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Reply to the 'Comment on "Intracellular stresses in patterned cell assemblies"' by D. Tambe et al., Soft Matter, 2014, 10, DOI: 10.1039/C4SM00597J

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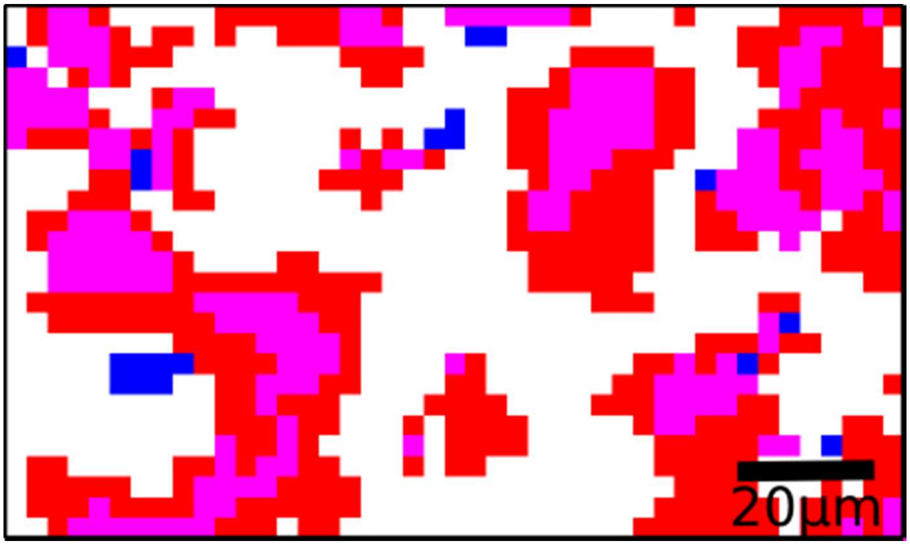
Validation of the method for calculating internal stresses as in Moussus et al, Soft Matter 2014, 10, 2414: cell/matrix stresses calculated from standard inversion methods (in red) colocalize with those derived from the proposed internal stresses (in blue).


20µm

Reply to the 'Comment on "Intracellular stresses in patterned cell assemblies"' by D. Tambe et al., Soft Matter, 2014, 10, DOI: 10.1039/C4SM00597J

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Tambe et al[1] proposed an original method to calculate intracellular stresses, that models cell monolayers as thin elastic materials. Based on this approach, Moussus et al[2] proposed a straightforward calculation of the internal stresses in cellular assemblies, valid either for a single cell or a cellular monolayer. As pointed by Tambe et al in their comment, this approach relies on the assumption that cell forces generate a displacement field that is continuously transmitted to the extracellular matrix. Under this assumption, the displacement field measured at the surface of the extracellular matrix can then be differentiated to calculate the stresses inside the cellular assembly. Tambe et al put this assumption into question, based on the assertion that cells only exert stresses at discrete adhesions sites, known as focal adhesions, so that elsewhere, there is *a priori* no contact, no stress and no continuity in the displacement field.

It is of no doubt that cellular stresses only transmit to the extracellular matrix at points of adhesion. However, determining the true cell contact region is very difficult, all the more on deformable substrates. Tambe et al[1] circumvents this issue by calculating intracellular stresses from the cell/matrix forces they compute as a first step. In principle, this approach is more reliable since the calculation of cell/matrix forces is not endangered by the limited knowledge of cell contact regions. However, practically, results shown by traditional traction force calculations, as obtained in[1] or in more resolved imaging[3,4] with different techniques, do not show any void regions in stresses as long as no assumptions are done on the regions where the forces apply[5]: cells pull on the matrix everywhere below them. This spread force field probably comes from the loss of information that originates from the regularization step, all the more that the optical resolution is low[5]. But it is this calculated force field which is employed in Monolayer Stress Microscopy (MSM) calculations[1]. So, in MSM, forces indeed apply everywhere. The displacement field is therefore continuous, meeting our working assumption. We can then argue that both MSM and our straightforward calculation are using the same hypothesis of continuity of the displacement field.

Going beyond this assumption would probably improve both methods. At the present time however, results based on this assumption are surprisingly consistent, showing that the error it brings does not exceed for instance the error that comes from the regularization step in cell/matrix force calculation. To prove it, we calculate back the corresponding stress field $\vec{T}$ that stresses the extracellular matrix from our direct intracellular stress calculation on a monolayer, using : $\mathrm{div}\sigma = \vec{T}/\mathrm{h}$, where h is the thickness of the cellular assembly. Fig. 1 shows a very good agreement with the traction force field calculated using Boussinesq equation. Comparing both calculations also enables us to calibrate our method and gives us a measurement of the Young's modulus times the thickness of the monolayer, Eh, which in this case proves to be around

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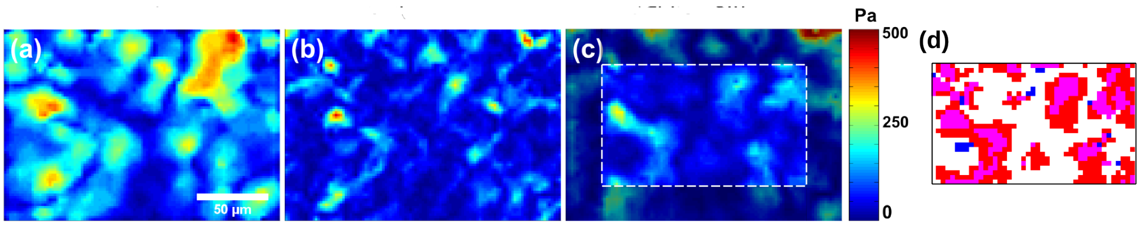

Figure 1 Consistency of the calculated cell to matrix stresses obtained from the intracellular stresses as calculated in[2], or from the Boussinesq equation using the measured displacement field on the top of the extracellular matrix. (a) Intracellular stresses in the monolayer calculated as in[2]. (b) Cell to matrix stresses calculated using $h\mathrm{div}\sigma$ from (a). (c) Cell to matrix stresses calculated using Boussinesq equation. Regions where the boundary conditions in Boussinesq equations have an influence are shaded. Eh has been optimized to 50 kPa.μm. (d) Superposition of the force fields from (b) (in blue) and (c) (in red). Only the stresses above 50 Pa are considered. Shaded areas in (c) are excluded. The pattern of stress from (b) colocalizes with the pattern of stress from (c), although (c) is more spread as expected from the regularization step.

50 kPa.μm (higher than the one initially used in[2]). In addition, we also believe that the sensitivity to the heterogeneity in the Young's Modulus would be equivalent in both methods (compare Fig. 4k in[6] and Eq. 2 in[2]).

Finally, we want to stress that avoiding two matrix inversions (which is mandatory in MSM) is really a gain of accuracy and rapidity as important errors are linked to these numerical processes which necessitate (direct or hidden) regularization techniques[5].

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