Microfluidic Measurements of Protein Biomarkers from Exhaled Breath

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BEAD-BASED ASSAYS

- Large surface area for capturing analytes from biological samples
  - >10^4 beads per sample
  - Amenable to protein or nucleic acid analytes
  - High capture efficiency
- Magnetic core of beads facilitates sample preparation
  - Beads can be controlled with a magnetic field
    - Easy to translate or capture with a simple magnet
- Low sample volume requirement (≤ 100 μL)
  - Amenable for point-of-care analyses

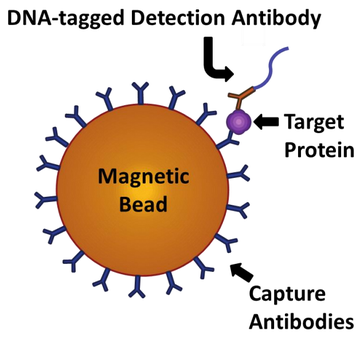

Figure 1. Illustration of an immunoPCR complex.

MICROWELL ARRAYS

- Compatible with bead-based assays
- Highly sensitive amplification-based detection
  - PCR and ELISA formats
- Enables single molecule detection
  - One bound target produces a locally high dye concentration (~1 μM) for facile fluorescence detection
- Digital detection mode provides LODs up to 1,000x superior to conventional bioassays
- Quantitation achieved by determining the percentage of active wells in the array
  - Correlates to the analyte concentration in the sample
    - Poisson statistics

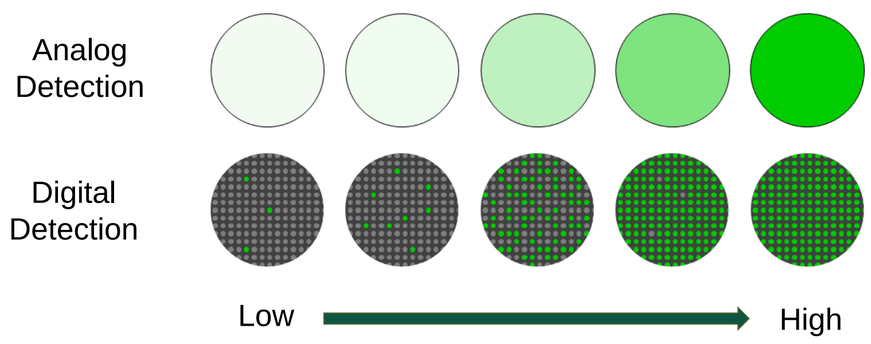

Figure 2. Illustration comparing a simulated fluorescent signal observed as a function of analyte concentration. Analog detection (top) yields a continuum response whereas digital detection (bottom) produces increasing numbers of individual "active" wells. Adapted from *Anal. Chem.* **2013**, *85*, 1258-1263.

MICROCHIP DESIGN

- Microwells etched into a silicon substrate
- A glass coverplate is etched to dictate channel height
- Wells densely packed into the array
  - ~10^6 wells/cm^2
- Wells engineered to hold only a single bead
  - 3.0 μm diameter regions for bead loading
- Bead loading regions separated from signal acquisition regions
  - 70 fL fluidic region adjoined for signal integration
- Prevents bead autofluorescence from interfering with assay signal

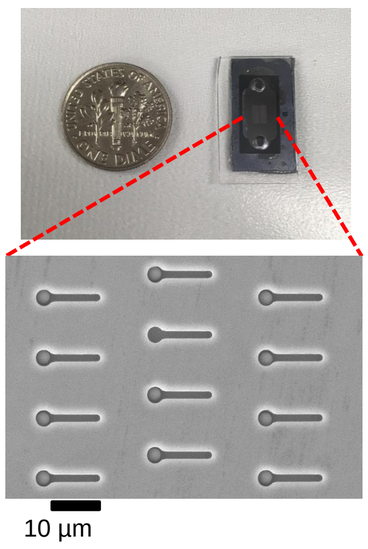

Figure 3. Photo of a microfluidic microwell array device. Inset shows a SEM image of beads loaded into the array. Adapted from *Anal. Bioanal. Chem.* **2020**, *412*, 6917-6926

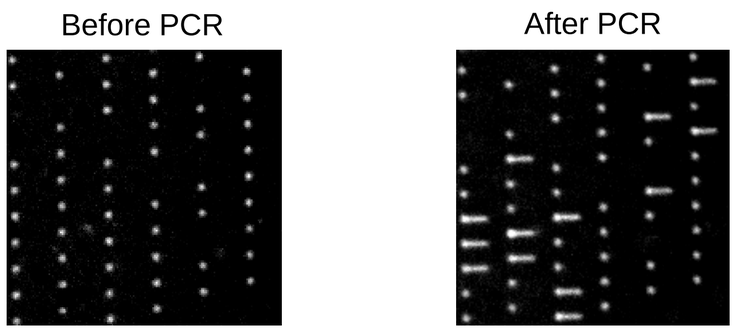

Figure 4. Images of a zoomed in region of a device before and after PCR amplification. Wells containing a single target biomarker exhibit intense fluorescence after PCR.

PROTEIN ASSAYS

- The cytokines GM-CSF, IL-6, IL-36γ were used as model proteins
  - Involved in inflammation pathways and upregulated in certain cancers
- Limits of detection lower than conventional assays
  - GM-CSF: 4 fM
  - IL-6: 30 fM
- Multiplexing achieved by encoding beads targeting different cytokines with different amounts of fluorescent dye
- Used a fluorescent protein for encoding
  - R-phycoerythrin
  - Small molecule dyes found to inhibit PCR amplification
- Achieved three distinct bead populations
  - No overlap in fluorescence between populations
- Encoded beads compatible with PCR
  - Beads encoded with 50 nM PE exhibited strong PCR amplification

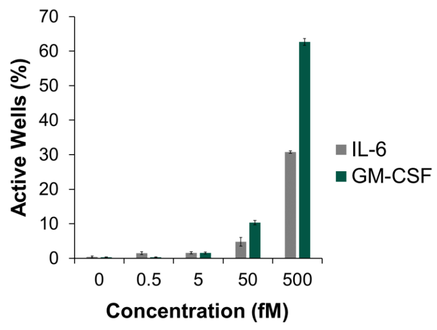

Figure 5. Standard curves for GM-CSF and IL-6 analyzed using iPCR.

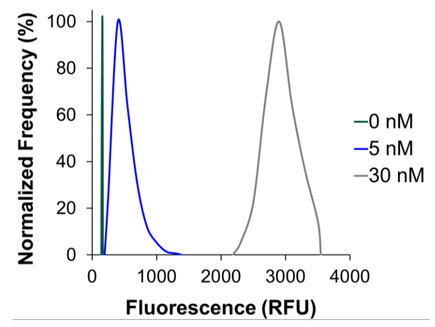

Figure 6. Fluorescence distributions from encoded bead populations.

EBC ANALYSIS

- Exhaled breath condensate (EBC) was collected in chilled vials
  - 100 μL collected from healthy volunteers
- Sample validation required to screen for saliva contamination
- Amylase assays showed that EBC contained no detectable amylase, unlike saliva
  - Verified that sample originated from the lungs, not the mouth

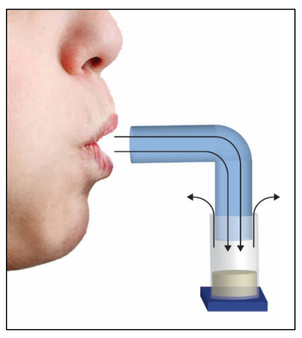

Figure 7. Exhaled breath condensate was collected for analysis.

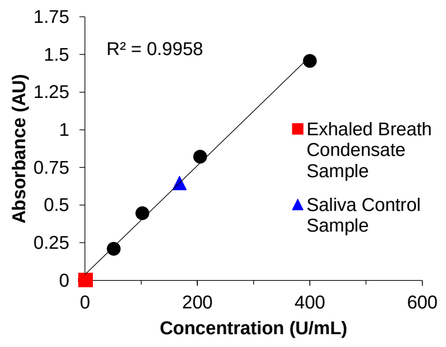

Figure 8. Amylase analysis to screen for saliva contamination.

- Multiplexed bead assays were conducted to measure each analyte in exhaled breath
  - Incubate 80 μL EBC with beads for capture
- Beads in each well were identified by measuring fluorescence at the decoding wavelength
  - Three encoded bead populations for three different target proteins
- Cytokines were quantified from EBC samples
  - GM-CSF: 110 fM
  - IL-6: 5 fM
  - IL-36γ: 400 fM
- Cytokines in EBC below the detection limit of conventional methods
  - LOD ELISA: 500 fM

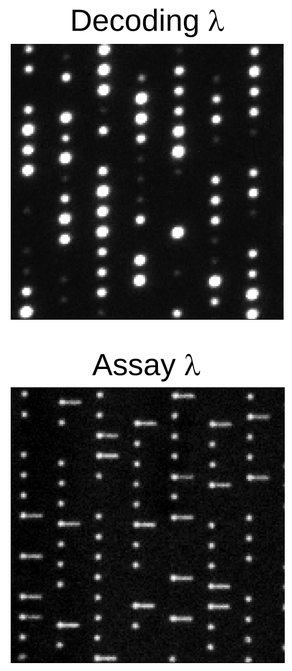

Figure 9. Images of the same region of a microwell array device imaged at the decoding (top) and assay (bottom) wavelengths.

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- Society for Analytical Chemists of Pittsburgh Starter Grant
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