Two-photon microscopy in biomedical application

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Outline

- Cell-cell interaction
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- Cell signal transduction
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  - 劉家聖
Cell-cell interaction
Multiphoton microscopy in life sciences

Table 1. Comparison of Methods Used to Study T Cell-APC Interactions

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Two-photon imaging of tissues (Bousso et al., 2002; Bousso and Robey, 2003; Mempel et al., 2004; Miller et al., 2002, 2003, 2004)</td>
<td>Provides real-time information about behavior of cells in tissue environments.</td>
<td>Difficulty in detecting signal deep in tissues limits the use of certain markers. Cannot simultaneously examine marker expression of cells being imaged.</td>
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<td>Immunofluorescence analysis of fixed tissue sections (Ingulli et al., 1997; Lind et al., 2001; Saiki et al., 2001; Schaefer et al., 2001).</td>
<td>Can use antibodies to identify different cell types. Provides information about location of specific cells within tissue environments.</td>
<td>Static information only.</td>
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<tr>
<td>Isolation of T cell and DC clusters from lymph nodes (Hommel and Kyewski, 2003)</td>
<td>Can use immunofluorescence and flow cytometric analyses to characterize cells within clusters.</td>
<td>Static information only.</td>
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<tr>
<td>Imaging of fixed T cell-APC conjugates (Freiberg et al., 2002; Monks et al., 1998)</td>
<td>High-resolutions analysis of proteins at T cell-ACP interface.</td>
<td>Static information only. Does not recapitulate normal cellular environment.</td>
</tr>
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</table>

Figure 3. Cellular Dynamics in the Lymph Nodes
(A) Schematic representation of a B cell follicle and of the T cell zone of lymph node. The mean velocities of the different cell types are indicated.
(B) Dynamics of antigen-independent and antigen-dependent T cell-DC contacts. The various proposed modes of T cell activation are shown. Data are summarized from Bousso and Robey, 2003; Mempel et al., 2004; Miller et al., 2004; Miller et al., 2002.

Multiphoton microscopy in life sciences

**A** Strategy for imaging thymocytes and stromal cells in thymic organ cultures

- CD4^+^CD8^+^ thymocytes from F5 TCR tg, class I def mice labeled with vital dye.
- Limiting thymic stromal cells from B6 (wt) mice labeled with vital dye.
- Excess unlabeled thymic stromal cells from MHC-mice.
- Reaggregate and culture in high oxygen.

**B** Strategy for imaging T cells and DC in lymph nodes

- Mature T cells labeled with green dye.
- Splenic dendritic cells (DC) labeled with red dye.
- Inject labeled DC.
- Isolate intact lymph nodes.
- Perform real-time 2-photon imaging (acquire optical slices 50-200 microns below tissue surface every 10-30 seconds).

Multiphoton microscopy in life sciences

Figure 2. Visualization of T Cell-APC Interactions in Tissue via Two-Photon Imaging

(A) Thymocyte: thymic stromal cell interactions during MHC recognition in RTOC. RTOCs were formed with F5 thymocytes (red), selecting wild-type stromal cells (green), and excess unlabeled MHC negative stromal cells. MHC recognition results in the selective accumulation of thymocytes around MHC-bearing stromal cells.
(B) T cell-DC contacts during priming in the lymph node. Imaging of intact lymph nodes containing P14 TCR CD8 T cells (green) and DC bearing their cognate antigen (red).

Real-time imaging of T-cell development

(a) GFP-expressing T-cell progenitors

Reseeding → 6–12 days → Thymocyte-depleted fetal thymic lobe (dye-labeled)

(b) CFP-expressing T cell progenitors from wild-type mice

Reseeding → 6–12 days → Thymocyte-depleted fetal thymic lobe

YFP-expressing T-cell progenitors from mutant mice

Current Opinion in Immunology

Real-time imaging of T-cell development

Cell signal transduction
Advantages of TPM in studying neuronal network

* Small photodamages-the excitation wavelength is 940nm
* Sub-micron resolution
* Study the electrical activity of 3D neuronal network
  Able to measure at depths of up to ~400um in light scattering tissues, such as neurons.

3D neuronal network - similar to real tissues in vivo
Optical Recordings of Action Potentials with TPM

The firing of Action potential with time at 1&2 (Courtesy from D.Dombeck)

Red : the recording from electrode
Green: the change of emission intensity

Record action potentials (Aps) at multiple sites thru voltage-sensitive dye

Offer the advantage to measure (Aps) of very small and fragile region
Understand Ap propagation thru axon
Understand the synaptic connection and efficacy
Monitor the exchange and processing of information from many individual neurons

What are other topics you can think of??

Functional imaging reveals rapid development of visual response properties in the zebrafish tectum
Using live FRET imaging to reveal early protein–protein interactions during T cell activation

Imaging in freely moving animals with a minature two photon microscope

- This figure shows a two photon microscope system
- (a) shows the schematic figure of implanted system mounted to a rats using fiber optics
- (b) *In vivo* imaging of dendrites in anesthetized head-restrained rats
- (c) high resolution imaging in freely moving rats

New developments in multiphoton microscopy *Hemchen and Denk*
Alzheimer’s disease *in vivo*

- Long term in vivo imaging through thinned skull (~20 μm thickness) in the neocortex of transgenic mice
- Plagues were stained in thioflavine S (red)
- Figure shows the growth of four distinguishable plague. (A,B,C,D)

*New developments in multiphoton microscopy* Hemchen and Denk
~ Thanks for your attention ~