

Project Page Link: <https://review.jove.com/account/file-uploader?src=20957818>

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

Yes

4.4.2., 4.5.1., 4.6.2.

Authors: Please create scope videos of the shots labeled as SCOPE and upload the files to your project page as soon as possible: <https://review.jove.com/account/file-uploader?src=20957818>

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

Videographer: Please film the computer screen for all shots labeled SCREEN

3. Filming location: Will the filming need to take place in multiple locations? **Yes**
11 floors (same building)

Current Protocol Length

Number of Steps: 20

Number of Shots: 55

Introduction

Videographer: *Obtain headshots for all authors available at the filming location.*

~~**REQUIRED:** What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Jan Rainey:** We investigate understudied spider silk proteins to understand their biophysical properties before and after self-assembly, enabling rational engineering of advanced silk-based biomaterials.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What are the current experimental challenges?~~

- 1.2. **Suad Rashid:** Current experimental challenges include purifying high-molecular-weight spidroins, solubilizing silk proteins for fiber formation, and controlling humidity and temperature during production.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What research gap are you addressing with your protocol?~~

- 1.3. **Hina Batool:** Our protocol overcomes the challenge of obtaining pure, consistent spider silk fibers by enabling individual silk production for accurate property characterization.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Sara Evans:** Our protocol allows us greater flexibility when choosing a protein. We can spin recombinant proteins that combine multiple silk types or fuse a silk protein to a biologically relevant peptide.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.5. **Jan Rainey, Professor, Dalhousie University:** (authors will present their testimonial statements live)
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

How does the video format of your article help you to share your work with the broader scientific community?

- 1.6. **Jan Rainey, Professor, Dalhousie University:** (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Hand-Pulling and Mounting of Spider Silk Fibers for Characterization

Demonstrator: Suad Rashid

2.1. To begin, prepare a C-shaped paper frame with a 1 by 0.5-centimeter window [1]. Place it on a glass slide [2]. Then, secure the upper portion of the frame to the slide using adhesive tape [3].

2.1.1. Talent cutting a C-shaped paper holder from 3 x 2 cm rectangular piece of paper.

2.1.2. Shot of the C-shaped holder being taped on a microscope slide.

2.1.3. Talent placing double-sided tape on either side of the 1 cm gap.

~~2.2. Affix transparent tape to one arm of the frame to create a hydrophobic surface for dope solution deposition [1]. Then attach double-sided tape to the opposite arm to secure the fiber [2].~~ NOTE: Not filmed

~~2.2.1. Shot of transparent tape being fixed to one arm of the frame.~~

~~2.2.2. Shot of double-sided tape being placed on the opposite arm of the frame.~~

2.3. Next, deposit ten to twenty microliters of spider silk protein spinning dope solution onto the transparent tape at room temperature [1]. Submerge a 200-microliter plastic pipette tip into the dope droplet [2] and draw it vertically out at a constant speed of approximately 6 millimeters per second to pull fibers [3].

2.3.1. Talent pipetting 10 to 20 μ L dope solution onto transparent tape

2.3.2. Shot of the pipette tip being submerged into droplet.

2.3.3. Shot of tip pulling fiber from solution.

2.4. Now, guide the hand-pulled fiber across the frame window [1], attach it to the double-sided tape, and allow it to dry at room temperature [2-TXT]. Secure both ends of the dried fiber with additional tape [3]. Focus on the portion of the fiber that spans the paper frame for subsequent characterization [4].

2.4.1. Talent guiding the hand-pulled fibre across the frame window.

2.4.2. Shot of the fibre being attached onto the window with double-sided tape.

2.4.3. Talent securing both ends of fiber with tape

2.4.4. Talent pointing to fibre spanning the paper frame. NOTE: Shots 2.3.3, 2.4.1 and 2.4.2 were combined as one shot.

3. Wet-Spinning and Post-Spin Processing of Synthetic Spider Silk Fibers

Demonstrators: Hina Batool

- 3.1. For wet spinning, centrifuge the spider silk dope solution at 7830 *rpm* for thirty minutes at room temperature [1] and collect the supernatant for fiber spinning [2].
 - 3.1.1. Talent placing a labelled tube with dope solution in a centrifuge.
 - 3.1.2. Shot of the supernatant being pipetted out of the centrifuged tube.
- 3.2. Load 50 to 80 microliters of spinning dope solution into a syringe that serves as a spinneret [1]. Secure the syringe in a syringe pump [2] and set the extrusion rate to 0.600 milliliters per hour using the pump controls [3].
 - 3.2.1. Talent drawing the dope solution into a syringe.
 - 3.2.2. Shot of the syringe being placed in a syringe pump.
 - 3.2.3. The pump extrusion rate is being set to six hundred microliters per hour.
- 3.3. Now prepare a coagulation bath comprising ninety-five percent ethanol and five percent deionized water [1]. Position the syringe pump so that the syringe needle is immersed in the coagulation bath [2]. Then attach the rollers to the spinning apparatus [3], adjust the roller speed using the motor control software [4], and turn on the necessary motors [5].
 - 3.3.1. Talent mixing ethanol and deionized water in a beaker.
 - 3.3.2. Shot of the syringe needle being submerged in the coagulation bath.
 - 3.3.3. Talent attaching rollers to the spinning apparatus.
 - 3.3.4. SCREEN: The roller speed being input into the instrument. *Videographer: Please film the computer screen for this shot*
 - 3.3.5. Talent switching on the motors.
- 3.4. After starting the syringe pump, use tweezers to guide the fiber forming in the coagulation bath [1]. To collect spun fibers, guide the forming fiber onto the first roller, ensuring the rotation matches the extrusion rate [2]. To perform post-spin stretching in the air, set roller 2 to rotate faster than roller 1 [3], guide the fiber from coagulation over roller 1 and collect it on roller 2 [4].
 - 3.4.1. Shot of tweezers being used to guide fibre formation.
 - 3.4.2. Shot of the fibre being guided onto the 1st roller.
 - 3.4.3. The roller 2 is being set to rotate faster than roller 1.
 - 3.4.4. Shot of the fiber being guided from roller 1 to roller 2.

- 3.5. To perform post-spin stretching after immersion in solvent, fill a solvent bath [1]. Guide the fiber from coagulation around roller 1 [2], then roller 3 in the solvent bath [3], and collect it on roller 2 in air, rotating faster than the other two rollers [4].

3.5.1. Talent filling a solvent bath with desired solvent.

3.5.2. Shot of fibre being guided around roller 1.

3.5.3. Shot of fiber being guided around roller 3 in the solvent bath.

3.5.4. Shot of fiber being guided around roller 2, rotating in air.

NOTE: Shots 3.6.2, 3.6.3, and 3.6.4 were combined as one shot.

4. Optical Evaluation and Tensile Testing of Individual Fibers

Demonstrator: Hina Batool

- 4.1. Remove a fiber from the roller it was collected on [1] and cut an approximately 2-centimeter-long segment [2].

4.1.1. Shot of a fibre being removed from the roller it was collected on.

4.1.2. Shot of the fibre being cut into a 2 cm long segment. **NOTE: Shots 4.1.1 and 4.1.2 were combined as one shot.**

- 4.2. Place the cut segment on the double-sided tape, aligning its long axis parallel to the holder's bottom edge [1], then secure it with masking tape on top of the double-sided tape, on both sides [2]. Firmly secure the bottom of the paper holder to the slide to prevent fiber movement during use, using additional tape [3]. Then, store the mounted fiber slides in a slide storage box until imaging or tensile testing [4].

4.2.1. Talent placing the cut segment on the double-sided tape.

4.2.2. Shot of the segment being taped with masking tape, on both sides.

4.2.3. Shot of the paper holder bottom being fixed to the slide with more masking transparent tape.

4.2.4. Talent placing slide in storage box.

- 4.3. To determine the diameter and evaluate any defects, examine the mounted fibers under an optical microscope [1]. Screen the entire fiber length for imperfections such as necking, twisting, bulging, or double fibers [2-TXT].

4.3.1. Talent placing the slide with fibre under an optical microscope.

4.3.2. SCOPE: Revised_SCOPE_4.3.2.mp4: 00:05:20

- 4.4. For fibers without defects, capture three micrographs at 10X (Ten-X) along the fiber's long axis, at the middle, and at each end [1].

- 4.4.1. SCOPE: Revised_SCOPE_4.4.1.mp4: 00:04:00:25
- 4.5. Use polarized light microscopy to assess molecular alignment. Examine the fiber with an inverted microscope featuring a rotatable stage and a ninety-degree polarizer. [1-TXT]. Capture an image of the fiber to evaluate its birefringence [2].
- 4.5.1. Talent placing the fiber slide under an inverted microscope.
- 4.5.2. SCOPE: Revised_SCOPE_4.5.2.mp4 00:12-00:20
- 4.6. For tensile testing with a home-built apparatus, detach the C-shaped paper frame holding the fiber from the slide [1] and secure it to a clamp attached to a weight [2].
- 4.6.1. Talent detaching the C-shaped paper frame from the slide.
- 4.6.2. Talent attaching it to a clamp with a weight.
- 4.7. Transfer the weighted clamp onto an empty analytical balance [1], then attach the top of the paper frame to a clamp connected to the syringe pump [2]. Cut one side of the paper holder to allow free fiber movement [3].
- 4.7.1. Talent placing the weighted clamp onto a empty analytical balance.
- 4.7.2. Talent attaching the top of the paper frame to a clamp connect to the syringe pump.
- 4.7.3. Shot of the one side of the paper holder being cut.
- 4.8. Now, tare the balance to zero out the mass of the weight and clamp [1]. Click **OK** in the balance control software [2], then press the Run button on the syringe pump [3].
- 4.8.1. Talent pressing Tare on the analytical balance.
- 4.8.3. SCREEN: **OK** is being clicked. *Videographer: Please film the computer screen for this shot* NOTE: 4.8.3 is moved here as per authors's request
- 4.8.2. Talent pressing the **Run** button on the syringe pump.
- 4.9. When the fiber breaks, press **Stop** on the syringe pump [1] and click **Yes** in the balance software to log weight versus time [2]. Perform FTIR spectroscopy to evaluate fiber-state secondary structure [3].
- 4.9.1. Talent pressing **Stop** button. NOTE: 4.9.1 and 4.9.2 are filmed in a single shot
- 4.9.2. SCREEN: The **Yes** option is being clicked. *Videographer: Please film the computer screen for this shot*
- 4.9.3. Talent in front of the computer looking at the FTIR spectra.

Results

5. Results

5.9. An ideal dope solution appeared completely clear and viscous without any visible precipitates, indicating full protein dissolution [1], whereas incomplete dissolution resulted in either a cloudy mixture [2] or visible particulate matter [3].

5.9.1. LAB MEDIA: Figure 7A.

5.9.2. LAB MEDIA: Figure 7C

5.9.3. LAB MEDIA: Figure 7B

5.10. Fibers produced without post-spin drawing showed a consistent diameter under optical microscopy [1], but those subjected to 4 times stretching in 40% ethanol exhibited a visibly thinner and more uniform morphology [2].

5.10.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the top-left panel labelled AS,*

5.10.2. LAB MEDIA: Figure 8A. *Video editor: Highlight the bottom-left panel labelled 4x-EtOH*

5.11. Higher birefringence in fibers subjected to 4 times ethanol stretching compared to the as-spun condition, indicating increased molecular alignment [1]. Defective fiber segments, such as kinks and irregular bends, were visible in some samples, rendering them unsuitable for analysis [2].

5.11.1. LAB MEDIA: Figure 8B. *Video editor: Highlight the bottom-right panel labelled 4x-EtOH*

5.11.2. LAB MEDIA: Figure 9. *Video editor: Please sequentially highlight A and then B*

5.12. Fibers drawn 4 times in ethanol demonstrated the highest tensile strength [1], while fibers spun in air 4 times exhibited brittle behavior with low extensibility [2].

5.12.1. LAB MEDIA: Figure 10. *Video editor: Highlight the dark blue curve labelled 4x-EtOH*

5.12.2. LAB MEDIA: Figure 10. *Video editor: Highlight the purple curve labelled 4x-air,*

5.13. FTIR spectra of silk fibers showed characteristic protein bands, including amide I peaks, consistent across conditions [1]. Second derivative analysis is useful for identifying key components of the amide I peak [2].

5.13.1. LAB MEDIA: Figure 11C and D.

5.13.2. LAB MEDIA: Figure 11 E and F *Video Editor: Please highlight the red curves in both graphs*

5.14. Deconvolution of the amide I region revealed a higher proportion of β (*beta*) -sheet content in the 4 times ethanol condition [1] compared to the as-spun state, regardless of analysis method used [2].

5.14.1. LAB MEDIA: Figure 11G and H *Video editor: please highlight the red curve corresponding to β -sheet in both graphs*

5.14.2. LAB MEDIA: Figure 11I and J . *Video editor: please highlight the red curve corresponding to β -sheet in both graphs*

1. Electroporation
Pronunciation link: <https://www.merriam-webster.com/dictionary/electroporation>
IPA: /ɪˌlek.troʊ.pəˈreɪ.ʃən/
Phonetic Spelling: ihˌlekˌtrohˌpuhˌrayˌshuhn
2. EBY100
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: eeˌbeeˌwhyˌoneˌzeroˌzero
3. Glycerol
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycerol>
IPA: /ˈɡlɪs.əˌrɔːl/
Phonetic Spelling: glisˌuhˌrawl
4. Erlenmeyer
Pronunciation link: <https://www.merriam-webster.com/dictionary/Erlenmeyer%20flask>
IPA: /ˈɜː.lənˌmaɪ.ə/
Phonetic Spelling: erˌlenˌmyˌer
5. Micropipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/micropipette>
IPA: /ˌmaɪ.kroʊ.paɪˈpet/
Phonetic Spelling: myˌkrohˌpieˌpet
6. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /ˌspek.troʊ.fəˈtɑː.mə.tə/
Phonetic Spelling: spekˌtrohˌfuhˌtomˌuhˌter
7. Optical density
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>
IPA: /ˈɑːp.tɪ.kəl ˈden.sə.ti/
Phonetic Spelling: opˌtihˌkuhl denˌsuhˌtee
8. Microcuvette
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: myˌkrohˌkyooˌvet
9. Conditioning buffer
Pronunciation link: <https://www.merriam-webster.com/dictionary/conditioning>

- IPA: /kən'dɪʃ.ənɪŋ 'bʌf.ə/
Phonetic Spelling: kuhn·dish·uh·ning buf·er
10. Sorbitol
Pronunciation link: <https://www.merriam-webster.com/dictionary/sorbitol>
IPA: /'sɔ:r.bə,tɔ:l/
Phonetic Spelling: sor·bih·tol
11. Electrocompetent
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: ih·lek·troh·kom·puh·tuhnt
12. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,ri:.sə'spend/
Phonetic Spelling: ree·suh·spend
13. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /,sen.trə.fju:'geɪ.fən/
Phonetic Spelling: sen·truh·fyoo·gay·shuhn
14. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /'plæz.mɪd/
Phonetic Spelling: plaz·mid
15. Linearized
Pronunciation link: <https://www.merriam-webster.com/dictionary/linearize>
IPA: /'lɪn.i.ə.raɪzd/
Phonetic Spelling: lin·ee·uh·ryzd
16. Cuvette
Pronunciation link: <https://www.merriam-webster.com/dictionary/cuvette>
IPA: /kju:'vet/
Phonetic Spelling: kyoo·vet
17. TRP1
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: tee·ar·pee·one
18. Fluorescence-activated cell sorting
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flɔ:'res.əns 'æk.tə,veɪ.tɪd sel 'sɔ:r.tɪŋ/
Phonetic Spelling: floor·ess·uhns ak·tuh·vay·tid sel sor·ting
19. Hemagglutinin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemagglutinin>
IPA: /,hi:.mə'glu:.tɪn.ɪn/
Phonetic Spelling: hee·muh·gloo·tuh·nin
20. Fluorescein isothiocyanate
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescein>

IPA: /ˌflɒr.əˈsiː.ɪn ˌaɪ.souˌθaɪ.ouˈsaɪ.ə.nɛɪt/

Phonetic Spelling: floor·uh·see·in eye·soh·thy·oh·sy·uh·nayt

21. Phycoerythrin

Pronunciation link: <https://www.merriam-webster.com/dictionary/phycoerythrin>

IPA: /ˌfaɪ.kou.əˈrɪθ.rɪn/

Phonetic Spelling: fy·koh·uh·rith·rin