Cell fluorescence photo-activation as a method to select and study cellular sub-populations grown in mechanically heterogeneous environments

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Review Timeline:

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| Submission Date            | 2020-10-30 |
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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 Dr. Dolega:

Your manuscript, entitled “Cell fluorescence photo-activation as a method to select and study cellular sub-populations grown in mechanically heterogeneous environments” has been seen by two referees whose verbatim comments are enclosed. Both referees felt that your findings, in principle, would be of interest to our MBC readership. However, both reviewers raised some important points that need to be addressed. In particular, evidence that the photoactivation does not change the transcriptome and a demonstration of this method in thicker samples would increase the significance of your approach. I encourage you to consider ways to address these concerns. Also, a brief discussion of the advantages and limitations of the photo-activation method should be included in the discussion. Some of this is nicely highlighted in Annex 1 but noting the salient points in the discussion is still useful. We would be happy to consider a revised manuscript that satisfies the joint concerns of the referees. Therefore, we look forward to receiving your revised manuscript, together with a letter indicating the changes you've made and your responses to the referees.

Sincerely,

Diane Lidke  
Monitoring Editor  
Molecular Biology of the Cell
Reviewer #1 (Remarks to the Author):

This short manuscript describes how a cell subpopulation can be tagged by photoactivating fluorescent proteins to be able to later sort them by FACS and perform transcriptomic analysis on the photolabeled population. The approach appears quite interesting although it is a bit difficult to judge its potential based solely on the data that are currently provided. Also, to strengthen some of the conclusions drawn by the authors, I would suggest to perform the following additional experiments.

1) The authors insist on the fact that their method is applicable for the tagging of subpopulations in 2D but also 3D samples (in particular in comparison to laser microdissection, see Annex S1.) Unfortunately, they show no evidence that their method is indeed applicable to 3D samples such as spheroids. It would be essential to demonstrate that the photolabeling they implemented allows to highlight a subpopulation of cells with good selectivity within the focal plane but also along the perpendicular axis. It would be interesting for example to show that, for a spheroid, one can highlight specifically cells located at the periphery or within the spheroid.

2) It would be important to assess more precisely how much the photoactivation step may perturb the cell physiology. Currently, the authors show that the illumination needed to photoconvert the PAmCherry does not induce DNA damage as judged by pH2AX staining. However, since they assess changes in the transcriptome, it would be more appropriate to show that this illumination step does not impact this transcriptome.

Also, as minor points, I suggest that the authors better explain how they estimate the diameter of the photoactivated area on figure 1C,D and also reformulate how they calculate the elongation parameter shown on Fig 2B.

Reviewer #2 (Remarks to the Author):

The manuscript by Aurielle and co-workers proposes a method allowing to isolate specific subpopulations of epithelial cells in a typical experiment involving mechanical manipulation allowing further follow up for e.g. gene expression studies. The method is based on using photoactivation of fluorescence in the desired subpopulation of cells that then can be harvest by FACS for targeted gene expression studies. A key appeal of the protocol is that it is sufficiently simple to allow this method to be applied to microscopes that can do the mechanical manipulation and the imaging that goes with it, without any expensive addition of kit and might therefore be attractive to a wider range of users.

I think that this is useful and have no major criticism. The data is well-presented and I like the comparison of the method to laser capture microscopy. The authors may wish to look at the few points I found below and revise the manuscript accordingly.

Mechanical manipulation followed by gene expression studies has been done a more comprehensive discussion of their method over others (not only laser capture) might be interesting?

Points:
I assumed that the greater the measured young's modulus value is the stiffer the measured component. Likewise I assumed that compressed tissues get stiffer. Figure 2B shows the calculated tissue elongation (how was that done? May be expand in the figure legend?) indeed suggesting compression of the tissue at the border. Figure 2C, however, shows a reduction in the young's modulus value for the cells that are assumed to be compressed in this area. Am I missing something? Does this make sense?

Fig1 shows the use of the field diagram to focus a circular spot for photoconversion, yet in the experiment, a different optical set up is used allowing photoconversion of a 400µm stripe (Fig 3A). The method and material needed for this set up are not detailed. This is important for the applicability of the method, however and should be detailed. It makes me wonder why this set up has not been the subject of the 'benchmarking analysis' in figure 1...

How efficient is the FACS? How many converted cells do you lose? Based on the fluorescent images, this information could be made available. Given that the authors discuss the low number of cells to be recoverable in their method by FACS. Is that possible at all?

Does this work for other cells than MCDK II?

There is no information of how long the photoconverted fluorescence can be traced, which would be useful as well.

If the format allowed, I would put Annex S1 into the main manuscript.

Fig 1D I assume the light intensity controlled by a neutral density filter, it might be desirable to measure the intensities at the
back aperture of the objective with a photospektrometer so the intensities become more comparable if people want to reproduce. Is that a concern?
Fig 1E should the grey values be normalised to the background, no?
Fig 1 F legend for false intensity colouring is missing

Fig 2 A. This can be improved to show the design of the box. Mark more clearly the surface on which the cells grow. This would help understand also the image in Fig 3B better
Fig 2 D say "above channel (stretched) for consistency with text

Figure 3 legend: “The graphical insert shows the photoactivated zones according to the position of the channel” not sure what this refers to, the dashed boxes in B? please clarify

Line 101: perhaps say with fully open field diaphragm?
Line 149: for compressed cells?
Line 154/155: “and changes occurring within the compressive gradient“ - there may be a gradient (does Figure 2B show that?)
Dear Dr. Dolega,

Thank you for revising your manuscript in response to the referees' recommendations. I am pleased to accept your manuscript for publication in MBoC. Congratulations to you and your colleagues.

Sincerely,
Diane Lidke
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Dolega:

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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We are pleased that you chose to publish your work in MBoC.

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

Reviewer #1 (Remarks to the Author):

The authors adequately addressed my comments. Therefore, I am happy to recommend the publication of this revised version of the manuscript.

Reviewer #2 (Remarks to the Author):

I think the manuscript has improved during the revision and I have no major criticism left.