

Bangalore Microscopy Course: 2016

National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, Sept. 18-25, 2016

Course Organizers

Ron Vale, Nico Stuurman, Kurt Thorn: University of California, San Francisco, USA

Satyajit Mayor, H. Krishnamurthy, Manoj V Mathew: NCBS, India

Jason Swedlow: University of Dundee, UK

Faculty:

Abhishek Kumar, National Institute of Biomedical Imaging and Bioengineering, USA

Christian Tischer, EMBL, Germany

Christoph F Schmidt, Georg August University, Gottingen, Germany

Jennifer Ross, UMass Amherst, USA

Joel S Silfies, Nikon Instruments Inc., USA

Maria Garcia-Parajo, ICFO-The Institute of Photonic Sciences, Spain

Nico Stuurman, University of California San Francisco, USA

Rahul Roy, Indian Institute of Science, India

Satyajit Mayor, National Centre for Biological Sciences, India

Stephen Ross, Nikon Instruments Inc., USA

Sudipta Maiti, Tata Institute for Fundamental Research, India

Support and Attendance by:

Andor, Carl Zeiss, CoolLED, DSS Imagetech, Hamamatsu, Leica, Nikon, Olympus, Photometrics, Towa Optics, Tokai Hit

Course Schedule

Sunday, Sept. 18: Opening Lecture, Poster Session

- 12:30-01:30pm** **Lunch**
Location: inStem Canteen
- 01:30-02:30pm** **Participants Registration**
Location: Southern Laboratories Complex (SLC) Reception
- 02:30-04:00pm** **Research Lecture 1:**
Opening remarks and lecture-Satyajit Mayor, NCBS
Location: Dasher (NCBS Auditorium), Southern Laboratories Complex (SLC)
- 04:00-08:00pm** **Poster session** by the students attending the course & Social
Location: Southern Laboratories Complex (SLC) Atrium
- 08:00-09:00pm** **Dinner**
Location: inStem Canteen

Monday, Sept. 19: Basics of microscopy: Microscope Light Path, Diffraction/Resolution, Phase and Polarization

- 08:30-01:15pm** **Didactic Lecture 1& Laboratory 1:**
Microscopy light path, Köhler illumination on rails, Identify Parts of a Microscope, Köhler Illumination on Stained Histology Slides
Samples: Bead Slides, Diatoms, Pond Water
- Lab leader:** Jennifer Ross, UMass Amherst, USA
Lab Instructors: Abhishek Kumar, Christian Tischer, Nico Stuurman, Rahul Roy, Stephen Ross and Joel Silfies
Lab Support: Rimple and Chandrima
Location: Raspuri
- 11:00-11:15am** **Coffee Break**
- 01:15-02:15pm** **Lunch**
Location: inStem Canteen

- 02:15-03:15pm** **Didactic Lecture 2:** Contrast Enhancement - Dark Field, Phase Contrast, Polarized Light, DIC –Stephen Ross, Nikon Inc., USA
Location: Dasher (NCBS Auditorium)
- 03:15-04:15pm** **Didactic Lecture 3:** Principles of Fluorescence and Fluorescence Microscopy - Nico Stuurman, University of California San Francisco, USA
Location: Dasher (NCBS Auditorium)
- 04:15-04:30pm** **Coffee Break**
- 04:30-05:30pm** **Research Lecture 2:** Rahul Roy, IISc, India
Location: Dasher (NCBS Auditorium)
- 05:30-07:30pm** **Laboratory 2:** Dark Field, Phase Contrast and DIC
Lab leader: Stephen Ross
Lab instructors: Abhishek Kumar, Christian Tischer, Nico Stuurman, Rahul Roy, Jennifer Ross and Joel Silfies
Lab Support: Vinaya and Rimple
Location: Raspuri
- 07:30-08:30pm** **Dinner**
Location: inStem Canteen
- 08:30-11:00pm** **Free time on Microscopes**

Tuesday, Sept. 20: Fluorescence Microscopy

- 09:00-11:00am** **Laboratory 3:** Fluorescence Lab A: Examine the fluorescence light path and dichroics/filter. Take images of fluorescence fixed specimens, Fluorescence beads for PSF and determining pixel shift
Lab leader: Rahul Roy
Lab instructors: Abhishek kumar, Christian Tischer, Nico Stuurman, Jennifer Ross, Stephen Ross and Joel Silfies
Lab Support: Chandrima and Vinaya
Location: Raspuri
- 11:00- 11:15am** **Coffee Break**
- 11:15-12:15pm** **Didactic Lecture 4:** Fluorescent dyes, Fluorescent Proteins and Selection of Fluorescent Probes- Rahul Roy, IISc, India
Location: Happus (LH1)
- 12:15- 01:15pm** **Lunch**
Location: inStem Canteen

- 01:15-03:15pm** **Laboratory 4:** Fluorescence Lab B: Fluorescence, Time Lapse, Cameras and projections of PSFs, Multi-wavelength Time Lapse Imaging of living cells.
Lab leader: Nico Stuurman
Lab instructors: Abhishek kumar, Christian Tischer, Jennifer Ross, Rahul Roy, Stephen Ross and Joel Silfies
Lab Support: Chandrima and Rimple
Location: Raspuri
- 03:15-04:15pm** **Didactic Lecture 5:** Image Analysis-Christian Tischer, European Molecular Biology Laboratory, Germany
Location: Happus (LH1)
- 04:15-04:30pm** **Coffee Break**
- 04:30-06:30pm** **Laboratory 5:** Image Processing/Analysis Workshop
Lab Leader: Christian Tischer, EMBL, Germany
Lab Support: Mugdha
Location: Raspuri
- 06:45-08:30pm** **Dinner** (For Faculty- Down Town Bangalore)
- 07:30-08:30pm** **Dinner** (For Others- inStem Canteen)
- 08:30-11:00pm** **Free time on microscopes**

Wednesday, Sept. 21: Optical Sectioning and Enhancing Resolution

- 08:30-09:30am** **Didactic Lecture 6:** Optical Detectors and Digital Image Acquisition-Nico Stuurman, University of California San Francisco, USA
Location: Happus (LH1)
- 09:30-10:30am** **Didactic Lecture 7:** Optical Sectioning Techniques- Confocal, Two Photon Excited Fluorescence, etc.- Sudipta Maiti, TIFR, India
Location: Happus (LH1)
- 10:30-10:45am** **Coffee Break**
- 10:45-11:45pm** **Didactic Lecture 8:** TIRF Microscopy-Joel Silfies, Nikon.
Location: Happus (LH1)
- 11:45-12:45pm** **Lunch**
- 12:45-04:00pm** **Laboratory 6:** Optical Sectioning Techniques (point scanning, line scanning, spinning disk).
2 x 1.5 hr modules

Lab Support: Raksha, Divya, Sunil, Chandrima, Chittaspandini, Charuhansini, Ananya, Aditya

1. Equipment: Olympus FV3000 point scanning Microscope
Location: CIFF, SLC
Lab Instructor: Olympus Application Specialist

2. Equipment: Olympus FV1000 point scanning Microscope
Location: CIFF, Eastern Laboratories
Lab Instructor: Olympus Application Specialist

3. Equipment: Leica SP8 point scanning microscope
Location: CIFF, SLC
Lab Instructor: Leica Application Specialist

4. Equipment: Andor spinning disk microscope
Location: Satyajit Mayor Lab, SLC
Lab Instructor: Darius

5. Equipment: Zeiss LSM 780 NLO point scanning microscope
Location: CIFF, SLC
Lab Instructor: Sudipta Maiti and Thomas

6. Equipment: Nikon A1R+ Confocal Microscope
Location: CIFF, SLC
Lab Instructor: Joel S Silfies

7. Equipment: Zeiss LSM 880–Point scanning microscope
Location: CIFF, Eastern Laboratories
Lab Instructor: Zeiss Application Specialist

8. Equipment: PerkinElmer Spinning Disk System
Location: CIFF, SLC
Lab Instructor: Joseph

04:00-04:15pm

Coffee Break

04:15-05:15pm

Research Lecture 3: Sudipta Maiti, TIFR, India
Location: Dasher (NCBS Auditorium)

06:00-7:00pm

Dinner
Location: inStem canteen

07:00-09:00pm Social and Cultural Event

Thursday, Sept. 22: Special Topics

09:00-10:00am Didactic Lecture 9: FRET Microscopy- Satyajit Mayor, NCBS, India.
Location: Happus (LH1)

10:00-11:00am Didactic Lecture 10: Fluorescence Microscopy-Based Methods to Study Protein Dynamics in Live Cells: from FRAP to FCS- Sudipta Maiti, TIFR, India
Location: Happus (LH1)

11:00-11:15am Coffee Break

11:15-12:15pm Didactic Lecture 11: Near-Field Approaches for Microscopy & Dynamics at the Nanoscale- Maria Garcia-Parajo, ICFO, Spain
Location: Happus (LH1)

12:15- 01:15pm Lunch

01:15-02:15pm Didactic Lecture 12: Optical Manipulation-Christoph Schmidt, Georg August University, Germany
Location: Happus (LH1)

02:15-05:30pm Laboratory 7: Specialized techniques 2 x 1.5 hr modules, distributed by student signup and selection. There will be 2 rotations of 1.5 hour each. Students can choose in advance and a rotation plan will be prepared.

Lab support: Raksha, Divya, Sunil, Amit, Chittaspandini, Charuhansini, Rimple, Ananya, Aditya, Debakshi

Technique 1: Integrating microscope systems and Configuring Automation Software for Microscopes

Equipment: Nikon Ti

Location: Raspuri

Lab Instructor: Stephen Ross , Joel Silfies& Nico Stuurman

Technique 2: Homo-Försters Resonance Energy Transfer (Homo-FRET)

Equipment: Nikon TIRF microscope with dual camera

Location: Mayor Lab, Southern Laboratories Complex (SLC)

Lab Leader: Satyajit Mayor

Lab Instructor: Joey

Technique 3: Fluorescence Correlation Spectroscopy (FCS)
Equipment: Zeiss LSM 780 NLO
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Sudipta Maiti and Thomas

Technique 4: Laser Micro Dissection and Catapulting
Equipment: PALM Microbeam Laser microdissection and catapulting-
Carl ZEISS
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Zeiss Application Specialist/Amit

Technique 5: TIRF Microscopy
Equipment: Olympus Multi color TIRF
Location: 2nd Floor, Southern Laboratories Complex (SLC)
Lab Instructor: Nishan

Technique 6: SIM and STORM Super Resolution Microscopy
Equipment: Nikon N-SIM/N-STORM
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Nikon Application Specialist/Neethu

Technique 7: Fluorescence Recovery After Photobleaching (FRAP)
Equipment: Perkin Elmer Spinning Disk System
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Joseph

Technique 8: Zeiss Airy Scanning Super Resolution Technique
Equipment: ZEISS LSM 800 with Airy scan Module
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: ZEISS Application Specialist

Technique 9: Olympus FV-OSR Super Resolution Microscopy
Equipment: Olympus FV3000
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Olympus Application Specialist

03:45-04:00pm **Coffee Break**

05:30-05:45pm **Coffee Break**

05:45-06:45pm **Didactic Lecture 13:** Super-Resolution Microscopy- Rahul Roy, IISc, India
Location: Happus (LH1)

06:45-07:45pm **Didactic Lecture 14:** Practical Aspects of Objectives and Lens Design -
Stephen Ross, Nikon Instruments Inc., USA
Location: Happus (LH1)

07:45-09:00pm **Dinner**
Location: inStem Canteen

09:00-11:00pm **Free Time on Microscope**

Friday, Sept. 23: Research Lectures, Specialized Techniques

09:00-10:00am **Research Lecture 4:** Jennifer Ross, UMass Amherst, USA
Location: Dasher (NCBS Auditorium)

10:00- 11:00am **Research Lecture 5:** Christoph Schmidt, GAU, Germany
Location: Dasher (NCBS Auditorium)

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

11:15-12:15pm **Research Lecture 6:** Maria Garcia-Parajo, ICFO, Spain
Location: Dasher (NCBS Auditorium)

12:15-02:30pm **Discussion over Lunch:** Small group discussions of students with faculty over lunch about their Research/microscopy interests.

02:30-05:45pm **Laboratory 8:** Specialized techniques
2 x 1.5 hr modules, distributed by student signup and selection. There will be 2 rotations of 1.5 hour each. Students can choose in advance and a rotation plan will be prepared.

Lab support: Raksha, Divya, Sunil, Amit, Chandrima, Chittaspandini, Charuhansini, Debakshi, Ananya, Aditya

Technique 1: Integrating microscope systems and Configuring Automation Software for Microscopes

Equipment: Nikon Ti

Location: Raspuri

Lab Instructor: Stephen Ross , Joel Silfies& Nico Stuurman

Technique 2: Homo-Försters Resonance Energy Transfer (Homo-FRET)

Equipment: Nikon TIRF microscope with dual camera

Location: Mayor Lab, Southern Laboratories Complex (SLC)

Lab Leader: Satyajit Mayor

Lab Instructor: Joey

Technique 3: Fluorescence Correlation Spectroscopy (FCS)
Equipment: Zeiss LSM 780 NLO
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Sudipta Maiti and Thomas

Technique 4: Laser Micro Dissection and Catapulting
Equipment: PALM Microbeam Laser microdissection and catapulting-Carl ZEISS
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Zeiss Application Specialist/Amit

Technique 5: TIRF Microscopy
Equipment: Olympus Multi color TIRF
Location: 2nd Floor, Southern Laboratories Complex (SLC)
Lab Instructor: Nishan

Technique 6: SIM and STORM Super Resolution Microscopy
Equipment: Nikon N-SIM/N-STORM
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Nikon Application Specialist/Neethu

Technique 7: Fluorescence Recovery After Photobleaching (FRAP)
Equipment: Perkin Elmer Spinning Disk System
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Joseph

Technique 8: Zeiss Airy Scanning Super Resolution Technique
Equipment: ZEISS LSM 800 with Airy scan Module
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: ZEISS Application Specialist

Technique 9: Olympus FV-OSR Super Resolution Microscopy
Equipment: Olympus FV3000
Location: CIFF, Southern Laboratories Complex (SLC)
Lab Instructor: Olympus Application Specialist

- 04:00-04:15pm** **Coffee Break**
- 05:45-07:00pm** **Graduation, Group Photo**
- 07:00-09:00pm** **Discussion over Dinner:** Small group discussions of students with faculty over dinner about their research/microscopy interests.
- 09:00-11:00pm** **Free Time on Microscopes**

Saturday, Sept. 24:

09:00-11:00am **Student Projects**

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

11:15-01:00pm **Projects**

01:00-02:00pm **Lunch**

02:00-04:00pm **Projects**

04:00-04:15pm **Coffee Break**

04:15-06:15pm **Projects**

06:00-10:00pm **Workshop Dinner and Social (Downtown Bangalore)**

Sunday, Sept. 25:

09:00-11:00am **Projects**

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

11:15-01:00pm **Projects**

01:00-02:00pm **Lunch**

02:00-04:00pm **Projects**

04:00-04:15pm **Coffee Break**

04:15-06:15pm **Project Presentation by Participants**

Projects

Project 1: Imaging Brain Slices

Guide: Kambadur Ananthamurthy and Sriram Narayanan

Lab Support: Aditya

Equipment: Beja Fry Multiphoton Microscope

Location: CIFF Southern Laboratories Complex (SLC) and Lab 1

Project 2: Fast and Isotropic imaging using diSPIM Microscopy

Guide: Neethu/Abhishek

Lab Support: Amit

Equipment: Home built diSPIM

Location: CIFF Southern Laboratories Complex (SLC)

Project 3: Image Processing

Guide: Christian Tischer

Lab Support: Mugdha

Location: Raspuri

Project 4: Diffusion law and its experimental executions

Guide: Thomas

Lab Support: Chittaspandini

Equipment: Zeiss 780 NLO

Location: CIFF Southern Laboratories Complex (SLC)

Project 5: Ultrafast single molecule TIRF microscope to follow dynamics of fluorescence conjugated Halo-GPI on membrane surface

Guide: Sangeeta Nath

Lab Support: Chandrima

Equipment: Nikon Ultrafast TIRF Microscope

Location: 1st Floor Clean Room, Southern Laboratories Complex (SLC)

Project 6: Expansion Microscopy

Guide: Aradhya Jain

Lab Support: Rimple/ Charuhansini

Equipment: Leica SP5 Confocal

Location: CIFF Southern Laboratories Complex (SLC)