

Bangalore Microscopy Course: 2012

National Centre for Biological Sciences, Tata Institute for
Fundamental Research, Bangalore

September 23-30, 2012

Course Organizers

Satyajit Mayor, H. Krishnamurthy and Manoj Mathew: National Centre for Biological Sciences,
Bangalore

Nico Stuurman and Kurt Thorn: University of California, San Francisco

Faculty:

- Satyajit Mayor, NCBS, India
- Sudipta Maiti, TIFR, India
- Roop Mallik, TIFR, India
- Kurt Thorn, UCSF, USA
- Nico Stuurman, UCSF and HHMI, USA
- Stephen Ross, Nikon Instrument Inc, USA
- Jan Ellenberg, European molecular Biological Laboratory, Germany
- Mike Davidson, University of Florida, USA
- Jason Swedlow, University of Dundee, UK
- Andreas Schönle, Max Planck Institute for Biophysical Chemistry, Germany
- Shalin Mehta, Marine Biology Laboratory, USA

Support and Attendance by:

Andor, Bitplane, Carl Zeiss, Coherent, DSS Imagetech, GE Healthcare, Icon Analytical, JH BIO,
Laser Science, Laser Spectra Services, Leica, Molecular Devices, Nikon, Olympus, Perkin Elmer,
Photometrics, Strand Life Sciences, Spectra Physics, Scientific Volume Imaging, TILL Photonics

Saturday, Sept. 22:

01:00-02:00pm **Lunch** for company folks, participants and faculty
Location: NCBS glass roof canteen

Sunday, Sept. 23:

Basics of microscopy: microscope light path, diffraction/resolution, phase and polarization.

08:00-08:30am **Registration**

08:30-09:00am **Inauguration:** Satyajit Mayor

09:00-10:00am **Didactic Lecture:** Microscopy light path, Koehler illumination, Diffraction/Resolution-
Speaker: Satyajit Mayor, NCBS/ Shalin Mehta, MBL
Location: 70 Seater Hall Southern laboratories

10:00-10:15am **Coffee Break**
Location: Next to 70 seater hall Southern laboratory

10:15-12:15pm **Laboratory:** Introduction to transmitted light microscopy: Koehler illumination and resolution. Identify parts of a microscope and light paths. Koehler illumination on stained histology slides. Exercise to look at resolution/diffraction with diatoms. Samples: Bead Slides, Diatoms, Pond Water.
Lab leader: Satyajit Mayor
Lab instructors: Kurt Thorn, Stephen Ross, Nico Stuurman, Jan Ellenberg, Shalin Mehta, Andreas Schönle, Mike Davidson, Roop Mallik and Company Application Specialists
Lab support: Joseph Jose Thottacherry
Location: Teaching Lab

12:15-01:15pm **Lunch**
Location: NCBS glass roof canteen

01:15- 02:15pm **Didactic Lecture:** Contrast enhancement I-Phase Contrast, Dark Field.
Speaker: Stephen Ross, Nikon Inc.
Location: 70 Seater Hall Southern laboratories

- 02:15-03:15pm **Didactic Lecture:** Contrast enhancement II-Polarized Light, DIC.
Speaker: Kurt Thorn, UCSF, USA
Location: 70 Seater Hall Southern laboratories
- 03:15-03:30pm **Coffee Break**
Location: Next to 70 seater hall Southern Laboratory
- 03:30-05:30pm **Laboratory:** Contrast enhancement:
Phase, DIC and Dark Field Microscopy.
Lab leader: Kurt Thorn
Lab instructors: Stephen Ross, Nico Stuurman, Satyajit Mayor, Mike Davidson,
Jan Ellenberg, Roop Mallik, Shalin Mehta, Andreas Schönle, and Company
Application Specialists
Lab support: Joseph Mathew K
Location: Teaching Lab
- 05:30-07:00pm **Research Lecture**
Speaker: Jan Ellenberg, EMBL
Location: Auditorium Southern laboratories
- 07:00-09:30pm **Poster and Social**
Location: Atrium Southern laboratories
- 08:30-9:30pm **Dinner** Dinner
Location: Southern laboratory Atrium
- 10:00-11:00pm **Free time on microscopes with faculty and company folks**
Location: Teaching Lab
(Coffee provided outside the teaching lab)

Monday, Sept. 24:
Fluorescence Microscopy

- 09:00-10:00am **Didactic Lecture:** Principles of fluorescence, fluorescent dyes,
fluorescent protein and the fluorescence light path
Speaker: Mike Davidson, University of Florida, USA
Location: 70 Seater Hall Southern laboratories
- 10:00-11:00am **Didactic Lecture:** Optical Detectors and Digital Image
Acquisition
Speaker: Nico Stuurman, UCSF, USA
Location: 70 Seater Hall Southern laboratories
- 11:00-11:15am **Coffee Break**
Location: Next to 70 seater hall Southern laboratory

- 11:15-1:15pm **Laboratory:** Fluorescence Lab A:

Examine the fluorescence light path and dichroic mirrors/filters.
Take images of fluorescence fixed specimens.
Fluorescence beads for psf and determining pixel shift
Lab leader: Stephen Ross
Lab instructors: Nico Stuurman, Kurt Thorn, Jason Swedlow, Jan Ellenberg, Sudipta Maiti, Andreas Schönle, Roop Mallik, Shalin Mehta and Company Application Specialist
Lab support: Parijat Sil
Location: Teaching Lab
- 01:15- 02:15pm **Lunch**
Location: NCBS glass roof canteen
- 02:15-03:15pm **Didactic Lecture:** Image Analysis
Speaker: Jason Swedlow, University of Dundee, UK
Location: 70 Seater Hall Southern laboratories
- 03:15-05:15pm **Laboratory:** Fluorescence Lab B:
Time Lapse and Cameras and 'z' projections of pfs
Multi-wavelength Time Lapse Imaging of living cells
Measure read-out noise and photon conversion factor of the camera
Lab leader: Nico Stuurman
Lab instructors: Stephen Ross, Kurt Thorn, Jason Swedlow, Sudipta Maiti, Mike Davidson, Andreas Schönle, Jan Ellenberg, Shalin Mehta and Company Application Specialists
Lab support: Joseph Jose Thottacherry
Location: Teaching Lab
- 05:15-05:30pm **Coffee Break**
Location: Outside Teaching Lab
- 05:30-06:30pm **Didactic Lecture:** High Content Screening
Speaker: Jan Ellenberg, EMBL
Location:70 Seater Hall Southern laboratories
- 06:30-07:30pm **Dinner**
Location: NCBS glass roof canteen
- 07:30-09:30pm **Image Analysis Workshop**
Lab Instructors: Jason Swedlow, Kurt Thorn, Nico Stuurman.
Location: CIFF Southern laboratories

09:30-11:00pm **Free time on microscopes**
Location: Teaching Lab
(Coffee/snacks outside teaching lab)

Tuesday, Sept. 25:

Optical Sectioning and enhancing resolution

09:00-10:00am **Didactic Lecture:** Optical sectioning techniques- Confocal, Two Photon Excited Fluorescence, etc.

Speaker: Sudipta Maiti, TIFR, India.

Location: 70 Seater Hall Southern laboratories

10:00-11:00am **Didactic Lecture:** Photo-activation, Photo-localization and Super-resolution imaging techniques, etc. Part-1

Speaker: Andreas Schönle, MPIbc, Germany

Location: 70 Seater Hall Southern laboratories

11:00-11:15am **Coffee Break**

Location: Next to 70 seater hall Southern laboratory

11:15-12:15pm **Didactic Lecture:** Photo-activation, Photo-localization and Super-resolution imaging techniques, etc. Part-2

Speaker: Andreas Schönle, MPIbc, Germany

Location: 70 Seater Hall Southern laboratories

12:15- 01:15pm **Lunch**

Location: NCBS glass roof canteen

01:15-05:15pm **Laboratory:** Optical Sectioning Techniques (point scanning, line scanning, spinning disk and TIRF).
4 x 1 hr modules

Lab leader: H Krishnamurthy

1) Equipment: Olympus FV1000 point scanning Microscope

Lab instructor: Kurt Thorn and Olympus application specialist

Lab support: Raksha K

Location: CIFF Southern laboratories

2) Equipment: Leica SP5 point scanning microscope
Lab instructor: Andreas Schönle and Leica application specialist
Lab support: Kiran K K
Location: CIFF Southern laboratories

3) Equipment: Andor spinning disk microscope
Lab instructor: Suvrajit Saha and Bruno Combettes (Andor)
Location: Satyajit Mayor Lab (Southern laboratories)

4) Equipment: Zeiss LSM 510 NLO point scanning microscope
Lab instructor: H Krishnamurthy and Zeiss application specialist
Lab support: Navya Jain
Location: CIFF Southern laboratories

5) Equipment: Zeiss LSM 5 Live- Line scanning system
Lab instructor: Riya and Zeiss application specialist
Lab support: Meera Nair
Location: CIFF Southern laboratories

6) Equipment: Olympus Cell TIRF microscope
Lab instructor: Subhashri, Gayathri M and Olympus application specialist
Location: Teaching Lab

7) Equipment: PerkinElmer Spinning Disk System
Lab instructor: Nico Stuurmann and Chris Johanson (PerkinElmer)
Lab support: Yatheendran K M
Location: CIFF Southern laboratories

8) Equipment: GE DeltaVision TIRF system
Lab instructor: Shivaprasad Bhuvanendran and Kathryn Buchanan (GE)
Lab support: Deepthy G Rao
Location: Teaching Lab

05:15-05:30pm

Coffee

Location: Next to 70 seater hall Southern laboratories

05:30-06:30pm

Didactic Lecture: FRET Microscopy

Speaker: Satyajit Mayor, NCBS

Location: 70 Seater Hall Southern laboratories

06:30-07:30pm

Research Lecture: Andreas Schönle

Location: Southern Laboratories Auditorium

07:30-08:30pm

Dinner

Location: NCBS glass roof canteen

08:30-11:30pm

Free time on microscopes with faculty/company folks

Location: Teaching Lab

(Coffee provided outside the teaching lab)

Wednesday, Sept. 26:

Special Topics: TIRF, FRAP, FCS, Polarization microscopy

09:00-10:00am

Didactic Lecture: "Fluorescence microscopy-based methods to study protein dynamics in live cells: from FRAP to FCS"-

Speaker: Sudipta Maiti, TIFR, India.

Location: 70 Seater Hall Southern laboratories

10:00-11:00am

Didactic Lecture: Optical Tweezers

Speaker Mallik, TIFR, Mumbai.

Location: 70 Seater Hall Southern laboratories

11:00-11:15am

Coffee Break

Location: Next to 70 seater hall Southern laboratories

11:15-12:15pm

Didactic Lecture: Polarization microscopy

Speaker: Shalin Mehta, MBL, USA

Location: 70 Seater Hall Southern laboratories

12:15-01:15pm

Lunch

Location: NCBS glass roof canteen

01:15-05:30pm

Laboratory: Specialized Techniques:

2 x 2 hr modules, distributed by student signup and selection. There will be 2 rotations of 2 hour each. Students can choose in advance and we will prepare a rotation plan.

1) Technnique: Integrating microscope systems

Equipment: Nikon Ti

Lab instructor: Nico Stuurman and Stephen Ross

Lab support: Parijath Sil

Location: Teaching lab

2) Technnique: Flourescence Recovery After Photobleaching (FRAP)

Equipment: LSM 5 Live

Lab instructor: Subhasri Ghosh and Zeiss Application specialist

Lab support: Navya Jain

Location: CIFF Southern laboratories

3) Technnique: Försters Resonance Energy Transfer (FRET)

Equipment: Nikon TIRF microscope with dual camera

Lab instructor: Riya Binil and Gayathri M

Lab support: Meera Nair

Location: Mayor Lab (Southern laboratories)

4) Technnique: Flourescence Correlation Spectroscopy (FCS)

Equipment: Zeiss 510 NLO with confocor

Lab instructor: SudiptaMaiti

Lab support: Suvrajith Saha

Location: CIFF (Southern laboratories)

5) Technnique: Mitosis Chromosome Dynamics

Equipment: LSM 5 10-405

Lab instructor: Jason Swedlow and Zeiss Application specialis

Lab support: Deepthy G Rao

Location: CIFF Training Centre (Northern Labs)

6) Technnique: Stimulated Emission Depletion (STED) Microscopy

Equipment: Leica SP5

Lab instructor: Andreas Schönle and Bettina Griesshaber
(Leica)

Lab support: Bhavyashree Suresh

Location: CIFF Southern laboratories

7) Technnique: Laser Micro Dissection and Catapulting system

Equipment: PALM Microbeam laser microdissection and catapulting-
Carl ZEISS

Lab instructor: Shivaprasad and Wolf-Dieter Schulz (Zeiss)

Lab support: Harikrishna

Location: CIFF Southern laboratories

8) Technnique: Optical Tweezers

Equipment: Thorlabs Optical Tweezer Kit

Lab instructor: Roop Mallik and Darius Köster

Location: Teaching Lab

9) Technique: Ground State Depletion (GSD) Super resolution
microscopy

Equipment: SR GSD Super resolution microscope-Leica

Lab instructor: Christian May (Leica)

Lab support: Raksha K

Location: CIFF training centre (Northern Labs)

10) Technique: Polarization microscopy

Equipment: LC Pol-Scope

Lab instructor: Shalin Mehta

Lab support: Amitabh Verma and Parijath Sil

Location: CIFF Southern laboratories

03:15-03:30pm

Coffee Break

Location: Teaching lab, Southern laboratory CIFF and CIFF training centre

03:30-05:30pm

Specialized techniques continued.

- 05:30-06:15pm **Research Lecture: Sudipta Maiti**
Location: Auditorium Southern laboratories
- 06:15-07:15pm **Dinner**
Location for participants: NCBS glass roof canteen
Location for faculty: Outside Campus
- 07:15-8:30pm **Non Commercial technical Presentations** by Company folks.
Location: Auditorium Southern laboratories
Program chair: Darius Köster
- 1) **Title:** “Fluorescent Imaging technologies in Translational Medical Research”
Speaker: Chris Johanson, PerkinElmer
- 2) **Title:** Technical Considerations for Rapid Live-cell Imaging
Speaker: Kathryn Buchanan, GE
- 3) **Title:** Real-time, Simultaneous multi channel TIRF Microscopy
Technique: Overcoming Optical Challenges
Speaker: Allen Na, Olympus
- 4) **Title:** Scientific Cameras - Things to Know
Speaker: Rachit Mohindra, Photometrics
- 5) **Title:** Andor Revolution DSD - Differential Spinning Disk : An innovative hybrid of spinning disk technology and structured illumination”
Speaker: Bruno Combettes, Andor
- 8:30-8:45pm **Coffee break**
Location: Next to Southern laboratory Auditorium
- 8:45-10:30pm **Non-commercial technical presentation continued**
- 6) **Title:** Laser micro dissection: bridging the gap between microscopy and molecular analyses
Speaker: Ulrich Sauer, Zeiss
- 7) **Title:** Gates open for improved confocal fluorescence and superresolution STED
Speaker: Bettina Griesshaber, Leica
- 8) **Title:** Ultrafast Lasers for Multimodal Non-Linear Imaging
Speaker: : Xuan Huang, Laser Science
- 9) **Title:** High Content Neuronal Toxicity Assays Using iPSC Derived Neurons
Speaker: Penny Tavormina, Molecular Devices

10) Title: Challenges of modeling a cell
Speaker: Luciano Lucas, Bitplane

11) Title: Lifecycle of an image in bio imaging.
Speaker: Nimisha Gupta, Strand Life Science

10:30-11:00pm **I manage:** Image Data software Demo
(coffee/snacks provided outside teaching lab)

Thursday, Sept. 27:

Research Lectures, Faculty – Student Group Discussions:

09:00-10:00am **Research Lecture:**
Speaker: Satyajit Mayor
Location: Auditorium Southern laboratories

10:00 –11:00am **Research Lecture:**
Speaker: Roop Mallik
Location: Auditorium Southern laboratories

11:00-11:15am **Coffee Break**
Location: Next to Southern laboratory Auditorium

11:15-12:15 am **Research Lecture:**
Speaker: Jason Swedlow
Location: Auditorium Southern laboratories

12:15-01:45pm Small group discussions of students with faculty over lunch about
Their research/microscopy interests.
(Packed lunch will be provided).

01:45-06:00pm **Laboratory:** x techniques- 2 x 2 hr modules, distributed
by student signup and selection.

Lab leader: Each instructor will prepare their own teaching plan,
setup and sample. There will be 2 rotations of 2 hour each.
Students can choose in advance and we will prepare a rotation
plan.

Lab instructors:

1) Lab: Integrating microscope systems

Equipment: Nikon Ti

Lab instructor: Nico Stuurman and Stephen Ross

Lab support: Parijath Sil

Location: Teaching lab

2) Technnique: Flourescence Recovery After Photobleaching (FRAP)
Equipment: LSM 5 Live
Lab instructor: Subhasri Ghosh and Zeiss application specialist
Lab support: Navya Jain
Location: **CIFF Southern laboratories**

3) Technnique: Försters Resonance Energy Transfer (FRET)
Equipment: Nikon TIRF microscope with dual camera
Lab instructor: Riya Binil
Lab support: Meera Nair
Location: **Mayor Lab (Southern laboratories)**

4) Technnique: Flourescence Correlation Spectroscopy (FCS)
Equipment: Zeiss 510 NLO with confocor
Lab instructor: SudiptaMaiti
Lab support: Suvrajith Saha
Location: **CIFF (Southern laboratories)**

5) Technnique: Mitosis Chromosome Dynamics
Equipment: LSM 510-405
Lab instructor: Jason Swedlow and Zeiss application specialist
Lab support: Deepthy G Rao
Location: **CIFF Training Centre (Northern Labs)**

6) Technnique: Stimulated Emission Depletion (STED) Microscopy
Equipment: Leica SP5
Lab instructor: Andreas Schönle and Bettina Griesshaber (Leica)
Lab support: Bhavyashree Suresh
Location: **CIFF Southern laboratories**

7) Technnique: Laser Micro Dissection and Catapulting system
Equipment: PALM Microbeam laser microdissection and catapulting-
Carl ZEISS
Lab instructor: Shivaprasad and Wolf-Dieter Schulz (Zeiss)
Lab support: Harikrishna
Location: **CIFF Southern laboratories**

8) Technnique: Optical Tweezers
Equipment: Thorlabs Optical Tweezer Kit
Lab instructor: Roop Mallik and Darius Köster
Location: **Teaching Lab**

9) Technique: Ground State Depletion (GSD) Super resolution microscopy
Equipment: SR GSD Super resolution microscope-Leica
Lab instructor: Christian May (Leica)
Lab support: Raksha K
Location: **CIFF training centre (Northern Labs)**

10) Technique: Polarization microscopy
Equipment: LC Pol-Scope
Lab instructor: Shalin Mehta
Lab support: Amitabh Verma and Parijath Sil
Location: **CIFF Southern laboratories**

03:45-04:00pm	Coffee Break Location: Teaching lab, Southern laboratory CIFF and CIFF training centre
04:00-06:00pm	Specialized techniques continued.
06:00-07:00pm	Lecture: Microscopy and Education Speaker: Mike Davidson
07:00-08:00pm	Dinner Location: NCBS glass roof canteen
08:00-10:00pm	Cultural Performance

Friday, Sept. 28: Equipment Demo and Student Projects

09:00-12:00pm	Demonstration of Equipments by company folks Location: Teaching lab, CIFF training centre and CIFF Southern Laboratories
12:00-01:00pm	Lunch Location: NCBS glass roof canteen
01:00-04:00pm	Student Projects
04:00-04:15pm	Coffee Break Location: Teaching Lab
04:15-08:00pm	Student Projects
08:00-09:00pm	Dinner Location: NCBS glass roof canteen
09:00 to 10:00pm	Free time on microscopes (coffee/snacks provided outside teaching lab)

Saturday, Sept. 29: Student Projects

09:00 – 11:00am	Student Projects
11:00 to 11:15am	Coffee Break Location: Teaching Lab
11:15 to 01:00pm	Student Projects
01:00-02:00pm	Lunch Location: NCBS glass roof canteen
02:00-04:00pm	Student Projects
04:00-05:30pm	Project presentation, graduation. Group photo. Location: Amphitheatre

(Coffee/Snacks Provided)

05:30-10:00pm

Souvenir Shopping/Dinner

Location: Down town Bangalore

For **September 28 and 29** a set of following 9 projects are provided.

- 1) **Project:** High Content Screening
Guide: Balaji Ramalingam
Lab support: Dhruv Raina
Equipment: Home built HCS system
Location: HCS facility Northern Labs
- 2) **Project:** Optical Tweezers
Guide: Roop Mallik and Darius Köster
Equipment: Thorlabs Optical Tweezers Kit
Location: Teaching Lab
- 3) **Project:** Microscope Automation using Micromanager
Guide: Nico Stuurman, Kurt Thorn
Lab support: Strand Engineers
Equipment: ASI modular infinity microscope kit
Location: Teaching Lab
- 4) **Project:** Super-resolution microscopy
Guide: Andréa's Schone
Lab support: Shivaprasad and Bettina Griesshaber/Christian May (Leica)
Equipment: Leica STED, Leica GSD
Location: CIFF Southern laboratories and CIFF Training Centre
- 5) **Project:** Imaging Brain Slices
 - a) Brain slice imaging
 - b) intrinsic signal imaging from an intact, living animal's brain**Guide:** Mehrab Modi, Parthasarathy, Aanchal Bhatia (Upinder Bhalla's Lab)
Equipment:
Location: CIFF Southern laboratories and Lab 13
- 6) **Project:** Axotomy and transport effects in *C.elegans*
Guide: Jyoti Dubey and Shika Ahlawat (Sandhya Kaushika's Lab)
Equipment: Laser micro surgery station
Location: Northern Labs (E-21)
- 7) **Project:** Ca⁺⁺ sensing imaging
Guide: Sumitha Chakraborty and Ramveer C
Equipment: Calcium imaging station
Location: CIFF Southern laboratories
- 8) **Project:** Locomotory Circuit in Drosophila Imaging
Guide: Aman Agarwal and Venkateshwara Reddy (Vijay Raghavan's Lab)
Equipment:
Location:

- 9) **Project:** Membrane dynamics
Guide: Shalin Mehta, Gayathri M
Lab support: Amitabh Verma
Equipment: Pol-Scope
Location: Teaching Lab

Sunday, Sept. 30: Free Time on Microscopes

09:00 - 01:00pm **Free Time on Microscopes**

01:00-02:00pm **Lunch**
Location: NCBS Canteen (Regular canteen lunch)