Inanovate, Inc.

Technology Review 2015

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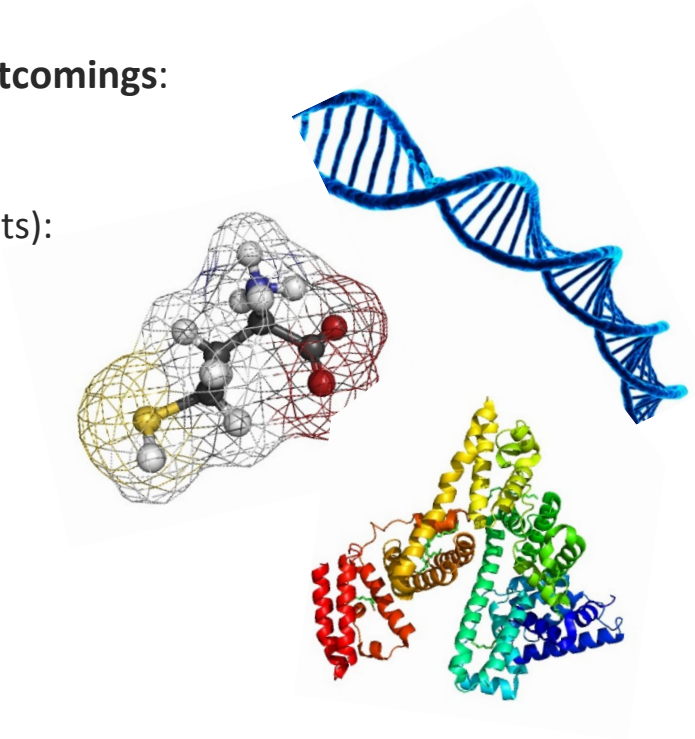
Advancing the science of biomarkers

- Detection and measurement of many different biomarkers (proteins and DNA) is critical for life science research and clinical diagnostics (biomarker market size was $20billion in 2014).
- Solution = Analyze multiple biomarkers in single test: **Biomarker Multiplexing**.
- But… Existing multiplexing technologies have several **shortcomings**:
  - Complex and costly devices
  - Limited range (target biomarkers split across several tests):
    - increases variability
    - Increases labor requirements
    - Increases sample use
    - Increases cost

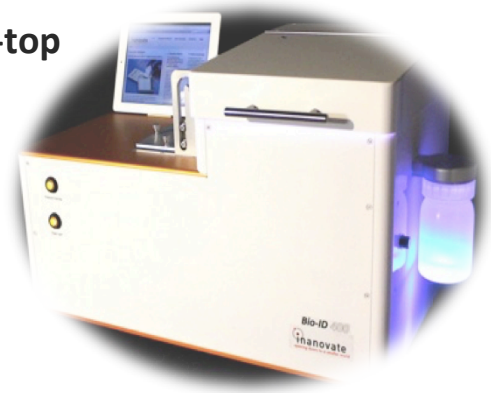


A better solution: The Bio-ID

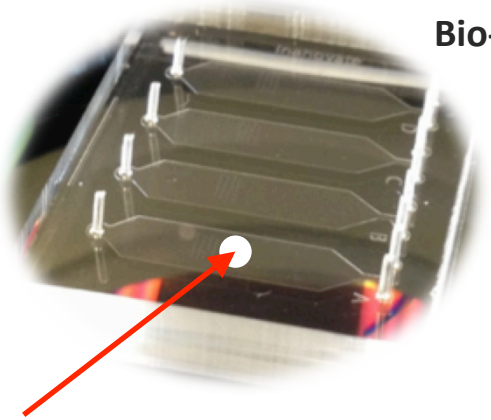
- Inanovate has developed a proprietary automated multiplexing platform: **The Bio-ID.**
- The Bio-ID can process virtually any mix of assays, in one multiplex, one run, and one dilution

= Better data. Less time. Less sample. Lower cost.

Bio-ID bench-top analyzer



Bio-ID disposable cartridge



Multiplex assay is contained in cartridge flow cell. Up to 300 assay spots may be printed per flow cell. Cartridge is inserted into analyzer to process assays.

The technology behind the Bio-ID

Longitudinal Assay Screening (LAS)

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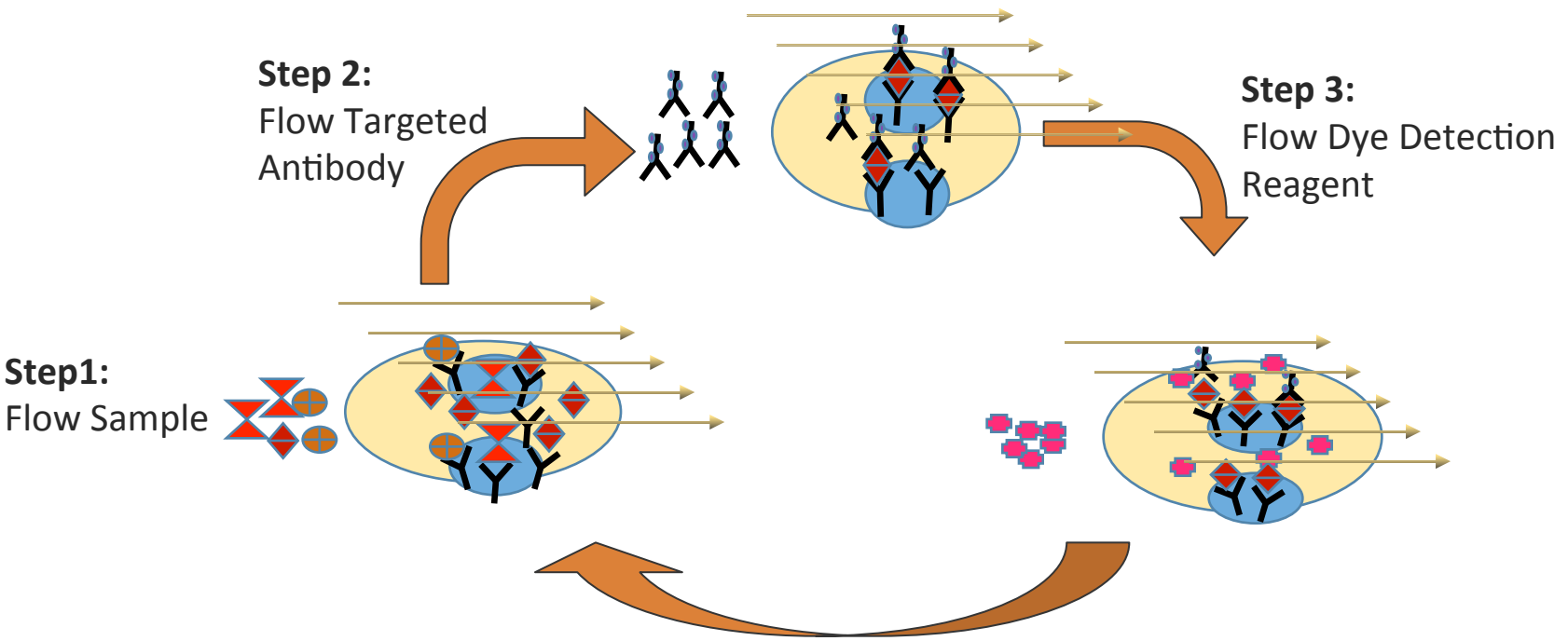
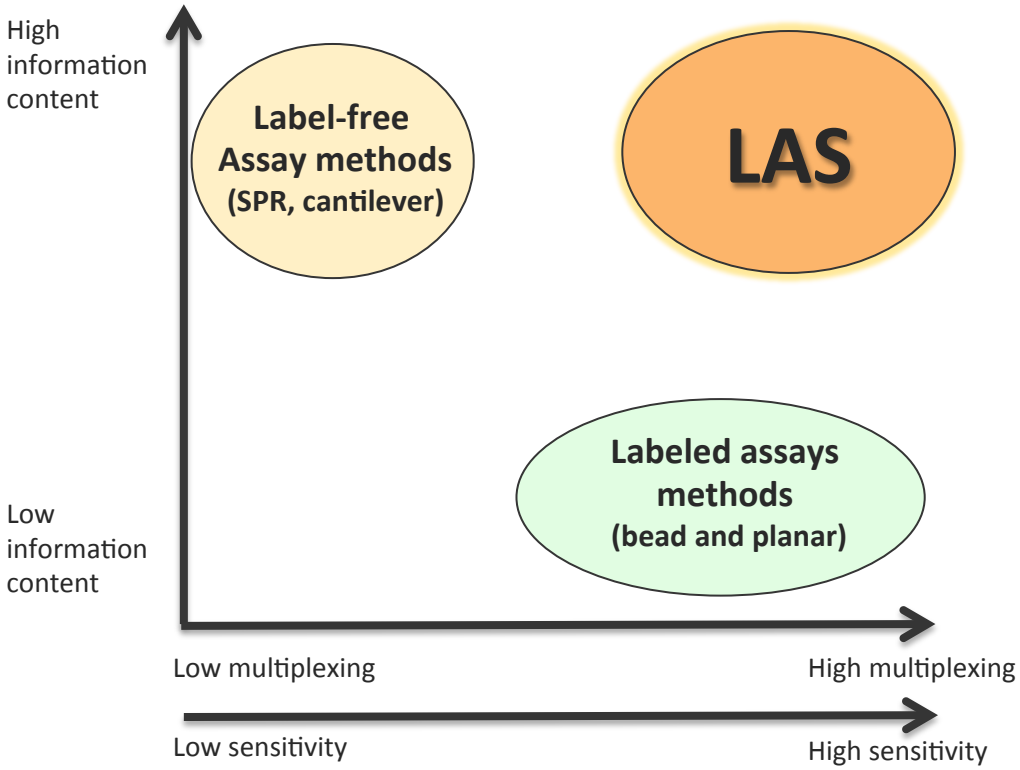
Longitudinal Assay Screening (LAS)

LAS is an assay method that performs multiple iterative 'mini-tests' in an automated micro-fluidic environment, generating information on each target biomarker throughout the duration of the assay: '**longitudinal data'**. This data adds an additional dimension to biomarker analysis.



Step 4: Detect fluorescence signal & **Repeat**

LAS: A new category of biomarker detection



LAS: Combining the best of the rest

- High multiplexing capacity
- High information content
- High sensitivity

LAS: Time-lapse photography for biomarkers

Existing multiplexing technologies such as bead or planar microarray approaches deliver users a single snap shot in time:

- Offers limited insights
- Can be highly variable
- Can miss important details
- Can lead to erroneous conclusions: is the man in the photo falling, tying his shoelace, or preparing to pitch?

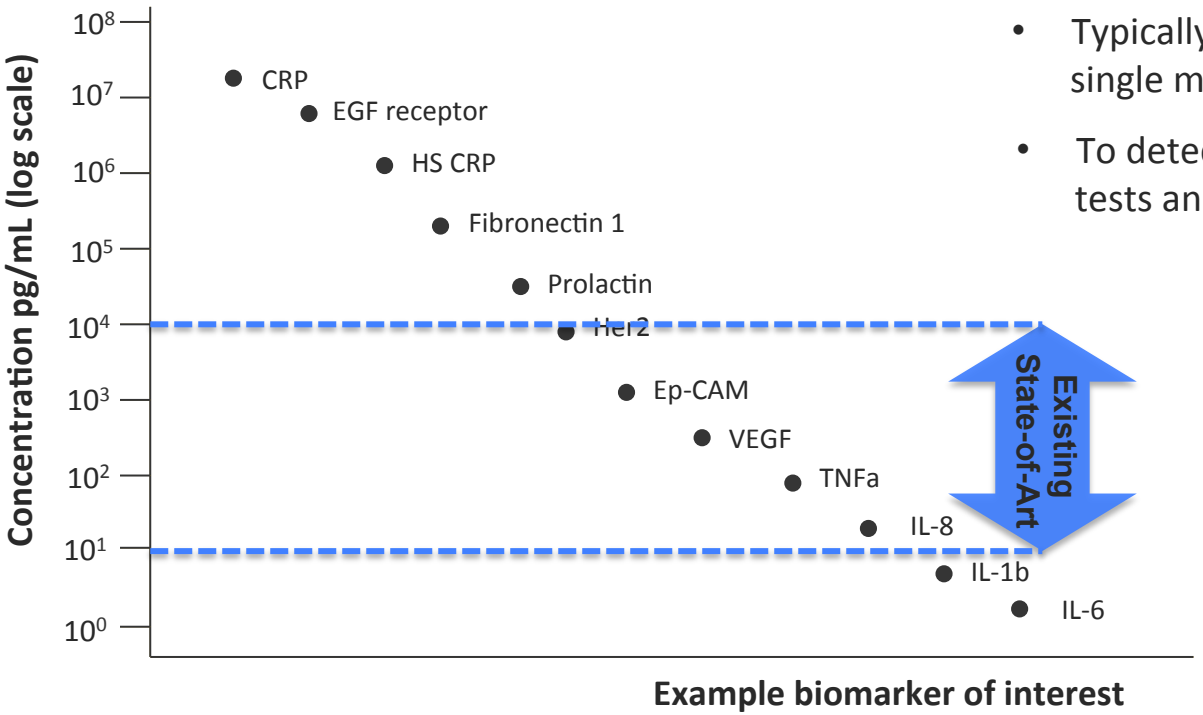
LAS

Inanovate's patented LAS method takes multiple images of biomarker interactions through time:

- LAS = time-lapse photography for biomarkers
- Offers a new dimension to biomarker analysis
- Increases depth and range of data
- More accurate and reliable insights & conclusions
- Better data. Less time. Less sample. Lower cost

The LAS difference

- Biomarkers can be present in patient samples at concentrations that vary by 8 logs (>10million fold).
- Biomarkers of the highest clinical interest are spread throughout this range (see below examples).



Existing multiplexing technologies:

- Typically measure across only 3 logs in a single multiplex test.
- To detect all biomarkers of interest, multiple tests and sample dilutions are run:
  - *Increases variability*
  - *Increases labor time*
  - *Increases sample use*
  - *Increases cost*

The LAS difference

- Biomarkers can be present in patient samples at concentrations that vary by 8 logs (10million fold).
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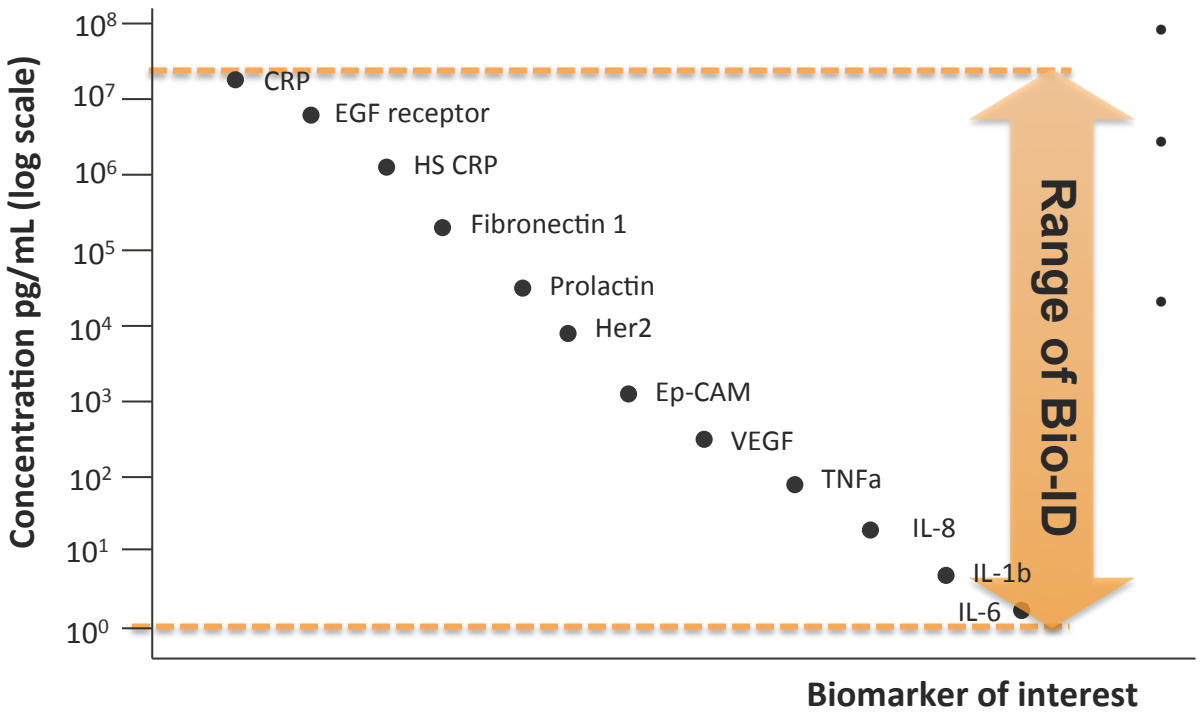

The Bio-ID

- Can measure across 7 logs in a single multiplex test.
- Can measure different types of biomarkers (antigens, antibodies, DNA etc..) in a single test.
- **Any assay, one multiplex, one run, one sample dilution**:
  - *Lowers variability*
  - *Improves work-flow*
  - *Lowers sample use*
  - *Lowers cost*

Summary advantages of LAS

- **Greatly expanded multiplexing range = Any assay, one multiplex, one run, one sample dilution**:
  - Lowers variability
  - Improves work-flow
  - Lowers sample use
  - Lowers cost

- **Time-course imaging of biomarker binding throughout a test = Improved data reliability and precision:**
  - Reduces cost, time and sample requirements for biomarker discovery
  - Improves accuracy of clinical tests

The Bio-ID platform

First platform to integrate LAS technology

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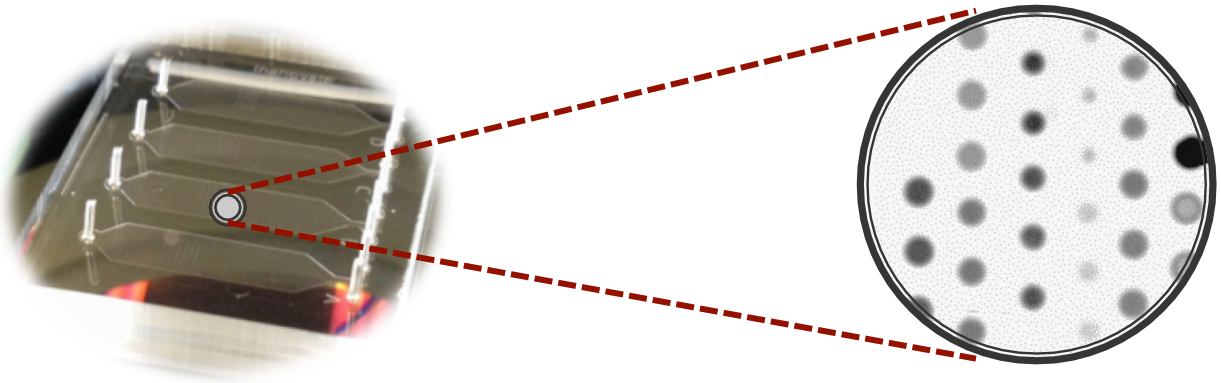
Key features and benefits

Planar, 2-D spot based, microarray format:

- High multiplexing capacity of up to 300 assays/spots per cell lowers cost per data-point and provides capacity for multiple intra-cell (intra-well) replicates and controls to improve data reliability.
- Glass surface with proprietary coating lowers printing variability and improves sensitivity.



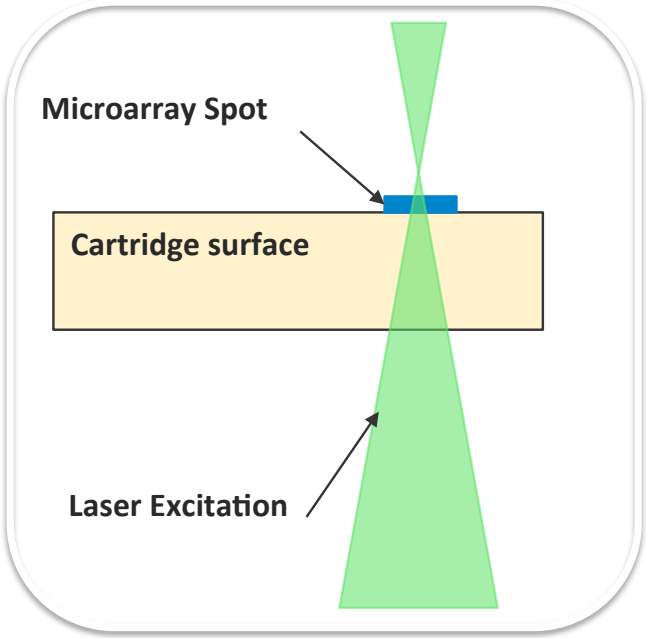
Planar array encased in low cost fluidic cartridge to provide closed processing environment:

- Enables precision fluid control and allows the iterative LAS method.
- Reduces sample and reagent volume requirements.
- Lowers per multiplex and per data-point costs.

Key features and benefits

Confocal laser based fluorescent detection:

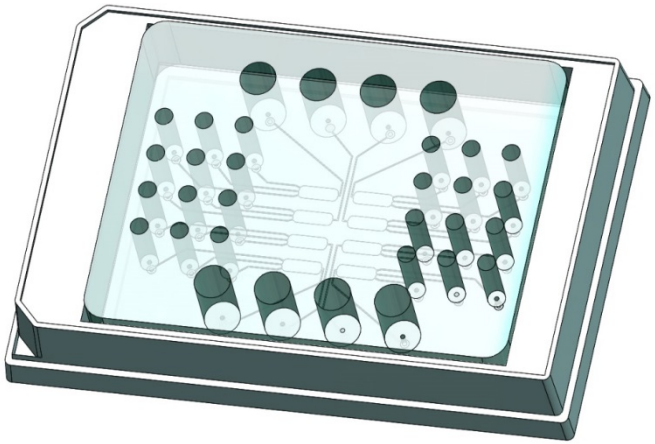
- **Flexible scanning**: Proprietary imaging methods help reduce variability from microarray printing procedures.
- **Automatic calibration**: Internal image standards allow instrument calibration before data collection, reducing run to run variability.
- **Confocal scanning**: Lowers background noise and improves sensitivity.
- **Environmentally robust**: Can operate under a variety of background light conditions without data compromise.

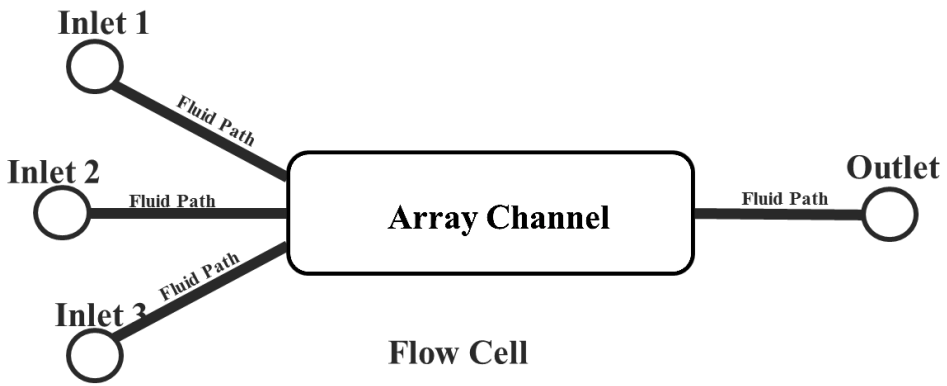


Key features and benefits

Precise, automated, dynamic fluidics control:

- Enables iterative LAS method.
- Limits operator error and improves data reliability.
- Enables users to process a variety of assay formats.
- Conforms to micro-titer plate format.
- Eliminates carryover and cross contamination between assays.



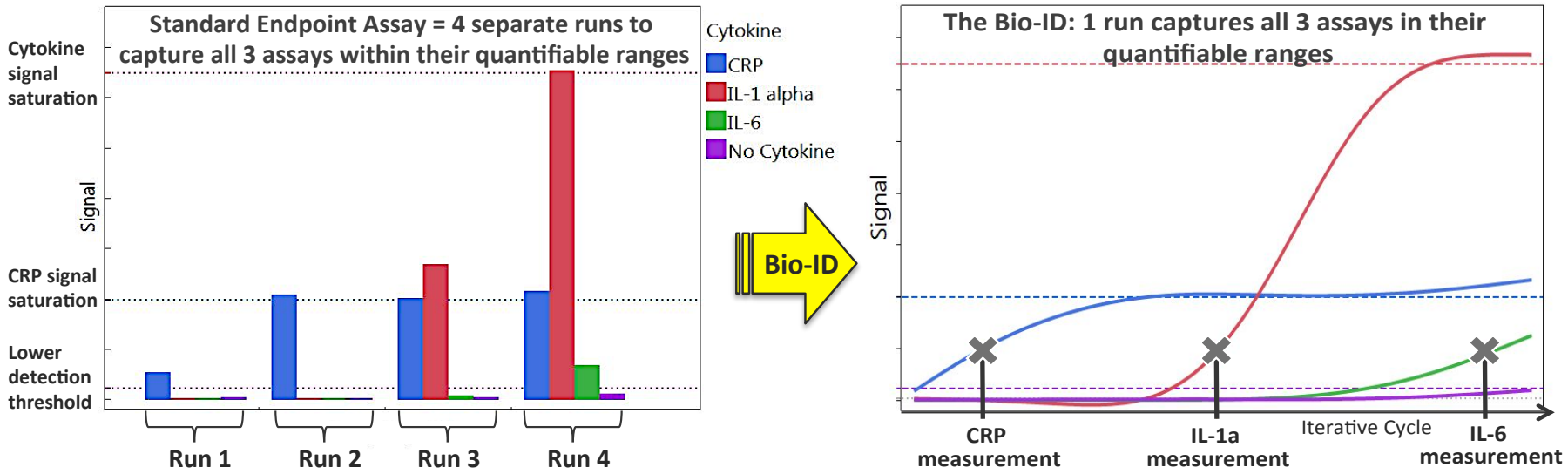


Flow Cell

Precise control of inlet pressure drives flow of each solution across the assay flow cell

Key features and benefits

Iterative, longitudinal data collection and processing:



Present state-of-art:

- High concentration assays reach saturation.
- Low concentration assays remain below detection limits.
- Limits multiplexing range to ~3logs.
- **One multiplex, 4 runs, 4 dilutions**

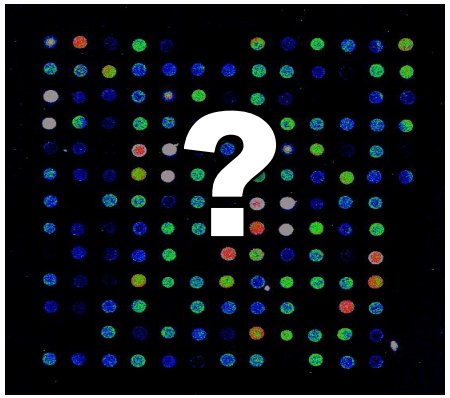
The Bio-ID:

- Collects and processes data throughout multiplex run.
- Each assay analyzed during optimal cycle for that assay.
- Extends multiplexing range to >6 logs.
- **One multiplex, one run, one dilution**

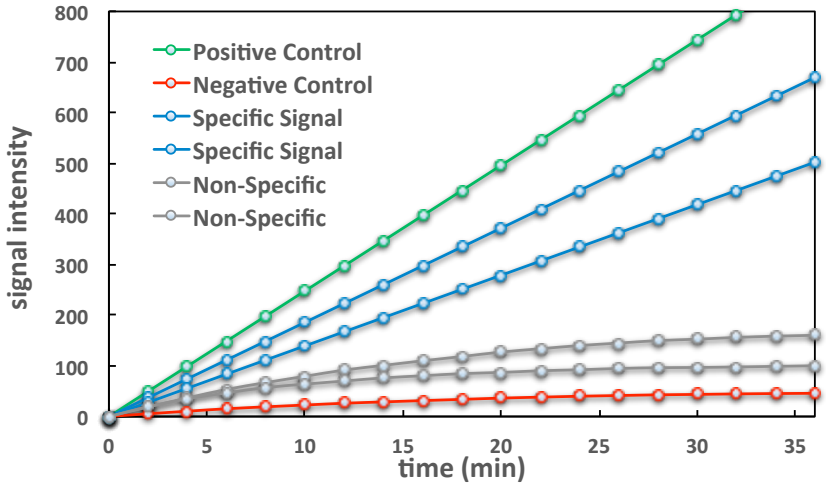
Key features and benefits

Iterative, longitudinal data collection and processing:

- Delivers high content data on target biomarkers.
- Enables trend analysis of low intensity signals, enhancing data reliability.
- Enables binding curve analysis and true/false signal discrimination for direct bind formats.



Left: Existing platforms rely on single signal intensity values. Non-specific or false signals increase the time and cost of biomarker programs, and reduce the accuracy of clinical tests.



Right: The Bio-ID generates iterative, longitudinal intensity profiles for each assay. Delivering high content data and an internal QC that improves data reliability. **Shortens biomarker discovery programs and improves reliability of diagnostic tests.**

Summary features and benefits

Assay Flexibility

- Precise & flexible automated image acquisition and fluidics enable the Bio-ID to run **multiplexes in multiple assay formats**:
  - ELISA formats: Direct, indirect, competition, EIA, reverse phase
  - Direct binding (Bmax and Kd determination)
  - Ligand competition assays

Extended Measurement Range

- Longitudinal data enables accurate quantitation across >6log range in a single multiplex. Enables development of clinically relevant multiplexes that can be run in a single sample dilution -- **any assay, one multiplex, one run, one dilution**.

Improved Data Quality

- High content, longitudinal data improves identification and discrimination of background and non-specific signals, resulting in **improved precision, accuracy, sensitivity and specificity**.

Case study data

The Bio-ID difference

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Case study 1: Multiplex, multi-modal ELISA

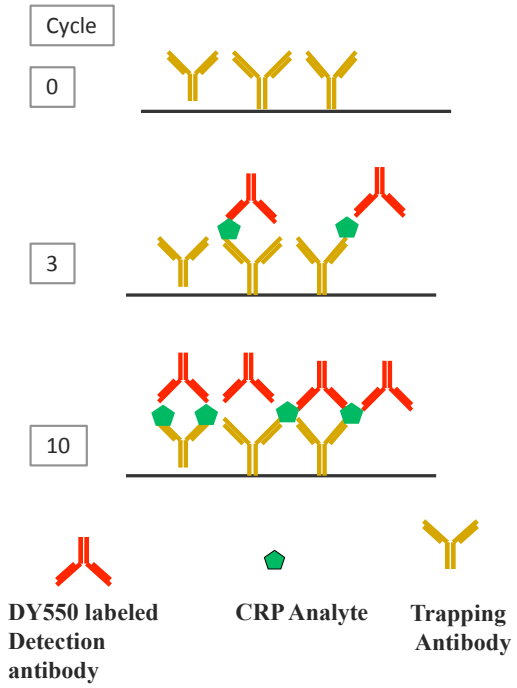
State-of-art for multiplexed ELISA assays:

- Assay specific sensitivity and dynamic range requirements lead to assays being split across multiple panels requiring multiple sample dilutions
- Background noise levels compromise sensitivity
- Analyte-dependent cross reactivity compromises data reliability

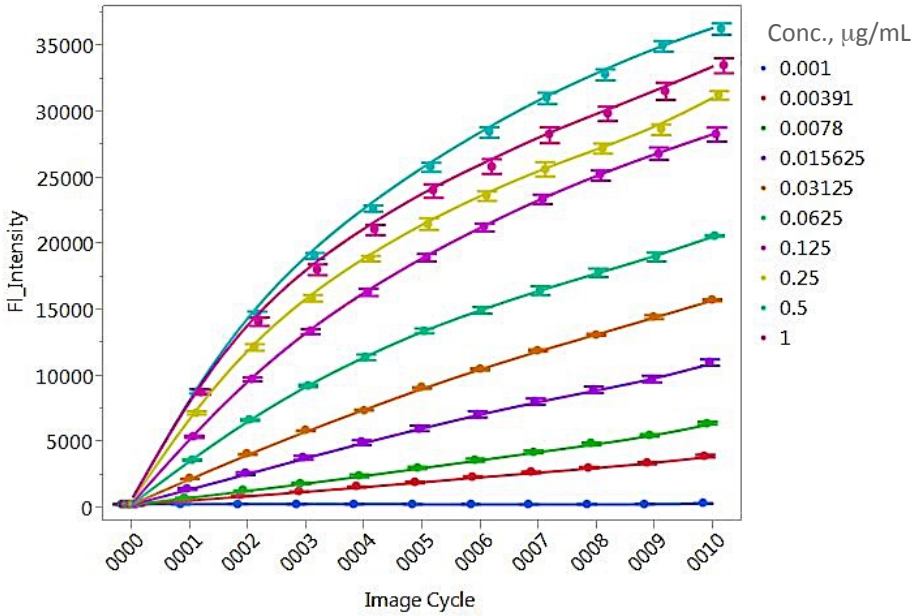
Multiplex ELISA assays on the Bio-ID:

- Assay specific sensitivity and dynamic range requirements can be satisfied through:
  - Longitudinal data acquisition and analysis
  - Dynamic fluidics control and image collection can process multiple assay formats in single multiplex test (**multi-modal**)
- Confocal imaging and cartridge surface chemistry ensures background noise level is very low – no need to block slides
- Segregated processing of different assay formats (in same run) can eliminate analyte dependent-cross reactivity

Direct ELISA format

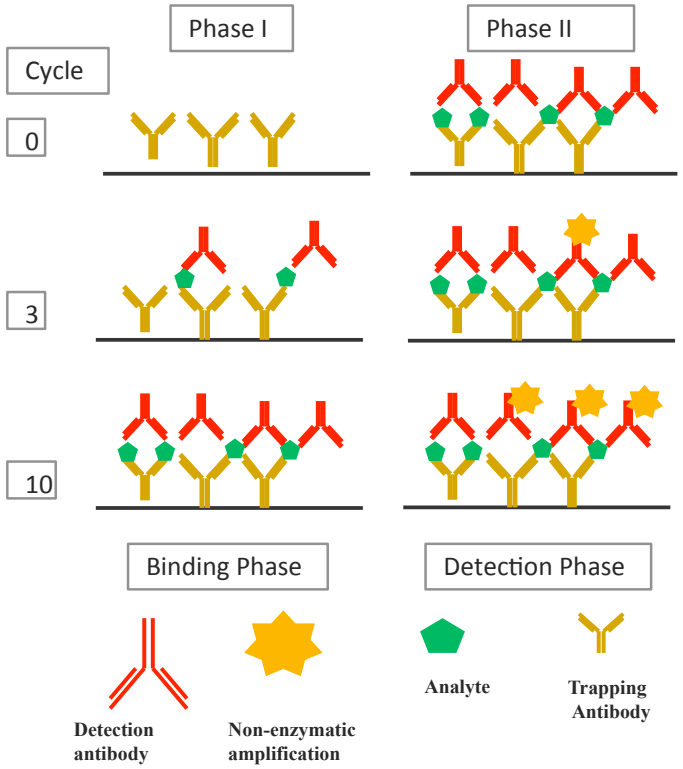


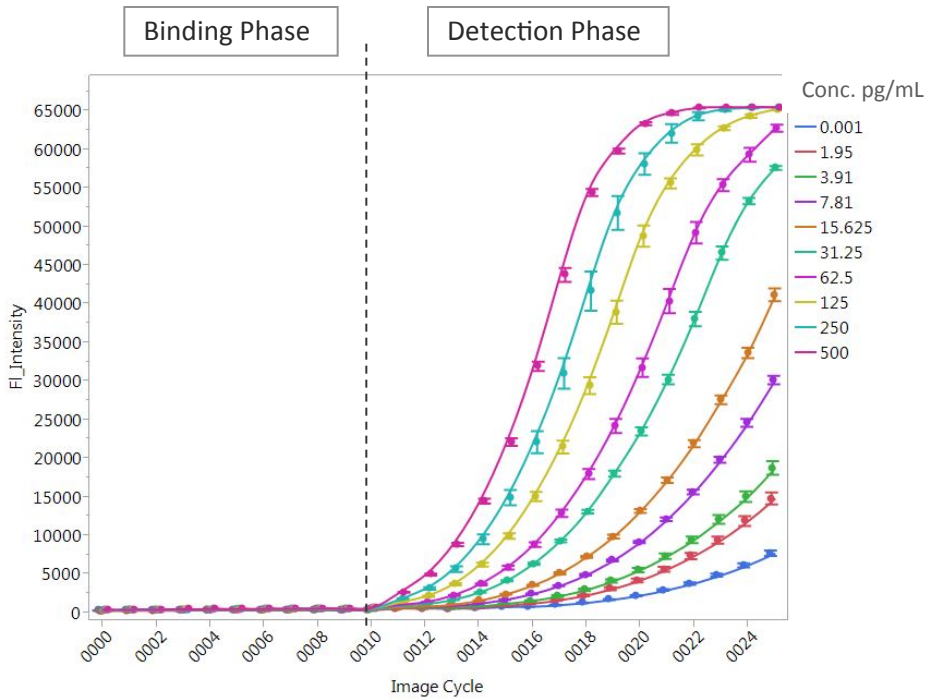
CRP – Direct detection with anti-human CRP-Dy550 Ab. Signal increases with cycle and concentration.



- Direct ELISA format optimal for high concentration analytes/biomarkers (> 1ng/mL)
- Dynamic range for CRP demonstration assay: 1 ng/mL – 1 mg/mL
- Rapid cycling and image capture process enables data acquisition in <20 mins machine time

Indirect ELISA format



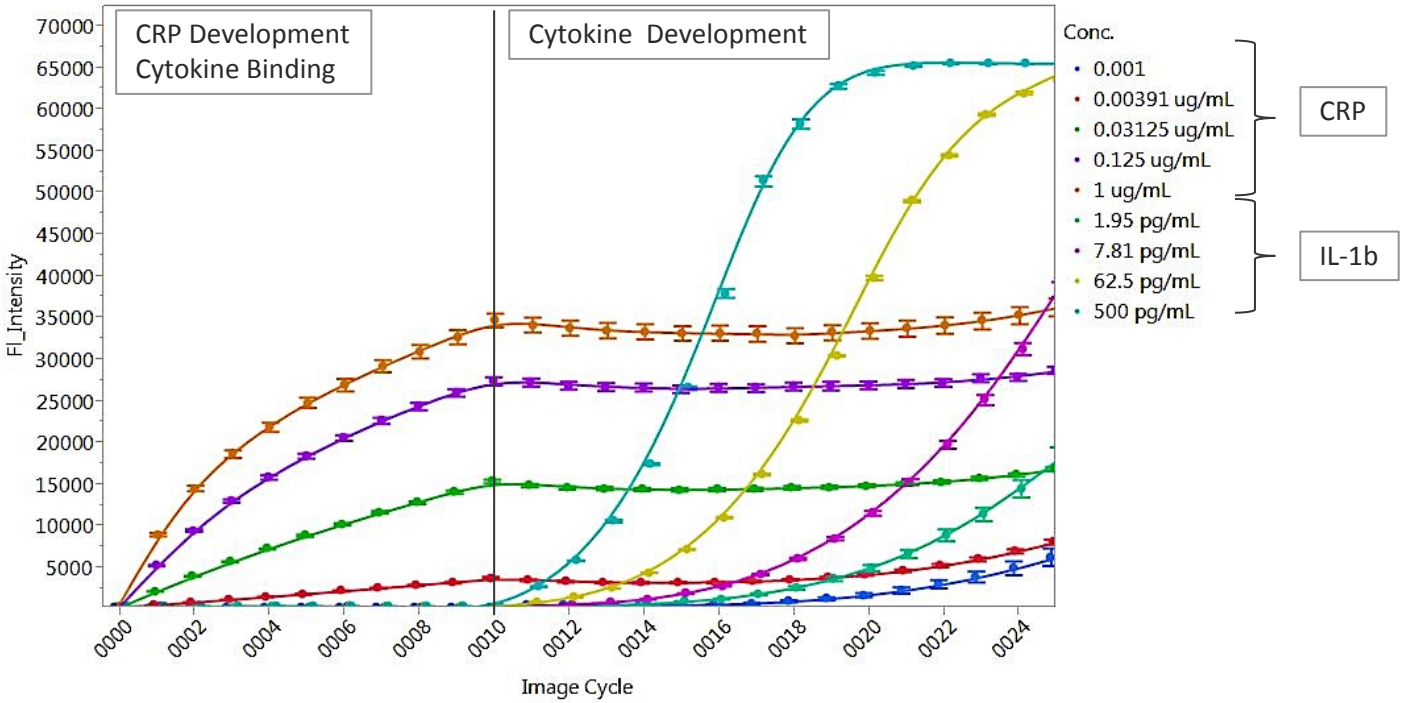


Cytokine detection: Signal increase with cycle and concentration.

- Indirect ELISA format optimal for low concentration analytes (< 1ng/mL)
- Dynamic range of demonstration assays: 1pg/mL to 1 ng/mL
- Rapid cycling and image capture enables data acquisition times equivalent to standard ELISA.

Multiplex, multi-modal ELISA: Putting it all together

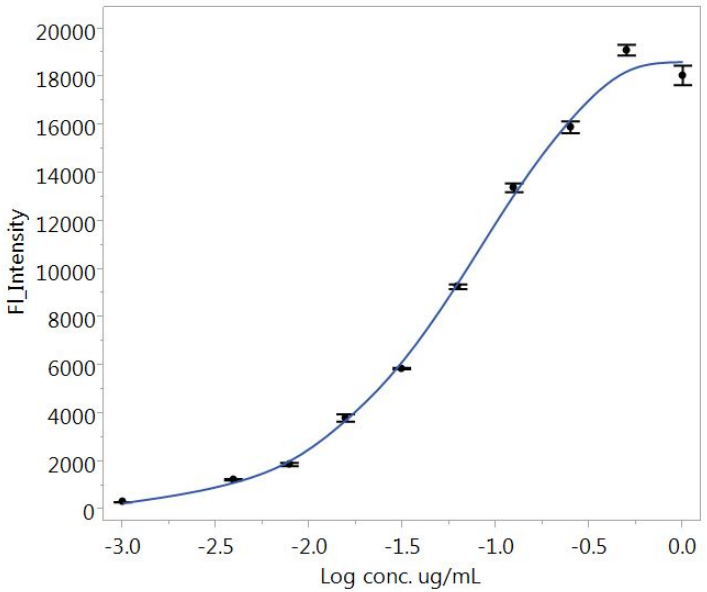
A 4-plex of CRP, IL-6, IL-1α, and IL-1β demonstrates the performance capabilities and advantages of the Bio-ID across different ELISA assay formats in a single multiplex



- CRP and cytokines are detected from the same sample but in different stages of the assay.
- **The extended multiplexing range (inter-assay dynamic range) in this example is 6 logs - 1,000 fold greater than existing state-of-the-art**.

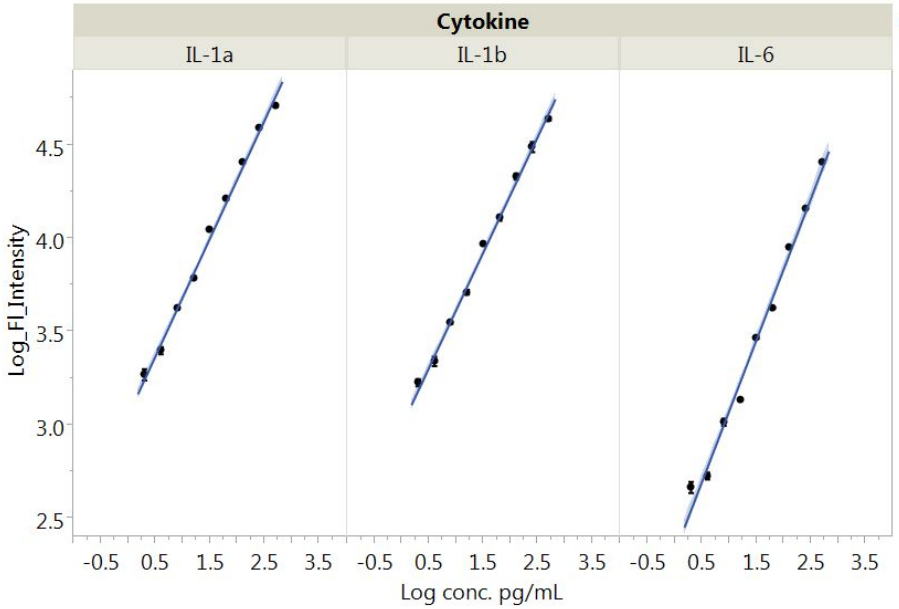
Multiplex, multi-modal ELISA: Precision, sensitivity & variability

CRP Direct detect ELISA



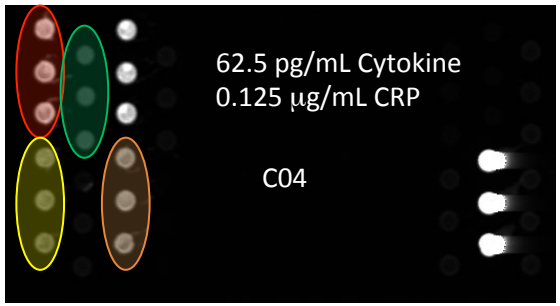
HLOQ: 1 ug/mL
LLOQ: 0.003 ug/mL
CV at LLOQ: <10%

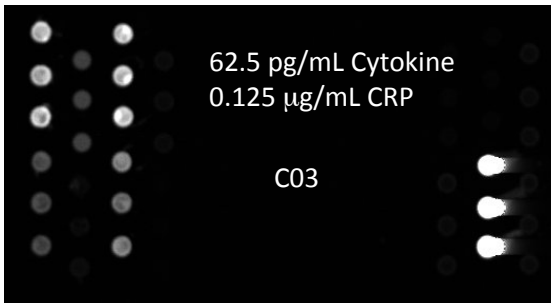
Cytokine Indirect ELISA

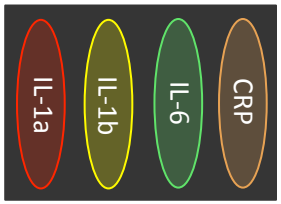


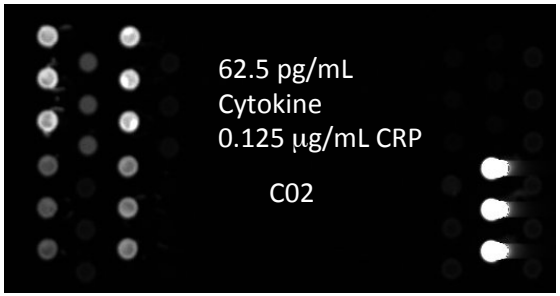
HLOQ: 1,000 pg/mL
LLOQ: 1 – 3 pg/mL
CV at LLOQ: <10%

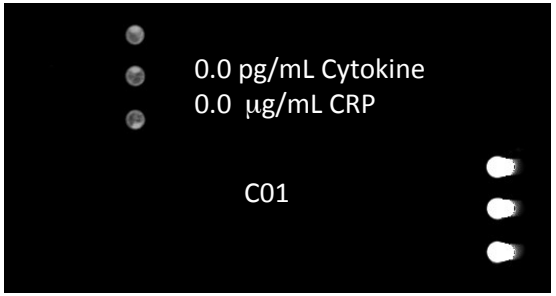
Multiplex, multi-modal ELISA: Variability data











	CV %		
Cytokine	**Intra-Cell**	**Inter-Cell**	**Inter-Chip**
CRP	< 5	6.8	<20
IL-1a	< 5	5.8	<20
IL-1b	5 – 8	11.1	<20
IL-6	< 2	2.5	<20

Multiplex, multi-modal ELISA: Performance summary and conclusions

Assay	LLOQ	CV (at LLOQ)	HLOQ	CV (at HLOQ)
CRP	<3ng/mL	8%	1,000ng/mL	6%
IL-1a	<3.8pg/mL	6%	1,000pg/mL	5%
IL-1b	<3.8pg/mL	8%	1,000pg/mL	8%
IL-6	3.8pg/mL	5%	1,000pg/mL	2%

- LLOQ and HLOQ are as **measured from assay runs, they are not extrapolated figures**.
- Wide inter-assay multiplexing range: No need for serial dilutions, saves money, time & sample.
- Multiple assay formats in one multiplex: Multiplexes with true clinical utility in one low cost test.
- Assays can be tuned for sensitivity based on time-course curve analysis: Improves sensitivity.
- Trend analysis increases reliability of low intensity signals, improving sensitivity.

Any assay, one multiplex, one run, one dilution

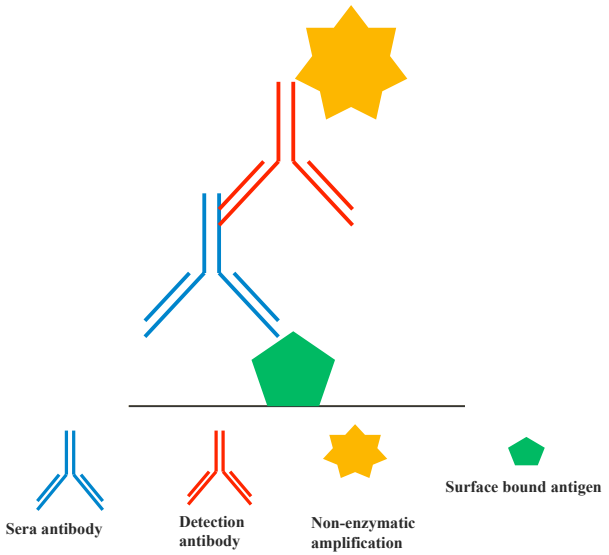
Case study 2: Multiplex serology assays

State-of-art for multiplexed serology assays:

- Background noise levels compromise sensitivity.
- Non-specific assay signals compromise data reliability.
- Dilution linearity and specificity are key performance metrics.

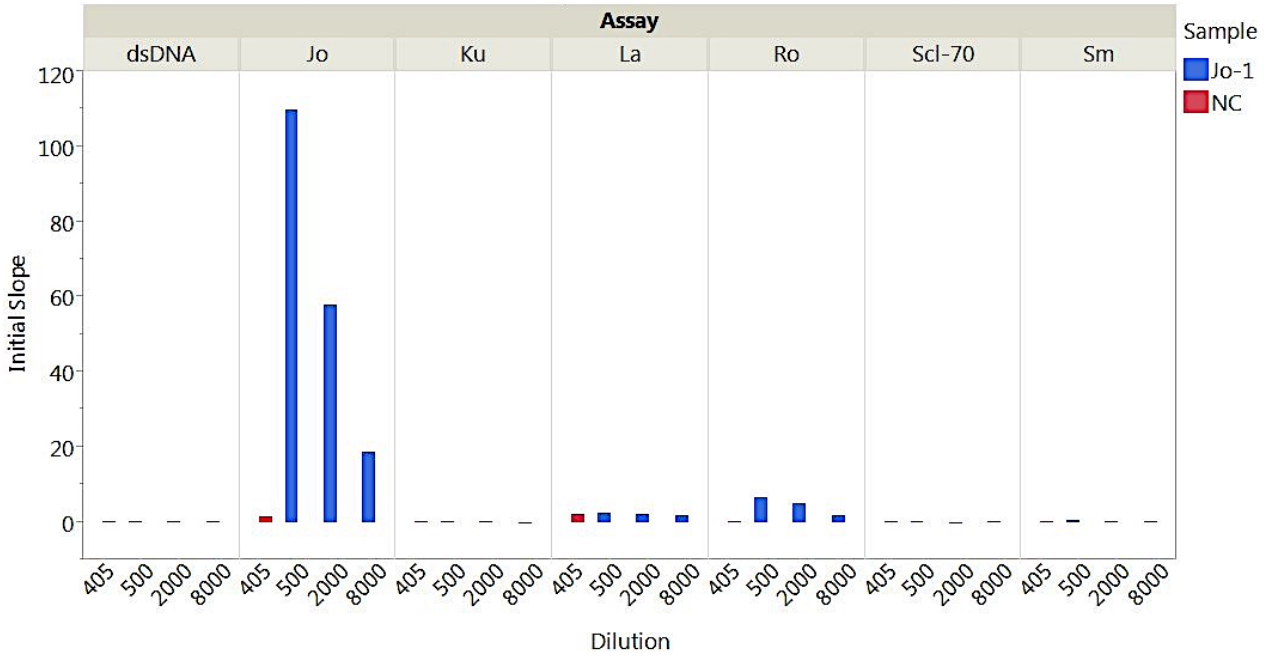
Multiplex serology assays on the Bio-ID:

- Direct ELISA format is optimal for use with serology assays.
- Low background enhances sensitivity.
- Time-course assay binding profiles can be used to identify non-specific signals:
  - Enhances data reliability.
  - Reduces cost and time of discovery programs and can improve accuracy of clinical tests.



Multiplex serology assay data: Specificity & dilution linearity

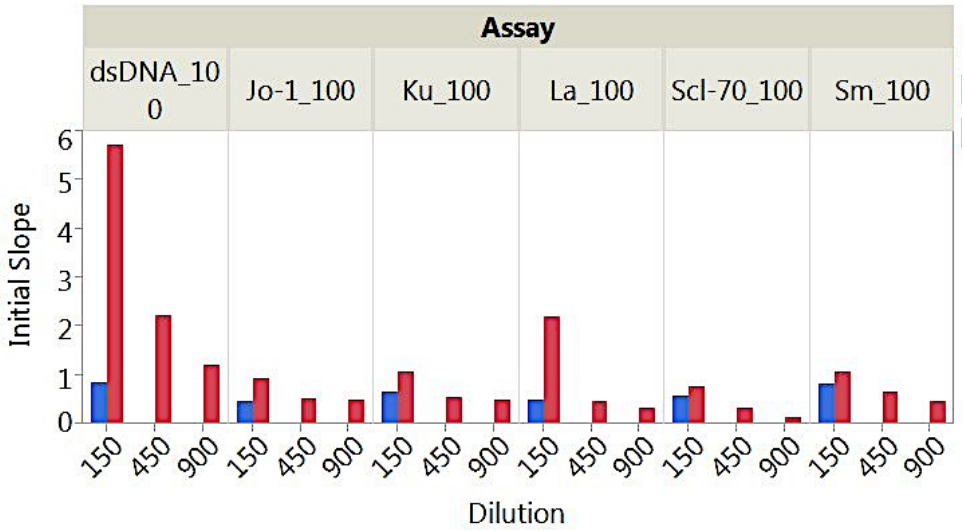
A 7-plex of Jo-1, Ku, SS-A (La), Ro, Scl-70, Sm and ds-DNA was developed and processed on the Bio-ID to demonstrate the performance capabilities and advantages across a serology multiplex.

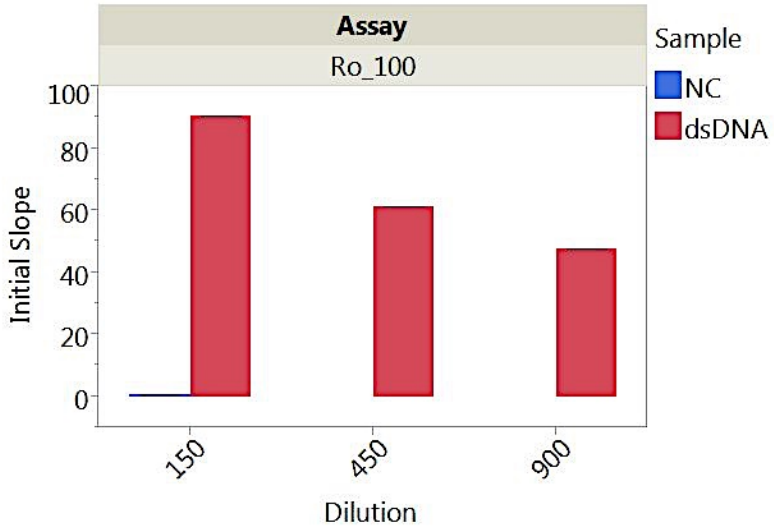


- AMLI Consensus Reference Panel: Reference Sample 7. The sample shown in the above figure contains Jo-1 at high concentration, and Ro at low concentration.
- High Specificity: The Bio-ID accurately identifies these autoantibody populations in human sera.
- High Sensitivity: Dilution linearity demonstrated.

Multiplex serology assay data: Specificity & dilution linearity

- AMLI Consensus Reference Panel: Reference Sample 10. Contains high levels of dsDNA, very high Ro, and low/medium La.





- Within the test sera, the autoantibodies were accurately identified while demonstrating dilution linearity.
- Further demonstrates the flexibility of the Bio-ID to precisely and efficiently run multiple assay formats.
- **Non-specific binding can be identified when assay is run in direct label mode,** shortening biomarker discovery and validation programs and improving reliability of diagnostic tests.

Case study 3: Direct bind assays

State-of-art for direct bind assays:

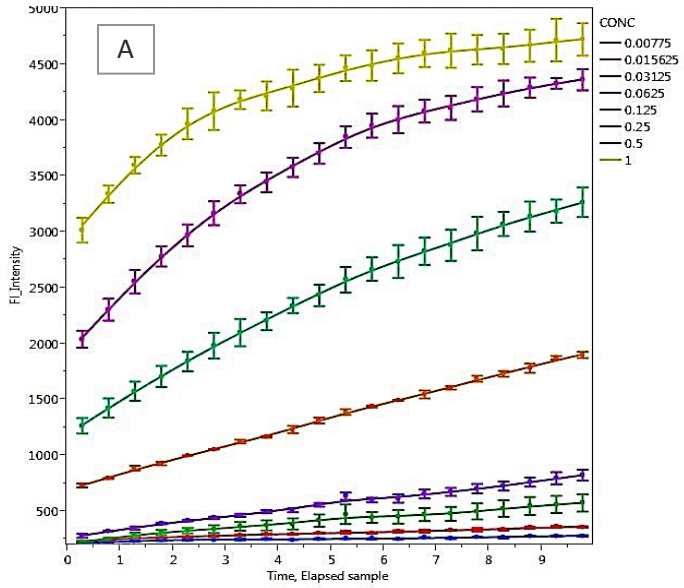
- Currently, multiplexed direct binding assays are largely SPR based.
- Both cost and sensitivity drawbacks.
- Level of multiplexing is low.

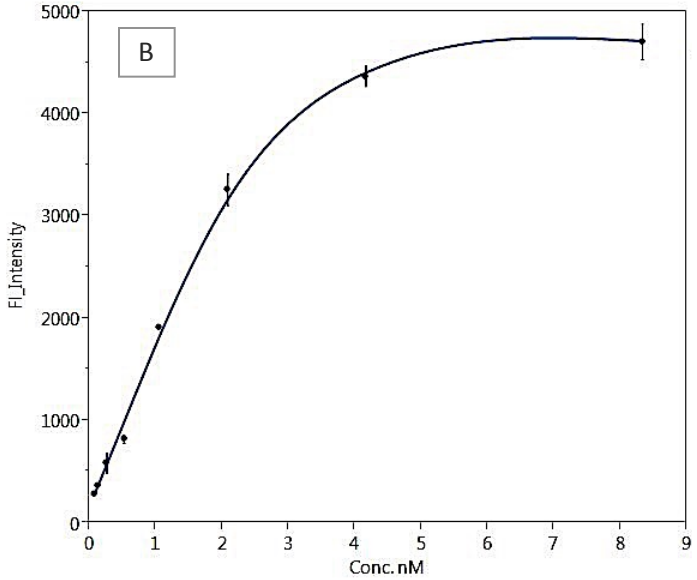
Direct bind assays on the Bio-ID:

- Assays can be discreet or can be continuous flow, supported by proprietary image capture methods.
- High multiplexing capacity of planar array formats.
- Bio-ID analyzer and cartridge substantially lower in cost than typical SPR devices.
- High sensitivity detection enables very fast assay processing (**<10mins**).
- Time-course assay binding profiles can be used to identify non-specific signals, improving data reliability.

Direct bind assay data: CRP binding to antibody target

Labeled CRP is flowed through the Bio-ID cartridge cells. At 30 second intervals bound ligand is detected using a proprietary imaging process, and the amount of bound CRP is measured.





Panel A represents a time course of binding at different concentrations of labeled CRP (mg/mL). Panel B represents a binding isotherm **measured at <u>5 minutes</u>.** The estimated Kd is 1.7 nM.

Case study 4: Competition assays

Current state-of-art for competition assays:

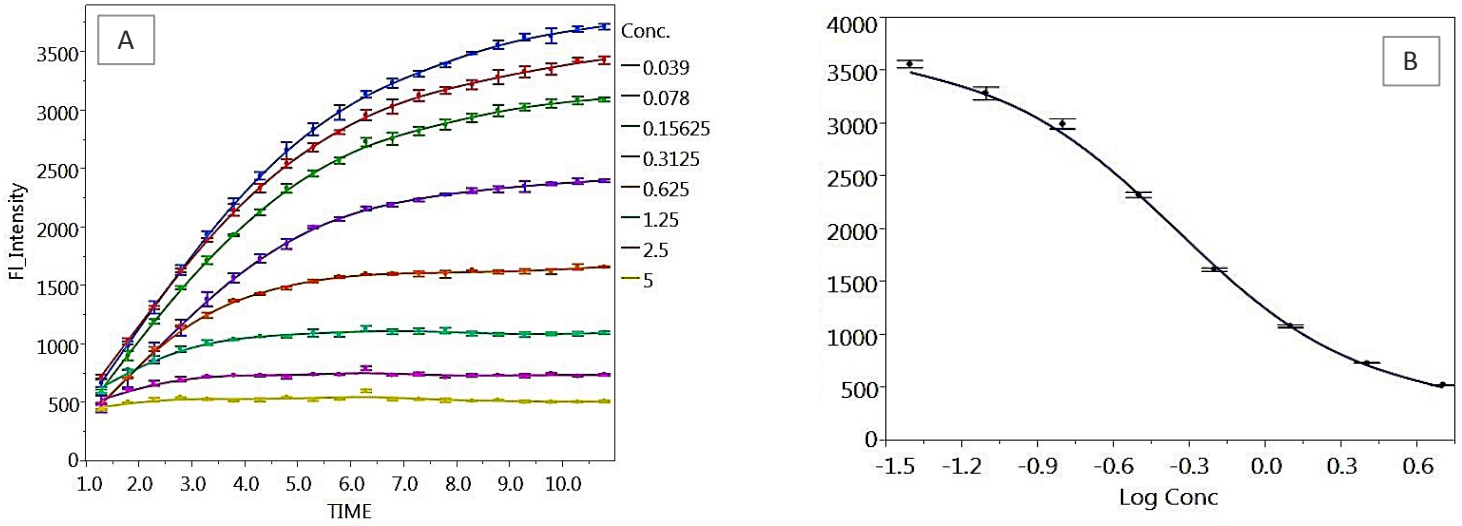
- Competition EIA assays are frequently used for detection of hormones and other metabolites, examples include:
  - Endocrine markers.
  - Eicosanoids, prostanoids and metabolites of arachadonic acid.
  - Fungal metabolites important in the food and food processing industry (e.g. alfatoxins).

Competition assays on the Bio-ID:

- The Bio-ID enables the development of multiplex competition assays for small molecules, metabolites, and hormones that are commonly determined using single-plex technology – lower cost per data-point.
- The Bio-ID's ability to directly monitor analyte/biomarker binding allows the development of **high precision, high sensitivity, multiplexed competition assays that can be processed in <10mins**.

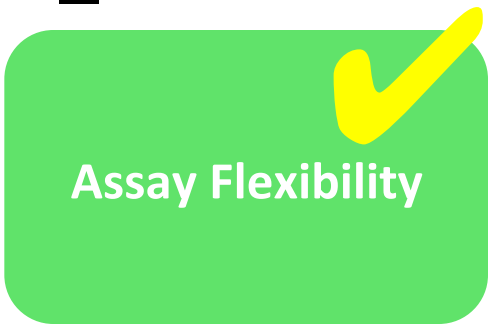
Competition assay data: CRP competition immunoassay

- Controlled fluidics, coupled to rapid and precise imaging allow for sensitive and specific assay development.
- Anticipated that most competition assays currently run on RIA or EIA can be placed on Bio-ID for precise, rapid, multiplexed processing.



Above figures: Competition assay using 4 nM DY550 labeled CRP and unlabeled CRP monitored in real time. Panel (A): Progress curves of CRP binding to antibody targets. Panel (B): Plot of Bmax in the presence of unlabeled CRP at **8 minutes.**

Summary features and benefits



- Precise & flexible automated image acquisition and fluidics enable the Bio-ID to run **multiplexes in multiple assay formats**:
  - ELISA formats: Direct, indirect, competition, EIA, reverse phase
  - Direct binding (Bmax and Kd determination)
  - Ligand competition assays

- Longitudinal data enables accurate quantitation across >6log range in a single multiplex. Enables development of clinically relevant multiplexes that can be run in a single sample dilution -- **any assay, one multiplex, one run, one dilution**.

- High content, longitudinal data improves identification and discrimination of background and non-specific signals, resulting in **improved precision, accuracy, sensitivity and specificity**.

Intellectual Property

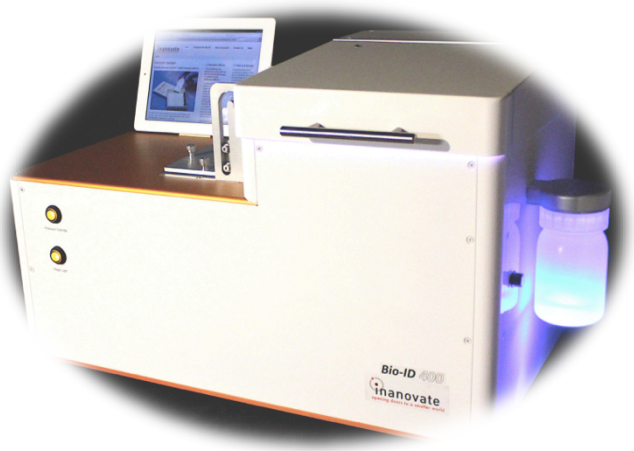
Inanovte's patent portfolio includes six patent families covering LAS and the Bio-ID platform. Each patent family is at varying stages of prosecution from issued patent through PCT applications. To stick with the prior analogy: '**Inanovate owns the IP landscape on 'time-lapse photography for biomarker detection'**.

Patent Title and Number	Status
Nano-Particle Biochip Substrates (US 11/729,510)	**Issued May '12**
Longitudinal Assay/An Effective Real-time Assay Platform (US 12/956,117)	**Issued Jan '15**
Capacitive Pumping & Flow Control (US 13/989,642)	Filed April '13
Method, System and Device for Analyte Detection and Measurement Using LAS (PCT/US14/24396)	Filed March '14
Cartridge Device for Processing Time-Resolved Assays (PCT/US14/24429)	Filed March '14
Analysis Methods of Time-Resolved Assay Data (PCT/US14/24415)	Filed March '14

Inanovate's IP council include Wolfe Greenfield & Sacks, and Hoffman Warnick

The Bio-ID

Any assay, one multiplex, one run, one dilution.



inanovate
Opening doors to a smaller world

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Exhibits: Application examples

Translating benefits into value

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Example 1: Biomarker discovery & validation

- Screening large panels of biomarkers in a reliable, time and cost efficient manner is the goal of researchers studying new biomarkers for diagnostic and/or therapeutic use.
- Current technologies:
  - Restrict users to screening a limited selection of biomarkers in any one multiplex - every assay in the multiplex has to conform to one assay format.
  - Limited multiplexing range restricts users to screening biomarkers present in samples at similar concentrations (~3 logs). Leads to requirement to run multiple sample dilutions – **adds time, cost and uses more sample**.
  - Limitations in multiplexing capacity leads to requirement for multiple sample runs to provide replicate data – **adds time, cost and uses more sample.**
- Bio-ID eliminates these restrictions and enables multiplex assays that directly address the biological and clinical needs of the researcher in a single panel that can be processed in a single run and single sample dilution.
- **Any assay, one multiplex, one run, one dilution.**

Example 1: Biomarker discovery & validation

Example: Inanovate inflammatory biomarker panel

	Existing competition*** (bead arrays)	Bio-ID***
Product format	3 x 96 well plates covering 60-biomarkers	1 x 8 channel cartridge with all 60-biomarkers per channel
Product price	$8,500	$1,200
# replicate runs required	2	1
# dilutions required	3	1
Sample volume requirement (µL)*	**150**	**10**
Cost per **sample data-point****	**$10**	**$2.50**

*Sample volume figures are representative of the typical volume of 'neat' sample required to screen across all 60 biomarkers.

'Sample data-point cost**' = cost of reliable data for each biomarker per sample. Accounts for replicate & serial dilution requirements.

***Estimated costs based on available commercial pricing information and internal cost analysis.

Impact for customer running 400 patient study:

Estimated cost saving = ~$175,000. Estimated sample saving = 10X. Estimtated labor saving = 5X.

Better data. Less time. Less sample. Lower cost.

Example 2: Multiplex diagnostic assays

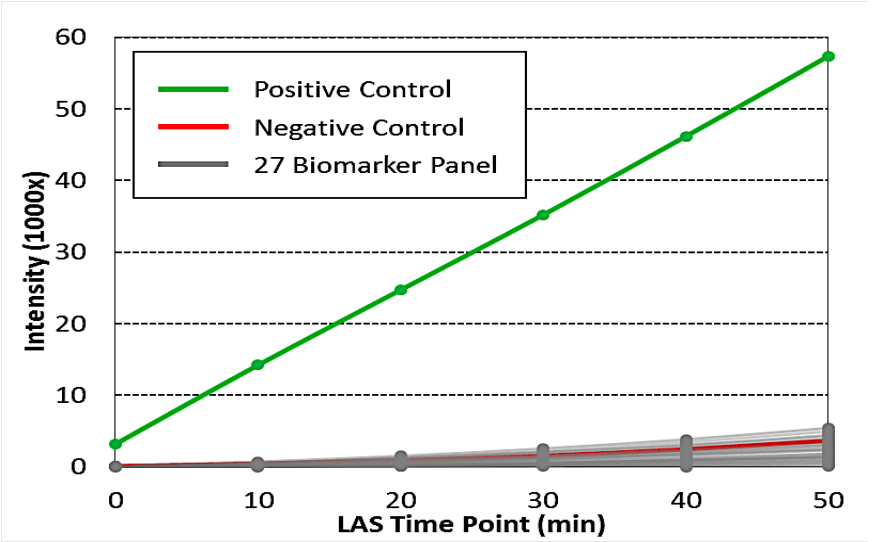
- Crescendo Biosciences, Vectra DA assay for evaluating rheumatoid arthritis patients.
- Current test encompasses 12 analytes arrayed on 3 separate plates (**3 tests requiring 3 runs**).
- The mixture of analytes is spread over discrete concentration ranges from ug/mL (CRP, SAA, VCAM); ng/mL (MMP-1 and MMP-3) to pg/mL (IL-6)
- The Bio-ID's extended multiplexing range and multi-modal assay design flexibility would enable all 12 markers to be analyzed in a single low cost, reliable test (**1 test, requiring 1 run**).
- **More reliable data. Less time. Less sample. Lower cost.**

Example 3: Serology discovery assays

- Auto-antibody biomarker discovery requires screening a broad range of un-validated serology assays.
- The Bio-ID can process all assays in a single multiplex, and use binding profile analysis to identify non-specific signals and eliminate from further analysis - addressing a major problem in the field.
- Shortens biomarker discovery programs and improves both accuracy and time to market for clinical tests.
- Forensic application opportunities: Identification and/or provenance of samples for drug testing.

Right: Example LAS data from a prostate cancer biomarker discovery program:

- End-point analysis across 160 patient samples & controls indicated 27 autoantibodies may discriminate prostate cancer from BPH.
- Multiple out-of-set sample runs on end point platform ultimately showed all markers to all be non-specific and clinically useless.
- LAS data showed all assay signals were result of non-specific binding with single sample runs (right figure).
- LAS's added dimension of biomarker data enables significant savings in time, money and sample.

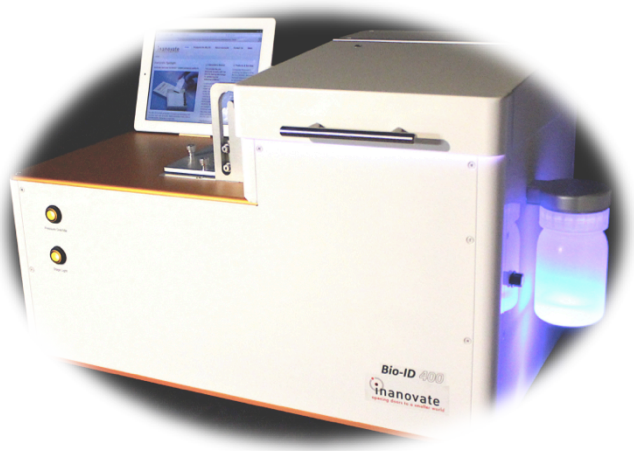


Example 4: Direct bind & competition assays

- Screening drug candidates against drug targets:
  - Screening of kinase inhibitors to establish activity profiles is presently achieved using single-plex activity assays or by TR-FRET.
  - Given the flexibility and multiplexing capacity of the Bio-ID, In principle the entire kinome can be reliably screened in a single run, on one low cost Bio-ID cartridge, a potentially disruptive technology application.
- Detection of multiple metabolites and competition EIA formats:
  - Rapid, reliable, low cost detection and analysis of multiple metabolites of interest that could displace HPLC or LC-MS.
  - Detection of arachadonic acid metabolites along with inflammatory cytokines.

The Bio-ID

Any assay, one multiplex, one run, one dilution.



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