Presentation transcript:

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Hi, my name is Tawab Karim I work in the lab of Carlas on the development of modular super resolution microscopes.

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Today I will talk about my work in the lab and I will do this by giving a brief introduction to what super resolution microscopy is. After that I will continue with different setups that were or are going to be developed. I will end this presentation with some projects that are currently in development.

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Super resolution microscopy is based on fluorescence microscopy. Fluorescence microscopy is where you illuminate your sample with a certain wavelength which will excite molecules which will fluoresce with another certain wavelength. The advantages of fluorescent microscopy is that you can differentiate between different proteins, due to fact that you can label them with a certain fluorescence. Another advantage is that you can do live cell microscopy which isn’t the case with electron microscopy for example.

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The disadvantage with fluorescence microscopy is that it is diffraction limited. This is a limit based on a physical property and thus cannot be broken. For light with a wavelength of 488nm the diffraction limit is around 250nm. Meaning if objects are closer then 250nm you are unable to differentiate them. Meaning thus looking at smaller object is physically impaired.

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This is where super resolution comes to save the day. By using localization methods you can determine the position of a molecule more precise then the diffraction limit allows. By taking multiple images from blinking molecules you get a slightly different “position back” By for example running a COM calculation you and adding up the results you can already improve the resolution of your image tremendously.

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EPI microscopy is the first type of microscope I will treat, while this is the most simple one. This type of microscope is based on a regular fluorescent microscope. In order to to super resolution microscopy we need a microscope first however. There are 3 possibilities to own a microscope. You can buy a pre build system, build one from standard components which are for example available from Thorlabs or you can design your own microscope. We have chosen to design our own and that is at the end of the line all the work I do. The reason we went with designing our own microscope instead of buying a pre build system is because a pre build system is not flexible enough for research purposes and if they are, they are immensely expensive. Standard components from Thorlabs was the methodology before, but due to the fact that they are discrete components the alignment process is very tedious and the components are very expensive as well. The main reason we went for designing our own components is because now we have the possibly to have a modular design which can be used in any type of microscope.

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And this is exactly what I did. I developed a microscope tower which has all the functionality we need from it and nothing more and nothing less. And by using smart designing techniques this tower can be used in any type of setup. On top of that, some design parameters are fixed which allows for modularity. There are still Thorlabs components used, but less then before.

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The next step was a TIRF setup, which stands for total internal reflection fluorescence. On the left you can see the illumination that is done using a regular epi microscope. In order to have TIRF you need to enter your objective at a certain position off-setted from the centre in order for it to fully internally reflect. If this is the case (depending on the objective you have) you can achieve an illumination depth of less then 400nm. In order to offset the excitation laser you thus need a linear stage in order to swap between epi and tirf microscopy.

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This module is at the moment in production. At its basics it’s a mirror which moves over a single axis. Because variation while aligning we determined we needed a translation of around 30mm and we needed the possibility to adjust the yaw of the mirror. This module is interchangeable with a fixed mirror. While if you position it at 0, you have your fixed mirror again. For TIRF microscopy one could use B splines in order to determine the position of the molecules. This is something Moses is working on.

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The next step in the development is the introduction of the digital micromirror device, also known as the DMD. A DMD is basically an array of small mirrors which can be rotated over a fixed angle. This allows for patterns in your illumination pattern.

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The DMD we use as an array of 2560x1600 pixels and each pixel has a length and width of 7.56 um. Because we are only interested in creating a pattern we can use a binary pattern. Which allows pattern generation with a frequency of up to 15kHz. This setup is currently being built by a msc student. The goal is also to slowly replace more and more Thorlabs comoponents with modular OM components.

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As I said, the DMD allows for the creation of patterns in your illumination path. And this allows on it’s turn for the use of SIM and or SIMFLUX. This is the software end which will be used on this setup. This is something that Jelmer works on for example.

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The next module to add to our microscope is where we are currently in our development. And that is the light sheet module. This module is for example very interesting for the NeuroDev Lab which is located at physics. They do research into the brain activity of zebrafish and while these aniimals are so small and you want to illuminiate only the part you are interested in, you need a very thin illumination pattern. One possibility is the AIM module, which conisists of 3 lenses which creates the light sheet.

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Another possibility to incorporate a light sheet module is via 3D tilt. This is the same principle as AIM but instead of illuminating

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