UNIVERSITY OF BOLOGNA – ARCES

SUMMARY OF MY MAIN ACTIVITIES

FILIPPO PICCININI

May 10, 2011

Who am I?

f.piccinini@unibo.it

First Name, Surname	**Filippo Piccinini**
Place of birth	**Forlimpopoli, FC, Italy**
Date of birth	**April 20, 1985**
Title	**Biomedical Engineer**
Email	**f.piccinini@unibo.it**
Web site	**www.filippopiccinini.altervista.org**

PH.D STUDENT
European Doctorate in Information Technology by

ARCES
Advanced Research Centre on Electronic Systems, University of Bologna

SUPERVISOR
Prof. Alessandro Bevilacqua

CO-TUTOR
Prof. Mauro Ursino

MAIN RESEARCH TOPIC
Biomedical Image Elaboration

ARCES

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My office

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My Professor's office

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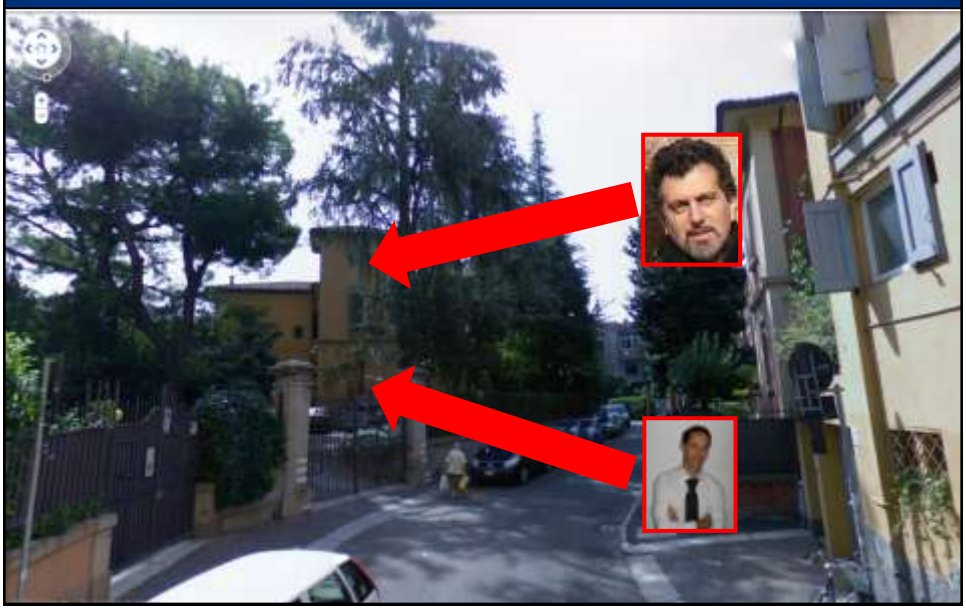

Biomedical engineering

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WEB SITE: http://www.ing2.unibo.it

Hospital partners

f.piccinini@unibo.it

IOR - BOLOGNA

BONE REGENERATION LABORATORY

IRST - MELDOLA

OPTICAL MICROSCOPE LABORATORY

CELL COLTURE LABORATORY

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

Your contact

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SUMMER SCHOOLS

- **ICVSS 2010 Computer Vision Summer School**
  Scicli (Ragusa), Italy, July 12-17, 2010

  F. Piccinini, A. Bevilacqua, A. Gherardi, L. Carozza, "***Accurate Mosaicing of Cell Images Acquired with Non Automated Equipment***". Poster to: ICVSS 2010 International Computer Vision Summer School, Scicli (Ragusa), Italy, July 12-17, 2010

- **CIMST 2010 Interdisciplinary Summer School on Bio-medical Imaging**
  ETH Zurich, Switzerland, September 6-17, 2010

  F. Piccinini, A. Bevilacqua, A. Gherardi, L. Carozza, E. Lucarelli, B. Dozza, "***Illumination Field Detection in Microscopic Images***". Poster to: 4th CIMST Interdisciplinary Summer School on Bio-medical Imaging, ETH Zurich, Switzerland, September 6-17, 2010.

Outline

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- Real time mosaicing
- Vignetting correction
- Microscope calibration
- Depth from focus
- Publications
- Contacts

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Real time mosaicing

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MOSAIC

A large image obtained stitching together two or many more images. A mosaic allows to extend the field of view of a camera, without losing resolution.

WHY MOSAICING IS IMPORTANT?

Real time mosaicing

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MOTIVATIONS OF MOSAICING

- To see a global condition of objects
- To store an instantaneous state of objects in contest
- To capture large objects of interest with a high resolution
- To perform time lapse experiments
- To perform high content analysis

MOTIVATIONS OF REAL TIME MOSAICING

- To acquire information of unexpected (tissue, cells) objects during a normal inspection
- To guide the operator during the navigation
- To get an immediate feedback when material is at one's disposal

Real time mosaicing

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BIOLOGICAL APPLICATIVE CONTEST

PROJECT ADVANCE

automatic non-invasive system based on high content analysis to detect and characterize vital mesenchymal stem cells in a spatio-temporal context

Collaboration with the Istituto Ortopedico Rizzoli (Bologna, Italy)

The Bone Regeneration Lab is the first in Italy to conduct a clinical trial of level 2 for utilization of Mesenchymal Stem Cells in regeneration medicine

GOAL

To study Mesenchymal Stem Cell's properties and behaviors to build biological pharmaceuticals to regenerate bones affected by tumors and other pathologies

Real time mosaicing

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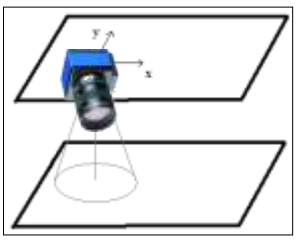
CONSTRAINTS

- Live cells for clinical uses ➔ cells cannot be manipulated
- Non automated light microscope

PROBLEMS

- Very low contrast typical of mesenchymal stem cells observed in phase contrast mode
- Holder is not perfectly perpendicular to the optical axis
- Not uniform illumination field distribution in the microscopic field of view



Real time mosaicing

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MATERIALS

- Microscope Nikon Eclipse TE2000-U equipped with Camera Nikon DXM1200F
- Cell cultures and histological samples

Real time mosaicing

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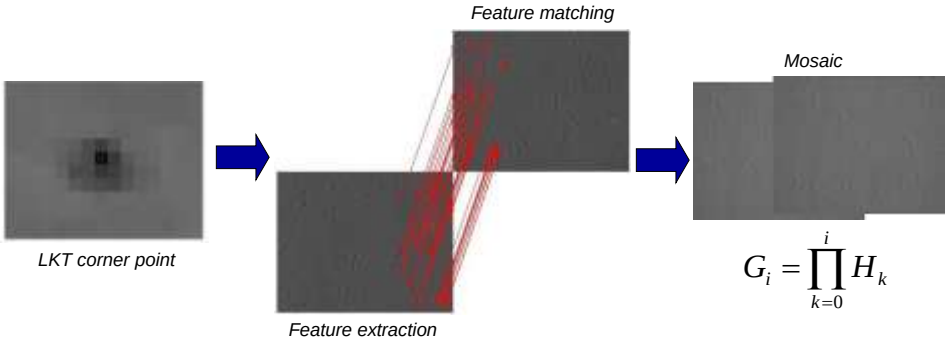
GEOMETRIC REGISTRATION

- Feature choice
- Lukas Kanade Tracker (LKT): corner point extraction and matching
- Transform matrix estimation



Real time mosaicing

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GEOMETRIC REGISTRATION

- Feature choice
- Lukas Kanade Tracker (LKT): corner point extraction and matching
- Transform matrix estimation

Original image

TRANSLATIVE	AFFINE	PROJECTIVE
$H = \begin{bmatrix} 1 & 0 & t_1 \\ 0 & 1 & t_2 \\ 0 & 0 & 1 \end{bmatrix}$	$H = \begin{bmatrix} a_{11} & a_{12} & t_1 \\ a_{21} & a_{22} & t_2 \\ 0 & 0 & 1 \end{bmatrix}$	$H = \begin{bmatrix} a_{11} & a_{12} & t_1 \\ a_{21} & a_{22} & t_2 \\ v^T{}_1 & v^T{}_2 & 1 \end{bmatrix}$
		
One corresponding point	Three corresponding points	Four corresponding points

Real time mosaicing

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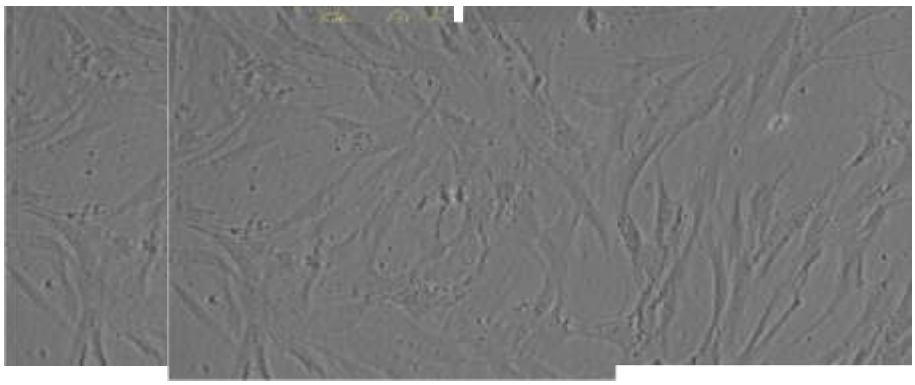
GEOMETRIC REGISTRATION

SUMMARY



Real time mosaicing

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PROBLEM

Seems between images can be markedly visible in stitching zone

Real time mosaicing

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PROBLEM

Seems between images can be markedly visible in stitching zone

In a first step blending can resolve problem of seems,
but it can bring to color inhomogeneity, ghost effect, movement effect, etc.

Real time mosaicing

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PROBLEM

Seems between images can be markedly visible in stitching zone

To completely resolve the problem the vignetting function due to uneven illumination field, lens, etc, must be studied…

Cell culture

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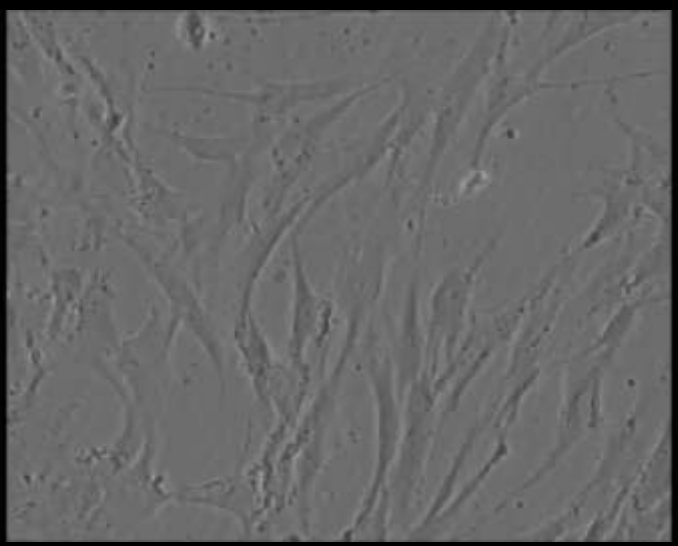

Courtesy of Dr. Ludovico Carozza

Histological sample

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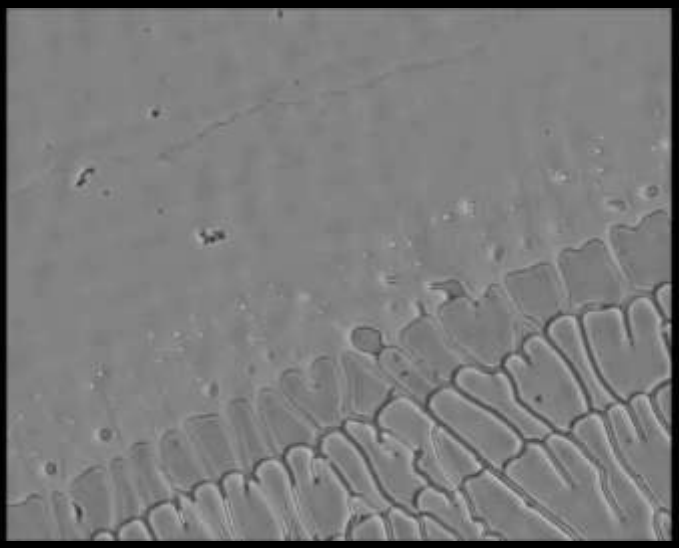

Courtesy of Dr. Ludovico Carozza

Outline

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Vignetting

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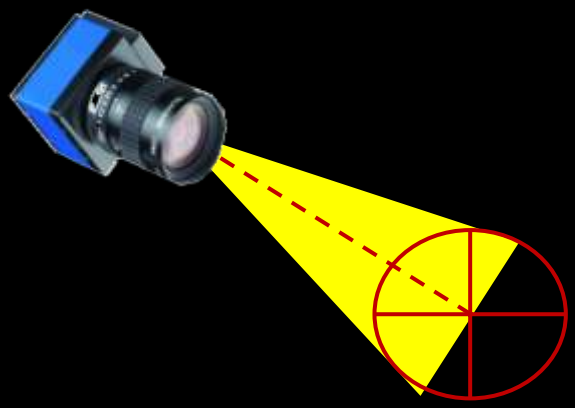
An effect of brightness attenuation away from the centre to the optical axis due to an uneven illumination field, lens, etc



Vignetting: a synthetic image

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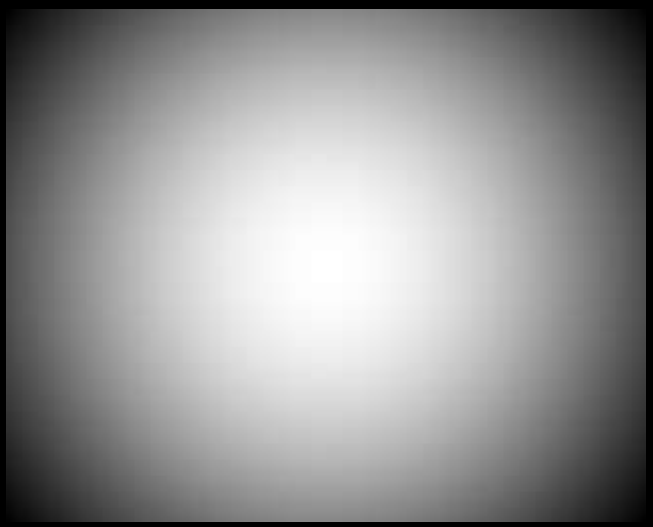

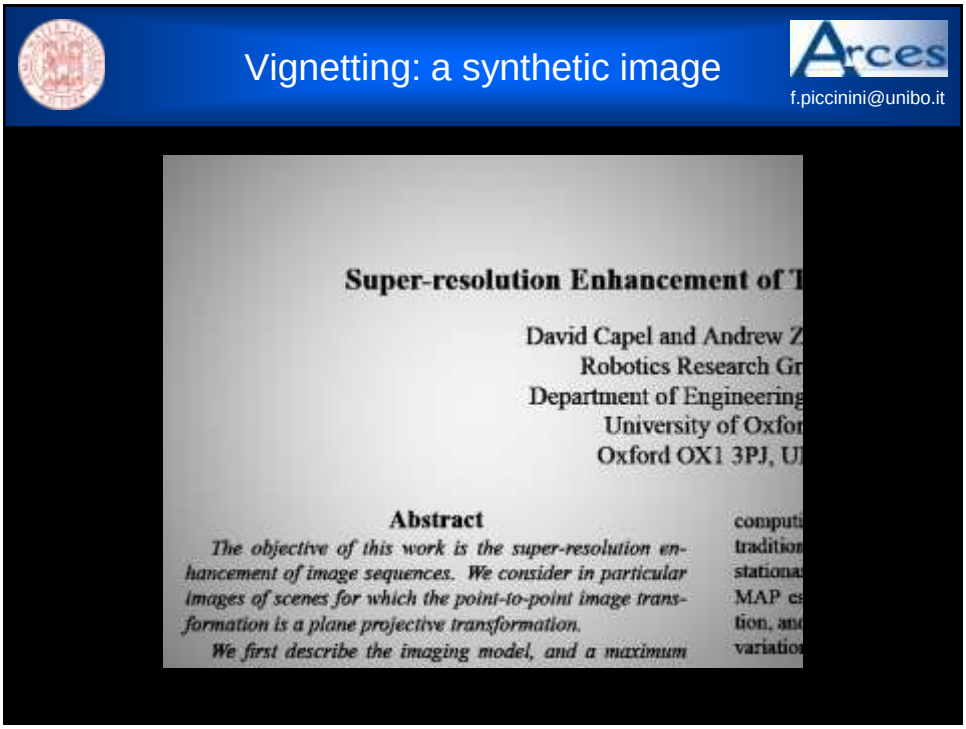
Vignetting: a synthetic image
Arces
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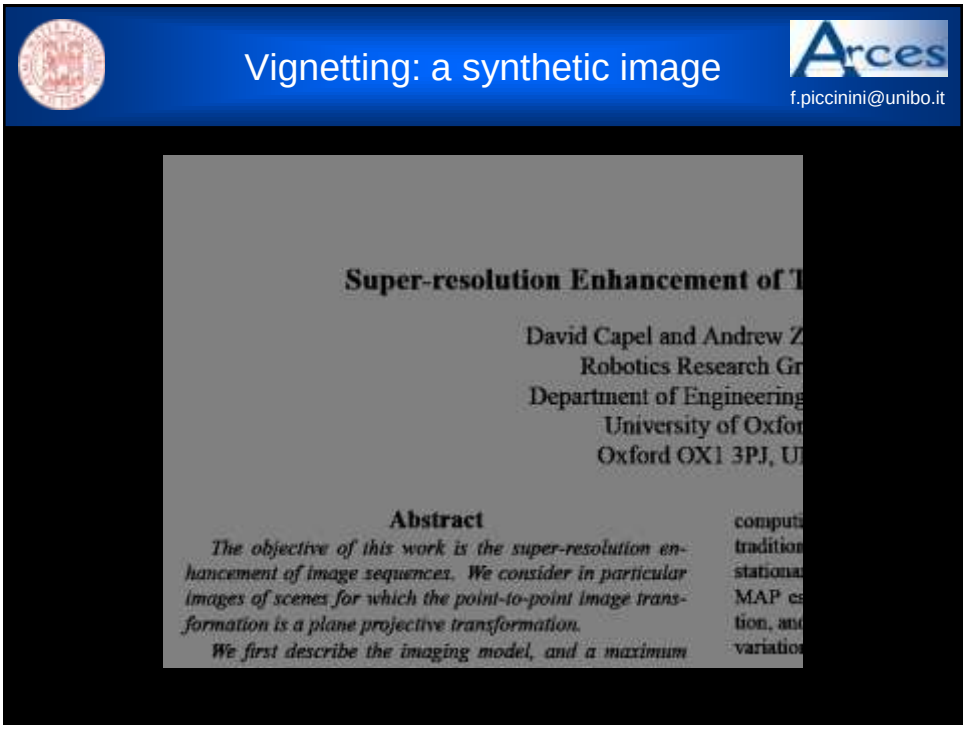
Super-resolution Enhancement of
David Capel and Andrew Z
Robotics Research Gr
Department of Engineering
University of Oxfor
Oxford OX1 3PJ, U
Abstract
The objective of this work is the super-resolution enhancement of image sequences. We consider in particular images of scenes for which the point-to-point image transformation is a plane projective transformation.
We first describe the imaging model, and a maximum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Vignetting: ideal case

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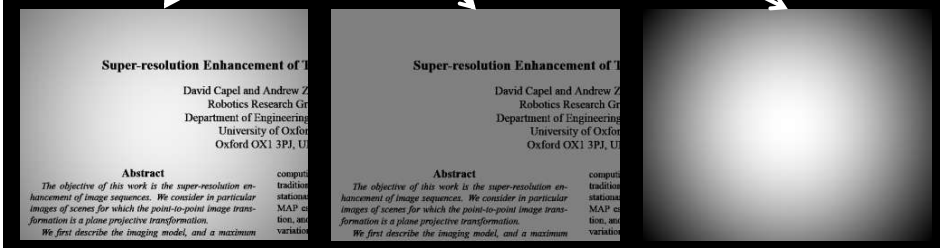
$$I = (F + B)\,IF$$



Vignetting: real case

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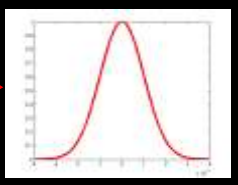
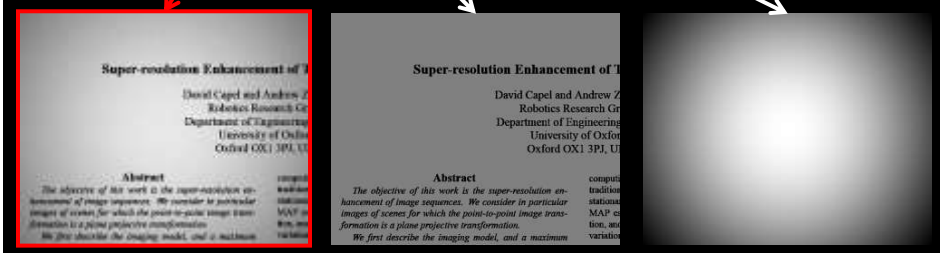
$$I = (F + B)\,IF + n$$





Vignetting: ideal case

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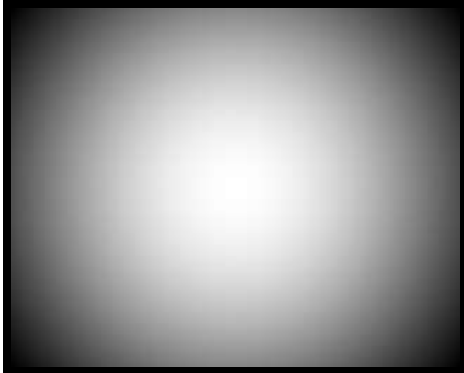
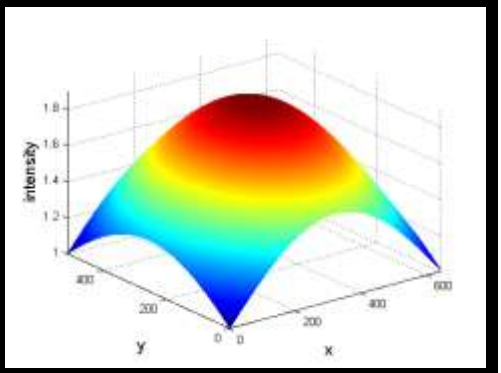
THEORETIC ILLUMINATION FIELD





Vignetting: ideal case

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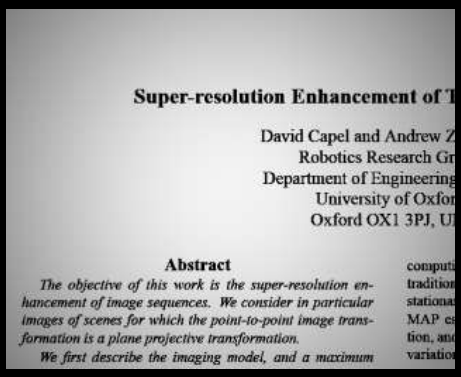
FLAT FIELD CORRECTION

1) $I = (F + B)\ IF$

2) $I_{ff} = I / IF = (F + B)$



Super-resolution Enhancement of T

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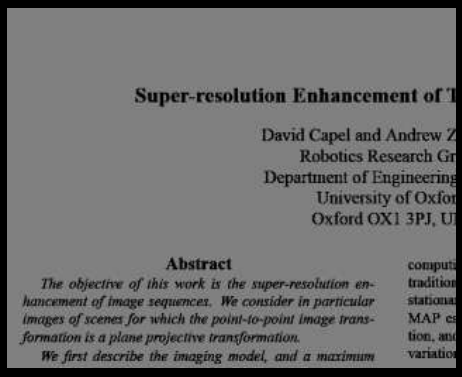

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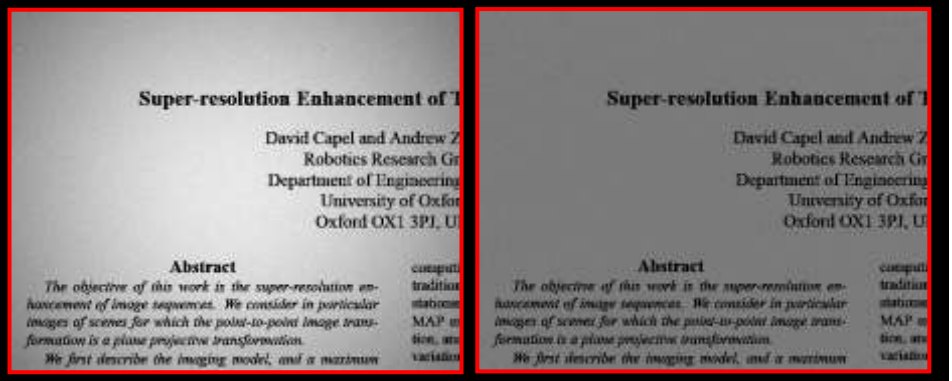
Vignetting: real case

f.piccinini@unibo.it

FLAT FIELD CORRECTION

1) $I = (F + B)\, IF + n$

2) $I_{ff} = I / IF = (F + B) + n'$



Vignetting

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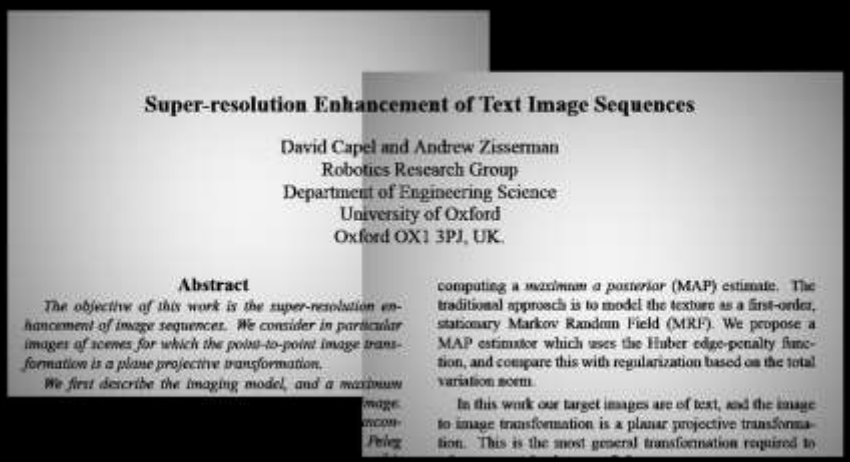

Vignetting

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Super-resolution Enhancement of Text Image Sequences

David Capel and Andrew Zisserman
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Department of Engineering Science
University of Oxford
Oxford OX1 3PJ, UK.

Abstract

The objective of this work is the super-resolution enhancement of image sequences. We consider in particular images of scenes for which the point-to-point image transformation is a plane projective transformation.

We first describe the imaging model, and a maximum

image.
incon-
Peleg

computing a *maximum a posterior* (MAP) estimate. The traditional approach is to model the texture as a first-order, stationary Markov Random Field (MRF). We propose a MAP estimator which uses the Huber edge-penalty function, and compare this with regularization based on the total variation norm.

In this work our target images are of text, and the image to image transformation is a planar projective transformation. This is the most general transformation required to

Vignetting

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David Capel and Andrew Zisserman
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In this work our target images are of text, and the image to image transformation is a planar projective transformation. This is the most general transformation required to

Vignetting - Flat field correction

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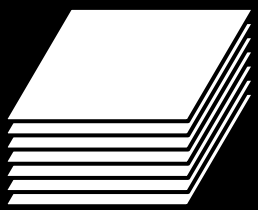
FLAT FIELD CORRECTION

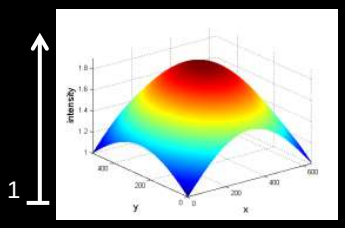
$I_{ff} = I / IF$

Typically, the illumination field is detected by an empty field (for example a white paper) acquired in advance and often temporally averaged (Light Field, LF)

I) LF = temporal mean (EF_i)



II) IF = LF/ min (LF)



Vignetting - Flat field correction

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FLAT FIELD CORRECTION

$I_{ff} = I / IF$

Typically, the illumination field is detected by an empty field (for example a white paper) acquired in advance and often temporally averaged (Light Field, LF)

PROBLEM:

To know the illumination field is not always possible, for examples in real time applications or when the acquisition device is not available

SOLUTION?

VIGNETTING FUNCTION FROM IMAGES' BACKGROUND REGIONS

Illumination field from background

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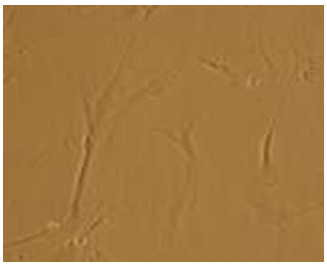
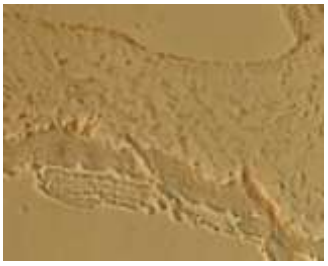
IN OPTICAL MICROSCOPY

Cell cultures and histological examinations cover the most relevant part of the biological routine examinations





In this kind of images the background (the culture's medium and the specimen support, respectively) can be considered as being uniform

Illumination field from background

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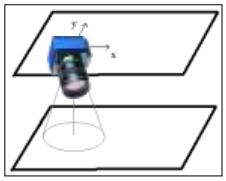
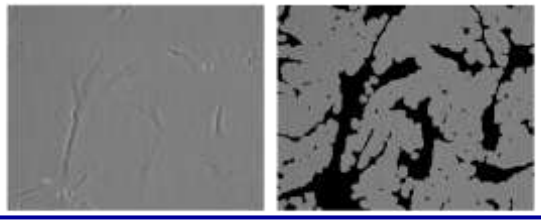
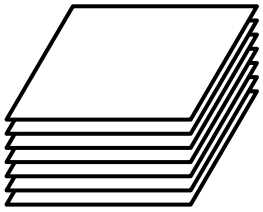
STAGE 1
image acquisition
and gray conversion



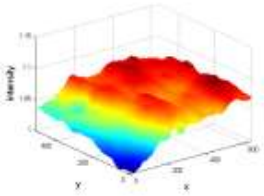
STAGE 2
background detection



STAGE 3
temporal
median filter



STAGE 4
illumination
field estimation



Illumination field from background

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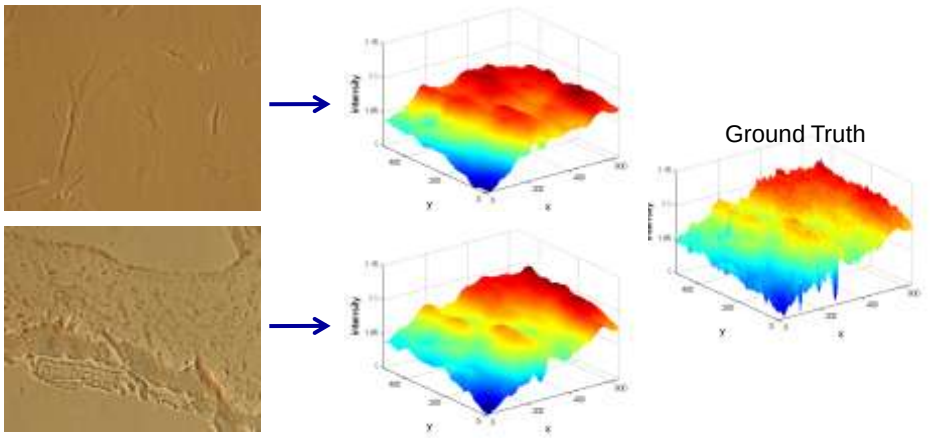
STAGE 4: illumination field estimation



Flat field correction

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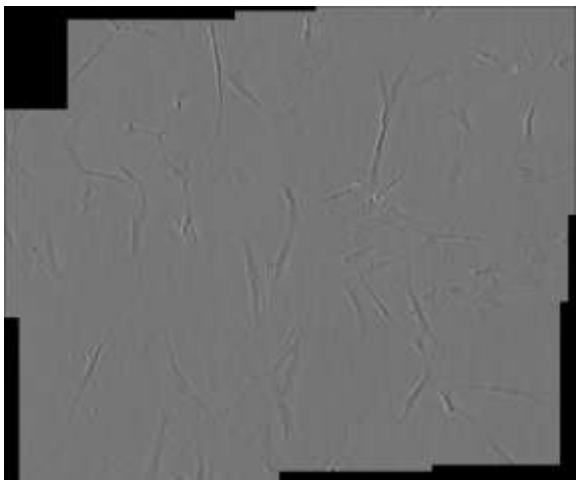

Mosaic with flat field correction

Flat field correction

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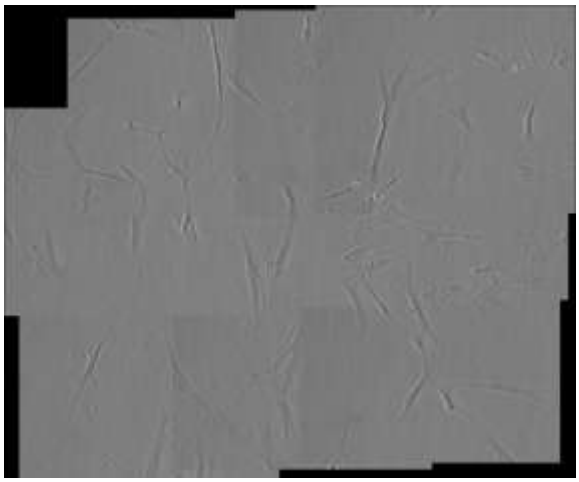
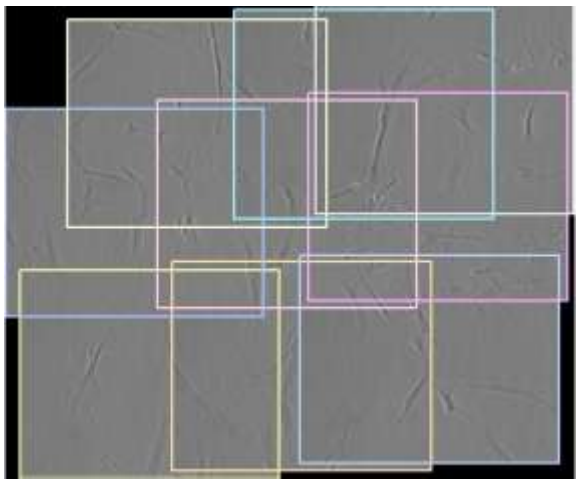


INPUT: 9 images (512 x 640). OUTPUT: mosaic (1162 x 1407)

Flat field correction

f.piccinini@unibo.it



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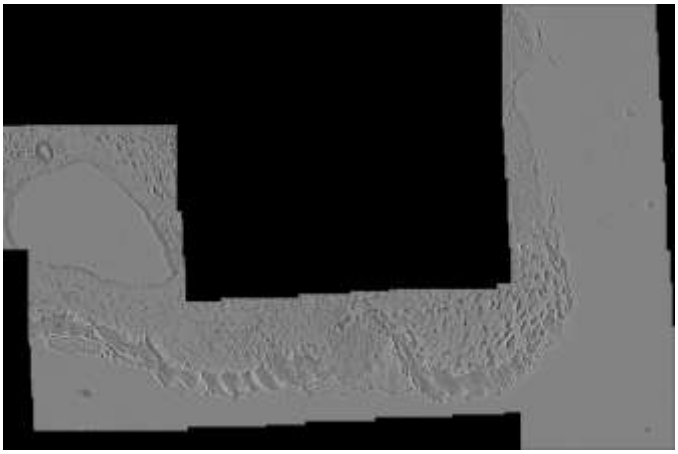

Mosaic with flat field correction

Flat field correction

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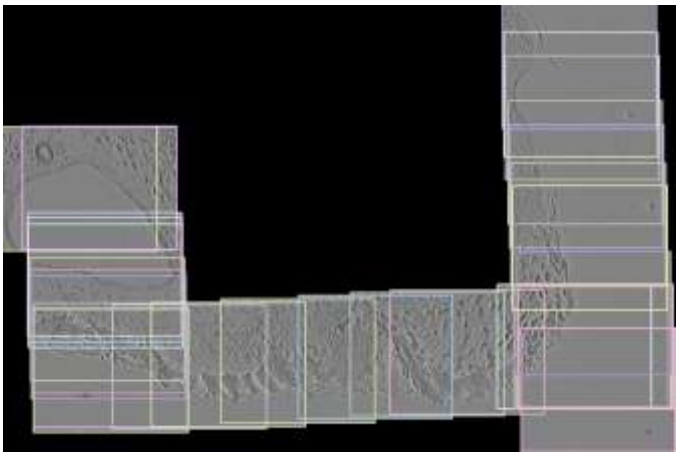

INPUT: 38 images (512 x 640). OUTPUT: mosaic (1847 x 2787)

Flat field correction – Error metrics

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- Root Mean Squared Error – RMSE

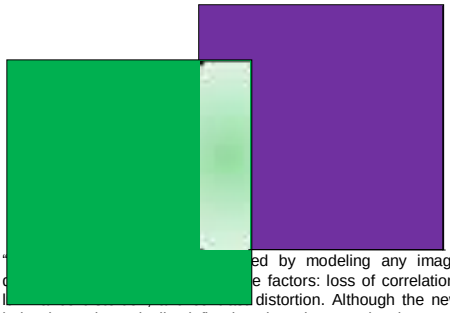
$$RMSE = \sqrt{\frac{\sum_x \sum_y (I(x,y) - R(x,y))^2}{N}}$$

- Signal to Noise Ratio – SNR

$$SNR = 10 * \log_{10} \frac{\sum_x \sum_y R(x,y)^2}{\sum_x \sum_y (I(x,y) - R(x,y))^2}$$

- Universal Quality Index – UQI (*)

$$UQI = \frac{4 * \sigma_{IR} * \bar{I} * \bar{R}}{(\sigma_I^{\,2} + \sigma_R^{\,2}) * (\bar{I}^2 + \bar{R}^2)}$$



"…ed by modeling any image …e factors: loss of correlation, … distortion. Although the new index is mathematically defined and no human visual system model is explicitly employed, our experiments on various image distortion types indicate that it performs significantly better than the widely used distortion metrics…"

(*) Zhou Wang and Alan C. Bovik, "*A Universal Image Quality Index*", IEEE Signal Processing Letters, Vol. 9, No 3, pp. 81-84, March 2002.

Flat field correction – Error metrics

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	RMSE	SNR	UQI
CELL w/o Flat Field	3.76	30.45	0.76
CELL Flat Field	2.08	35.10	0.90
HISTOLOGICAL w/o Flat Field	4.75	29.29	0.93
HISTOLOGICAL Flat Field	3.25	32.02	0.96

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Flat field correction – Error metrics

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Color vignetting correction

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R G B images

Color vignetting correction

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Input: RGB image not flat field → $\text{Image}_{RGB}\text{IN} = f(R,G,B)$

RGB to GRAY conversion → $GRAY = 0.299*R + 0.587*G + 0.114*B$

Flat field correction → $\frac{GRAY}{IF} = 0.299*\frac{R}{IF} + 0.587*\frac{G}{IF} + 0.114*\frac{B}{IF}$

Output: RGB image flat field → $\text{Image}_{RGB}\text{OUT} = f(\frac{R}{IF},\frac{G}{IF},\frac{B}{IF})$

ALGORITHM

```
INPUT: RGB image not flat field
    for each RGB channels {
        New channel = Old channel / Illumination Field
    }
OUTPUT: RGB image flat field
```

Color vignetting correction

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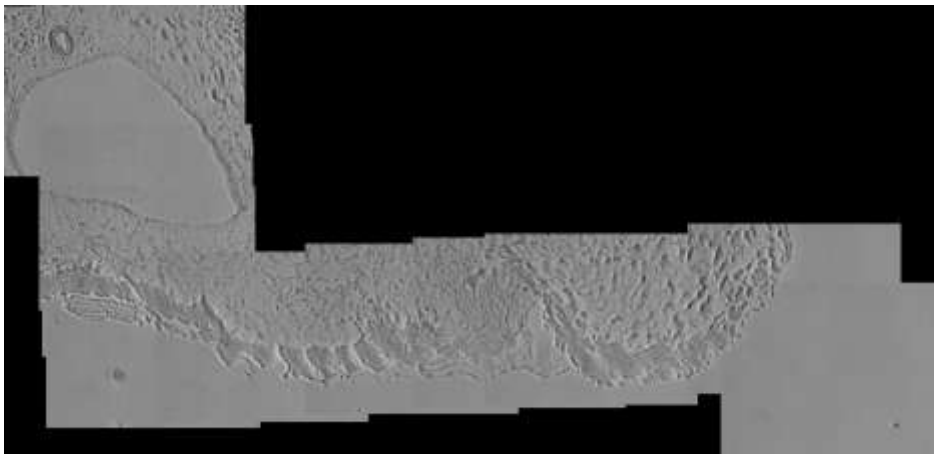

GRAY mosaic without flat field correction

Color vignetting correction

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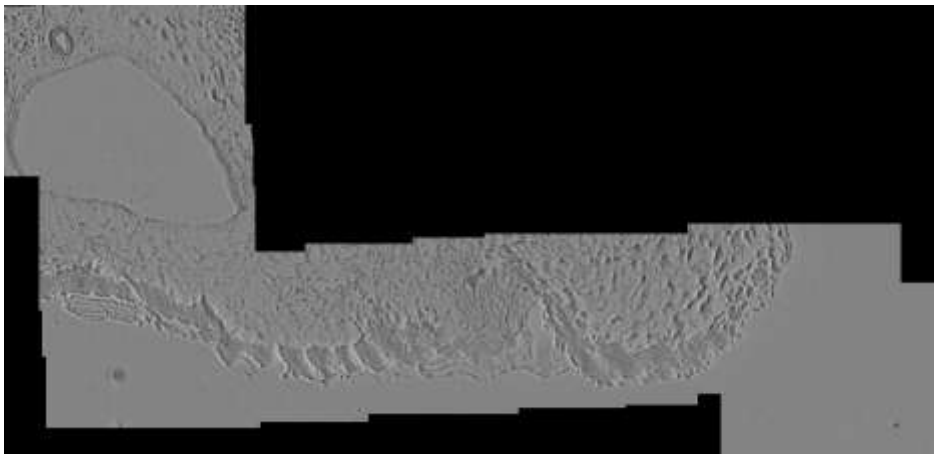

GRAY mosaic with flat field correction

Color vignetting correction

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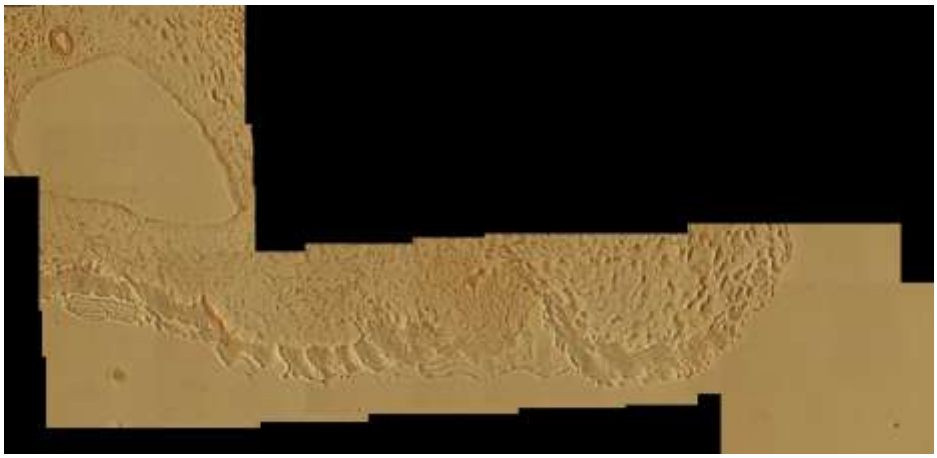

RGB mosaic without flat field correction

Color vignetting correction

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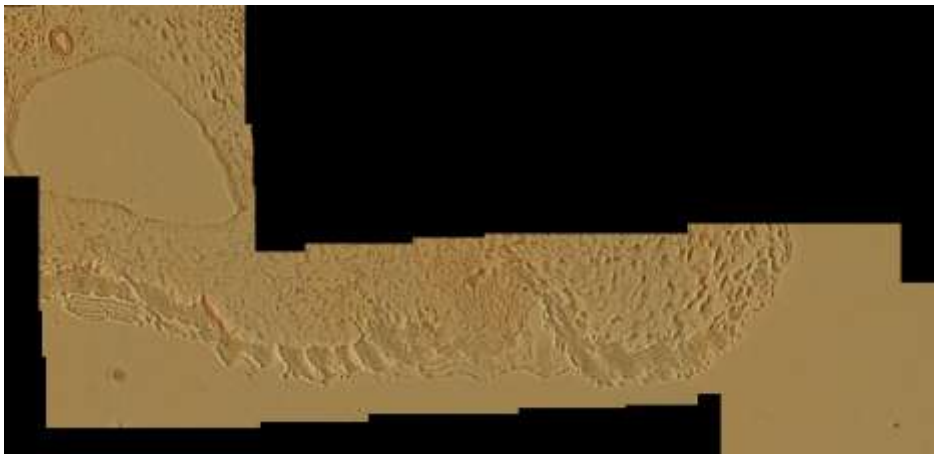

RGB mosaic with flat field correction

Saturation

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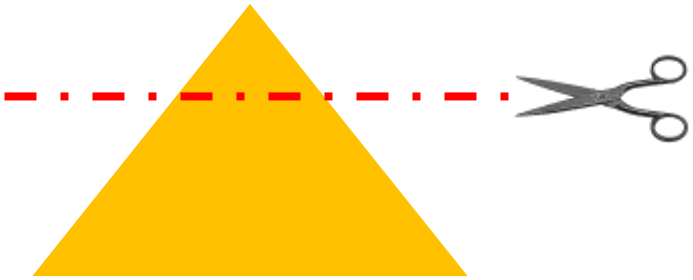
DEFINITION: image in saturation

Image with some regions in which the intensity values are all at the maximum value

PROBLEM

- Where image is in saturation you cannot understand the original curve



Saturation

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DEFINITION: image in saturation

Image with some regions in which the intensity values are all at the maximum value

PROBLEM

- Where image is in saturation you cannot understand the original curve
- When one or several channels are in saturation the color flat field correction generates incoherent colors

IMPORTANT NOTE

If there is even a channel not in saturation, the RGB to GRAY conversion generates a output image not in saturation and, in this case, there aren't any problems with the GRAY flat field correction.

Correction with saturation

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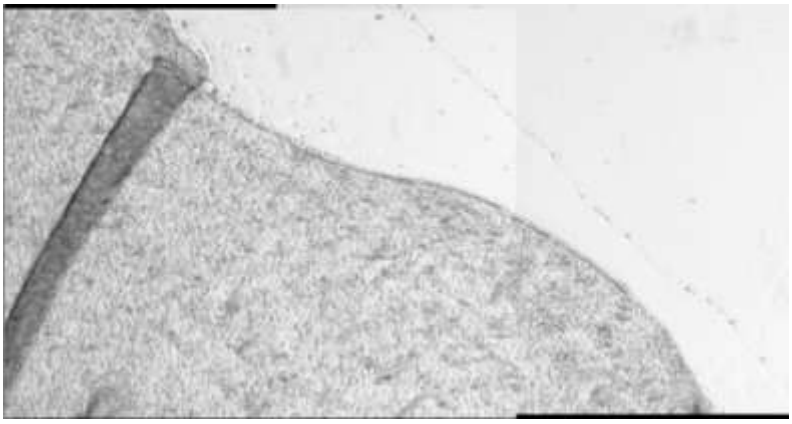
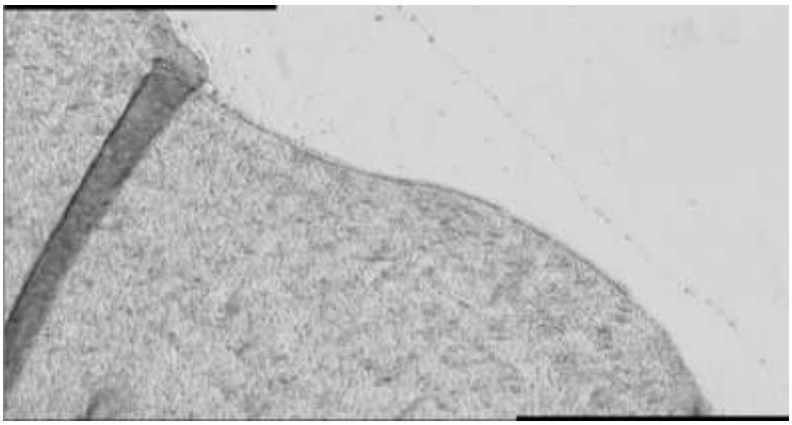


GRAY mosaic without flat field correction

Correction with saturation

f.piccinini@unibo.it



GRAY mosaic with flat field correction

Correction with saturation

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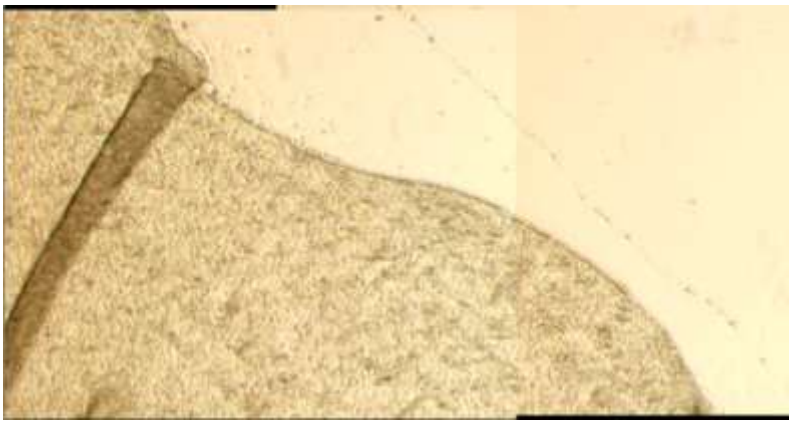

RGB mosaic without flat field correction

Correction with saturation

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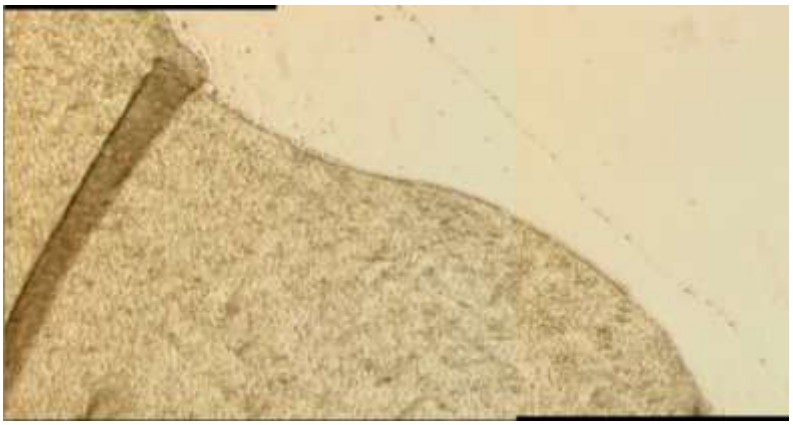

RGB mosaic with flat field correction

Correction with saturation

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IDEA

Use a dynamic remapping color LUT (Look Up Table) to force that for each gray level there is only a RGB conversion color.

PRACTICAL SOLUTION

During mosaicing operation, when is detected a image in saturation, LUTs are calculated by comparing flat field mosaic in gray and in RGB (with incoherent colors due to the flat field correction applied on images in saturation). One LUT is referred to background regions and the other LUT is referred to foreground region. For each GRAY level in each region, one and only one RGB conversion is permitted. To select the conversion, a mean value is calculated for each channel, of the RGB mosaic, with the same GRAY conversion. LUT are dynamical improved with the new images added to the mosaic .

…
10
11
15
…

LUT of the background

…	…	…
10	10	10
13	10	11
25	10	15
…	…	…

Correction with saturation

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ALGORITHM

```
when RGB image in saturation is added to mosaic {
    for each intensity levels in GRAY mosaic {
        LUT_fg  = a look up table from foreground is calculated
        LUT_bg = a look up table from background is calculated
    }
    RGB mosaic is remapped using the two LUT
}
after for each new RGB image added to mosaic {
    if there are new intensity levels in the flat field GRAY image {
        LUT_fg  is improved
        LUT_bg is improved
    }
    RGB image is remapped using the two LUT
}
```

Correction with saturation

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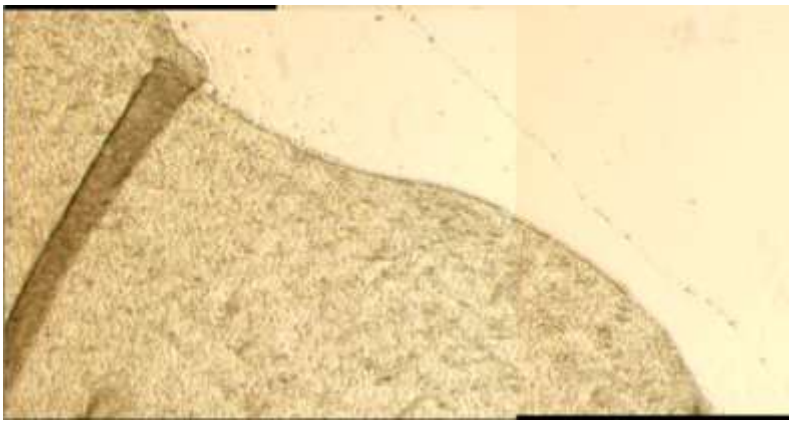

RGB mosaic without flat field correction

Correction with saturation

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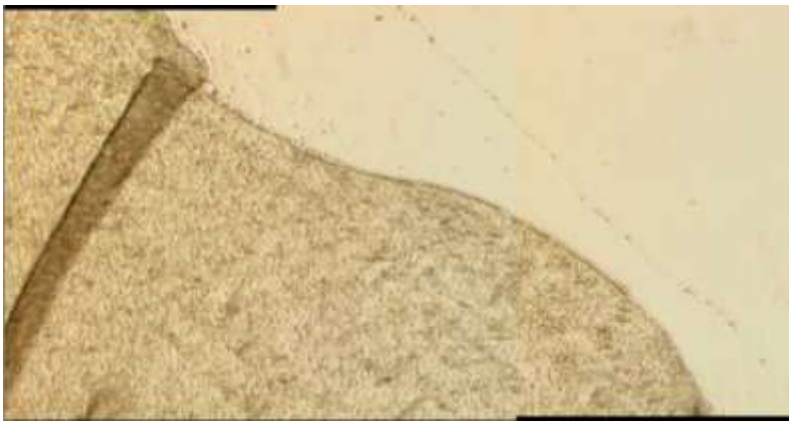

RGB mosaic with flat field correction and without saturation correction

Correction with saturation

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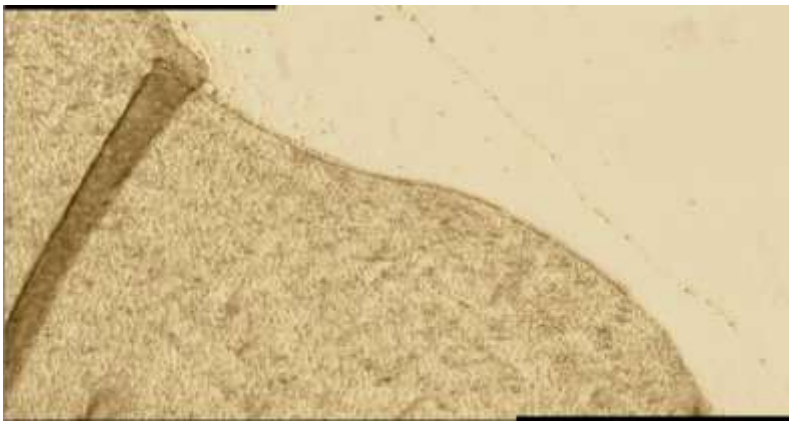

RGB mosaic with flat field correction and saturation correction

Outline

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- Real time mosaicing
- Vignetting correction
- Microscope calibration
- Depth from focus
- Publications
- Contacts

Microscope calibration

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MOTIVATIONS:

- To know the real size of the observed objects
- To understand if something in microscope it is wrong

NORMAL PROCEDURE:

Typically, when you buy a microscope with a camera, you could ask to the selling company to calibrate the system to understand the conversion factor “µm/pixel”. It is commonly done using a set of reference gold standard grids of which they know very accurately the size of each different square.

PROBLEMS:

- Every time you change something in the setup (e.g.: move the camera) of the system the calibration should be redone
- High cost
- Time to call the technician of the selling company with the grid

OUR IDEA:

To use a secondary reference object to calibrate ourselves systems composed by microscope and camera. It permits to save money and time.

Microscope calibration

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HYCOR KOVA GLASSTIC SLIDE

Disposable microscope slide made of optically clear plastic. Designed for microscopic examination of urine and other body fluids. Commonly used to count cells thanks to the printed counting grid.

UNIT PRICE: only 1 dollar!!!

Microscope calibration

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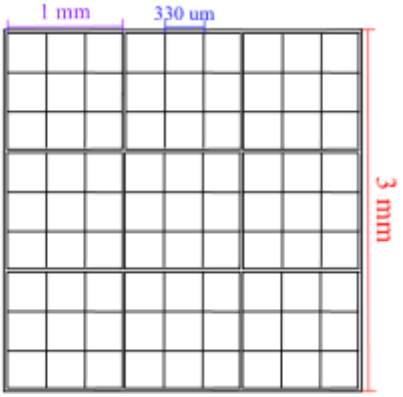
SIZE DIMENSIONS:

After a correspondence of about 7 email and several telephone calling with the selling company, and a verification test conducted with a microscope calibrated with a gold standard, we have prove that:

- smallest squared grid dimensions between line centroids: 330 µm ± 6%
- lines dimension: 25 µm ± 10%



Microscope calibration

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METHOD

- For each microscope's objective
- Whenever microscope's setup change

Take a image of a Kova Slide grid → Measure the pixel size of a small square → Calculate the conversion coefficient (CC) "µm/pixel"

Objective 5x	Objective 10x	Objective 20x	Objective 40x
			
CC = 1,24 ± 10%	CC = 0,62 ± 10%	CC = 0.31 ± 10%	CC = 0,16 ± 15%

Microscope calibration

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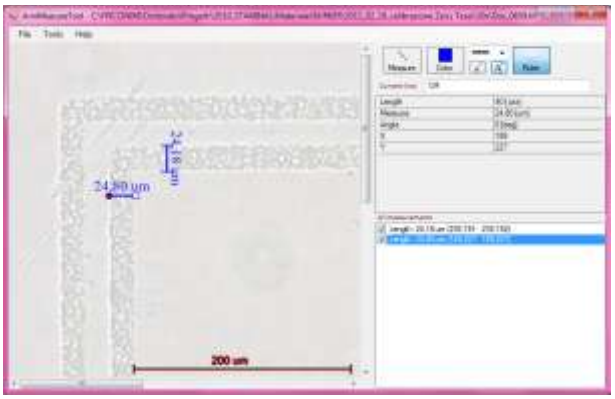
C# SOFTWARE: ANN MEASURE TOOL

User friendly image editor used to:

- Calibrate microscope system through a reference object
- Measure and label image object



Courtesy of Dr. Alessandro Gherardi

Outline

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- Real time mosaicing
- Vignetting correction
- Microscope calibration
- Depth from focus
- Publications
- Contacts

Depth from focus

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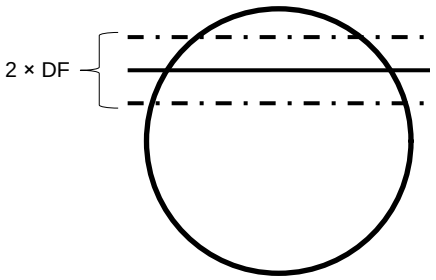
DEPTH OF FOCUS (DF)

the distance over which the image plane can be displaced while a single object plane remains in acceptably sharp focus. Knowing several characteristics of camera and lenses is theoretically possible to calculate the DF.

http://www.microscopyu.com/tutorials/java/depthoffield/index.html



Depth from focus

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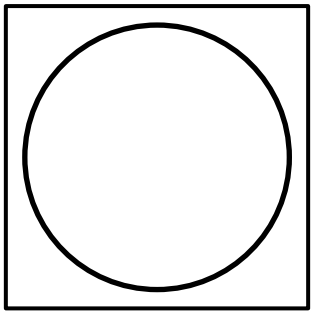
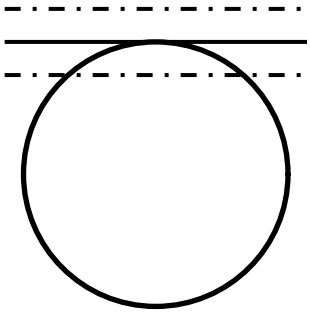
OBJECT RECONSTRUCTION

Starting from a images' stack of the same object, where images are acquired at different depth of plane (Z-plane), if the Z distance between two subsequent image is less that DF, is possible use the stack to reconstruct the single 2D image completely sharp of the object.



2D RECONSTRUCTED IMAGE



Depth from focus

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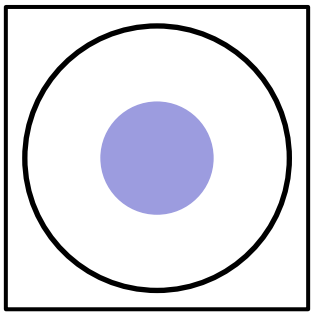
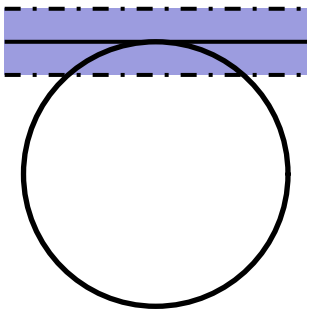


2D RECONSTRUCTED IMAGE



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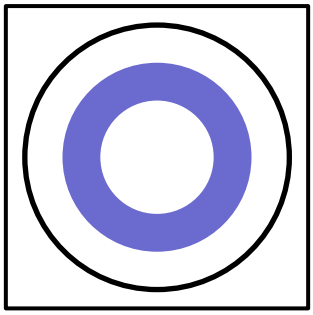
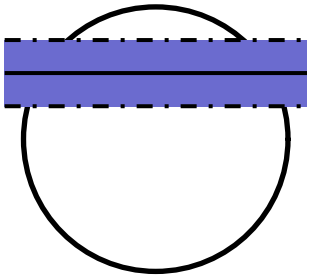


2D RECONSTRUCTED IMAGE



Depth from focus

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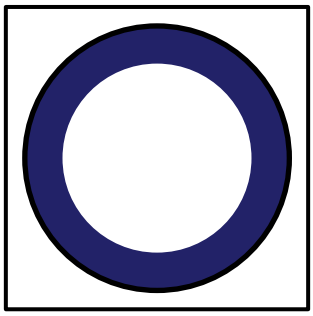
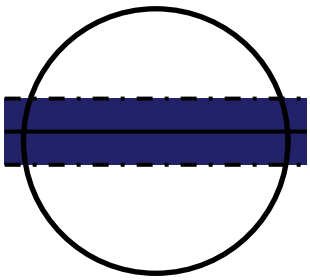


2D RECONSTRUCTED IMAGE



Depth from focus

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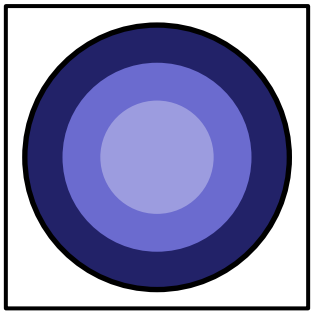
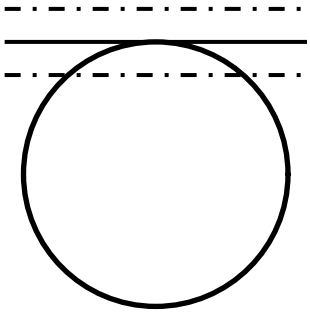


2D RECONSTRUCTED IMAGE



Depth from focus

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BIOLOGICAL APPLICATIVE CONTEST

PROJECT STAMINAL

Characterization of stem cells through automatic analysis of microscopic images in preclinical therapy.

Collaboration with the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, IRST (Meldola, FC, Italy)

The IRST Pre-Clinical Pharmacology Lab is the first to isolate of stem cells from normal lung tissue of adult humans and to obtain spheroids.

GOAL

To study spheroids properties and behaviors to build solid biological model, useful to study treatment and drugs' effects against cancer diseases.

Depth from focus

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PROBLEM

The obtained spheroids have a diameter much greater of the depth of focus. A single 2D image acquired with optical microscopy is not sufficient to observe a spheroid with all boundary regions sharp.

SOLUTION

2D plane spheroid reconstruction starting from a stack of images in which depth difference between Z-plane of two subsequent images are less that value of depth from focus of the system.

MATERIALS

Spheroid cultures and microscope system compose by non-automated inverted Zeiss Axiovert 200 microscope and Zeiss Axiocam MRc camera. The micrometric microscope holder's shift is of 1 µm (from data sheet). For each observed spheroid we have acquired a sequence of several images in which depth between Z-planes is 5 µm.

Depth from focus

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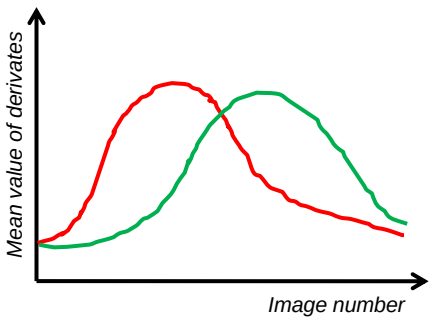
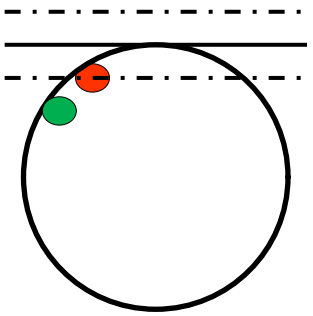
ALGORITHM

While an object's region is in focus, it is sharp and well defined. When the focus is lost the region became smooth. Starting from a stack of images acquired at different Z-plane positions, to understand the image in which an object's region is at the best focus, we can search in the stack the maximum of derivatives mean value of the small region.



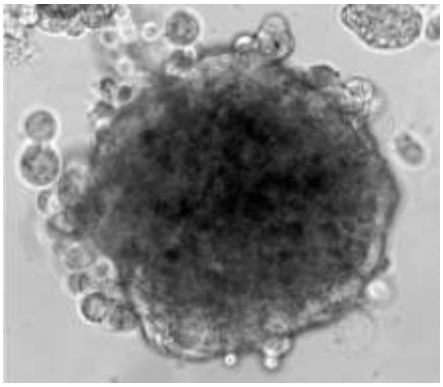


Depth from focus

f.piccinini@unibo.it

REAL CASE

- Sequence of 45 images relative to a spheroid of lung cancer cells.
- Each images is acquired moving holder of step of 5 µm in Z.



Depth from focus

f.piccinini@unibo.it

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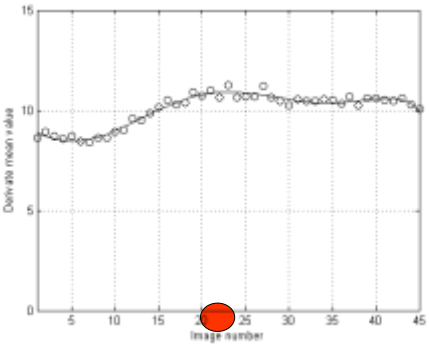
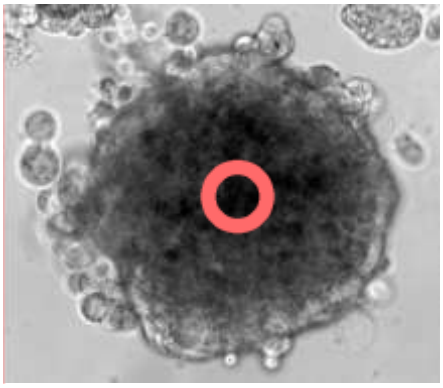
- Sequence of 45 images relative to a spheroid of lung cancer cells.
- Each images is acquired moving holder of step of 5 µm in Z.





Depth from focus

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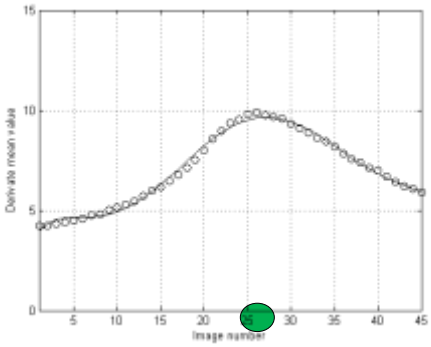
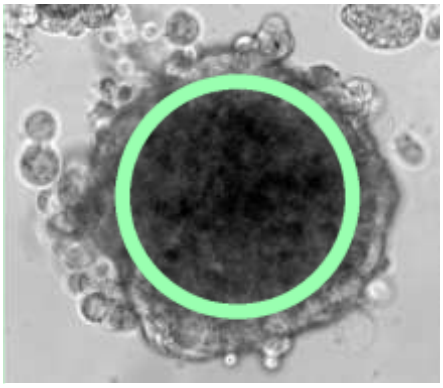
REAL CASE

- Sequence of 45 images relative to a spheroid of lung cancer cells.
- Each images is acquired moving holder of step of 5 µm in Z.





Depth from focus

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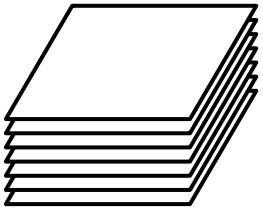
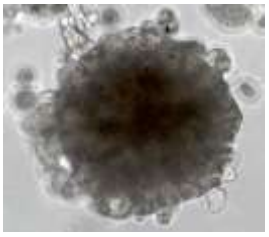
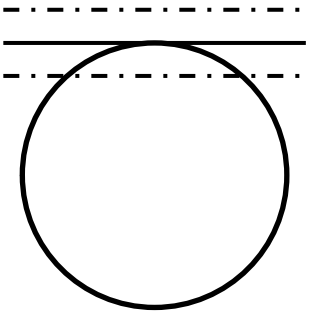
INPUT

Images stack







ALGORITHM PIPELINE

Derivatives mask calculated for each image → Strong spatial mean filter on each derivatives mask → Temporal maximum value for each X Y position → Mask of indices referred to images with the maximum values → Strong spatial mean filter on the mask of indices → 2D image reconstructed with values copied from images' stack according to the mask of indices

Depth from focus

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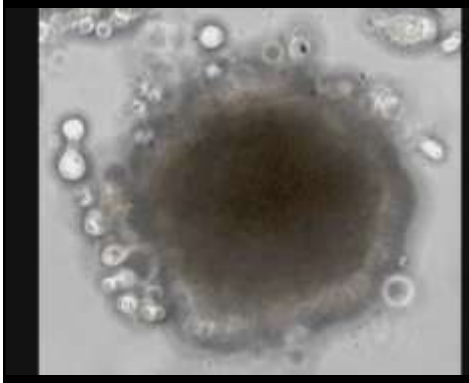
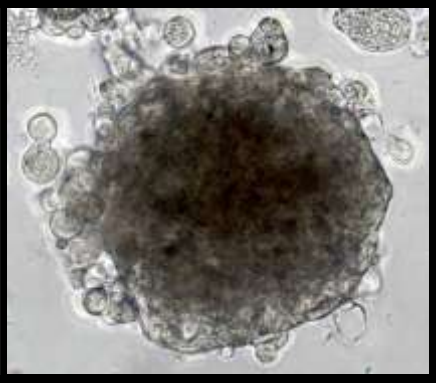
INPUT:
IMAGES STACK



OUTPUT:
OUR METHODS



Depth from focus

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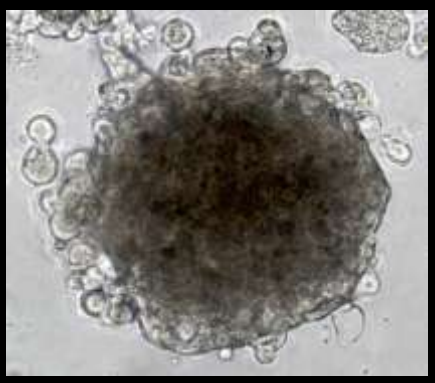
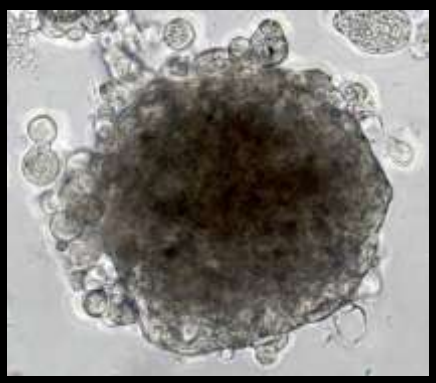
OUTPUT:
OTHER METHODS



OUTPUT:
OUR METHODS



MICHAEL UMORIN IMAGEJ PLUGIN
http://imagej.nih.gov/ij/plugins/stack-focuser.html

Depth from focus

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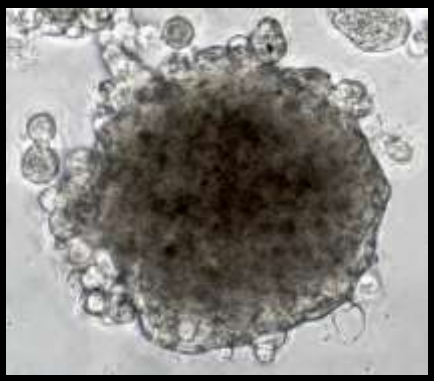
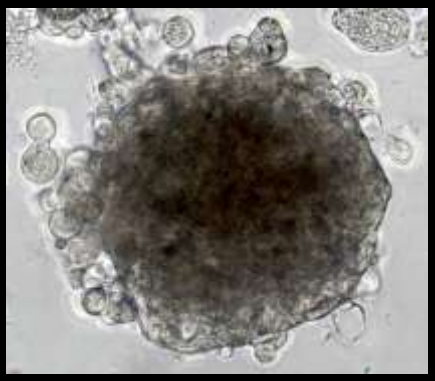
OUTPUT:
OTHER METHODS



OUTPUT:
OUR METHODS



MICHAEL UNSER (EPFL) IMAGEJ PLUGIN
http://bigwww.epfl.ch/demo/edf/index.html

Outline

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- Real time mosaicing
- Vignetting correction
- Microscope calibration
- Depth from focus
- Publications
- Contacts

Publications

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- A. Bevilacqua, F. Piccinini and A. Gherardi, "***Vignetting correction by exploiting an optical microscopy image sequence***", submitted to the 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS), Boston, USA, August 30 – September 3, 2011.
- A. Bevilacqua and F. Piccinini, "***Is an empty field the best reference to correct vignetting in microscopy?***", submitted to the 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS), Boston, USA, August 30 – September 3, 2011.
- A. Gherardi, A. Bevilacqua, and F. Piccinini, "***Illumination field estimation through background detection in optical microscopy***", IEEE Symposium on Computational Intelligence in Bioinformatics and Computational Biology (CIBCB), Paris, France, April 11-15, 2011, pp. 49-54.
- L. Carozza, A. Bevilacqua, and F. Piccinini, "***An Incremental Method for Mosaicing of Optical Microscope Imagery***", IEEE Symposium on Computational Intelligence in Bioinformatics and Computational Biology (CIBCB), Paris, France, April 11-15, 2011, pp. 55-60.

IN PREPARATION FOR SUBMITTION TO JOURNAL OF MICROSCOPY

A. Bevilacqua, F. Piccinini and A. Gherardi, "***Vignetting correction by exploiting an optical microscopy image sequence***", extension of article submitted to the 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS).

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- Prof. Alessandro Bevilacqua, email: alessandro.bevilacqua@unibo.it
- Computer Vision Group, University of Bologna: lab and web

http://www.ing2.unibo.it/Ingegneria+Cesena/Studenti/Laboratori

http://cvg.deis.unibo.it/

THANK YOU!