

Wrinkling Substrates for Cell Contraction Analysis

- PRODUCT DESCRIPTION -

The wrinkling substrates are designed to provide semi-quantitative information on cell contractile activity. The technology is based on the concept that contractile cells produce large deformations on elastic substrates that are visible in transmission light microscopy as "wrinkles" - analogous to wrinkling a sheet of paper with your hand. The method is related to traction force microscopy that, in contrast, requires high resolution microscopy, substrate surface markers, and complex computational analysis of contraction-induced deformations.

Fields of application:

- Wrinkling substrates are principally used to visualize and measure contraction of adherent cells *in vitro*. The mechanical properties of the substrate are adapted to the contractile force of fibroblastic and muscular cells.
- Wrinkling substrates can be used to visualize and measure contraction of tissue samples (e.g. muscle fibers, cross-sectioned blood vessels, contractile glands, organoids, etc.)
- Wrinkling substrates can be used to identify contractile cells in a heterogenous population by combining with immunofluorescence microscopy.
- Wrinkling substrates can be used to measure cell/tissue contraction in response to drug treatments over time, over concentration series, and to compare contraction between conditions (e.g., contractile versus non-contractile fibroblasts).
- Note: Contractile forces developed by single epithelial, endothelial, inflammatory, and adaptive immune cells during adhesion and migration are typically too small to be analyzed with this technology.

Key Features:

- Wrinkling substrates have been extensively tested for cell culture.
- Wrinkling substrates are sterile and shipped/stored in dry conditions.
- Wrinkling substrates are easy to use for cell culture and subsequent analysis. They consist of a petri dish support, provided with a ~80 μm -thick wrinkling silicone layer and protein coating.
- Wrinkling substrates are available in single-well $\varnothing 35$ mm dishes with either plastic or glass coverslip bottom support.
- To promote cell attachment, wrinkling substrates are ready-to-use and pre-coated with bovine gelatin (2 $\mu\text{g}/\text{cm}^2$). Substrates cannot be ordered without protein coat.
- Wrinkling substrates are completely transparent, and cells with wrinkles can be visualized using standard transmission light microscopes (e.g., phase contrast, DIC) even at low magnification (e.g., 10x or 20x objectives). Minor reduction in optical quality may occur due to air inclusions in the bulk material. Inclusions do not affect substrate properties or cell growth.
- Wrinkling substrates are compatible with most standard molecular or cell biology techniques: immunofluorescence, immunohistochemistry, protein analysis (e.g., Western blotting, and RNA/DNA extraction). Note that particular care must be taken to scrape material off the deformable soft surface; recommended is trypsinization for cell harvesting (see below).

Wrinkling Substrates for Cell Contraction Analysis

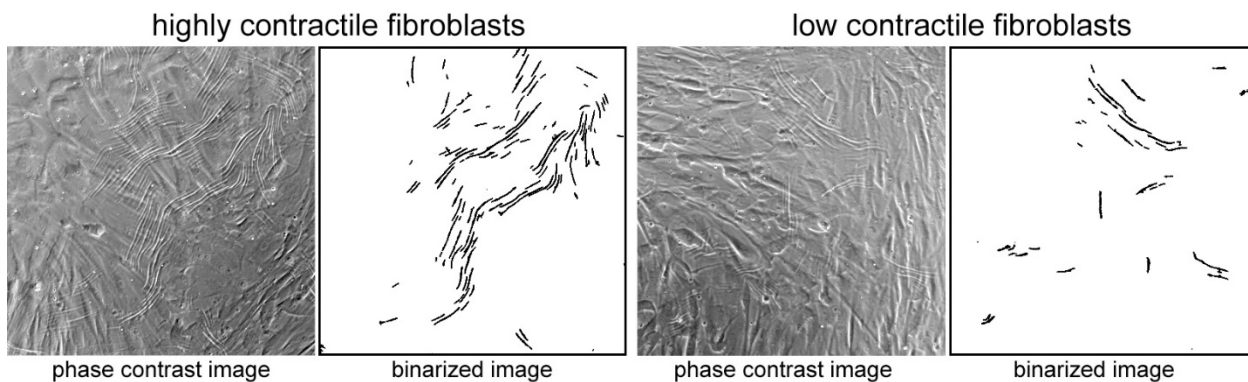
- INSTRUCTIONS OF USE -

How to use Wrinkling substrates

- Seed cells as on any other culture surface.
- Depending on cell attachment efficacy and forces developed by your cells, wrinkles may be observed as early as cells attach and spread (1-3 hours).
- Typically, wrinkles would be assessed starting after overnight or 1-day cell growth.
- Wrinkles elevate over the substrate level by 1-5 μm and it is pivotal to use properly configured light microscopes for their detection. For phase contrast microscopy, make sure to use the correct phase ring/objective combinations and perform the Koehler technique. For DIC, make sure to use the correct polarizer/objective combinations.

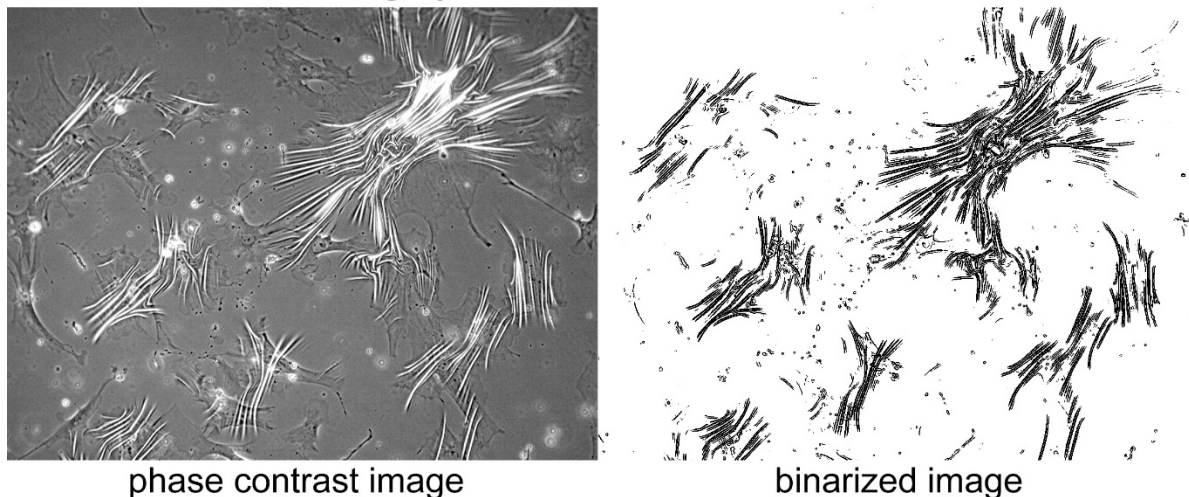
This is what you should expect under a well set-up culture microscope:

35 mm plastic bottom format, cell culture microscope, 10x objective, phase contrast:



35 mm glass bottom format, cell culture microscope, 20x objective, phase contrast:

highly contractile fibroblasts



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- ANALYSIS -

Wrinkle quantification:

- Association of wrinkles with cells indicates their contractile activity. We recommend two basic protocols for quantification:
 - o Count the number of cells producing wrinkles and relate to total cell number (% of contractile cells). This method is suitable to compare different cell populations or different treatment groups across different wells.
 - o Use image analysis programs (e.g., the open software ImageJ/Fiji) to threshold for bright wrinkles. Then binarize the image and calculate the image area covered by wrinkles. This method is very effective to quantify changes in wrinkle intensity/number during a treatment (before/after) over one image position. Combined with cell normalization it can also be used to compare separate wells.
 - o The number of wrinkles per cell is not a good representation of cell force.

Combination with other techniques:

Immunohistochemistry:

- Wrinkling substrates can be used for any standard immunostaining technique using MeOH, EtOH, PFA, or acetone fixation. We recommend to adding 0.02% TX-100 to all buffers used for antibody dilutions and washing steps.
- With careful handling, wrinkles may be preserved during and after fixation if cells do not relax in the procedure.
- Wrinkles will be lost when substrates are mounted with mounting medium and coverslips.

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Cell Lysis:

- Cell scraping with rubber policemen to recollect protein, RNA or DNA is possible but, due to the softness of the substrates must be performed with greater care than on plastic. We recommend as alternatives to scraping: a) cell trypsinization and lysis of the pellet after spinning or b) addition of the lysis buffer to adherent cells and incubation under vigorous rocking. No interference has been reported between the substrate material and standard protein/DNA/RNA extraction buffers.
- Wrinkles will be lost upon any mechanical procedure and/or cell detachment.

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- TECHNICAL DATA -

Storage and handling:

- Wrinkling substrates can be stored at room temperature or at 4°C, best protected from light.
- Storage of up to 3 months will have no influence on substrate wrinkling capacity.
- Due to the protein coat, longer storage is not recommended.
- Please use wrinkling substrates before the date indicated on the packaging (DLU).
- Do not expose wrinkling substrates to UV light which will change the mechanical properties and thus sensitivity of the material to contractile forces.
- Be aware that the nature of the contraction measurement requires the wrinkling substrates to be soft; surfaces are thus vulnerable to mechanical insult (e.g., scraping off cells).

Sterilization:

- Wrinkling substrates are sterile.
- Wrinkling substrates are **for Research Use Only**

Wrinkling substrate thickness:

- The thickness of the wrinkling substrate layer is ~80 µm. Total thickness of the culture dish bottom depends on the format:
- Ø35 mm with plastic bottom support have a total thickness of ~1100 µm.
- Ø35 mm dishes with glass bottom support; total thickness 210-250 µm.

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Additional notes for microscopy:

- The thickness of the plastic support (~1 mm) does not allow use of short distance high-resolution objectives. Long distance high-resolution objectives may be used.
- For live studies with these formats, use of water-immersion objectives and upright microscopy is recommended for best image quality.
- For high magnification and inverted microscopy (typically 40x immersion objectives), Ø35 mm dishes with glass bottom support are recommended.