

Micropatterned Adhesion Sites for Spheroid Cultivation Under Flow

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Abstract

The Need

- Switch from 2D to 3D applications in recent years due to higher validity of the experimental outcome concerning *in vivo* relevance.
- Spheroid generation, cultivation and observation over long time period combined with high quality imaging often laborious

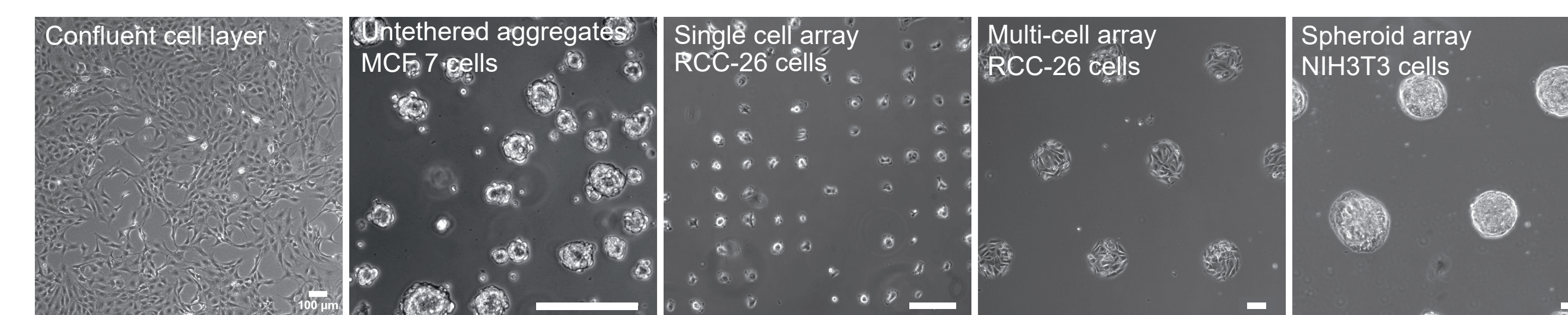
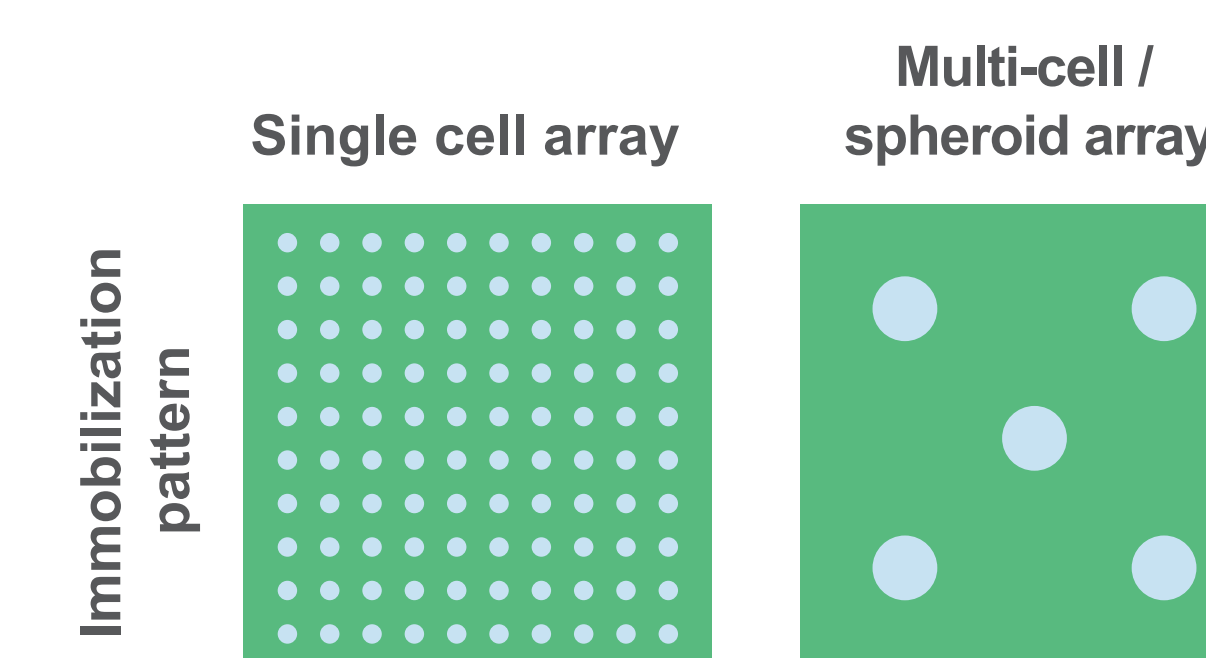
Our Solution

- Micropatterned adhesion ligands on highly passivated coverslip combined with a perfusable channel system
- Generation of homogeneously distributed spheroids within one channel either by seeding single cells or immobilization of preformed aggregates.
- Perfusion at low flow rates enables homogenous nutrient supply as well as metabolite analysis together with toxicological screenings

Defined Cancer Cell Arrays on a Micropattern

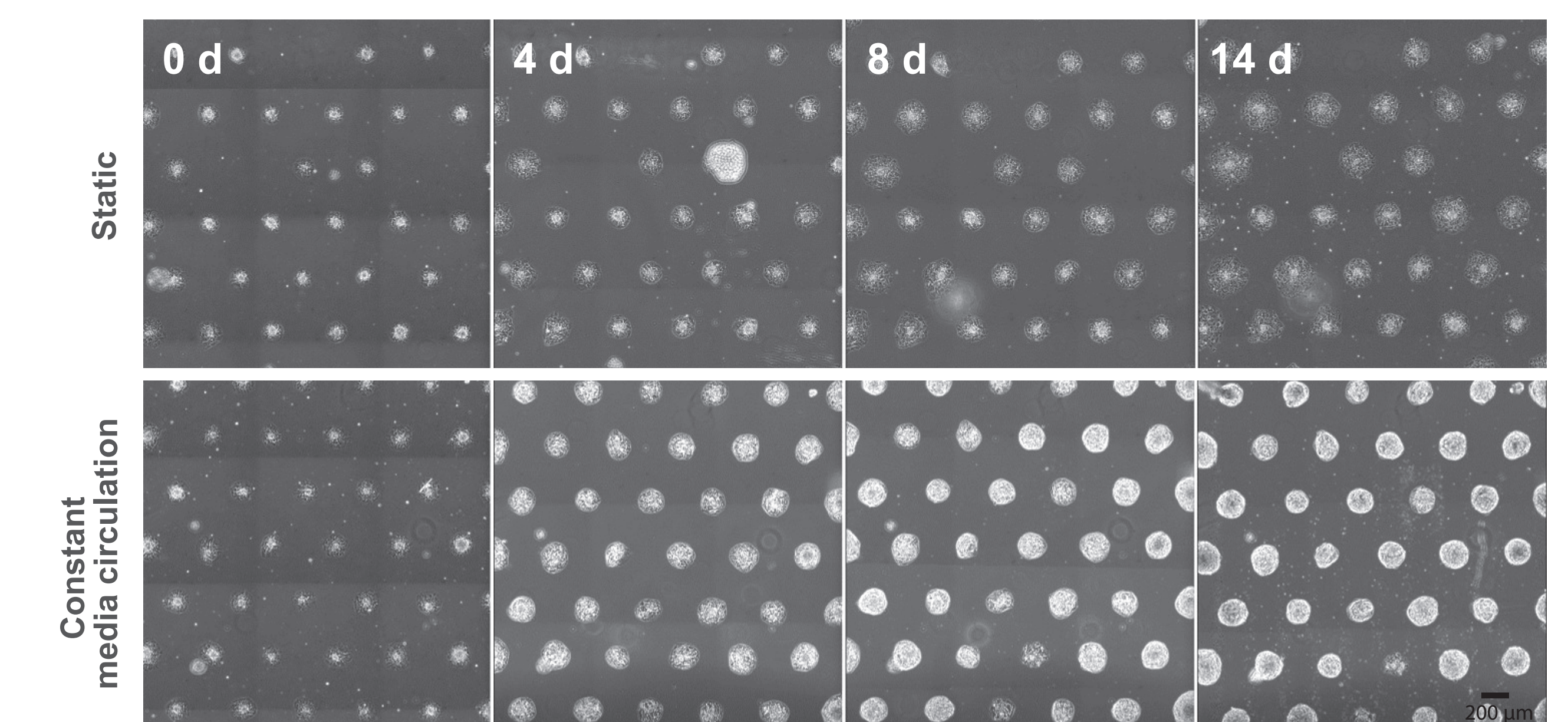
Using the micropatterning technology, cells are immobilized (adhered/tethered) on spots of different sizes offering the formation of single cell, multi cell or cell aggregate arrays.

In contrast to nonstructured cell assemblies, these arrays allow a detailed optical analysis of the same cells/aggregates over a long time period.



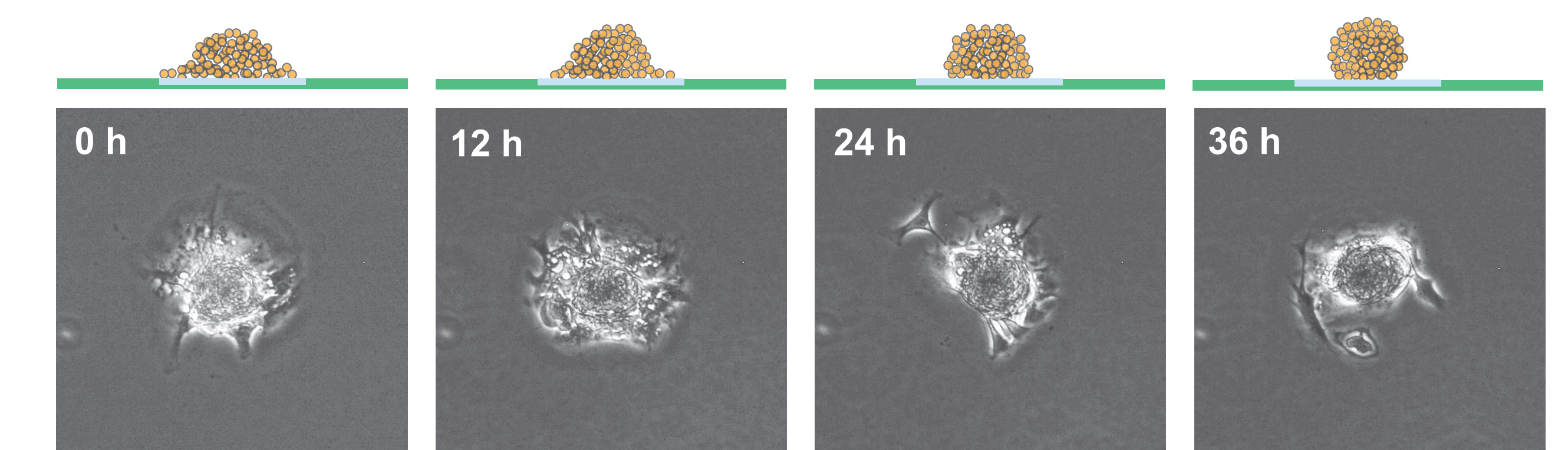
Spheroid Culture Under Constant Media Circulation

By creating micropatterns within a μ -Slide VI^{0.4} Luer, aggregates on the pattern can be constantly supplied with fresh media by perfusing the system with the ibidi Pump System.



Fibroblasts (3T3) under flow conditions at 3 dyn/cm² compared to the static control, cultured in the μ -Slide VI^{0.4} Luer for 14 days.

By applying a constant media flux around the cell aggregates, spheroids become more compact and round.



Fibroblast (3T3) aggregates under flow conditions at 3 dyn/cm² form a compact and round spheroid over time.

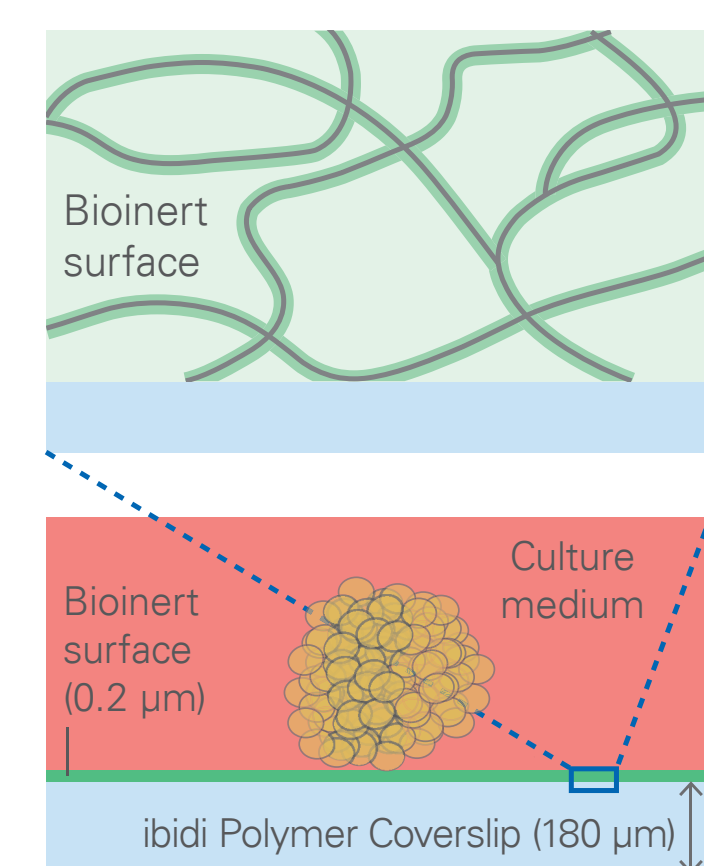
Micropatterning on the Bioinert Surface

The Bioinert Principle

- Thin polyol hydrogel layer, covalently bound to the ibidi Polymer Coverslip #1.5

Features

- Biologically inert—no cell or protein adhesion
- Long-term stable
- Ready-to-use
- Highest optical quality for imaging

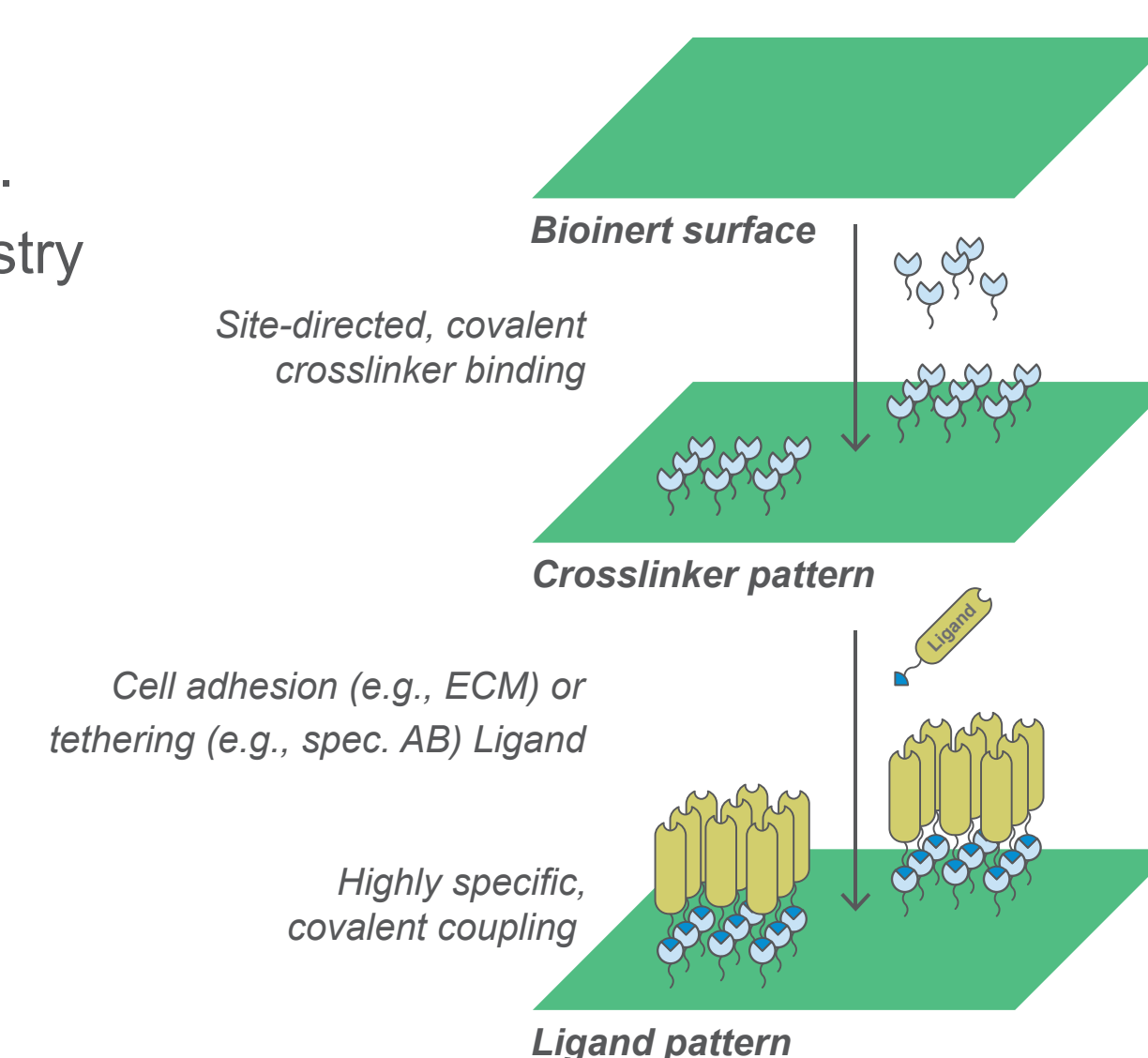


Functionalization

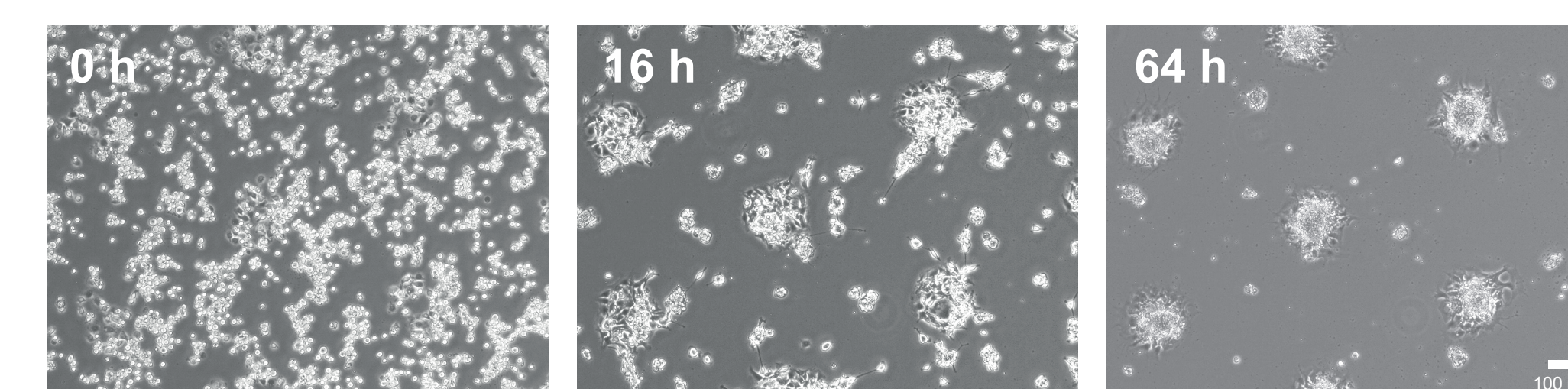
- Specific cell adhesion/tethering for weeks.
- Custom-specific adhesion via click chemistry
- Defined cell and molecule immobilization

Optics

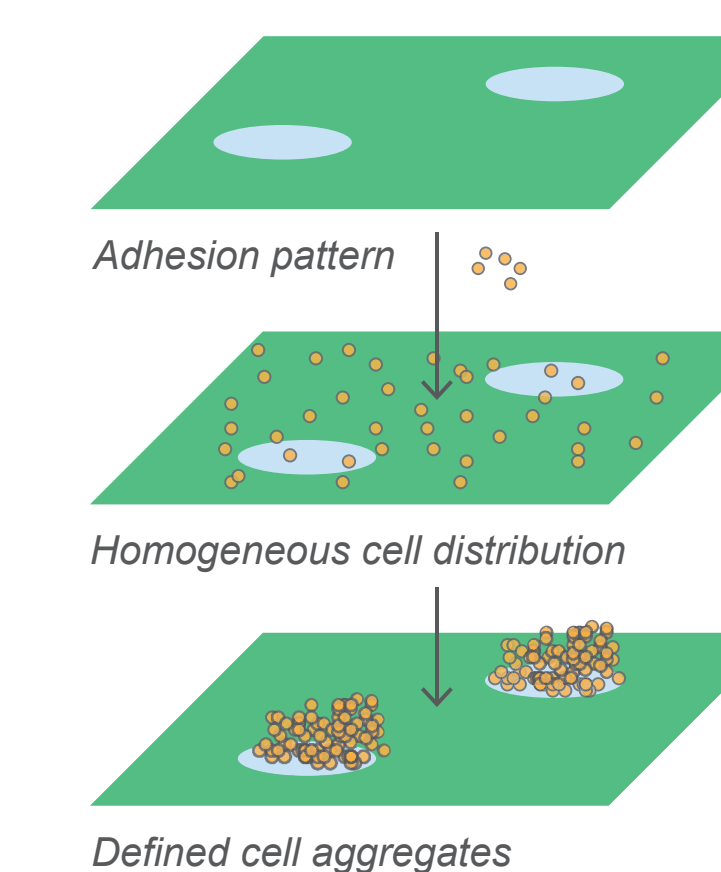
- Very low autofluorescence
- No visibility of μ -Patterns in brightfield
- Optional μ -Pattern fluorescence



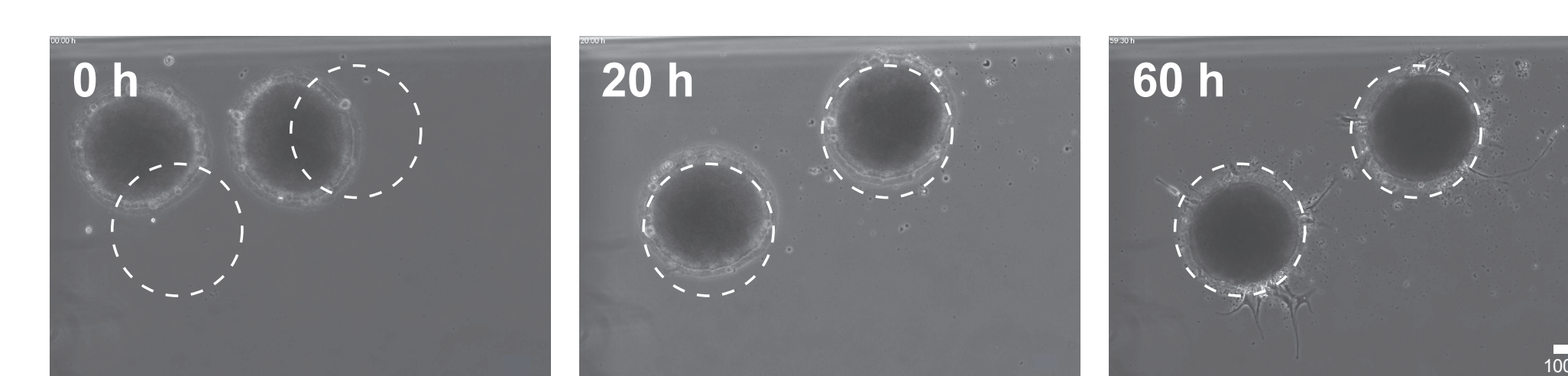
Spheroid Generation From Single Cells



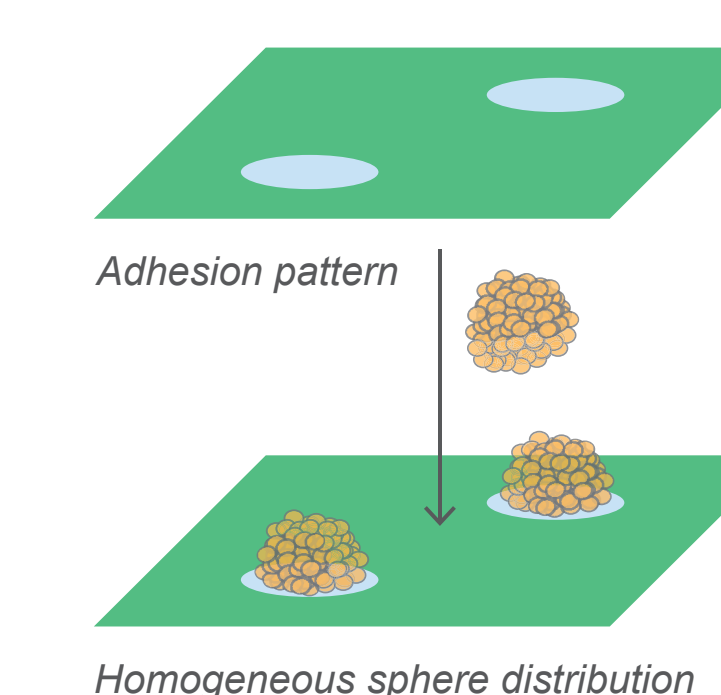
Spheroid generation: Defined 3T3 cell aggregates form on a μ -Pattern (200 μ m) in the μ -Slide VI^{0.4} Luer after single cells were flushed in the channel.



Immobilization of Preformed Spheroids/Organoids



Spheroid immobilization: Preformed 3T3 cell aggregates were stably localized on a μ -Pattern (dashed line, 200 μ m) in the μ -Slide VI^{0.4} Luer.



Outlook

- Toxicological screenings in perfused spheroid cultures
- Tethering cell aggregates to patterns by specific antibody interactions
- Combining spheroid cultivation on patterns with invasion studies by overlaying an ECM matrix
- Develop reversible binding to retrieve aggregates for further analysis