世界真細小 --- 顯微鏡
Small World by Microscopy

香港浸會大學數學系
吳國寶
Michael Ng
2014 The Nobel Prize in Chemistry

- Eric Betzig, William Moerner and Stefan Hell won the Nobel Prize in chemistry for developing new methods that let microscopes see finer details than they could before.

- The three scientists were cited for "the development of super-resolved fluorescence microscopy," which the Royal Swedish Academy of Sciences said had bypassed the maximum resolution of traditional optical microscopes.

- "Their ground-breaking work has brought optical microscopy into the nano-dimension," the academy said.
Why?

- Science is based on observations (観察).
- Of all the instruments used in the study of biology, it is the microscope that has contributed most to the furthering of knowledge in the subject.
- Vision is the most precious of the human senses.
- The alleviation of weak eyesight and the extension of the power of normal vision would have been priorities in scientific endeavors.
History

- Things seen under a larger angle appear larger, those under a smaller angle appear smaller, and those under equal angles appear equal (Euclid, 300 BC) → Refraction

- Letters, however tiny and obscure, are seen much larger and clearer through a glass ball filled with water (Seneca, 62 AD).

- Roger Bacon (1220–92) experimented with lenses, or what are now known as lenses, and concluded that they would be useful for aiding vision. In his Opus majus written in 1266-67 he wrote:

  If a man looks at letters or other small objects through the medium of a crystal or of glass or of some other transparent body placed above the letters, and it is the smaller part of a sphere whose convexity is toward the eye, and eye is in the air, he will see the letters much better and they will appear larger to him.
Roger Bacon’s hand-drawn diagram showing his rule of refraction of light. Light rays from the object (Resvisa) are refracted by a convex transparent material so that the image (Imago) seen by the eye (Oculus) is larger than the object. This photograph is of the original diagram in Bacon’s hand-written manuscript of 1267.
Microscope

The invention of the telescope and that of the microscope are interwoven. Both of these instruments came into existence about 1600, the exact date is not known.

The telescope was immediately recognized as a valuable instrument for military endeavors, since it would allow one to spy on the enemy as if they were much closer, and also from outside the range of their guns.

The earliest record of the existence of a microscope is contained in a letter written by Constantijn Huygens in London on 30 March 1622 and addressed to his parents in the Netherlands. In a paragraph written in Huygens told his parents that he had purchased a “lunette de Drebbel” as he called the microscope. In 1622 the word “microscope” had yet to be coined as a name for the instrument.

Constantijn Huygens (1596-1687) was a Dutch poet, who served his country as a diplomat. He was fluent in several languages, an accomplished musician, a connoisseur of paintings, and had a good knowledge of science. He was the father of the eminent Dutch astronomer, mathematician and physicist Christiaan Huygens.
Microscopic Observations

The earliest records of microscopic observations date from 1625 and 1630 and are investigations of a bee and a weevil by Federico Cesi and Francesco Stelluti. These records of observations pre-date the oldest microscopes extant. The microscope used for these observations was probably made by Galileo, since it is known that in 1624 Galileo sent a microscope to Cesi in Rome.

Robert Hooke (1635-1703) used a single-lens microscope for some of the observations reported in Micrographia, the first classic treatise on microscopic studies (Hooke, 1665).

Lamp allows optimal viewing with controlled artificial light under any conditions of natural light both day and night.
Other Observations

- The microscope was used extensively for research in Italy, the Netherlands and England. Marcelo Malpighi in Italy began the analysis of biological structures beginning with the lungs. Robert Hooke's Micrographia had a huge impact, largely because of its impressive illustrations. The greatest contribution came from Antonie van Leeuwenhoek who discovered red blood cells and spermatozoa and helped popularise microscopy as a technique. On 9 October 1676, Van Leeuwenhoek reported the discovery of micro-organisms.
Microscope with two convex Lens
The Nobel Prize in Physics 1953: Frits (Frederik) Zernike "for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope"

The Nobel Prize in Physics 1986: Gerd Binnig and Heinrich Rohrer "for their design of the scanning tunneling microscope“

The Nobel Prize in Physics 1986: Ernst Ruska "for his fundamental work in electron optics, and for the design of the first electron microscope“

The Nobel Prize in Chemistry 1982: Aaron Klug "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid-protein complexes"

The Nobel Prize in Chemistry 2014: Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy"
Phase Contrast Microscope

- The same cells imaged with traditional bright field microscopy (left) and with phase contrast microscopy (right). These structures were made visible to earlier microscopists by staining, but this required additional preparation and killed the cells.
Illustration of phase shift. The horizontal axis represents an angle (phase) that is increasing with time.
Phase Contrast Microscope

- When light waves travel through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength dependent and may give rise to colors. Photographic equipment and the human eye are only sensitive to amplitude variations.

- Without special arrangements, phase changes are therefore invisible. Yet, phase changes often carry important information.

- The basic principle to make phase changes visible in phase contrast microscopy is to separate the illuminating background light from the specimen scattered light, which make up the foreground details, and to manipulate these differently.
Fluorescence Microscopy

- Fluorescence microscopy is currently the most important tool for visualizing biological structures at the subcellular scale.

- The combination of fluorescence, which enables a high imaging contrast, and the possibility to apply molecular labelling, which allows for a high imaging specificity, make it powerful imaging modality.

- Excitation light of a certain wavelength is projected onto the specimen via the objective lens of the microscope. The light is absorbed by the fluorescent labels and reemitted at a larger wavelength.
Main Challenge

- Diffraction is the slight bending of light as it passes around the edge of an object. The amount of bending depends on the relative size of the wavelength of light to the size of the opening. If the opening is much larger than the light's wavelength, the bending will be almost unnoticeable.

- Diffraction pattern of red laser beam made on a plate after passing a small circular hole in another plate

\[ d = \frac{\lambda}{2 \cdot n \cdot \sin \theta} \]

- \( d \) is the resolvable feature size
- \( \lambda \) is the wavelength of light,
- \( n \) is the index of refraction of the medium being imaged in, and
- \( \theta \) is the half-angle subtended by the optical objective lens.
Limit of Resolution

The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities. An example of this important concept is presented in the figure below (Figure 1), where point sources of light from a specimen appear as Airy diffraction patterns at the microscope intermediate image plane.

The limit of resolution of a microscope objective refers to its ability to distinguish between two closely spaced Airy disks in the diffraction pattern. Three-dimensional representations of the diffraction pattern near the intermediate image plane are known as the point spread function, and are illustrated in the lower portion of Figure 1. The specimen image is represented by a series of closely spaced point light sources that form Airy patterns and is illustrated in both two and three dimensions.
Spatial Resolution of Biological Imaging Techniques

- Small Molecule: 1 nm
- Protein: 10 nm
- Virus: 100 nm
- Bacteria: 1 μm
- Cell: 10 μm
- Hair: 100 μm
- Ant: 1 mm
- Mouse Brain: 1 cm
- Mouse: 10 cm

- PET
- MRI and Ultrasound
- Optical Coherence Tomography
- Widefield and TIRF Microscopy
- Confocal Microscopy
- 4Pi and i3M
- High Resolution Structured Illumination
- Ground State Depletion (GSD)
- Saturated Structured Illumination (SSIM)
- Stimulated Emission Depletion (STED)
- PALM, FPALM and STORM
- Near-Field (NSOM)
- Electron Microscopy

Figure 1
Main Challenge

- This is sufficient for imaging many subcellular structures, it is insufficient for providing an image of the molecular machinery that underlies the functioning of the cell.

- Electron microscopy can reveal image detail on the order of nanometers but does not allow live-cell imaging nor efficient specific labelling.
A Simple Illustration

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
A Simple Illustration

<table>
<thead>
<tr>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
<th>$X_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_5$</td>
<td>$X_6$</td>
<td>$X_7$</td>
<td>$X_8$</td>
</tr>
<tr>
<td>$X_9$</td>
<td>$X_{10}$</td>
<td>$X_{11}$</td>
<td>$X_{12}$</td>
</tr>
<tr>
<td>$X_{13}$</td>
<td>$X_{14}$</td>
<td>$X_{15}$</td>
<td>$X_{16}$</td>
</tr>
</tbody>
</table>
A Simple Illustration

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
4 Equations and 16 Unknowns
Not solvable (many solutions)

\[ X_1 + X_2 + X_5 + X_6 = 7 \]
\[ X_3 + X_4 + X_7 + X_8 = 8 \]
\[ X_9 + X_{10} + X_{13} + X_{14} = 7 \]
\[ X_{11} + X_{12} + X_{15} + X_{16} = 8 \]
A Simple Illustration

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
4 Equations and 12 Unknowns
Not solvable (many solutions)

\[
\begin{align*}
X_2 + X_3 + X_6 + X_7 &= 9 \\
X_4 + X_8 &= 3 \\
X_{10} + X_{11} + X_{14} + X_{15} &= 9 \\
X_{12} + X_{16} &= 3
\end{align*}
\]
A Simple Illustration

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4 Equations and 12 Unknowns
Not solvable (many solutions)

\[ X_5 + X_6 + X_9 + X_{10} = 9 \]
\[ X_{13} + X_{14} = 3 \]
\[ X_7 + X_8 + X_{11} + X_{12} = 9 \]
\[ X_{15} + X_{16} = 4 \]
A Simple Illustration

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Observations
4 Equations and 9 Unknowns
Not solvable (many solutions)

\[ X_6 + X_7 + X_{10} + X_{11} = 12 \]
\[ X_{14} + X_{15} = 4 \]
\[ X_8 + X_{12} = 2 \]
\[ X_{16} = 2 \]
16 Equations and 16 Unknowns
Solvable (why ?)

\begin{align*}
X_1 + X_2 + X_5 + X_6 & = 7 & X_2 + X_3 + X_6 + X_7 & = 9 \\
X_3 + X_4 + X_7 + X_8 & = 8 & X_4 + X_8 & = 3 \\
X_9 + X_{10} + X_{13} + X_{14} & = 7 & X_{10} + X_{11} + X_{14} + X_{15} & = 9 \\
X_{11} + X_{12} + X_{15} + X_{16} & = 8 & X_{12} + X_{16} & = 3 \\
X_5 + X_6 + X_9 + X_{10} & = 9 & X_6 + X_7 + X_{10} + X_{11} & = 12 \\
X_{13} + X_{14} & = 3 & X_{14} + X_{15} & = 4 \\
X_7 + X_8 + X_{11} + X_{12} & = 9 & X_8 + X_{12} & = 2 \\
X_{15} + X_{16} & = 4 & X_{16} & = 2 
\end{align*}
## Recovered Values

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Localization-based superresolution microscopy. (a) The schematic shows a subcellular structure (a microtubule network) that is uniformly labeled with specific fluorophores. (b) In conventional imaging, all of the fluorophores in the sample are simultaneously excited. Due to the resolution limit of a fluorescence microscope, the resulting widefield image is poorly resolved and fails to reveal the underlying structure in the sample. (c) In localization-based superresolution microscopy, the imaging conditions facilitate activation of random subsets of fluorophores that are typically spatially well separated. These fluorophores are then localized with subpixel precision and their coordinates are used to create a superresolution image of the sample. (d) The resulting superresolution image provides fine structural information of the sample that is not accessible through a widefield image. (e) The comparison of a practical widefield image and a superresolution image. In (e), the size bar is 2 μm. In all other panels, size bars are 300 nm.
The principle behind PALM. A sparse subset of PA-FP molecules that are attached to proteins of interest and then fixed within a cell are activated (A and B) with a brief laser pulse at $\lambda_{\text{ac}} = 405 \text{ mm}$ and then image data $\lambda_{\text{exc}} = 561 \text{ mm}$ until most are bleached (C).

Eric Betzig et al. Science 2006;313:1642-1645
Conclusion

- Biology (Observations) $\rightarrow$ Physics (Principles and Techniques) $\rightarrow$
  Biology (small-scale Observations) $\rightarrow$ Chemistry (Fluorescence Techniques) $\rightarrow$
  Biology (nano-scale Observations) $\rightarrow$ Mathematics (Models, Algorithms and Computation)
References

David Bardell, The invention of the microscope, BIOS 75(2) 78-84, 2004.


http://www.microscopyu.com/articles/phasecontrast/phasemicroscopy.html

http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html

Slide is available at http://www.math.hkbu.edu.hk/~mng
Experiment

- Use mobile phone to construct a microscope, and a convex lens
- Mobile phone magnification is based on image processing technique on digital image
- Convex lens can be used to optical effect to magnify the object
Thank you very much!