Adaxial epidermis of onion (*Allium cepa*) bulb scale was used to investigate relationships between cell deformations accompanying plasmolysis or deplasmolysis, mechanical properties of cell walls, and cell geometry. Onion epidermis is weakly attached to underlying parenchyma. Thus, tissue stresses do not occur in this tissue and it is easy to isolate without cells damage. Plasmolysis and deplasmolysis of onion epidermal cells leads to complete removal or restoration of tensile stresses in the cell walls, respectively, which are accompanied by cell wall deformation.
The following working hypotheses were tested:

**Hypothesis 1:** Plasmolysis and deplasmolysis of adaxial onion epidermal cells is accompanied by decrease or increase, respectively, of cell volume and surface area of outer periclinal cell wall.
**Hypothesis 2:** The extent of volume and area deformations accompanying plasmolysis and deplasmolysis depends on: maturity of onion from which the epidermal cells come from; amount of osmotic pressure modification; cell shape and size.
**Hypothesis 3:** Deformation of dead cells, accompanying the osmotic treatment, depends on the deformation of adjacent cells.
**Hypothesis 4:** Deformation anisotropy of outer periclinal cell walls, accompanying plasmolysis, depends on cell geometry, stress anisotropy in the wall of turgid state cell, and anisotropic mechanical properties of the wall.
First, epidermal cells, the walls of which were stained with Propidium Iodide (PI), were observed in confocal microscopy. PI staining additionally allowed to assess cell viability, because cells with nuclei stained by PI are often considered as dead. Next, using images from confocal microscopy and MorphoGraphX software, deformations accompanying osmotic treatment of epidermal cells were calculated. On this basis it was found, that most of volumetric deformations in cells with PI unstained nuclei were according to expectations: plasmolysis and deplasmolysis was accompanied by cell volume decrease and increase, respectively (the first working hypothesis was confirmed).
Next, factors affecting these deformations were searched for. Volumetric deformations accompanying plasmolysis and deplasmolysis were compared in cells coming from young and mature onions, which were incubated in hypotonic or isotonic PI solution before plasmolysis. The measurements showed that volumetric deformations are higher in cells coming from young onions, the walls of which are probably thinner and more elastic than cell walls in mature onion epidermis. Higher volume deformations were also found when osmotic pressure modification was higher: the change from hypotonic to hypertonic solution is higher than from isotonic to hypertonic solution. Moreover, the extent of volumetric deformations were related to cell shape and size (the second working hypothesis was confirmed).
Volumetric deformations accompanying plasmolysis and deplasmolysis of cells with PI stained nuclei were also studied. Contrary to the assumption about the cell death, it was found that the deformations of some of these cells were similar to deformations of cells with PI unstained nuclei (i.e. plasmolysis was accompanied by cell volume decrease, deplasmolysis – by the increase). From studies on yeast cells (*Saccharomyces cerevisiae*) it is known that transient membrane permeability may accompany osmotic treatments, and PI can reach living cell nucleus. Perhaps osmotic treatment causes similar membrane permeability in onion epidermal cells, therefore a part of cells with PI stained nuclei was in fact alive and thus deformed as expected. However, deformations of other cells with PI stained nuclei were different: plasmolysis was accompanied by cell volume increase and deplasmolysis – by decrease. In order to explain such deformations, for each cell with PI stained nuclei, a portion of its perimeter, which was adjacent to cells with PI stained nuclei, was assessed. On this basis it was found that the observed deformations accompanying plasmolysis of dead cells (volume increase) is likely a passive deformation resulting from deformation of alive adjacent cells (the third working hypothesis was confirmed).
Next, areal deformations of outer periclinal walls accompanying osmotic treatment were studied. For most of cells areal deformations were as expected (i.e. plasmolysis was accompanied by wall area decrease and deplasmolysis – increase), but the extent of these deformations was very low (likely close to the measurement error). Nevertheless, the analysis of optical transverse sections by the same cells before plasmolysis, after plasmolysis and after deplasmolysis, shows that despite the

low areal deformations, osmotic treatment is accompanied by changes in outer periclinal wall curvature similar to those accompanying pumping and deflation of a mattress.
In the second part of studies we examined wrinkles, which are visible in Nomarski microscopy, which appear on the protoplast facing cell wall surface after the stress removal. The arrangement of wrinkles was analyzed in order to assess the anisotropy of the outer periclinal wall deformation accompanying plasmolysis. It is known that wrinkle orientation is perpendicular to the direction of maximal deformation. The analysis showed that wrinkle arrangement is not uniform within a single cell wall, but similar in central part of different cells. The wrinkle arrangement near the cell tip depends on the tip shape. These results show that deformation of outer periclinal walls, accompanying plasmolysis, is anisotropic and the direction of maximal shrinking in the central part of cells is transverse to the cell axis.
Next, cellulose fibrils arrangement was examined on the protoplast facing wall surface, i.e. the surface where wrinkles appear, for central parts of cells (Atomic Force Microscopy), and across the whole wall thickness in central parts of cells and variously shaped cell tips (polarized light microscopy). The aim of these studies was to check if the anisotropy of areal wall deformation depends on anisotropic mechanical properties of cell wall, which result from cellulose fibrils arrangement. The results showed that the deformation
anisotropy does not depend on the cellulose fibrils arrangement, but rather on geometry of the cell (the fourth working hypothesis was partly confirmed).