1st　Journal review

Construction of nonlinear optical microscopy for clinical application

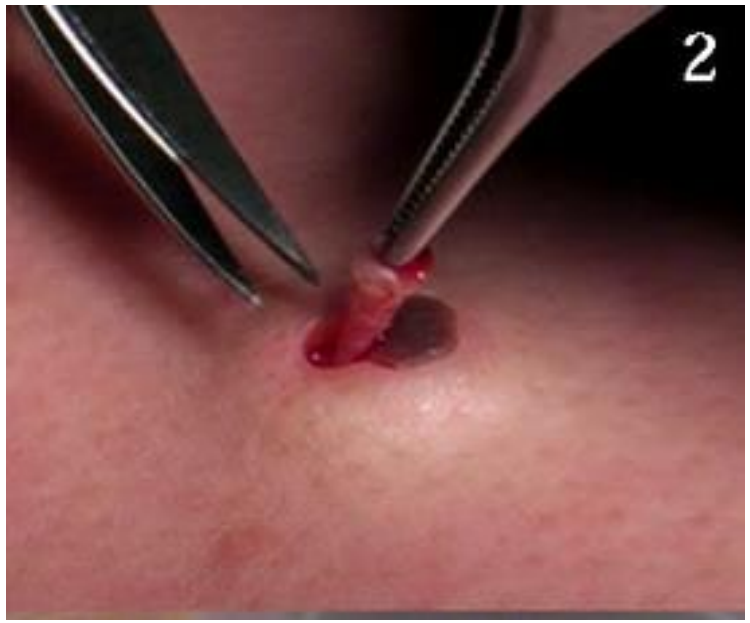
2015/06/17　M2 Atsuta

Nonlinear optical microscopy

In diagnosis, the invasive sampling of physical biopsy takes risks of trauma, infection, hematoma, and hemorrhage

⇒ Nonlinear optical microscopy

It is non-invasive and non-contact ,
so physical biopsy diagnosis is NOT needed on measurement using nonlinear optical microscopy. (SHG microscopy etc..)

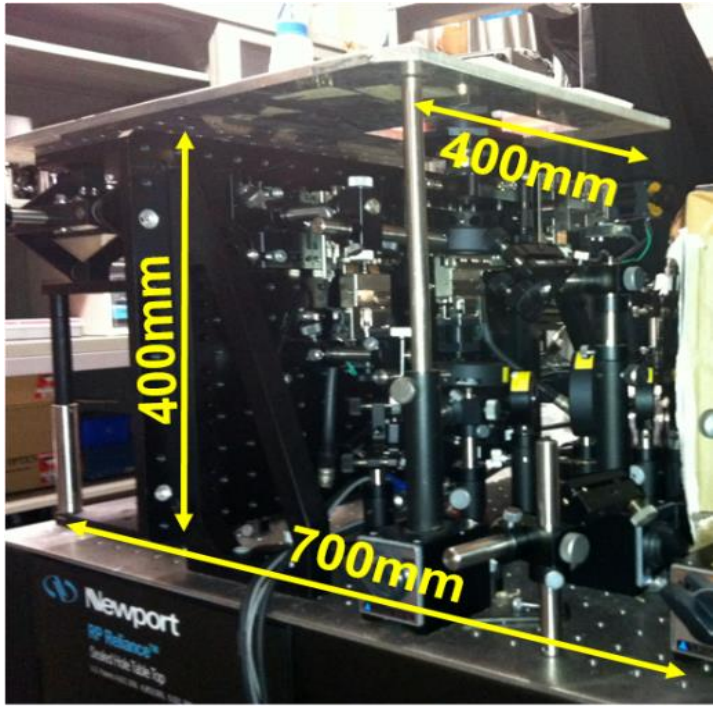


http://www.momohime-medical.com/

Nonlinear optical microscopy

For clinical application…

Traditional laboratory microscopes are large scale, inflexible, and free-space beam delivery.
So they are limited its applications.



Optical microscopes need flexible fiber-optical probe

① C. Bechtela, et al. “Large field of view MEMS-based confocal laser scanning microscope for fluorescence imaging” Optik.**125**,876 (2013)

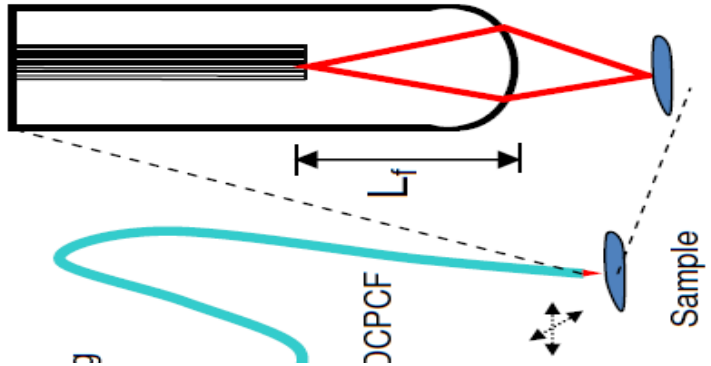
② S. Tang, et al. ”Design and implementation of fiber-based multiphoton endoscopy with microelectromechanical systems scanning” Journal of Biomedical Optics.**14**, 034005 (2009)

(Fibroblast)
③B. A. Danowski **“**Fibroblast contractility and actin organization are stimulated by microtubule inhibitorsl” Journal of Cell Science .**93**, 255 (1990)

① “Large field of view MEMS-based confocal laser scanning microscope for fluorescence imaging”

Introduction Fluorescence confocal laser scanning microscopy

F-LSM is now a widely used and powerful imaging technique in fundamental biological and medical research.



Recently…

Fibre scan microscopy :

○ thin probe system ⇒ locally imaging for cell etc…

× restricted field of view ⇒ unsuitable for skin imaging

space saving, moderately prized sys-tem with a large field of view

Using MEMS mirror

the MEMS mirror

f_{slow}= 200 Hz , f_{fast}= 1.336kHz

The mirror deflect ±5[deg] D = 2mm

twice the total mirror scan angle of 2φ, and the smallest resolvable spot size given by

$$\Delta\varphi = \mathrm{k}\,\lambda\,\frac{1}{D_{eff}}$$

Here, k : denoting the aperture shape factor

D_{eff} : effective mirror diameter

The number Nθ of resolvable spots for the scanner is given by the ratio of the total optical scan angleθ

$$N_\theta = \frac{\theta\ D_{eff}}{k\ \lambda}$$

Here, λ= 520nm

$$N_\theta \approx 990$$

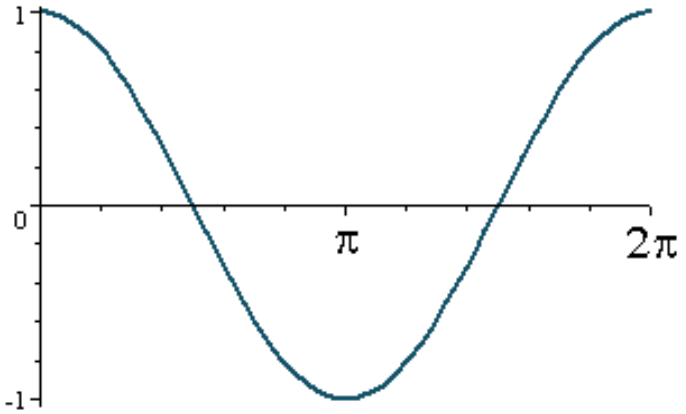
the MEMS mirror

f_{slow}= 200 Hz , f_{fast}= 1.336kHz

The mirror deflect ±5[deg] D = 2mm

MEMS mirror scan the spot very fast…

So, pixel dwell time has a great impact on the number of photons available for detection.



$$t = \frac{1}{\pi f_{fast} N_{\theta}}$$

π : the period of one line scan

If, number of photons unavailable for detection,
(As resonant frequency f_{fast} can't change.)
We must change to large number of pixel or high peak power to increase photons.

Field of view

Spot diameter and the Rayleigh range

$$\omega_0 = 0.5k\ \lambda_{ex}\ f/\# = 0.49\ \mu m$$

$$Z_R = \frac{\pi}{\lambda_{ex}}(\omega_0)^2 = 1.54\ \mu m$$

It wants to expand the beam system to 7mm for aperture diameter.
As M = 7, Field of view is

$$d_{im} = 2\ f\ \tan(2\frac{\varphi}{\mathrm{M}}) = 500\ \mu m$$

Here, ϕ represents the scanning angle of 5 °

Optical design

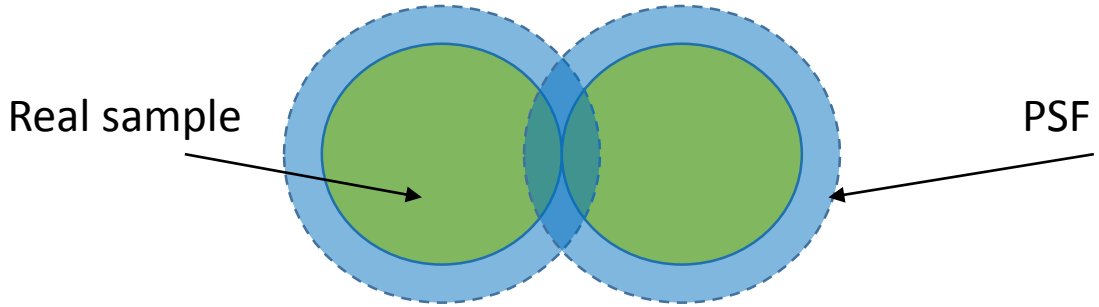
The strehl ratio

It represents the smallness of aberration

0.8 is the diffraction limit

Point Spread Function (PSF)

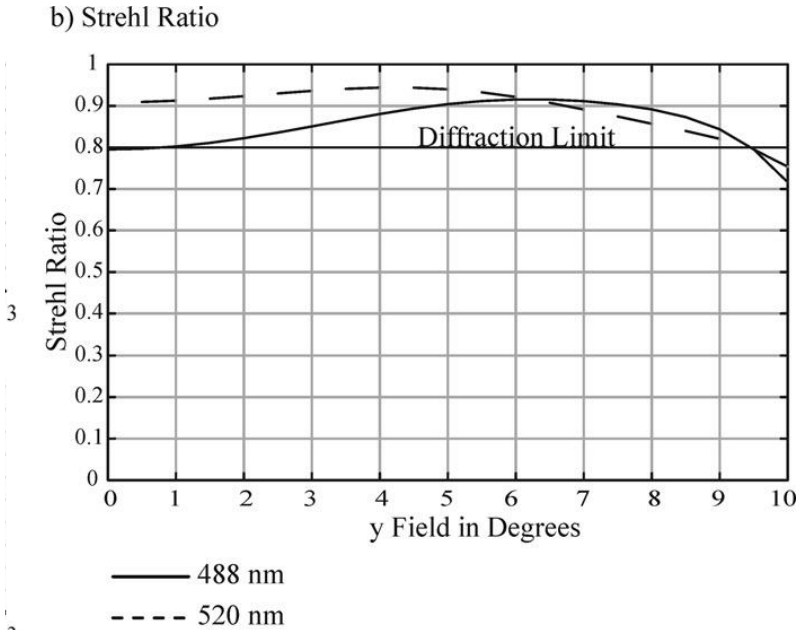
(Image) = (light spot) + (PSF)

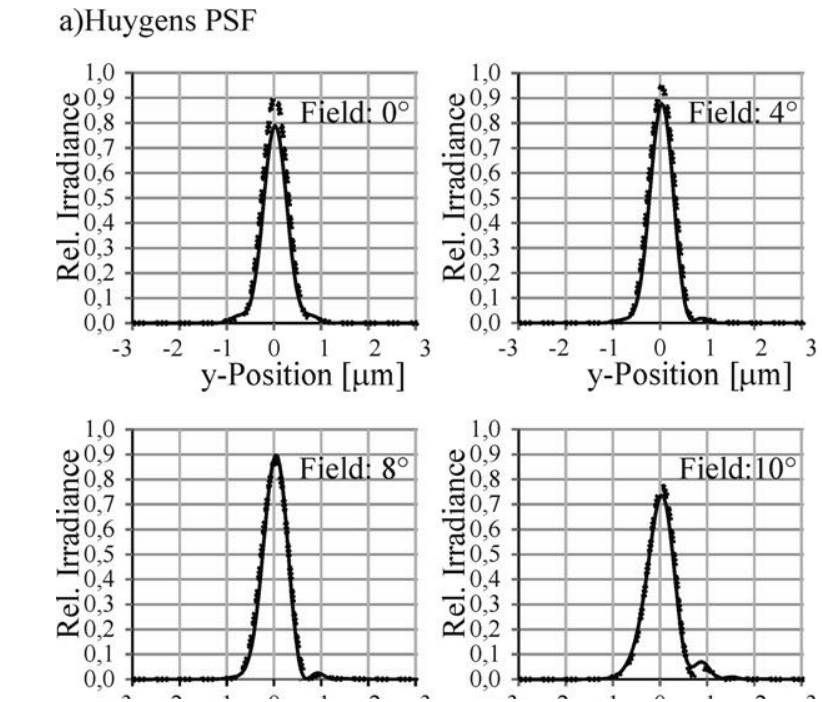


the maximum angle of 10◦

the spot size is around 1.2µm.

This can identify until 6µm.





Wave aberration from OPD(optical path Difference)

in tangential and sagittal direction

Coma aberration occurs between the scan lens and MEMS mirror

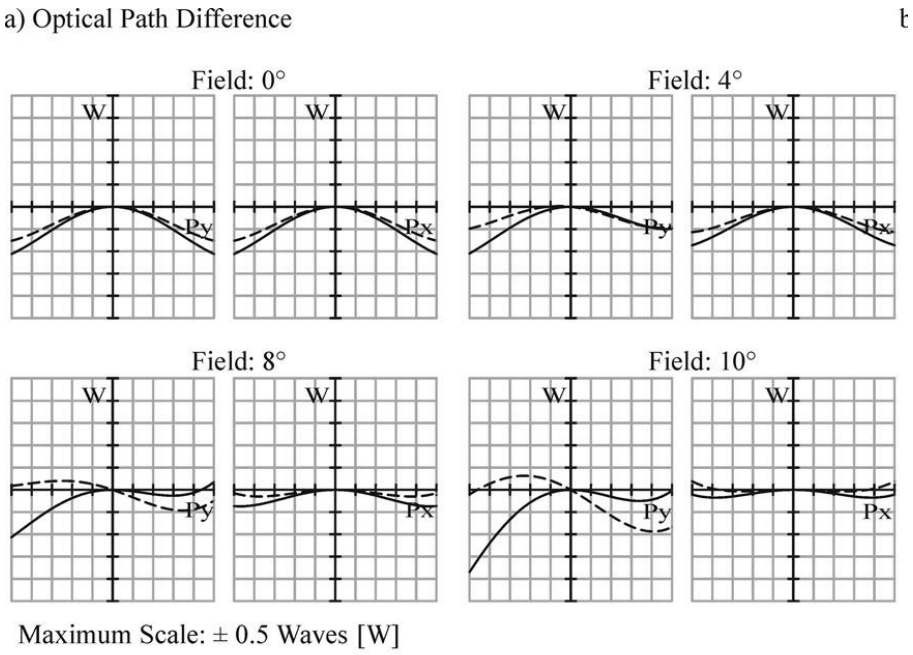
Field curvature

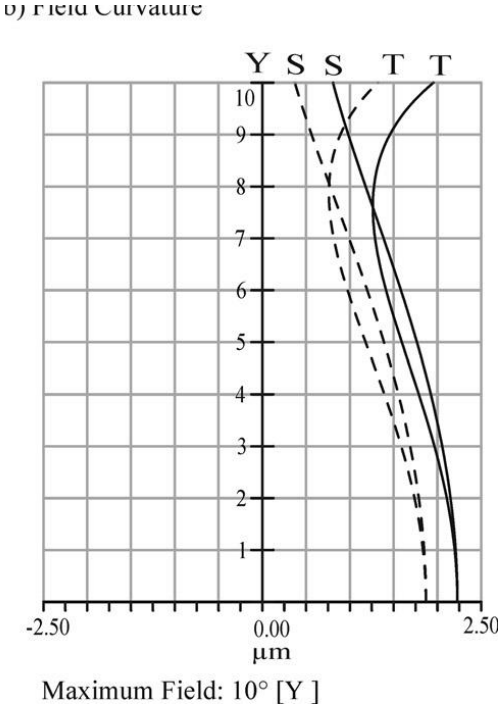
Field curvature in tangential and sagittal direction

coma aberration > spherical aberration.

And another characteristic of the system important to look at is the field curvature

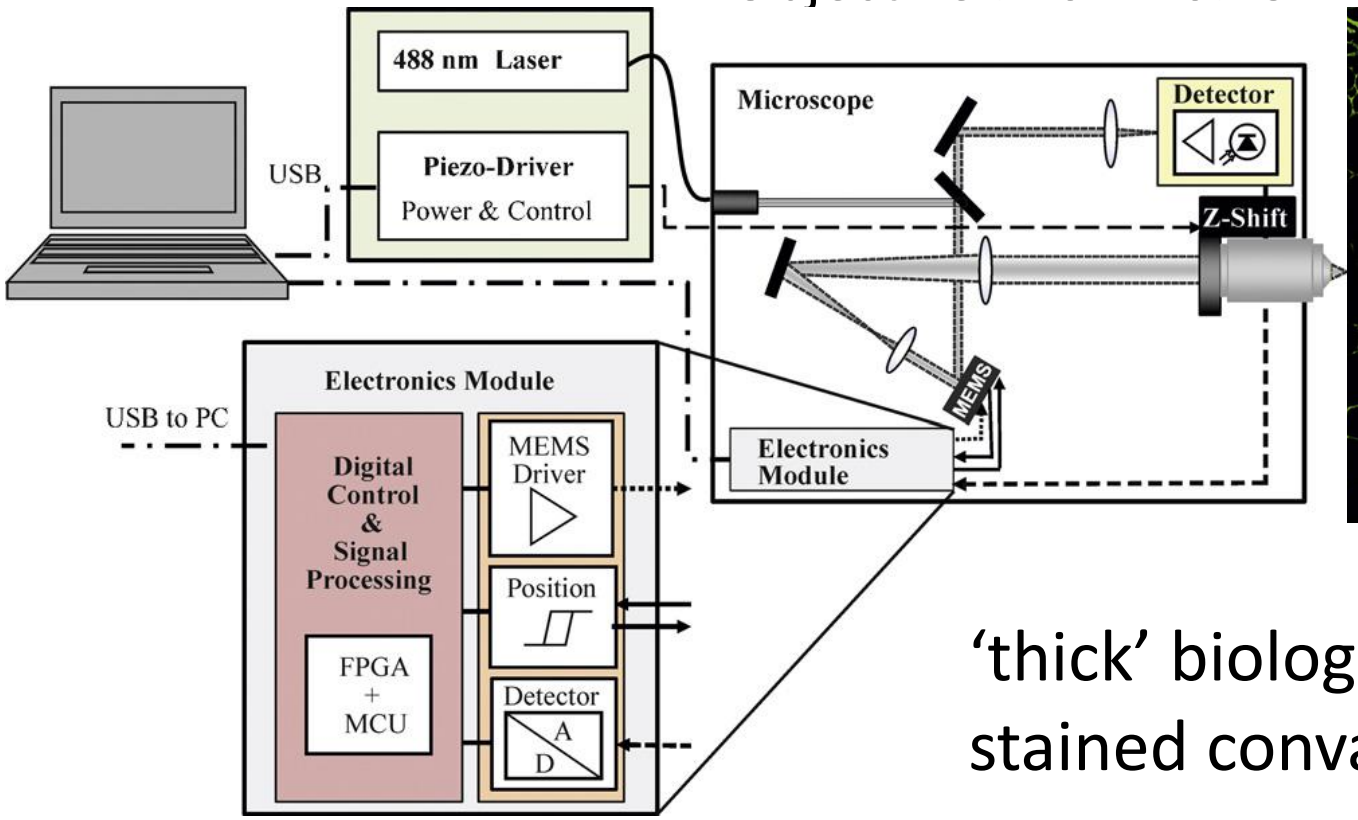
In total these aberrations restrict the maximum scanning angle to ±10◦. ⇒ this design is expected to application of cell imaging.

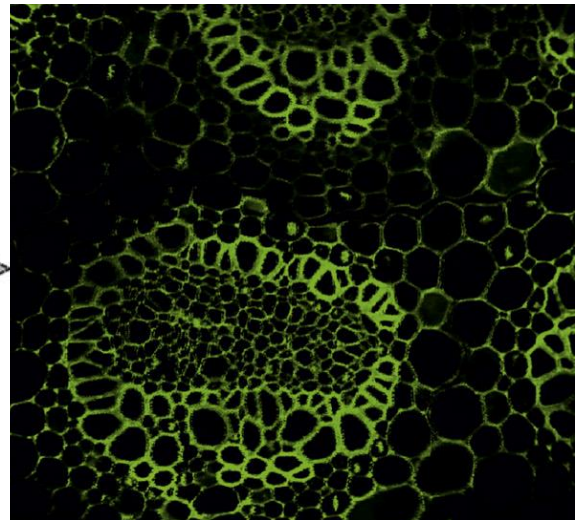


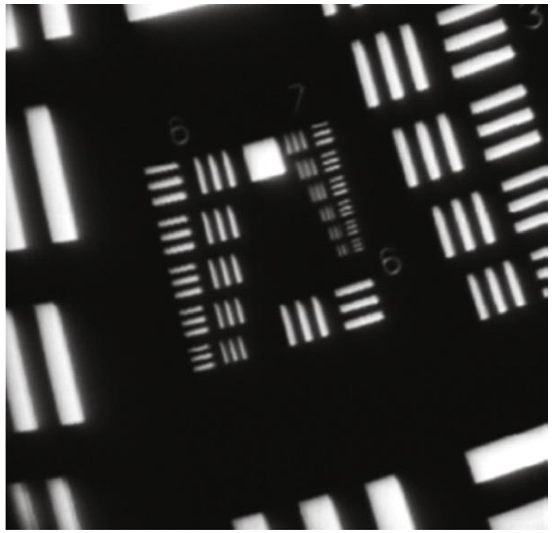


Setup and results

Objective : 20 × 0.75







‘thick’ biological samples of fluorescent stained convalaria are imaged

In TC, group 7 has 228 line pairs/mm, corresponding to a line width of 2.2µm.

summary

Confocal fluorescence laser scanning microscopes are well established and widely used in biological and medical research.

First measurements have shown a lateral and axial resolution of 2.2µm and 3µm, respectively with a field of view of 500µm × 500µm.

②”Design and implementation of fiber-based multiphoton endoscopy with microelectromechanical systems scanning”

Introduction

Delivering femtosecond pulses through fibers and designing miniature scanning probes are two challenges in MPM endoscopy.

These problems were solved by
DCPCF(Double-cladding photonic crystal fibers) and MEMS mirror.

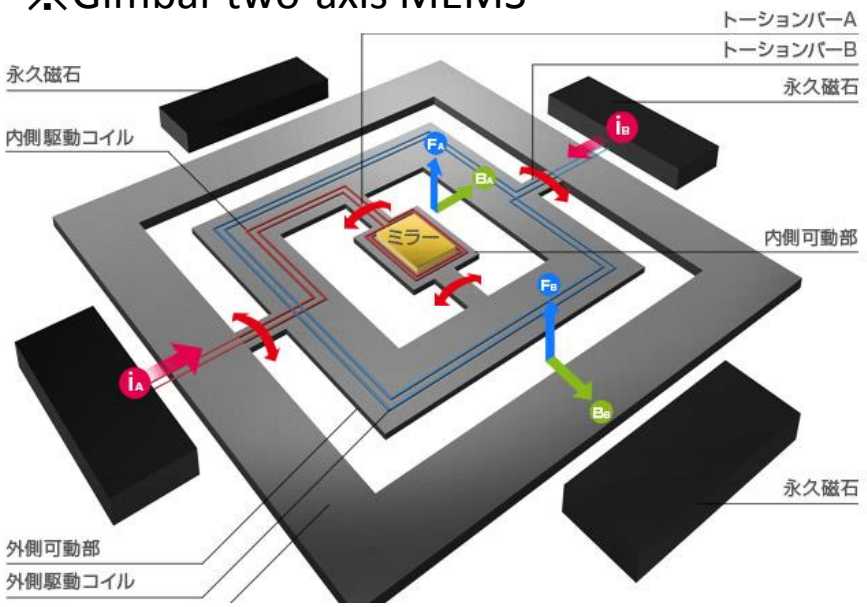
① Comparing SMF, hollow-core PBF, and DCPCF
② Three configurations of probe design are discussed,
and their advantages and disadvantages are compared.

Setup:

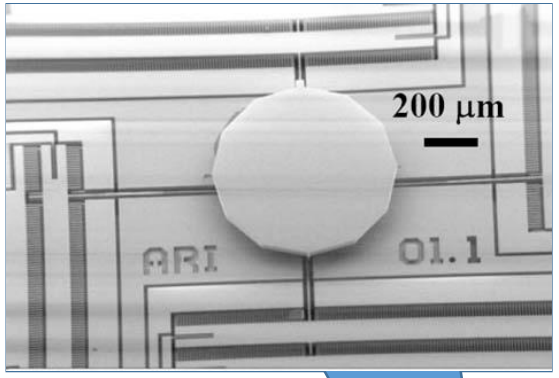
MEMS mirror

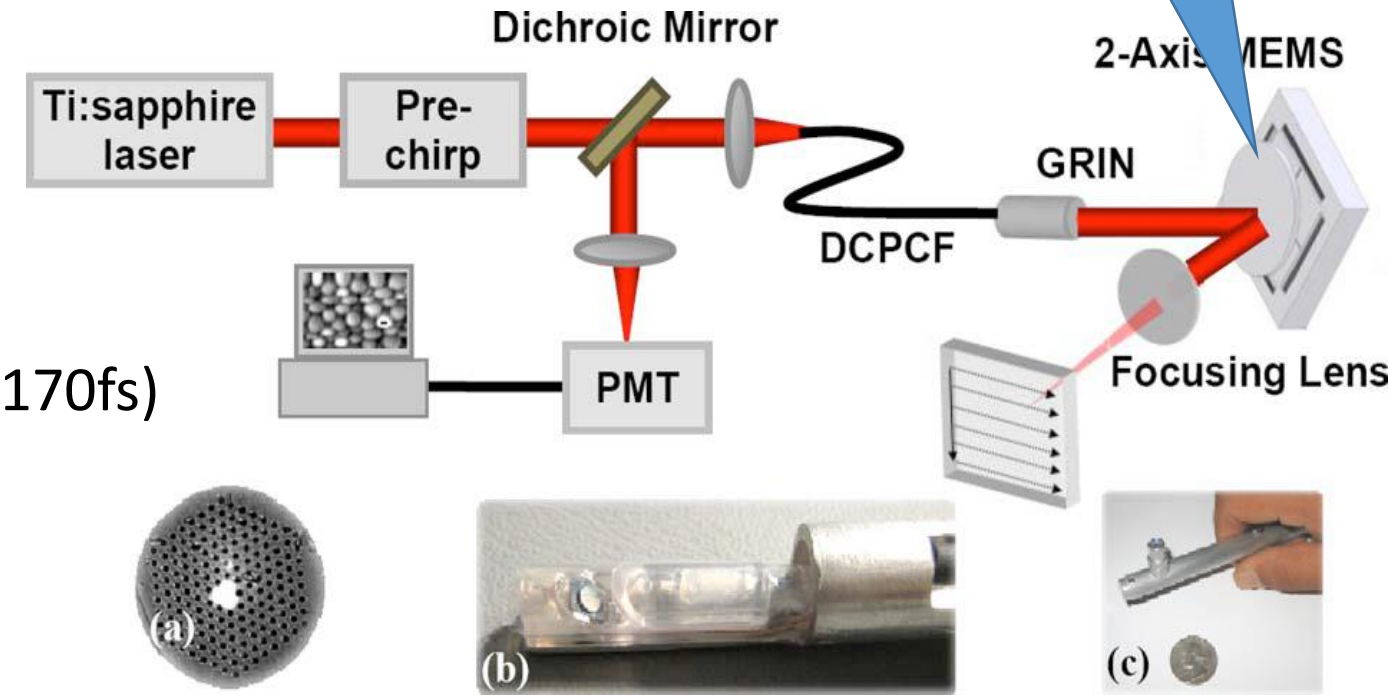
Gimbal-less
Mirror diameter 2mm
Al coat (reflectance 80%)

※Gimbal-two-axis MEMS



A gimbal-less two-axis MEMS



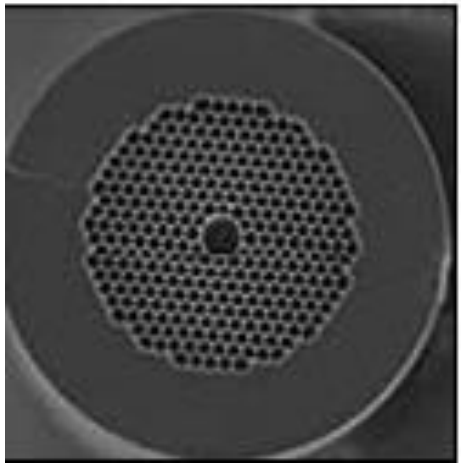


The light source,
Ti: S laser (790nm, bandwidth 10nm,
pulse width 170fs)

Objective (5 ×, NA = 0.1)
Coupling efficiency of 30%

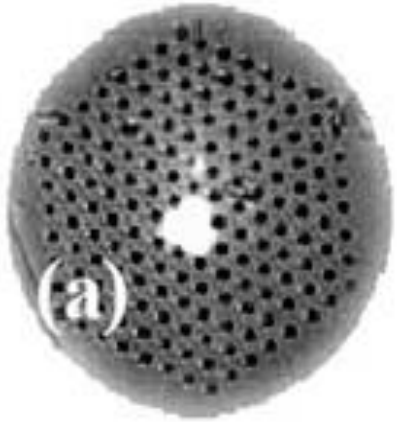
Fiber delivery

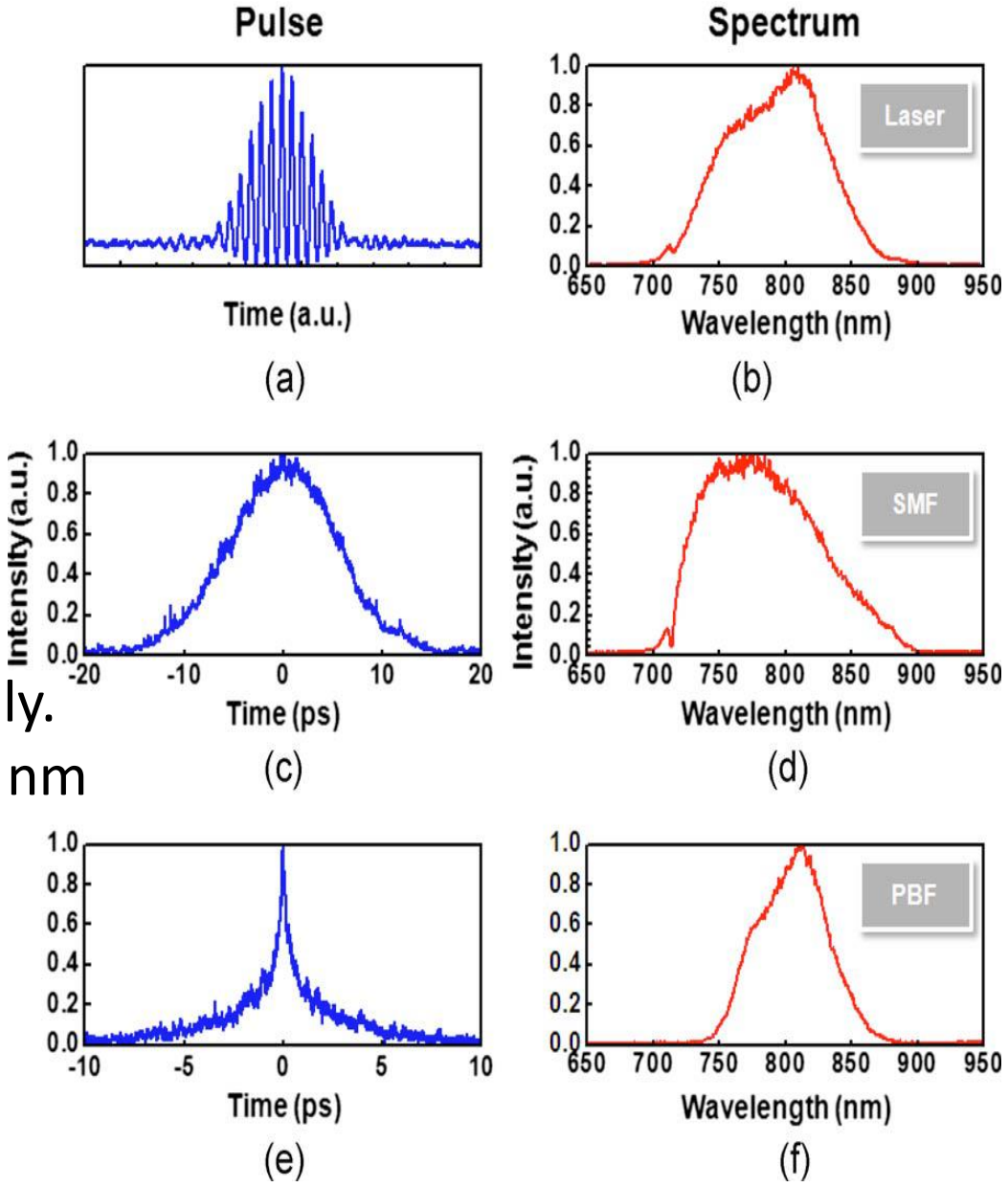
(c)(d)SMF
Signal attenuation is negligible
Laser bandwidth increase



(e)(f)Hollow-core PBF
~20 times lower dispersion than SMF
Inside low-loss window, attenuation is low
but outside the window, attenuation increases rapidly.
(window: 90 nm around a center wavelength of 800 nm

⇒DCPCF
Signal attenuation is negligible
A good collection efficiency with core and the inner clad





Design:

Design 1　the simplest case

○ easy alignment and packaging as well as size efficiency

× long WD : poor resolution at the focal point.

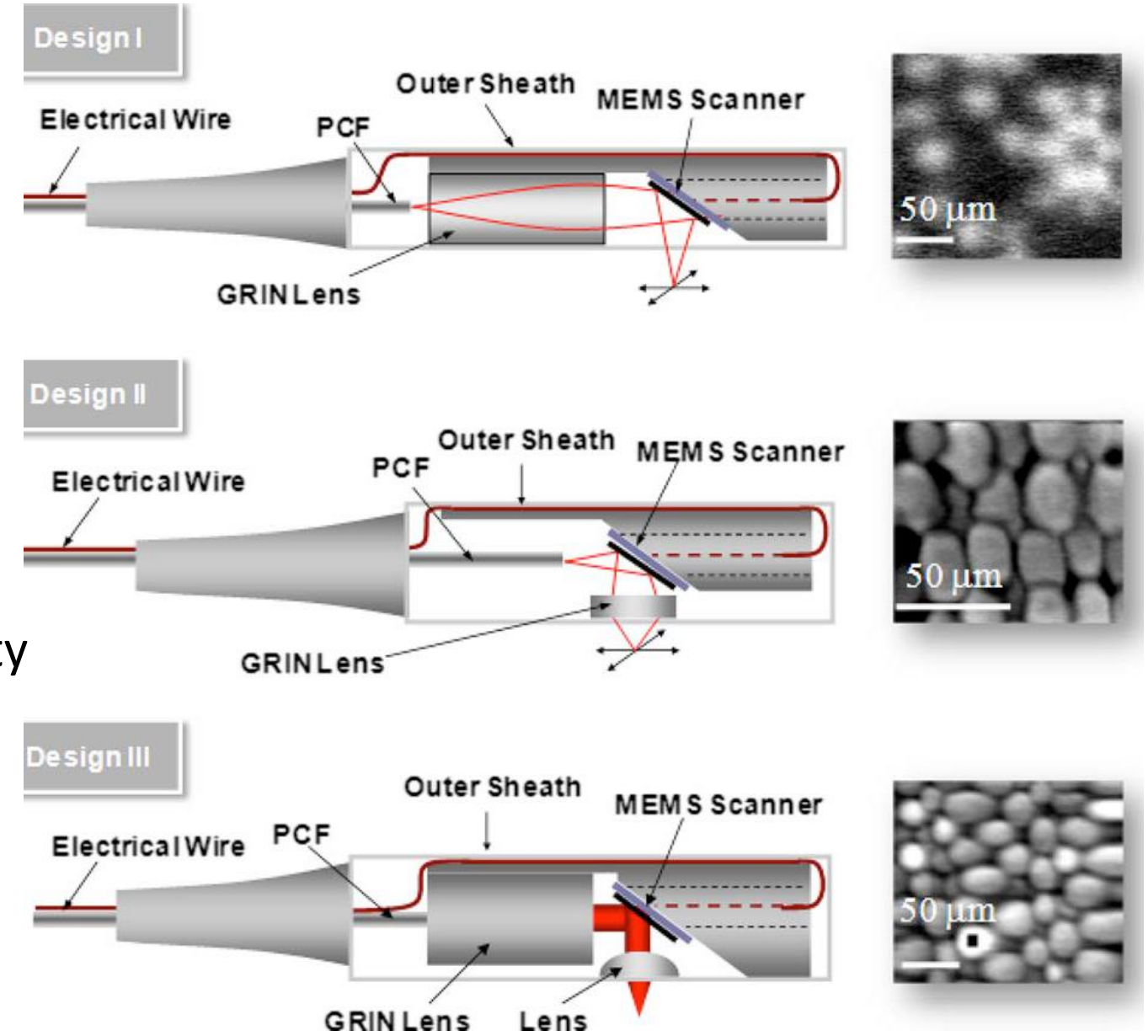
Design 2

○ short WD

× beam are restricted ⇒ lack of flexibility

Design 3

○ provide a collimated beam ⇒ good flexibility

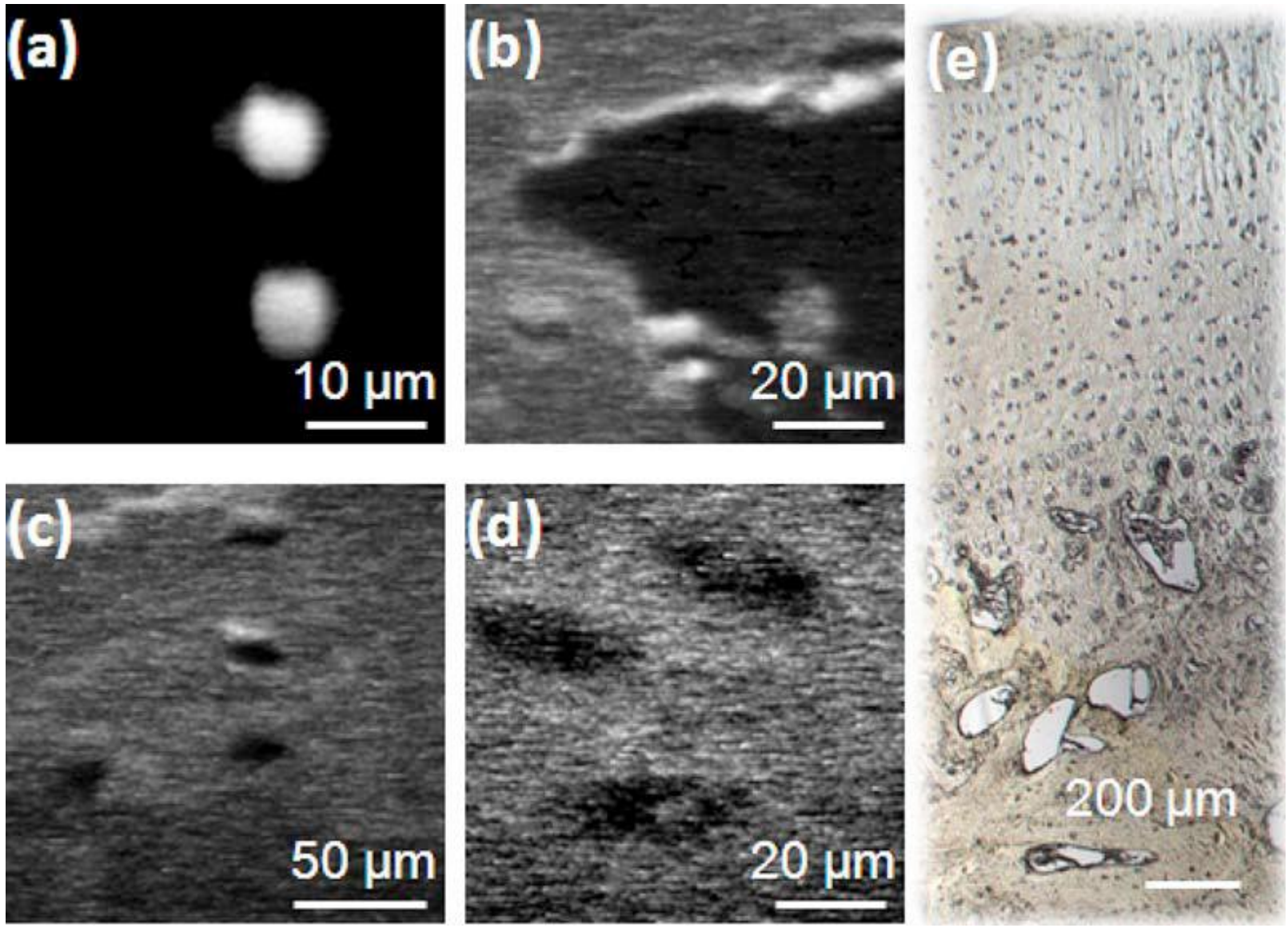


Result

The Fluorescence image
128 * 128 pixels
Frequency, 64Hz and 0.25Hz
(multiphoton fluorescence
is low signal)

Sample :
(a) 6μm beads;
(b) bone structure
(c) and (d) chondrocytes;

(e) white light microscope photo showing the bovine knee joint cartilage sample.



summary

Compares the three types of performance of the fiber, it was demonstrated pulses propagating and high collection efficiency ability of DCPCF.

- Compares the three probe design, it was found configuration that provides flexibility for optimum imaging performance and packaging with collimating and focusing lens.

③“Fibroblast contractility and actin organization are stimulated by microtubule inhibitorsl”

Introduction

The locomotion of fibroblasts and other tissue cells results from their exertion of contractile 'traction‘ forces on objects and materials

⇒ These cells traction are known to be produced by a cytoplasmic actin(SF : stress fiber) and myosin network

Several lines of evidence have implicated microtubules as playing some controlling role in the motility of tissue cells ;

microtubule-depolymerizing drugs : cause regression of growth

⇒In cancer cells, to act as an anti-cancer agent

In this study…

performed to clarify whether the microtubules to determine what role by exerting traction.

method:

・*C3H/10T1/2 mouse embryo fibroblasts*

Eagle's MEM with Hanks' salts, supplemented with 10 % fetal calf serum, penicillin, streptomycin, and 7-5mM-Hepes.

Cells were removed from tissue culture flasks by a brief treatment with trypsin-EDTA solution, and plated onto either glass coverslips or silicone rubber substrata.

Cells were used for experimentation 1-5 days after plating.

Experimental equipment

Time-lapse filming

Kodak Plus-X Reversal film, using a Sage time-lapse apparatus attached (Olympus)

Immunofluorescence

・Zeiss 63 X, 1-4 n.a. Planapo objective on a Zeiss IM-35 inverted microscope equipped for epi-fluorescence.

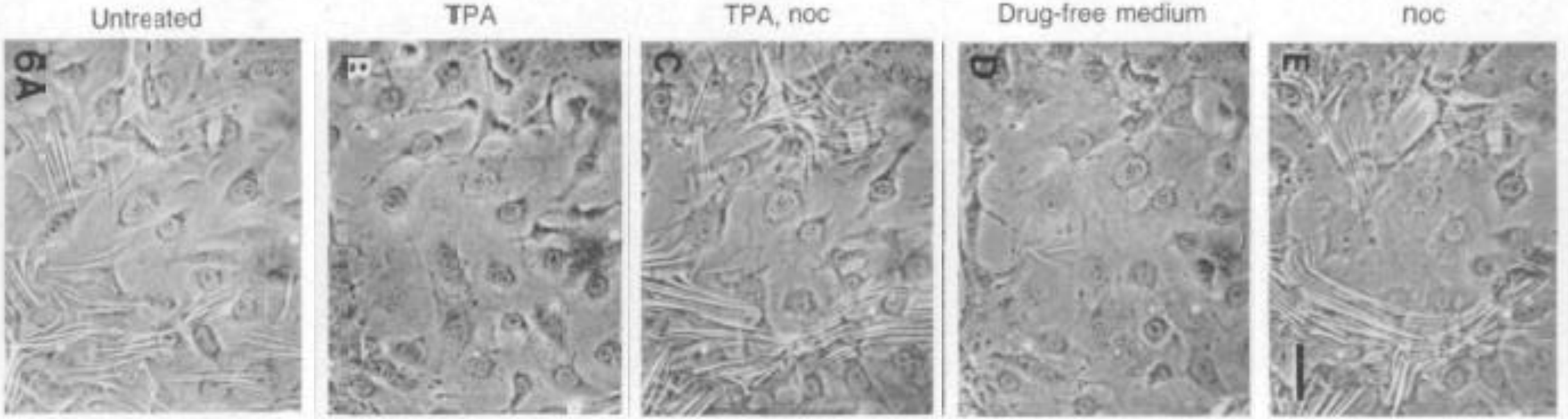
・Single-labeling of f-actin and Double-labelling of actin and tubulin:

Result and Discussion

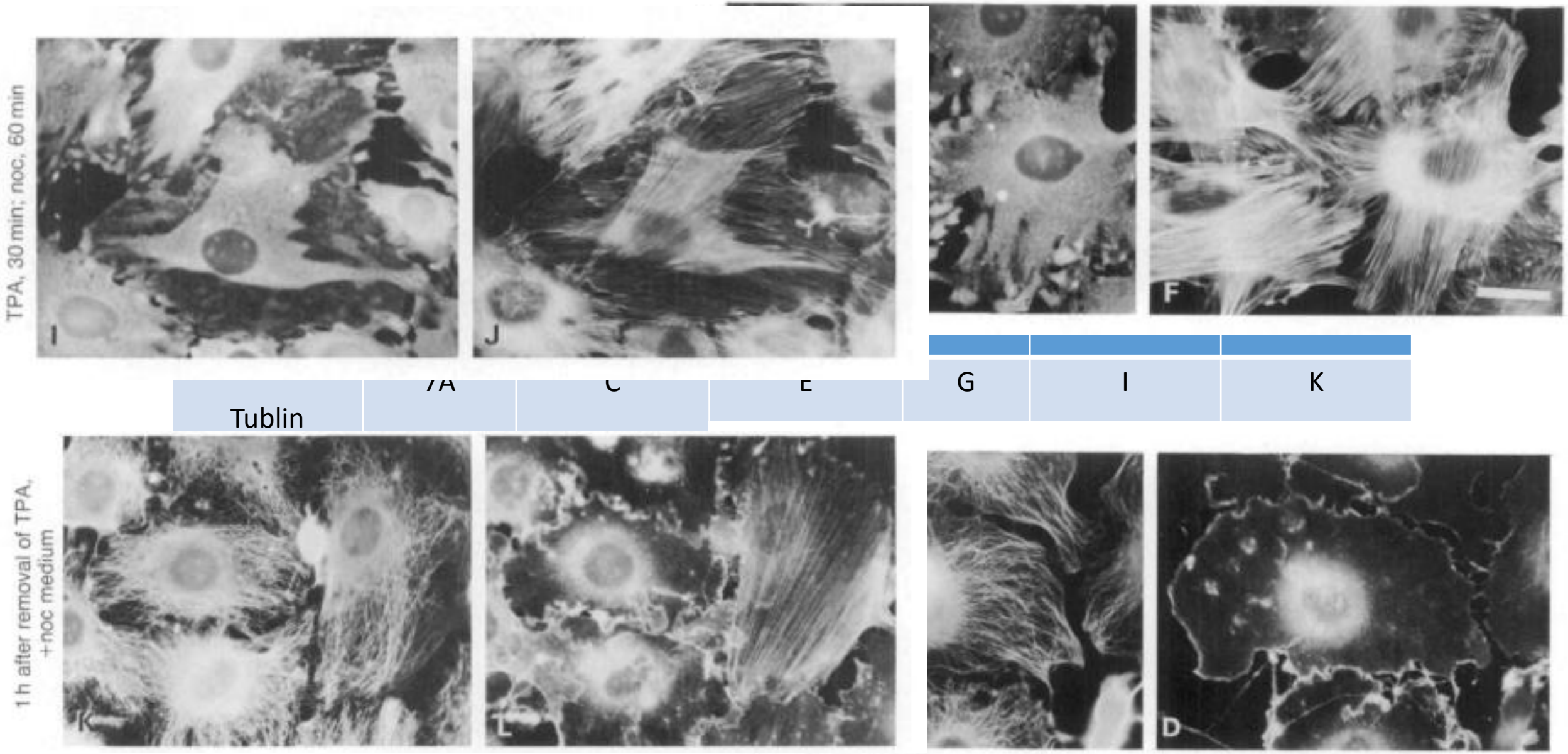
Tractive force is evaluated by the number and size of wrinkles in the rubber.

TPA : Tumor marker (But it is normal cell, too)
Noc : Stabilizers

Colcemid : anticancer drug
VB (vinblastine)



Result and Discussion



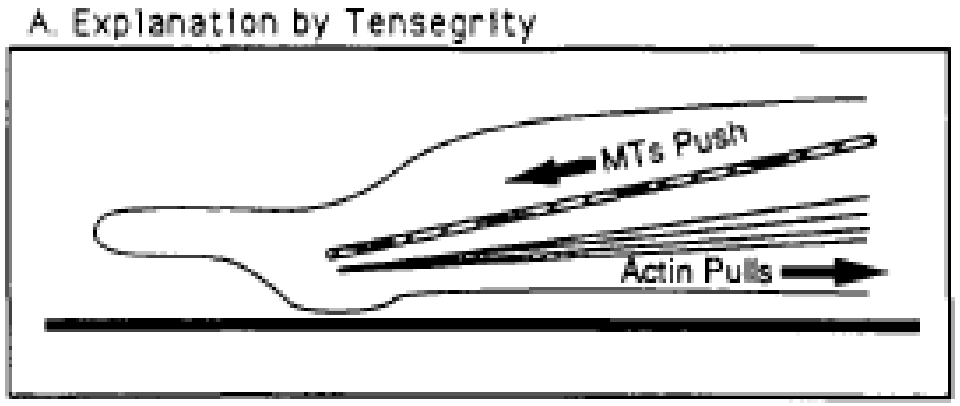
Tublin	7A	C	E	G	I	K

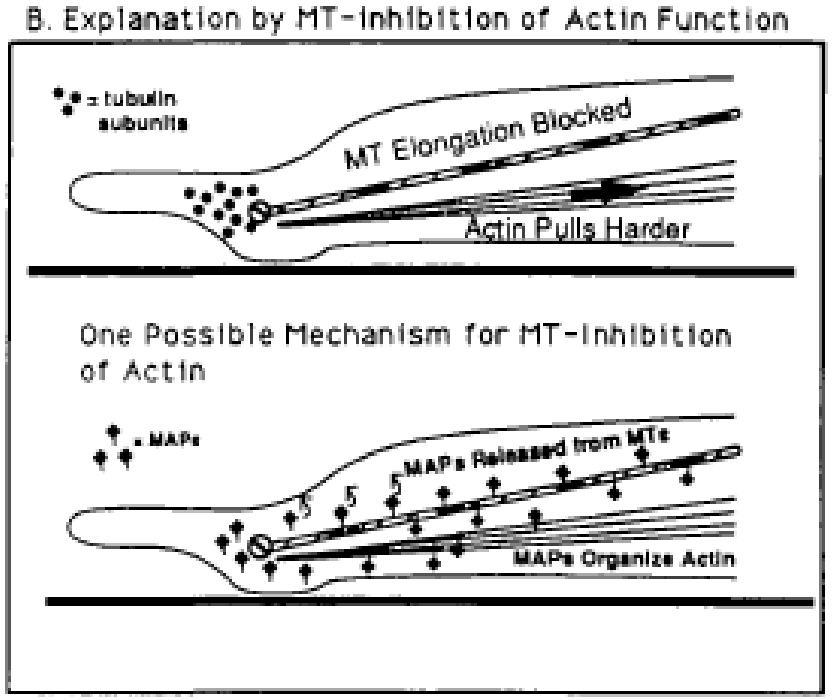
summary

Rather than a gradual change of fibroblasts, abrupt and change
Recovery of stress fibers was unexpected.

Cytoplasmic Microtubules, it is to exert a large pressing force, partially offset the pull of actin stress fibers.

Microtubule-associated protein is secreted, it interacts with actin SF.





my opinion

- MEMS mirror can be expected wide field of view.
- There is a need for strict design for further miniaturization.
- Old study of fibroblast cells was determined by appearance
  ⇒ Quantitative evaluation is necessary

That’s all.