Lab 6
Polylactide: Synthesis, Tacticity and Properties
Report Due: *In Lecture*, Wednesday, April 26

Introduction

Polylactide is a commercial, biodegradable polymer that is produced for a variety of packaging, biomedical, and other materials applications. As the infrastructure for extracting lactide monomer from natural resources and for polymerizing lactide into polylactide is built, this polymer will likely displace polymers made from petroleum feedstocks for a variety of applications. L-Lactide monomer is currently distilled from corn biomass, which naturally contains L-lactic acid, oligo(L-lactide) and other precursors which are driven to cyclic L-lactide by the distillation process. The distilled L-lactide is then polymerized in the presence of tin 2-ethylhexanoate to give poly(L-lactide), PLLA.

L-lactic acid   poly(L-lactide)   S,S-lactide
                                                                (L-lactide)
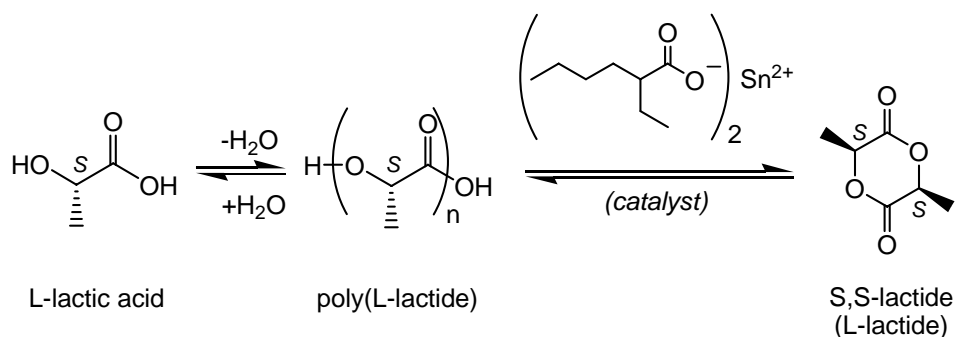
The second reaction of this scheme accounts for both the distillation of L-lactide monomer (in the forward direction, driven partly by evaporation of the product) and the formation of PLLA (in the reverse direction). The ΔH_p and ΔS_p for the polymerization reaction are both negative, and ΔG_p is close to zero; as a result, the polymer is thermodynamically favored at low temperatures and monomer is favored at high temperatures. PLLA is naturally hydrolyzed back to monomer by composting or by organisms that consume the polymer.

The predominant stereoisomer found in nature is L-lactide. However, during the lactide formation process, epimerization of the chiral center can occur and the L-lactide can become contaminated with both *meso*-lactide and D-lactide. When mixtures of L-lactide, *meso*-lactide and D-lactide are copolymerized, the stereochemical purity of the resultant polymer is compromised (i.e. not all of the stereocenters in the polymer have the same configuration). This leads to a material that is more difficult to crystallize and, if the level of impurity is high enough, the material can be completely amorphous (non-crystalline). As Gruber and colleagues point out,[1] keeping a high level of crystallinity is important for the mechanical performance of the material. However, PLLA that is too crystalline can yield a brittle product, and so sometimes stereodefects are intentionally incorporated to improve the polymer's mechanical properties. In any case, the degree of stereochemical purity in the polymer product is important for commercial applications.

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[1] Drumright, R. E.; Gruber, P. R.; Henton, D. E. *Adv. Mater.* **2000**, *12*, 1841.

In this experiment, you will perform the ring-opening polymerization of L-lactide with various amounts of added racemic lactide added. (Racemic lactide is a statistical mixture of 25% L-lactide, 25% D-lactide, and 50% *meso*-lactide. In this mixture, the relative numbers of *R* and *S* stereocenters is exactly 1:1.) This will create occasional single and double stereodefects in the polymer that can be quantified by a variety of analytical techniques. At the low fractions of *rac*-lactide you will be using, one would imagine that most of these would be isolated single and double *R*-defects in an excess of *S*-polymer.

SSSSSSSSSSS	*SSRSSSSSSRS*	*SSSSRRSSSSSS*
PLLA *homopolymer*	*single stereodefects:* *contaminated with* *meso*-lactide	*pairs of stereodefects:* *contaminated with* *D-lactide*

Both types of stereodefects shown above are observed in commercial PLLA polymer. There are also a few complexities to the polymer stereoregularity keep in mind:

1. Transesterification (chain transfer to polymer) processes can randomize the as-synthesized polylactide chains, thus redistributing the stereochemical defects.

2. The "copolymerization" of these two lactide monomers probably isn't an ideal random copolymerization ($r_1 = r_2 = 1$), and therefore the defects may not be distributed randomly.

3. Some polymerization catalysts lead to epimerization of the chiral centers, thus randomizing their absolute configuration.

So, the stereoregularity of the polymer product is not solely determined by the stereochemistry of the monomers used. Nevertheless, in your polymer, the number of defects should be roughly dictated by the amount of racemic lactide impurity added.

^{1}H NMR can be used to quantify the number of *R* defects in a nearly all-*S* polymer, because local variation in tacticity results in measurable variation in chemical shift.[2] In an NMR study of defects in PLLA, researchers at Cargill and the U determined that the local monomer sequence *SSSSRS*, containing a single *R* defect, showed a characteristic resonance at $\delta = 5.21$ ppm in a 500 MHz decoupled NMR spectrum, whereas the stereoregular *SSSSSS* sequence showed a resonance at $\delta = 5.17$ ppm.[3,4] (The defective *SSSSRS* sequence has tacticity notation *iiiss*, where *i* refers to an isotactic *SS* dyad and *s* refers to a syndiotactic *RS*/*SR* dyad.) In addition, double stereodefects showed two characteristic ^{1}H resonances at $\delta = 5.22$ and $\delta = 5.23$ ppm.

i *i* *i* *s* *s*	*i* *i* *i* *s* *i*	*i* *s* *i* *s* *i*
S S S S R S	S S S S R R	S S R R S S
single stereodefect $\delta = 5.21$ *ppm*	*double stereodefect* $\delta = 5.22$ *ppm*	*double stereodefect* $\delta = 5.23$ *ppm*

[2] Odian, G. *Principles of Polymerization*; Wiley: New York, 1991; chapter 8.
[3] Thakur, K. A. M.; Kean, R. T.; Hall, E. S.; Kolstad, J. J.; Lindgren, T. A.; Doscotch, M. A.; Siepmann, J. I.; Munson, E. J. *Macromolecules* **1997**, *30*, 2422.
[4] Thakur, K. A. M.; Kean, R. T.; Hall, E. S.; Doscotch, M. A.; Munson, E. J. *Anal. Chem.* **1997**, *69*, 4303.

In your NMRs, you may or may not be able to integrate the δ = 5.21-5.23 ppm signals independently; however, taken together, these peaks indicate how much stereodefect is present in your polymer.

Polarimetry can also be used to quantify stereochemical defects in PLLA, though it cannot distinguish single and double defects. For a stereochemically pure polymer sample, the observed optical rotation observed in a polarimeter (α) is defined by the specific rotation $\left[\alpha_D^{25}\right]_0$, the cell path length l, and the concentration c:

$$(6.1) \qquad \alpha = \left[\alpha_D^{25}\right]_0 lc$$

This specific rotation is labeled "D" because it is usually measured at the atomic D line of a sodium lamp source at 589 nm. For PLLA, $\left[\alpha_D^{25}\right]_0$ has been reported to be -156°,[5] and you will confirm this by measuring the rotation of your pure PLLA polymer. The optical purity of a PLA polymer can then be determined by measuring the optical rotation of the impure sample and comparing it to this specific rotation.

$$(6.2) \qquad \text{optical purity (\%)} = \frac{N_S - N_R}{N_S + N_R} = \frac{\left[\alpha_L^{25}\right]}{\left[\alpha_L^{25}\right]_0} \times 100$$

In this lab, you will also study the effects of stereodefects on the crystallization of PLLA. Pure PLLA is a highly crystalline polymer, but increasing amounts of R-stereocenters inhibit the crystallization of the polymer. This effect can be observed by measuring the amount of heat required to melt the crystalline polymer; the more heat that is required, the more crystalline the polymer is. In PLLA, stereodefects have also been shown to depress the crystalline melting temperature (T_m). You will be performing this thermal measurement by differential scanning calorimetry (DSC), an instrumental technique that measures heat flow into a material.[6] DSC is an extremely common technique for measuring the temperature and enthalpy of phase transitions in materials. In the case of polymers, DSC can be used to determine glass transition temperatures (T_g) and crystallization/melting temperatures (T_c and T_m). At any temperature where no phase change is occurring, the flow of heat into a material (sometimes labeled ΔT) is determined by the material's heat capacity (C_p), the heating rate (q), and an instrumental calibration factor (K):

$$(6.3) \qquad \Delta T = \frac{qC_p}{K} .$$

Over most temperature ranges the DSC instrument measures a constant heat flow because heat capacity remains constant. A glass transition is, by definition, a phase change which alters the heat capacity of the material but does not have a transition enthalpy. So, at a material's T_g, the DSC trace shows a first-order inflection. However, at a material's crystalline melting temperature (T_m) the DSC instrument must overcome an enthalpy of fusion (ΔH_f), which results in a second-order peak in the DSC trace. The area underneath the peak corresponds directly to ΔH_f for the sample.

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[5] Sarasua, J. R.; Prudhomme, R. E.; Wisniewski, M.; Leborgne, A.; Spassky, N. *Macromolecules* **1998**, *31*, 3895.

[6] For more information on DSC, see Macrogalleria's DSC webpage, (http://www.pslc.ws/macrog/dsc.htm).

Because most crystalline polymers are actually only partially crystalline, they display only a fraction of the theoretical maximum ΔH_f. The degree of crystallinity in a polymer sample can be calculated by comparing the ΔH_f measured by DSC with the maximum ΔH_f^0 obtained by other (indirect) methods.

$$(6.4) \qquad \text{degree of crystallinity} \approx \frac{\Delta H_f}{\Delta H_f^0}$$

For PLLA, a number of values for ΔH_f^0 have been reported.[5] In this lab, you will use the value $\Delta H_f^0 = 93$ J/g.

Components of a DSC trace. The trace above would be obtained in the heating direction; in the opposite, cooling direction, $\Delta T < 0$ and transitions have opposite signs.

You will also be examining the effects of stereodefect on the crystallization of PLLA using polarized optical microscopy.[7] In polarized optical microscopy, a polymer sample is imaged between two polarizing films. When the polarization axes of the two films are oriented at a 90° angle to one another, the illuminating light cannot ordinarily pass through to the eyepieces or camera. However, if the material between the crossed polarizers exhibits some order, birefringence in the sample will cause the light to pass. As a result, growing spherulites of ordered, crystalline material can be distinguished from the surrounding disordered melt by characteristic bright and dark patterns against a dark background.

The growth of these crystalline spherulites can be imaged by time-lapse digital photography. The rate of growth (G) of a spherulite is defined as dr/dt, where r is the radius of the spherulite. A plot of r vs. t usually gives a linear plot with slope G,[8] until the measured spherulites grow into one another. There are two conflicting influences on how G varies with temperature: viscosity and undercooling. The viscosity of the sample determines how quickly molecules can organize themselves at the crystalline front, and because viscosity increases with decreasing temperature, low temperatures slow crystallization. However, the difference between the sample temperature and the crystallization temperature ($T_c - T$), or "undercooling", thermodynamically drives the growth of crystals. In this case, temperatures that are too high ($T_c \approx T$) also suppress crystallization. As a result of these two competing effects, the rate at which polymer crystals grow is usually maximized tens of degrees below T_c. Stereodefects will also impact the rate at which PLA crystallizes from the melt. You will image the effect of stereodefects on crystallization kinetics for your PLLA in this lab, by imaging the growth rate of spherulites for different materials on a temperature-controlled microscope stage.
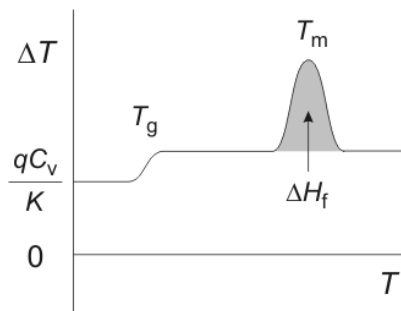
Experimental

Polymerization of pure and impure L-lactide (Mar 30/31)

The goal of this experiment is to synthesize polylactide by ring-opening polymerization.

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[7] For more information on polarized light microscopy, see MicroscopyU's interactive webpage (http://www.microscopyu.com/articles/polarized/polarizedintro.html). You may also want to check out the other pages at the MicroscopyU site to learn about materials microscopy.

[8] "Nucleation and Growth Kinetics: Measurements", in Schultz, J. M. *Polymer Crystallization*; Oxford University Press: New York, 2001; chapter 7.

- Check out two small stirbars from the stockroom, and pick up two vials with black phenolic caps from the TA's.

- In addition to polymerizing a sample of pure L-lactide, you will have been assigned a ratio of L-lactide to *rac*-lactide monomer to copolymerize. For each of these samples, weigh out 3 g *total* lactide and place it into a vial. Label each vial appropriately.

- Use a microsyringe to add 0.1 mL of a 0.2 M solution of tin 2-ethylhexanoate initiator in methylene chloride to each vial.

- Place the vials in the oven, set at 170 °C, *without their caps* for 3 minutes. By this time the methylene chloride should have evaporated and the lactide mixture should melt. If not, you may put the vials back in for 2 more minutes.

- Using tongs or gloves, remove the vials from the oven. Tightly cap the vials and then shake them vigorously for 30 seconds. (This helps disperse the tin.) Place the capped vials back into the oven at 170 ºC and leave them for 1 h. The polymerization is sensitive to temperature, so try to follow TA's instructions on keeping the oven door closed and on placement of your vial in the oven.

- Remove the vials from the oven and let them cool at room temperature for 5 minutes. Open the vials and add 10 mL chloroform and a small magnetic stir bar to each. VERY loosely recap the vials and place them on a magnetic stirrer/hot plate. VERY gently warm the mixture to dissolve the polylactide. You will need to rapidly stir the mixture until the entire polymer sample has dissolved. This can take up to 30 minutes.

- Let the solution cool back to room temperature and precipitate the polymer by pouring it into a beaker containing 30 mL methanol. If you see solid material remaining in your vial, repeat the previous step with 10 mL more chloroform. Recover the polymer using a Büchner funnel vacuum filtration apparatus. Dry the material as best you can on the filter, and measure the yield of your product.

- Put 0.2 g of each polymer into a separate aluminum pan and melt the samples using a hot plate. Be careful not to touch the polymers with your fingers. Once the polymer samples have melted, check to make sure that the normal oven has been set to 100 ºC, and put your pans into the oven for 30 minutes to anneal. After annealing, save these samples separately from your bulk material for next week's DSC experiments.

- Dissolve another 0.1 g of each of your polymers in 1 mL THF, and filter the solutions through teflon syringe filters into aluminum pans. Cover each pan with aluminum foil and label it, and punch a few small holes in the foil to allow your solution to evaporate. Place these pans in the hood to dry overnight; you will use the dried material in the optical microscope experiment.

- Dry the remainder of your polymers in labeled aluminum pans in the vacuum oven. You will pick these samples up next week.

^{1}H-NMR spectroscopy of polylactide: Determining tacticity (Apr 6/7)

The TA's will collect two NMR spectra for each of your two polymer samples. One of these spectra will be a typical NMR with adjacent protons coupled to each other in the normal way. In the second, however, the TA's will "decouple" the α-protons from the methyl protons in your PLA by selectively irradiating the methyl resonances ($\delta = 1.6$ ppm). This serves to simplify the α-

proton (δ = 5.2 ppm) region of the spectrum by turning the quartets that you might expect there into singlets. A consequence of this method is that all useful information in the irradiated, δ = 1.6 ppm region is lost. If you see anything at δ = 1.6 ppm, ignore it.

- Check out two NMR tubes from the stockroom.

- Prepare ^{1}H-NMR samples by dissolving 30 mg of PLA in 1 mL of $CDCl_3$. It is important to have a low concentration to control the broadening of the peaks in the NMR spectrum due to increased solution viscosity.

- A TA will collect a decoupled ^{1}H NMR spectrum of each of your PLA samples, and the data can be worked up as for previous labs. In particular, you will want to closely examine the δ = 5.3-5.0 ppm region of the NMR spectrum, as this is where the information on the stereochemistry of the α-proton is. Use reference 2 (above) to guide your analysis of this region of the NMR spectrum; what types of stereodefects do the peaks in this region correspond to?

- When you print out your NMR spectra, make sure you print out both a full (δ = 10-0 ppm) spectrum as well as a closeup of the δ = 5.3-5.0 ppm region for each sample.

Polarimetry (Apr 6/7 or 13/14; see schedule)

- Weigh out 100 mg of each of your polymer samples into a small, tared beaker and record the exact mass.

- Add 10 mL of chloroform to each beaker to dissolve the polymer.

- Have the TA show you the polarimeter.

- Once the polymer is dissolved place enough of the solution into the 50 mm sample cell to nearly fill it, making sure not to touch the ends of the cell.

- Check that there are no bubbles in the cell, as they will disturb the readings.

- Enter the concentration of your sample into the control panel on the top of the instrument. (Note: the concentration entered is [(mg/ml)*0.1].)

- With nothing in the sample chamber, lift and close the door. Wait for a reading to appear then press [zero]. Repeat this step two times.

- After the instrument is zeroed, place the sample cell into the chamber. Make sure that the end of the cell is pressed up against the left side of the chamber.

- Once the sample cell is in place, lift and close the door and wait for a reading. Record the number displayed. Repeat this three times.

- Clean the cell with acetone, making sure not to touch the ends of the cell.

Differential scanning calorimetry (Apr 6/7 or 13/14; see schedule)

Caution: Never touch the PLA samples or crucibles with your hands. Oils from your fingers will alter the results of the DSC analysis. Use tweezers and a razor blade to hold and cut your sample.

- Weigh out 3-10 mg of your PLA sample. Accurately weighing out the samples is essential to accurate results; in order to obtain accurate DSC data you must weigh your samples to the nearest 1/10 of a milligram.

- Place your annealed sample at the bottom of a 40 μL aluminum crucible. Use the crucible press to secure the top to the crucible. Place your crucible in a properly labeled vial until it is time to run your sample.

- *DSC instrument instructions:* If at any time you enter an incorrect number you can press the "CH" key to reenter the correct number. If you realize you entered an incorrect number after you've moved on to the next step, you must press the "Reset" button and start over from the beginning.

  Step-by-step instructions (the TA will explain all of the parameters in detail)

DSC Display	**Press**
DSC 35 °C	Arrow up then 8
Scan parameters	Run
Start Temp	0 then Run
Rate °K/min	20 then Run
End Temp	200 then Run
Time iso min	Run
Plot cm 10	Run
Range FS mw 10	Run
Offset %90	Run
Pan type 1/2 1	Run
Limit m W 0	Run
Content	Run
Auto limit 0/1 0	1 then Run
Start Temp °C	100 then Run
End Temp °C	195 then Run
Baseline Type 8	7 then Run
Plot Cm 10	Run
Plot Mode 101	Run
ΔH Spec J/g 290	93 then Run
DSC Content °C	Run
Indent#	Group# and L-lactide %
Weight	Enter weight in mg then Run
Insert T °C	Run
Insert Sample	Insert your sample then Run
Start T °C	Your trial has begun

- For each PLA sample, you should measure both T_g and T_m (peak temperature) if possible.

Polarized optical microscopy (Apr 6/7 or 13/14; see schedule)

You will be performing this experiment in groups of four. The group should collect a complete set of variable-temperature images on one PLLA sample, and then collect as much data as possible on both optically impure samples.

- The TA will help you familiarize yourself with the polarizing microscope. Make sure you can identify the polarizer, analyzer, condenser, and objective. In addition, make sure you understand how to control the temperature of the hot stage, and how to insert a sample into the viewing area.

- You will be using two programs, Pixera Viewfinder and Pixera Studio, to save images. On the computer, make sure that both programs are open, and that there are no images loaded in Studio. With the Viewfinder program active, practice moving a sample of crystalline imidazole around the viewing area. *Notice that the area imaged by the camera is smaller that the view in the eyepieces; as a result, sometimes it helps to use the eyepieces for rough positioning of features into the camera's view.* Rotate the sample stage to see the effect of polarizer and analyzer angle on the features of the crystal.

- In Viewfinder, select **Options** -> **Customize…** -> **Capture Destination**. Make sure that both the "Save Captures to Disk" and "Send to Pixera Studio" boxes are checked. Make sure your target directory is set to "C:\chem5223\", and give your image files an appropriate prefix. The software will automatically append a sequential, three-digit number to this prefix to name every bitmap image you take. Hit **OK**.

- With the heat stage temperature set near room temperature, place the stage micrometer in the stage, in the viewing column, and focus. The micrometer has a set of ruled lines that you will use to calibrate distances in your other images.
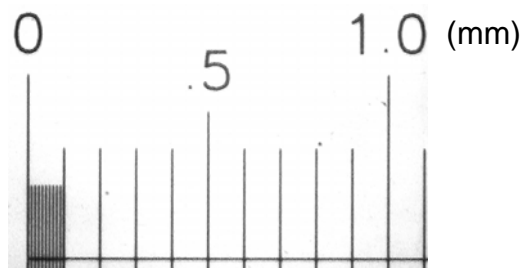
Find these lines in the eyepieces, and then center and straighten them (horizontal/vertical) in the camera view. *The micrometer is polarized so that you can see it in between the crossed polarizers. Make sure that the objective does not touch the hot stage while you are adjusting the focus; this will damage the optics.*

- Focus, and take a picture by either (a) pressing the "camera" button at the bottom left or (b) pressing **Enter**. The picture should pop up in the Studio window, and the filename should end with a "1". Bring Viewfinder back to the front of the desktop, and remove the micrometer from the heat stage.

- Sprinkle a very small amount of your filtered PLLA onto the last third of a 3" x 1" microscope slide, and then cover this with another slide. Be careful to keep the slides as free of dust as possible; you can help this by wearing gloves while you prepare your samples, and by keeping the box of unused slides closed.

- Set the hot stage to 120 °C. Wait until the stage reaches the set temperature before continuing.

- Heat your sample slide on a hot plate, pre-set to ~190 °C, in order to melt the polymer solid. A minute or so after you see your material melt, press down on the hot cover slip with something other than your finger to spread the liquid material.

- Immediately slip your slide sandwich into the hot stage on the microscope. As your material cools, move your sample around and try to find spherulitic nuclei in your sample using the eyepieces. As soon as you center a few spherulites nucleating in the Viewfinder window, focus as best you can, and immediately take an image. Continue to take images in Viewfinder every 20 seconds or so, and record the run time of each image. If you don't ever observe spherulites, make another sample and try again. If you still don't observe spherulites at this point, cool the stage to 90 °C (instead of 120 °C), make another sample, and try again. If you don't observe spherulites at this point, it may be because your polymer does not crystallize.

- Continue to collect images until the spherulites have grown into one another. Even if one side of a spherulite has stopped growing, you can always use a different direction to determine the radius, so keep imaging until nothing is growing. All of your images should pop up in Studio. Once you are finished, you can enter Studio, check to make sure nothing wrong has happened in the images, and close them.

- Repeat these instructions for all of the group's samples. (You may want to use different filename prefixes for your different runs.)

- Once you are finished, write your files to CD-R. Put a blank CD-R disk into the CD-RW drive. Windows should ask you what you would like to do with the CD; select the Nero CD burning software. On the right panel, find your files in the C://chem5223/ directory. Select all of your files that you would like to save, and drag them into the left (CD-R) panel. When you have selected all the files you want, select the "burn" icon at the top to create your CD.

Image analysis:

- In order to obtain radii r for your growing spherulites, you will have to open your image files in an image analysis program that allows you to make diagonal distance measurements. The image viewer that comes with Microsoft Windows won't do this, but the NIH-supported freeware program ImageJ will. (Program available in the Microcomputer Lab in 176 Kolthoff, or free download from http://rsb.info.nih.gov/ij; you will also need the latest version of the Java Runtime Environment, J2SE 5.0 JRE, from http://java.sun.com/j2se/1.5.0/download.jsp. If you use WebCT, you probably already have this version on your computer.) You can also do this in most commercial image editing programs such as Adobe Photoshop. The instructions below are written for ImageJ.

- First, open the image of your stage micrometer in ImageJ. In the ImageJ Toolbar, select the Straight Line tool. Click-and-drag a line between two of the micrometer lines. Information about your line, including angle and length in pixels, are located underneath the Toolbar.

- Calculate a conversion factor to turn micrometer image pixels into real µm.

- Using the same Straight Line tool, you should now be able to measure spherulite radii r in µm for each of the images you collected. Try to pick the center point of each spherulite as consistently as you can, and you may want to make multiple measurements for different spherulites in each image for accuracy.

Lab Writeup (due *In Lecture*, April 26)

For this report, you will be responsible for providing both a structural and a logical framework for your experiments—meaning your report should not only have all the appropriate sections and data, but it should also say something about stereodefects in polylactide and what effects those defects have on the spectroscopic, optical and thermal properties of PLA. You will probably find some conclusions on these subjects in the references given in these instructions—does your data agree? What characteristics of crystalline polymers are your results consistent with?

Be particularly critical of your Experimental section—would someone who did not have my instruction sheet be able to reproduce your experiments based on your description alone? However, do not simply copy the Experimental, as there are many details that aren't described there.

I am available for comments on this report, and you are welcome to bring drafts to me anytime up until Monday, April 24th to ask how I feel about what you've written; I promise to help you improve your manuscript. (You may also want to speak with the writing tutors at the Online Writing Center or Center for Writing.) Good luck!

In your report, include the following parts:

- Polarimetry data, and an independent calcuation of what fraction of R stereocenters you have based on this data. Do things match up?
- NMR data, both coupled and decoupled. What does the decoupled spectrum tell you about the tacticity of your polymer?
- DSC data. Show traces.
- Polarized microscope data. Show at least three images from one of your time series to demonstrate what the growing crystallites looked like; make sure these images include scale bars. Do different domains in the same experiment grow at the same or at different rates? In addition, graph the growth data for domain growth at different temperatures.
- How did stereodefect impact the crystallization kinetics in your material? Does this match results from the literature?