Supplementary information

TECHNICAL EXPLANATIONS

For experienced users we develop two technical points alluded to in the main text.

Regarding condition number

Imagine one is solving two equations with two unknowns (a, b). In the mathematical plane (a, b), each equation has solutions represented by a line, and the solution of the system is the point (a_0, b_0) where these lines intersect. If at (a_0, b_0) both lines are nearly perpendicular to each other, it means that the equations provide really independent information, and the solution (a_0, b_0) is determined with precision and robustness. When the lines representing the equations intersect with a very acute angle, they are not truly independent, and the value of (a_0, b_0) is sensitive to the exact positioning of the lines, to noise and to solver errors. The condition number generalises that idea to several equations with many unknowns (Brodland et al., 2014; Ehsandar, 2015; Veldhuis et al., 2015; Mashburn, 2015).

Regarding out of equilibrium situations

To generalise Eqs. (1,2) to out of equilibrium situations, it helps to understand their origin. These equations do not result from Newton's second law of motion, that of inertia, which applies to systems with a mass. Here the vertices and junctions have a negligible mass, and whether they are accelerated or not does not change the mechanical balance. In fact, Eqs. (1,2) result from Newton's third law of motion, namely the equality of action and reaction (here between cell contour and cytoplasm), which always holds, whether at or out of equilibrium (Viennot and Décamp, 2020). In out of equilibrium situations, the equations should include dissipative interactions such as arising from viscosity.

ASSESSMENT ON MOUSE EMBRYOS OF 3D STRESS INFERENCE PIPELINE

To assess the various steps of the pipeline used in practice, here we conducted 3D stress inference and micropipette aspiration on preimplantation mouse embryos (8- and 16-cell stage). Pipettes can measure tensions of contact surfaces between outer cells and the medium, hereafter noted t_{cm} and called *outer tension* for brevity. We first analysed the images (Fig. S1a) presented in Veldhuis et al. (2017). Our procedure (Fig. 7) consists in manually segmenting cells, then rotating the reconstructed embryo to visualize a section perpendicular to each three-cell edge, and extract angles; tensions are then automatically inferred. We inferred a consistently higher outer tension t_{cm} compared to the cell-cell tension $t_{cc}/2$ at 90 min and 240 min post division during compaction (Fig. S1b). This difference between t_{cm} and $t_{cc}/2$ increased with time during compaction, consistent with previous study (Maître et al., 2015). We found no correlation between pipette tension and inferred tension between individual cells in the same embryo (Fig. S1c). This could be due to the small range of measured tension in the same embryo that is too small to be resolved with the stress inference detection precision. Alternatively, we note that the micropipette aspiration itself incurs some experimental errors. The resolution of the applied pressure in the setup is about 10 Pa, which translates to an error of pipette tension in the range of 30 to 50 pN/μm. This could generate the intra-embryo variability in pipette tension in Fig. S1c.

We next studied the outer tension of "reduced embryos" (Maître et al., 2015; Chan et al., 2019). Briefly, embryos at the 4-cell stage were washed with pronase to remove the *zona pellucida*. The embryos were then washed in calcium-free medium, and the dissociated blastomeres (1/4 embryos) were cultured to reach the 16-cell stage, which we term the 4/16 embryos. Similarly, 8/16 embryos were made by aggregating two dissociated blastomeres at the 4-cell stage and cultured to reach the 16-cell stage.

Performing stress inference on these reduced embryos, we inferred consistently higher outer tensions compared to cell-cell tensions, for both the 4/16 and 8/16 embryos (Fig. S1d, h), consistent with the case of full embryos (Maître et al., 2015). This demonstrates that 3D stress inference can detect region-specific differences in tension within a tissue, at different developmental stages and with different cell geometries.

We observed a better correlation between pipette tension and inferred tension in the 8/16 embryos (Fig. S1i) than in the 4/16 embryos (Fig. S1f). This could be due to the too small range of pipette tensions in the 4/16 embryos. We had previously shown that the outer tension correlates with cell stretching in early mouse embryos (Chan et al., 2019). Hence, the small variability of pipette tensions in 4/16 embryos may be due to the fact that the outer cells in these embryos are more equally stretched (Fig. S1e) while those of the 8/16 embryos are stretched to various different degrees (Fig. S1g).

We had previously shown that the reduced embryos have higher outer tensions compared to that of the full embryos, due to the cells being more stretched (Chan et al., 2019). We therefore investigated whether this trend can be captured by 3D stress inference. Indeed, we inferred a higher outer tension (normalised to the cell-cell tension) in the 4/16 embryos, compared to that of the 8/16 embryos (Fig. S2b). This is consistent with the measured pipette tensions (Fig. S2a), which further validates the robustness of 3D stress inference on a statistical level.

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Supplementary Figures

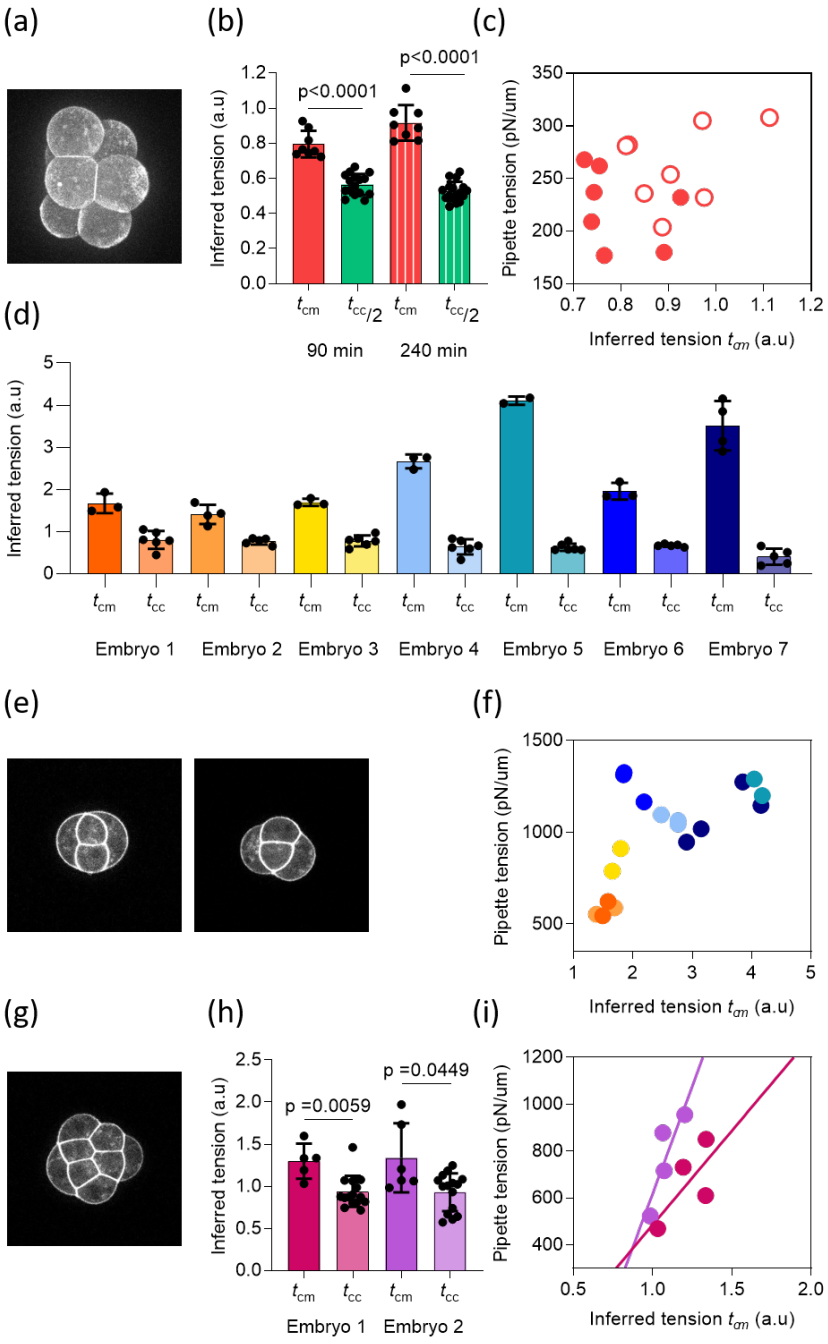

Fig. S1. Stress inference detects spatial and temporal changes of tension in early mouse embryos on a statistical level. (a) 8-cell stage mTmG mouse embryo. (b) Plot of inferred outer tensions t_{cm} and cell-cell tensions t_{cc} between inner cells at 90 min and 240 min post division time (8-cell stage). One embryo per timepoint was analyzed, containing each 8 cells with 21 junctions each. (c) Plot shows a lack of correlation between measured and inferred outer tensions in the same embryo measured at early (close circles) and late (open circles) 8-cell stage. (d) Plot of inferred outer tensions and cell-cell tensions in 4/16 embryos. Stress inference can detect the difference between the two tensions in the same embryo. 7 embryos were analyzed, containing each 4 cells which represent between 9 and 11 junctions per embryo. (e) 4/16 mTmG embryos. (f) Plot shows a lack of correlation between the measured and inferred outer tensions within the same 4/16 embryo. Each curve corresponds to one embryo, pipette tension was measured for 2, 3 or 4 cells per embryo according to their accessibility. (g) 8/16 mTmG embryos. (h) Plot of outer tensions and cell-cell tensions in 8/16 embryos. Stress inference again detects the difference between tensions in the same embryo. Two embryos were analyzed, with 8 cells and 21 junctions per embryo. (i) Plot shows a correlation between the measured and inferred outer tensions within the same 8/16 embryo.

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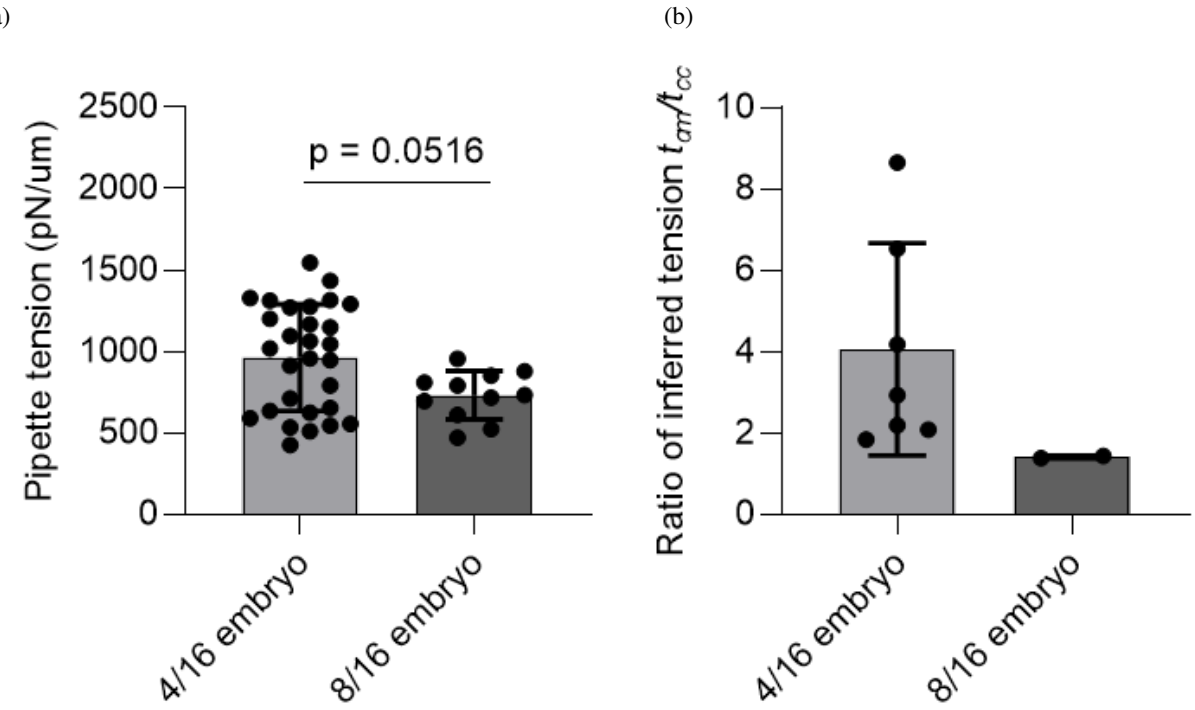

Fig. S2. 4/16 mouse embryos have higher outer tensions compared to that of 8/16 embryos, as measured by micropipettes (a) and inferred by stress inference (b). In (b), each data point indicates the average outer tension t_{cm} normalised to the average cell-cell tension t_{cc} per embryo. 4/16 embryos: 28 cells and 63 interfaces in 7 embryos; 8/16 embryos: 16 cells and 65 junctions in 2 embryos.