Vysens Application Note (AN) #1

Document Information

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1. Title

Measurement of LNCaP–anti-Her2 interaction under increasing shear using CellVysion with a continuous ligand density gradient

2. Objective of This Measurement

This application note describes the measurement of LNCaP–anti-Her2 interaction under increasing shear conditions using the Vysens CellVysion system with a continuous ligand density gradient.

3. Biological System

In this measurement, the interaction between a human prostate cancer cell line and a surface-immobilized antibody was examined.

Cell type
LNCaP (human prostate cancer cell line)

Target / ligand
Anti-Her2-Biotin antibody

Material origin
LNCaP cells were purchased from ATCC.

Relevant characteristics
The LNCaP cell line has been reported to express approximately 30,000 Her2 antigens per cell (literature data).

4. Experimental Setup

The experiment was performed using a streptavidin-functionalized SPR sensor surface on which a continuous ligand density gradient was generated.

Sensor surface
SPCV SAHC200M SPR sensor prism (CytoVysion compatible, Xantec Bioanalytics)

Surface coating

200 nm streptavidin-derivatized linear polycarboxylate hydrogel (medium density)

Functionalization

A continuous anti-Her2-Biotin ligand density gradient was generated on the streptavidin-coated surface.

Measurement conditions

- Running buffer: PBS + 1% BSA + 0.25% EDTA (0.5M v/v) + 0.01% Tween-20
- Cell concentration: 2,2 × 10^6 cells/mL
- Sedimentation phase: 10 minutes
- Automatically increasing shear flow applied after sedimentation
- Temperature: room temperature

5. Measurement Procedure

The sensor was inserted into the CellVysion system and primed using running buffer and MilliQ water. The optimal SPR angle was set automatically and tuned manually prior to calibration.

Calibration was performed by injecting a calibration solution with a refractive index difference of 945 RU with respect to running buffer, allowing pixel intensities to be converted to RU values. The calibration run was recorded using ThorCam software.

Subsequently, a continuous anti-Her2-Biotin gradient was generated by injecting 90 µL of a 5 µg/mL antibody solution into the cuvette. The gradient formation was recorded for approximately 10 minutes.

After rinsing the system with running buffer, 50 µL of LNCaP cell suspension was injected (2,2 million cells/mL). A sedimentation phase of 10 minutes allowed cells to settle onto the surface. Following sedimentation, an automatically increasing shear flow was applied.

The entire run was recorded using the ThorCam software and analyzed using the CellVysion Analysis Software. The tipping point was identified based on pixel position in the SPR image (using ImageJ software), and the corresponding ligand density was calculated in RU.

6. Data Output

The measurement produced multiple data outputs describing calibration, gradient formation, and cell retention under shear.

Output types

- Reflectivity versus time during calibration
- Ligand density gradient profile (RU per pixel)
- SPR image showing cell distribution after shear
- Ligand density value at the identified tipping point (RU)

Number of measurements

Single measurement (n = 1)

Example figures

1. Calibration reflectivity plot

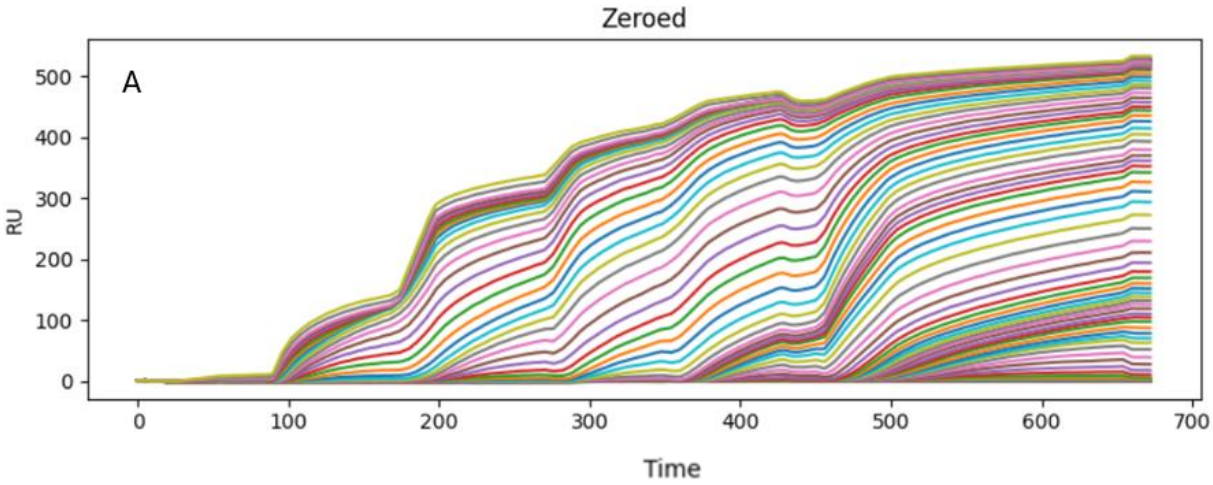

Figure 1. Calibration of the sensor surface to calibrate all pixel intensities and transfer to RU values.

2. Gradient generation curve

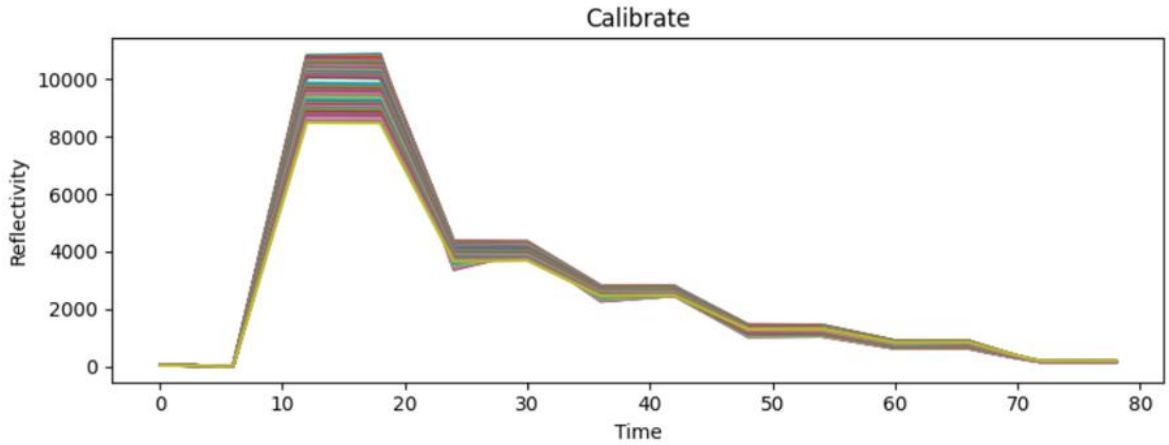

Figure 2. Anti-Her2-Biotin continuous ligand density gradient generation. Process of gradient generation plotted in time.

3. Final ligand density per pixel

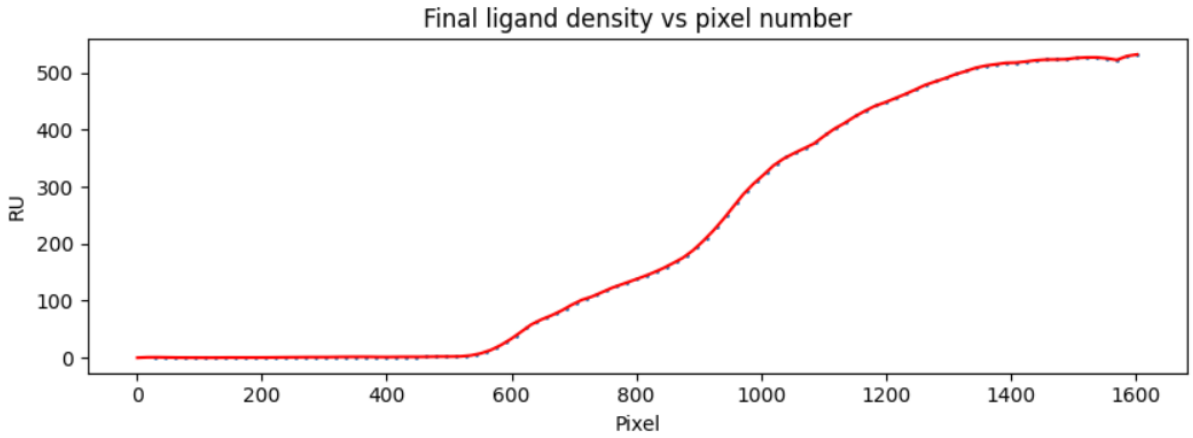

Figure 3. Anti-Her2-Biotin continuous ligand density gradient generation. Final ligand density on the sensor surface plotted per pixel.

4. SPR image with tipping point location

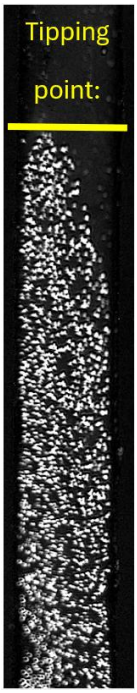

Figure 4. Cell Avidity of LNCaPs on an anti-Her2 ligand density gradient. Tipping point is visualized in the SPR image where LNCaPs remain bound under flow.

7. Technical Observations

During gradient generation, a continuous variation in ligand density was observed across the sensor surface. Ligand density varied along the pixel axis as recorded in the gradient profile.

After cell injection and sedimentation, cells were visible on the surface in the SPR image. During increased shear, unbound or loosely bound cells detached from the sensor surface.

A transition point was observed at a specific pixel position where LNCaPs remained bound under flow. The anti-Her2-Biotin density at this position was 3.7 RU.

8. Limitations

The measurement requires viable cell suspension and a functional streptavidin-coated SPR surface.

A continuous gradient must be successfully generated prior to cell injection.

The measurement was performed under defined shear conditions only.

The data presented represents a single measurement without replicates.