

*Final publishable summary report***REMEDI     Resolution Enhanced Microscopy for Medical Diagnostics*****Executive summary***

Measurements on a nanometre-scale require nanometre-stability on the time-scale of the measurement. Conventional light-microscopes are not well suited for the stability requirements of super-resolution, which critically depends on the vibration susceptibility and the thermal behaviour of all opto-mechanical components, including immersion fluids connecting objective and sample.

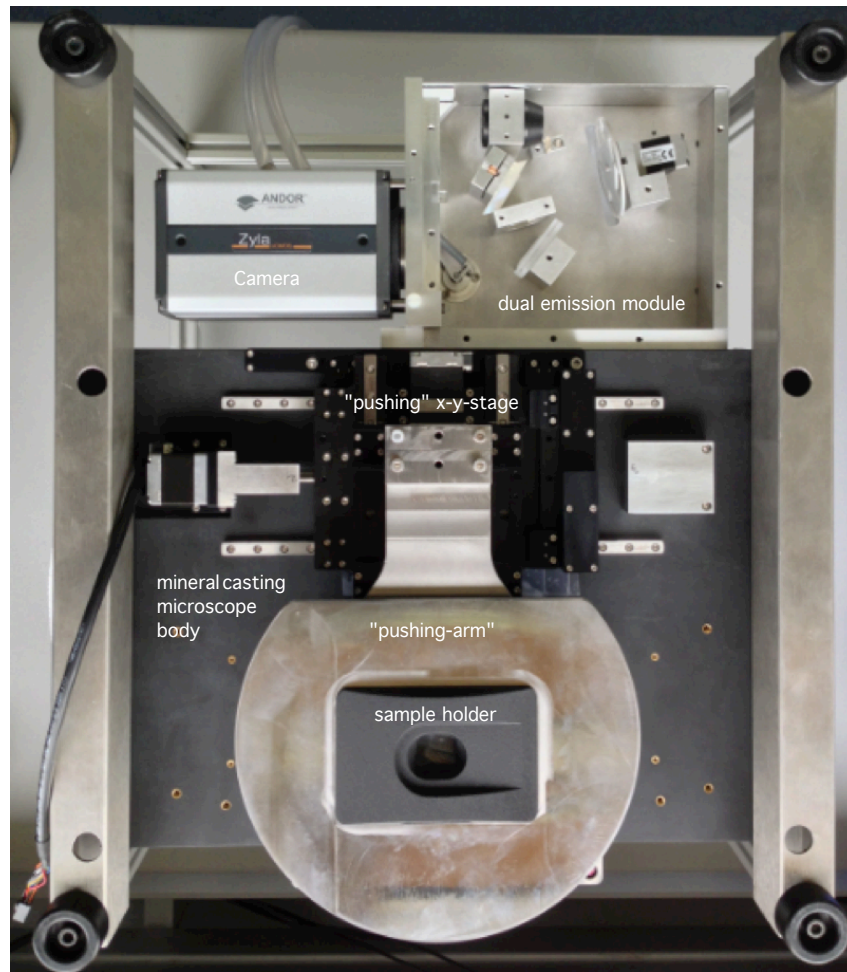
The consortium has therefore developed a novel microscope platform concept, employing new materials with maximal inherent vibration damping capabilities, minimal or matched thermal expansion of all critical parts and, where all these measures were not sufficient, new concepts for feedback-controlled dynamic realignments. As the major enemy of nanometre stability are thermal drifts and perturbations, measures were taken to maintain a constant temperature (to 0.01°) for sample, objective and all system components involved in keeping or changing the relative position of sample and objective.

With this approach we were able to reduce short-term instabilities to levels where one could barely measure them. However, single-molecule localisation-based microscopy techniques require extended image acquisition times. We thus set out to reduce the time needed for achieving sufficient localisation accuracy in order to avoid long-term drifts, which are very hard to control in routine medical diagnostics facilities. Shortening image acquisition times was addressed by employing novel sCMOS camera technology in combination with read-out schemes tailored especially for their application in single molecule localisation imaging.

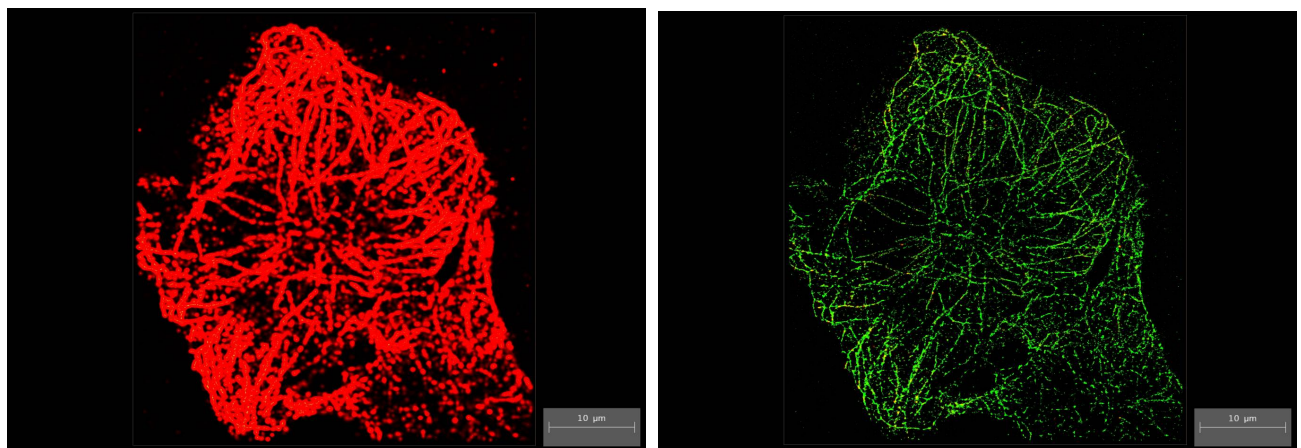
The consortium addressed resolution enhancement not only in two, but also in three dimensions. The large chip-size of the new sCMOS camera allows projecting two images obtained at different focal positions next to each other onto a single chip, and by using suitable algorithms the differential-focus recordings allow reducing the vertical position accuracy down to 20 nm levels, too. Moreover, in order to increase selectivity, each of the two images, separated by their different z-position, can be split into two emission colours and projected, side by side, on the large sCMOS chip.

Technology development was rounded up by soft- and firmware development for single molecule localisation and FPGA-based image pre-processing. Our goal was to extract super-resolution images online while thousands of localisation images are being gathered. To this end we have developed new single molecule localisation algorithms and have embedded them into the system software.

Validation was performed using two key microscopy applications: 1) diagnostic/experimental pathology using the expression of important receptors in breast cancer and 2) tumour immunology using the example of lipid raft-associated signal transduction in lymphoma cells.



**Fig. 1:** Second generation REMEDI setup with high-stability push & shove x-y-stage, optimized focus drive (not shown) dual emission module and Andor Zyla sCMOS camera.



**Fig. 2:** REMEDI imaging of tubulin structure in a dendritic cell. Left side: at conventional low resolution (red channel), right side: at super-resolution composed of 1.5 billion localizations (green channel). Scale bar represents 10  $\mu\text{m}$ .