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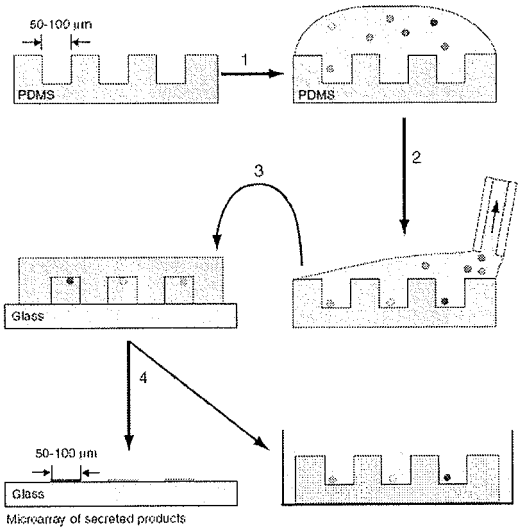


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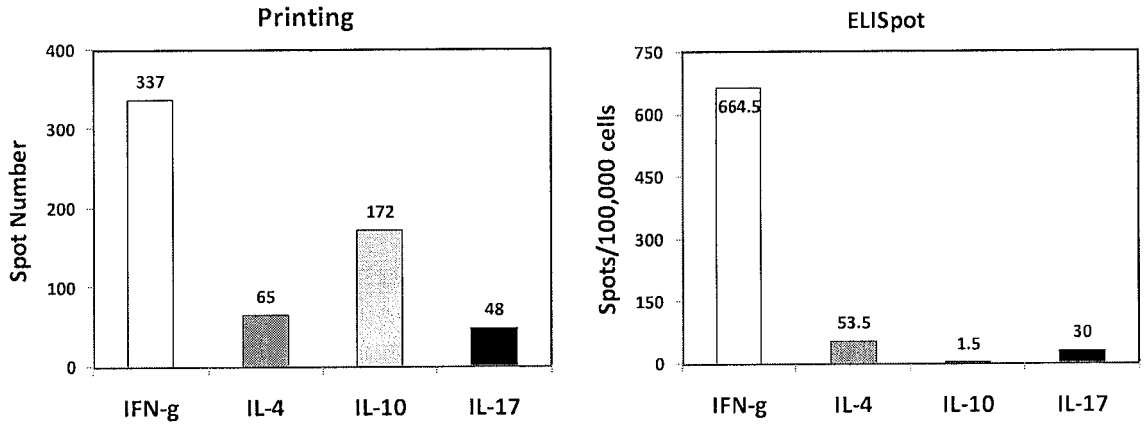


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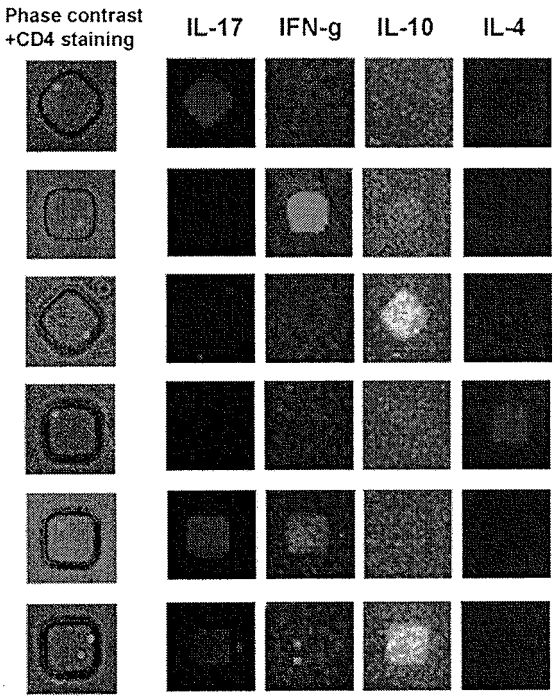


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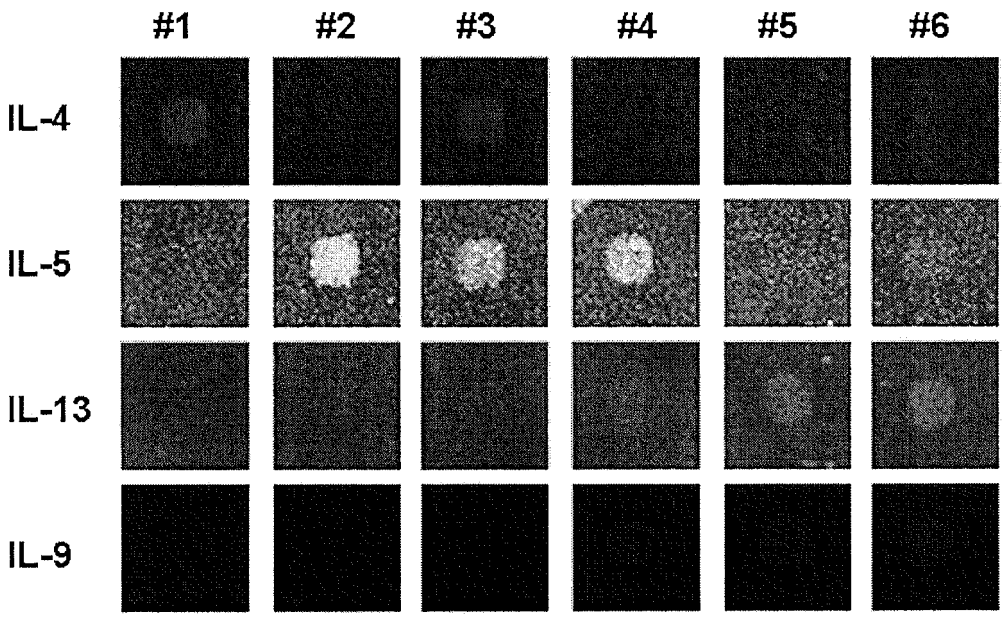


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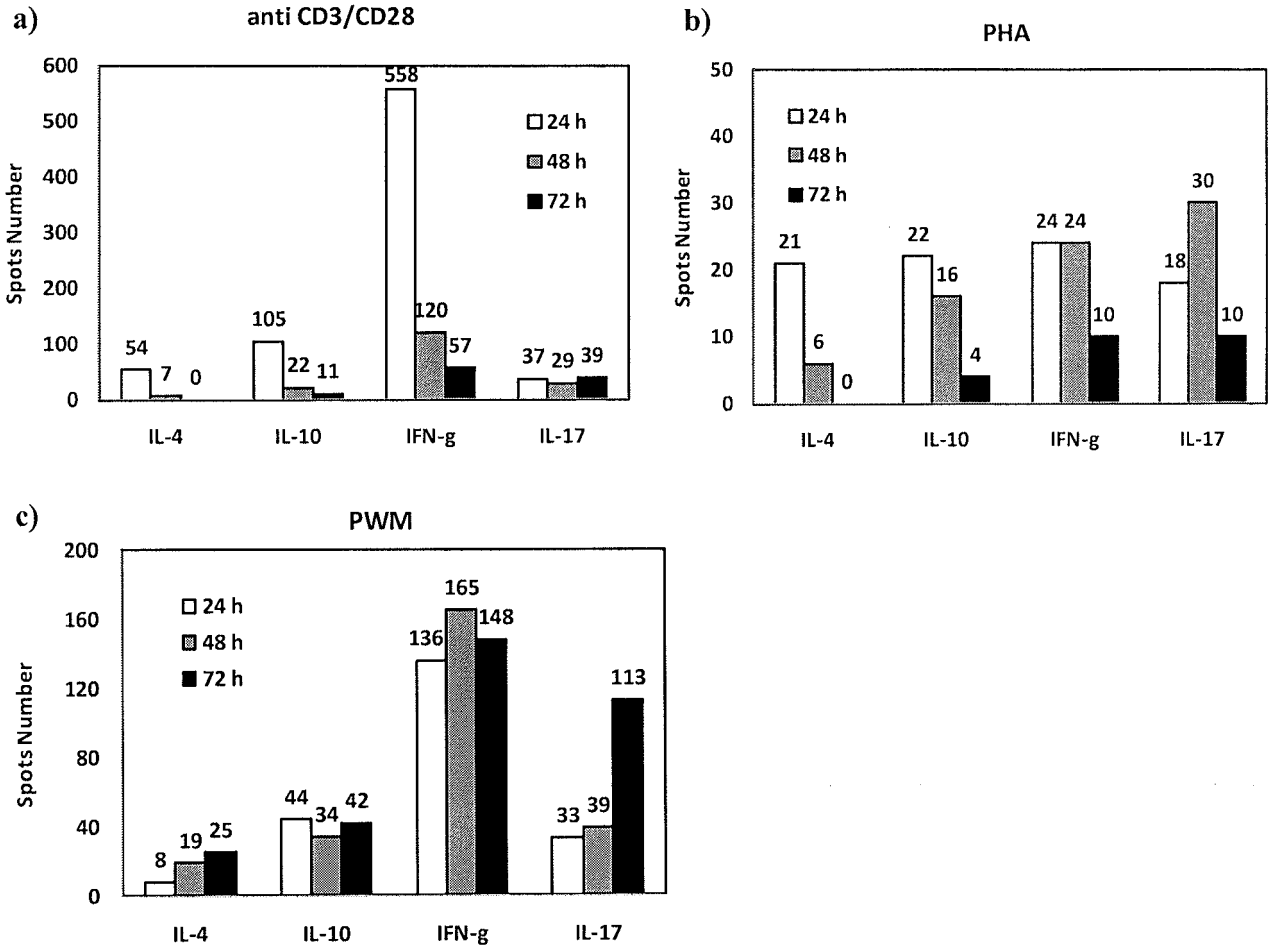

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7
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22
11
558
120
57
37
29
39
24 h
48 h
72 h
IL-4
IL-10
IFN-g
IL-17
b)
PHA
Spots Number
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30
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21
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48 h
72 h
IL-4
IL-10
IFN-g
IL-17
c)
PWM
Spots Number
200
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120
80
40
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8
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24 h
48 h
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IL-4
IL-10
IFN-g
IL-17

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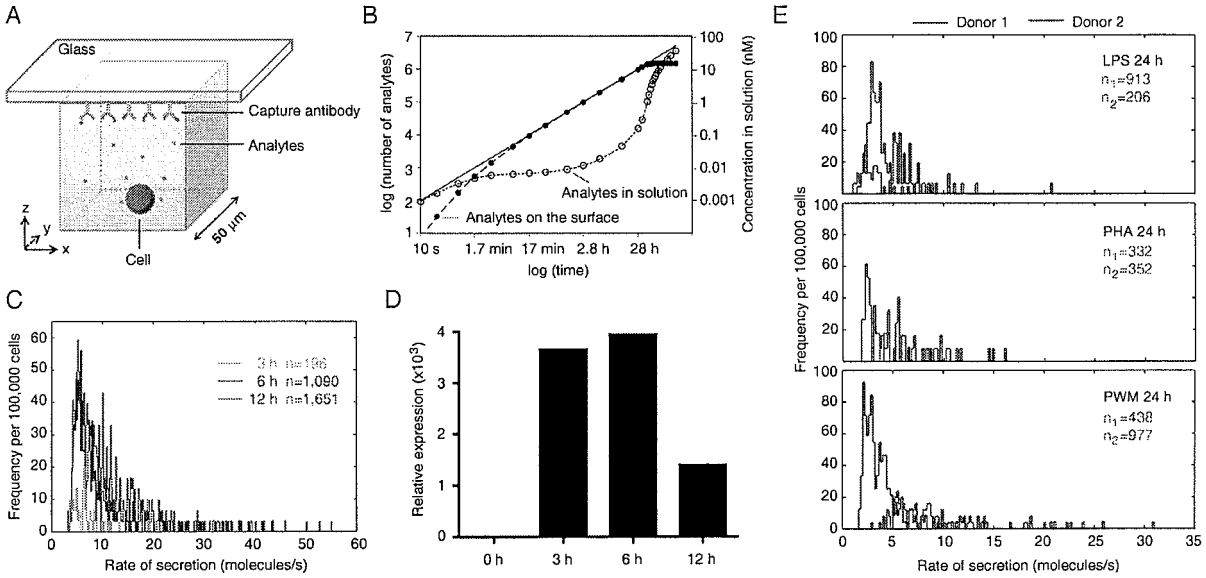


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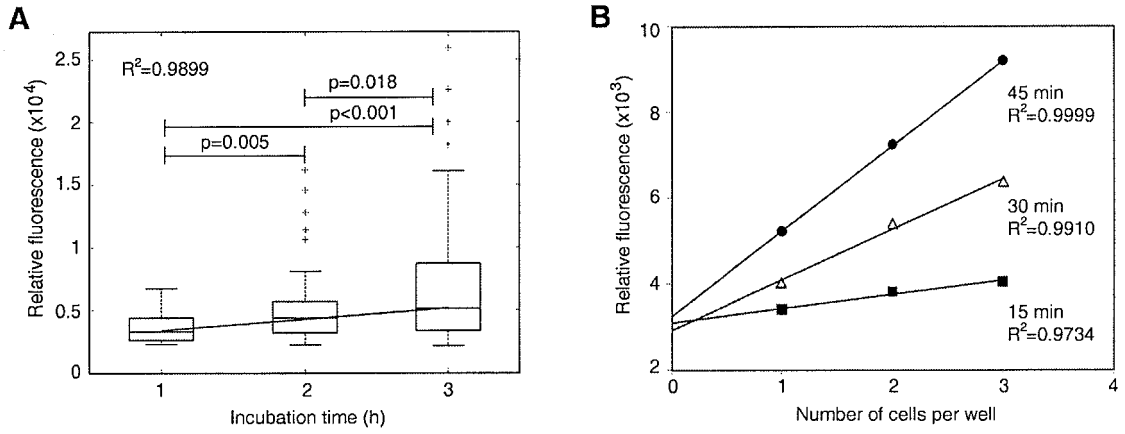


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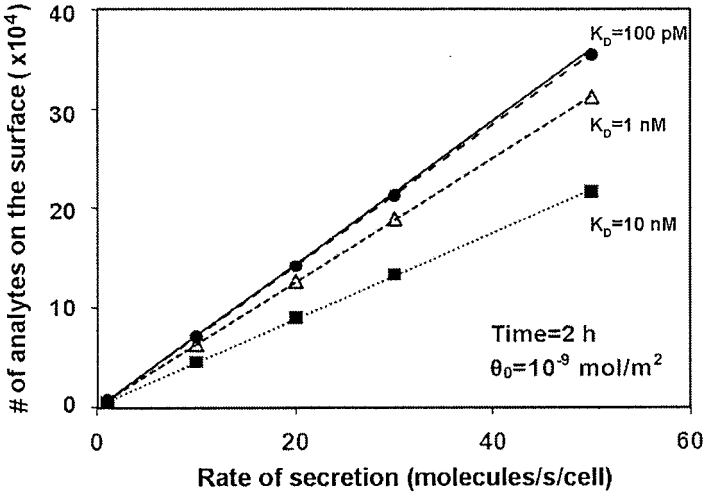


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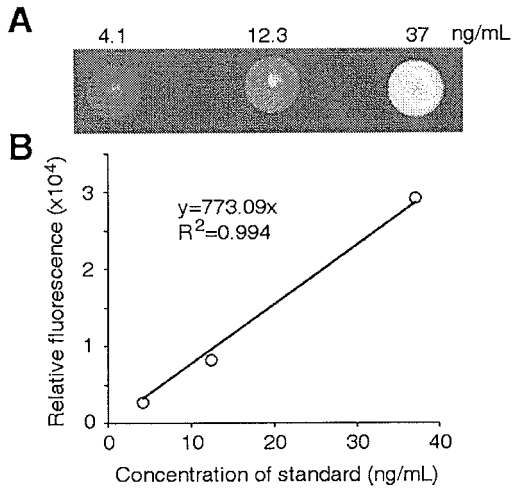


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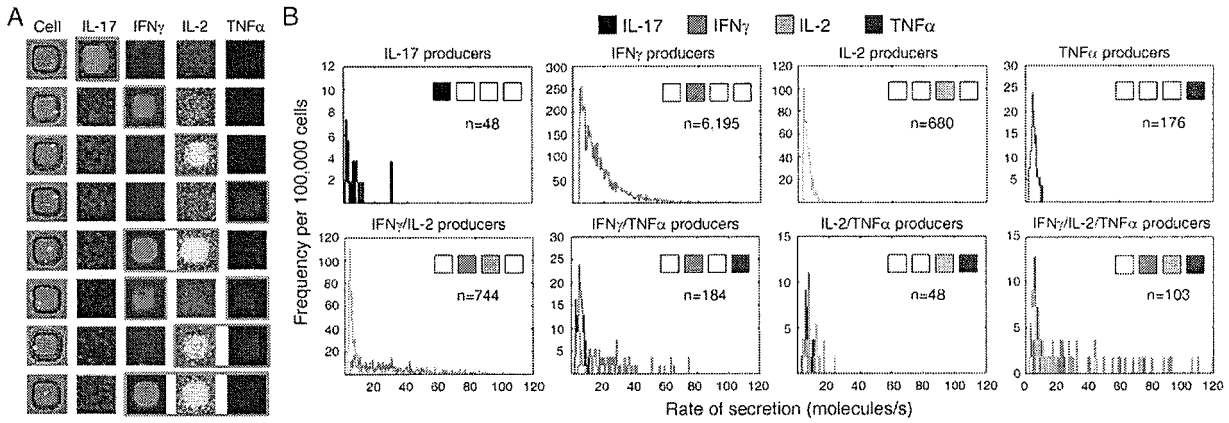


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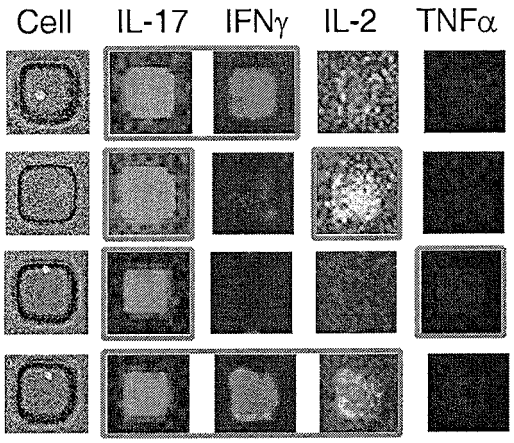


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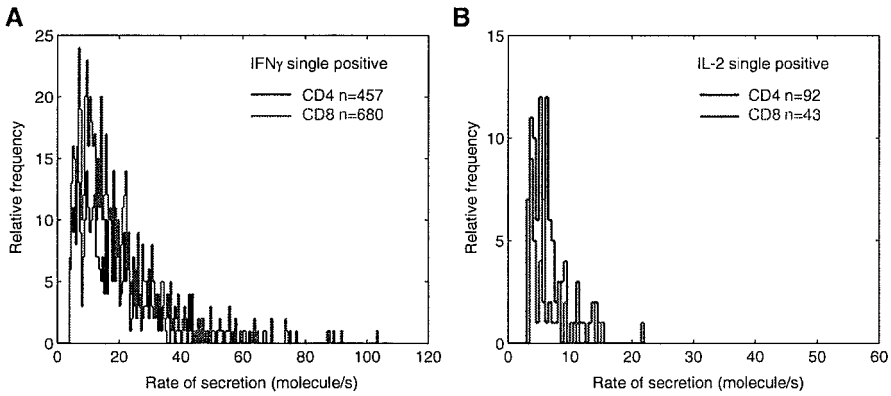


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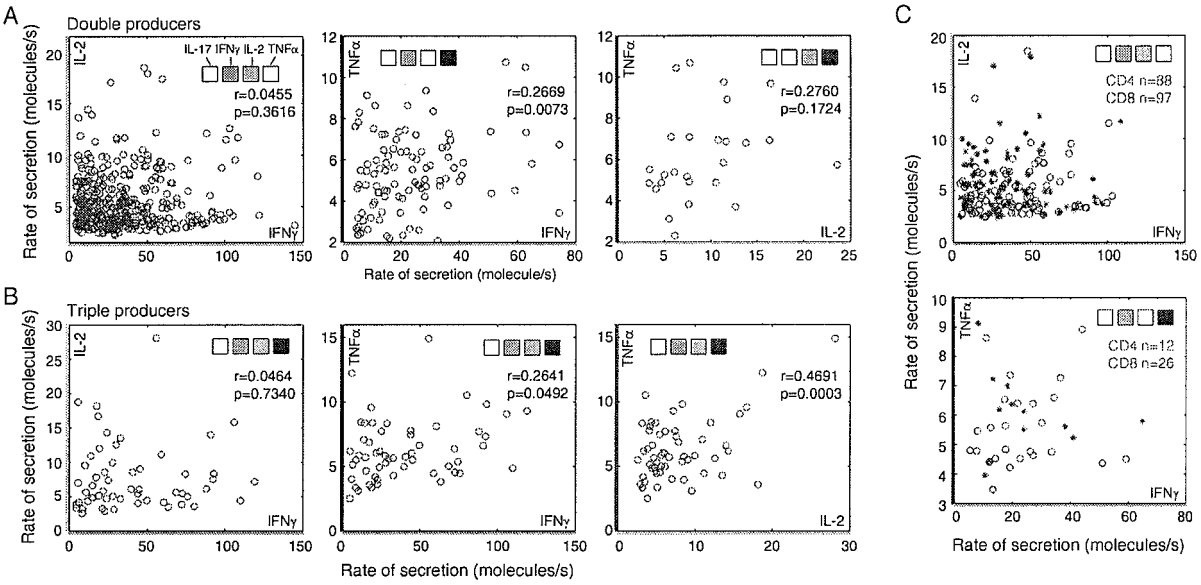


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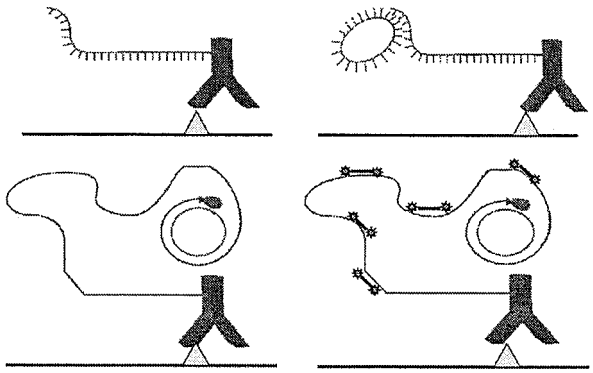


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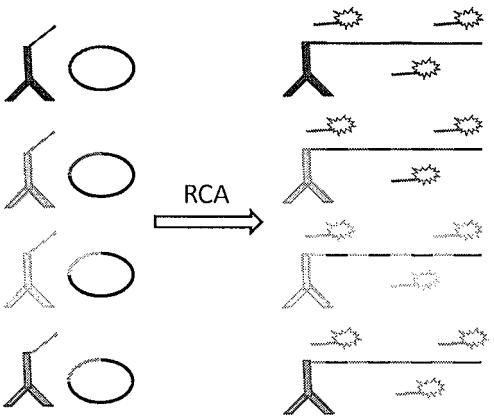


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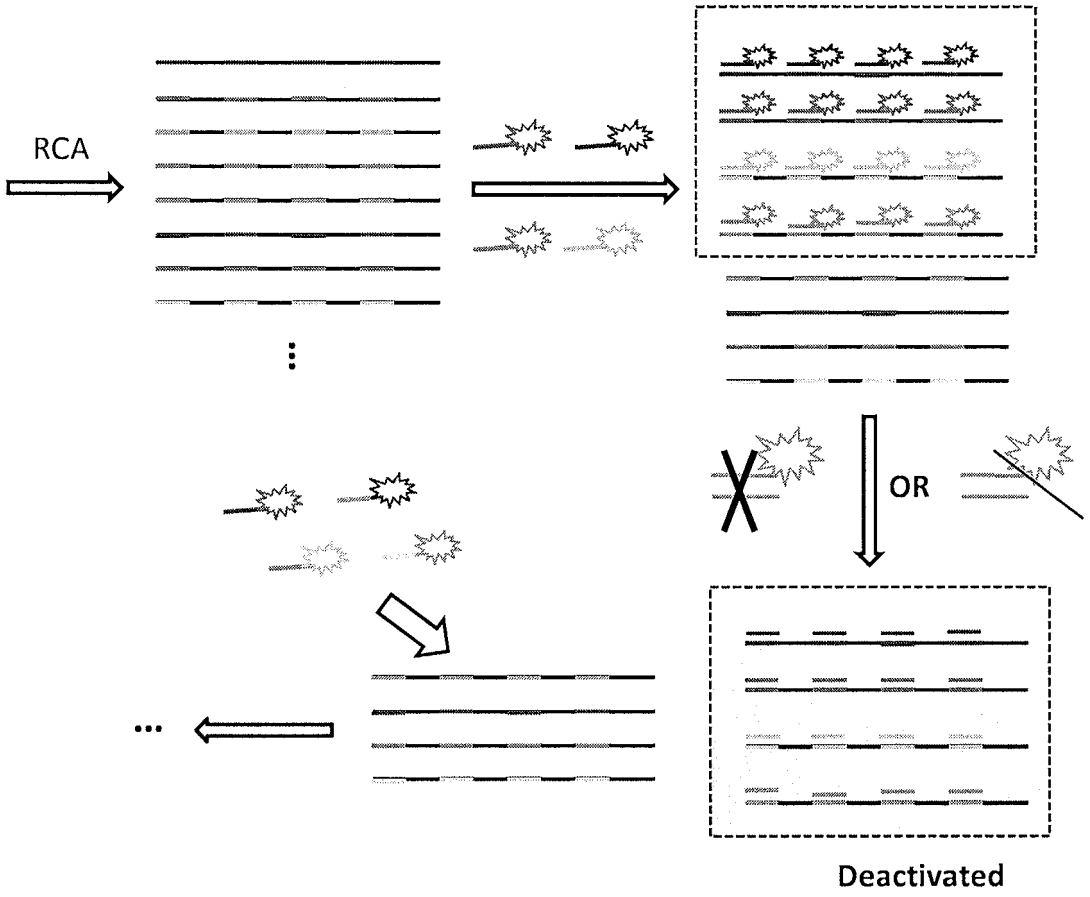
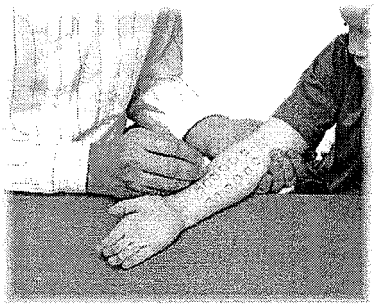


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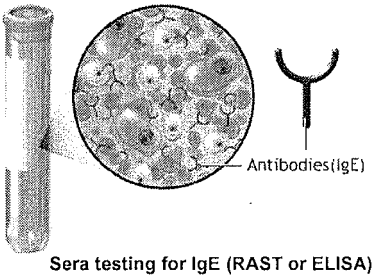
The Problem

Current Allergy Testing



Percutaneous (Skin Prick) Testing

The blood test measures the levels of allergy antibody, or IgE, produced when your blood is mixed with a series of allergens in a laboratory

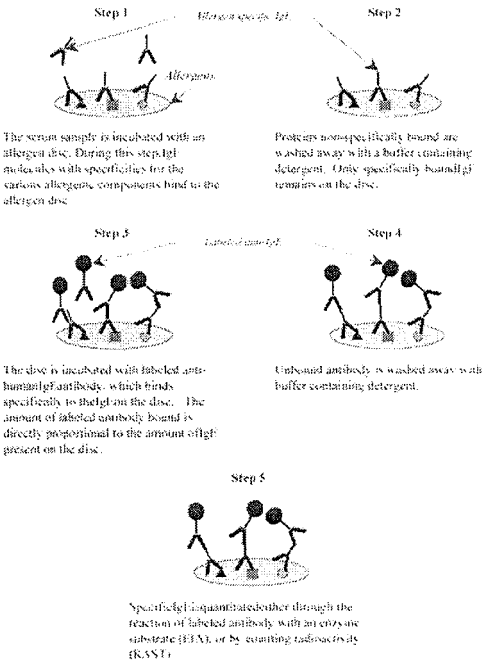


Sera testing for IgE (RAST or ELISA)

Skin testing
- Multiple pin pricks with panel of allergens
- Measures short-term and long-term immune responses
- *Qualitative* assessment of superficial inflammation
- *Traumatic experience* for infants and parents
- Often, *single data point* in child's history

Immunosorbent assays
- Minimally invasive blood draw (5 mL sample)
- Measures serum titers of IgE antibodies
- *Complicated* by pan-specificity of antibodies and elevated levels from other conditions (HIV, skin disease, parasites)
- *Not predictive*—single parameter
- Correlation over time challenging

Food Challenge
- Double blind test with various foods (some that are suspected of inducing a reaction) are given to patient
- Cannot be used on patients with history of severe (anaphylactic) reactions



Immunological Basis of Allergy

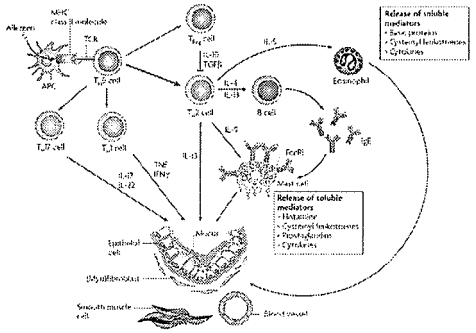
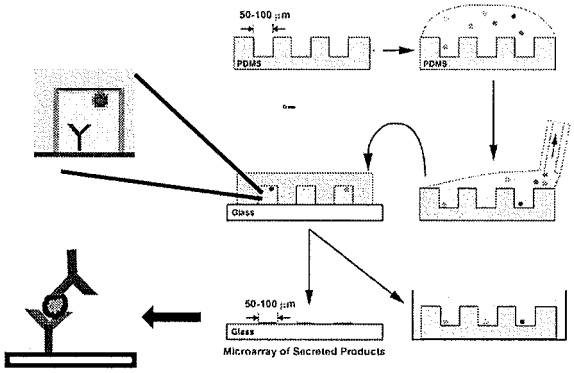


Figure 18.

Implementation

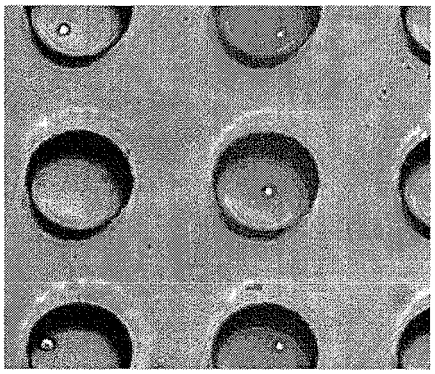
Microengraving



Schematic of the Microengraving Method

How it Works

- Cells, suspended in media, are deposited onto a large array of microwells molded on a poly(dimethylsiloxane) slab, and allowed to settle from suspension in the microwells at a density of ≤1 cell/well.
- The loaded microwells are then inverted onto a glass slide coated with a specific capture reagent (e.g., anti-cytokine).
- After an incubation period, the microwells are removed and applied to a second glass slide coated with a different capture reagent (e.g., anti-IgG and anti-IgM).
- The resulting microarrays are interrogated with fluorescently labeled reagents for detection and laser-based fluorescence scanners.
- After printing, the cells in the wells can be stained *in situ* for subsequent imaging by Immunofluorescence.



Nature Biotechnology, 24, 703-707 (2006)
Clinical Immunology, 129, 10-18 (2008)
Proc. Natl. Acad. Sci. USA, 105, 17902-17907 (2008)
J. Immunol. Methods, in press (2008)

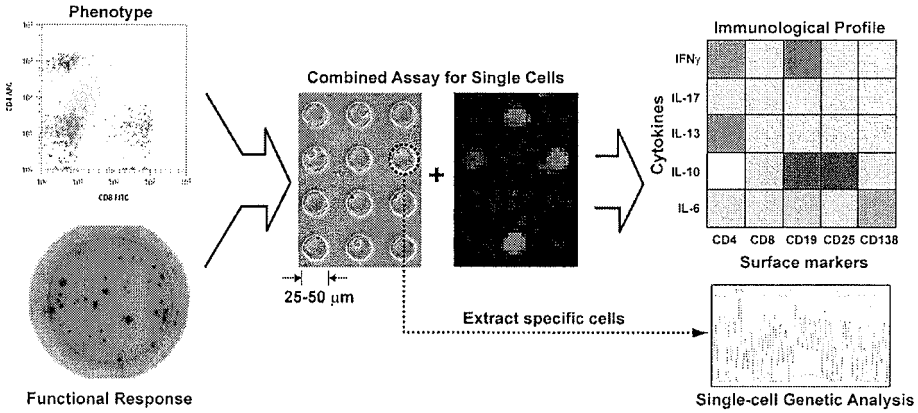
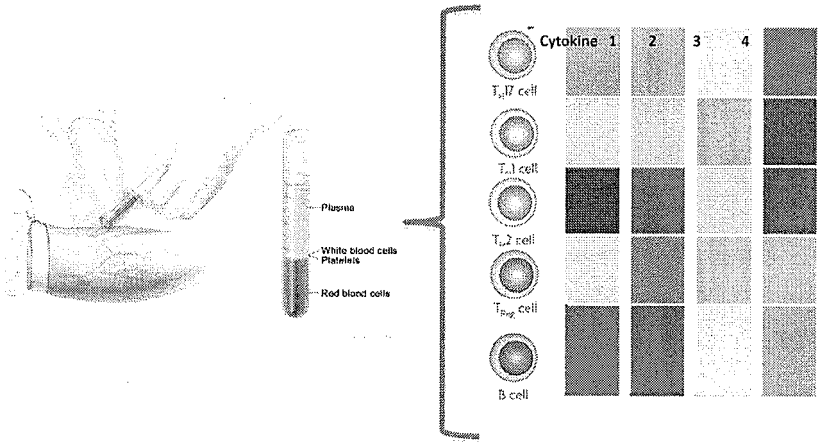


Figure 19.

The Solution

Profiling the Allergic Response



Elucidating the Unique Immunological Profile of an Allergic Individual

- Whole blood from a patient is drawn, and cells of interest are isolated.
- Cells are stimulated with allergens and are used for microengraving.
- The resulting cellular microarray is read as an "allergy-array" which provides specific data concerning reactivity to the allergen(s) of interest.

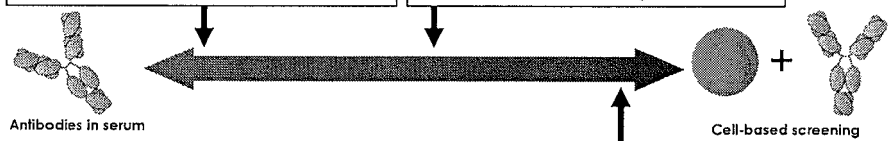
Advantages of this Diagnostic:

- Small blood volumes are needed for a comprehensive allergic profile, so pediatric/infant allergy testing is possible.
- Diagnostics are performed over time to assess a patients reactivity over time, as well as potentially measure the efficacy of immunotherapy.
- Data from the "allergy-array" are used to predict cross-reactivity to related allergens.

Specific IgE capture
(Hitachi, Alerchek, Immulite)
- Restricted number of allergens
- Not scalable ($2 / allergen)

ImmunoCAP
(Phadia, QuestDiagnostic)
- High sensitivity
- Small library (< 20 allergens)
- Large volumes (5 mL sample)
- Not definitive – follow-up necessary
- No data on cells

ELISPOT assays
(AID, MAbTech)
- Data on ab-producing cells,
- Tests large allergen library
- No data on types of ab-producing cells
- Low sensitivity and throughput
- ~250,000 cells per allergen



Improved Allergy Diagnosis
- Combined assay for cells and IgE/IgG_4
- *High throughput + high content*
- Digital sensitivity
- Low volumes, low costs
- *~10,000 cells per allergen*

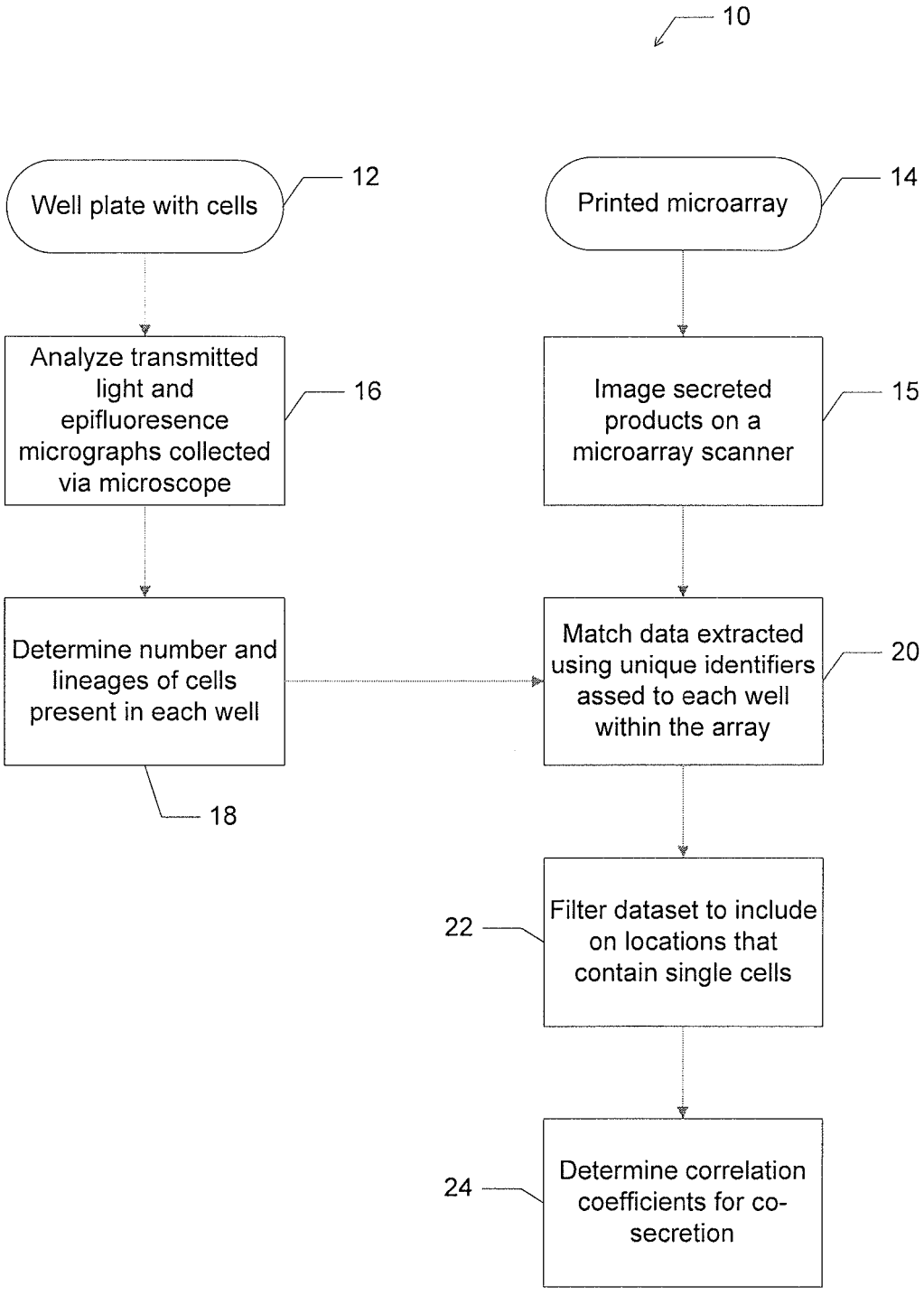

10
Well plate with cells
12
Printed microarray
14
Analyze transmitted light and epifluoresence micrographs collected via microscope
16
Image secreted products on a microarray scanner
15
Determine number and lineages of cells present in each well
18
Match data extracted using unique identifiers assed to each well within the array
20
22
Filter dataset to include on locations that contain single cells
24
Determine correlation coefficients for co-secretion

Figure 20

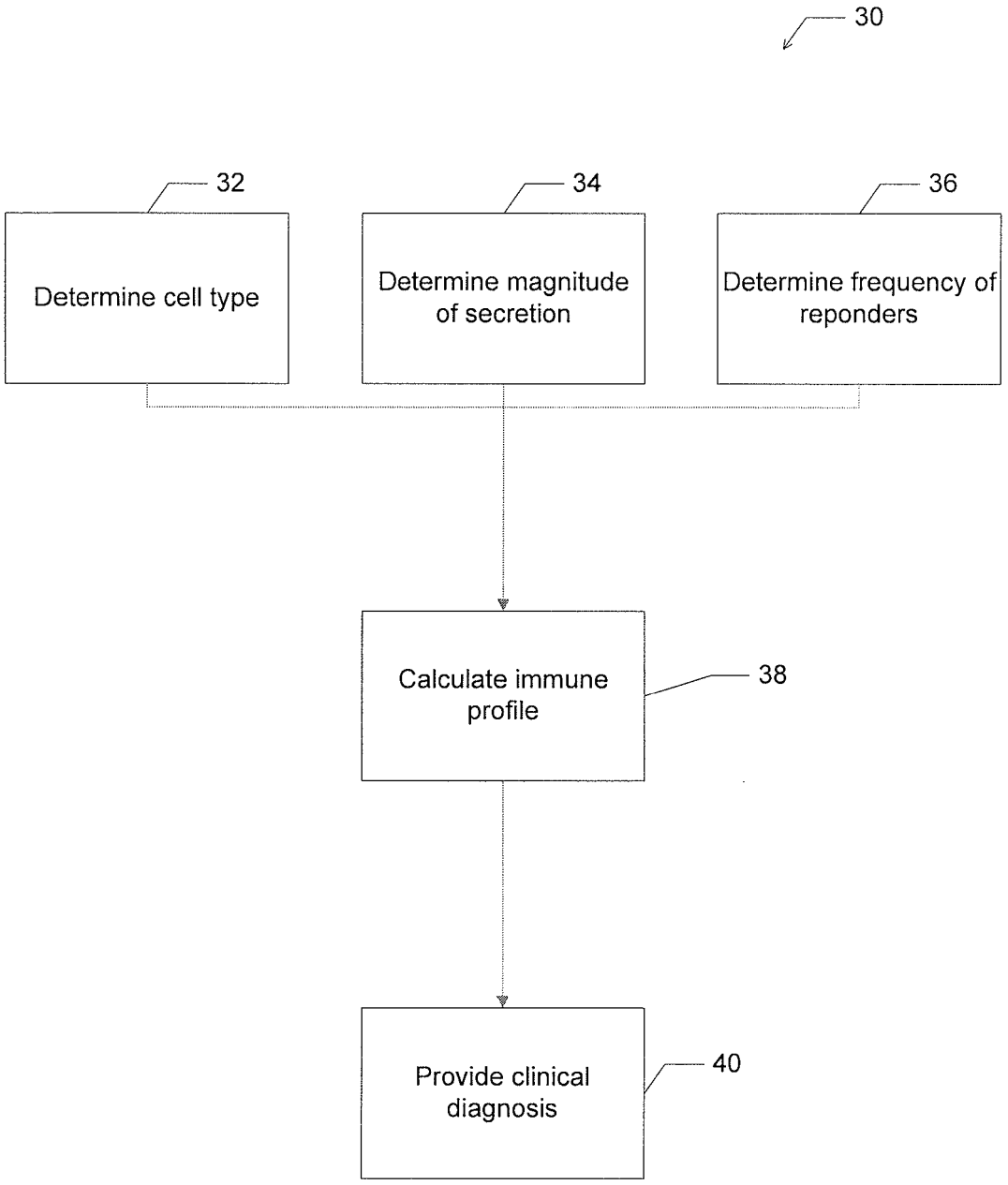

30
32
34
36
Determine cell type
Determine magnitude of secretion
Determine frequency of reponders
Calculate immune profile
38
Provide clinical diagnosis
40

Figure 21