Imaging Methods in Mechanosensing, a Historical Perspective and Visions for the Future

Kirill Lavrenyuk, Daniel Conway, and Kris Dahl

Corresponding author(s): Kris Dahl, Carnegie Mellon University

Review Timeline:

| Event                        | Date       |
|------------------------------|------------|
| Submission Date              | 2020-10-28 |
| Editorial Decision           | 2020-11-30 |
| Revision Received            | 2021-03-11 |
| Accepted                     | 2021-03-11 |

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
Dear Prof. Dahl:

two reviewers did a great service for you providing very detailed comments. Both theirs, and mine, opinions are: 1) the review addresses an exciting and timely topic (and for this reason I am not rejecting the manuscript but rather giving you the opportunity to drastically revise it). 2) The review is very poorly written (I normally try not to make comments like that, but in this case - the PI has to thoroughly re-write and edit the text, rather than leaving the task to the student). 3) Pay special attention to feel the sections, other than the historical section, with more substance. 4) Pay attention to logical connections between consecutive sections - right now the review reads like a few disconnected pieces. If you do all that, I will send the manuscript to both reviewers for the second look.

Sincerely,

Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Dahl,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.
In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The review is very topical and authored by two experts in the field. However, there are substantial issues with the review. These occur at all levels, including the clarity of the overall message, inaccurate summaries of pertinent literature, a severe lack of citations, and overall poor writing (or at least incomplete editing). The central premise of the review is important, but substantial editing (likely including a very substantial re-write) will be required to make this manuscript suitable for publication.

MAJOR CONCERNS
1. Overall, the quality of the writing is a serious defect in this manuscript and needs to be improved substantially. There are many sentences that do not make sense. There are a large number of terms that are either nonstandard, undefined, or misused. There are large areas of the text that contain no citations, and some citations seem to be in error. Perhaps a professional editor could aid in the refinement of the manuscript. I have placed a large list of issues in the minor comments, so if the authors are allowed to resubmit the review, they can fix them without having to respond to each point-by-point.

2. At times, the overall premise of the review is unclear. The title seems to suggest that the manuscript will focus on optical methods for studying mechanosensing. However, the manuscript quickly dives into a discussion of mechanical models. The discussion of models is overly reliant on tensegrity, even from a historical perspective. Tensegrity is a concept from architecture/sculpture. It is incapable of explaining sub-cellular dynamics/rearrangements (as incorrectly stated on line 54), certainly makes no predictions about mapping cell fates (as incorrectly stated on line 60), and (while it is a valuable concept in the context of mechanotransmission) has little to do with explaining mechanosensing. These points must be fixed or clarified. Additionally, how the discussion of models relates to optical approaches needs to be clarified or the title needs to be changed.

3. The authors seem to imply that a model of cell mechanics could "capture the diverse biomechanical environment of human tissue" and would also help infer molecular-scale mechanisms...
of mechanotransduction. This seems beyond the scope of any traditional, existing, or even conceived model. The authors are describing an inherently multi-scale problem that would span sub-nanometer to meter length scales and micro-second to hour (at least) time scales while incorporating details regarding hundreds to thousands of proteins and/or protein states. None of the complexity of this task is discussed. Additionally, it is not clear to this reviewer how the discussed optical techniques would even begin to accomplish this daunting task. Most of the discussed techniques don't even measure the same thing (i.e. complex shear modulus, cell stress generation, protein-specific molecular-scale forces, contribution of motors to intracellular fluctuations...). For the review to have a clear main point, the authors must provide a more clear definition of what they mean by cell mechanics model as well as explain how the optical measurements have either led to the development and/or refinement of the models.

4. The authors spend a great deal of time discussing the response of cells to substrate stiffness, and how this could be understood through a cell mechanical model. Currently, most ideas in rigidity sensing involve clutch models (see work of Odde and Roca-Cusachs). A discussion of these models would strengthen the review as well as enable a discussion of molecular processes and the multi-scale nature of the problem.

MINOR CONCERNS

Due to the poor writing/editing, there are a plethora of minor concerns. I have listed the most serious here. The authors should not take this as an exhaustive list and should consider re-evaluating every sentence and every citation (or lack thereof) in the manuscript.

1. In line 47: the authors assert that tensegrity demonstrates that "biological phenomenon were inherently downstream of mechanics". This reviewer does not understand how tensegrity does this. More explanation is needed.
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32. Line 195-198: This sentence needs editing.

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36. Line 234-235: This sentence is not true. Many tension sensors loose/change their structure during loading. In fact, that is the basis of the FN-based sensor being discussed in the next
sentence. Also, see the villin-head piece tension made by Grashoff and the DNA-based structures made by T.J. Ha, Chris Chen, and Khalid Salaita all alter their structure reversibly with force.

37. Line 237: The "dynamic range of extracellular matrix proteins" is not a standard term and needs to be defined.

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43. Like 262: The term "cellular junctions" should be replaced, as tension sensors have been used in a variety of sub-cellular compartments, include the focal adhesions, cytoskeleton, and nucleus.

44. Line 266-270: The authors are conflating several topics here. First, focal adhesions are generally not referred to as junctions. Also, the description of the data from Grashoff et al is flawed. Disassembly of focal adhesions correlated with the progressive loss of force on vinculin. A sudden drop of force preceding disassembly, as described in the text, was not observed. Also, vinculin is a soluble protein, so it is not subject to endocytosis. To the knowledge of this reviewer, vinculin tension has not been studied in the context of endocytosis. This section should be rewritten.

45. Line 278: For the compression of sensors to affect the force measurements, both compressive and tensile forces must act with the same optical volume. This may or may not be true in all cases. This section should be re-written to acknowledge this limitation.

46. Like 279: The meaning of "cell density" is not clear. Do the authors mean the concentration of the protein on the cell surface or number of cells in a given area? If the authors mean the latter, a greater explanation of how this effects FRET is required.

47. Line 286: The authors appear to have misinterpreted the study completed in reference 39. Those authors did indeed demonstrate discrepancies between the behavior of the sensors in vitro and within cells. However, they also developed a means for calibrating the sensors in a cellular environment. These additional efforts need to be mentioned to provide a complete picture.

48. Line 288: The pertinence of the forces across integrin being 1-5 pN to the measurement of the vinculin tension is not clear. Talin has up to 12 binding sites for vinculin, one might expect forces of approximately 36 pN on an integrin.

49. Line 289: What conflicting results are being discussed is not clear.

50. Line 293: The point of the section entitled "Unified theory of cell mechanics" is not clear. The manuscript only really discusses a single model, tensegrity, so talking about model reconciliation seems inappropriate. Also, there does not seem to be much more stated than, there is a not a unified model. The end of this sections just seems to be the acknowledgement that tissues are different from individual cells, which is of course true. Citations in this section would greatly aid the reader. Perhaps the authors can refine the text to make their point clearer?

51. Line 299: What the term "cellular landscape" means is unclear. How is "sufficiency" of the models determined?

52. Line 300: What the term "simple models" is referencing is unclear. Do you mean tensegrity? Mechanical circuits? Something else?

53. Line 308: Again, it is unclear how a grouping of experimental techniques will lead to development of more advanced models.

54. Line 312: How any of the topics discussed would lead to a "fundamentally new way to develop therapeutics" is unclear. The authors should remove this line or provide substantially more detail.

55. Line 315: Are the authors proposing this organization into three broad categories for the first
time? If so, greater detail is required. If not, a citation is required.

56. Line 326: There are a variety of targets for mechanosensitive cell signaling pathways. ROCK and its inhibitor fasudil, for instance. Cynthia Reinhart-King has an excellent review on this topic. This section should be re-written to address this point.

57. YAP and TAZ should both be capitalized.

58. Line 352: Citations are needed.

59. Line 354-356: Exactly what the authors are saying is not clear. What does "mechanosensing methods" mean? Do you mean traction force? What does "cellular structures" mean in this context. This reviewer believes that the author is suggesting that drugs that might have been developed for another target may actually have affects on mechanosesning pathways, but is not sure.

Reviewer #2 (Remarks to the Author):

This review manuscript from Dahl and co-workers is provides the review with a historical perspective, current discussion of optical-based force detection with protein sensor, and concludes with a short future looking section. Aside from the historical section, the other sections seem short and under-developed. Some of the questions posed in the paper are not clearly answered (see below). Generally speaking, it seemed like three separate papers with awkward transitions. More specific comments are shown below; while I like the big picture questions, the paper largely does not focus on them, and that seems to be a big weakness. All are addressable though.

1. Figures in the paper are mislabeled. There are 2 Figure 1s. Figure 2 is shown last in the manuscript (page 26). Not all of the panels in each figure appear sequentially, e.g. Figure 2B then 2A.

2. Organization of the paper is not very clear. The authors end the intro by saying that there will be some historic discussions of mechanobiology, then more modern mechanotransducers and finally future medical applications. Perhaps the latter occurs in the final paragraph(s) but it is not clear what the separation is for the other sections. More explicit section headings would help.

3. The big questions at the beginning of the paper (last part of the introduction) were very nice and I felt that they'd be cornerstone of the paper. However, they are barely acknowledged. For example, the question about "meaningful medical applications" is not answered except perhaps in the last paragraph of the manuscript. The first question about mechanical signaling integrating together at the whole cell level is another hugely important question. We've watched a lot of pathways (circa Shu Chien/Roger Tsien, Martin Schwartz, etc), but I didn't get a sense that this question was answered either. These are VERY good questions; I just did not get a sense that they were answered.

4. The final paragraph is particularly odd. It does not conclude the manuscript really but goes off on a tangent about drugs (which could be related to the big questions at the outset of the paper) but it does not conclude the thoughts of the authors. Rather it left me wondering if I had missed a page of text in reading. The last sentence does help but up to that point, I have know idea where the authors are going. That paragraph should be much more introspective.

Minor:

1. I would disagree with the notion that 3D TFM is prohibitively difficult. Chris Chen and colleagues did it a decade ago (Legant et al; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3056435/). While
the method has not caught on for a variety of reasons, I think that saying it is basically "too hard to
do" sells short the method. Precisely because morphogenesis, etc require 3D is a reason to make
3D TFM more accessible!

2. Formatting for Table 1 was messed up and I could not easily tell where the PRO's and CON's began/ended.
Response to Reviewer Comments

Reviewer #1 (Remarks to the Author):

MAJOR CONCERNS
1. Overall, the quality of the writing is a serious defect in this manuscript and needs to be improved substantially. There are many sentences that do not make sense. There are a large number of terms that are either nonstandard, undefined, or misused. There are large areas of the text that contain no citations, and some citations seem to be in error. Perhaps a professional editor could aid in the refinement of the manuscript. I have placed a large list of issues in the minor comments, so if the authors are allowed to resubmit the review, they can fix them without having to respond to each point-by-point.

Thank you for your close reading of our text to point out these major concerns. We have made significant edits to every section of the review to address readability concerns as well as citation errors. We have added a significant number of references regarding existing ideas in the field that we discuss.

2. At times, the overall premise of the review is unclear. The title seems to suggest that the manuscript will focus on optical methods for studying mechanosensing. However, the manuscript quickly dives into a discussion of mechanical models. The discussion of models is overly reliant on tensegrity, even from a historical perspective. Tensegrity is a concept from architecture/sculpture. It is incapable of explaining sub-cellular dynamics/rearrangements (as incorrectly stated on line 54), certainly makes no predictions about mapping cell fates (as incorrectly stated on line 60), and (while it is a valuable concept in the context of mechanotransmission) has little to do with explaining mechanosensing. These points must be fixed or clarified. Additionally, how the discussion of models relates to optical approaches needs to be clarified or the title needs to be changed.

We thank reviewer #1 for this comment. We have addressed this issue by including several references from Donald Ingber in which he develops the idea of the tensegrity model and how it would have been possible in his view to address the issues of sub-cellular dynamics and cell fate. Although his argument is not sufficiently mechanistic (as we have noted to the text), he does explicitly make the argument for how this model impacted these phenomena which we feel is important to address in a historical context. Further in the paper, we have added several other more recently developed models (clutch-motor model for cellular structures, and soft-glassy model of the cell as a material explanation of cellular properties) to contrast and update the reader on the best existing explanations for biomechanical cellular phenomena.

3. The authors seem to imply that a model of cell mechanics could "capture the diverse biomechanical environment of human tissue" and would also help infer molecular-scale mechanisms of mechanotransduction. This seems beyond the scope of any traditional, existing, or even conceived model. The authors are describing an inherently multi-scale problem that would span sub-nanometer to meter length scales and micro-second to hour (at least) time scales
while incorporating details regarding hundreds to thousands of proteins and/or protein states. None of the complexity of this task is discussed. Additionally, it is not clear to this reviewer how the discussed optical techniques would even begin to accomplish this daunting task. Most of the discussed techniques don't even measure the same thing (i.e. complex shear modulus, cell stress generation, protein-specific molecular-scale forces, contribution of motors to intracellular fluctuations...). For the review to have a clear main point, the authors must provide a more clear definition of what they mean by cell mechanics model as well as explain how the optical measurements have either led to the development and/or refinement of the models.

This is an important point. We have added several sections [151-162, 282-295] discussing new, more accurate mechanical models of the cell developed since tensegrity. In both cases, one model [151-162] was developed by imaging approaches as should be clear in the review, and the other was refined by the addition of sub-cellular imaging [282-295]. Finally, in the “Unified Theory…” [465-510] we discuss how despite measuring different biophysical phenomena, they should be in broad agreement with one another. The complex sheer modulus measured by rheological approaches is indicative of the cytoskeletal state of the cell. We discuss similarly cell stress generation and protein-specific molecular-scale forces in relation to the cytoskeleton. Thus it should be possible to predict the average behavior of cell stresses and molecular forces related to the cytoskeleton from rheological measurements, and predict rheological properties in a model that accurately integrate forces across the cell.

4. The authors spend a great deal of time discussing the response of cells to substrate stiffness, and how this could be understood through a cell mechanical model. Currently, most ideas in rigidity sensing involve clutch models (see work of Odde and Roca-Cusachs). A discussion of these models would strengthen the review as well as enable a discussion of molecular processes and the multi-scale nature of the problem.

This is an excellent suggestion, and we have added a section [151-162] with a discussion of Chan and Odde. Similarly, we have added more discussion in [181-201] addressing other groups who have discovered motor-clutch phenomena in other mechanotransducing proteins. A discussion of the connection between molecular dynamics and cellular behavior has also been added in [151-162] as well as in the “Unified Theory of Cell Mechanics” section.

MINOR CONCERNS
Due to the poor writing/editing, there are a plethora of minor concerns. I have listed the most serious here. The authors should not take this as an exhaustive list and should consider re-evaluating every sentence and every citation (or lack thereof) in the manuscript.

For this reviewers list of “Minor Concerns” all concerns have been addressed in the text, as indicated by the line-through.

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2. Line 49: "mechanical elements" is not defined.
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46. Line 279: The meaning of "cell density" is not clear. Do the authors mean the concentration of the protein on the cell surface or number of cells in a given area? If the authors mean the latter, a greater explanation of how this effects FRET is required.

47. Line 286: The authors appear to have misinterpreted the study completed in reference 39. Those authors did indeed demonstrate discrepancies between the behavior of the sensors in vitro and within cells. However, they also developed a means for calibrating the sensors in a cellular environment. These additional efforts need to be mentioned to provide a complete picture.

48. Line 288: The pertinence of the forces across integrin being 1-5 pN to the measurement of the vinculin tension is not clear. Talin has up to 12 binding sites for vinculin, one might expect forces of approximately 36 pN on an integrin.

49. Line 289: What conflicting results are being discussed is not clear.

50. Line 293: The point of the section entitled "Unified theory of cell mechanics" is not clear. The manuscript only really discusses a single model, tensegrity, so talking about model reconciliation seems inappropriate. Also, there does not seem to be much more stated than, there is not a unified model. The end of this sections just seems to be the acknowledgement that tissues are different from individual cells, which is of course true. Citations in this section would greatly aid the reader. Perhaps the authors can refine the text to make their point clearer?

51. Line 299: What the term "cellular landscape" means is unclear. How is "sufficiency" of the models determined?

52. Line 300: What the term "simple models" is referencing is unclear. Do you mean tensegrity? Mechanical circuits? Something else?

53. Line 308: Again, it is unclear how a grouping of experimental techniques will lead to development of more advanced models.

54. Line 312: How any of the topics discussed would lead to a "fundamentally new way to develop therapeutics" is unclear. The authors should remove this line or provide substantially more detail.

55. Line 315: Are the authors proposing this organization into three broad categories for the first time? If so, greater detail is required. If not, a citation is required.

56. Line 326: There are a variety of targets for mechanosensitive cell signaling pathways. ROCK and its inhibitor fasudil, for instance. Cynthia Reinhart-King has an excellent review on this topic. This section should be re-written to address this point.

57. YAP and TAZ should both be capitalized.

58. Line 352: Citations are needed.

59. Line 354-356: Exactly what the authors are saying is not clear. What does "mechanosensing methods" mean? Do you mean traction force? What does "cellular structures" mean in this context. This reviewer believes that the author is suggesting that drugs that might have been developed for another target may actually have affects on mechanosensing pathways, but is not sure.
Reviewer #2 (Remarks to the Author):

MAJOR:

This review manuscript from Dahl and co-workers is provides the review with a historical perspective, current discussion of optical-based force detection with protein sensor, and concludes with a short future looking section. Aside from the historical section, the other sections seem short and under-developed. Some of the questions posed in the paper are not clearly answered (see below). Generally speaking, it seemed like three separate papers with awkward transitions. More specific comments are shown below; while I like the big picture questions, the paper largely does not focus on them, and that seems to be a big weakness. All are addressable though.

1. Figures in the paper are mislabeled. There are 2 Figure 1s. Figure 2 is shown last in the manuscript (page 26). Not all of the panels in each figure appear sequentially, e.g. Figure 2B then 2A.

   Thank you for pointing out these errors in the manuscript. We have addressed the issue by correctly labeling all figures and referencing them in numerical order as they appear throughout the work.

2. Organization of the paper is not very clear. The authors end the intro by saying that there will be some historic discussions of mechanobiology, then more modern mechanotransducers and finally future medical applications. Perhaps the latter occurs in the final paragraph(s) but it is not clear what the separation is for the other sections. More explicit section headings would help.

   We have corrected the arrangement of the manuscript to flow from introducing the oldest technique, and oldest model to newest technique and newest model. Continuing, the section looking forward to the translatability of the field. Finally, we tie the paper together by considering how the historical influences drive the field towards discoveries of both biologically and clinical relevance.

3. The big questions at the beginning of the paper (last part of the introduction) were very nice and I felt that they'd the be cornerstone of the paper. However, they are barely acknowledged. For example, the question about "meaningful medical applications" is not answered except perhaps in the last paragraph of the manuscript. The first question about mechanical signaling integrating together at the whole cell level is another hugely important question. We've watched a lot of pathways (circa Shu Chien/Roger Tsien, Martin Schwartz, etc), but I didn't get a sense that this question was answered either. These are VERY good questions; I just did not get a sense that they were answered.

   We have re-addressed these questions listed in lines [86 – 92] in the context of the manuscript to provide answers. We feel that the first question listed “How do molecular structures…” is addressed throughout the text by examining how different technologies have contributed to our mechanical understanding of the cell. Question two, “Can we reformulate…” is addressed through the section titled “Unified theory…” explicitly where we address how to reconcile the
presented models ([151-162] & [282 – 295]) to generate a more comprehensive, unified theory [465-510]. Question 3, “Are mechanical models…” is addressed through the section “A Brief Look Forward” by examining a mechanosensitive pathway that is directly responsible for transcription, as well as secondary biomechanical impacts of existing medicines not previously characterized.

4. The final paragraph is particularly odd. It does not conclude the manuscript really but goes off on a tangent about drugs (which could be related to the big questions at the outset of the paper) but it does not conclude the thoughts of the authors. Rather it left me wondering if I had missed a page of text in reading. The last sentence does help but up to that point, I have know idea where the authors are going. That paragraph should be much more introspective.

This is an excellent point, and upon re-examining the text, it would seem that the current organization of the manuscript seems more complete. The past history of the technologies is juxtaposed recent advances in mapping mechanosensitive pathways as well as introducing the above listed approaches as a means of probing drug efficacy. Finally, it leads into the concluding section of the article regarding the unified theory of cellular mechanics to tie up the manuscript and address the questions posed in the introduction.

MINOR:
1. I would disagree with the notion that 3D TFM is prohibitively difficult. Chris Chen and colleagues did it a decade ago (Legant et al; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3056435/). While the method has not caught on for a variety of reasons, I think that saying it is basically "too hard to do" sells short the method. Precisely because morphogenesis, etc require 3D is a reason to make 3D TFM more accessible!

We have added a brief discussion of the literature cited as well as adjusted the language with which we discuss the feasibility of 3D TFM. Moreover we have added that work is being done to increase the accessibility and flexibility of this approach.

2. Formatting for Table 1 was messed up and I could not easily tell where the PRO's and CON's began/ended.
We have addressed this by re-formatting the table, highlighting where the PRO’s and CON’s are respectively.
Dear Prof. Dahl:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

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Dear Prof. Dahl:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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