ACQUIFER Smart Imaging: An interface for remote controlling and unsupervised feedback microscopy on the Imaging Machine

High content screening is routinely employed to automatically acquire multi-dimensional image datasets at fixed positions within wells of microtiter plates. While this is sufficient for many applications, it imposes rather inflexible screening workflows as imaging positions are pre-defined by users, often regardless of specimen location or sample characteristics. This can lead to acquisition of unnecessary datasets, omission of features of interest and could hinder more complex assays that would depend on real-time analysis of image data.

Smart imaging describes methods for the (semi-)automated recognition and analysis of structures of interest within samples and the subsequent data acquisition only from these interesting regions. The analysis and decision making is usually performed by external software packages that interface with the microscope's soft- and hardware to remote control devices and modify imaging workflows depending on sample characteristics.

Smart imaging can massively improve **higher magnification assays,** as the limited field of view of higher magnification objectives often leads to omission of structures of interest at pre-defined imaging positions.. Regions of interest (e.g. certain phenotype) can be detected by external image processing routines and selectively imaged. This also enables **tissue specific imaging** in large specimen (e.g. zebrafish), as the tissues or organ of interest can be automatically identified and imaged at high resolution. By restricting acquisition to interesting structures, smart imaging techniques **save time, data volumes and resources** in large screening projects. Moreover, it **enables complex assays** like organ-specific screening in large specimen, which is not feasible by conventional high-content screening methods.

The **ACQUIFER Imaging Machine** offers an easy-to-use smart imaging interface for unsupervised feedback microscopy. Via the **ACQUIFER script language** virtually any external software (e.g. Fiji/ImageJ, any programming languages) that can produce text based output can be readily interfaced with the screening microscope. This script based communication between external software and imaging device enables **unsupervised feedback microscopy** without the necessity of complex hardware communication protocols or dedicated third-party control software.

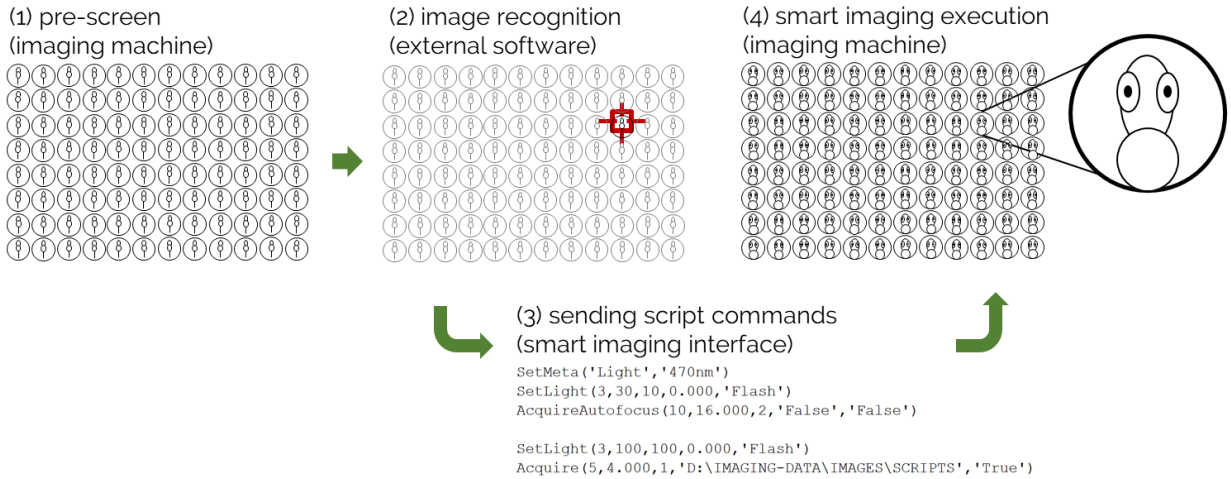

Figure 1: Schematic illustration of Smart Imaging screening workflows on the ACQUIFER imaging machine. (1) Pre-screen data is captured usually at lower resolution to image large field of views. (2) An image recognition step is carried out to detect features of interest such as the position of interesting structures. A vast range of external software can can be used to develop customized image analysis tools. (3) The external software sends scripts written in the ACUQIFER script language to the smart imaging interface which are subsequently transferred to the ACQUIFER Imaging Machine controller. (4) Smart imaging scripts are integrated and executed within running screening experiment, e.g. centring and higher resolution imaging of regions of interest.

The ACQUIFER Smart interface enables unsupervised feedback microscopy and automated decision making for complex high content screening experiments on the ACQUIFER Imaging Machine.

ACQUIFER Smart Imaging features:

- Intuitive ACQUIFER script language for command and control
- Use scripts and scriptlets to remote control the Imaging Machine
- Interface with your own external image processing routines
- Compatible with any external software capable of producing text output
- Open Software interface for developers
    - Easy-to-use file system based interface to the Imaging Machine
    - TCP/IP interface for more advanced application developments
- Control imaging workflow and hierarchical data structures

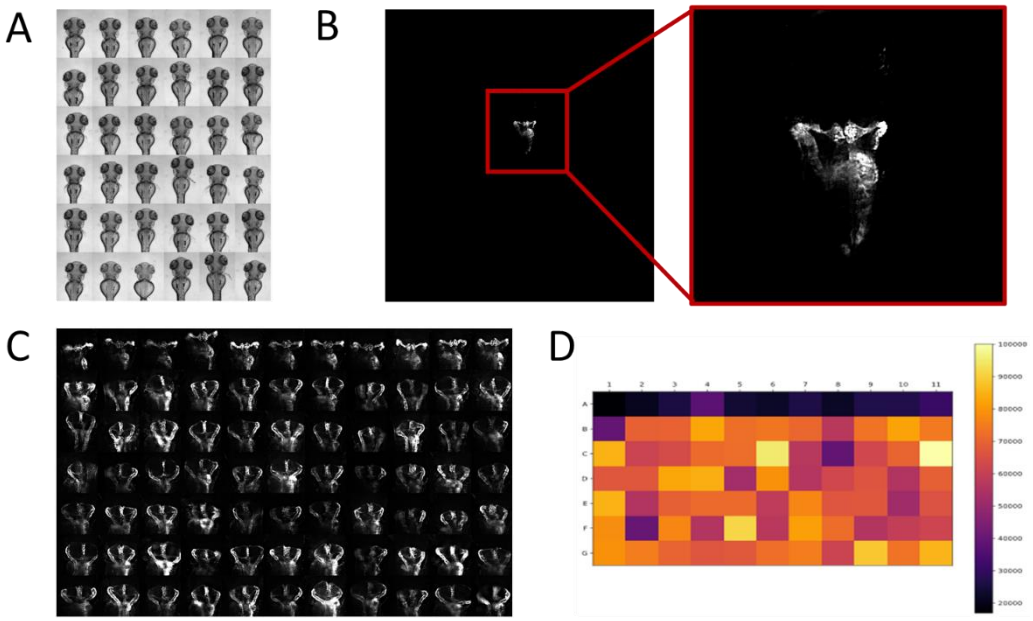

Figure 2: A whole organism smart imaging workflow for high resolution imaging of zebrafish embryonic kidneys. Illustrative example to demonstrate the usage of the ACQUIFER Smart Imaging interface for organ- and tissue specific imaging in zebrafish disease models. (A) Zebrafish embryos are consistently mounted in agarose coated microtiter plates generated using 3D printed orientation tools. (B) Kidney regions are detected within low resolution pre-screen data using a Fiji macro. Smart Imaging scriptlets are generated by Fiji and transferred and executed via the Smart Imaging interface of the ACQUIFER Imaging Machine leading to higher resolution datasets of pronephric areas. (C) Detailed visualization of kidney regions can be readily obtained for entire 96-well multiwell plates using Smart Imaging enabling the scoring of wildtype and disease kidney phenotypes. (D) Smart Imaging data allows the subsequent automated quantitative analysis and phenotypic scoring. Image courtesy of Gunjan Pandey, MSCA-ITN fellow at DITABIS AG / University Children's Hospital Heidelberg. This project has received funding from the European Union's Framework Programme for Research and Innovation Horizon 2020 (2014-2020) under the Marie Skłodowska Curie Grant Agreement No.642937.

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