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Title: Field-Deployable Lens-Free Imaging Platform for Rapid Label-Free Analysis of Natural Killer Cell Activation

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? less than 100 m

Current Protocol Length
Number of Steps: 25
Number of Shots: 55 (15 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Samir Kumar:** Our goal is to rapidly assess immune function by quantifying NK cell activation in real time using label-free, lens-free imaging to improve immune monitoring.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What significant findings have you established in your field?

1.2. **Samir Kumar:** We have shown that the innate immunity index or I^3 reliably distinguishes between the NK activity of healthy and immunocompromised donors and correlates strongly with cytokine and flow cytometry markers.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What research gap are you addressing with your protocol?

1.3. **Samir Kumar:** The existing NK cell assays require labels, bulky instruments, and long incubation times. Our LSIT platform fills this gap by enabling fast, affordable, label-free profiling using simple optoelectronics.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Samir Kumar:** Our label-free LSIT platform differentiates NK cell activation at the single cell level within thirty seconds using shadow parameters, eliminating the need for staining and costly flow cytometry.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What research questions will your laboratory focus on in the future?

1.5. **Samir Kumar:** We will extend LSIT to monitor T and B cell activation and integrate deep learning algorithms for high-throughput classification of different immune cell states.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.3.1*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Review Board of Korea University Anam Hospital

Protocol

2. Natural Killer Cell Isolation and Activation

Demonstrator: Inha Lee

2.1. To begin, remove the kits for natural killer or NK cell isolation from the refrigerator **[1]** and allow them to come to room temperature between 15 and 28 degrees Celsius **[2]**.

2.1.1. WIDE: Talent opening a refrigerator and taking out the kits.

2.1.2. Talent placing the kits on a bench.

2.2. Prepare the required equipment by placing a magnetic separator, pipettes with sterile tips, **[1]** and set an incubator or heating block to 37 degrees Celsius **[2]**.

2.2.1. Talent arranging the magnetic separator, pipettes with sterile tips on the workbench.

2.2.2. Talent setting the temperature to 37 degrees Celius on the heatblock or incubator.

2.3. Using a pipette, add 0.5 milliliters of whole blood to the green-capped reaction tube containing the antibody cocktail **[1]**. Gently mix the contents by pipetting up and down the tube 5 to 6 times **[2]**, then incubate the tube for 5 minutes **[3]**.

2.3.1. Talent pipetting whole blood into a green-capped tube.

2.3.2. Talent gently pipetting the green-capped tube.

2.3.3. Talent placing the green-capped tube in a rack and starting a 5-minute timer.

2.4. Transfer the entire contents of the green-capped tube into the red-capped separation tube 1 using a pipette **[1]** and gently mix by pipetting the tube 5 to 6 times **[2]**.

2.4.1. Talent transferring the contents into a red-capped tube.

2.4.2. Talent pipetting the red-capped tube.

2.5. Place the red-capped tube on the magnetic separator and incubate at room temperature for 10 minutes **[1]**.

2.5.1. Talent placing the red-capped tube on the magnetic separator and starting a 10-

minute timer.

2.6. While keeping the magnet in place, use a pipette to transfer approximately 1.5 milliliters of the supernatant into the purple-capped separation tube 2 **[1]**. Gently mix the contents by pipetting the tube 5 to 6 times **[2]**.

2.6.1. Talent transferring the supernatant into a purple-capped tube while the red-capped tube remains on the magnet.

2.6.2. Talent gently pipetting the purple-capped tube.

2.7. Now, place the purple-capped tube on the magnetic separator and incubate for 10 minutes **[1]**.

2.7.1. Talent placing the purple-capped tube on the magnetic separator and starting a 10-minute timer.

2.8. While the magnet is still in place, transfer approximately 1 milliliter of the supernatant into a recovery tube with a gray lid using a pipette **[1]** and carefully mix the contents by pipetting the tube **[2]**.

2.8.1. Talent transferring the supernatant into a gray-lidded tube while keeping the purple-capped tube on the magnet.

2.8.2. Talent gently pipetting the gray-lidded tube.

2.9. Next, add 100 microliters of the isolated natural killer cell suspension into the Vehicle tube **[1]** and another 100 microliters into the activation stimulator cocktail or ASC tube **[2]**.

2.9.1. Talent pipetting 100 microliters of NK cell suspension into the labelled “Vehicle tube”.

2.9.2. Talent pipetting 100 microliters of NK cell suspension into the labelled “ASC tube”.

2.10. Gently mix the contents of both tubes using a vortex mixer to ensure even distribution **[1]**. Incubate the tubes in the heating block or incubator set at 37 degrees Celsius for 1 hour **[2]**.

2.10.1. Talent gently mixing both the Vehicle and ASC tubes by vortex mixing.

2.10.2. Talent placing the tubes into a 37-degree Celsius incubator and setting a 1-hour timer.

3. Imaging the Cells with the LSIT Platform for NK Cell Activity Assessment

Demonstrator: Hojin Cheon

3.1. Switch on the LSIT *(L-S-I-T)* platform **[1-TXT]**.

3.1.1. Talent reaching to power on the LSIT platform. **TXT: LSIT: Lens-free Shadow Imaging Technology**

3.2. Launch the LSIT Capture software **[1]**, log in by entering your ID and password **[2]**. Then, select **Calibrate** to initiate calibration mode **[3]**.

3.2.1. SCREEN: 68834-Screenshot.mp4 00:00–00:10

3.2.2. SCREEN: 68834-Screenshot.mp4 00:11–00:20 and 00:23-00:24

3.2.3. SCREEN: 68834-Screenshot.mp4 00:25–00:30

3.3. Press the **Open** button on the touchscreen or use the physical sliding door button to open the drawer **[1]**. When the drawer opens, remove the calibration slide and store it **[2]**.

3.3.1. Talent pressing **Open** on the screen or using physical button; drawer slides open.

3.3.2. Talent removing the calibration slide and placing it in a storage box.

3.4. Now, click **Set background** to calibrate the optical intensity **[1]**.

3.4.1. SCREEN: 68834-Screenshot.mp4 00:35–00:40

3.5. Replace the calibration slide in the drawer **[1]** and close the drawer **[2]**.

3.5.1. Talent placing the calibration slide back into the drawer.

3.5.2. Talent pressing **Close** or sliding the door shut.

3.6. Then, click **Start calibration** to complete the process **[1]**.

3.6.1. SCREEN: 68834-Screenshot.mp4 02:32–02:35 and 02:59-03:00

3.7. Remove an assay slide from its pouch, label it with the sample data before placing it on a clean, flat surface **[1]**. Pipette 10 microliters of the vehicle sample into channels A and B **[2]** and then pipette 10 microliters of the ASC-stimulated sample into channels C and D **[3]**.

3.7.1. Talent unsealing an assay slide pouch, labeling the slide with a marker.

3.7.2. Talent pipetting the vehicle sample into channels A and B of the assay slide.

3.7.3. Talent pipetting the ASC-stimulated sample into channels C and D of the assay slide.

3.8. Now, press **Open** to eject the drawer **[1]**, remove the calibration slide **[2]**, and place it in a storage box **[3]**.

3.8.1. Talent pressing **Open** on the touchscreen or using physical button.

3.8.2. Talent lifting the calibration slide from the drawer.

3.8.3. Talent placing the slide in the storage box.

3.9. Insert the prepared assay slide into the drawer **[1]** and press **Close** to secure it **[2]**.

3.9.1. Talent inserting the assay slide into the drawer.

3.9.2. Talent pressing **Close** or sliding the drawer shut.

3.10. Go back to the **Main Menu** and select the **NK Cell Activity** and **Test** from the main screen **[1]**. Enter the sample ID or scan the barcode to load the sample profile **[2]**. Then, press **Capture** to begin image recording **[3]**.

3.10.1. SCREEN: 68834-Screenshot.mp4 03:05–03:19

3.10.2. SCREEN: 68834-Screenshot.mp4 03:19–03:29

3.10.3. SCREEN: 68834-Screenshot.mp4 03:48–04:00

3.11. After capturing the image, click on **Analyze** to begin processing **[1]**. Review the NK cell activity percentage and cell count displayed on the screen **[2]**. Download the results to a USB drive or print them using the touchscreen menu **[3]**.

3.11.1. SCREEN: 68834-Screenshot.mp4 04:00–04:08

3.11.2. SCREEN: 68834-Screenshot.mp4 04:49–05:00

3.11.3. SCREEN: 68834-Screenshot.mp4 05:25–05:30

4. Cell Counting and Post-Analysis Clean-Up

Demonstrator: Hojin Cheon

4.1. Select **Cell counting** and **Test** on the LSIT main interface **[1]**. Load 10 microliters of the new sample onto a fresh assay slide channel using a pipette **[2]**. Insert the slide into the drawer, close the drawer using the touchscreen or button **[3]**, and enter the sample ID on the system **[4]**.

4.1.1. SCREEN: 68834-Screenshot.mp4 05:45–05:53

4.1.2. Talent pipetting 10 microliters of the sample into a new channel on a clean assay slide.

4.1.3. Talent inserting the assay slide into the drawer, pressing **Close.**

4.1.4. Talent entering the sample ID.

4.2. Enter the dilution factor on the touchscreen if applicable **[1]** and press **Counting** to begin cell quantification **[2]**. View the results displayed as cells per microliter on the screen **[3]**.

4.2.1. Show the dilution factor input field and talent entering the value.

4.2.2. SCREEN: 68834-Screenshot.mp4 06:02–06:05 and 06:28-06:30

4.2.3. SCREEN: 68834-Screenshot.mp4 06:40–06:47

4.3. After analysis, open the drawer by pressing the **Open** button or toggling the physical switch **[1]**. Remove the used slide from the drawer using gloved hands **[2]**.

4.3.1. Talent pressing **Open** or using the switch; drawer slides open.

4.3.2. Talent carefully lifting out the used assay slide and discarding it in the designated waste container.

4.4. Finally, place the calibration slide back into the drawer to protect the sensor **[1]** and close the drawer using the touchscreen or the physical toggle switch **[2]**.

4.4.1. Talent retrieving the calibration slide from the storage box and placing it in the drawer.

4.4.2. Talent pressing **Close** or sliding the drawer shut.

Results

5. Results

5.1. The LSIT platform, with integrated software, quantitatively assessed NK cell activation by comparing the CSP values of activated and unstimulated control cells **[1]**. It provided the immunity report with a summarized statistical table **[2]**.

5.1.1. LAB MEDIA: Figure 5A and B

5.1.2. LAB MEDIA: Figure 5C

5.2. ASC stimulation induced clear morphological changes in NK cells, including increased size and cytoplasmic complexity, as seen in Hema-3–stained cytospins after 30 minutes and 2 hours **[1]**.

5.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the "ASC"-stimulated cells*.

5.3. Shadow images showed ASC-stimulated cells had visibly more complex diffraction patterns compared to vehicle controls **[1]**, with extracted parameters indicating increased peak-to-peak distance, cytoplasmic granularity and combined shadow parameter **[2]**.

5.3.1. LAB MEDIA: Figure 2B. *Video editor: Focus on the "ASC"-stimulated shadow images in the lower row. These have a blue background*.

5.3.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the bars for "ASC" in all 3 graphs of 2C*.

5.4. Combined shadow parameter values allowed clear classification between Healthy donors **[1]** and Cancer patients **[2]**, with minimal overlap in single-cell distributions of activated and non-activated NK cells **[3]**.

5.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the box plot for " HD".*

5.4.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the box plot for " CP".*

5.4.3. LAB MEDIA: Figure 2E. *Video editor: Highlight red points (activated)* .

5.5. Healthy donors showed significantly higher Innate Immunity Index values **[1]** than cancer patients following ASC stimulation **[2]**.

5.5.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the scatter plot for HD group*.

5.5.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the scatter plot for CP group*.

Pronunciation Guide:

1. Immunocompromised

Pronunciation link:
https://www.merriam-webster.com/dictionary/immunocompromised
IPA: /ˌɪm.jə.noʊˈkɑːm.prəˌmaɪzd/
Phonetic Spelling: im-yuh-noh-KOM-pruh-myzd

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2. Cytokine

Pronunciation link:
https://www.merriam-webster.com/dictionary/cytokine
IPA: /ˈsaɪ.t̬oʊˌkaɪn/
Phonetic Spelling: SYE-toh-kine

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3. Flow Cytometry

Pronunciation link:
https://www.merriam-webster.com/dictionary/cytometry
IPA: /floʊ saɪˈtɑː.mə.tri/
Phonetic Spelling: floh sy-TAH-muh-tree

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4. Optoelectronics

Pronunciation link:
https://www.merriam-webster.com/dictionary/optoelectronics
IPA: /ˌɑːp.toʊ.ɪˌlɛkˈtrɑː.nɪks/
Phonetic Spelling: op-toh-ee-lek-TRAH-niks

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5. Incubator

Pronunciation link:
https://www.merriam-webster.com/dictionary/incubator
IPA: /ˈɪŋ.kjəˌbeɪ.tɚ/
Phonetic Spelling: IN-kyuh-bay-ter

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6. Microliter

Pronunciation link:
https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪ.krəˌliː.t̬ɚ/
Phonetic Spelling: MY-kroh-lee-ter

7. Vehicle (scientific usage: "control vehicle")

Pronunciation link:
https://www.merriam-webster.com/dictionary/vehicle
IPA: /ˈviː.ɪ.kəl/
Phonetic Spelling: VEE-ih-kul

8. Vortex (as in vortex mixer)

Pronunciation link:
https://www.merriam-webster.com/dictionary/vortex
IPA: /ˈvɔːrˌtɛks/
Phonetic Spelling: VOR-teks

9. Assay

Pronunciation link:
https://www.merriam-webster.com/dictionary/assay
IPA: /ˈæˌseɪ/
Phonetic Spelling: A-say

10. Cytoplasmic

Pronunciation link:
https://www.merriam-webster.com/dictionary/cytoplasmic
IPA: /ˌsaɪ.toʊˈplæz.mɪk/
Phonetic Spelling: SYE-toh-plaz-mik

11. Morphological

Pronunciation link:
https://www.merriam-webster.com/dictionary/morphological
IPA: /ˌmɔːr.fəˈlɑː.dʒɪ.kəl/
Phonetic Spelling: mor-fuh-LAH-jih-kul

12. Cytospin

Pronunciation link:
https://www.howtopronounce.com/cytospin
IPA: /ˈsaɪ.toʊˌspɪn/
Phonetic Spelling: SYE-toh-spin

13. Granularity

Pronunciation link:
https://www.merriam-webster.com/dictionary/granularity
IPA: /ˌgrænjʊˈlɛrəti/
Phonetic Spelling: gran-yoo-LAIR-uh-tee

14. Anam (as in Korea University Anam Hospital)

Pronunciation link:
https://www.howtopronounce.com/anam
IPA: /ˈɑː.nɑːm/
Phonetic Spelling: AH-nahm

15. Metaimmunetech

Pronunciation link:
No confirmed link found
IPA (constructed): /ˌmɛt.ə.ɪˈmjuː.nˌtɛk/
Phonetic Spelling: MET-uh-ih-MYUN-tek

16. Gastroenterology

Pronunciation link:
https://www.merriam-webster.com/dictionary/gastroenterology
IPA: /ˌgæs.troʊˌɛn.təˈrɑː.lə.dʒi/
Phonetic Spelling: gas-troh-en-tuh-RAH-luh-jee

17. Hepatology

Pronunciation link:
https://www.merriam-webster.com/dictionary/hepatology
IPA: /ˌhɛp.əˈtɑː.lə.dʒi/
Phonetic Spelling: hep-uh-TAH-luh-jee

18. Immunity

Pronunciation link:
https://www.merriam-webster.com/dictionary/immunity
IPA: /ɪˈmjuː.nə.ɾi/
Phonetic Spelling: ih-MYOO-nuh-tee

19. Diffraction

Pronunciation link:
https://www.merriam-webster.com/dictionary/diffraction
IPA: /dɪˈfræk.ʃən/
Phonetic Spelling: dih-FRAK-shun

20. Calibration

Pronunciation link:
https://www.merriam-webster.com/dictionary/calibration
IPA: /ˌkæl.əˈbreɪ.ʃən/
Phonetic Spelling: kal-uh-BRAY-shun