九州大学 エネルギーウィーク 2022
Energy Week 2022

九州大学 KYUSHU UNIVERSITY

Q-PIT Kyushu University Platform of Inter-/Transdisciplinary Energy Research

微小流路による微小管集団運動の制御

Control of Microtubules Collective Motion by Microflow Channel

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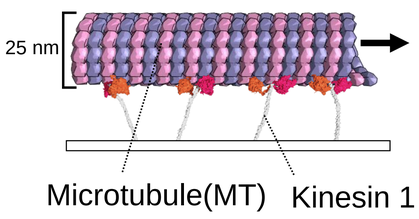
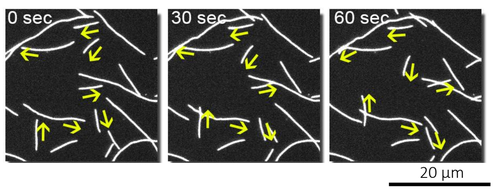
Introduction

In vitro gliding assay

+ATP (Chemical energy)

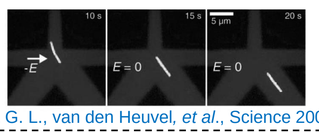




Kinesin motor protein propels MTs by converting the chemical energy of ATP into mechanical work.

ATP driven micro transportation devices

T. Fischer, *et al.*,Nat. Nanotechnol. 2009

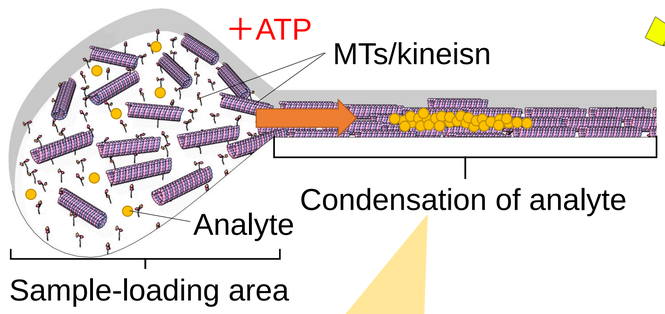


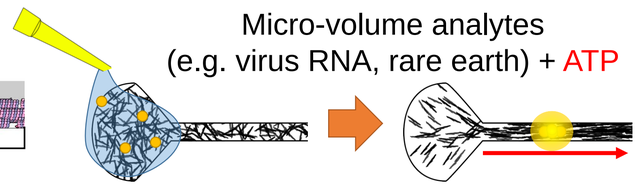
M. G. L., van den Heuvel, *et al*., Science 2006

MT/kinesin system can transport and organize material at their size scales at high energy efficiency.

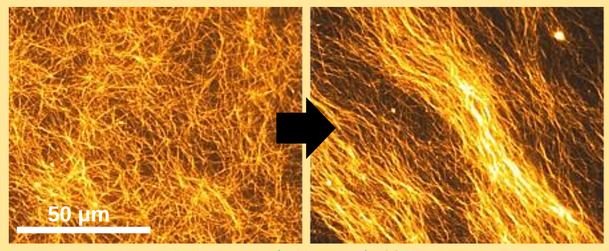
Microflow channel can guide moving direction of MTs.

Micro-biosensor device activated by ATP





Concentration and detection of diluted target analytes by MT local accumulation induced by collective motion



D. Inoue, et al., Nanoscale 2015

Collective motion

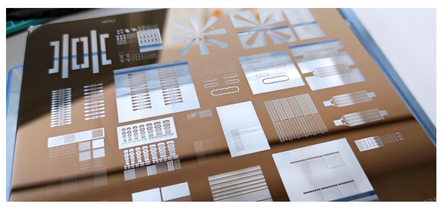
Highly-dense gliding MTs autonomously organize directed flow and increase their local density.

Aim of project

To develop a microdevice to concentrate analytes by controlling microtubule collective motion.

Materials & Methods

Photomask (Toppan)



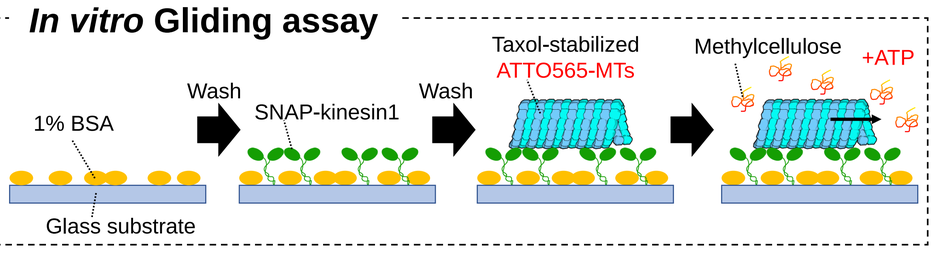
Cheap LED UV light (Jaxaman)

Wavelength: 365 nm
Radiance: 1600 mW/sr/m^2

Available resins to make microflow channel

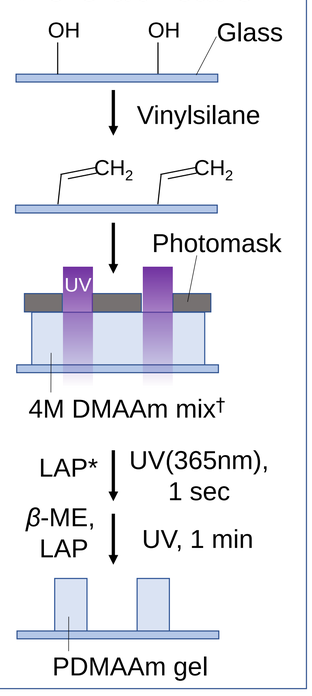
Materials	Descriptions
N,N-dimethylacrylamide (DMAAm)	**Nonionic, neutral hydrogel** Less interaction with proteins. Low toxicity unlike acrylamide. Weak bond with glass substrate.
Norland Optical Adhesive (NOA61)	**UV responsive epoxy resin** Less steps for preparation Strong bond with glass substrate Non-specific interaction with proteins.

In vitro Gliding assay



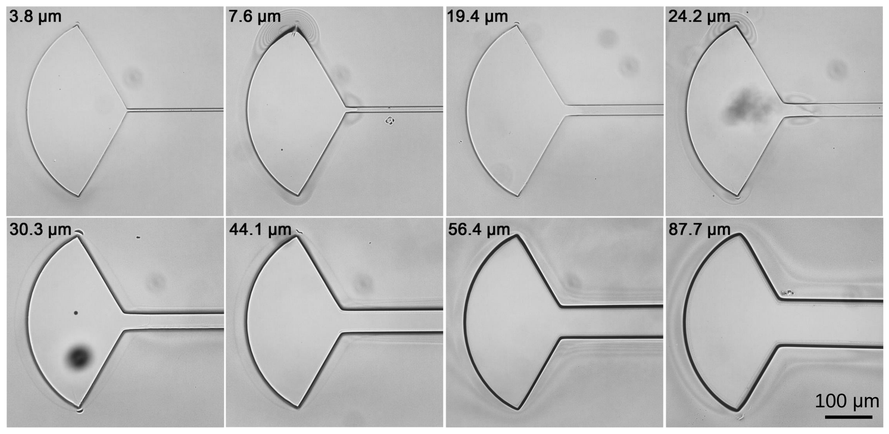
Experiment 1: Poly(N,N-dimethylacrylamide) gel

Microfabrication

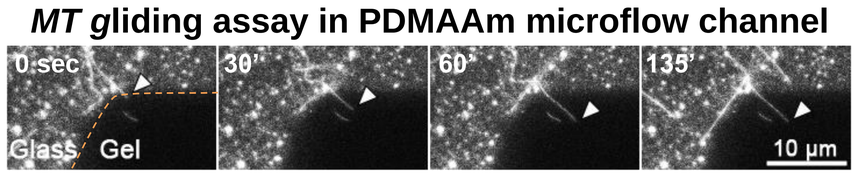


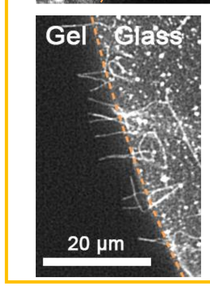
†4M DMAAm, 4mol% MBAA, 0.05mol% LAP, 53.6% Glycerol

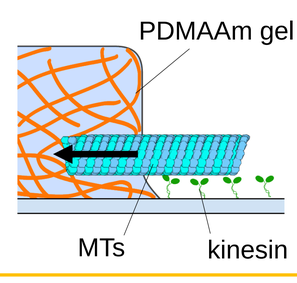
*LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate)
→ Water-soluble photoinitiator to start radical polymerization of the gel



MT gliding assay in PDMAAm microflow channel



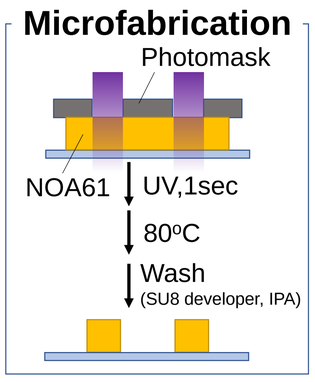


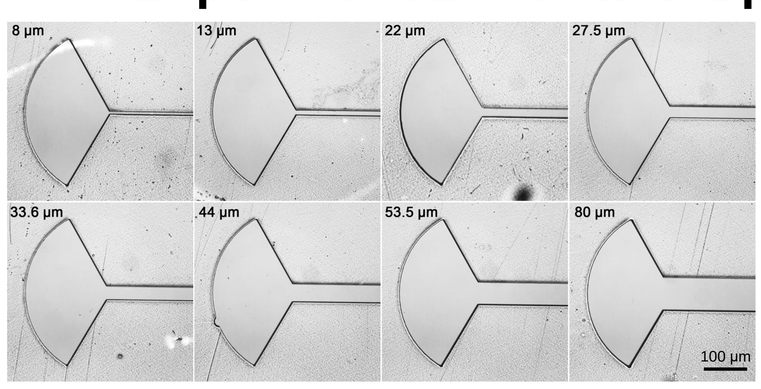


MTs stuck in the soft hydrogel wall.
→Gel is not suitable Material to guide MTs.

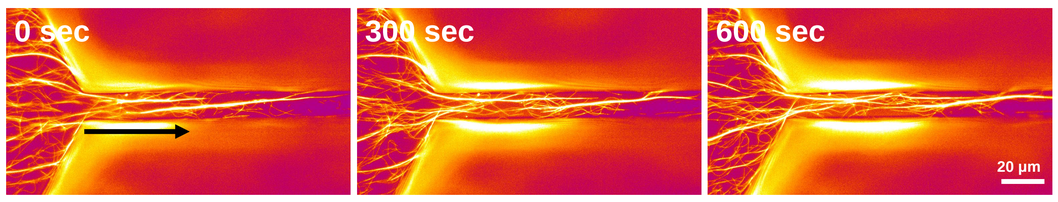
Experiment 2: Norland Optical Adhesive 61 (NOA61)

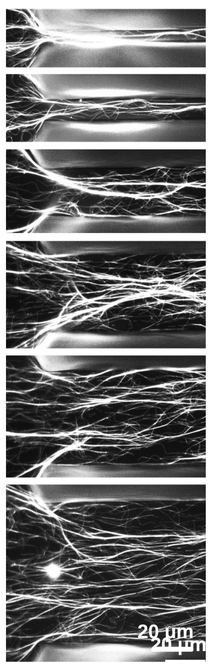
Microfabrication

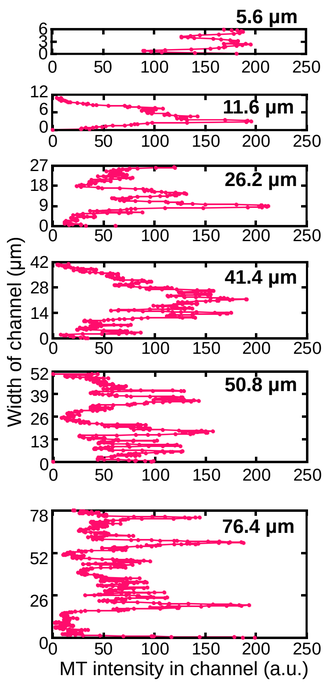




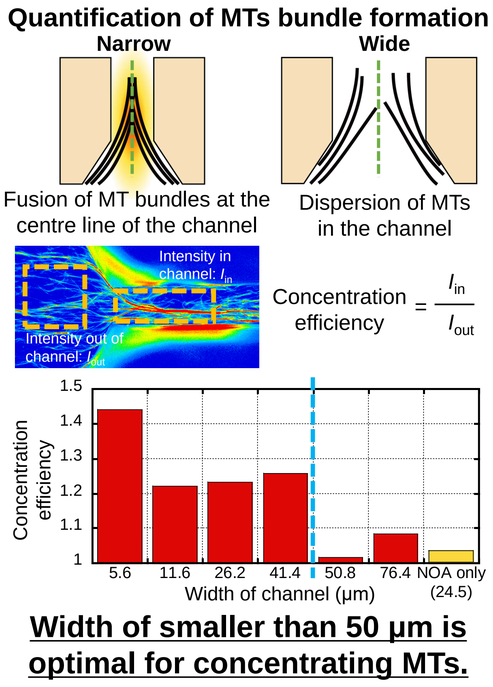
Dense bundle formation of MTs in microflow channel





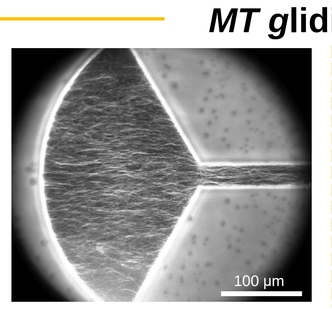


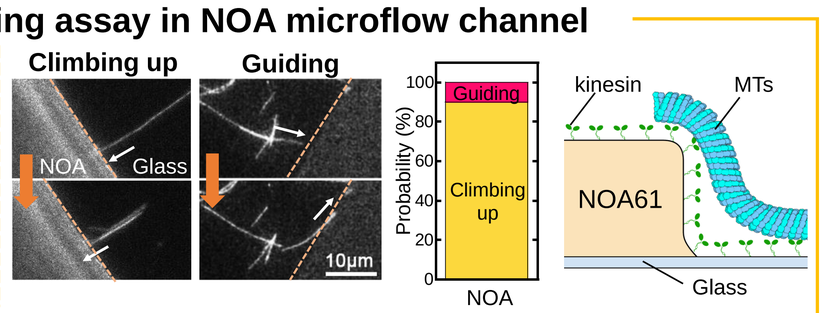
Quantification of MTs bundle formation



Width of smaller than 50 μm is optimal for concentrating MTs.

MT gliding assay in NOA microflow channel

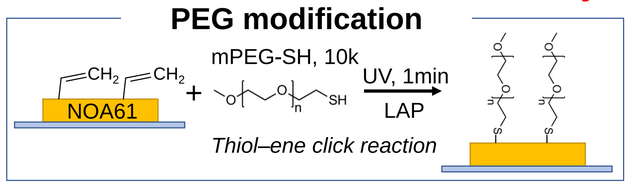


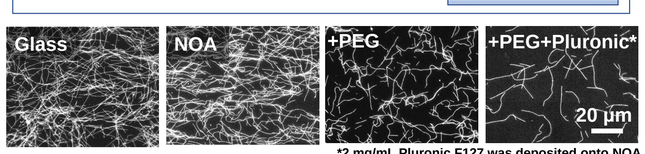


90% MTs climb up NOA wall where kinesin is absorbed→ NOA can't guide MTs.

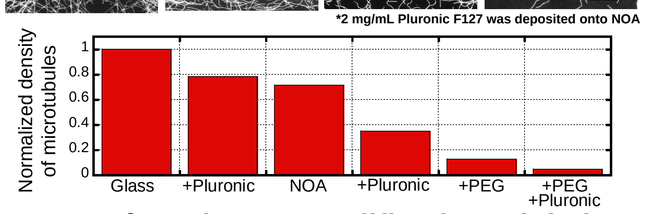
Passivation of NOA surface by using PEG & Pluronic F127

PEG modification



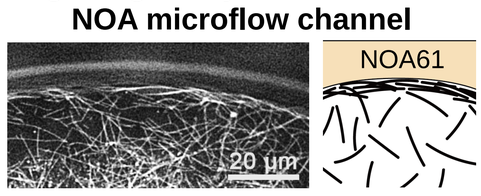


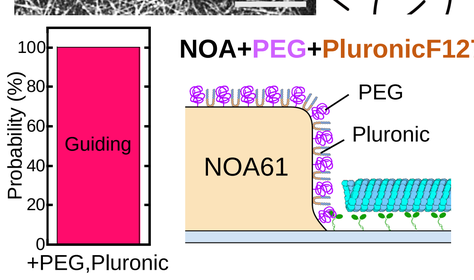
*2 mg/mL Pluronic F127 was deposited onto NOA



PEG + PluronicF127 modification minimizes kinesin-MTs absorption onto NOA.

MT gliding assay in PEG-Pluronic-NOA microflow channel





NOA+PEG+PluronicF127

PEG & Pluronic modification of NOA enables to guide MTs at 100%.

Summary

- PDMAAm gel was not available to guide gliding MTs.
- Modification of NOA61 with PEG and PluronicF127 improved guiding probability of MTs from 10% to 100%.
- Microflow channel with a certain width (<50 μm) increased the density of MTs and assemble dense MT bundles.

Next plan: Demonstration of molecular sensing of small volume analytes

Acknowledgement:

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