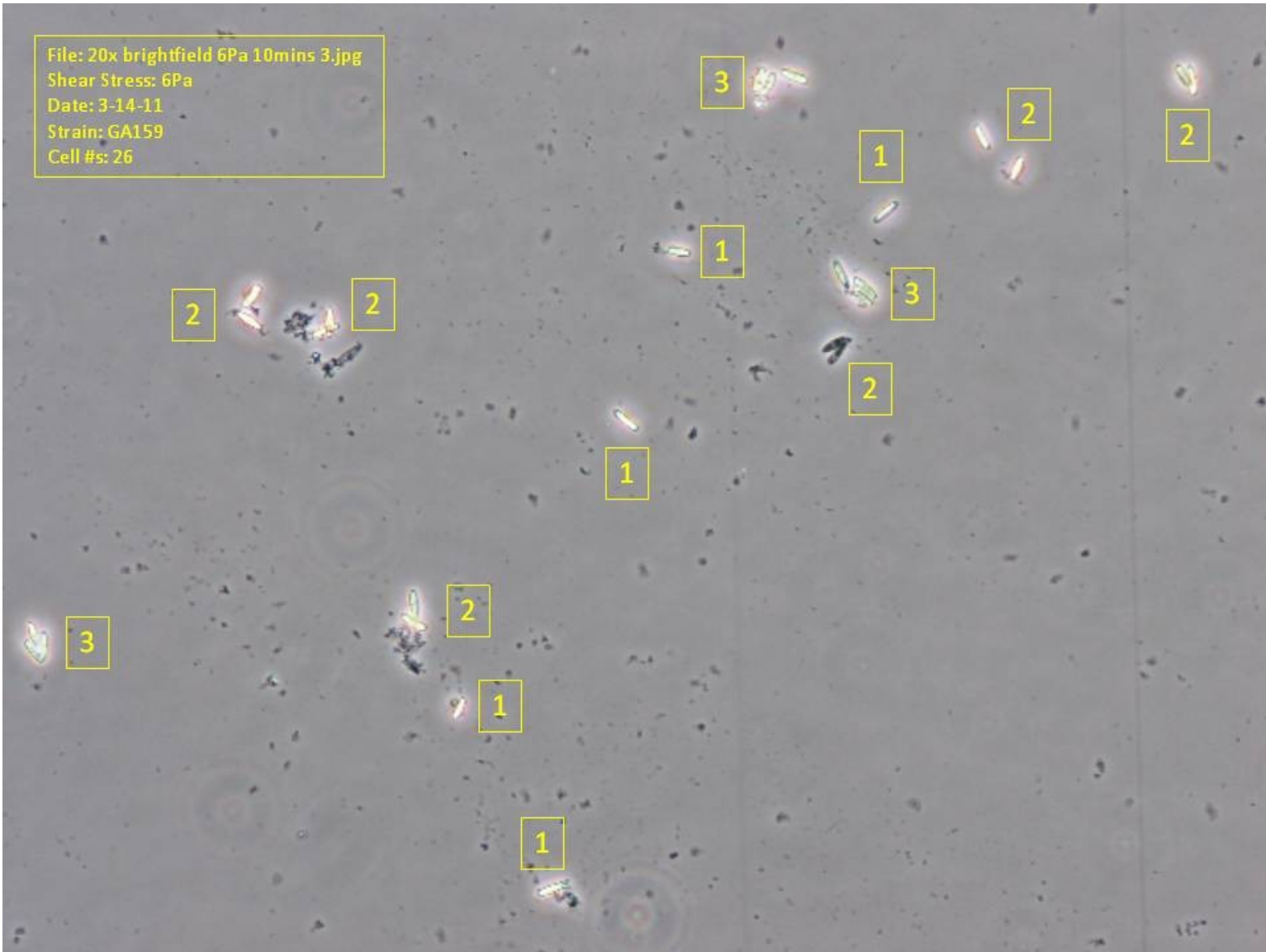
File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 3-14-11  
Strain: GA159  
Cell #s: 12



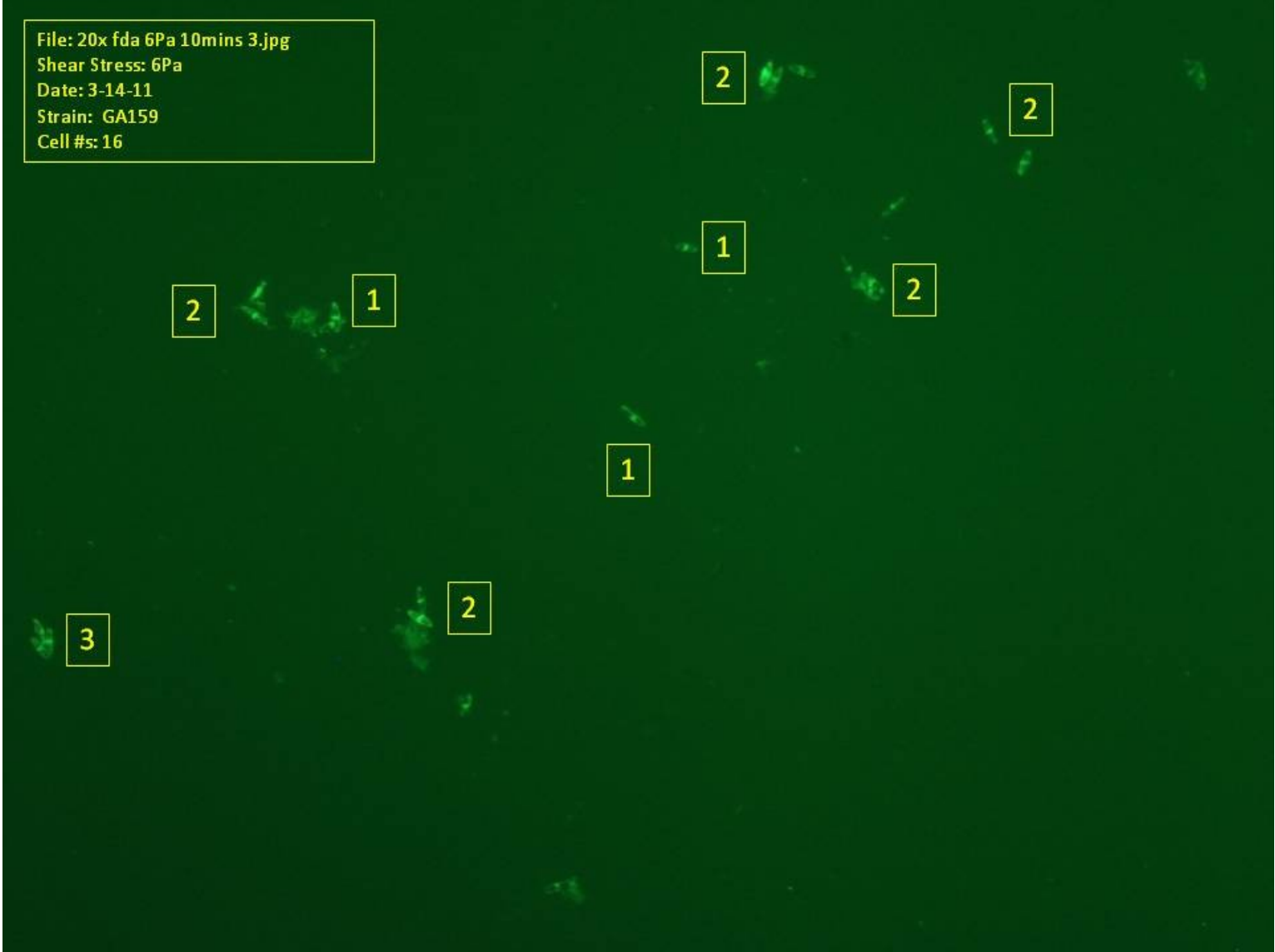


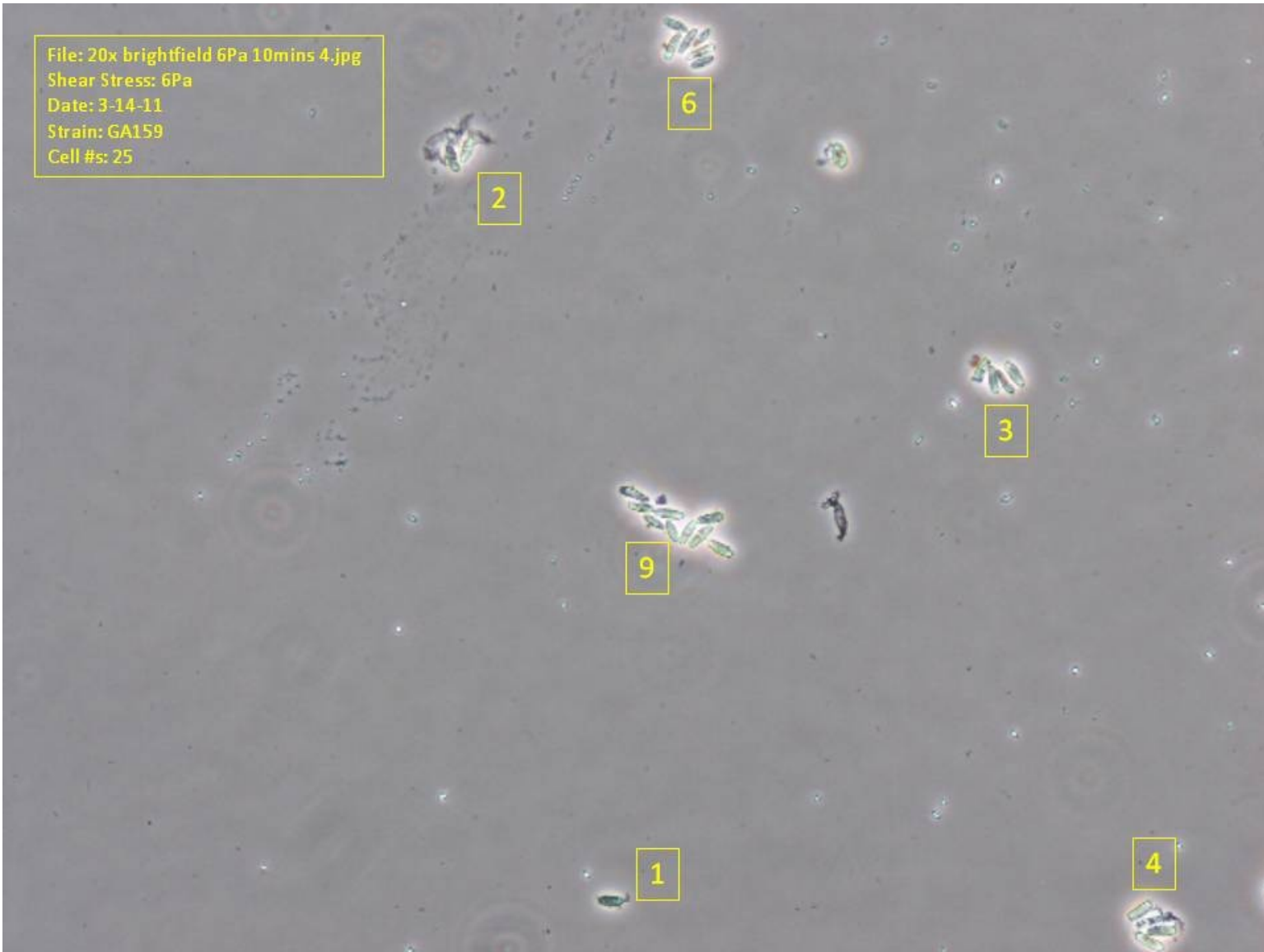






File: 20x fda 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 3-14-11  
Strain: GA159  
Cell #s: 16





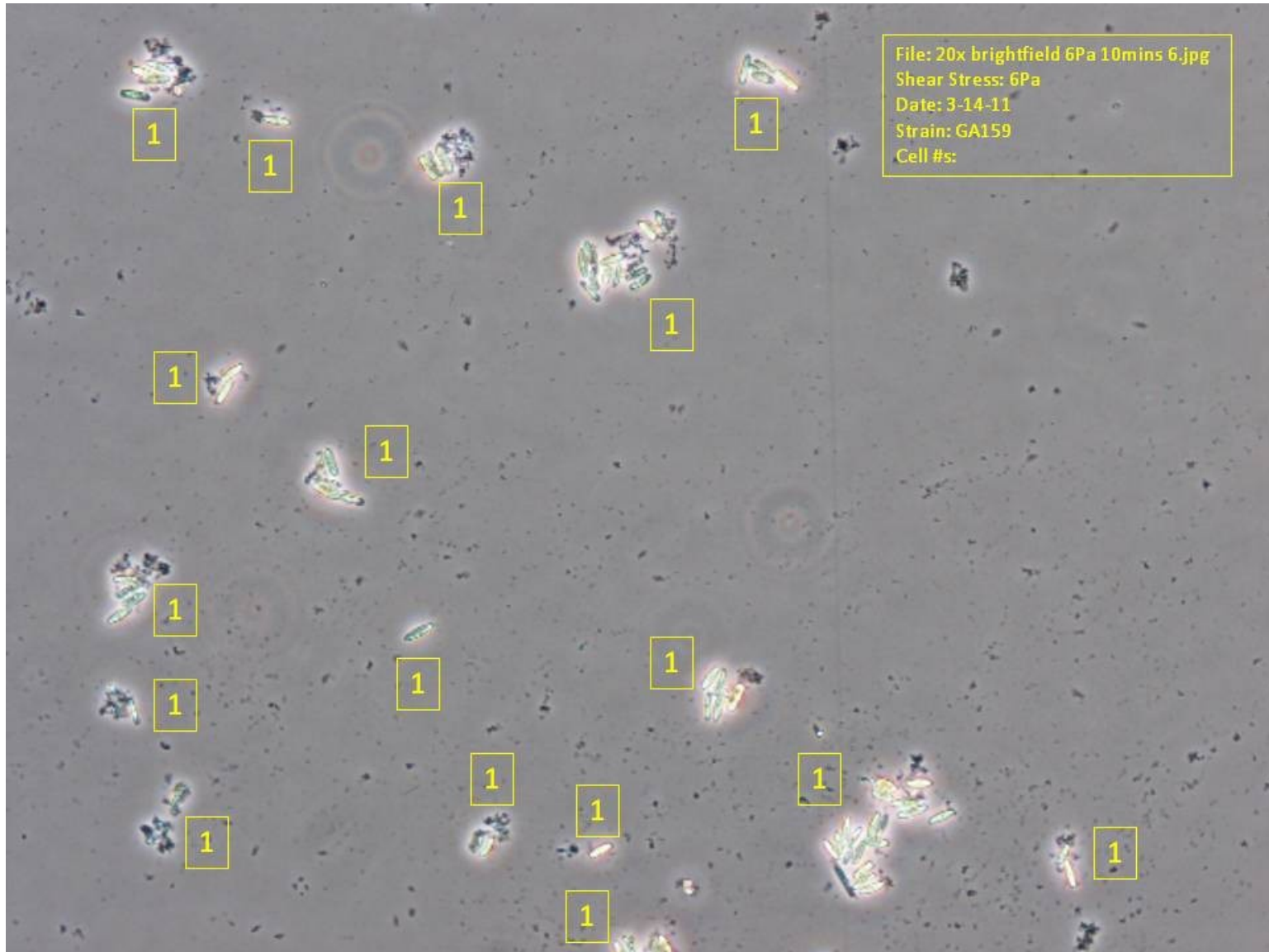




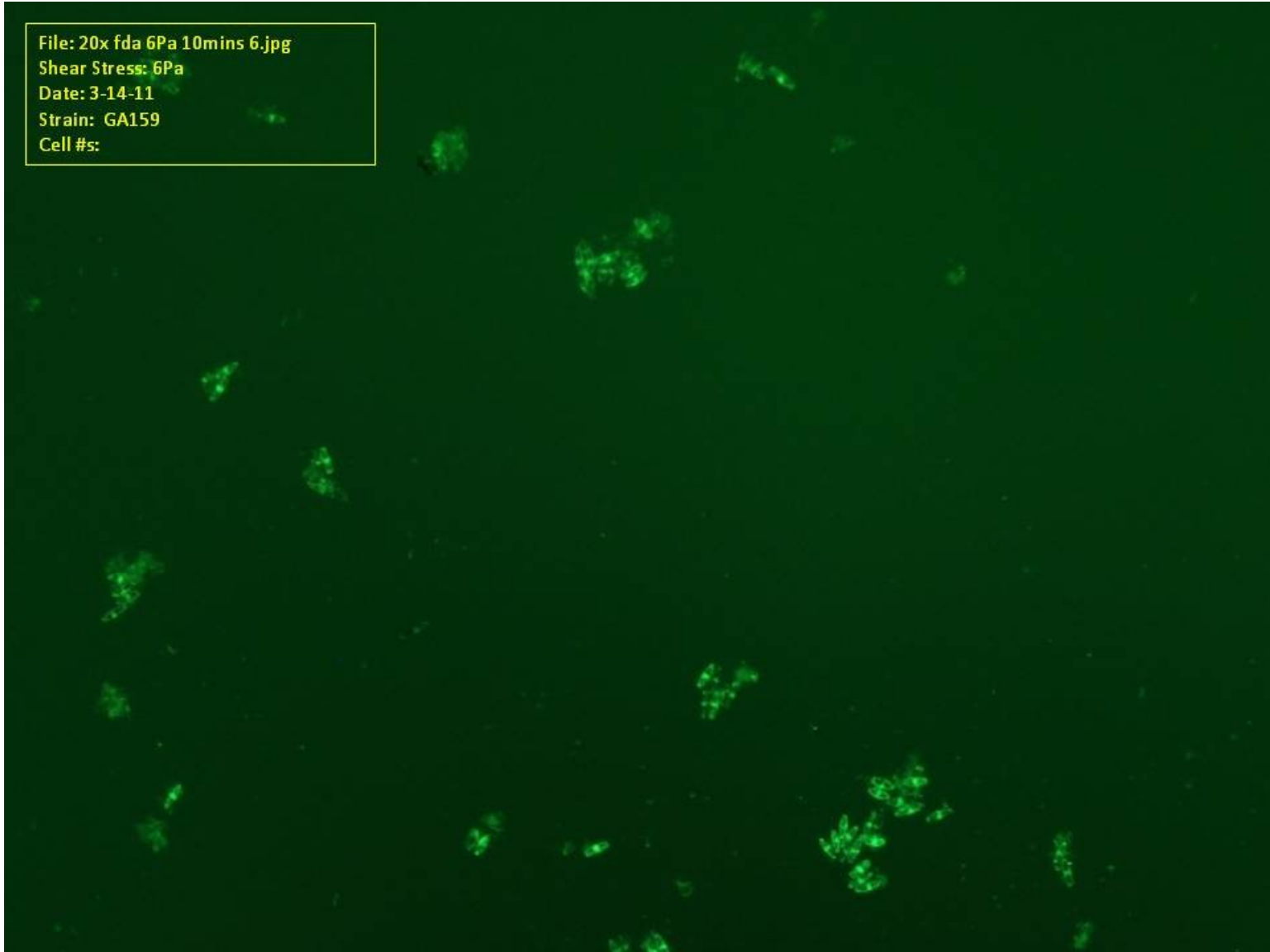
File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 3-14-11  
Strain: GA159  
Cell #s: 17

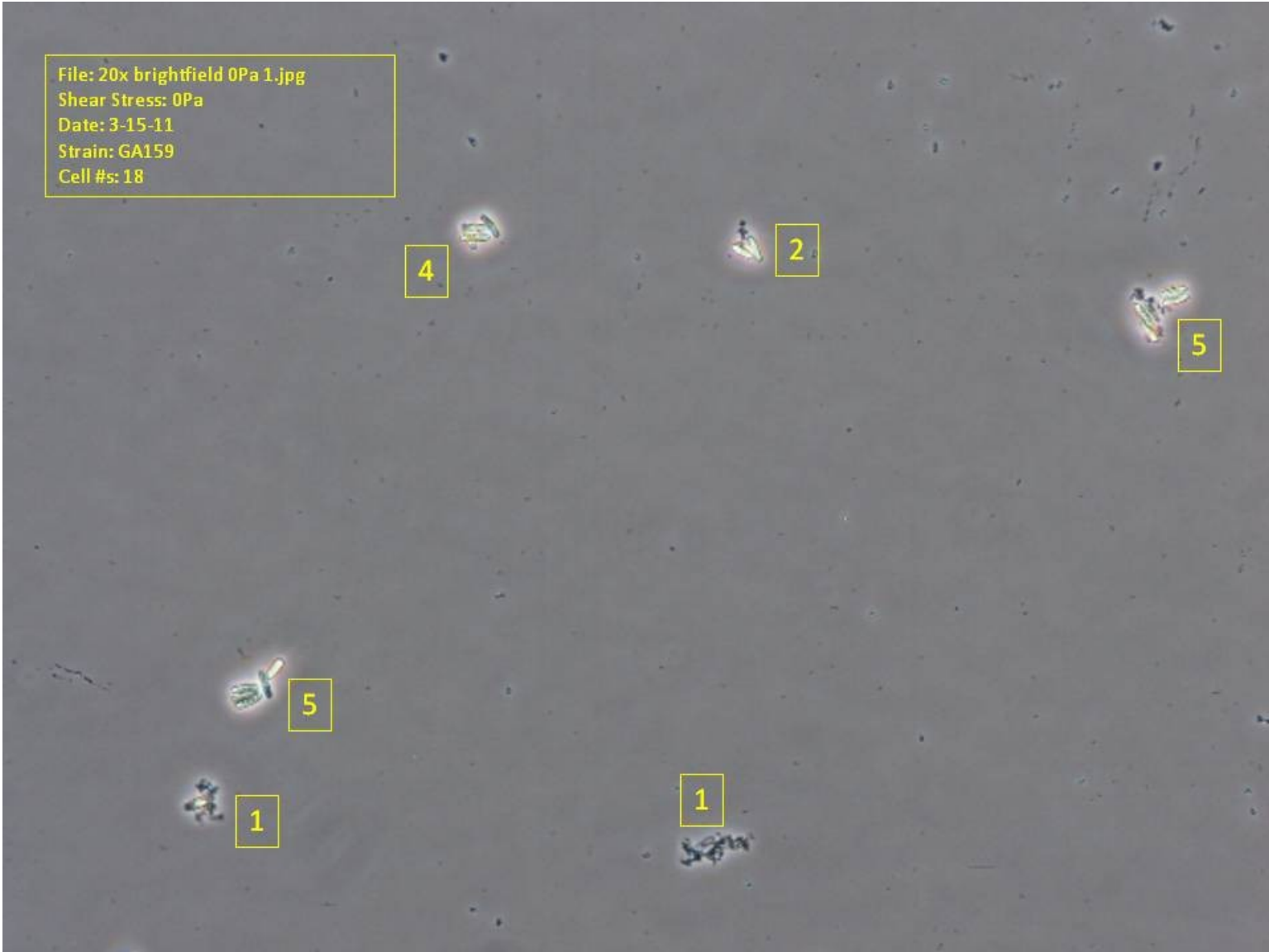






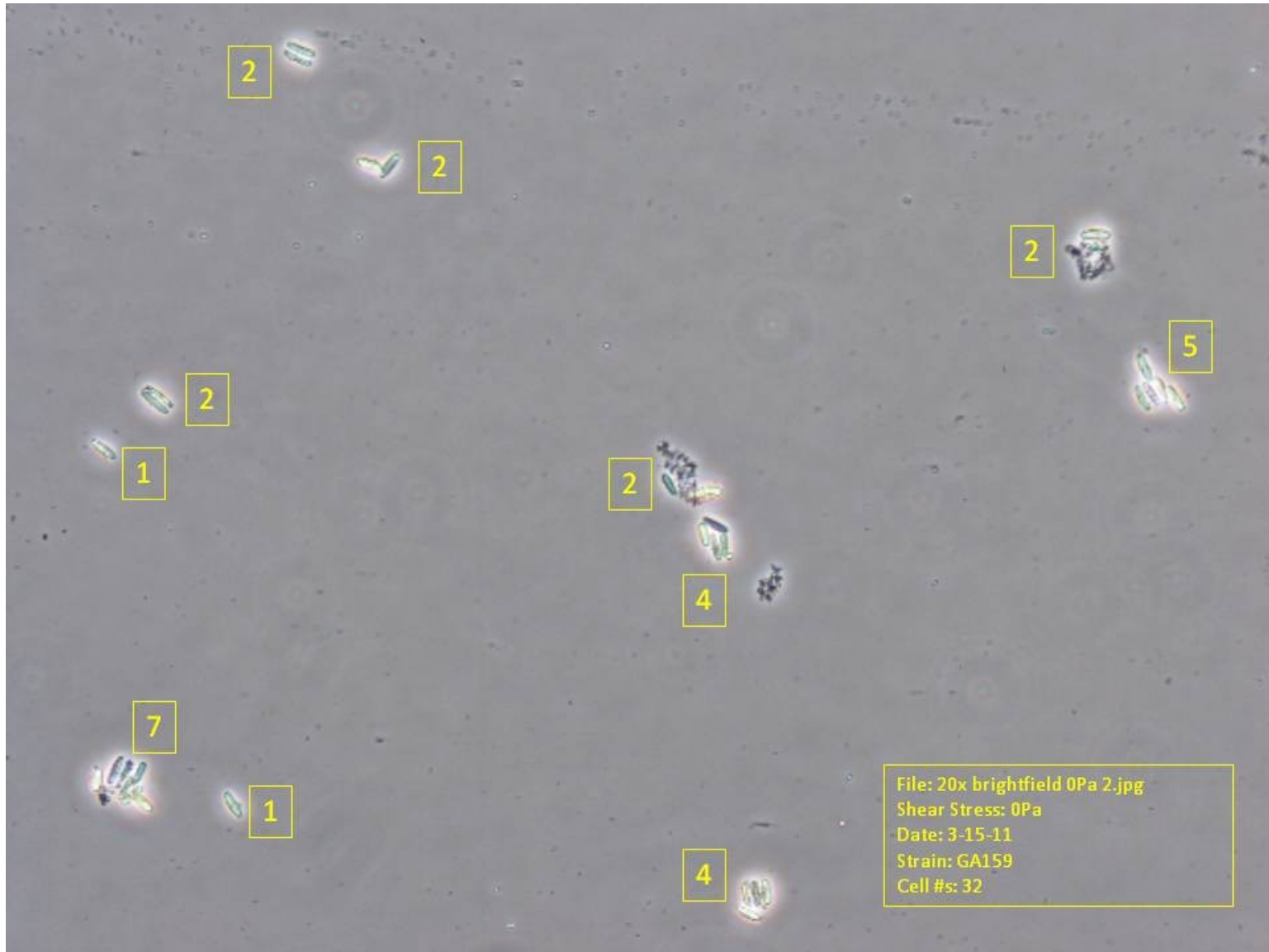
File: 20x fda 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 3-14-11  
Strain: GA159  
Cell #s:



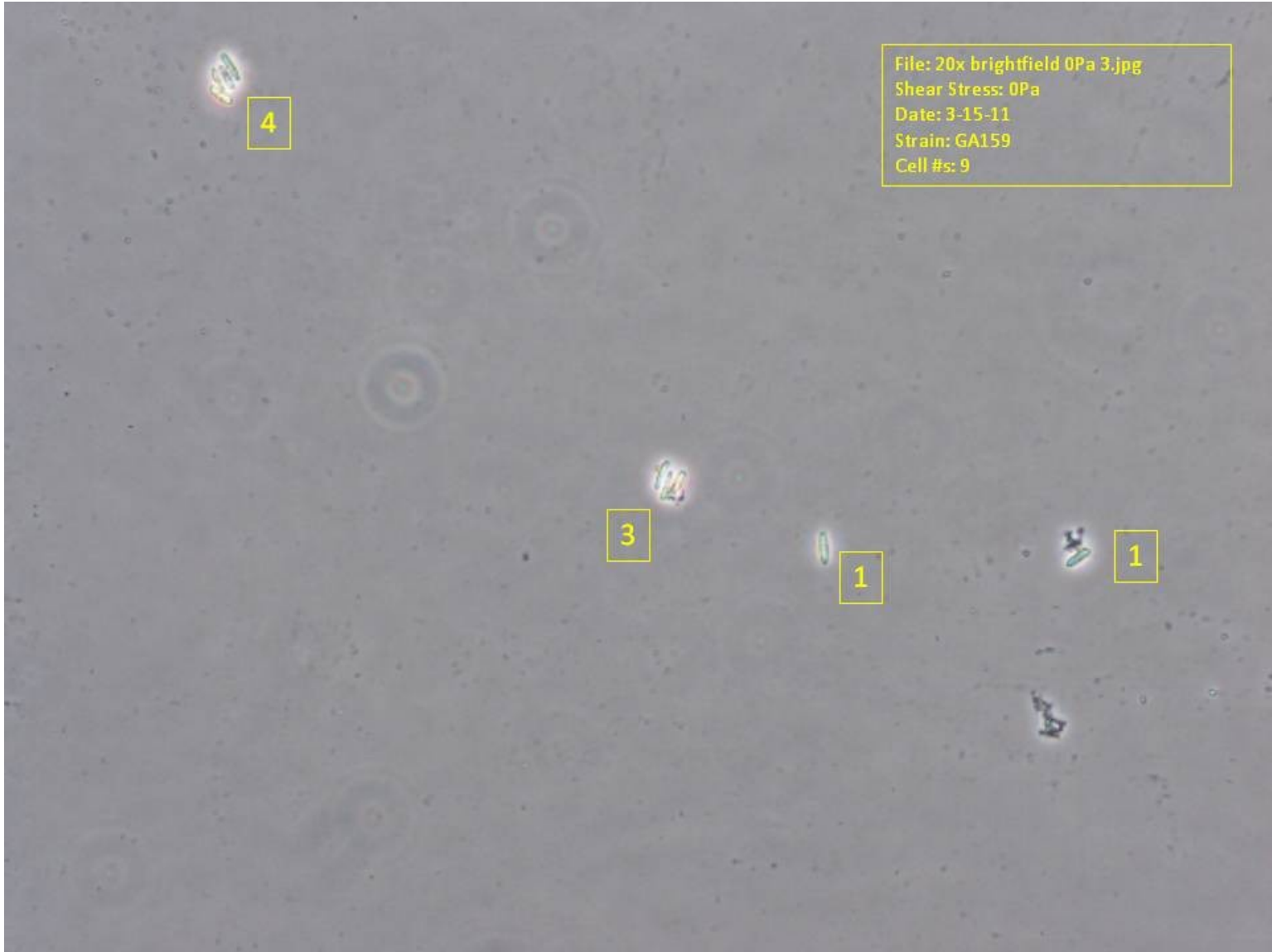


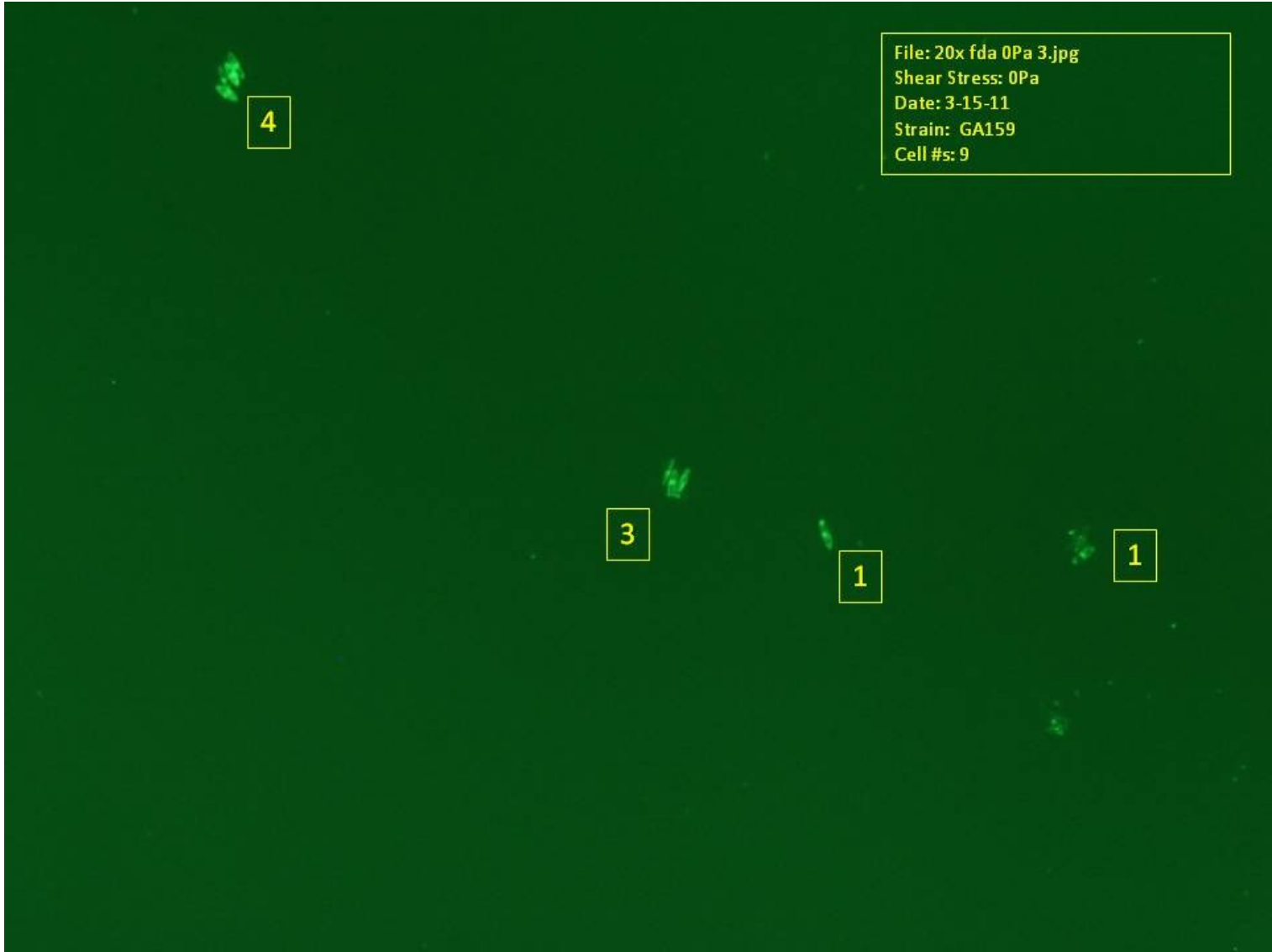
File: 20x fda 0Pa 1.jpg  
Shear Stress: 0Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 15



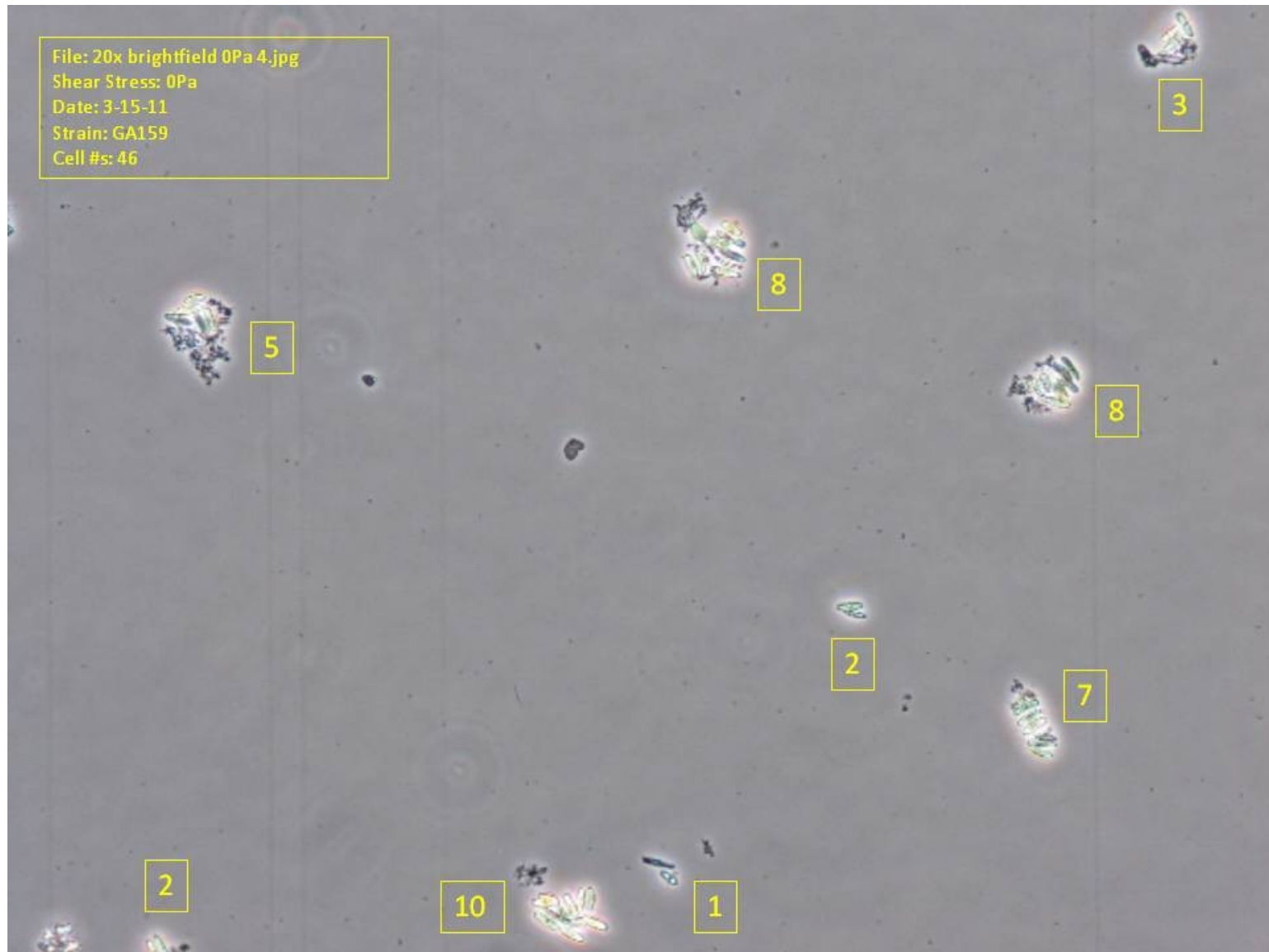


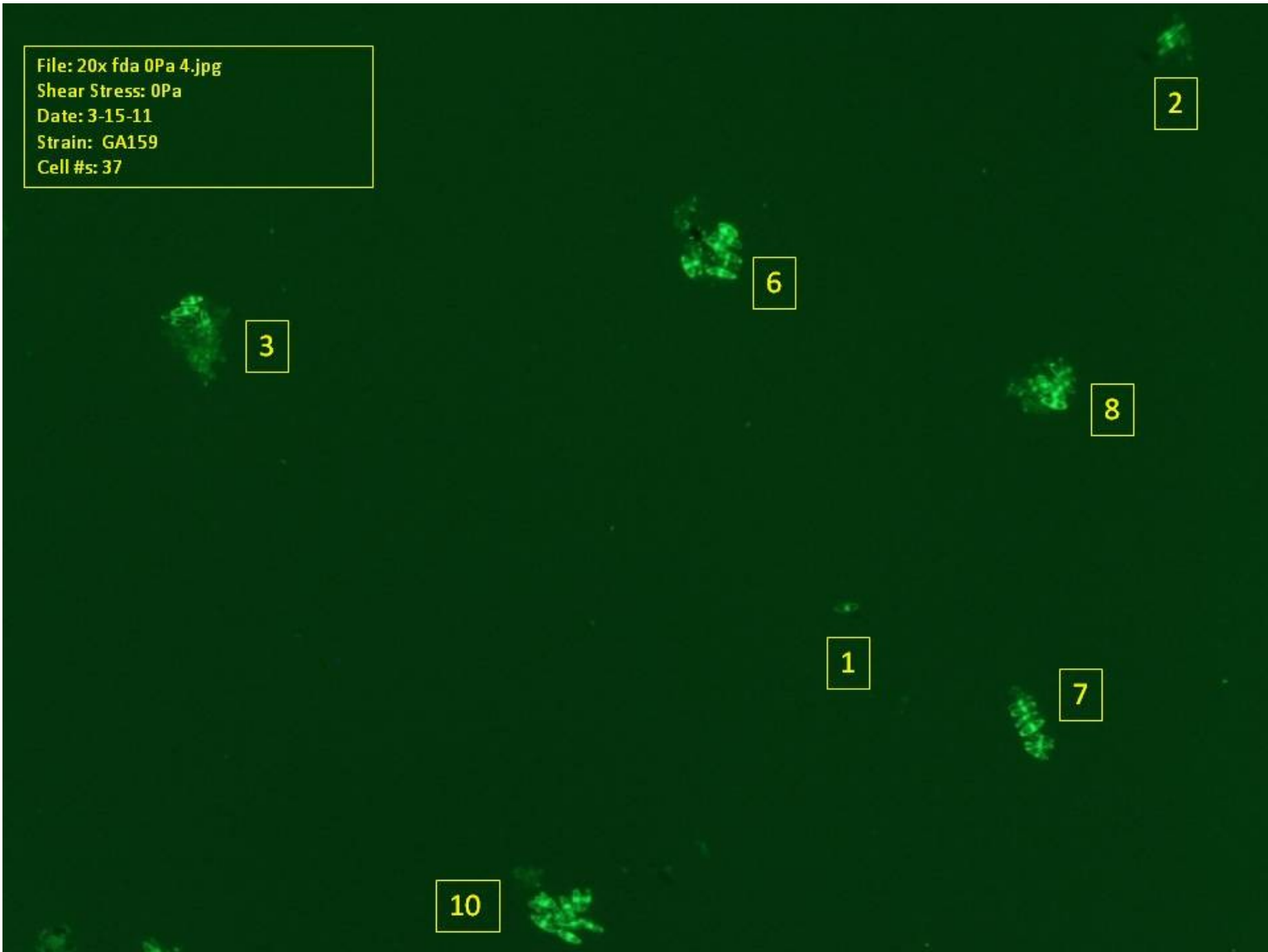


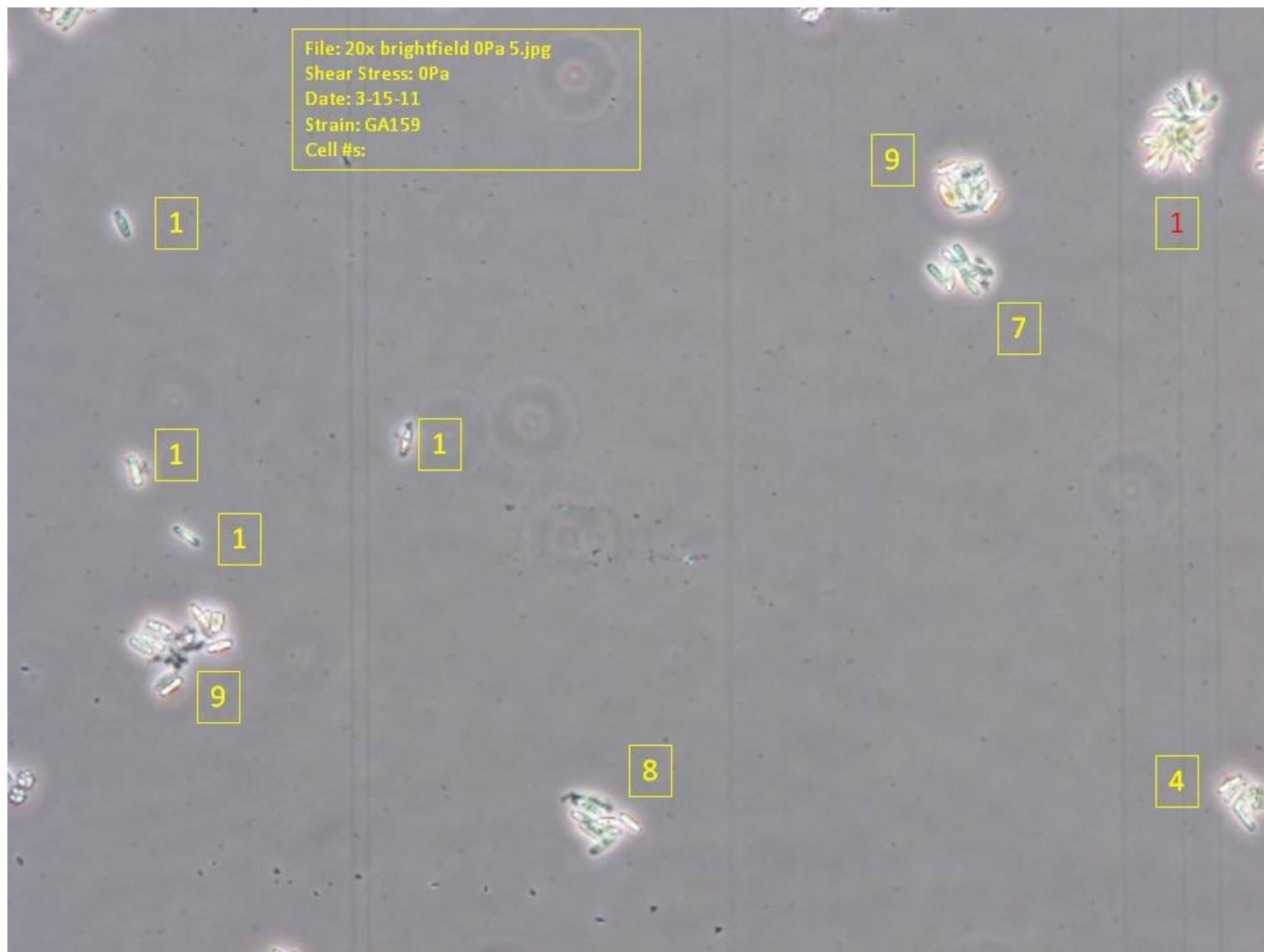




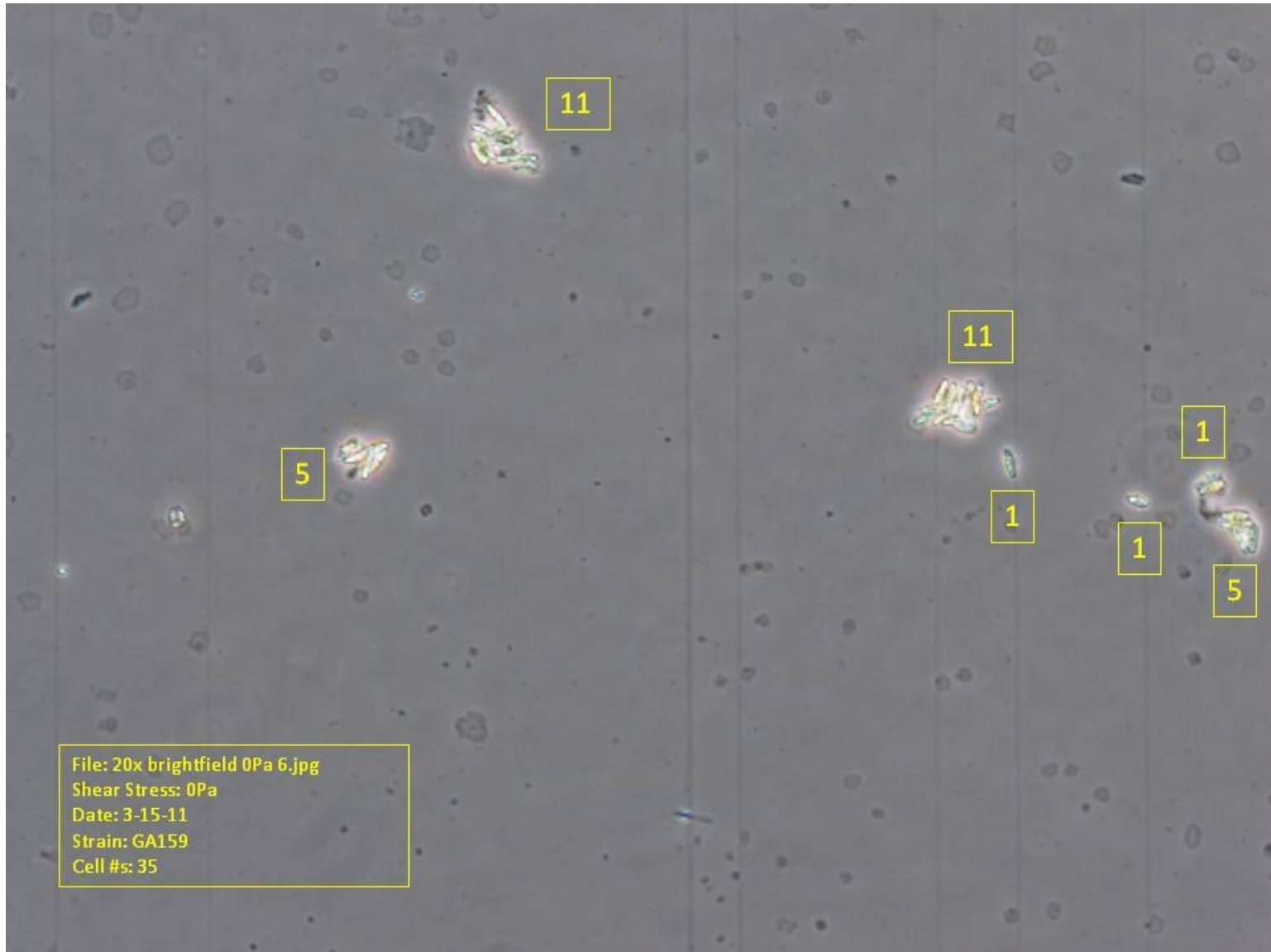














File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 5



File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 4





File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 18



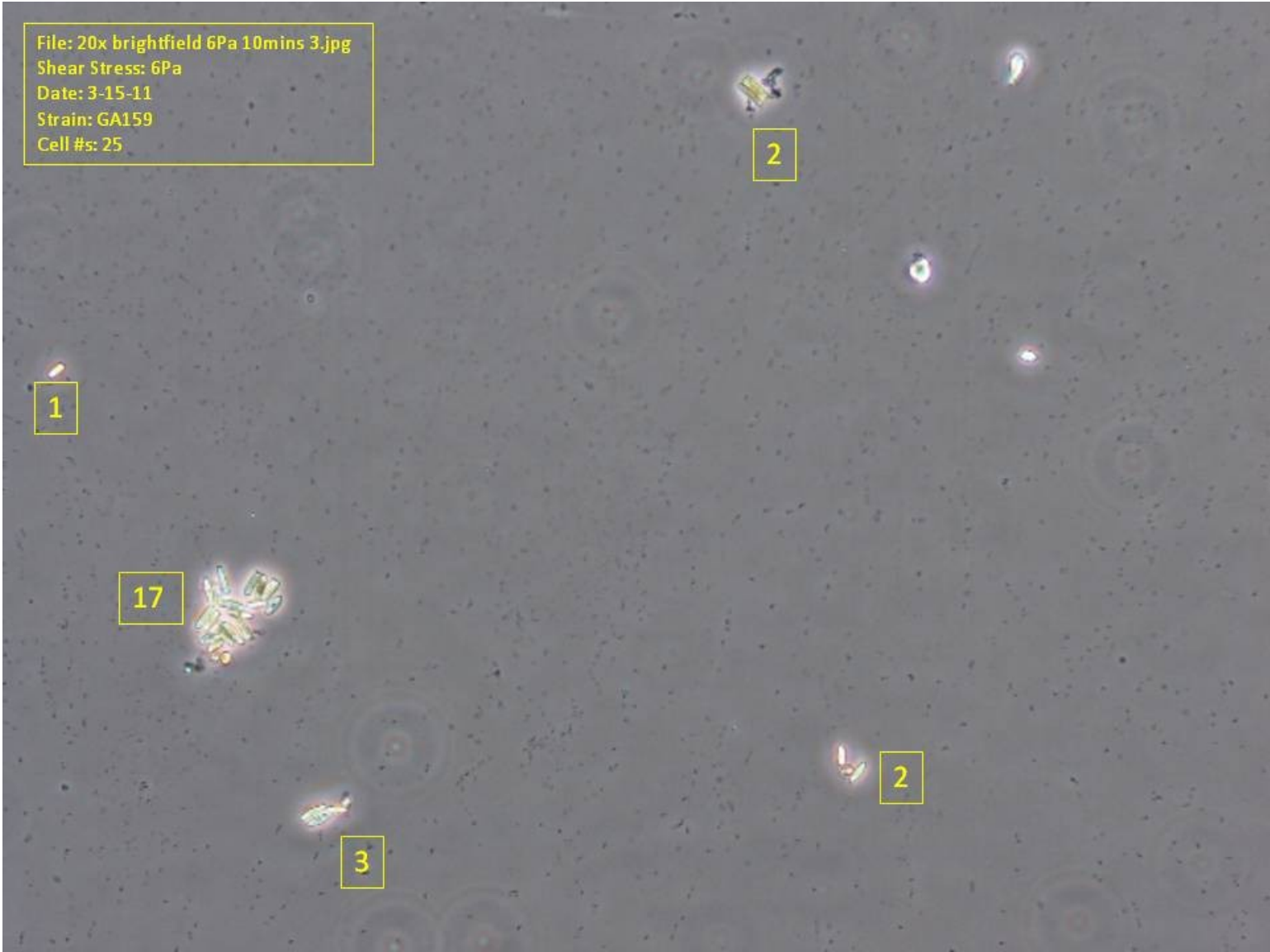
File: 20x fda 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 11

2

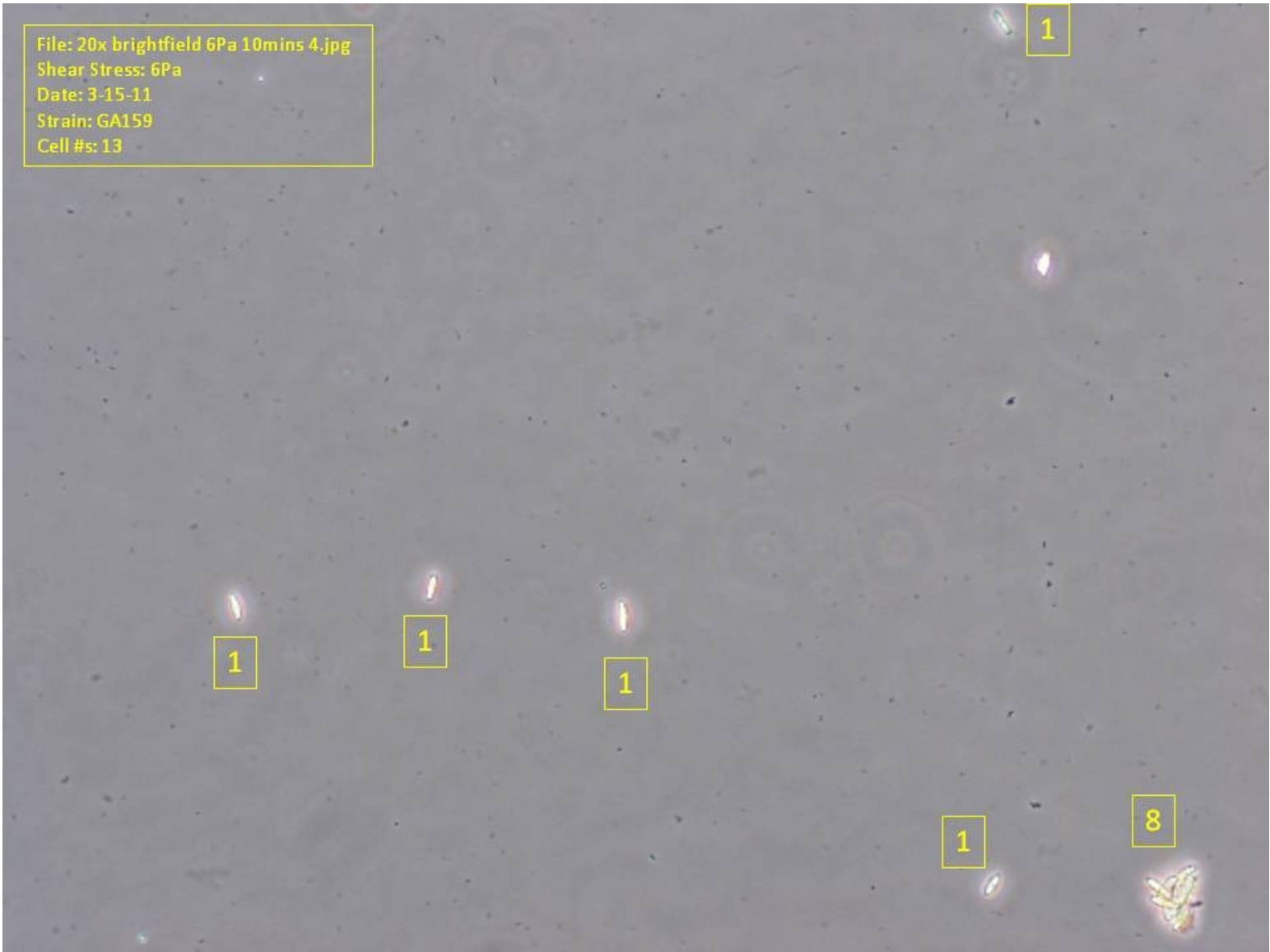
6

3

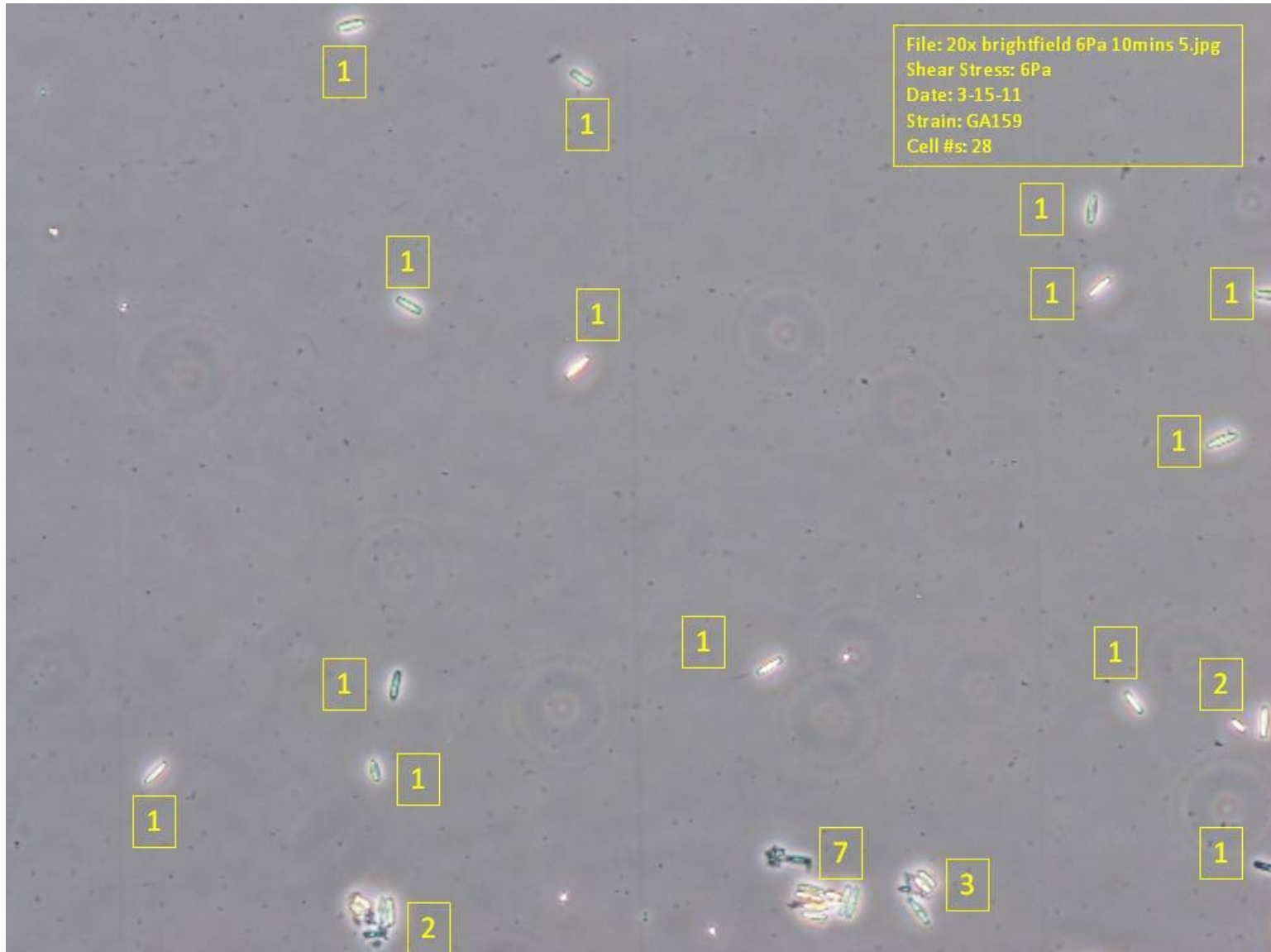
File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 25

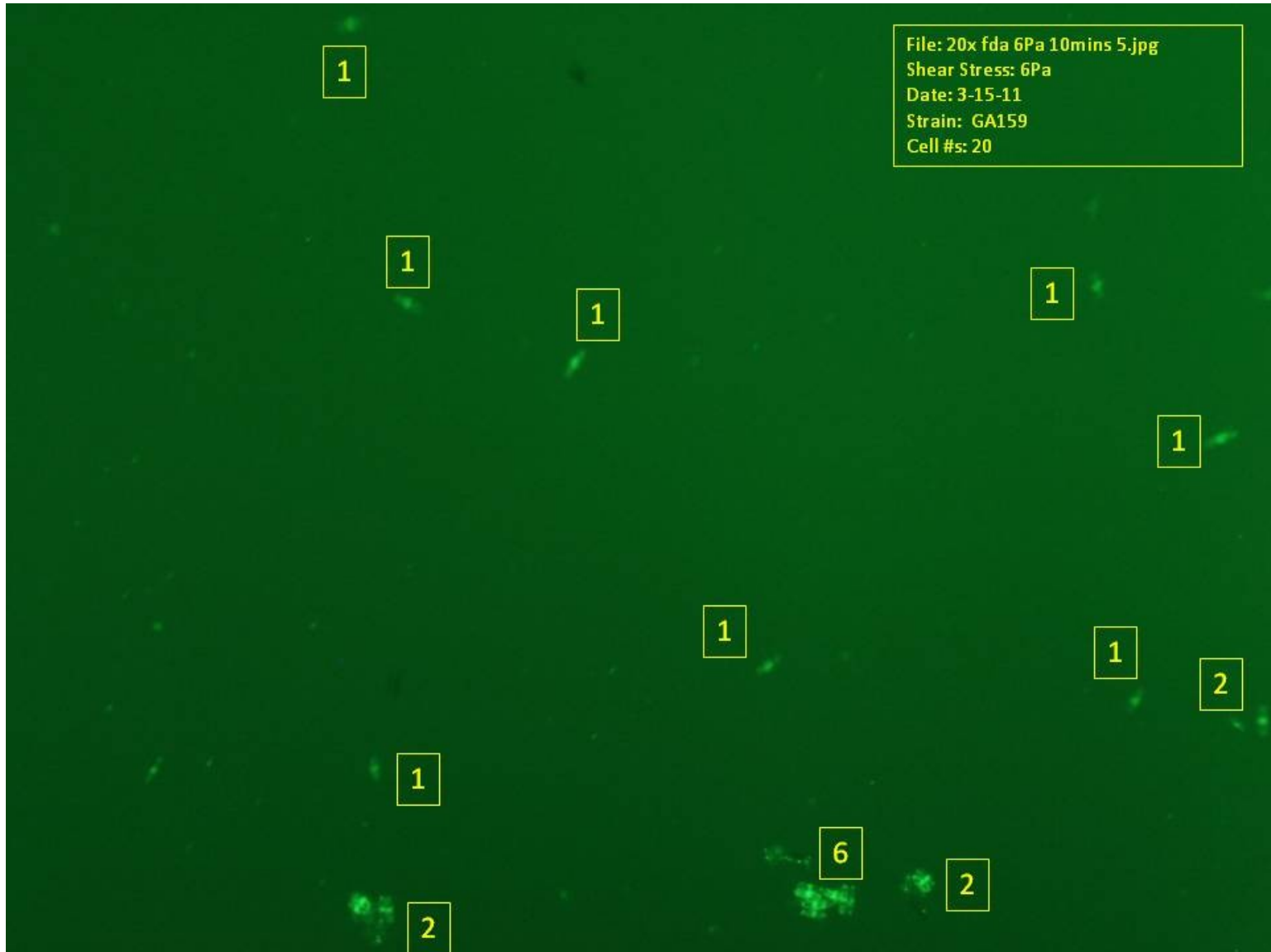










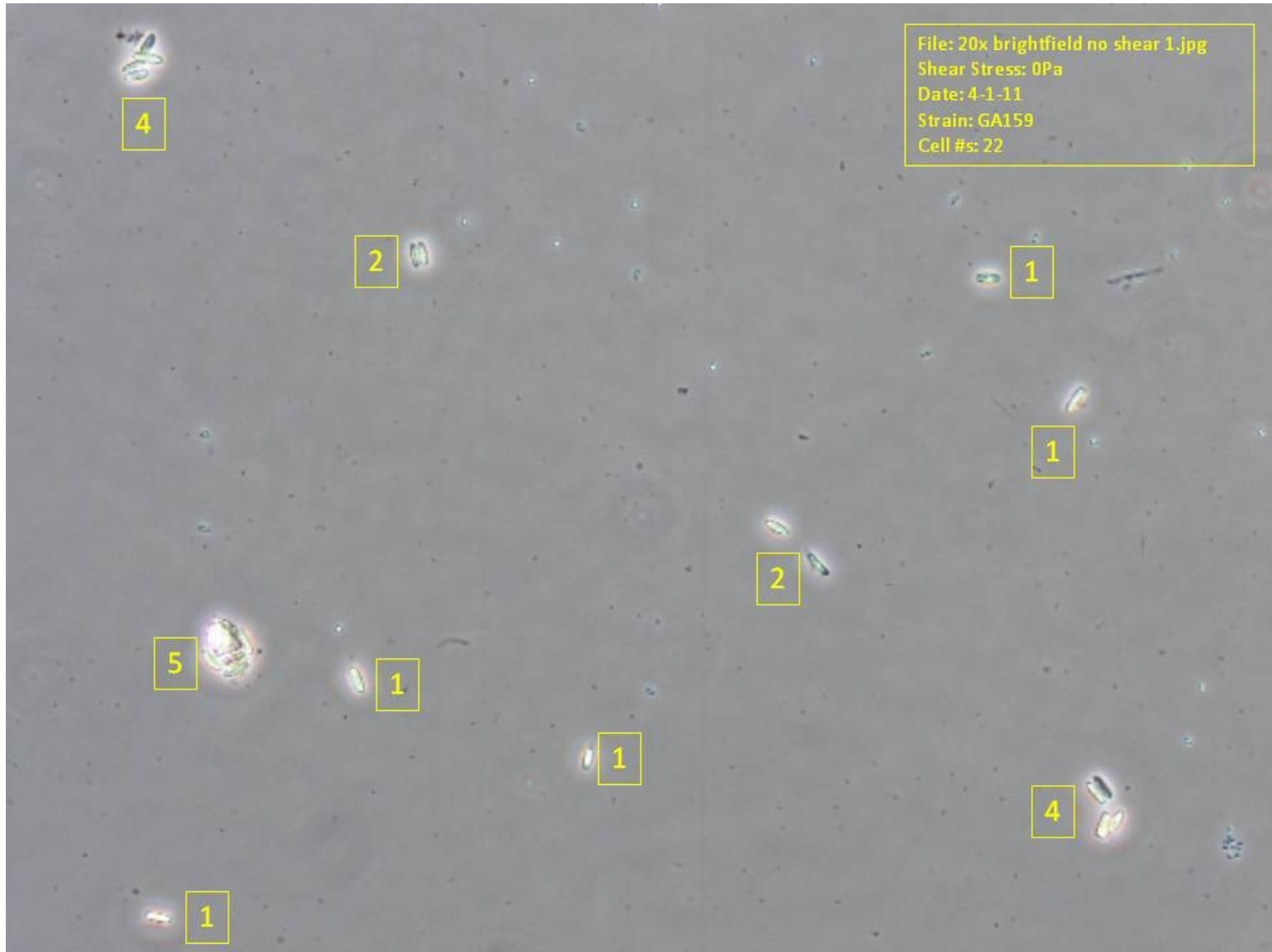


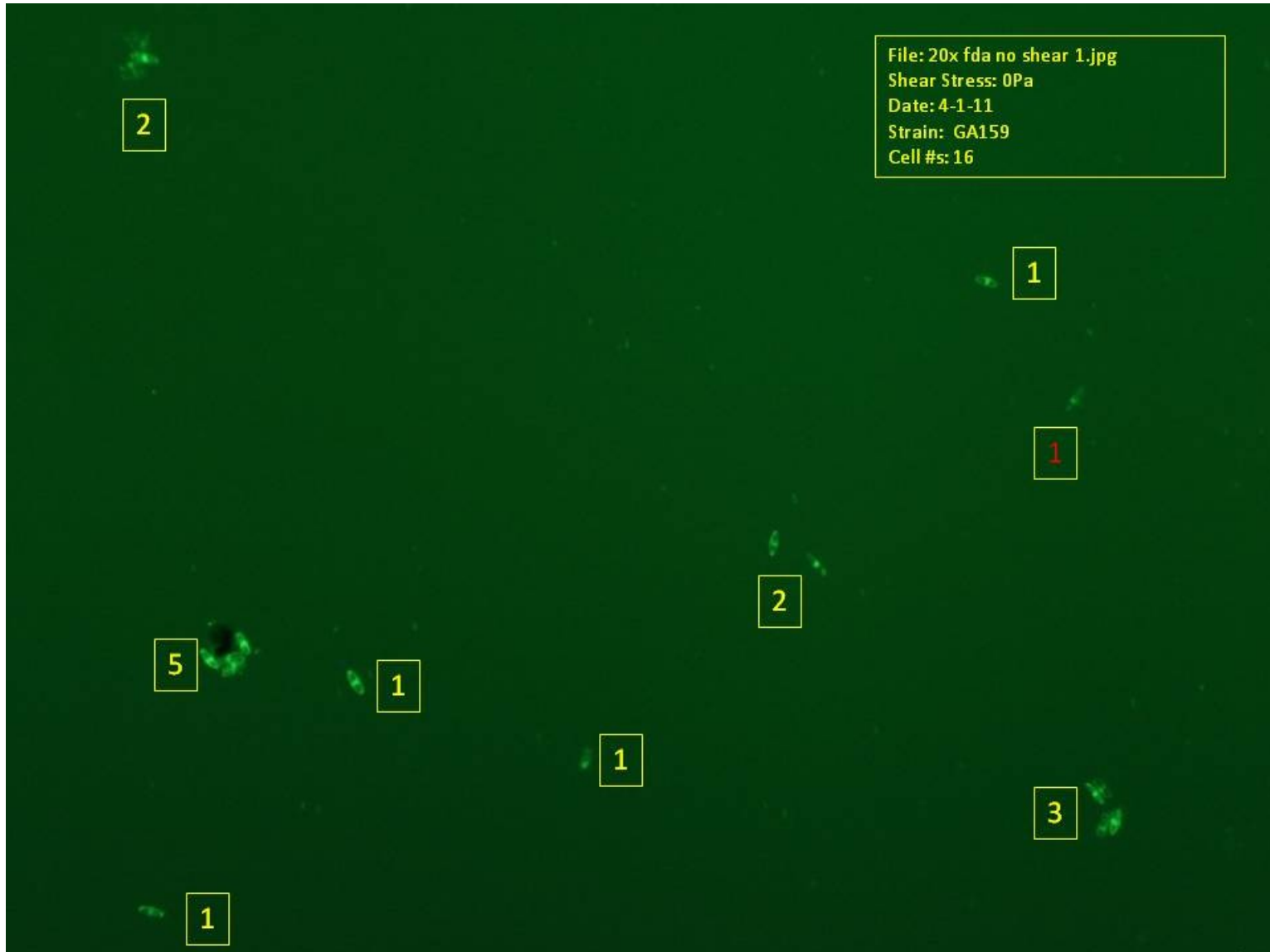


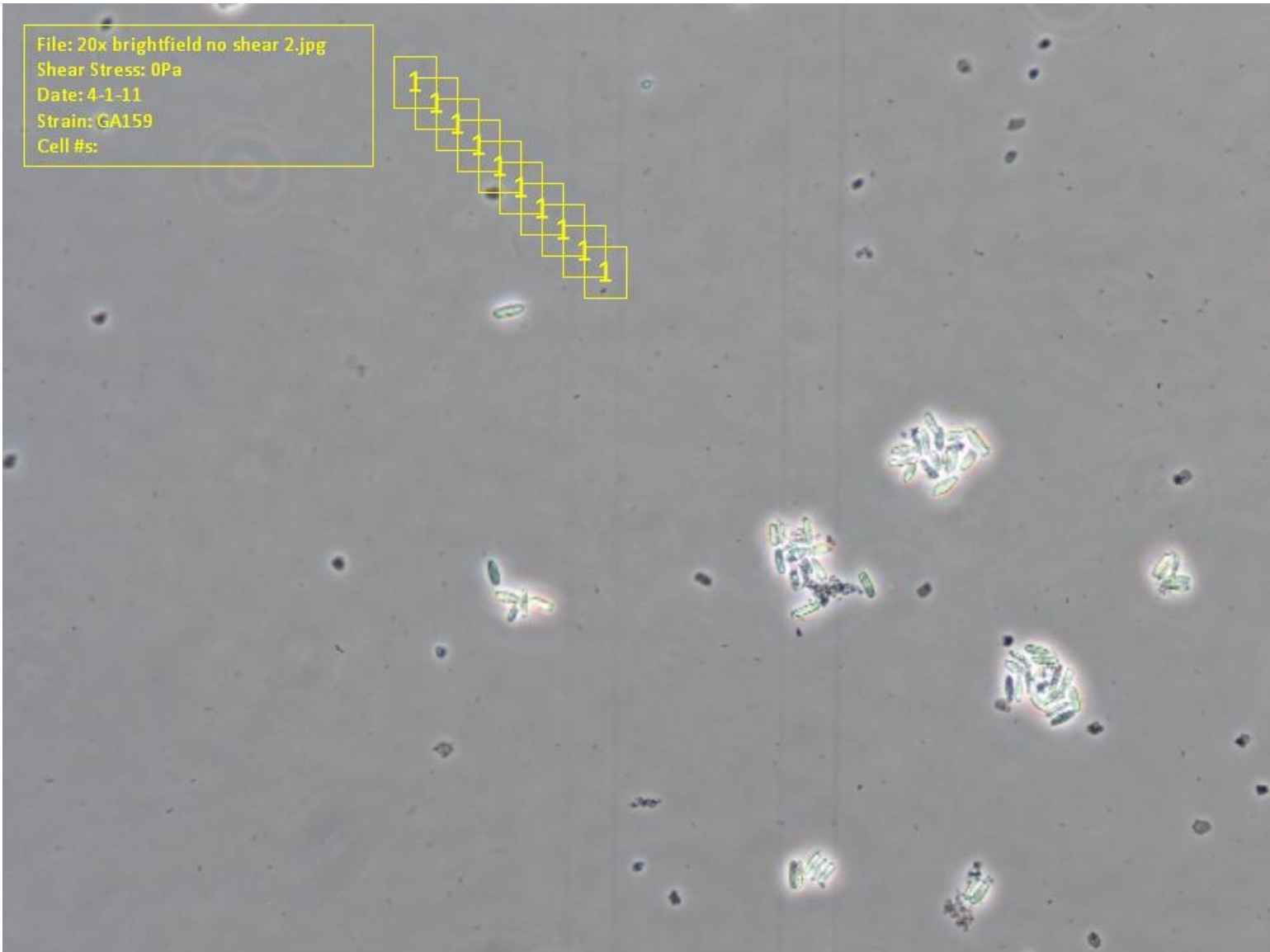
File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 3-15-11  
Strain: GA159  
Cell #s: 17



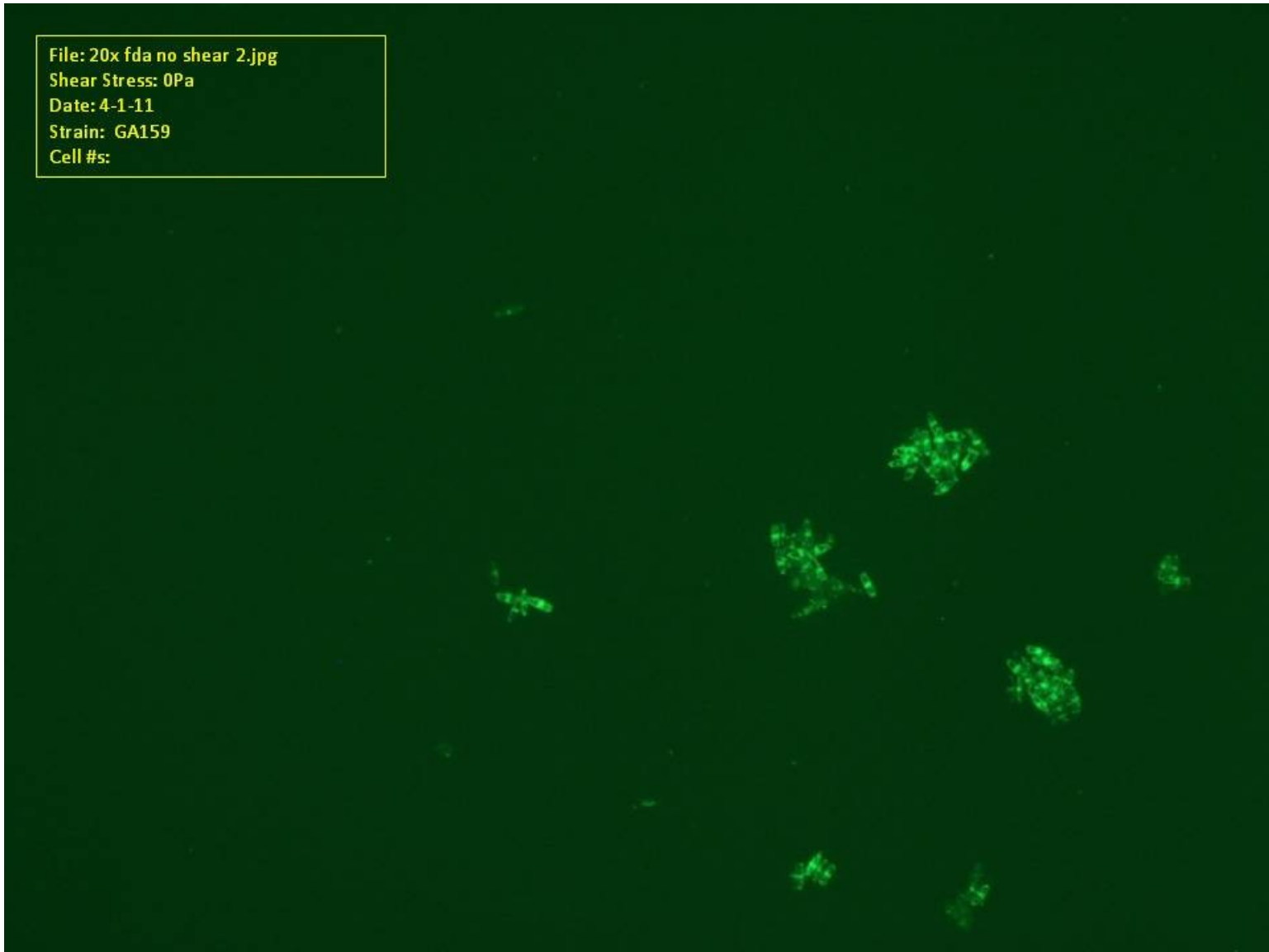


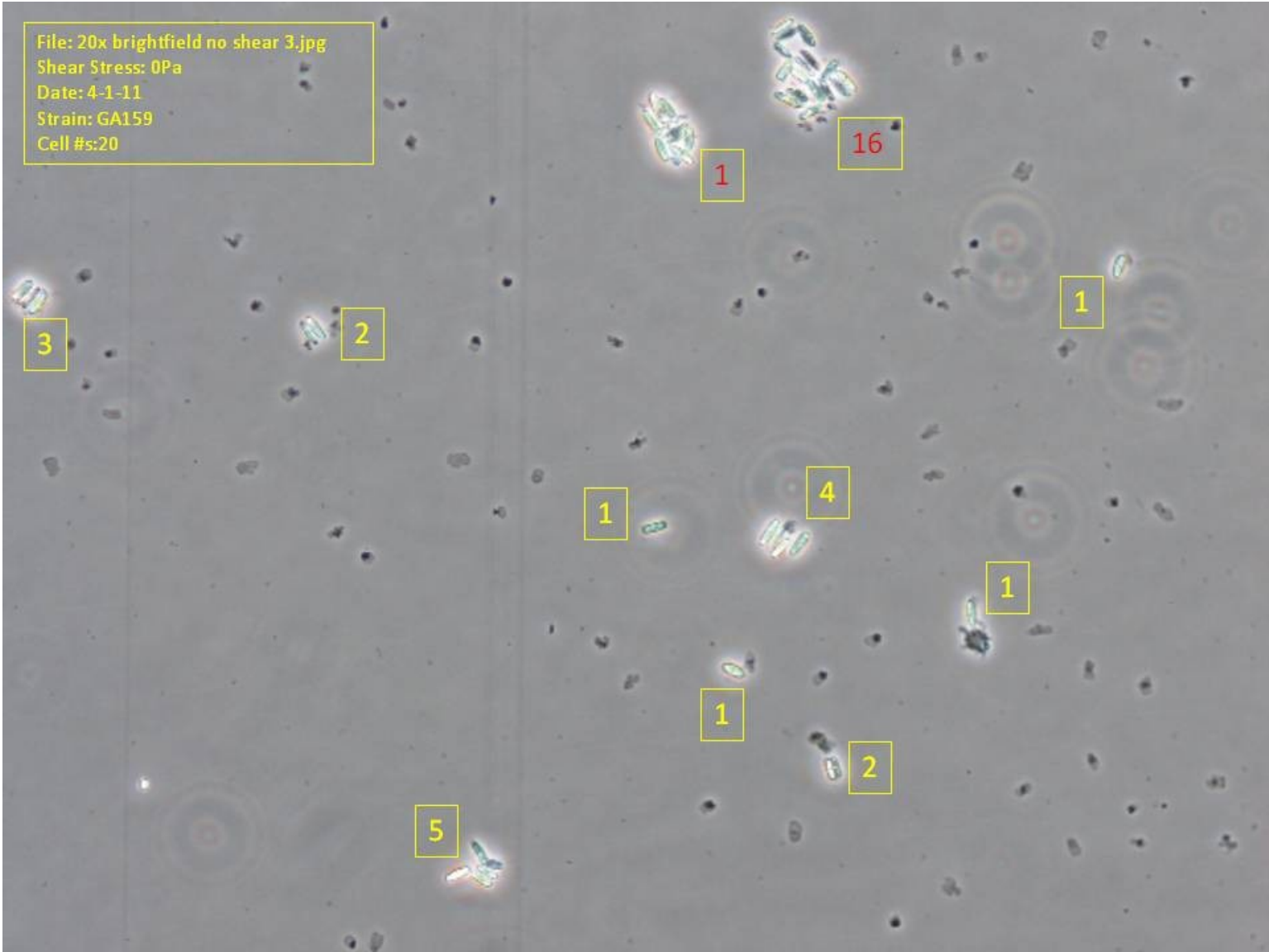


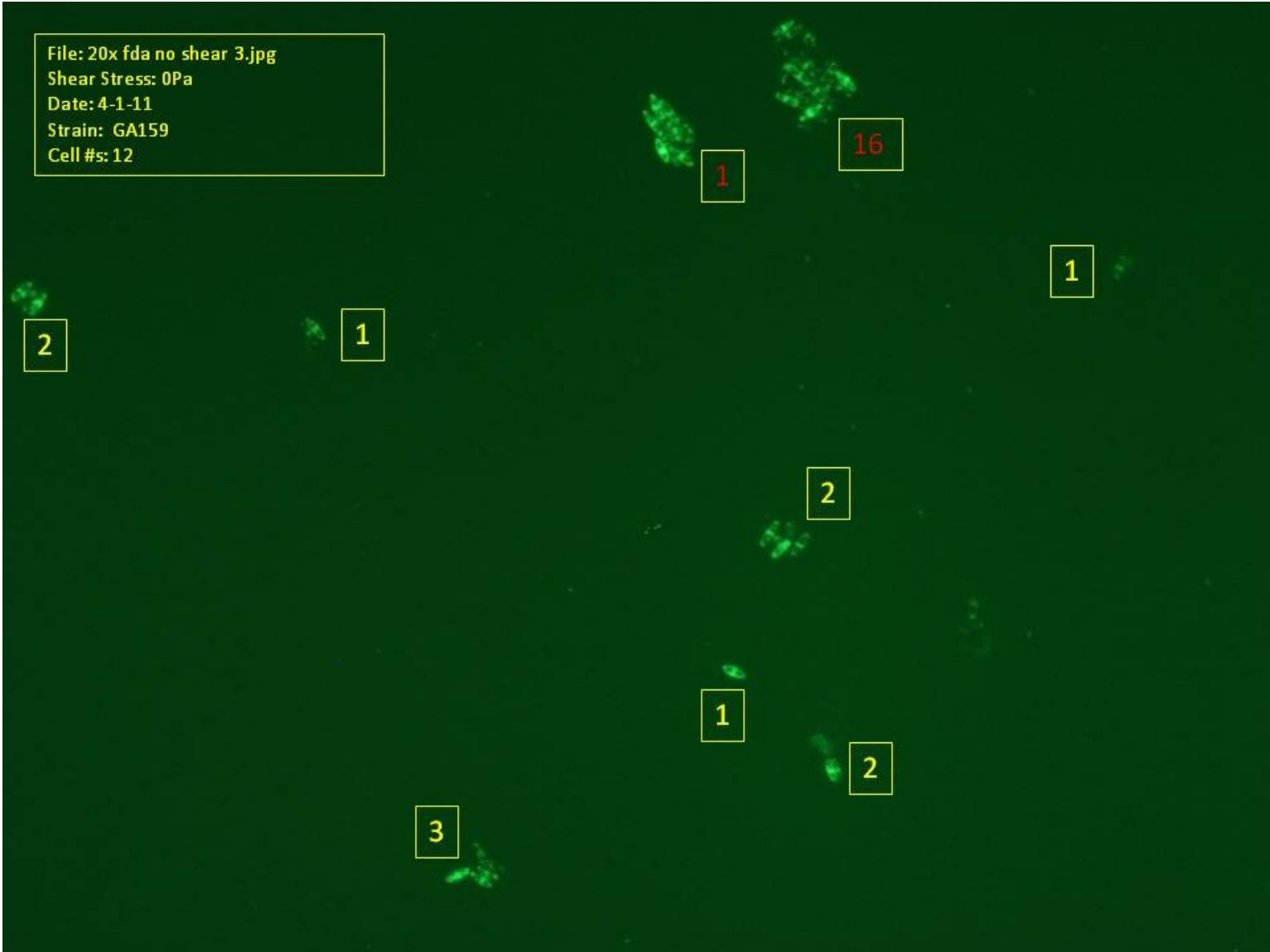




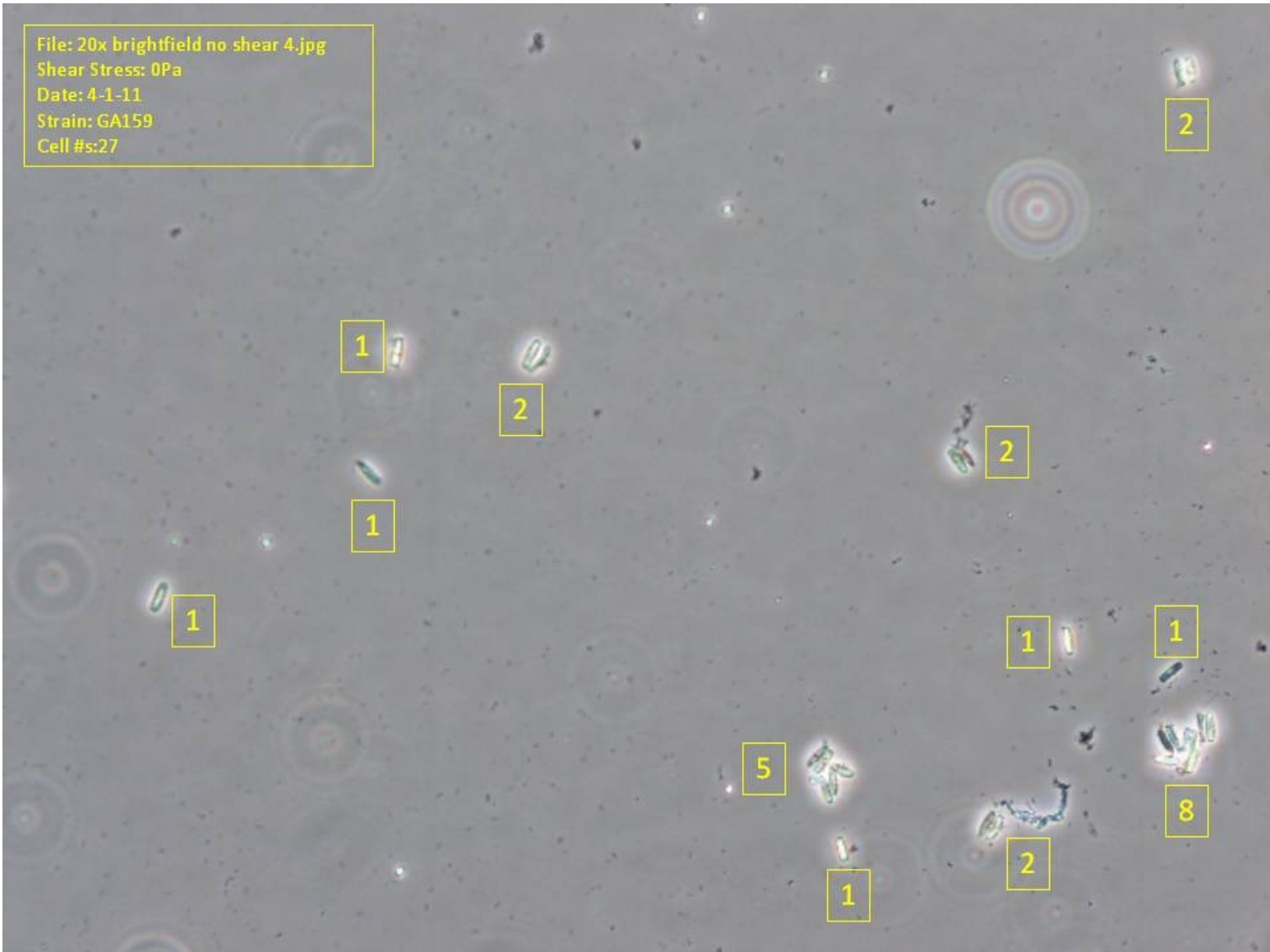
File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: GA159  
Cell #s:





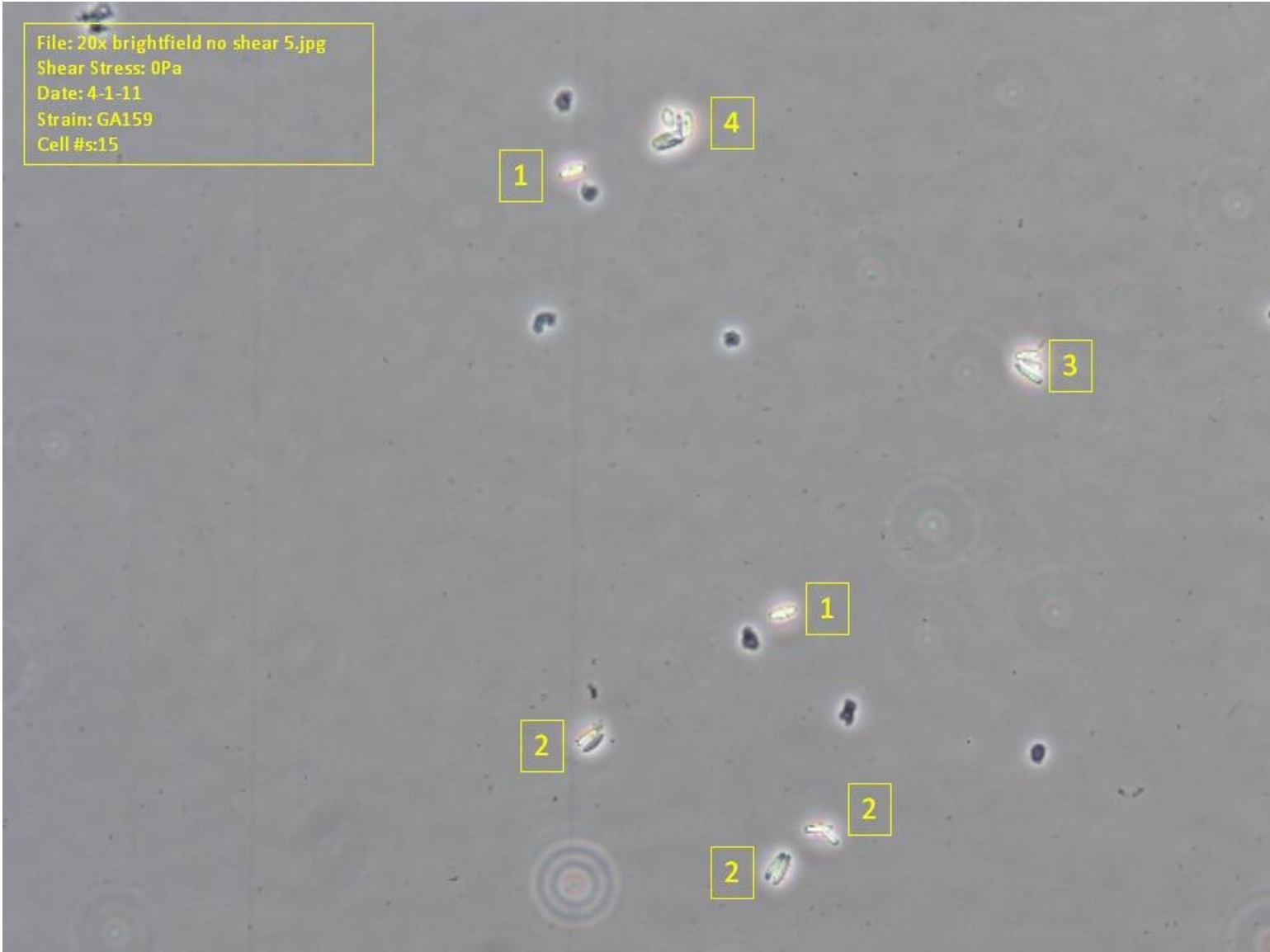




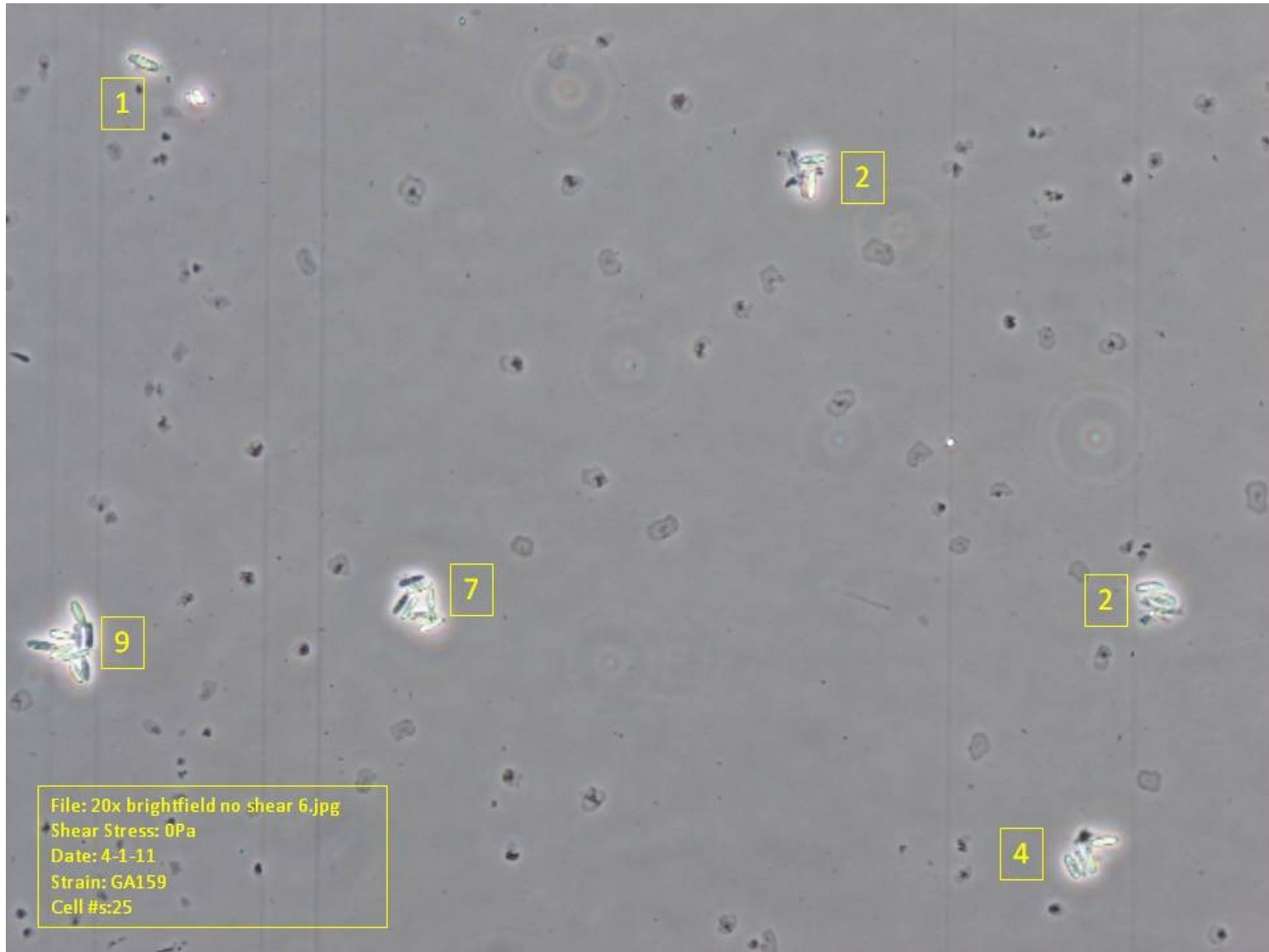




File: 20x brightfield no shear 5.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: GA159  
Cell #s:15

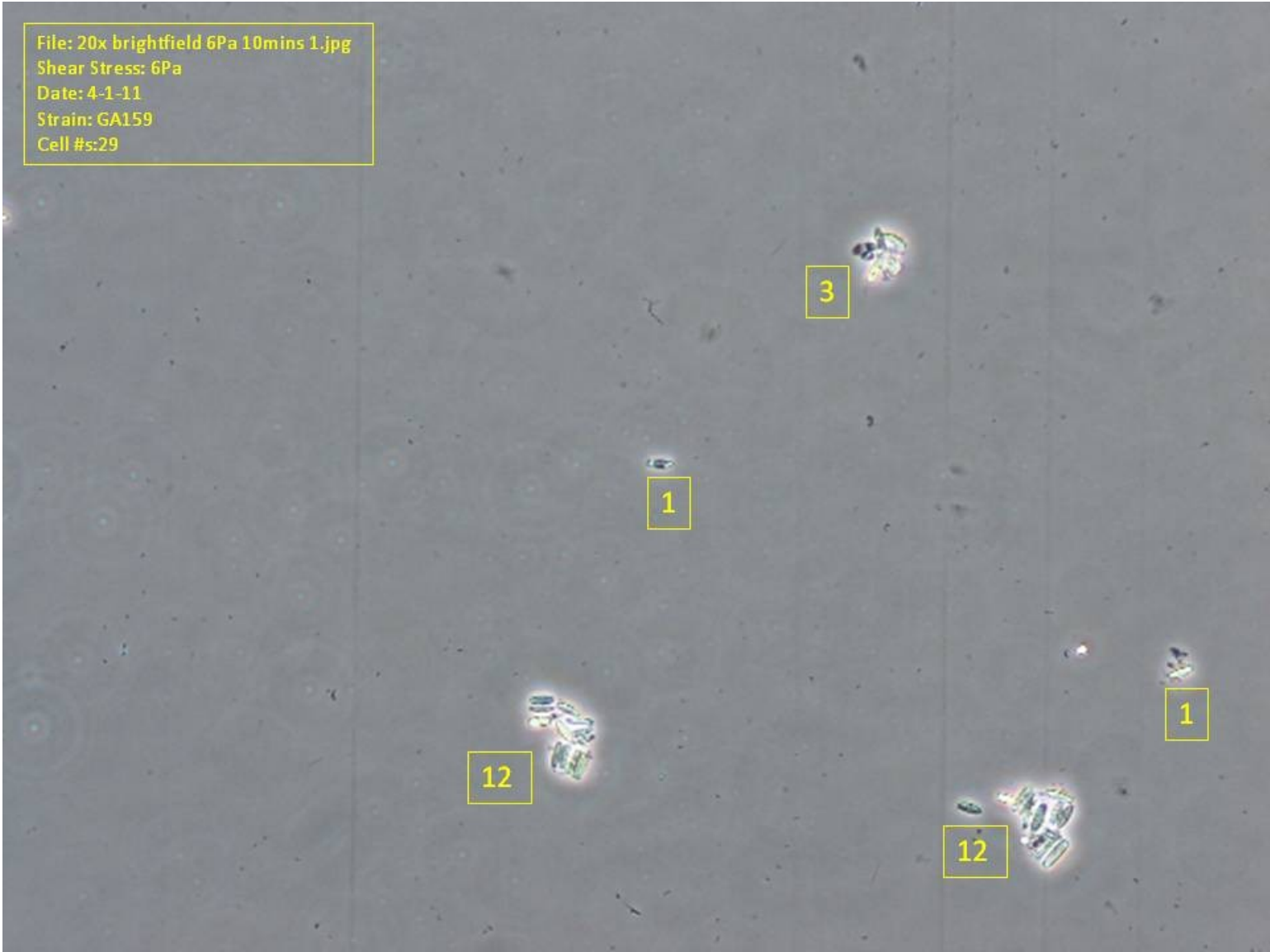




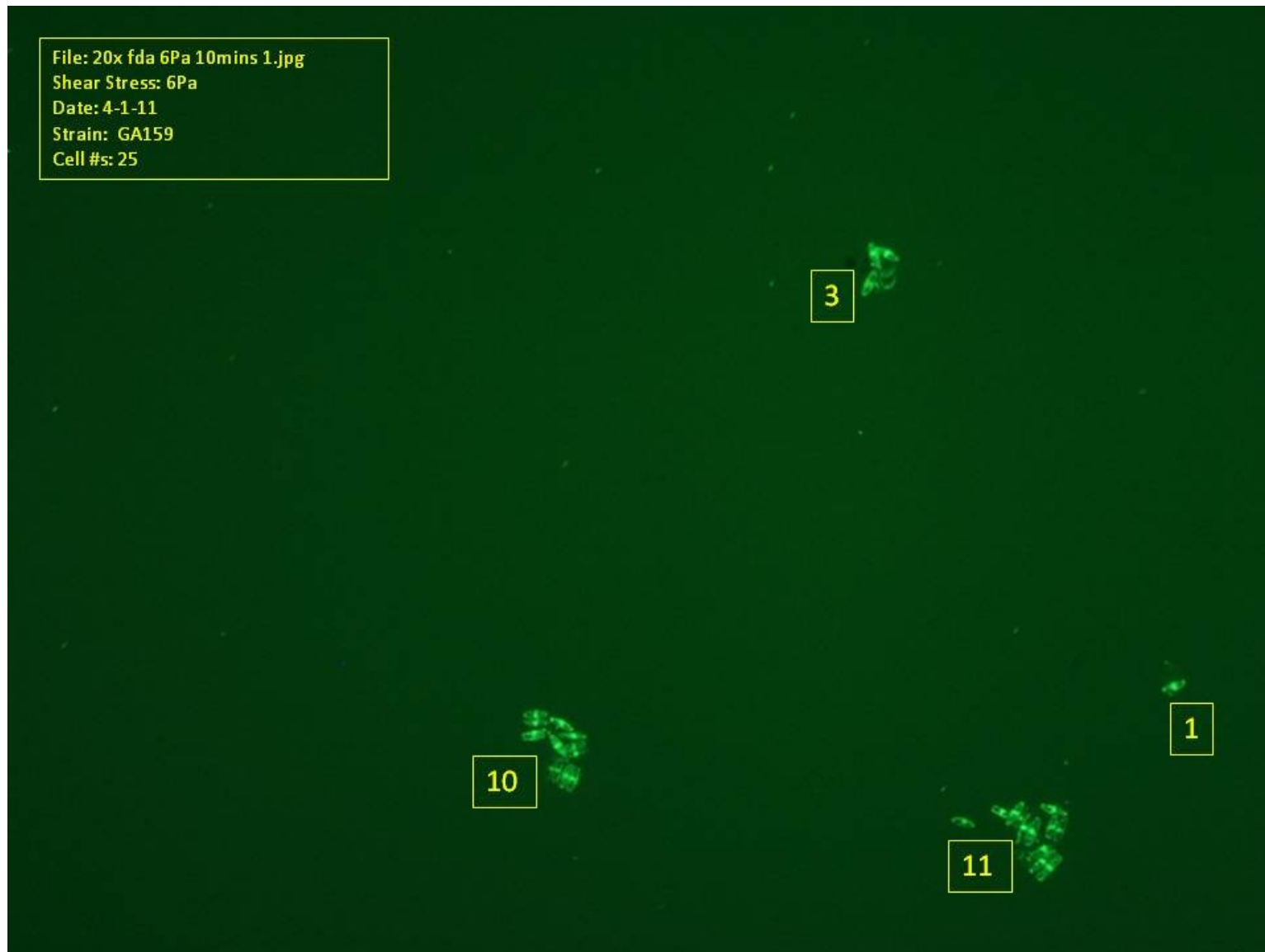




File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: GA159  
Cell #:s:29

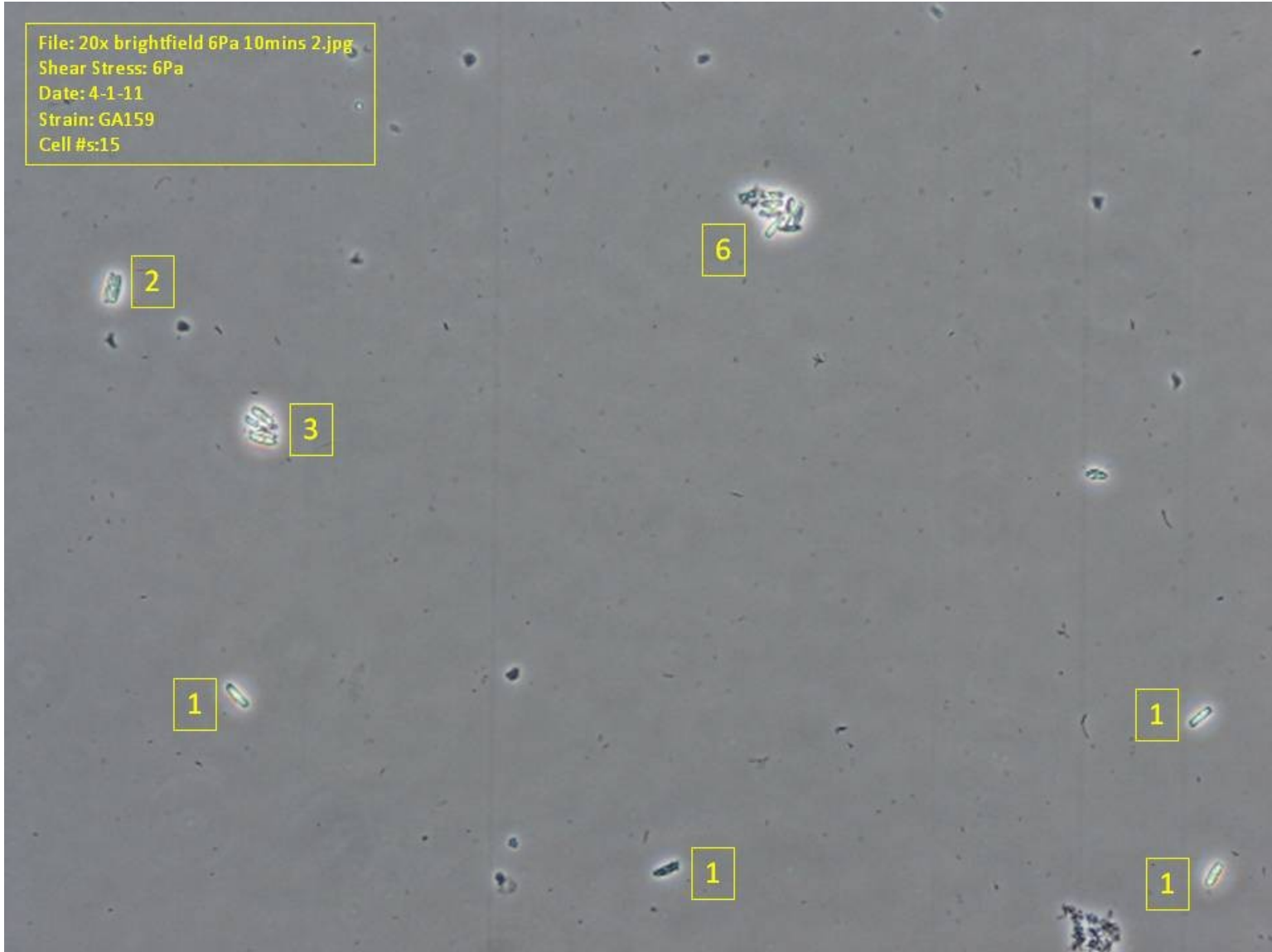


File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: GA159  
Cell #s: 25

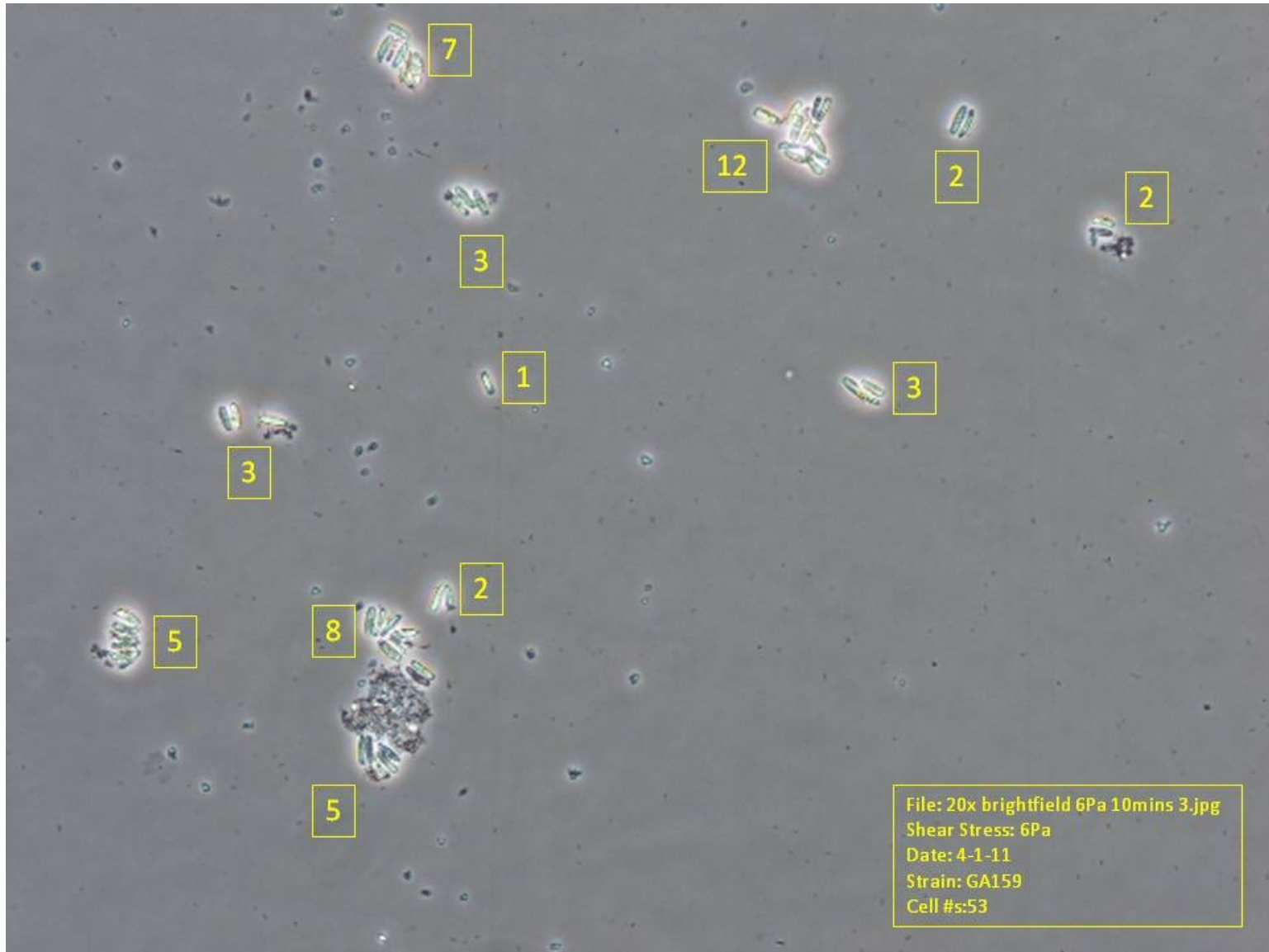


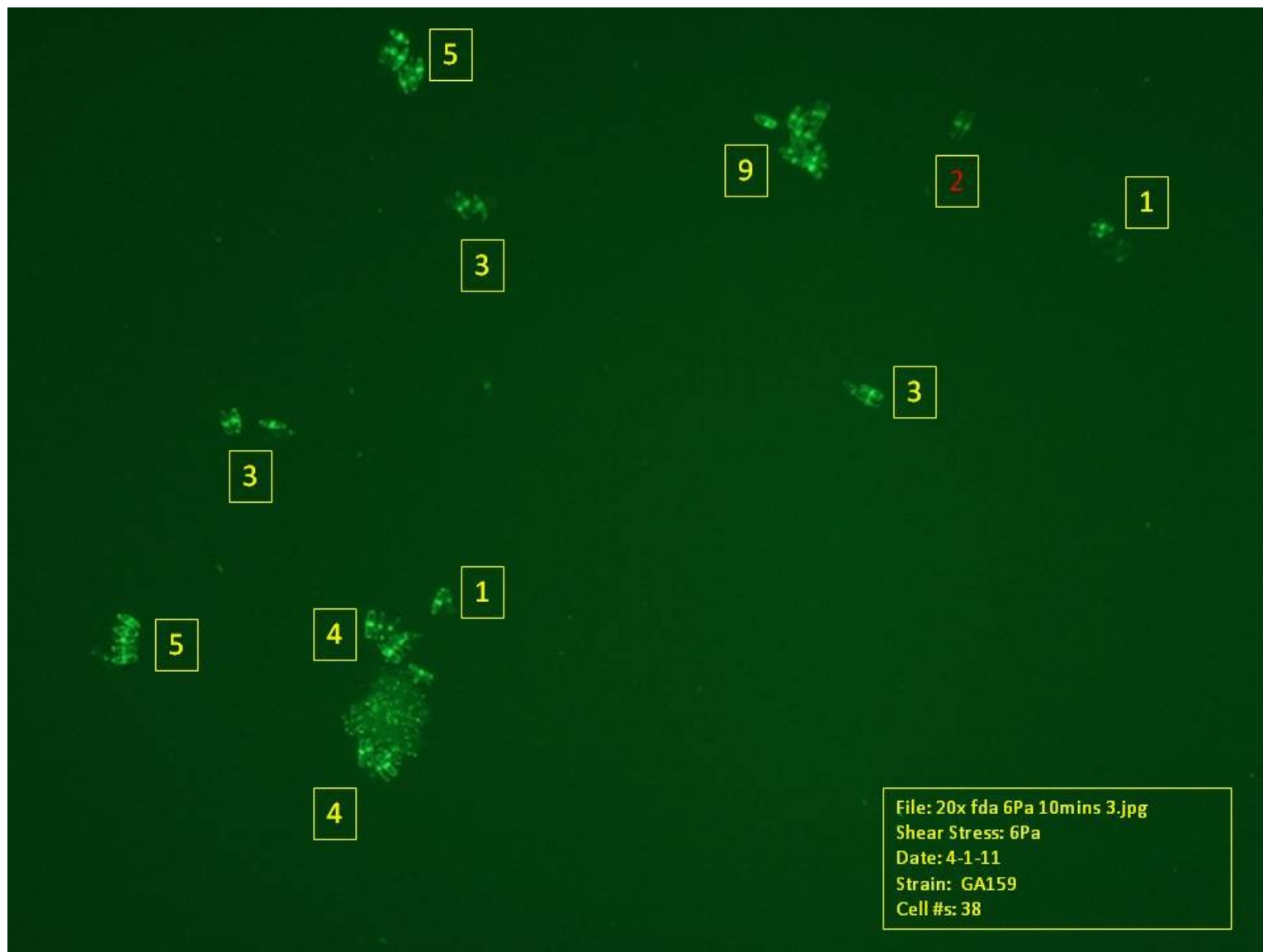


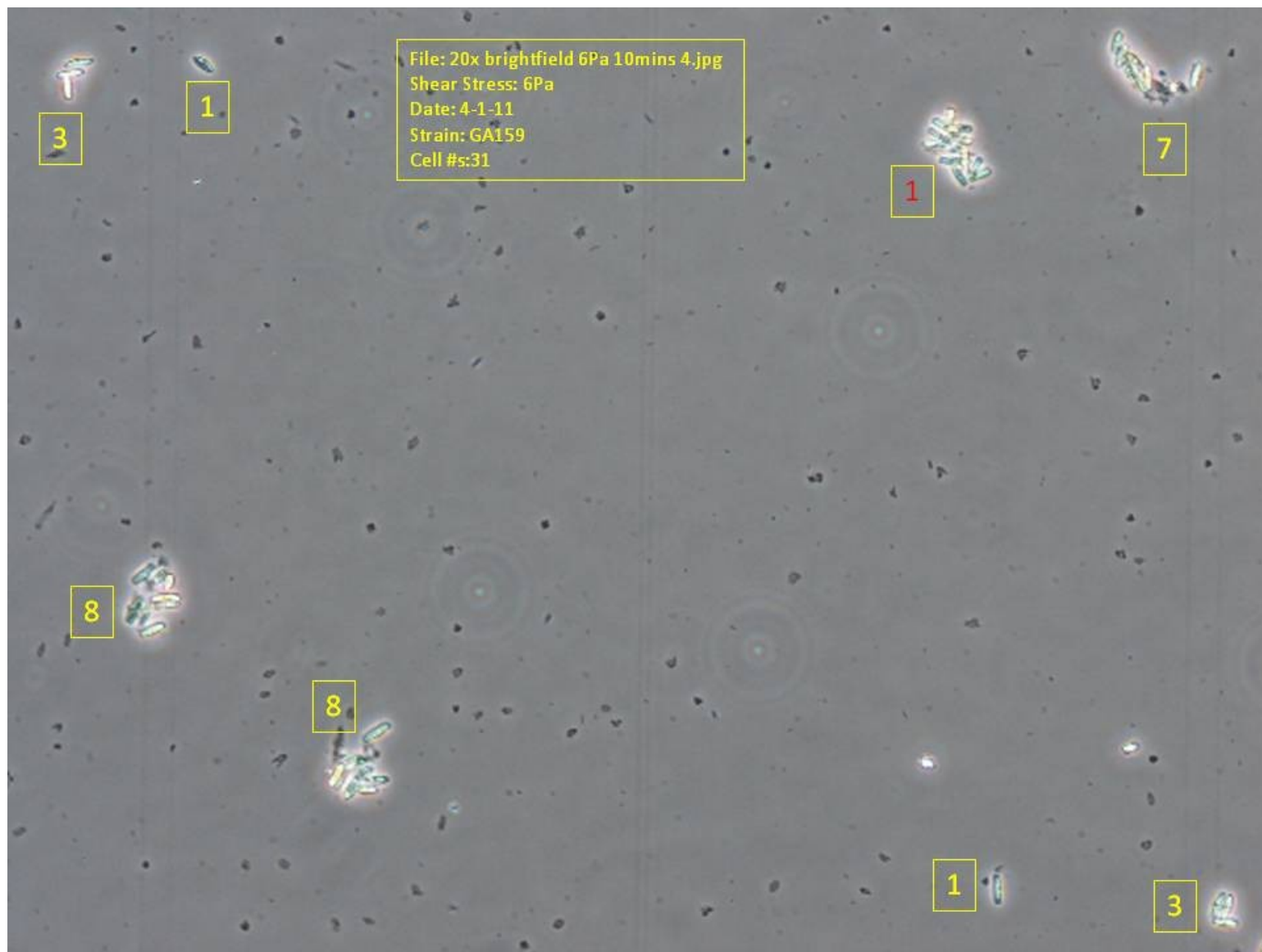
File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: GA159  
Cell #s:15



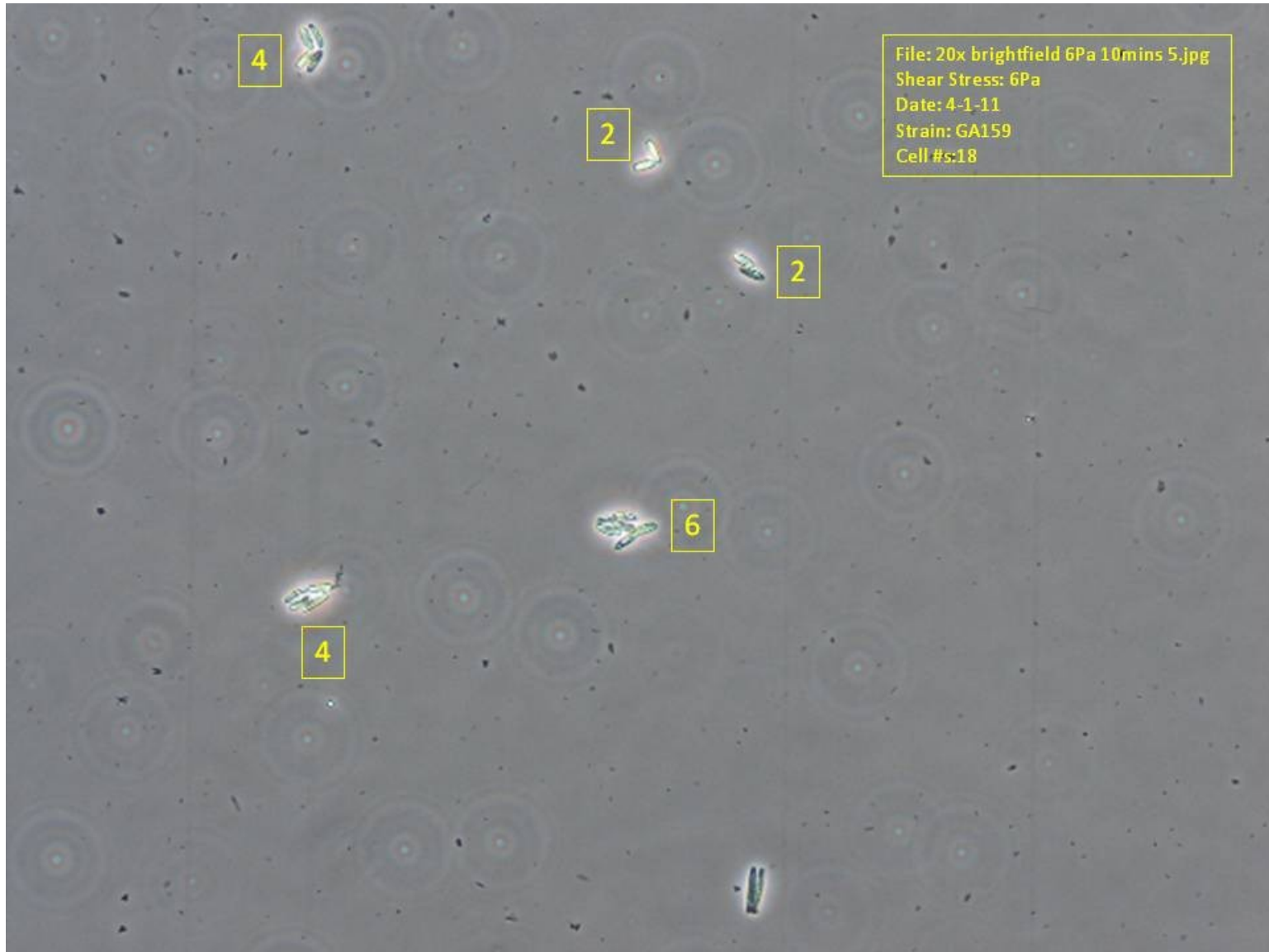








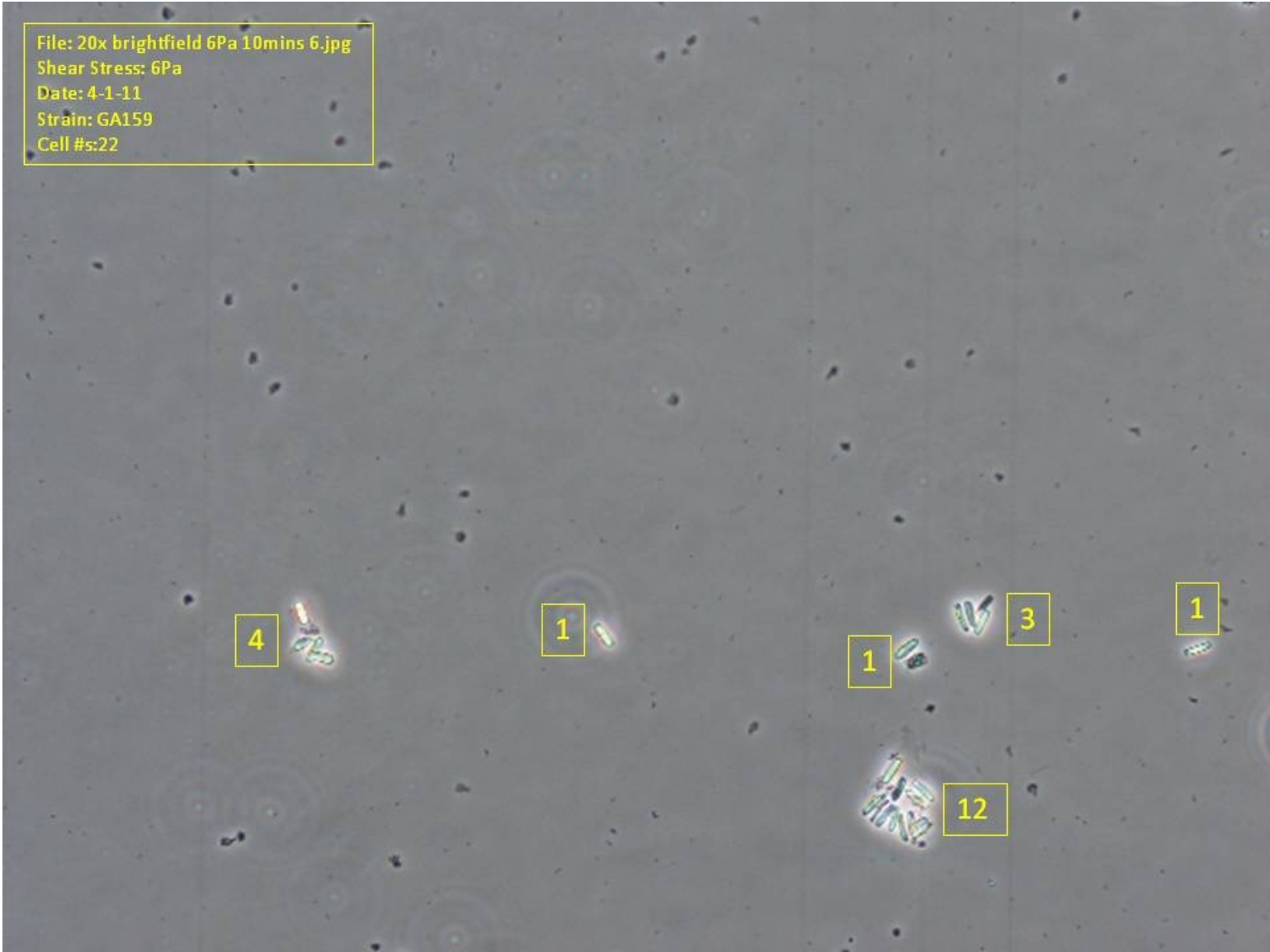








File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: GA159  
Cell #:22



File: 20x fda 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: GA159  
Cell #s: 12

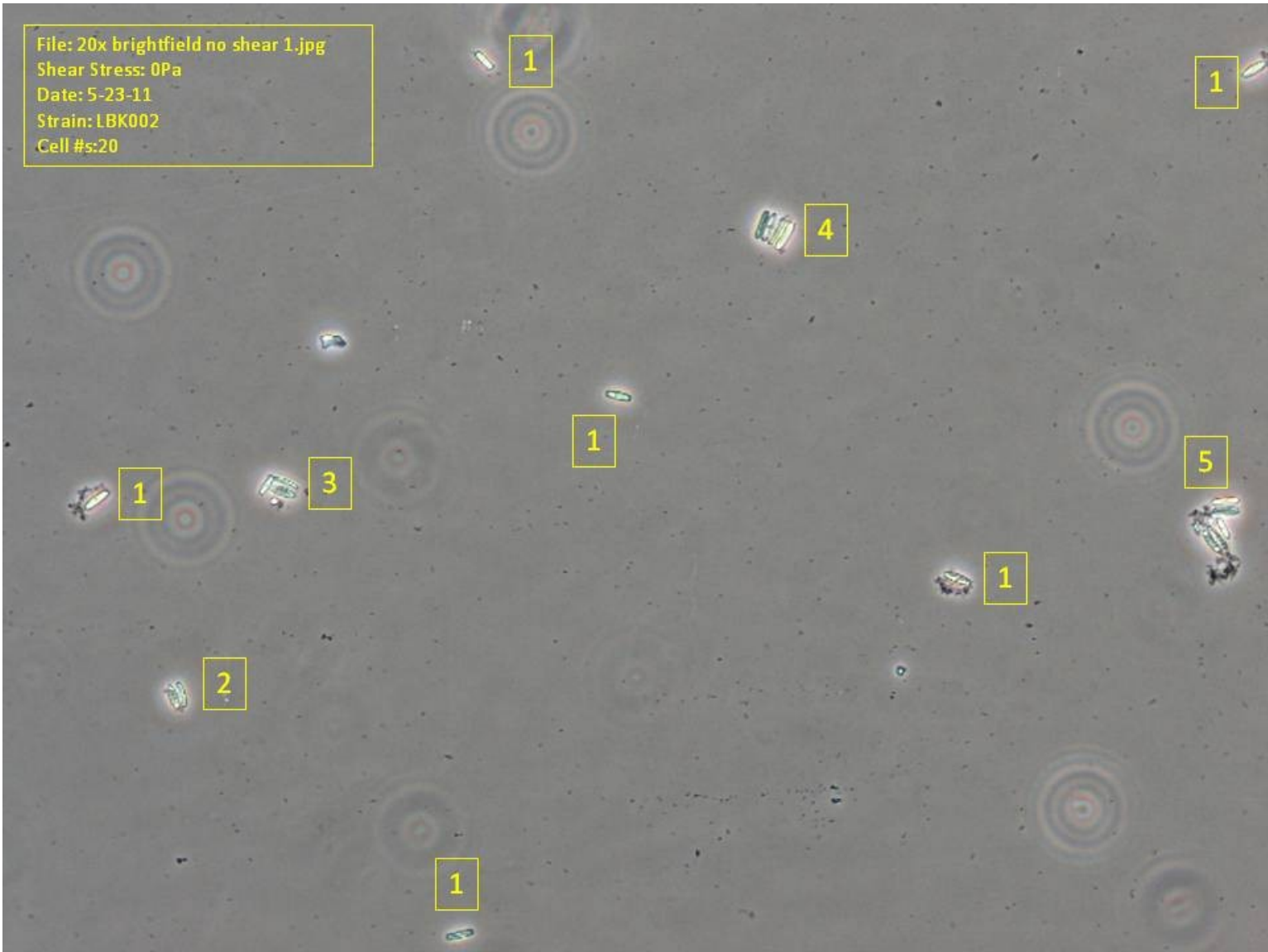


## APPENDIX B

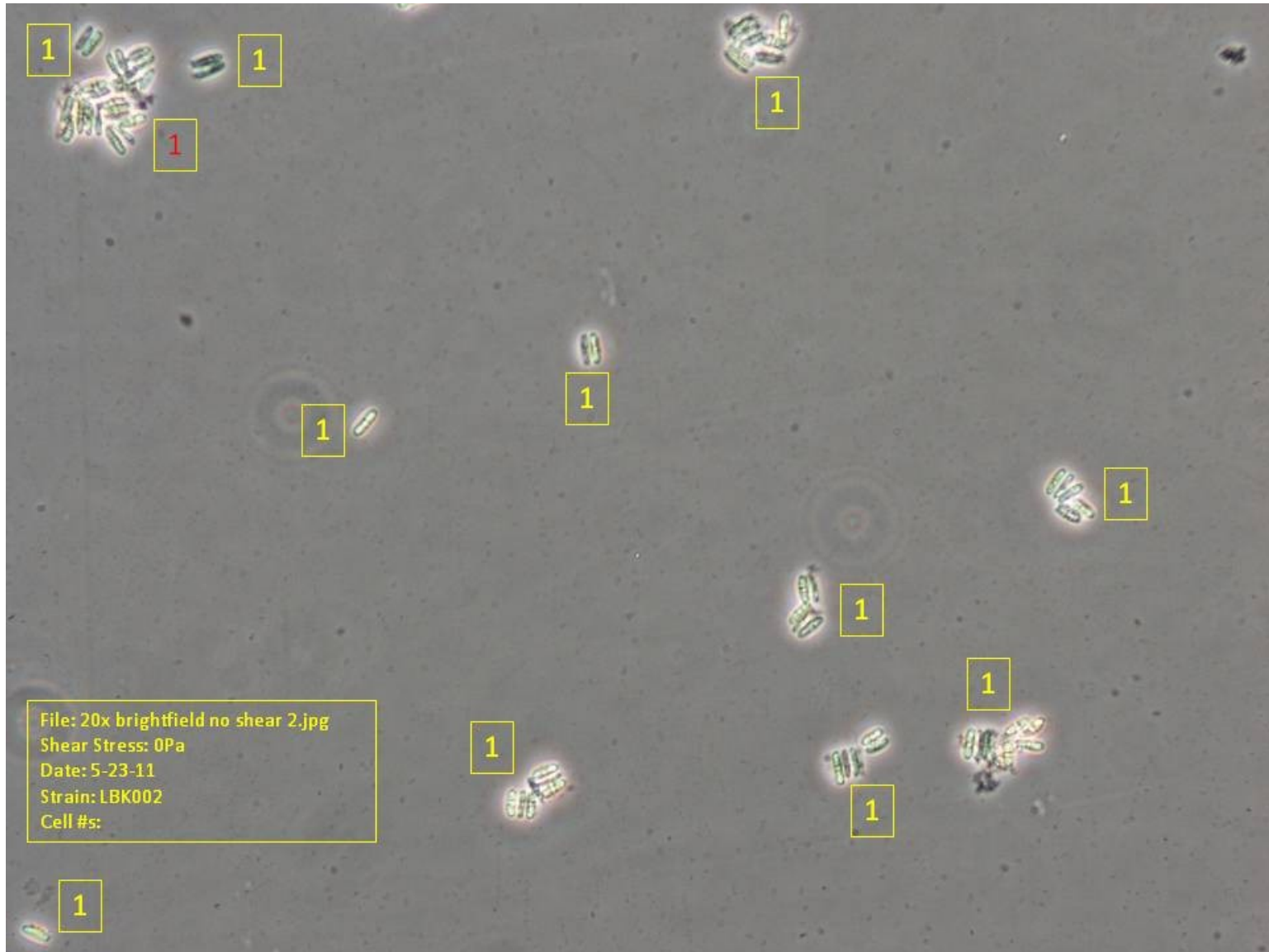
The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK002, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	20	20			32	30	39	36	40	35	25	22	91.67%	6.08%	-0.01014
	6	26	23	13	13	20	17	27	23	25	19			85.59%		
Rep 2	0	52	47	23	20	38	34	46	40	42	37			88.56%	6.14%	-0.01023
	6	49	39	23	20			43	34	29	26	38	31	82.42%		
Rep 3	0	26	24	45	40	39	35	16	12	22	19			87.84%	9.08%	-0.01514
	6	85	71	26	21	23	18	18	16	41	26			78.76%		
														<b>Avg.</b>	<b>7.10%</b>	
														<b>Std. Dev.</b>	<b>1.72%</b>	

The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.

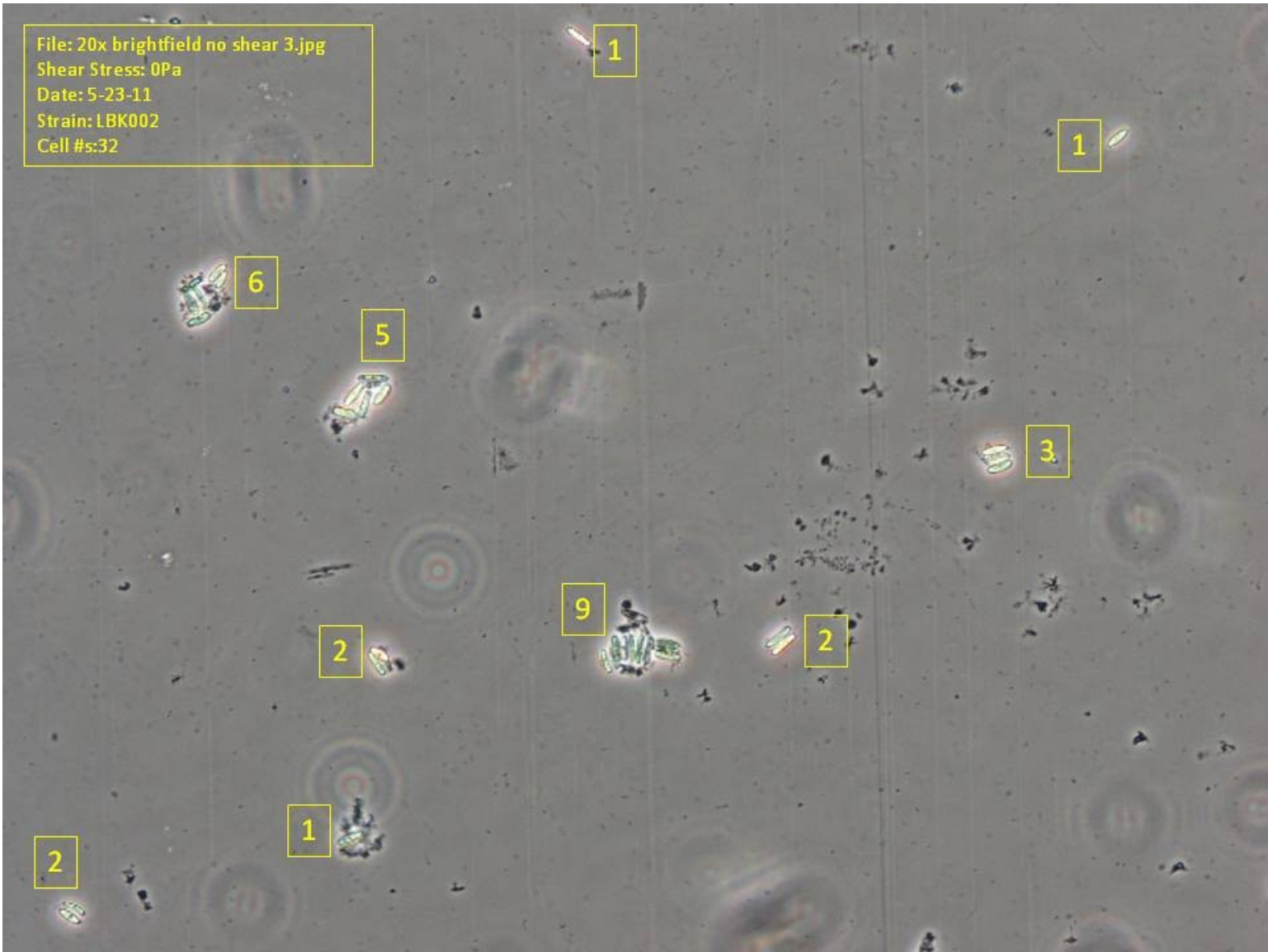






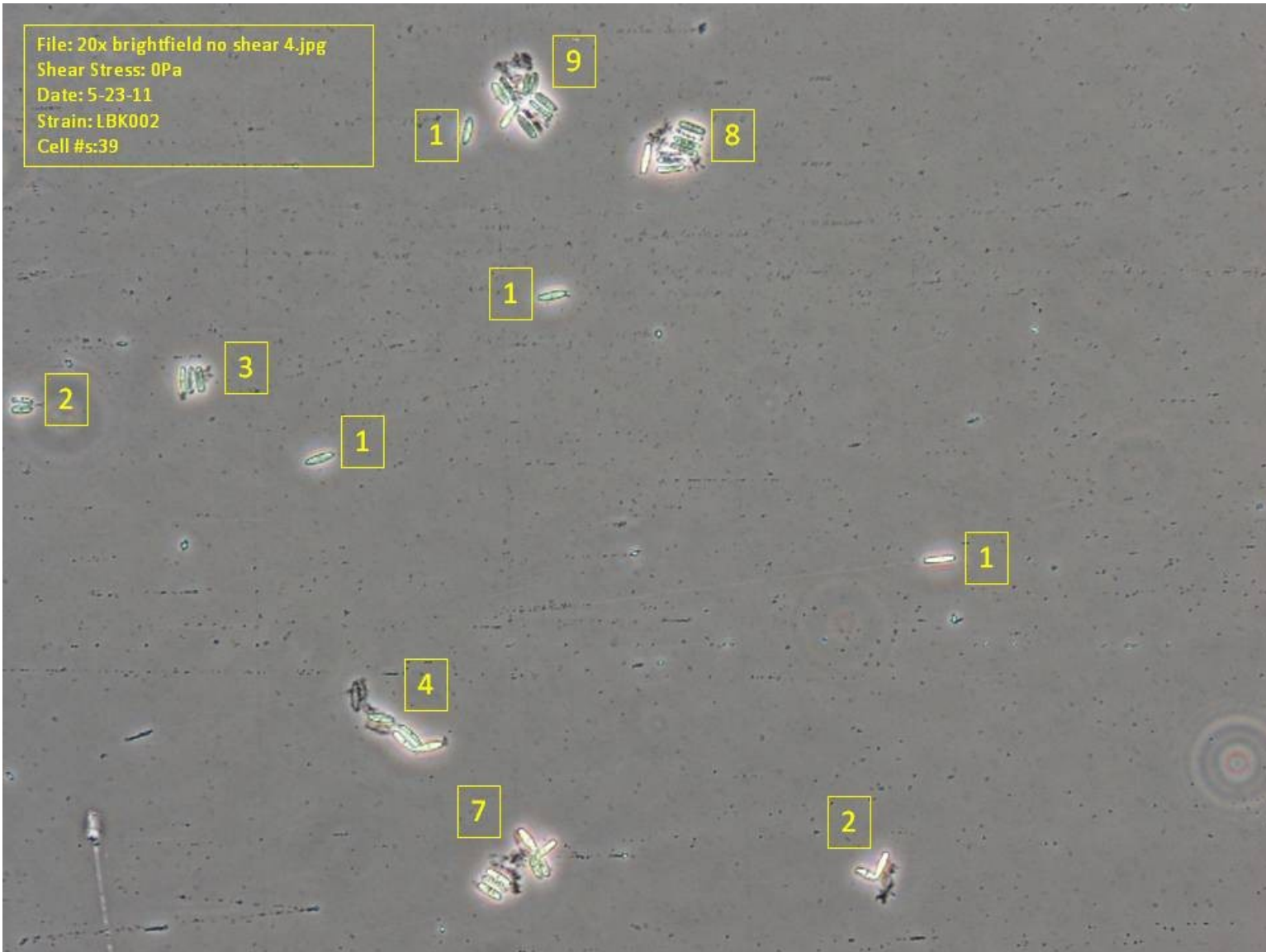


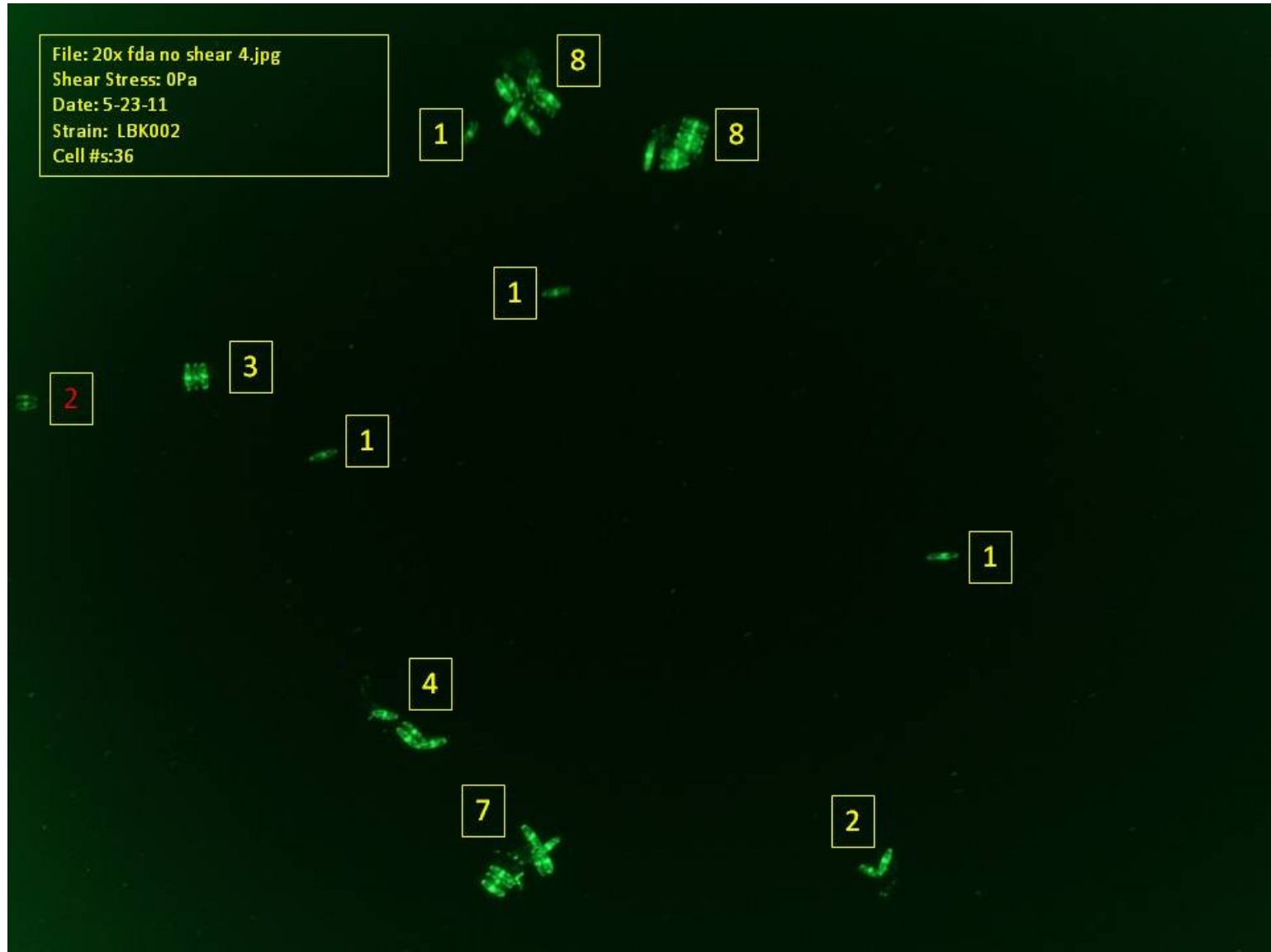
File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:

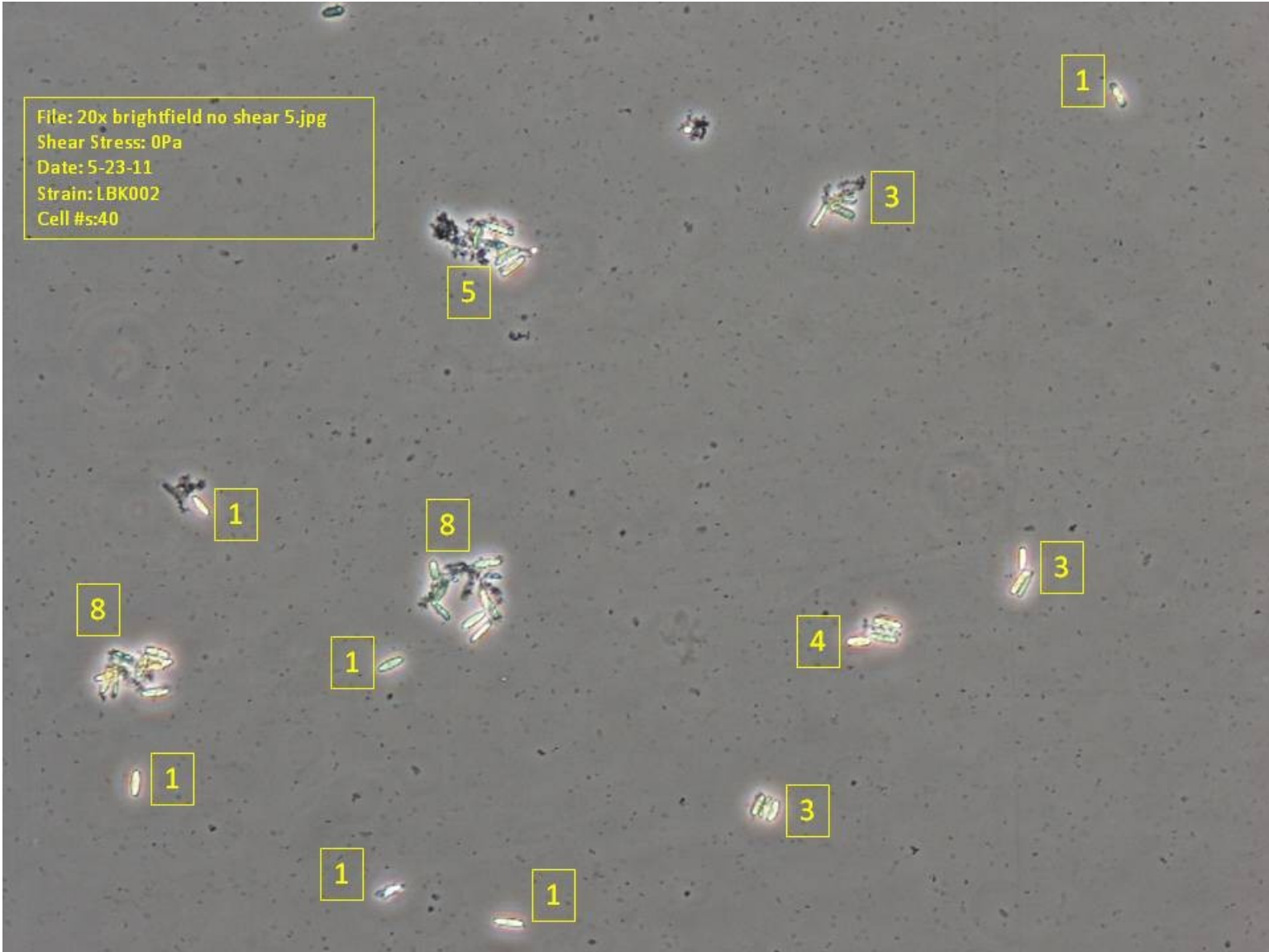


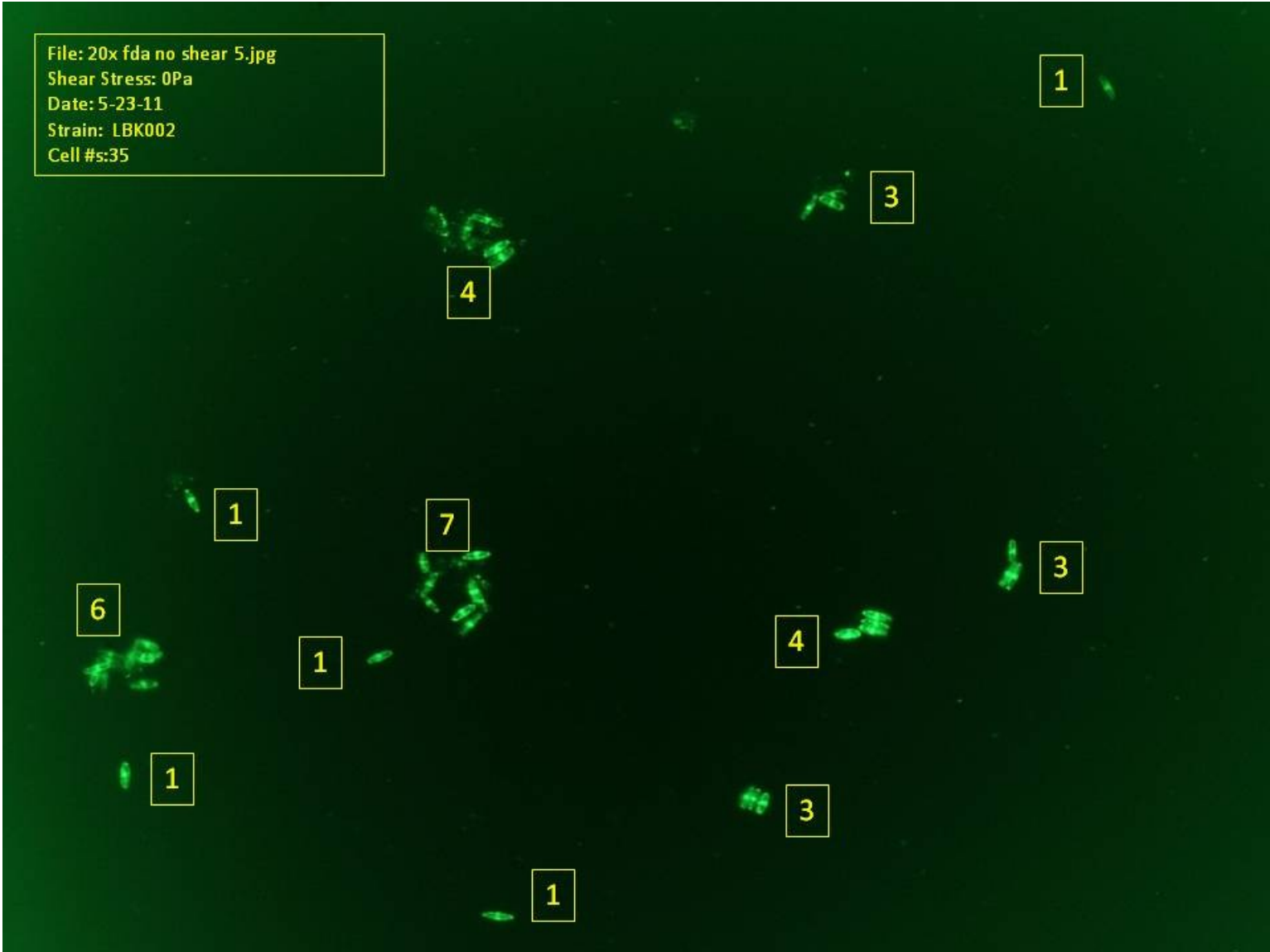








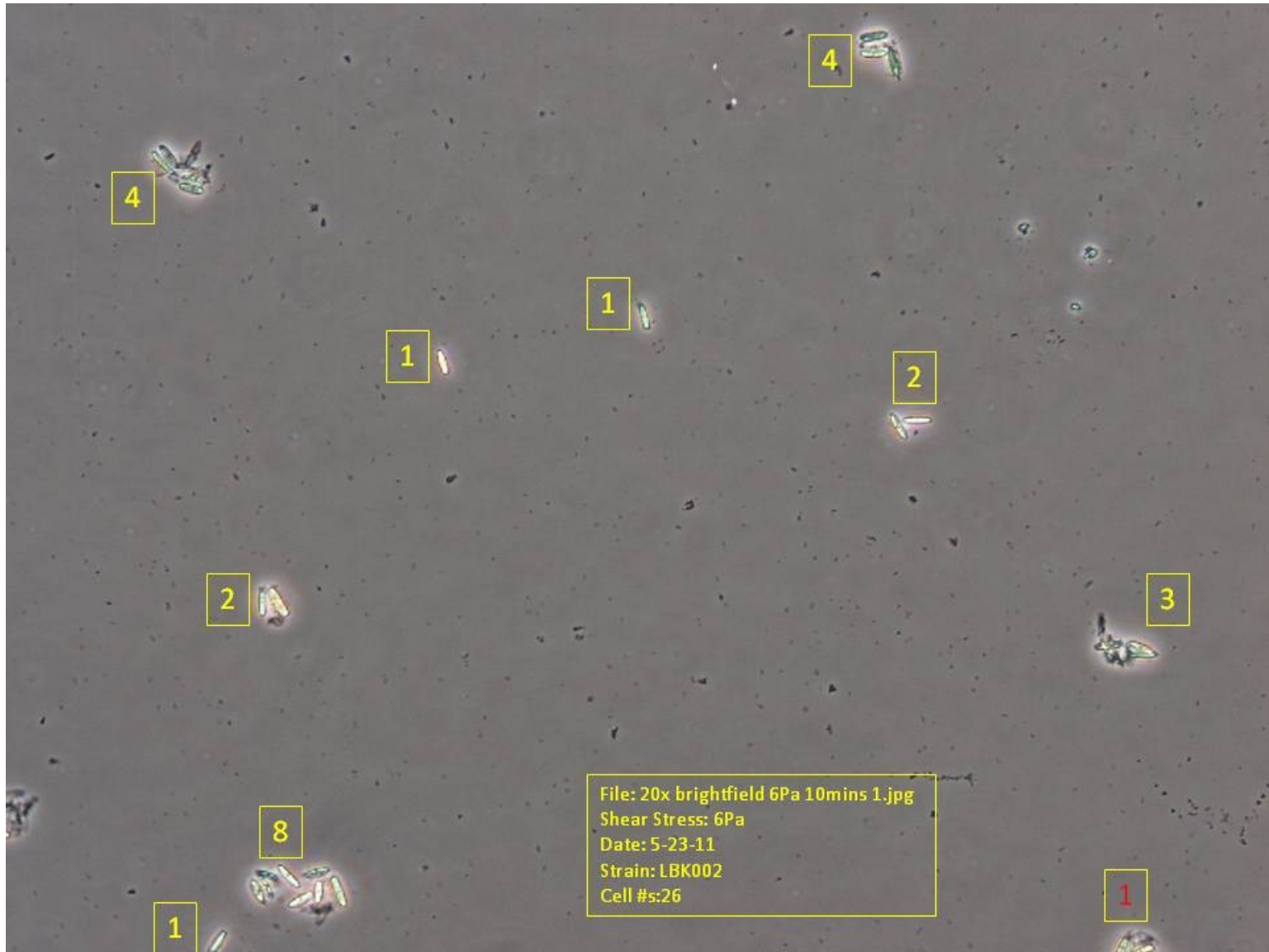




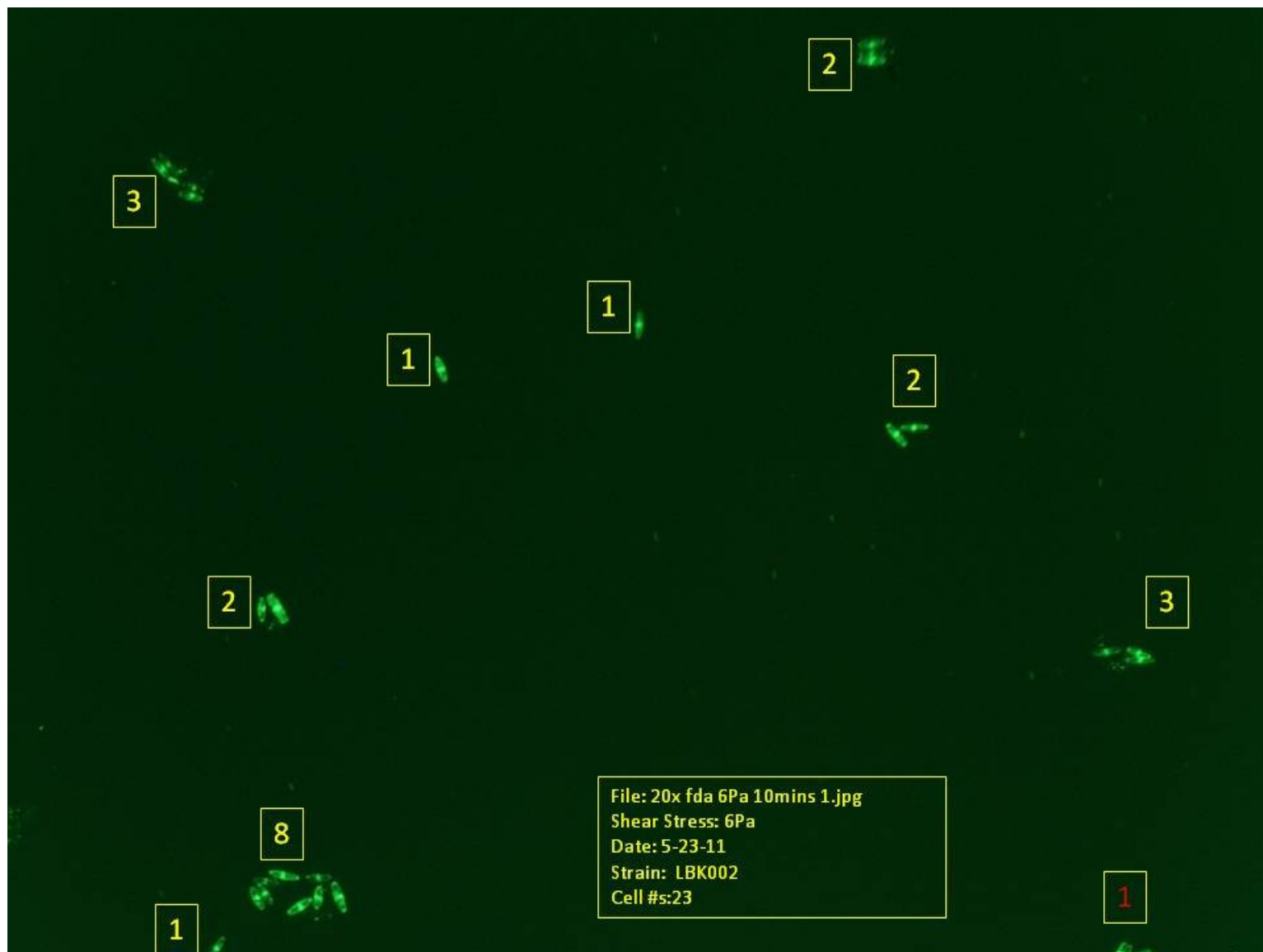
File: 20x brightfield no shear 6.jpg  
Shear Stress: 0Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:25

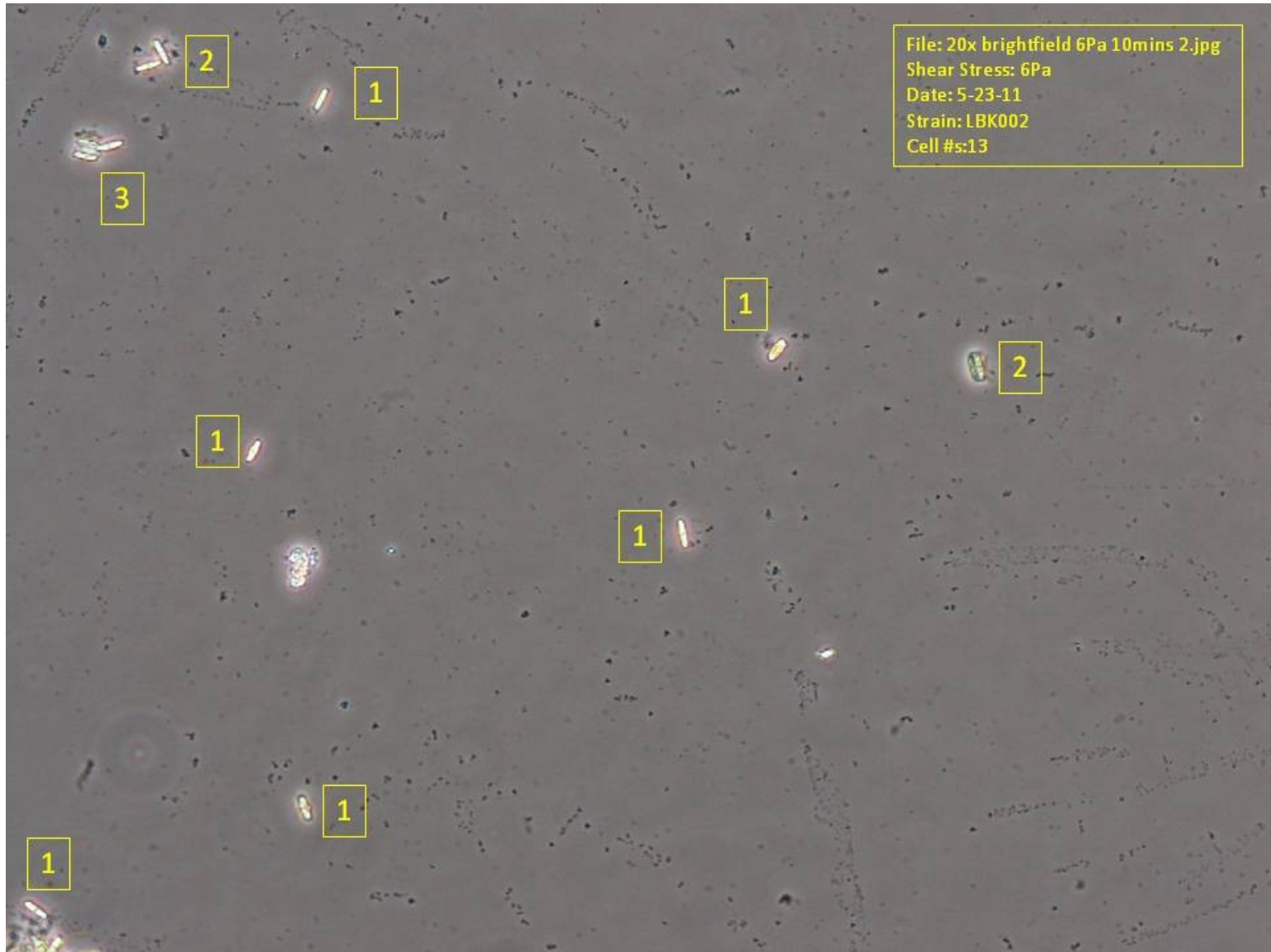


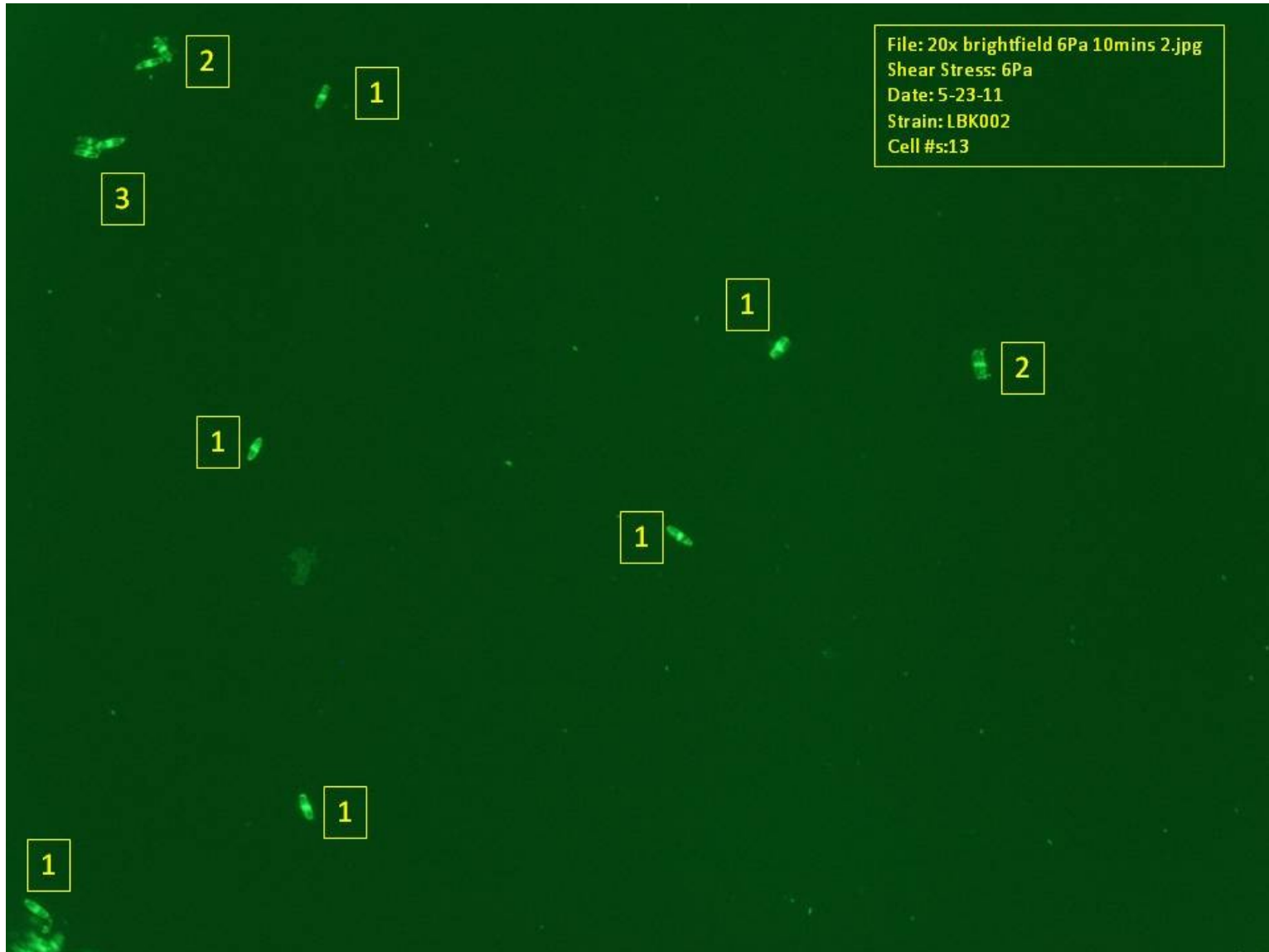




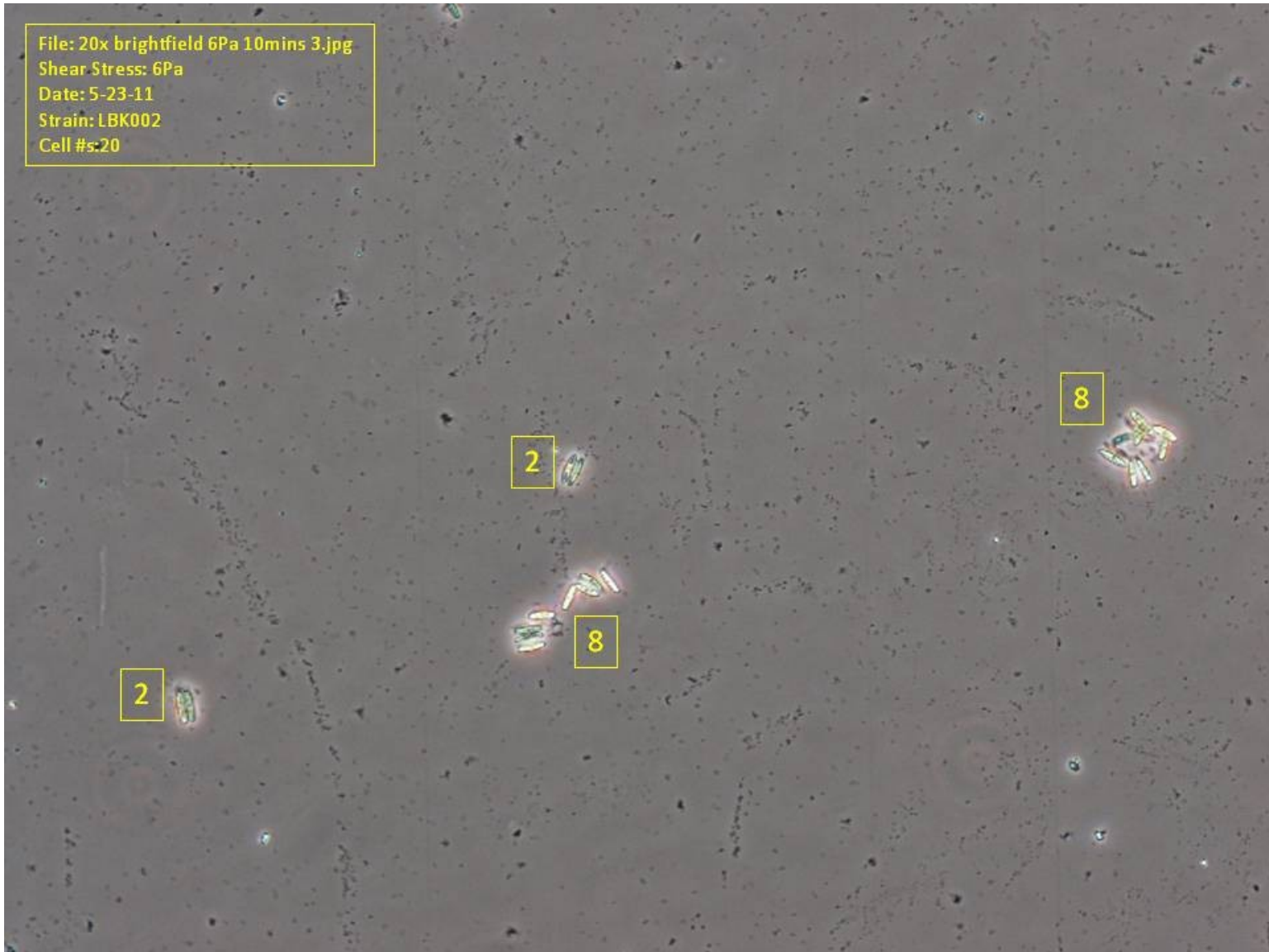






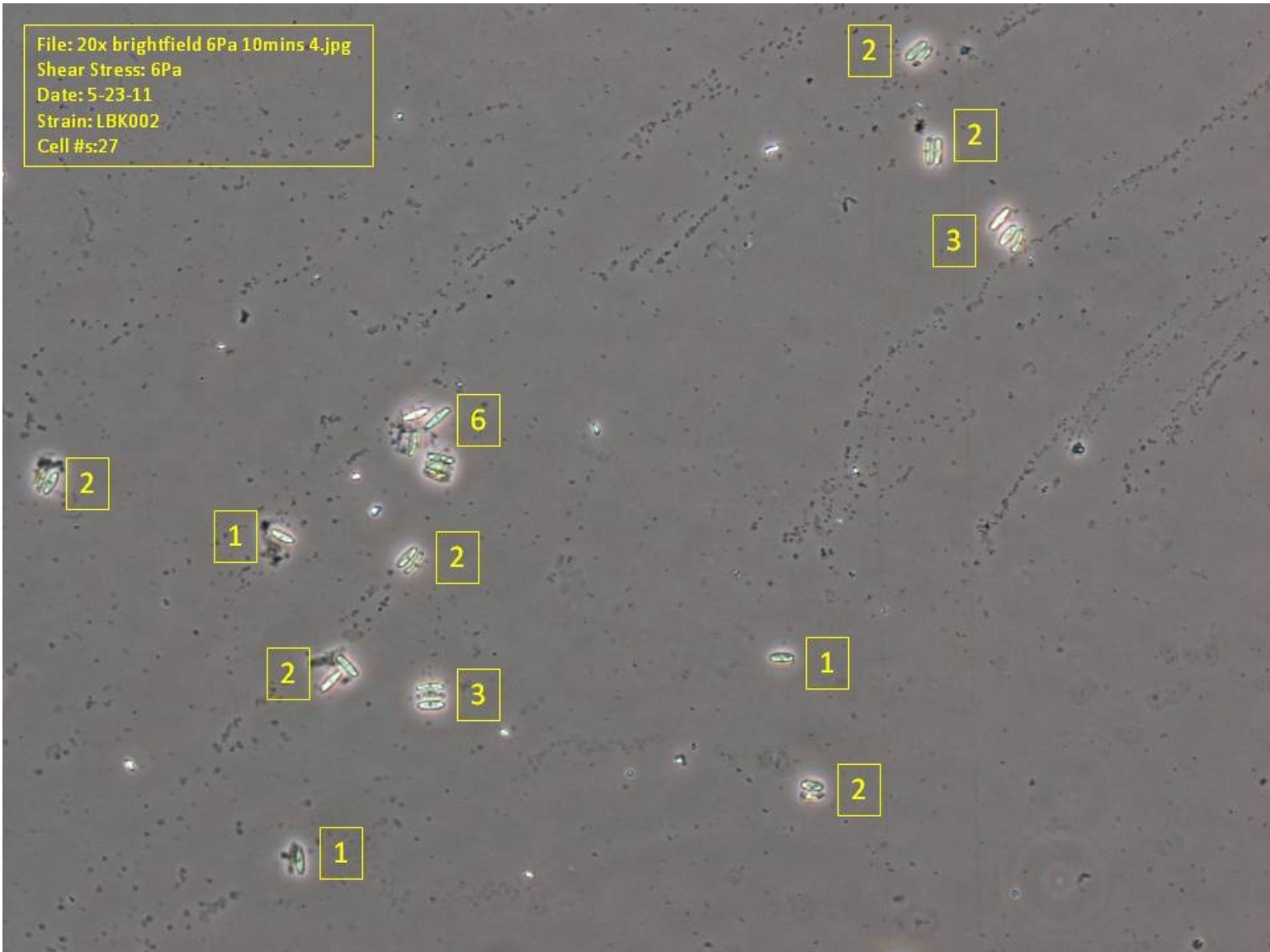


File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:20



File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:17

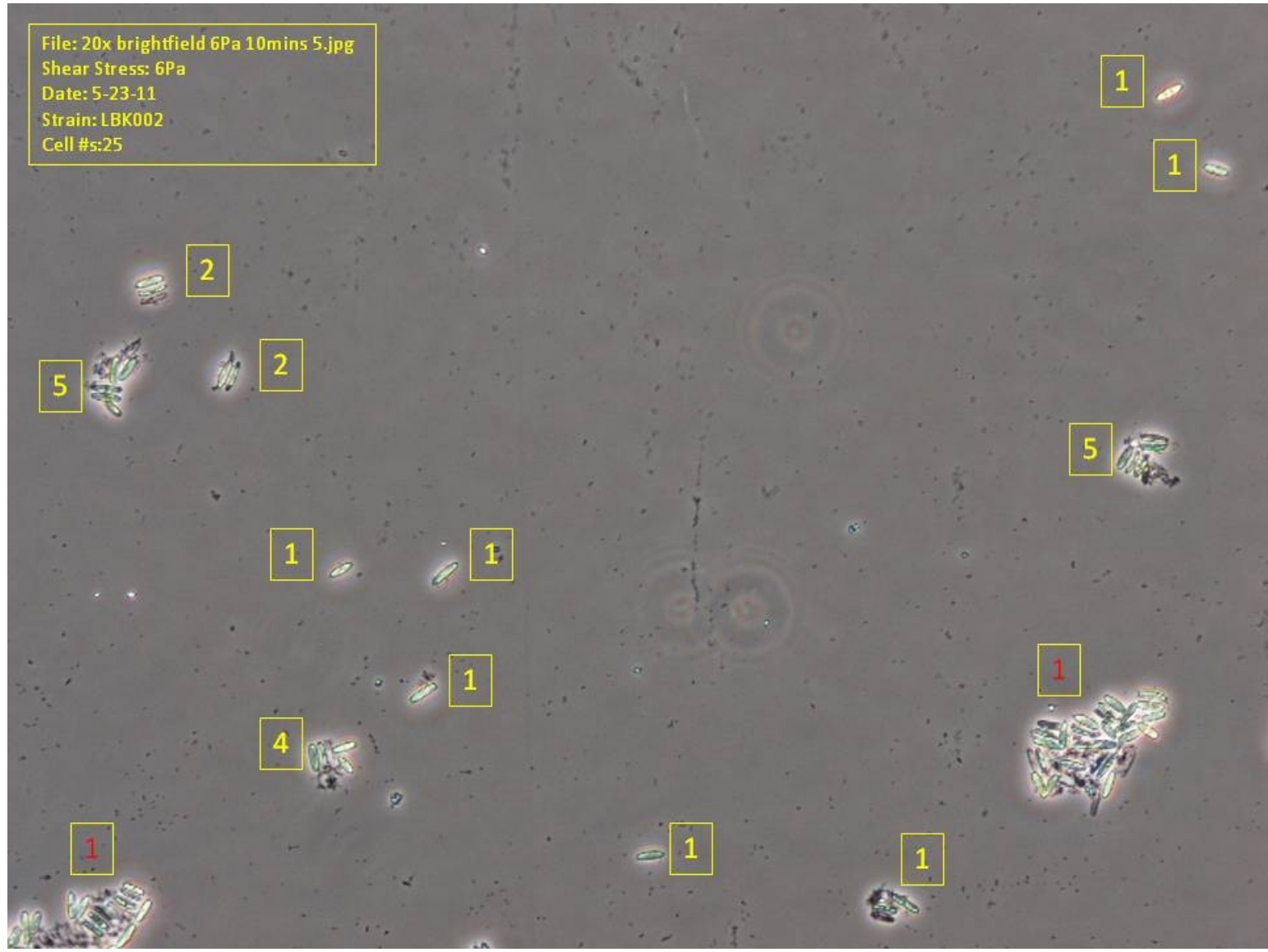




File: 20x brightfield 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:23



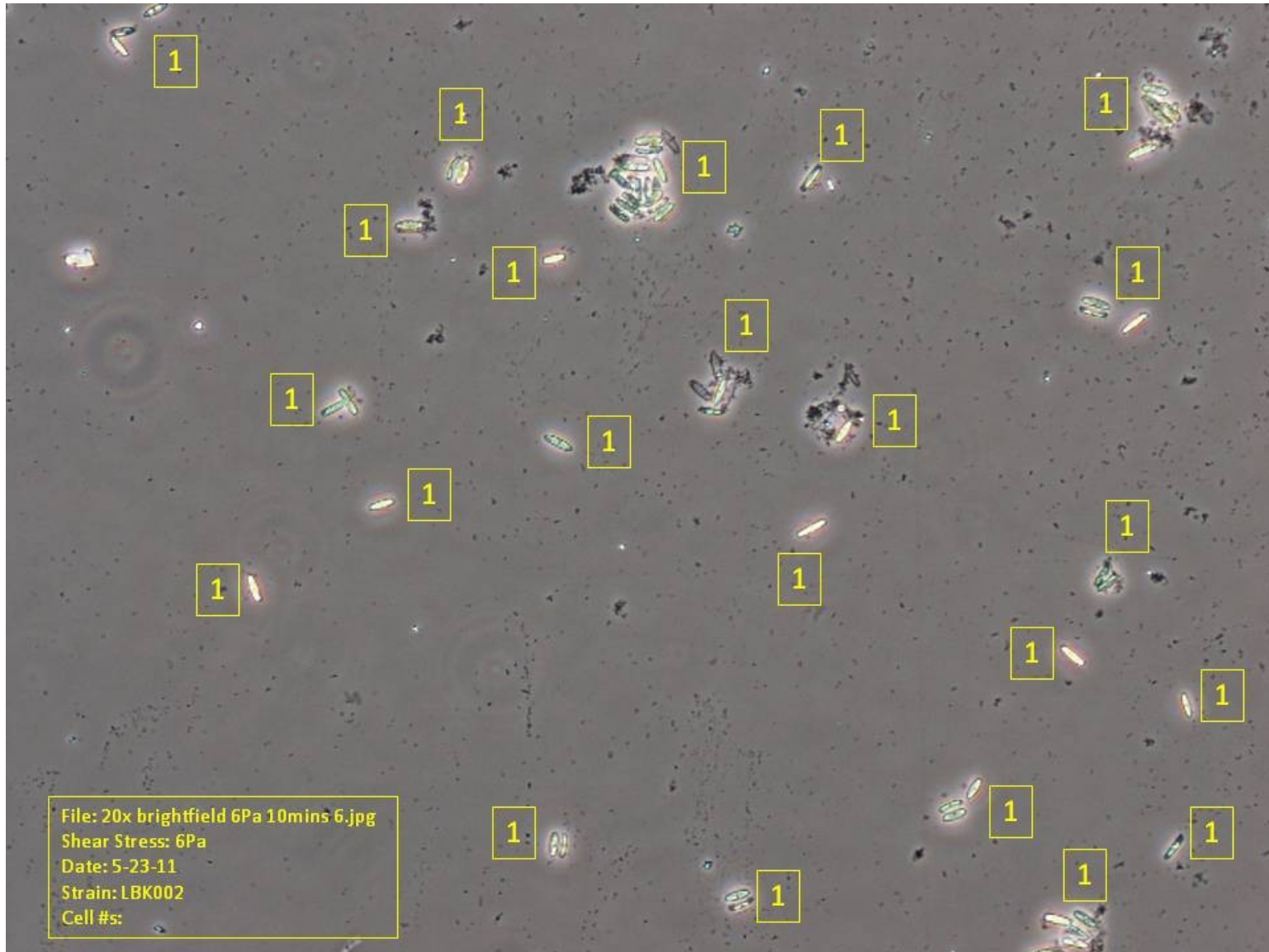
File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #:s:25



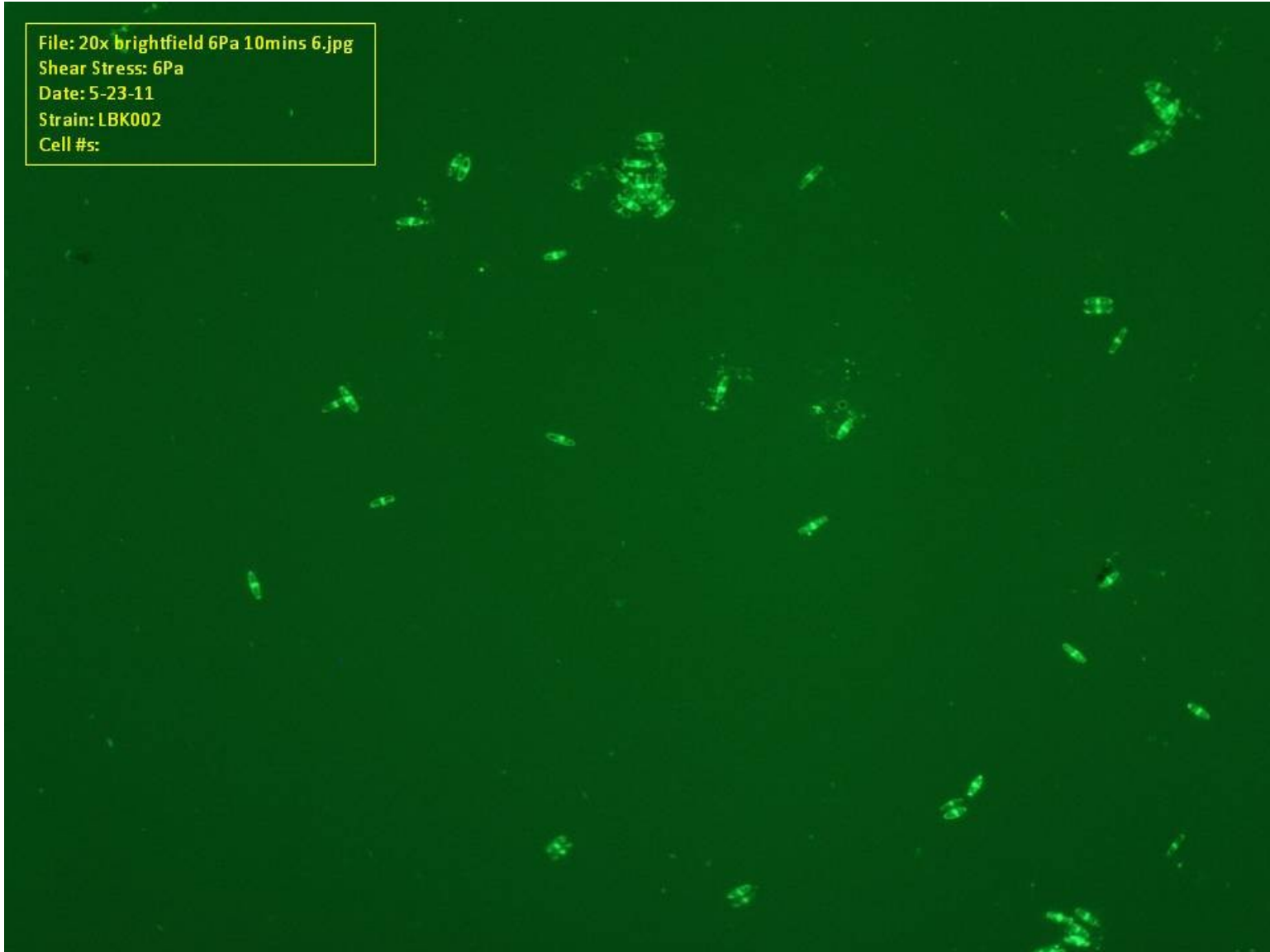


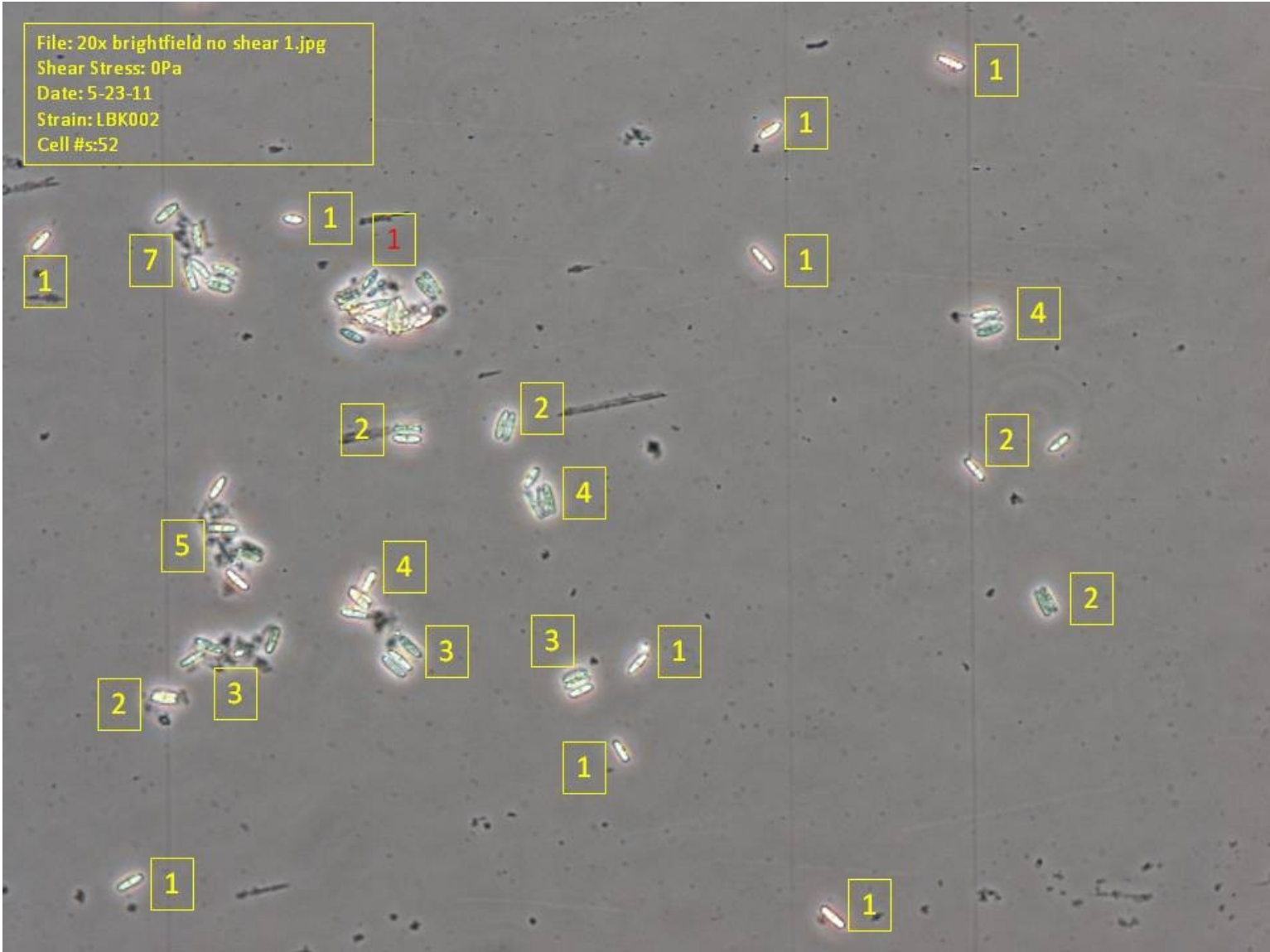
File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:19

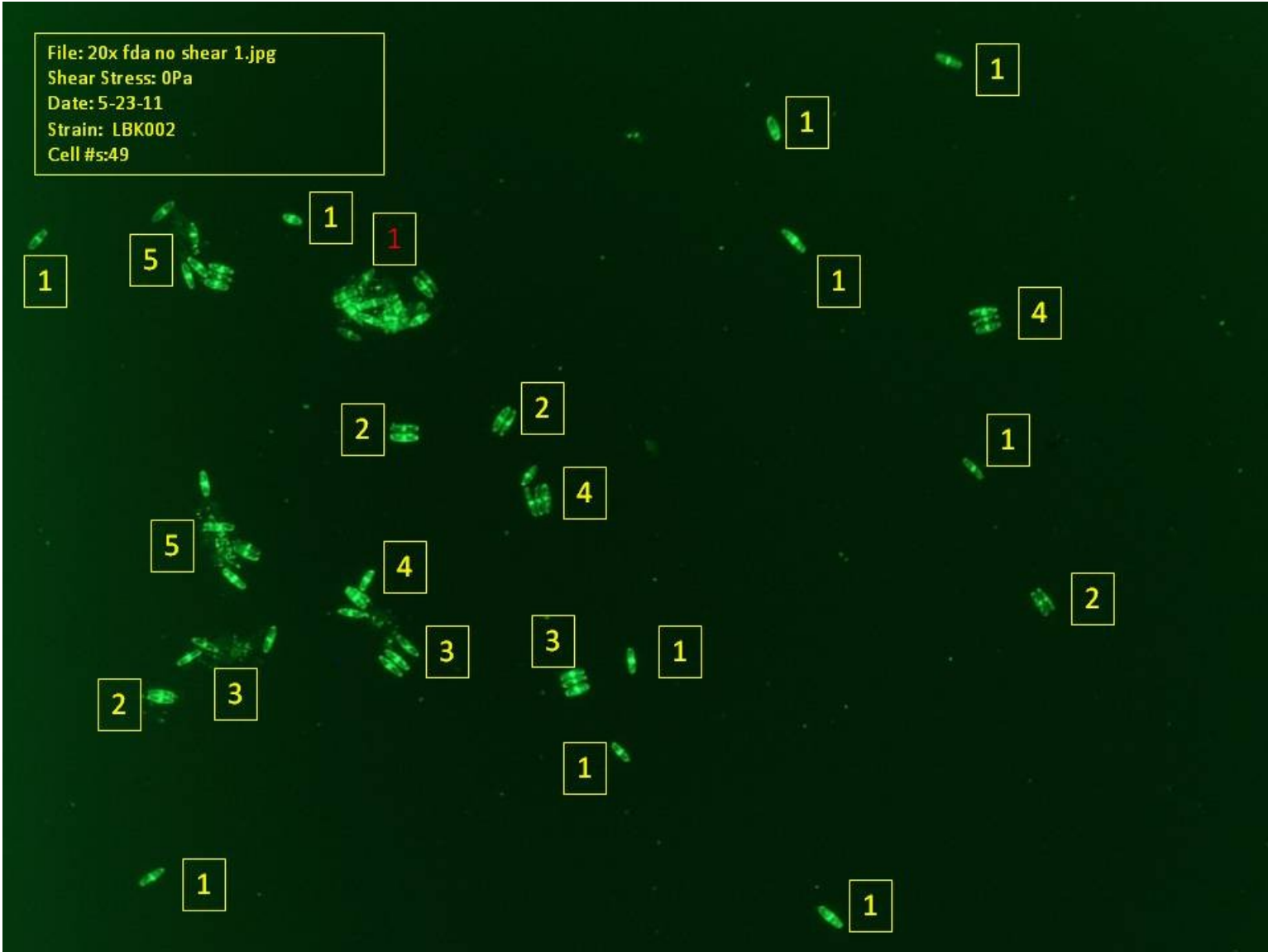


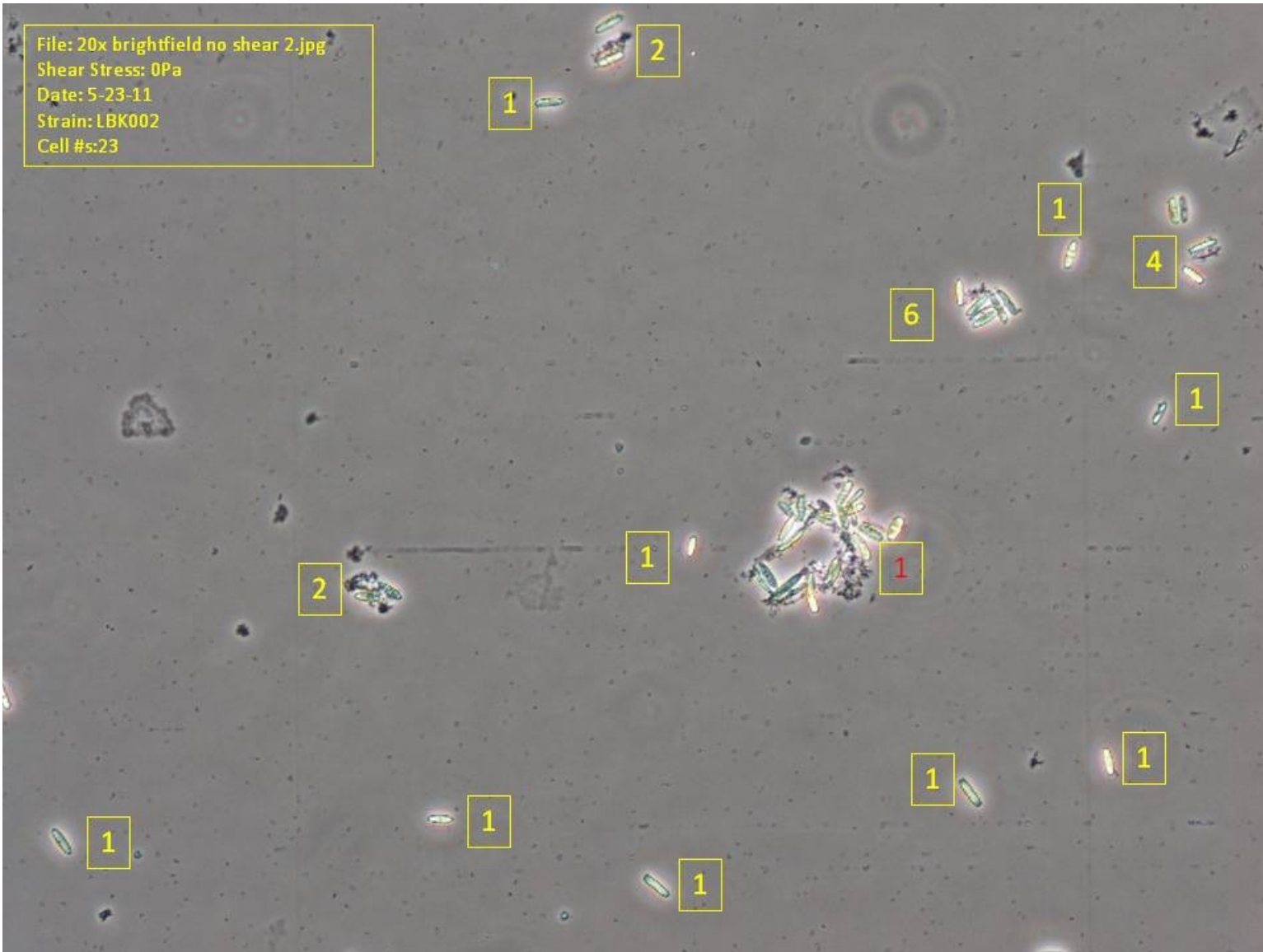


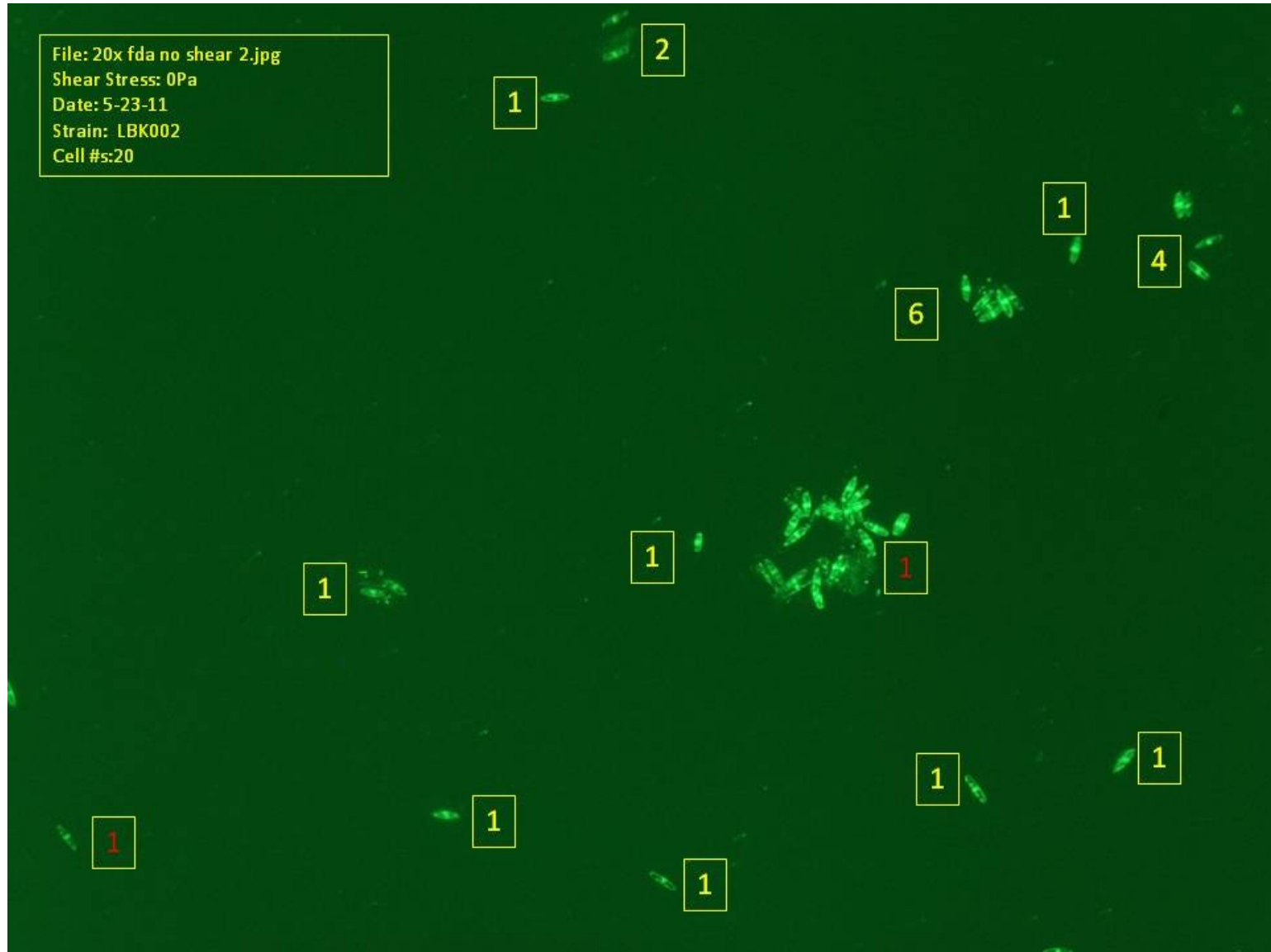
File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:

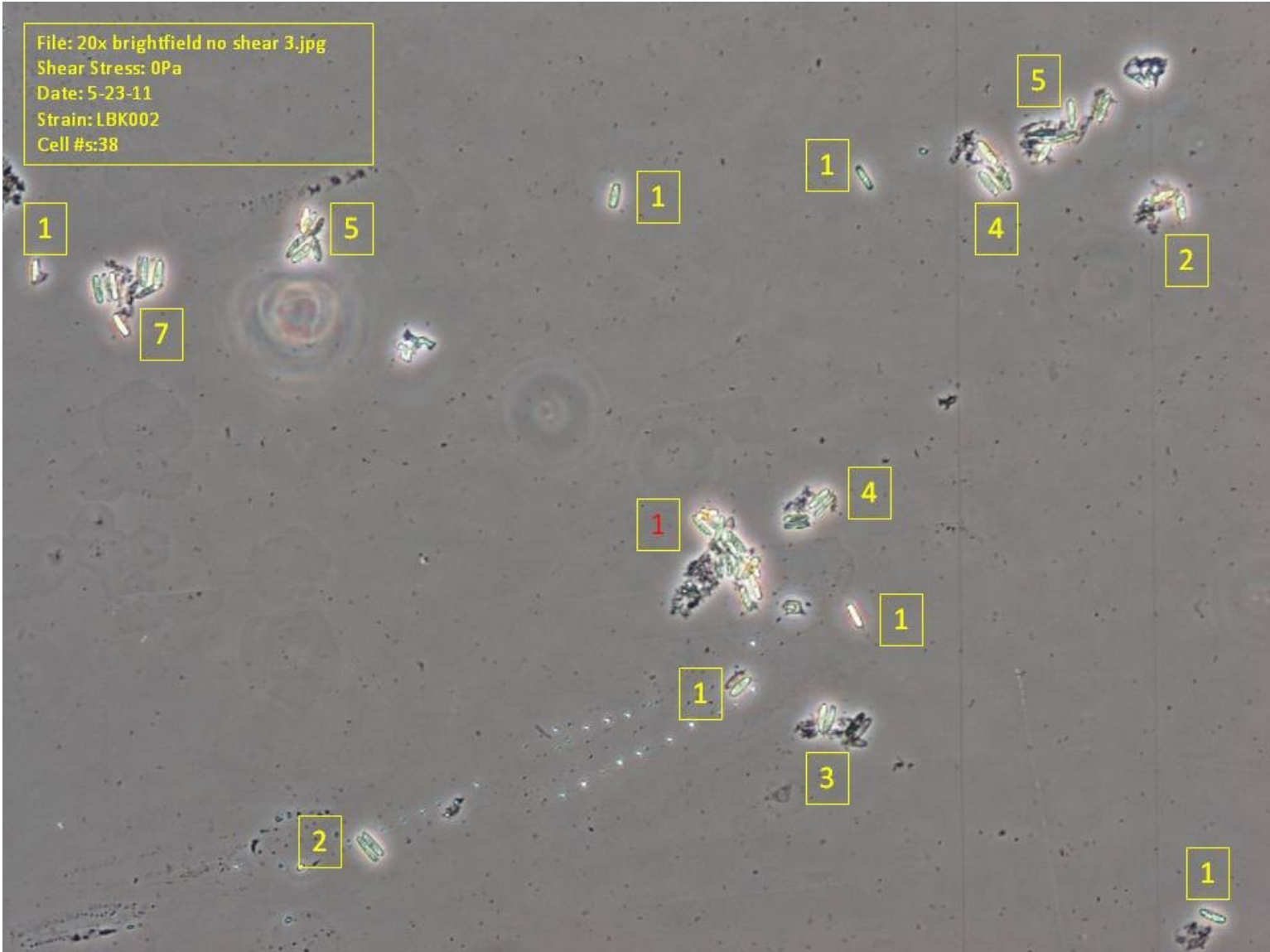




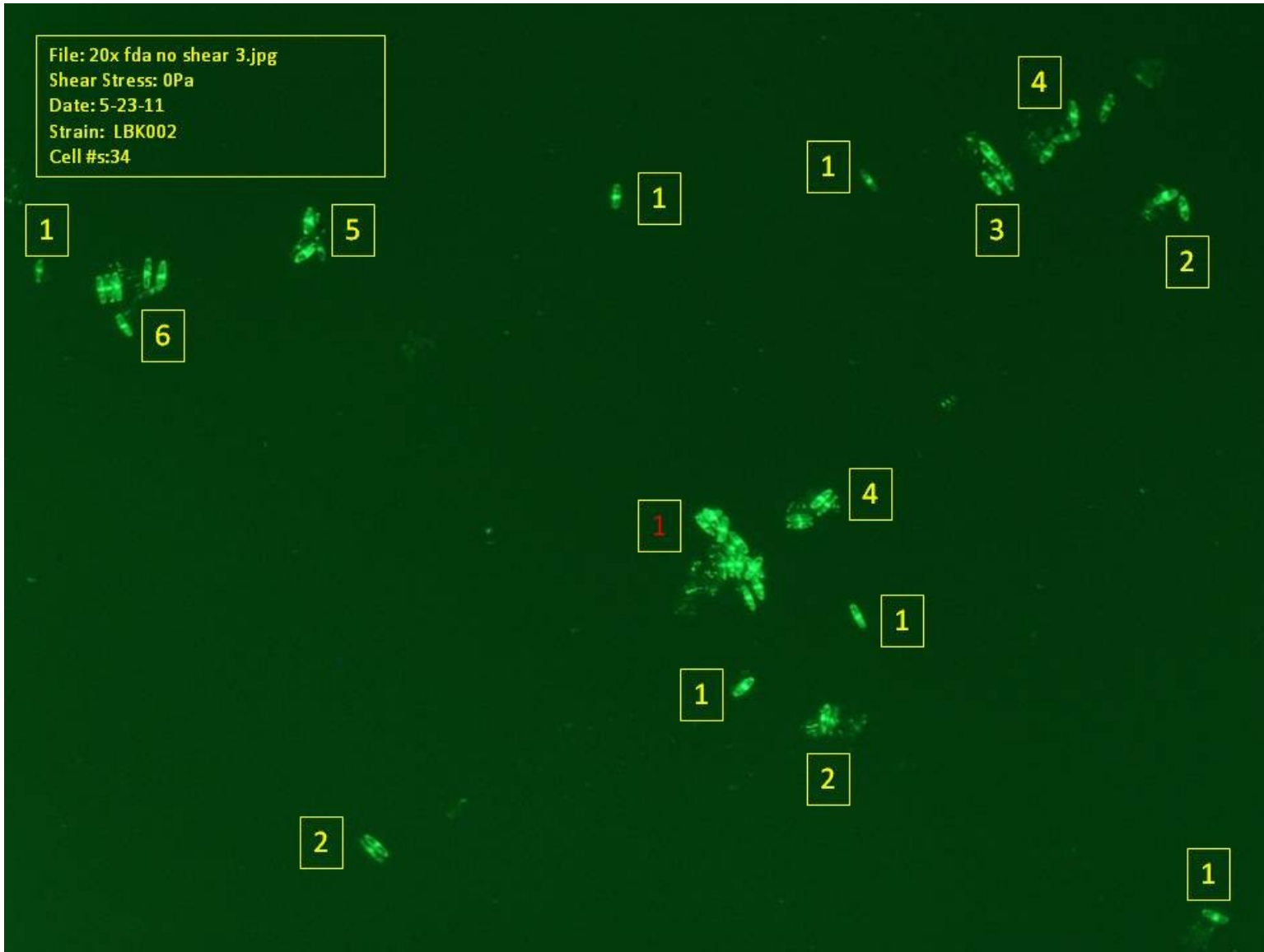


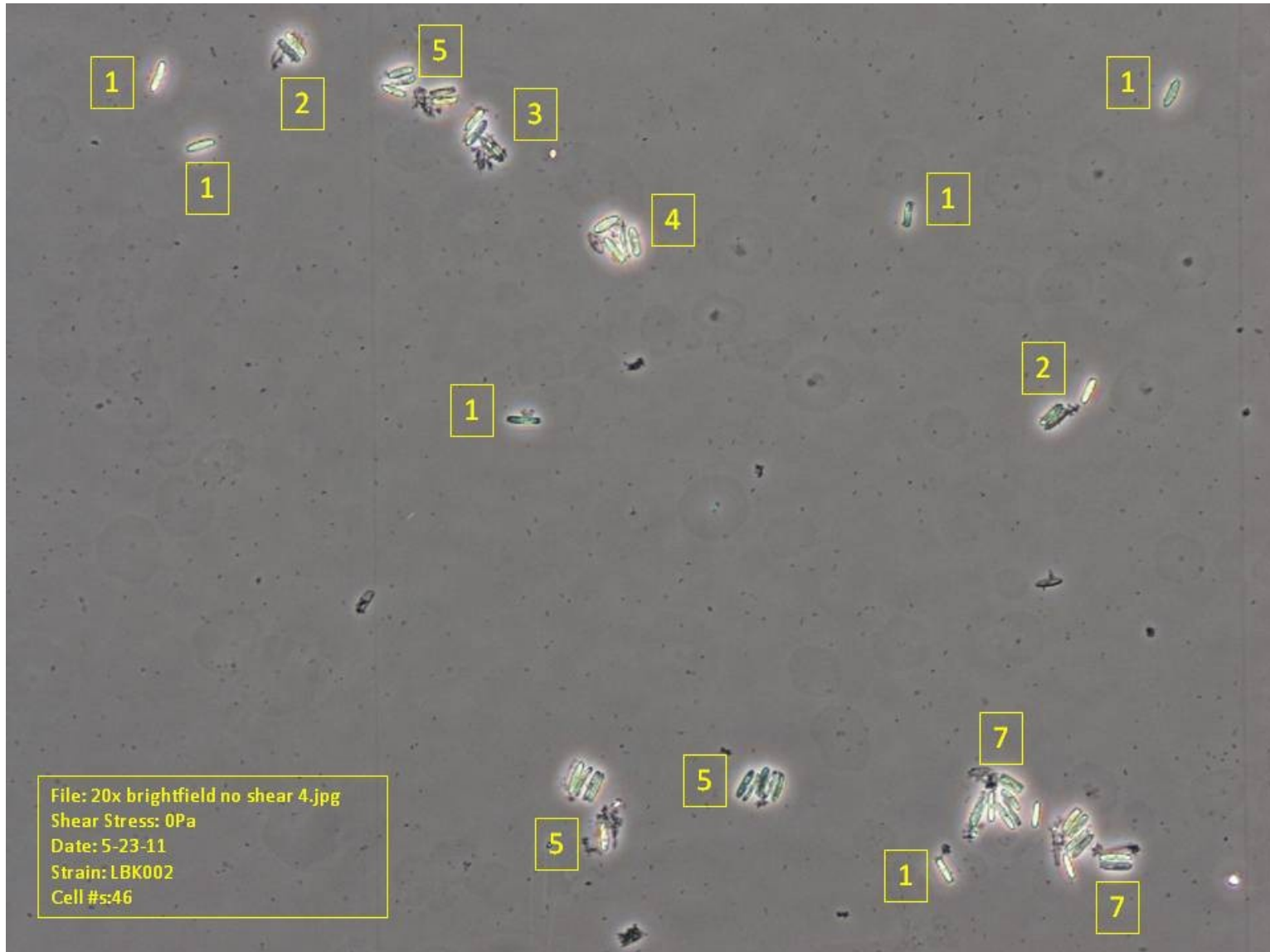


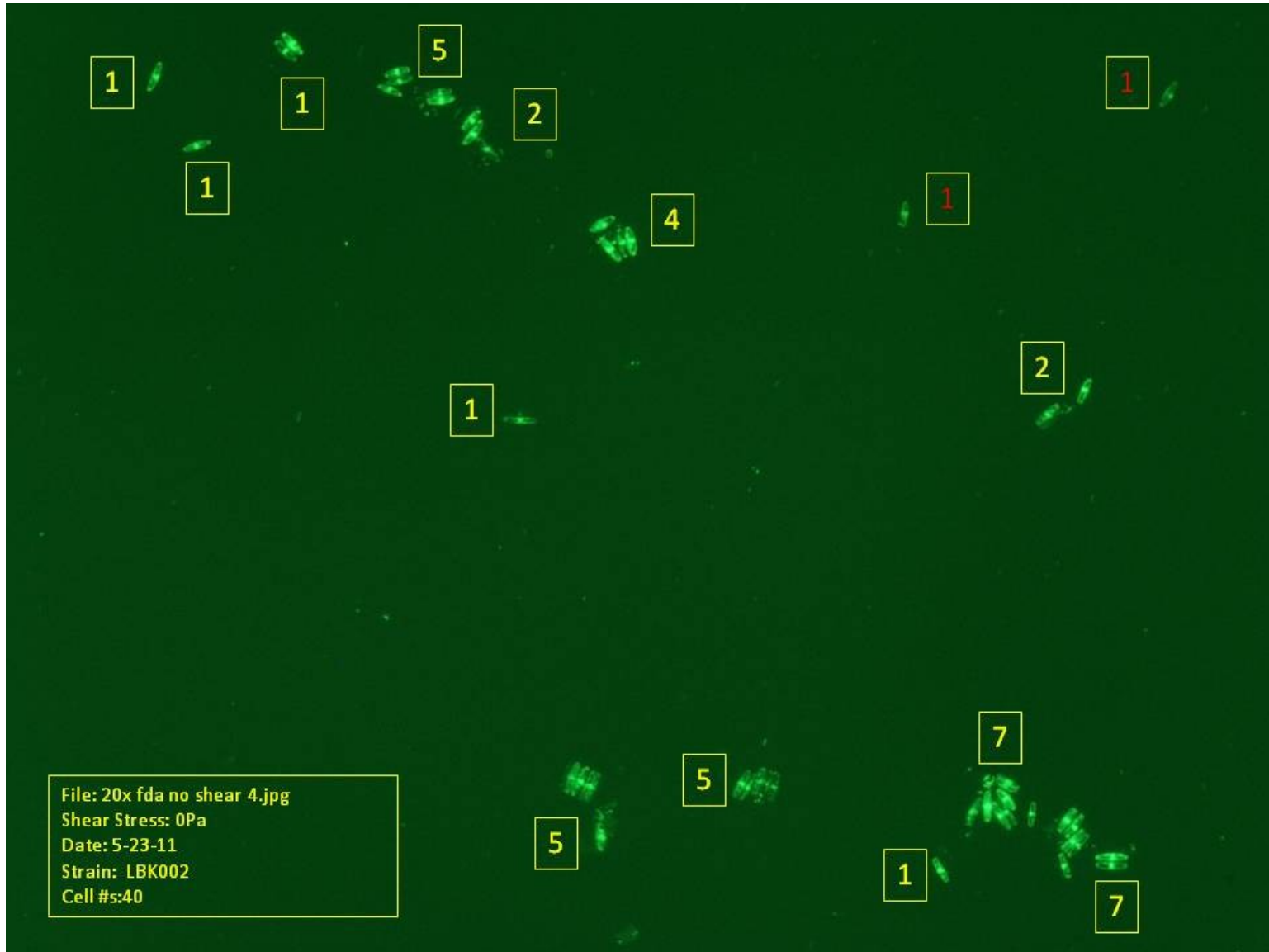


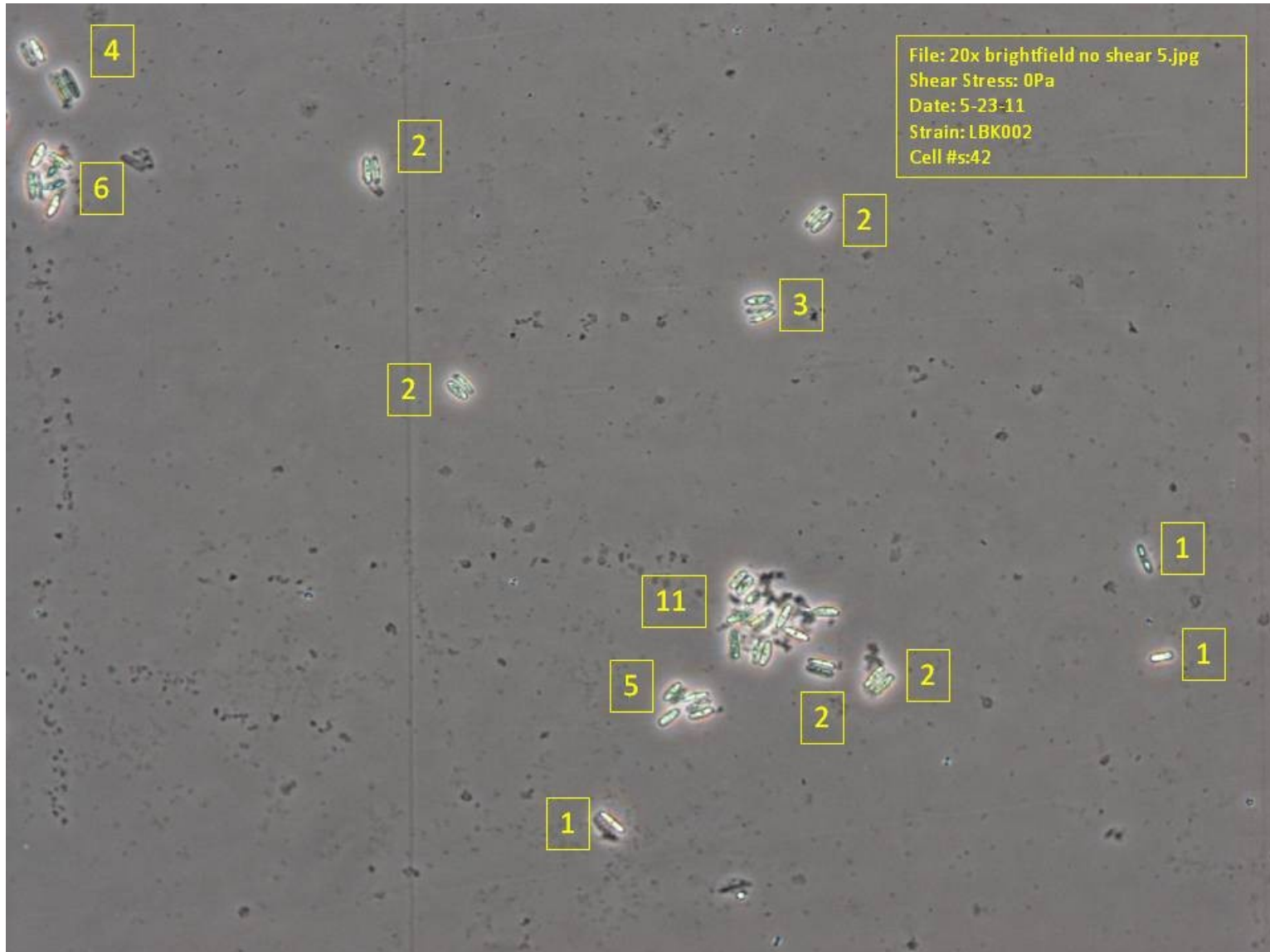


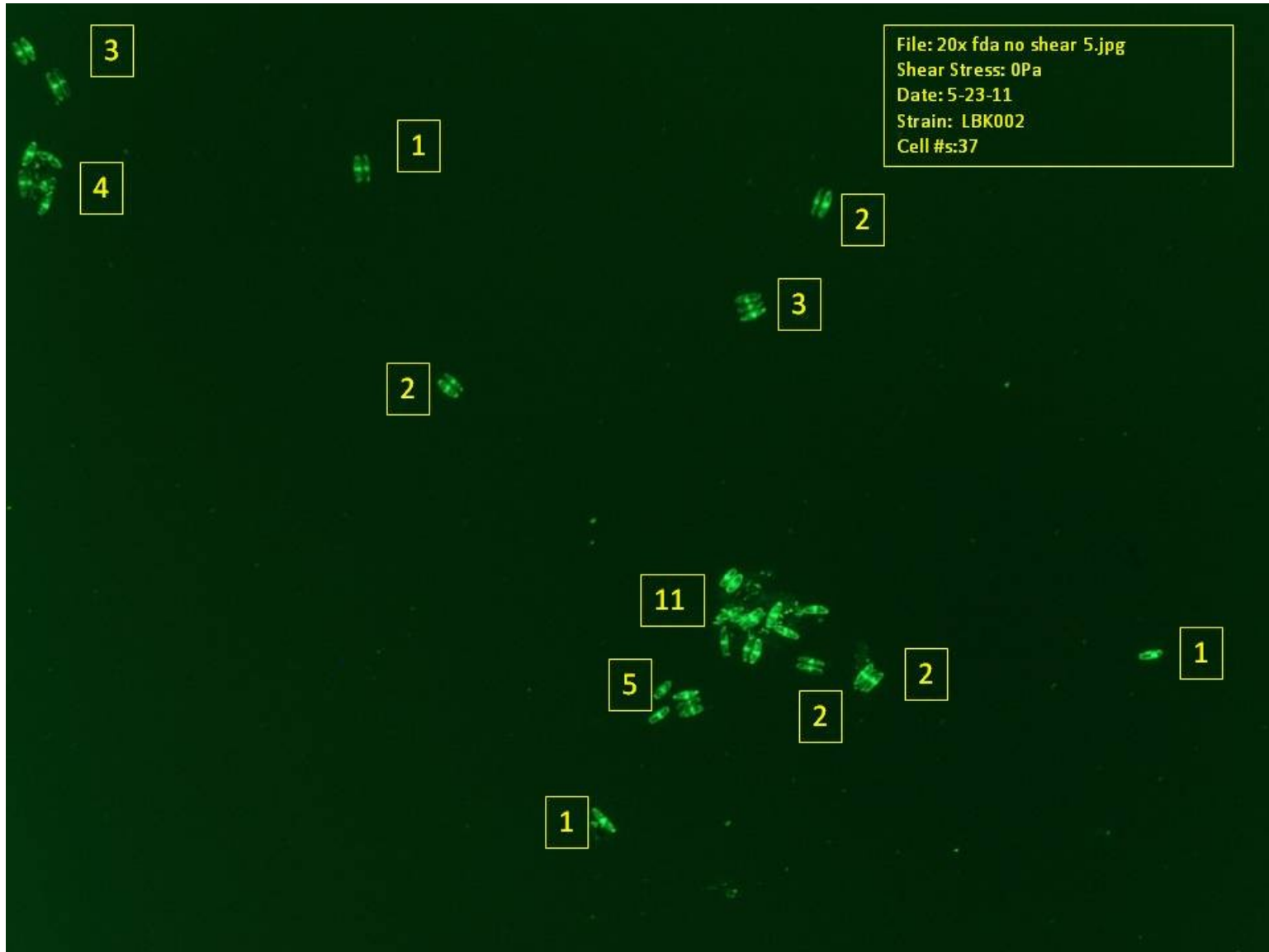


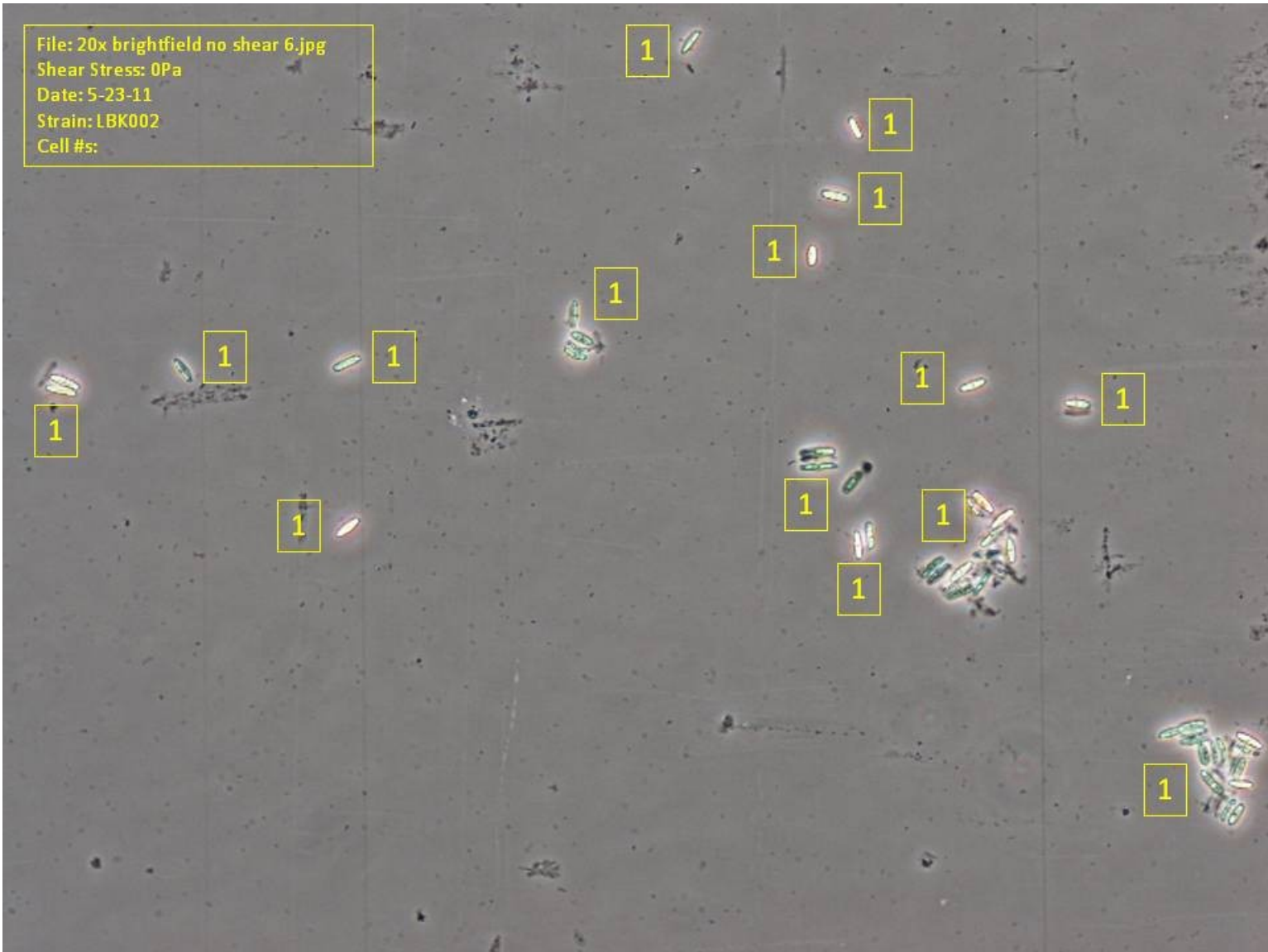




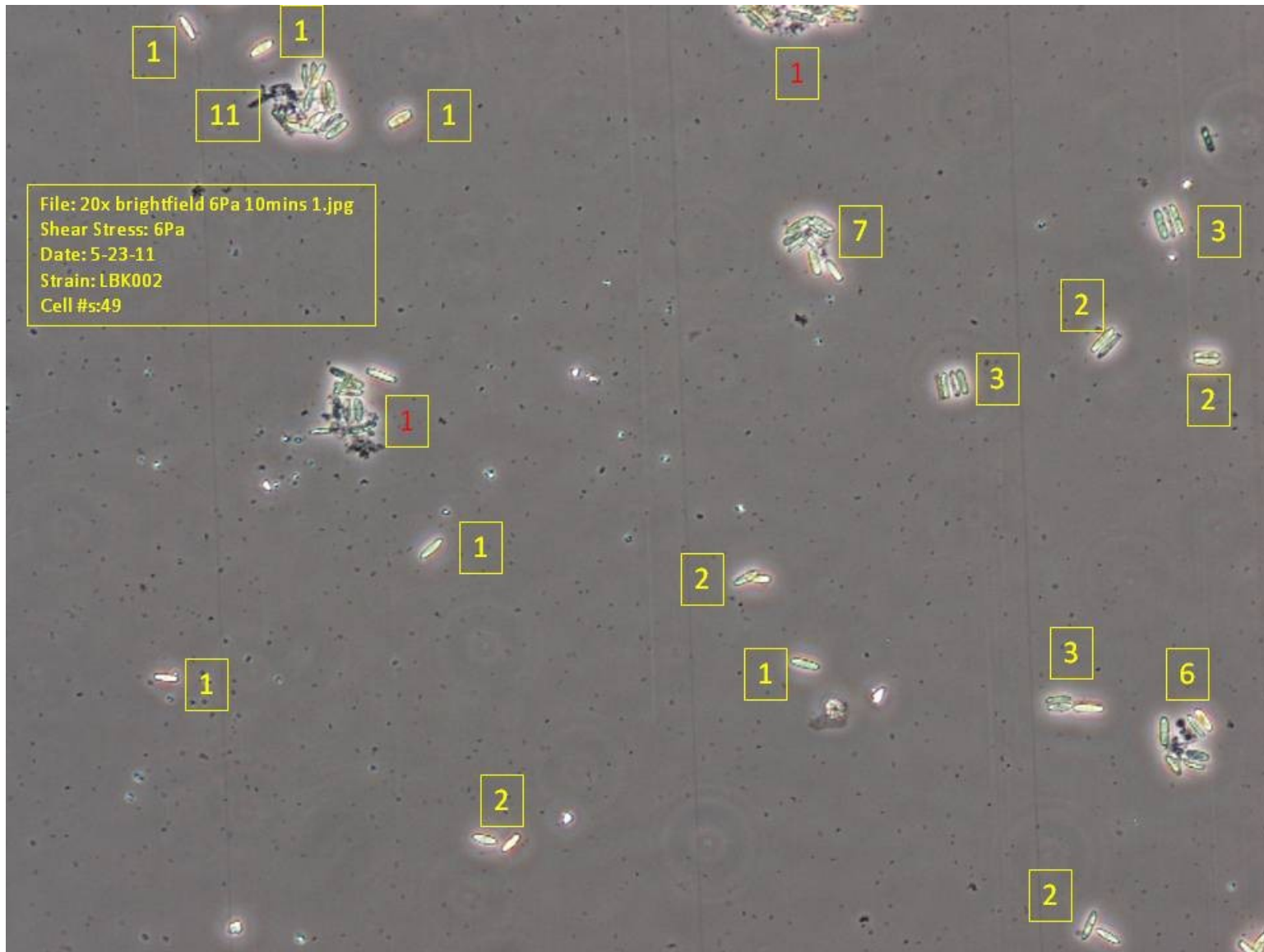




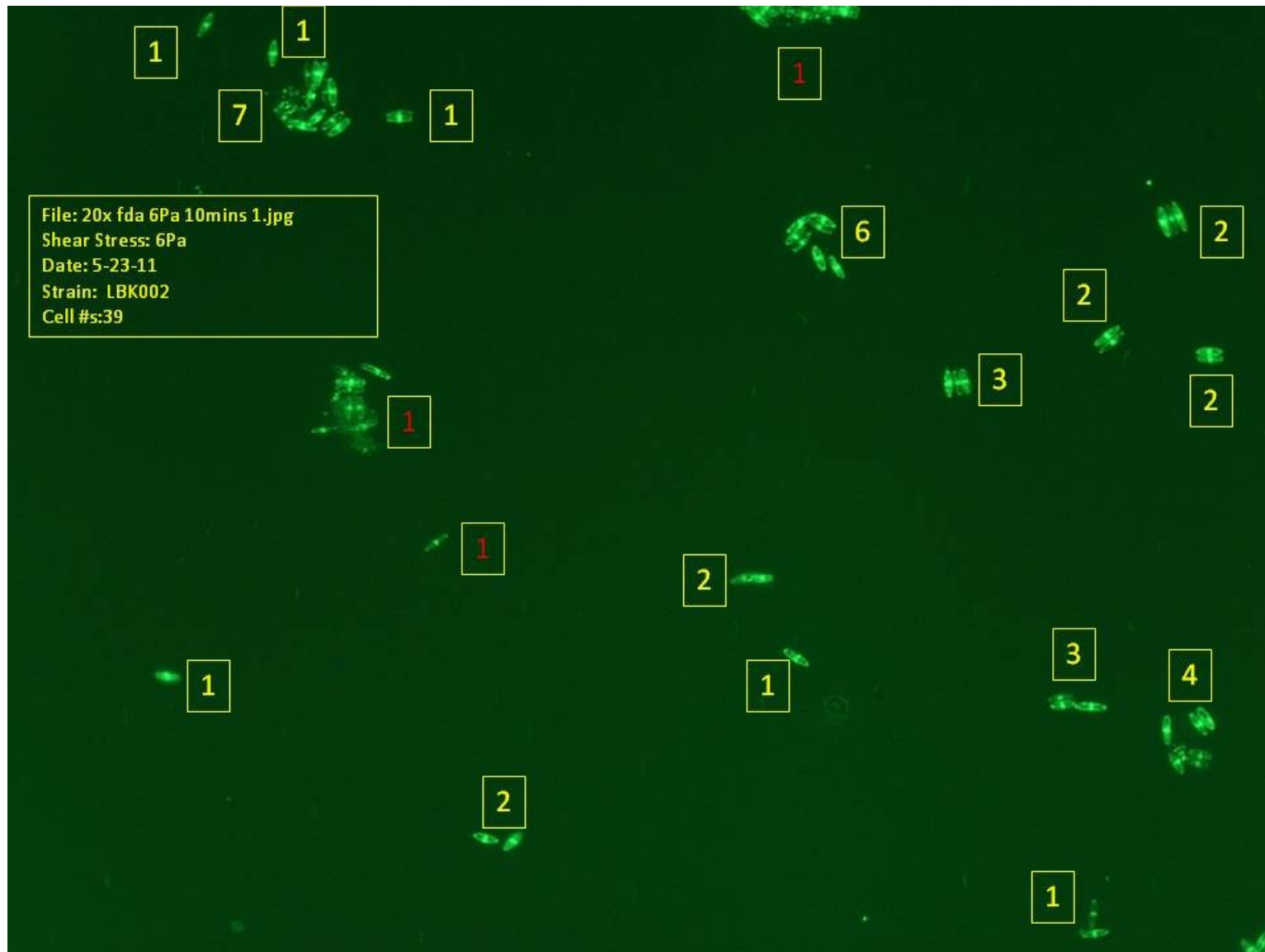


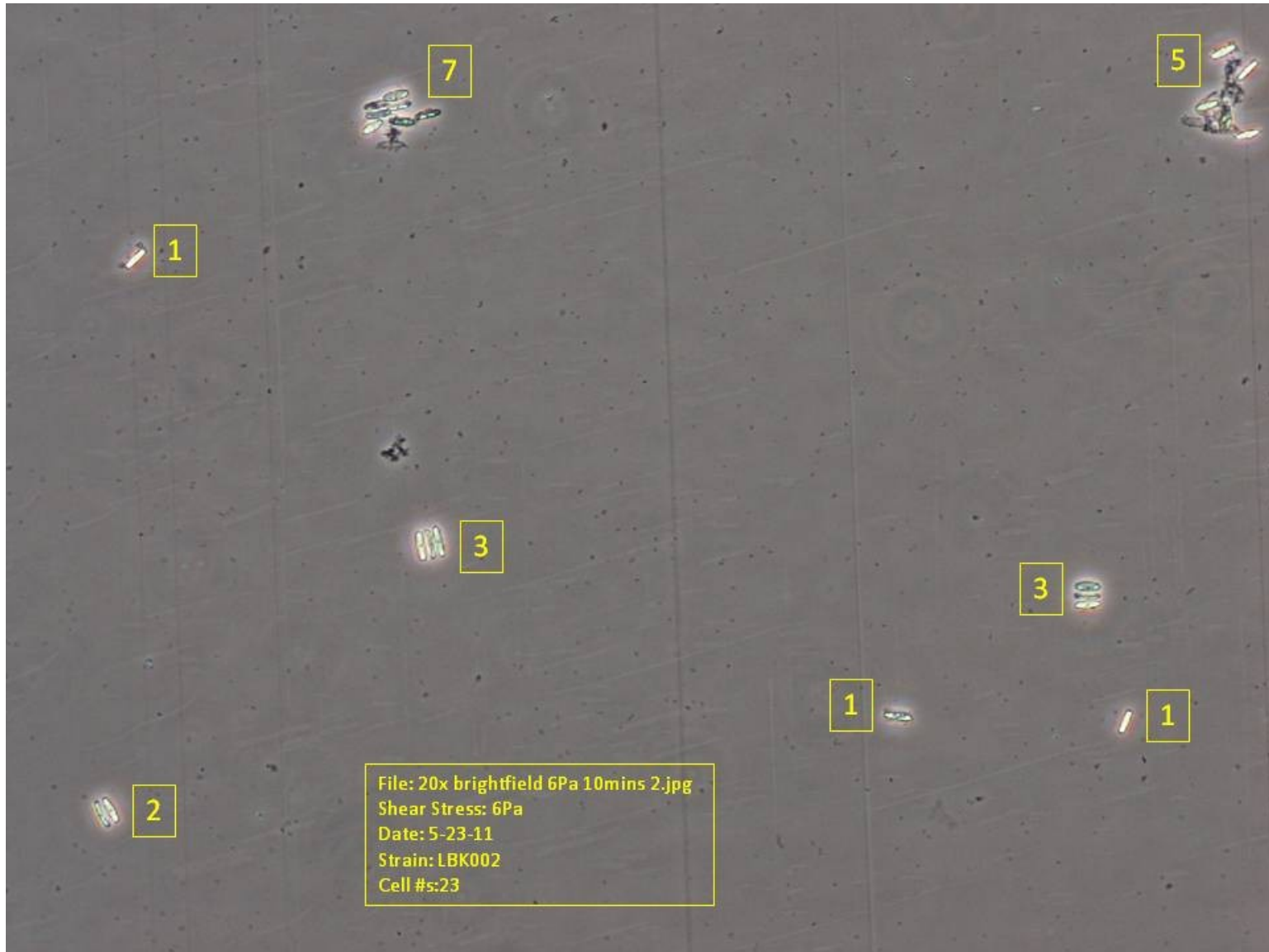




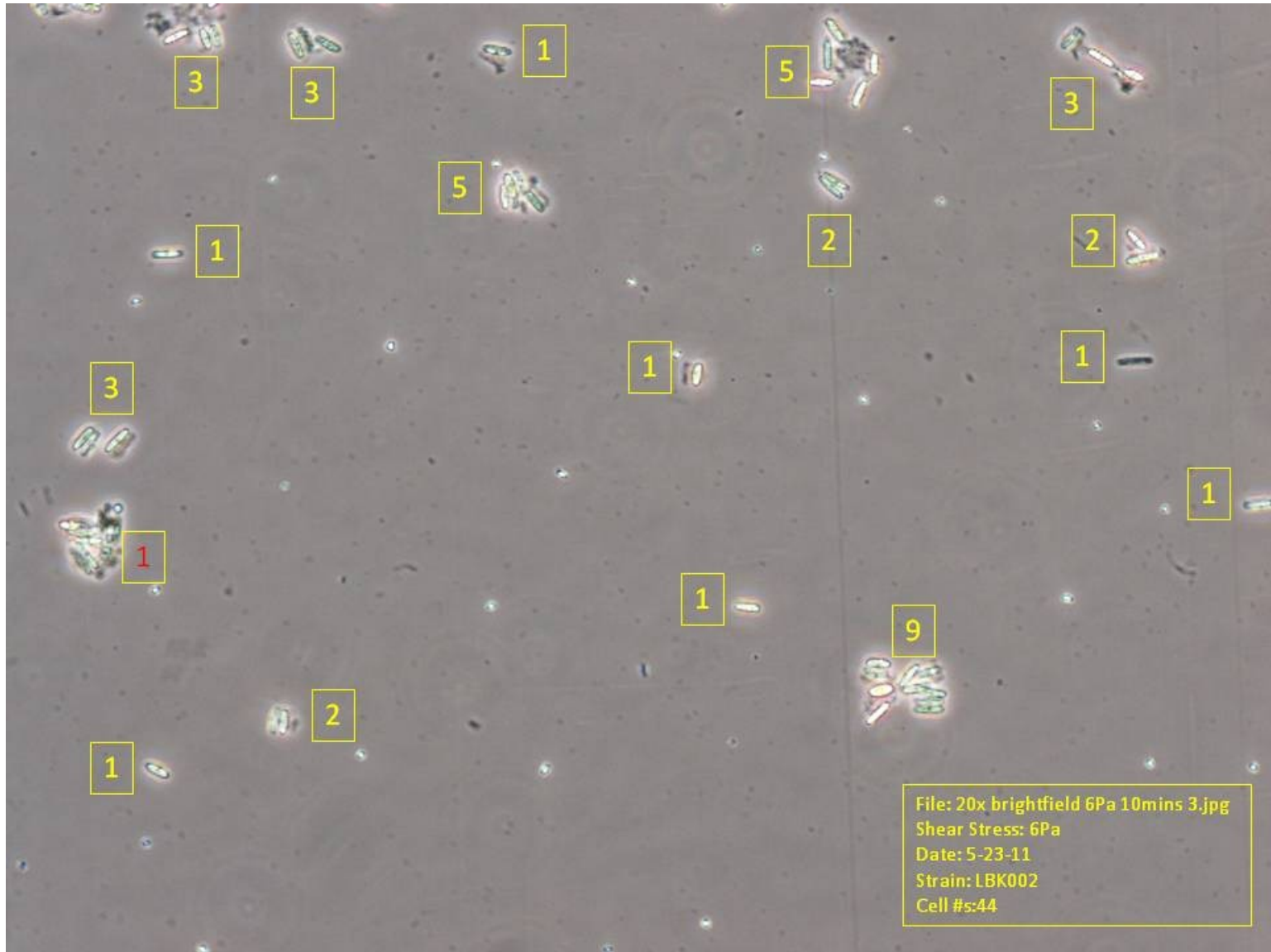


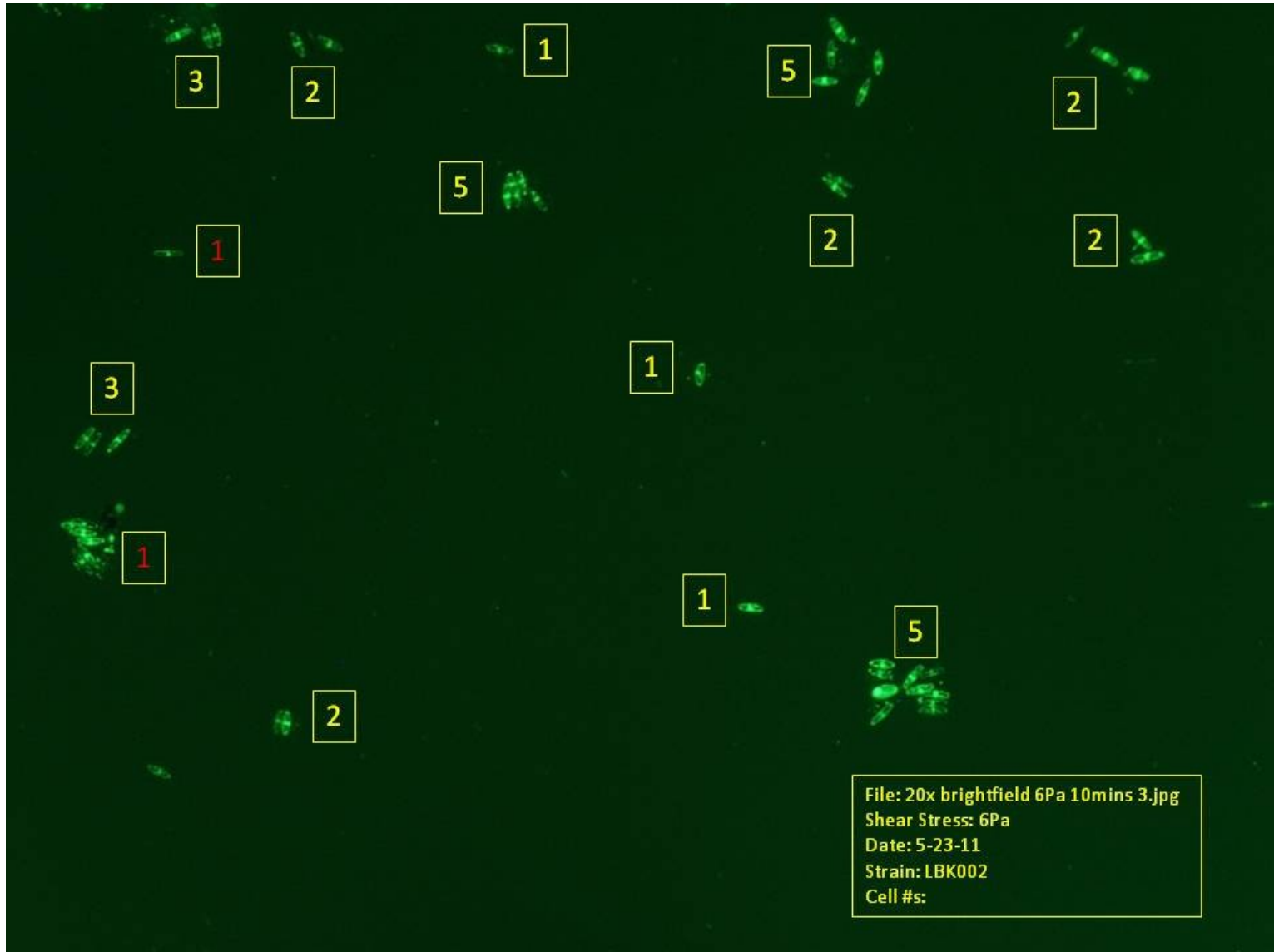


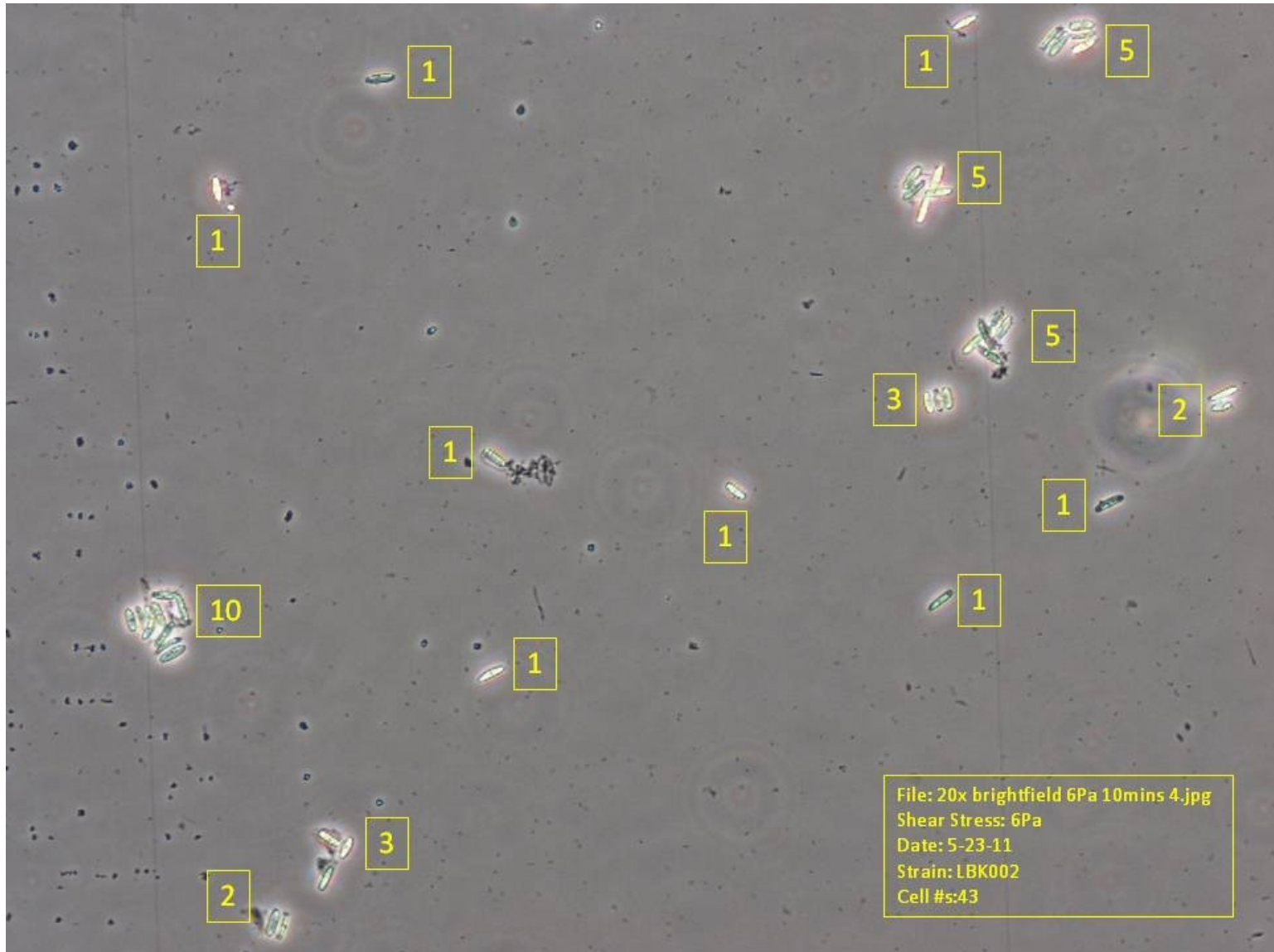


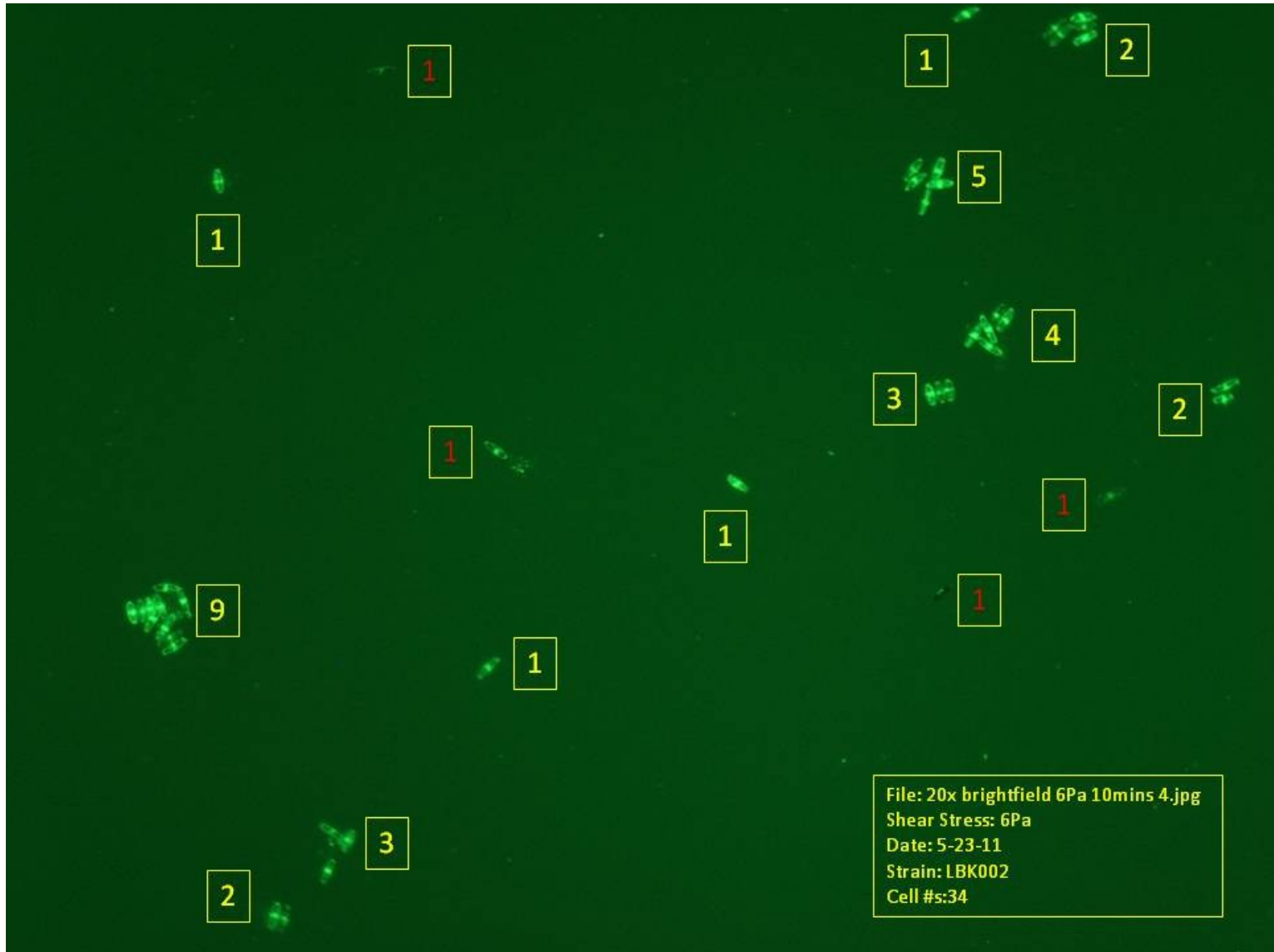


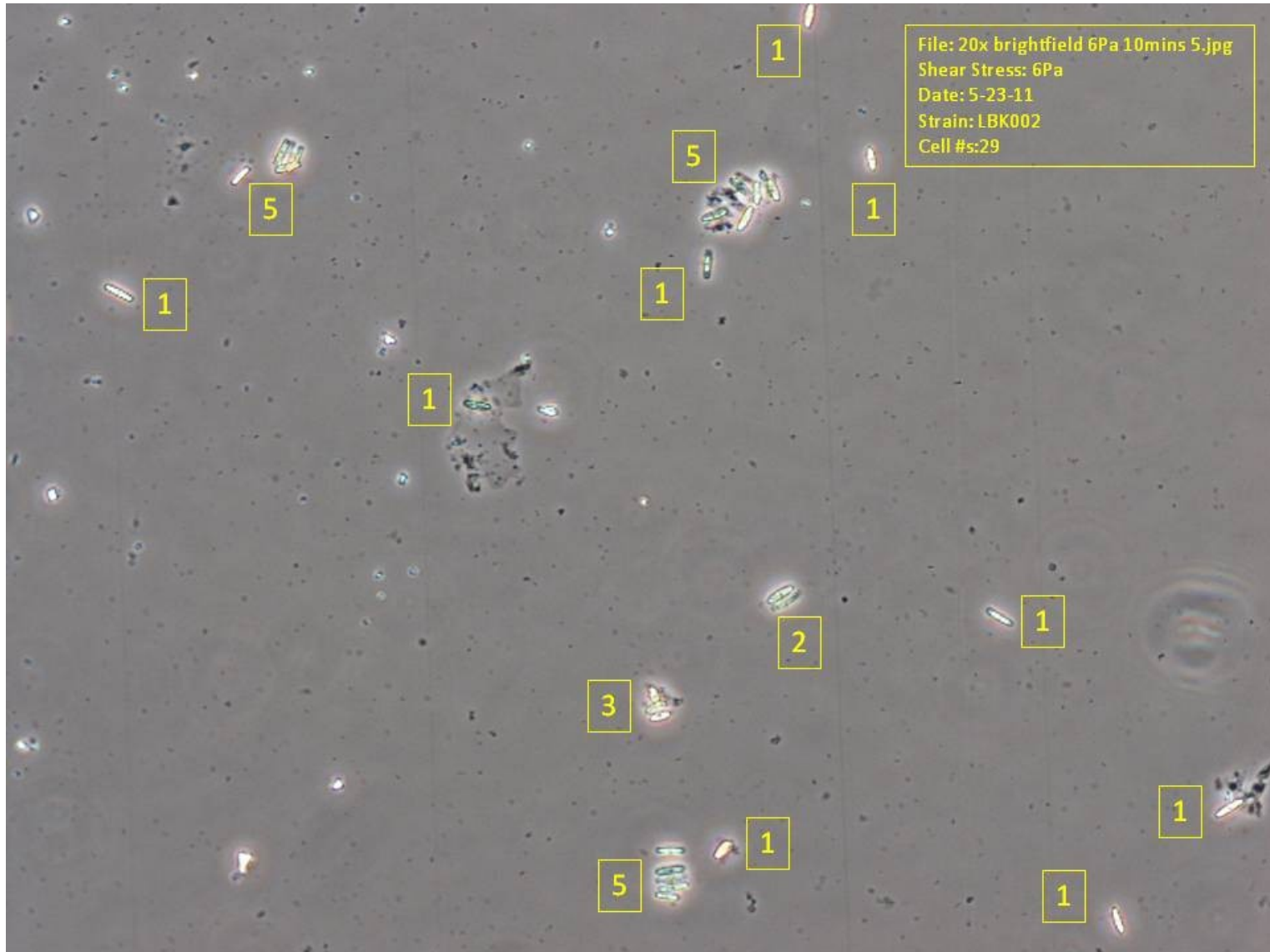




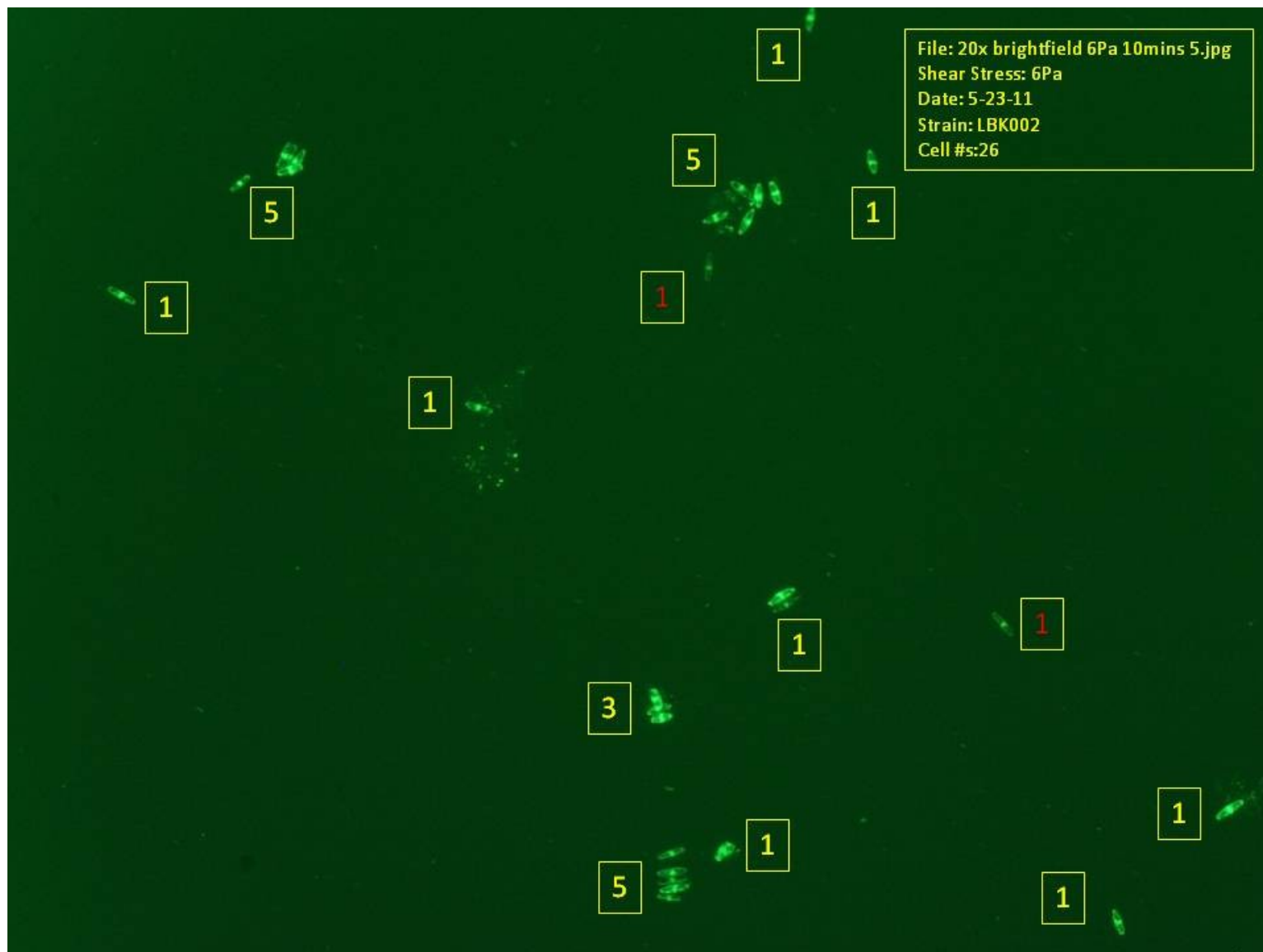


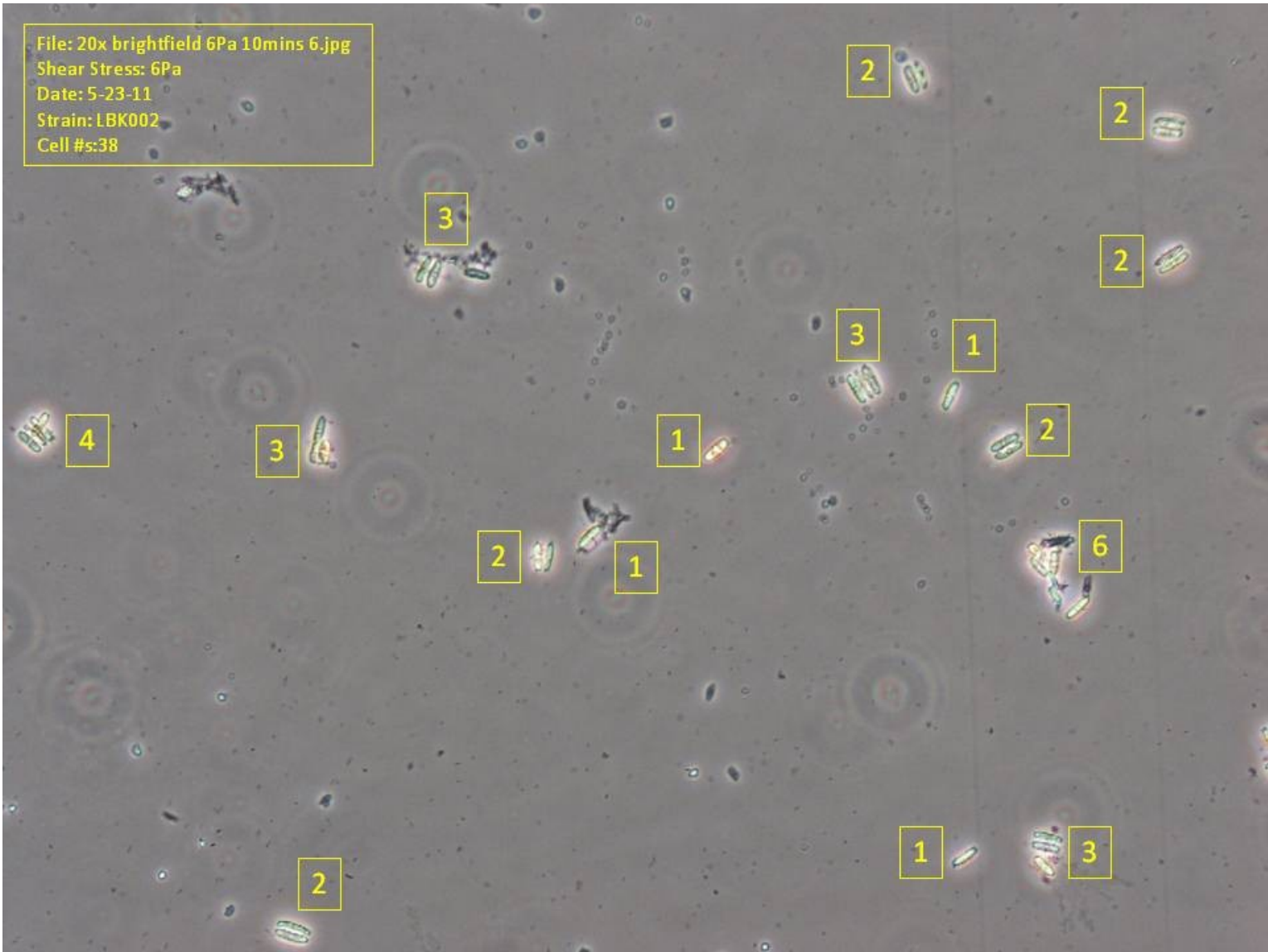




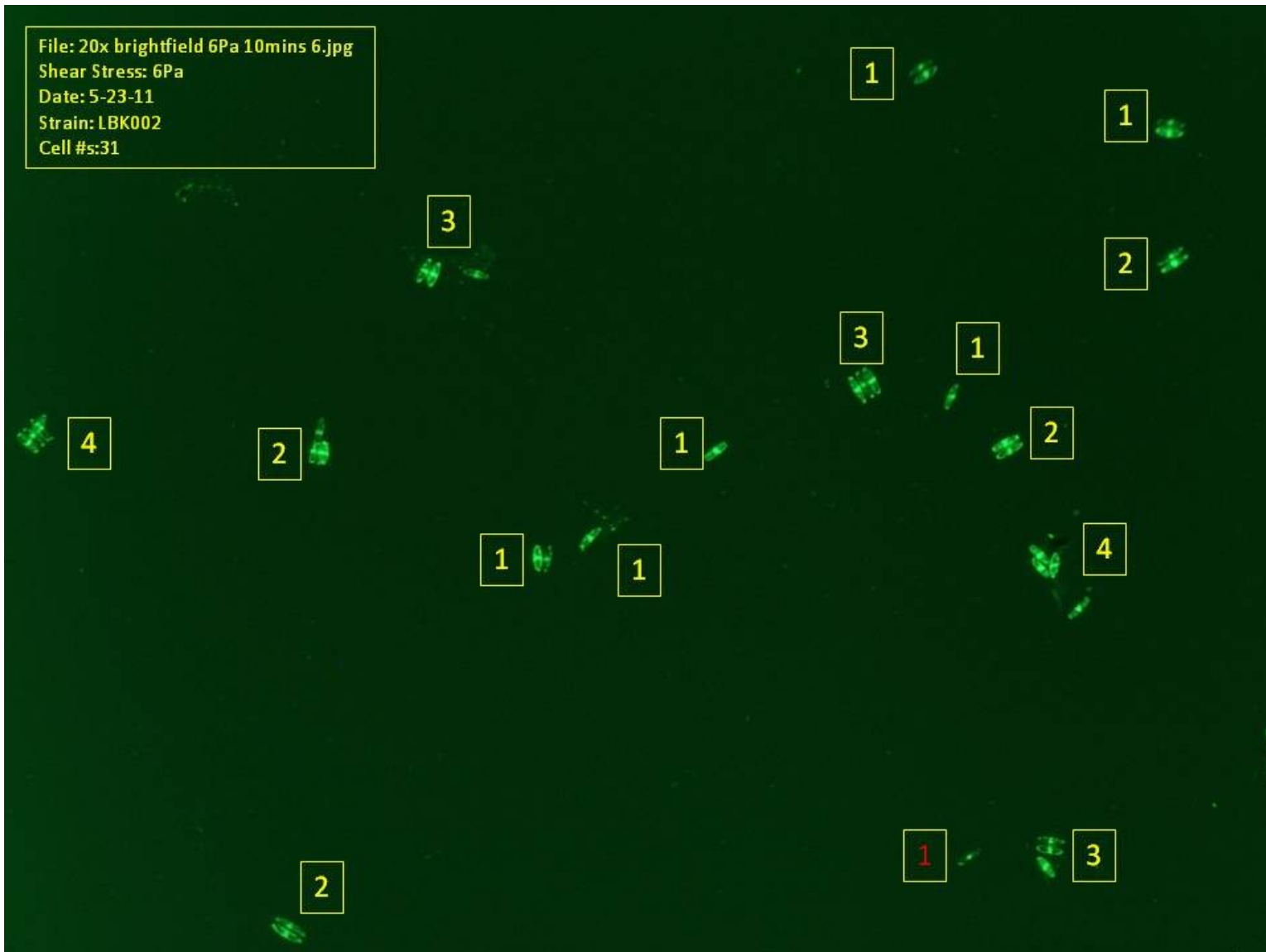




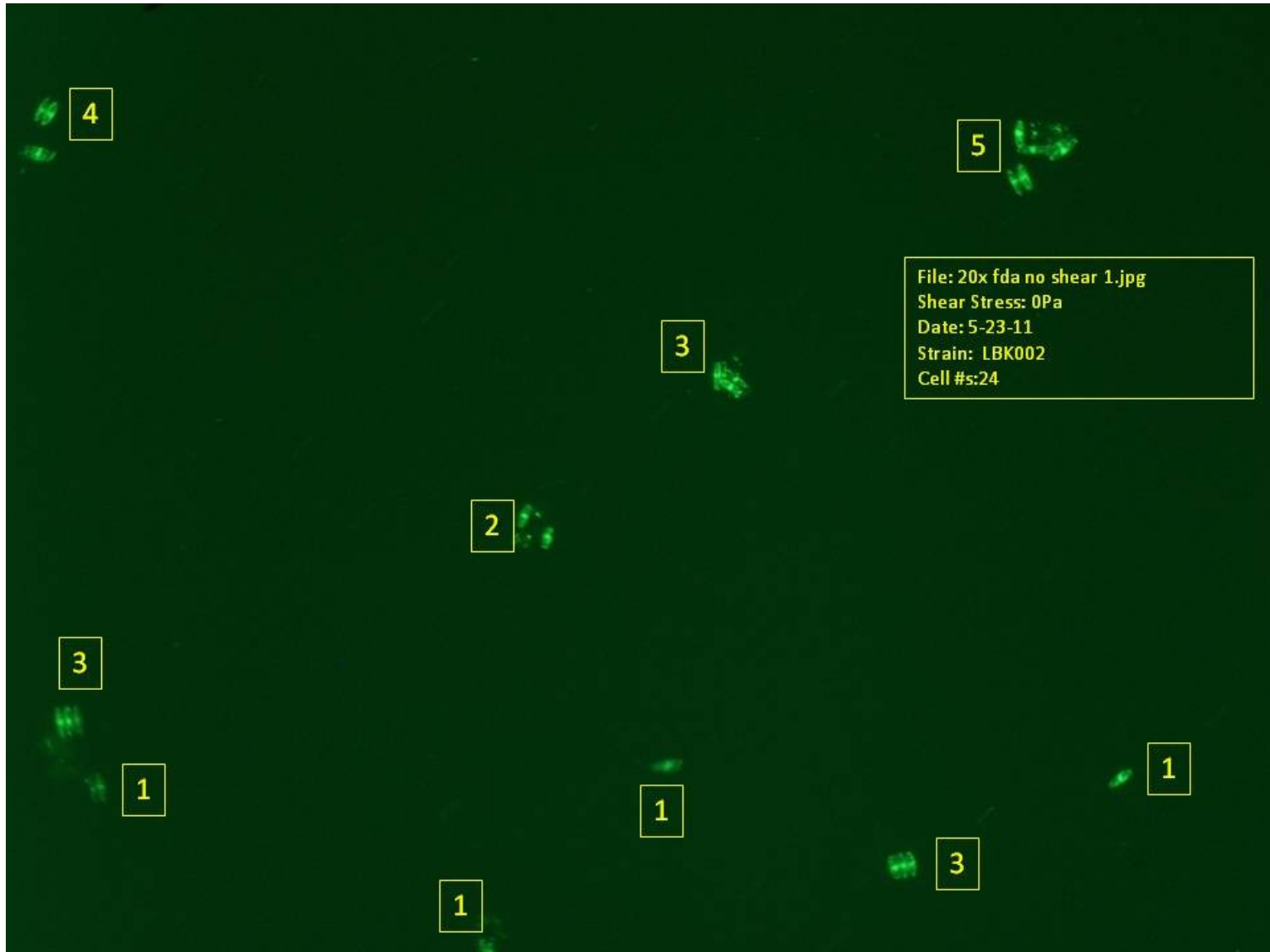


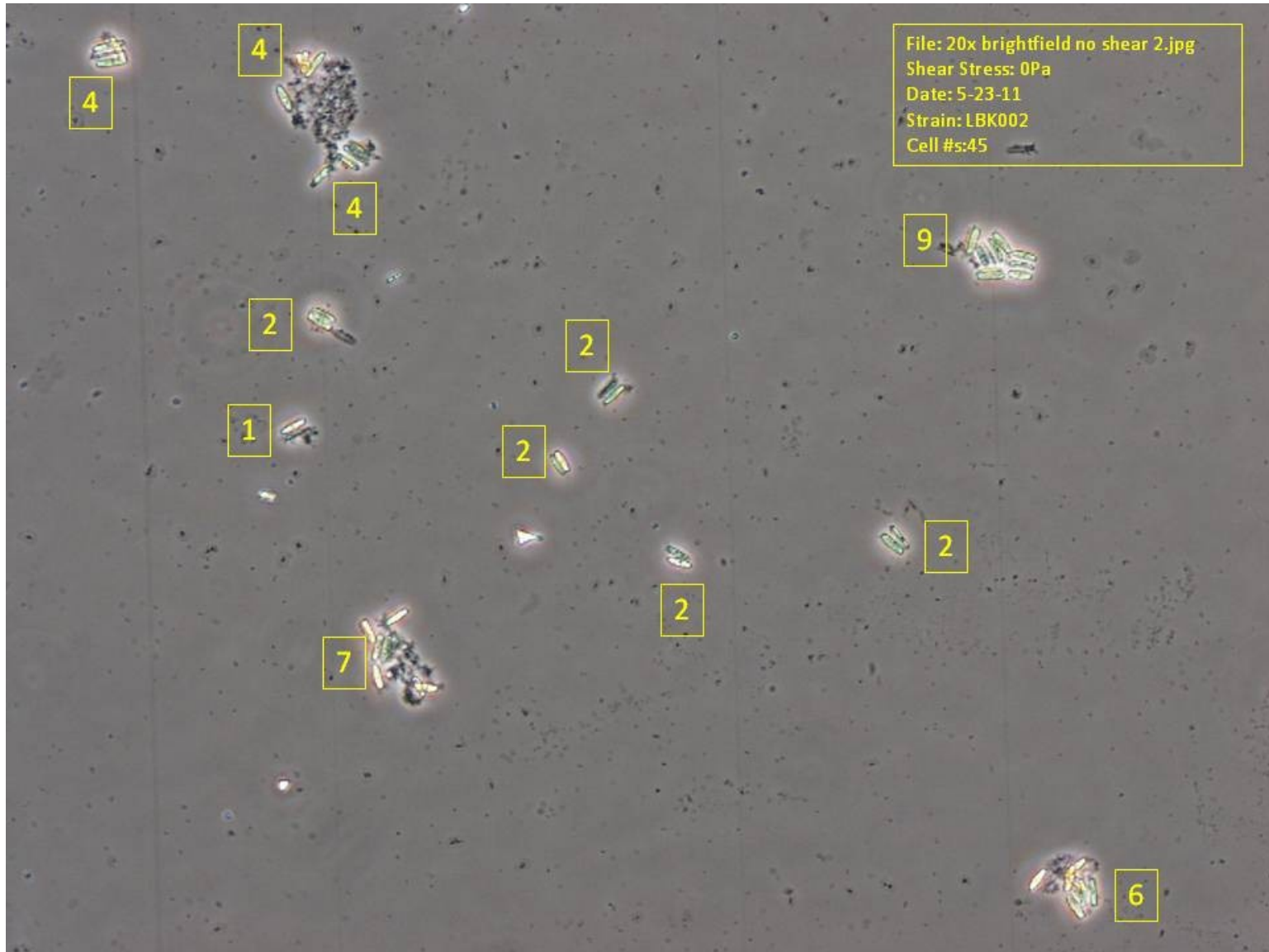


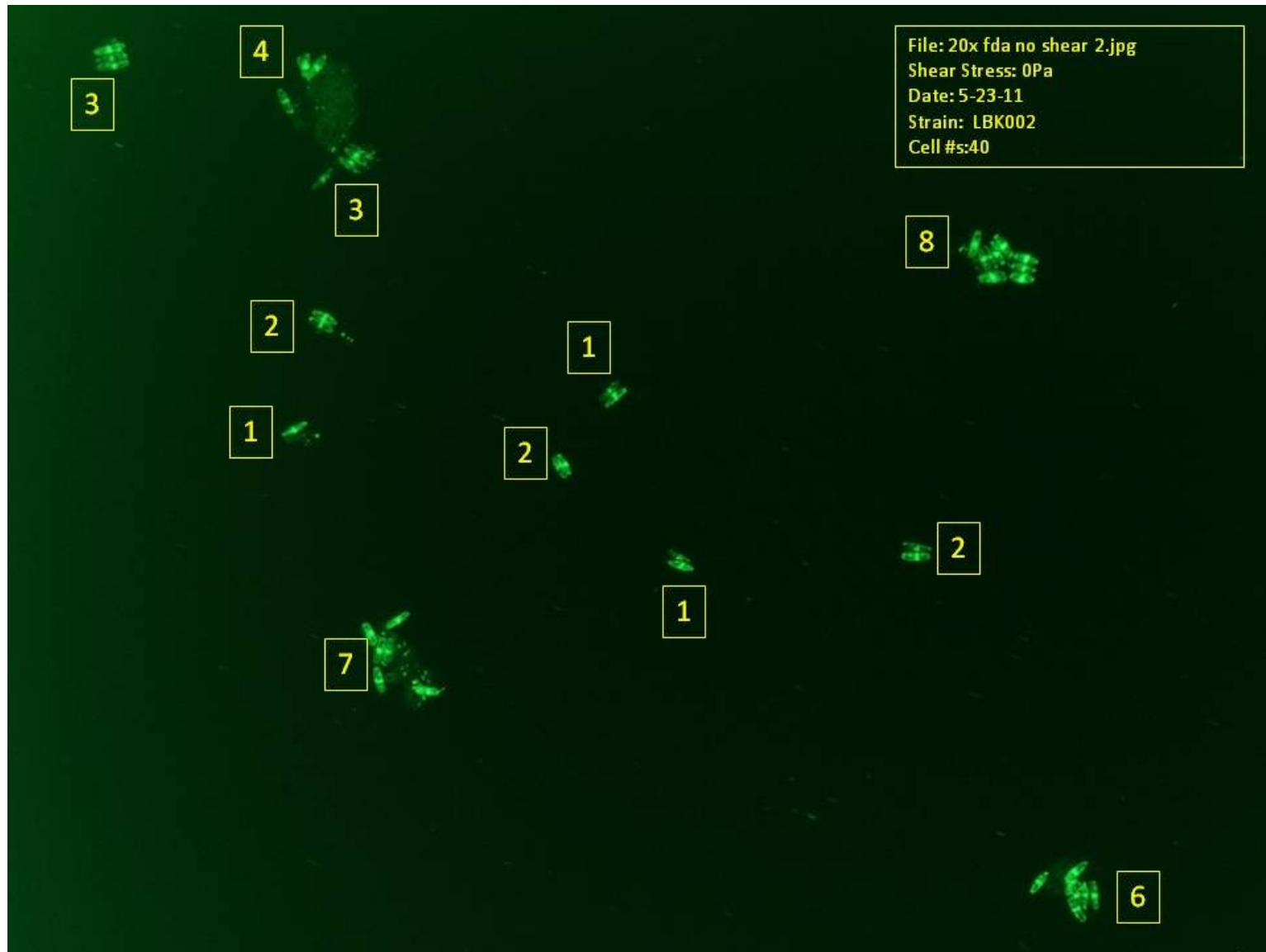
File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:31

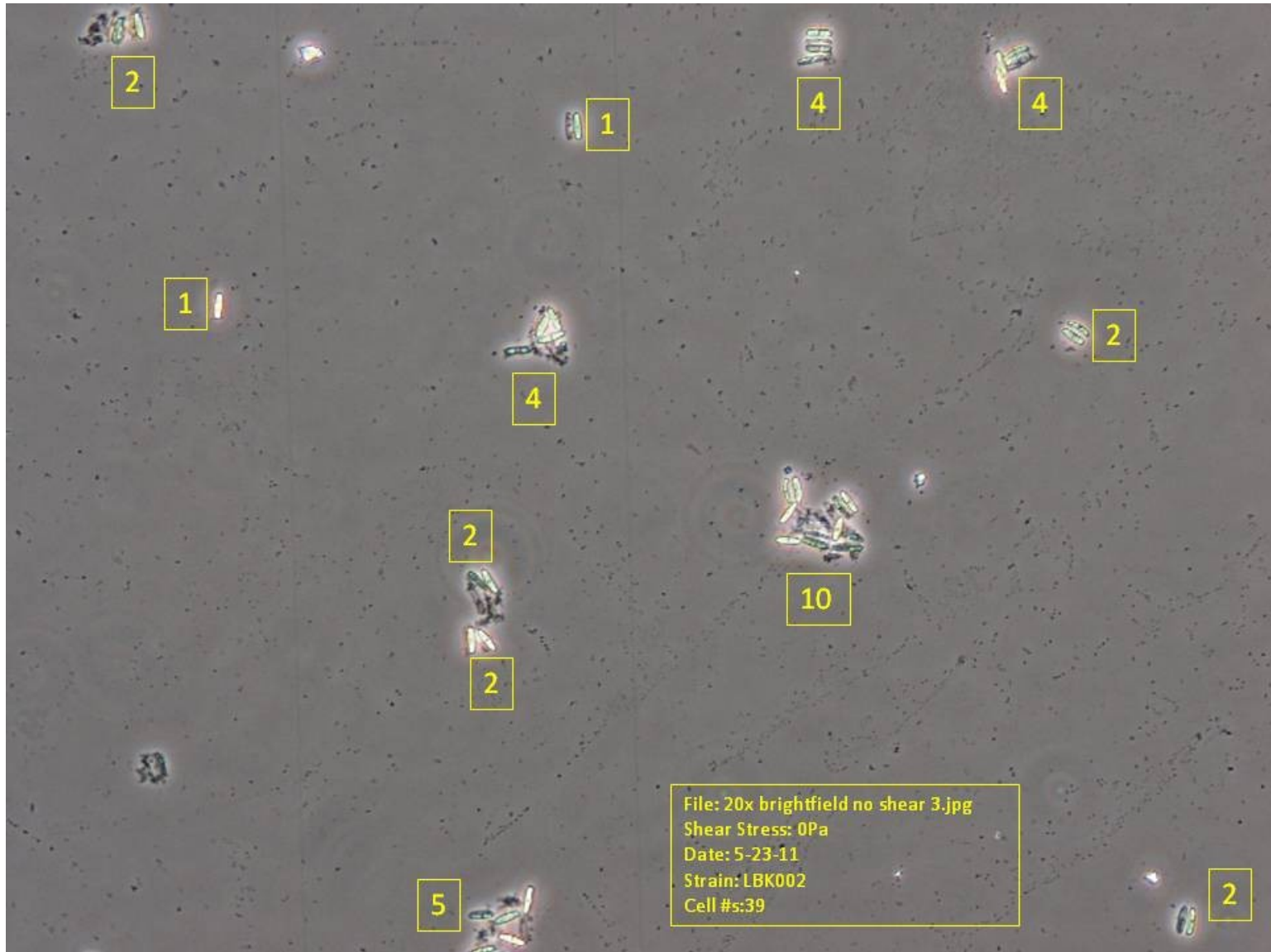






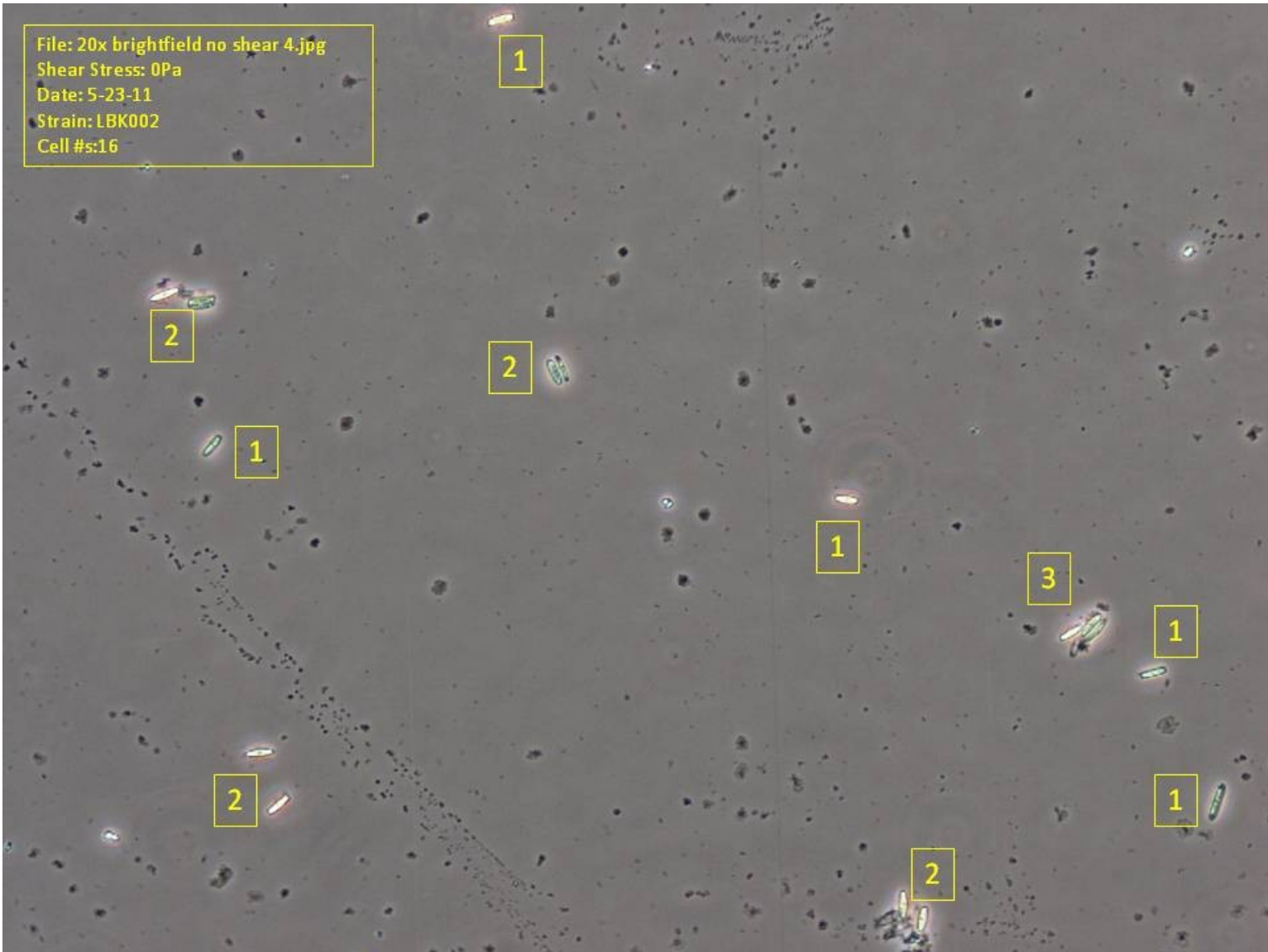




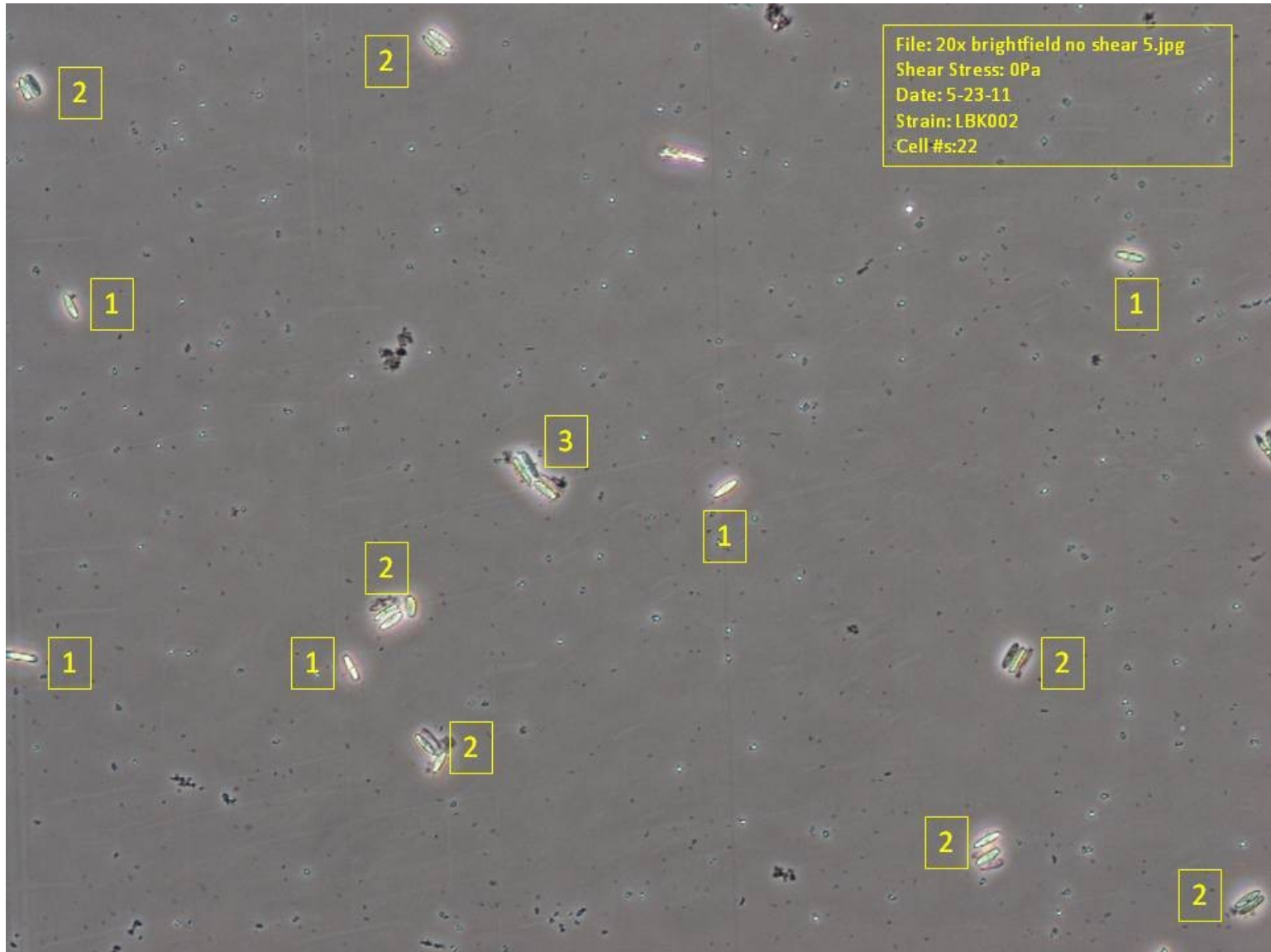


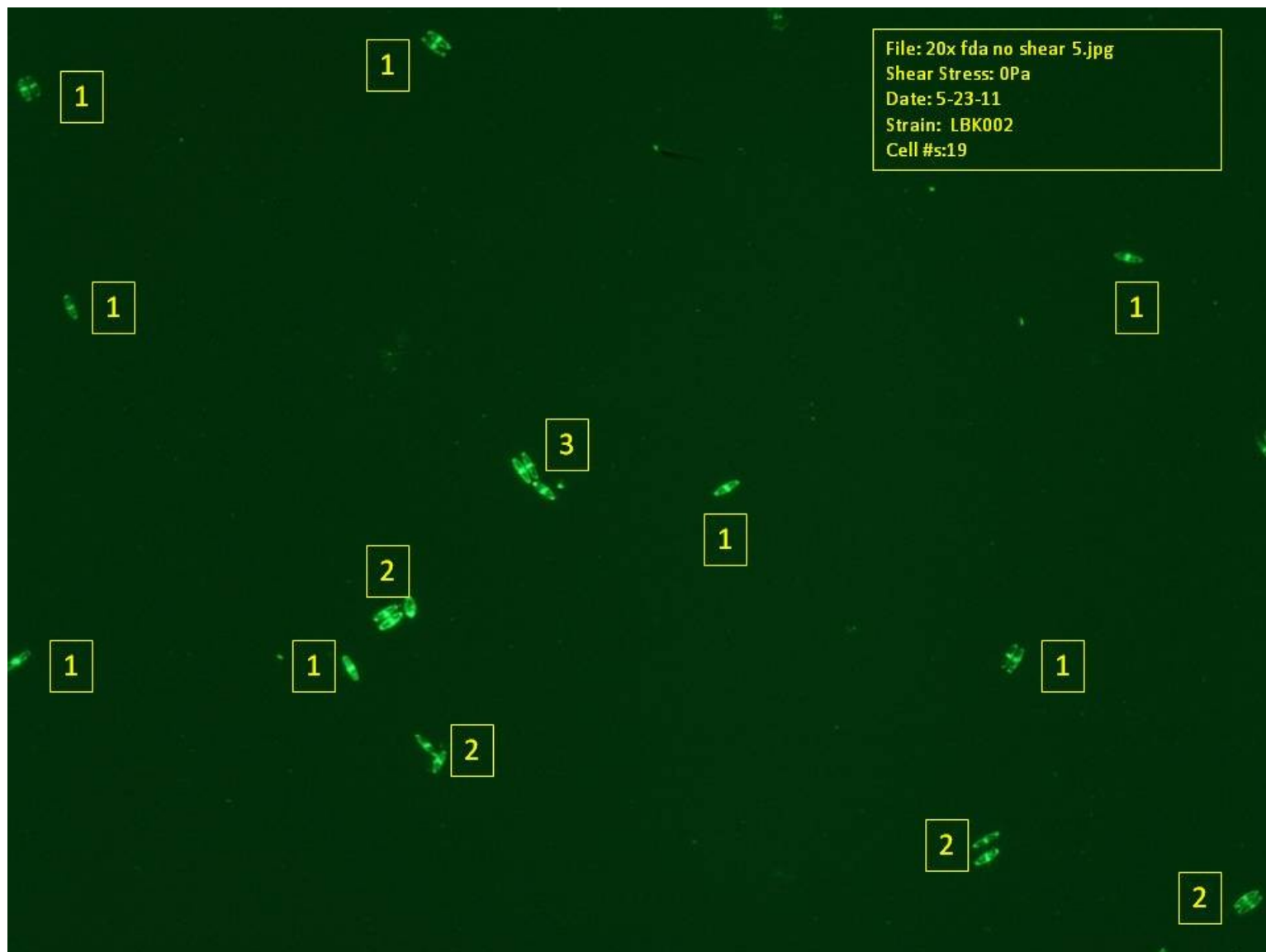




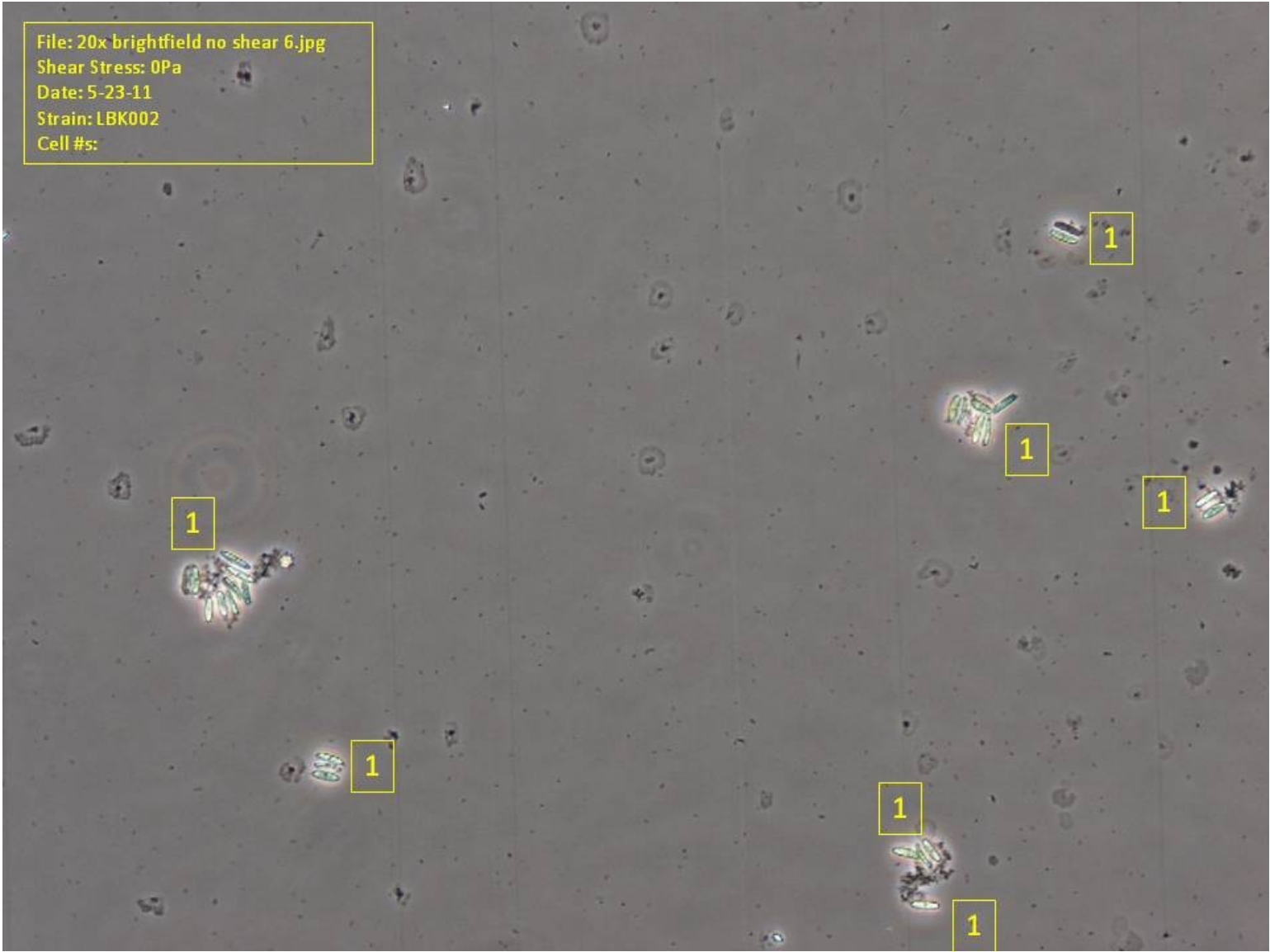




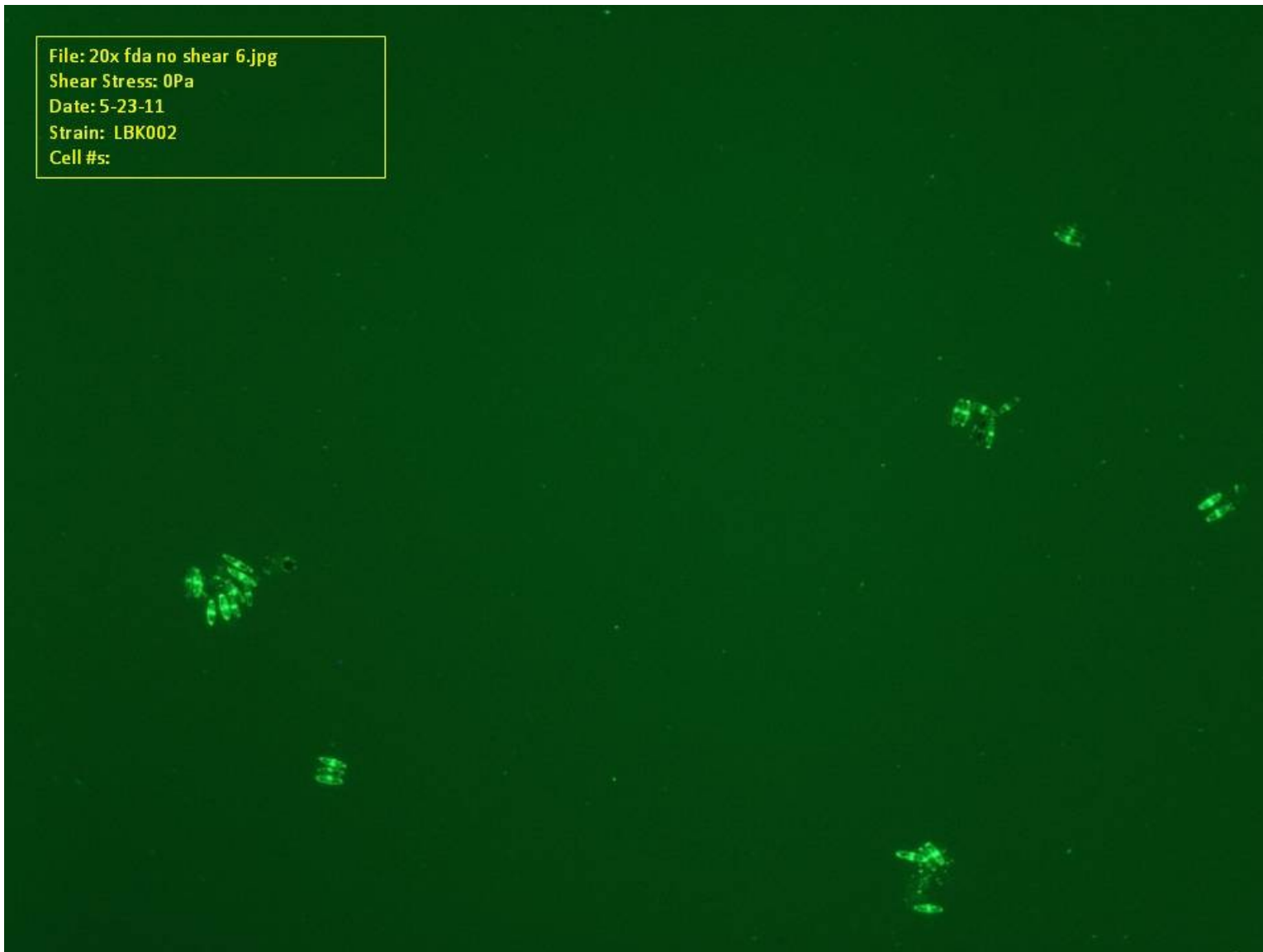


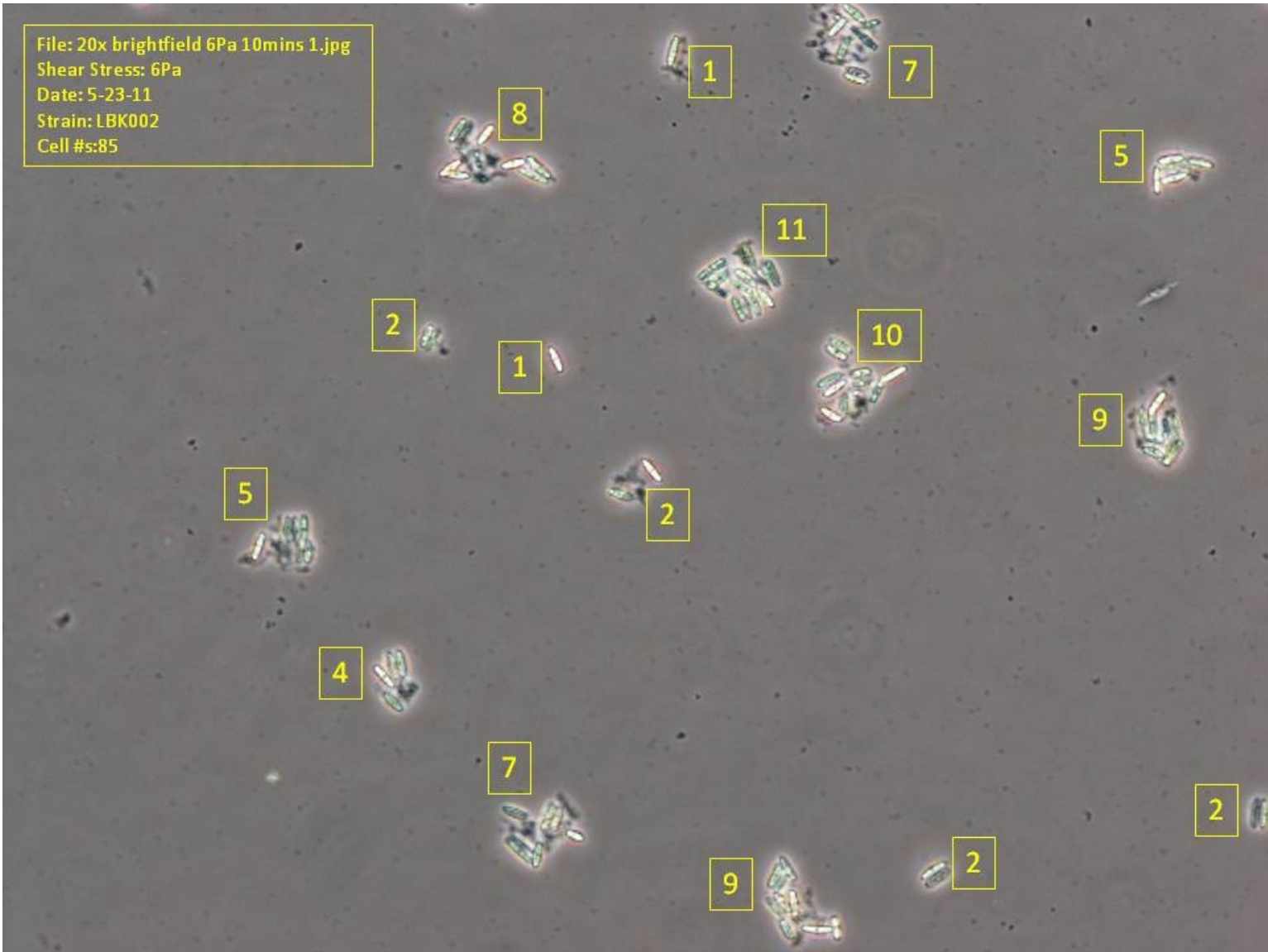


File: 20x brightfield no shear 6.jpg  
Shear Stress: 0Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:

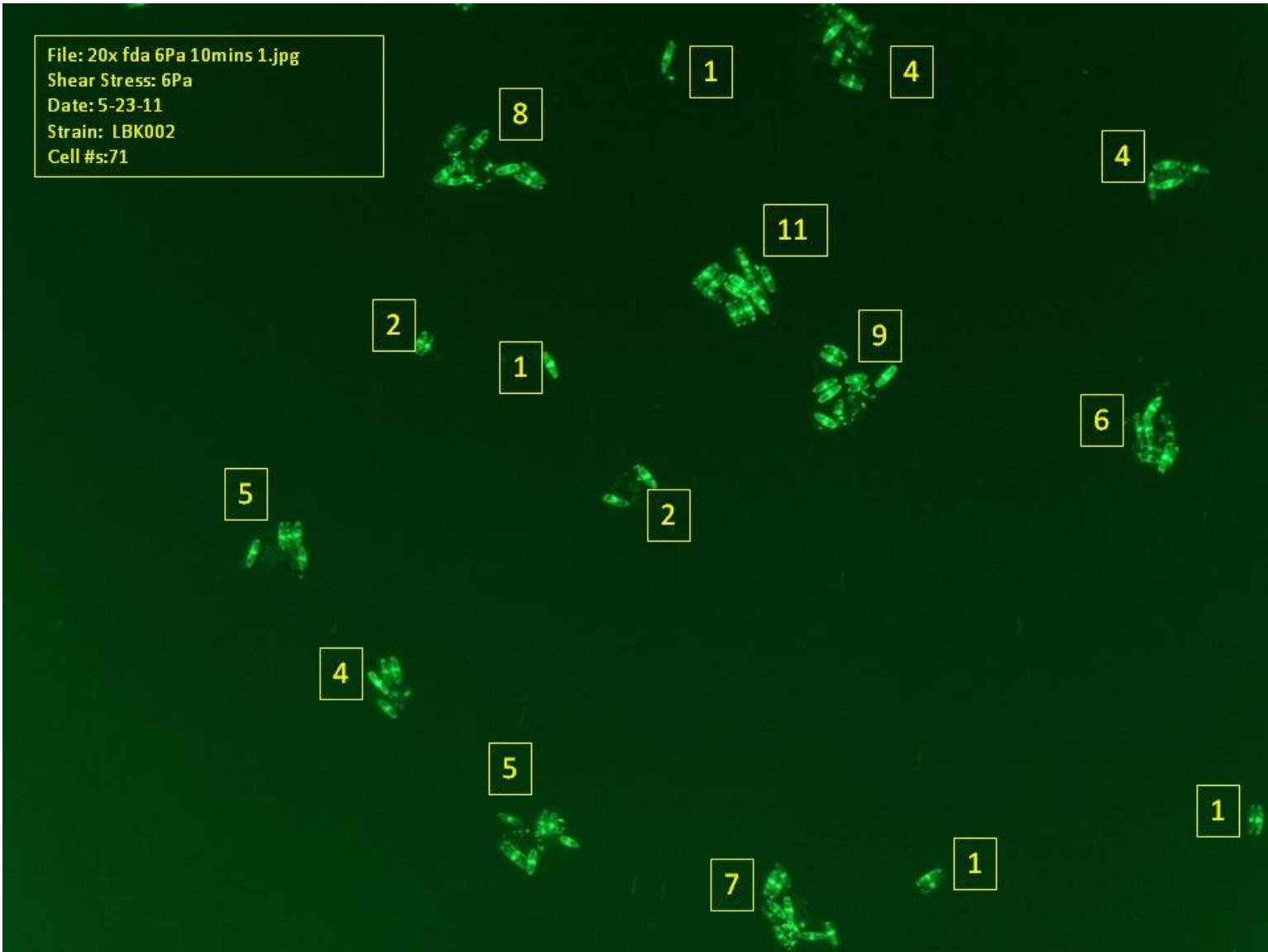


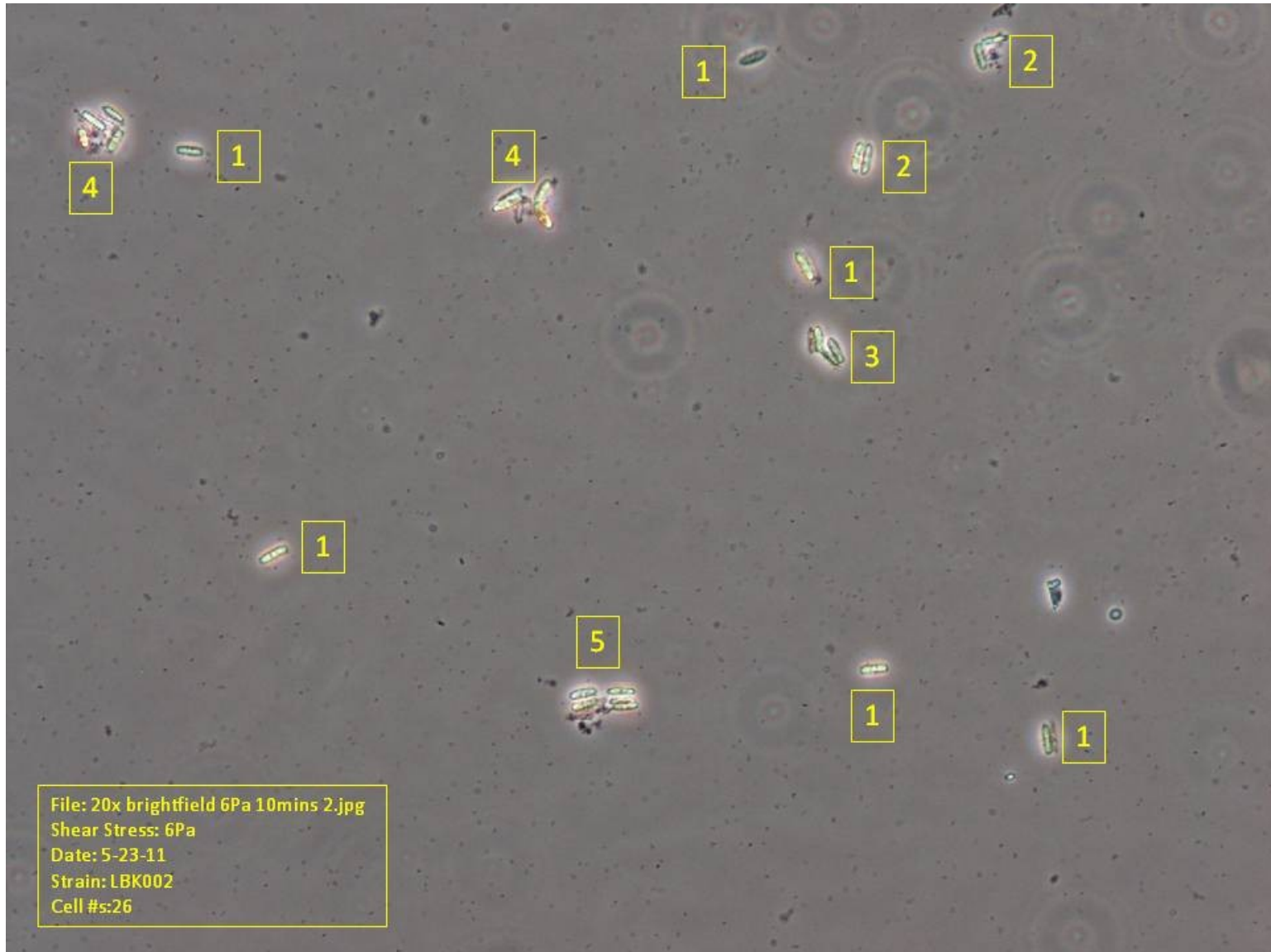
File: 20x fda no shear 6.jpg  
Shear Stress: 0Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:

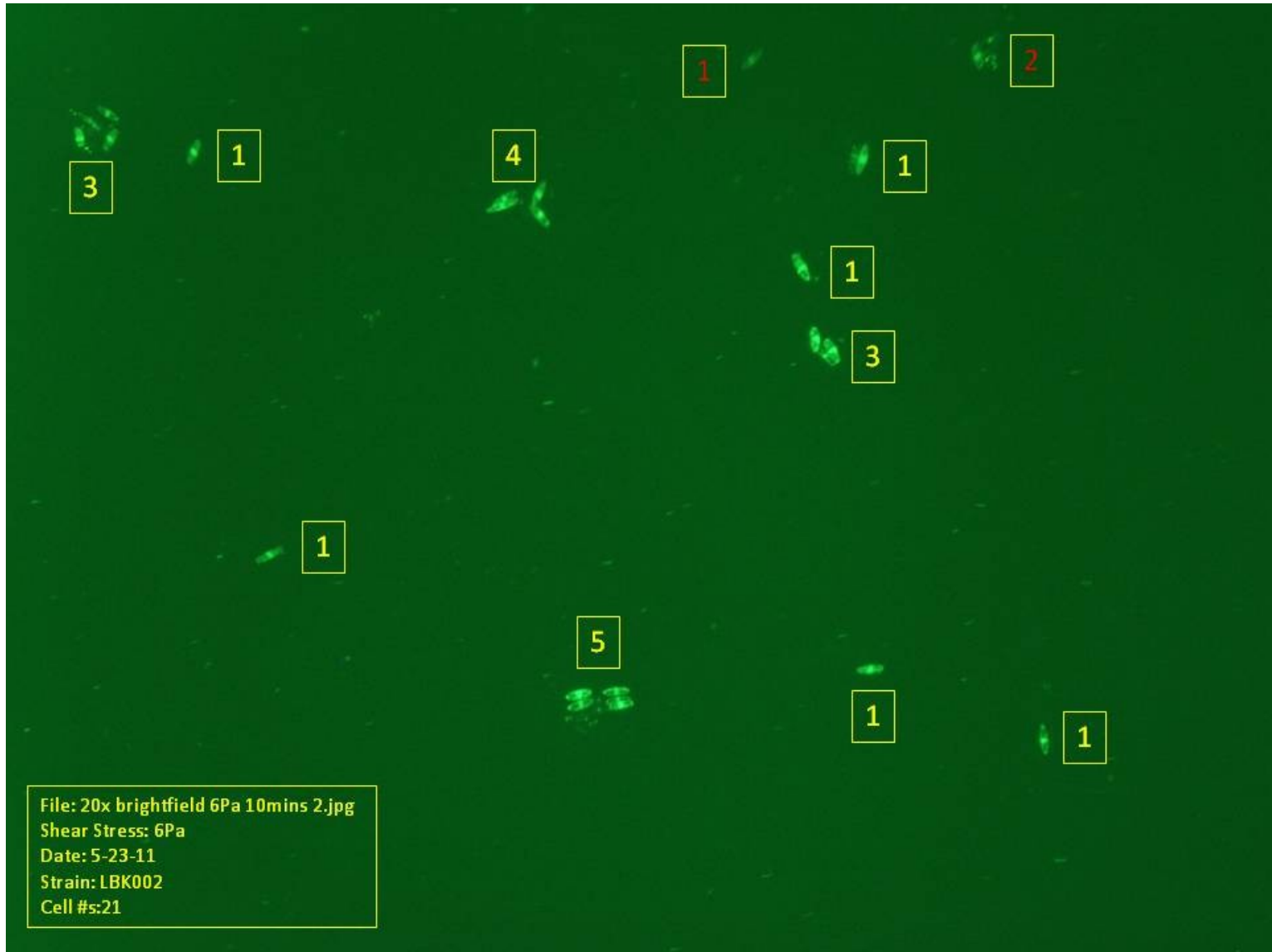




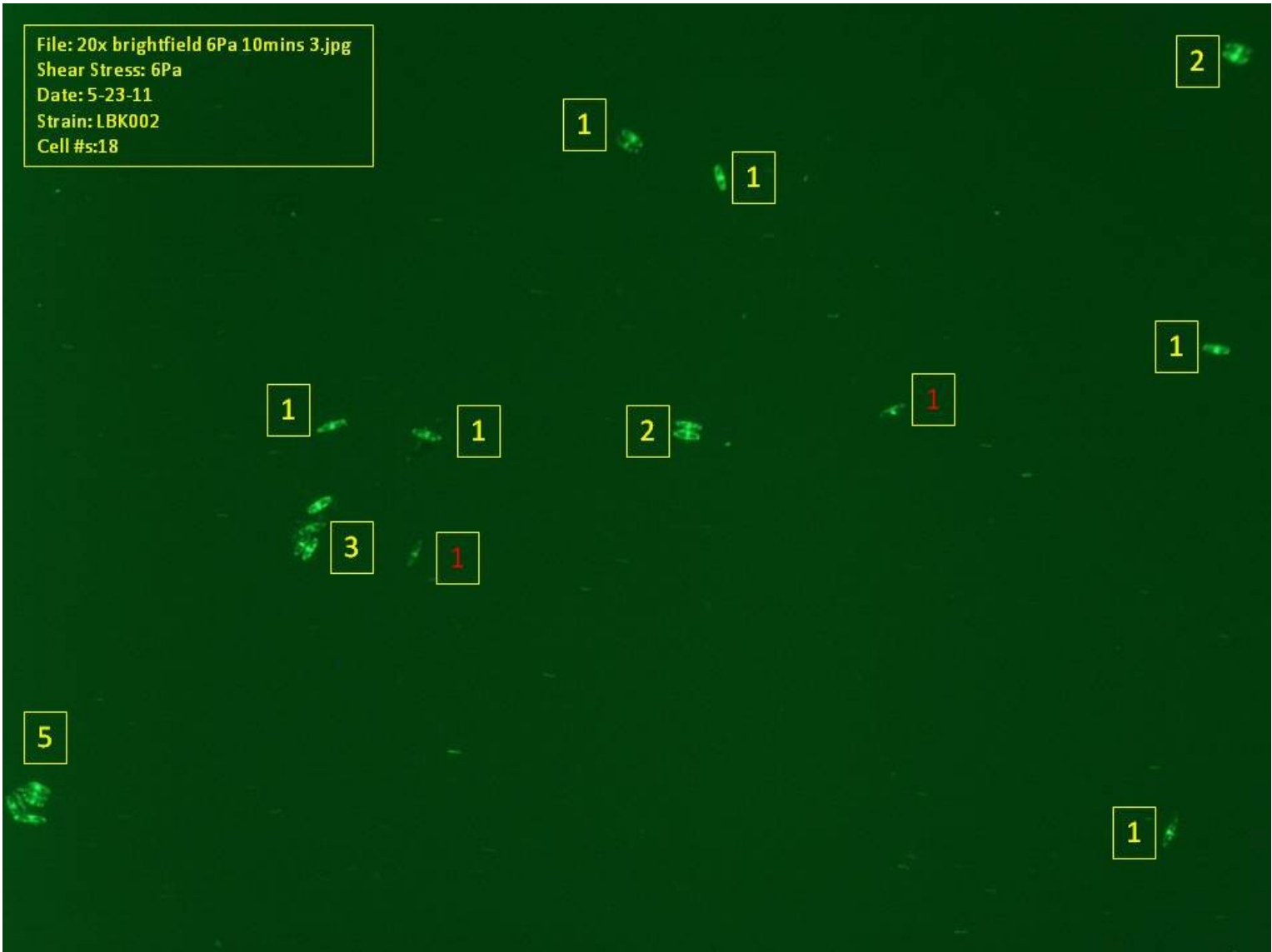


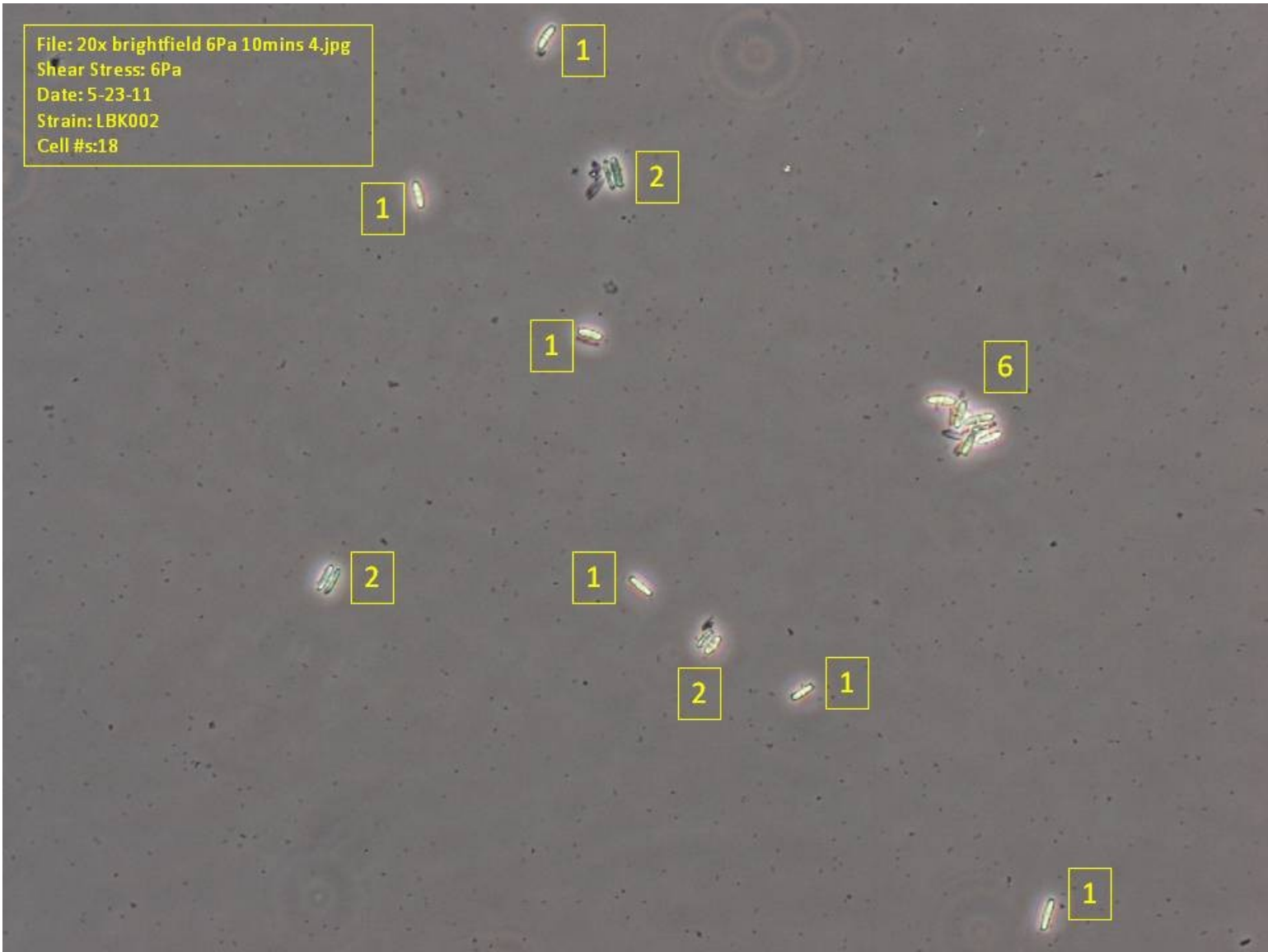




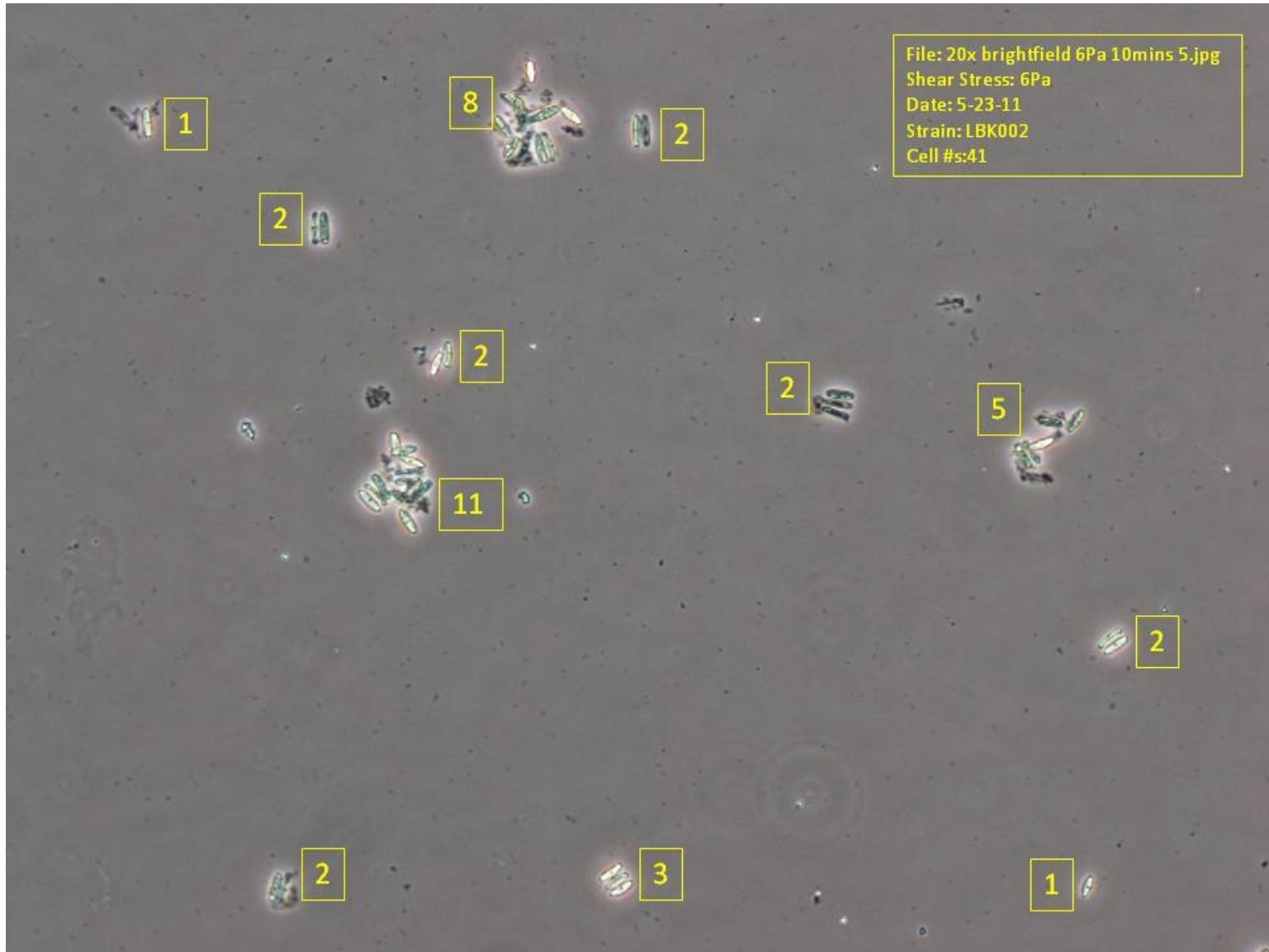




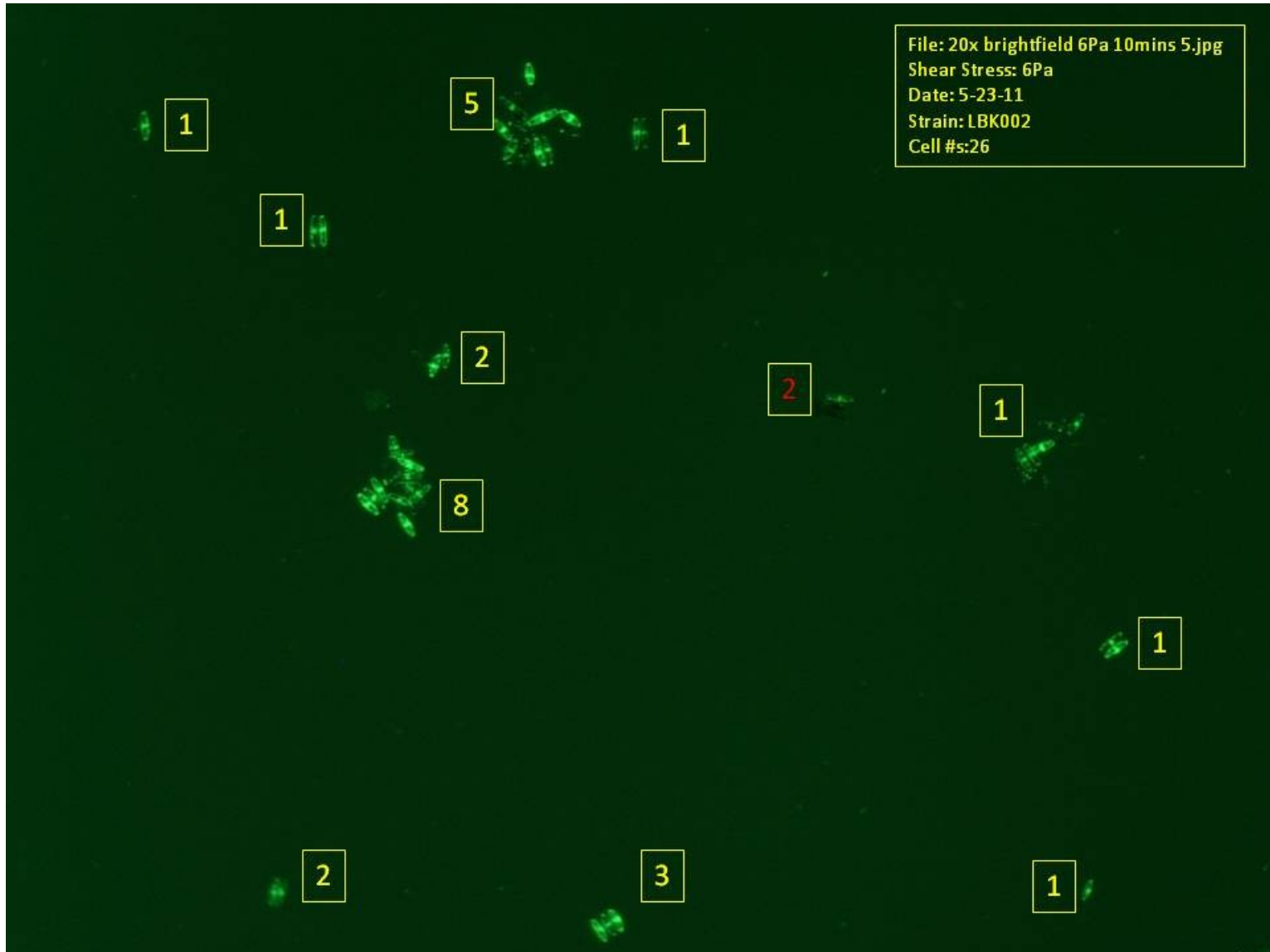






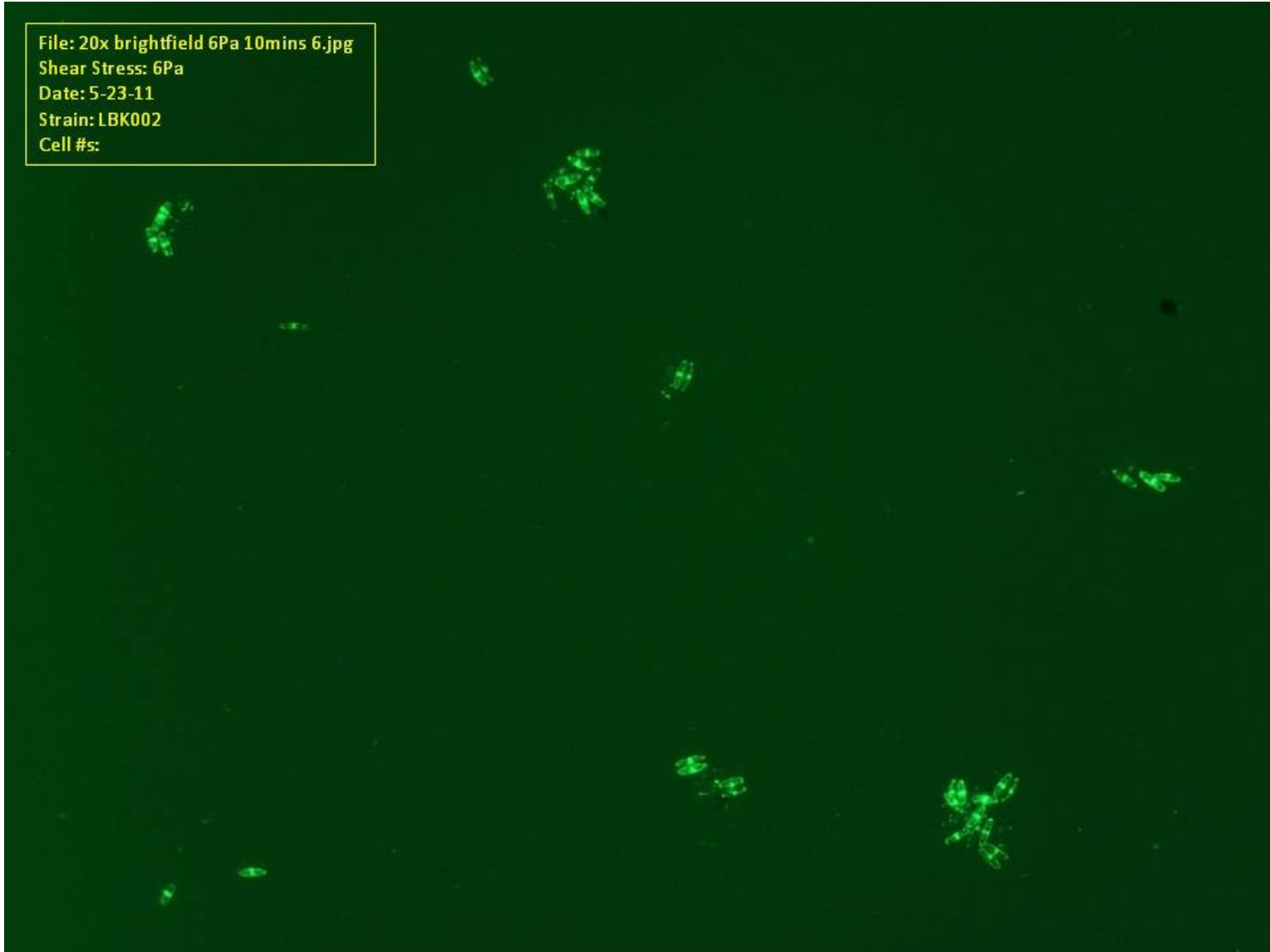








File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-23-11  
Strain: LBK002  
Cell #s:

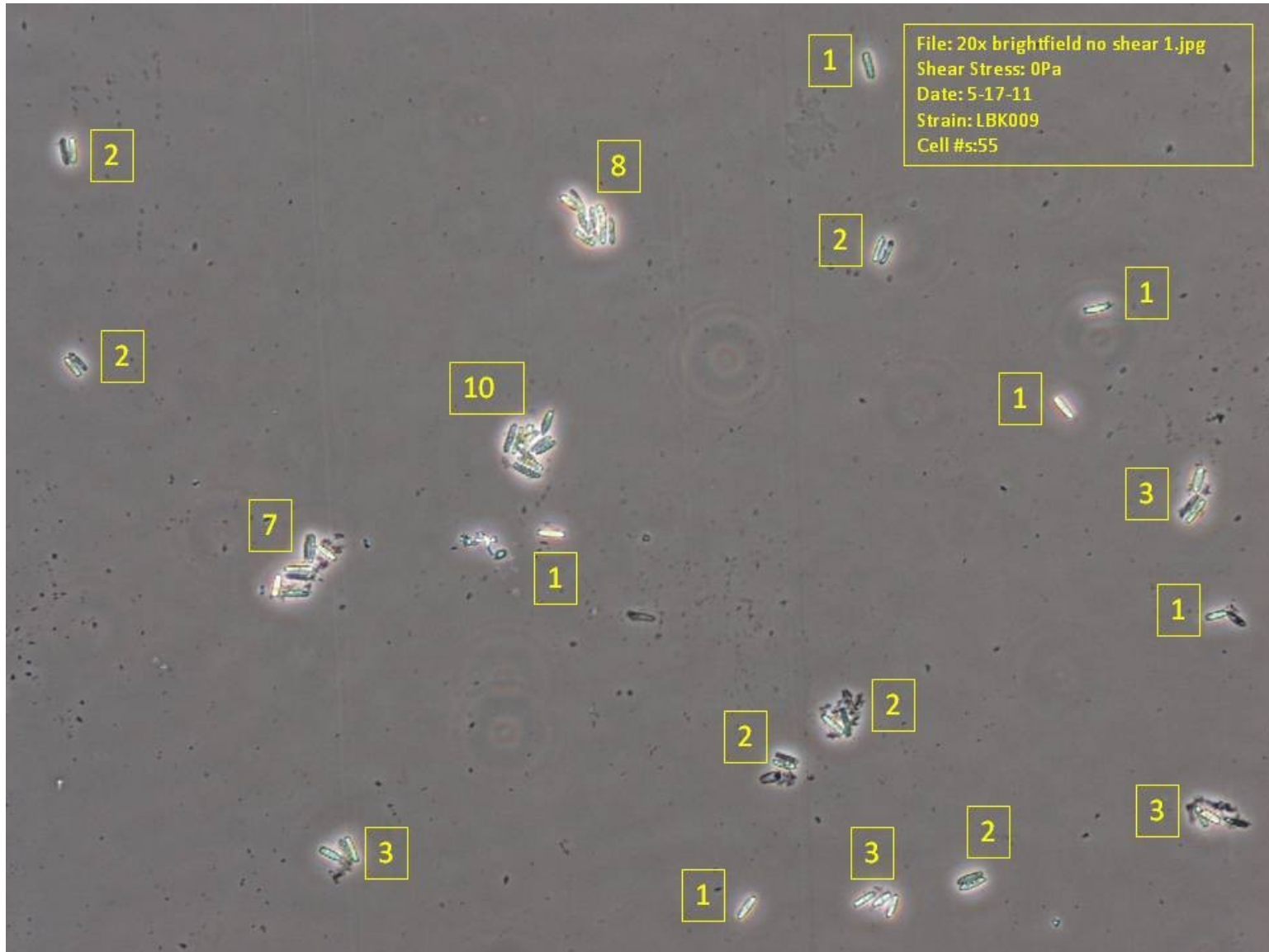


## APPENDIX C

The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK009, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	55	44	23	19	30	26	42	30	38	27	26	19	77.10%	0.89%	-0.00149
	6	24	19	27	20			47	36	59	38	70	60	76.21%		
Rep 2	0	49	41	52	41	37	28	44	36	24	22			81.55%	6.14%	-0.01023
	6	52	38	90	71	30	25	26	19	42	28			75.42%		
Rep 3	0	62	55	48	35	46	35	27	22	40	31			79.82%	12.55%	-0.02091
	6	48	34	31	24	18	13	38	23			30	17	67.27%		
														<b>Avg.</b>	<b>6.53%</b>	
														<b>Std. Dev.</b>	<b>5.84%</b>	

The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.

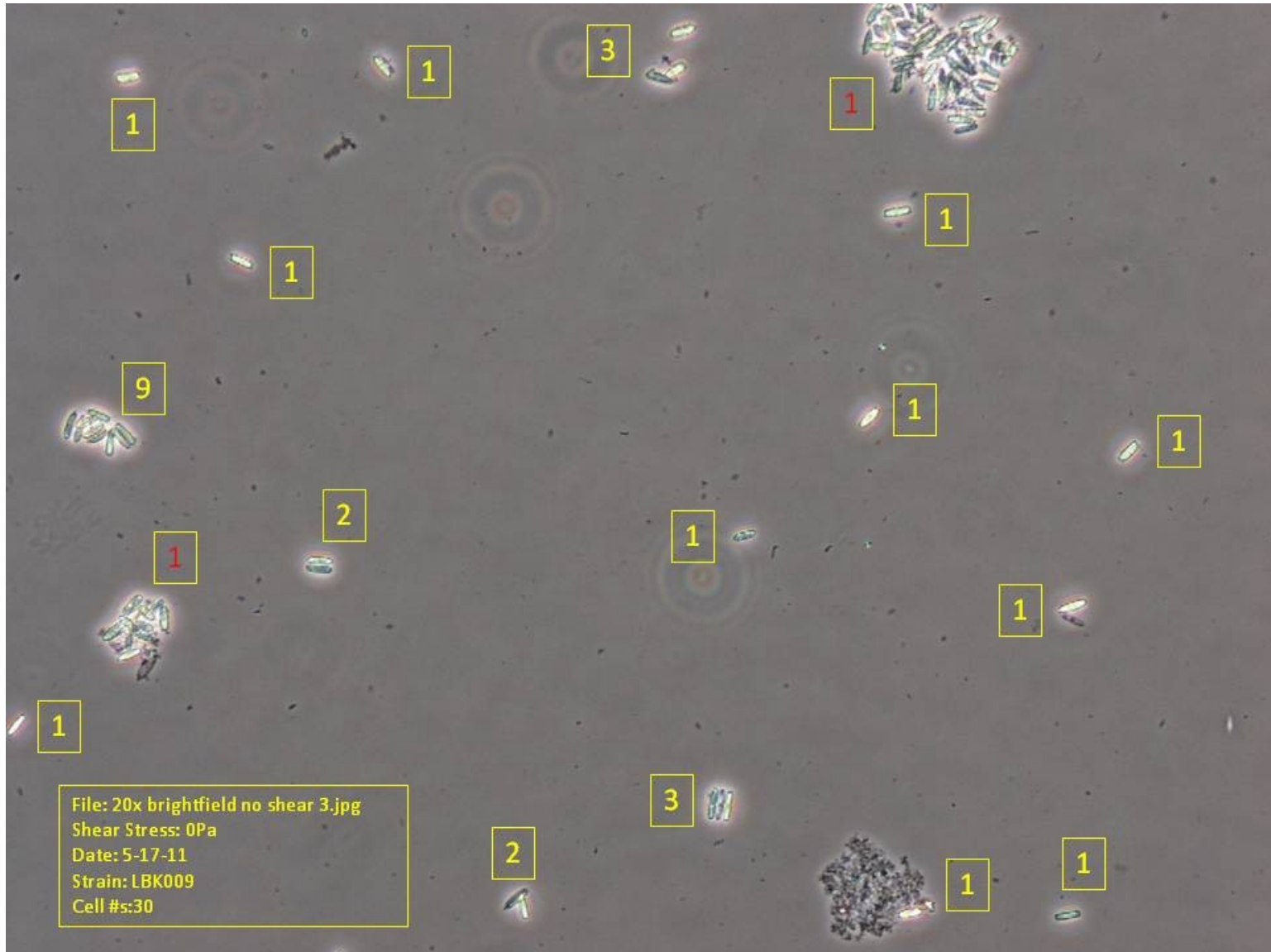




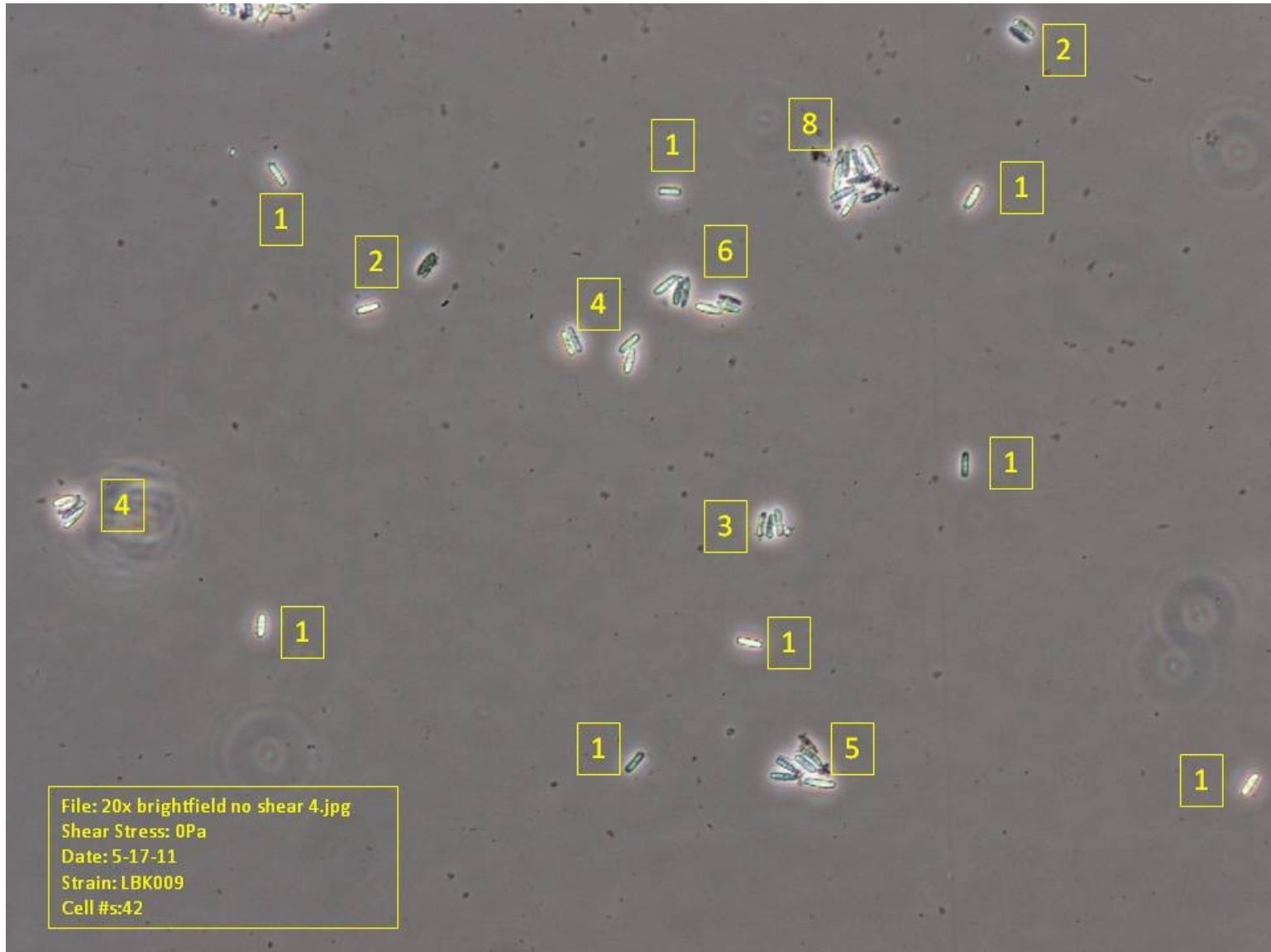


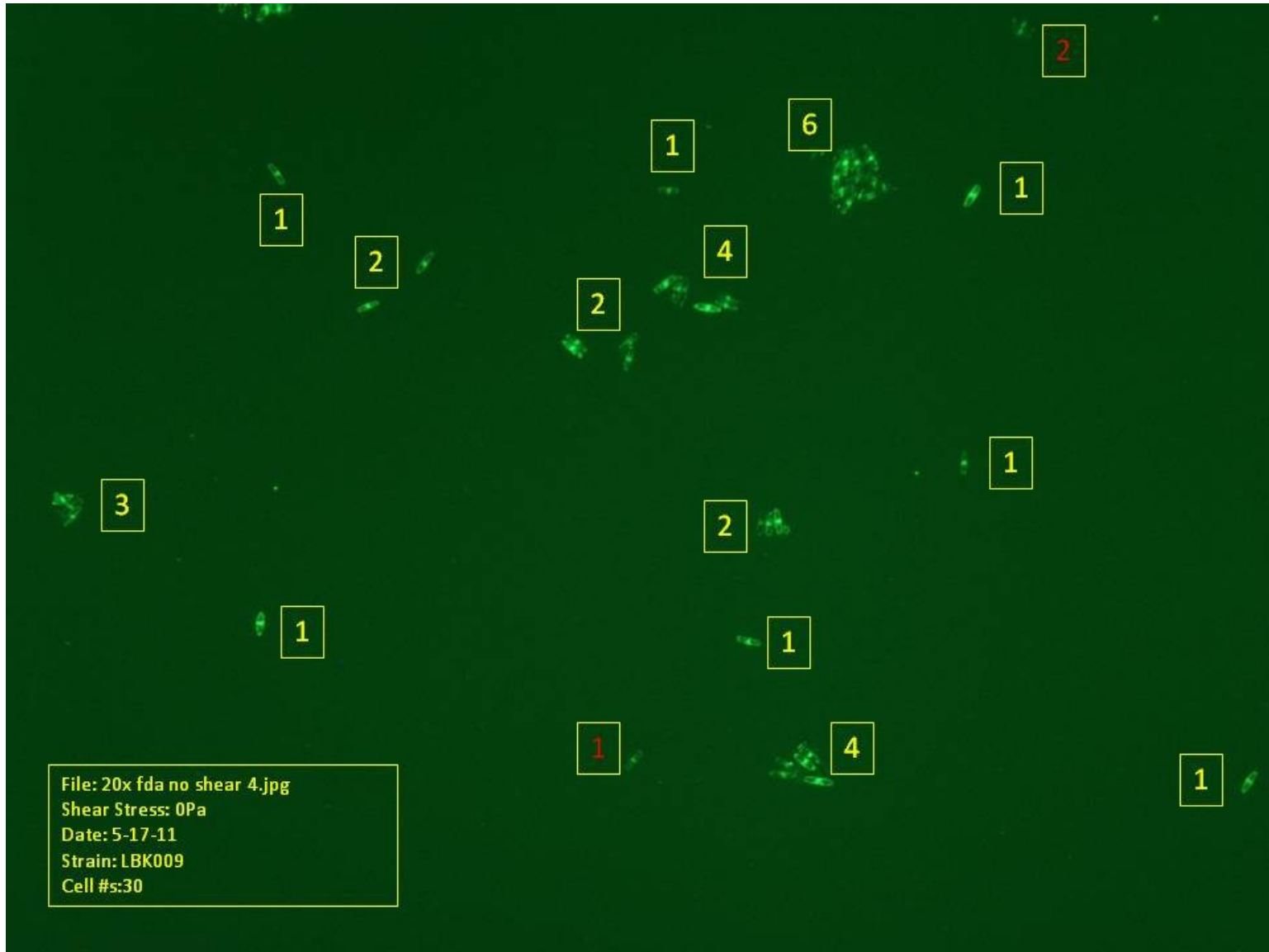


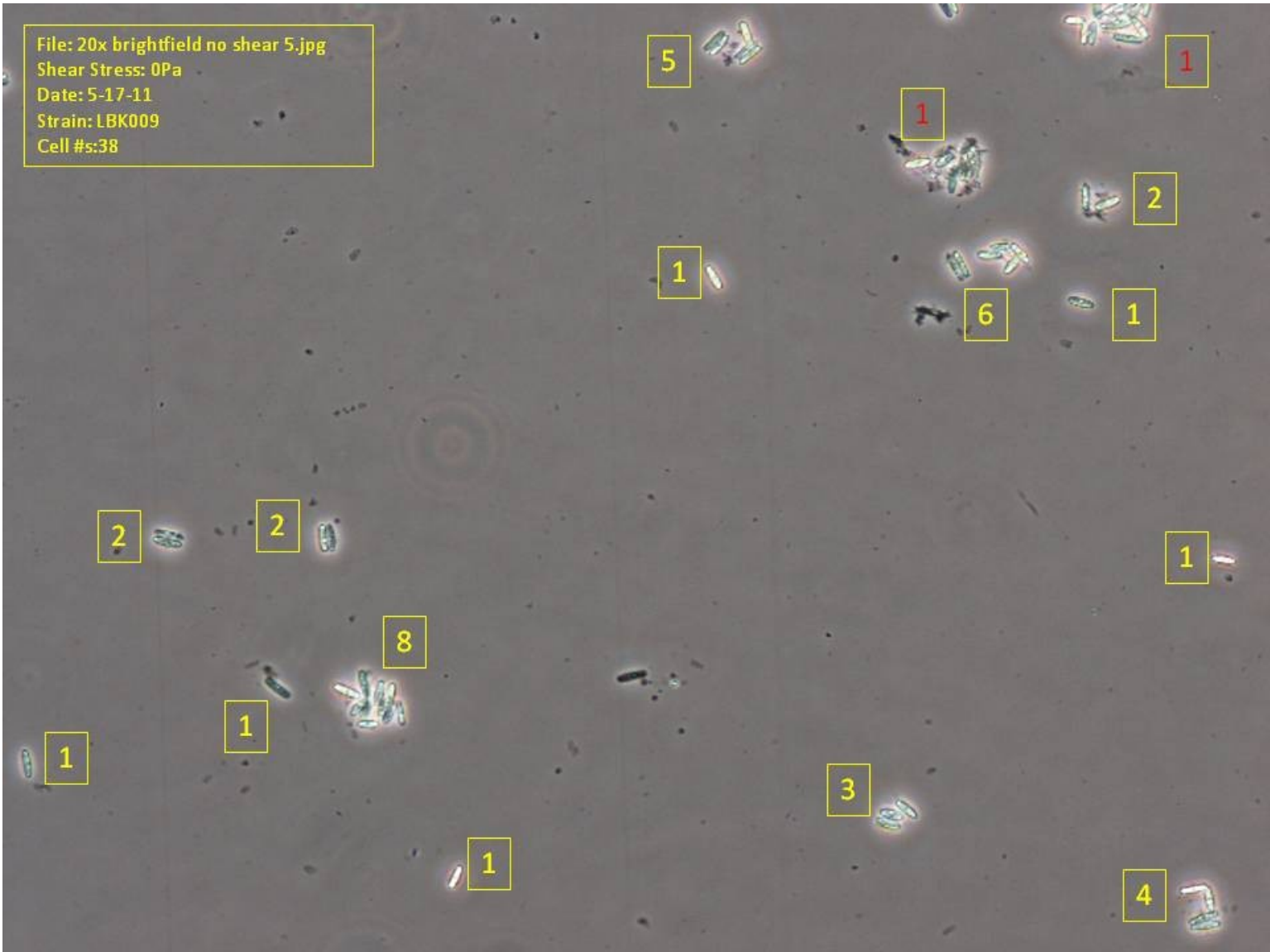


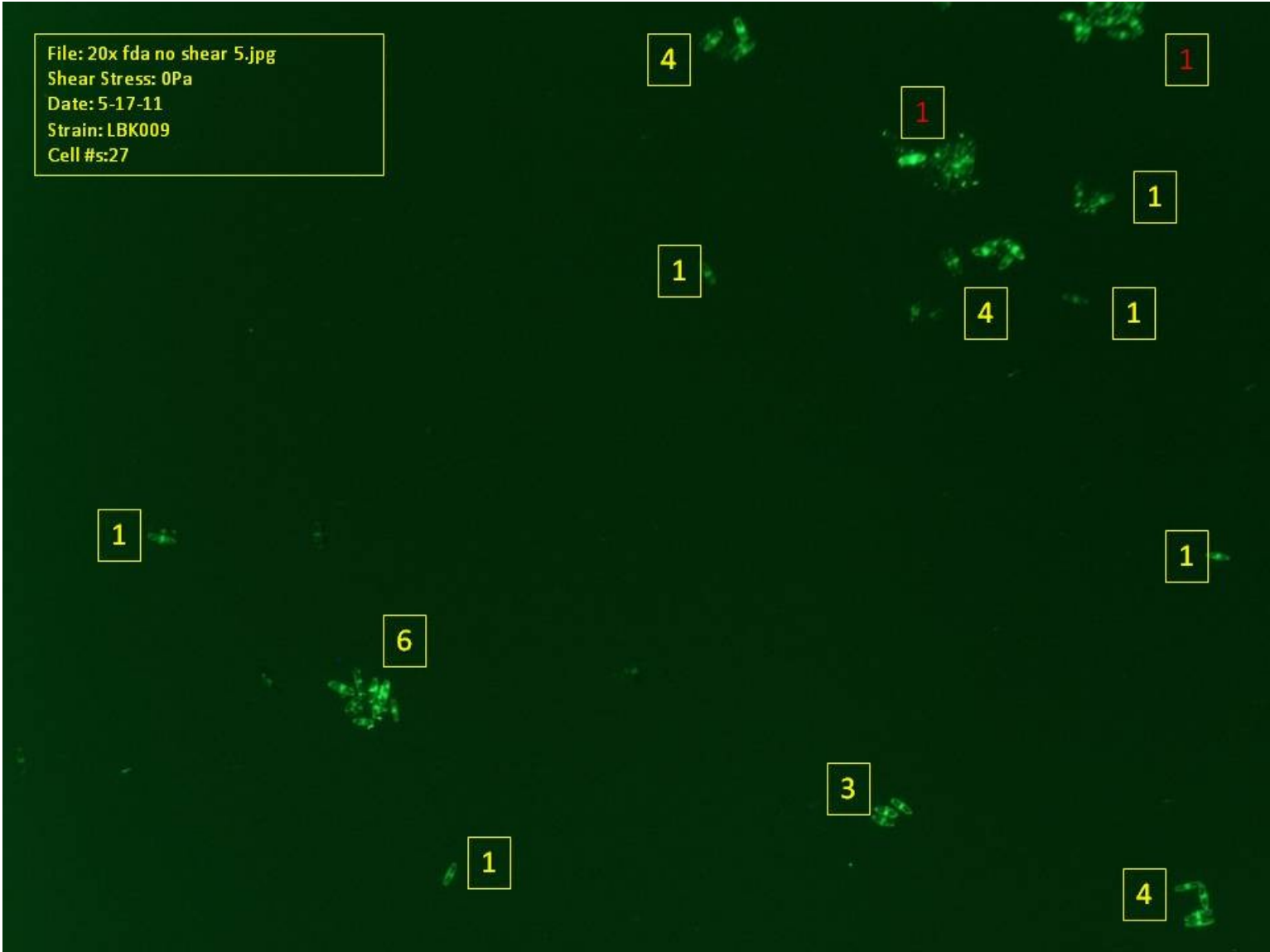


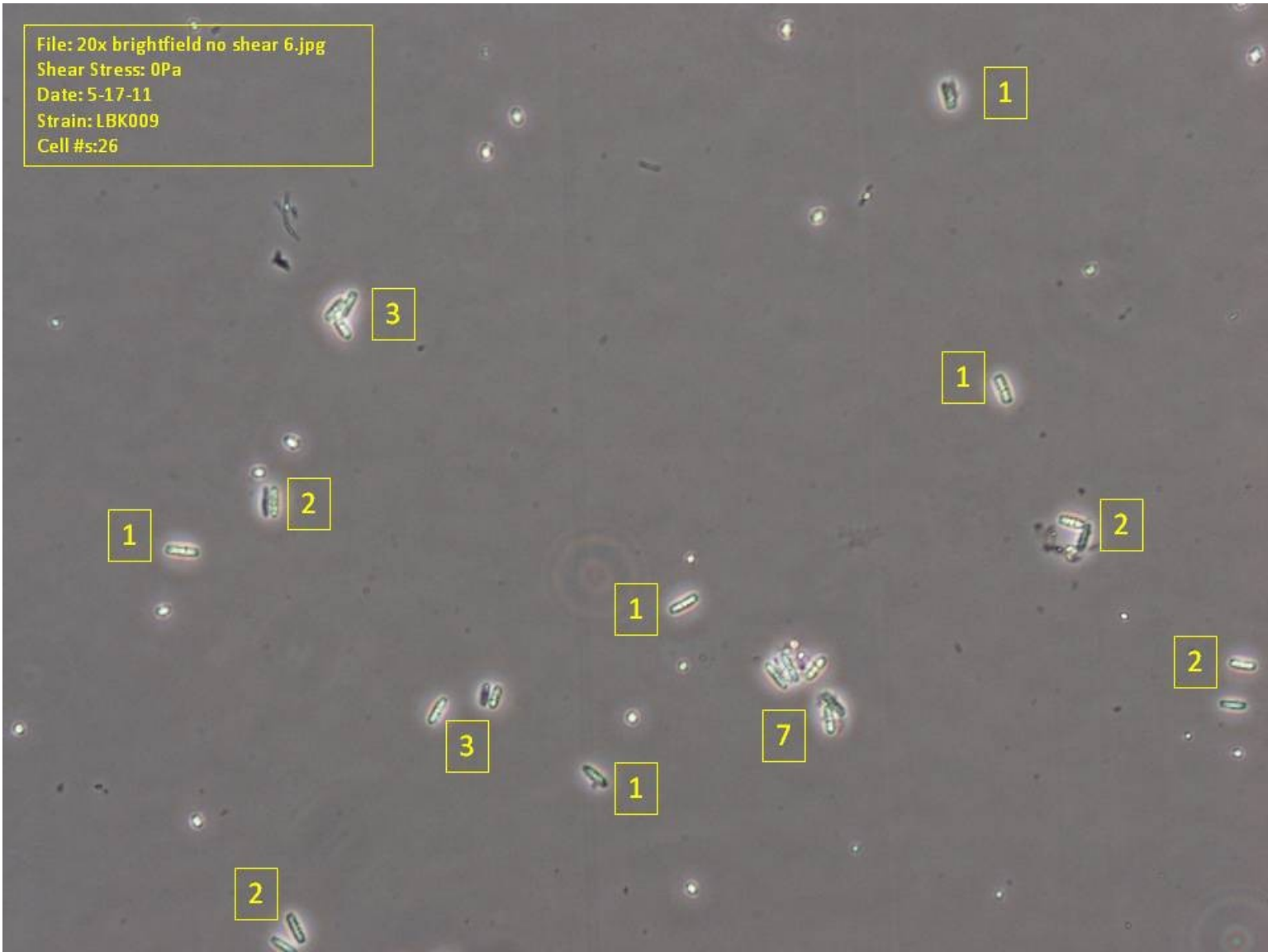








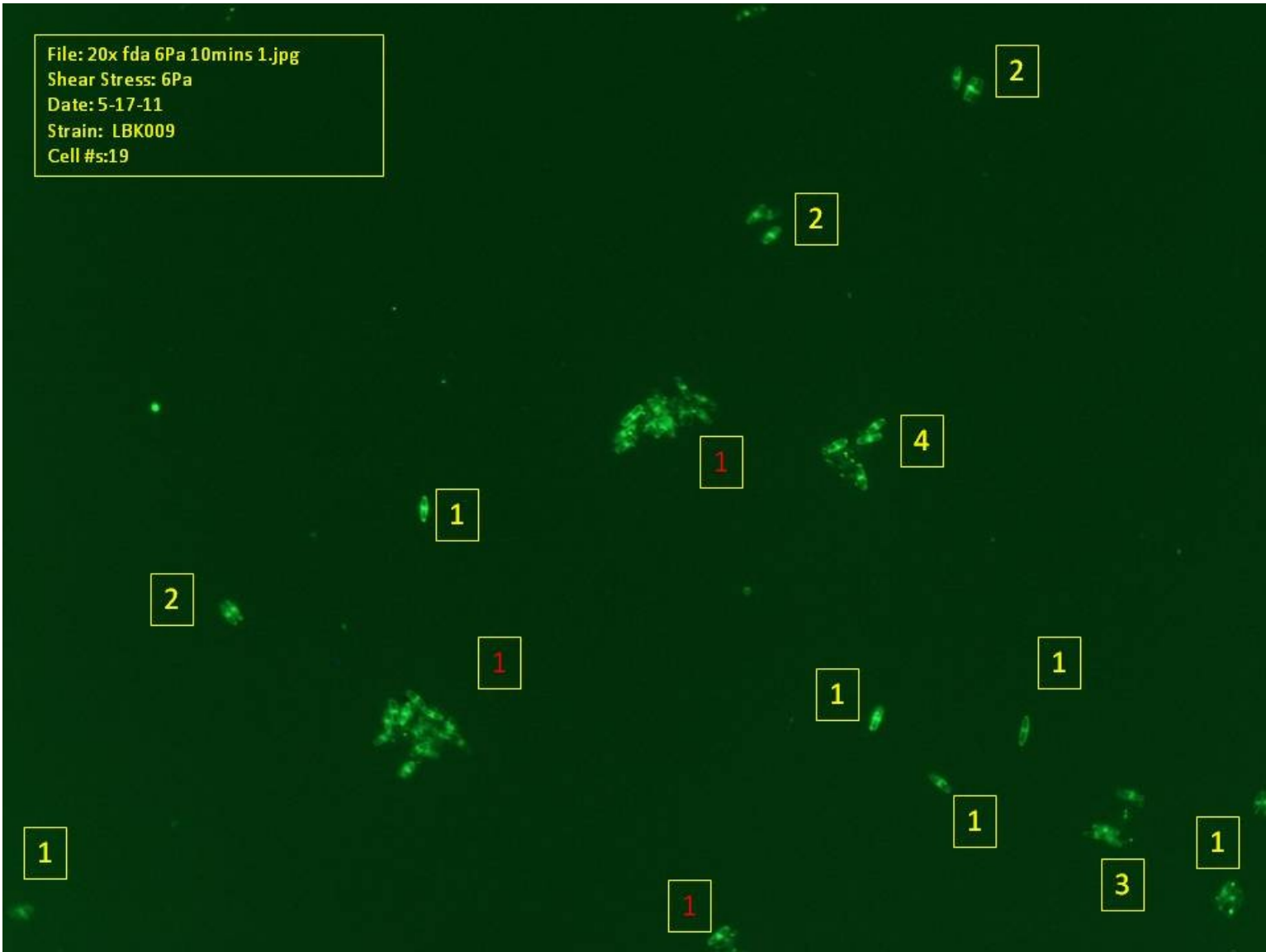


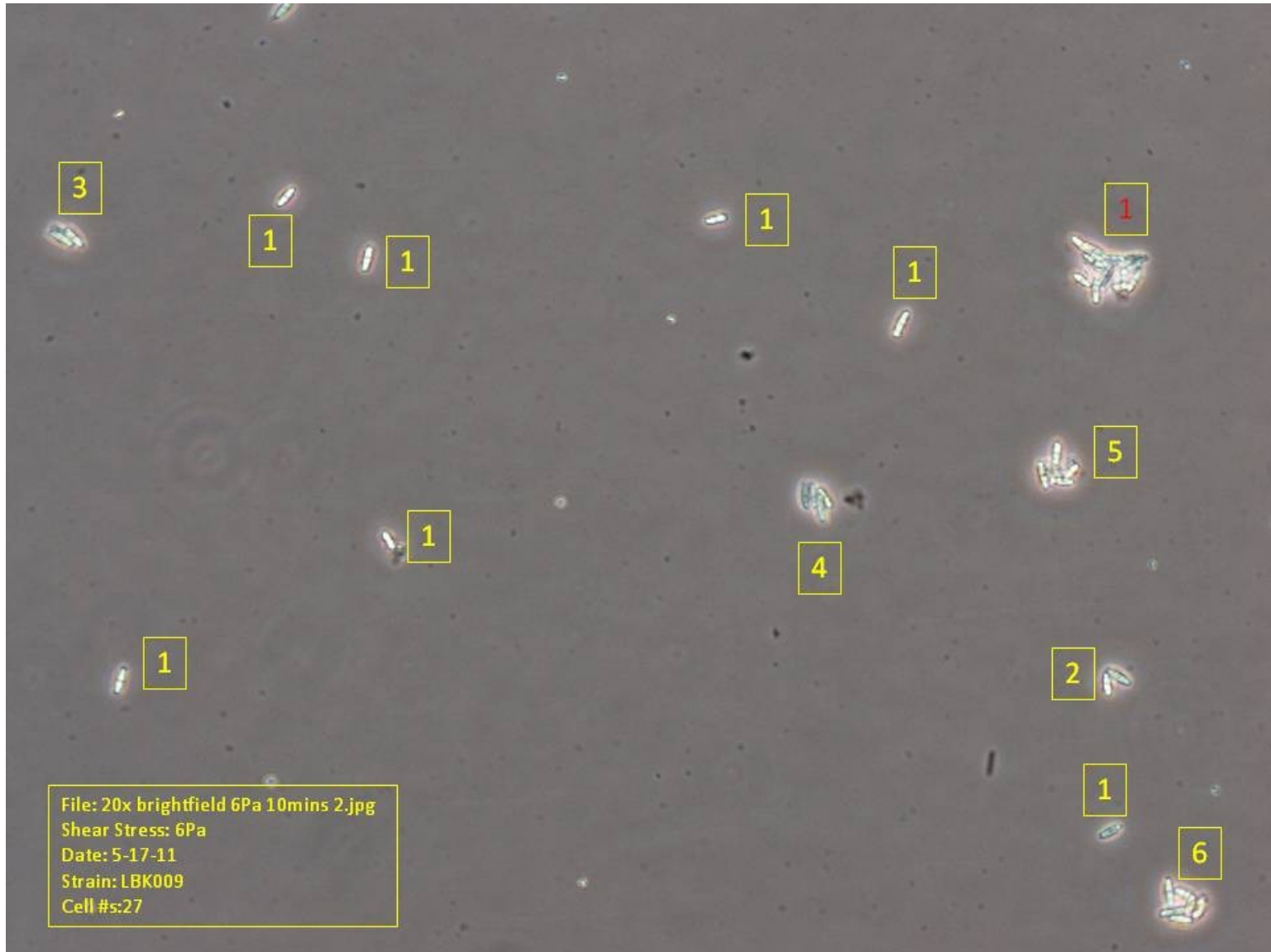




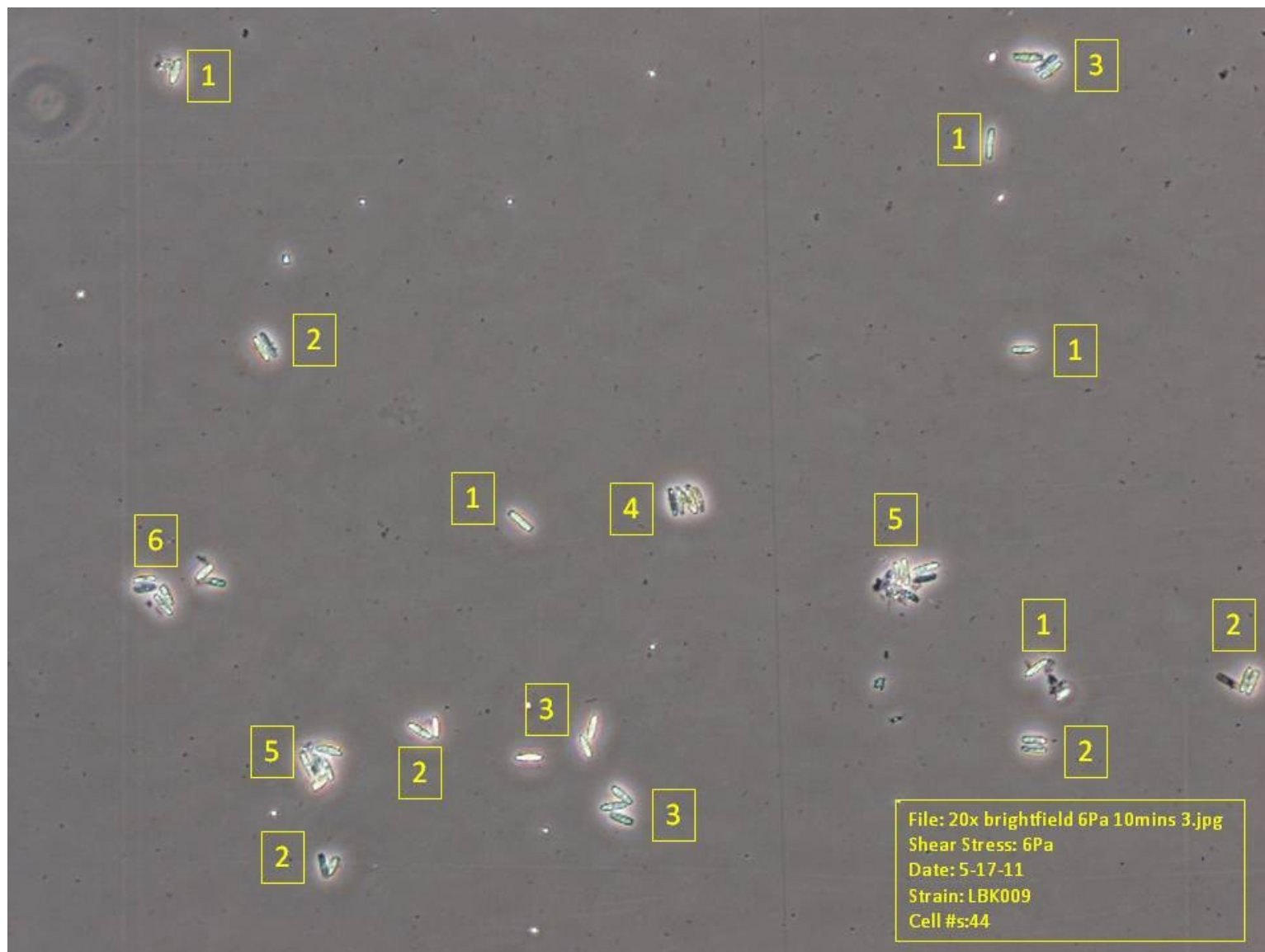


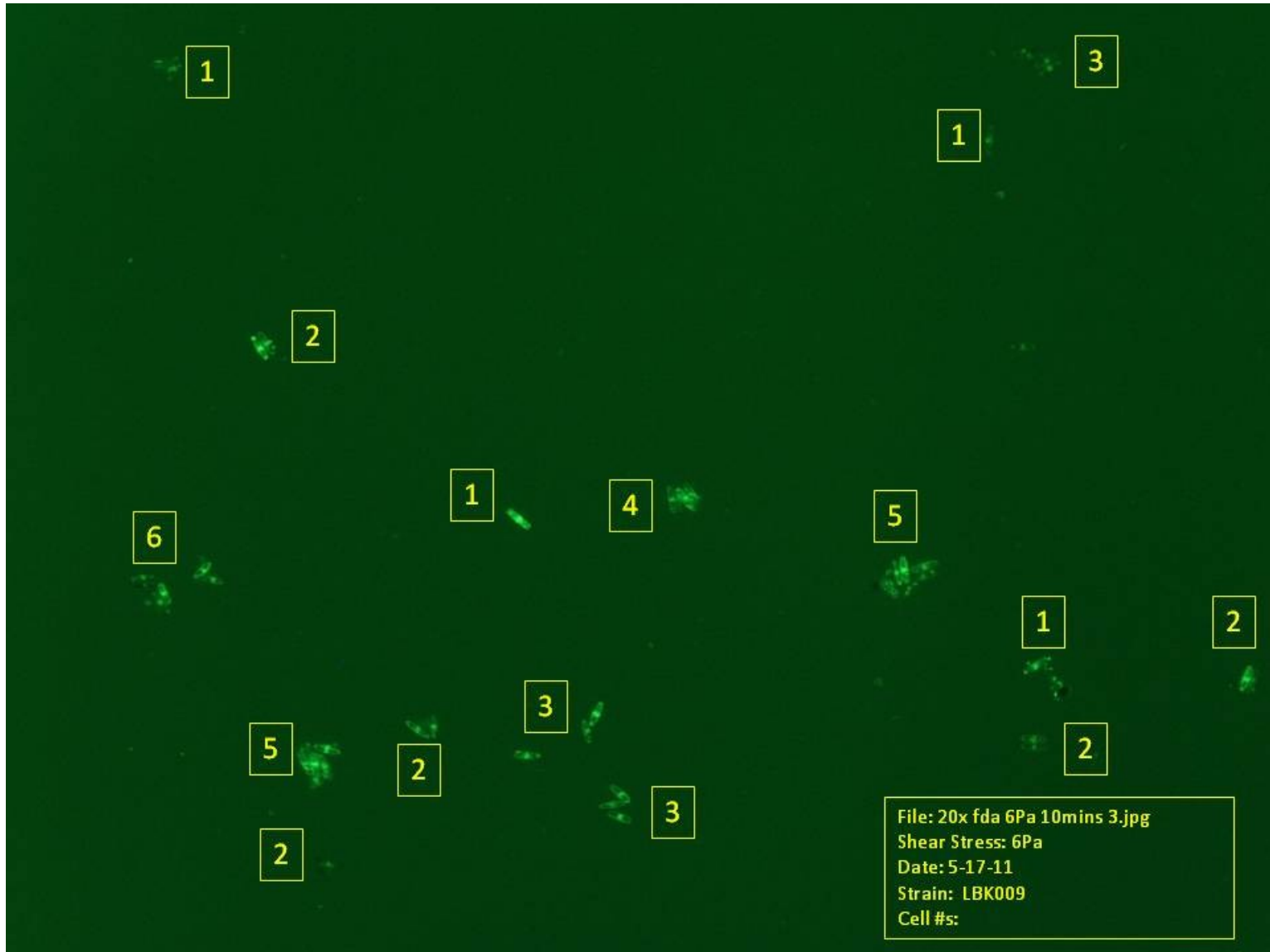


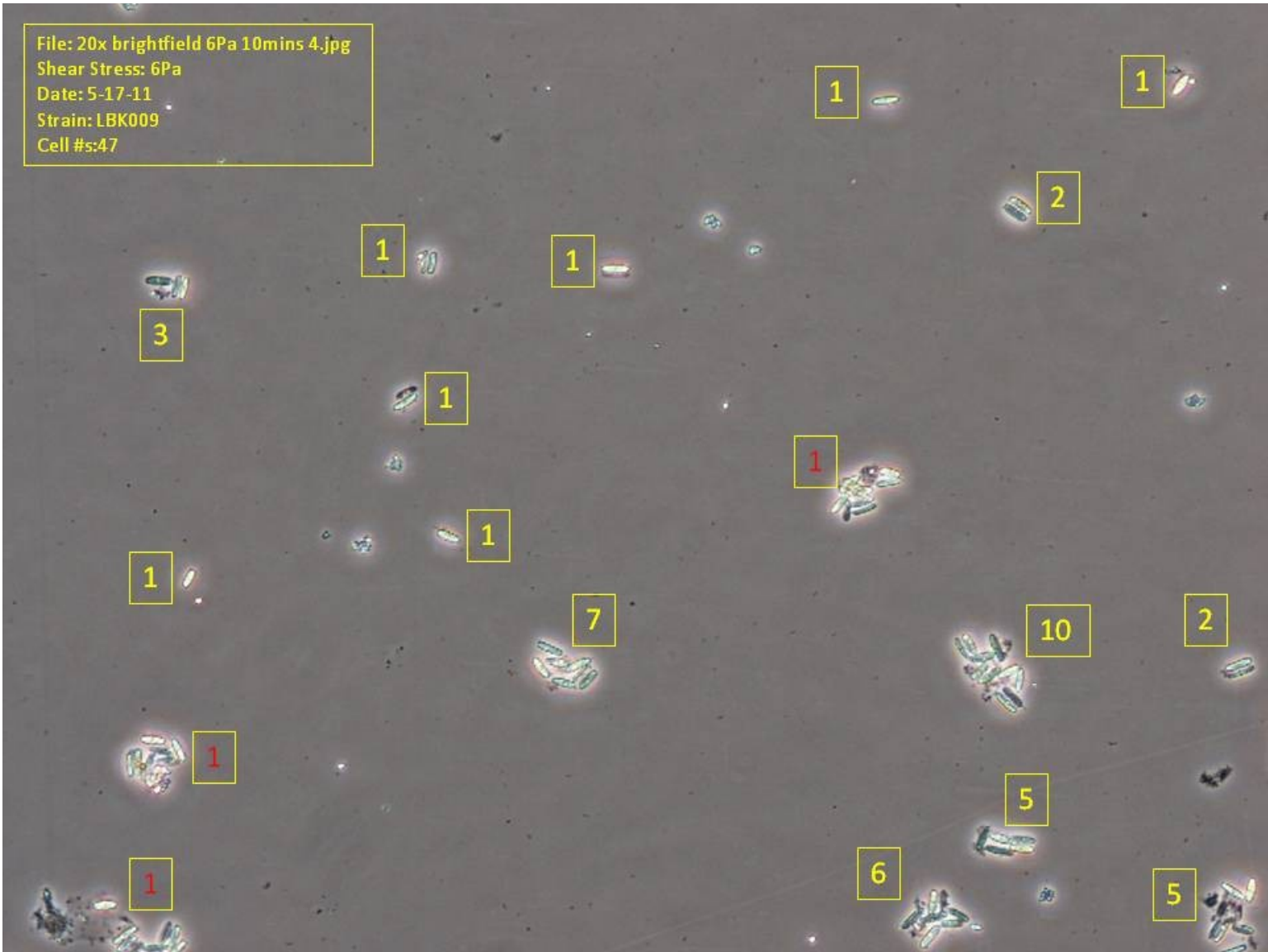








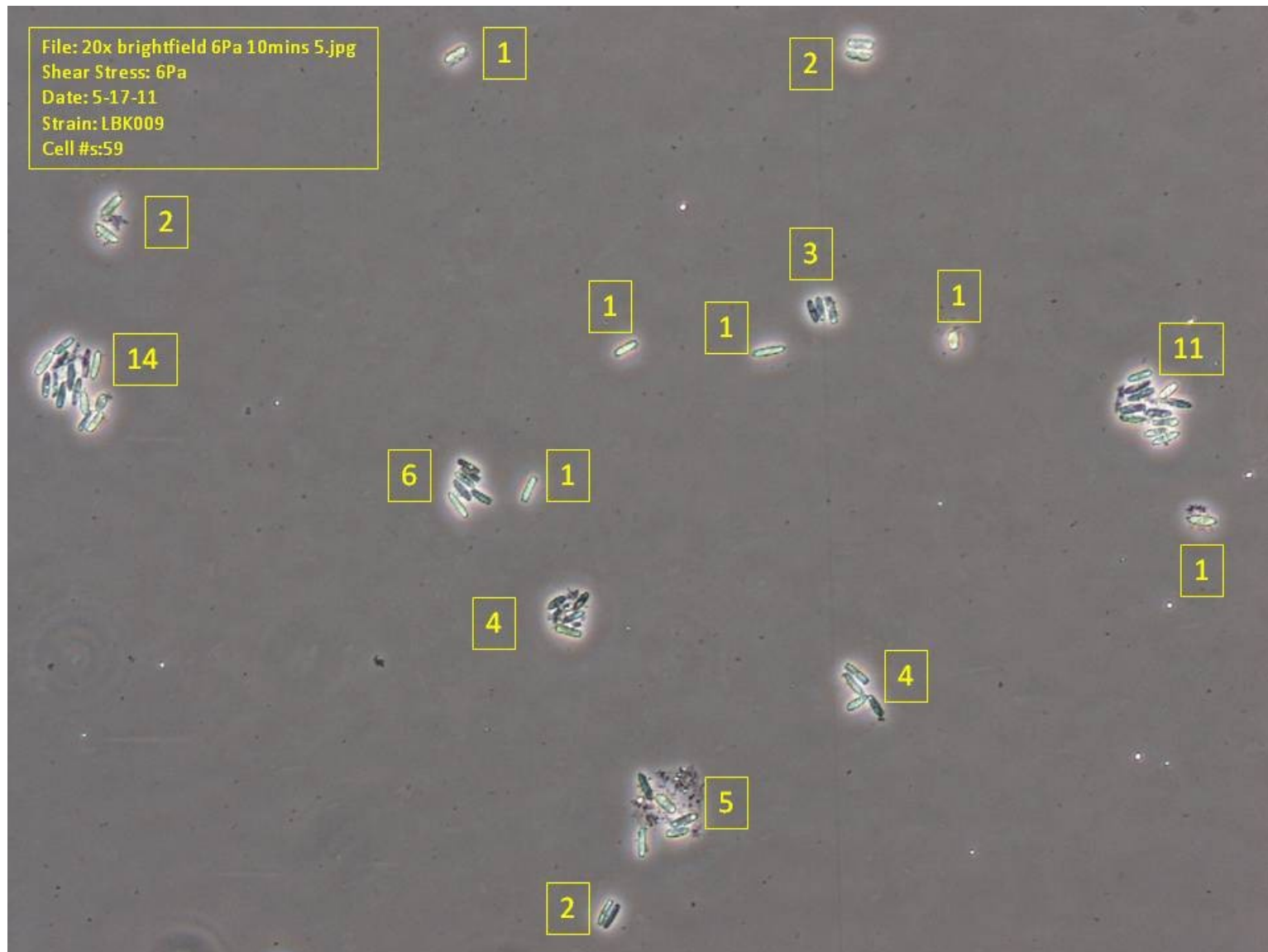




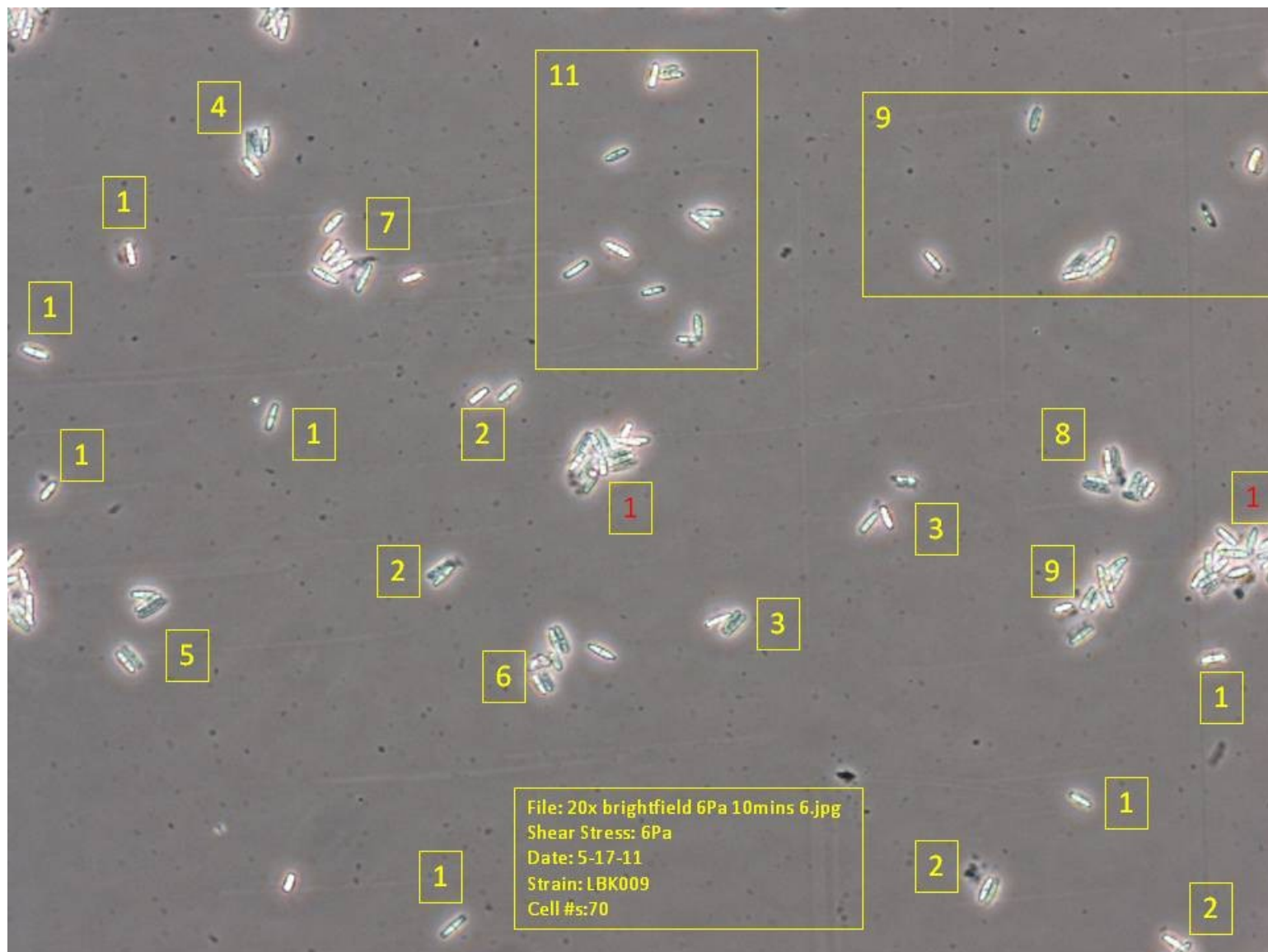


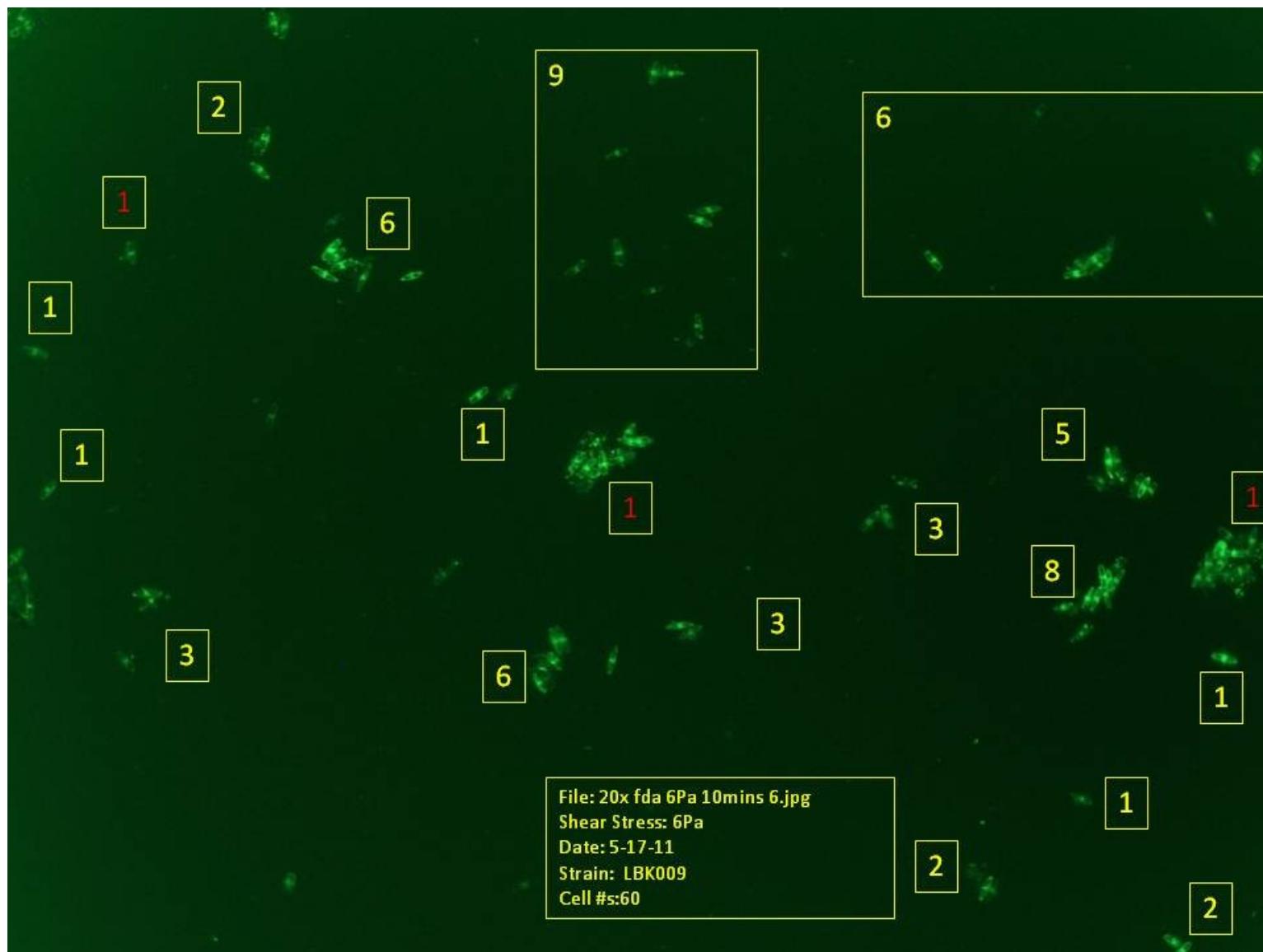


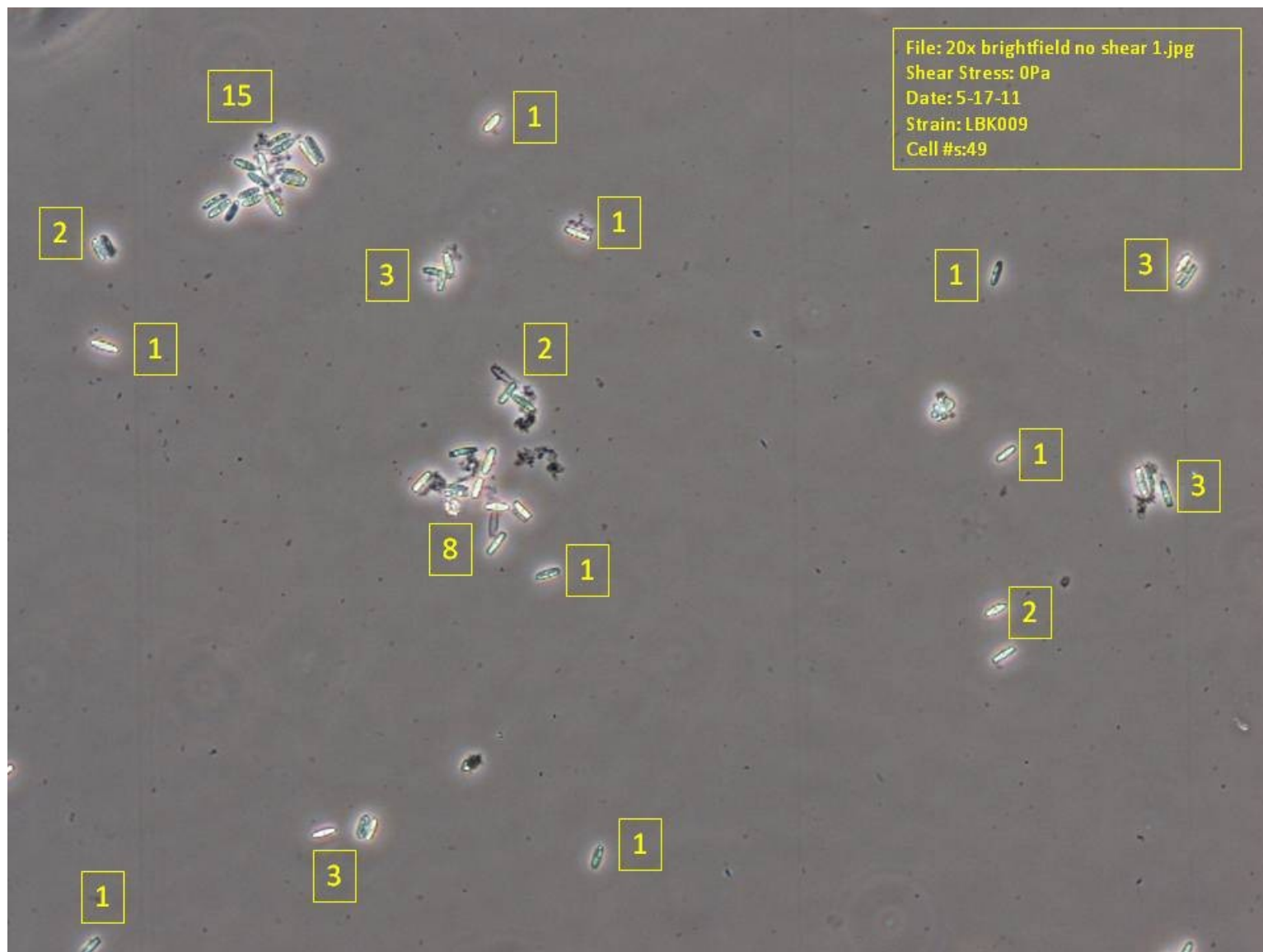
File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-17-11  
Strain: LBK009  
Cell #s:59

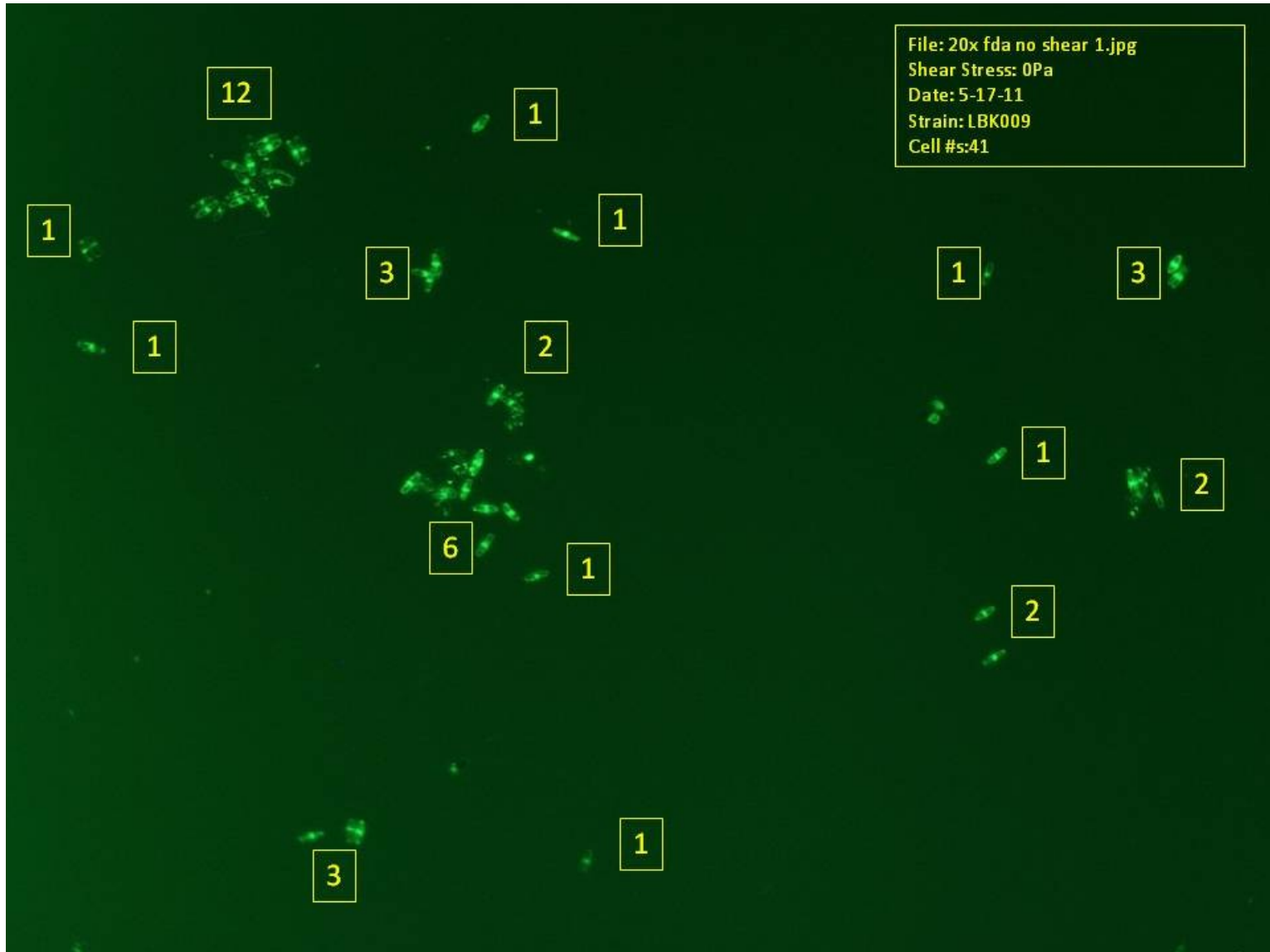


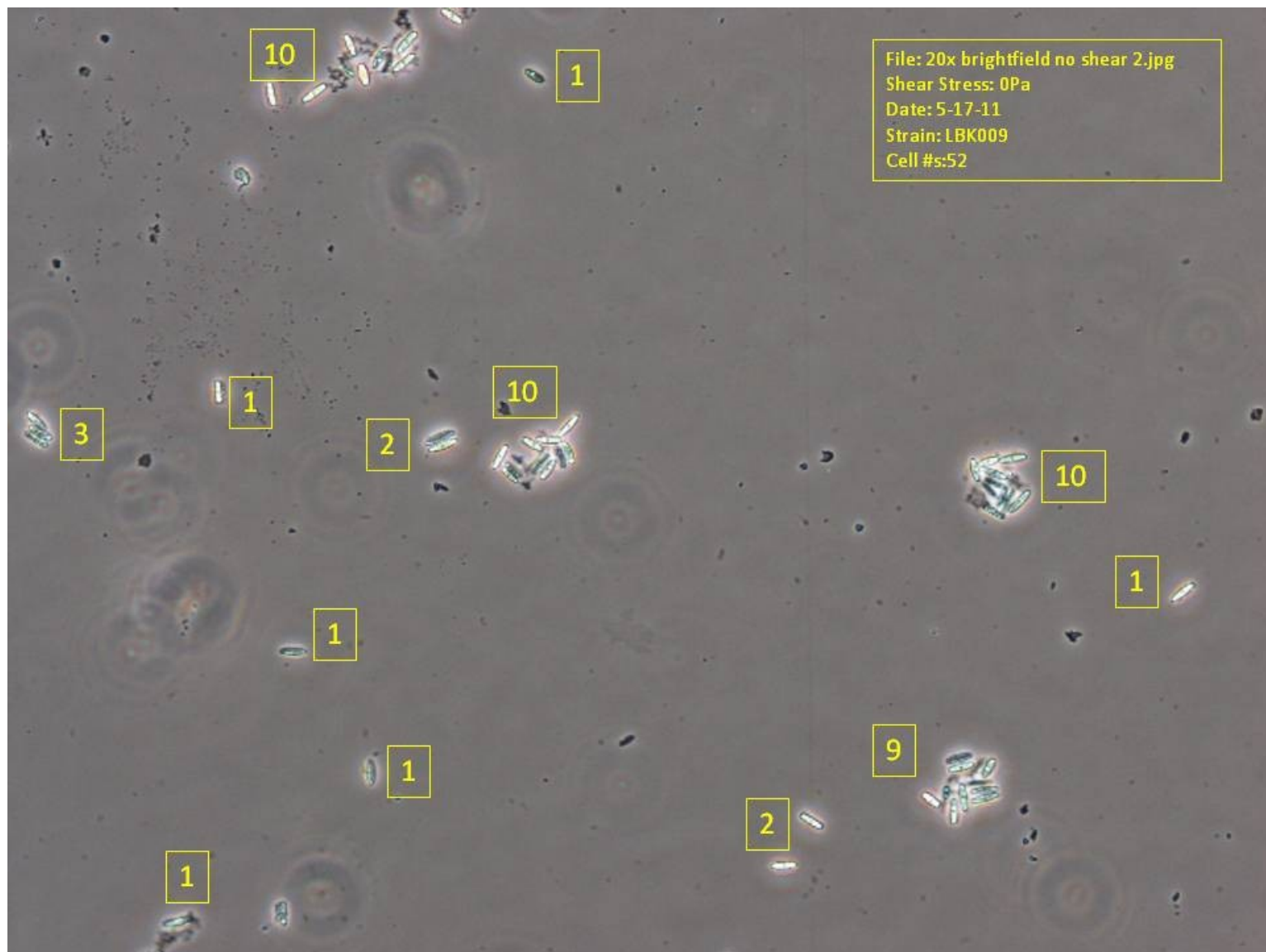






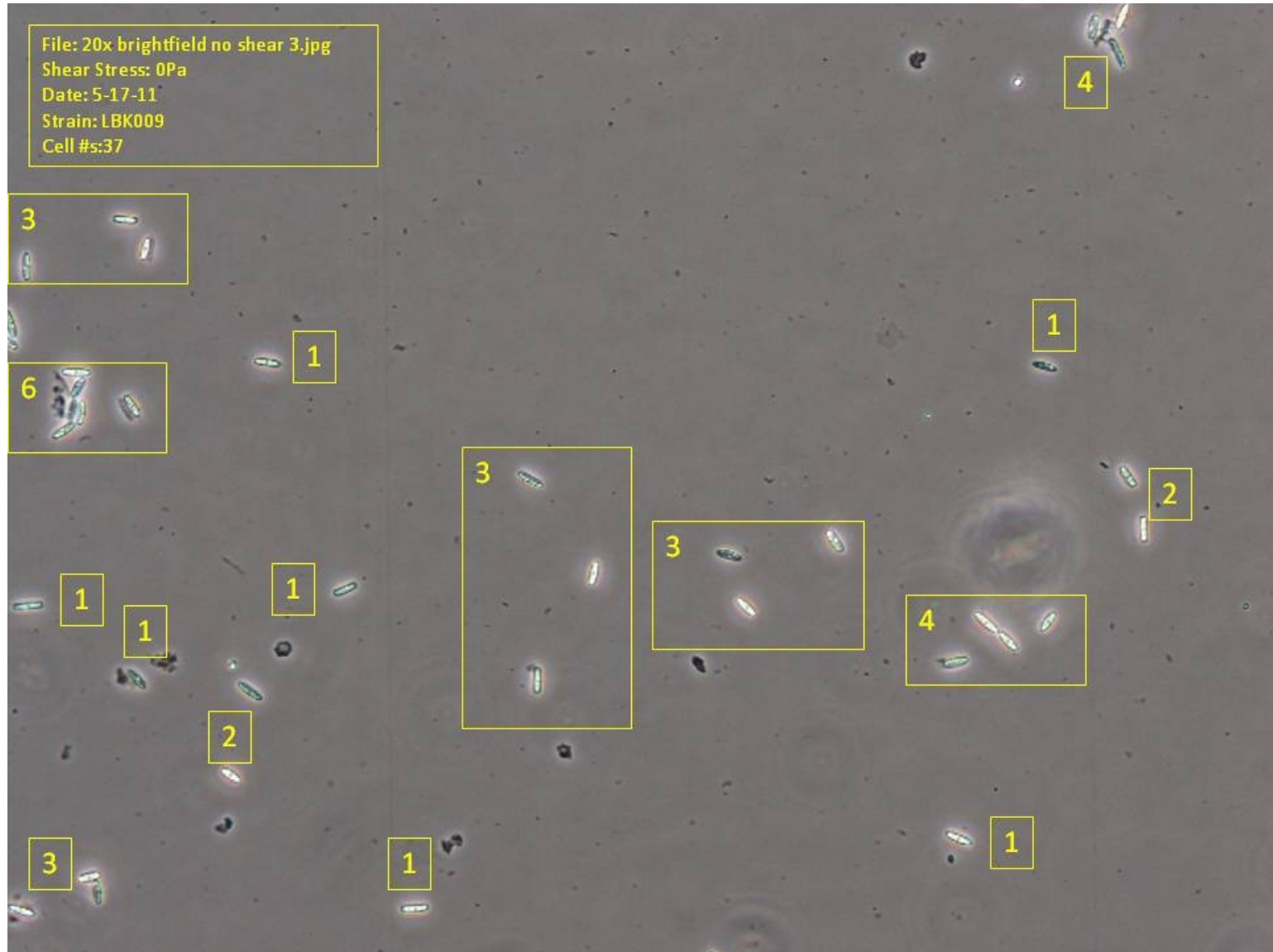


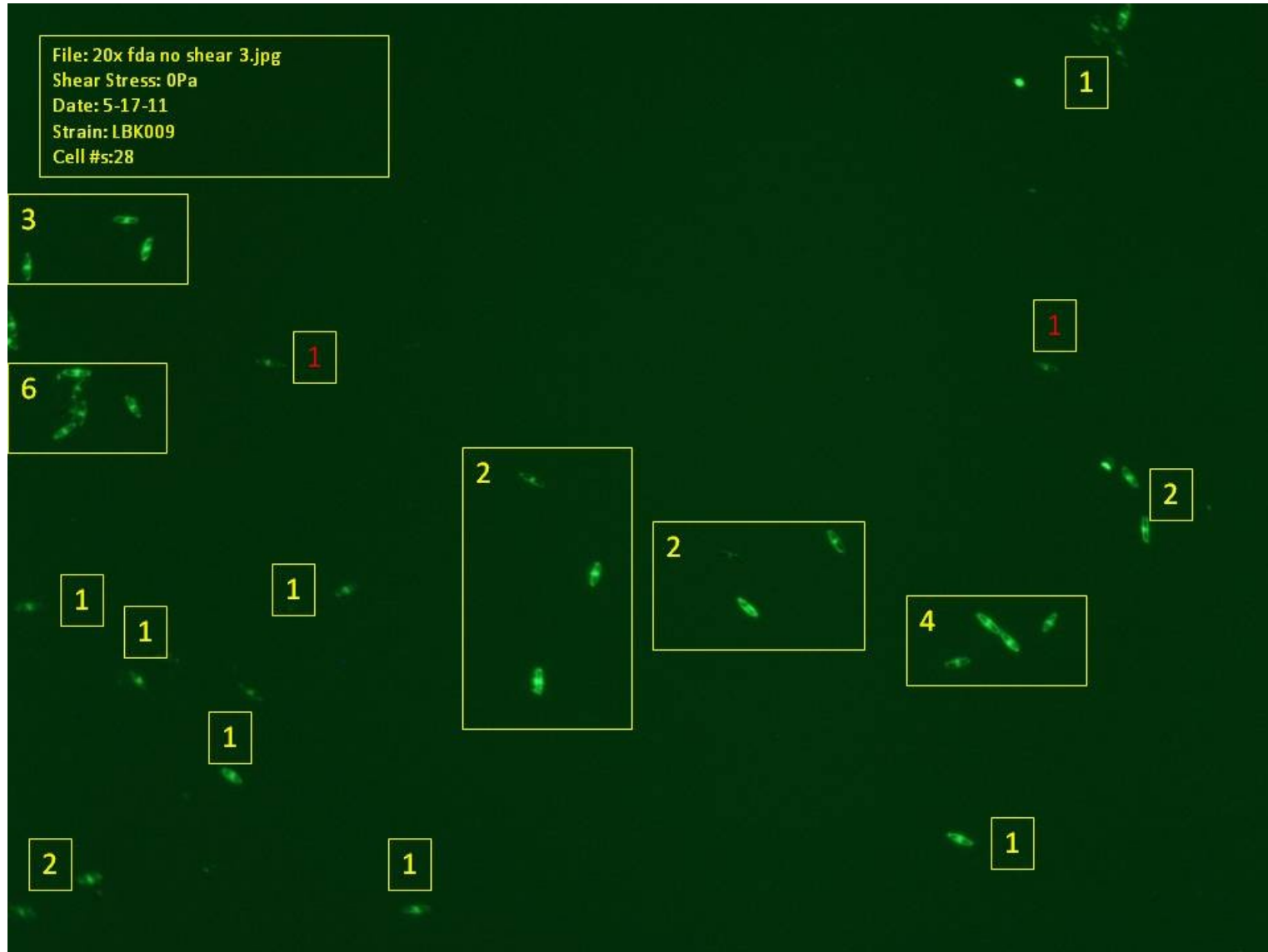


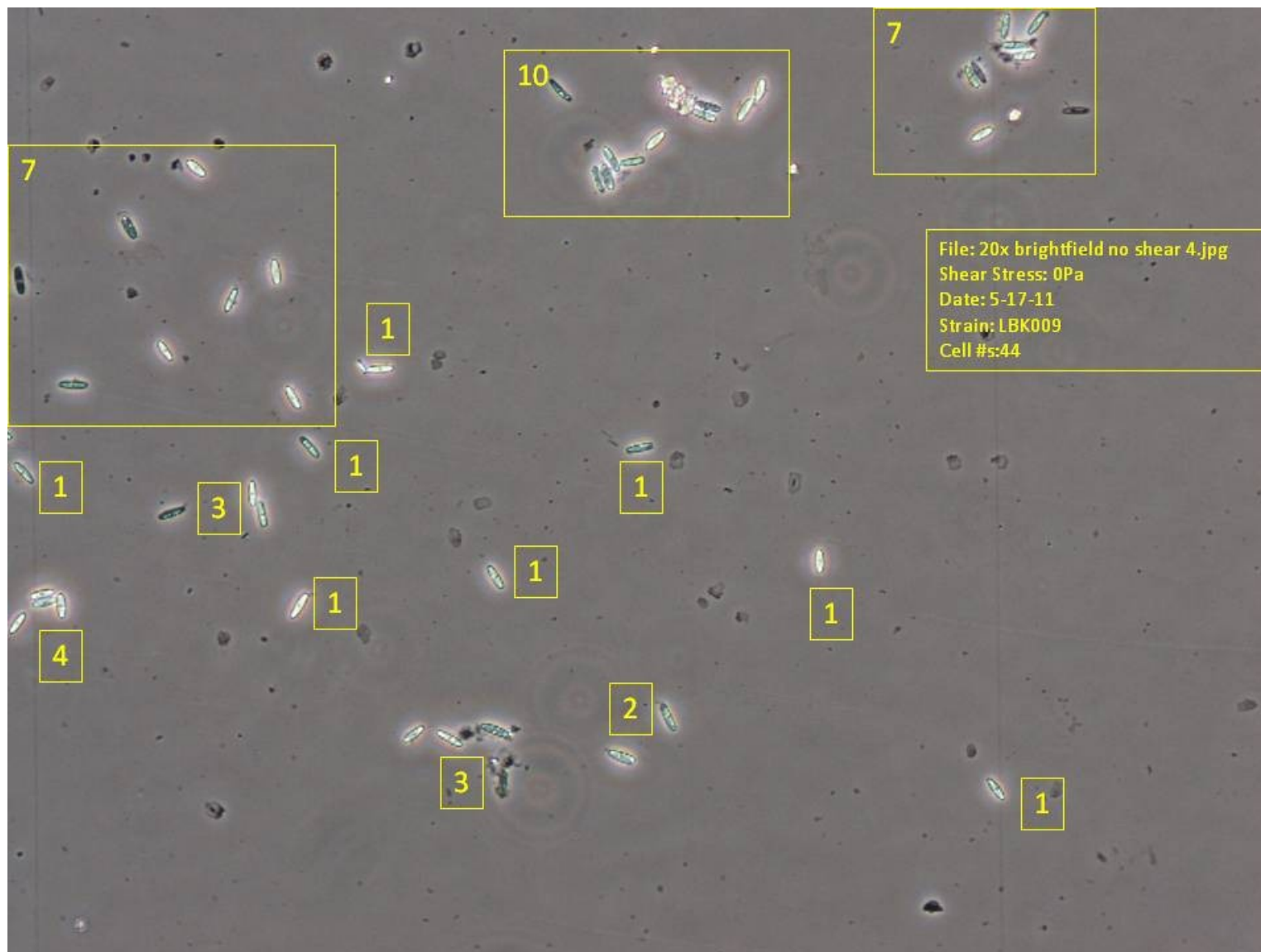


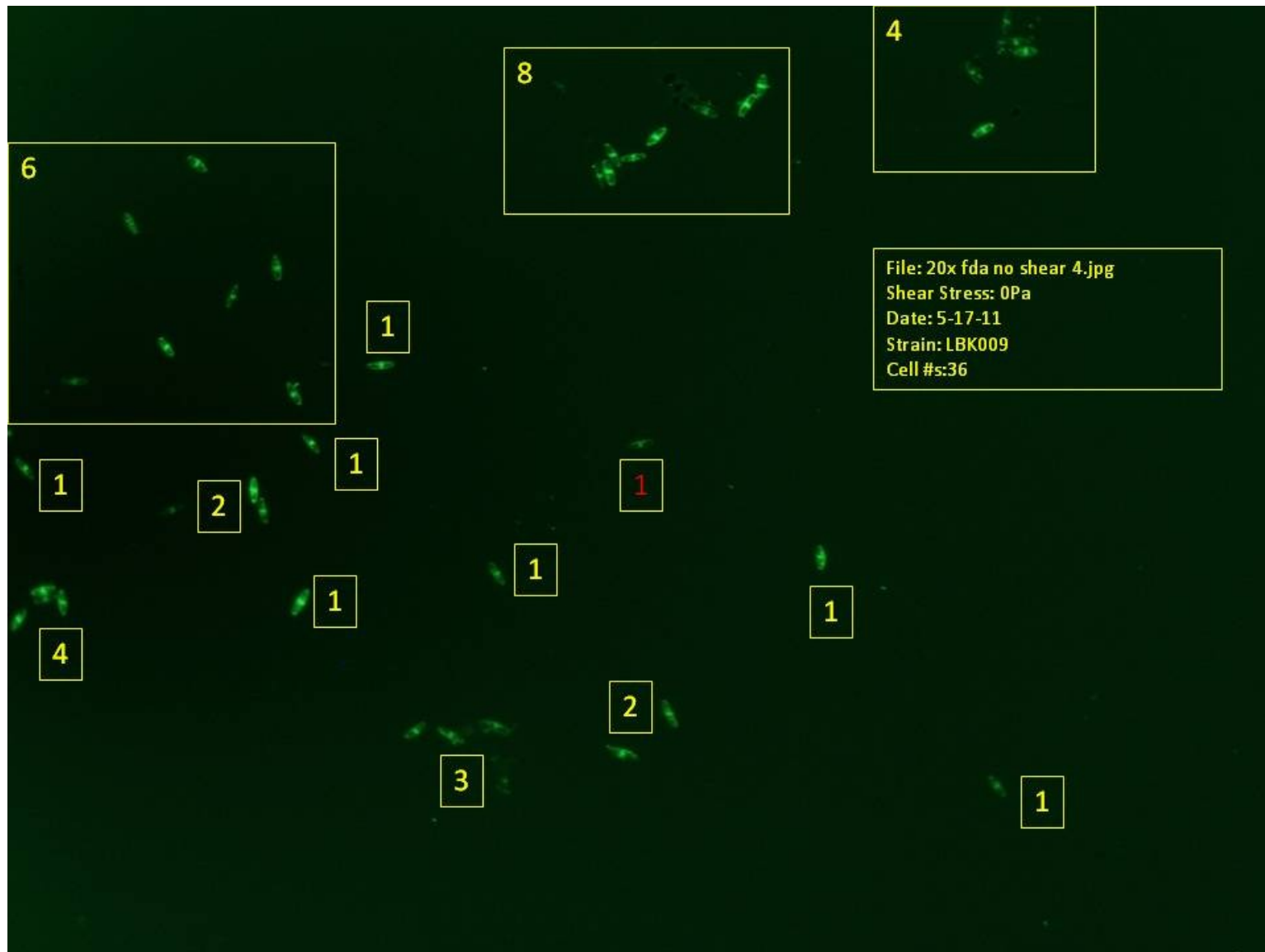


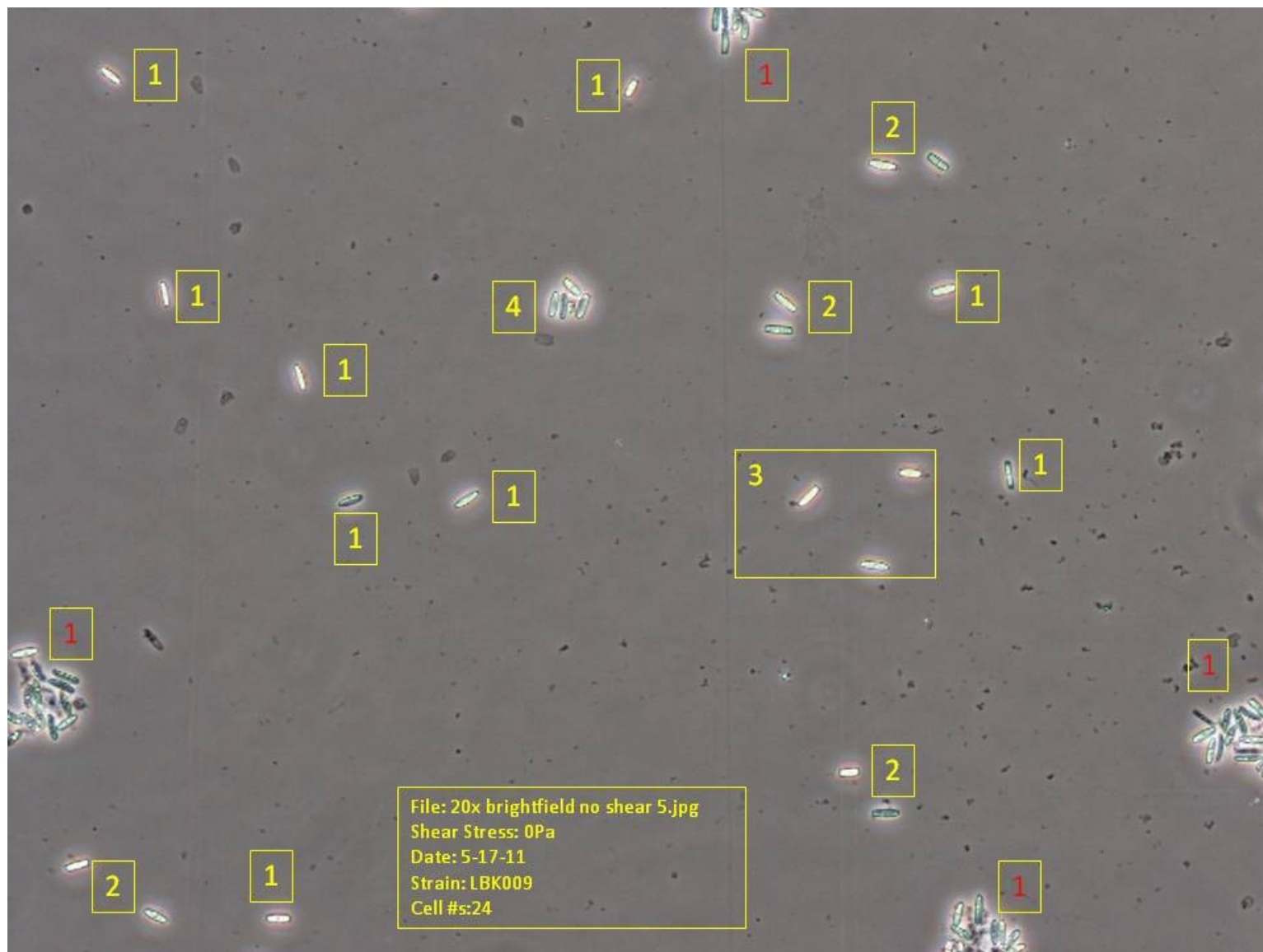




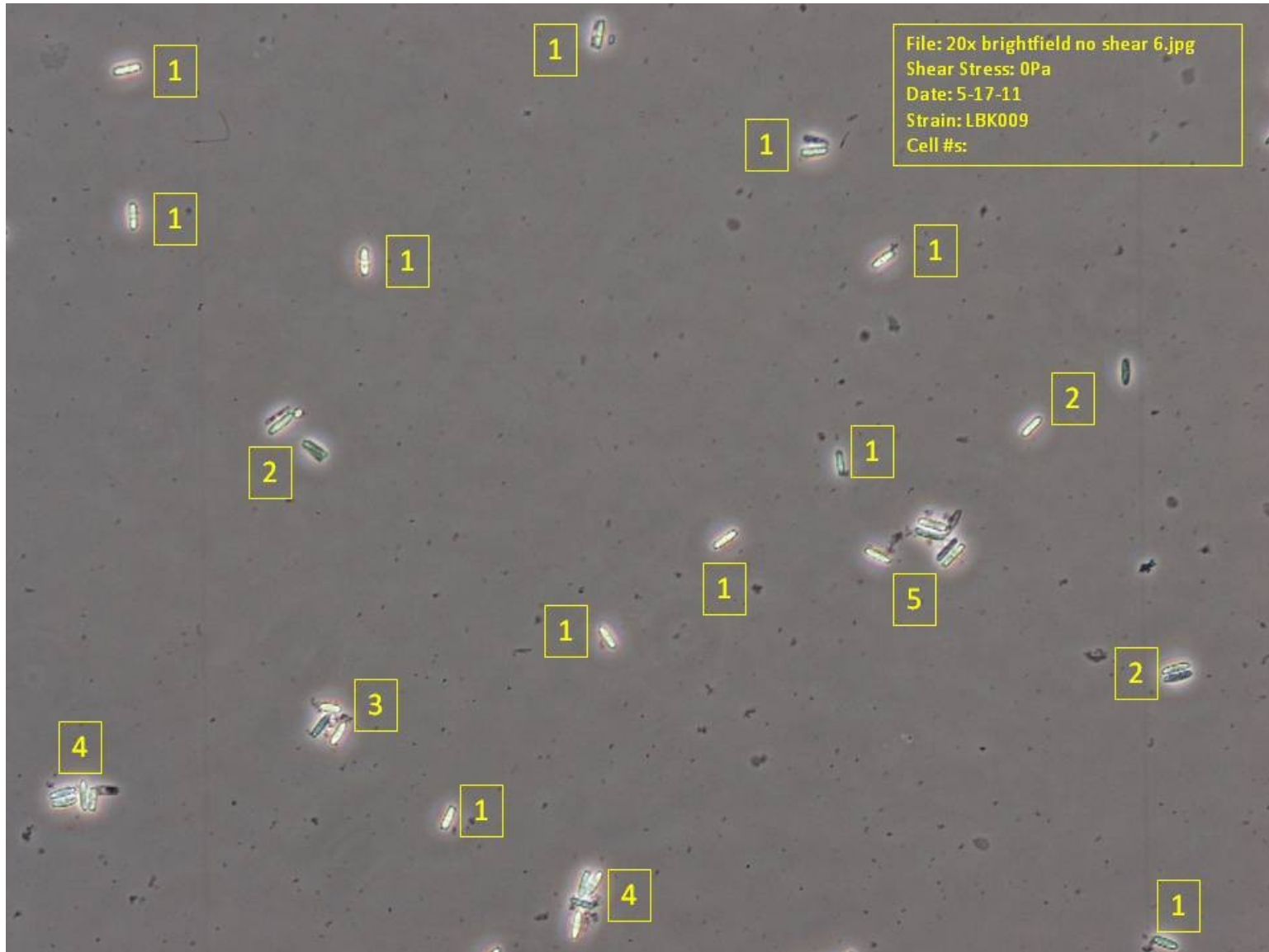


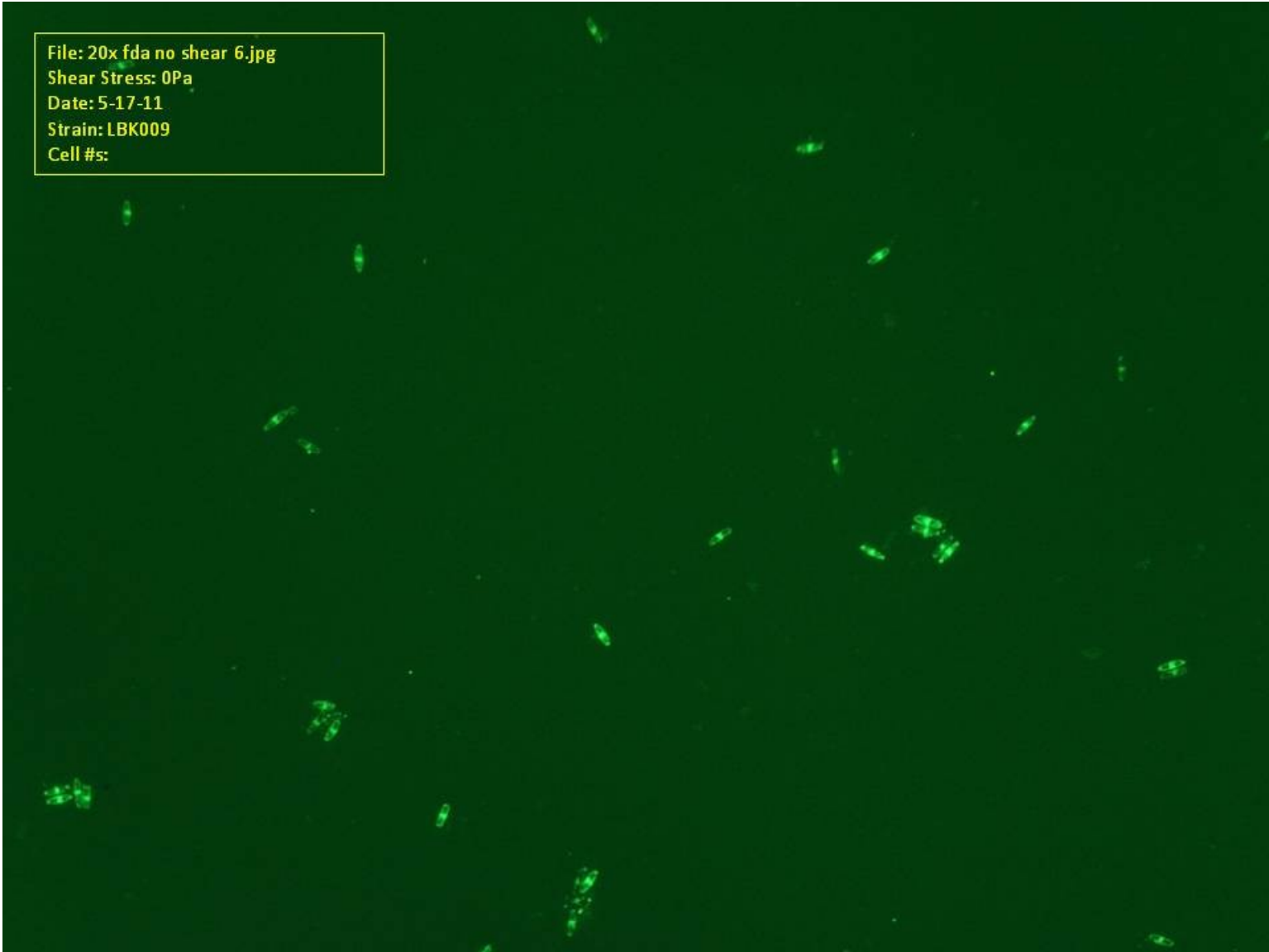




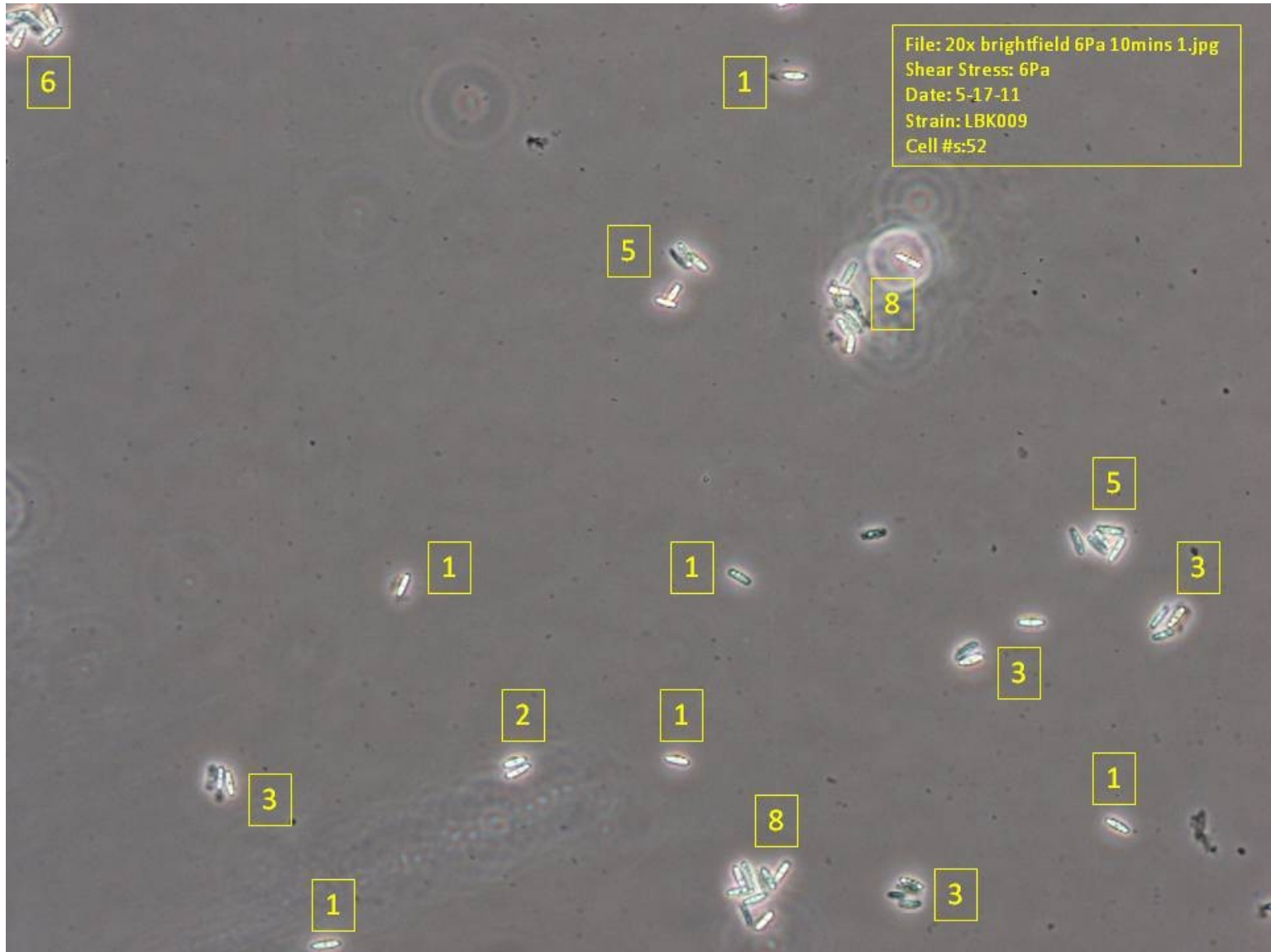


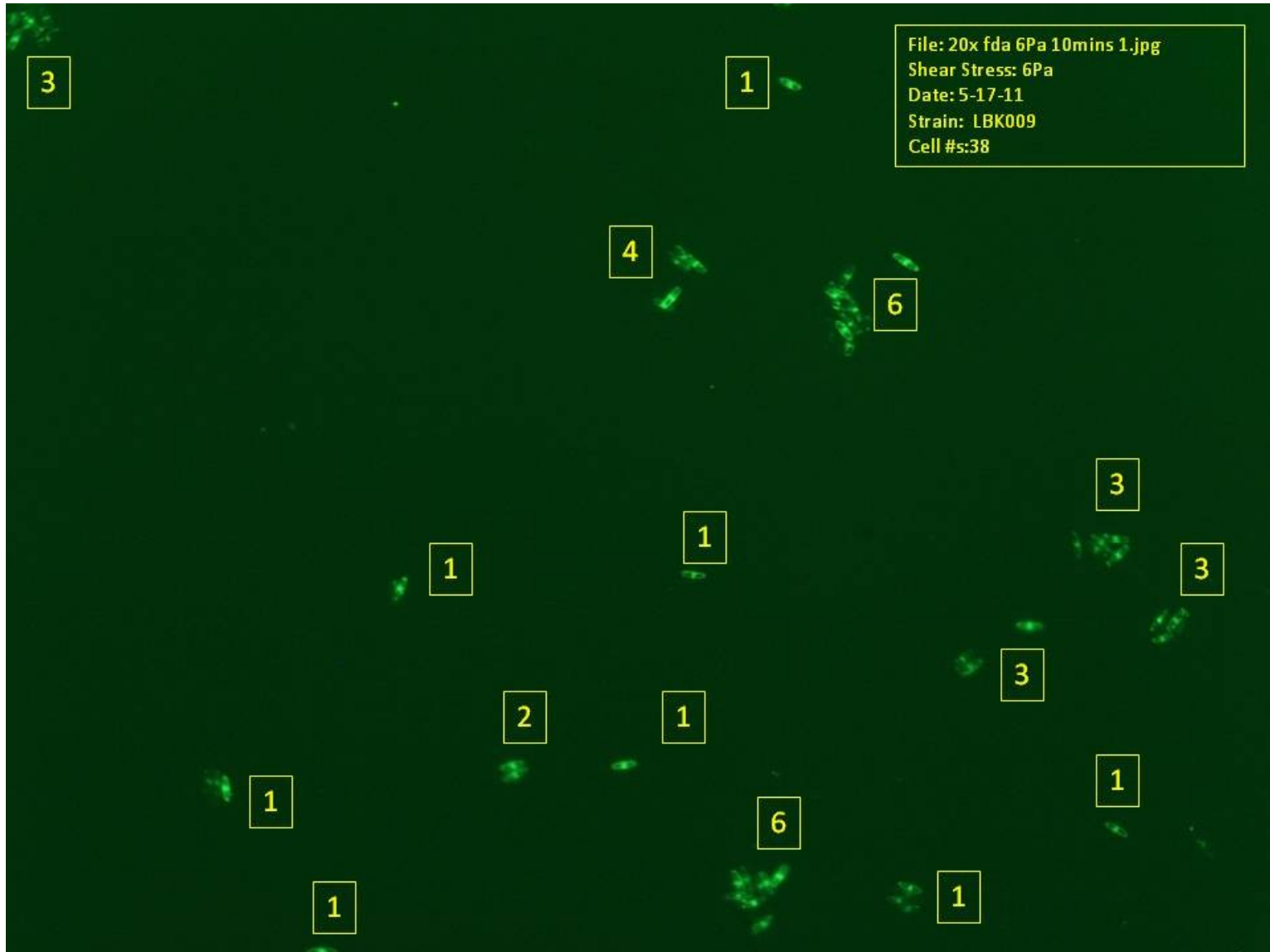


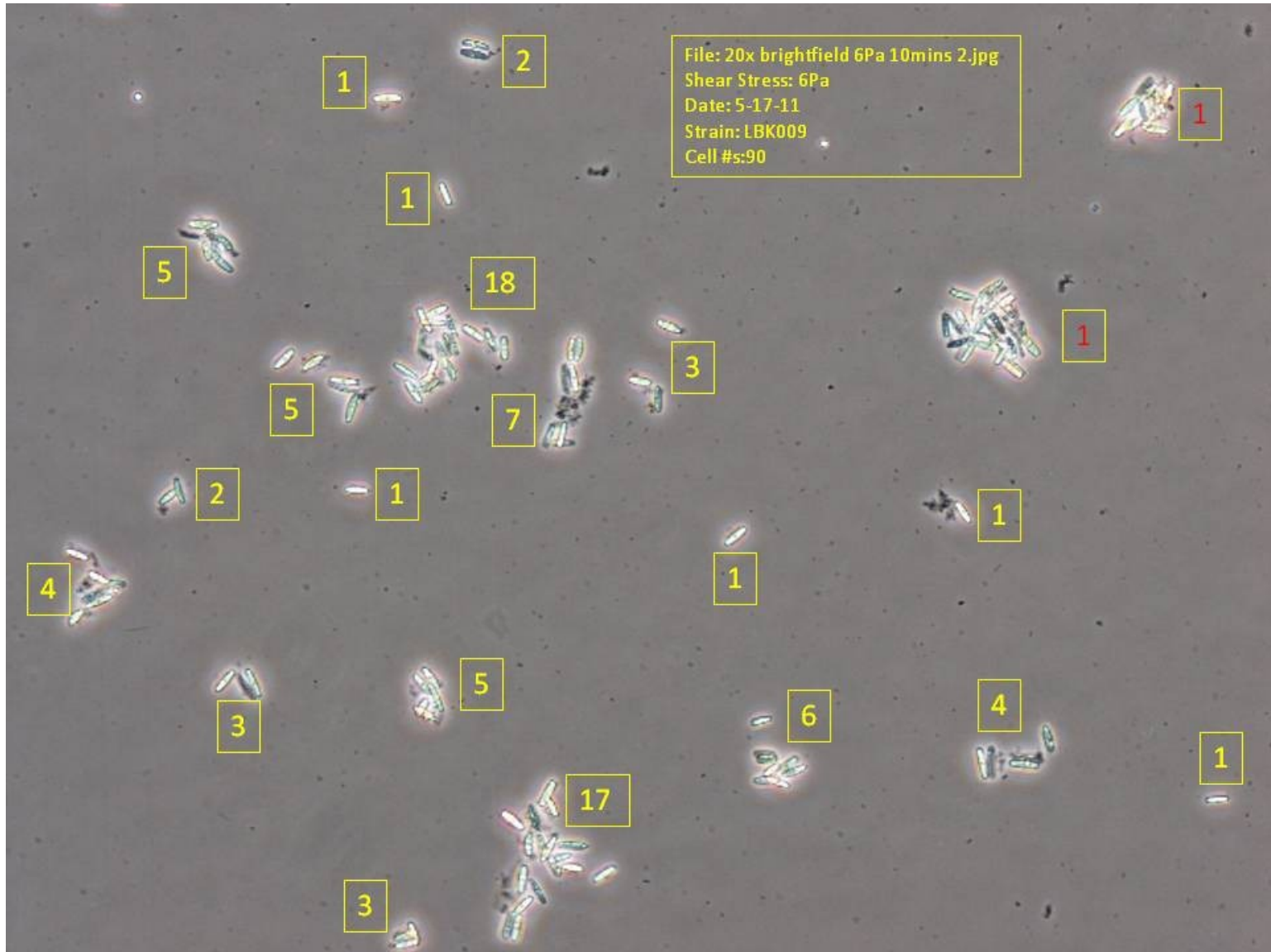


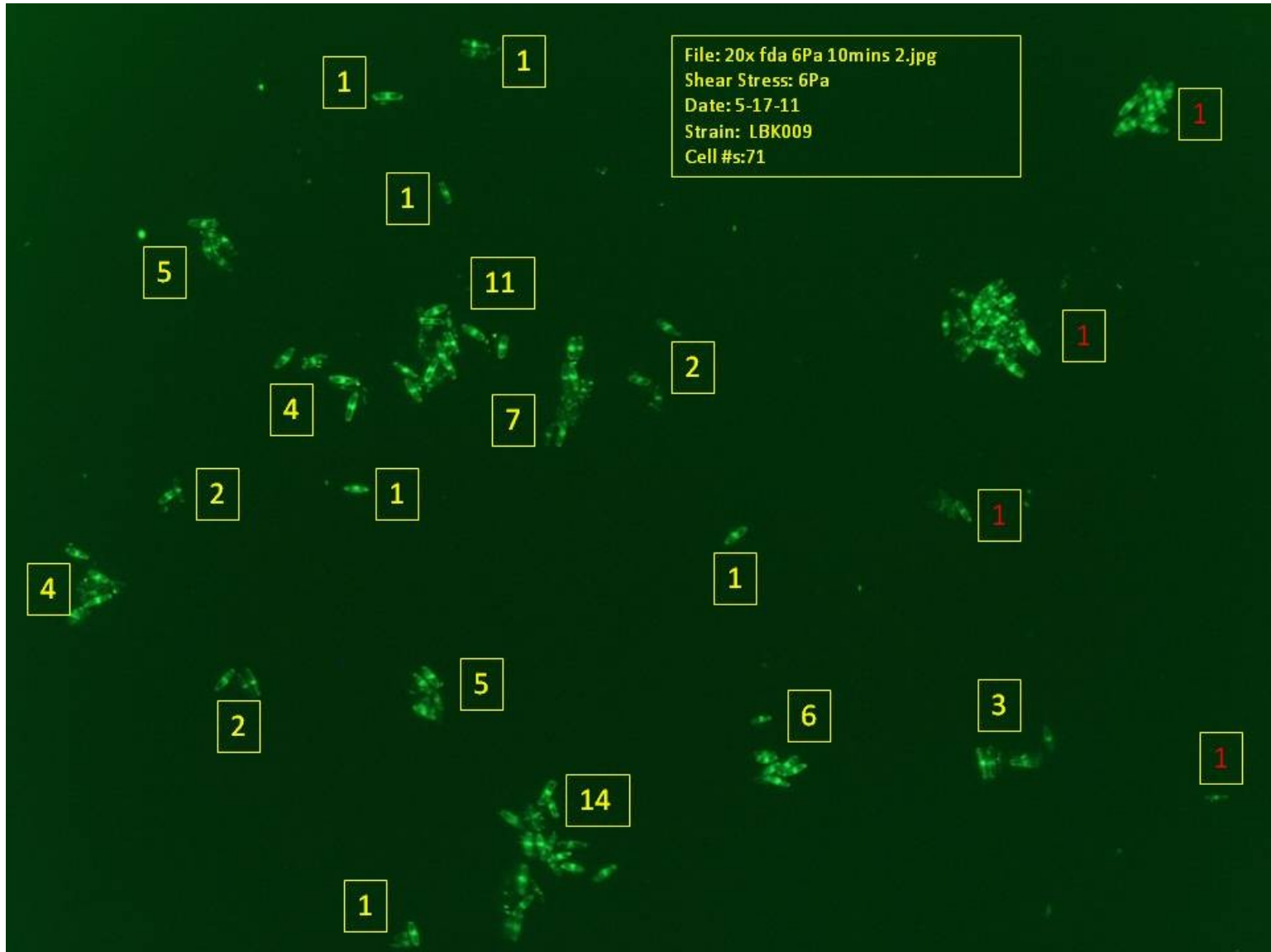


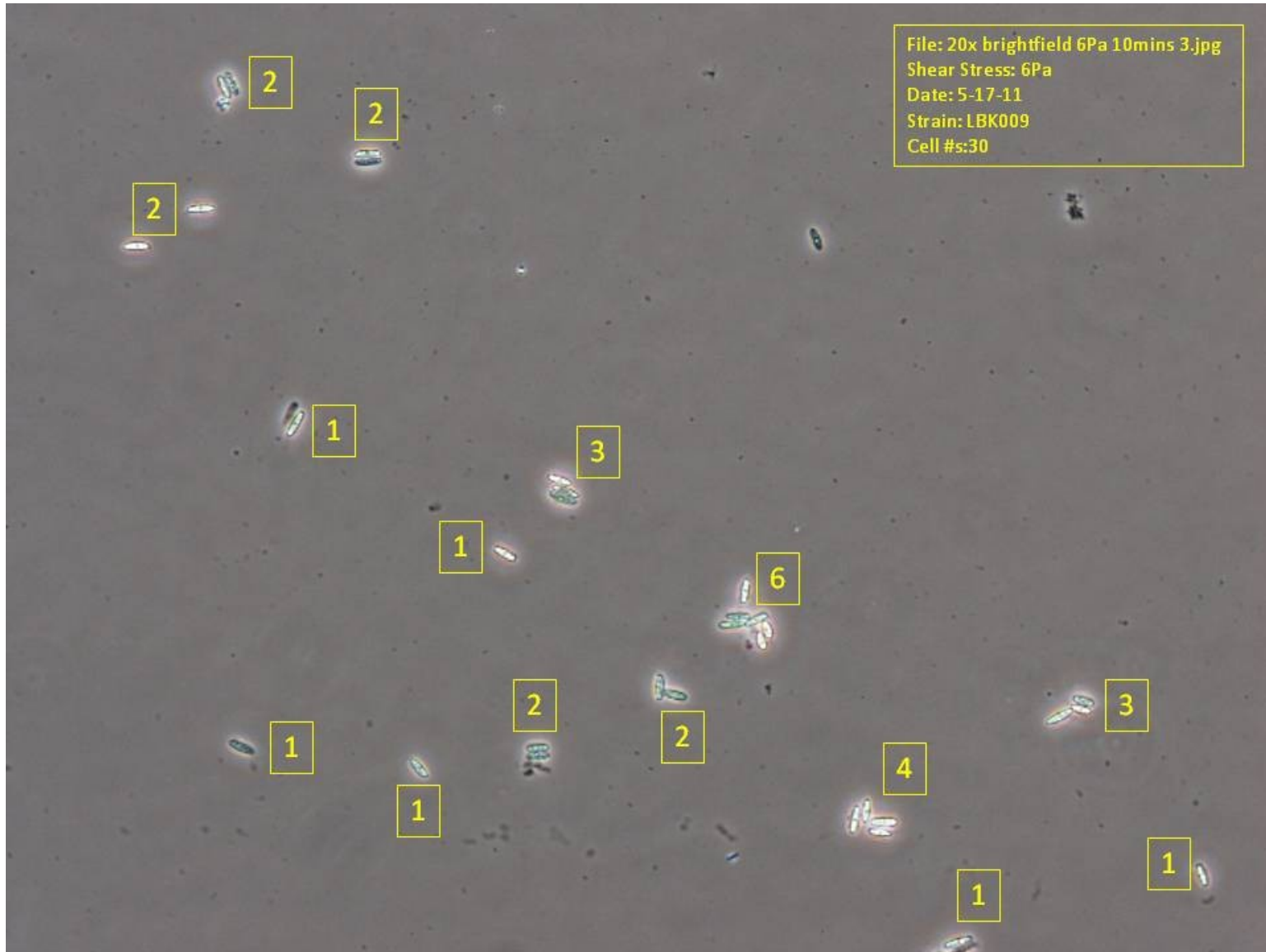


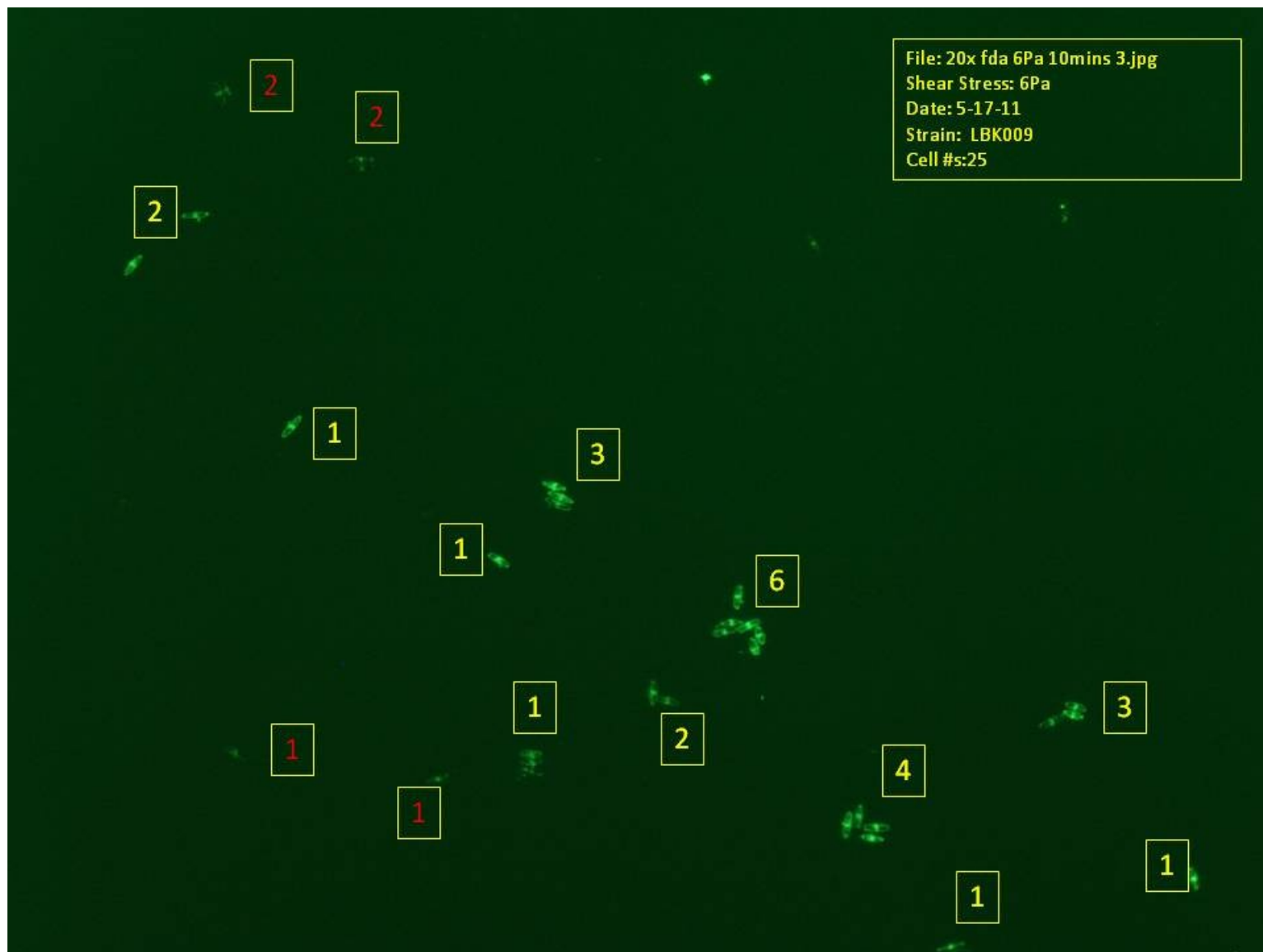


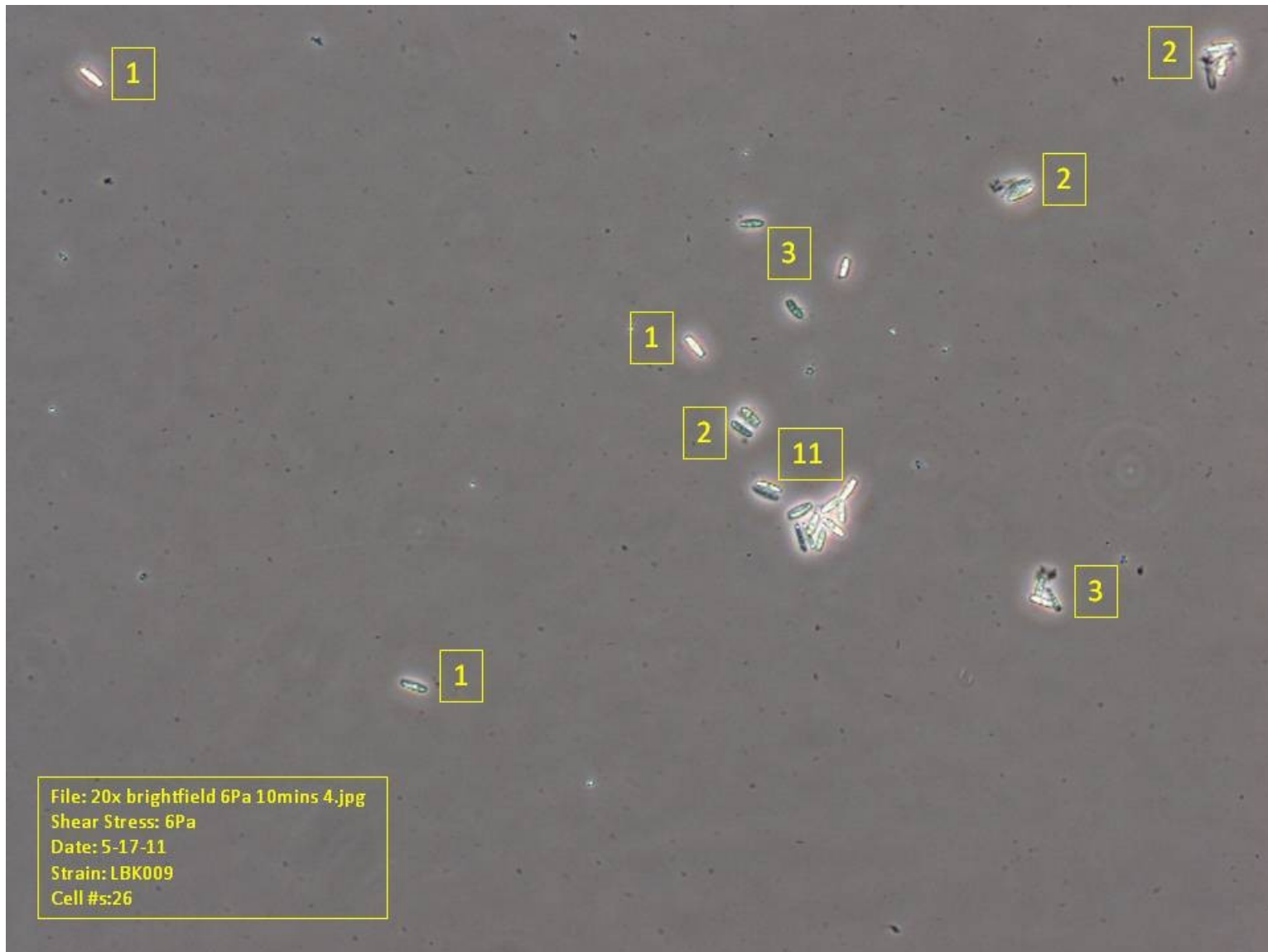






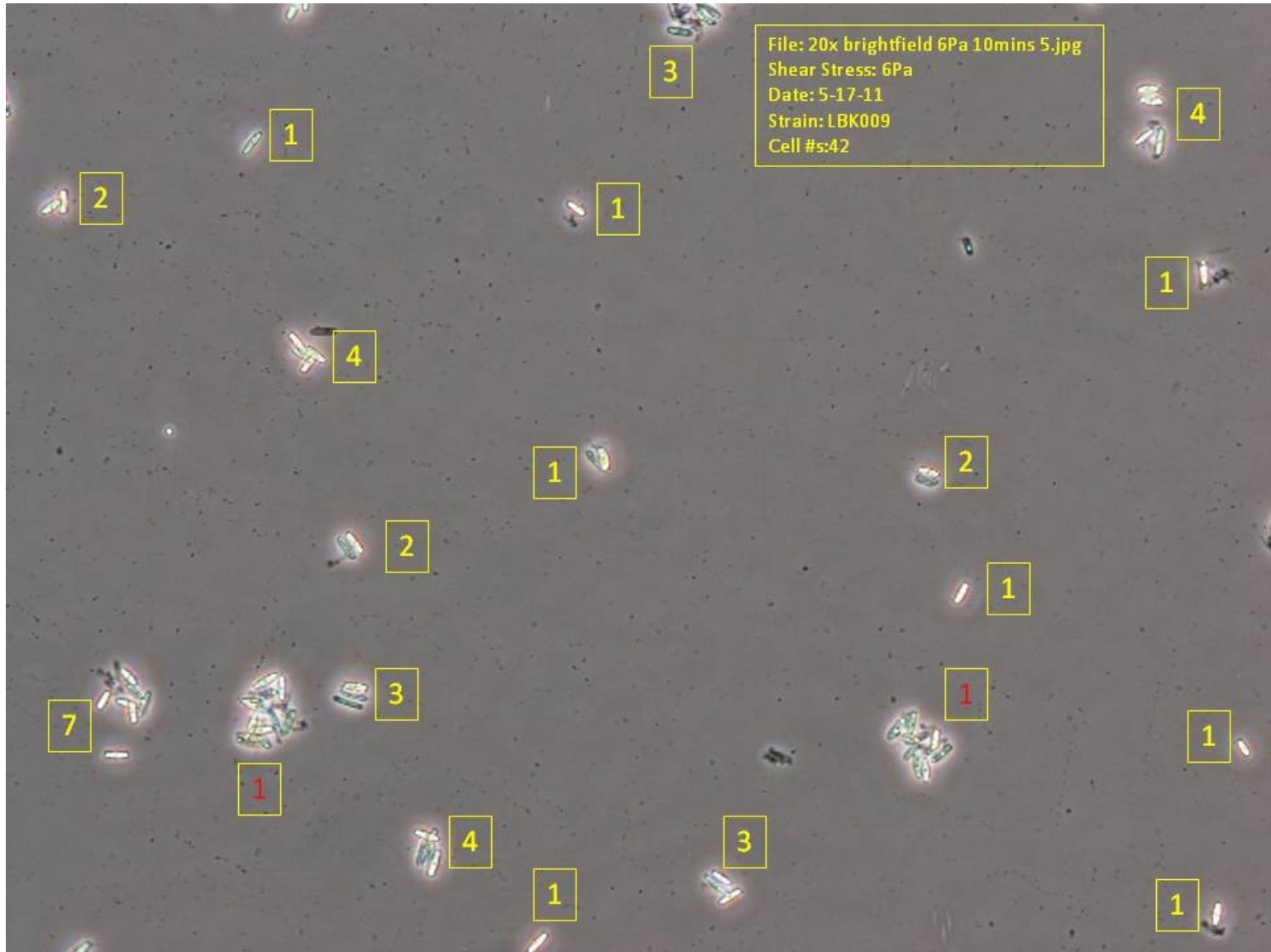


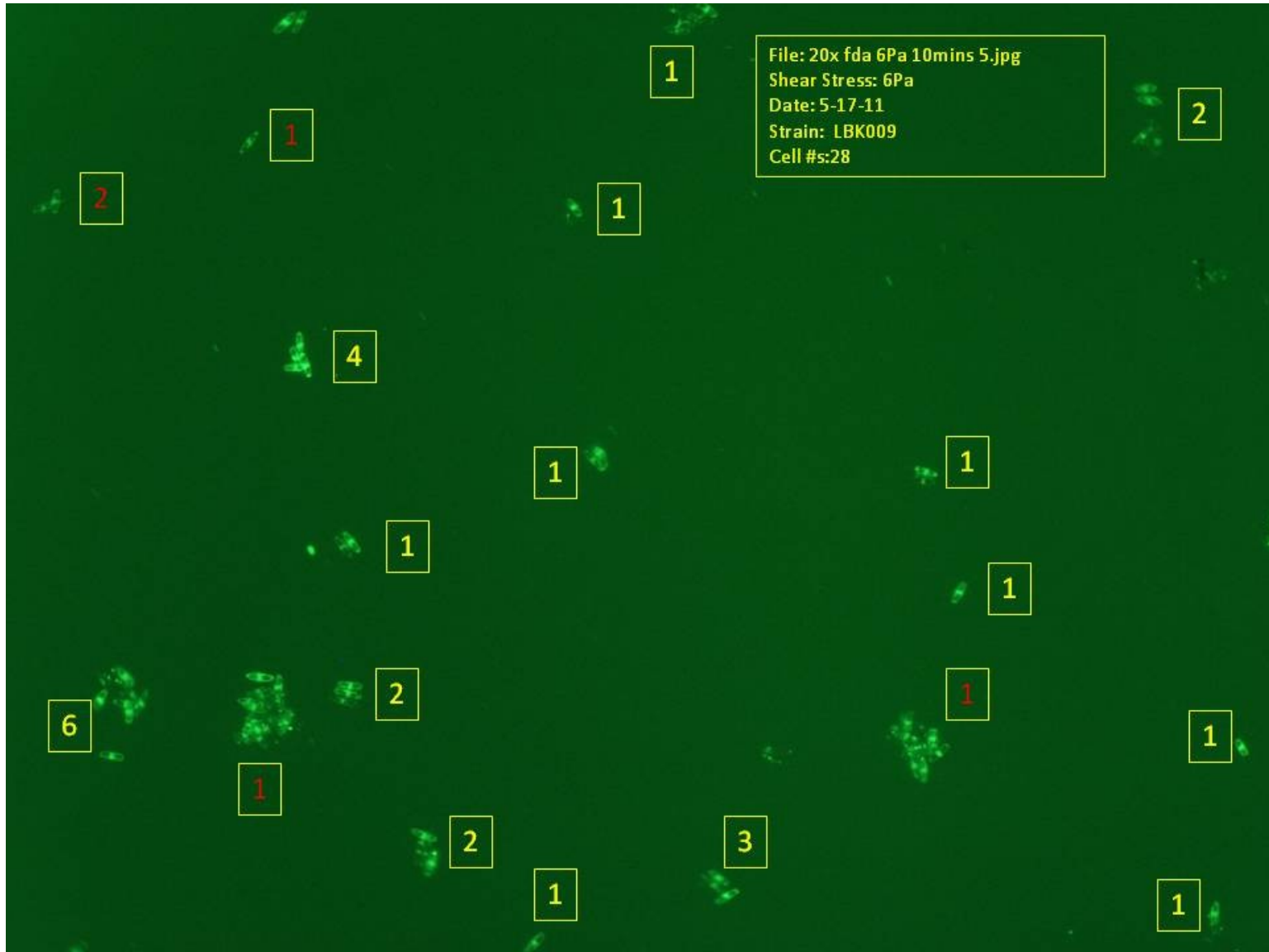


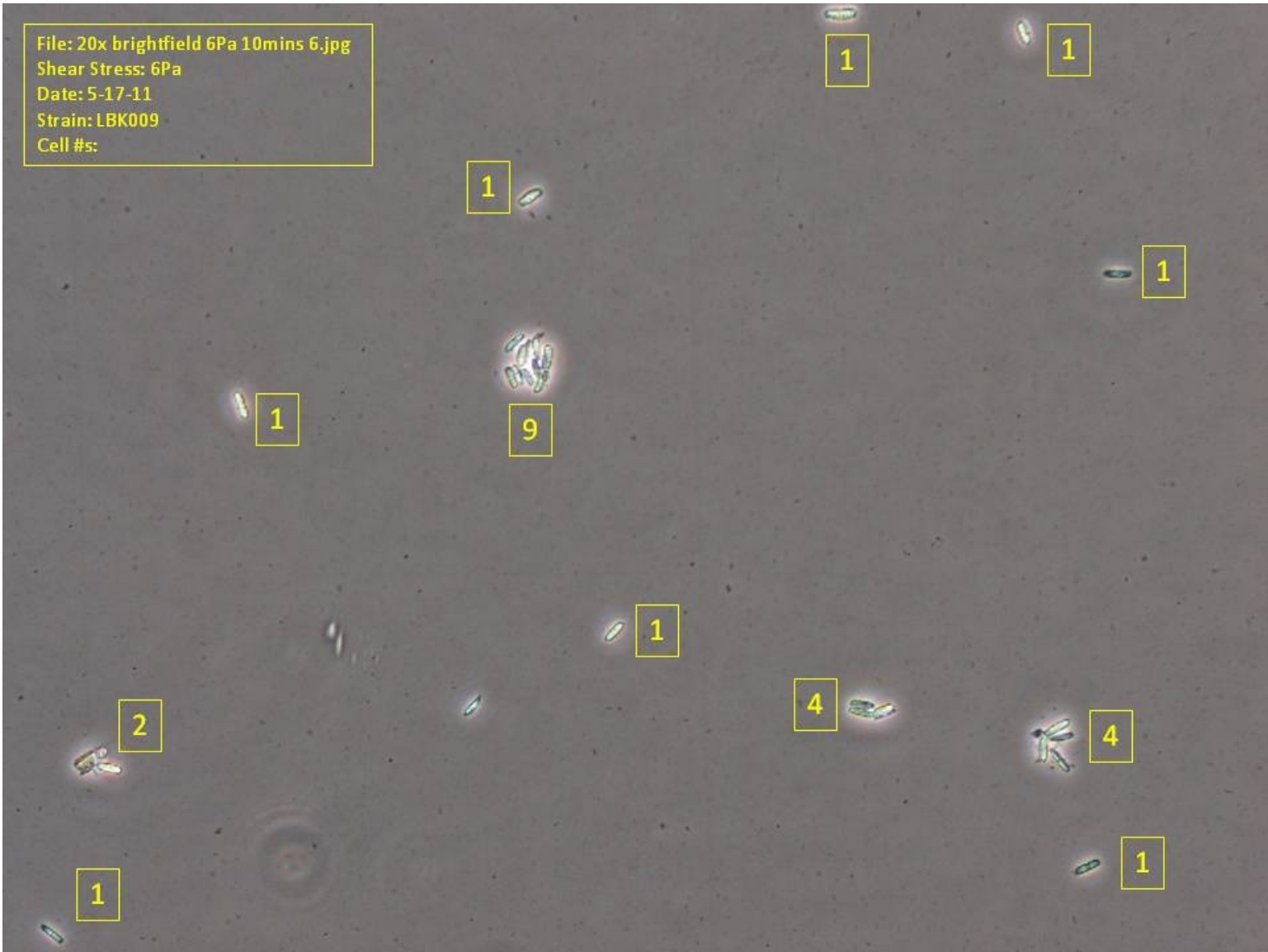


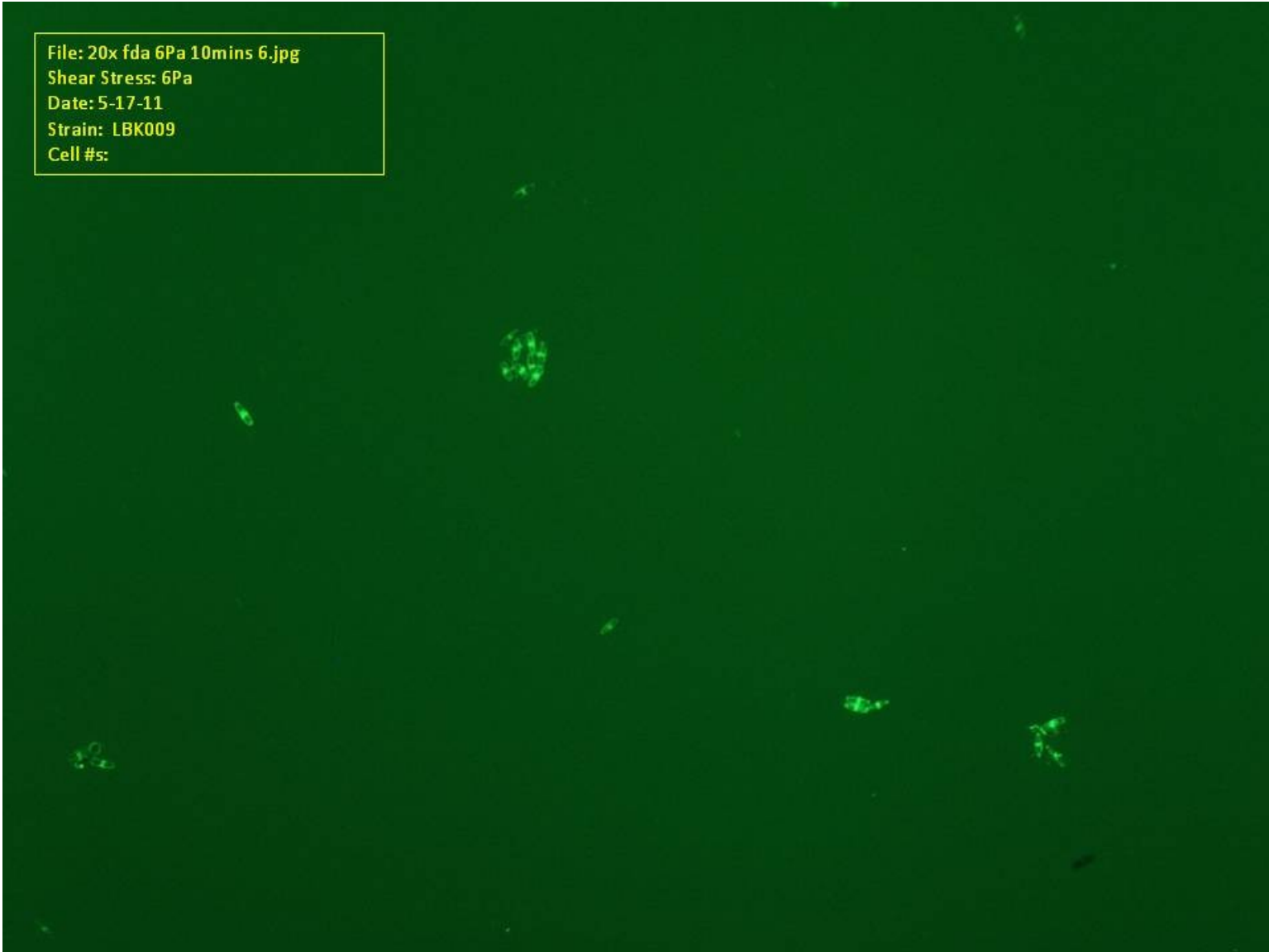






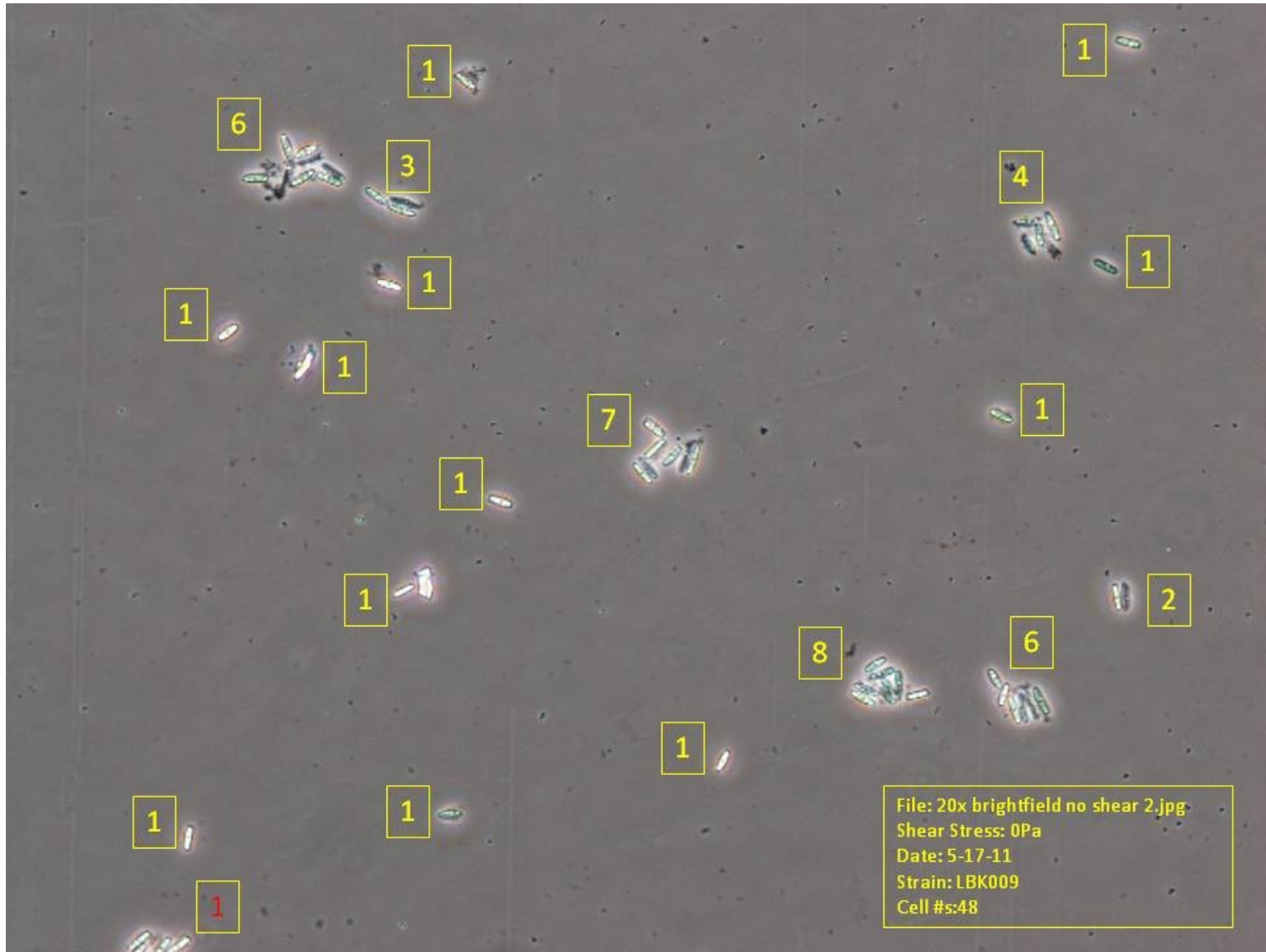


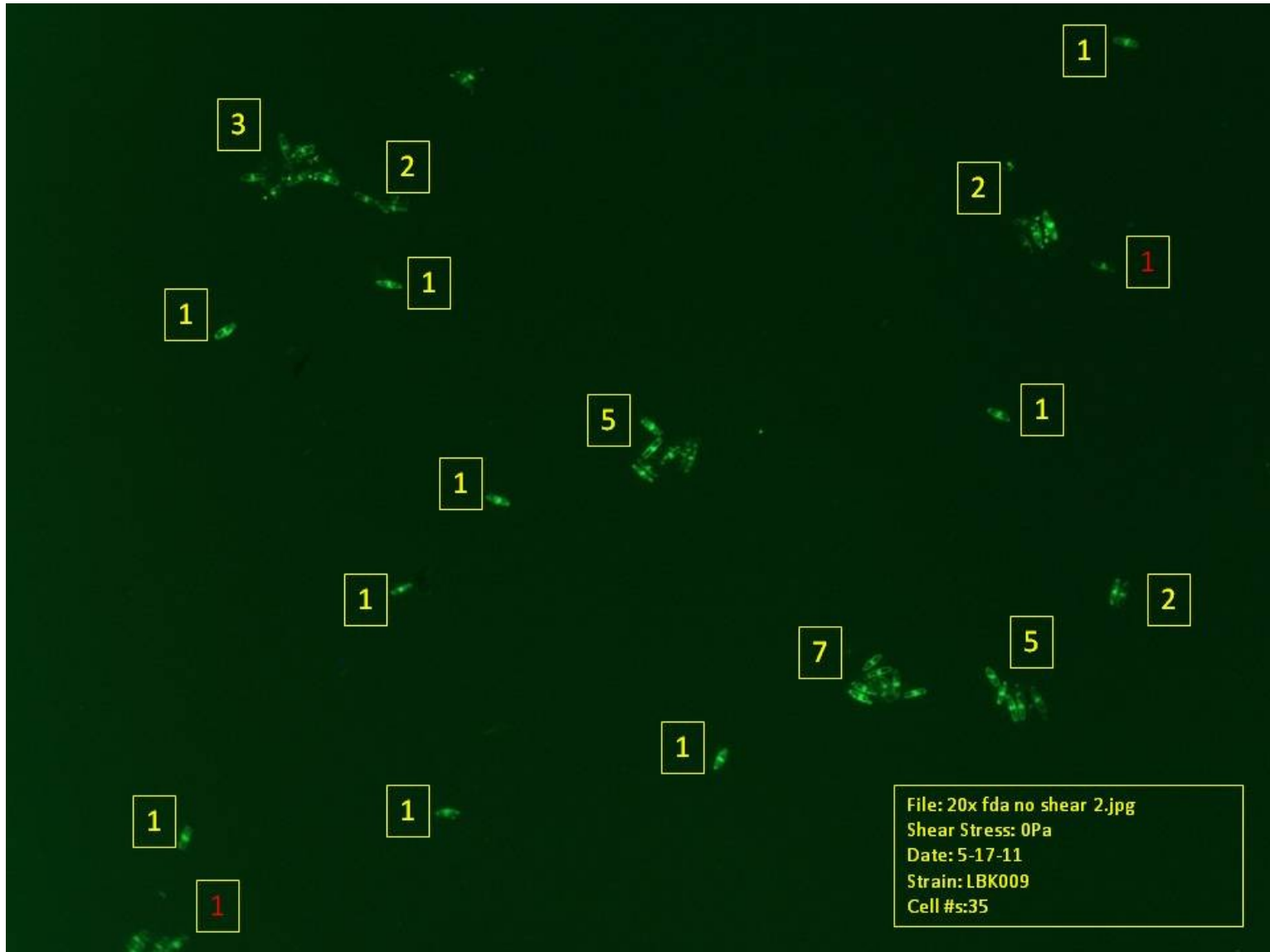




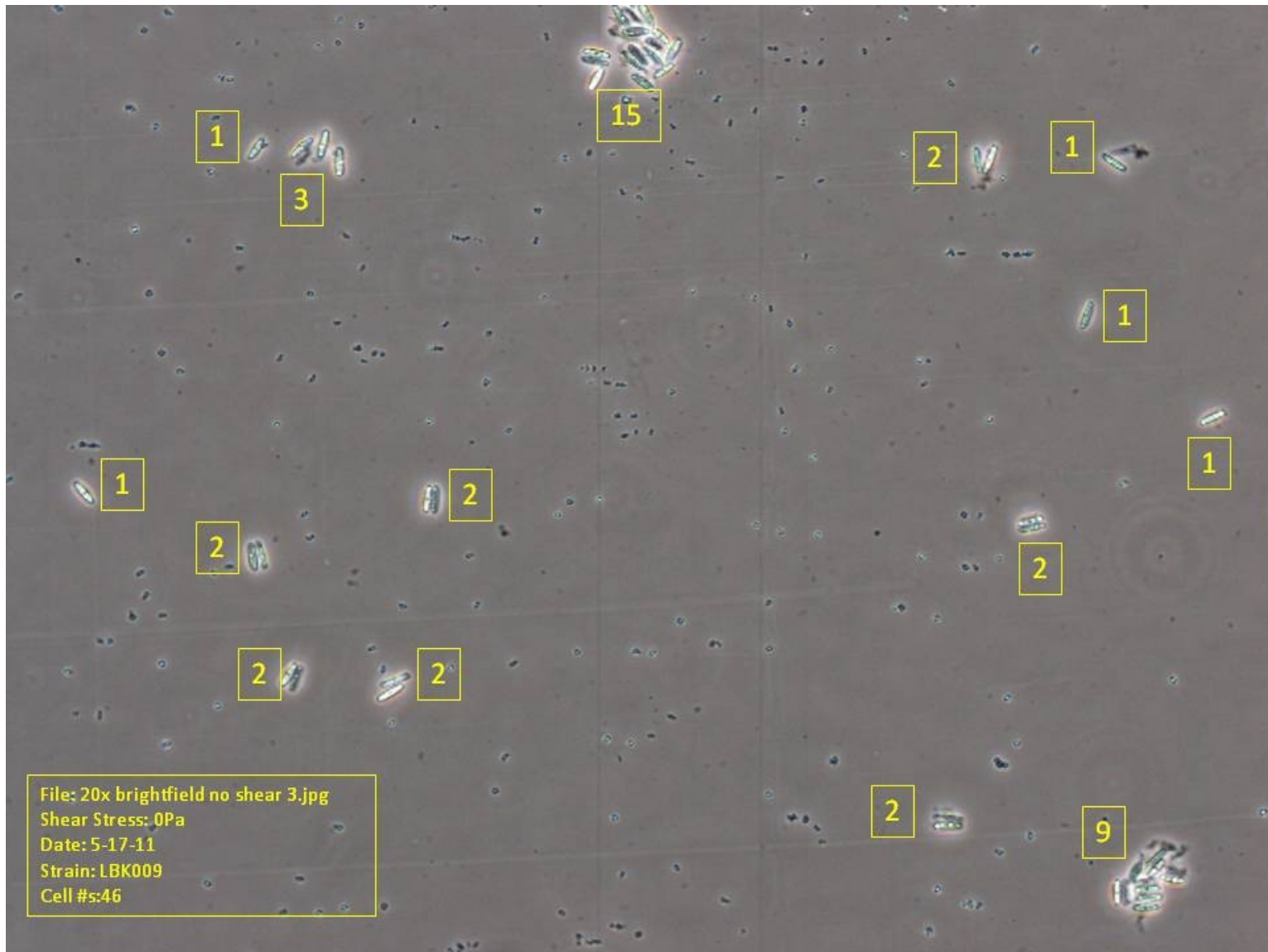




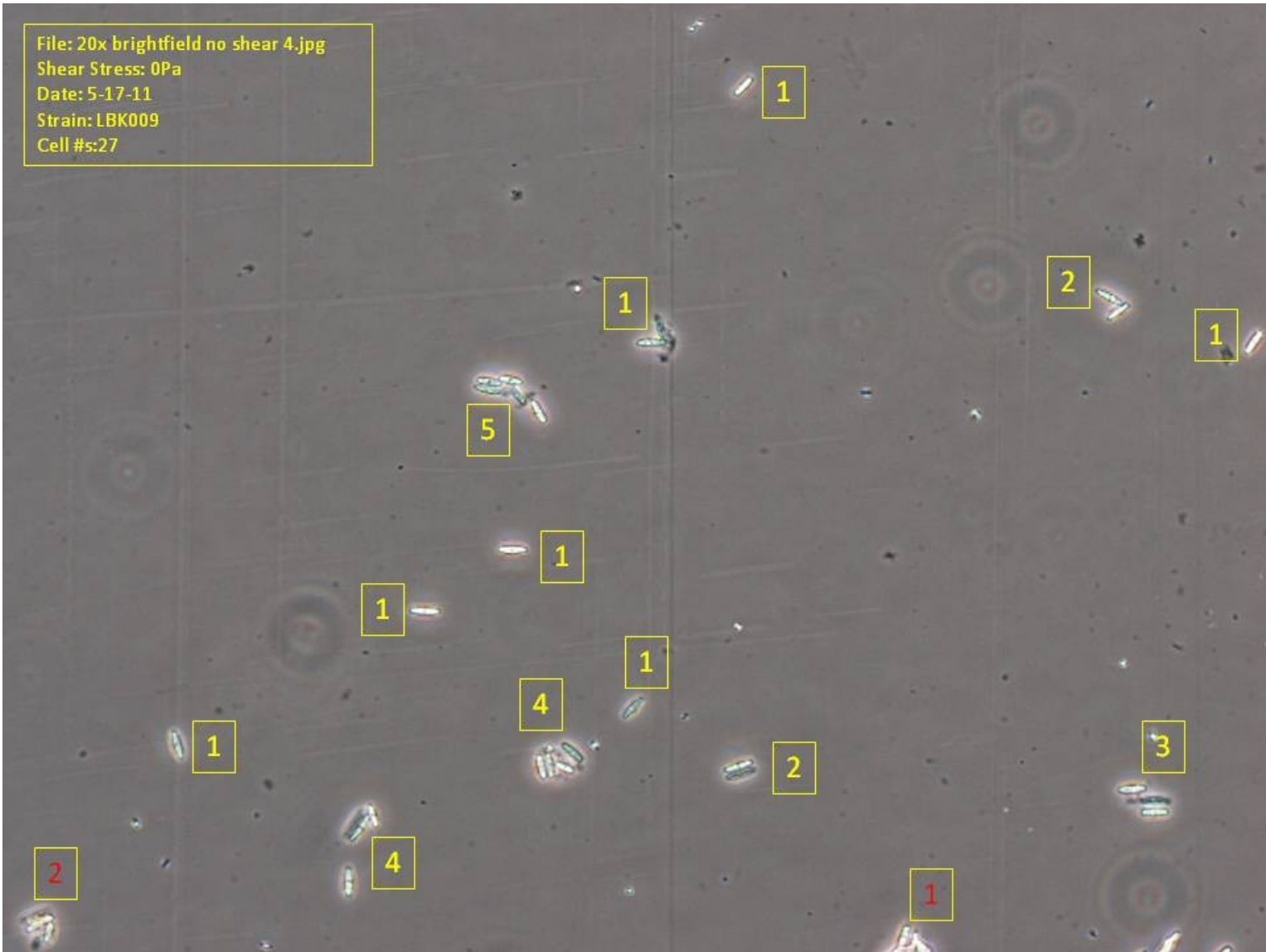






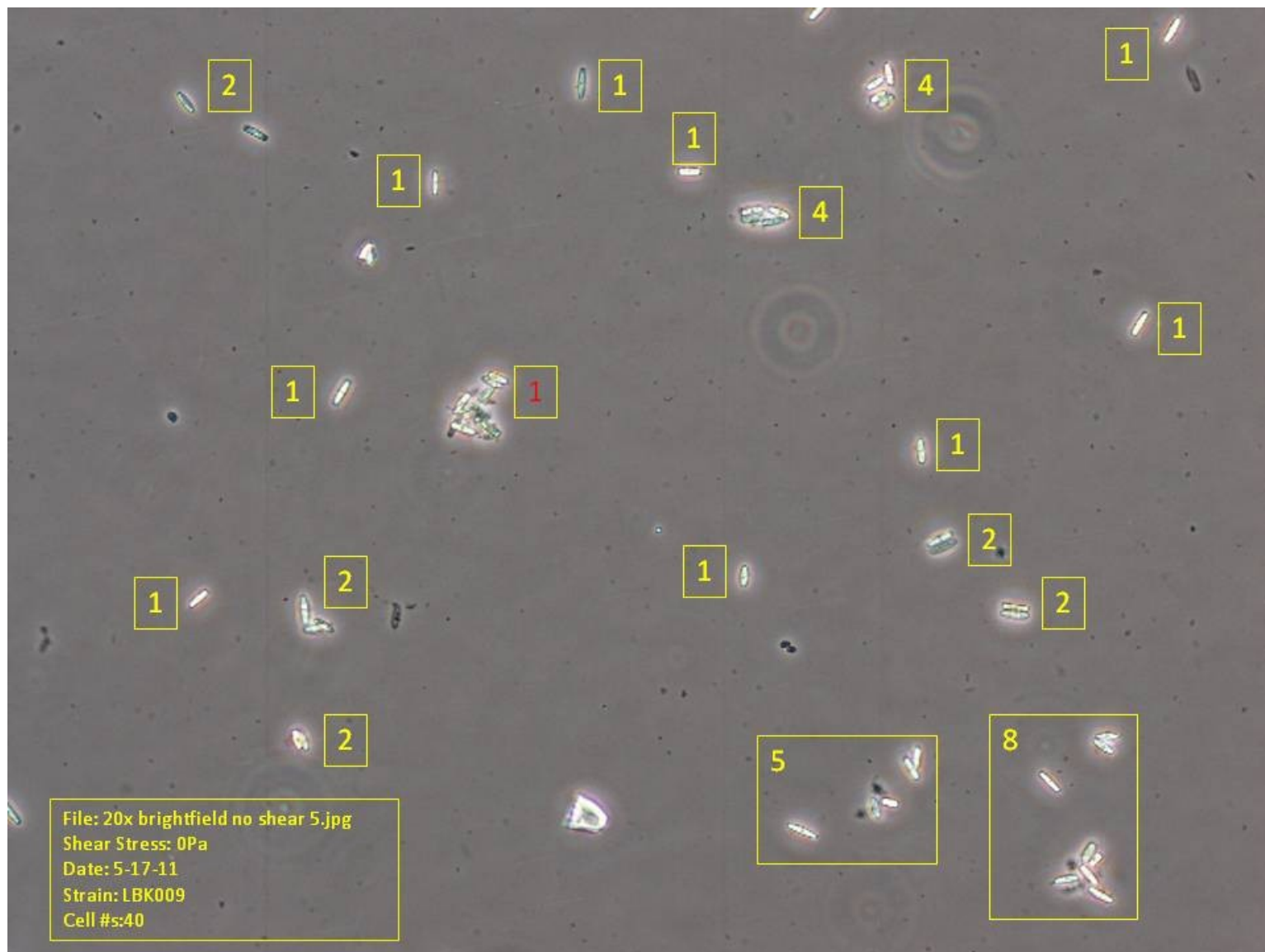


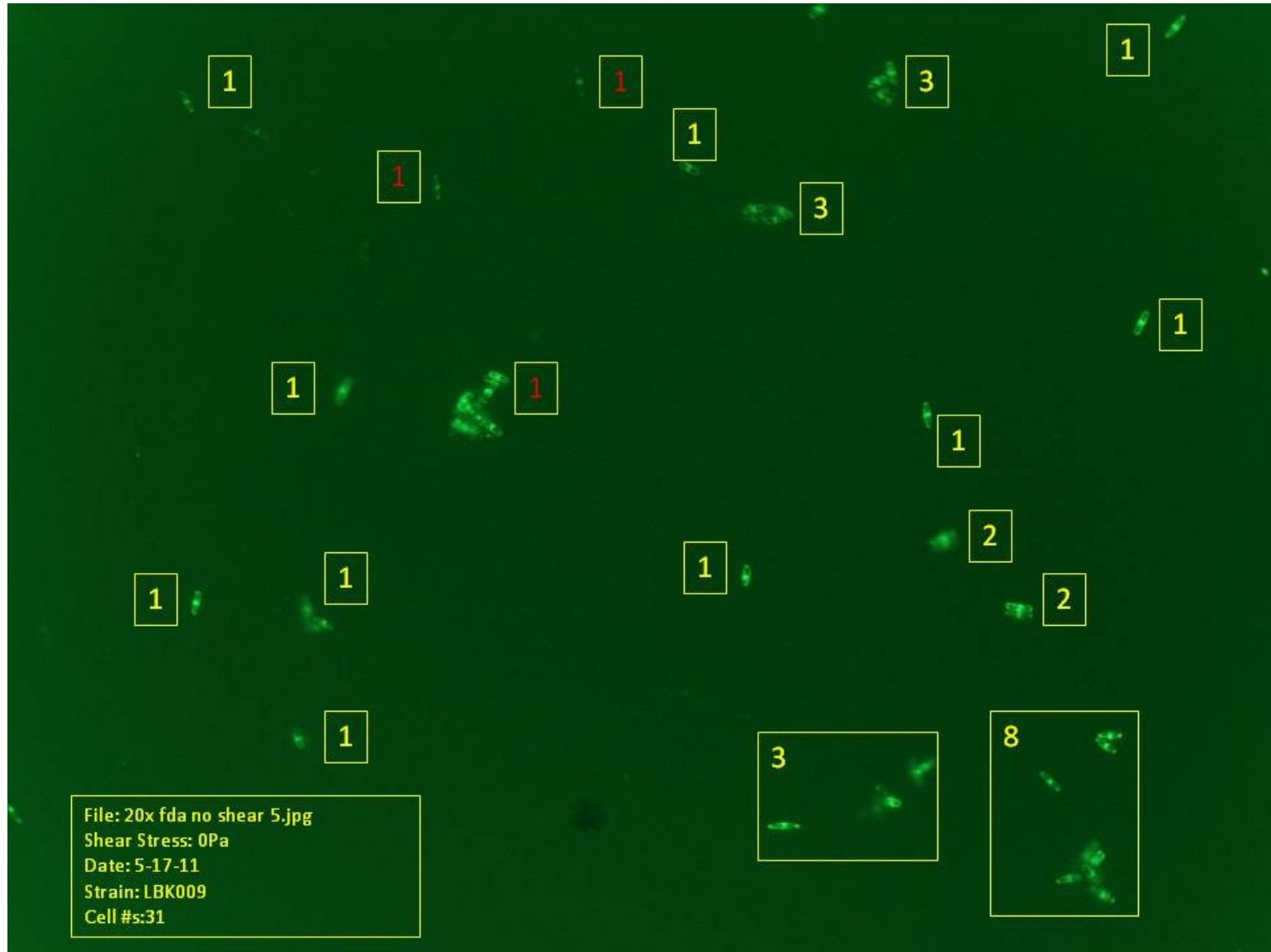


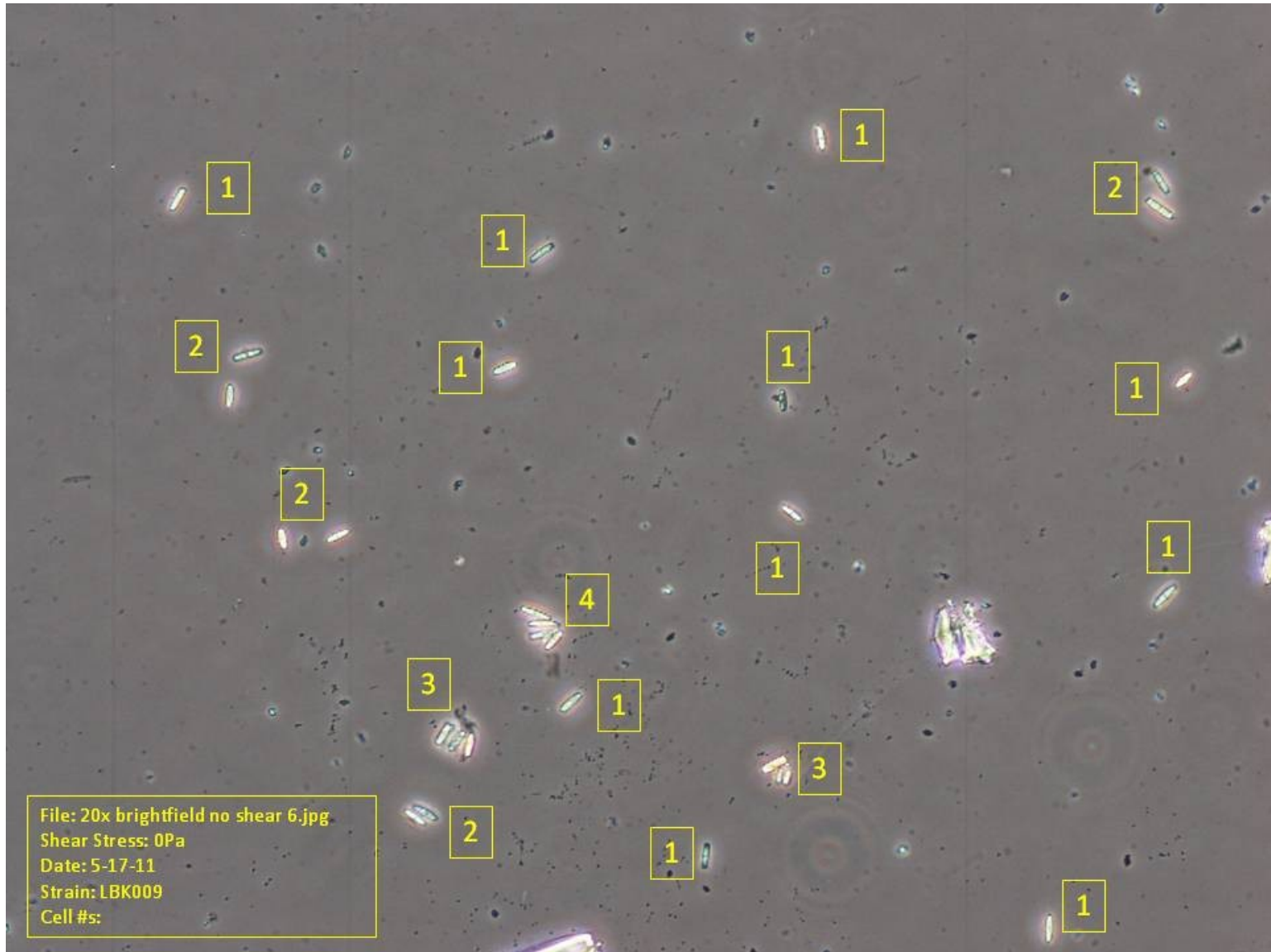


File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-17-11  
Strain: LBK009  
Cell #s:22



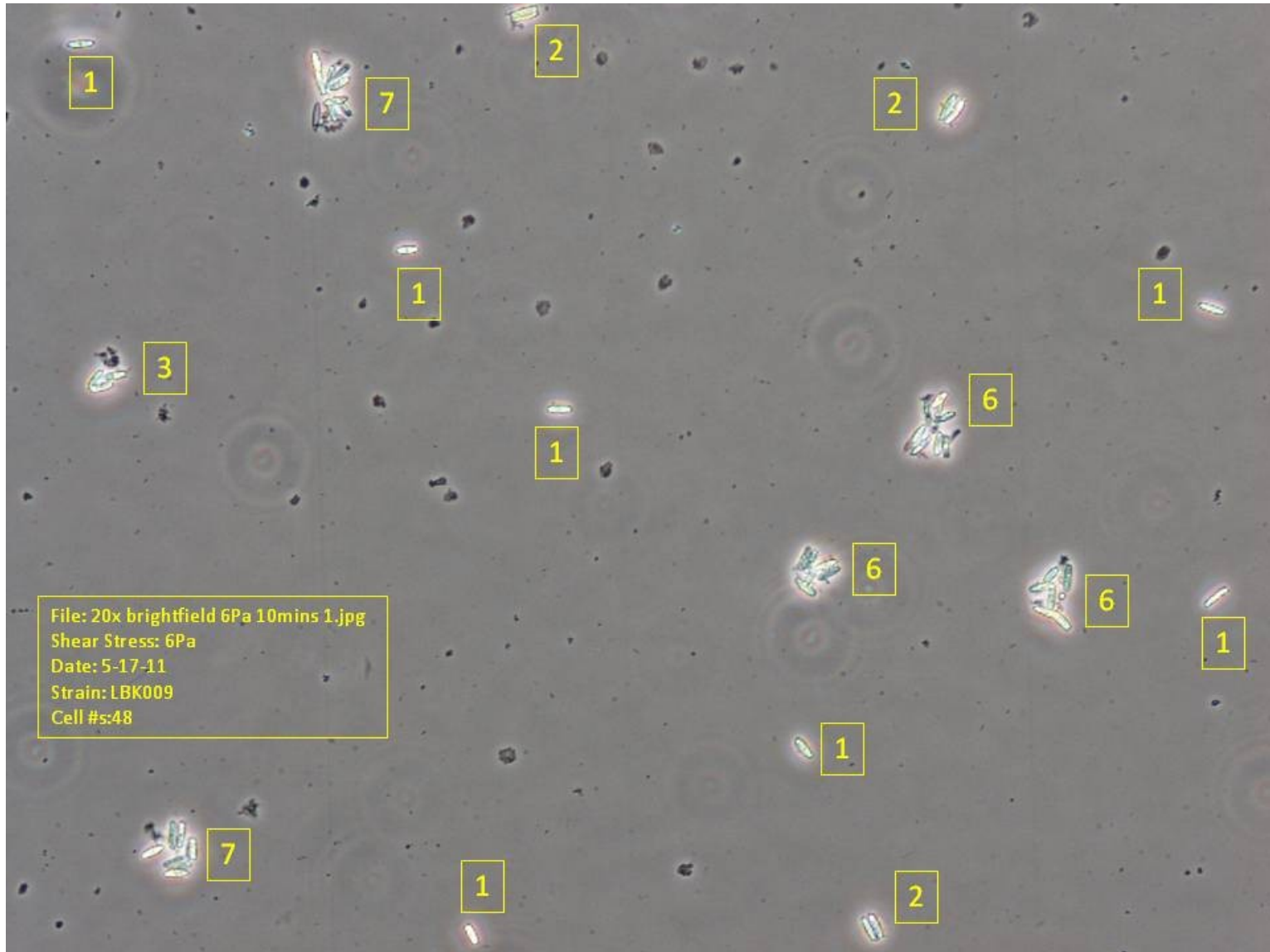










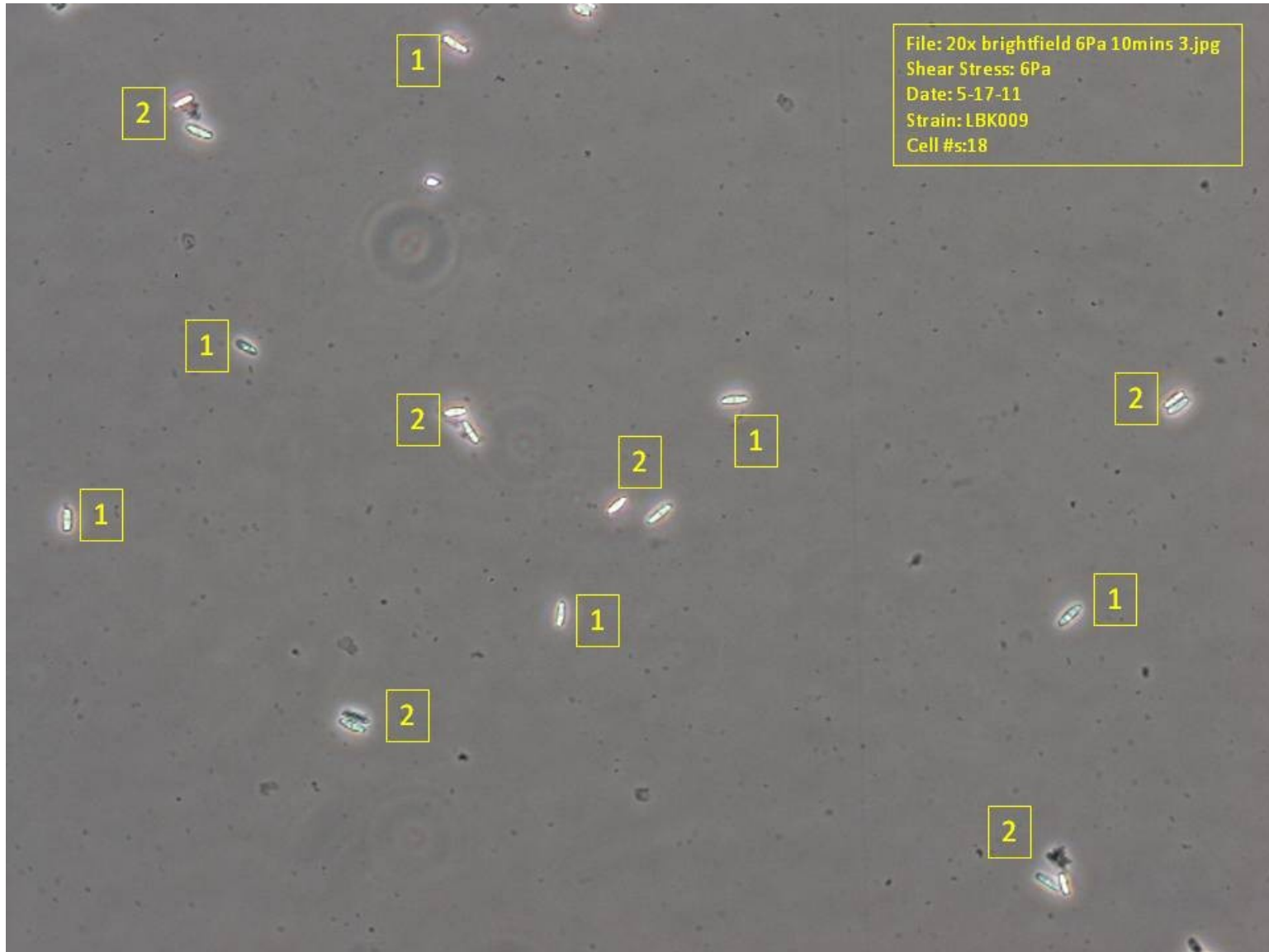




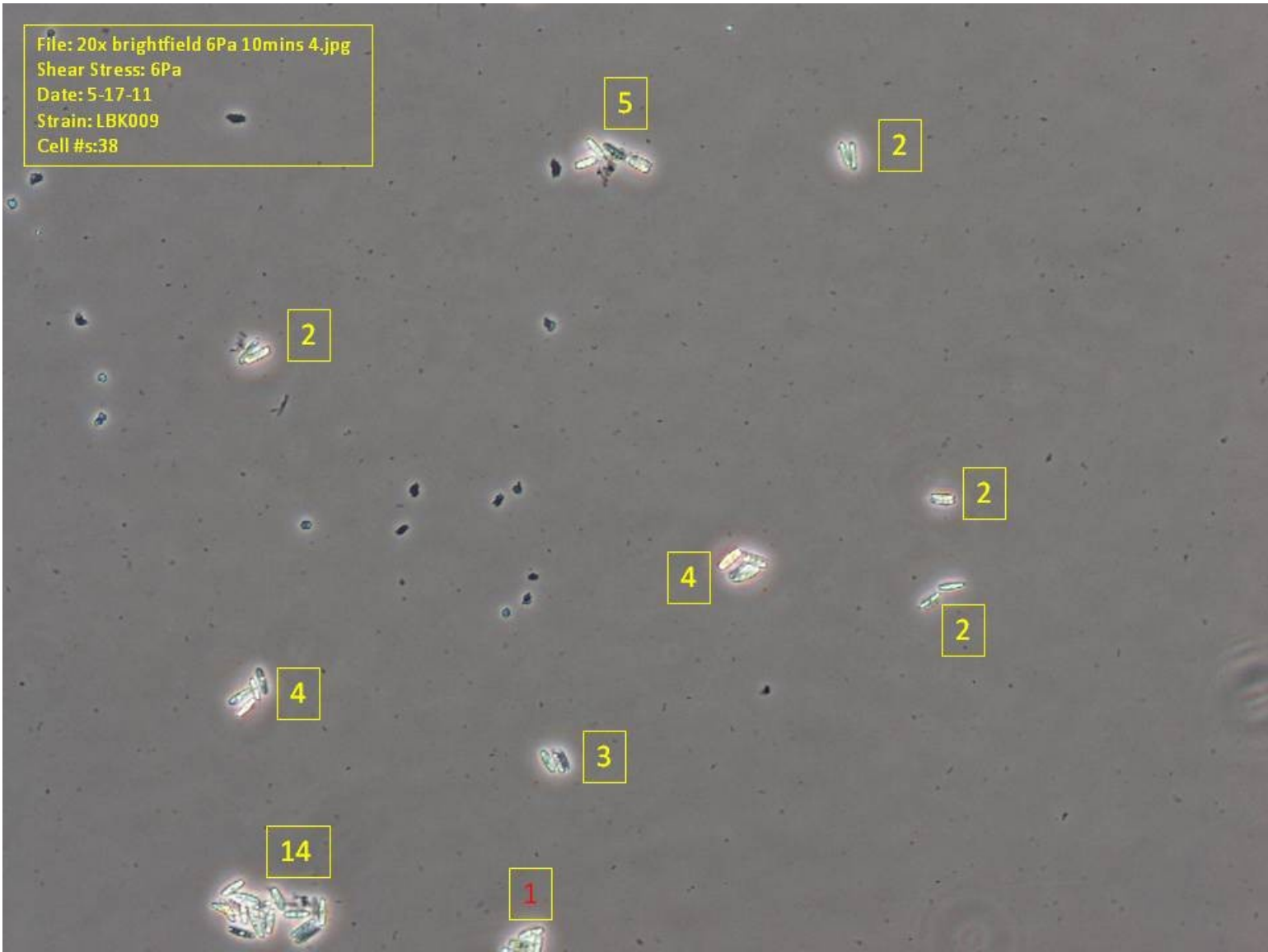
File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 5-17-11  
Strain: LBK009  
Cell #s:31





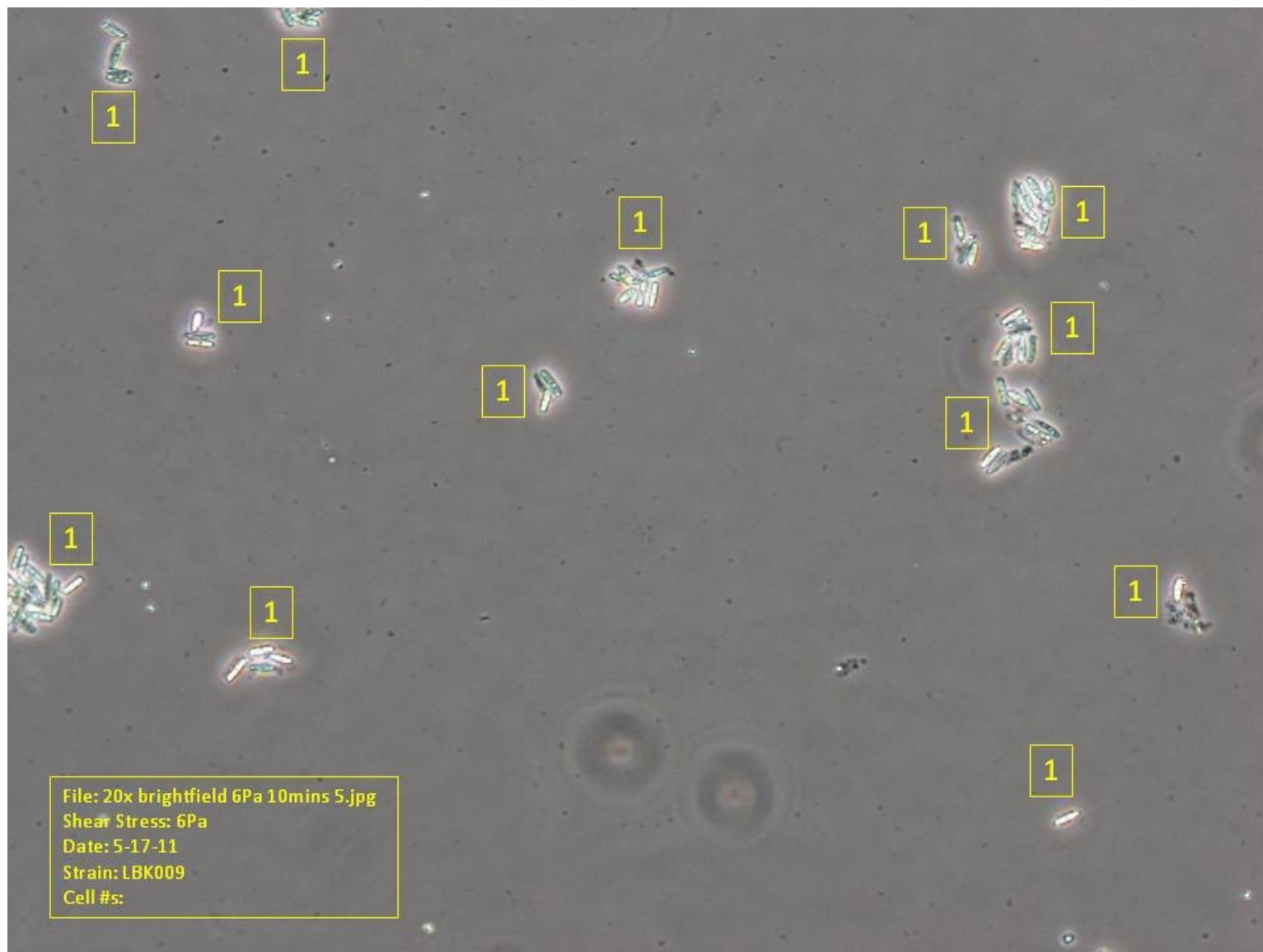


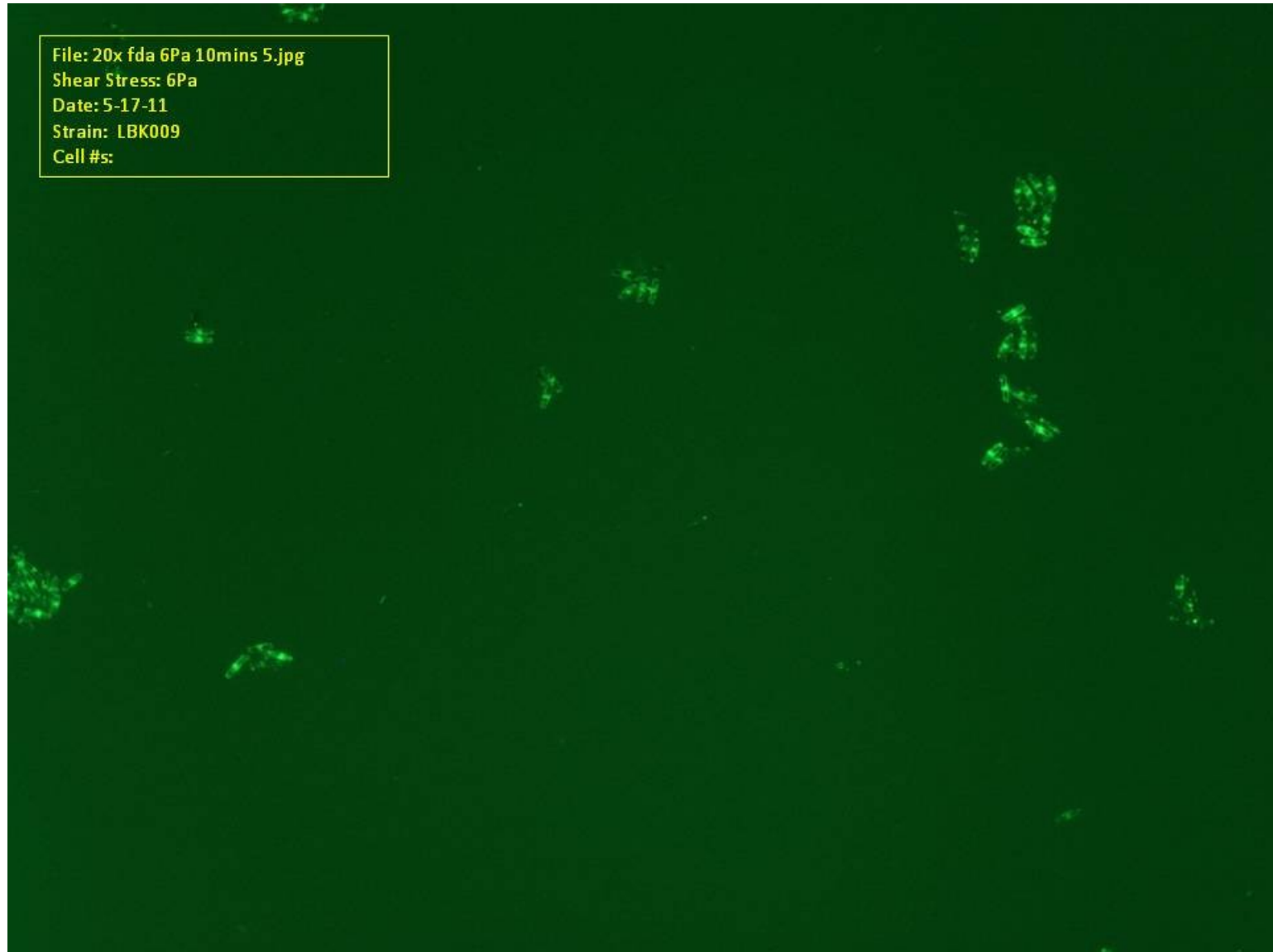




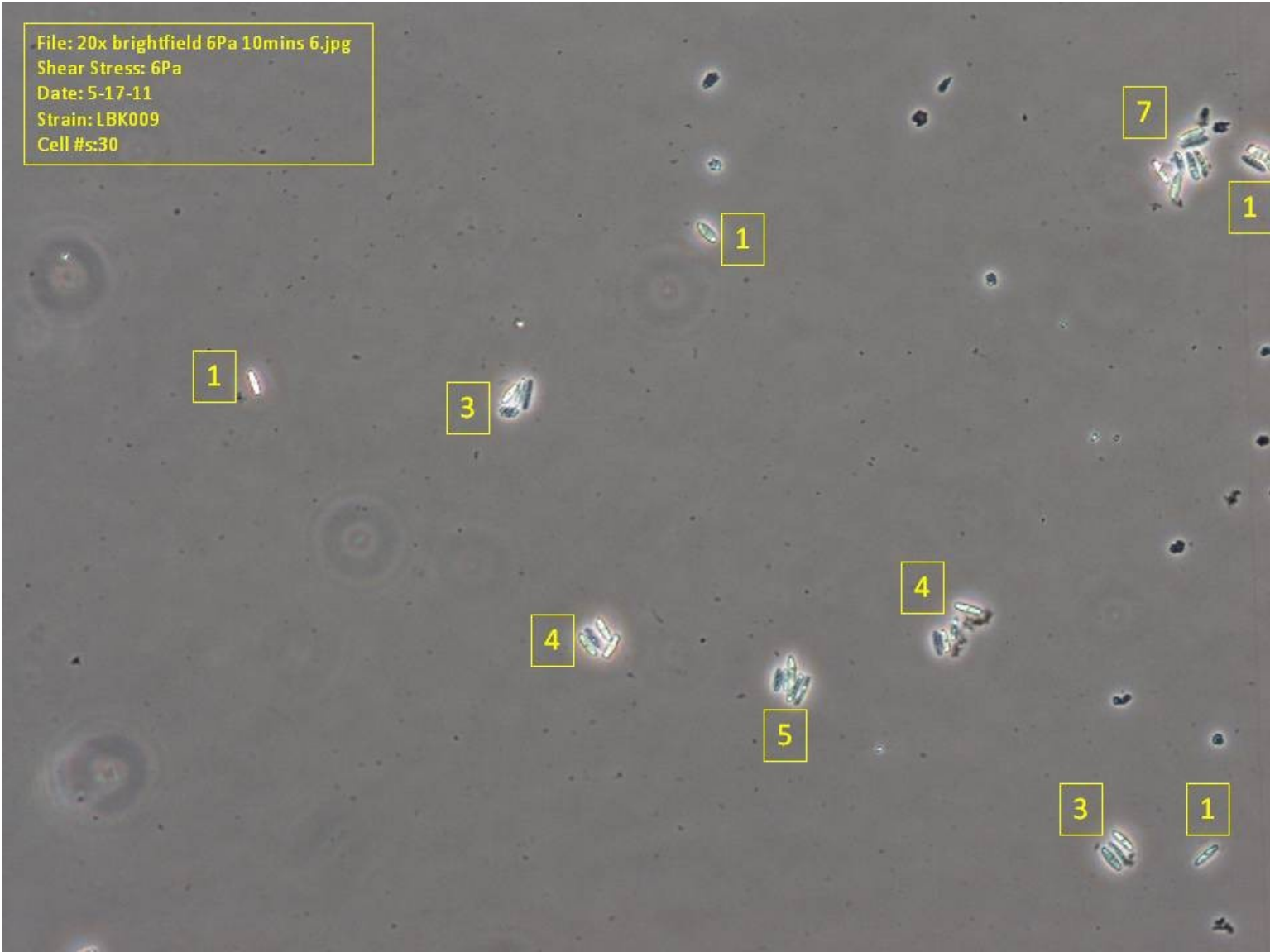


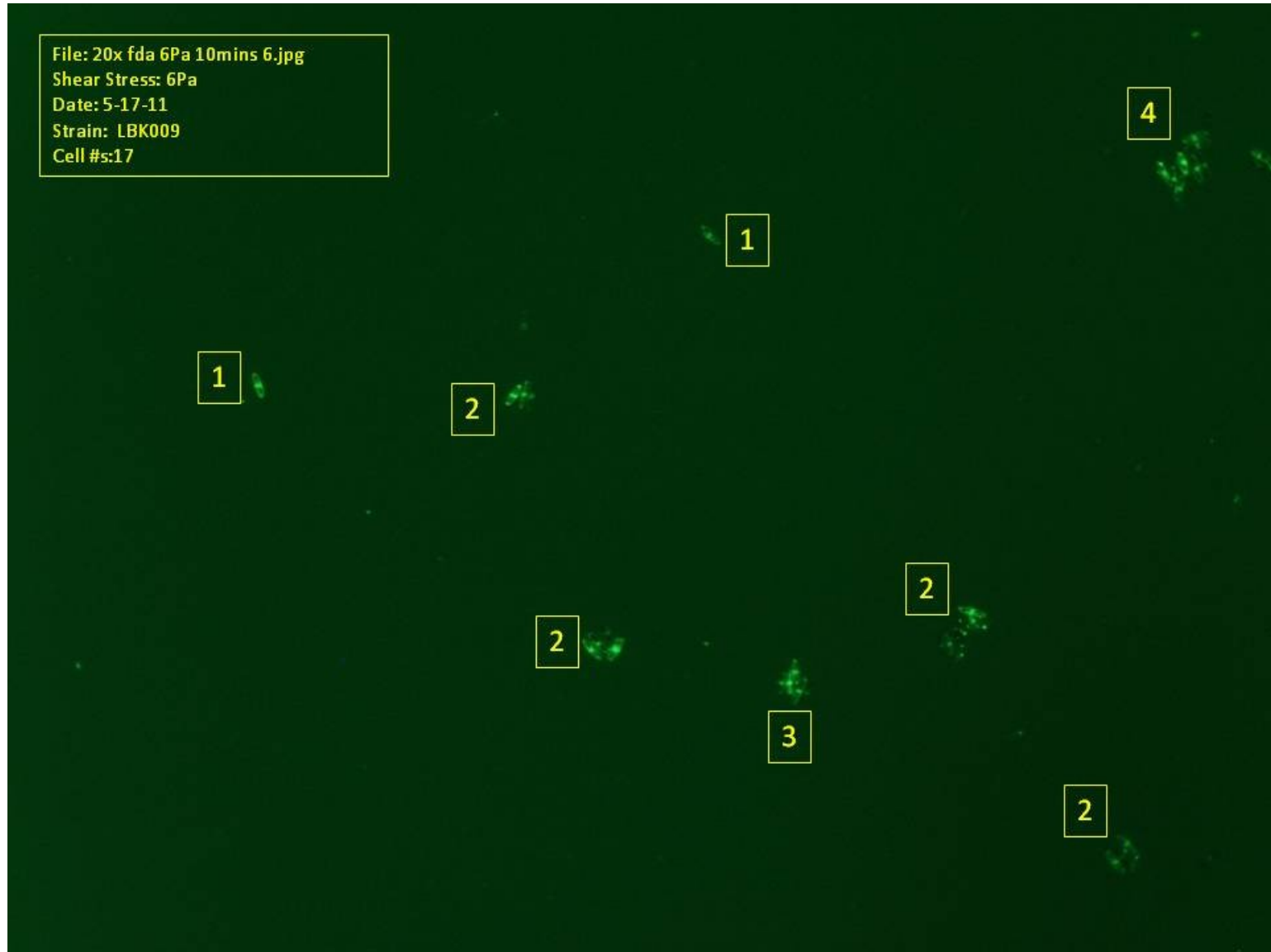






File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-17-11  
Strain: LBK009  
Cell #:s:30



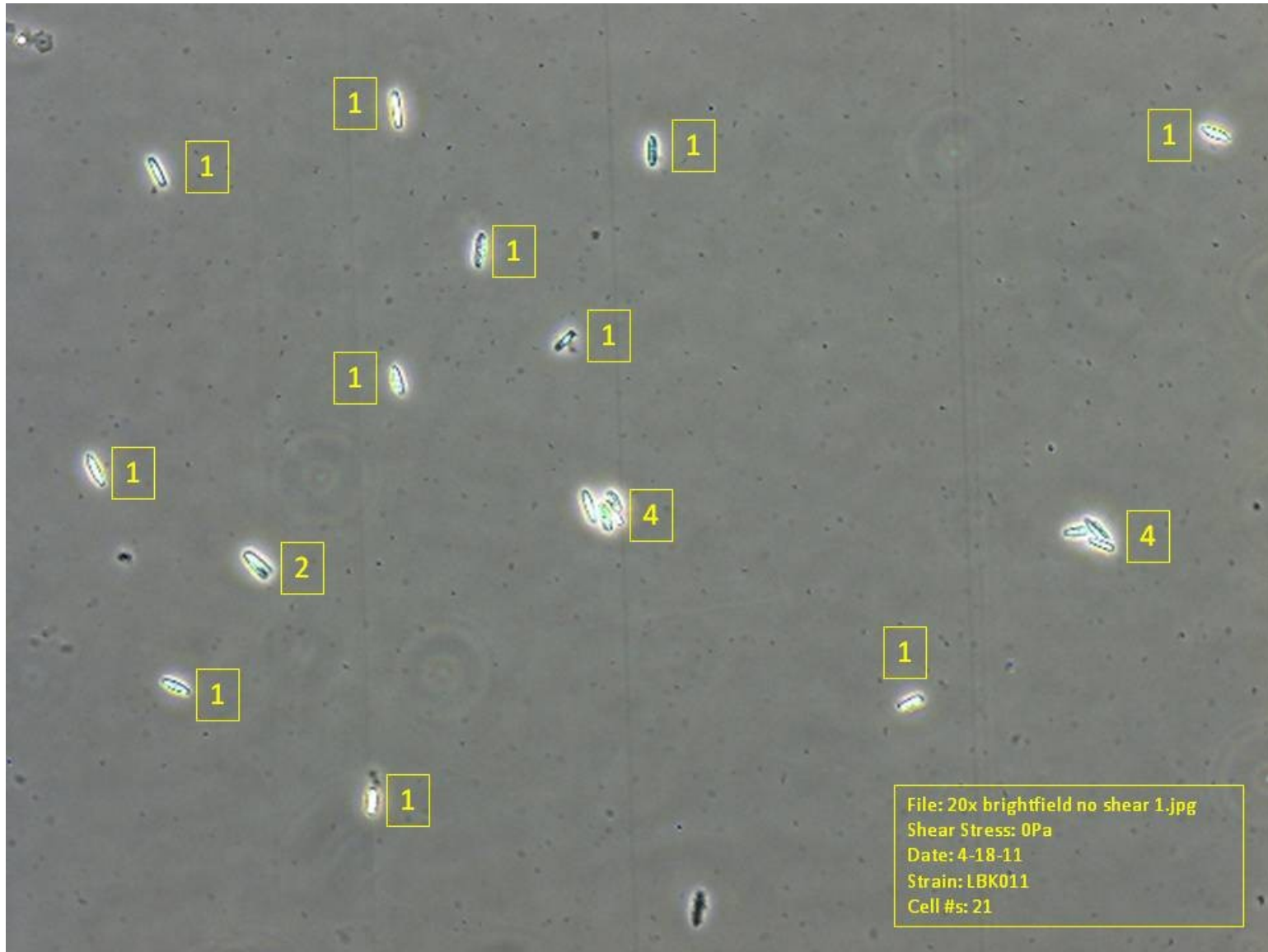


## APPENDIX D

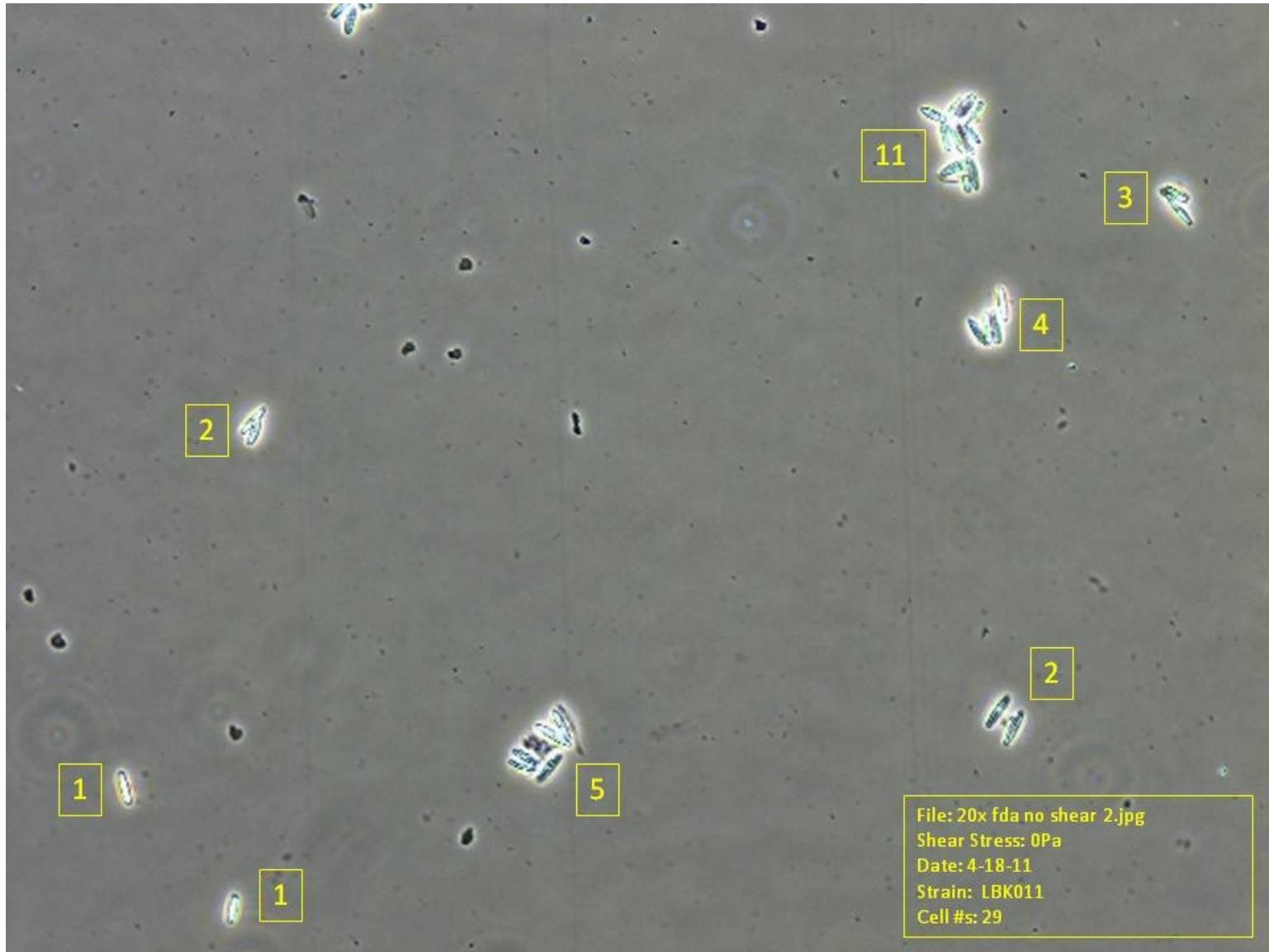
The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK011, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Average Cell Viability	Average Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	21	18	29	21	12	9	23	14	14	7	7	2	66.98%	13.49%	-0.02249
	6	15	7	26	16			15	10	11	4	19	9	53.49%		
Rep 2	0	10	8	25	20	15	12					23	12	71.23%	6.60%	-0.011
	6	17	13	22	16	8	6	18	5	17	13			64.63%		
Rep 3	0			18	15	15	9	34	26	17	8	18	9	65.69%	13.31%	-0.02218
	6	14	7	17	10	24	12	20	9	30	17			52.38%		
														<b>Avg.</b>	<b>11.13%</b>	
														<b>Std. Dev.</b>	<b>3.93%</b>	

The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.

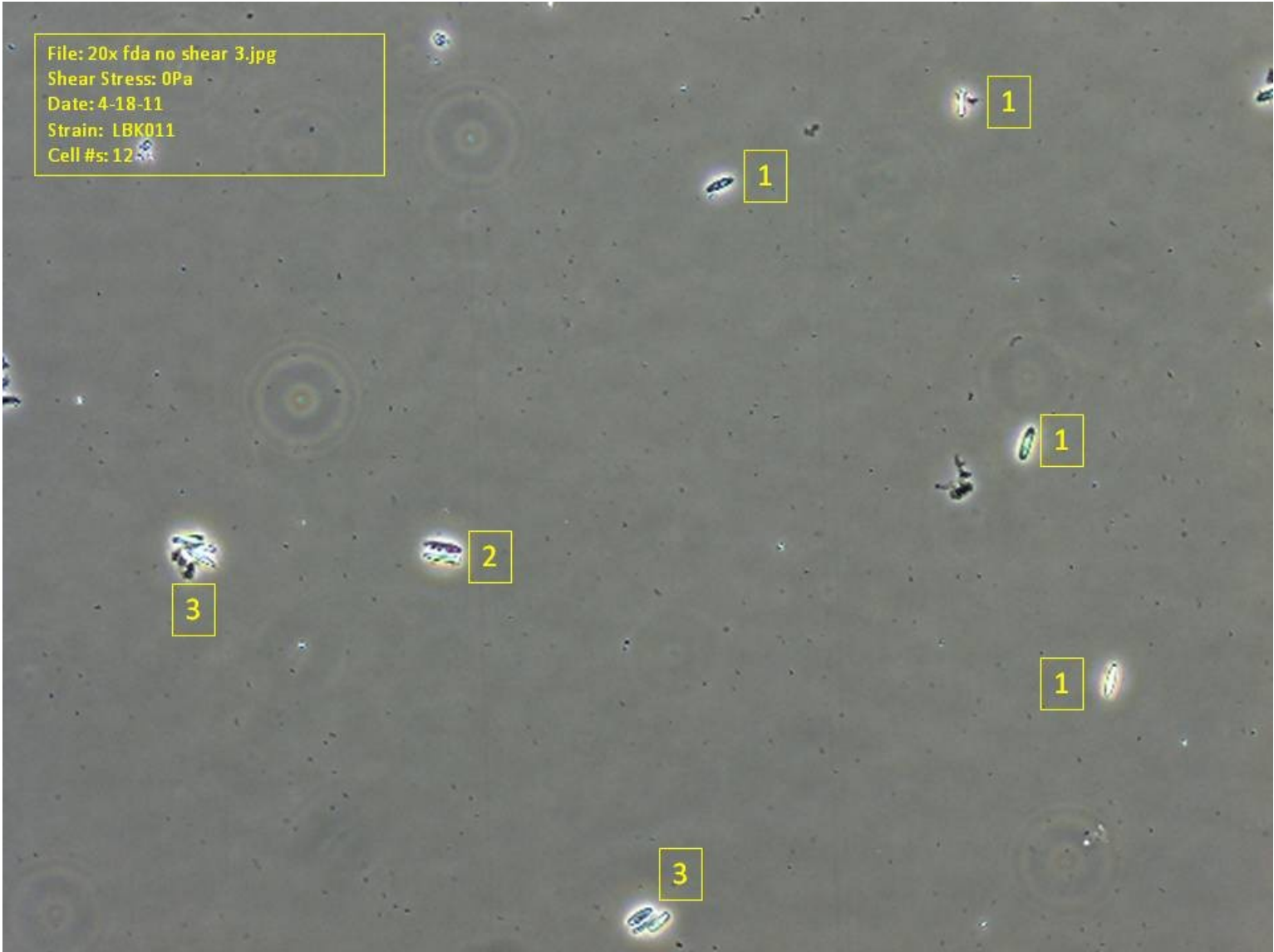




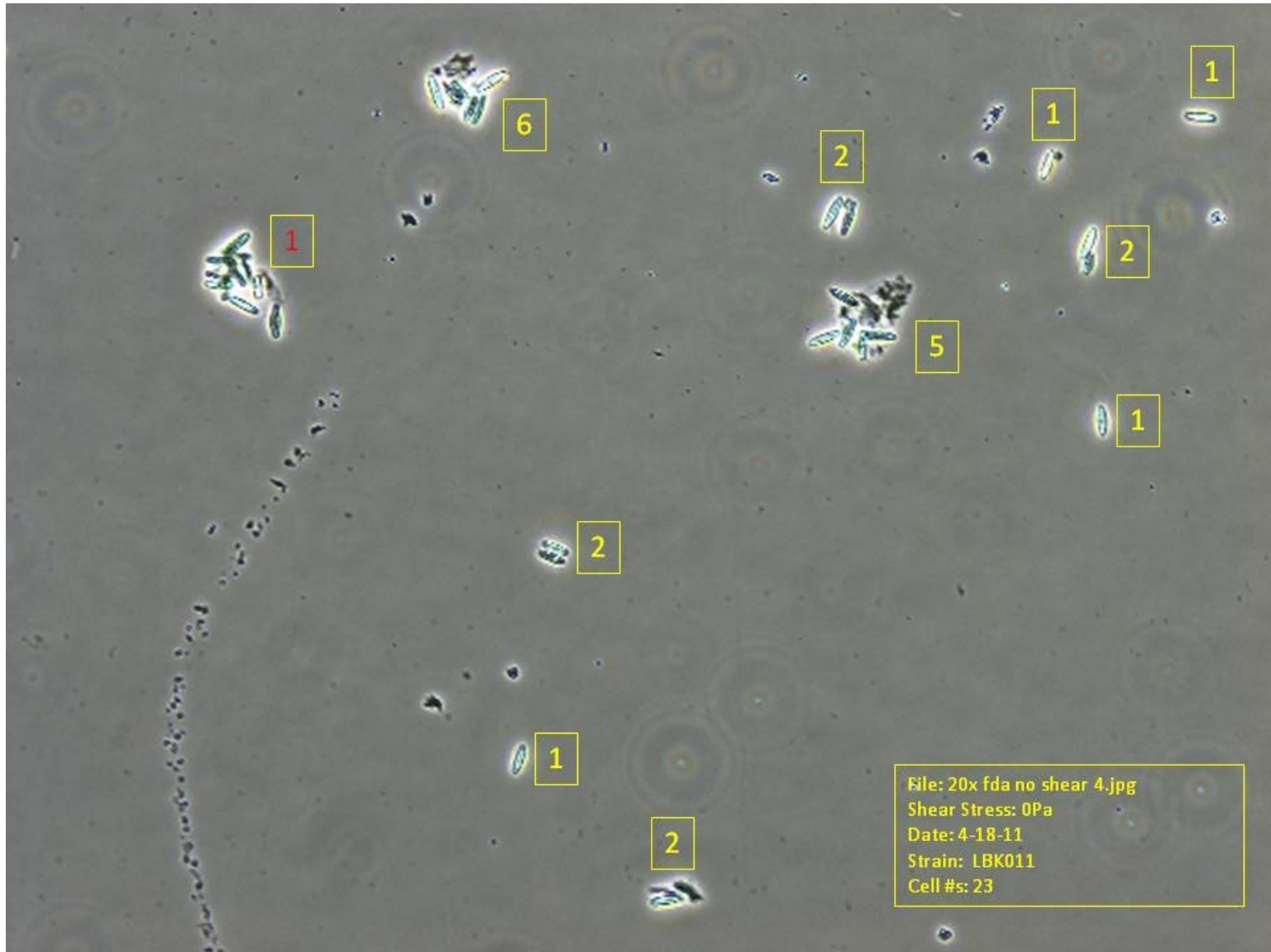


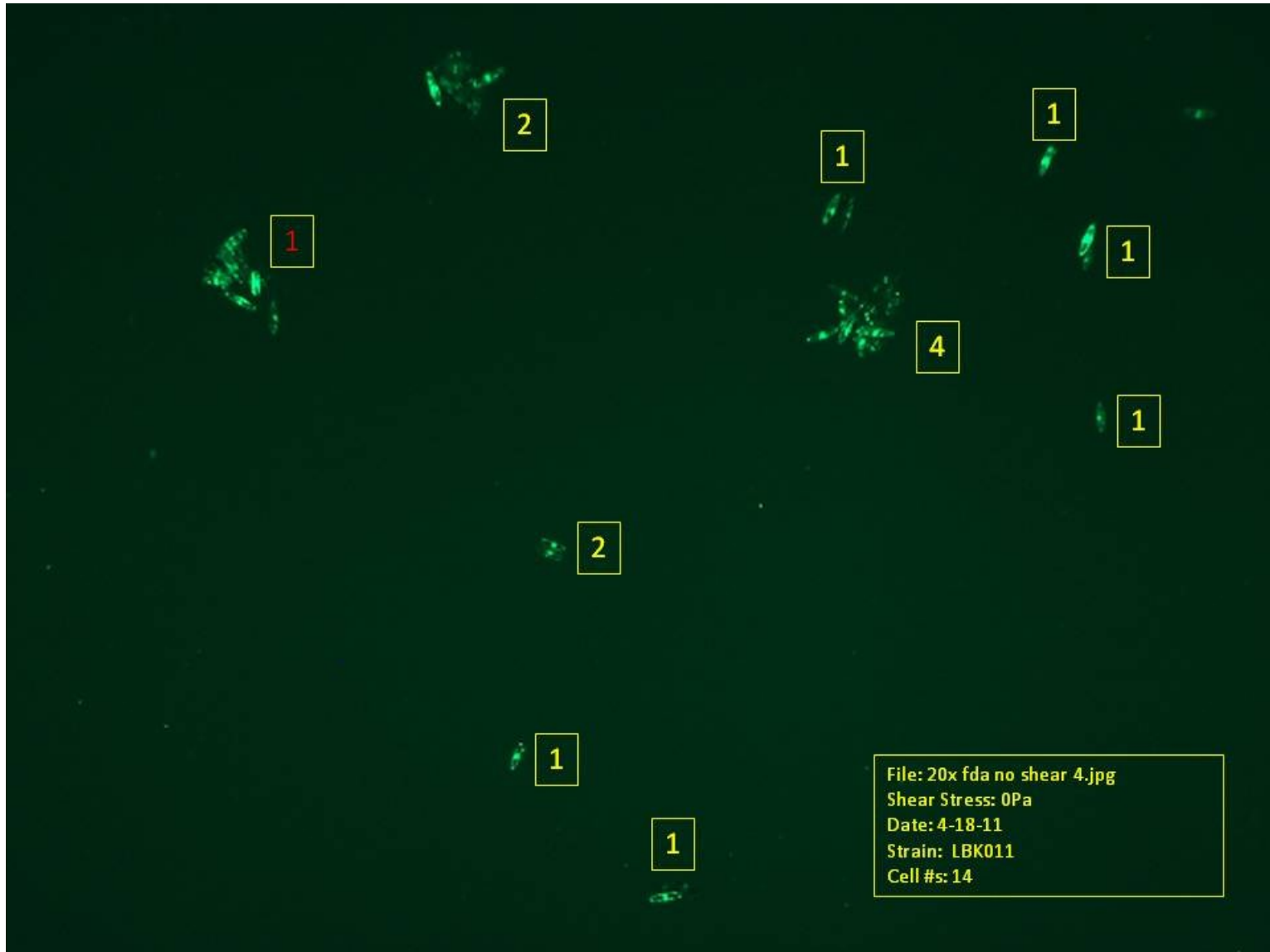


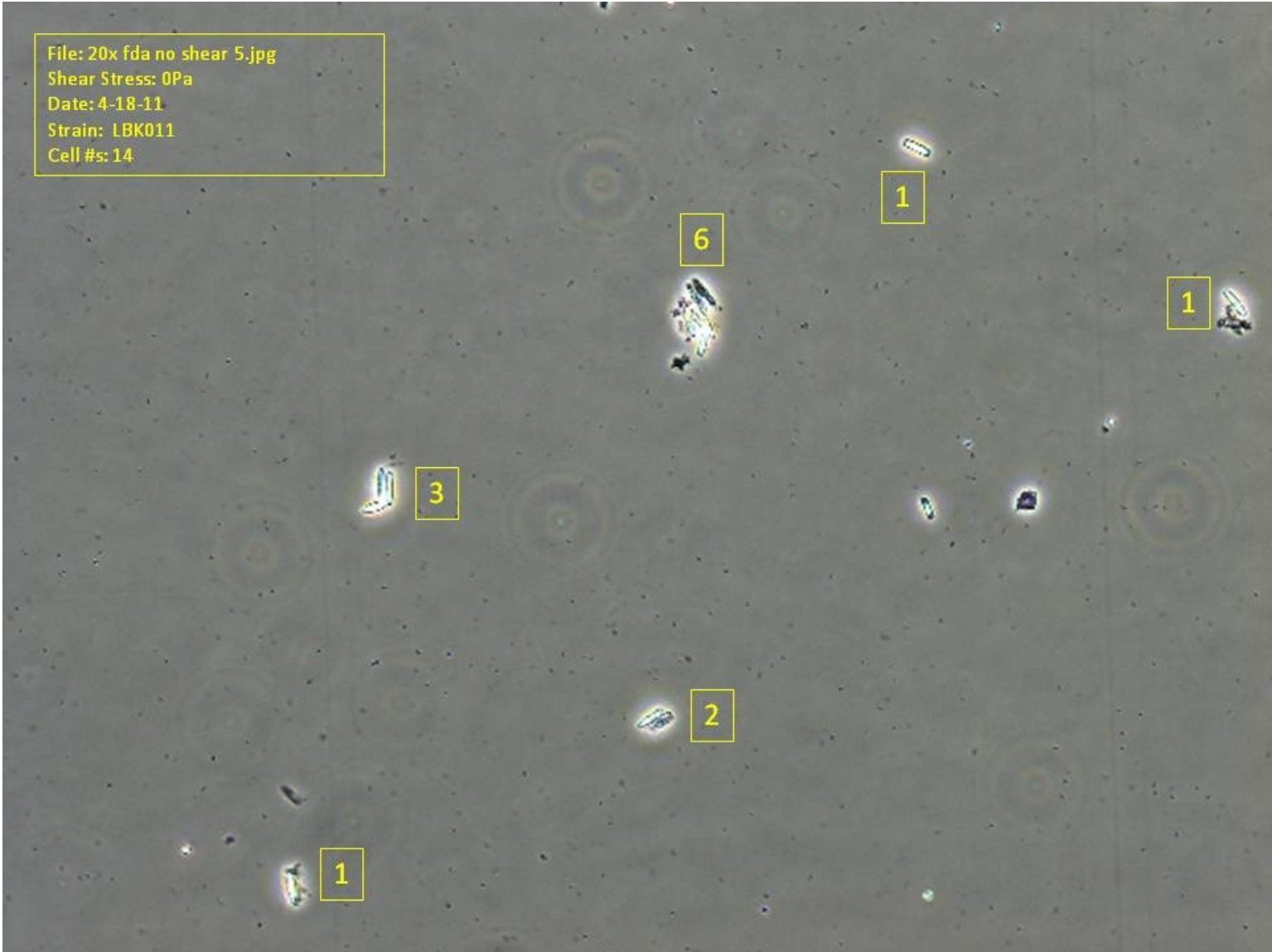






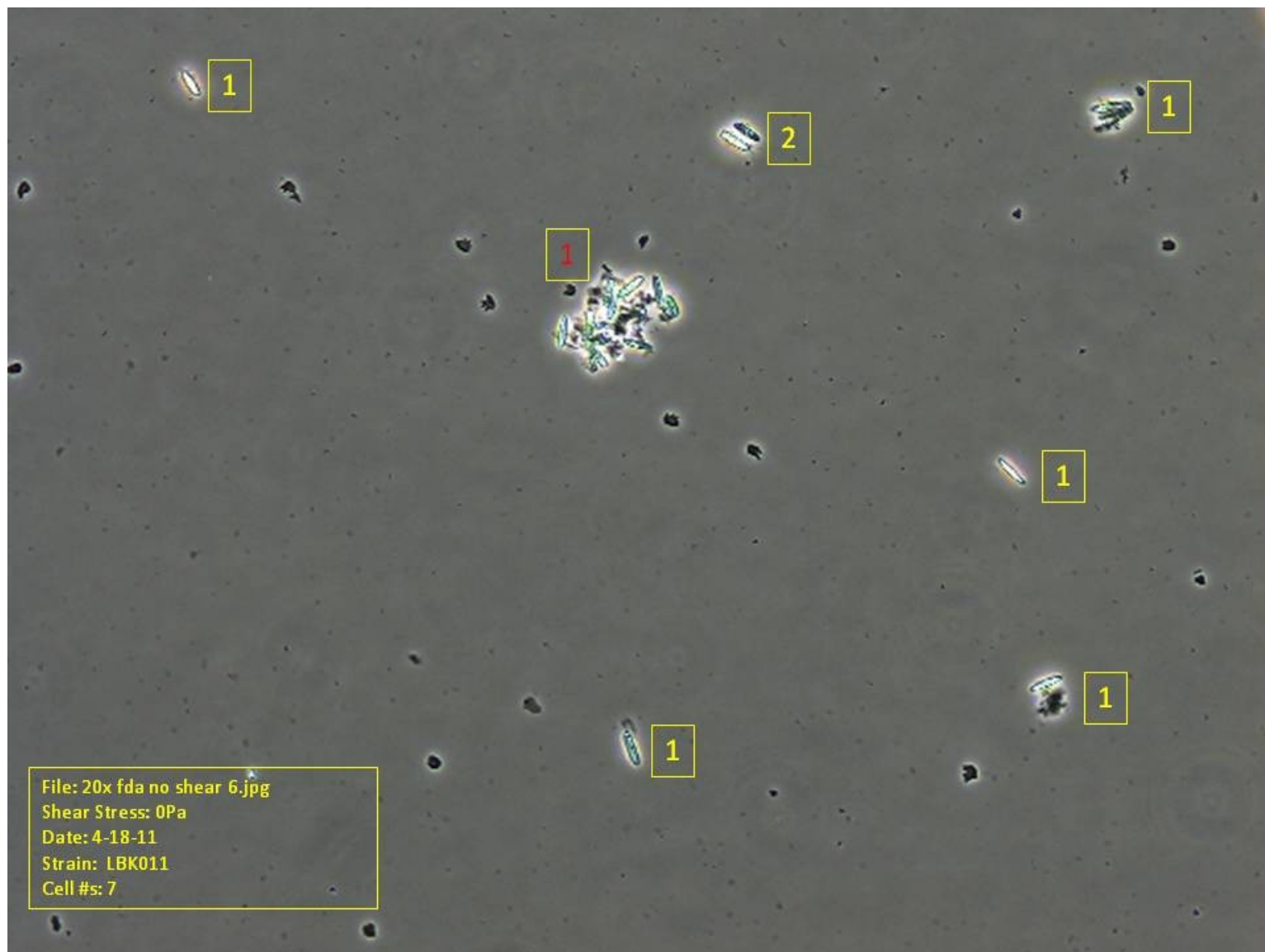






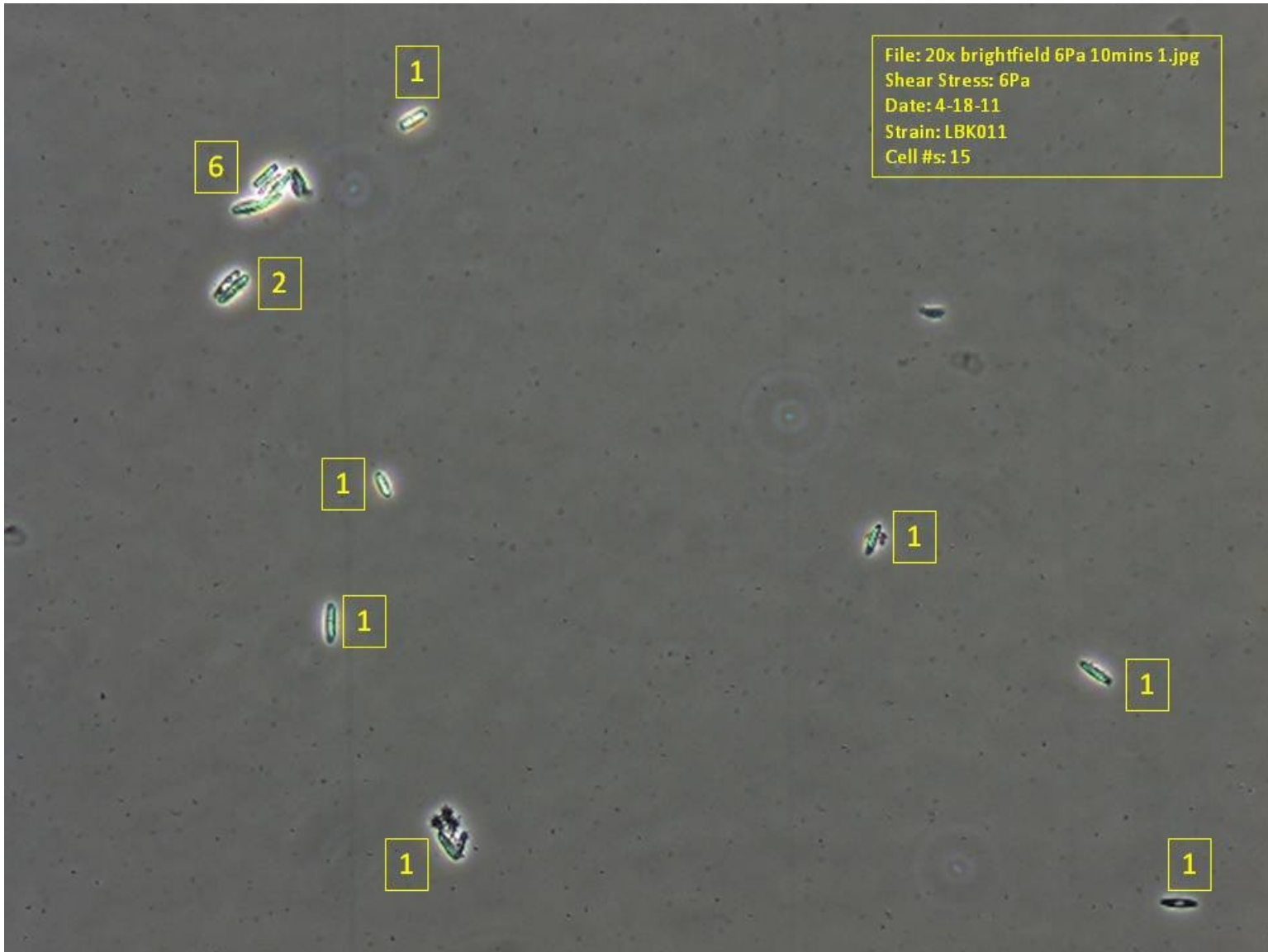
File: 20x fda no shear 5.jpg  
Shear Stress: 0Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 7

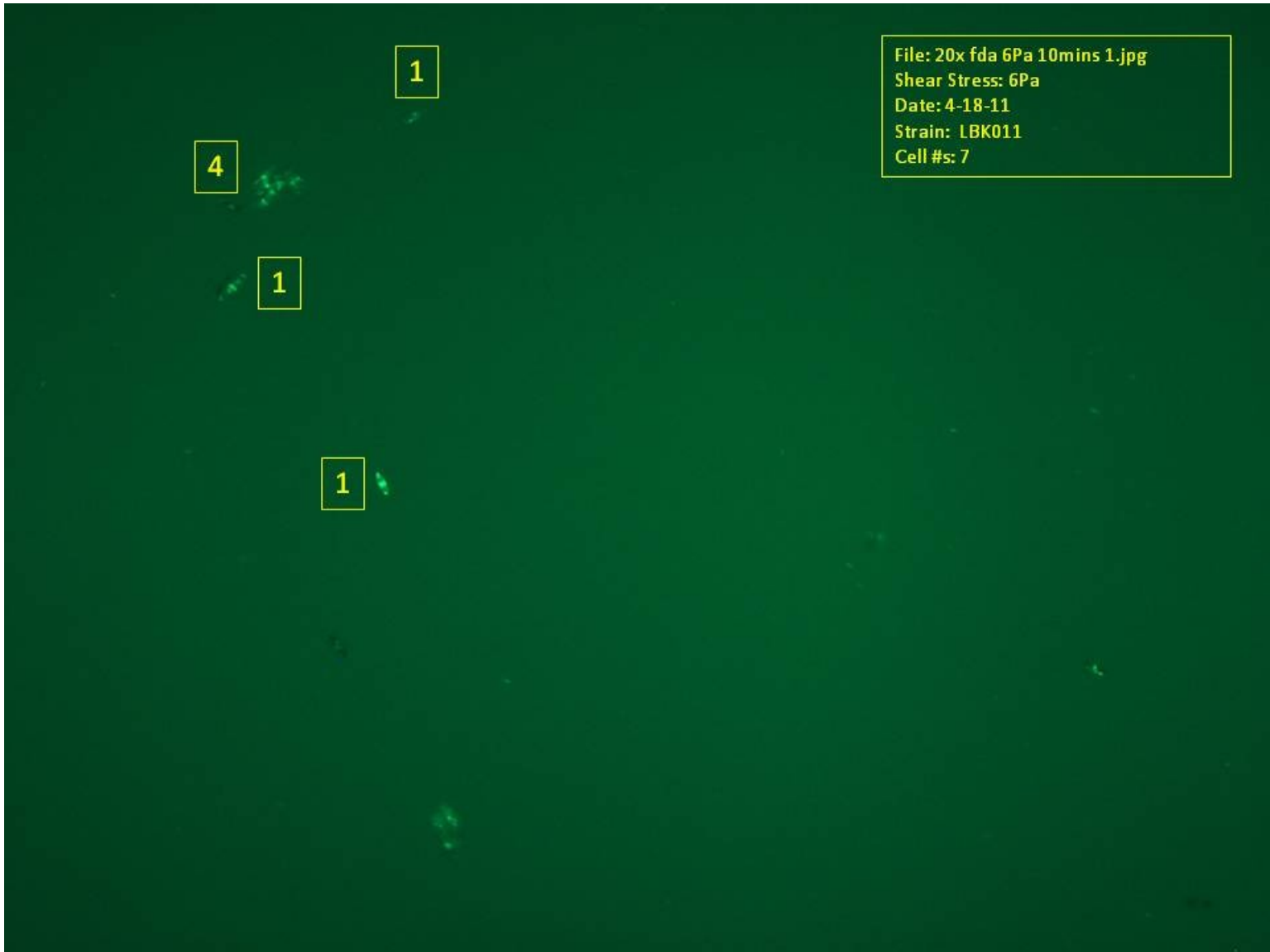


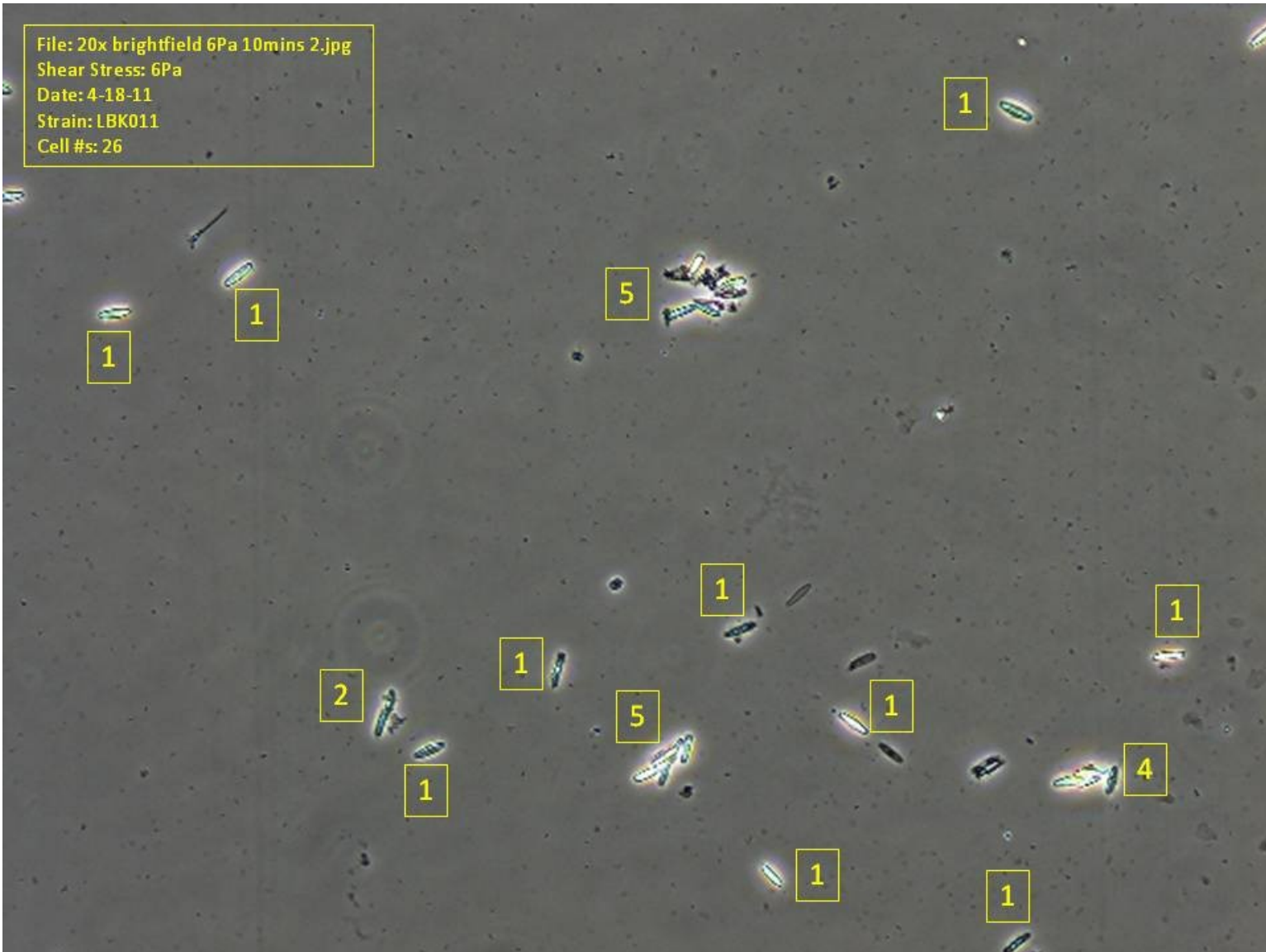


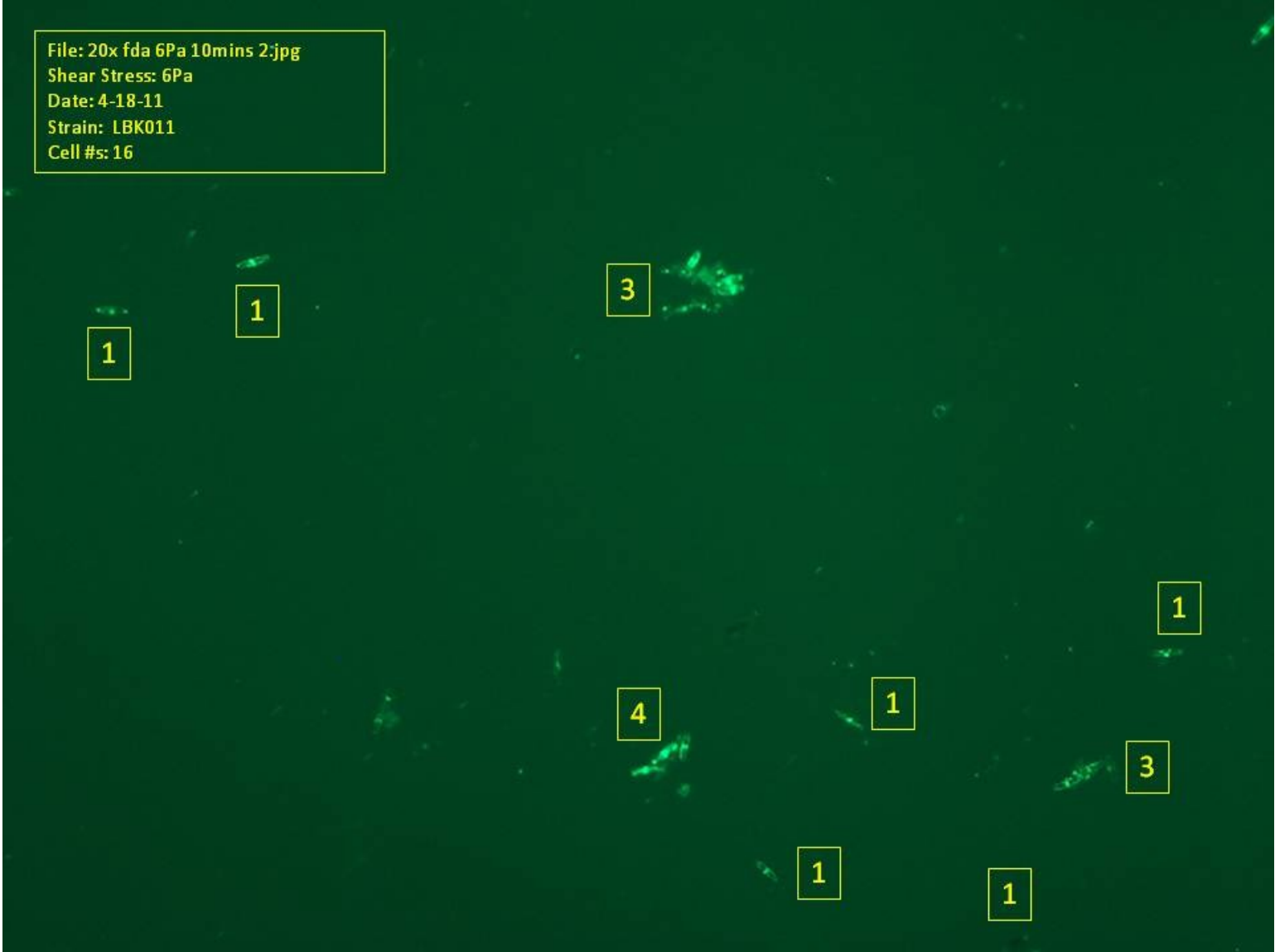




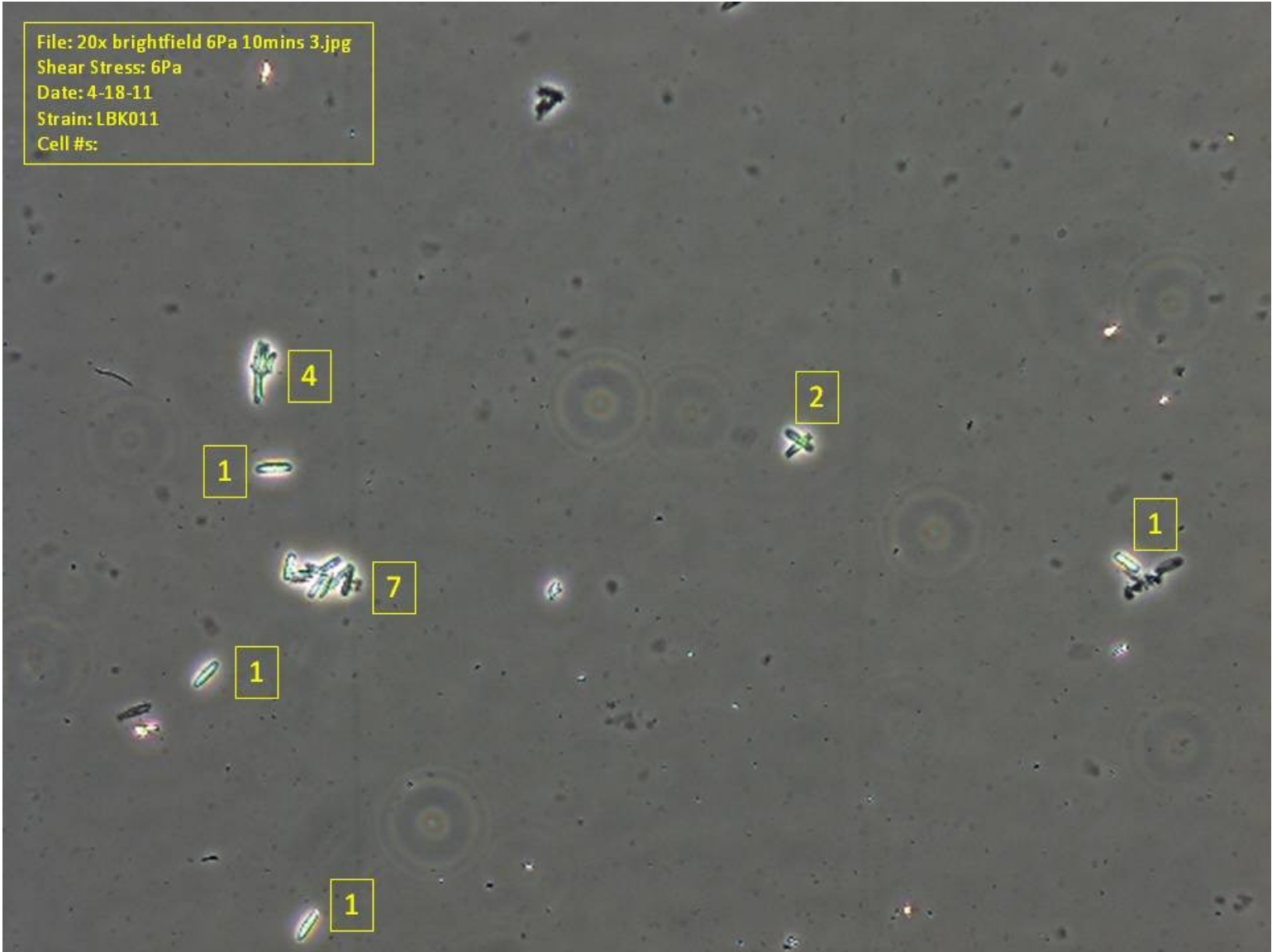




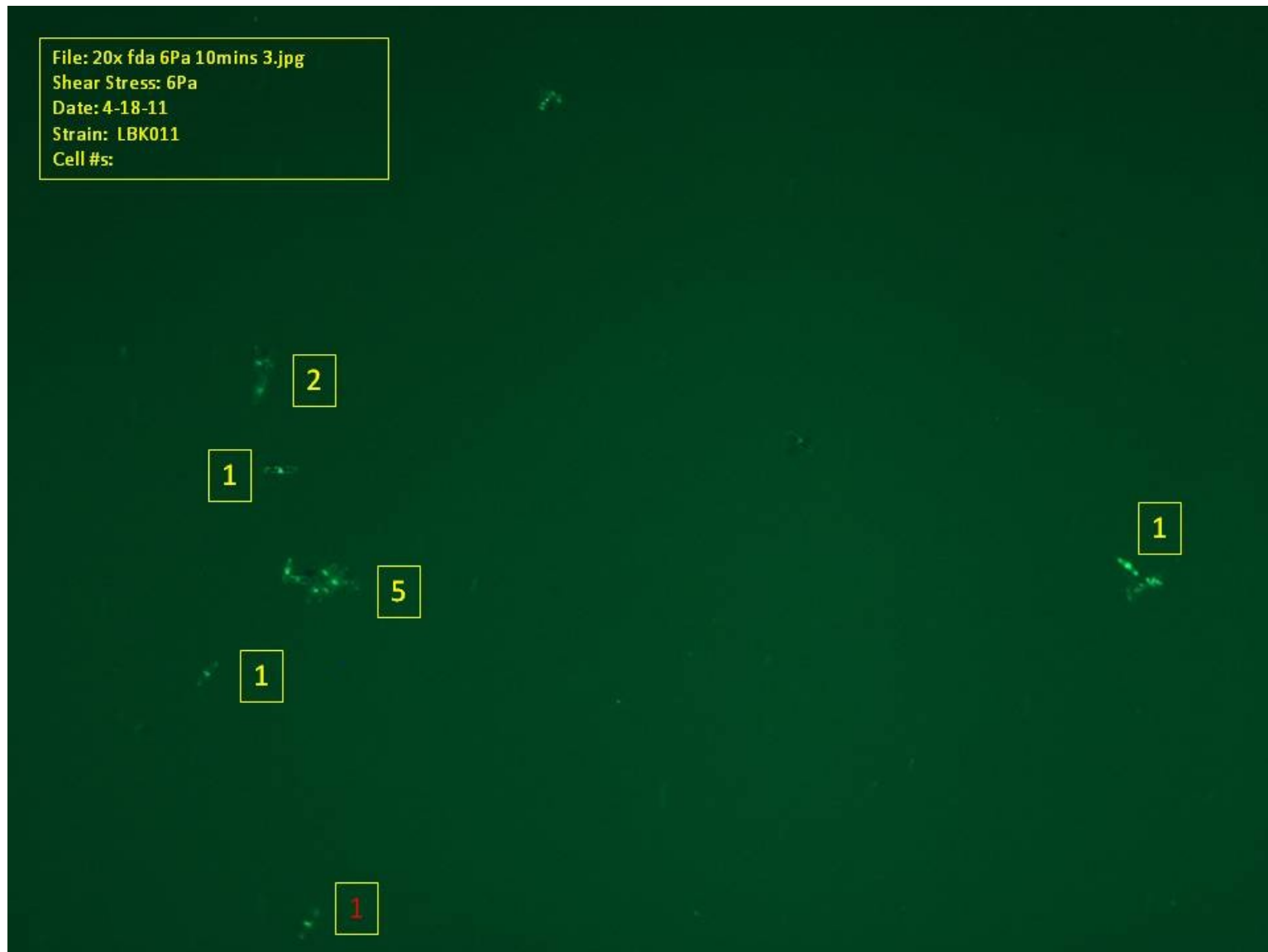


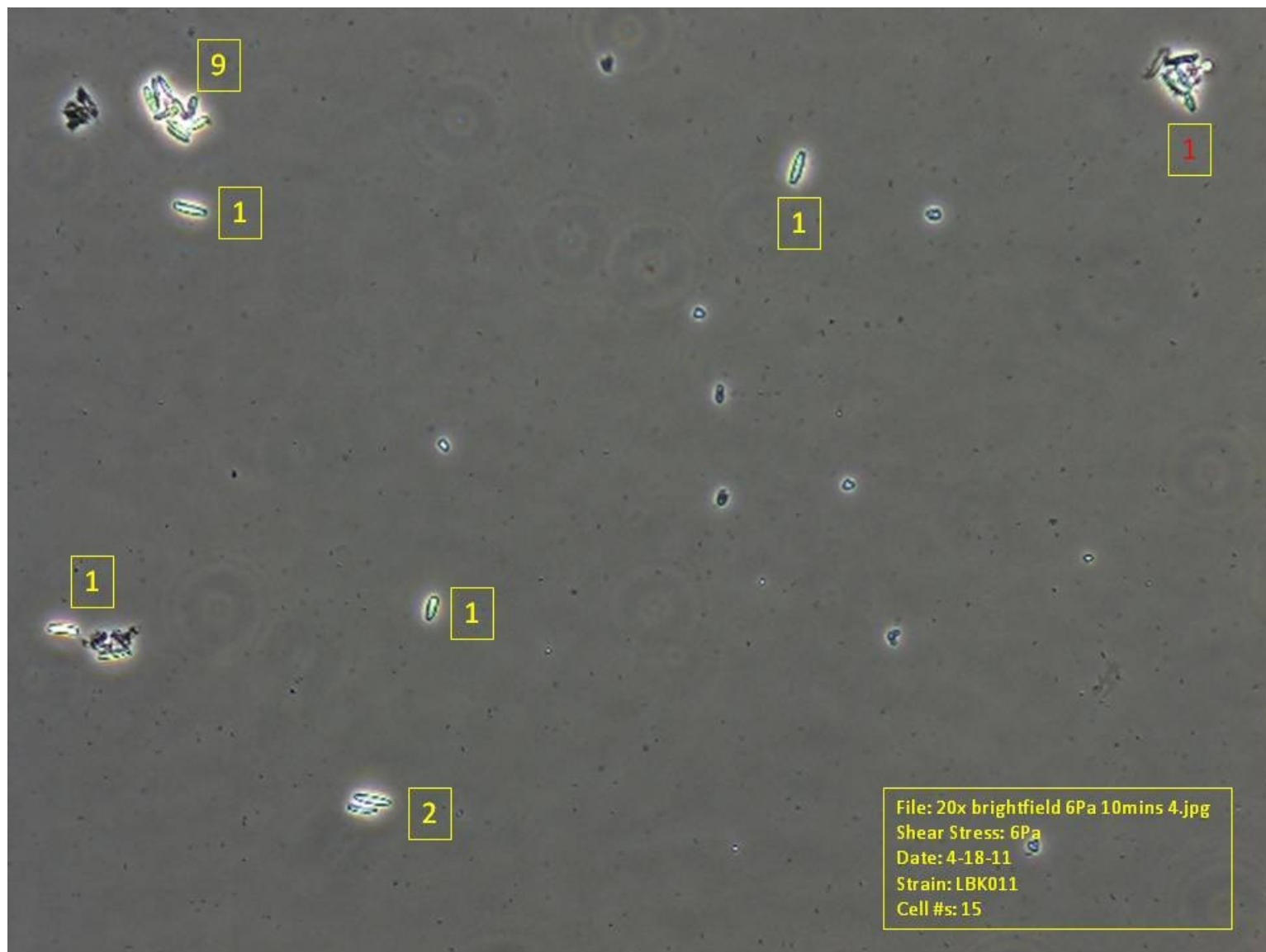


File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s:



File: 20x fda 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s:

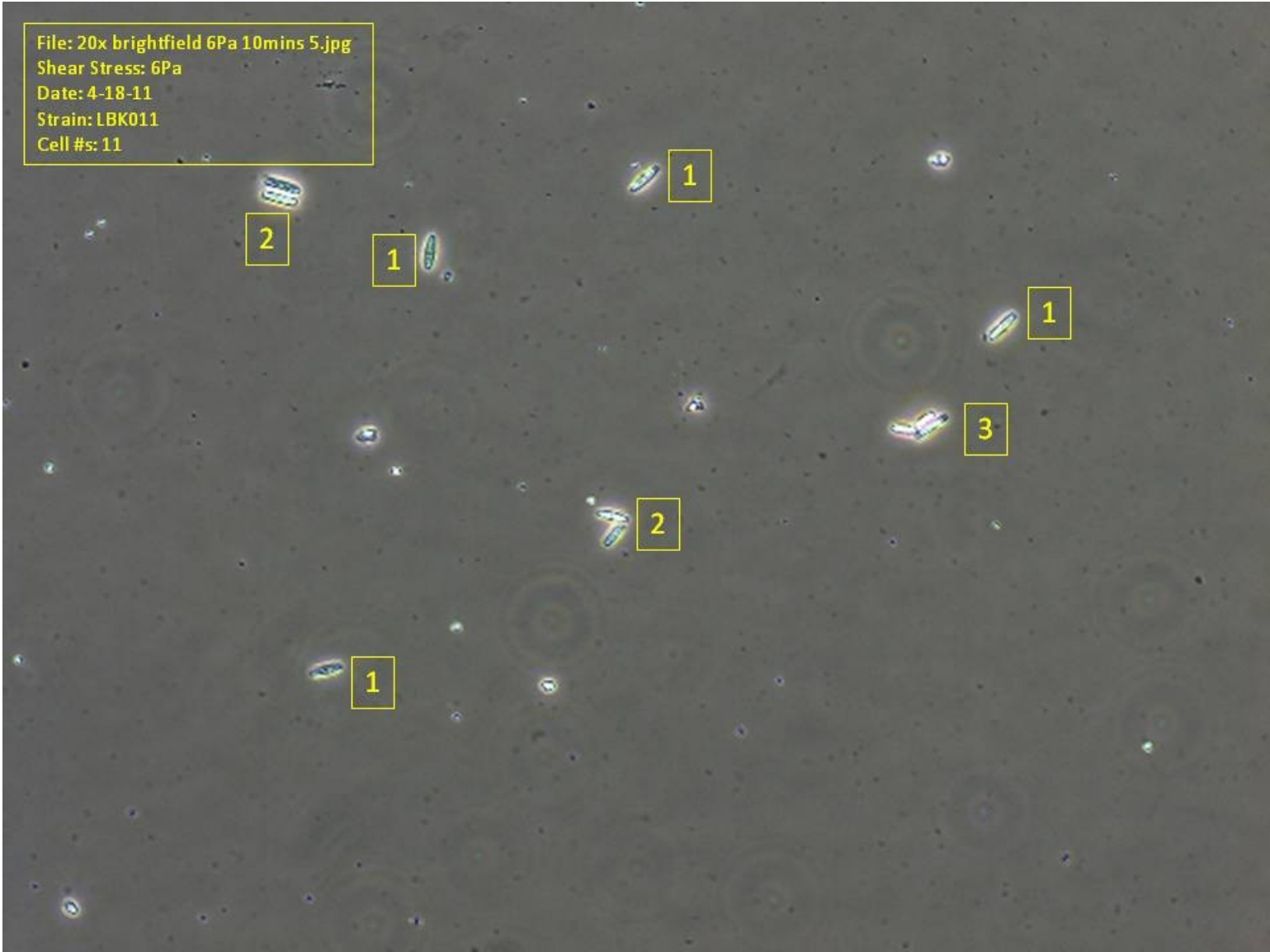






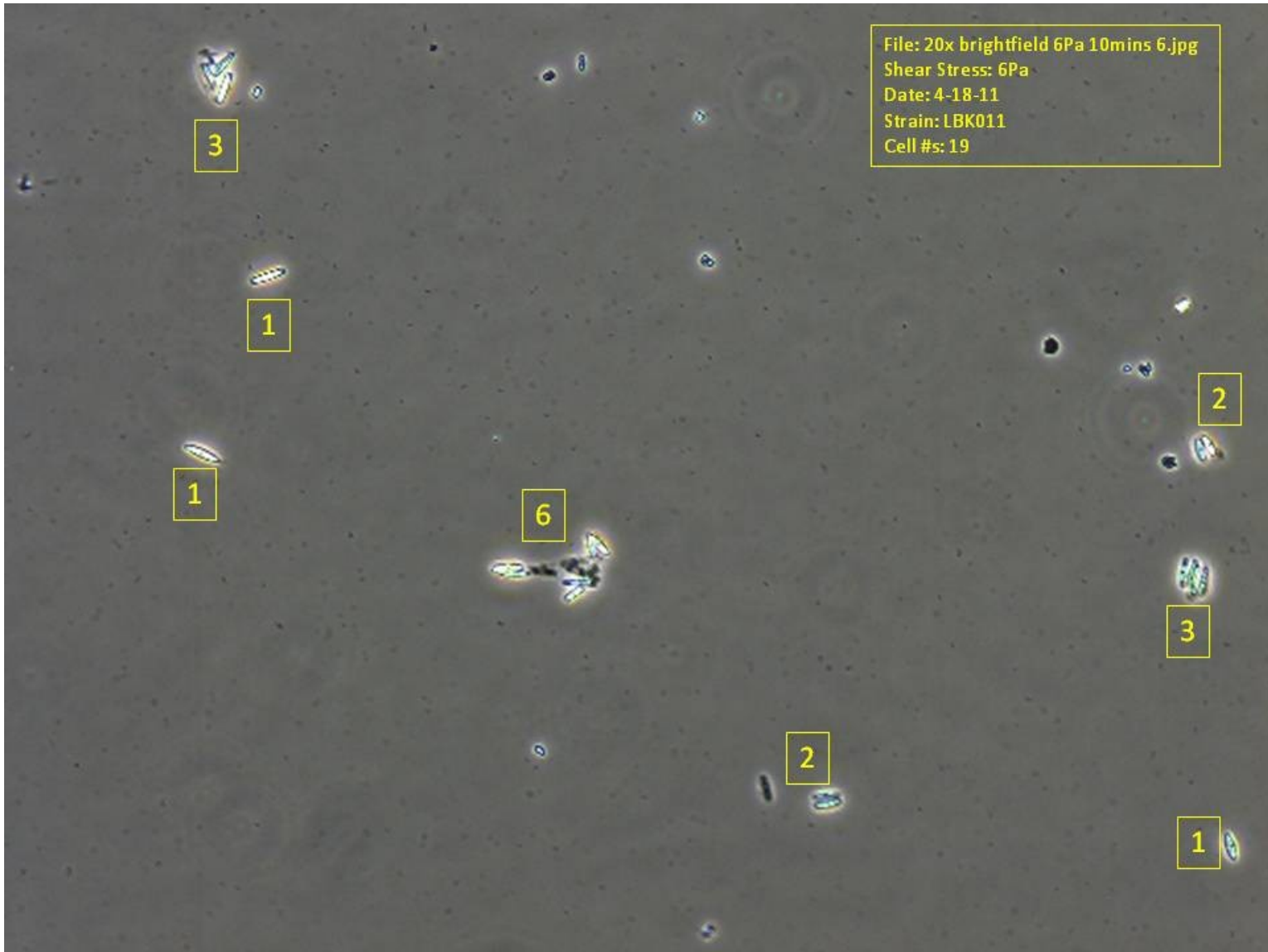


File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 11

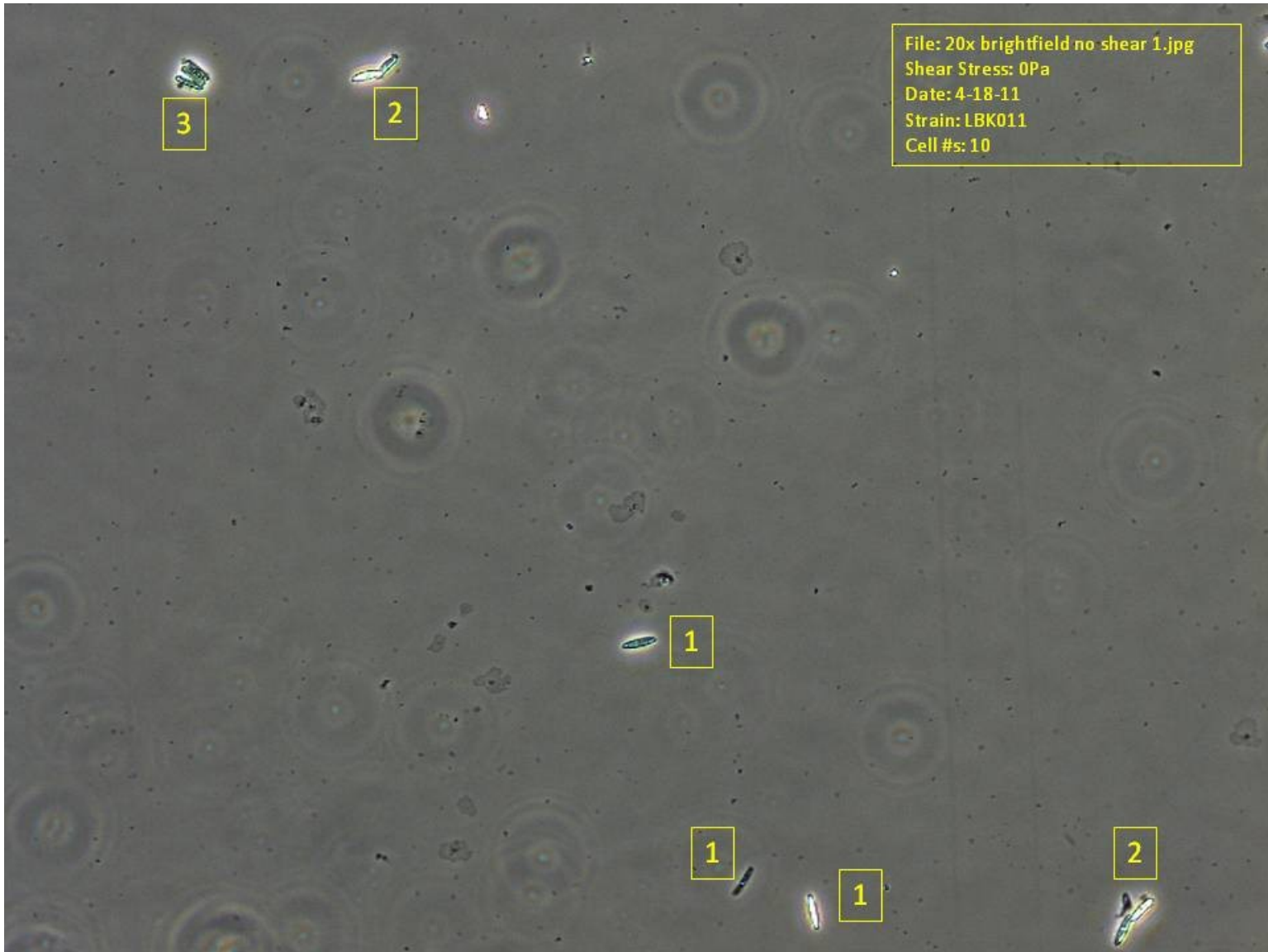


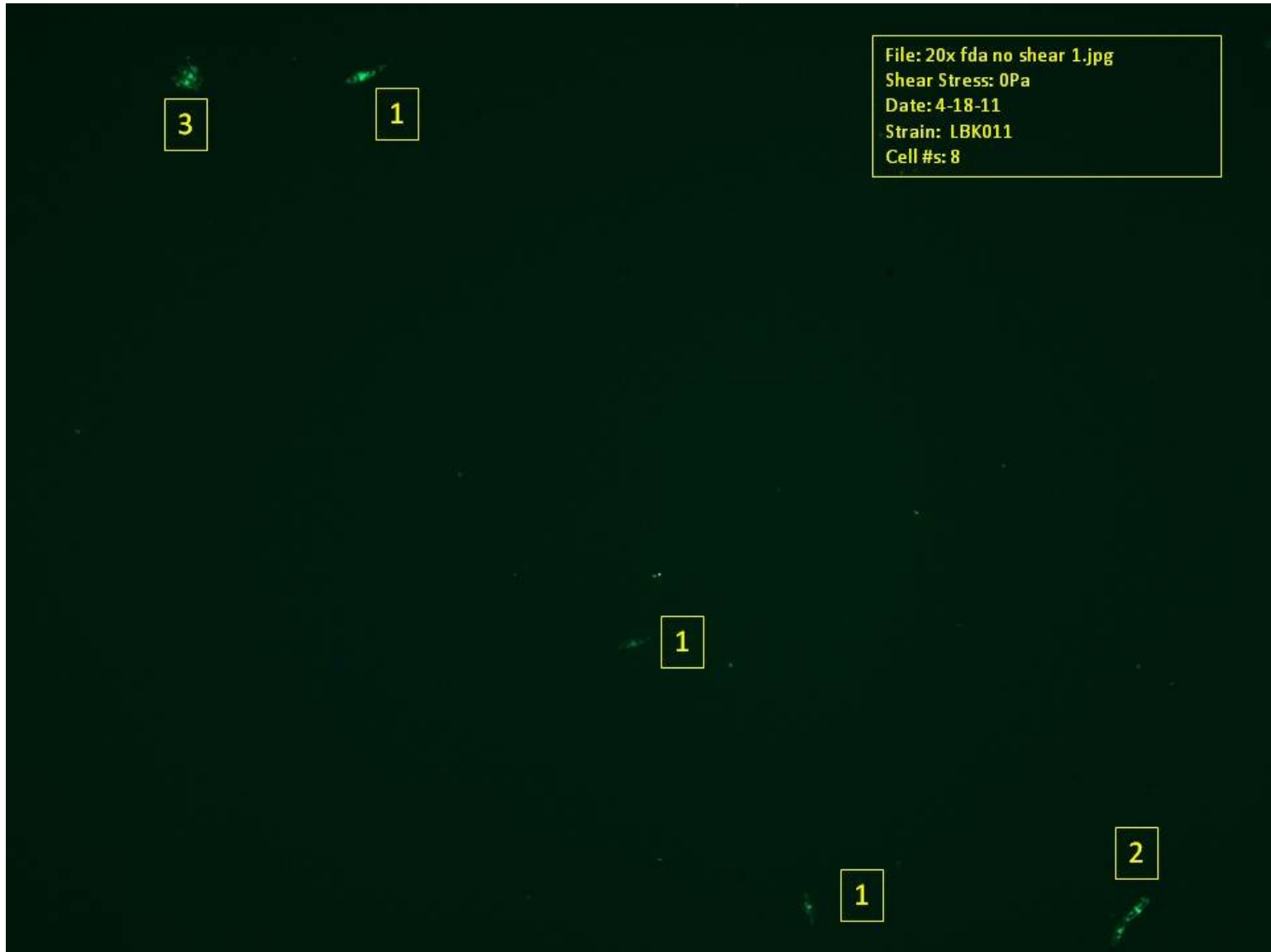
File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 4

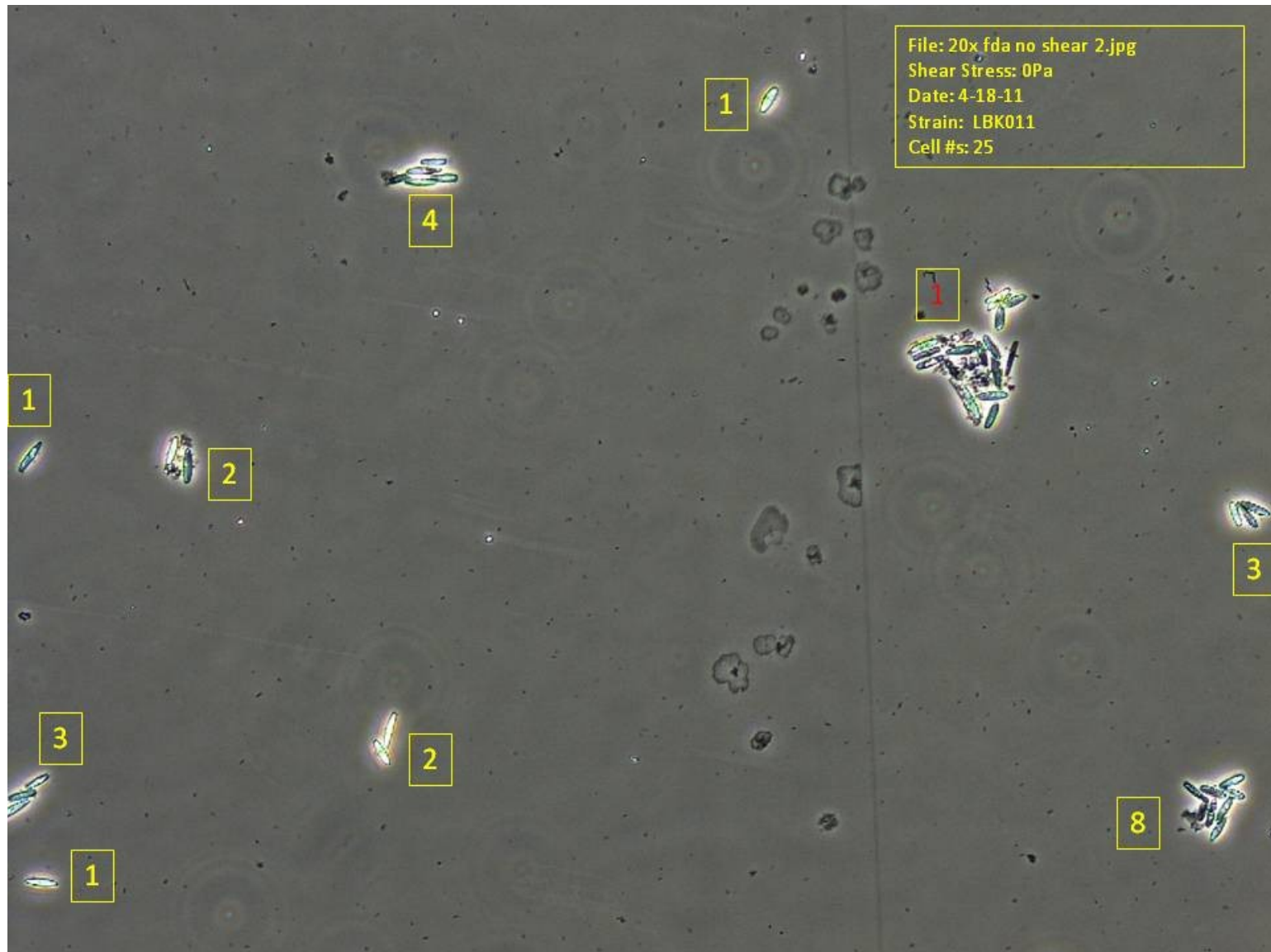






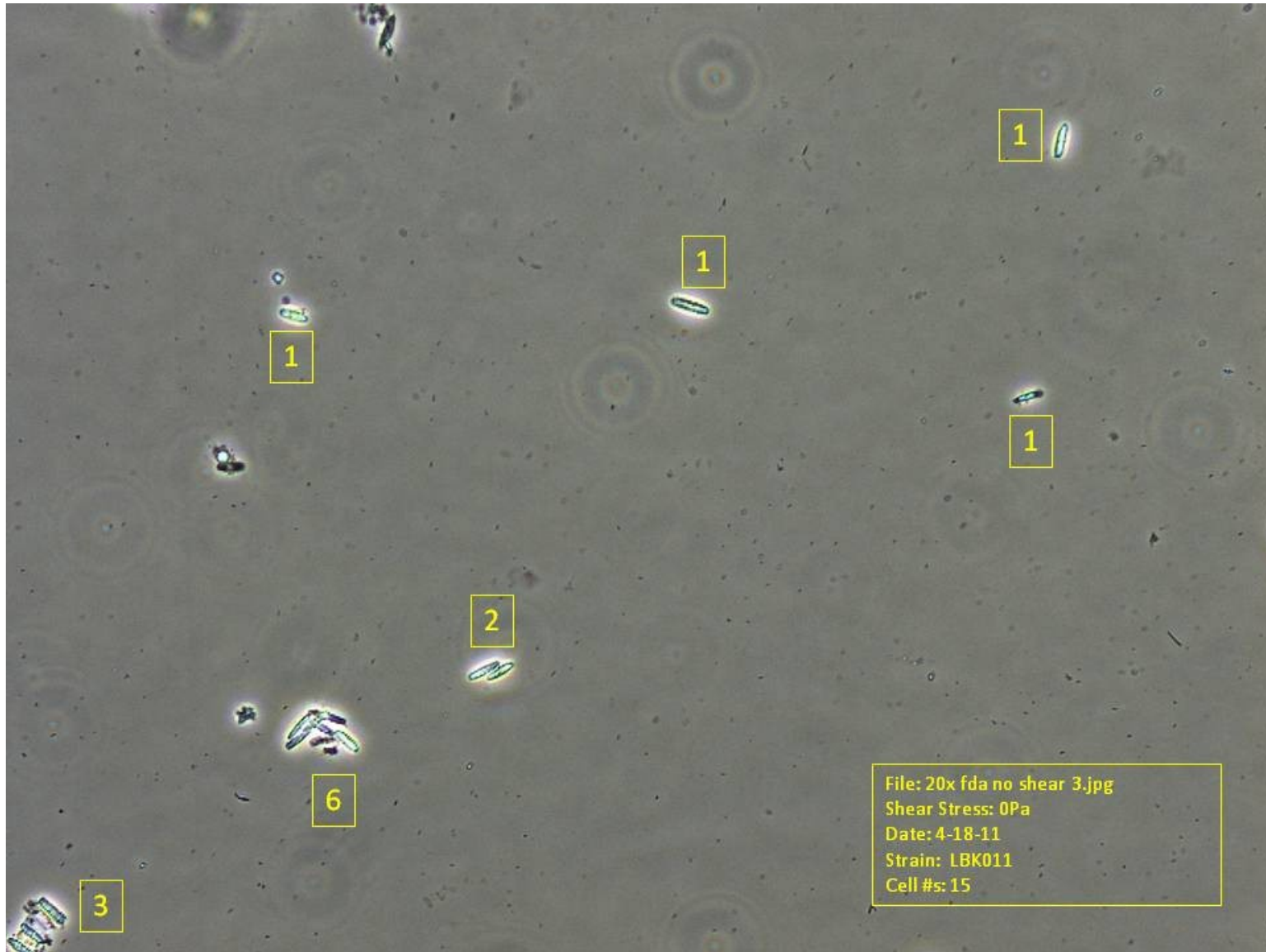




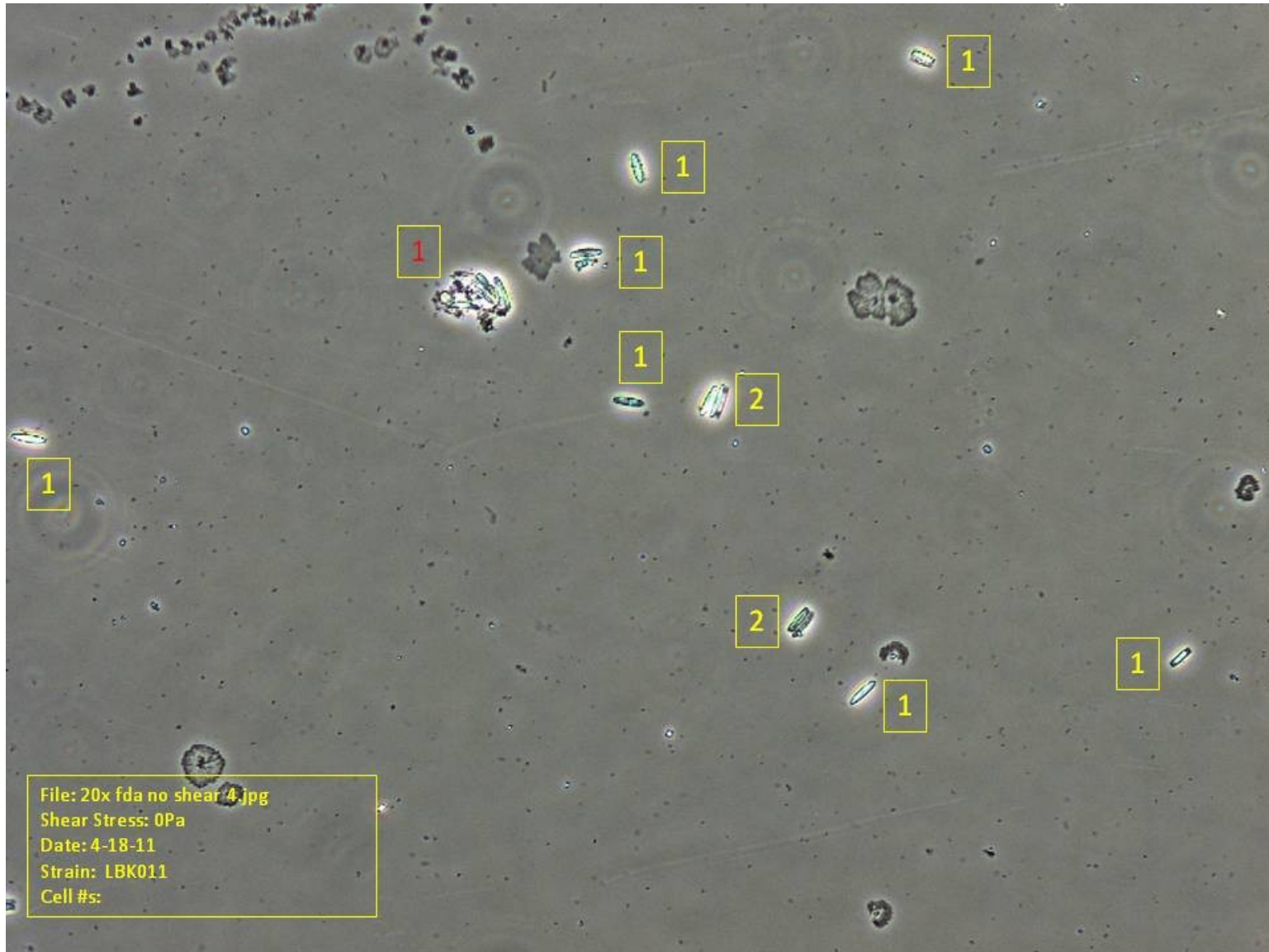




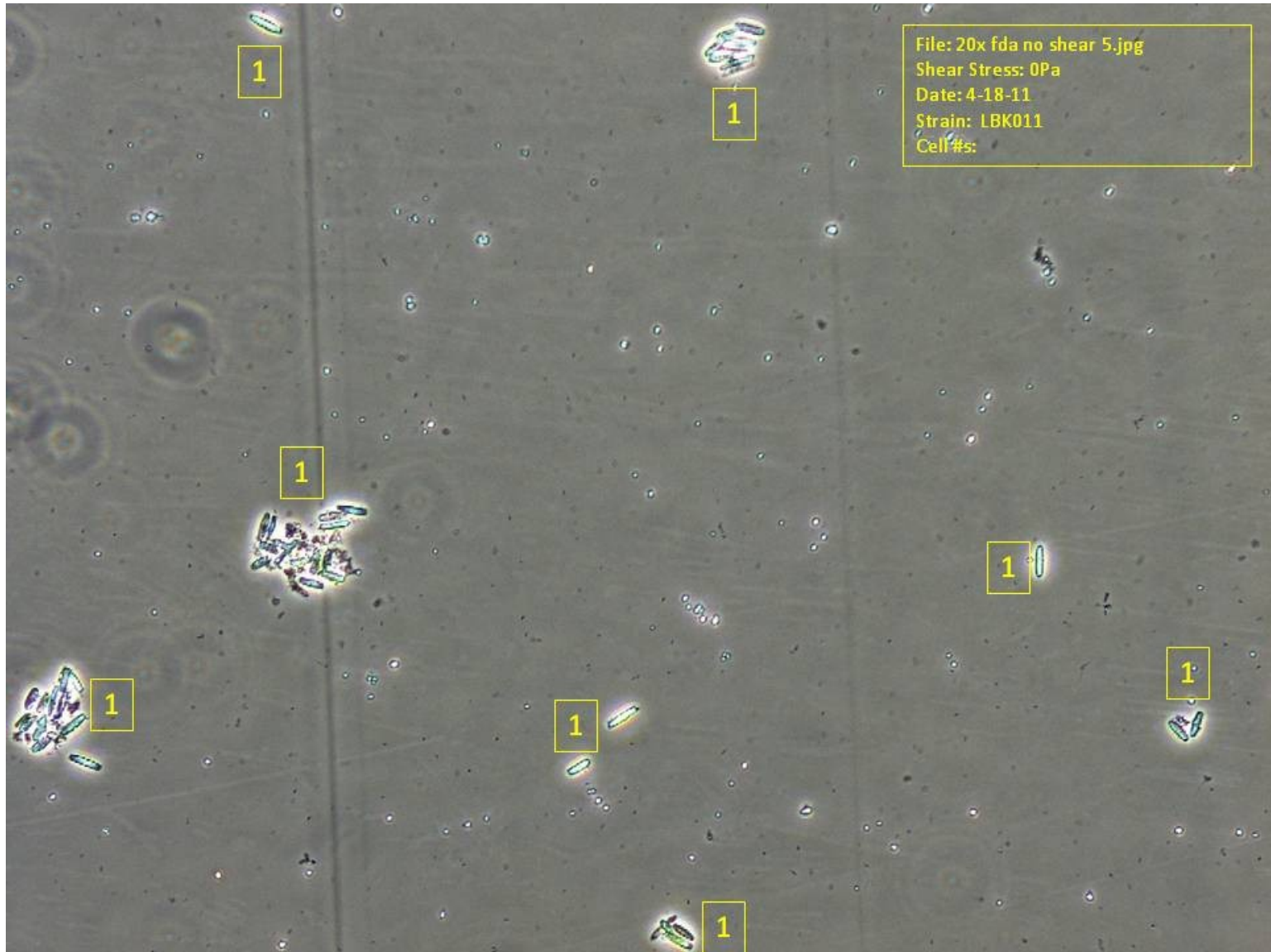


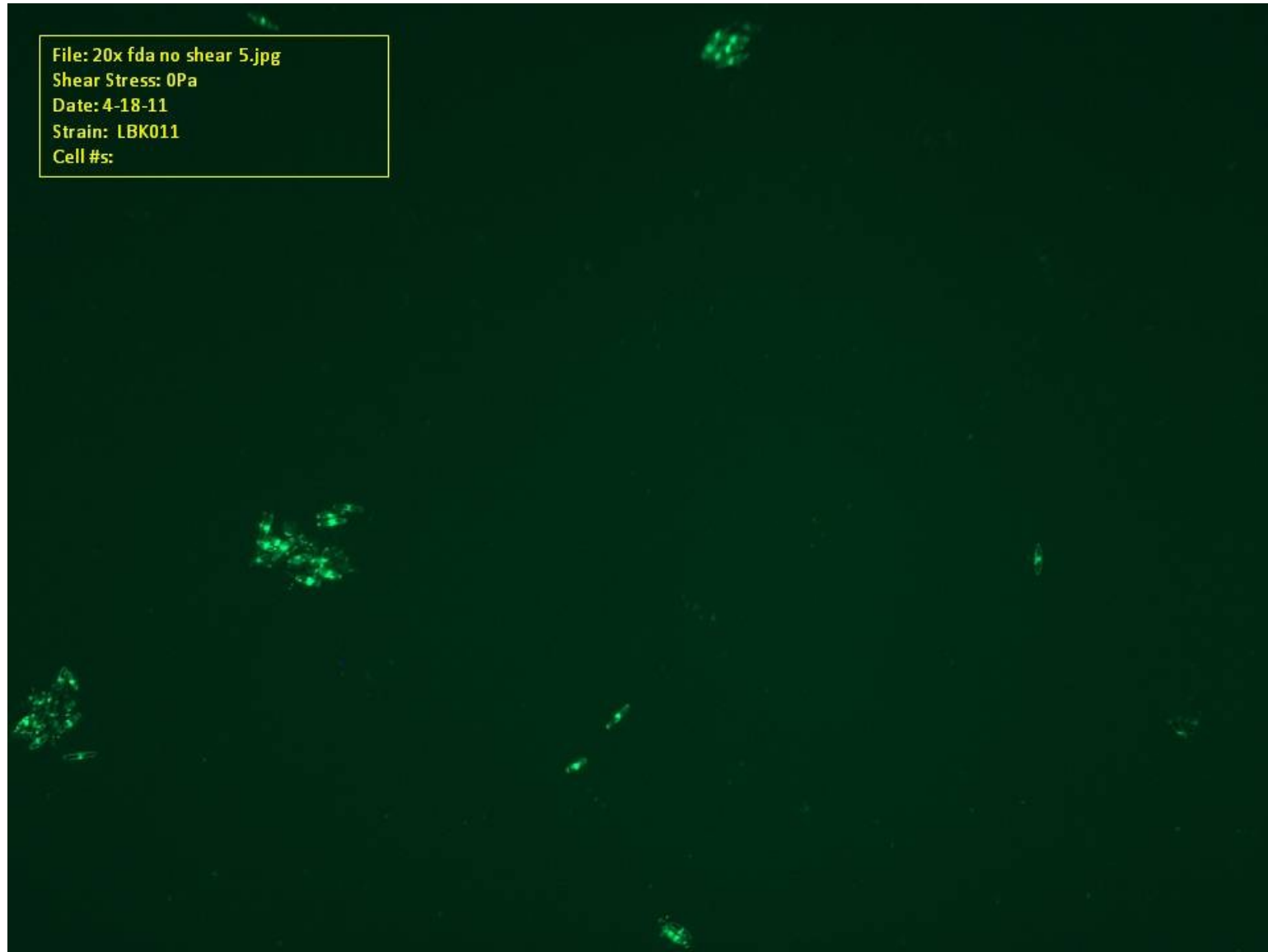


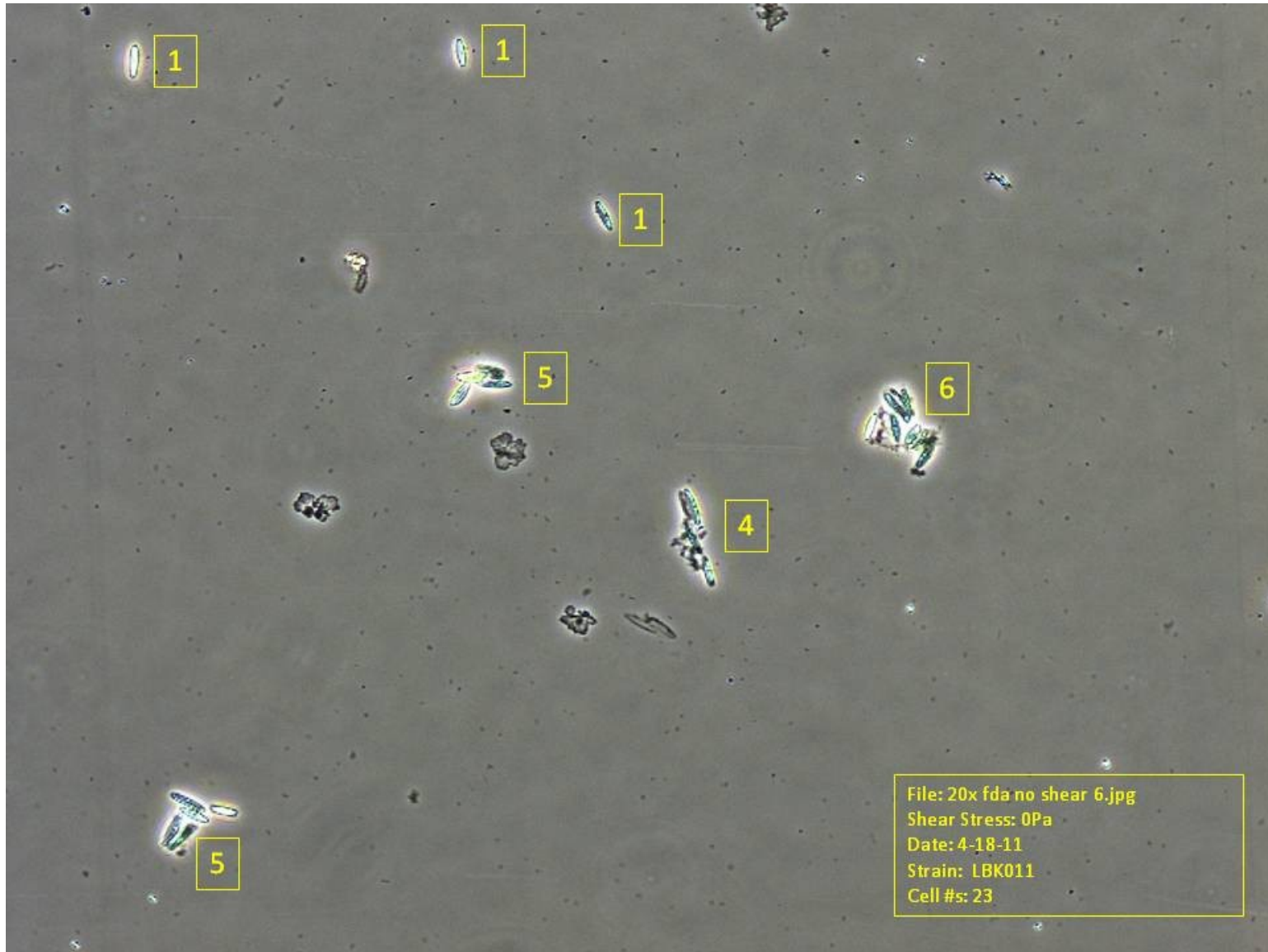








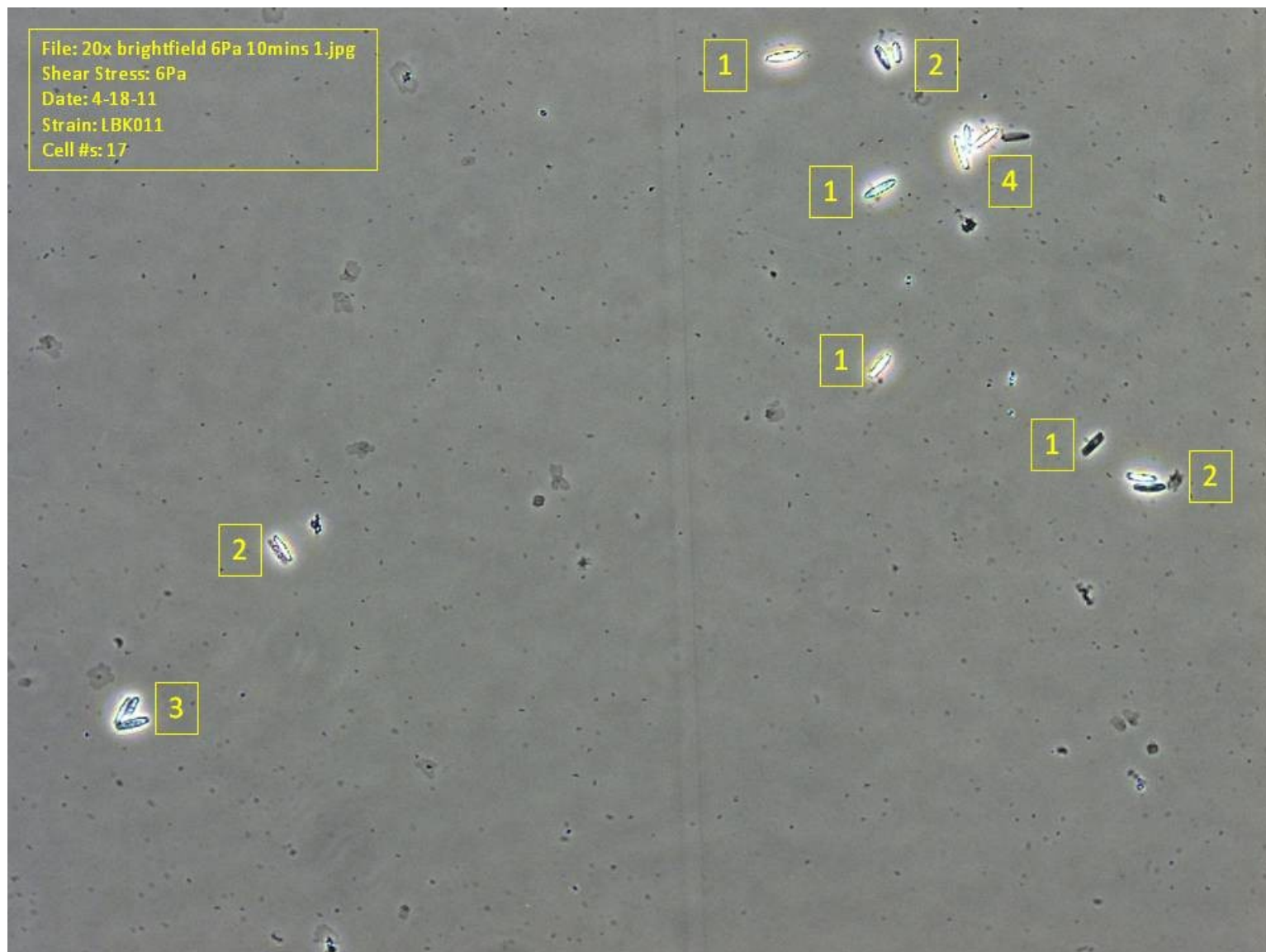






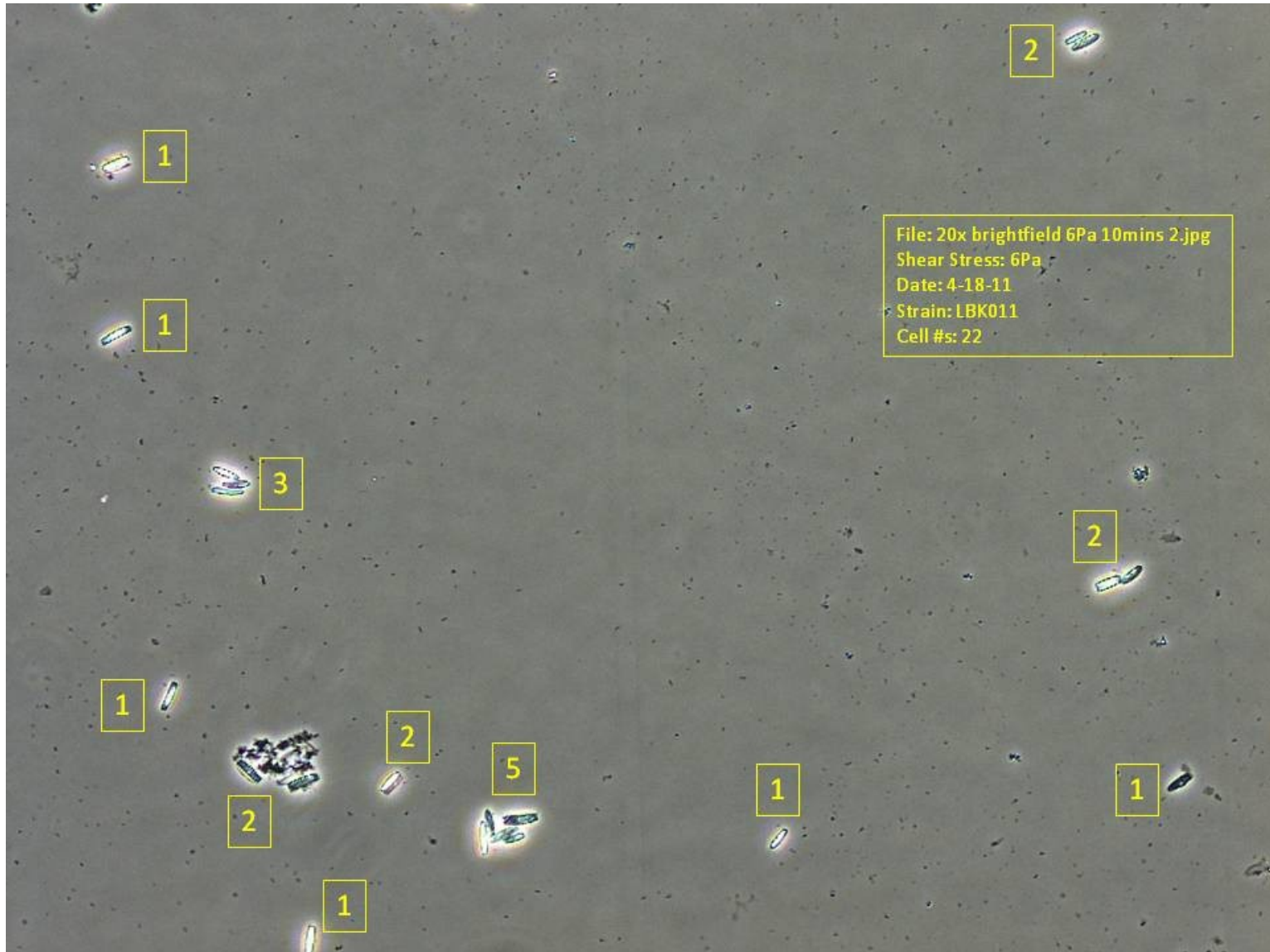


File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 17



File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 13



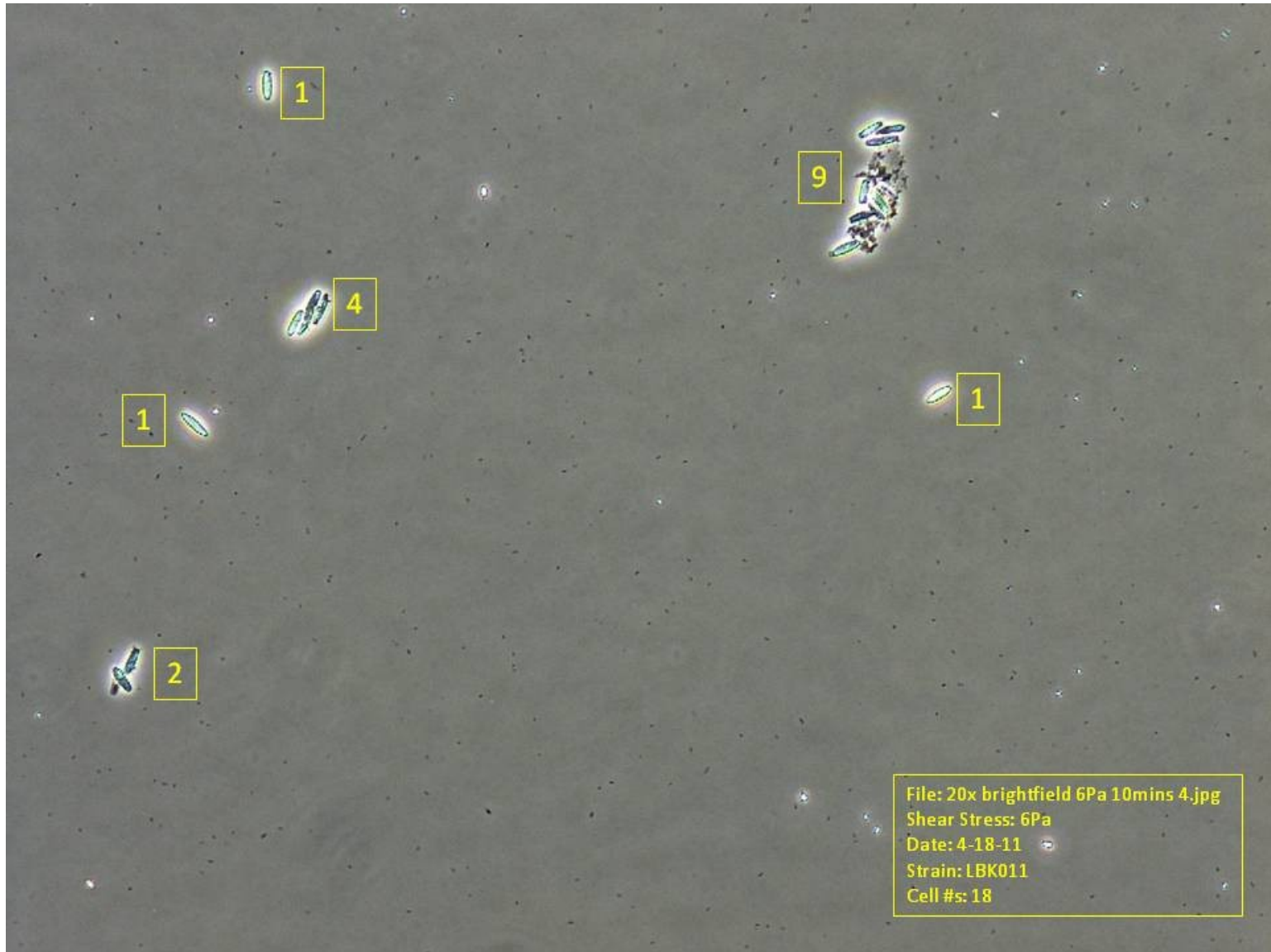




File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 8



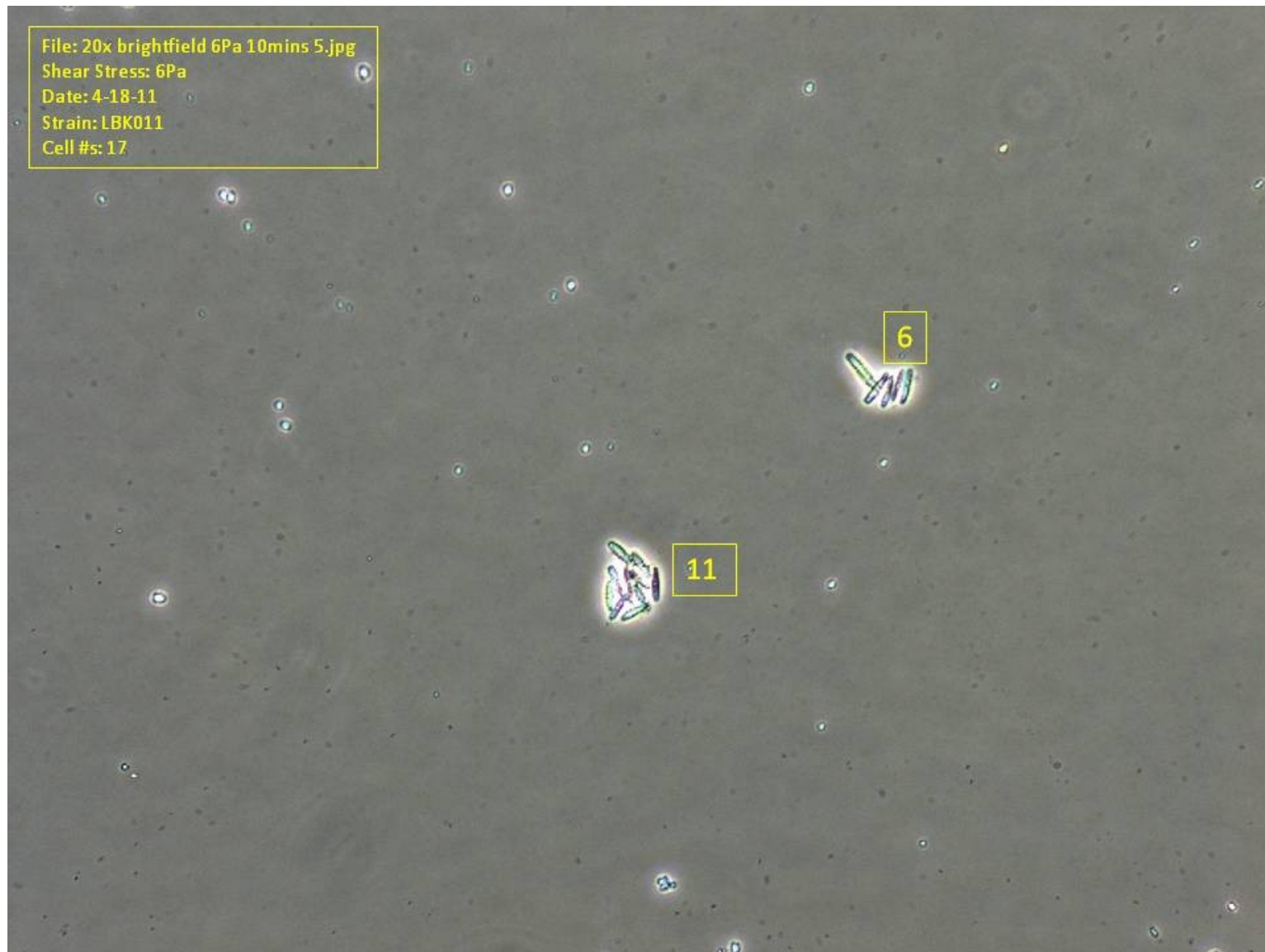






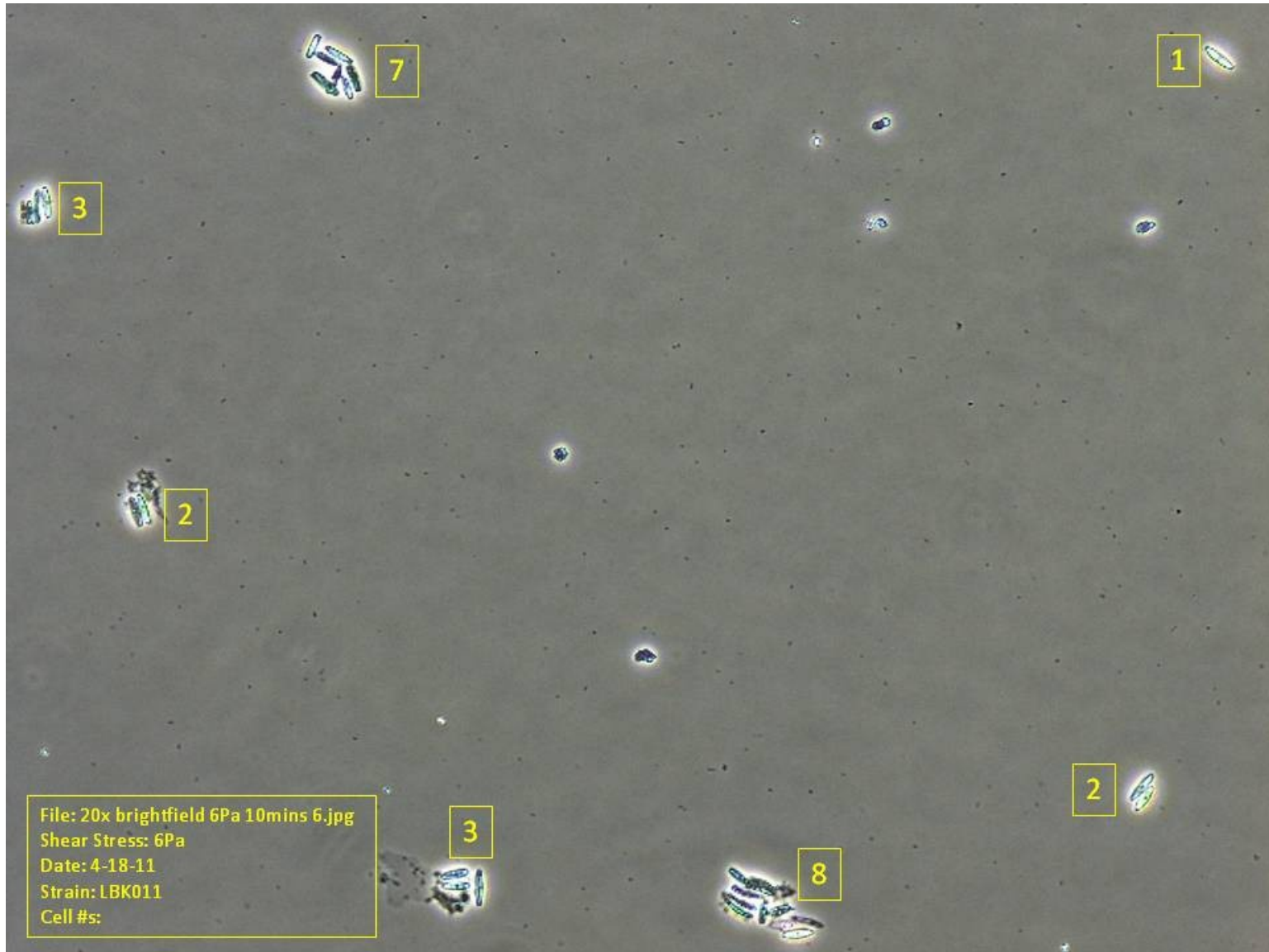


File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #: 17



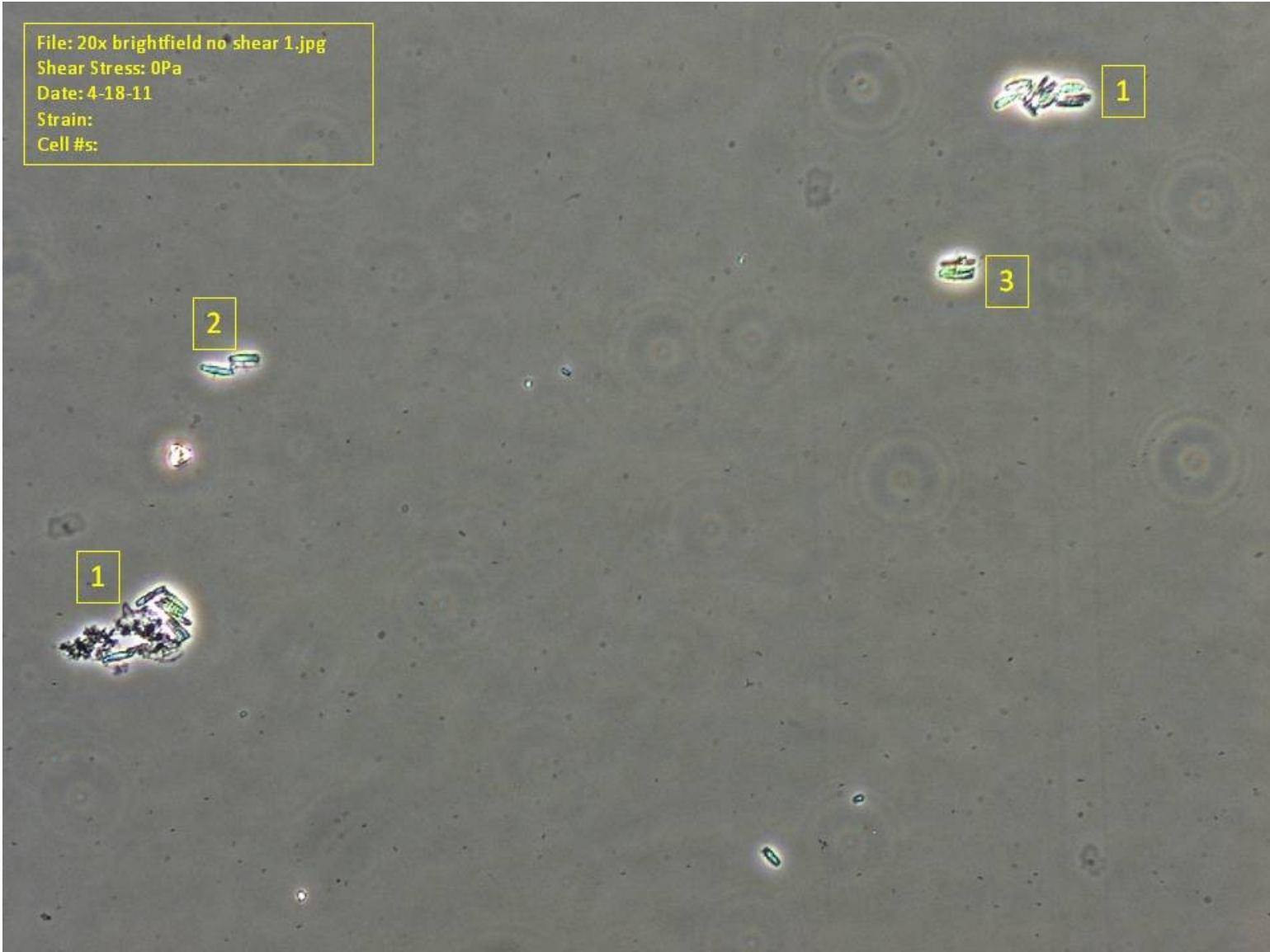
File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 13



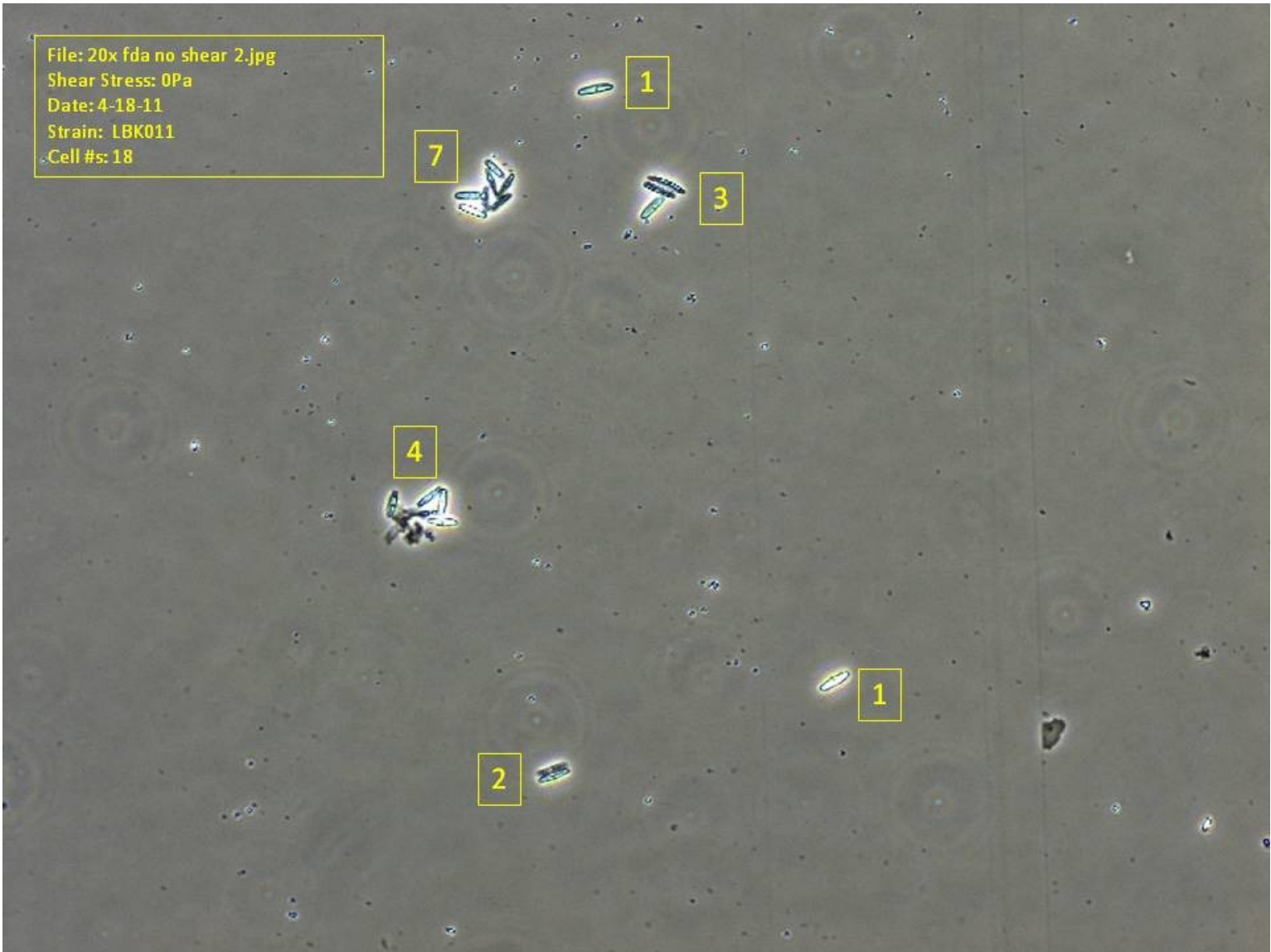




File: 20x brightfield no shear 1.jpg  
Shear Stress: 0Pa  
Date: 4-18-11  
Strain:  
Cell #s:









File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 15

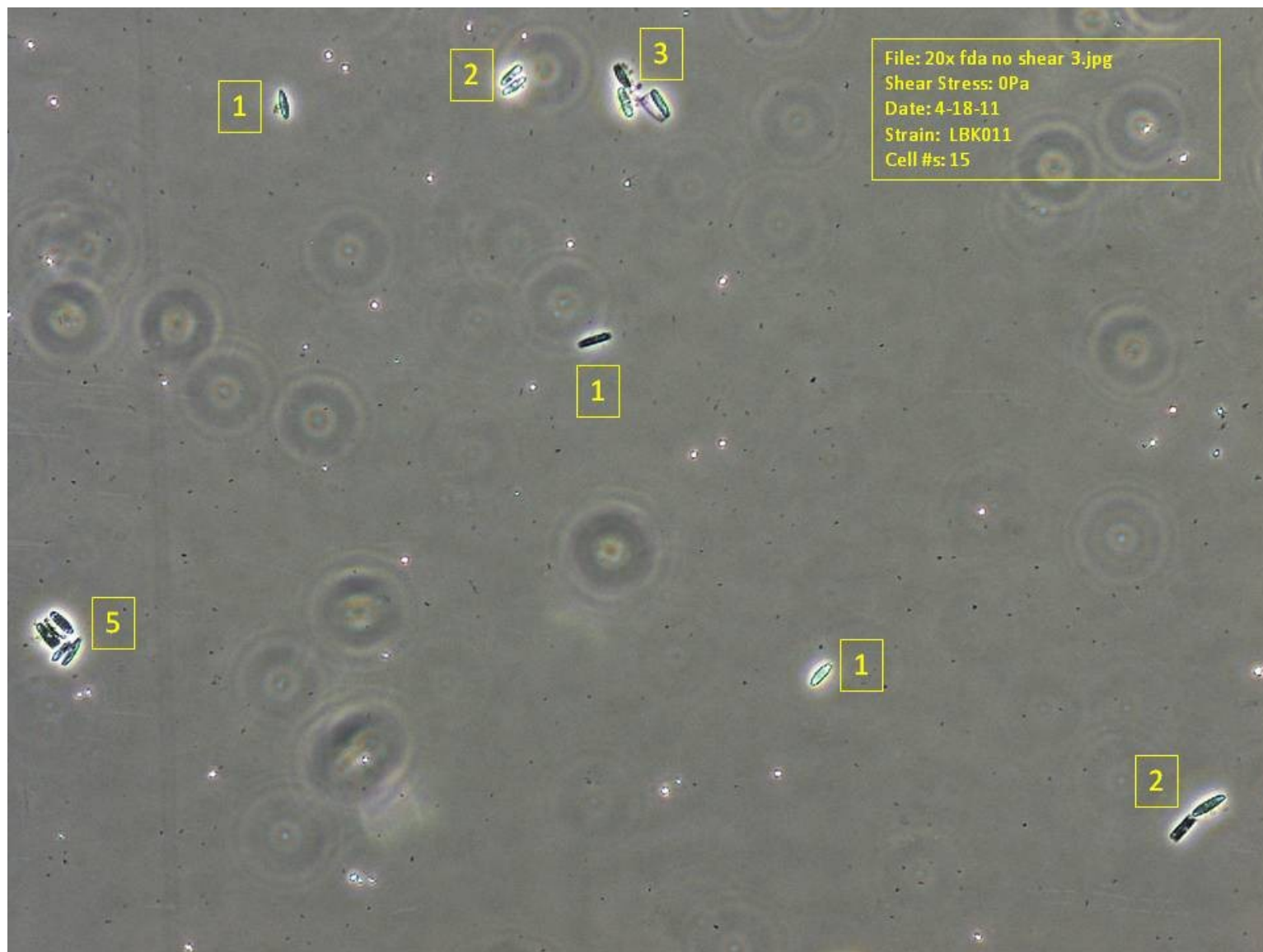
7

3

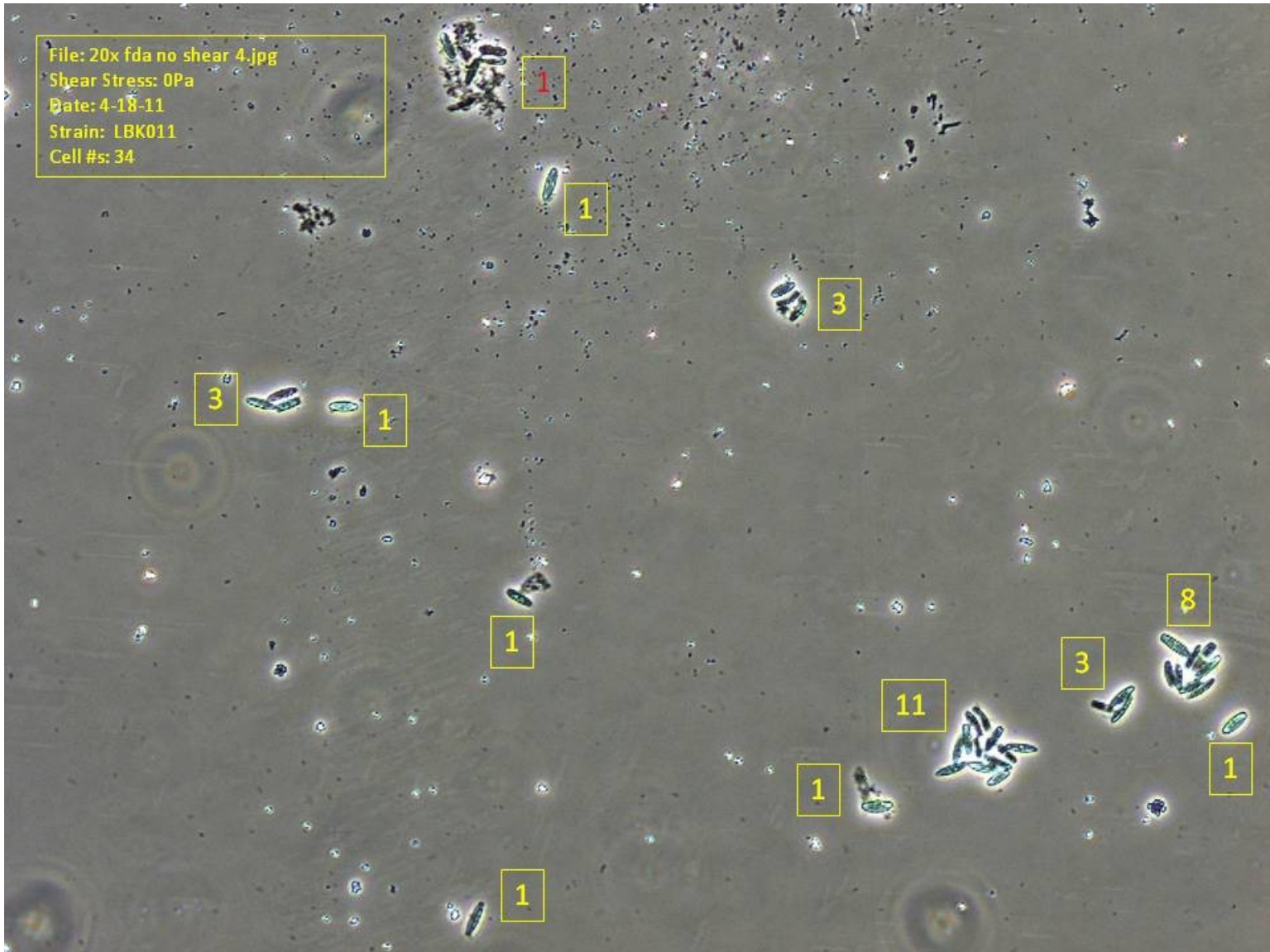
3

1

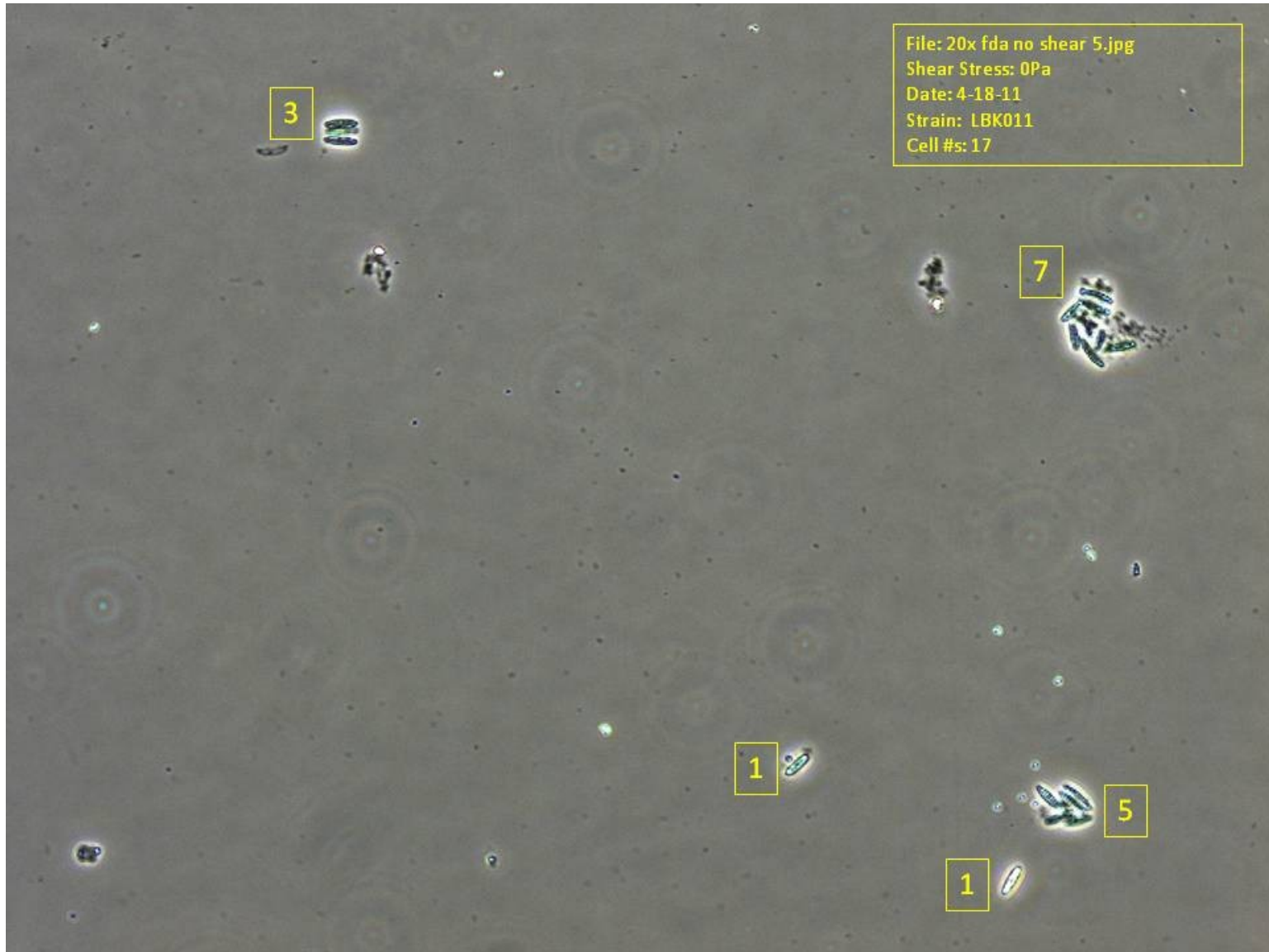
1



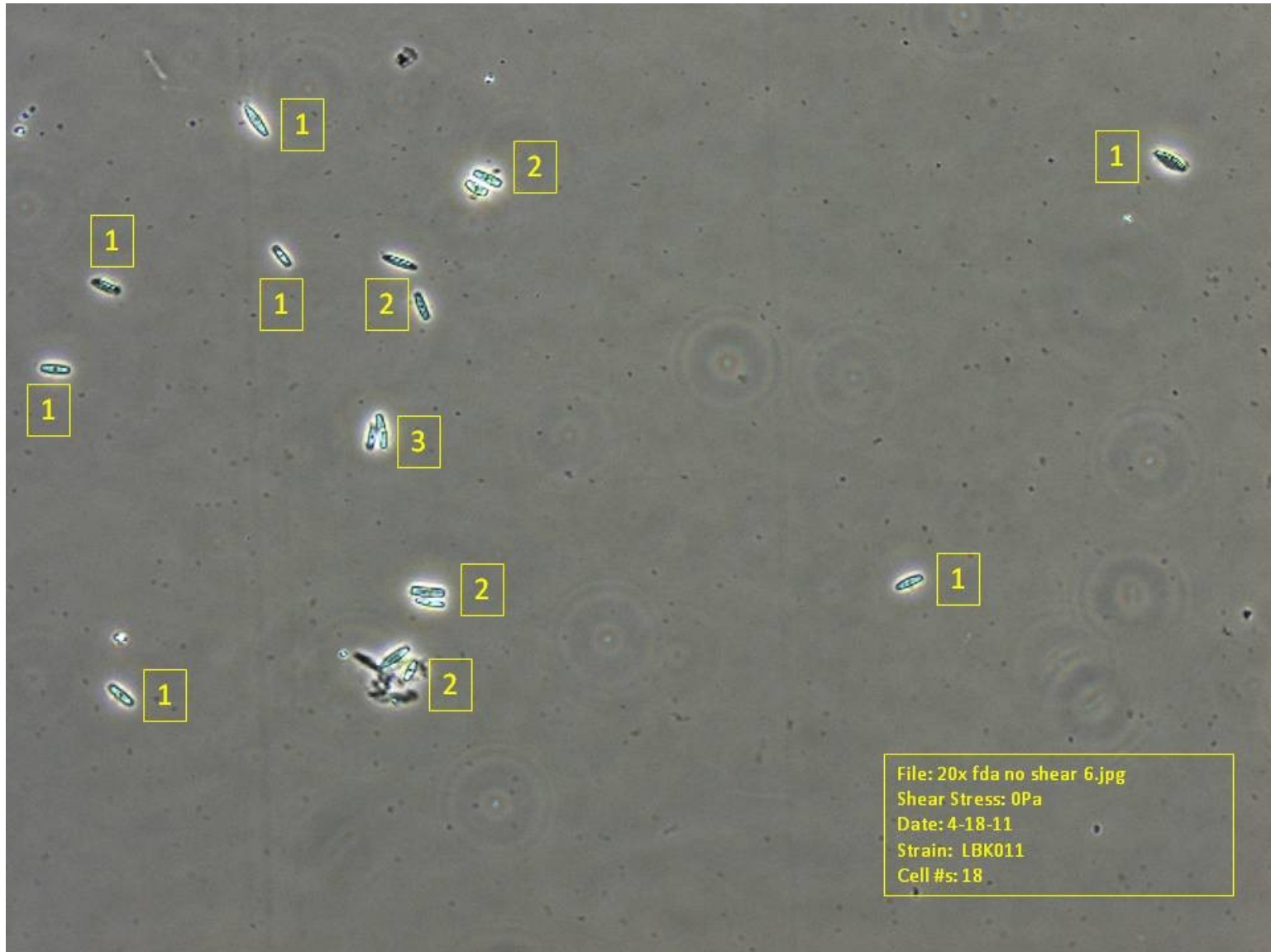






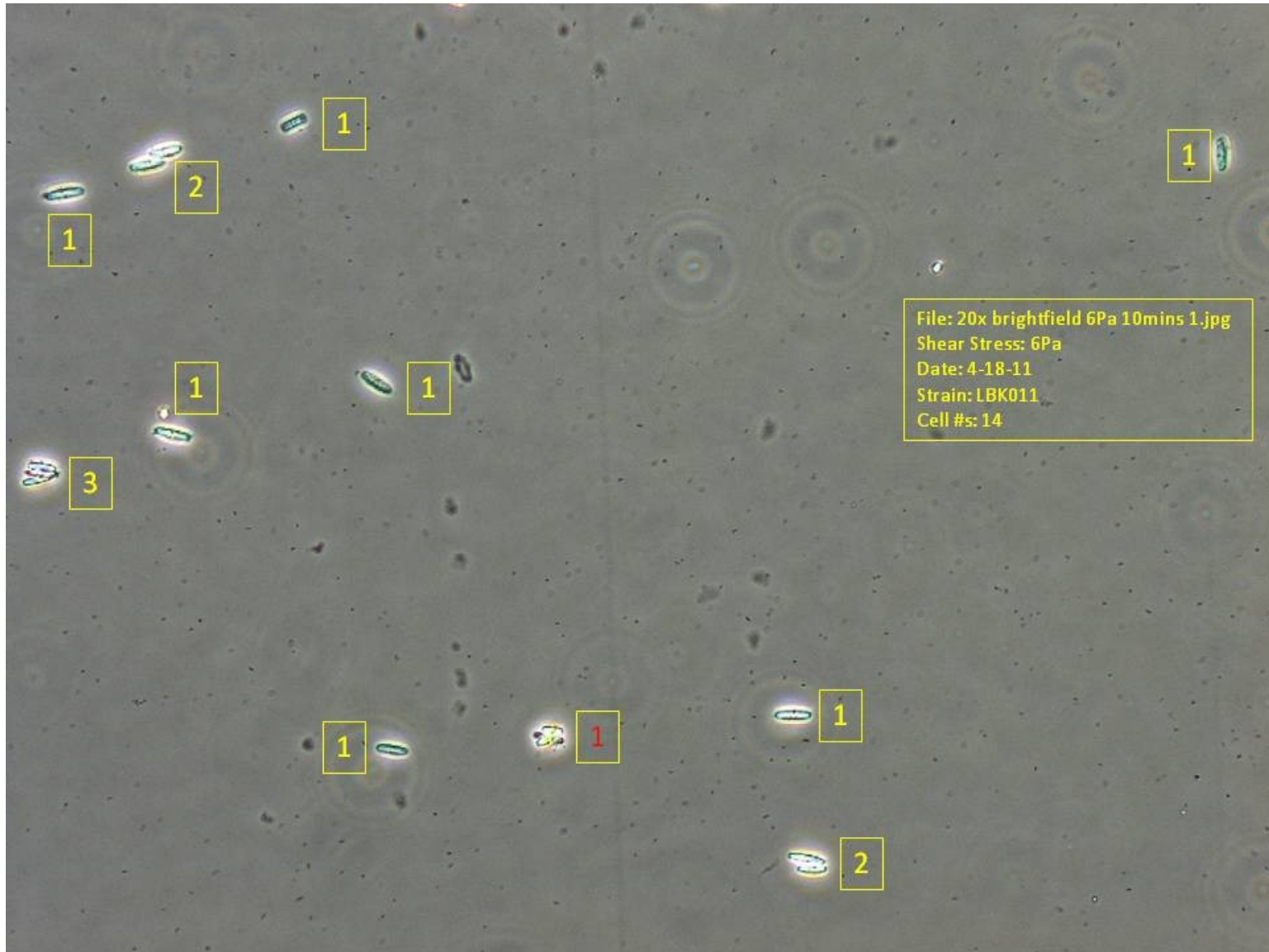






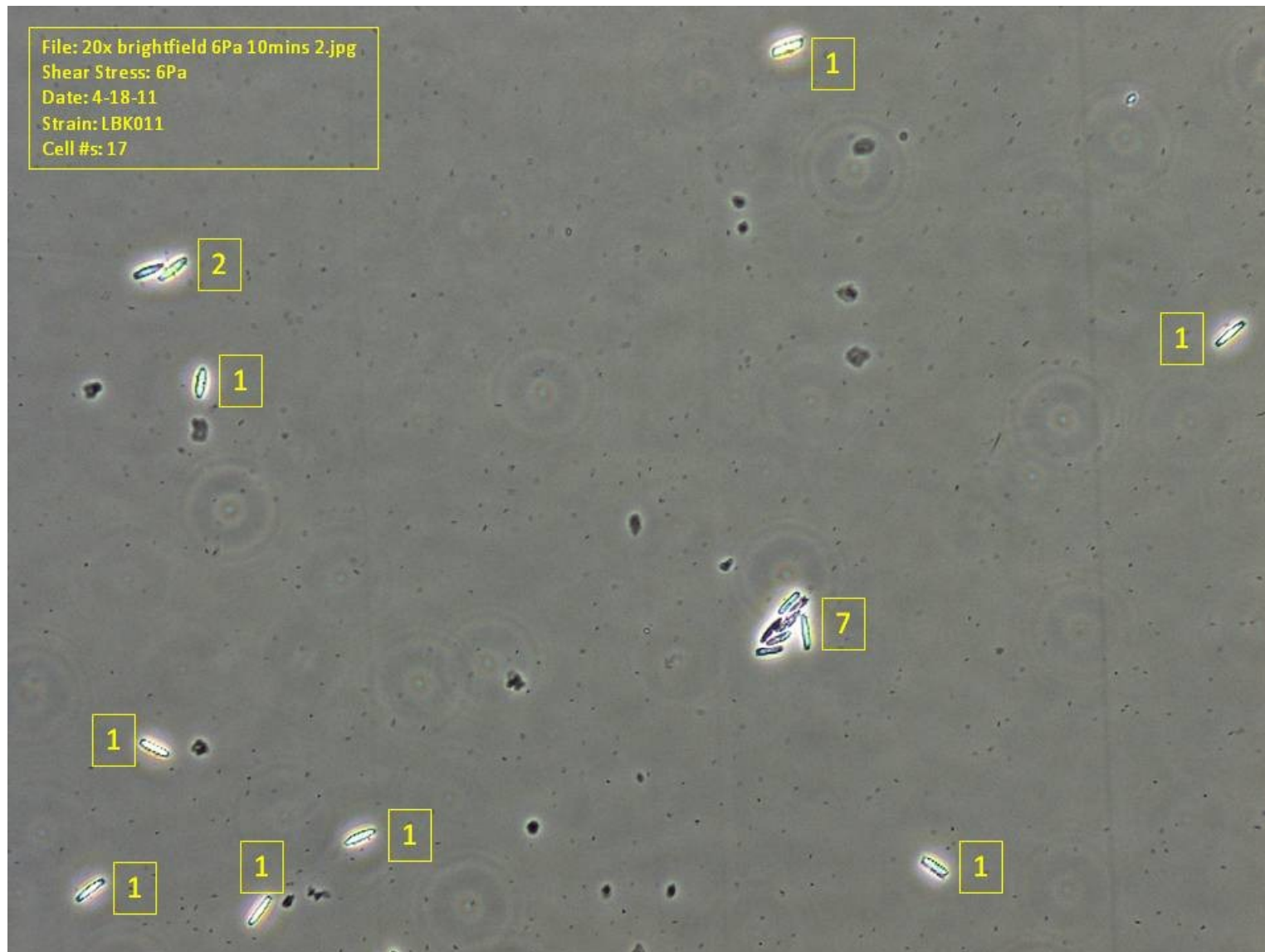




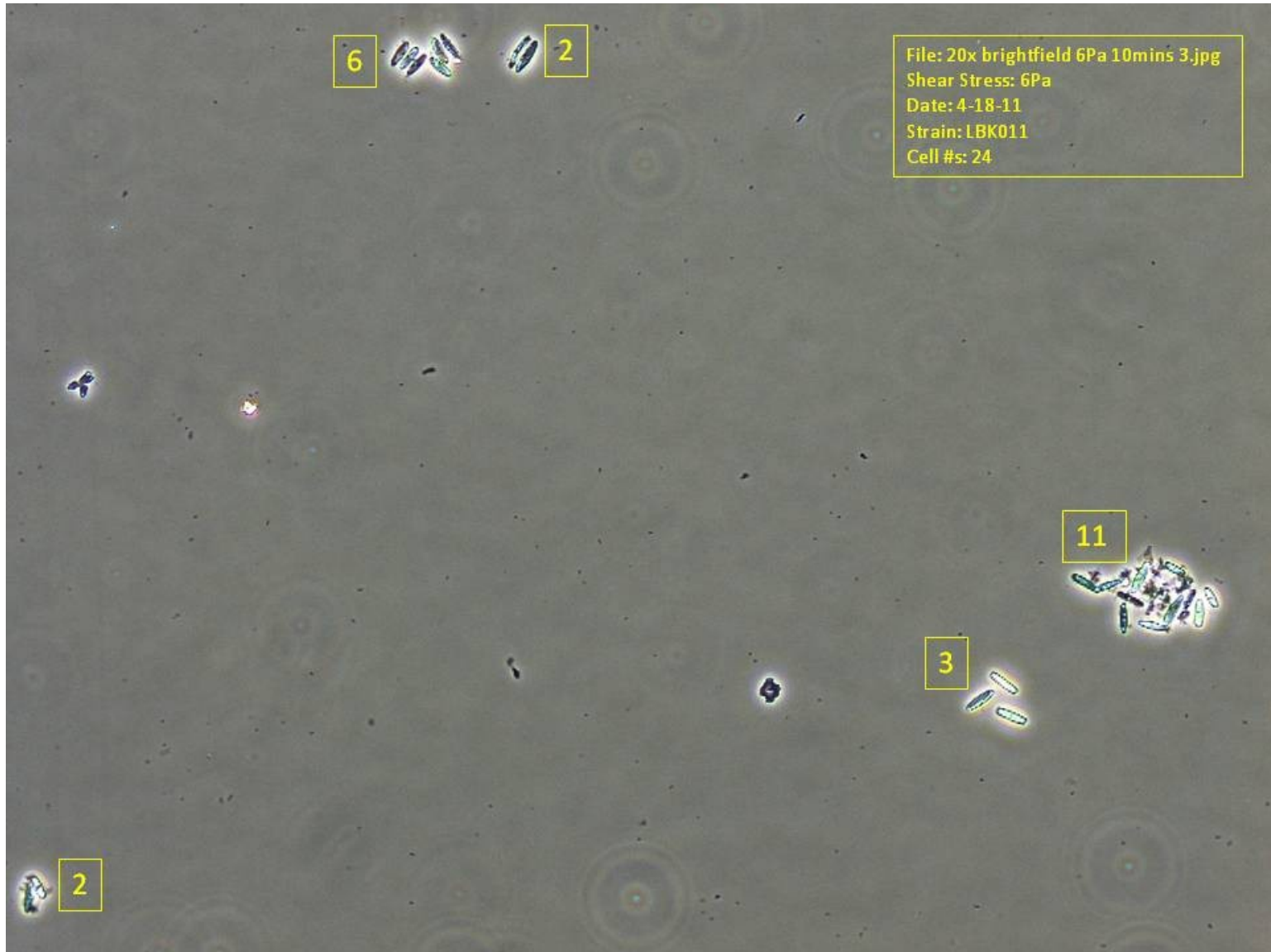


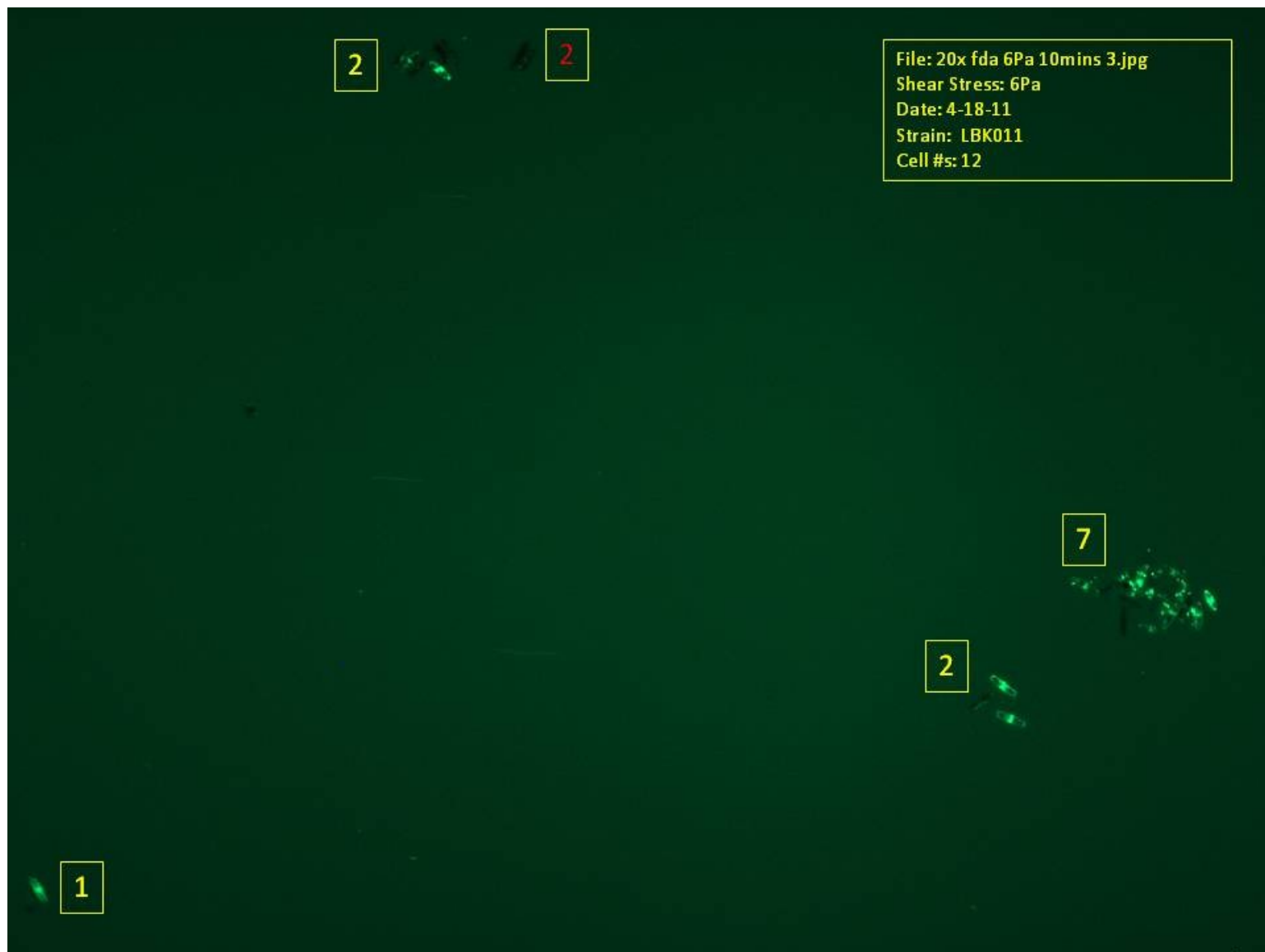


File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 17

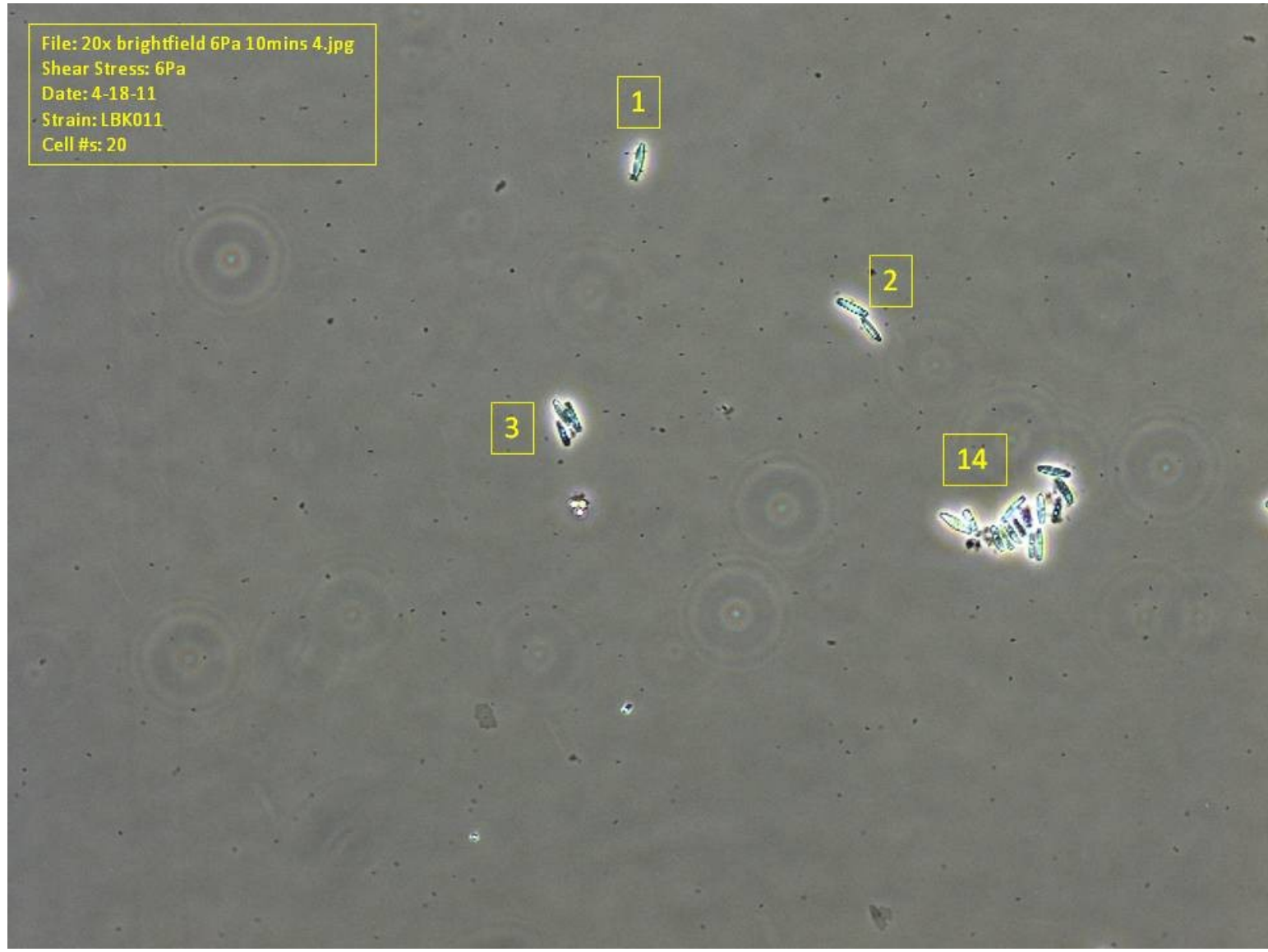








File: 20x brightfield 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #: 20





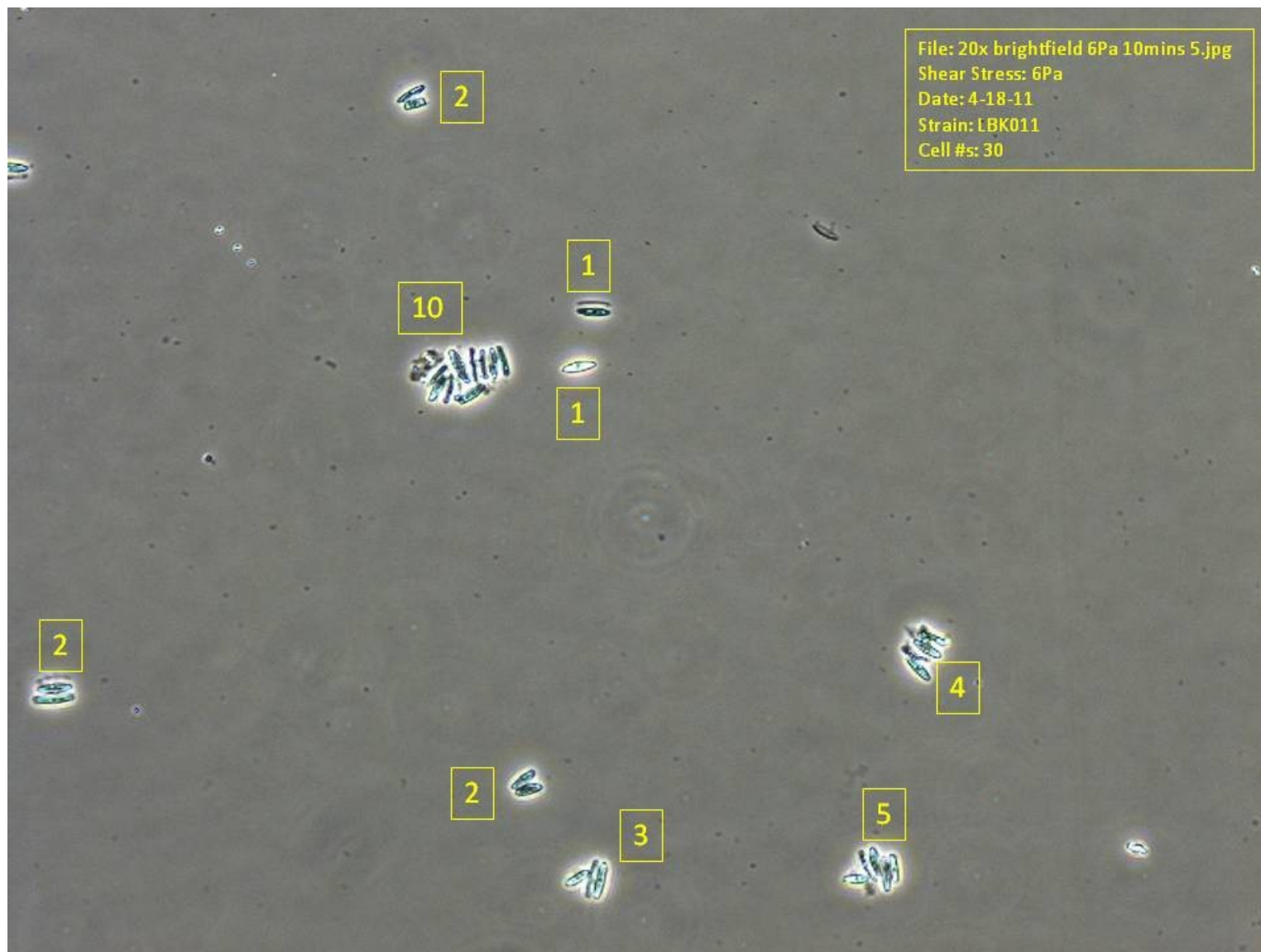
File: 20x fda 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s: 9

1

2

3

9





File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s:



File: 20x fda 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 4-18-11  
Strain: LBK011  
Cell #s:

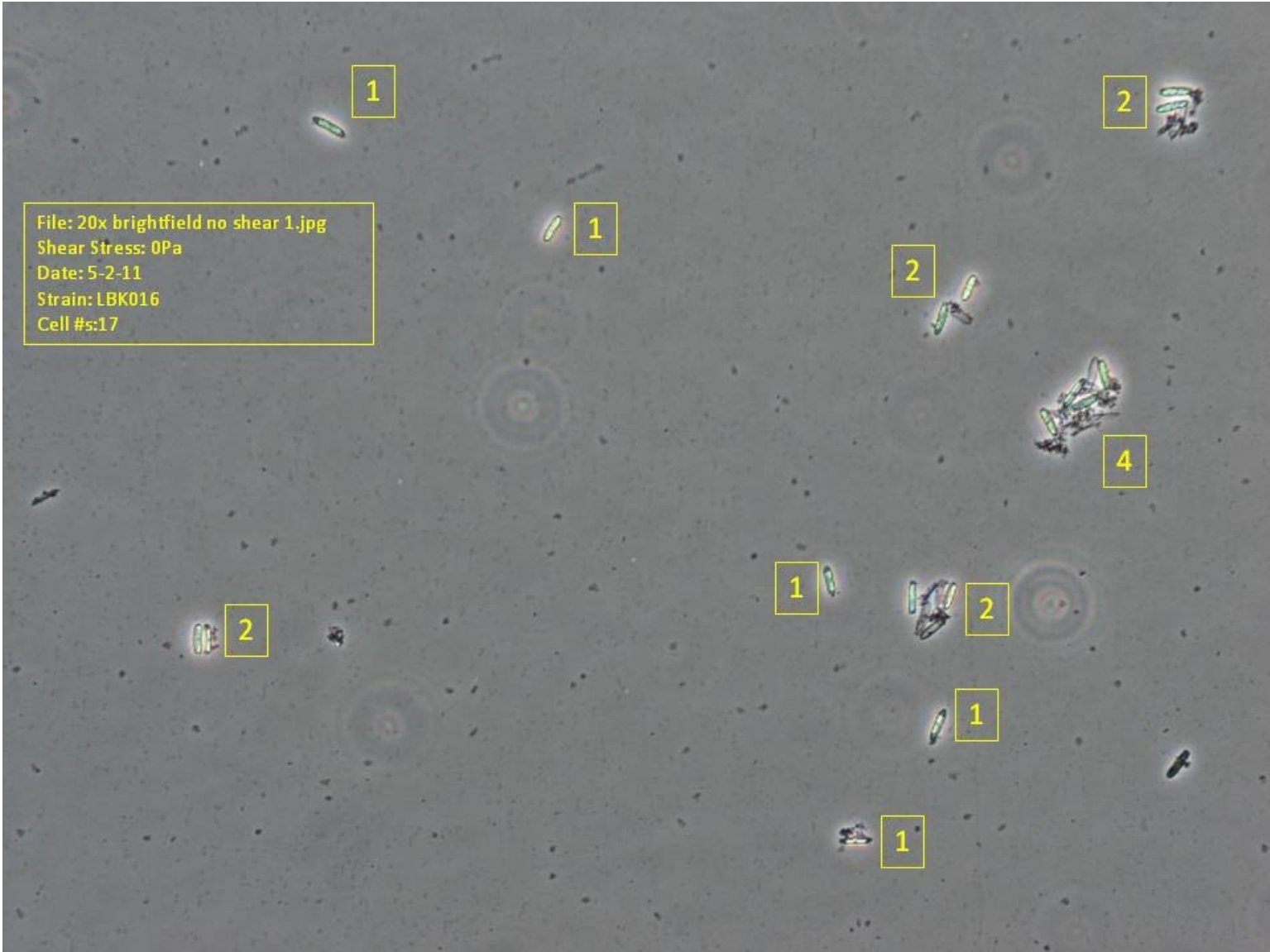


## APPENDIX E

The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK016, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	17	15	19	17	31	24	21	18	30	24			83.05%	3.70%	-0.00617
	6	19	18	16	13	28	21	17	13	12	8			79.35%		
Rep 2	0	15	11	20	16			29	28	19	18	22	22	90.48%	3.63%	-0.00606
	6	19	16	17	14	32	28	27	25	19	16			86.84%		
Rep 3	0	13	10			15	12	17	15	32	28	23	18	83.00%	1.52%	-0.00253
	6	22	17	26	23	23	18	16	11	21	19			81.48%		
														<b>Avg.</b>	<b>2.95%</b>	
														<b>Std. Dev.</b>	<b>1.24%</b>	

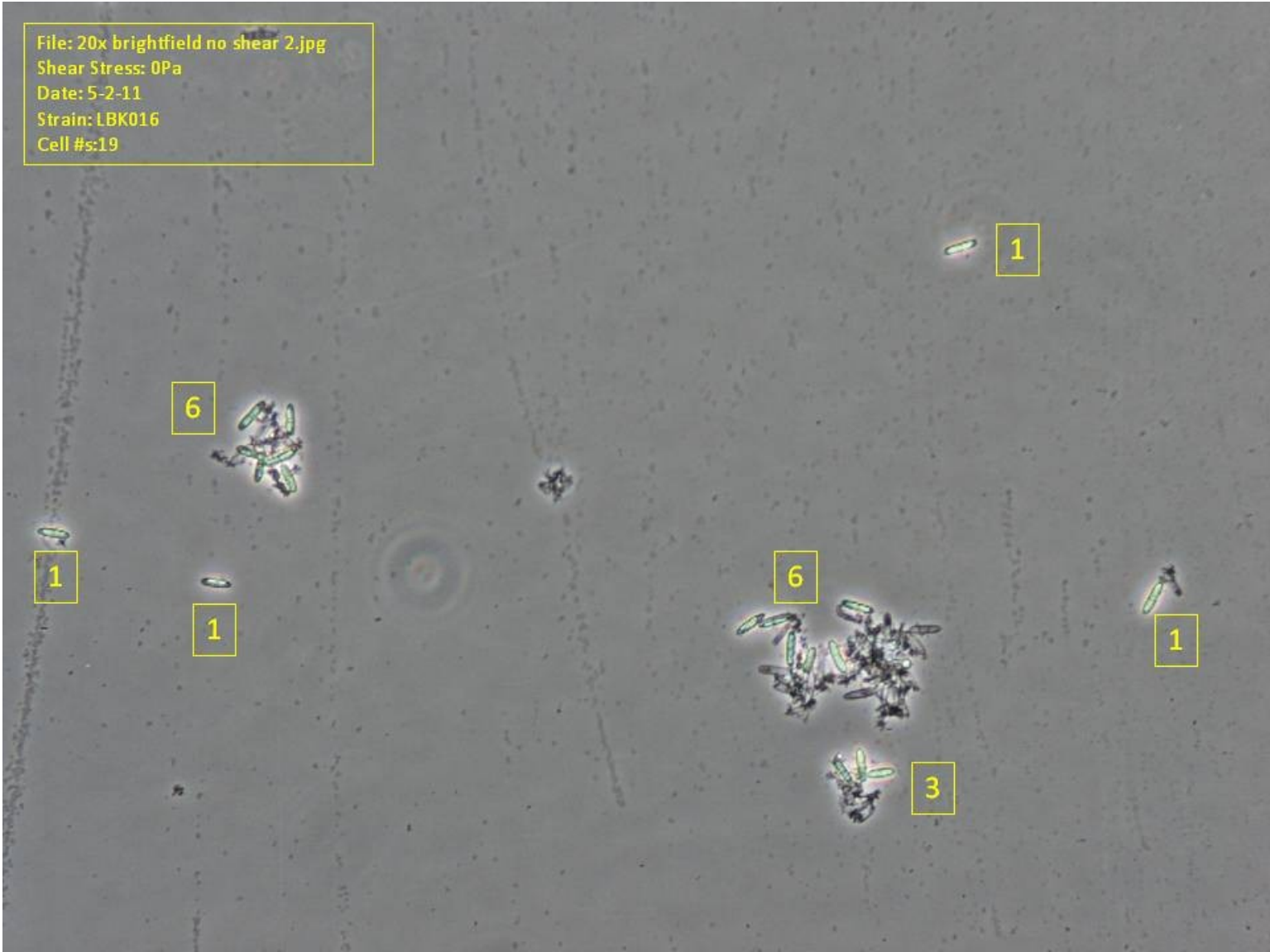
The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.



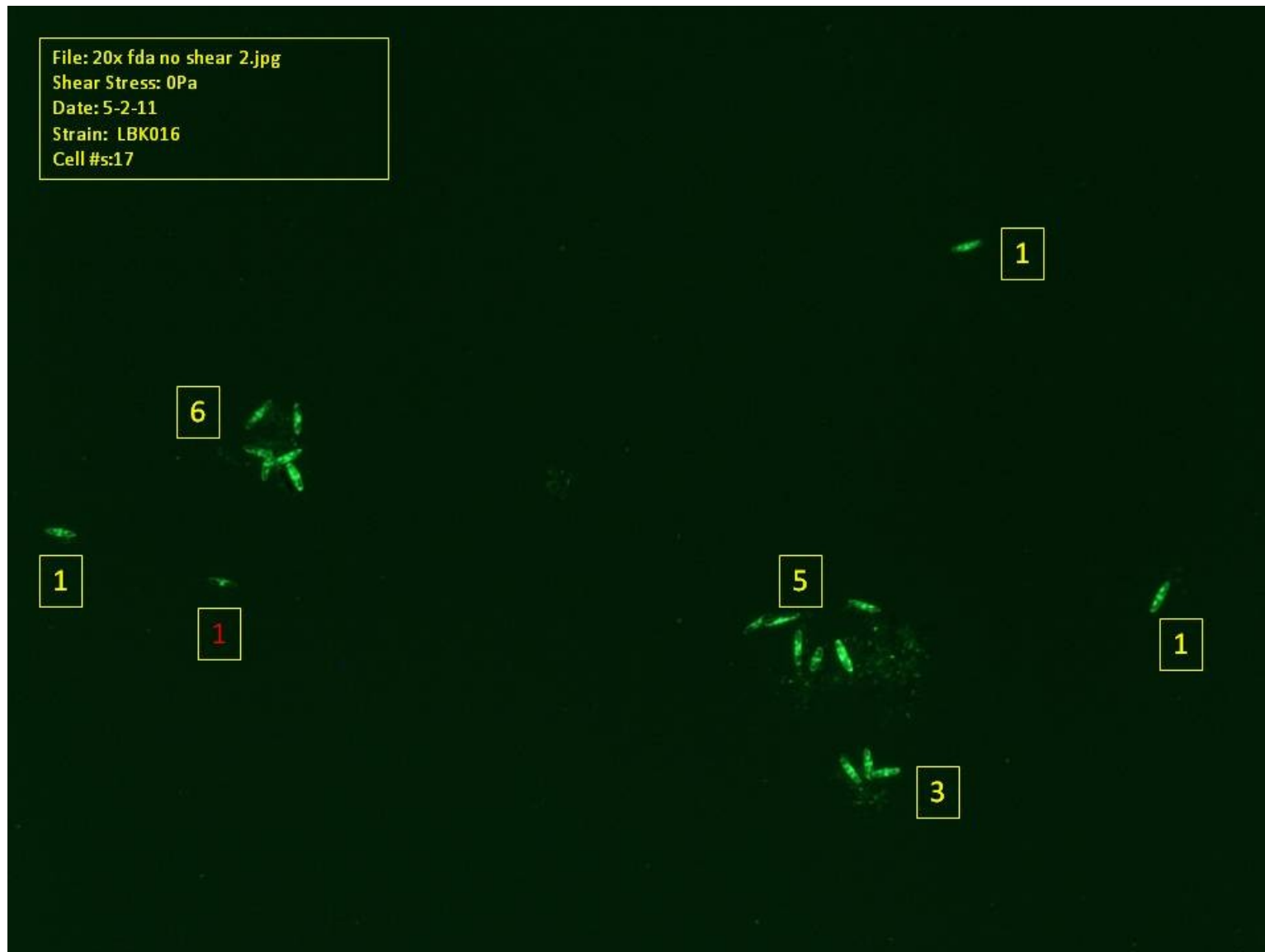


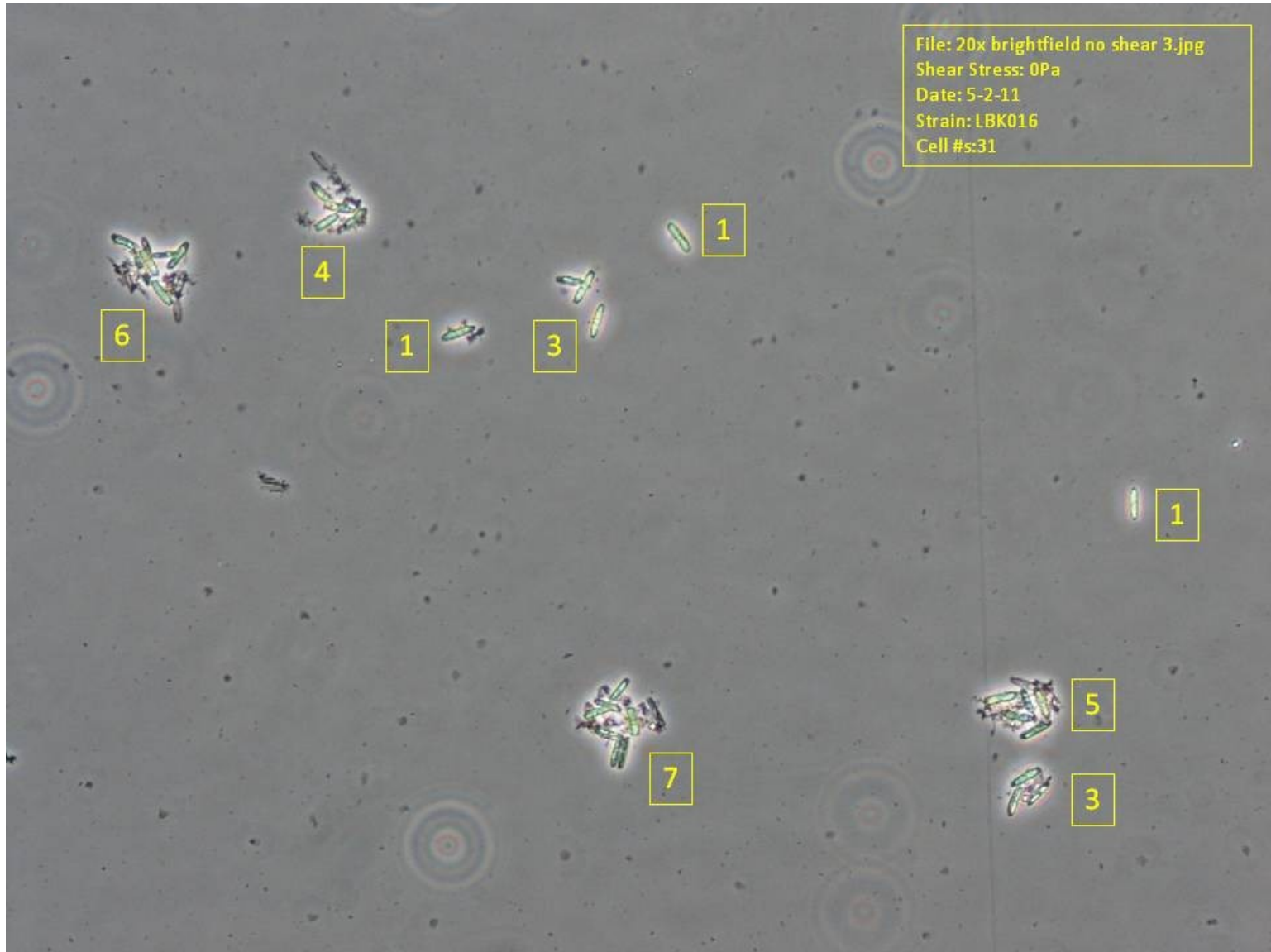


File: 20x brightfield no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK016  
Cell #:s:19

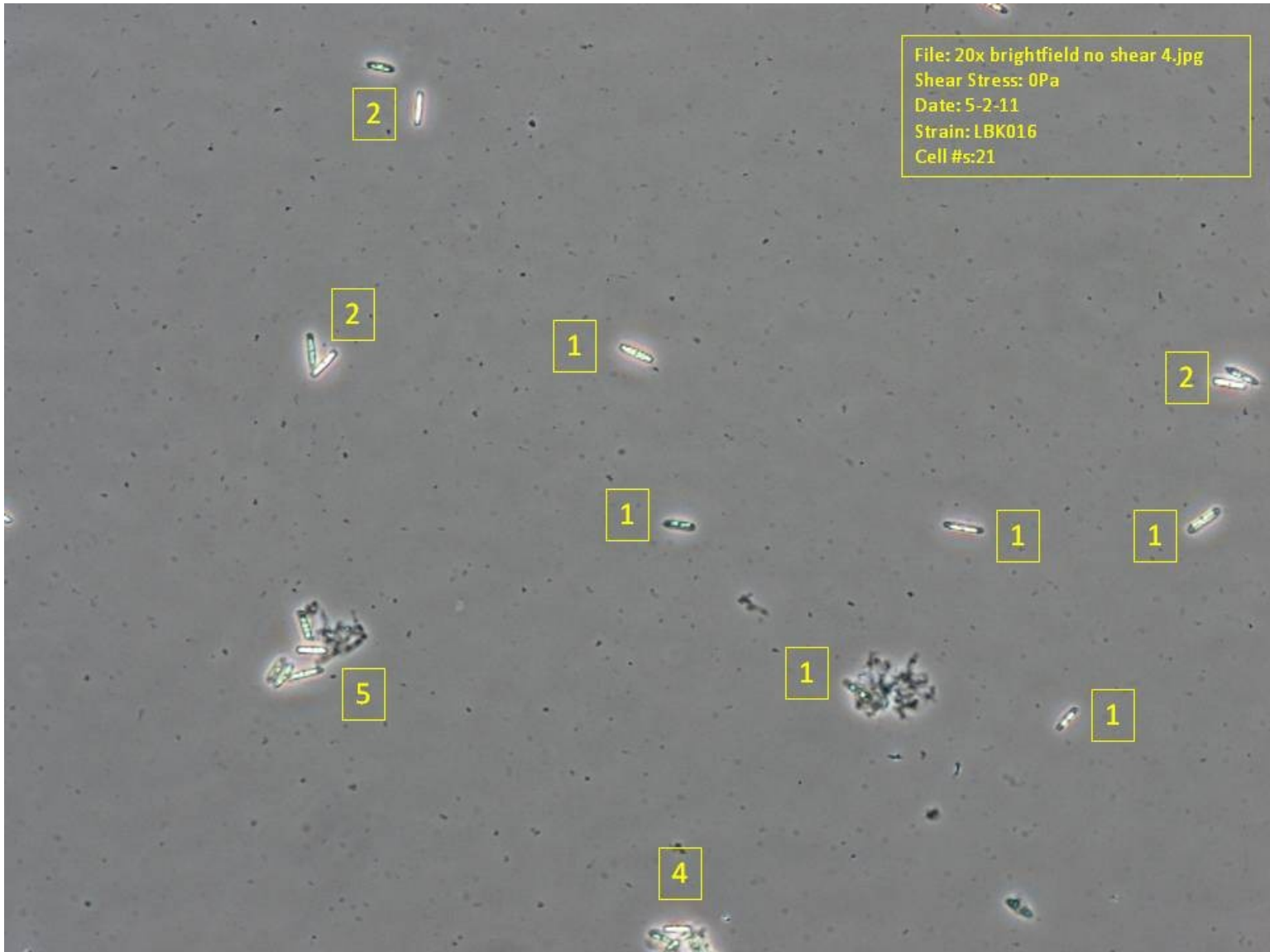


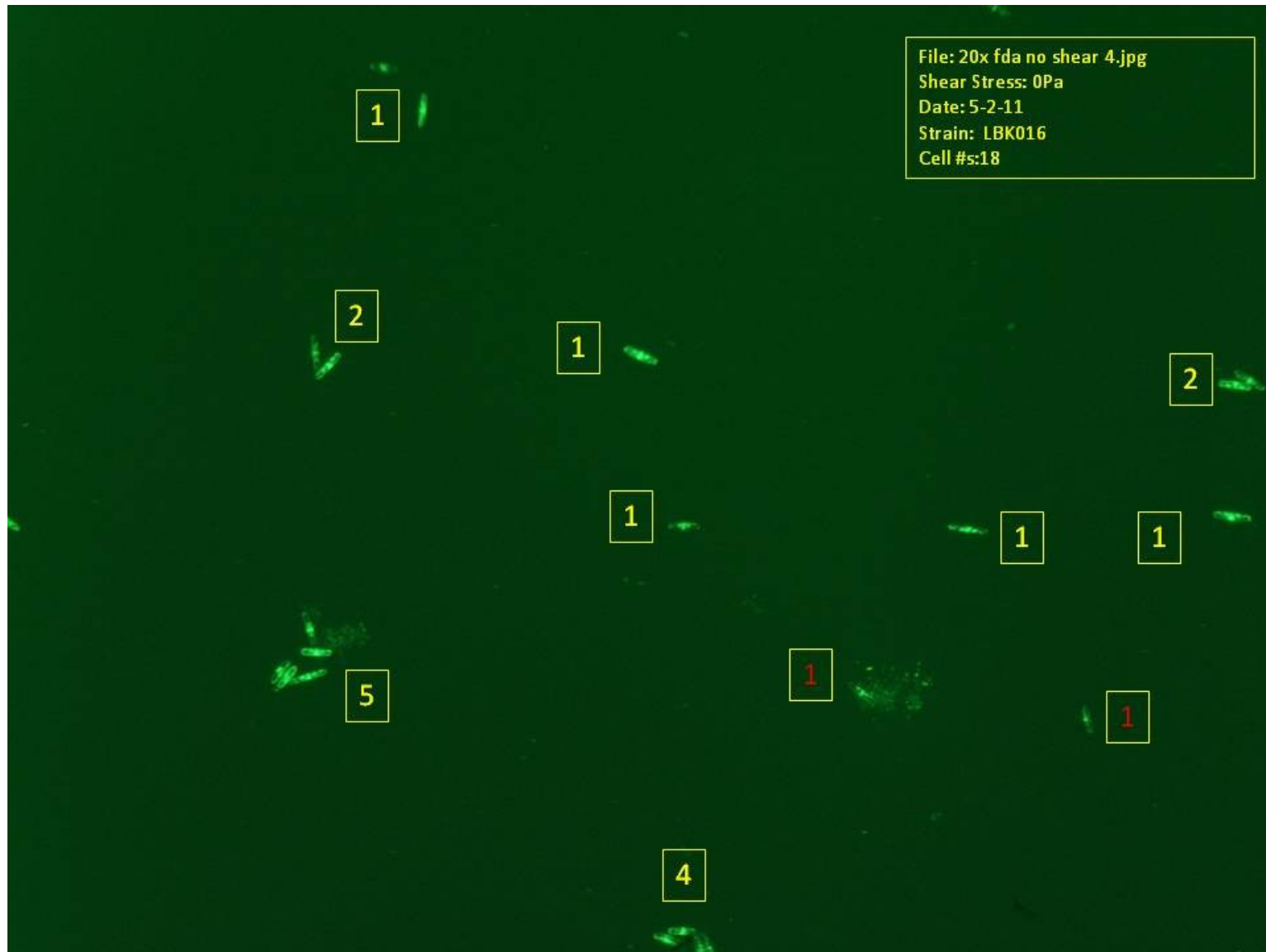
File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK016  
Cell #:17

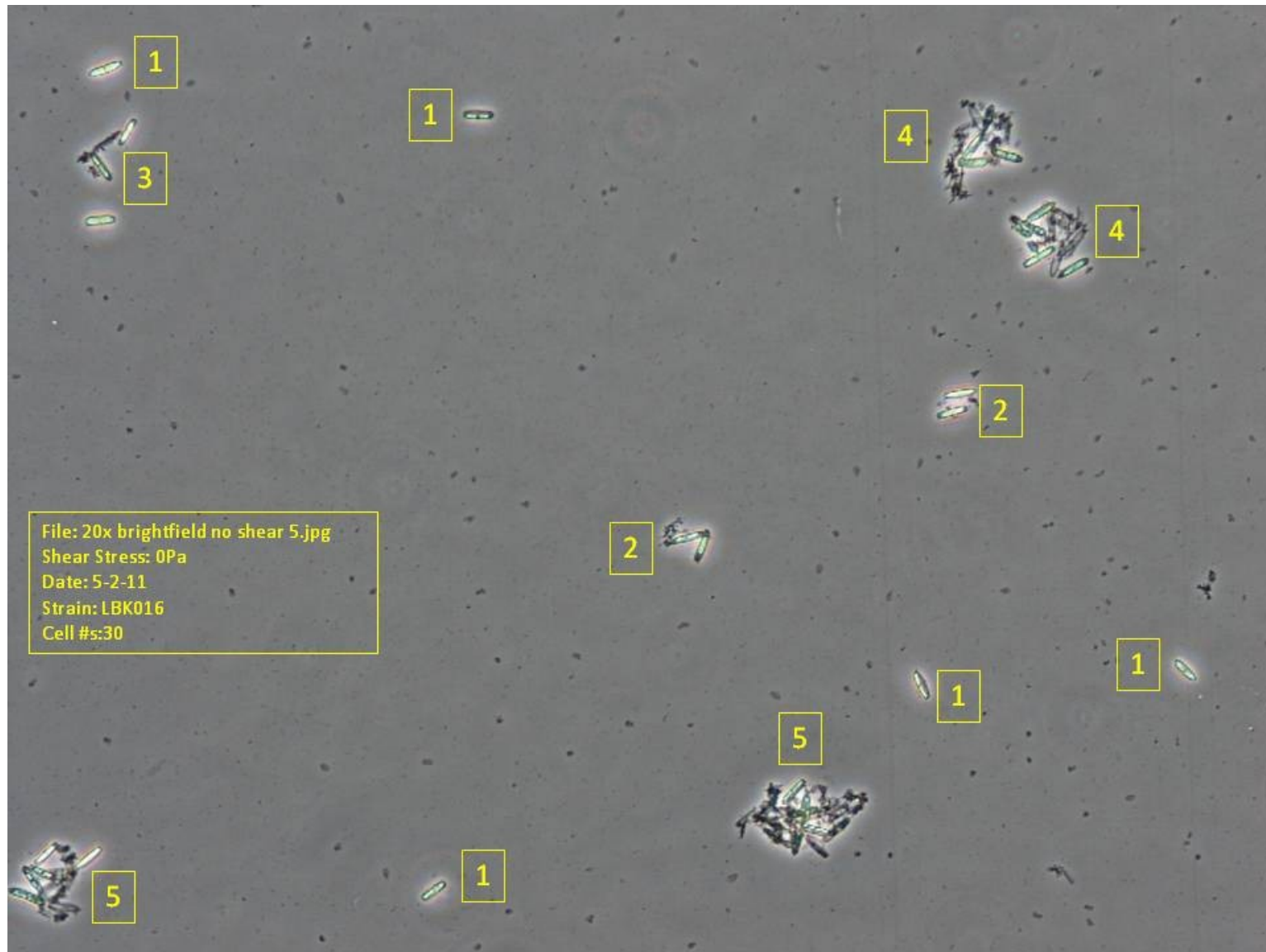


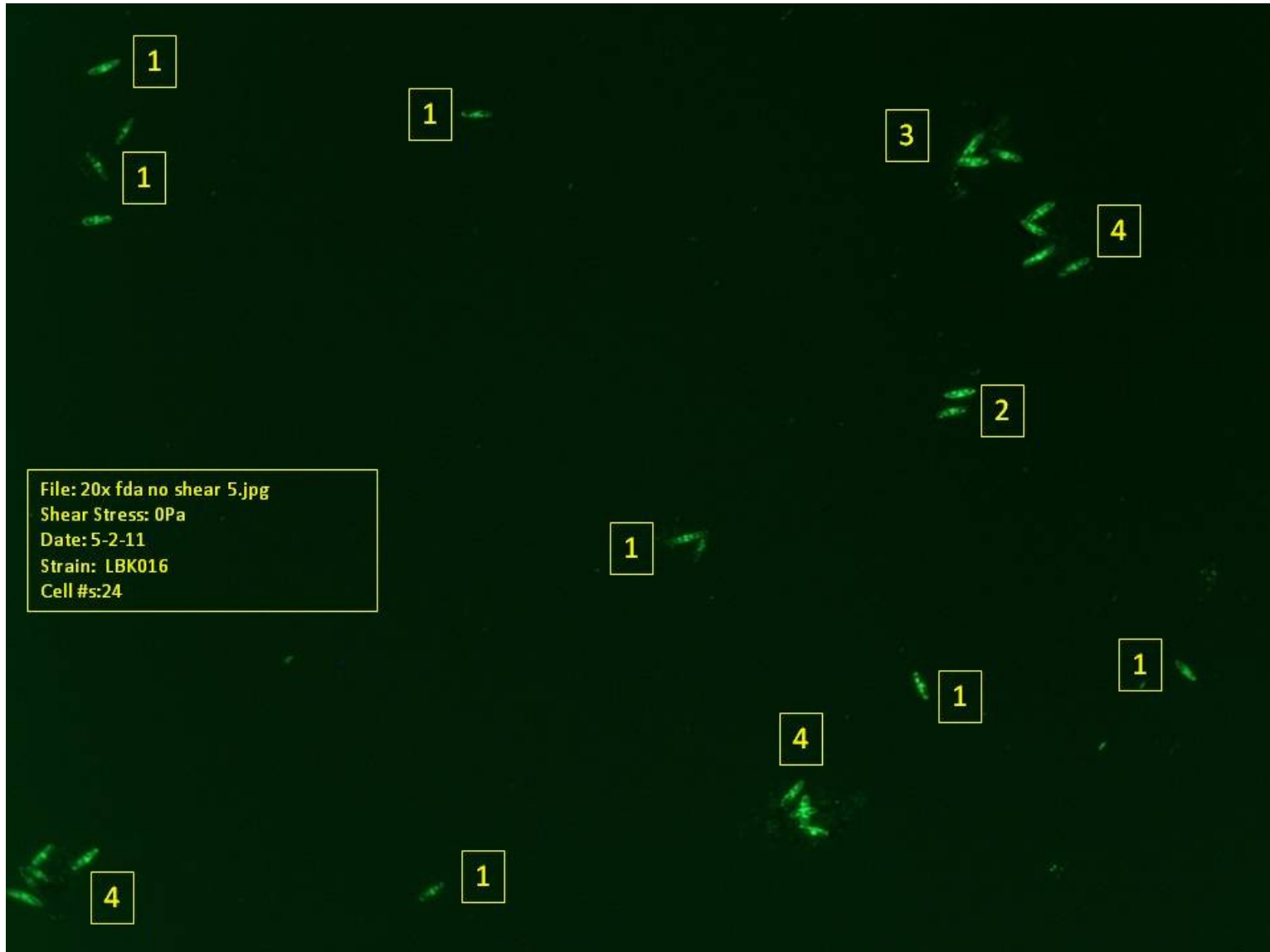




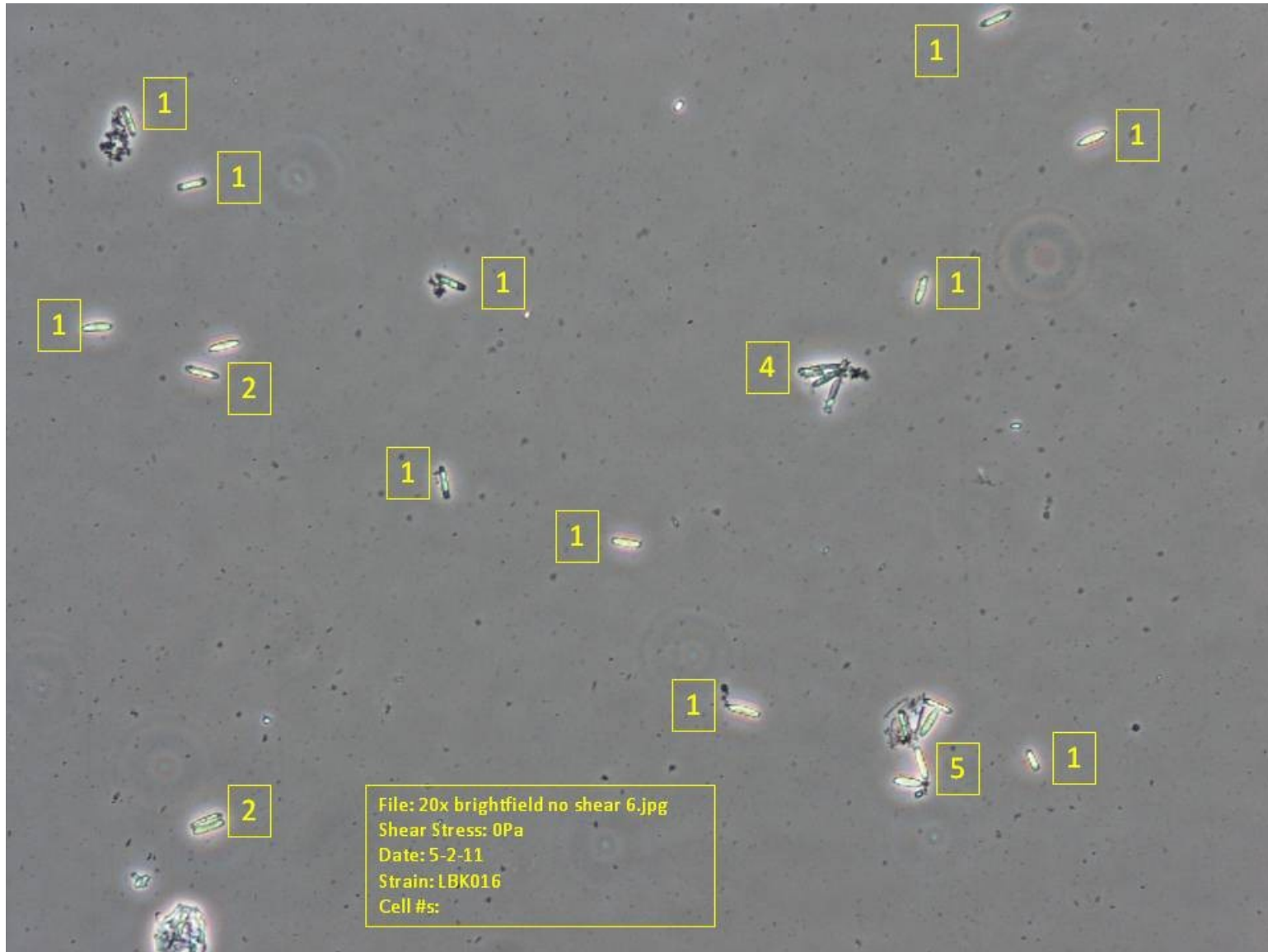


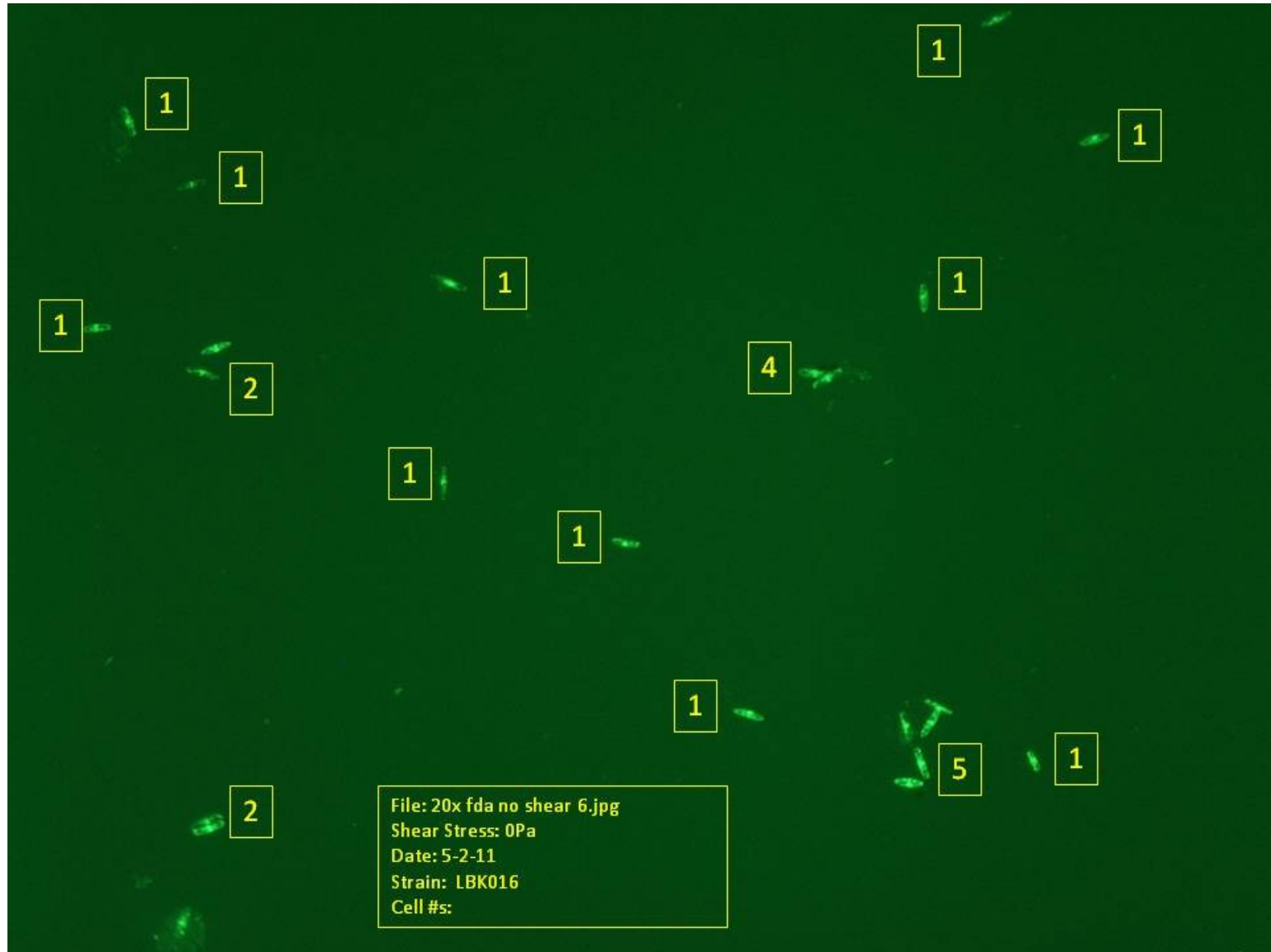


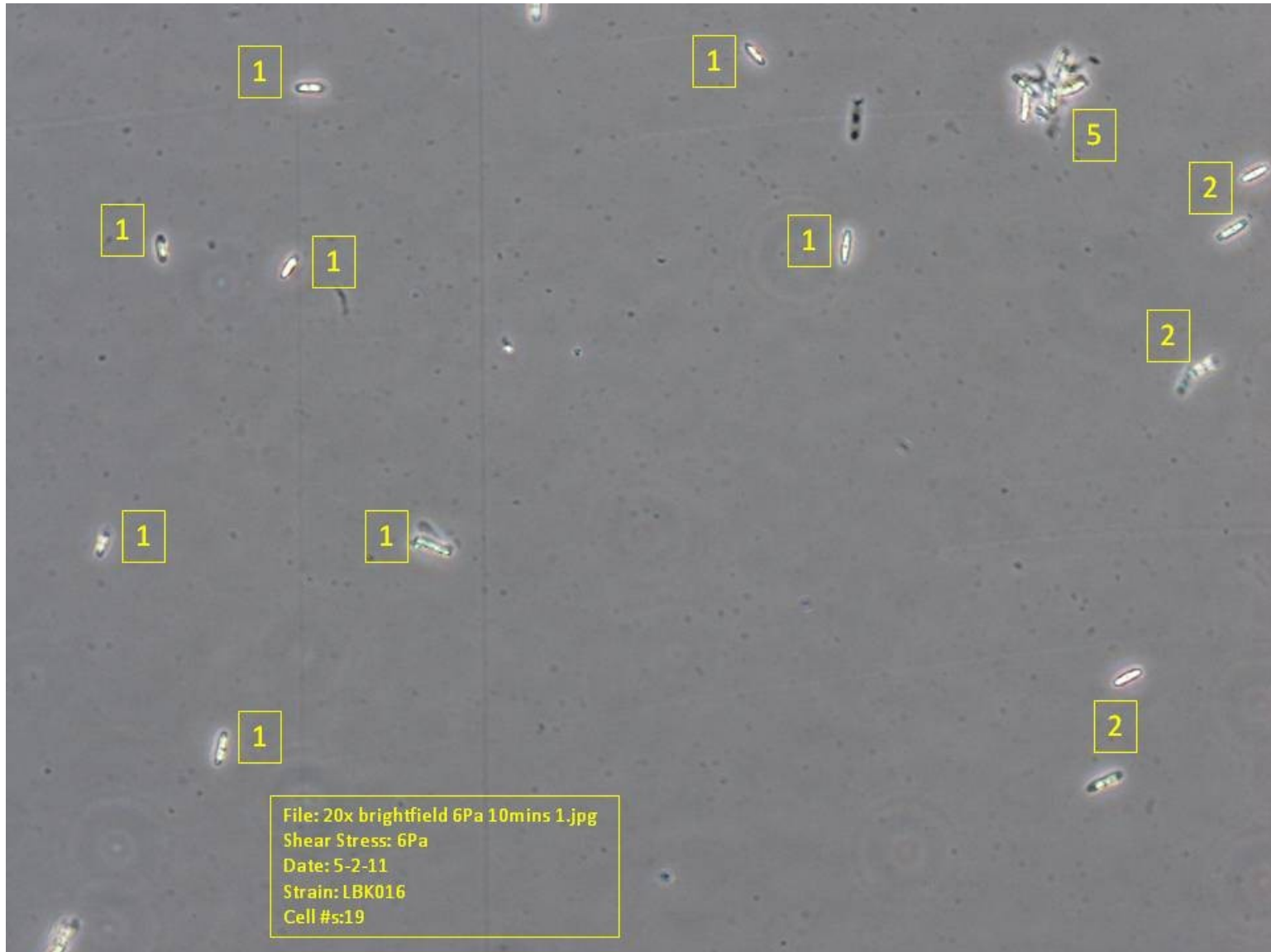




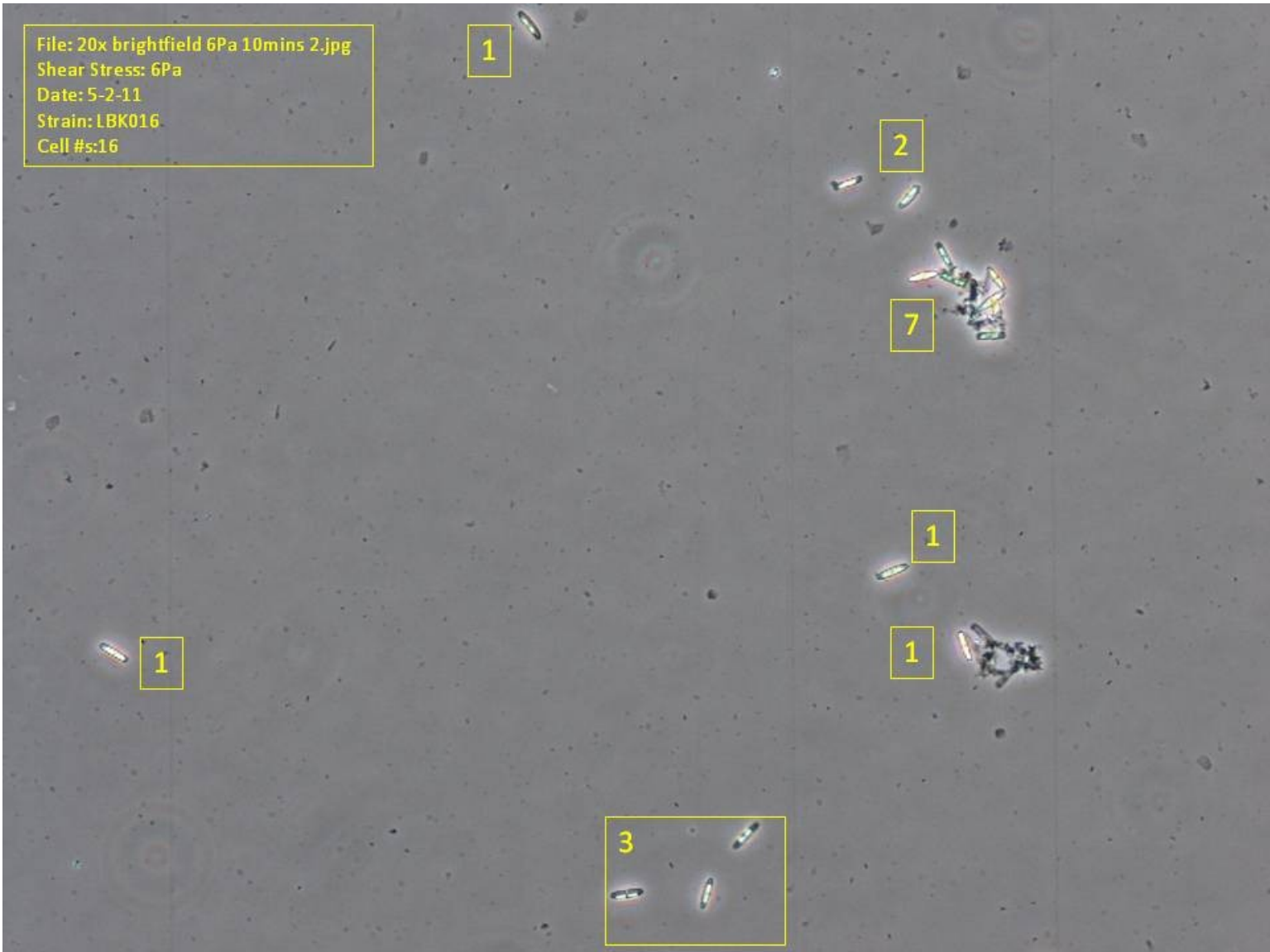




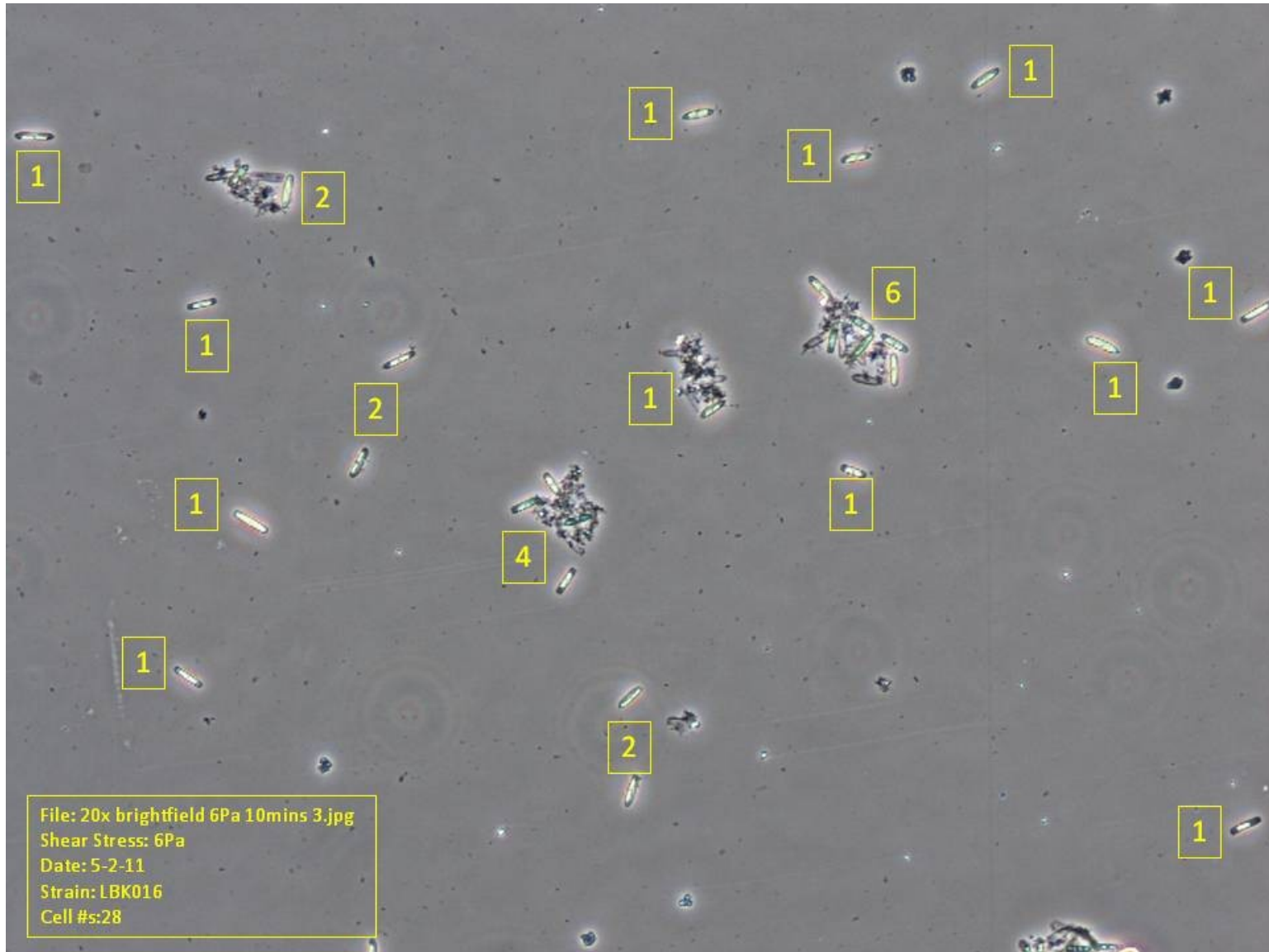






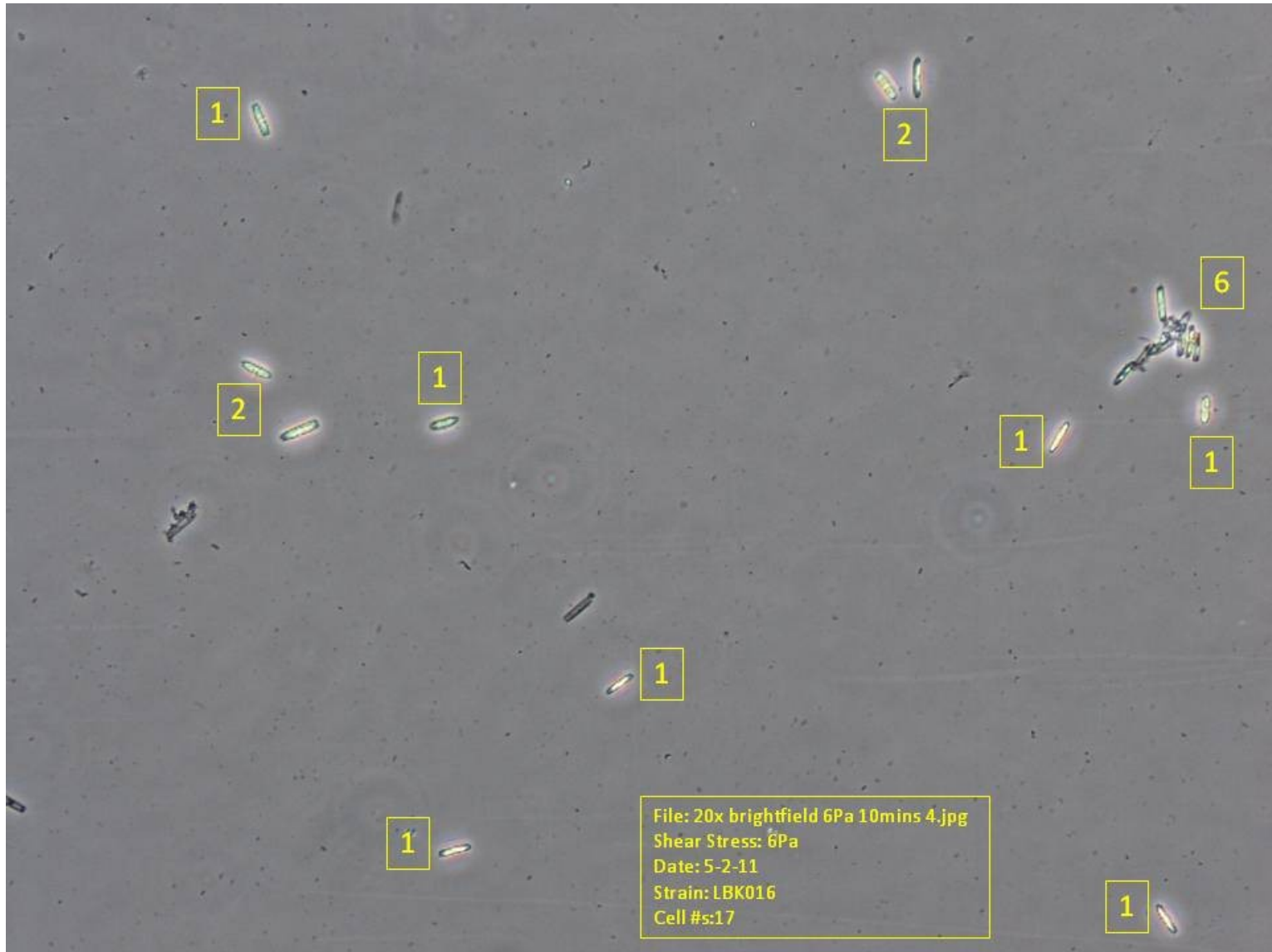


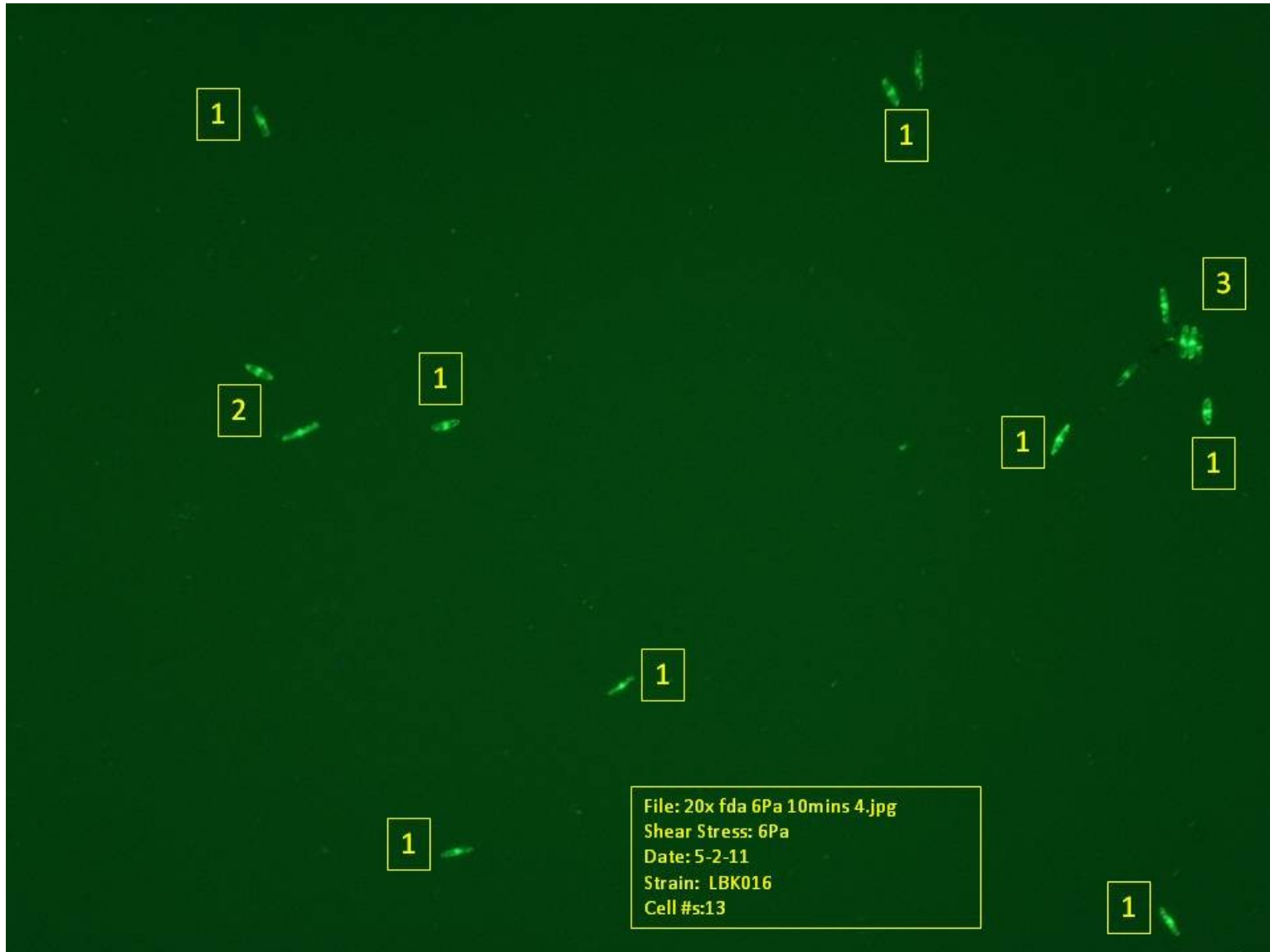


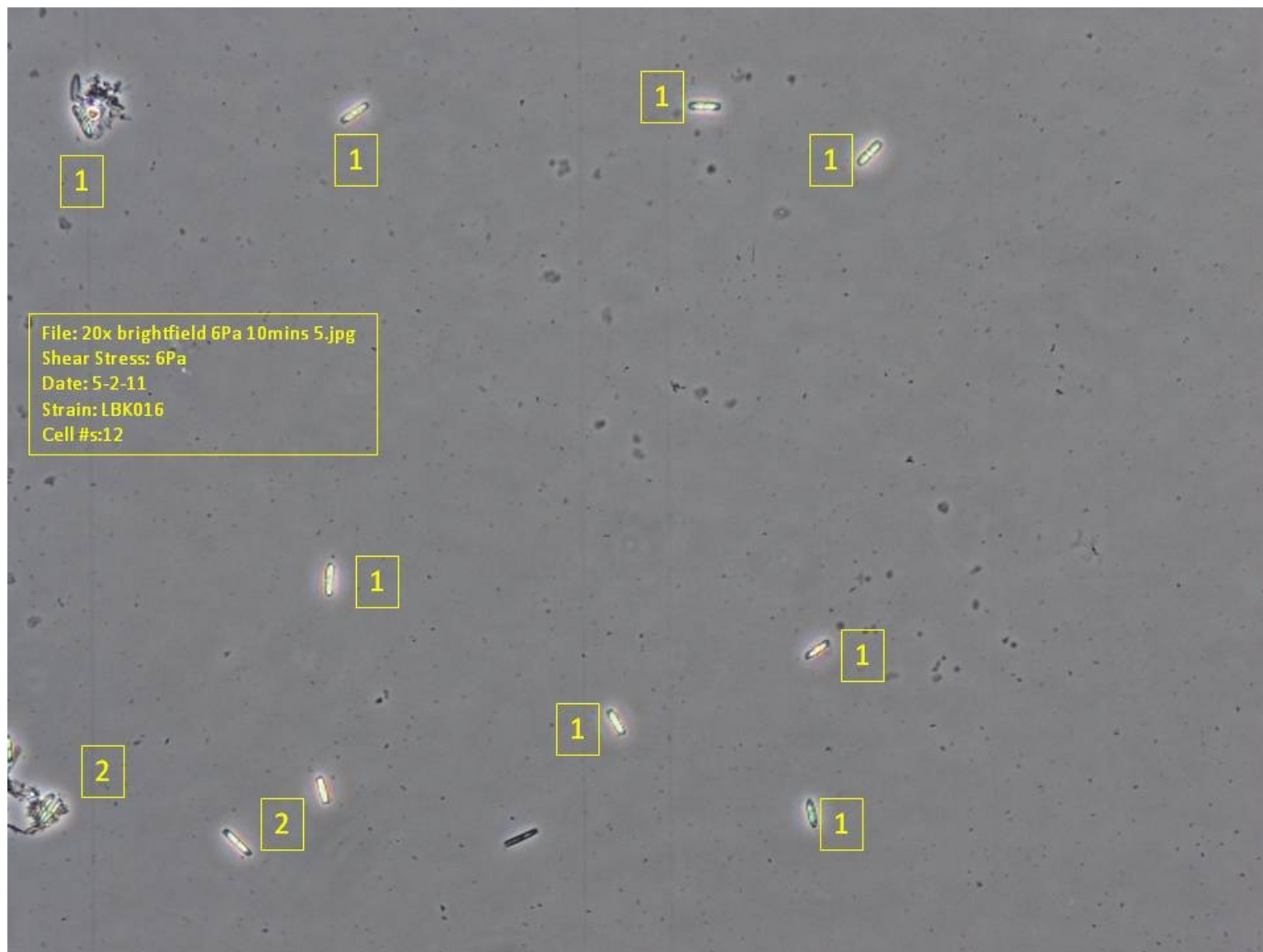




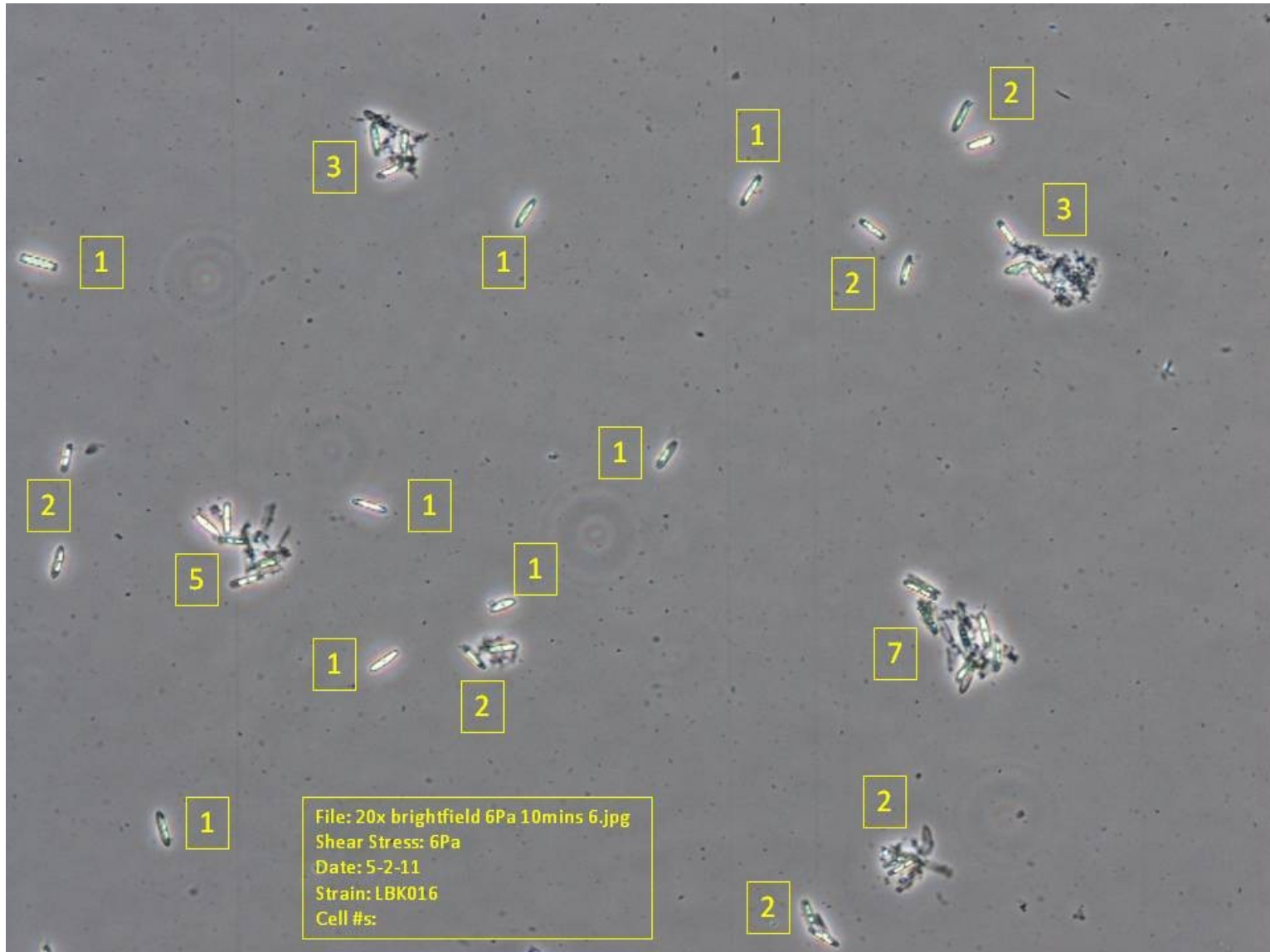


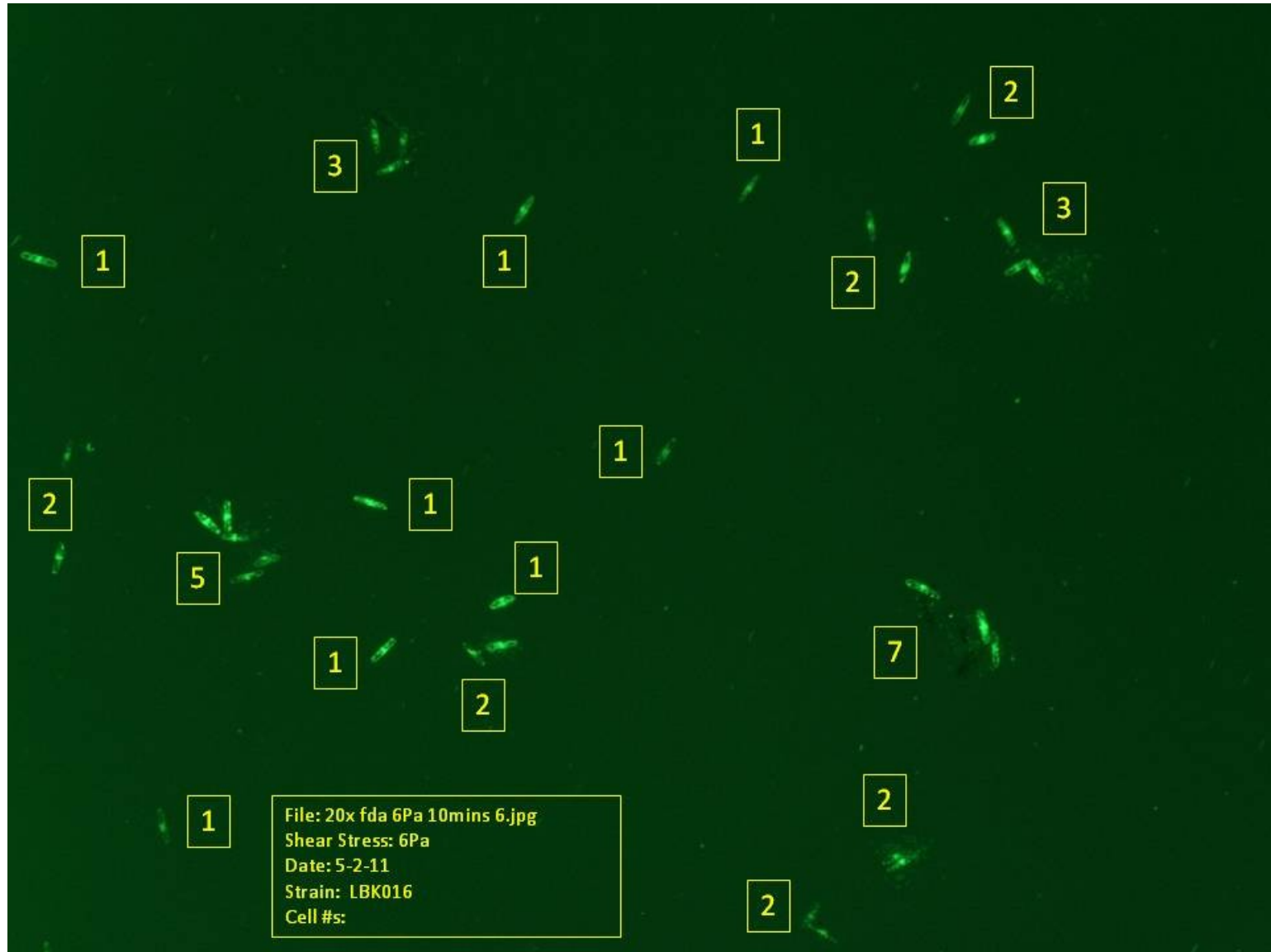


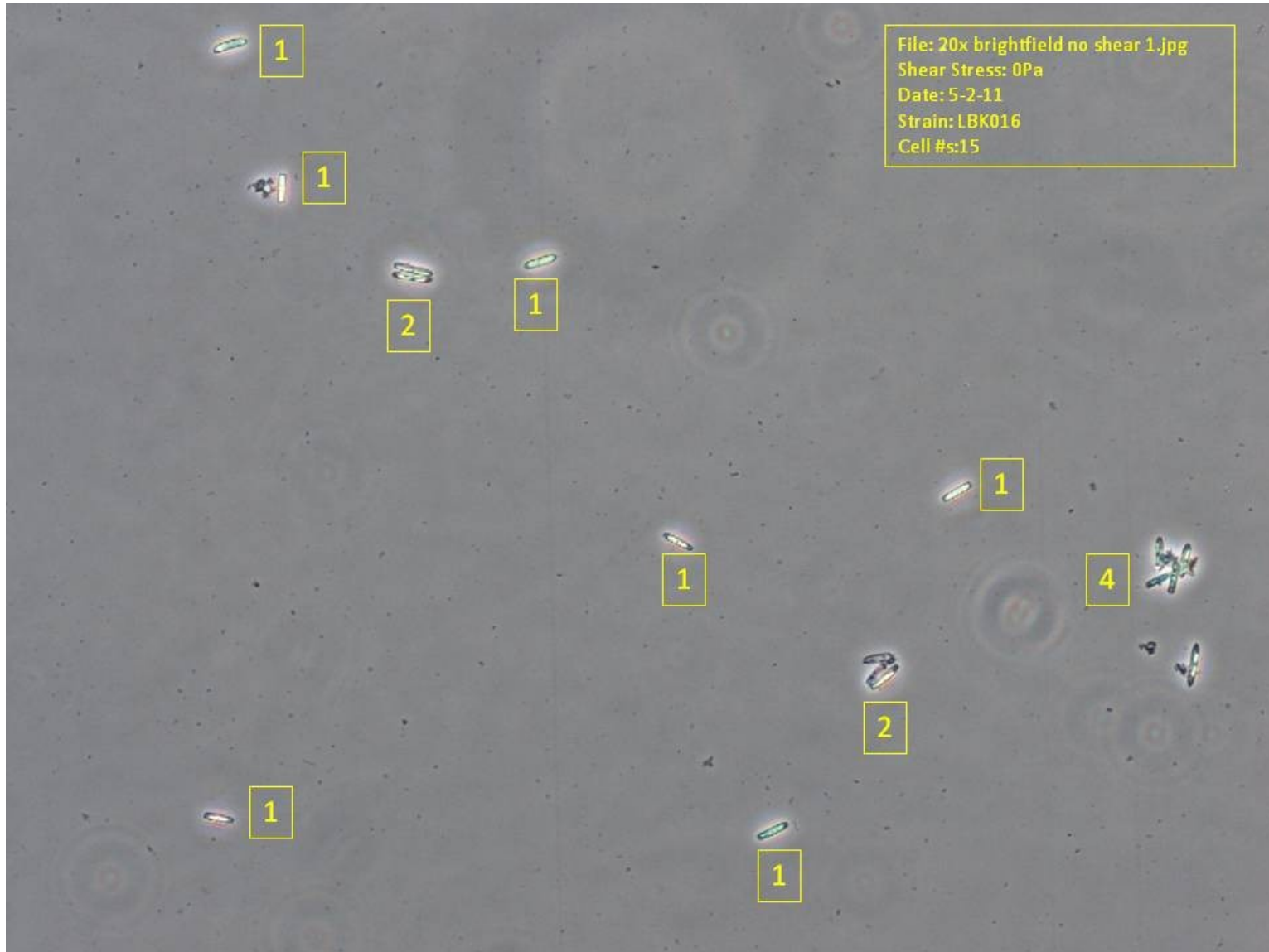






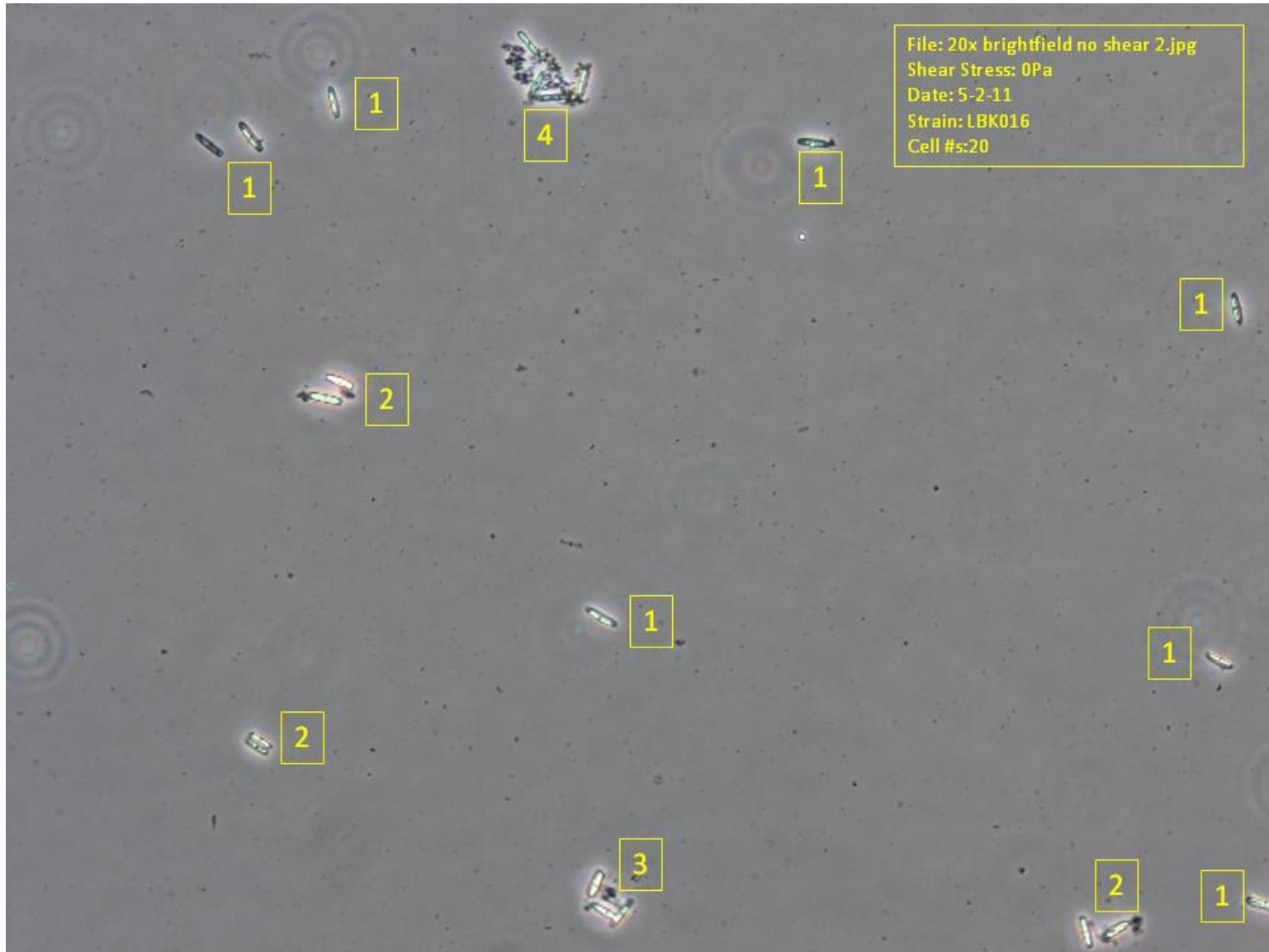




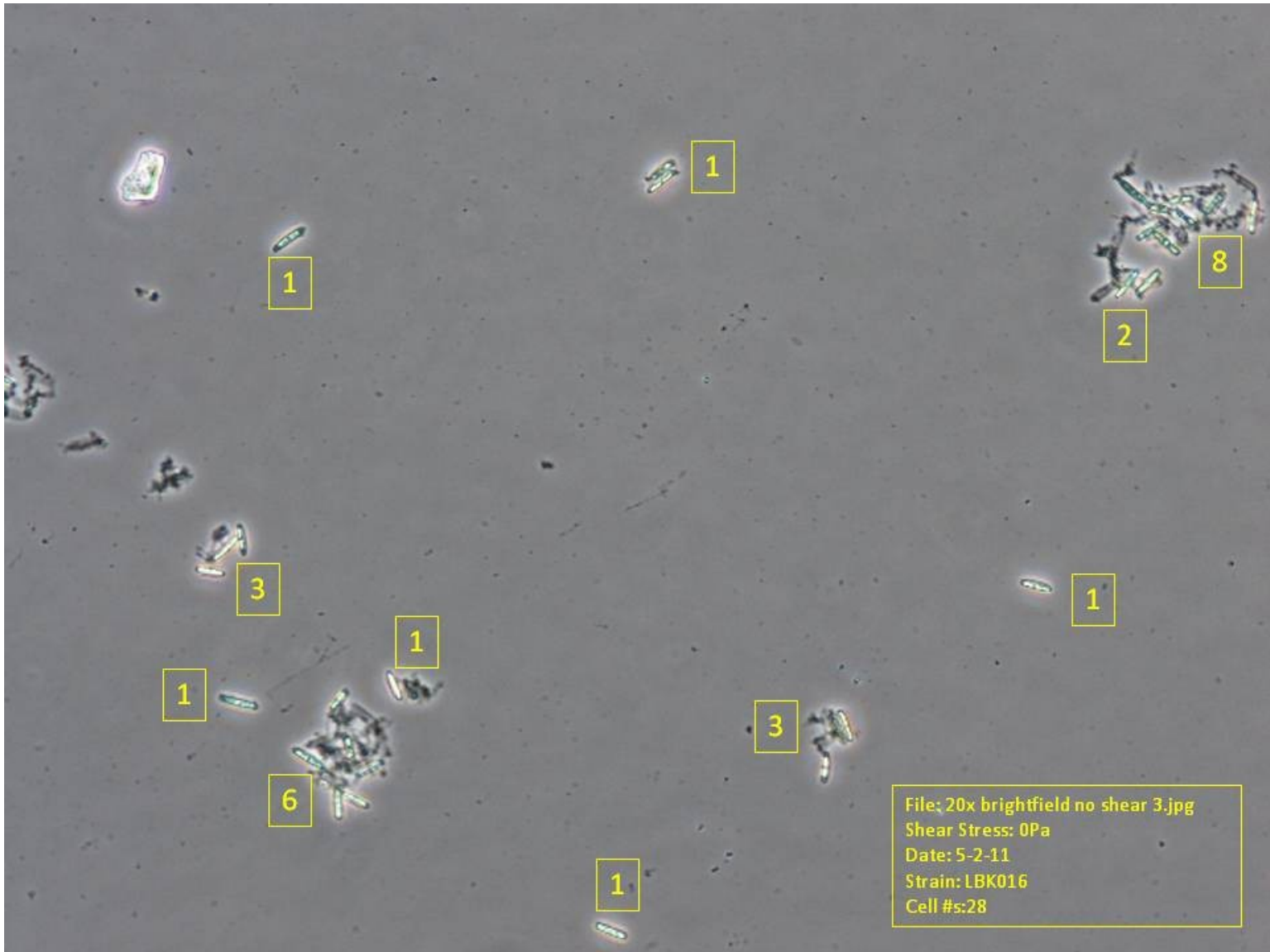




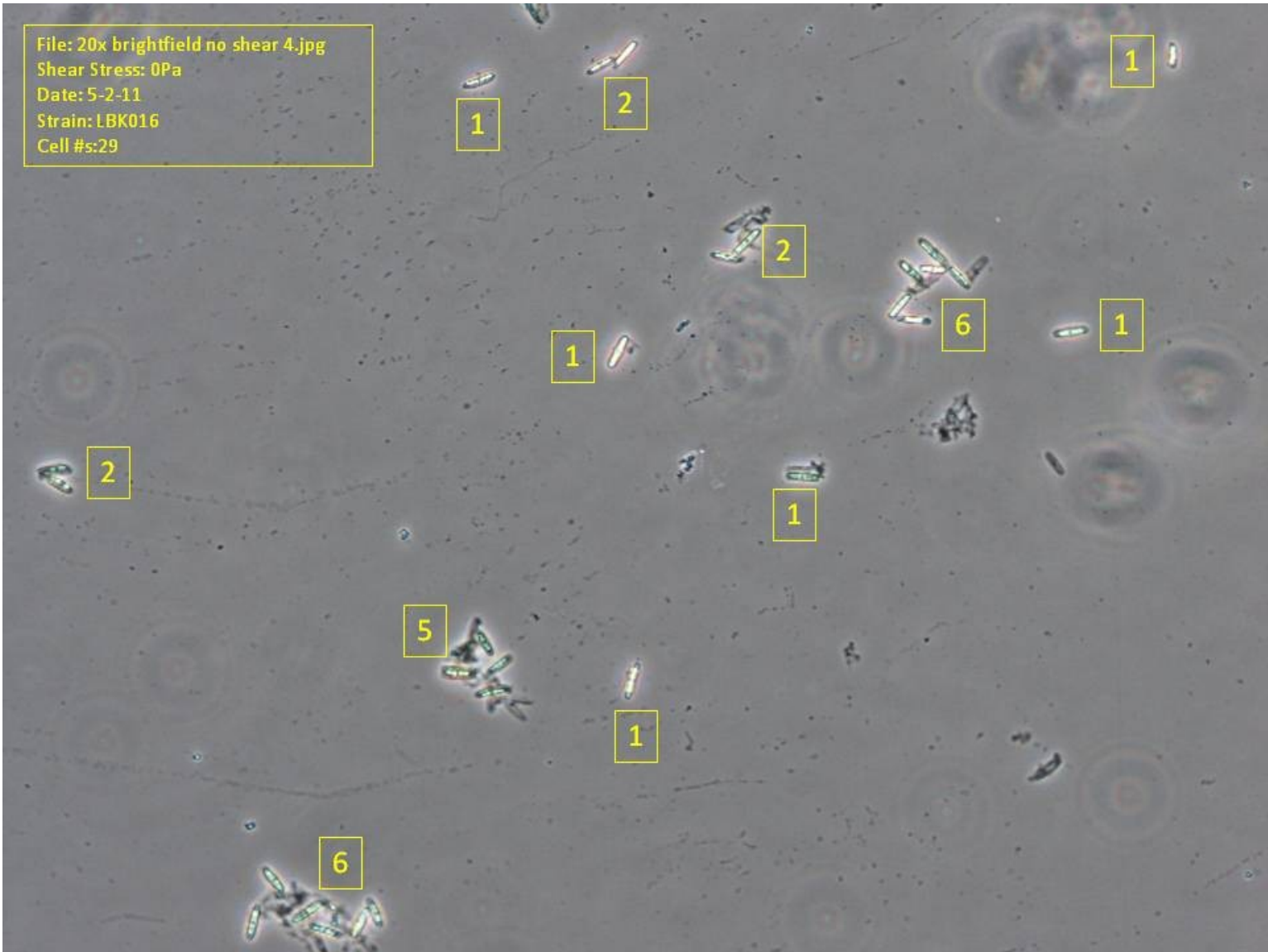




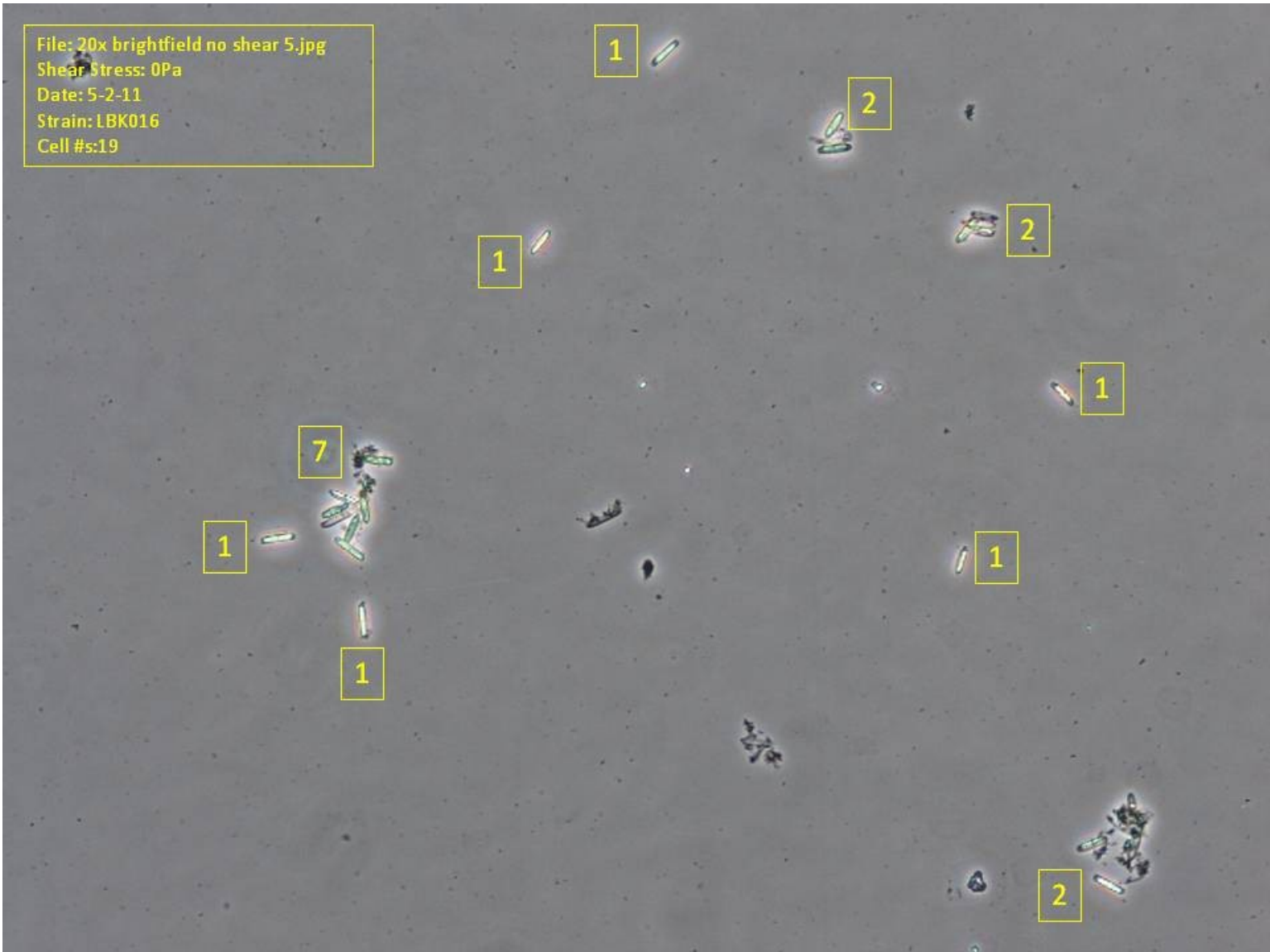


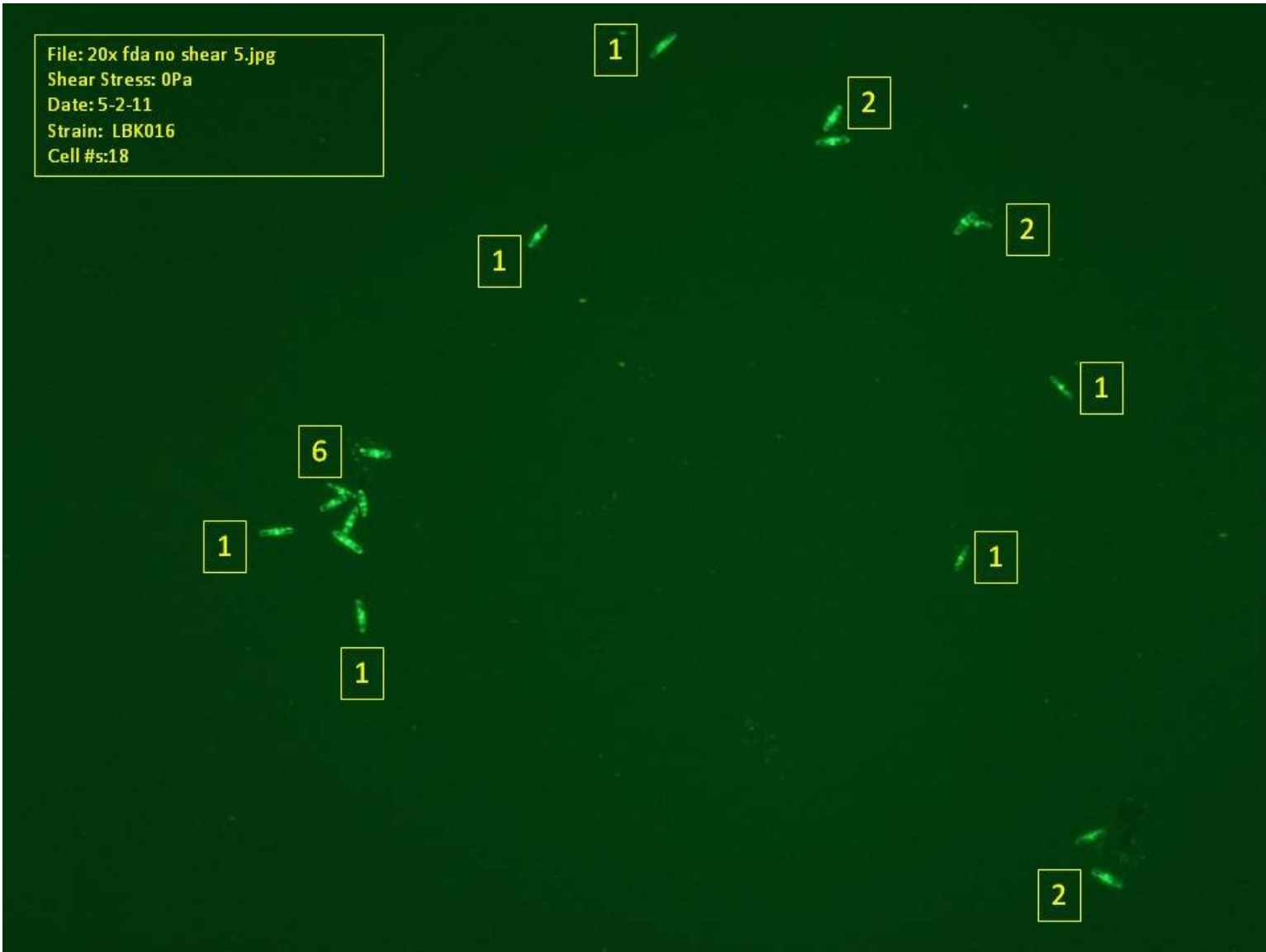




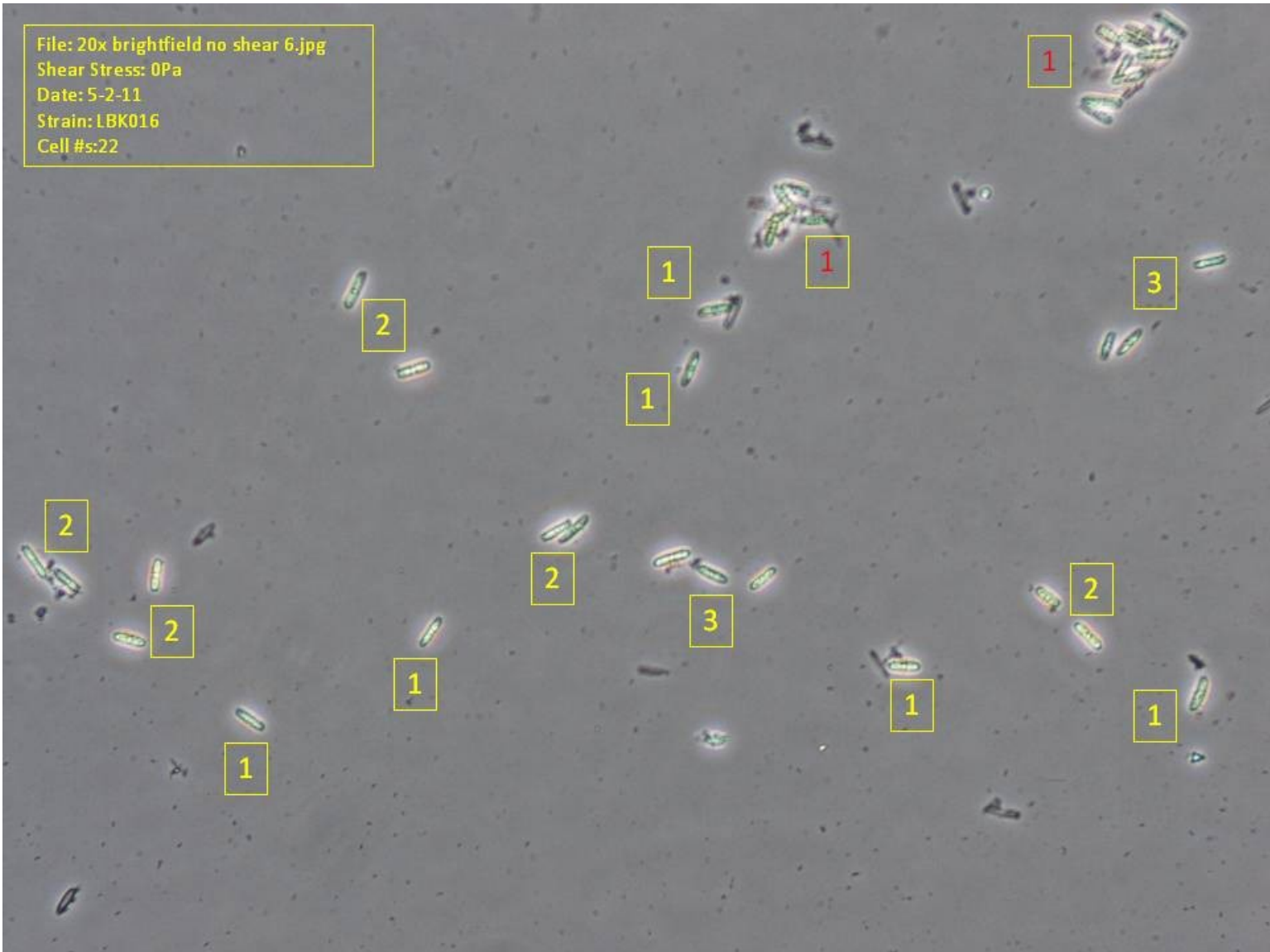


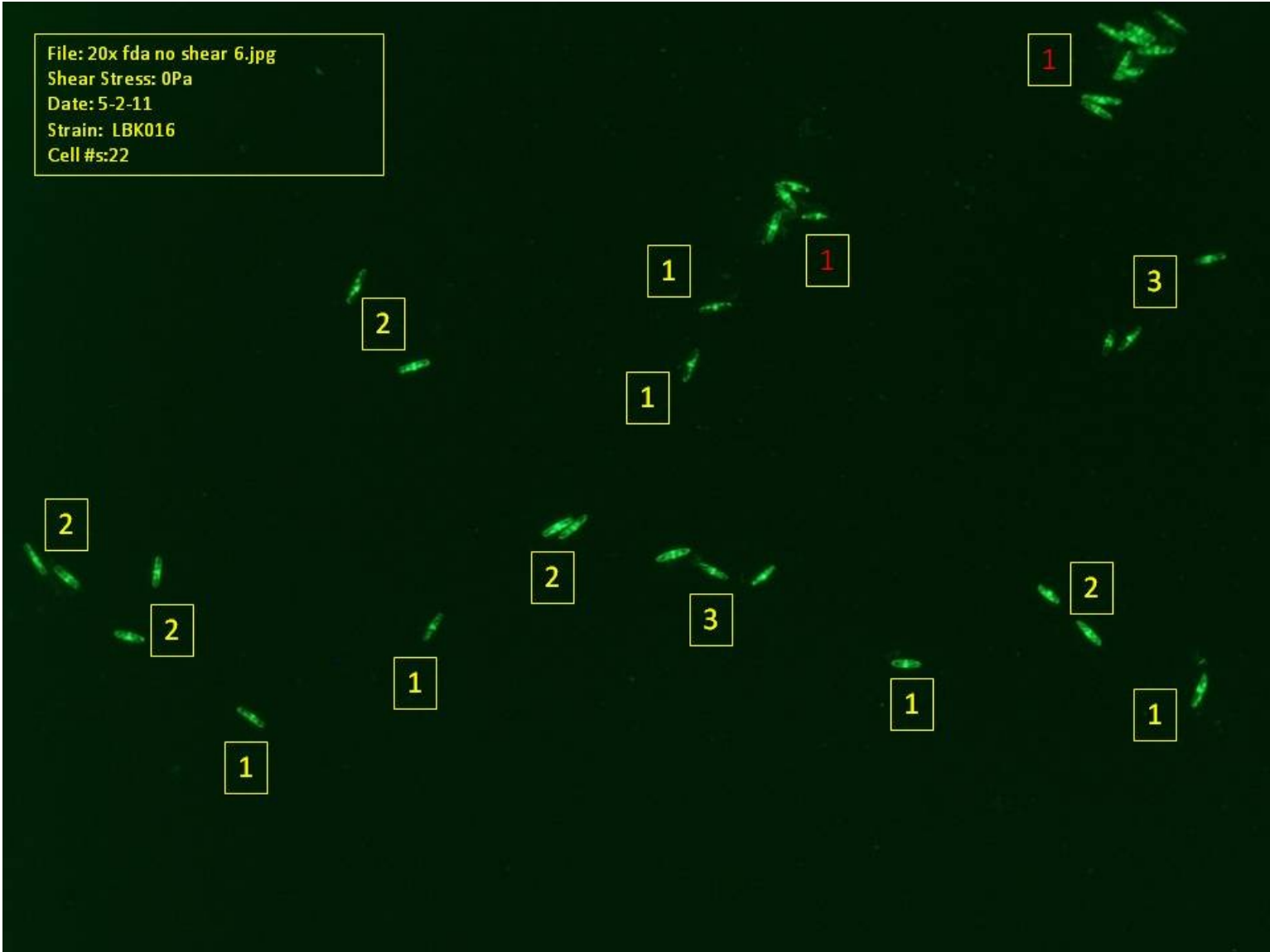


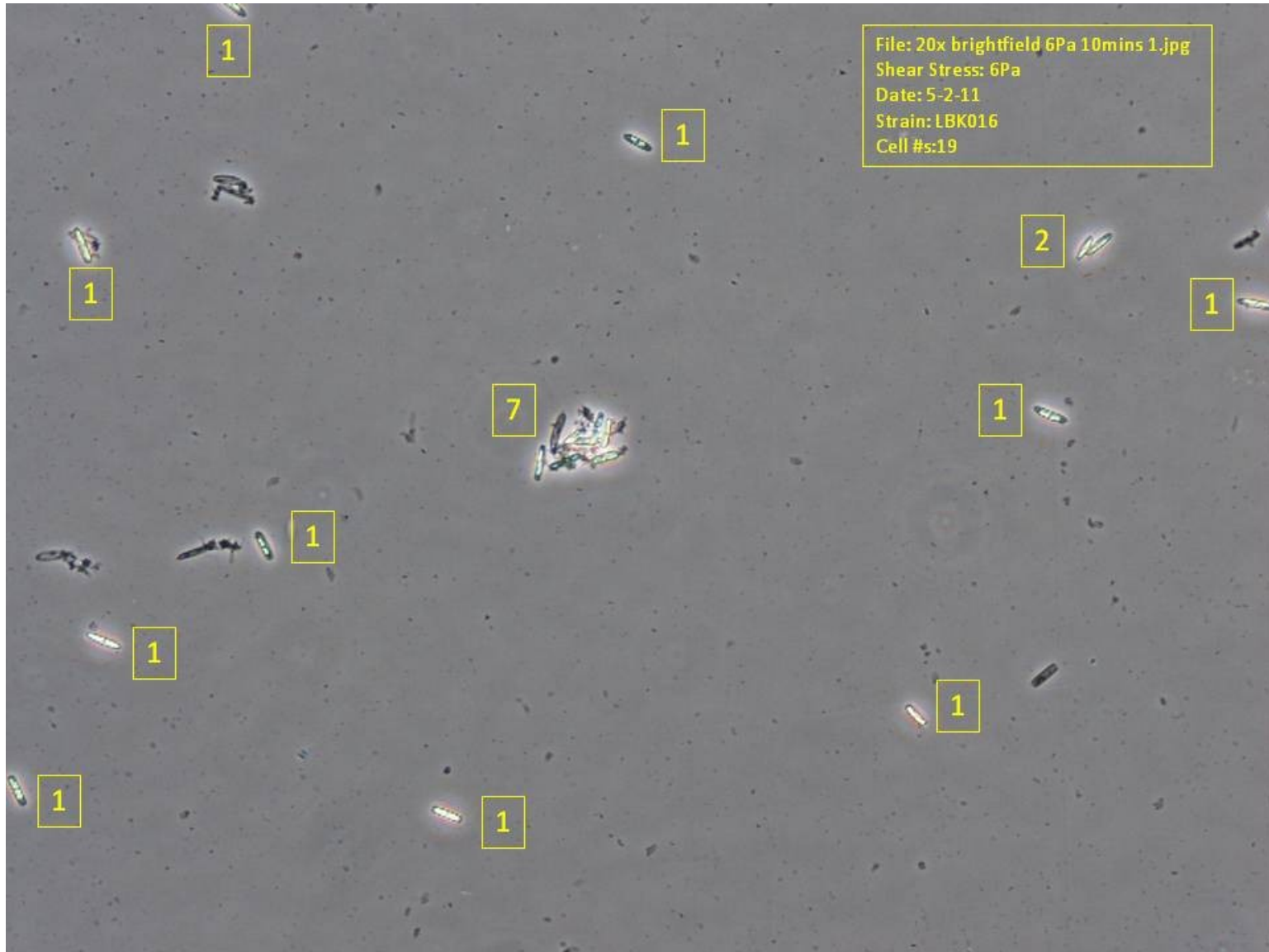




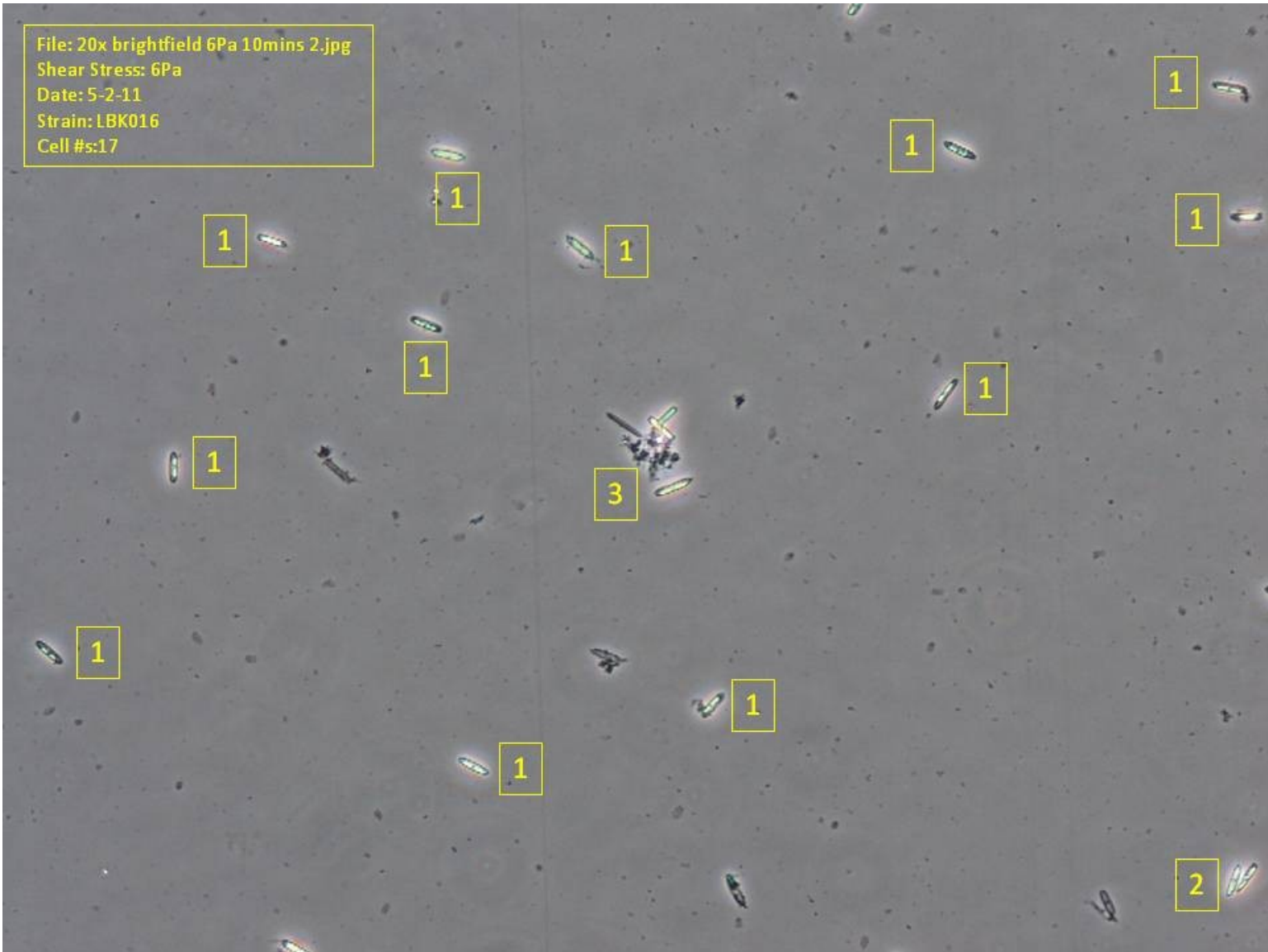


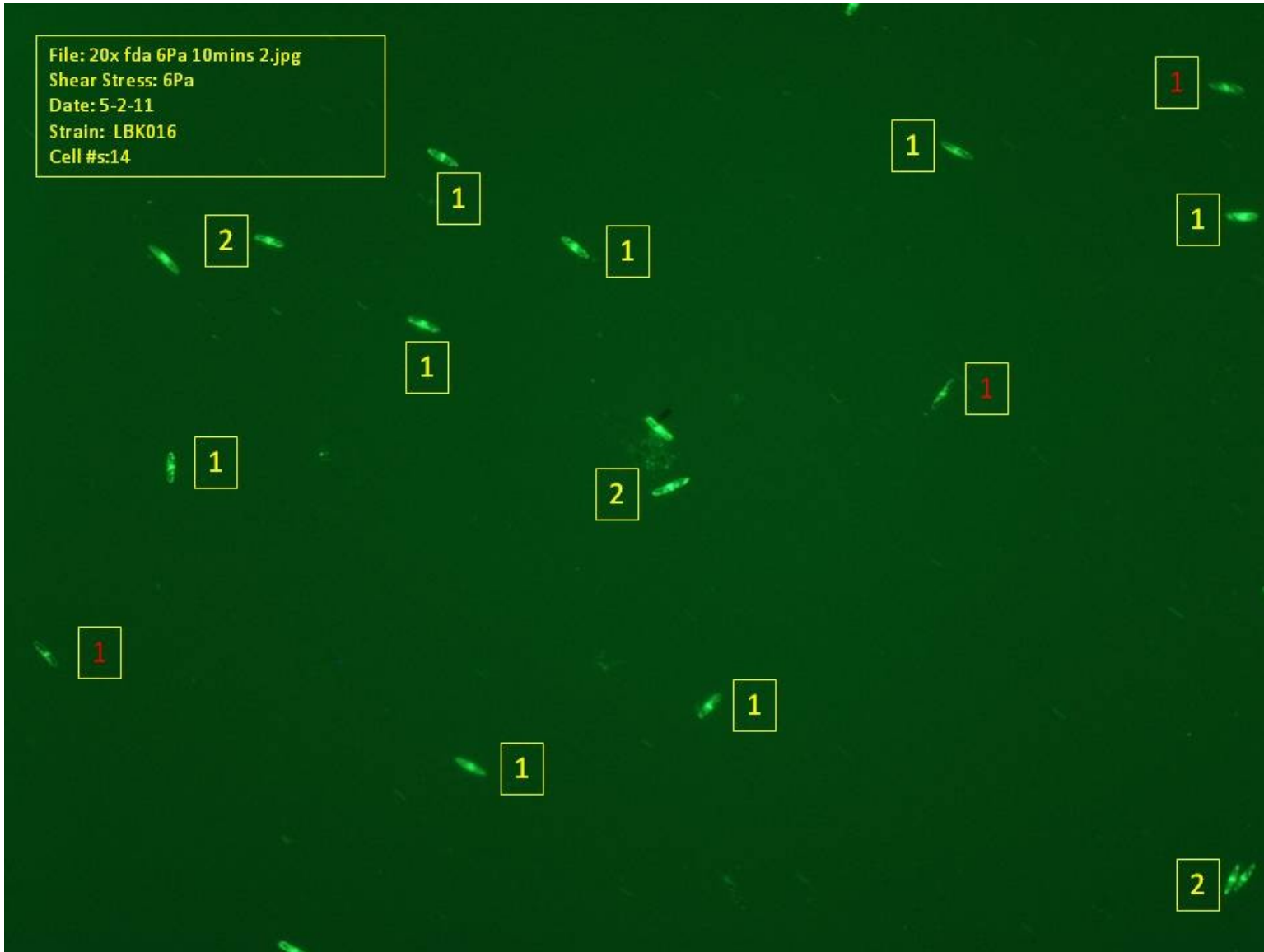


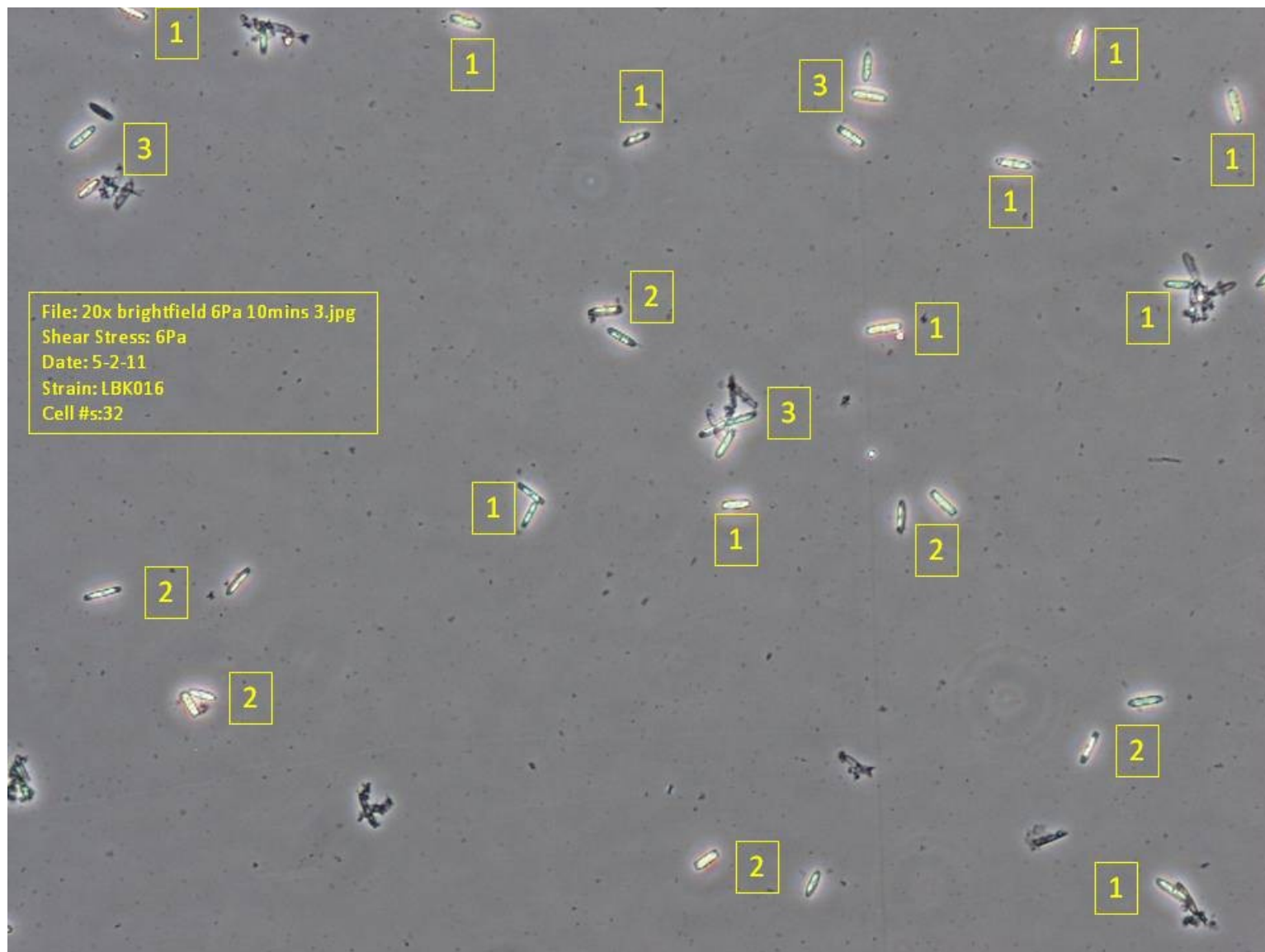






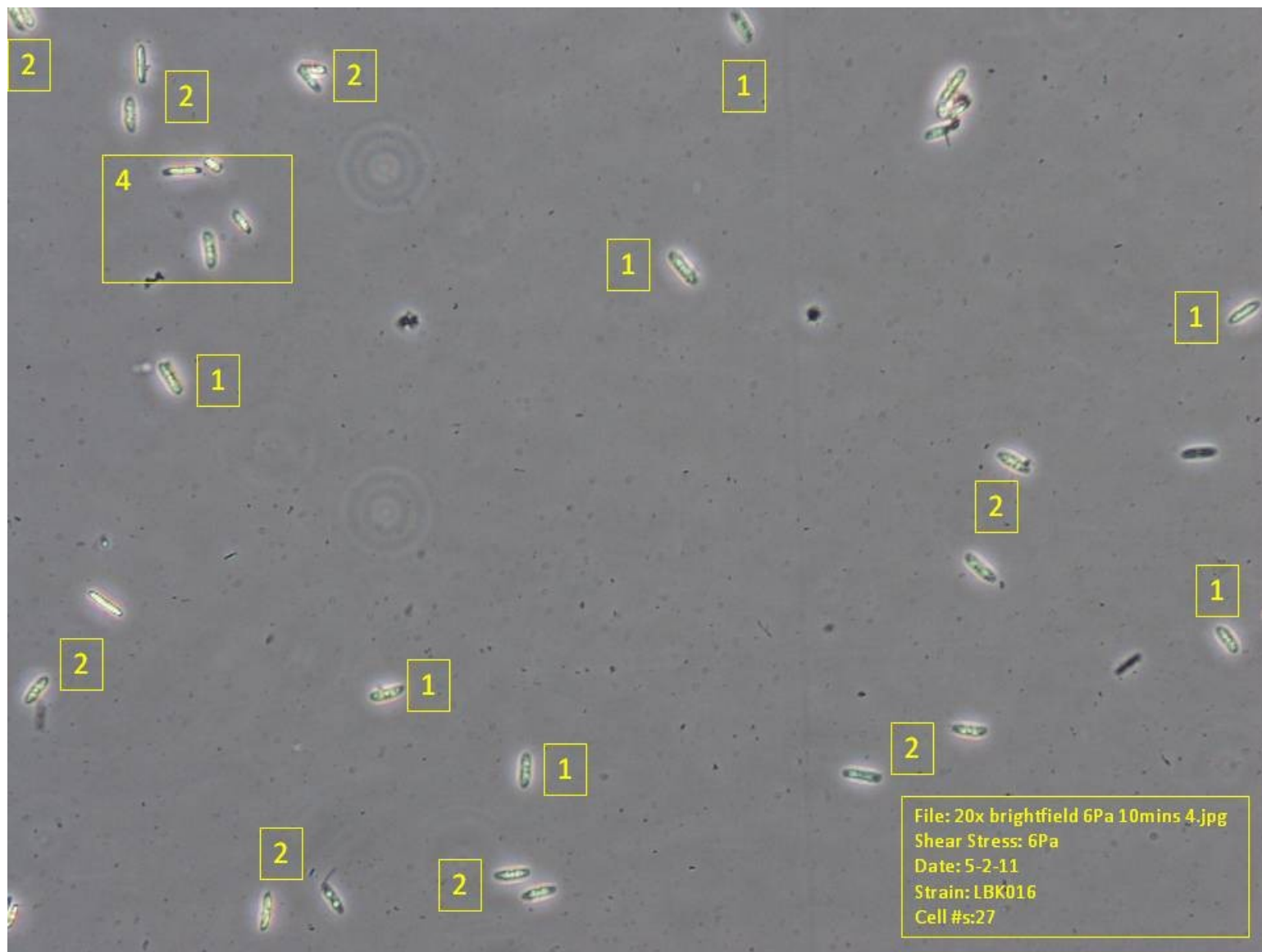


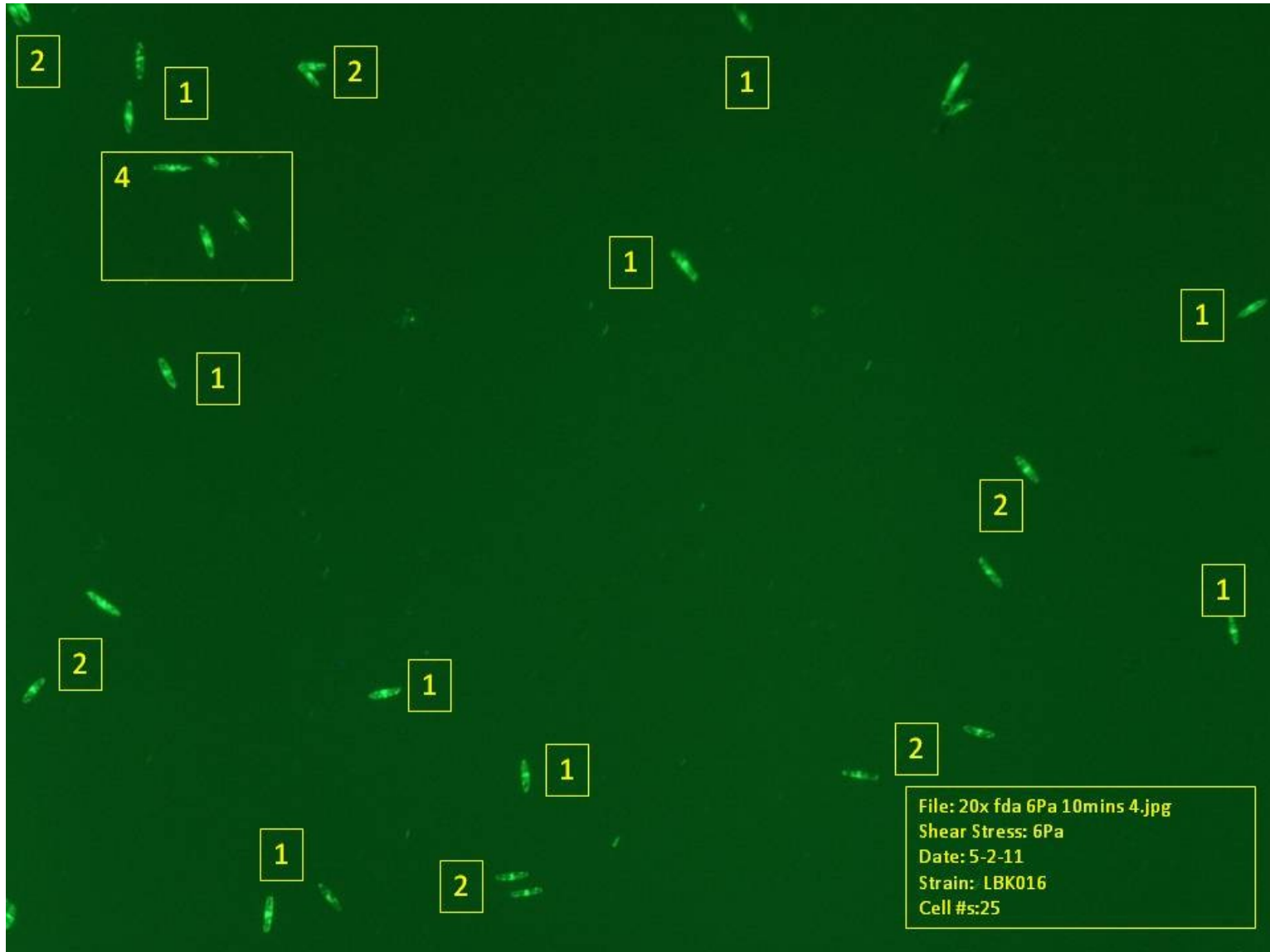


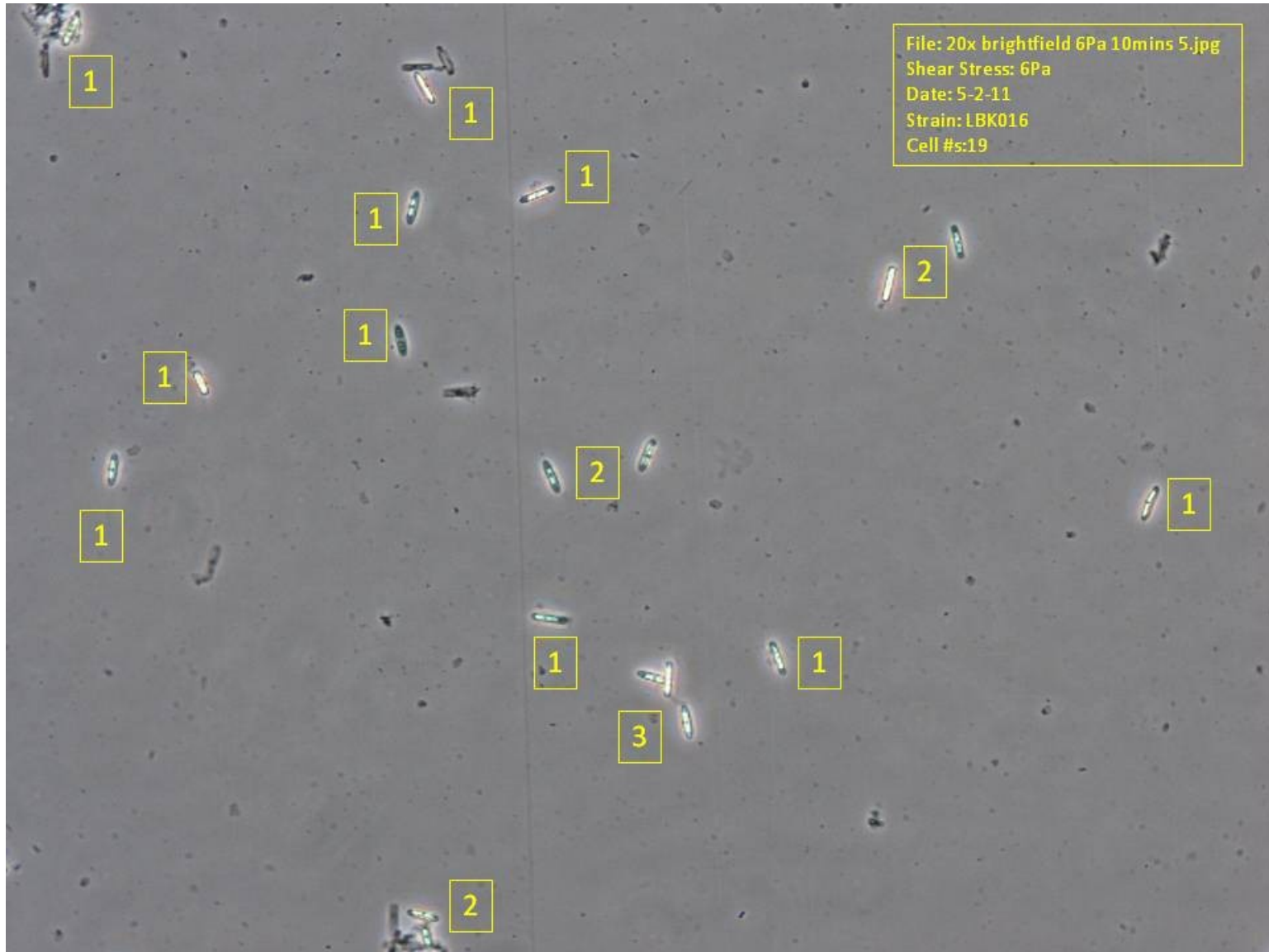


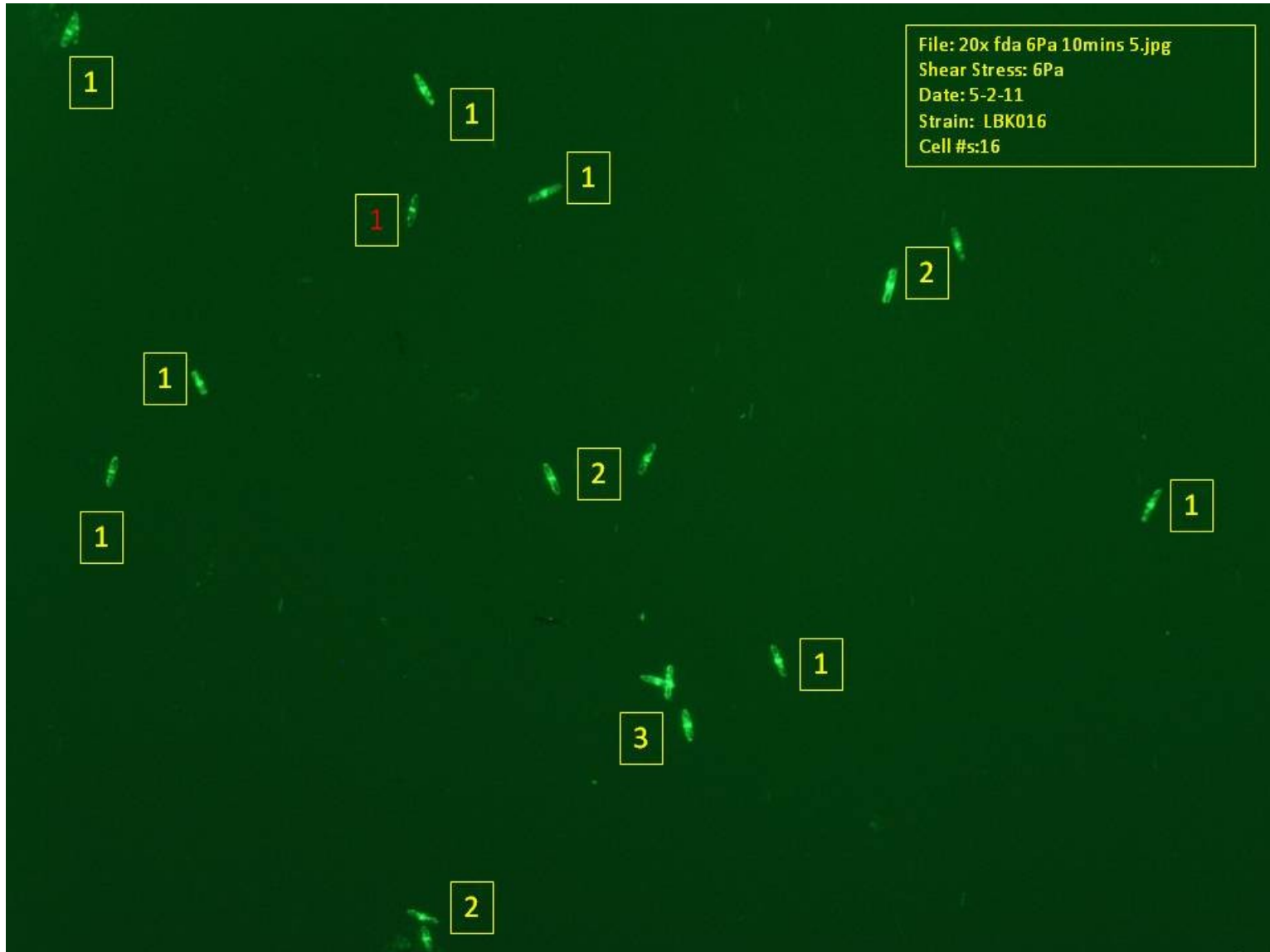


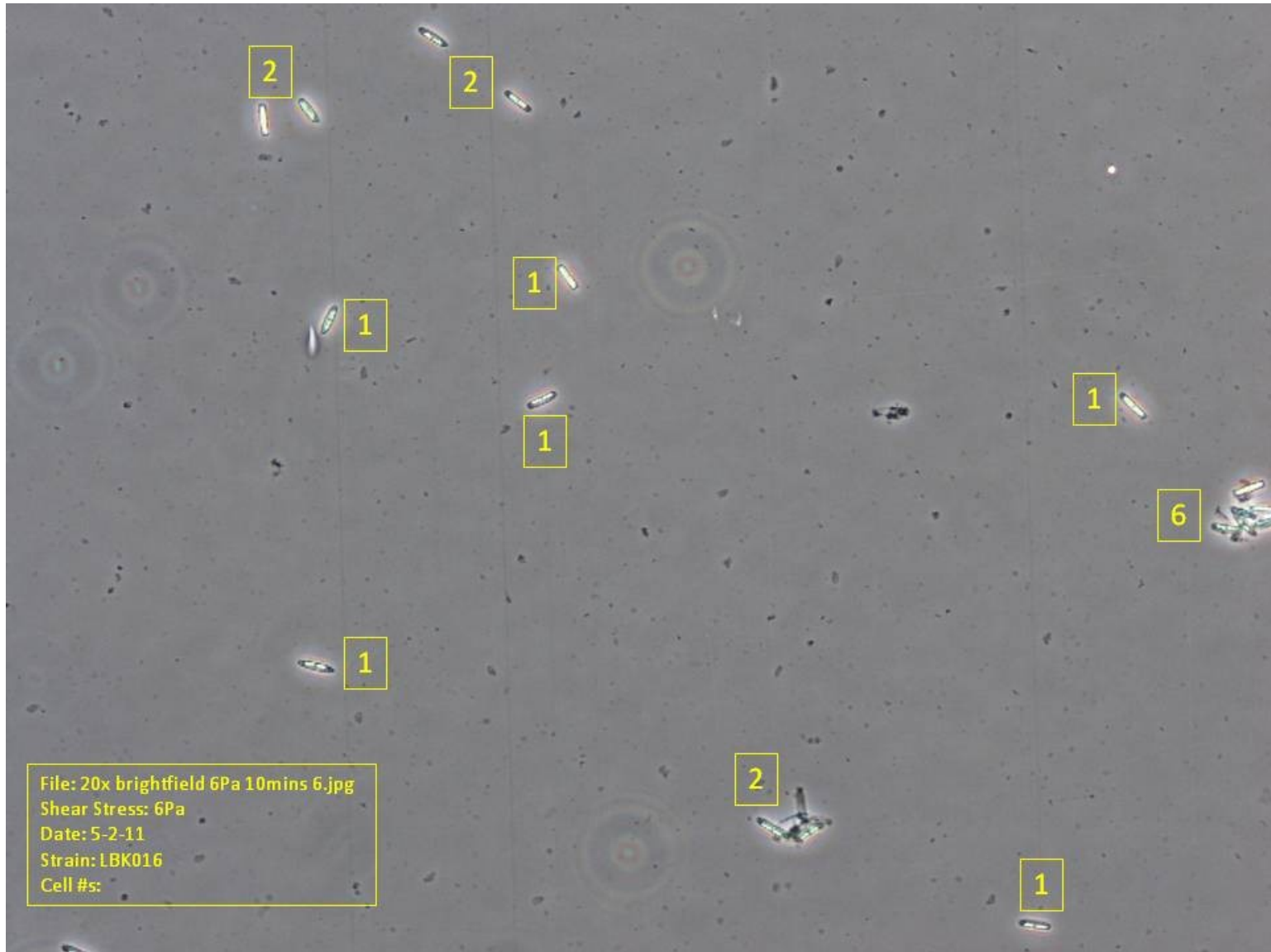




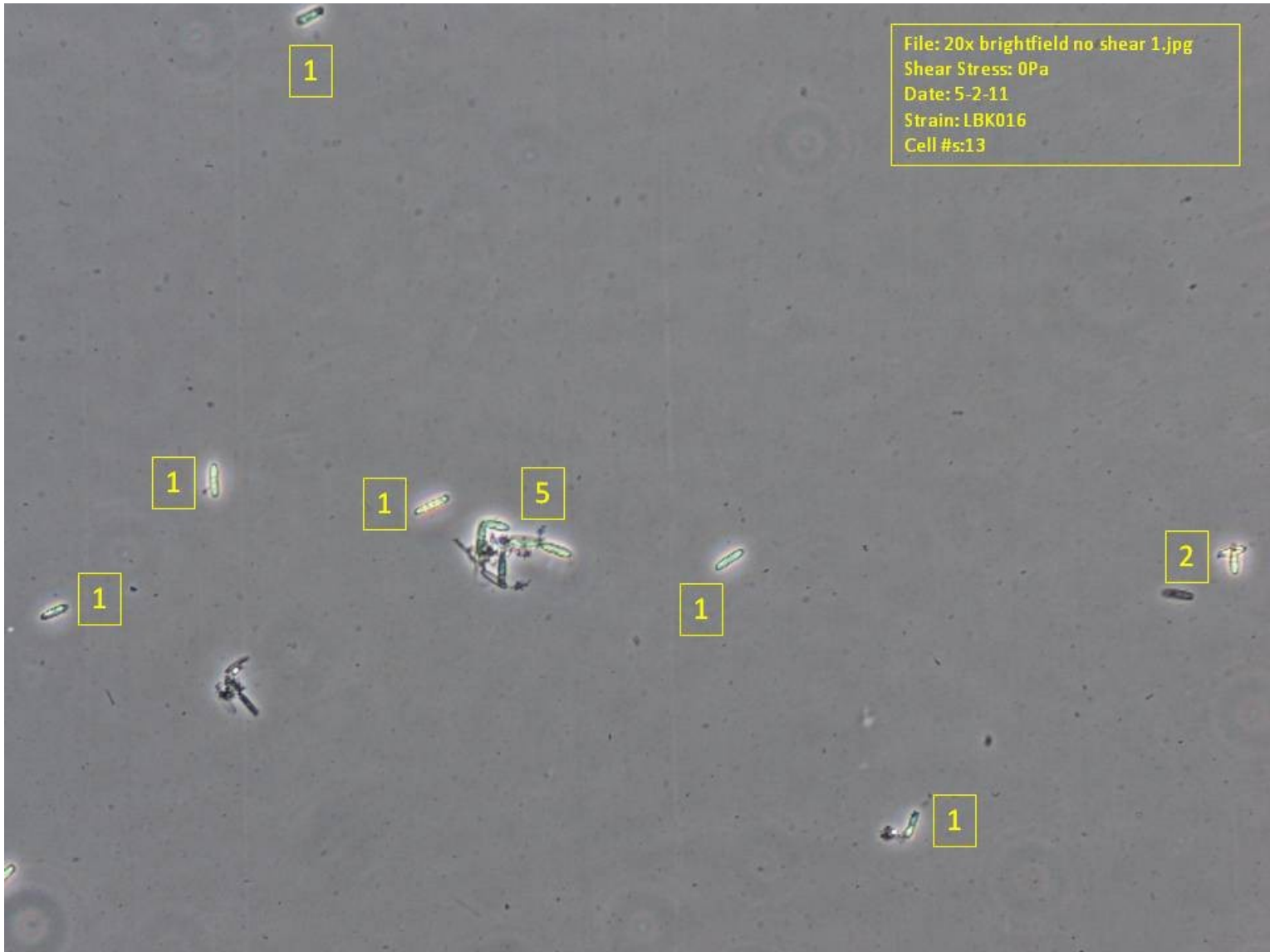










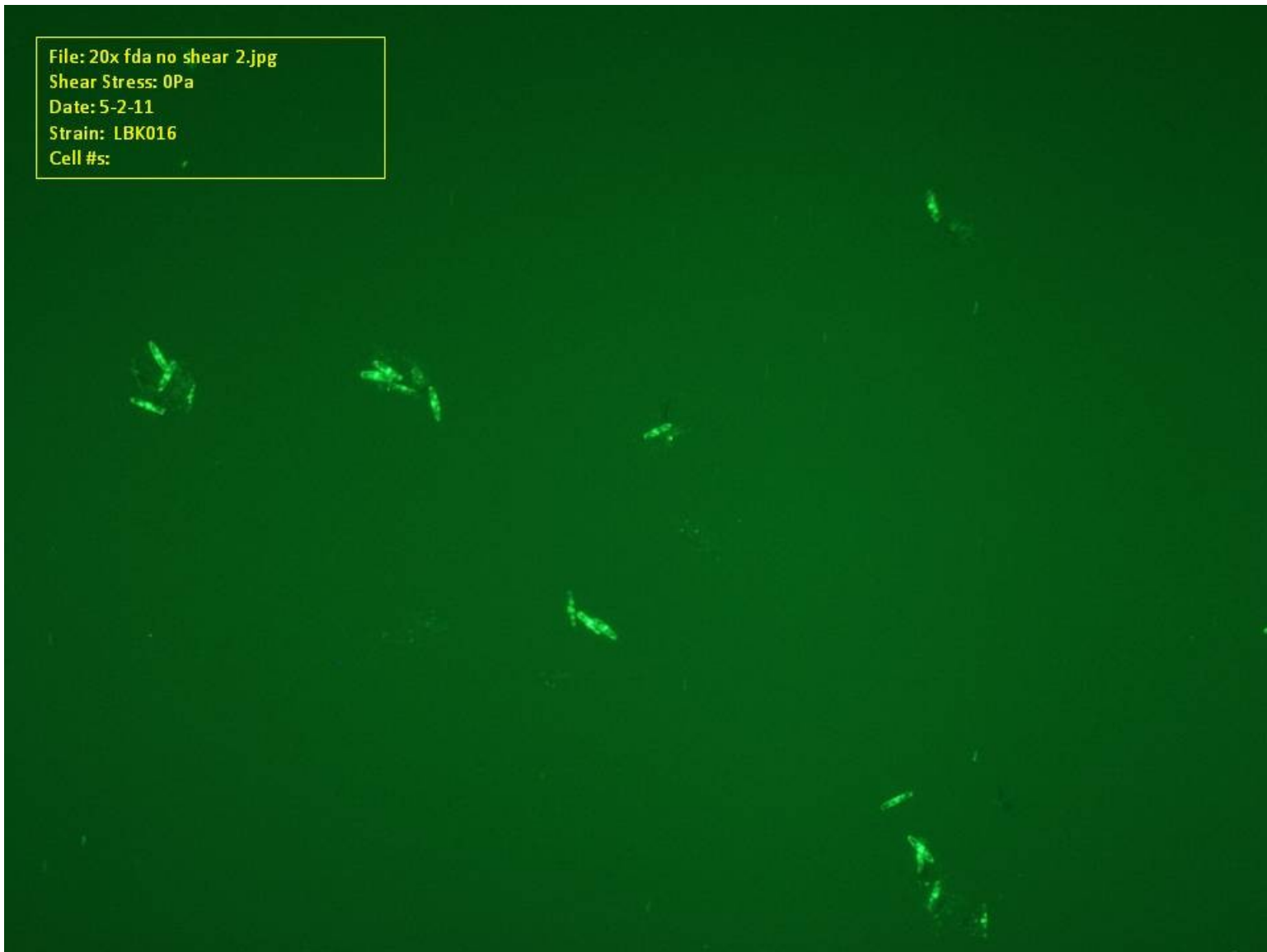


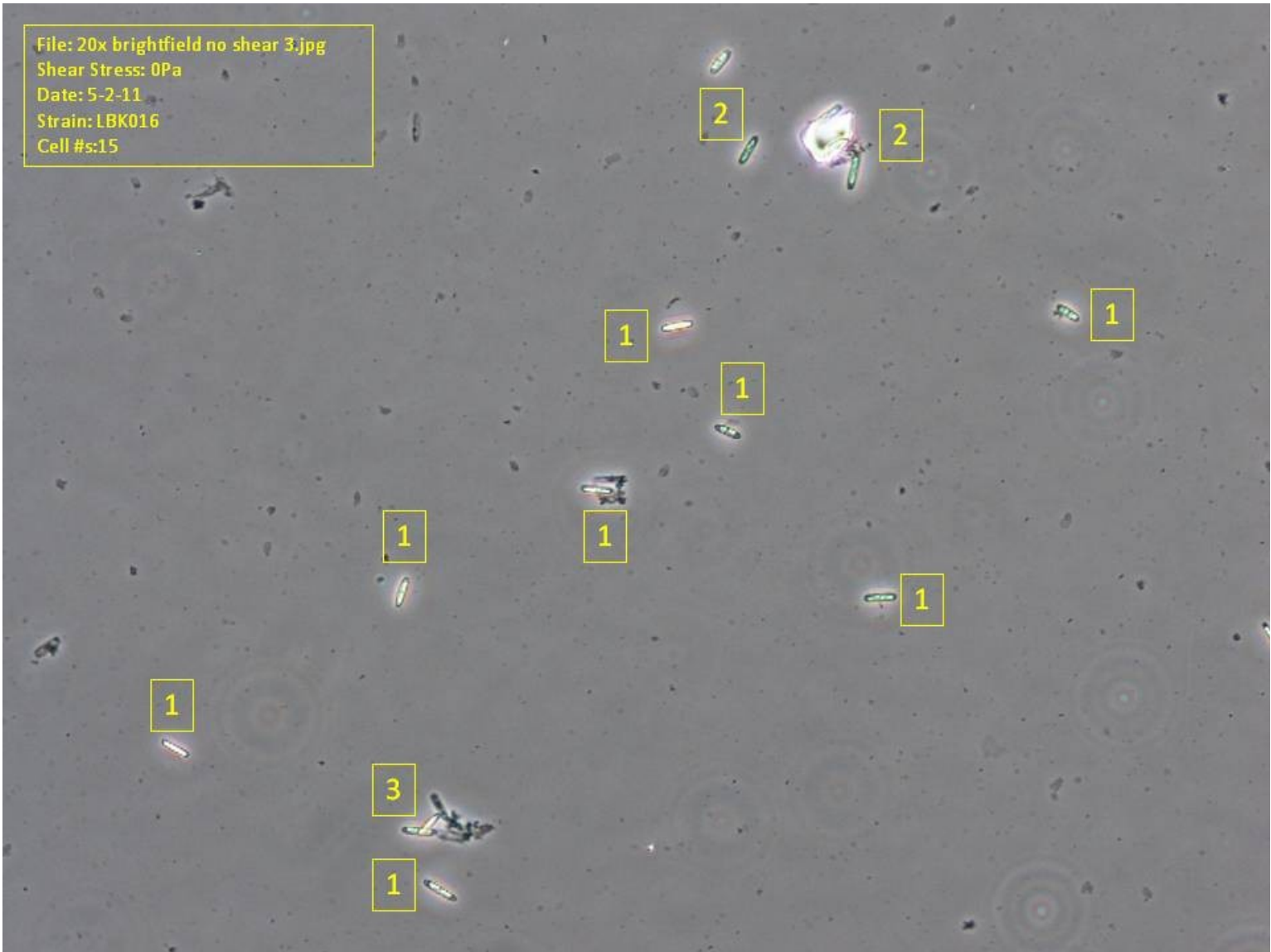


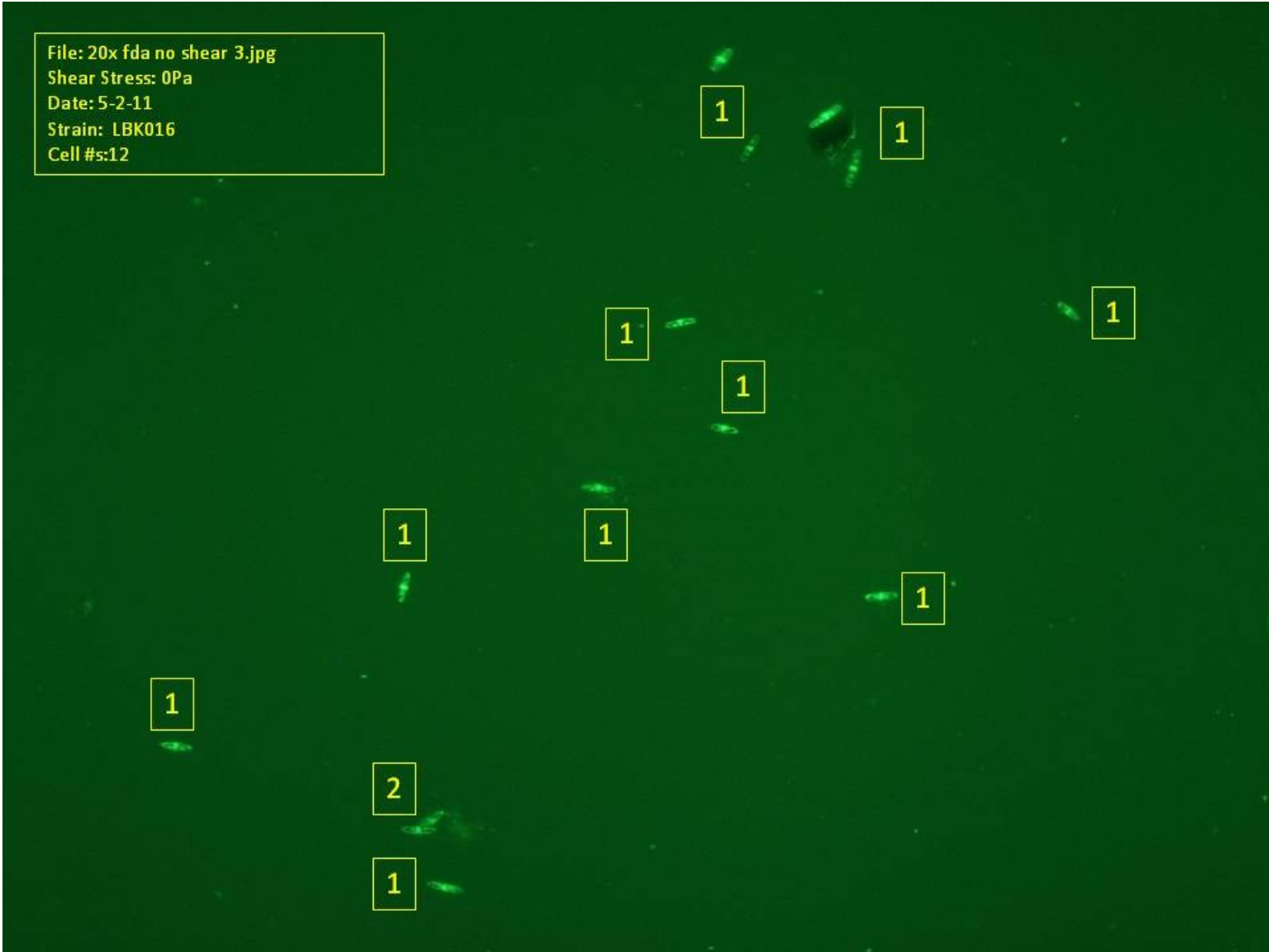


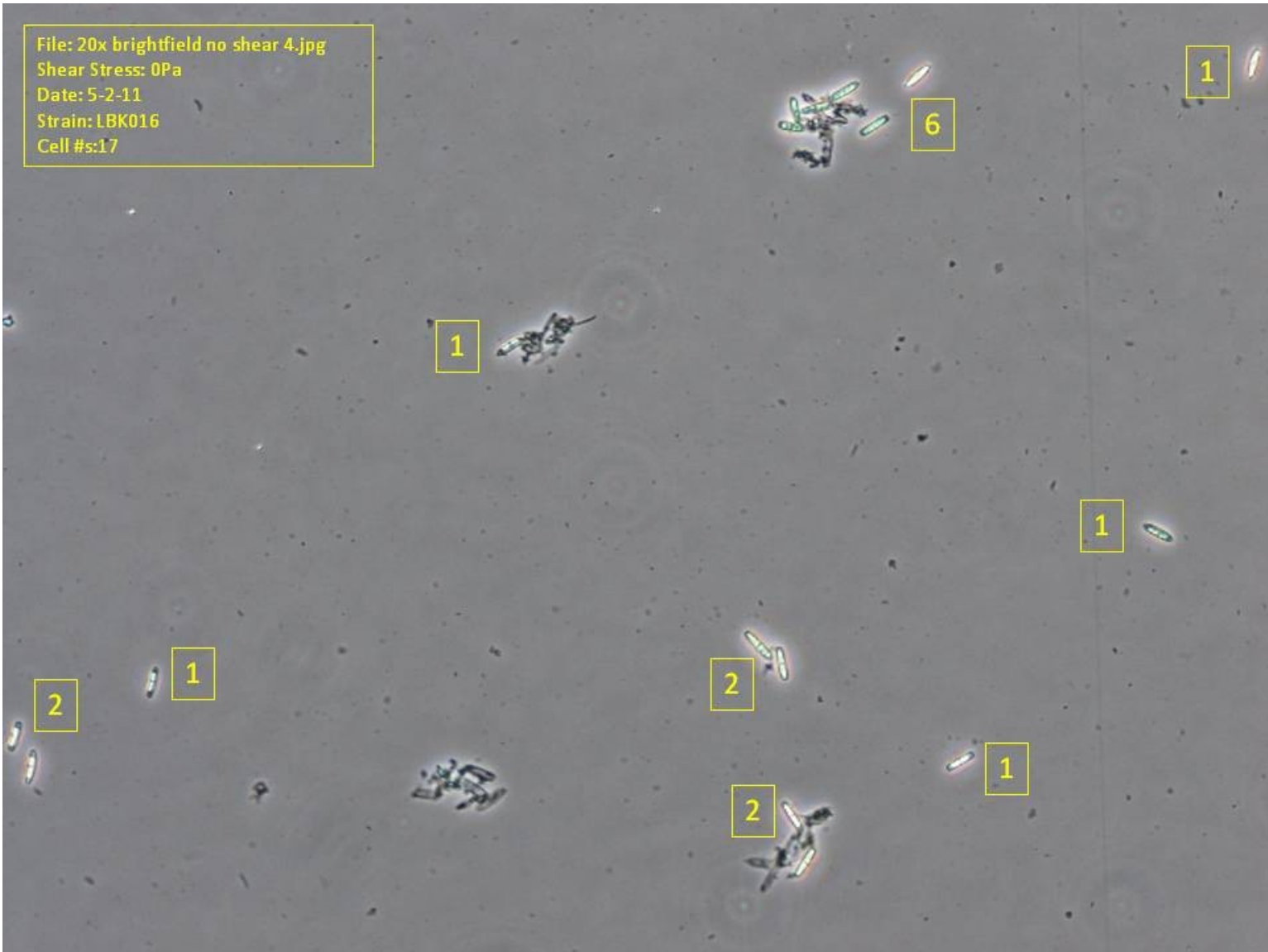


File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK016  
Cell #s:

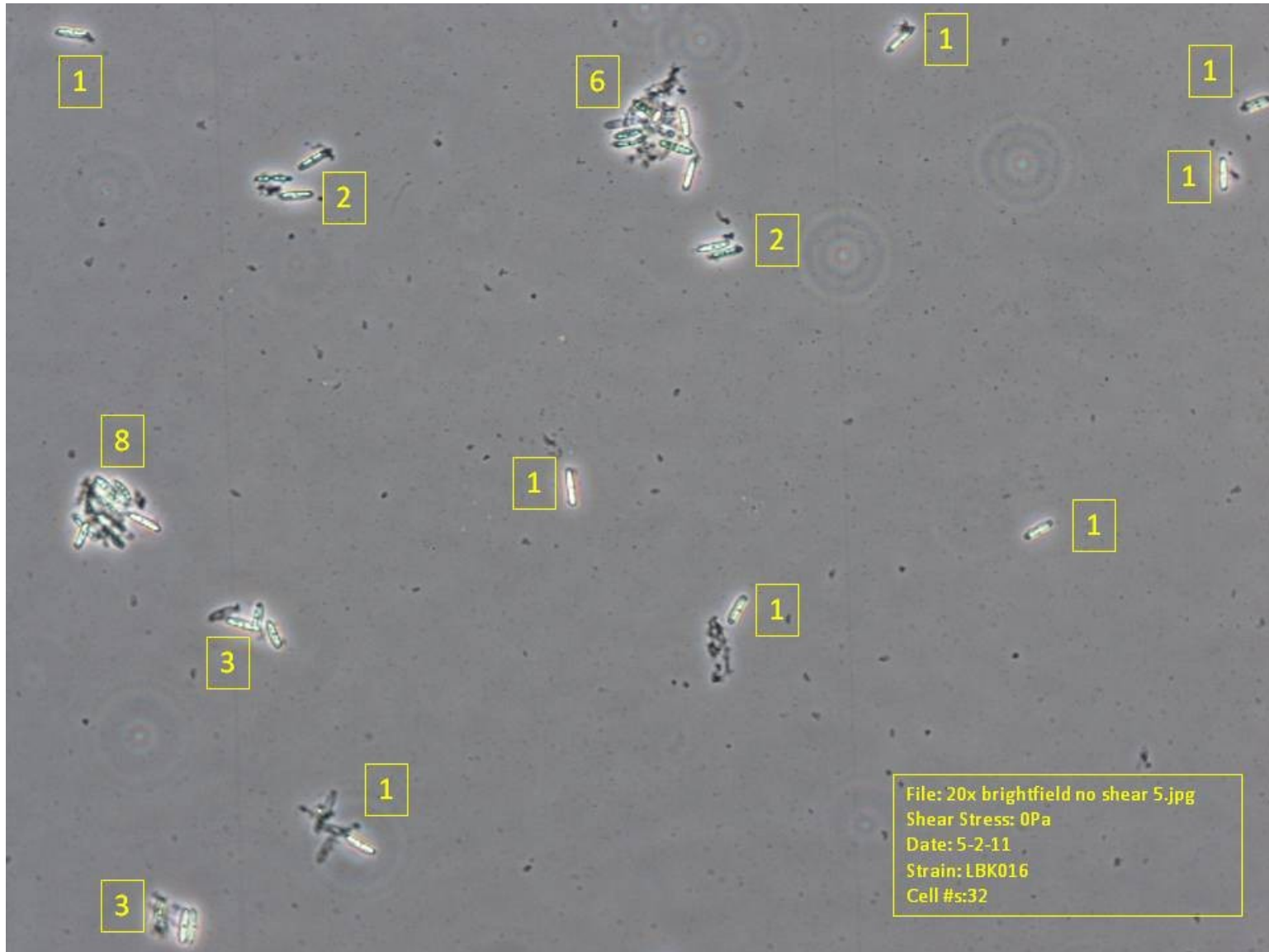










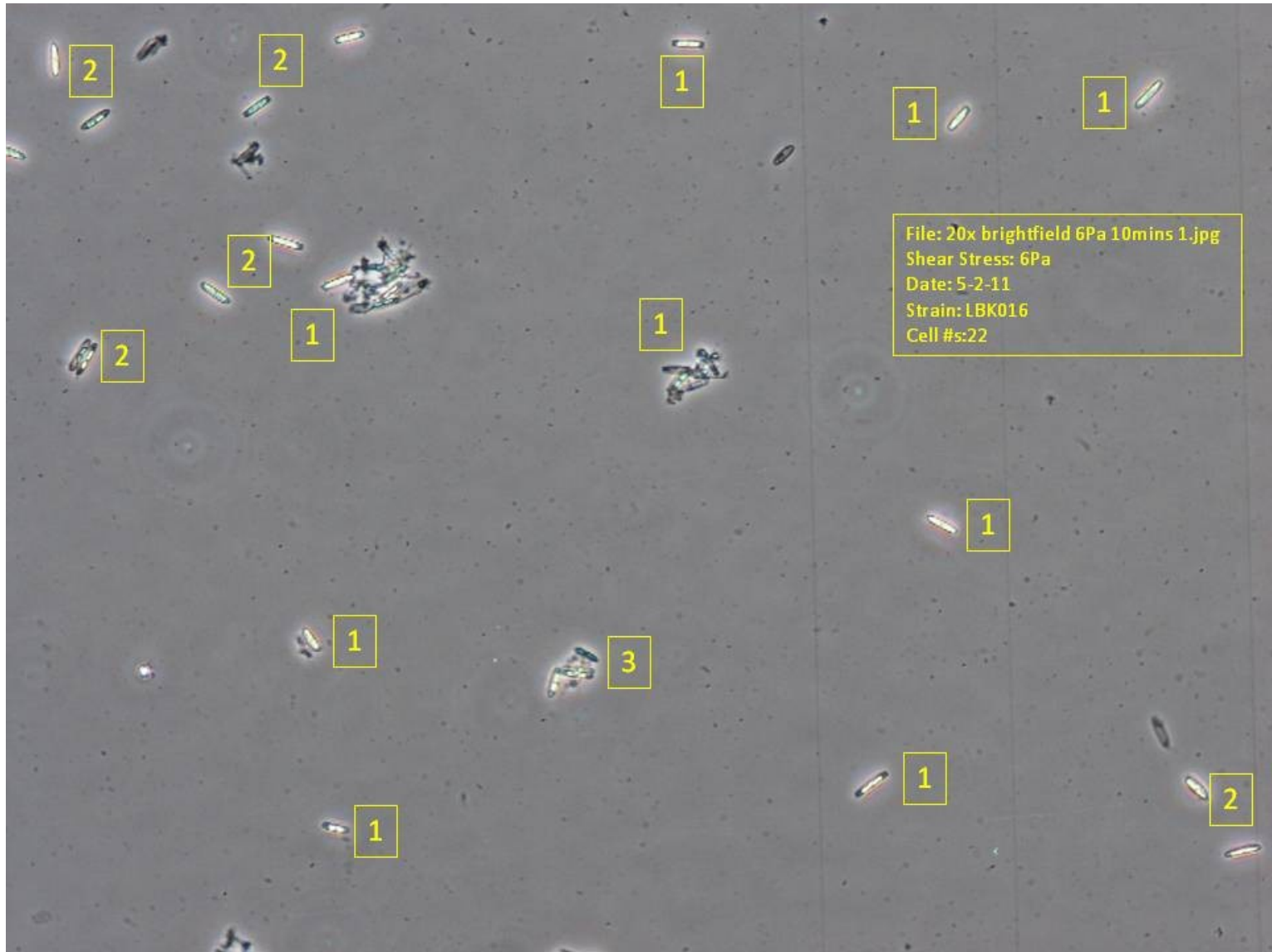


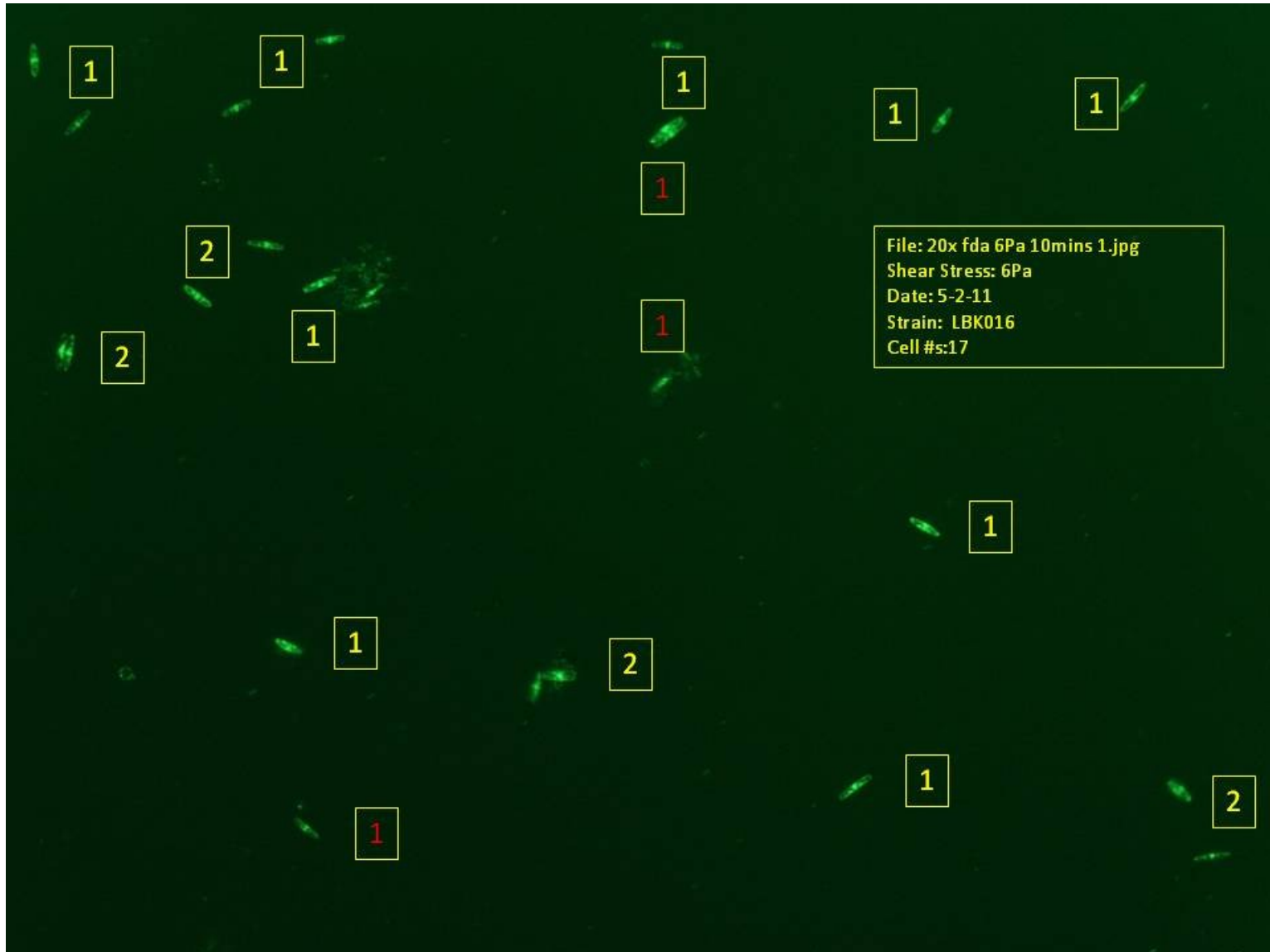


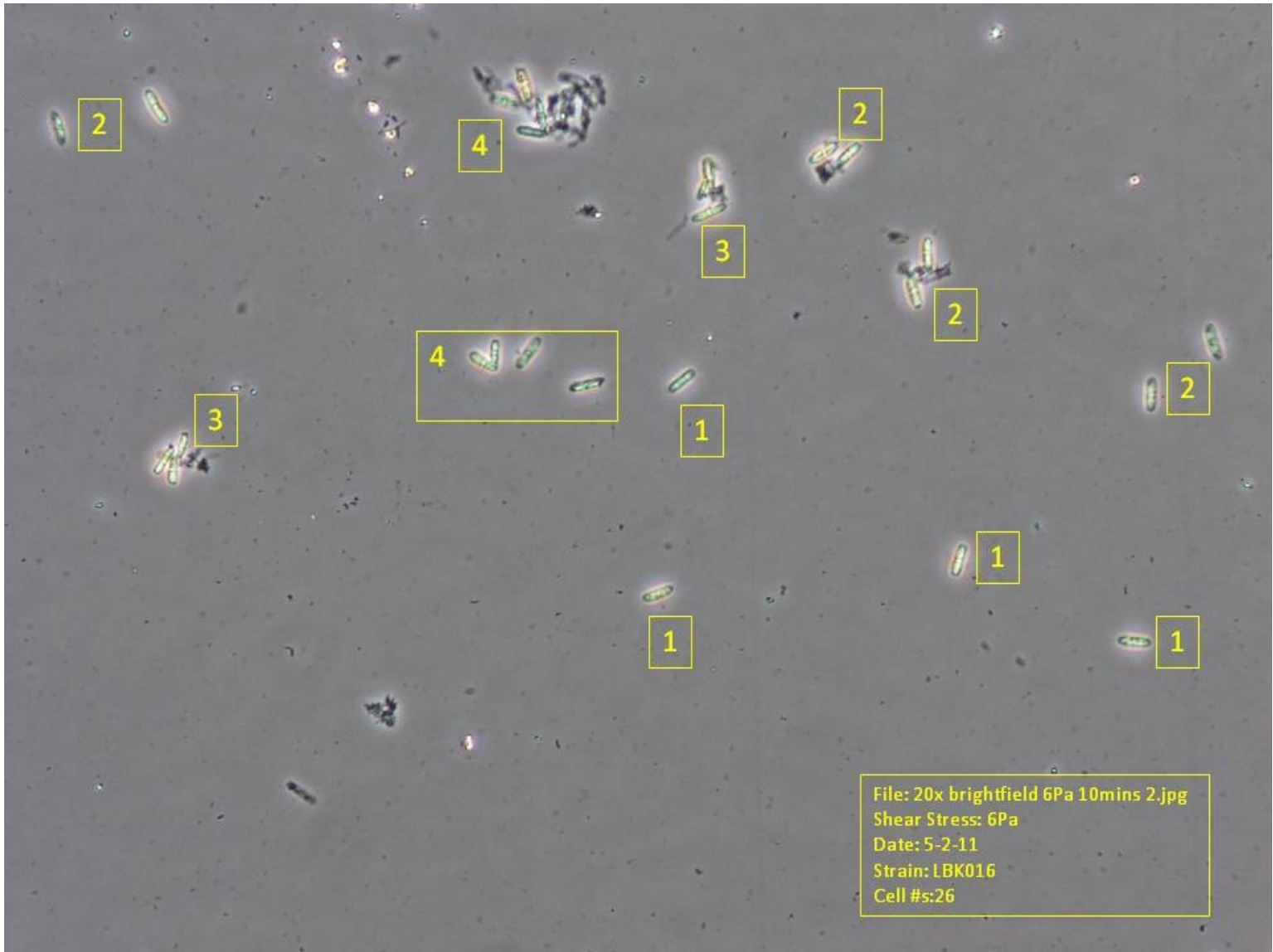






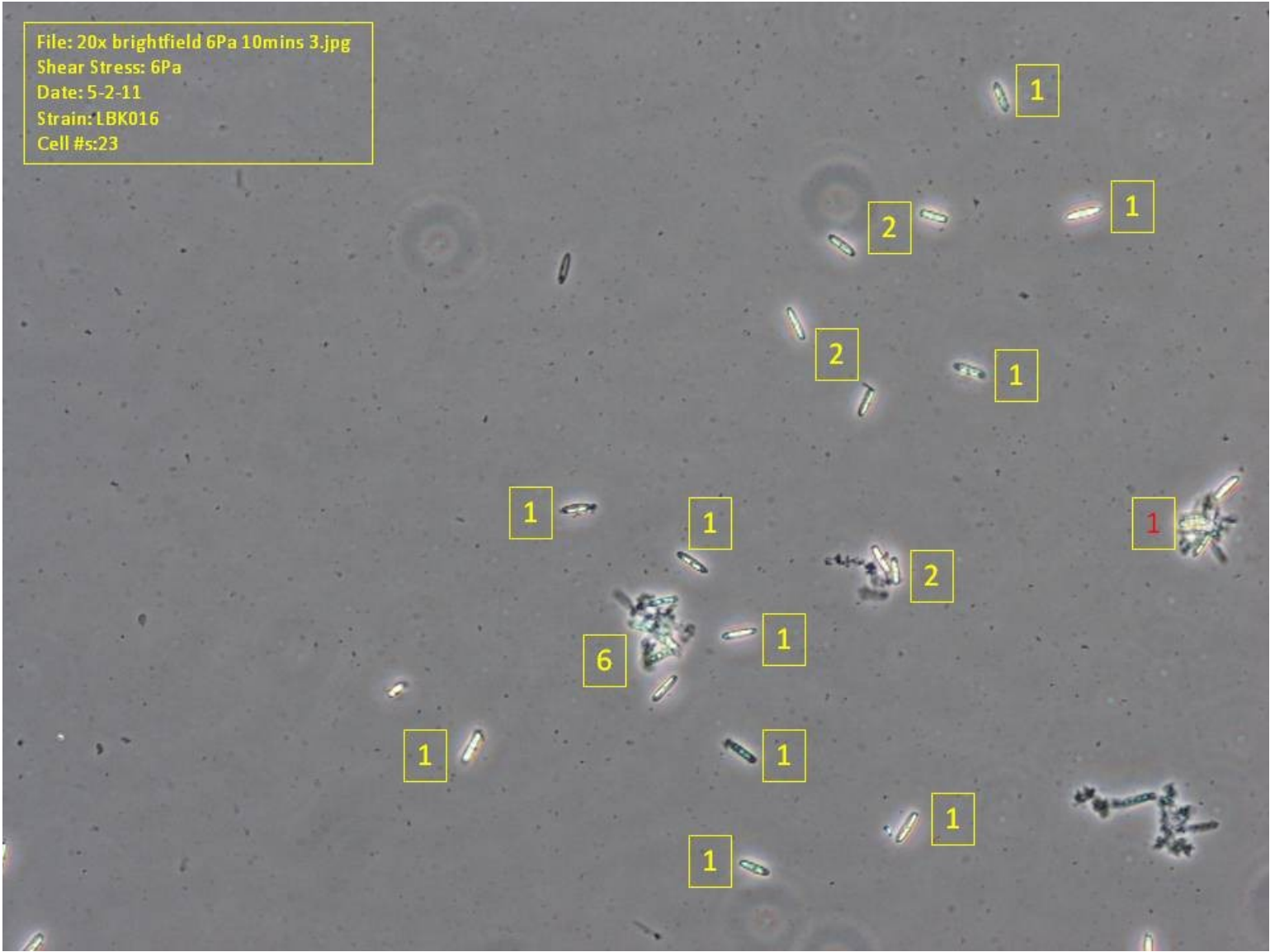






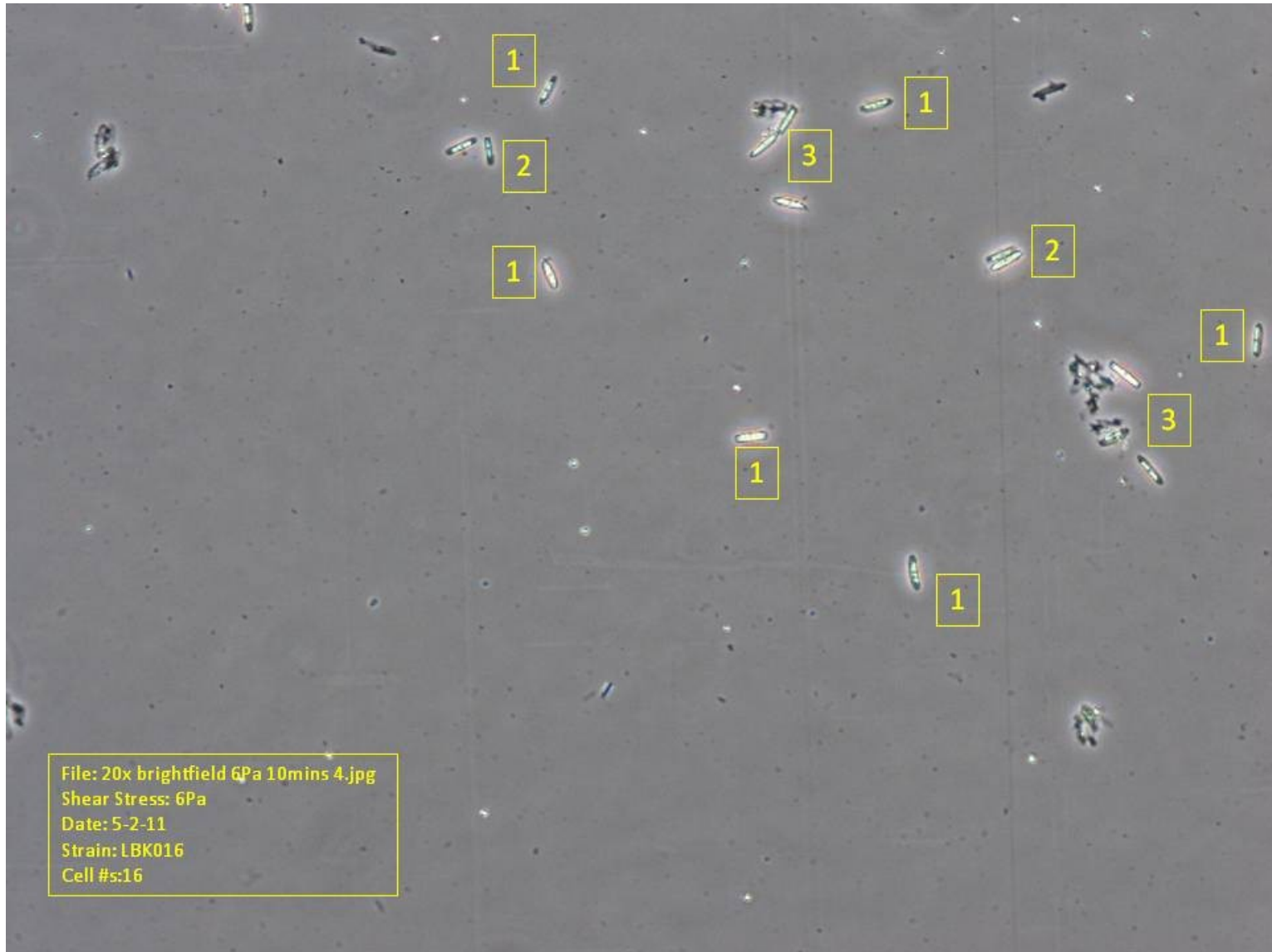


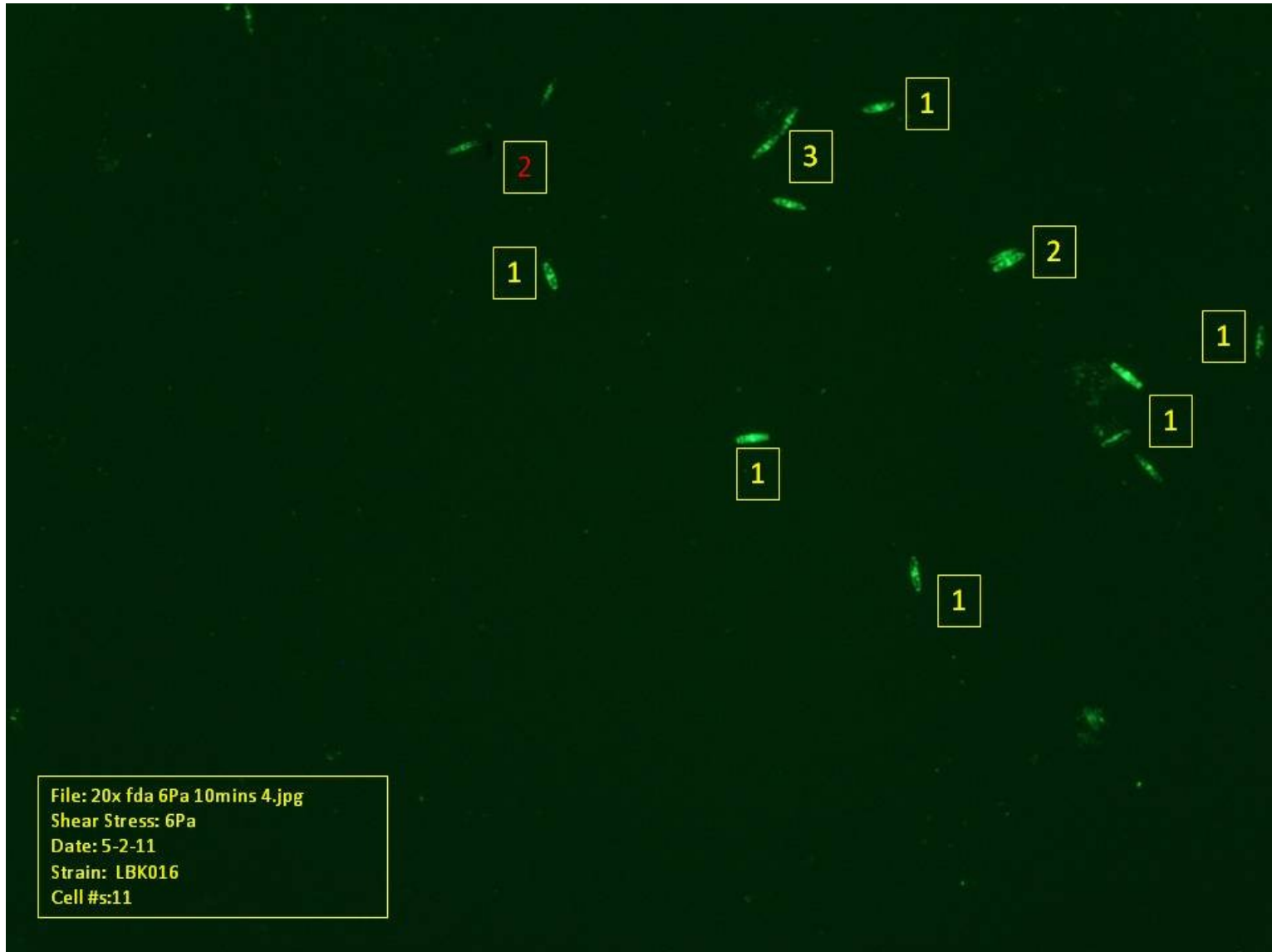
File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK016  
Cell #:s:23

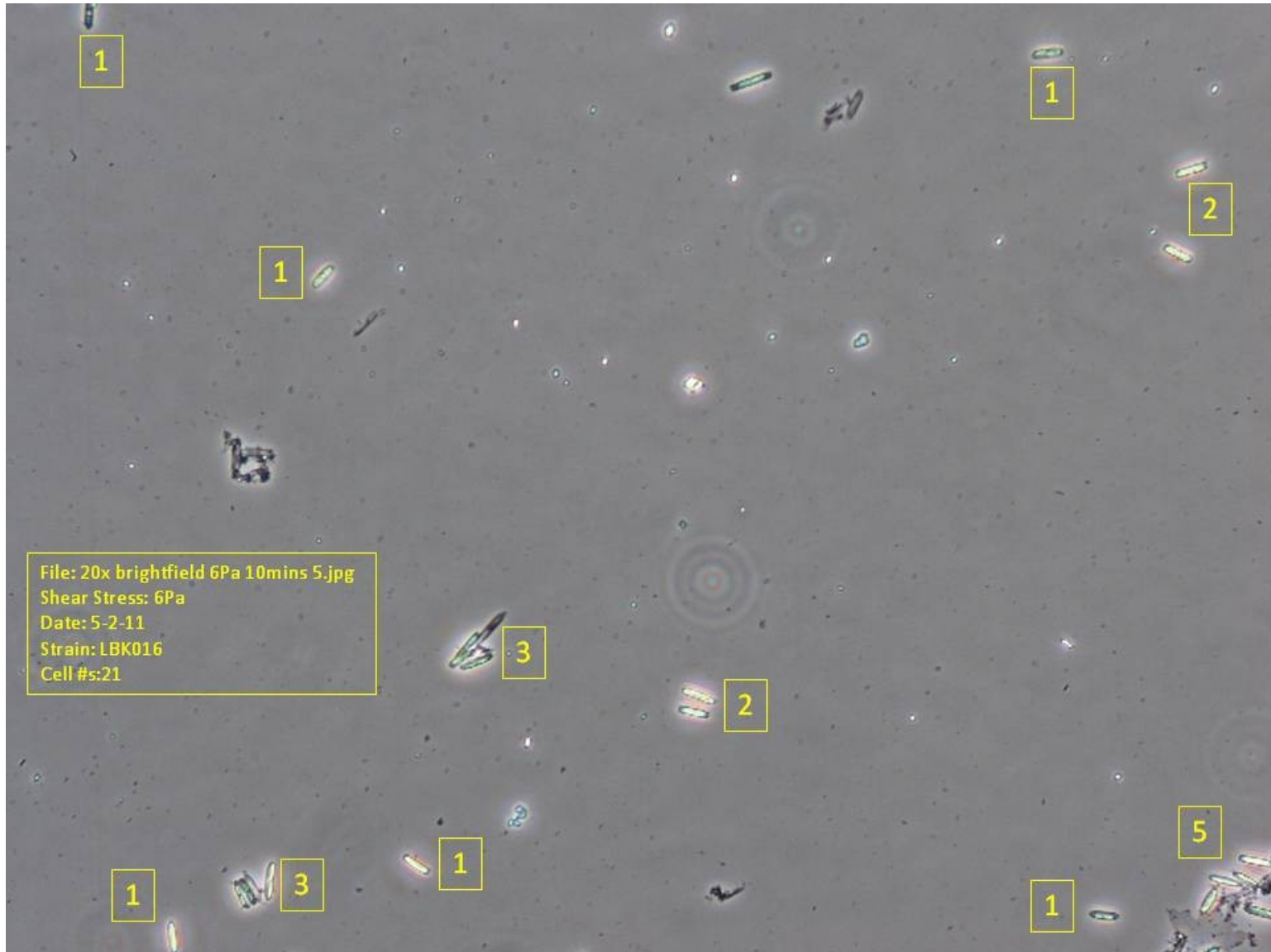


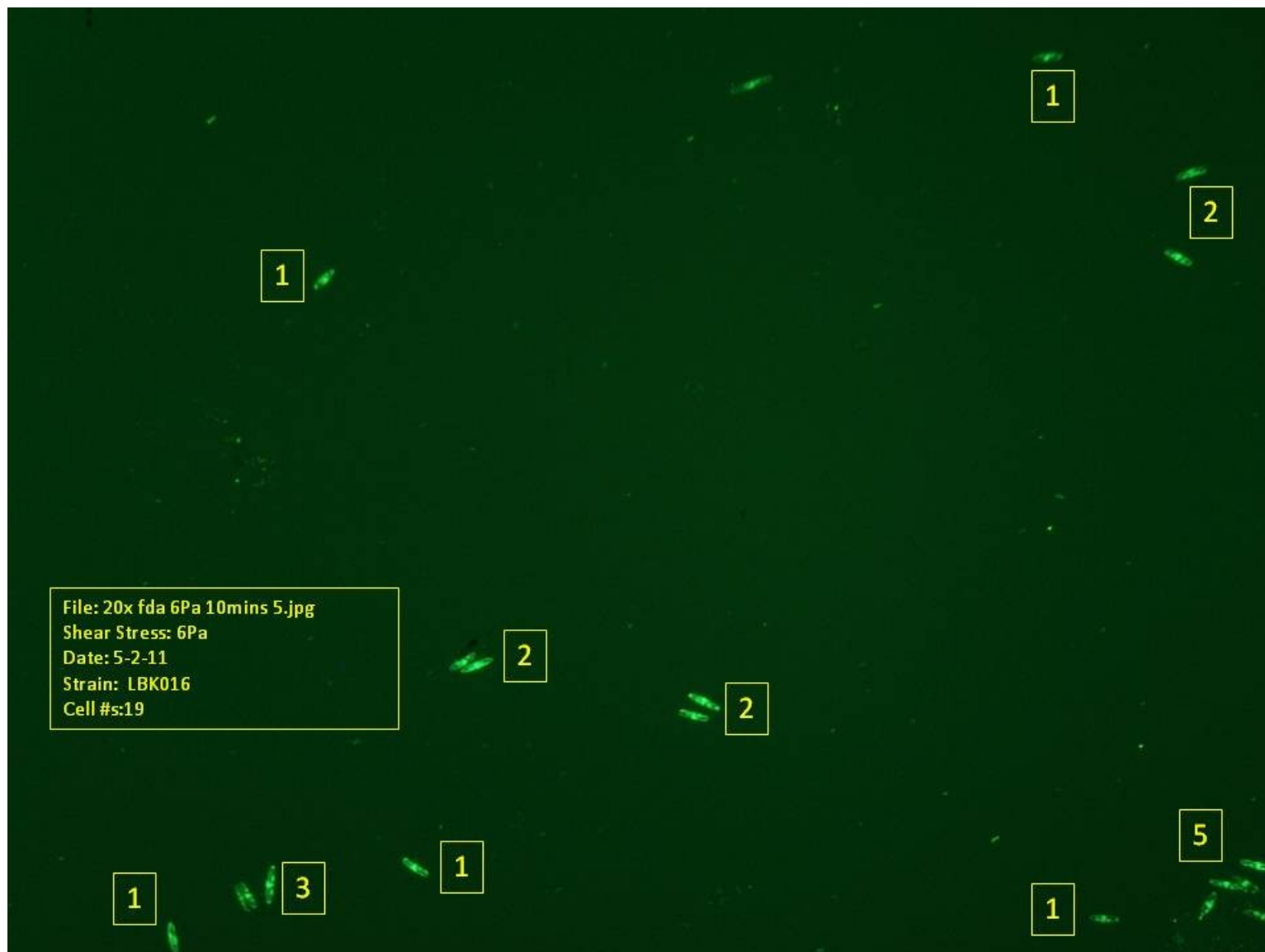












File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK016  
Cell #s:





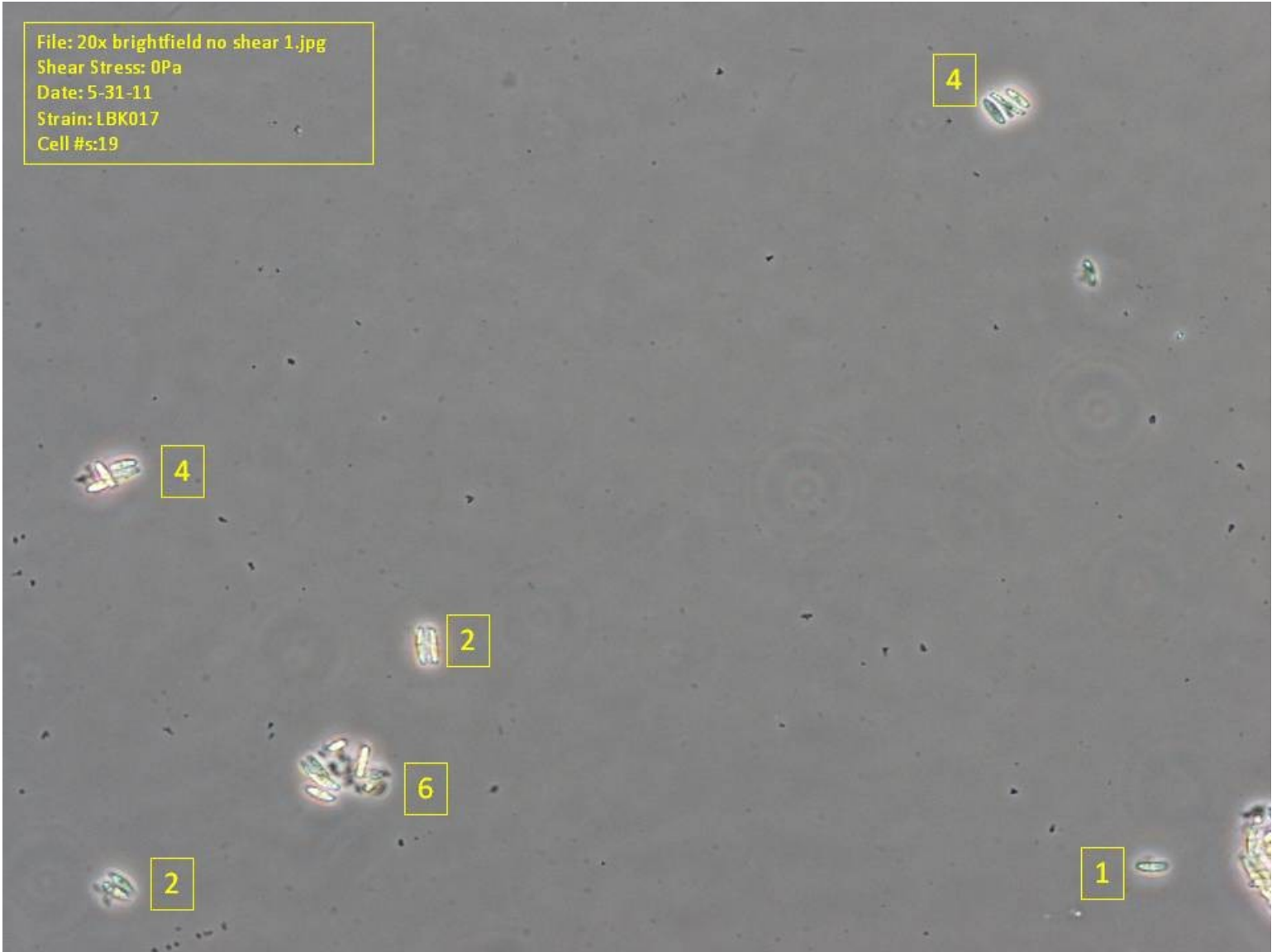
## APPENDIX F

The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK017, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	19	17	20	17	14	13			14	11	10	7	84.42%	8.01%	-0.0133518
	6			36	29	26	20	42	31	40	29	34	27	76.40%		
Rep 2	0	17	15	17	15					15	13	34	28	85.54%	5.68%	-0.0094684
	6	22	19	34	26	33	28			27	20	28	22	79.86%		
Rep 3	0	26	24	20	17	18	15	15	11			13	11	84.78%	4.41%	-0.0073575
	6	27	25	34	30	30	24	17	16	18	12	37	24	80.37%		
														<b>Avg.</b>	<b>6.04%</b>	
														<b>Std. Dev.</b>	<b>1.82%</b>	

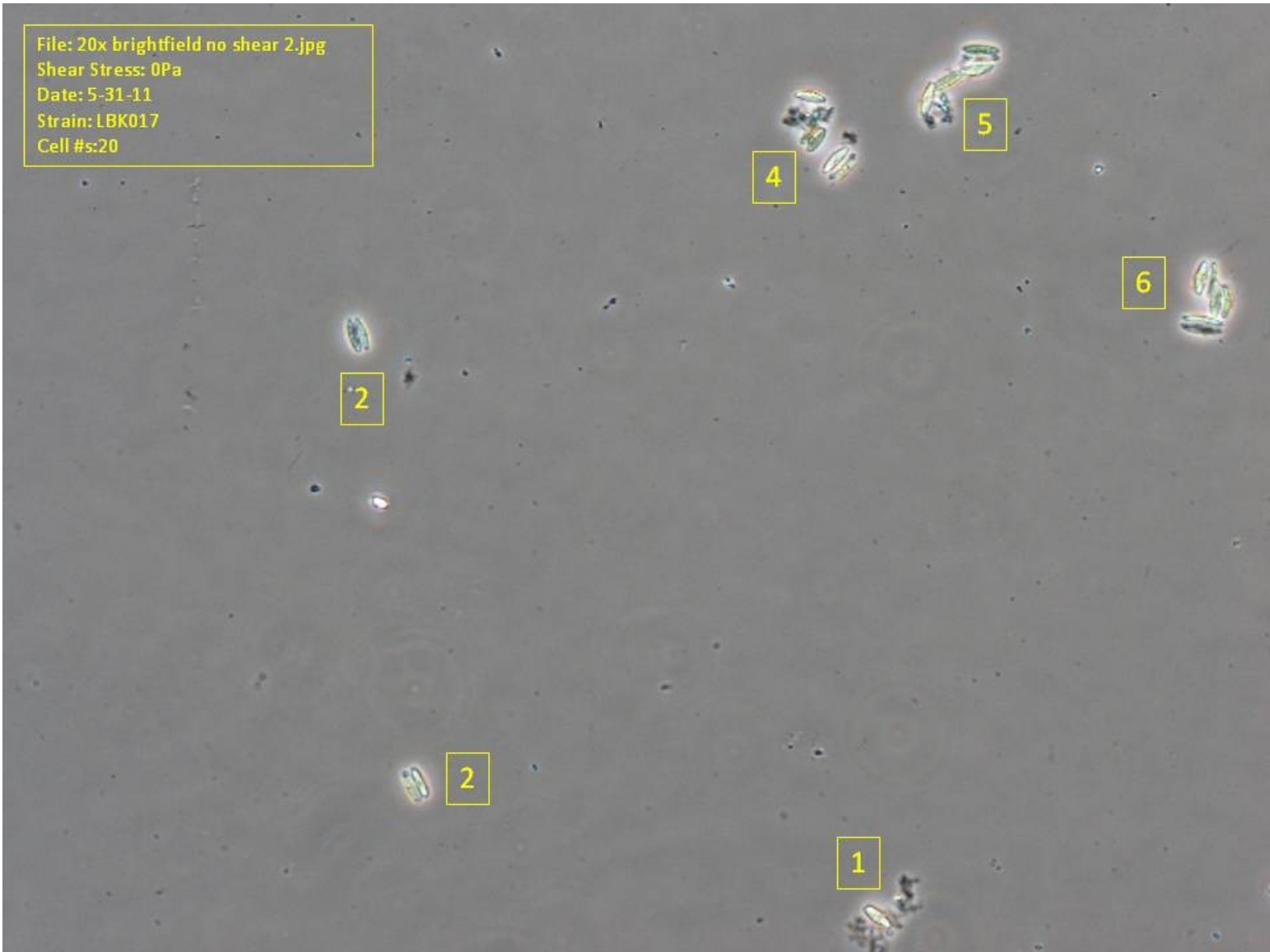
The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.

File: 20x brightfield no shear 1.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:19



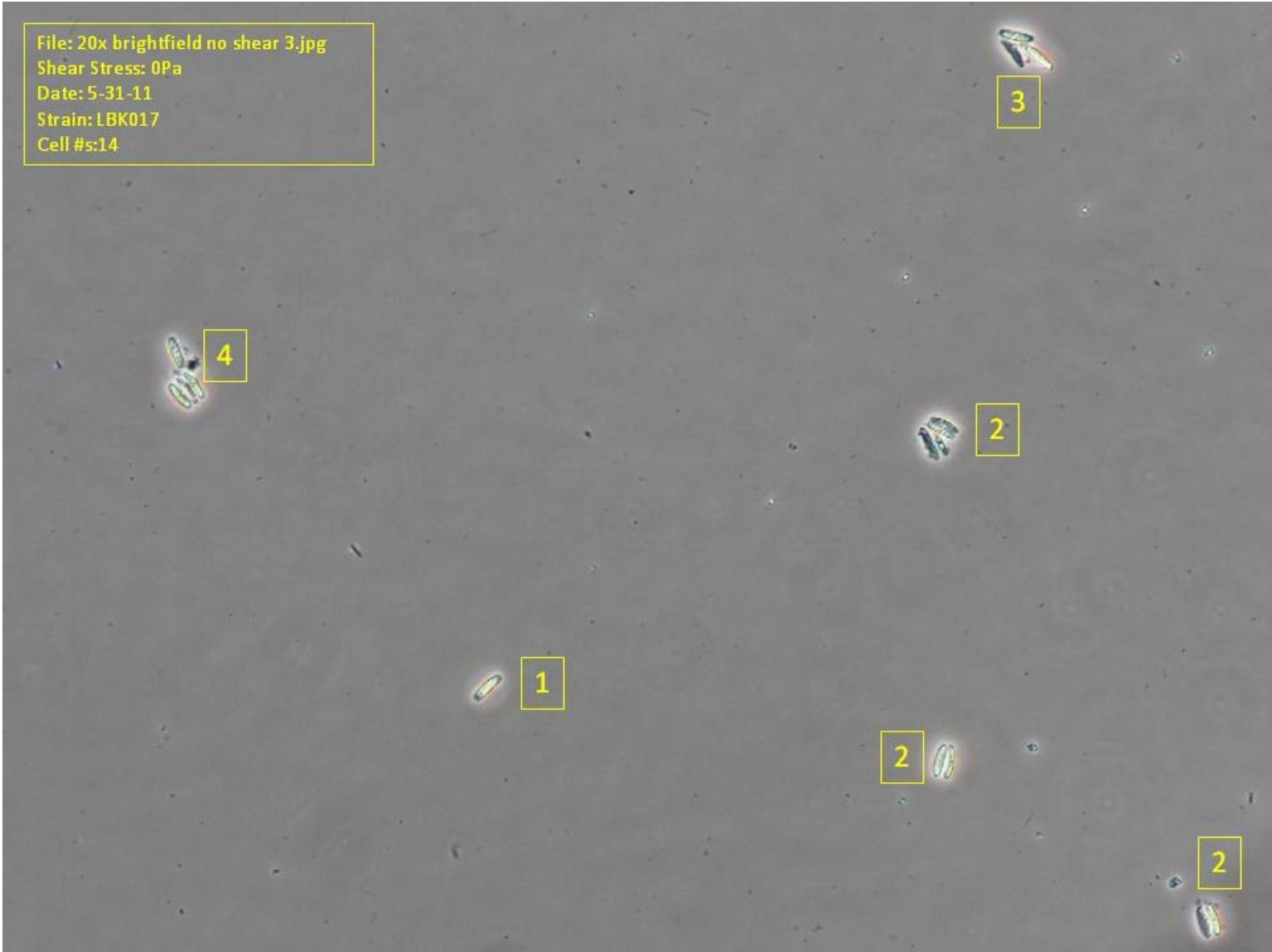


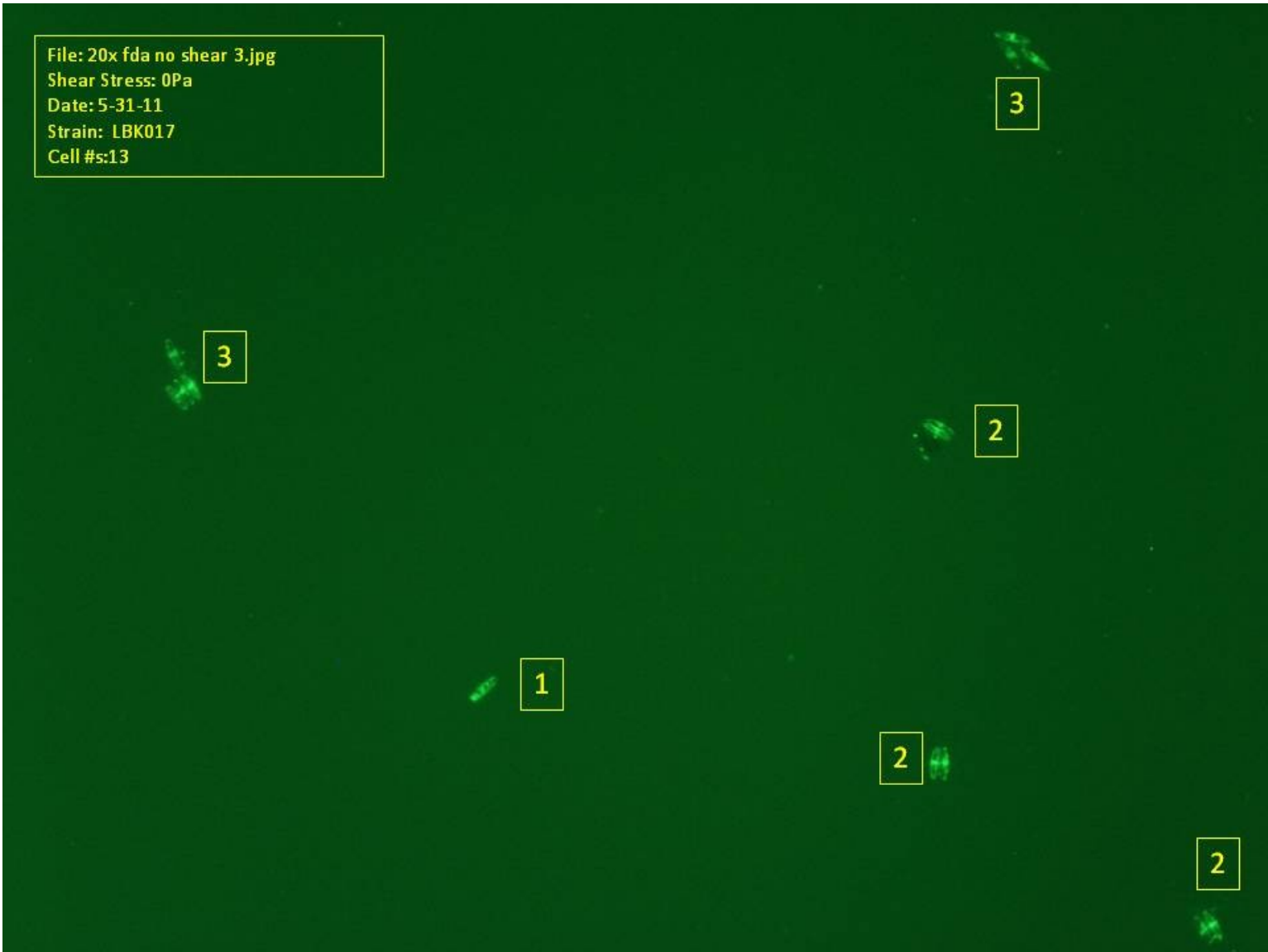


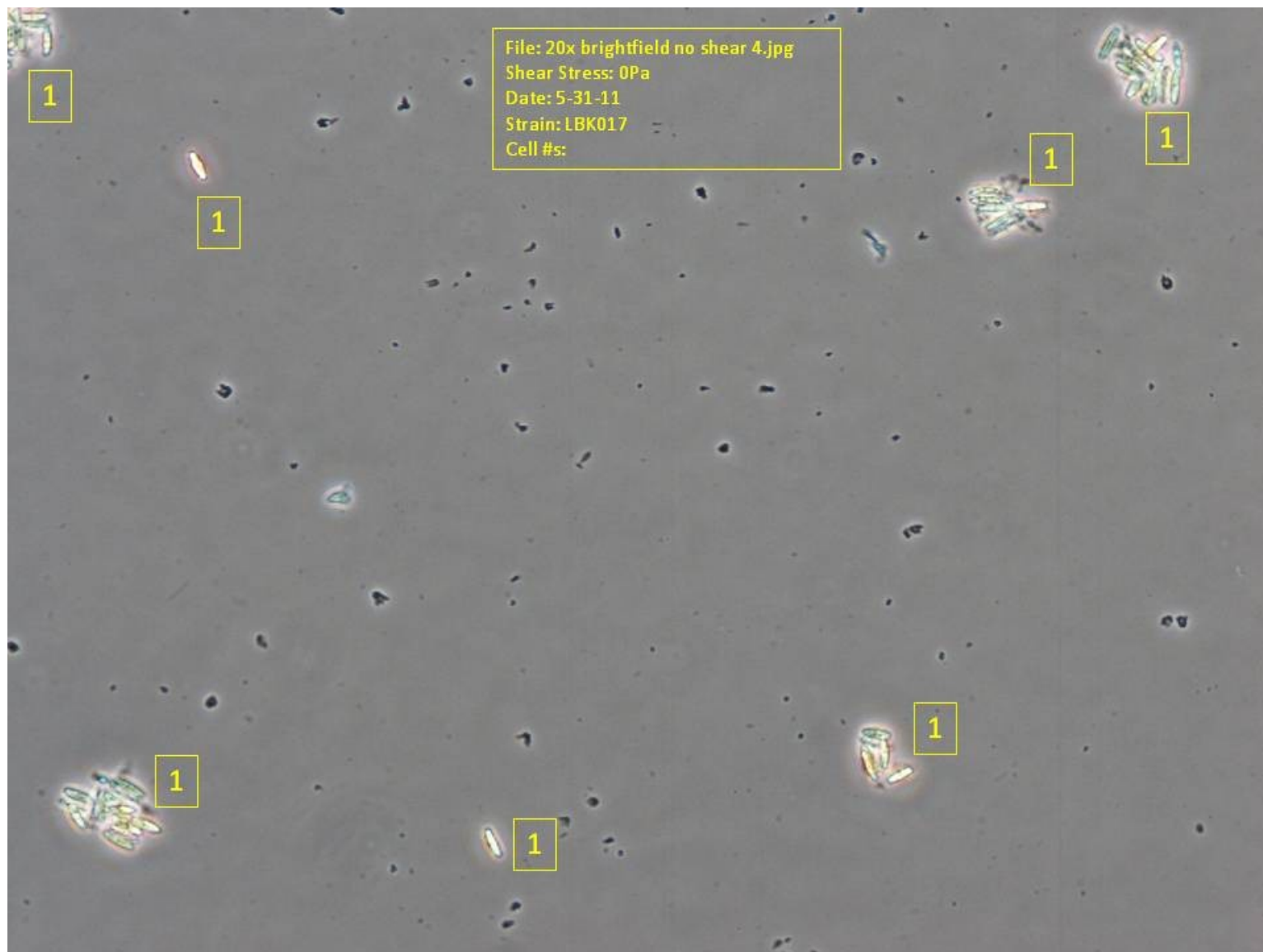


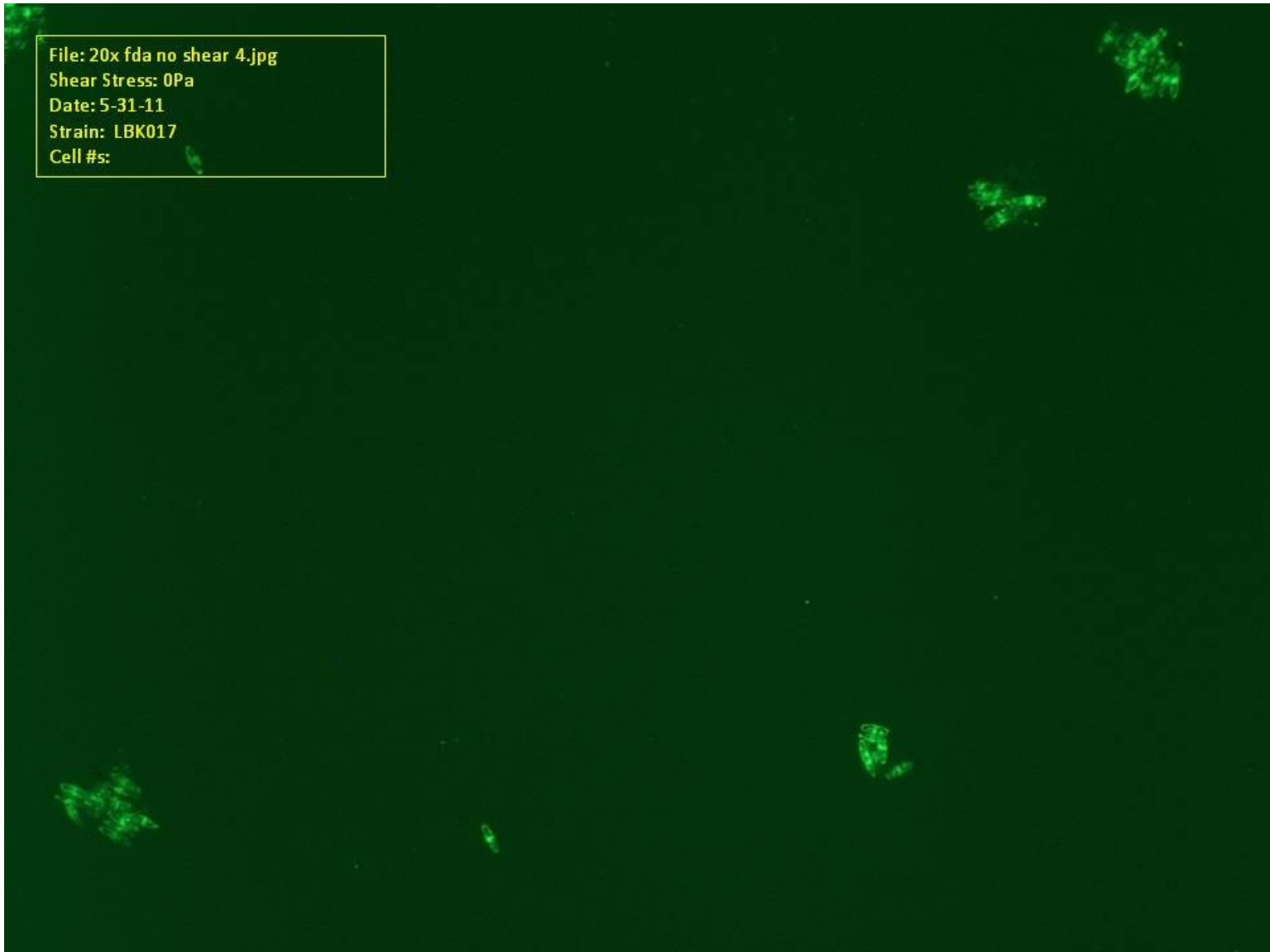


File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:14

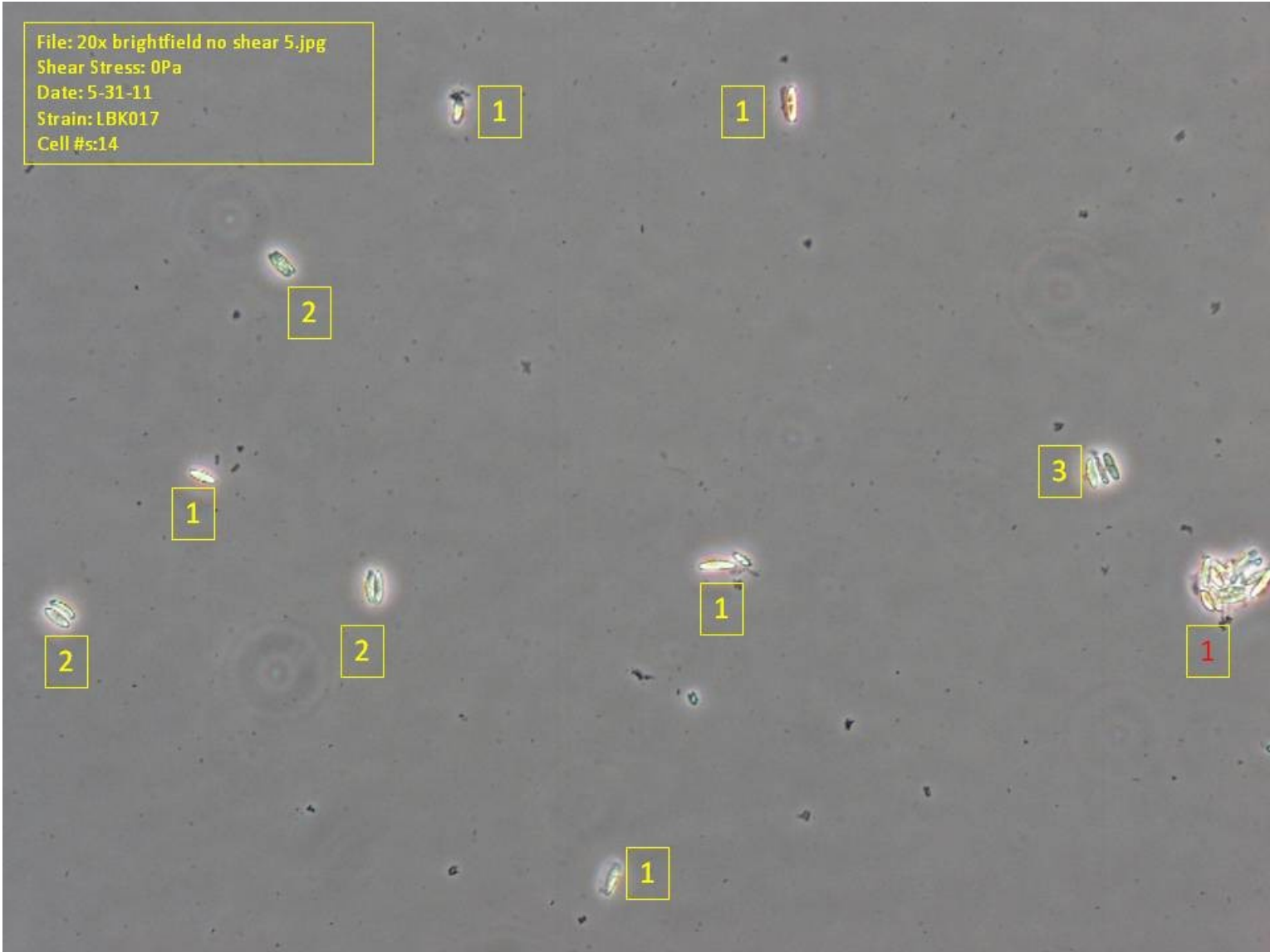






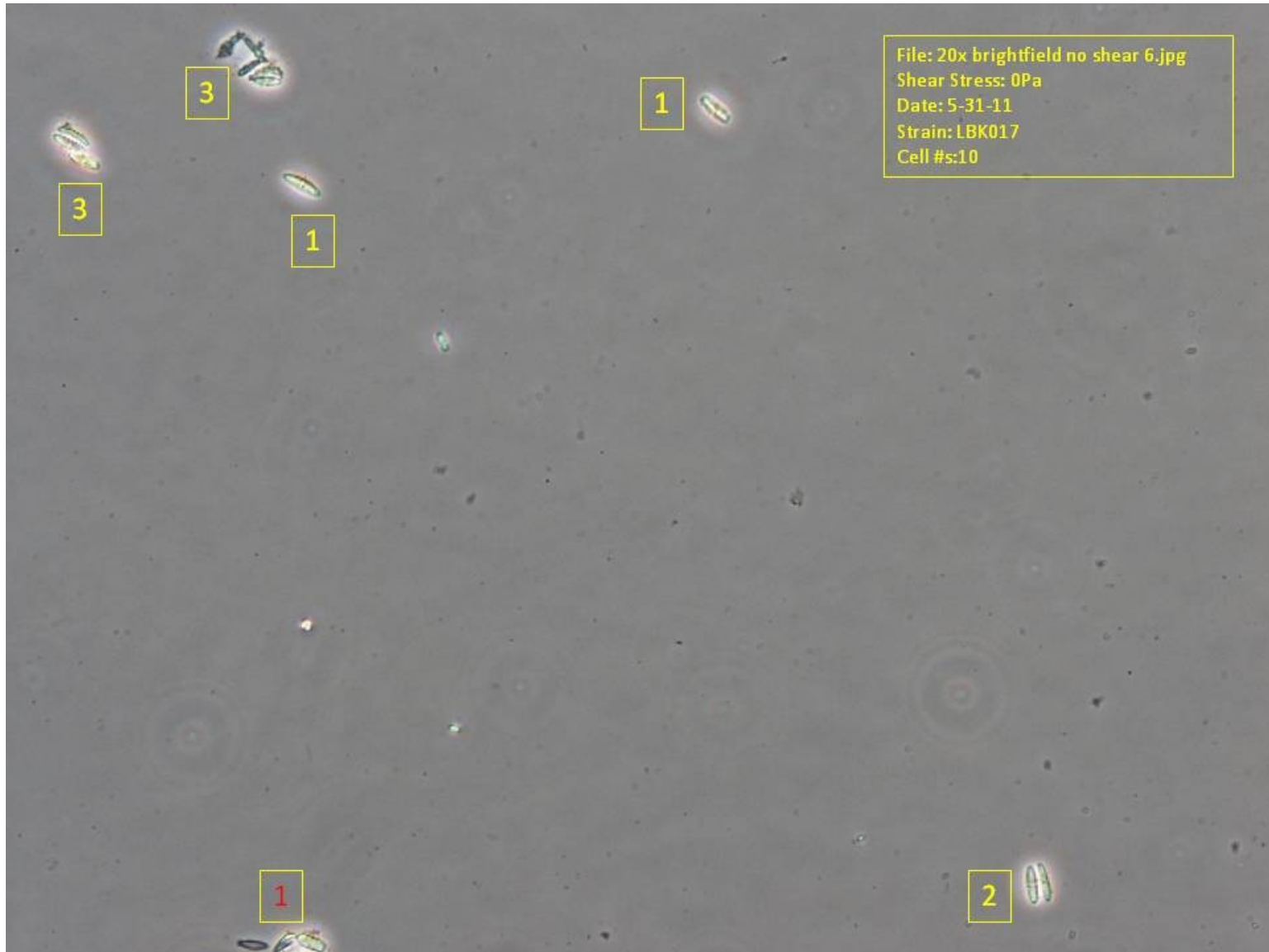


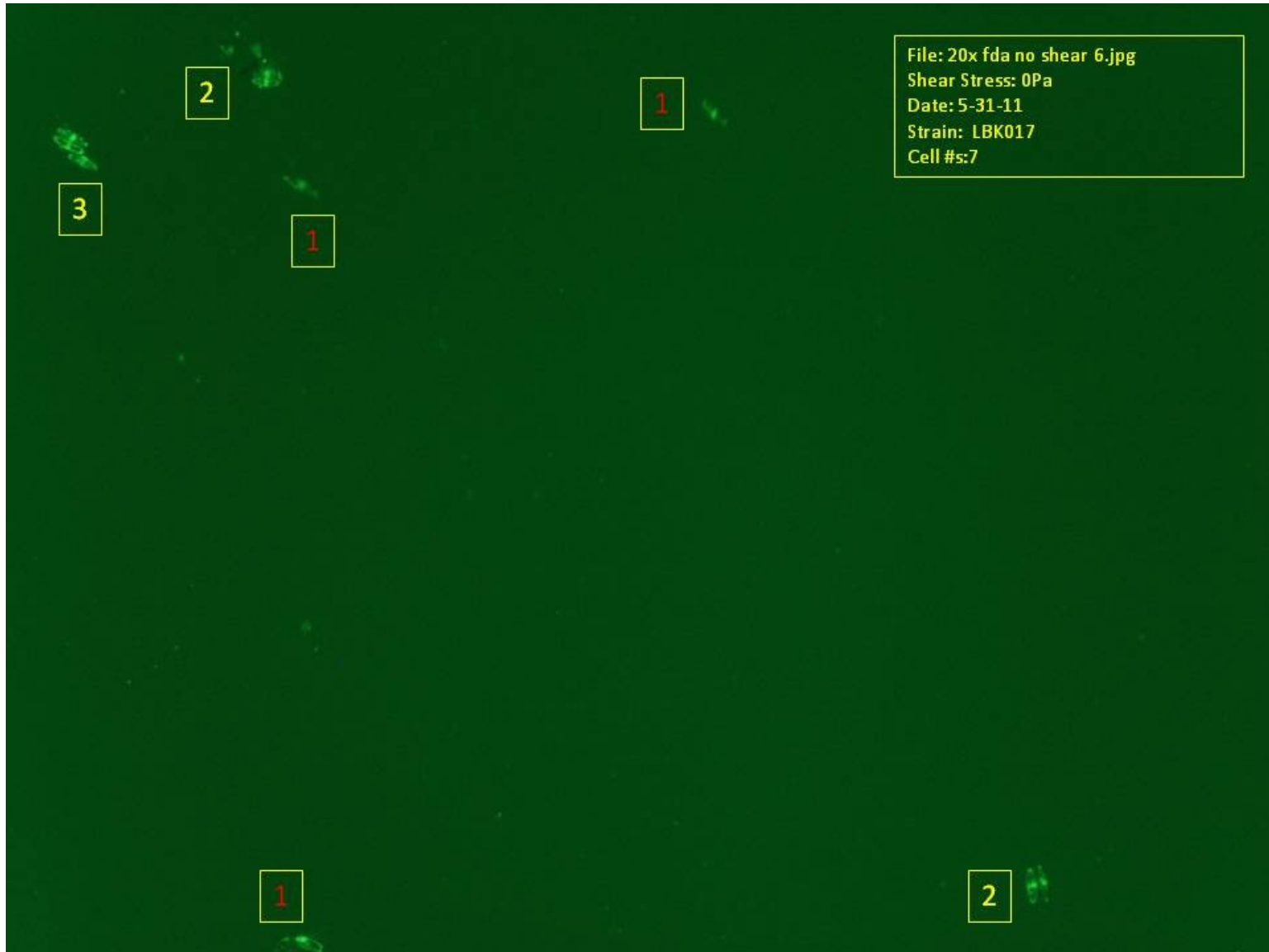
File: 20x brightfield no shear 5.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:14

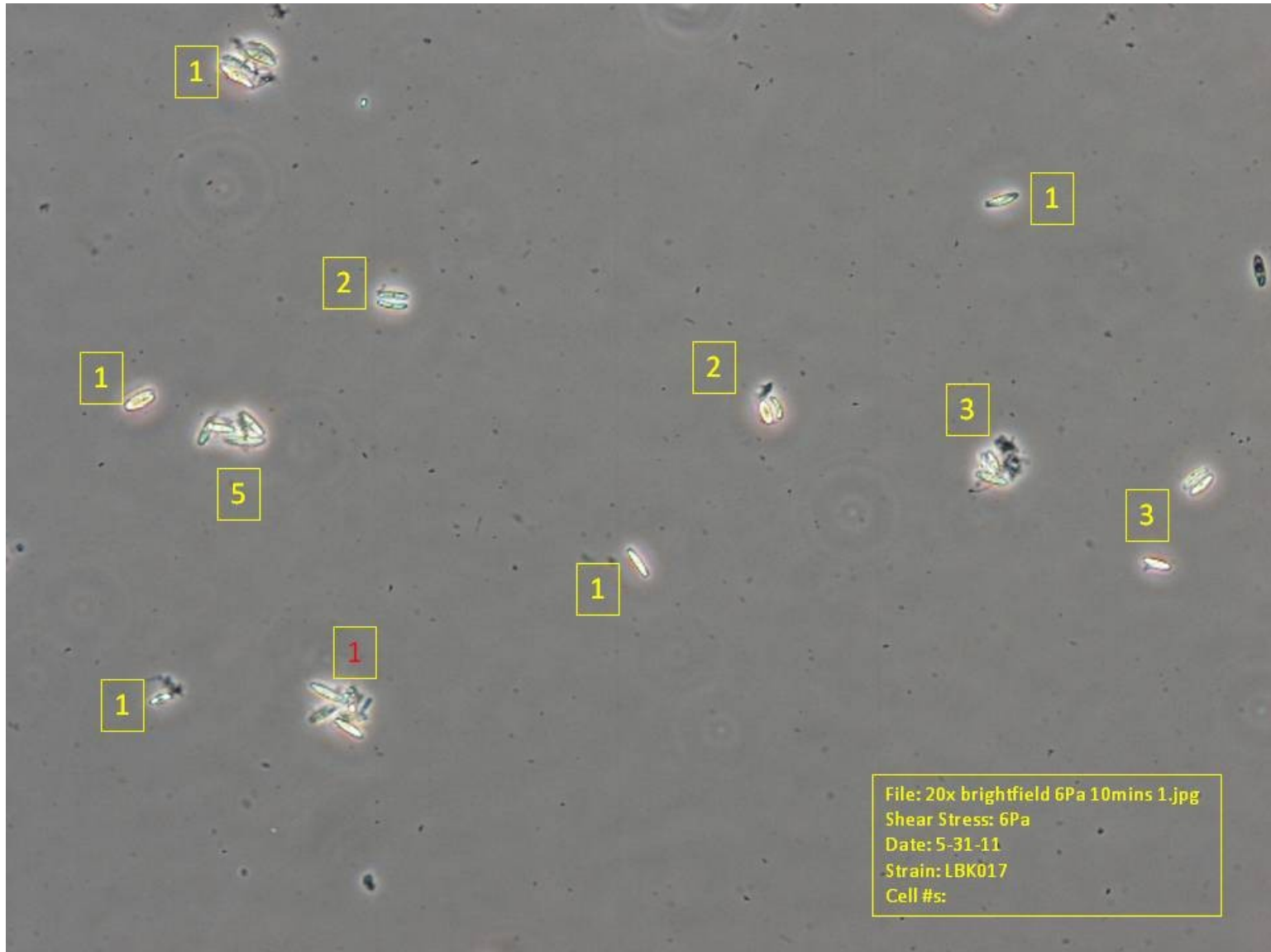


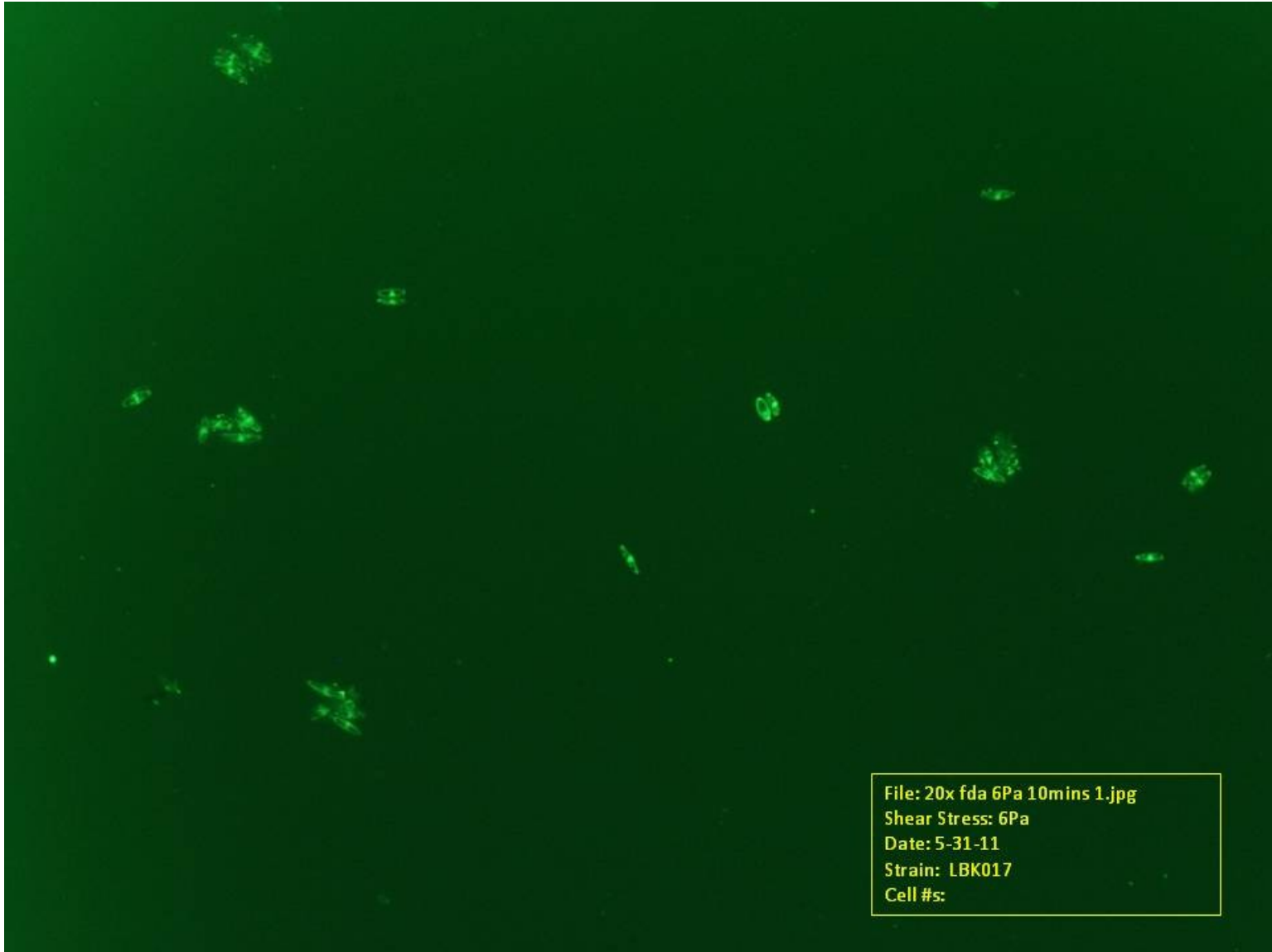


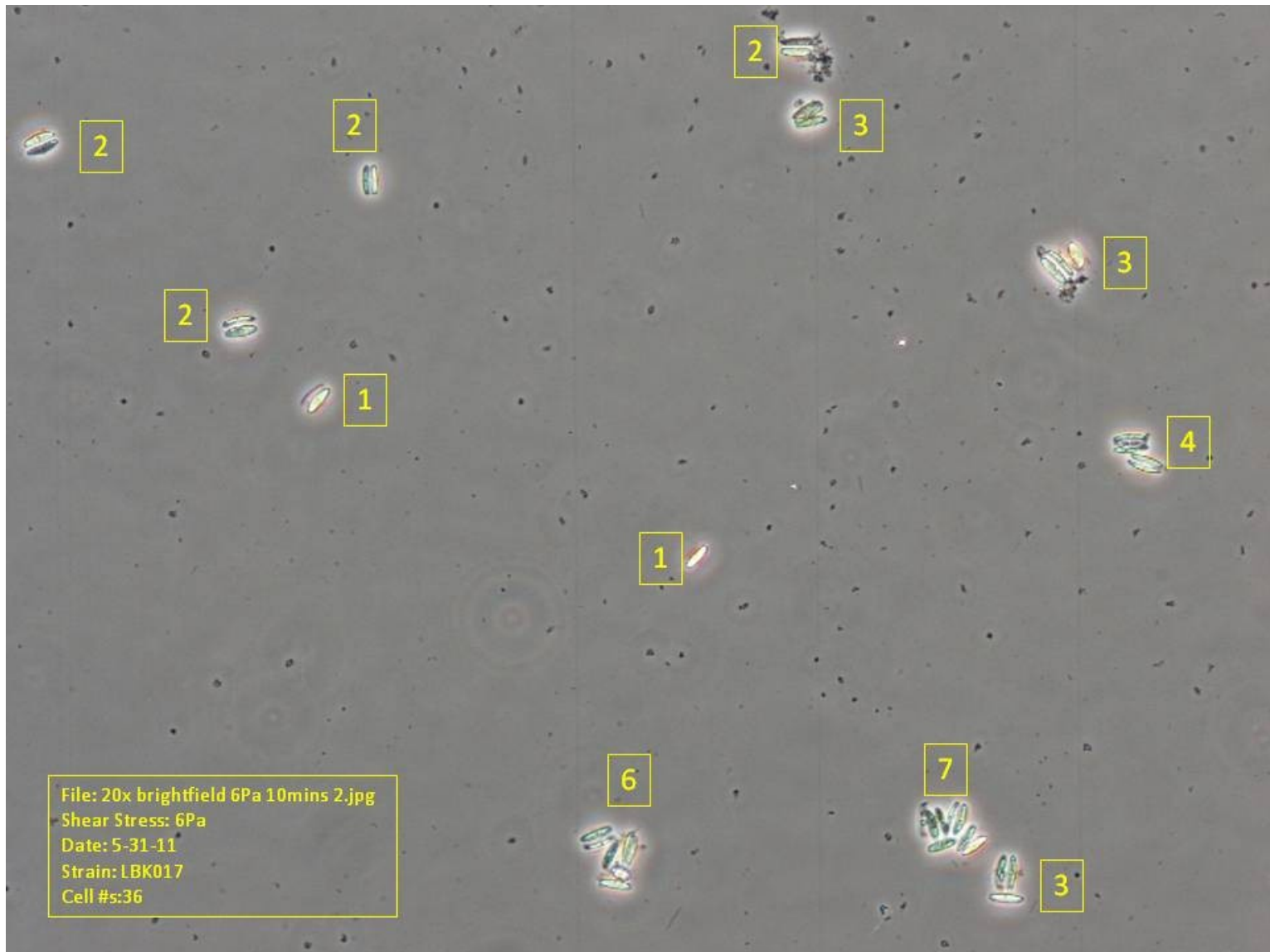


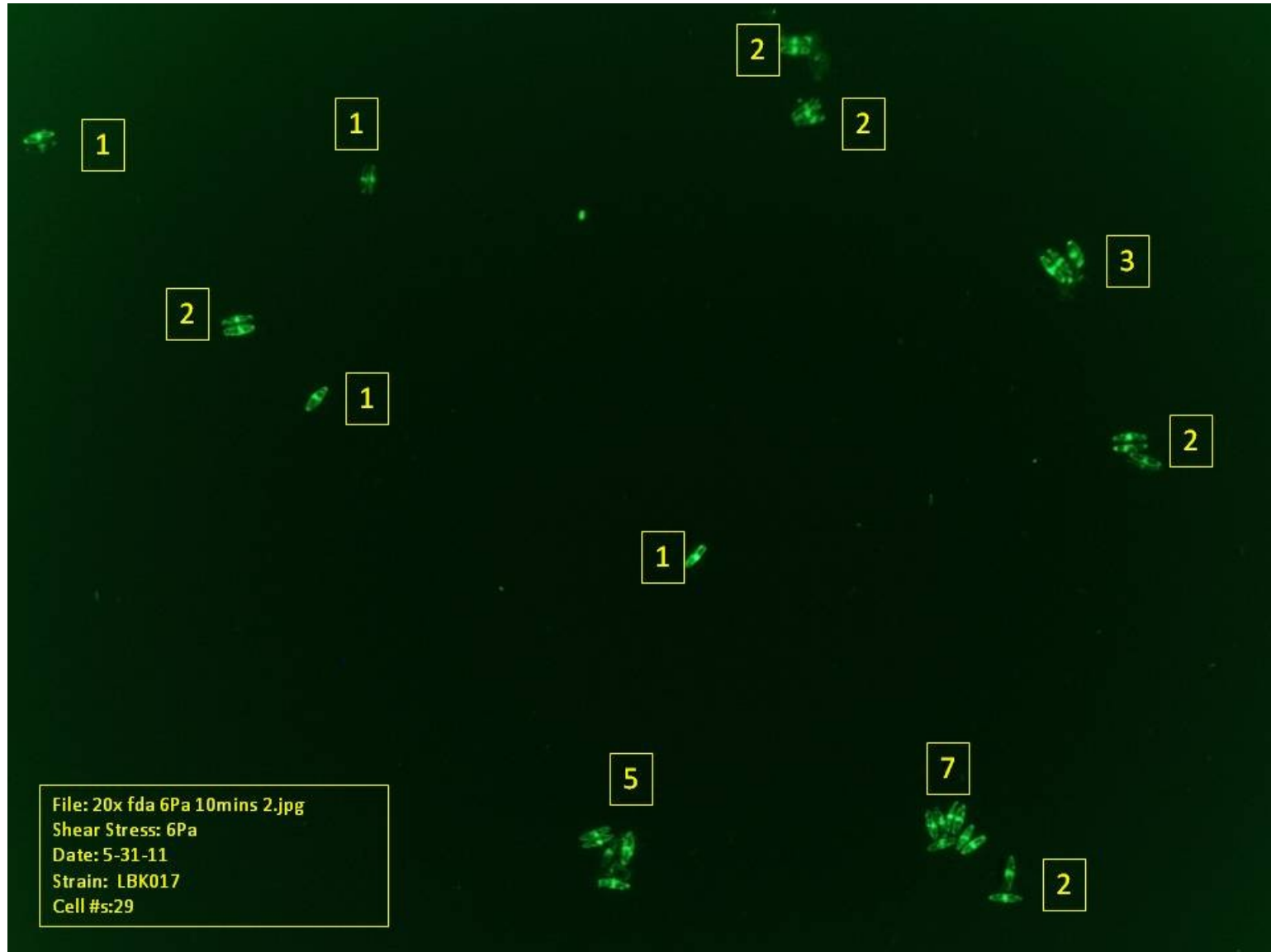




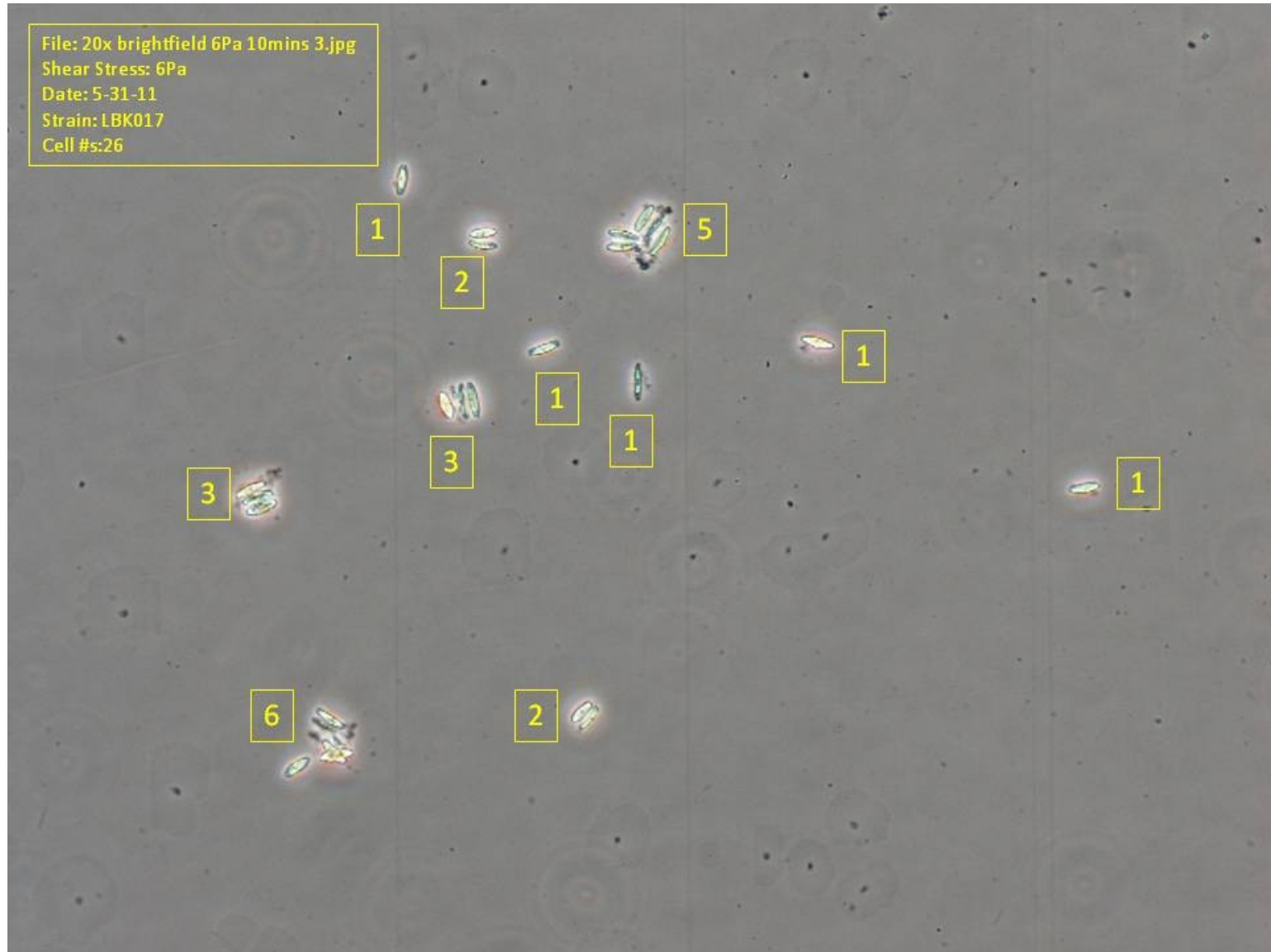






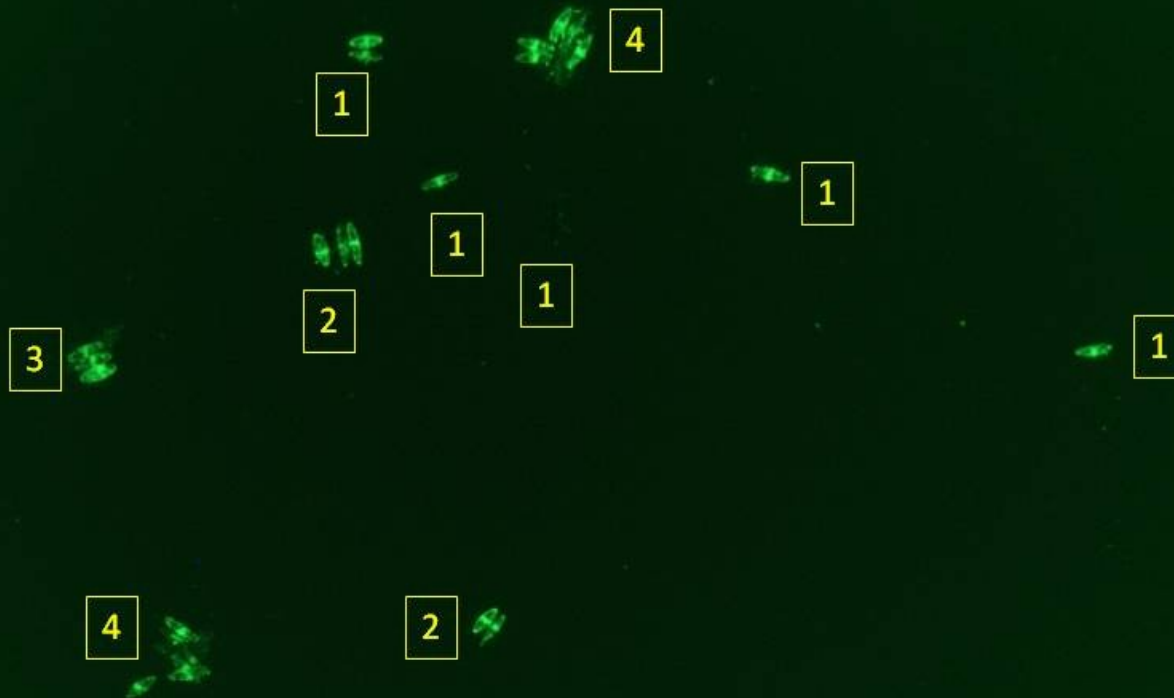


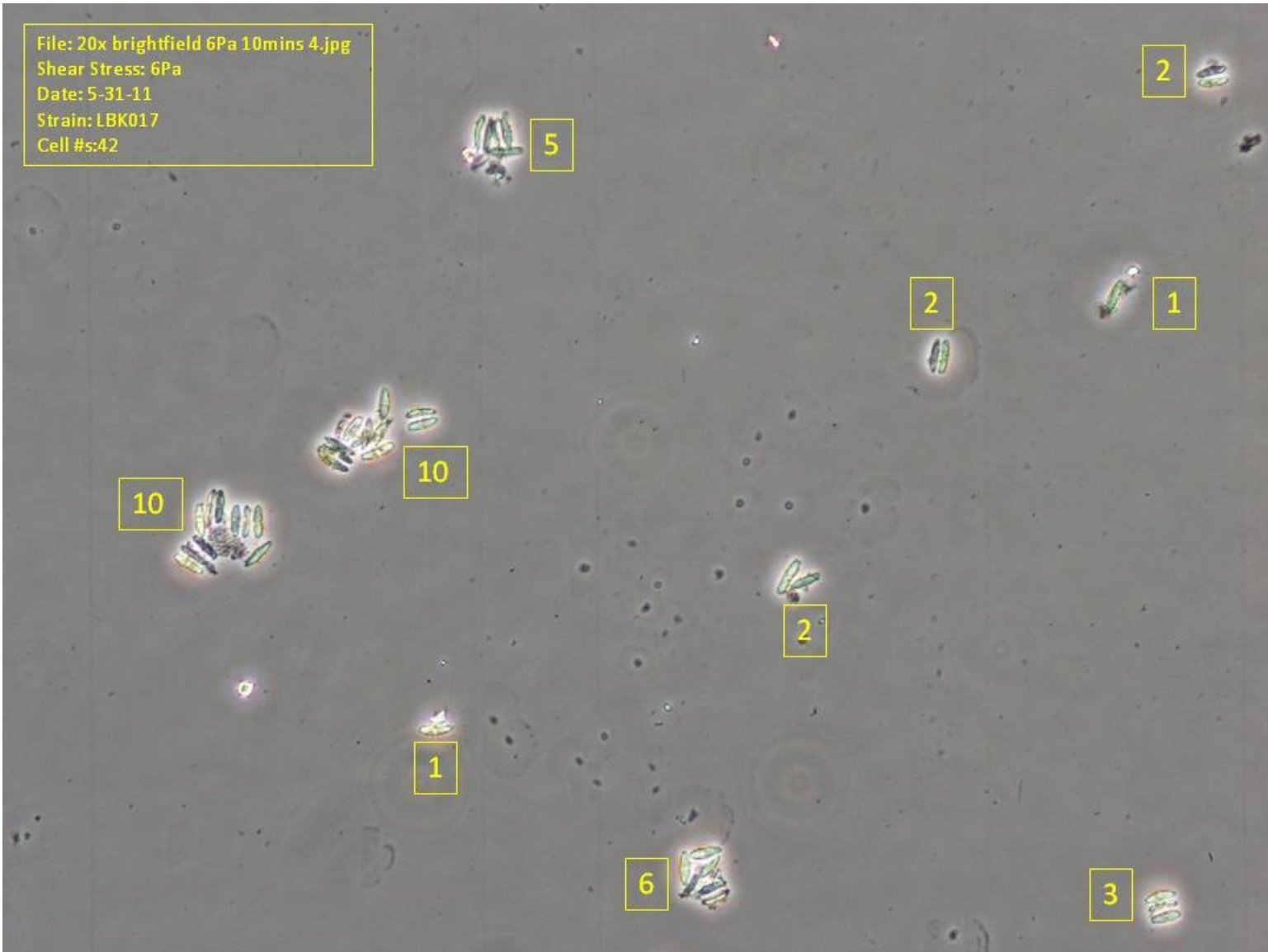
File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #:s:26

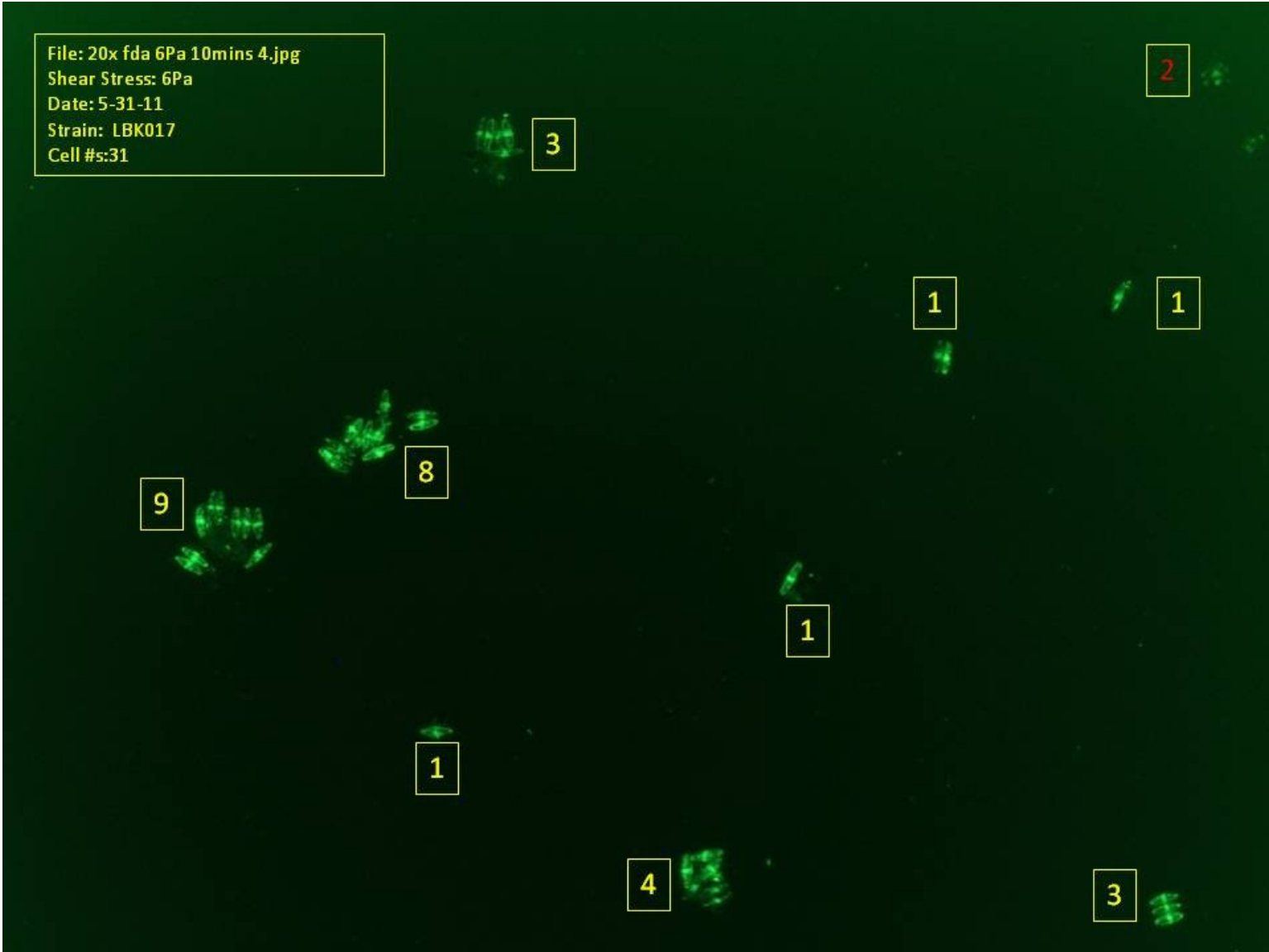


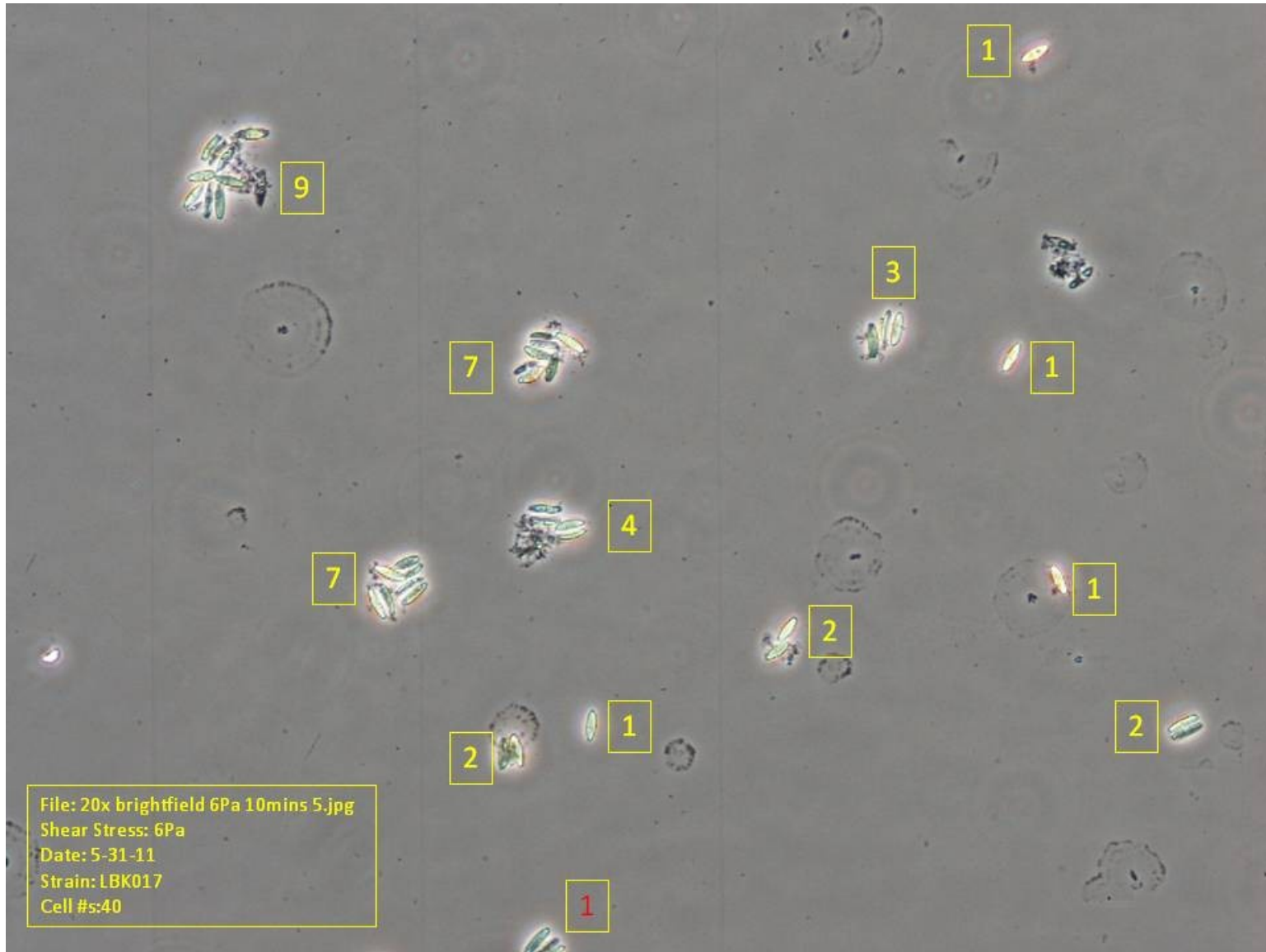


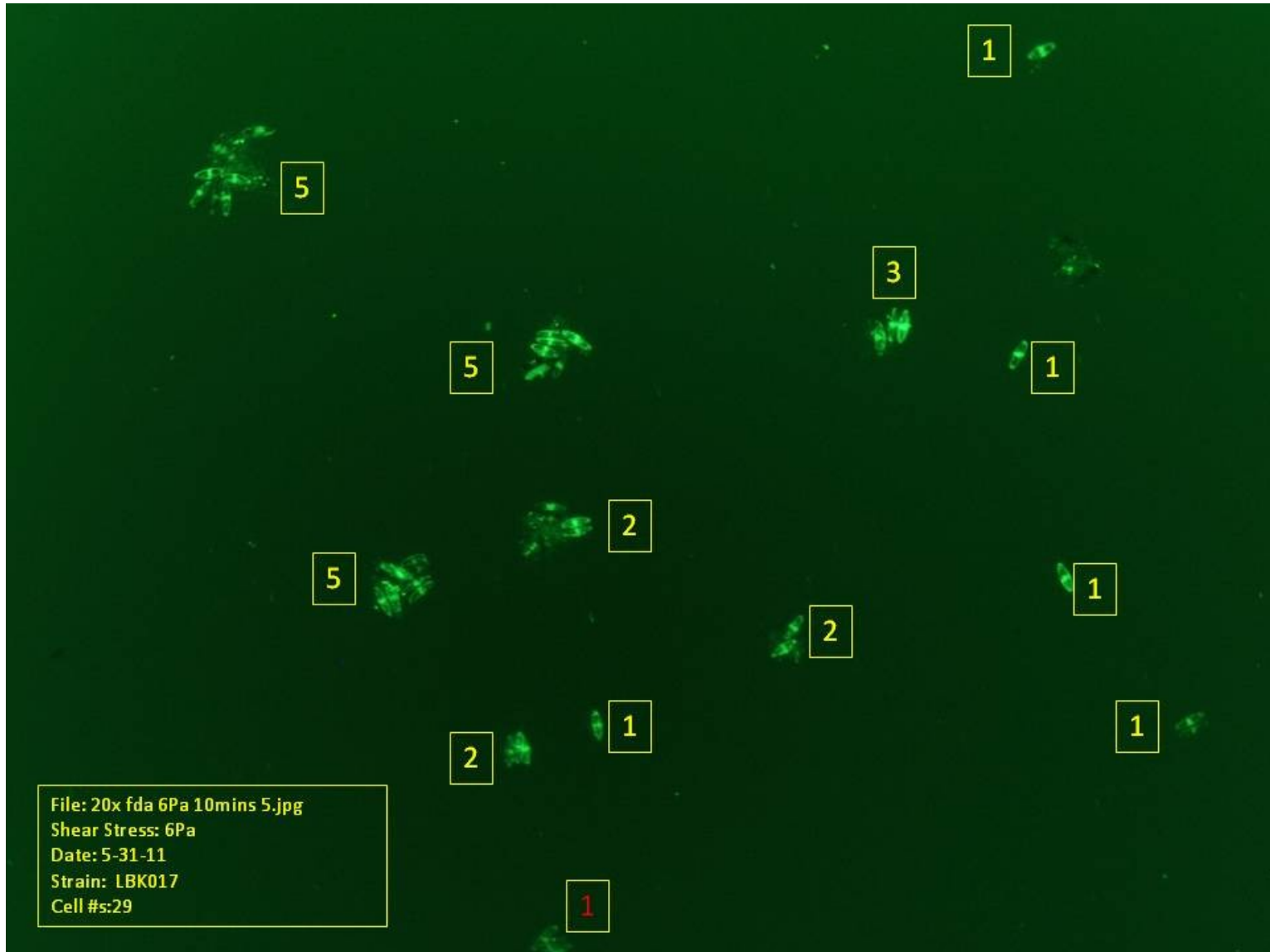
File: 20x fda 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:20

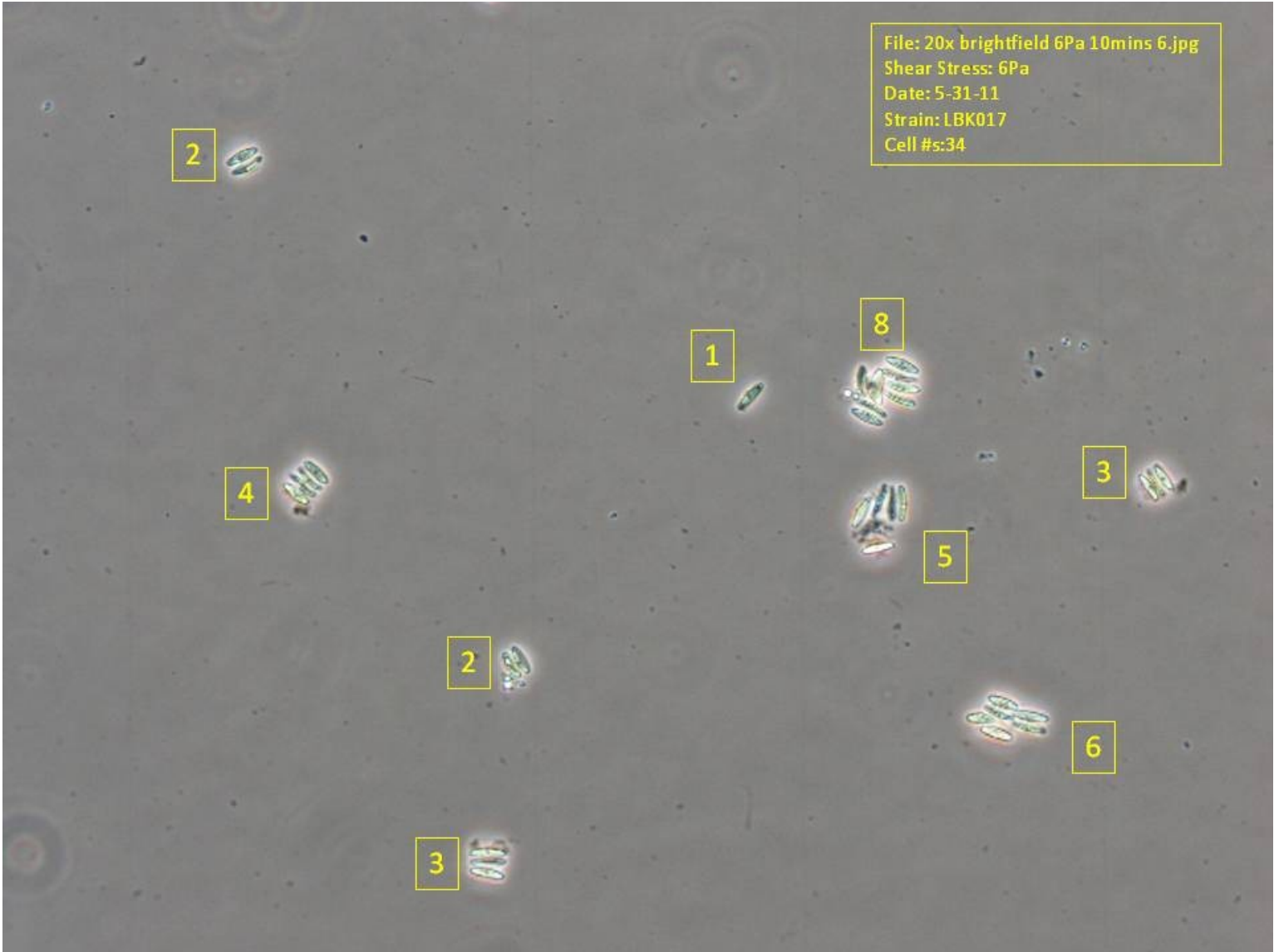


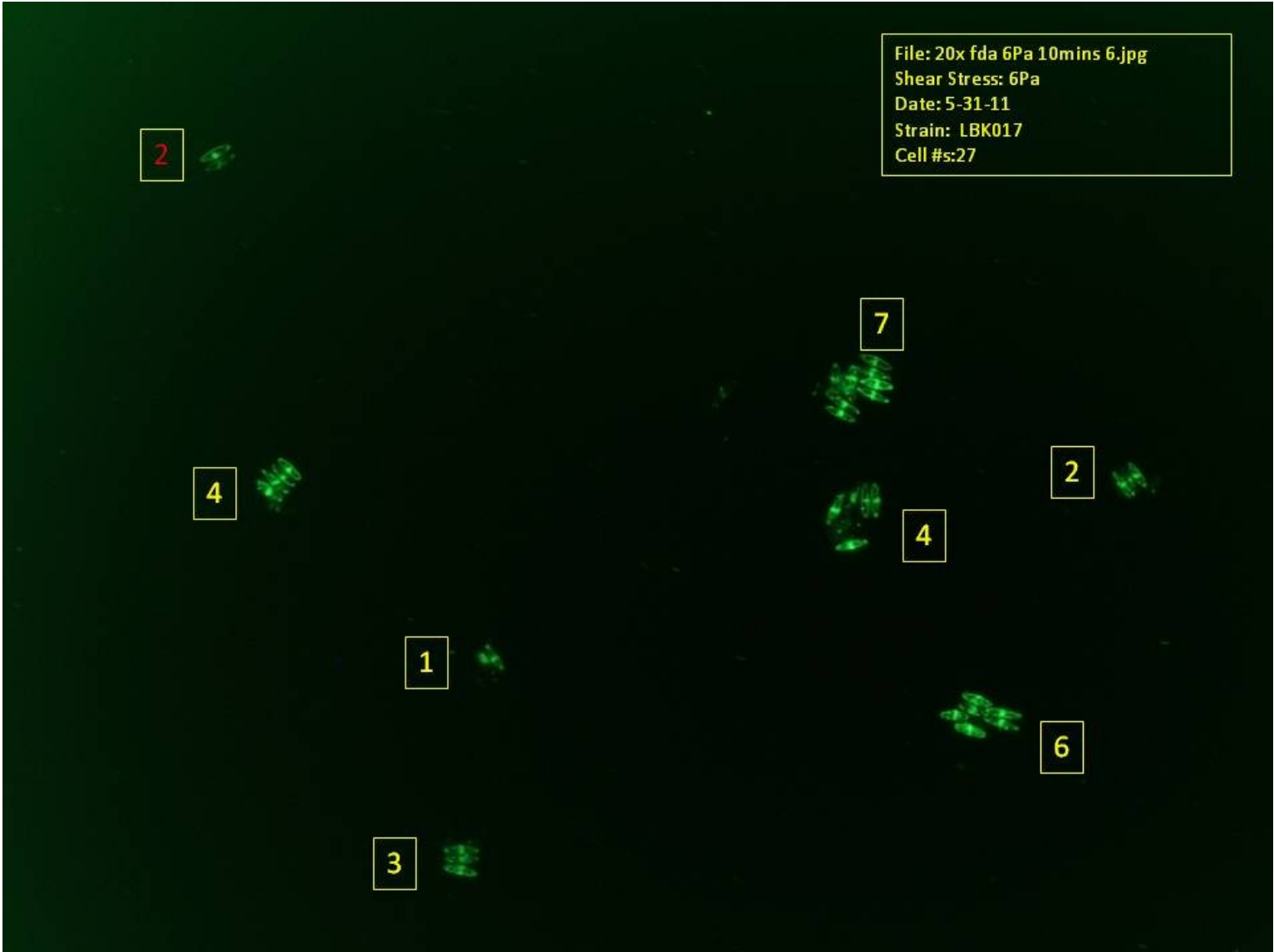








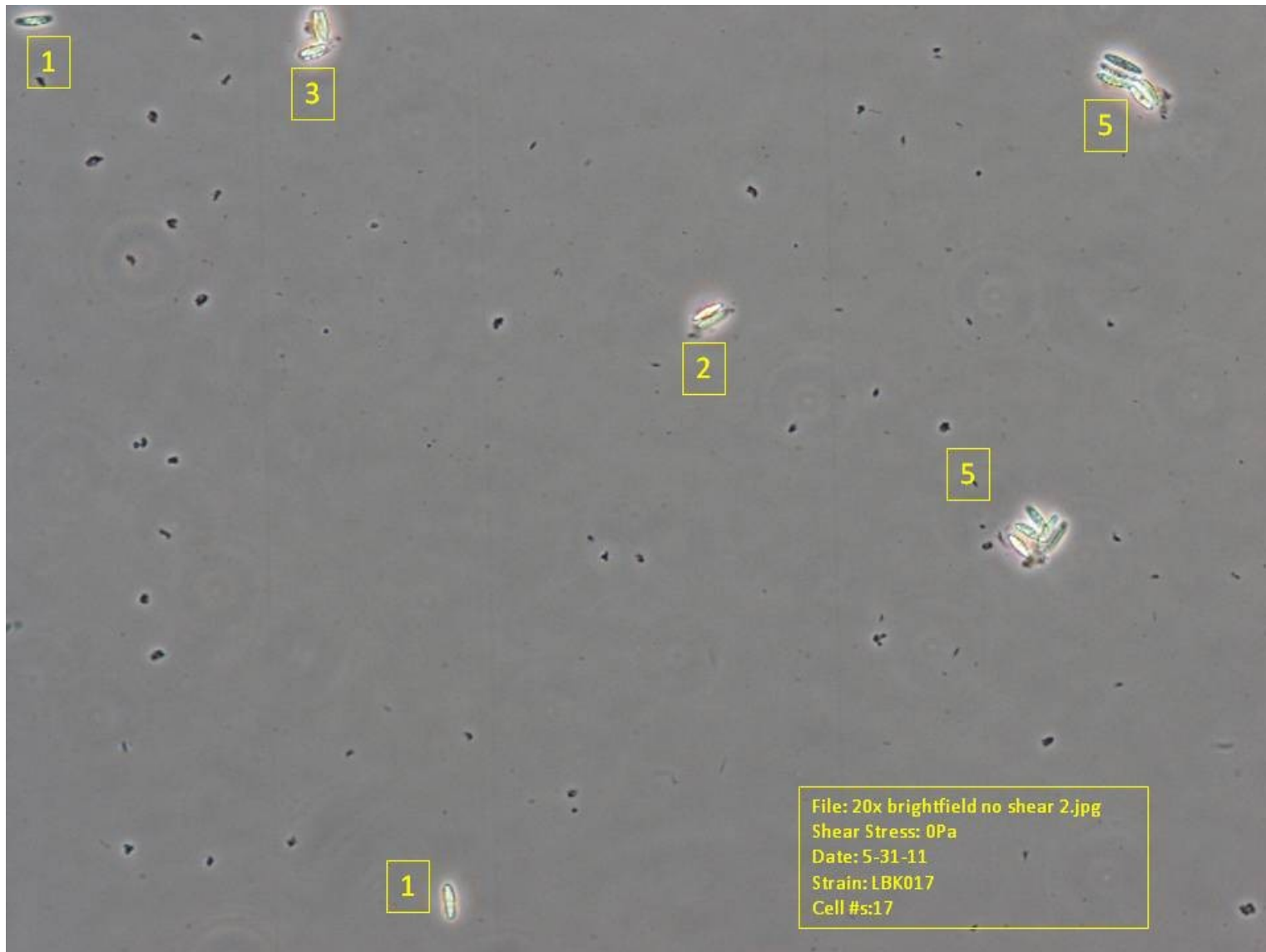










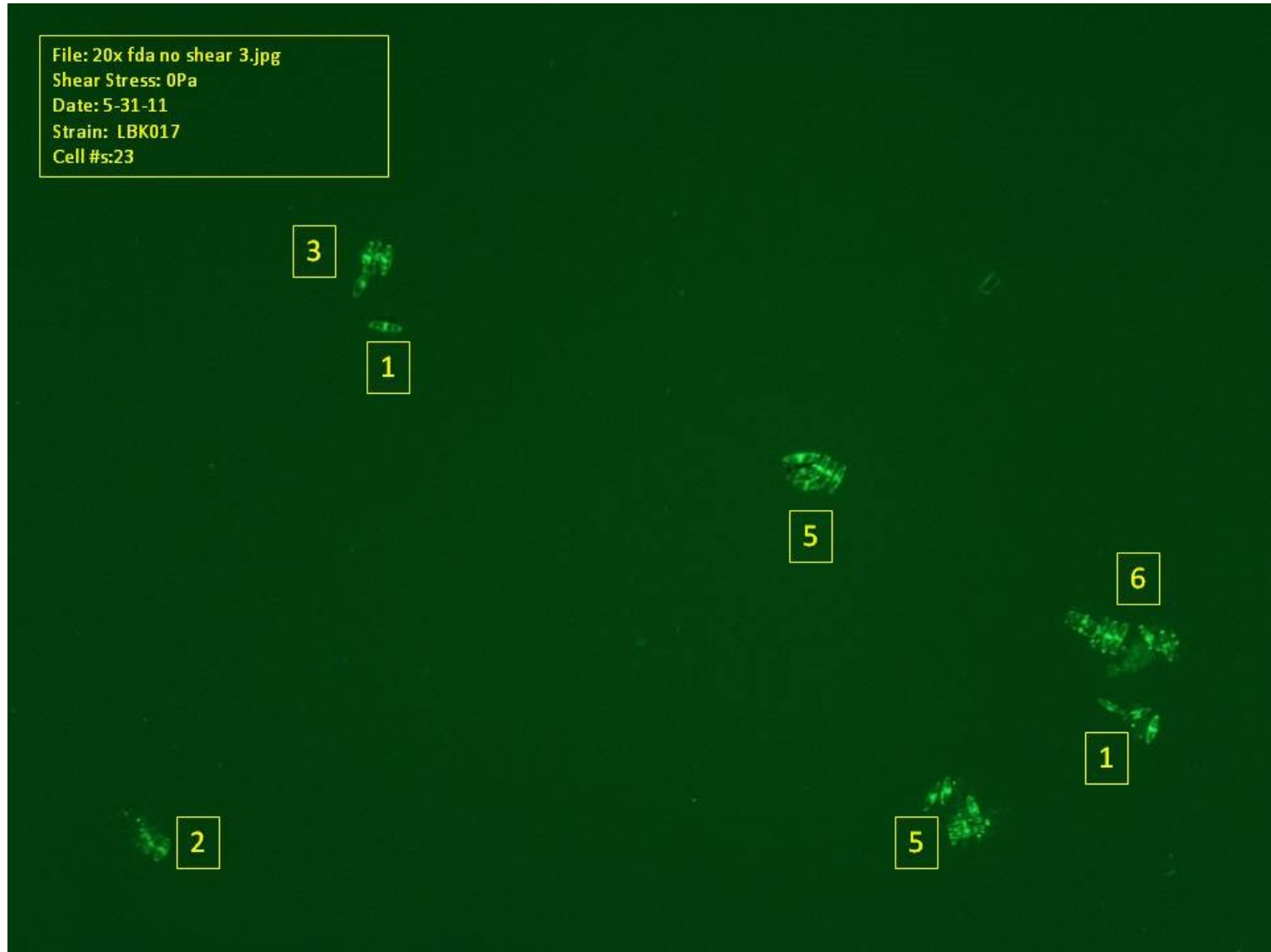




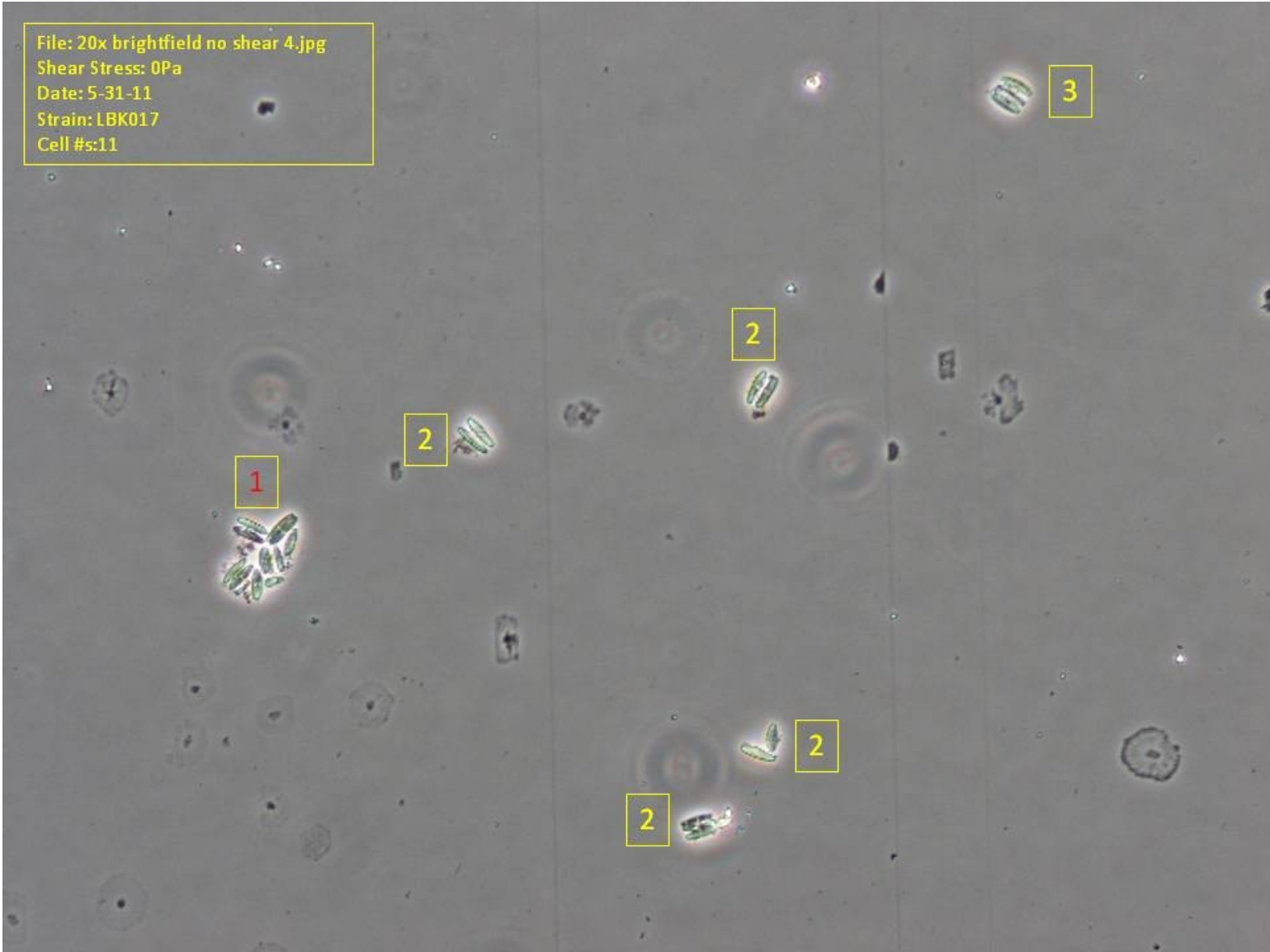
File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:31



File: 20x fda no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:23



File: 20x brightfield no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:11



File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:

1

2

2

3

2

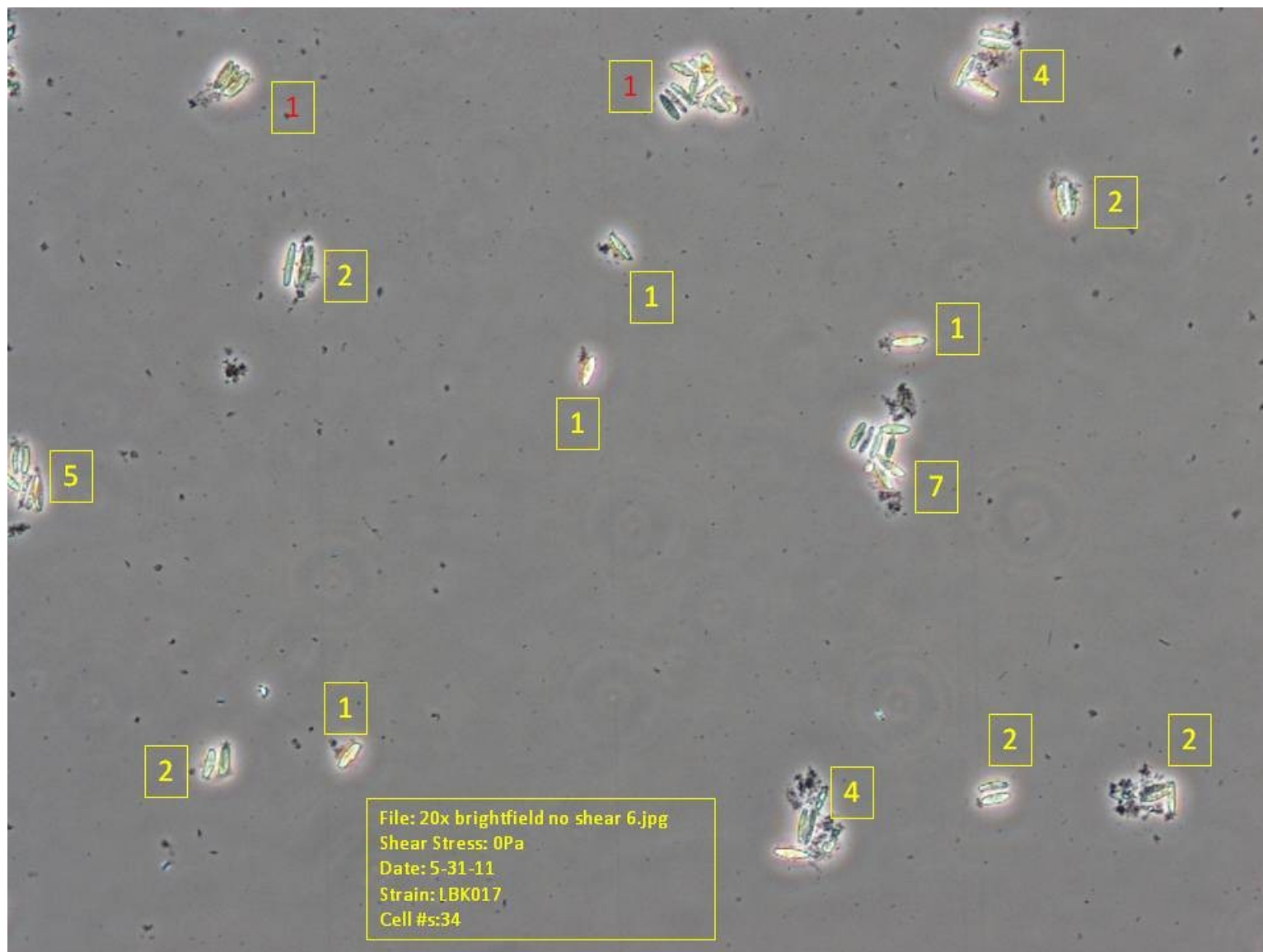
2

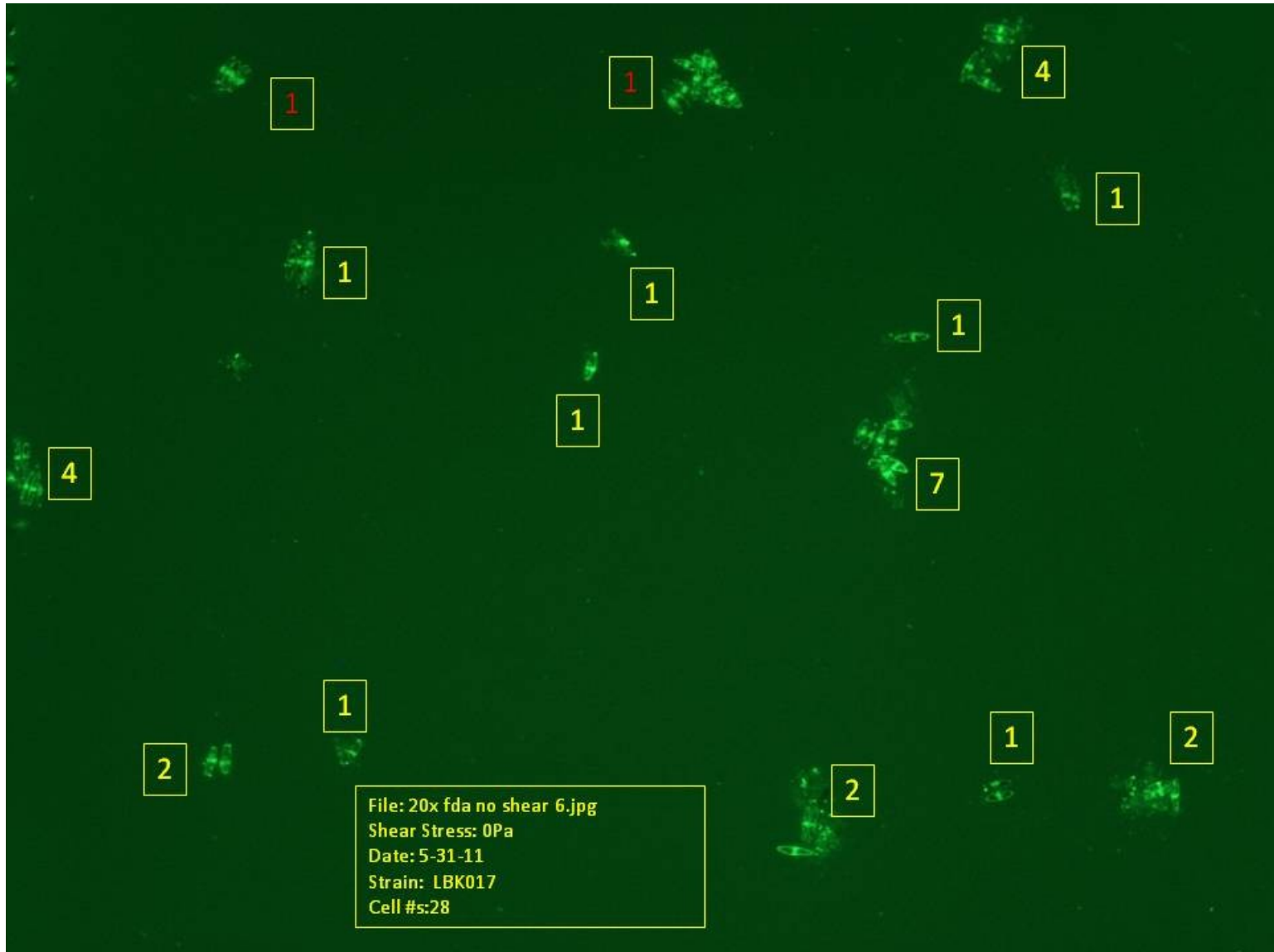
File: 20x brightfield no shear 5.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:15



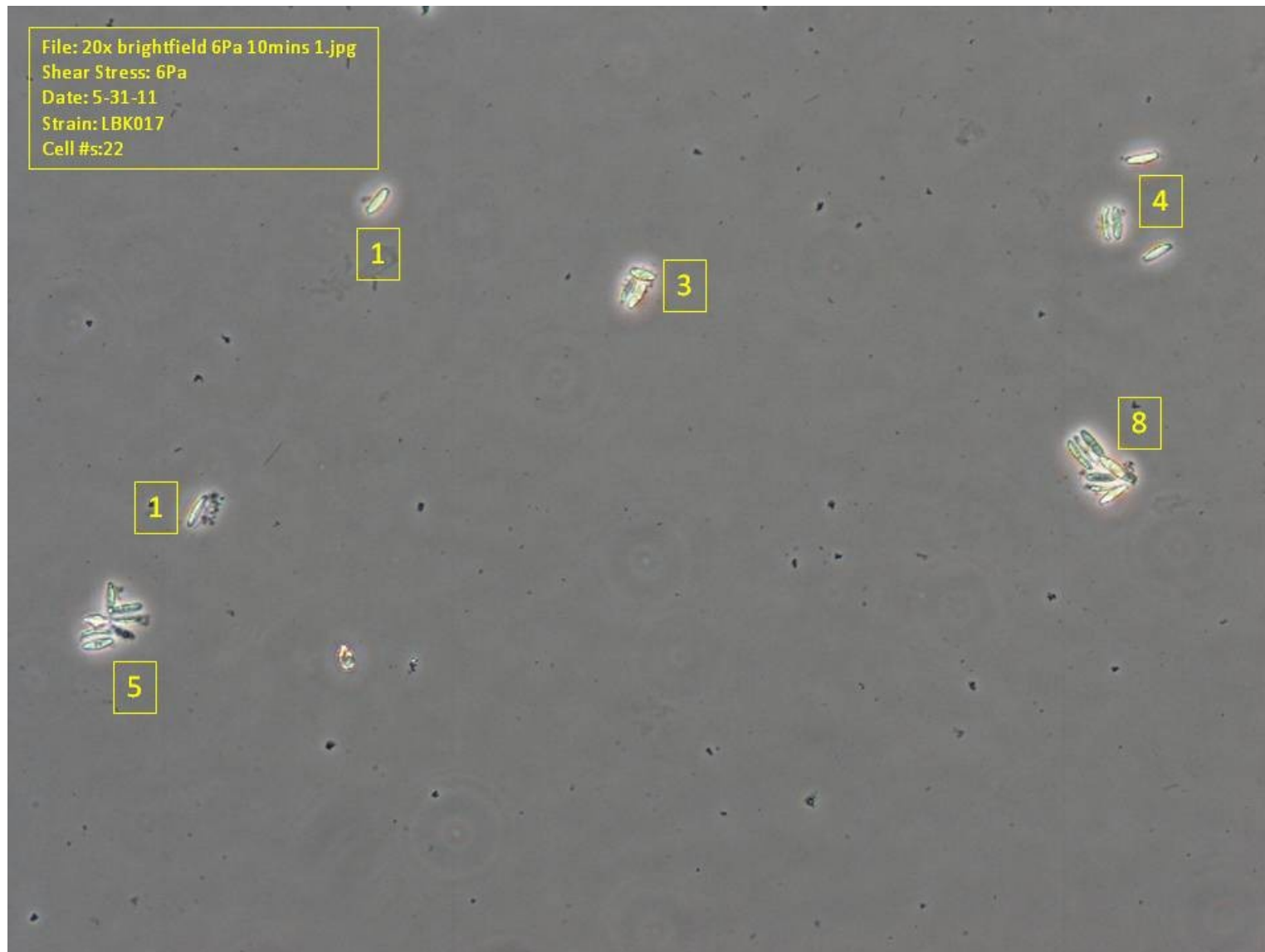








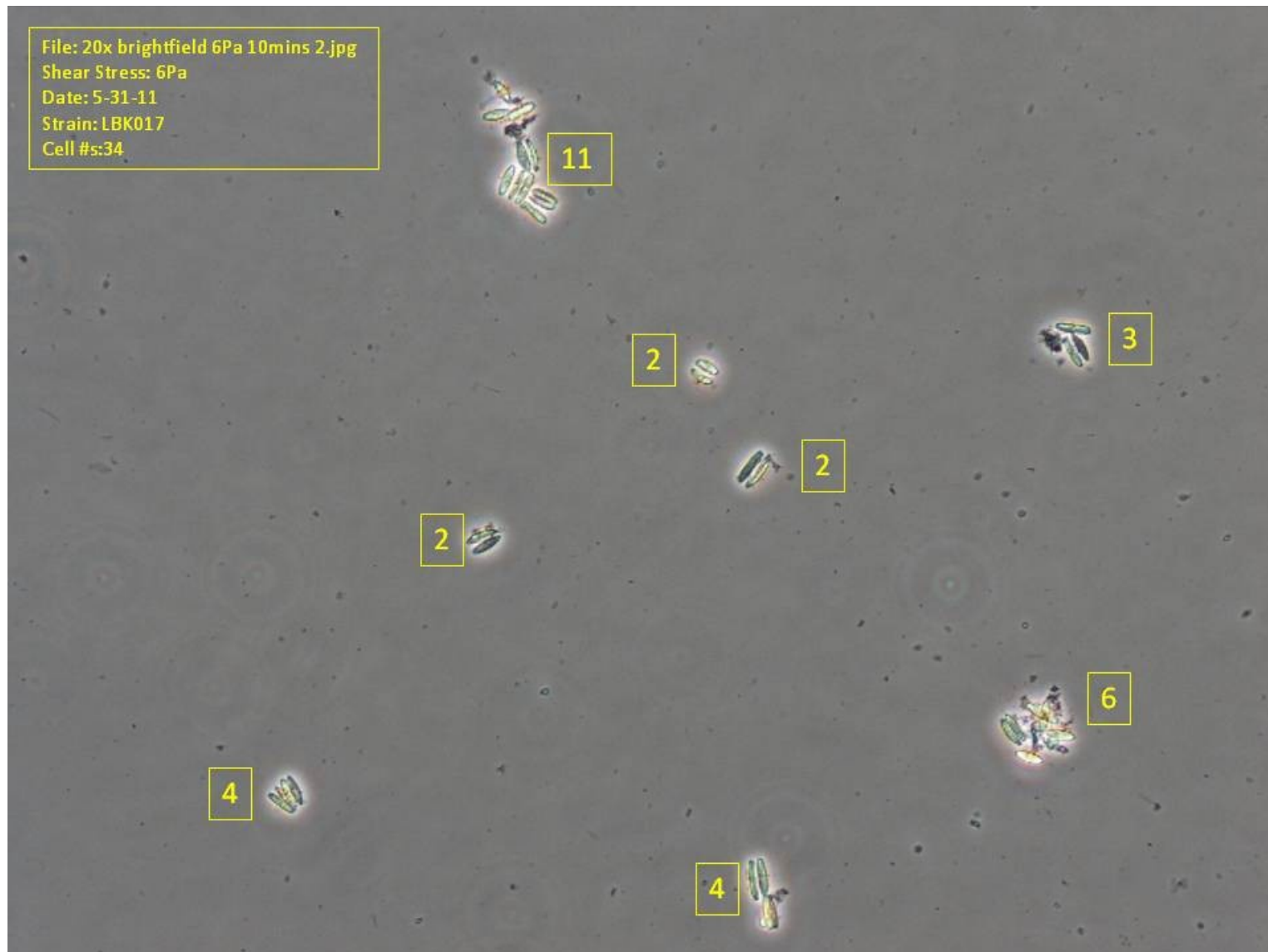
File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:22



File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:19

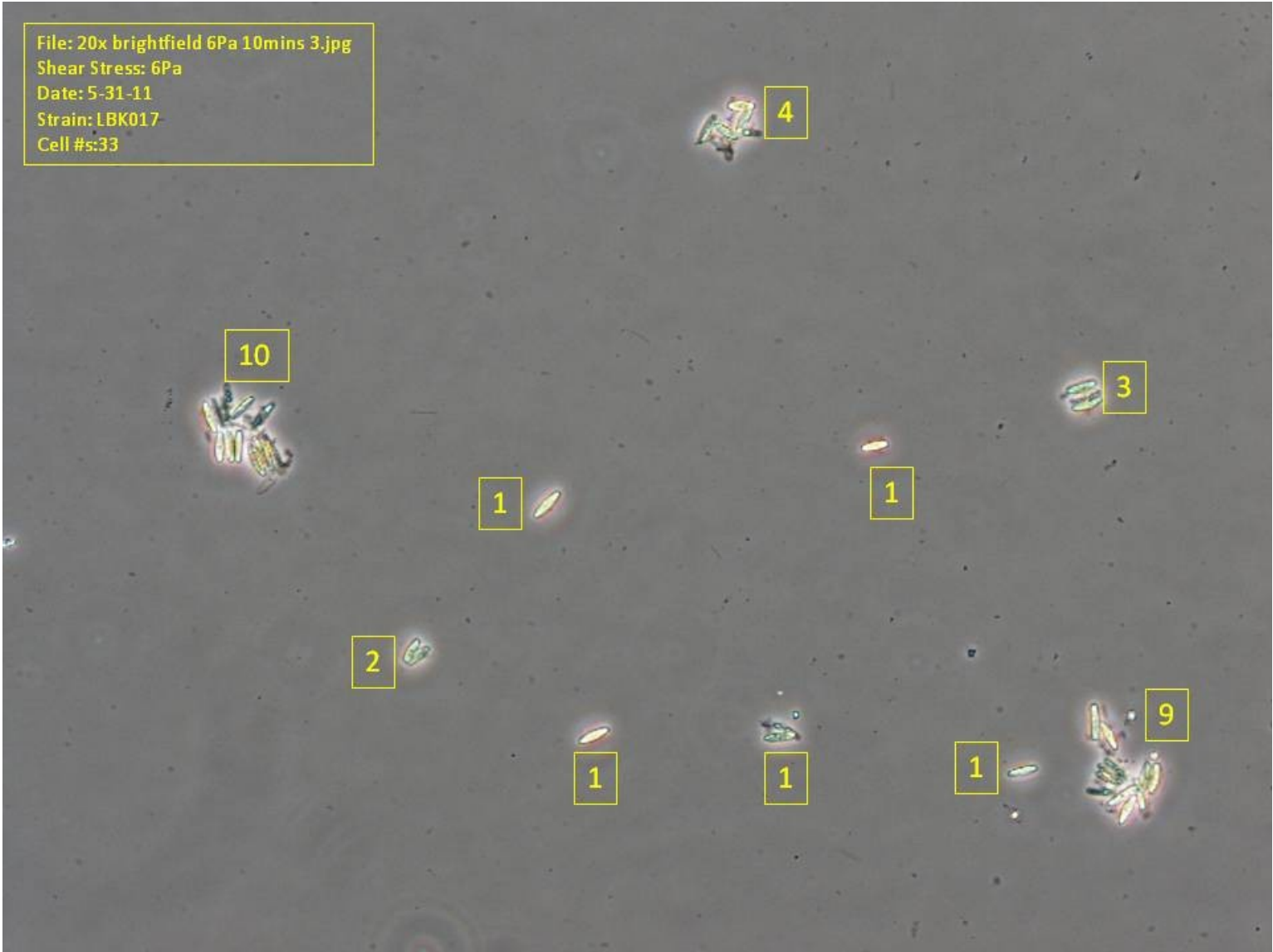


File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:34



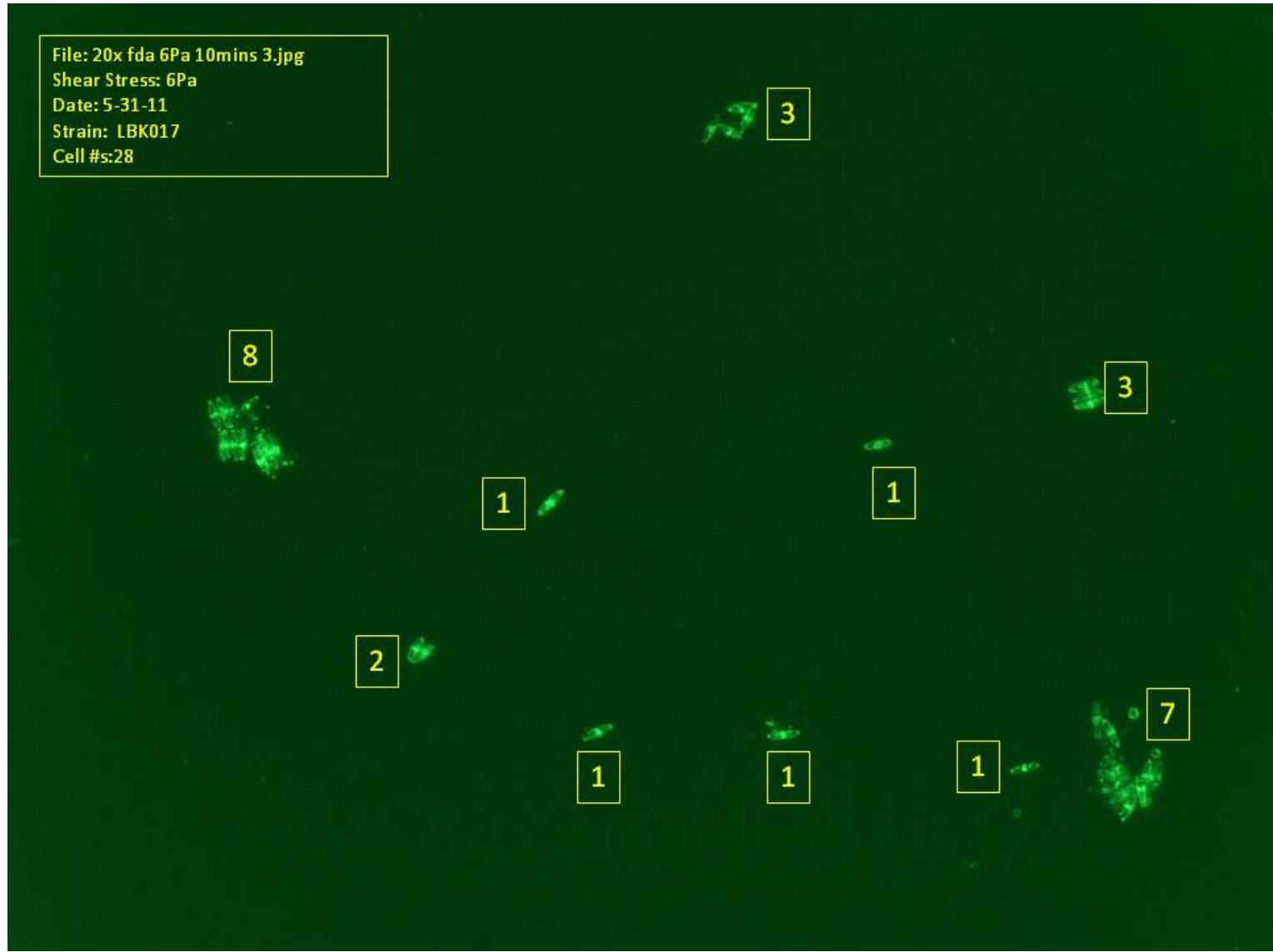


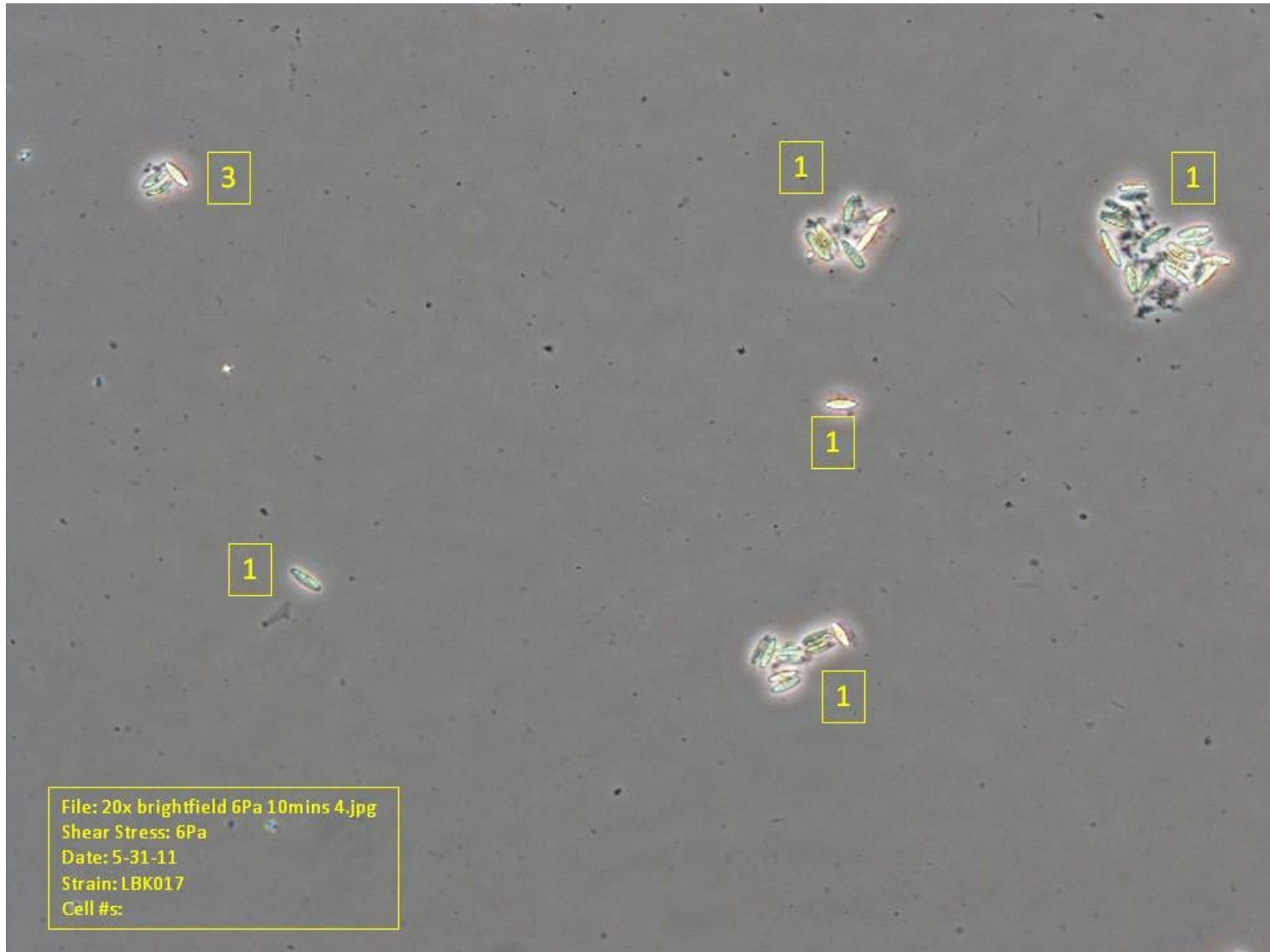
File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:33



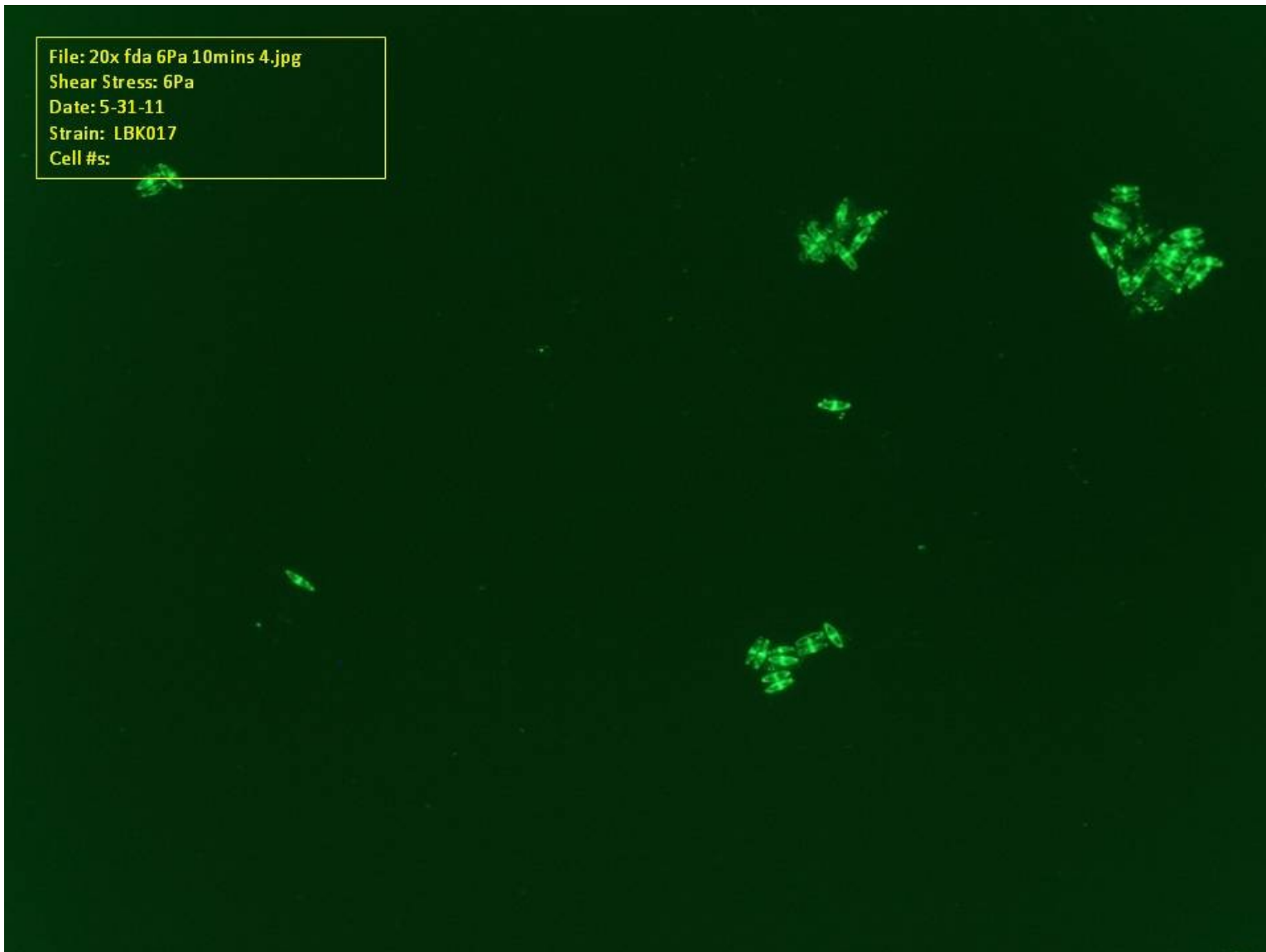


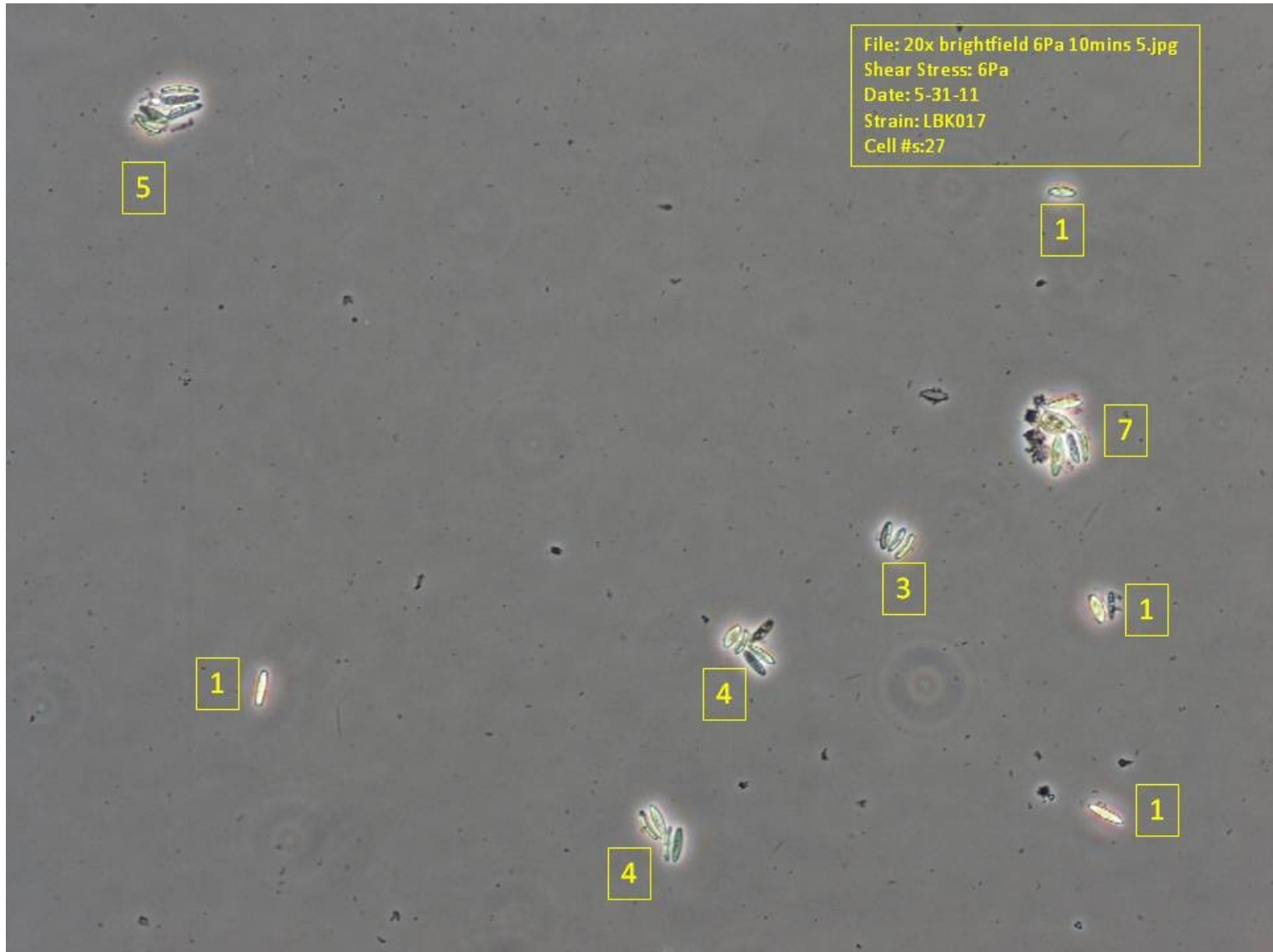
File: 20x fda 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:28

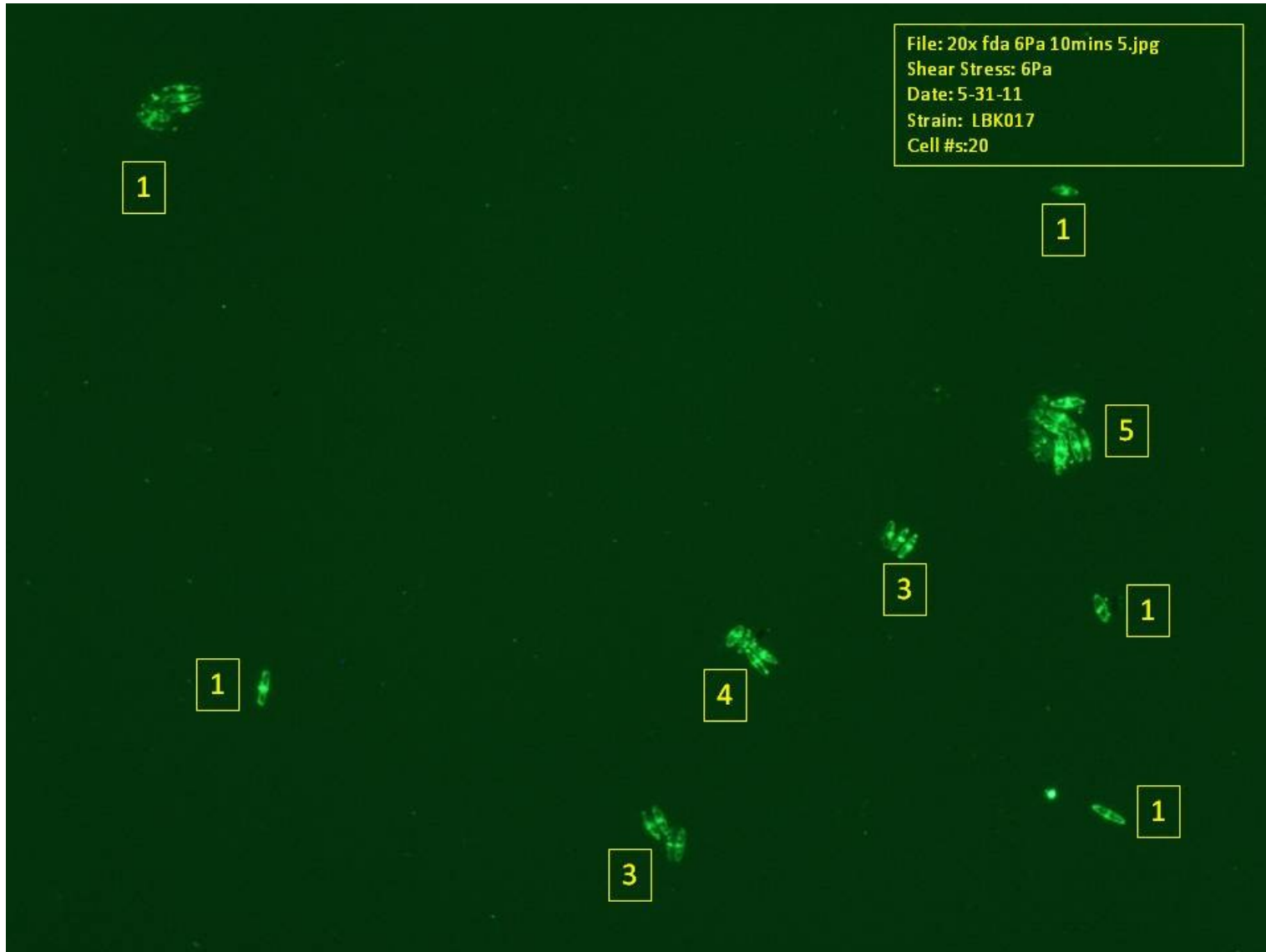


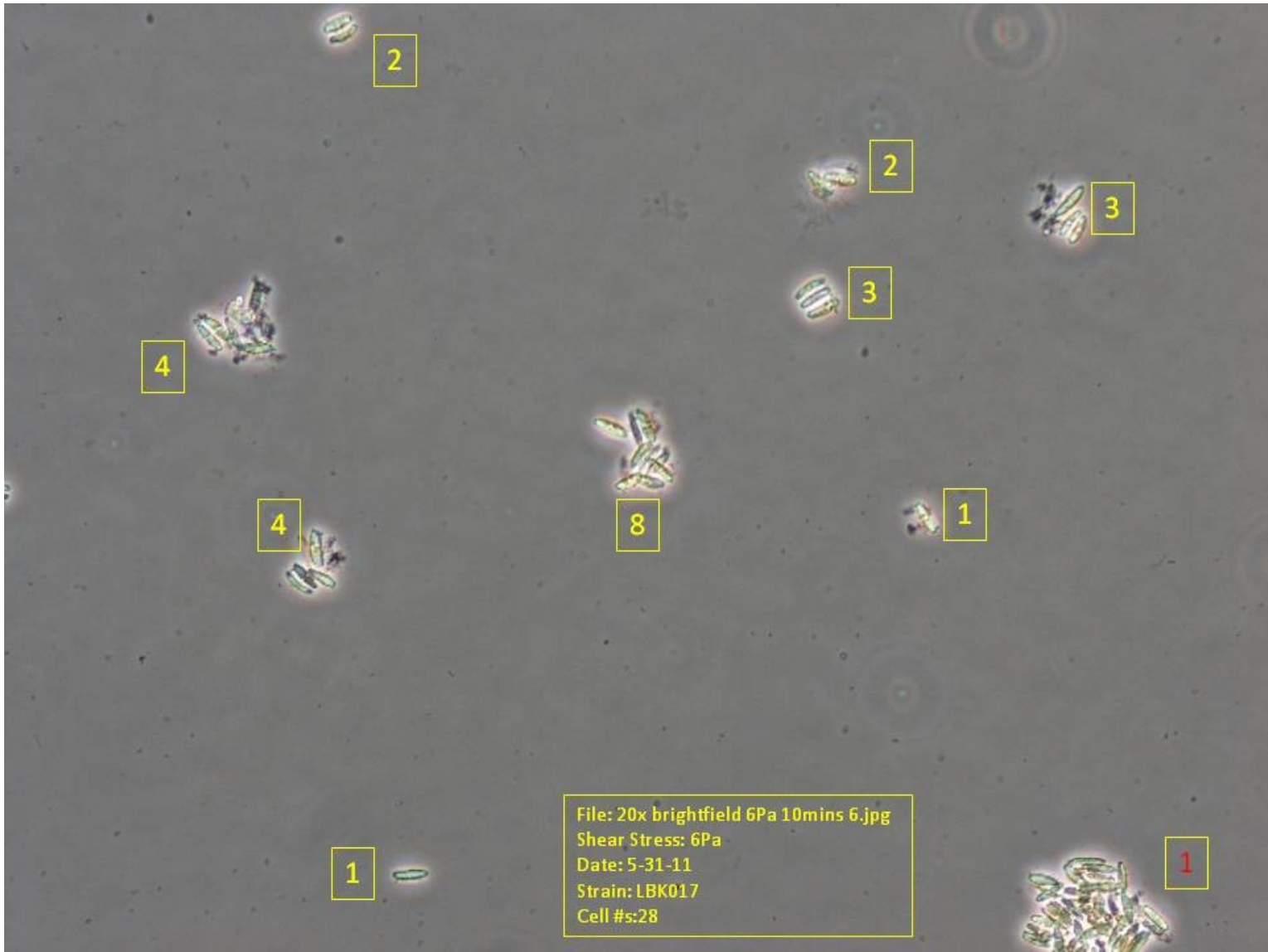


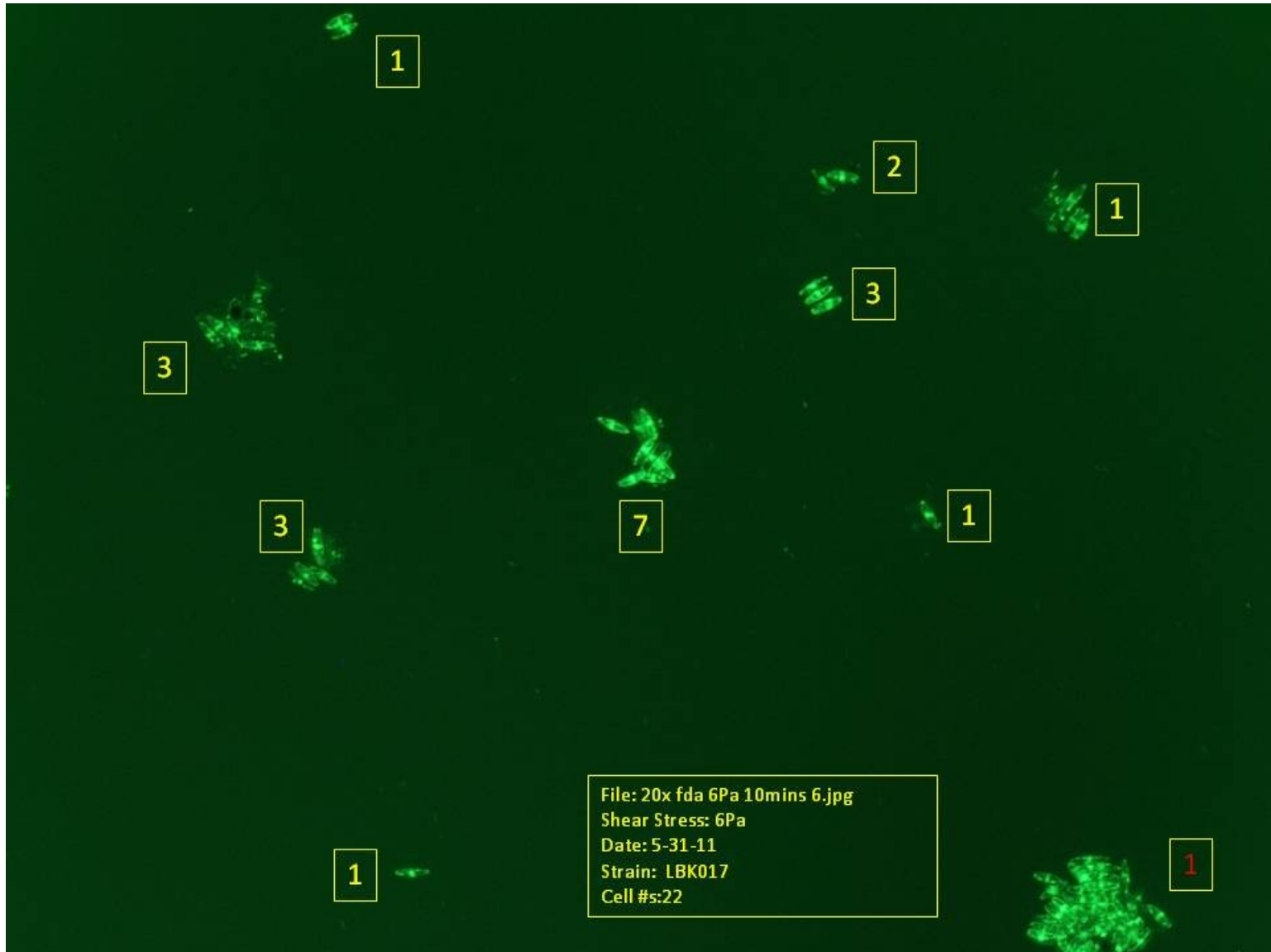
File: 20x fda 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:

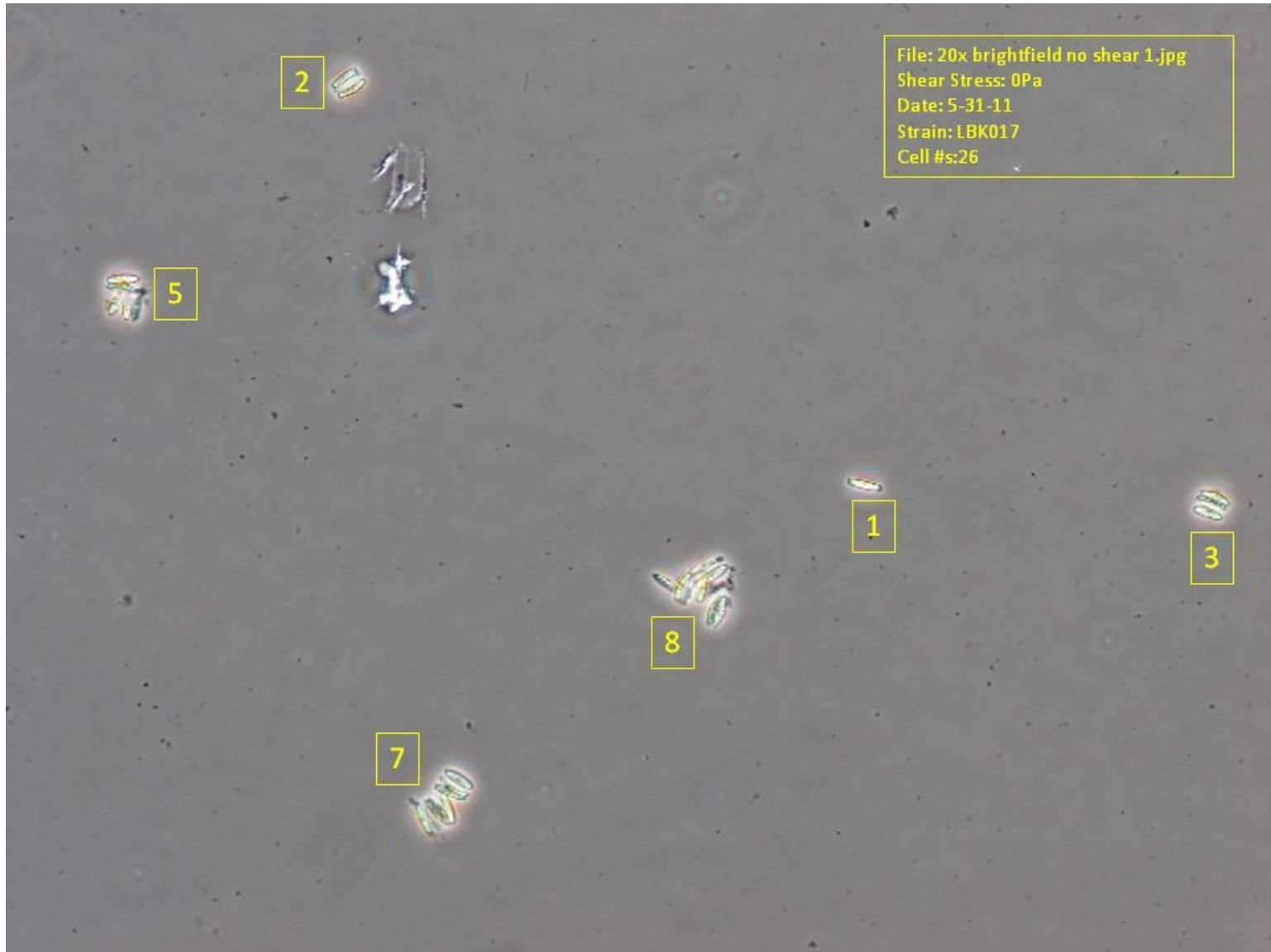






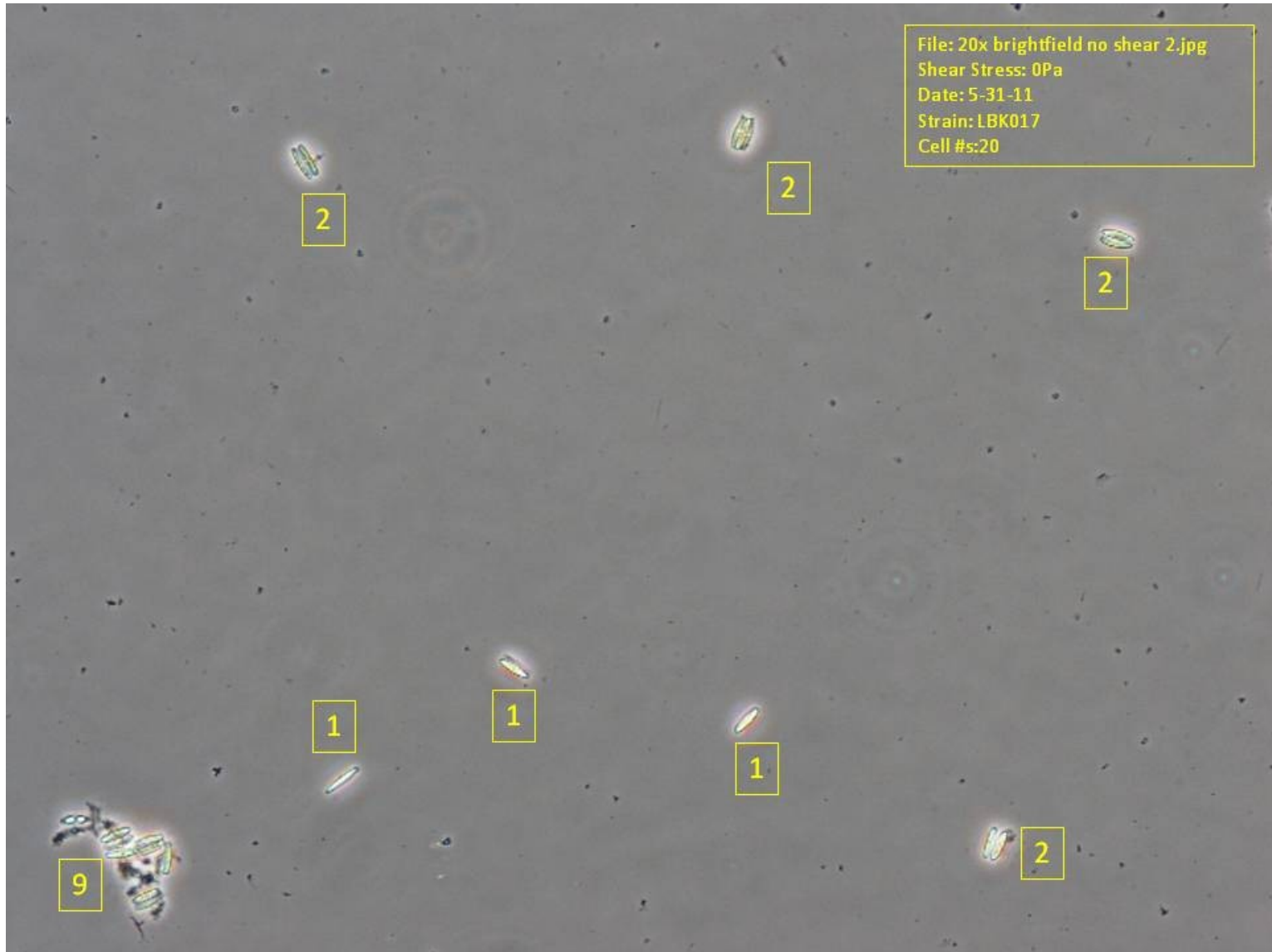






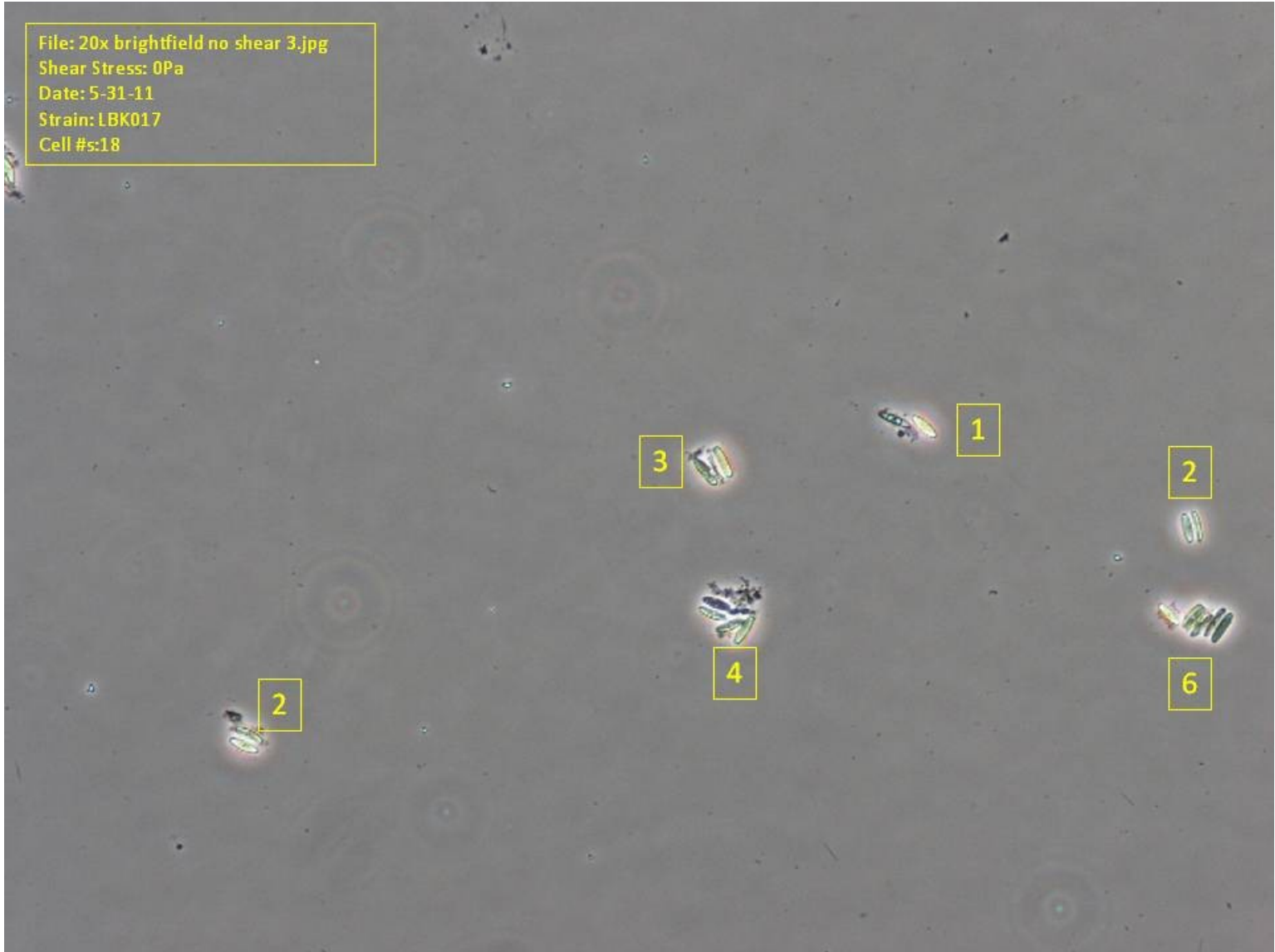




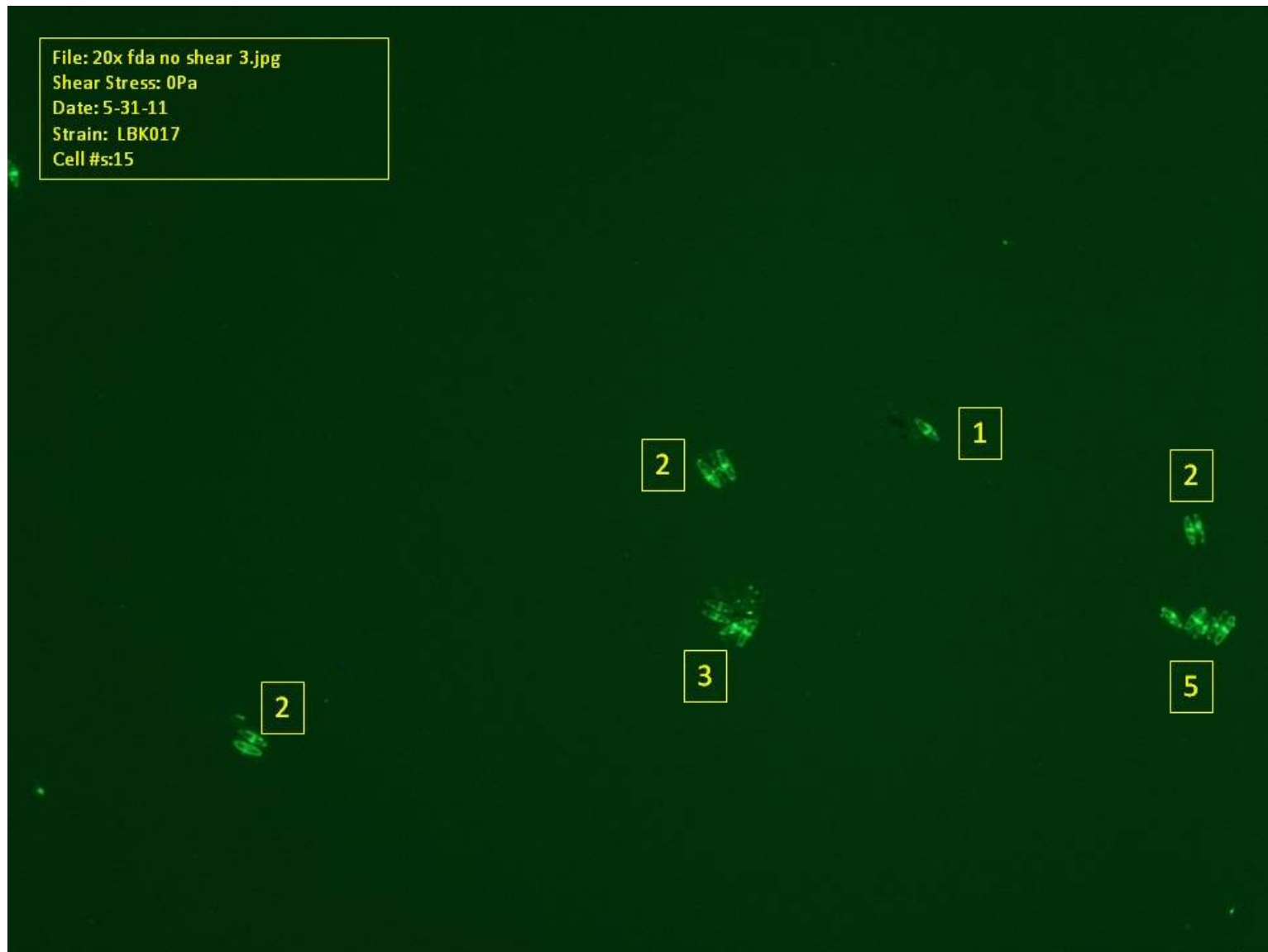




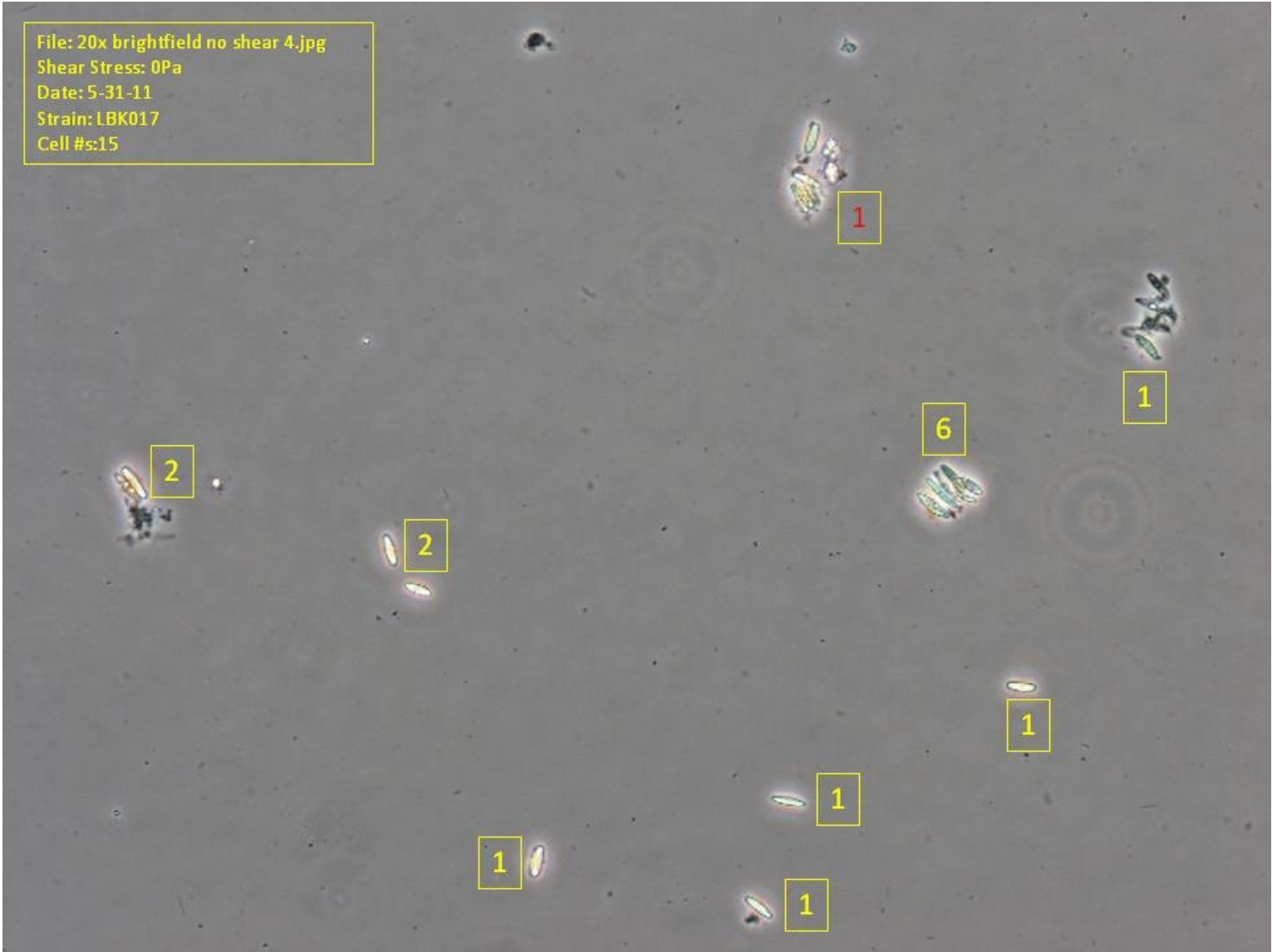
File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #:s:18



File: 20x fda no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:15



File: 20x brightfield no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:15



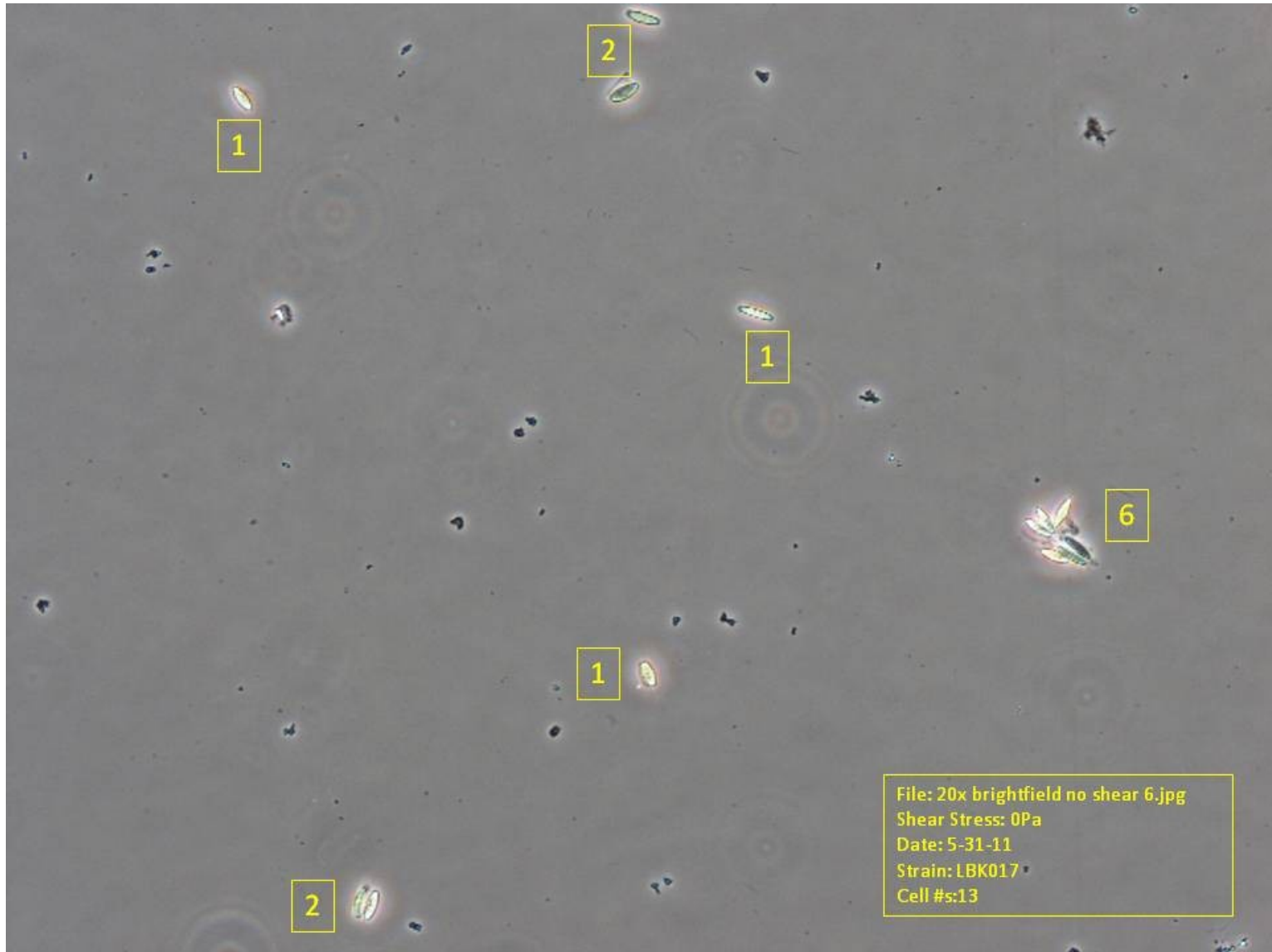


File: 20x brightfield no shear 5.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:14

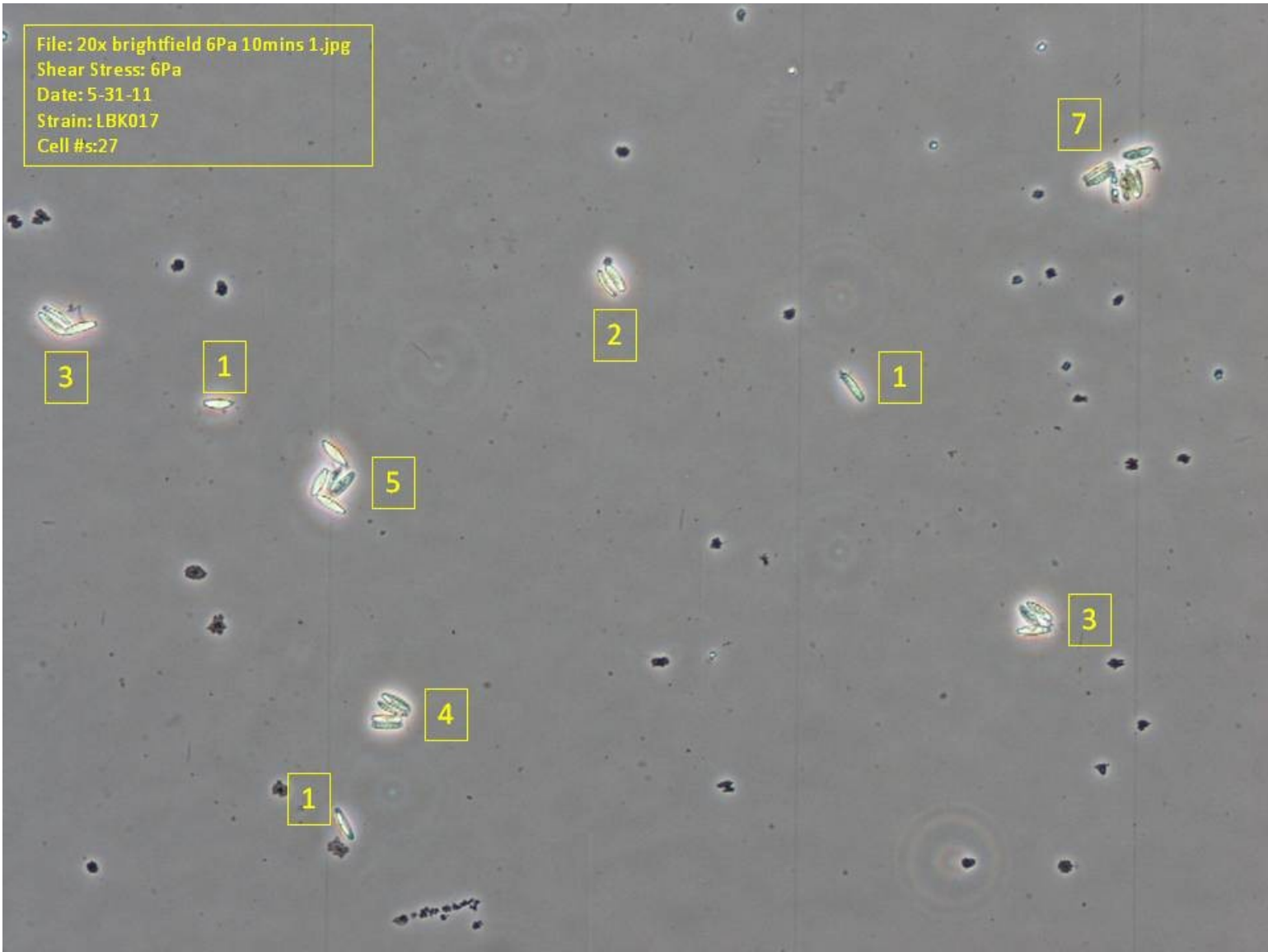




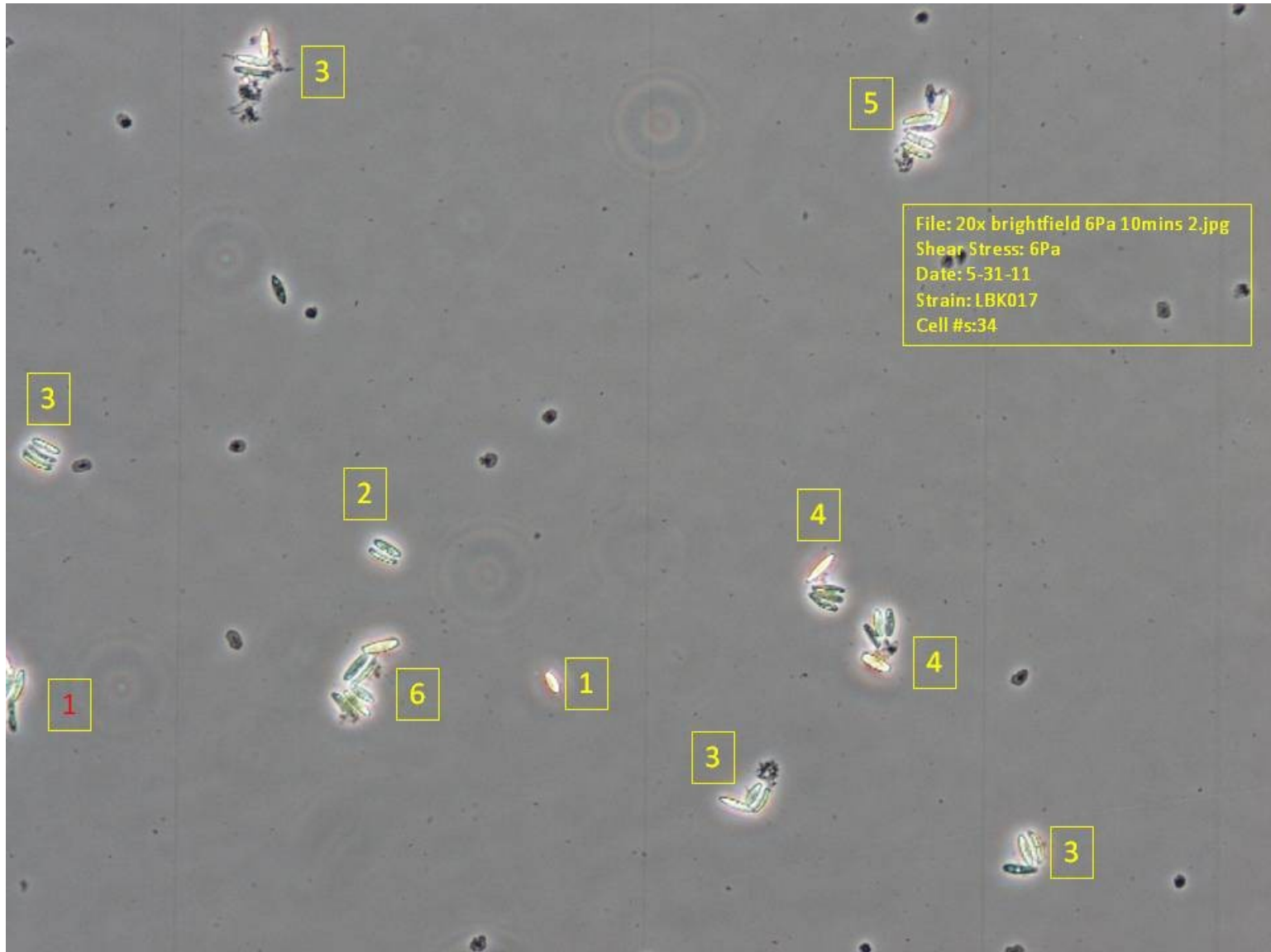


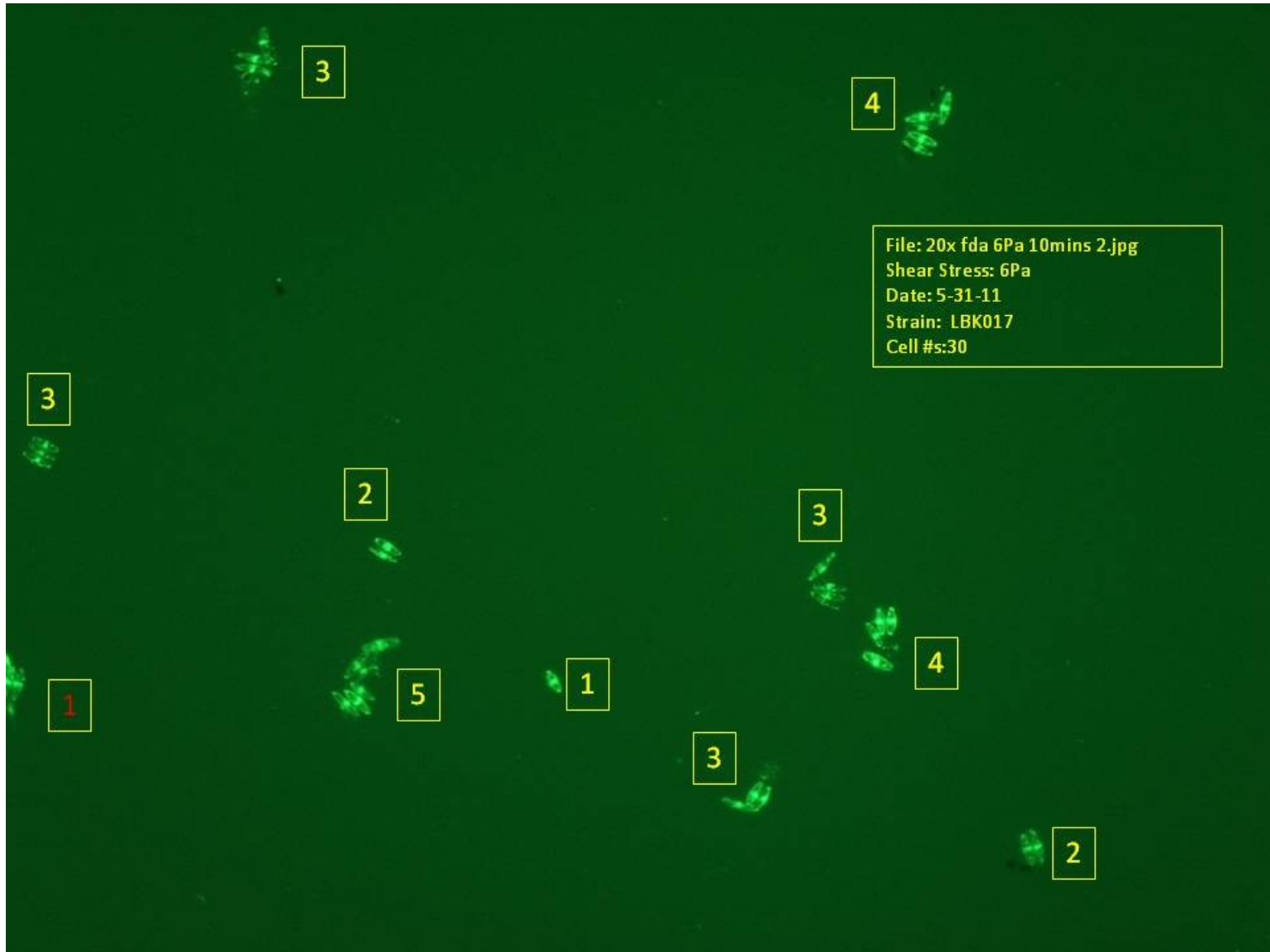


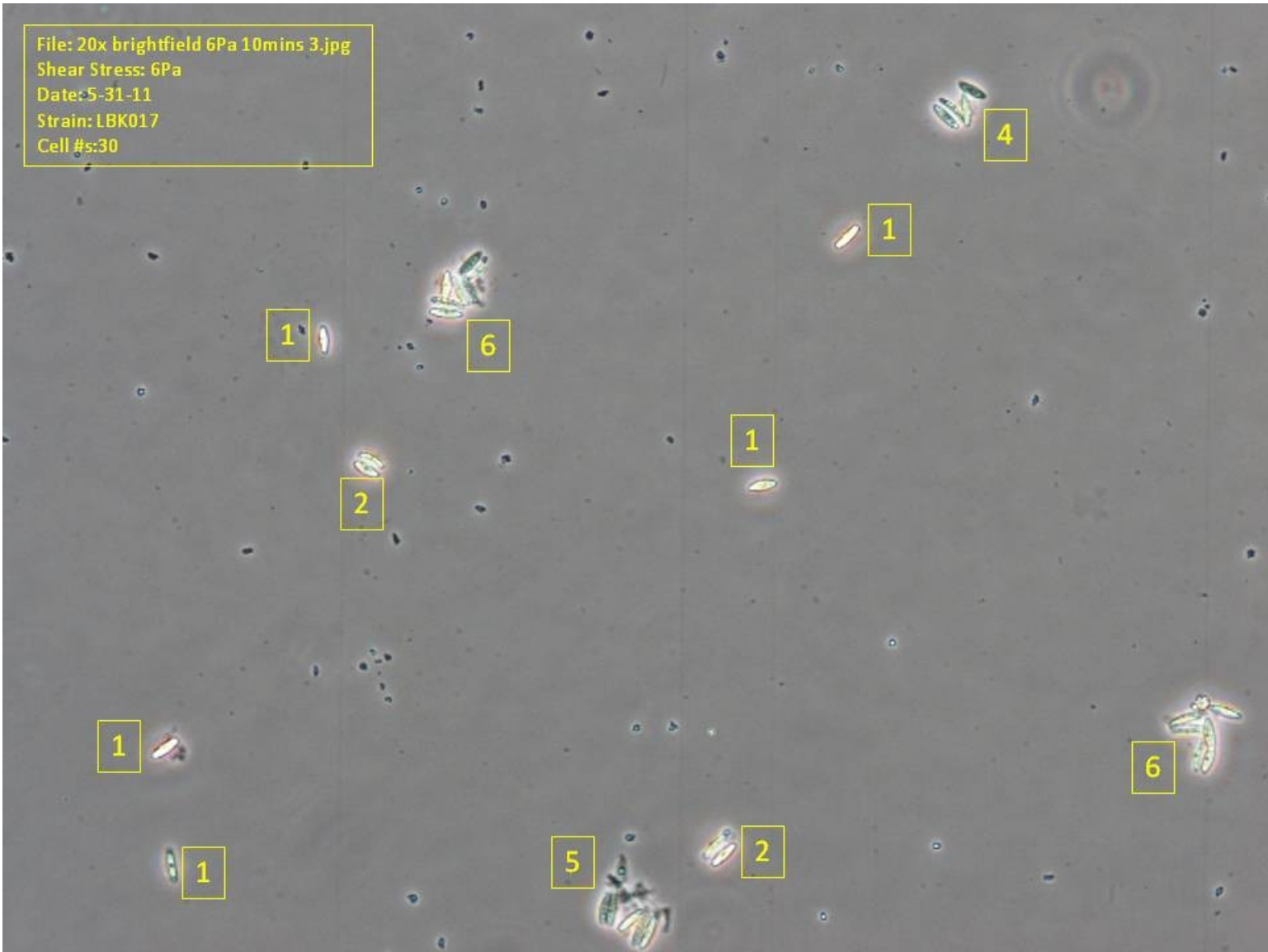






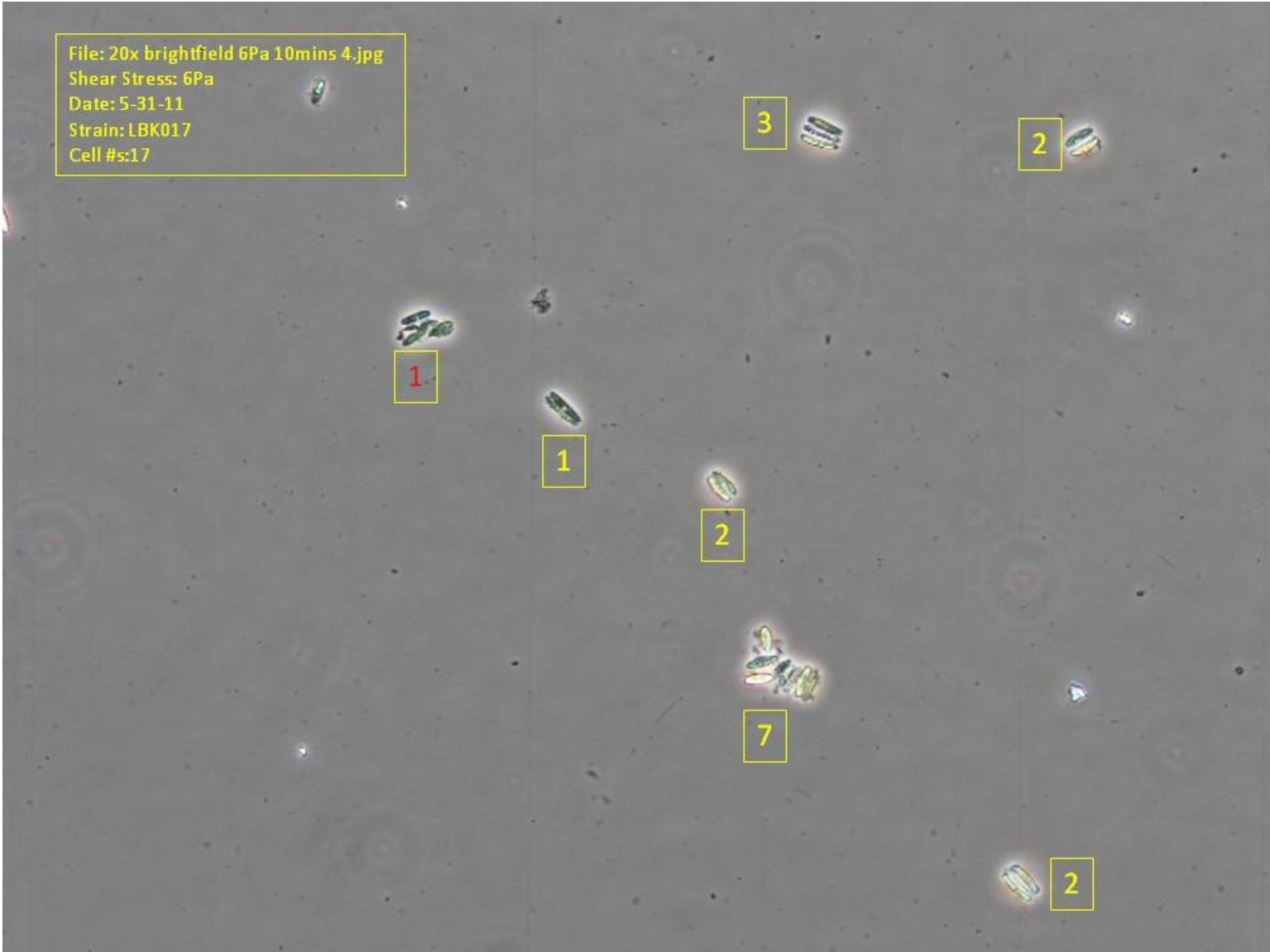






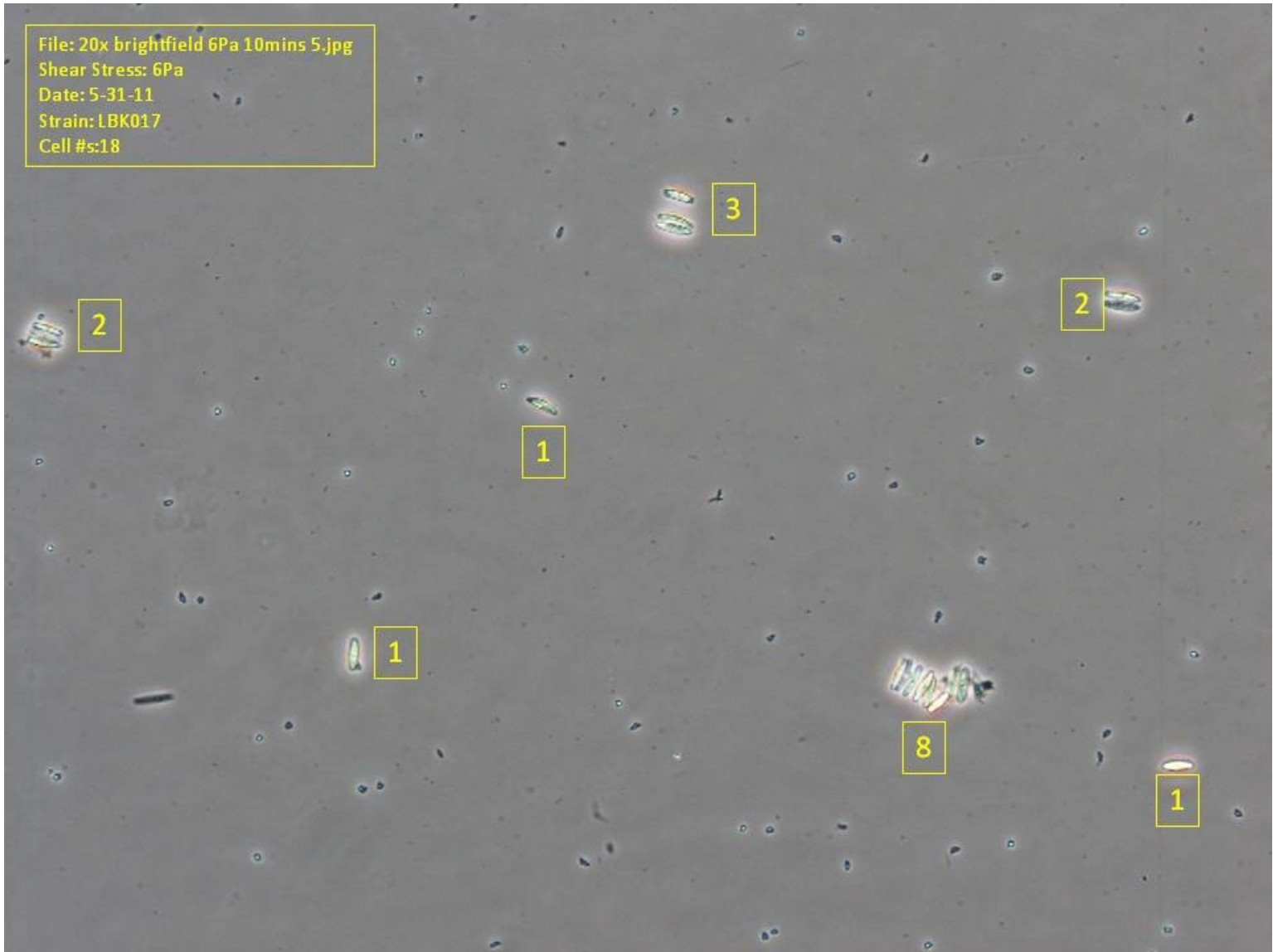






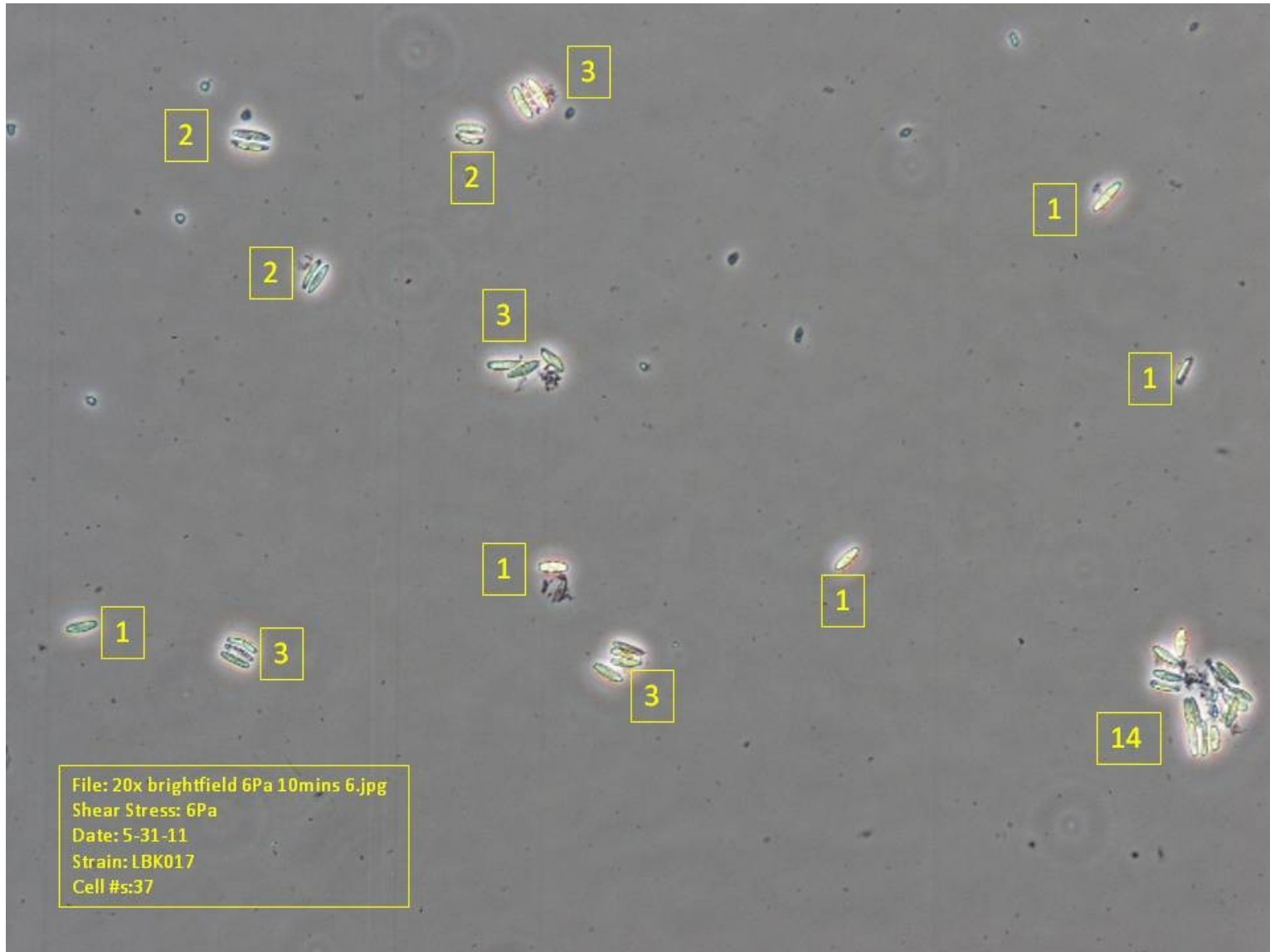


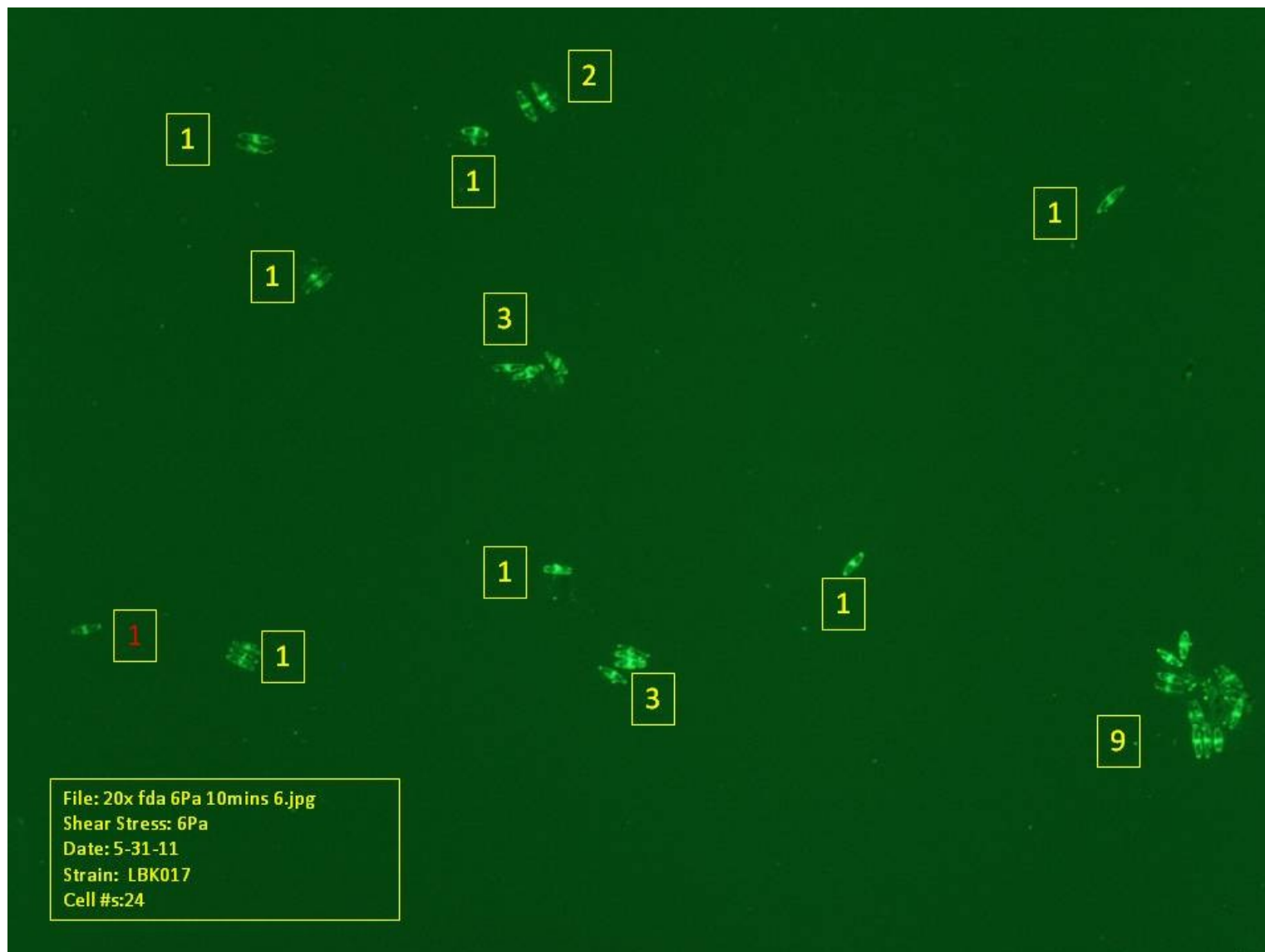
File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:18



File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK017  
Cell #s:12







## APPENDIX G

The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK018, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

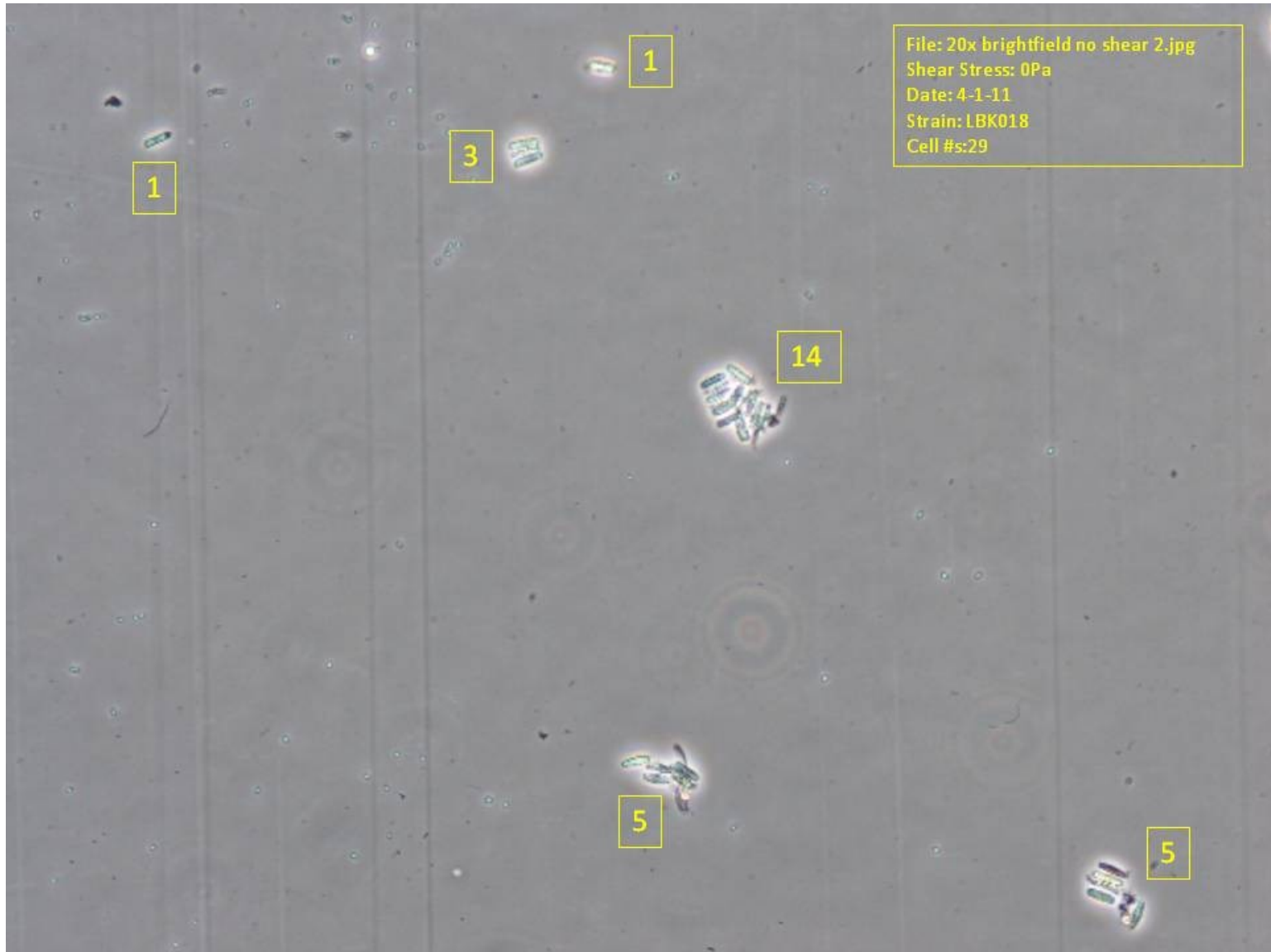
	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	28	24	29	27	19	14			11	9	12	6	80.81%	4.25%	-0.00708
	6	38	34	38	29	13	11	23	14	16	10			76.56%		
Rep 2	0	12	11	12	10	26	25	18	15	7	4			86.67%	17.19%	-0.02865
	6	26	23	19	14	15	8	12	5	23	16			69.47%		
Rep 3	0	12	12	14	11	28	23	19	14	18	17			84.62%	21.65%	-0.03609
	6	19	13	7	5	25	16	15	9	15	8			62.96%		
														<b>Avg.</b>	<b>14.36%</b>	
														<b>Std. Dev.</b>	<b>9.04%</b>	

The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.











File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:19

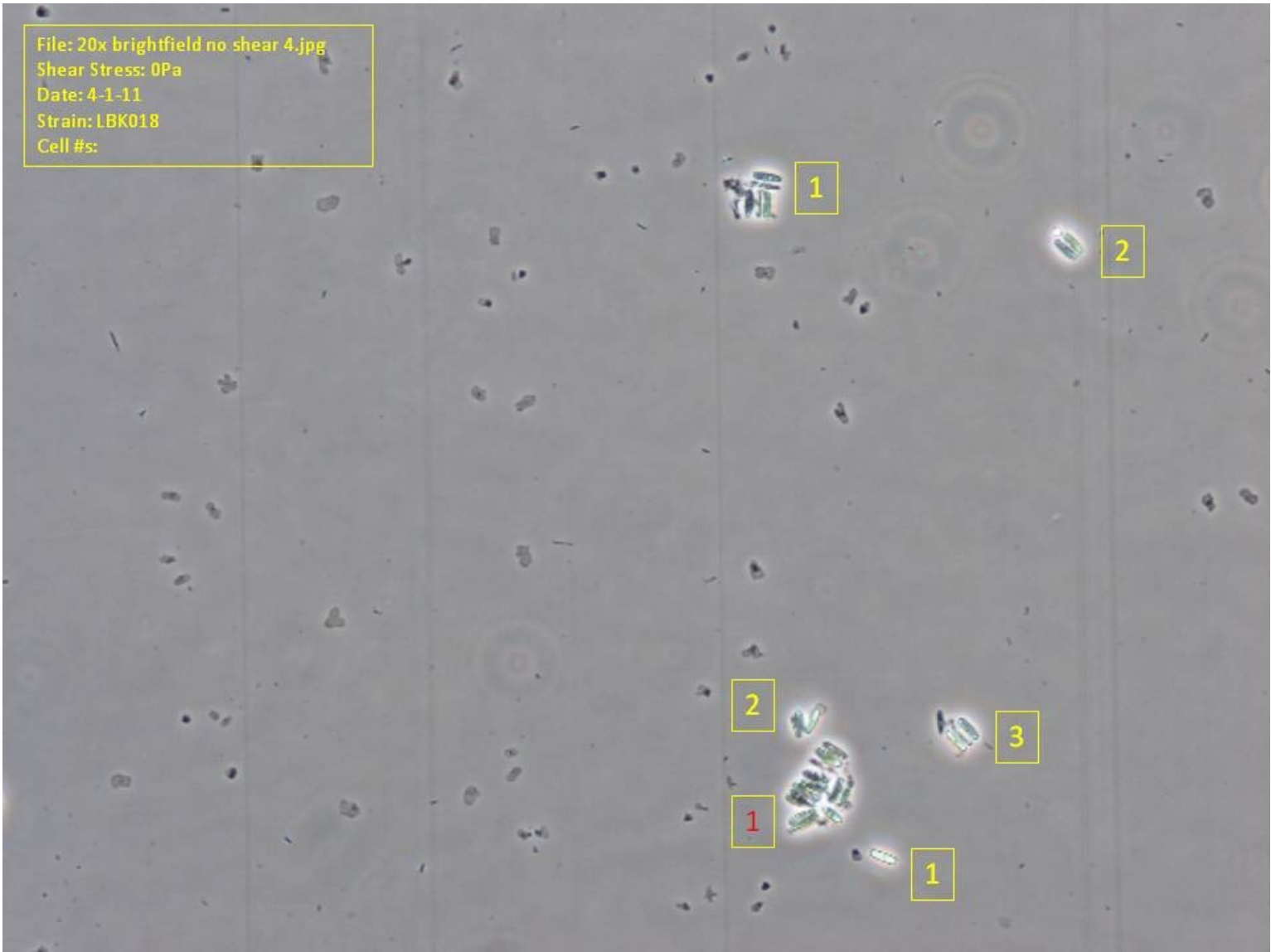


File: 20x fda no shear 3.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 14

5

8

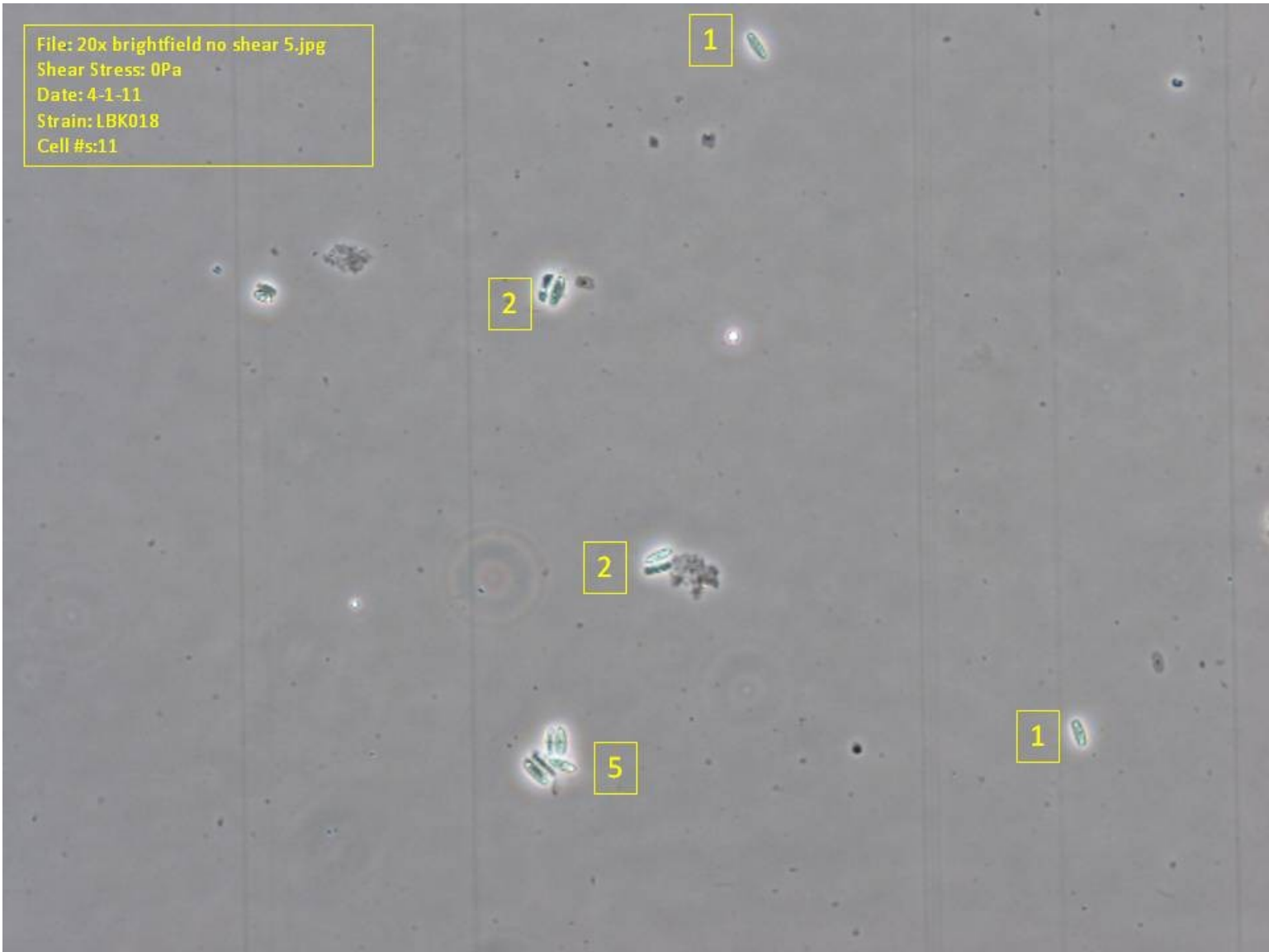
1



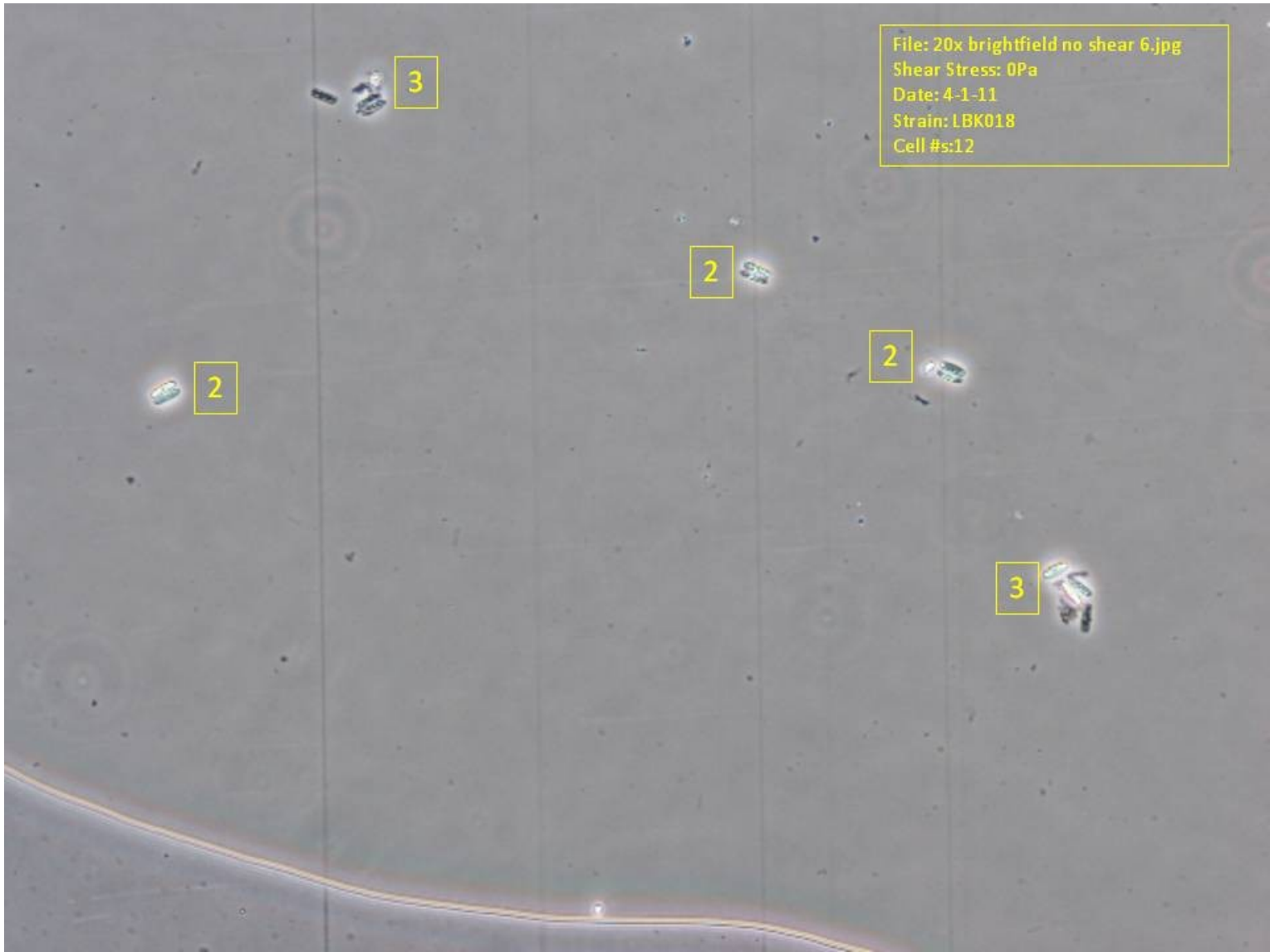
File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:

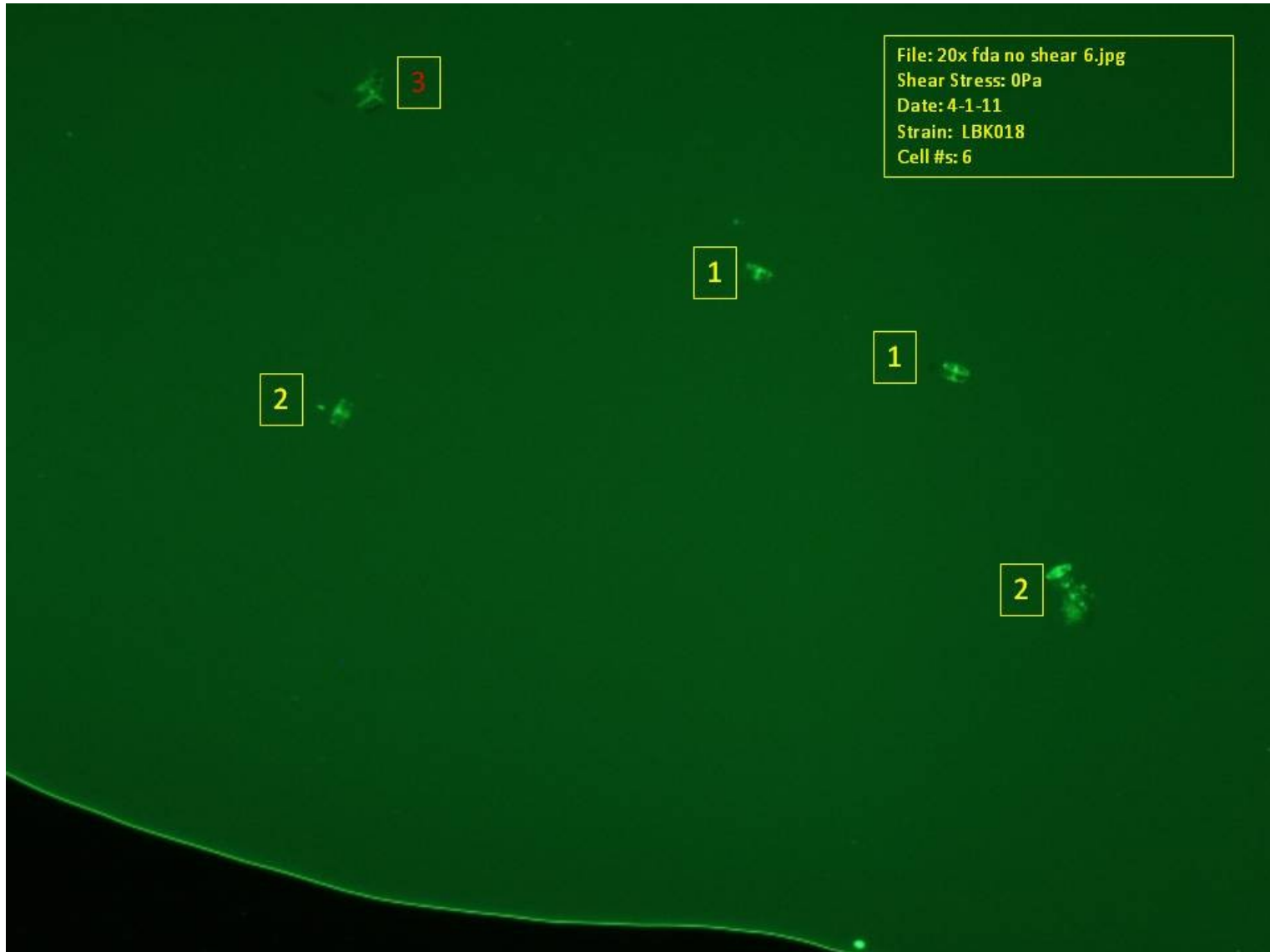




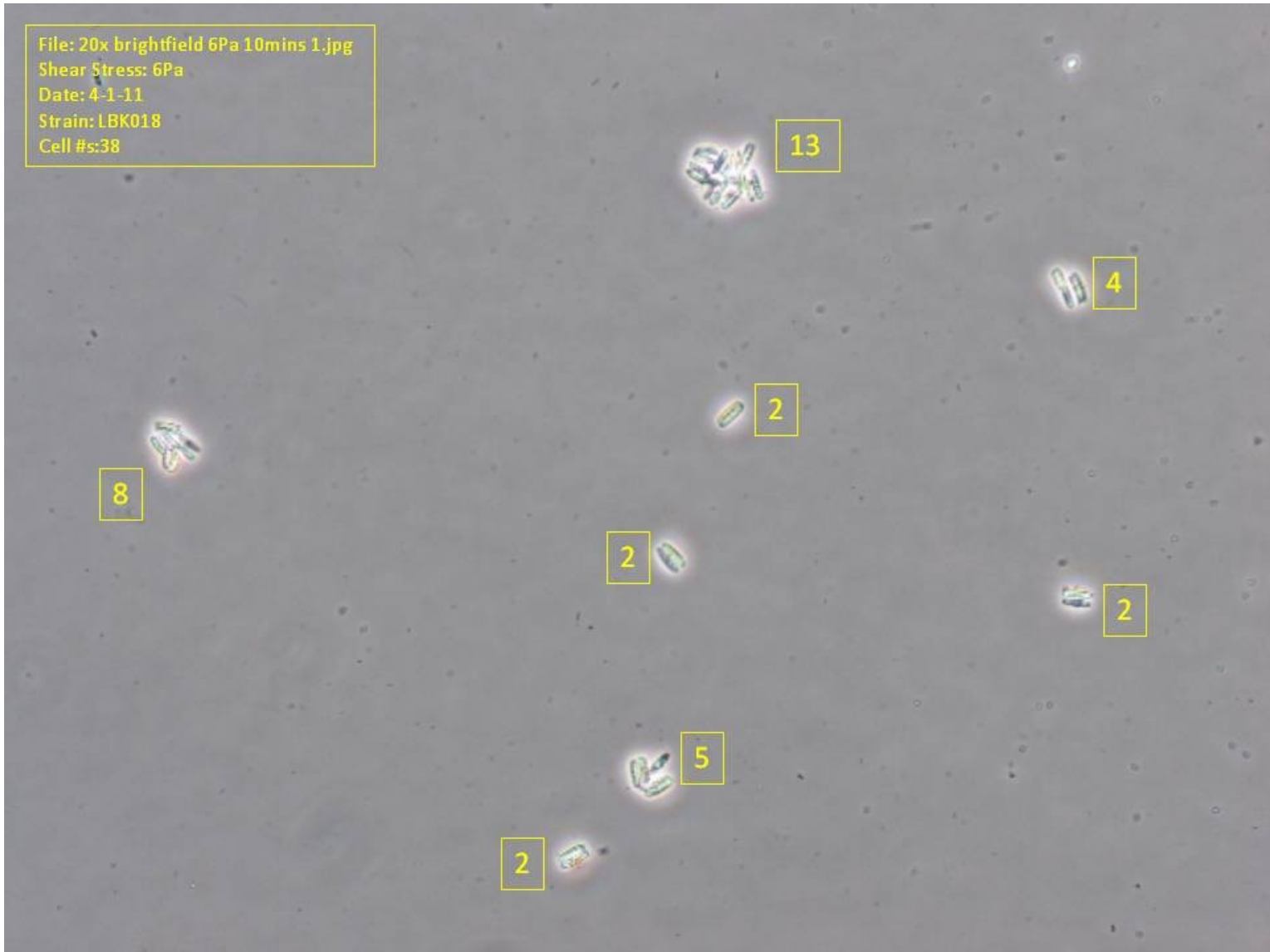








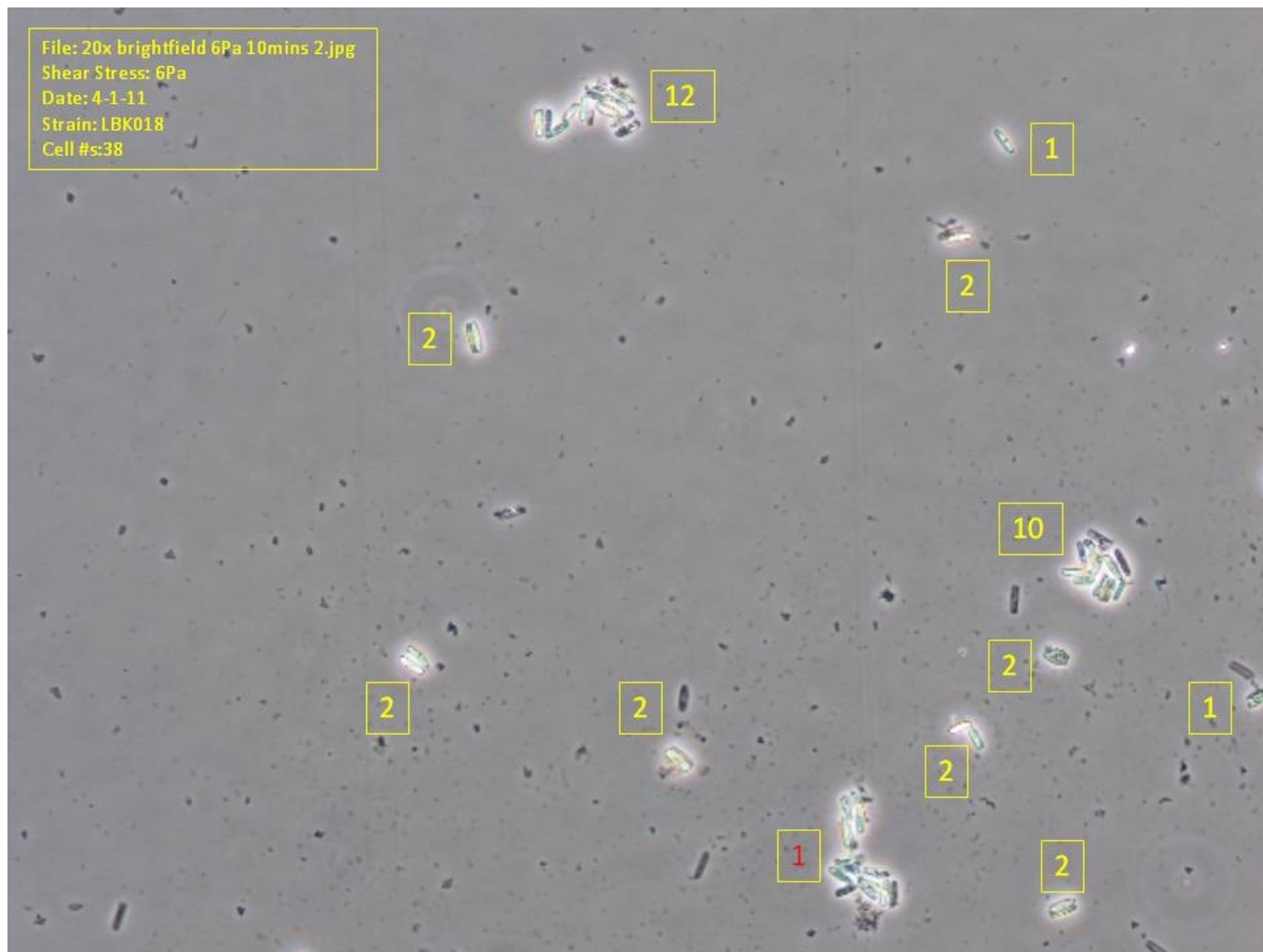
File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:38

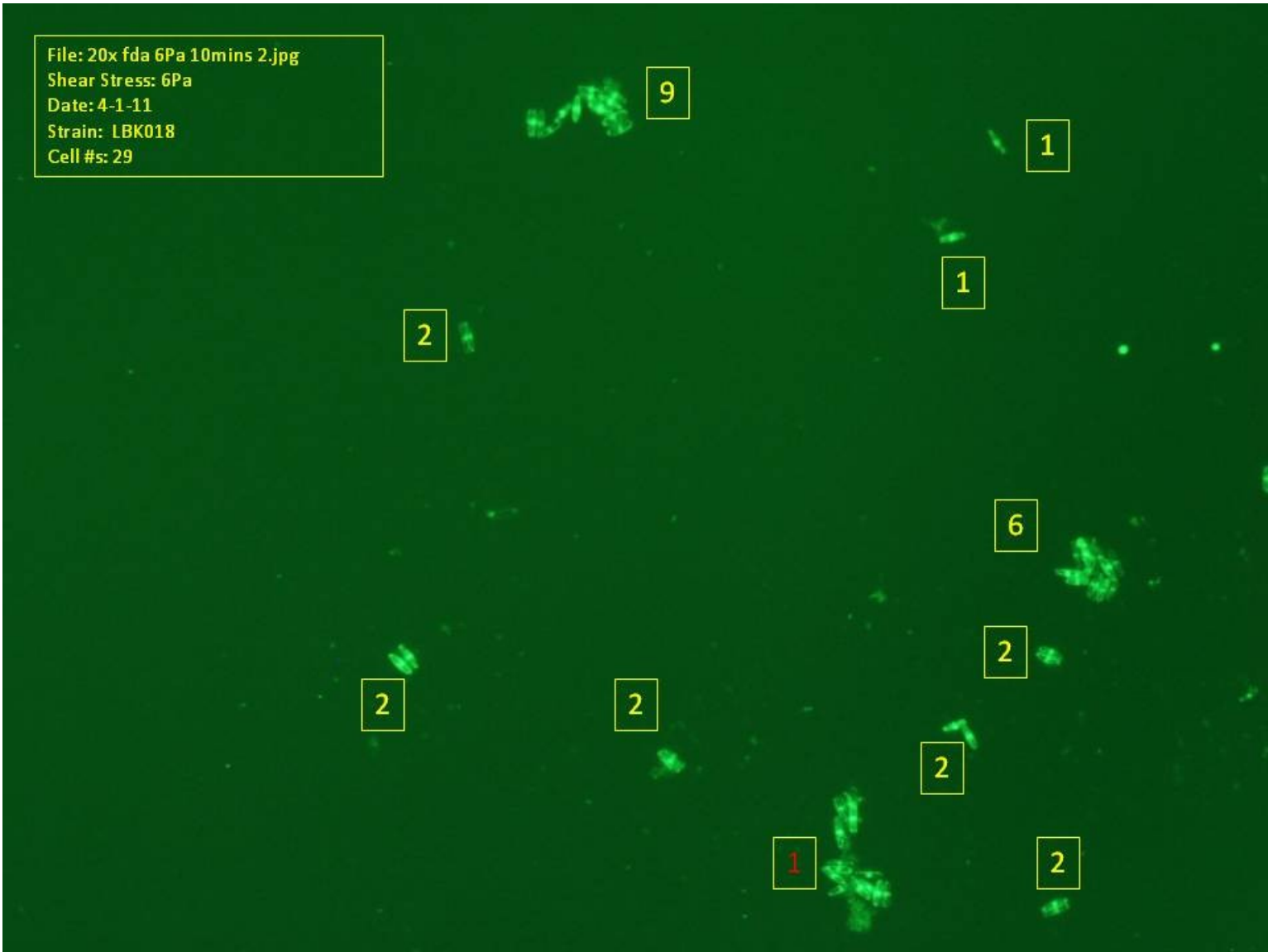


File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 34

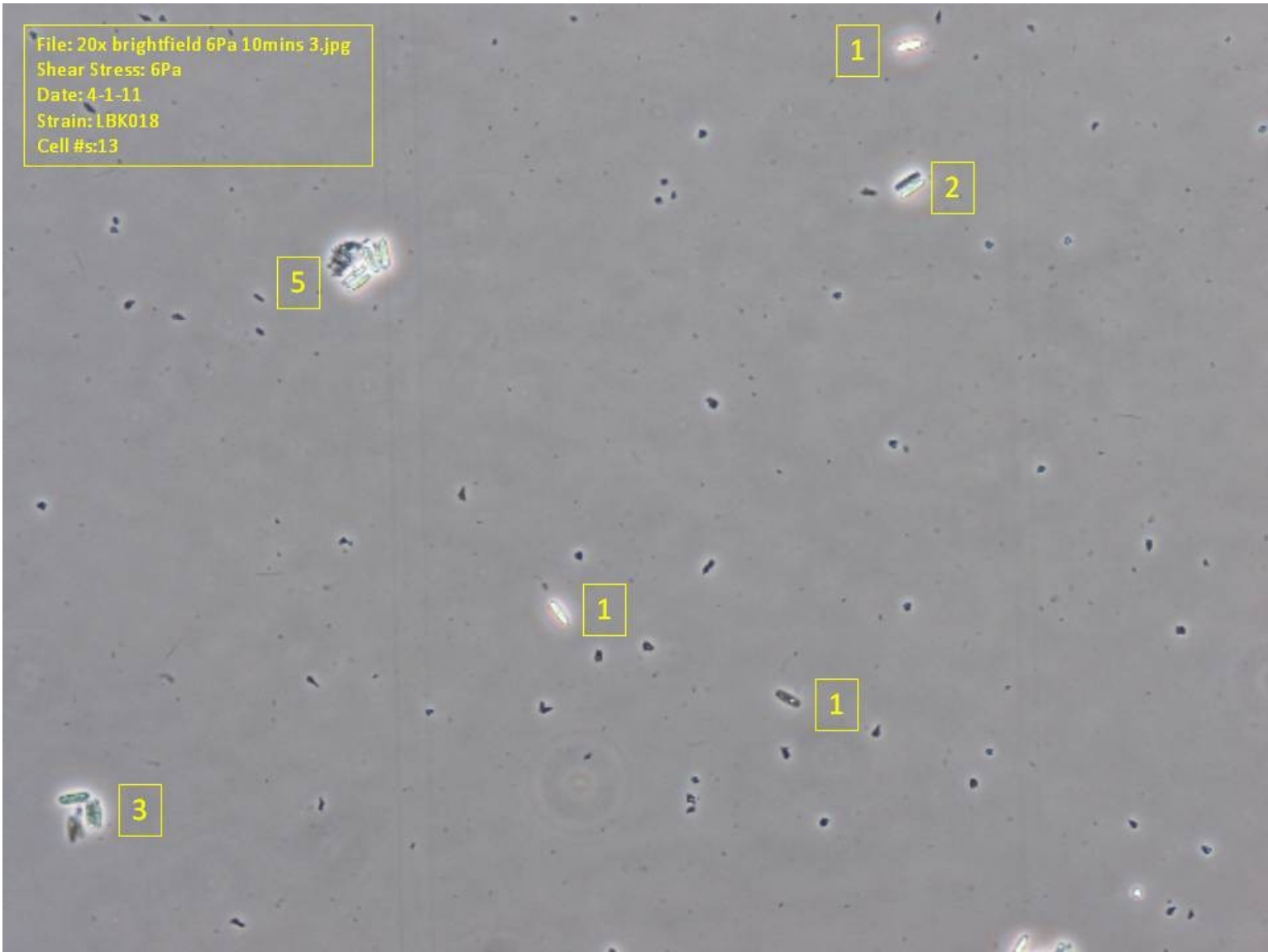


File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:38

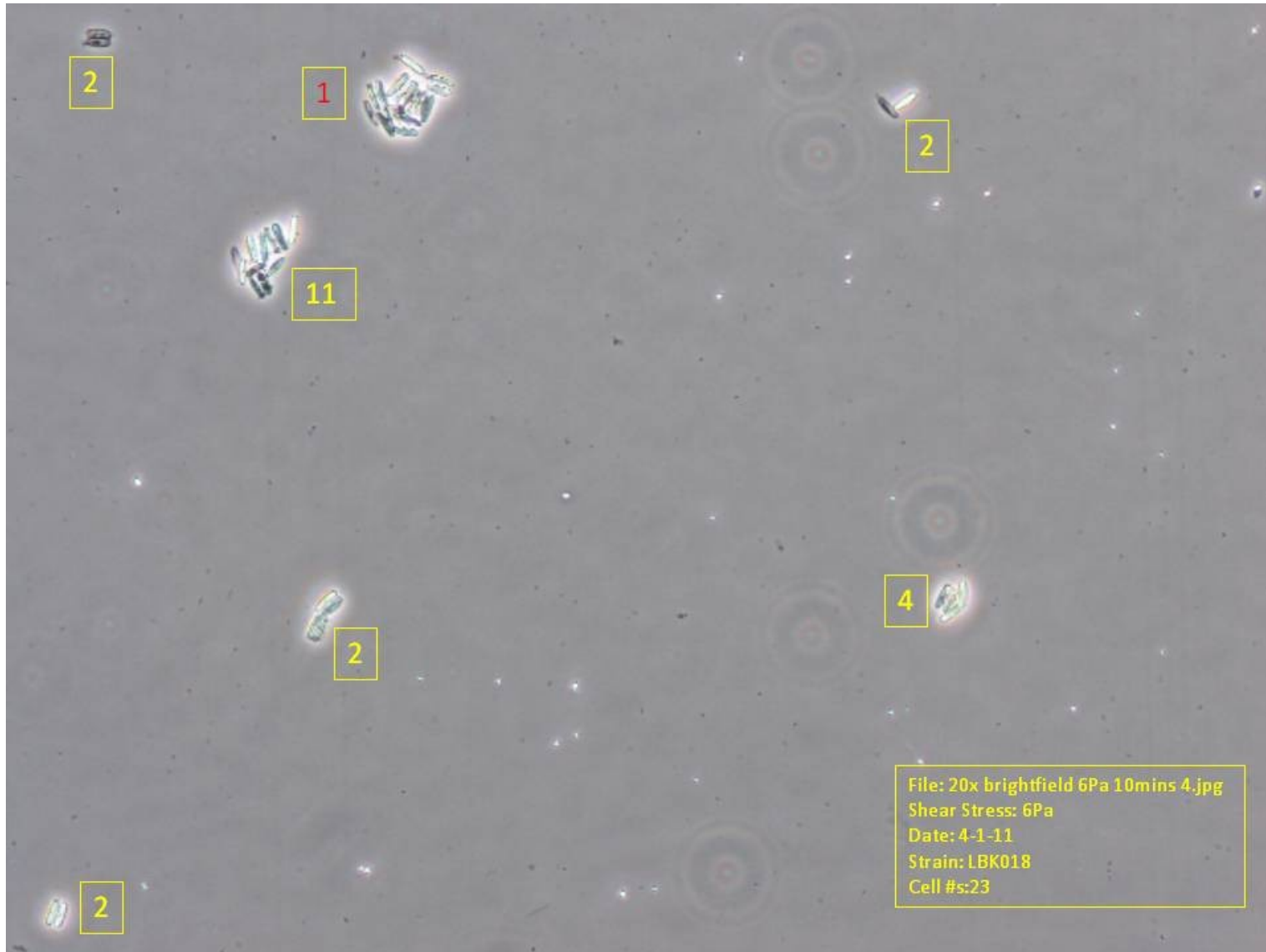






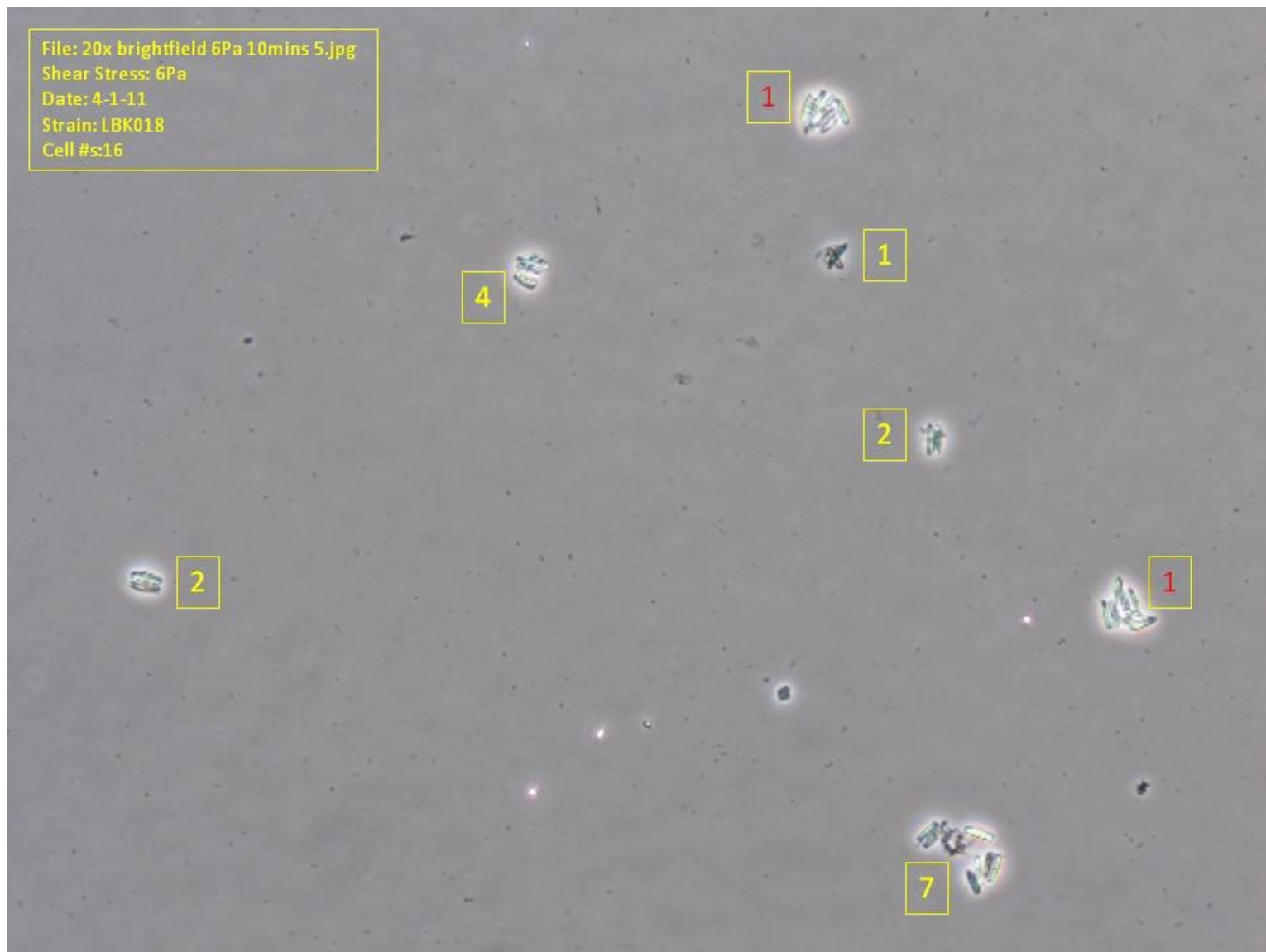




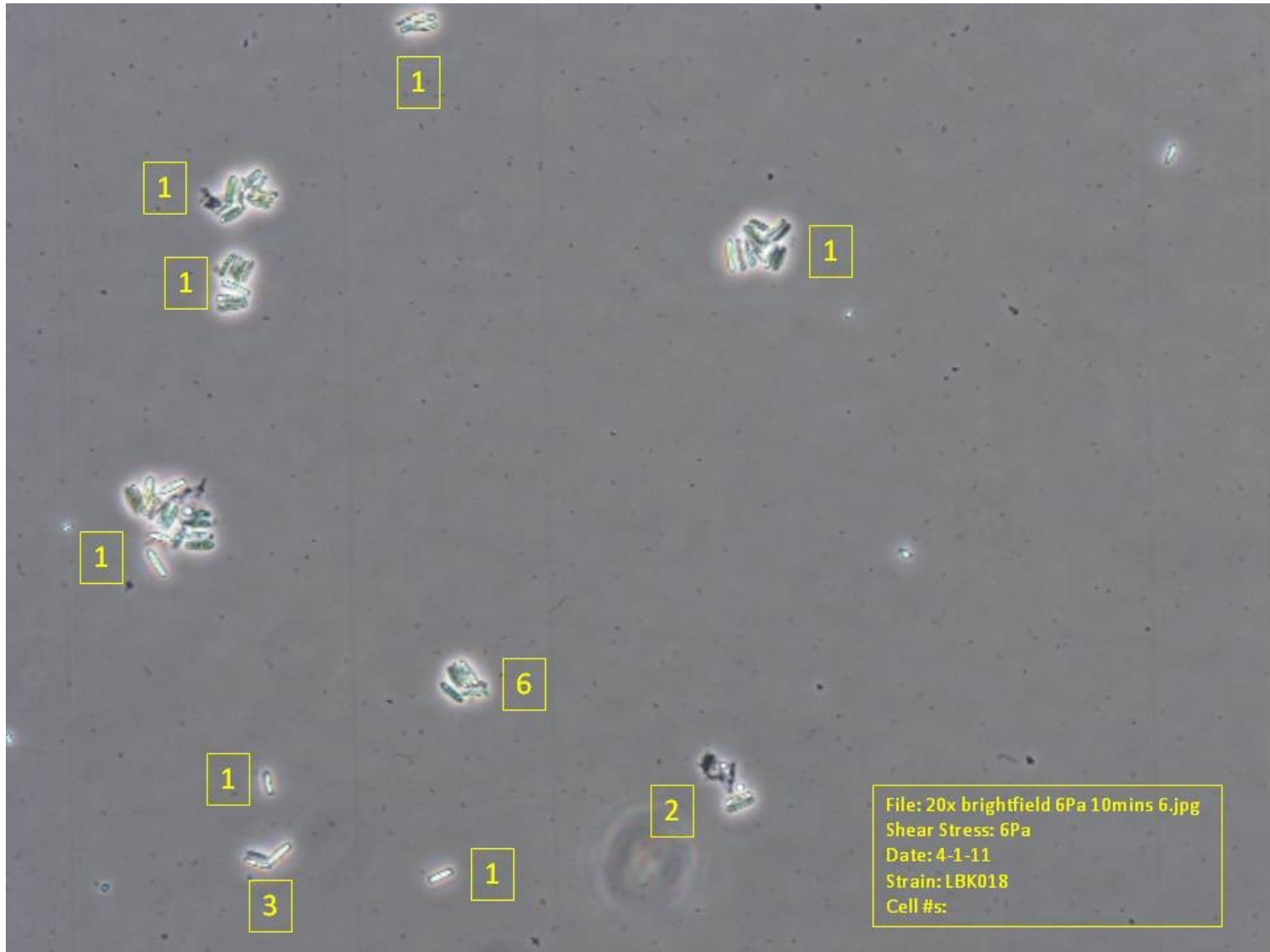


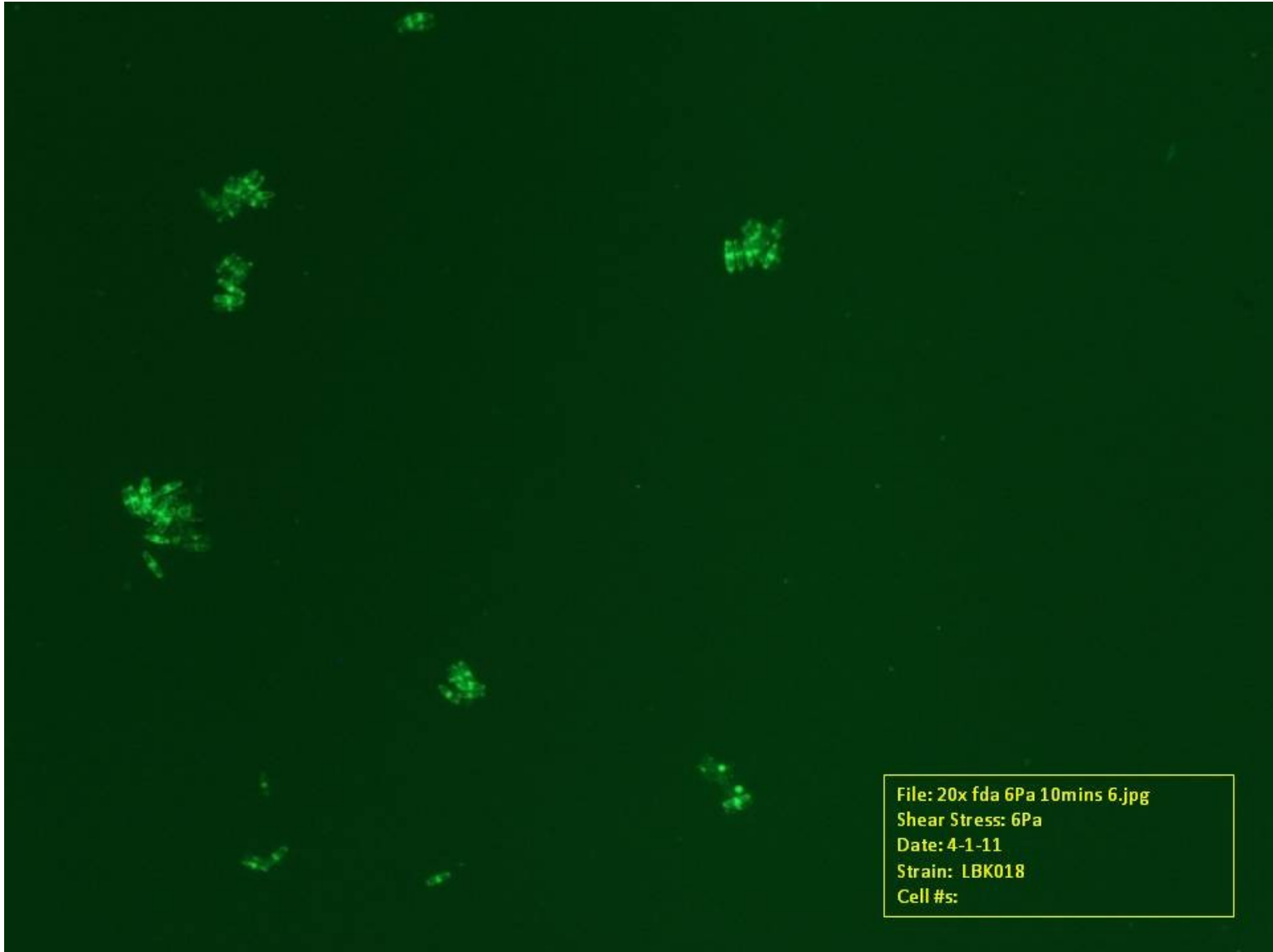


File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:16

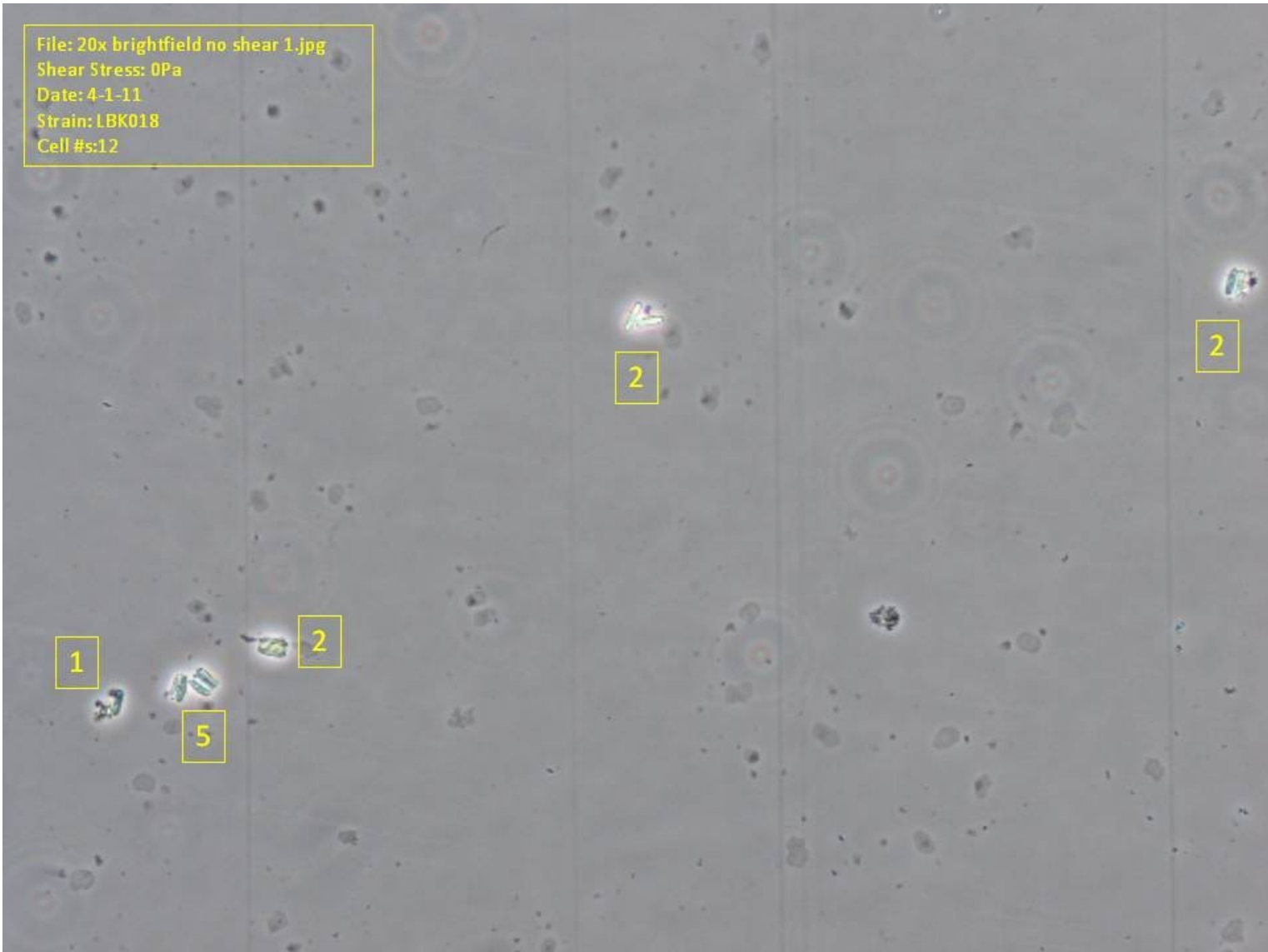






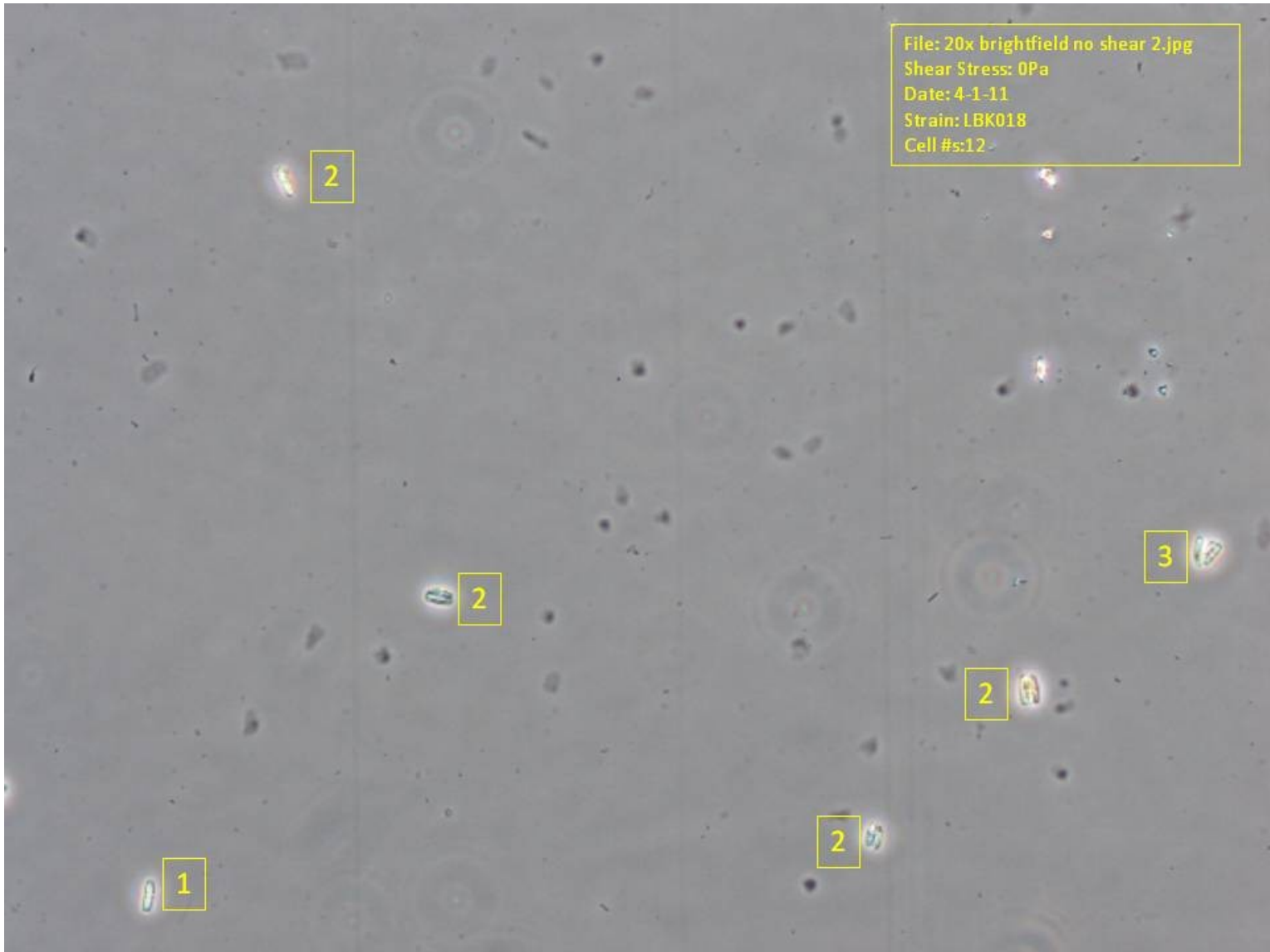




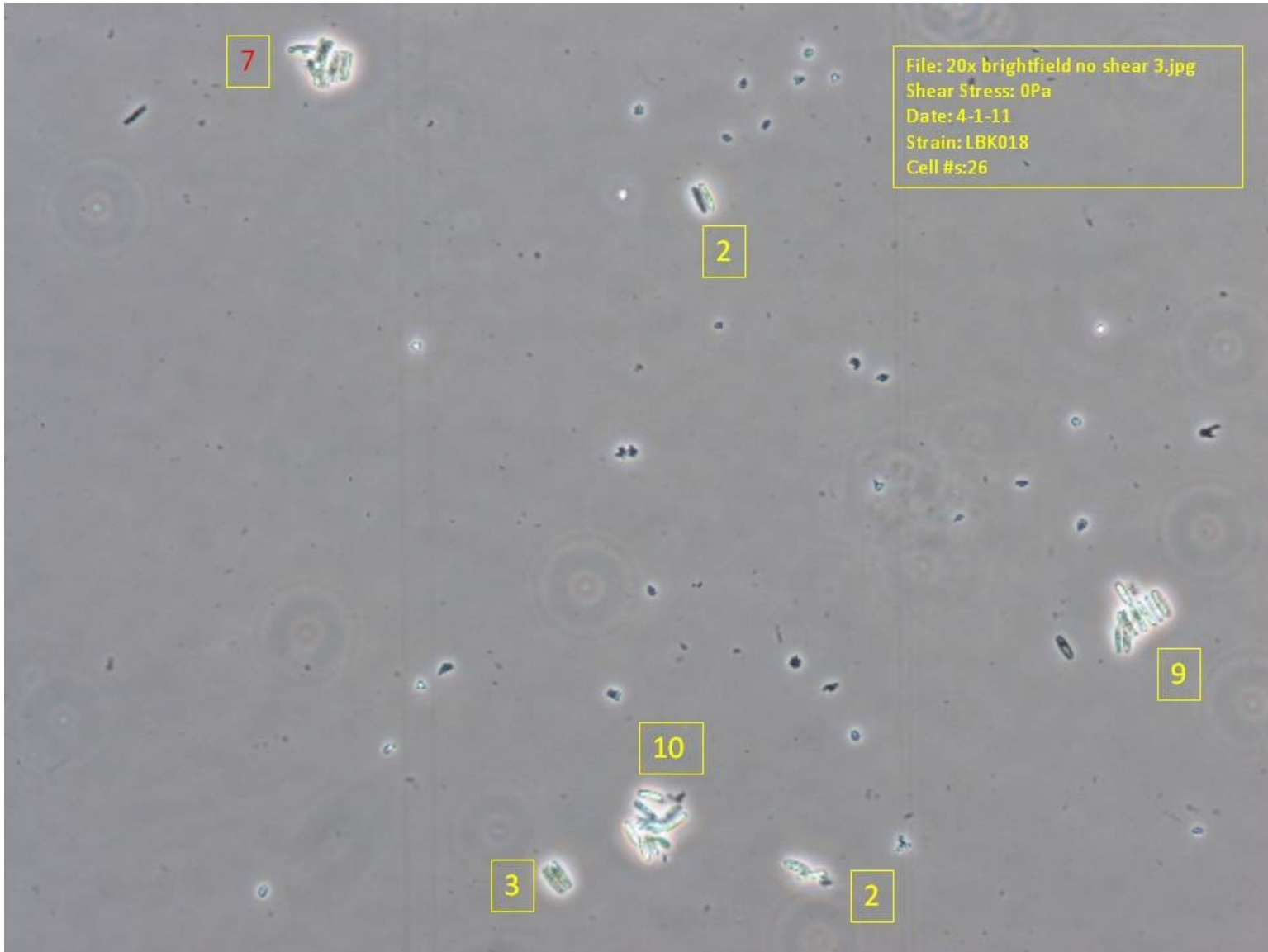


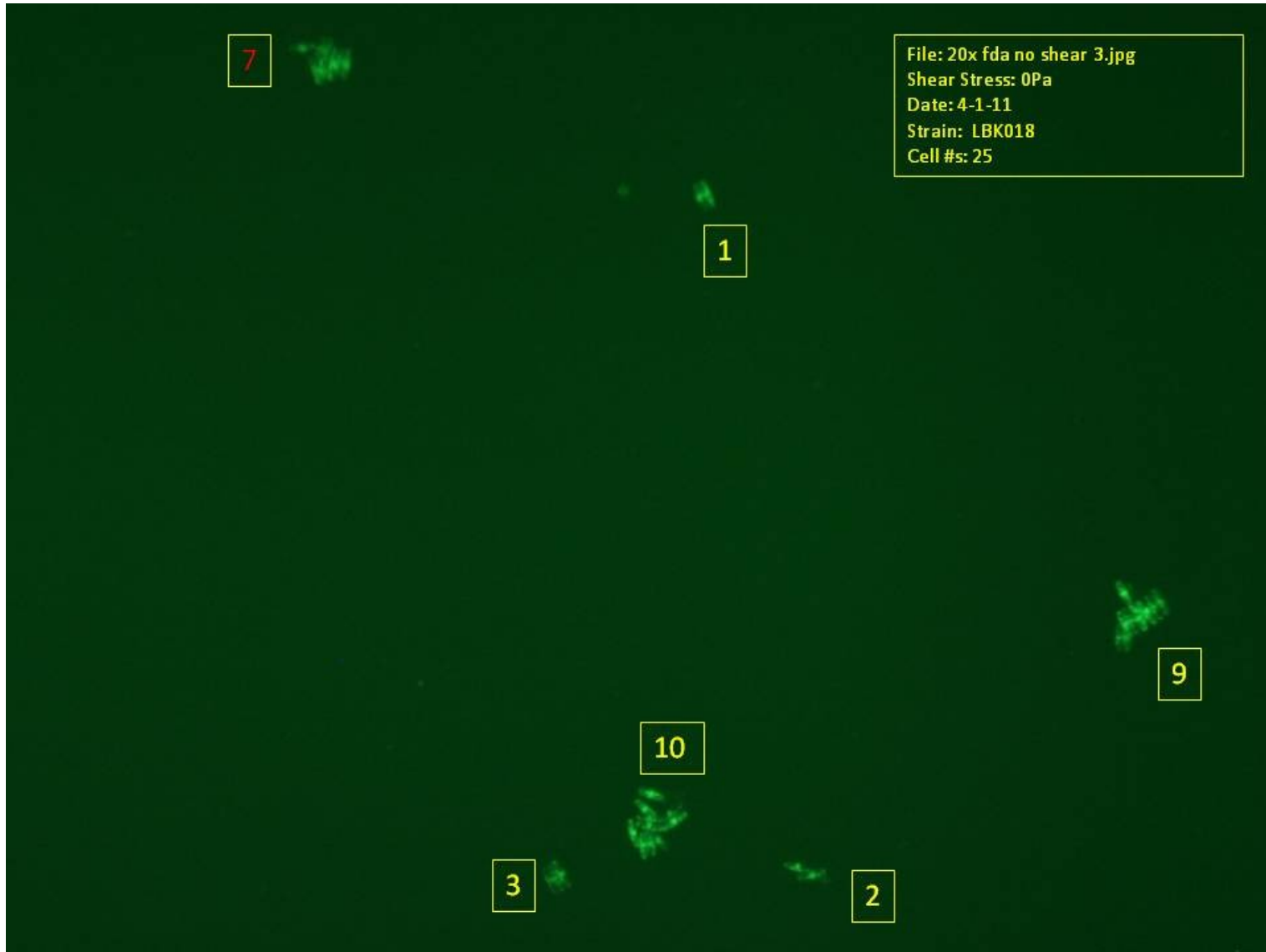
File: 20x fda no shear 1.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 11



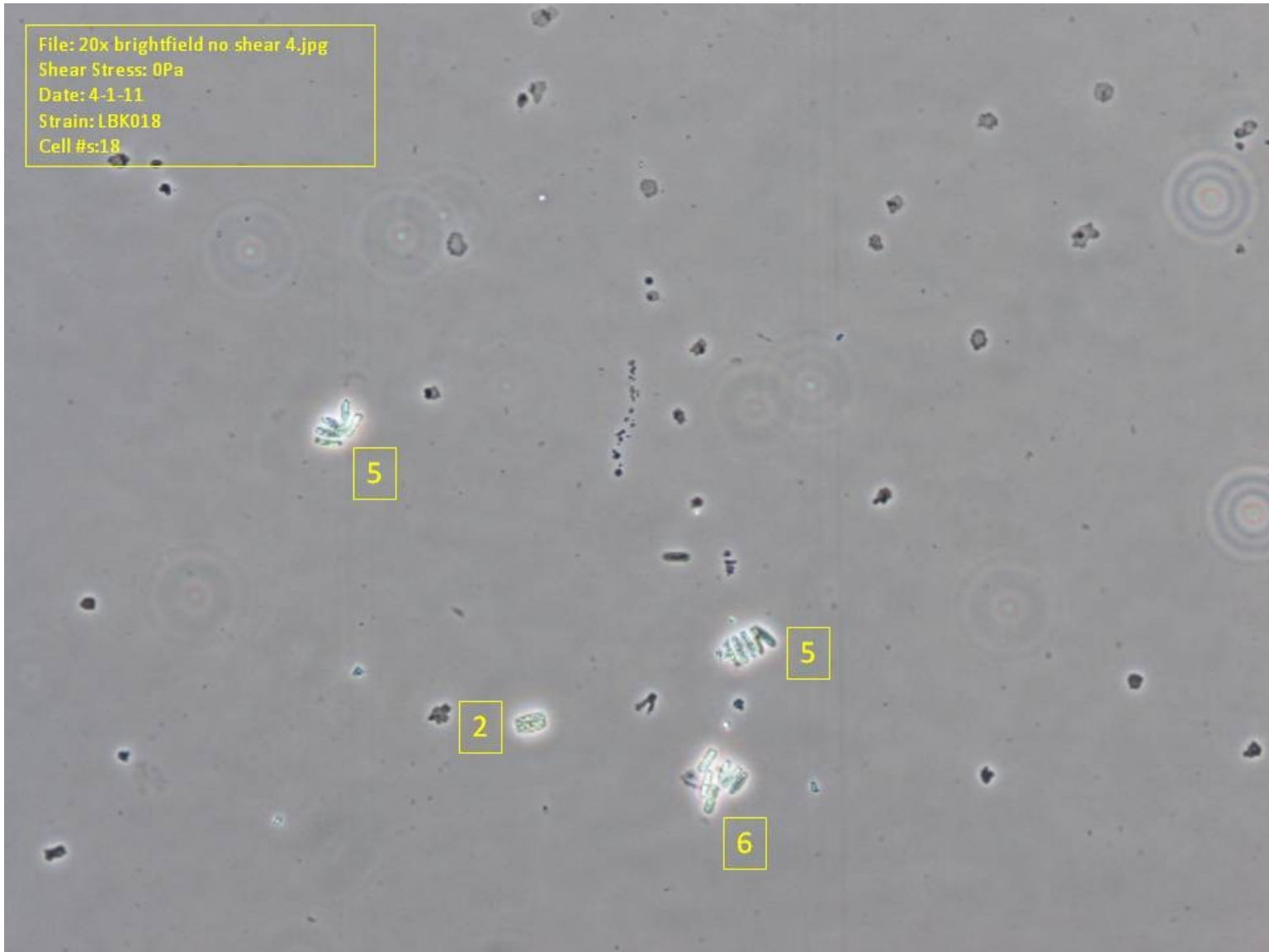








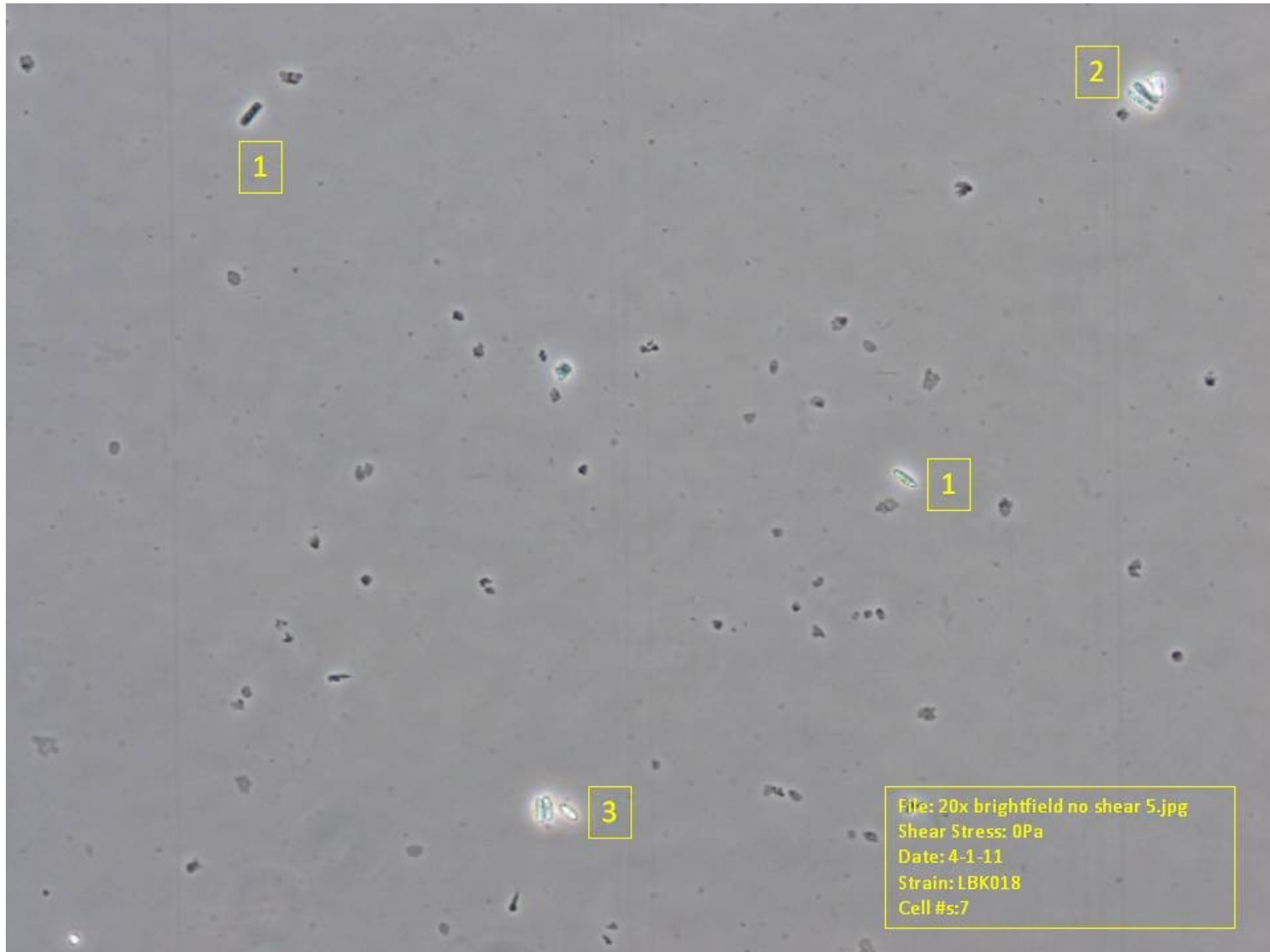
File: 20x brightfield no shear 4.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s:18



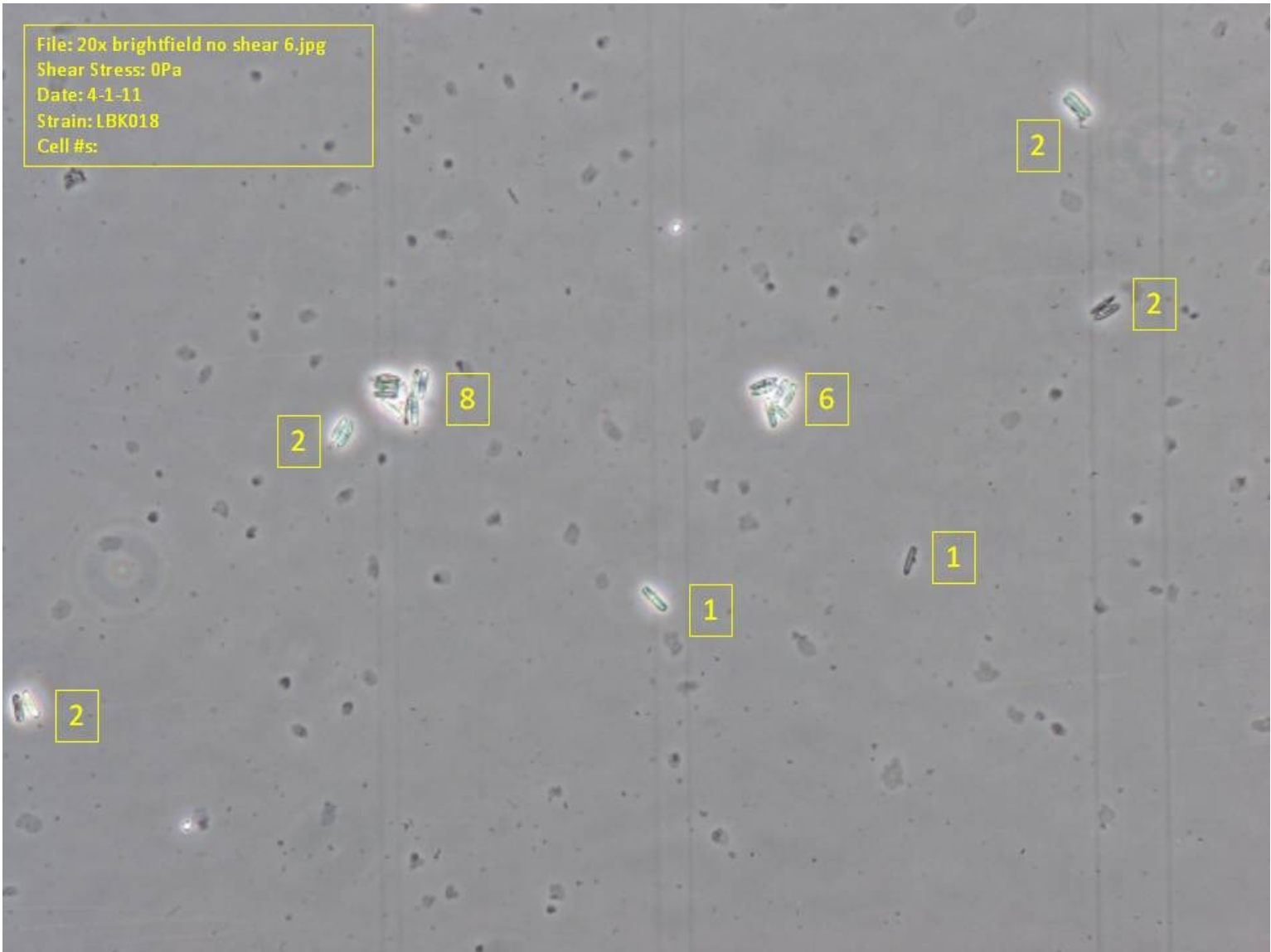
File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 15



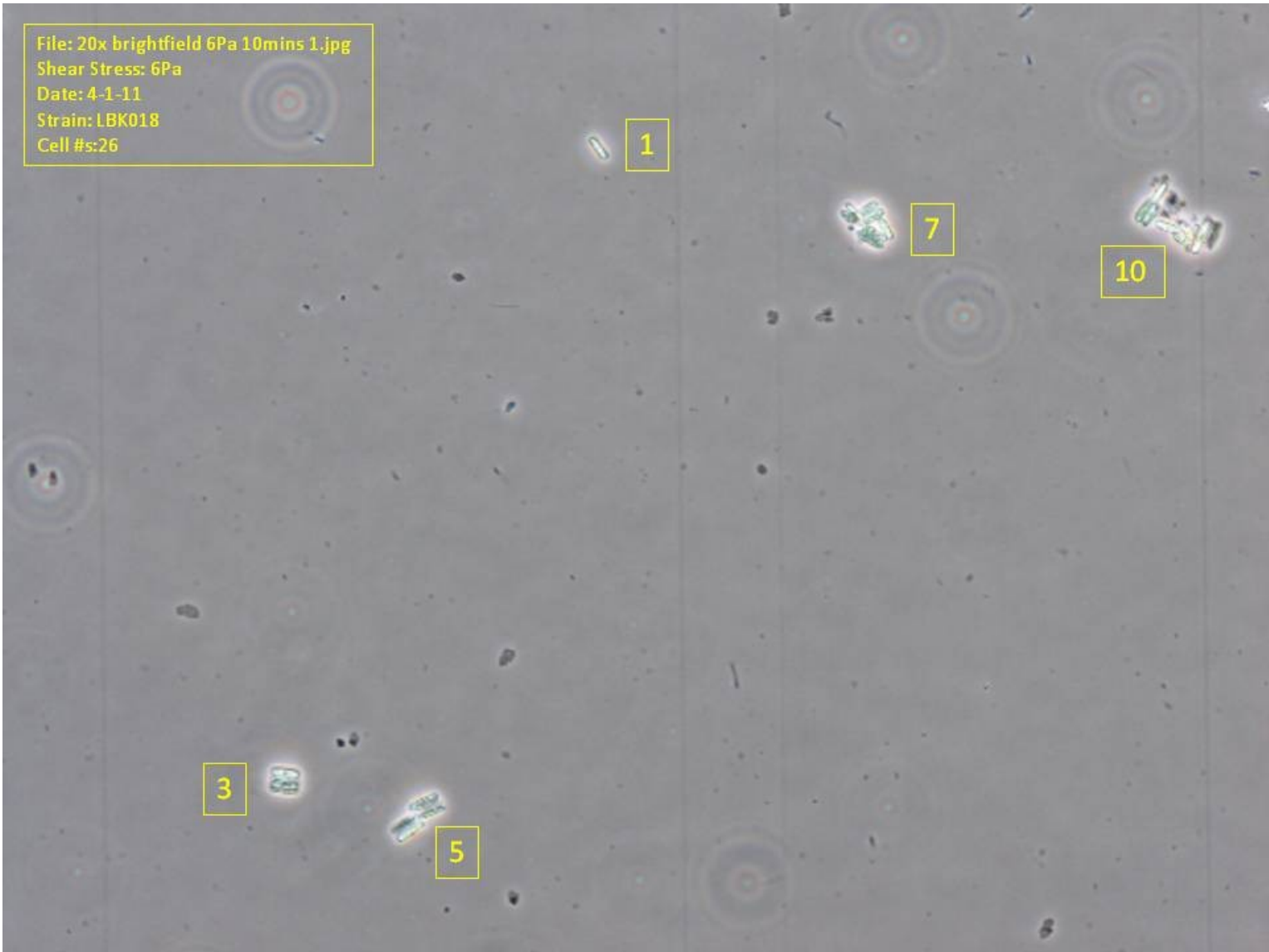




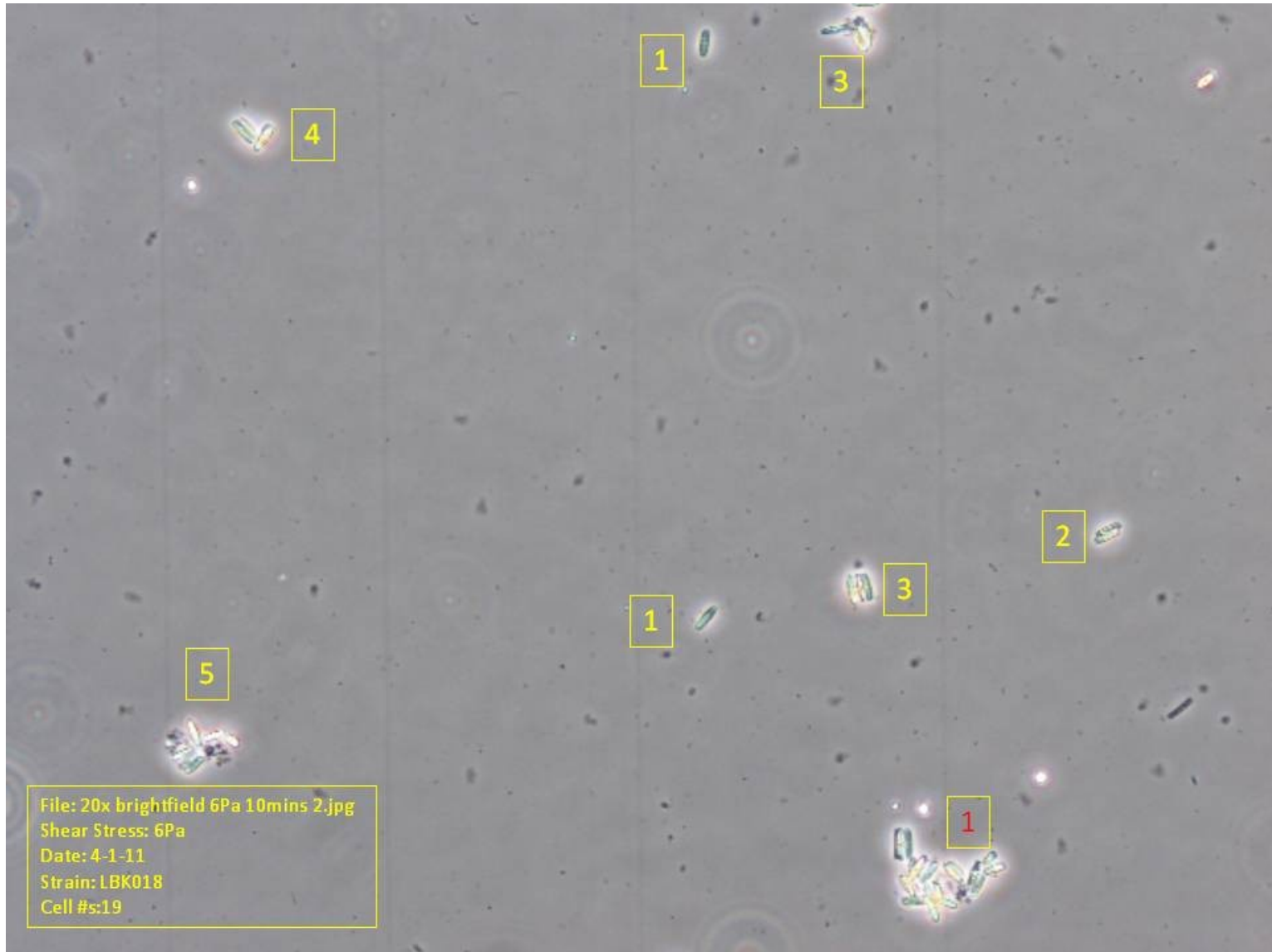






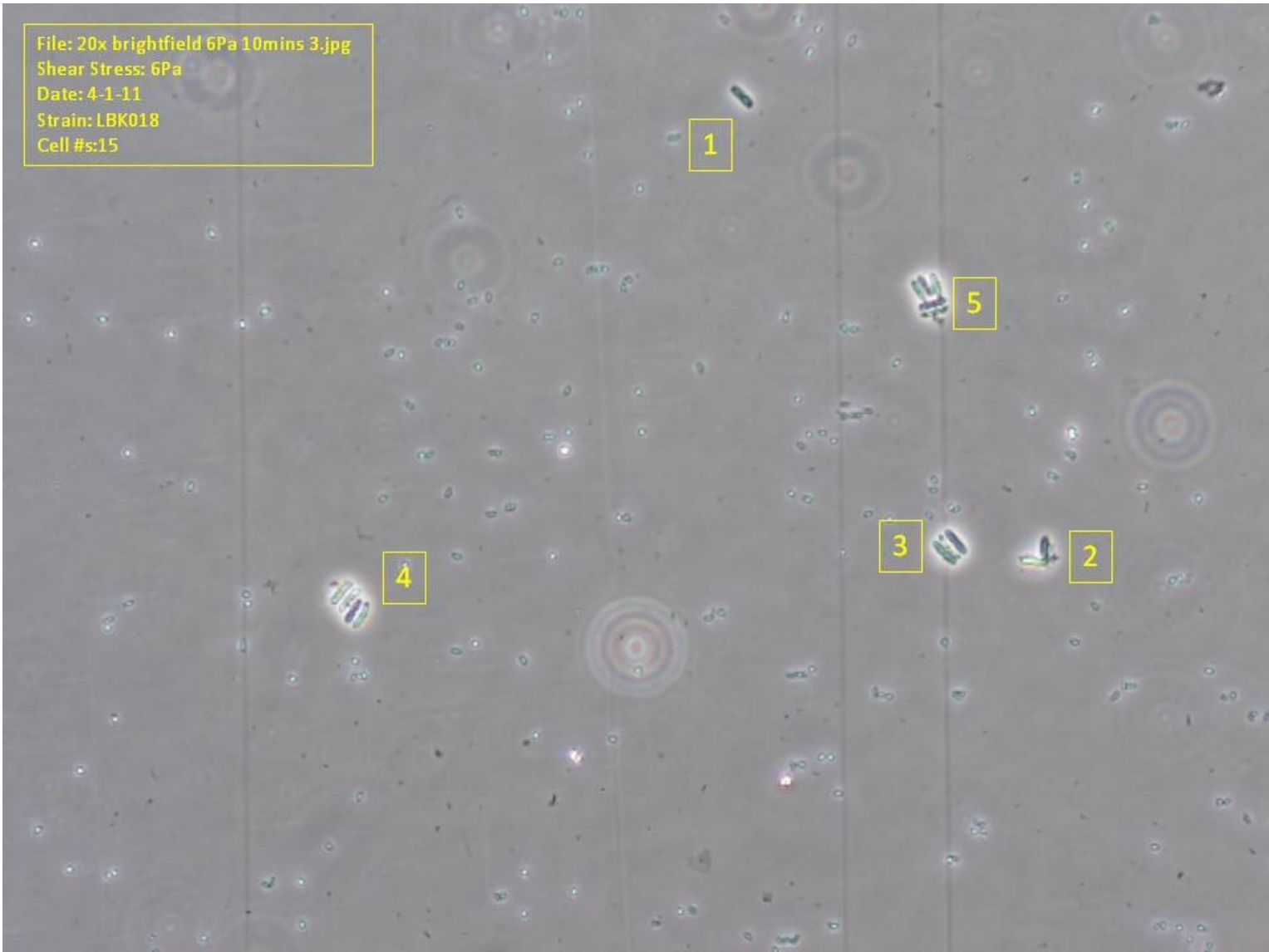






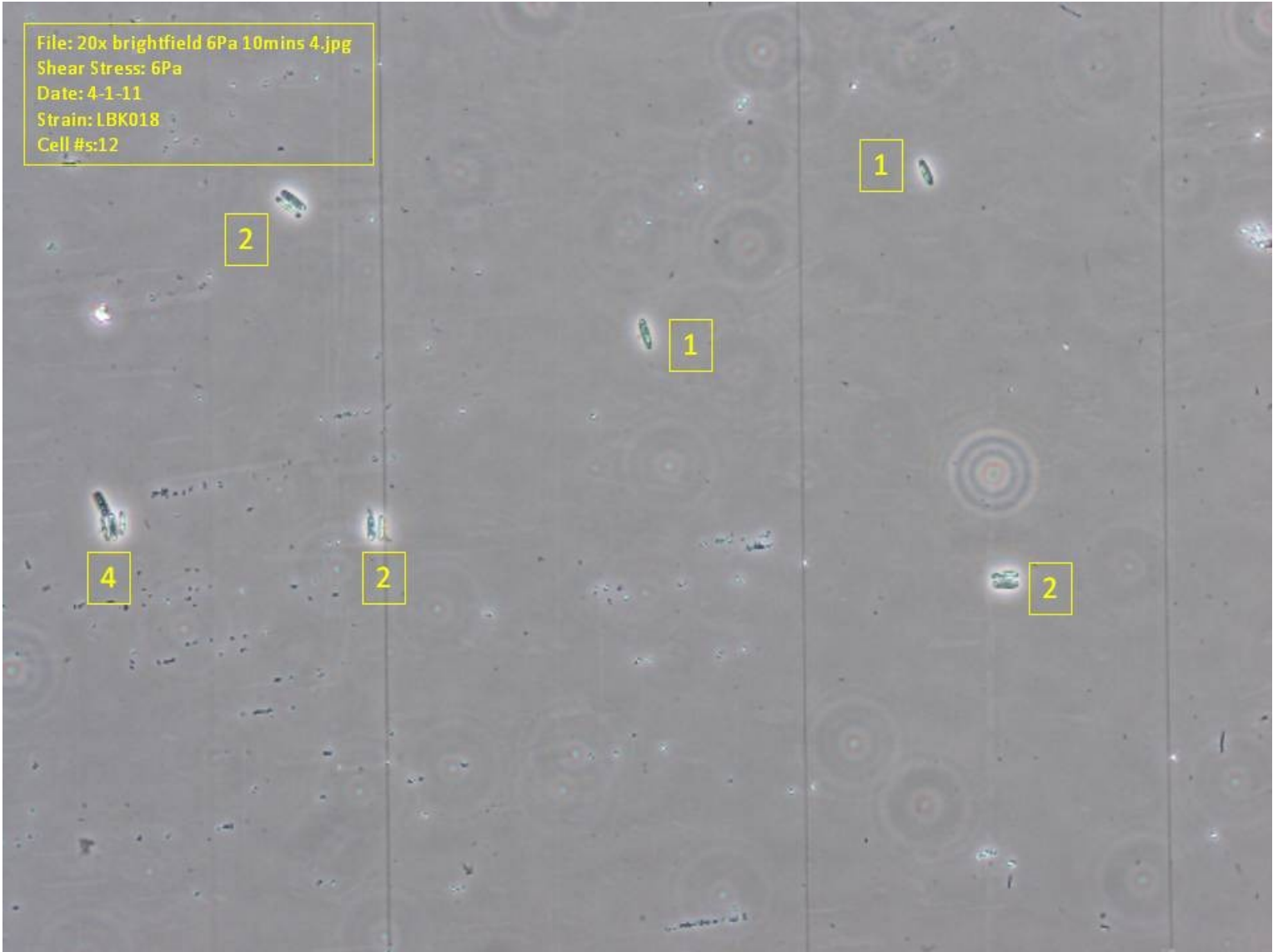






File: 20x fda 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 8





File: 20x fda 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 4-1-11  
Strain: LBK018  
Cell #s: 5

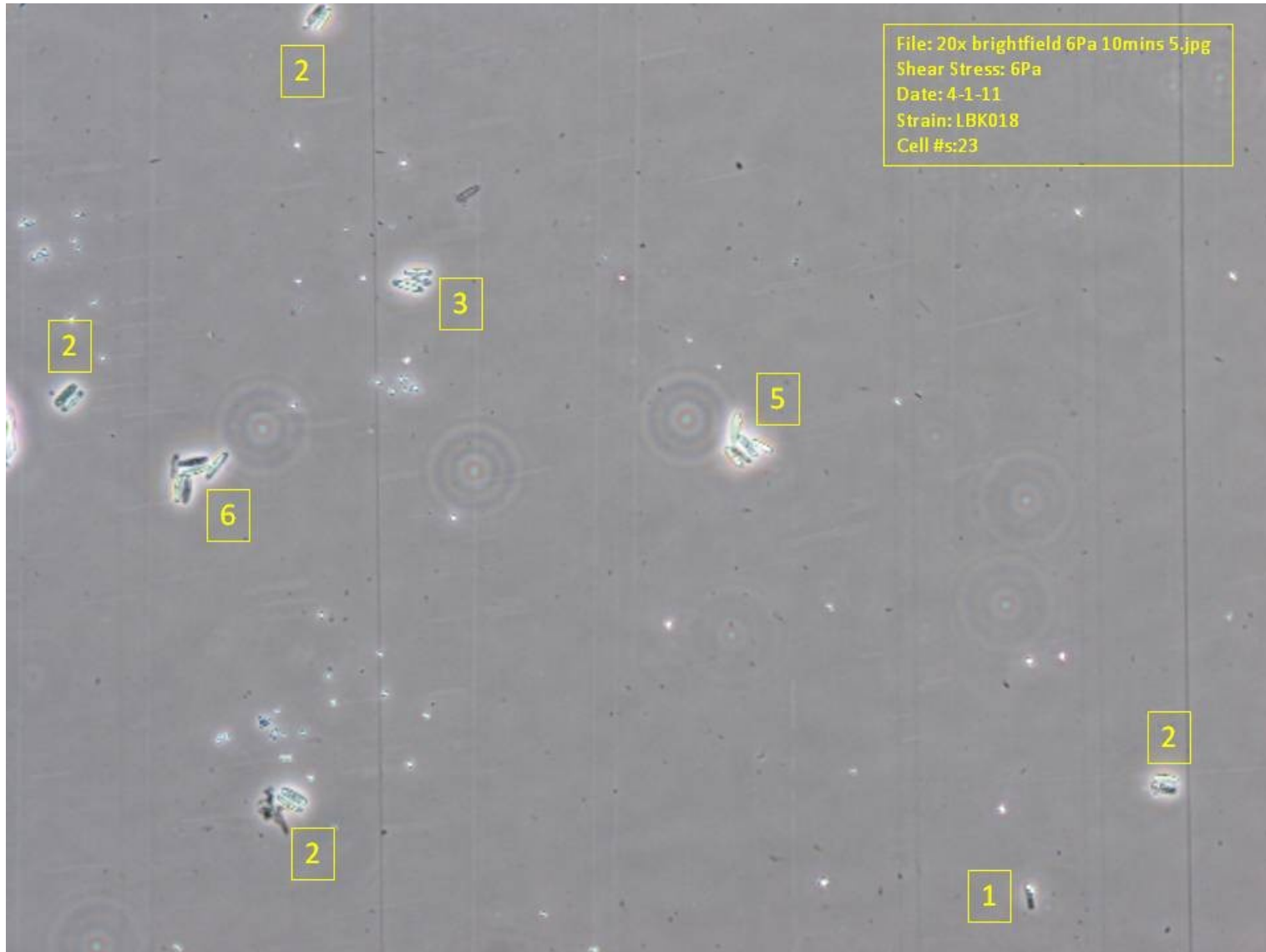
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1

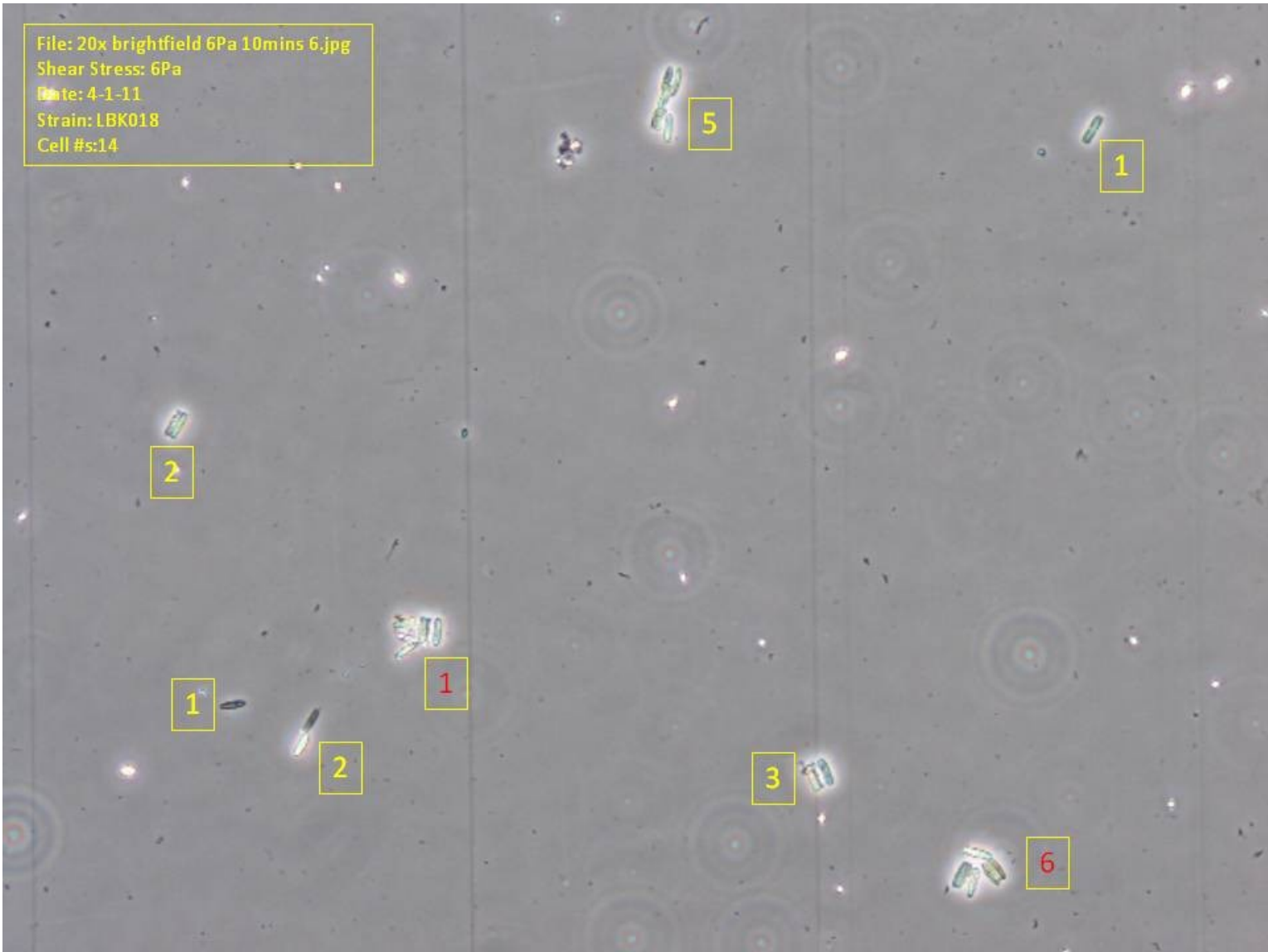
1

1

1

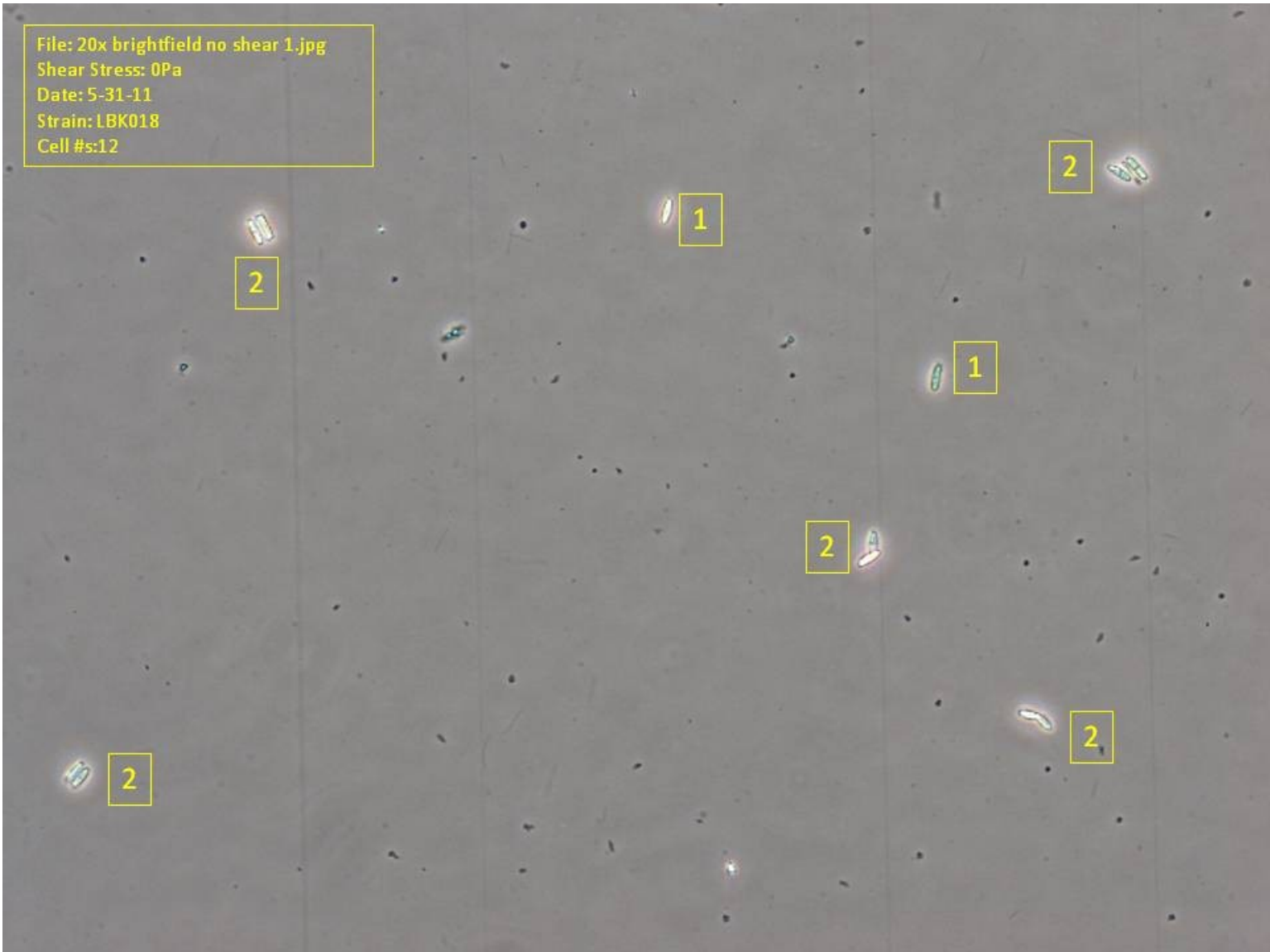










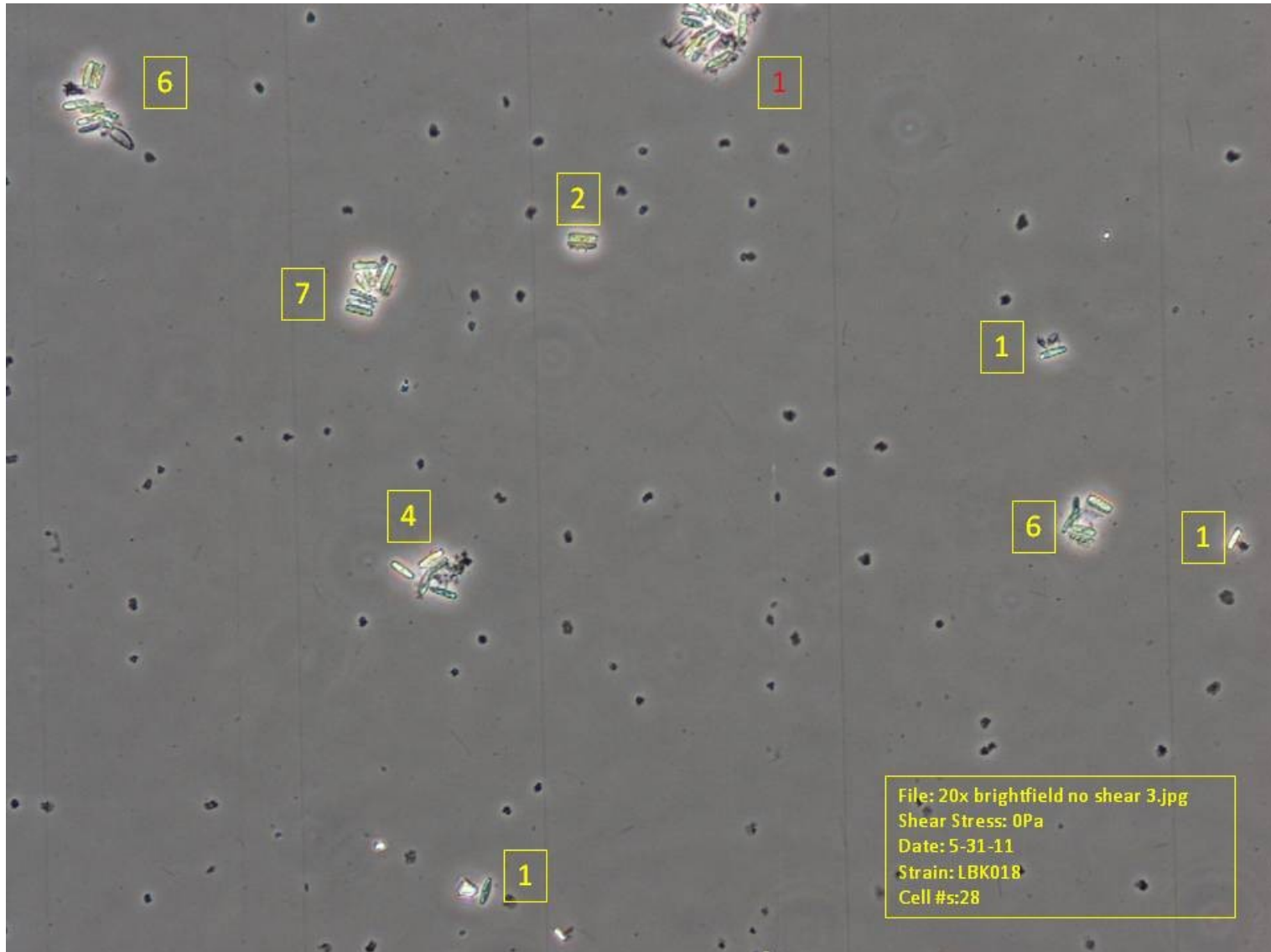




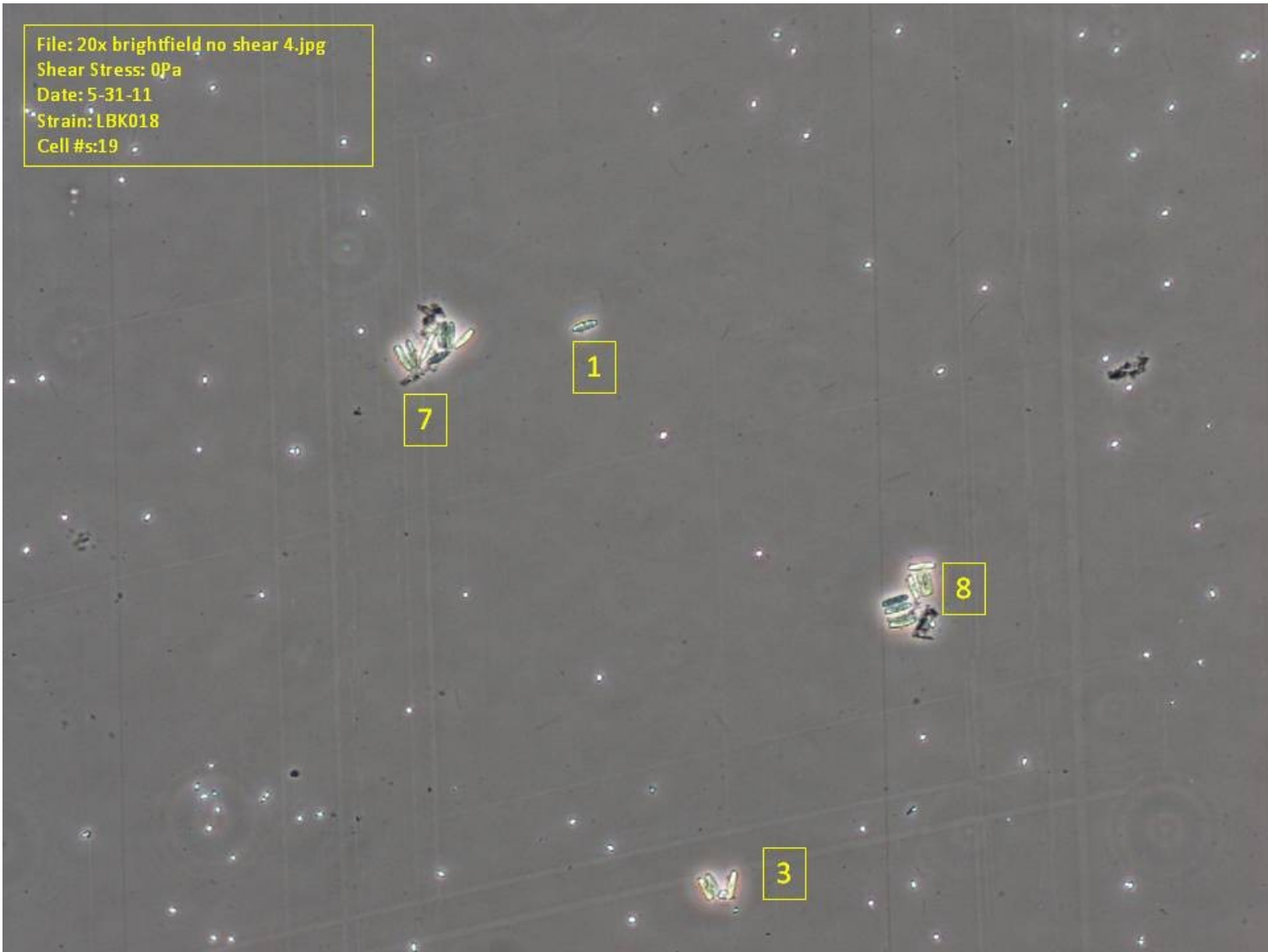
File: 20x brightfield no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK018  
Cell #:s:14











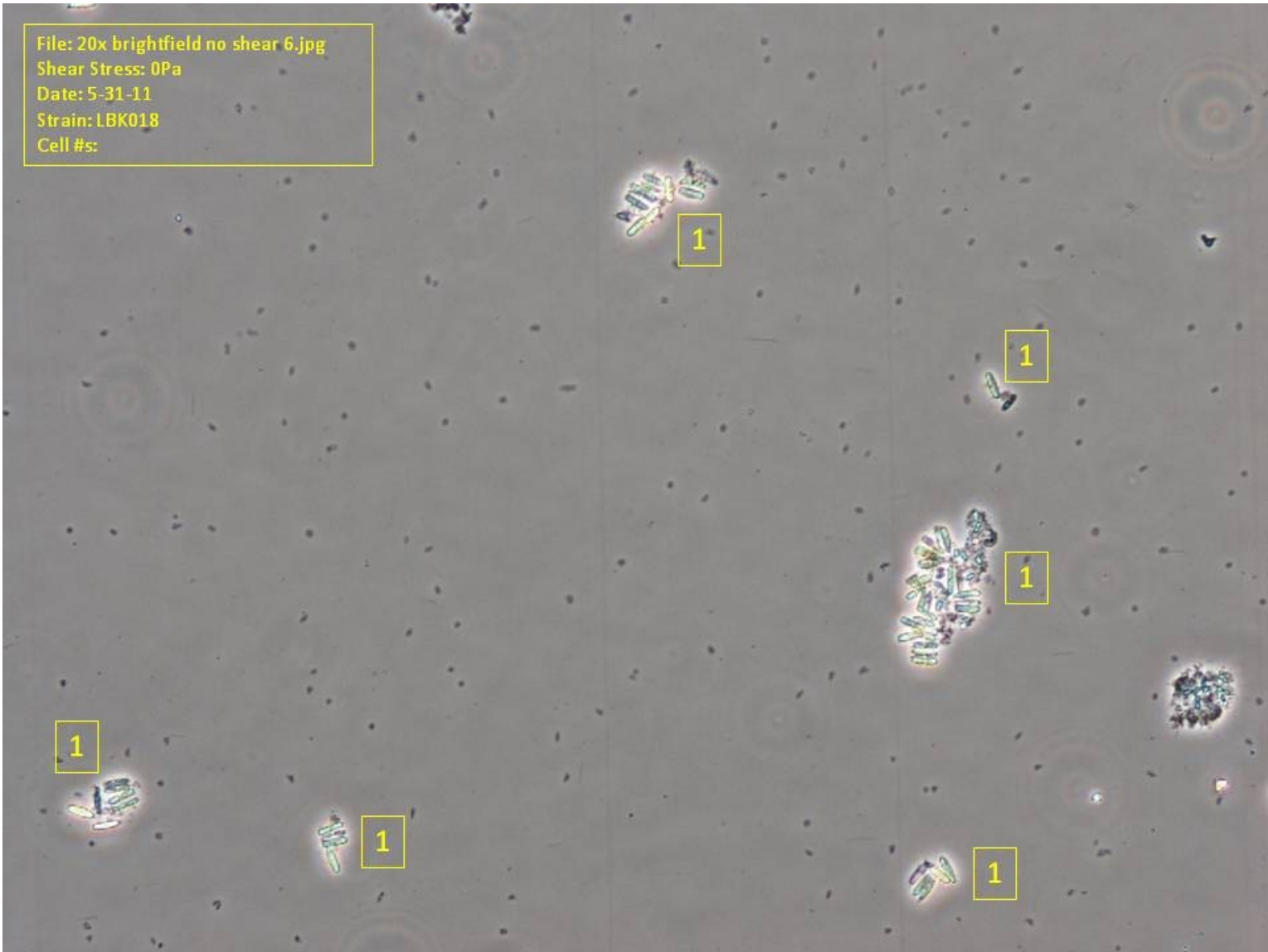
File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK018  
Cell #s:14

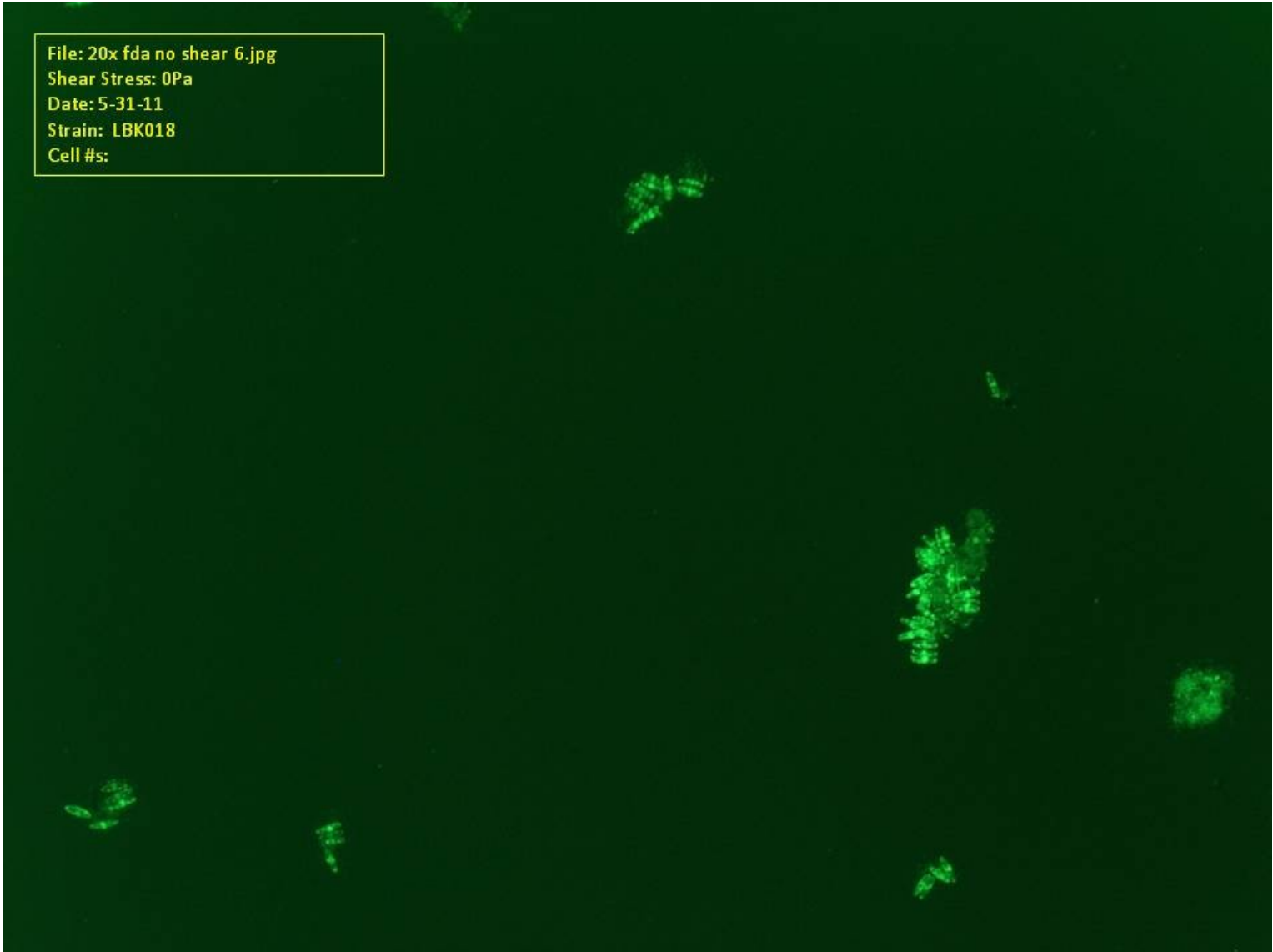


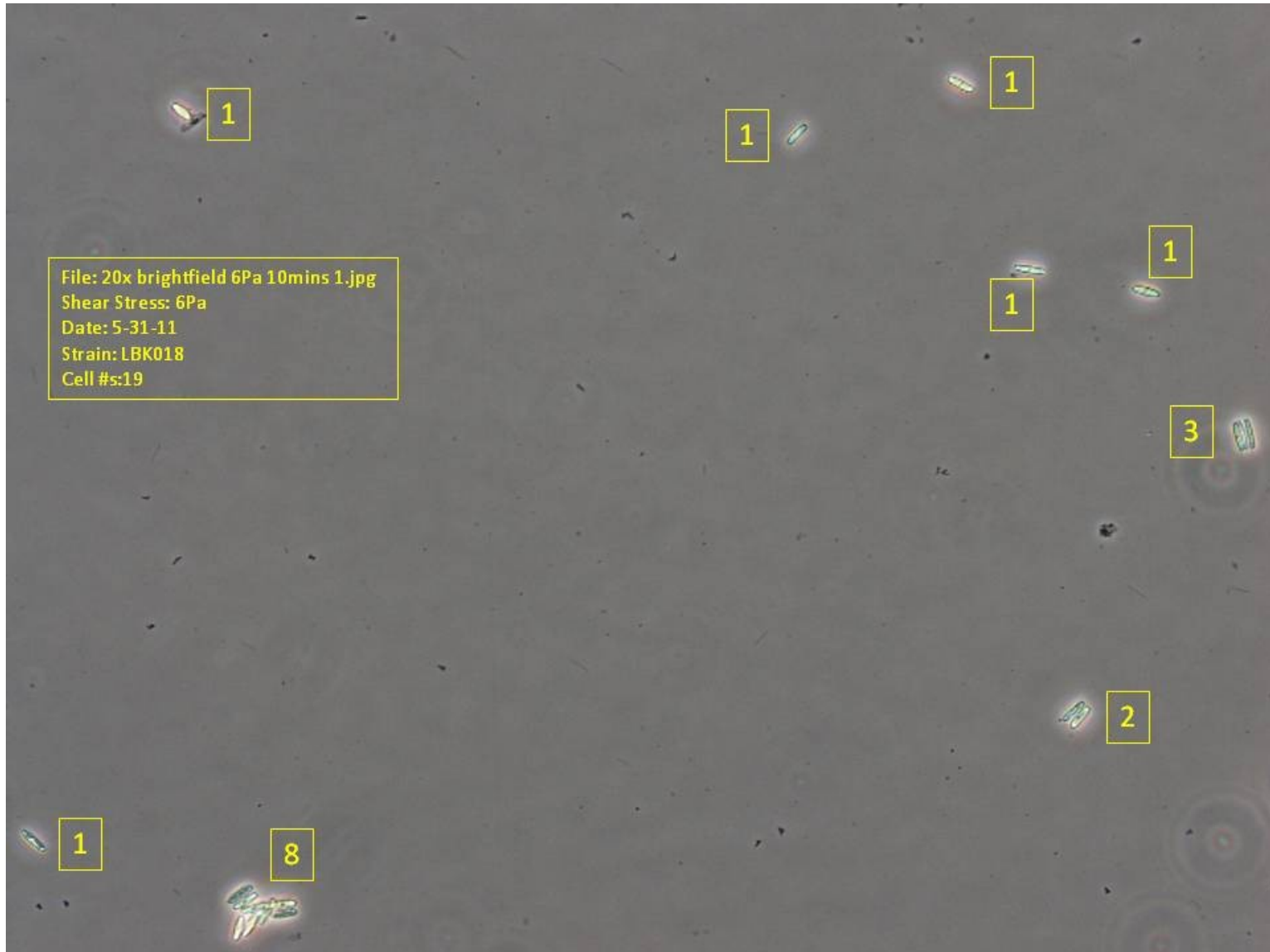


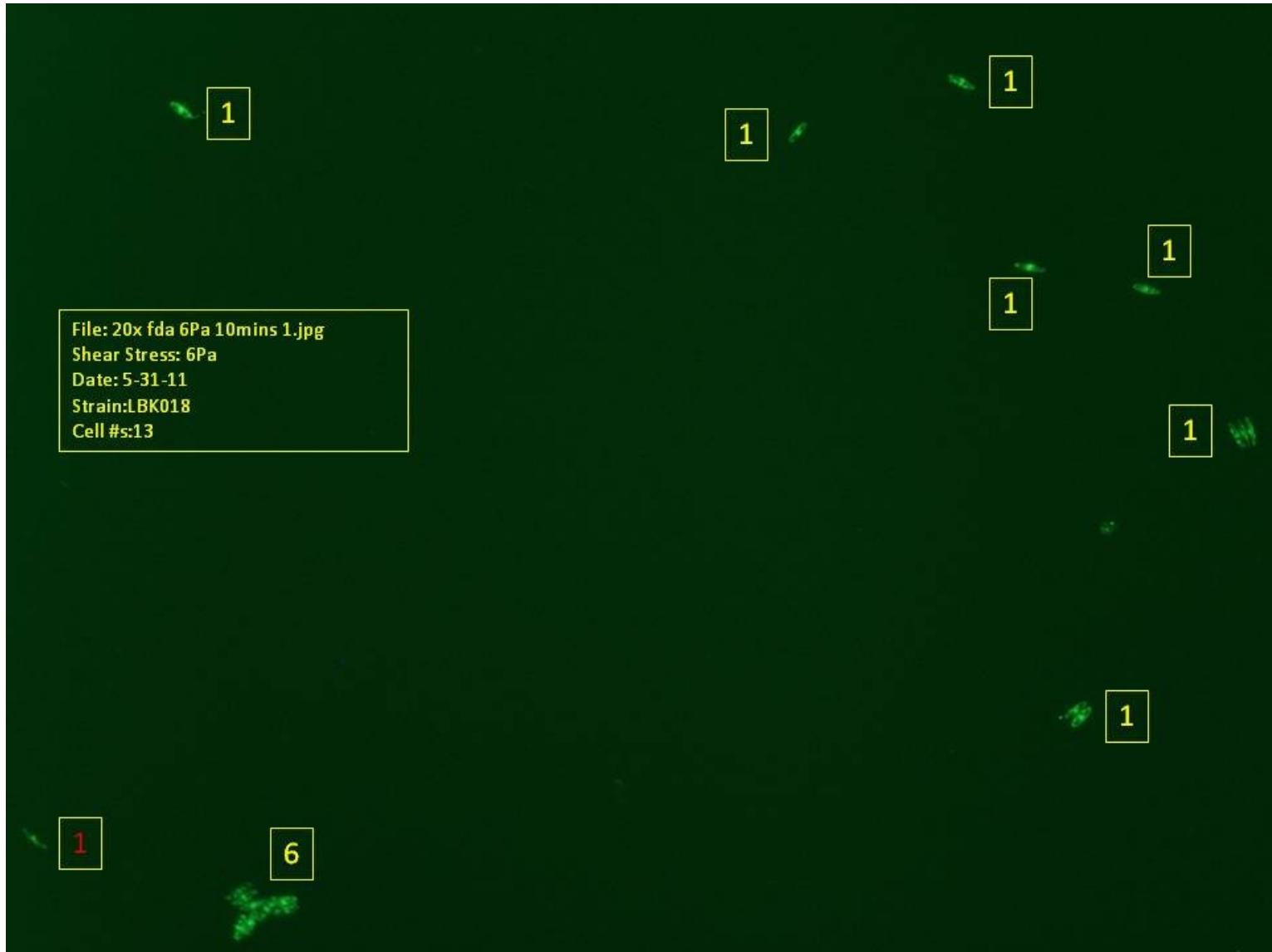




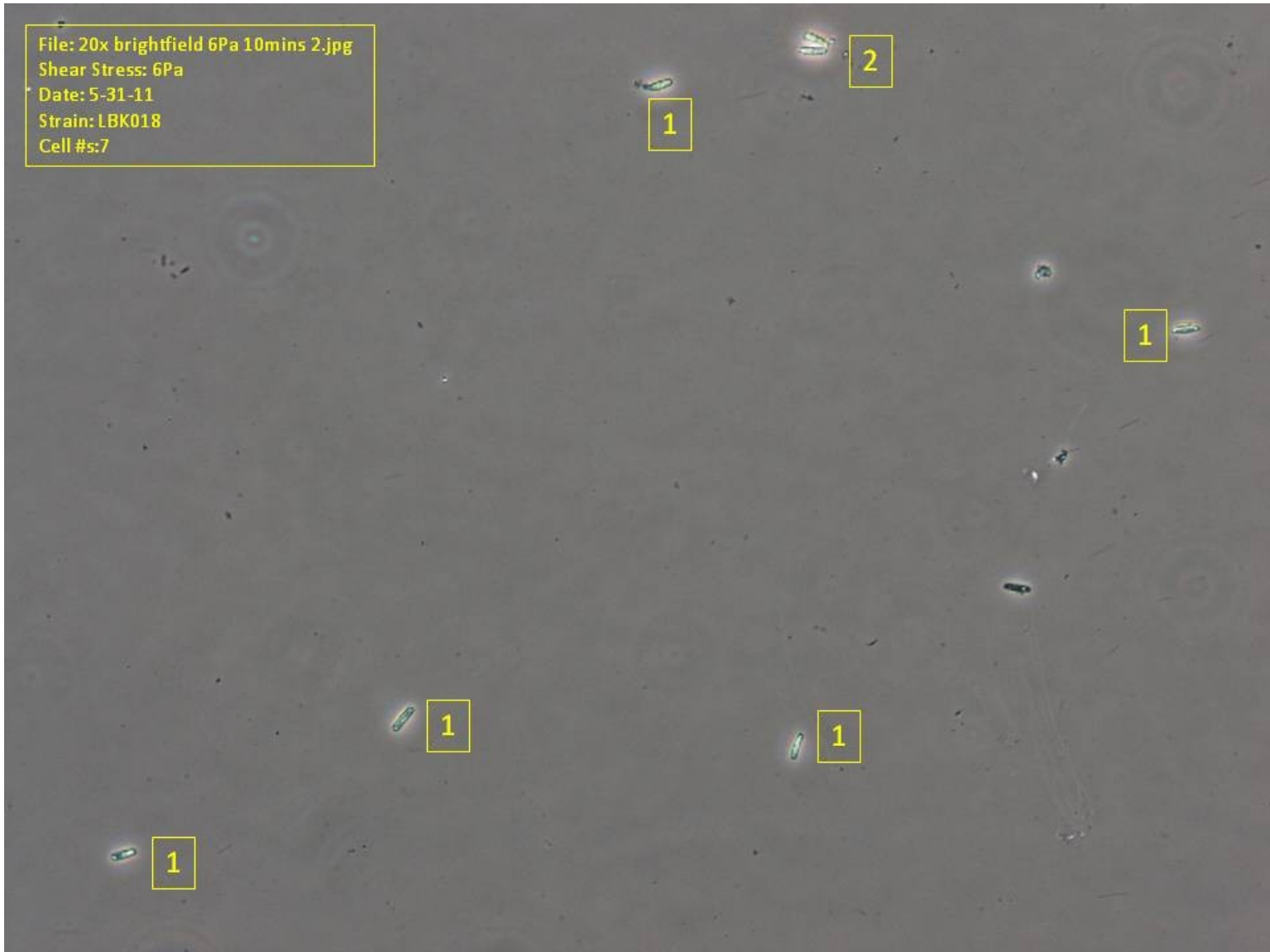






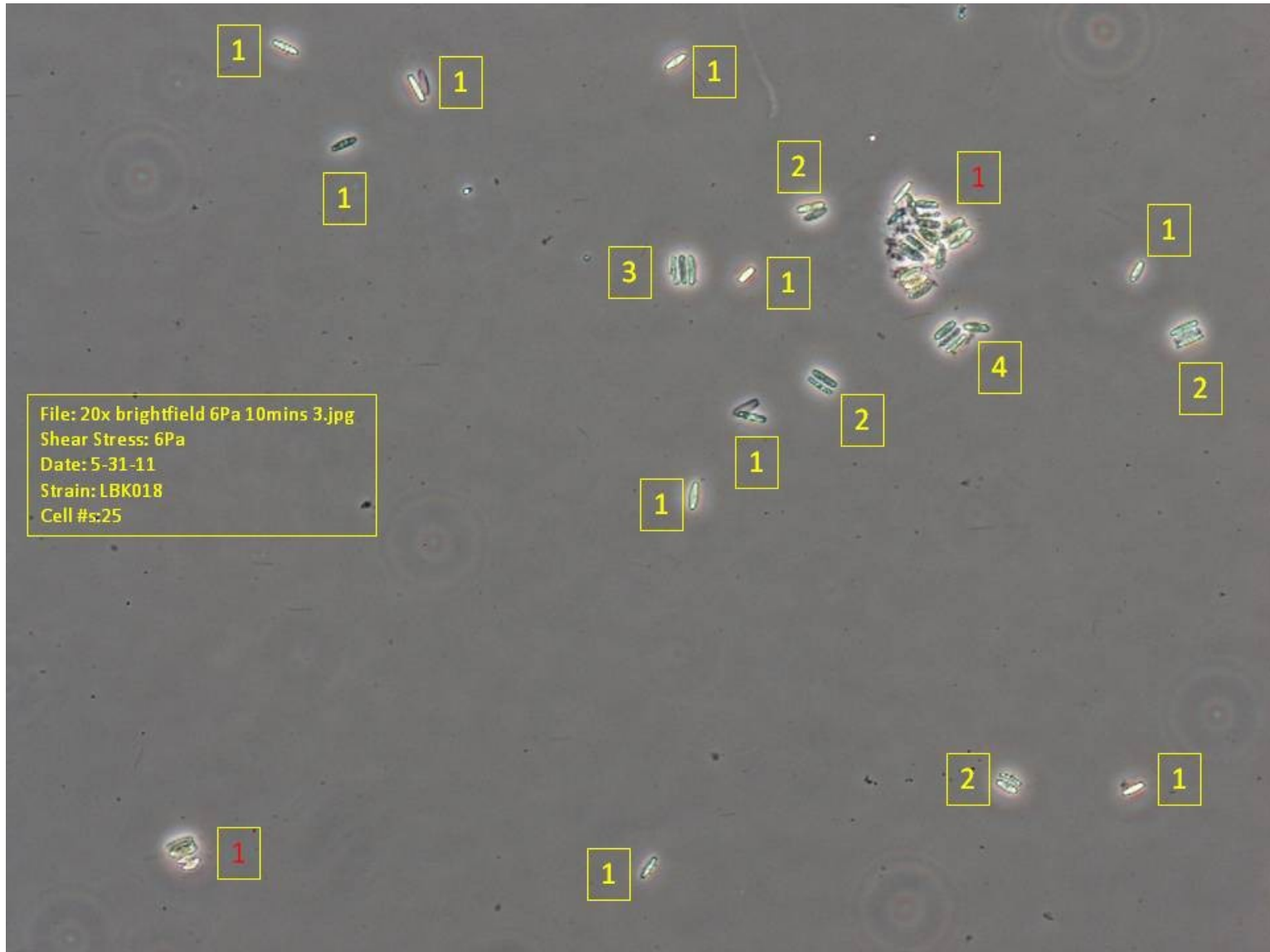


File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK018  
Cell #s:7











File: 20x brightfield 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK018  
Cell #s:15



File: 20x fda 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain:LBK018  
Cell #s:9

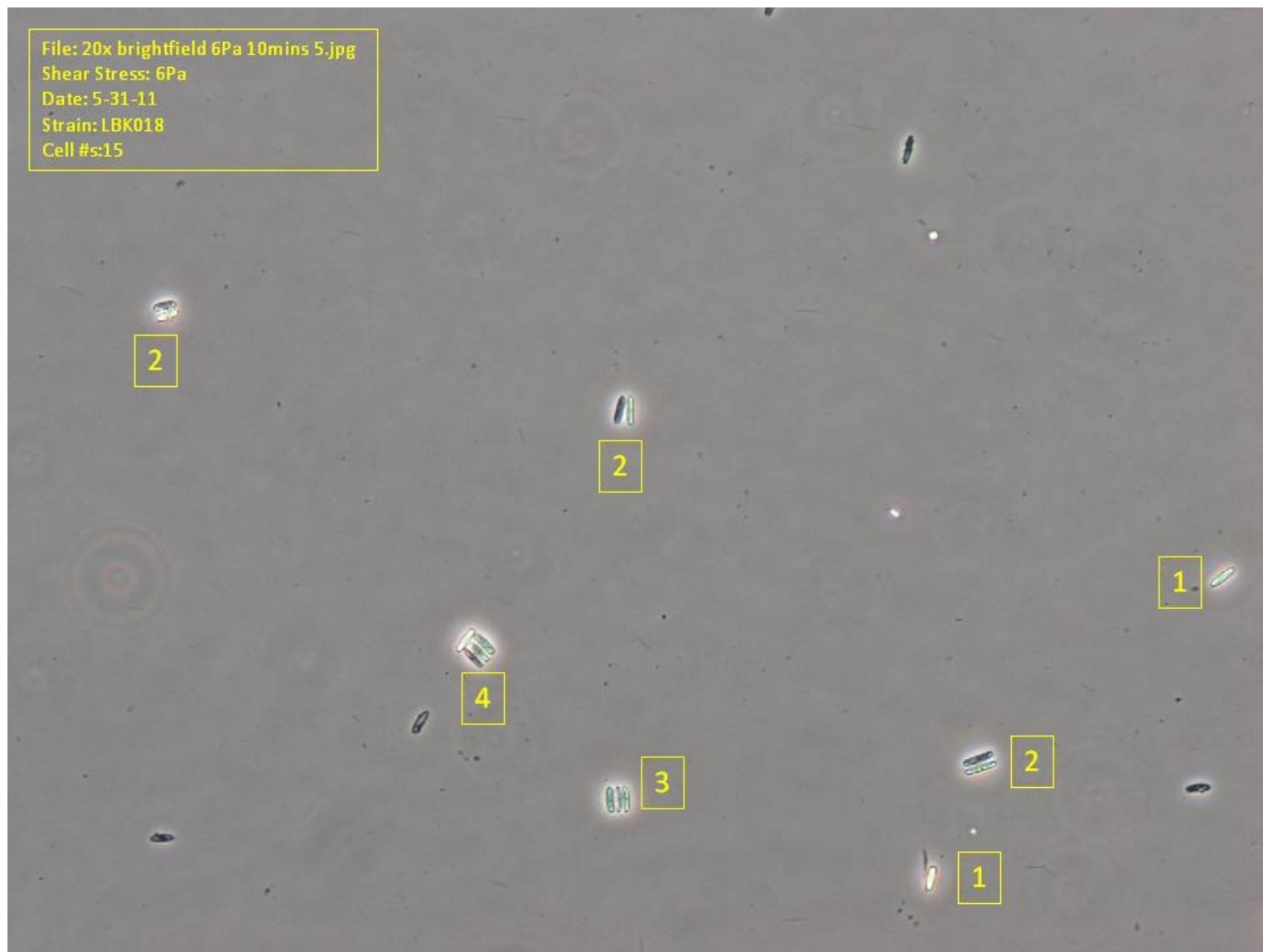
2

5

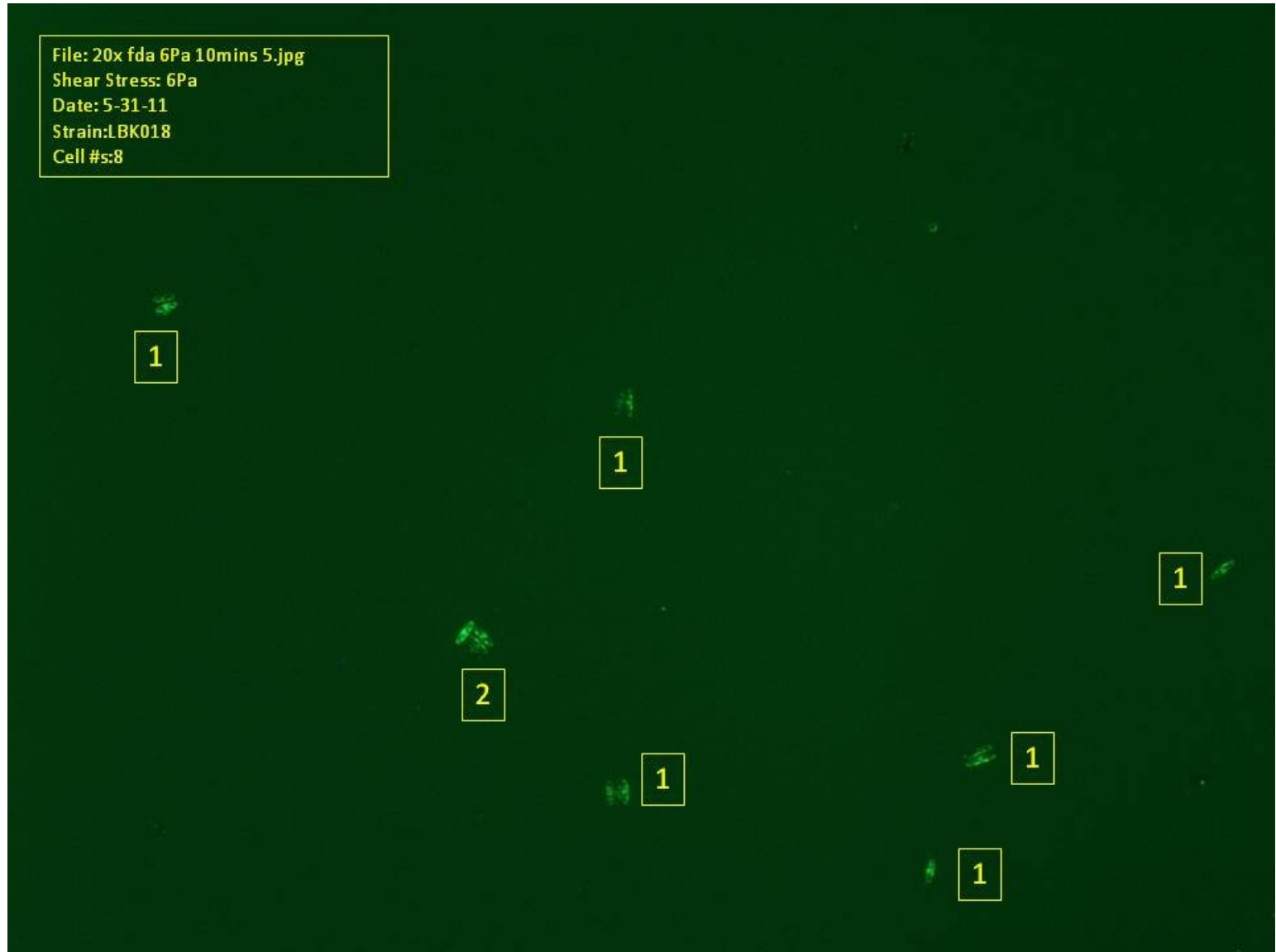
2

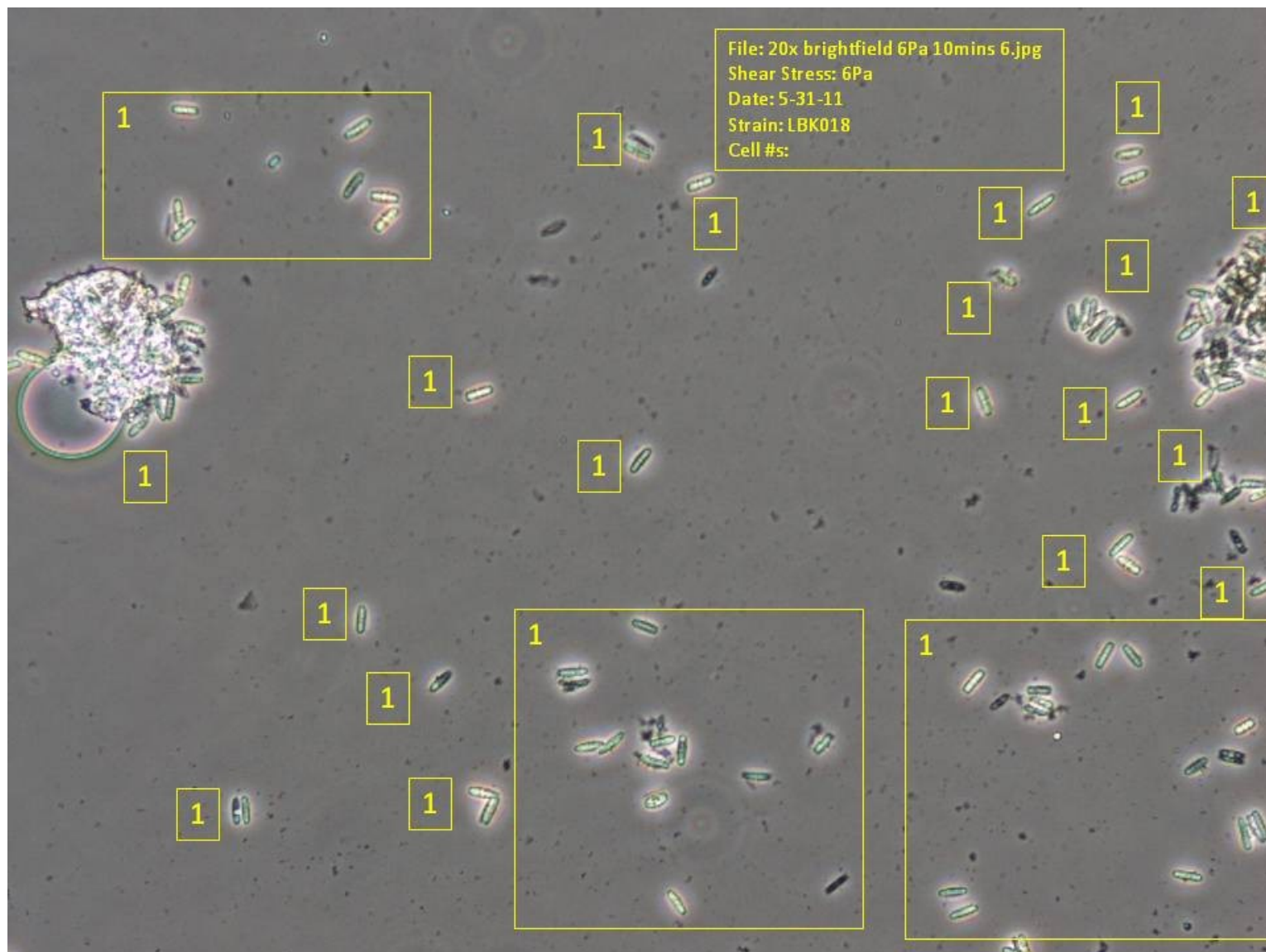
2

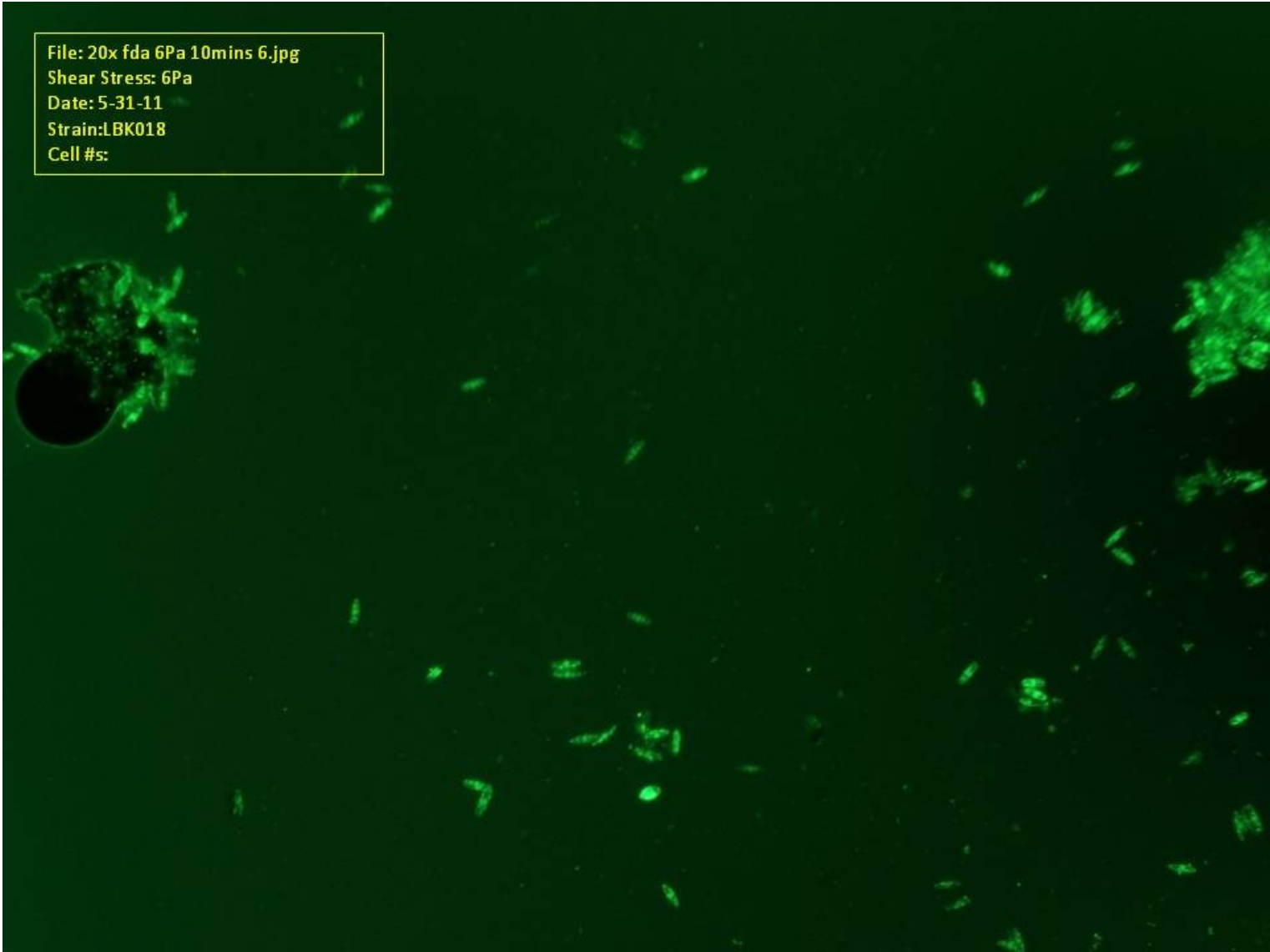
File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK018  
Cell #:s:15



File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain:LBK018  
Cell #s:8









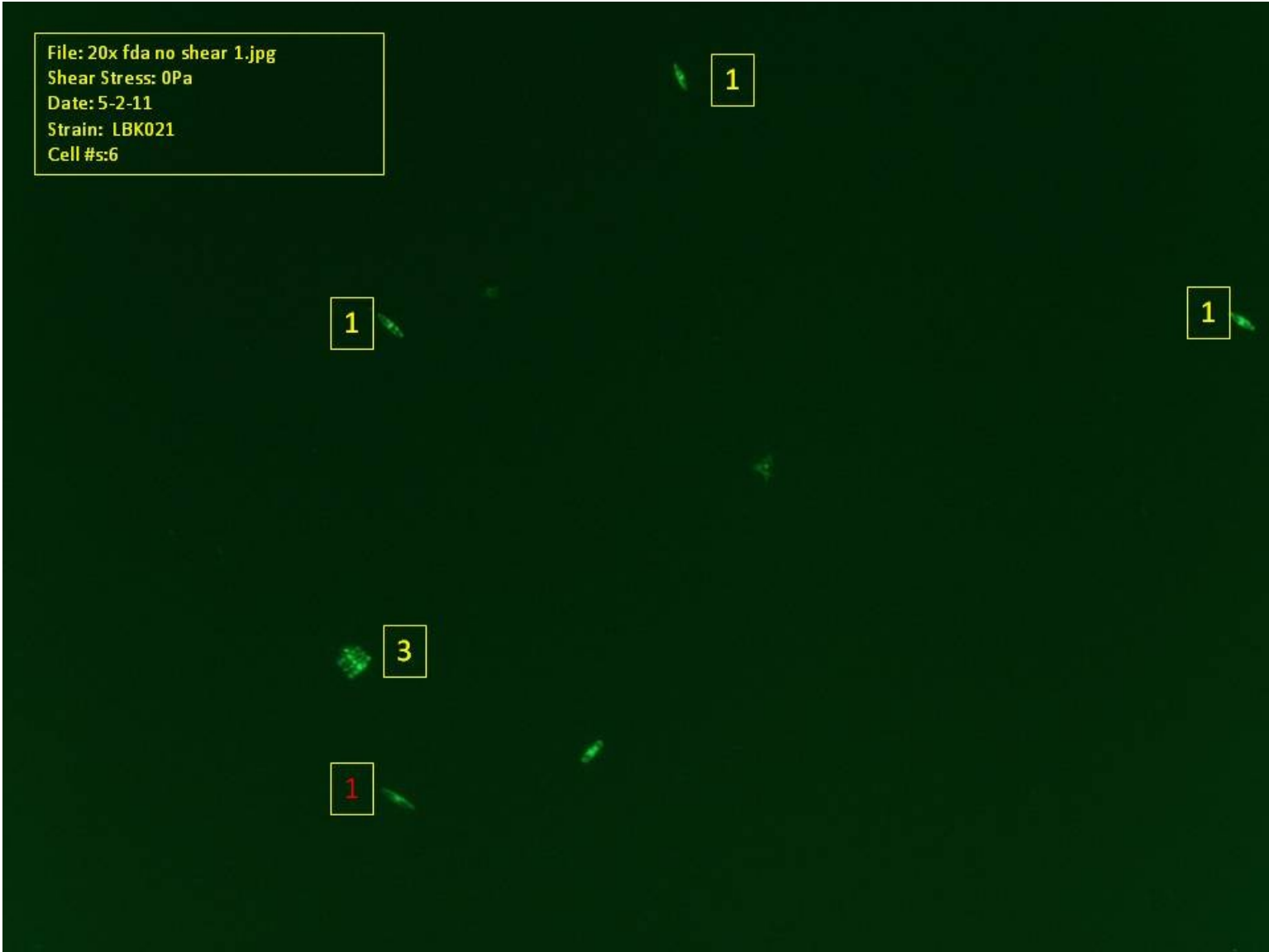
## APPENDIX H

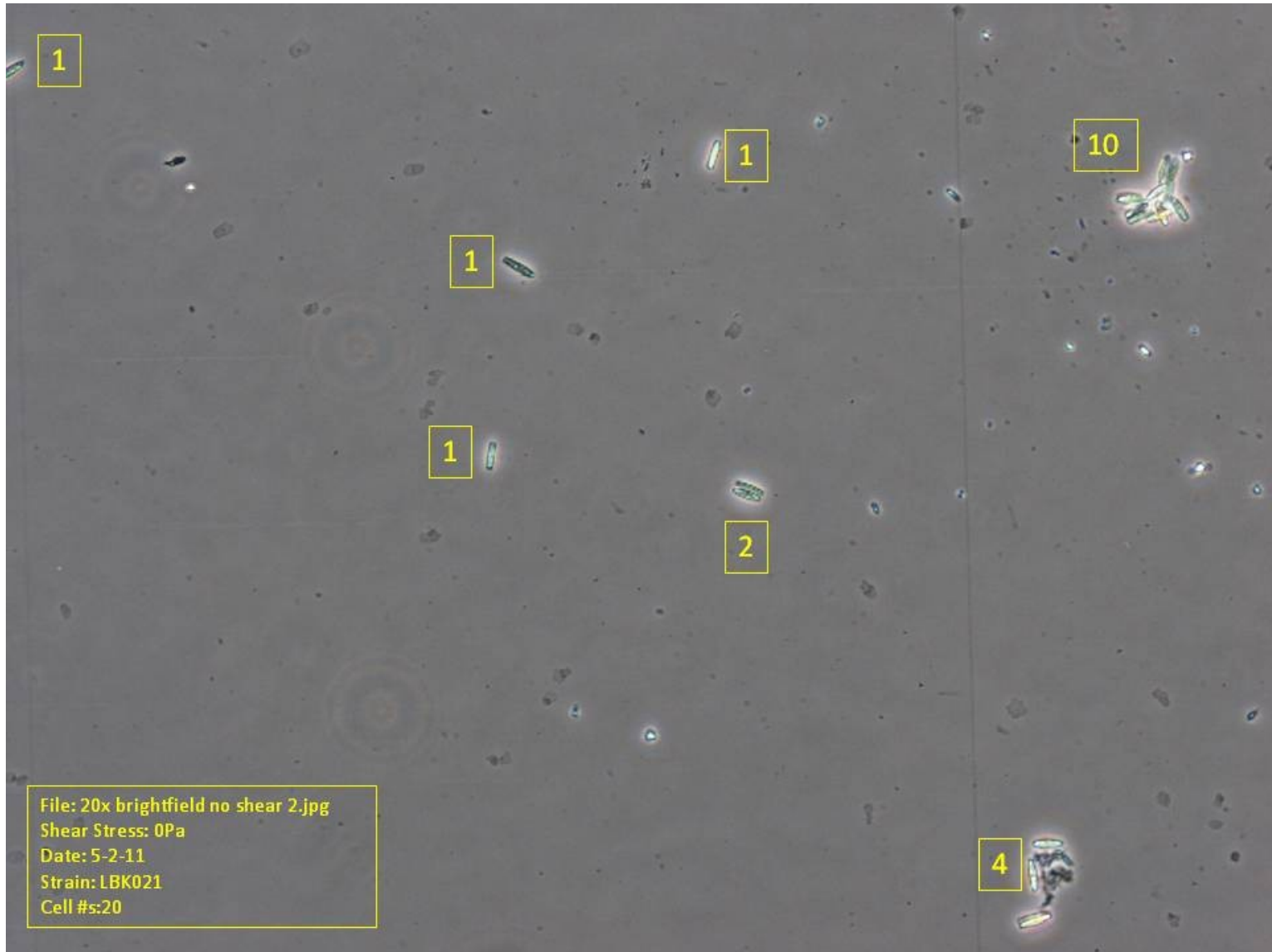
The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK021, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	7	6	20	16	18	14	24	21			12	6	77.78%	1.71%	-0.00285
	6	21	19	38	30	17	10	13	9	28	21			76.07%		
Rep 2	0	14	12	15	14	9	7	13	8	11	8			79.03%	1.35%	-0.00224
	6	17	14	27	19	32	30	16	13	29	18			77.69%		
Rep 3	0	23	20	11	8	11	9	13	11	10	7	15	9	77.11%	0.84%	-0.00140
	6	20	14	24	21	20	15	24	15	30	25			76.27%		
														<b>Avg.</b>	<b>1.30%</b>	
														<b>Std. Dev.</b>	<b>0.44%</b>	

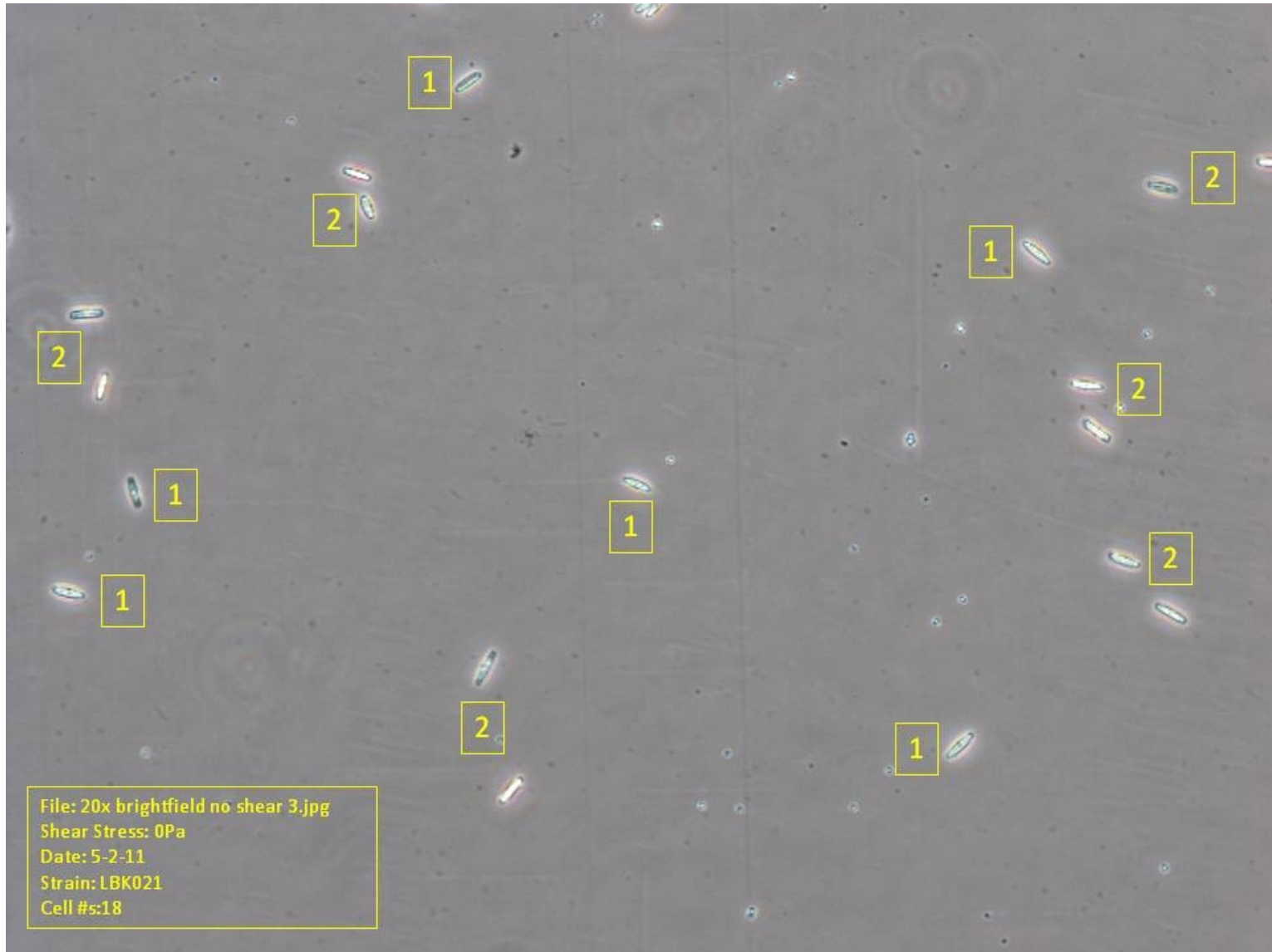
The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.



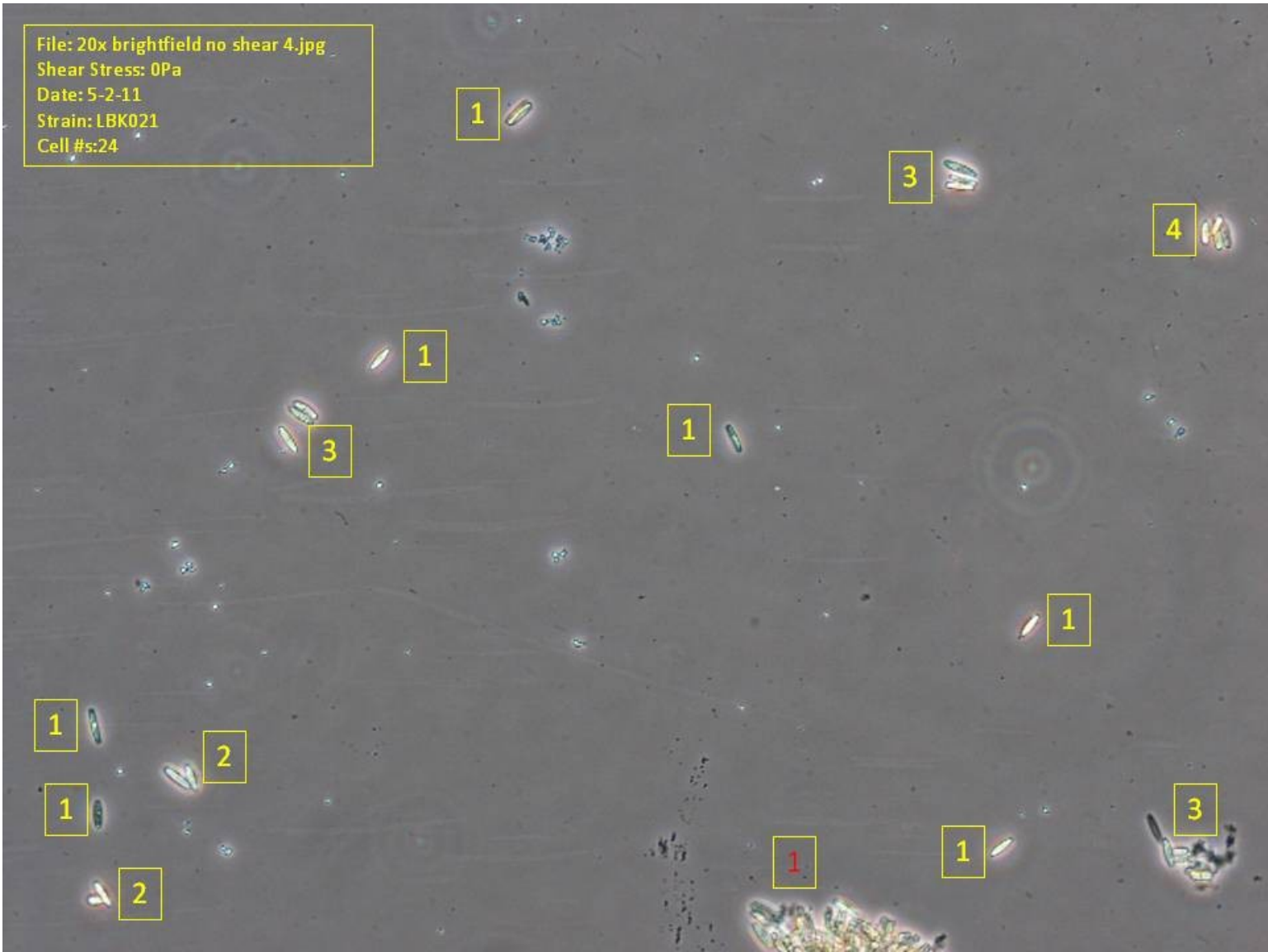






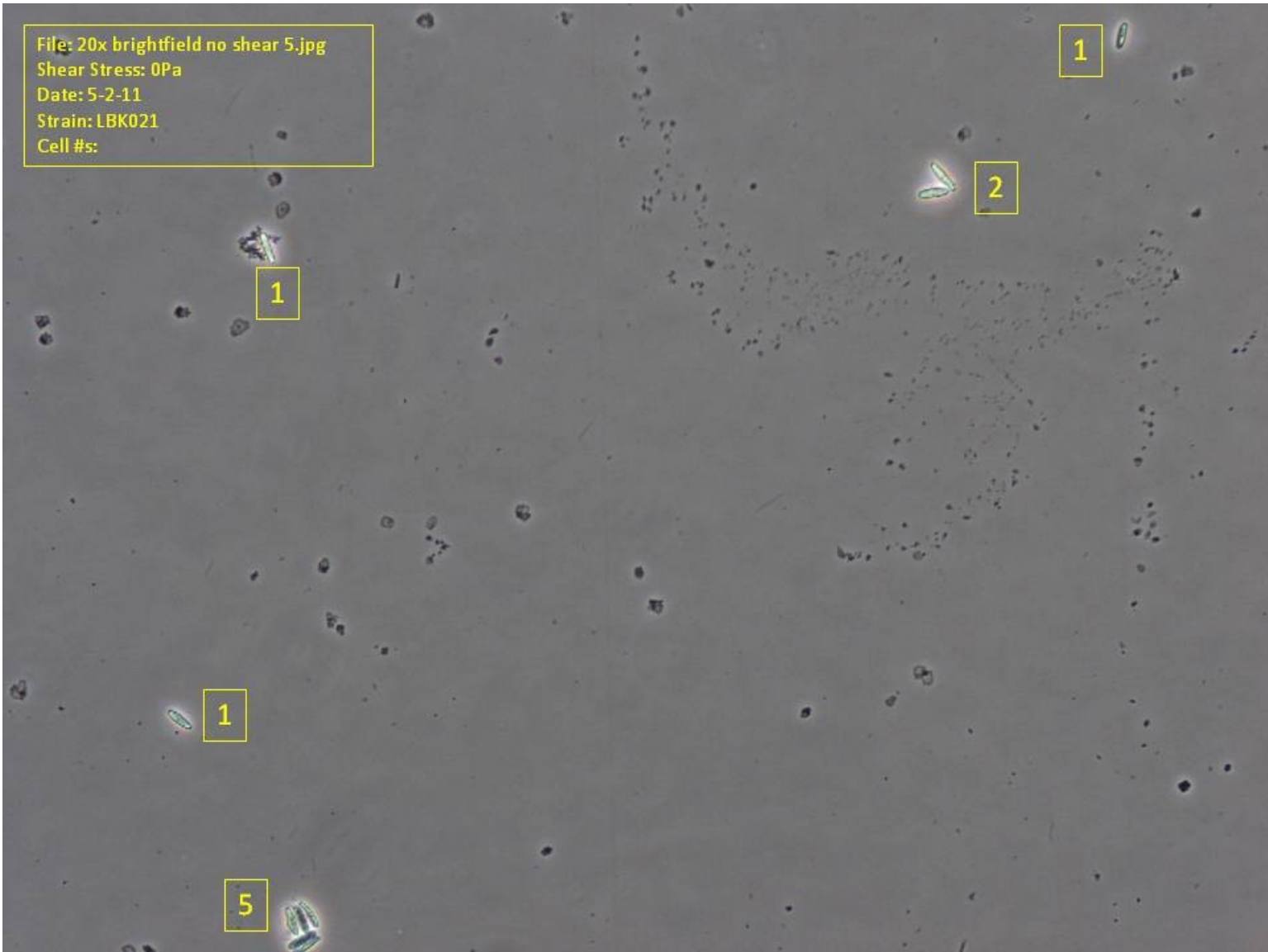




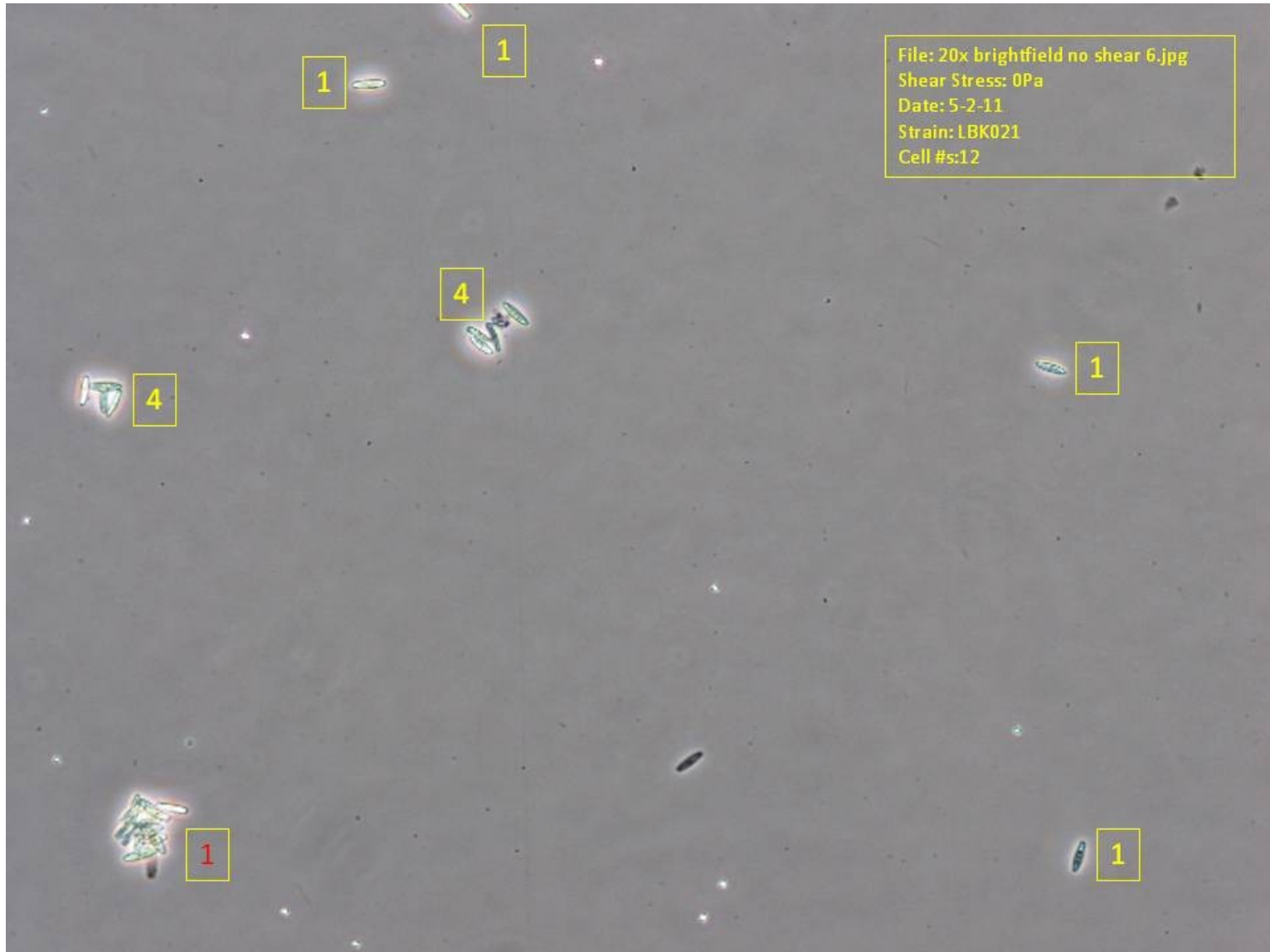






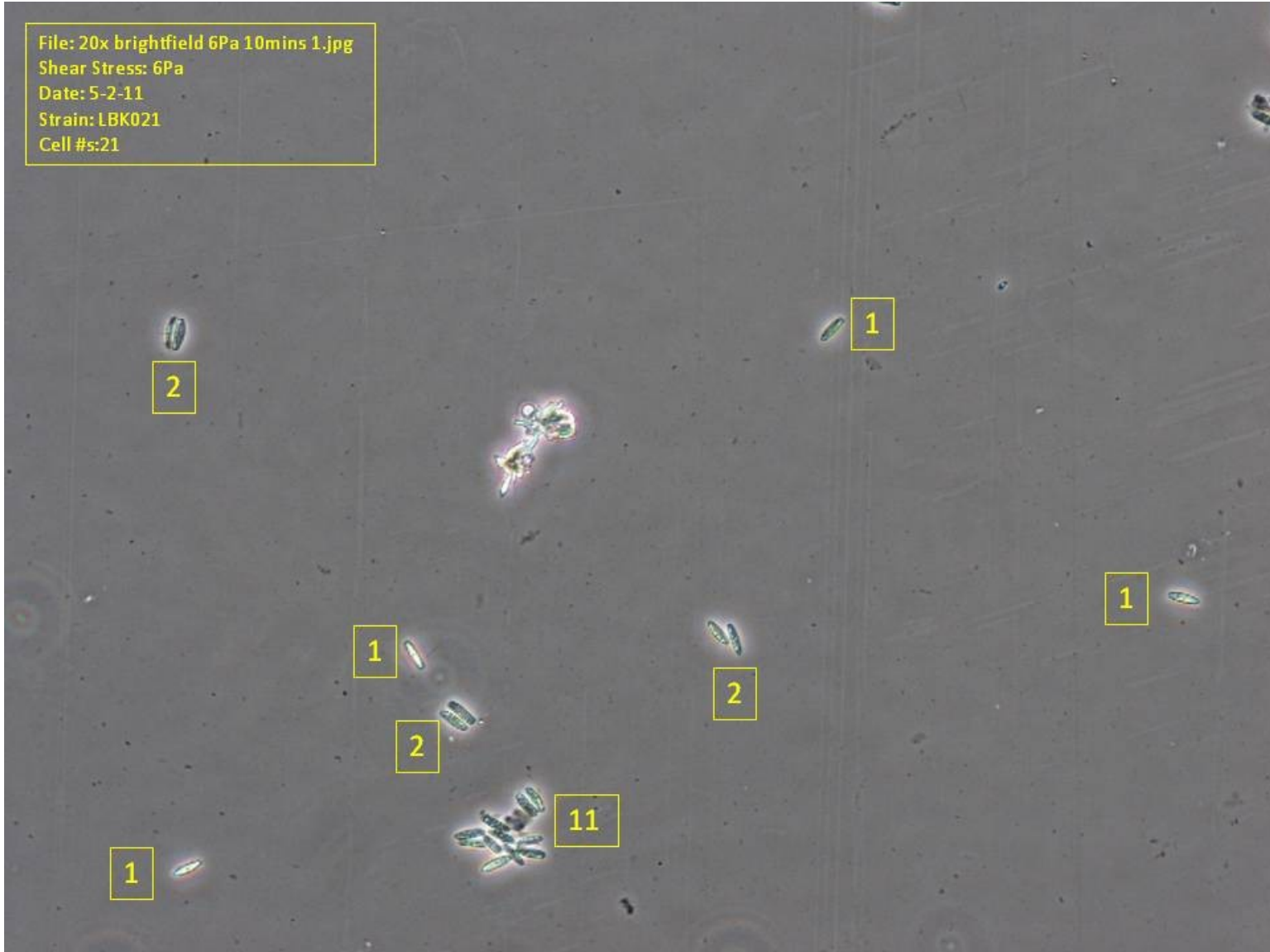




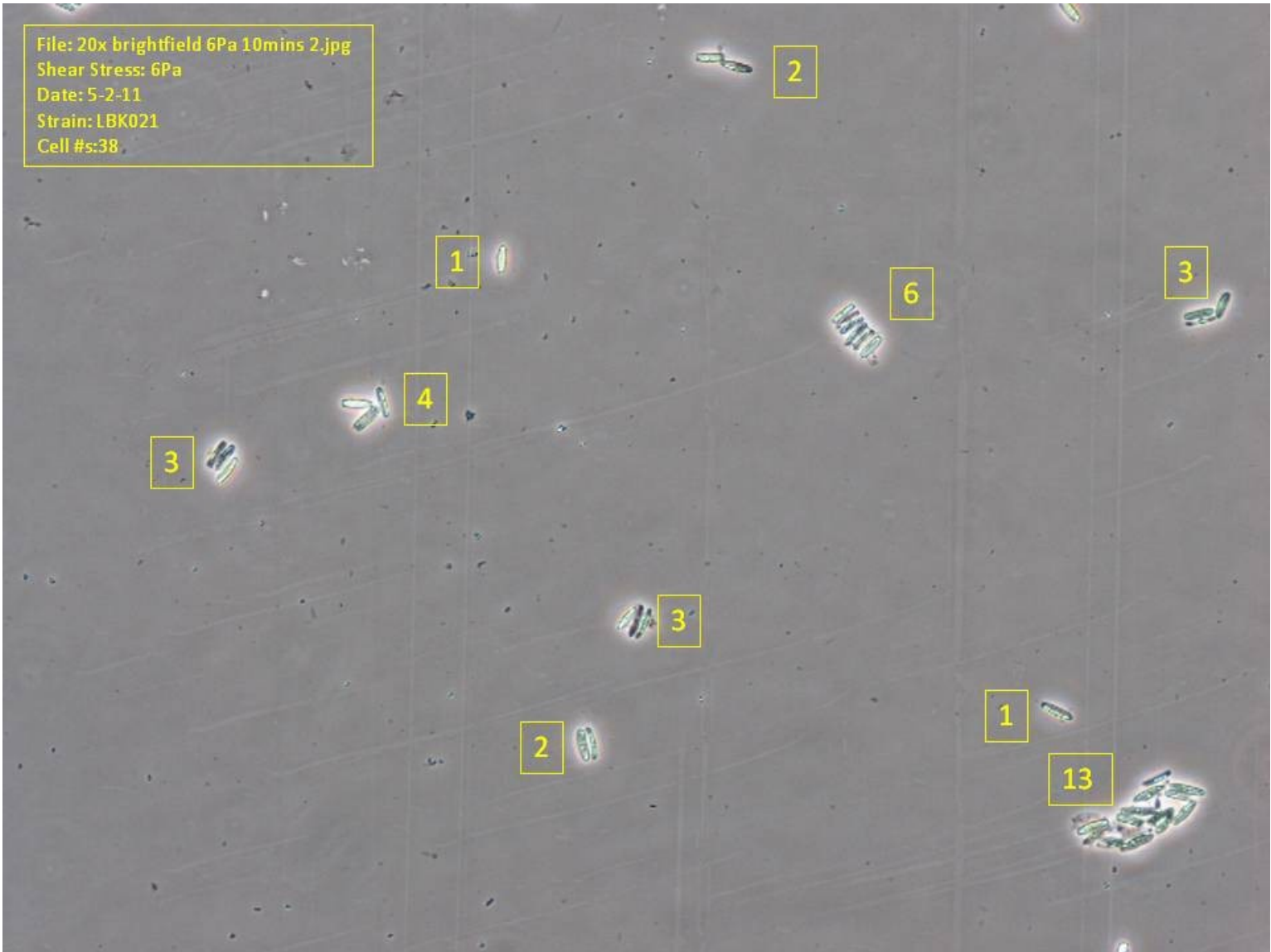




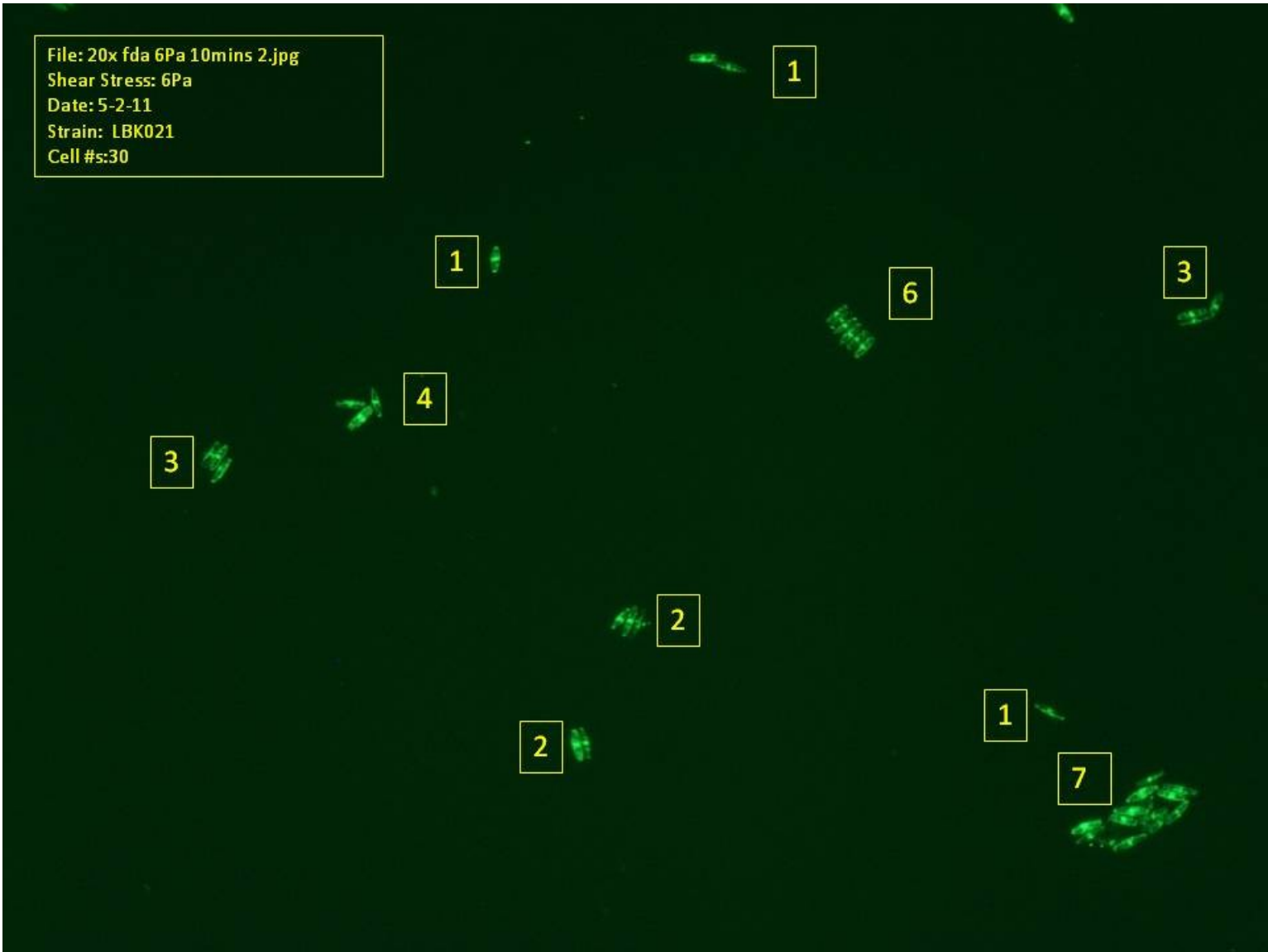
File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:s:21

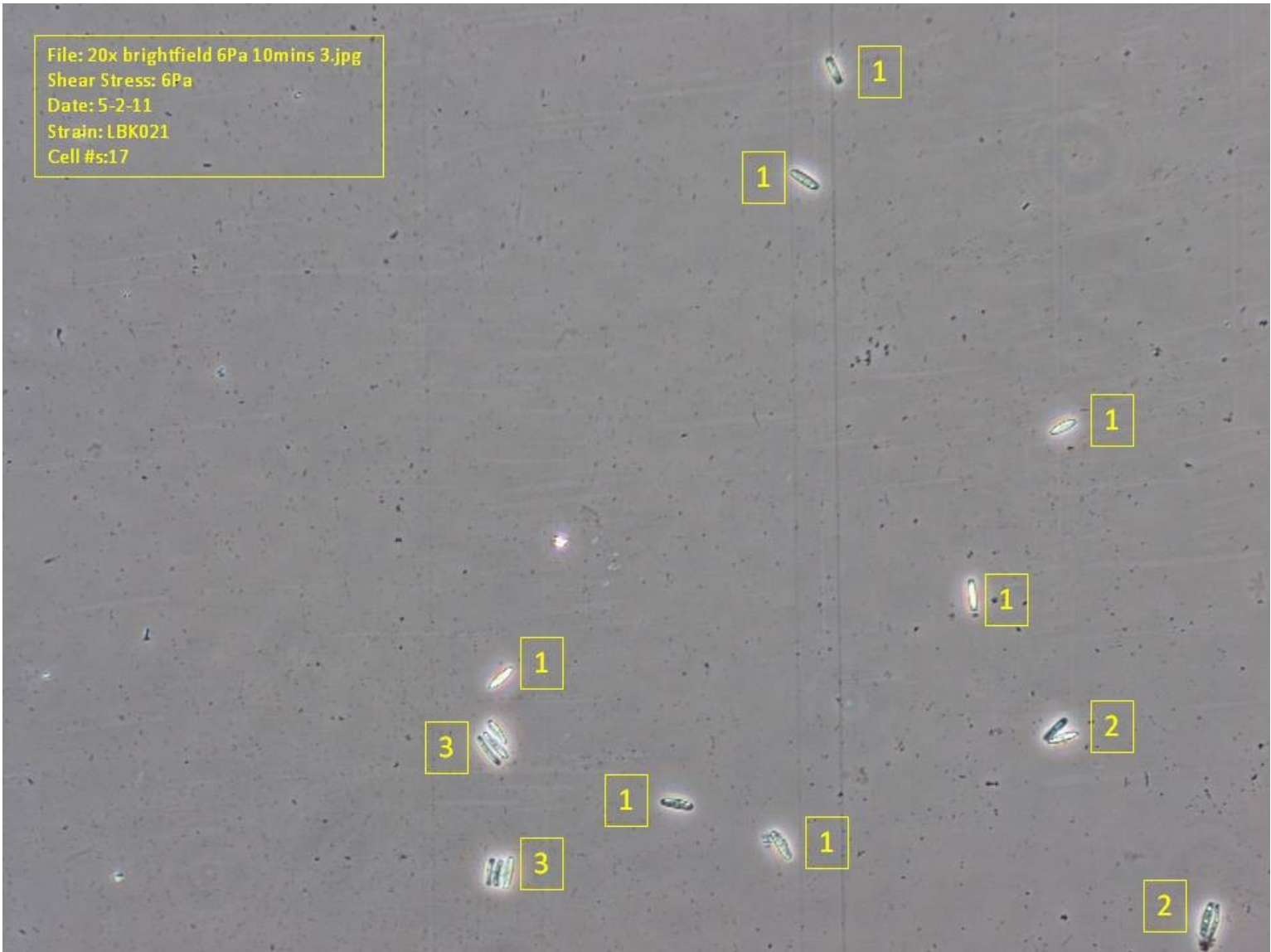




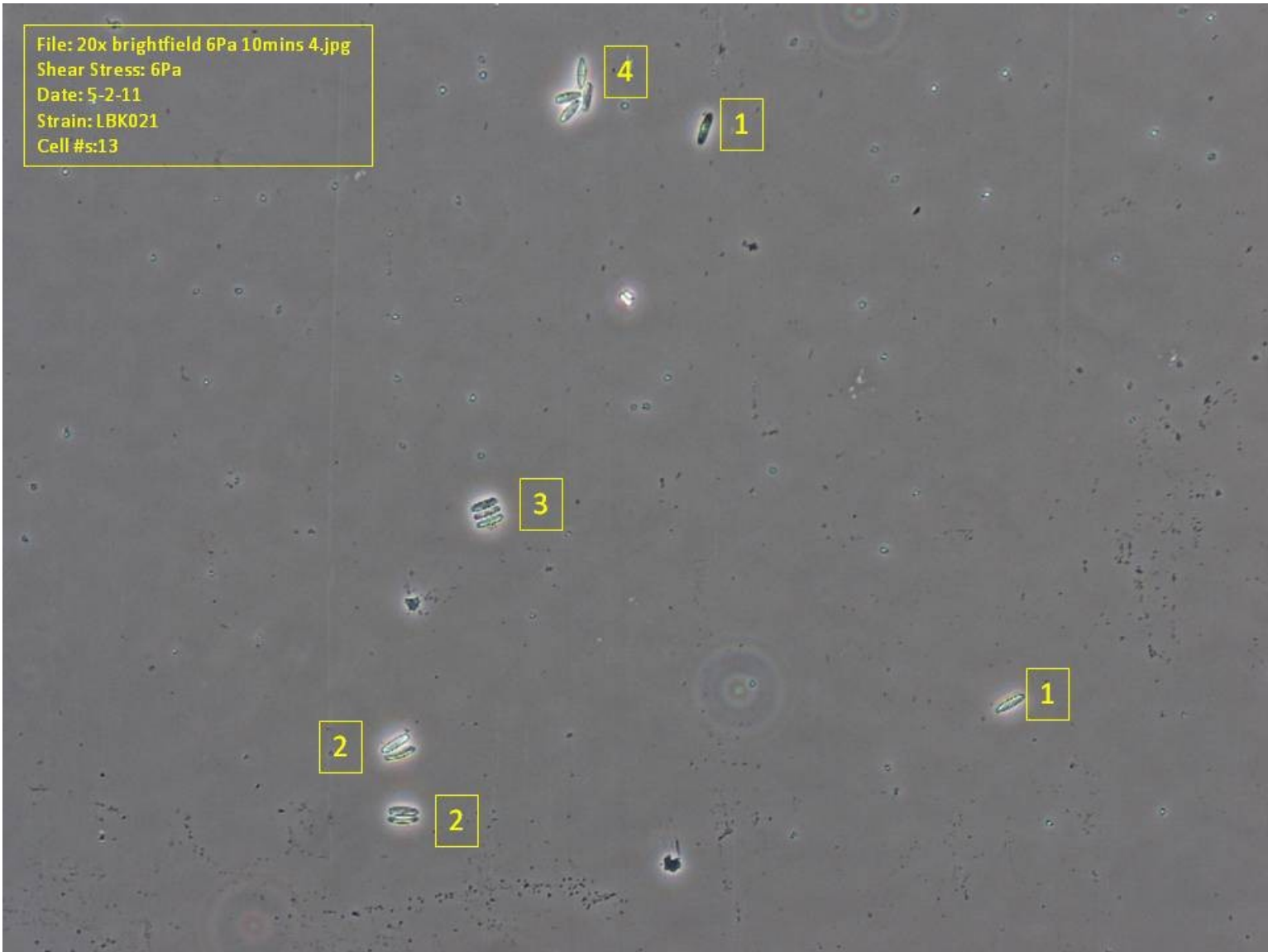




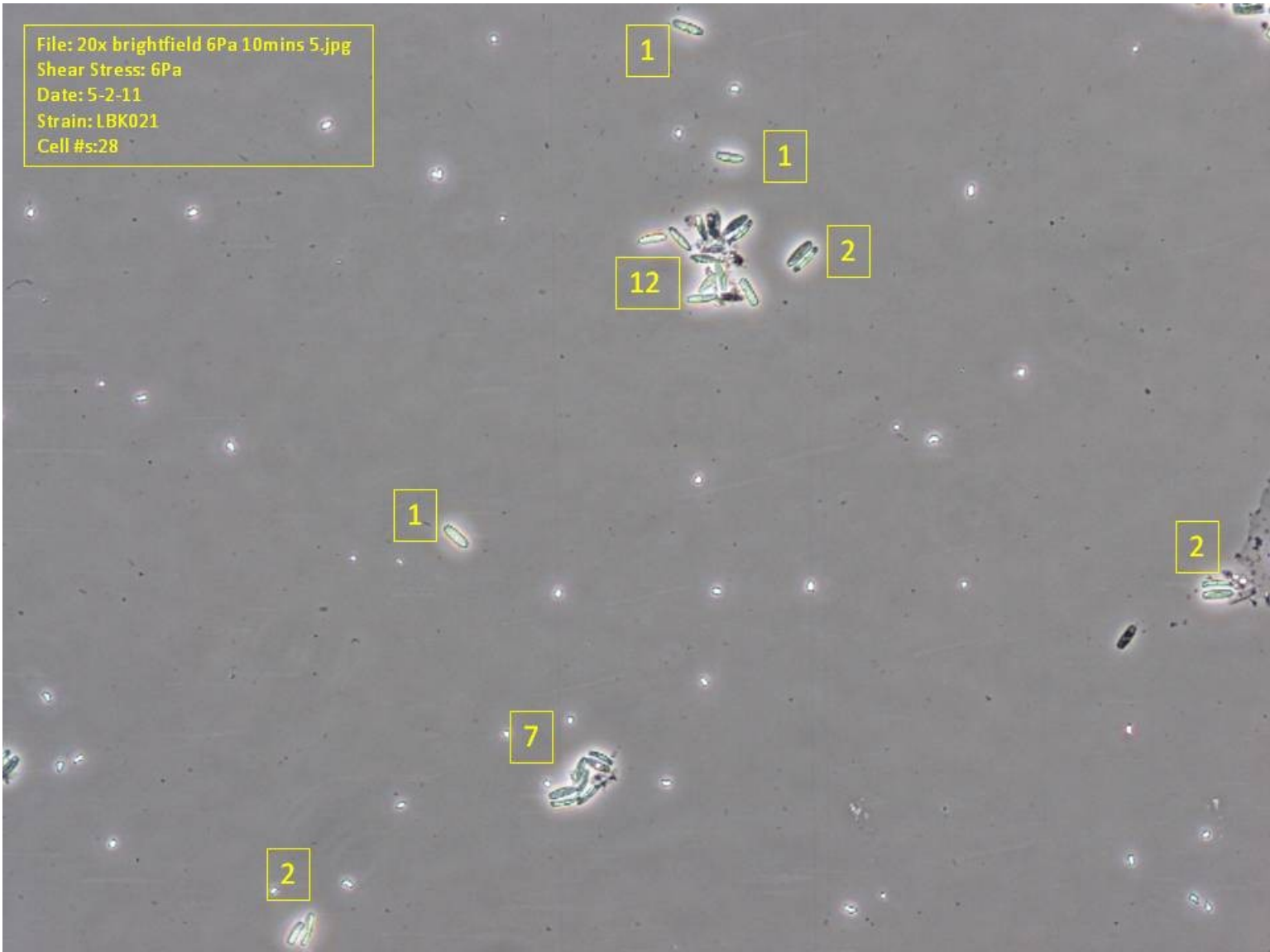




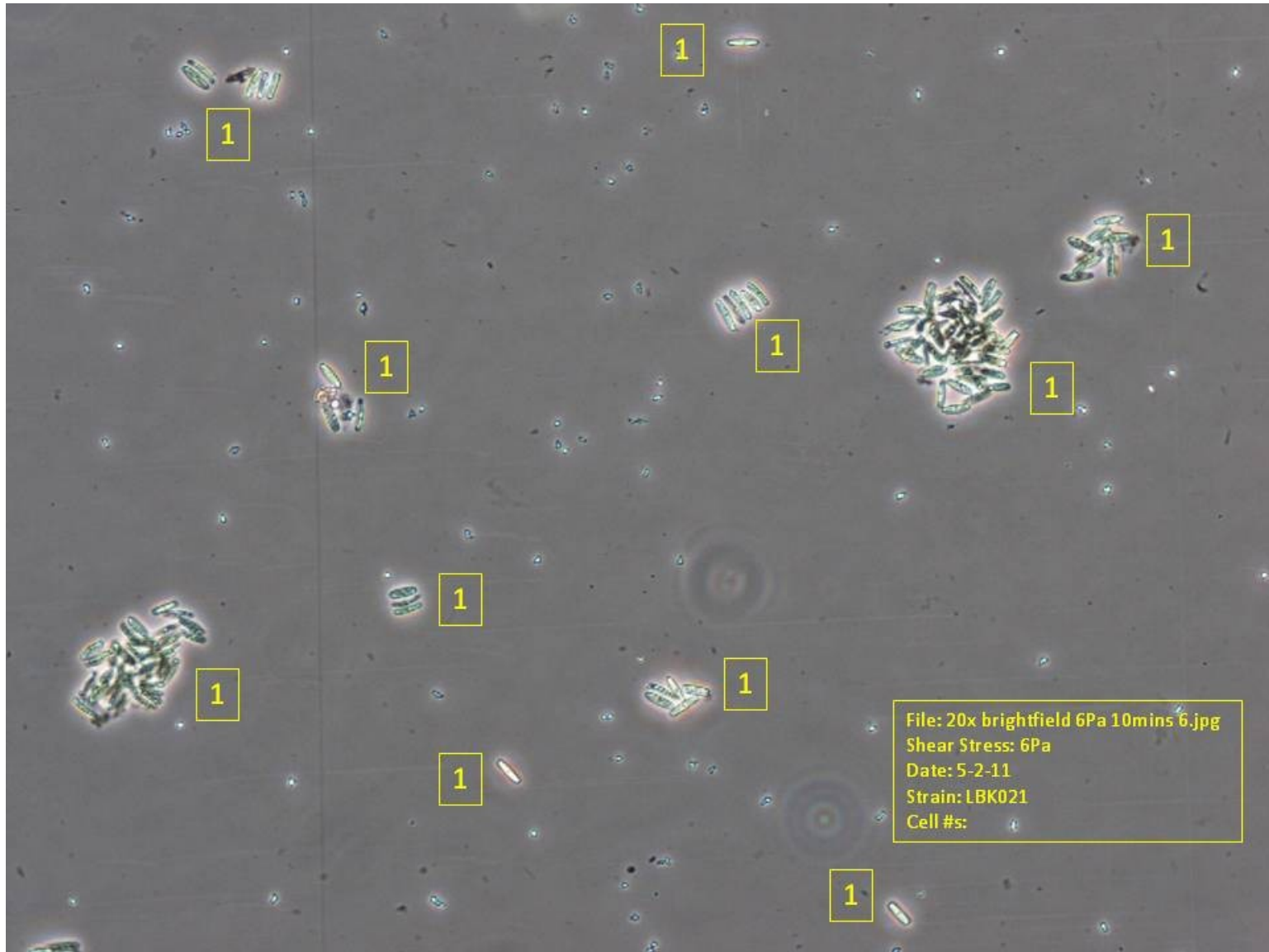




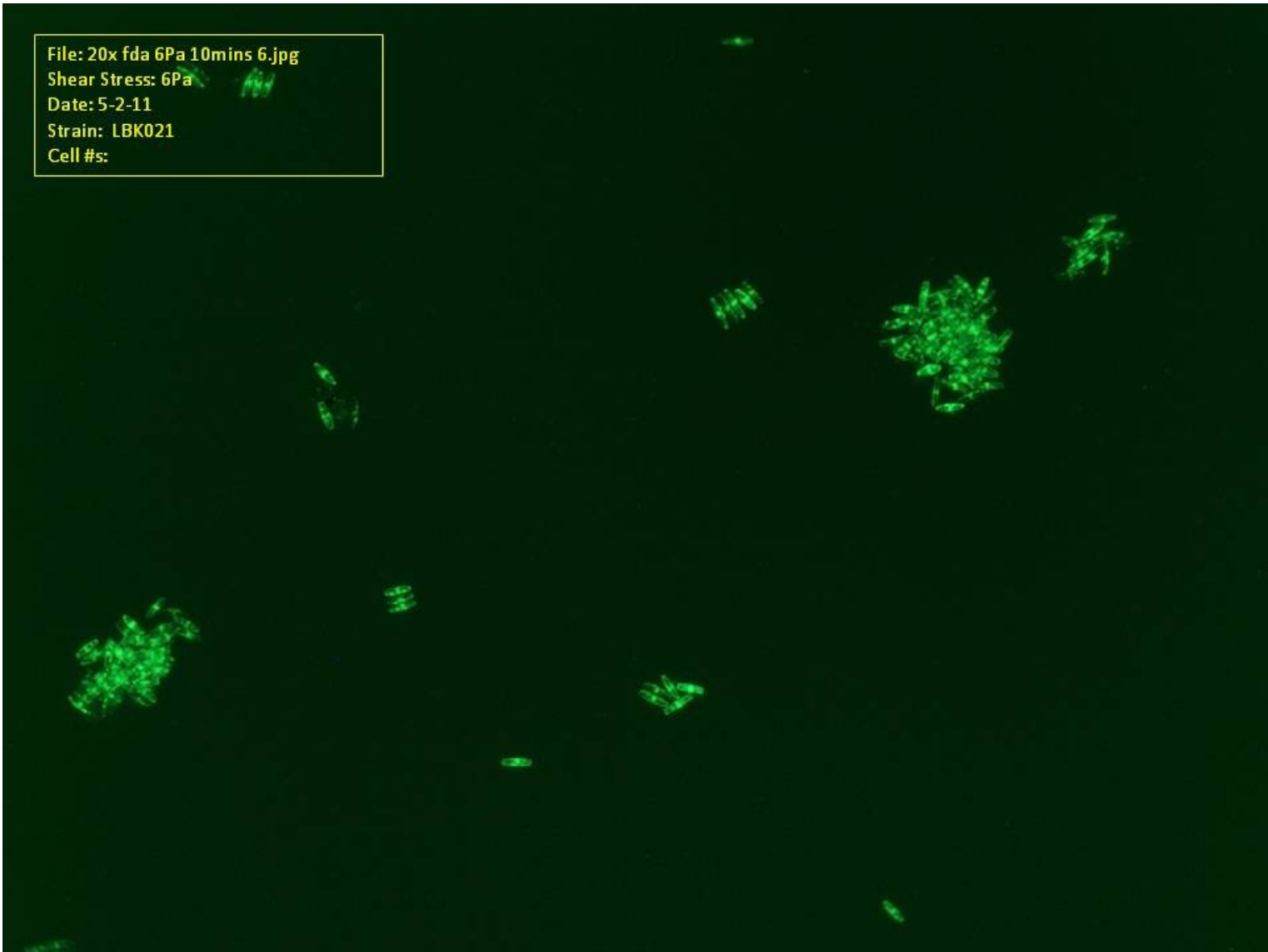




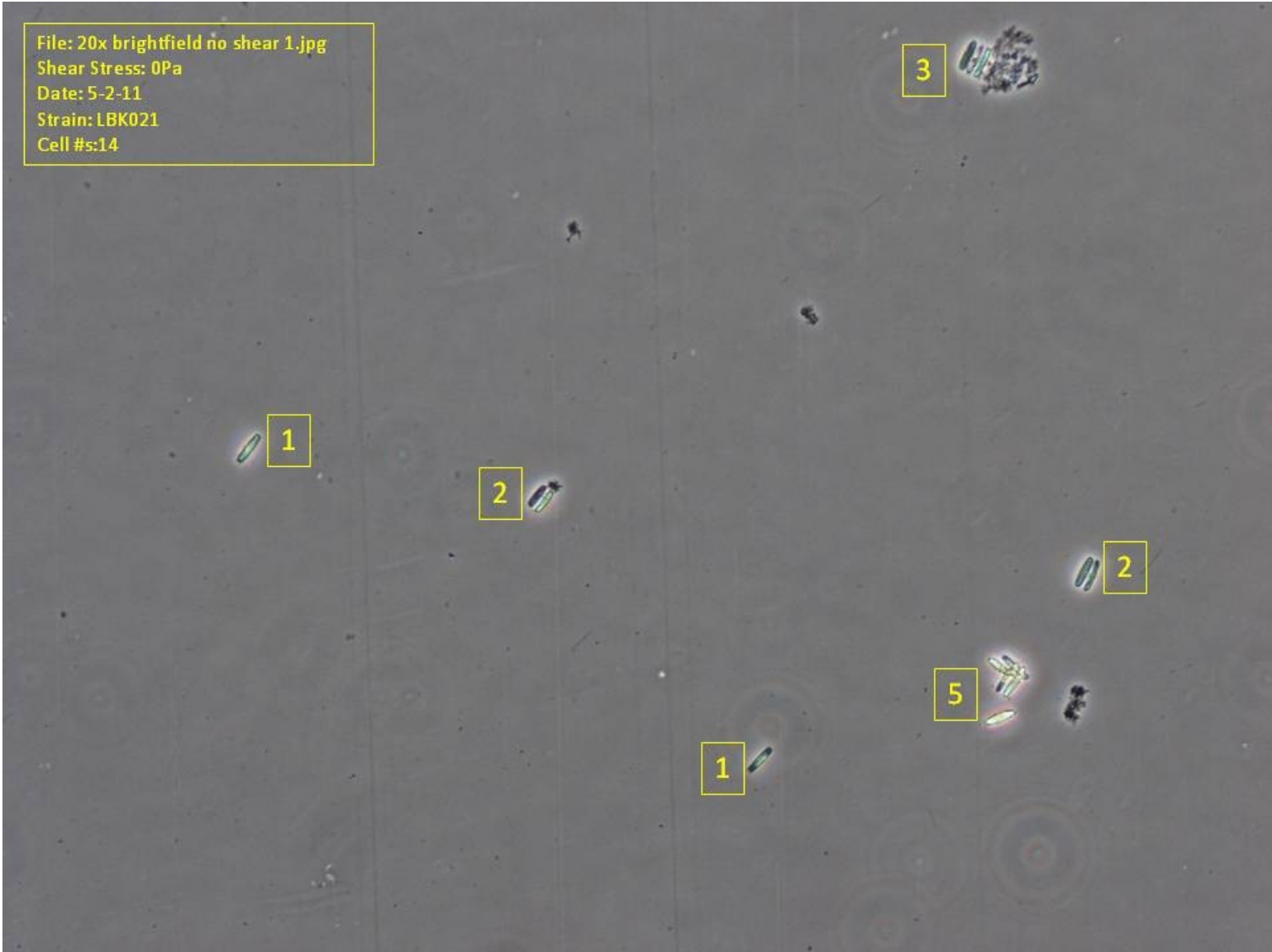


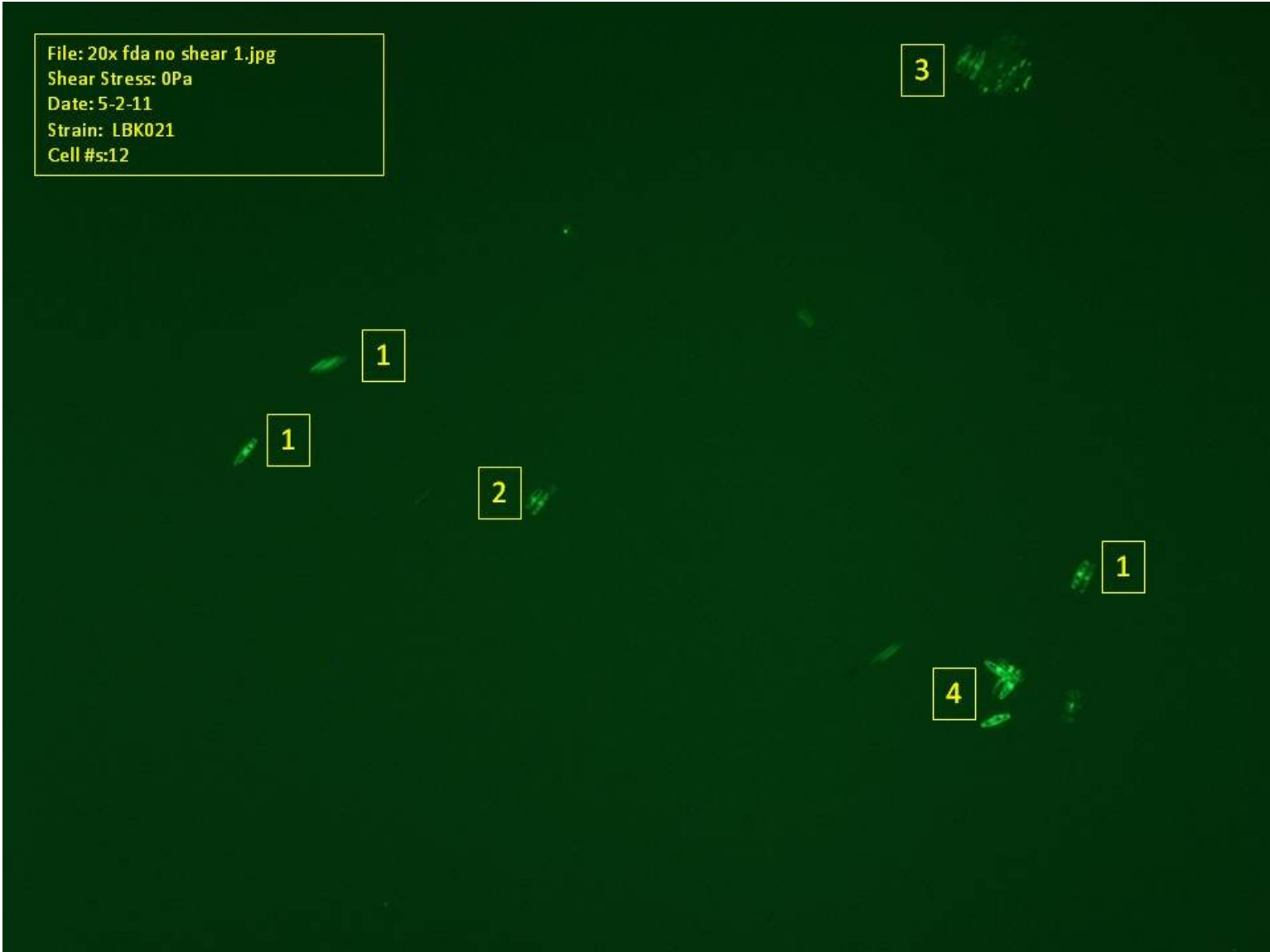


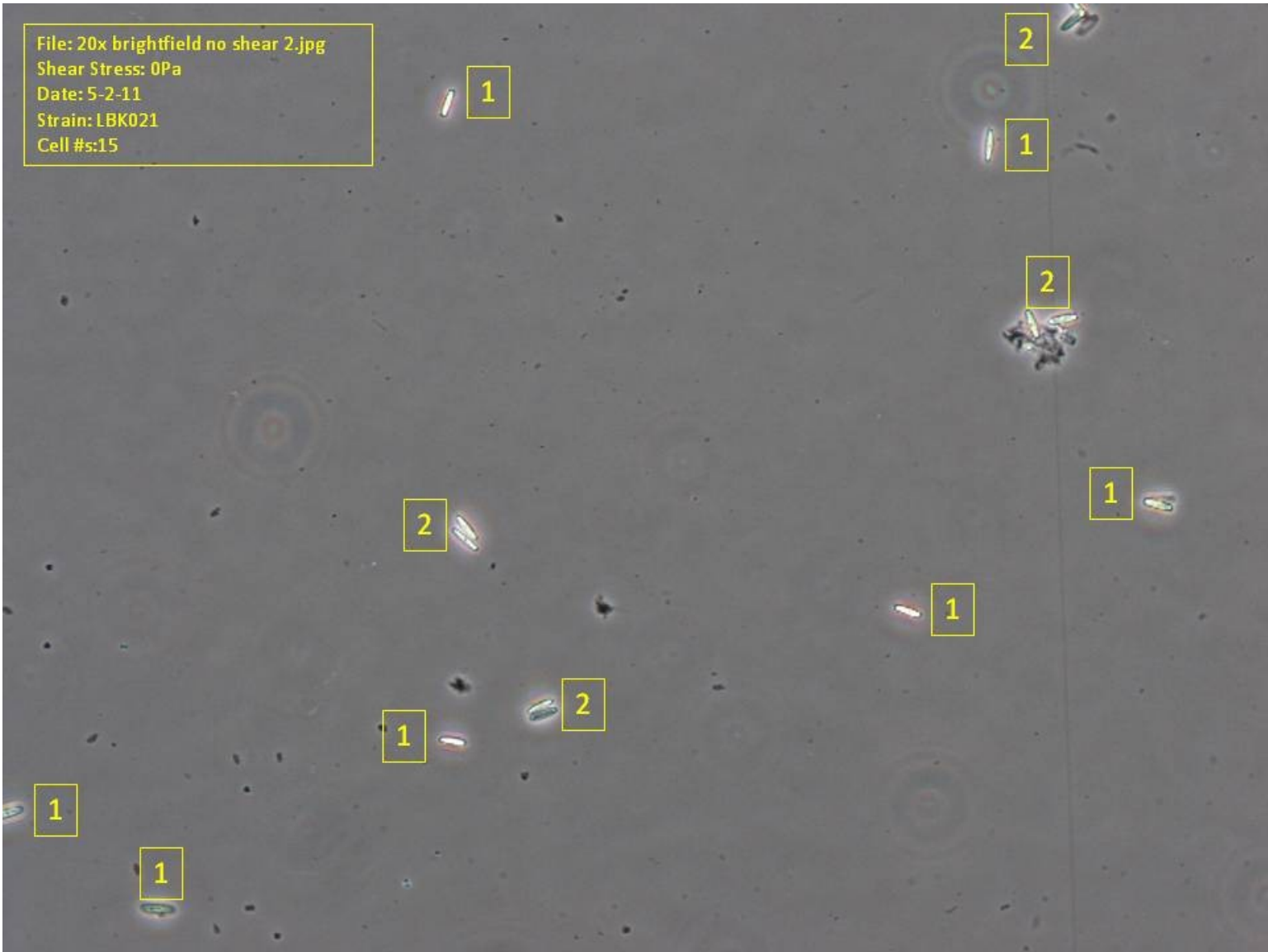




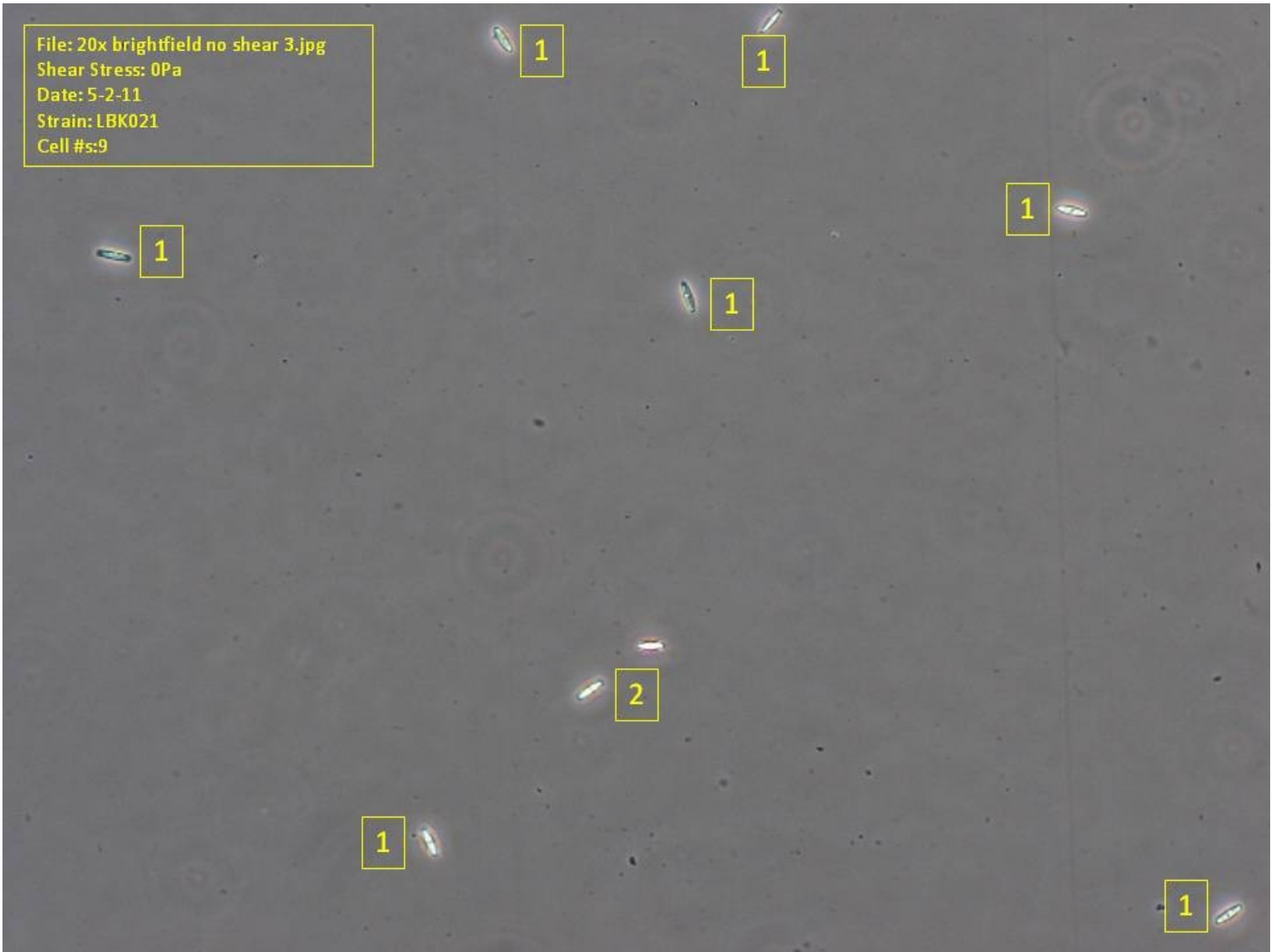
File: 20x brightfield no shear 1.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:14



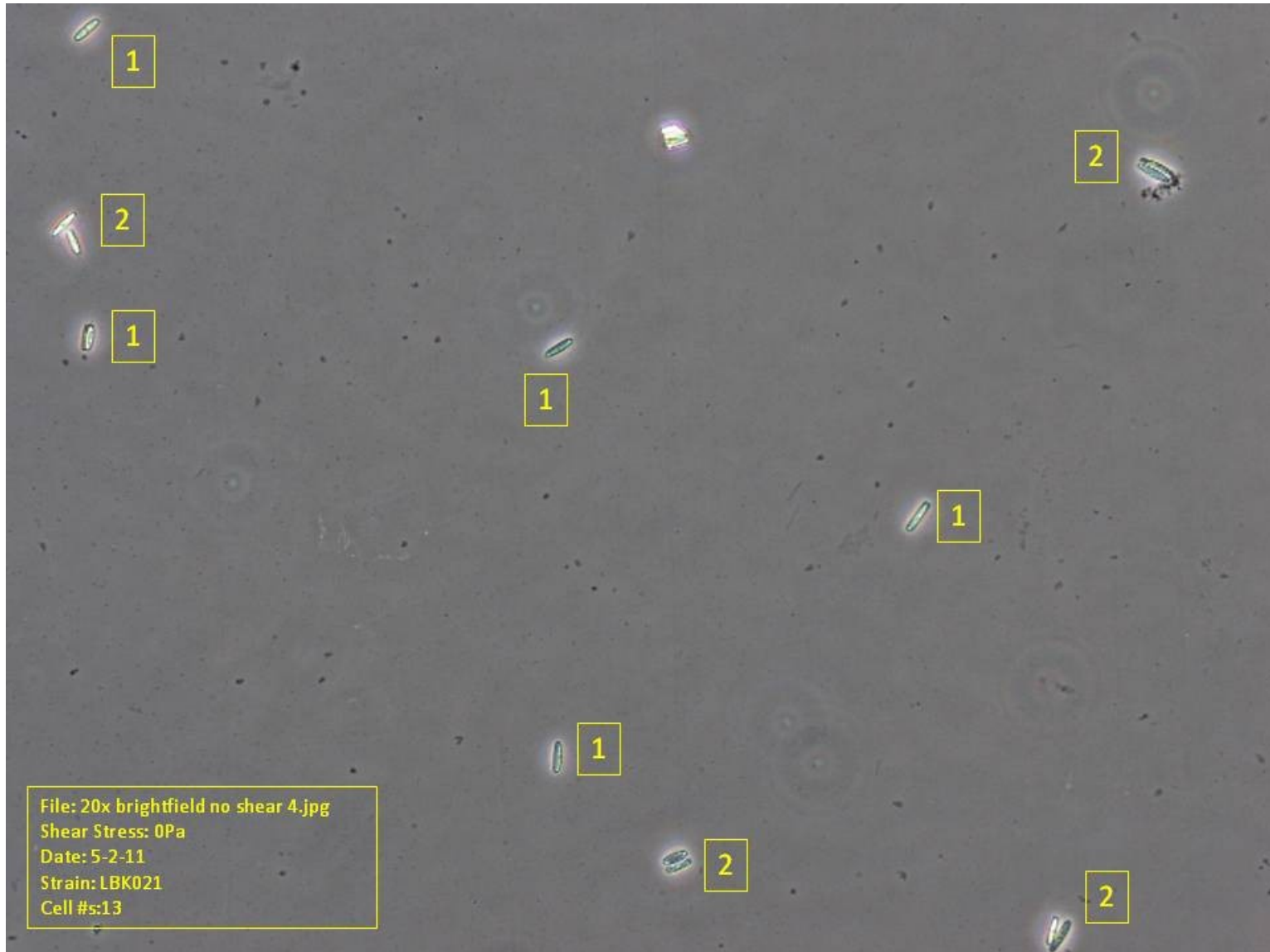










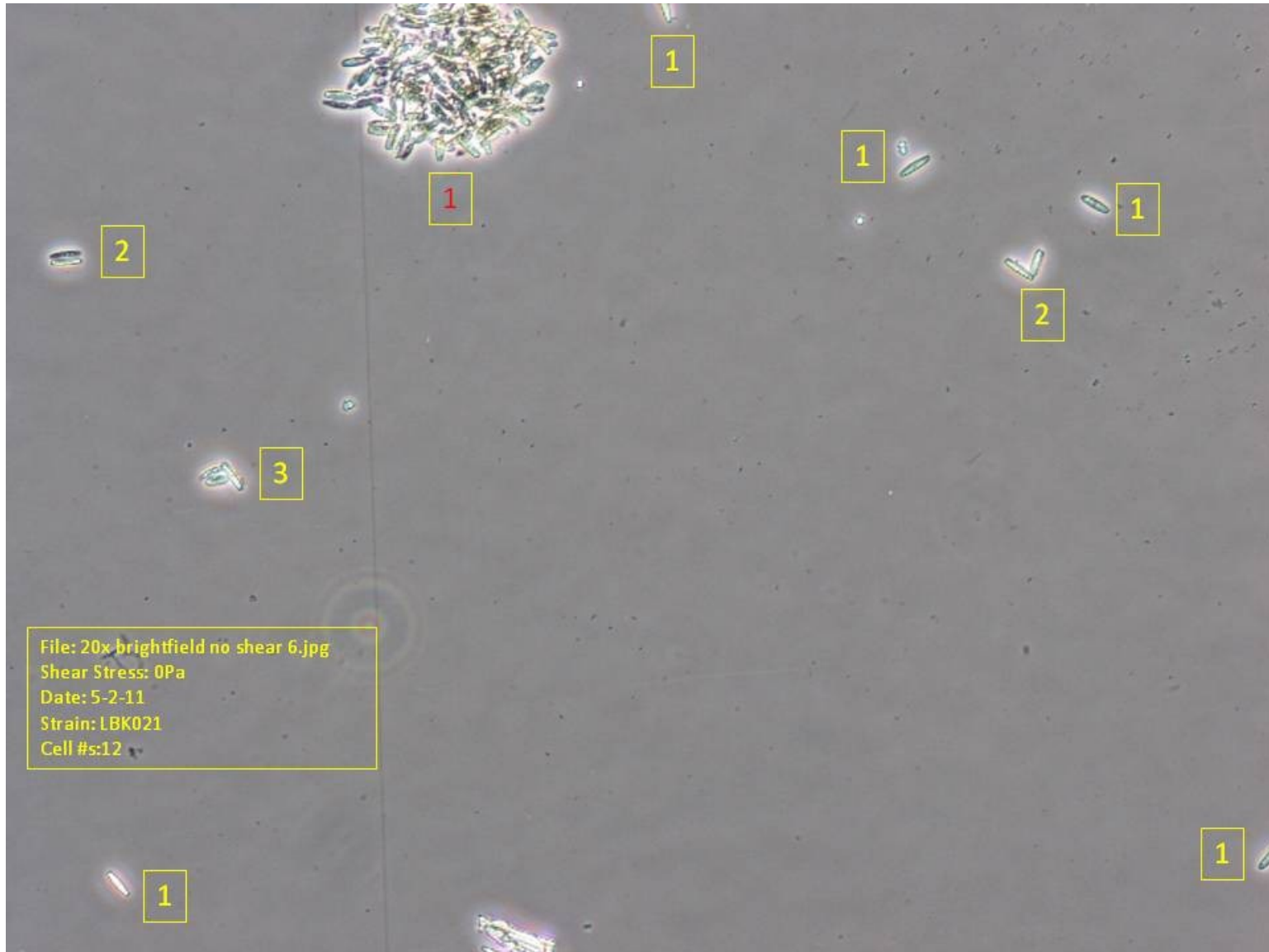






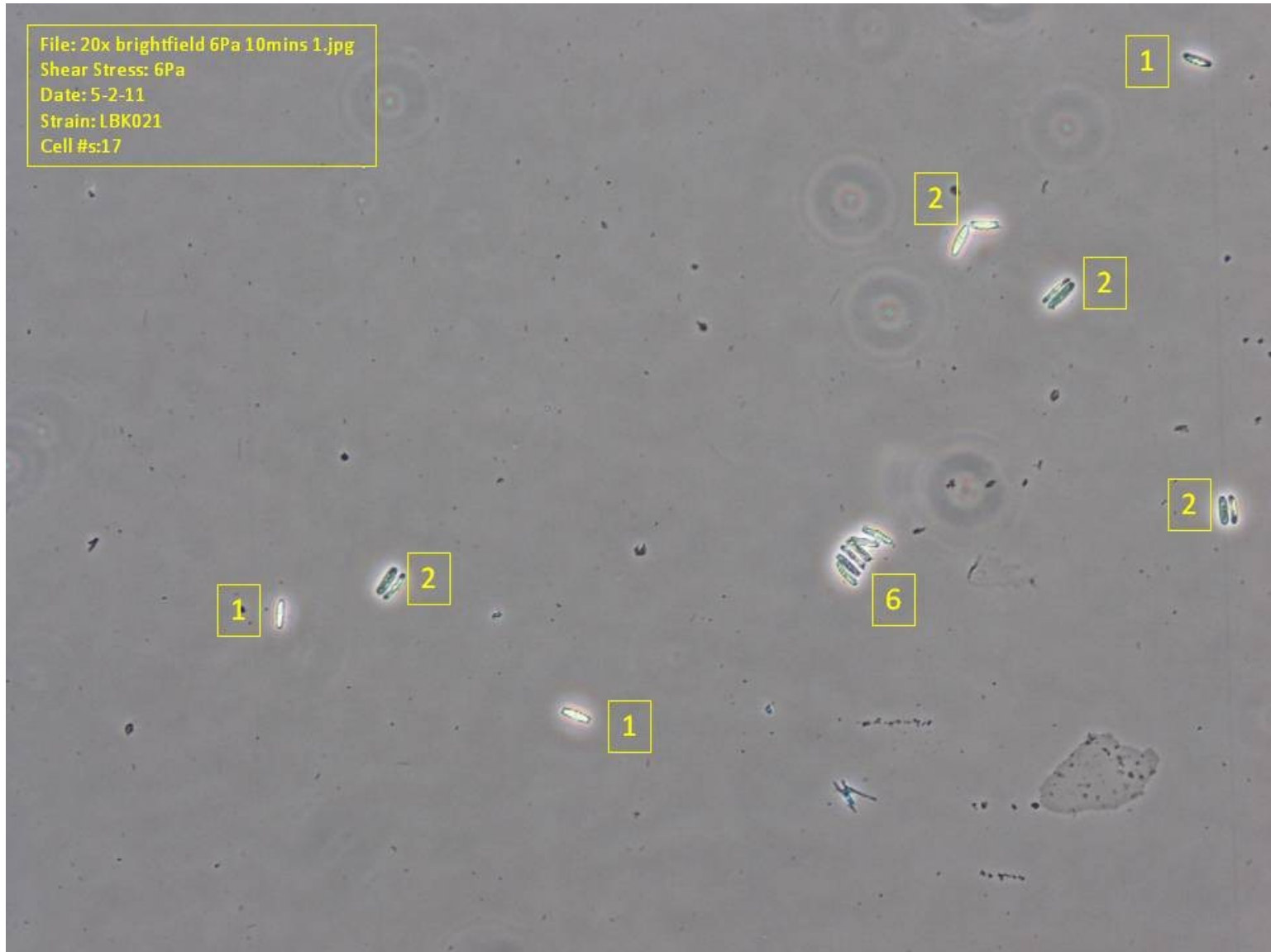




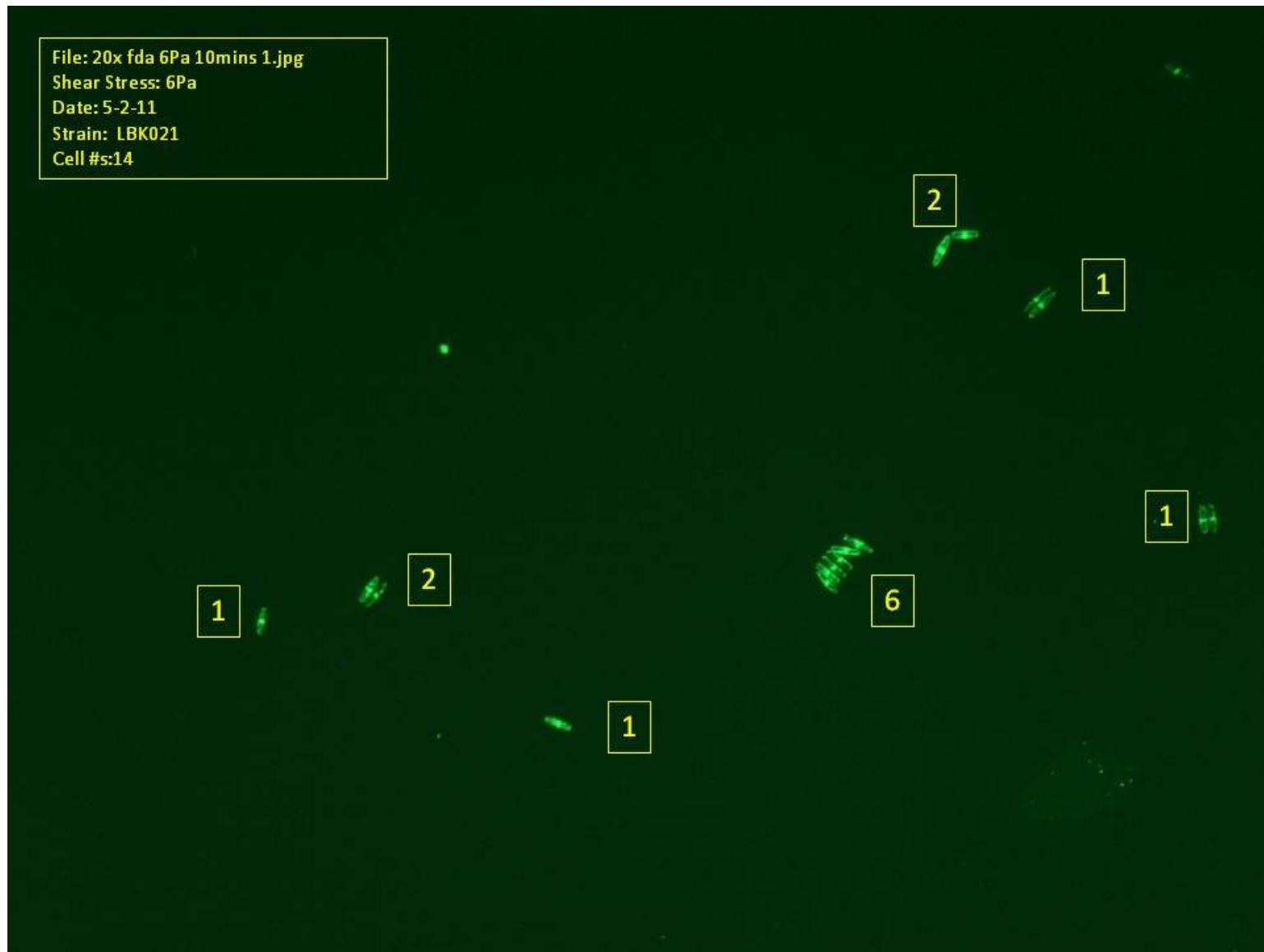


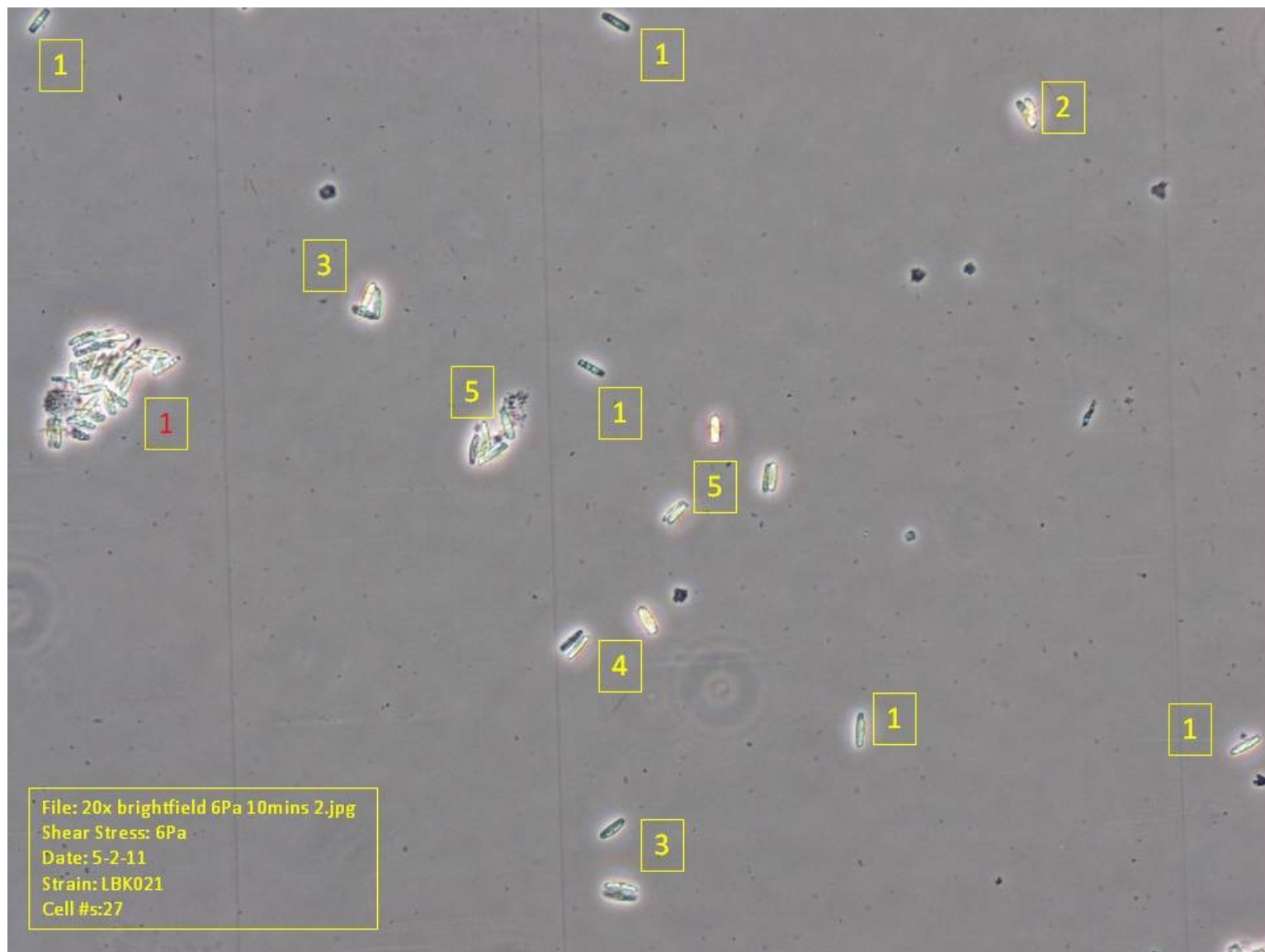


File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:17

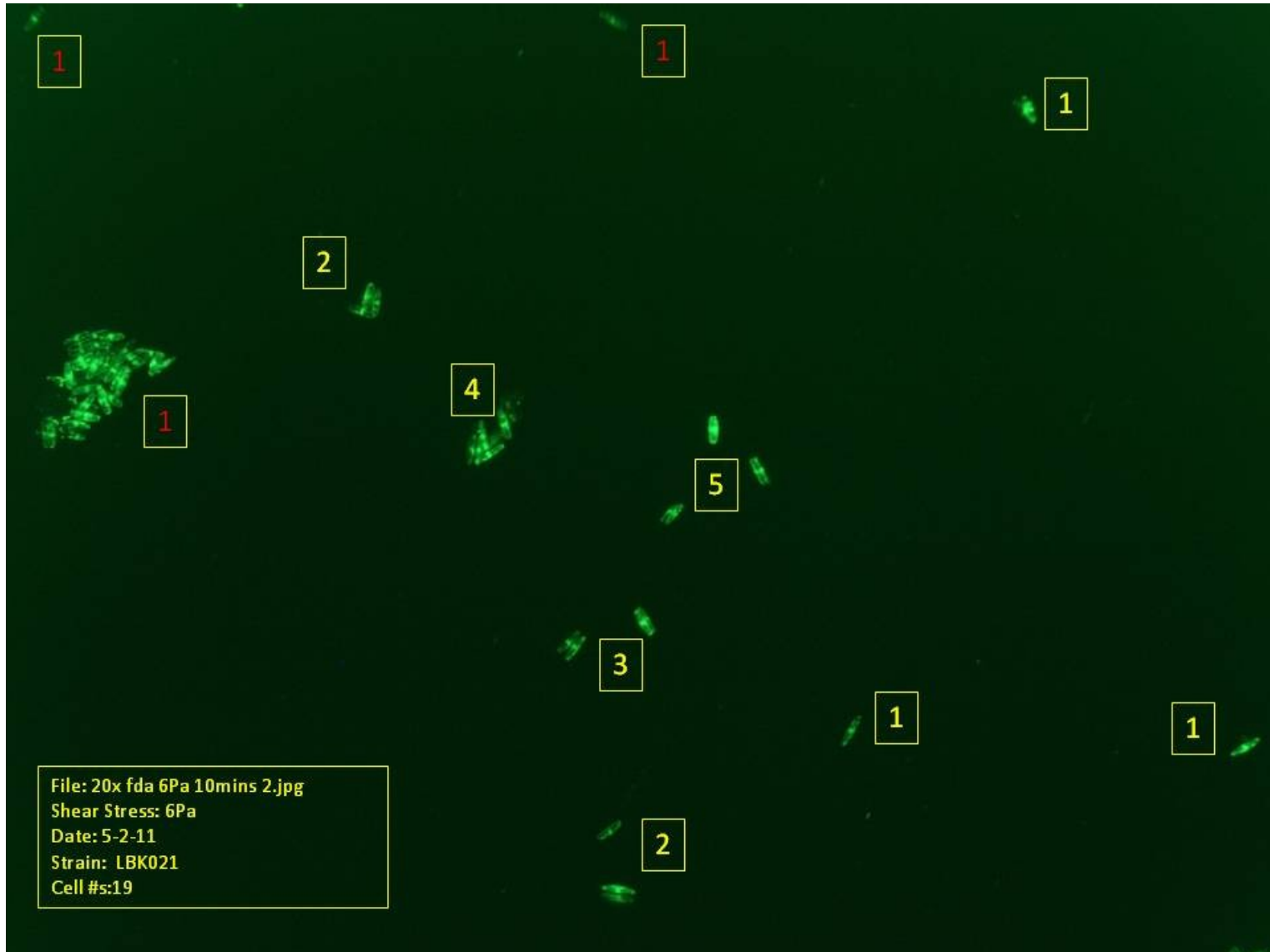


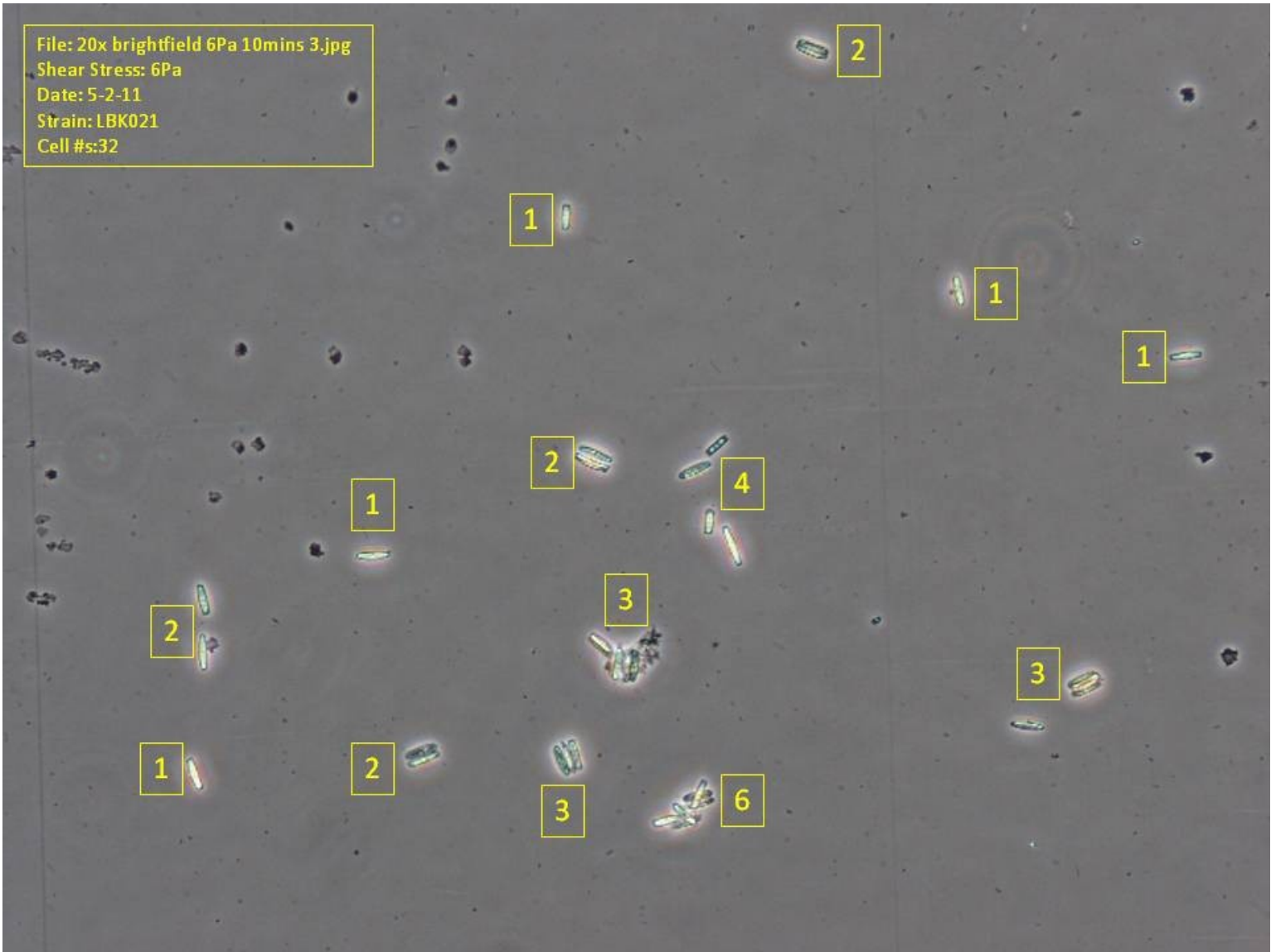
File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:14

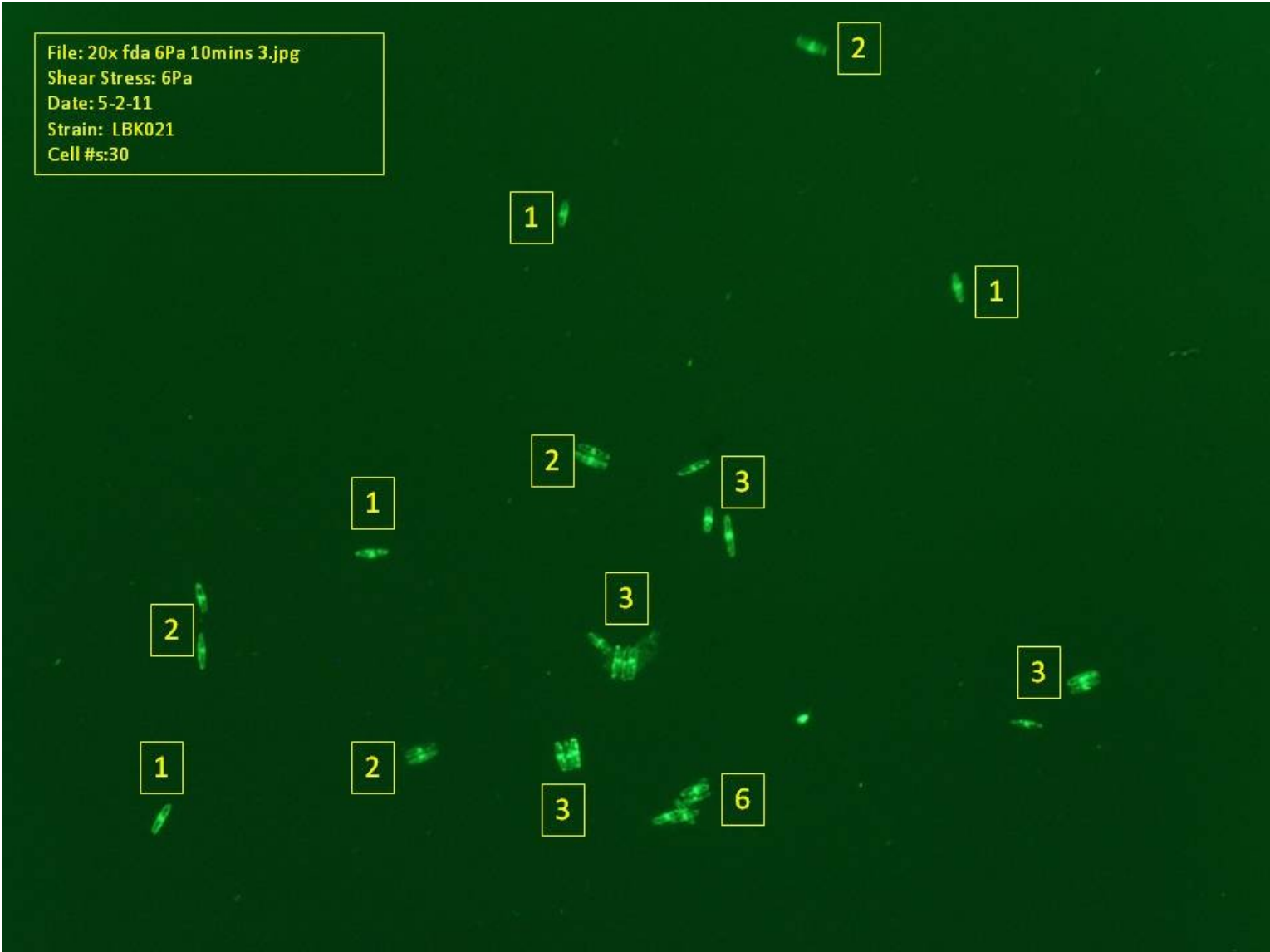


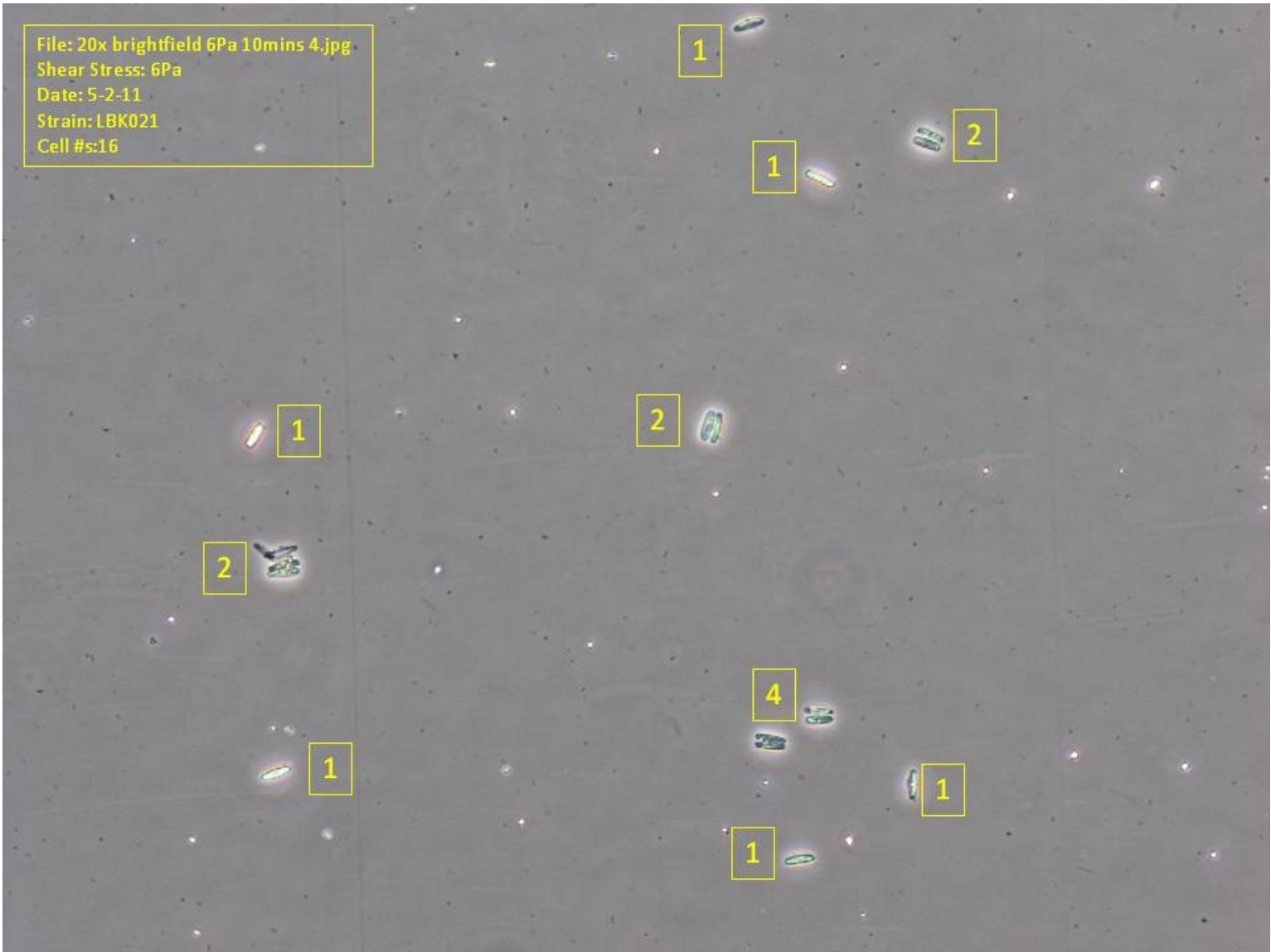


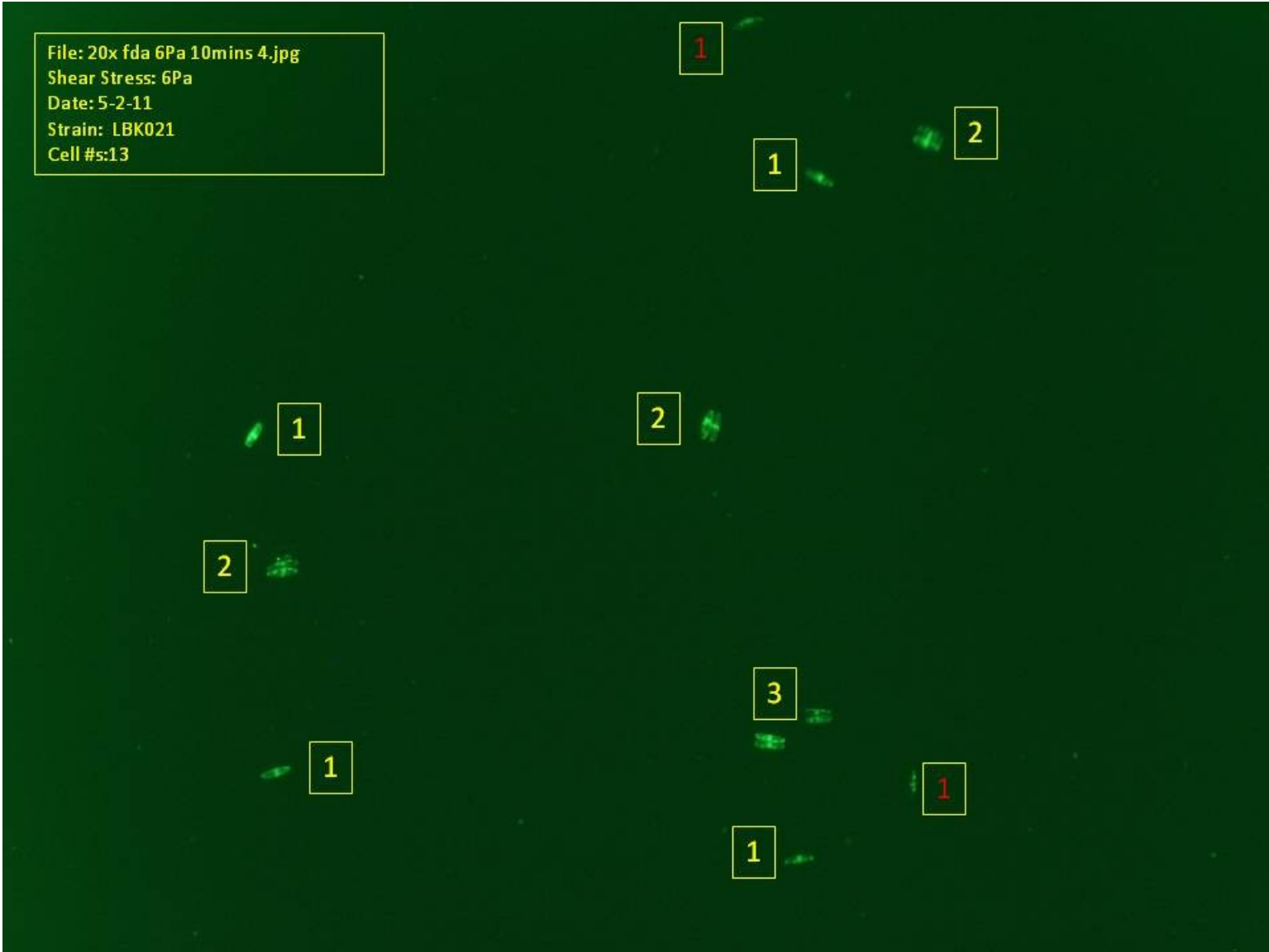


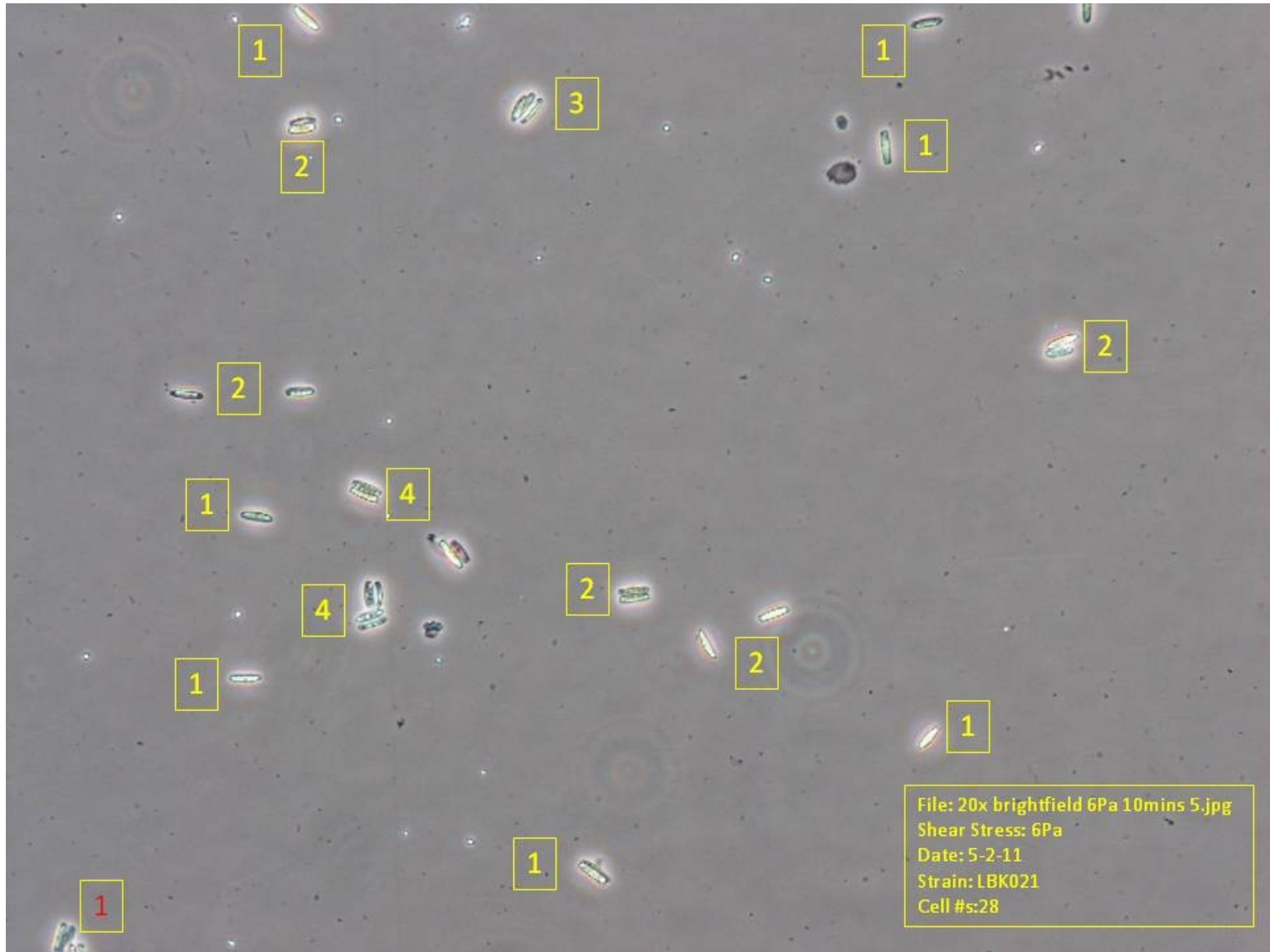


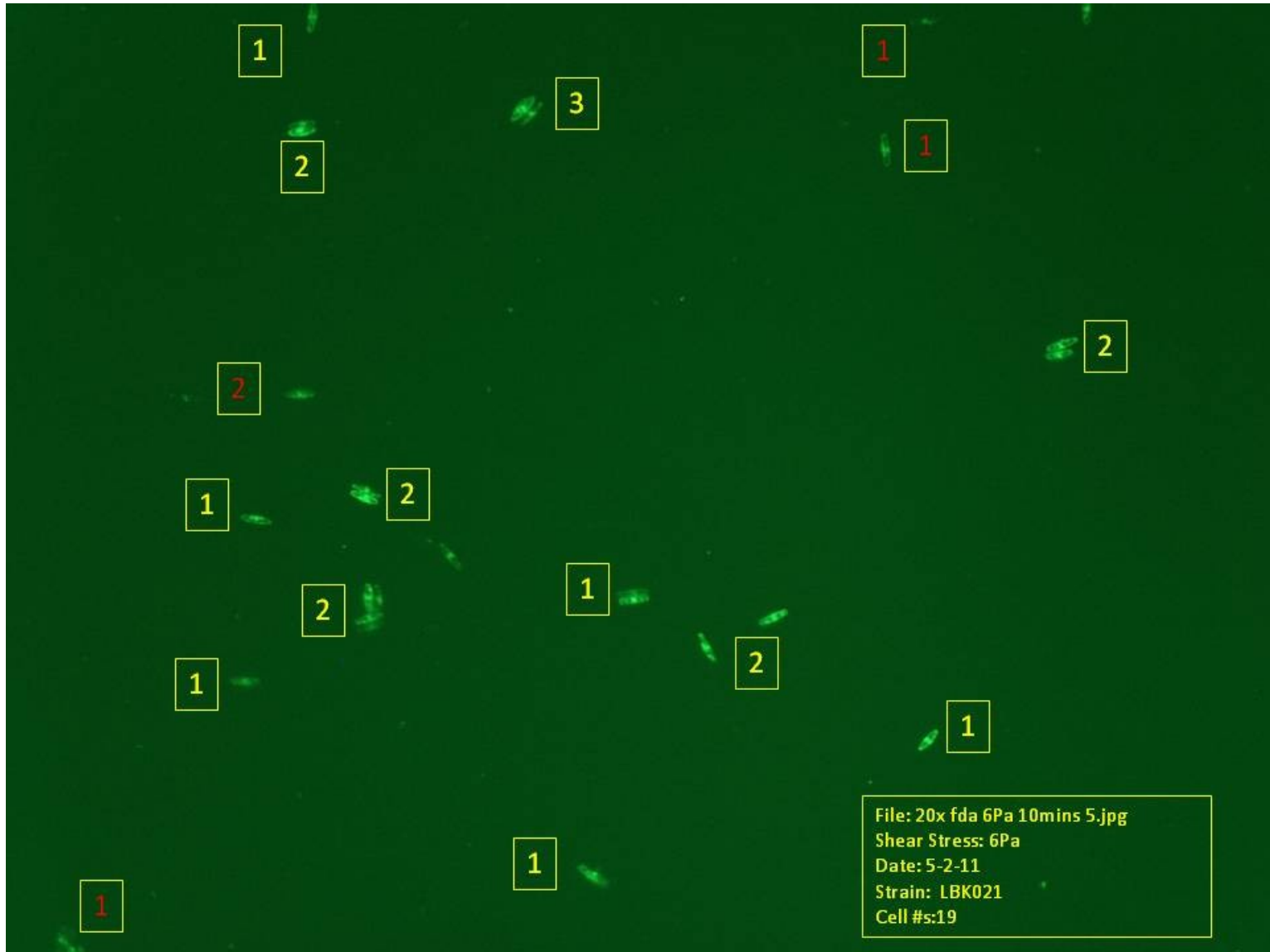


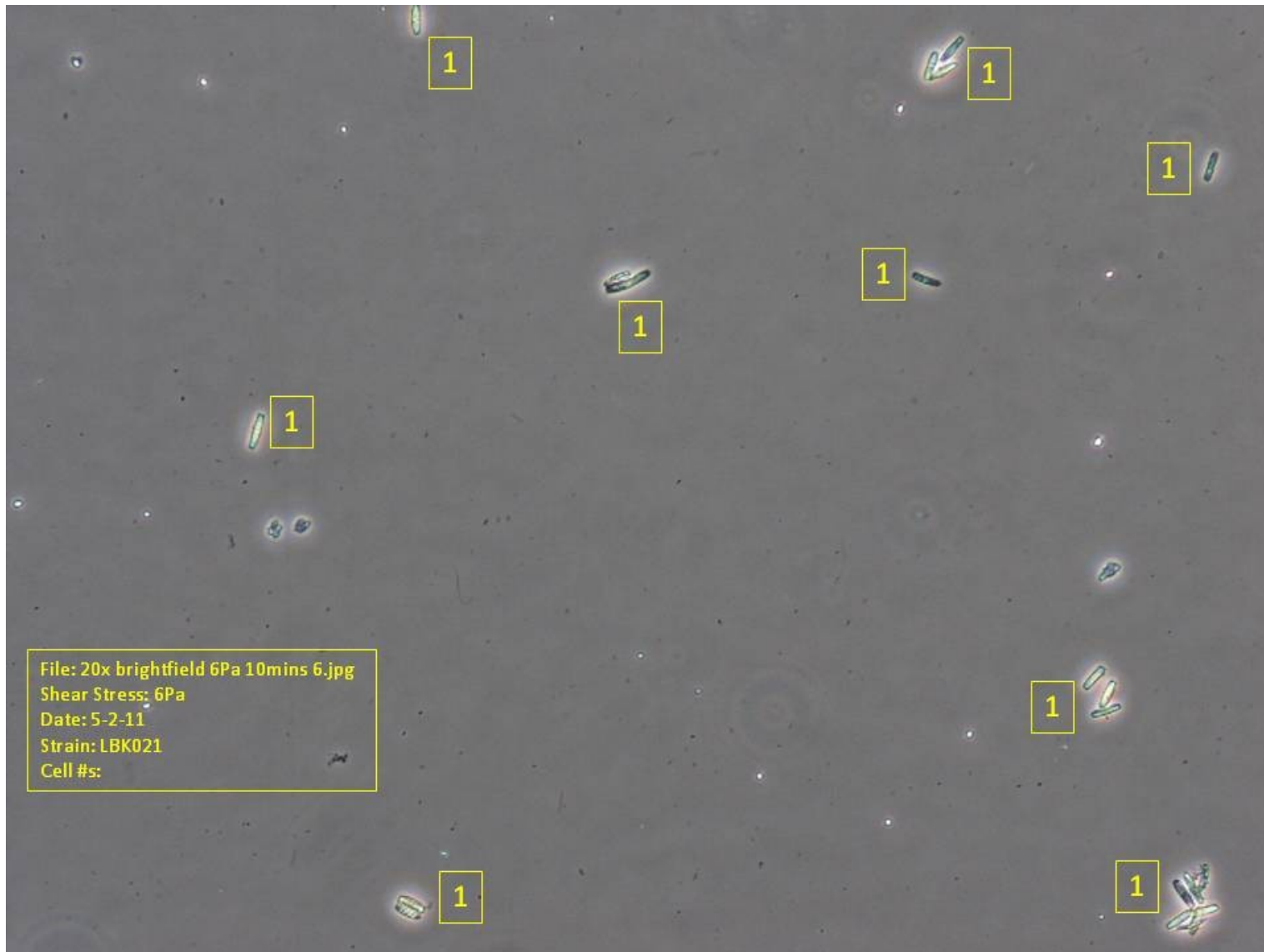






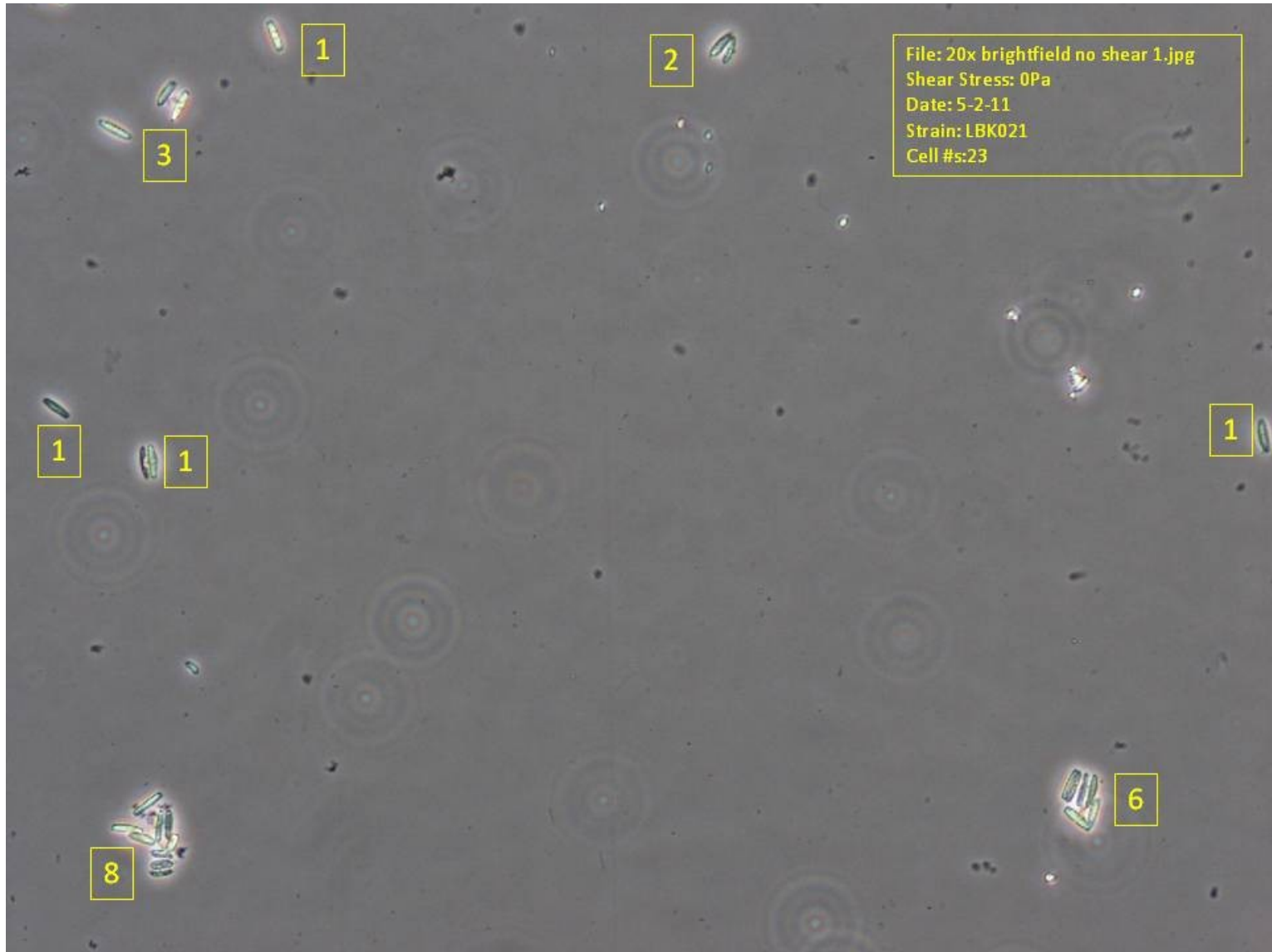


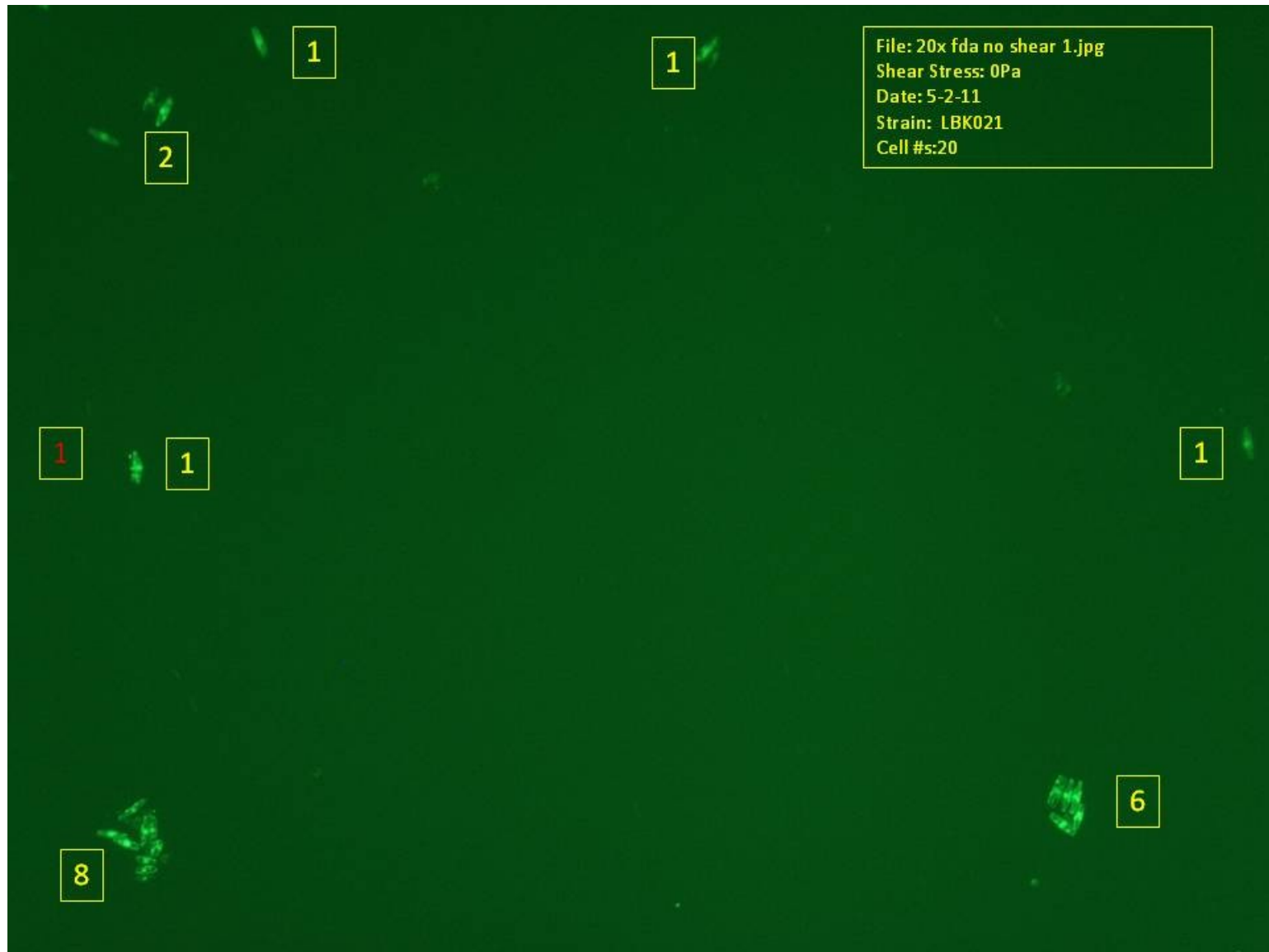




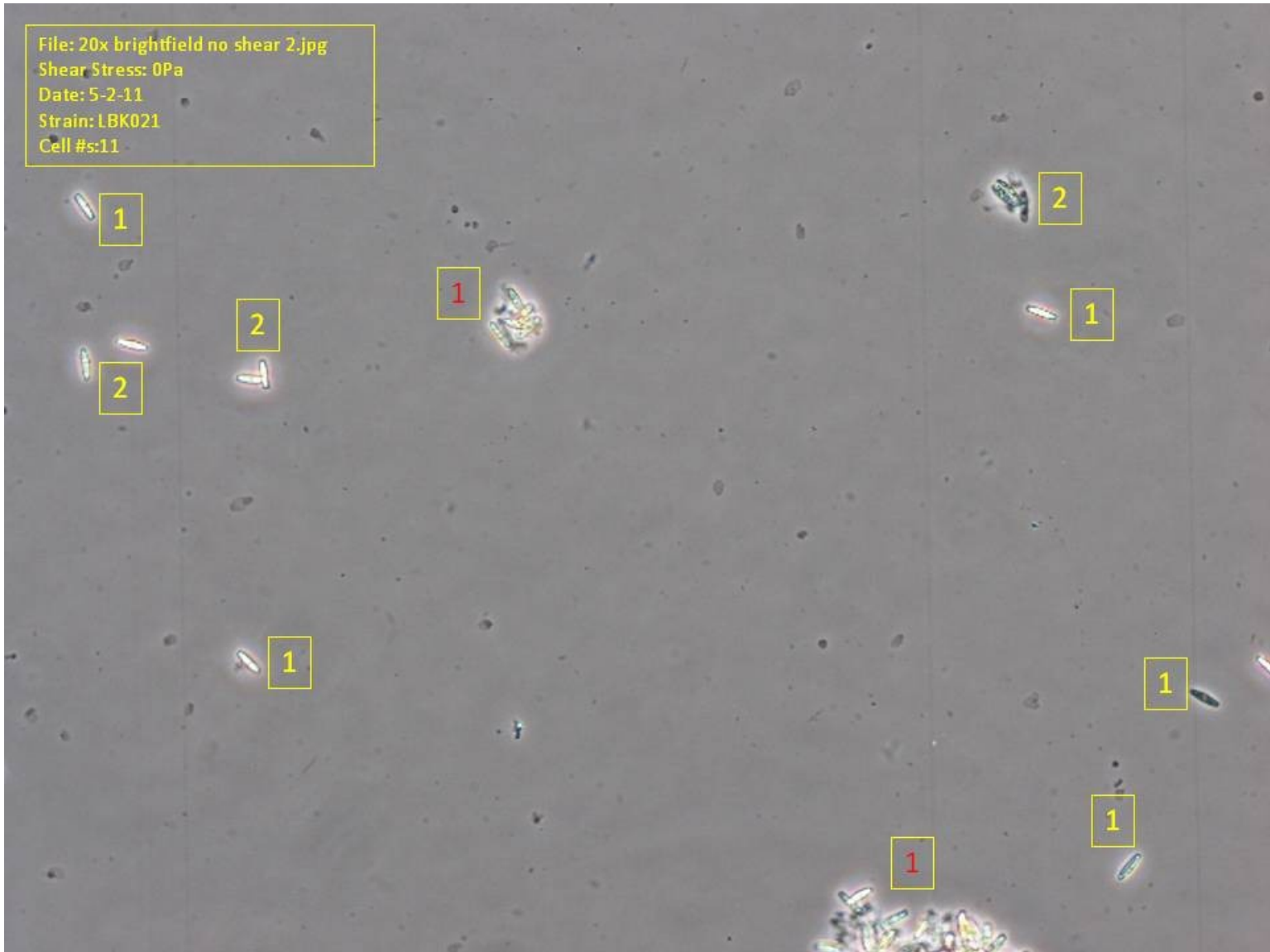




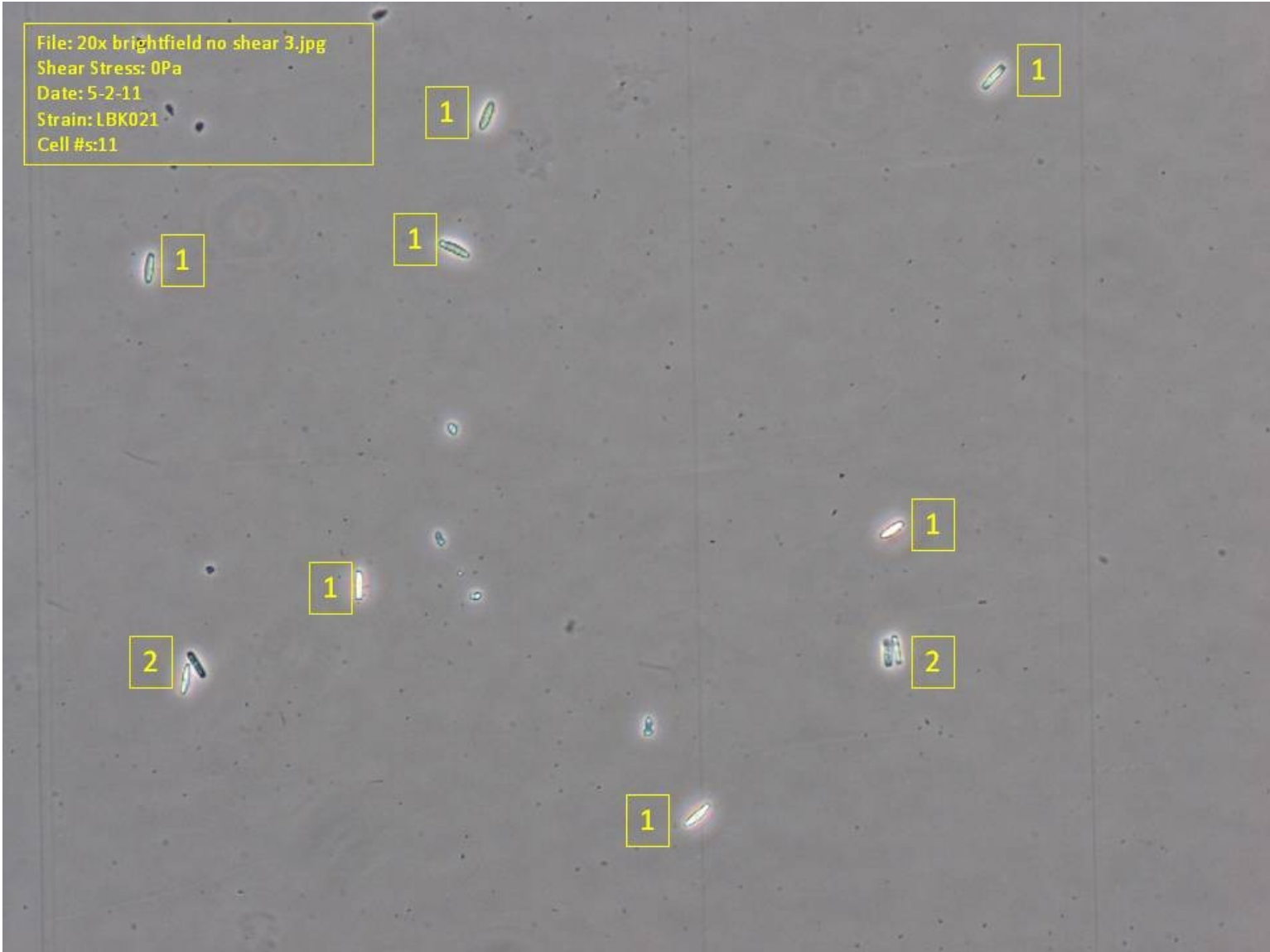




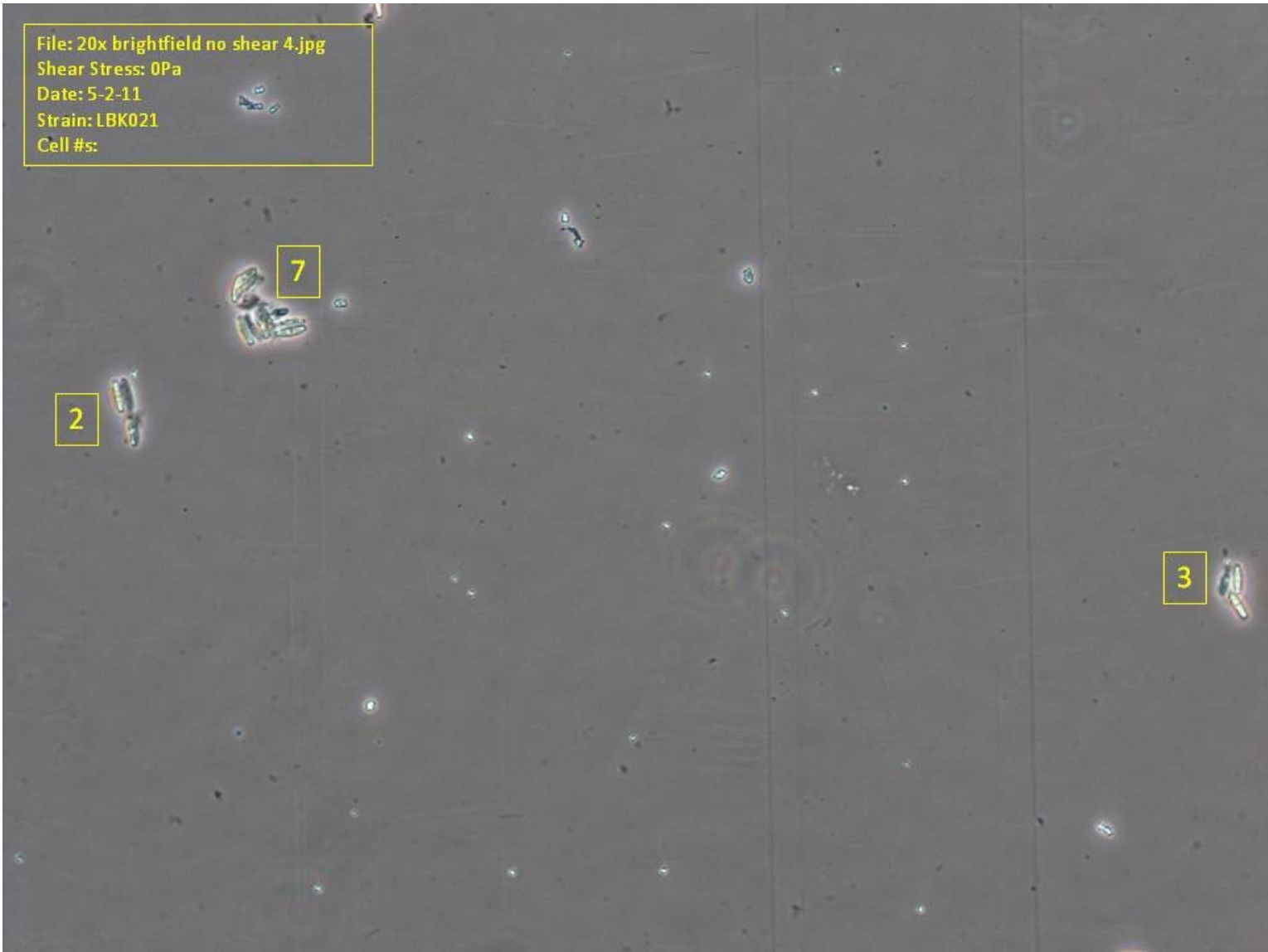
File: 20x brightfield no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:11











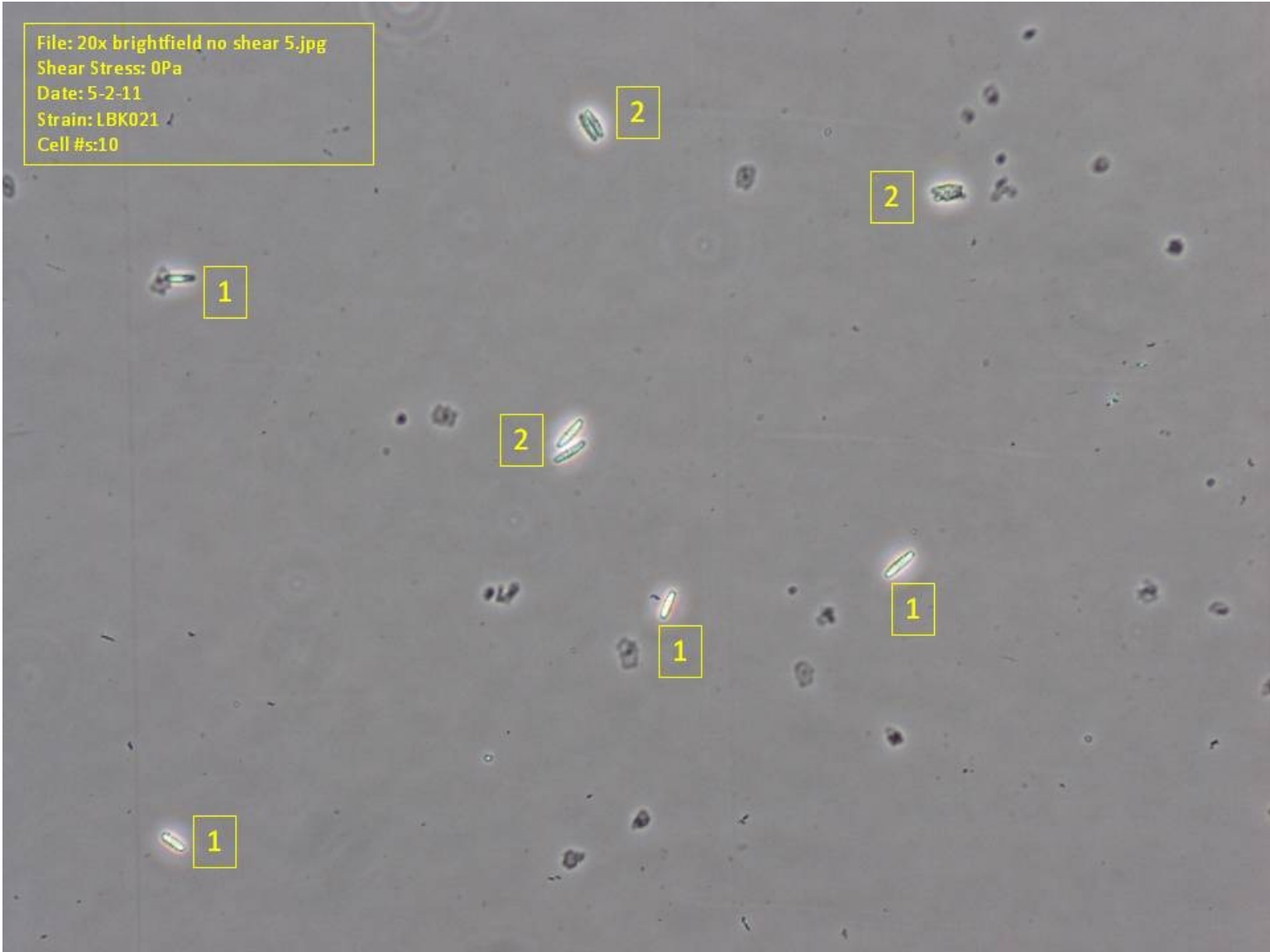


File: 20x fda no shear 4.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:

2

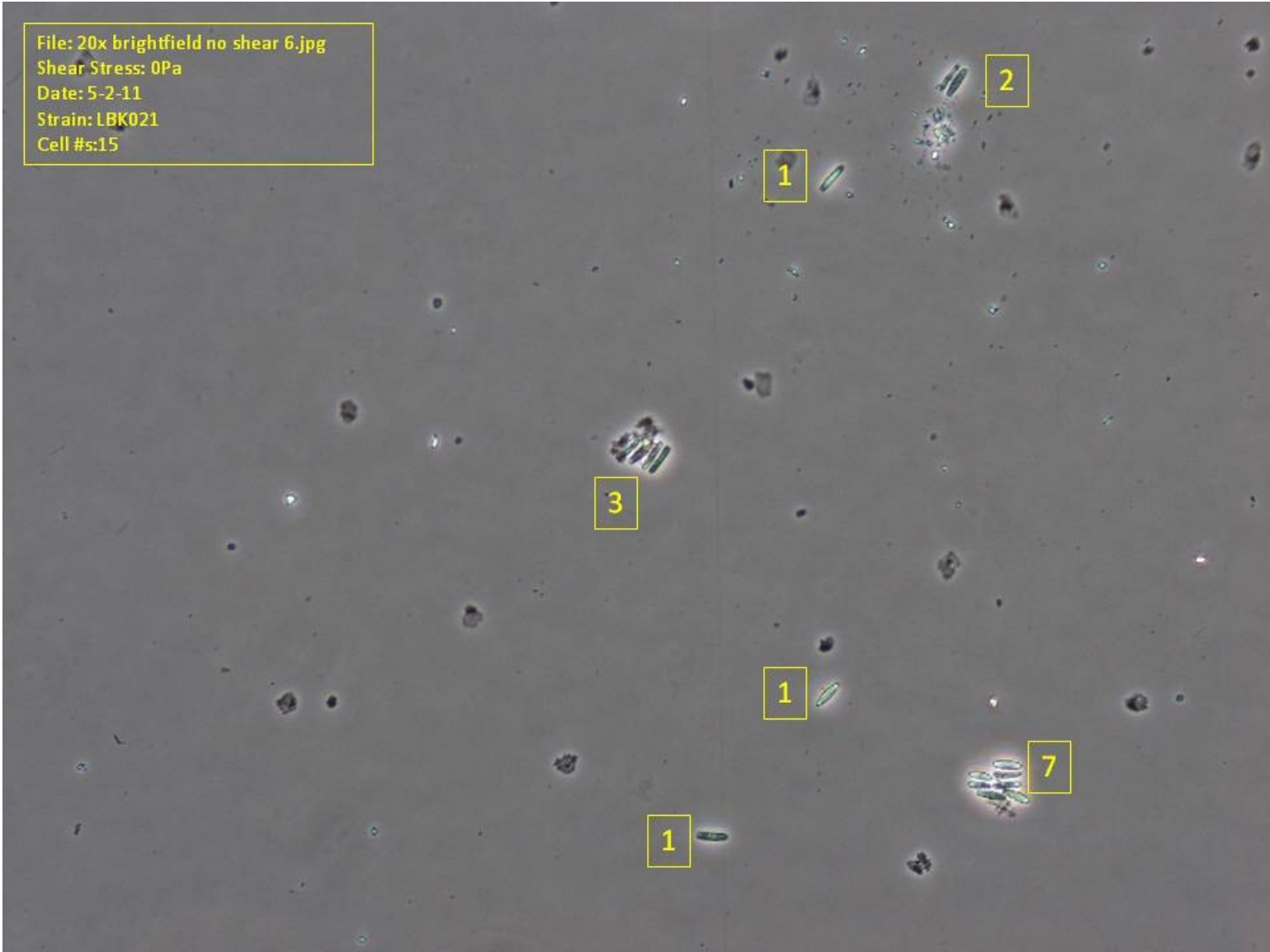
6

3



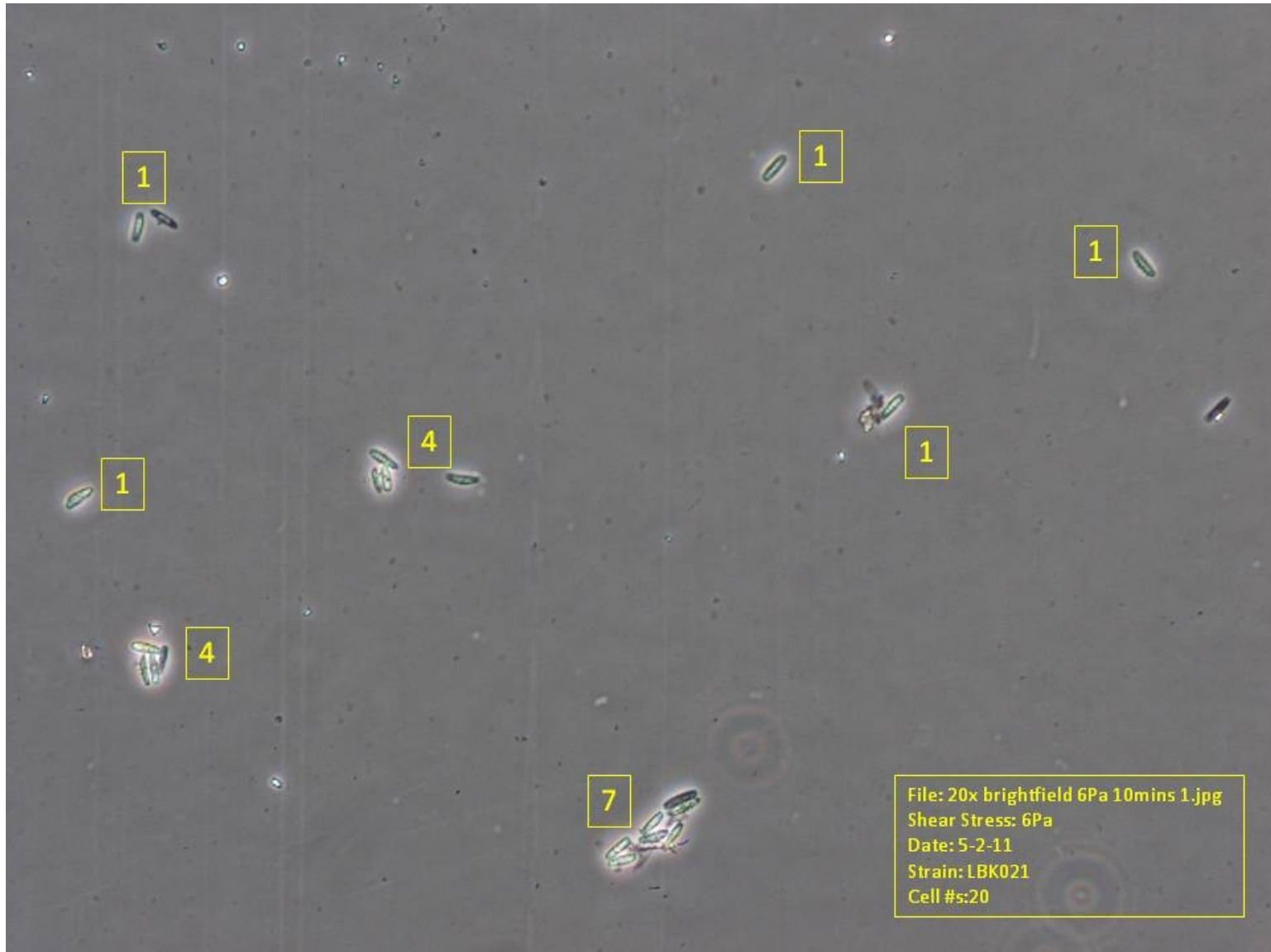


File: 20x brightfield no shear 6.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:s:15



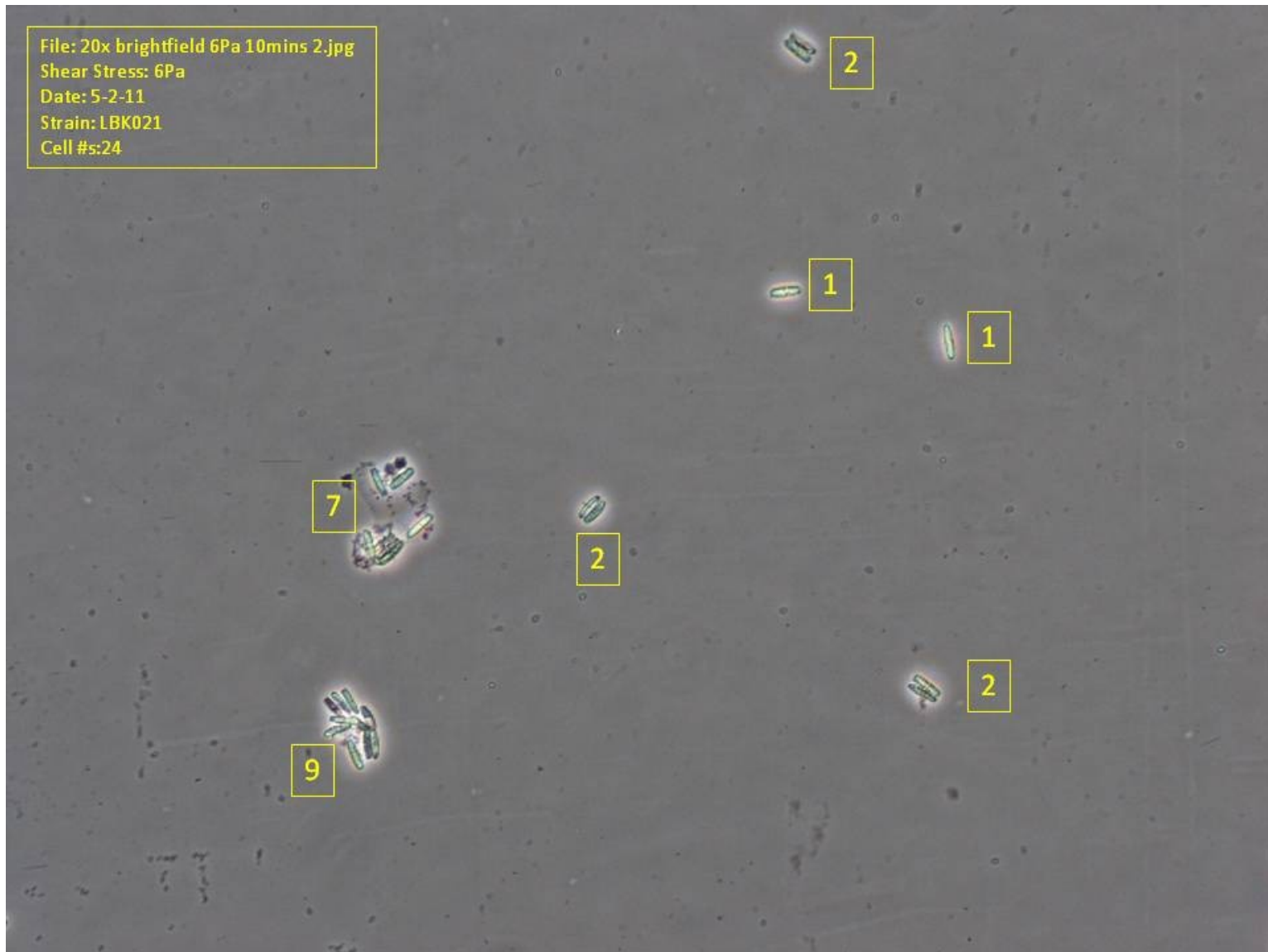
File: 20x fda no shear 6.jpg  
Shear Stress: 0Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:9



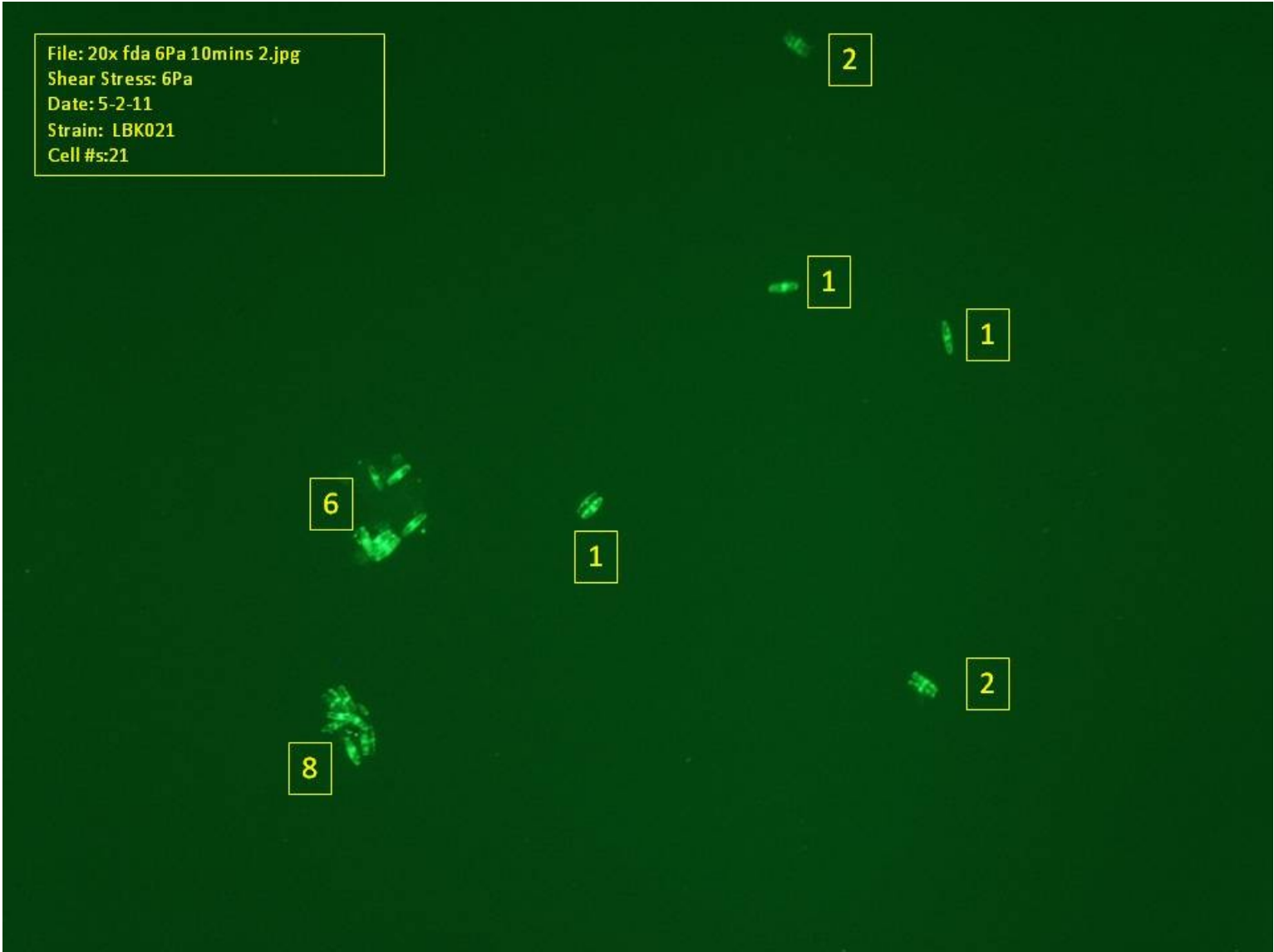




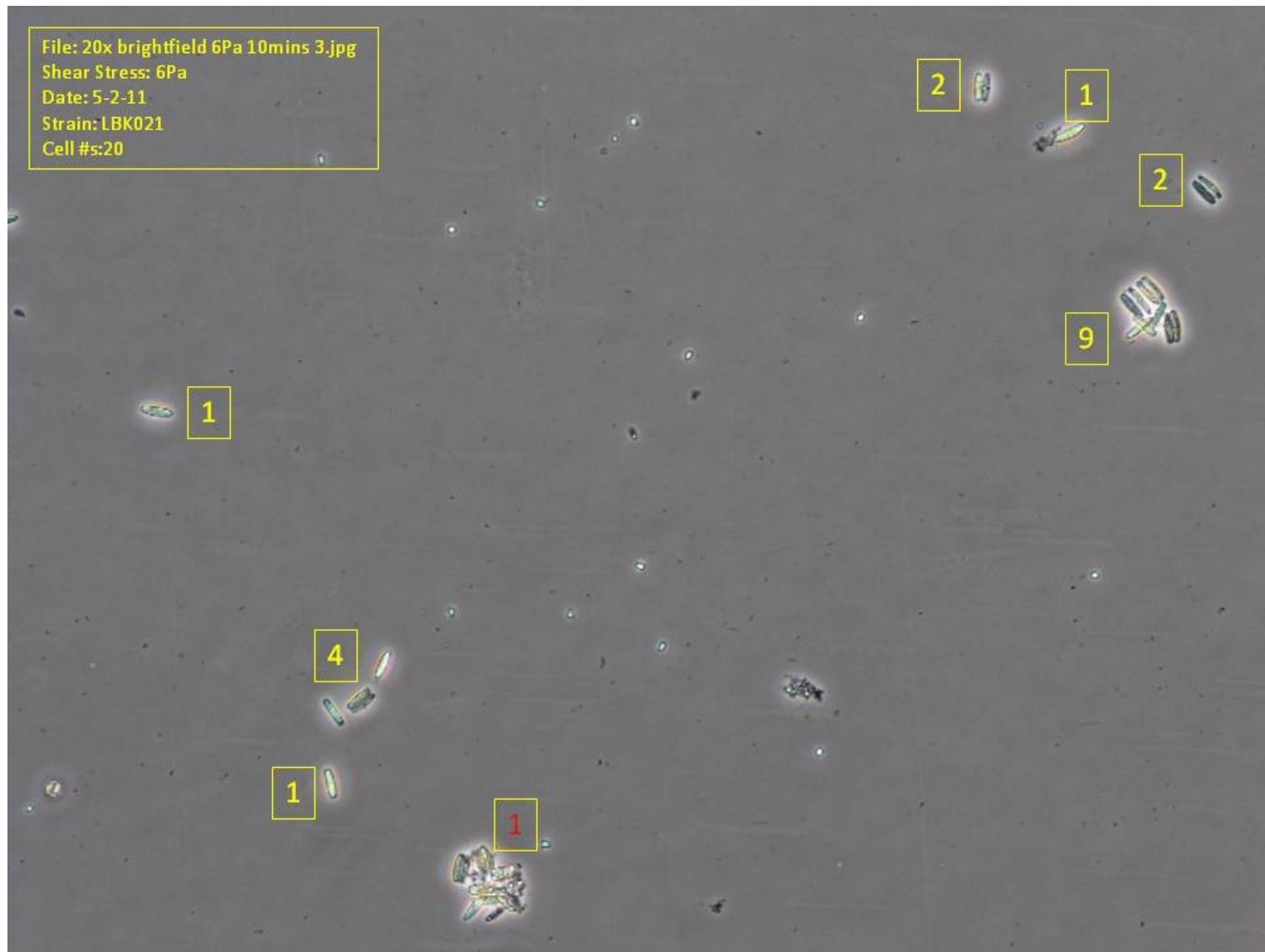
File: 20x brightfield 6Pa 10mins 2.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:s:24





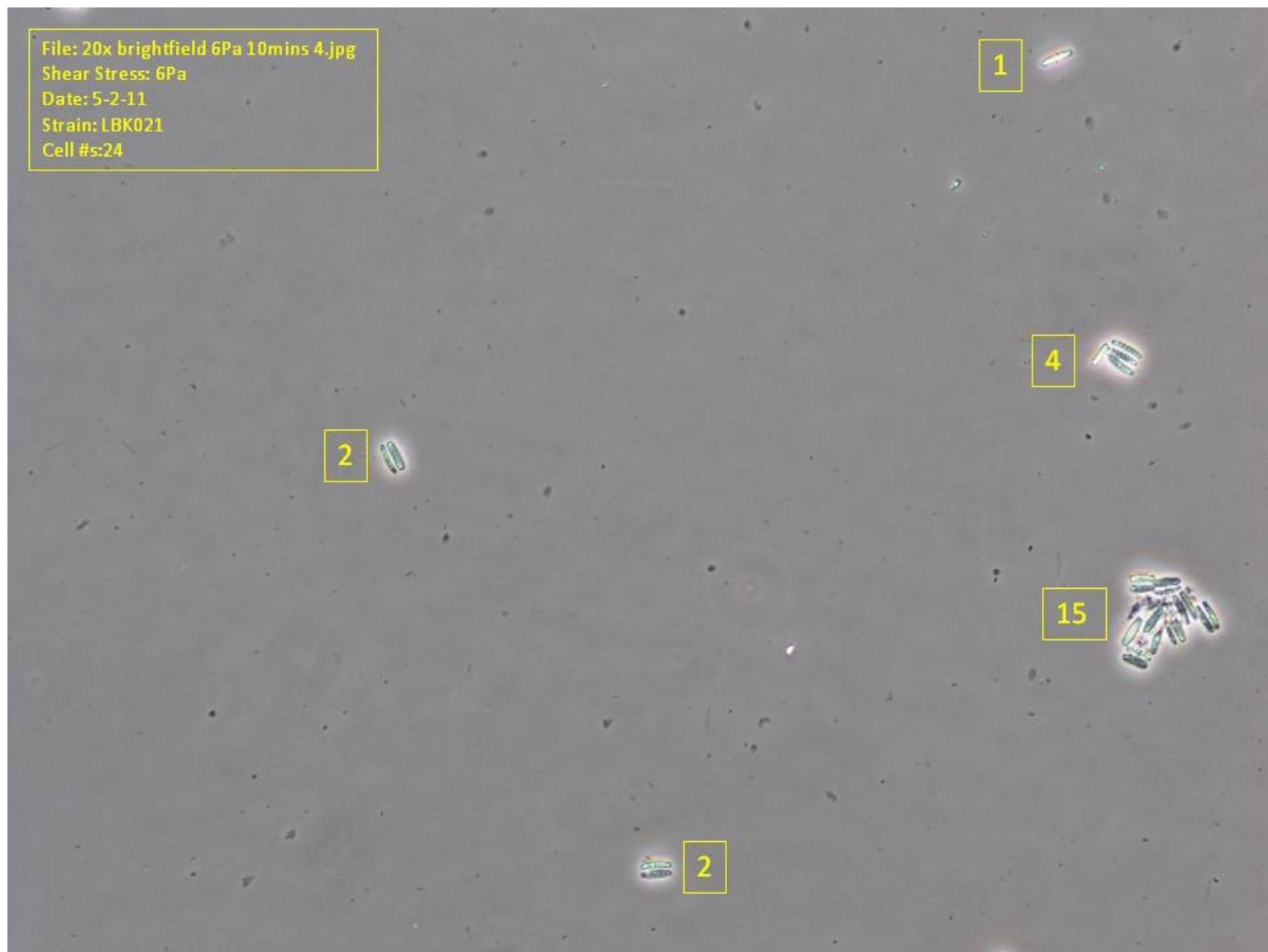


File: 20x brightfield 6Pa 10mins 3.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:20





File: 20x brightfield 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #:s:24



File: 20x fda 6Pa 10mins 4.jpg  
Shear Stress: 6Pa  
Date: 5-2-11  
Strain: LBK021  
Cell #s:15

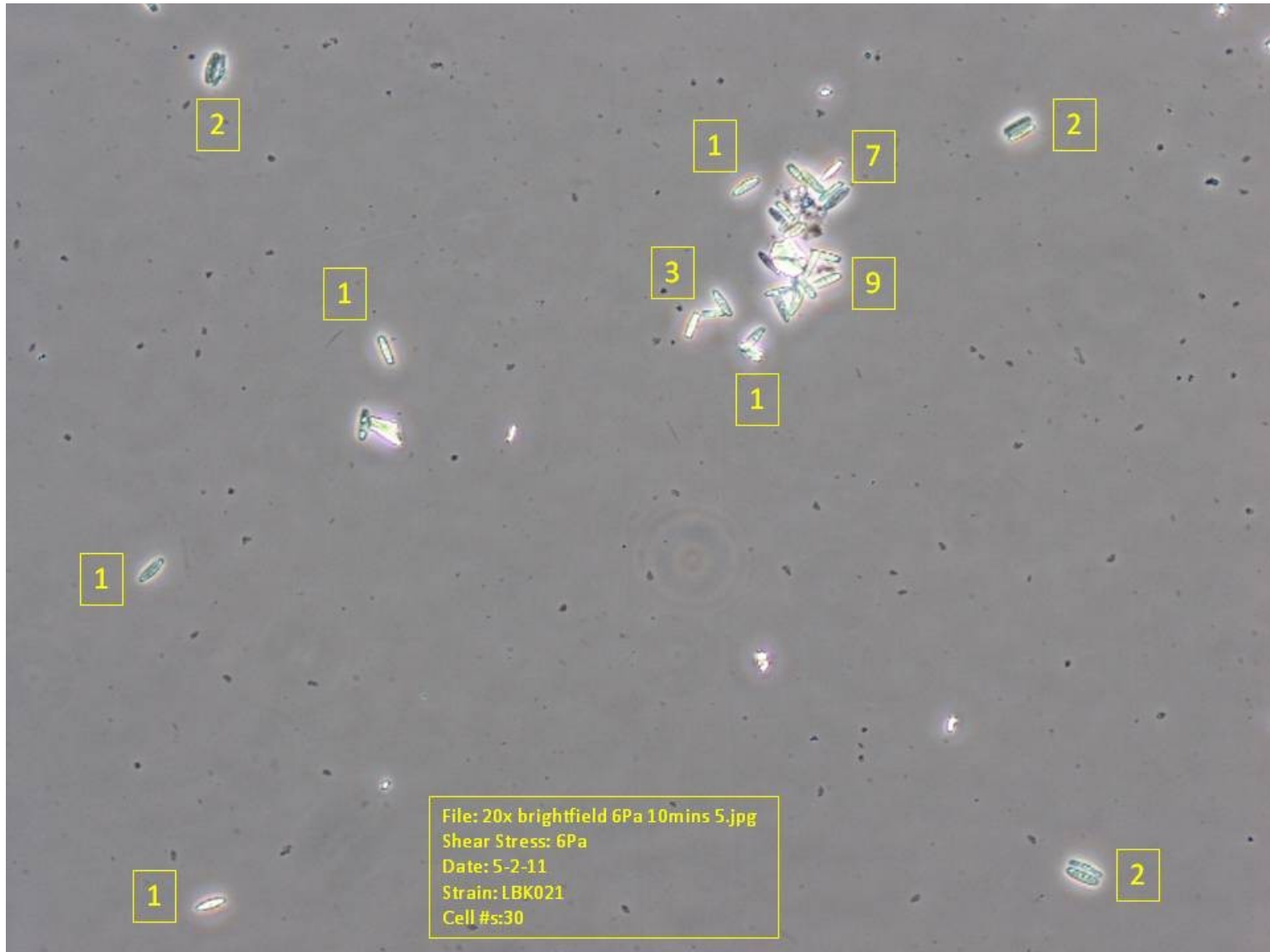
1

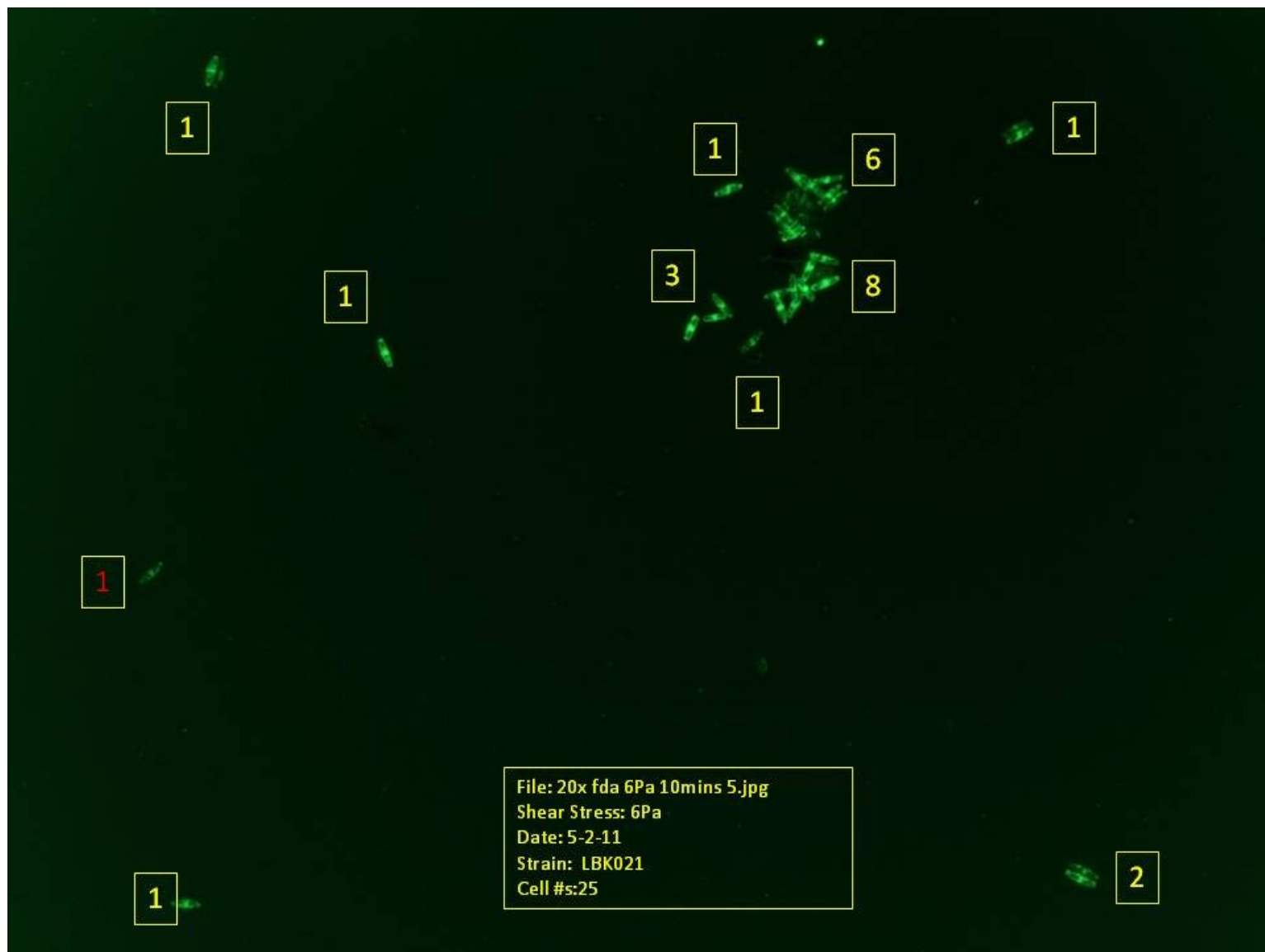
1

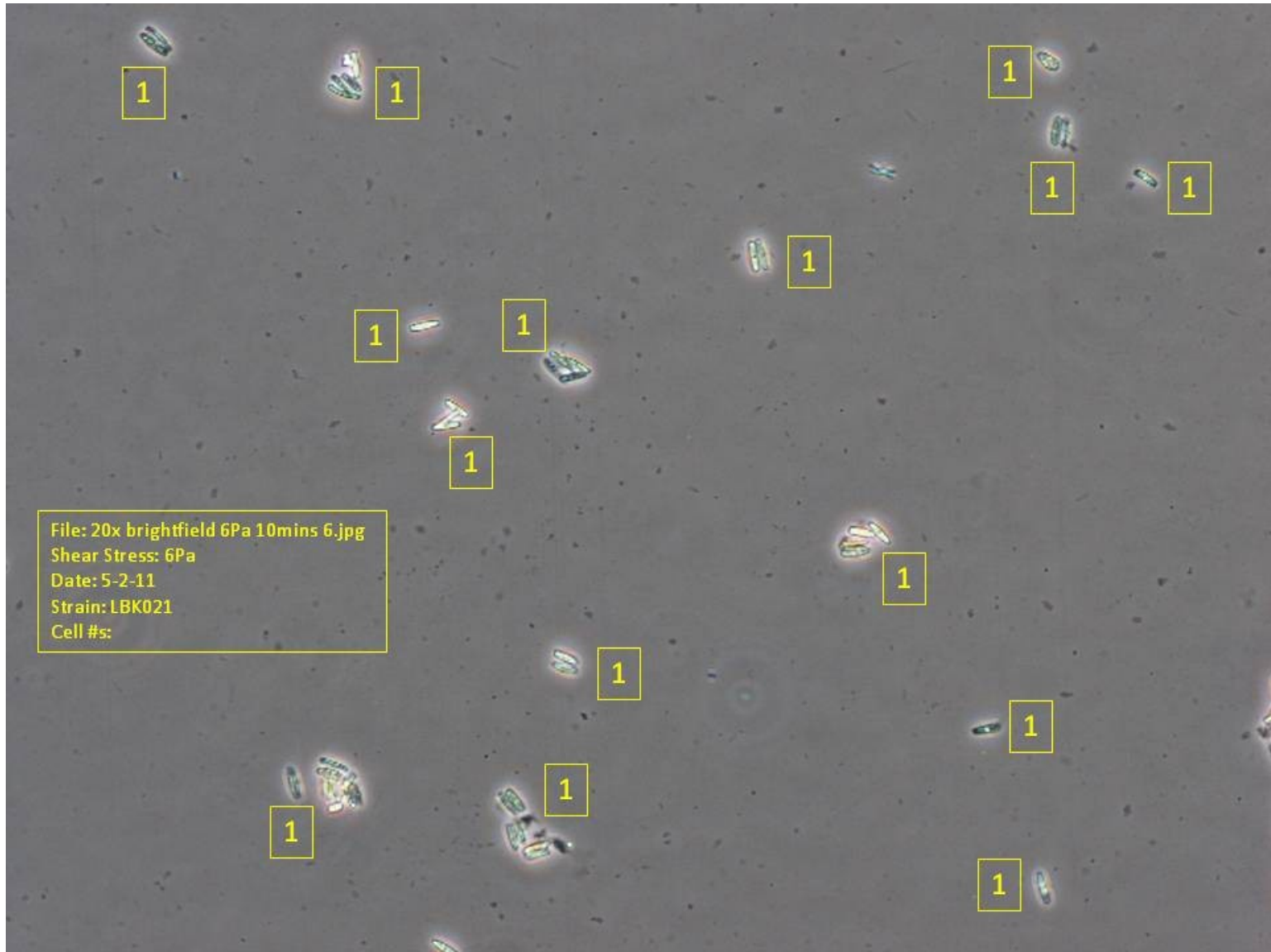
3

9

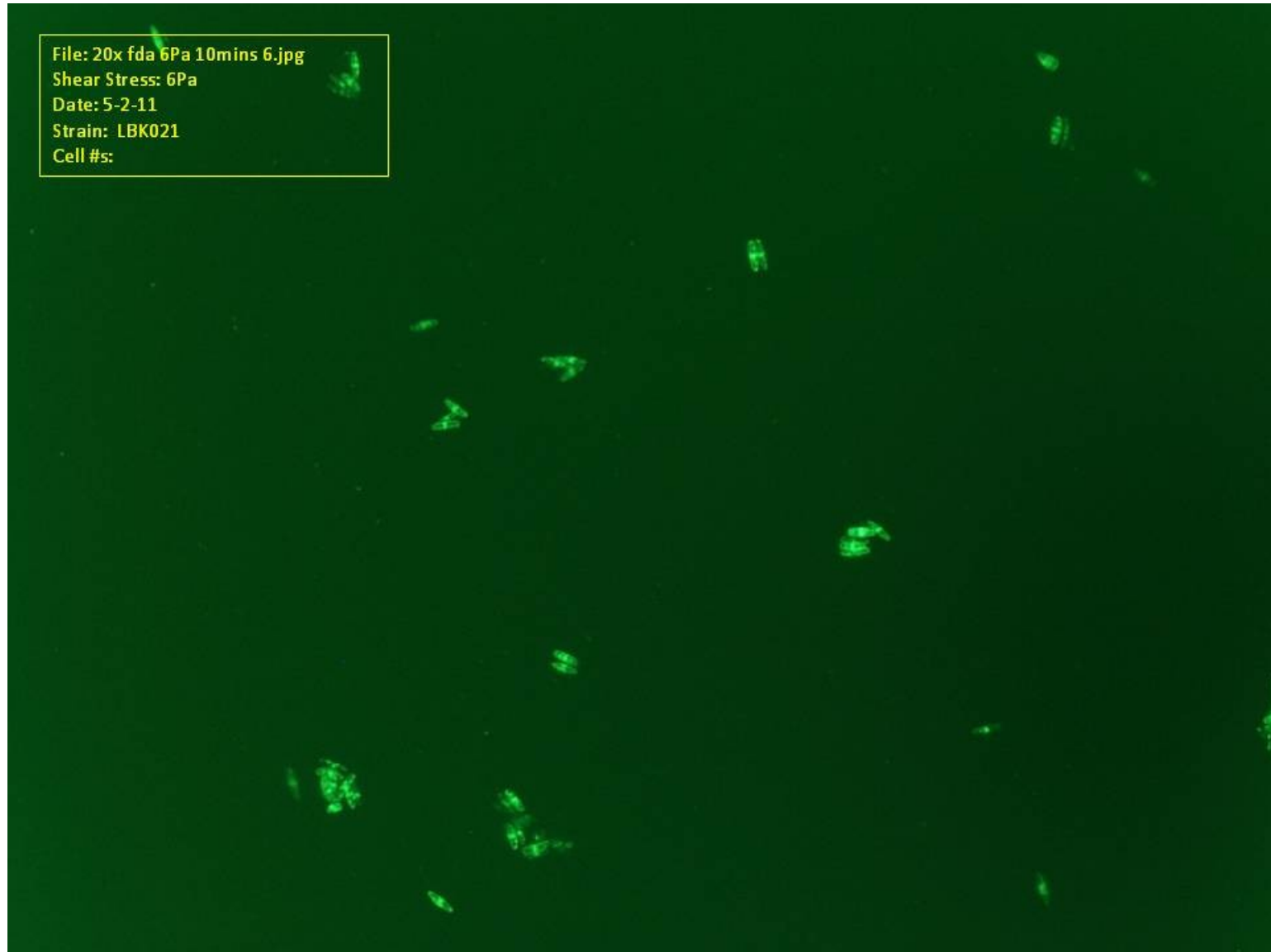
2











## APPENDIX I

The following table is the three replicate cell counts for a *Nitzschia Sp.* diatom, referred to as LBK023, at no shear stress and six Pascals of shear stress. This table provides the total cell counts under white light and the corresponding live cell count as seen under fluorescent light after staining with fluorescein diacetate. An average cell viability was calculated at each shear stress level and a corresponding average decrease between no shear and six Pascals of shear stress. The slope calculated from the average cell viability numbers was used to statistically determine if shear stress impacted cell viability. An average decrease was calculated to determine which of the nine species was most resistant to shear stress. As a note, if a site does not have counts either five sites had already been counted so the sixth was unnecessary or the site was not able to be counted due to clarity of the image.

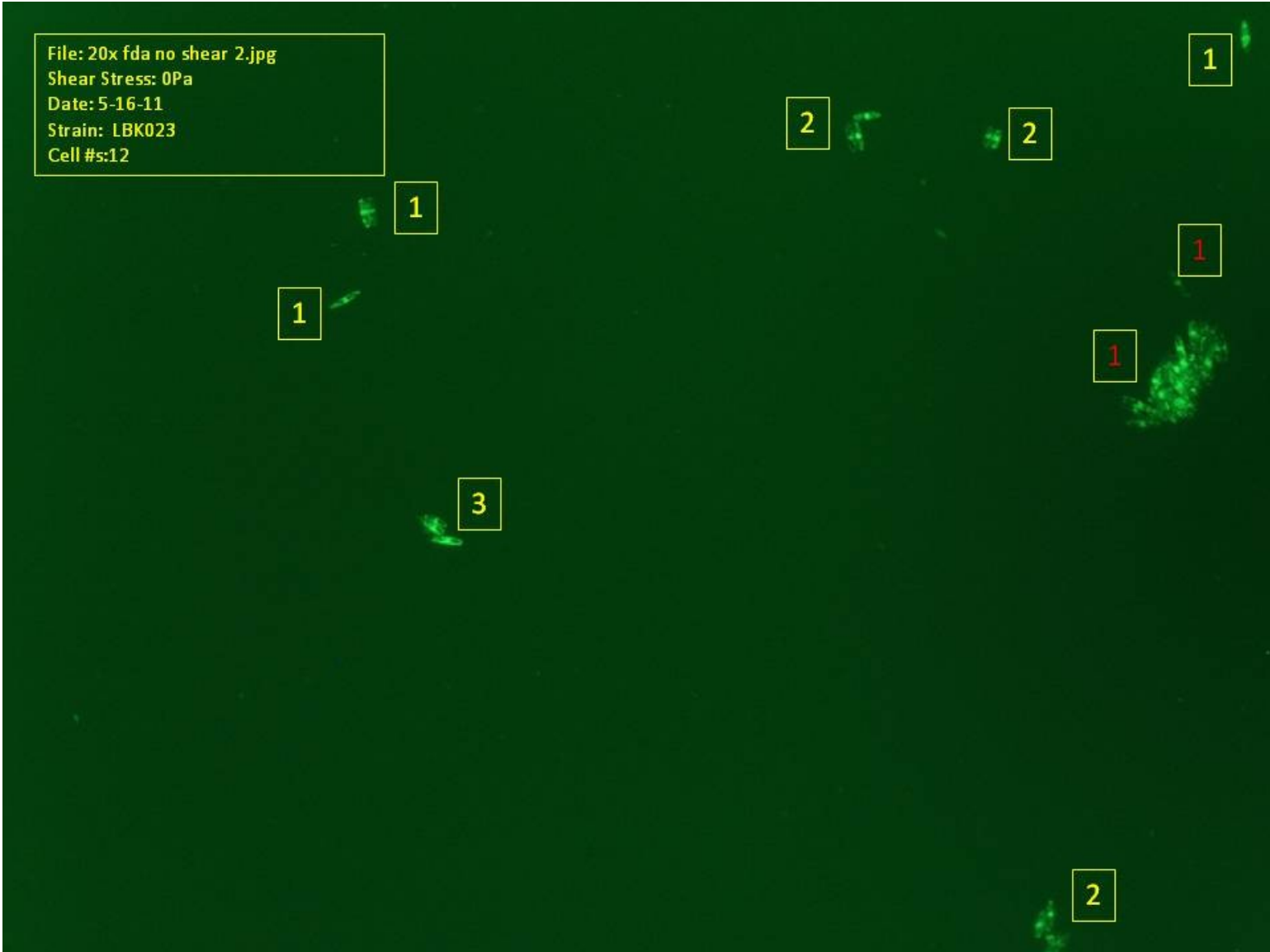
	Shear Stress (Pa)	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Avg. Cell Viability (%)	Avg. Decrease	Slope
		Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells	Total Cells	Live Cells			
Rep 1	0	7	6	17	12	8	6	10	6			9	7	72.55%	4.59%	-0.00765
	6	11	10	15	11	31	20	13	7			33	22	67.96%		
Rep 2	0	13	11	7	6	13	10			10	8			81.40%	11.65%	-0.01941
	6	33	28	13	9	23	13	24	19	15	8	11	6	69.75%		
Rep 3	0	10	10	27	26	27	23	23	20	12	10	10	6	87.16%	15.36%	-0.02560
	6	15	11	13	10			11	8	10	7	29	20	71.79%		
														<b>Avg.</b>	<b>10.53%</b>	
														<b>Std. Dev.</b>	<b>5.47%</b>	

The counts in the above table come from the images following. The order for these images is the white light image followed by its paired fluorescent light image. The species name, date, shear stress level, and cell count are located in a box on the image. The images are grouped in twelves, with the first group corresponding to the no stress test of replicate one; the six Pascal shear stress level of replicate one follows. This pattern is repeated for replicates two and three.









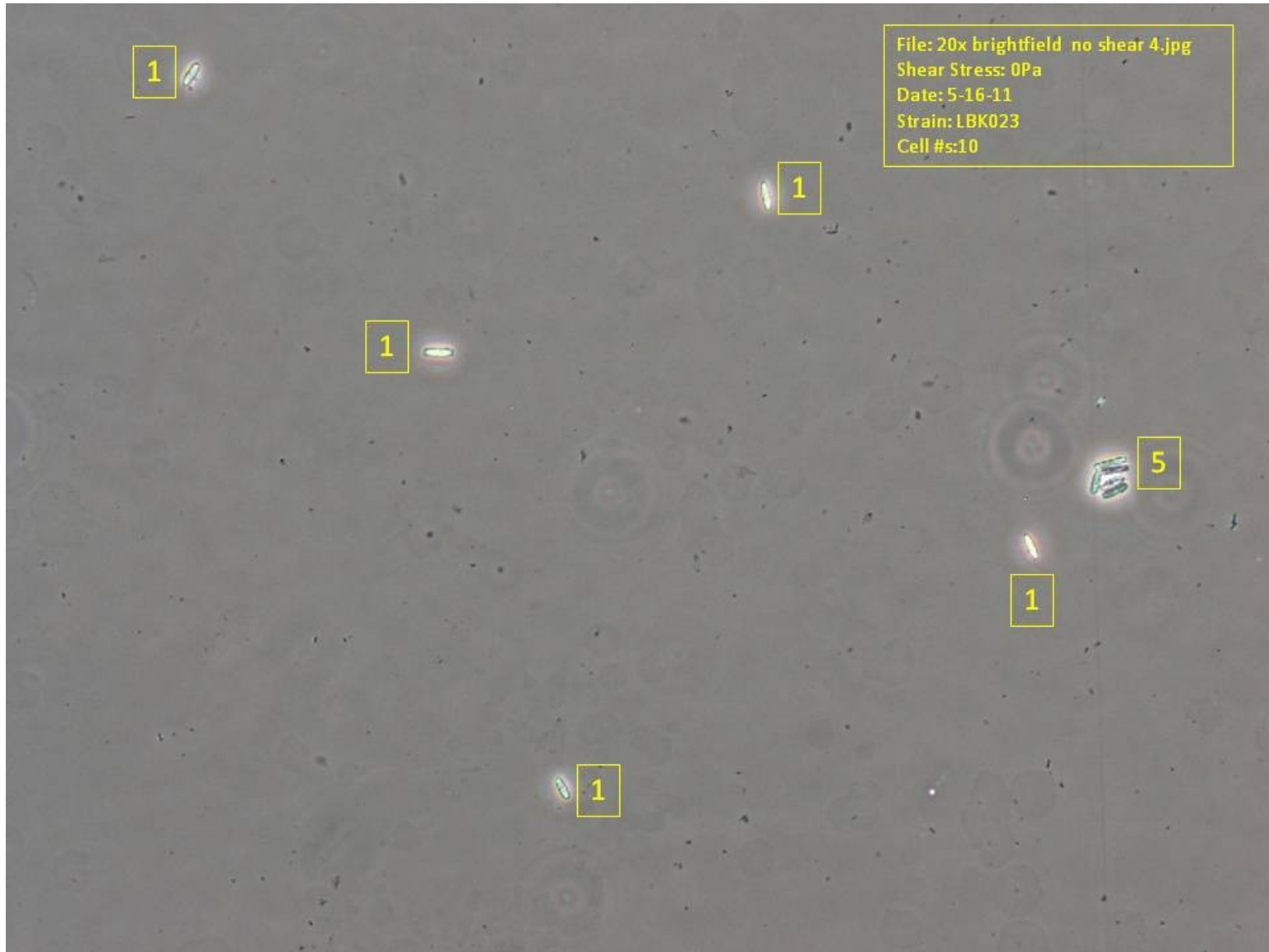
File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #s:8



File: 20x fda no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #:6

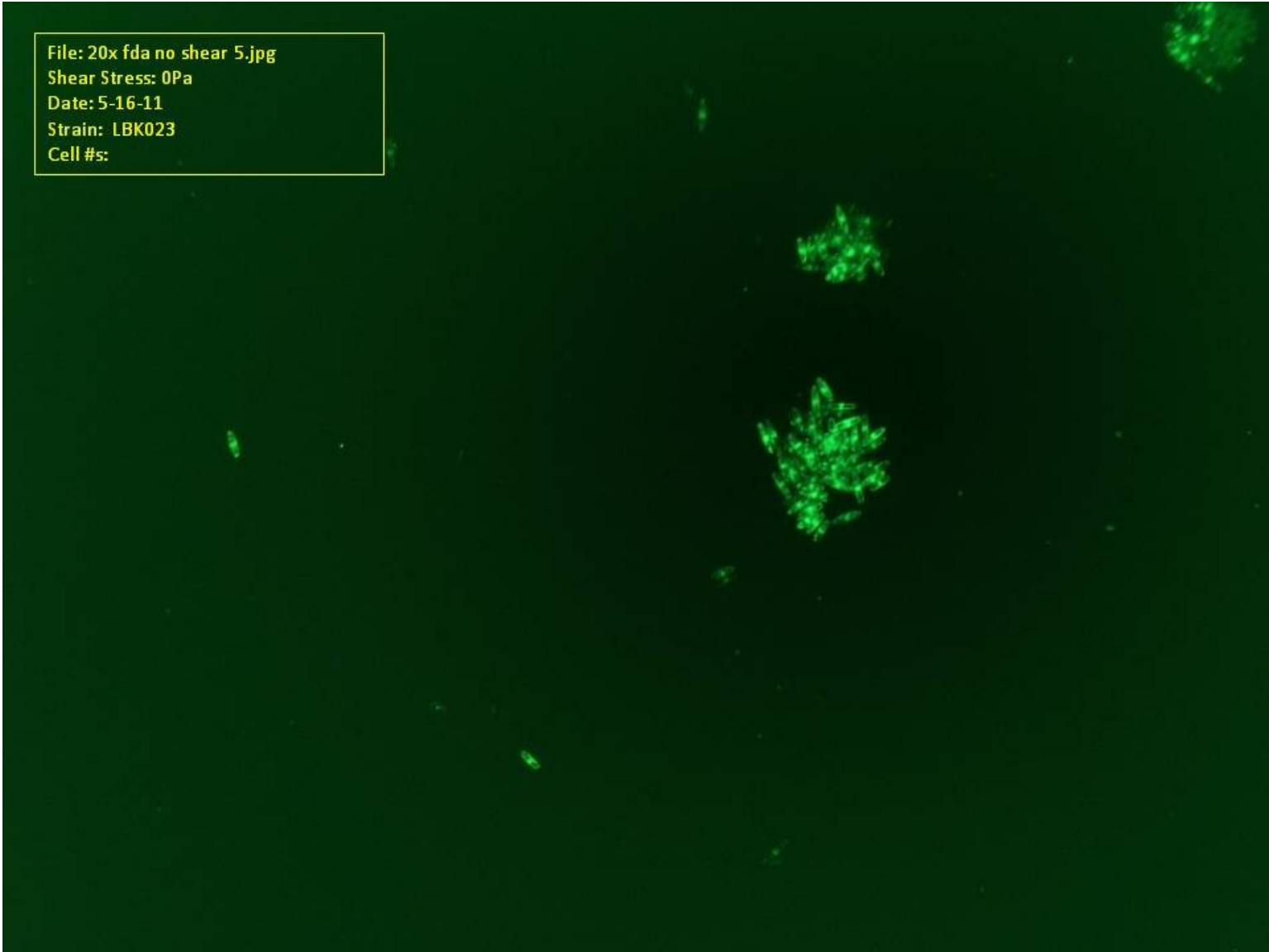


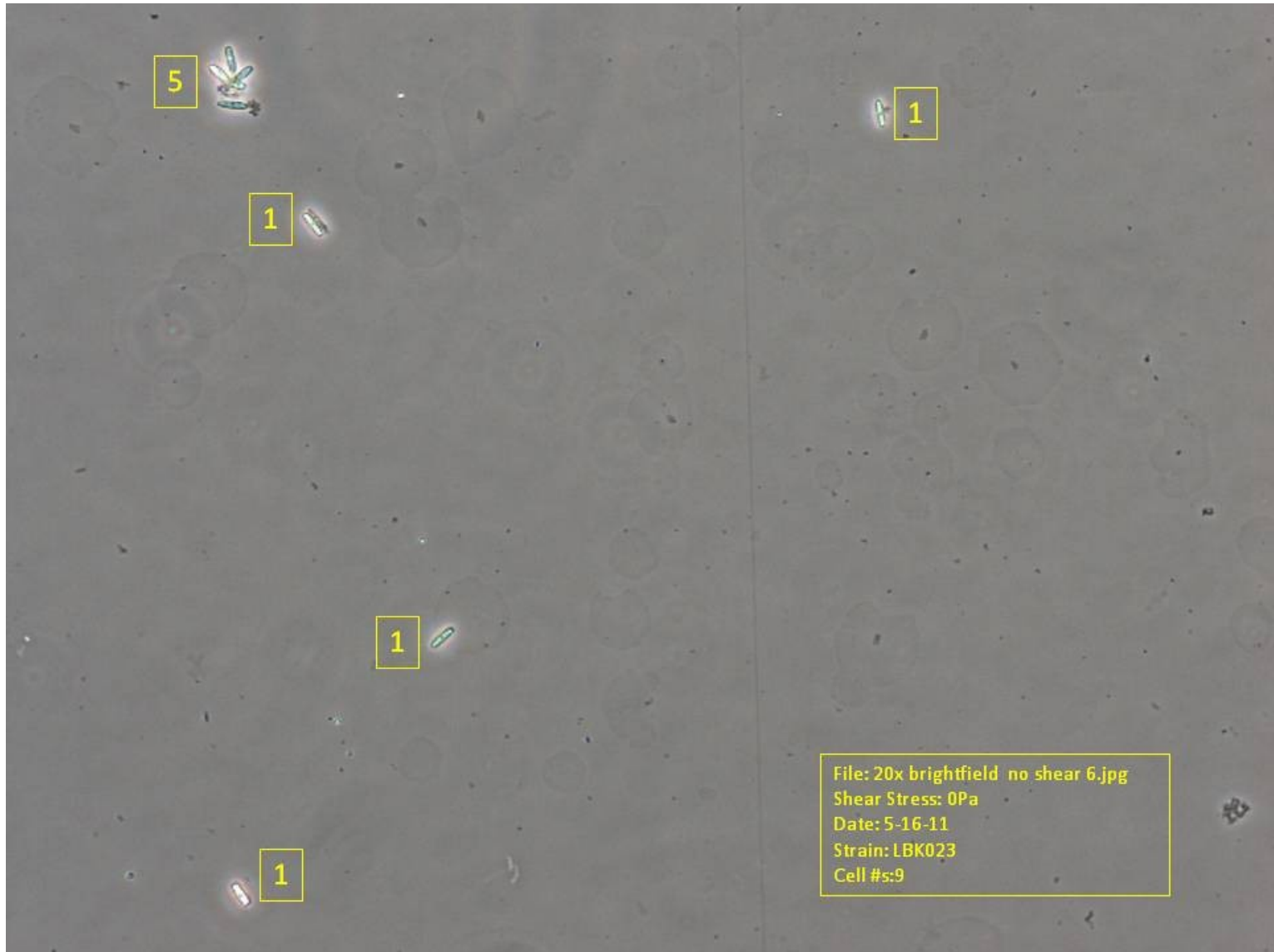




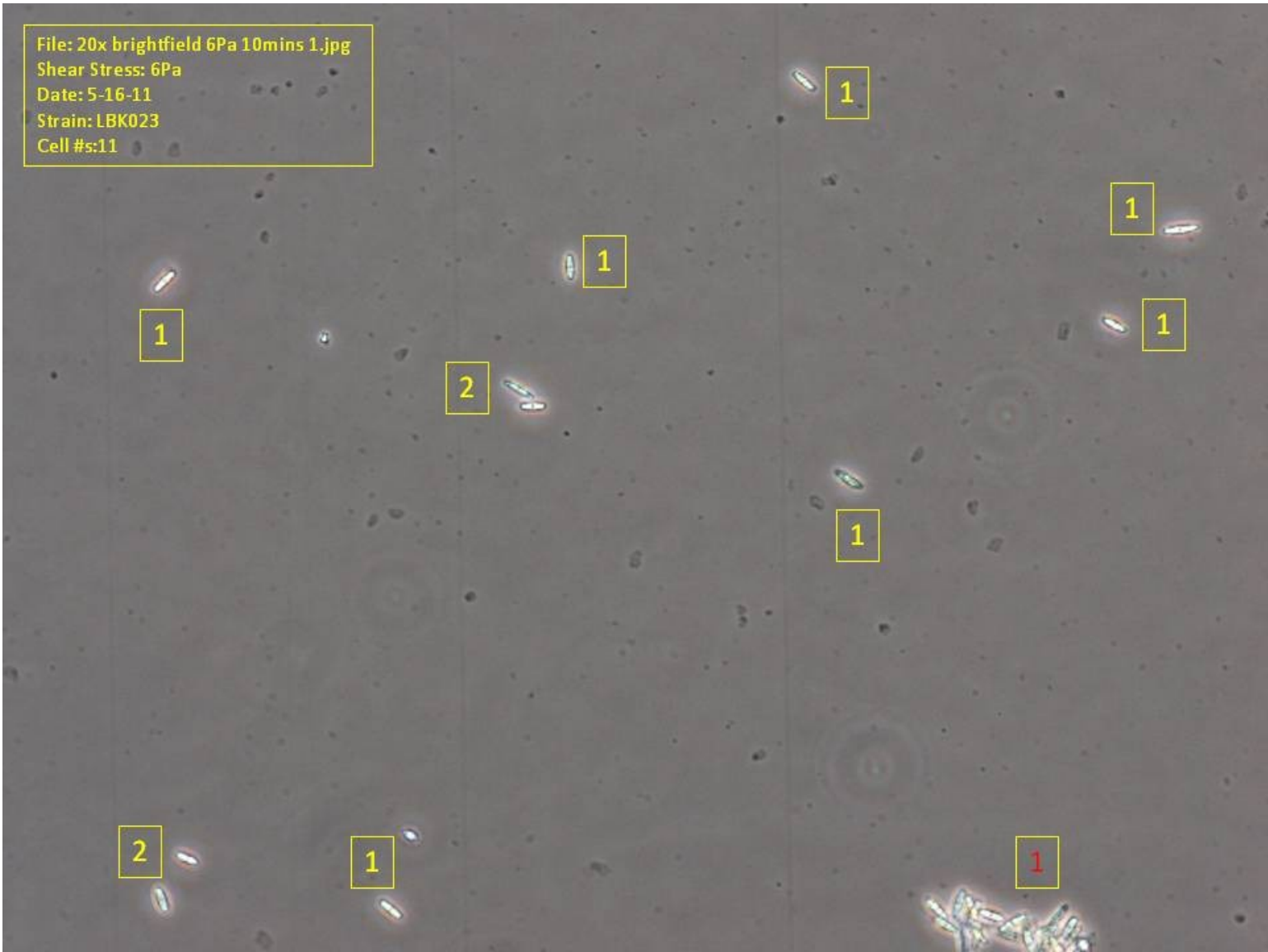






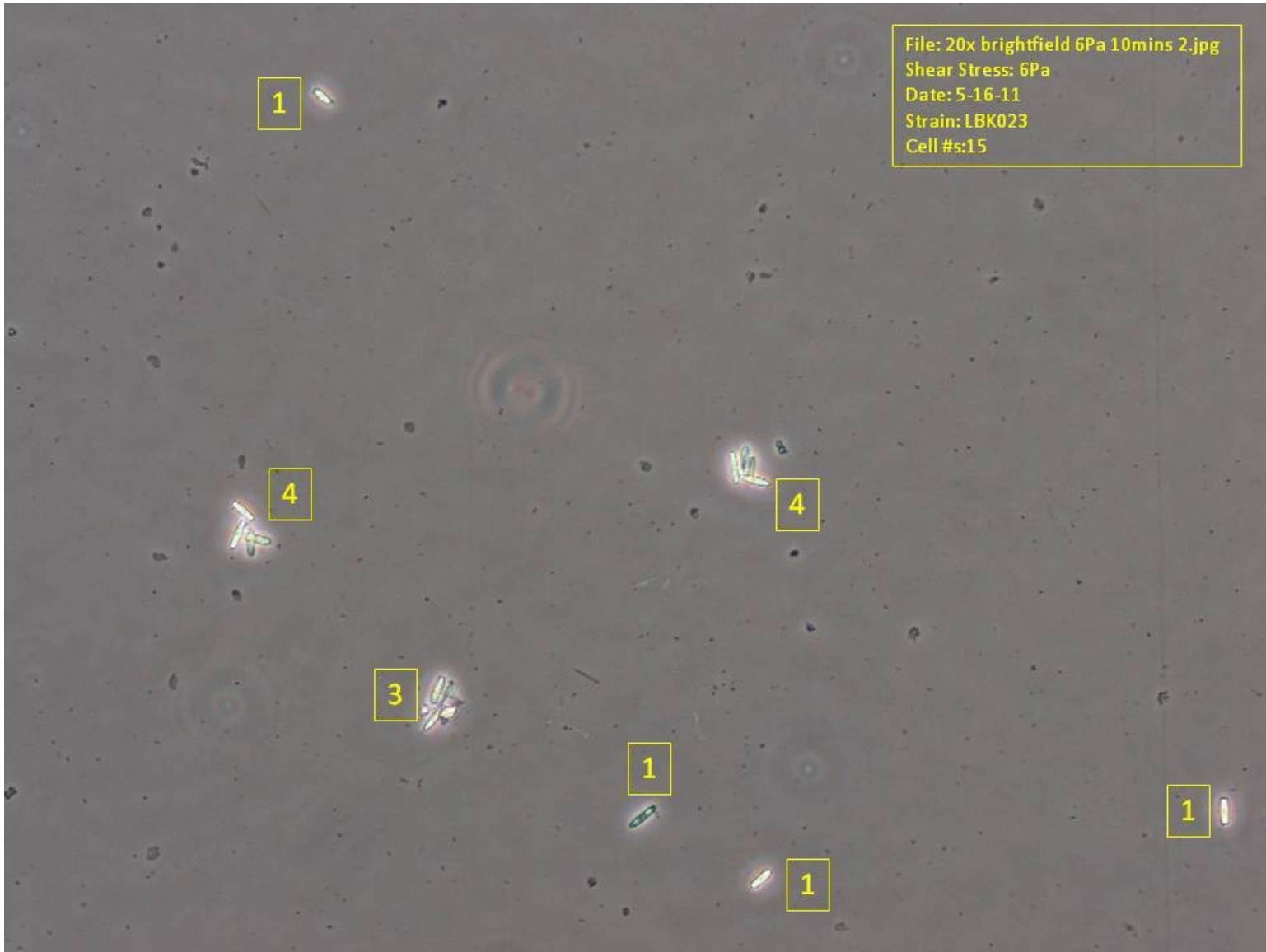




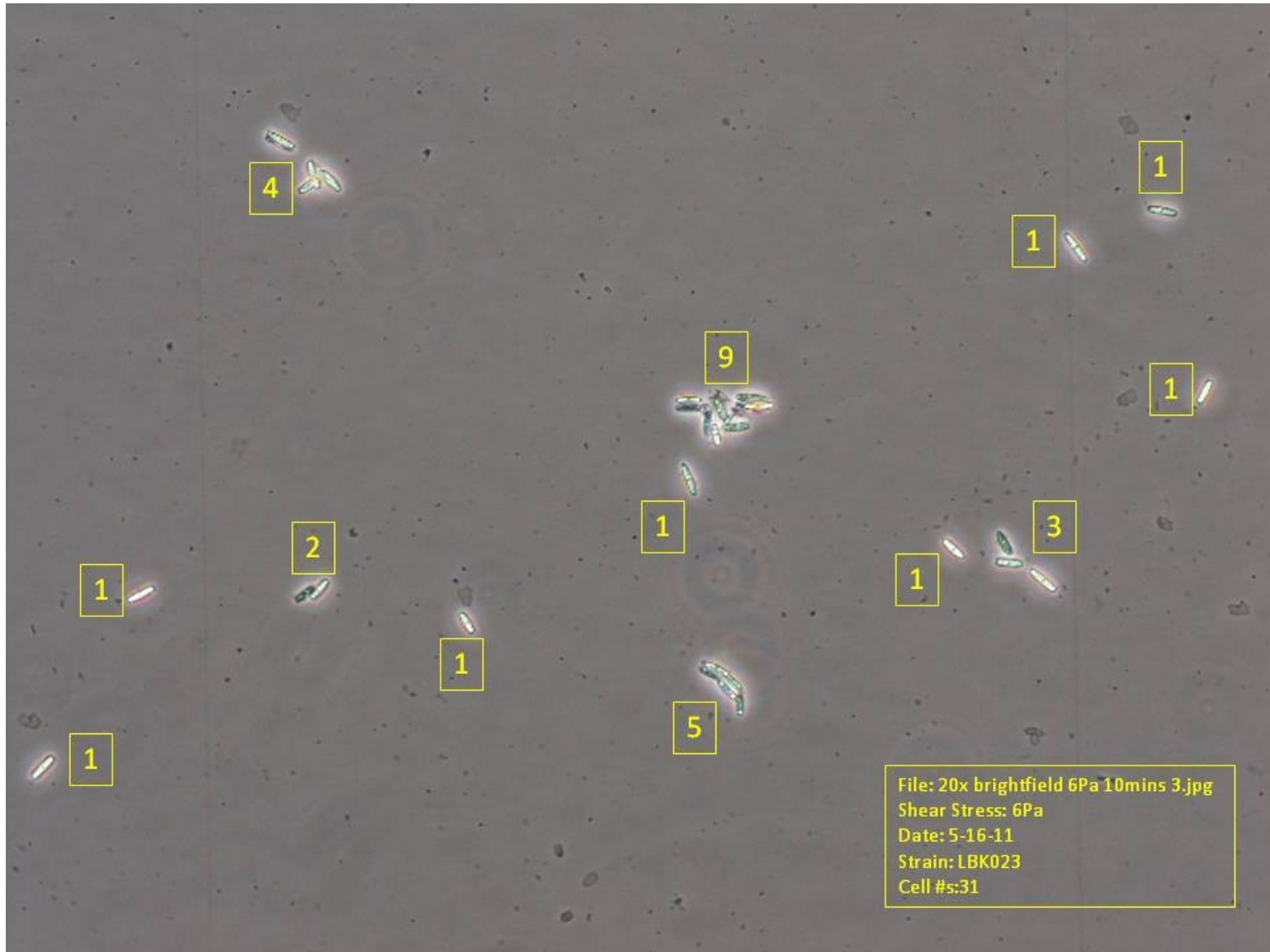


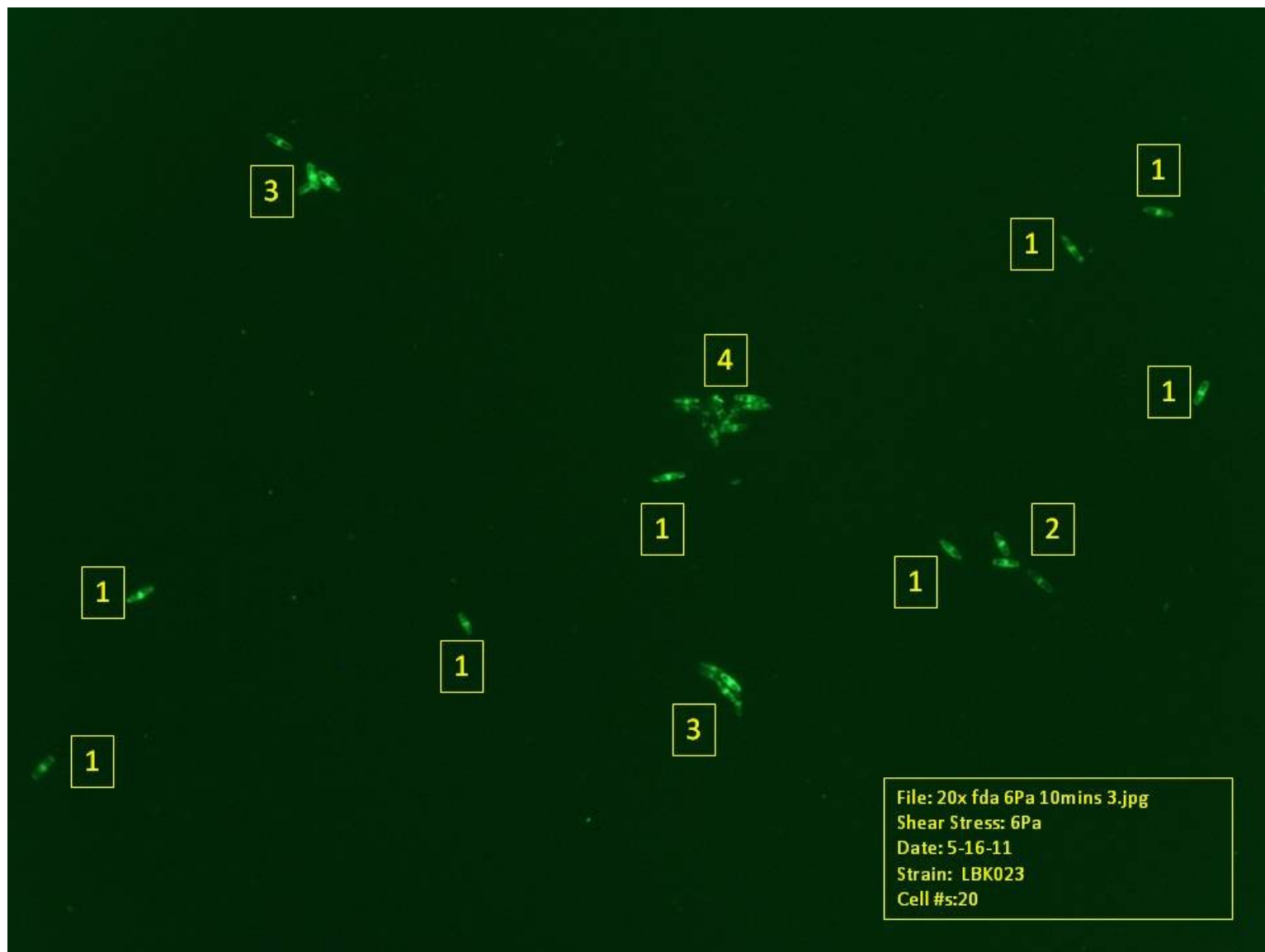


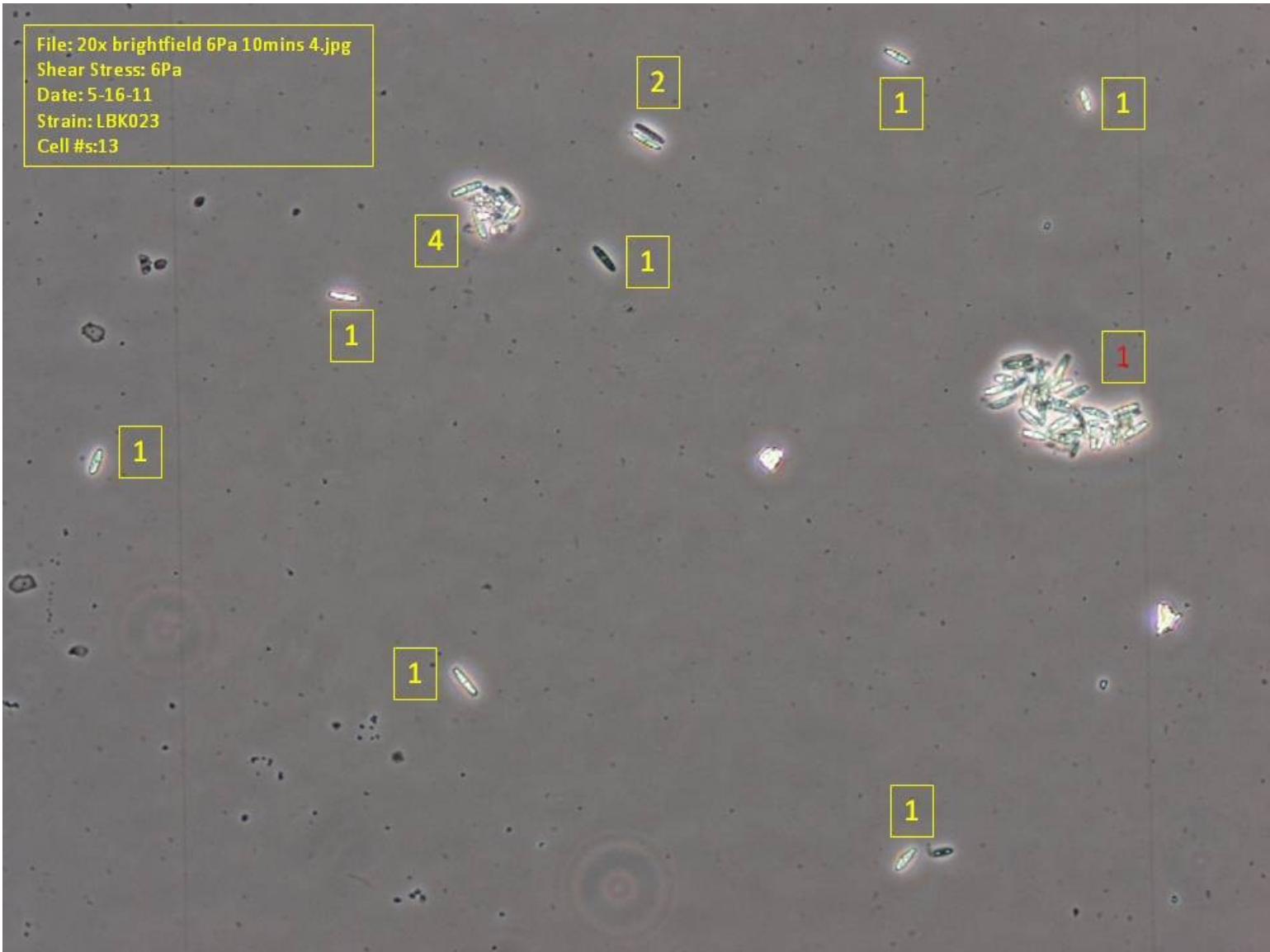


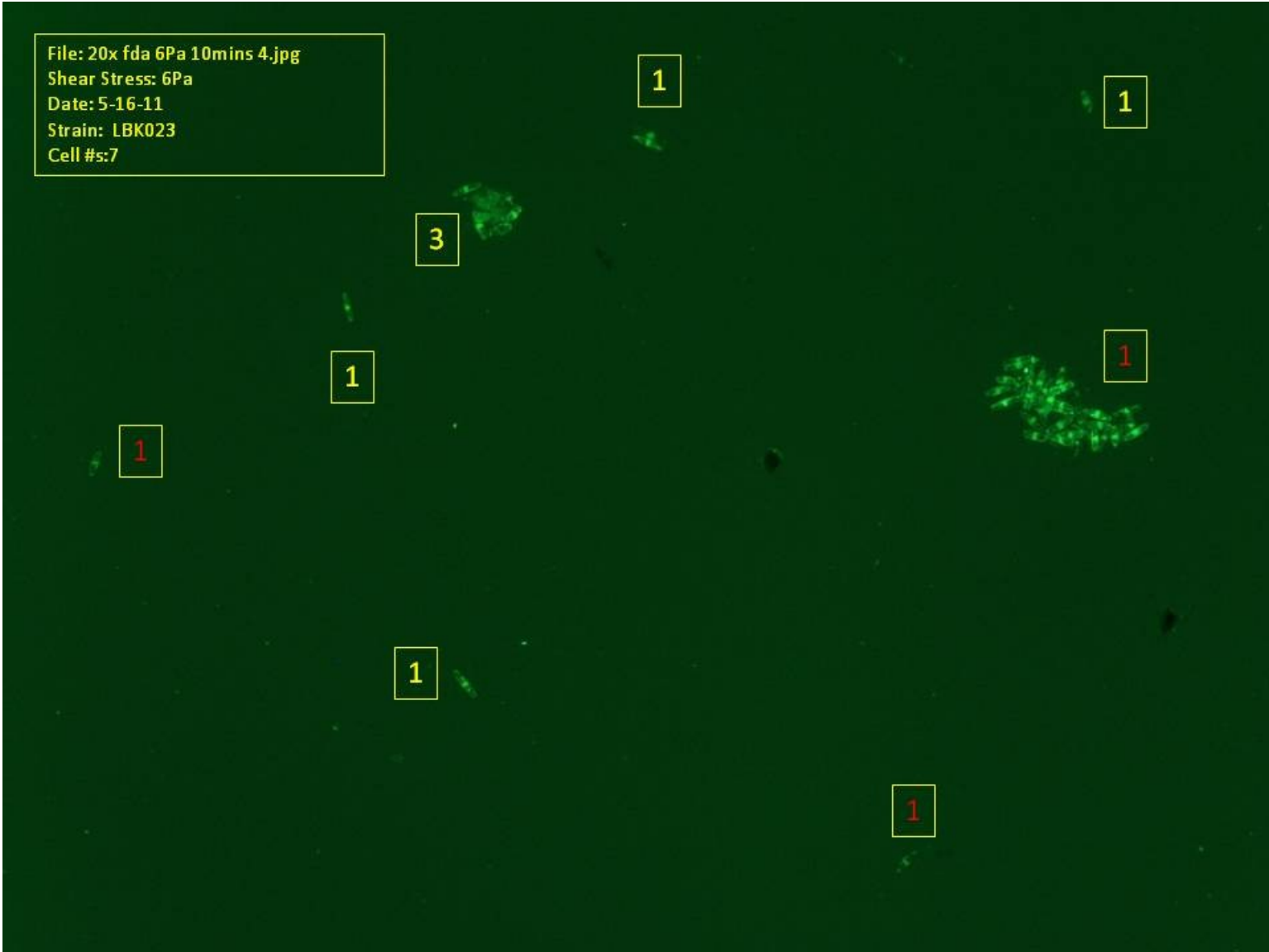


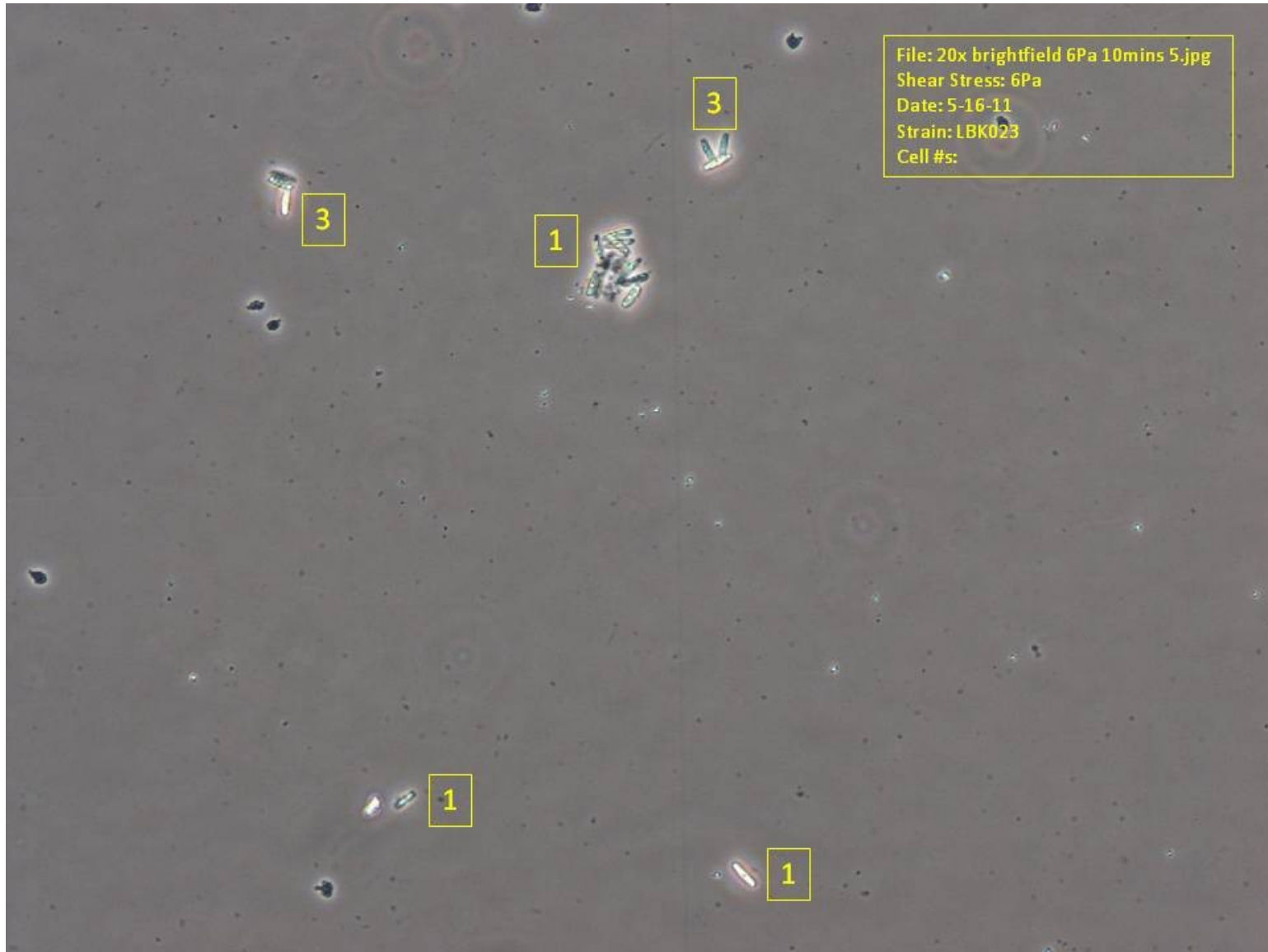




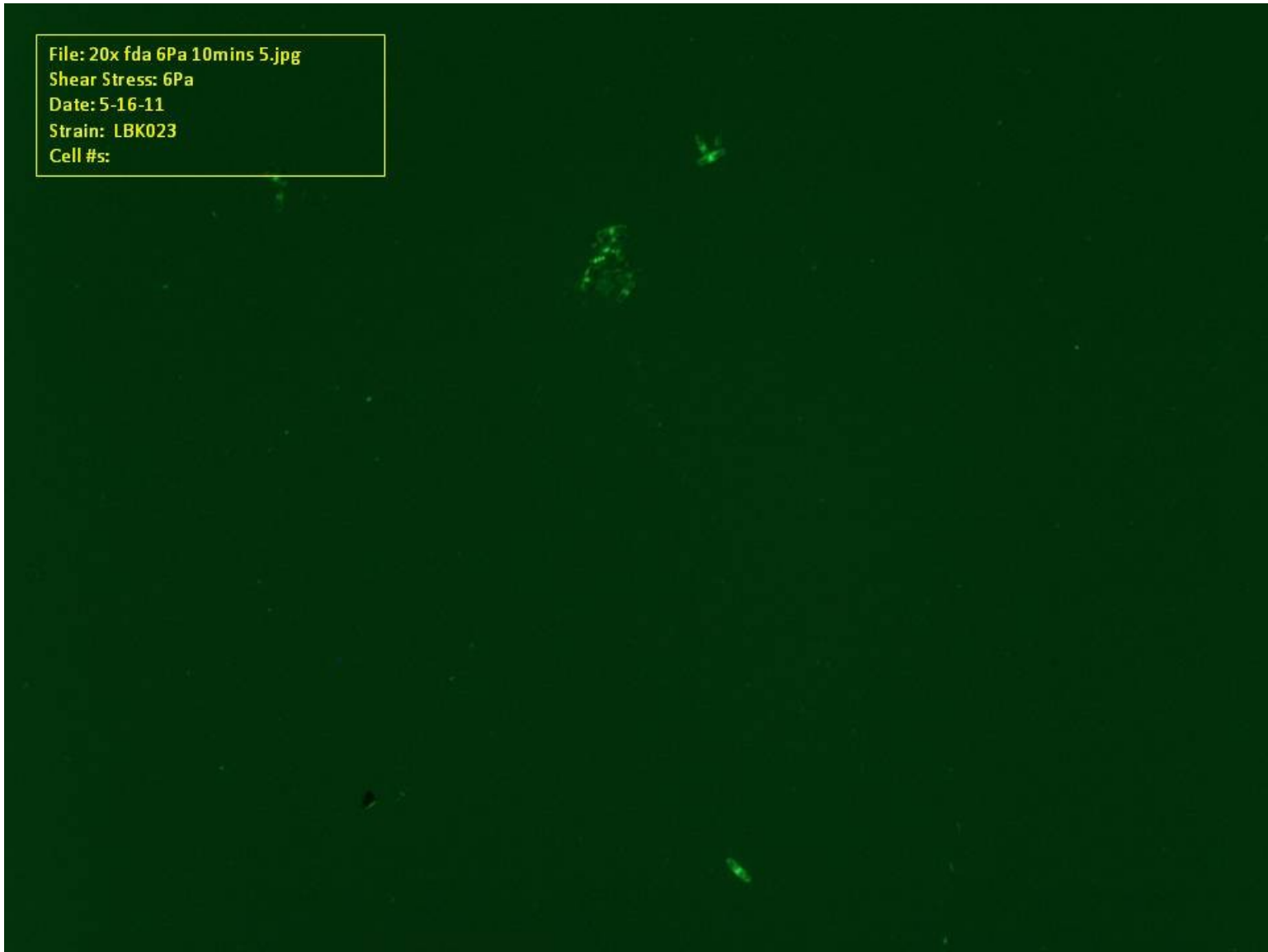




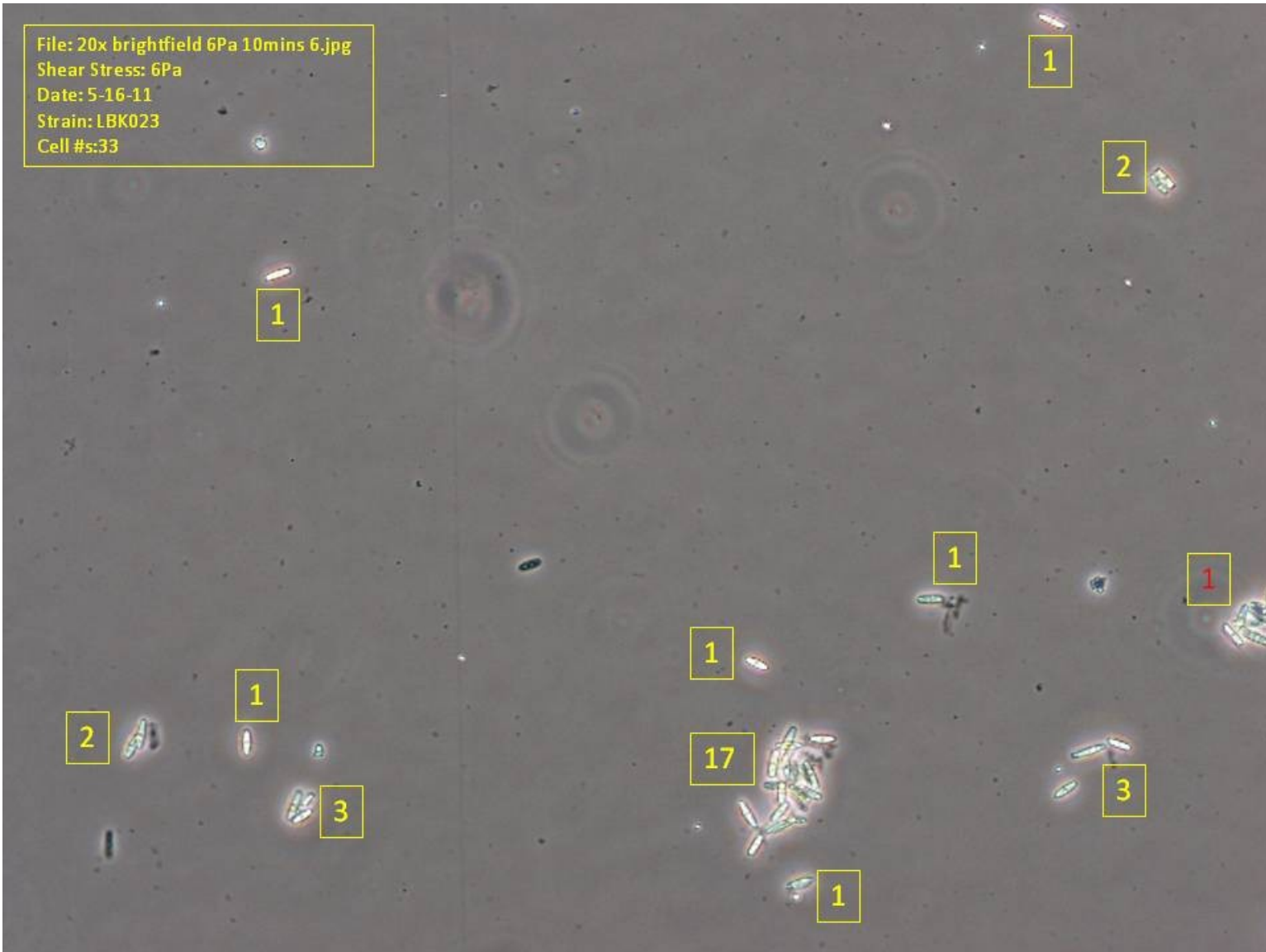




File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #s:

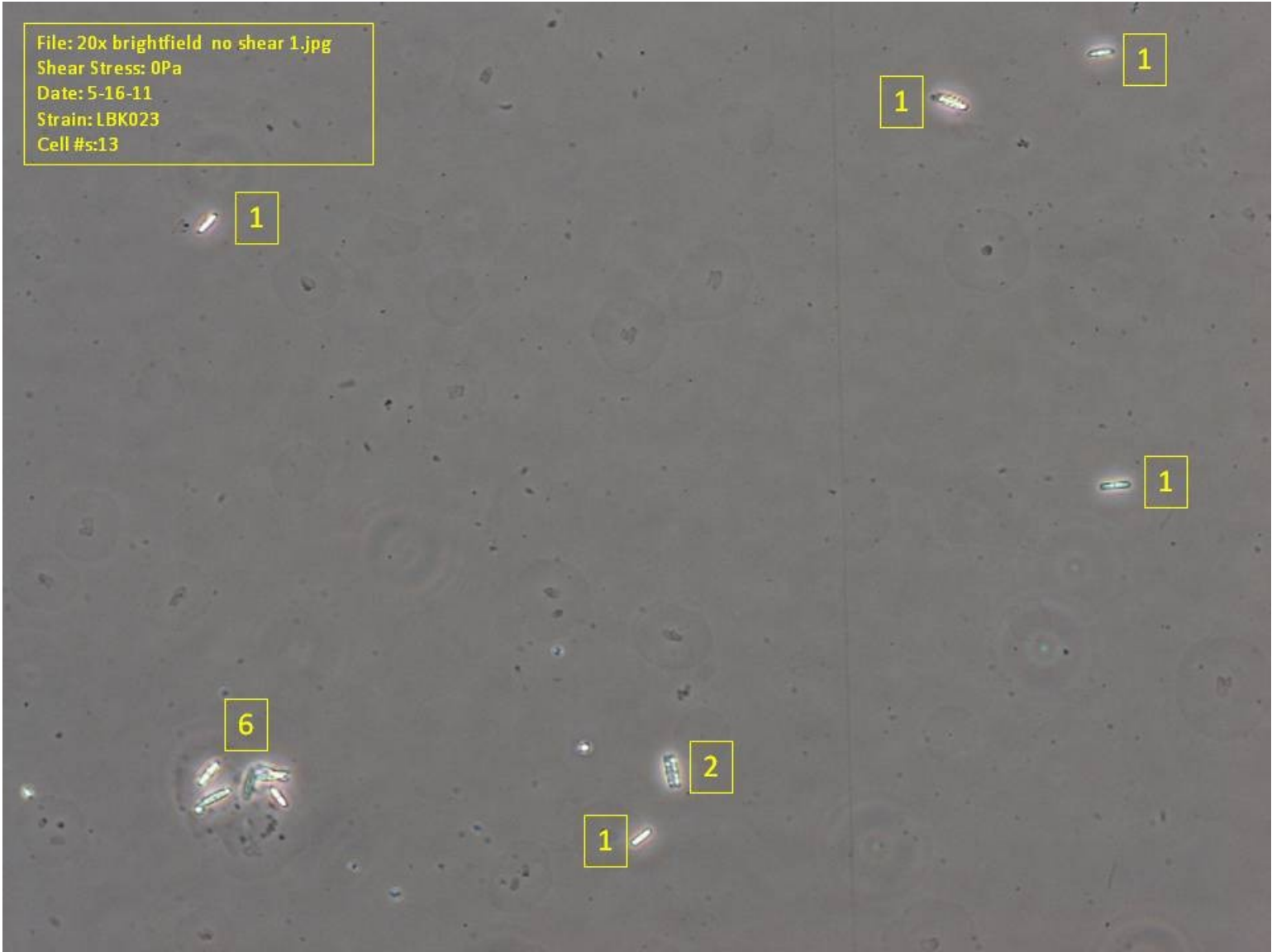






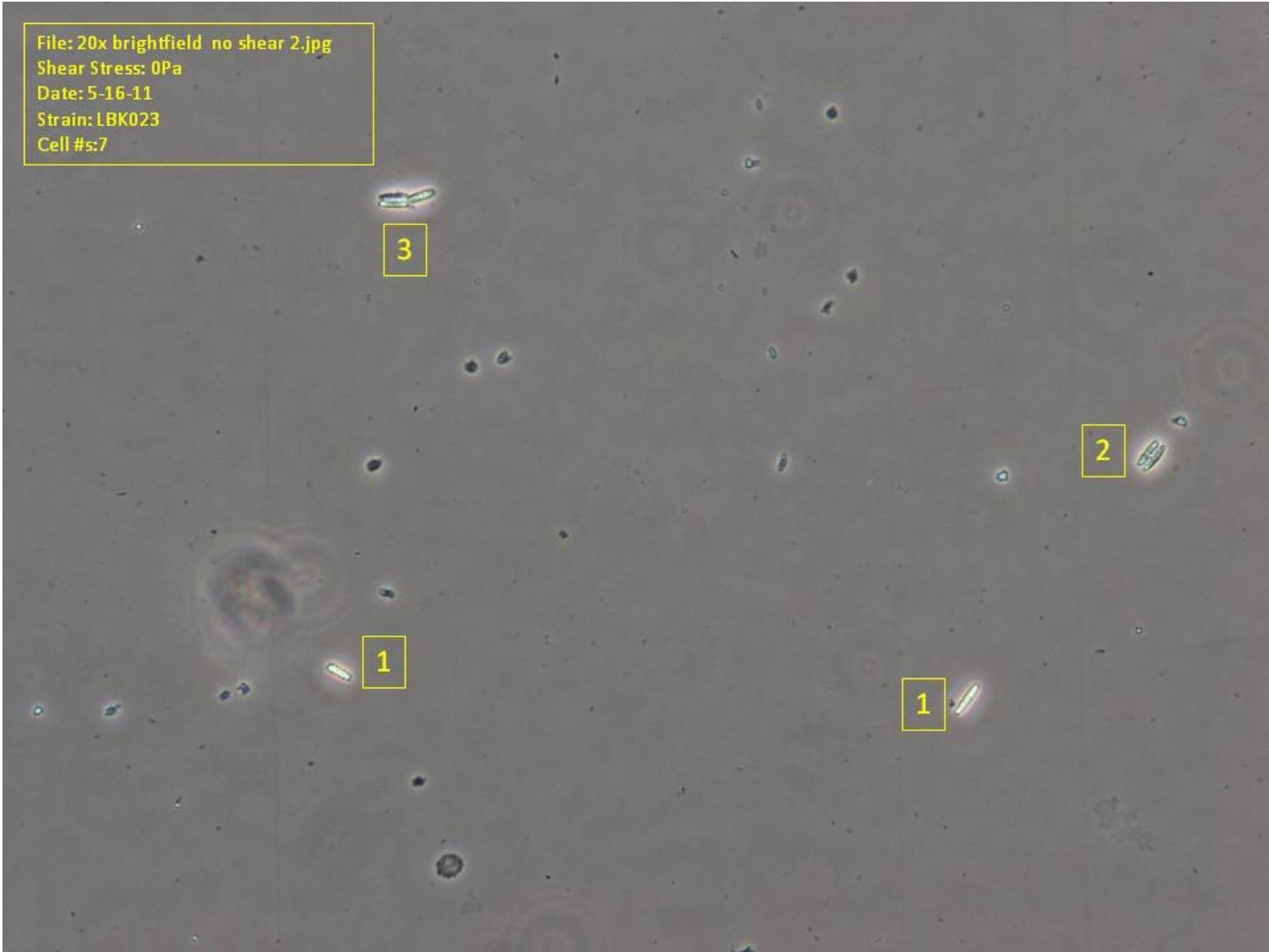


File: 20x brightfield no shear 1.jpg  
Shear Stress: 0Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #s:13





File: 20x brightfield no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #:7



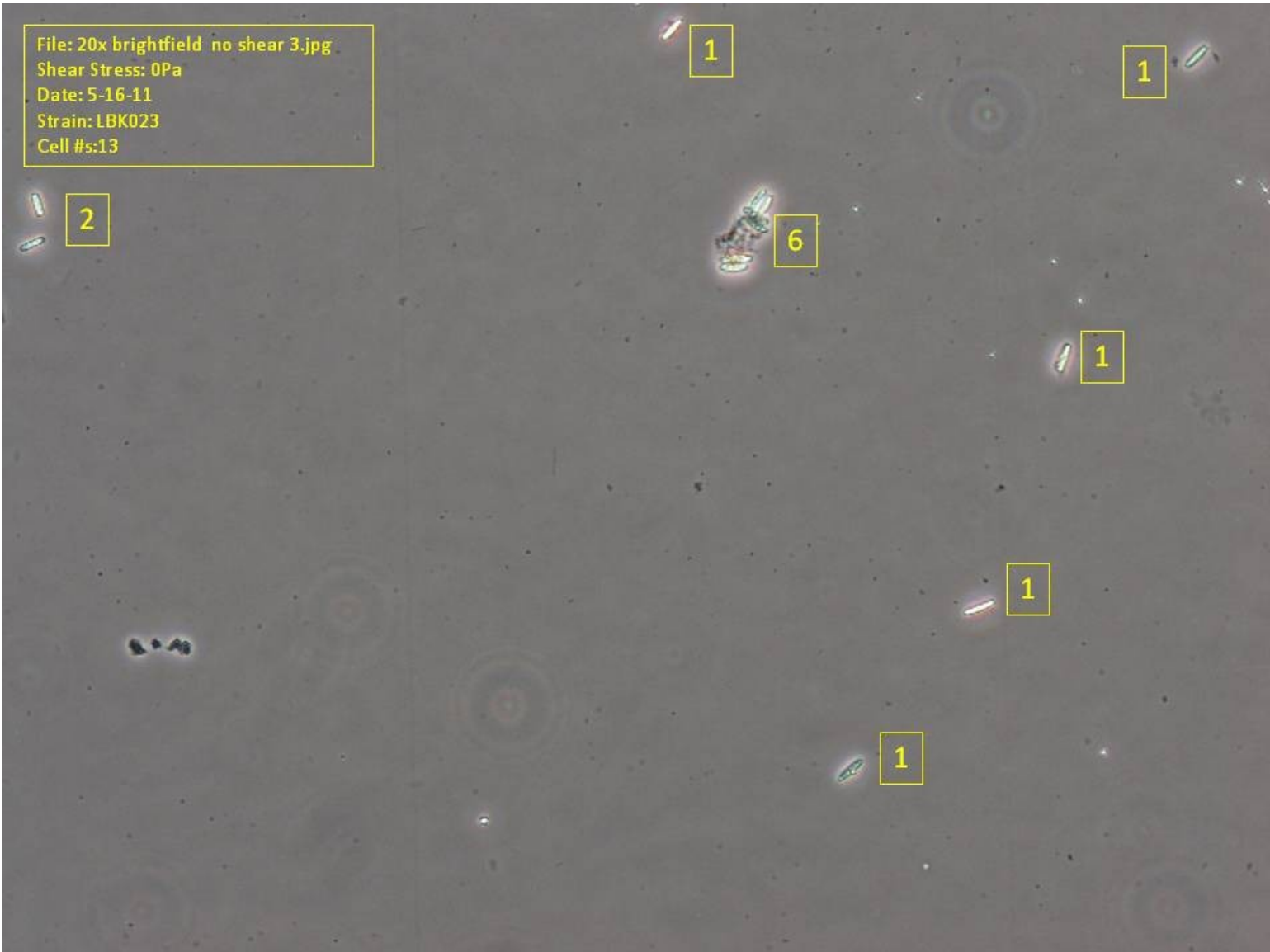
File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #:6

2

2

1

1





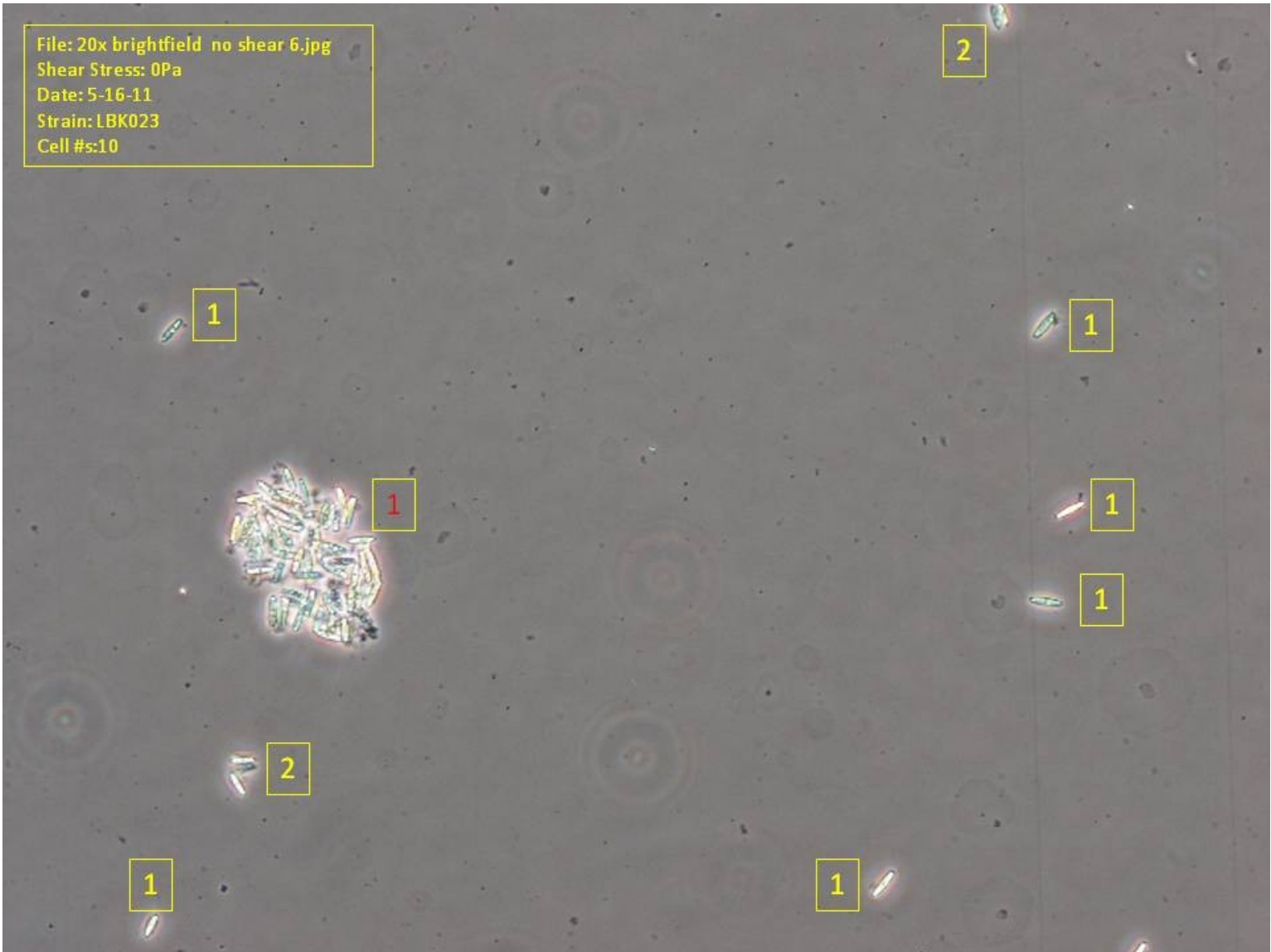












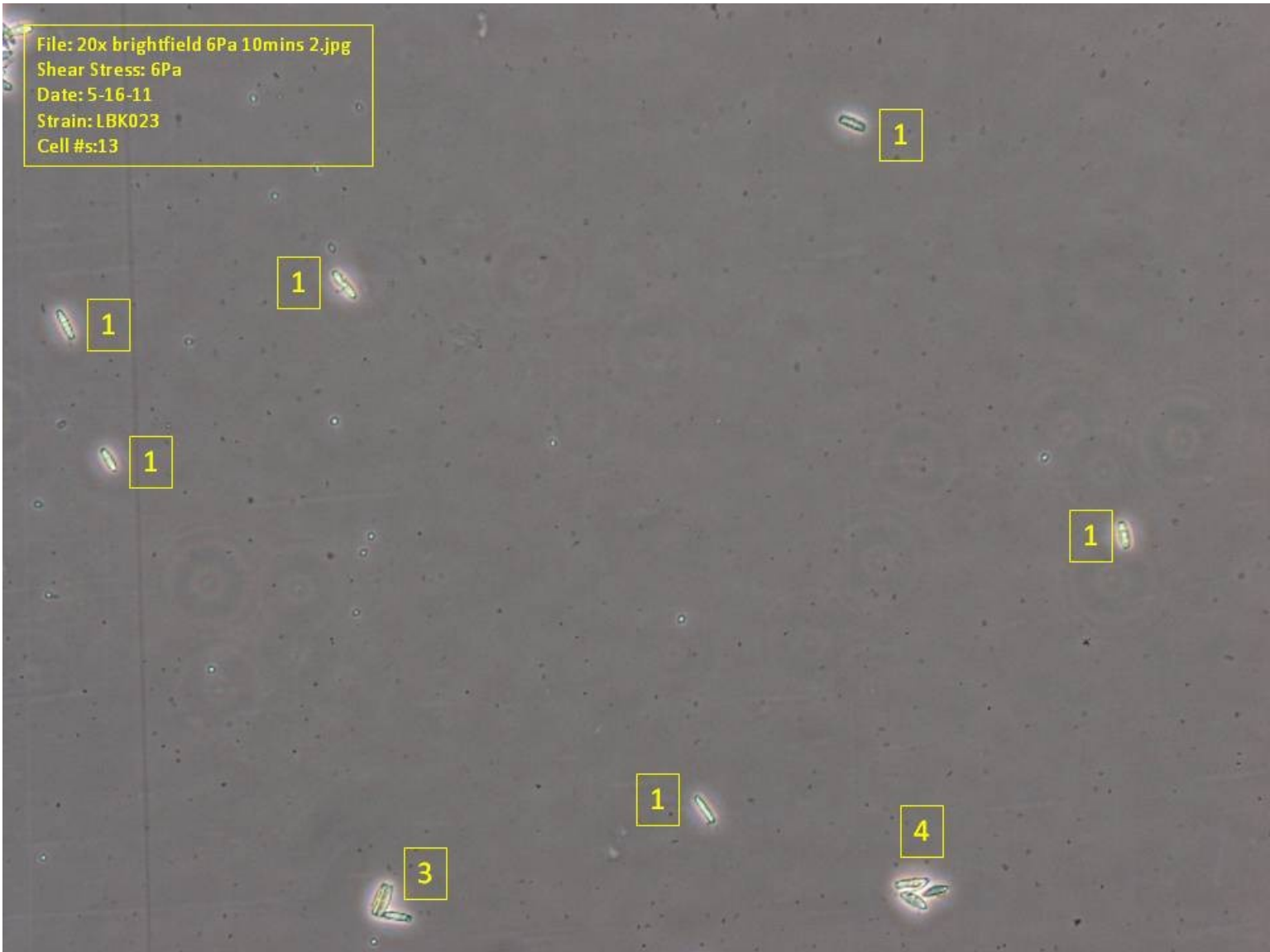


File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #s:33

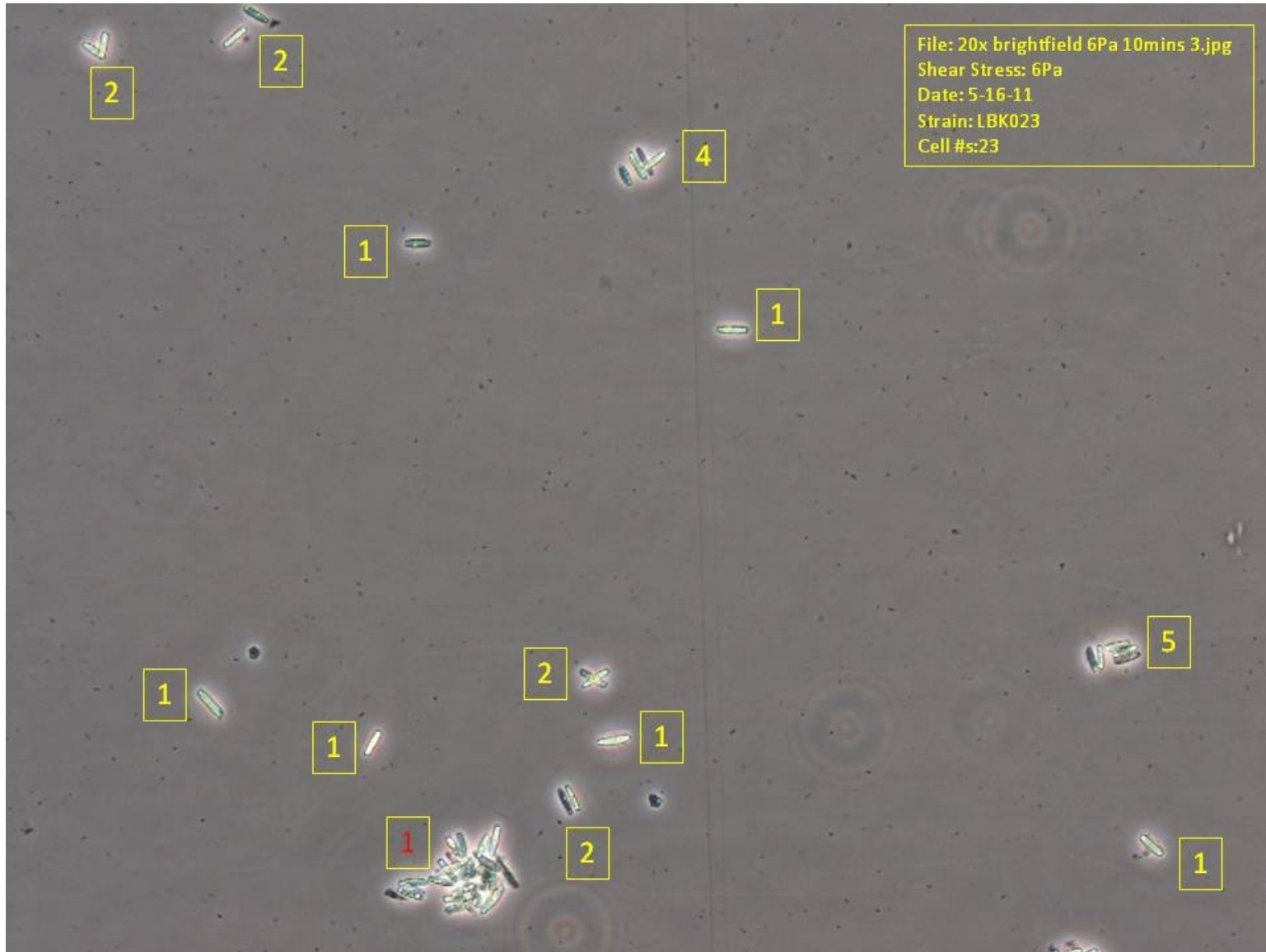


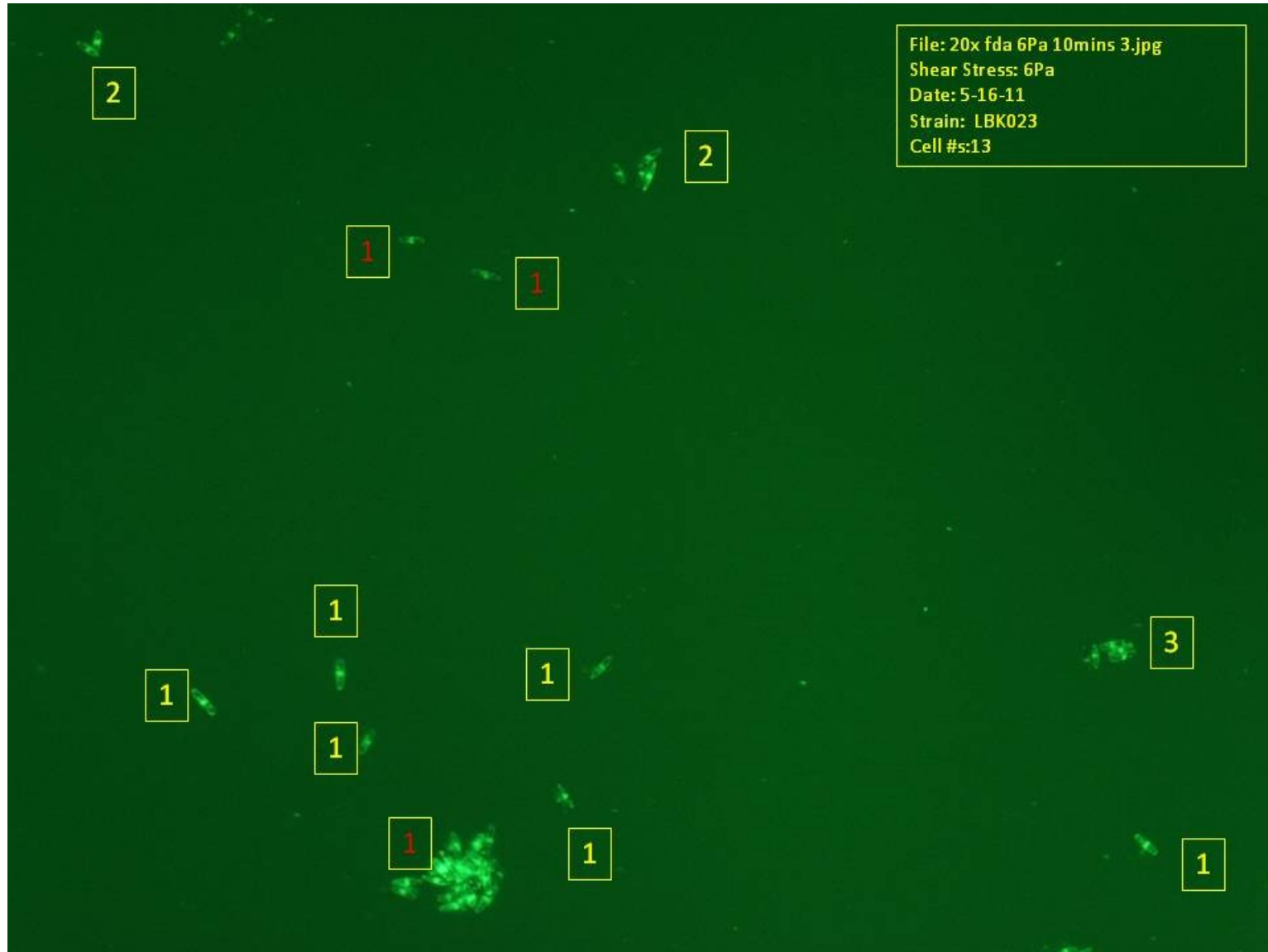


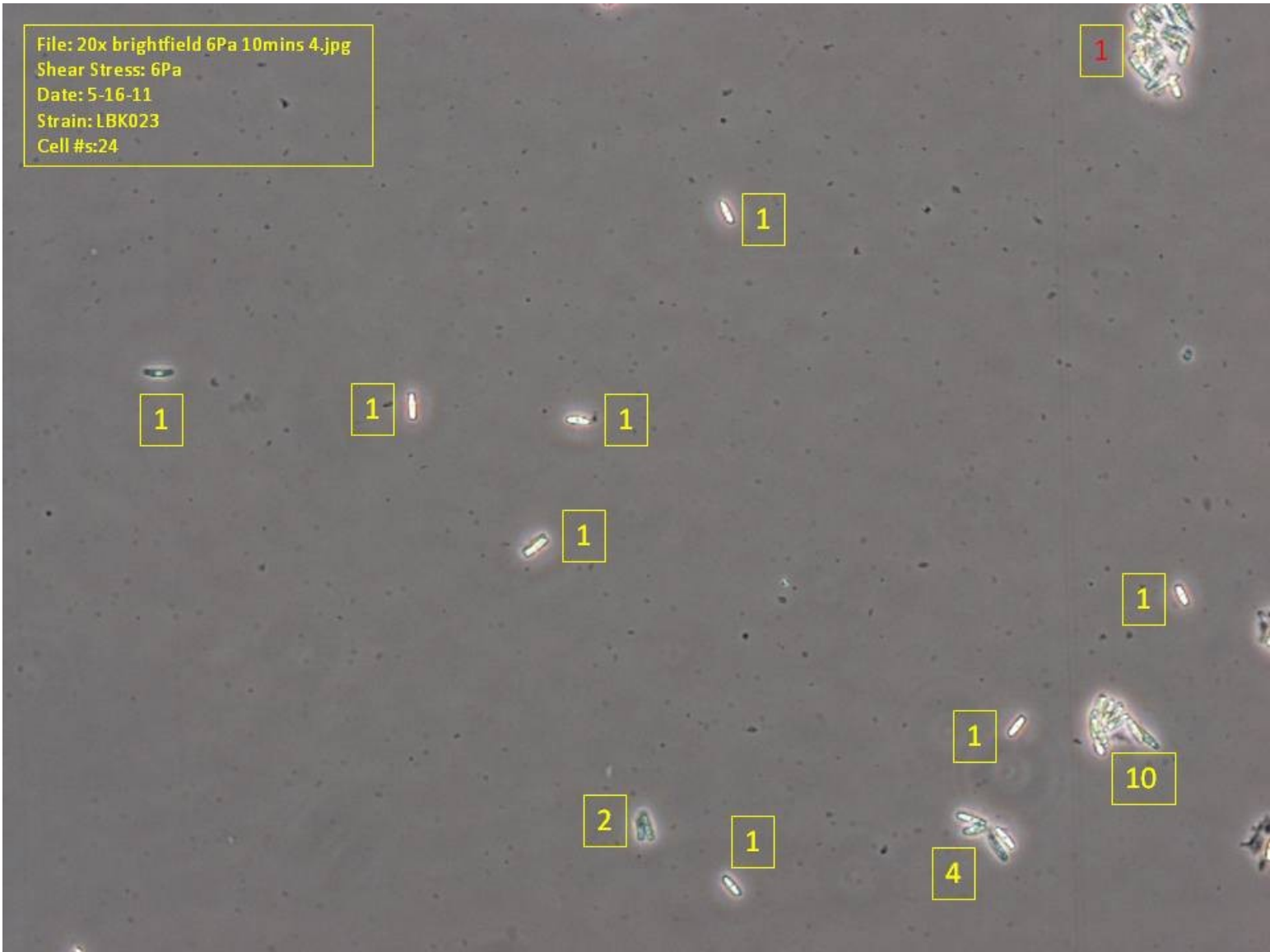




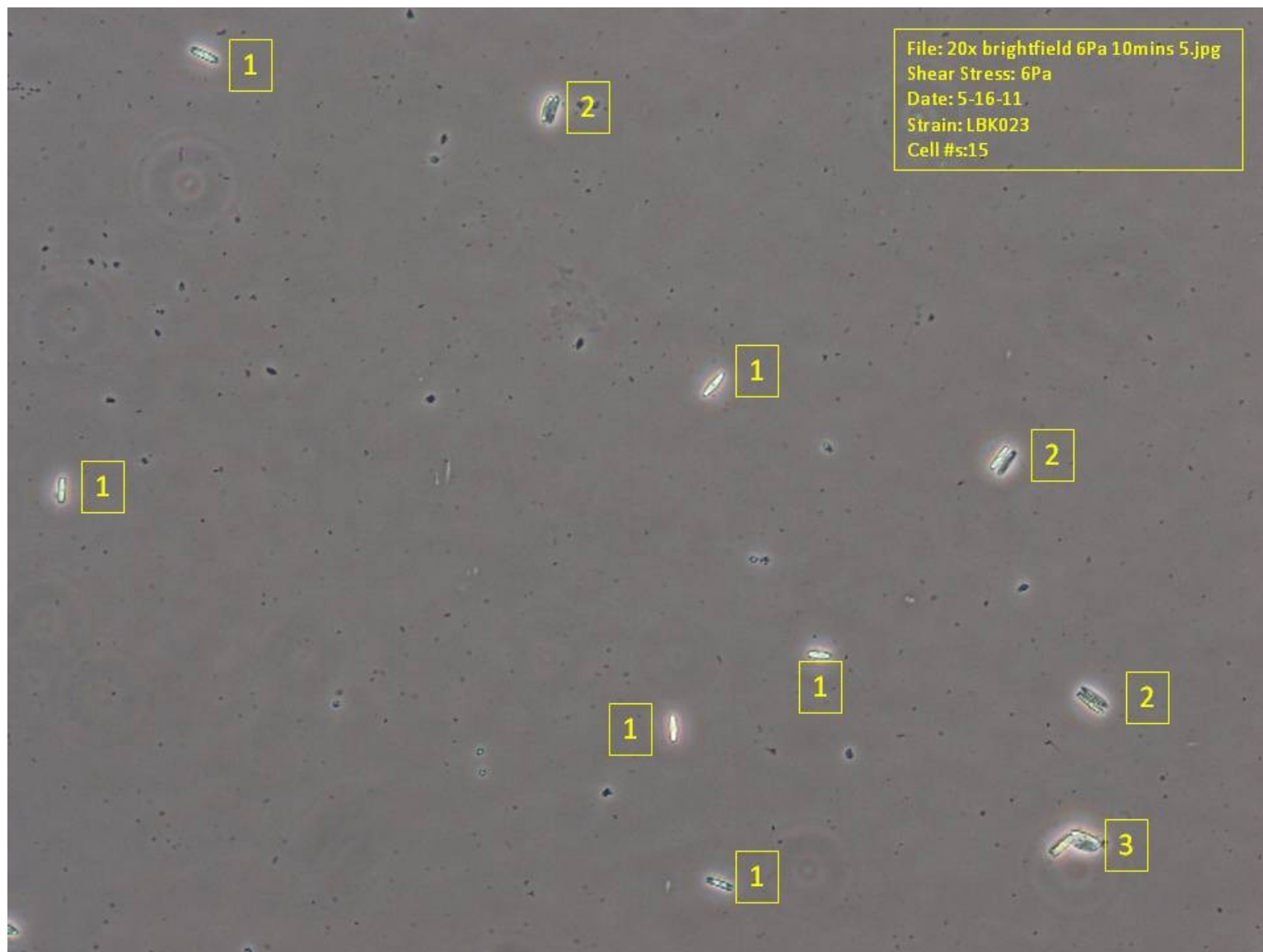








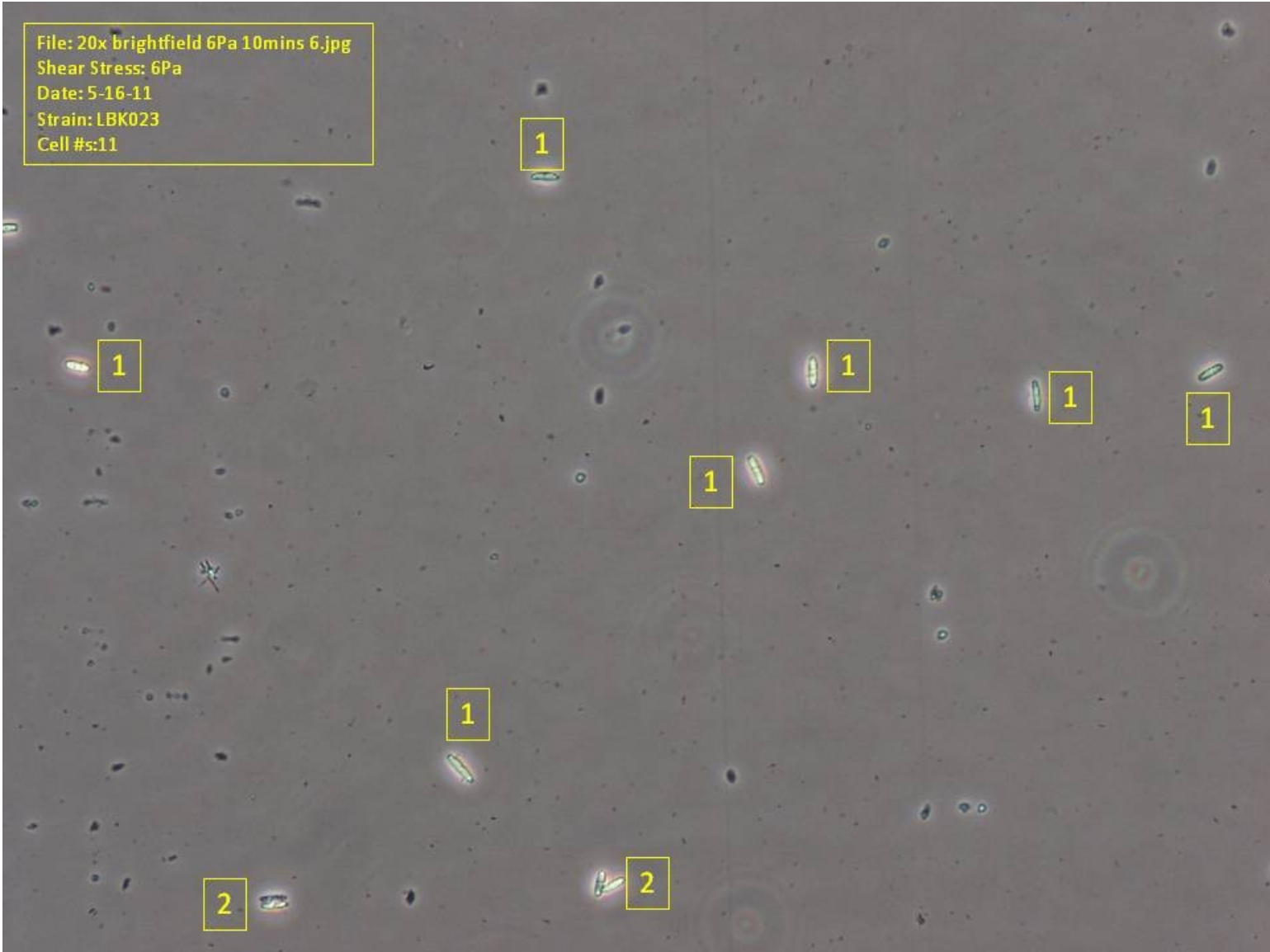








File: 20x brightfield 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #s:11



File: 20x fda 6Pa 10mins 6.jpg  
Shear Stress: 6Pa  
Date: 5-16-11  
Strain: LBK023  
Cell #:6

1

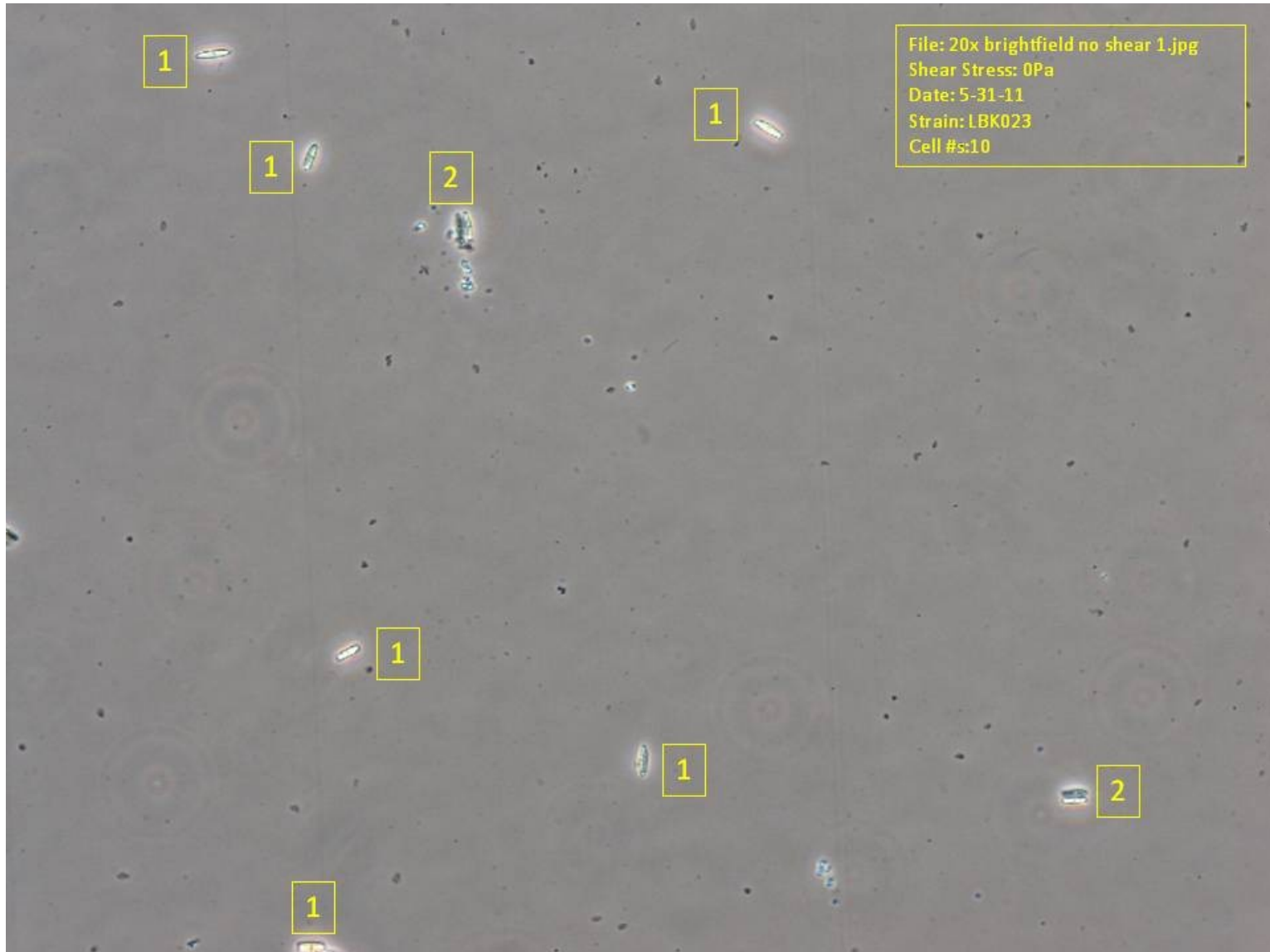
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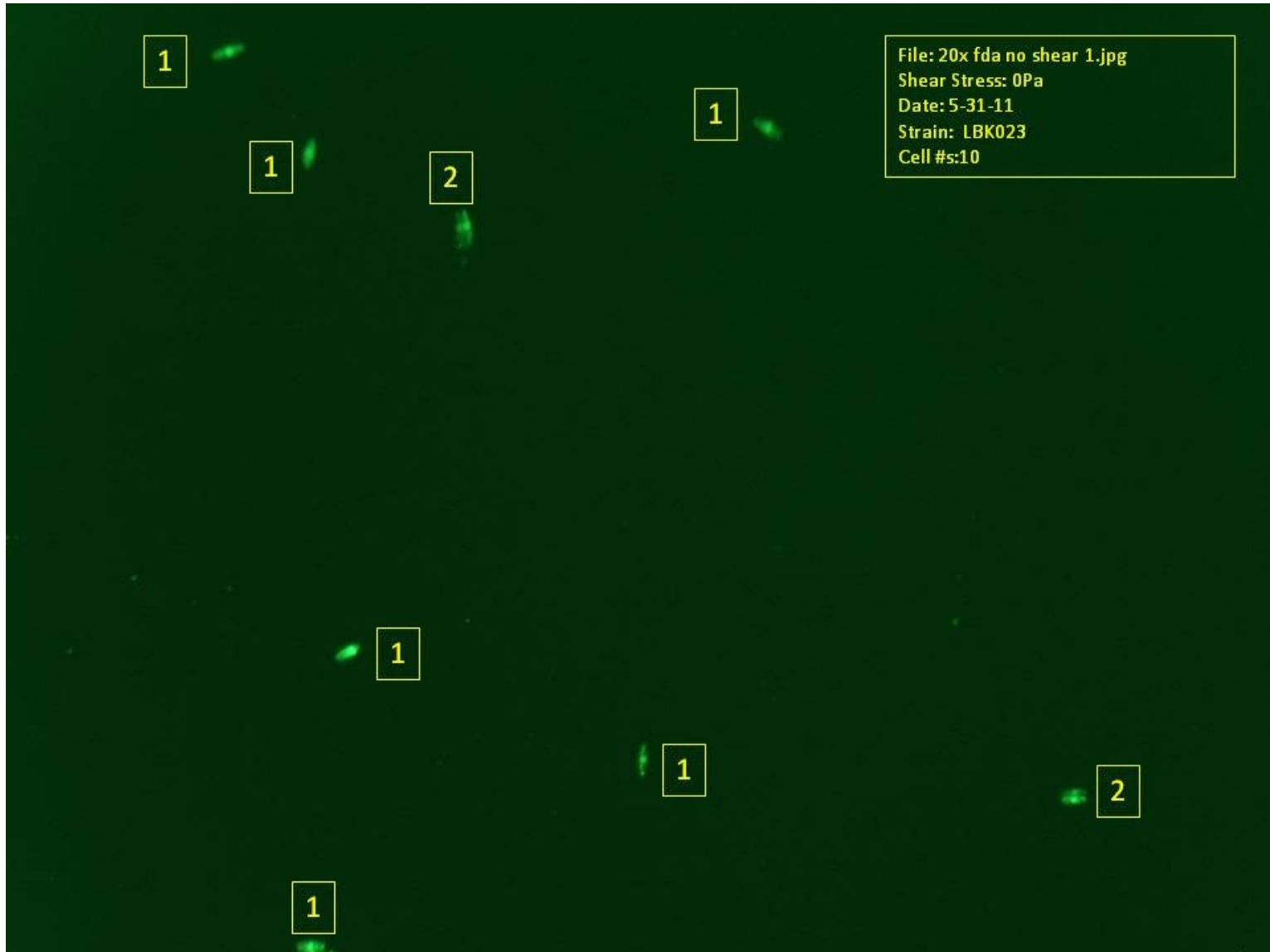
1

1

1

1

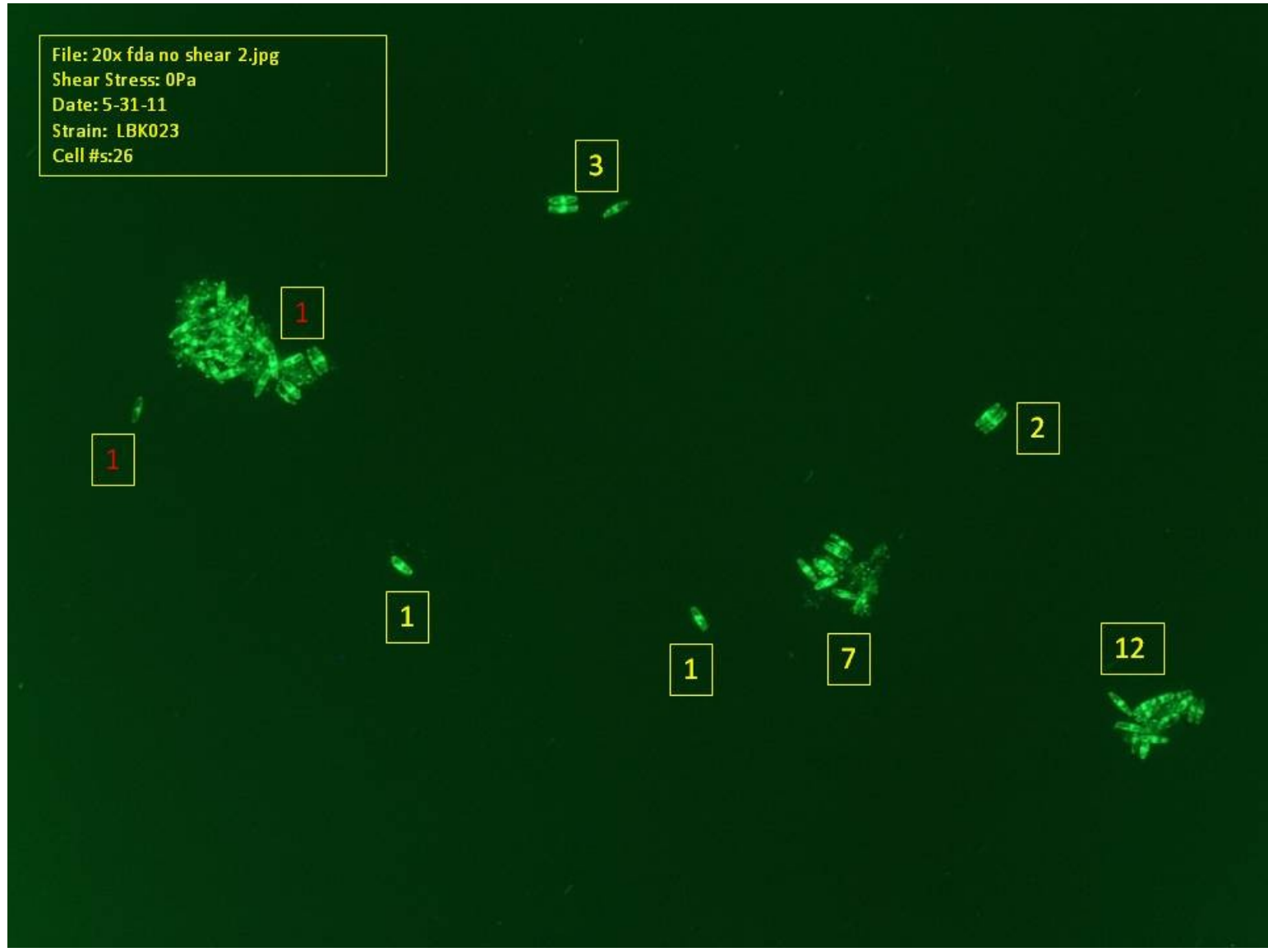




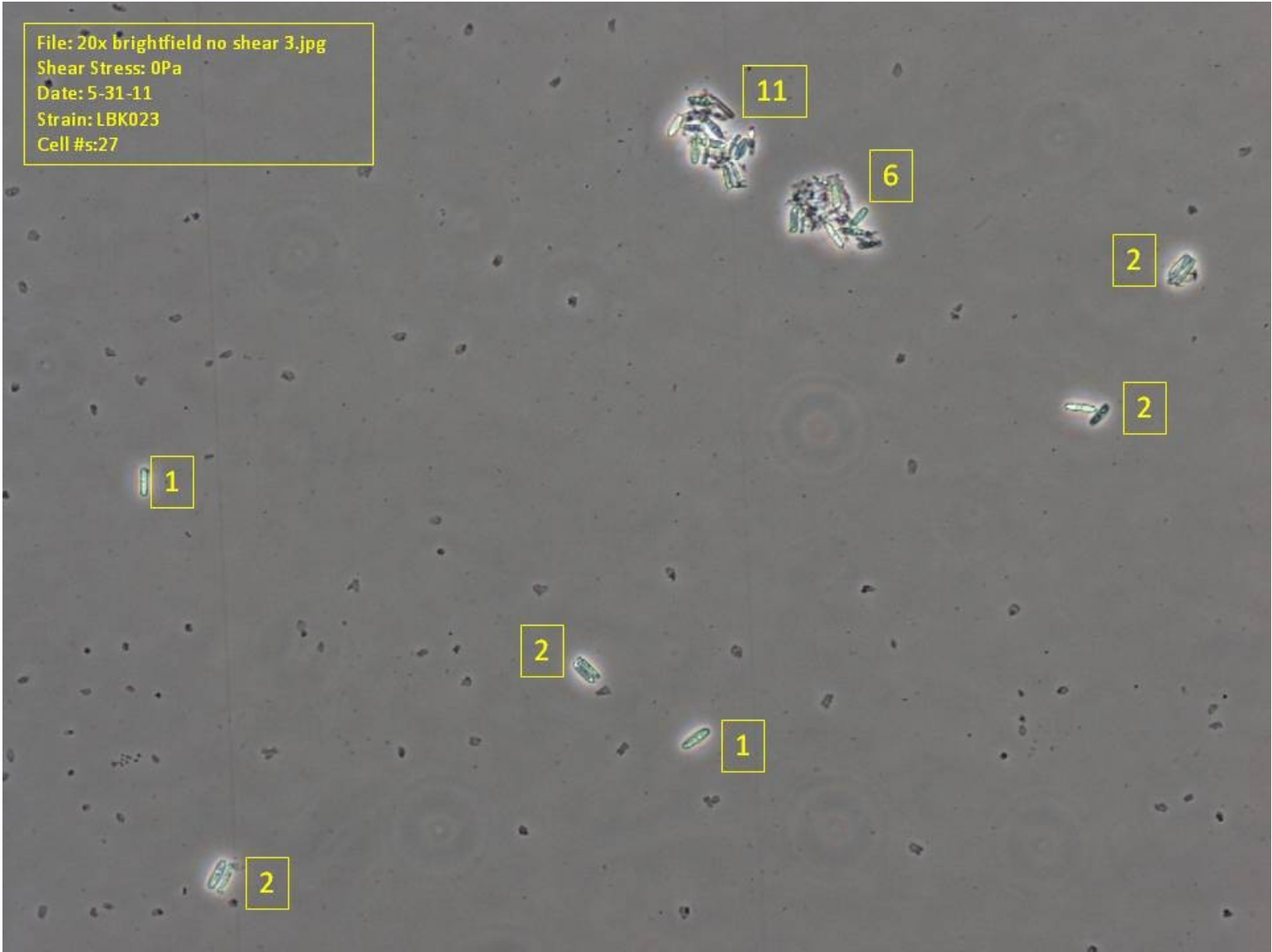
File: 20x brightfield no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:27

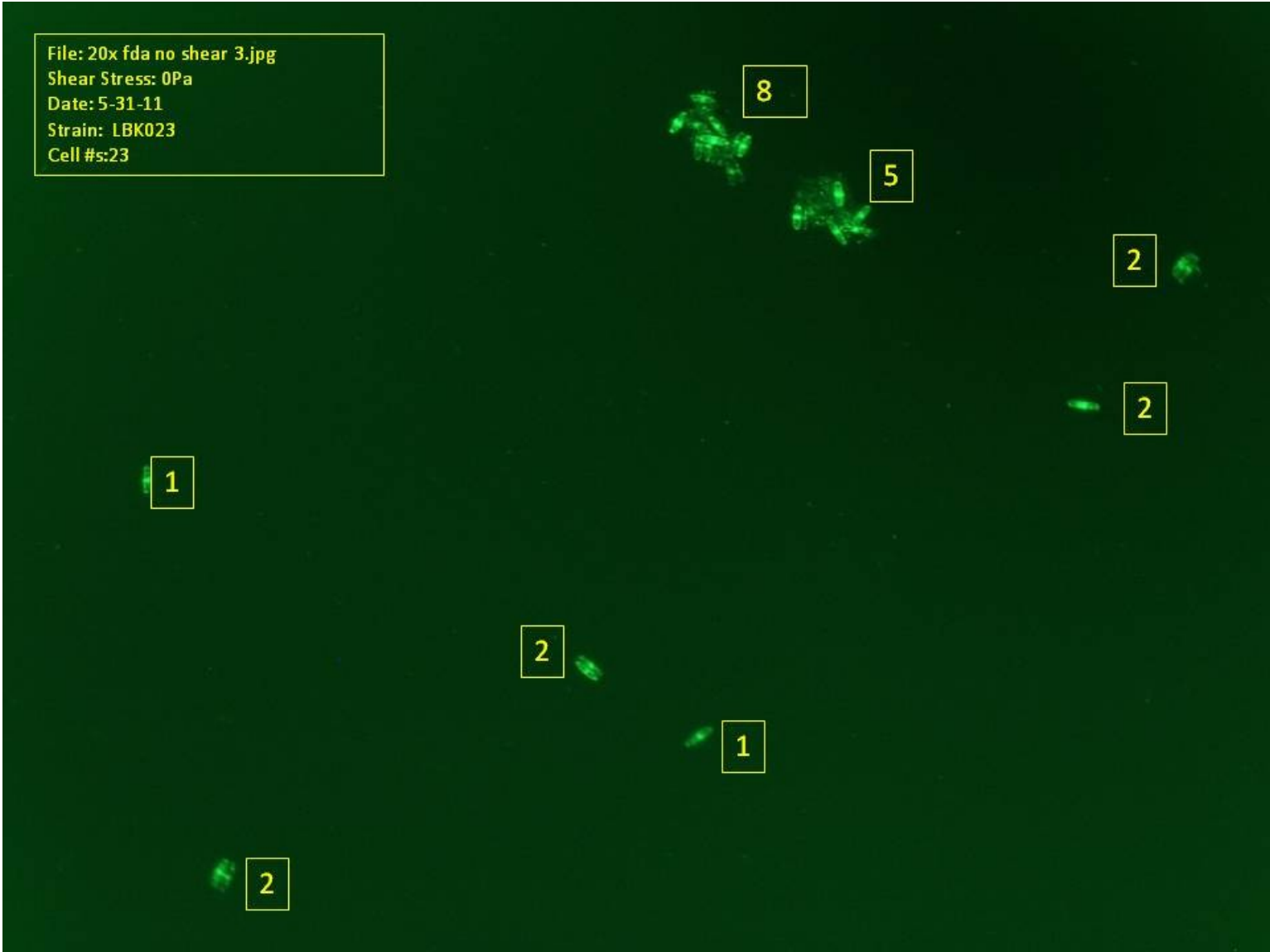


File: 20x fda no shear 2.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:26

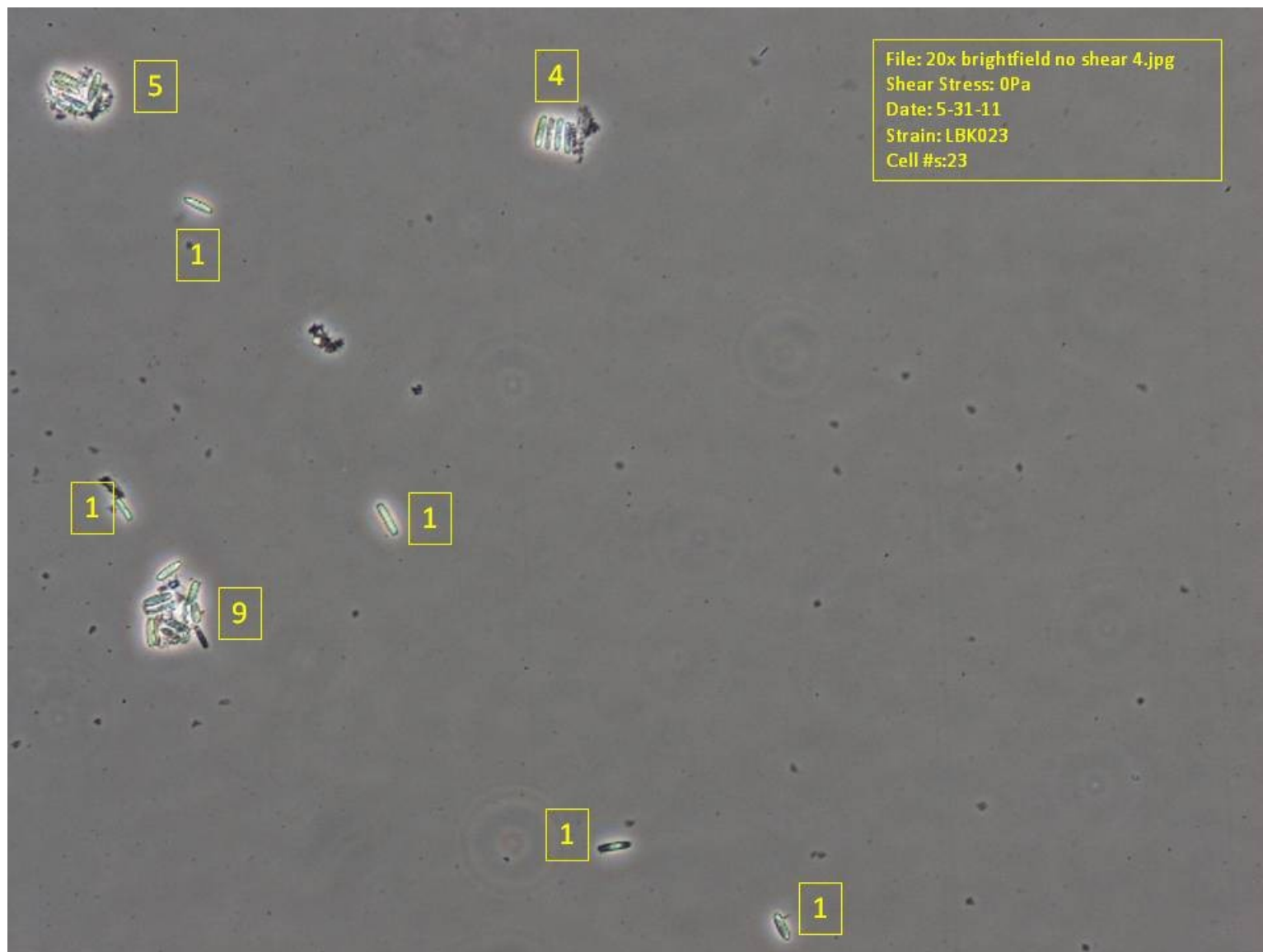


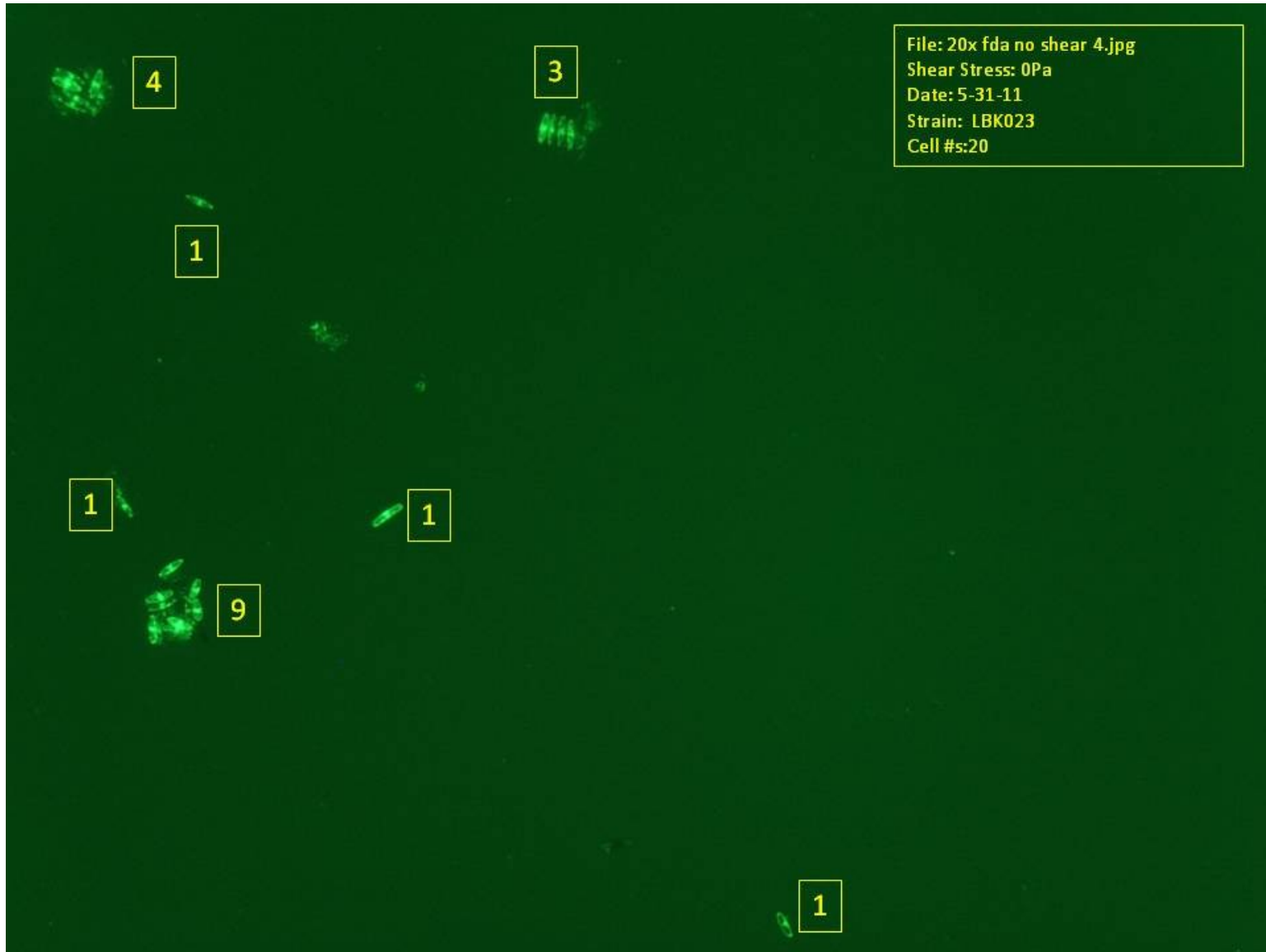
File: 20x brightfield no shear 3.jpg  
Shear Stress: 0Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:27

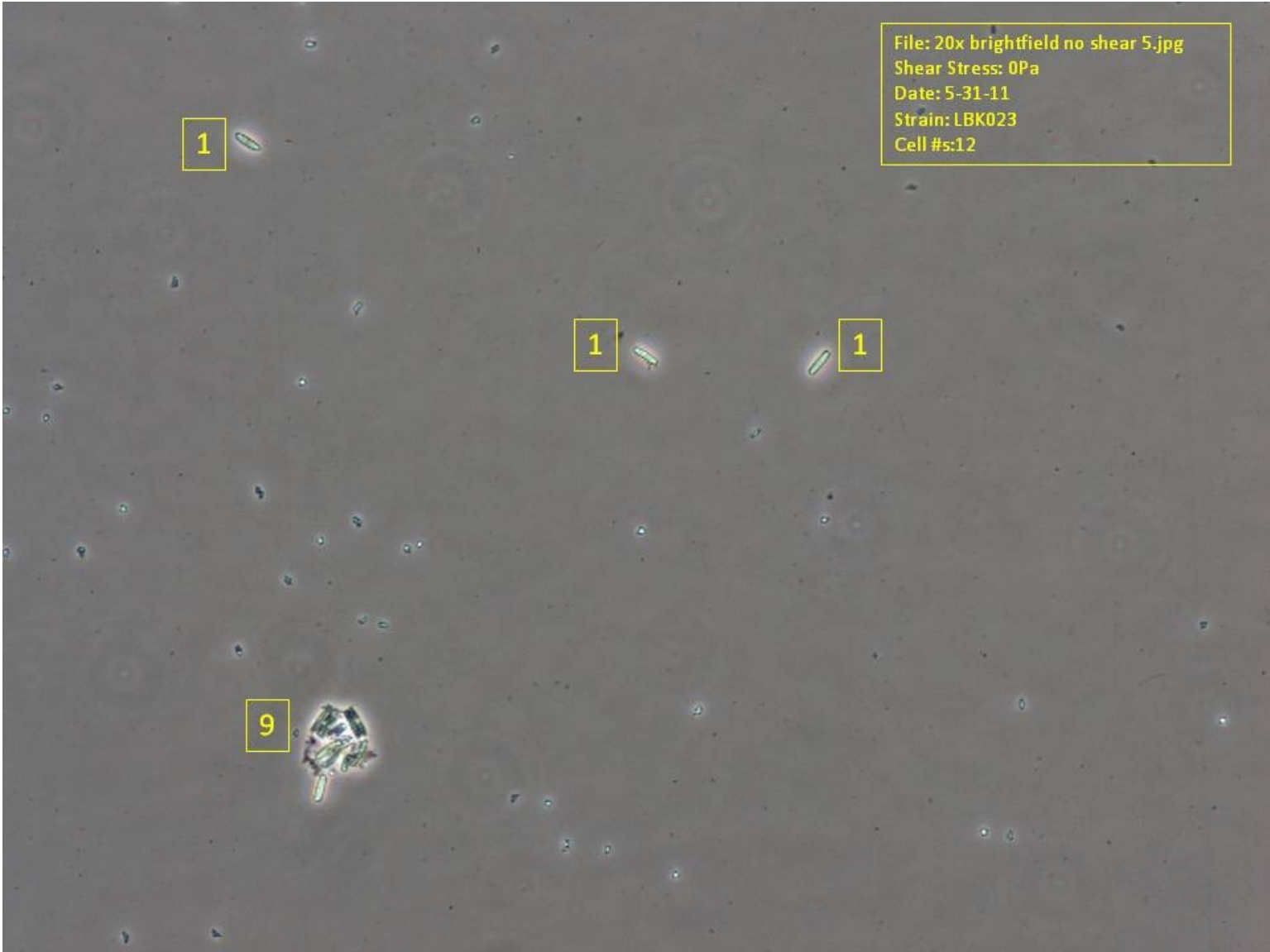




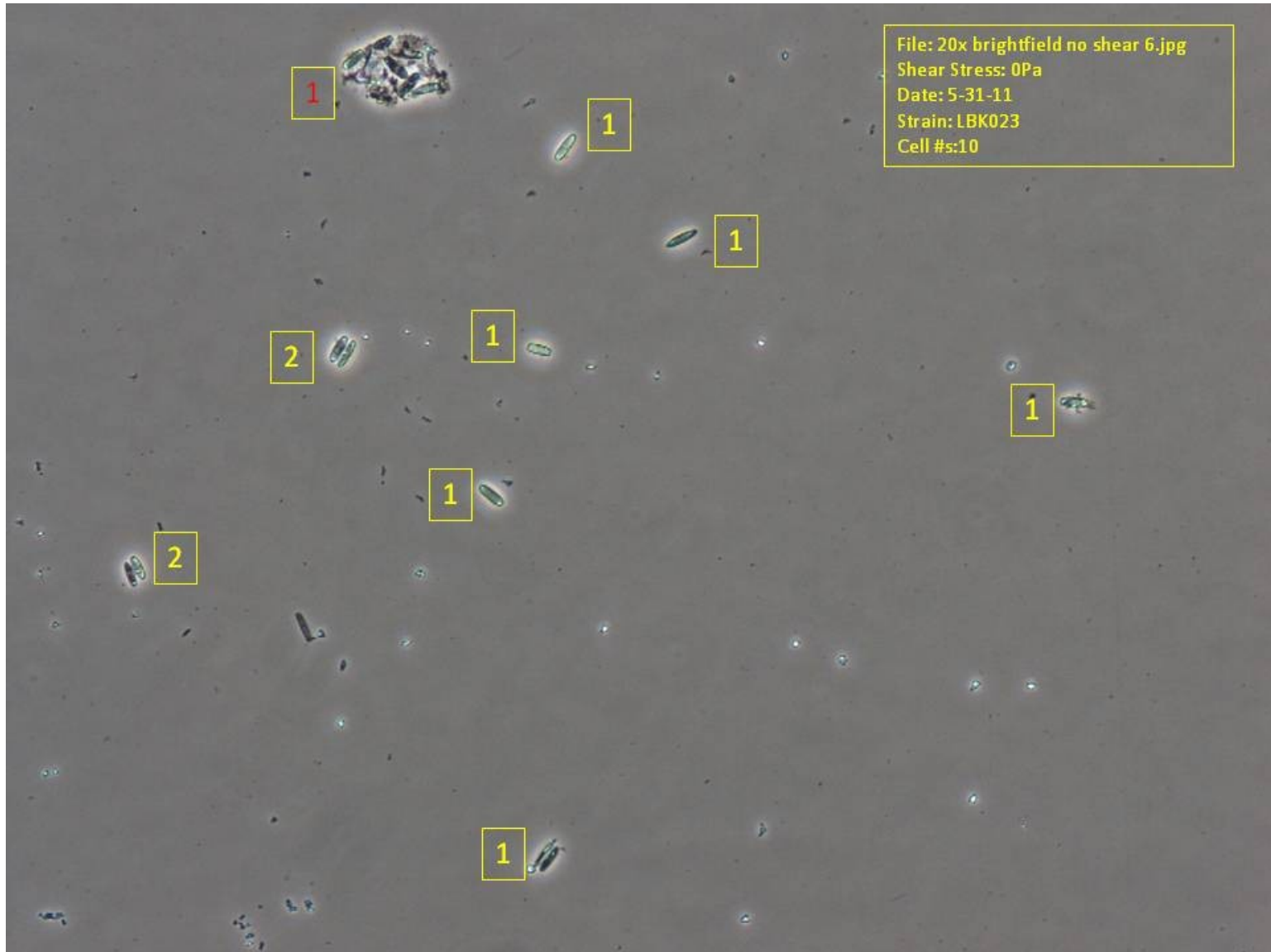






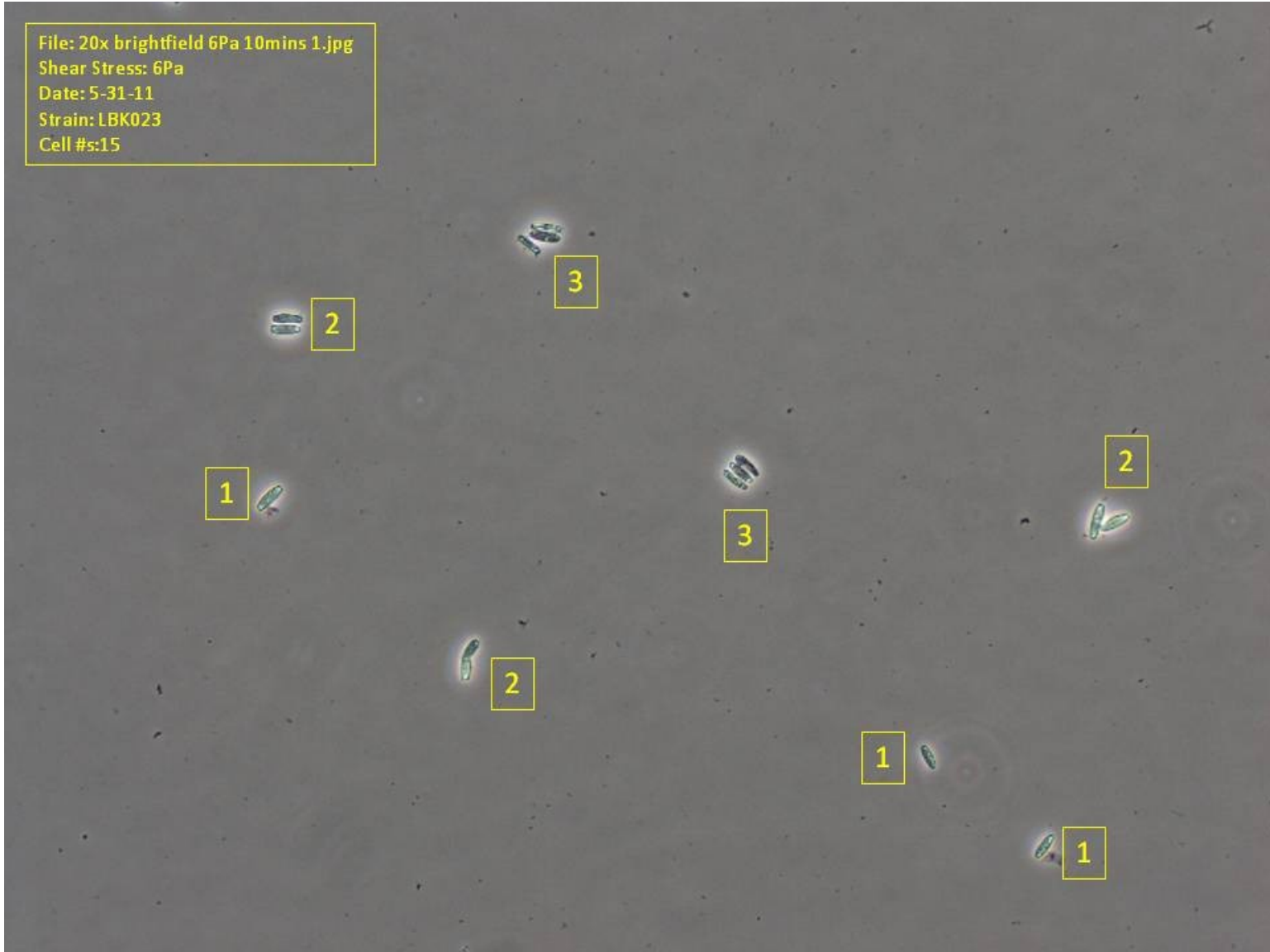








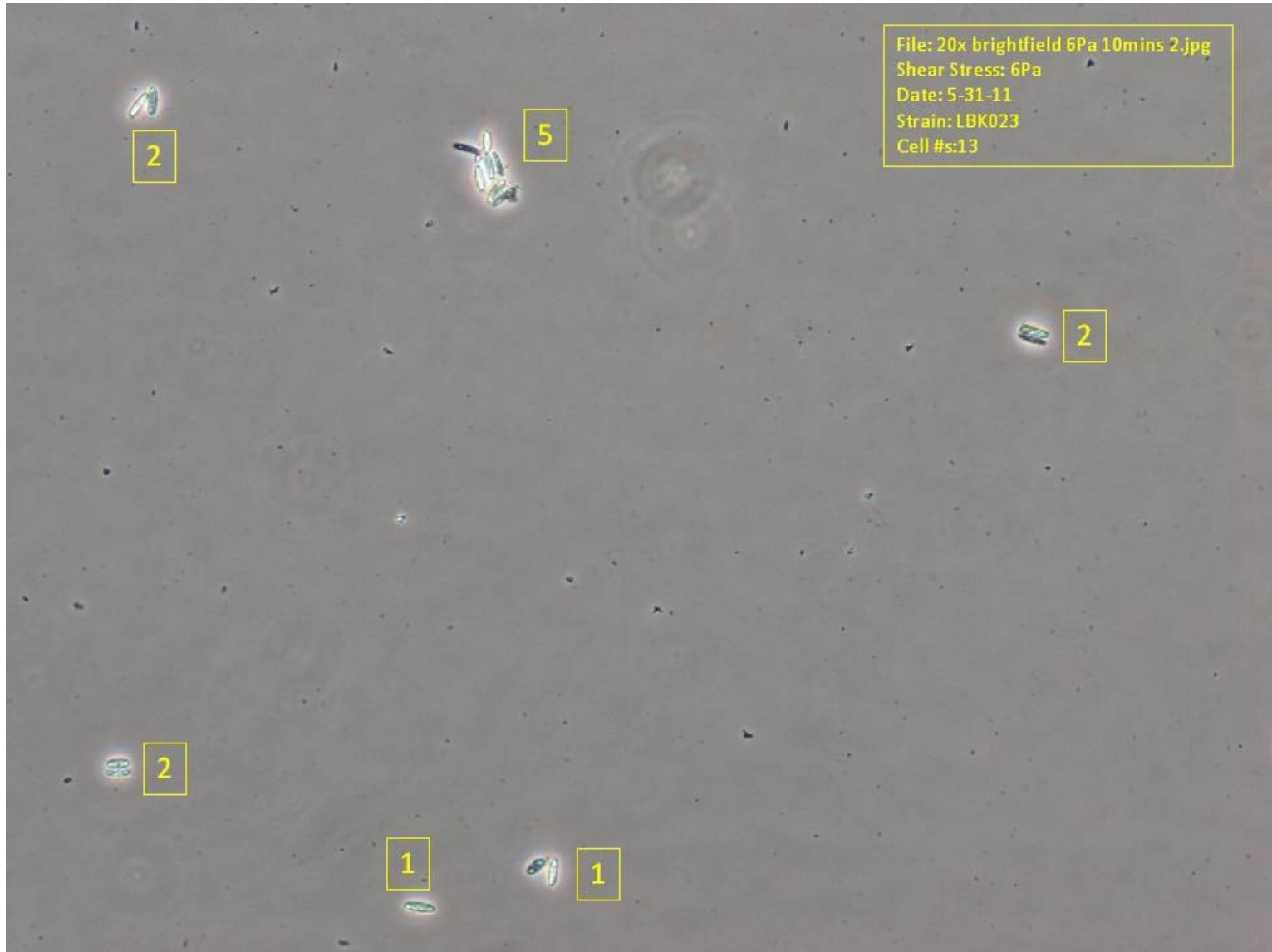
File: 20x brightfield 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:15



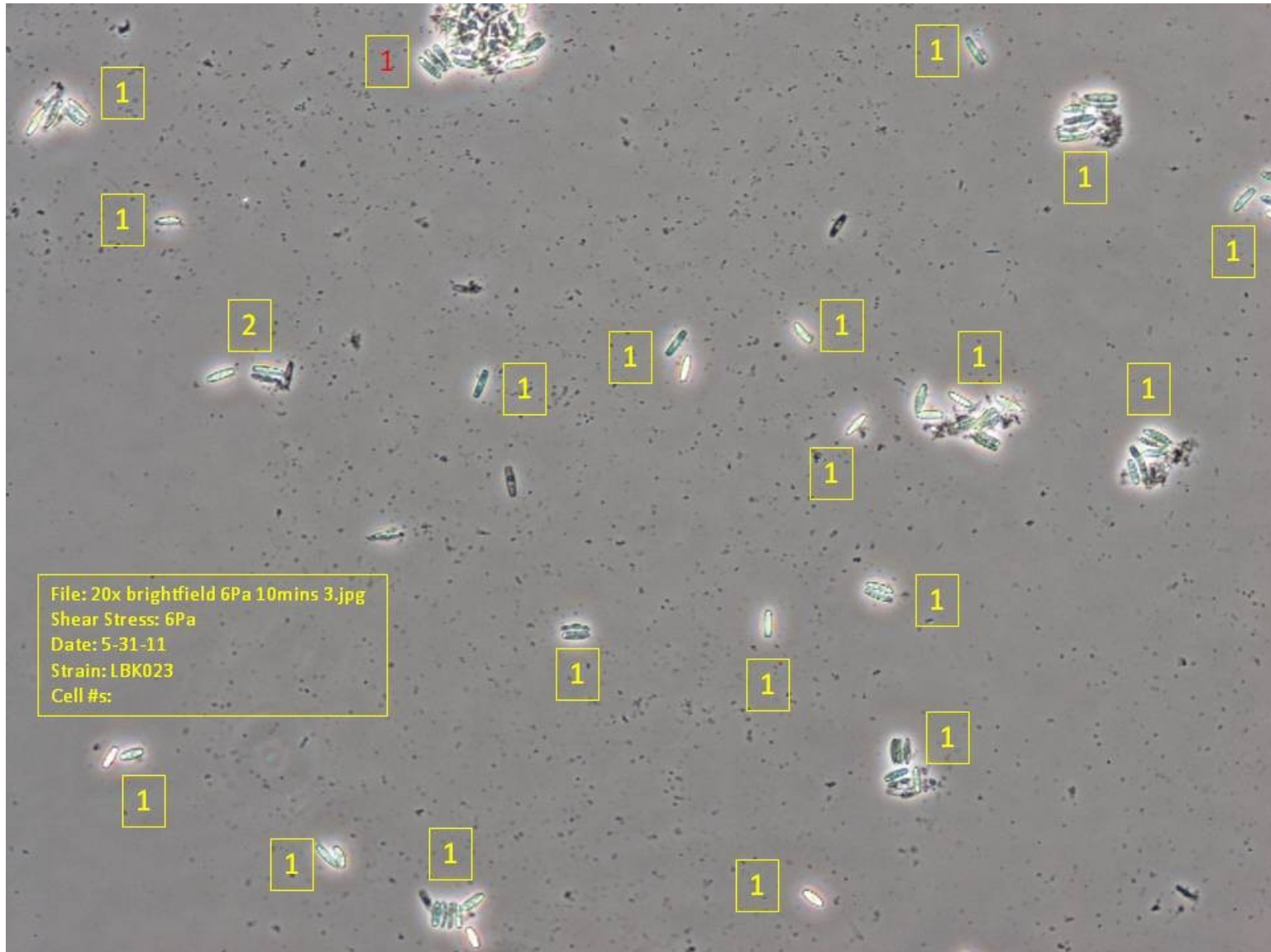
File: 20x fda 6Pa 10mins 1.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:11

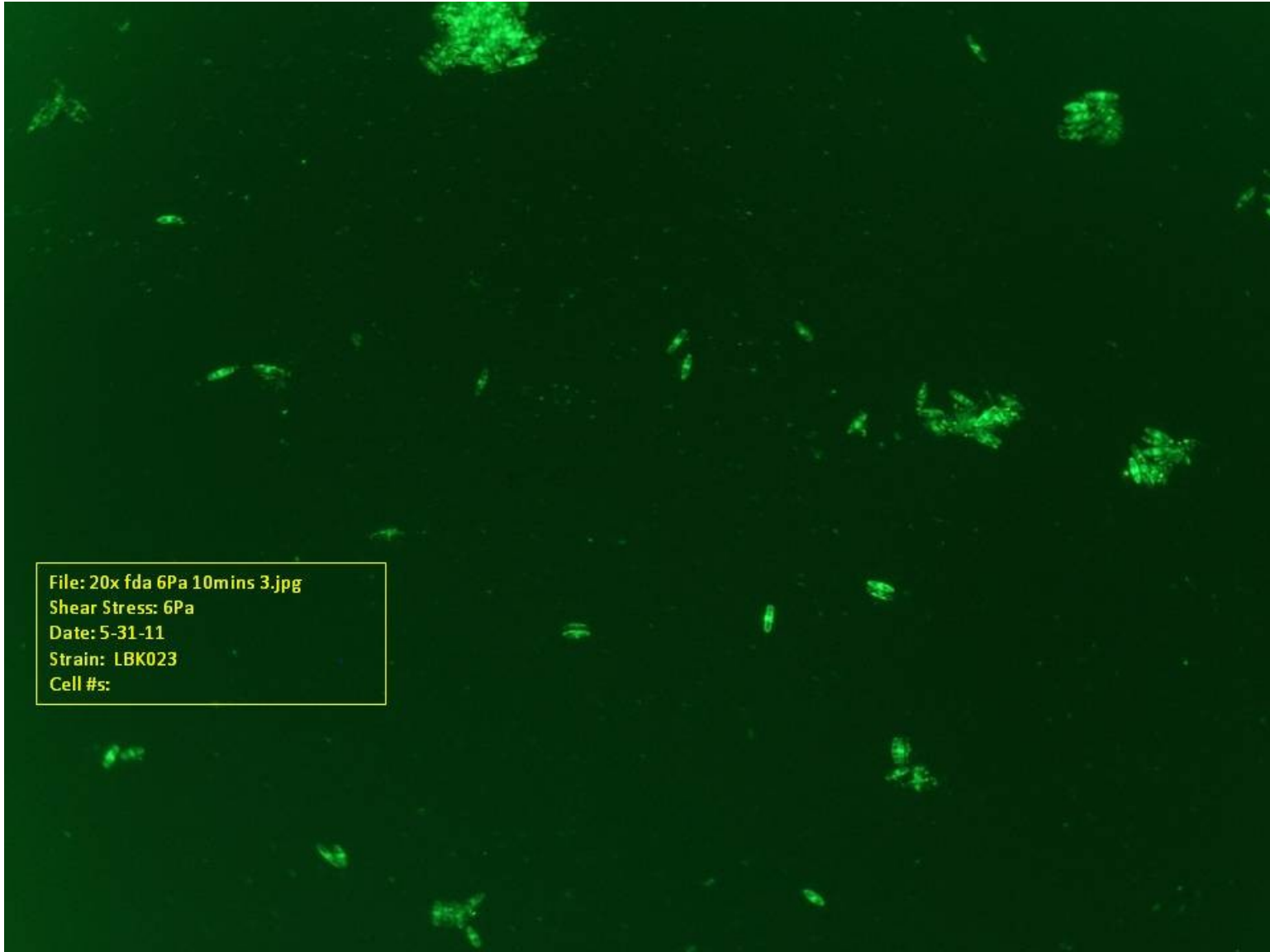


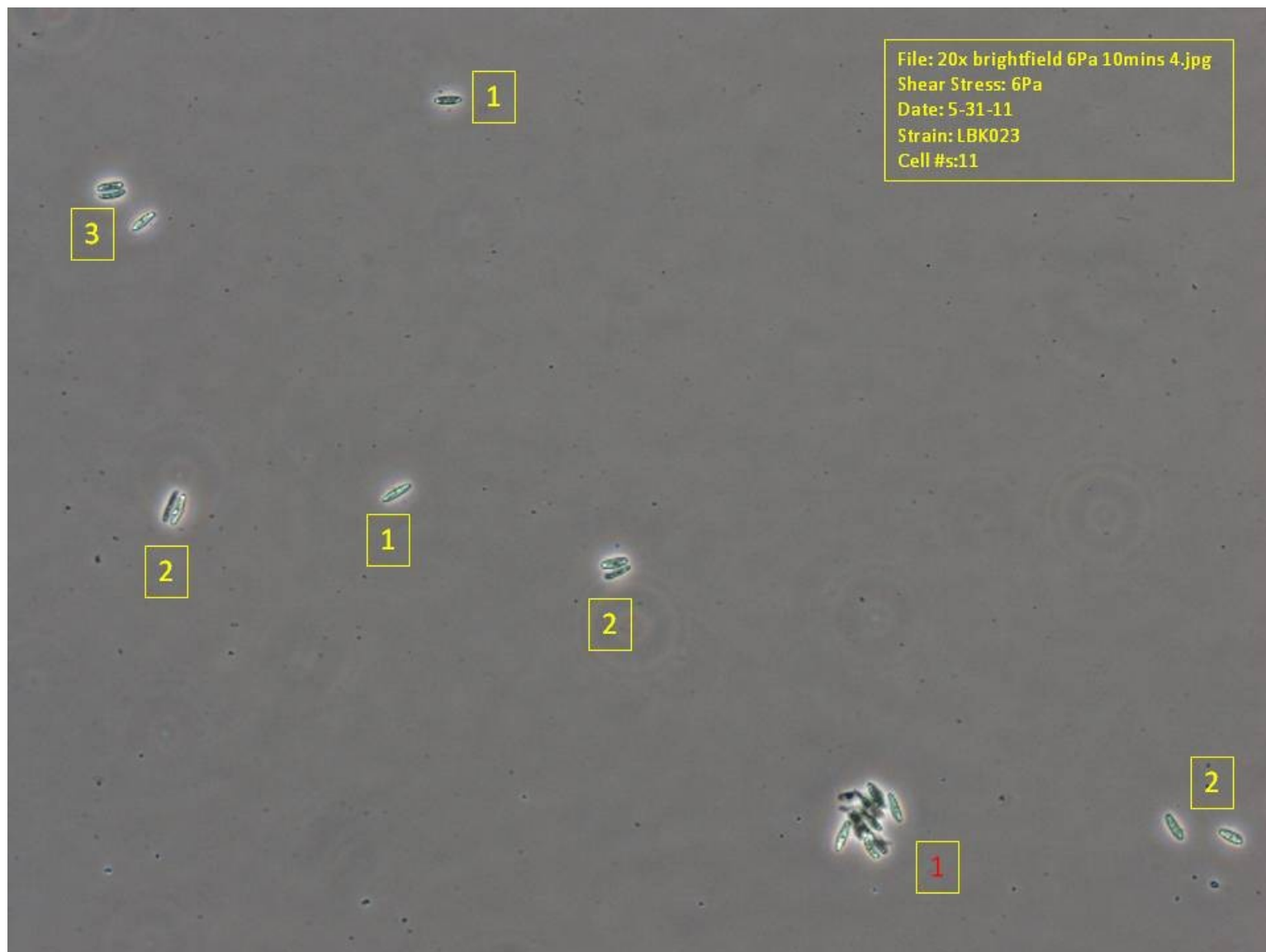






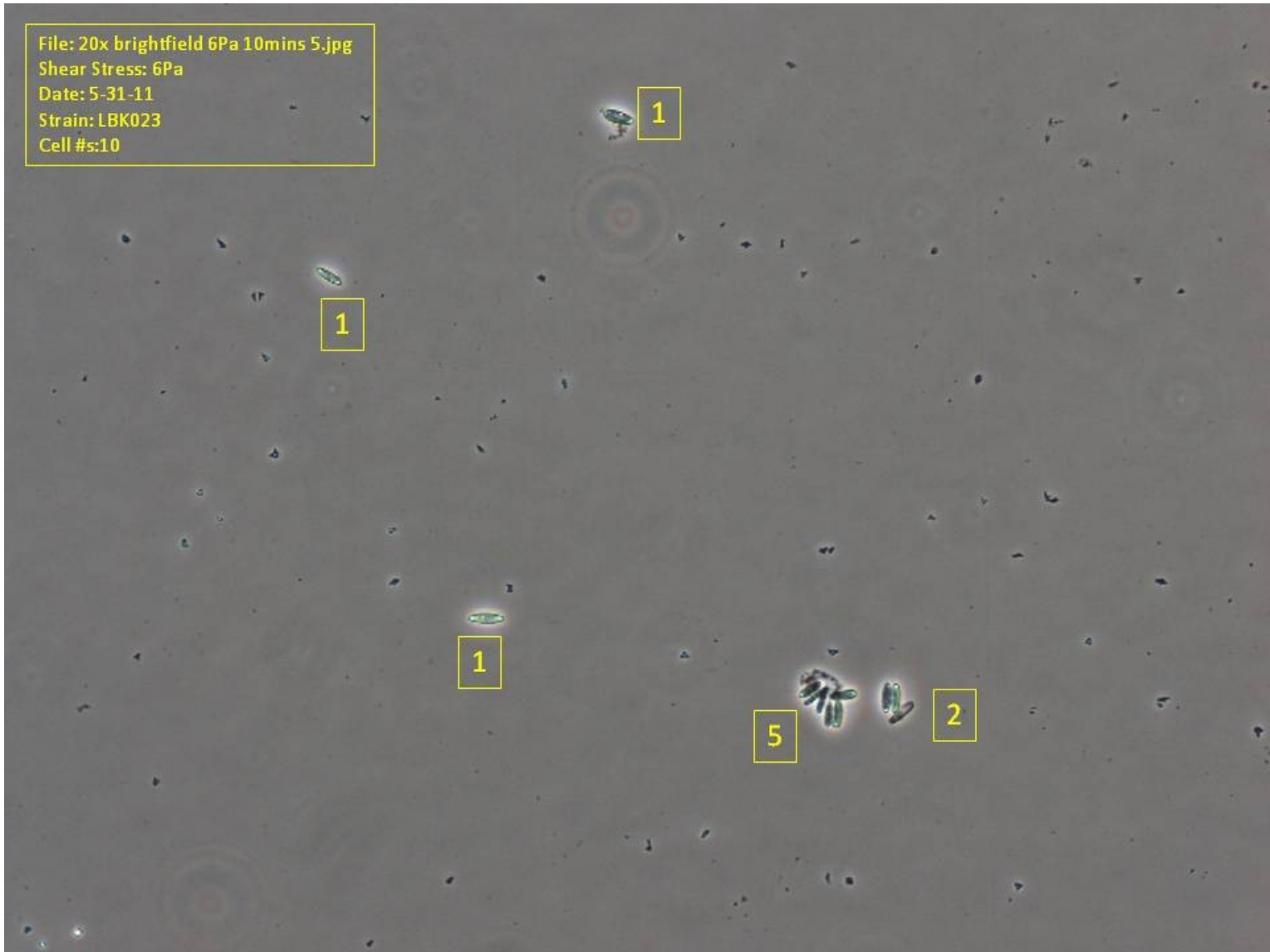








File: 20x brightfield 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #s:10



File: 20x fda 6Pa 10mins 5.jpg  
Shear Stress: 6Pa  
Date: 5-31-11  
Strain: LBK023  
Cell #:7





