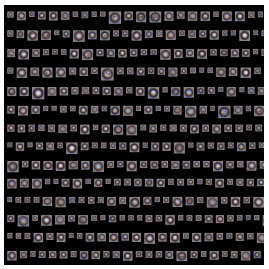
Nov 27, 2018

Flow Imaging Microscopy of Elastin-like Polymers



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the methods used by the Balog lab at the University of New England to evaluate elastin-like polymer microparticles using the FlowCam.

Guidelines

The protocol workflow is as follows:

1. Sample preparation (< 1 h)
2. Flow cell cleaning (1+ h, variable)
3. Instrument setup (< 1 h)
4. Focusing (< 1 h)
5. Running your sample (variable, ~20 min per run)
6. Initial post-processing (< 10 min)

Materials

This protocol was written for the following instrument and software versions:

FlowCam VS-IV-C B
VisualSpreadsheet v. 4.7.6

Before start

Perform all necessary calculations to determine the total volume of sample needed, the amount of ELP required, and the volume of water or buffer needed to resuspend ELP at the desired concentration. It is recommended to wait until the flow cell is cleaned and focused before preparing the sample.

Expect to try different concentrations to find the optimal conditions for your sample.

Use a syringe filter to sterilize solvents.

Acquaint yourself with both the FlowCam manual and the white papers available on the Fluid Imaging website to help guide your experimental design and methods development.

Sample Preparation

1 Prepare a bucket of ice for keeping samples, solvents, and focus beads cold.

2 Weigh out the desired mass of freeze-dried ELP required, typically 0.8-1.5 mg per experiment.

3 Resuspend ELP in the previously calculated volume of solvent. Help the ELP go into solution by alternately chilling on ice and pipetting up and down until solid material is no longer visible. Sample may remain on ice during cleaning and focusing procedures but should warm to room temperature at least 10 min before it is run.

Note

Example calculations:

Desired concentration: ~1.4 mg/mL
Desired volume: at least 1 mL

Added 1.12 mL of sterile-filtered, ice cold H_2O to 1.6 mg of freeze-dried ELP.

Flow Cell Cleaning

4 Visually inspect the 50 µm flow cell for damage. If there are no apparent cracks, proceed to step 5.

Intact 50 µm flow cell

5 Sonicate the flow cell in DI water for 180 s.

Flow Cell Cleaning - Additional Procedures (optional)

6 If the flow cell is not clean, fill the flow cell with isopropyl alcohol and sonicate again. Repeat this step as many times as necessary until the flow cell is clean.

Note

In desperate times, the flow cell can be cleaned with a detergent solution. However, the presence of detergents may affect protein behavior, so care should be taken to rinse flow cells thoroughly after washing with detergents.

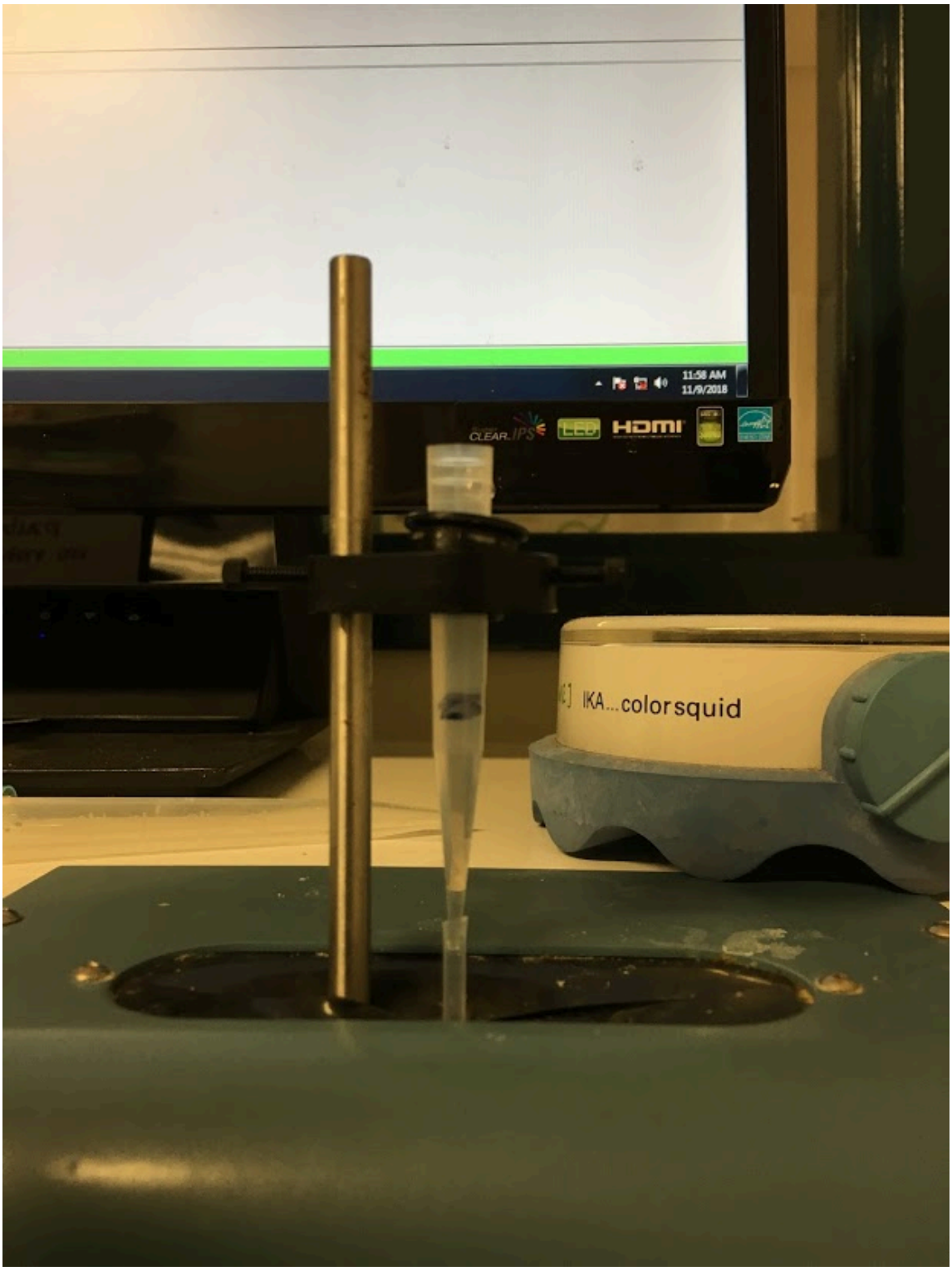
Instrument Setup

7 Turn on the computer, then the FlowCam. Open the Visual Spreadsheet software.

8 Install the 20X objective, 20X collimator, and the 0.5 mL syringe according to instructions in the FlowCam manual.

9 Install the flow cell according to instructions in the FlowCam manual.

10 Place a 1 mL pipette tip on the top of the flow cell tubing. Make a mark about halfway up the pipette tip using a Sharpie.



1 mL pipette tip with marker line

11 Add DI water to the pipette tip until the liquid level is above the mark.

Note

Unless the flow cell is being removed for cleaning or storage, add liquid as necessary to keep the liquid level above this mark during operation!

12 In Visual Spreadsheet, go to Setup > Pump > Prime. Aspirate 0.5 mL of water.

13 Repeat step 6 two more times.

Note

It is important to aspirate the flow cell at the beginning of each experiment to determine whether there are flow issues. Visually confirm that the liquid level is going down during aspiration. Check for tiny bubbles in the tubing downstream of the flow cell or air pockets in the syringe. If water is not flowing, the flow cell may be broken or there could be something blocking the flow. Return to step 5 for additional cleaning procedures.

14 Within the Prime window, click Evacuate Syringe.

Focusing

15 Click the Setup|Focus icon to open the Setup and Focus Mode viewer window. This shows you what the camera sees. Inspect the area for contamination, bubbles, or anything else that might be problematic. Return to the cleaning procedure in step 5 if needed.

Note

Imperfections in the glass may or may not be an issue. Wait to see if they get mistakenly captured as particles.

16 When the flow cell is clean, add water and aspirate again to confirm that the flow is acceptable.

17 Return to the Setup and Focus Mode window. Add 100 µL of focus beads. Go to the Pump Control window and set the flow rate to 0.030 mL/min. Visually confirm that beads are flowing through the flow cell.

18 Manually focus on the beads by very gently adjusting the position of the flow cell.

19 In the Setup and Focus Mode window, go to Tools > Autofocus to open the Auto Focus Assistant. Follow the steps provided. A pop-up will appear telling you whether the Autofocus is successful.

Note

If the Autofocus is unsuccessful, add more beads and try again.

Running Your Sample

20 Add the solvent your sample is resuspended in (not the sample!) to the pipette tip. Go to Setup > Pump > Prime. Aspirate 0.5 mL.

21 Add 100 µL of your sample to the pipette tip.

22 Click the Context icon to choose your context settings. Set the minimum ESD diameter to 2 µm.

An example context file is attached. It can be opened and viewed with a text editor.

11-02-2018-Buffer#4-Run#1.post.ctx

23 Click the AutoImage icon and follow the instructions provided. Select "Stop when user terminates experiment or fluid is aspirated." Assign the run a name using the following format or similar:

date - solvent - run number

24 Click "Save" to automatically start the run.

Note

The first run should be considered a "practice run" to identify potential problems. In addition to flow issues, this is when you can determine whether imperfections in the glass are being detected as particles. Also, look at the Capture Y vs Capture X scatter plot to confirm that particles are being captured across the entire area. Inspect the particle edge trace and binary image overlays to determine whether particles are being captured properly, and adjust particle capture and segmentation threshold context settings accordingly.

25 After the run is complete, aspirate again, adding solvent if necessary.

26 Add another 100 µL of sample and repeat steps 22-24 as many times as desired.

27 After you are finished running samples, add DI water and aspirate again.

28 Remove the flow cell from the flow holder and repeat step 4 (sonication bath).

Note

Make sure to return the flow cell to the box labeled "proteins only."

Initial Post-Processing - Bead Removal

29 In VisualSpreadsheet, open the .lst file for the experiment. Click the "Open View" button to open the View window.

30 Select three images of beads.

31 Go to Filter > Like selected particles (Statistical). This will re-sort all images by their filter score based on similarity to the selected images.

32 Manually select all images of beads. Go to Edit > Delete Images Selected.

33 Go to File > Save As List... to save as a new .lst file.