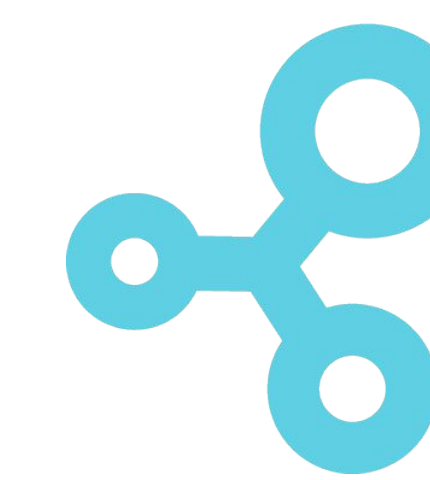


Rapid Hybridoma Screening with Automated Sample Preparation and Digital SPR Analysis



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Introduction

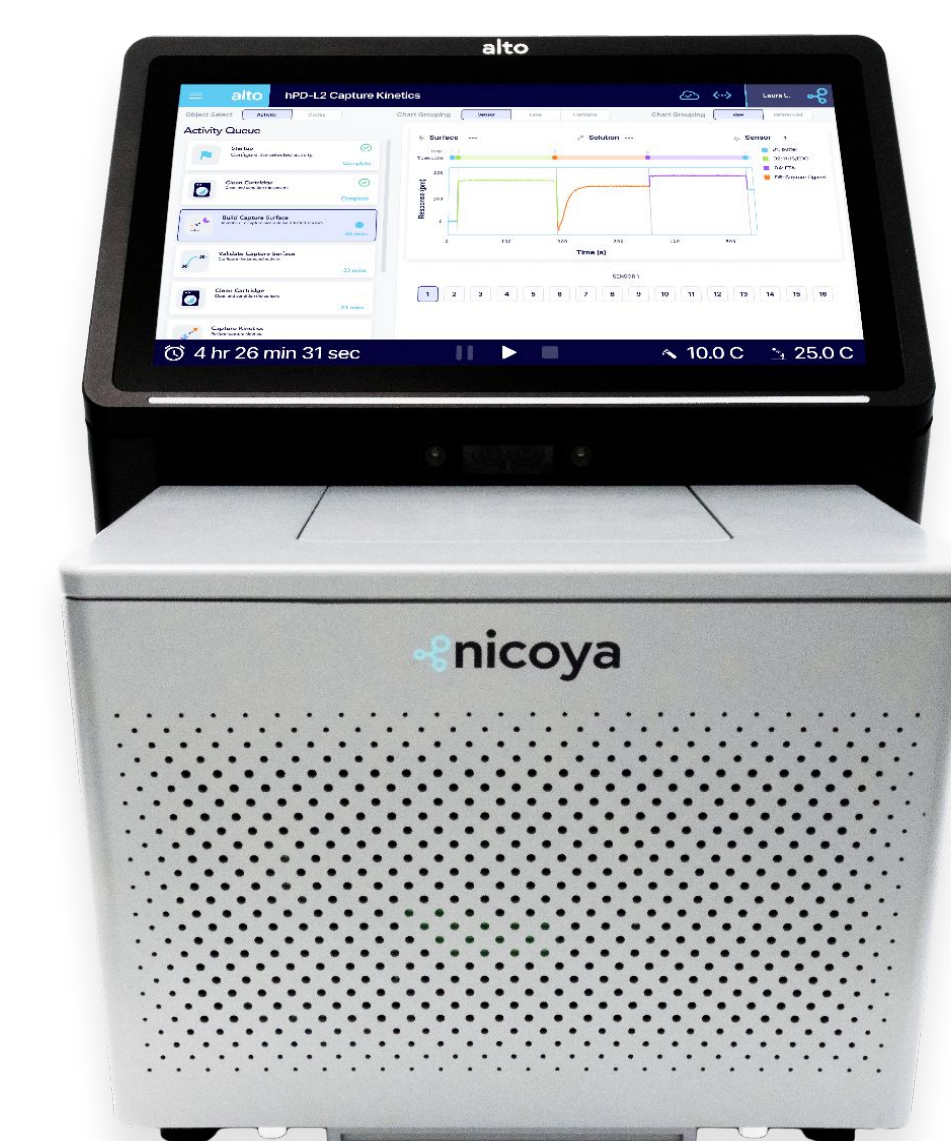
Alto™ is the world's only Digital SPR™ instrument that uses digital microfluidics (DMF) to handle samples. This allows all SPR sensors, samples, buffers and reagents needed for an experiment to be contained in a disposable, automation-compatible cartridge, making Alto the only fully automatable SPR instrument.

Here, we show how Alto seamlessly integrates with the Opentrons Flex™ and Brooks Automation PF400 robot arm to rapidly screen through hybridomas in supernatant with only a few minutes of user intervention.

Nicoya's analysis software automatically analyzes the data from each interaction to identify and provide single concentration kinetics on hits.

Alto™ Digital SPR™: Label-free binding analysis in a droplet

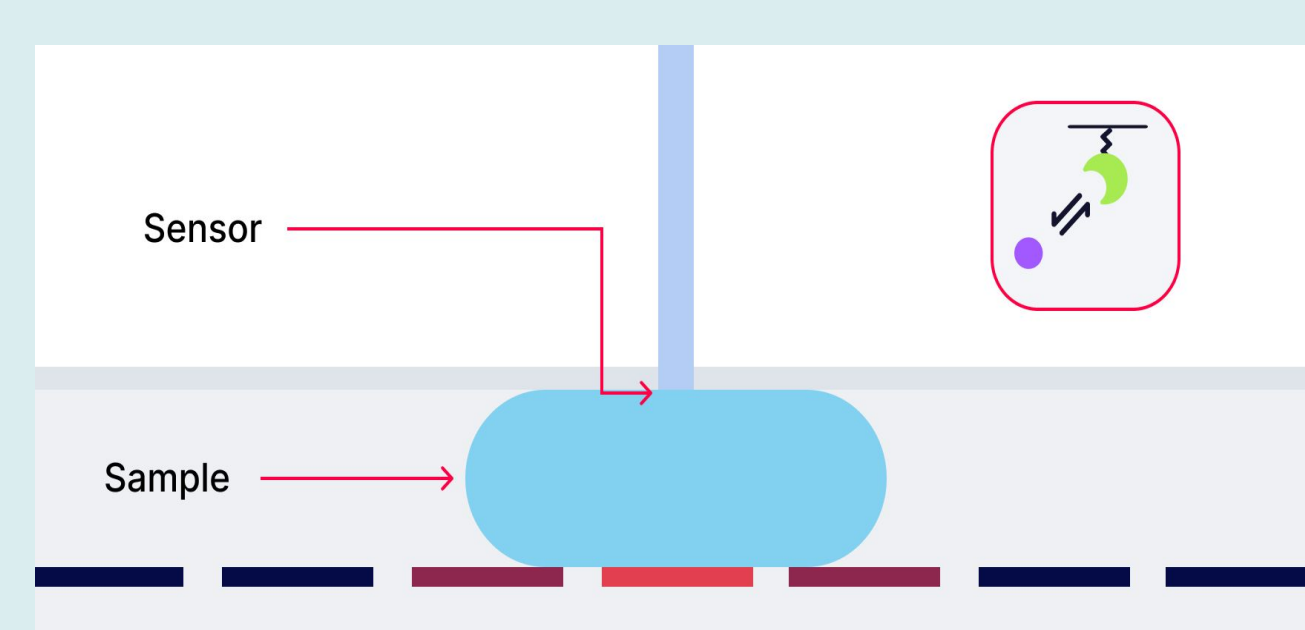
Nicoya's Alto system uses digital microfluidics (DMF) to deliver automatically diluted sample droplets to SPR sensors for effortless real-time characterization of biomolecular interaction analysis including quantitation, screening, epitope binning and binding kinetics.



- 16 independently addressable channels for high throughput.
- Complete kinetics analysis using only 2 μ L of crude or purified sample.
- No manual dilutions, tagging, degassing, cleaning, or strenuous assay set up.
- Powerful screening data visualization and analysis software.

What is DMF? (Digital Microfluidics)

DMF is a liquid-handling technology capable of accurately controlling and manipulating discrete nanoliter-sized droplets across an array of electrodes. The fluidics are contained within a disposable cartridge, allowing Alto to overcome the major limitations associated with increasingly complex fluidic systems present in traditional label-free instruments.



Automated Cartridge Preparation

Traditional SPR instruments are difficult to automate due to their complex and error-prone fluidic systems. Any error with the fluidics within the scheduled runs can be detrimental for subsequent assays, particularly when using crude samples.

Alto's cartridge-based fluidics overcome these issues and make it an ideal SPR instrument for screening and automation integration. Operating as an integrated workcell, Nicoya's Alto, the Opentrons Flex and the Brooks PF400 robot arm enable rapid, hands-free screening of up to 2,000 leads/week.

Figure 1 shows how the Alto Automation Suite can be configured to screen through over 200 hybridoma leads in crude sample in 32 hours.

Figure 1: A) Diagram showing the different components of the automated Alto workcell.

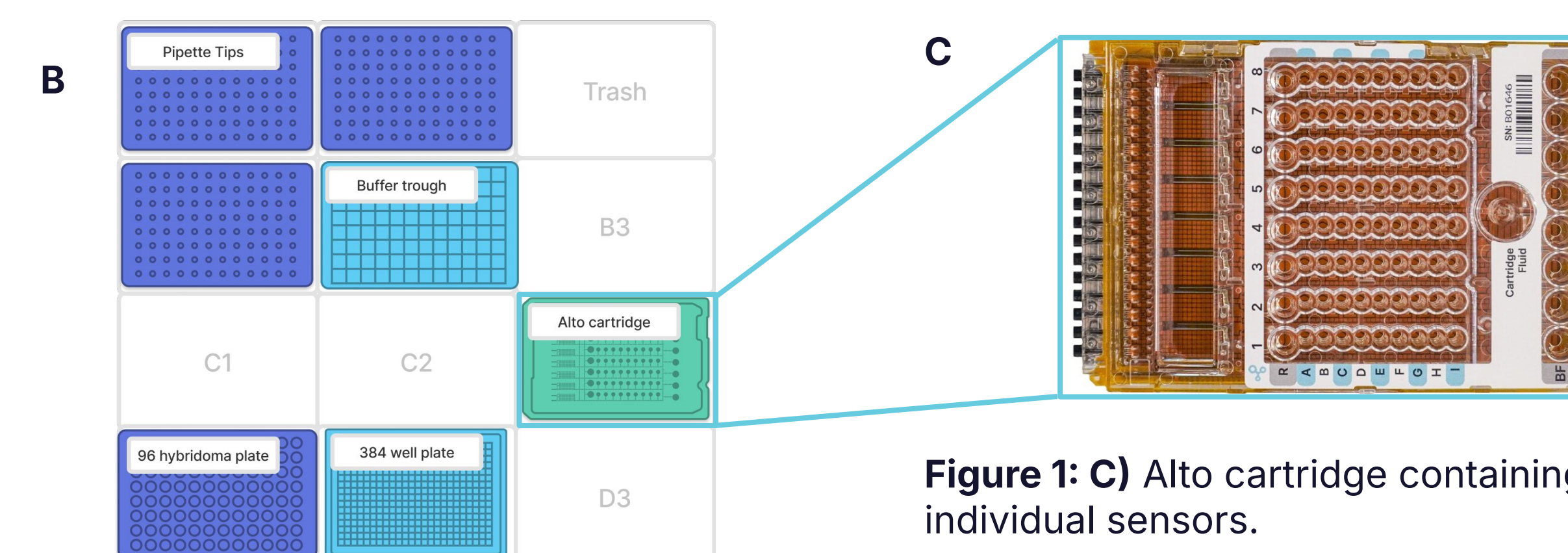
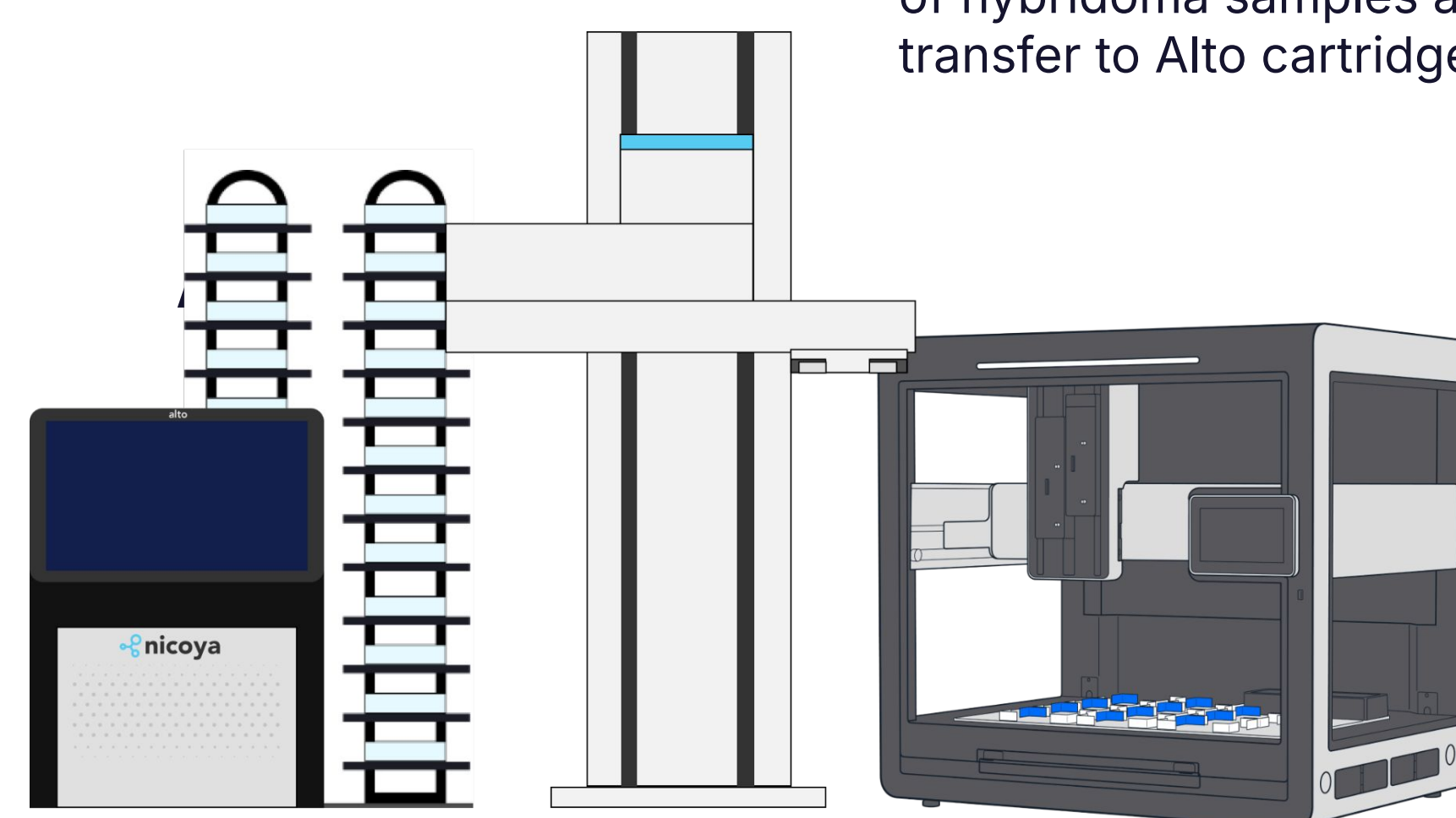


Figure 1: B) Diagram showing the deck configuration of the Opentrons Flex for preparation of hybridoma samples and transfer to Alto cartridge.

Figure 1: C) Alto cartridge containing 16 individual sensors.

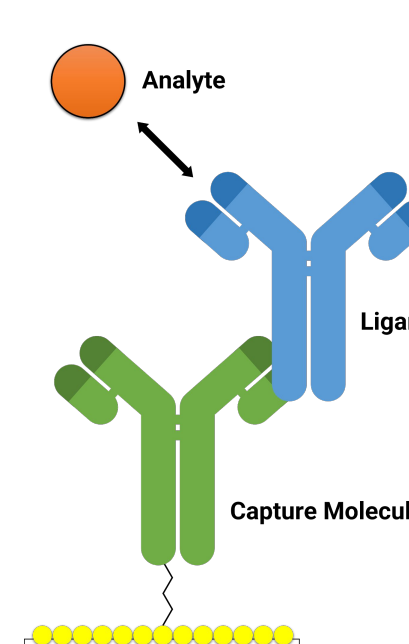
Steps

1. The PF400 robot arm transferred a cartridge from the plate hotel to slot C3 of the Opentrons Flex.
2. Using the Opentrons Flex, samples in supernatant were transferred from the hybridoma plate to the 384-well plate and diluted 2x with buffer from the well trough.
3. Samples, reagents and buffers were transferred to the Alto Cartridge™ according to the sample layout .csv file obtained from the User Portal.
4. The PF400 transferred the cartridge to the Alto.
5. The capture screening assay was initiated on the Alto.
6. Once the experiment was completed, the PF400 removed the cartridge from the Alto.

Hybridoma Screening

Real-time, label-free binding analysis is routinely used to screen hybridoma clones for high affinity antibodies prior to further expansion and subsequent characterization. Alto allows for rapid, automated, SPR-quality screening and characterization of biologic lead libraries with simple, pre-configured capture screening and binding kinetics characterization assays to select lead molecules.

Figure 2 shows the capture screening assay, where an anti-human VHH was immobilized to the sensor surface. The hybridomas were used as ligands, with their target, H5N1 Hemagglutinin (HA), as the analyte.



Antibodies generating binding responses greater than 40 RUs were considered hits, and those with responses below 20 RUs are non-binders. Anything in between were considered intermediate binders.

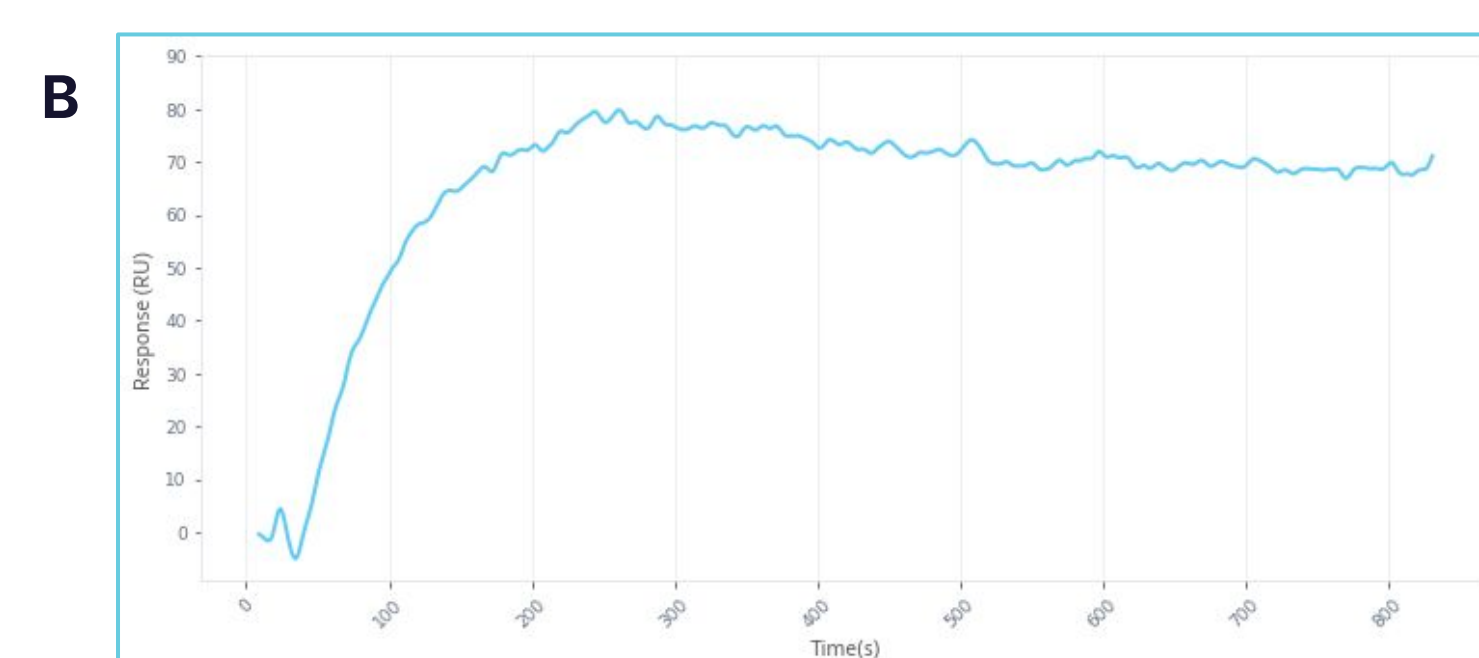
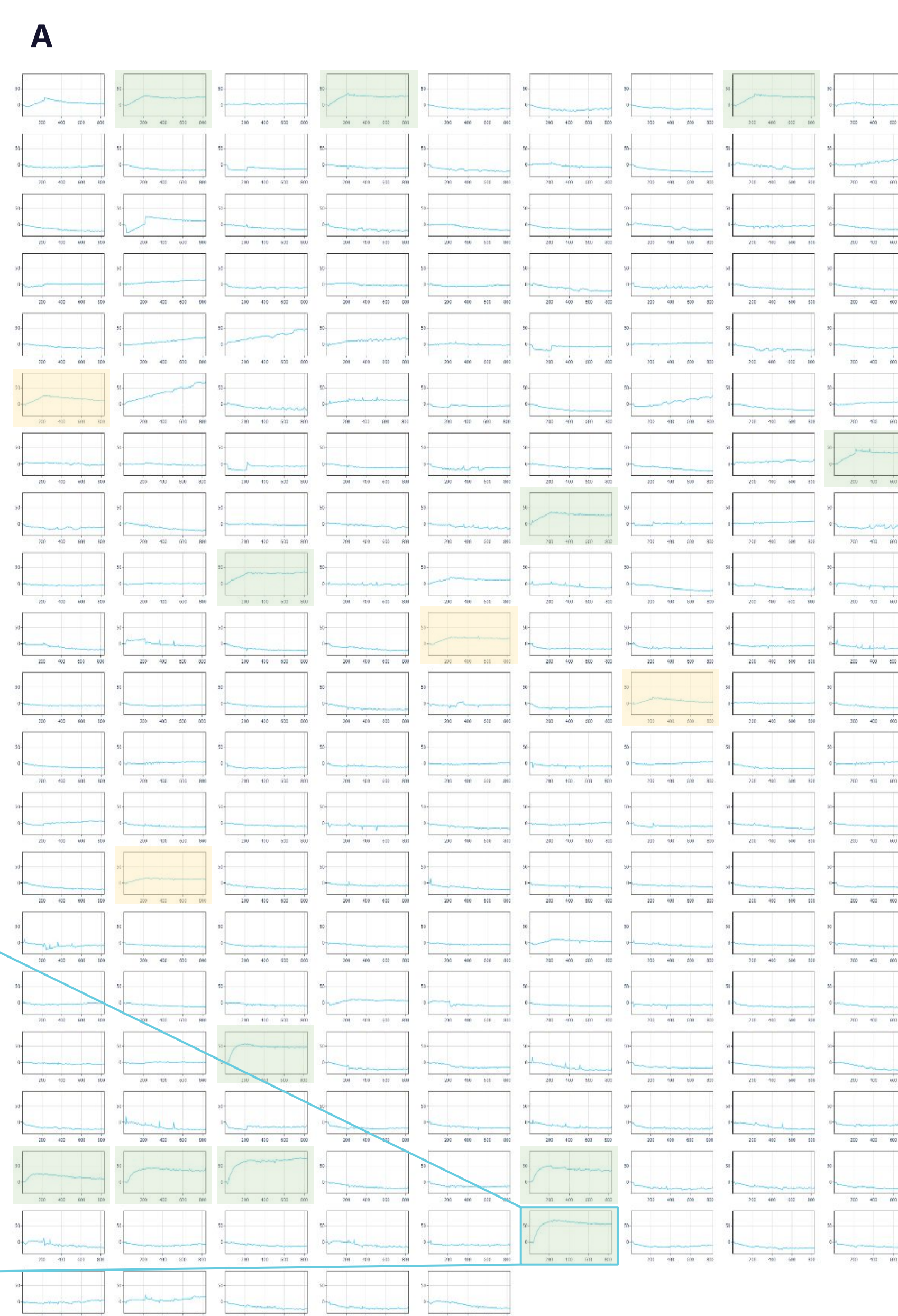


Figure 2: B) Sample sensorgram showing binding of the target to one of the captured mAbs showing a hit.

Figure 2: A) Grid view of all the binding curves measured between each antibody (ligand) and H5N1 HA. The 12 hits are highlighted in green, while the 4 intermediate hits are highlighted in yellow. Other interactions did not generate a sufficient binding response and are considered non-binders.



Conclusion

Alto's API and cartridge-based fluidic handling make it the first SPR instrument to be fully compatible with automation. This is demonstrated in this study where the Alto Automation Suite is used to rapidly screen hundreds of antibodies in crude sample.

In this study, 4 cartridges were run to screen over 180 antibodies in 32 hours. Each cartridge was prepared with the Opentrons Flex, and loaded into Alto with the Brooks PF400 robot arm, showing how the Alto Automation Suite™ takes the human labor and error out of affinity measurements.

The automated workflow demonstrated is a powerful addition to drug discovery labs, enabling screening of thousands of candidates per week with minimal hands-on time.



Learn how Alto can take your discoveries to the next level. Visit our website or contact us:

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