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Imaging centers spread across the globe – partnerships between cooperation and academia ((biotech highlight))

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Review

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Imaging centers spread across the globe – partnerships between cooperation and academia ((biotech highlight))

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Keywords: Light microscopy / Live imaging / Imaging facility

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Abbreviations: **NIC, Nikon Imaging Center;**

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5 In the last eight years, Nikon Imaging Centers have been set up as collaborations between
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7 University Institutes and the Nikon Cooperation on science campuses across the world. This
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9 article summarizes the philosophy of the partnership between cooperation and academia, and
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11 how the Nikon Imaging Centers operate as light microscopy facilities within the research
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13 communities they are part of.
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15 16 **1. The Nikon Imaging Centers as collaboration between Nikon and Academia** 17

18 There are currently 8 Nikon Imaging Centers world wide, three in the United States, two in
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20 Asia and three in Europe. The first Nikon Imaging Center (NIC) was opened in 2001 at
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22 Harvard Medical School (Boston, MA, USA). It was the idea of Marc Kirschner, who was
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24 head of the Cell Biology Department at that time. He and his colleagues in the department had
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26 already pioneered live microscopy for the quantitative understanding of cell and
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28 developmental questions. So the idea was not to bring microscopes to needy scientists, who
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30 have never seen a microscope equipped with a digital camera, but to use the synergy of
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32 scientific excellence and cutting edge equipment. This collaboration was a big success with
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34 the scientific community at Harvard Medical School and has encouraged the formation of
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36 more NICs. In the meantime, 7 more NICs were created all over the world based on this
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38 working formula. The NIC at the University of Heidelberg, Germany, opened in September
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40 2005. Next came the Nikon Imaging Center at Hokkaido University (2005), Oxford
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42 University (2006), the University of California San Francisco (2006), the Singapore
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44 Bioimaging Consortium (2007), the Institut Curie in Paris (2007), and Northwestern
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46 University in Chicago (2008).
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50 I have witnessed the early NIC at Harvard Medical School (NIC@HMS) as a post-doctoral
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52 fellow and I have planned and directed the NIC at the University of Heidelberg (NIC@HD)
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2 during the last 3 years. Based on my experience as both a user and staff of the NIC, I will try
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4 to outline the philosophy of the NICs and what they bring to the scientific community.

5
6 In all cases, these centers are set up as a partnership between a University Institute and the
7
8 Nikon Cooperation. The directors and staff of the facilities are hired by the University, and
9
10 the centers are embedded in the scientific community door to door with research labs.

11
12 The NICs are built on local initiative and serve the needs of the scientific community they
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14 operate in. The NIC@HD was initiated by Thomas Holstein at the Institute of Zoology and
15
16 Jörg Kukulies from Nikon, Germany. After one and a half years in the Zoology Institute it is
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18 now located at the new Bioquant building. Like other light microscopy facilities, the NICs
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20 main mission is to make equipment accessible to a large number of scientists, and to provide
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22 guidance in using it. Currently, the NIC@HD is serving 240 active users from more than 90
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24 research groups. The type and number of equipment is negotiated between the academic
25
26 partner and the local Nikon branch. We started with 5 systems and then rapidly agreed to add
27
28 more as the demands grew. After its first 3 years the NIC@HD now offers 9 imaging setups
29
30 (see Table 1) and we have considerably diversified the range of possible applications.

31
32 Visitors to the NICs are often impressed by the abundance of equipment and cannot help to
33
34 wonder whether there is a catch somewhere as the equipment is loaned to the University free
35
36 of charge. Are these centers just “outlets” of Nikon, where the University provides the sales
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38 location? Is the staff trying to talk users into buying equipment? This is not how the Nikon
39
40 Imaging Centers operate. Nevertheless, it is clear that the local Nikon branches and Nikon
41
42 Japan have endorsed the NICs in their global marketing strategy and it is important to them,
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44 that the NICs are showcases for Nikon’s imaging products. They are set up prominently in
45
46 research hot spots, where a large number of postdocs and future group leaders become
47
48 familiar with Nikon systems. However, the “displayed” products are put to the acid test as
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50 users work on them more than 10 hours a day. The NICs operate within an environment
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52 where users have the choice: Heidelberg has an astounding number of advanced microscope
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2 systems at both the University and other research centers. An enormous advantage for the
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4 researchers at NICs is the support that is added to the instrumentation. The instruments are
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6 kept in top working condition and there is always somebody who knows how to operate their
7
8 advanced functions. Frequently, this is not the case when instruments are acquired by
9
10 individual groups.

11 The exchange of experience between facility users and the NIC staff also involves the product
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13 specialist of Nikon. This way, Nikon learns about new applications and imaging demands.

14
15 The good relation with the Nikon specialists helps the facility to operate and maintain the
16
17 highly sophisticated equipment. The relation between academic staff and Nikon employees is
18
19 generally relaxed and frank. A main reason for this might be the agreement between
20
21 University and Nikon that the NICs will not purchase microscopes from Nikon's main
22
23 competitors. This way the Nikon specialists are not in the position where they have to
24
25 "advertise" their products and thus they share a lot more information with the NIC staff. In
26
27 turn, the NIC staff is trying to give critical feedback to improve setups. At the NIC@HD we
28
29 have given a lot of input directly to programmers during the early development of the NIS-
30
31 Elements software.

32
33 In addition to Nikon, other companies also contribute equipment to the NICs. These are often
34
35 local alliances and differ from NIC to NIC. In the case of the NIC@HD, Hamamatsu
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37 Photonics is sponsoring cameras, the German company AHF Analysentechnik provides
38
39 filters, the Dutch company Scientific Volume Imaging is bringing its worldwide renowned
40
41 deconvolution package to the NIC@HD. Perkin Elmer also operates as a major contributor, as
42
43 one of the two spinning disk systems on a Nikon inverted microscope is sponsored by them.

44
45 And the second one? It was bought through a German Research program grant, as well as the
46
47 LaVision Biotec 2-photon system on the Nikon fix stage microscope (Table 1). It is not
48
49 uncommon, that sponsored and bought equipment is hosted in the NICs, as the foremost
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51 interest of the facility is to provide a comprehensive choice of equipment. For the grant
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2 holders it is attractive to put their instruments within an existing facility. The choice of what
3
4 type of sponsored equipment should be part or the Imaging Center is made by the directors
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6 and steering committee of the NIC together with the companies who join the effort.
7
8 Technically, the equipment is loaned and does not become property of the University. This
9
10 works as a big advantage for the academic partner. Equipment is replaced when advanced
11
12 models become available. In the last 3 years we have exchanged all the inverted microscopes
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14 from TE2000 to the new Ti series with automated xy-stages and hardware focus. The TIRF
15
16 setup was completely motorized, and we added 3 entirely new systems. As the systems are
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18 loaned, planning is much more flexible. Often, new innovations make existing instruments
19
20 completely unpopular to users and upgrading the instrument is sometimes not possible or
21
22 extremely costly. NICs have the opportunity to test whether an instruments suits the needs of
23
24 the scientific community and can otherwise negotiate for a replacement. Of course, there is a
25
26 down side to the exchange of instrumentation as it contradicts the fundamental principle to
27
28 “never change a running system”. Installing a new system usually means a lot of trouble
29
30 shooting and extra work for the staff and the new setup is sometimes met by initial skepticism
31
32 on the user side. Even changing just the software on a single microscope station can mean that
33
34 50 users have to be retrained.

35 36 **2 The equipment**

37
38 The dream for both users and staff would be to have one type of microscope that covers all
39
40 applications, or at least have every setup driven by a single software package. Unfortunately,
41
42 doing microscopy is more like traveling: you have to change the means of transport all the
43
44 time. Let us assume you want to look at actin dynamics in a moving cell. The obvious choice
45
46 for a 3D time lapse study is a spinning disk confocal microscope (Fig. 1 and Fig. 2A-D), yet
47
48 even finer details of the actin cortex can be imaged with total internal reflection fluorescence
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50 microscopy (TIRFM; Fig. 2E). Then again, fluorescence recovery after photobleaching
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(FRAP) experiments have to be done on a laser scanning confocal microscope, unless the spinning disk has a photo bleaching unit. If the cellular dynamics need to be monitored in tissue, this might require the use of a 2-photon microscope, which allows to generate fluorescence signals in deeper cell layers. Most of the systems at the NIC@HD (see Table 1) are confocal microscopes or systems that also generate optical sections like the TIRFM and the 2 photon setup. In cases where specimens are flat, wide field fluorescence microscopes with sensitive cameras provide a valuable alternative as they offer an excellent signal to noise ratio for weak fluorescence signals.

Imaging of live cells or entire organisms is a big challenge. To keep an organism alive on stage while preventing it from moving out of view is just one problem. Being able to visualize the signal over an extended time period without phototoxicity or photobleaching is another.

There is no general solution, given that each experiment has different priorities (speed, resolution ...) and problems (poor signal, thick specimen...). Live imaging and *in-vivo* imaging in particular requires much more sophisticated instrumentation [1] and more optimization by the researcher. Ironically, the resulting images are poorer in resolution and blurry because of noise and/ or autofluorescence in the tissue. Yet compared to the crisp images of fixed specimen, their beauty lays in the temporal information and the physiological relevance. For this reason, the NIC@HD puts a lot of effort in optimizing live experiments together with users. Most microscopes can be equipped with heated chambers and CO₂ incubation.

3 The software that drives the microscopes

Using different equipment also means that users will encounter different types of software. Even though we have endorsed the Nikon NIS-Elements software at the NIC@HD and work with Nikon microscopes only, we are currently using 3 additional software packages for image acquisition (VLOCITY from Improvision, ImSpector Software from LaVision Biotec,

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1
2 Nikon Biostation software). The quality of the software is key to the overall performance of
3
4 the system. Nowadays it is possible to perform microscopy without oculars (*e.g.* on the Nikon
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6 Biostation, see Table 1), but not without using software to drive the different hardware
7
8 components and sensors. The automation of microscope components in combination with a
9
10 smart software allow to store microscope settings and recall them with one mouse click. The
11
12 facility staff can prefabricate imaging protocols and pass them on to users. This way, even
13
14 researchers without profound knowledge of microscopy can perform complicated imaging
15
16 routines, which were previously the privilege of the experts.

17
18 In addition to the microscope setups, the NICs provide additional extra computers for data
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20 evaluation, storage of data on servers, specialized software for data rendering and
21
22 deconvolution. While the NIC@HD relies on commercial software in the facility, we also use
23
24 and teach ImageJ for image analysis [2], simply because it is a non-proprietary software
25
26 which is available to users in their labs, at home, and in their future career.

27 28 **4 Service to the scientific community**

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30 In many respects the NICs run like other light microscopy facilities. They provide equipment,
31
32 training of researchers, maintenance of microscopes and the logistics that make it possible to
33
34 share instrumentation among many people [3, 4]. Different from other core facilities, light
35
36 microscopy facilities have quickly moved away from “full service” where staff would acquire
37
38 images for researchers. Instead providing a working environment that allows researchers to
39
40 perform cutting edge microscopy has been proven to be much more successful. This way,
41
42 researchers become “users” empowered to generate and improve imaging results. The by far
43
44 most time consuming task for the NIC staff is to train and advise users. In their very
45
46 resourceful article on light microscopy facilities, the staff of the light microscopy facility at
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48 the Max Planck Institute for Cell Biology and Genetics, Dresden, devote a whole paragraph to
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50 users [4]. They state: “Users are what it is all about; they can be a blessing and a curse! Good
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2 users push staff for assistance with cutting-edge applications, provide valuable feedback about
3 the state of equipment, and are key to recognizing new trends...Bad users are selfish monsters
4 who expect the equipment to be in top working condition when they want to use it, but give
5 no thought to the condition in which they leave it". Little needs to be added to this angled but
6 apt characterization, though I should mention that all shades between good and bad exist.
7
8 Also, in quantitative terms, the ratio between good and bad is something like 100:1. As a
9 consequence, the staff gets used to good users very quickly and is then annoyed at the
10 occasional black sheep.

11
12 In all the NICs, the researchers are trained individually, on their own samples. This is
13 demanding for the staff, as they do not show (or show off with) beautiful samples they
14 already know, but try to optimize settings together with the researchers. For this reason, most
15 staff members are PhD qualified and often have a post-doctoral or other professional research
16 experience. In order to optimize the imaging experiment, the staff must know the microscope,
17 but also get a quick understanding of the sample. It is ridiculous to painfully enhance the
18 sensitivity of the sensor, when it would have been very easy to increase the expression level
19 of a GFP construct, or to improve the staining protocol. Experienced staff can save the users a
20 lot of trouble not related to the microscope directly. They help in setting up live experiments,
21 getting expression levels right, discriminating autofluorescence from real signal. This does
22 not mean that for all imaging experiments, solutions are straightforward. There are live
23 specimen, which are very dim and nevertheless very interesting. Also, an application may
24 demand extremely quick acquisition and requires that the staff optimize the instrument for
25 sensitivity, speed and resolution.

26
27 The projects at the NIC@HD range from simple morphological analysis to experiments where
28 imaging is used as a read-out of physiological activity in the cell (*e.g.* Calcium Imaging, FRET-
29 sensor) and protein interaction (*e.g.* FRAP and fluorescent resonance energy transfer). To setup a
30 successful experiment detailed knowledge about all imaging components is required [5] and as for
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1 every scientific experiment appropriate controls need to be incorporated. Otherwise, serious
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3 misinterpretation might occur, for example crosstalk between channels might be taken as
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5 colocalization of two markers. To see researchers jump to more demanding imaging techniques
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7 without an understanding of the basics has a disheartening effect on the staff of the imaging facilities
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9 [6]. It is their job to make researchers aware of pitfalls, but ultimately the interpretation of the results
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11 falls into the responsibility of the researchers. To promote and deepen the understanding of principles
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13 in microscopy and discuss advanced applications the NICs also provide courses on a regular basis,
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15 which are extremely popular with PhD students.
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18 5 Outlook

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20 The research conducted at the NIC@HD over the last three and a half years has contributed to around
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22 50 peer-reviewed research papers. Given the delay between data acquisition and publication this is
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24 likely to rise steeply during the next years. The projects are very diverse and it is only possible to list
25
26 a few examples (Table 1). Looking back on the last three years, we were able to exchange and
27
28 improve setups, making new applications possible (fast FRAP experiments, simultaneous
29
30 photoactivation and acquisition). Other experiments have become much more productive, (e.g. time-
31
32 lapse acquisition is now done with hardware focus and multi-point acquisition). To take part in the fast
33
34 progress of light microscopy-based research, the NICs depend on the dedication of both Nikon and
35
36 the University to continuously invest into the NICs. They rely on Nikon's sustained commitment to
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38 provide hardware and new innovations. As we are increasingly combining high content with high
39
40 throughput imaging, we need high resolution microscopes which can perform fast and parallel
41
42 acquisition without compromising on sensitivity. To evaluate the resulting experimental data, smart
43
44 ways to handle and analyze the large data sets need to be developed by University IT specialists. It is
45
46 the task of the NIC staff to integrate knowledge and pass it on to users. Each of the NICs worldwide
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48 has its own focus of expertise, but systematic exchange of information amongst the NICs and other
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50 imaging centers is a largely untapped resource, and will help to develop solutions to common
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2 problems and foster the development of novel imaging solutions.
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6 *I would like to thank Nikolas Herold for image data shown in Fig. 2 and Günther Gerisch for*
7 *providing Dictyostelium, labeled yeast and experimental support for data displayed in Fig. 1.*

8
9
10 *Thanks go to Takeharu Nagai (NIC@HU), Jennifer Waters (NIC@HMS) and the Imaging*
11 *Facilities Dresden, especially Britta Schroth-Diez for discussions on light microscopy*
12 *facilities and to Georg Stöcklin for critical reading of the manuscript.*
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18 *The author has declared no conflict of interest.*

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Figures and Tables

Figure 1. Actin dynamics in the amoebae of *Dictyostelium discoideum* captured by spinning disc confocal microscopy in 3D and time. Cells expressing a construct of an actin-binding protein, GFP-LimE (green), show that actin accumulates at the cortex (c), around intracellular vacuoles (v) and prominently builds up in filopodia (f) and endocytic cups. Actin polymerization drives endocytosis (en) of solid particles like yeast (A) or allows for bulk uptake of liquid (B), a process called macropinocytosis (mp). (A) Cell in the process of engulfing a yeast (red, TRITC stained). (B-D) *Dictyostelium* cells undergoing spontaneous shape changes, which have been reconstructed in space (36 z-slices) and time (as indicated). The z-stacks were deconvolved with a theoretical PSF using **Huygens** 3.0 to enhance localization accuracy. (B) Single optical slice through the middle of cells. (C) Maximum projection. (D) Angled projections (renderings) of different time points. Scale bars are **10** μm .
Image: Ulrike Engel.

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Figure 2. T-cell line expressing GFP live-act (green) to highlight actin dynamics. The cells settle on a substrate covered with modified HIV virus particles (red). (A-D) Live imaging on a spinning disk confocal microscope (21 z-slices, 0.15 μm spacing, time as indicated). (A) 3D view of the cell in a side projection (B) orthogonal maximum projection. (C) Optical xy-slice and reconstructed xz- and yz-slices allow to determine the exact position of a virus particle that has bound to the cell (white arrow head). (D) Insert detail (3 μm length) is magnified to

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show the movement of virus particle (white arrow head) with actin flow (time in seconds). (E)
 TIRFM of cell attaching to substrate. Only the actin cytoskeleton directly in contact with the
 substrate is visible and makes it possible to discern actin bundles at the cortex. Scale bars are
 10_μm. Images: N. Herold, University of Heidelberg.

Table 1. Equipment and Applications at the NIC@HD

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Number of setups	Type of Instrument	Short Description	Applications
2	Laser scanning confocal microscopes (LSCM) with standard and spectral detectors: Nikon C1Si and Nikon A1RSi on Nikon Ti inverted microscope with automated xy-stage and hardware focus.	Special features in addition to standard LSCM: Nikon A1R with galvano- and resonant scanner allows for frame rates of 30 fps and for photoactivation while acquiring images. Fast z-acquisition with a piezo-based z-stepper. The C1 is equipped with controlled-light exposure microscopy, (CLEM [7]), which reduces phototoxicity. 7 Laser lines cover the range of 405- 640nm Plan Apo objectives with chromatic correction from UV	Fixed and live morphology in 3D and 4 channels [e.g. 8, 9]. Comparative immunocytochemistry, such as analysis of knockout mice phenotypes [10]. FRAP experiments [11]. Photactivation in hippocampal neurons. FRET [12] and FRET-based sensors [13] and other ratio imaging. <i>In-vivo cell</i> migration in cancer models.

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		to red.	
2	Perkin Elmer UltraView ERS spinning disk confocal microscopes on Nikon Ti/Te2000 inverted microscope with Prior automated xy-stage H117.	Yokagawa CSU22-based spinning disk confocal microscope for real time confocality equipped with xy-table and piezo-based z-stepper. Laser lines cover the range of 405 to 640 nm. Images are mainly recorded with high numerical aperture lenses (<i>e.g.</i> 100x Plan Apo VC) onto an EM-CCD. Special feature: Photokinesis unit allows for FRAP and Photoactivation experiments in region of interests.	Mostly subcellular structure at high spatial and temporal resolutions, such as cytoskeletal dynamics in neuronal outgrowth, actin dynamics during virus binding (Fig. 2A-E), virus particle production [16], actin dynamics (Fig. 1) and organelle propulsion in <i>Dictyostelium</i> [9]. Force measurement in malaria parasites. Live experiments in cells and invertebrate animals with genetically encoded Ca ²⁺ and redox sensors [13].
1	LaVision Biotec 2-photon microscope (TRIM scope) on	2-photon laser setup on fix-stage microscope equipped with dipping lenses for	Dynamic morphology or physiological sensors (<i>e.g.</i> Ca ²⁺) in thick specimen.

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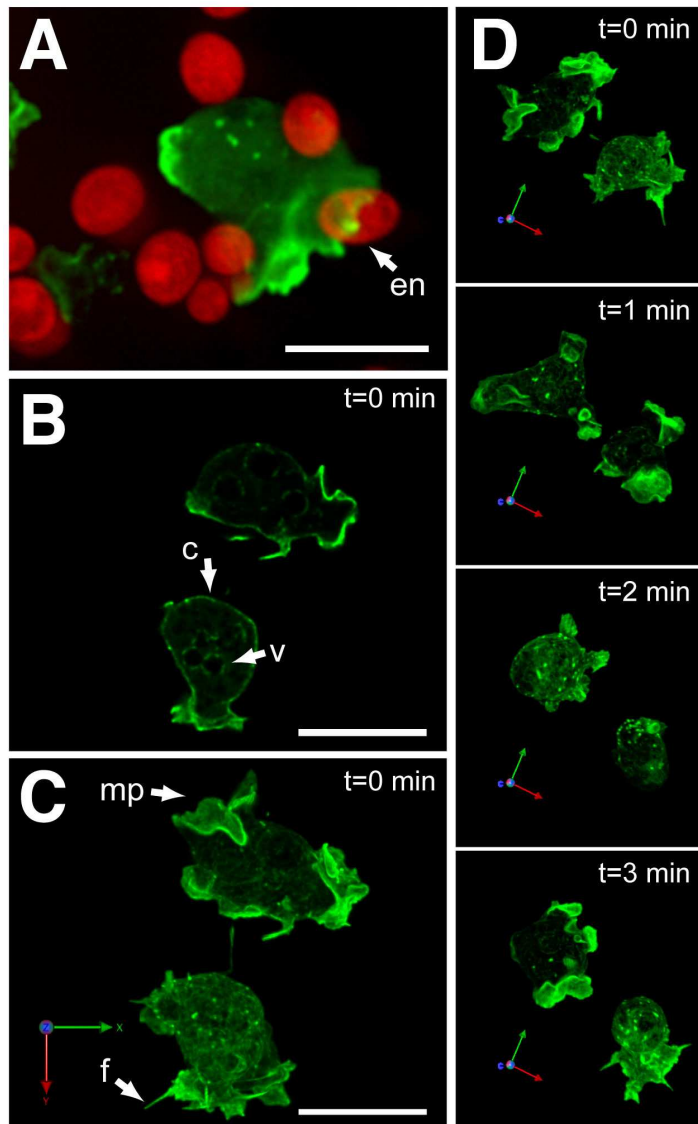
	Nikon FN-1 fix stage microscope.	physiological microscopy. The Femto-second pulses range from 700- 1150 nm and allow for excitation of blue to red emitting dyes. The signal is detected on descanned photo multiplier tubes. A special feature is the multi-beam scanning and fast detection on 2 cameras.	Acute brain slices, small embryos or <i>in-vivo</i> imaging in mice.
1	Nikon Total Internal Reflection Microscope (TIRFM) equipped with Sutter fast filter wheels for TIRF or epi illumination multi-channel time lapse.	Objective TIRF (with 60x or 100x Plan 1.49) on inverted fully automated microscope with hardware focus, automated xy-stage and sensitive cameras (Orca-AG or back-illuminated EM-CCD). TIRF illumination (488 and 561 nm lasers) and wide field fluorescence can be combined.	Receptor clustering, focal adhesion dynamics, vesicle fusion, virus cell interaction (see Fig. 2E). Microtubule cell cortex interactions during migration [6]. Ca ²⁺ imaging in TIRF (Fluo-4) and epifluorescence mode (Fura-2).
1	Nikon 90i automated upright microscope	Fully automated upright fluorescence microscope equipped with fluorescence,	Immunofluorescence for overview and high resolution.

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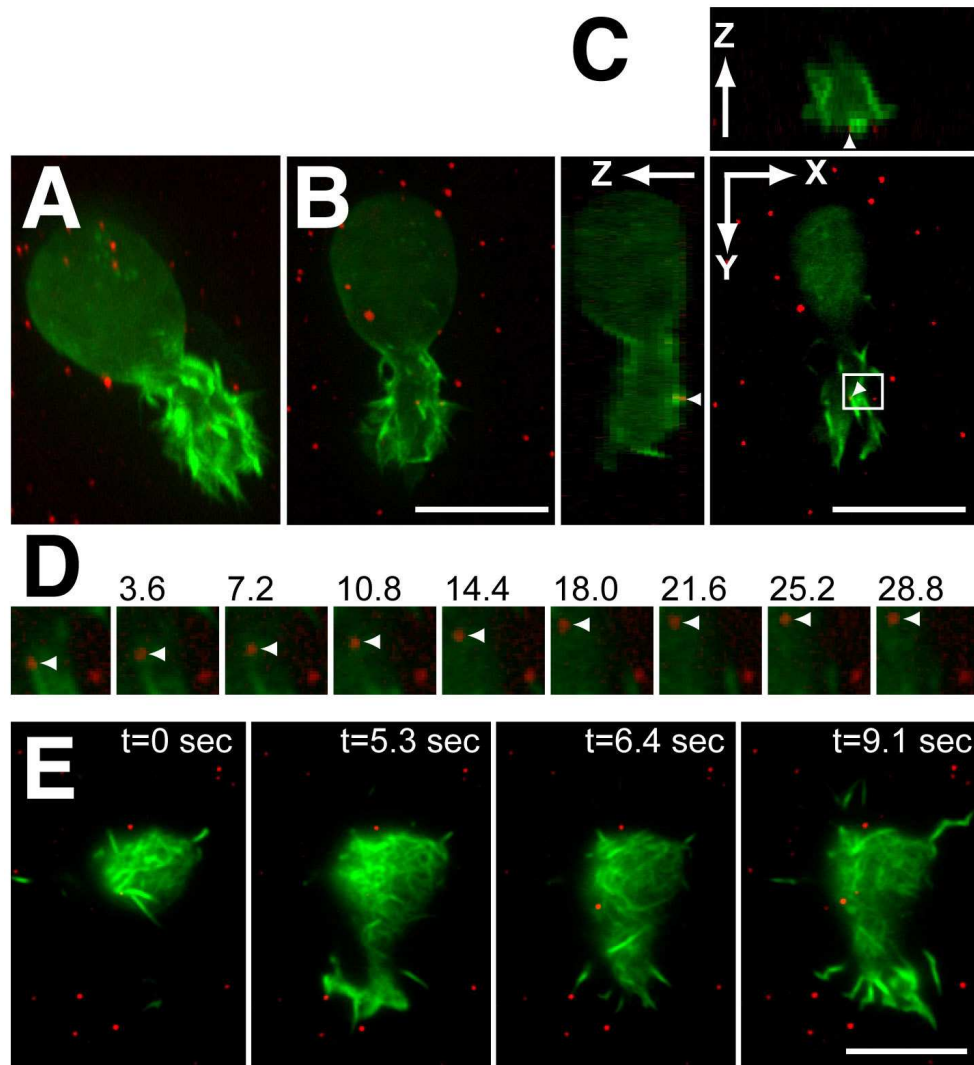
		differential interference contrast and Plan Apo objective series.	Automated acquisition of large sections (stitching) [17].
1	Nikon multi-zoom microscope AZ100	Fluorescence and differential interference contrast on zoom microscope for magnifications 5x-400x	<i>In-situ</i> hybridization and immunofluorescence of whole mounts. Nerve outgrowth in organotypic slices.
1	Nikon Biostation	Environmentally enclosed timelapse microscope with multi-fluorescence and multi-point acquisition.	Long term imaging over several days: cells cycle studies, RNAi phenotypes, malaria infection of liver cells.

Figure 1



106x182mm (300 x 300 DPI)

Figure 2



121x148mm (300 x 300 DPI)